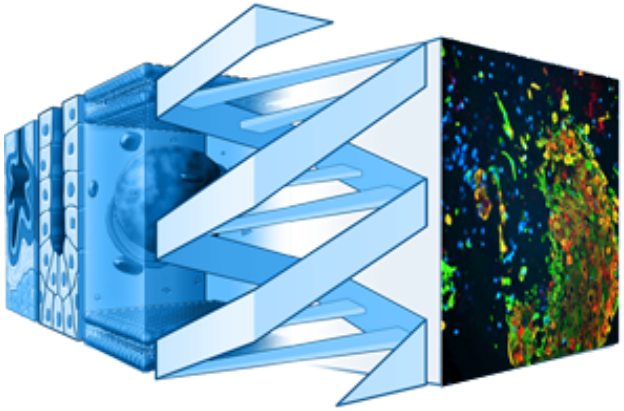

OHSU CENTER FOR SPATIAL SYSTEMS BIOMEDICINE

BioSciences

BERKELEY LAB
Lawrence Berkeley National Laboratory

LAWRENCE BERKELEY NATIONAL LABORATORY: LIFE SCIENCES DIVISION

Integrating OMERO into a correlative microscopy workflow: successes/struggles

Damir Sudar and many others

OME workshop - June 5, 2014

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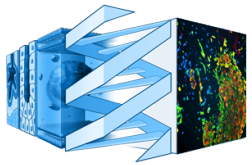
Oregon Health & Science University, Lawrence Berkeley National Laboratory, Quantitative Imaging Systems, Inc.



OMERO use: Research context

- OHSU: iLEM for Multiscale Structural Epigenomics
- LBNL: Integrated Bioimaging Initiative
  - Drosophila Gene Localization Atlas
  - Next Generation BioImaging project
  - Integrated Imaging of Microbial Community Response to External Threats
- OHSU: Various drug screens, RNAi aberration screens, and microenvironment screens

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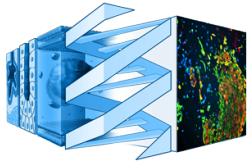
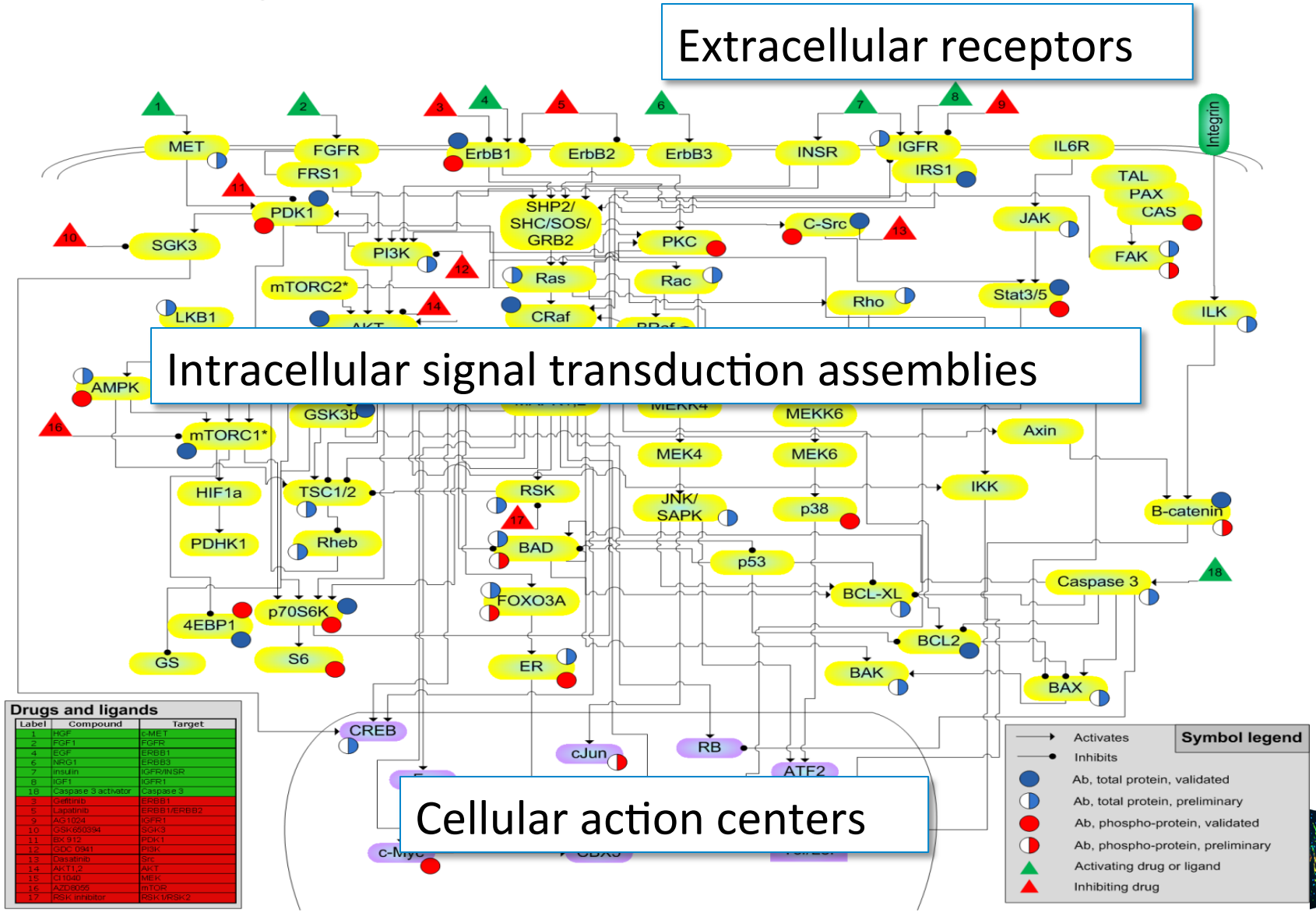


iLEM motivation: Understanding regulatory signal transduction

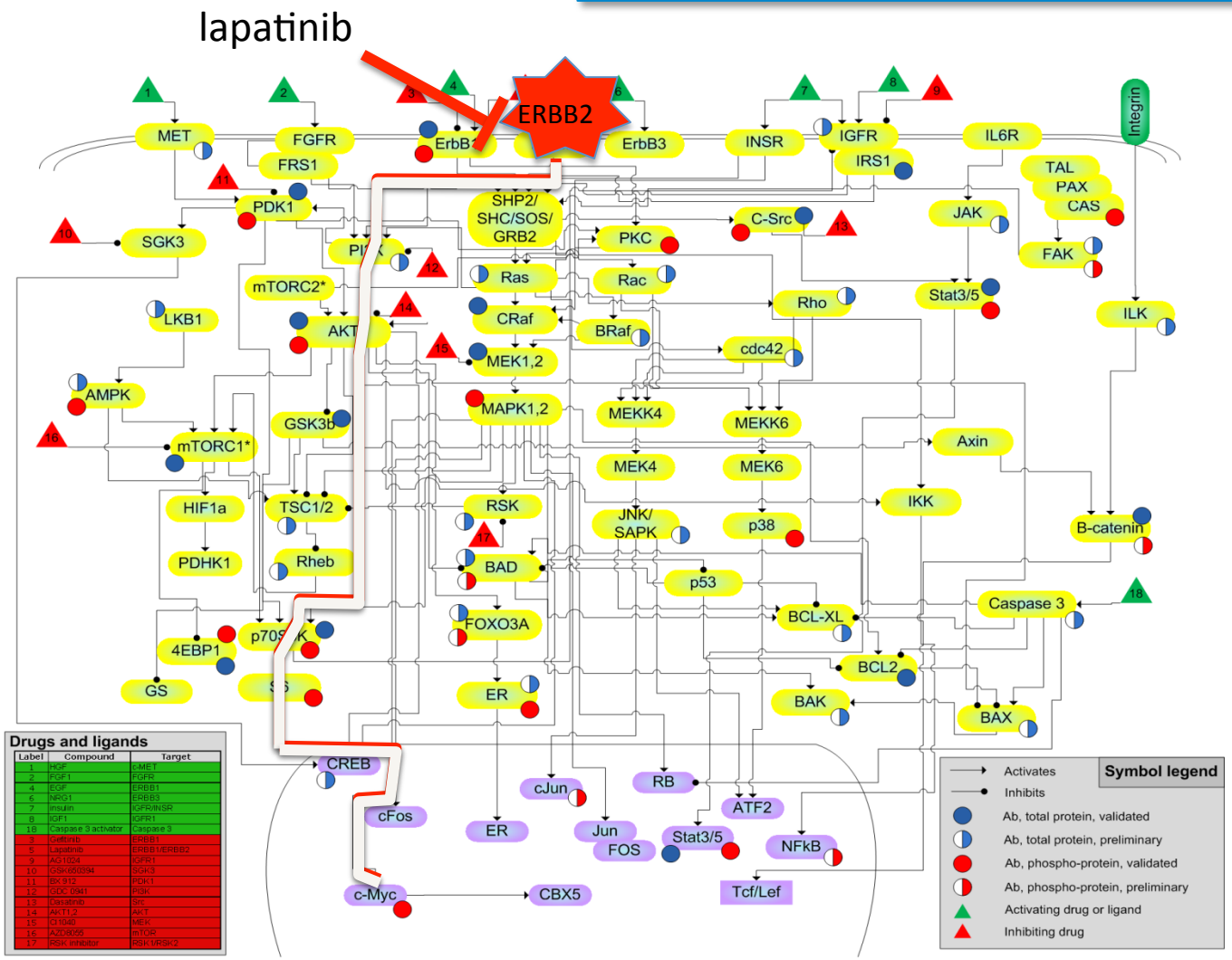
Elucidating the roles of the nano- and microenvironments



Prototype problem

RTK signaling in breast cancer

Our goal in cancer treatment is to develop therapies to correct the defect



RTK – Receptor tyrosine kinase

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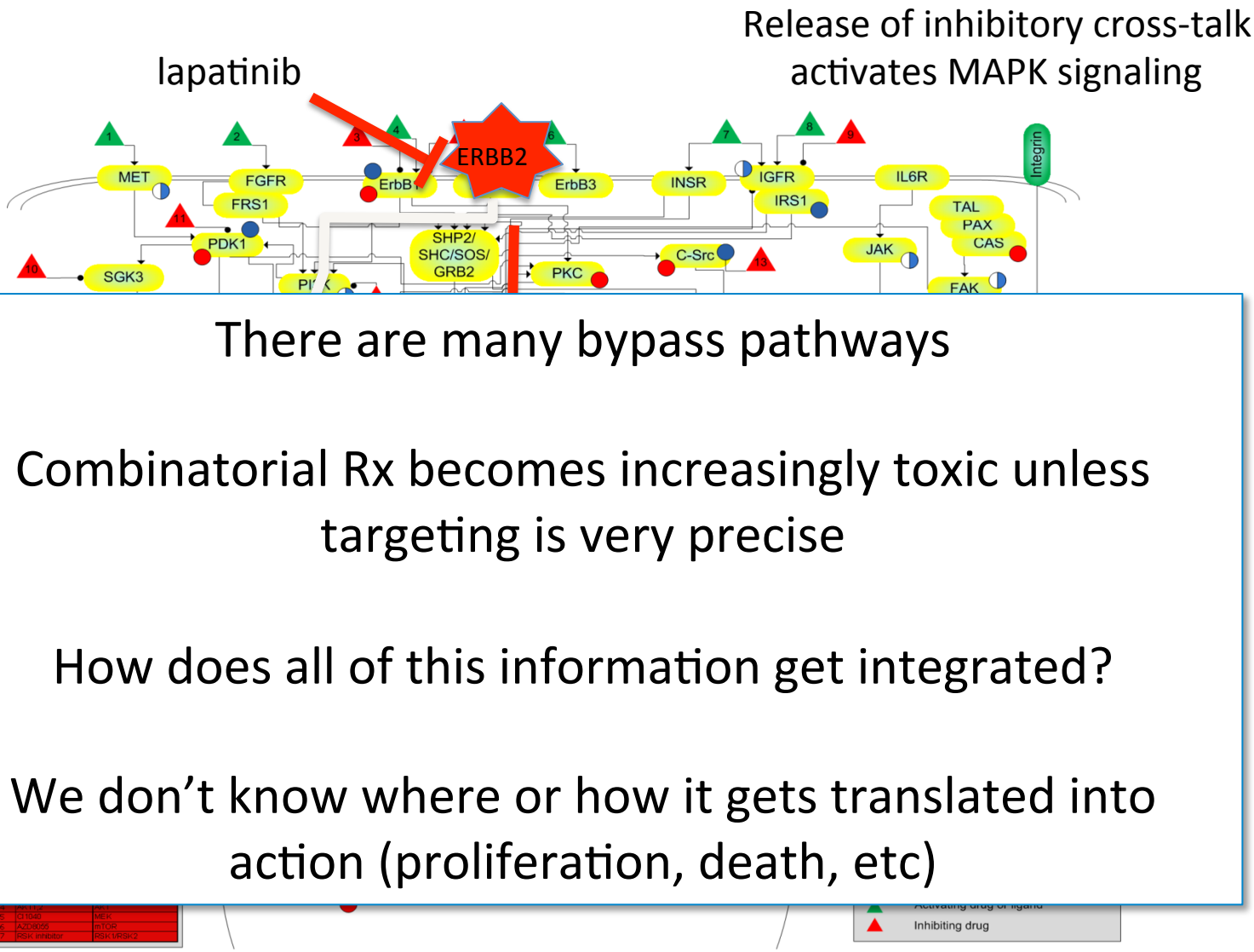
Activation of bypass mechanisms can attenuate response



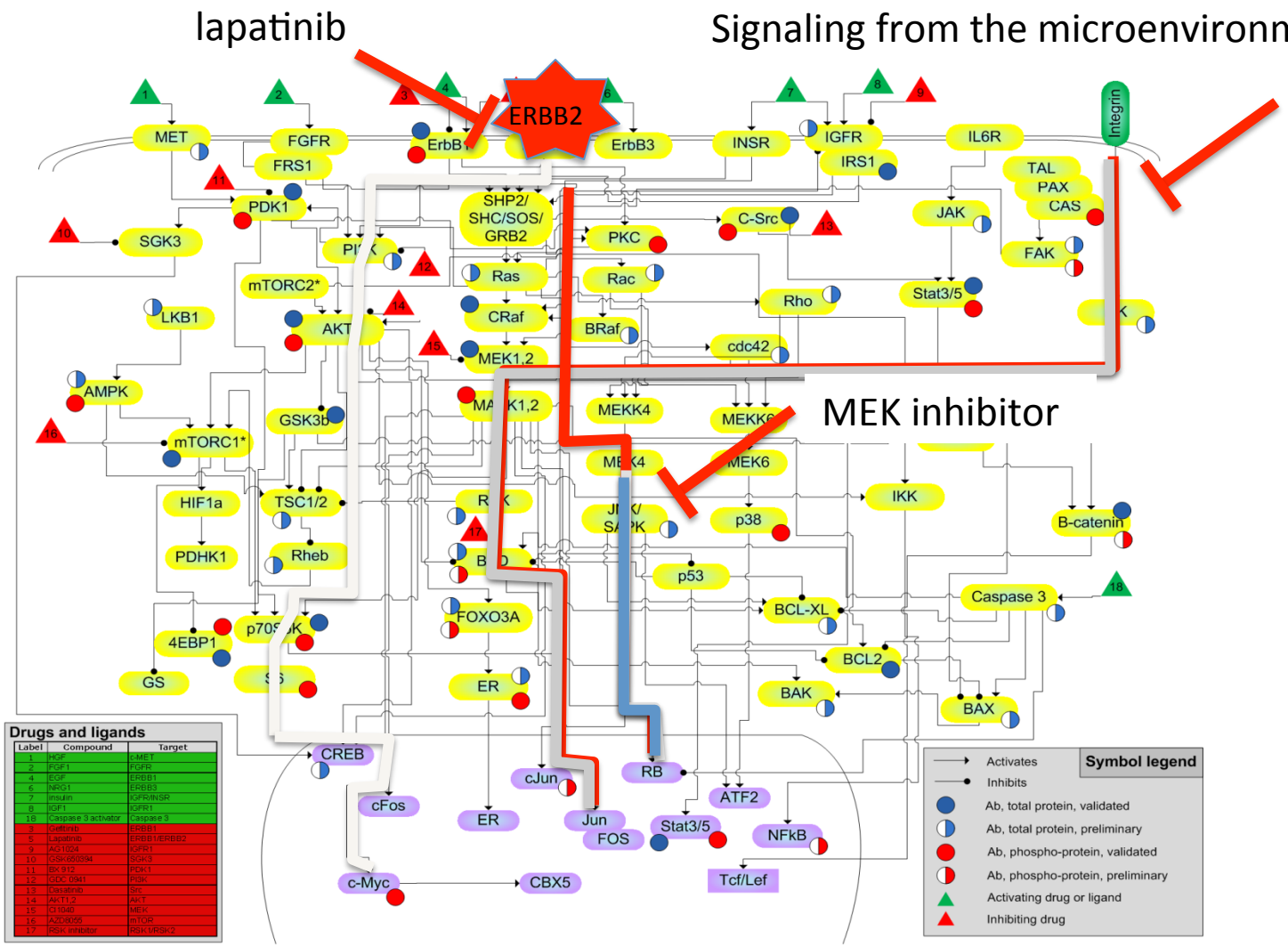
There are many bypass pathways

Combinatorial Rx becomes increasingly toxic unless targeting is very precise

How does all of this information get integrated?

We don't know where or how it gets translated into action (proliferation, death, etc)

Signaling from the microenvironment also can bypass the inhibitor



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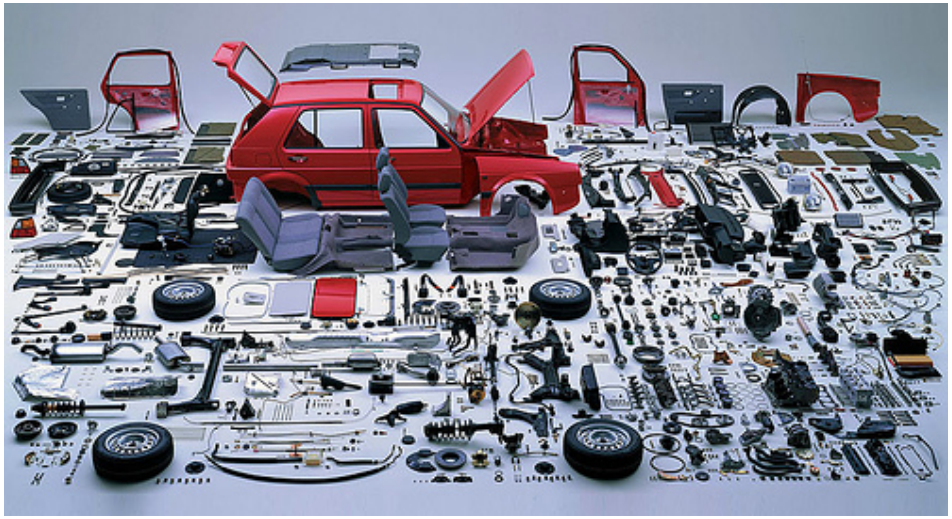
We need to know how the parts are assembled in order to fully understand function (and response to therapy)

Moving from the parts list to the assembly manual



You can't understand something from looking at the parts

You need to know how they work together

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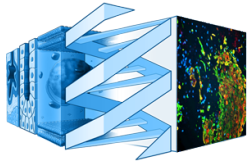
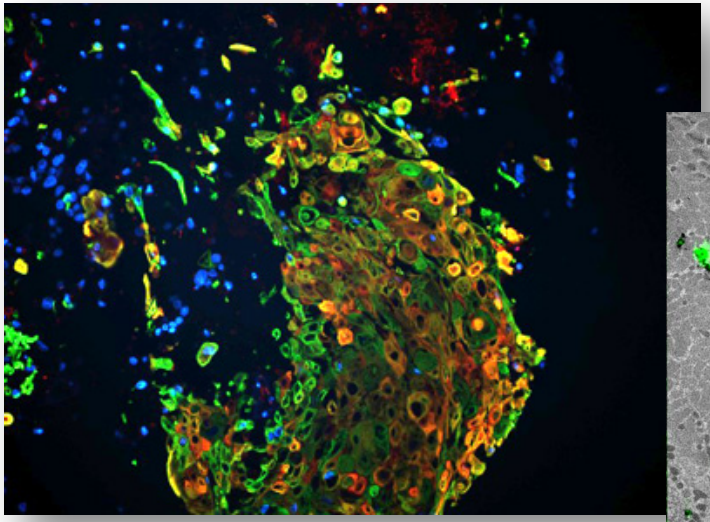
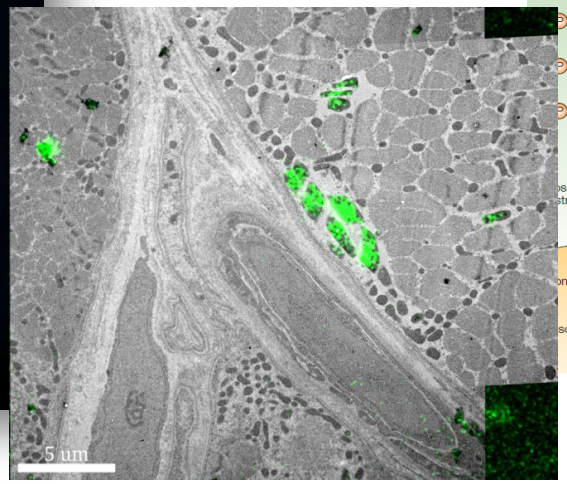


These studies motivate the development of strategies to visualize pathways in situ

Defining the microenvironment

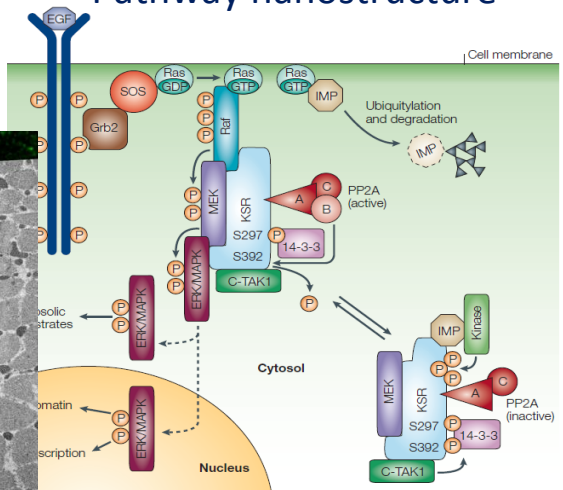


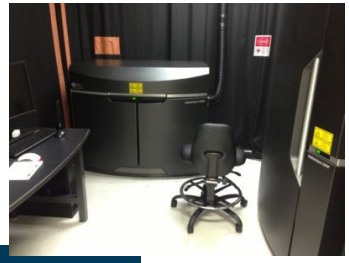
Basal breast cancer CK5/6 green, CK17 orange, CD44 red, DAPI blue



iLEM will define sub-cellular and micro-environmental context

Pathway nanostructure





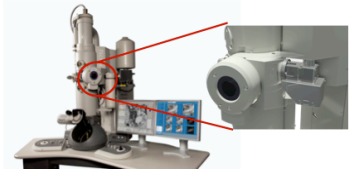
OMX

Olympus Scan^R

Tecnai iLEM ™

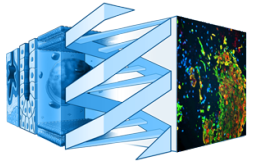
Tecnai iLEM™

The Integrated Light and Electron Microscope; a fast, accurate, and automated solution for correlative light and electron microscopy.



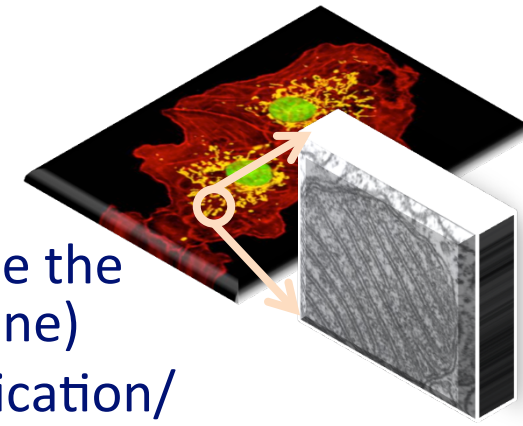
FEI CorrSight

FEI Helios



Correlative Microscopy

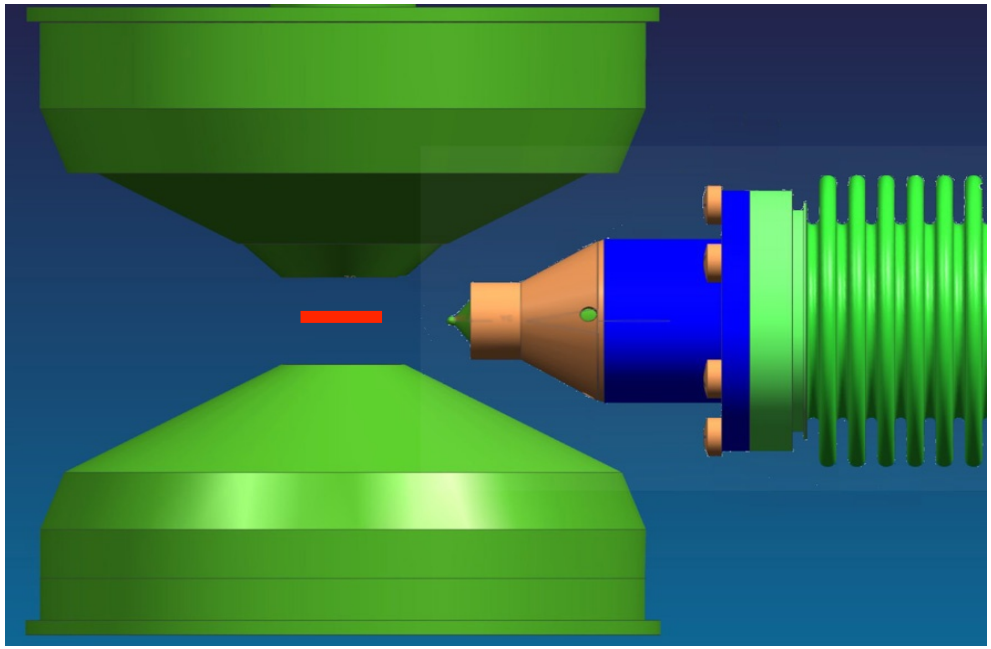
- Find specific cells in a large field of view
- Find specific areas in a cell
  and zoom in to study these at the ultrastructural level

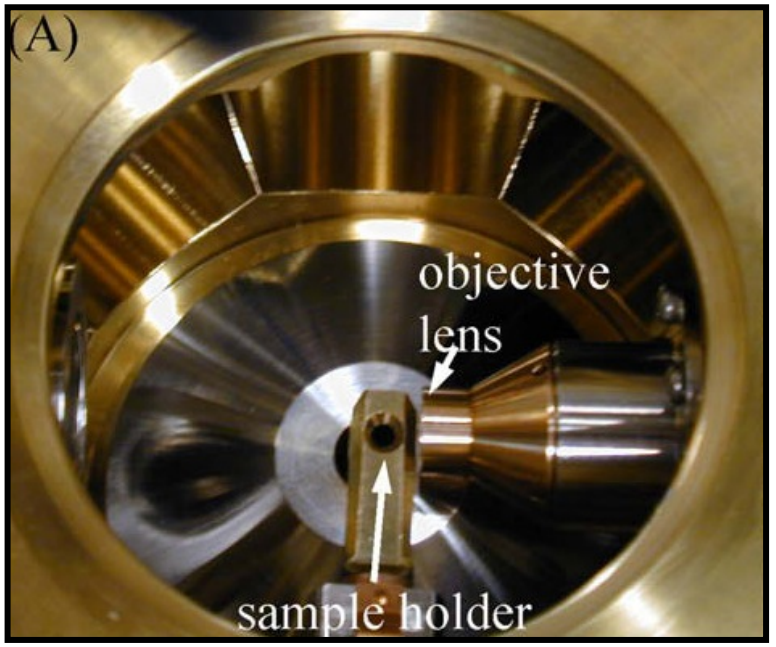


- How?
  - Use two different microscopes and correlate the information (two machines or combine in one)
  - Use a machine that has a very large magnification/ resolution range and multimodal imaging

Truly Integrated Light and Electron Microscopy (iLEM)

Agronskaia AV et al., J Struct Biol. 2008

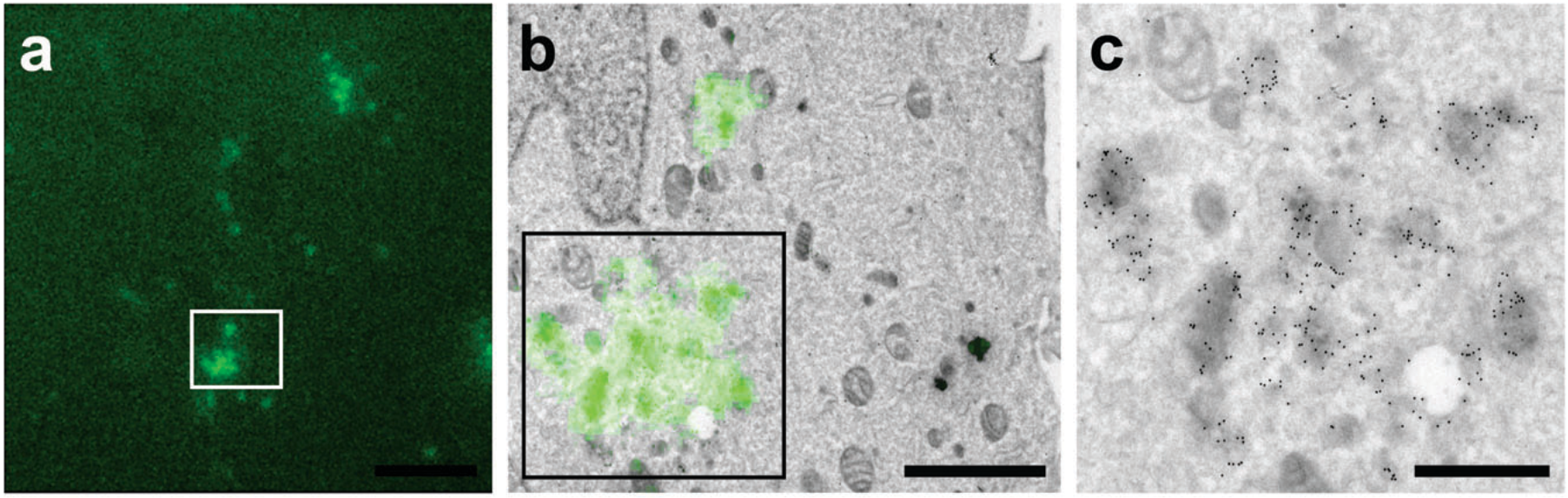




Optimal Navigation Tool for correlative microscopy

- Faster correlation between FM and EM
- Less potential contamination (cryo)
- No compromise on EM performance, decline in FM resolution

Sample prep is critical and difficult to optimize



THP-1 cells fixed with VIS2FIX$_{FS}$ and IF+IG labeled for LAMP-2 (a lysosomal protein)

Karreman and Donselaar et al., Traffic 2011

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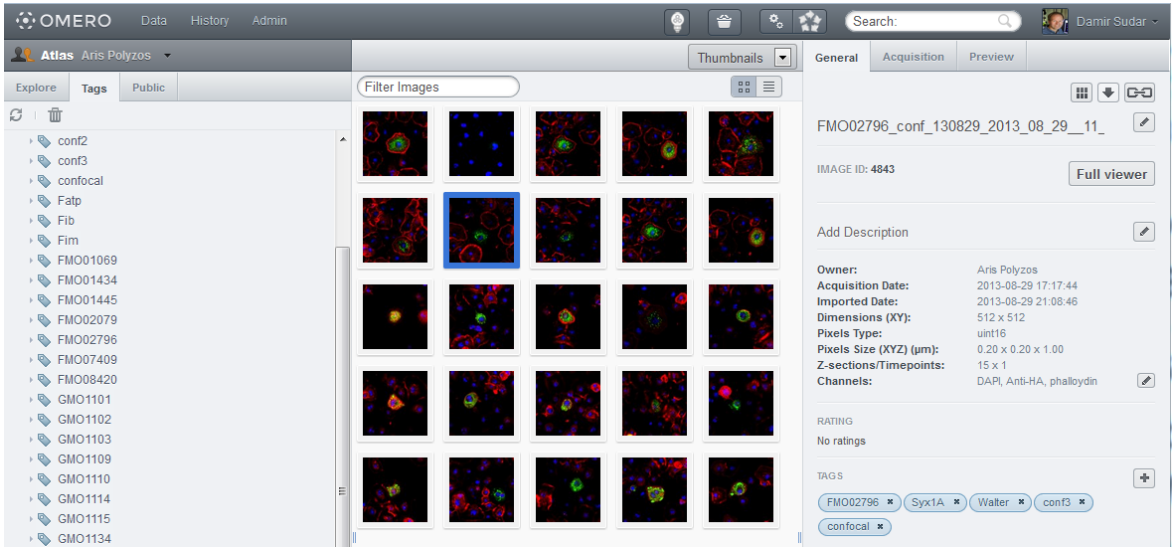
Managing the image data, metadata, and experimental parameters

- Image data and image metadata: OMERO
- Experimental metadata: ISA-Tab & various Minimum Information formats (MIARE, MIACA,)



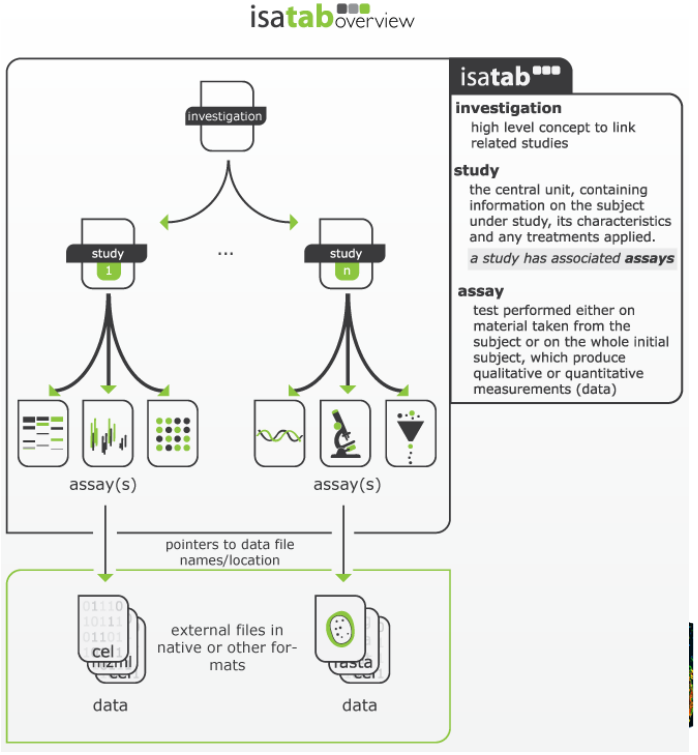


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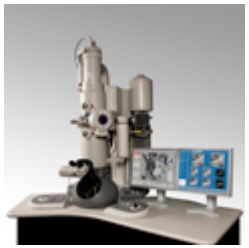
Image acquisition workflow

Titan Krios TEM

TeraStation

Helios FIB/SEM

TeraStation



Tecnai w/ iCorr

TeraStation

Scan^R and other light microscopes

Local disk

OMERO server
w/ 10TB

Utrecht - Netherlands

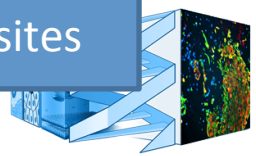
FEI™ Explore. Discover. Resolve.

Eindhoven - Netherlands

LBNL

UCB

Other sites



Our experience

- Likes:
  - Easy setup (after dealing with OHSU firewall issues)
  - Excellent retrieval performance: inside/outside OHSU
  - Fully user-initiated image uploads and image retrieval
  - Good support for our image/experiment types
- Wishes:
  - Support for rich experimental metadata (e.g. ISA-Tab)
  - Bulk image download
  - Import using OMERO.web
  - Deeper folder structure

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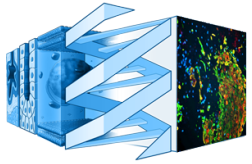
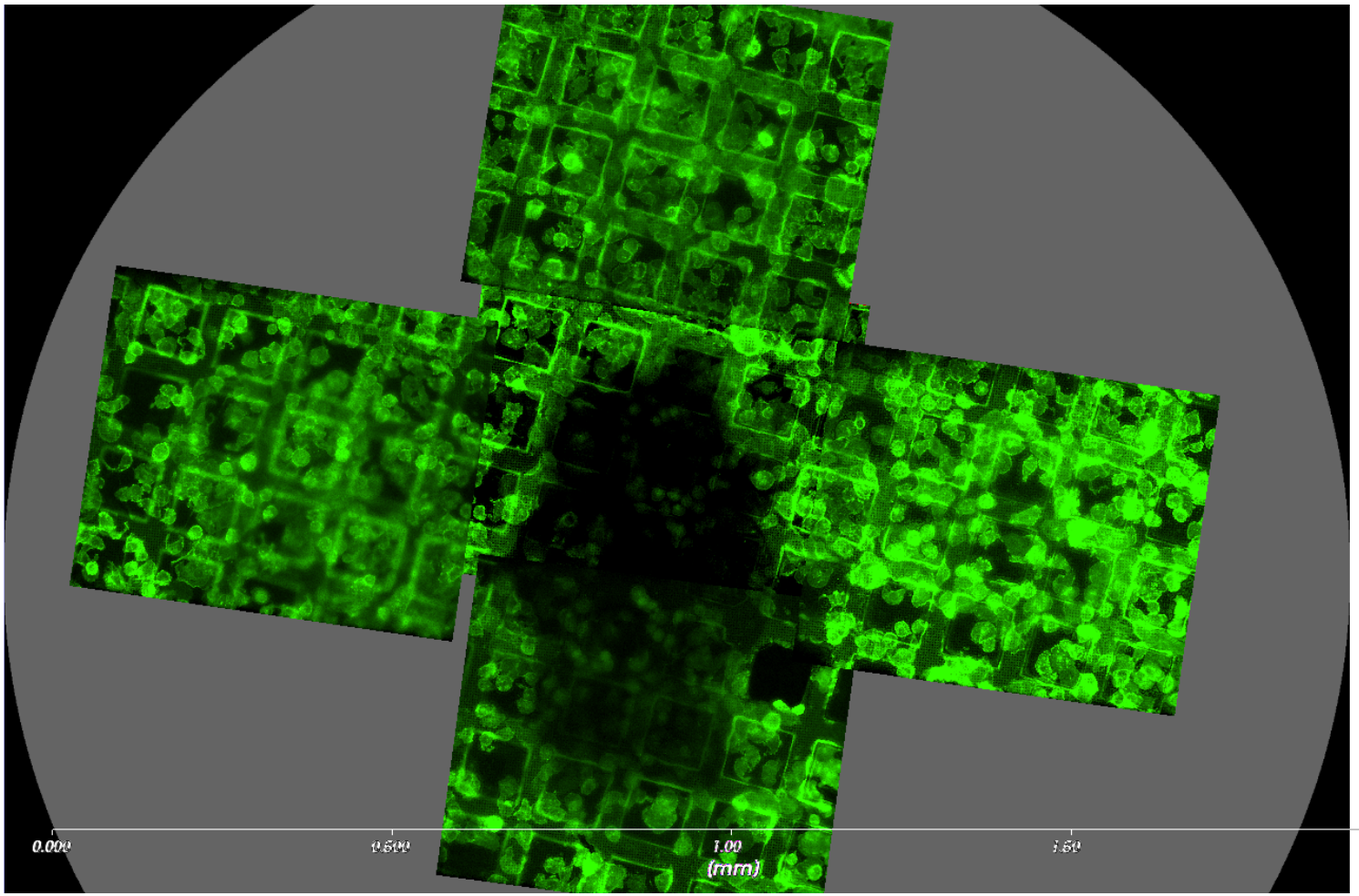


2D iLEM workflow w/ iCorr



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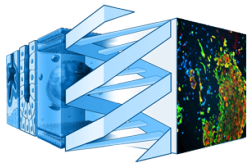

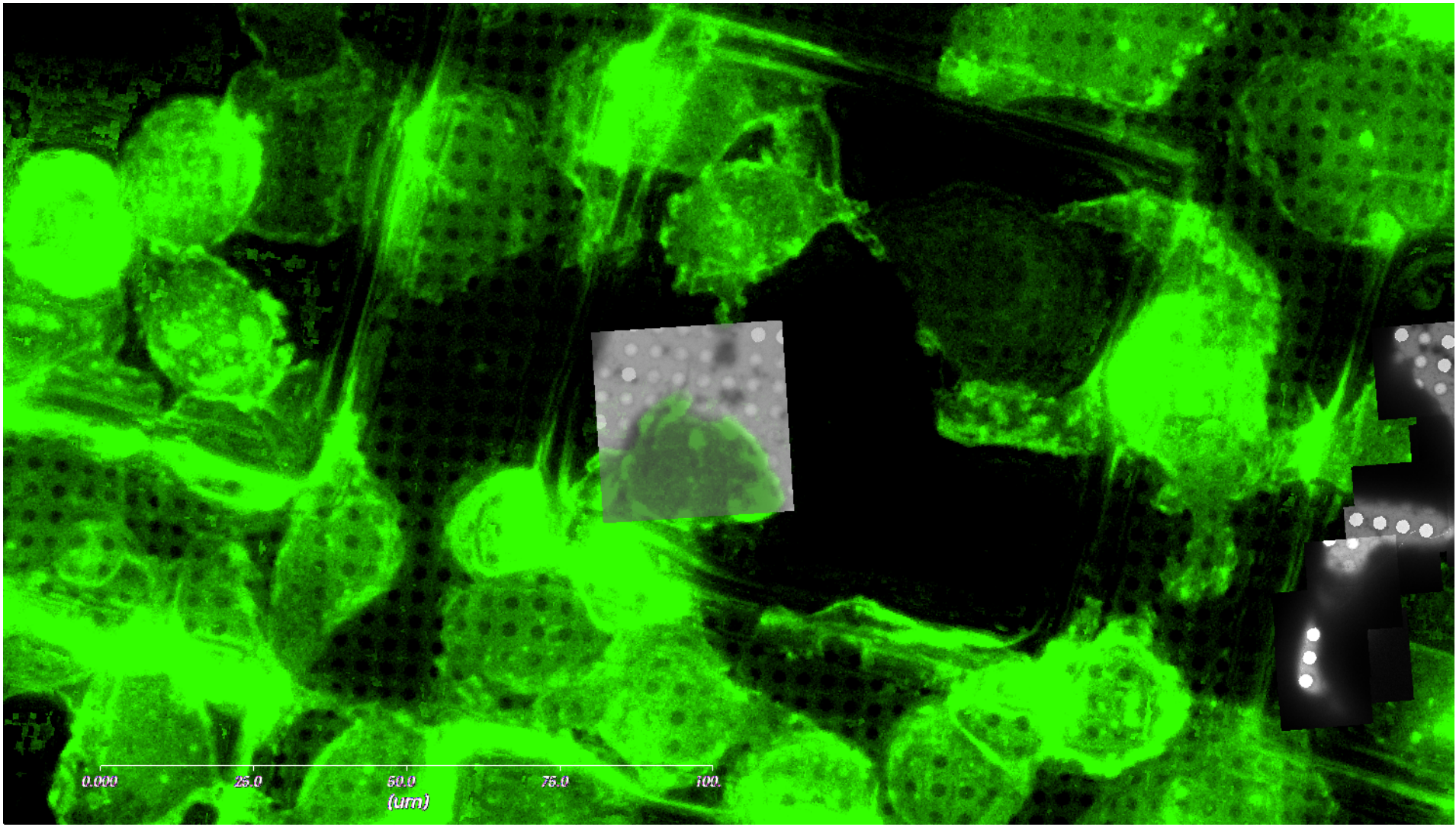

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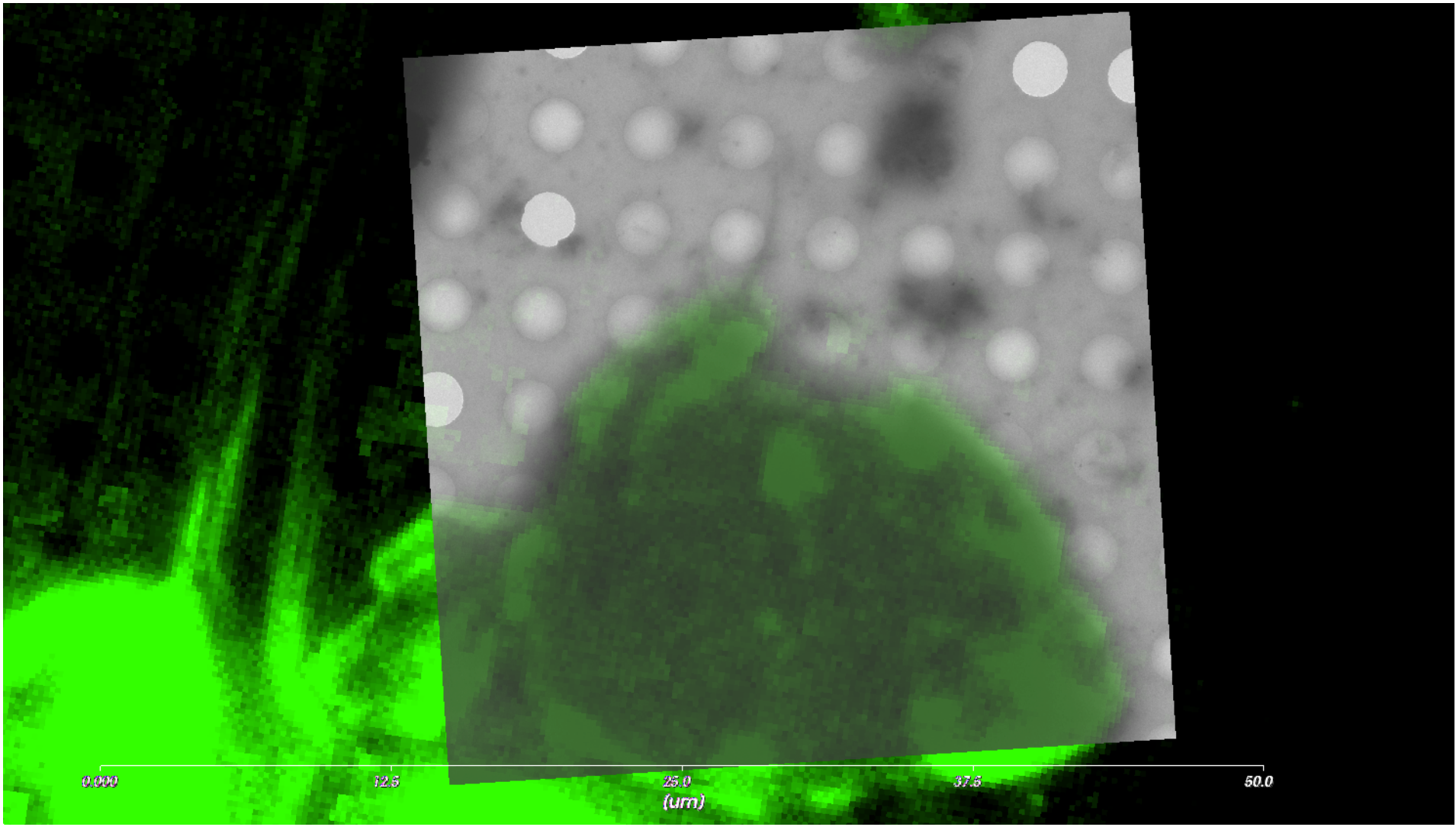

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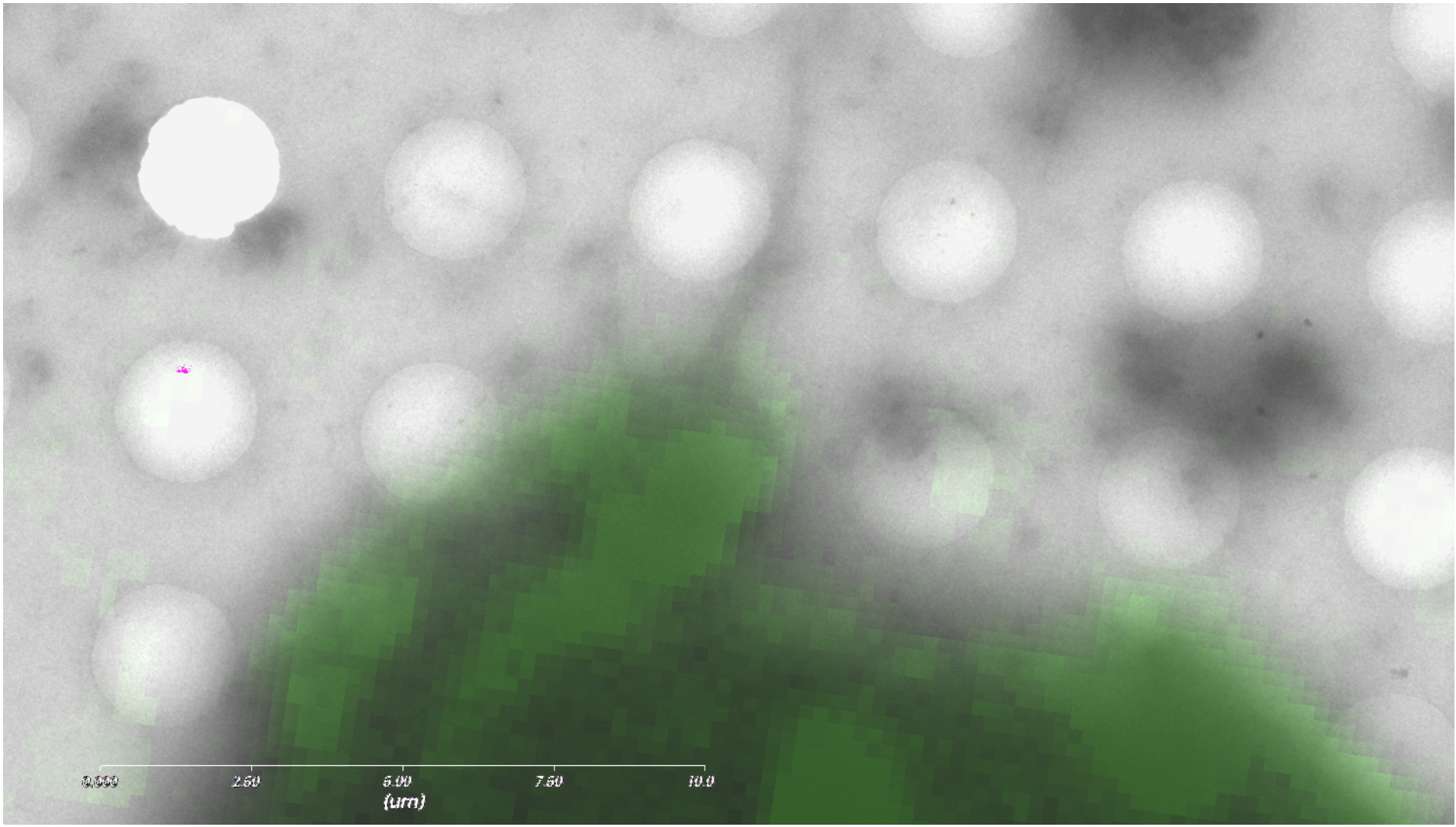

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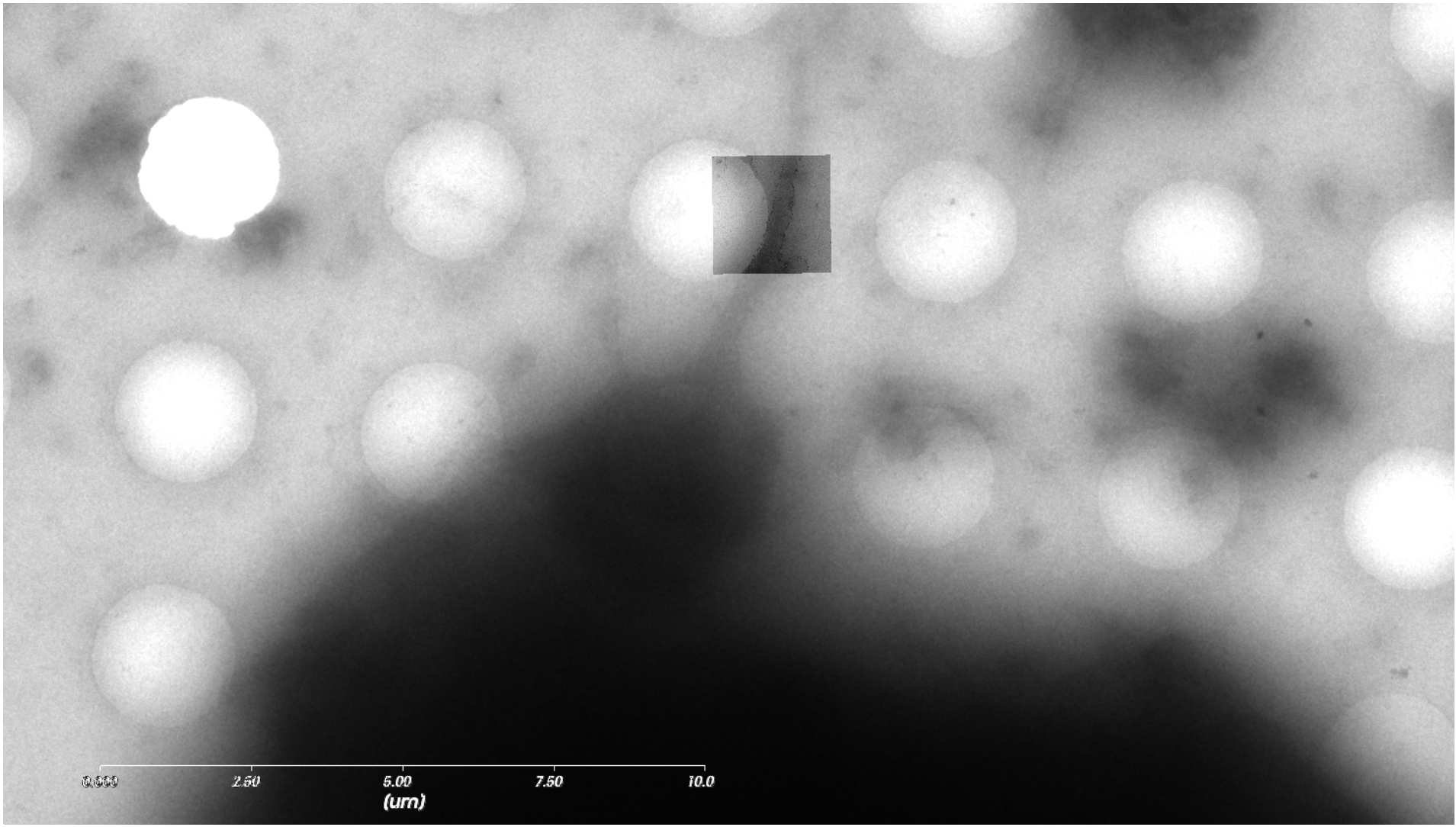

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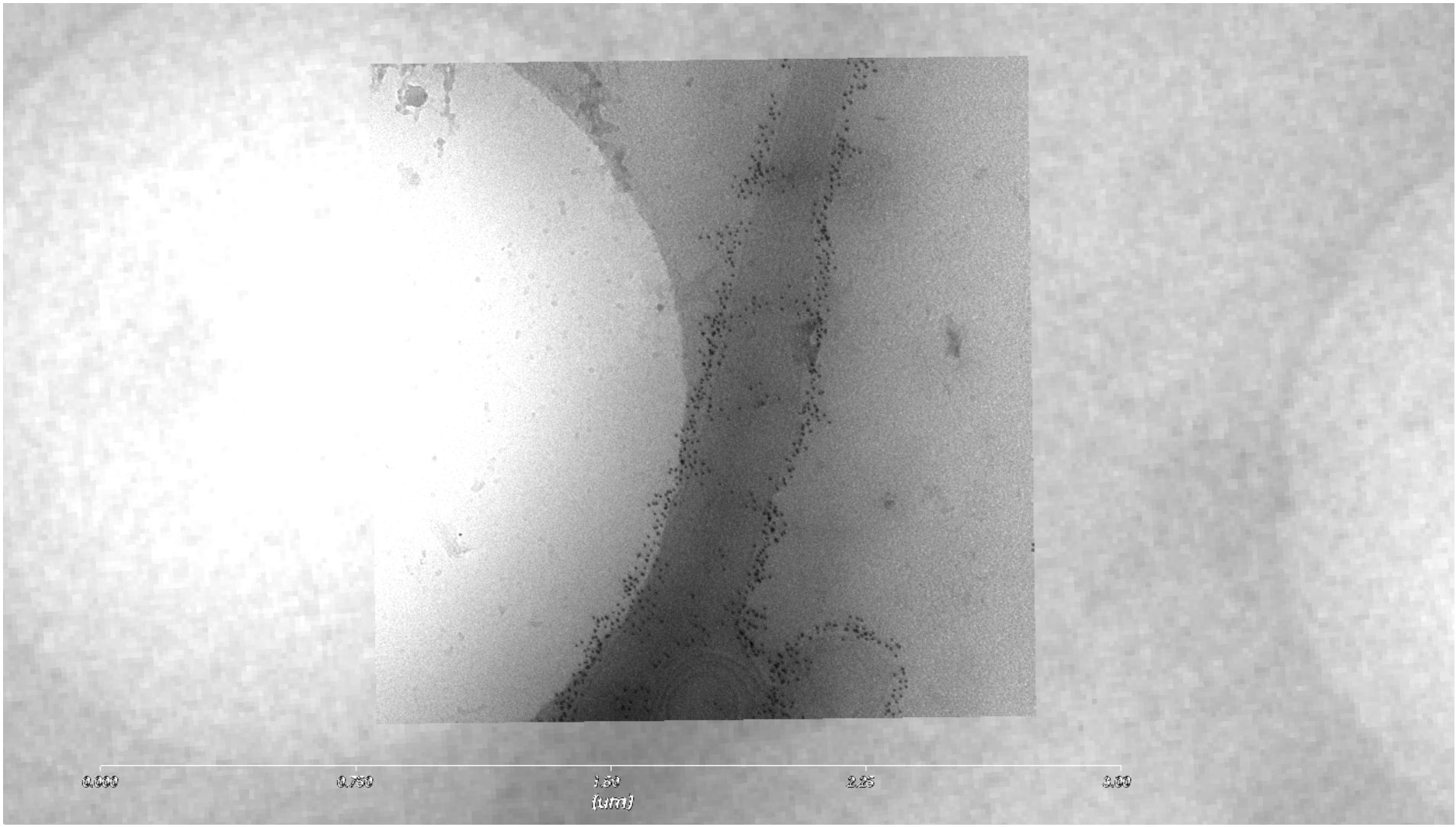

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3D iLEM workflow w/ FIB/SEM

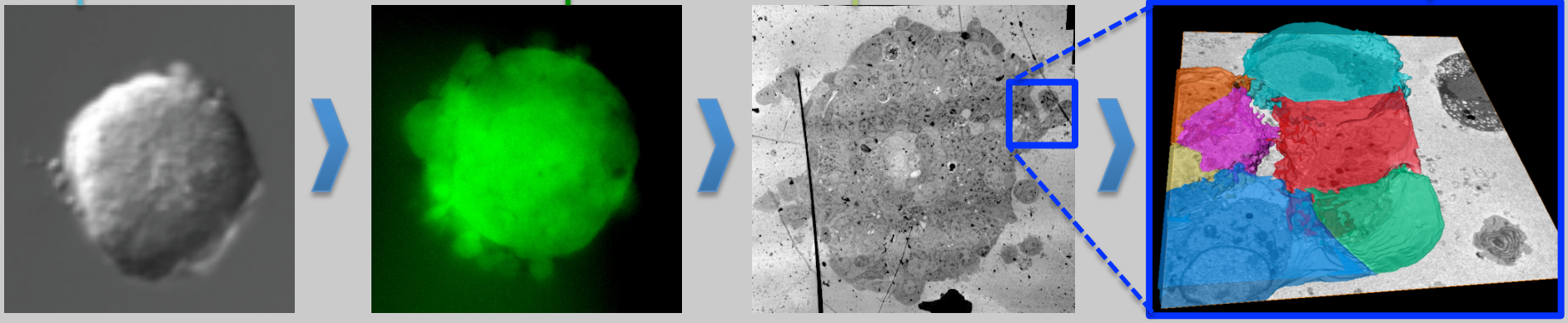
Integrating molecular information and structural detail with nanometer precision

- ✓ Multicellular growth (Organovo)
- ✓ Printing wide spectrum of tissues
- ✓ Disease oriented research
- ✓ Live cell imaging

- ✓ Modular fluorescent probes
- ✓ Tracking during growth
- ✓ Super resolution capable
- ✓ EM sample prep compatible

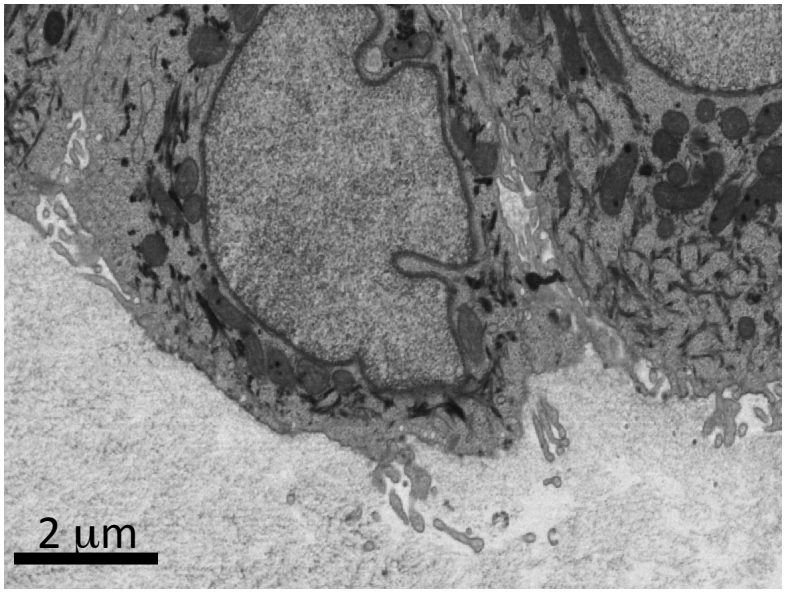
- ✓ Cryo-immobilization (HPF & plunge)
- ✓ Preservation of probes in EM prep
- ✓ Multiple EMs (Krios, Helios, iCorr)
- ✓ MAPS correlation of SEM data

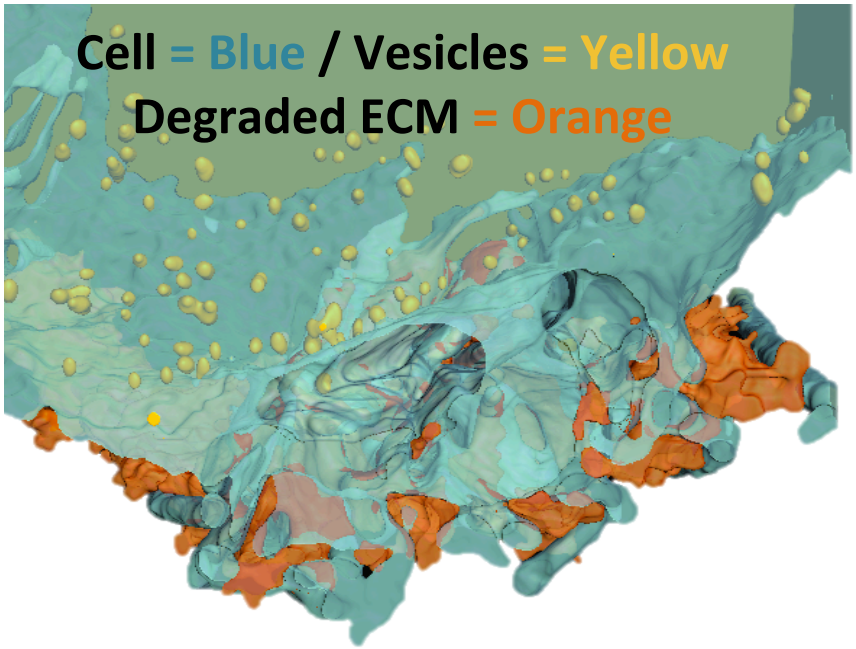
- ✓ Development of Integrative Software
- ✓ 3D rendering & reconstruction
- ✓ Precision analysis of 3D data
- ✓ Mapping molecular information into 3D

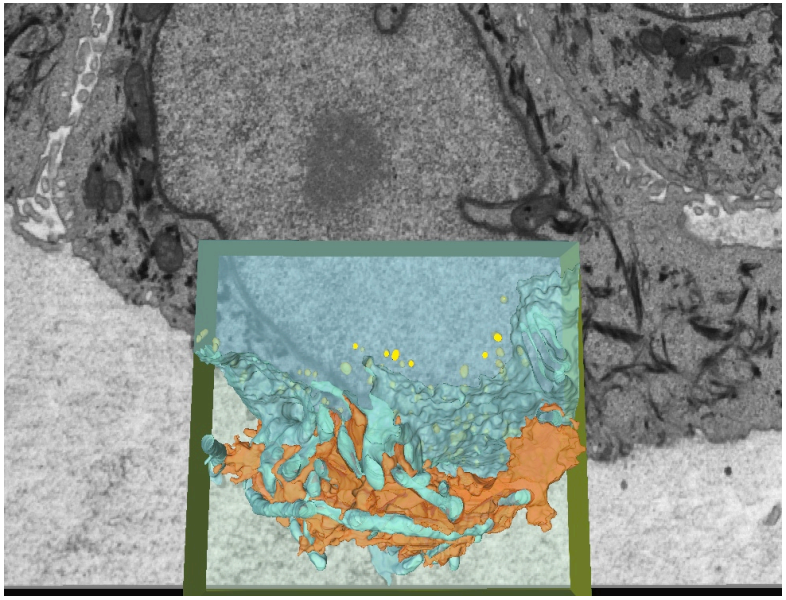


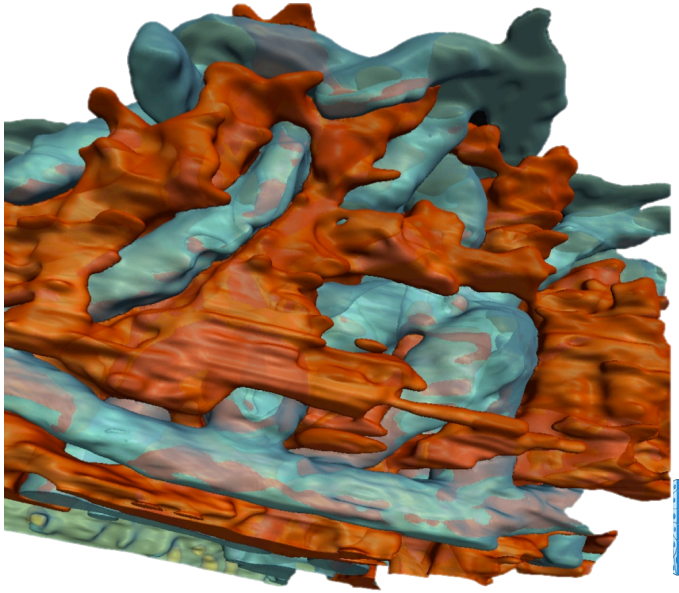
Integrating molecular information and structural detail with nanometer precision

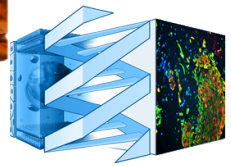
Helios Nanolab 650
FIB-SEM











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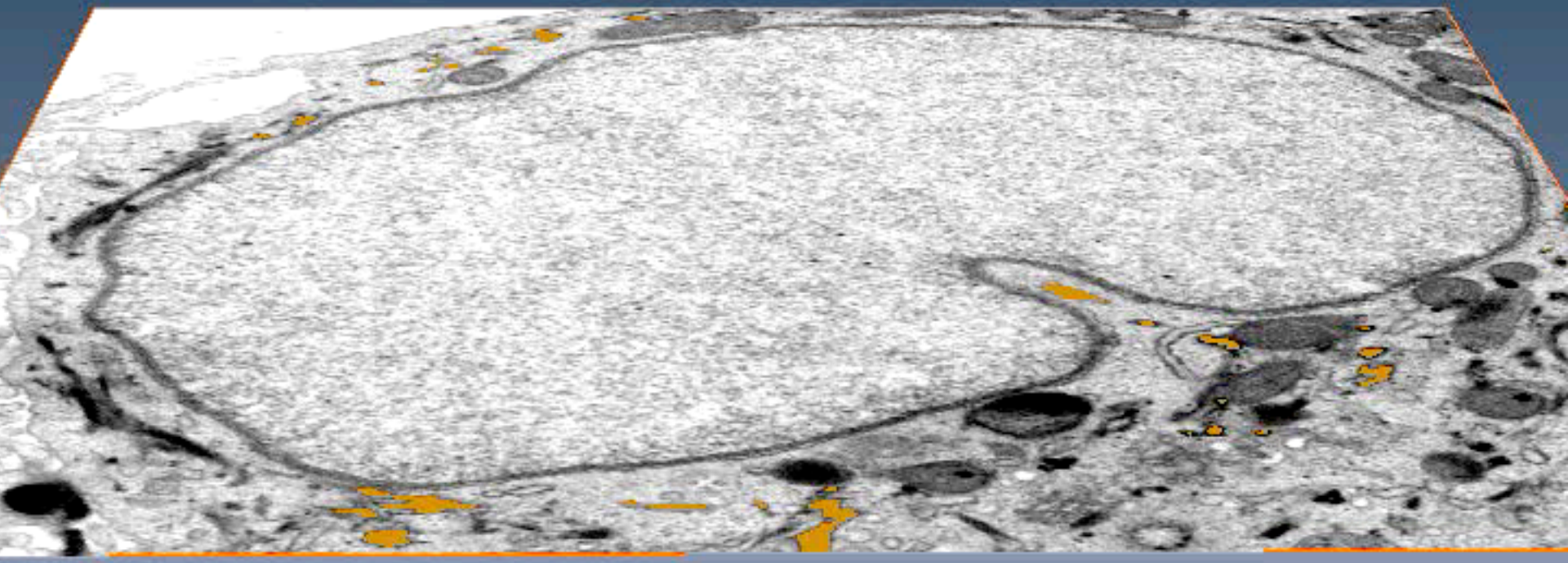

Our experience

- Likes:
  - Large images are handled well
  - Scripting
  - Flexible data access options
- Wishes:
  - Registration information between imaging modalities i.e. a Real-World coordinate system
  - Tiled/chunked storage of nD data in multiple scales
  - Automated segmentation methods

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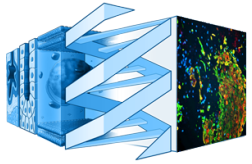
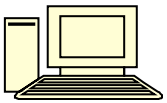


Image analysis/visualization workflow

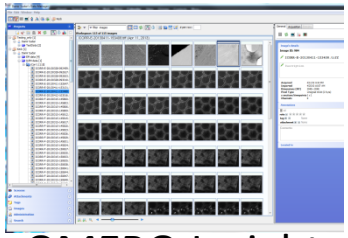


Local workstation

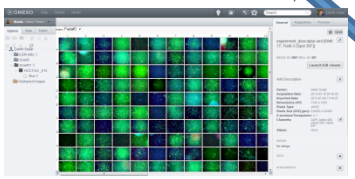
Local disk

HT cluster

Utrecht - Netherlands

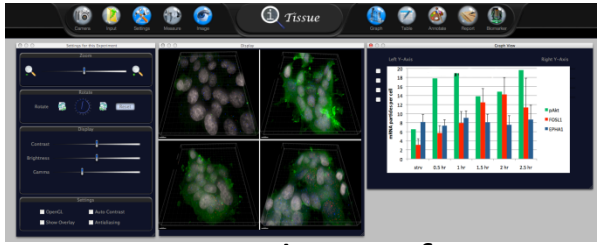


OMERO.Insight

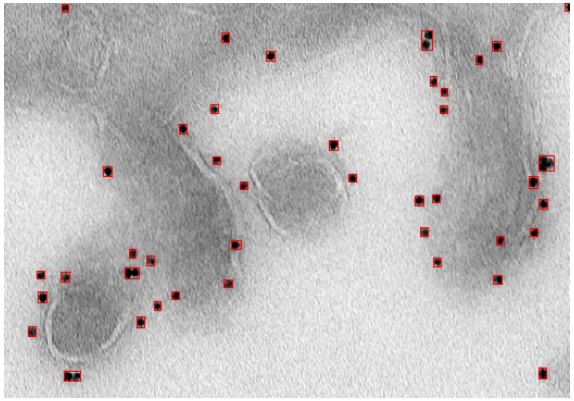


OMERO.web

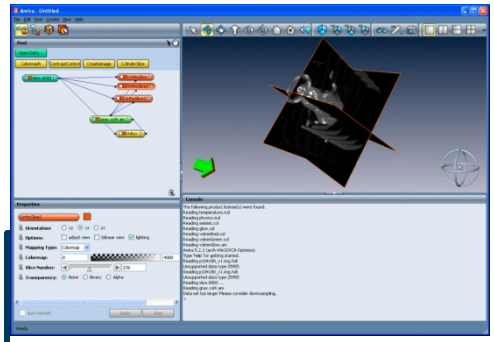
OMERO server
w/ HDF tables



QI image analysis software



Custom software (Java, C++, ImageJ/Fiji)



AMIRA and other FEI software

LBNL

QI

LUMC

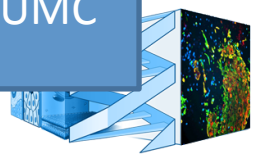
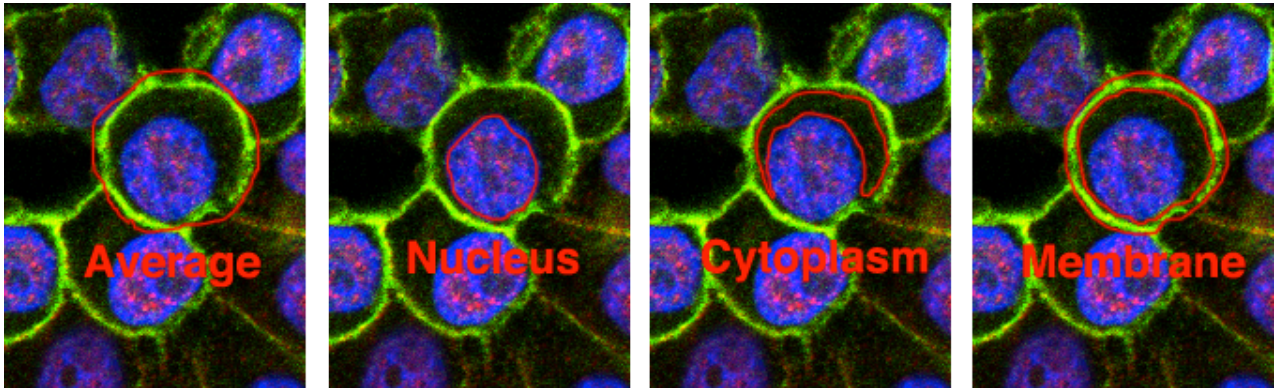


Image analysis and visualization

- Find individual cells
- Find sub cellular regions: Nucleus, Cytoplasm, Membrane
- Measure biomarker expressions in each region
- Relate channels: e.g. pAkt versus total Akt
- Show statistics & relate statistics to morphology of cells and tissue
- Compare over time and perturbation conditions



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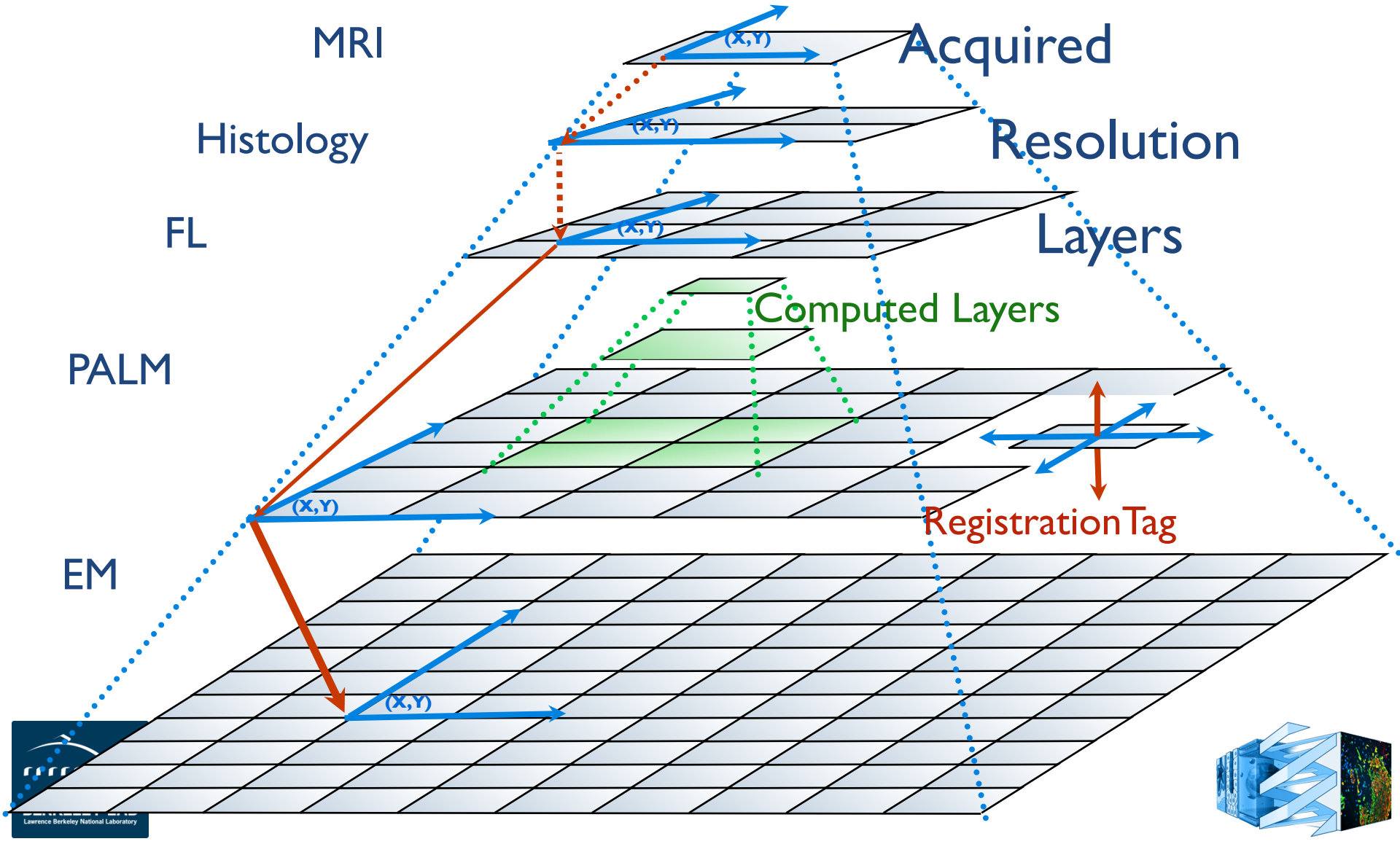
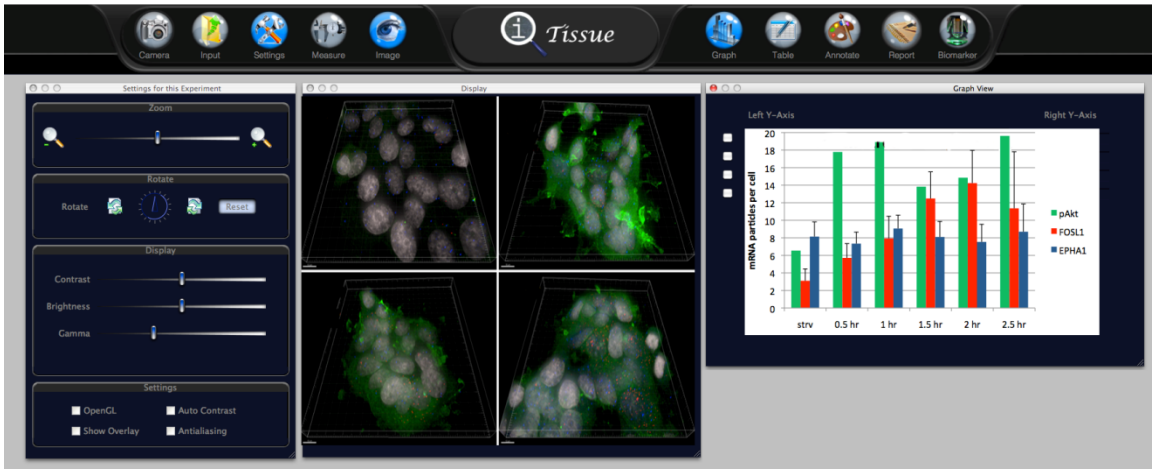
MultiScale Viewer





Qi Fluorescence Biomarker Quantitation

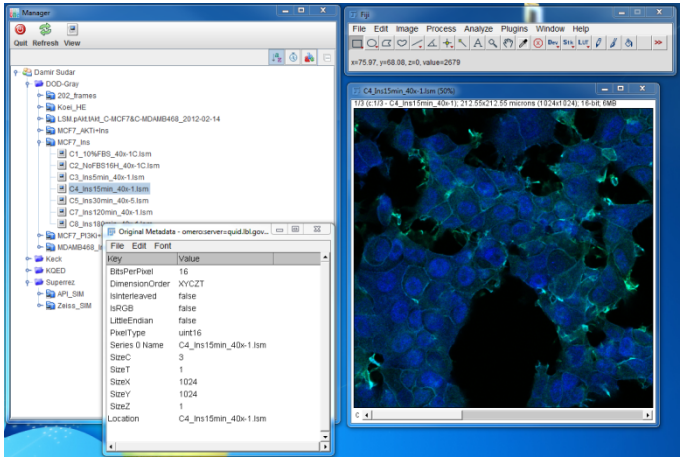
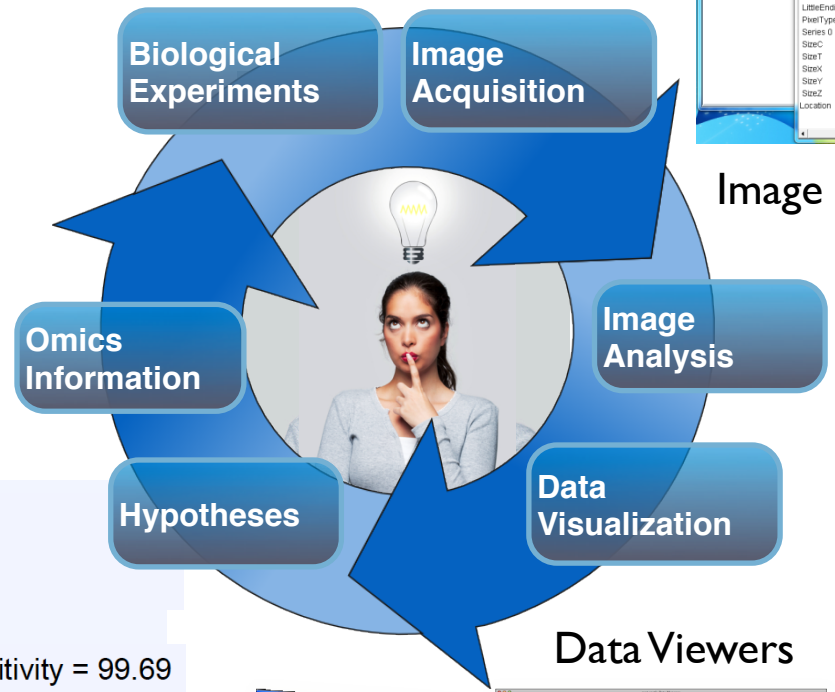
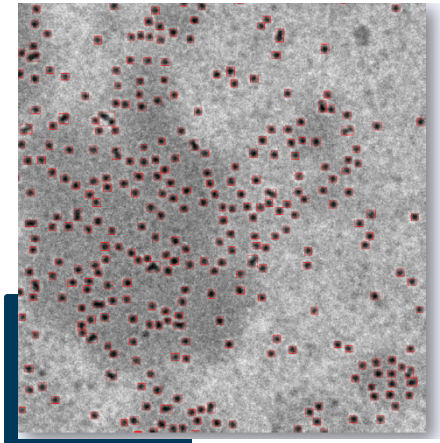


Image & Metadata Database



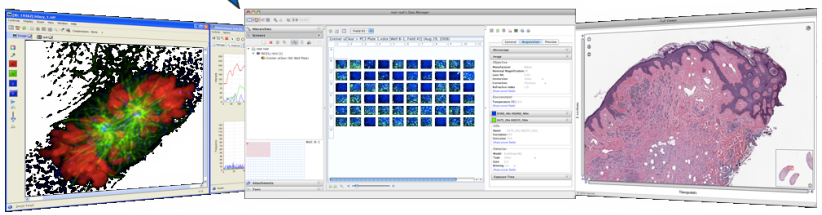
EM Image Analysis

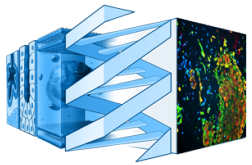


Sensitivity = 99.69

Specificity = 98.66

Data Viewers





Our experience

- Likes:
  - Good Python support
  - Access to original data via “Archived” or “File paths”
  - HDF support for feature data
- Wishes:
  - Improved Java and C++ API support and documentation
  - Well defined infrastructure to initiate server-side processing on HPC cluster
  - Easy use/display/searching of the HDF data
  - Abstracted shape descriptions of objects in close relationship with pixel/voxel descriptions

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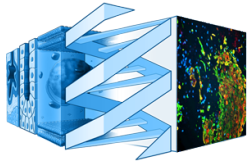

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And the OME team!!!

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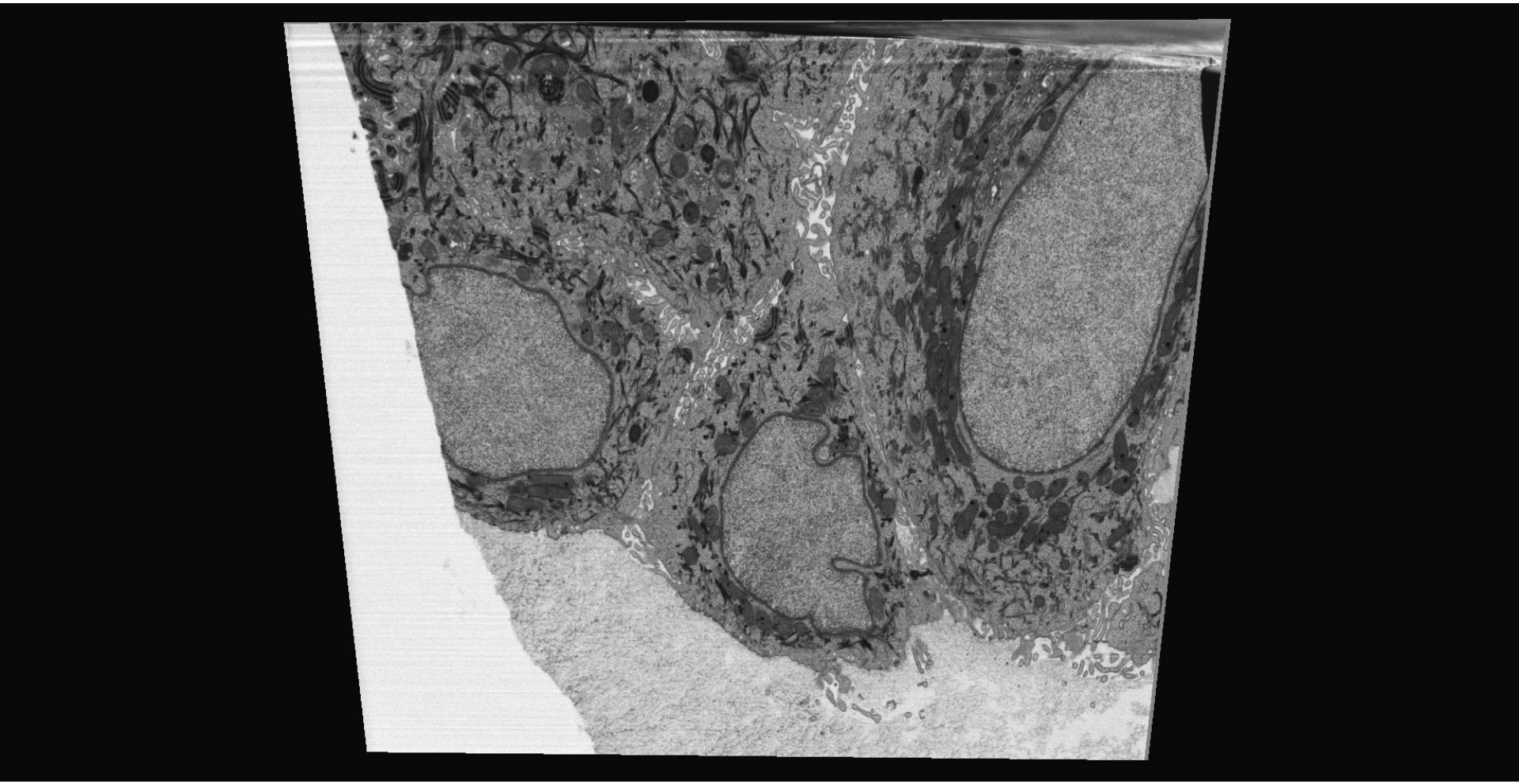

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