Enhancing fluorescence microscopy with deep learning

Adapted from Weigert et al., *Nature Methods* (2018)
‘Content-aware’ restoration (CARE)

Hari Shroff
hari.shroff@nih.gov
October 19, 2021
Workflow for image denoising

RCAN: ‘residual channel attention network’
Better at preserving high resolution information than alternative neural networks

Collaboration with SVision (Bellevue, WA) => 3D RCAN
Chen et al, Nature Methods 2021
https://github.com/AiviaCommunity/3D-RCAN (we’ve used Biowulf, desktops, STRIDES/cloud)
Denoising => operate super-resolution microscope indefinitely?

U2OS transfected with Mito-GFP
Imaged with iSIM, prohibitively high SNR
360 W/cm²

Normalized Fluorescence Intensity

Frames

DL
High SNR iSIM

2.2 W/cm²
2600 Volumes (!)
1 Volume = 24 planes
Every 5.6 s
Total ~50,000 images for 4 hours
Denoising => operate super-resolution microscope indefinitely?

Green: Mitochondria (Mito GFP) Red: Lysosome (Lamp1-Apple)
1 volume = 12 slices (0.25 µm z step), time interval: 5.1 s, 300 volumes
RCAN for resolution enhancement, confocal volumes -> STED volumes

SiR DNA, live MEF cells
RCAN model trained on 22 matched confocal/STED volumes (fixed)
RCAN for iSIM-> expansion microscopy, living cells

Obvious resolution enhancement, particularly in z
‘Chaining’ RCANs to de-aberrate, isotropize, and super-resolve light-sheet data

Pan-nuclear marker, live *C. elegans* embryo, 1.1 NA detection
‘Chaining’ RCANs to de-aberrate, isotropize, and super-resolve light-sheet data

Pan-nuclear marker, live *C. elegans* embryo, 1.1 NA detection

Triple RCAN output – most gentle way to attain super-resolution?
Gentle 4D super-resolution imaging in living embryos

Pan-nuclear marker, live *C. elegans* embryo, 1.1 NA detection

Triple RCAN output – most gentle way to attain super-resolution?

100 volumes
Current paradigm

Microscope

Imaging model

Data

Addition to the paradigm?

Microscope

Imaging model

Data
Challenges and Opportunities

*How much of what we see can we believe?*

*How much can spatial resolution or SNR be improved for a given training set?*

*How linear are the results?*

*How much (or little) training data is required for a given application?*

*To what extent can ‘hybrid’ optical / computational methods bypass inherent physical limitations of the microscope?*

*Can image reconstruction that is pleasing or useful to the human eye be bypassed, instead producing valid scientific inferences directly from the raw data?*

*How do we get these methods in the hands of biologists?*

*What training programs and hardware need to be in place so that more scientists can use these approaches?*
Thanks!