To: MDDT Program Coordinator

RE: **MDDT Qualification Submission**

Dear Sir/Madam: January 23, 2017

Please accept this **MDDT pre-qualification proposal.** The MDDT being proposed is a **Clinical Outcome Assessment** called **eeDAP, an evaluation environment for digital and analog pathology**. It is being submitted by Brandon D. Gallas in the FDA/CDRH's Division of Imaging, Diagnostic, and Software Reliability (DIDSR) in the Office of Science and Engineering Laboratories (contact information below). We are sharing the MDDT qualification effort and experience with the WSI Working Group (https://nciphub.org/groups/wsi_working_group).

The Context of Use for eeDAP is as follows:

eeDAP is a Clinical Outcome Assessment used in reader studies for whole slide imaging premarket submissions (PMA or 510k deNovo) to compare the accuracy or reproducibility of pathologist evaluations of digital images on a display to those of glass slides on a microscope. The pathologist evaluations of patient tissue are the clinical outcomes. The accuracy or reproducibility of the pathologist evaluations is the clinical outcome assessment; this assessment reflects image quality.

The eCopy is an exact duplicate of the paper copy.

Sincerely,

Brandon Gallas

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MDDT proposal for eeDAP: evaluation environment for digital and analog pathology

23 January 2017

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2 Product Description

eeDAP is being proposed as an MDDT for Clinical Outcome Assessments (COA). eeDAP is an evaluation environment for digital and analog pathology. eeDAP is a software and hardware platform for designing and executing digital and analog (microscope) pathology studies where the digital scan of a glass slide, or whole slide image (WSI) is registered to the real-time view of the corresponding glass slide on the microscope. This registration allows for different pathologists to evaluate the same fields of view (FOVs) in digital mode or in microscope mode. Consequently, it is possible to reduce or eliminate a large source of variability in comparing these modalities in the hands of the pathologist: the FOVs (the tissue) being evaluated. In fact, the current registration precision of eeDAP allows for the evaluation of the same individual cell in both domains. As such, a study can be designed where pathologists are asked to evaluate a preselected list of individual cells or specific FOVs in the digital mode and with the microscope. Consequently, paired observations from co-registered FOVs are collected allowing for a tight comparison between WSI and optical microscopy.

A reader study with eeDAP is intended to evaluate the scanned image, not the clinical workflow of a pathologist or lab. Instead of recording a typical pathology report, eeDAP enables the collection of explicit evaluation responses (formatted data) from the pathologist corresponding to very narrow tasks. This approach removes the ambiguity related to the range of language and the scope that different pathologists use in their reports.

Reader studies utilizing eeDAP are meant to focus on tasks related to specific histopathology features. Since certain image features can challenge image quality properties (color fidelity, focus quality, and depth of field), reader studies with tasks based on features can provide valuable information for the assessment of WSI and its role in clinical practice. eeDAP allows for the formulation of different types of tasks, many of which are currently available in eeDAP: free-text, integer input for counting tasks, a slider in a predefined range for a confidence scoring task (ROC task, receiver operating characteristic task), check boxes of specific

categories for a classification task, and marking the image for a search task. These simple tasks can be customized with moderate MATLAB programming skills to suit the study's specific purposes.

In what follows, we provide a quick summary of the eeDAP hardware and software. More details can be found in a paper by Gallas et al. [1].

2.1 eeDAP Hardware

The eeDAP hardware includes a microscope with a camera (mounted for simultaneous viewing with the eyepiece), a motorized stage (programmable with a stage controller and joystick), and computer with a monitor as shown in Figure 2.1. There is also a reticle in the microscope eyepiece. The reticle in the eyepiece is also synthesized in software; virtual reticle marks are superimposed on the digital WSI image and on the camera image. The reticle serves two purposes. The reticle marks allow for the localization of specific features during image registration. The reticle marks are also used to help reduce the area of the FOV or to specify individual cells to evaluate (create a *study* FOV).

eeDAP supports usb and FireWire (IEEE 1394) cameras. The camera pixel sizes tested are smaller than 10um. At 40x magnification, this corresponds to camera pixels that cover areas smaller than 0.25 x 0.25 um^2; in the common parlance, we say that the camera resolution is 0.25 um/pixel. Regarding the motorized stage, eeDAP supports communications with Ludl and Prior stages. The step size for the stages tested are smaller than 0.1 um. The computer needs to be a Windows 7 operating system fast enough with memory and space to manage large WSI images (~10GB). Additionally, the computer must be able to communicate with the camera (usb or IEEE 1394) and with the stage (RS-232). There are no specific requirements of the display, and in fact, the display may be considered a component of the WSI system to be evaluated.

2.2 eeDAP Software

The eeDAP software is made up of graphical user interfaces (GUIs) written in MATLAB [2]. Using eeDAP does not require a full licensed version of MATLAB. It can be run as a precompiled stand-alone application. The precompiled stand-alone application requires that the free MATLAB compiler runtime (MCR) library be installed [2]. The software uses the Bio-formats library to read digital WSI images and extract FOVs [3]. Normally the resolution of 40x WSI images is about 0.25 um/pixel. The Bio-formats library supports several proprietary WSI image formats such as svs, ndpi and tiff. Please note that the Bio-formats library was not used in earlier versions of eeDAP; specifically, it was not used in the version described in the paper by Gallas et al. [1]. The change to Bio-formats was made because it is better supported, it works on 64-bit systems, and it is available under the GNU public "copyleft" licenses.

The eeDAP software is made up of three GUIs: **study initialization, global image registration, and data collection**. During **study initialization**, eeDAP reads a study-specific input file. The input file contains the filenames of the WSIs, hardware specifications, and the list of tasks with corresponding FOV locations that will be interpreted by the pathologist. At the end of the study initialization, eeDAP extracts all the WSI FOVs for fast access and transforms the colors so that the image viewed in eeDAP is the same as the image viewed on the scanner-specific viewer.

Global image registration is only done if eeDAP is run in MicroRT mode. The global image registration is equivalent to finding the mathematical relationship between the stage coordinates and WSI coordinates. It allows the stage to move to a location that is the same as the WSI location. The global image registration requires three anchors, three pairs of stage-WSI registered coordinates. Each anchor is generated by a local registration: an (x,y) stage coordinate and an (x,y) WSI coordinate that correspond to the same specimen location. The local registration is an interactive process with the study administrator. The study administrator

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- Camera
- Microscope
- Moving stage with multiple slides
- Stage controller
- Joystick for stage control
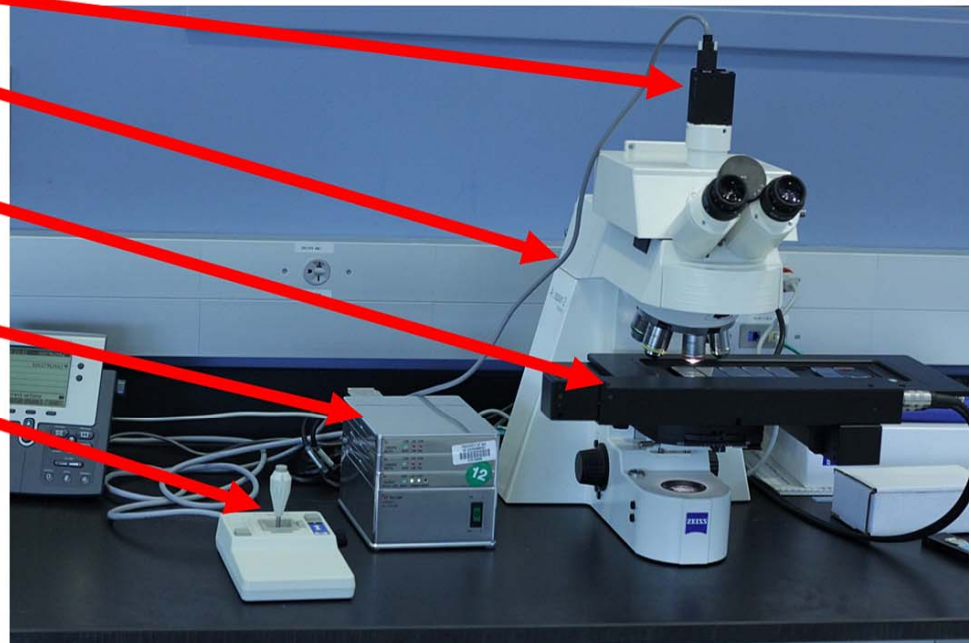- Computer and monitor not shown

Figure 1: The eeDAP hardware: microscope, camera, computer-controlled stage with joystick, and a computer with monitor (not shown).

navigates the microscope to a landmark, takes a snapshot of the microscope FOV with the microscope mounted camera, and eeDAP records the stage coordinates. Then the study administrator clicks near the corresponding location in the WSI image displayed in the GUI. Figure 2.2 shows global registration GUI and the camera image of a landmark location chosen by the study administrator. It also shows the corresponding location in the WSI image; the location the study administrator should click on next. eeDAP then finds the local registration by maximizing the match of the camera image and the patch of WSI selected by the study administrator.

Once we have solved for the global registration between the WSI image and the stage (via the camera image), we have to register the camera image and the eyepiece. This is an interactive process with the study administrator. While looking through the microscope, the study administrator navigates the stage so that a reticle mark lands at a precise location. After clicking on the GUI to record that location, the study administrator navigates the stage so that the virtual reticle mark superimposed on the camera image lands on the same precise location and clicks on the GUI to record that location. The difference in the two positions determines the shift needed to register the eyepiece view and the camera view.

The **data collection** GUI is the same for digital and microscope modes. The GUI shows the WSI FOV and has interfaces for collecting the pathologist's responses. The difference between modes is that, when run in digital mode, the pathologist sits at the computer and interacts with the data collection GUI. In microscope mode, a study administrator sits at the computer and interacts with the data collection GUI. Meanwhile, the pathologist is engaged with the microscope. The pathologist speaks his or her responses for the study administrator to enter into the GUI. The study administrator also has the responsibility of verifying that the microscope is accurately registered.

Figure 2.2 shows the data collection GUI with the WSI patch and the corresponding camera image. In both of these images are virtual reticle marks, though they are difficult to see in the figure. The study administrator verifies accurate registration by comparing the images and the virtual reticle marks. If registration is not accurate, there are buttons to perform an automated local registration of the current FOV. Finally, the FOV that the pathologist sees in the microscope is round and it is larger than what they see on the GUI, but they are supposed to be the same. The mismatch comes from a slight misuse the term FOV. We should really say that eeDAP allows for pathologists to evaluate the exact same *study* FOVs in both modalities. A study FOV is based on the reticle.

For the cameras and stages tested, we are able to repeatably and reliably register the WSI image and the glass slide so that the pathologists can evaluate the same FOVs in both modes.

3   Context of Use

3.1   COU Statement

eeDAP is a Clinical Outcome Assessment used in reader studies for whole slide imaging premarket submissions (PMA or 510k deNovo) to compare the accuracy or reproducibility of pathologist evaluations of digital images on a display to those of glass slides on a microscope. The pathologist evaluations of patient tissue are the clinical outcomes. The accuracy or reproducibility is the clinical outcome assessment; this assessment reflects image quality.

3.2   The device or product area for which the MDDT is to be qualified.

eeDAP is to be qualified for the evaluation of WSI systems, also known as virtual microscopy systems, which can digitize whole slides at microscopic resolution in a short period of time [4]. WSI systems are part
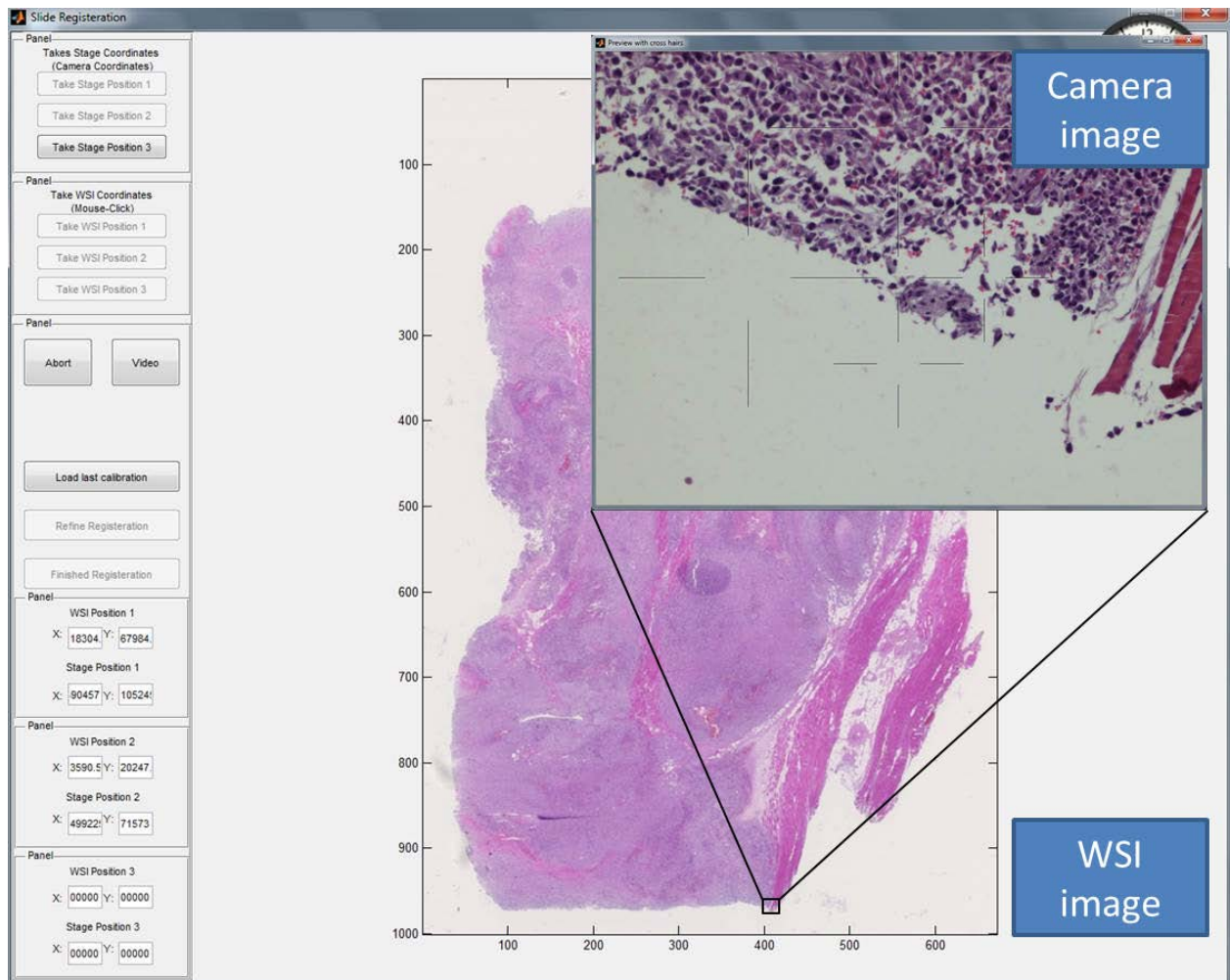
Figure 2: Screen shot of the image registration GUI, including the window showng the camera image.
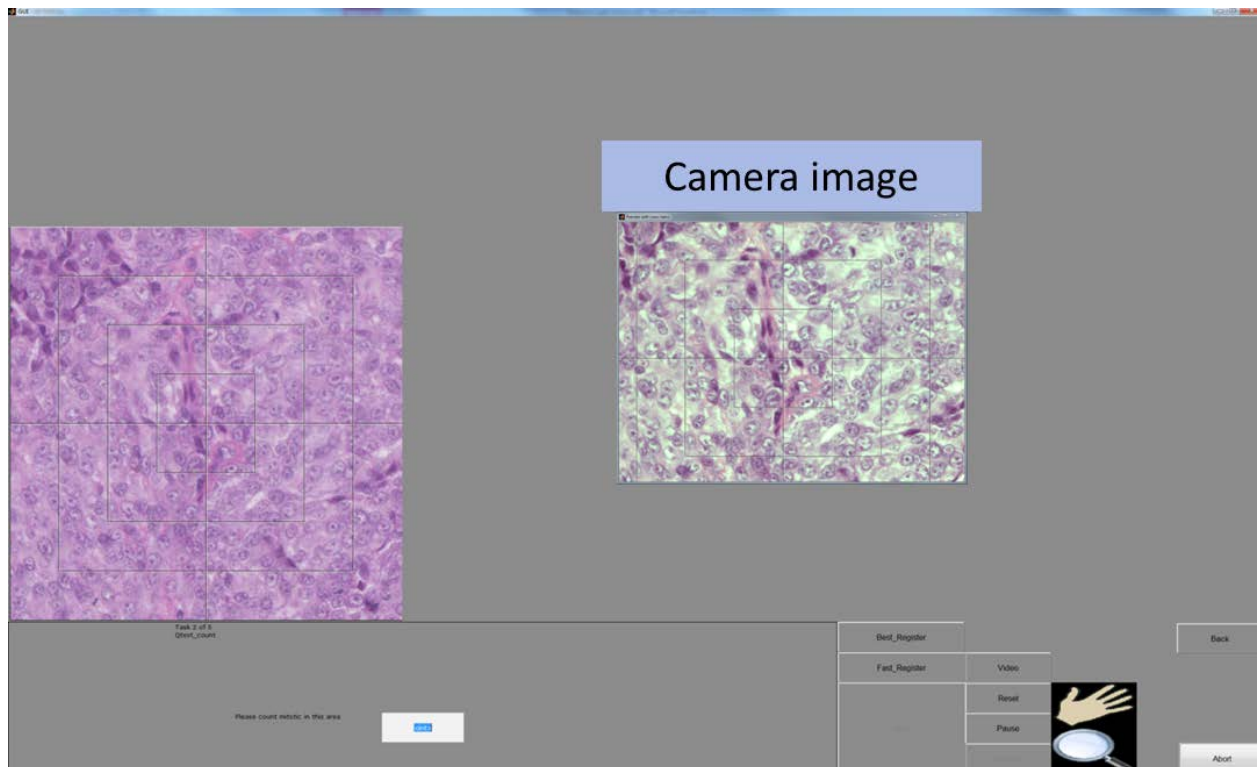
Figure 3: Screenshot of eeDAP data collection GUI with camera image of microscope view. In both of these images are virtual reticle marks, though they are difficult to see in the figure. The reticle marks help verify registration and to define a study FOV.

of the vision for digital pathology (DP). DP incorporates the acquisition, management, and interpretation of pathology information, including WSIs. The imaging chain of a WSI system consists of multiple components including the light source, optics, motorized stage, and a sensor for image acquisition. WSI systems also have embedded software for identifying tissue on the slide, auto-focusing, selecting and combining different fields of view (FOVs) in a composite image, and image processing (color management, image compression, etc.). Details regarding the components of WSI systems can be found in a paper by Gu and Ogilvie [5].

The potential public impact of WSI systems are well documented and include telepathology, digital consultation and slide sharing, pathology education, indexing and retrieval of cases, and the use of automated image analysis [6, 7, 8].

In addition to WSI systems, eeDAP could be used in the development and evaluation of WSI image analysis tools and CAD algorithms (computer aided diagnosis, detection, prognosis, etc.). Examples of such tools include:

- Color standardization/normalization

- Segmentation of tumors and other tissue structures

- Automated detection of different cell types and biomarkers

- Image retrieval

- Decomposing images containing multiple biomarkers (multiplex images)

- Diagnosis and prognosis, subtyping, and staging of cancer and non-cancer diseases

We do not plan to qualify eeDAP for the development of WSI systems or the development and evaluation of these adjunct products in this proposal in order to keep the COU focused. However, if eeDAP is qualified for the evaluation of WSI systems, it should also have value for the additional uses. A future MDDT proposal can consider expanding the COU if needed.

The stage of WSI development is advanced. WSI devices have received approval elsewhere in the world (European Union, UK and Ireland, and Canada.) In the United States, however, WSI devices are available for research and education, but they have not been approved for clinical use. The challenge for FDA approval is that there is not a clear pathway to approval. There is guidance for the technical assessment [9], but there is not guidance for the reproducibility/feature studies or the clinical trial. However, the FDA has communicated an outline for the clinical trial [10].

The stage of development of WSI image analysis tools and CAD algorithms is broad. There are likely applications that are ready for clinical use and applications that are still in the early stages of development. For the applications early in the development cycle, it is expected that the approval of WSI systems will lead to a substantial increase in data (images + annotations + outcomes). Consequently, it is expected that the pace of image analysis development will increase and more applications will be ready for clinical use in the near future.

3.3 The stage(s) of device development (e.g., early feasibility study, pivotal study, etc.) the MDDT will support

eeDAP is to be qualified to provide data for the analytic validation of a WSI system as is expected in a premarket submission. The specific regulatory path has not been established yet, but it is likely PMA or

510k deNovo. For analytic validation, the manufacturer must demonstrate that the WSI system creates digital images accurately and reliably for interpretation in the hands of the pathologist. Analytic validation complements the clinical performance studies and the technical performance assessment [9]: the technical performance of the WSI system and the components in the imaging chain, from image acquisition to image display. As the WSI technology matures, analytic validation has the potential to play a larger role in the approval process. Specifically, it is possible that analytic performance could be shown to be effective surrogate for clinical performance. If true, analytic performance could support the approval or clearance of WSI devices with a clinical study. Furthermore, it is possible that technical performance could predict analytic performance in the future as we understand both better. If true, technical performance could ultimately support the approval or clearance of WSI devices without analytic or clinical studies.

3.4 The specific role of the MDDT (for clinical uses this includes the study population or disease characteristics, as well as specific use – diagnosis, patient selection, clinical endpoints).

The rold of eeDAP is to be a platform for the design and execution of studies of pathologists performing task-based evaluations of tissue and cell features that are critical to diagnosis or differential diagnosis of disease. Such studies characterize image quality and the accuracy and reliability of the digital WSI images in the hands of the pathologist. When there is a reference (truth) result for the task, the endpoints of these studies characterize pathologist accuracy. When a reference result is not available (too burdensome or destroys the tissue), the endpoints of these studies measure the precision or reproducibility of pathologist evaluations: reader agreement from a single scan can provide a measure of precision, and reader agreement across multiple scans on the same or different WSI scanners can provide measures of reproducibility. Bias, correlation (or concordance), and percent agreement are typical agreement measures that can be used.

It is not always clear what level of accuracy and reproducibility is "good enough" for the analytic validation. This is why it is extremely useful to compare the accuracy and reproducibility of WSI evaluations to those from the glass slide on the microscope. This comparison controls for task difficulty and case selection. eeDAP allows for such a comparison, and it does so in a way that tightly reduces pathologist variability and correlates the results across the modalities (WSI and microscope), making for an efficient study in statistical and practical terms. Pathologist variability is reduced because all the pathologists evaluate the same FOVs. The results across the modalities are correlated because the pathologists are evaluating the same FOVs *in both modalities*. Reducing pathologist variability clearly improves statistical precision, and better precision allows studies with fewer resources. Regarding correlations, consider the following property of variances: $\mathrm{var}\,(A - B) = \mathrm{var}\,(A) + \mathrm{var}\,(B) - 2 \times \rho \sqrt{\mathrm{var}\,(A)\,\mathrm{var}\,(B)}$. Here we see that as the correlation ρ increases, the variance of the difference decreases, improving statistical precision and allowing smaller studies.

4 Advantages of eeDAP

The advantage of using eeDAP compared to typical clinical evaluation protocols is that eeDAP studies are fast and customizable, use fewer resources, and yield performance results that are more precise and reproducible.

The speed comes from the fact that FOVs are queued up and automatically presented to the pathologist; there is no need to search an entire slide. Additionally, the pathologist evaluations are recorded electronically in lock-step with the presentation of the FOVs. As such, there is little concern for transcribing errors.

Fewer resources (slides) are needed because the sampling unit is the FOV instead of the slide. Thanks to biological variability, the FOVs can often sample a diverse set of presentations of the feature (detectability, counts, orientations, morphologies, or classes). Precision and reproducibility are improved because pathologists are basing their evaluations on the same tissue.

Regarding precision and reproducibility, current practices lead to extremely noisy data. Specifically, let's consider the clinical protocol for mitotic counting as recommended by Smedly et al. [11]. The mitotic index, "should be determined by counting the number of mitotic figures in 10 consecutive hpf [sic. high-powered FOVs] commencing in the area of highest mitotic activity for oral and lip neoplasms and in random fields for cutaneous neoplasms." This protocol was followed in a study by collaborators at the NIH (Mark Simpson PI). As part of the study, the pathologists saved annotations of the FOVs that they used while evaluating the WSI. Figure 4 shows FOVs selected by different pathologists. There is very little overlap in the FOVs; pathologists are counting different mitotic figures from different tissue. Figure 4 shows the within-reader correlation of counts made using the microscope compared to the counts made using the WSI. Each point shows the counts from the same reader evaluating the same case in the two modes, and each count is the sum of 10 FOVs. It is clear that this data yields a lot of variability. The highlighted points show some extreme differences, and the highlighted band qualitatively identifies the variation in counts from H&E 40X WSI images when 10 counts are observed on the microscope.We expect to show that eeDAP will significantly reduce this variability by forcing pathologists to count mitotic figures in the same tissue.

5 Disadvantages of eeDAP

There are two disadvantages of eeDAP worth mentioning. First, when collecting data in digital mode, the image is displayed with Matlab. As such, eeDAP doesn't evaluate the native image viewer's human factors and workflow components. Also, there is currently no panning or zooming.

The other main disadvantage is that eeDAP is primarily designed for tasks that can be done on a finite FOV. The finite FOV may be at different magnifications, but eeDAP is not designed for complicated tasks that explore the WSI and aggregate different features. This disadvantage, however, keeps the focus of eeDAP on evaluating image quality.

6 Strength of Evidence

Tool Validity: Does the available data adequately support the validity of the measurement? Does the MDDT measure reliably and accurately? Depending on the tool type, this may include analytical, clinical, and construct validity, sensitivity, specificity, accuracy, precision, repeatability, external validity, reduction of bias, verification of the constitutive model, uncertainty quantification, numerical convergence, etc.

Plausibility: Is it scientifically plausible that the measurements obtained through use of the MDDT are related to the true outcome of interest? Is there a causal path or mechanistic explanation to connect the MDDT to the outcome?

Extent of Prediction: What data are available to demonstrate a predictive relationship between the MDDT and the true outcome of interest? What is the strength of that predictive relationship? Is the prediction repeatedly demonstrated in multiple studies or as a class effect? If relevant, is the conclusion (that the effect of treatment on the measurement obtained using the MDDT predicts the outcome of interest) supported by credible information?
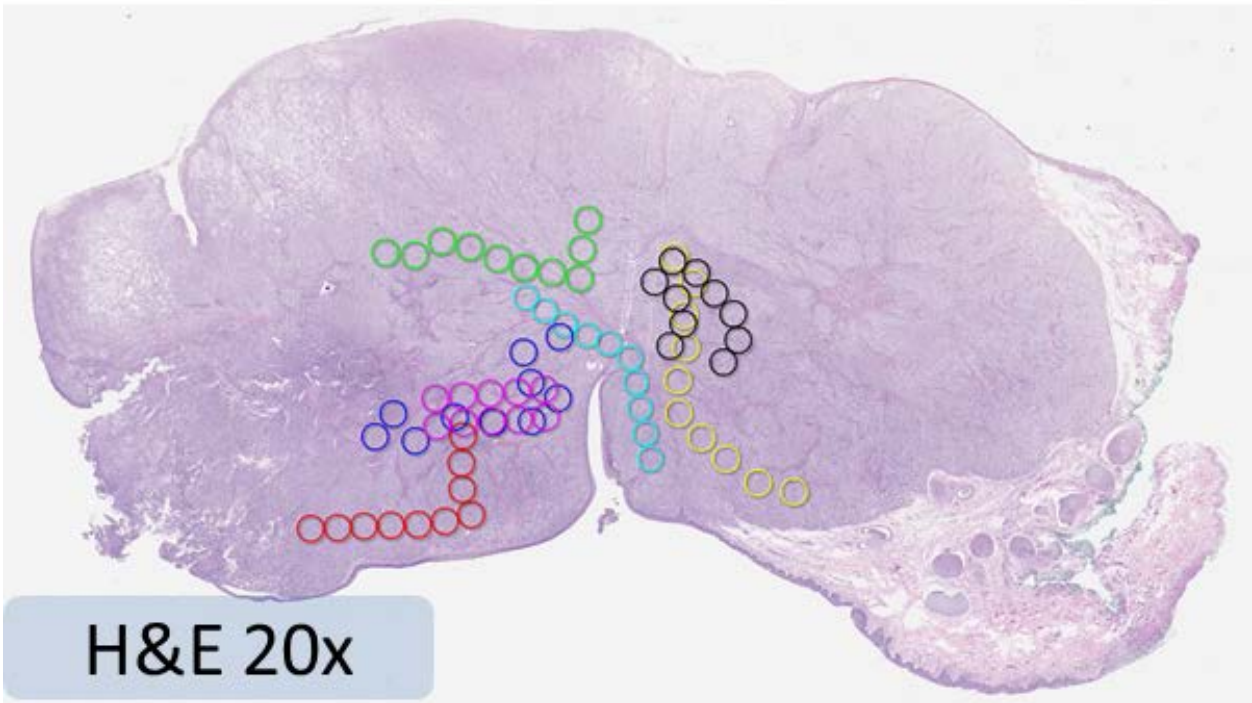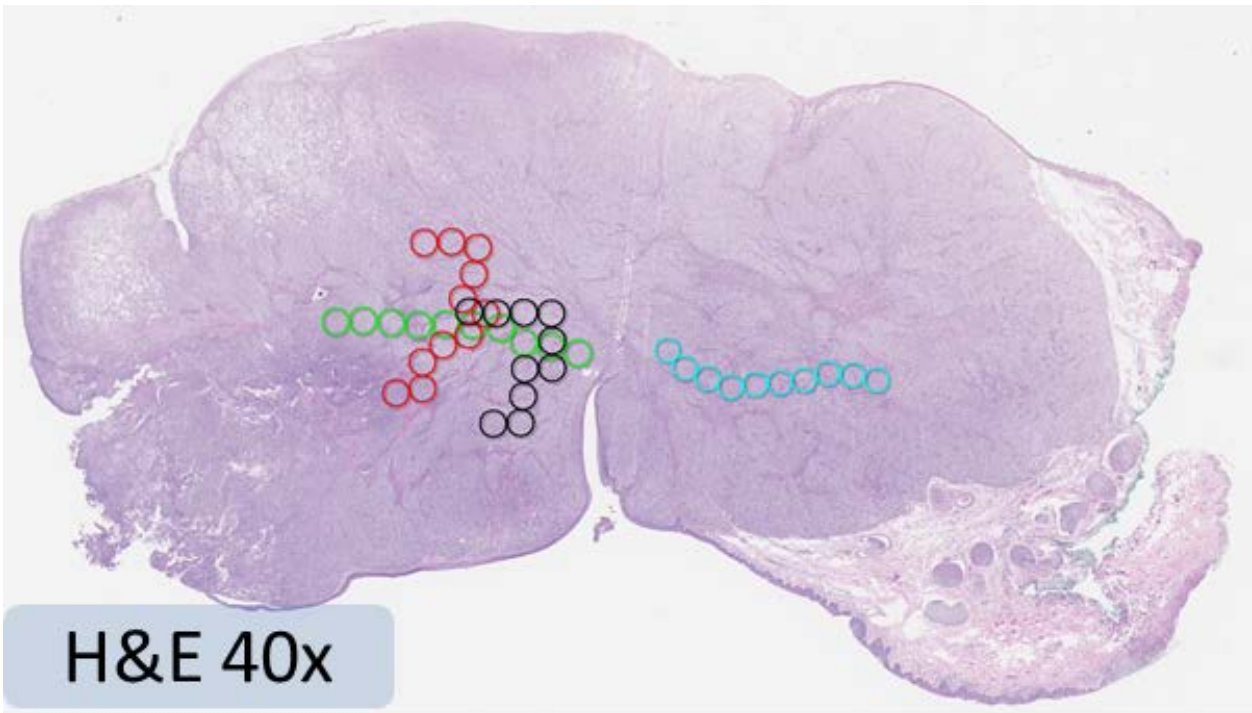
Figure 4: These two images show 10 FOVs selected by different readers while performing a mitotic counting task. The top image corresponds to counts taken at 20x and the bottom image corresponds to counts taken at 40x.
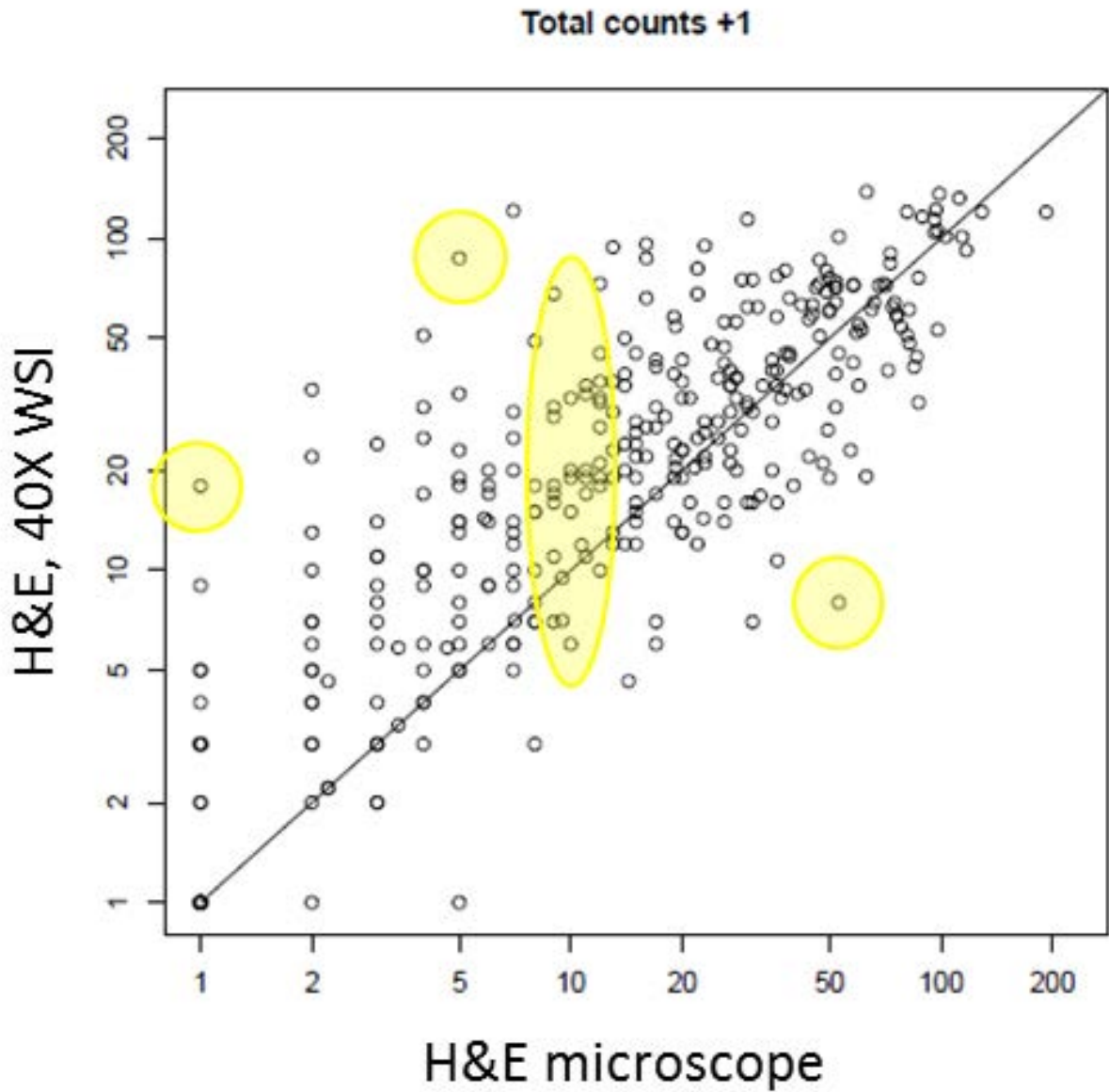
Total counts +1

Figure 5: This figure shows the within-reader correlation of counts of mitotic figures made on the microscope and made on the WSI. Each point shows the counts from the same reader evaluating the same case on the two modalities. Each count is the sum of 10 FOVs, following the clinical protocol. Note that this plot is a log-log plot, and the subsequent coefficient of variation is very large. The highlighted points show some extreme differences, and the highlighted band qualitatively identifies the variation in counts from H&E 40X WSI images when 10 counts are observed on the microscope.

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Capture: Does the MDDT fully capture the aggregate effect of the intervention on the true outcome of interest? Does the MDDT account for every major effect of the intervention? Are there available data which call this into question?

If our MDDT proposal is accepted, we will develop a full submission with supporting evidence of benefits to using eeDAP for the context of use.

eeDAP can be used to evaluate the performance of pathologists to find, classify, enumerate, and otherwise perform a feature-based task. The validity of the task is not disputable, but the relevance (extent of prediction and capture) to the device evaluation depends on whether or not the task is relevant to the clinical task or somehow stresses relevant imaging characteristics.

In some cases, the extent of prediction will be very strong. For example, an image analysis algorithm may be designed to find mitotic figures. In this case, a study with eeDAP could be created to explicitly evaluate the performance of the algorithm by collecting pathologist classifications on candidate cells and evaluating the algorithm's true-positive and true-negative rates.

In other cases, the extent of prediction will not be as strong or direct. A single eeDAP feature study may only demonstrate adequate image quality related to one task or one imaging characteristic and additional tasks may be needed to demonstrate adequate image quality related to other tasks or other imaging characteristics. We believe that a few different feature studies could demonstrate effectiveness that would be expected to generalize to a full and comprehensive intended use statement. For example, counting mitotic figures is a task that stresses the resolution of the WSI system, as fine details of the nucleus and chromosomes are needed to find and classify cells as mitotic figures. Also, identifying lesion tissue at low magnification is a task that stresses color resolution and dynamic range and could be designed for lower magnification. Tasks such as these may be considered as surrogates to *all* clinical tasks, and it will be the aggregate of evidence on the performance on such tasks that will adequately demonstrate effectiveness of a WSI system, an image analysis tool, or a CAD.

At a technical level, we need to validate that the registration process is accurate and the color representation in eeDAP is the same as would be in the proprietary WSI system-specific viewer.

7 Plan to get data

The main evidence that we plan to collect will be in the form of task-based studies of tissue features. At least one study will compare a mitotic counting study with eeDAP to the mitotic counting study following the clinical protocol mentioned above. We plan to demonstrate that pathologist evaluations taken with eeDAP are more reproducible than pathologist evaluations taken following a standard clinical method. In another study, additional evidence that we plan to collect will demonstrate that differences in imaging characteristics can be identified using eeDAP. For example, we intend to artificially degrade an imaging characteristic (resolution, focus, color) and conduct a study using eeDAP to show that the performance of pathologists is worse on the degraded system than on the baseline system.

For the evidence of technical validity we plan to design a study to characterize the accuracy of the image registration process and the color fidelity. For the color fidelity evaluation, we plan to follow the methods outlined previously [1].

8 Consent to Public Disclosure and Use

We hereby authorize FDA to make public sufficient information to support use of the qualified MDDT and for the general public to use and rely on data generated using the MDDT in gaining FDA clearance or

approval of other devices.

In fact, we are publicly sharing the submission process (documents and drafts, and communications with CDRH program staff) with the WSI working group at their NCIPhub group page `https://nciphub.org/groups/wsi_working_group`. The group page is open to the public and membership is open to anyone that requests it. Additionally, the compiled eeDAP software and all source code are shared on Github with HTML and PDF versions of user manuals [12]. The only prerequisite software for the compiled eeDAP software is the MATLAB runtime compiler [2], which is free to download and use.

References

[1] Brandon D. Gallas, Marios A. Gavrielides, Catherine Conway, Adam Ivansky, Tyler Keay, Wei-Chung Cheng, Jason Hipp, and Stephen M. Hewitt. Evaluation environment for digital and analog pathology (eedap): a platform for validation studies. *J Med Img*, 1(3):037501, 2014. doi: 10.1117/1.JMI.1.3.037501. URL `http://spie.org/Publications/Journal/10.1117/1.JMI.1.3.037501`.

[2] MathWorks. *MATLAB Runtime Comiler*. Natick, MA, US, Accessed 12/20/2016. URL `https://www.mathworks.com/products/compiler/mcr.html`.

[3] The Open Microscopy Environment. *Bio-Formats Documentation*. Dundee, UK, Accessed 12/20/2016. URL `https://www.openmicroscopy.org/site/support/bio-formats5.3/`.

[4] Marcial García Rojo, Gloria Bueno García, Carlos Peces Mateos, Jesús González García, and Manuel Carbajo Vicente. Critical comparison of 31 commercially available digital slide systems in pathology. *Int J Surg Pathol*, 14(4):285–305, Oct 2006. doi: 10.1177/1066896906292274. URL `http://dx.doi.org/10.1177/1066896906292274`.

[5] Jiang Gu and Robert W. Ogilvie, editors. *Virtual microscopy and virtual slides in teaching, diagnosis, and research*. CRC Press, Boca Raton, FL, 2005.

[6] Liron Pantanowitz, Paul N Valenstein, Andrew J Evans, Keith J Kaplan, John D Pfeifer, David C Wilbur, Laura C Collins, and Terence J Colgan. Review of the current state of whole slide imaging in pathology. *J Pathol Inform*, 2:36, 2011. doi: 10.4103/2153-3539.83746. URL `http://dx.doi.org/10.4103/2153-3539.83746`.

[7] Ronald S. Weinstein, Anna R. Graham, Lynne C. Richter, Gail P. Barker, Elizabeth A. Krupinski, Ana Maria Lopez, Kristine A. Erps, Achyut K. Bhattacharyya, Yukako Yagi, and John R. Gilbertson. Overview of telepathology, virtual microscopy, and whole slide imaging: prospects for the future. *Hum Pathol*, 40(8):1057–1069, Aug 2009. doi: 10.1016/j.humpath.2009.04.006. URL `http://dx.doi.org/10.1016/j.humpath.2009.04.006`.

[8] Shaimaa Al-Janabi, André Huisman, and Paul J. Van Diest. Digital pathology: current status and future perspectives. *Histopathology*, 61(1):1–9, Jul 2012. doi: 10.1111/j.1365-2559.2011.03814.x. URL `http://dx.doi.org/10.1111/j.1365-2559.2011.03814.x`.

[9] FDA CDRH. *Guidance for Industry and FDA Staff - Technical Performance Assessment of Digital Pathology Whole Slide Imaging Devices*. Maryland, 2016. URL `http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM435355.pdf`.

[10] Nicholas Anderson. *Regulatory Challenges and Opportunities for Digital Pathology*, Accessed 12/20/2016. URL `https://nciphub.org/resources/1927`.

[11] R. C. Smedley, J. Lamoureux, D. G. Sledge, and M. Kiupel. Immunohistochemical diagnosis of canine oral amelanotic melanocytic neoplasms. *Vet Pathol*, 48(1):32–40, Jan 2011. doi: 10.1177/ 0300985810387447. URL `http://dx.doi.org/10.1177/0300985810387447`.

[12] Brandon D Gallas. *eeDAP v4p0: Evaluation Environment for Digital and Analog Pathology*. Division of Imaging and Applied Mathematics, CDRH, FDA, Silver Spring, MD, 2016. URL `https://github.com/DIDSR/eeDAP/releases`.

Evaluation environment for digital and analog pathology: a platform for validation studies

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Evaluation environment for digital and analog pathology: a platform for validation studies

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Abstract. We present a platform for designing and executing studies that compare pathologists interpreting histopathology of whole slide images (WSIs) on a computer display to pathologists interpreting glass slides on an optical microscope. eeDAP is an evaluation environment for digital and analog pathology. The key element in eeDAP is the registration of the WSI to the glass slide. Registration is accomplished through computer control of the microscope stage and a camera mounted on the microscope that acquires real-time images of the microscope field of view (FOV). Registration allows for the evaluation of the same regions of interest (ROIs) in both domains. This can reduce or eliminate disagreements that arise from pathologists interpreting different areas and focuses on the comparison of image quality. We reduced the pathologist interpretation area from an entire glass slide (10 to 30 mm^2) to small ROIs (<50 μm^2). We also made possible the evaluation of individual cells. We summarize eeDAP's software and hardware and provide calculations and corresponding images of the microscope FOV and the ROIs extracted from the WSIs. The eeDAP software can be downloaded from the Google code website (project: eeDAP) as a MATLAB source or as a precompiled stand-alone license-free application. © The Authors. Published by SPIE under a Creative Commons Attribution 3.0 Unported License. Distribution or reproduction of this work in whole or in part requires full attribution of the original publication, including its DOI. [DOI: 10.1117/1.JMI.1.3.037501]

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1 Introduction

Digital pathology (DP) incorporates the acquisition, management, and interpretation of pathology information generated from a digitized glass slide. DP is enabled by technological advances in whole slide imaging (WSI) systems, also known as virtual microscopy systems, which can digitize whole slides at microscopic resolution in a short period of time. The potential opportunities for DP are well documented and include telepathology, digital consultation and slide sharing, pathology education, indexing and retrieval of cases, and the use of automated image analysis.[1–3] The imaging chain of a WSI system consists of multiple components including the light source, optics, motorized stage, and a sensor for image acquisition. WSI systems also have embedded software for identifying tissue on the slide, auto-focusing, selecting and combining different fields of view (FOVs) in a composite image, and image processing (color management, image compression, etc.). Details regarding the components of WSI systems can be found in Gu and Ogilvie[4] There are currently numerous commercially available WSI systems as reviewed by Rojo et al. in terms of technical characteristics.[5]

A number of studies (many cited in Refs. 6 and 7) have focused on the validation of WSI systems for primary diagnosis, with findings generally showing high concordance between glass slide and digital slide diagnoses. A common drawback of current validation studies of WSI systems is that they sometimes combine diagnoses from multiple pathology tasks performed on multiple tissue types. Pooling cases can lead to the undersampling of clinical tasks as discussed in the study by Gavrielides et al.[8] It can also dilute differences in reader performance that might be specific to certain tasks. Another issue from current validation studies is that agreement was typically determined by an adjudication panel comparing pathology reports from the WSI and microscope reads head-to-head. Guidelines are sometimes developed for defining major and minor discrepancies, but there is a considerable amount of interpretation and judgment required of the adjudication panel as the pathology reports are collected as real-world, sign-out reports (free text). Additionally, the focus of most validation studies is on primary diagnosis, with minor emphasis on related histopathology features that might be affected by image quality. The quantitative assessment of a pathologist's ability to evaluate histopathology features in WSI compared to the microscope would be useful in identifying possible limitations of DP for specific tasks. Related work includes the study of Velez et al.[9] where discordances in the diagnosis of melanocytic skin lesions were attributed to difficulty in identifying minute details such as inflammatory cells, apoptosis, organisms, and nuclear atypia. Finally, studies focusing on primary diagnosis do not typically account for differences in search patterns or FOV reviewed by observers. The selection of different areas to be assessed by different observers has been identified as a source of interobserver variability.[10]

In this paper, we present an evaluation environment for digital and analog pathology that we refer to as eeDAP. eeDAP is a software and hardware platform for designing and executing digital and analog pathology studies where the digital image

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is registered to the real-time view on the microscope. This registration allows for the same regions of interest (ROIs) to be evaluated in digital mode or in microscope mode. Consequently, it is possible to reduce or eliminate a large source of variability in comparing these modalities in the hands of the pathologist: the FOV (the tissue) being evaluated. In fact, the current registration precision of eeDAP allows for the evaluation of the same individual cell in both domains. As such, a study can be designed where pathologists are asked to evaluate a preselected list of individual cells or groups of cells in the digital mode and with the microscope. Consequently, paired observations from coregistered FOV are collected allowing for a tight comparison between WSI and optical microscopy.

A reader study with eeDAP is intended to evaluate the scanned image, not the clinical workflow of a pathologist or lab. Instead of recording a typical pathology report, eeDAP enables the collection of explicit evaluation responses (formatted data) from the pathologist corresponding to very narrow tasks. This approach removes the ambiguity related to the range of language and the scope that different pathologists use in their reports. At the same time, this approach requires the study designer to narrow the criteria for cases (slides, ROIs, cells) to be included in the study set.

Reader studies utilizing eeDAP can focus on the evaluation of specific histopathology features. Since certain features challenge image quality properties such as color fidelity, focus quality, and depth of field, such reader studies can provide valuable information for the assessment of WSI and its role in clinical practice. The presented framework allows for the formulation of different types of tasks, many of which are currently available and customizable in eeDAP: free-text, integer input for counting tasks, a slider in a predefined range for a confidence scoring task (ROC task, receiver operating characteristic task), check boxes of specific categories for a classification task, and marking the image for a search task. Figure 1 shows the examples of the GUI presentation for two scoring tasks that we have explored: on the left, the reader would be asked to provide a score between 1 and 100 reflecting their confidence that the cell within the reticle is a plasma cell [in hematoxylin and eosin (H&E) stained, formalin-fixed, paraffin-embedded colon tissue], whereas on the right, the reader would provide a score reflecting their confidence that the cell within the reticle is a mitotic figure (in H&E stained, formalin-fixed, paraffin-embedded sarcoma).

In this paper, we outline the key software and hardware elements of eeDAP. First, we discuss the eeDAP software as a publicly available resource and describe software specifications and requirements. We next talk about the tone reproduction curves that characterize eeDAP and the native viewers: the curves showing the lightness in the output image given the transmittance of the input slide. In Sec. 2.3, we summarize the local and global registration methods that are key to pairing ROIs across the digital and microscope domains. In Sec. 2.4, we provide the key hardware specifications that eeDAP requires and then demonstrate the differences in FOVs and image sizes between the two domains: the digital image and the glass slide. These calculations and corresponding representative images help to provide a sense of scale across the digital and analog domains. Finally, we talk about reticles and their important role in narrowing the evaluation area to a small ROI or an individual cell.
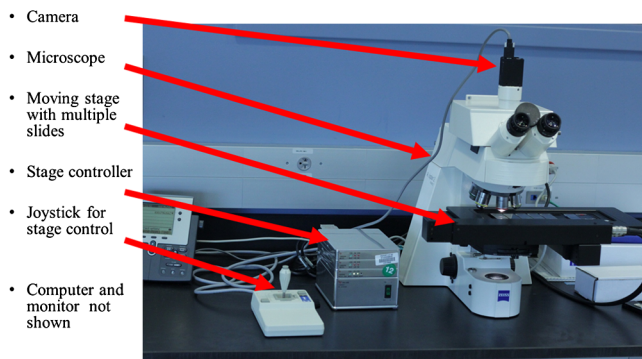
2 Methods

In this section, we summarize the key elements of the eeDAP software and hardware. The eeDAP software is made up of three graphical user interfaces (GUIs) written in MATLAB (Mathworks, Natick, Massachusetts).

The first interface establishes the evaluation mode (Digital or MicroRT) and reads in the study input file. The input file contains the file names of the WSIs, hardware specifications, and the list of tasks with corresponding ROI locations that will be interpreted by the pathologist. Each ROI is defined by a location, width, and height in pixel coordinates of the WSI, and all are automatically extracted on the fly from the WSIs named.
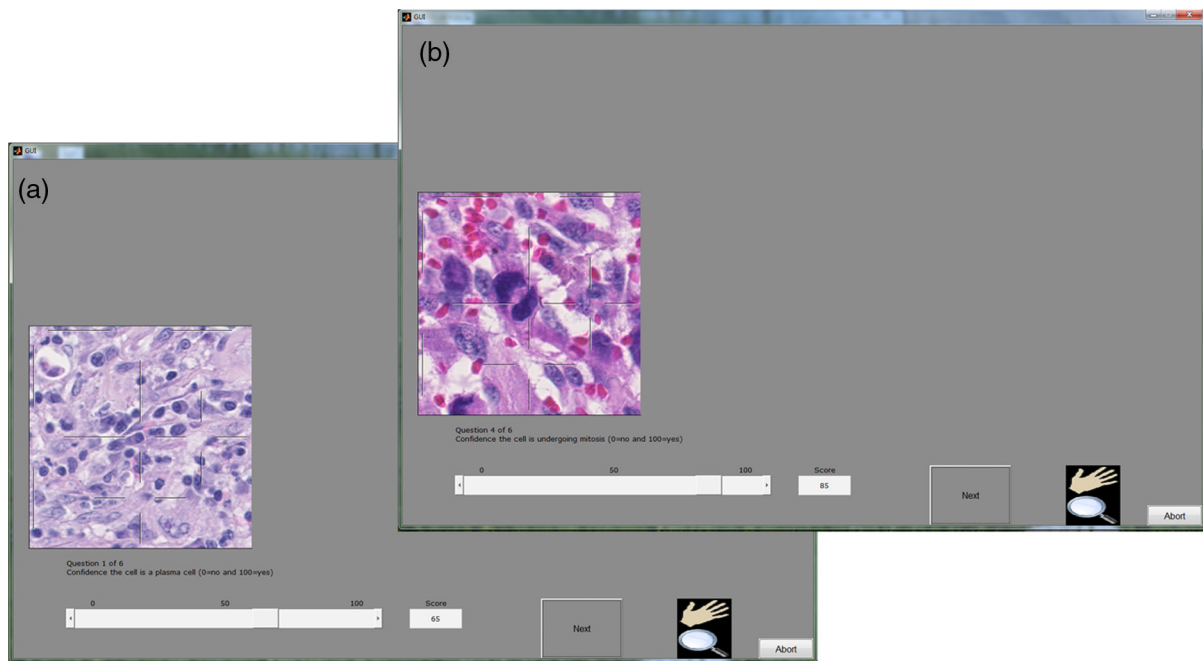
Fig. 1 Here are the two windows, each showing the eeDAP presentation of a slider task: the image on the left is of colon tissue, the image on the right is of sarcoma.

- Camera
- Microscope
- Moving stage with multiple slides
- Stage controller
- Joystick for stage control
- Computer and monitor not shown

Fig. 2 The evaluation environment for digital and analog pathology (eeDAP) hardware: microscope, camera, computer-controlled stage with joystick, and a computer with monitor (not shown).

There are installation requirements that make the ROI extraction possible from the proprietary WSI file formats. We also discuss a color gamut comparison between eeDAP and a native WSI viewer (a viewer designed by a WSI scanner manufacturer).

The second interface is executed only for studies run in the MicroRT mode. This interface globally registers each WSI to its corresponding glass slide. For each global registration of each WSI, a study administrator must interactively perform three local registrations. The local and global registration methods are described in Sec. 2.3.

The third interface runs the study in accordance to the list of tasks given in the input file. If the study is run in the Digital mode, the pathologist views the ROIs on the computer display in the GUI and enters the evaluations therein. If the study is run in MicroRT *mode*, the pathologist views the ROIs through the microscope (calibrated for Köhler illumination) and is responsible for any focusing in the *z*-plane. Although the pathologist is engaged with the microscope in the MicroRT mode, the study administrator is viewing the ROIs on the computer display in the GUI and enters the evaluations there as dictated by the pathologist. The study administrator also monitors a live camera image of what the pathologist sees through the microscope. This allows the study administrator to confirm and maintain a high level of registration precision in MicroRT mode.

The eeDAP hardware consists of an upright brightfield microscope, a digital camera, a computer-controlled stage with a joystick, a computer monitor, and a computer (see Fig. 2). The microscope requires a port for mounting the camera that allows for simultaneous viewing with the eyepiece. eeDAP currently supports a Ludl controller and compatible *xy* stage, and an IEEE 1394 FireWire camera communicating according to a DCAM interface (RGB, 8 bits-per-channel, minimum width 640, minimum height 480). Setup instructions and example specifications can be found in the user manual.

Below we summarize how these components are used in registration and how the WSI and real microscope image appear to the pathologist. We also identify an important part of the microscope, the reticle. The reticle is housed in the microscope eyepiece. One reticle that we use identifies ROIs in the microscope FOV and another points at individual cells.

2.1 eeDAP Availability and Technical Requirements

The software component of eeDAP is publicly available as MATLAB source code or as a precompiled stand-alone license-free MATLAB application.[11] Running eeDAP source code

requires the MATLAB image acquisition toolbox and the installation of third party software to extract ROIs from WSIs. WSIs are often extremely large (several GB) and are stored as large layered TIFF files embedded in proprietary WSI file formats. eeDAP uses ImageScope, a product of Aperio (a Leica Biosystems Division) to read images scanned with WSI scanners from Aperio (.svs) and other formats, including .ndpi (Hamamatsu). ImageScope contains an ActiveX control named TIFFcomp that allows for the extraction and scaling of ROIs. A consequence of using TIFFcomp is that the MATLAB version must be 32 bits.

The precompiled stand-alone application requires that the MATLAB compiler runtime (MCR) library be installed. It is important that the version of the MCR correspond exactly to that used for the stand-alone application (refer to the user manual).

2.2 Tone Reproduction Curves

Manufacturers of WSI scanners typically provide software for viewing their proprietary file formats. These viewers may include specialized color management functions. In fact, we observed color differences when we viewed .ndpi images with the native Hamamatsu viewer (NDP.view) side-by-side with the Aperio viewer (ImageScope) and MATLAB (with the Aperio ImageScope Active X component TIFFcomp). In an attempt to understand the native viewer and correct for these differences (so that we can show the images as they would be seen in the native viewer), we considered the image adjustments that may have caused them. From these, we observed that the images appeared the same in the three viewers when we adjusted the gamma setting. To confirm our observations, we measured the tone reproduction curves of NDP.view (gamma = 1.8 and gamma = 1.0) and ImageScope (no adjustments made; equivalent to the MATLAB).

Following the work of Cheng et al.,[12] we measured the transmittance of the 42 color patches of a color phantom (film on a glass slide, see Fig. 3). Using an FPGA board, we then retrieved the sRGB values of a Hamamatsu scanned image of the color phantom from the NDP.view with gamma set to 1.8 (default), gamma set to 1.0 (turning off the gamma adjustment), and ImageScope (default, no gamma correction). We then converted the sRGB values to the CIELAB color space and plotted the normalized lightness L^* channel against the normalized transmittance. The results (Fig. 3) supported our visual observations:

- There is good agreement between the tone reproduction curves of NDP.view with gamma = 1.0 and ImageScope.
- The tone reproduction curve of NDP.view with gamma = 1.0 appears to be linearly related to transmittance.
- The tone reproduction curve of NDP.view with gamma = 1.8 appears to be 1/1.8 gamma transformation of transmittance.
- The default images displayed by NDP.view and ImageScope (and MATLAB by equivalence) differ only in the gamma setting.

2.3 Registration

eeDAP uses registration to link the stage (glass slide) coordinates to the WSI coordinates. eeDAP has two levels of registration: global and local. The global registration is equivalent to
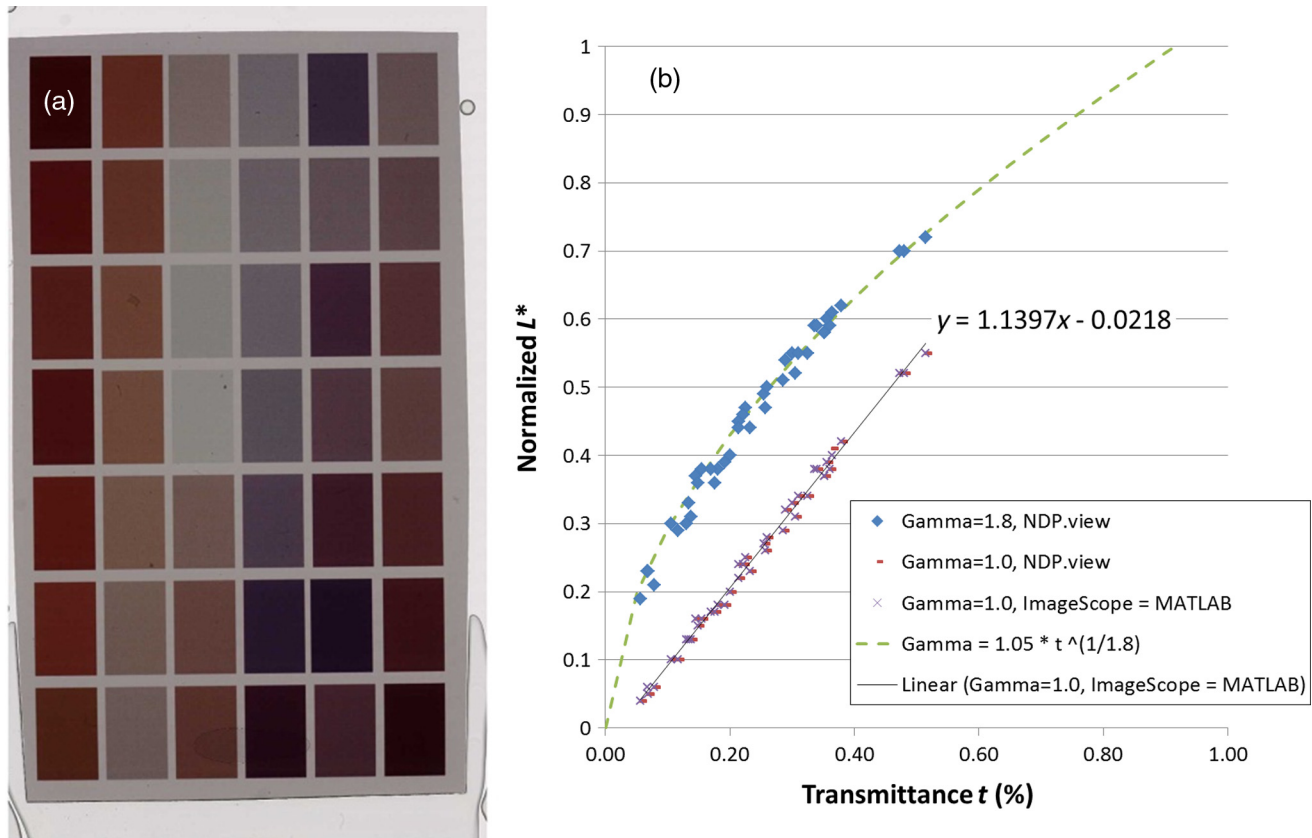
Fig. 3 (a) Hamamatsu scanned image of a color phantom (film on a glass slide). (b) The transmittance of the 42 color patches plotted against the normalized lightness L^* in the CIELAB color space (derived from the average sRGB values in a patch).

finding the transformation between the stage and WSI coordinates. The global registration requires three anchors, three pairs of stage-WSI registered coordinates. Each anchor is generated by a local registration: a (x, y) stage coordinate and a (x, y) WSI coordinate that correspond to the same specimen location.

eeDAP conducts two levels of global registration: low and high resolutions. Low resolution corresponds to microscope magnifications such as 2×, 5×, and 10×; the entire WSI image is scaled to fit in the GUI. High resolution registration corresponds to the microscope magnifications such as 20× and 40×; the low-resolution registration results are used to limit the amount of the WSI shown in the GUI, sequentially zooming in on the location of the low-resolution anchors.

eeDAP uses local registration for two purposes. The first purpose is to support global registration as discussed. The second purpose is to maintain a high level of registration precision throughout data collection. During our pilot studies, we observed that the precision of the global registration was deteriorating as the stage moved throughout the study. Therefore, we implemented a button that could be pressed during data collection that could register the current microscope view to the current task-specific ROI. The current level of precision appears to allow for the reliable evaluation of individual cells. Technical details of local and global registrations are provided below.

2.3.1 Local registration

A local registration is accomplished by taking a snapshot of the microscope FOV with the microscope-mounted camera and by

finding a search region containing the corresponding location in the WSI (see Fig. 4). The search region is identified by the study administrator and avoids searching the entire (very large) image. A local registration yields a (x, y) coordinate in the WSI and a (x, y) coordinate on the microscope stage that identify the same location.

The camera image contains some amount of specimen on the glass slide and is labeled by the (x, y) coordinate of the current stage position. See, for example, the "Preview with cross hairs" window labeled "Camera image" depicted in Fig. 4. The camera image has three channels (RGB) and must be at least 640 × 480. The physical size of a (square) camera pixel is given by the manufacturer specifications. This size divided by any magnification by the microscope (objective × camera adapter) determines the camera's spatial sampling period in units of the specimen.

We extract a patch of the WSI image (RGB) that is larger than and contains the same content as captured by the camera. See, for example, the image labeled "WSI image" depicted in Fig. 4. The WSI's spatial sampling period (often referred to as the WSI resolution) is given by the manufacturer specifications in units of the specimen and is often recorded in the WSI image.

An ROI extracted from a WSI image can be rescaled (interpolated) to have the same sampling period as the camera image using the ratio of the sampling periods. In other words, the number of pixels before and after rescaling is determined by
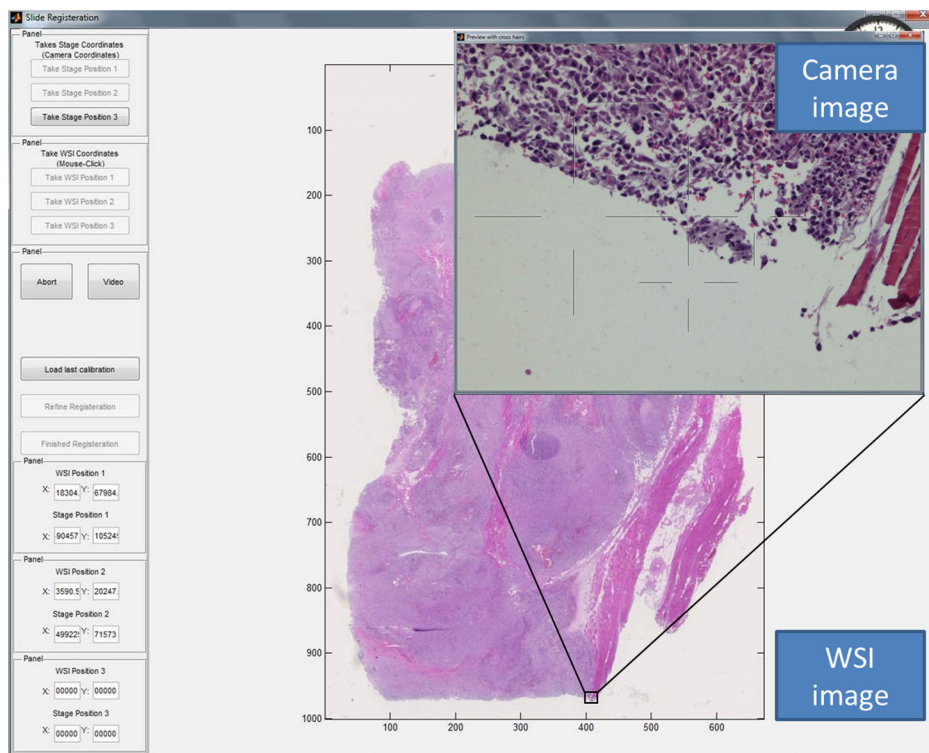
Fig. 4 Screen shot of the registration interface including the real-time microscope field of view (FOV) as seen with the mounted camera ("Camera image").

[corresponding number of camera pixels]

$$= \frac{\text{WSI sampling period}}{\text{camera sampling period}} [\text{number of WSI pixels}].$$

Given the camera image $c(x, y)$ and the WSI image $d(x, y)$ at the same scale, we perform normalized cross-correlation to find the Δx, Δy shift that best registers the two images. In other words, we find the Δx, Δy that maximize the following sum:

$$\frac{1}{n} \sum_{x,y} \frac{(c(x, y) - \bar{c})(d(x + \Delta x, y + \Delta y) - \bar{d})}{\sigma_c \sigma_d},$$

where the sum is over the n pixels in the camera image, (x, y) indexes the pixels in the image, Δx, Δy is the shift in pixels, and $\bar{c}, \sigma_c$ and $\bar{d}, \sigma_d$ are the average and standard deviation of the elements of $c(x, y)$ and $d(x, y)$ considered in the sum.

2.3.2 Global registration

Global registration is done for each WSI in the input file and corresponding glass slide on the microscope stage. Each global registration is built on three local registrations. The three local registrations yield three pairs of coordinates that define the transformation (the change of basis) between the coordinate system of the WSI (image pixels) and the coordinate system of the stage (stage pixels).

Let the three pairs of coordinates be given by $(x_i^{\text{WSI}}, y_i^{\text{WSI}})$, $(x_i^{\text{stage}}, y_i^{\text{stage}})$ for $i = 1, 2, 3$. Given these pairs, we define the two coordinate systems with the following matrices:

$$M_{\text{WSI}} = \begin{bmatrix} x_2^{\text{WSI}} - x_1^{\text{WSI}}, & x_3^{\text{WSI}} - x_1^{\text{WSI}} \\ y_2^{\text{WSI}} - y_1^{\text{WSI}}, & y_3^{\text{WSI}} - y_1^{\text{WSI}} \end{bmatrix},$$

$$M_{\text{stage}} = \begin{bmatrix} x_2^{\text{stage}} - x_1^{\text{stage}}, & x_3^{\text{stage}} - x_1^{\text{stage}} \\ y_2^{\text{stage}} - y_1^{\text{stage}}, & y_3^{\text{stage}} - y_1^{\text{stage}} \end{bmatrix}.$$

Then given a new location in the WSI coordinate system $[x_{\text{new}}^{\text{WSI}}, y_{\text{new}}^{\text{WSI}}]^t$, we can determine the corresponding location in the stage coordinate system with the following transformation:

$$\begin{bmatrix} x_{\text{new}}^{\text{stage}} \\ y_{\text{new}}^{\text{stage}} \end{bmatrix} = M_{\text{stage}} M_{\text{WSI}}^{-1} \left(\begin{bmatrix} x_{\text{new}}^{\text{WSI}} \\ y_{\text{new}}^{\text{WSI}} \end{bmatrix} - \begin{bmatrix} x_1^{\text{WSI}} \\ y_1^{\text{WSI}} \end{bmatrix} \right) + \begin{bmatrix} x_1^{\text{stage}} \\ y_1^{\text{stage}} \end{bmatrix}.$$

In words, we first shift the new point according to the origin in the WSI coordinate system $(x_1^{\text{WSI}}, y_1^{\text{WSI}})$. Next, we map the point from the WSI coordinate system to the standard one with M_{WSI}^{-1} and then map it to the stage coordinate system with M_{stage}. Finally, we shift the point according to the origin in the stage coordinate system $(x_1^{\text{stage}}, y_1^{\text{stage}})$. Consequently, the location of each ROI for each task given in the input file can be accessed in the WSI coordinate system or the stage coordinate system.

The study administrator determines each local registration by navigating the microscope with the joystick to an appropriate anchor, taking the camera image, and then approximately identifying the corresponding anchor in the WSI. An appropriate anchor is one that can be recognized in the WSI image and is surrounded by one or more salient features. Salient features increase the likelihood of a successful registration; repetitive features and homogeneous regions do not. Additionally, global registration is better when the set of three anchors are widely separated; encompassing the entirety of the tissue is best. The most challenging aspect in finding the appropriate anchors is navigating the microscope stage

with the joystick, focusing on the specimen, and determining the corresponding location in the WSI image.

In Fig. 4, we see the "Camera image" and the "WSI image." The study administrator has clicked on the WSI image to indicate where in the WSI to search for the camera image. A patch of the WSI image is extracted from the WSI at the full scanning resolution, the patch is scaled to the resolution of the camera, and a local registration produces the shift that identifies the corresponding WSI location to pair with the current stage location.

2.4 Comparing FOV and Image Sizes

In the following, we provide the key hardware specifications that eeDAP requires and demonstrates the calculation of different FOVs and image sizes. These calculations provide the relationships regarding scale across the digital and analog domains.

2.4.1 Microscope FOV

An important parameter of an optical microscope is the field number (FN); it is the diameter of the view field in millimeters at the intermediate image plane, which is located in the eyepiece. The FN is a function of the entire light path of the microscope starting with the glass slide, through the objective, and ending at the intermediate image plane in the eyepiece; the FN is often inscribed on the eyepiece. To get the FOV in units of the specimen being viewed, we divide the FN by the objective magnification. We currently have an Olympus BX43 microscope (FN = 22 mm) and a Zeiss Axioplan2 Imaging microscope (FN = 23 mm). At 40× magnification due to the objective, the FOV covered in the specimen plane is given by

- Olympus FOV at 40×

    - diameter = 22/40 = 0.550 mm,
    - area = 0.2376 mm,

- Zeiss FOV at 40×

    - diameter = 23/40 = 0.575 mm,
    - area = 0.2597 mm.

The FN can also be used to determine the perceived size of the microscope image at an effective viewing distance of 25 cm. The 25 cm effective viewing distance is a design convention[13] that is not well documented or well known. The perceived size is then simply the FN times the eyepiece magnification. Since the eyepieces on both microscopes above have 10× magnification, the perceived diameters of the intermediate images are 22 cm (Olympus) and 23 cm (Zeiss) at the effective viewing distance of 25. This corresponds to a visual angle (subtended angle of object at the eye) equal to $2 \times \arctan(23/(2*25)) \approx 50$ deg. In Fig. 5, we show what the microscope FOV looks like for the sarcoma slide scaled to fit the page.

2.4.2 Size of scanner images

We have access to two WSI scanners: a Hamamatsu Nanozoomer 2.0HT and an Aperio CS. They both operate at 20× and 40× magnification equivalent settings with similar sampling periods:

- 0.4558 μm at 20× and 0.2279 μm at 40× (Hamamatsu);

- 0.5000 μm at 20× and 0.2500 μm at 40× (Aperio).

The 40× Hamamatsu scanned images we have been using for pilot studies have $123,008 \times 82,688$ pixels (10 GB) and $39,680 \times 51,200$ pixels (2 GB). By multiplying the number of pixels by the sampling period, we get the size of the images in units of the specimen on the glass slide. These images correspond to the image areas of 28.0 mm × 18.8 mm and 9.0 mm × 11.7 mm. We have been extracting 400×400 ROI patches that show 0.092 mm × 0.092 mm patches of the specimen (area ≈ 0.0084 mm^2) for our most recent pilot study, which is 3.2% of the microscope FOV.

The size of a patch seen by a pathologist depends on the computer monitor pixel pitch (distance between pixels). For a computer monitor with a 258-μm pixel pitch, the display size of a 400×400 patch is 10.32 cm × 10.32 cm (area ≈ 106 cm^2). If we assume a viewing distance of 25 cm from the computer monitors (to match the effective viewing distance in the microscope), we can compare the image size of the ROI on the computer
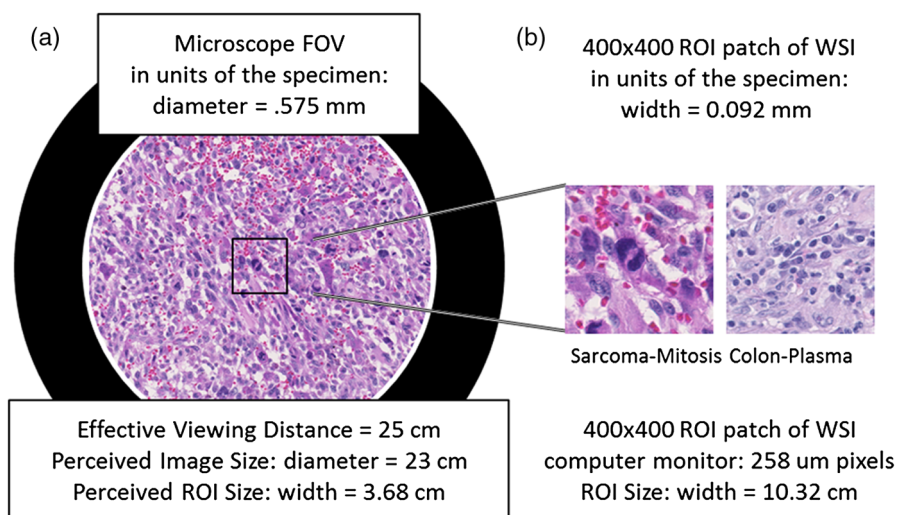
Fig. 5 The two images in this figure depict the relative sizes of the microscope image as seen through the eyepiece at 40× (a) and a 400×400 regions of interest patch from a whole slide image as seen on a computer monitor with 258-μm pixels at a viewing distance of 25 cm (b).
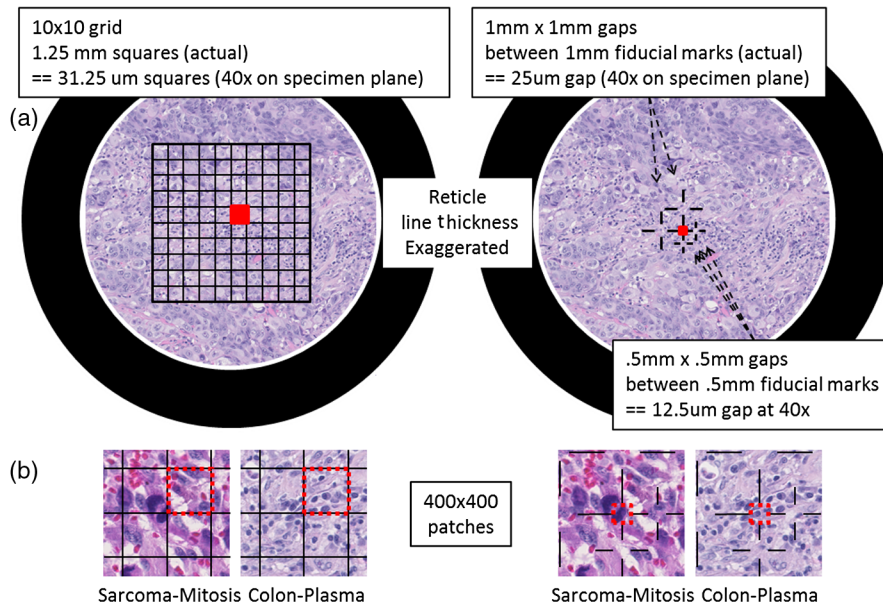
Fig. 6 Reticles (line thicknesses exaggerated) as seen through simulated microscope FOV (a) and 400×400 patches as they appear in eeDAP (b). The red squares in the simulated microscope FOVs and the red-dash boundary squares in the 400×400 patches indicate the "evaluation" areas. In the 400×400 sarcoma patch on the left, a majority of the obvious mitotic figure falls in the grid square to the left of the evaluation square. In the 400×400 sarcoma patch on the right, the central cross hairs point to the obvious mitotic figure. In the 400×400 colon patch on the left, there are several plasma-cell candidates in the "evaluation" square. In the 400×400 colon patch on the right, the cross hairs point to a single plasma-cell candidate to be evaluated.

monitor to the microscope perceived image size. Figure 5 shows the relative sizes of the two views side by side, demonstrating the apparent magnification of the specimen area in the displayed 400×400 patches.

2.4.3 *Size of camera images*

We currently have a Point Grey Flea2 color camera (FL2G-50S5C-C) that has a default output format of 1024×768 with 6.9-μm pixels. This format corresponds to 2×2 binning of a camera with a native pixel size of 3.45 μm. At $20\times$ magnification ($40\times$ objective times $0.5\times$ camera adapter), the spatial sampling period in units of the specimen plane corresponds to 0.345 μm (6.9/20) and the camera FOV is 0.353 mm $\times$ 0.265 mm (area = 0.0234 mm^2), which is about 36% of the microscope FOV.

2.5 *Reticles*

Reticles are pieces of glass that are inserted at the intermediate image plane in the eyepiece. They contain fine lines and grids that appear superimposed on the specimen. Reticles help to measure features or help to locate objects. The current version of eeDAP uses them to narrow tasks to very small regions and individual cells, allowing for an expansion of capabilities. In Fig. 6, we depict reticles as seen through the microscope (line thickness exaggerated) and as they appear in eeDAP (400×400 patches). These reticles are described below and were studied in two feasibility reader studies for their functionality.

In the first feasibility study, we used a reticle containing a 10×10 grid with squares that are 1.25 mm on a side (Klarmann Rulings: KR-429). At $40\times$, these squares are 31.25 μm on a side in the specimen plane. When running in Digital mode, eeDAP

digitally creates a reticle mask to create the same effect as the real reticle in the microscope. The instructions for this study were to score the reticle square that was immediately above and to the right of the center cross (red squares in Fig. 6). Identifying the center cross in the 10×10 grid in MicroRT mode is challenging; it is accomplished by rotating the eyepiece as the center cross remains fixed. Additionally, the instructions to score a square were to score the cell that was most likely the target (mitotic figure or plasma cell as shown in Fig. 1), considering cells with at least half their nuclei in the square.

In the second (similar) feasibility study, we used a custom reticle that has fiducials that point to gaps (Klarmann Rulings: KR-32536). Two gaps are 1 mm $\times$ 1 mm and three gaps are 0.5 mm $\times$ 0.5 mm. At $40\times$, these gaps are 25.0 μm and 12.5 μm on a side. The instructions for this study were much more direct: score the cell at the center of the center fiducials (red squares in Fig. 6).

3 Results and Discussion

We have been using pilot studies to identify weaknesses and future improvements needed for eeDAP and the general study design. The main weakness that we identified was that the registration precision throughout data collection was not good enough: pathologists were not evaluating identical ROIs. We have addressed this in the current generation of eeDAP by incorporating the ability to do a local registration for every ROI during data collection. We have also created a custom reticle that allows us to point at individual cells. This reduces ambiguity and disagreements due to evaluations based on multiple different cells within an ROI.

We also observed that the .ndpi WSI images appeared darker when viewing with eeDAP (and ImageScope) compared to

viewing with the native viewer, NDP.view. Through observation and subsequent measurement, we determined that the difference was a simple gamma adjustment, and we implemented a color look-up table to make this and any other color adjustment possible with eeDAP.

Our pilot studies emphasized the need for reader training. We found that pathologists needed to develop a level of comfort in scoring individual candidate cells, as this is not a part of a pathologist's typical clinical work flow. This is especially true when we asked for a 101-point confidence rating instead of a yes–no decision. Consequently, we are focusing our efforts to creating training on the cell types and scorings. Training on cell types may include Power Point slides that contain verbal descriptions of typical features and sample images. Training may also include eeDAP training modules: the training modules may elicit scores of the typical features as well as the overall score and then provide feedback in the form of the scores of experts.

As we move beyond pilot studies to pivotal studies, we need to investigate and establish several methods and protocols to reduce the variability between the pilot study and the pivotal study, to reduce variability during a pivotal study, and to allow for a study to be replicated as closely as possible. Methods and protocols are needed on the following issues:

- Computer monitor QA/QC and calibration, including color

  - It is understood in radiology that poor-quality displays can result in misdiagnosis, eye-strain, and fatigue.[14] As such, it is common in the practice and evaluation of radiology to control, characterize, and document the display conditions. This culture has led to the creation of standards that treat displays.[15] This issue is not yet fully enabled and appreciated within the culture of DP practice or evaluation. Study reports do very little to describe the display characteristics and calibration, with recent work being the exception.[8] However some groups, including the International Color Consortium, are filling the void and addressing the challenging issue of display and color calibration.[16–18]

- Slide preparation.

  - It is well known that there is significant variability in tissue appearances based on processing, sectioning, and staining differences and this variability leads to variability in diagnosis.[19] Protocols for slide preparation are a part of standard lab practice and are changing with increased automation, driving standards in this space.[20]

- Tissue inclusion/exclusion criteria, including methods to objectively identify candidate cells for the evaluation task.

  - Identifying inclusion/exclusion criteria for study patients (or in the current context, their tissue) is needed to convey the spectrum of the tissue being used, and thus the trial's generalizability and relevance.[21,22] Given the tissue, when the task is to evaluate individual cells, it is important to not bias the selection process. For our work, we intend to first

identify the entire spectrum of presentations, not just presentations that are easy in one modality or another (as might result from pathologist identified candidates). Once the entire spectrum of presentations is identified, there may be reasons to subsample within to stress the imaging system evaluation and comparison. Future work may include the incorporation of algorithms for the automated identification of candidate cells to be classified or histopathological features to be evaluated. Such algorithms may be less biased and more objective in creating the study sets.

Finally, a coherent analysis method is needed that does not require a gold standard, since one is typically not available for the tasks being considered. To address this need, we are investigating agreement measures, such as concordance, that compare pathologist performance with WSI to conventional optical microscopy. The goal is to develop methods and tools for multi-reader, multicase analysis of agreement measures, similar to the methods and tools for the area under the ROC curve[23] and the rate of agreement.[24]

4 Conclusions

In this paper, we presented the key software and hardware elements of eeDAP, a framework that allows for the registration and display of corresponding tissue regions between the glass slide and the WSI. The goal was to remove search as a source of observer variability that might dilute differences between modalities. The software part of eeDAP can be downloaded from the google code website (project: eeDAP) as a MATLAB source or as a precompiled stand-alone license-free application.[11] This software can be paired with the required hardware (microscope, automated stage, and camera) and used to design and execute reader studies to compare DP to traditional optical microscopy.

References

1. L. Pantanowitz et al., "Review of the current state of whole slide imaging in pathology," *J. Pathol. Inf.* **2**, 36 (2011).
2. R. S. Weinstein et al., "Overview of telepathology, virtual microscopy, and whole slide imaging: prospects for the future," *Hum. Pathol.* **40**(8), 1057–1069 (2009).
3. S. Al-Janabi, A. Huisman, and P. J. Van Diest, "Digital pathology: current status and future perspectives," *Histopathology* **61**(1), 1–9 (2012).
4. J. Gu and R. W. Ogilvie, Eds., *Virtual Microscopy and Virtual Slides in Teaching, Diagnosis, and Research*, CRC Press, Boca Raton, Florida (2005).
5. M. G. Rojo et al., "Critical comparison of 31 commercially available digital slide systems in pathology," *Int. J. Surg. Pathol.* **14**(4), 285–305 (2006).
6. L. Pantanowitz et al., "Validating whole slide imaging for diagnostic purposes in pathology: guideline from the College of American

Pathologists Pathology and Laboratory Quality Center," *Arch. Pathol. Lab. Med.* **137**(12), 1710–1722 (2013).

7. A. R. Jara-Lazaro et al., "Digital pathology: exploring its applications in diagnostic surgical pathology practice," *Pathology* **42**(6), 512–518 (2010).

8. M. A. Gavrielides et al., "Observer performance in the use of digital and optical microscopy for the interpretation of tissue-based biomarkers," *Anal. Cell. Pathol.*, Epub ahead of print (2014).

9. N. Velez, D. Jukic, and J. Ho, "Evaluation of 2 whole-slide imaging applications in dermatopathology," *Hum. Pathol.* **39**(9), 1341–1349 (2008).

10. P. J. van Diest et al., "A scoring system for immunohistochemical staining: consensus report of the task force for basic research of the EORTC-GCCG. European Organization for Research and Treatment of Cancer-Gynaecological Cancer Cooperative Group," *J. Clin. Pathol.* **50**(10), 801–804 (1997).

11. B. D. Gallas, *eeDAP v2.0: Evaluation Environment for Digital and Analog Pathology (code.google.com/p/eedap)*, Division of Imaging and Applied Mathematics, CDRH, FDA, Silver Spring, Maryland (2013).

12. W.-C. Cheng et al., "Assessing color reproducibility of whole-slide imaging scanners," *Proc. SPIE* **8676**, 86760O (2013).

13. H. G. Kapitza *Microscopy from the Very Beginning*, 2nd ed., Carl Zeiss, Jena, Germany (1997).

14. D. Hirschorn, E. Krupinski, and M. Flynn, "Displays," in *IT Reference Guide for the Practicing Radiologist* (2013).

15. J. T. Norweck et al., "ACR-AAPM-SIIM technical standard for electronic practice of medical imaging," *J. Digit Imaging* **26**(1), 38–52 (2013).

16. ICC, "ICC White Paper 7: The Role of ICC Profiles in a Colour Reproduction System, International Color Consortium," Reston, VA (2004), http://www.color.org/ICC_white_paper_7_role_of_ICC_profiles.pdf (27 10 2014); P. Green, Ed., *Color Management: Understanding and Using ICC Profiles*, Wiley, New York, NY (2010).

17. E. A. Krupinski et al., "Observer performance using virtual pathology slides: impact of LCD color reproduction accuracy," *J. Digit Imaging* **25**(6), 738–743 (2012).

18. A. Badano et al., "Consistency and standardization of color in medical imaging: a consensus report," *J. Digit Imaging*, Epub ahead of print (2014).

19. K. H. Allison et al., "Understanding diagnostic variability in breast pathology: lessons learned from an expert consensus review panel," *Histopathology* **65**(2), 240–251 (2014).

20. *I/LA28-A2: Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays; Approved Guideline*, 2nd ed., Clinical and Laboratory Standards Institute, Wayne, Pennsylvania (2011).

21. P. M. Bossuyt et al., "Toward complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative," *Am. J. Clin. Pathol.* **119**(1), 18–22 (2003).

22. K. F. Schulz et al., "Consort 2010 statement: updated guidelines for reporting parallel group randomised trials," *BMJ* **340**, c332 (2010).

23. B. D. Gallas et al., "A framework for random-effects ROC analysis: biases with the bootstrap and other variance estimators," *Commun. Stat. A* **38**(15), 2586–2603 (2009).

24. W. Chen and A. Wunderlich, *iMRMC_Binary v1.0_Beta1: A Matlab Software Package for Simulating, Analyzing, and Sizing a Multi-Reader Multi-Case Reader Study with Binary Assessments*, Division of Imaging and Applied Mathematics, CDRH, FDA, Silver Spring, Maryland (2014).

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MATLAB Runtime

Run compiled MATLAB applications or components without installing MATLAB

The MATLAB Runtime is a standalone set of shared libraries that enables the execution of compiled MATLAB applications or components on computers that do not have MATLAB installed. When used together, MATLAB, MATLAB Compiler, and the MATLAB Runtime enable you to create and distribute numerical applications or software components quickly and securely.

To download and install the MATLAB Runtime:

1. Click the version and platform that corresponds to the application or component you are using.

   Note: you can find this information in the `readme.txt` file that accompanies the application or component.

   > ⚠ **Note:** R2014a-2016a does not support macOS Sierra 10.12. If you choose to run any of these versions of the MATLAB Runtime on this unsupported macOS version, you might need to install a patch to fix an incompatibility issue. Learn more to determine if this incompatibility impacts you. We strongly recommend that you do not run any version of the MATLAB Runtime older than R2014a on macOS Sierra 10.12.

Release (MATLAB Runtime Version#)	Windows	Linux	Mac
R2016b (9.1)	64-bit	64-bit	Intel 64-bit
R2016a (9.0.1)* *	64-bit	64-bit	Intel 64-bit
R2015b (9.0)*	32-bit / 64-bit	64-bit	Intel 64-bit
R2015aSP1 (8.5.1)	32-bit / 64-bit	64-bit	Intel 64-bit
R2015a (8.5)	32-bit / 64-bit	64-bit	Intel 64-bit
R2014b (8.4)	32-bit / 64-bit	64-bit	Intel 64-bit
R2014a (8.3)	32-bit / 64-bit	64-bit	Intel 64-bit

R2013b (8.2)	32-bit / 64-bit	64-bit	Intel 64-bit
R2013a (8.1)	32-bit / 64-bit	64-bit	Intel 64-bit
R2012b (8.0)	32-bit / 64-bit	64-bit	Intel 64-bit
R2012a (7.17)	32-bit / 64-bit	32-bit / 64-bit	Intel 64-bit
R2011b and earlier***	Open MATLAB and run the command `mcrinstaller` to locate MATLAB Runtime for redistribution with your compiled components.		

* MATLAB Runtime 9.0, for R2015b, is intended to work with MATLAB 8.6, which is also R2015b.

* * MATLAB Runtime 9.01, for R2016a, is intended to work with MATLAB 9.0, which is also R2016a.

* * * Online versions of MATLAB Runtime are only available for releases after R2012a. Older releases of MATLAB Runtime were shipped with MATLAB Compiler. If you do not have MATLAB, please request the Runtime from the MATLAB Compiler user who created the deployed package.

2. Save the MATLAB Runtime installer file on the computer on which you plan to run the application or component.

3. Double click the installer and follow the instructions in the installation wizard.

See the MATLAB Runtime Installer documentation for more information.

The version of the MATLAB Runtime is tied to the version of MATLAB.

     United States

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Bio-Formats Documentation

The following documentation is split into four parts. *About Bio-Formats* explains the goal of the software, discusses how it processes metadata, and provides other useful information such as version history and how to report bugs. *User Information* focuses on how to use Bio-Formats as a plugin for ImageJ and Fiji, and also gives details of other software packages which can use Bio-Formats to read and write microscopy formats. *Developer Documentation* covers more indepth information on using Bio-Formats as a Java library and how to interface from non-Java codes. Finally, *Formats* is a guide to all the file formats currently supported by Bio-Formats.

- *About Bio-Formats*
- *User Information*
- *Developer Documentation*
- *Formats*

Bio-Formats 5.3.2 uses the *June 2016* release of the OME Model.

Bio-Formats is a community project and we welcome your input. You can find guidance on *Reporting a bug*, upload files to our QA system for testing, and contact us via our mailing lists or forums. Further information about how the OME team works and how you can contribute to our projects is in the Contributing Developer Documentation.

Critical Comparison of 31 Commercially Available Digital Slide Systems in Pathology

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Advances in new technologies for complete slide digitization in pathology have allowed the appearance of a wide spectrum of technologic solutions for whole-slide scanning, which have been classified into motorized microscopes and scanners. This article describes technical aspects of 31 different digital microscopy systems. The most relevant characteristics of the scanning devices are described, including the cameras used, the speed of digitization, and the image quality. Other aspects, such as the file format, the compression techniques, and the solutions for visualization of digital slides, (including

diagnosis-aided tools) are also considered. Most of the systems evaluated allow a high-resolution digitization of the whole slide within about 1 hour using a ×40 objective. The image quality of the current virtual microscopy systems is suitable for clinical, educational, and research purposes. The efficient use of digital microscopy by means of image analysis systems can offer important benefits to pathology departments.

Keywords: virtual microscopy; whole slide scanning; motorized microscope

The term *virtual microscopy* is used to describe the acquisition and storage of microscopy digital images. The digital images may consist of a selected area or the whole histology or cytology slide, without being limited to just one or a few regions of interest. Virtual microscopy therefore allows access to all regions of interest within a sample slide by using a personal computer (PC) or digital device without use of the microscope.[1]

Virtual microscopy is a general concept that includes different aspects related to this process spanning from the image acquisition to the visualization systems. Because the digital image is not less real than the one provided by the optical microscope, we believe that the term *virtual microscopy* is not an accurate term, and we prefer the term *digital microscopy*. Virtual microscopy systems are currently capable of complete digitization of the histology and cytology slides, a process known as whole-slide imaging (WSI). Nowadays, they are available in multiple formats and commercial solutions.

The history of these virtual devices is rather recent. The first virtual microscopy system was described in 1997 by the Computer Science Department at University of Maryland and the Pathology Department at Johns Hopkins Hospital, Baltimore, Maryland.[1,2] To the best of our knowledge, there are no recent reviews on the state of the art for possible virtual microscopy solutions. In 2003, the European Organization for Research and Treatment on Cancer (EORTC) published the results of a poll on virtual

microscopy systems to that date (3D Histech, Aperio, Histkom, InterscopeTech, Leica/Van Hopplynus, Olympus, Nikon, Samba, Syncroscopy, Trestlecorp, Zeiss and Zem).[3]

The objective of this work is to provide a comparative description of 31 solutions available on the market that are able to perform a whole slide digitization or assistance in complete slide review for anatomic pathology applications, together with other related products.

Digital Systems Classification

Digital imaging devices may be divided into 2 classes according to their purpose: the digital microscope (scan the whole slide) and diagnosis-aided systems.

The main objective of digital microscopes or WSI devices is to build digital slides. They are capable of digitizing slides at high magnifications. Diagnosis-aided systems are designed to help with the detection of the region of interest, and some are able to quantify biomedical signals. We include in this review those systems capable of whole-slide scanning or areas-of-interest scanning, at least with low magnification, and not just taking static images from a microscopic field.

By using a criterion based on the components of the device, it is possible to distinguish 2 groups: motorized microscopes and scanners. We have included within the group of motorized microscopes those in which the functionality and original components remain, such as eyepieces, multiple lenses, (motorized revolver), position, and spotlight control. The second main component of these systems is the camera joined up to the microscope. Another essential component is the software that controls the microscope and the camera. The progressive method, in which the final image is composed frame by frame, is the most common procedure used to scan the slides.

Slide scanners include components similar to those used in automated microscopes but with some modifications, such as absence of eyepieces and absence of position and focus control. In this way, slide scanners become special devices for virtual microscopy. The scanners are closed systems. They are open only to introduce the slide tray to be scanned. The scanners are controlled by a PC, usually placed inside the scanner box, which includes an antivibration system. Table 1 shows a classification schema of digital microscopy devices.

Components of the Whole Slide Digitization Systems

All high-resolution virtual microscopy devices are composed of an optical microscope system, an acquisition system (photography), software that controls the scan process, and a digital slide viewer. Other optional components include the slide feeder or the image-processing program.

Cameras

The camera that takes the images is one of the critical components when analyzing the quality and speed of digital microscopy solutions. It is therefore very important to know the camera characteristics in detail.

Usually, these cameras have a charged coupled device (CCD) sensor that provides an analogue signal. Digital cameras convert analogue signals into digital. The main characteristic when analyzing digital camera quality is the image resolution or CCD size (number of pixels the sensor is able to detect) (Table 2).

Most digital cameras are connected to the PC through a FireWire port (LifeSpan Alias, Olympus SIS, Slide, Zeiss Mirax Scan). This may require the use of card adapters. Clarient ACIS uses the Matrox Meteor-II /multichannel card. The Hamamatsu C9600 NanoZoomer and Aperio ScanScope T2 use a CameraLink connection. The correction lens, located in the adaptor between the camera and the microscope, is ×1.0 (LifeSpan Alias, Olympus SIS .slide, Zeiss Mirax Scan) or ×0.63 (Zeiss Mirax Scan, Samba Naviqap). Some systems use an F-mount (LifeSpan Alias, Aperio ScanScope).

Stage

The high resolution and fast stages used in virtual microscopy are able to move at speeds of about 32 mm/s (Zeiss Mirax Scan), 38 mm/s (Aperio ScanScope T2), 41.22 mm/s (LifeSpan Alias), or even 180 mm/s (Olympus SIS .slide). The stage accuracy is about 1 μm to 3 μm, although some types are able to get an accuracy or minimum distance of 0.002 μm to 0.015 μm for the z-axis and 0.25 μm for the x-axis and y-axis (Syncroscopy SyncroScan and Montage Explorer, LifeSpan Alias, Bacus BLISS, Olympus SIS .slide). The most frequently used stages are

Table 1. Classification of Digital Microscopy Solutions

1. Digital microscopes (WSI): virtual slides creation	
A. Automated Microscopes (based on the following models)	Bacus BLISS (Olympus BX61; Zeiss Axioplan 2)
	LifeSpan Alias (Leica DM LA)
	Nikon Eclipse E600FN with EclipseNet-VSL
	Olympus SIS .slide (Olympus BX51)
B. Scanners	
Progressive Scanning	Nikon Coolscope with EclipseNet-VSL
	Zeiss Mirax Scan
	Zeiss Mirax Desk
Lineal Scanning	Aperio ScanScope T2
	Aperio ScanScope CS
Area transference (Time Delay and Integration - TDI)	Hamamatsu C9600 NanoZoomer
Optical matrix	DMetrix DX-40
C. Software and components for virtual slides management	Apollo Telemedicine ASAP Imaging and PathPACS
	Aurora mScope
	Fairfield PathSight
	MicroBrightField Virtual Slide System
	Samba Naviqap
	Syncroscopy SyncroScan
	SlidePath Digital Slidebox
	Trestle MedMicroscopy and Xcellerator
	Tribvn ICS WF
	VMscope
2. Diagnosis aided systems: image analysis and telepathology	
A. Automated Microscopes	Applied Imaging Ariol
	BioGenex iVision
	BioGenex GenoMx VISION
	CytoCore InPath Slide Based Test
	Imstar Pathfinder Morphoscan with E-Mage VS (virtual slide)
	Leica AS TPS2 (frozen section studies)
	MetaSystems Metafer
B. Scanners	
Progressive scanning	Clarient ACIS
	CyTyc ThinPrep Imaging System
Cytometry by laser	Compucyte iColor and Compucyte iCyte

Daedal 106004 model (Aperio ScanScope T2), Leica CTR MIC (LifeSpan Alias), and Maerzhauser scan 100*80 (Olympus SIS .slide). The stage of a motorized microscope may be controlled by means of a joystick (Olympus SIS .slide) or the Leica SmartMove (LifeSpan Alias).

Coolscope) or multispectral channels (LifeSpan Alias). The Zeiss Mirax Scan built-in white light has a complementary color temperature filter. It allows the use of dark field or fluorescence, similarly to Olympus SIS .slide, Hamamatsu NanoZoomer (since August 2006), and Applied Imaging (AI) Ariol.

Optical Equipment—Illumination

The illumination method most frequently used is the halogen lamp (100 W in Olympus SIS .slide). In the scanners, it can be an internal light source (EKE 150 W, Aperio ScanScope T2) or an external one (EKE 150 W, Aperio ScanScope CS). Other systems use light-emitting diodes (LEDs), with white light (Nikon

Hardware

Concerning the computer hardware, most solutions are based on workstations with 2 microprocessors (Xeon), 2.8 GHz to 3.6 GHz, and 4 gigabytes of RAM. The operative system used by the control devices and the workstations is usually Windows XP Professional (Microsoft, Redmond, Wash).

Table 2. Cameras Used in Some Slide Digitization Systems

Device	Model	CCD-CMOS Size	Sensor Pixel Size (μm)	Resolution (μm/pixel) ×20/×40 objective
Aperio ScanScope T2	Basler L301 kc (CCD)	Color, scanned 3-lineal RGB, 3 × 2098 pixels	14 ×14	0.47/0.23
Aperio ScanScope CS	Basler L301 kc (CCD)	Color, scanned trilateral RGB, 3 × 2098 pixels	14 × 14	0.5/0.25
Applied Imaging Ariol	Redlake MegaPlus ES 4.0/E (monochrome CCD)[a]	Interline CCD 2048 × 2048 pixels	7.4 × 7.4	0.368/0.184
Bacus BLISS	CCD	3 CCD Color 752 × 480 pixels	—	—/—
Clarient ACIS	Sony 3 CCDs 60 fps	Progressive scanning	—	0.0625 μm²/ pixel (×60)
DMetrix DX-40	DMetrix CMOS			
DM5760	24 mega-pixels	—	0.47/—	
Hamamatsu C9600 NanoZoomer	3 CCDs-TDI	4096 × 64 pixels	8 × 8	0.46/0.23
LifeSpan Alias	Diagnostic Instruments SPOT Xplorer 4MP Mono CCD	Monochromatic, 2048 × 2048 pixels (15.16 mm²)	7.4 × 7.4	0.37/0.185
Nikon Eclipse E600FN	Nikon DXM1200F (CCD) with Bayer mask	2/3" CCD. 3,840 × 3072 pixels	6.45 × 6.45	—/0.45
Nikon Coolscope	Nikon DS-5M	2/3" CCD. 2560 × 1920 pixels	8 × 8	—/—
Olympus SIS .slide	CCD. Peltier-cooled with Bayer mask. 12 bits/color	2/3" CCD. 1376 × 1032 pixels	6.45 μm × 6.45 μm	0.32/0.16
Zeiss Mirax Scan	Allied Vision Technologies Marlin F-146C	1392 × 1040 progressive CCD	4.65 μm × 4.65 μm	0.23/0.12 (optional: 0.32/0.16)

a. A range of cameras can be adapted, from 1392 × 1040 pixels (Jai CV-M4+ and CoolSnapEs), to 2048 × 2048 pixels (Redlake ES 4.0/E).

The recommended way for managing the storage is using centralized (enterprise) hospital storage servers. If the pathology department has to have its own storage, the following configuration is recommended: 6 disks, each of 300 gigabytes; 10k rpm hot swap for a total of 3.8 TB.

High-Resolution Monitors

All virtual microscopy solutions include a flat thin-film transistor (TFT) monitor, sized from 20 inches to 23 inches. These screens must be high resolution, such as Apple M9179 Cinema (Apple Computers, Inc, Cupertino, Calif), 30-inch liquid crystal display (LCD) TFT screen of 2560 × 1600 pixels, with 200-ppp resolution. This screen size allows a visual field 4 times larger than the classic microscope field of view. The Apple monitor is optional with Zeiss Mirax Scan, and it has a contrast ratio of 400:1 and a pixel pitch of 0.25 mm.

Aperio ScanScope includes 23-inch HP 2335 LCD monitors (Hewlett Packard, Palo Alto, Calif) (1920 × 1200 pixels, distance pixel 0.258 mm, contrast

500:1). Olympus SIS .slide includes a 20.1-inch NEC LCD2080UX+ monitor (Irving, Texas) (1600 × 1200 pixels, distance pixel of 0.255 mm, contrast 450:1). Bacus BLISS also includes a 20.1-inch LCD monitor.

The Digitization Process or Slide Scanning

Different aspects should be taken into account to evaluate the digitization process, such as the digitization speed, the maximum sample size, the focus quality, the digitization at different planes, the methods for scanning and image assembling, and the formats used to store the scanned samples. Table 3 includes some relevant aspects related to well-known digitization systems.

Digitization Speed

The digitization speed or total scanning time is one of the most important aspects to consider before

Table 3. Main Characteristics of Whole Slide Scanning Systems

Device	Slide Feeder	Viewer (Proprietary/Web)	Objective Lens	Image Stitching
Aperio ScanScope T2	120 (BCR)[a]	ImageScope/Zoomifyer	Olympus ×20/0.75 Plan Apo or ×40/0.75 Plan Fluor[d]	Stitching or ×40/0.75
Aperio ScanScope CS	5 (BCR)[a]	ImageScope/Zoomifyer	Olympus ×20/0.75 Plan Apo (×40 with "doubler").[e]	Stitching
Applied Imaging Ariol	50 (BCR)[a]	Web browser & Zoomifyer	Olympus (×1.25, ×5, ×10, ×20 and ×40)[f]	Stitching
Bacus BLISS	No	WebSlide viewer/Browser (applet java o Active X)	Olympus o Zeiss (×1.25, ×10, ×20 and ×40)	Tiling
Clarient ACIS	100 (BCR)[a]	—	Olympus ×4, ×10, ×20, ×40, ×60	—
DMetrix DX-40	40 (slipstream)	Digital Eyepiece	Matrix with ×80 objective lens, with N.A. = 0.65	Stitching
Hamamatsu C9600 NanoZoomer	210 (BCR)*	Fairfield PathSight File Viewer	Proprietary	Tiling during scanning
LifeSpan Alias	300 (BCR)[a] optional	Applet Java—Active X	Leica ×40 PL Fluotar 0.75 NA (and ×2.5, ×5, ×10, ×20, and ×63)	Stitching or Tiling
Nikon Coolscope	160 (Cool-Loader) optional	EclipseNet/Web DB (Bacus at USA)	×5, ×10, ×20 and ×40 or ×2, ×4, ×20, and ×40 (low-mag)	Stitching
Nikon Eclipse E600FN	No	EclipseNet/Web DB	Nikon CFI60 Plan Fluor ×40, CFI60 Plan Apochromat ×20, CFI60 Plan Fluor ×4	Stitching
Olympus SIS .slide	SL50, with BCR[a] or stage 4 slides optional	OlyVIA[c]/Zoomifyer	Olympus 40 UPLSAPO, ×2 PLAPON, ×10, ×20 (to 6)	Tiling
Zeiss Mirax Scan	300 (BCR)[a] optional	Mirax Viewer (free and full featured versions)[b]/applet Java	Carl Zeiss Plan-Apochromat ×40 NA 0.95 or Zeiss Plan-Apochromat ×20, NA 0.8	Stitching (optional tiling)

a. BCR = includes a bar code reader.
b. Full featured version is 2,500 Euros/5 users license. The free viewer has every essential tool for digital slide viewing and only the additional application modules are missing, such as 3D reconstruction, TMA evaluation, and on-line teleconsultation.
c. OlyVIA (Olympus Viewer for Imaging Applications)
d. An optional ×40 Plan Apo may be used, but due to the extremely short working distance, some user interaction may be required for initial "macro focus," depending on glass slide thickness.
e. Optional objectives: ×40/0.75 Plan Fluor, or ×40/0.90 Plan Apo
f. Any objective may be used including ×60 and ×100.

choosing among these systems. An objective evaluation is quite difficult because it is dependent on factors such as:

- The area size to be scanned.
- The objective lens used (×20 or ×40). DMetrix DX-40 is able to take 3,000 images/s for the ×80 objective.
- The camera CCD size.
- The motorized stage model.
- The required time on the previsualization stage: panoramic view, selection of area of interest and focusing method.

- The number of focusing points needed. Slides with irregular surface require a higher number of points, which reduces the scanning speed.
- The number of planes at the z-axis to be digitized.
- The speed to obtain data from the camera to the PC and from the PC to the storage device.

For those devices with a slide feeder, the time to upload and download a slide is about 6 to 8 seconds. The total time, including the code bar reading, is around 15 seconds.

Tables 4 and 5 show the scanning time with ×20 and ×40 objective lens, respectively, sorted by

Table 4. Required Time for Slide Scanning or Digitization With a ×20 Objective

Device	Area 10 × 10 mm	Area 15 × 15 mm	Whole: 25 × 50 mm
DMetrix DX-40	—	—	58 sec (ultra-speed mode)
Aperio ScanScope CS	2 min	2:45 min	4 min
Syncroscopy SyncroScan	—	—	5 min
Hamamatsu C9600 NanoZoomer	1 min	2.5 min	10 min
MicroBrightField	1 to 3 min	2 to 7 min	10-40 min
MetaSystems Metafer	—	—	11-14 min
Aperio ScanScope T2	2 min	5 min	16-30 min
Trestle MedMicroscopy	—	—	16 min
Zeiss Mirax Scan	3.5 min[a]	2:25-6:45 min[b]	15 min
Olympus SIS .slide	2.5 min	6 min	30 min
Fairfield Imaging PathSight	4 min	8 min	47 min
Applied Imaging Ariol	7 min	16 min	1 h 30 min
LifeSpan Alias	8.5 min	20 min	1 h 40 min
Samba Naviqap	—	—	2 h 22 min
Nikon Eclipse E600FN	—	—	4 h to 12.5 h
Imstar Pathfinder E-Mage VM	—	—	5 min (5 mm²)

a. Fluorescent scanning: 10 × 10 mm, ×20 objective, 0.23 μm/pixel, 3 channels with the following exposure times: 52, 100, and 48 milliseconds, 40:00 minutes.
b. With a ×0.63 camera adapter and focusing every 1 mm, scanning time is 3:05 minutes; if focus is made every 2 mm, scanning time is reduced to 2:25 minutes.

Table 5. Required Time for Slide Scanning or Digitization With a ×40 Objective

Device	Speed (mm²/s)	Area of 10 × 10 mm	Area of 15 × 15 mm	Whole: 25 × 50 mm
Hamamatsu C9600 NanoZoomer	0.35	4 min	9 min	40 min
Aperio ScanScope T2	0.66	6 min	13 min	1 h 15 min
Zeiss Mirax Scan	0.35	12.5-31 min	39 min	1 h 45 min
Olympus SIS .slide	0.18	11 min	25 min	1h 46 min
MicroBrightField	0.07-0.52	3.5-22 min	7.5-50 min	40 min-4 h 38 min
Applied Imaging Ariol	0.23	28 min	64 min	6 h
LifeSpan Alias	0.05	35 min	80 min	7 h 28 min
Nikon Coolscope	—	1 h	2 h 30 min	12 h 30 min

ascending order, according to the information provided by the manufacturer.

Slide Holder and Maximum Sample Size

All of the evaluated devices work with conventional slides 1 × 3 inches (25 × 75 mm) × 1 mm thick. Some systems, such as the first version of the Nikon Coolscope, do not work properly with round corner slides, whereas this is the type of slide recommended by Zeiss Mirax Scan.

The total scanning surface is constrained to the motorized stage used and to the histologic slide type. The scanners or motorized microscopes are usually able to go through all the slide width (27 mm), but usually they only go through the 60% to 70% of the slide length (50 mm); that is, they avoid the label area. The slide label is obtained by a special camera (Aperio ScanScope, LifeSpan Alias) or read by a code bar device (Zeiss Mirax Scan). For bar code reading with the Aperio ScanScope T2, we have obtained the best results using 2D Datamatrix code-bar of 8 points, generated with Tissue-Tek AutoWrite Glass Slide Printer. LifeSpan Alias can read multiple barcode formats

The Aperio ScanScope CS is able to work with large format slides (2 × 3 inches). This option is also available in Olympus SIS .slide.

The maximum scanning area of Aperio ScanScope T2 is 22.9 mm × 54.9 mm; for Aperio ScanScope CS, it is about 26.3 mm × 56.1 mm (50 mm × 57.1 mm for large slides); and for Zeiss Mirax Scan, it is about 27 mm × 50 mm.

The Mirax Scan digitization system is available with 2 configurations: Mirax Scan, for automatic digitization (several 50-slide cassettes), and Mirax Desk, a cheaper option intended for manual slide scanning.

Concerning the slide thickness, those slides mounted between 2 pieces of glass because of a break can be digitized with a conventional motorized microscope (Bacus BLISS, Olympus SIS .slide), but usually not with scanners such as Aperio ScanScope.

To hold the specimen slide, a mechanical attachment (Bacus BLISS), simple lateral pressure (Olympus SIS .slide), or vacuum system (LifeSpan Alias) can be used.

Focus Quality

The quality of the focusing may be evaluated by the following parameters:

- The focus range of movement (150 μm for Zeiss Mirax Scan.
- The time for adjusting the focus (0.1 second for Zeiss Mirax Scan).
- Minimum distance (least step) (0.15 μm for Zeiss Mirax Scan).
- The method applied to distance measurement: infrared laser light of 780-nm wavelength (Zeiss Mirax Scan) or the triangulation method, point by point (Aperio ScanScope T2).
- Movement resolution at the z-axis (0.3 μm with objective ×20 for Zeiss Mirax Scan and 0.1 μm for Aperio ScanScope T2 with objective ×40).

The number of focusing points (focusing map) may be manual or automatically set in Aperio ScanScope T2 and CS, AI Ariol, and Hamamatsu C9600 NanoZoomer. With this last device, it is also possible to adjust the number of focus points in the batch mode. Olympus SIS slide uses several options to choose different focusing algorithms.

Systems based on motorized microscopes (Table 1) are particularly suitable to scan focus difficult areas within the slide because they allow higher control over the stage position. However, scanners may also
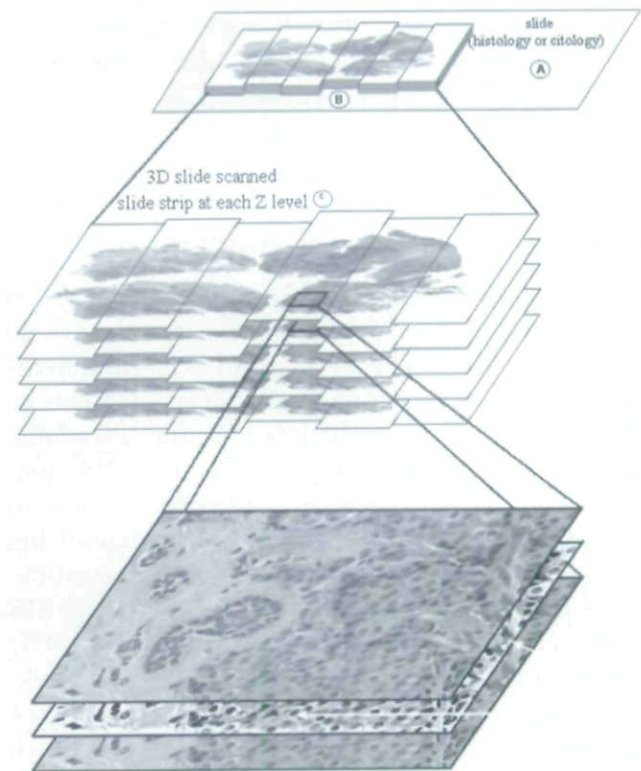

Figure 1. Multiple focus planes digitization from a histology slide.

have an observation mode that allows the focus to be manually adjusted over a set point.

Multiple Planes Digitization Through the Z-Axis

Visualizing multiple planes is a requirement for pathologists in cases such as thick tissue slides or cytology slides with 3-dimensional (3D) clusters. Therefore, the scanning system should be able to digitize different focused planes through the z-axis in a way similar to that used by pathologists with the microscope fine focus control (Figure 1).

Different systems are able to provide the digitization through the z-axis, at least on one area of the slide. These are the Aperio ScanScope CS (Remote Revisit), Bacus BLISS, LifeSpan Alias, Nikon Coolscope with EclipseNet-VSL, and Hamamatsu NanoZoomer (since August 2006).

It is sometimes possible to obtain a perfect focus image even if the system is not able to produce z-axis

scanning by means extended focal imaging (EFI), which is optimal focus over all points (Olympus SIS .slide). Syncroscopy SyncroScan also integrates a similar system for plane focusing.

Whole Slide Scanning Method and Stitching

The first step of the digitization process is to decide whether it is necessary to scan the whole slide or only arbitrarily selected areas, or automatically detected tissue or cytology fields. This can be achieved using a previsualization tool (available in most systems), by taking an overview slide picture with a digital camera or webcam (Aperio ScanScope, Zeiss Mirax Scan), or by digitalizing the whole slide at the lowest available magnification (×2.5 for LifeSpan Alias; ×2 for Olympus SIS .slide; ×1.25 for Bacus BLISS and AI Ariol). The Hamamatsu C9600 NanoZoomer uses an additional high-resolution camera (1344 × 1024 Interline CCD) to create the slide map. It is a quick process that usually takes less than 15 seconds (Zeiss Mirax Scan, Olympus SIS .slide).

It is also possible to manually select several regions of interest (Olympus SIS .slide, and AI Ariol) or draw up the estimated shape of one or several regions to be scanned (LifeSpan Alias, Nikon EclipseNet VS).

The second step is to adjust the focus point (focus map) for the selected region (see Focus Quality) and adjust image settings, such as white balance.

The third step is the scan. This process consists on taking different fields of the original slide and joining the different strips (virtually on the screen or in a real file on the PC) to create a seamless virtual slide. This process can be set manually or automatically (batch mode or one touch) in all described systems, as well as in a mixed way, available in NDP Scan 1.2 software of the Hamamatsu NanoZoomer, where the user corrects the focus options or the regions of interest that the system uses in batch mode (Figure 2).

The acquisition of microscopic fields is usually square-by-square, from the slide's upper left corner to the lower right one (Figure 3A). Thus, the final image is a mosaic composed of multiple files. Other systems use linear cameras (Aperio ScanScope), where the acquisition file corresponds to a strip set of the same length than the slide or area of interest width (Figure 3B).
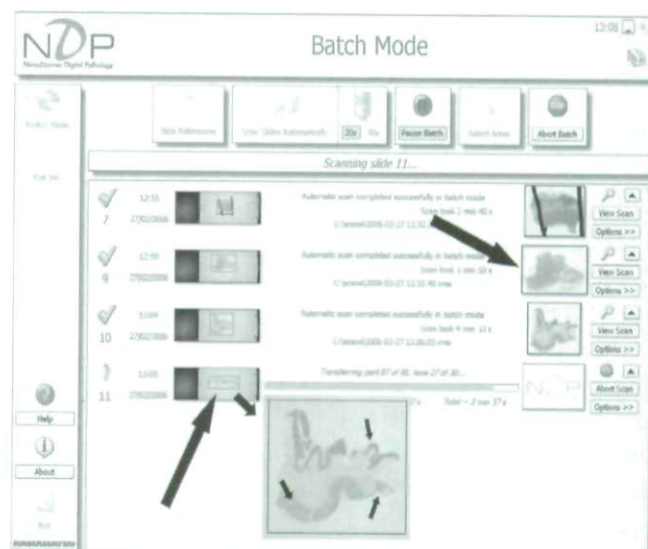
Figure 2. Bath mode in Hamamatsu NanoZoomer allows automatic region of interest selection and focusing points and manual adjustments for editing and amending failed scans.

The first method (square matrix acquisition) takes a high number of image frames (tiles). For instance, it is estimated that for an area of 15 mm × 10 mm at ×40, the Olympus SIS .slide needs about 1253 pictures.

The use of monochromatic cameras (LifeSpan Alias, and AI Ariol) requires three scanners (R, G, B) for each area. At 2048 × 2048 pixels CCD (LifeSpan Alias), each acquisition will be 4 MB in size, therefore 12 MB per frame.

Aperio ScanScope uses a lineal scanner and adjusts the focus from one line to the next at a high frequency rate. Moreover, the line scanned is always at the optical axis, thus avoiding 2D optical aberrations. At a ×20 objective, each uncompressed tagged image file format (TIFF) file with a scanned image stripe is 200 mega-pixels in size when Aperio ScanScope is used.

The assembling process of the slide squares may be done in 2 different ways:

1. Mechanical adjustment: Tiling the borders of each fragment. This method is available in most systems.
2. Software adjustment: Stitching between the images. The images are acquired with some overlapping between them and the common regions are matched by software. This method
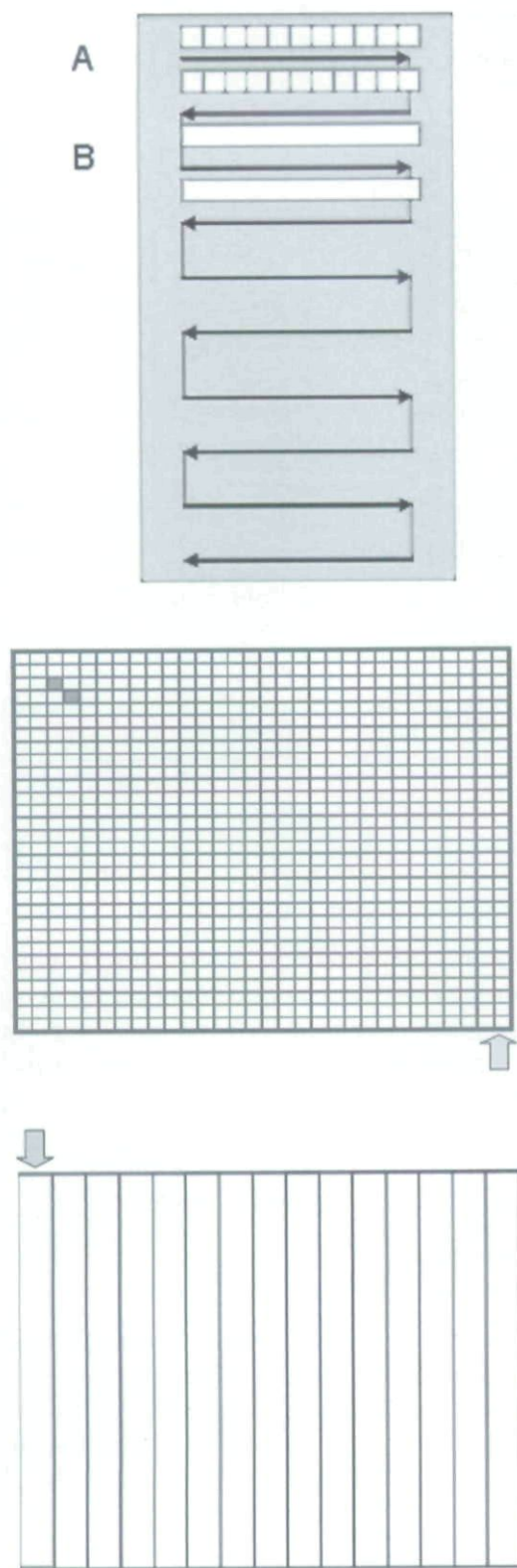
Figure 3. (A) Scanning based on the sequential microscope individual fields capture (squared matrix) where an image is obtained by each field. (B) Lineal scanning, where each file groups all fields within a line.

is available in Aperio ScanScope, AI Ariol, DMetrix, LifeSpan Alias, and Zeiss Mirax Scan.

File Formats

The final result obtained after a digital slide has been created may be physically as follows:

- Multiple files—usually thousands of Joint Photographic Experts Group (JPEG) files— in one or several folders. Usually, each folder corresponds to a different magnification (SlidePath, Bacus Bliss). It is also possible to generate files with bitmaps without compression (LifeSpan Alias). These files are particularly useful for image analysis.[4]
- Several files with one or multiple resolutions (usually JPEG). This is the method used by Zeiss Mirax Scan and Zoomifyer viewer.[5]
- A single compressed file (JPEG2000, JPEG).

It is possible to obtain a single file with multiresolution information. When this solution is implemented, all the information, including the panoramic image or thumbnail and the captures to different resolutions, is stored in a single physical file. Often, the structure of these files is pyramidal[6] and may be TIFF (Aperio ScanScope), JPEG2000, Flashpix (MicroBrightField Virtual Slide), or other (VSI extension in Olympus SIS. slide).

Table 6 summarizes the various compression techniques and file formats used by different manufacturers. To facilitate transmission through communications networks, high-resolution images are stored partitioned in small chunks (240×240 pixels in Aperio ScanScope T2 and CS).

The maximum compression for JPEG2000 is usually double than that of JPEG at the same percent compression ratio, but it requires double computational time. Therefore, some solutions, like Aperio ScanScope CS, use JPEG2000 compression by hardware.

Digital Slide Visualization and Processing

Ideally, the movements to be carried out by the pathologist when visualizing a virtual slide on the

Table 6.   Compression Methods and File Formats

Device	Default Compression Method	JPEG2000 Compression Available	Other Optional Compression Methods	Available Uncompressed Format	File Type	File Size ×40
Aperio ScanScope T2	JPEG (libjpeg)	Lossless (1:20). Matrox Imaging algorithm	LZW (lossless)	TIFF	.SVS (modified TIFF 6.0, pyramidal)	1.5 GB
Aperio ScanScope CS	JPEG (libjpeg)	By hardware	LZW (lossless)	TIFF	.SVS (modified TIFF 6.0, pyramidal)	1.5 GB
AI Ariol	JPEG	Yes	No	BMP, JPEG2000, and PNG	JPEG	—
Bacus BLISS	JPEG	No	—	No	JPEG	—
Hamamatsu C9600 NanoZoomer	JPEG	No	No	Under development	JPEG	2 GB
LifeSpan Alias	JPEG o JPEG2000	Yes (Aware Jpeg2000 SDK)	JPEG	TIFF	.TIF	2 GB
Nikon EclipseNet-VSL	JPEG	Yes (max 350 MB)	No	TIFF, BMP	.VSL (JPEG) and :JP2 (JPEG2000)	2 GB (JPEG)
Olympus SIS .slide	CMW (Leadtools Wavelet compressed 1:15)	Yes (with or without loss)	JPEG	TIFF, but not RAW	.VSI (pyramidal, up to 9 resolutions)	1.5 GB
Zeiss Mirax Scan	JPEG	Third party (VMscope)	No	BMP, PNG	.DAT (JPG)	1.5 GB

screen should be the same as those made when a conventional microscope is used. They are:

- X-axis and y-axis movements → lateral and vertical displacement through the screen.
- Objective shifting or zoom.
- Z-axis displacement (focusing and focus planes).
- Other functions.

X-Axis and Y-Axis Movements

One of the main problems in digital microscopy systems is the low screen refreshment during the horizontal and vertical displacements. This is due to the large amount of data that need to be transferred between the different parts of computer (central processing unit, hard disk, graphics card, memory) or through the communication network. It is therefore possible to watch during this process how the pieces of each slide's region start uploading into the screen. This effect may be quite disturbing for pathologists, who are used to going quickly through the slide with only the limitation of their eyes' accommodation.

To avoid this problem, the proposed solution is partitioning large images into small pieces according to the required magnification and buffering adjacent pieces (prefetching) in the viewer. In most cases, the microscopy viewer includes a thumbnail image to get a picture of the entire slide (Figure 4). Some solutions bring this image into a resizable window (LifeSpan Alias, Olympus SIS .slide).

Pointer and Tracking Devices

The mouse is not a comfortable device for pathologists to use to review the slide because they are used to a wheel system to control the fine movements and focus. It is therefore likely that devices similar to the SmartMove, designed by Leica Microsystems (Figure 5), will become popular in the near future.

Objective Shifting or Zoom

The virtual slide viewers allow pathologists to work in a way similar to that used with conventional microscopes, by using ×4, ×10, ×20, ×40, or ×60
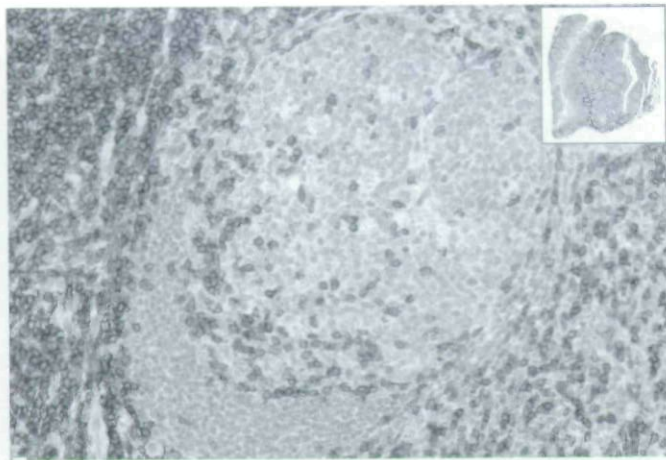
Figure 4. The thumbnail image (slide map) at the right upper corner, allows identifying the zone to be seen into detail (CD3 in normal tonsil).

Figure 5. SmartMove controller by Leica Microsystems.

objectives. In addition, most viewers have additional tools to facilitate the everyday work, such as:

- Continuous sliding bar that allows adjustment of the zoom in a flexible way.
- Digital zoom to quickly visualize at higher magnifications small selected regions of interest.
- Opening 2 windows of the same slide at different magnifications.

Z-Axis Displacement (Focusing and Focus Planes)

Histology slides, and especially cytology slides, may require the capture of multiple z-planes to get a perfectly focused image. It is particularly interesting to obtain a complete view of some structures, such as papillary structures on cytology smears.

In general, digitization of multiples planes at the z-axis is usually available for motorized microscopes but not for scanners. However, Aperio ScanScope CS has an option called "Remote Revisit," that allows for the capture a set of z-planes ("z-stack") composed of small regions of interest within the digital slide that can be selected with a remote connection to the scanner. This allows focusing on real-time selected areas from the slide. Those z-stack images are kept on the server together with the high-resolution 2D image. It is during the visualization process that the z-stack images are overlapped and matched all together.

Others Functions

Simultaneous and synchronized displacement on multiple windows: This is an especially useful tool that allows movements performed on one slide to be reproduced in all the other opened slides. This function helps to compare different sections from the same paraffin block stained with different markers, even if the sections have undergone rotation and translations. This option is available on several described systems, such as Aperio ScanScope (Aperio SmartSync), Zeiss Mirax Scan, Olympus SIS .slide, and AI Ariol. The link between multiple scans may be used by the Ariol system to define the regions for capturing and analyzing over all of the linked slides. For example, the hematoxylin and eosin slide may be used to select the tumor regions on all slides in an immunohistochemistry panel.

Track of visiting areas: Some viewers (Zeiss Mirax Scan) keep track of the visited regions within the slide.

Digital bookmarks: It is possible to include bookmarks on digital slides, facilitating the retrieval of interesting positions in subsequent case reviews. These bookmarks can be in text or in graphic format (ie, arrows, circles). The bookmark list with labeling options should allow a viewer to go directly to the right magnification and coordinates of the slide with a simple click of the mouse. Another interesting option, not always available, is identifying the author

of the bookmark. Aperio ScanScope, Zeiss Mirax Scan and Olympus SIS .slide include digital bookmark options.

Teleconsultation and conferencing: Virtual slides can be visualized and commented on simultaneously by several pathologists. This virtual "multihead microscope" allows all users to review the same areas when one of the users takes control of the session, or different pathologist can review different parts of the same slide at the same time. Moreover, discussions and comments are available by means of a dialog window. With session control tools, it is possible to assign a role to each participant of the conference.

Remote conference tool: This is available on several systems, such as Aperio ScanScope, Bacus BLISS, Zeiss Mirax Scan, and Olympus SIS .slide. This tool facilitates the use of digital slides in multicenter quality assurance protocols.[7] ScanScope CS and Ariol, and Mirax Scan allow remote logging onto the system, and running the system using Remote Desktop over the Internet.

Image Analysis: Apart from the Tissue Micro-Array (TMA) specific modules described below, some of the digitization solutions (Mirax Scan, Aperio ScanScope) have modules to conduct quantitative analyses of regions of interest within digital slides,[8] such as quantification of positive immunohistochemistry pixel detection, positive nucleus, cellular membrane detection and micrometastasis detection. Except for specialized solutions (AI Ariol, Clarient ACIS), the image analysis provided by most of virtual slides solutions is limited to some edition, visualization, and management options. It is also possible, however, to perform image analysis in digital slides with other compatible software (Olympus AnalySIS).

Three-dimensional reconstruction: Zeiss Mirax Scan (3D Mirax) has an optional module that gives the possibility of creating 3D reconstructions from virtual slides.

Software for Tissue-Micro Arrays: Tissue Micro-Array slides require specific management tools. For this reason, some manufactures have integrated specific TMA modules, such as Aperio TMALab or SIS .slide. Specific solutions, such as AI Ariol and Clarient ACIS, can be more suitable for TMA management.

File Format Export Options: The digital image visualization module must be able to manipulate them (ie, rotate, resize, contrast modification, filtering) and, above all, it must be able to export them in different formats such as JPEG, TIFF (without compression), and raw format. For instance, Olympus SIS .slide is able to export the slide to a standard

Web page, generating multiple JPEG, each one of 512 × 512 pixels, that may be read by Zoomifyer.

The resulting files in the exporting options, which can include annotations, are good-quality images, and they are most valuable for scientific papers, congress presentations, or pathology reports. For example, the ImageScope viewer from Aperio is able to provide images of 1.676 × 926 pixels (96 ppp).

Available Systems on the Market

Bacus Bliss
http://www.bacuslabs.com/

Bacus BLISS is a pioneer system of virtual slides. Besides a good image quality, Bacus BLISS incorporates numerous tools for virtual slides management and visualization via Web, also used by other manufactures. Contact: Bacus Laboratories, Inc, 410 Eisenhower Lane N, Lombard, IL 60148; phone: 630-424-9750; fax: 630-424-9754; e-mail: info@bacuslabs.com.
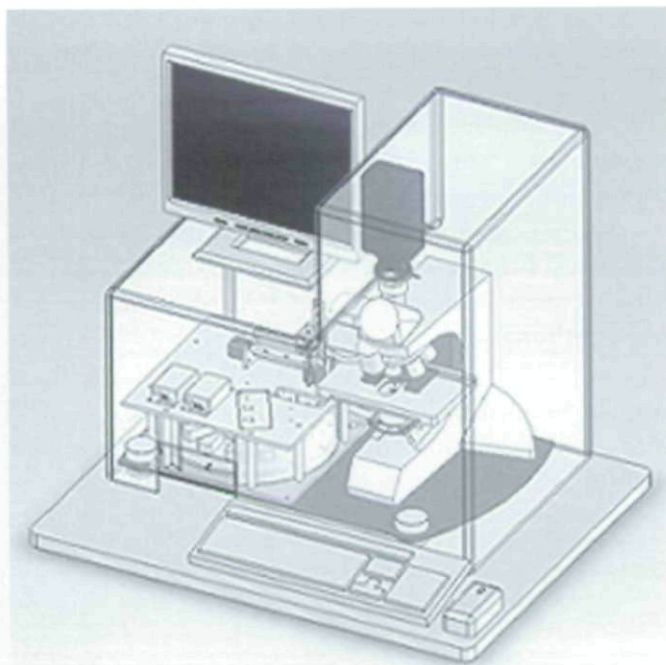
The image gallery is available at the Web site http://66.106.109.242/blipathology/index.html (it requires Java installation).

LifeSpan Biosciences Alias
http://www.lsbio.com/products/automatedimage capture/

The main characteristic of LifeSpan Alias is the high image quality, due to the numerous modifications the manufacturers have done to the Leica microscope. The system includes an illumination system based on 4 colors LED (RGB + amber). A recent modification of ALIAS was the addition of the 63x objective. Contact: LifeSpan Biosciences Inc, 2401 4th Ave,

Suite 900, Seattle WA 98121; phone: 206-374-1175; fax: 206-464-1723; e-mail: Rajesh.Krishnan@LifeSpanBiosciences.com; Web page: http://www.lsbio.com/.

Olympus-Soft Imaging System .slide
http://www.soft-imaging.net/rd/english/3412.htm

This system is easy to use and provides a very good image quality. Its outstanding software includes numerous modules (ie, slide review, conferencing, measurements). Since 2004, Soft Imaging System (SIS) is part of Olympus Europe. The contact address is local to each country by Olympus Optical: http://www.olympus-global.com/en/global/

A gallery of digital SIS slides is at http://www.dotslide.soft-imaging.de/

Nikon Eclipse E600FN with EclipseNet-VSL
http://www.nikon-instruments.com/uk/products/imaging.html and http://www.lim.cz/index.php?lang=en&inc=enet_vsl

This system is proposed by Nikon for a complete slide digitization. The digital slides are kept on database of a workstation or in any other network PC. EclipseNet performs all the capture and storage processes. The EclipseNet Web DB database allows reviewing the slides using a web browser. EclipseNet VSL works with all Nikon digital cameras (see also Nikon Coolscope below).
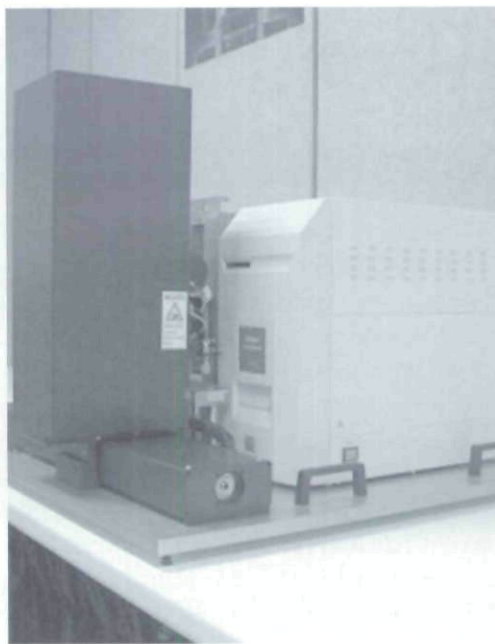
In the near future, EclipseNet will be replaced by Nikon Imaging Software (NIS) Elements, the Nikon worldwide software platform. At present, NIS does not include an instrument for creating or managing virtual slides.

Nikon Coolscope with
EclipseNet-VSL or Bacus Coolscope VS
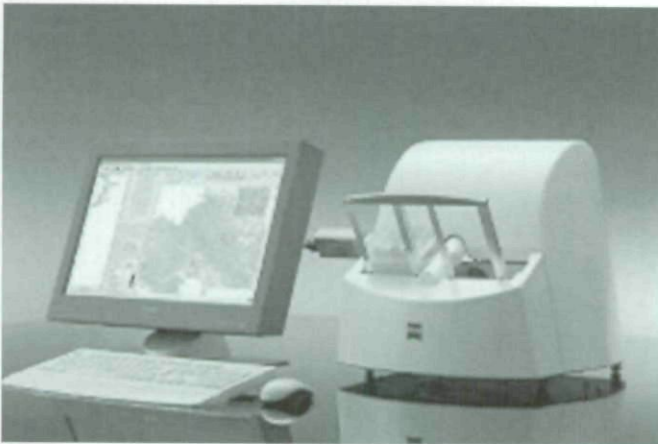http://www.coolscope.com/ and http://www.eclipsenet.info/

Nikon Coolscope digital microscope is a compact system that includes the functionalities of an optical microscope and a PC. It allows transferring images from slides using standard hypertext transfer protocol (http) and file transfer protocol (ftp). In the United States it is provided with software from Bacus (Coolscope VS). This system is also available in Europe as an alternative to EclipseNet VSL. EclipseNet VSL is able to drive Coolscope and also a microscope with a scanning stage. The latter option allows more flexibility for lens selection; for instance, using oil immersion lenses is not available for the Coolscope. The Coolscope slide loader (Cool-loader) is designed for telepathology purposes, not for VSL creation. Contact (international): http://www.nikon-instruments.jp/eng/contact/ContactTop.aspx

The LUCIA Net VSL digital slide gallery is available at http://www.lim.cz/webdb/index.php?dsn=SampleDatabase&dbid=27&jmeno=guest, or sample databases at http://www.lim.cz/index.php?lang=en&inc=webdb.

Carl Zeiss Mirax Scan and Mirax Desk
http://www.zeiss.de/mirax

Mirax Scan is based on the Hi-Scope (http://www.3dhistech.com/) developed in Hungary by Dr Bela Molnar's team, from Semmelweis University at Budapest. The system is able to digitalize automatically a large number of sample slides. This is one of the few systems with fluorescent scanning capabilities. Up to 10 filter cubes can be mounted in Mirax Scan. The obtained virtual slide is always a folder with several files with .dat extension. A Software Development Kit will be soon available to access the slide data by third-party programmers. A smaller model Mirax Desk is also available. It is equivalent

to Mirax Scan in speed, resolution, and image quality in bright field, but has no fluorescence and it can only scan one slide.

More information is available at the Web site. A contact form and international section is at http://www.zeiss.com/explore.

Several examples are available at the 3D Histech Web sites: http://www.3dhistech.com/viewer2/; Zeiss', http://www.zeiss.de/mirax; and PathoNet site: http://www.pathonet.org/

Aperio ScanScope T2 and CS
http://wwww.aperio.com/ and http://www.scanscope.com/

The Aperio Company has 2 different ScanScope scanners: T2 and CS. They are remarkable for their good quality/speed ratio, the efficient virtual slides data base management, including tools like Workflow Manager, and for the easy publication of virtual slide both on the Internet and the hospital intranet. The company has released a new pathology information system, called Spectrum, to help the integration of gross study images, microscopy digital slide images, reports, clinical histories, and other associated documents and images.
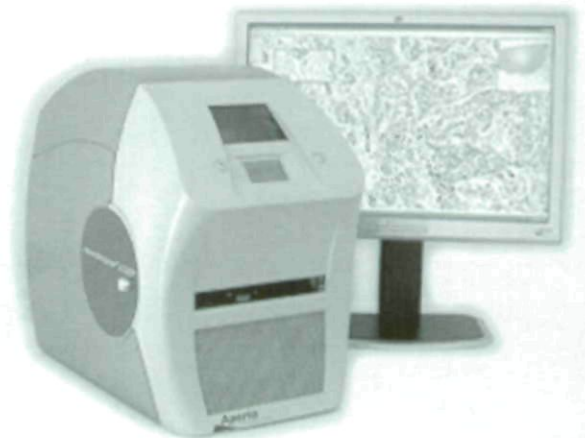
Aperio Technologies Headquarters is at 1430 Vantage Court, Suite 106, Vista, CA 92081; phone: 760-539-1100; fax: 760-539–1116; e-mail: info@ aperio.com. The European office address is Charwell House, Wilsom Rd, Alton, Hampshire GU34 2PP, UK; phone: +44-0-1420-540-271; e-mail: europeinfo@ aperio.com.

The image gallery is available at http://images2 .aperio.com/ and http://images.scanscope.com/.

Other examples are available at California Medical Center at California University on Davis (http://ccm .ucdavis.edu/imagearchive/), Leeds University (http:// www.virtualpathology.leeds.ac.uk/), and Hospital General de Ciudad Real (http://www.hgcr.es/html/).

Hamamatsu C9600
NanoZoomer Digital Pathology
http://jp.hamamatsu.com/products/node.do?dir=/ap plication/medical/pa195&lang=en&ext=html

This is a fast slide scanner that can handle more than 200 slides. Files obtained with NanoZoomer can be published in Web sites using Bacus WebSlide software. Contact e-mail: salesmaster@hq.hpk.co.jp.

Sample images scanned with Hamamatsu NanoZoomer are available at Bacus Laboratories Web site at http://www.bacuslabs.com/blog/products/ WebSlideBrowser.html

Dmetrix DX-40
http://www.dmetrix.net/

This innovative device, developed at Tucson, Arizona, is defined as an "array microscope" because it includes an 80-lens or miniature objectives array combined in the same scanner. In this fashion, the whole slide image is obtained in one shot (9,10). Contact: DMetrix, Inc, 1141 West Grant Rd, Suite 100, Tucson, AZ 85705

Apollo Telemedicine
http://www.apollotelemedicine.com/

Apollo Telemedicine software is used by MicroBrightField and DMetrix solutions. Some of their products are ASAP Imaging, to share virtual slides and work in a remote way with the same slide, facilitating the second opinion (consult); Apollo LIVE for videoconference; and PathPACS for storage management and virtual slide retrieving. An image gallery is available at http://216.204.84.52/
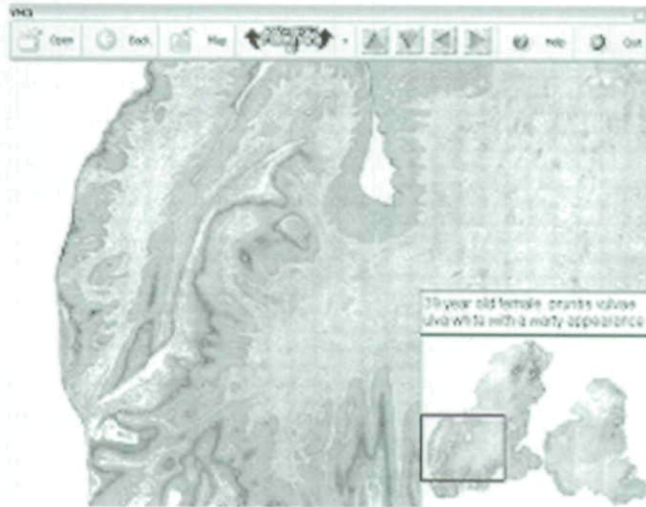
Aurora mScope
http://www.mscope.net

Aurora MSC is a company specialized in software for pathology image management. Its products are compatible with different scanners, including Aperio, Trestle, and Nikon, among others. Some of the modules available are Scanner Interface Manager for image generation, file conversion, data base, and work list management; Distributed Digital Slide Server, and image server optimized for the use of digital slides, using a wavelet compression which is 25% more efficient than JPEG; Laboratory Image Workflow Manager for images follow-up from their generation, including storage and integration within the pathology workflow process; and Authoring and Publishing Server with educational tools in pathology. Contact: Aurora MSC, 505 University Ave, Suite 1603. Toronto, Ontario M5G 2P1, Canada; e-mail: rwilk@mscope.net.

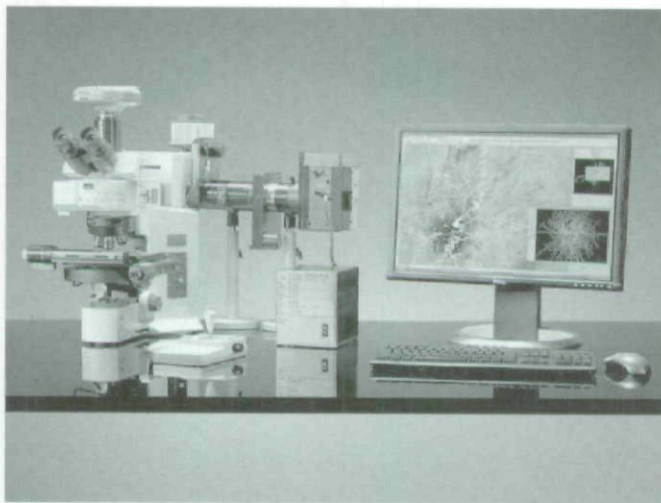## Fairfield PathSight
http://www.fairfield-imaging.co.uk/

PathSight is a flexible system, capable of both semi-automatic and automatic working modes. The java-based viewer is free. Fairfield is a British company funded on 1989 located at Nottingham, UK, and since 1995 it has belonged to Medical Solutions Group (e-mail: info@medical-solutions.co.uk). They provide an image gallery at http://www.fairfield-imaging .co.uk/fairfield/mikewells/ and http://interpath1.uio .no/telemedisin/

MicroBrightField Virtual Slide System

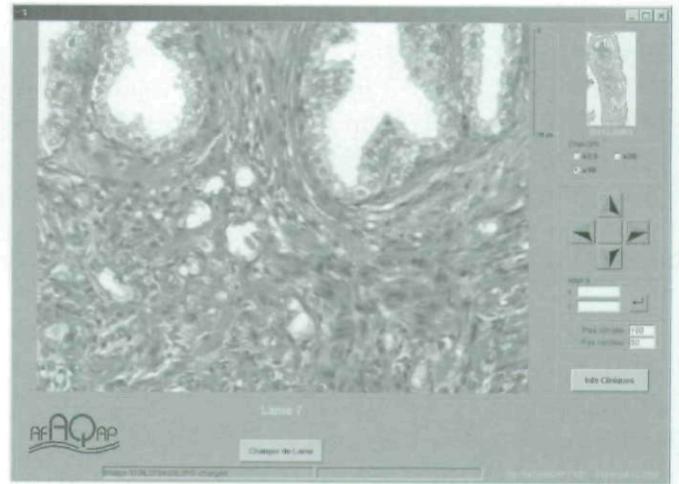http://www.microbrightfield.com/products/virtual
slide/whitepaper.html

Virtual Slide System is a software module to be added to microscopy solutions of some manufactures, such as Neurolucida or Stereo Investigator. Contact (Europe): MicroBrightField Europe, E.K. Matthissonstrasse 6, D-39108 Magdeburg, Germany; phone: +49-391-732-6989; fax: +49-391-732-6989; e-mail: rbraul@online.de.

Samples of virtual slides can be found at Neuroinformatica Web (http://neuroinformatica.com/) and Virtual Slidebox (http://www.path.uiowa.edu/virtualslidebox/).

Samba Naviqap

http://www.sambatechnologies.com/ProduitsUS1.htm

Samba uses the automatic microscopy workstation AcCell, with a control PC that may be combined with a digital slide server (PDB2000). Contact: Samba Technologies, Zirst, 53 chemin du Vieux Chêne, 38240 Meylan, France; phone: +33-0-476-04-00-50; fax: +33-0-476-04-15-98; e-mail: samba@sambatechnologies.com.

An example of a virtual slide is at http://www.molecular-dx.com/1_investors/3_samba/40_virtual SlideViewer.html

Syncroscopy SyncroScan

http://www.syncroscopy.com/syncroscopy/syncro
scanshort.asp

SyncroScan is a system based on software for automatic microscopy that is possible to install on different microscopes. Syncroscopy Europe Office contact: Beacon House, Nuffield Rd, Cambridge CB4 1TF, United Kingdom; phone: +44-0-1223-727127; fax: +44-0-1223-727101; e-mail: eurosales@syncroscopy.com; international: intlsales@syncroscopy.com.

Some examples of digital slides are at the Web site http://www.syncroscopy.com/syncroscopy/microscopy .asp

SlidePath Digital Slidebox

http://www.slidepath.com/

The Digital Slidebox is a management system for digital slides that allows users to create their own Web-based pathology resource, with application for external quality assurance. Contact: SlidePath, The Innovation and Enterprise Centre, Dublin City University, Dublin 9, Ireland; phone: +353-0-1-700-7576; fax: +353-0-1-700-7555; e-mail: info@SlidePath.com.

Trestle MedMicroscopy and Xcellerator

http://www.trestlecorp.com/medmicro_sysdiagram .asp and http://www.trestlecorp.com/Xcellerator.asp

The Trestle MedMicroscopy software is used combined with motorized microscopes, such as Olympus BX-40 or Olympus BX-50 (11). Moreover, Xcellerator is a set of software toolkits for digital image archiving and management. It may incorporate tools for image analysis such as those developed by BioImagene (http://www.bioimagene.com). Contact: Trestle Corporation, 199 Technology Dr, Suite 105, Irvine, CA 92618; phone: 800-823-3203, 949-673-1907; fax: 949-673-1058; e-mail: info@trestlecorp.com.

Tribvn ICS WF
http://www.tribvn.com/tribvn/med/stations.htm

The French company Tribvn has developed a module for virtual slides that can also be used in its telepathology platform, TeleSlide. These systems may be used with several devices, either motorized microscopes or scanners (Aperio ScanScope, Nikon Coolscope) and are able to export proprietary files to JPEG2000 format. Contact: Tribvn 39, rue Louveau. 92320 Châtillon, France; phone: +33-0-1-55-58-05-20; fax: +33-0-1-55-58-05-30; e-mail: info@tribvn.com; Web page: http://www.tribvn.com/

VMscope
http://www.vmscope.com/

The VMscope GmbH was founded in 2004 by members of the University Hospital Charité in Berlin. Its products are VM Slide Server, which provides the virtual slides for fast transmission over Internet, and VM Slide Explorer for the client side. It offers different services, such as slide scanning, slide databases, and publishing slides on the Internet, with several authoring tools (Learning Portal, VM TMA Module). The VM Slide Converter creates virtual slides in the standard format JPEG2000 from the data format of several slide scanners, such as Zeiss Mirax. Contact: VMscope GmbH, am Campus Charité Mitte, Schumannstr 20-21, 10117 Berlin, Germany; phone: ++49-0-30-450-536188; fax: ++49-0-1212-579320-483; e-mail: info@vmscope.de.

BioGenex iVISION and GenoMx VISION
http://www.biogenex.com/

In addition to scanning facilities, iVISION includes automated imaging analysis, enabling the user to capture images and quantify results in immunohistochemistry and in situ hybridization applications. The GenoM VISION System is designed for tissue microarray core image acquisition, management (archiving, retrieval, and cataloging), and analysis. Contact: BioGenex Headquarters. 4600 Norris Canyon Rd. San Ramon, CA 94583; phone: 1-925-275-0550, 1-800-421-4149 (in the US); fax: 1-925-275-0580; e-mail: info@biogenex.com.

CytoCore InPath Slide Based Test
http://www.molecular-dx.com/2_laboratorians/2_inpath/270_inCell_HPV.html

Formerly Molecular Diagnostics, this company has developed a screening for cervical dysplasia and
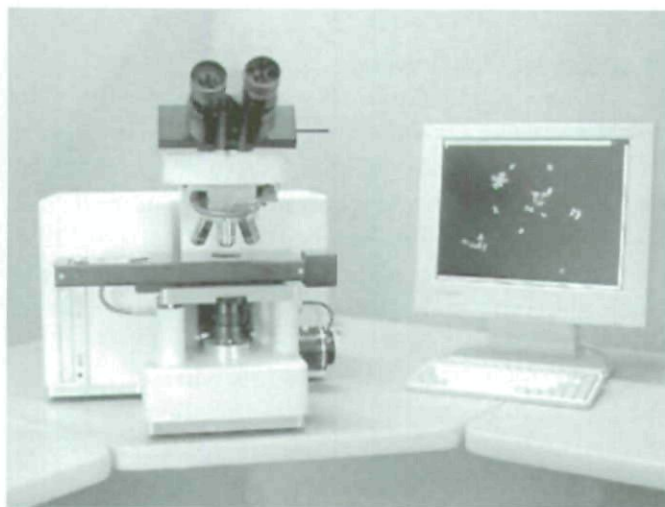
cervical cancer screening technology that includes an analysis of cytology slides through use of an automated instrument for detection of any material indicative of "not normal." Contact: CytoCore, Inc, 414 N Orleans St, Suite 800, Chicago, IL 60610; phone: 312-222-9550; fax: 312-222-9580; e-mail: info@molecular-dx.com.

Imstar Pathfinder Morphoscan and E-Mage
http://www.imstar.fr/products/pathology/emage/

Imstar Pathfinder provides automated assessment of molecular cell markers in cytology and histology, including tissue arrays. Two modules are available: E-Mage VS for creation of virtual slides, and E-Mage VM (virtual microscope) for interactive browsing of selected area of virtual slide images. Contact: IMSTAR SA, 60 rue Notre Dame-des-Champs 75006 Paris, France; phone: +33-0-1-42-34-93-70; fax: +33-0-1-46-34-51-57; e-mail: info@imstar.fr.

Leica AS TPS2
http://www.leica-microsystems.com/
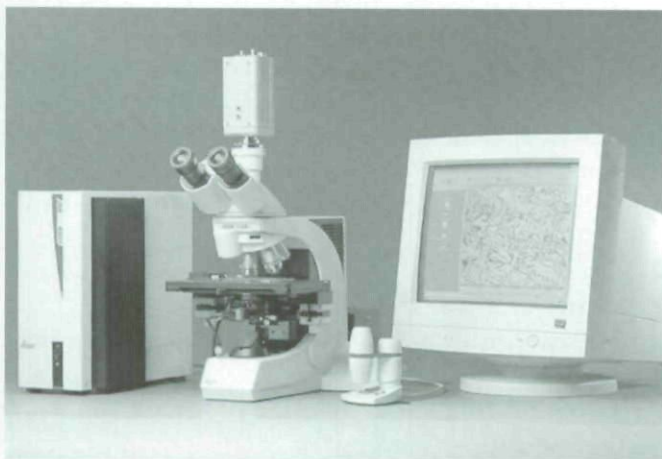
Leica AS TPS2 is a telepathology system especially designed to obtain a second opinion and providing telepathology facilities for frozen section diagnosis. It is neither a virtual slide system nor an automatic image analysis solution, but we have included this system because of its multiple integrated tools (patient-related case data, telepathology case database, remote control, shared pointer, and video and audio conference, amongst others). Contact: Leica Mikrosysteme Vertrieb GmbH, Lilienthalstr 39-45, D-64625 Bensheim, Germany; phone: +49-6251-136 0; fax: +49-6251-136-155. Contact Web address: http://www.leica-microsystems.com/contact.

MetaSystems Metafer
http://www.metasystems.de/
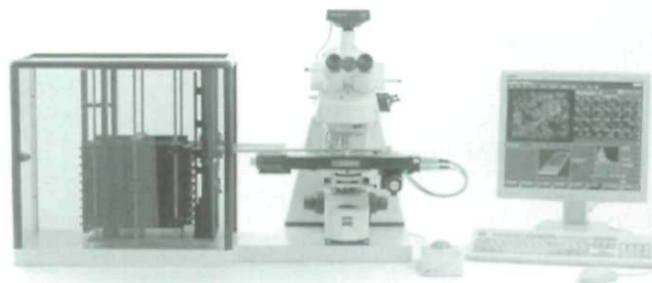
This company has converted an automatic metaphase finder into a "multipurpose scanning platform" that can be of use also in pathology, for instance, in fluorescence in situ hybridization (FISH) imaging. These are two examples: the MetaCyte module analyzes cells in single cell preparations and in tissue sections, and the RCDetect module finds rare cells. Contact: Robert-Bosch-Str 6, 68804 Altlussheim, Germany; phone: +49-6205-39610; fax: +49-6205-32270; general e-mail: info@metasystems.de.

Applied Imaging Ariol
http://www.aicorp.com/products/02path.htm

Ariol is a system for automatic image analysis. It has been developed to quantify immunochemical and FISH techniques. Thus, it allows using conventional microscopy and fluorescence.[12] A complete TMA package is available. The Sanger Institute (http://www.sanger.ac.uk/) is the UK reference site.

In some countries, this system is distributed by Olympus resellers. Corporate offices (sales for Americas and Pacific Rim): Applied Imaging Corporation, 120 Baytech Dr, San Jose CA 95134-2302; phone:

+1-408-719-6400; fax: +1-408-719-6401; e-mail: info@aicorp.com. International operations (sales for Europe, Middle East and Africa): Applied Imaging International, Ltd, Bioscience Centre, Times Square, Scotswood Rd, Newcastle upon Tyne NE1 4EP, United Kingdom; phone: +44-191-202-3100; fax: +44 191 202 3101; e-mail: info@aii.co.uk.

Examples of bright field and fluorescent TMA analysis are available at http://www.sanger.ac.uk/Teams/Team86/icc_group_intro.shtml

Clarient ACIS (Chromavision)
http://www.chromavision.com/product/acis1.htm

ACIS stands for Automated Cellular Imaging System, and it was developed by Chromavision. ACIS has been designed for immunohistochemistry image analysis.[13] In July 2005, Dako and Clarient signed an ACIS development and distribution agreement. Clarient Headquarters: Clarient, Inc. 31 Columbia, Aliso Viejo, CA 92656; phone: 949-443-3355, ext 295; fax: 888-443-3345; e-mail: info@clarientinc.com.

CyTyc ThinPrep Imaging System
http://www.cytyc.com/lab/lab_cervical_cancer_thinprepimaging.shtml

This solution is designed for gynecologic cytology and it allows processing of up to 300 slides per day. The system only stores the coordinates of the interesting or abnormal areas, together with the slide identification. Contact: Cytec Corporation. 85 Swanson Rd, Boxborough, MA 01719; phone: 1-888-ThinPrep. European headquarters' e-mail: uk@cytyc.com.

Compucyte iColor and Compucyte iCyte
http://www.compucyte.com/icolor.htm and http://www.compucyte.com/icyte.htm
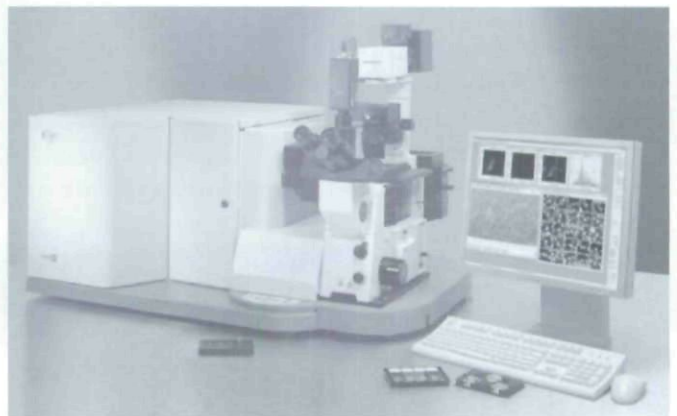
iColor allows simultaneous analysis of fluorescent and chromatically-stained specimens. iColor combines functions of flow cytometry, fluorescence image analysis, and immunohistochemistry.

The iCys Research Imaging Cytometer first performs scanning (with simultaneous acquisition of multiple-fluorescence and bright-field laser-scatter images) and analysis of the slides, and then the

microscope stage may be automatically moved to the location of any features of interest. Headquarters: CompuCyte Corporation, 12 Emily St, Cambridge, MA 02139; phone: +1-617-492-1300; fax: +1-617-577-4501; e-mail: salesinfo@compucyte.com.

Conclusions

After analyzing the main features of the anatomic pathology slide digitization systems available on the market, we conclude that these systems are able to scan a digital slide using the highest image quality available (objective ×40) in about 1 hour. These digital slides may be used for diagnosis, digital records, second opinion, medical education, professional training, quality assurance, image analysis, and research. Most of the reviewed systems can be provided with some software for TMA analysis.

Digital slides allow the retaining of a permanent file of the slide that avoids daily problems such as breakage, loss, or fading of stain and fluorescent signals.

Some devices include warnings about their use being "only for research and not for clinical diagnosis purposes," even when their commercial material includes descriptions about their use in clinical field. The US Food and Drug Administration may take action when this practice is detected.[14]

Future systems should improve some technical aspects, such as the scanning speed, the necessary bandwidth on communication networks, large requirements for storage, user interfaces (which are also different from the conventional microscope), improvement of focusing, and detection of tissue or cytology areas. Many of the systems are not suitable for use with polarization light (amyloid or crystals detection), but in the near future, most systems will incorporate the necessary filters.

Another drawback to current systems is their high cost, usually between 60 000 to 180 000 Euros, ($75 000 to $230 000), except Nikon Coolscope with a lower price of about 15 000 Euros ($20 000).

We believe that current technology is allowing a progressive shift towards a complete microscopic image digitization in pathology. Even if today's systems are suitable only for the digitization of a certain number of selected cases, in the near future, all the technical problems are likely to be solved, and all cases studied by the pathology department will be digitized. Keeping in mind all the presented difficulties, during the First Virtual Slide Congress on the Internet, our group confirmed that pathologists find themselves quite comfortable working with virtual slides.[15] Many other authors have confirmed the utility of virtual slides.[16]

It is already possible to integrate links to the virtual slide into the pathology final reports. The pathologist may sign the forms at the same time the corresponding images are visualized. This facilitates a double-checking and validation (text and images) before the final report is sent.

The intellectual process of analyzing and interpreting pathology images to provide a final diagnostic is one of the fundamental aspects of the pathologist's work; therefore, both image and report always must include the name of the pathologist and department where that intellectual work has been done. Only those images corresponding to validated and signed reports should be available in the patient's clinical record.

The enterprise-centralized and automated storage is the best option and should be based in what is called the Picture Archiving and Communication Systems, which will permit an efficient way of seeking pathology images. This will be possible, thanks to the Digital Imaging and Communications in Medicine (DICOM) image format, which is being adapted to be used also for pathology images.

Acknowledgments

References

1. Ferreira R, Moon B, Humphries J, et al. The virtual microscope. *Proc AMIA Annu Fall Symp.* 1997;449-453. Available from: http://citeseer.ist.psu.edu/ferreira97 virtual.html. Accessed June 8, 2006.
2. Afework A, Beynon MD, Bustamante F, et al. Digital dynamic telepathology—the virtual microscope. *Proc AMIA Symp.* 1998;912-916. Available from: http://citeseer .ist.psu.edu/afework98digital.html. Accessed June 8, 2006.
3. The EORTC Tumor Bank. European Organisation for Research and Treatment of Cancer (EORTC). Available from: http://www.eortc.be/vtb/tumorbank/. Accessed June 8, 2006.

4. Costello SP, Johnston DJ, Dervan PA, O'Shea DG. Development and evaluation of the virtual pathology slide: a new tool in telepathology. *J Med Internet Res.* 2003;5:e11. Available from: http://www.jmir.org/2003/2/e11/. Accessed June 8, 2006.

5. Galvez J, Munn RJ, Garbutt AW, Cardiff RD. The development of the mouse models of human cancer consortium image archives. Advancing pathology informatics, imaging, and the Internet (APIII 2002). Scientific Session and E-Poster Session Abstracts. *Arch Pathol Lab Med.* 2003;127:806. Available from: http://arpa.allenpress.com/arpaonline/?request=get-toc&issn=1543-2165&volume=127&issue=7. Accessed June 8, 2006.

6. García Rojo M, Bueno García G, González García J, Carbajo Vicente M. Preparaciones digitales en los servicios de Anatomía Patolorgica (I). Aspectos básicos de imagen digital. *Rev Esp Patol.* 2005;38:69-77. Available from: http://www.pgmacline.es/revpatologia/volumen38/vol38-num2/38-2n02.htm. Accessed June 8, 2006.

7. Patel AA, Gil-Bertson J, Dhir R, Becich MJ. Virtual quality assurance protocol: a quality assurance protocol for pathology slides from multiple institutes. advancing pathology informatics, imaging, and the Internet (APIII 2002). Scientific Session and E-Poster Session Abstracts. *Arch Pathol Lab Med.* 2003;127:804-805. Available from: http://arpa.allenpress.com/arpaonline/?request=get-toc&issn=1543-2165&volume=127&issue=7. Accessed June 8, 2006.

8. Olson AH, Eichhorn O, Saligrama K. Algorithmic processing of high resolution virtual slides. Advancing practice, instruction, and innovation through informatics (APIII 2004): Scientific Session and E-Poster Abstracts. *Arch Pathol Lab Med.* 2005;129:819-820. Available from: http://arpa.allenpress.com/arpaonline/?request= get-toc&issn=1543-2165&volume=129&issue=6. Accessed June 8, 2006.

9. Weinstein RS, Descour MR, Liang C, et al. An array microscope for ultrarapid virtual slide processing and telepathology. Design, fabrication, and validation study. *Hum Pathol.* 2004;35:1303-1314. Available from: http://dx.doi.org/10.1016/j.humpath.2004.09.002. Accessed June 8, 2006.

10. Weinstein RS. Innovations in medical imaging and virtual microscopy. *Hum Pathol.* 2005;36:317-319.

11. Hitchcock CL, Hitchcock LE. Three years of experience with routine use of telepathology in assessment of excisional and aspirate biopsies of breast lesions. *Croat Med J.* 2005;46:449-457. Available from: http://www.cmj.hr/2005/46/3/15861526.pdf. Accessed June 8, 2006.

12. Habib I. Automated microscope slide analysis in pathology. Los Angeles, CA: IVD Technology; 2005. Available from: http://www.devicelink.com/ivdt/archive/05/05/001.html. Accessed June 6, 2006.

13. Divi RL, Dragan YP, Pitot HC, Poirier MC. Immunohistochemical localization and semi-quantitation of hepatic tamoxifen-DNA adducts in rats exposed orally to tamoxifen. *Carcinogenesis.* 2001;22:1693-1699. Available from: http://carcin.oxfordjournals.org/cgi/content/full/22/10/1693. Accessed June 8, 2006.

14. Gutman SI. Warning letter. Rockville, MD: Food and Drug Administration; 2003. Available from: http://www.fda.gov/foi/warning_letters/g3970d.htm. Accessed June 8, 2006.

15. Gonzalez J. Digital microscopy applications in the Virtual Pathology Congress (http://www.conganat.org). SEAP regional meeting. Ciudad Real: Sociedad Española de Anatomía Patológica; 2006. Available from: http://www.seap.es/regional/clm/. Accessed June 8, 2006

16. Gilbertson JR, Ho J, Anthony L, Jukic DM, Yagi Y, Parwani AV. Primary histologic diagnosis using automated whole slide imaging: a validation study. *BMC Clin Pathol.* 2006;6:4. Available from: http://www.biomedcentral.com/1472-6890/6/4. Accessed June 8, 2006.

Review Article

Review of the current state of whole slide imaging in pathology

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Abstract

Whole slide imaging (WSI), or "virtual" microscopy, involves the scanning (digitization) of glass slides to produce "digital slides". WSI has been advocated for diagnostic, educational and research purposes. When used for remote frozen section diagnosis, WSI requires a thorough implementation period coupled with trained support personnel. Adoption of WSI for rendering pathologic diagnoses on a routine basis has been shown to be successful in only a few "niche" applications. Wider adoption will most likely require full integration with the laboratory information system, continuous automated scanning, high-bandwidth connectivity, massive storage capacity, and more intuitive user interfaces. Nevertheless, WSI has been reported to enhance specific pathology practices, such as scanning slides received in consultation or of legal cases, of slides to be used for patient care conferences, for quality assurance purposes, to retain records of slides to be sent out or destroyed by ancillary testing, and for performing digital image analysis. In addition to technical issues, regulatory and validation requirements related to WSI have yet to be adequately addressed. Although limited validation studies have been published using WSI there are currently no standard guidelines for validating WSI for diagnostic use in the clinical laboratory. This review addresses the current status of WSI in pathology related to regulation and validation, the provision of remote and routine pathologic diagnoses, educational uses, implementation issues, and the cost-benefit analysis of adopting WSI in routine clinical practice.

Key words: Consultation, diagnosis, digital, education, frozen section, imaging, informatics, telepathology, validation, virtual microscopy, whole slide imaging

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INTRODUCTION

Whole slide imaging (WSI), also commonly referred to as "virtual" microscopy, involves the digitization or scanning of glass slides to produce "digital slides" for viewing by humans or subjecting them to automated image analysis. The creation of digital slides is intended to simulate light microscopy. Since the introduction of whole slide scanners almost a decade ago (around 1999), WSI technology has evolved to the point where digital slide scanners are currently capable of producing high-resolution digital images within a relatively short time. Scanning of slides at multiple magnifications and focal planes (so-called z axis) is also possible. Compared

to static digital images, WSI have been shown to be more beneficial for educational and some diagnostic purposes.[1] However, there appear to be several technical and logistical barriers to be overcome before WSI becomes a widely accepted modality in the practice of Pathology. For example, current scanning technology does not satisfactorily accommodate thick smears and three-dimensional cell groups in cytopathology.[2,3]With tissue sections, scanners are currently unforgiving when encountering tissue folds, bubbles and poor staining of material to be scanned.[4] Unless significant modifications to workflow are made centered around digital pathology (e.g. automation, continuous flow processes, quality of the histology presented to the WSI devices), placing WSI systems in the clinical pathology laboratory has been shown to stress the system in terms of reliability and throughput.[5]

In the United States, regulatory issues regarding digital pathology are also in flux. The Food and Drug Administration (FDA) convened a panel hearing in October 2009 that focused on how best to regulate whole slide digital imaging systems used for primary pathologic diagnosis. At present, there are unclear regulatory standards related to image capture and display, validation, and clinical use of WSI. This review addresses the current status of WSI regulation and validation and the use of WSI for remote and routine pathologic diagnosis and education. We also discuss implementation issues and cost-benefit considerations.

REGULATION AND VALIDATION

In the United States, federal regulations set forth in the Food, Drug and Cosmetic Act of 1938 and the Medical Device Amendments of 1976 provide the FDA with limited authority over medical devices. Some of these devices are subject to premarket review through 510(k) premarket notification process or premarket approval application (PMA). These US federal regulations pertain primarily to manufacturers of whole slide digital imaging systems, and potentially also to laboratories that incorporate WSI in diagnostic services. The FDA convened a panel hearing in October 2009 that focused on how best to regulate WSI systems that are to be used for primary diagnosis in surgical pathology.[6] Details of the events and debates of this FDA advisory panel meeting are available on *The Daily Scan* blog.[7]While WSI systems are clearly medical devices subject to FDA regulation, there are a number of open issues the FDA will need to address before the regulatory environment is clarified:

Will the FDA choose to regulate these devices, or exercise discretion on the grounds that they are similar to conventional microscopes, which the FDA has chosen not to regulate?

If regulation is contemplated, will it be applied to entire WSI systems or will WSI components be regulated separately (i.e., image capture, image storage and manipulation, display screens, other aspects of the user interface, specialized software functions)? [Figure 1]

How will regulation be applied to care models in which components of WSI are purchased and operated by different entities (e.g., image capture in one facility, image hosting and manipulation in a second, and interpretation in a third facility)?

Will regulatory approval of WSI cover all types of diagnostic work, or will some tissue types, disciplines, analyses, or diagnostic entities be excluded? Current WSI approval, for example, is limited to HER2/neu, estrogen receptor (ER) and progesterone receptor (PR) analysis.[8]

In addition to FDA requirements, the Clinical Laboratory Improvement Amendments (CLIA) impacts clinical laboratories using WSI systems. If used in a clinical laboratory for an application not explicitly cleared or approved by the FDA, an argument can be made that the laboratory is employing a laboratory-developed test (LDT) and is subject to CLIA validation requirements pertaining to LDTs. Finally, professional and scientific standards require pathologists to assume responsibility for the methods they employ in the care of patients, including WSI.

How should WSI be validated? Validation is traditionally defined as confirmation, through the provision of objective evidence, that the requirements for a specific intended application have been fulfilled. In the case of a clinical laboratory test the validation process must take into account the purpose for which a test is intended, performance claims that the test must meet to be suitable for the intended application, and an assessment of the risks that may prevent the test from serving its intended purpose. Tests themselves are said to be validated after all of the individual performance claims appropriate for the clinical application are found to be valid. Performance claims can be of a number of types, including claims about analytic bias, reproducibility, suitability of certain
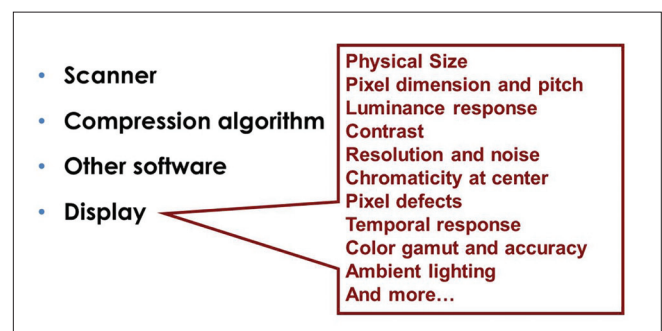
Figure 1: Qualities of a digital display device. WSI systems can be regulated as a whole, or individual components such as displays can be regulated separately

specimen types, and turnaround time. Claims can concern the accuracy of diagnosis or the accuracy of an individual measurement (e.g., tumor size).

It is impossible to use the scientific method to affirmatively prove that a claim is valid. "Validity" is a matter of informed judgment. Reasonable people may differ over the degree of assurance required or the types of procedures that should be performed to assess a claim, and may have different views about the types of claims that should be tested to consider a test fit for a particular use. Table 1 lists some of the specific validation issues raised by WSI. Although limited validation studies have been published using WSI,[9-11] no generally-accepted standard guidelines are available to validate WSI for diagnostic use in the clinical laboratory. Evaluators must consider a range of issues that include sample size and statistical power, separating pathologist performance issues from device performance issues, the scope of cases to include in a challenge set, whether the set should be "enriched" with difficult cases, washout (time interval before asking a pathologist to review the same diagnostic material), the time it takes pathologists to become facile with WSI instruments, and the setting in which validation is assessed. Table 2 lists one of the authors' (PNV) personal preferences for WSI validation.

PRIMARY FROZEN SECTION DIAGNOSIS AND TELEPATHOLOGY

WSI in recent years has been effectively utilized by several groups for telepathology, including primary frozen section diagnosis and secondary/tertiary teleconsultation.[12-20] The advantages of using WSI for this purpose include access to an entire digitized slide or even an entire case (set of slides), automated scanning, the high resolution of images available for review, rapid interpretation time, and the ability to exploit simultaneous viewing (teleconferencing). The University Health Network (UHN) in Ontario, Canada has extensive experience using WSI for telepathology.[21,22] UHN is a multi-site academic institution in downtown Toronto, comprising the Princess Margaret Hospital (PMH), Toronto Western Hospital (TWH) and Toronto General Hospital (TGH) which houses UHN's consolidated pathology department. TWH has no on-site pathologist and is located approximately one mile to the west of TGH. It is also the only UHN site where neurosurgery is performed, generating up to 10 frozen sections in a typical week. Sending a single pathologist to TWH to cover this small volume of frozen sections, most of which come from neurosurgery, created several challenges including delays in regular case sign-out at TGH, delays in carrying out academic responsibilities at TGH and no possibility of consulting with colleagues on difficult frozen sections. The latter issue created the risk of compromised diagnostic accuracy

Table 1: Issues to consider in the validation of WSI for routine diagnostic application

- Separating the device from the practitioner
- Pathologist experience (in practice and with the device)
- Washout and validation setting
- Types of data generated
- Measuring accuracy
- Measuring bias
- Measuring precision (intra-rater, inter-rater, and inter-instrument)
- Sample size
- Generalizability of findings

Table 2: Preferences for WSI validation for routine diagnostic application

- Measure intra-observer bias and precision
- Use general pathologists with defined device experience
- Utilize high-quality display
- Enrich the case sample (stack with difficult cases)
- Washout period > 2 weeks
- Analyze each parameter separately (e.g., tumor type, tumor grade, etc.)
- 80% power to detect 10% difference in bias or precision
- Generalize to all specimens except hematology, cytology, and dermatopathology

and/or unnecessarily deferred frozen section diagnoses. Telepathology was identified as a viable solution to these challenges and has been in use at UHN for over seven years.

At UHN, a team that consisted of a pathologist, a senior histotechnologist and an information technology (IT) support person was formed in 2003 to select a digital pathology vendor, validate the system to be used for frozen section diagnosis, train new users and carry out due diligence that included consultation with the medical malpractice insurance provider, development of a protocol for approval by UHN's Medical Advisory Committee and engagement of the surgeons at TWH. After an 18-month development period, the system went live in November of 2004 initially using a robotic microscope (Leica TPS2, Leica Microsystems) for making frozen section diagnoses at TWH in the absence of an on-site pathologist. The robotic microscope was used until October 2006 to report 350 frozen sections. While the robotic system was found to provide diagnostic accuracy that was equivalent to a light microscope, it typically took 10 min to review a single frozen section slide and produced total turnaround times (TAT) of > 20 min. This created challenges with respect to meeting CAP accreditation benchmarks for TAT.

In September 2006, UHN began parallel testing between the robotic microscope and a WSI platform (Aperio ScanScope CS). After only 30 cases, it was apparent that WSI was going to provide superior image quality, a user

experience that more closely replicated light microscopy than the robotic device and a four to fivefold reduction in the amount of time required to review a frozen section slide. The TAT (time from receiving tissue to calling the surgeon with a diagnosis) was approximately 15 min for WSI versus 20 min per single block frozen section using the robotic microscope. Since October 2006, UHN pathologists have used WSI to make over 1800 primary frozen section diagnoses in the absence of an on-site pathologist. WSI has provided diagnostic accuracy that is equivalent to that experienced with light microscopy and facilitates the reporting of single block frozen sections with total TATs in the range of 14 to 16 min. They have experienced a 5% deferral rate with at least two pathologists reviewing the case before a deferred diagnosis is given, a quality measure that is not possible with a lone on-site pathologist reporting frozen sections by light microscopy.

Several factors have contributed to the success of the UHN program including a well-defined clinical application in the form of a small volume of neuropathology frozen sections, an uncomplicated frozen section workflow where most cases involve single pieces of tissue < 10 mm in size, an implementation period of approximately 18 months that allowed all team members to build confidence in the system and a team approach involving pathologists, histotechnologists, IT support staff, vendors and surgeons committed to making the program work. It has been the UHN experience that consistently high-quality frozen section slides produced by a skilled histotechnologist is an absolute requirement in order to have image quality that is sufficient to allow reliable frozen section diagnoses to be made via WSI [Figure 2]. System failure, requiring a pathologist to travel from TGH to TWH to report a frozen section, has occurred on six occasions (0.3% of cases) with a 15-min delay in TAT for the affected cases. The WSI failures included an unexpected hospital network shutdown (one case), moving the scanner to another network drop in the frozen section room associated with a loss of connectivity due to an IP address problem (one case), scanner failing to scan small (~2 mm) pale pieces of edematous brain tissue (two cases; the problem was resolved by adjusting the scanner gains to create a "faint slide" scanning protocol), excess mounting media on a frozen section slide that fouled the scanner objective requiring a thorough cleaning of the scanner objective and stage (one case), and a burned out light bulb in scanner light source (one case). The UHN has found WSI technology to be safe, accurate and reliable for making frozen section diagnoses in settings where there is no on-site pathologist. Successful implementation requires: effective planning and communication, a willingness to adjust old routines without compromising quality, and histotechnologists who are able to provide consistently high-quality frozen section slides.

ROUTINE PATHOLOGICAL DIAGNOSIS

WSI is increasingly being used in the day-to-day practice
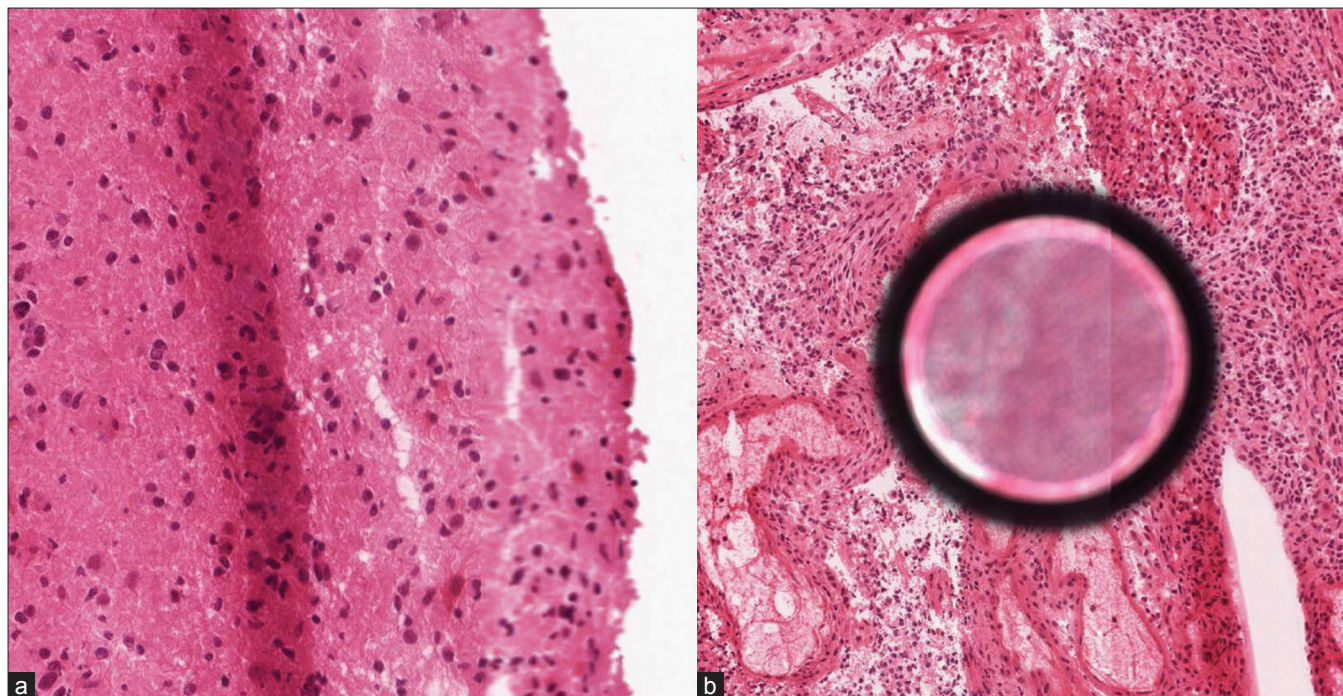
Figure 2: This figure shows two examples to illustrate the impact of suboptimal frozen section slides on image quality generated by WSI devices. (a) A diffuse astrocytoma with a tissue fold in the center of the field is shown that has caused the edge of the section (right edge) to be out of focus, (b) A high-grade astrocytoma with a large air bubble under the coverslip

of surgical pathology, particularly for teleconsultation. Digitized slides have been used for certain quality assurance practices, such as obtaining second opinions. However, the question on most pathologists' minds is whether WSI will be utilized for making routine pathologic diagnoses, ushering in the era of the "slideless" laboratory. The adoption of digital pathology has been slower than the adoption of digital images in radiology. This is partly related to the fact that pathology digital data is acquired in a slightly different manner from that in radiology. Although both disciplines require an imaging modality to collect primary data,[23] in radiology, images begin as *digital* data whereas pathology images have to be converted from an *analog* substrate into a digital format. Other differences between radiology and pathology digital imaging are the picture archiving systems (i.e., Picture Archiving and Communication System or PACS) and associated standards (e.g., Digital Imaging and Communications in Medicine or DICOM) available for radiology, larger file size and associated metadata of pathology digital image files, and workflow efficiencies in radiology.[23,24]Some of the barriers to the adoption of digital pathology images are related to the performance, workflow efficiency, infrastructure, integration with other software, and exposure to digital images.[25] Despite significant increases in technology, current adoption of WSI in the clinical space has been restricted and limited largely to niche practices.

The general pathology laboratory at Kalmar County Hospital in Kalmar, Sweden, is unique in that for around two years they have been digitizing all of their glass slides.[26] They scan around 60,000 histopathology slides per year, and over 75% of their histopathology diagnostic work is performed using digital pathology. Their impetus to go "slideless" was related to ergonomics as well as the need to network with colleagues in a country where there was a shortage of pathologists. Essential requirements for their success included: full integration with the digital pathology system and laboratory information system (LIS), reliable scanning, running the slide scanner continually with limited use of lab personnel, and good image quality. Obtaining consultations on their difficult cases in a timely manner was greatly facilitated through digital slide sharing and conferencing. More institutions are following suit; for example, a clinical trial at the University of Pittsburgh Medical Center (UPMC) evaluating the feasibility of signing out a high volume of surgical pathology cases using only digitized slides is currently underway.

Rendering routine pathologic diagnoses using WSI is feasible if the images truly represent an accurate digital reproduction of the scanned glass slide which can be saved, archived, reviewed and later retrieved without degradation of the image. Moreover, apart from integration with the LIS, the routine use of WSI in

pathology laboratories will require seamless connectivity over broadband networks, efficient workstations, cost-effective storage solutions, and standards-based informatics transactions for integrating information with WSI.[27,28] It is difficult to think of WSI for diagnostic purposes without considering the rest of the electronic medical record. It seems unlikely that pathologists will render diagnoses without access to additional medical information. One of the reasons for reported discrepancies between digital and glass slide diagnoses is attributed to inadequate clinical data, apart from other factors such as image quality, missed tissue on the digital slide and the pathologists' lack of experience using a WSI system.[29] It was demonstrated in one telepathology study using a virtual slide system that the correct diagnosis was made in 66% of cases without clinical data provided compared to a correct diagnosis of 76% with clinical data provided.[17] Therefore, in order for WSI to become an accepted diagnostic modality the provision of adequate medical information (e.g. gross pathology description, prior pathology reports, clinical history, etc.) will need to be weaved into the imaging system. Additional concerns that have yet to be satisfactorily addressed relate to malpractice and liability issues, as well as reimbursement for technical services related to producing the WSI.

Digital slides offer several advantages over glass slide review in terms of fidelity of the diagnostic material, portability, ease of sharing and retrieval of archival images, and ability to make use of computer-aided diagnostic tools (e.g. image algorithms).[30] Image analysis tools can automate or quantify with greater consistency and accuracy than light microscopy.[31] WSI has also permitted new business models of care in pathology. One such example is the virtual immunohistochemistry service provided by large national laboratories. After the remote reference laboratory performs technical staining and slide scanning services, the referring pathologist is provided with full access to these immunostained slides for their interpretation or referral to a teleconsultant. This has allowed some pathology practices to re-capture a portion of the reimbursement for professional interpretation services that has previously been diminished by these business practices. In the near future, the adoption of standards, validation guidelines, automation of workflow, creation of new revenue streams, and nuances of clinical digital practice will likely dictate a new standard of care for primary pathologic interpretations.

EDUCATION, TUMOR BOARDS AND PRE-SENTATIONS

WSI has gained tremendous acceptance for education, at tumor boards, and for presentations. WSI are

much more interactive than glass slides, easy to share anywhere at any time, and can help standardize training material. The successful use of WSI in undergraduate medical education and pathology resident training has been highlighted by several authors,[32-38] including the creation of digital slide teaching sets.[39-41] Unlike glass slide teaching sets, digital slides will not fade, break or disappear. Digital slides also offer the ability to standardize images, permit annotation, and can provide a wide case range for trainees, including rare cases. Digital teaching sets that can be accessed on a server over a network are available to multiple users, and can be developed to contain test modules for trainees. Not surprisingly, many medical schools are abandoning the light microscope. Collaboration among students is easier with WSI, and this technology supports the creation of a virtual-slide laboratory in medical schools. WSI also allows one to track how users view, pan and zoom around a WSI.[42,43] This function has been shown to be particularly helpful with respect to tutoring and assessing trainees [Figure 3], as well as for the development of image processing tools.

WSI have also had a positive impact on pathologists presenting cases at tumor boards in several institutions.[44,45] This is because WSI offers higher quality images with annotation, greater educational value for clinicians, involves less preparation time than photographing cases, and permits real-time flexibility (e.g. easy to add on cases, perform side-by-side viewing, and gives access to the entire slide which allows one to answer "on-the-spot" questions). WSI has also permeated into other areas such as E-education, virtual workshops, digital images in pathology journals and for proficiency testing.[46-48]

APPLICATIONS AND CAVEATS FOR SERVICE IMPLEMENTATION

In order to integrate WSI into routine practice, an infrastructure needs to be developed in the pathology department. This infrastructure consists of: (i) hardware for scanning slides, storing the scanned images, transmission of the images to pathologists, and the interfaces necessary to display the images and report interpretations; and (ii) the software to facilitate the workflow of the image movement, display, and reporting of the results. Following development of the internal infrastructure, the addition of remote teleconsultation requires that other features be considered in the system. These include security of protected patient information, process validation, as well as regulatory, medico-legal, and billing issues all to be added to the software overlay.

Figure 3: Search maps of WSI of inflammatory skin biopsies. Using a "light" version of SlideTutor a user's interaction with the digital image is recorded. The green highlighted areas represent the areas of the image that were viewed (search map). The search maps of three different residents are shown at different magnifications. Images courtesy of Dr. Claudia Mello-Thoms, Department of Biomedical Informatics and Department of Radiology, University of Pittsburgh, USA

And finally, when telconsultations are coming from outside the institution's firewall, engagement of IT resources in order for systems to "talk" to one another successfully.

There are a number of methods for receipt of WSI teleconsultation cases. For institutions communicating cases regularly, a secure permanent connection such as a virtual private network (VPN) is an optimal solution in terms of security. For ad hoc consultation cases, coming from a variety of remote sites, internet transmission and security may be enabled via a variety of commonly used encryption modalities. It is implicit that devices and image formats must be compatible across institutions. In order to facilitate consultations from pathologists at outside institutions to this subspecialty-based pathology practice at the Massachusetts General Hospital (MGH) in Boston, devices and software were "agnostized" and thereby able to process raw images in any format. Remote sites scan slides and enter clinical and demographic information using the MGH department's website. Images are queried via their software and directed first to a "hot seat" review station where they are then triaged to the appropriate subspecialist consultant, who also has the ability to share the images with other intranet users. Finalized cases are reported in the same system.

As already alluded to above, the advent of rapid whole slide scanning has several applications. In fact, the use of WSI for primary diagnosis and rapid teleconsultation is now not only possible, but may be preferred over routine microscope-based tasks. However, barriers to widespread adoption of WSI for teleconsultation that still need to be overcome include the high cost of scanning devices, validation of the process of interpretation of WSI for primary diagnosis (all specimen types may not perform similarly), the potential for FDA regulation, and legal issues related to teleconsultation across states and internationally.

EFFICIENCIES AND COSTS

While the advantages of WSI for digital pathology are well established,[27,49] formal evaluation of the parameters that impact the costs and benefits of various digital pathology activities based on WSI have not been rigorously evaluated. Analyses based on cost have traditionally focused on direct costs (for both hardware and software) and indirect costs (support personnel), while evaluations of the opportunities provided by WSI have usually focused on operational factors such as ease of use, scalability, etc. However, analyses of this nature largely ignore a fundamental workflow issue in diagnostic surgical pathology that is part of routine practice, namely that the histological sections on glass slides that are a necessary and intrinsic component of

diagnostic surgical pathology must be produced as part of any WSI process.

The department of pathology at the Washington University School of Medicine in St. Louis, MO in the USA developed a rigorous "value-added" approach that focuses on specific operational measures (cost, time, and accuracy), and the various clinical settings in which they can provide enhancement, to determine the settings in which WSI is able to improve surgical pathology practice.[50] The perspective for their value-added analysis is a tertiary care medical center surgical pathology practice characterized by a large volume of high-complexity cases; a subspecialty emphasis sign-out model; multiple sign-out areas; numerous training programs; and an academic pathology department. The results of their value-added approach depend upon this practice setting.

Value-added is defined by purely operational measures, specifically cost savings, time savings, or improvements in accuracy. Value-added can be assessed on a number of different scales. While the value-added approach described below focuses largely on the analysis-related patient care activities, WSI also adds value to educational activities and research. Some aspects of digital pathology based on WSI are specifically not value-added in the Washington University practice setting. For example, the mere capability of being able to produce a digital image that can be used for primary diagnosis (digital sign-out) in and of itself is not value-added, since the diagnosis based on the routine histological section is already possible from conventional light microscopy. However, aspects of digital sign-out that are not value-added in this tertiary care model may well provide a benefit in other practice settings, such as support of subspecialty consultation or the opportunity to view special stains produced by outside laboratories.

Overall, WSI as a tool for complete diagnostic sign-out was not yet economically viable. However, there were five specific areas in which WSI provided capabilities that were found to enhance the pathology practice at Washington University, which were either superior to currently existing workflow processes, or were unavailable at the time [Table 3]. Using these five specific capabilities,

Table 3: Specific added benefits of WSI

- WSI of selected slides from cases submitted in consultation
- Directly enhanced patient care through the availability, portability, and permanence of the images for patient care conferences
- Provision of a QA function
- WSI of slides that will be destroyed by ancillary testing
- WSI of slides that will be sent out
- WSI of legal cases
- WSI of cases for digital image analysis

the pathologists identified several areas in which WSI did not necessarily improve diagnostic accuracy, but nonetheless improved patient care. First, the use of WSI of selected slides from cases sent in consultation provided them with the opportunity to enhance patient care by allowing an immediately-available permanent record of the slides to guide frozen section diagnosis at the time of subsequent definitive excision; for comparison at sign-out of subsequent excision or post-therapy specimen; for presentation at patient care conferences; in QA activities; and so on. Second, WSI of selected slides sent to other institutions as requested or required by their policies for patient care, or slides encumbered by medico-legal proceedings, provided a permanent record for use in patient care activities even though their department lost control of the original glass slides. Third, WSI of original H and E slides that would be destroyed as part of ancillary testing made it possible to retain the diagnostic content of the slides; given the demonstration that molecular tests can be performed on nucleic acids collected from glass slides, the electronic record of slides produced by WSI will likely become more important. Fourth was the use of WSI for digital image analysis (e.g., HER-2/neu analysis) to support emerging slide-based diagnostic paradigms.

In their evaluation of WSI at Washington University, it became clear that the faculty and trainees at their institution varied in their comfort level and experience with the various software packages for image analysis, and also showed marked variation in their willingness to incorporate digital image analysis into their routine practice. The faculty members were unanimous in their unwillingness to incorporate a digital imaging process requiring that they move back and forth between different software packages; many staff were unwilling to have two computer monitors so that both software packages could be open at the same time; and the faculty demanded that any WSI process was operational both locally and remotely. In collaboration with several vendors, they therefore pursued a model of one-stop-shopping in which a seamless interface was created between the imaging software (Aperio Spectrum) and their LIS (Cerner Copath). Development of this new functionality required both system architecture design and new software code, and was associated with a significant additional investment in time and money. Implementation of this interface had an overall cost of approximately $70,000 ($27,000 for software development for the Aperio interface and the CoPath HL7 interface; $45,000 for purchase of the underlying CoPath Advanced Bar Coding and Tracking (AB and T) module. The need for development of this custom interface emphasizes additional hidden costs that are often overlooked in the evaluation of the utility of WSI in routine pathology workflow. Off-the-shelf hardware and software packages, regardless of the vendor, have generic functionality and

integration into specific practice environments may likely require custom software changes.

The aforementioned value-added approach appears to have been successful in identifying settings at Washington University in which WSI added utility to the surgical pathology practice, based on several metrics:

Number of scans. The number of cases scanned per year has shown consistent growth (at least 33% per year over the last three years).

Acceptance. Although faculty and trainee acceptance is difficult to measure directly and objectively, faculty and trainee demands for IT support for use of WSI via remote access by laptop computers, iPads (and similar tablets), and iPhones (and other smart phones) are interpreted as evidence that their faculty and trainees are integrating WSI into their routine workflows.

Expanded utilization. The initial value-added approach identified WSI of slides seen in consultation as an enhancement to patient care; interest from faculty to extend WSI to include select in-house cases is interpreted as evidence of the increasing recognition of a role for WSI in patient care activities.

CONCLUSION

Digital pathology systems offer pathologists an alternate, emerging mechanism to manage and interpret information. They offer increasingly fast and scalable hardware platforms for slide scanning with software that facilitates remote viewing, slide conferencing, archiving and image analysis. Initially deployed and validated largely within the research and biopharmaceutical industries, WSI is increasingly being implemented for direct patient care. Improvements in image quality, scan times and image-viewing browsers will hopefully allow pathologists to more seamlessly convert to digital pathology, much like our radiology colleagues have done before us. However, WSI creates both opportunities and challenges. While there are clearly successful niche applications of WSI technology for clinical, educational and research purposes, it is evident that several areas still require attention and/or careful consideration before more widespread clinical adoption of WSI takes place. These include regulatory issues, development of standards of practice and validation guidelines, workflow modifications, as well as defining situations where WSI technology will really improve practice in a cost-effective way. Current progress concerning these and other issues, along with improving technology, will no doubt pave the way for increased adoption over the next decade, allowing the pathology community as a whole to harness the true potential of WSI for patient care. The digital decade will likely redefine how pathology is practiced and the role of the pathologist.

COMPETING INTERESTS

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AUTHORS' CONTRIBUTIONS

All authors contributed to the writing, editing and review of this manuscript.

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REFERENCES

1. Pantanowitz L. Digital images and the future of digital pathology. J Pathol Inform 2010;1pii:15.
2. Pantanowitz L, Hornish M, Goulart RA. The impact of digital imaging in the field of cytopathology. Cytojournal 2009;6:6.
3. Wilbur DC. Digital cytology: Current state of the art and prospects for the future. Acta Cytol 2011;55:227-38.
4. Bautista PA, Yagi Y. Improving the visualization and detection of tissue folds in whole slide images through color enhancement. J Pathol Inform 2010;1:25
5. Gilbertson J, Yagi Y. Histology, imaging and new diagnostic work-flows in pathology. Diagn Pathol 2008;Suppl 1:S14.
6. Lusky K. Where will FDA land on whole-slide digital? CAPToday 2009;23:12:1.
7. FDA advisory panel meets, debates digital pathology. The Daily Scan. Blog posted by Ole Eichhorn Oct 26 2009. Available from: http://blog.aperio.com/2009/10/fda-advisory-panel-meets-debates-digital-pathology.html [Last accessed on 2011 Jun 20].
8. Pantanowitz L. Automated HER2 digital image analysis. Adv Lab 2007;16:80.
9. Gilbertson JR, Ho J, Anthony L, Jukic DM, Yagi Y, Parwani AV. Primary histologic diagnosis using automated whole slide imaging: A validation study. BMC Clin Pathol 2006;6:4.
10. Nielsen PS, Lindebjerg J, Rasmussen J, Starklint H, Waldstrøm M, Nielsen B. Virtual microscopy: an evaluation of its validity and diagnostic performance in routine histologic diagnosis of skin tumors. Hum Pathol 2010;41:1770-6.
11. Jukić DM, Drogowski LM, Martina J, Parwani AV. Clinical examination and validation of primary diagnosis in anatomic pathology using whole slide digital images. Arch Pathol Lab Med 2011;135:372-8.
12. Costello SS, Johnston DJ, Dervan PA, O'Shea DG. Development and evaluation of the virtual pathology slide: A new tool in telepathology. J Med Internet Res 2003;5:e11.
13. Furness P. A randomized controlled trial of the diagnostic accuracy of internet-based telepathology compared with conventional microscopy. Histopathology 2007;50:266-73.
14. Słodkowska J, Pankowski J, Siemiatkowska K, Chyczewski L. Use of the virtual slide and the dynamic real-time telepathology systems for a consultation and the frozen section intra-operative diagnosis in thoracic/pulmonary pathology. Folia Histochem Cytobiol 2009;47:679-84.
15. Fallon MA, Wilbur DC, Prasad M. Ovarian frozen section diagnosis: Use of whole-slide imaging shows excellent correlation between virtual slide and original interpretations in a large series of cases. Arch Pathol Lab Med 2010;134:1020-3.
16. Williams S, Henricks WH, Becich MJ, Toscano M, Carter AB. Telepathology for patient care: What am I getting myself into? Adv Anat Pathol 2010;17:130-49.
17. Massone C, Peter Soyer H, Lozzi GP, Di Stefani A, Leinweber B, Gabler G, et al. Feasibility and diagnostic agreement in teledermatopathology using a virtual slide system. Hum Pathol 2007;38:546-54.
18. Słodkowska J, Chyczewski L, Wojciechowski M. Virtual slides: Application in pulmonary pathology consultations. Folia Histochem Cytobiol 2008;46:121-4.
19. Wilbur DC, Madi K, Colvin RB, Duncan LM, Faquin WC, Ferry JA, et al. Whole-slide imaging digital pathology as a platform for teleconsultation: A pilot study using paired subspecialist correlations. Arch Pathol Lab Med 2009;133:1949-53.
20. Jakate S. Application of virtual microscopy in consultation practice of gastrointestinal and liver pathology. J Pathol Inform 2010;1:16.
21. Evans AJ, Chetty R, Clarke BA, Croul S, Ghazarian DM, Kiehl TR, et al. Primary frozen section diagnosis by robotic microscopy and virtual slide telepathology: The University Health Network Experience. Hum Pathol 2009;40:1070-81.
22. Evans AJ, Kiehl TR, Croul S. Frequently asked questions concerning the use of whole-slide imaging telepathology for neuropathology frozen sections. Sem Diag Pathol 2010;27:160-6.
23. Montalto MC. Pathology RE-imaged: The history of digital radiology and the future of anatomic pathology. Arch Path Lab Med 2008;132:764-5.
24. Hipp JD, Fernandez A, Compton CC, Balis UJ. Why a pathology image should not be considered as a radiology image. J Pathol Inform 2011;2:26.
25. Patterson ES, Rayo M, Gill C, Gurcan MN. Barriers and facilitators to adoption of soft copy interpretation from the user perspective: Lessons learned from filmless radiology for slideless pathology. J Pathol Inform 2011;2:1.
26. Thorstenson S. Digital pathology system: Case study. Advance Lab 2010;19:69.
27. Weinstein RS, Graham AR, Richter LC, Barker GP, Krupinski EA, Lopez AM, Erps KA, Bhattacharyya AK, Yagi Y, Gilbertson JR. Overview of telepathology, virtual microscopy, and whole slide imaging: prospects for the future. Hum Pathol 2009;40:1057-69.
28. Daniel C, Rojo MG, Klossa J, Mea VD, Booker D, Beckwith BA, et al. Standardizing the use of whole slide images in digital pathology. Comput Med Imaging Graph. 2011 [In press].
29. Jara-Lazaro AR, Thamboo TP, Teh M, Tan PH. Digital pathology: Exploring its applications in diagnostic surgical pathology practice. Pathology 2010;42:512-8.
30. Feldman MD. Beyond morphology: Whole slide imaging, computer-aided detection, and other techniques. Arch Pathol Lab Med 2008;132:758-63.
31. Friedberg RC, Pantanowitz L. Practice evolution: Decentralized computer-assisted immunohistochemical image analysis. Arch Pathol Lab Med 2009;133:597-600.
32. Boutonnat J, Paulin C, Faure C, Colle PE, Ronot X, Seigneurin D. A pilot study in two French medical schools for teaching histology using virtual microscopy. Morphologie 2006;90:21-5.
33. Bruch LA, De Young BR, Kreiter CD, Haugen TH, Leaven TC, Dee FR. Competency assessment of residents in surgical pathology using virtual microscopy. Hum Pathol 2009;40:1122-8.
34. Chen YK, Hsue SS, Lin DC, Wang WC, Chen JY, Lin CC, et al. An application of virtual microscopy in the teaching of an oral and maxillofacial pathology laboratory course. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;105:342-7.
35. Foster K. Medical education in the digital age: Digital whole slide imaging as an e-learning tool. J Pathol Inform 2010;1:14.
36. Harris T, Leaven T, Heidger P, Kreiter C, Duncan J, Dick F. Comparison of a virtual microscope laboratory to a regular microscope laboratory for teaching histology. Anat Rec 2001;265:10-4.
37. Weaker FJ, Herbert DC. Transition of a dental histology course from light to virtual microscopy. J Dent Educ 2009;73:1213-21.
38. Helin H, Lundin M, Lundin J, Martikainen P, Tammela T, Helin H, et al. Web-based virtual microscopy in teaching and standardizing Gleason grading. Hum Pathol 2005;36:381-6.
39. Conran R, Fontelo P, Liu F, Fontelo M, White E. Slide2Go: A virtual slide collection for pathology education. AMIA Annu SympProc 2007:918.
40. Li L, Dangott BJ, Parwani AV. Development and use of a genitourinary pathology digital teaching set for trainee education. J Pathol Inform 2010;1.pii:2.
41. Sharma G, Chenevert J, Dangott BJ, Muse A, Duboy JD, Pantanowitz L, et al. Development of a head and neck pathology slide teaching set for trainee education. J Pathol Inform 2010;1:38.
42. Treanor D, Lim CH, Magee D, Bulpitt A, Quirke P. Tracking with virtual slides: A tool to study diagnostic error in histopathology. Histopathology 2009;55:37-45.

43. Mello-Thoms C, Pantanowitz L, Parwani A, Ho J, Sharma G, Crowley R. Analysis of digital slide exploration characteristics of expert pathologists. J Pathol Inform 2010;1:22.
44. Heffner S. Streamlining tumor board reviews. AdvLab 2008. p. 20.
45. Tecotzky R. Digital pathology enhances hospital's tumor board meetings. MLO 2009. p. 60.
46. Dee FR, Lehman JM, Consoer D, Leaven T, Cohen MB. Implementation of virtual microscope slides in the annual pathobiology of cancer workshop laboratory. Hum Pathol 2003;34:430-6.
47. Rosai J. Digital images of case reports and other articles. Int J Surg Pathol 2007;15:5.
48. Marchevsky AM, Wan Y, Thomas P, Krishnan L, Evans-Simon H, Haber H. Virtual microscopy as a tool for proficiency testing in cytopathology: A model using multiple digital images of Papanicolaou tests. Arch Pathol Lab Med 2003;127:1320-4.
49. Hedvat CV. Digital microscopy: past, present, and future. Arch Pathol Lab Med 2010;134:1666-70.
50. Isaacs M, Yates S, Clermont W, Rossi J, Pfeifer JD. Implementation of whole slide imaging in surgical pathology: A value added approach. J Pathol Inform 2011 [In press].

Special Section on Telepathology

Overview of telepathology, virtual microscopy, and whole slide imaging: prospects for the future☆

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Summary Telepathology, the practice of pathology at a long distance, has advanced continuously since 1986. Today, fourth-generation telepathology systems, so-called virtual slide telepathology systems, are being used for education applications. Both conventional and innovative surgical pathology diagnostic services are being designed and implemented as well. The technology has been commercialized by more than 30 companies in Asia, the United States, and Europe. Early adopters of telepathology have been laboratories with special challenges in providing anatomic pathology services, ranging from the need to provide anatomic pathology services at great distances to the use of the technology to increase efficiency of services between hospitals less than a mile apart. As to what often happens in medicine, early adopters of new technologies are professionals who create model programs that are successful and then stimulate the creation of infrastructure (ie, reimbursement, telecommunications, information technologies, and so on) that forms the platforms for entry of later, mainstream, adopters. The trend at medical schools, in the United States, is to go entirely digital for their pathology courses, discarding their student light microscopes, and building virtual slide laboratories. This may create a generation of pathology trainees who prefer digital pathology imaging over the traditional hands-on light microscopy. The creation of standards for virtual slide telepathology is early in its development but accelerating. The field of telepathology has now reached a tipping point at which major corporations now investing in the technology will insist that standards be created for pathology digital imaging as a value added business

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proposition. A key to success in teleradiology, already a growth industry, has been the implementation of standards for digital radiology imaging. Telepathology is already the enabling technology for new, innovative laboratory services. Examples include STAT QA surgical pathology second opinions at a distance and a telehealth-enabled rapid breast care service. The innovative bundling of telemammography, telepathology, and teleoncology services may represent a new paradigm in breast care that helps address the serious issue of fragmentation of breast cancer care in the United States and elsewhere. Legal and regulatory issues in telepathology are being addressed and are regarded as a potential catalyst for the next wave of telepathology advances, applications, and implementations.

1. Background

Two previous Telepathology Symposiums have been published in *Human Pathology*, in 1997 and 2001 [1,2]. Each of these symposiums included articles that have become "classics" in the field of telepathology and are among the most widely cited articles in the telepathology literature. The first Telepathology Symposium, in 1997, marked the 10th anniversary of the publication of the first articles in English, using the term "telepathology" [3,4]. The second Telepathology Symposium marked our entry into the 21st century, in which information technologies will play an ever increasing role in health care [5-10].

We now mark the 20th anniversary of the field of telepathology. Interest has increased as telepathology, and several of its enabling technologies, such as virtual microscopy and whole slide imaging, are being commercialized by a new wave of companies. Hundreds of virtual slide scanners have been sold by more than 30 commercial vendors.

The telepathology literature has shown steady growth as well. A PubMed search on the term telepathology, in December 2008, listed 628 telepathology articles in this National Library of Medicine database. There were 39 publications in medical journals in the year 2008 alone. These have originated from laboratories in many countries. Several monographs have been published on telepathology [9,10]. These provide detailed coverage of the telepathology field and its intellectual underpinnings, as well as overviews of areas ripe for both telepathology research and clinical implementations. The PubMed database lists only 3 articles on telepathology published before the year 1990, all from a single laboratory Ronald S. Weinstein in Chicago, IL [3,4,11]. A few other early publications from the same laboratory are in the computer science and engineering literature but not in the PubMed database [12-14]. This third *Human Pathology* Telepathology Symposium is expanded in scope and includes interrelated articles on telepathology, virtual microscopy, and whole slide digital imaging.

The original definition of telepathology was "the practice of pathology at a long distance" [3,4]. What was meant by "long distance" was a distance of many miles. Today "long" is taken to mean longer than any distance at which the light microscope system operator can control the microscope "hands-on." For practical purposes, this means any distance bridged by some telecommunications system, ranging from a few meters to a distance half-way around the world. The basic definition is essentially unchanged today. It is irrelevant whether telepathology is practiced using static images, virtual slides, whole slide images, or the images generated from a robotically controlled motorized light microscope [2]. Telepathology is about the practice of pathology at a distance by pathologists [9,15]. It encompasses all of the elements of a pathology histopathology consultation including the generation of a written report, quality control, and quality assurance (QA) of all of the processes of light microscopy, the gathering and interpretation of patient information, and, where needed, consultation with the patient's other physicians [9,10].

"Whole slide imaging," a relatively new term, is a technique with 2 components: the creation of digital images of the entire area of a glass histopathology or cytopathology slide, and the viewing of such a large digital image slide using a virtual slide viewer [10,16]. Whole slide imaging, taken alone, is not the practice of pathology or telepathology, although some authors have used the term as slang for the practice of telepathology. In the United States, this is to be discouraged for a very practical reason. A rapidly growing list of payors in the United States reimburse for telepathology services [15]. They correctly regard telepathology as being under the telemedicine umbrella, requiring the same hospital credentialing, the same medical licensure, and the same level of QA. Those agencies that reimburse for telepathology understand that physicians are providing a service comparable to that provided by more traditional methods [17]. They reimburse for pathology services at a distance, just as they reimburse for radiology services at a distance when they reimburse for teleradiology. At this late date, substituting term "whole slide imaging" for "telepathology" would unnecessarily complicate current efforts to make telepathology services universally reimbursable in the United States.

"Virtual microscopy" is the technology that attempts to emulate traditional light microscopy using digital image files (ie, virtual slides) manipulated on a computer screen using microscope emulator software. Typically, developers of virtual microscopy systems create a "presentation layer" for computers enabling the virtual microscope operator to perform the control functions ordinarily handled with a traditional light microscope, including positioning of the objective lens relative to a histopathology slide and

adjustments for contrast and brightness, using the functionality of a computer mouse. Virtual microscopy computer controls may include iconic representations of light microscope components such as objective lenses. For example, the operator of a virtual slide viewer may see graphic representations of 0.6×, 2×, 4×, 20×, and 40e× objective lenses and select the magnification of choice by clicking on one of these icons with a computer mouse. The system responds by displaying the virtual slide at the selected magnification. The aim is to make the virtual microscopy seem user-friendly for traditionally trained hands-on light microscopists [9,18,19].

Finally, in current usage, the term "virtual microscopy" is not a synonym for telepathology any more than light microscopy would be analogous to the practice of pathology. Therefore, in this Telepathology Symposium, we will use the terms "virtual microscopy" and "whole slide imaging" to describe specific digital imaging modalities.

Thus, the terms "static image," "whole slide image," "dynamic," or "virtual slide" are used as modifiers for the word telepathology to identify digital image acquisition modalities used for telepathology. For example, the term "virtual slide telepathology" is extensively used in this overview.

This Telepathology Symposium includes 8 original articles, in addition to this overview.

1.1. Evolution of standards-based telepathology workstations

The diagnostic workstation serves as the organizing principle for the integration of pathology services and health information technologies. The widespread implementation of telepathology will benefit from the development of telepathology standards and the establishment of a standard-based industry for manufacturing interoperable telepathology workstations [9,10,20-22]. The tasks involved in creating the framework for such telepathology standards are somewhat daunting. A strong case can be made for encouragement of the development and implementation of telepathology standards sooner than later. Otherwise, telepathology could remain in the shadow of teleradiology for years to come.

The process of digital imaging in pathology involves a series of operations, each contributing to the quality of the final image that is displayed on the computer screen. The operations include preimaging steps including sample preparation and staining by a histology laboratory; optical image formation by a virtual slide scanner, digital image sampling by the sensor (eg, camera) of the imager, postprocessing of the digital information, image compression, transmission of the digital image file across a telecommunication network, and display of the digital image file on the pathologist's video display [9,10,21]. Each operation would benefit from some level of industry-wide standardization.

There is extensive literature on digital imaging [9,10,16,19,21-23]. Each step of that process is fairly well understood, as recently discussed elsewhere [9,10]. Experts understand that this multistep process is hard to standardize or perhaps even to understand fully at this time. The overarching principles for developers of pathology imaging standards are the following: (1) systems should be able to share digital image files; (2) the standards should allow the transmission of information on baseline colors and recommended display parameters; (3) the digital images should be useful to the pathologist, not necessarily better or worse than direct examination of a slide under the light microscope; (4) a mechanism to evaluate image quality objectively should be available; (5) mechanisms to adjust and correct for major and minor problems with tissue processing should be developed and be practical in their implementation; and (6) protocols should be in place to make sure that data are not corrupted. It is essential to have end-to-end fidelity of imaging data. Public *standards* organizations should support pathologists in the development of such standards. Standards should be easy to understand, adopt, follow, and advance, like a roadmap including terminologies, color, resolution, quality, and data formats [16].

Many new and useful functions and technologies have been developed recently for pathology slide digital imaging. There is confusion among words. Definitions are not clear to everyone, and a word can be used in different ways by different individuals. For example:

> "Z-stack" versus "multi layer" versus "extended focus."
> "Resolution" versus "sampling period" versus "magnification."

These nomenclature issues will be resolved with time and expanding usage.

The color management issues in pathology are challenging. Radiology does not have to deal with most of these color management issues, which has been a big advantage for teleradiology system developers. In addition to the general challenges of display, image acquisition and software issues shared by radiologists, for pathologists, the staining of the locally produced histopathology slide is yet another factor to be dealt with under the rubric of color management. Using spectral analysis and proper calibration, color reproduction and stain standardization by digital imaging are possible [10]. The methodology for this aspect of color management has been developed by several groups, but such methodology may be challenging to support at the institutional level.

Macbeth Color Checkers are routinely used in telemedicine practices to adjust the color of video monitors (Fig. 1, left). At the Massachusetts General Hospital, a protocol has been established to manage the color standardization of virtual microscopy systems in a way that is easy and practical. A Macbeth Color Checker slide was made in-house, based on a previous study of how this could be delivered to a user and an imaging device. Because a
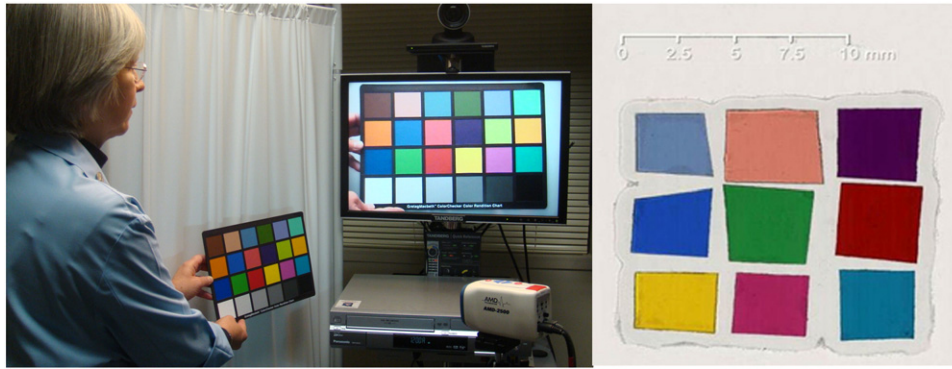
Fig. 1 Left, Telemedicine case manager at the Arizona Telemedicine Program, Tucson, AZ, using a handheld Macbeth Color Checker to compare the color output of an AMD-2500s General Examination camera (lower right) with the video image on a Tandberg video monitor. Color adjustments can be made using controls on the camera, the video monitor, or in the room lighting. Color fidelity is very important for teledermatology among other imaging medical specialties. The Macbeth Color Checker is an array of 24 printed color squares, which include spectral simulations of light and dark skin, foliage, and so on (50). It is used for precise color balance for digital photography. It was scientifically designed to help determine the true color balance of any color rendition system. Right, Macbeth Color Checker on a slide. Yagi adaptation of the Macbeth Color Checker for virtual slide telepathology system calibration. The transparent miniaturized Macbeth Color Checker is mounted on a glass slide and is scientifically designed to allow for adjusting color balance in video microscopy systems. The miniaturized Macbeth Color Checker slide can be used to calibrate either virtual slide telepathology scanners or viewers (Y. Yagi, 2009, unpublished photograph).

standardized color chart, such as a Macbeth Color Checker, would be relatively expensive ($60/sheet) to give to all staff, a handmade color chart slide turned out to be a reasonable approach. A color chart slide was made and tested in-house (Fig. 1, right). Operationally, the color chart slide is scanned and compared with the color on the video display and the original slide. The user also checks the color chart on the Web. If a user feels the displayed video image is suboptimal, a telephone call would be made to a help desk for assistance in correction of the color problem. Sources of color problems can be the standard slide, the scanner, or the virtual slide viewer among others (Yagi, unpublished data, 2009).

Virtual slide scanners can also introduce other problems. A number of scanners are available from various vendors, offering ever faster scanning speeds and higher image quality [24,25]. However, there are still some issues that may need to be solved before implementation occurs in clinical environments on a widespread basis. Stability, focus, and consistency of image quality can be problematic with the early virtual slide scanners. Slide quality, focusing, and compression at the virtual slide scanner have an influence on image quality. At the user end, it is not easy to improve the focus algorithm and the compression algorithm, other than the compression ratio [10].

Experience has shown that it is generally possible to improve the slide quality in the local histopathology laboratory of telepathology service users. Some histopathology laboratories routinely produce glass histopathology slides of very high quality. In other laboratories, histopathology slide preparation problems can include paraffin section wrinkles, variations in thickness across the entire tissue section, and bubbles in the mounting media among others

[9]. Thin, flat histopathology sections yield better digital image quality [16]. Working with the histology and immunohistochemistry laboratories and establishing QA/QC (quality control) for virtual slide imagining are important for telepathology clinical services [9].

Critical components in all virtual slide scanners are light microscope optics and illumination systems [24,25]. Most vendors use single optical axis instruments. Array microscopes offer a much larger field of view. This can be leveraged into faster virtual slide scanning times [24,25].

Optical resolution is a function of the wavelength of light used and the numerical aperture of the lens system (Resolution = (f) wavelength / 2 NA). When illuminating light in a whole slide scanner is not conditioned correctly with filters, there is a tendency for the wavelength to shift to longer values (more red) because of the characteristics of the lamps in common use. Most microscopes correct for this with a neutral density filter for brightness and a blue filter (depending on the light source) for color correction. Some scanners have multiple objective lenses, and other scanners have a zoom lens. The recent incorporation of LEDs for illumination in the DMetrix Ultrarapid Virtual Slide Scanners (DMetrix, Inc, Tucson, AZ) provides an attractive alternative approach.

There is a need to reconsider definitions of "resolution" in the context of digital imaging pathology. However, as a practical rule of thumb, 20× means 0.46 to 0.50 μm/pixel, and 40× means 0.23 to 0.25 μm/pixel [10,24].

With regard to digital image standards, the current primary standards organization for clinical image sharing is Digital Imaging and Communications in Medicine (DICOM). A creation of the American College of Radiology and National Electrical Manufacturers Association, its main

purpose is the sharing of clinical images (and related information) in a clinical context [9,10].

In 2005, DICOM established a special working group (Working Group 26) to develop extensions to the standard for telepathology imaging and pathology in general. So far, Working Group 26 has developed 1 balloted (accepted) DICOM supplement and is working on a second. The first, Supplement 122 ("Specimen Module and Revised Pathology SOP Classes"), describes sharing of clinical and process information about the "specimen that is the subject of an image" such as processing, staining, and so on. Supplement 122 describes a way of sharing this information so that the digital slide image can be interpreted between systems.

The second supplement, Supplement 145 ("Whole Slide Microscopic Image Information Object Definition and Service/Object Pair Classes"), defines sharing digital images between systems. The supplement has not yet been balloted and therefore is not yet an accepted part of DICOM. The details are well beyond the scope of this overview, but the basic approach is as follows: each whole slide image is divided into small "tiles," and an index is created that relates each tile to the other tiles. The result is a long, indexed stack "series" of image tiles (very much the way DICOM handles computed tomography scans). Information is also available on the "acquisition context," including the illumination source, filters, magnification, and lenses, as well as color encoding, file formats, compression, and so on. Although Supplement 145 will have a mechanism to share this information, it will be up to the acquisition devices to provide this information and the display devices to use it.

Presently, there are 2 significant limitations on single-image objects within DICOM, which may need to be overcome for virtual slide telepathology. First, DICOM image objects pixel dimensions are stored as unsigned 16-bit integers, for a maximum value of 64K, inadequate for virtual slide telepathology. Second, DICOM image objects data size are stored as unsigned 32-bit integers, for a maximum value of 2 GB. This may need to be adjusted upward for some virtual slide telepathology applications.

Information about the DICOM standard, including the text of Supplement 122, the minutes of Working Group 26, and how to join the working group, are available at http://medical.nema.org/.

With this as background information regarding the current status of the development of digital imaging standards and, in turn, telepathology virtual slide imaging standards, we have included in this Telepathology Symposium a consideration of topics related to medical imaging workstation design and function. Radiology leads other medical imaging specialties in the area of workstation design and testing. Therefore, current information on teleradiology workstations will serve as the point of departure for this discussion.

Telepathology lags well behind teleradiology in terms of defining pathology workstation specifications and developing workstation standards. Whereas standards have been critical to the current diffusion of teleradiology into the main stream of radiology practice, the development of such standards is in their infancy for pathology. Color, resolution, compression, and video monitor specifications are infrequently mentioned in the telepathology literature. Standards that would foster interoperability of telepathology systems by different vendors are under development. Telepathology systems from different vendors are not yet interoperable.

To jumpstart our consideration of the important topic of telepathology workstation design, a researcher with keen interest in both teleradiology workstations and telepathology workstations was asked to summarize the current state-of-the art in workstation design and to provide context for pathologists interested in exploring the literature on medical imaging workstations [26]. Elizabeth A. Krupinski, PhD, is a cognitive psychologist who is a Research Professor at the University of Arizona and who works in a large radiology imaging group with a distinguished background. Historically, digital radiology was invented in the Radiology Imaging Research Group's Laboratory of the Department of Radiology at the University of Arizona College of Medicine, in Tucson, AZ, in 1973 [27], decades before Dr Krupinski joined the group. Doctor Krupinski's research in this productive, radiology imaging research laboratory is related to physician imaging workstation design. A number of the radiology workstation design issues overlap with those of telepathology workstations. Doctor Krupinski's research on digital pathology imaging has involved work on the pathologist-computer interface [20,28,29]. It is also noteworthy that Dr Krupinski was one of the first investigators to obtain extramural federal funding for telepathology research from the National Institutes of Health's relatively new National Institute for Biomedical Imaging and Bioengineering (NIBIB). The NIBIB could become an important source of funding for digital pathology research in the future. Doctor Krupinski also has broader interests in the field of telemedicine and is Past President of the American Telemedicine Association. Her article in this Telepathology Symposium provides a roadmap to the future of telepathology workstation design research based on lessons learned from teleradiology [26]. A related, important topic that deserves consideration is the Picture Archiving and Communications System (PACS). For lack of space, we will limit our consideration to a few sentences for now. Implementing a pathology PACS strategy at the local, regional, or even national level creates a major undertaking. During implementation, PACS system could be challenging to individual pathologists because they often require changes in workflow, in work habits, and the acquisition of new skill sets. Another PACS implementation barrier is the high cost of such systems. Most of the savings are "soft" and not directly traceable back to the pathology department. On the other, hand, the implementation of PACS may be encouraged by national reimbursement policies or other federal initiatives in the foreseeable future.

1.2. Virtual microscopy in education

Education might be considered the first so-called killer application for virtual microscopy and virtual slide tele-pathology (Fig. 2).

Doctor Fred Dee, at the University of Iowa, has 2 contributed articles in this Telepathology Symposium on the topics of education and testing [18,30]. From our perspective, Dr Dee has been our National Library of Medicine's "go-to person" for conceptualizing, developing, evaluating, manufacturing, and hosting public teaching sets of virtual pathology slides for years. His group produces and hosts user-friendly, high-quality virtual slide teaching sets that are available over the Internet. In doing so, Dr Dee has achieved a larger purpose and helped define the use of the Internet as an educational tool in pathology. Doctor Dee has the well-deserved reputation of being a master educator and continues to explore various uses of virtual microscopy in a number of different training settings. He keeps his keen eyes on the future of virtual microscopy and, often, makes the future happen.

Doctor Dee's first article provides an up-to-date survey of the current uses of virtual microscopy for pathology education and testing [18]. His thoughts on the growing use of virtual microscopy in US medical schools are authoritative. This will be of particular value to medical school faculty members at the "have not" medical schools who may benefit from being armed with such information to convince their own reluctant curriculum committee to make an investment in virtual microscopy.

Could conventional light microscopes eventually become extinct in medical school student pathology laboratories? They have already disappeared from the University of Arizona. As a result of this trend to dismantle traditional light microscopy laboratories, we were told that this year's crop of new pathology residents in the United States included recruits who used light microscopes, hands-on, for the first time, the first day of their pathology residencies. That must have surprised the old timers! Seemingly, we are past the tipping point in the transition from light microscopy to virtual microscopy in medical student education.

As a potential downside to the implementation of virtual microscopy, could the ease of access to high-quality histopathology virtual slides turn out to be a Faustian proposition for medical school pathology departments? Although virtual microscopy can provide easy access to high-quality histopathology materials, does the removal of traditional light microscopy laboratory physical facilities from medical schools destabilize what was previously the academic pathology department's medical student education
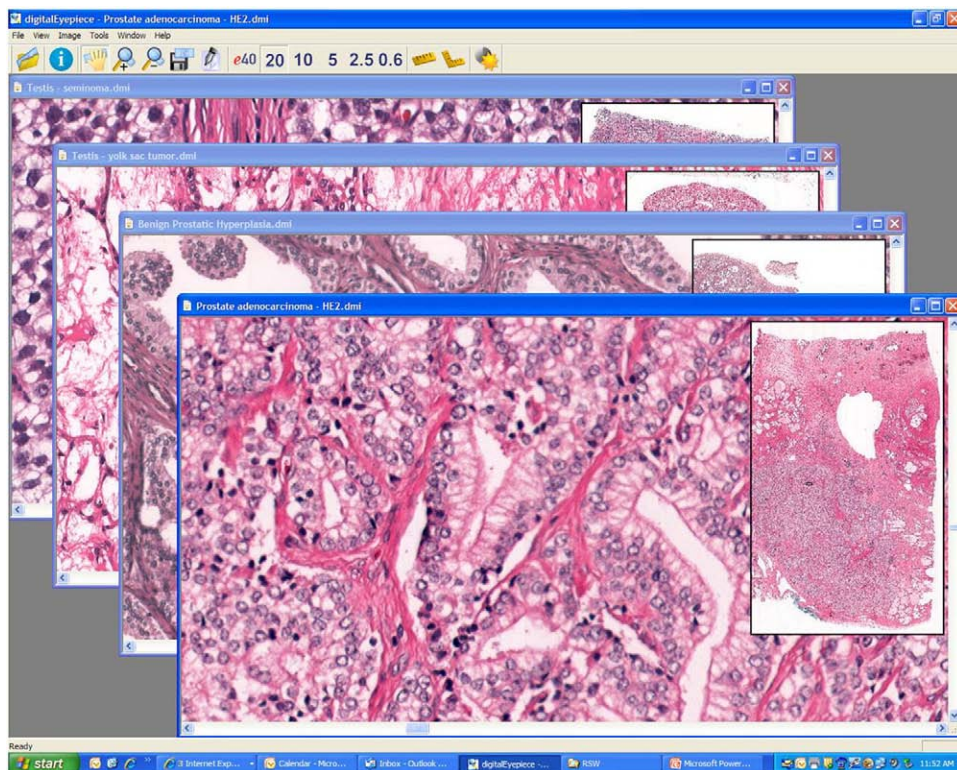
Fig. 2 DMetrix Digital Eyepiece virtual slide viewer showing a cascade of virtual slides used in the Genitourinary Block in the preclinical curriculum at the University of Arizona College of Medicine, in Tucson, AZ. A thumbnail image overlays each higher magnification virtual slide histopathology field. Images, back to front, are of seminoma, yolk sac tumor, prostatic hyperplasia, and prostate adenocarcinoma. Clicking on any of the 4 large images, with a mouse, brings that virtual slide to the foreground for navigation and examination.

franchise? Does the introduction of transparency in histopathology teaching have ramifications for nursing and pharmacy students wishing to study histopathology for the first time? Would interprofessional training, as championed by the United States Academy of Science's Institute of Medicine, actually benefit from having virtual histopathology go "open source." One way or the other, for academic pathology departments, virtual microscopy could be a transformational technology [7,8].

The article by Bruch et al [30], describes a promising new application of virtual microscopy in pathology resident training. The study may seem modest at first reading but, upon closer examination, it is elegant and even groundbreaking.

Doctor Dee and his colleagues have developed a 20-question surgical pathology competency test using virtual slides as test materials. Their results are thought provoking. As an example, from the learning curve for residents shown in the article, we might infer that the first few months of surgical pathology training, when the learning curve is the steepest, may be critically important in more than one way. Pathology residency program directors should take note and might want to insure that the very best surgical pathologist role models in their departments (with respect to expertise, quality of reports, professionalism, and so on) are on service when lifelong professional surgical pathology skills, habits, and attitudes become ingrained, during the "minting" of the future practitioners of pathology in their first few months on the surgical pathology service.

The resident competency assessment tool created and assessed by Fred Dee's group also provides a persuasive rationale for developing a full library of Web-based competency assessment tools. Dee and his colleagues show that pathology subject competency can be meaningfully assessed in a stepwise fashion. This is a practical and now validated use for virtual microscopy in resident education. Hopefully, their work is the first step toward the creation of a comprehensive set of Web-based pathology competency tests that will become readily available. It may also be a step in the direction of creating competency-based resident training programs in which scheduling through rotations and calendering are finally uncoupled. Whereas rigid, calendar-based scheduling is obviously very practical, competency-based scheduling is desirable and could be computer enabled [31]. Rate of the progression through such a curriculum could be influenced by the use of supplemental Web-based courseware by motivated residents to accelerate or decelerate through a competency-based curriculum [31].

Bibliographies of the 2 articles contributed by Fred Dee and his colleagues in Iowa can serve as useful guides to additional articles validating uses of virtual microscopy in education. This is an added benefit of publishing these articles back-to-back in this Telepathology Symposium.

1.3. Institutionalization of sustainable robotic and virtual slide telepathology services

Two invited articles, one by Dr Bruce E. Dunn and his collaborators at the Department of Veterans Affairs Medical Center in Milwaukee, WI, and the other by Dr Andrew Evans and his surgical pathology colleagues in Toronto, Canada, provide interesting contrasts between 2 successful, active, telepathology services [32,33]. Dr Dunn and Dr Evans are both outstanding pathologists who have been directing sustainable telepathology services for years. Both are excellent observers who can sense what takes place in their own parallel universes, at their service user-sites some distance away. They both demonstrate, once again, the value of careful observation of what goes on in a surgical pathology service. Pathology residents, with a service bent, often ask what projects are available for them for research. Although not necessarily the intent of their respective articles, Dr Dunn and Dr Evans show that the service laboratory environments, in which pathologists work every day, can be worth studying in-depth. Residents might be reminded that an important research tool can be the careful quantitating of the mundane metrics in a functioning hospital laboratory.

Dunn and Evans have different pathology service models, each with its own challenges, which were successfully addressed by the implementation of telepathology. Doctor Dunn's telepathology service bridges hundreds of miles [32]. Doctor Evan's telepathology site is within walking distance of his office [33].

Doctor Dunn is headquartered in VISN 12 (Veterans Integrated Service Network 12 in the upper midwestern United States) of the US Department of Veterans Affairs and the University of Wisconsin in Milwaukee. He was an early adopter of robotic telepathology, in 1996. Now regarded as a pioneer in telepathology, he turned a difficult pathology service coverage issue at the small Iron Mountain Veterans Affairs Hospital 240 miles north of Milwaukee, on the upper peninsula of Michigan, into a "win-win" opportunity for VISN 12 [34-39].

Dunn's serious challenge was to head off the closure of the Iron Mountain Medical Center for lack of laboratory coverage and other support services. The stakes were high. Closure of any rural hospital has harsh implications for its geographically isolated population. Bruce E. Dunn, MD—bright, innovative, energetic, and seemingly ever optimistic—is also a natural problem solver. He tackles big problems with exuberance, goodwill, and professionalism and has the personal staying power necessary to succeed with a complex, multi-institution challenge. Doctor Dunn was tasked with coming up with a solution that would improve pathology coverage at the Iron Mountain hospital and thus keep the hospital's doors open.

In 1996, robotic telepathology was still in an early stage in its development as the potential driver of a new clinical service. Because this is a new clinical activity for the Department of Veterans' Affairs, successful institutionaliza-

tion of the technology would require monitoring services on an ongoing basis with a carefully designed QA program. Then, there is the personal commitment part of the story. To make this work, Dr Dunn was willing to drive from Milwaukee to Iron Mountain and back, monthly, for medical staff activities indefinitely into the foreseeable future. This is a 480-mile roundtrip drive, through snow storms in the winter months in northern Michigan.

Iron Mountain, MI, is the ski jumping capital of the United States. Its airport is closed for months in the winter due to deep snow and blizzard conditions. The initiation of this project was a heroic undertaking, driven by Dr Dunn's desire to keep this small rural hospital open. This required a clear vision for the future on Dr Dunn's part, an appreciation of quality issues in the practice of surgical pathology, a remarkable work ethic on his part, and that of his coworkers, and the willingness to be the program's champion. The effort requirements would be high and the commitment would be long term, but the rewards would come from saving a rural veterans' hospital from closure. Anybody who deals with the trials and tribulations for rural hospitals in America knows how critically important it is to keep these institutions viable.

Doctor Dunn's robotic telepathology program has been in service for 12 years. It has expanded into a regional program [32]. For years, on a semiregular schedule, Dr Dunn drove from Milwaukee to Iron Mountain and back, month in and month out, for troubleshooting, for making certain that things were running well, and for assuring that the medical staff members at Iron Mountain were satisfied with the timelines and quality of the pathology services. Equally important, he put this human face on a distant laboratory program by becoming an insider at medical staff meetings in the rural Iron Mountain facility. The doctors and laboratory workers at Iron Mountain enjoyed chatting about their weekend hunting and fishing triumphs with the Milwaukee telepathologists using the videoconferencing features built into their Apollo Telepathology System. The physician's assistant at Iron Mountain, who performed the video-supervised grossing of surgical pathology specimens from the start, eventually became certified as a medical technologist by distance education, using the telepathology system's built-in video-conferencing feature.

What else makes Dr Dunn's clinical research on telepathology noteworthy? A pathologist and microbiologist by training, who is well known in the clinical microbiology community for his research on *Helicobacter pylori*, Dr Dunn is a scholar with a sharp eye and an unusual talent for quantitating things. Over the past dozen years, Dr Dunn and his colleagues have amassed an unusually large amount of information on the practice of robotic telepathology, ranging from data on the technical performance of their robotic telepathology system to data on human factors involved in being a telepathologist [32,35-37]. His Milwaukee group's diagnostic accuracy using robotic telepathology for primary diagnoses provides a baseline for future diagnostic accuracy studies [32,37]. He has carefully documented telepatholo-

gists' profiles, including practice patterns of novices and seasoned pathologists, viewing times for robotic telepathology slides, diagnostic accuracy, learning curves for users of the robotic telepathology technology, and comparative studies on individual pathologist error rates. Robotic telepathology was the first use of a robotic interface for a telemedicine application.

The article by Dunn et al, in this Telepathology Symposium, is his third article in this Telepathology Symposium series [32,36]. It summarizes the experiences of the Milwaukee VA group using robotic telepathology for the primary diagnosis of over 10,000 surgical pathology cases. It is noteworthy that Dunn's group still does same-week glass slide overreads for all cases. Their perceived need for a glass slide overread is based, at least partly, on quality concerns. We wonder if there is a "creature of habit" element here, or perhaps it is inconvenient to amend or rewrite a Standing Operating Procedure in the US Department of Veterans Affairs.

It is noteworthy that, unlike iteration, Dunn's work, some published studies on diagnostic accuracy of telepathology have used slide readers who were unqualified to participate in the clinical research. They had very little prior experience with digital imaging at the time of the study. The use of training sets for digital pathology diagnostic accuracy studies has been very uneven. Some studies have even used pathology residents as case readers. This is often not high quality research. It is a telltale sign of unacceptable study design, unless, of course, the aim is to specifically study residents' performance. Diagnostic accuracy studies aimed at evaluating new medical imaging technologies should refrain from using residents or fellows as the slide readers. Results of resident or fellow-based studies could distort meta-analysis of telepathology diagnostic accuracy studies down the line. At the very least, the slide readers for diagnostic accuracy studies should be Board-certified pathologists with suitable prior experience in digital imaging pathology.

The Dunn group is careful with regard to paying attention to the issues of slide reader competency. The studies by the Dunn group generally meet this recommended Board-certified pathologist requirement. Their diagnostic accuracy findings are therefore credible, in part, because the pathologists who participated in their QA programs are qualified.

Creating an early sustainable robotic telepathology service in the United States represents a commendable achievement by Dr Dunn. The Dunn Veterans Affair's group was honored for their work on the Milwaukee–Iron Mountain program by the Vice President of the United States, Al Gore, who came to Milwaukee on March 26, 1996, and presented Dr Dunn with the Vice President's "Hammer Award" for excellence in innovation. That day, Dr Dunn and his team at the Department of Pathology at the Veterans' Affairs Medical Center in Milwaukee were in the national spotlight. Video clips of the award ceremony show a very proud Vice President Gore, a strong advocate of information technologies, presenting Dr Bruce E. Dunn with a "Hammer Award" commemorative

plaque. That day, the large Milwaukee audience enthusiastically applauded the work of 2 "information superhighway visionaries," Bruce E. Dunn, MD, and Al Gore, the Vice President of the United States.

In marked contrast to the Milwaukee–Iron Mountain Program, the next article in the Telepathology Symposium describes a telepathology service bridging city blocks, not hundreds of miles [33]. Doctor Evans and 4 uropathologist colleagues at the University of Toronto (Toronto, Canada) had responsibility for covering a neurosurgical frozen section service at a freestanding neurological institute, less than a mile away from their university hospital's main laboratory in downtown Toronto. In their situation, the geographic challenge shrinks from Dr Dunn's long haul coverage of a very remote hospital pathology laboratory hundreds of miles away to servicing a demanding, subspecialty neuropathology frozen section service, a 15- to 20-minute walk away. One of the Toronto on-service pathologists' recurring questions was, "Do I walk or do I drive the short distance? Is it worth the time to get in a car to drive to a hospital down the street to respond to a request for an intraoperative frozen section examination?" Telepathology again provided a solution. Doctor Evan readily acknowledges that an added incentive came from the relative limited expertise in neuropathology of a group of uropathologists, otherwise diagnosing kidney, urinary bladder, prostate, and testis cases most of the time but being asked to cover a demanding neuropathology frozen section service at another hospital. The ready availability of immediate second opinions within their group and group decision making for a teleneuropathology frozen section service was a significant benefit for this group [33].

The Telepathology Symposium article by Andrew Evans, MD, PhD, and his colleagues in Toronto includes a meticulous description of the transformation of a robotic telepathology group practice into a virtual slide telepathology group practice. Doctor Evans initially adopted robotic dynamic telepathology, largely because it was the gold standard for telepathology at the time [38-40]. The practice migrated to virtual slide telepathology after carefully comparing their options, robotic telepathology versus virtual slide telepathology, and then setting the stage for shifting from one telepathology technology to a next-generation system.

The study by Evans et al [33] describes in detail their experience making this transition. They make interesting observations that are relevant to broader issues related to technology adaptation and diffusion, and they also discuss a number of related topics including in-service training for a new technology and strategies for gaining user acceptance of virtual slide telepathology by surgeons.

1.4. University teaching hospital QA program

The next 2 articles on innovative telepathology patient service applications are contributed by our group at the University of Arizona in Tucson, AZ. Historically, the Department of Pathology at the University of Arizona has been engaged in anatomic pathology innovations and in using telepathology for second opinions and QA since 1993 [41-45]. The telepathology services stem, in part, from the then Department Head's (RSW) involvement directing a large national QA program when he served as Director of the Central Pathology Laboratory of National Bladder Cancer Group from 1982 to 1988 [9]. The Central Pathology Laboratory at Rush Medical College in Chicago, IL, was charged with responsibility for rereviews of urinary bladder cancer specimens from patients entered on National Cancer Institute–funded multi-institution bladder cancer clinical protocols. He had become deeply concerned over the significant negative impact of interobserver variability among pathologists involved with clinical trials in the United States. In 1985, he began to explore the possibility of using "telepathology," a term he coined, as a solution [3,4]. He had done his pathology training at the Massachusetts General Hospital, 1965-1970, when a pioneering multispeciality telemedicine service was initiated and in the national spotlight. To him, telepathology was an option worth exploring.

Doctor Weinstein was Chairman of Pathology at Rush Medical College in Chicago, from 1975 to 1990, where he invented robotic telepathology. He relocated to Arizona in 1990, as Chair, and his new department began deploying telepathology in 1993, with the creation of an international static image telepathology second-opinion service [41,42]. More than 250 consultations were provided to hospitals in rural Arizona, Mexico, and China (Table 1). This involvement with the Arizona International Telemedicine Program's telepathology program was a learning experience for the Department of Pathology [41-43]. They defined both the value and the limitations of static image telepathology and established diagnostic accuracy standards that have stood the test of time [43,44]. There was 88% overall concordance between static image telepathology written reports and subsequent glass slide rereviews, and 96% concordance between static image telepathology diagnoses and diagnoses

Table 1 Arizona telepathology services [a]

Modality	Class [b]	QA	Second opinions	Frozen sections	Deferred cases [c]
Static image telepathology	2A	–	239	–	17
Robotic dynamic telepathology	3B	3064 [d]	81	142	228
Virtual slide telepathology	5C	329	–	–	–
Total		3393	320	142	245

[a] University of Arizona telepathology services, initial 4000 cases, 1993-2008.
[b] Weinstein Classification of Telepathology System [2].
[c] For special studies, such as immunohistochemistry, or for glass slide review.
[d] Includes some second-opinion cases.

on the same glass slides on rereview by conventional light microscopy for clinically significant diagnoses [43]. Overall diagnostic accuracy of audited, original surgical pathology reports from the rural hospitals, as compared with the QA diagnoses based on our glass slide rereviews, was approximately 83%. The Arizona group concluded that a static image telepathology second-opinions service represented an improvement in diagnostic accuracy when compared with the client institution's own laboratory's written surgical pathology reports. The largest discrepancy rates were for the static image telepathology cases received by the Arizona International Telemedicine Program on surgical pathology cases from Mexico and China.

Two limitations of static image telepathology became apparent. First, errors in static image diagnoses were most often due to the original microscopic field selections of the local pathologists. On rereview of the glass slides forwarded to the laboratory in Tucson, other histopathology fields showed more important diagnostic information than fields selected for static imaging fields. A second challenge arose from the mismatch between the sophistication of our University pathologists' diagnoses and the locally available therapeutic options in Mexico or China. Frequently, these international referring institutions lacked access to the therapies that would be considered to be standard treatments in the United States. Such mismatches in diagnoses and available therapies lead to considerable frustration, especially for the Chinese patients whose physicians in China requested the telepathology second-opinion consultations but were then disappointed that the telepathology service could not ship them the recommended drugs. This type of mismatch may partially account for the perceived under-utilization of some international static image telepathology services [9,10]. We learned that there is little, if anything, to be gained from offering false hope or—of even greater concern—creating a crisis in trust of the patient's physician when recommended therapies cannot be delivered. International telepathologists, working with patients in developing countries, are reminded that there are also many disparities in the availability, level, and quality of health care services within the United States as well.

The University of Arizona's Department of Pathology initiated robotic telepathology second-opinion services at several rural hospitals in Arizona in 2001. Many cases originating from these rural sites were successfully diagnosed using our robotic telepathology systems (Apollo Telemedicine, Fairfax, VA). An additional type of robotic telepathology service was initiated in Arizona in the year 2002. Robotic telepathology-based second-opinion surgical pathology QA services were packaged for rural hospital laboratories in weekly, regularly scheduled, 1 hour blocks. A group of 4 telepathologists at the University of Arizona, in Tucson, cover the QA service on a rotating basis. Complex or unusual cases can be rereviewed by additional University-based subspecialty pathologists. By the end of 2008, 3064 robotic telepathology QA reports had been issued. Glass slide

overreads on quality control cases for the services showed that the diagnostic accuracy for the service was between 98% and 99%. These surgical telepathology QA services are ongoing, providing access to university-quality subspecialty pathology services at 2 Arizona rural communities (Table 1). User satisfaction with the service has been high. One rural solo-practice pathologist said, "I no longer feel that my heart is being squeezed at the end of each workday."

From the perspective of the University of Arizona telepathology service providers, they feel that providing Arizona's rural practitioners with easy access to a large subspecialty pathology group practice is desirable service and professionally rewarding. From the perspective of rural physicians, turnaround times for obtaining second opinions and expert consultations are reduced. However, over time, some rural hospital administrators may begin to take such services for granted and regard them as an entitlement, which is a downside risk because expectations may not be met if the bar continually rises.

Against this backdrop of extensive experience with rural telepathology for years, the University of Arizona pathology faculty established 2 additional new, overlapping, urban virtual slide telepathology services in 2004 [42,45-48].

The University of Arizona pathology faculty, in Tucson, covers the surgical pathology services at 2 financially competing University Hospitals: one, a flagship university hospital processing 20,000 surgical pathology specimens, and the second, a smaller, former county hospital accruing about 2000 surgical pathology specimens per year. The Department of Pathology at the University Medical Center has a tradition of holding a daily 2:00 PM QA conference at which surgical pathology cases representing all new cancer cases, difficult cases, and rare cases are examined by 1 to 4 staff pathologists, fellows, residents, and medical students seated at a 14-headed light microscope [44,48]. Now, the glass histopathology slides from surgical pathology cases requiring QA at the smaller hospital are immediately scanned into telepathology virtual slides and are inserted into the workflow of the 2:00 PM QA conference at the larger hospital for virtual slide telepathology analysis and STAT reporting of results to the smaller facility (Fig. 3). As reported in this Telepathology Symposium, an analysis of 329 consecutive QA cases verified that the QA reviews by virtual slide telepathology are highly accurate and result in the immediate revision of surgical pathology reports containing discrepancies [46].

The second article describes the laboratory component of an innovative rapid breast care service [47,49]. It includes a subset of QA virtual slide telepathology breast cases described elsewhere in this Telepathology Symposium but included in this article in a different context, namely, the providing of immediate second opinions on surgical pathology specimens from a rapid breast care service [47,49,50]. This innovative service bundles telemammography, telepathology, and teleoncology into a same-day "virtual" point-of-care service. It has been implemented to
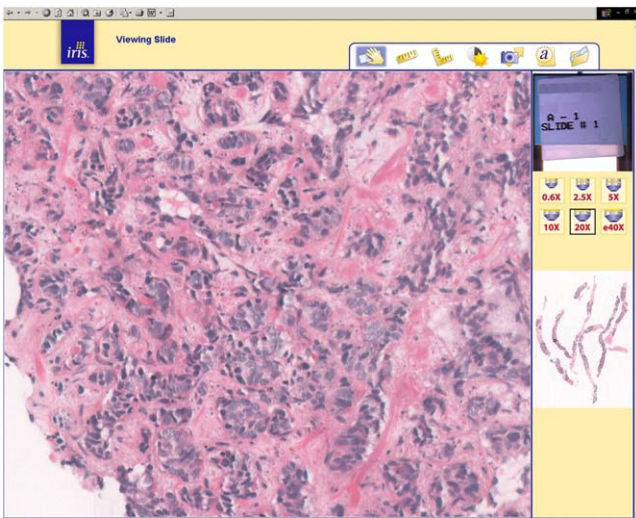
Fig. 3 DMetrix Iris virtual slide viewer as used for surgical pathology QA services at the University Medical Center in Tucson, AZ. With this viewer, magnification selection is based on clicking, with a computer mouse, on iconic representations of microscope lenses in the right panel. The magnification of the histopathology virtual slide is displayed by a box around the specific objective lens, showing the ×20 objective lens in this example. The thumbnail image of a histopathology of breast core biopsies is also visualized in the right panel. The large window displays invasive breast carcinoma at ×20 magnification. This breast core biopsy was rapidly processed. The diagnosis was communicated to the patient, by her oncologist, the same day as the breast core biopsy procedure.

reduce the fragmentation of breast care services and to alleviate patient stress (Fig. 3 and Fig. 4).

1.5. Telepathology reality check

The final article in this Telepathology Symposium is entitled "Medicolegal aspects of telepathology"[17].

We made it the last article in the Telepathology Symposium over concerns that the mere glance at the title by a practicing pathologist might induce some kind of an anaphylactic reaction. Among the dreaded calls a pathologist gets is one from the secretary buzzing him or her in an office to say, "There's a lawyer on the line."

Doctor Keith Kaplan, a pathologist at the Mayo Clinic, deserves credit for capturing the attention of Stanley T. Leung, MD, JD, who is that rare bird, the "doctor-lawyer," who has his feet firmly planted in both professions and was willing to research a legal issue out of professional curiosity. Their article clearly reflects Keith Kaplan's input because the topics emphasized in the article are topics that almost anyone in the laboratory outreach business would recognize as reasonably familiar territory. A number of these topics are relevant to traditional decentralized laboratory practices as well.

For many academic pathologists, the business underpinnings of their daily pathology service activities can be a

mystery and may even appear to be hostile territory. It may be relatively easy for a department chair or a laboratory director to want to say "get over it" to their academic pathology faculty, but usually they will not get over it. In fact, some academic pathologists are prone to obsess over the risks of new technologies. For some, this may be because they are protected from any need to use business or legal lingo and thinking, in their own academic world, where all that is required is mastery of billing codes for surgical pathology. For those pathologists who are already desensitized to legal affairs and are experienced in the business of pathology and medicine, the article by Leung and Kaplan may actually mitigate their instinctive technophobia that might otherwise accompany their entrance into the digital pathology and telepathology worlds. The more likely reaction pathology business people will have to this article will be, "I'm glad that someone finally sorted all this out." As a practical matter, handing a copy of the article by Leung and Kaplan to one's laboratory practices' own lawyers could save individual pathology practices a bundle of money.

Excellent legal research is now available for everyone. With respect to these authors, some other innovators spend their professional lives seemingly floating on the meniscus between their own dreamworld visions of the future and everyday reality. Doctor Kaplan is one of those early adopters of technology who appreciate quality scholarship while extending his own deep intellectual roots in the bedrock of the real world. He likes technology but wants road maps to follow, mile by mile, as he enters other people's "virtual" worlds. This is a very good thing and makes for a
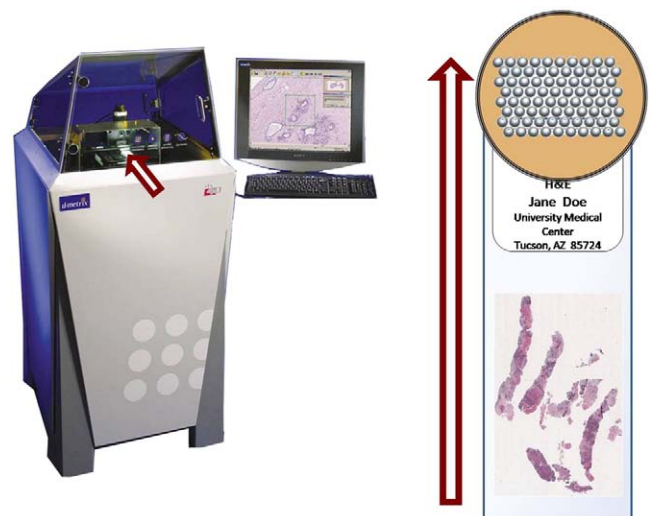
Fig. 4 Left, Dmetrix-DX40 Ultrarapid Virtual Slide Scanner. Arrow points to its lenslet array ensemble. Right, Concept rendering of a DMetrix lenslet array, overlaying a glass histopathology slide of core biopsies. In a DMetrix Virtual Slide Scanner, a 3-layered lenslet array moves in relation to a glass slide (ie, down the slide, as shown in this illustration) at the rate of 3 mm/s. A sensor would be mounted on the lenslet array ensemble and would perform digital imaging at approximately 3000 frames per second. In actual use, the slide is inverted 180 degrees.

great pathology blogger as well as a solid contributor to the hardcopy pathology literature. This valuable article reflects Dr Kaplan's concerned professionalism regarding this ever-expanding realm of digital pathology. It is appropriate that Dr Leung and Dr Kaplan cap off this Telepathology Symposium with their own brand of reality testing. Admittedly, going from this Symposium's lofty articles on new applications to a legal briefing may resemble dropping from a balloon at 5000 ft to ground level in a matter of minutes. On the other hand, there is always something nice about getting home safely. Thank you Dr Leung and Dr Kaplan for this safe landing.

In addition, we thank all of the authors of the HUMAN PATHOLOGY Telepathology Symposium for their contributions to what will hopefully become part of the "classic" literature in the telepathology field.

References

[1] Weinstein RS, Bhattacharyya AK, Graham AR, Davis JR. Telepathology: a ten-year progress report. HUM PATHOL 1997;28:1-7.

[2] Weinstein RS, Descour MR, Liang C, Bhattacharyya AK, Graham AR, Davis JR, et al. Telepathology overview. From concept to implementation. HUM PATHOL 2001;32:1283-99.

[3] Weinstein RS. Prospects for telepathology. (Editorial). HUM PATHOL 1986;17:433-4.

[4] Weinstein RS, Bloom KJ, Rozek LS. Telepathology and the networking of pathology diagnostic services. Arch Pathol Lab Med 1987;111:646-52.

[5] Weinstein RS. The futurist meets the twenty-first century. (Editorial). HUM PATHOL 2000;31:1-2.

[6] Weinstein RS. Innovations in medical imaging and virtual microscopy. (Editorial). HUM PATHOL 2005;36:317-9.

[7] Weinstein RS. The S-Curve framework. Predicting the future of anatomic pathology. Arch Pathol Lab Med 2008;132:739-42.

[8] Weinstein RS. Time for a reality check. Arch Pathol Lab Med 2008;132:777-80.

[9] Kayser K, Szymas J, Weinstein RS. Telepathology and telemedicine: communication, electronic education and publication in e-Health. Berlin: VSV Interdisciplinary Medical Publishing; 2005. p. 1-257.

[10] Kayser K, Molnar B, Weinstein RS. Digital pathology virtual slide technology in tissue-based diagnosis, research and education. Berlin: VSV Interdisciplinary Medical Publishing; 2006. p. 1-193.

[11] Weinstein RS, Bloom KJ, Rozek LS. Telepathology: long distance diagnosis. Am J Clin Pathol 1989;9(Suppl):539-42.

[12] Weinstein RS, Bloom KJ, Rozek LS. Telepathology: system design and specifications. SPIE Proc Visual Commun Image Process 1987;845:404-7.

[13] Bloom KJ, Rozek LS, Weinstein RS. ROC curve analysis of super high resolution video for histopathology. SPIE Proc Visual Commun Image Process 1987;845:408-12.

[14] Weinstein RS, Bloom KJ, Rozek LS. Static and dynamic imaging in pathology. IEEE Proc Image Manage Commun 1990;1:77-85.

[15] Barker G, Krupinski EA, Larsen T, Erps K, Weinstein RS. Pay per view. The Arizona Telemedicine Program's billing results. Telemedicine J E-Health 2001;7:287-91.

[16] Yagi Y, Gilbertson JR. Digital imaging in pathology: the case for standardization. J Telemed Telecare 2005;11:109-16.

[17] Leung ST, Kaplan KJ. Medicolegal aspects of telepathology. HUM PATHOL 2009;40:1137-42.

[18] Dee FR. Virtual microscopy in pathology education. HUM PATHOL 2009;40:1112-21.

[19] Molnar B, Berczi L, Ficsor L, Varga V, Tagscherer A, Tulassay Z. Digital slide and virtual microscopy-based routine and telepathology evaluation of gastrointestinal biopsy specimen. In: Kumar S, Dunn BE, editors. Telepathology. Berlin Heidelberg: Springer-Verlag; 2009. p. 5-18.

[20] Krupinski E, Weinstein RS, Bloom KJ, Rozek LS. Progress in telepathology: system implementation and testing. Adv Pathol Lab Med 1993;6:63-87.

[21] Klaus K, Gortler J, Goldmann T, Vollmer E, Hufnagl P, Kayser G. Image standards in tissue-based diagnosis (diagnostic surgical pathology). Diagn Pathol 2008;3:17.

[22] Demichelis F, Della Mea V, Forti S, Dalla Palma P, Beltrami CA. Digital storage of glass slides for quality assurance in histopathology and cytopathology. J Telemed Telecare 2002;8:138-42.

[23] Glatz-Krieger K, Spornitz U, Spatz A, Mihatsch MJ, Glatz D. Factors to keep in mind when introducing virtual microscopy. Virchows Arch 2006;448:248-55.

[24] Weinstein RS, Descour MR, Liang C, Richter L, Russum WC, Goodall JF, et al. Reinvention of light microscopy. Array microscopy and ultrarapidly scanned virtual slides for diagnostic pathology and medical education. Virtual microscopy and virtual slides in teaching, diagnosis and research. Boca Raton, FL: CRC Press; 2005. p. 9-35.

[25] Weinstein RS, Descour MR, Liang C, Barker G, Scott KM, Richter LY, et al. An array microscope for ultrarapid virtual slide processing and telepathology. Design, fabrication, and validation study. HUM PATHOL 2004;35:1303-14.

[26] Krupinski EA. Virtual slide telepathology workstation-of-the-future: lessons learned from teleradiology. HUM PATHOL 2009;40:1100-11.

[27] Bashsur RL, Shannon GW. History of telemedicine evolution, context, and transformation. New Rochelle, NY: Mary Ann Liebert, Inc; 2009. p. 258-68.

[28] Krupinski EA, TIllack AA, Richter L, Henderson JT, Bhattacharyya AK, Scott KM, et al. Eye-movement study and human performance using telepathology virtual slides. Implications for medical education and differences with experience. HUM PATHOL 2006;37:1543-56.

[29] Weinstein RS, Bloom KJ, Krupinski EA, Rozek LS. Human performance studies of the video component of a dynamic telepathology system. Zentral Pathol 1992;138:381-436.

[30] Bruch LA, De Young BR, Kreiter CD, Haugen TH, Leaven TC, Dee FR, et al. Competency assessment of residents in surgical pathology using virtual microscopy. HUM PATHOL 2009;40:1122-8.

[31] Gorstein F, Weinstein RS. Rethinking pathology residency training and education. (Editorial). HUM PATHOL 2001;32:1-3.

[32] Dunn BE, Choi H, Recla DL, Kerr SE, Wagenman BL. Robotic surgical telepathology between Iron Mountain and Milwaukee Department of Veterans Affairs Medical Center: a twelve year experience. HUM PATHOL 2009;40:1092-9.

[33] Evans AJ, Chetty R, Clarke BA, Croul S, Ghazarian DM, Kiehl TR, Ordonez BP, Ilaalagan S, Asa SL. Primary frozen section diagnosis by robotic microscopy and virtual slide telepathology: the university health network experience. HUM PATHOL 2009;40:1070-81.

[34] Dunn BE, Chejfec G, Weinstein RS. Progress toward development of a full-service, telepathology-based laboratory. Cell Vision 1996;3:463-6.

[35] Dunn BE, Choi H, Almagro UA, Recla DL, Krupinski EA, Weinstein RS. Routine surgical pathology in the Department of Veterans Affairs: experience-related improvements in pathologist performance in 2200 cases. Telemed J 1999;5:323-37.

[36] Dunn BE, Almagro UA, Choi H, Sheth NK, Arnold JS, Recia DI, et al. Dynamic-robotic telepathology: Department of Veterans Affairs feasibility study. HUM PATHOL 1997;28:8-12.

[37] Dunn BE, Choi H, Recla DL, Wisniewski TR. Telepathology as an essential networking tool in VISN 12 of the United States Veterans Health Administration. In: Kumar S, Dunn BE, editors. Telepathology. Berlin Heidelberg: Springer-Verlag; 2009. p. 19-30.

[38] Nordrum I, Engum B, Rinde E, Finseth A, Ericsson H, Kearney M, et al. Remote frozen section service: a telepathology project in Northern Norway. HUM PATHOL 1991;22:514-8.

[39] Eide TJ, Nordrum I. Frozen section service via the tele-network in Northern Norway. Zentrall Pathol 1992;138:409-12.

[40] Kaplan KJ, Burgess JR, Sandberg GD, Myers CP, Bigott TR, Greenspan RB. Use of robotic telepathology for frozen-section diagnosis: a retrospective trial of a telepathology system for intraoperative consultation. Mod Pathol 2002;15:1197-204.

[41] Weinstein RS, Bhattacharyya A, Halliday BE, Yu YP, Davis JR, Byers JM, et al. Pathology consultation service via the Arizona-International Telemedicine Network. Arch Anat Cytol Pathol 1995; 43:219-26.

[42] Bhattacharyya AK, Davis JR, Halliday BE, Graham AR, Leavitt SA, Martinez R, et al. Case triage model for the practice of telepathology. Telemed J 1995;1:9-17.

[43] Halliday BE, Bhattacharyya AK, Graham AR, Davis JR, Leavitt SA, Nagle RB, et al. Diagnostic accuracy of an international static-imaging telepathology consultation service. Hum Pathol 1997;28:17-21.

[44] Weinstein LJ, Epstein JI, Edlow D, Westra WH. Static image analysis of skin specimens: the application of telepathology to frozen section evaluation. Hum Pathol 1997;28:30-5.

[45] Krupinski EA, Weinstein RS, Rozek LS. Experience-related differences in diagnosis from medical images displayed on monitors. Telemed J 1996;2:101-8.

[46] Graham AR, Bhattacharyya AK, Scott KM, Lian F, Grasso LL, Richter LC, Henderson JT, Lopez AM, Weinstein RS. Virtual slide telepathology for an academic teaching hospital surgical pathology quality assurance program. Hum Pathol 2009;40:1129-36.

[47] Lopez AM, Graham AR, Barker GP, Richter LC, Krupinski EA, Lian F, Grasso LL, Miller A, Kreykes LN, Henderson JT, Bhattacharyya AK, Weinstein RS. Virtual Slide Telepathology Enables an Innovative Telehealth Rapid Breast Care Clinic. Hum Pathol 2009;40:1082-91.

[48] Weinstein RS. Risks and rewards of pathology innovation: the academic pathology department as a business incubator. Arch Pathol Lab Med 2009;133:580-6.

[49] Weinstein RS, Lopez AM, Barker GP, Krupinski EA, Descour MR, Scott KM, et al. The innovative bundling of teleradiology, telepathology and teleoncology services. IBM Syst J 2007;46:69-84.

[50] http://scien.stanford.edu/class/psych221/projects/05/joanmoh/checker2.html.

REVIEW

Digital pathology: current status and future perspectives

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During the last decade pathology has benefited from the rapid progress of image digitizing technology. The improvement in this technology had led to the creation of slide scanners which are able to produce whole slide images (WSI) which can be explored by image viewers in a way comparable to the conventional microscope. The file size of the WSI ranges from a few megabytes to several gigabytes, leading to challenges in the area of image storage and management when they will be used routinely in daily clinical practice. Digital slides are used in pathology for education, diagnostic purposes (clinicopathological meetings, consultations, revisions, slide panels and, increasingly, for upfront clinical diagnostics) and archiving. As an alternative to conventional slides, WSI are generally well accepted, especially in education, where they are available to a large number of students with the full possibilities of annotations without the problem of variation between serial sections. Image processing techniques can also be applied to WSI, providing pathologists with tools assisting in the diagnosis-making process. This paper will highlight the current status of digital pathology applications and its impact on the field of pathology.

Keywords: digital archiving, education, image processing, slide scanning, telepathology, virtual microscopy, whole slide images

Abbreviations: CAD, computer-aided diagnosis; DICOM, Digital Imaging and Communications in Medicine; FISH, fluorescence *in situ* hybridization; HER2, human epidermal growth factor receptor 2; QA, quality assurance; TMA, tissue microarrays; WSI, whole slide images

Introduction

Interpreting images of tissues and cells at a resolution higher than the naked human eye is the core of pathology. For a long time the microscope has been the only available instrumentation to this end, over centuries providing live images at increasing resolution through ever improving-optics.[1]

During the last decades, optical pathology has gradually changed[2] by the introduction of digital cameras producing still images and microscope-mounted video cameras that allow live examination of slides (dynamic images). These still or dynamic images can be transferred by the means of network connections to remote sites to be assessed by another pathologist, commonly called telepathology.[3,4] This has found applications such as teleconsultation and frozen section diagnosis.[5]

Approximately a decade ago, further improvements of these techniques resulted in the creation of digital slide scanners.[6] These slide scanners produce whole slide images (WSI, also called digital or virtual slides) that combine the advantages of images from live cameras (whole slide access) and digital cameras (high resolution).[1]

WSI are explored using an image viewer, which enables the examination of digital slides in a manner comparable to the use of a conventional microscope in three aspects: first, WSI can be explored at different

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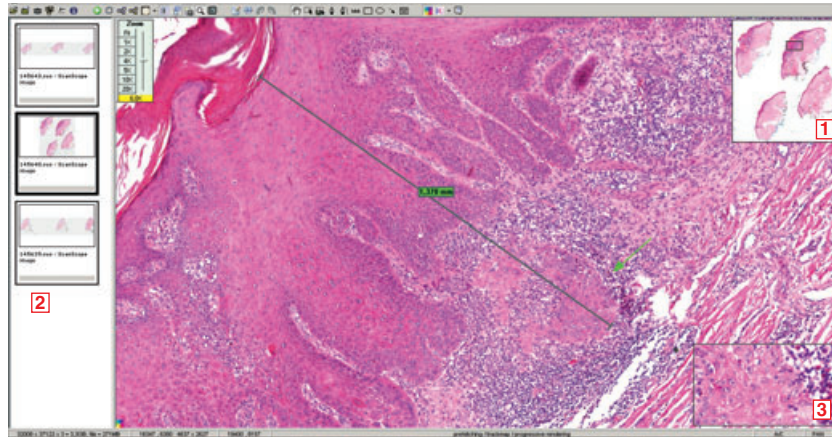
Figure 1. Screenshot from a whole slide image as seen in Aperio's ImageScope viewer application. The presence of a navigation (overview) in the upper right side (1) of the screen provides orientation within the slide shown. The other slides of the same case are presented in the panel on the left side (2) of the screen, which can be explored directly. Annotations can be placed on the slide (for example, the arrow in the image presented above) and measurements can be performed easily (e.g. the line length shown in the image above, but also the area and lengths of boxes and circles which can be drawn on the slide can be measured). The current location of the cursor on the image is magnified further in the magnification window (3).

magnifications, with the additional advantage of in-between magnifications, if provided by the viewer software. Secondly, navigation of the slides in each direction is possible. Thirdly, some scanners allow scanning more than one focus plane, thereby even allowing focusing up and down.[7–11] Furthermore, WSI have several virtues over conventional slides:

• Image viewers are able to show an overview image together with the high(er)-power view, resulting in better orientation within the slide when viewing at high(er) magnification and more easy navigation to other regions of interest.

• Image viewers can display several slides side by side, so the examiner can compare structural details between slides or compare easily different stains of the same tissue area.

• WSI can be made available instantaneously to multiple examiners from all over the world through the internet without the need for a microscope.

• Focusing is carried out during scanning, necessitating less user interaction.

• The quality of WSI is constant over time.

• WSI can be used directly for automated image analysis and morphometry.

• WSI can be integrated within the electronic patient records, together with other images. Figure 1 shows a screenshot of a WSI as it is seen with an image viewer.

Slide scanners

There are major differences between the different manufacturers and types of slide scanners. One major

difference is the capacity; some can be loaded with only one slide, others with several hundred slides per scanner load. They also use different acquisition techniques, the two major ones being line scanning, which is performed by continuous precise movement of a stage[1,12] or by using a regular CCD camera that acquires square image tiles one by one.[1,13] At the end of the scan, these lines or tiles are stitched together, generating the final output image representing the slide.[12,14,15]

Scanners are either supplied with one objective (further magnification is conducted by adding a ×2 additional lens) or supplied with more objectives, having different magnifications and numerical apertures. Scanners with multiple objectives are supplied mainly with objectives of maximum magnification of ×40, although the DMetrix DX-40 is supplied with a ×80 objective.[16]

Table 1 shows a summary of some more scanner features and their different implementations between slide scanners. Some scanners are able to scan at multiple focus layers. By stacking those images together they provide a three-dimensional (3D) image stack. Although the scan time increases linearly with the number of layers, this can be beneficial for cytological specimens, frozen sections and other thick specimens where the pathologist needs to inspect the cellular architecture at different planes. Further, mitoses recognition is easier when multiple focus layers are available.

Scanners equipped with special fluorescence illumination optics, light sources and more sensitive image acquisition sensors are provided by different vendors. These scanners are able to scan fluorescently labelled

Table 1. Essential slide scanner features and the extreme ends of implementation in slide scanners from different vendors and different types

Feature	Alternative 1	Alternative 2
Available magnifications	One fixed objective (possibly with post-magnification)	Different objectives (sometimes even extendible)
Focusing technique	Placing different focus points on tissue areas	Continuously focusing
Image file format	Open format (can be standard, such as jpeg 2000 or DICOM with jpeg (2000) compression)	Closed format (often proprietary)
Image acquisition technique	Linear scanning/line scanning	CCD camera
z-stack acquisition	Yes	No
Fluorescence	Yes	No

DICOM, Digital Imaging and Communications in Medicine.

cell and tissue samples and convert them to high-resolution colour digital slides. Fluorescent digital imaging provides the opportunity to store fluorescently stained slides permanently, eliminating the problem of stains fading over time. These fluorescent WSI can also be utilized for automated image analysis, such as for fluorescence *in situ* hybridization (FISH).

Several factors determine the quality and usefulness of the final WSI as experienced by the end user:[7,13,17]

• The quality of the tissue itself (e.g. preservation state) and the technical quality of the original slide (e.g. leaked glue, scratches, tears, irregular mounting, the quality of staining and the amount of text scribbling).

• The image acquisition technique of the slide scanner that is defined by the method of focusing, colour management, white balancing and contrast.

• Post-processing of the scanned slides: the accuracy of stitching and degree of compression.

• Completeness of the scan (all tissue pieces on the original slide should be present on the WSI). To avoid scanning and storing unnecessary regions, some algorithm is often applied to scan only the area of interest.

• Image-handling issues that are determined by the viewer (smooth scrolling, the ability to use various magnifications) or the information technology (IT) infrastructure (short access time).

• The quality of the computer screen or projector used to display the images. Factors influencing the perception of digital slides include, but are not limited to, the resolution of the screen, the accuracy of colour presentation, brightness and contrast.

Because of the high resolution needed and the inherent colour information present in each slide, the size of each scan is between a few megabytes up to several gigabytes, depending mainly on the amount of tissue present on the slide.[1] Different techniques exist to reduce this image size, for example reducing the scan area with algorithms to detect tissue areas and compression of the final image.[1,18,19] The time needed to scan each slide is dependent on the size of tissue present on it, the time to handle the physical glass slide inside the scanner, speed of focusing and processing of the output. For example, performing a whole slide scan (25×50 mm^2) at ×20 takes 58 s for the Dmetrix (in ultra-speed mode) scanner, while it takes 4 min for an Aperio ScanScope CS (as provided by the manufacturers).[16] Performing scanning for slides with areas of 15×15 mm at ×40 will take between 9 and 80 min, depending on the scanner type. A recently introduced scanner from Philips claims to scan a slide area of 15×15 mm at ×40 in <50 s.

IT infrastructure

After a slide has been scanned it should be made available to the users, and the images should be linked in some way to a laboratory management or reporting system. To achieve this, barcodes on the slide are often used. Either 1D barcodes or 2D barcodes are suitable for this.

To store WSI, some type of storage infrastructure is needed. The total amount of required storage space is dependent on the defined purposes of whole slide scanning. Storing a limited amount of WSI for consultation, research or educational purposes may not require mass storage capacity. However, large-scale scanning, for example when routinely scanning all produced slides in a medium-sized laboratory, already requires a huge storage environment of up to 40 terabytes per year, excluding backup.[1] Depending on

the retrieval characteristics of the end-users, ultra-fast fibre channel hard disks are required. Eventually (depending mainly on the pricing), flash-based solid-state drives will provide fast access, as they have a low access time and low latency. Because not all images are needed to be available instantaneously, older images might be archived to slower (but cheaper) storage media, such as tape.

The quality of the display monitors affects digital slides examination significantly. The display resolution is the most important parameter, which determines the image quality and the size of the viewed field. For example, monitors having a resolution of 1600×1200 pixels show only 21% of the corresponding field under the conventional microscope.[20] Other parameters, such as colour calibration, contrast and brightness, also have an effect on the perceived image quality.

The network speed potential limits the speed of image retrieval and must be sufficient for continuous streaming of image files. Usually, 100 Mbit connections will be sufficient. Most image viewers incorporate efficient strategies for retrieving images; instead of downloading the complete image file, only the request area of interest and adjacent sections are fetched from file storage. This information is cached for fast retrieval in later requests. Also, some viewers first show low-resolution tiles while fetching the high-resolution tiles.

When the same WSI needs to be available to multiple users at the same time (e.g. for digital practical sessions or during slide courses), specially tuned accelerator servers may be required for even more strategic caching strategies.

At the time of writing, most slide scanner manufacturers use their own file format. Some are even proprietary; some are based on other standards, such as jpeg 2000 (J2k). The former is obviously a big disadvantage to end-users, who are often forced to install multiple viewers when exchanging images, and hinders market penetration of digital microscopy. Some propose to use the jpeg 2000 format as a standard.[18,21] In radiology, Digital Imaging and Communications in Medicine (DICOM) is the standard file format used for storing and exchanging images. The DICOM committee recently (August 2010) succeeded in finalizing a supplement to extend the DICOM standard to support WSI. This is an important development which all vendors, hopefully, will take seriously and comply with.

Applications

One can think of many applications using digital slides in pathology, but they can basically be grouped into four different main applications: (i) education, (ii) diagnostics, (iii) research and (iv) archiving.

EDUCATION

Traditionally, education in the field of cell and tissue pathology has been based on glass slides and thus relied on conventional microscopy using double- or multi-headed microscopes.[17,22] However, the multiheaded microscope limits the number of students able to access it. For a long time, next to live viewing of glass slides, static images in the form of diapositives have been used in presentations. The next stage was using static digital images that could be incorporated into teaching software, supplemented with annotations. Since WSI have become available, teaching was probably among the first applications of WSI.[22,23] WSI provide exactly the same image to teacher and students, can be made available to an unlimited number of students at the same time (even remote) and thereby function as a scalable multiheaded microscope,[10] circumvent the unavoidable variation between serial sections from tissue blocks and provide full possibilities for annotations, links and incorporating questions, videos and sound clips. Taking full advantage of these virtues requires, however, a professional software environment such as PathXL (i-Path, Belfast, UK) or Digital Slidebox (Slidepath, Dublin, Ireland). Also, complete training programs, including digital slides with annotations and questionnaires and online testing programs for pre- and postgraduates are provided by several companies.

The use of digital slides for education also has some disadvantages: students no longer learn to use the microscope[24,25] which can, however, be learned later if necessary, and knowledge on the role of cells and tissues in disease is more important than the skill of handling a microscope. Further, digital education then depends fully on the well-functioning IT infrastructure, and any failure or slow performance of the system will severely affect the teaching process. Lastly, the resolution provided by WSI from a good scanner is lower than when examining glass slides under a good microscope, but still more than good enough for teaching students.

Virtual microscope laboratories have been applied successfully in several universities around the world.[26] At the University Medical Center Utrecht, digital microscopy teaching was implemented gradually starting in 2007. The students quickly accepted WSI for teaching, liked it better than the conventional microscopy, and their performance in examinations did not decrease with the use of WSI-based teaching.[25] These results are comparable to those from other universities,

such as the University of Iowa and the University of Basel.[7,23,24]

DIGITAL DIAGNOSTICS

With the availability of WSI, obstacles associated with the previous static and live systems (bias and error in selecting the images from microscopic fields for diagnosis in static system and low image resolution of the dynamic system) have been overcome.[3] The progress in image resolution of WSI,[27] scanning speed and user friendliness of the viewers has made true digital slide-based diagnostics feasible in several ways:

• Consultations for difficult or rare cases: digital consultation can be performed within hours versus days to weeks for cases sent through regular mail. At UMC Utrecht, we have implemented a server for digital consultation (http://www.slideconsult.com) where anyone having a WSI and an internet connection can upload a case for digital consultation with one of our pathologists. This server was implemented using mScope clinical software (Aurora MSC, Montreal, Canada). It is possible to discuss cases online, where one becomes the master who can navigate through the image while the other participant(s) see these movements live on their screen.

• Slide conferences and panels, which is a special form of consultation. Super-specialized pathologists in specific areas of pathology traditionally meet physically on a regular basis to discuss cases. Using the software as described above, panel members no longer need to travel to meet physically and can view and assess cases remotely by WSI, or participate in a virtual panel as described in the previous bullet-point. Several slide panels in the Netherlands exchange their images digitally and discuss them online through our server. A screenshot from the pathology slide panel is shown in Figure 2.

• Telerevision and quality assurance (QA): it is common practice to revise the relevant pathology material for referred patients. Again, shipping slides through regular mail is slow and slides may be lost or damaged. Conducting this digitally speeds up the revision process dramatically, and obviates the need of sending slides. Some hospitals perform digital QA conferences on a daily basis to revise difficult, rare and new cancer cases from other hospitals. Experience from the University of Arizona Pathology Faculty showed that QA by WSI telepathology was extremely accurate and also allowed direct revision of the discrepant cases.[28,29] Another study for assessing the usefulness of WSI for QA programs also showed that QA can be carried out efficiently with WSI.[30] For clinical trials where the patient's material often needs to be revised by an expert pathologist before randomization this would also work very well. The same
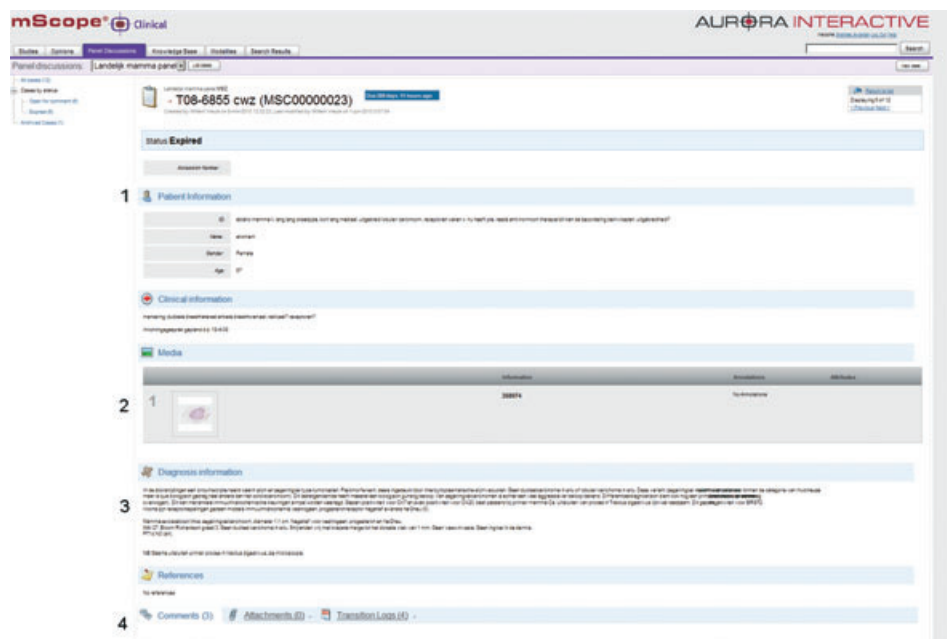
Figure 2. Screenshot from http://www.slideconsult.com. The slide shows the panel module, where the (registered) pathologists have access to digital slides and can render diagnosis from a distance. (1) Clinical information about this case, (2) the uploaded digital slide(s) for consultation, (3) diagnosis of the specialist pathologist who submitted the case and (4) comments from the other panel members.

software system as described above can be used to accommodate this.

• Frozen sections diagnosis: still or dynamic telepathology systems have been used to facilitate the evaluation of frozen sections for a long time, especially for hospitals without pathology department.[5,31–34] The estimated average diagnostic accuracy of frozen section telepathologic diagnosis using old systems (especially dynamic and hybrid) is approximately 95–96%.[35] Using the WSI, pathologists were able to increase diagnostic accuracy and reduce the time required to complete the diagnosis.[36] Another study showed that WSI were superior to a conventional dynamic telepathology system in terms of usability and turnaround time. Reduction in the time of diagnosis and better image quality were the main two reasons for preferring telepathology using WSI.[35]

• Image analysis: automated image analysis will enhance diagnostic efficacy in histopathology. Because inspection of WSI is probably slightly more time-consuming than conventional slides,[37] the creation of programs for detection of regions of interest will be advantageous and speed up the workflow, especially if those areas of interest could be computed before the pathologist sees the image. To this end, grid computing would probably be needed to be able to apply several algorithms to WSI (computing might take a long time because of WSI resolution).[38] Software for computerized quantification of immunohistochemically stained WSI to improve the objective assessment of the immunoreactivity is available from several scanner vendors. Such software estimates colour intensity relative to control cells. Using this information they categorize the staining as 0+, 1+, 2+ or 3+. Examples of dedicated (non-scanner vendor) software packages for tissue quantification are Definiens TissueStudio (Definiens, Munich, Germany) and AQUA (HistoRx, Branford, CT, USA). The most commonly seen application of image analysis-based quantification of immunohistochemical stains is for human epidermal growth factor receptor 2 (HER2)/neu quantification.[39,40] Some of these applications have clearance by the USA Food and Drug Administration (FDA), such as the Automated Cellular Imaging System (ACIS III), which has approval for their Hercep test, oestrogen receptor (ER) and progesterone receptor (PR) applications.[41] Particularly for HER2 scoring in breast cancer it has been shown that WSI-based image analysis provides a higher concordance rate with FISH than inspection by eye and lowers inter-observer variability.[42] Other current applications include assessment of the percentage of ER-, PR- and Ki67-positive nuclei.

The same principles can be applied to the quantitative assessment of tissue microarrays (TMA), where multiple tiny histological specimens are placed on the same slide to be assessed for immunoreactivity or gene amplification. Examples of scanners that are able to perform TMA analysis are ACIS, GenoMX and Ariol SL-50.[43]

• Upfront digital diagnostics: the current state of technology already allows conducting upfront digital diagnostics. However, this is still unusual, probably related to the fact that handling WSI still takes more time than conventional slides, and the currently insufficient validation of WSI-based diagnostics. Initial evaluation of the diagnostic accuracy on WSI showed a high correlation with glass slide diagnosis in breast, pulmonary, gastrointestinal tract and prostate specimens.[44] Further validation is ongoing in different places in the world. WSI allows pathologists to work remotely, such as from home or from any location around the world. Further, conglomerates of smaller pathology laboratories may begin to super-specialize when cases are easily available through WSI.

RESEARCH

For research purposes, digital slides can be used for viewing, storing annotations and measuring (most WSI viewers support measuring areas and lengths). Also, image processing algorithms as described in the previous paragraph can be used, and many new ones are continuously being developed. Easy exchange of (annotated) images is a major advantage. Scoring TMAs can be easier, as the grid of the cores can be assessed and individual cores can then be presented as a perfect array and viewed and analysed individually. Some biobanks systematically include WSI of banked cases for documentation (http://www.tubafrost.org).

DIGITAL ARCHIVING

For many years the storage of microscopic information in pathology has been in the form of glass slides. However, this is not without problems, such as the required large storage rooms with fortified floors, the fragile nature of the glass slide, fading of the stain over time, and finally the labour and logistical issues involved with ongoing storing and retrieving glass slides during which they regularly become misplaced.[45] A fully digital slide archive would have many advantages:[1]

• WSI are saved permanently with constant quality.
• Easy retrieval of cases for teaching, research, clinicopathological conferences and quality assurance.

• The same case can be accessed by different observers at the same time.

• WSI can be integrated into the pathology report and the hospital information system.

The more widespread the digital archiving across laboratories, the higher the potential gain; for example, for telerevision. Although local digital archives could be interfaced, there would certainly be economy of scale if larger (even nationwide) storage facilities served different laboratories. Archived digital slides are a huge data warehouse containing a great deal of information, especially when linked to the original reports containing diagnostic information. Future developments in the area of automated image analysis and correlating this to, for example, clinical outcome, might provide better insights into disease processes.

Future perspectives

During the last decade pathology has benefited hugely from the progress of information technology. The innovation of digital pathology has opened new challenges where whole-slide examination on computer screens has become possible for several applications in pathology. The applications and use of WSI are expected to increase steeply over the next decade, also related to anticipated developments.

The large number of the slides for daily diagnosis in pathology requires high-speed scanners. Fortunately, new scanners are becoming available that can scan slides with a tissue area of 15×15 mm at $\times 40$ in <1 min.

Besides the required speed increase to facilitate upfront diagnostics, the image quality also needs to improve. Some vendors are currently selling scanners that have continuous focusing mechanisms which will prevent unfocused areas in the WSI. The option to perform *z*-scanning to simulate focusing and scanning of fluorescent slides will probably become more common.

WSI have been used in many aspects of pathology and are generally well accepted. The use of digital slides for teleconsultation, telerevision, frozen section diagnoses and quality assurance is expected to increase over the next few years. Upfront WSI-based diagnostics is currently validated in different centres and is expected to be successful, especially when viewers become more user-friendly. Obtaining FDA approval will definitely help, as well as standards for image storage and optimal IT infrastructure that support its routine use.[20]

The validation of their use for daily pathology practice and standardization of the image format will have a great impact on pathology and health-care systems. In September 2010, an extension to the DICOM file format was accepted by the DICOM committee to support storage of WSI. Adaptation to this standard by scanner vendors is now anticipated.

Hopefully, the used of WSI in education will yield generations of pathologists who are more familiar with the use of WSI. In addition, the application of digital archiving is found to be a solution for permanent slide storage with constant quality (especially for fluorescent slides), plus the advantage of easy retrieval for research purposes, education and revision. However, storage costs are still a limiting factor, although these are expected to drop steeply.

Progress in bandwidth of mobile connections may soon allow accessing WSI on PDAs, Apple's iPad or similar. Hopefully, software for this will soon be available.

Conclusion

We expect the next decade in digital pathology to bring several developments. First, we anticipate further improvements in scanning speed and image acquisition techniques, which will lead to scan speeds at $\times 40$ below 30 s. This will allow a setup where slides can be scanned before they leave the laboratory, and will also facilitate *z*-scanning without major impact on performance. This is the way forward for upfront digital diagnostics, as the scanning delay can then be neglected and image analysis algorithms can be run in the background. Full integration of scanners into the laboratory workflow where, for example, a conveyor belt-like setup takes slides through a stainer and coverslipper and then through the scanner, would be a breakthrough. We expect improvements in compression algorithms (e.g. the development of 3D compression for reducing file size of *z*-scans), in storage solutions that will become faster and cheaper, and in software to access WSI on PDAs or Apple's iPad. Further, other research projects are focusing on the development of algorithms aiding in detection of, for example, mitotic figures, micro-organisms, metastases in lymph nodes, quantitative analysis of immunohistochemical stains and perhaps even automated 'diagnosis' of the cases for QA, often called computer-aided diagnosis (CAD). Such algorithms can run on those images in the background and guide the pathologist to areas of interest (for example with a high mitotic count or possible metastases or microorganisms). Moreover, 3D reconstruction of serial WSI may provide novel insights and better orientation within a given section. This has been tried for colorectal biopsies, which resulted in better detection of small intestinal polyps.[46]

References

1. Huisman A, Looijen A, van den Brink SM, van Diest PJ. Creation of a fully digital pathology slide archive by high-volume tissue slide scanning. *Hum. Pathol.* 2010; **41**; 751–757.
2. Weinstein RS. Innovations in medical imaging and virtual microscopy. *Hum. Pathol.* 2005; **36**; 317–319.
3. Teodorovic I, Isabelle M, Carbone A *et al.* TuBaFrost 6: virtual microscopy in virtual tumour banking. *Eur. J. Cancer* 2006; **42**; 3110–3116.
4. Weinstein RS. Prospects for telepathology. *Hum. Pathol.* 1986; **17**; 433–434.
5. Baak JP, van Diest PJ, Meijer GA. Experience with a dynamic inexpensive video-conferencing system for frozen section telepathology. *Anal. Cell. Pathol.* 2000; **21**; 169–175.
6. Gilbertson JR, Patel AA, Yagi Y. Clinical slide digitization–whole slide imaging in clinical practice. In Gu J, Ogilvie RW eds. *Virtual microscopy and virtual slides in teaching, diagnosis and researches.* Boca Raton: Taylor & Francis, 2005; 225–240.
7. Glatz-Krieger K, Spornitz U, Spatz A, Mihatsch MJ, Glatz D. Factors to keep in mind when introducing virtual microscopy. *Virchows Arch.* 2006; **448**; 248–255.
8. Schrader T, Niepage S, Leuthold T *et al.* The diagnostic path, a useful visualisation tool in virtual microscopy. *Diagn. Pathol.* 2006; **1**; 40.
9. Romer DJ, Yearsley KH, Ayers LW. Using a modified standard microscope to generate virtual slides. *Anat. Rec. B New Anat.* 2003; **272**; 91–97.
10. Gongora JH, Barcelo HA. Telepathology and continuous education: important tools for pathologists of developing countries. *Diagn. Pathol.* 2008; **3**(Suppl. 1); S24.
11. Dee FR, Lehman JM, Consoer D, Leaven T, Cohen MB. Implementation of virtual microscope slides in the annual pathobiology of cancer workshop laboratory. *Hum. Pathol.* 2003; **34**; 430–436.
12. Coleman R. Can histology and pathology be taught without microscopes? The advantages and disadvantages of virtual histology *Acta Histochem.* 2009; **111**; 1–4.
13. Glatz-Krieger K, Glatz D, Mihatsch MJ. Virtual slides: high-quality demand, physical limitations, and affordability. *Hum. Pathol.* 2003; **34**; 968–974.
14. Westerkamp D, Gahm T. Non-distorted assemblage of the digital images of adjacent fields in histological sections. *Anal. Cell. Pathol.* 1993; **5**; 235–247.
15. Silage DA, Gil J. Digital image tiles: a method for the processing of large sections. *J. Microsc.* 1985; **138**; 221–227.
16. Rojo MG, Garcia GB, Mateos CP, Garcia JG, Vicente MC. Critical comparison of 31 commercially available digital slide systems in pathology. *Int. J. Surg. Pathol.* 2006; **14**; 285–305.
17. Rocha R, Vassallo J, Soares F, Miller K, Gobbi H. Digital slides: present status of a tool for consultation, teaching, and quality control in pathology. *Pathol. Res. Pract.* 2009; **205**; 735–741.
18. Krupinski EA. Virtual slide telepathology workstation-of-the-future: lessons learned from teleradiology. *Semin. Diagn. Pathol.* 2009; **26**; 194–205.
19. Staniszewski W. Virtual microscopy, data management and image analysis in Aperio ScanScope system. *Folia Histochem. Cytobiol.* 2009; **47**; 699–701.
20. Treanor D, Jordan-Owers N, Hodrien J, Wood J, Quirke P, Ruddle RA. Virtual reality Powerwall versus conventional microscope for viewing pathology slides: an experimental comparison. *Histopathology* 2009; **55**; 294–300.
21. Tuominen VJ, Isola J. The application of JPEG2000 in virtual microscopy. *J. Digit. Imaging* 2009; **22**; 250–258.
22. Romer DJ, Suster S. Use of virtual microscopy for didactic live-audience presentation in anatomic pathology. *Ann. Diagn. Pathol.* 2003; **7**; 67–72.
23. Dee FR. Virtual microscopy in pathology education. *Hum. Pathol.* 2009; **40**; 1112–1121.
24. Dee FR, Meyerholz DK. Teaching medical pathology in the twenty-first century: virtual microscopy applications. *J. Vet. Med. Educ.* 2007; **34**; 431–436.
25. Klein Klouwenberg P, Valkenburg M, ten Cate O, Bootsma A, van Dijk M. Traditionele versus virtuele microscopie bij het onderwijs in de histopathologie. *Tijdeschrift voor medische onderwijs* 2009; **28**; 261–268.
26. Merk M, Knuechel R, Perez-Bouza A. Web-based virtual microscopy at the RWTH Aachen University: didactic concept, methods and analysis of acceptance by the students. *Ann. Anat.* 2010; **192**; 383–387.
27. Weinstein RS, Descour MR, Liang C *et al.* An array microscope for ultrarapid virtual slide processing and telepathology. Design, fabrication, and validation study. *Hum. Pathol.* 2004; **35**; 1303–1314.
28. Weinstein RS, Graham AR, Richter LC *et al.* Overview of telepathology, virtual microscopy, and whole slide imaging: prospects for the future. *Hum. Pathol.* 2009; **40**; 1057–1069.
29. Lopez AM, Graham AR, Barker GP *et al.* Virtual slide telepathology enables an innovative telehealth rapid breast care clinic. *Semin. Diagn. Pathol.* 2009; **26**; 177–186.
30. Ho J, Parwani AV, Jukic DM, Yagi Y, Anthony L, Gilbertson JR. Use of whole slide imaging in surgical pathology quality assurance: design and pilot validation studies. *Hum. Pathol.* 2006; **37**; 322–331.
31. Weinstein MH, Epstein JI. Telepathology diagnosis of prostrate needle biopsies. *Hum. Pathol.* 1997; **28**; 22–29.
32. Della MV, Cataldi P *et al.* Image sampling in static telepathology for frozen section diagnosis. *J. Clin. Pathol.* 1999; **52**; 761–765.
33. Kaplan KJ, Burgess JR, Sandberg GD, Myers CP, Bigott TR, Greenspan RB. Use of robotic telepathology for frozen-section diagnosis: a retrospective trial of a telepathology system for intraoperative consultation. *Mod. Pathol.* 2002; **15**; 1197–1204.
34. Nordrum I, Engum B, Rinde E *et al.* Remote frozen section service: a telepathology project in northern Norway. *Hum. Pathol.* 1991; **22**; 514–518.
35. Evans AJ, Chetty R, Clarke BA *et al.* Primary frozen section diagnosis by robotic microscopy and virtual slide telepathology: the University Health Network experience. *Semin. Diagn. Pathol.* 2009; **26**; 165–176.
36. Tsuchihashi Y, Takamatsu T, Hashimoto Y, Takashima T, Nakano K, Fujita S. Use of virtual slide system for quick frozen intra-operative telepathology diagnosis in Kyoto, Japan. *Diagn. Pathol.* 2008; **3**(Suppl. 1); S6.
37. Kayser K, Gortler J, Bogovac M *et al.* AI (artificial intelligence) in histopathology – from image analysis to automated diagnosis. *Folia Histochem. Cytobiol.* 2009; **47**; 355–361.
38. Gortler J, Berghoff M, Kayser G, Kayser K. Grid technology in tissue-based diagnosis: fundamentals and potential developments. *Diagn. Pathol.* 2006; **1**; 23.
39. Bloom K, Harrington D. Enhanced accuracy and reliability of HER-2 ⁄ neu immunohistochemical scoring using digital microscopy. *Am. J. Clin. Pathol.* 2004; **121**; 620–630.

40. Slodkowska J, Filas V, Buszkiewicz E *et al.* Study on breast carcinoma Her2∕neu and hormonal receptors status assessed by automated images analysis systems: ACIS III (Dako) and ScanScope (Aperio). *Folia Histochem. Cytobiol.* 2010; **48;** 19–25.

41. Rojo MG, Bueno G, Slodkowska J. Review of imaging solutions for integrated quantitative immunohistochemistry in the pathology daily practice. *Folia Histochem. Cytobiol.* 2009; **47;** 349–354.

42. Minot DM, Kipp BR, Root RM *et al.* Automated cellular imaging system III for assessing HER2 status in breast cancer specimens: development of a standardized scoring method that correlates with FISH. *Am. J. Clin. Pathol.* 2009; **132;** 133–138.

43. Cregger M, Berger AJ, Rimm DL. Immunohistochemistry and quantitative analysis of protein expression. *Arch. Pathol. Lab. Med.* 2006; **130;** 1026–1030.

44. Wilbur DC, Madi K, Colvin RB *et al.* Whole-slide imaging digital pathology as a platform for teleconsultation: a pilot study using paired subspecialist correlations. *Arch. Pathol. Lab. Med.* 2009; **133;** 1949–1953.

45. Gabril MY, Yousef GM. Informatics for practicing anatomical pathologists: marking a new era in pathology practice. *Mod. Pathol.* 2010; **23;** 349–358.

46. Wu ML, Varga VS, Kamaras V *et al.* Three-dimensional virtual microscopy of colorectal biopsies. *Arch. Pathol. Lab. Med.* 2005; **129;** 507–510.

Technical Performance Assessment of Digital Pathology Whole Slide Imaging Devices

Guidance for Industry and Food and Drug Administration Staff

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Preface

Public Comment

You may submit electronic comments and suggestions at any time for Agency consideration to http://www.regulations.gov . Submit written comments to the Division of Dockets Management, Food and Drug Administration, 5630 Fishers Lane, Room 1061, (HFA-305), Rockville, MD 20852. Identify all comments with the docket number [FDA-2015-D-0230]. Comments may not be acted upon by the Agency until the document is next revised or updated.

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Technical Performance Assessment of Digital Pathology Whole Slide Imaging Devices

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Guidance for Industry and Food and Drug Administration Staff

This guidance represents the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff or Office responsible for this guidance as listed on the title page.

I.   Introduction

FDA is issuing this guidance to provide industry and agency staff with recommendations regarding the technical performance assessment data that should be provided for regulatory evaluation of a digital whole slide imaging (WSI) system. This document does not cover the clinical submission data that may be necessary to support approval or clearance. This document provides our suggestions on how to best characterize the technical aspects that are relevant to WSI performance for their intended use and determine any possible limitations that might affect their safety and effectiveness.

Recent technological advances in digital microscopy, in particular the development of whole slide scanning systems, have accelerated the adoption of digital imaging in pathology, similar to the digital transformation that radiology departments have experienced over the last decade. FDA regulates WSI system manufacturers to help ensure that the images intended for clinical uses are reasonably safe and effective for such purposes. Essential to the regulation of these systems is the understanding of the technical performance of the WSI system and the components in the imaging chain, from image acquisition to image display and their effect on pathologist's diagnostic performance and workflow. Prior to performing non-technical analytical studies (i.e., those using clinical samples) and clinical studies to evaluate a digital imaging system's performance, the manufacturer should first determine the technical characteristics that are relevant to such performance for its intended use and determine any possible limitations

37   that might affect its safety and effectiveness. This guidance provides recommendations
38   for the assessment of technical characteristics of a WSI device.
39
40   FDA's guidance documents, including this guidance, do not establish legally enforceable
41   responsibilities. Instead, guidances describe the Agency's current thinking on a topic and
42   should be viewed only as recommendations, unless specific regulatory or statutory
43   requirements are cited. The use of the word *should* in Agency guidance means that
44   something is suggested or recommended, but not required.
45

II.   Background

47
48   For over a hundred years, the reference method for the diagnosis of cancer and many
49   other critical clinical conditions has been histopathological examination of tissues using
50   conventional light microscopy. This process is known as surgical pathology in the
51   United States.
52
53   In surgical pathology, patient tissue from surgery, biopsy or autopsy goes through a
54   process that includes dissection, fixation, embedding, and cutting of tissue into very thin
55   slices which are then stained, for example by the hematoxylin and eosin (H&E) protocol,
56   and permanently mounted onto glass slides. The slides are examined by a pathologist
57   under a light microscope by dynamically adjusting the focus and using different
58   magnifications. By integrating their interpretations obtained by microscopic examination
59   of the tissue from all slides pertaining to a case, pathologists arrive at a diagnosis of the
60   case.
61
62   WSI refers to the digitization of the stained entire tissue specimen on a glass slide. The
63   glass slide is still prepared and stained just as for conventional light microscopy.
64   Depending on the system used, various magnifications, scanning methodologies,
65   hardware, and software are employed to convert the optical image of the slide into a
66   digital whole slide image. With WSI, the pathologist views the image on a computer
67   monitor rather than through the microscope oculars.
68

III. Scope

70
71   This document provides guidance regarding only the technical performance assessment
72   of WSI systems for regulatory evaluation. WSI systems are defined here as those
73   consisting of (a) an image acquisition subsystem that converts the content of a glass slide
74   into a digital image file, and (b) a workstation environment for viewing the digital
75   images. If not otherwise specified, the term "image" in the context of whole slide
76   imaging refers to a pyramid structure consisting of multiple images at different
77   resolutions. The baseline image has the highest resolution. This guidance is applicable
78   for surgical pathology tasks performed in the anatomic pathology laboratory. It is
79   intended to provide recommendations to industry and FDA staff regarding only the
80   technical performance assessment data needed for the regulatory evaluation of a WSI
81   device. This document is not meant to provide guidance for special stain techniques or

82 fluorescence imaging or for the non-technical analytical studies (utilizing clinical
83 samples) or pivotal clinical studies necessary to support safety and effectiveness, nor
84 does this guidance alone suffice to demonstrate safety and effectiveness of WSI systems.
85 Interpretation of WSI images on mobile platforms is beyond the scope of this guidance.
86

IV. Policy

88
89 The following subsections of this section describe the technical performance assessment
90 data FDA believes will facilitate the regulatory evaluation of a WSI device.
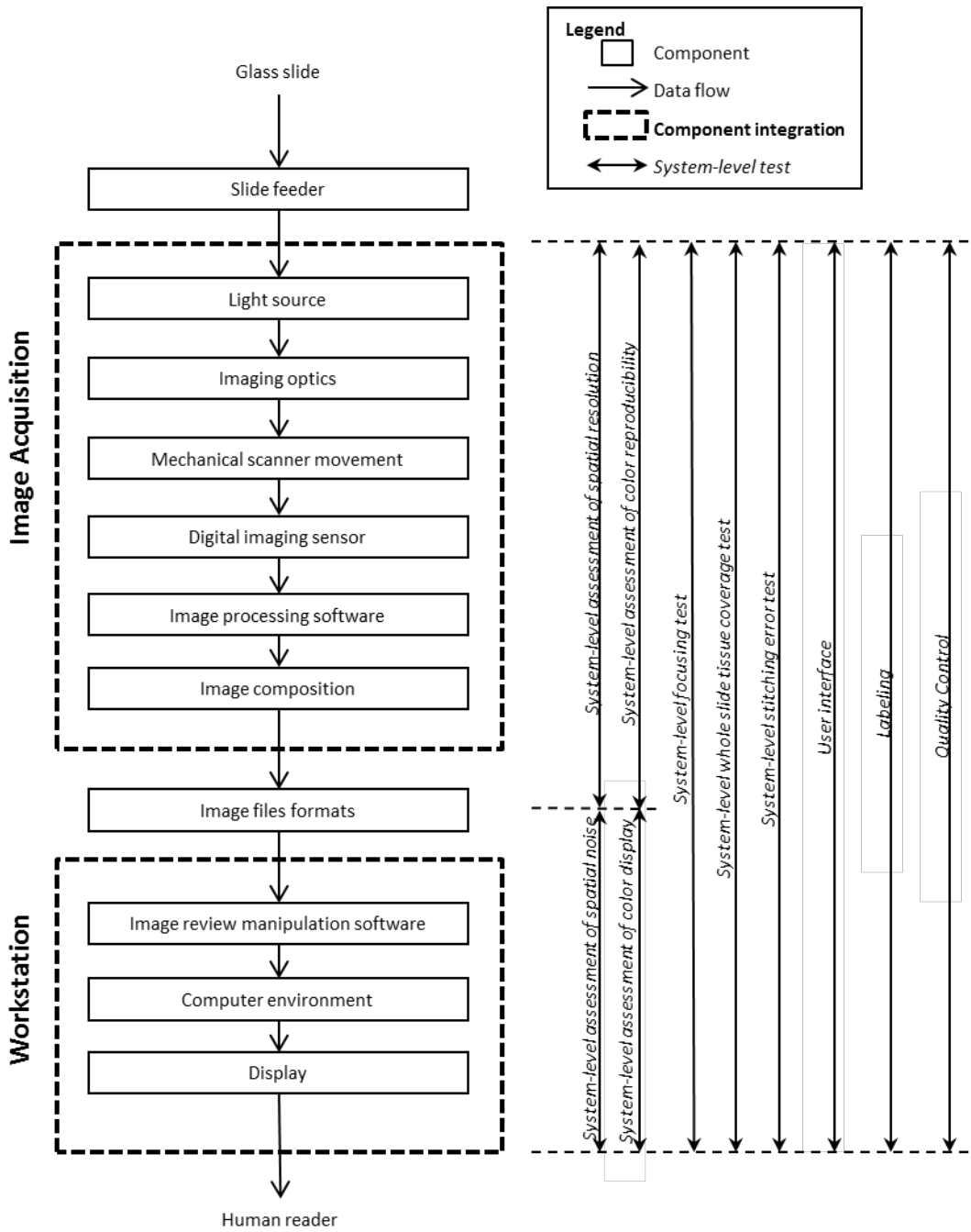91

IV(A). Description and Test Methods for Each Component

93
94 This subsection details the descriptions and the test methods at the component level that
95 should be included in the technical performance assessment of a WSI device. For
96 purposes of this guidance only, a component is a piece of hardware, software, or a
97 combination of hardware and software that processes the image signals flowing through
98 the imaging chain. The concept of a component is based on the transformation of the
99 image signals. For example, the digital imaging sensor is a hardware device that converts
100 optical signals into digital signals. The image composition component is a software
101 program that stitches sub-images together to form a whole slide image. A component
102 and a physical device need not be in close physical proximity. For example, the light
103 source component and the image optics component are usually tightly coupled within the
104 same device, while the display calibration data is often distributed in both the color
105 profile in the computer environment component and the on-screen display settings in the
106 display component.
107
108 The components in a WSI device can be grouped in two subsystems: image acquisition
109 and image display. The image acquisition subsystem digitizes the tissue slide as a digital
110 image file. The image display subsystem converts the digital image file into optical
111 signals for the human reader. In the paradigm of telemedicine, the digital image file can
112 be electronically sent to a remote site for reading, so the image acquisition subsystem and
113 the image display subsystem do not need to be physically coupled. Methods for
114 independently testing the image acquisition and display subsystems are described in
115 Section IV(B).
116
117 Sponsors should provide a block diagram of the components found in the WSI system in
118 the premarket submission. A chart indicating the relationship among the components and
119 the test methods utilized for the specific system characterization should also be provided.
120 Diagram 1 on the following page is offered as an example block diagram of typical
121 components found in current WSI systems. The components of a particular WSI system
122 might not include all of those listed in the diagram or may include additional
123 components. Sponsors are encouraged to provide additional diagrams, illustrations, and
124 photographs of their devices as part of their submissions.
125
126

127 **Diagram 1: Example block diagram of typical components found in current WSI**
128 **systems**
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168   ## IV(A)(1).   Slide Feeder
169
170   ### IV(A)(1)(a).   Description
171
172   The slide feeder is the mechanism(s) used to introduce the slide(s) to the scanner. For the
173   slide feeder, sponsors should provide the following information, if applicable:
174   - Configuration of the slide feed mechanism (a physical description of the
175   equipment)
176   - Slide configuration (physical description of the slide (i.e., custom or
177   commercial off-the-shelf))
178   - Number of slides in queue (carrier)
179   - Class of automation (e.g., robotics, pneumatics, etc.)
180   - User interaction
181   - Hardware (e.g., loading of slides into carrier)
182   - Software (e.g., does the system recognize the number of slides or is this
183   specified by the user)
184   - Feedback (e.g., alarms, notifications, etc.)
185   - Failure Mode and Effects Analysis (FMEA) (including severity,
186   likelihood, mitigations, etc.)
187
188   ## IV(A)(2).   Light Source
189
190   ### IV(A)(2)(a).   Description
191
192   The light source, including the light guide, generates and delivers light to the slide being
193   imaged. The two major components are the lamp and condenser. For the light source,
194   sponsors should provide the following information and specifications, if applicable:
195   - Lamp
196   - Bulb type (e.g., halogen, xenon arc, LED)
197   - Manufacturer and model
198   - Wattage
199   - Spectral power distribution
200   - Expected lifetime
201   - Output adjustment control (electrical/electronic/mechanical)
202   - Optical filter(s)
203   - Type (e.g., heat blocking, polarization, neutral density, diffusing)
204   - Manufacturer and model
205   - Expected intensity variation (coefficient of variation)
206   - Over the duration of scanning a single slide
207   - Over the course of a single workday
208   - Over the lifetime of the device
209   - Expected spectral variation
210   - Over the duration of scanning a single slide
211   - Over the course of a single workday
212   - Over the lifetime of the device
213   - Capability of tracking intensity and spectral degradation with lifetime

214   •   Condenser
215   o   Illumination format (e.g., Kohler, critical)
216   o   Manufacturer and model
217   o   Numerical aperture
218   o   Focal length
219   o   Working distance
220
221   **IV(A)(2)(b).**   **Test Method**
222
223 The following steps should be used to measure the spectral distribution of light incident
224 on the slide. Position the input of a calibrated spectrometer or monochromator at the
225 plane where the slide would be placed, centered on the illumination spot from the
226 condenser. If desired, the light can be coupled into the spectrometer via light guide (e.g.,
227 fiber optic cable) or an integrating sphere. The measurement aperture should be at least
228 as large as the anticipated field of view on the slide at the lowest magnification of the
229 imaging optics. The wavelength accuracy and relative spectral efficiency of the
230 spectrometer or monochromator in the wavelength range of 360-830 nm should be
231 calibrated prior to measurements and reported. Plots of the measured spectrum with at
232 least 10 nm spectral resolution should be provided, using radiometric units (e.g., spectral
233 irradiance in $W/cm^2/nm$, spectral radiance in $W/sr/cm^2/nm$).
234

IV(A)(3). Imaging Optics

235
236
237   **IV(A)(3)(a).**   **Description**
238
239 The imaging optics comprises the microscope objective and auxiliary lens(es) (e.g., tube
240 lens), which optically transmit an image of the tissue from the slide to the digital image
241 sensor. Sponsors should provide the following information and specifications, if
242 applicable:
243   •   Optical schematic with all optical elements identified from slide (object plane) to
244   digital image sensor (image plane)
245   •   Microscope objective
246   o   Manufacturer
247   o   Type
248   o   Magnification
249   o   Numerical aperture (NA)
250   o   Focal length
251   o   Working distance
252   •   Auxiliary lens(es)
253   o   Manufacturer
254   o   Lens type
255   o   Focal length
256   •   Magnification of imaging optics: ISO 8039:2014 *Optics and optical instruments*
257   *— Microscopes — Magnification*
258
259

260   **IV(A)(3)(b).   Test Methods**
261
262   Sponsors should conduct the following tests in conformance with the International
263   Standards, if applicable:
264   • Relative irradiance of imaging optics at image plane per ISO 13653:1996 *Optics*
265   *and optical instruments – General optical test methods - Measurement of relative*
266   *irradiance in the image field*
267   • Distortion per ISO 9039:2008 *Optics and photonics — Quality evaluation of*
268   *optical systems —Determination of distortion*
269   • Chromatic aberrations per ISO 15795:2002 *Optics and optical instruments —*
270   *Quality evaluation of optical systems — Assessing the image quality degradation*
271   *due to chromatic aberrations*
272

273   ## IV(A)(4).   Mechanical Scanner Movement
274
275   **IV(A)(4)(a).   Description**
276
277   The mechanical scanner addresses the physical characteristics of the stage upon which
278   the glass slide is affixed. The key components include stage configuration, movement,
279   and control. This information is relevant whether it is only the stage that is moving and
280   the optics are stationary, or if there is movement on all axes. For the mechanical scanner,
281   sponsors should provide the following information and specifications, if applicable:
282   • Configuration of the stage (a physical description of the stage)
283   o Stage size
284   o Stage manufacturer and model number
285   o Stage material (e.g., anodized aluminum)
286   o Single multi-axis or multiple stacked linear stages (manufacturer and
287   model number)
288   o Type of guides or ways (e.g., bearings)
289   o Sample retention mechanism (slide holder)
290   • Method of movement of the stage (e.g., stepper motor, servomotor, piezomotor,
291   etc., coupled with belt, ball-screw, lead-screw, etc.)
292   o Movement resolution for XY-axes
293   o Movement in Z-axis
294   o Speed range
295   o Travel distance
296   o Maximum scanning area
297   o Localization and reading of bar code labels
298   • Control of movement of the stage
299   o Open or closed loop operation
300   o Positional accuracy (calibration) and repeatability
301   ▪ Lost motion compensation (e.g., backlash)
302   o Physical control (e.g., joystick) for single-slide, non-batch mode
303   o Selection of area to be scanned (in accordance to image composition
304   software)
305   ▪ whole slide

306   ▪   automatically determined area with tissue content
307   •   Failure Mode and Effects Analysis (FMEA) (including severity, likelihood,
308   mitigations, etc.)
309
310   **IV(A)(4)(b).**   **Test Method**
311
312 Sponsors should demonstrate the mechanical performance of the stage with respect to
313 positional repeatability and accuracy on all relevant axes, in accordance with ISO 230-
314 2:2014 Test code for machine tools—Part 2: *Determination of accuracy and*
315 *repeatability of positioning numerically controlled axes.*
316

IV(A)(5).   Digital Imaging Sensor

318
319   **IV(A)(5)(a).**   **Description**
320
321 The digital image sensor is an array of photosensitive elements (pixels) that convert the
322 optical signals of the slide to digital signals, which consist of a set of values
323 corresponding to the brightness and color at each point in the optical image. Please
324 provide the following information and specifications:
325   •   Sensor type (e.g., CMOS, CCD) and manufacturer
326   •   Pixel information/specifications
327   o   Number and dimensions of pixels
328   o   Design of color filter array
329   ▪   Configuration of color filter array
330   ▪   Spectral transmittance of color filter mask
331   •   Responsivity specifications
332   o   Relative response versus wavelength
333   o   Linearity
334   o   Spatial uniformity
335   •   Noise specifications
336   o   Dark current level (electrons per second)
337   o   Read noise (electrons)
338   •   Readout rate (e.g., pixels per second, frames per second)
339   •   Digital output format (e.g., bits per pixel, bits per color channel)
340
341   **IV(A)(5)(b).**   **Test Methods**
342
343 Sponsors should conduct the following tests in conformance with the corresponding
344 International Standards, if applicable:
345
346   •   Opto-electronic conversion function per ISO 14524:2009 *Photography —*
347   *Electronic still-picture cameras — Methods for measuring optoelectronic*
348   *conversion functions (OECFs)*
349   •   Noise measurements per ISO 15739:2013 *Photography — Electronic still-picture*
350   *imaging — Noise measurements*
351

352   ### IV(A)(6).   Image Processing Software
353
354   ### IV(A)(6)(a).   Description
355
356   Image processing software refers to the embedded software components of the image
357   acquisition device. It typically includes control algorithms for image capture and
358   processing algorithms for raw data conversion into the digital image file. Sponsors
359   should provide the following information and specifications, if applicable:
360   - Exposure control
361   - White balance
362   - Color correction
363   - Sub-sampling
364   - Pixel-offset correction
365   - Pixel-gain or flat-field correction
366   - Pixel-defect correction
367
368   ### IV(A)(6)(b).   Resources
369
370   See the guidance entitled "*Guidance for the Content of Premarket Submissions for*
371   *Software Contained in Medical Devices*"
372   (http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocument
373   s/ucm089543.htm) for the information that should be provided.
374
375   ### IV(A)(7).   Image Composition
376
377   ### IV(A)(7)(a).   Description
378
379   Image composition is a step present in systems that produce whole slide images as
380   opposed to individual fields of view. Whole slide scanning is typically performed in
381   accordance with the positioning of a stage that moves in submicron steps. At each
382   location of the stage movement, an image of the field of view is acquired. Images can be
383   acquired with a degree of overlapping (redundancy) between them to avoid gaps in data
384   collection. Images can also be acquired at different depths of focus followed by the
385   application of focusing algorithms. At the end of this process, all acquired images are
386   combined (stitched) together to create a composite high resolution image. There are a
387   number of features that can affect this process, and they are listed below. Sponsors
388   should provide a description of these features, if applicable:
389   - Scanning method
390   - Single objective or multiple miniature objectives in an array pattern
391   - Scanning pattern: square matrix acquisition (tiling), line scanning, etc.
392   - Overlap between scanned regions
393   - Merging algorithms that stitch the aligned images together into a
394   composite image file. Such algorithms may employ functions to align
395   adjacent fields of view in accordance to the scanning pattern, overlap, etc.

396   o   Automatic background correction functions to eliminate the effect of non-
397   uniformities in the microscope's illumination and image merging
398   procedure. These non-uniformities if not corrected might create visible
399   borders (seams and stitch lines) between the adjacent fields of view.
400   •   Scanning speed: time to scan the whole slide. This time is dependent on selected
401   magnification, and the amount of tissue on the glass slide.
402   •   Number of planes at the Z-axis to be digitized (stack depth)
403
404   **IV(A)(7)(b).   Test Methods**
405
406 Testing for image composition can be performed on a system level using special
407 calibration slides (such as grid patterns) that can test for line uniformity and focus
408 quality. Sponsors should provide the following outputs for these tests, if applicable:
409   •   Images of digitized calibration slides
410   •   Analysis of focus quality metrics
411   •   Analysis of coverage of the image acquisition for the entire tissue slide
412

413 ## IV(A)(8).   Image Files Formats

414
415   **IV(A)(8)(a).   Description**
416
417 The final result from image acquisition can be a whole slide image consisting of a stack
418 of all acquired fields of view and magnifications during WSI. The complete digitized
419 image file usually occupies between 1-20 gigabytes of storage space depending on the
420 sample and the magnification of the objective lens used. Images can then be stored in a
421 number of ways and formats. Sponsors should provide the following information:
422   •   Compression method (e.g., the wavelet-based JPEG2000 compression standard or
423   TIFF)
424   •   Compression ratio: ratio of uncompressed to compressed file size. This metric
425   should be provided along with descriptive information on the data it was
426   measured from, since compression ratio is dependent on the content of the data
427   applied to.
428   •   Compression type: lossless or lossy compression
429   •   File format: can be formats easily accessible with public domain software such as
430   JPEG or TIFF, or can be proprietary formats only accessible with specific vendor
431   viewers. The file format depends on the file organization and related use.
432   •   For systems that interact with DICOM-compliant software and hardware,
433   sponsors should provide a DICOM compatibility report.
434   •   File organization:
435   o   Single file with multi-resolution information (pyramidal organization)
436   o   Stack of files at different magnifications
437
438
439

440 ### IV(A)(9). Image Review Manipulation Software
441
442 #### IV(A)(9)(a). Description
443
444 For the image review manipulation software, sponsors should provide the following
445 information, describing software features, if applicable.
446 - Continuous panning (moving in x-y space) and pre-fetching (buffering adjacent
447   images to speed up panning time)
448 - Continuous zooming (magnification)
449 - Discrete Z-axis displacement
450 - Ability to compare multiple slides simultaneously on multiple windows
451 - Ability to perform annotations
452 - Image enhancement such as sharpening functions
453 - Color manipulation, including color profile, white balance, color histogram
454   manipulation, and color filters
455 - Annotation tools
456 - Tracking of visited areas and annotations
457 - Digital bookmarks (revisit selected regions of interest)
458 - Virtual "multihead microscope" (this is when multiple pathologists
459   simultaneously review the same areas remotely)
460
461 #### IV(A)(9)(b). Resources
462
463 See the guidance entitled "*Guidance for the Content of Premarket Submissions for
464 Software Contained in Medical Devices*"
465 (http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocument
466 s/ucm089543.htm) for additional information on this subject.
467
468 ### IV(A)(10). Computer Environment
469
470 #### IV(A)(10)(a). Description
471
472 Computer environment refers to the workstation, including both hardware and software
473 components, that retrieves the digital image file and drives the display for the user to
474 review the images. Sponsors should provide the following information and
475 specifications, if applicable:
476 - Computer hardware
477 - Operating system
478 - Graphics card
479 - Graphics card driver
480 - Color management settings
481 - Color profile
482 - Display interface (e.g., DVI or DisplayPort)
483

484   **IV(A)(11).   Display**

485

486   **IV(A)(11)(a).   Description**

487

488 The final stage of a WSI system is the display component that presents the scanned image
489 to the pathologists for reading. Technically, display refers to the optoelectronic device
490 that converts the digital image signals in the RGB space into optical image signals. For
491 the display, sponsors should provide the following information and specifications, if
492 applicable:

493   •   Technological characteristics of the display device (e.g., in-plane switching LCD
494   panel with TFT active-matrix array with fluorescent backlight)
495   •   Physical size of the viewable area and aspect ratio
496   •   For transmissive displays, backlight type and properties including temporal,
497   spatial, and spectral characteristics
498   •   Frame rate and refresh rate
499   •   Pixel array, pitch, pixel aperture ratio and subpixel matrix scheme (e.g., chevron,
500   RGBW)
501   •   Subpixel driving to improve grayscale resolution (e.g., spatial and temporal
502   dithering)
503   •   Supported color spaces
504   •   Display Interface
505   •   User controls of brightness, contrast, gamma, color space, power-saving options,
506   etc. via the on-screen display (OSD) menu
507   •   Ambient light adaptation including the ambient light sensing method,
508   instrumentation, and software tool description
509   •   Touch screen technology including method, functionality, and any calibration or
510   periodical re-tuning requirements
511   •   Color calibration tools (sensor hardware and associated software), color profile,
512   and method for color management
513   •   Frequency and nature of quality-control tests to be performed by the user and/or
514   the physicist with associated action limits.

515

516   **IV(A)(11)(b).   Test Methods**

517

518   •   ***User controls:*** Modes and settings of the display undergoing testing should be
519   specified, including brightness, contrast, gamma, white point, color space, etc.
520   See *2.1 Modified-Performance Modes, IDMS 1.03*.
521   •   ***Spatial resolution:*** Measurements of the transfer of information from the image
522   data to the luminance fields at different spatial frequencies of interest typically
523   done by reporting the modulation transfer function. Non-isotropic resolution
524   properties should be characterized properly by providing two-dimensional
525   measurements or measurements along at least two representative axes. See *7.7*
526   *Effective Resolution, IDMS 1.03*.

527   •   ***Pixel defects (count and map):*** Measurements (counts) and location of pixel
528   defects. This is typically provided as a tolerance limit. Pixel defects can interfere
529   with the visibility of small details in medical images. See *7.6 Defective Pixels,*
530   *IDMS 1.03.*
531   •   ***Artifacts:*** Evaluate for image artifacts such as ghosting and/or image sticking
532   from displaying a fixed test pattern for a period of time. See *4.6 Artifacts and*
533   *Irregularities, IDMS 1.03.*
534   •   ***Temporal response:*** Measurements of the temporal behavior of the display in
535   responding to changes in image values from frame to frame. Since these
536   transitions are typically not symmetric, rise and fall time constants are needed to
537   characterize the system. See *10.2.3 Gray-to-Gray Response Time, IDMS 1.03.*
538   •   ***Maximum and minimum luminance (achievable and recommended):***
539   Measurements of the maximum and minimum luminance that the device outputs
540   as used in the application under recommended conditions and the achievable
541   values if the device is set to expand the range to the limit. See *2.4 Vantage-Point*
542   *Suite of Measurement, IDMS 1.03.*
543   •   ***Grayscale:*** Measurements of the mapping between image values and the
544   luminance. See *6.1 Grayscale, IDMS 1.03.*
545   •   ***Luminance uniformity and Mura test:*** Measurements of the uniformity of the
546   luminance across the display screen. See *8.1.2 Sampled Vantage-Point Uniformity*
547   *and 8.2.3 Mura Analysis, IDMS 1.03.*
548   •   ***Stability of luminance and chromaticity response with temperature and lifetime***
549   •   ***Bidirectional reflection distribution function:*** Measurements of the reflection
550   coefficients of the display device. Specular and diffuse reflection coefficients can
551   be used as surrogates for the full bidirectional reflection distribution function. See
552   *11.12 Diagnostic: Characterizing Hemisphere Uniformity, IDMS 1.03.*
553   •   ***Gray Tracking:*** Chromaticity at different luminance levels as indicated by the
554   color coordinates in an appropriate units system (e.g., CIE *u'v'*). See *AAPM Task*
555   *Group 196 Report.*
556   •   ***Color scale:*** Color coordinates of primary and secondary colors as a function of
557   the digital driving level and their additivity. See *6. Gray- and Color-Scale*
558   *Measurement and 5.4 Color-Signal White, IDMS 1.03.*
559   •   ***Color gamut volume:*** See *5.31 Volume-Color-Reproduction Capability, IDMS*
560   *1.03.*
561
562   **IV(A)(11)(c).   Resources**
563
564 Those interested in learning more about these types of display considerations should
565 consider reading:
566
567   •   *IDMS 1.03 - Information Display Measurements Standard Version 1.03,*
568   *International Committee for Display Metrology, Society for Information Display,*
569   *www.icdm-sid.org*
570
571   •   E. Samei, A. Badano, D. Chakraborty, K. Compton, C. Cornelius, K. Corrigan,
572   M. J. Flynn, B. Hemminger, N. Hangiandreou, J. Johnson, M. Moxley, W.

13

573   Pavlicek, H. Roehrig, L. Rutz, J. Shepard, R. Uzenoff, J. Wang, and C. Willis,
574   *Assessment of display performance for medical imaging systems, Report of the*
575   *American Association of Physicists in Medicine (AAPM) Task Group 18*,
576   Technical Report, AAPM (April 2005).
577
578 • IEC 62563-1:2009, *Medical electrical equipment – Medical image display*
579   *systems – Part 1: Evaluation methods*
580
581 • Amendment 1 to IEC 62563-1: *Medical image display systems – Part 1:*
582   *Evaluation methods*
583
584 • The guidance entitled "*Guidance for Industry and FDA Staff: Display Accessories*
585   *for Full-Field Digital Mammography Systems-Premarket Notification (510(k))*
586   *Submissions*"
587   (http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceD
588   [ocuments/ucm107549.htm](http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm107549.htm)).
589

590 # IV(B).   System-level Assessment

591
592 This subsection details the test methods at the system level that should be included in the
593 technical performance assessment of a WSI device. In this guidance, *system* refers to a
594 series of consecutive components in the imaging chain with clearly defined, measureable
595 input and output. For example, a system-level test can be designed for the image
596 acquisition subsystem, the image display subsystem, or a combination of both. The goal
597 of system-level tests is to assess the composite performance of a series of consecutive
598 components in the imaging chain. System-level tests should be conducted when the
599 component-level tests are either unfeasible or unable to capture the interplay between
600 components.
601
602 The common framework of the system-level tests described in this section is to compare
603 the system under test with an ideal system based on the same input, and then report the
604 difference between their outputs quantitatively. Designing such a system-level test
605 typically involves the following steps: (1) define the scope of the system and its input and
606 output, (2) define the input, which in most cases is a test target or phantom, (3) measure
607 the input to establish the ground truth that would be generated by an ideal system, (4)
608 measure the output of the system under test, and (5) calculate the errors between the truth
609 and the output with a quantitative metric. The framework of a typical system-level test is
610 shown in Diagram 2. Notice that the *ideal system* is a hypothetical device that generates
611 the perfect output with respect to the objective of the test such as color or focus. The
612 purpose of the ideal system is to define the intended behavior of the system under test.
613 The ideal system does not need to be implemented. Instead, the ideal system should be
614 simulated by a test method that establishes the truth of the input phantom.
615
616
617
618

619   **Diagram 2: Framework of a typical system-level test.**
620
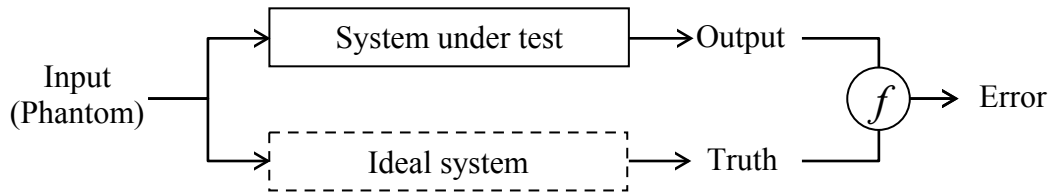
626   # IV(B)(1).   Color Reproducibility
627
628   ### IV(B)(1)(a).   Description
629
630   Color reproducibility is one of the key characteristics of a WSI system. The color
631   characteristics are determined by every component in the imaging chain. Therefore, the
632   color characteristics might be best evaluated at the system level. Color reproducibility
633   indicates the accuracy and precision of the color transformation from the tissue sample on
634   the slide to the image on the display. The colors of the tissue specimen should be
635   accurately and precisely reproduced on the display based on the color reproduction intent,
636   which should be clearly defined and justified by the sponsor.
637
638   ### IV(B)(1)(b).   Test Methods
639
640   The WSI system should be tested with a target slide. The target slide should contain a set
641   of measurable and representative color patches. Ideally the color patches should have
642   similar spectral characteristics to stained tissue. The color patches should include a
643   grayscale ramp for evaluating the grayscale response. The truth of the color patches
644   should be measured with proper apparatuses separately.
645
646   For each color patch, the intended color (i.e., the expected output color based on the color
647   reproduction intent defined by the Sponsor) should be calculated based on the truth of the
648   color patches.
649
650   The target slide should be scanned and displayed by the WSI system. The output color of
651   each color patch should be measured from the display.
652
653   The three datasets – truth, intended color, and output color – should be compared and
654   analyzed. The sponsor should provide a rationale if the intended color is different from
655   the truth.
656
657
658
659
660
661
662

15

Diagram 3: Framework of the system-level color reproducibility test.
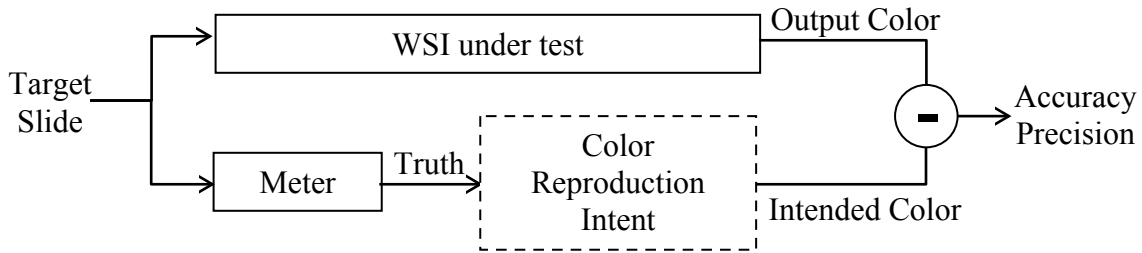
IV(B)(1)(c).　　Resources

Useful references on the subject of color reproducibility can be found at the International Color Consortium website http://www.color.org.

IV(B)(2).　Spatial Resolution

IV(B)(2)(a).　　Description

Spatial resolution is another key characteristic of a WSI system. The goal of this system-level test is to evaluate the composite optical performance of all components in the image acquisition phase (i.e., from slide to digital image file).

IV(B)(2)(b).　　Test Methods

The following test is recommended for assessing spatial resolution of the image acquisition phase:

- Resolution and spatial frequency response: ISO 12233:2014(E) — Photography — Electronic still picture imaging — Resolution and spatial frequency responses.

IV(B)(3).　Focusing Test

- The quality of focus in WSI can be affected by a number of inter-related factors, including the scanning method and approaches for constructing a focus map. Due to a trade-off between the number of focus points and the overall speed of the scanning process, focusing is typically based on a sample of focus points, determined automatically (auto-focus) or manually by the user. Since tissue can have uneven depth, auto-focus algorithms are needed to detect and adjust for different depths of focus.

- Data demonstrating that the focus quality is acceptable, even in the presence of uneven tissue, should be provided. Such data with proper justification could be derived from a phantom study, from clinical data, or both in a complementary fashion. The technology of phantom construction for testing focus is under development and this guidance will be updated as such technologies become available. Sponsors could attempt to build their own phantoms for testing depth

709   of focus for their device. Alternatively, sponsors could provide experimental data
710   using clinical tissue slides. Sampling of cases for such an experiment should be
711   enriched for uneven tissue cases within a range representative of typical
712   laboratory output.   Alternative approaches for assessing the focus quality of a
713   WSI will be considered along with proper justification. In addition, the following
714   specifications should be provided, if applicable:

- o Focus method: auto-focus for high-throughput or user-operated focus points
- o Instructions for the selection of manual focus points (if applicable), including number of focus points and location in relation to a tissue sample
- o Metrics used to evaluate focusing and description of methods to extract them
- o Methods for constructing focus map from sample focus points

Diagram 4: Framework of the system-level focusing test.
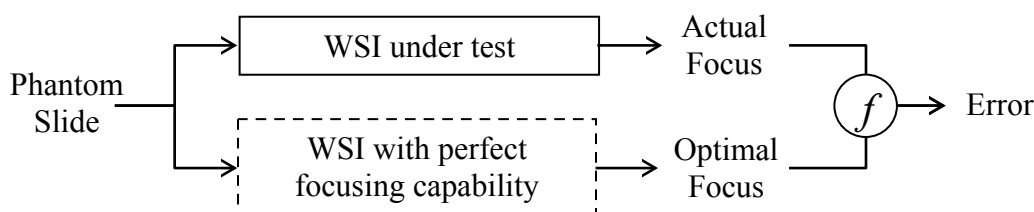
IV(B)(4).   Whole Slide Tissue Coverage

IV(B)(4)(a).   Description

During the scan phase, WSI systems usually skip blank areas where tissue is absent in order to reduce scan time and file size. The purpose of the whole slide tissue coverage test is to demonstrate that all of the tissue specimen on the glass slide is included in the digital image file.

IV(B)(4)(b).   Test Method

Sponsors should include a test that demonstrates the completeness of the tissue coverage. Sponsors should describe the test method and include the following items:

- Selection of the input tissue slide
- How to determine the complete coverage of the input tissue slide
- How to measure the actual coverage of the WSI output
- Calculate the ratio of the actual to complete coverage

Diagram 5: Framework of the system-level whole slide tissue coverage test
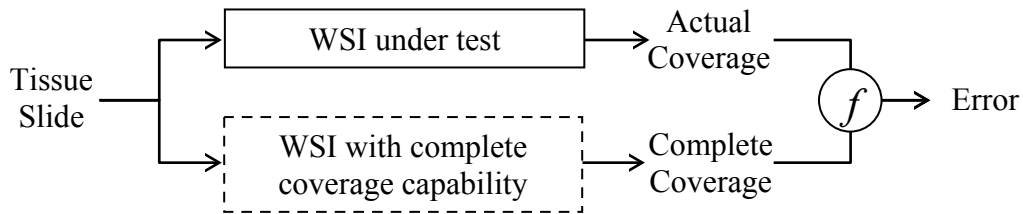
IV(B)(5).   Stitching Error
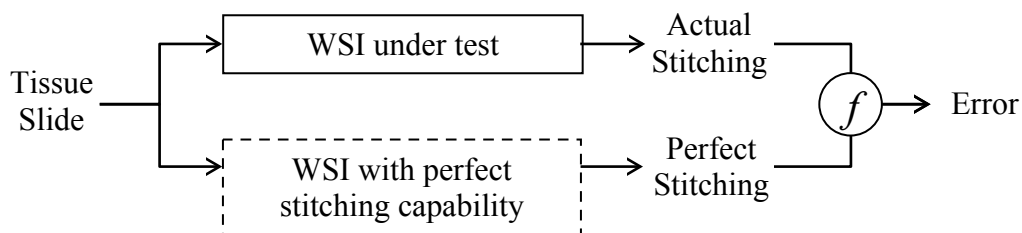
IV(B)(5)(a).   Description

Stitching is the technique that enables a WSI system to combine thousands of sub-images into a single whole-slide image. Although during the scanning process a certain amount of overlapping between adjacent sub-images is maintained for alignment purposes, successful stitching relies on the texture present in the overlapped area. When the stitching algorithm fails to align two sub-images seamlessly, the error may or may not be perceivable by the human reader depending on whether noticeable stitching artifacts are generated. Therefore, a system-level test should be conducted when assessing the stitching quality of the WSI system.

IV(B)(5)(b).   Test Methods

Sponsors should include a test that evaluates the stitching errors and include the following items:
- Selection of the input test slide
- Method for sampling of the stitching boundaries where stitching errors might occur
- How to determine the ideal stitching as the ground truth
    - For example, the region of the stitching boundaries can be re-imaged in one shot such that there is no stitching artifact.
- How to evaluate quality of the actual stitching based on the perfect stitching
    - For example, compare the image of stitching boundaries with the perfect one that does not have stitching artifact. The difference between these two images can be used as a figure of merit of the stitching quality.

Diagram 6: Framework of the system-level stitching error test

18

801
802 ## IV(B)(6).   Turnaround Time
803
804 ### IV(B)(6)(a).   Description
805
806 Turnaround time is the time required by the WSI system to execute a particular user
807 operation such as panning/zooming where the software and I/O (input/output) devices
808 retrieve image data, execute the computation, and refresh the image on the display. The
809 turnaround time starts when the user enters a command via a keyboard stroke or a mouse
810 click/movement and finishes when the image is completely updated on the display.
811 Turnaround time is important for a WSI system when fast and repetitive panning
812 operations are performed during a search task, which is delay-free in an optical
813 microscope. Prolonged, unpredictable turnaround time may impact the user's diagnostic
814 performance. The user interface should properly prompt the user when the operation is
815 incomplete and the requested image is not available . The turnaround time may vary
816 greatly depending on the user-requested operation, image content, data size/location,
817 computer workload, display size, etc. The sponsor should report the typical turnaround
818 time as well as the test method and test conditions.
819

820 # IV(C).   User Interface
821
822 ## IV(C)(1).   Description
823
824 The user interface covers all components and accessories of the WSI system with which
825 users interact while loading the slides and acquiring, manipulating, and reviewing the
826 images. It also includes preparing the system for use (e.g., unpacking, set up,
827 calibration), and performing maintenance. Elements of the user interface have been
828 noted in many of the preceding sections and include two broad categories:
829 - Options through which the user operates the WSI system, such as:
830   - Software menu options (e.g., scanning parameters)
831   - Physical controls (e.g., clips on the slide feeder)
832   - Connectors and connections (e.g., cables connecting system components)
833 - Information presented to the user through
834   - Visual displays (e.g., scanned image, software menus)
835   - Sounds (e.g., tone played when scanning completed)
836   - Instructions (e.g., software users' manual)
837   - Labels
838

839 ## IV(C)(2).   Test Methods
840
841 It is recommended that the analysis to identify the use-related hazards of the WSI system
842 include the consideration of use errors involving failure to acquire, perceive, read,
843 interpret, and act on information from the WSI system correctly or at all and the harm
844 that could be caused by such errors. A human factors/usability validation test should be
845 performed to demonstrate that representative users of the WSI system can perform
846 essential tasks and those critical to safety under simulated use conditions.

847
848 When selecting participants for validation testing, sponsors should carefully consider user
849 capabilities and expectations that could potentially impact the safe and effective use of
850 the WSI system. Examples of items that should be considered, if applicable, include
851 visual acuity and type of vision correction and the impact of expectations formed from
852 prior experience with other systems (e.g., optical microscope).
853
854 When selecting the critical tasks to be evaluated, sponsors should incorporate all known
855 use related errors and problems from similar devices (devices having similar
856 technological characteristics and indications for use) into the validation testing.
857 Consideration also should be given to whether task performance changes over time, and
858 if test duration needs to account for user fatigue. Examples might include a user altering
859 a task sequence in response to fatigue from repetitive image selection and manipulation
860 with mouse or keyboard.
861
862 When creating the simulated use conditions for validation testing, special consideration
863 should be given to the location of the WSI system primary workstation, its components,
864 their arrangement and how their locations affect user performance. Examples of location
865 considerations might include multiple monitors, a monitor with sub-optimal display
866 settings, or glare on a monitor from indoor lighting.
867
868 A human factors/usability validation test report should generally include the information
869 found in Table 1.
870
871 **Table 1: Items a Human Factors/Usability Validation Test Report Should Include**
872

Section	Contents
1	Intended device users, uses, use environments, and training • Intended user population(s) and critical differences in capabilities between multiple user populations • Intended uses and operational contexts of use • Use environments and key considerations • Training intended for users and provided to test participants
2	Device user interface • Graphical depiction (drawing or photograph) of device user interface • Verbal description of device user interface
3	Summary of known use problems • Known problems with previous models • Known problems with similar devices

		• Design modifications implemented in response to user difficulties
4	User task selection, characterization and prioritization	
	• Risk analysis methods • Use-related hazardous situation and risk summary • Critical tasks identified and included in HFE/UE validation tests	
5	Summary of formative evaluations	
	• Evaluation methods • Key results and design modifications implemented • Key findings that informed the HFE/UE validation testing protocol	
6	Validation testing	
	• Rationale for test type selected (i.e., simulated use or clinical evaluation) • Number and type of test participants and rationale for how they represent the intended user populations • Test goals, critical tasks and use scenarios studied • Technique for capturing unanticipated use errors • Definition of performance failures • Test results: Number of device uses, success and failure occurrences • Subjective assessment by test participants of any critical task failures and difficulties • Description and analysis of all task failures, implications for additional risk mitigation	
7	Conclusion A statement to the effect that "The <device name/model> has been found to be reasonably safe and effective for the intended users, uses and use environments" should be included under the following conditions: • The methods and results described in the preceding sections support this conclusion. • Any residual risk that remains after the validation testing would not be further reduced by modifications of design of the user interface (including any accessories and the Instructions for Use (IFU)), is not needed, and is outweighed by the benefits that	

		may be derived from the device's use.

873
874 Recommended methods for performing a human factors/usability validation test are
875 described in the resources listed in section IV(C)(3) entitled "Resources" directly below.
876 The goal of testing is to assure that users can operate the WSI system successfully for the
877 intended uses without negative clinical consequences to the patient and that potential use
878 errors or failures have been eliminated or reduced.
879
880 ### IV(C)(3).   Resources
881
882 FDA recognizes standards published by national and international organizations that
883 apply human factors engineering/usability engineering (HFE/UE) principles to device
884 design and testing. The recognized standards listed below provide suggestions on
885 conducting an analysis of use-related hazards and a human factors/usability validation
886 test to assess the safety and effectiveness of the final device design.
887
888 • ISO 14971:2007, Medical Devices – Application of Risk Management to Medical
889   Devices: Provides systematic process to manage the risks associated with the use
890   of medical devices.
891 • AAMI/ANSI HE75:2009, *Human Factors Engineering – Design of Medical*
892   *Devices*: Comprehensive reference of recommended practices related to human
893   factors design principles for medical devices.
894 • IEC 62366-1:2015, *Medical devices – Application of usability engineering to*
895   *medical devices:* Describes the process to conduct medical device usability testing
896   and incorporate results into a risk management plan.
897 In addition, FDA has published guidance with human factors related recommendations to
898 assist manufacturers and facilitate premarket review. The guidance entitled "Guidance
899 for the Content of Premarket Submissions for Software Contained in Medical Devices"
900 (http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocument
901 [s/ucm089543.htm](http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm089543.htm)). This guidance document provides recommendations to industry
902 regarding premarket submissions for software devices, including stand-alone software
903 applications and hardware-based devices that incorporate software. It includes test
904 methods to assure that the software conforms to the needs of the user and to check for
905 proper operation of the software in its actual or simulated use environment.
906

907 ## IV(D). Labeling
908
909 The premarket application must include labeling in sufficient detail to satisfy the
910 requirements of 21 CFR Part 801 and 21 CFR 809.10. The labeling includes
911 supplementary information necessary to use and care for the WSI system such as
912 instruction books or direction sheets and software user manuals.
913

914 Although instructions, labeling, and training can influence users to use devices safely and
915 effectively, they should not be the primary strategy used to control risk. Modification of
916 the user interface design is a more effective approach to mitigate use-related hazards.
917
918 ## IV(D)(1). Test Methods
919
920 It is recommended that studies on labeling and training be conducted separately from
921 other human factors/usability validation testing. Human factors/usability validation
922 testing should be conducted with the final version of the labeling and related materials.
923 Timing and content of training should be consistent with that expected of actual users.
924
925 ## IV(D)(2). Resources
926
927 FDA has published several guidance documents on labeling to facilitate premarket
928 review and assist manufacturers.
929 - The guidance entitled "Labeling - Regulatory Requirements for Medical Devices"
930   (http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/
931   GuidanceDocuments/UCM095308.pdf).
932   - This publication covers labeling issues that device manufacturers,
933   reconditioners, repackers, and relabelers should consider when a product
934   requires labeling. Labeling includes adequate instructions for use,
935   servicing instructions, adequate warnings against uses that may be
936   dangerous to health, or information that may be necessary for the
937   protection of users.
938 - The guidance entitled "Device Labeling Guidance #G91-1 (blue book memo)"
939   (http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceD
940   ocuments/ucm081368.htm).
941   - This guidance is intended to ensure the adequacy of, and consistency in
942   device labeling information. It is intended for use by industry in preparing
943   device labeling.
944 - The guidance entitled "Human Factors Principles for Medical Device Labeling"
945   (http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/
946   GuidanceDocuments/UCM095300.pdf).
947   - This report presents the principles of instruction, human factors, and
948   cognitive psychology that are involved in designing effective labeling for
949   medical devices.
950

951 # IV(E). Quality Control
952
953 Sponsors should provide information on the quality control procedures, including
954 frequency and testing methods to be performed by the laboratory technologists and/or
955 field engineers with associated quantitative action limits. Discussions of tests for
956 constancy should include discussions of the slide feeder and scanning mechanisms,
957 coverage of the entire tissue slide, the bar code reader, the light source, the imaging
958 sensor device, and the calibrations at the component and system level. A detailed quality
959 control manual should be provided.

Regulatory Challenges and Opportunities for Digital Pathology

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Center for Devices and Radiological Health

Disclaimer

The views expressed during this presentation are those of the presenter and do not necessarily reflect the policy or position of the US FDA or the US government.
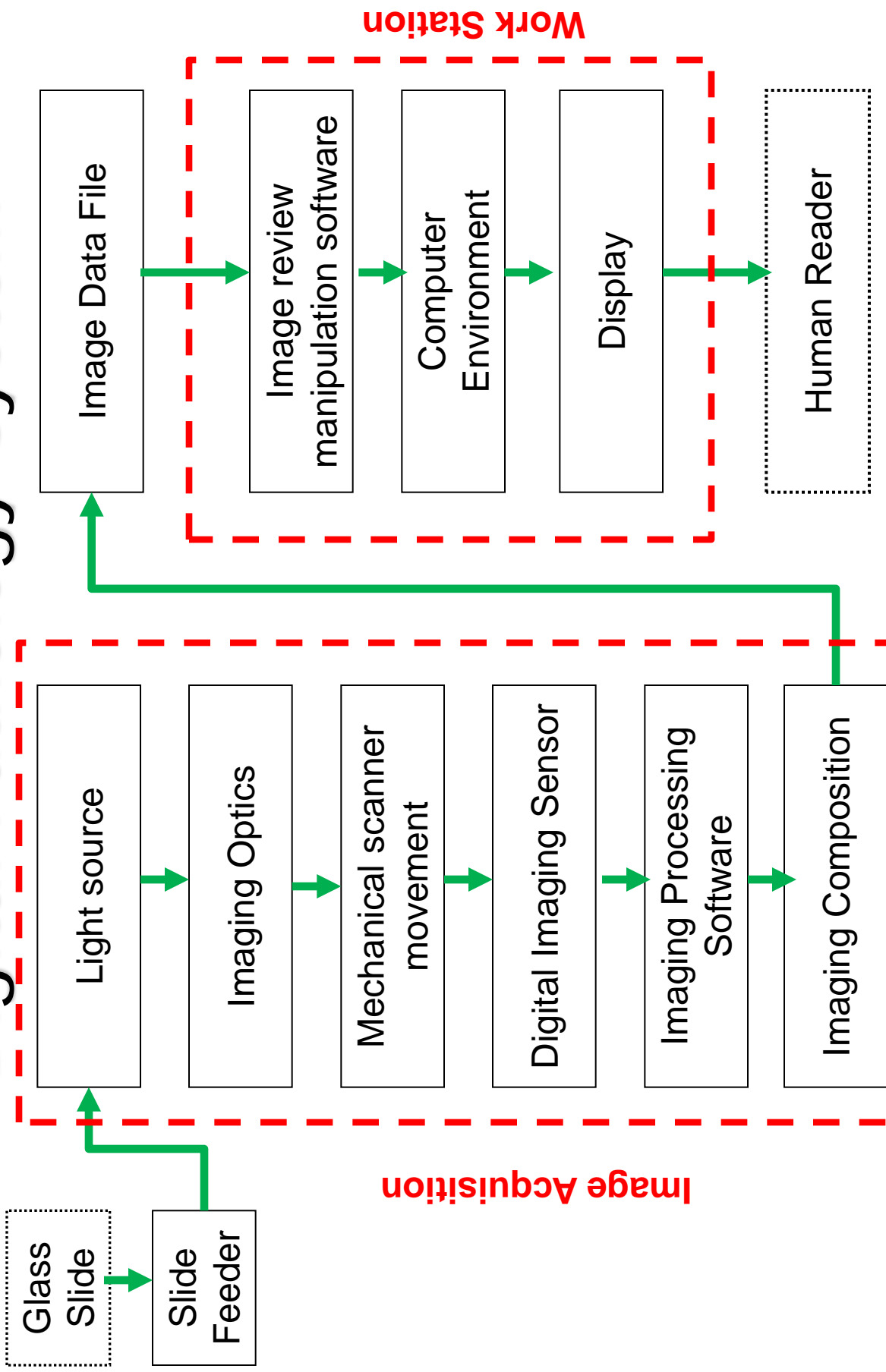
Please visit FDA website for device advice

Digital Pathology vs Virtual Microscopy

- Digital Pathology
  - A dynamic, image-based environment that enables the acquisition, management and interpretation of pathology information generated from a digitized glass slide

- Virtual Microscopy
  - Practice of converting glass slides into digital slides for reviewing on a computer screen, typically over a network
  - Scanning/zooming operations, optical resolution, visual magnification, and focus similar to a microscope

Digital Pathology System

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FDA

Work Station

Image Data File → Image review manipulation software → Computer Environment → Display → Human Reader

Image Acquisition

Light source → Imaging Optics → Mechanical scanner movement → Digital Imaging Sensor → Imaging Processing Software → Imaging Composition

Glass Slide → Slide Feeder

Whole Slide Image (WSI)

- Whole Slide Image (WSI)

  – A digitized histopathology glass slide created on a slide scanner

  – The digitized glass slide represents a high-resolution replica of the original glass that can then be manipulated through software to mimic microscope review and diagnosis

  – Also referred to as a virtual slide

- Whole Slide Imaging

  – The acquisition process of creating a virtual slide or whole slide image on a slide scanner

Intended Use of WSI Systems

- Intended Use – Intended for primary surgical pathology diagnosis in lieu of optical microscopy

  – Not an adjunct

- Indications for Use – Broad applications

  – Different organ systems

  – Different diseases/conditions/cases (e.g., simple vs complicated, common vs rare)

  – Different specimen types (e.g., cytology preps vs biopsies)

  – Different stains (e.g., H&E, special stains)

  – Different users (e.g., generalists vs specialists)

  – Different clinical settings (e.g., intranet vs internet access)

FDA Considerations for WSI Validations

- Components vs System
  - Technical assessment of individual components vs Characterization of integrated systems or subsystems

- Non-Clinical vs Clinical
  - Technical vs non-technical (e.g., human elements)

- Clinical Representation vs Statistical Power
  - Number of organs vs numbers of cases per organ
  - Number of readers vs number of cases
  - Consecutive/representative cases vs enrichment cases
    - Different organ systems or diseases/conditions
  - →Claims vs limitations

- Premarket vs Postmarket

Technical Assessment of WSI System

- Does the system function accurately and reliably in image acquisition and processing processes?

- Levels of Testing
  - Components
  - Integrated subsystems
  - Complete system

- Methodology of Testing
  - Test materials
  - Testing methods

- Product specifications and limitations

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Analytical Validation of WSI System

- Does the system output digital images accurately and reliably for interpretation in the hands of the intended users with various sources of variability?

  – Precision

  – Instrument-to-Instrument Reproducibility

  – Reader-to-Reader Reproducibility

  – Feature Studies

    • Accuracy and reproducibility in identification of histological features critical to diagnosis or differential diagnosis of diseases

10

Feature Study for WSI Validation

- Objective
  - Accuracy and precision of pathologist identification of a set of challenging histological features of interest using WSI

- Experiment Design
  - 20 histopathological features "in their natural environment" (e.g., psammoma bodies, tumor margins, micrometastases)
  - Each feature selected from ≥3 different organ systems
  - WSI scanning at a magnification consistent with the power at which the feature is typically identified by pathologists (40x or 60x).
  - Multiple (≥3) sites/scanners and readers

Clinical Validation of WSI System

- Does WSI system allow intended users to make diagnosis of surgical pathology specimens as accurately and reliably as optical microscopy?
  - Serious consequences to public health if misdiagnosis caused by suboptimal images

Clinical Study Design

- Overview
  - 4 Clinical study sites
  - 1 Scanner at each site → 4 scanners in total
  - 4 Readers (pathologists) at each site → 16 readers in total
    - Generalists vs specialists representative of intended use population
  - ~2,000 cases representing multiple organ systems
    - Single-slide cases (~1,500) vs multi-slide case

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Example List of Study Cases

EXAMPLE OF PROPOSED WSI STUDY BY ORGAN, DIAGNOSIS AND PROCEDURE:
(TOTAL 2000 CASES FOR THIS EXAMPLE)

We encourage the sponsor to include rare and unusual diagnoses (as many as 5%) in the larger (>100) groups

#oS = Number of Slides per Case; CNB = Core Needle Biopsy; TUR = Transurethral Resection; LEEP = Loop Electrosurgical Excision Procedure

ORGAN	# OF CASES	SUBTYPES (procedures)		#oS	Notes
BREAST	300		Benign/Atypical CNB	1	
		50	Benign/Atypical Lumpectomy	Multiple	
		50	In-Situ Carcinoma CNB	1	1 slide for CNB; 1-5 slides for Lumpectomy
		50	In-Situ Carcinoma Lumpectomy	Multiple	
		50	Invasive Carcinoma CNB	1	
		50	Invasive Carcinoma Lumpectomy	Multiple	
PROSTATE	300	120	Benign Core Bx	1	
		30	Benign Resection	Multiple	1 slide for Core Bx; More than 1 slide for Resection
		120	Adenocarcinoma Bx	1	
		30	Adenocarcinoma Resection	Multiple	
LUNG/BRONCHUS/Larynx/oral cavity/nasopharynx	100	25	Benign/Inflammatory Bx Only	1	1 slide for Bx; At least 1 of tumor and 1 of bronchial margin for Resection
		25	Dysplasia Bx Only	1	
		30	Carcinoma Bx	1	
		20	Carcinoma Resection	Multiple	
COLORECTAL	150	50	Benign/Inflammatory Bx	1	1 slide for Bx; At least 1 of tumor and 1 of margins for Resection (Nodes - consider excluding as nodes are tested separately)
		50	Adenomas Including Severe Dysp Bx	1	
		40	Adenocarcinoma Endoscopic Bx	1	
		10	Adenocarcinoma Resection	Multiple	
GE Junction	100	50	R/O Barrett's/Dysplasia Bx	1	1 slide for Bx
		50	Non-Neoplastic/Inflammatory Bx	1	

Clinical Study Design

- Overview
  - 4 Clinical study sites
  - 1 Scanner at each site → 4 scanners in total
  - 4 Readers (pathologists) at each site → 16 readers in total
    - Generalists vs specialists representative of intended use population
  - ~2,000 cases representing multiple organ systems
    - Single-slide cases (~1,500) vs multi-slide case
  - Each pathologist makes diagnosis of each case under optical microscope and WSI
    - Special stains slides, if available, may be provided upon request
  - Expert panel diagnosis or original signout as the truth
  - Primary Endpoint: Non-inferiority in diagnosis error rates

Risk Consideration for Digital Pathology

- Potential risks to patients vs mitigations

- Intended use
  - Primary diagnosis vs adjunctive
  - Screening in asymptomatic vs monitoring in diagnosed

- Representation of a glass slide
  - Area of coverage (e.g., FOVs vs complete scan)
  - Quality (e.g., color, depth, resolution)

- Degree of automation in quality controls and interpretation

- Degree of separation from glass slides

Risk-Based Device Classification

- Class I: Common, Low-Risk Devices
    - ➤General Controls
    - ➤Most exempt from premarket submission

- Class II: Moderate Risk Devices
    - ➤Special controls
    - ➤Premarket notification (510(k))
    - ➤Substantial equivalence to a predicate

- Class III: Complex, High-Risk Devices
    - ➤Premarket Approval (PMA)

17

Class III: High Risk

- Intended to support or sustain human life or prevent impairment of human health, or presents a potential unreasonable risk of illness or injury

- Serious harm to patients with an incorrect result from an IVD

  - Cancer screening tests
  - Cancer diagnosis
  - Oncology companion diagnostics

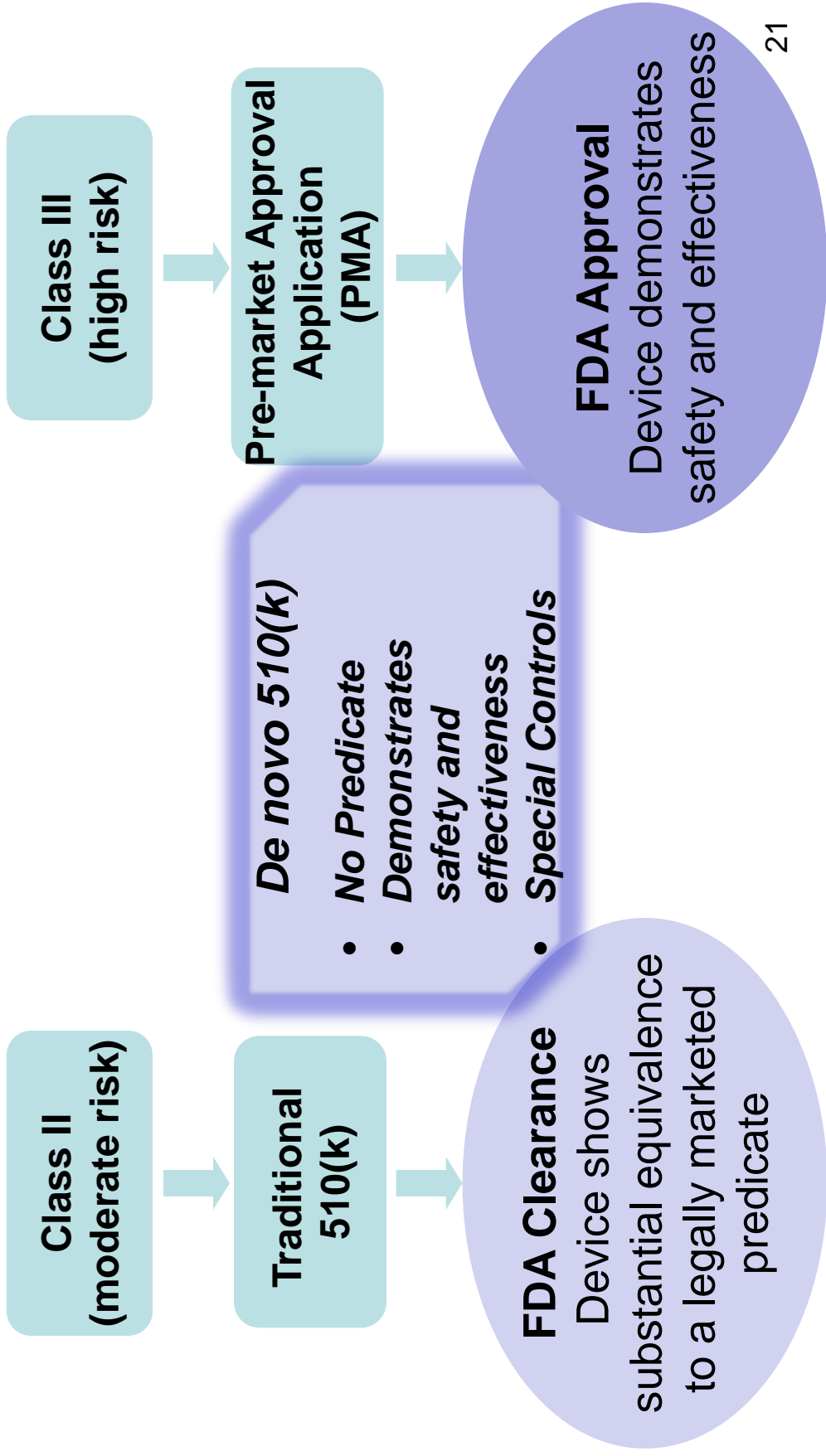- Premarket Application [PMA]

18

Cervical Cytology Screening Device

- Hologic ThinPrep® Imaging System (P020002): …assist in primary cervical cancer screening of ThinPrep Pap Test slides for the presence of atypical cells, cervical neoplasia, including its precursor lesions (LSIL, HSIL), and carcinoma as well as all other cytologic criteria as defined by 2001 Bethesda System: Terminology for Reporting Results of Cervical Cytology…

- BD FocalPoint™ Slide Profiler (P950009): …intended for use in initial screening of cervical cytology slides… identifies up to 25% of successfully processed slides as requiring no further review… also identifies at least 15% of all successfully processed slides for a second manual review…to detect slides with evidence of squamous carcinoma and adenocarcinoma and their usual precursor conditions….

- Class III (Procode: MNM)

19

Unclassified Devices

- Class III by default

- Can be Class I
  - If sufficient information exists to determine that the application of general controls are sufficient to provide reasonable assurance of the safety and effectiveness

- Can be Class II
  - If sufficient information exists to determine that the special controls would provide reasonable assurance of its safety and effectiveness

- 513(g) or de novo

Regulatory Processes

Class III (high risk) → **Pre-market Approval Application (PMA)** → **FDA Approval** — Device demonstrates safety and effectiveness

De novo 510(k)
- *No Predicate*
- *Demonstrates safety and effectiveness*
- *Special Controls*

Class II (moderate risk) → **Traditional 510(k)** → **FDA Clearance** — Device shows substantial equivalence to a legally marketed predicate

21

Special Controls

- Used to mitigate the risks to patients

- Sufficient information to establish Special Controls

  – Promulgation of performance standards

  – Development and dissemination of guidelines

  – Labeling requirements or other appropriate actions

  – Postmarket surveillance / Patient registries

  – For a device intended "for a use in supporting or sustaining human life, the Secretary shall examine and identify the special controls, if any, that are necessary to provide adequate assurance of safety and effectiveness and describe how such controls provide such assurance"

- De novo or Premarket Notification [510(k)]

22

WSI as a Candidate for *De Novo*

- Is there sufficient information for Special Controls?

  - Performance standards
    - Technical, non-clinical, clinical studies

  - Labeling requirements
    - Training
    - Subgroup analysis: Limitations of WSI?
    - Access to glass slides?

  - Postmarket surveillance
    - Postmarket studies, Patient registries?

  - Change controls
    - Component replacement: Technical specifications?
    - When to submit a new 510(K)?

Significant Changes or Modifications

- 21 CFR 807.81(a)(3) ….The following constitute significant changes or modifications that require a premarket notification:

(i) A change or modification in the device that could significantly affect the safety or effectiveness of the device, e.g., a significant change or modification in design, material, chemical composition, energy source, or manufacturing process.

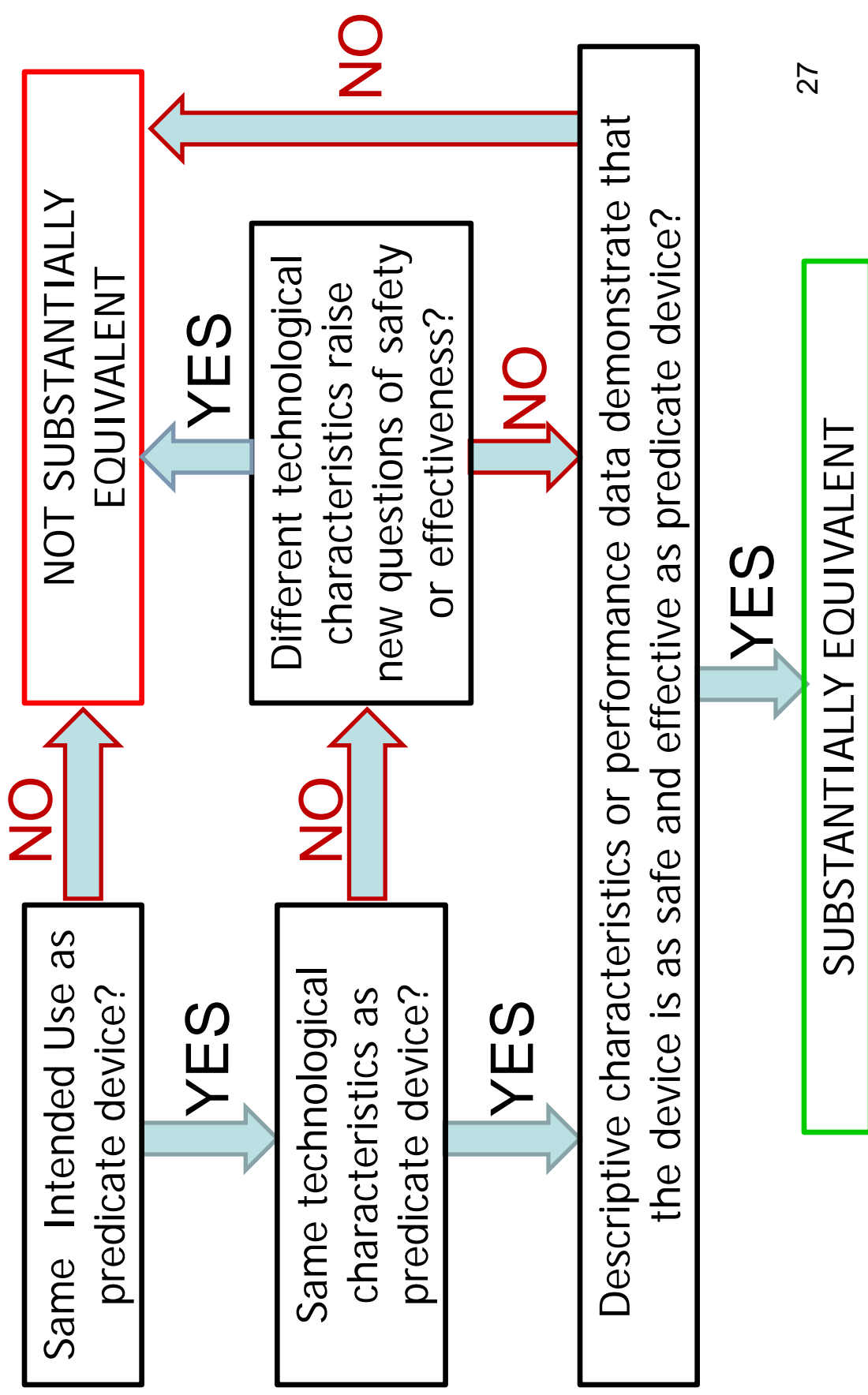(ii) A major change or modification in the intended use of the device.

Risk Assessments

- Use an accepted method of risk assessment (e.g., ANSI/AAMI/ISO 14971) for TPLC
  - Initiating hazards, failure modes, or circumstances
  - Sequences of events leading to a hazardous situation
  - Likelihood of such situations arising
  - Likelihood of the hazardous situations leading to harm
  - Nature of the harm that could result
- Considerations for Risk Assessments
  - Risk likelihood or probability
  - Risk severity
  - Device effectiveness

25

When to submit a 510(k)

- Is the same method or protocol, described in the previous 510(k), used to support the change?

- Does the change affect the use of the device?

- Does a risk assessment of the changed device identify any possible new or increased risks?

- Are clinical data necessary to evaluate safety or effectiveness for purposes of determining substantial equivalence?

- Do design verification and/or validation activities produce any unexpected issues of safety or effectiveness?

Substantial Equivalence (510(k))

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Same Intended Use as predicate device?

YES →

NO ↑ → NOT SUBSTANTIALLY EQUIVALENT

Same technological characteristics as predicate device?

YES →

NO ↑ → Different technological characteristics raise new questions of safety or effectiveness?

YES → NOT SUBSTANTIALLY EQUIVALENT

NO → Descriptive characteristics or performance data demonstrate that the device is as safe and effective as predicate device?

NO → NOT SUBSTANTIALLY EQUIVALENT

YES → SUBSTANTIALLY EQUIVALENT

Future Considerations

- Intranet, internet, mobile apps
  - Access vs fidelity vs confidentiality (cybersecurity)
- Computer-aided interpretation
  - Primary diagnosis vs adjunctive
  - FOVs vs WSI
- Clinical truth
  - No more diagnosis under optical microscopy
  - Sub-optical features
- Workflow
  - Record retention requirement vs storage/retrieval cost
  - Multiplex functionality and customized applications

Case Study: IHC Imaging Systems

- 21 CFR §864.1860 (Class II; Procode: NQN, NOT, OEO)

  – Imaging Devices for Digital Read/Imaging Analysis

  - GenASIs HiPath IHC Family (140957...)
  - Aperio ePathology eIHC IVD System/ScanScope® XT (k141109...)
  - Ventana Virtuoso™ System (k130515, k121516, k122143...)
  - BioImagene PATHIAM System with iScan (k080910)
  - ChromaVision Automated Cellular Imaging System (k032113)
  - Applied Imaging Ariol™ (k031715)

  – Assay kits

  - anti-HER2/neu (4B5, HercepTest™), anti-ER (SP1, 1D5), anti-PR (1E2, PgR 636), anti-Ki67 (30-9, MIB1), anti-p53 (DO-7)

  – Not all imaging devices cleared for use with all assay kits

A Pathologist's Struggle

"…As the breast marker analysis workflow is currently structured, pathologists would sign into the image analysis database in either RUO or IVD mode. Switching between modes requires re-logging in…

I sign out about 10 breast analysis cases a day. That means that I will have to log in to the system up to 20 times to analyze and sign the reports.

… Double logins will force pathologists to sign out all cases under RUO mode, in my opinion…

… not only do I have to login again to sign out the same case, but after I re-login, I have to search for the case from the list waiting in the cue. This takes time and is inefficient. Breast cancer cases are difficult to sign out correctly. The H&E needs to be carefully reviewed to look for normal ducts (this information should appear on the report, indicating the presence of an internal control). Areas of tumor need to be carefully selected for analysis. Stopping and starting signouts could mean not just a delay in signing out (due to human error), but important details in the H&E could be missed when evaluating subsequent stains. Matching up areas of interest is also important. I could easily see a situation where I would have to sign into a case a third time if I discovered an area of staining in an RUO antibody scan that should have been selected for the IVD antibody…"

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FDA "Open Channel" Concept

" …..we will allow a device manufacturer to create a partition for an end user to customize their IHC imaging analysis algorithm based on the parameters that the device manufacturer has fully validated. For instance, if and only if a device manufacturer receives FDA clearance for an algorithm that can detect and enumerate nuclear staining of an FDA-cleared IHC assay, may an "open channel" be made available for an end user to customize the algorithm for detection and enumeration of nuclear staining of an FDA-cleared or otherwise analytically validated IHC assay.   The report however should clearly state that the customized algorithm has not been cleared or reviewed by FDA and it should be apparent to the end user when they are working in the "open channel" environment (e.g., different colored background, etc.)…."

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Take Home Messages

- Digital pathology devices including WSI regulated by FDA based on intended use

- FDA has published the final guidance for technical assessment of WSI system for primary diagnosis

- FDA has outline WSI validation studies for sponsors
  - Clinical study to validate WSI for a broad intended use (i.e., primary diagnosis in lieu of optical microscopy)
  - Feature study to supplement non-clinical & clinical studies

- FDA is considering WSI as candidate for *de novo*

- FDA is proposing "open channel" for imaging devices

- Please consult FDA via the pre-submission process[2]

U.S. Food and Drug Administration
Protecting and Promoting Public Health

Resources

- Technical assessment of WSI system draft guidance
  - http://www.fda.gov/ucm/groups/fdagov-public/@fdagov-meddev-gen/documents/document/ucm435355.pdf
- Cybersecurity draft guidance
  - http://www.fda.gov/downloads/medicaldevices/deviceregulationandguidance/guidancedocuments/ucm356190.pdf
- CDRH device advice
  - http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/
- Pre-submission guidance
  - http://www.fda.gov/downloads/medicaldevices/deviceregulationandguidance/guidancedocuments/ucm311176.pdf
- Division of Industry and Consumer Education (DICE)
  - 800-638-2041/301-796-7100

Thank you

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Prognostic Markers for Canine Melanocytic Neoplasms: A Comparative Review of the Literature and Goals for Future Investigation

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What is This?

Prognostic Markers for Canine Melanocytic Neoplasms: A Comparative Review of the Literature and Goals for Future Investigation

R. C. Smedley[1], W. L. Spangler[2], D. G. Esplin[3], B. E. Kitchell[4], P. J. Bergman[5], H.-Y. Ho[4], I. L. Bergin[6], and M. Kiupel[1,7]

Abstract

Many studies have evaluated various prognostic markers for canine melanocytic neoplasms either as primary or secondary goals; however, design, methodology, and statistical validation vary widely across these studies. The goal of this article was to evaluate and compare published canine melanocytic neoplasm studies in relation to the principals established in the Recommended Guidelines for the Conduct and Evaluation of Prognostic Studies in Veterinary Oncology. Based on this evaluation, we determined which parameters currently have the most statistically supported validity for prognostic use in canine melanocytic neoplasia. This information can also be used as part of evidence-based prospective evaluations of treatment regimens. Additionally, we highlight areas in which the current data are incomplete and that warrant further evaluation. This article represents an initiative of the American College of Veterinary Pathologists' Oncology Committee and has been reviewed and endorsed by the World Small Animal Veterinary Association.

Keywords

canine, melanoma, prognosis

Melanocytic neoplasms are commonly diagnosed in dogs, and malignant melanoma is reportedly the most common canine oral malignancy.[3,13,15,16,36,44,47] Despite the prevalence of these neoplasms, one review states that "there is no single diagnostic technique capable of differentiating benign from malignant melanocytic neoplasms or of predicting survival time."[44] Another study stated that the "behaviour of melanocytic neoplasms is a continuous spectrum ranging from strictly benign to highly malignant. Thus, the borderline between the 2 categories may be rather broad."[37]

The primary foundation literature that describes the morphology and biological behavior of melanocytic neoplasms in dogs and cats begins with veterinary publications of the late 1950s and early 1960s. In these early studies, some of which were written by medical or dental pathologists,[17,33] the morphology of neoplasms was often described as being malignant or benign without specifically defining the anatomic basis for that distinction. The behavior of these neoplasms was similarly determined anecdotally from necropsy reports, but there was often no correlation between specific histological criteria of malignancy and the metastatic lesions observed.[7]

It has been an accepted observation that canine melanocytic neoplasms vary widely in biological behavior. Although many studies have evaluated various prognostic markers for canine

melanocytic neoplasms, either as primary or secondary goals, few true prognostic studies exist. Additionally, there are no universally accepted criteria to prognosticate canine melanocytic neoplasms. It is therefore unfortunate that potentially invalid conclusions of both early and current studies regarding prognosis of melanocytic neoplasms have become widely cited in routine neoplasia pathology practice. Such studies are referenced regardless of whether the conclusions are adequately

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supported by well-designed methods with sufficient case numbers, an appropriate reference population, specific inclusion and classification criteria, uniform modalities of therapy, adequate statistical analysis, and complete clinical outcome assessment, including survival data. Furthermore, it is often difficult to compare prognostic studies because of different classification systems, different groupings of neoplasms such as by location or inclusion of both canine and feline neoplasms, and different methodologies for evaluating specific parameters such as mitotic index (MI) or growth fraction. Most published reports are retrospective studies, and few validate outcome and survival conclusions with postmortem findings. Without an accurate prognosis, appropriate recommendations regarding primary and/or adjunct therapy for dogs with melanocytic neoplasms cannot be made.[19]

Prior to determining an accurate prognosis, an accurate diagnosis must be established. The lack of pigmentation and the tremendous cellular variability of some canine melanocytic neoplasms may pose significant challenges to the pathologist. Specific histological criteria such as junctional activity and/ or finding nests of neoplastic cells within the overlying epithelium can help support a diagnosis of an amelanotic melanocytic neoplasm;[43,44] however, ultimate confirmation requires positive labeling with immunohistochemical markers that have been shown to have high specificity for these neoplasms, such as Melan-A, PNL-2, and tyrosine reactive proteins 1 and 2.[8,9,12,25,36,41,43,44,46] Some prognostic studies have included amelanotic melanocytic neoplasms within a case series without providing proper evidence that these neoplasms are truly of melanocytic origin.[25,32,37] The accidental inclusion of soft tissue sarcomas or other neoplasms into a case series of melanocytic neoplasms may substantially alter the prognostic significance of the parameters under investigation.

In this work, we conducted a detailed literature review of canine melanocytic neoplasia publications and evaluated the different postulated prognostic classification schemes according to the recently published Recommended Guidelines for the Conduct and Evaluation of Prognostic Studies in Veterinary Oncology.[49] These guidelines were initiated by the ACVP Oncology Committee and reflect the current consensus opinion of veterinary pathologists and oncologists on how best to assess the prognostic classification systems used to characterize canine neoplasms. Based on these criteria, we determined which prognostic parameters have been demonstrated to have statistical significance according to these standards (Table 1). Additionally, we have provided recommendations for the prognostication of canine cutaneous and digit melanocytic neoplasms and for oral and lip melanocytic neoplasms (Table 2). Ocular melanocytic neoplasms will not be discussed owing to insufficient material.

The results and conclusions of each evaluated study are described under the relevant parameter headings, and the consensus significance of each parameter is summarized. The main categories of parameters include: signalment, clinical staging/ response to treatment, neoplasm location, gross morphologic features, histological features, and molecular biologic attributes.

Throughout this article, the term "melanocytoma" will be used to refer to a benign neoplasm of melanocytic origin, whereas the term "malignant melanoma" will be used to refer to a malignant neoplasm of melanocytic origin according to the current World Health Organization (WHO) nomenclature,[15] regardless of the terminology employed in the study being discussed. The term "melanocytic neoplasms" will be used when it is unknown whether the lesions are benign or malignant and when it is known that both benign and malignant neoplasms are being referenced together. Junctional activity refers to proliferation of neoplastic cells at the dermo-epidermal junction (Fig. 1).[15,16] The term "junctional activity" has often been used incorrectly to describe the presence of intraepithelial neoplastic cells. Intraepithelial neoplastic cells are a distinct histological feature of many melanocytic neoplasms, and the term "compound neoplasm" is used to describe a neoplasm that has both an epidermal (Fig. 2) and dermal component.[15,16] Dermal neoplasms are confined to the dermis.[15,16]

Signalment

Although many prognostic studies have recorded the breed of affected dogs, only one study has shown that breed may have some prognostic significance. This study reported that more than 75% of melanocytic neoplasms exhibited benign behavior in some breeds (Doberman Pinscher and Miniature Schnauzer), whereas in other breeds (Miniature Poodle), more than 85% of melanocytic neoplasms were malignant.[4] Both cutaneous and oral melanocytic neoplasms were included in that study.[4] Statistical analysis was not performed for this prognostic factor, however. In another report, Golden Retrievers, Labrador Retrievers, and Cocker Spaniels represented one-third of the dogs in the study, but there was no statistically significant difference in breed predisposition reported.[19]

Sex has not been demonstrated to be a prognostic factor for dogs with melanocytic neoplasms of any site. Whereas earlier reports state that male dogs have a higher frequency of melanoma than female dogs,[44] several more recent studies have not reported any significant sex differences in terms of survival.[1,2,11,19,20,28,29,32,35,40,42,45]

In general, it would appear that malignant melanocytic neoplasms are more common in older dogs. The mean age of dogs with benign melanocytic neoplasms was 8.1 ($n = 86$) years, whereas the mean age of dogs with malignant melanocytic neoplasms was 11.6 ($n = 71$) years in one study.[4] However, patient age does not have definitive prognostic significance. A few studies have reported that dogs with oral malignant melanomas are generally older than dogs with cutaneous malignant melanomas,[31,42] but age has not been shown to be correlated with survival for oral neoplasms in most studies.[2,19,29,31,35,42,45] One study stated that age negatively influenced survival of dogs with melanocytic neoplasms arising from the skin, feet, and lips.[45] Age is a difficult prognostic factor to evaluate, as older dogs are more likely to suffer intra- and postoperative complications and slower recovery from surgery. Older animals are also more likely to have additional life-limiting comorbid conditions. One report

Table 1. Significance of Prognostic Factors for Canine Melanocytic Neoplasms Based on Location

Prognostic Factor	Oral Neoplasms	Lip Neoplasms	Digit Neoplasms	Other Cutaneous Neoplasms
Signalment	No	No	No	No
Weight of dog	No	No	No	No
Stage of disease	Yes	NE	NE	NE
Lymph node metastasis	No[c]	NE	NE	NE
Distant metastasis	Yes	Yes	Yes	Yes
Size of neoplasm	Possible	Possible	Possible	Possible
Symmetry of neoplasm	IN	IN	IN	IN
Morphologic classification (benign vs malignant)	Variable[b]	Variable[b]	Variable[b]	Variable[b]
Nuclear atypia	Yes	Yes	Yes	Yes
Mitotic index	Yes	Yes	Yes[a]	Yes
Cell type	No	No	No	No
Cellular pleomorphism	No	No	No	No
Degree of pigmentation	Yes	Yes	Yes[a]	Yes
Junctional activity	No	Yes	Yes	Uncertain
Intraepithelial neoplastic cells	No	No	No	No
Ulceration	No	NE	Yes[a]	Yes
Level of infiltration/invasion	Possible	NE	Yes[a]	Yes
Lymphatic invasion	Yes	NE	NE	Yes
Necrosis	Possible	Possible	Possible	Possible
Inflammation	Possible	Possible	Possible	Possible
Completeness of excision	IN	IN	IN	IN
Ki67 index	Yes	Yes	Yes[a]	Yes
Proliferation index	IN	IN	IN	IN
Expression of Melan-A, S-100, vimentin, NSE	No	No	No	No
DNA ploidy	Possible	NE	NE	Possible
MCC and MVD	IN	IN	IN	IN
Plasma VEGF	IN	IN	IN	IN
Response to treatment	IN	IN	IN	IN

[a] Only 12-digit neoplasms were included in the Laprie et al. study, and they were grouped together with the other cutaneous neoplasms.
[b] Dependent on which classification system used.
[c] Only limited data available.
Abbreviations: IN, insufficient data available; NE, site not specifically examined; Possible, limited data support that this factor has prognostic significance for this location.

stated that age significantly affected survival of dogs with malignant oral neoplasms of various cell lines of origin, including malignant melanomas, that were treated by partial mandibulectomy ($P < .001$).[27]

There is no evidence to suggest that the weight of the affected dog has any prognostic significance.[19,35]

Clinical Staging and Response to Treatment

Stage of disease has been shown to be significantly associated with clinical and survival outcome for dogs with oral malignant melanoma.[19,20,29] In one study, the authors staged dogs with oral malignant melanomas according to the then-current WHO system for oral neoplasia and found no statistically significant difference for remission length or survival times using this method.[19] These authors proposed an alternative system for staging of canine oral malignant melanomas based on neoplasm size, location within the oral cavity, and mitotic index.[19] They reported statistically significant differences in remission length and survival time for different stages using this alternative system.[19]

It seems intuitive that evidence of metastasis would indicate a poor prognosis. Perhaps this is the reason many studies have reported metastatic rates, but very few have adequately evaluated it as a prognostic factor. In one study, metastasis was a significant negative determinant of patient survival for all neoplasm locations.[45] Another study reported that for oral malignant melanomas, the absence of distant metastasis at the time of surgery was statistically significant for longer remission lengths and survival times; however, it was found to be of little consequence, as these substrata were overrepresented in their respective groups.[19] Only 2 studies specifically examined regional lymph node metastasis as a prognostic factor.[19,35] Unlike distant metastasis, regional lymph node metastasis had no prognostic value for remission length, "time to first event," or survival time ($P > .05$) for dogs with oral "malignant" melanomas.[19,35] It should be noted that only neoplasms histologically designated as malignant were included in these latter 2 studies, thus there was no reference population.

Based on these data, it can be concluded that evidence of visceral metastasis is associated with a poor prognosis for canine melanocytic neoplasms of any site. Additional studies that include benign and malignant melanocytic neoplasms from various

Table 2. Recommendations for Prognostication of Canine Melanocytic Neoplasms

Location	Oral/Lip Melanocytic Neoplasms		Cutaneous/Digit Melanocytic Neoplasms
Distant metastasis	Poor prognosis		Poor prognosis
Lymphatic invasion	Poor prognosis		Poor prognosis[a]
Mitotic index	10 consecutive fields starting in area w/highest mitotic activity		10 random fields
	Avoid areas of ulceration for both methods		
	< 4/10 hpf	Favorable prognosis	< 3/10 hpf
	≥ 4/10 hpf	Poor prognosis	≥ 3/10 hpf
Nuclear atypia[b]	% atypical nuclei in 200 cells counted		Subjective assessment
	< 30%	Favorable prognosis	< 20%
	≥ 30%	Poor prognosis	≥ 20%
Degree of pigmentation	Subjective assessment		
	% Pigmented cells	Scale 0 (no pigment) to 2 (high pigment)	
	≥ 50%	Favorable prognosis	2
	< 50%	Uncertain prognosis	0 to 1
Presence of ulceration	No prognostic significance		Poor prognosis
Level of infiltration/invasion	Shallow w/no bone lysis	Favorable prognosis	Limited to dermis
	Deep w/possible bone lysis	Poor prognosis	Extends beyond dermis
Ki67 index	Average number of positive nuclei per grid (5 hpf grid areas counted)		% of positive nuclei in 500 cells counted
	Avoid areas of ulceration and inflammation and assess highest staining areas for both methods		
	< 19.5	Favorable prognosis	< 15%
	≥ 19.5	Poor prognosis	≥ 15%

[a] Parameter was not specifically examined for neoplasms of the digit.
[b] Parameter should be assessed in epithelioid predominant neoplasms and in spindloid neoplasms with sufficiently observable nuclear detail.

anatomic sites are needed in order to make definitive conclusions regarding lymph node metastasis as a prognostic factor.

It is difficult to draw sound conclusions regarding the effectiveness of various treatment modalities for canine melanocytic neoplasms for 2 main reasons. First, few clinical studies provide adequate description of the histological criteria used to diagnose a neoplasm as malignant. Neoplasm location alone should not be used to classify a melanocytic neoplasm as being malignant or benign. As discussed in the following, a subset of oral and lip melanocytic neoplasms has been identified that do not exhibit malignant behavior.[2,10,45] Thus, attempts to separate these neoplasms from true malignant melanomas must be performed before treatment strategies can accurately be evaluated. Without knowing the criteria used to categorize the neoplasms as malignant, it is not possible to accurately interpret the survival data. Second, many clinical studies have staged dogs based on the WHO recommended guidelines and compared historically published survival times for each stage with the survival times of the dogs under study in a given case series. Based on this system, stage I dogs have neoplasms that are < 2 cm in diameter, stage II dogs have neoplasms that are 2 to < 4 cm in diameter, stage III dogs have

neoplasms that are ≥ 4 cm in diameter and/or there is evidence of lymph node metastasis, and stage IV dogs have evidence of distant metastasis. Extreme caution must be used when historically published survival times are used as comparators, because each study set of neoplasms can be extremely different, and, as stated above, many studies have not used strict histological criteria to select cases for inclusion. The location, histological features, and clinical parameters, in addition to the overall quality of the study, all need to be considered when choosing an appropriate reference set of melanocytic neoplasms for comparison. For example, in a study by Esplin, the mean survival time for dogs with oral or lip melanocytic neoplasms was 22.7 months, but this study included only histologically well-differentiated melanocytic neoplasms.[10] It would be erroneous to use this mean survival time for statistical analysis in a study set of neoplasms that included any melanocytic neoplasm other than histologically well-differentiated melanocytic neoplasms from the oral cavity or lip. Another concern with studies that use stage to classify cases is whether or not the staging classification system really has prognostic significance. As discussed above, Hahn et al. reported that there was no statistically significant difference for remission length or survival time using the
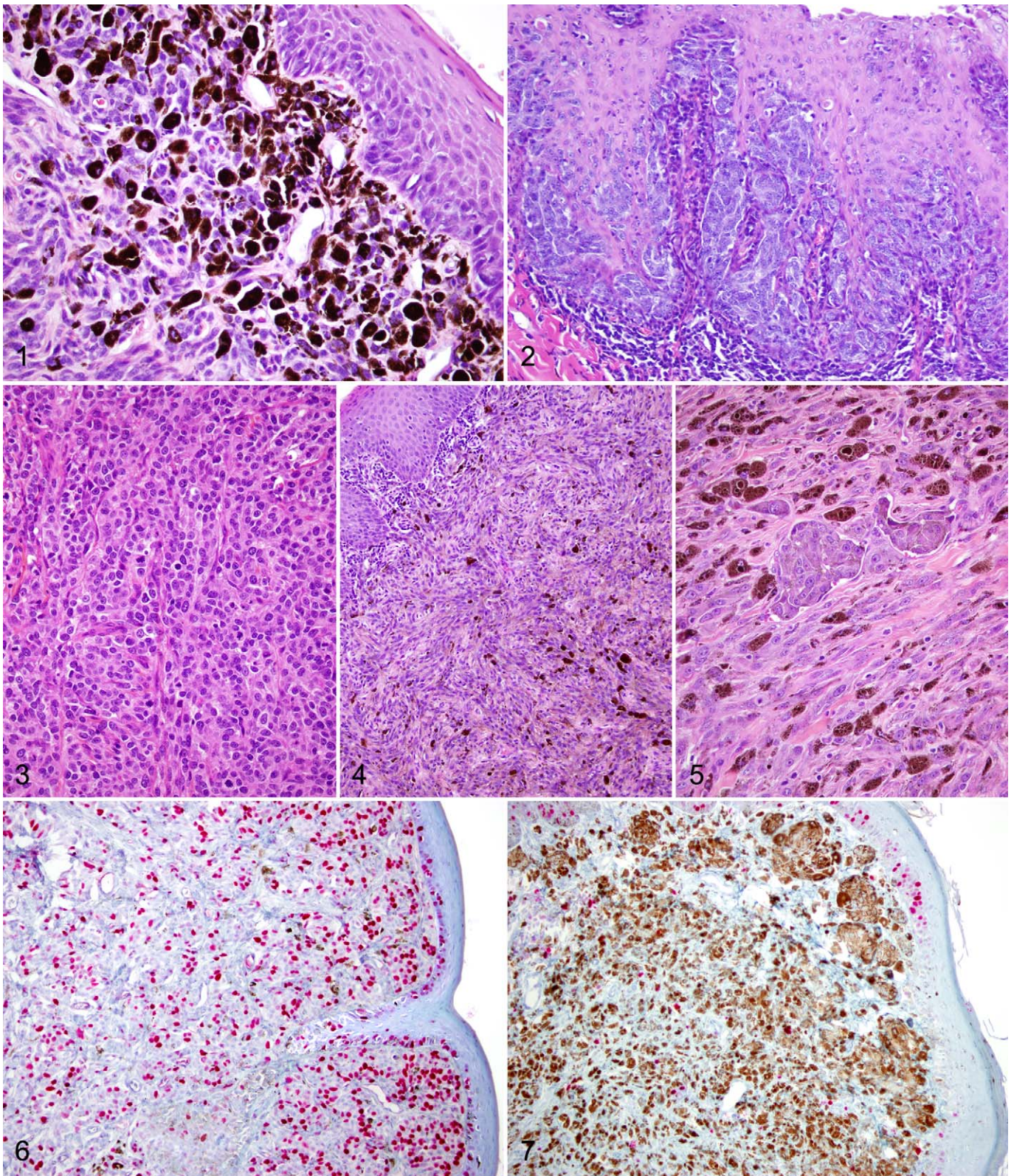
Figure 1. Canine oral melanocytic neoplasm. Pigmented neoplastic melanocytes extend to the dermo-epidermal junction. Hematoxylin and eosin. **Figure. 2.** Canine oral amelanotic melanocytic neoplasm. Intraepithelial nests of neoplastic melanocytes are present within the mucosal epithelium. Hematoxylin and eosin. **Figure 3**. Canine oral amelanotic melanocytic neoplasm, epithelioid variant. Neoplastic melanocytes are polygonal/epithelioid and arranged in cords and nests. Hematoxylin and eosin. **Figure 4**. Canine oral melanocytic neoplasm, spindloid variant. Neoplastic melanocytes are spindloid and arranged in streaming bundles. Hematoxylin and eosin. **Figure 5**. Canine oral melanocytic neoplasm,

WHO staging system for oral melanocytic neoplasms, but that an alternative staging system did have prognostic significance.[19] Additionally, the WHO staging system for oral melanocytic neoplasms is based largely on primary neoplasm size, which has not definitively been proven to have prognostic significance. Also, as discussed by Bergman (2007), the size of the neoplasm has not been standardized to the size of the patient and histological criteria are not incorporated into this system.[3] For these reasons, well-designed prospective studies with standardized clinical staging, histopathologic diagnoses, treatment protocols, follow-up, and statistical evaluation are greatly needed to accurately evaluate various treatment modalities for canine melanocytic neoplasms of various body sites.[47]

Neoplasm Location

Perhaps more than any other parameter, neoplasm location has been historically relied on to predict the biological behavior of melanocytic neoplasia. Numerous studies have evaluated location in this context.[1,2,4,5,10,19,20,28,31,32,36,37,42,45] Historically, melanocytic neoplasms of the oral cavity and lip have been considered to have a poor prognosis and neoplasms of the skin have been considered to have an overall favorable prognosis.[3,4,5,19,36,44] The statement that all oral melanocytic neoplasms should be considered malignant is commonly found throughout the literature and has become dogma.[3,4,5,13,14,36,44] Although numerous studies have indeed reported that a high percentage of melanocytic neoplasms from the oral cavity exhibit malignant behavior,[4,5,31,32] generalized prognostic statements based on neoplasm location have been refuted by more recent studies that have identified a subset of oral and lip melanocytic neoplasms with a more favorable prognosis.[2,10,42,45]

Melanocytic neoplasms of the oral cavity had the shortest median survival time (147 days) when compared with those of the feet and lips (676 days) and to those from the skin (725 days) in one study.[45] However, whereas 92% of the oral melanocytic neoplasms had been classified as malignant in the original biopsy report, metastasis or recurrence was observed in only 59% of the cases.[45] Case follow-up intervals for this study ranged from 12 months to 4 years. Independently, another study demonstrated that heavily pigmented oral and lip neoplasms composed of well-differentiated melanocytes with a low mitotic index of ≤ 3 per 10 high-power fields (hpf) had a favorable prognosis.[10] Ninety-five percent of dogs with neoplasms meeting these criteria either survived the duration of the study period (51 months) or died of other causes (23.4 months mean survival time).[10] The risk of neoplasm-associated death was the same for melanocytic neoplasms of the lip as compared with lesions at other locations within the oral cavity.[10] In a third study, approximately one-third of dogs with melanocytic neoplasia of the lip survived at least 1 year after surgical removal, despite the majority of neoplasms having a malignant histological appearance, which was not specifically defined.[42] Of dogs with ''benign''-appearing melanocytic lip neoplasms, 9 of 10 (90%) survived at least 1 year in that study.[42] A major difficulty in comparing studies that have examined melanocytic neoplasms of the lip is that many authors do not differentiate between lip neoplasms that arise from the haired skin and those that arise from the mucosa. Differences in survival times may be a result of the type of lip neoplasms (haired skin vs mucosa) present in the various studies.

For cutaneous melanocytic neoplasms, digital neoplasms have been considered to have a worse prognosis than those from elsewhere on the skin. Neoplasms of the digit had a lower 2-year survival rate (56%) when compared with lesions of other cutaneous sites (83.8%) in one study; however, this difference was not significant because of the small number of cases evaluated.[28] Others have independently reported 1-year survival rates for digital neoplasms of 44%[21] and 42%[30] and 2-year survival rates of 11%[21] and 13%.[30] In another study, 74% of melanocytic neoplasms of the feet and lips had originally been reported to be malignant, but only 38% of those neoplasms actually demonstrated malignant behavior.[45] On the other hand, 59% of melanocytic neoplasms of the skin were originally reported as malignant, but only 12% actually exhibited malignant behavior.[45] There was also a subset of cutaneous melanocytic neoplasms that exhibited malignant behavior that would have been predicted to be benign based on current microscopic criteria for prognostication.[45] As a consequence of low mortality among dogs with cutaneous neoplasms, the positive predictive value of the mathematical model used by the authors was a ''virtual coin toss (54.5%),'' suggesting that additional prognostic factors should be sought when evaluating cutaneous melanocytic neoplasms.[45] Since amelanotic melanocytic neoplasms were excluded from the study, the relative percentages of benign melanocytic neoplasms at each of the anatomic sites may be higher in this particular study population than that of the general population. In contrast, one study found that the prognosis following histologic diagnosis of a malignant melanoma was considered poor, regardless of the primary neoplasm site, although only 20 dogs were included in this study.[37] In an additional study, 45.8% of dogs (11 of 24) with cutaneous and 42.9% of dogs (6 of 14) with nailbed melanocytic neoplasms survived at least 1 year after surgical removal of the neoplasm.[42] In another study, although a higher proportion of neoplasms on the feet were histologically malignant, the behavior of malignant neoplasms on the foot also did not vary greatly from

Figure 5 (continued). mixed variant. Both neoplastic spindloid melanocytes arranged in streaming bundles and nests of neoplastic epithelioid melanocytes are present. Hematoxylin and eosin. **Figure 6** Canine oral poorly melanotic malignant melanoma. A large number of neoplastic melanocyte nuclei show positive nuclear labeling for Ki67, consistent with a high growth fraction. Immunohistochemistry using an alkaline phosphatase detection system with a vector red chromogen, counterstain, hematoxylin. **Figure 7**. Canine cutaneous melanocytoma. A small number of neoplastic melanocyte nuclei show positive nuclear labeling for Ki67, consistent with a low growth fraction. Immunohistochemistry using an alkaline phosphatase detection system with a vector red chromogen, counterstain, hematoxylin.

that of lesions arising from other skin locations.[5] However, the exact location on the foot (eg, digit, nail bed, pad, dorsal surface) was not indicated in this report.

Taken as a whole, these results support that anatomic location is indeed an important and integral prognostic parameter for canine melanocytic neoplasia. Melanocytic neoplasms in and around the mouth are, more often than not, aggressive in their nature, although a subset of oral and lip melanocytic neoplasms exhibits benign behavior. In haired skin, excluding feet, the proportion of benign-to-malignant melanocytic neoplasms is reversed when compared to oral neoplasms. However, cutaneous neoplasms with malignant behavior are more difficult to distinguish histologically from benign neoplasms than are oral or lip neoplasms. The real and current challenge for pathologists examining canine melanocytic neoplasms is the accurate and consistent recognition of benign oral neoplasms and malignant cutaneous ones.

Gross Morphologic Features

There is conflicting information in the current literature regarding the prognostic significance of the size of melanocytic neoplasms. In one study that included canine melanocytic neoplasms from various anatomic sites, increasing neoplasm size or volume was a significant negative determinant of patient survival.[45] Two additional studies found a negative correlation between increasing neoplasm volume and survival time in dogs with oral malignant melanomas.[19,35] This parameter was significantly related to "time to first event," development of pulmonary metastasis, and survival time in a set of 111 canine oral malignant melanomas based on univariate and multivariate Cox regression analysis.[35] Hahn et al. reported that dogs with oral malignant melanomas of less than 8 cm³ had significantly longer remission lengths and survival times.[19] Again, neither of these 2 studies included a reference population. Several other studies have not identified a correlation between lesion size and clinical outcome.[5,20,27,31,37,42,48] Because of the conflicting reports, the practical value of this parameter as a prognostic indicator is still questionable. Further evaluation of this parameter is required, using a statistically determined threshold value for comparison to clinical outcome and survival data.

Symmetry has not been assessed as a prognostic factor for canine melanocytic neoplasms. Aronsohn et al. recorded the symmetry of the lesion for 14 melanocytomas of the distal extremities but did not assess this parameter for the 14 malignant melanomas reported in that study.[1] No conclusions were made with regard to prognosis.

Histological Features

The histological characteristics of benign and malignant melanocytic neoplasms in dogs were defined in detail in 1974 in the WHO International Histological Classification of Tumours of Domestic Animals.[50] Early reports were often descriptive and lacked correlation of histological features with clinical outcome. Consequently, there are conflicts in the literature when these descriptive criteria are used to predict outcome. Some reports show high correlation between histological appearance and clinical outcome,[4,28,37,47] whereas others state that histological appearance is of no prognostic value,[20] and a few studies have shown variable results.[5,42] A major complicating factor is that histological evaluation of atypia is somewhat subjective. Traditionally, the mitotic index is the only histological parameter to which a numerical value is assigned. Some argue that even mitotic index is not totally objective owing to variations in field selection, size and number of fields, number of counted cells, degree of pigmentation, or whether bleaching was performed. Another challenge is that histological features are sometimes combined with nonhistological features, such as neoplasm size or location, when assessing neoplasm behavior, which makes it difficult to determine the relative contribution of histological appearance to the predictive accuracy. Here we review the following histological parameters for their ability to predict prognosis of canine melanocytic neoplasms: morphologic classification (also referred to in the literature as histological diagnosis or cytologic features); nuclear atypia; predominant cell type (eg, spindloid, epithelioid, mixed); cellular pleomorphism; mitotic index; degree of pigmentation; junctional activity; intraepithelial neoplastic cells; ulceration; level of infiltration or invasion; necrosis; inflammation; and margin evaluation.

Morphologic Classification

Many studies use morphologic criteria to classify melanocytic neoplasms as benign or malignant. Although morphologic classification appears to be helpful in predicting prognosis, different classification schemes are often used in different studies, making comparisons difficult. Most studies have evaluated the prognostic significance of individual criteria, such as nuclear atypia or cellular pleomorphism, among other features, whereas some studies have evaluated these features under a broader assessment of "morphologic classification."[4,5,20,28,37,42,47]

An early report used multiple criteria described in a previous study to confirm malignancy in a set of 121 oral and pharyngeal melanocytic neoplasms.[7,47] These features were presence of pigment, degree of tissue infiltration, neoplasm-cell emboli in vessels, and metastatic deposits in regional lymph nodes. Nuclear pleomorphism and abundant mitoses, although the specific numbers were not given, were present in almost every case.[47] This study appeared to profile highly aggressive melanocytic neoplasms, as 52 of 54 necropsied dogs (96%) displayed evidence of metastasis or local recurrence.[47] Thus, it was not designed to evaluate the discriminatory power of these parameters between benign and malignant neoplasms. Additionally, details regarding classification criteria were not provided, the univariate significance of individual prognostic factors was not considered, and no statistical analysis was performed.

One study histologically classified melanocytic neoplasms as malignant or benign according to the WHO classification system available at that time.[5,50] This classification was based on criteria established in human medicine that had not yet been

shown to be prognostically significant in dogs. Fifty-nine dogs with histologically benign neoplasms had a median survival time of 110 weeks, and within a 2-year follow-up period, 7 of these 59 (12%) dogs died.[5] Seventy-five dogs with histologically malignant neoplasms had a median survival time of 30 weeks, and in the same 2-year observation period, 53 of these 75 (71%) dogs died.[5] The study was unable to correctly classify a small number of neoplasms (about 10%) that appeared histologically benign but exhibited malignant behavior.[5] Furthermore, the median survival time of dogs with histologically malignant cutaneous melanomas was 70 weeks, with only 15 of 33 (45%) dogs dying from the neoplasm within a 2-year period.[5] This result raises a question about the validity of this classification to correctly identify malignant melanomas of the skin. When the same classification system was used in another study of 169 melanocytic neoplasms from various locations, the authors reported an overall accuracy rate of 89% to correctly identify the biological behavior based on the morphologic classification.[4] Nevertheless, 14 neoplasms were behaviorally benign but histologically malignant. Eight of these lesions arose in skin (4 from the digit), 4 were ocular, and 2 were of the oral cavity. These neoplasms reportedly exhibited at least 1 histological criterion of malignancy, which was usually cellular pleomorphism with or without a high mitotic index.[4]

In another study, melanocytic neoplasms were classified as malignant or benign based on cell size and shape, nuclear size and shape, chromatin pattern, prominence of nucleoli, and lack of pigment.[42] These features were subjectively assessed. The study included 27 histologically malignant nail bed neoplasms, 58 histologically malignant skin neoplasms, and 80 lip neoplasms that were a mix of both histologically benign and malignant lesions. One-year outcomes were known for 84 cases (46/80 lip neoplasms, 14/27 nail bed neoplasms, and 24/58 haired-skin neoplasms). Thirty-two dogs had lip neoplasms that were histologically classified as malignant, yet 10 of these 32 (31%) dogs survived at least 1 year. Fourteen dogs had lip neoplasms that were histologically classified as benign. Of this group of dogs, 10 had lesions arising from the mucous membrane and 4 were from the haired skin. Twelve of these 14 dogs were neoplasm free for at least a year. Almost half of the dogs with histologically malignant skin or nail bed neoplasms survived over 1 year after neoplasm removal. The author concluded that traditional histological criteria of malignancy are useful, but a single feature or features correlating with outcome could not be identified.[42] Regression analysis was not performed in that study.

Regression analysis was performed in another study that classified neoplasms based on features of cellular pleomorphism and atypia, defined only as nuclear pleomorphism, nuclear hyperchromasia, mitotic figures, and microscopic infiltrative nature, which were used as cytologic features of malignancy.[37] The authors used an empirically determined cutoff point of more than 2 mitoses per 10 hpf to differentiate benign from malignant neoplasms. This histological classification method significantly correlated with survival ($P < .001$).[37] However, the study population was small (27 total neoplasms from various locations) and included both dog and cat cases that were not separated from one another for statistical assessment. Neoplasms were not separated by anatomic location, and the follow-up time to determine survival was only 6 months. Additionally, this study combined mitotic index and infiltrative nature in the histological classification, making it difficult to compare the results of this study to other publications.

Histological classification, based largely on mitotic index, correctly predicted the biological behavior in 63 of 68 cutaneous neoplasms (93%) in another study.[28] Dogs with histologically benign neoplasms had a statistically significant longer 2-year survival rate than dogs with malignant neoplasms ($P < .0001$).[28]

Nuclear Atypia

Since classification by nuclear atypia relies on observer assessment and not a quantitative measure, highly specific and defined criteria for atypia are very important to maintain reproducibility and minimize inter-observer variation. When assessed by strict criteria, nuclear atypia was highly correlated with outcome for canine melanocytic neoplasms arising at any anatomic site in pigmented epithelioid-predominant neoplasms.[45] Furthermore, a specific threshold value could be statistically determined.[2,45] Well-differentiated neoplastic cells are defined as having a small nucleus with a single, centrally oriented nucleolus and minimal clumping of chromatin.[45] Condensed strands of nuclear chromatin commonly extend from the nucleolus to the nuclear membrane, with condensation of chromatin along the inner surface of the membrane. In sections of cells lacking a nucleolus, the chromatin is fine and evenly dispersed at the periphery of the nucleus. More undifferentiated neoplastic cells are characterized by larger nucleoli of less regular shape.[45] They are eccentrically located in the nucleus and often multiple. In some cases, multiple nucleoli haphazardly connect to the inner surface of the nuclear membrane by thin strands of chromatin and give the appearance of a coarsely vacuolated nucleus.[45] In the defining study, nuclear atypia was subjectively evaluated on a decile scale from 1 to 10, representing the estimated percentage of nuclei involved (ie, 0 = no nuclear atypia, 1 = 1-9% involved nuclei, 2 = 10-19% involved nuclei, 3 = 20–29% involved nuclei, and beyond, using this ordinal scale to a final score of 10.).[45] A variation on this method was used by Bergin et al., who used the same criteria semiquantitatively by assessing atypia in 200 counted cells within each oral and lip neoplasm.[2] This method was applied to all epithelioid and mixed neoplasms as well as to 4 of 5 spindloid variants that had sufficiently observable nuclear detail.[2] The 2 studies had comparable results but established slightly different threshold values for malignant behavior. One determined a threshold of ≥ 5 ($\geq 40\%$)[45] and the other determined a threshold of ≥ 4 ($\geq 30\%$) for oral melanocytic neoplasms.[2] This variation may be a result of remaining interobserver differences and slightly different statistical methods for establishing a threshold. When classified by nuclear atypia, 86.3% of the oral and lip melanocytic neoplasms were correctly classified with respect to outcome at 1 year.[2] Prognostic evaluation based on both nuclear atypia and MI had an overall correct behavioral classification of

81% for melanocytic neoplasms of the feet and lips.[45] A statistically predictive model using a nuclear atypia cutoff of >3 (> 20%) gave a sensitivity of 80%, a specificity of 94.4%, a positive predictive value of 54.5%, a negative predictive value of 98.2%, and an overall correct classification of 93.3% for cutaneous melanocytic neoplasms.[45]

Despite these results, nuclear atypia scored in increments of 10 is time consuming to assess and still potentially susceptible to interobserver variation. Assessing the percentage of nuclei exhibiting atypia in 200 counted cells is somewhat more objective and less time consuming, but still perhaps too rigorous for routine diagnostic pathology. It remains to be seen whether the same criteria can be applied in a more subjective way (assessment as mild, moderate, severe) and still retain comparable sensitivity and specificity. An additional drawback is the inability to define nuclear atypia criteria in neoplasms with insufficient nuclear detail, such as those predominantly composed of spindle, whorled type, or signet-ring cells.[45]

One study specifically evaluated anisokaryosis as a prognostic factor for cutaneous melanocytic neoplasms and found that it was associated with a significantly shorter survival time ($P < .0001$).[28] Anisokaryosis was determined subjectively on a scale of 1 to 4, 1 being low and 4 being marked, thereby making it difficult to reproduce the reported results. Three additional studies of oral and/or cutaneous neoplasms did not find a correlation between nuclear atypia and neoplasm behavior.[31,40,42] Possible reasons for this discrepancy include a low number of cases, a lack of neoplasms with a favorable outcome in the dataset, and less defined criteria for atypia.

Mitotic Index

MI is one of the factors that has been most commonly evaluated for prognostic utility in canine melanocytic neoplasia.[1,2,5,10,19,20,28,31,36,40,42,45] It has been shown to be a useful prognostic factor for melanocytic neoplasms of both the skin and oral cavity, including the lip,[2,5,19,31,40,45] although it does not appear to be as useful as nuclear atypia or growth fraction.[2,31,45] MI has been defined in many different ways, including: the average number of mitotic figures per hpf with variable numbers of fields counted, a range of numbers of mitotic figures per hpf (eg, 0-2 mitoses per hpf), and the number of mitotic figures per 10 hpf. MI has also been assessed in different ways, such as the number of mitotic figures counted in a certain number of random fields, or in a certain number of fields within an area of high mitotic activity. This variability in reporting criteria makes it difficult to compare results. When determining the mitotic index in 2 studies, areas underlying ulceration were avoided,[2,28] and in 3 other studies an MI of 0 was assigned to all areas where the nuclei were obscured by pigment.[5,10,19] Assigning an MI of 0 to heavily pigmented areas was supported by identification of no to rare mitotic figures in bleached samples of such areas in one study.[10] To avoid invalid comparisons, it is critical for the reader to pay close attention to how the MI is reported in each study and to refer to the original study.

In multiple studies, the MI was determined by counting the number of mitoses in 10 consecutive non-overlapping hpf with commencement in an area of high mitotic activity.[2,44,45] According to this method, oral and lip melanocytic neoplasms with ≥ 4 mitoses per 10 hpf have been associated with an increased risk of patient death within 1 year of diagnosis.[2] This threshold value had a sensitivity of 90% and a specificity of 84%.[2] To the best of our knowledge, this report provides the only statistically determined threshold value shown to have prognostic significance. This value is similar to the findings in a descriptive evaluation of 69 well-differentiated, highly pigmented lip and oral melanocytic neoplasms with a favorable outcome. In that study, none of these well-differentiated neoplasms had an MI > 3 per 10 hpf.[10] In another study, the MI was determined to be a significant negative determinant of patient survival for oral melanocytic neoplasms.[45] The cutoff value for MI in oral neoplasms in this study[45] (14 per 10 hpf) dramatically differed from the cutoff for oral neoplasms in the Bergin et al. study[2] (≥4 per 10 hpf), despite use of part of the same dataset. This finding may be a result of different methods of statistical evaluation and significantly larger numbers of well-differentiated neoplasms in one study.[2]

For cutaneous melanocytic neoplasms, the MI was demonstrated to be a significant negative determinant of patient survival in 3 studies.[5,28,45] The MI ($P ≤ .001$) provided a strong indication of eventual clinical outcome for melanocytic neoplasms in one of these studies; however, a predictive model based on the MI could not be constructed.[45] In contrast, an MI ≥3 per 10 randomly selected hpf, with avoidance of areas underlying ulceration, was shown to be statistically correlated with a low 2-year survival rate in the other 2 studies.[5,28] In one of these studies, the histologic diagnosis of cutaneous melanocytic neoplasms was correlated with MI in 67 of 68 cases.[28] Based on multivariate analysis using a Cox regression model, increasing MI was highly correlated with poorer prognosis.[28] The empirically determined cutoff value of ≥ 3 per 10 hpf had a predictive value of 91% (62 of 68 cases were accurately predicted) for survival and was significantly correlated with the Ki67 index ($r = 0.596$; $P < .0001$).[28] In the second study, there was a significant difference ($P < .01$) in survival over a 2-year study period for dogs with a cutaneous melanocytic neoplasm in which the MI was < 3 per 10 hpf (median survival of 104 weeks) versus dogs with melanocytic neoplasms with a MI of ≥ 3 per 10 hpf (median survival of 30 weeks).[5]

In 3 other studies, the MI was determined by the average number of mitoses per single hpf in various numbers of consecutive fields.[19,31,40] In one study, the MI was based on the average number of mitotic figures per hpf determined by counting the number of mitoses in 20 randomly selected 450 μm-diameter high-magnification fields in a set of oral malignant melanomas.[19] Neoplasms were grouped into 2 categories–those with an MI ≤ 3 and those with an MI > 3 mitoses per single hpf based on separation analysis. Twelve dogs had neoplasms with an MI ≤ 3 per hpf and had significantly longer remission lengths and survival times than did other dogs.[19] Twenty neoplasms in this study had an MI that was > 3 mitoses per hpf.[19] In another study, the MI was

defined as the average number of mitoses per hpf (40×) in 3 fields counted.[31] The average MI for dogs that were still alive at 1 year of follow-up, with no evidence of recurrence or metastasis, was 2.6 mitoses per hpf. The average MI for dogs that were dead within 1 year was 13.4 mitoses per hpf ($P = .001$).[31] There was also a strong correlation between Ki67 index and mitotic counts ($r = 0.706$; $P = .0001$).[31] Neoplasms were not separated by anatomical location in this study, which included both oral and cutaneous neoplasms.[31] In the third study, the average number of mitotic figures per single hpf was determined, after counting mitoses in 10 hpf in each neoplasm.[40] The average MI was 3.33 mitoses per hpf in this small series of 10 oral malignant melanomas with osteocartilaginous differentiation. In this study, 4 neoplasms with the highest mitotic indices recurred 60 days after diagnosis, and 3 neoplasms with the lowest MI did not recur during the follow-up period.[40] Thus, in these 3 studies,[19,31,40] the reported MI for melanocytic neoplasms with a poor prognosis would be comparable to > 30, 134, or 33.3 mitoses per 10 hpf, respectively. These values are significantly higher than those reported by other investigators,[2,5,28,45] but only melanocytic neoplasms histologically designated as "malignant" were evaluated in the latter 3 studies.[19,31,40]

A few reports, however, have not found a prognostic significance of the MI for melanocytic neoplasms at specific anatomic locations.[5,20,36,42] Three studies did not find a statistically significant correlation between MI and survival rates for dogs with oral melanocytic neoplasms,[5,20,36] although oral neoplasms tended to have a higher MI than those in the skin in one of these reports.[5] Kaplan-Meier analysis showed a trend toward differing survival times of dogs with neoplasms of different mitotic indices in another study; however, these results were not statistically significant.[36] A separate study, using an MI cutoff of < 3 mitoses per hpf or ≥ 3 mitoses per hpf, did not find a prognostic significance of MI for lip, skin, and nail bed melanocytic neoplasms that had been histologically classified as malignant.[42] The cutoff point in this study was selected based on prior reports, rather than statistically determined, at a similar high value as reported in 2 previous studies.[19,31] The difference in results may be a reflection of this high cutoff point, which is much higher than those used in most other studies.[2,5,28,45]

Predominant Cell Type

Melanocytic neoplasms are most commonly composed of 1 of the following 3 cell types: (1) epithelioid or polygonal (Fig. 3); (2) spindloid or fibromatous (Fig. 4); and (3) mixed epithelioid and spindloid (Fig. 5).[5,19,28,31,32,36,37,45] Other less commonly described cell types include: whorled type;[5,31,45] cellular;[5] balloon cell;[45] signet ring;[45] clear cell;[36] and an adenomatous/papillary type mentioned in one report.[36] Several studies have examined the predominant cell type as a potential prognostic parameter for canine cutaneous and oral or lip melanocytic neoplasms, but none has found a statistically significant correlation with survival.[1,5,19,28,31,32,37,45] Only 3 studies have suggested that cell type may be related to biologic behavior, but no association with survival was demonstrated.[5,31,32]

Cellular Pleomorphism

A few studies have evaluated cellular pleomorphism as a prognostic factor, but poor characterization of this term in the literature makes it impossible to draw meaningful conclusions about its prognostic significance. Cellular pleomorphism may encompass such characteristics as cell size, cell shape, pigmentation, and nuclear features, among other factors. One study found that cytologic features did not correlate with clinical outcome for the histologically malignant melanomas evaluated.[42] Another study, which did not specifically define cellular pleomorphism, did not find any prognostic value for this feature in terms of remission length or survival time ($P > .05$) for canine oral malignant melanomas.[19] A third study evaluated the presence of giant cells as a prognostic factor but did not find a statistically significant association with survival.[45]

Degree of Pigmentation

As previously noted, it can be difficult to accurately diagnose an amelanotic melanocytic neoplasm. Furthermore, objectively measuring the degree of pigmentation has been shown to be equally difficult. Nevertheless, several studies have attempted to examine the degree of pigmentation as a potential prognostic marker for canine melanocytic neoplasms to varying degrees.[1,2,5,19,20,28,32,36,42] Thus far, all of these studies have only subjectively determined the amount of pigmentation in melanocytic neoplasms and have not been able to identify threshold levels that could be statistically evaluated, with the possible exception of "high" pigment, assessed as at least 50% pigmented cells in one study.[2]

Although it would intuitively seem that well-differentiated neoplasms would be more highly pigmented than less differentiated neoplasms, with accordingly better prognoses, several studies have not found a significant correlation between degree of pigmentation of melanocytic neoplasms from various body sites with survival outcomes.[5,19,20,32,36,42] Conversely, in a descriptive (nonprognostic) study consisting of well-differentiated, highly pigmented oral and lip neoplasms, 95% of the dogs were either alive at the end of the study (mean survival 23 months) or died of unrelated causes.[10] This finding was statistically corroborated in a separate study in which oral and lip melanocytic neoplasms with high pigment (subjectively assessed as ≥ 50%) had significantly longer survival times compared to all other pigment categories (none, low, or moderate).[2] Despite the fact that increasing pigment generally correlated with better survival in this study, only high pigment could be used to classify neoplasms (high negative predictive value).[2] Low or no pigmentation did not reliably predict a poor outcome.[2] Similarly, another report showed that pigmentation was an independent prognostic factor ($P = .0007$) for cutaneous melanocytic neoplasms and heavy pigmentation had a positive influence on survival time ($P < .0001$).[28] Pigmentation was subjectively scored on a scale from 0 (no pigment) to 2 (highly pigmented).[28] Even though these studies support an

association between a high degree of pigmentation and longer survival times, this parameter cannot be reliably assessed, as there are currently no objective measures of this parameter nor validated cutoff points for comparative evaluation. In addition, the prognosis of neoplasms with lesser degrees of pigmentation cannot be accurately determined based on this parameter alone.

Junctional Activity

Junctional activity is a common feature of melanocytic neoplasms and an important feature to support the diagnosis of an amelanotic melanocytic neoplasm. A limited number of studies have examined the prognostic significance of junctional activity. One study demonstrated junctional activity as an independent prognostic factor ($P = .0239$) for cutaneous melanocytic neoplasms, and its occurrence was associated with a longer survival time ($P = .0046$).[28] In contrast, junctional activity negatively influenced survival for feet and lip neoplasms but was not statistically significantly associated with survival for skin neoplasms in another study.[45] Furthermore, in 2 studies of canine oral melanocytic neoplasms, an association of junctional activity with survival time was not found.[19,45] However, the results of these studies may be skewed, because poorly differentiated amelanotic melanomas without junctional activity might not be classified as melanomas on routine histopathologic examination and would not be included in the study set. Thus, there is no evidence of prognostic significance of junctional activity for oral melanocytic neoplasms, but its significance for dogs with cutaneous neoplasms is uncertain.

Intraepithelial Neoplastic Cells

Only one study has examined the prognostic significance of intraepithelial neoplastic cells, and no statistically significant association with survival was found for melanocytic neoplasms arising from any anatomic location.[45] One study reported that 9 of 10 dogs with canine oral malignant melanomas with osteocartilaginous differentiation had evidence of intraepithelial neoplastic cells; however, no conclusions were made in regard to this parameter.[40] Thus, based on currently available information, the presence of intraepithelial neoplastic cells does not appear to be a useful prognostic factor.

Ulceration

Many oral neoplasms are ulcerated as a result of perpetual trauma in the oral cavity regardless of cell of origin. One would therefore intuit that this factor would be of little prognostic importance for oral melanocytic neoplasms. For canine oral malignant melanomas, one study found that ulceration was of no prognostic value for remission length or survival time ($P > .05$).[19] Another study reported that 9 of 10 dogs with osteocartilaginous differentiation of oral malignant melanoma had evidence of ulceration.[40] There is some support for this factor as a prognostic marker for cutaneous melanocytic neoplasms in

one study, however.[28] In that study, ulceration of cutaneous melanocytic neoplasms was associated with a significantly shorter survival time ($P = .0023$) and was shown to be an independent prognostic factor ($P = .0065$).[28] In contrast, another study did not find a correlation between ulceration and clinical outcome for lip, cutaneous, or nail bed melanocytic neoplasms.[42] However, ulceration was examined only in histologically malignant neoplasms in that study;[42] thus, there was no reference population.

Level of Infiltration/Invasion

Several studies have examined the level of infiltration or invasion of surrounding tissues for utility as a prognostic factor.[1,19,28,31,32,35-37] When only malignant melanomas (oral and cutaneous) are evaluated, the level of stromal infiltration does not appear to be related to survival time.[19,31,42] Two studies reported that radiographic evidence of bony lysis did not affect outcome in dogs with oral malignant melanomas.[27,48] Another study, however, did find that a lack of bone lysis observed on skull radiographs was statistically associated with longer "times to first event" and longer survival based on both univariate and multivariate analysis for dogs with oral "malignant melanomas."[35] Also, when this factor was evaluated in a series of melanocytic neoplasms that contained both benign and malignant neoplasms, it appeared to be related to survival time.[28,37] For cutaneous melanocytic neoplasms, one study showed that lesions strictly limited to the dermis, and therefore of shallow depth, had a positive influence on survival time ($P < .0001$), and deep infiltration was shown to be an independent prognostic factor ($P = .0012$).[28] Another study showed that invasive growth was significantly negatively correlated with survival ($P = .024$) of dogs and cats with melanocytic neoplasms from various anatomic locations.[37] This study had a short follow-up time of only 6 months.[37] The definition of infiltration in some studies is ill defined, and thus it is difficult to draw meaningful conclusions regarding this factor.[32]

Vascular invasion, whether of blood or lymphatic vessels, is generally regarded as the gold standard for designating a melanocytic neoplasm as being malignant,[15,44] and lymphatic vessel invasion was reported to have prognostic significance in one study that included both cutaneous and oral melanocytic neoplasms.[31] In that study, the authors reported statistically significant differences in the course of disease between neoplasms with lymphatic invasion and those without, as demonstrated by survival curves ($P = .0144$).[31]

Necrosis

The presence of necrosis was negatively related to survival in a study set of 389 melanocytic neoplasms containing both benign and malignant lesions from various locations,[45] but it had no correlation with survival among a set of 38 malignant melanomas from various locations in another study.[31] In the larger study, the P values for intralesional necrosis varied among the

different sites (mouth, feet and lip, skin), but were significantly negatively correlated with survival for all sites.[45] The smaller study reported that the degree (extensive, moderate, or absence) of necrosis was not significantly related to survival.[31] However, no reference population was included, as only primary "malignant" melanomas were examined.[31] Thus, based on the results of the larger study, it would appear that the presence of necrosis does have prognostic significance, but this feature is difficult to measure objectively, specific cutoff values have not been determined, and its presence may represent different biological processes such as surface necrosis owing to trauma and deeper necrosis owing to ischemia.

Inflammation

The presence of inflammation is another potential prognostic parameter that is difficult to objectively measure. Studies that have examined this parameter have reported conflicting results.[19,31,45] One could postulate the presence of neoplasm infiltrating lymphocytes as being a favorable prognostic feature, as these lesions are frequently the target of specific active immunotherapy approaches. One study recorded the degree of lymphocytic and mononuclear cell infiltration within primary malignant melanomas from various body sites and compared this feature to Ki67 expression and survival.[31] The authors found that the presence of inflammation was not significantly related to either growth fraction or patient outcome.[31] Another study confirmed these findings and showed that the degree of inflammation, specifically lymphocytic infiltration, did not have prognostic value for remission length or survival time ($P > .05$) for canine oral malignant melanomas.[19] However, a large study showed that P values for deep inflammation varied among different anatomical locations (mouth, feet and lips, skin) of neoplasms, but were negatively and significantly correlated with survival in all sites.[45] This study evaluated both benign and malignant melanocytic neoplasms, whereas the 2 previously described studies included only malignant melanocytic neoplasms. Although deep intratumoral inflammation significantly influenced survival in a negative manner in the large study,[45] the significance of this feature requires confirmation in a prospective trial, and prognostic cutoff values should be established.

Margin Evaluation

Complete surgical excision is recommended for all melanocytic neoplasms; however, there is little, if any, support for this recommendation in the published literature.[48] Only 2 studies have examined margin evaluation as a prognostic factor, and both studies included only malignant melanocytic neoplasms.[19,42] Treatment by radical surgical excision of oral malignant melanomas resulted in longer remissions and survival times than did conservative surgical excision, without resection of underlying bone, in one of these studies;[19] however, extension of neoplastic cells to the surgical margins, or lack of extension, did not predict outcome for the lip, oral, or

cutaneous neoplasms in either study.[19,42] Surgical removal of the digit does appear worthwhile, however, with 6 of 14 dogs with histologically malignant digital melanoma living at least 12 months in one study.[42] Henry et al. also showed that surgical removal of digital neoplasms had a positive influence on survival.[21] This study included 64 digital neoplasms of various cell lines of origin, only 10 of which, however, were classified as malignant melanomas.[21] Authors of another study, which included various types of oral neoplasms, including malignant melanomas, stated that careful preoperative assessment and gross evidence of complete surgical removal do not consistently ensure a low prevalence of local recurrence; however, they did report that lack of extension of neoplastic cells to the surgical margins was an important prognostic factor.[48] Based on the current incomplete and contradictory information in the literature, further investigation of this parameter in a prospective manner is clearly needed. Nevertheless, complete and wide excision is desirable whenever possible, despite the current inability to use this parameter prognostically.

Molecular Prognostic Parameters

Various molecular parameters that are routinely used for prognostication of different neoplastic entities in human medicine, especially those assessed via immunohistochemical evaluation,[23] have been tested in canine neoplasms. In general, molecular tests have the advantage of being semiquantitative and more objective than traditional histological assessment. Thus, they are generally less affected by interobserver variation. The following molecular parameters have been examined to varying degrees in the recent veterinary literature for their ability to predict the prognosis of canine melanocytic neoplasms: growth fraction measured by Ki67 labeling; cell-cycle phase index (PI) measured by either bromodeoxyuridine (BrdU) or proliferating cell nuclear antigen (PCNA) labeling; expression of Melan A/MART-1, S-100, vimentin, and neuron-specific enolase (NSE); expression of p53, PTEN, Rb, p21 (waf-1), and p16 (ink-4a); DNA ploidy; mast cell count (MCC); microvessel density (MVD); and expression of vascular endothelial growth factor (VEGF). These parameters are reviewed below.

Growth Fraction

Tumor growth fraction, assessed by immunohistochemical labeling for Ki67, has been evaluated as a prognostic factor in several recent studies.[2,28,31,37,38,40] Some studies erroneously refer to the Ki67 index as the proliferation index rather than indicating growth fraction.[28,31,40] In order to be most accurate, the Ki67 index in this article will always be referred to as growth fraction, or simply as Ki67 index.

Although each of these retrospective studies used different methods to measure and report Ki67 index, they all have shown that this parameter has prognostic significance for canine melanocytic neoplasms.[2,28,31,37,40] The Ki67 index has been shown to be significantly different between benign and malignant

melanocytic neoplasms, with increasing values negatively correlated with survival.[2,28,31,37] One study demonstrated that the mean Ki67 index was significantly higher in oral melanocytic neoplasms from dogs that died within 1 year of diagnosis (Fig. 6) than the mean for neoplasms from dogs that were still alive at 1 year ($P < .0005$).[2] This study reported a negative correlation with survival by Cox regression analysis and efficacy as a diagnostic test by ROC analysis (area under the curve = 0.887, 95% confidence interval [CI] = 0.807-0.968).[2] ROC analysis was also used to establish a threshold value for Ki67, and the classification capabilities of this value were tested by Kaplan-Meier survival analysis.[2] The Ki67 labeling index for each neoplasm was determined by manually counting the number of positively labeled neoplastic cell nuclei within the area of a 1 mm^2 optical grid reticle at $400\times$ using a standard light microscope and a cell counter. Five grid areas within the areas of highest labeling were counted and averaged to determine the Ki67 labeling index. Areas under regions of ulceration were avoided. The threshold value was statistically determined to be 19.5 positive nuclei per grid reticle. The sensitivity and specificity of this threshold value as a prognostic marker in that study population was 87.1% and 85.7%, respectively. The positive predictive values, with respect to outcome at 1 year, were 86.3% when classified by the Ki67 threshold, 82.5% when classified by nuclear atypia, and 79% when classified by mitotic index. Kaplan-Meier survival analysis showed that the survival curves for dogs with a Ki67 index < 19.5 and dogs with a Ki67 index $\geq$ 19.5 were significantly different ($P <.0001$). Ki67 correlated with mitotic index, nuclear atypia, and pigment (inversely) with respect to survival, and the areas transcribed by the ROC curves (measure of test efficacy) were similar for Ki67, nuclear atypia, and mitotic index. Ki67 and nuclear atypia had somewhat better test performance than mitotic index, as indicated by tighter 95% CI for the AUC.[2]

A Ki67 threshold value capable of predicting survival rates in cutaneous melanocytic neoplasms has also been determined and is expressed as a percentage of positive labeling cells.[28] This study evaluated labeling for Ki67 in 68 cutaneous neoplasms, 12 of which were from the digit. Only 3 of these 12 digital neoplasms involved the ungual epithelium. In neoplasms with a focal immunoreactivity pattern, nuclei with weak to strong labeling for Ki67 were counted in these aggregated areas; in neoplasms with a diffuse staining pattern, counting was performed in randomly selected fields.[28] Similar to the study by Bergin et al., positive nuclei were counted at $400\times$ with the help of an eyepiece graticule, and areas under regions of ulceration were avoided.[28] Counting was performed without knowledge of neoplasm outcome. One observer calculated ratios for both 500-cell counts and 1,000-cell counts; the second observer calculated a ratio for 1,000-cell counts only. There was no statistically significant difference between the percentages for 500 cells and 1,000 cells, and there was little interobserver variation for the 1,000-cell counts. Thus, counting 500 cells was considered adequate. Empirically, it was noted that none of the biologically benign neoplasms had an index greater than 15% (Fig. 7), so this threshold level was

evaluated in regard to survival using Kaplan-Meier survival curves. There was a statistically significant ($P < .0001$) lower survival rate for dogs with neoplasms with a Ki-67 index greater than 15%. MI and Ki67 index were also shown to be significantly correlated ($r = 0.596$; $P < .0001$). Although MI and histological criteria were shown to be significantly correlated with survival, the percentage correct classification of the Ki67 index (97%) was higher than that of MI (91%) and histological criteria (93%).[28] It should be remembered that predictive values and percent correct classification are population-dependent parameters as opposed to sensitivity and specificity or AUC from an ROC curve, which are relatively population-independent. The latter were not reported for this study.

The 2 studies described above are by far the most clinically useful studies for canine oral[2] and cutaneous[28] melanocytic neoplasms, respectively. Both studies used manual counting techniques to determine Ki67 index that are easily applied in a diagnostic setting. Even though one study reported the Ki67 index as the number of positive nuclei per grid reticle[2] and the other study reported it as a percentage,[28] the authors of the first study suggested that the threshold levels were similar. The authors stated that for the grid reticle method, each grid field typically contains between 100 and 150 cells, and counting 5 grids would be roughly comparable to counting 500 cells.[2] Thus, the threshold for oral or lip melanocytic neoplasms of 19.5 or more positively staining cells out of a mean 125 cells/grid would be equivalent to 15.6%, similar to the 15% cutoff in the cutaneous melanocytic neoplasia study.[2,28] This assertion necessitates subsequent direct comparison. Nevertheless, these 2 studies have similar qualitative findings that are supported by high case numbers, extensive statistical analysis, and survival data.

Three additional studies have examined Ki67 index less rigorously but reached similar conclusions.[31,37,40] One study evaluated Ki67 index as a percentage of positive labeling nuclei per 600 cells in each of the 10 canine oral melanomas with osteocartilaginous differentiation included in their study.[40] It is unclear how they selected the 600 cells (eg, randomly vs within areas of highest staining). A threshold value of 30% for Ki67 index was empirically determined but was not statistically evaluated with respect to survival, likely because of the small dataset and descriptive nature of the report.[40] Two studies have employed computerized quantitative image analysis systems to evaluate Ki67 labeling, which may be of limited use in routine diagnostic settings.[31,37] One of these did not determine a specific threshold value, but instead, separated canine melanocytic neoplasms from various body sites into quartiles based on their Ki67 index and assessed the difference in survival between dogs with values above and below the median.[31] Dogs with values above the median value had statistically lower survival times than those with values below the median.[31] The study concluded that Ki67 index showed good predictive value, as shown by survival curves.[31] Ki67 index strongly correlated with mitotic counts ($r = 0.706$; $P = .0001$) and was statistically higher in oral malignant melanomas than in cutaneous ones.[31] High Ki67 index displayed a borderline correlation with lymphatic vessel invasion.[31] There was no correlation between histologic cell

types (eg, epithelioid, spindloid) of malignant melanomas and survival, but there were some differences in growth fraction among neoplasms with different cell types. Epithelioid, spindloid, and mixed malignant melanomas had a significantly higher growth fraction than melanocytomas, whereas whorled-type malignant melanomas had a Ki67 index that was not statistically different with respect to benign lesions, and thus dogs with whorled-type malignant melanomas had a better prognosis compared with dogs with malignant melanomas of other cell types.[31] Age, neoplasm thickness, stromal invasion, degree of atypia, and presence of inflammation or necrosis were not significantly related to Ki67 index and survival time.[31] The second quantitative image analysis study evaluated growth fraction (Ki67 index) and phase index, as measured by PCNA labeling in melanocytic neoplasms from various body sites of both dogs and cats.[37] An increasing Ki67 index was significantly correlated with decreasing survival time ($P = .027$) via Cox regression analysis, but a specific threshold value for neoplasm growth fraction was not determined.[37] There was a significant correlation between Ki67 index and PCNA labeling ($r = 0.69$, $P < .001$).[37] Additionally, Ki67 index significantly correlated with the macroscopic infiltrative aspect of the neoplasm at the time of surgical treatment ($P = .01$), but no correlations were found with cell type or neoplasm size.[37]

Based on the above evidence, Ki67 index is a statistically valid prognostic marker for canine melanocytic neoplasms. In a diagnostic setting, the Ki67 index for cutaneous melanocytic neoplasms should be determined according to the method of Laprie et al. (percentage positive cells of 500 cells counted),[28] and the Ki67 index for oral and lip melanocytic neoplasms should be determined according to the method of Bergin et al. (average number of positively staining neoplastic cells per 1 mm^2 optical grid reticle at $400\times$),[2] as outlined above. This index is especially helpful for melanocytic neoplasms that exhibit both prognostically favorable and poor histological parameters, or so-called "gray zone" cases. Furthermore, some neoplasms that exhibit "histological criteria of malignancy," but a low growth fraction have been identified and shown to have longer survival times than would have been expected based on histological features alone.[2,28] One study identified a subgroup of cutaneous neoplasms that were classified as benign based on the strict histological criteria described in that study, but that exhibited malignant behavior.[45] In another study, one subset of lip neoplasms with a histologically benign appearance exhibited malignant behavior, and a subset of lip neoplasms with a malignant histological appearance exhibited benign behavior.[42] Ki67 index is more objective and less subject to interobserver variation than histological atypia and mitotic index, although this characteristic should ideally be tested prospectively and with direct comparison of results from different observers.

Phase Index

Only 2 studies have examined cell cycle phase index (PI) as a prognostic indicator of canine melanocytic neoplasms and

neither used methods that could easily be applied in a diagnostic setting.[37,52] One study evaluated the BrdU labeling index as a measure of PI in a set of 23 oral neoplasms of different types, including 1 malignant melanoma.[52] This $n = 1$ for melanocytic neoplasms does not allow for any conclusions to be drawn. Since evaluation of BrdU requires intravenous injection of BrdU to dogs prior to surgery for mass removal, this method is impractical for routine clinical use. Another study used immunohistochemical labeling for PCNA and quantitative image analysis to determine the PI in 20 random fields.[37] Because of its long half-life, PCNA can be detected in cells throughout the cell cycle, but it is produced only in late G1 and throughout the S phase of the cycle.[6,37] The authors found that the PI differed significantly between the histologically malignant and benign melanocytic neoplasms from various sites of both dogs and cats ($P < .001$).[37] The correlation between Ki67 and PCNA labeling was significant ($r = 0.69$, $P < .001$), but unlike Ki67, PCNA labeling was not statistically related to survival duration ($P = .445$).[37]

PI appears to have some prognostic significance for melanocytic neoplasms; however, no specific conclusions or recommendations can be made from these limited studies. Additional investigation of this parameter using a larger number of canine neoplasms from both oral and cutaneous sites, with complete survival data, is needed to more fully evaluate the prognostic potential of PI. Also, this parameter should be measured using a method that can easily be applied in a diagnostic setting, such as determining immunohistochemical labeling for PCNA in a manner similar to the methods used for growth fraction.

Expression of Melan A/MART-1, S-100, Vimentin, and NSE

Immunohistochemical labeling for Melan-A/MART-1, S-100, vimentin, and NSE has been used in the diagnosis of melanocytic neoplasms,[8,9,12,25,36,43] but their value as prognostic markers is rather limited. Only one study has evaluated these antibodies for their potential use as prognostic markers for canine melanocytic neoplasms.[25] There did not appear to be any correlation between the labeling properties of vimentin, S-100, or NSE and the behavior of the melanocytic neoplasms in this study. The authors did, however, report that intensity of Melan A/MART-1 expression was significantly ($P < .03$) correlated with a favorable outcome.[25] This study used a small number of cases and did not perform survival analysis. In contrast, another study found that strong labeling for Melan-A in a primary neoplasm did not predict benign behavior of that neoplasm.[36] Based on our current knowledge, it seems unlikely that labeling for any of these markers can be used to predict the prognosis of canine melanocytic neoplasms.[44]

Expression of p53, PTEN, Rb, p21 (waf-1), and p16 (ink-4a)

Two studies have examined the expression of p53,[26,39] and one also examined the expression of PTEN, Rb, p21, and p16 in relatively small numbers of canine melanocytic neoplasms from

various sites.[26] Abnormalities in the expression or localization of these proteins were found in a proportion of the neoplasms, but there were no significant differences between benign and malignant neoplasms and there was no correlation with survival.[26,39]

DNA Ploidy

Few studies have examined DNA ploidy for prognostic significance in canine melanocytic neoplasms. Differences in DNA ploidy and other morphometric variables have been shown between benign and malignant melanocytic neoplasms, but no correlation with survival time has been demonstrated.[4,38] The methodology is not easily applied for melanocytic neoplasms, since heavily pigmented neoplasms are difficult to evaluate and accurate interpretation of acceptable histograms is difficult.[4] Furthermore, assessment of DNA ploidy is labor intensive and not easily applied in a diagnostic setting.[4] Thus, use of this parameter for prognostication is not recommended at this time.

Mast Cell Count and Microvessel Density

Only one study has examined microvessel density (MVD) and the number of mast cells within the neoplasms (mast cell count, MCC) as prognostic parameters for canine melanocytic neoplasia.[32] The study concluded that MCC and MVD were significantly correlated and that high MCC and MVD in canine melanocytic neoplasms were associated with a poor prognosis.[32] Cutoff values of median MCC and MVD were used to divide dogs into high and low groups for both parameters and compared to survival for a portion of the study population. Statistically, MCC was found to be a better prognostic marker than MVD ($p < .01$); however, both MCC and MVD at the lesion periphery, as well as centrally, were shown to be independent prognostic factors.[32] The study was based on a small sample population, follow-up data were available for only 18 cases, and all 18 neoplasms exhibited malignant behavior. A reference population of benign neoplasms was not included in the study. Further evaluation of MCC and MVD, with regard to survival outcomes, must be performed using a larger study population that includes both benign and malignant neoplasms, further stratified by anatomic neoplasm location, before substantial conclusions can be made regarding their utility as prognostic parameters.

Vascular Endothelial Growth Factor

In human cancer patients, high plasma vascular endothelial growth factor (VEGF) levels have been correlated with a poor prognosis.[22,24] One study measured plasma VEGF in 70 dogs with various neoplasms, only 6 of which were of melanocytic origin.[51] The authors found a significant difference in plasma VEGF levels between the various types of neoplasms.[51] The lowest levels were found in dogs with epulides and the highest levels were found in dogs with oral melanocytic neoplasms.[51] There was no comparison of plasma VEGF to survival. No conclusions can be drawn regarding the prognostic significance of this marker for canine melanocytic neoplasms at this time, though evaluation of this parameter for its prognostic significance may be worthwhile.

Conclusions

Based on review of the current literature in terms of the Recommended Guidelines for the Conduct and Evaluation of Prognostic Studies in Veterinary Oncology,[49] we identified the prognostic parameters that have the most statistically demonstrable prognostic significance based on published survival times for canine cutaneous and digit melanocytic neoplasms and for oral and lip melanocytic neoplasms (Table 2) to date. In our recommendations, a favorable prognosis relates to expected survival times longer than 1 year and a poor prognosis relates to an expected death as a result of melanocytic neoplasia within less than 1 year post diagnosis for all melanocytic neoplasms. For specific survival times and clinical outcomes for each parameter, the reader should refer to the sections in this article specific to that parameter and the original study being cited. Only the most significant factors are discussed in the following.

When attempting to predict the biological behavior of a particular melanocytic neoplasm, accurate determination of melanocytic origin of the neoplasm is crucial prior to prognostication. Since amelanotic melanocytic neoplasms are difficult to distinguish from soft tissue sarcomas, which commonly exhibit an aggressive biological behavior, inaccurate differentiation will influence the validity of any prognostic study. According to the current literature, IHC labeling with a combination of Melan-A, PNL2, TRP-1, and TRP-2 as single antibodies, or as a cocktail, provides the highest sensitivity for detecting amelanotic melanomas while maintaining 100% specificity.[8,9,12,25,36,41,43,44,46] Other commonly used antibodies, such as S-100 or MiTF, are highly sensitive for detecting amelanotic melanocytic neoplasms, but they lack the specificity necessary to provide an accurate diagnosis.[9,18,34,41,43,44]

In general, oral melanocytic neoplasms have a worse prognosis than cutaneous neoplasms, and those on the lip or digit have a worse prognosis than cutaneous melanocytic neoplasms at other sites. However, as several studies[2,10,28,42,45] have demonstrated, location alone cannot be used to predict prognosis for a given patient, as there are definitely exceptions to all generalizations. Some factors have prognostic significance for neoplasms of a particular site, but not for neoplasms arising at other locations (Table 1). Distant metastasis is indicative of a poor prognosis for all melanocytic neoplasms regardless of their location. It would also seem that lymph node metastasis would be associated with a poor prognosis; however, only 2 studies have compared this factor to prognosis, and both examined only oral "malignant" melanomas.[19,35] Neither study identified any association between regional lymph node metastasis and remission length, "time to first event," or survival time ($P > .05$), but no reference population was included.[19,35] Thus, additional studies that include benign and malignant neoplasms from various anatomic locations are

needed for definitive conclusions regarding lymph node metastasis as a prognostic factor.

Although there is some reported evidence that neoplasm size or volume has prognostic significance, because of conflicting results in the literature, recommendations regarding this parameter cannot be made at this time. Additional studies using a statistically determined threshold value with comparisons to complete survival data are needed before significant conclusions can be drawn.

Histological classification of canine melanocytic neoplasms as benign or malignant has generally been associated with clinical outcome and survival. However, "morphologic classification" as an individual prognostic parameter is difficult to evaluate in the current literature, as different classification criteria have been applied.

Histological evaluation of nuclear atypia has a high positive predictive value for epithelioid-predominant melanocytic neoplasms[2,45] and for spindloid neoplasms with sufficiently observable nuclear detail[2] in terms of prognosis. Significant nuclear atypia is associated with a poor prognosis, and minimal or no atypia is associated with a favorable prognosis;[2,45] however, assessment of this parameter is subject to interobserver variation, resulting in different specificities and sensitivities. Rigorous description and standardization of the criteria for nuclear atypia provide some measure of reproducibility.[2,45] Therefore, the degree of nuclear atypia should be evaluated for melanocytic neoplasms based on the criteria described by Spangler and Kass (2006), as follows:

Well-differentiated or typical melanocytic neoplastic cells[45]

- small nucleus
- single, centrally oriented nucleolus
- minimal clumping of chromatin
- may have condensed strands of nuclear chromatin extending from the nucleolus to the nuclear membrane
- condensation of chromatin along the inner surface of the membrane
- cells that lack a nucleolus have fine and evenly dispersed chromatin at the periphery of the nucleus

Poorly differentiated neoplastic cells (atypical)[45]

- larger nucleoli of less-regular shape that are eccentrically located in the nucleus
- often multiple nucleoli
- in some cases, multiple nucleoli are haphazardly connected to the inner surface of the nuclear membrane by thin strands of chromatin and give the appearance of a coarsely vacuolated nucleus.

Although the original evaluation was based on an incremental scale from 1 to 10, signifying the subjectively estimated percentage of nuclei involved,[45] such a rigorous grading scale is unlikely to be applied in a routine diagnostic setting. A statistically similar, but more practical method for oral and lip melanocytic neoplasms is to use a threshold value for semiquantification of nuclear atypia of $\geq 30\%$.[2]

The MI should be determined by counting the number of mitotic figures in 10 consecutive hpf commencing in the area of highest mitotic activity for oral and lip neoplasms[2,45] and in random fields for cutaneous neoplasms.[28] Areas under ulceration should be avoided. MI should be evaluated in bleached tissue sections to ensure that the melanin within the cell does not obscure the mitoses, and mitotic figures need to be distinguished from small chromatic fibroblast nuclei within the interstitium. The MI should be reported as the number of mitoses per 10 hpf.[2,15,28,45] Dogs with cutaneous neoplasms that have an MI ≥ 3 per 10 hpf[5,28] and dogs with oral or lip neoplasms that have an MI ≥ 4 per 10 hpf[2] are expected to have shorter survival times. At least 50% of dogs with cutaneous melanocytic neoplasms with an MI ≥ 3 per 10 hpf were dead by 7 months in 2 studies.[5,28] Approximately 80% of dogs with oral or lip melanocytic neoplasms with an MI ≥ 4 per 10 hpf were dead by 1 year in another study.[2] Dogs with melanocytic neoplasms with an MI less than those values, for each respective site, are expected to have longer survival times.[2,5,28] Less than 10% of dogs with cutaneous melanocytic neoplasms with am MI < 3 per 10 hpf died within a 2-year follow-up period in 2 studies.[5,28] Less than 10% of dogs with oral or lip melanocytic neoplasms with an MI < 4 per 10 hpf died within a 1-year follow-up period in one study.[2]

The presence of inflammation and/or necrosis should not be used to determine prognosis. Although both have been associated with a poor prognosis for melanocytic neoplasms at any site,[45] both are difficult to objectively evaluate, and no cutoff values have been determined.

Pigmentation should be evaluated but not used as a sole predicting factor. Although a high degree of pigmentation is associated with a favorable clinical outcome in both cutaneous and oral melanocytic neoplasms,[2,10,28] outcome is not predictable in oral neoplasms with moderate, low, or no pigmentation.[2] When paired with assessment of nuclear atypia, the degree of pigmentation has greater prognostic significance for oral and lip melanocytic neoplasms.[2] Dogs with highly pigmented, well-differentiated oral or lip neoplasms were found to have longer post diagnosis survival times that approached 2 years.[10]

One study demonstrated lymphatic invasion as a negative prognostic factor for oral and cutaneous melanocytic neoplasms.[31] Even though lymphatic invasion was not specifically evaluated as a prognostic factor for melanocytic neoplasms of the lip or digit in that study,[31] vascular invasion is considered by some as the best indicator of malignancy.[15]

The presence of ulceration has been associated with a poor prognosis in one study, which included cutaneous neoplasms from various sites including 12 digital neoplasms.[28] Deep infiltration/invasion has also been shown to be associated with a poor prognosis for cutaneous melanocytic neoplasms,[28] and possibly for oral melanocytic neoplasms.[35,37] Junctional activity has been associated with shorter survival times for melanocytic neoplasms arising on the feet or lips,[45] but its

significance for cutaneous melanocytic neoplasms at other sites is uncertain because of conflicting reports.[28,45]

Because of the inherent subjectivity in histological evaluation, we recommend that more than 1 parameter be considered to classify melanocytic neoplasms as benign or malignant. If these features conflict with one another, a neoplasm should be diagnosed as a melanocytic neoplasm and both the favorable and the poor prognostic factors should be discussed.

No criteria have yet been established to allow for completely objective assessment of histological parameters for prognostic purposes in a routine laboratory setting. In contrast, the Ki67 index, as a measure of the growth fraction of neoplastic cells, is a highly objective test that has a higher predictive value than histologic criteria.[2,28] Assessment of Ki67 index is recommended for all melanocytic canine neoplasms and is especially useful for cases with conflicting histological parameters, or so-called "grey zone" cases. Based on the current literature, Ki67 index has been statistically shown to have prognostic significance using 2 separate methods for oral and lip melanocytic neoplasms and for cutaneous and digit neoplasms. For oral and lip melanocytic neoplasms, the Ki67 index should be reported as the average number of positively labeled nuclei per 1 mm^2 grid reticle at $400\times$ by counting the number of positive nuclei in 5 grid areas.[2] Neoplasms with a Ki67 index ≥ 19.5 are expected to have a poor prognosis (Fig. 6), and neoplasms with an index less than 19.5 are expected to have a favorable prognosis.[2] For cutaneous melanocytic neoplasms, the Ki67 index should be reported as a percentage of positive-staining nuclei per 500 cells.[28] Neoplasms with a Ki67 index $\geq 15\%$ are expected to have a poor prognosis, and neoplasms with a Ki67 index $<15\%$ (Fig. 7) are expected to have a favorable prognosis.[28] For both methods, counting should commence in an area with high labeling, but areas of inflammation and areas underlying ulceration should be avoided.[2,28] Although Bergin et al. reported Ki67 index as the average number of positive nuclei per 1 mm^2 grid reticle, Laprie et al. also used a grid reticle for assistance in counting 500 cells. It would be ideal to be able to report Ki67 index in the same manner regardless of location; however, the current literature does not allow for this. Thus, unless additional prognostic studies are performed to determine statistically valid threshold values using one or both of these methods in melanocytic neoplasms from various sites, the Ki67 index should be determined as the average number of positive nuclei per 1 mm^2 grid reticle for oral and lip melanocytic neoplasms and as the percentage of positive nuclei in 500 cells for cutaneous and digit melanocytic neoplasms. The use of grid reticles is becoming more common in diagnostic pathology because it allows for standardization of field area between different microscopes. They are inexpensive and can easily be inserted into most microscopes. Thus, diagnostic laboratories should consider acquiring at least one optical with a grid reticle that can be shared between pathologists. Assessing the average number of positive cells per grid within 5 grid areas is less labor intensive and less prone to error compared to counting 500 neoplastic cells.

Although it is impractical to accurately predict, on an individual basis, the biological behavior of melanocytic neoplasms by applying elaborate numeric criteria to specific histological features,[45] evaluation of nuclear atypia and MI, in combination with Ki67 index and clinical features, will maximize the percentage of correctly classified neoplasms. An accurate prognosis is becoming more and more important in veterinary oncology, as various treatment options are now available for specific types of neoplasms and many more clients are pursuing these options. These treatments are: often costly; may involve radical surgeries or costly radiotherapy, chemotherapy, or immunotherapy; and may have significant or yet unknown side effects. Thus, it is important to identify which melanocytic neoplasms require additional therapies and which will likely be cured by excision alone.

None of the previously published studies met all the standards outlined by the Recommended Guidelines for the Conduct and Evaluation of Prognostic Studies in Veterinary Oncology.[49] We based our recommendations regarding prognostic factors for canine melanocytic neoplasms on the studies that came the closest to meeting these standards. Very few studies had adequate sample numbers, strict selection criteria, and detailed statistical analysis with comprehensive clinical outcome and survival data. A major difficulty in veterinary prognostic studies is to accurately evaluate survival data, as many pets are euthanized, rather than die, as a result of neoplastic disease. Often, it is even difficult to associate the cause of euthanasia with the progression of neoplastic disease. Some dogs with melanocytic neoplasms may be euthanized as a result of deterioration of health secondary to neoplastic disease, others may be euthanized because of management issues in patient care that the owner is reluctant or unable to manage, and others may be euthanized for completely unrelated medical comorbidities. It is also often difficult to determine a cause of death, even in dogs that die a natural death, as necropsy is rarely performed. Additionally, none of the published studies specifically stated the expected magnitude of each factor's contribution or the expected prognosis of the population. Also, few studies specifically defined a reference population. In some cases, this information could be inferred by the reader. Another major pitfall is that many of the studies that have examined various treatments for dogs with melanocytic neoplasms have failed to use rigorous inclusion and exclusion criteria and well-defined end point evaluation. Thus, few conclusions can be drawn from those studies. All of the studies examined here were retrospective. Clearly, well-designed prospective studies are greatly needed to confirm the validity of the described prognostic parameters. Future canine melanocytic neoplasm prognostic studies should use the Recommended Guidelines for the Conduct and Evaluation of Prognostic Studies in Veterinary Oncology[49] to aid in study design. An effort should be made to design more rigorous prospective trials, especially to evaluate specific treatment protocols that would allow a more targeted therapy approach.

References

1. Aronsohn MG, Carpenter JL: Distal extremity melanocytic nevi and malignant melanomas in dogs. JAAHA **26**:605–612, 1990.
2. Bergin IL, Smedley RC, Esplin DG, Spangler WL, Kiupel M: Prognostic evaluation of Ki67 threshold value in canine oral melanoma. Vet Pathol **48**:41–53, 2011.
3. Bergman PJ: Canine oral melanoma. Clin Tech Small Anim Pract **22**:55–60, 2007.
4. Bolon B, Calderwood Mays MB, Hall BJ: Characteristics of canine melanomas and comparison of histology and DNA ploidy to their biologic behavior. Vet Pathol **27**:96–102, 1990.
5. Bostock DE: Prognosis after surgical excision of canine melanomas. Vet Pathol **16**:32–40, 1979.
6. Bravo R, Macdonald-Bravo H: Changes in the nuclear distribution of cyclin (PCNA) but not its synthesis depend on DNA replication. EMBO J **4**:655–661, 1985.
7. Brodey RS: A clinical and pathologic study of 130 neoplasms of the mouth and pharynx in the dog. Am J Vet Res **21:**787–812, 1960.
8. Cangul IT, van Garderen E, van der Linde-Sipman JS, van den Ingh TSGAM, Schalken JA: Canine balloon and signet-ring cell melanomas: a histological and immunohistochemical characterization. J Comp Path **125**:166–173, 2001.
9. Choi C, Kusewitt DF: Comparison of tyrosinase-related protein-2, S-100, and Melan-A immunoreactivity in canine amelanotic melanomas. Vet Pathol **40:**713–718, 2003.
10. Esplin DG: Survival of dogs following surgical excision of histologically well-differentiated melanocytic neoplasms of the mucous membranes of the lips and oral cavity. Vet Pathol **45:**889–896, 2008.
11. Finocchiaro LM, Glikin GC: Cytokine-enhanced vaccine and suicide gene therapy as surgery adjuvant treatments for spontaneous canine melanoma. Gene Ther **15**:267–276, 2008.
12. Giudice C, Ceciliani F, Rondena M, Stefanello D, Grieco V: Immunohistochemical investigation of PNL2 reactivity of canine melanocytic neoplasms and comparison with Melan A. J Vet Diag Invest **22**:389–394, 2010.
13. Goldschmidt MH: Benign and malignant melanocytic neoplasms of domestic animals. Am J Dermatopathol **7(Suppl):** 203–212, 1985.
14. Goldschmidt MH: Pigmented lesions of the skin. Clin Dermatol **12:**507–514, 1994.
15. Goldschmidt MH, Dunstan RW, Stannard AA, von Tscharner C, Walder EJ, Yager JA: Histological classification of epithelial and melanocytic neoplasms of the skin of domestic animals. *In:* World Health Organization International Histological Classification of Neoplasms of Domestic Animals, 2nd series, Volume III, pp. 38–40. Armed Forces Institute of Pathology, Washington DC, 1998.
16. Goldschmidt MH, Hendrick MJ: Neoplasms of the skin and soft tissues. *In:* Neoplasms in Domestic Animals. ed. Meuten DJ, 4th ed., pp. 78–82. Iowa State University Press, Ames, IA, 2002.
17. Gorlin, RJ, Barron CN, Chaudhry AP, Clark JJ: The oral and pharyngeal pathology of domestic animals: a study of 487 cases. Am J Vet Res **20:**1032–1061, 1959.
18. Granter SR, Weilbaecher KN, Quigley C, Fletcher CD, Fisher DE: Microphthalmia transcription factor: not a sensitive or specific marker for the diagnosis of desmoplastic melanoma and spindle cell (non-desmoplastic) melanoma. Am J Dermatopathol **23:**185–189, 2001.
19. Hahn KA, DeNicola DB, Richardson RC, Hahn EA: Canine oral malignant melanoma: prognostic utility of an alternative staging system. J Sm Anim Pract **35:**251–256, 1994.
20. Harvey HJ, MacEwen EG, Braun D, Patnaik AK, Withrow SJ, Jongeward S: Prognostic criteria for dogs with oral melanoma. JAVMA **178:**580–582, 1981.
21. Henry CJ, Brewer WG Jr, Whitley EM, Tyler JW, Ogilvie GK, Norris A, Fox LE, Morrison WB, Hammer A, Vail DM, Berg J: Canine digital tumors: a veterinary cooperative oncology group retrospective study of 64 dogs. J Vet Intern Med **19:**720–724, 2005.
22. Hyodo I, Doi T, Endo H, Hosokawa Y, Nishikawa Y, Tanimizu M, Jinno K, Kotani Y: Clinical significance of plasma vascular endothelial growth factor in gastrointestinal cancer. Eur J Cancer **34:**2041–2045, 1998.
23. Idikio HA. Immunohistochemistry in diagnostic surgical pathology: contributions of protein life-cycle, use of evidence-based methods and data normalization on interpretation of immunohistochemical stains. Int J Clin Exp Pathol **3:**169–176, 2010.
24. Jin-no K, Tanimizu M, Hyodo I, Nishikawa Y, Hosokawa Y, Endo H, Doi T, Mandai K, Ishitsuka H: Circulating platelet-derived endothelial cell growth factor increases in hepatocellular carcinoma patients. Cancer **82:**1260-1267, 1998.
25. Koenig A, Wojcieszyn J, Weeks BR, Modiano JF: Expression of S100a, vimentin, NSE, and melan A/MART-1 in seven canine melanoma cell lines and twenty-nine retrospective cases of canine melanoma. Vet Pathol **38:**427–435, 2001.
26. Koenig A, Bianco SR, Fosmire S, Wojcieszyn J, Modiano JF: Expression and significance of p53, Rb, p21/*waf-1*, p16/*ink-4a*, and PTEN tumor suppressors in canine melanoma. Vet Pathol **39:**458–472, 2002.
27. Kosovsky JK, Matthiesen DT, Marretta SM, Patnaik AK: Results of partial mandibulectomy for the treatment of oral tumors in 142 dogs. Vet Surg **20(6):**397–401, 1991.
28. Laprie C, Abadie J, Amardeilh MF, Net JL, Lagadic M, Delverdier M: MIB-1 immunoreactivity correlates with biologic behaviour in canine cutaneous melanoma. Vet Dermatol **12:**139–147, 2001.

29. MacEwen EG, Kurzman ID, Vail DM, Dubielzig RR, Everlith K, Madewell BR, Rodriguez CO Jr, Phillips B, Zwahlen CH, Obradovich J, Rosenthal RC, Fox LE, Rosenberg M, Henry C, Fidel J: Adjuvant therapy for melanoma in dogs: results of randomized clinical trials using surgery, liposome-encapsulated muramyl tripeptide, and granulocyte macrophage colony-stimulating factor. Clin Cancer Res **5:**4249–4258, 1999.

30. Marino DJ, Matthiesen DT, Stefanacci JD, Moroff SD: Evaluation of dogs with digit masses: 117 cases (1981-1991). J Am Vet Med Assoc **207:**726–728, 1995.

31. Millanta F, Fratini F, Corazza M, Castagnaro M, Zappulli V, Poli A: Proliferation activity in oral and cutaneous canine melanocytic tumours: correlation with histological parameters, location, and clinical behaviour. Res Vet Sci **73:**45–51, 2002.

32. Mukaratirwa S, Chikafa L, Dliwayo R, Moyo N: Mast cells and angiogenesis in canine melanomas: malignancy and clinicopathological factors. Vet Dermatol **17:**141–146, 2006.

33. Mulligan RM: Melanoblastic neoplasms in the dog. Am J Vet Res **22:**345–351, 1961.

34. Ohsie SJ, Sarantopoulos GP, Cochran AJ, Binder SW: Immunohistochemical characteristics of melanoma. J Cutan Pathol **35:**433–444, 2008.

35. Proulx DR, Ruslander DM, Dodge RK, Hauck ML, Williams LE, Horn B, Price GS, Thrall DE: A retrospective analysis of 140 dogs with oral melanoma treated with external beam radiation. Vet Radiol Ultrasound **44:**352–359, 2003.

36. Ramos-Vara JA, Beissenherz ME, Miller MA, Johnson GC, Pace LW, Fard A, Kottler SJ: Retrospective study of 338 canine oral melanomas with clinical, histologic, and immunohistochemical review of 129 cases. Vet Pathol **37:**597–608, 2000.

37. Roels S, Tilmant K, Ducatelle R: PCNA and Ki67 proliferation markers as criteria for prediction of clinical behaviour of melanocytic tumours in cats and dogs. J Comp Pathol **121:**13–24, 1999.

38. Roels SL, Van Daele AJ, Van Marck EA, Ducatelle RV: DNA ploidy and nuclear morphometric variables for the evaluation of melanocytic neoplasms in dogs and cats. Am J Vet Res **61:**1074–1079, 2000.

39. Roels S, Tilmant K, Ducatelle R: p53 expression and apoptosis in melanomas of dogs and cats. Res Vet Sci **70:**19–25, 2001.

40. Sanchez J, Ramirez GA, Buendia AJ, Vilafranca M, Martinez CM, Altimira J, Navarro JA: Immunohistochemical characterization and evaluation of prognostic factors in canine oral melanomas with osteocartilaginous differentiation. Vet Pathol **44:**676–682, 2007.

41. Sandusky GE, Carlton, WW, Wightman KA: Immunohistochemical staining for S100 protein in the diagnosis of canine amelanotic melanoma. Vet Pathol **22:**577–581, 1985.

42. Schultheiss PC: Histologic features and clinical outcomes of melanomas of lip, haired skin, and nail bed locations of dogs. J Vet Diagn Invest **18:**422–425, 2006.

43. Smedley RC, Lamoureux J, Sledge DG, Kiupel M: Immunohistochemical diagnosis of canine oral amelanotic melanocytic neoplasms. Vet Pathol **48:**32–40, 2011.

44. Smith SH, Goldschmidt H, McManus PM: A comparative review of melanocytic neoplasms. Vet Pathol **39:**651–678, 2002.

45. Spangler WL, Kass PH: The histologic and epidemiologic bases for prognostic considerations in canine melanocytic neoplasia. Vet Pathol **43:**136–149, 2006.

46. Sulaimon, SS, Kitchell BE, Ehrhart EJ: Immunohistochemical detection of melanoma-specific antigens in spontaneous canine melanoma. J Comp Pathol **127:**162–168, 2002.

47. Todoroff RJ, Brodey RS: Oral and pharyngeal neoplasia in the dog: a retrospective survey of 361 cases. JAVMA **175:**567–571, 1979.

48. Wallace J, Matthiesen DT, Patnaik AK: Hemimaxillectomy for the treatment of oral tumors in 69 dogs. Vet Surg **21:**337–341, 1992.

49. Webster JD, Dennis MM, Dervisis N, Heller J, Bacon NJ, Bergman PJ, Bienzle D, Cassali G, Castagnaro M, Cullen J, Esplin DG, Peña L, Goldschmidt MH, Hahn KA, Henry CJ, Hellmén E, Kamstock D, Kirpensteijn J, Kitchell BE, Amorim RL, Lenz SD, Lipscomb TP, McEntee M, McGill LD, McKnight CA, McManus PM, Moore AS, Moore PF, Moroff SD, Nakayama H, Northrup NC, Sarli G, Scase T, Sorenmo K, Schulman FY, Shoieb AM, Smedley RC, Spangler WL, Teske E, Thamm DH, Valli VE, Vernau W, von Euler H, Withrow SJ, Weisbrode SE, Yager J, Kiupel M: Recommended guidelines for the conduct and evaluation of prognostic studies in veterinary oncology. Vet Pathol **48:**7–18, 2011.

50. Weiss E, Frese K: Histological classification and nomenclature of tumours of the skin. Bull World Health Organ **50:**79–100, 1974.

51. Wergin MC, Kaser-Hotz B: Plasma vascular endothelial growth factor (VEGF) measured in seventy dogs with spontaneously occurring tumours. In Vivo **18:**15–20, 2004.

52. Yoshida K, Yanai T, Iwasaki T, Sakai H, Ohta J, Kati S, Ishikawa K, Lackner AA, Masegi T: Proliferative potential of canine oral epulides and malignant neoplasms assessed by bromodeoxyuridine labeling. Vet Pathol **36:**35–41, 1999.

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Current updates

1. New hardware supported:

- Prior Stage (ProScan III)
- USB 3.0 camera
- Klarman Rulings reticle (KR-871)

2. User manual overhaul.
3. New workflow updates:

- "Use offset button" is added during registration to avoid camera and eyepiece registration.
- "Hide reticle" and "Show reticle" buttons are added to the task collection gui.

4. Improve "Best Registration" button performance.
5. Improve Camera_stage_review utility application interface and "take image" button.

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