Decoding heterogeneous tumor MR signals:
MR histology and cytometric feature mapping connects 2D pathology and in vivo MRI of sarcomas

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INTRODUCTION

MR imaging presents multiple advantages for tumor analysis, including superior soft tissue contrast and multiple contrast options. Metrics such as the apparent diffusion coefficient (ADC) have garnered much attention as a potential biomarker for tumor status. Tumor MRI/pathology studies have demonstrated a relationship between tissue cellularity and ADC, but reports are inconsistent. We are working to develop a mechanism for reliable comparison of "gold standard" 2D histopathological sections with MRI images. The ability to perform these analyses on large datasets will provide valuable insight into the biology behind the MR signal.

OBJECTIVES

1. Construct a digital space with resources to register multi-resolution, multi-parametric imaging data.
2. Generate a pipeline to compare tumor pathology slides and MR data, including in vivo MRI and ex vivo MR histology (MRH).

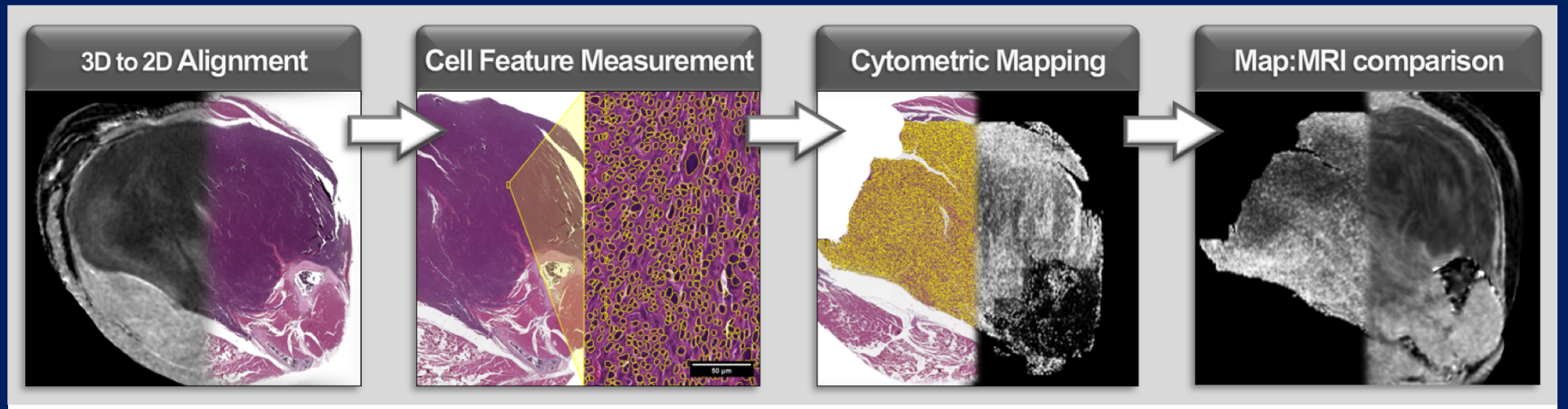

Figure 1. Schematic for co-registration of high-resolution 3D MRH with cytometric property maps derived from 2D H&E histology slides. Demonstration of the four phases over which correlative MR studies are performed: (1) Orienting MR to H&E slides by selection of the appropriate 2D plane in the MR images, followed by non-linear landmarks-based alignment; (2) Implementation of a multi-step machine-learning algorithm for nuclear segmentation over entire histology slides; (3) Measurement of segmented nuclei and generation of quantitative cytometric feature maps; (4) Correlative studies of tumor MR signal and cytometric features.

References:
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2. Chen L, Liu M, Bao J et al. (2013) The correlation between apparent diffusion coefficient and tumor cellularity in patients: a meta-analysis. PLoS One 8: e79008.
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5. Schmidt UW, M.; Broaddus, C.; Myers, G. (2018) Cell Detection with Star-Convex Polygons. In Medical Image Computing and Computer Assisted Intervention Society conference, pp. 8. Granada, Spain.
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1. Linear & landmarks-based Image Registration

Methods

Registration is performed using 3D Slicer in the "Image Space" on the following image types (Fig.2):

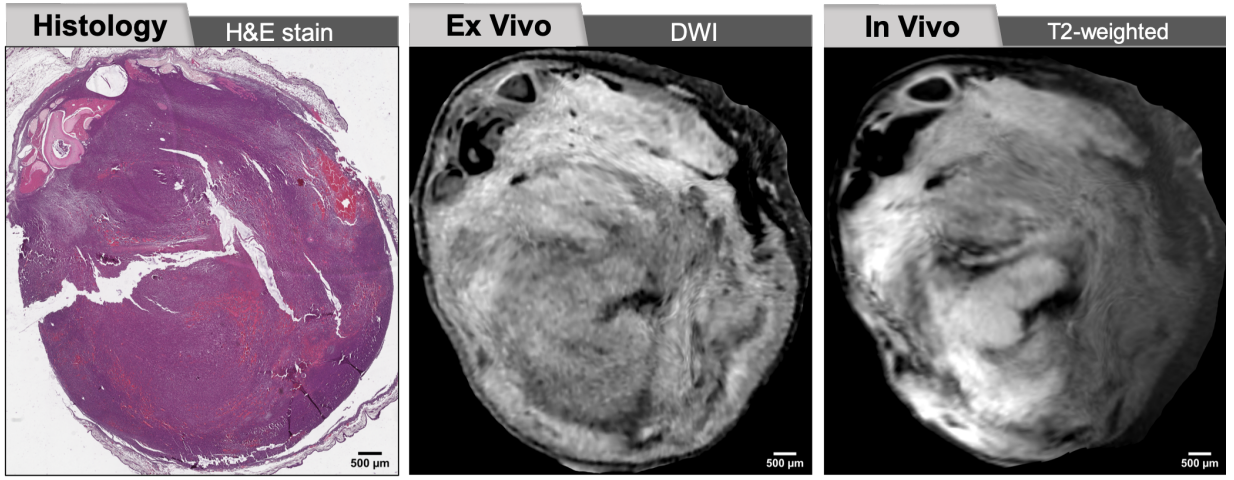
- H&E tumor cross sections (2D; digitized 40X), downsampled to 2.0 μm^2 for alignment (~200-300 MB)
- MRH images (3D isotropic) at 50 μm^3 (~300-400 MB)
- In vivo MRI images (3D) at 100 X 100 X 300 µm (~10-50 MB)

MRH to histology alignment:

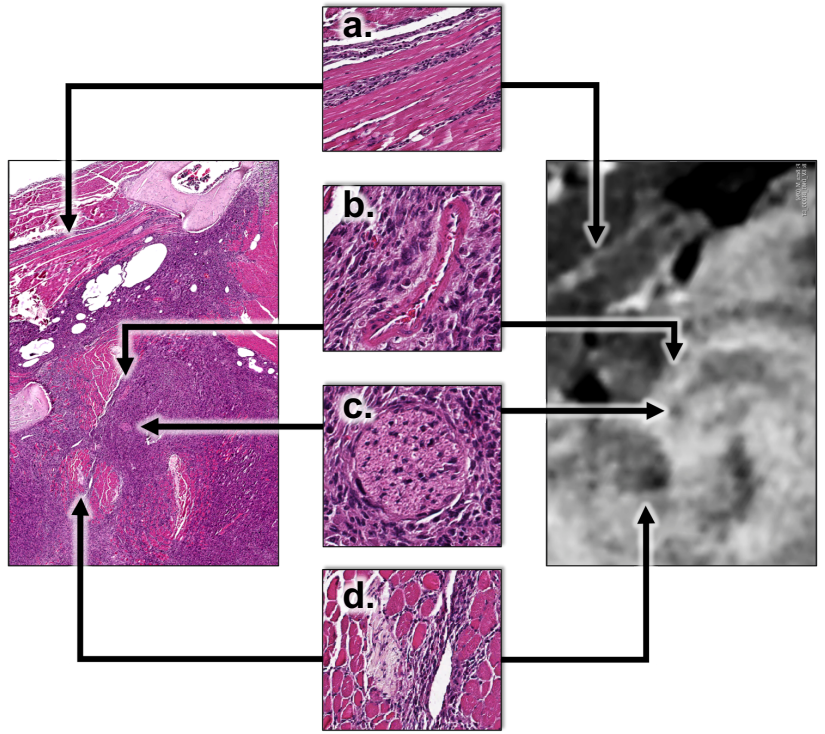
1. **Rotational (linear) alignment**
   - 3D isotropic MRH images are rotated against a fixed pathology slide until the corresponding slice is obtained.
2. **Tissue shrink**
   - The selected MRH slice is linearly resized to correct for tissue shrinkage during histological prep.
3. **Landmarks-based (non-linear) alignment**
   - 80-120 landmarks are placed on anatomical structures visible in high-resolution images (Fig. 3).
   - A non-linear transformation matrix is generated based on the identified landmarks and is applied to all MRH images in the dataset.

In Vivo MRI to histology alignment:

1. Alignment of 3D in vivo MRI to 3D MRH via linear rotation and landmark-based alignment.
2. MRH-aligned in vivo images are registered to the histology via transformations determined in the above steps.



◀ **Figure 2. Multi-resolution, multi-parametric image registration of sarcoma tissues.** Displayed are the registered images of a sarcoma cross-section, including 2D histology stained with H&E (left), an ex vivo MRH diffusion-weighted image at b-value of 500 and 50um isotropic resolution (middle), and an in vivo bias-corrected T2-weighted image (right).



◀ **Figure 3. High-resolution landmarks-based alignment of MRH and 2D histology.** MRH images (grayscale, right) were aligned to H&E pathology images (left). The high resolution of ex vivo MRH facilitates identification of fine structures normally seen in pathological images, including tumor-infiltrated muscle (a), vasculature (b), nerve structure (c), and cartilage (d).

Innovations & Advancements

- The "Image Space" environment facilitates storage, handling, and registration of large, multi-modality datasets without sacrificing resolution.
- The high spatial resolution of MRH images provide structural information relative to tissue histology that in vivo MRI is not able to reasonably resolve.
- We have demonstrated that MRH images are an excellent conduit for registration, with morphological detail for comparison with histology, and signal patterns which speak to in vivo MRI.

2. Measurement of Cytometric Characteristics

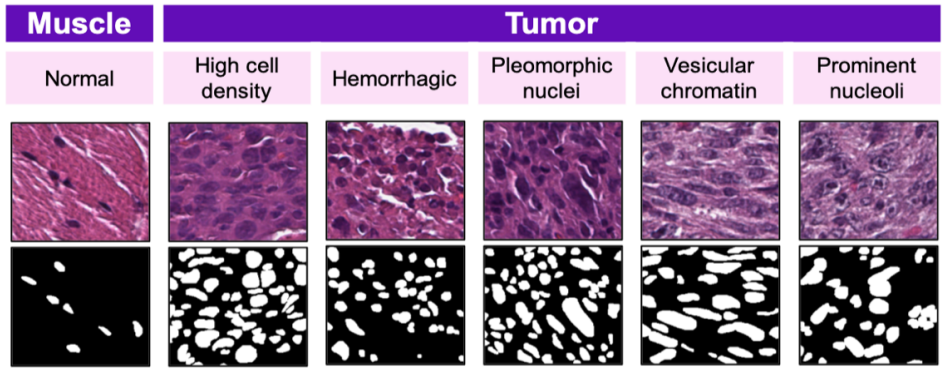
Methods

Automated segmentation of nuclei in H&E slides of sarcomas is met with an abundance of challenges, including vastly differing morphologies (Fig. 4) that render older segmentation methods (e.g. watershed) ineffective.

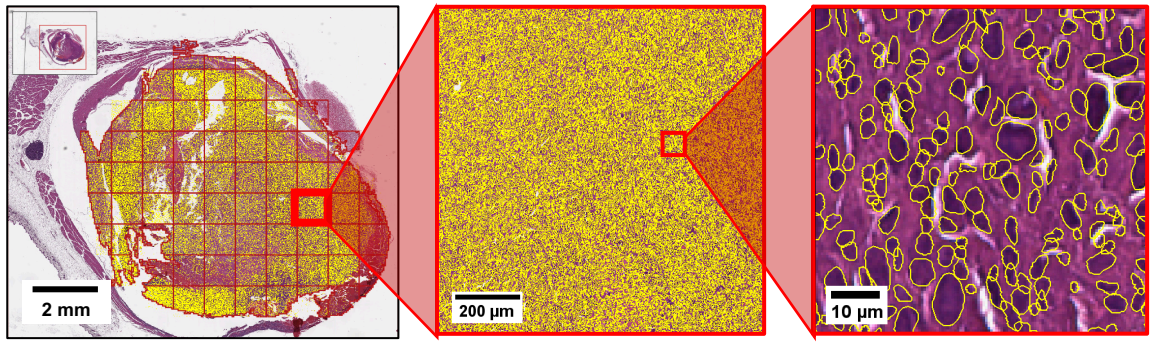
Multi-step processing for automated nuclear segmentation:

- Optical density images derived from the H&E slides in QuPath were exported to FIJI in tiles at full resolution.
- A house-built FIJI macro was used to bulk process all tiles from a sample. Processing included a series of filters, followed by ROI generation using the StarDist plugin for FIJI.
- Overlays of nuclear ROIs were imported back into QuPath.

Cytometric measurements were determined for each nucleus detected across entire samples (typically > 1 million detections; Fig. 5). In this pilot study, approximately 40 features were measured per nucleus, with some key features of interest listed in Table 1.



▲ **Figure 4. Nuclear segmentation over whole H&E slides.** Binary nuclear segmentations are shown in a variety of nuclei/tissue conditions present in the sarcoma dataset.



◀ **Figure 5. Automated nuclear segmentation of whole-slides.** Processing of tiles in FIJI produces overlays that can be imported into QuPath, as shown here on an entire histological slide of sarcoma tissue.

Table 1. Samples of measurable cytometric properties over whole-slide samples

Cytometric Property	Calculation	General Description	Demonstration
Detection Count	$\frac{\#\ nuclei}{area}$	The detection count is a measure of how many nuclei were detected in a given area (i.e. nuclear/cellular density)	
Delaunay Mean Distance	*Average internuclear distance (µm)*	Via Delaunay triangulation, the mean distance from one nuclei to each of its closest neighbors.	
Delaunay Ratio	$\frac{Del.min\ distance}{Del.\max distance}$	When considering all nuclei adjacent to a given nucleus, the Delaunay ratio describes the anisotropy of neighbor-to-neighbor distances in a tissue.	

Cytometric Property	Calculation	General Description	Demonstration
Nuclear Circularity	$4\pi\left(\frac{area}{perimeter^2}\right)$	Nuclear circularity describes how close a defined nucleus is to a circle, where a value of 1 indicates a perfect circle.	
Nuclear Mean Hematoxylin Value	*Mean hematoxylin intensity value*	Nuclear mean hematoxylin values for detected nuclei describe staining intensity across the entire nuclear area.	
Nuclear Area	*Area of defined nucleus*	Nuclear area describes the size of a segmented nucleus in μm^2	

Innovations & Advancements

- We have built a bulk-processing macro for implementation in FIJI which produces nuclear segmentations at full resolution for the entire area of a pathology H&E slide. Methods for further improving the current segmentation algorithms are currently underway.
- QuPath freeware within the “Image Space”, facilitates measurement of a variety of features for each nucleus over an entire slide (e.g. ~40 measurements/nucleus. ~1-2 million nuclei/slide).

3. Generation of Cytometric Property Maps

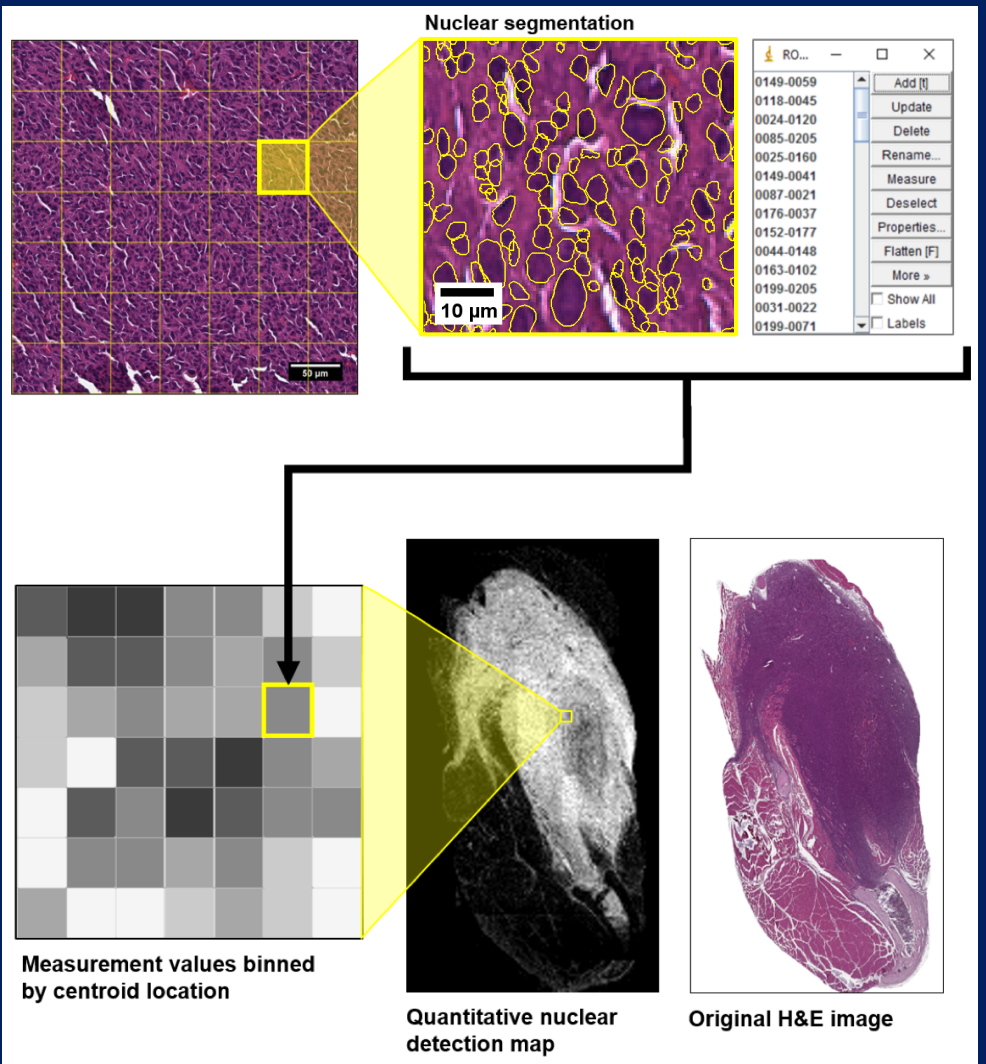
Methods

To compare MR signal to a variety of cytometric features, we have written code in MATLAB to create spatially-correct, quantitative maps of cell properties (Fig. 6).

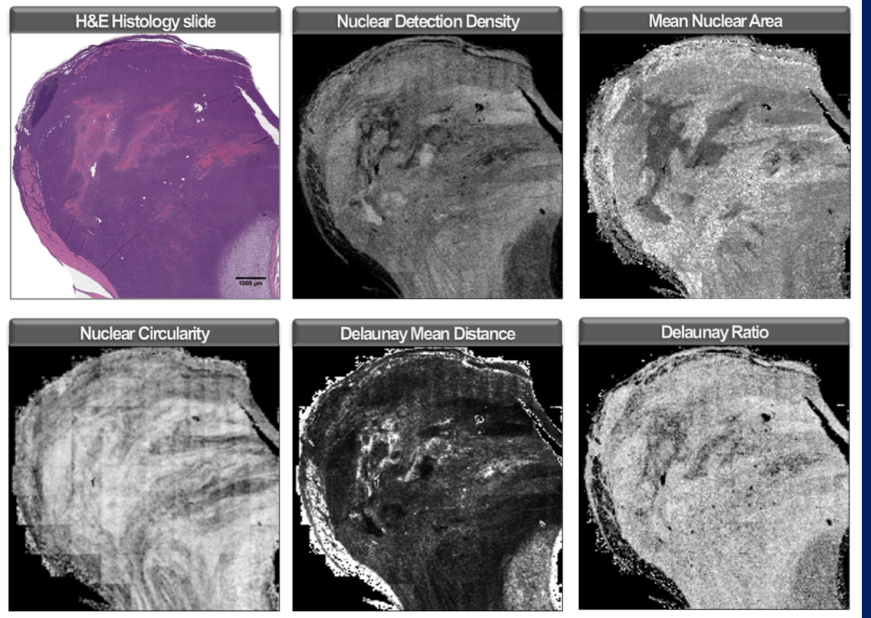
Generally, the operations performed by the program are as follows:

1. Upload measurement spreadsheet generated by QuPath.
2. Based on slide dimensions of the, create a grid with bins of a specified size (in this case, 50 μm^2).
3. Sort each detected nucleus into the appropriate bin based on the location of its centroid.
4. For a given cytometric property (e.g. nuclear circularity), calculate the outputs for each bin, such as mean value and variance.
5. Generate a .tif image in which the bin values are represented as pixels, and the scale of gray values is known.

The resulting images are spatially matched to the original H&E image (Fig. 7). Thus, both MRH and in vivo MR images can be compared to the resulting maps via previously executed alignments (step 1 of the pipeline).



▲ **Figure 6. Generating quantitative cytometric feature maps of whole-slide pathology images.** Following automated nuclear segmentation, cytometric feature maps were created by sorting nuclear measurements into spatial bins based on their centroid location. Mean values and variance were calculated for each bin and represented in grayscale. The resulting images were quantitative cell feature maps that could be compared to histology-registered MR images.



▲ **Figure 7. Examples of generated cytometric maps.** Examples of quantitative cytometric property generated from an H&E slide (top left) of sarcoma tissues.

Innovations & Advancements

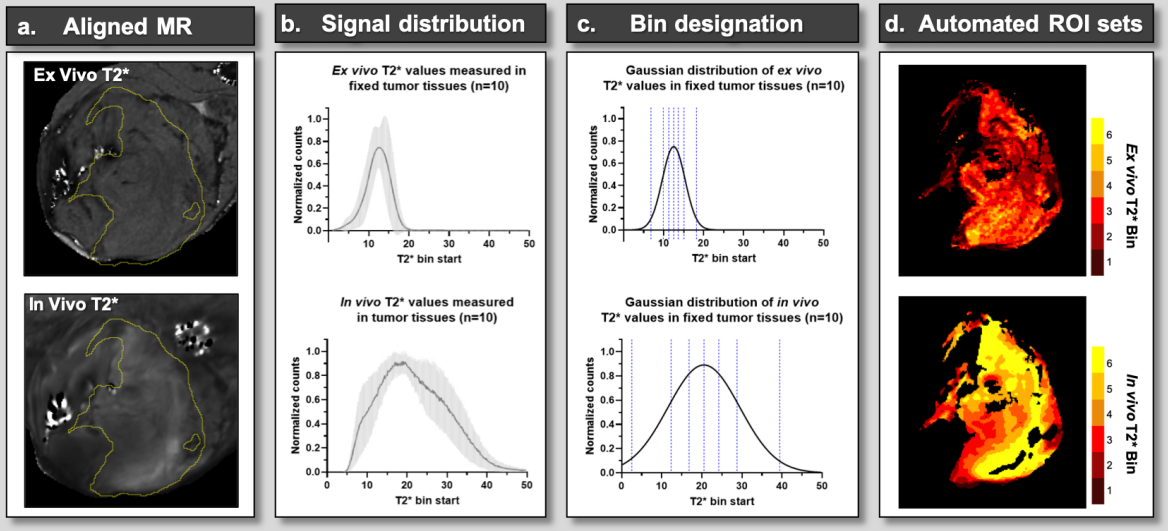
- We have written code in MATLAB which is capable of sorting and computing a variety of features measured from nuclei across an entire histology slide.
- This code generates grayscale images of a known scale which are spatially related to the histology image, and therefore can be registered to MR images from the same tissue sample.
- This code is being utilized in a pilot study of sarcoma (n=10) with multiple slides taken per tumor.
- We are currently developing an online tool based on this code for easy public access and use.

4. Correlative comparison of maps with MR signal

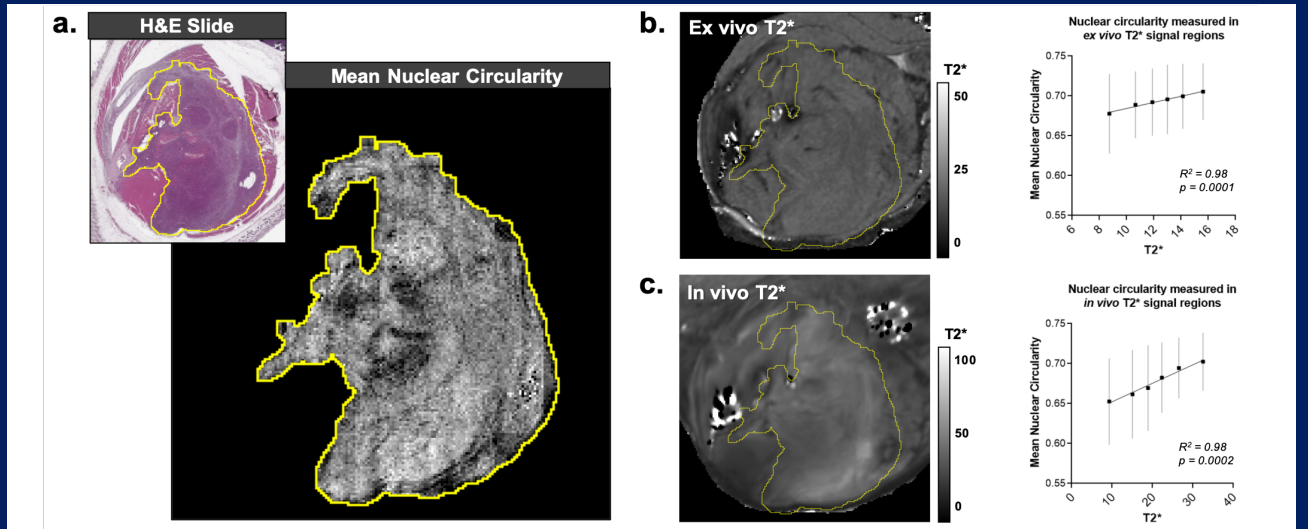
Methods

We have written an ImageJ plugin that generates ROI sets in histology-aligned sarcoma MR images. Sarcomas (n=10) were segmented (in vivo and ex vivo) to generate ADC and T2* distribution curves. Values between ±2σ of the Gaussian mean were divided into 6 bins. The defined bins were programed into the macro for automatic ROI detection (Figure 8).

For each image, the macro generates an ROI set and applies it to each cytometric property map of the sample. Measurements are made for each threshold and reported as mean cytometric property value and measured MR signal value. In this way, cytometric properties can be compared to MR signal over whole-tissue samples in a single efficient step (Figure 9).



▲ **Figure 8. Automated intra-tumor thresholding of T2* images.** Examples ex vivo (top) and in vivo (bottom) histology-aligned sarcoma images in which tumor is delineated in yellow (a). In vivo and ex vivo signal was measured in all tumors (n=10) and curves were normalized (b). Signal bins were determined based on the Gaussian fit of the tumor curves, with bin boundaries designated in blue (c). Shown is the application of determined T2* bins on the sample sarcoma images, with each automatically-generated ROI shown as a colored bin (d).



▲ **Figure 9. Automated comparison of cytometric property maps and MR signals.** A map of nuclear circularity for the same sample is given, with the H&E image for reference (a). Tumor is delineated with yellow. Correlations between nuclear circularity and MR T2* maps are shown, including ex vivo T2* (b) and in vivo T2* (c).

Innovations & Advancements

- We have automated the process of ROI generation within MR dataset, as well as the measurement of said ROIs over the entire collection of cytometric property maps.
- We are able to begin identifying relationships between cytometric features and MR signal in large datasets which include multiple contrasts and resolutions.

Conclusions

Within the “Image Space”, we can register large datasets of multi-contrast, multi-resolution data. We have created a multitude of tools which compliment freely available software, and have built a pipeline for registration of whole-slide histology with in vivo MRI, using MRH as a conduit. Further, we can generate data for entire studies, without being limited by data size or resolution. Thus, we have provided the groundwork for understanding heterogenous tumor MR signals based on the tissue pathology.