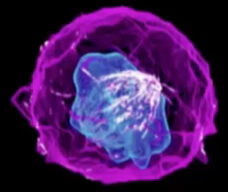
ALLEN INSTITUTE *for*
CELL SCIENCE

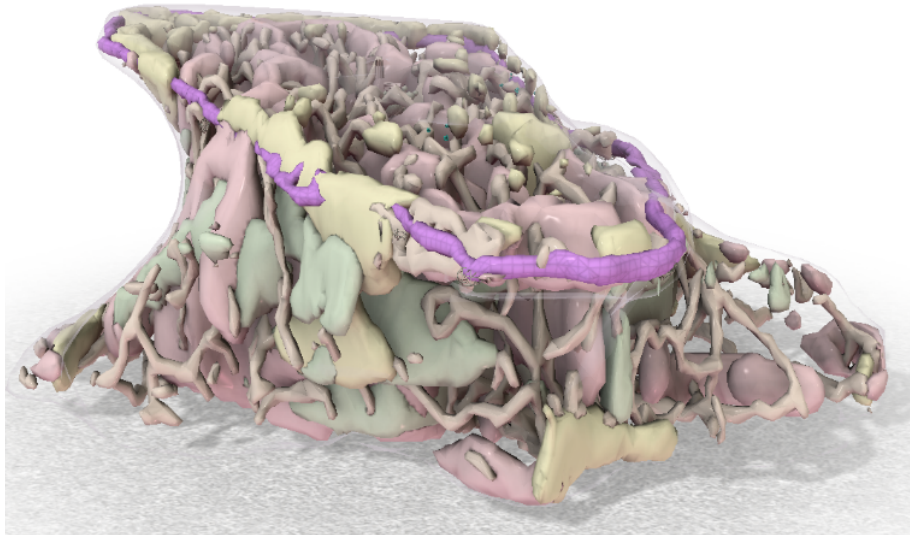


Animation, segmentation and statistical modeling of biological cells using microscopy imaging and GPU compute

Jianxu Chen (Scientist, Assay Development)

Look at a cell and know what it is doing

…what it did



what it will do…

cell organization ⇄ **function**

Basic science

- How to define and categorize cells to create a meaningful and useful cell atlas?
- What are the principles of cell organization?
- How do cells transition between states?

Differentiation/regeneration

Pathology/disease

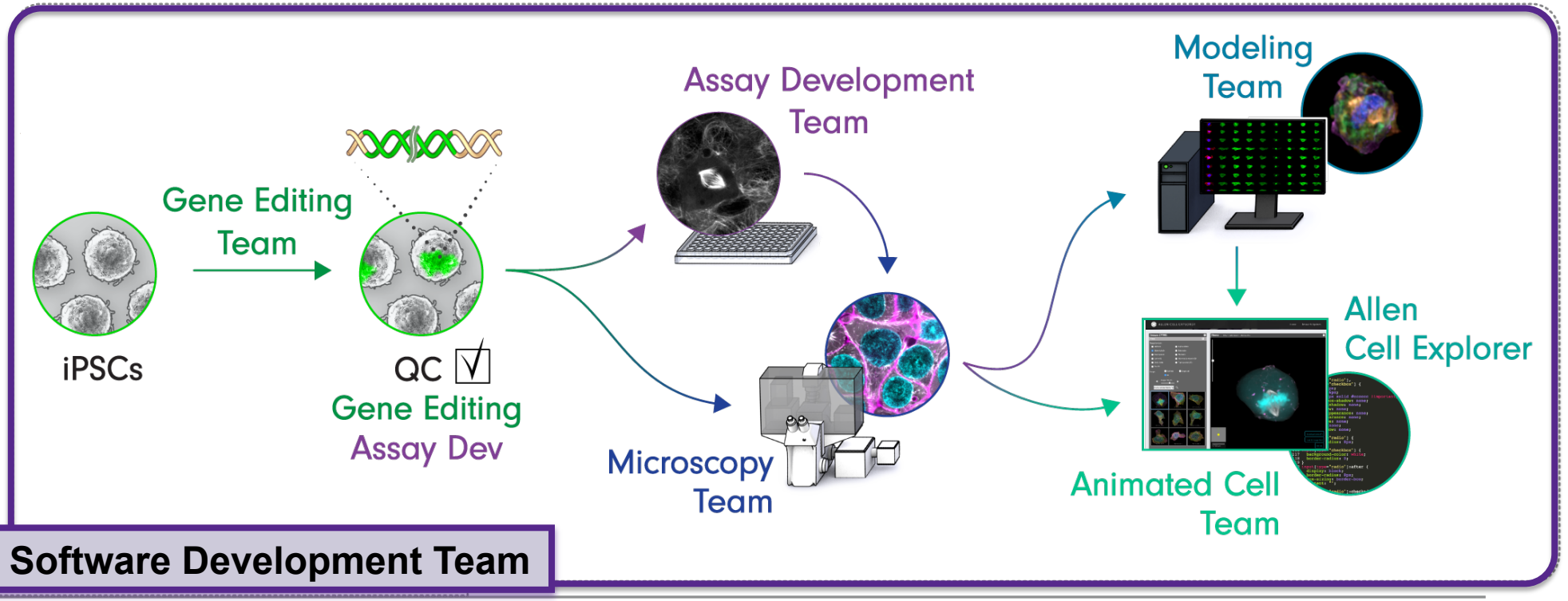
Holistic approach via 3D live cell imaging!

Create *and understand* a "state space" of human stem cell signatures

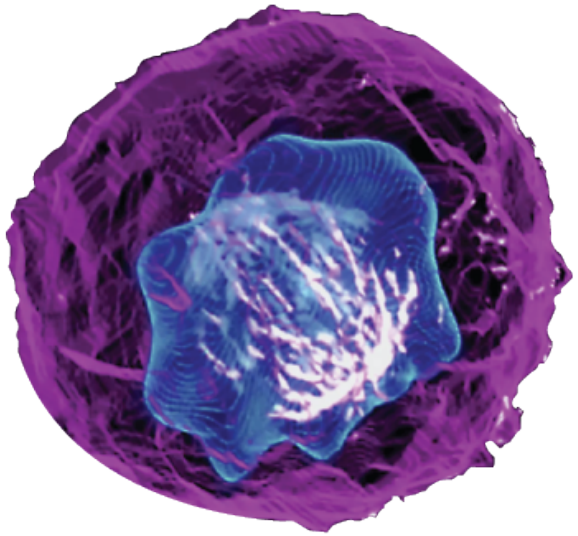
The Allen Institute for Cell Science organization and values

~4 years
~65 people

Institute values
- Team science
- Open science
- Community engagement



The Allen Cell Explorer Tool Kit



Cell creator

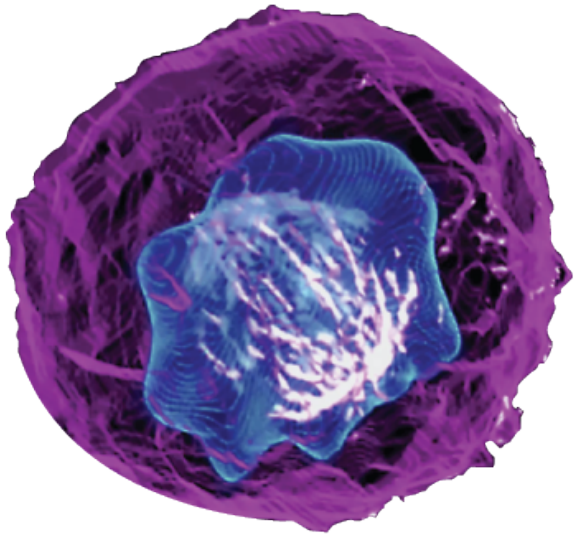
Cell image generator

Cell image analyzer

Cell image visualizer

Cell image simulator

The Allen Cell Explorer Tool Kit



Cell creator

Cell image generator

Cell image analyzer

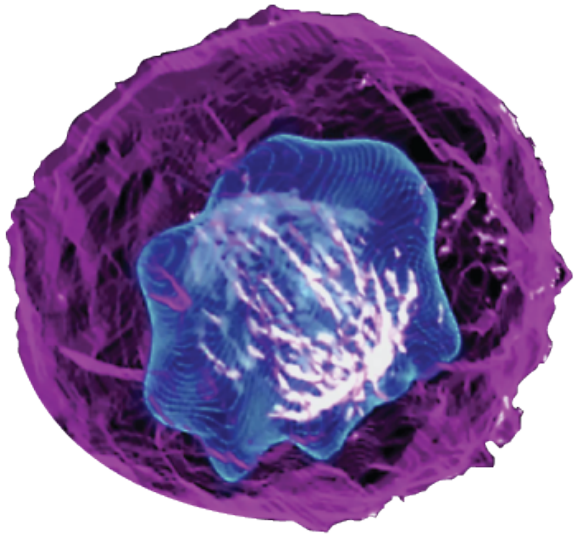
Cell image visualizer

GPU based

Cell image simulator

GPU computing opens a new door for research in cell biology.

The Allen Cell Explorer Tool Kit



Cell creator

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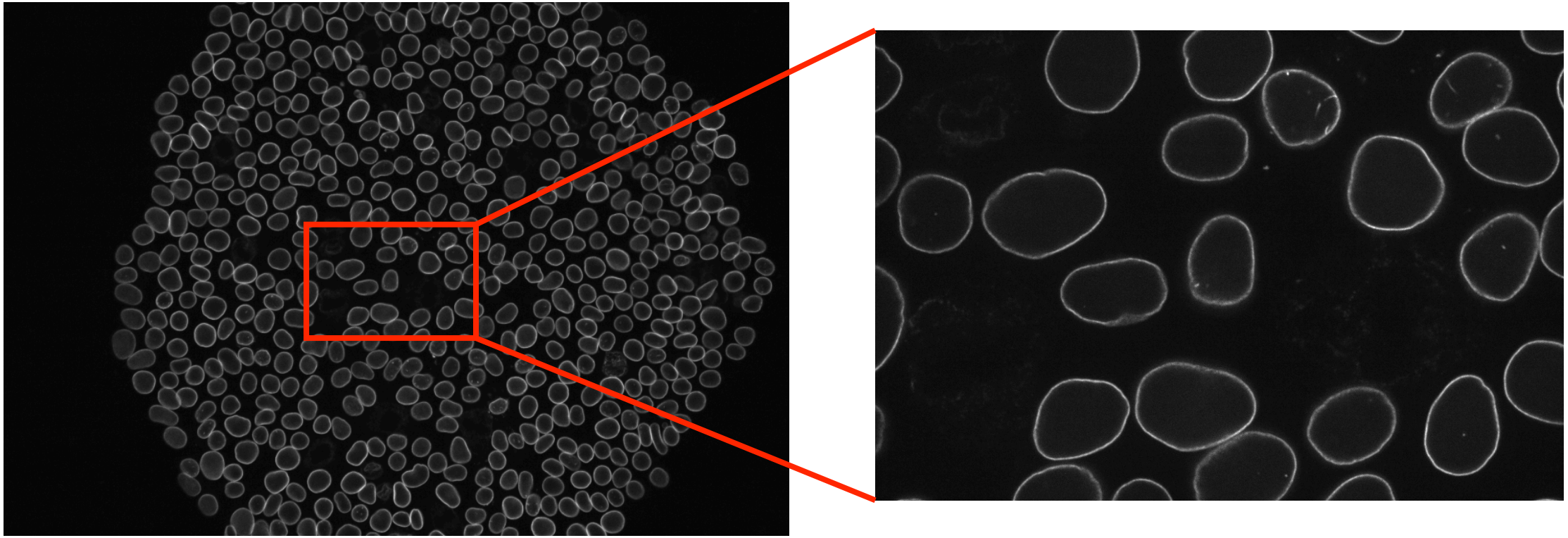
Cell image simulator

Building computational transfer functions on 3D light microscopy images

All images are 3D

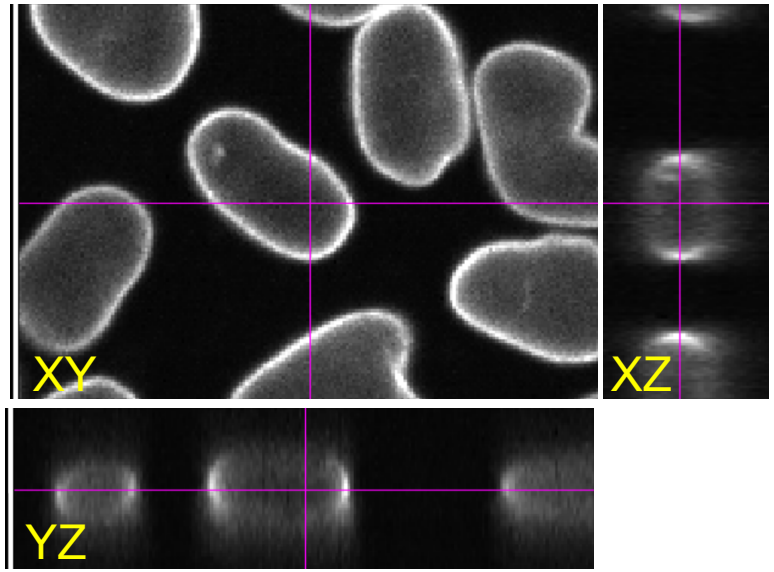


20x (0.8 NA) spinning disk microscopy image

100x (1.25 NA) spinning disk microscopy image

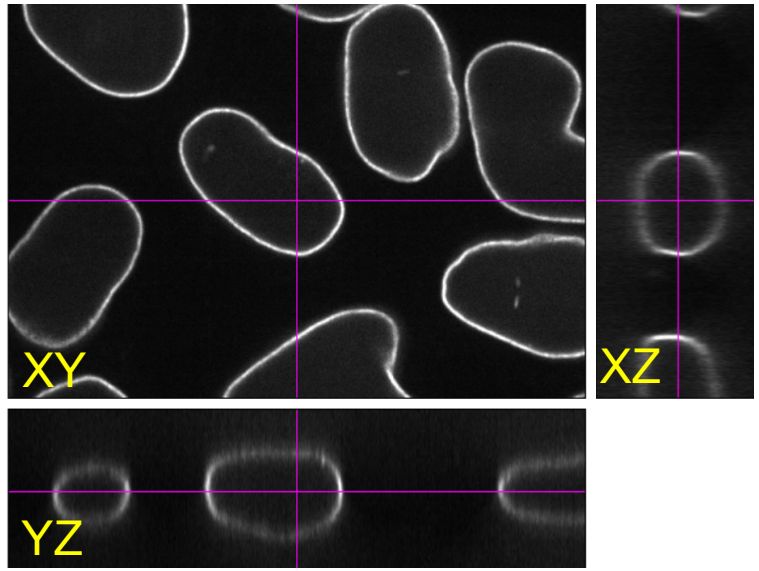
Comparison of lamin B1 at different magnifications/resolutions

lamin B1 at 20x 0.8NA
(rescaled to same size as 100x)



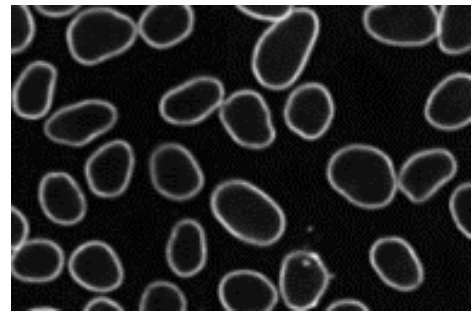
Pros	Larger field of view (FOV), low phototoxicity, long timelapse (~2 days)
Cons	Low resolution, hard to extract accurate nuclear shape, esp. in Z

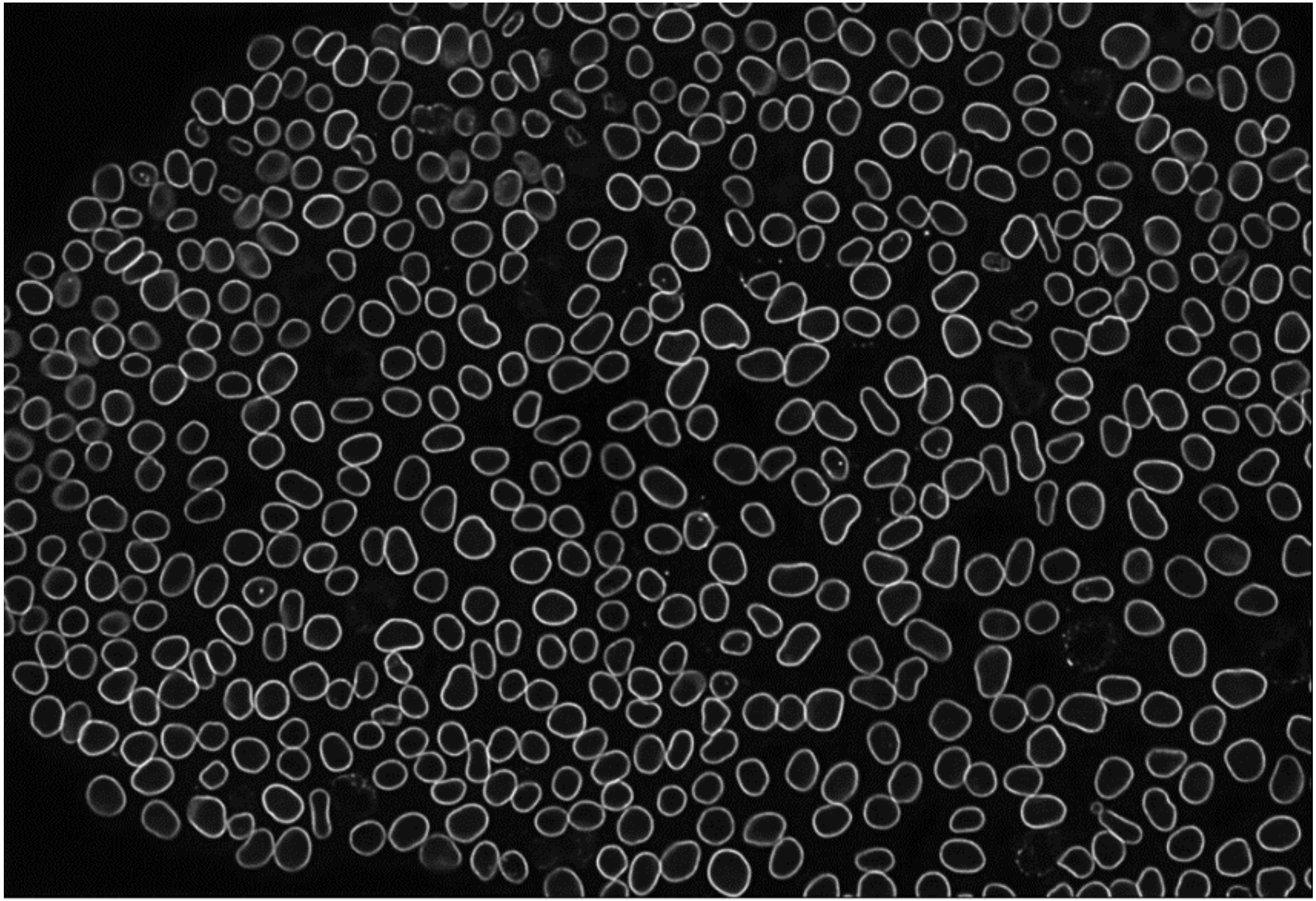
lamin B1 at 100x 1.25NA



Pros	High resolution for accurate nuclear shape measurement
Cons	Smaller FOV, only short timelapse (~2hr) due to phototoxicity

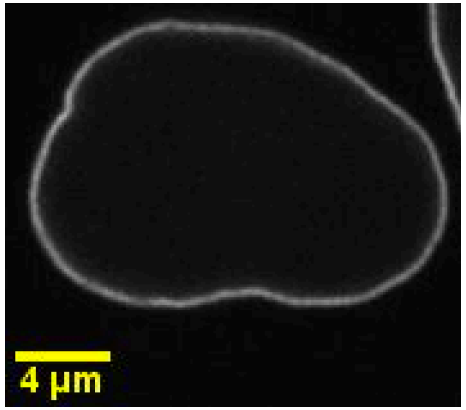
Can we transform images?



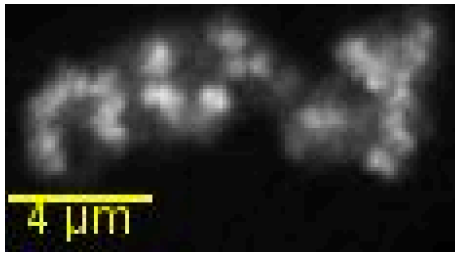


Transfer functions can be …

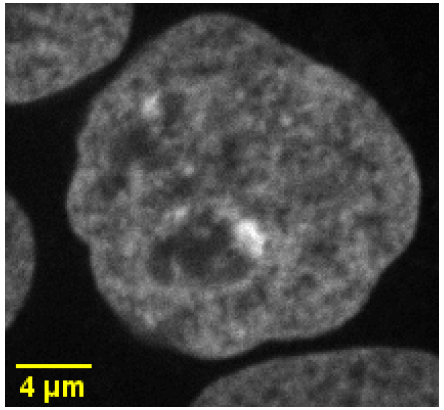
different intracellular structures with different features:



lamin B1
(nuclear envelope)



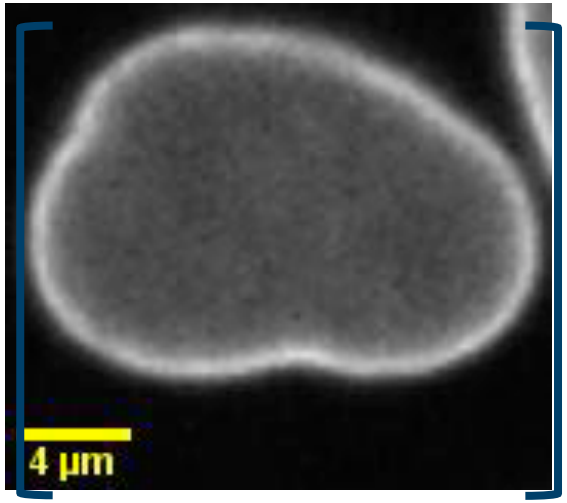
fibrillarin
(nucleolus)



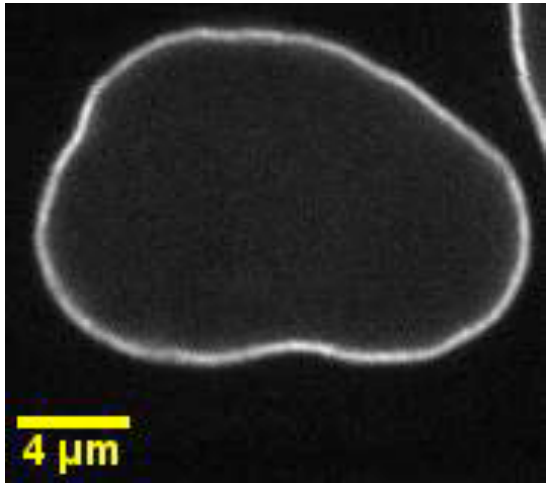
histone H2B
(histones)

Transfer functions can be …

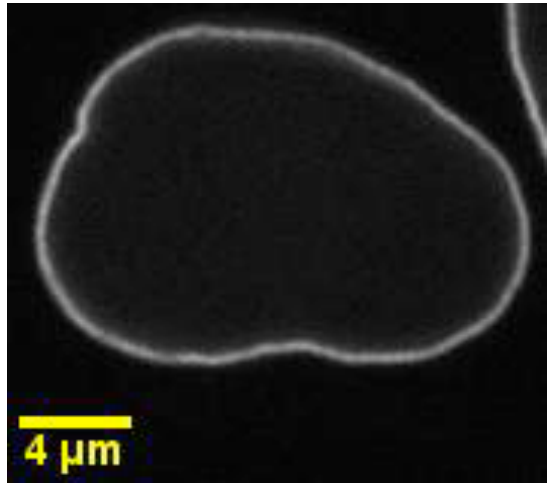
different intracellular structures with different features:



real 20x, 0.8NA
(rescaled to 100x)



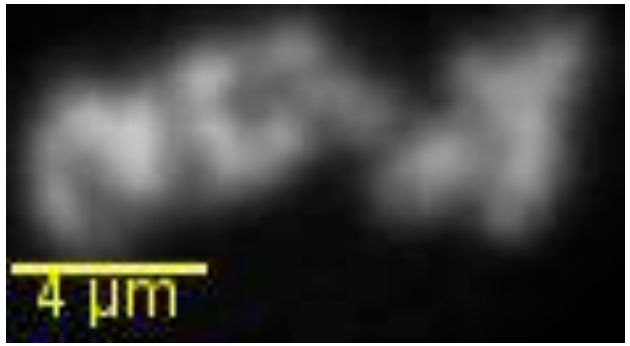
predicted 100x,
1.25NA



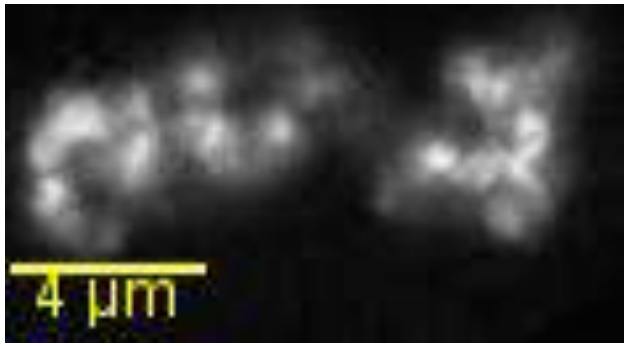
real 100x,
1.25NA

Transfer functions can be …

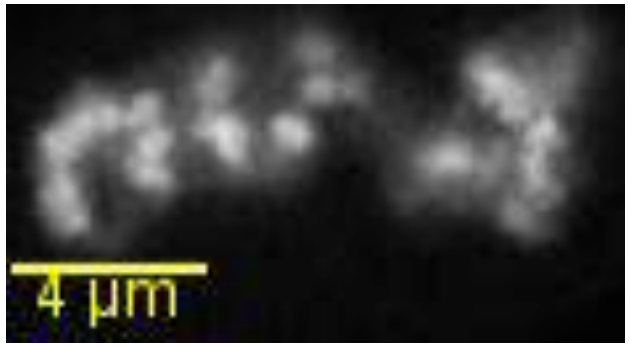
different intracellular structures with different features:



real 20x, 0.8NA
(rescaled to 100x)



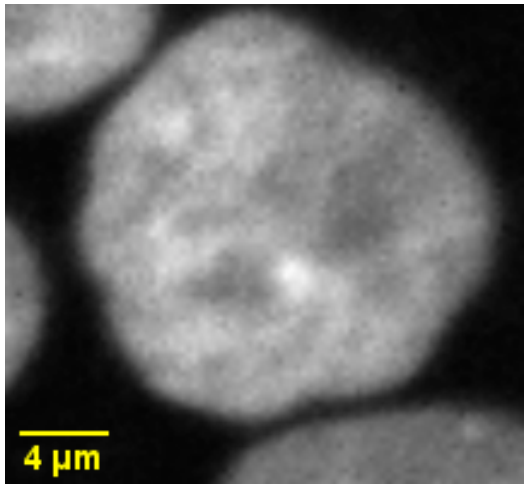
predicted 100x,
1.25NA



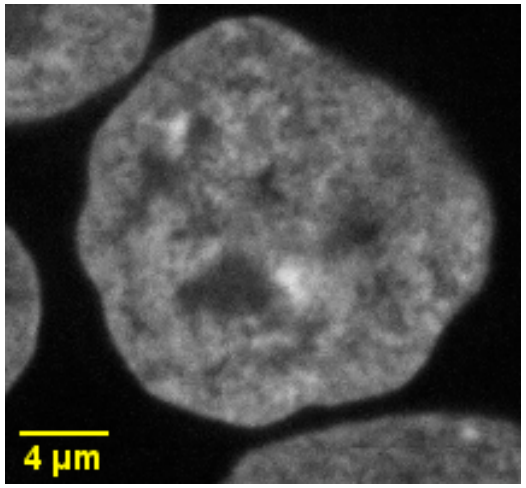
real 100x,
1.25NA

Transfer functions can be …

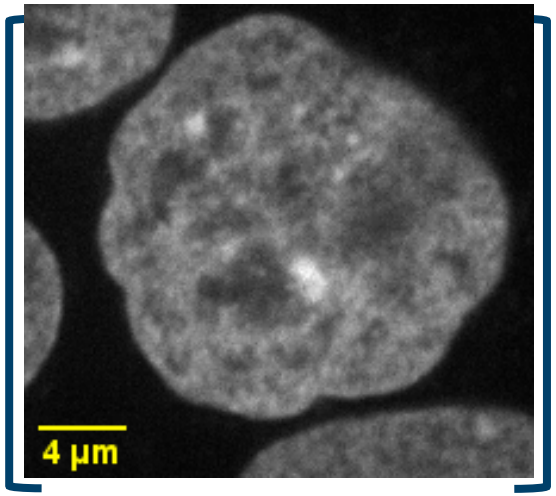
different intracellular structures with different features:



real 20x, 0.8NA
(rescaled to 100x)



predicted 100x,
1.25NA

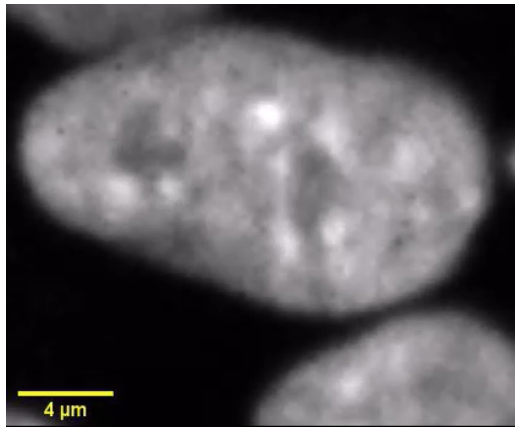


real 100x,
1.25NA

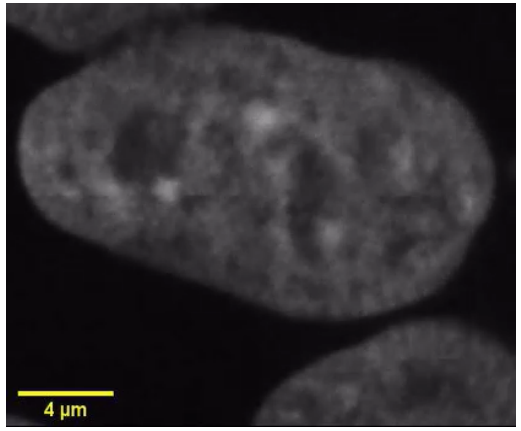
Transfer functions can also be …

Daisy-chained for further improvement:

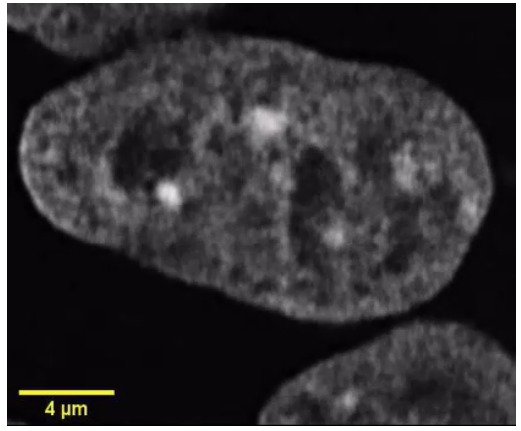
Real 20x spinning disk image
(rescaled to the same size as AiryScan FAST)



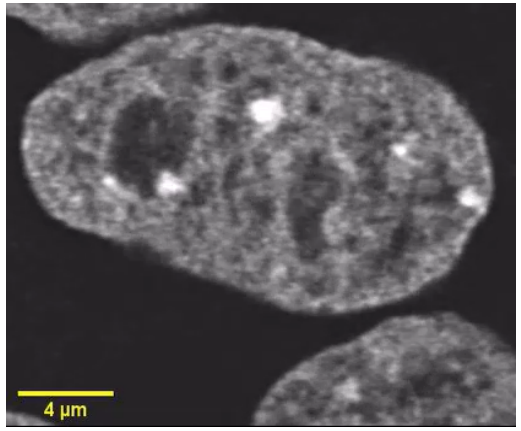
predicted 100x spinning disk image
(rescaled to the same size as AiryScan FAST)



predicted ZEISS LSM 880 AiryScan FAST image*



real ZEISS LSM 880 AiryScan FAST image

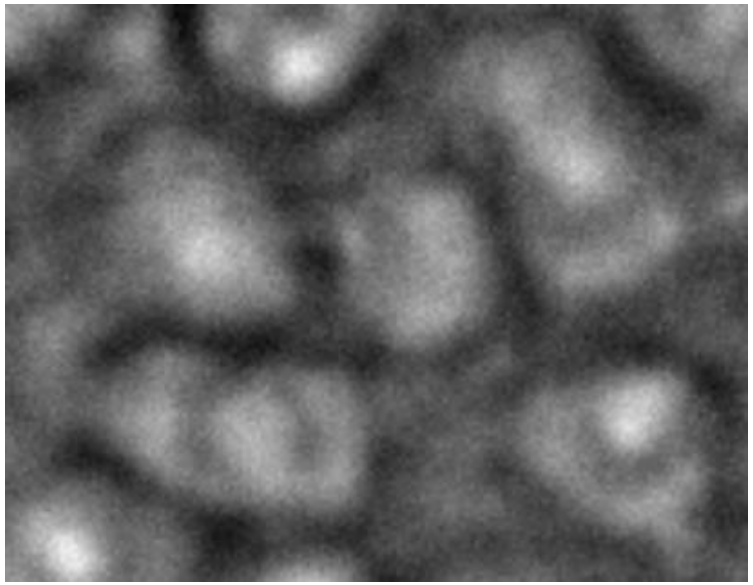


histone H2B

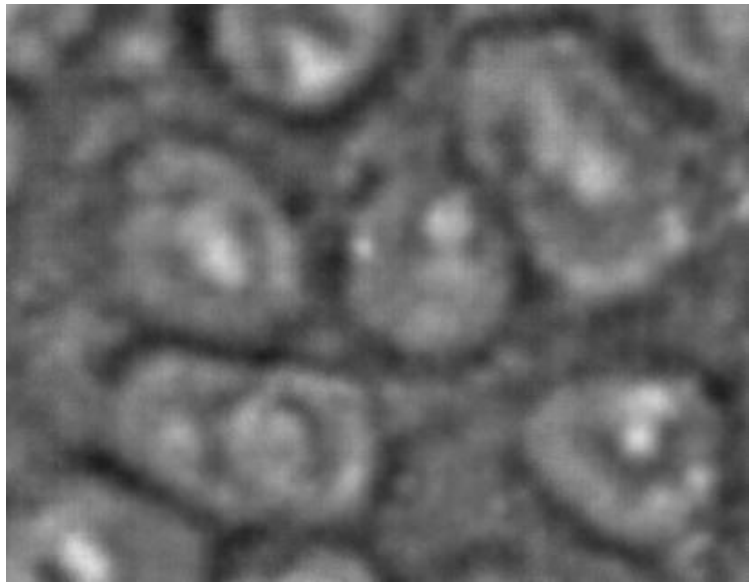
* laser scanning confocal microscopy with ~1.5x enhanced resolution

Transfer functions can also be …

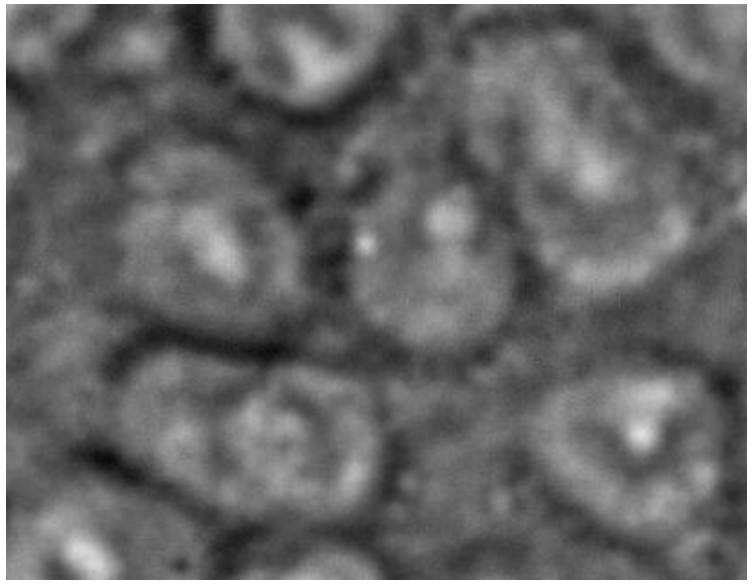
on bright-field images



real 20x, 0.8NA
(rescaled to 100x)



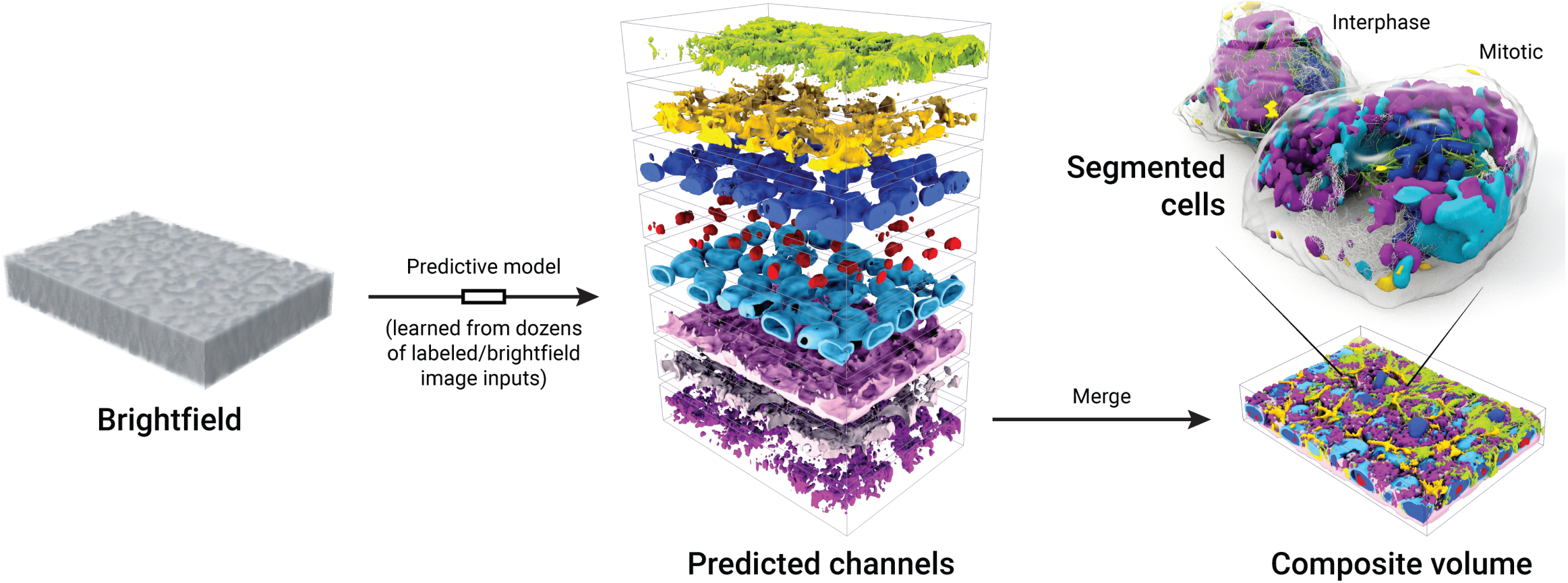
predicted 100x, 1.25 NA



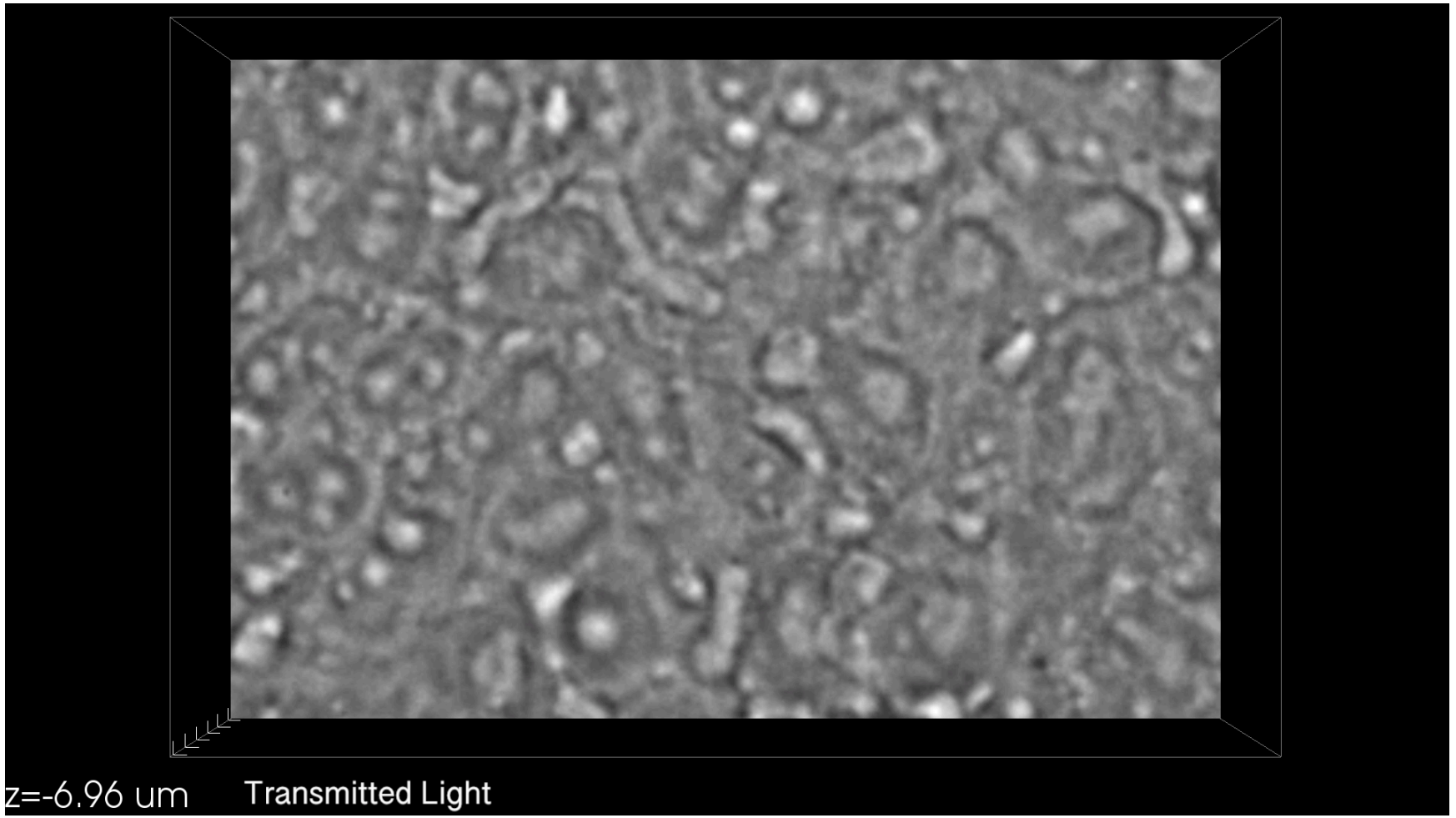
real 100x, 1.25 NA

A special cell image generator:

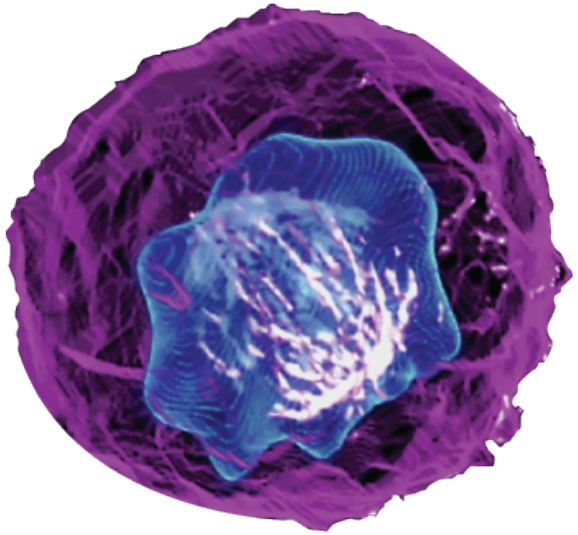
‘Label-free’ deep learning approach predicts fluorescence images of intracellular structures from 3D bright-field images



“Label-free” allows biologists to see through the cells without fluorescent dyes:



The Allen Cell Explorer Tool Kit



Cell creator

Cell image generator

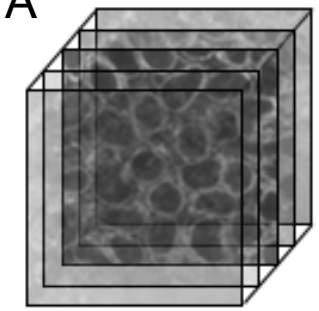
Cell image analyzer

Cell image visualizer

Cell image simulator

The Allen Cell Structure Segmenter: an open-source toolkit for segmenting 3D intracellular structures in fluorescence microscopy images

A

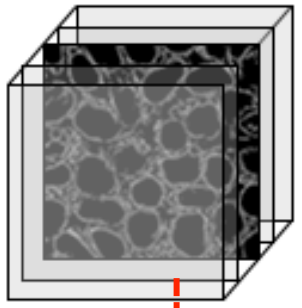


Original single channel 3D image stack

Pre-processing

Core Segmentation Algorithms

Post-processing



3D binary image stack

analysis or models

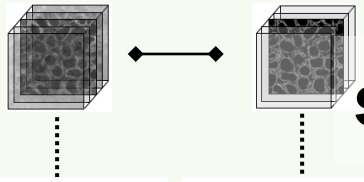
jupyter + python™

Classic Image Segmentation Workflow

B

improve accuracy and robustness

Training data



Fluorescent images

Ground truth images

sorting/merging

Deep learning model

Iterative Deep Learning Workflow

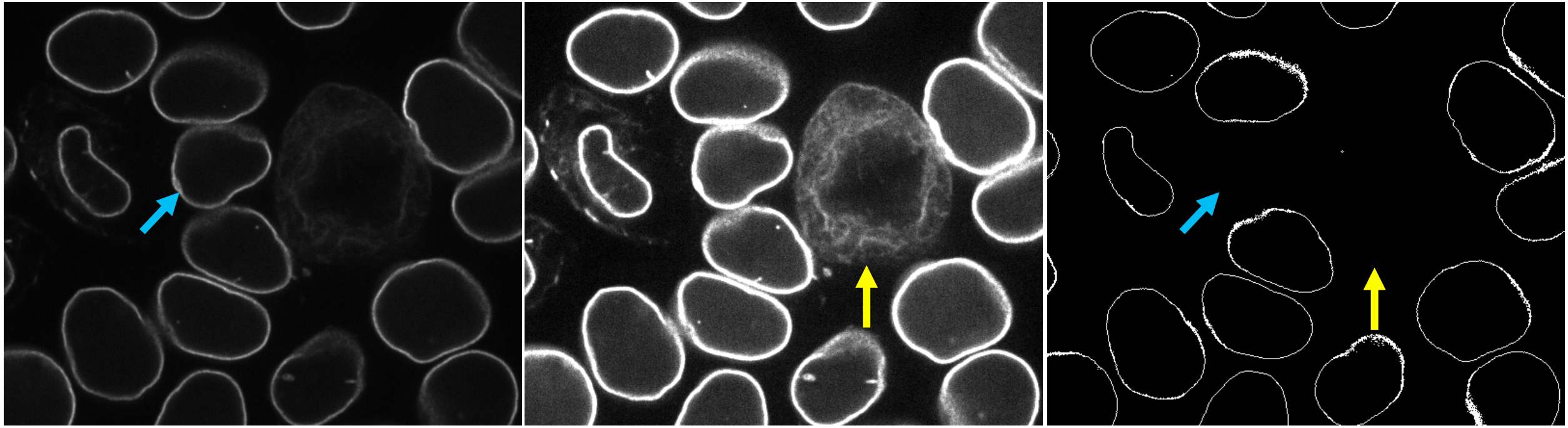
www.allencell.org/segmenter

Applying the iterative deep learning workflow to create a robust lamin B1 segmentation model

Original lamin B1

Adjusted contrast

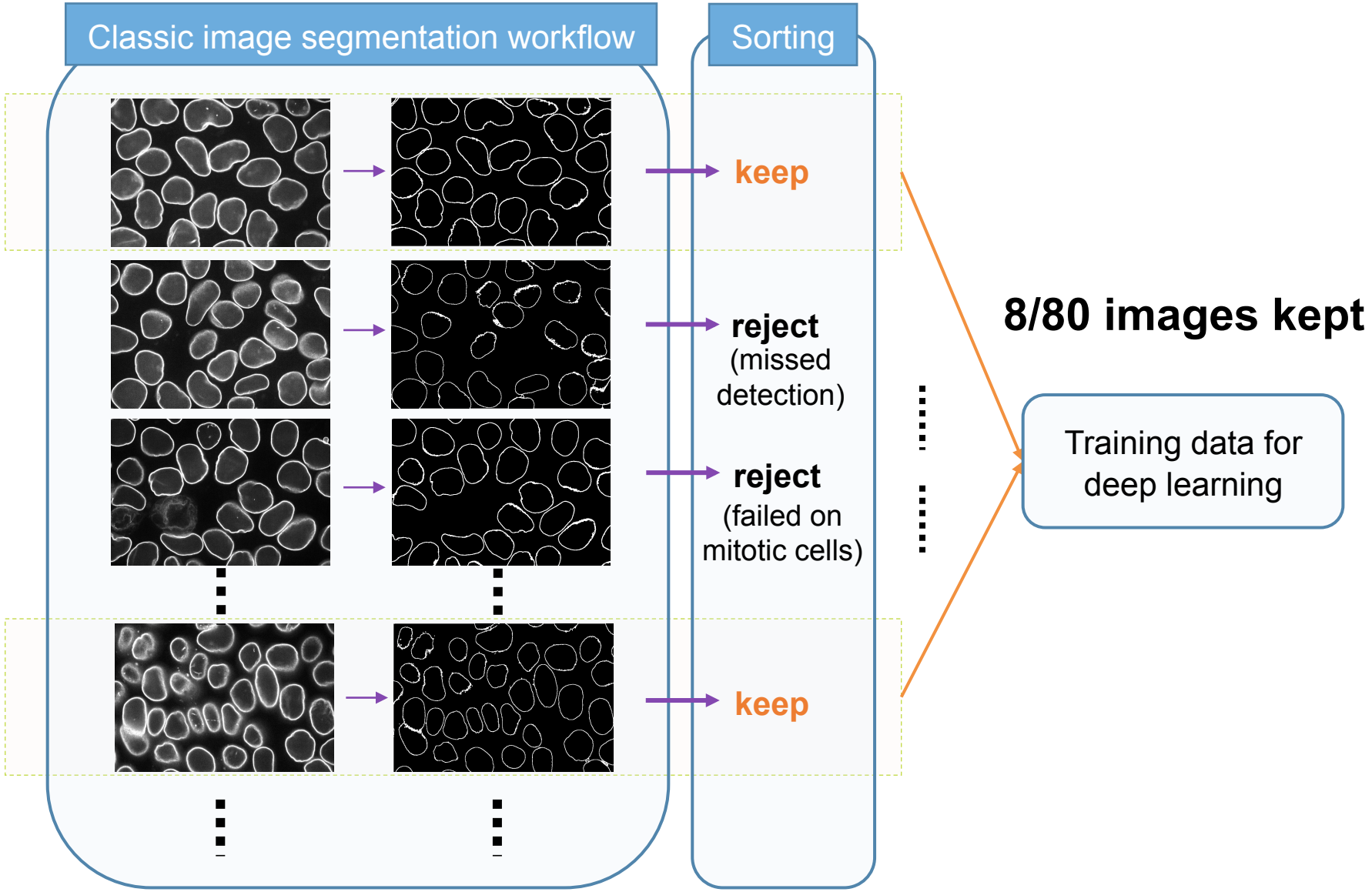
Lamin B1 interphase specific classic segmentation workflow



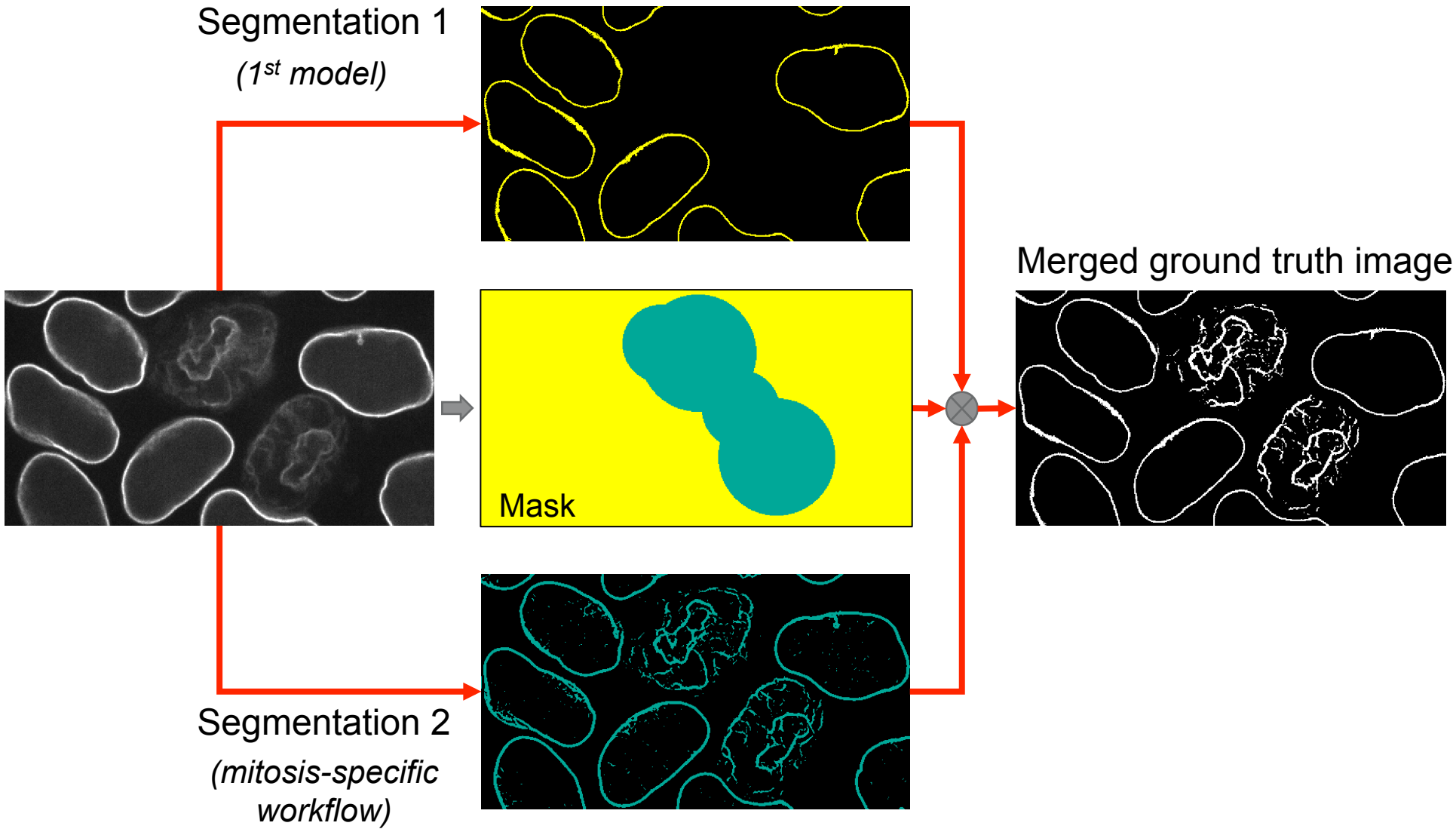
→ Error in interphase lamin B1

→ Error in mitotic lamin B1

Applying iterative deep learning: *sorting* initial segmentation results



Applying iterative deep learning: *merging* segmentation results

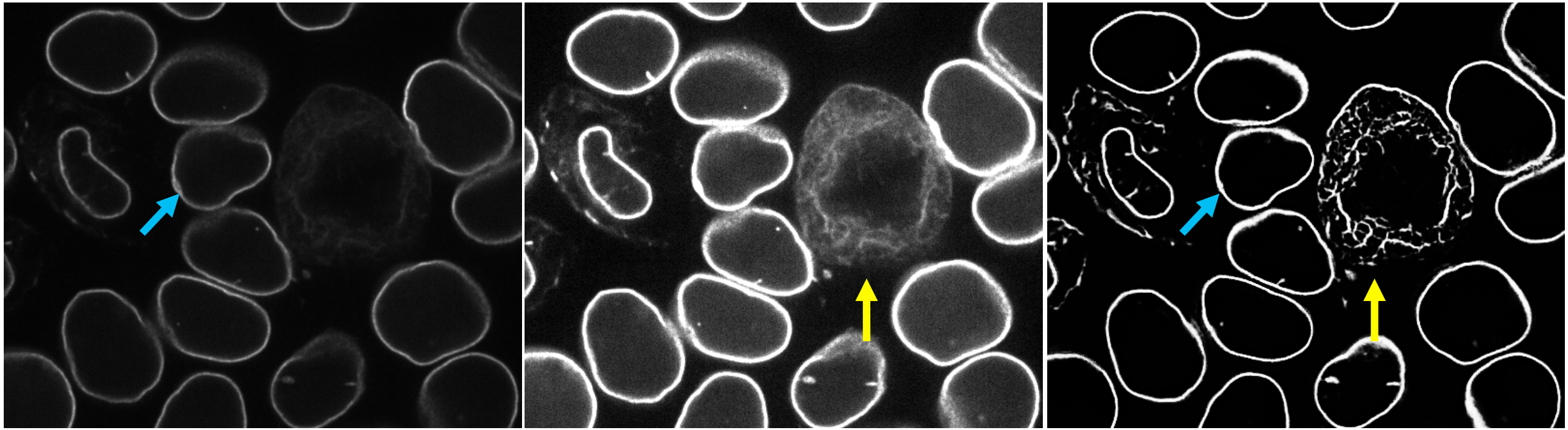


Applying the iterative deep learning workflow to create a robust lamin B1 segmentation model

Original lamin B1

Adjusted contrast

Lamin B1 interphase specific classic segmentation workflow

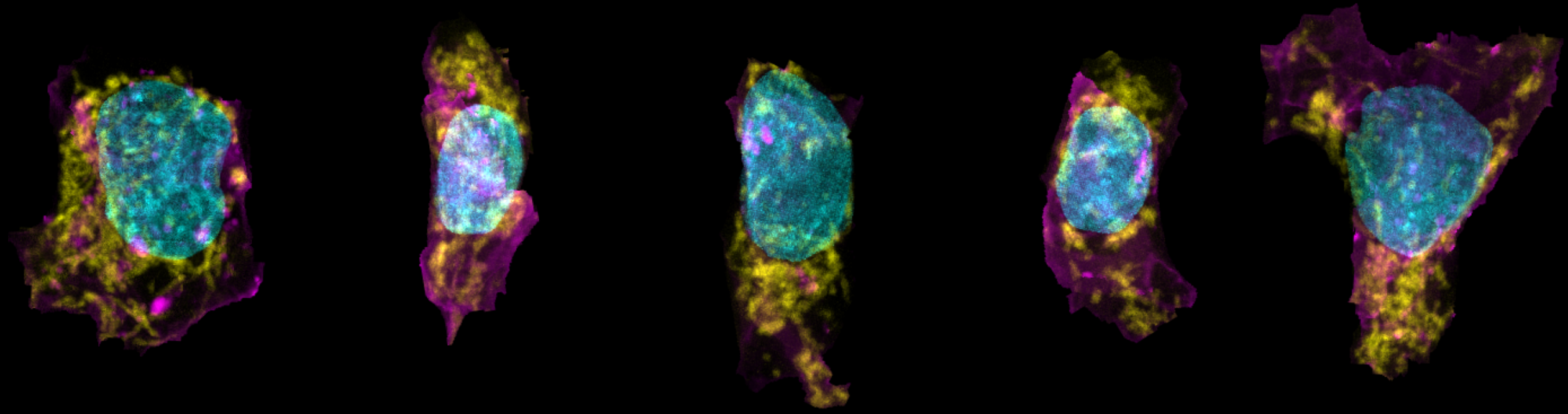


With sorting – interphase detection

With merging – mitotic detection

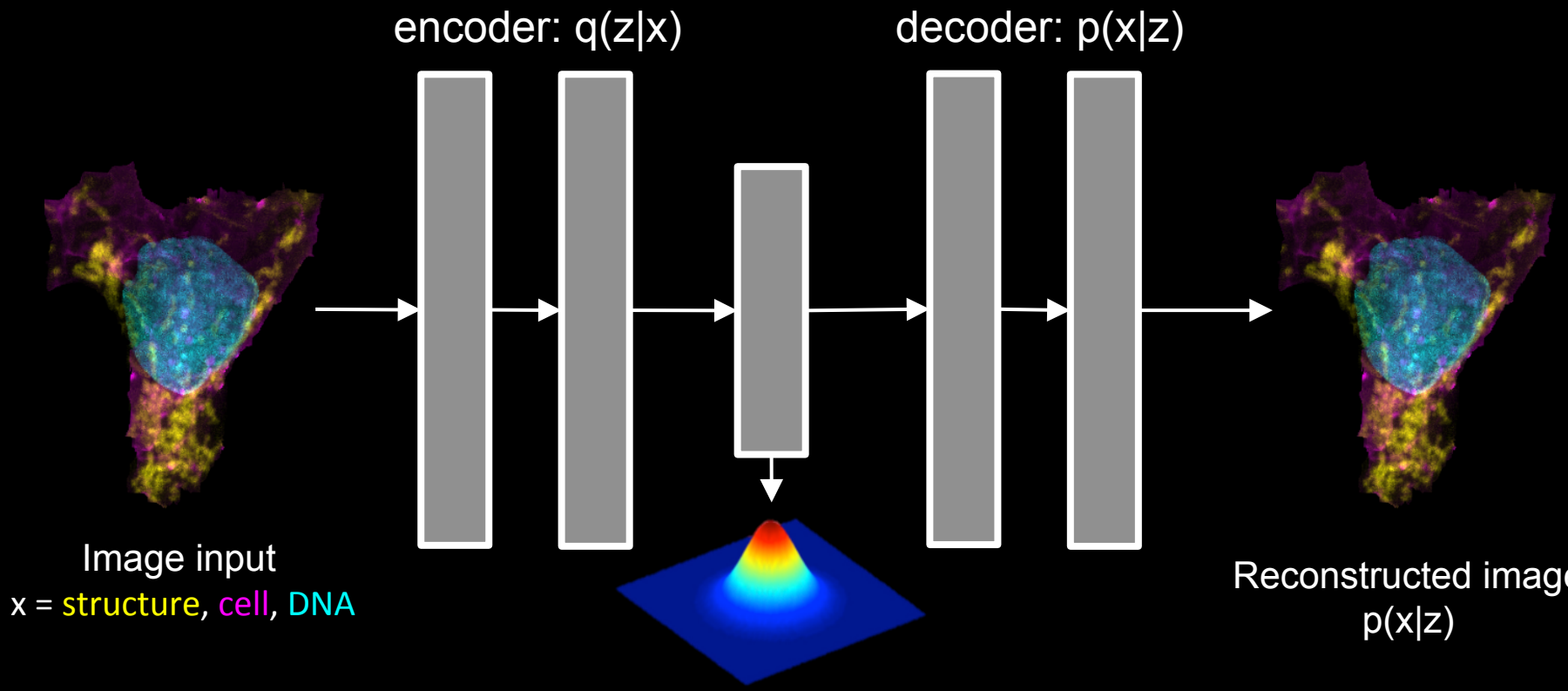
But, single cell image analysis is more than just segmentation ...

Modeling relationships between structures….



If we see a **cell** and **DNA** pattern, how much does that tell me about the **mitochondria**?

Variational autoencoders learn probabilistic representations of data

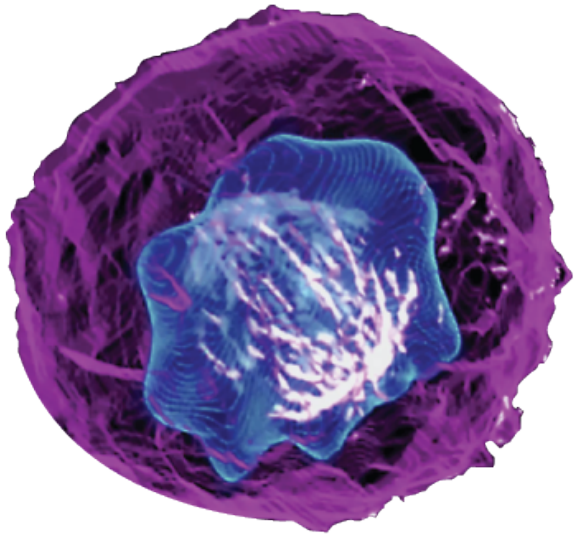


$$\ln p(x) \geq L(x) = E[\ln p(x|z)] - KL(q(z|x) \mid p(z))$$

How well did I reconstruct the image?

How complex was my representation

The Allen Cell Explorer Tool Kit



Cell creator

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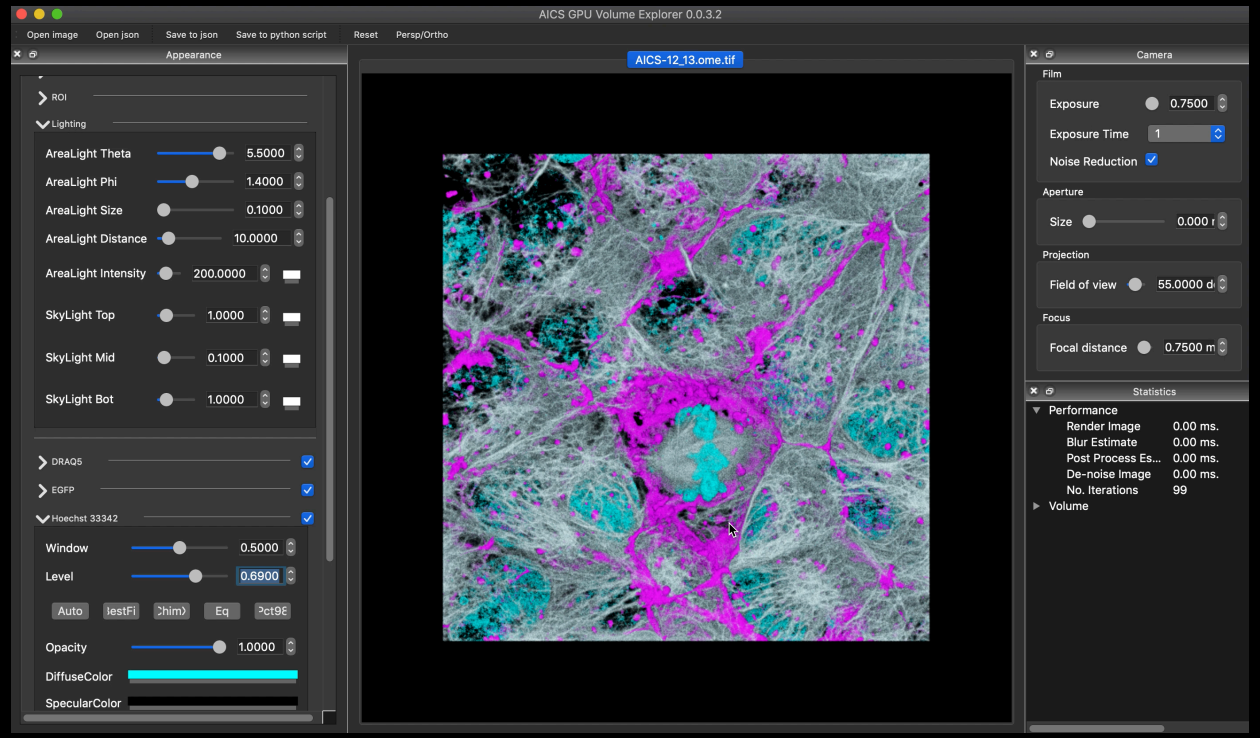
Cell image simulator

Path-trace rendering of 3D microscope images with AGAVE

Get AGAVE: allencell.org/software-and-code.html



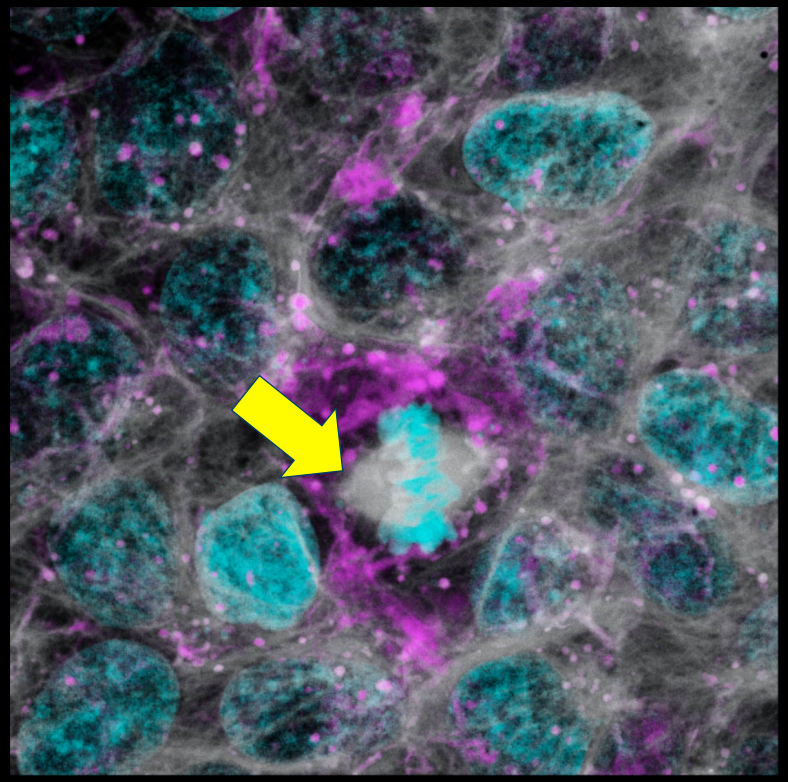
Advanced GPU Accelerated Volume Explore (AGAVE)

Desktop application for Linux, Windows, and MacOS

Cell data integration: enhancing 3D interpretability

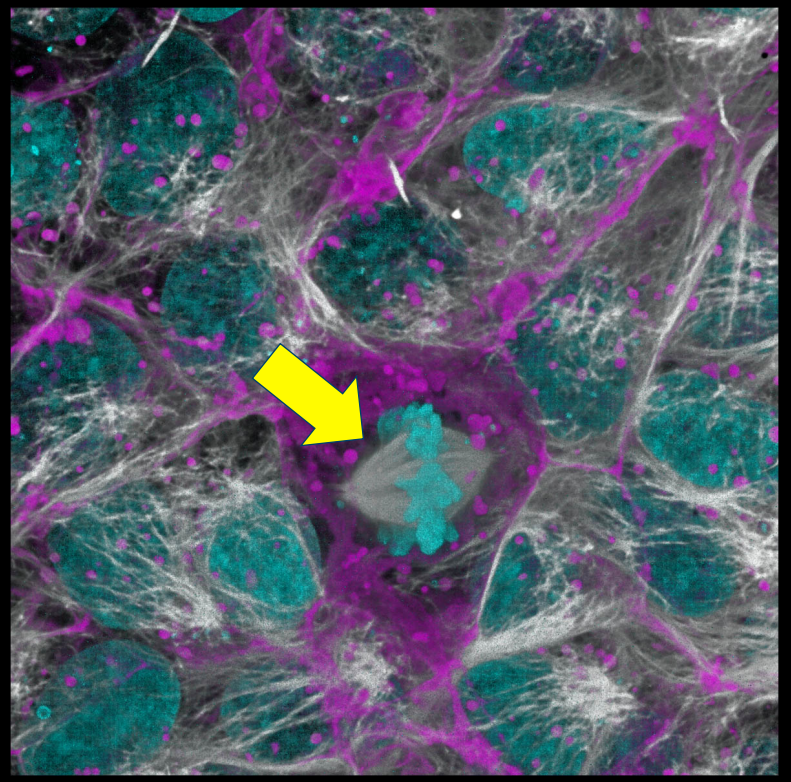
Advanced GPU Accelerated Volume Explore (AGAVE)

Classic

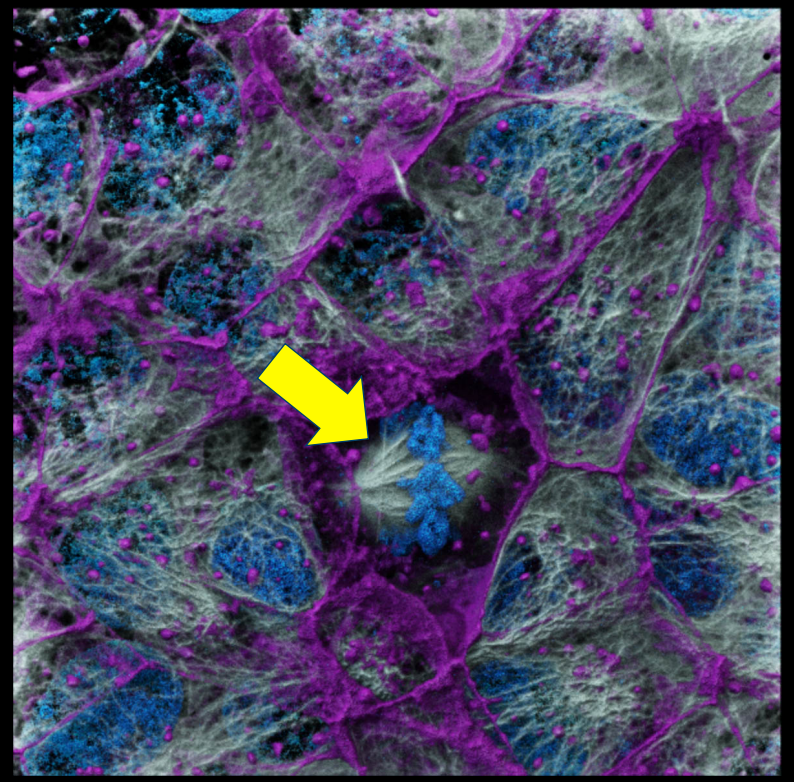


Standard volume rendering

AGAVE



AGAVE transparent path-tracing

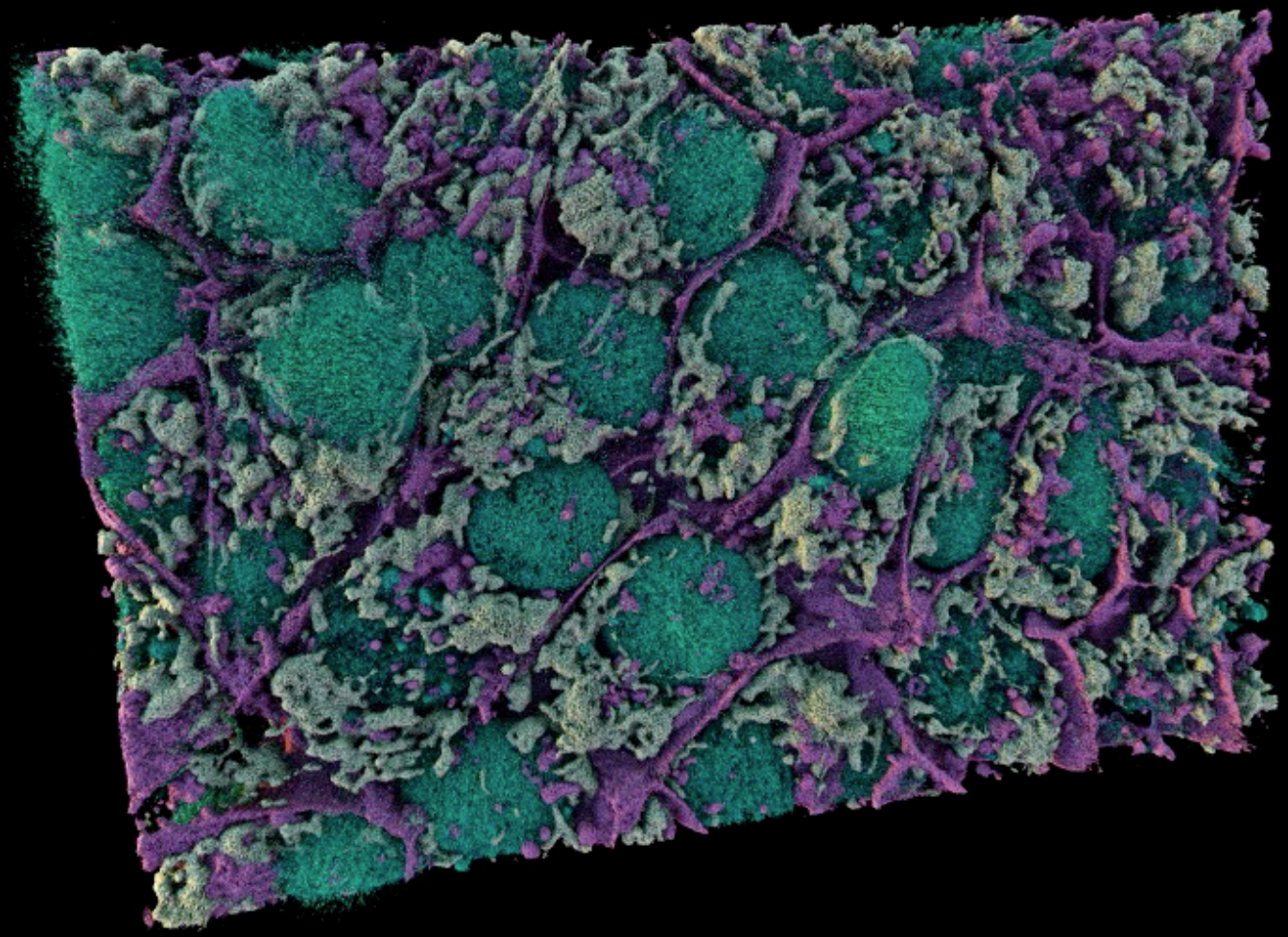


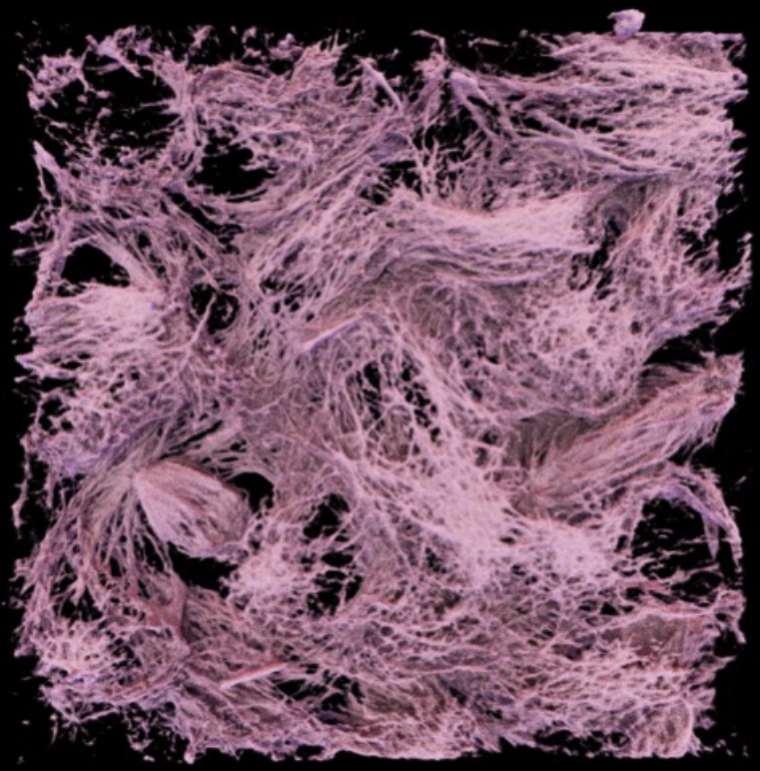
AGAVE cinematographic path-tracing

Cell membrane dye | DNA dye | GFP-tagged structure

Why go to the computational expense of pathtracing?

Contributions beyond ambient occlusion: shadow casting can clarify topology

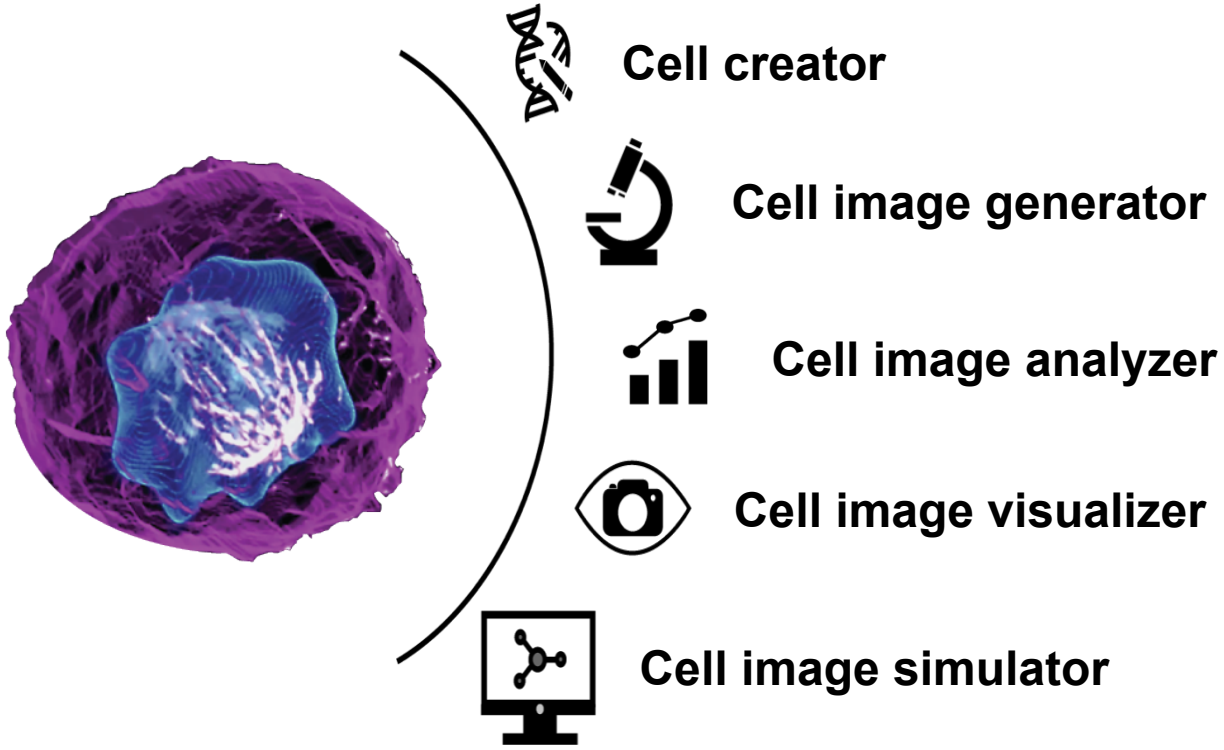




Time series with AGAVE via Python

Get AGAVE: allencell.org/software-and-code.html

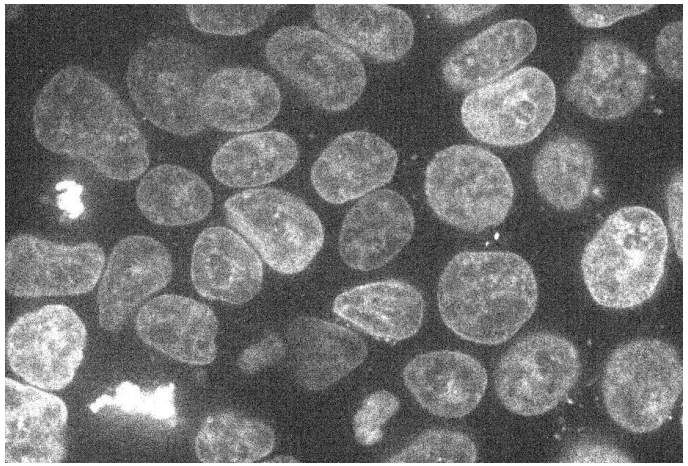
Lattice light sheet timelapse of lamin B1 rendered with AGAVE

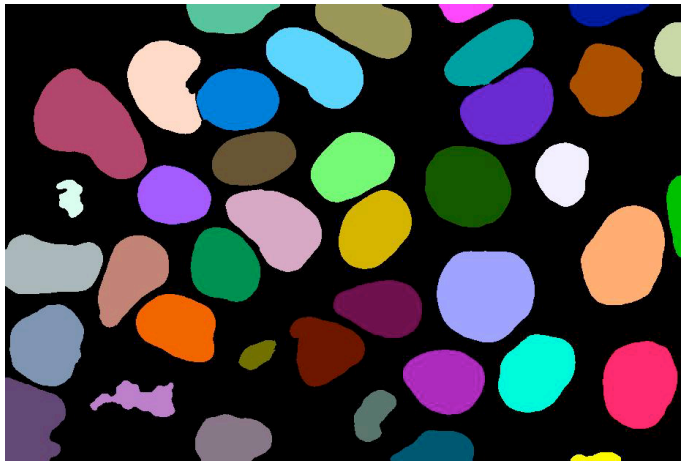


GPU computing greatly facilitates cell biology research, but …

Applying deep learning here requires new ways of thinking, especially in 3D microscopy images.

Example 1: Nuclear segmentation

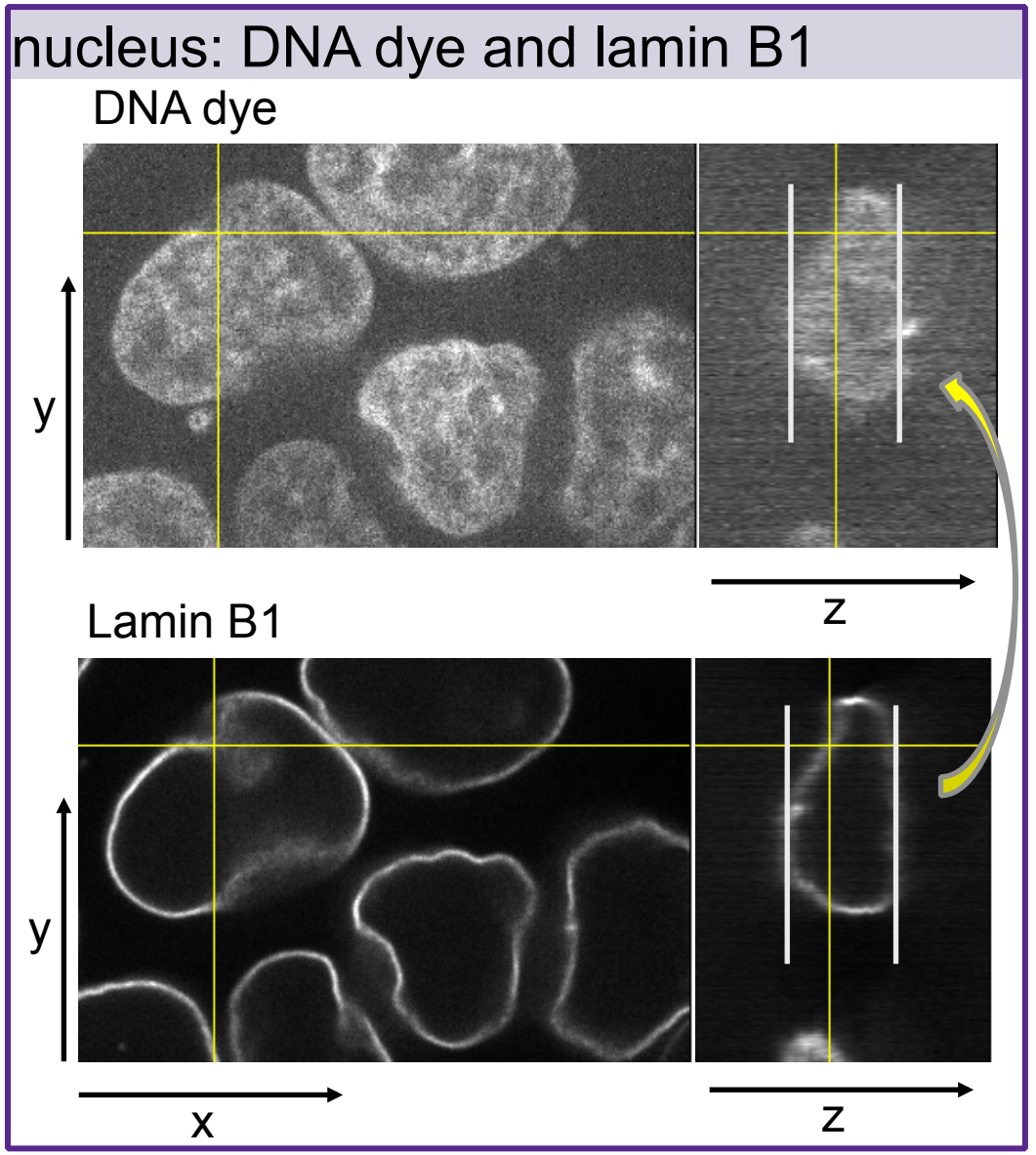




Will annotating 100 images in 3D + V-Net solve the problem?

Beyond 3D manual annotation …

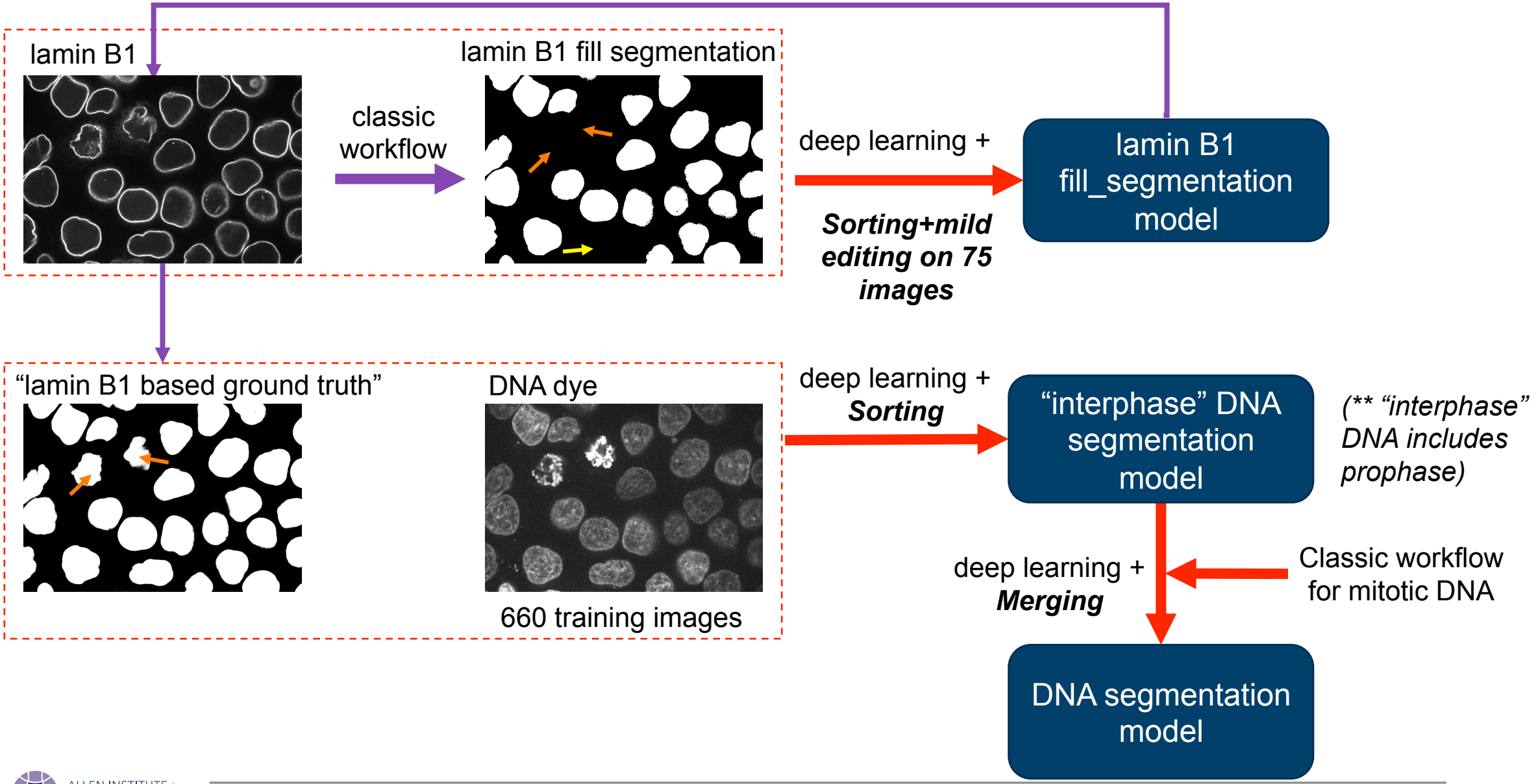
take advantage of specialized biological experiments to obtain “more biologically correct” ground truth for training



Training Assay:

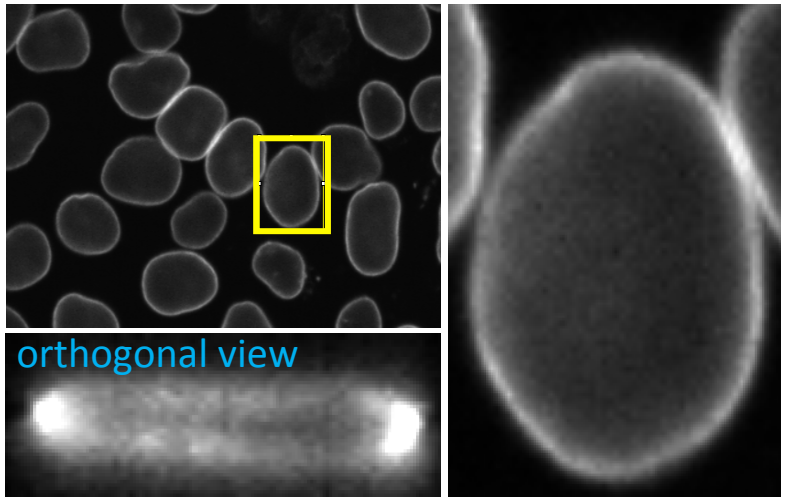
- **Suppose we have a “main” biological assay (e.g., larger scale pipeline) to study a biological problem.**
- **The *Training Assay* approach = developing auxiliary biological assays (smaller scale and specialized) to train deep learning models so that we can extract more accurate information from the main assay.**

Segmentation of DNA dye via a lamin B1 *Training Assay*:

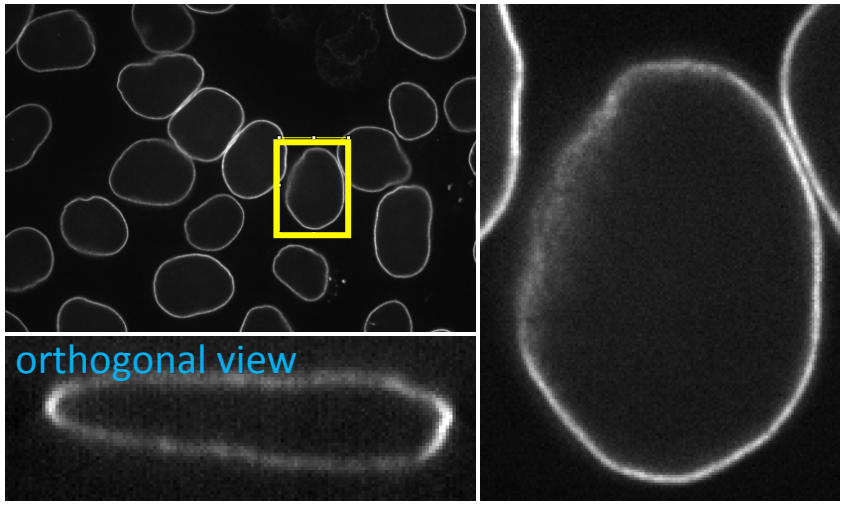


Example 2: Re-visit lamin B1 20x → 100x transfer function

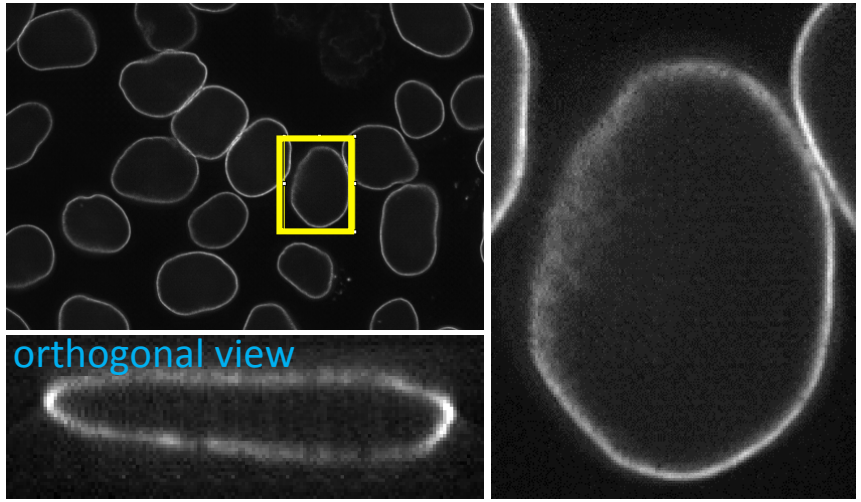
real 20x/0.8NA microscopy image



real 100x/1.25NA microscopy image



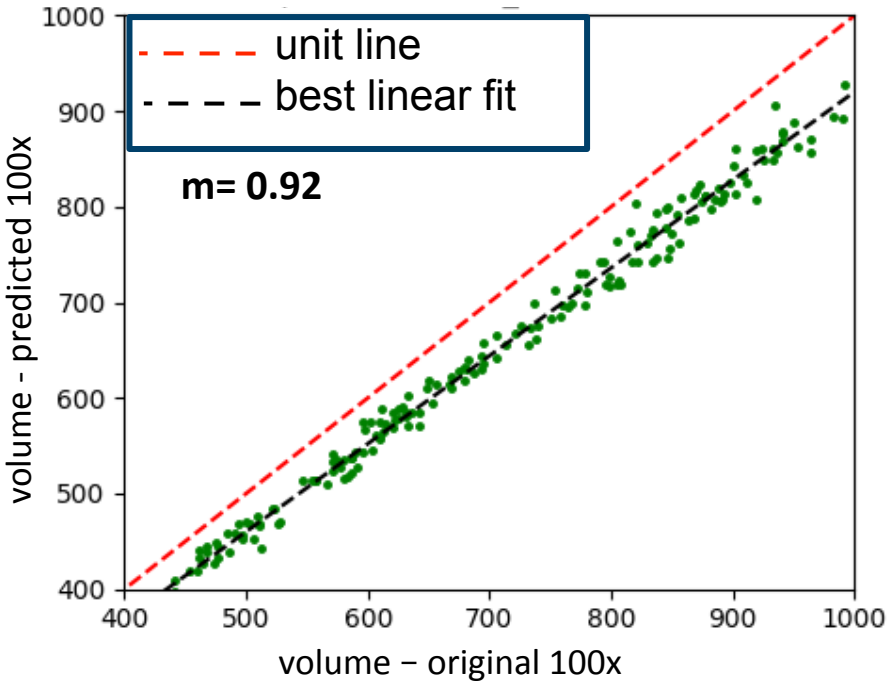
predicted 100x/1.25NA image



Method: Conditional Generative Adversarial Network (cGAN)

Looking real ≠ Biologically correct

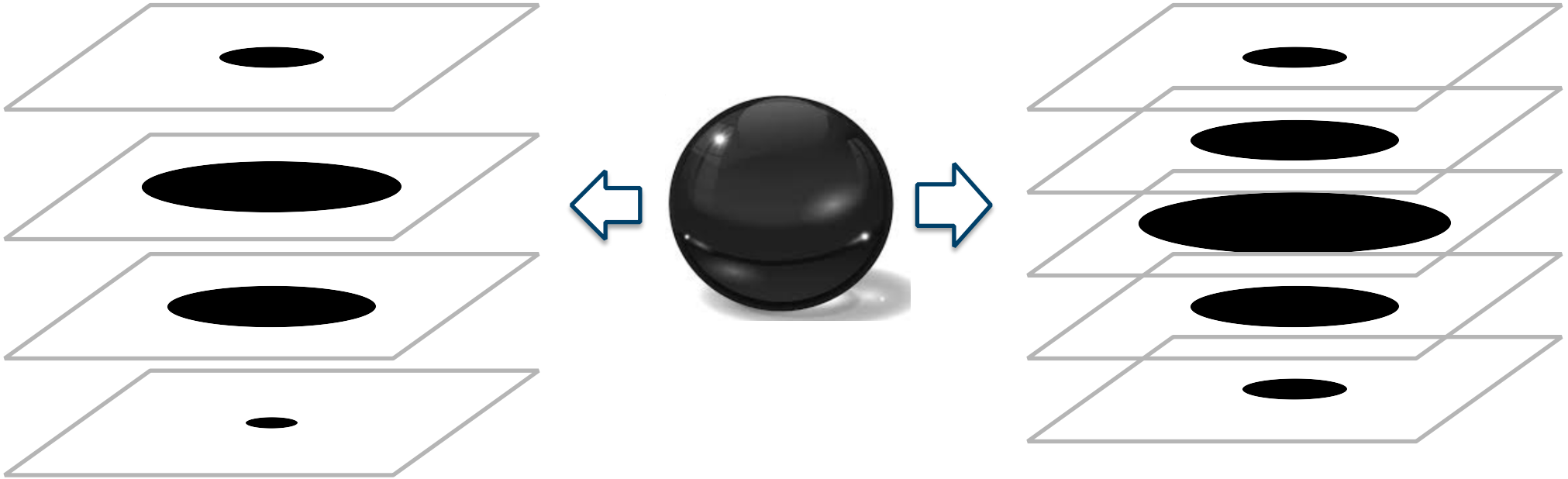
Biology-driven quantitative validation:



~8% error in measured nuclear volume

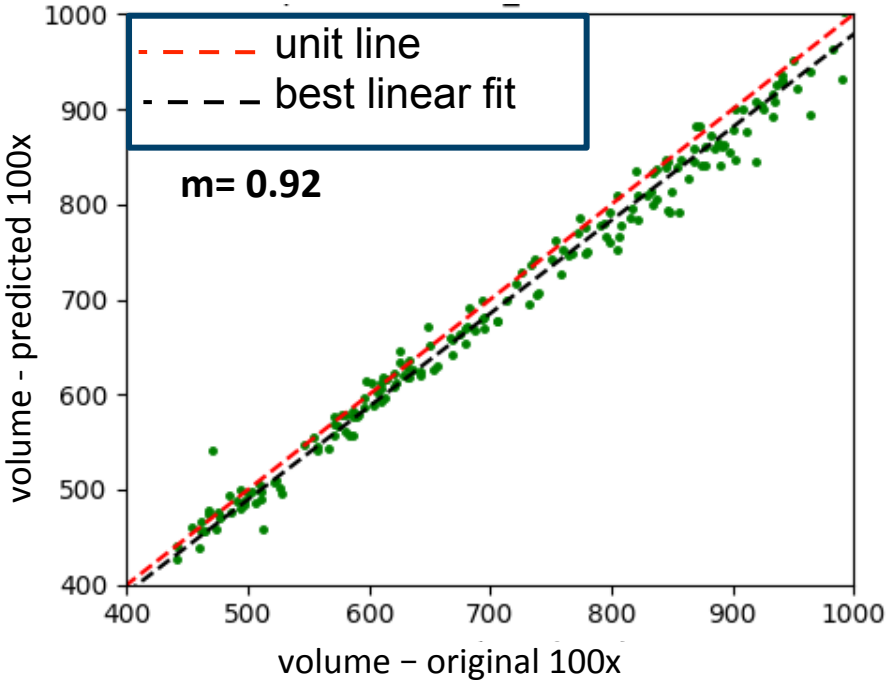
Why?

It is not easy to have well-aligned trained pairs



Misalignment = Z-spacing diff + registration error + (modality diff)

An improved deep learning model

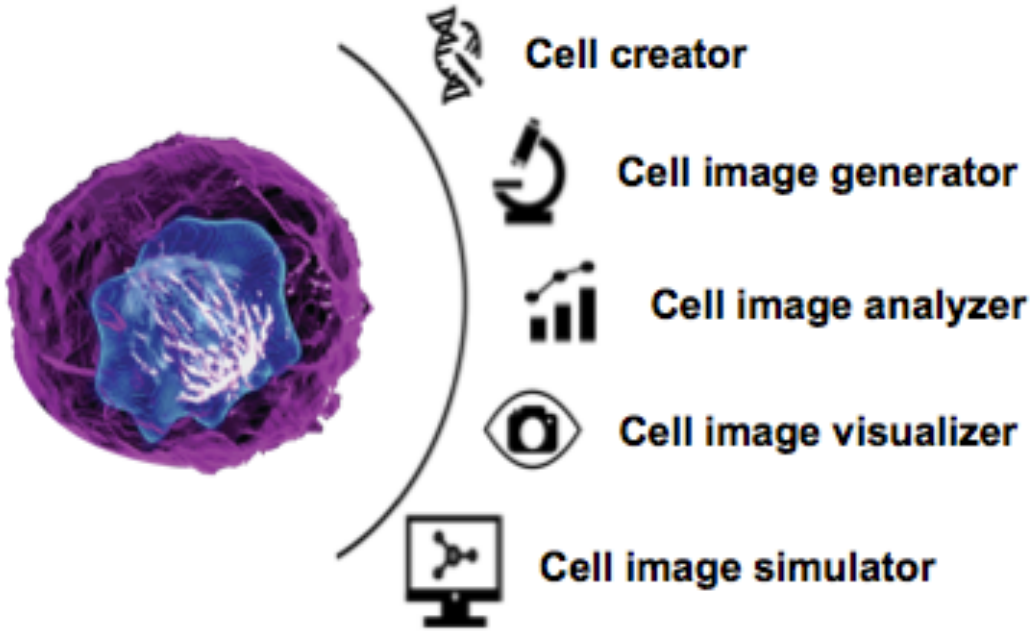


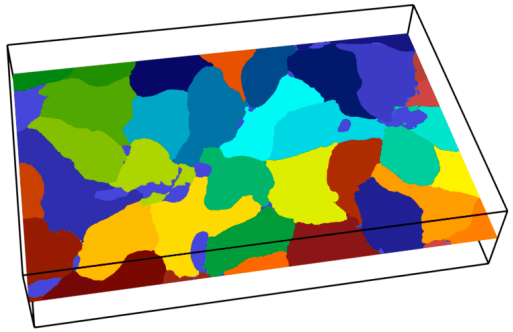
from 8% to 2% error

We introduce a new Auto-Align module (based on spatial transformer network) in cGAN to automatically handle the potential misalignment in training data.

Summary

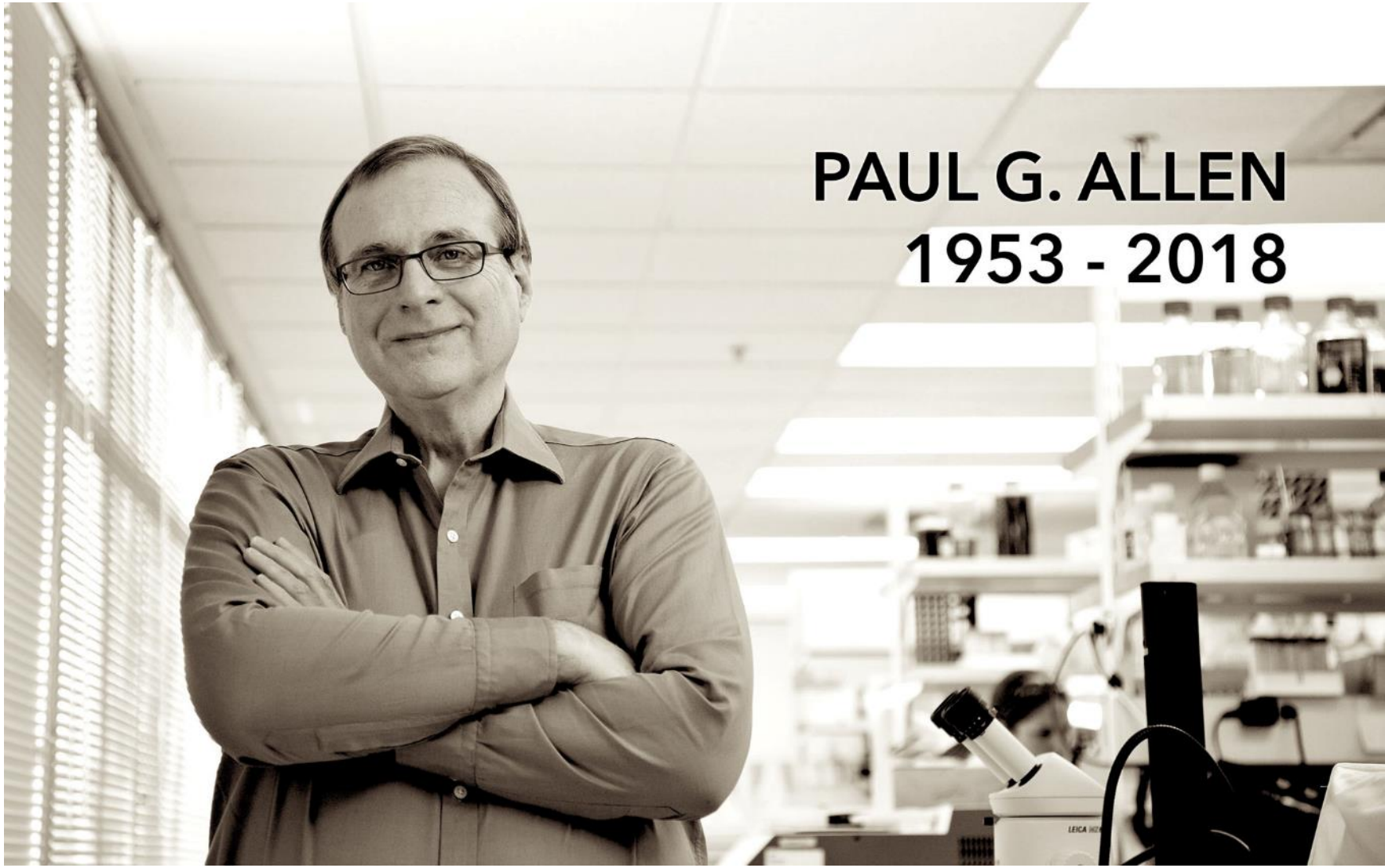
At the Allen Institute for Cell Science, we build GPU compute solutions with special 3D microscopy image adaptions for animation, segmentation, statistical modeling, and more to advance cell biology research.





THANK YOU

We wish to thank the Allen Institute founder, Paul G. Allen, for his vision, encouragement and support



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