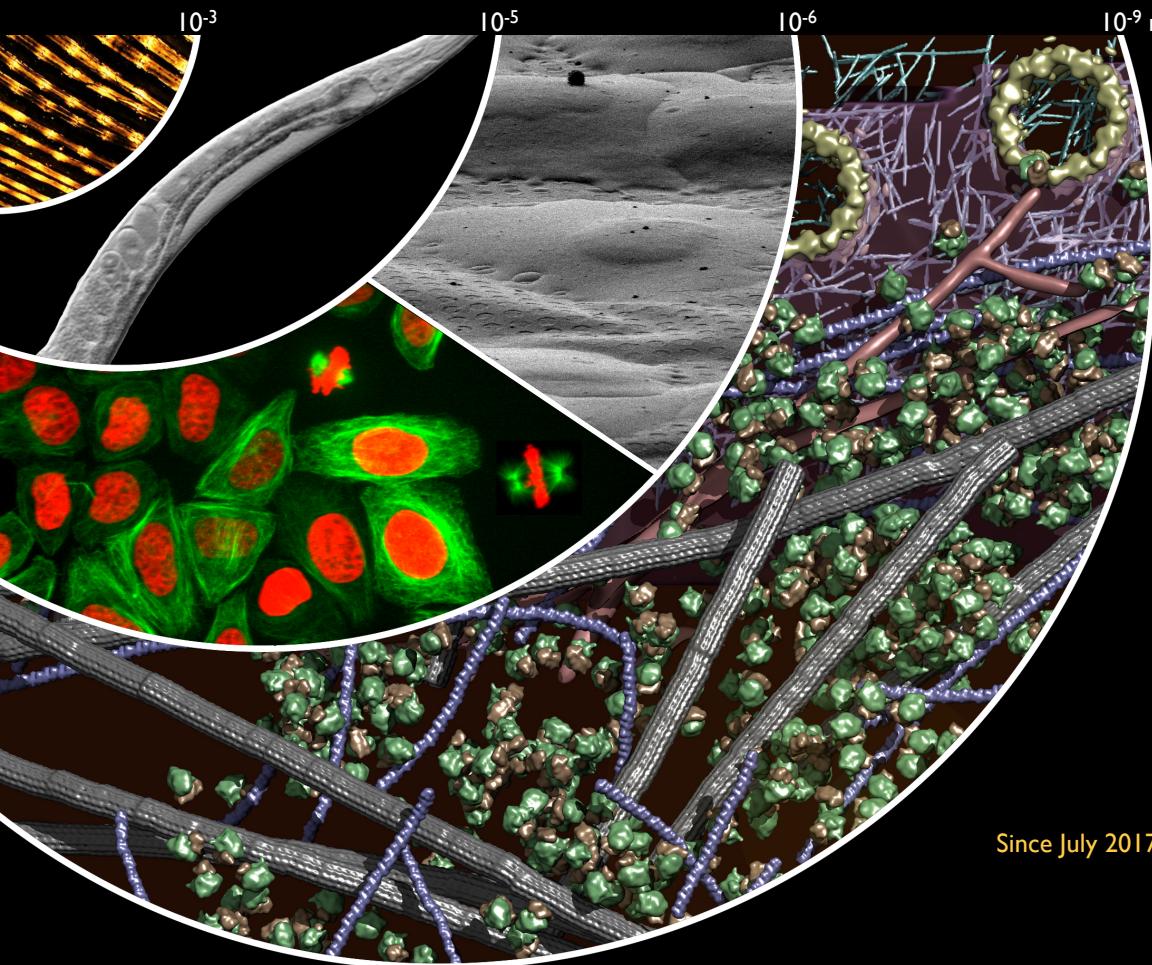
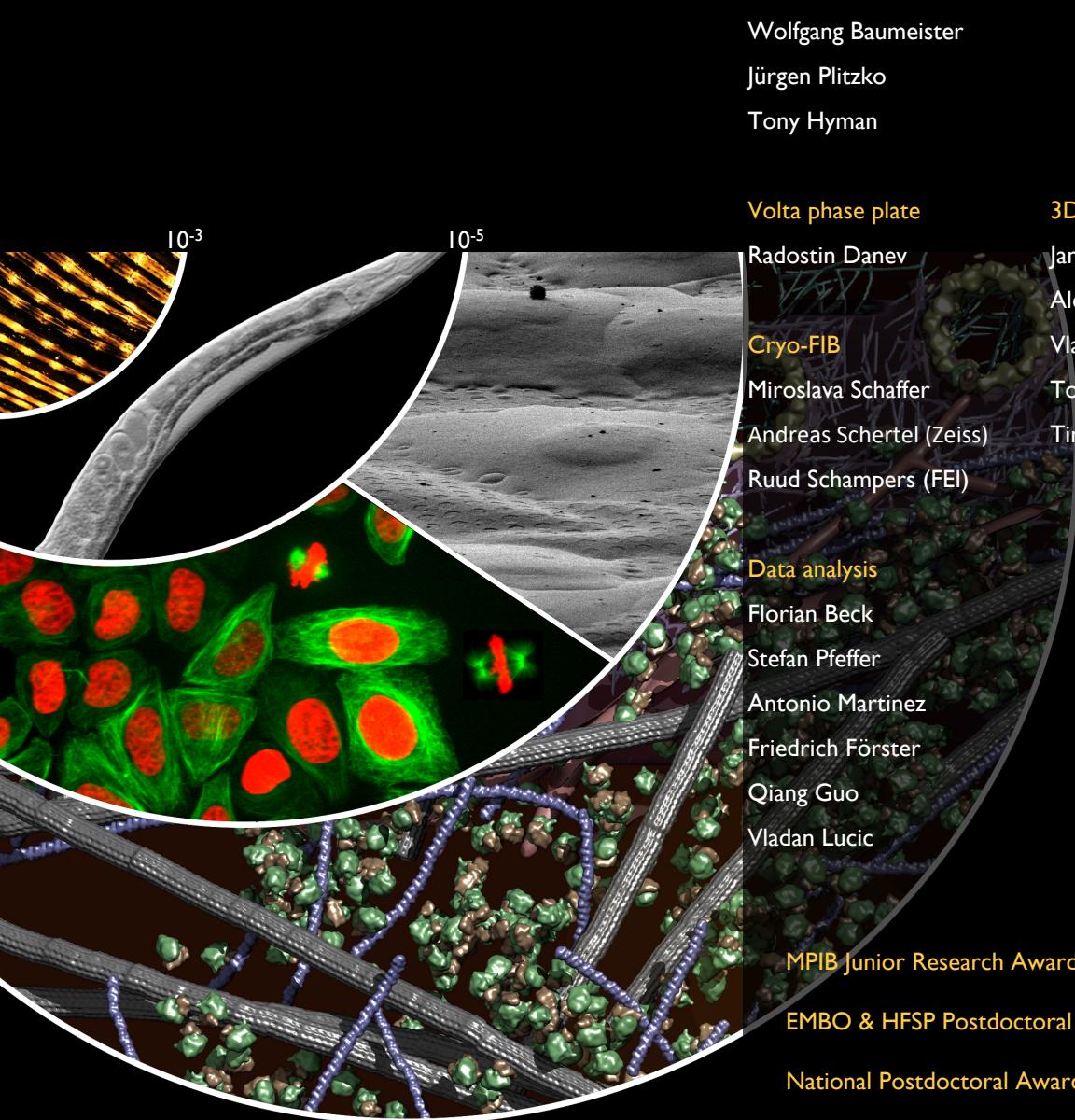


Site Specific Cryo-FIB Preparations Aimed at *in-cell* Structural Biology



Julia Mahamid

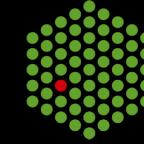
Since July 2017: Structural & Computational Biology, EMBL, Heidelberg



Wolfgang Baumeister

Jürgen Plitzko

Tony Hyman



3DCellPhase

My Group

Edoardo D'Imprima

Sara Kathrin Goetz

Mauricio Toro-Nahuelpan

Fergus Tollervey

Jenny Sachweh

Liang Xue

Ievgeniia Zagorij

Xiaojie Zhang

Associated Postdocs (EIPODs)

Mainak Bose

Irene De Teresa Trueba

Kar Ho Herman Fung

Yuki Hayashi

Cecilia Perez-Borrjero

Volta phase plate

Radostin Danov

Cryo-FIB

Miroslava Schaffer

Andreas Schertel (Zeiss)

Ruud Schampers (FEI)

Data analysis

Florian Beck

Stefan Pfeffer

Antonio Martinez

Friedrich Förster

Qiang Guo

Vladan Lucic

3D Correlation

Jan Arnold

Alex de Marco

Vladan Lucic

Tobias Mayer

Tim Laugks

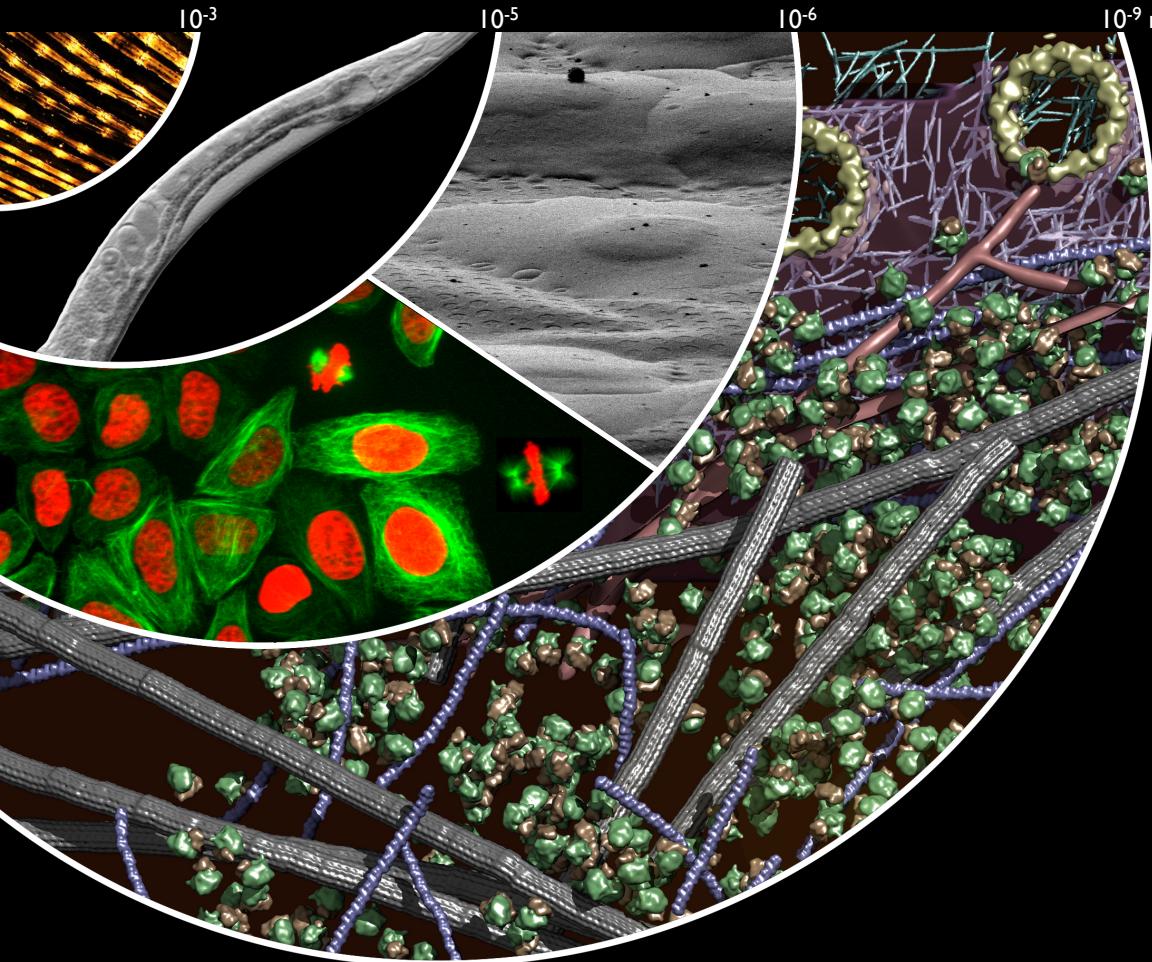
MPIB Junior Research Award

EMBO & HFSP Postdoctoral Fellowships

National Postdoctoral Award for Women in Science – The Weizmann Institute of Science

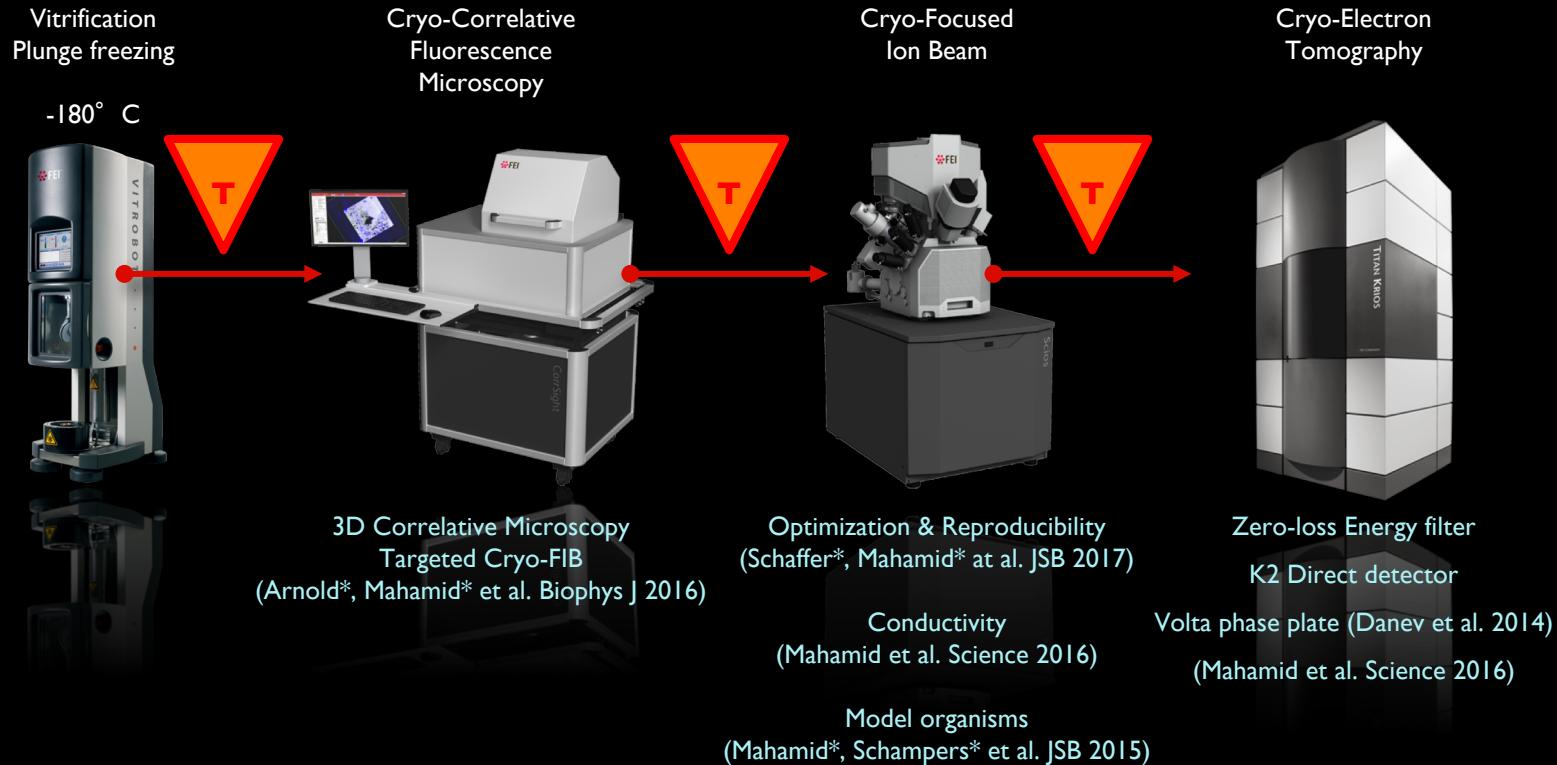
Leica for Cryo-CLEM

Molecular Views into Cellular Function by *in situ* Cryo-Electron Tomography

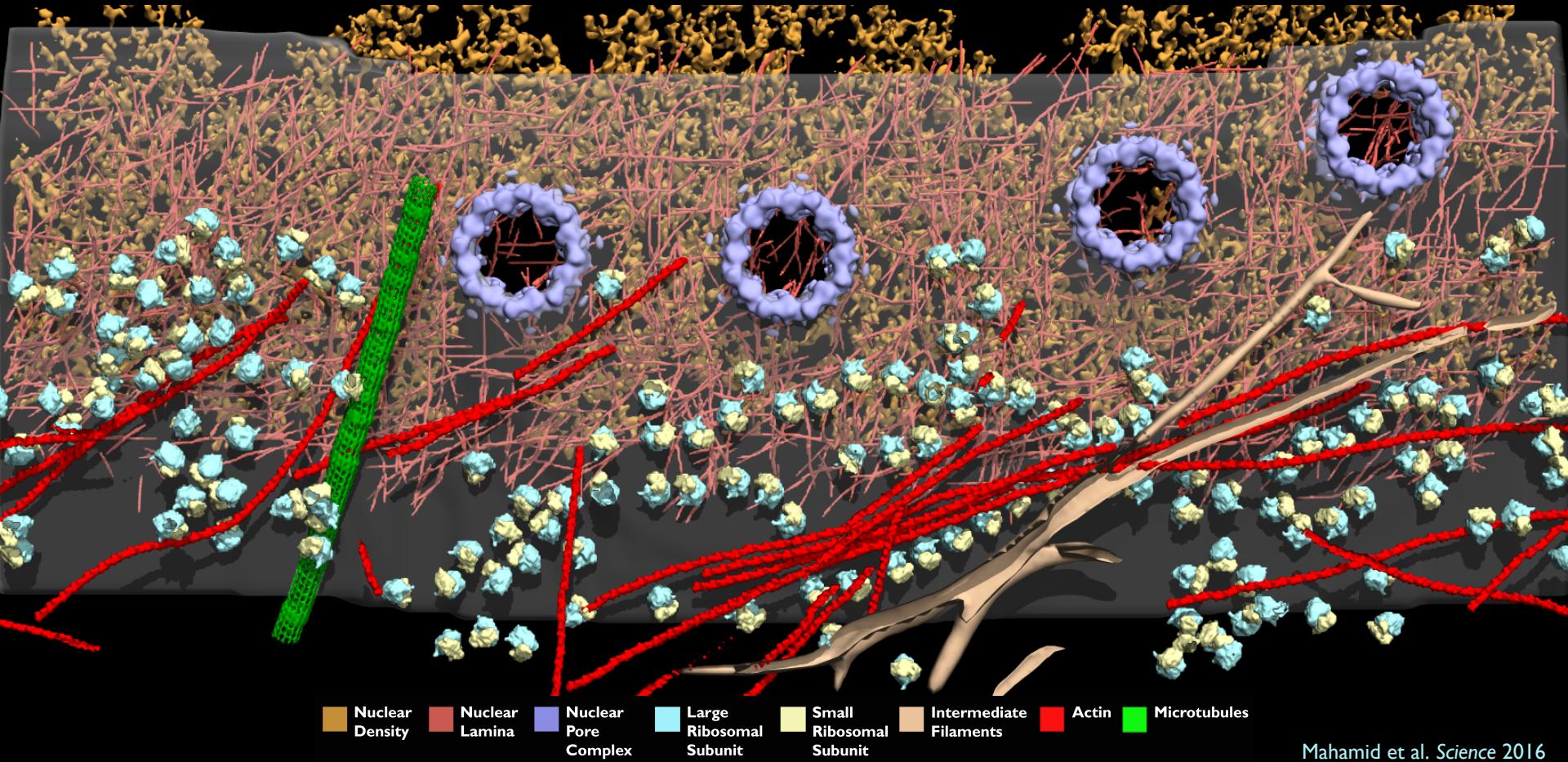


- Samples that have to be studied in their **hydrated state** to ensure structural preservation
- Suitable **sample thickness** for ‘electron transparency’ & to obtain molecular resolution
- Sensitivity to **ionizing radiation**
- Low-contrast due to weakly scattering building blocks
- Data mining and **quantitative interpretation**

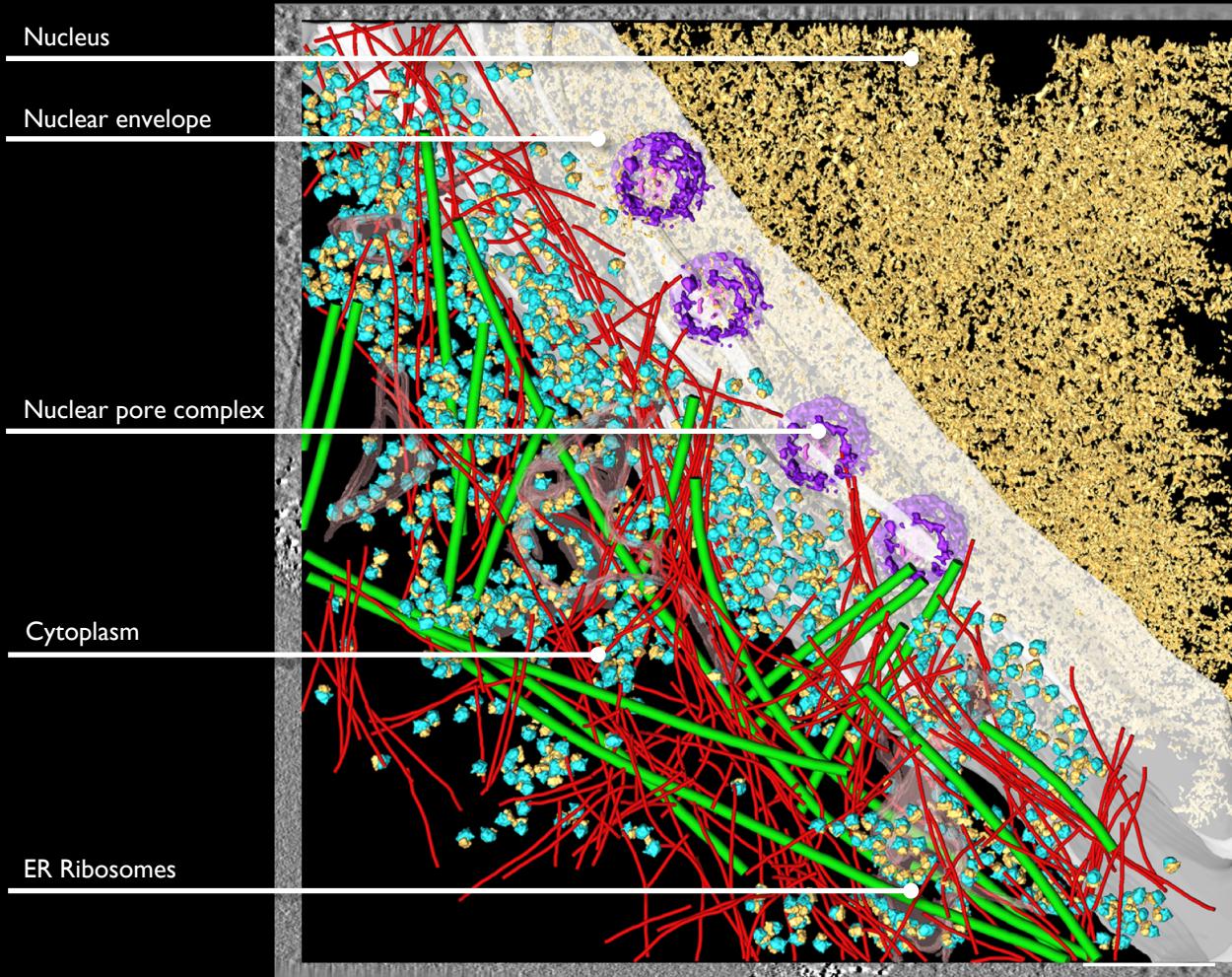
Cellular Cryo-Electron Tomography: Molecular Views into Cellular Function



Visualizing the Molecular Sociology at the HeLa Cell Nuclear Periphery



Intracellular Landscapes: Cryo-ET with VPP



Titan Krios, 300 kV

K2 direct detector & VPP

Pixel size: 0.421 nm

Tilt range: -50° to 60°

Tilt Increment: 2°

Defocus: -0.5 μm

Series total dose: 60 e-/Å²

Alignment of tilt series by feature tracking

No CTF Correction

No Dose weighting

'Segmentation'

Template matching

(Frangakis et al. PNAS 2002)

Filament tracing

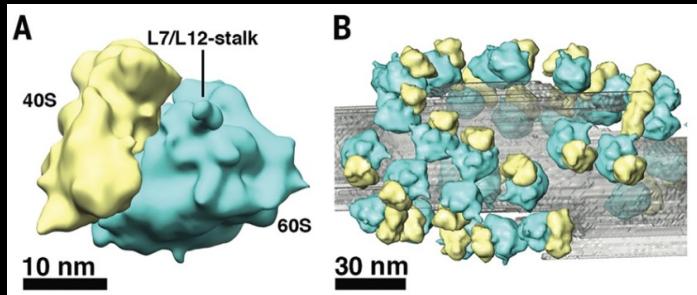
(Rigort, JSB 2012; Weber, JSB 2012)

Subtomogram averaging

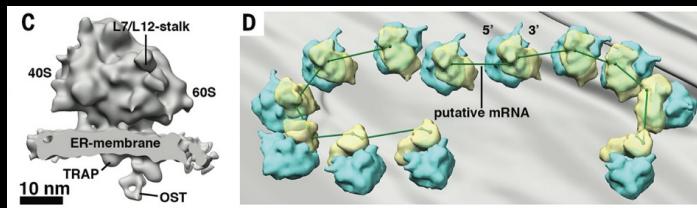
(Förster, Hegerl. *Methods in Cell Biology* 2007)

3D analysis of Ribosomes and Polysomes

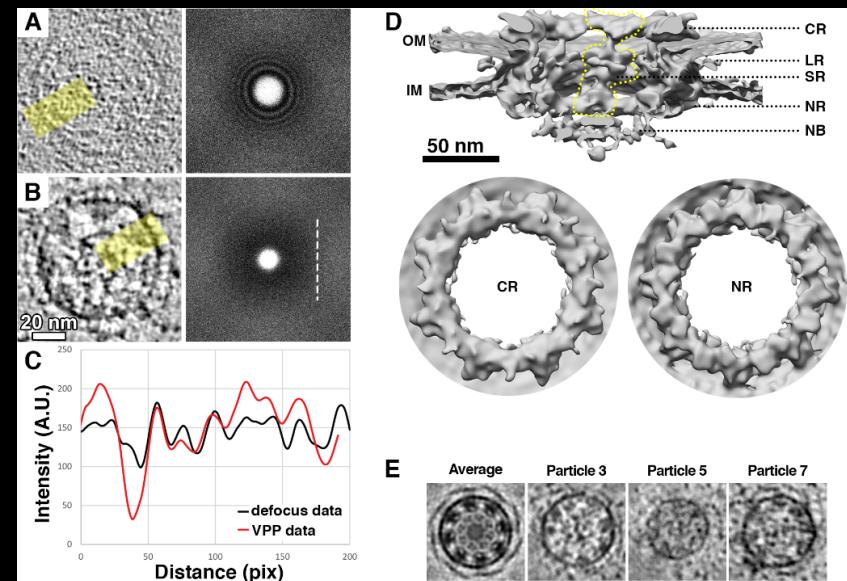
Cytoplasmic Ribosomes, 900 particles: 28 Å @ 0.33 FCS



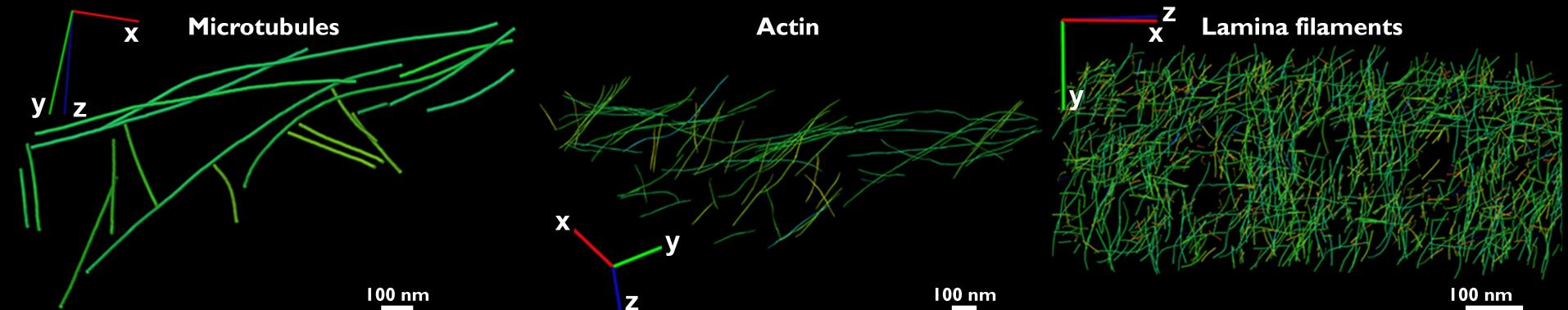
ER-bound Ribosomes, 140 particles: 35 Å @ 0.33 FCS



Plasticity of the Nuclear Pore Complex



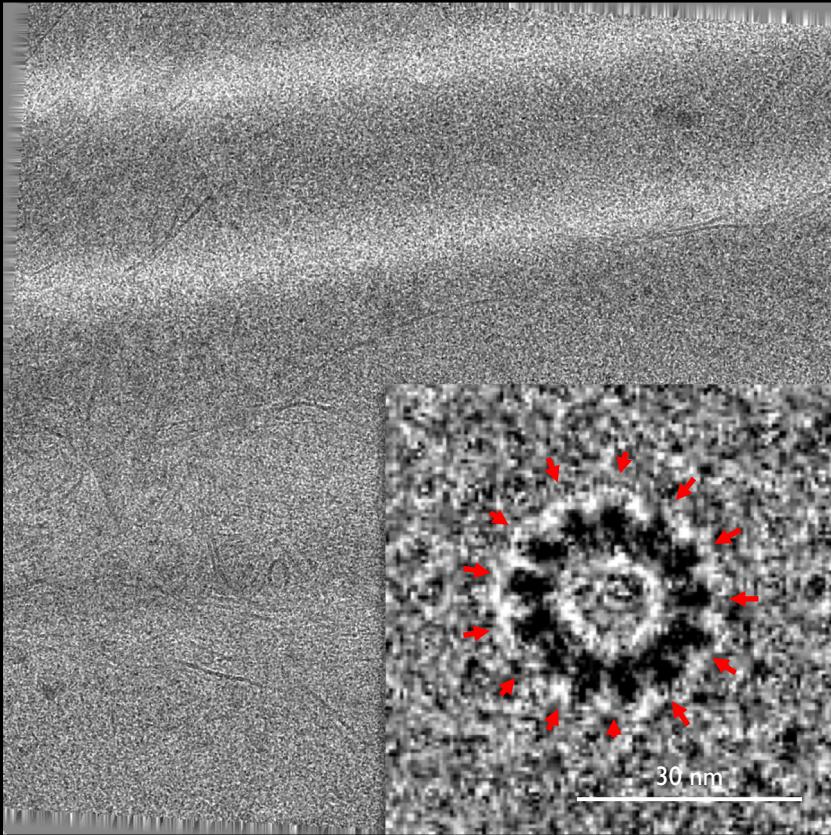
Analysis of Cellular Filaments



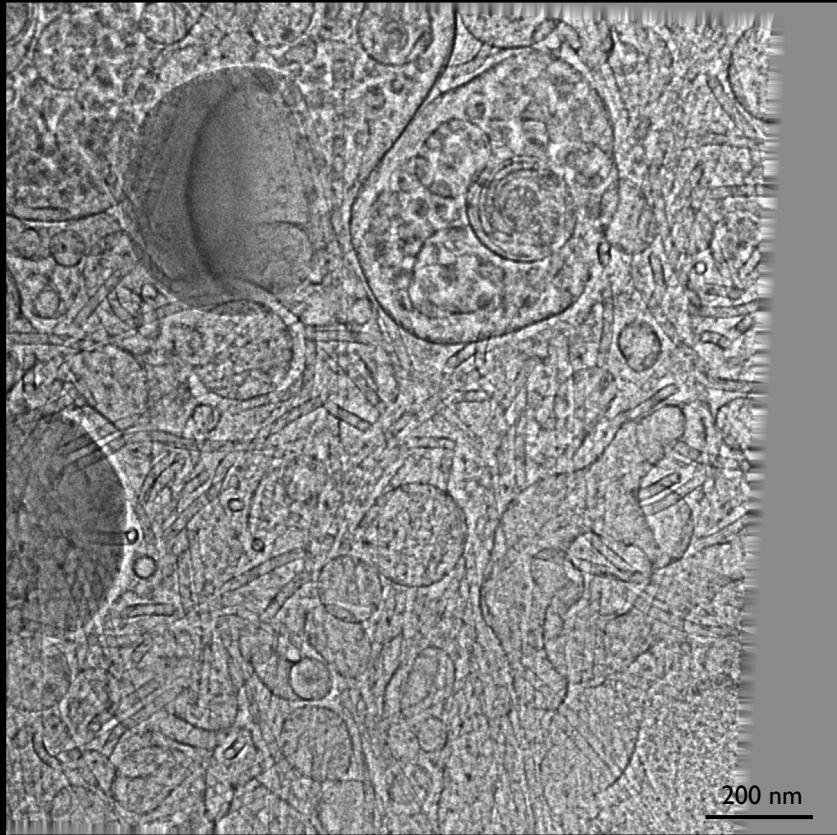
The putative lamina filaments produce a surprisingly high elastic modulus of ~ 190 MPa, matching measurements on whole nuclei attributed to lamin A (Schape et al. 2009)

Cellular Cryo-Electron Tomography: Tilt series

Defocus -6 μm



Phase-plate, defocus -0.5 μm



Titan Krios, 300 kV

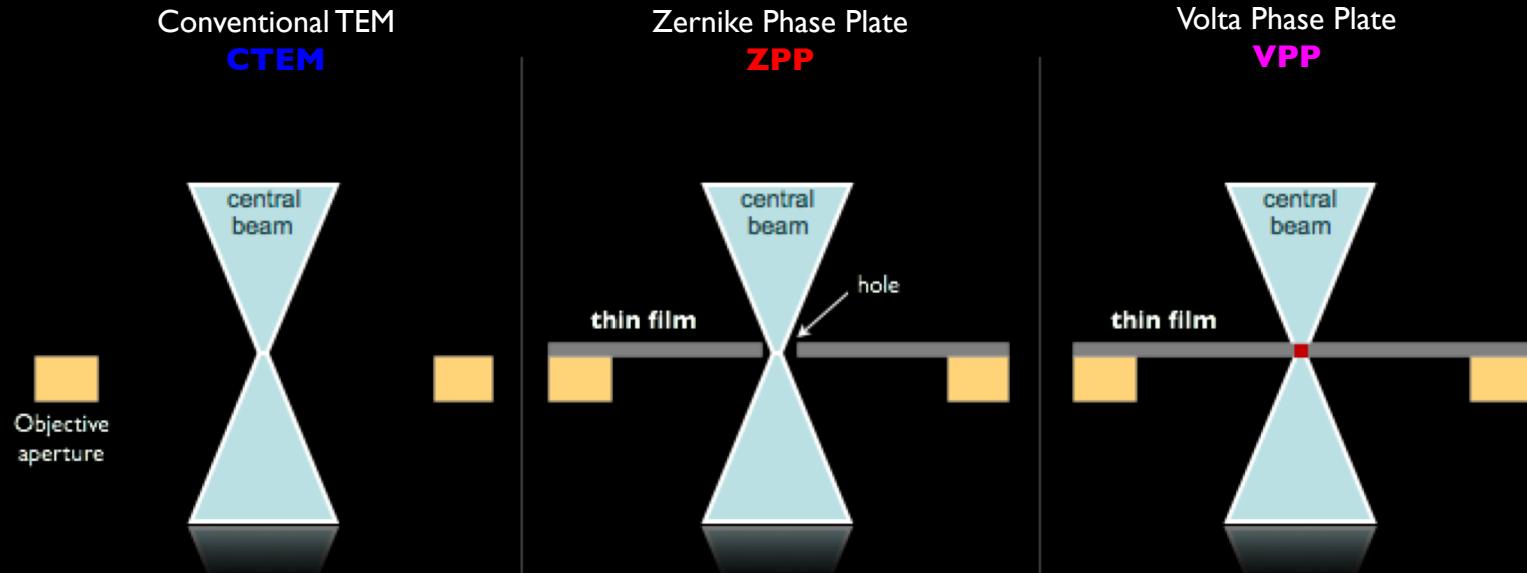
K2 direct detector

Pixel size: 0.53 nm

Series total dose: $\sim 60 \text{ e-}/\text{\AA}^2$

with Radostin Danev, MPI of Biochemistry, Martinsried

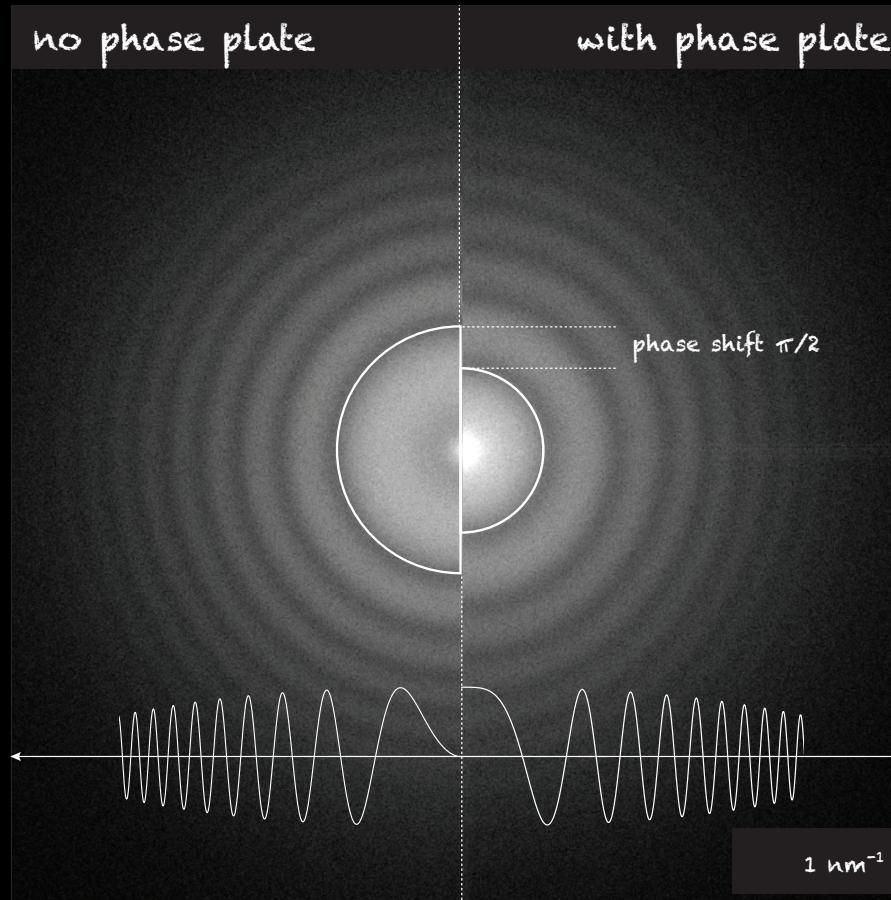
Phase Contrast TEM: Phase Plates



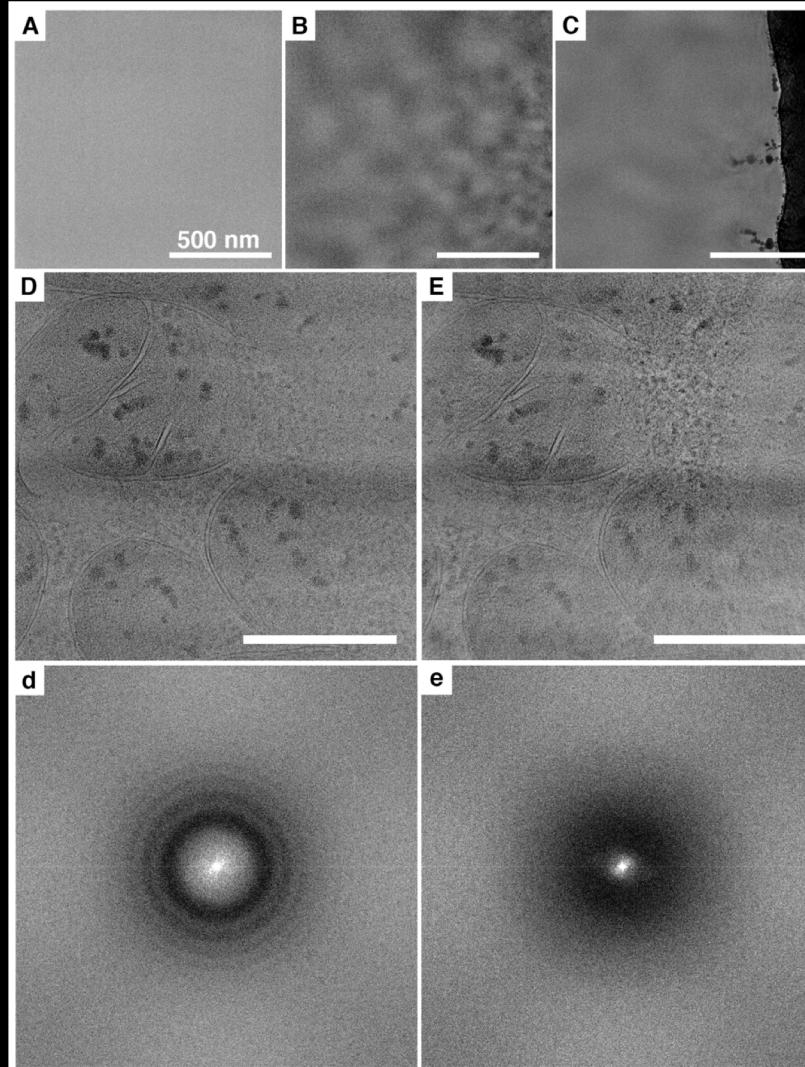
Dai et al. Visualizing Virus Assembly
Intermediates Inside Marine
Cyanobacteria. *Nature* 2013

Danev et al. Volta potential phase plate for in-focus phase contrast transmission electron microscopy. *PNAS* 2014

Phase Contrast TEM: Volta Phase Plate



Cellular Cryo-Electron Tomography: Conductivity

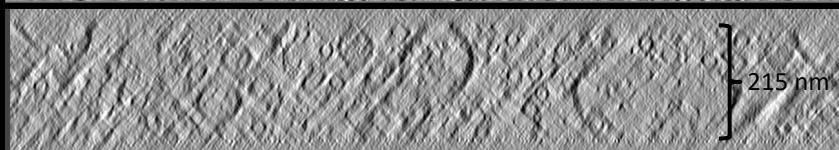
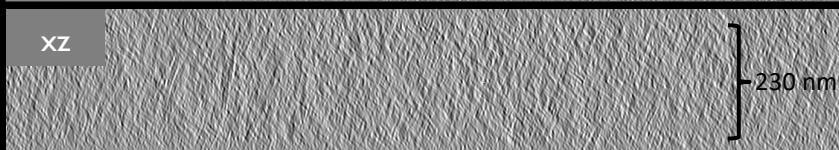
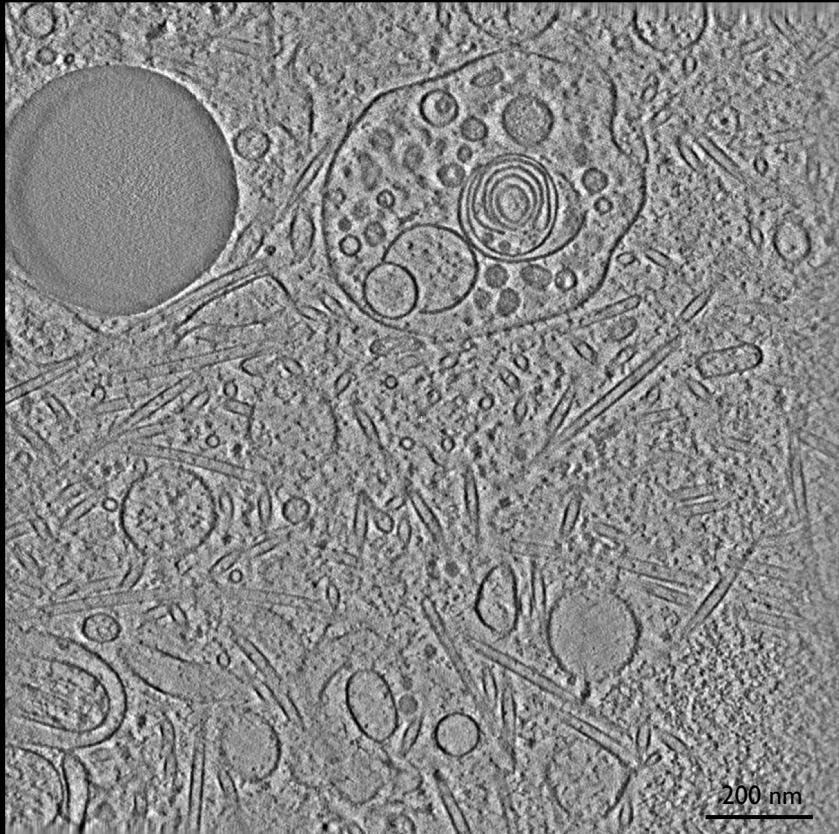


Cellular Cryo-Electron Tomography: Volumes

Defocus -6 μm

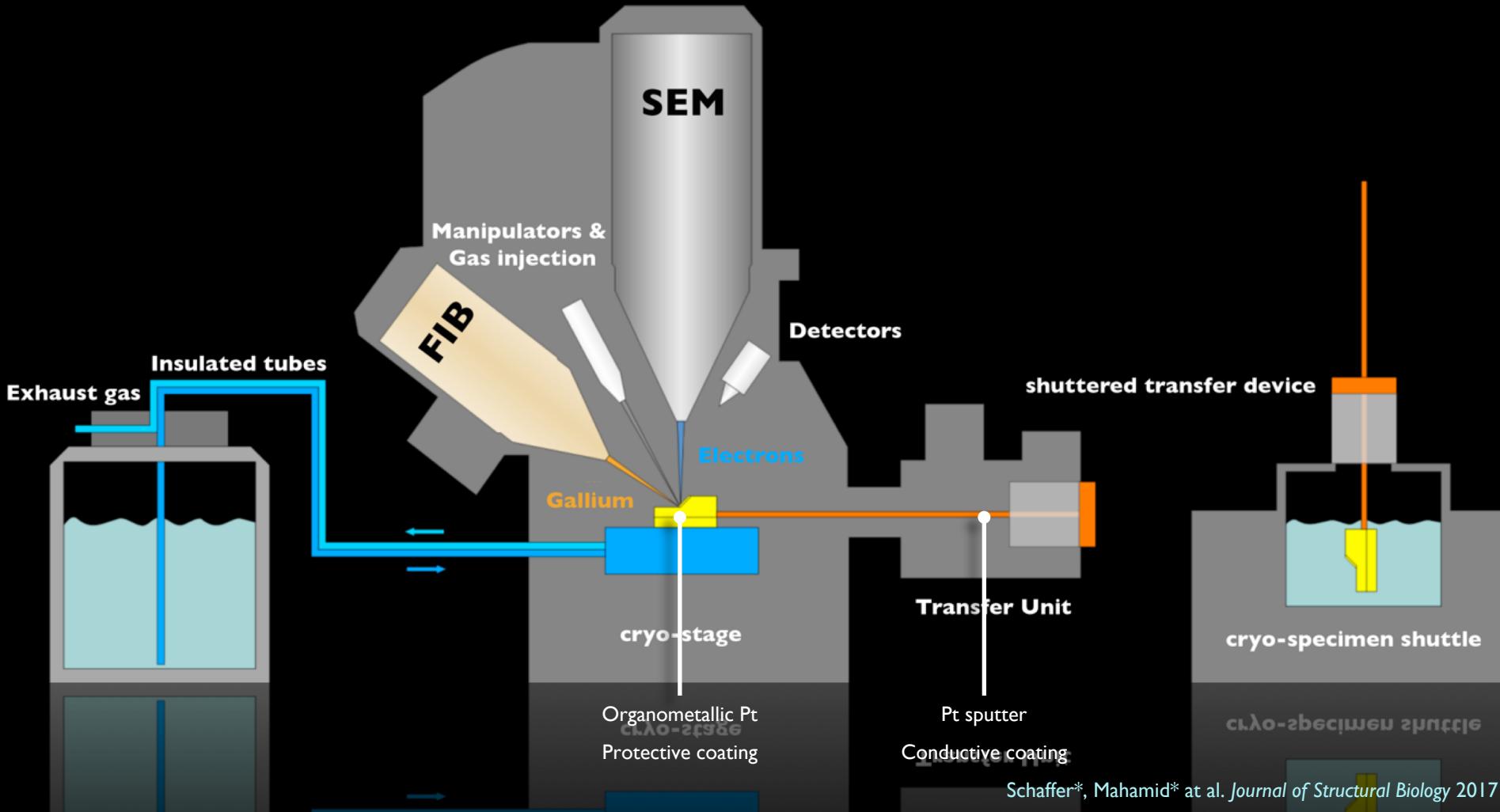


Phase-plate, defocus -0.5 μm

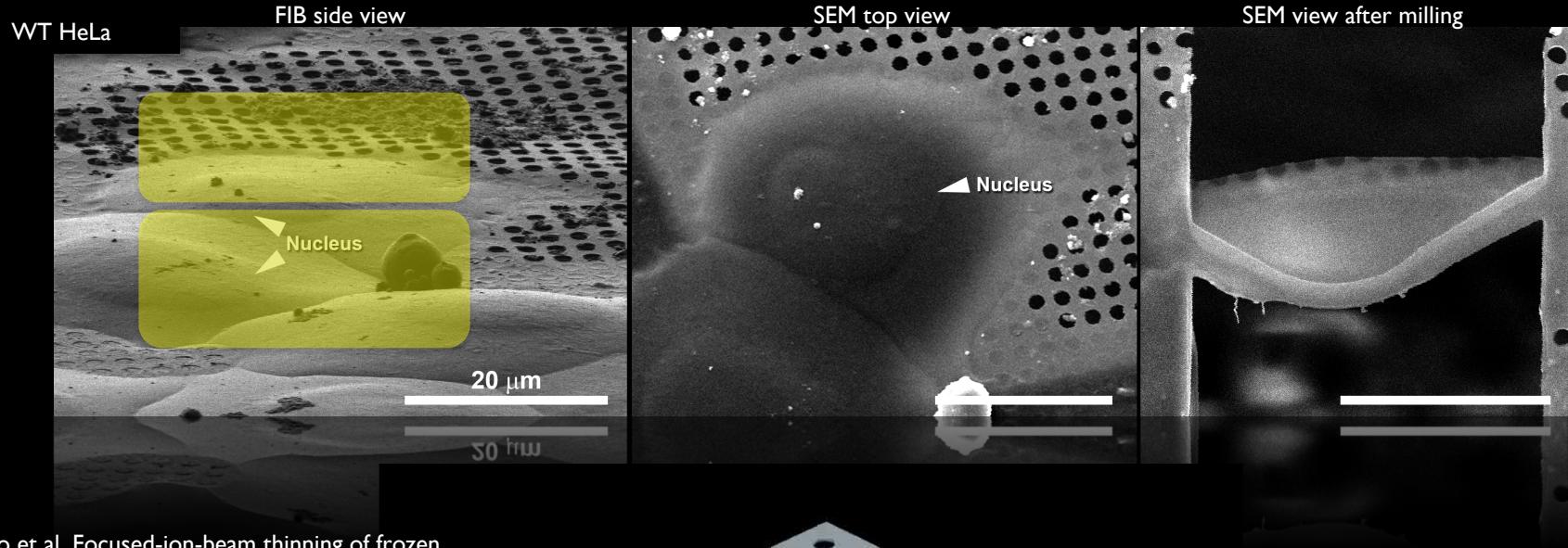


With Radostin Daney

Small Dual Beam: Cryo-FIB Setup

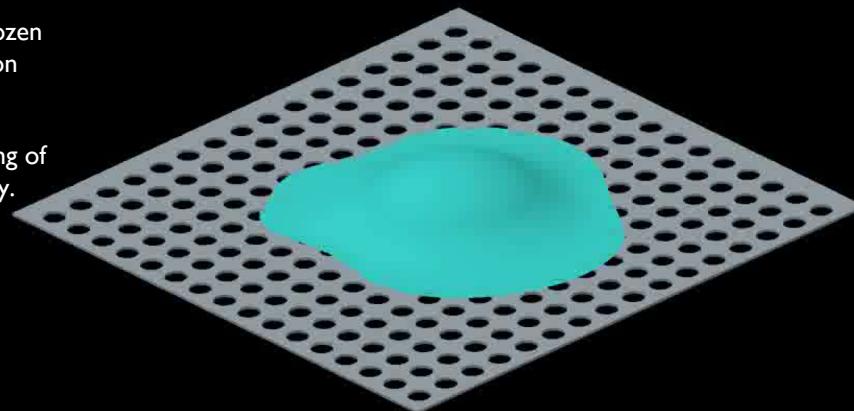


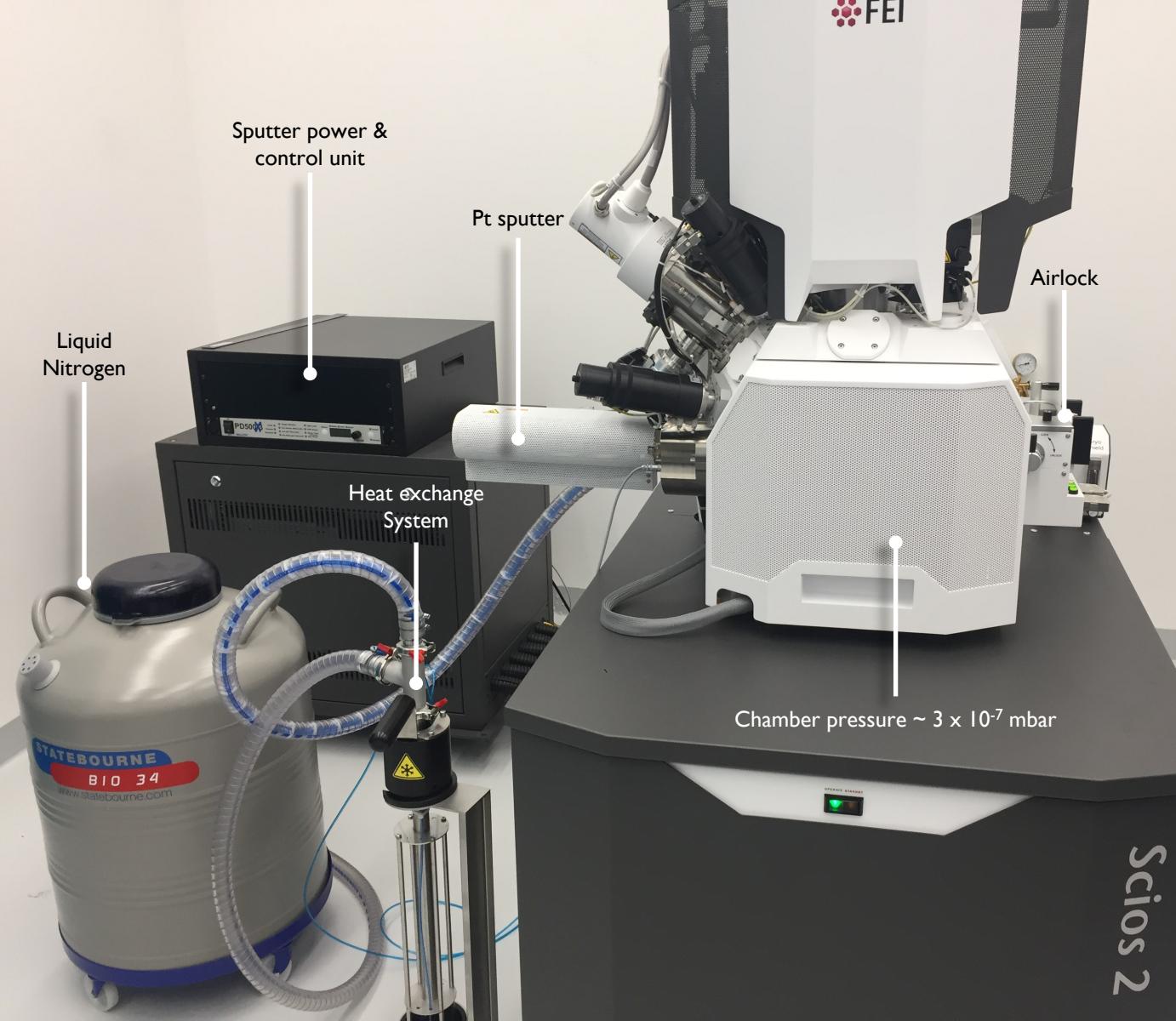
Cryo-FIB: Opening a ‘Window’ into the Cell



Marko et al. Focused-ion-beam thinning of frozen hydrated biological specimens for cryoelectron Microscopy. *Nature Methods* 2007

Rigort et al. Focused ion beam micromachining of eukaryotic cells for cryo-electron tomography. *PNAS* 2012

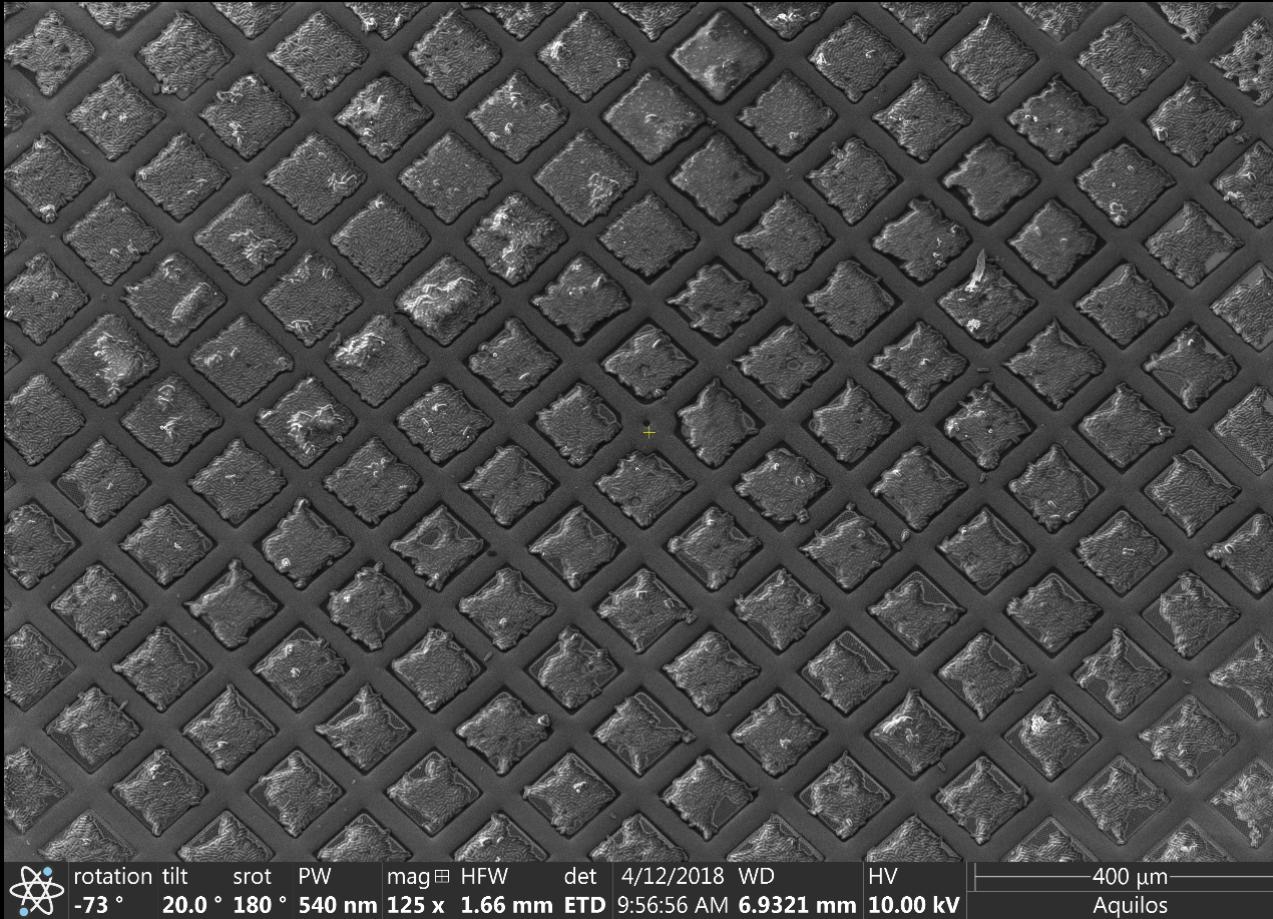




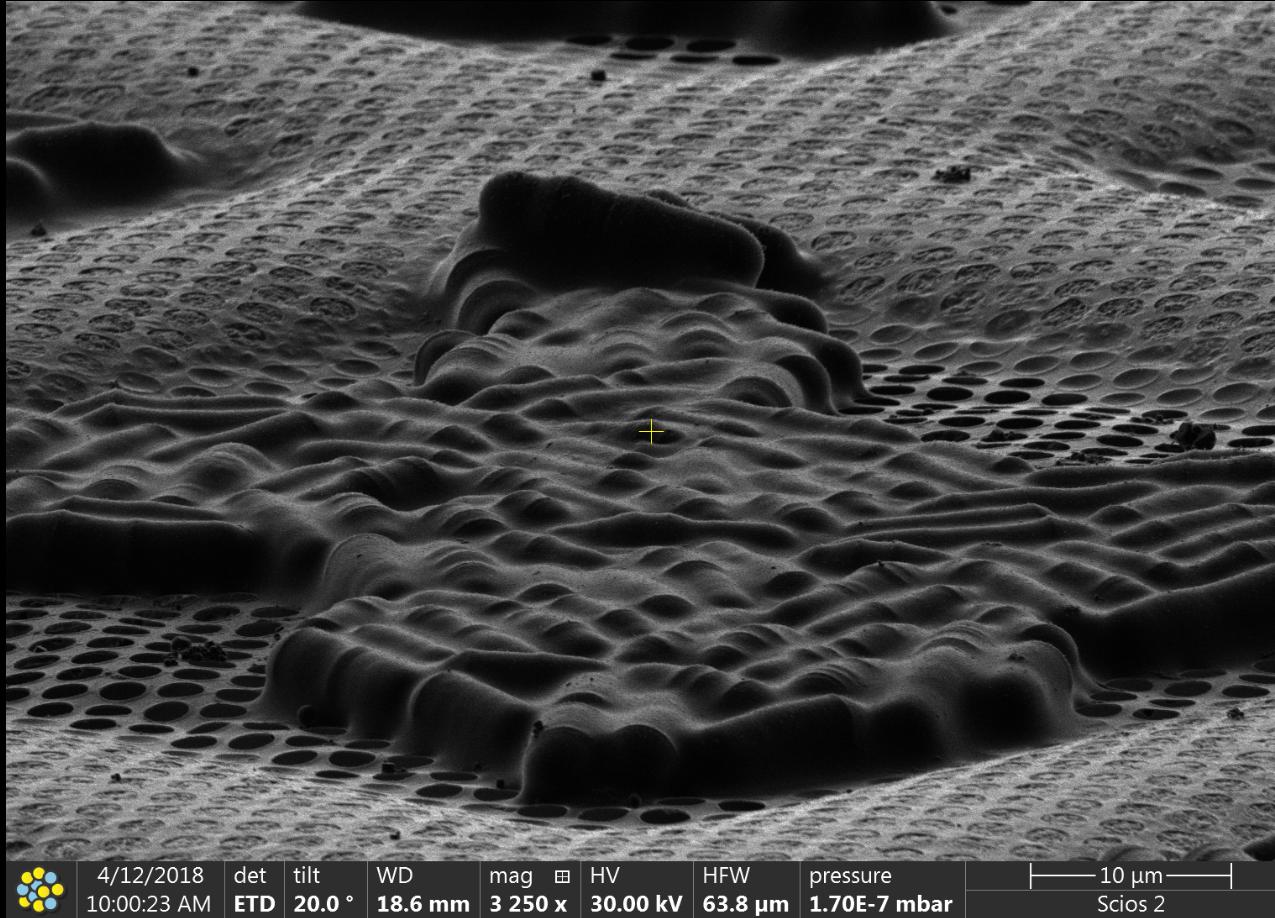
Scios2/Aquilos

Delivered end of June, 2017

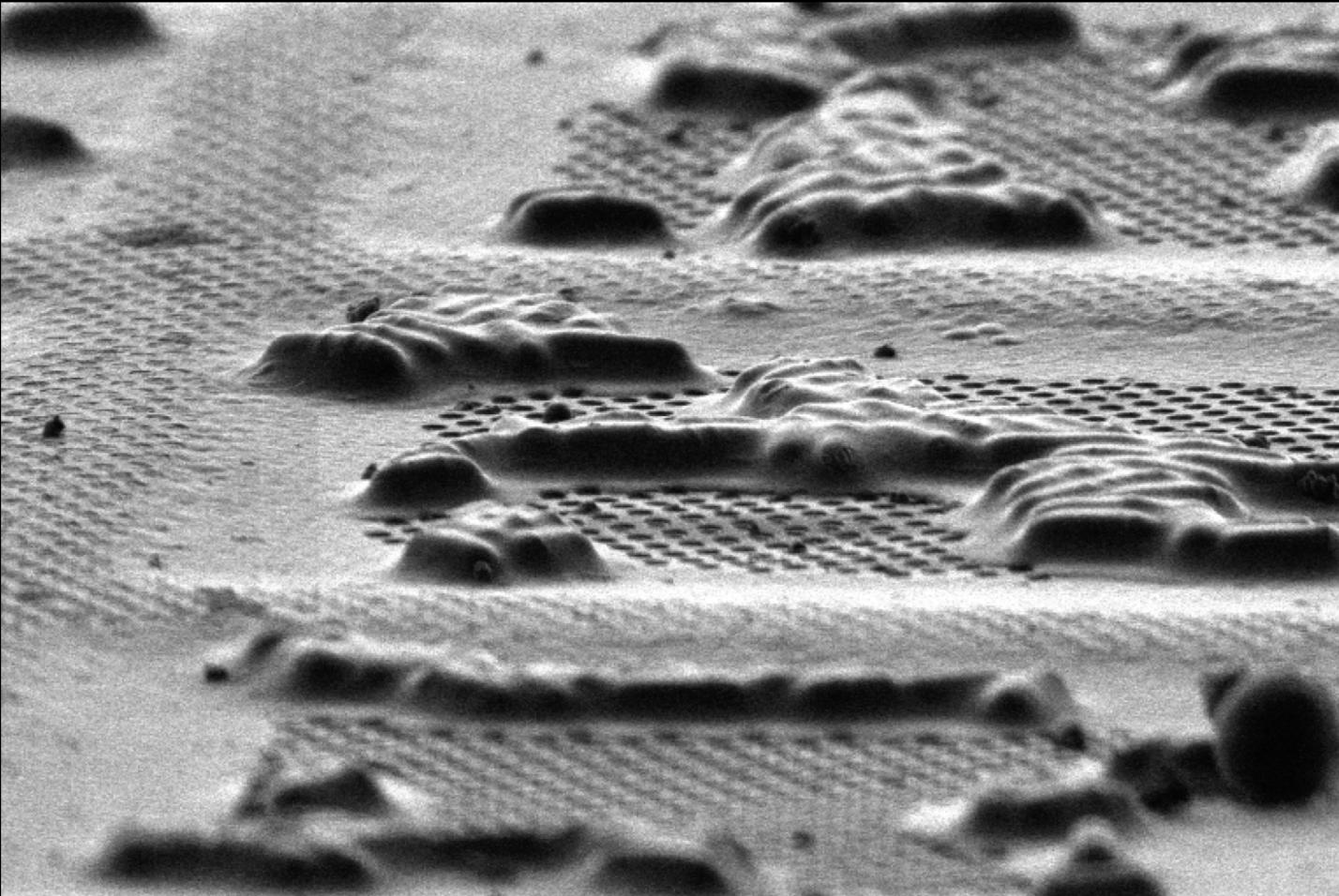
Sc. pombe frozen by Leica plunger: The (almost) Perfect Grid



Sc. pombe frozen by Leica plunger: The (almost) Perfect Grid



Sc. pombe frozen by Leica plunger: The (almost) Perfect Grid

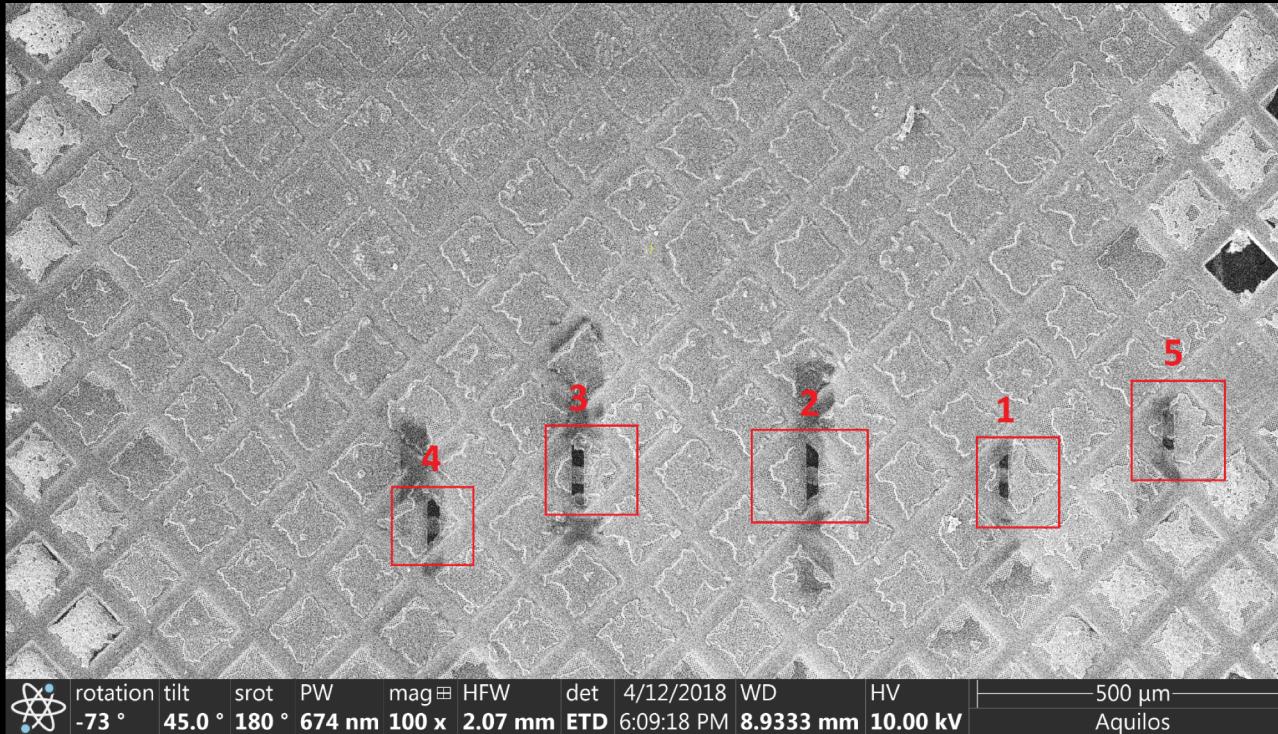


Sc. pombe frozen by Leica plunger: The (almost) Perfect Grid

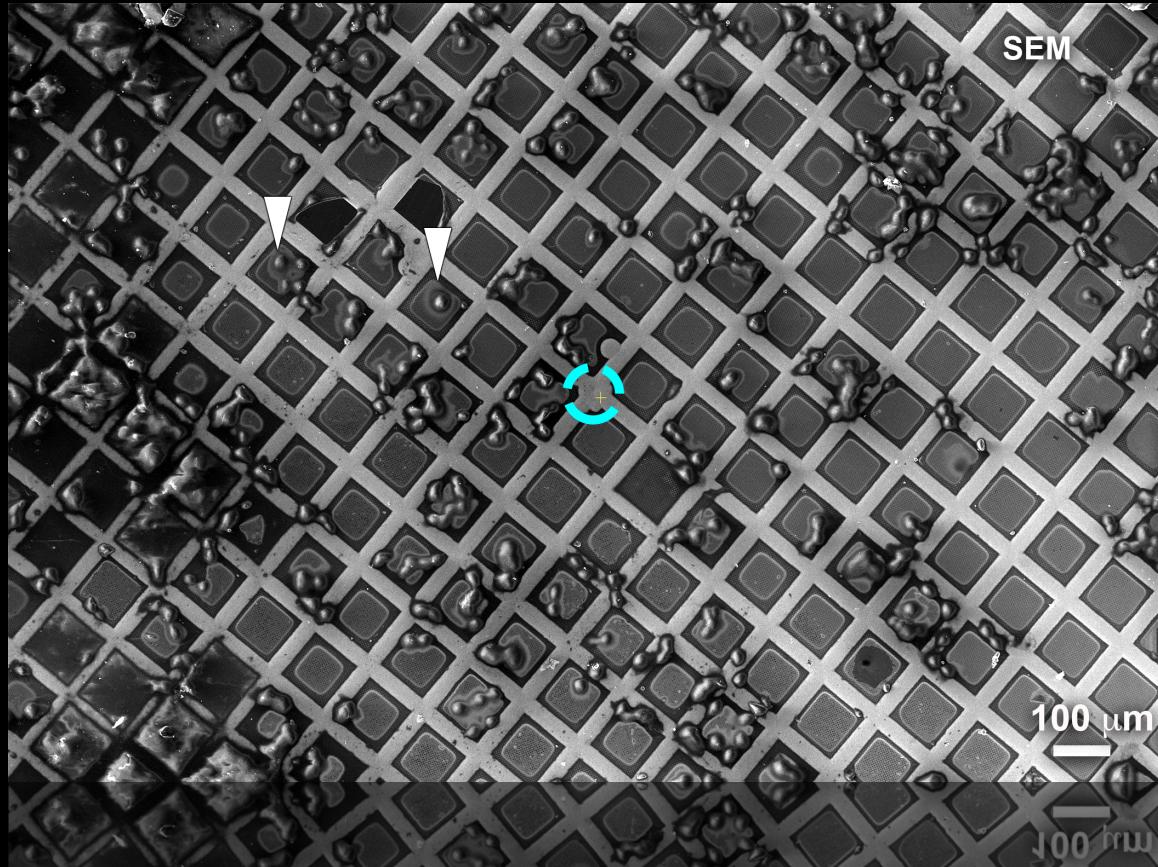
Ready for development of automated procedures::

Step wise rough milling of 5-8 lamella (down to 1000-500 nm)

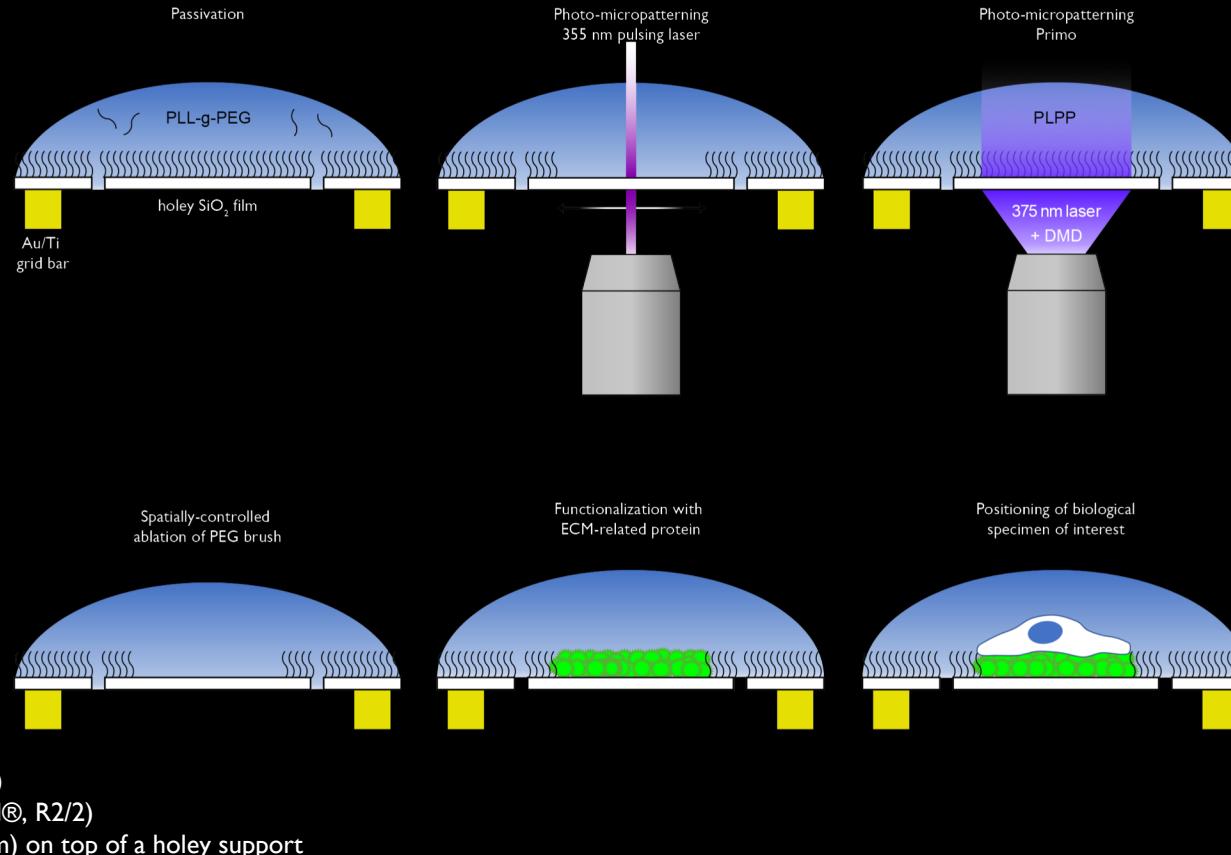
Manual polishing to desired thickness (300-50 nm)



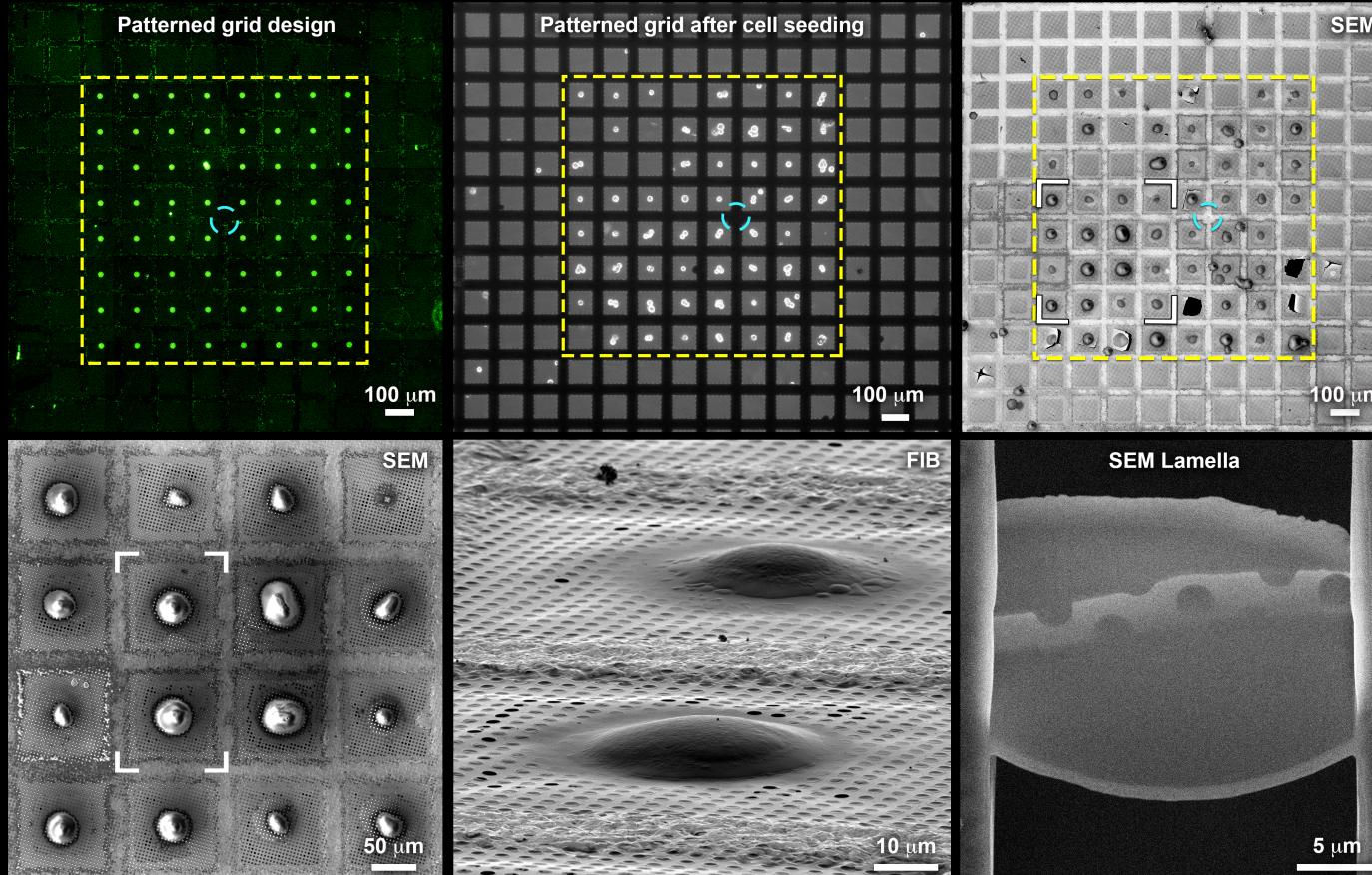
Tailoring Cryo-Electron Microscopy Grids by Photo-Micropatterning



Tailoring Cryo-Electron Microscopy Grids by Photo-Micropatterning



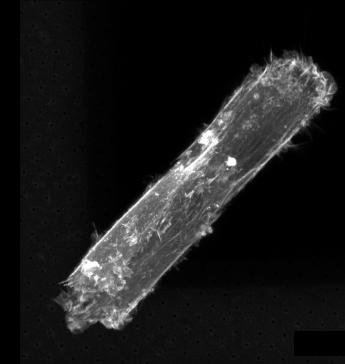
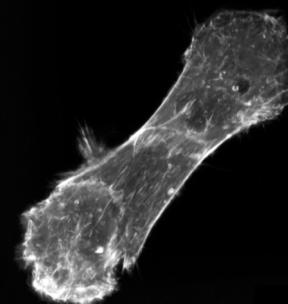
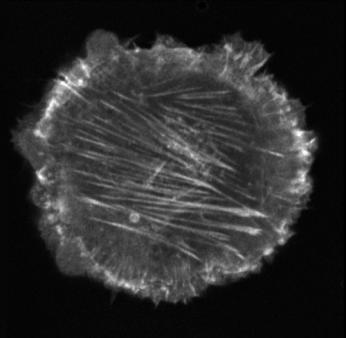
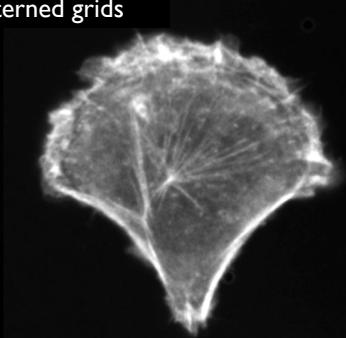
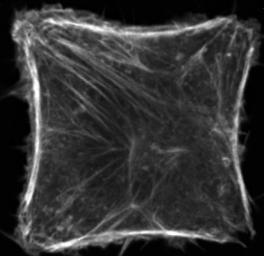
Tailoring Cryo-Electron Microscopy Grids by Photo-Micropatterning



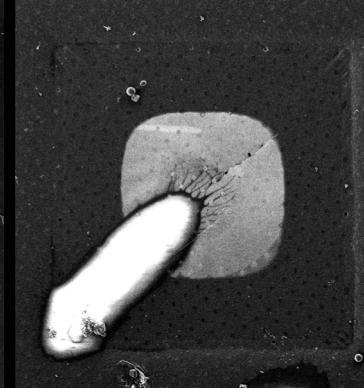
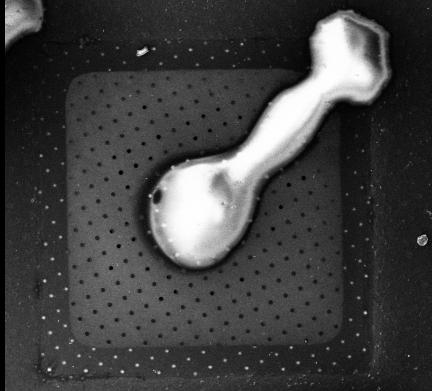
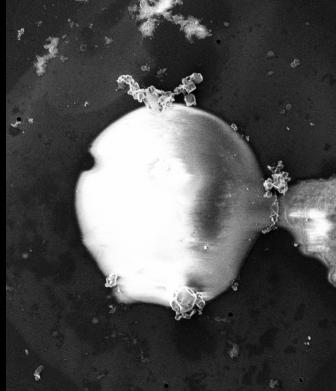
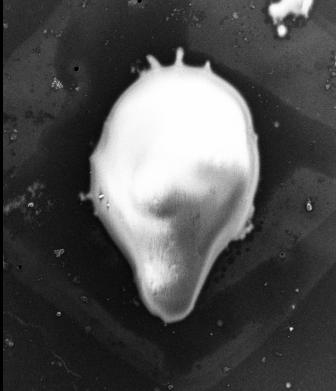
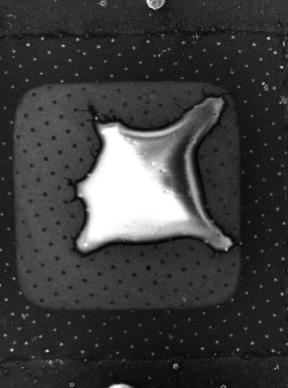
Micropatterning: Beyond Simple Positioning



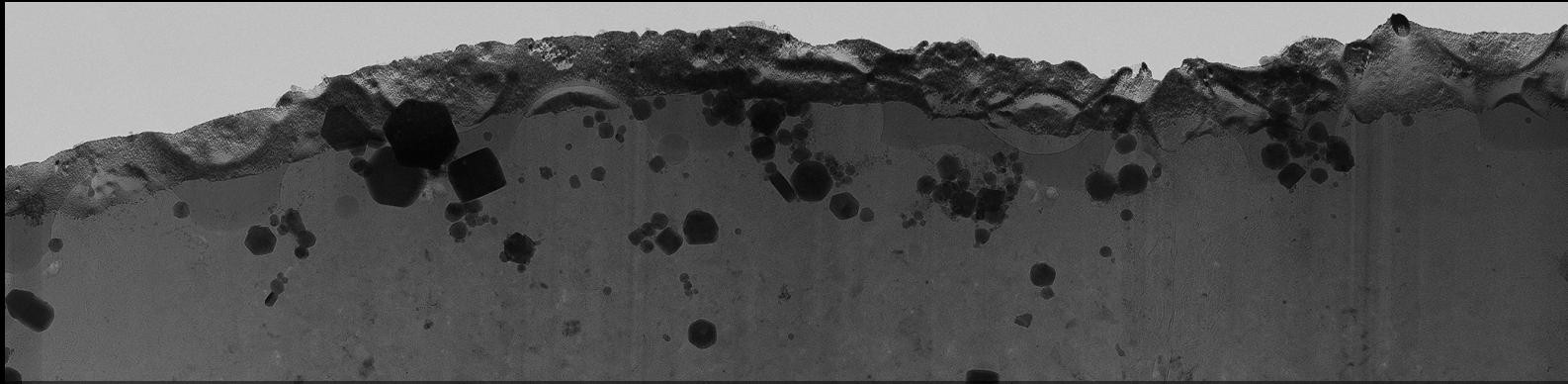
Lifeact:GFP live-cell imaging on patterned grids



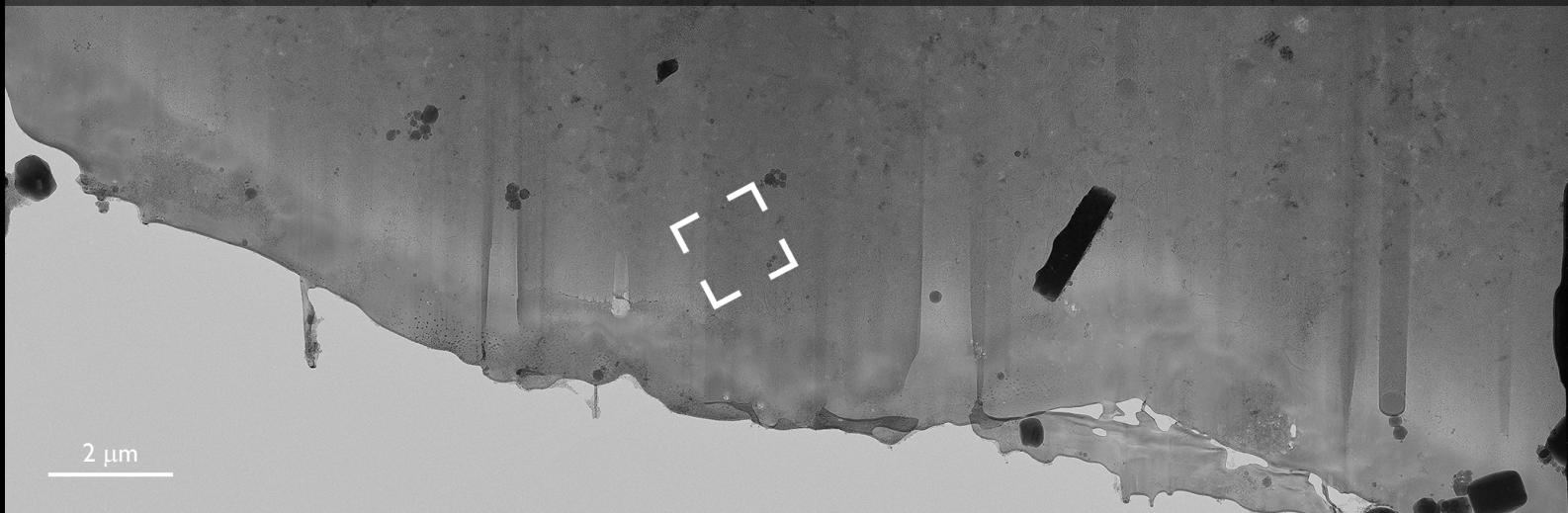
SEM of patterned grids



Cryo-FIB: Final Lamella

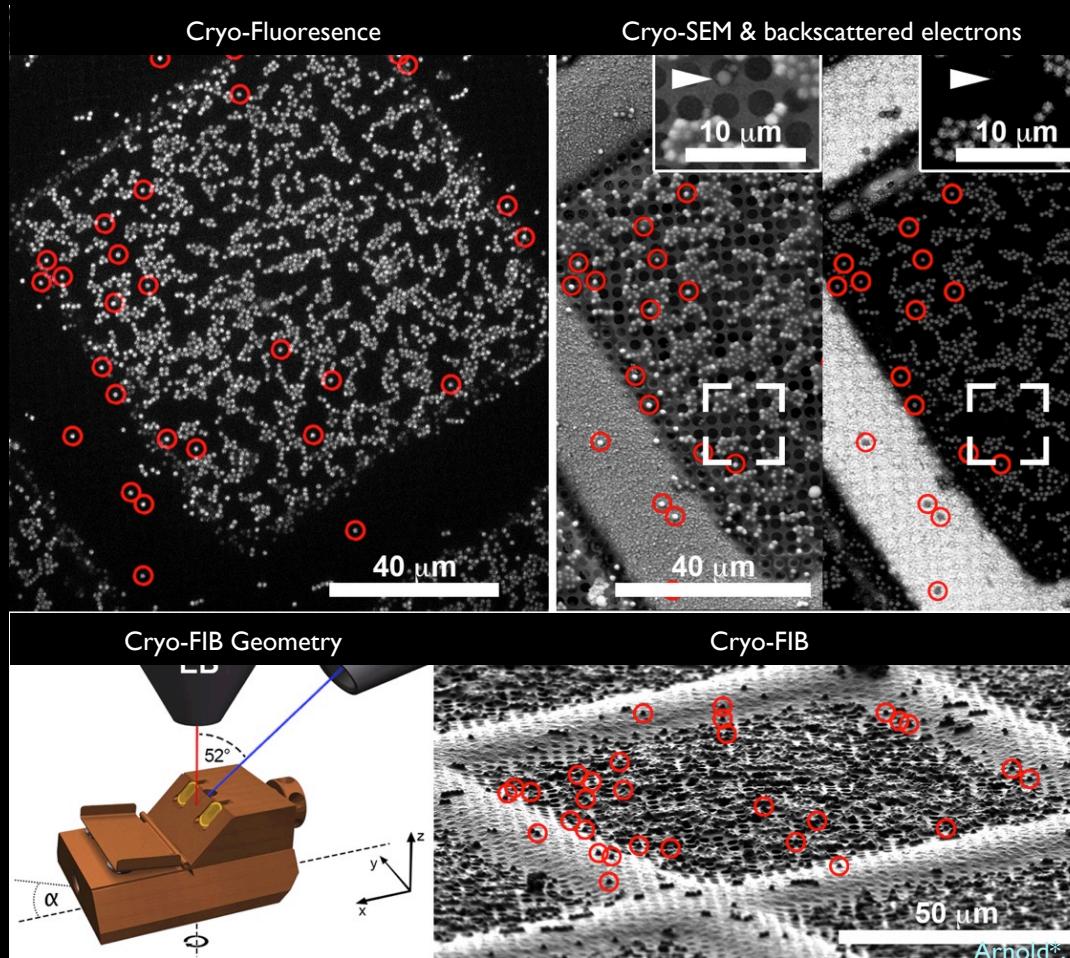


Whole cell volume : $300 \mu\text{m}^3 \rightarrow 0.1\% \text{ covered BUT } 99.9\% \text{ missing}$

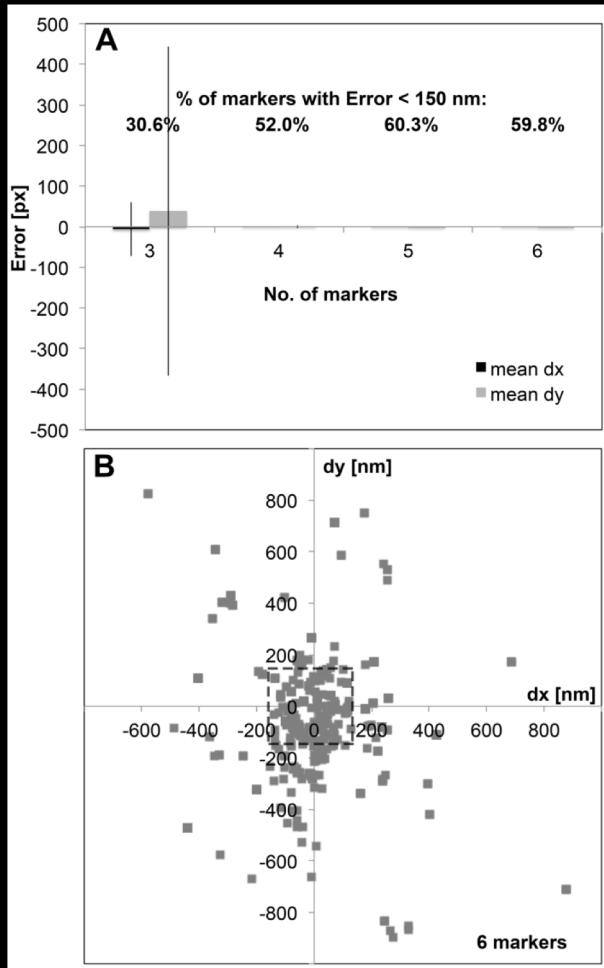


2 μm

3D Correlative Cryo-FLM & EM: Fiducial Markers

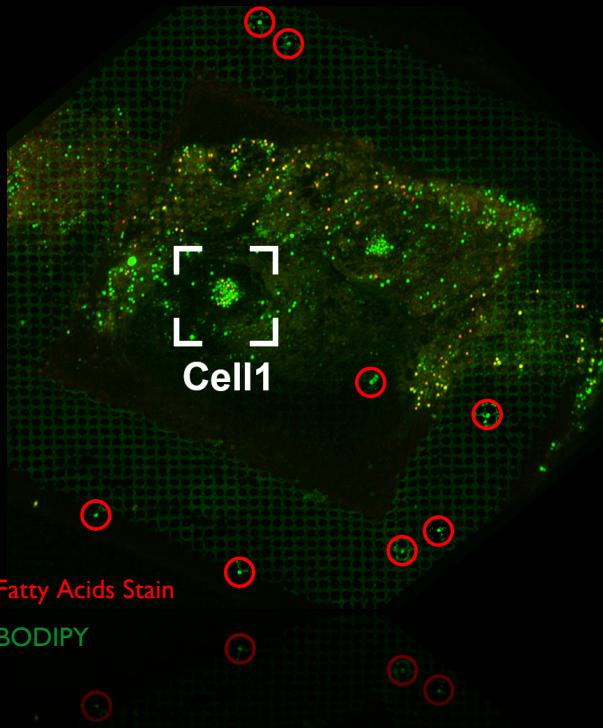


3D Correlative Cryo-FLM & EM: Correlation Accuracy

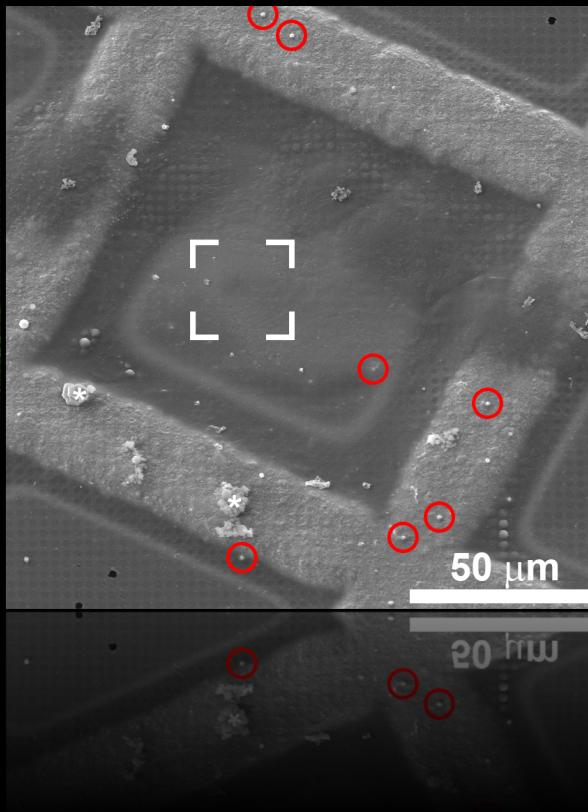


3D Correlative Cryo-FLM & EM: Targeted FIB Milling in Cellular Samples

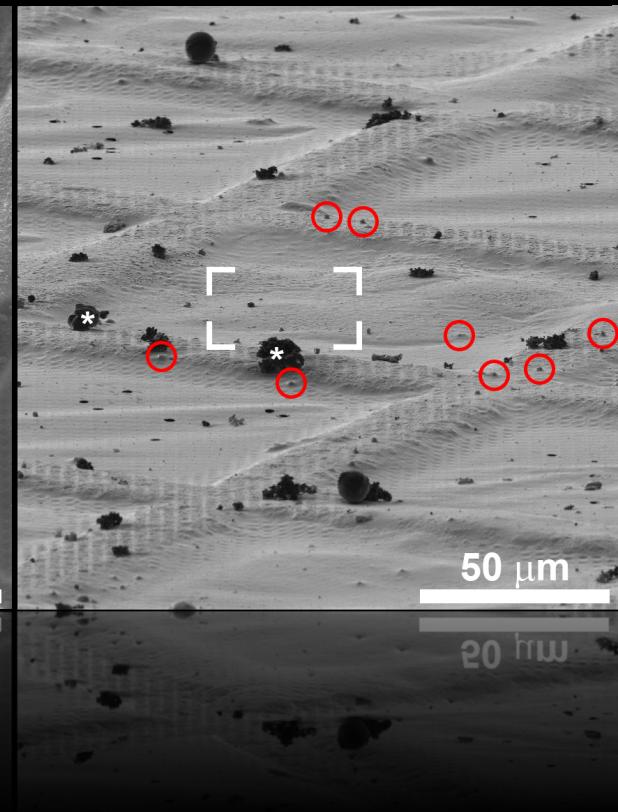
Cryo-fluorescence: MIP of spinning disk confocal



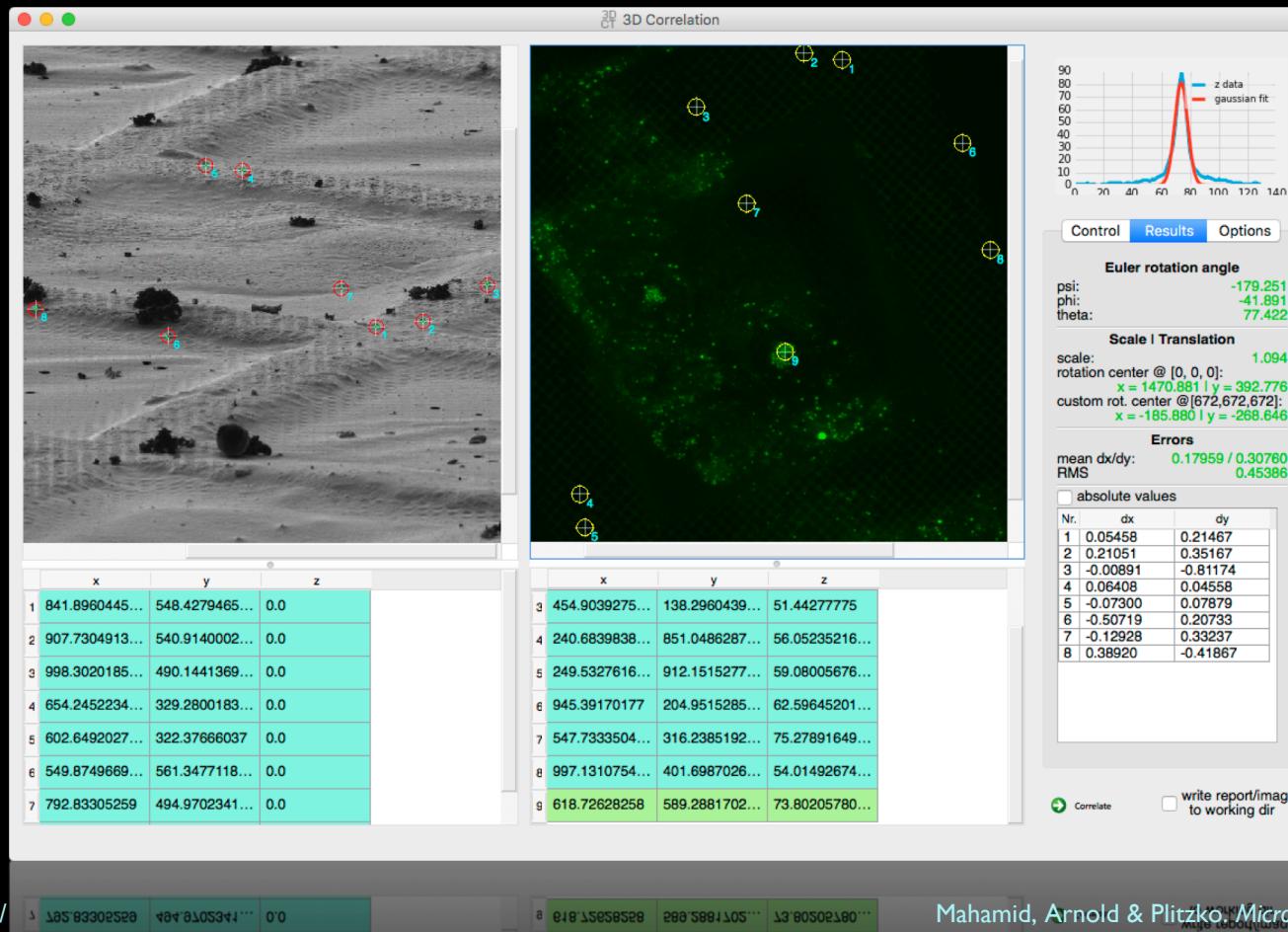
SEM view



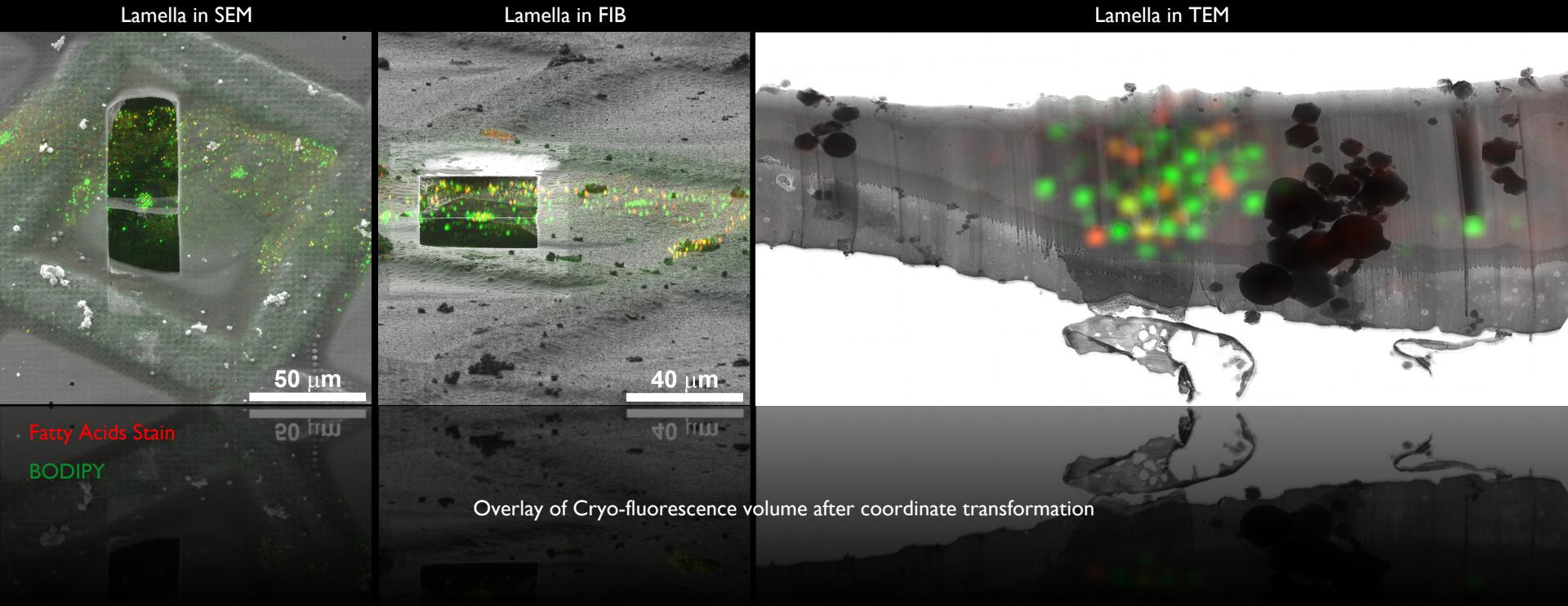
FIB view



3D Correlative Cryo-FLM & EM: Online Targeted FIB Milling

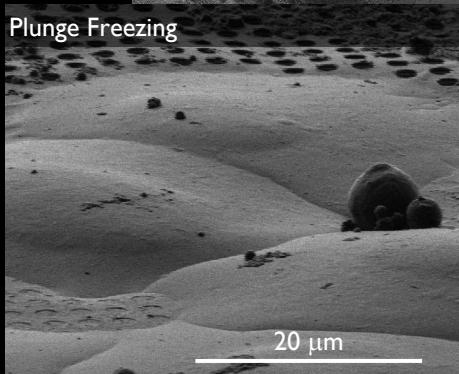


3D Correlative Cryo-FLM & EM: Targeted FIB Milling and TEM Navigation

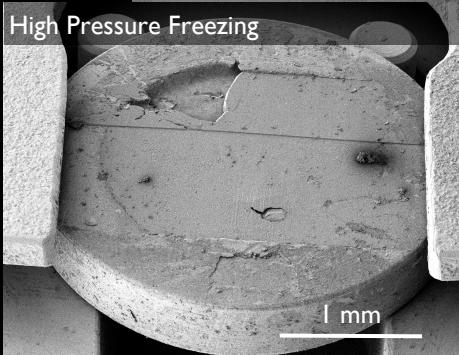


Most Important: VITRIFICATION

Plunge Freezing



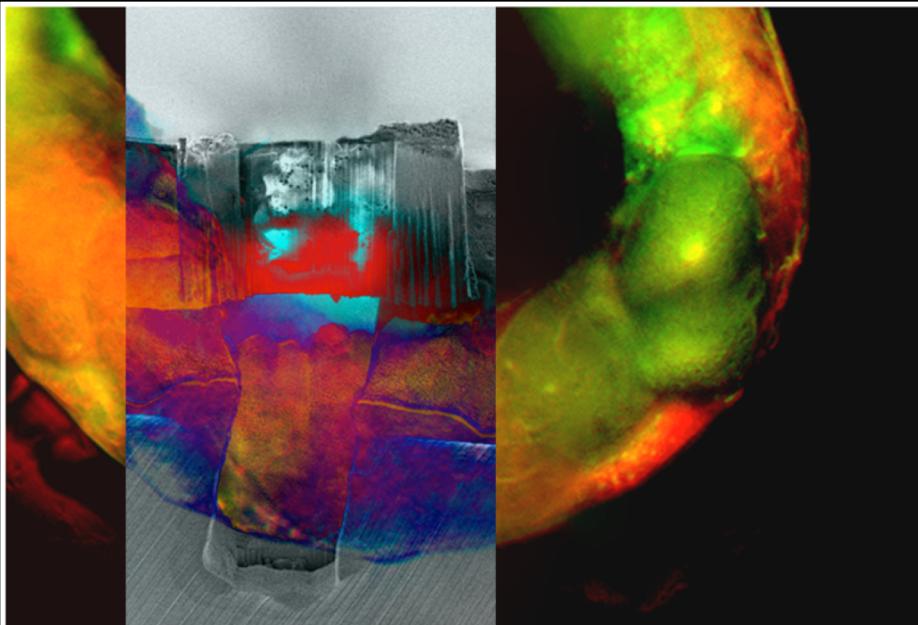
High Pressure Freezing



2 μm

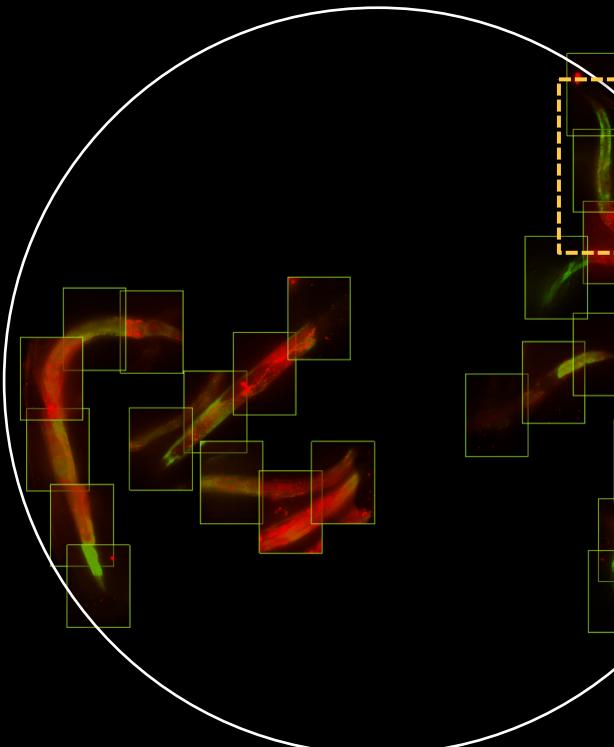


Cryo-FIB Lift-out: ‘Biopsies’ on the Micron Scale

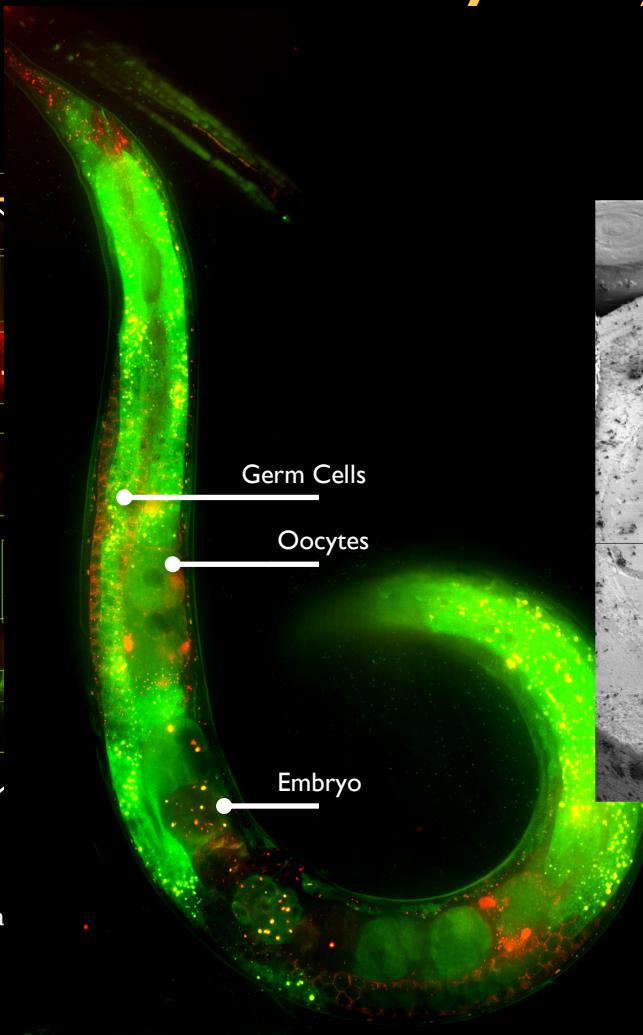


New & Improved Version:
Miroslava Schaffer, In Collaboration with Kleindiek

Cryo-FIB Lift-out: Guided by Cryo-Confocal LM

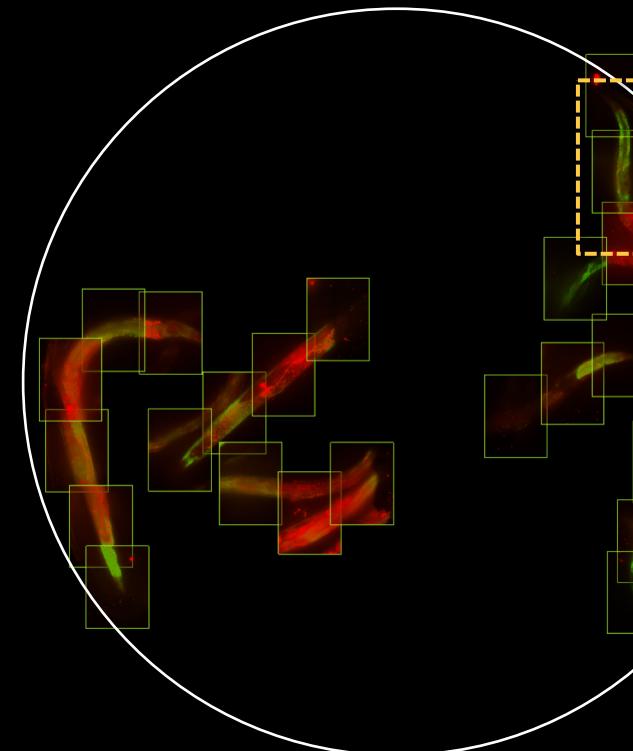


High pressure frozen *C. elegans* worms – regular PLA
Cryo-planed – Ultramicrotomy
Cryo-Confocal fluorescence

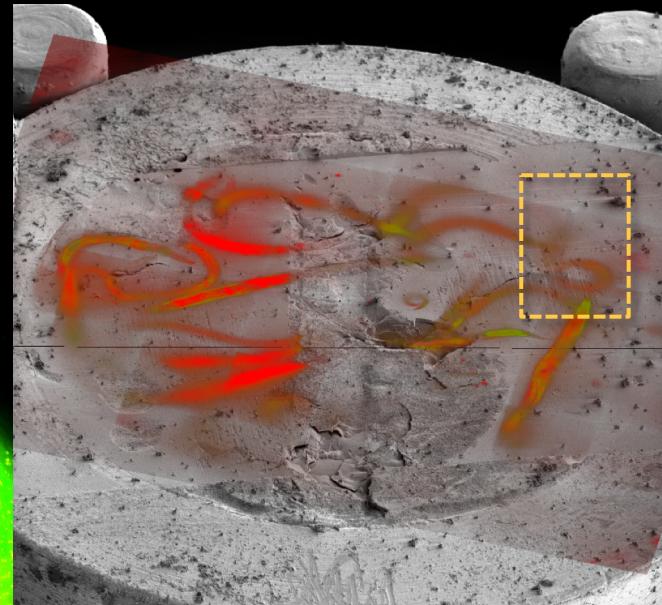
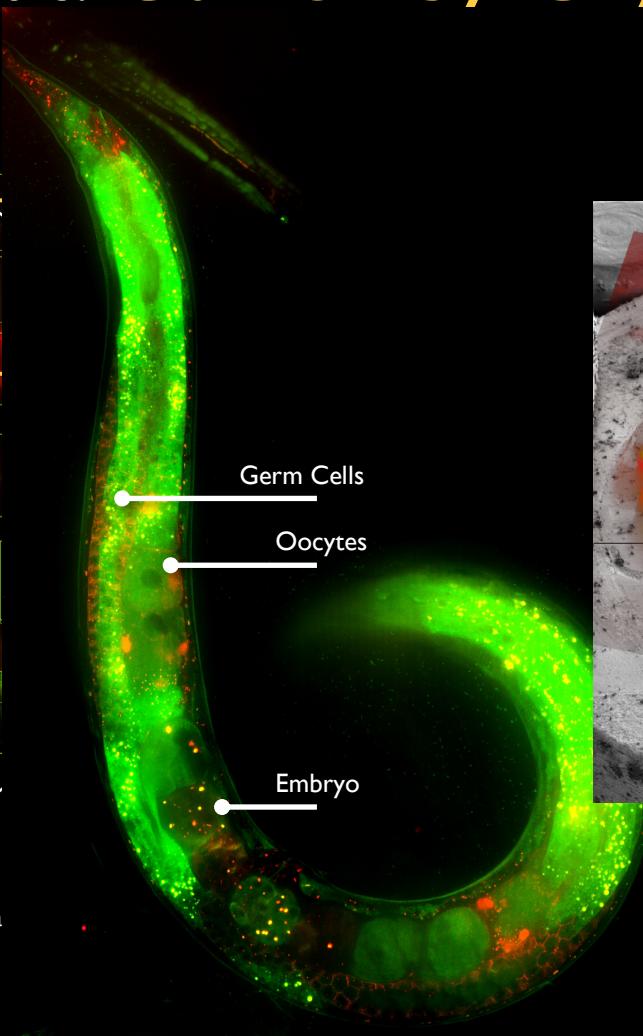


2D Correlation

Cryo-FIB Lift-out: Guided by Cryo-Confocal LM



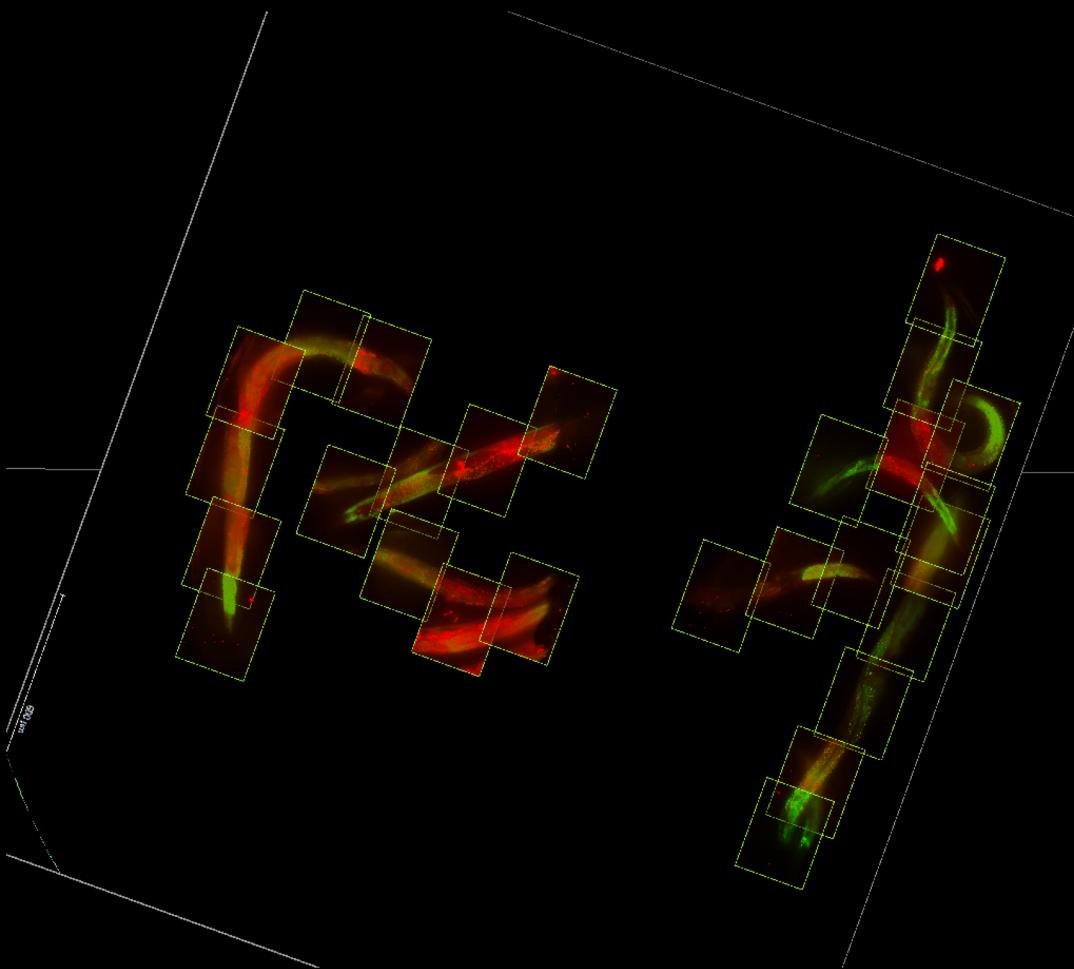
High pressure frozen *C. elegans* worms – regular PLA
Cryo-planed – Ultramicrotomy
Cryo-Confocal fluorescence



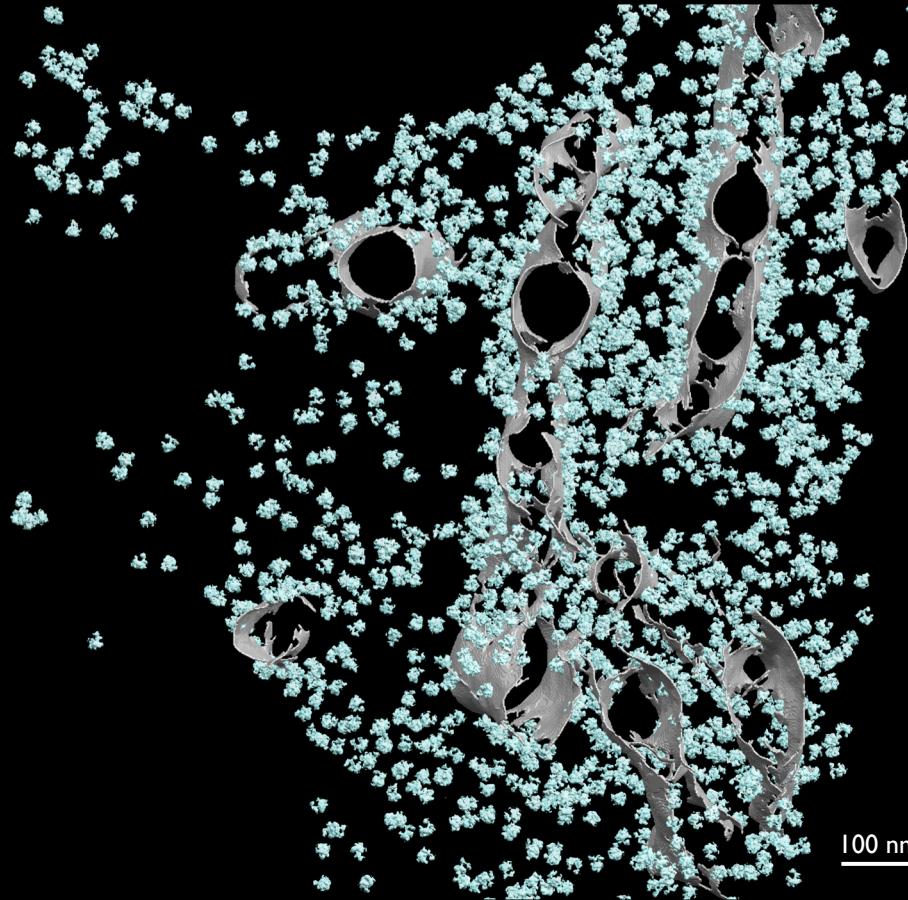
1 mm

2D Correlation

Cryo-FIB Lift-out: The Magic

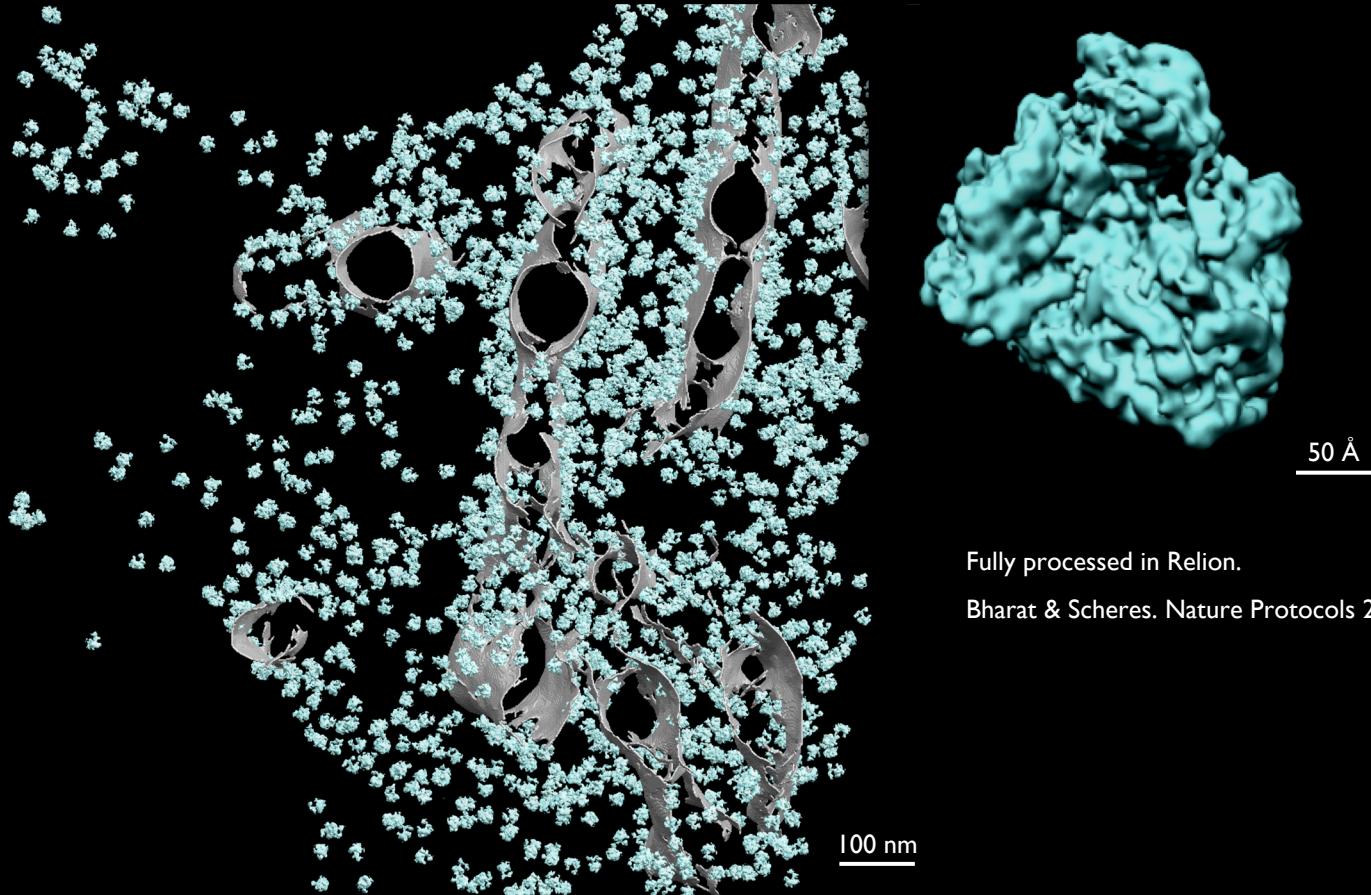


Cryo-FIB Lift-out: *C. elegans* Molecular Landscapes



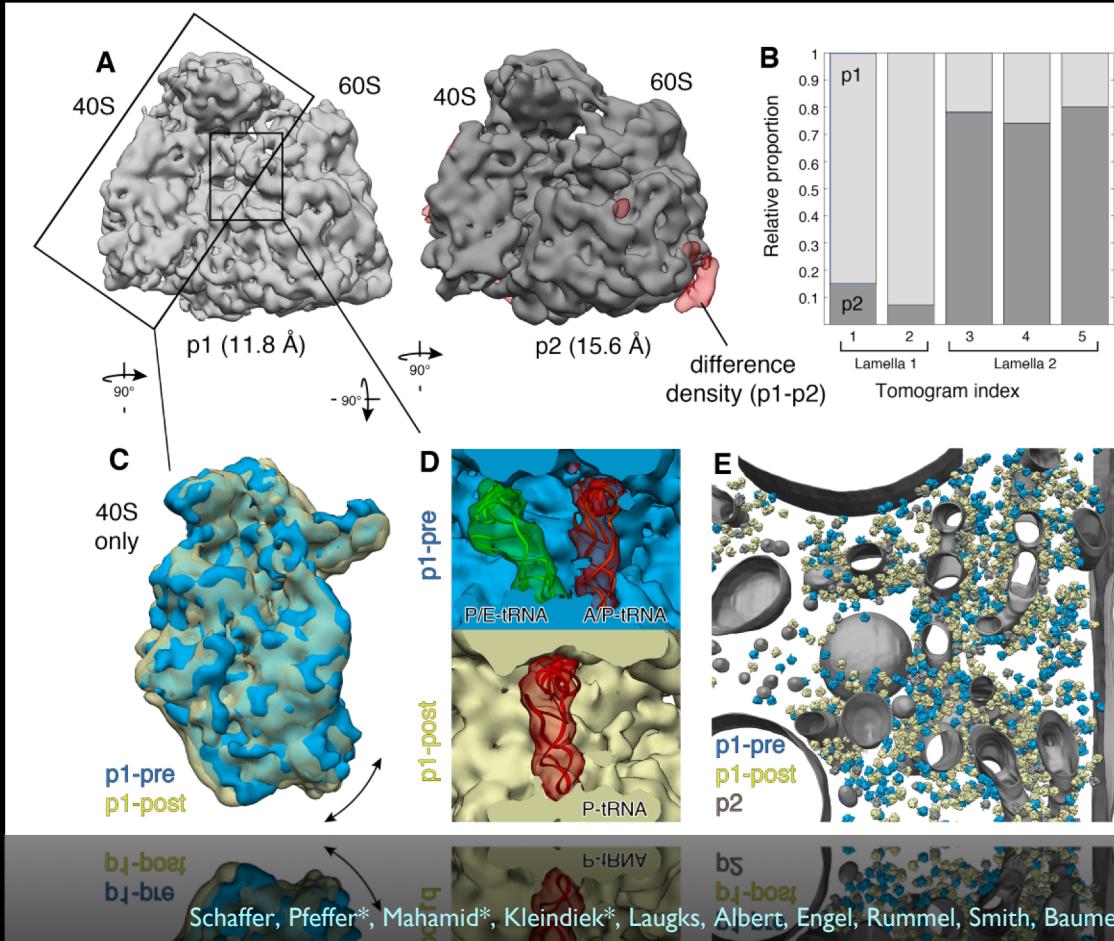
Titan Krios, 300 kV
K2 direct detector
Pixel size: 0.34 nm
Tilt range: 30° to -60°
Target defocus: 5 µm
Series total dose: 100 e-/Å²

Lift-Out Lamellae Provide Molecular-resolution: 80S *C. elegans* Ribosomes

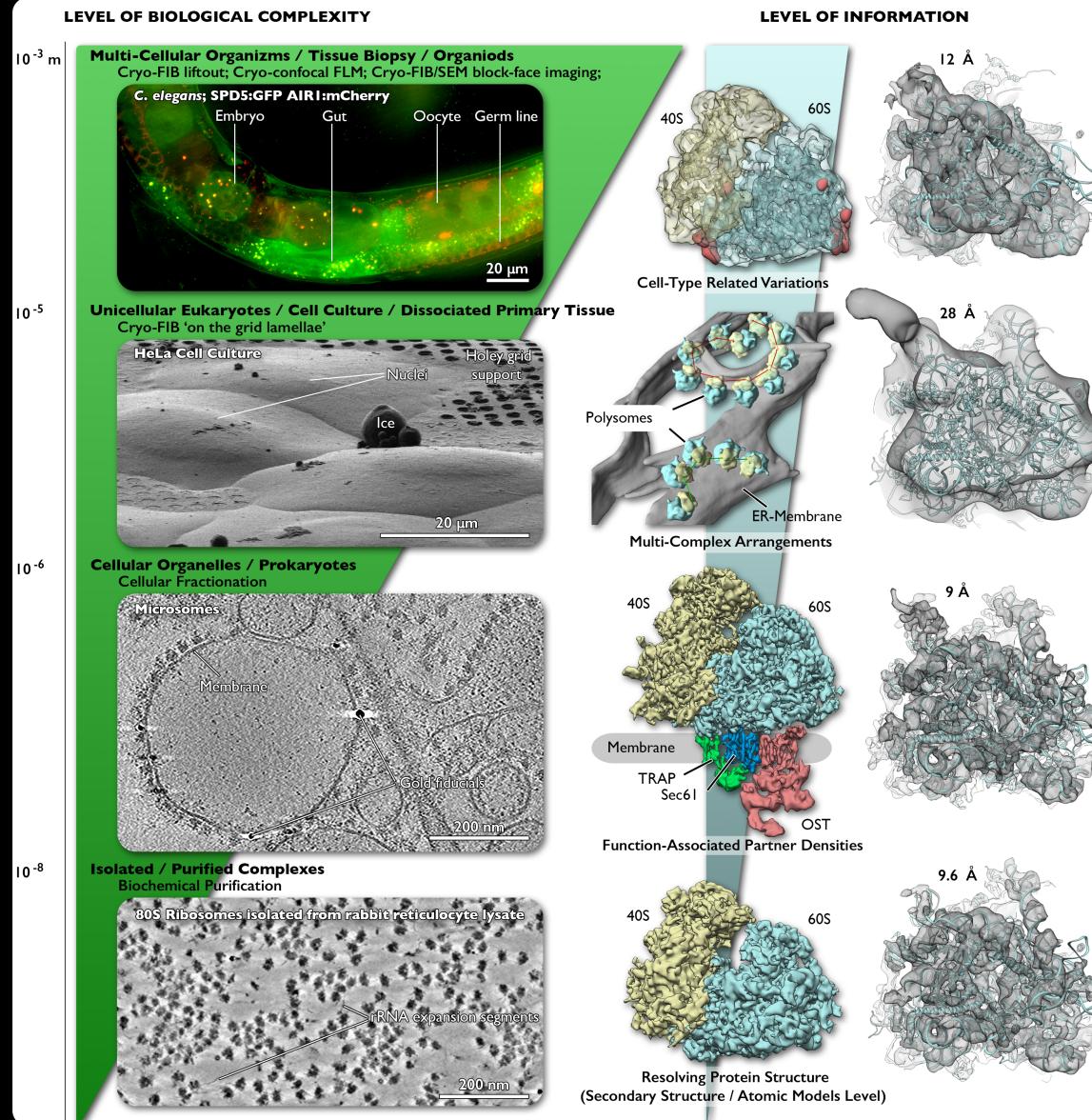


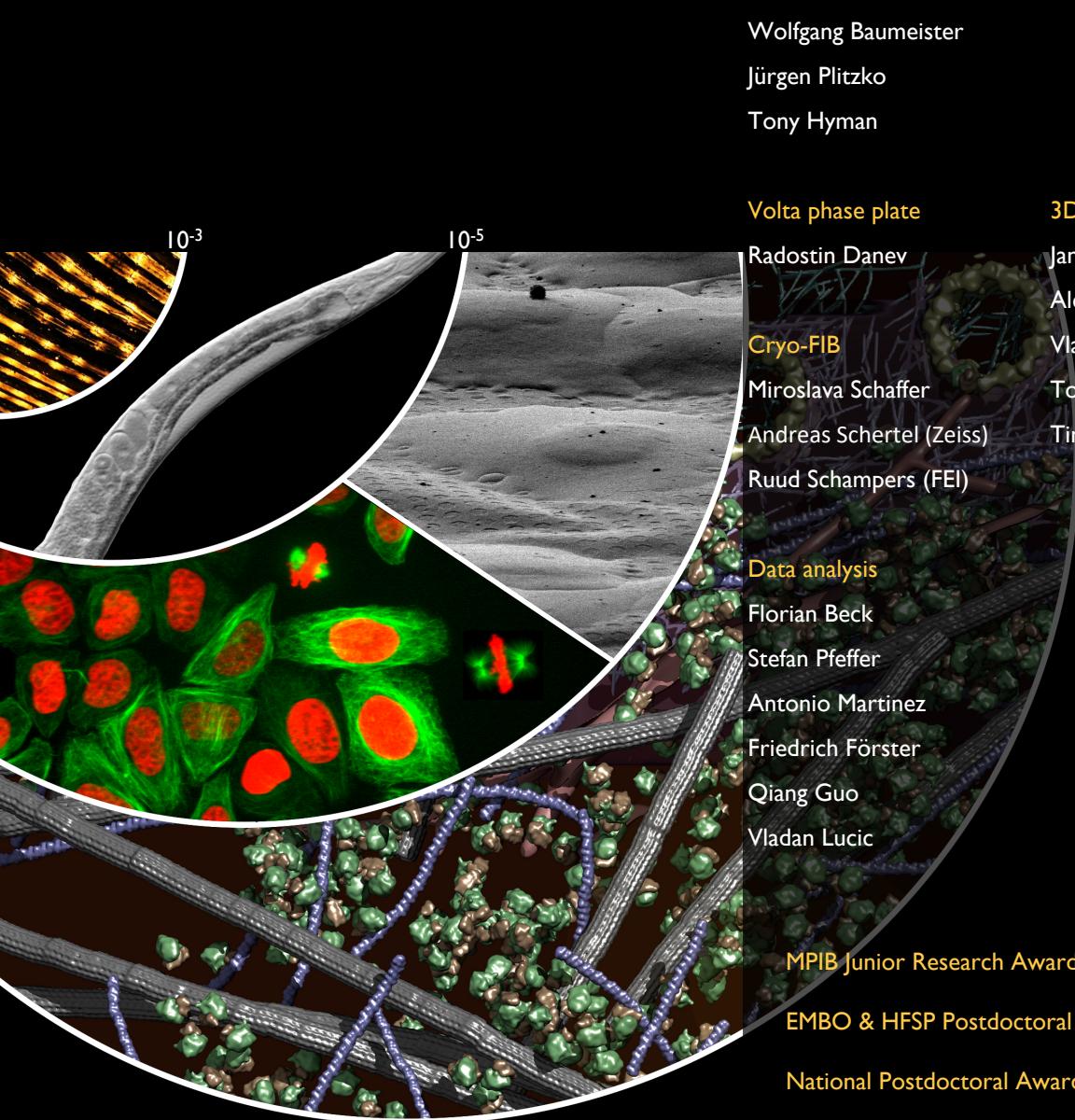
Fully processed in Relion.
Bharat & Scheres. Nature Protocols 2016

Lift-Out Lamellae Provide Molecular-resolution: 80S *C. elegans* Ribosomes



Schaffer, Pfeffer*, Mahamid*, Kleindiek*, Laugks, Albert, Engel, Rummel, Smith, Baumeister & Plitzko. *Nature Methods* 2019.

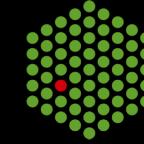




Wolfgang Baumeister

Jürgen Plitzko

Tony Hyman



Volta phase plate

Radostin Danev

Cryo-FIB

Miroslava Schaffer

Andreas Schertel (Zeiss)

Ruud Schampers (FEI)

Data analysis

Florian Beck

Stefan Pfeffer

Antonio Martinez

Friedrich Förster

Qiang Guo

Vladan Lucic

MPIB Junior Research Award

EMBO & HFSP Postdoctoral Fellowships

3D Correlation

Jan Arnold

Alex de Marco

Vladan Lucic

Tobias Mayer

Tim Laugks

My Group

Edoardo D'Imprima

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Mauricio Toro-Nahuelpan

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Xiaojie Zhang

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Leica for Cryo-CLEM