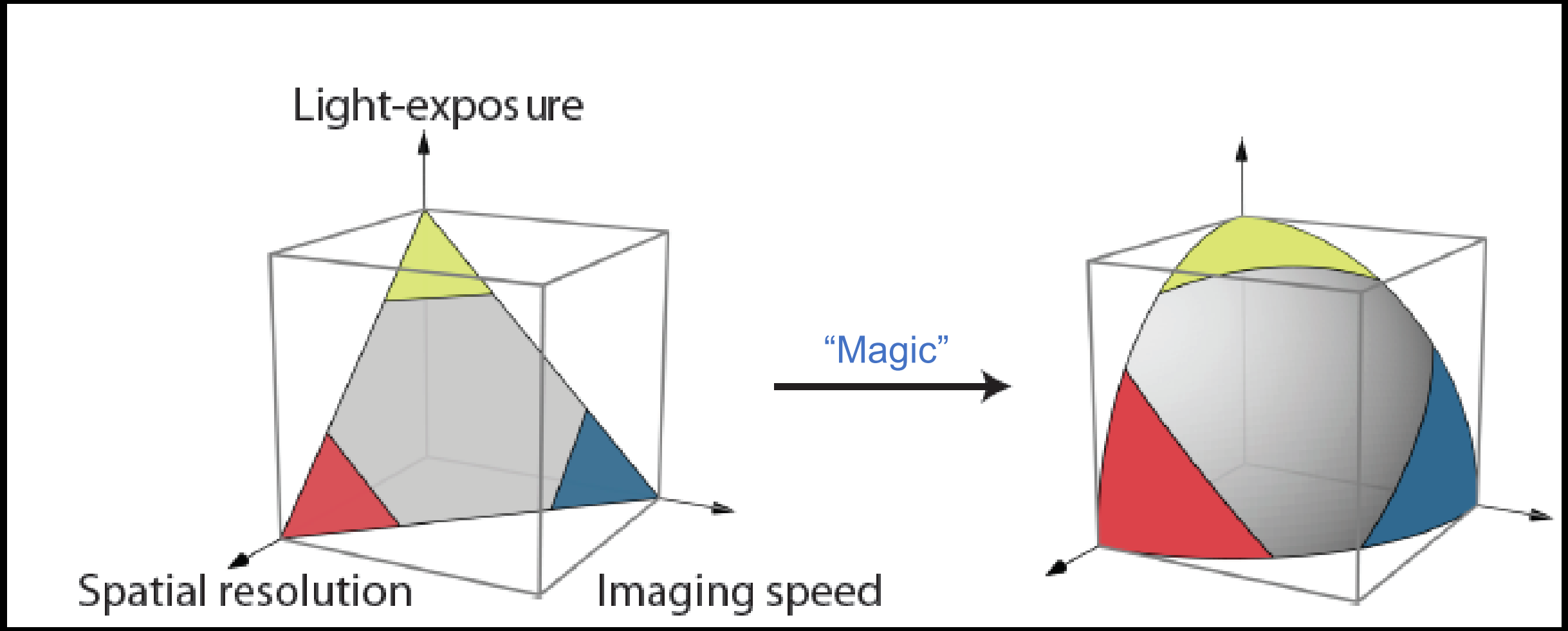
Enhancing fluorescence microscopy with deep learning



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

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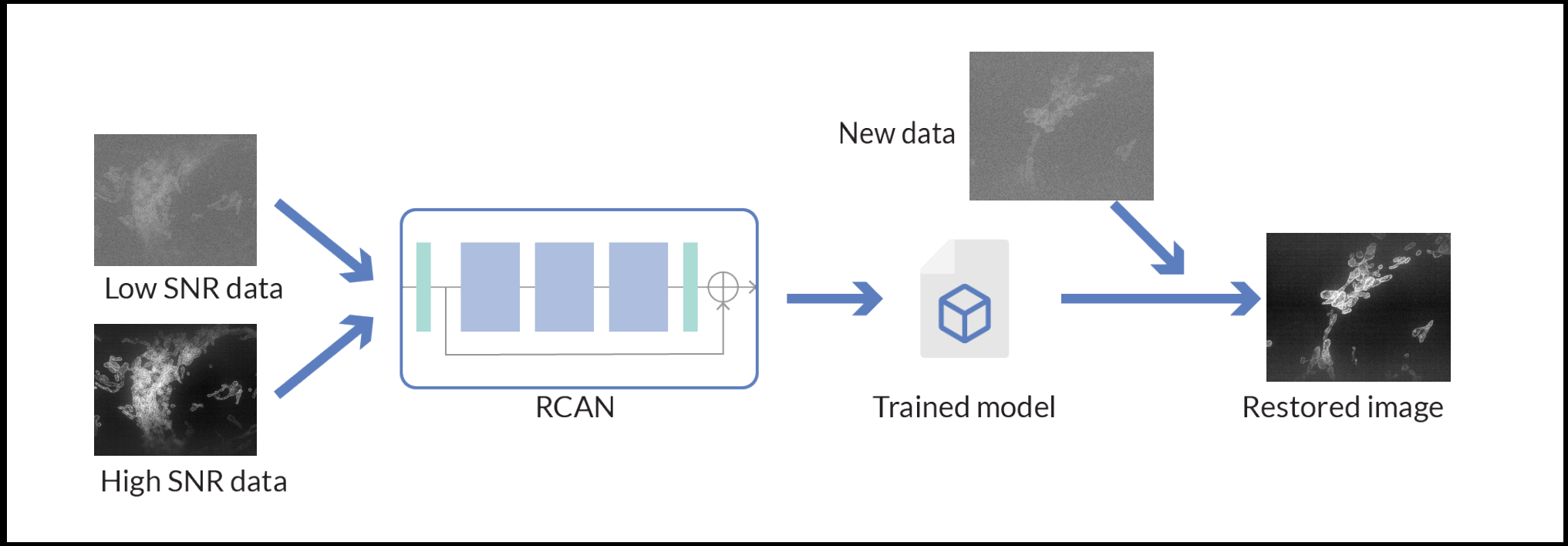
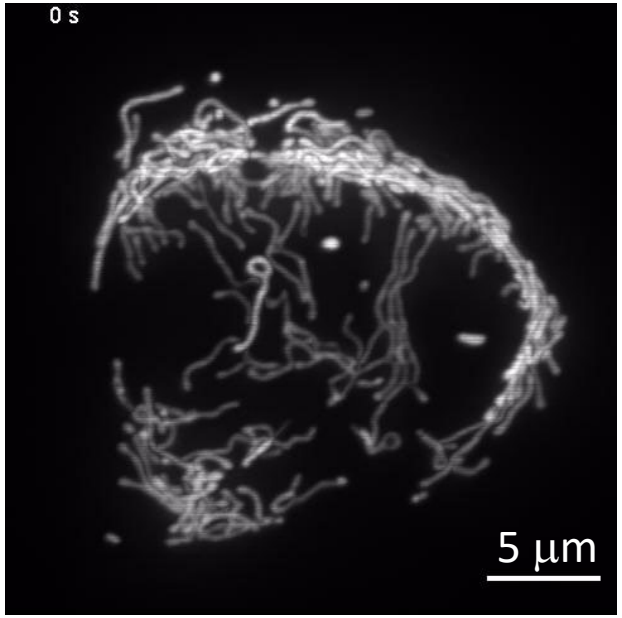
Workflow for image denoising



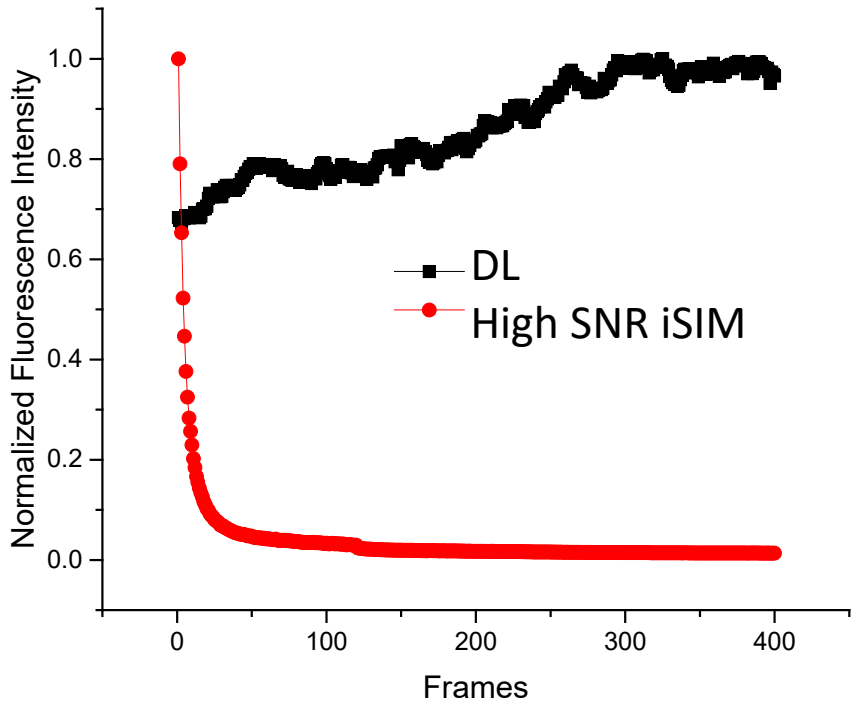
RCAN: 'residual channel attention network'
Better at preserving high resolution information than alternative neural networks
Zhang *et al.* arXiv:1807.02758v2 (2018)

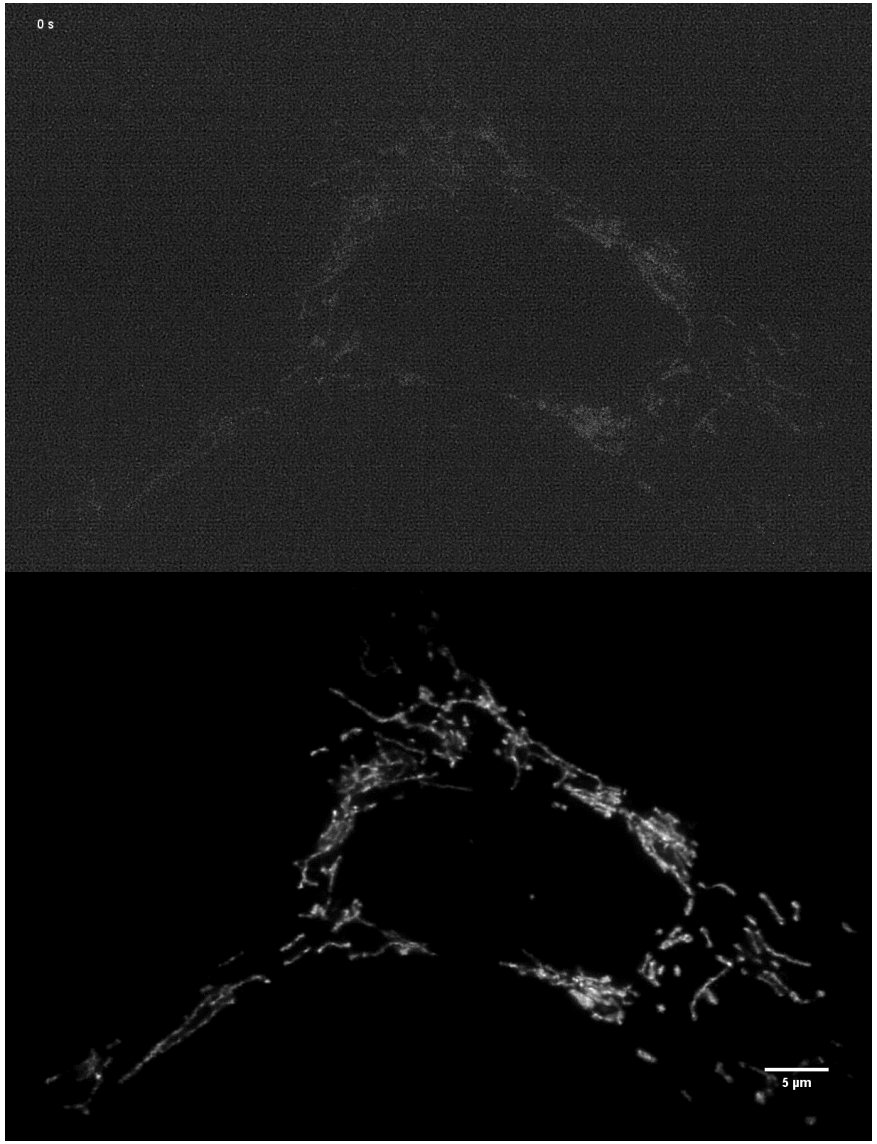
Collaboration with SVision (Bellevue, WA) => 3D RCAN
Chen et al, *Nature Methods* 2021
https://github.com/AiviaCommunity/3D-RCAN (we've used Biowulf, desktops, STRIDES/cloud)

Denoising => operate super-resolution microscope indefinitely?



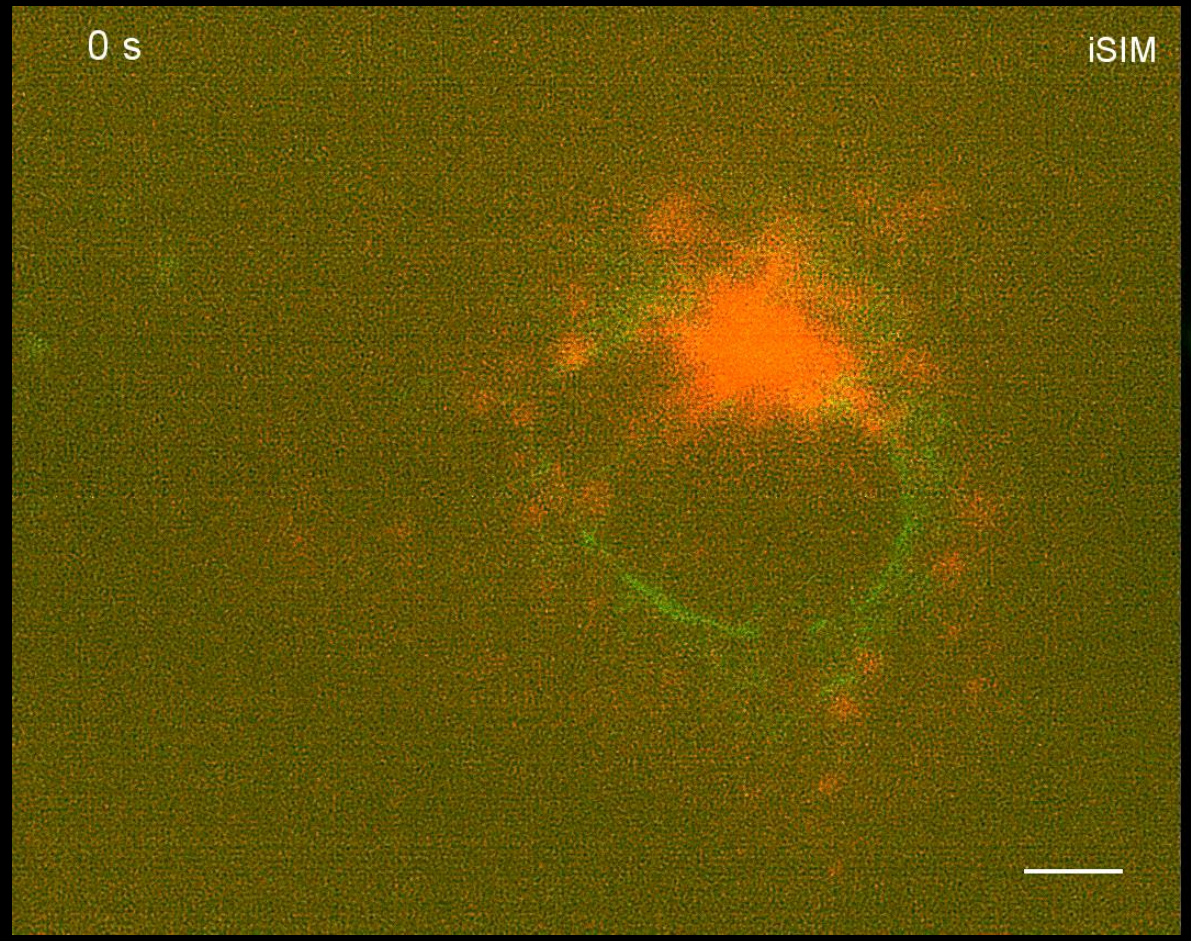
U2OS transfected with Mito-GFP
Imaged with iSIM, prohibitively high SNR
360 W/cm^2

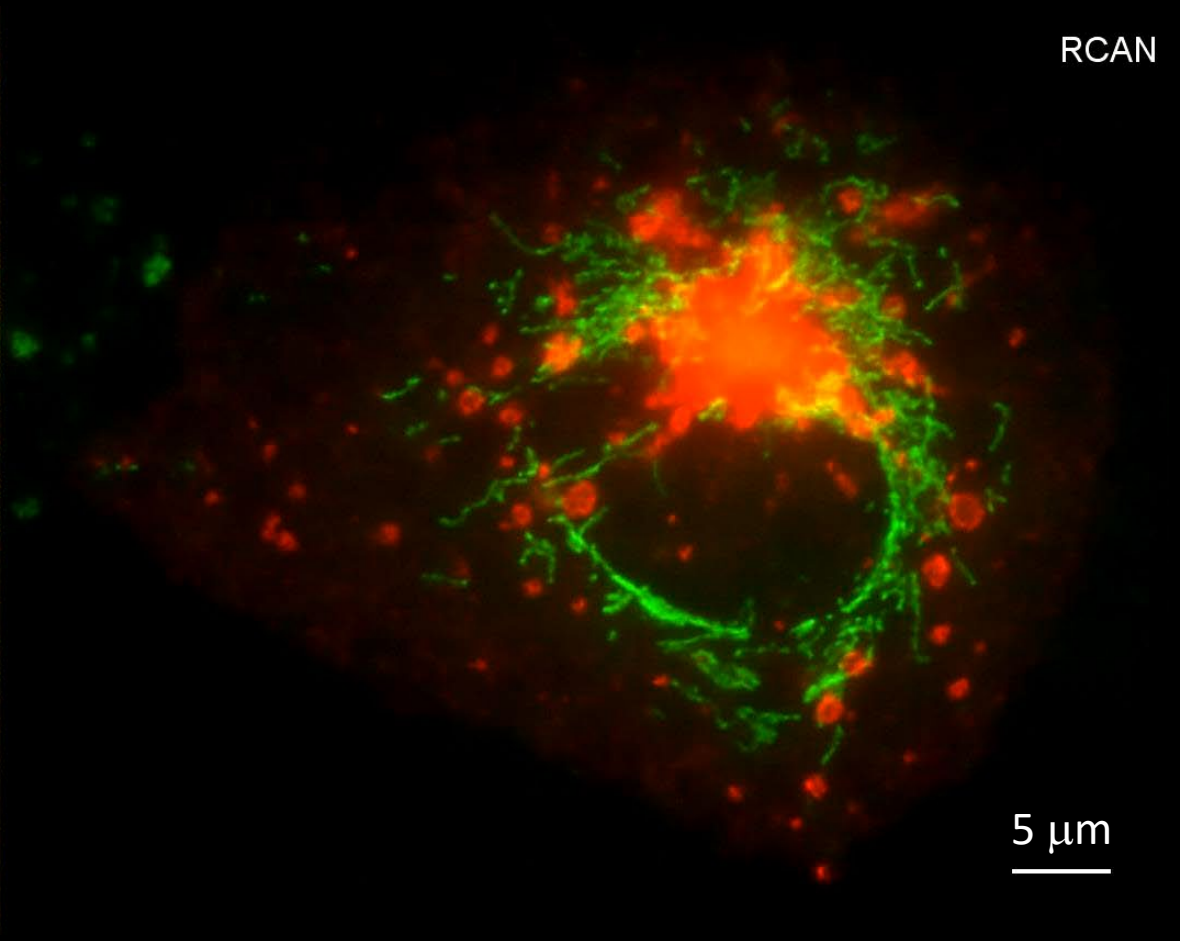




2.2 W/cm^2
2600 Volumes (!)
1 Volume = 24 planes
Every 5.6 s
Total ~50,000 images for 4 hours

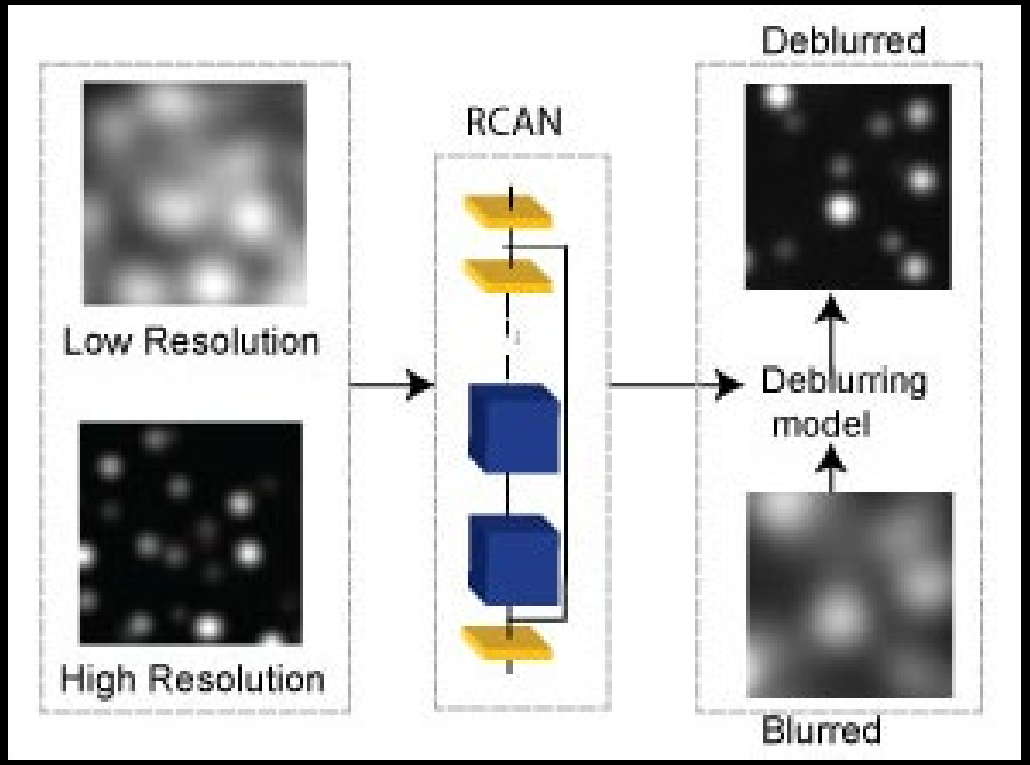
Denoising => operate super-resolution microscope indefinitely?

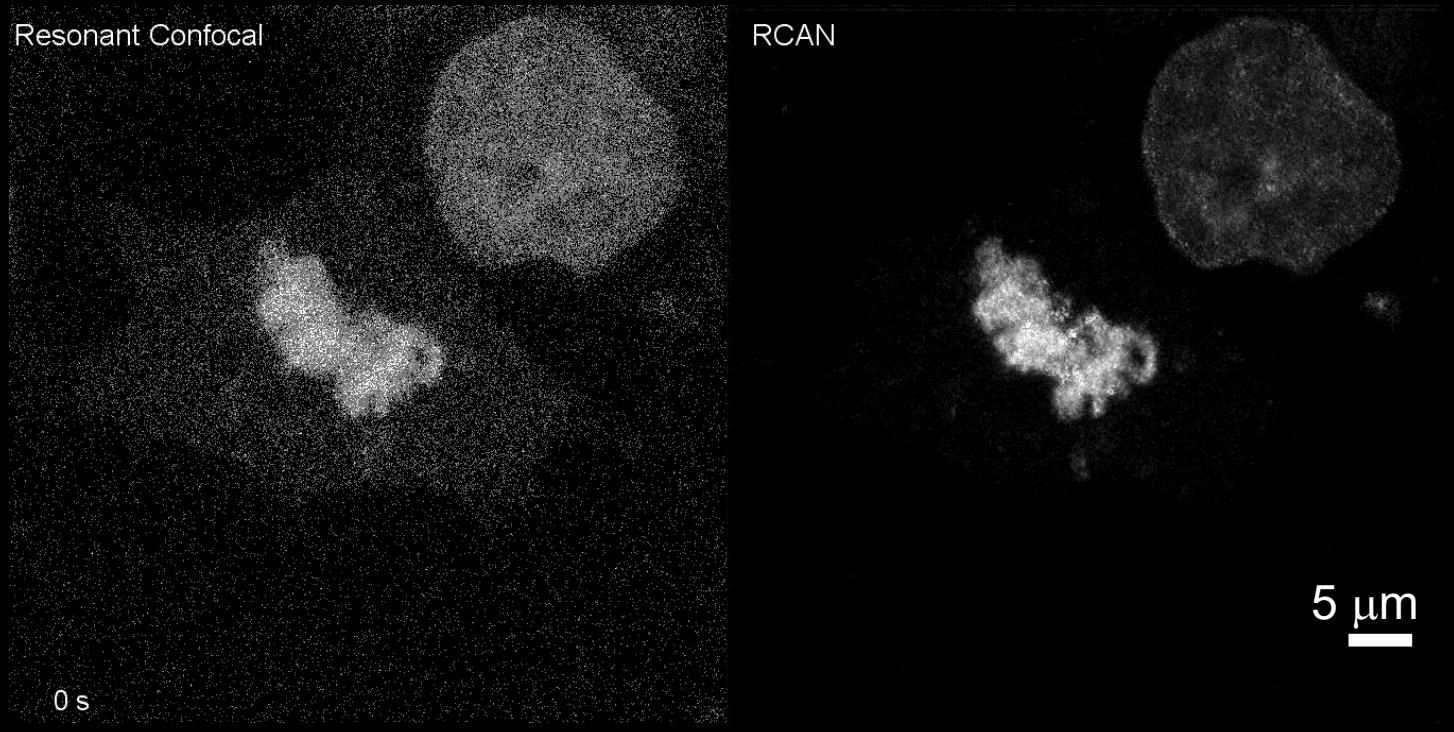




Green: Mitochondria (Mito GFP) Red: Lysosome (Lamp1-Apple)
1 volume = 12 slices (0.25 μm z step), time interval: 5.1 s, 300 volumes

RCAN for resolution enhancement, confocal volumes -> STED volumes



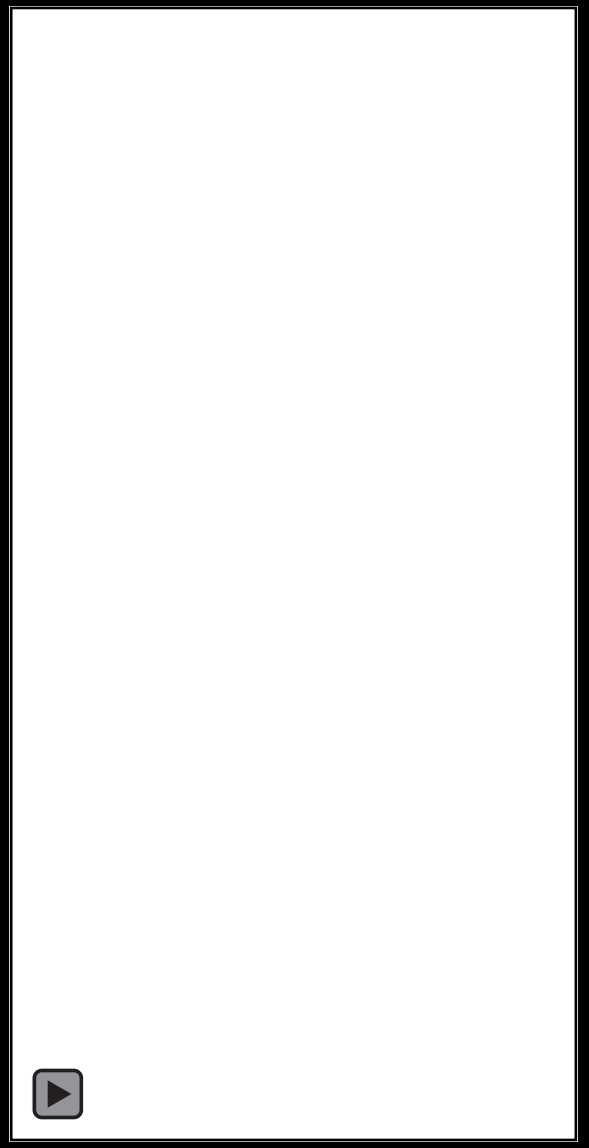


SiR DNA, live MEF cells
RCAN model trained on 22 matched confocal/STED volumes (fixed)

RCAN for iSIM-> expansion microscopy, living cells

iSIM decon

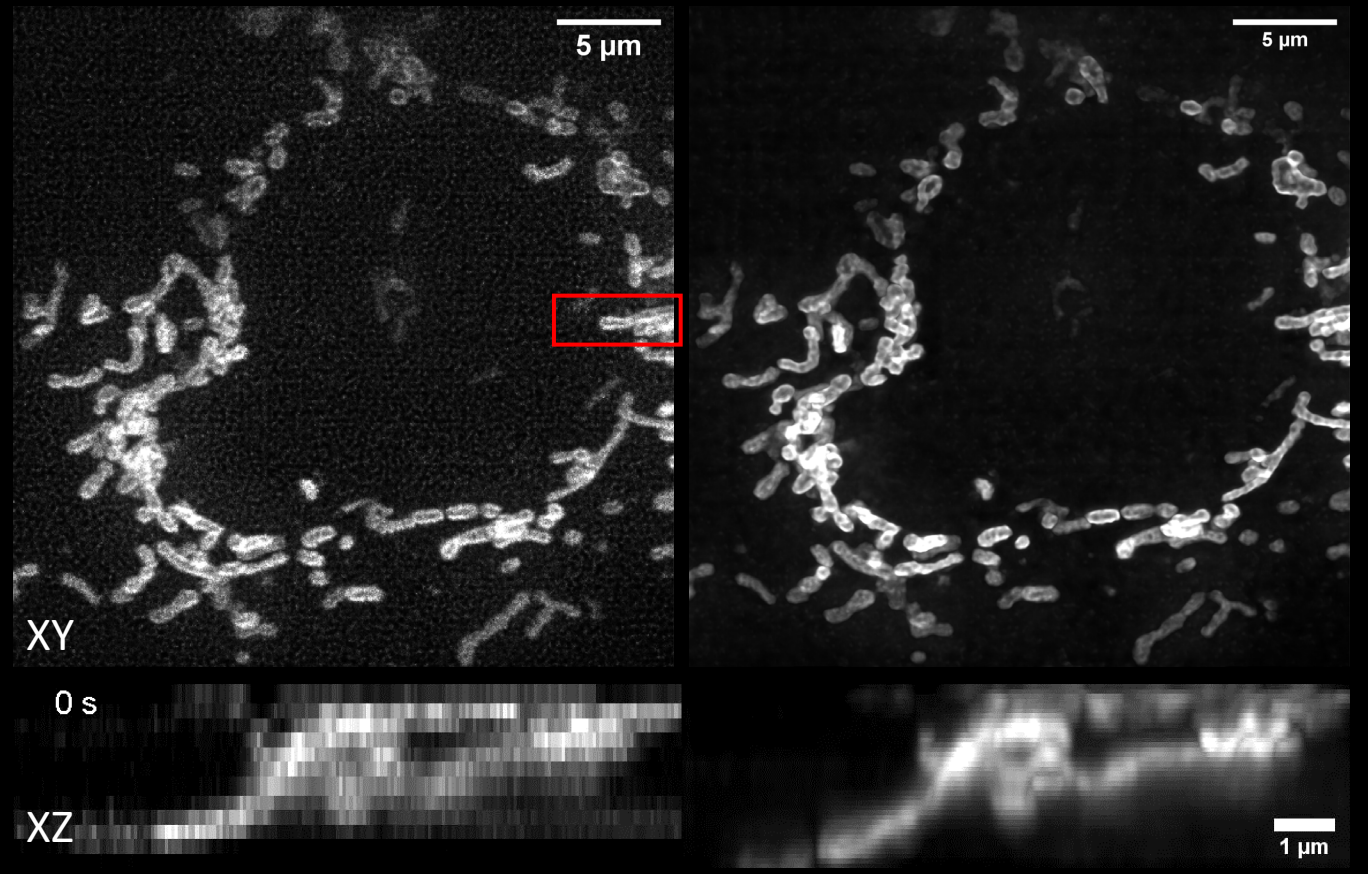
RCAN



EGFP-EMTB, Jurkat T cell, 1 vol/5 s

iSIM decon

RCAN



mEmerald-Tomm20, U2OS 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

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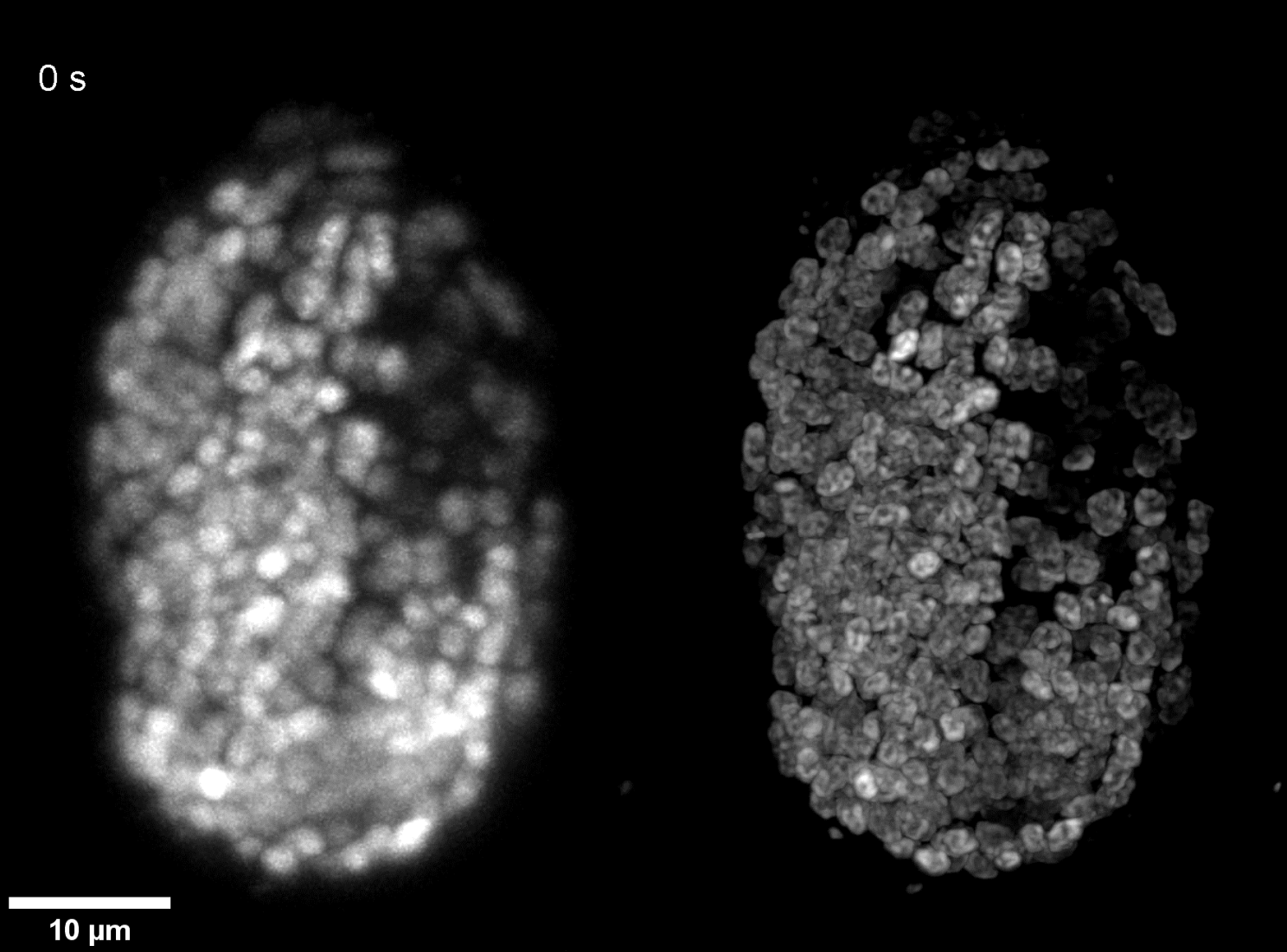
Triple RCAN output – most gentle way to attain super-resolution?

Gentle 4D super-resolution imaging in living embryos



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

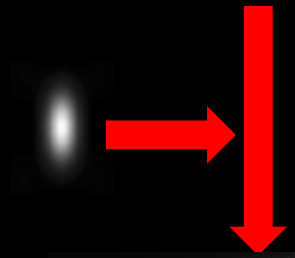
Triple RCAN output – most gentle way to attain super-resolution?

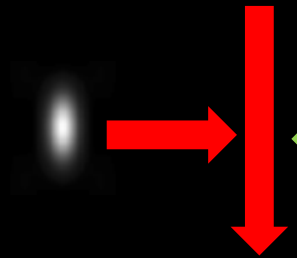
Current paradigm

Addition to the paradigm?

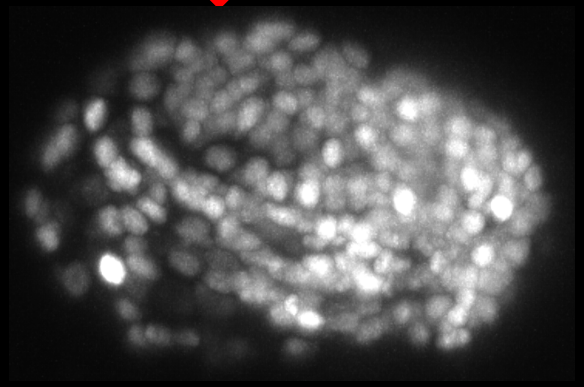
Microscope

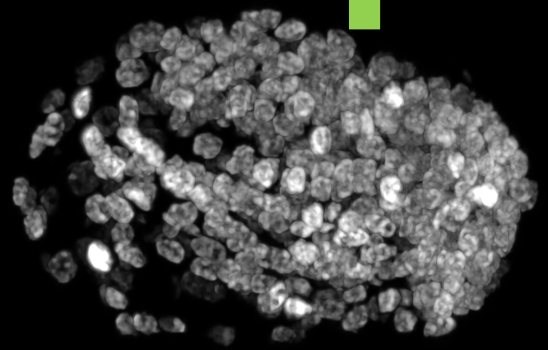
Imaging model





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!