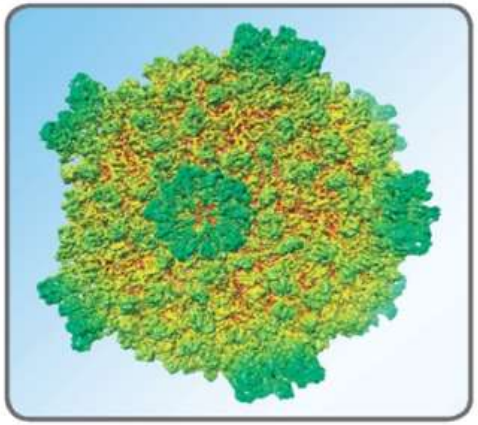
Correlative Light and Electron Microscopy

Kristian Wadel

FEI Life Sciences

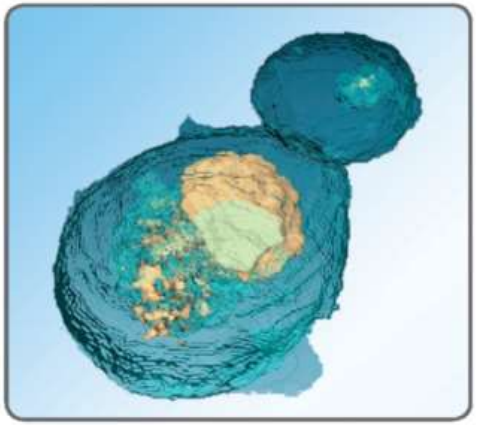
Structural Biology Solutions
Visualize life at the 3D molecular level



3.88 Å structure of Cytoplasmic Polyhedrosis virus by cryo-electron microscopy

Courtesy of Xuekui Yu, Lei Jin & Z. Hong Zhou, University of California, Los Angeles, USA

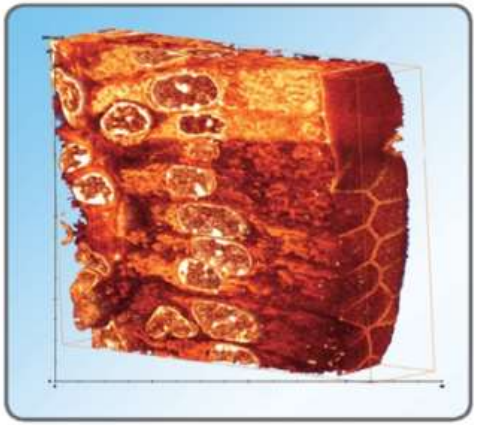
Cellular Biology Solutions
Discover life's cellular architecture in 3D



Volume rendering of the three-dimensional architecture of a dividing yeast cell

Courtesy of Sriram Subramaniam, National Institutes of Health, Bethesda, USA

Tissue Biology Solutions
Connect life's ultrastructure to the mesoscopic scale

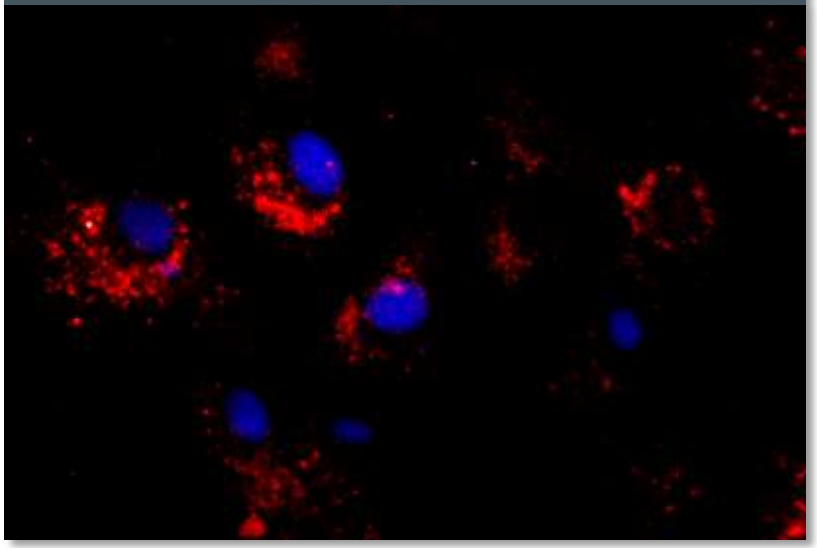


Mouse intestine epithelial tissue imaged 50 x 50 x 10 micron using a pixel size of 25 nm and section thickness of 40 nm

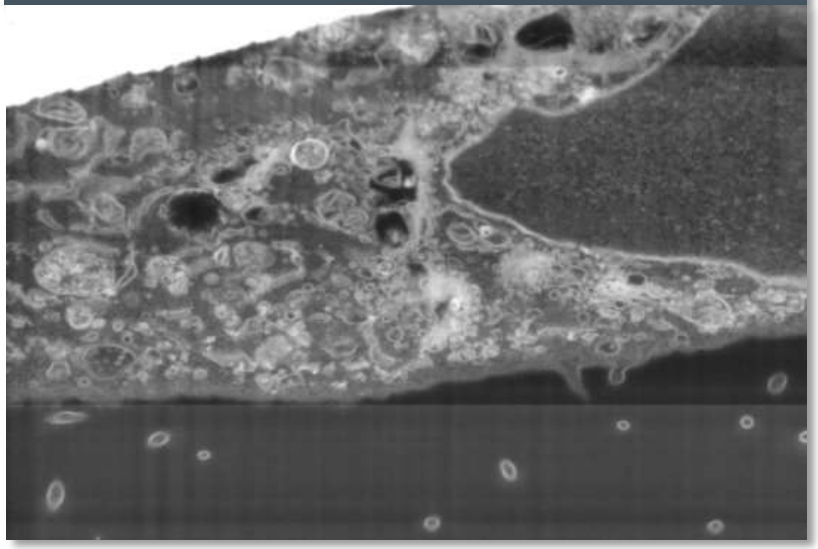
Courtesy of Paul Matsudaira, Dept of Biological Sciences, National University of Singapore

Motivation for CLEM

Light Microscopy
- Dynamics (e.g. live samples)
- Fluorescent probes/labels
- Limited resolution
- Large Field of View

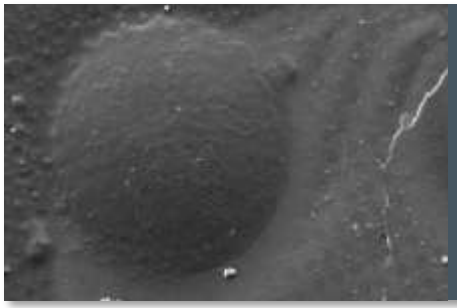


Electron Microscopy
- Embedded/Frozen samples
- 2D/3D structural imaging
- High resolution
- Small Field of View

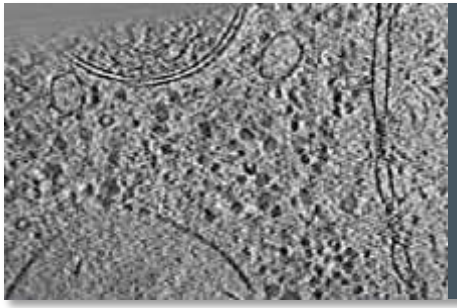


The importance of scale

Small organisms and tissues: millimeters



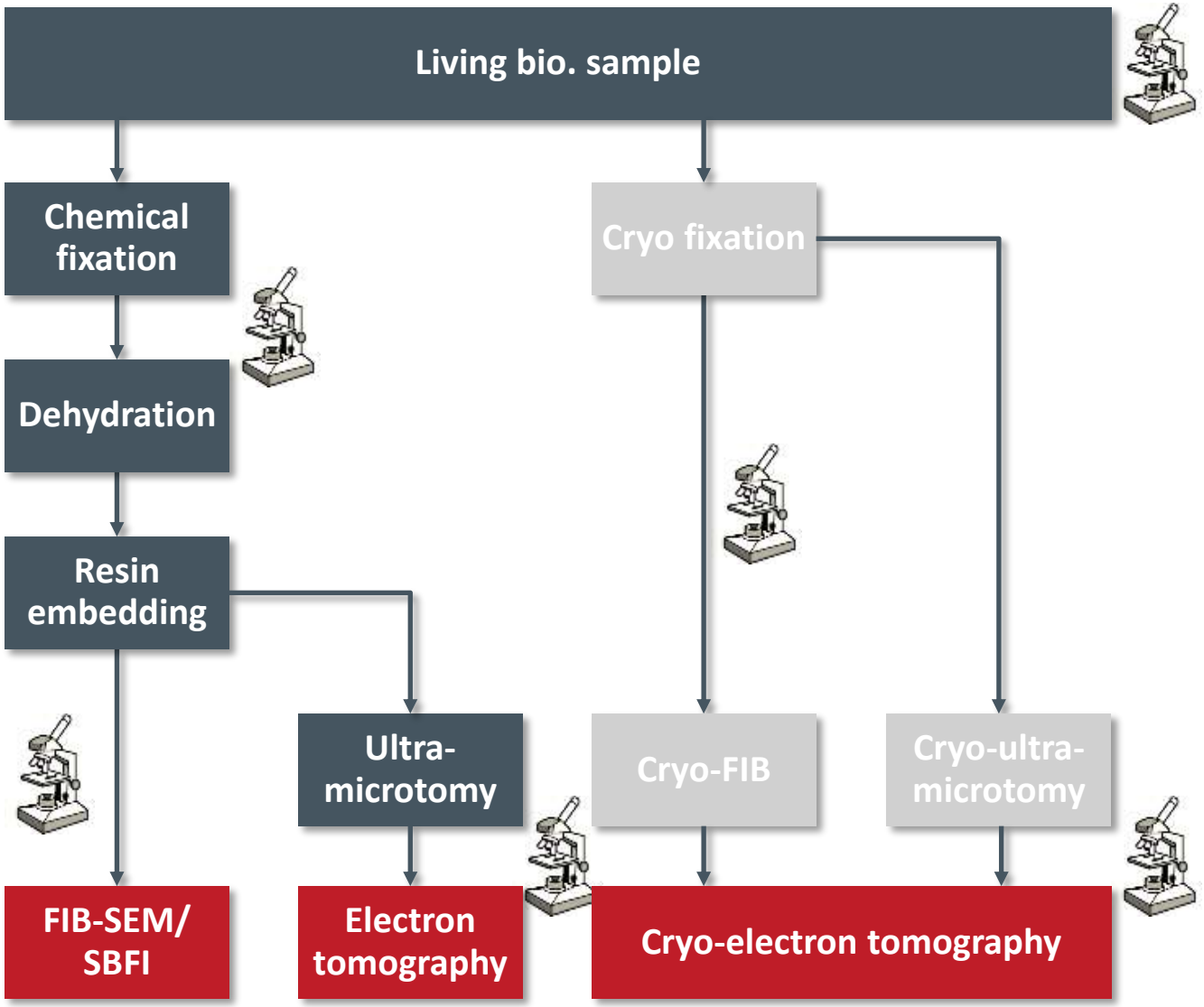
Cell: ≈300 µm^3



Tomogram: ≈0.4 µm^3

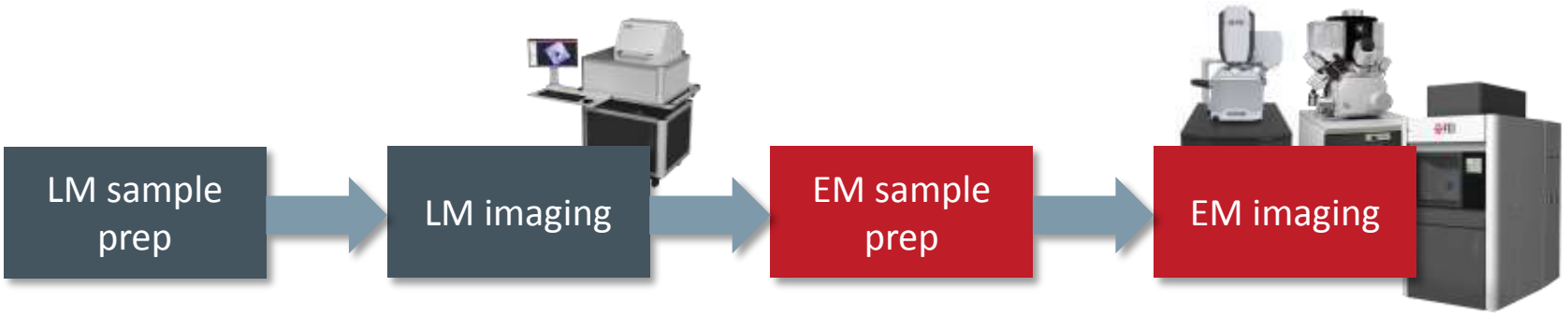
Top image: Courtesy of D. McCarthy, University College London
Middle and bottom: Courtesy of J. Mahamid, J. Plitzko and W. Baumeister, MPI for Biochemistry

Sample preparation workflows

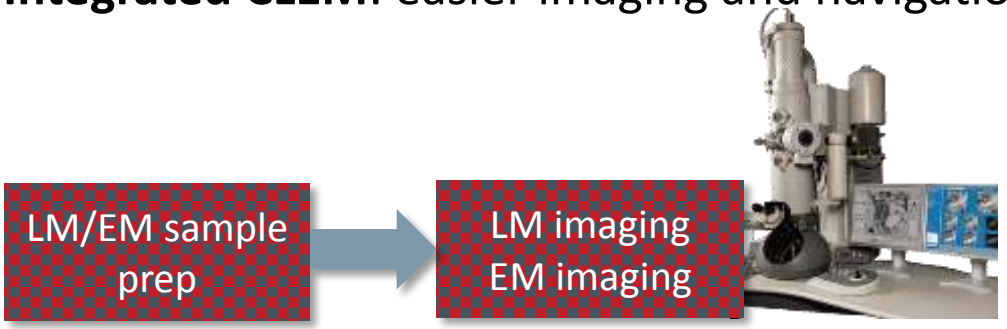


Two approaches for CLEM

Sequential CLEM: flexibility in LM imaging and EM labeling and staining



Integrated CLEM: easier imaging and navigation, maximum sample protection



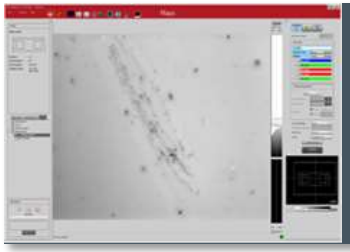
Solutions for CLEM from FEI

iCorr

- **Integrated CLEM** on dual-modality TEM

CorrSight

- Dedicated light-microscopy system for **sequental CLEM** workflow



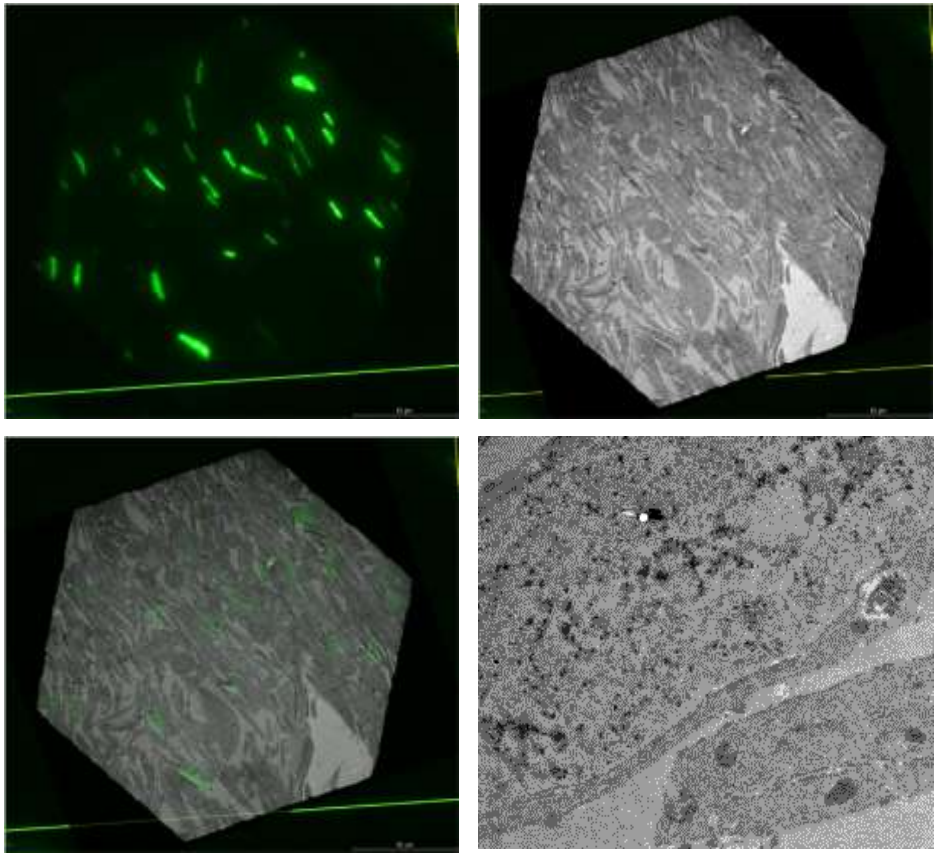
MAPS & Amira

- MAPS: Unified software interface for navigation and acquisition on LM & SEM/SDB & TEM soon
- Amira: Visualize and register 3D LM & EM data

iCorr

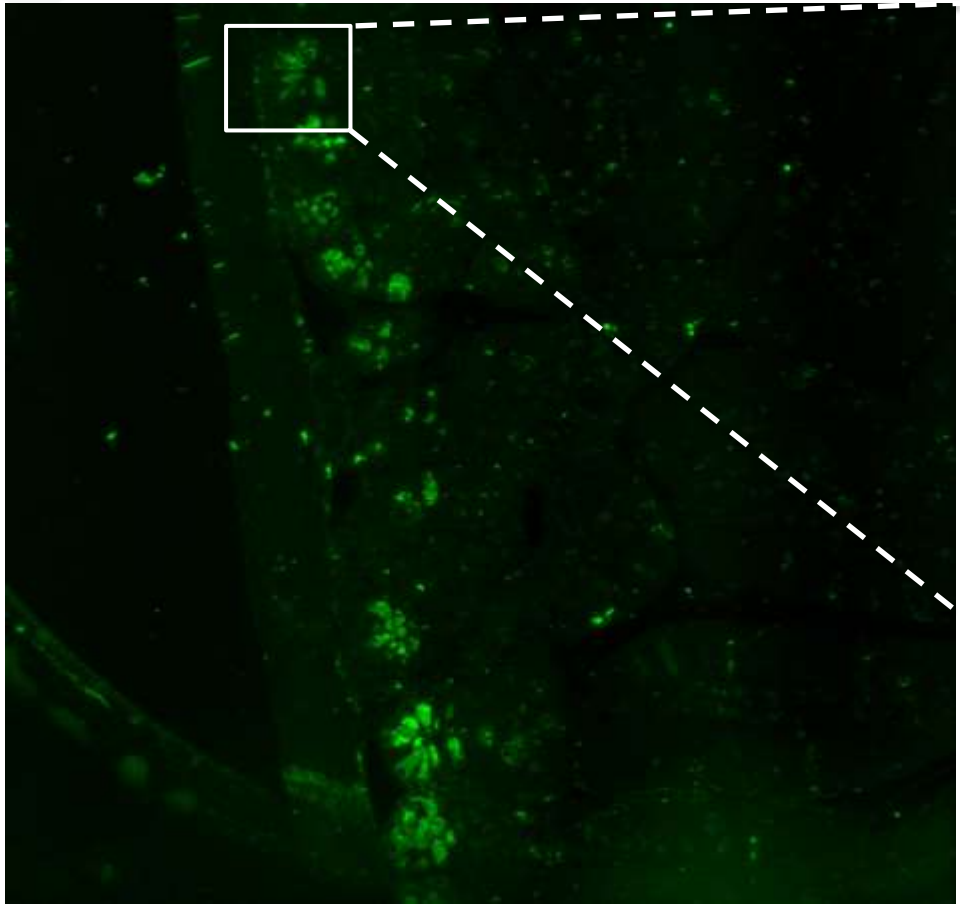
iCorr- intuitive navigation in correlative workflow

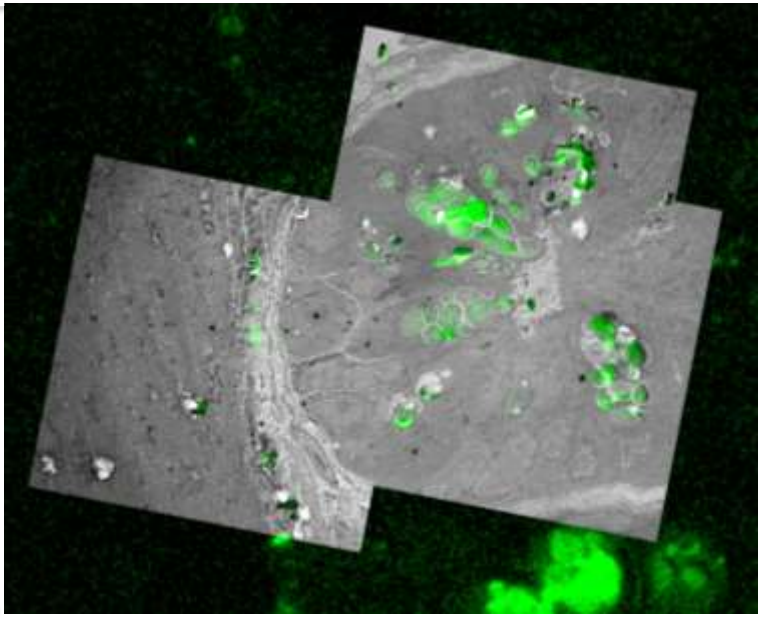
- Optimal navigation tool, fully integrated in the TEM
- Special 15x 0.5 NA objective
- Maximum sample protection, no transfer of samples between instruments
- Instant image overlay and scaling of optical and electron microscopy data



Courtesy of M. Karreman (EMBL) and E. van Donselaar (University Utrecht)

iCorr: Immunolabeling

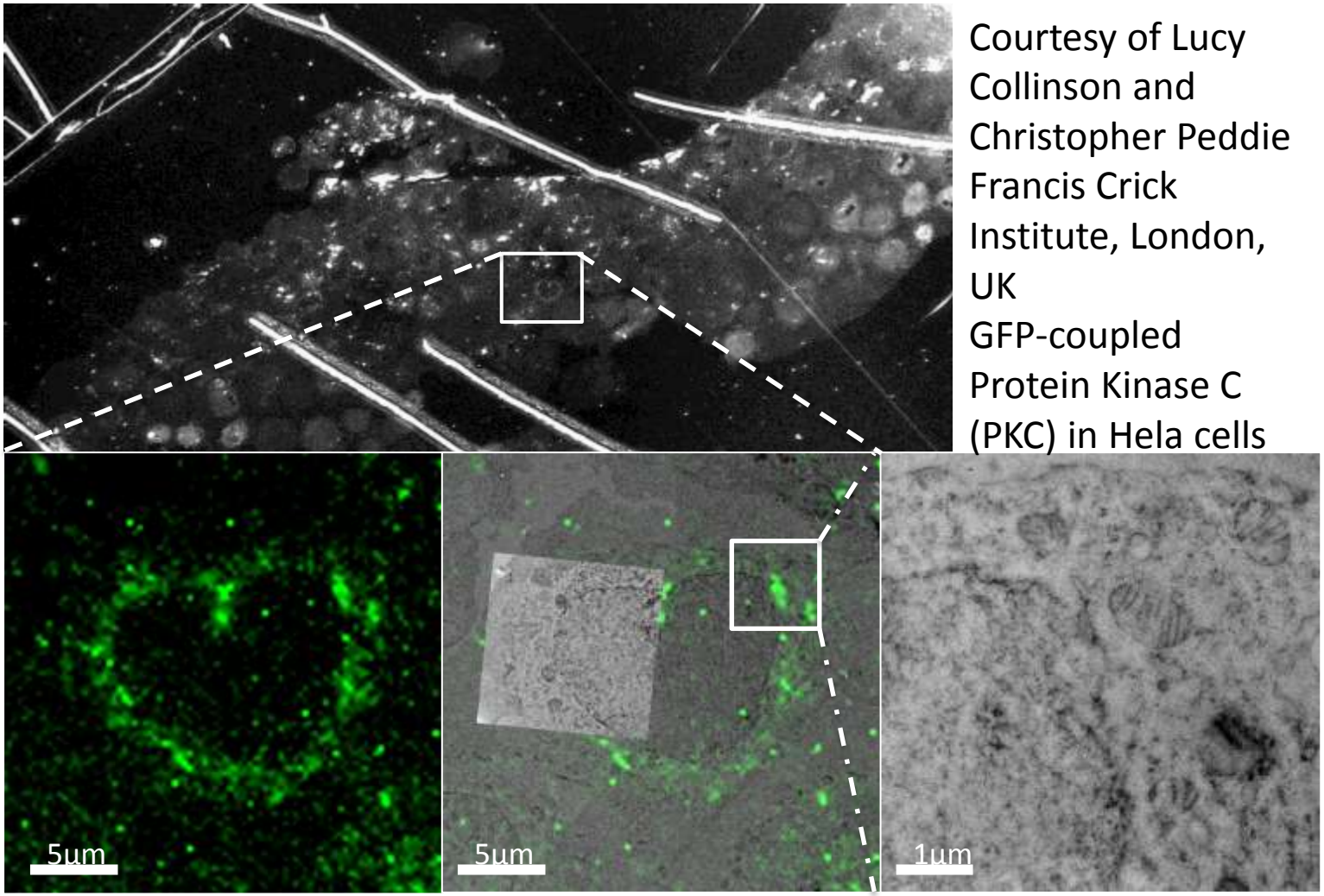




Courtesy of Nicholas Smith and Melissa Wong, Oregon Health and Science University.

Mouse intestinal tissue (Paneth cells), immuno-labeled for lysozyme

iCorr: GFP labeling



Courtesy of Lucy Collinson and Christopher Peddie
Francis Crick Institute, London, UK
GFP-coupled Protein Kinase C (PKC) in Hela cells

CorrSight & MAPS

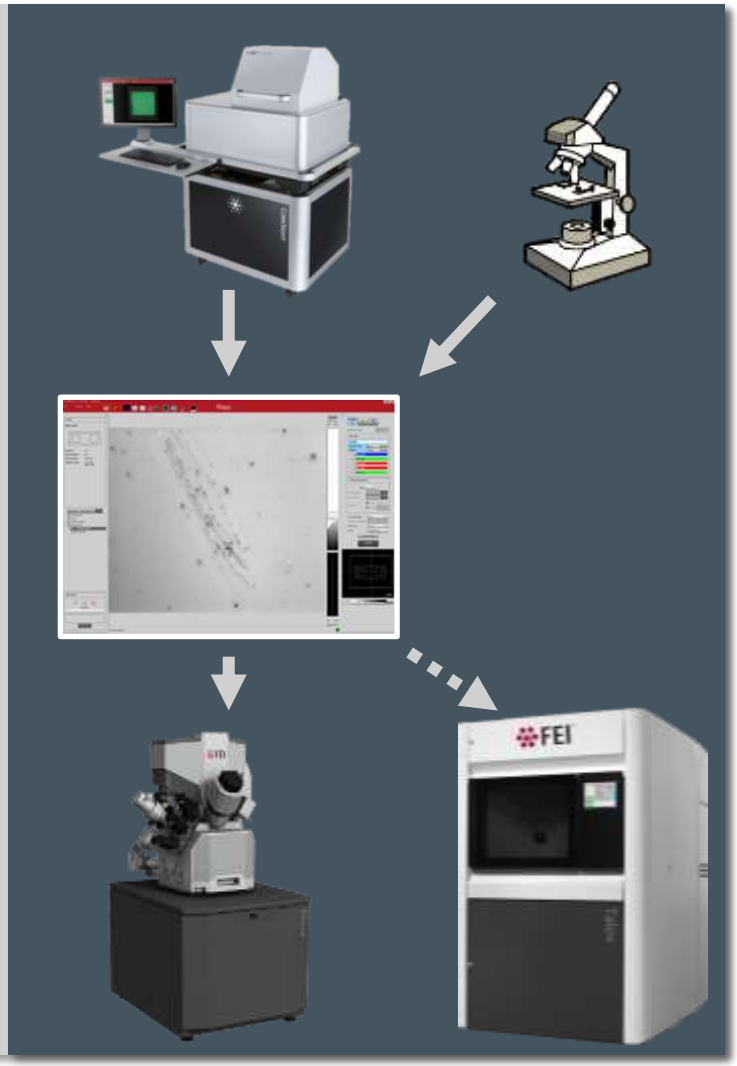
CorrSight: microscope, sample prep and navigation tool

Unique modular concept:

- From wide-field fluorescence to spinning disk confocal depending on the need
- Immobile sample stage allowing complex sample environments
- Easy exchange of sample environments for different experiments

MAPS: A unified software interface for CLEM

- MAPS bridges the CorrSight and SEM/SDBs
- Makes navigation and image correlation fast and intuitive
- Provides tiling and stitching
- Open software platform can import and correlate any image
- Will be available for TEMs as well



Prescreening of EM samples

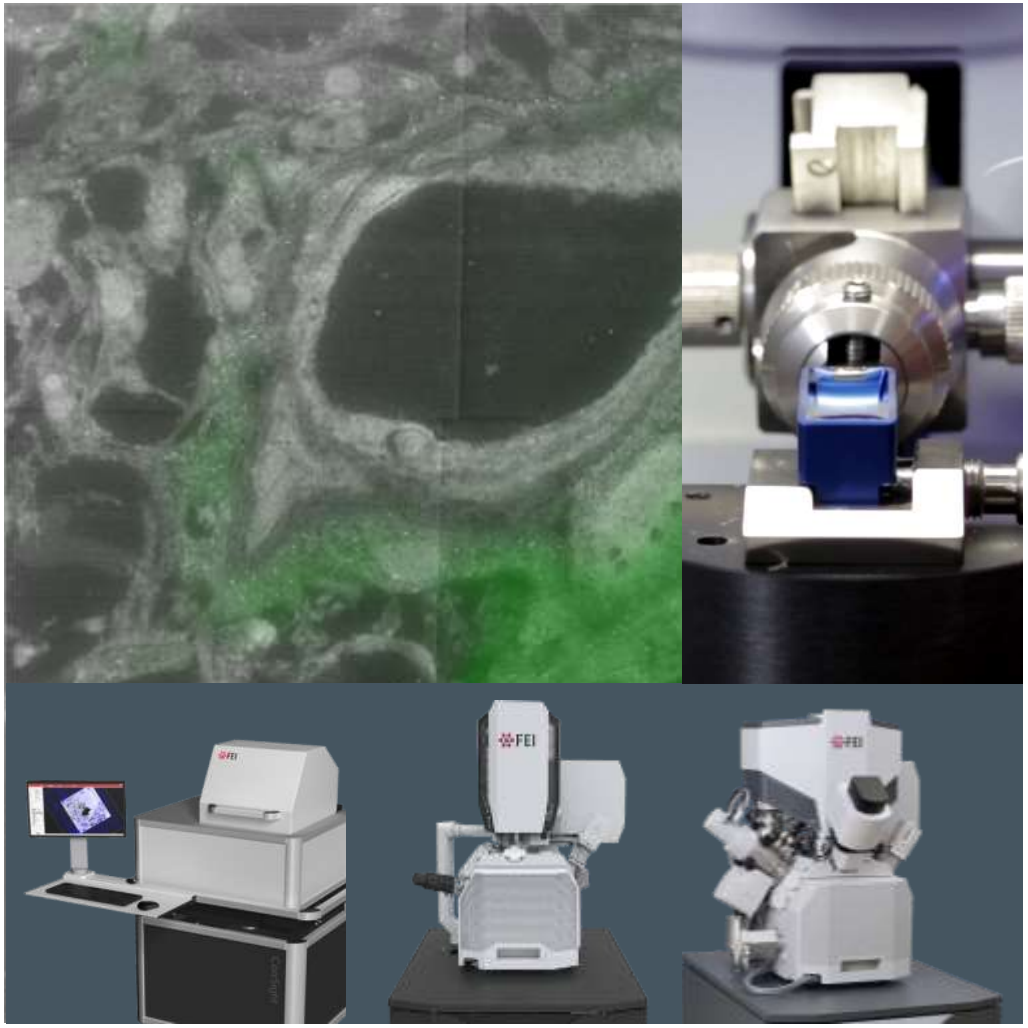
Experimental steps

Grow cells/tissue
with fluorescent label

Embedding sectioning

LM imaging to Localize ROIs
(CorrSight screen)

EM acquisition on ROIs
(SDB/SEM/VS)

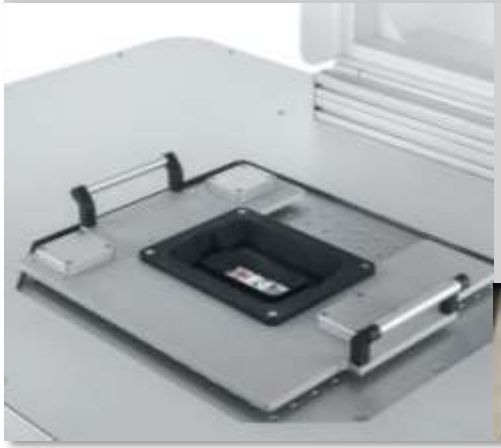


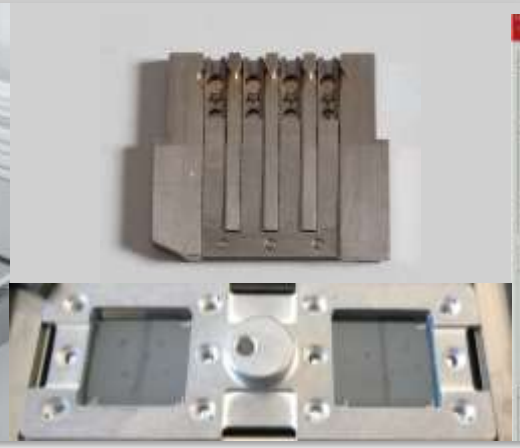
Courtesy of C. Loussert-Fonta and B. Humbel, UNIL

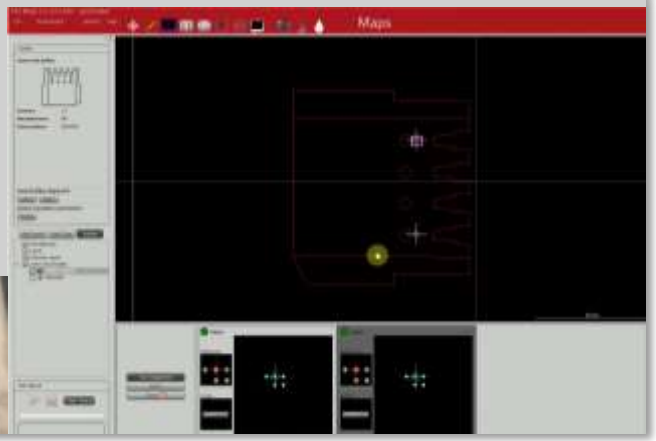
Correlative sample holders for automated fiducial-based correlation

Automated scanning of multiple samples to identify promising areas

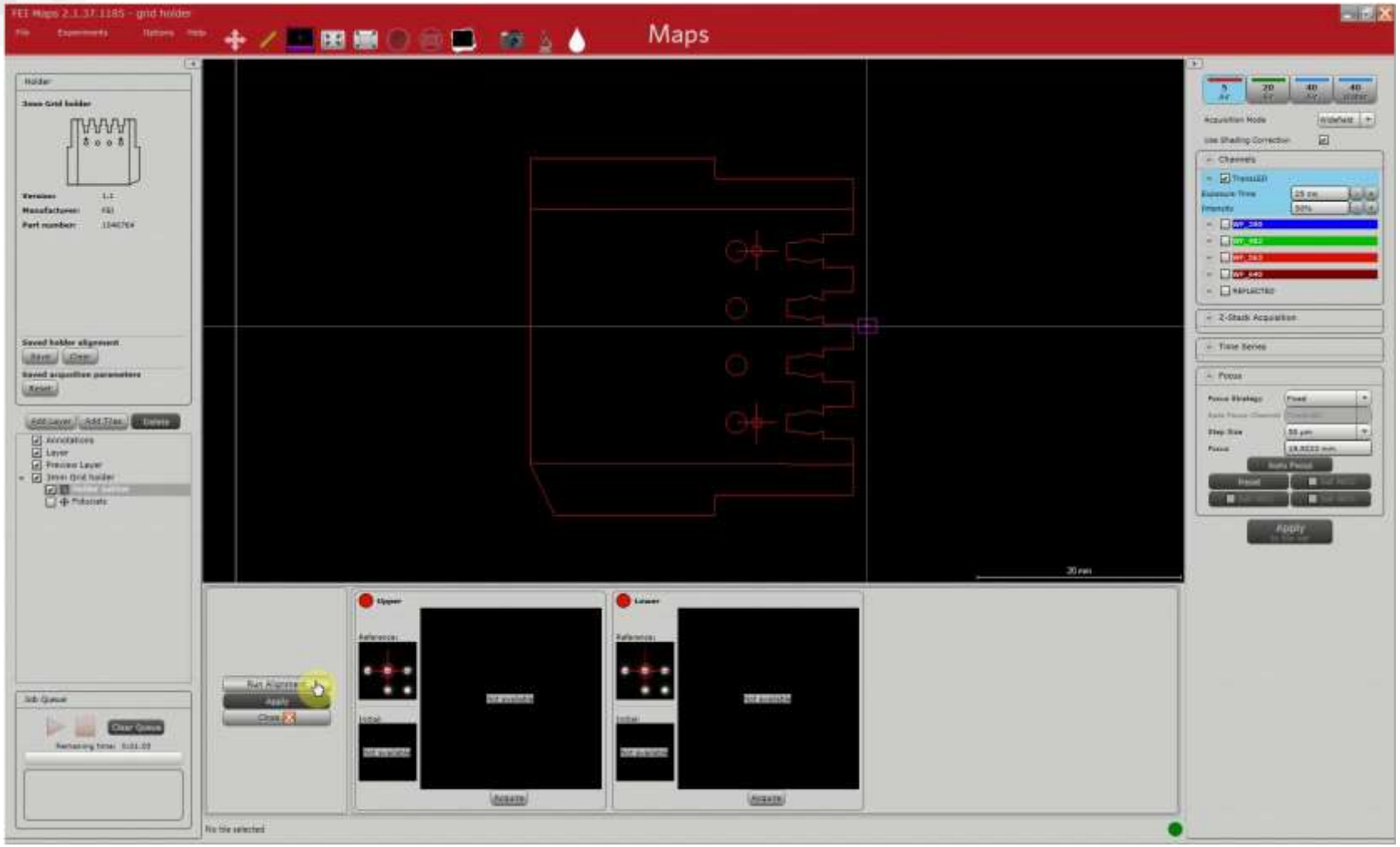
- Sample holders **directly compatible** with CorrSight and SEMs
- Available for a variety of sample formats
  - **ITO** slides
  - TEM **grids**
- Fiducials for **automated correlation**





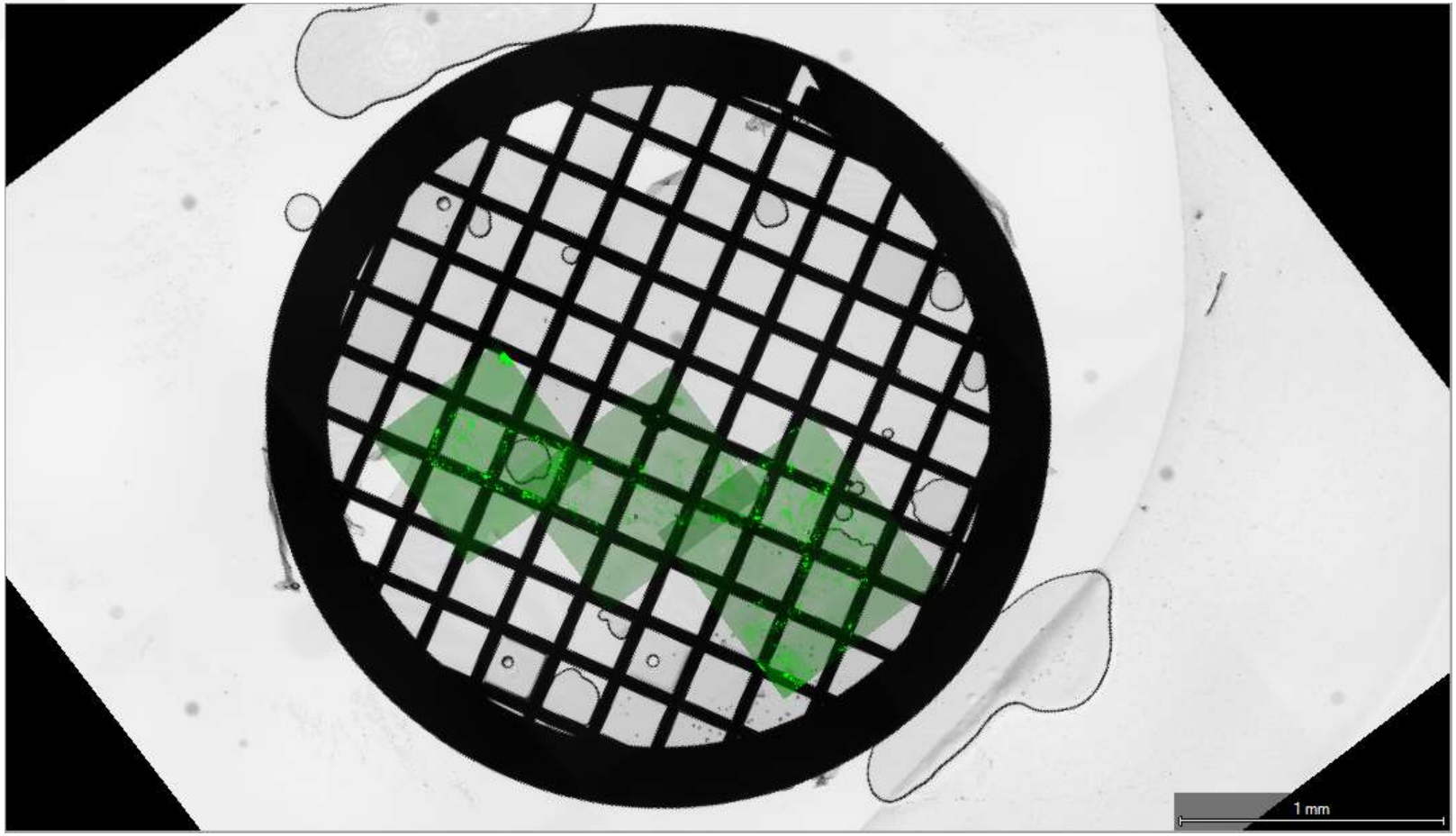


Automatic fiducial-based alignment



LM & EM data acquisition and correlation using MAPS

STEM imaging of astrocytes in Tokuyasu-prepared brain sections



Data courtesy of Celine Loussert-Fonta & Bruno Humbel

Unil
UNIL | Université de Lausanne
HEC Lausanne

From live-cell imaging to 3D ultrastructure

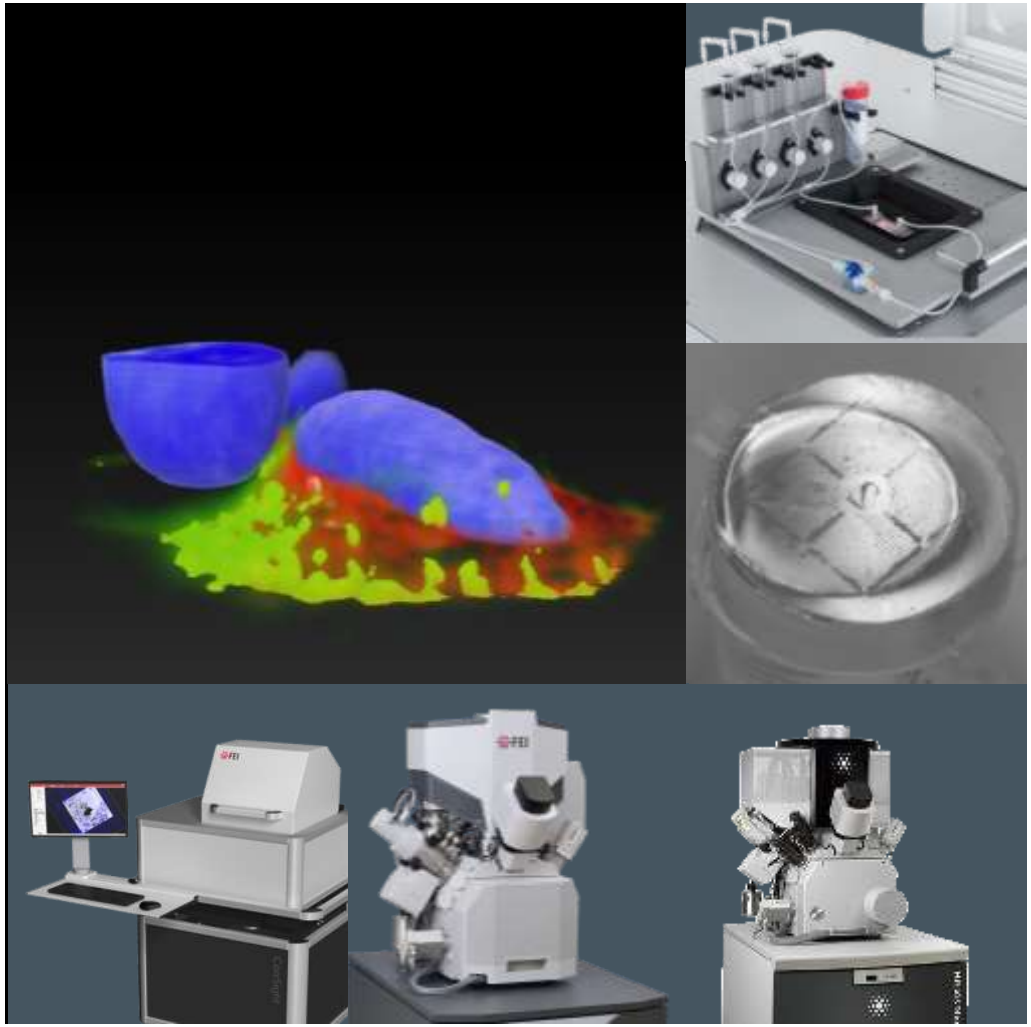
Experimental steps

Cell culture ibidi µ-slides

LM imaging to identify areas of interest; in-situ fixation, staining, embedding

LM/EM with MAPS
3D serial sections acquisition on area of interest wit SDB/SEM/VS

Reconstruction & visualization of 3D serial section dataset with AMIRA



Courtesy of S. Kwon and C. López, OHSU

CorrSight Live

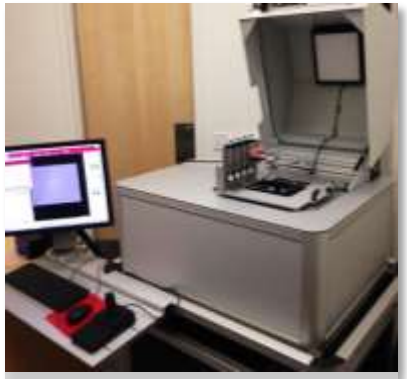
Sample environment

- Perfusion
- Heating
- CO_2 incubation

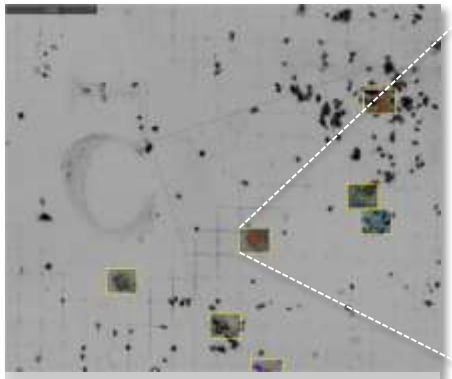
Microfluidic chamber

- Open wells allowing **easy handling** of different samples
- Closed by foil for the experiment to allow controlled **closed perfusion**
- **Optical quality bottom** (170 µm thickness)
- **Grid coordinate system** imprinted on the bottom

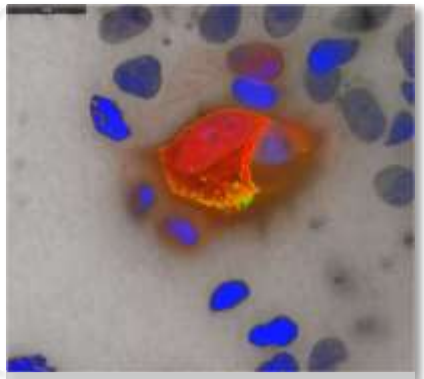
CorrSight Live: imaging and fixation of MCF-7 adherent breast cancer cells



Live cell imaging and µ-fluidics module



MAPS tiling/stitching large area overview – transmitted light and fluorescence microscopy



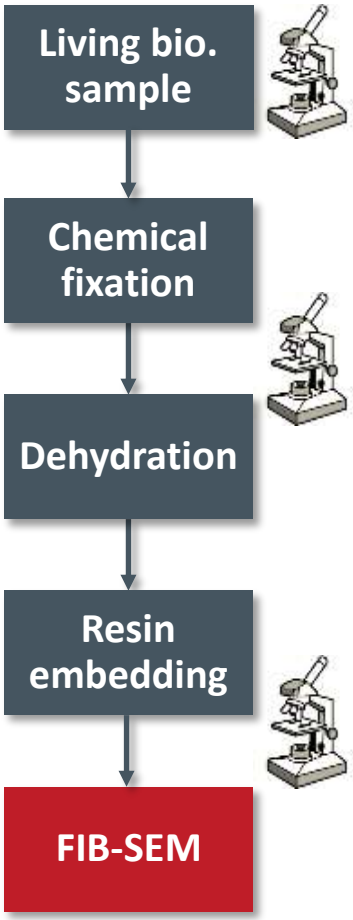
Area of interest identified by fluorescence microscopy

fixation, staining, resin-embedding directly on µFluidics module

Courtesy of S. Kwon and Claudia López

OREGON HEALTH & SCIENCE UNIVERSITY

OHSU: Sample preparation workflow



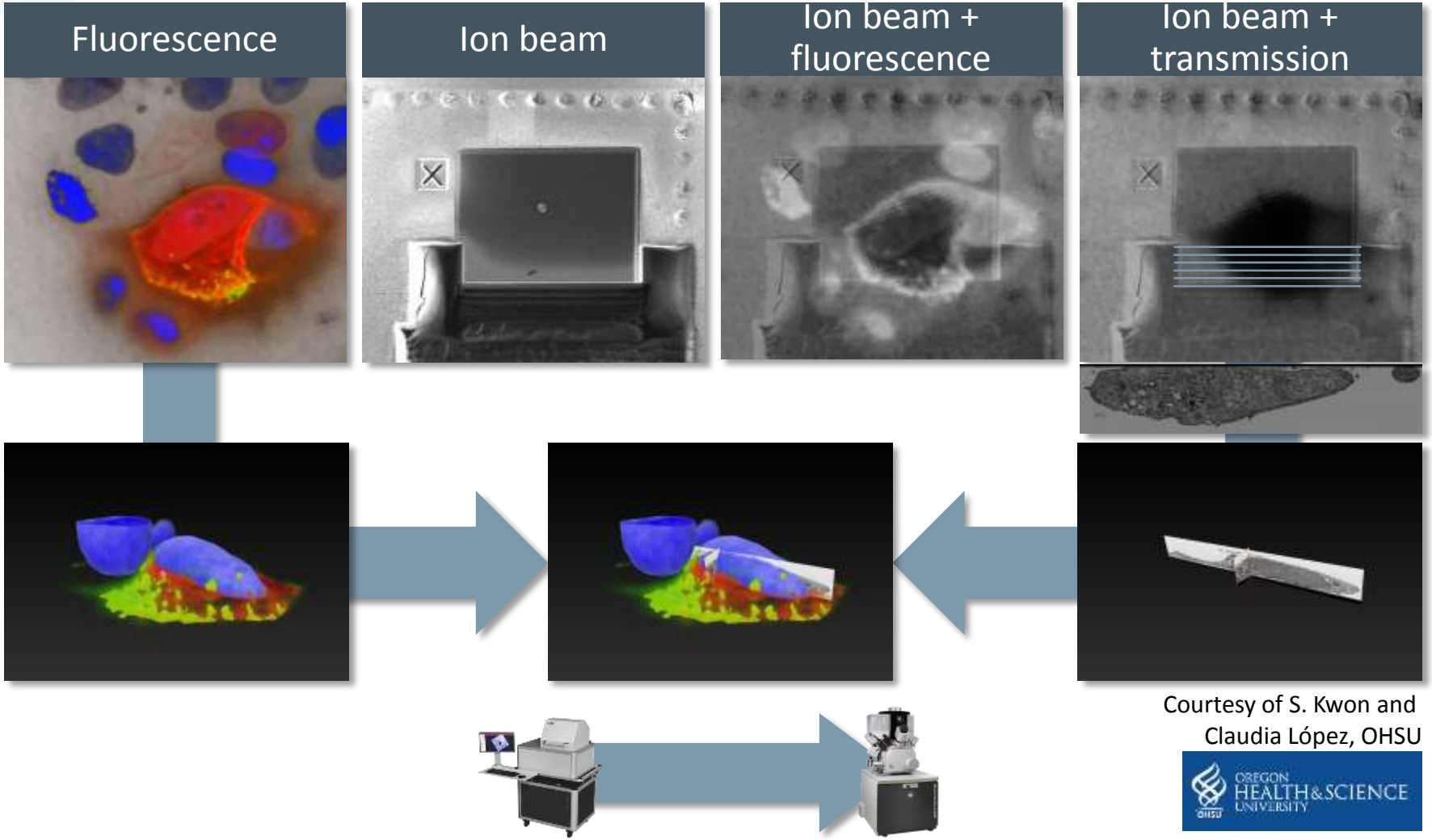
Cell fixation, imaging, post-fixation, and staining protocol

1- Cells are fixed in 4% Paraformaldehyde in PBS (pH 7) for 30'. Cultured cells are then washed with PBS (pH 7) using the same microfluidic flow rate.
2- Cells are then washed with PBS (pH 7) using the same microfluidic flow rate.
3- Cells are post-fixed in Karnovsky's fixative (2.5% Paraformaldehyde, 2.5% Gluteraldehyde in 100 mM cacodylate buffer pH 7.2) for 30' at room temperature.
4- Cells are then washed with 100 mM cacodylate buffer (pH 7.2).
5- Cells are then stained with 2% tannic acid in 100 mM cacodylate buffer pH 7.2 for 30' at room temperature.
6- Cells are then washed with 100 mM cacodylate buffer (pH 7.2).
7- Cells are then stained with 2% Osmium tetroxide in 100 mM cacodylate buffer pH 7.2 with 0.8% K3Fe(Cn)6 (potassium ferocyanide) for 30' at room temperature.
8- Cells are then washed with dH2O.
9- Cells are then stained with 7% uranyl acetate in dH2O for 30' at room temperature
10- Cells are then washed with dH2O.
11- Cells are then dehydrated by incubating with solutions of 25% acetone, 50% acetone, 95% acetone, and 100% acetone, each for 10' at room temperature. The final 100% step should be done twice.
12- Cells are then incubated with a 1:1 EPON:acetone solution at room temperature overnight.
13- Cells are then incubated with 100% EPON (~50 µl per well) overnight at room temperature.
14- After the overnight incubation, the EPON was exchanged for fresh solution and the microslide is incubated at 60° C overnight.
15- After complete polymerization of the microslide well, each well can be ejected from the slide by bending the slide and pushing the polymerized well from underneath.
16- Each polymerized well "puck" can now be processed for SEM imaging.

3D electron microscopy: FIB-SEM/DualBeam

- Using focused ion beam for automated sectioning and imaging of the freshly cut block face
- Walk-away acquisition of volumes (200 μm^3)
- Curtaining artifacts on block face
- Best axial resolution (3nm)
- Lateral resolution limits set by image acquisition times

3D CLEM on plastic-embedded MCF-7 adherent breast cancer cells



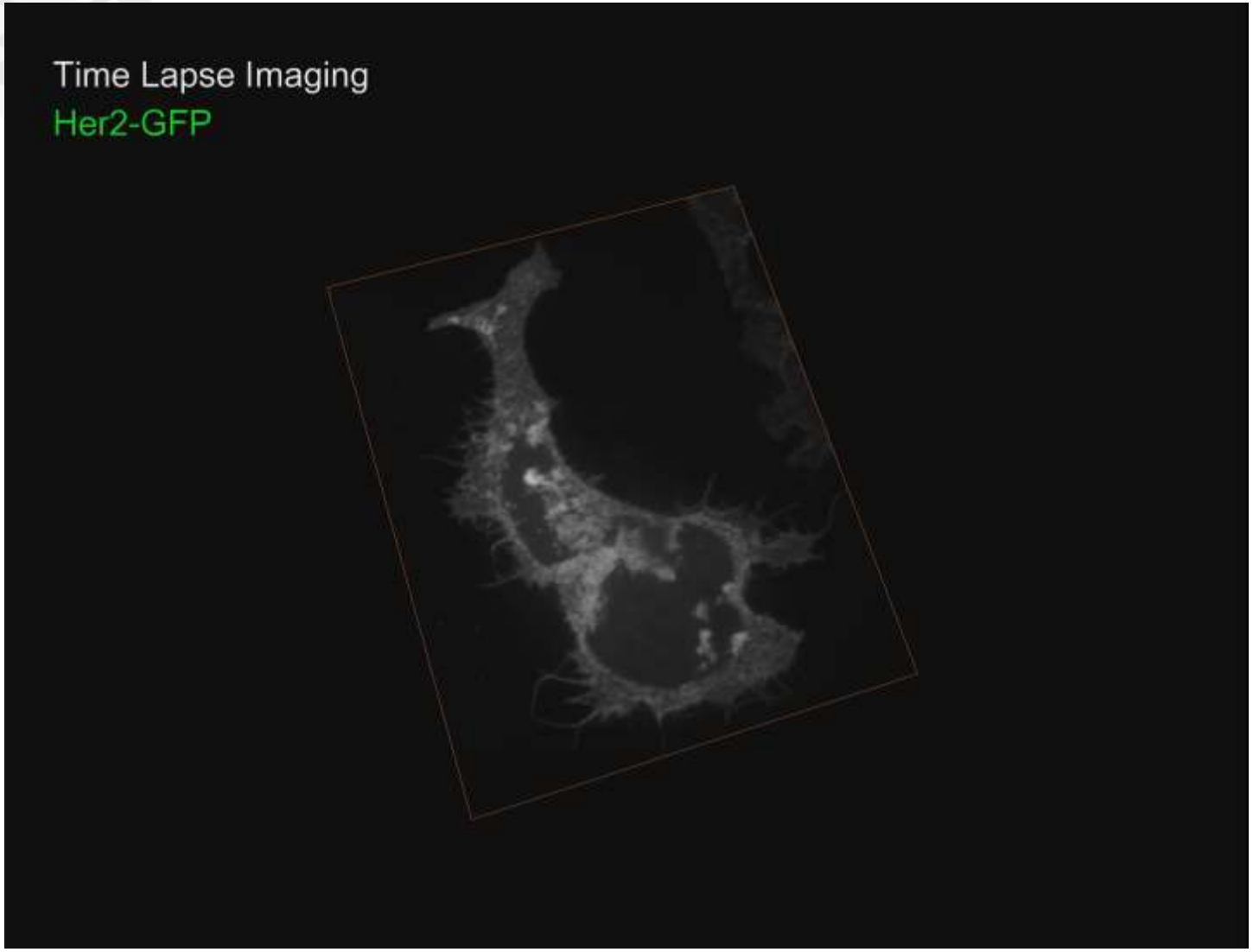
Courtesy of S. Kwon and Claudia López, OHSU

Explore. Discover. Resolve.

TechnoInfo

FEI™

MCF-7 adherent breast cancer cells



Courtesy of S. Kwon
and
Claudia López

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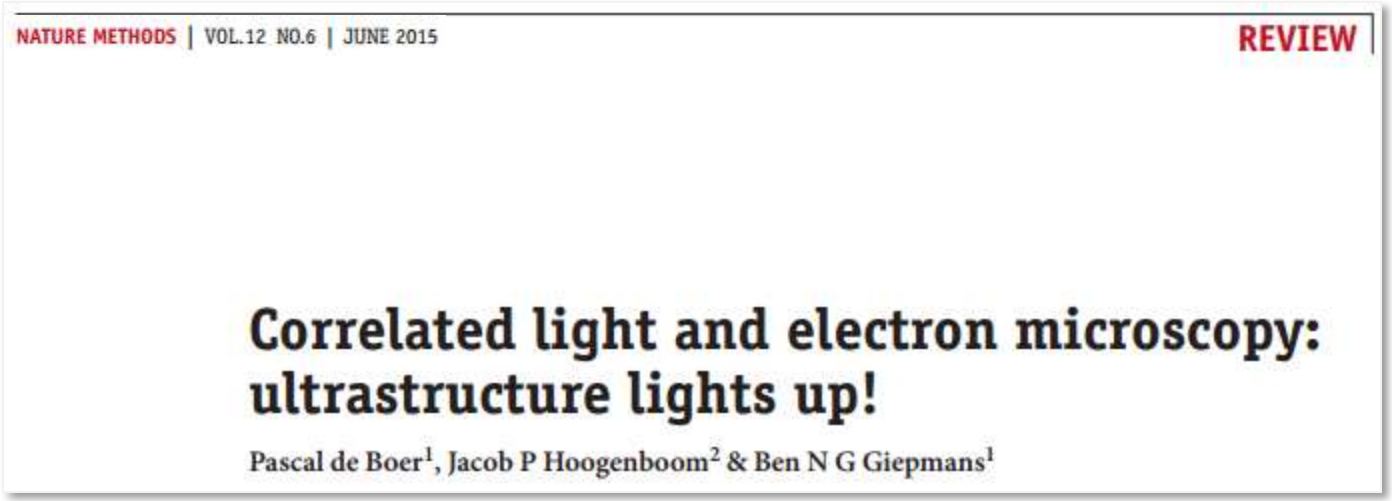
Further reading…



NATURE METHODS | VOL.12 NO.6 | JUNE 2015

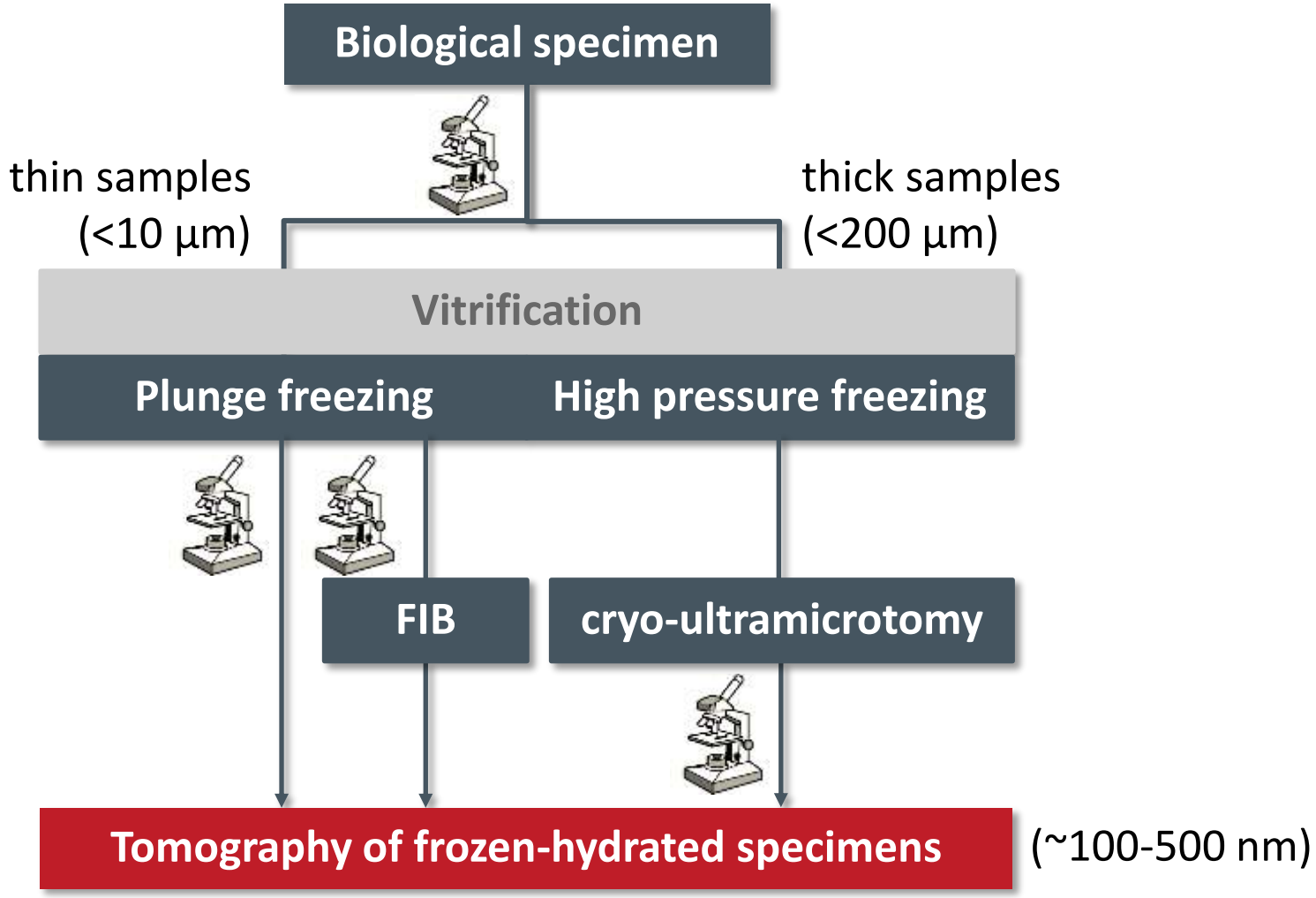
REVIEW

Correlated light and electron microscopy: ultrastructure lights up!

Pascal de Boer[1], Jacob P Hoogenboom[2] & Ben N G Giepmans[1]

Selected volume tomography (cryo-electron tomography)

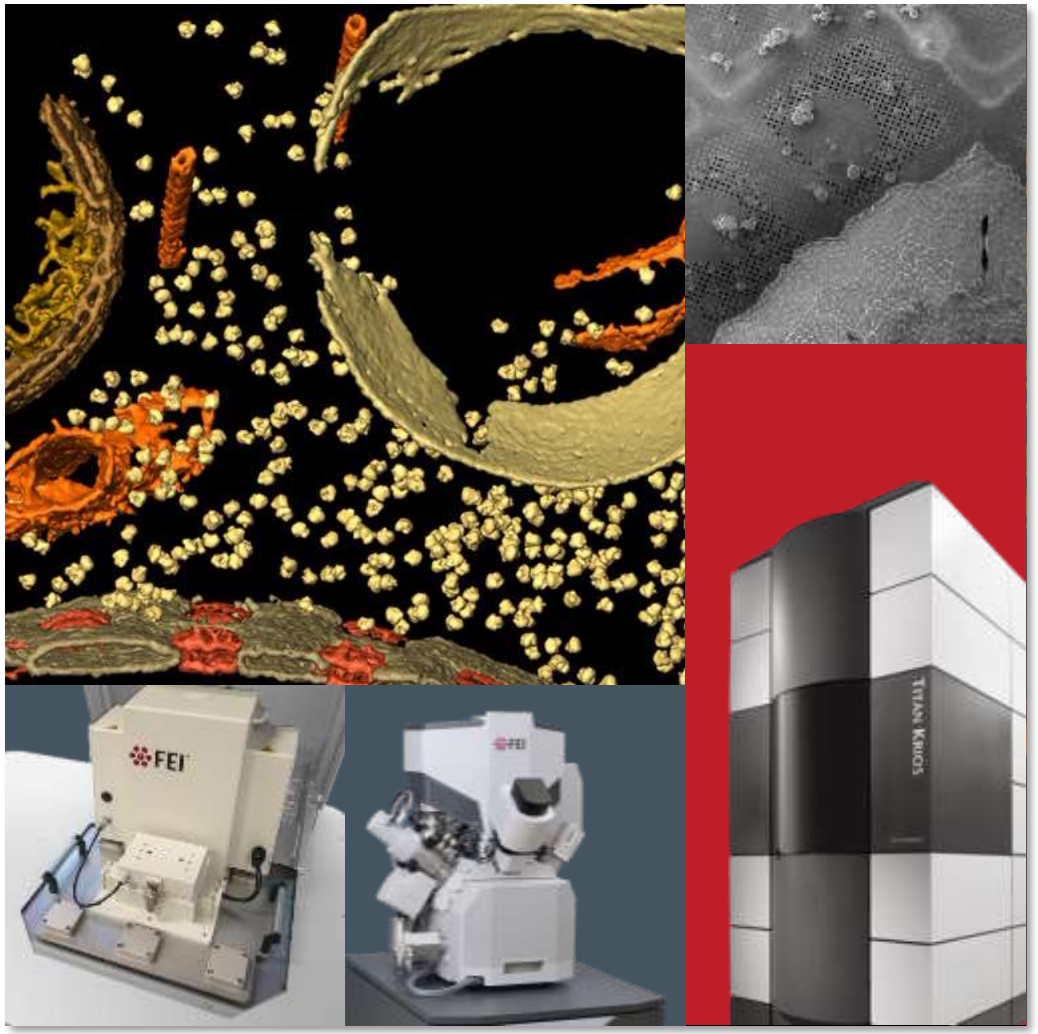
Cryo-electron-tomography workflow



Adapted from Rigort et al., Methods in Cell Biology Vol. 111 (2012)

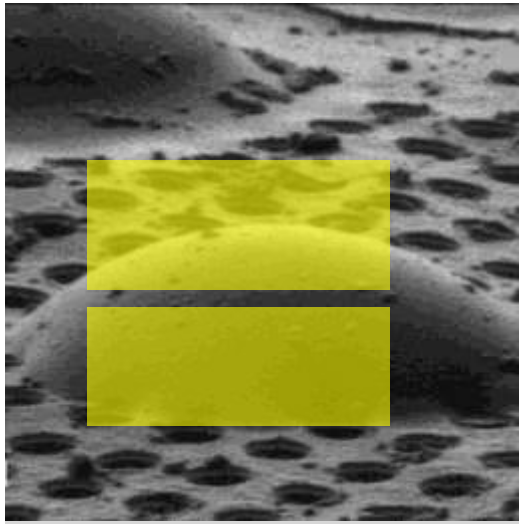
Experimental steps

- Cell culture on EM grids
- Plunge freezing
- Autogrid mounting
- Cryo-LM
- Cryo-FIB milling
- Cryo-electron tomography
- Reconstruction & visualization with AMIRA

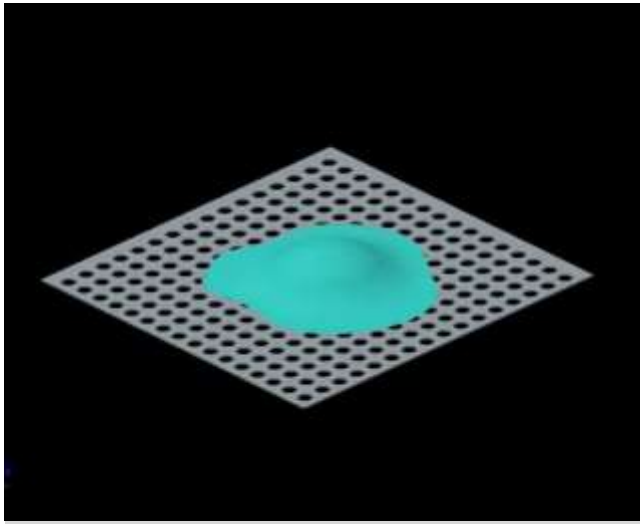


Courtesy of W. Baumeister and J. Plitzko, MPI for Biochemistry

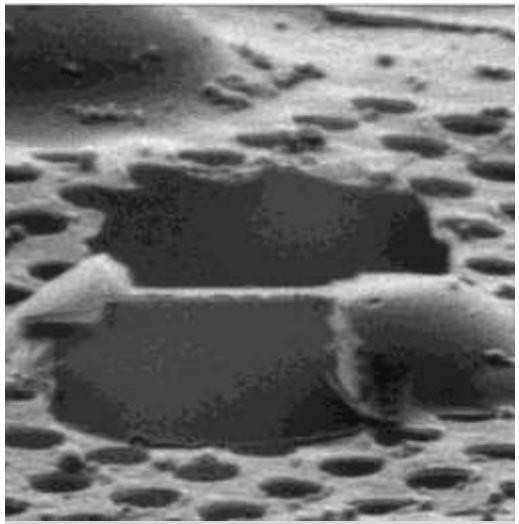
Cryo-FIB milling of lamella



Define milling area (ion beam image)



A lamella of 80-350 nm supported by the remains of the cell is created

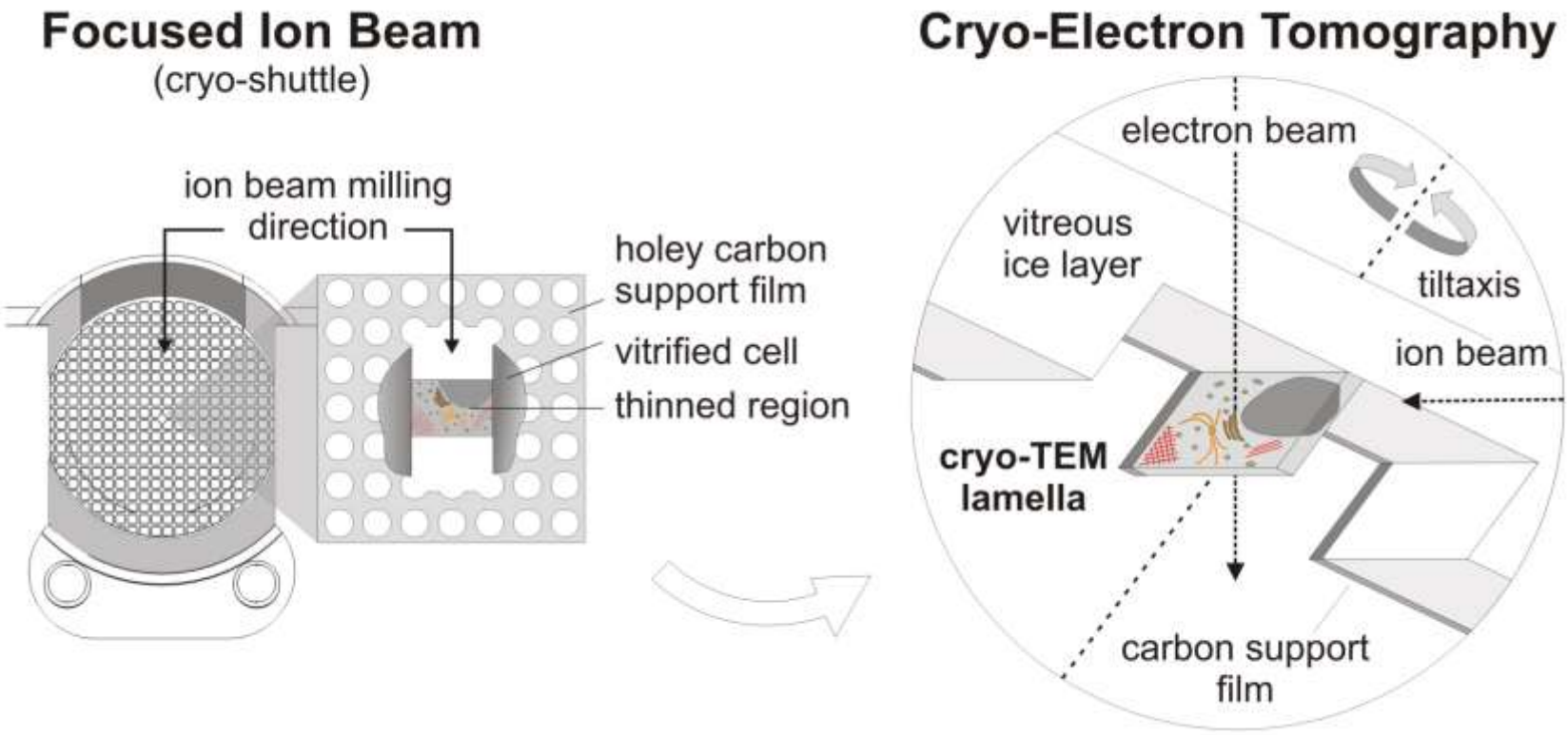


Resulting lamella (ion beam image)

Courtesy of W. Baumeister and J. Plitzko

Max Planck Institute of Biochemistry Martinsried, Germany

Cryo-FIB milling

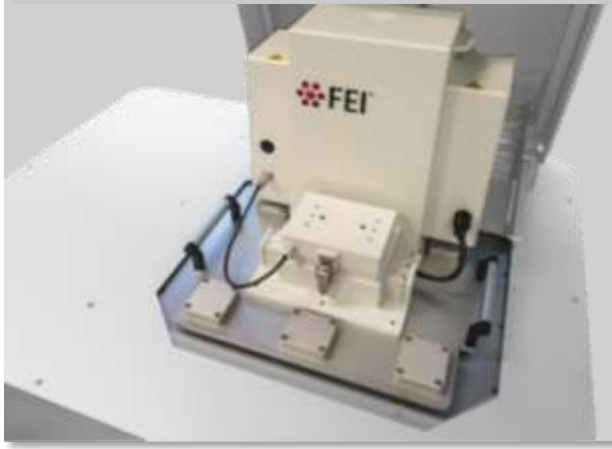


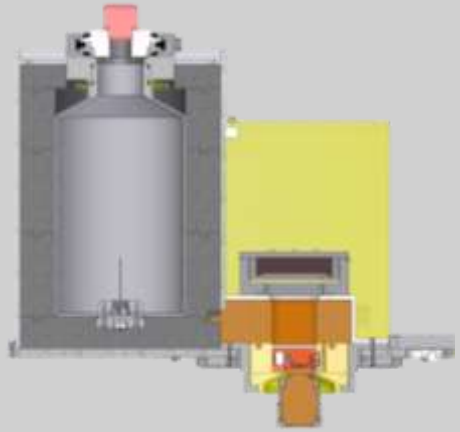
Rigort et al., Proc Natl Acad Sci USA (2012) Mar 20;109(12):4449-54.

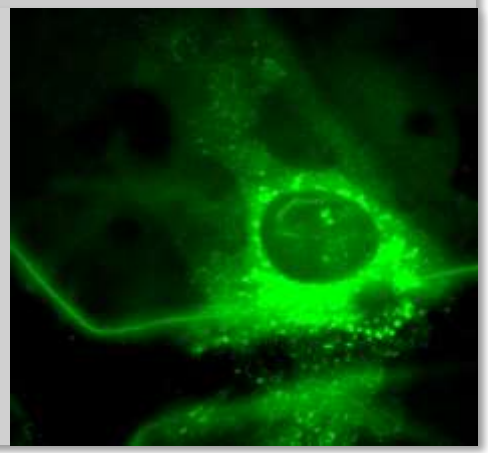
Cryo-light microscopy

CorrSight cryo

- **No LN_2 pump** needed
- Up to **2 grid positions** in a fixed geometry
- Compatible with **40x/0.9 NA** objective
- **No condensation** / frost
- Samples pre-mounted on shuttles for **quick and safe exchange**
- Works with **all CorrSight imaging modes**: transmission, widefield fluorescence, SI, spinning disk confocal

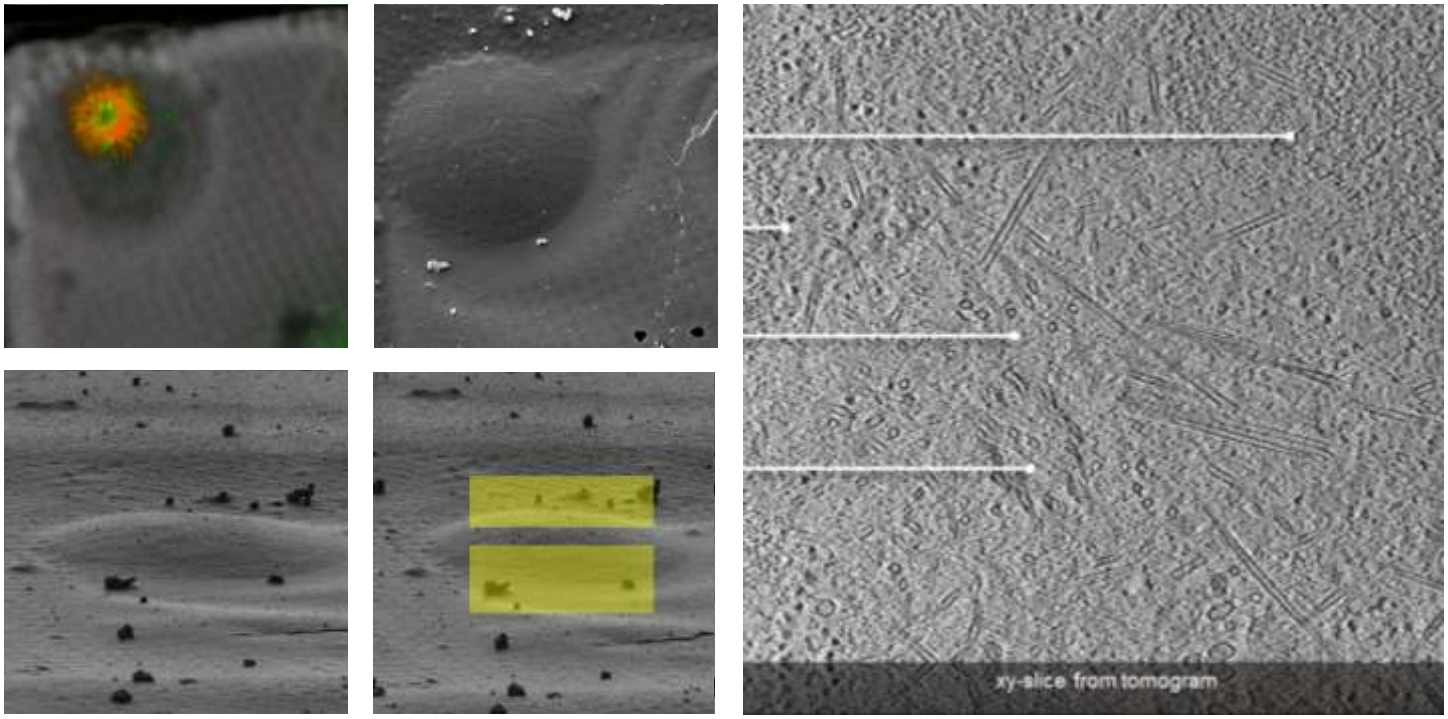






Courtesy of Dorit Hanein, Sanford Burnham

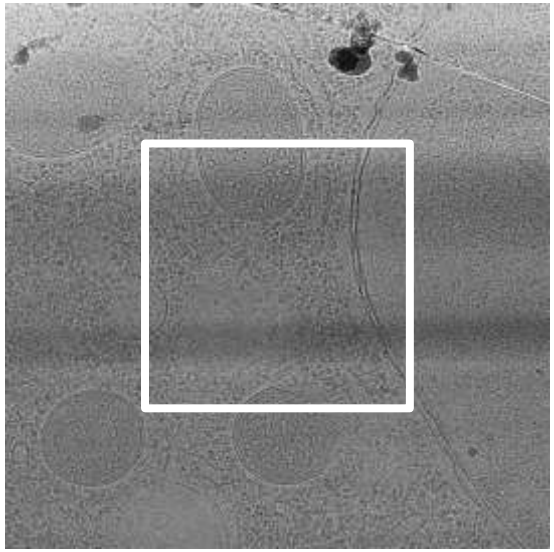
Imaging of HeLa cells: from cryo-light microscopy to cryo-TEM, through cryo-FIB-milling



Courtesy of J. Mahamid and J. Plitzko, MPI for Biochemistry

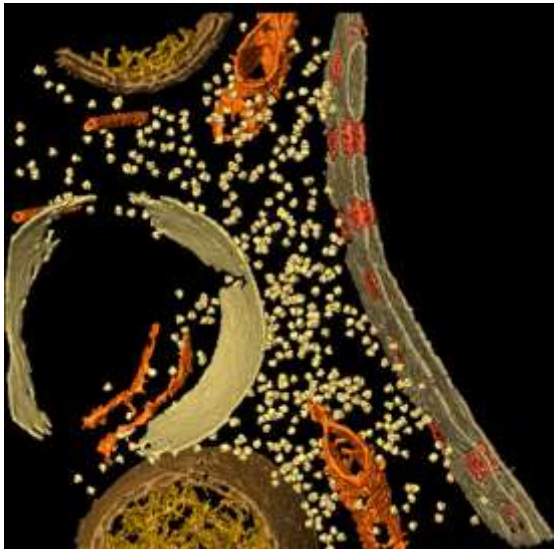
Cryo-ET of FIB-milled lamella

TEM projection



3D reconstruction

Surface rendering



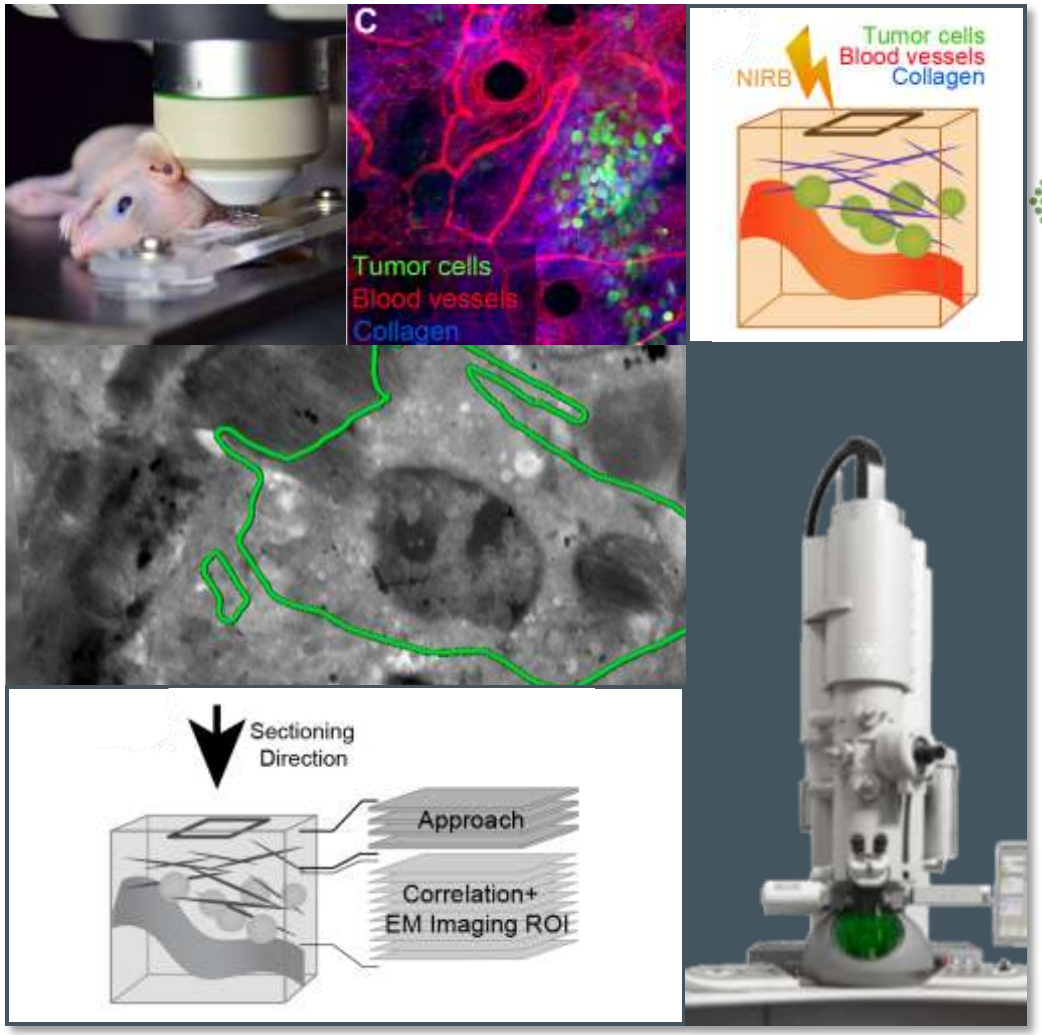
Courtesy of J. Plitzko

Max Planck Institute of Biochemistry Martinsried, Germany

Image registration of 3D CLEM data using Amira

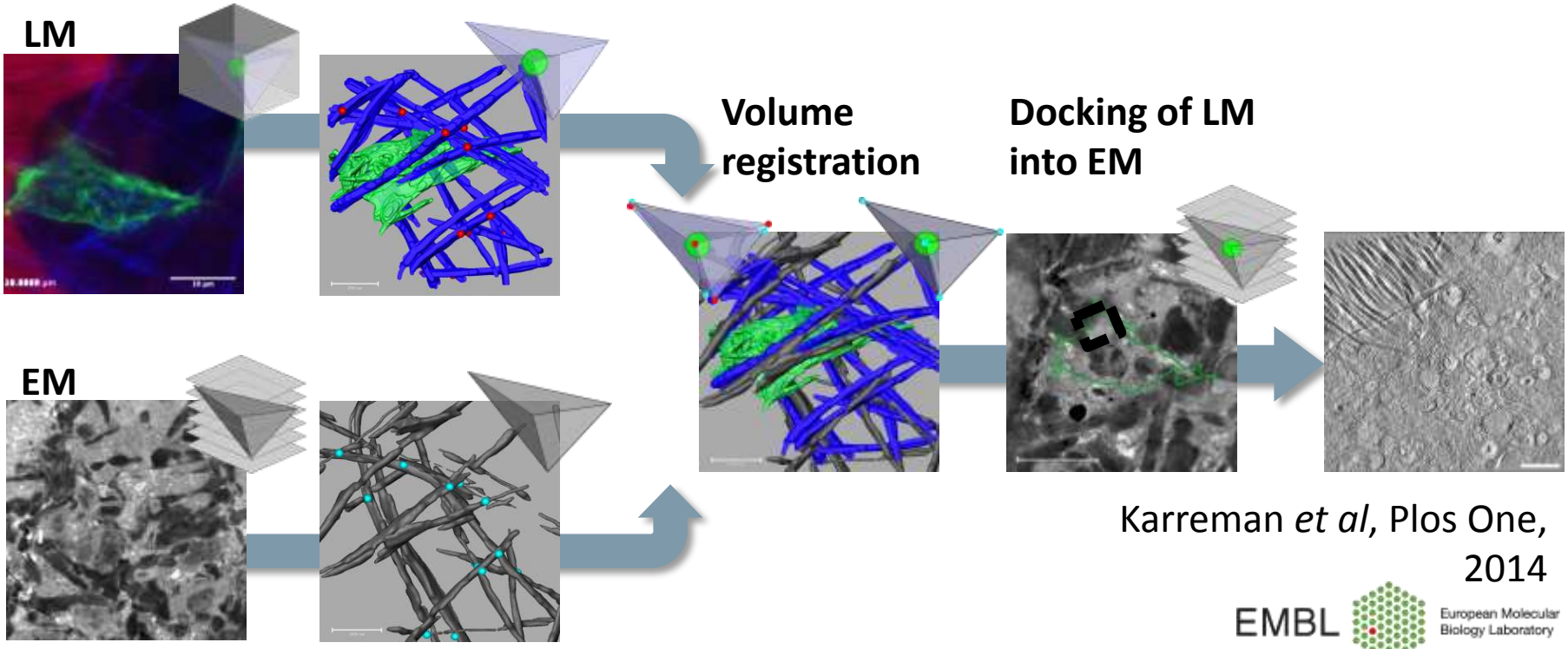
Intravital 2-photon to high-resolution EM

- Intravital 2p
- NIRB marking of the ROI
- Sample preparation for EM / ultramicrotomy
- TEM imaging
- Image registration
- Tomography



Karreman *et al*, Plos One, 2014

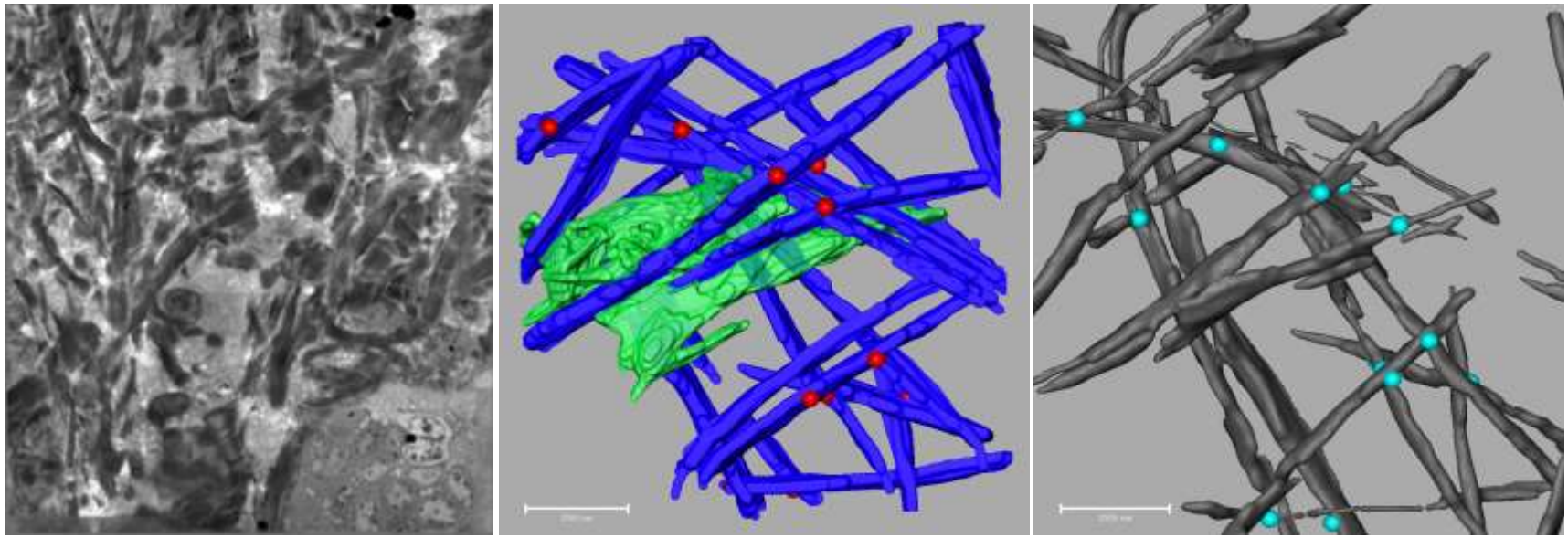
Using registered volumes for targeted tomography



Karreman *et al*, Plos One, 2014

EMBL European Molecular Biology Laboratory

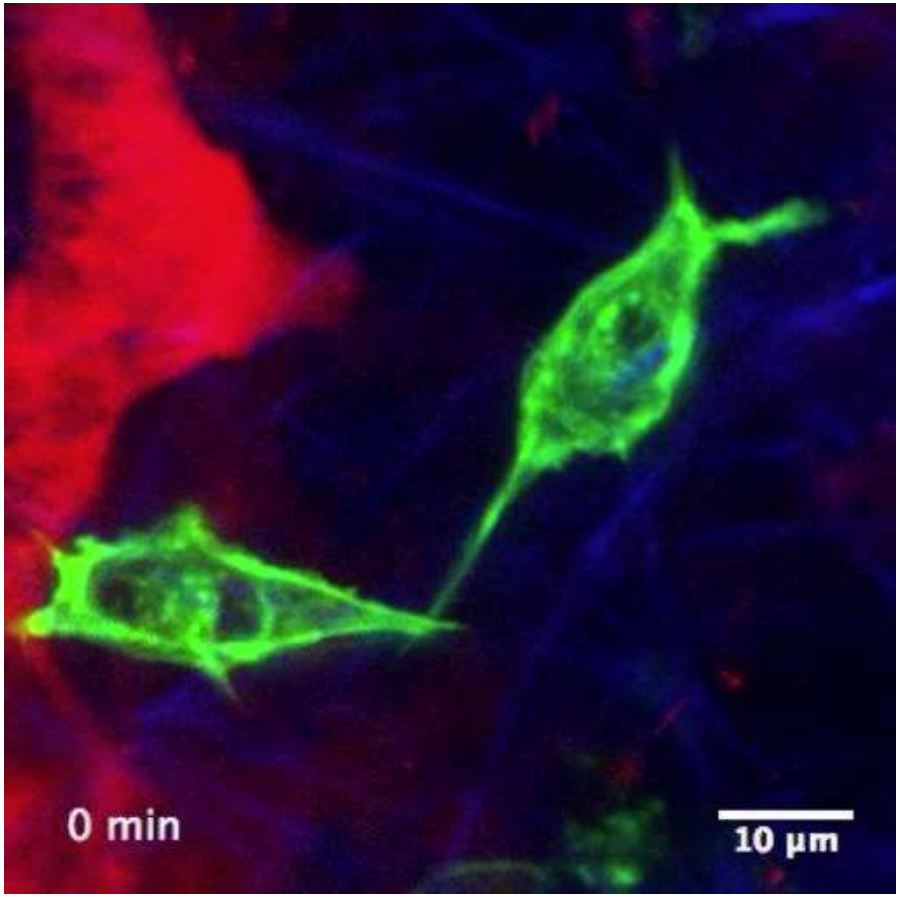
Segmentation of collagen as basis for landmark registration



Karreman *et al*, Plos One, 2014

EMBL European Molecular Biology Laboratory

Intravital imaging to electron tomography



Karreman *et al*,
Plos One, 2014

EMBL European Molecular Biology Laboratory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