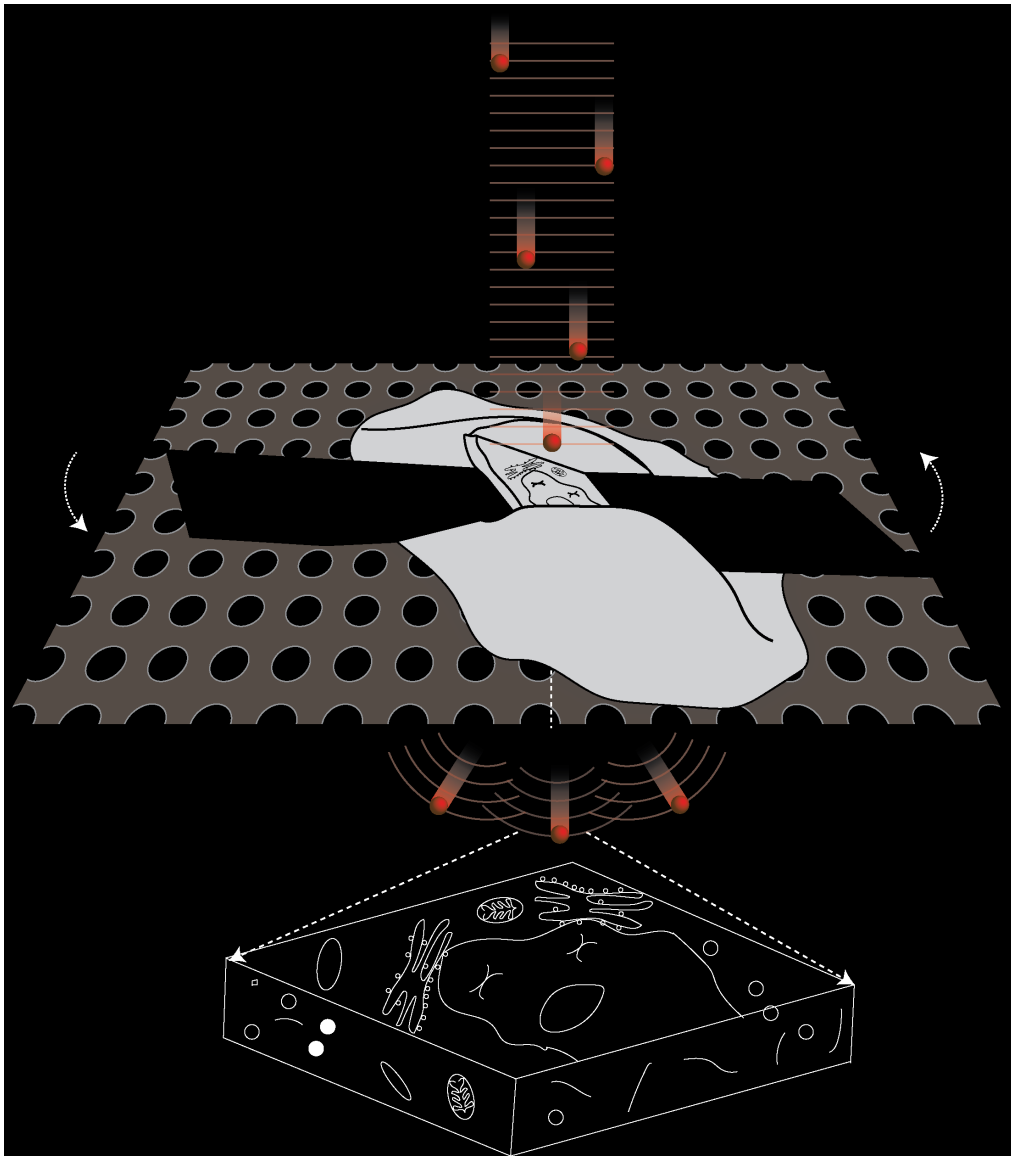
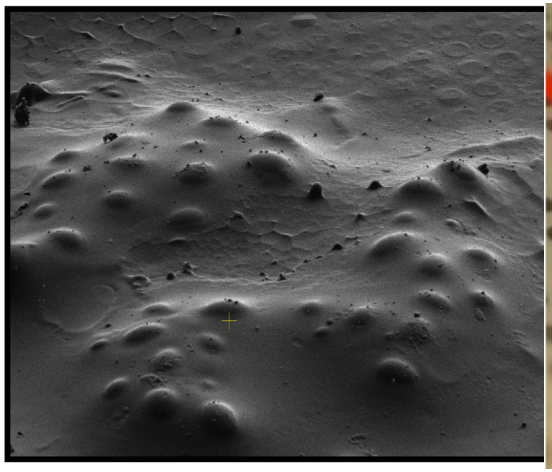
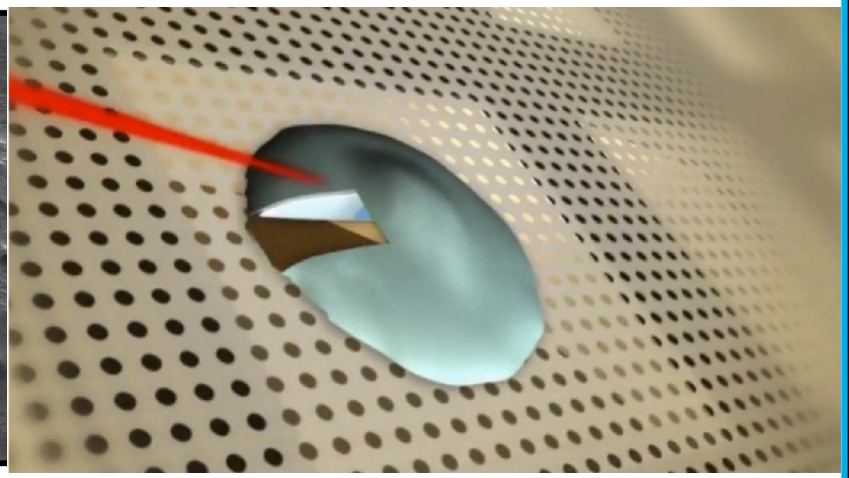


Cryo focused ion beam (FIB) milling of biological samples

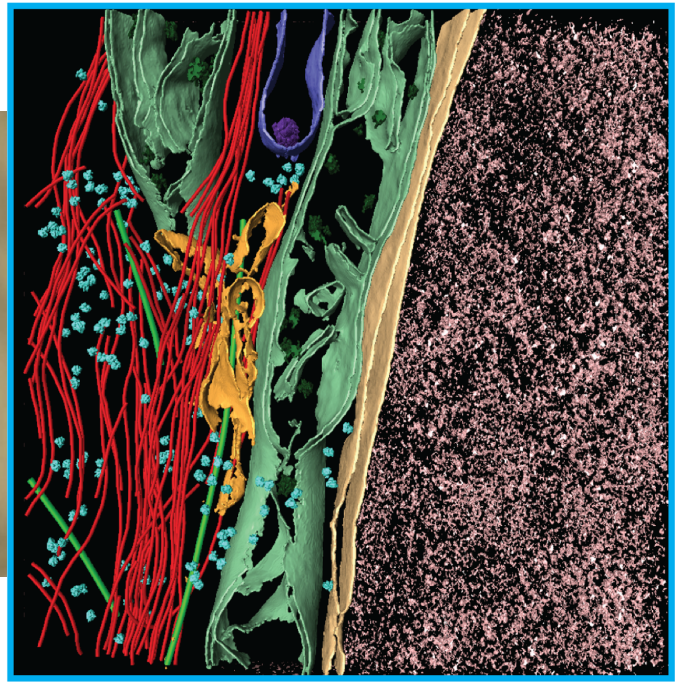
by:
Digvijay Singh
Damon Runyon Fellow at Villa Lab

Cryo-Focused ion beam (cryo-FIB) milling for opening windows into cells





Thermo Fisher



Vitrification

Rapid-freeze cells in culture to preserve structures

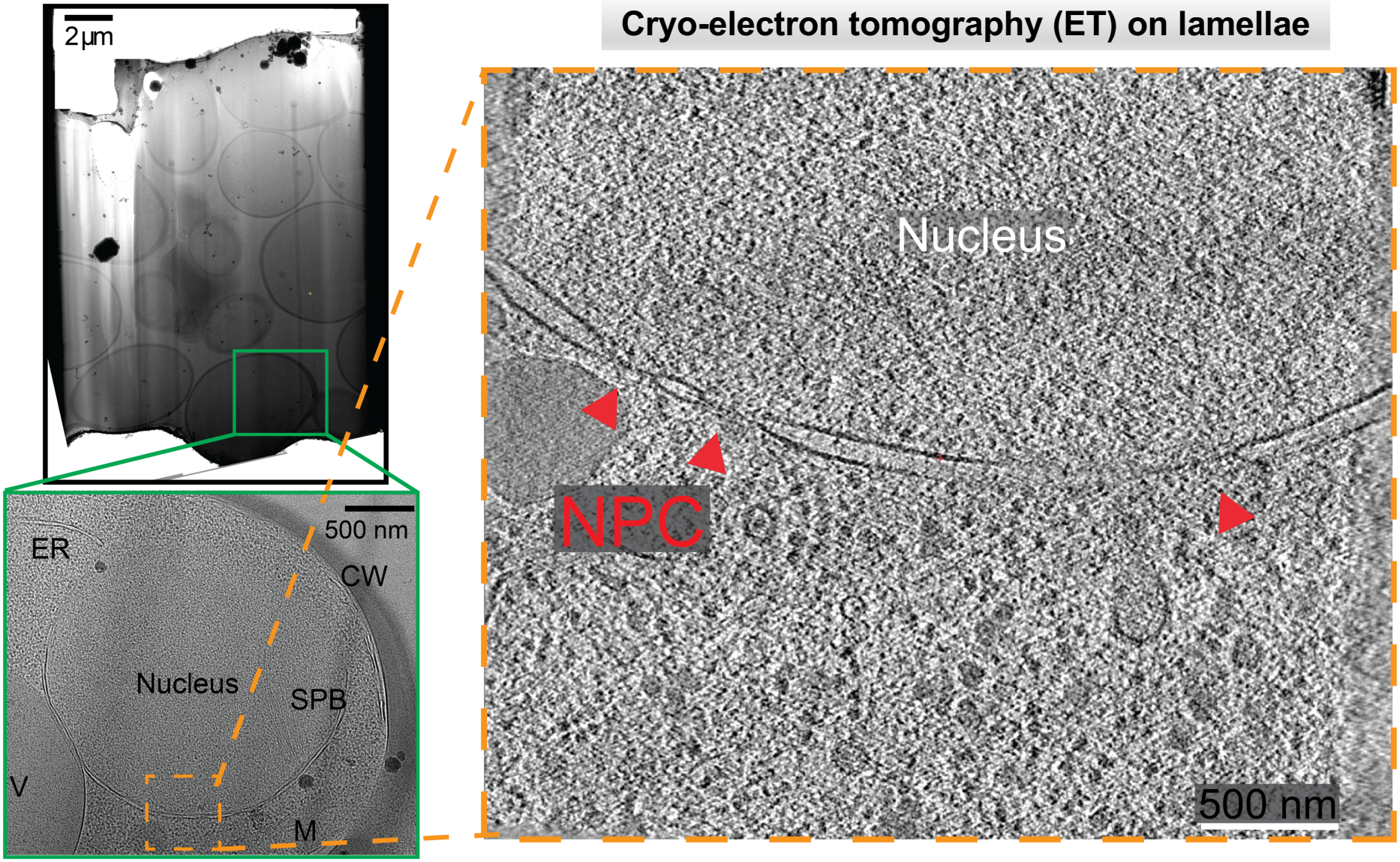
Cryo-FIB milling

Micro-machine thin lamella out of cell(s)

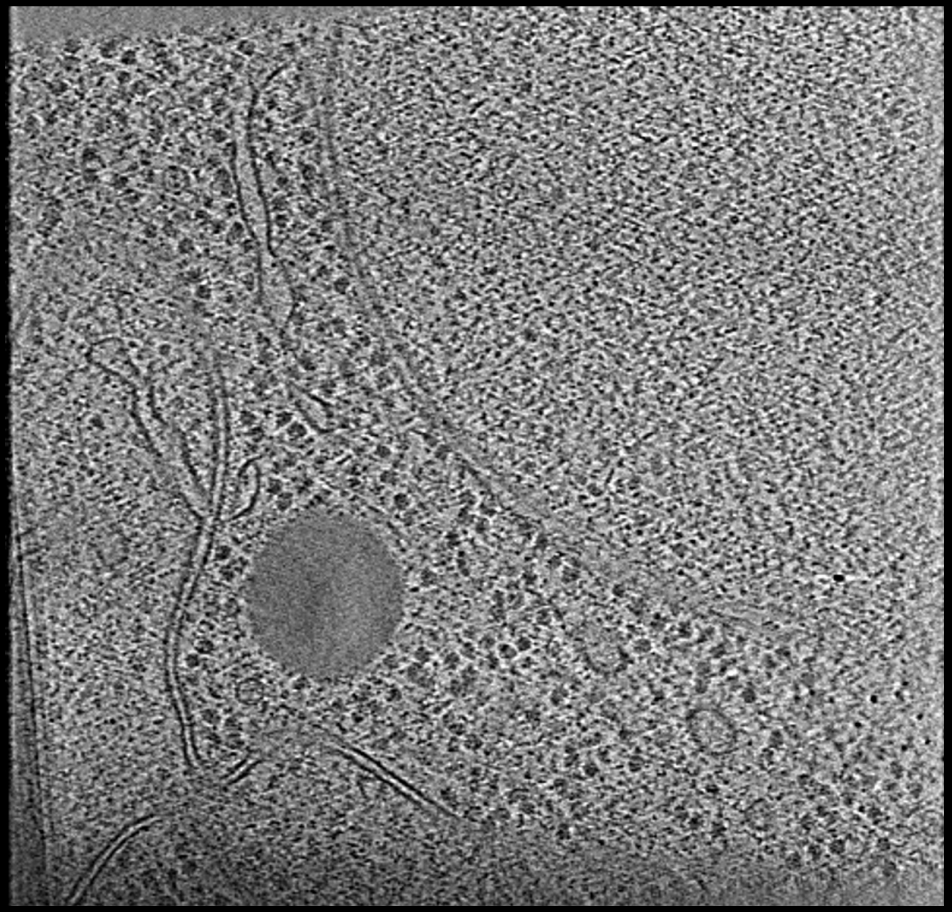
Cryo-electron tomography (cryo-ET)

3D reconstruction of molecular landscapes in-situ

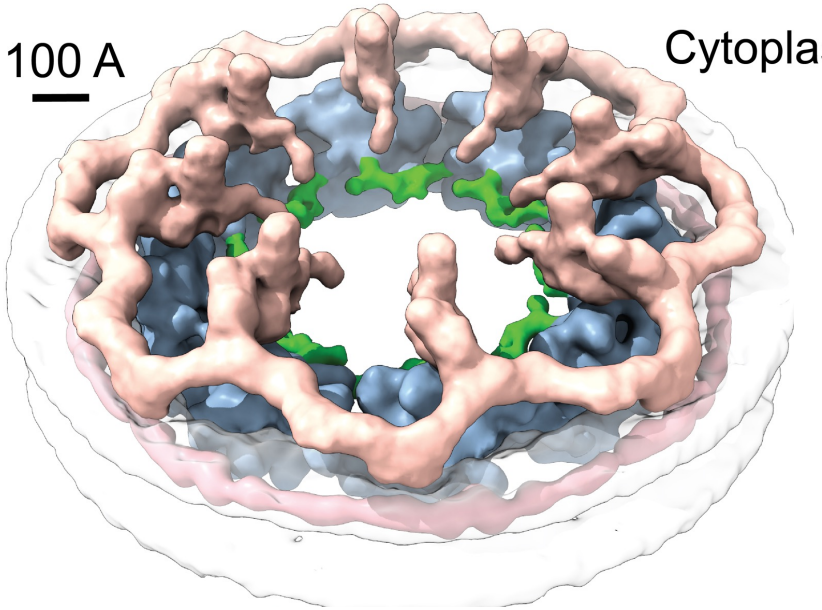
Wagner, Watanabe, Schampers, **Singh**, Persoon, Schaffer, Fruhstorfer, Plitzko & Villa.
Nature Protocols.


Cryo-electron tomography (ET) on lamellae
2 µm
500 nm
ER
CW
Nucleus
SPB
V
M
Nucleus
NPC
500 nm

Molecular structures in cellular context

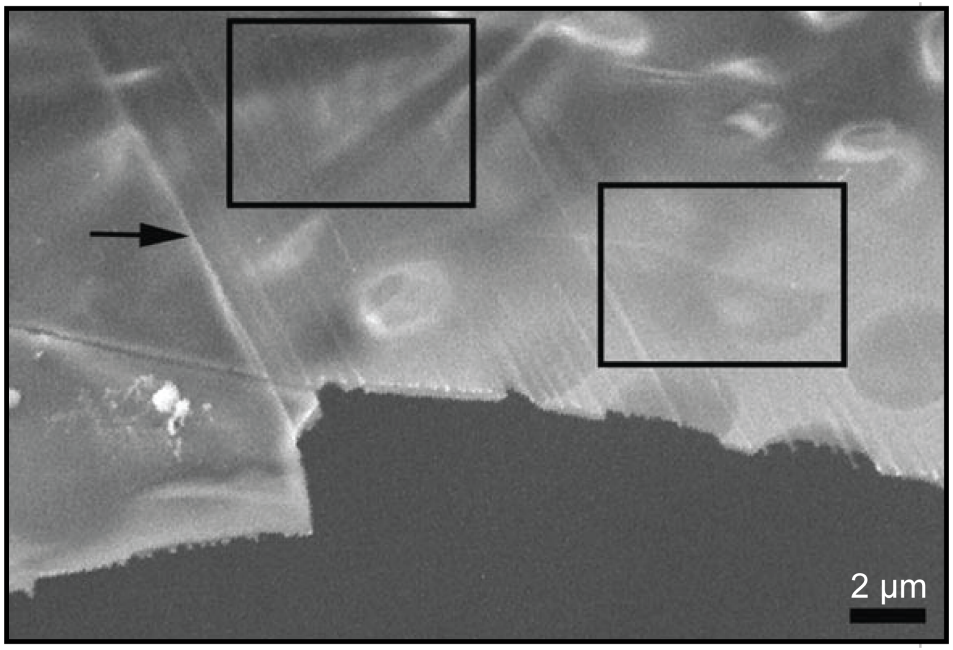


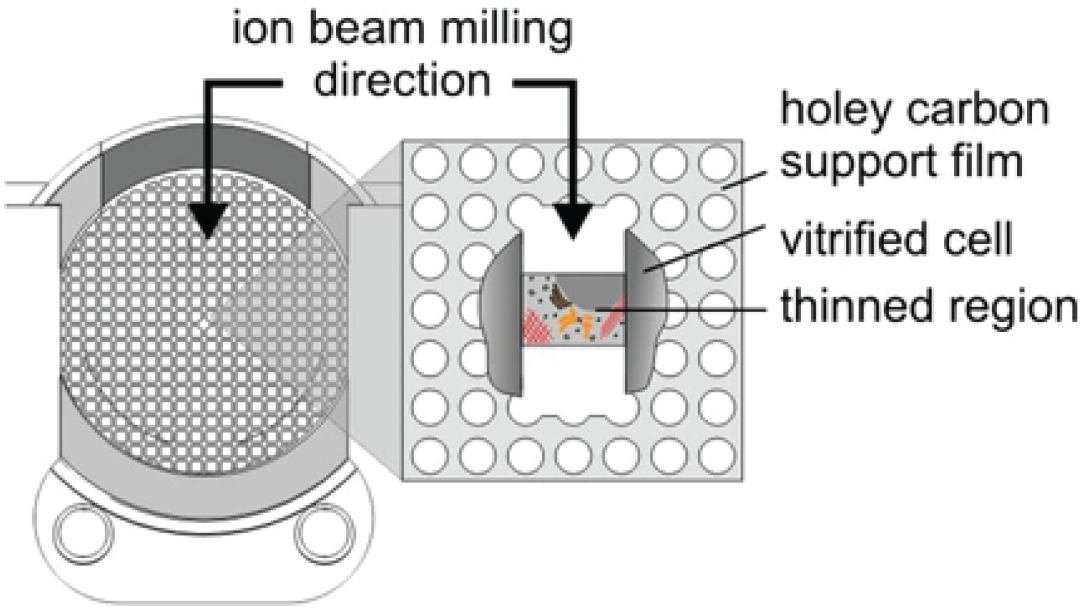
Co-axial rings of the Nuclear Pore complex



Akey, Singh, Ouch, Echeverria *et al.* Cell (2022)

A short (& incomplete) history of cryo-FIB milling





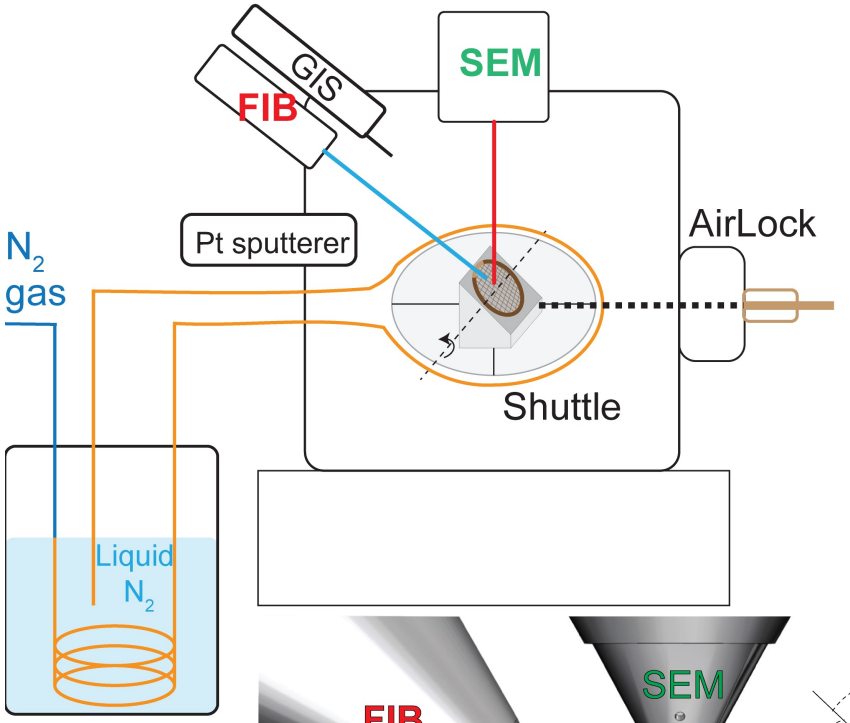
Marko *et al.* Nature Methods (2007)

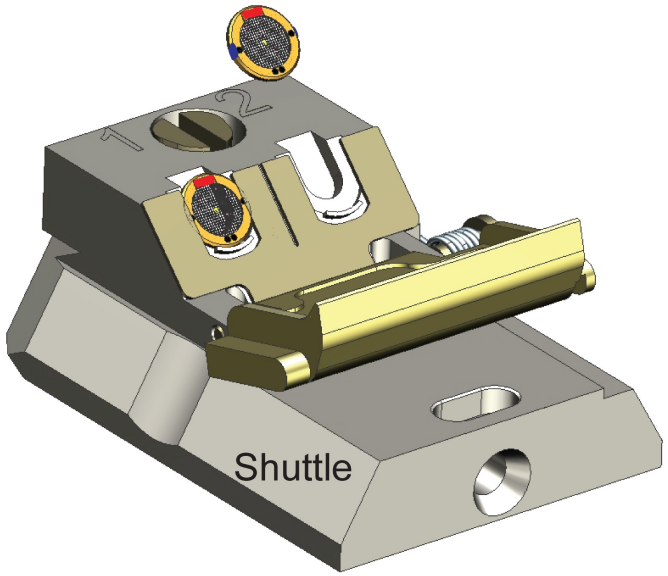
Rigort *et al.* PNAS (2012)

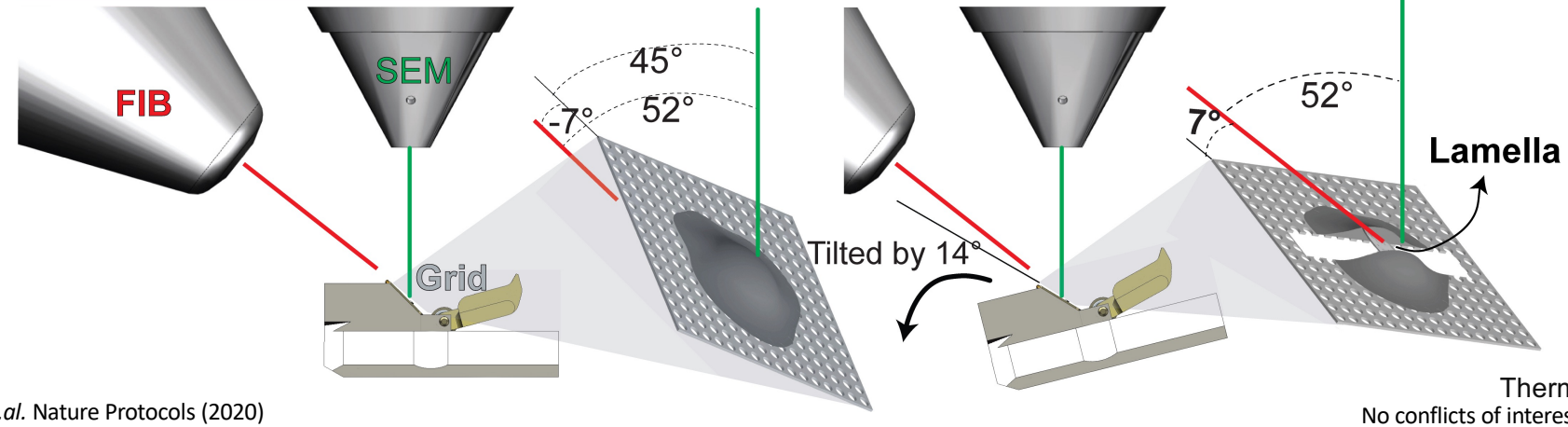
Wang *et al.* J. Struct. Biol. (2012)

De Winter *et al.* J. Struct. Biol. (2013)

Schematic of dual-beam for cryo-FIB milling



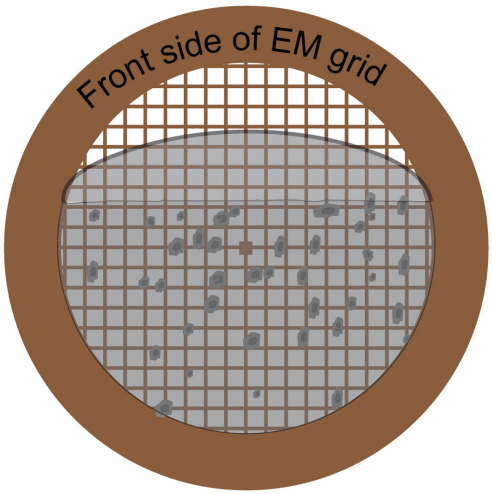


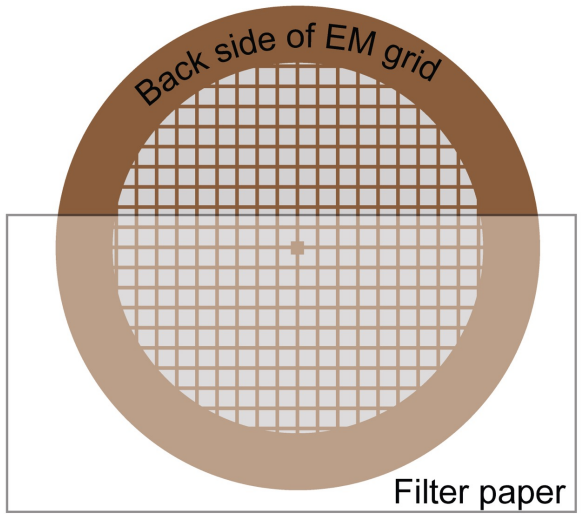


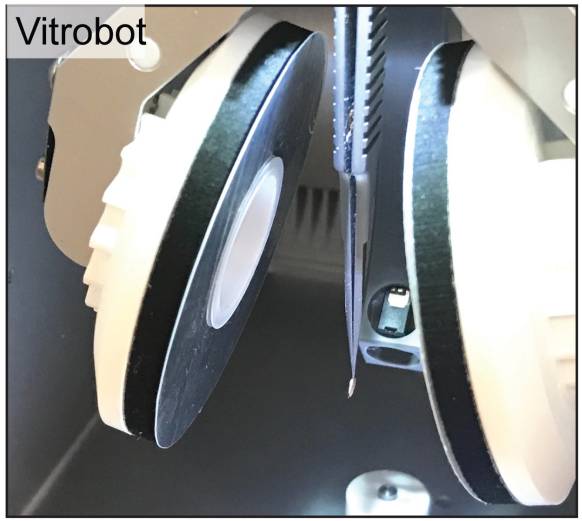
Wagner, Watanabe *et.al.* Nature Protocols (2020)

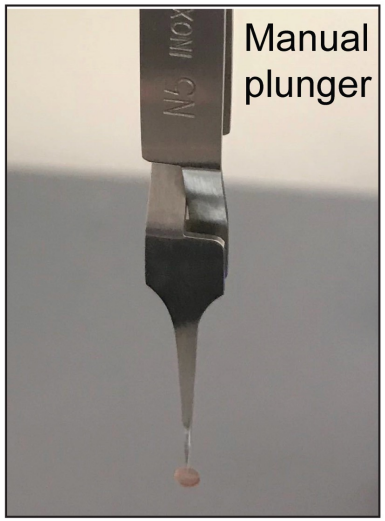
Thermo Fisher
No conflicts of interest with them

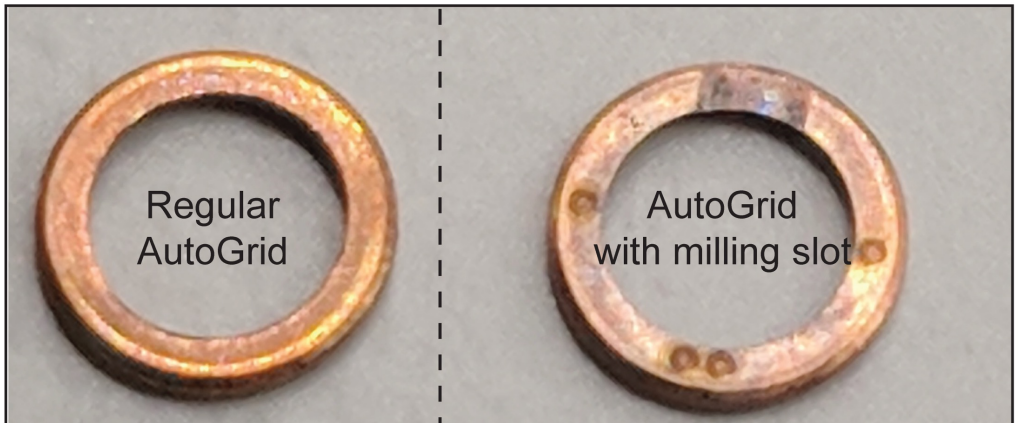
Vitrification of cellular samples for cryo-FIB milling

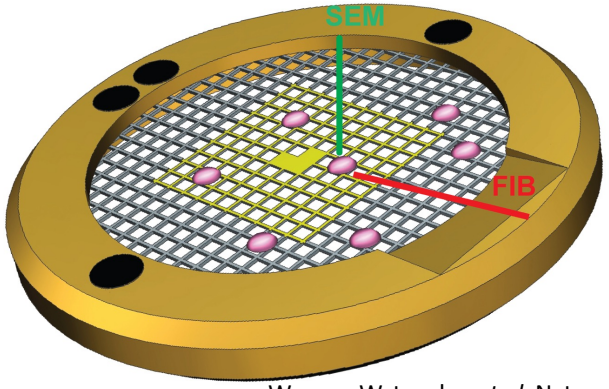






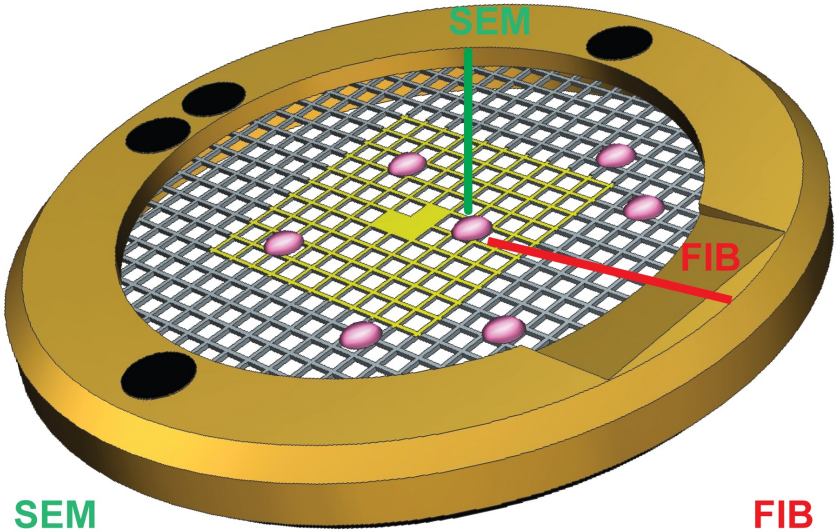




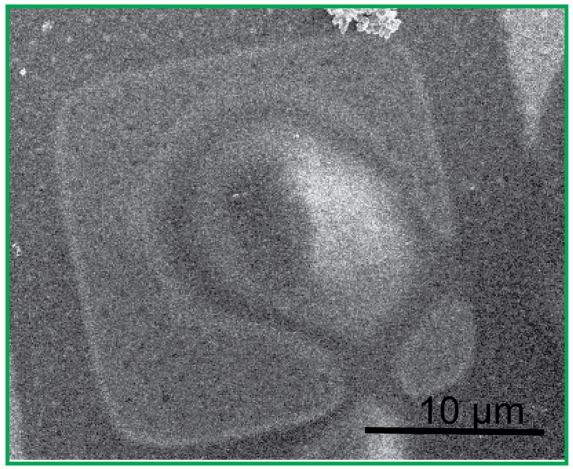


Wagner, Watanabe *et.al.* Nature Protocols (2020)

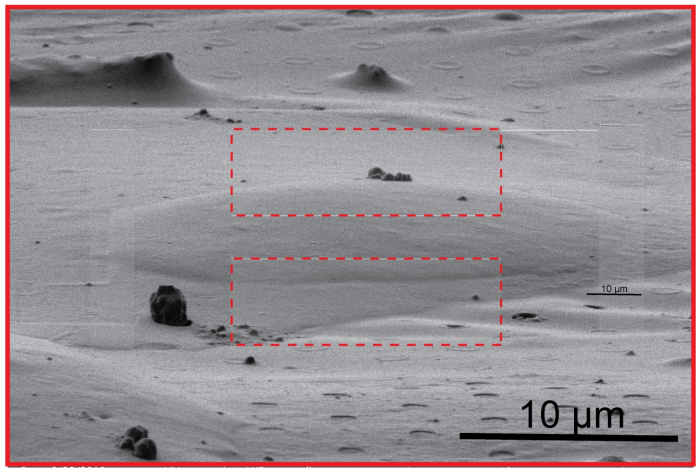
SEM and FIB views



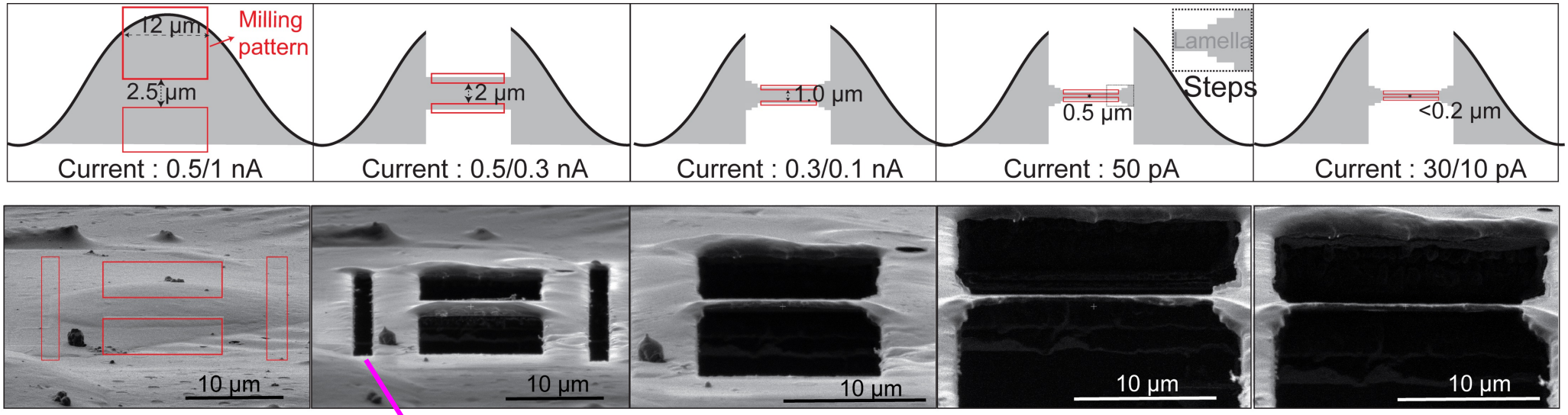
SEM



FIB



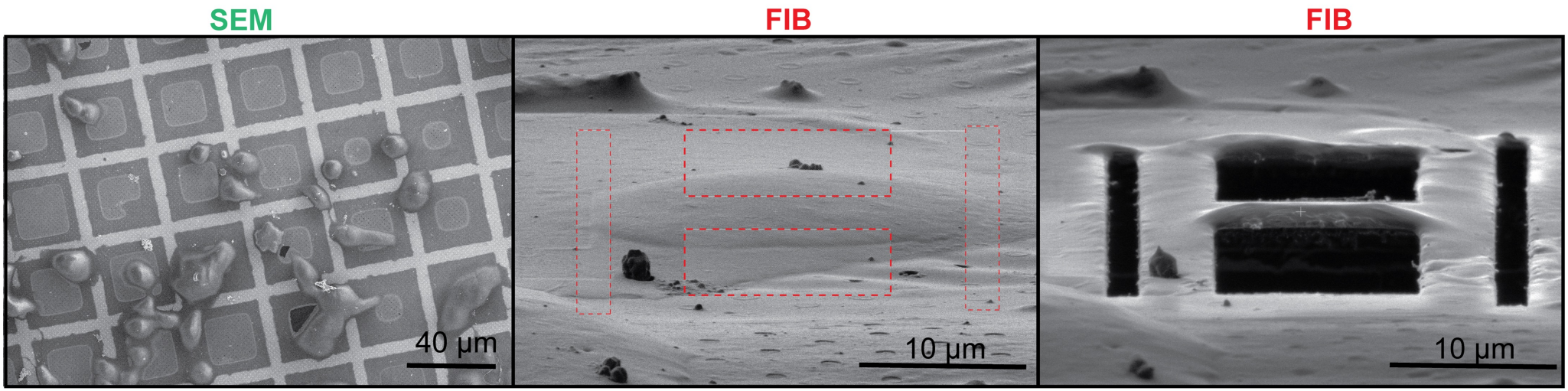
Progressive cryo-FIB milling for fragile cellular samples



Expansion joints help relieve stresses in the lamella and improves its stability

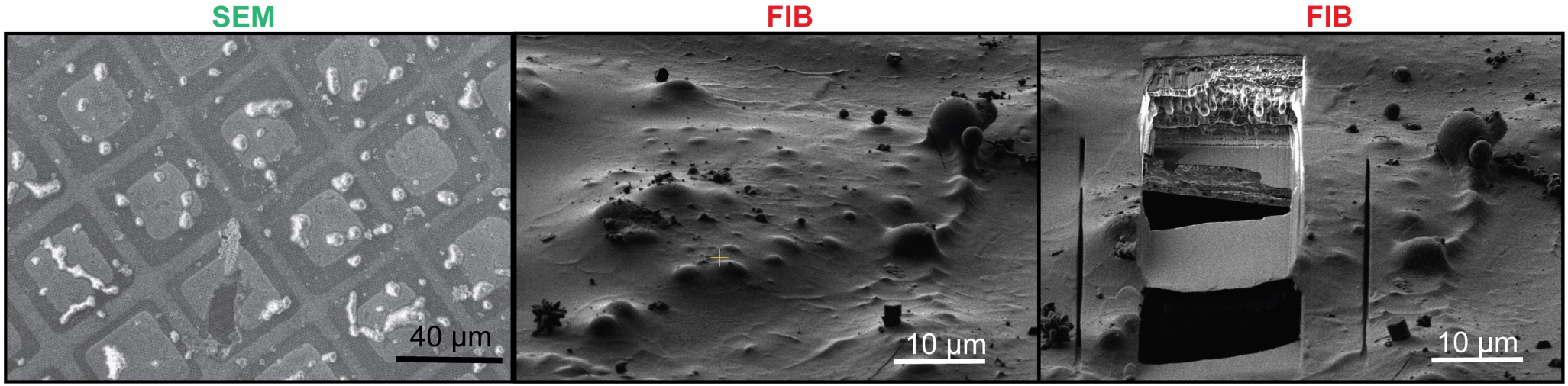
Wolff *et.al. J. Struct. Biol.* (2019)

Ideal mammalian cell samples for cryo-FIB



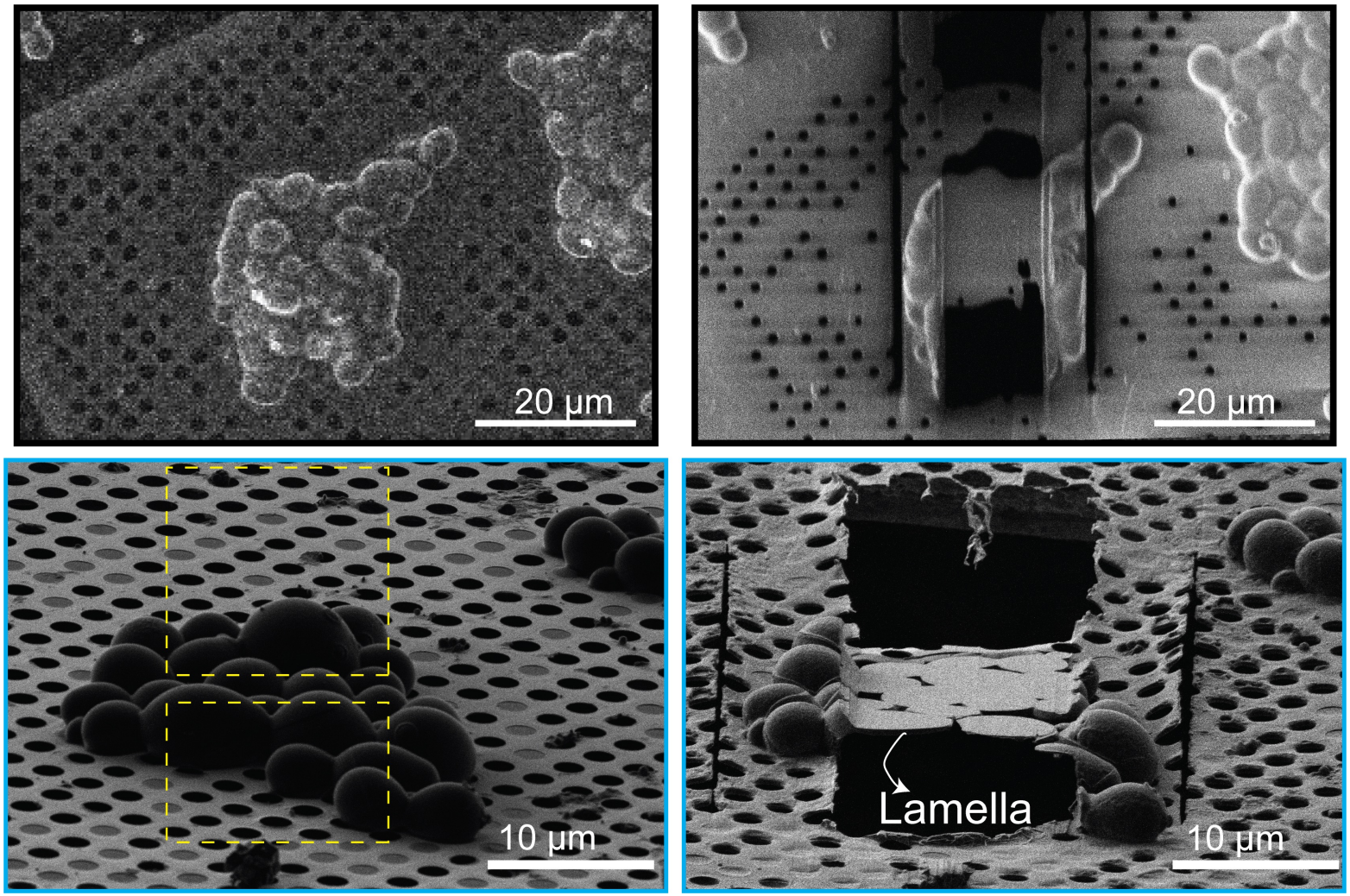
- Ideal density ~1 cell in middle of grid squares.
- Cells near grid bars can't be milled.
- Balance between good vitrification & hydration.
- Bigger clump of cells (>1) likely to have bad vitrification deep-down.

Ideal yeast samples for cryo-FIB

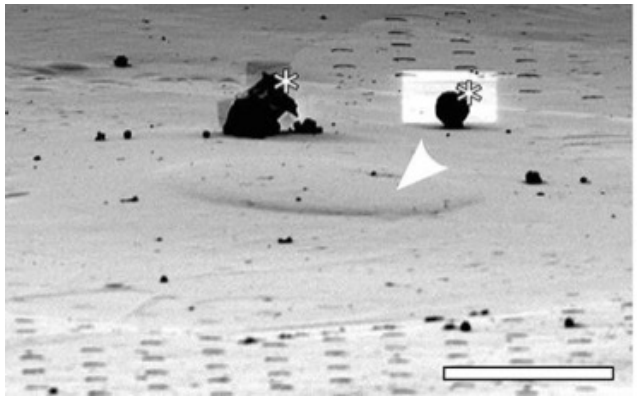


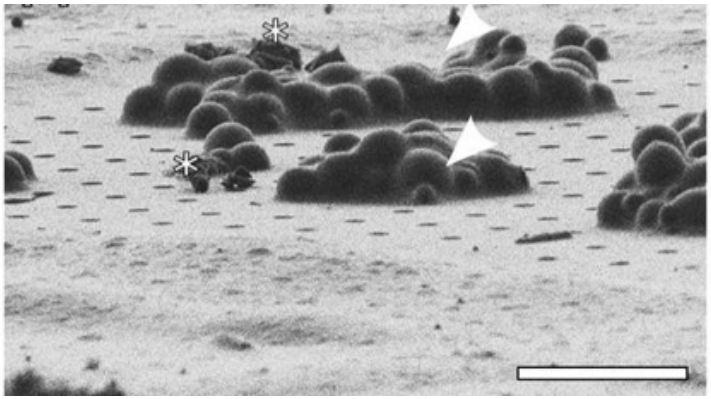
- Ideal density: clumps of 7-20 cells in middle of grid squares.
- Yeast clumps ’protrude’ out of the grid surface, making their identification in FIB view easier.
- Certain cells with cell-wall can withstand low-hydration.

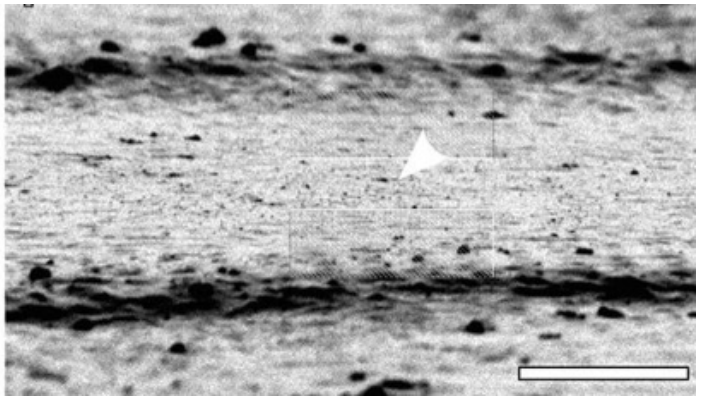
Cells with cell-wall provide wider blotting range



Mammalian vs. Yeast vs. Bacteria



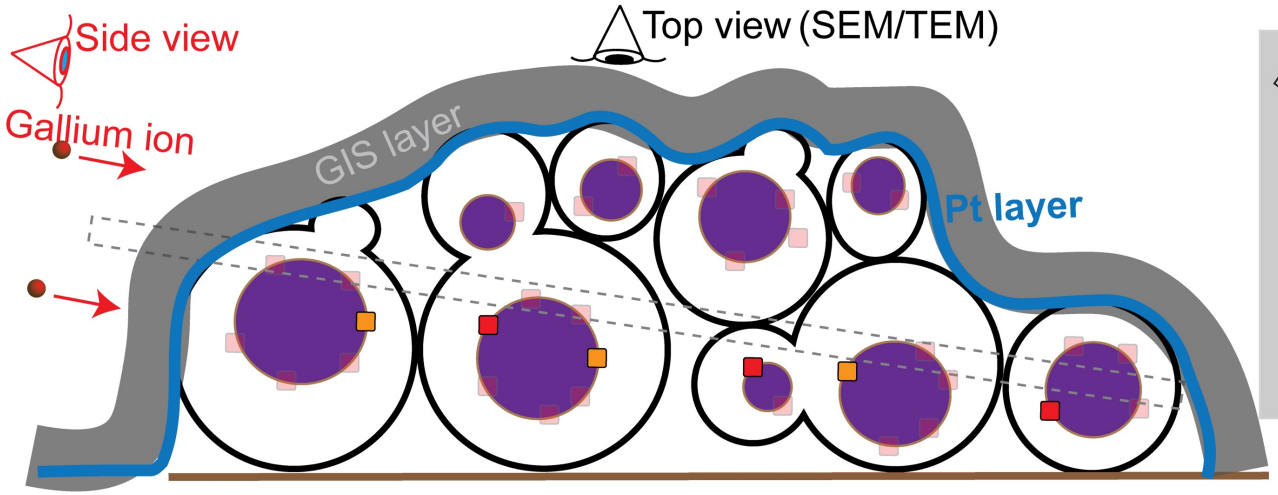


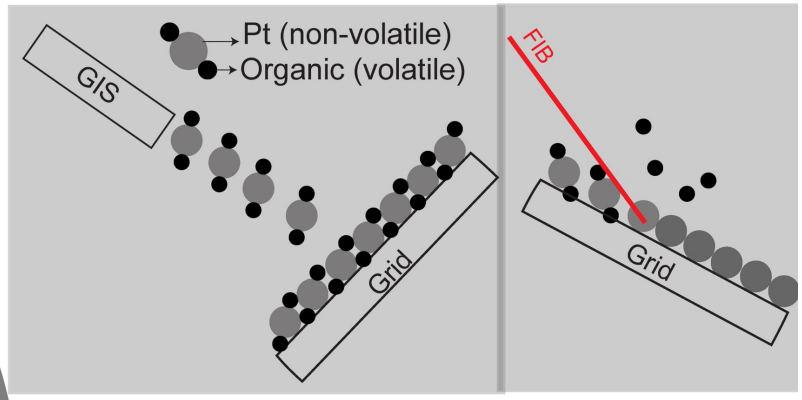


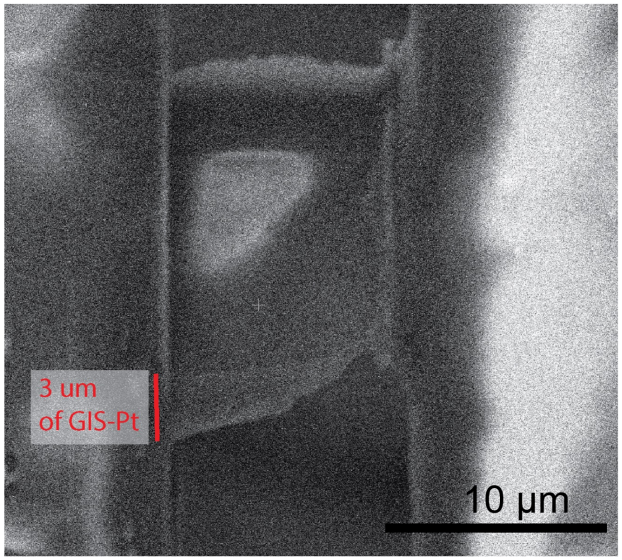
- Ideal density for bacteria: Monolayer of bacterial cells covered in vitreous ice.
- Difficult identification in FIB views.

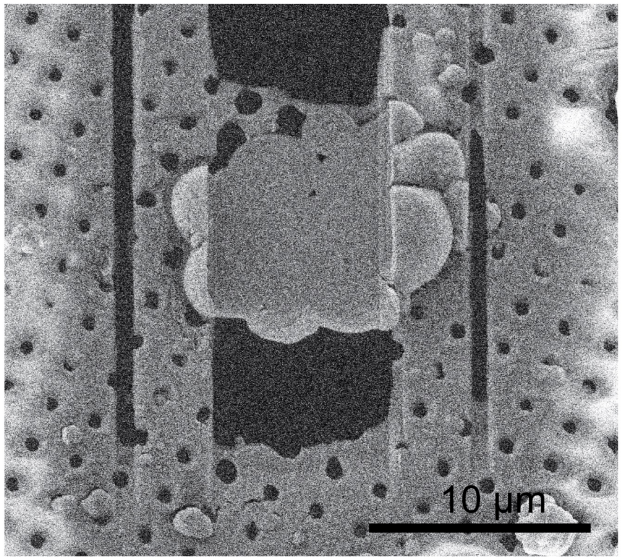
Lam *et. al.* Methods Mol Bio. (2021)

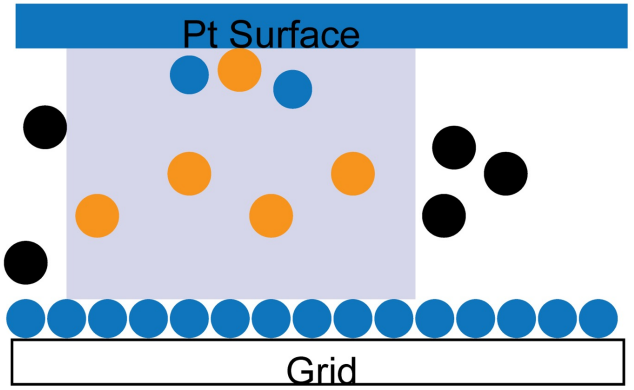
Imparting protection and conductivity to cellular samples



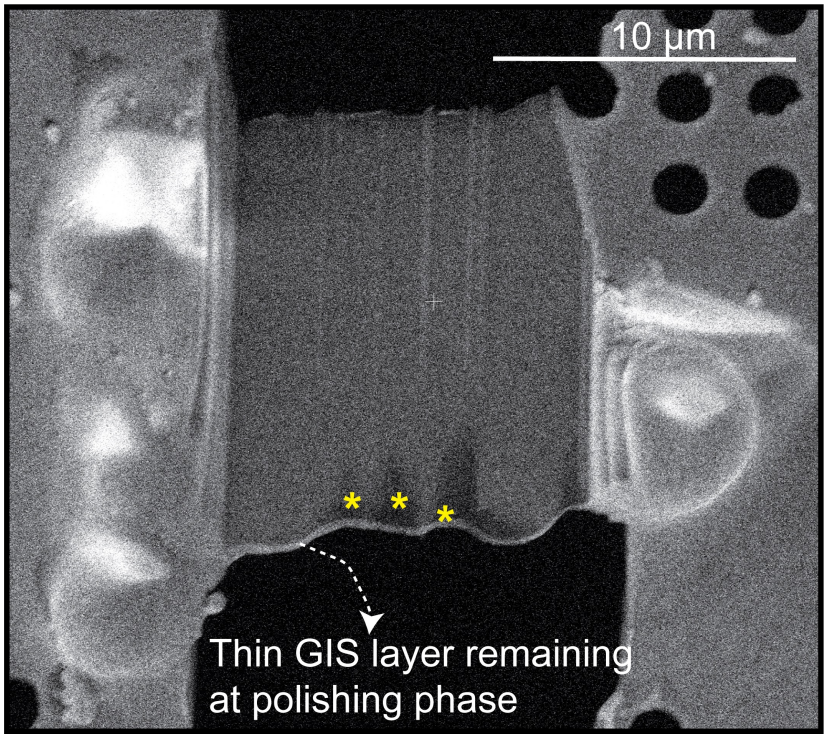




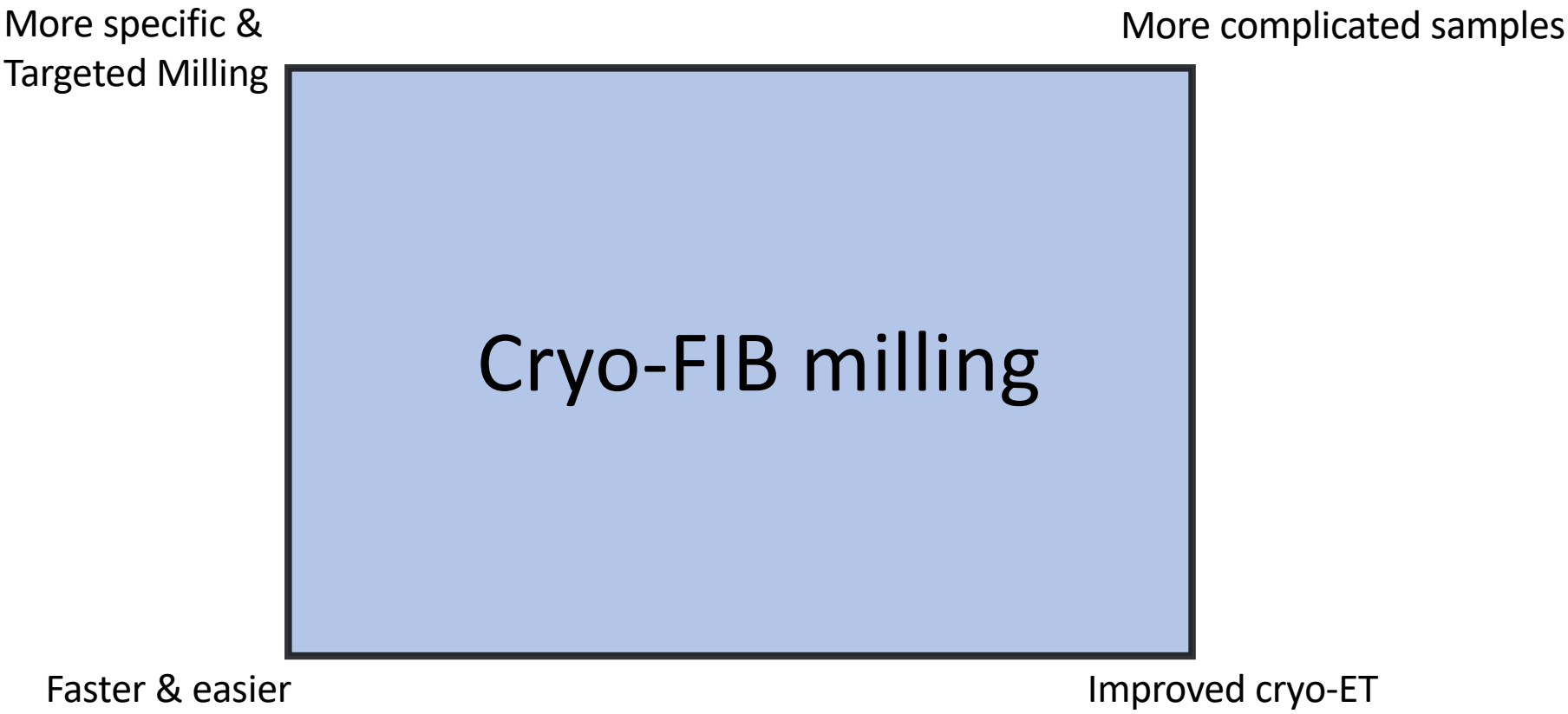




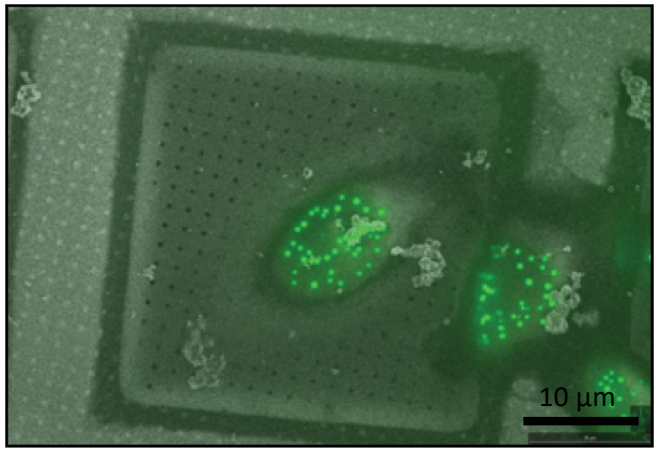
SEM contrast helps with gauging the thickness of the lamella



Techniques for better FIB-milling assisted cryo-ET



Cryo-fluorescence to identify regions of interest for targeted milling

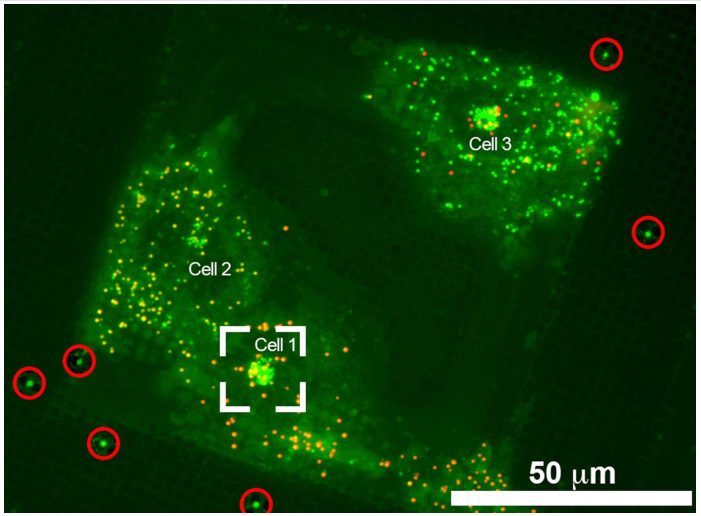


Yu *et al*. Science (2021)

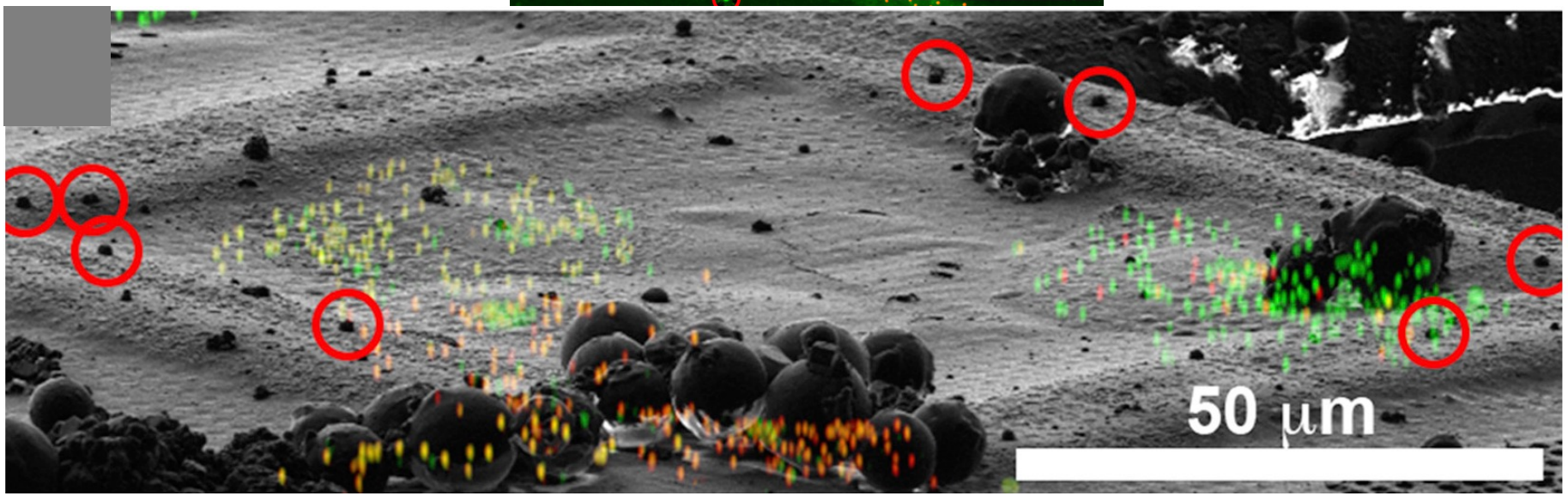
Leica

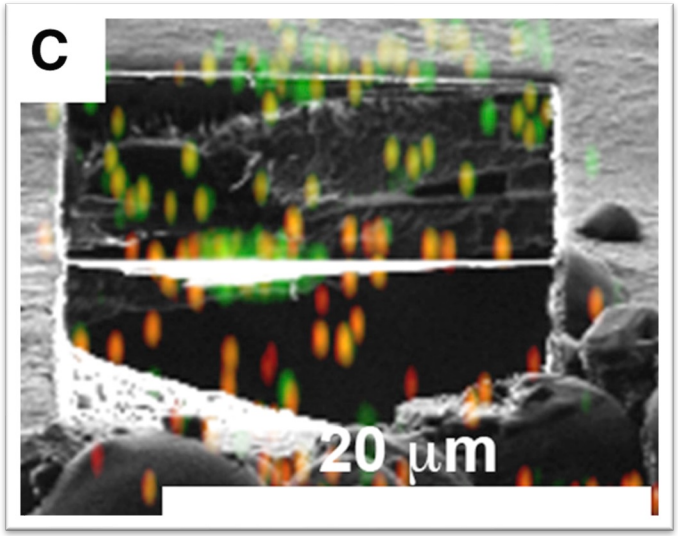
No conflicts of interest with them

Getting the targets of interest within the lamellae

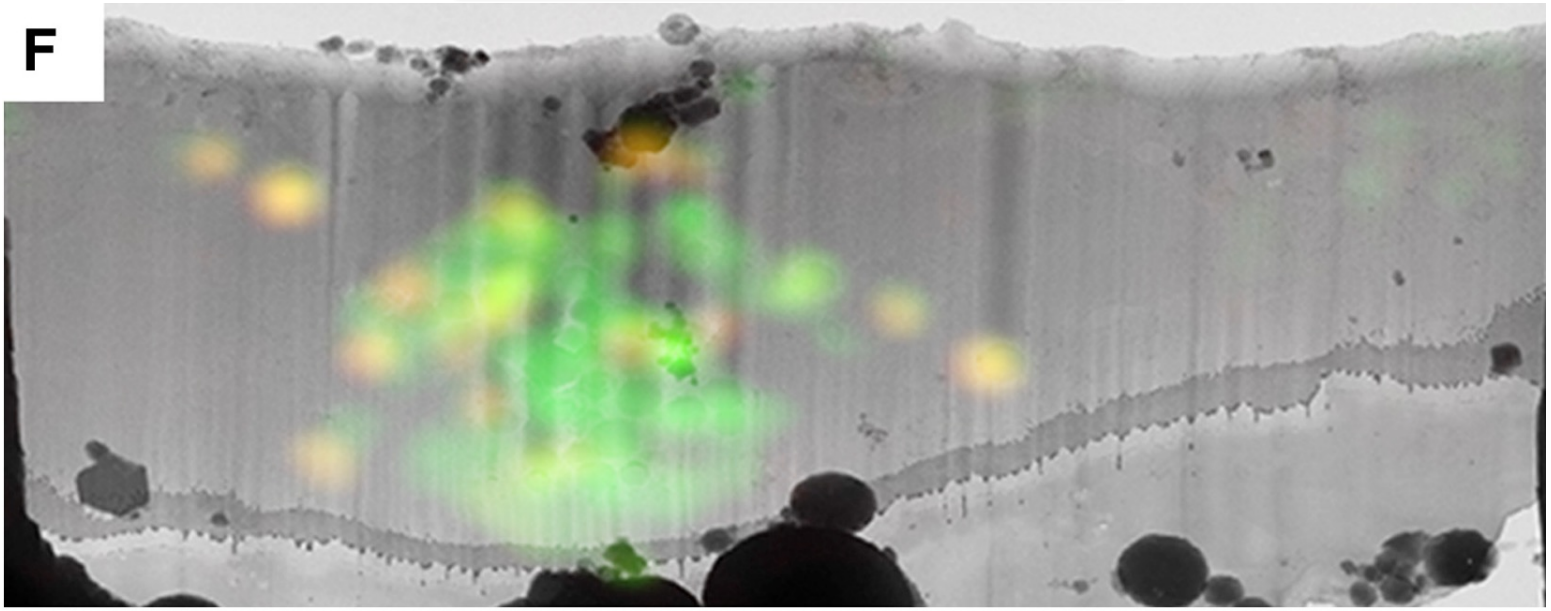


Arnold *et al.* (2016)



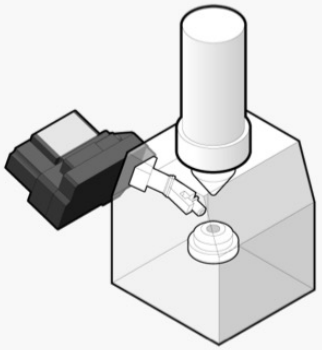


Arnold *et al.* (2016)



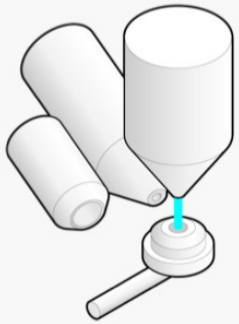
Fluorescence inside the dual-beam

1. Load sample in cryo-FIB/ SEM/FLM.

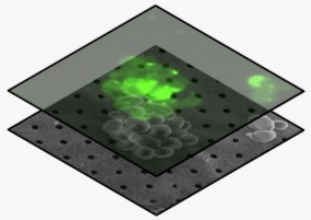


2. Move to FLM position and capture FLM image.

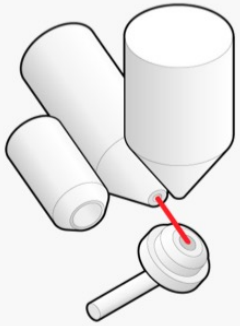
3. Move to SEM position and capture SEM image.



4. Image correlation.

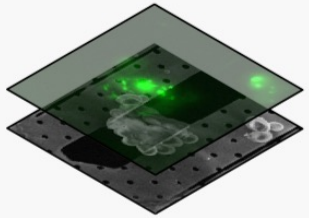


5. Move to a region of interest (ROI) based on the FLM image.



6. FIB mill lamella.

7. After milling: verify if fluorescence (from ROI) is still present.

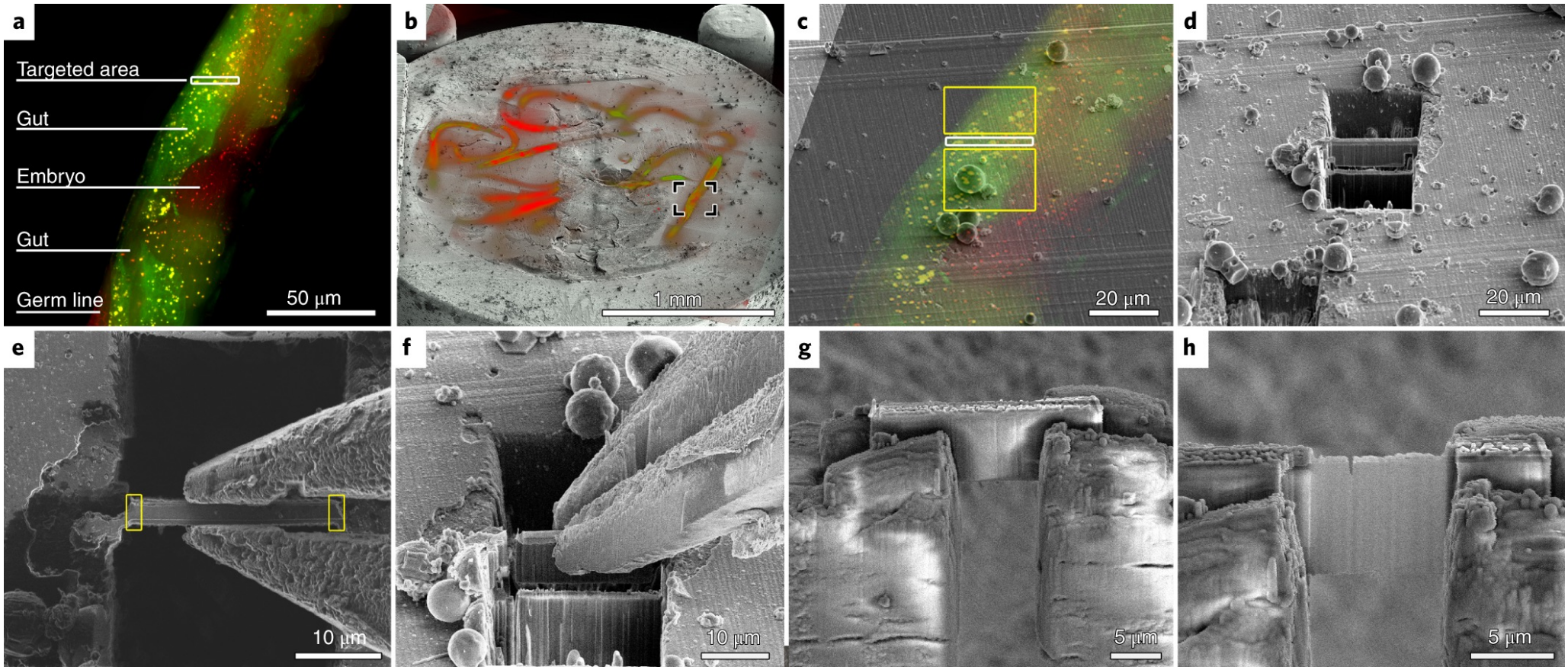


Gorelick *et al.* Elife (2019)

delmic

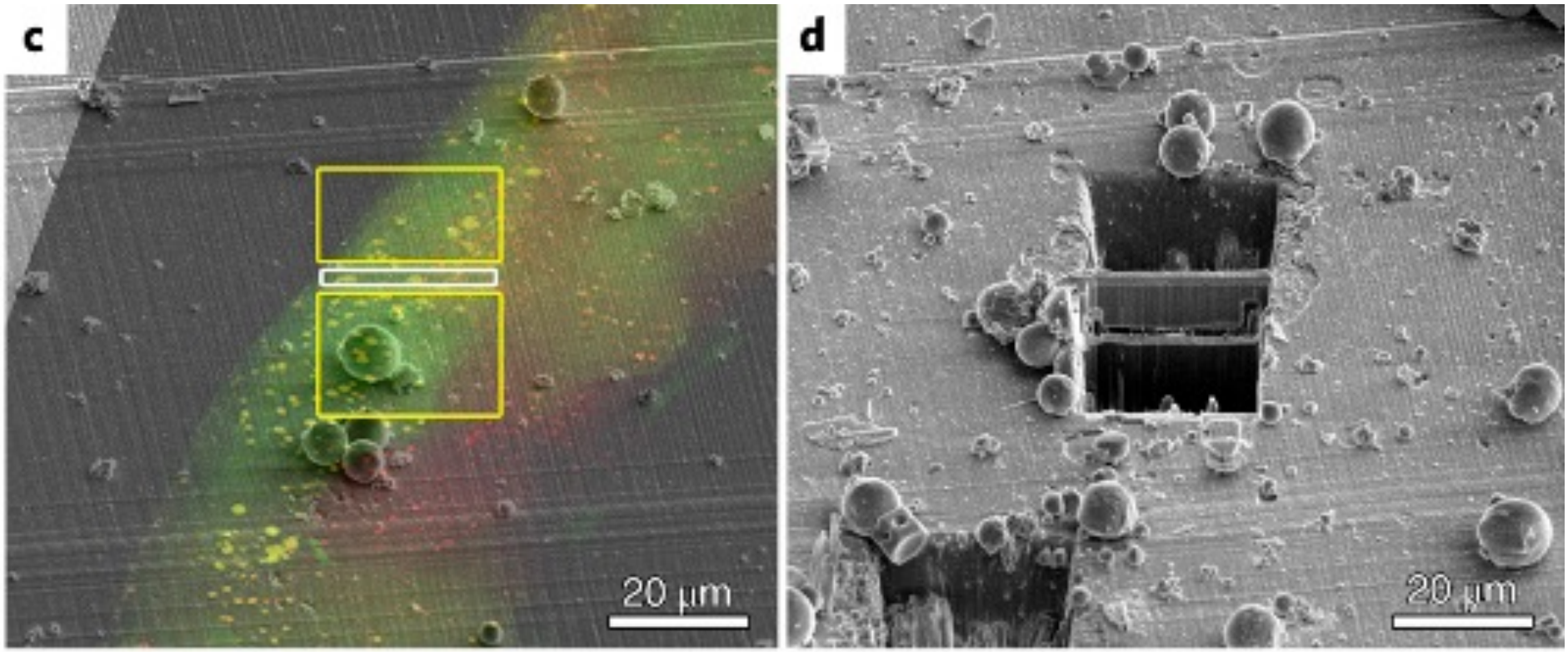
No conflicts of interest with them

Bringing tissues and other thick samples within reach of cryo-ET



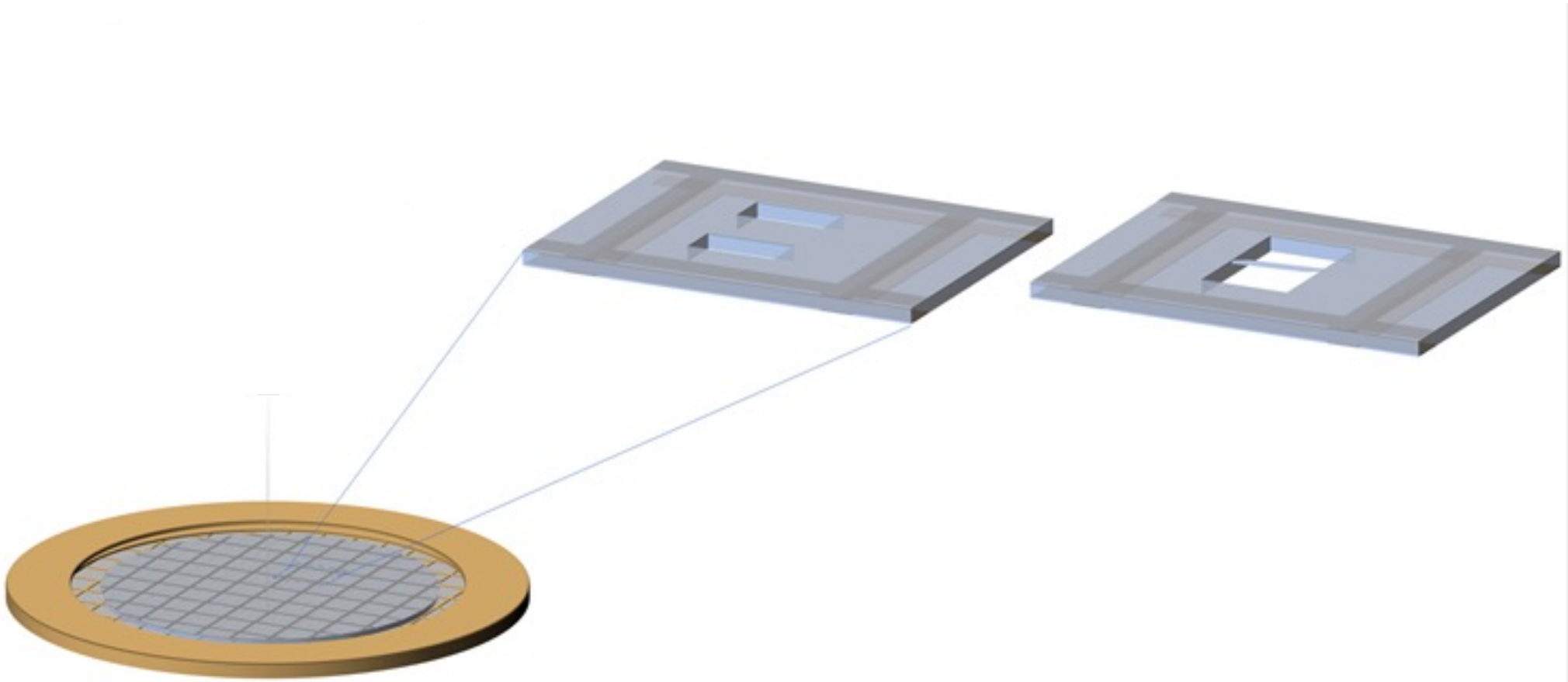
Schaffer et al. (2019)

Lift out to circumvent poor Z- resolution



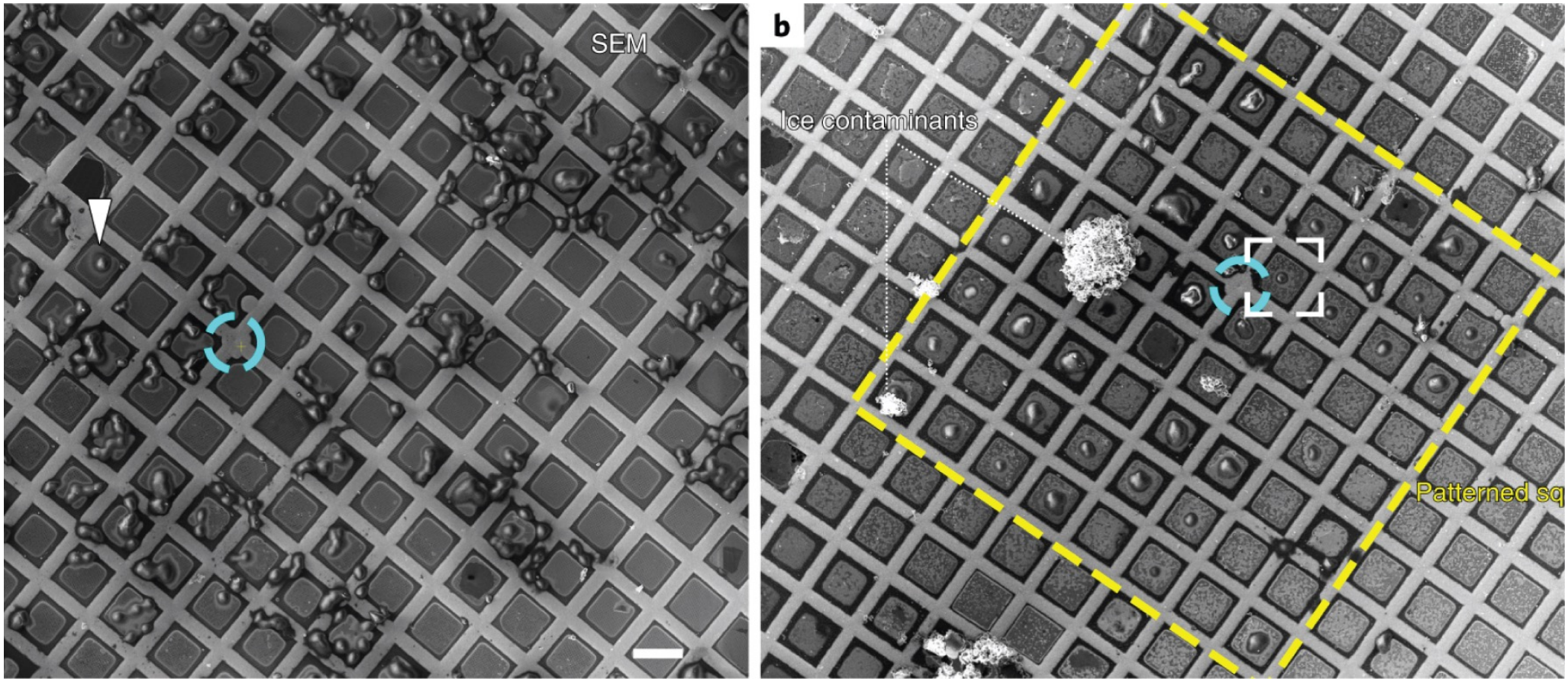
Schaffer *et al.* (2019)

Waffle method for high-pressure frozen and thicker samples



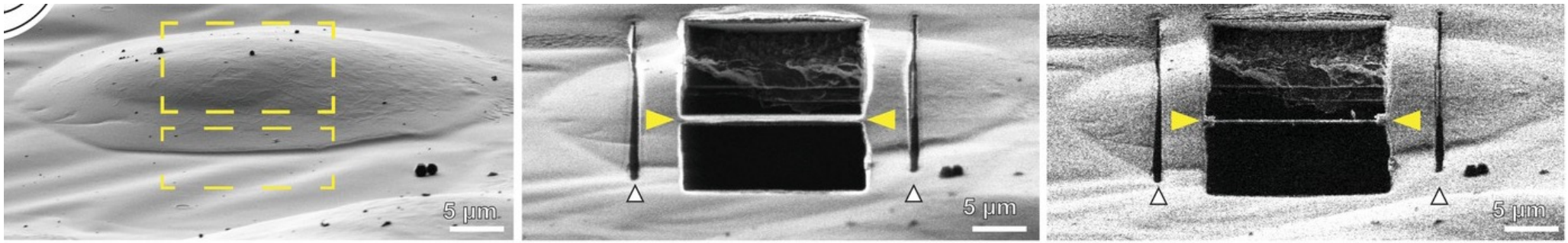
Kotaro *et al.* Nature Comm (2022)

Surface patterning for desired placement of cells on the grid



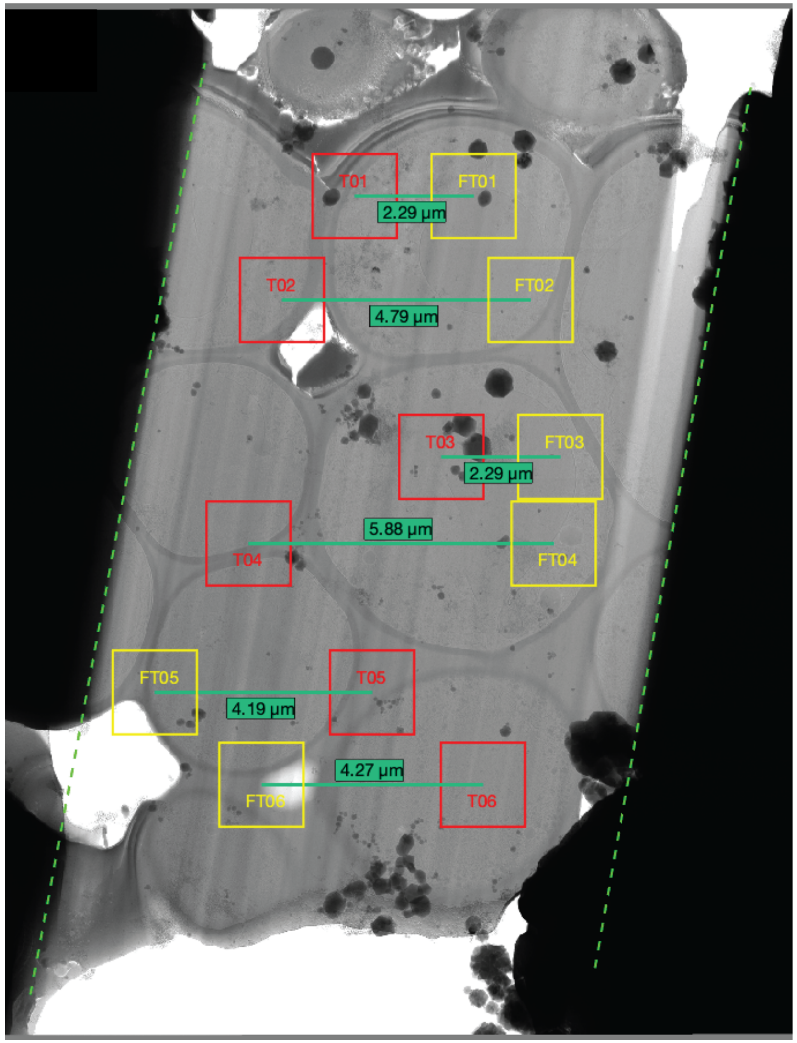
Toro-Nahuelpan *et al.* Nature Methods (2020)

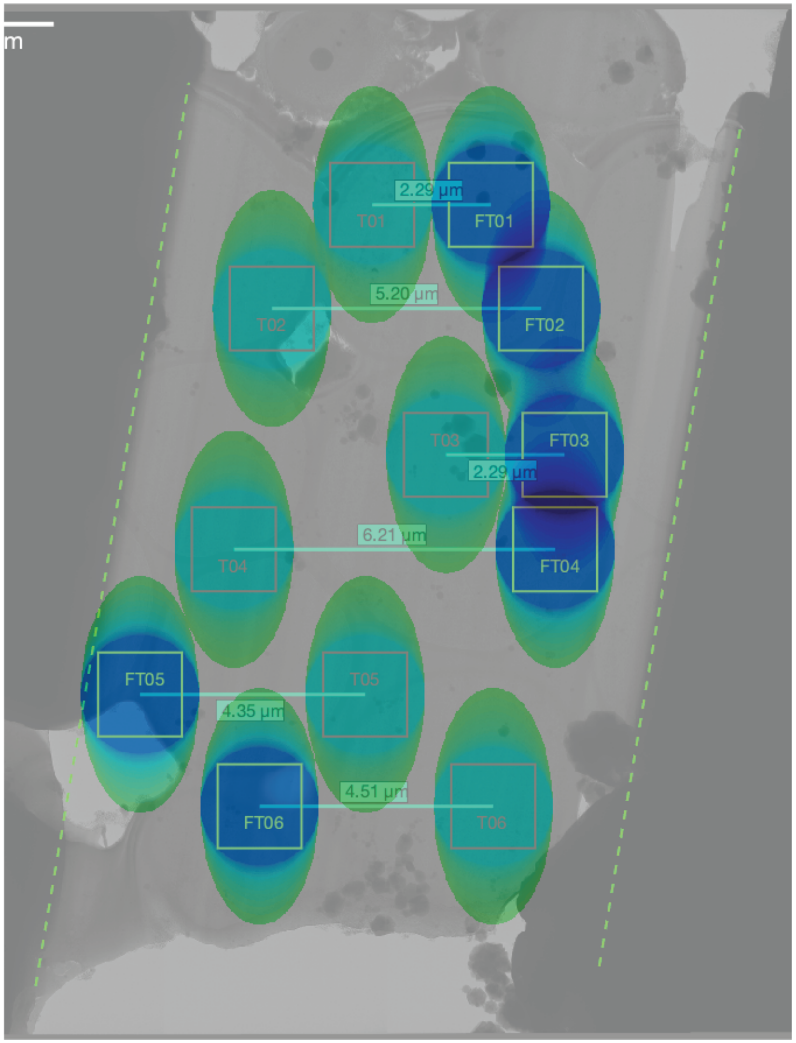
Automated milling



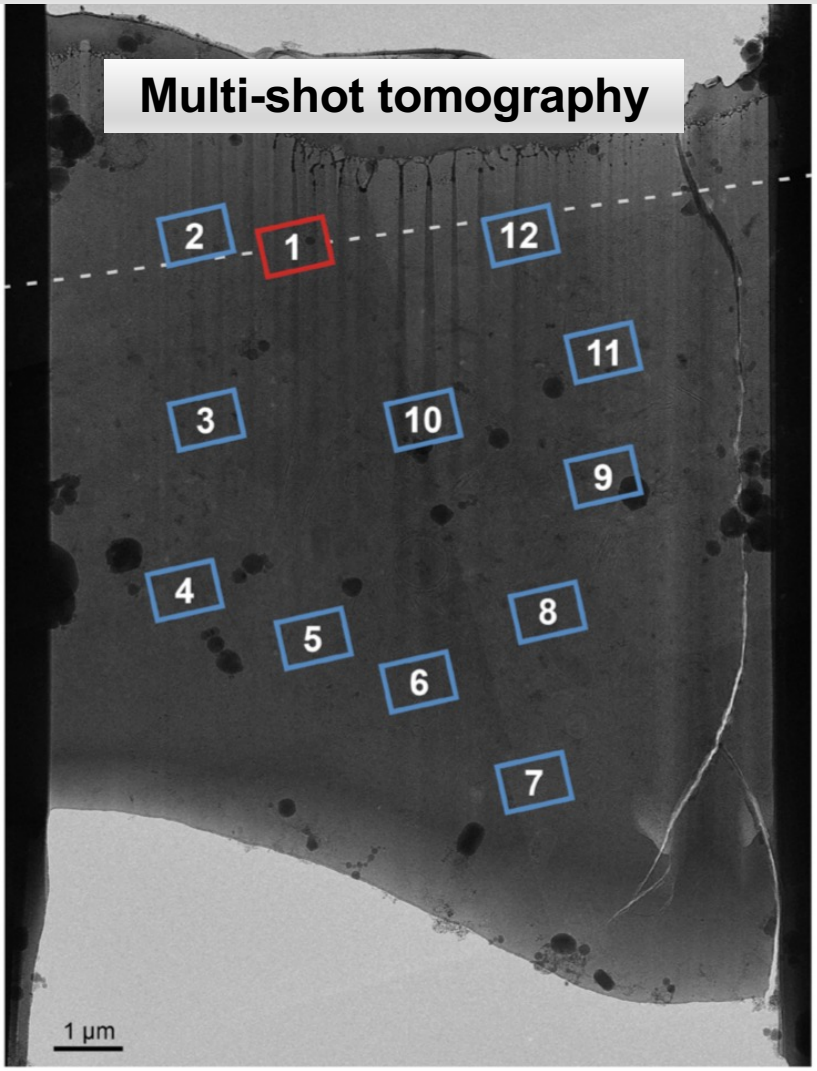
Klumpe *et al.* (2021)
Buckley *et al.* (2020)
Zachs *et al.* (2021)
Tacke *et al.* (2021)
Dutka *et al.* (2019)

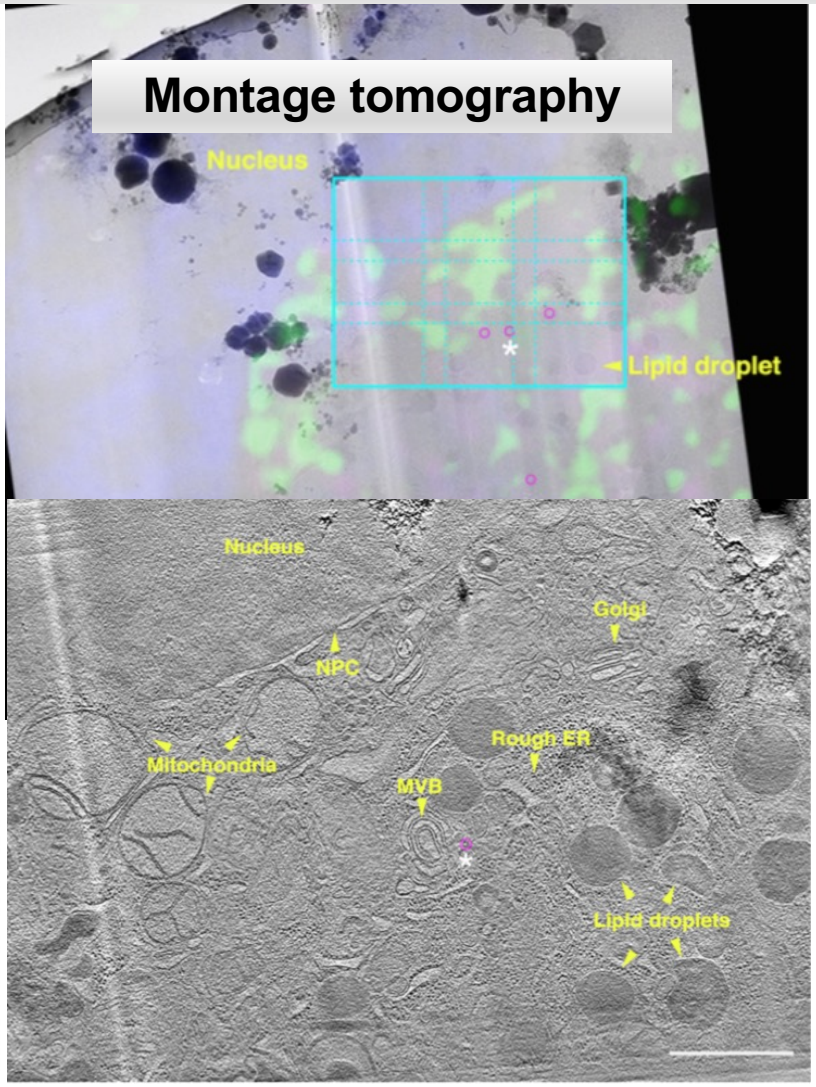
Cryo-ET on lamellae





High throughput cryo-ET





Eisenstein et al. (2022)

Yang et al. (2021)

Peck et al. (2021)

Thank you!