Allen Cell Toolkit

Open-source resources and tools for visualizing, interrogating and modeling 3D cell behavior

University of Toronto
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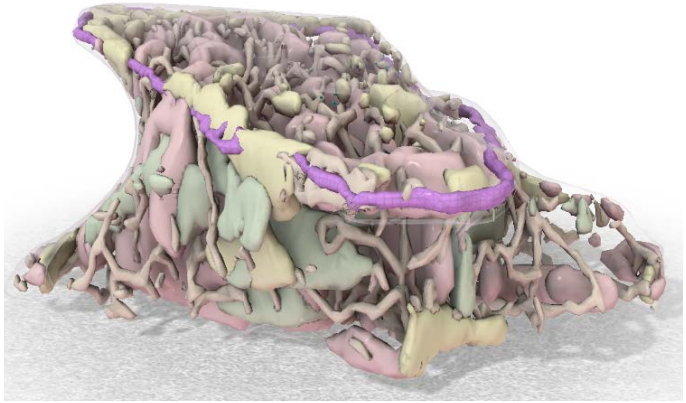
Allen Institute for Cell Science

Look at a cell and know what it is doing

…what it did



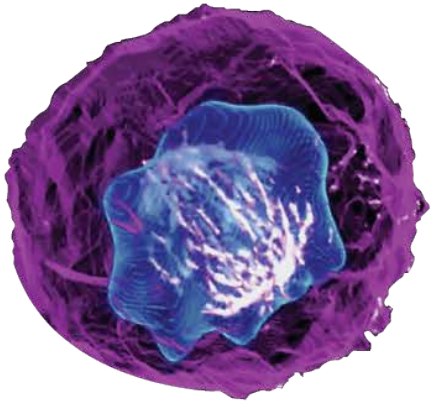
what it will do…

cell organization ⇄ function

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

Holistic approach via 3D live cell imaging

Building the Allen Cell Explorer Tool Kit



An emerging suite of tools and workflows for visualizing, interrogating, and modeling cell behaviors in 3D with use cases for research and education

Cell designer - Allen Cell Collection

Cell image generator
- Automated microscopy platform
- Image Collection

Cell image analyzer - 3D Allen Cell and Structure Segmenter

Cell image visualizer
- AGAVE
- Integrated Cell Models

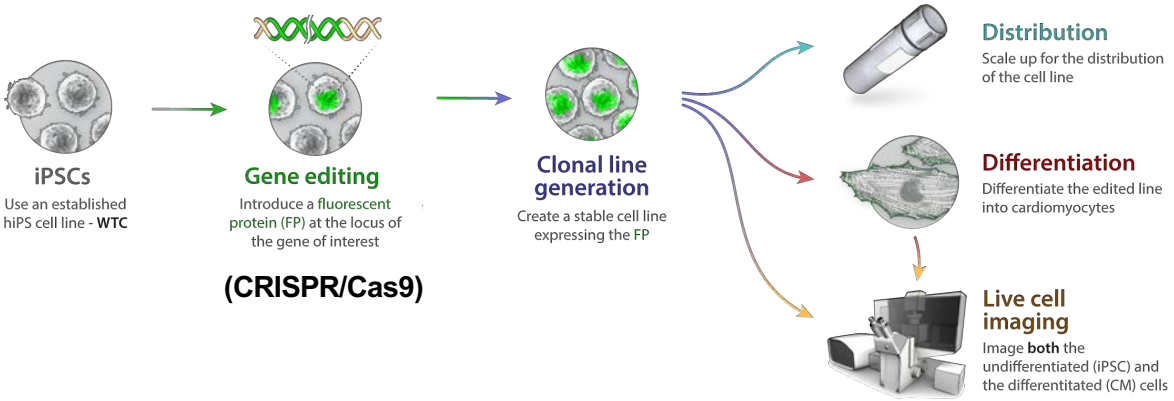
Cell image simulator - Simularium^{beta}

allencell.org

Allen Cell Collection - a suite of FP-tagged hiPSC lines representing major structures of the cells

Community engagement to select key proteins to tag

	Structure	Gene/Protein
1	Adhesions	Paxilin
2	Microtubules	Alpha tubulin
3	Nucleus	LaminB1
4	Mitochondria	Tom20
5	Cell-cell junctions	Desmoplakin
6	Actin	Beta actin
7	ER	Sec61B
8	Nucleolus	Fibrillarin
9	Golgi	ST6Gal1
10	Centrosome	Centrin2



- Generate a collection of high quality clonal FP-tagged iPSC lines
  - Enable live imaging of major structures in stem cells and CMs
- Develop editing strategy, workflows and QC methods
- Release cell lines to the research community

addgene
The nonprofit plasmid repository

Coriell Institute
For Medical Research

The Allen Cell Collection- isogenic FP-tagged hiPSCs for live imaging

	Structure	Protein	FP	Status
1	Matrix adhesions	Paxillin	EGFP	Available
2	Microtubules	Alpha-tubulin	mEGFP	Available
3	Nuclear envelope	Lamin B1	mEGFP	Available
4	Mitochondria	Tom20	mEGFP	Available
5	Desmosomes	Desmoplakin	mEGFP	Available
6	Actin filaments	Beta-actin	mEGFP	Available
7	Endoplasmic reticulum (ER)	Sec61 beta	mEGFP	Available
8	Nucleolus (Dense Fibrillar Component)	Fibrillarin	mEGFP	Available
9	Actomyosin bundles	Non-muscle myosin heavy chain IIB	mEGFP	Available
10	Tight junctions	Tight junction protein ZO-1	mEGFP	Available
11, 12	Cytoplasm	Safe harbor	mEGFP	Available
13	Centrosome	Centrin-2	mTagRFP-T	Available
14	Microtubules	Alpha-tubulin	mTagRFP-T	Available
15, 16	Golgi	**Sialyltransferase 1**	mEGFP	Available
17	Lysosome	LAMP-1	mEGFP	Available
18	Autophagosomes	Autophagy-related protein LC3 B	mEGFP	Available
19, 20	Endosome	**Ras-related protein Rab-5A**	mEGFP	Available
21	Peroxisomes	Peroxisomal membrane protein PMP34	mEGFP	Available
22	Plasma membrane	Safe harbor, CAAX domain	mTagRFP-T	Available
23	Gap junctions	Connexin-43	mEGFP	Available
24	Nucleolus (Granular Component)	Nucleophosmin	mEGFP	Available
25	Nuclear pores	Nucleoporin Nup153	mEGFP	Available
26	Histones	Histone H2B type 1-J	mEGFP	Available
27	Adherens junctions	Beta-catenin	mEGFP	Available
28	Paraspeckles/stress Granules	RNA-binding protein FUS	mEGFP	Available
29	Cohesin	SMC protein 1A	mEGFP	Available
30	ER/Nuclear envelope	**Sec61 beta/lamin B1**	mEGFP/mTag RFP-T	Available
31	Transcription factor	Transcription factor SOX-2	mEGFP	Available
32	Nucleolus dual	**Fibrillarin/nucleophosmin**	mEGFP/mTag RFP-T	Available
33	CM - Sarcomeric thin filament	Troponin I, slow skeletal type	mEGFP	Available
34	CM - Sarcomeric thick filament	MLC-2a (early)	mEGFP	Available
35	CM - Sarcomere M-line	Titin	mEGFP	Available
36	Sarcoplasmic reticulum/Endoplasmic Reticulum	SERCA2	mEGFP	Available
37	CM - Sarcomeric thick filament	MLC-2v (late)	mEGFP	Available
38	CM - Sarcomeric z-disc	Alpha-actinin-2	mEGFP	Available

- ~51 FP-tagged isogenic iPSC lines (WTC-11)
  - Major structures, signaling, cardio-specific, multi-edits
  - Mostly mono-allelic (1 structure tagged/line)
  - Extensive QC
- Sharing cell lines, plasmids, and methods
  - Cell lines – Coriell Institute
  - Plasmids – Addgene
  - Distribution to stem cell cores at major institutes
  - Methods and tutorials - MBoC, Stem Cell Reports, JoVE
  - Allencell.org

SCAN ME

Bolded- *both mono and bi-allelic lines*
Red *– Cardiomyocyte-specific edits*
Blue *- dual tagged lines*

Allen Cell Collection (allencell.org/cell-catalog)

Cell Catalog

The catalog below provides a listing of our completed cell lines, as well as those in progress. Click a row in the catalog table to see images and quality control data related to each completed line.

Cell Catalog **update April 21, 2021**

We have one new fluorescently tagged hiPSC line available in the Allen Cell Collection. The line is a single edited, nuclear line in which telomeres are visualized via mEGFP-tagged TRF2.

Nuclear line:

- TERF2-mEGFP-mono

The Allen Cell Collection (below) now has 48 high quality-certified fluorescently tagged hiPSC lines that target 38 key cellular structures and substructures available to help your research program.

Allen Institute for Cell Science

Lab Plasmids

Video Tutorials and Methods

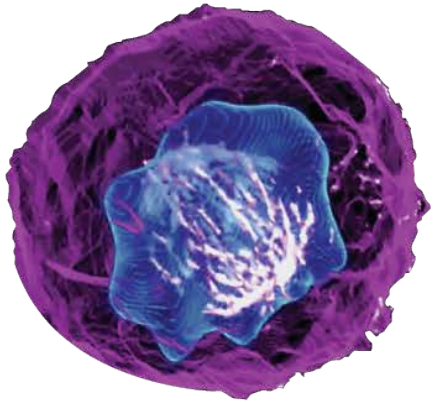
Allen Cell Methods

Instructional Videos for Success in the Lab

To ensure researchers have success working with our human induced pluripotent stem cell lines in their lab, researchers from our teams highlight nuanced techniques and helpful tips while demonstrating various laboratory protocols.

Read our paper on Systematic gene tagging using CRISPR/Cas9 in human stem cells to illuminate cell organization in Molecular Biology of the Cell (MBoC)

Building the Allen Cell Explorer Tool Kit



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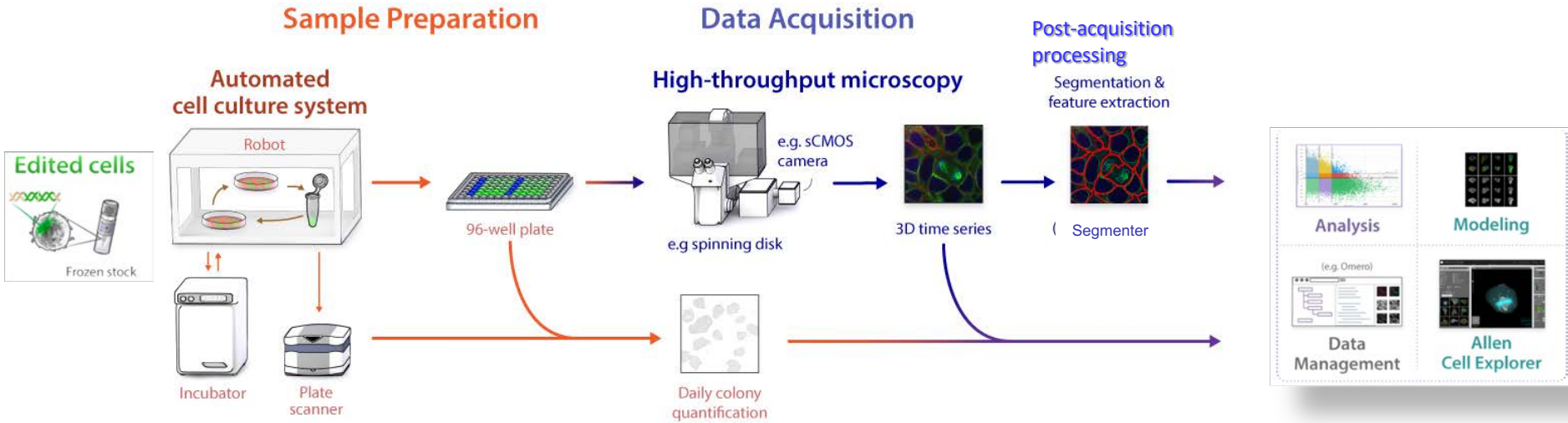
allencell.org

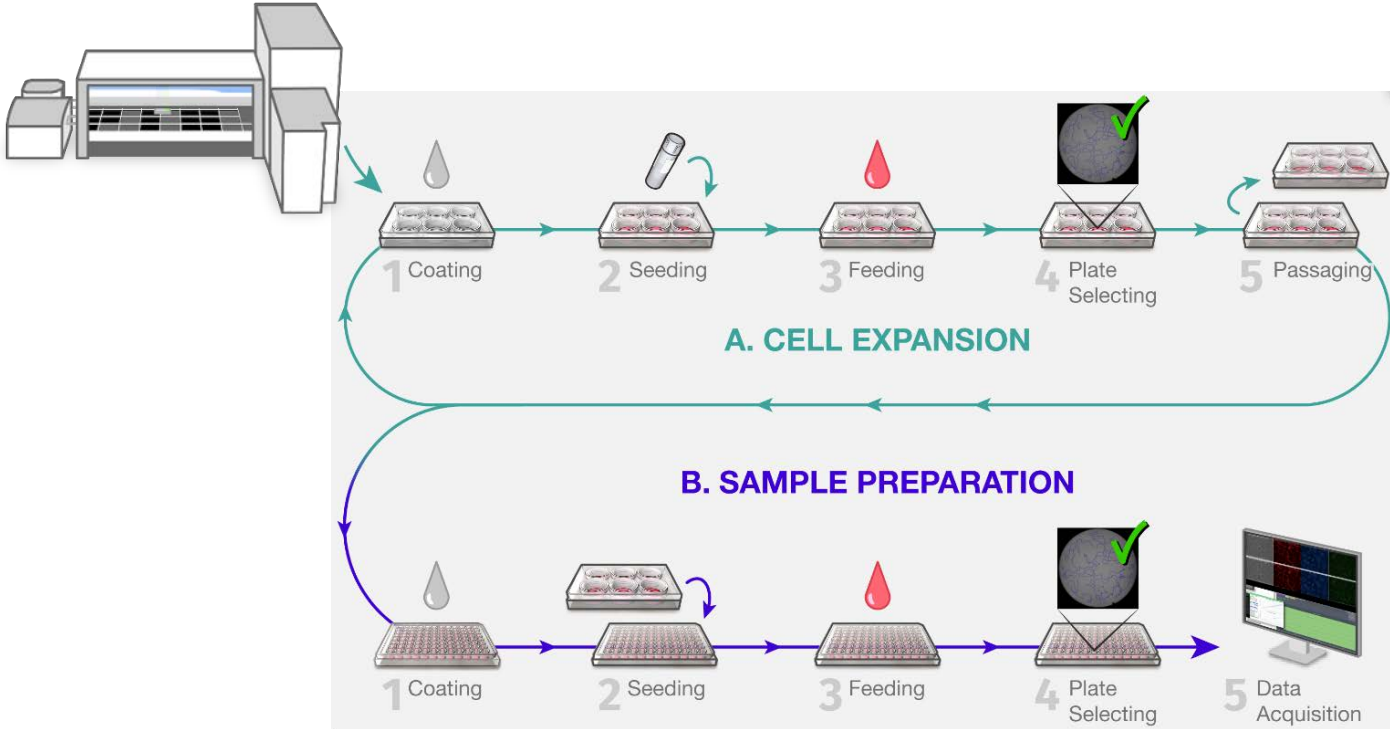
Image data acquisition pipeline



Automated Cell Culture System

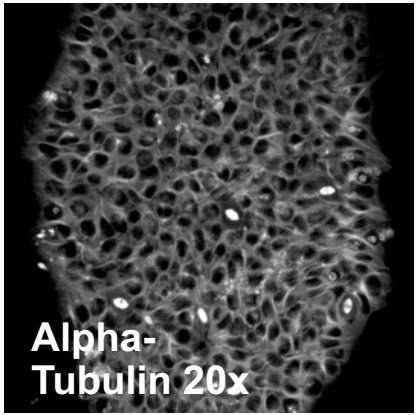
Coston M.E., et. al., *bioRxiv* 11/2020

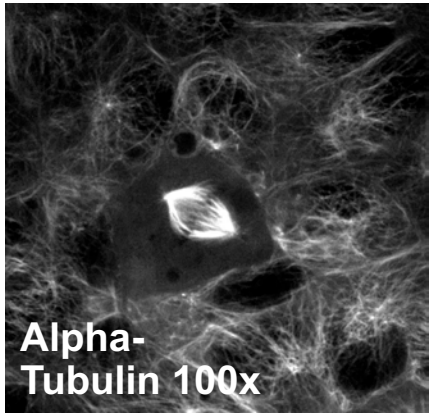
Automated Cell Culture System

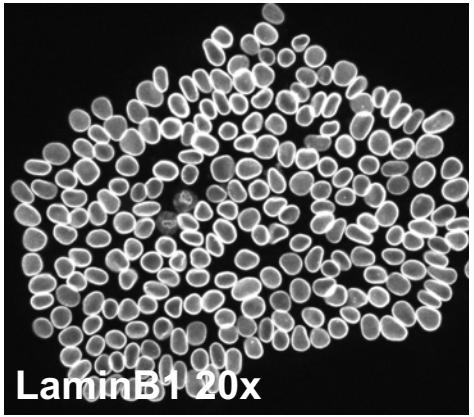


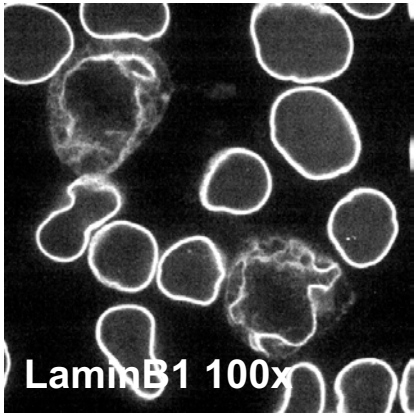
Coston M.E., et. al., *bioRxiv* 11/2020

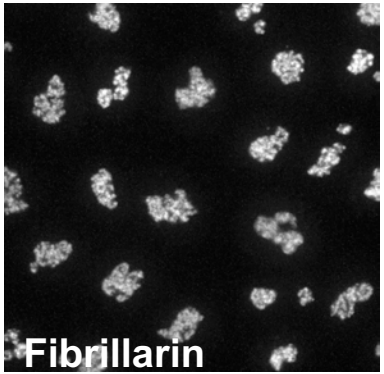
Acquiring Our Image Collection

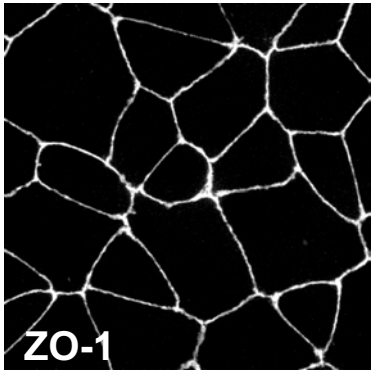


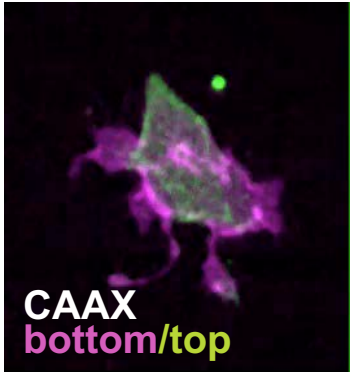






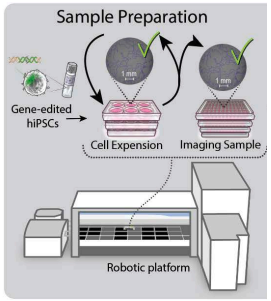






An automated microscopy platform provides live, 3D single cell data at scale

Reproducible, scalable 3D live cell imaging



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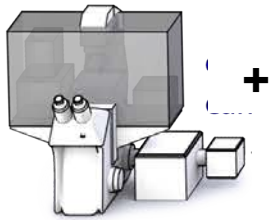

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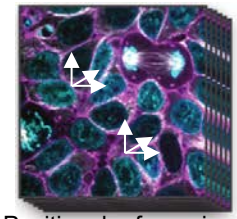
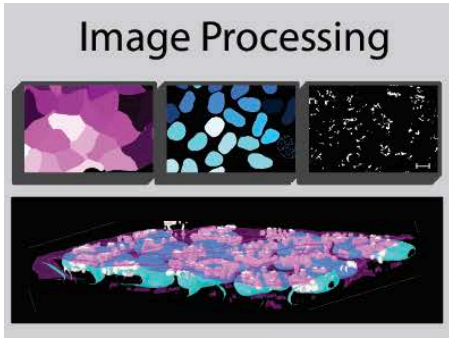
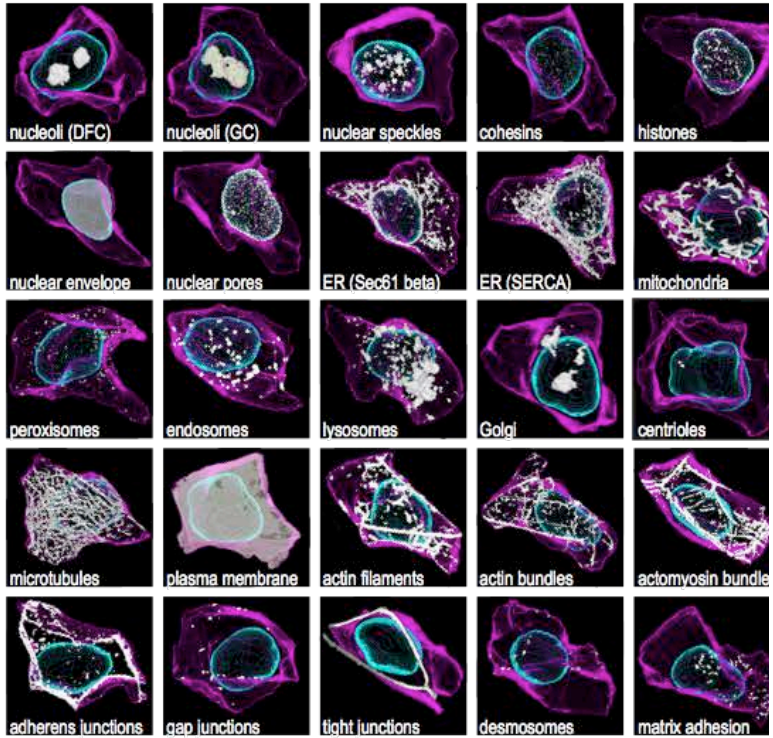


Positional referencing
membrane & DNA/nucleus

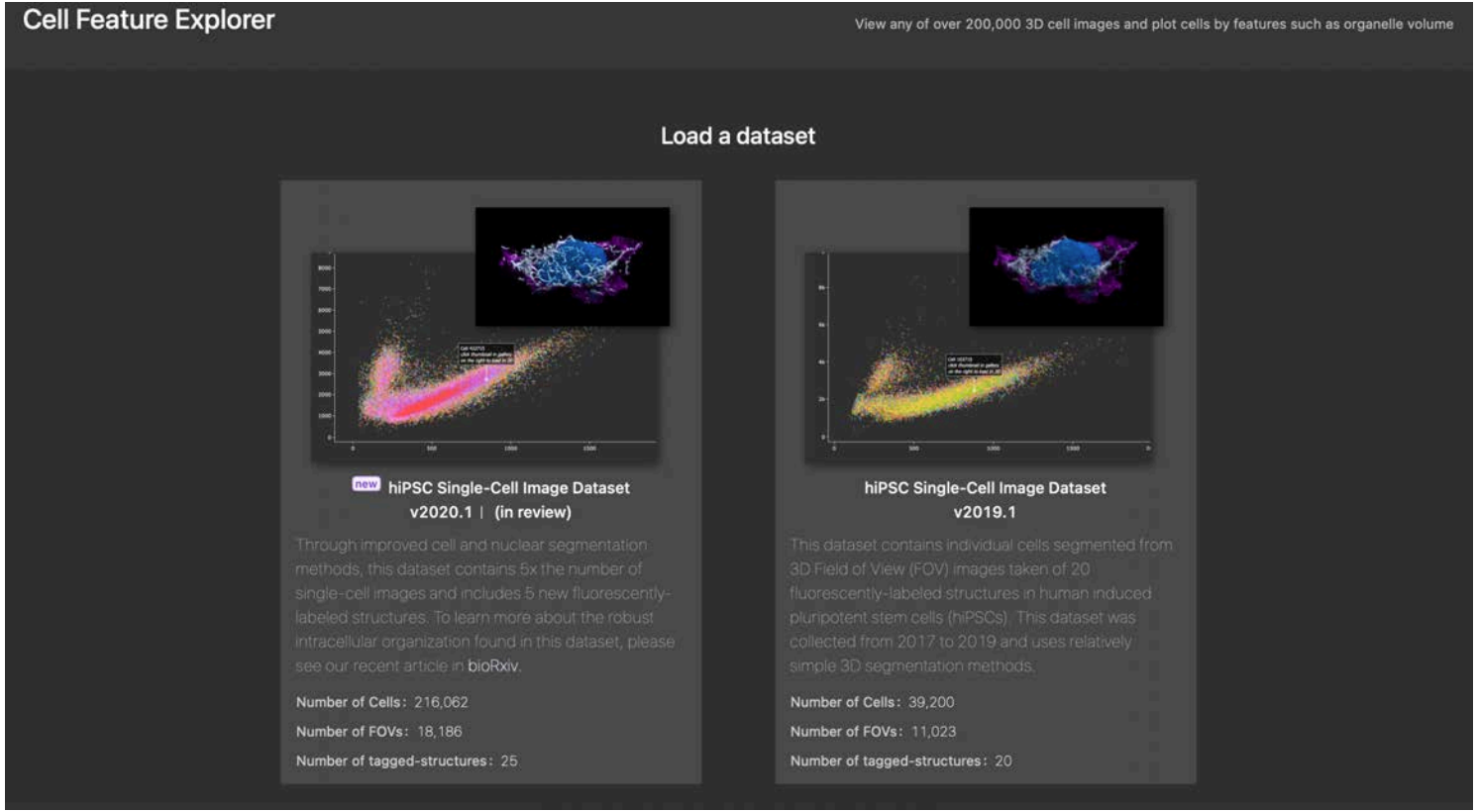
+



Individually segmented cells

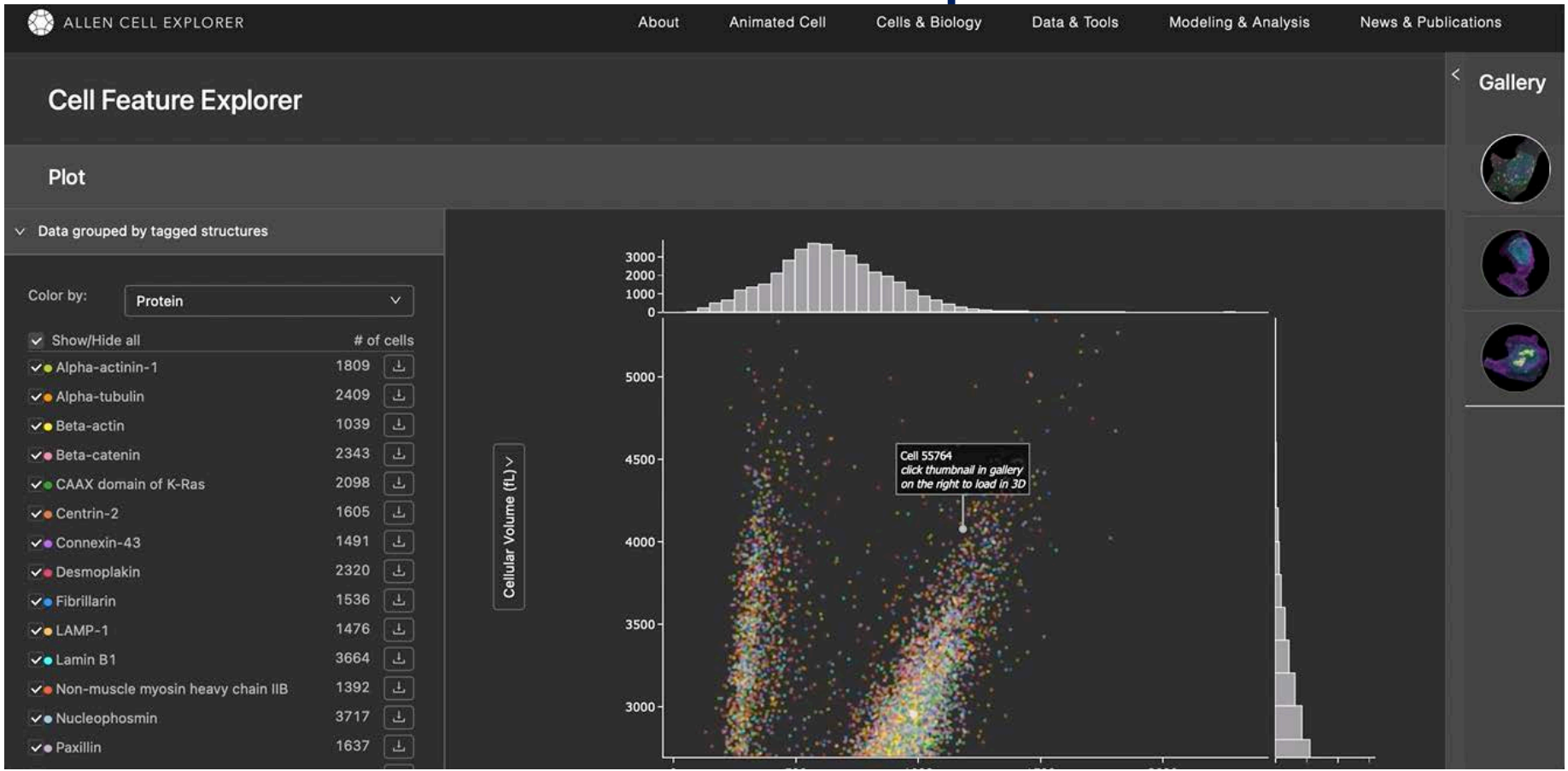


The Cell Feature Explorer

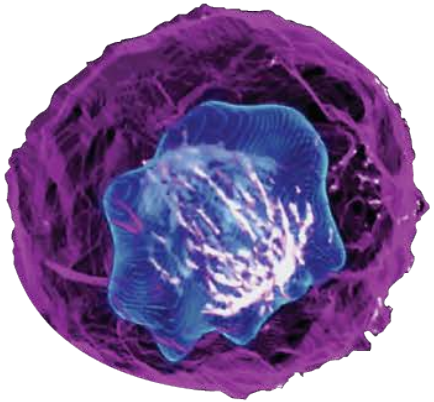


hIPS-derived cardiac dataset coming this summer!

The Cell Feature Explorer



Building the Allen Cell Explorer Tool Kit



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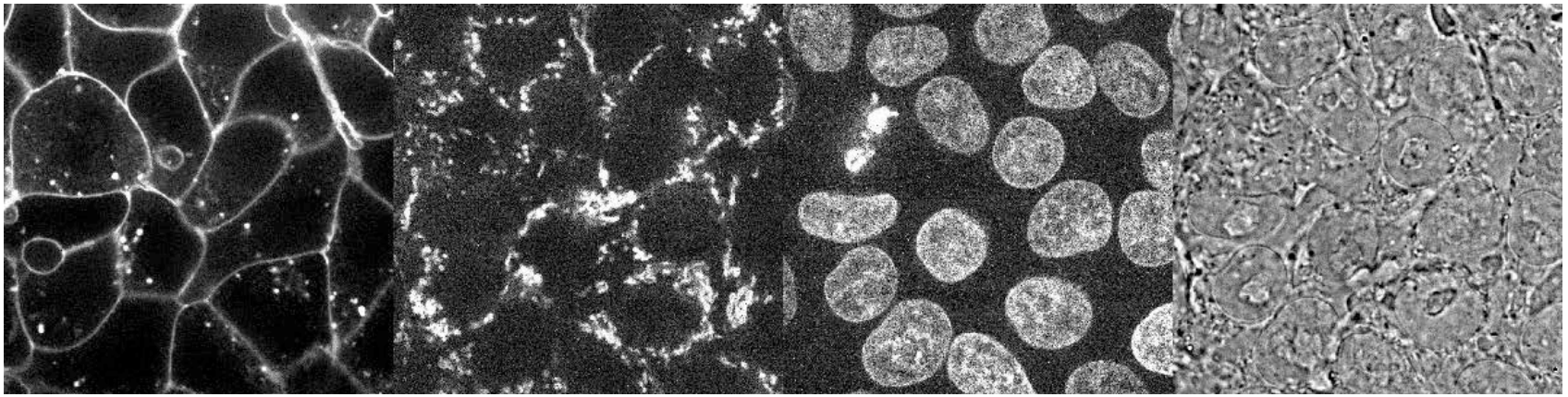
allencell.org

An emerging suite of tools and workflows for visualizing, interrogating, and modeling cell behaviors in 3D with use cases for research and education

We Created an Integration Assay for Gene-edited hiPSCs

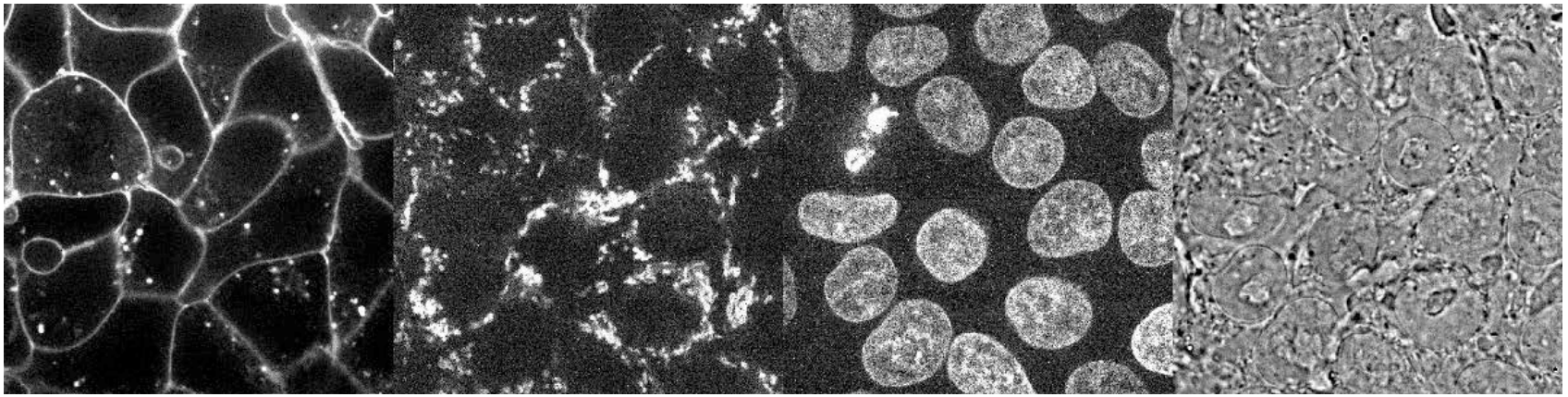
cell membrane	mitochondria	DNA (nucleus)	Brightfield
CellMask Deep Red	**Tom20-mEGFP**	**Hoechst**	transmitted light image



- **Live 3D imaging of over 18,000 fields of views of hiPSCs for 29 structures and counting**

How to create a scalable 3D cell integration assay for gene-edited hiPSCs?

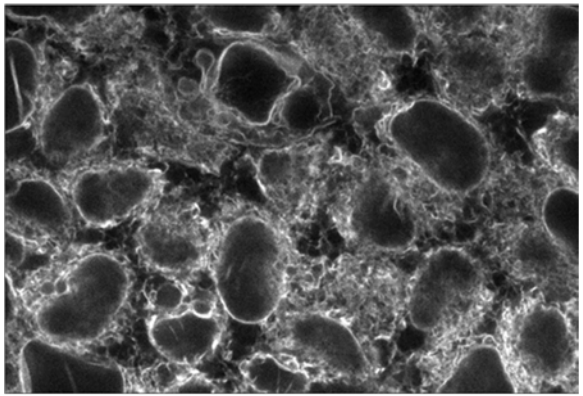
cell membrane	mitochondria	DNA (nucleus)	Brightfield
CellMask Deep Red	**Tom20-mEGFP**	**Hoechst**	transmitted light image



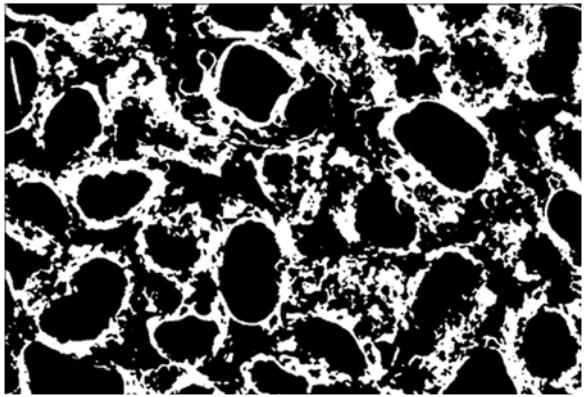
- **Live 3D imaging of over 18,000 fields of views of hiPSCs for 29 structures and counting**
- **Need a tool that enables robust and accurate 3D segmentation of cells, nuclei and structures**

The Allen Cell & Structure Segmenter

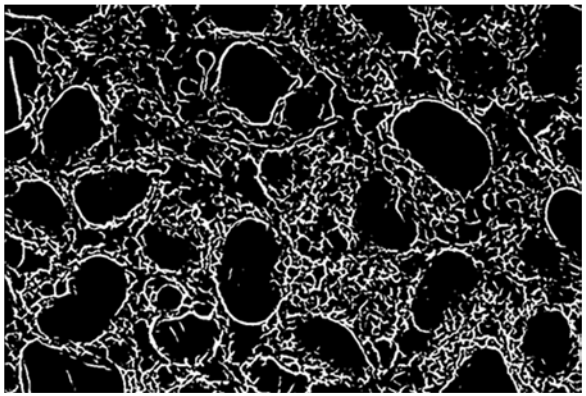
Goal: extract the most high-quality & biologically informative segmentations in 3D from optical microscopy possible allencell.org/segmenter



Input 3D image stack
(ER via Sec61 beta)



Threshold segmentation
(local adaptive threshold)



Segmenter classic
workflow: SEC61B

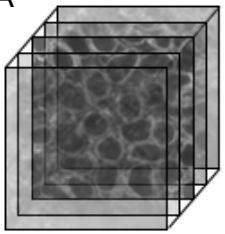
Note: All images are 3D. A middle z-slice is shown.

bioRxiv preprint (Chen J, et. al., Dec. 13., 2020)

The Allen Cell & Structure Segmenter:

An open-source toolkit for segmenting 3D intracellular structures in fluorescence microscopy images

A

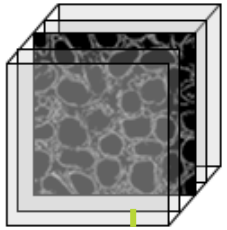


Original single channel 3D image stack

Pre-processing

Core Segmentation Algorithms

Post-processing



3D binary image stack

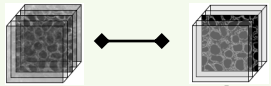
analysis or models

jupyter + python

Classic Image Segmentation Workflow

B

Training data



Fluorescent images

Ground truth images

Deep learning model

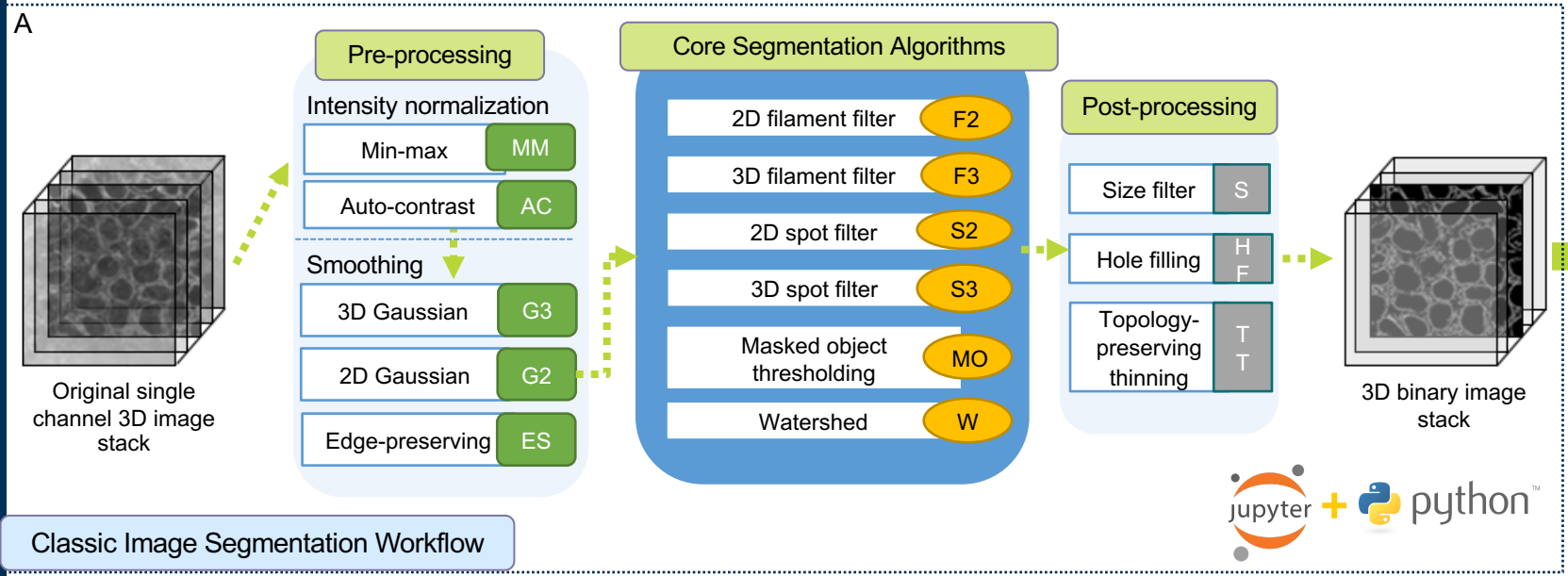
sorting/merging

Iterative Deep Learning Workflow

allencell.org/segmenter

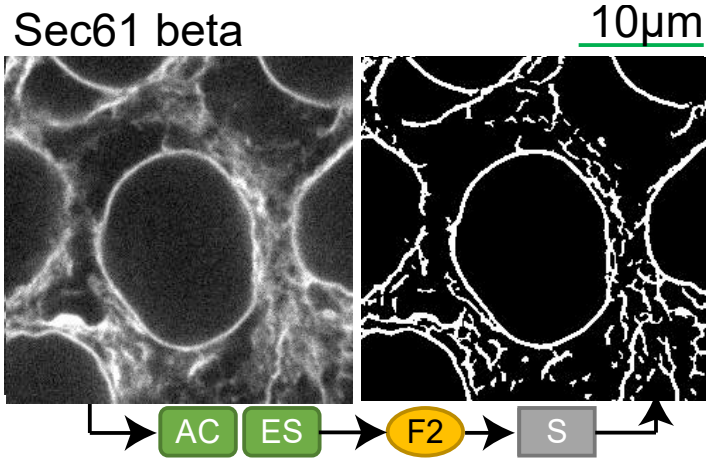
The Allen Cell & Structure Segmenter:

The Classic Segmentation Workflow

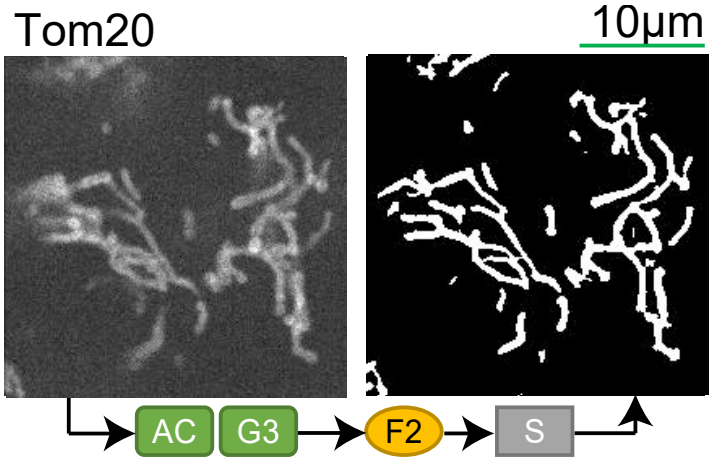


Intracellular structure classic segmentation workflow

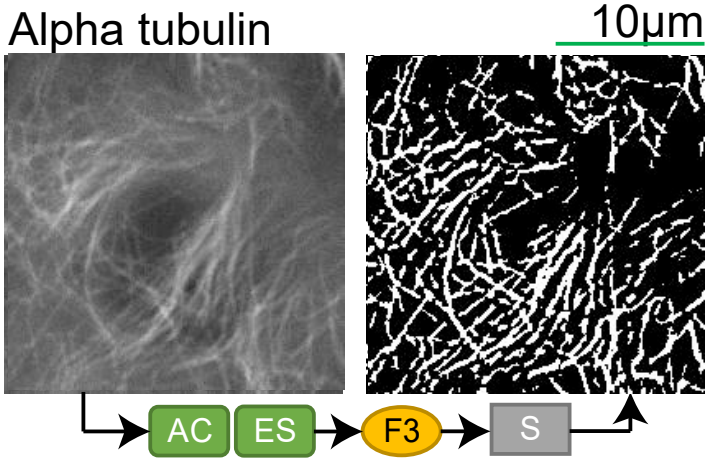
Sec61 beta



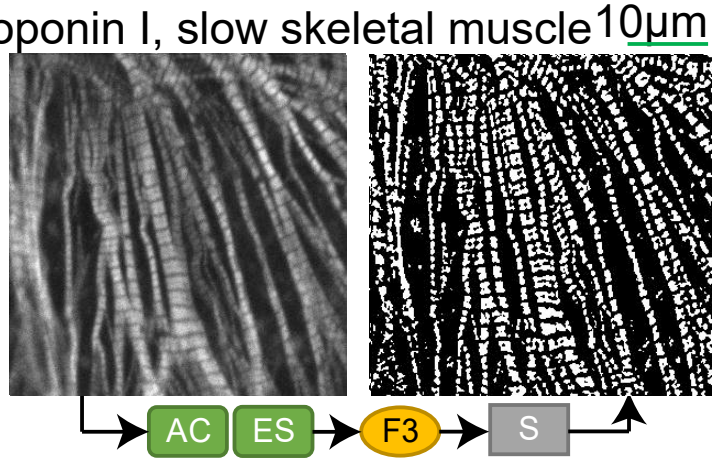
Tom20



Alpha tubulin



Troponin I, slow skeletal muscle



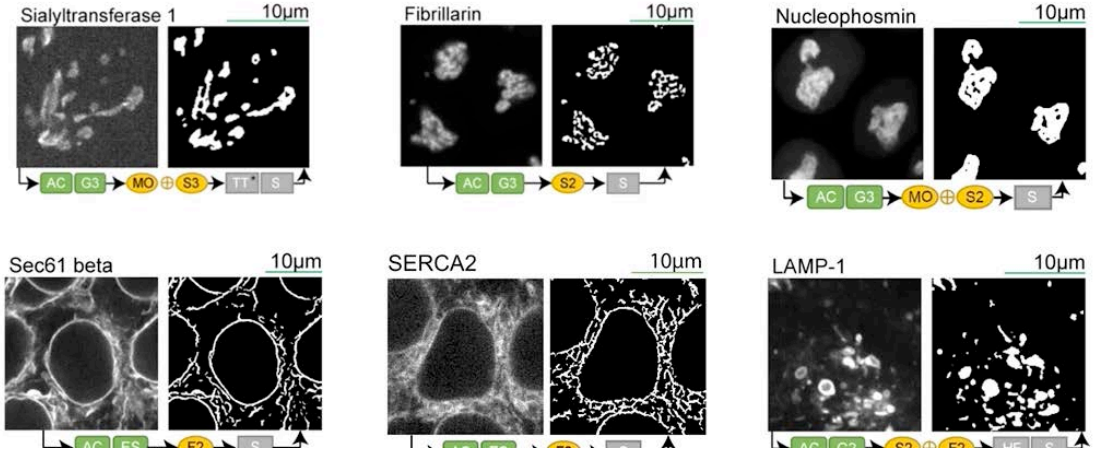
Created a Lookup Table for Intracellular Structure Classic Segmentation Workflow

algorithm symbolic legend

- MM — Min-max normalization
- AC — Auto-contrast normalization
- G2 — 2D Gaussian smoothing
- G3 — 3D Gaussian smoothing
- ES — Edge-preserving smoothing
- F2 — 2D filament filter
- F3 — 3D filament filter
- S2 — 2D spot filter
- S3 — 3D spot filter
- MO — Masked object thresholding
- W — Watershed
- S — Size filter
- HF — Hole filling
- TT — Topology-preserving thinning

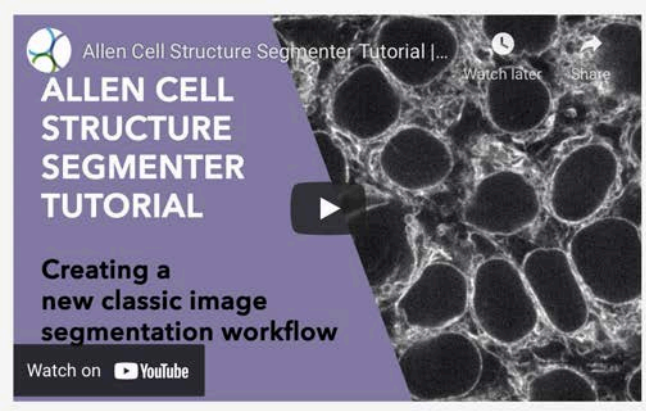
Lookup table of classic image segmentation worksflows for 28 intracellular structure localization patterns

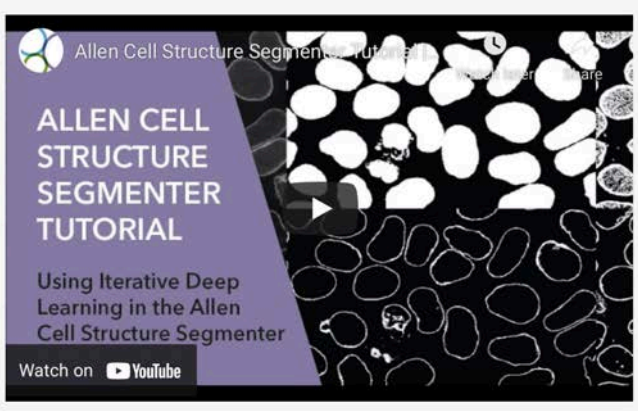
Click on thumbnail to view video



“Find your Morphology Match”

Making the Segmenter User-friendly





Jupyter Notebooks & Tutorials

Napari plugin App
coming June 30th
napari.org

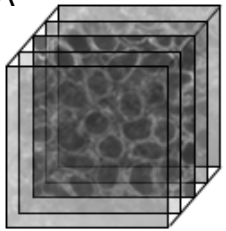
Learn more at
allencell.org/segmenter

SCAN ME

Questions:
forum.allencell.org

Napari Allen Cell & Structure Segmenter Plugin

A



Original single channel 3D image stack

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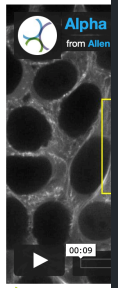
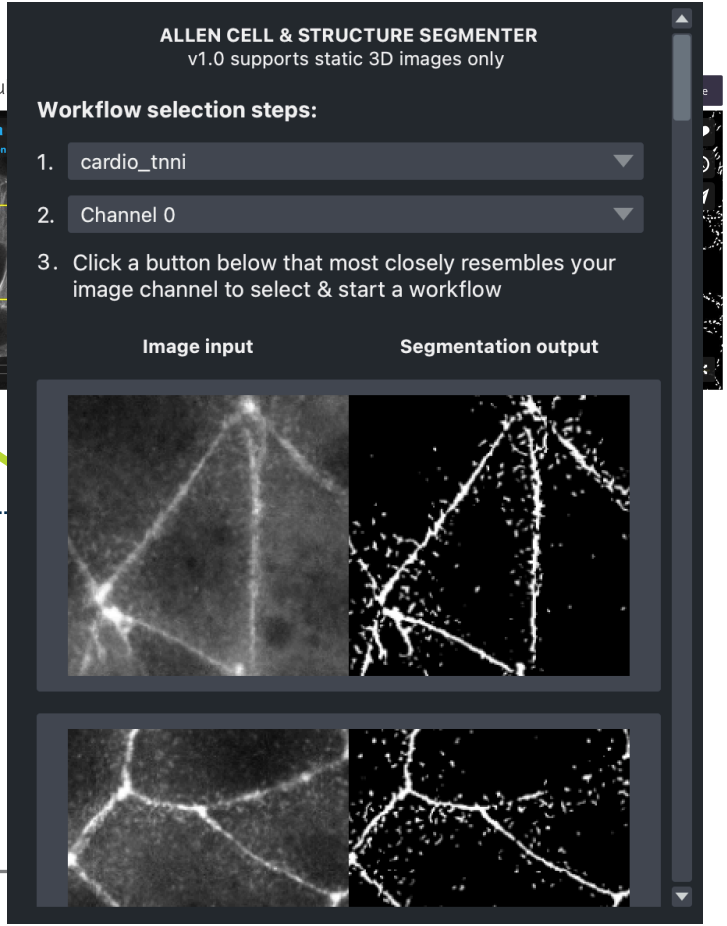
Alpha tubu

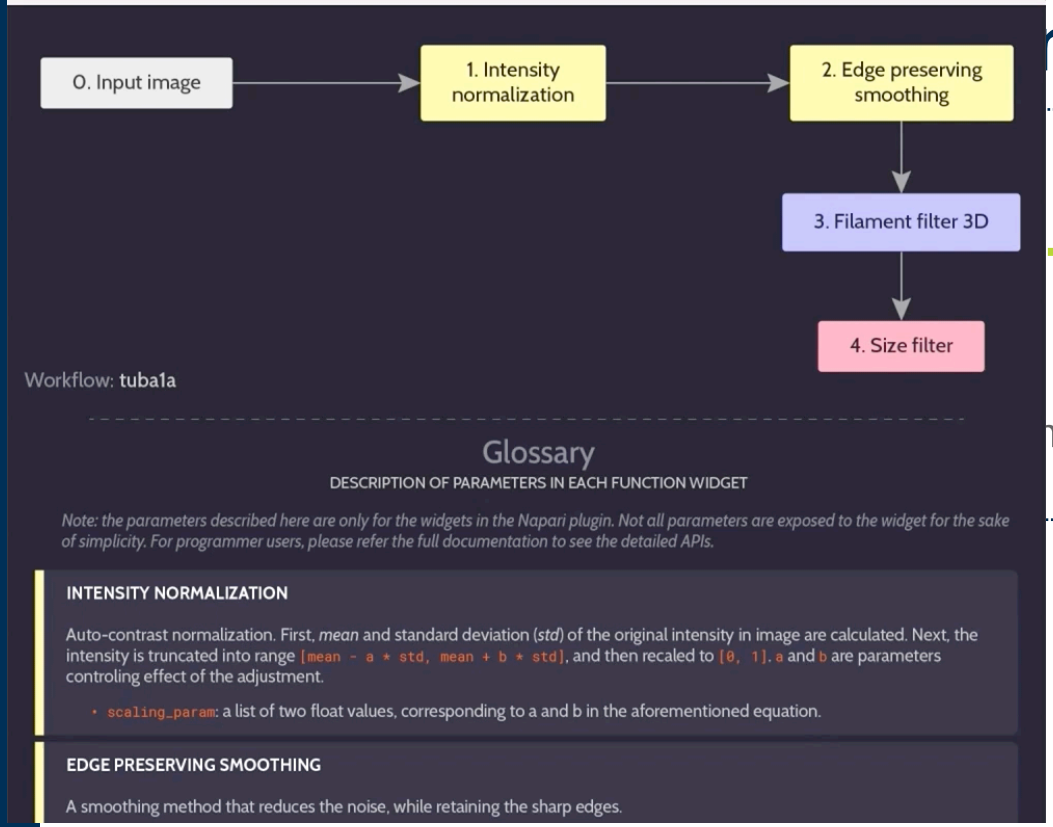


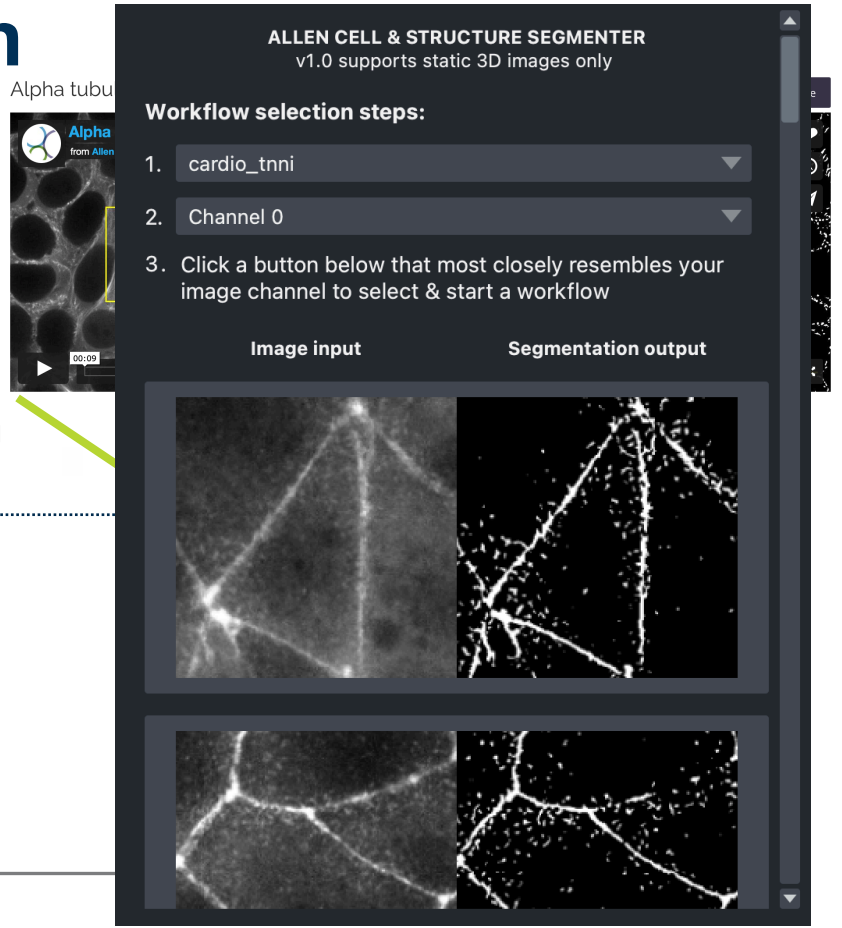
jupyter + pyth

Classic Image Segmentation Workflow



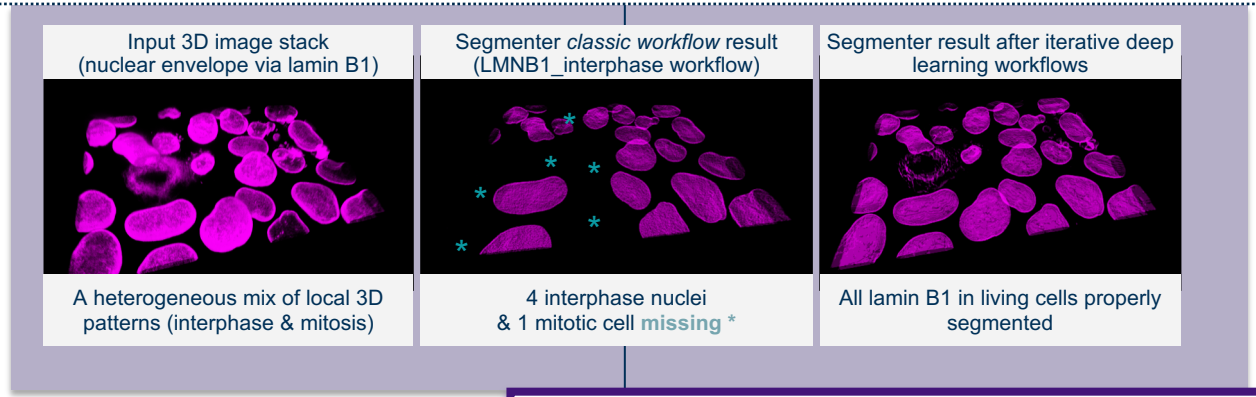
Napari Allen Cell & Structure Segmenter

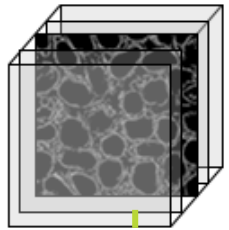




The Allen Cell & Structure Segmenter:

combines classic image segmentation & deep learning





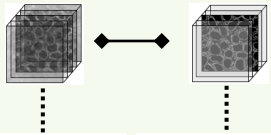
3D binary image stack

analysis or models

B

improve accuracy and robustness

Training data

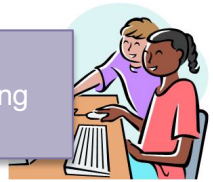


Fluorescent images

Ground truth images

Deep learning model

Sorting/Merging



Iterative Deep Learning Workflow

allencell.org/segmenter

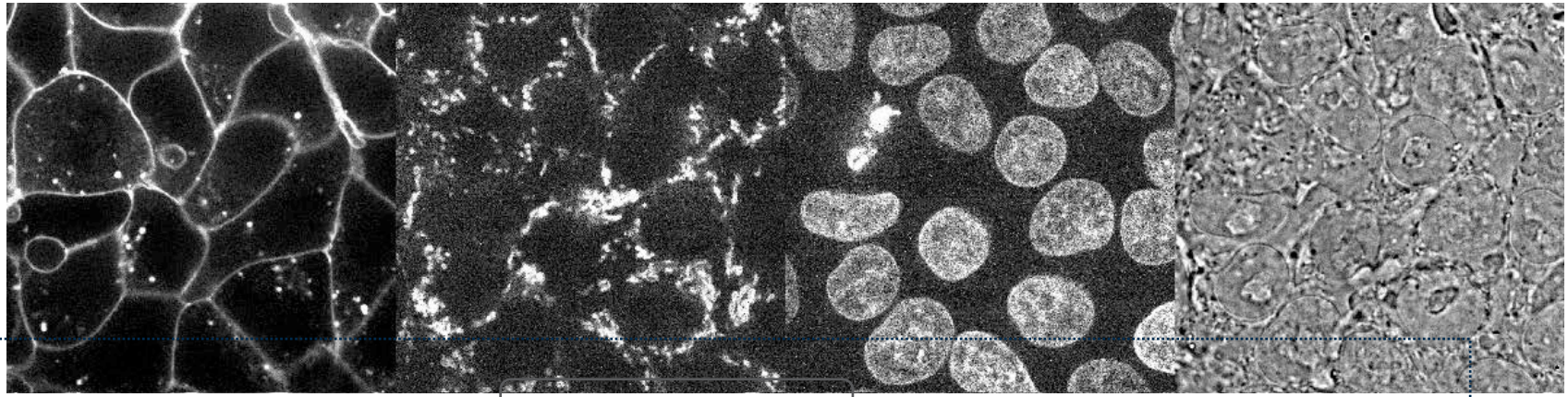
Scalable cell integration assay for gene-edited hiPSCs

cell membrane
CellMask Deep Red

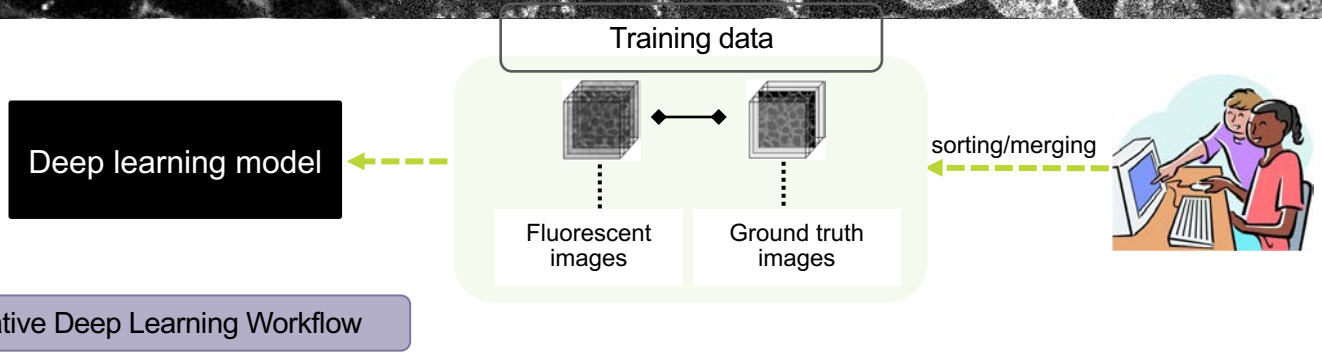
mitochondria
Tom20-mEGFP

DNA (nucleus)
Hoechst

Brightfield
transmitted light image



B



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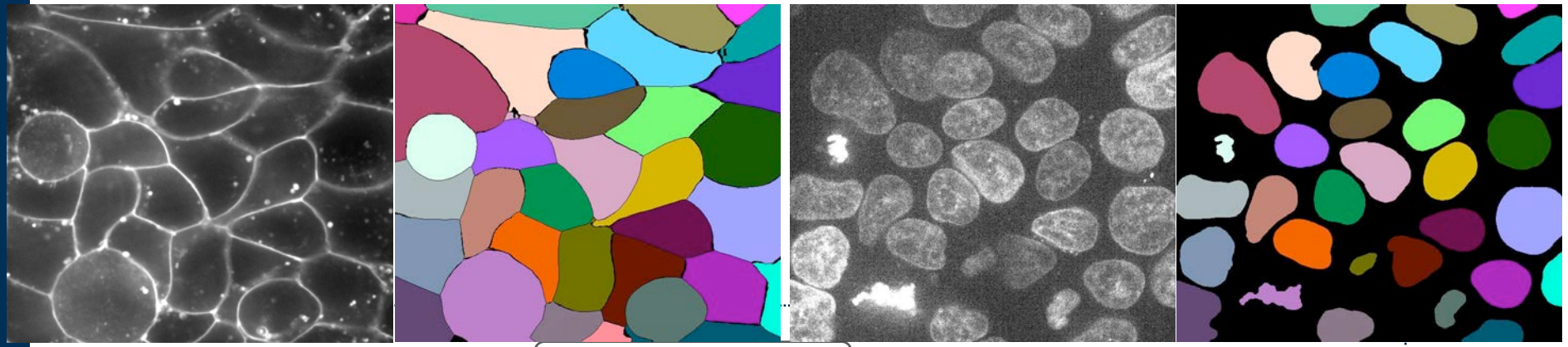
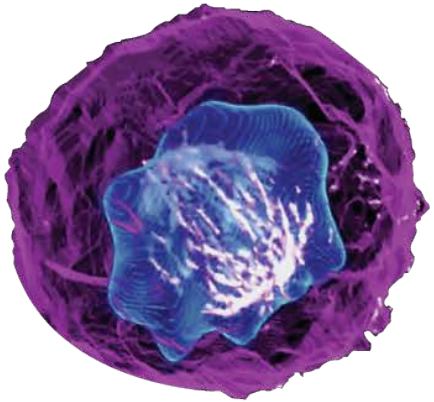


- Iterative deep learning combined with ‘training assays’ can improve segmentation accuracy, see *bioRxiv* preprint (Chen J, et. al., Dec. 13., 2020)
- Created a scalable 3D cell integration assay
- Achieved the robust segmentation of >18,000 fields of view to get >215,000 single cell images for analysis
- **Napari Allen Cell & Structure Segmenter plugin *available June 30th, 2021***

Building the Allen Cell Explorer Tool Kit



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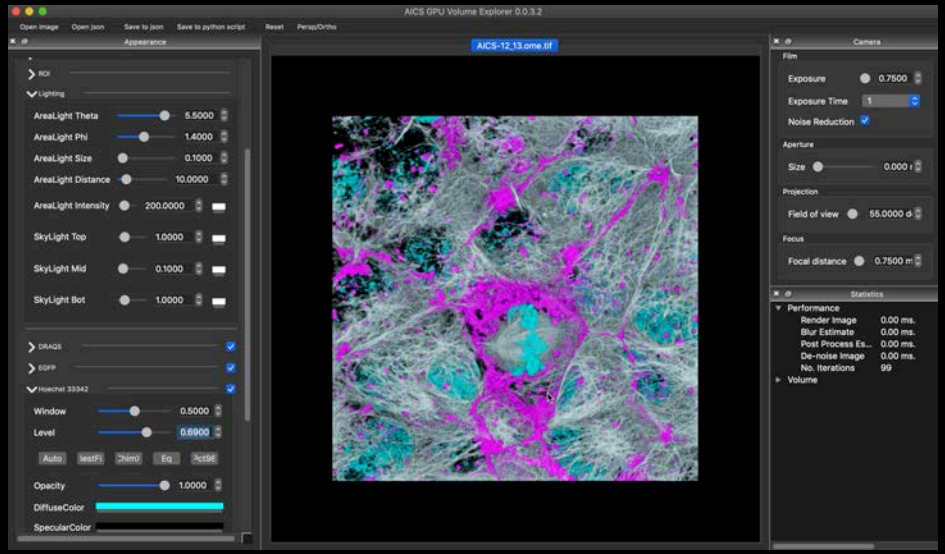
Cell image simulator - Simularium[beta]

An emerging suite of tools and workflows for visualizing, interrogating, and modeling cell behaviors in 3D with use cases for research and education

allencell.org

Path-trace rendering of 3D microscope images with AGAVE

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



Advanced GPU Accelerated Volume Explorer (AGAVE)

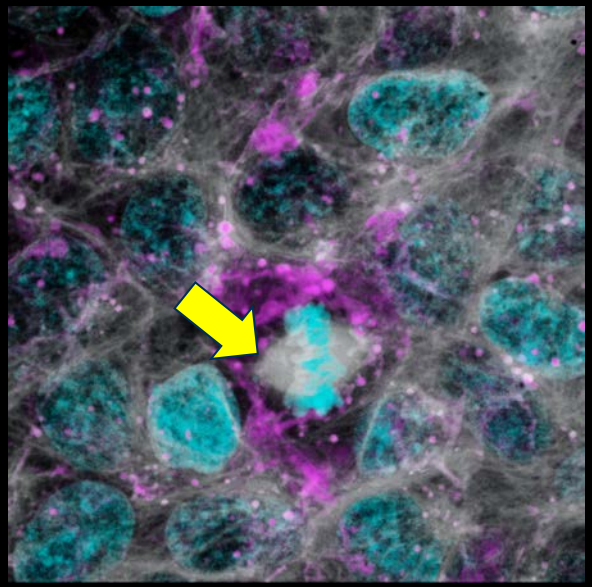
Desktop application for Linux, Windows, and MacOS

Cell data integration: enhancing 3D interpretability

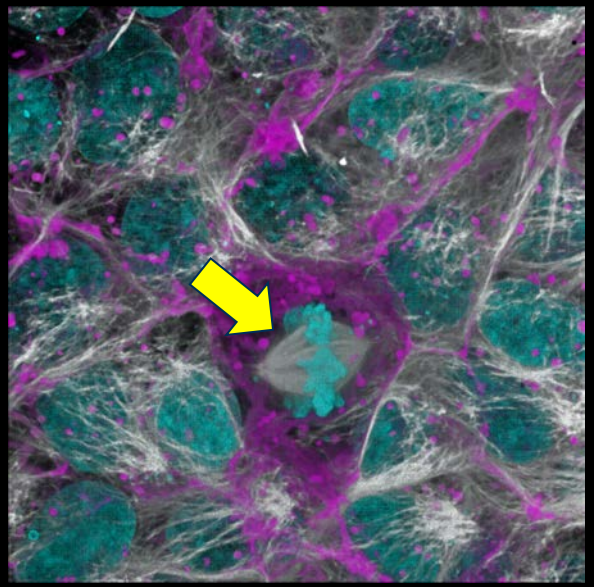
Advanced GPU Accelerated Volume Explore (AGAVE)

Classic

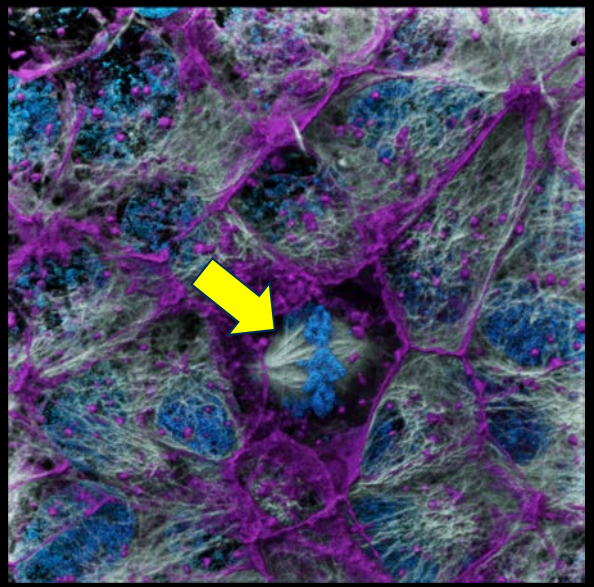
AGAVE



Standard volume rendering



AGAVE transparent path-tracing

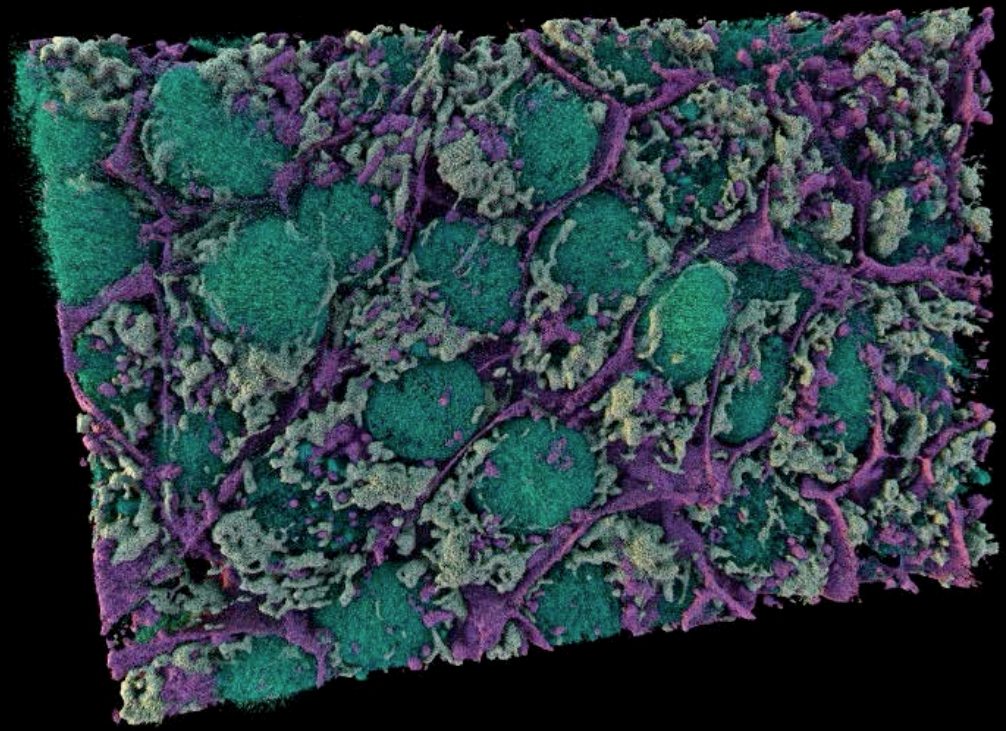


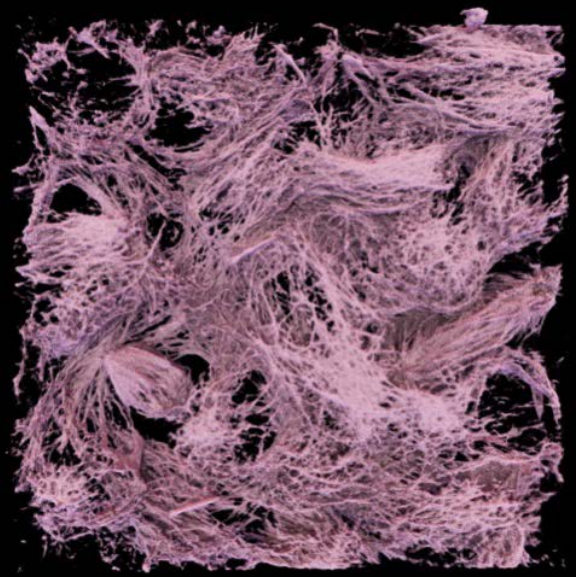
AGAVE cinematographic path-tracing

Cell membrane dye | DNA dye | mEGFP-tagged structure

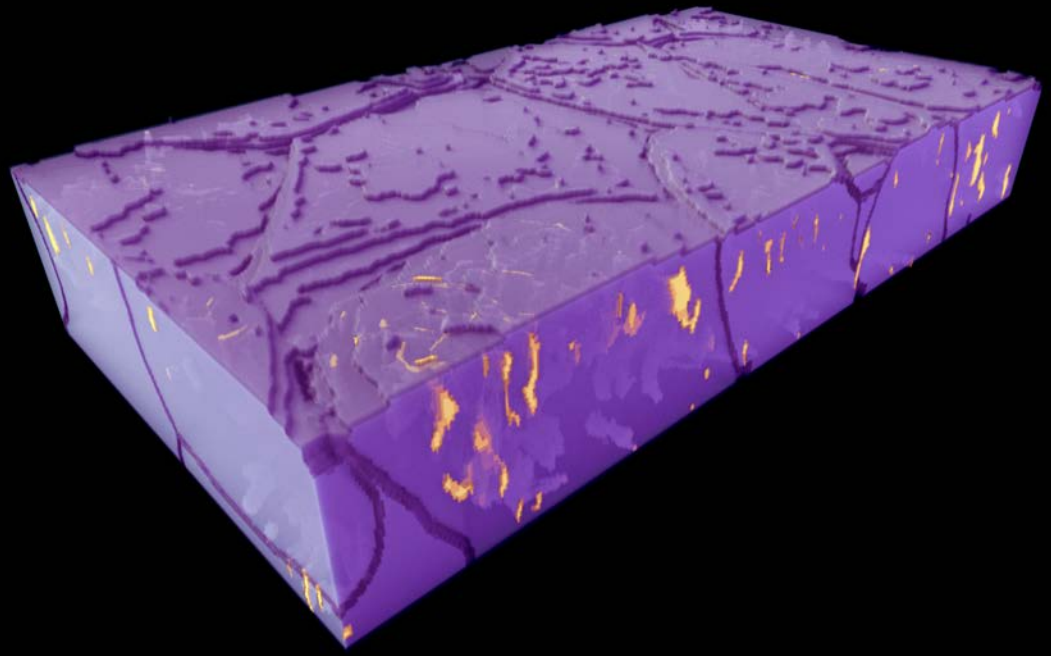
Why go to the computational expense of pathtracing?

Contributions beyond ambient occlusion: shadow casting can clarify topology





AGAVE can help understand segmentation results

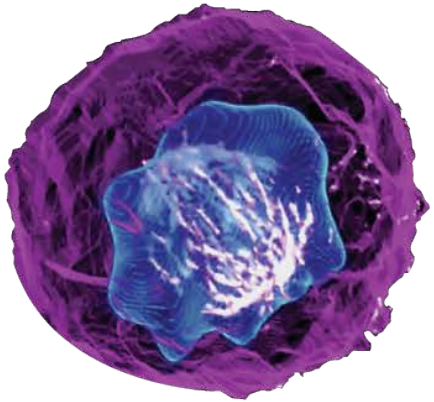


before training assay

after training assay

- help finding errors in 3D
- help comparing 3D effect of parameters
- help understand topology/morphology in 3D

Building the Allen Cell Explorer Tool Kit



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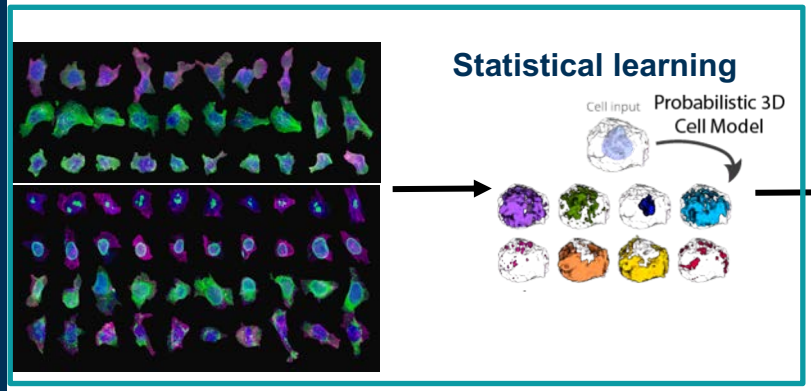
allencell.org

Building an ‘integrated cell’

How can we build a complete ‘Integrated Cell’ from images of a cells tagged with 1 or 2 structures at a time?

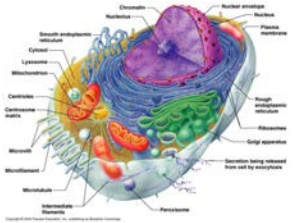


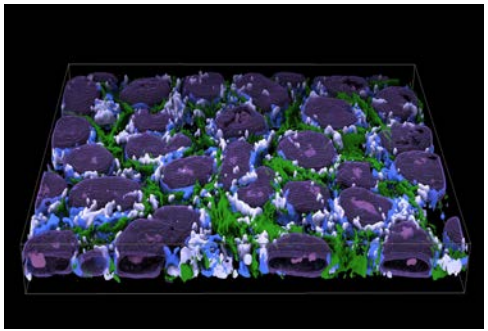
Statistical learning

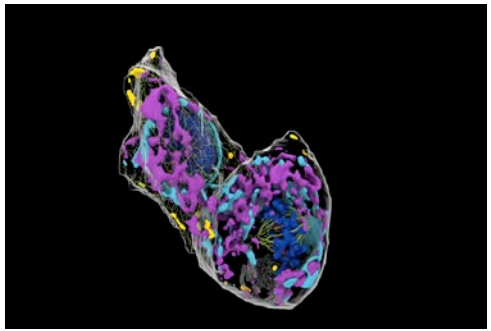
Spatial integration
Predictive modeling

Label-free approach

brightfield

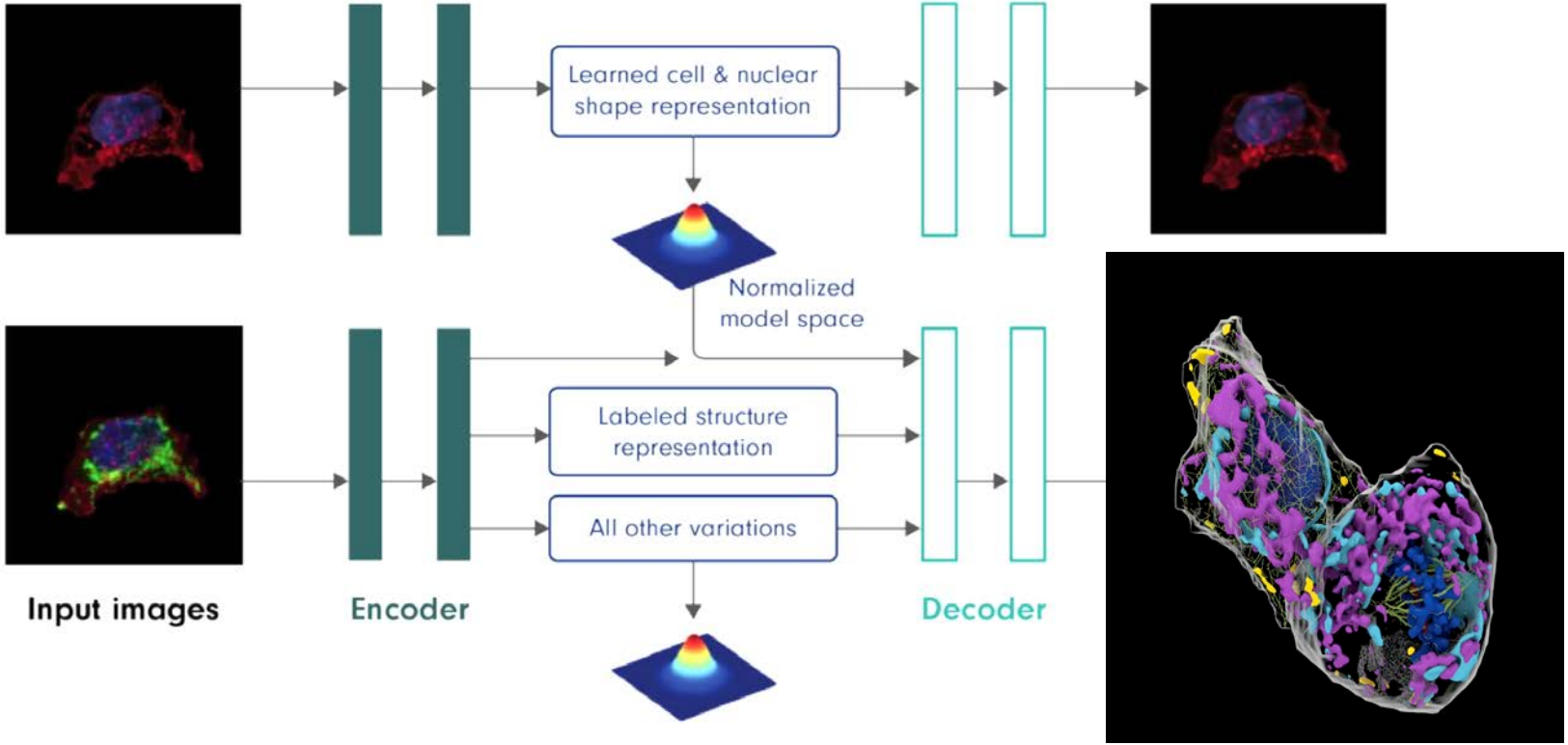




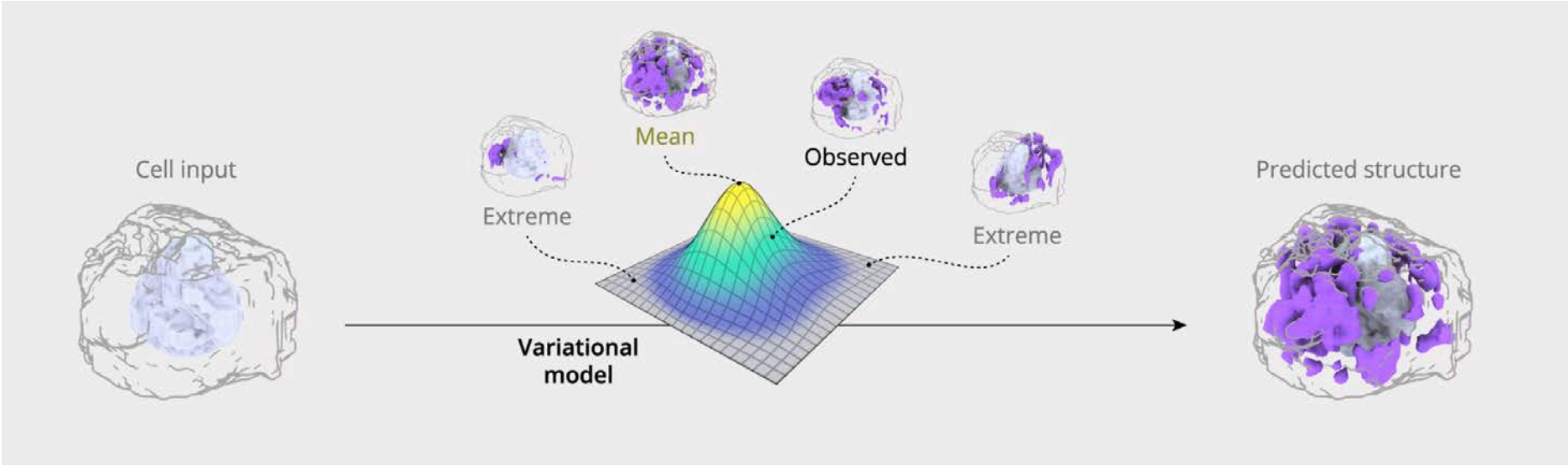


- ***Establish correlations***
- ***Generate hypothesis***
- ***Follow cell states***

Statistical Learning via Autoencoders



Statistical Learning via Autoencoders



coming soon on ***bioRxiv***

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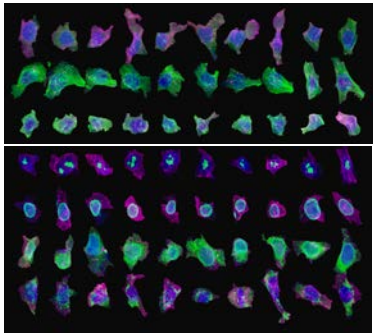
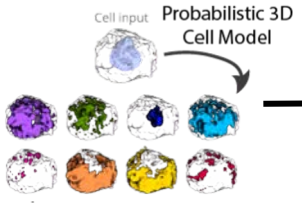


Statistical learning



Spatial integration
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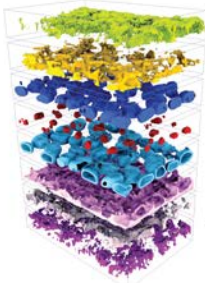
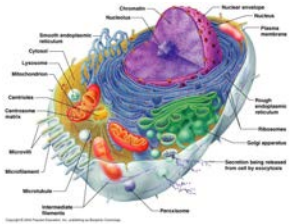
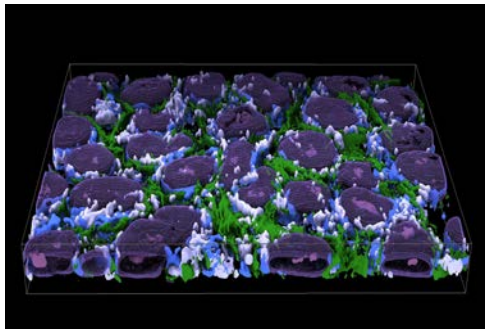
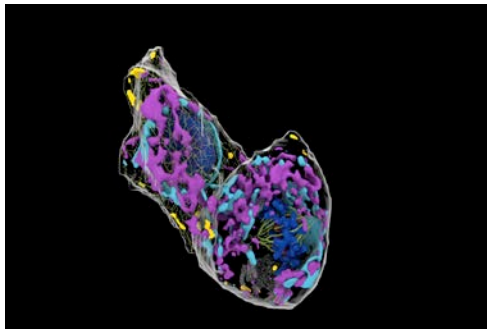
Label-free approach



brightfield







- ***Establish correlations***
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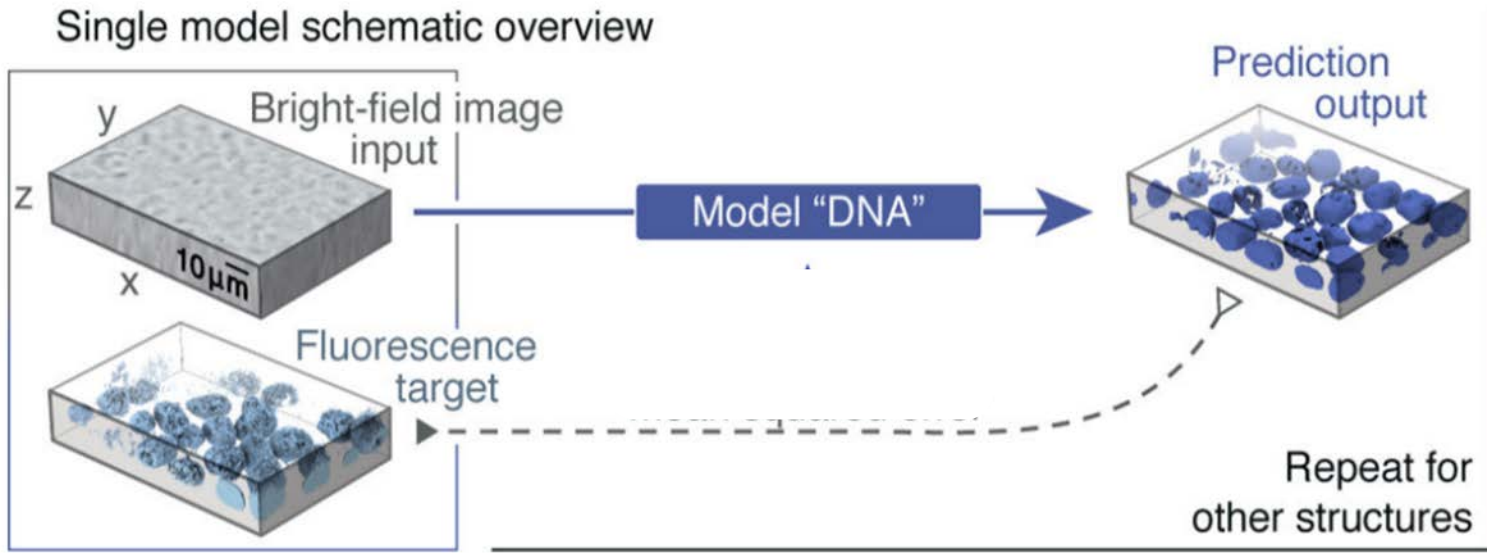
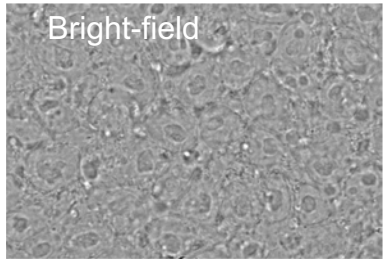
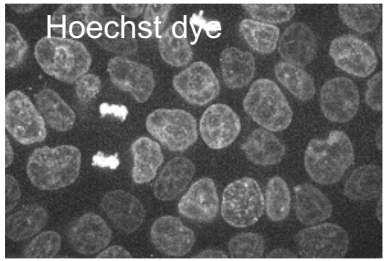
Label-Free Method



Label-free prediction of cellular structures

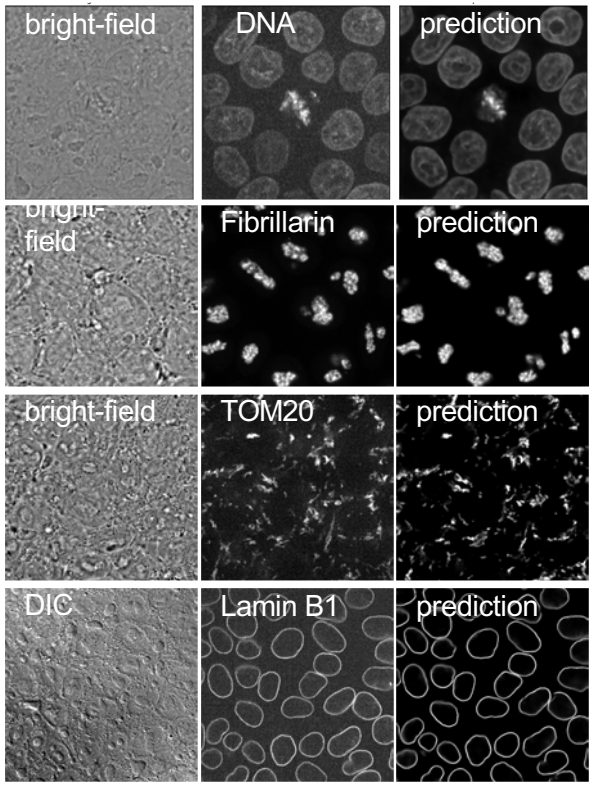


Training on DNA



Computer learns the features in the bright field image that correspond to the fluorescence

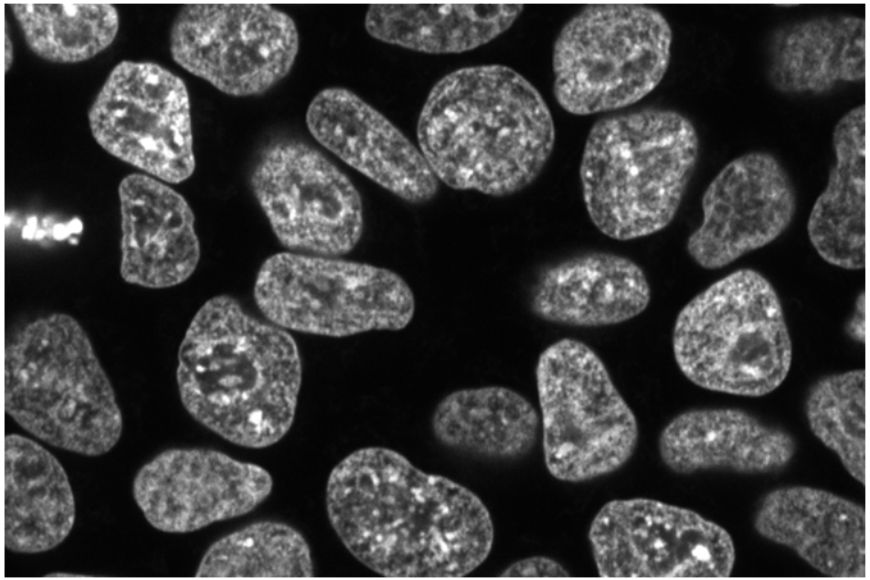
"U-net"



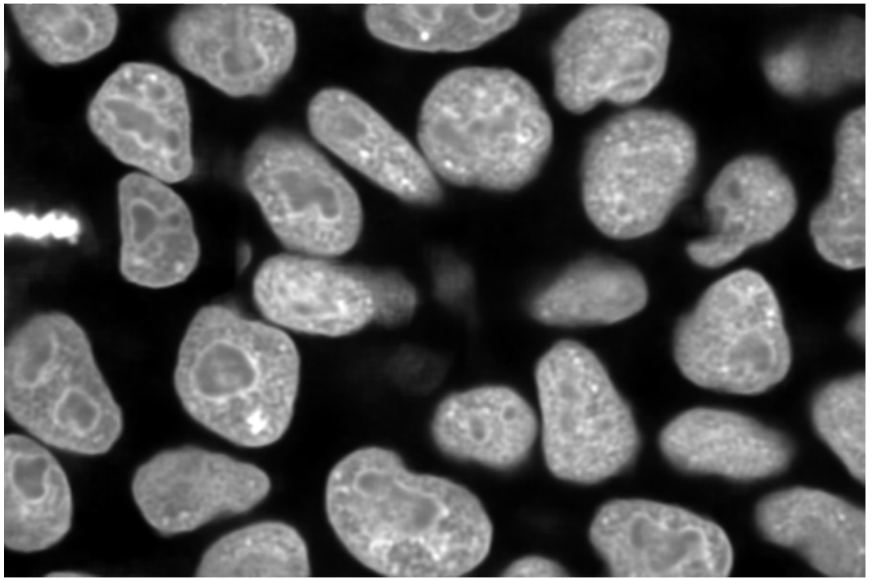
- Code available on GitHub
- Integrating label-free approaches with real data

Ounkomol, et al. Nature Methods 2019

Nuclear Structure Prediction



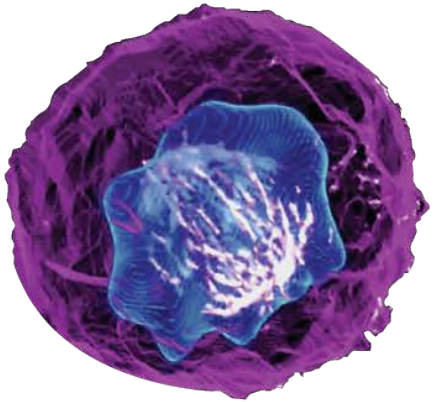
Real H2B



Label-free prediction

Current focus is the shape, not texture.

Allen Cell Explorer Tool Kit (allencell.org)



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Cell image simulator
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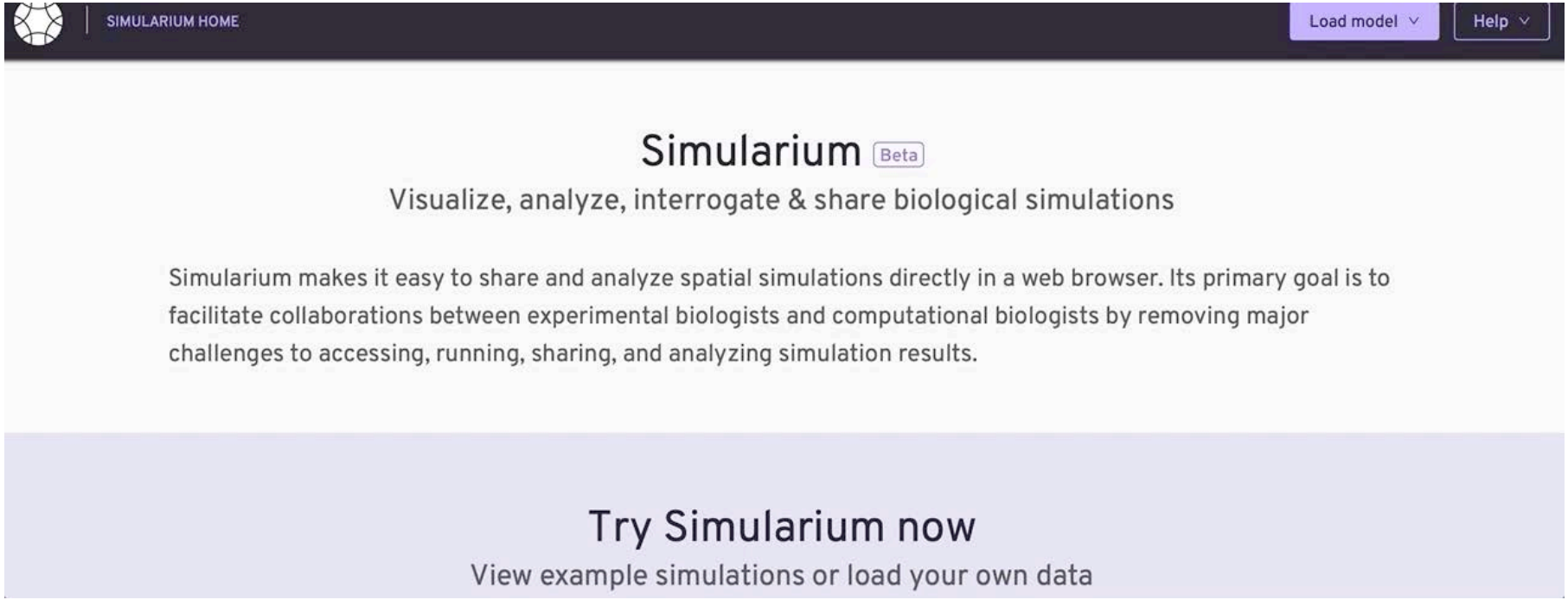
Simularium[Beta]

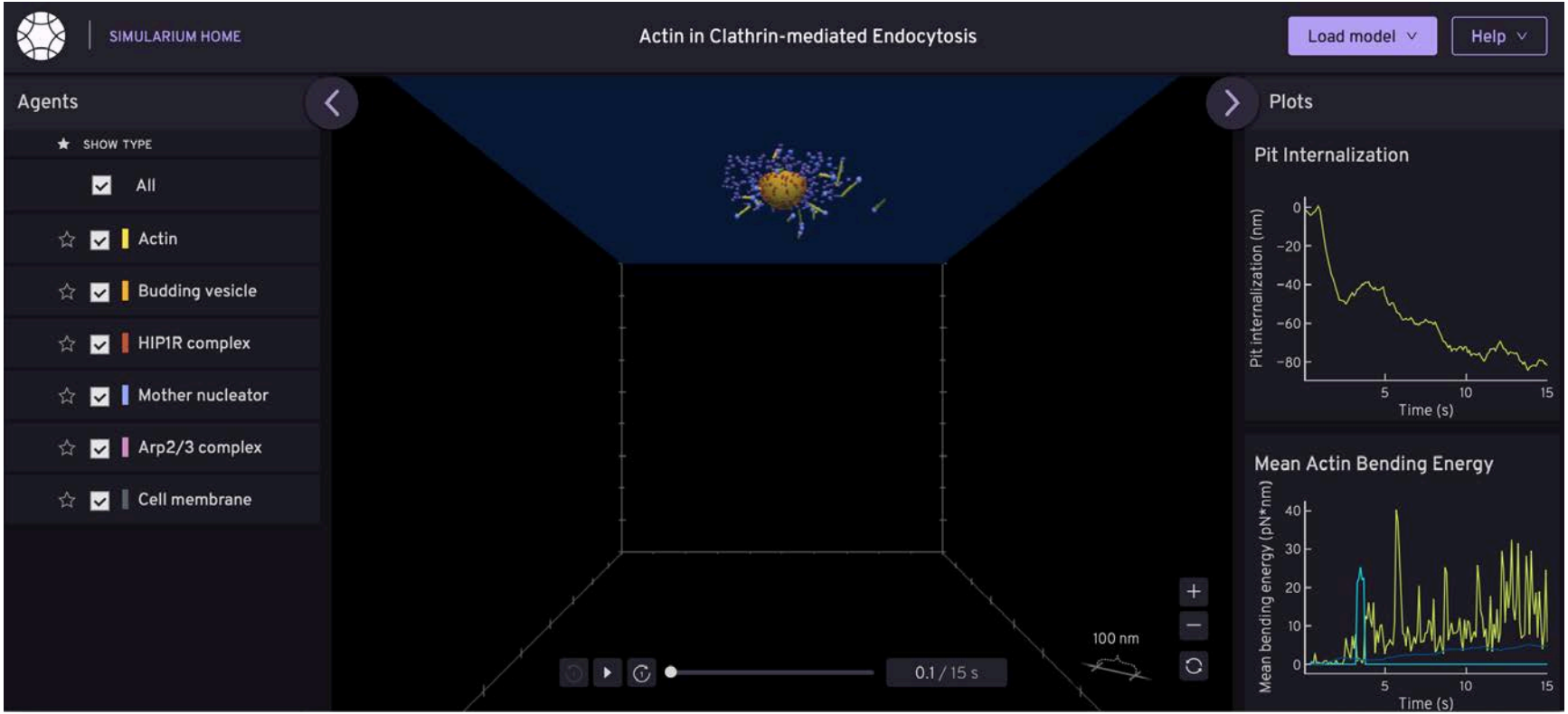
Visualize, analyze, interrogate & share biological simulations



simularium.allencell.org

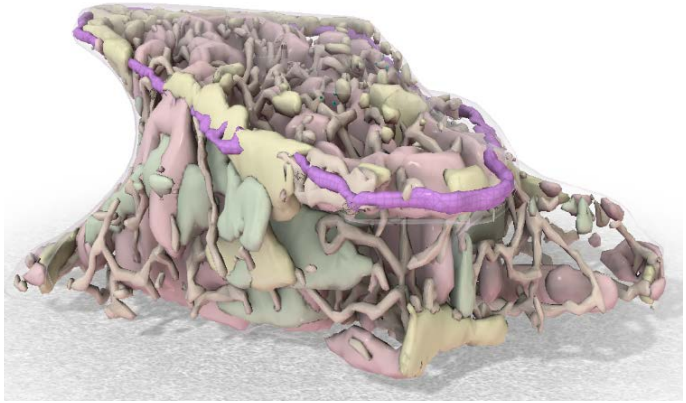
Try Simularium[beta]

View example simulations or load your own data



Tools to Visualize, Analyze and Model Cell Behavior

…what it did



what it will do…

cell organization ⇄ function

Create and understand a “state space” of human stem cell signatures

Holistic approach via 3D live cell imaging

Allen Cell Discussion Forum

Welcome to the Allen Cell Discussion Forum, the official community site for Allen Institute for Cell Science! This forum is a place for learning, helping, and sharing experiences of using our publicly-available cell lines, plasmids, genomic data, software, and analytic tools.
Get started: read our new user guide and know our community guidelines.

Gene-editing

Human iPSC gene-editing techniques, tips, & issues

hiPSC Culture

Human iPSC culture, differentiation techniques, tips, & issues

Imaging Software & Code

Label-free, cell segmentation, integrated cell modeling, and machine learning techniques, tips, & issues

Education

Cell biology educational resources, use-cases, & tips

More Info

Community Ground Rules & Privacy Statement

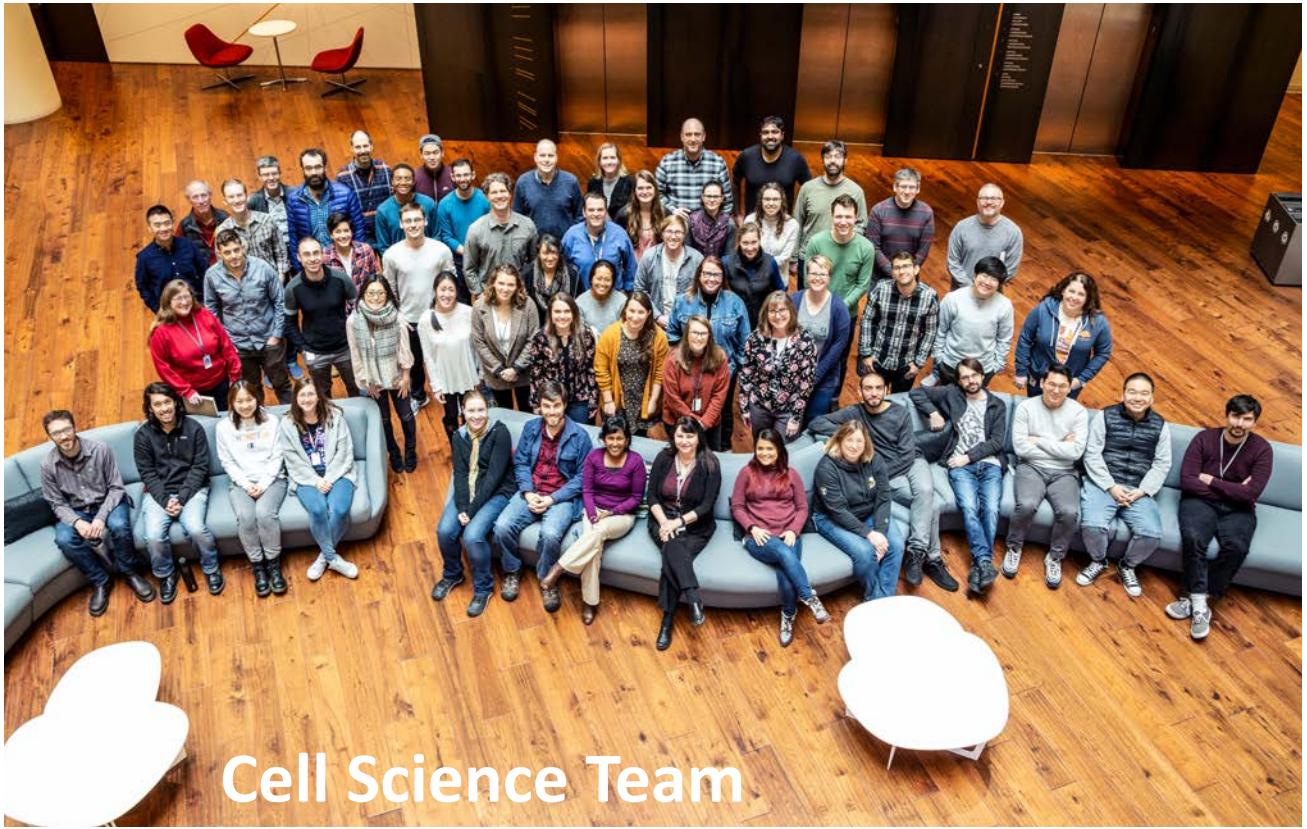
allencell.org

Full website of the Allen Institute for Cell Science including pages for Gene-editing Methods and Videos & Tutorials

Thank you

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

And a special thanks to our supporting partners at Coriell Institute and Addgene.



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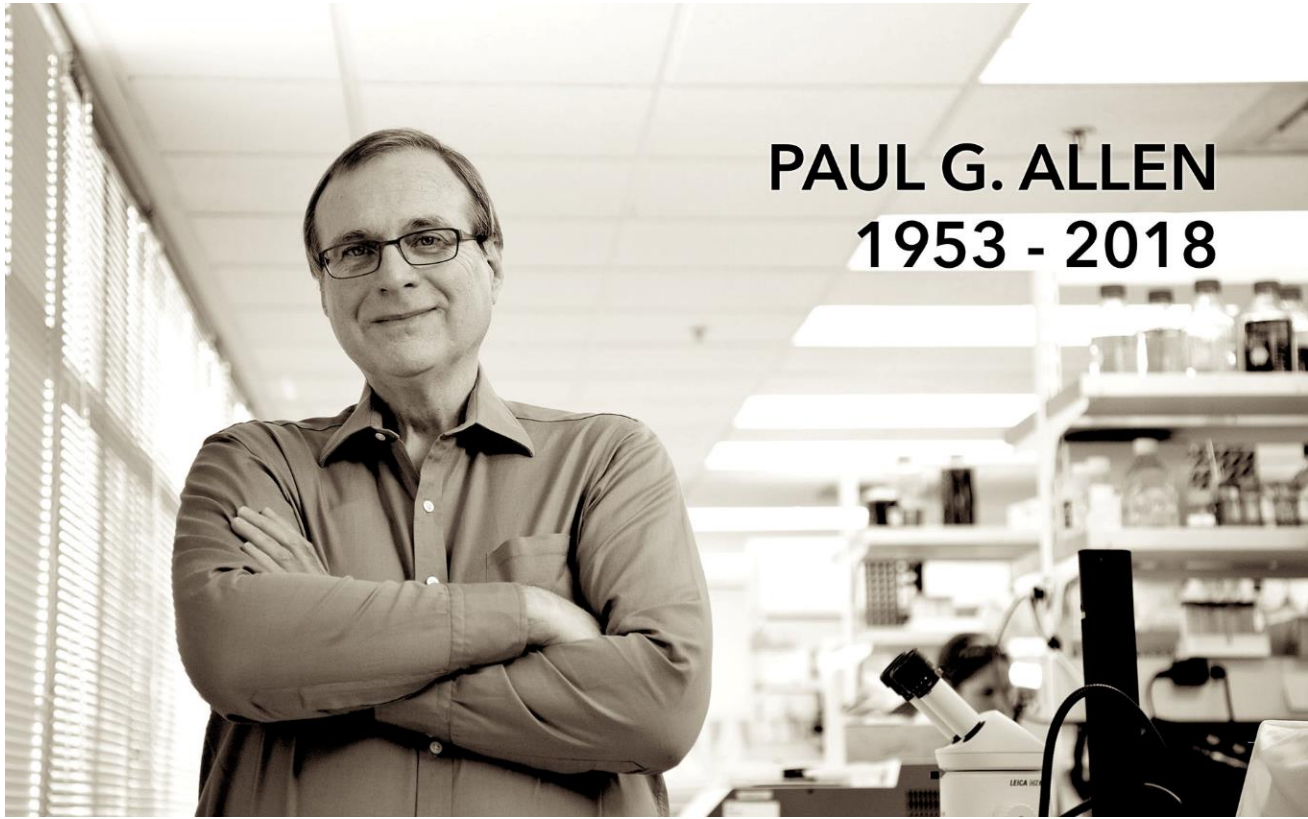

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