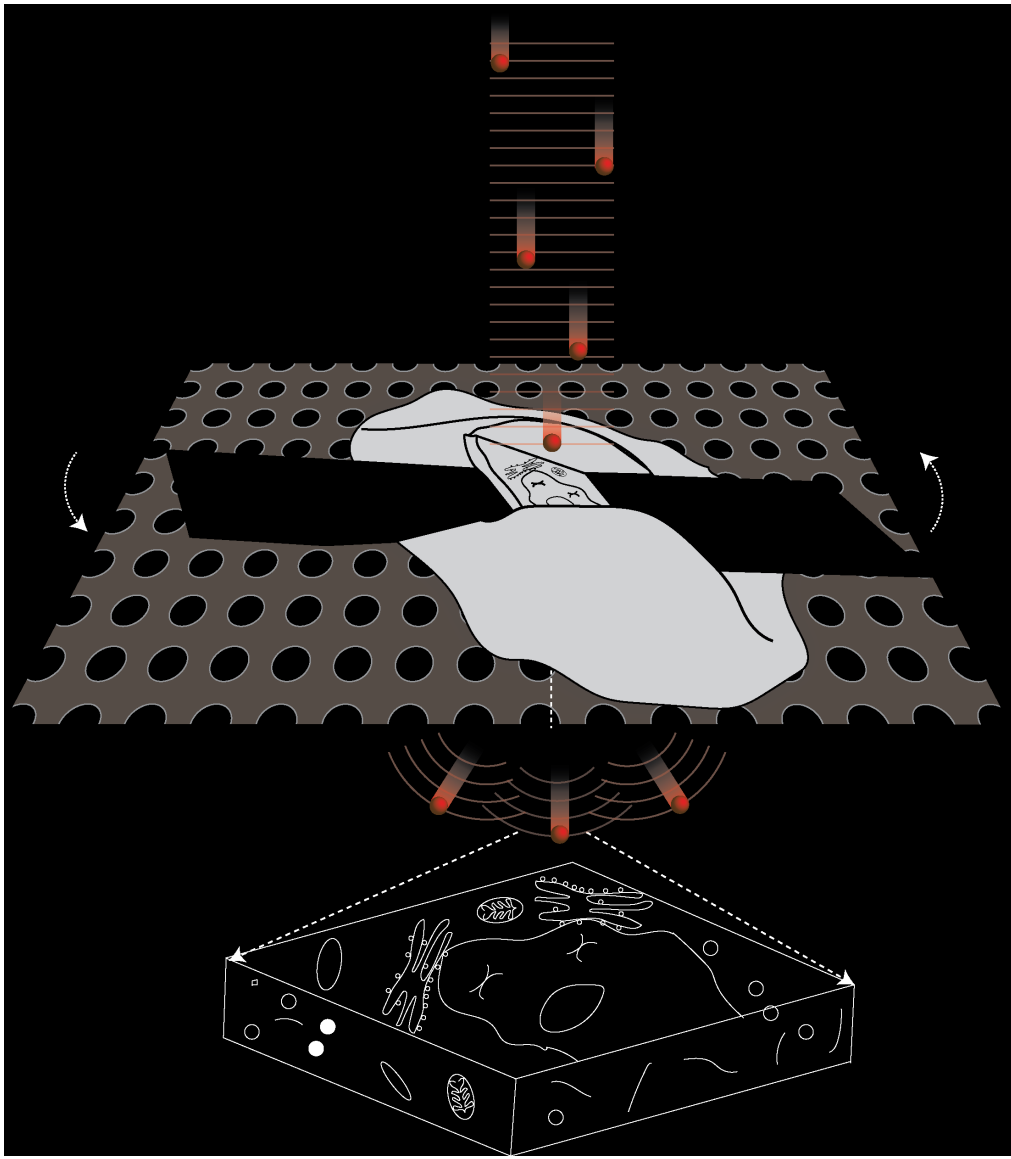
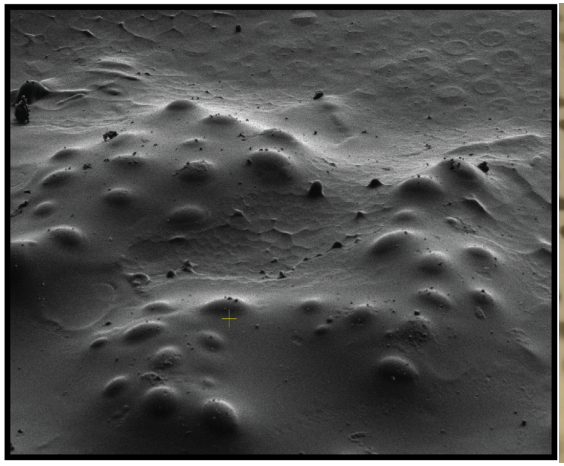
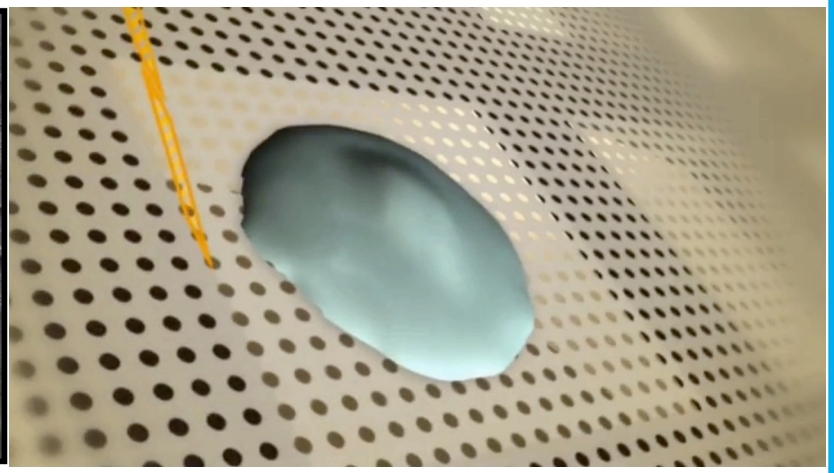


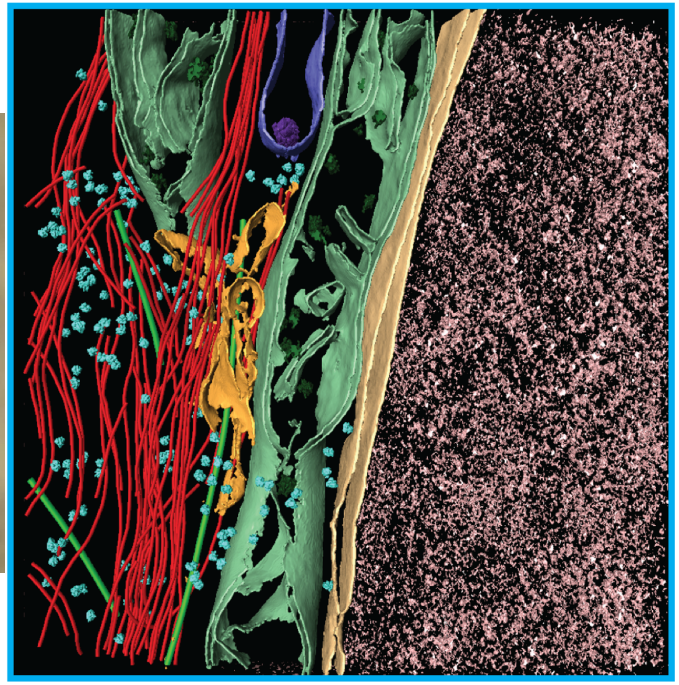
Micromachining of biological samples for molecular photography inside them

by:
Digvijay Singh
Damon Runyon Fellow
Villa Lab, UC San Diego

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







Vitrification

Rapid-freeze cells in culture to preserve structures

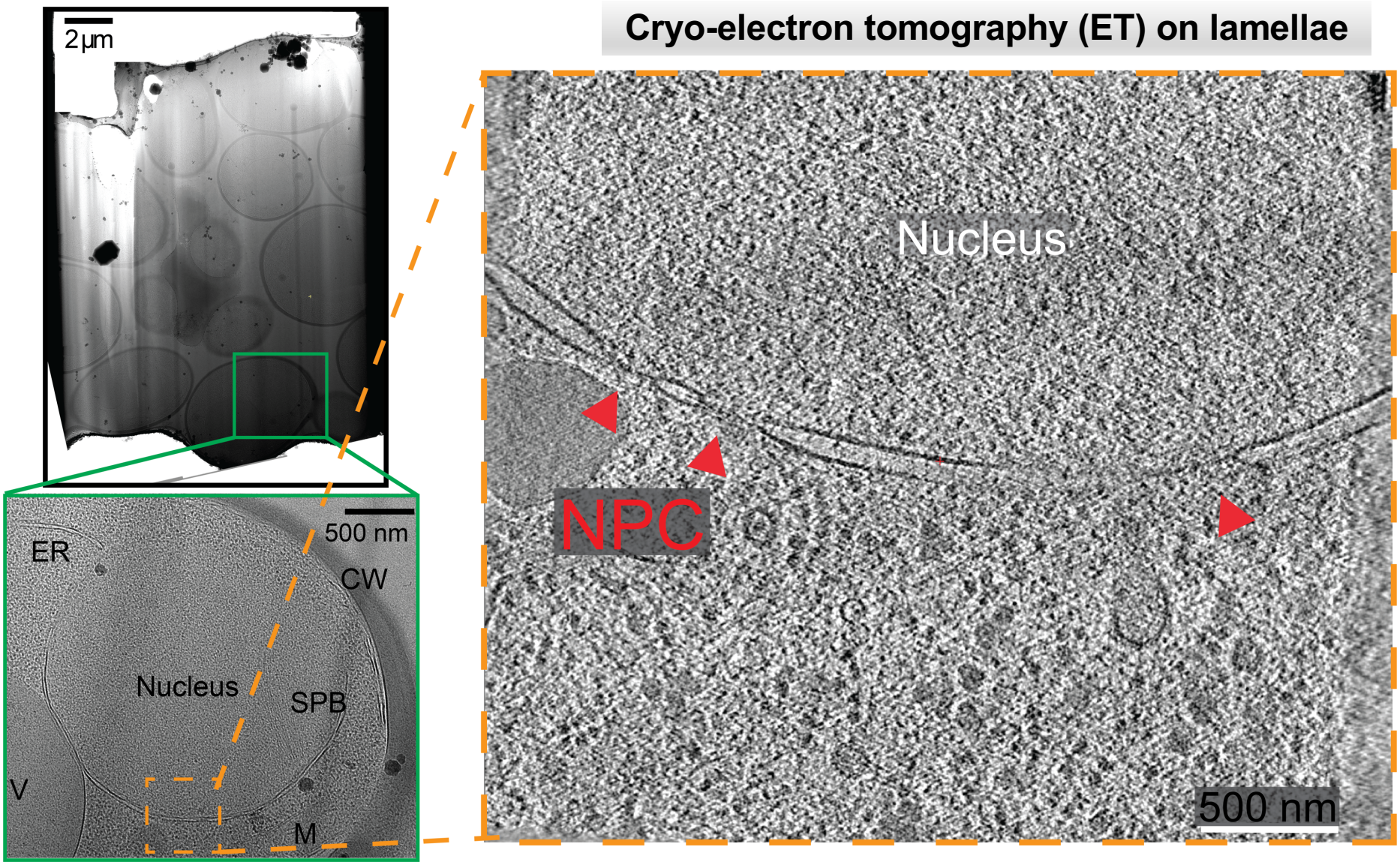
Cryo-FIB milling

Micro-machine thin lamella out of cell(s)

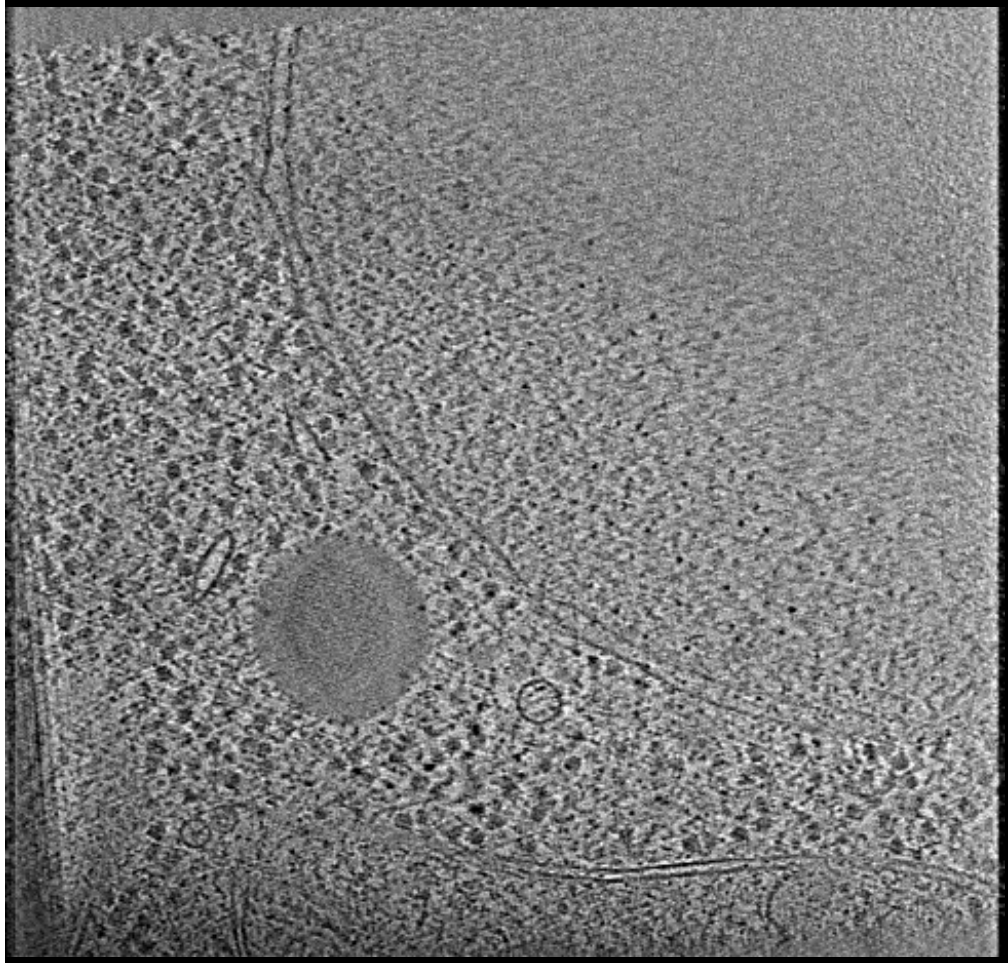
Cryo-electron tomography (cryo-ET)

3D reconstruction of molecular landscapes in-situ

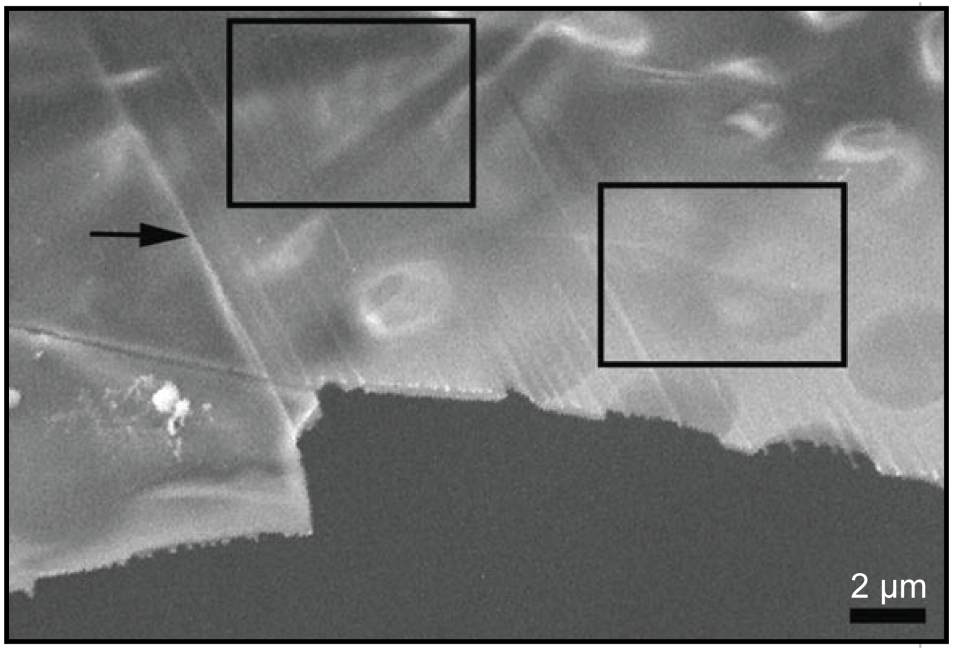
Wagner, Watanabe *et.al.* Nature Protocols (2020)

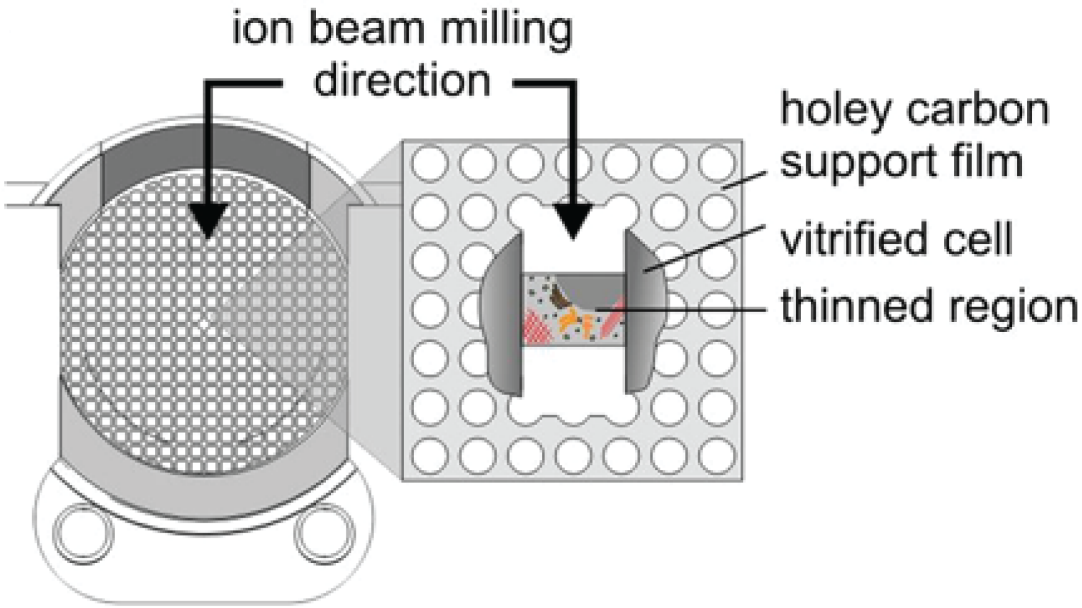

Cryo-electron tomography (ET) on lamellae
2 µm
500 nm
ER
CW
Nucleus
SPB
V
M
Nucleus
NPC
500 nm

Molecular structures in cellular context



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





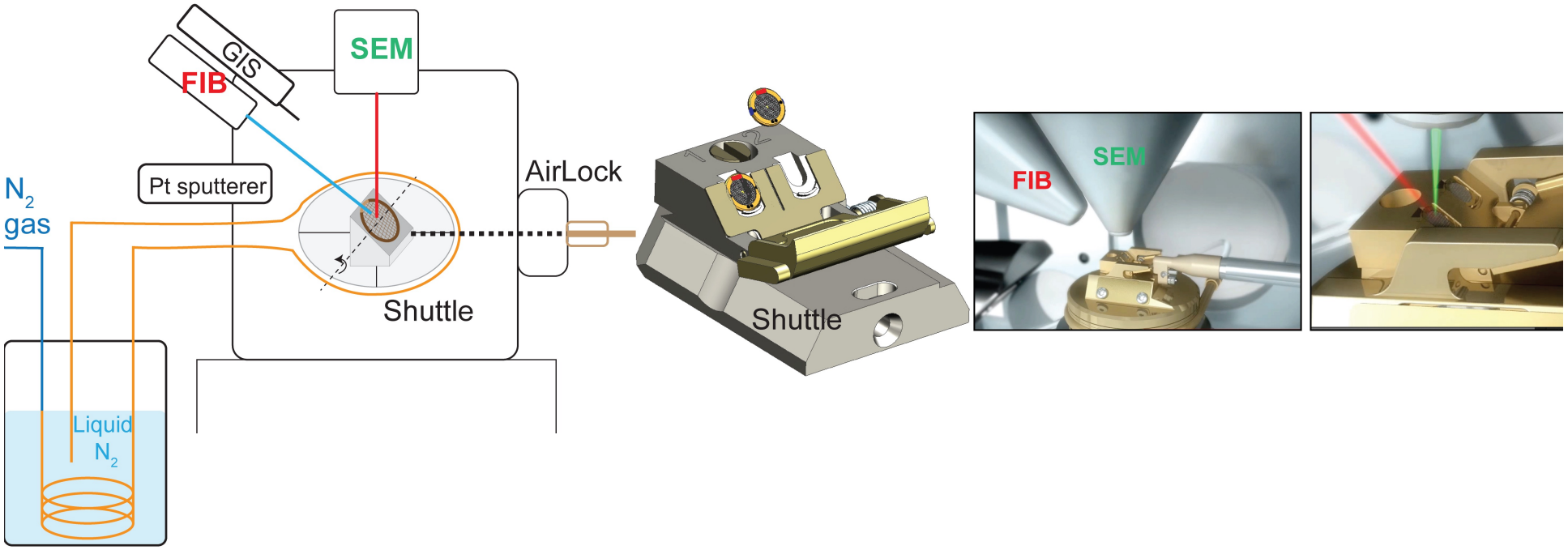
Marko *et al.* Nature Methods (2007)

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

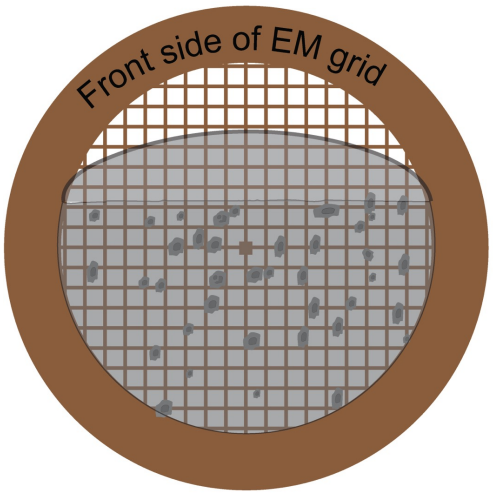


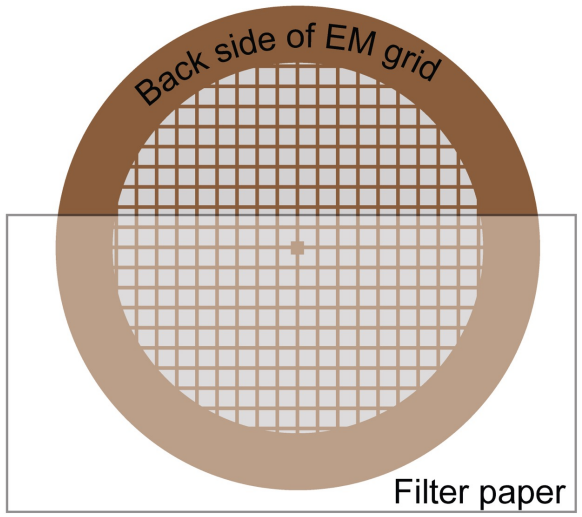
Wagner, Watanabe *et.al.* Nature Protocols (2020)

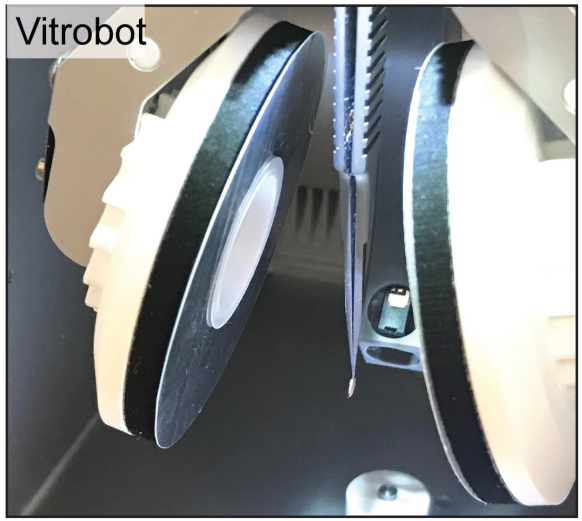
Thermo Fisher

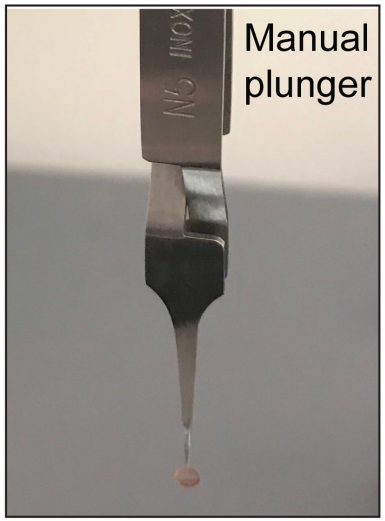
No conflicts of interest with them

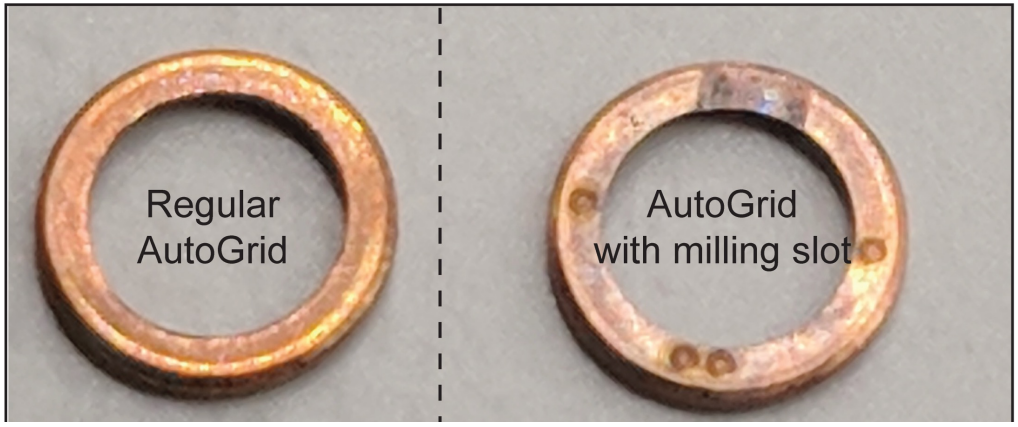
Vitrification of cellular samples for cryo-FIB milling

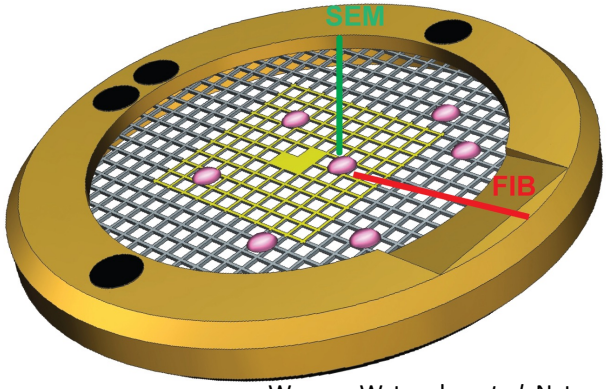






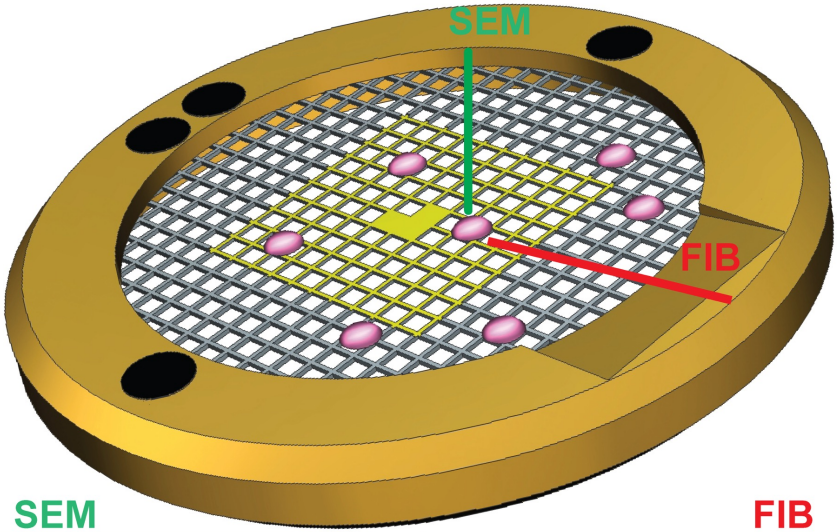




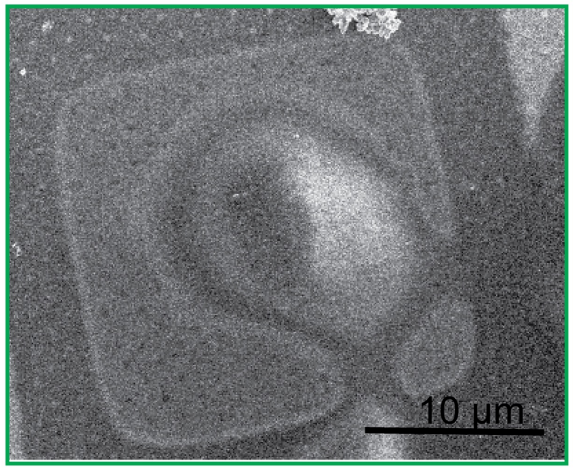


Wagner, Watanabe *et.al.* Nature Protocols (2020)

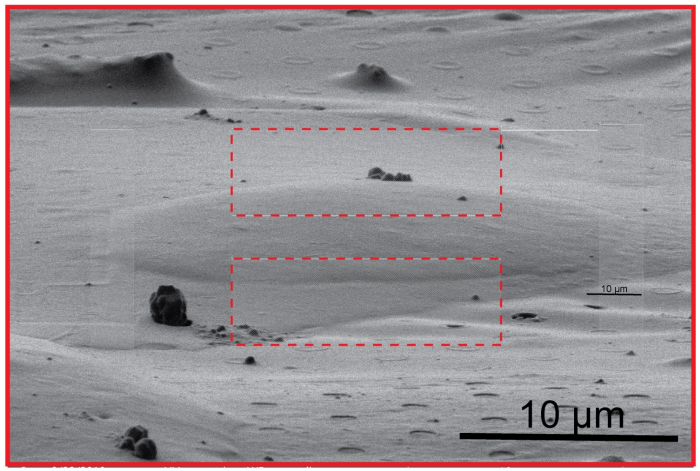
SEM and FIB views



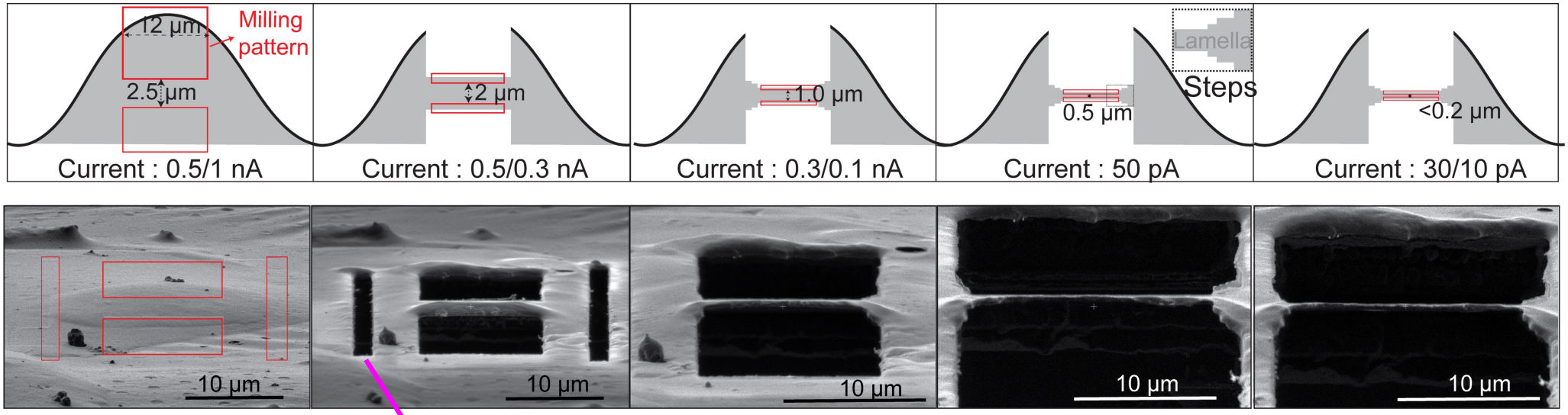
SEM



FIB



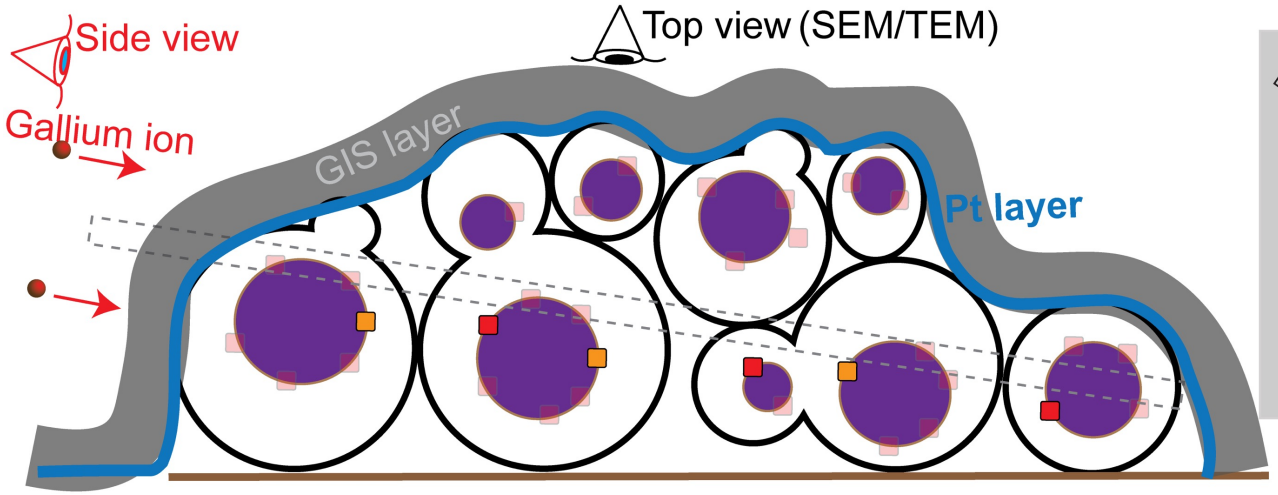
Progressive cryo-FIB milling for fragile cellular samples

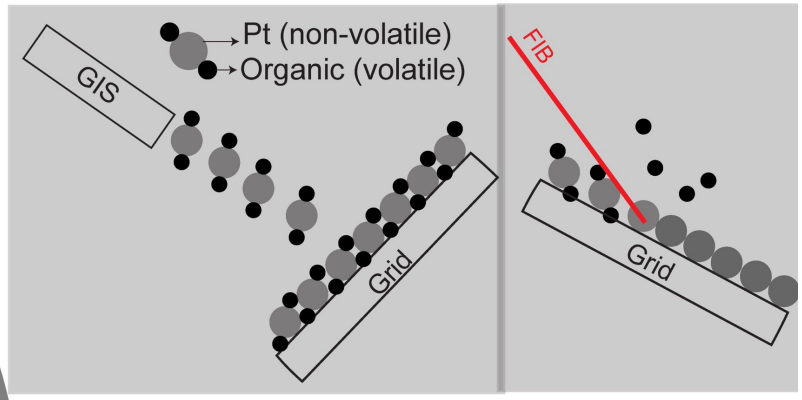


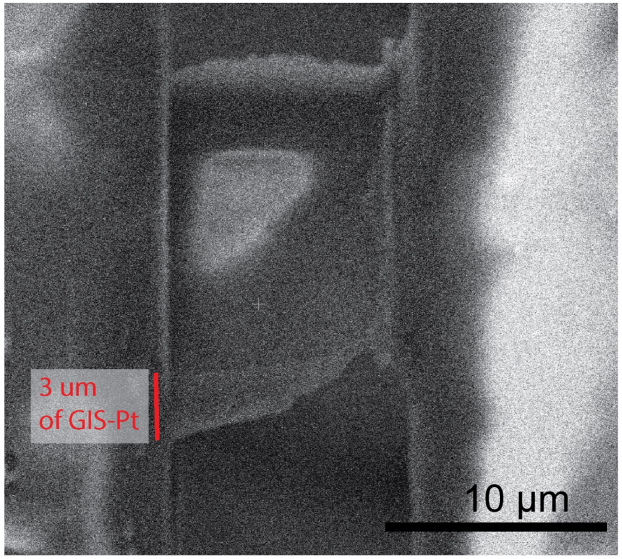
Expansion joints help relieve stresses in the lamella and improves its stability

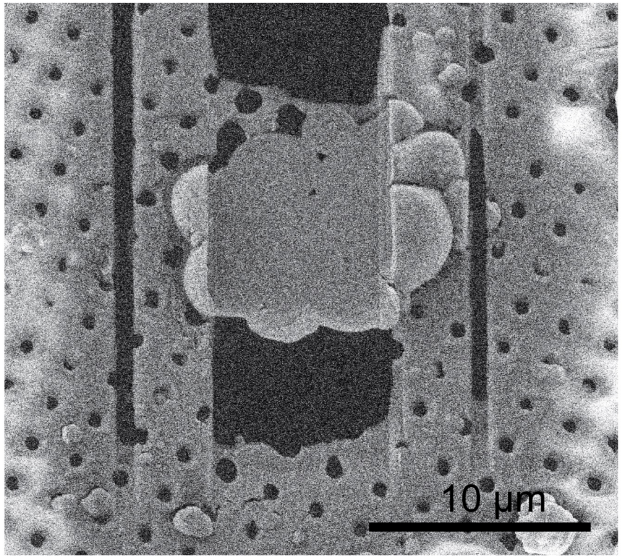
Wolff *et.al. J. Struct. Biol.* (2019)

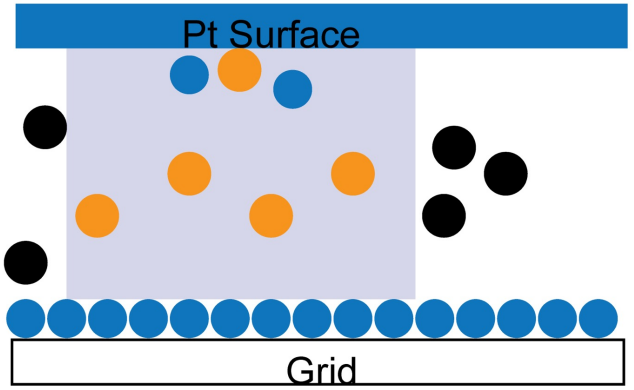
Imparting protection and conductivity to cellular samples



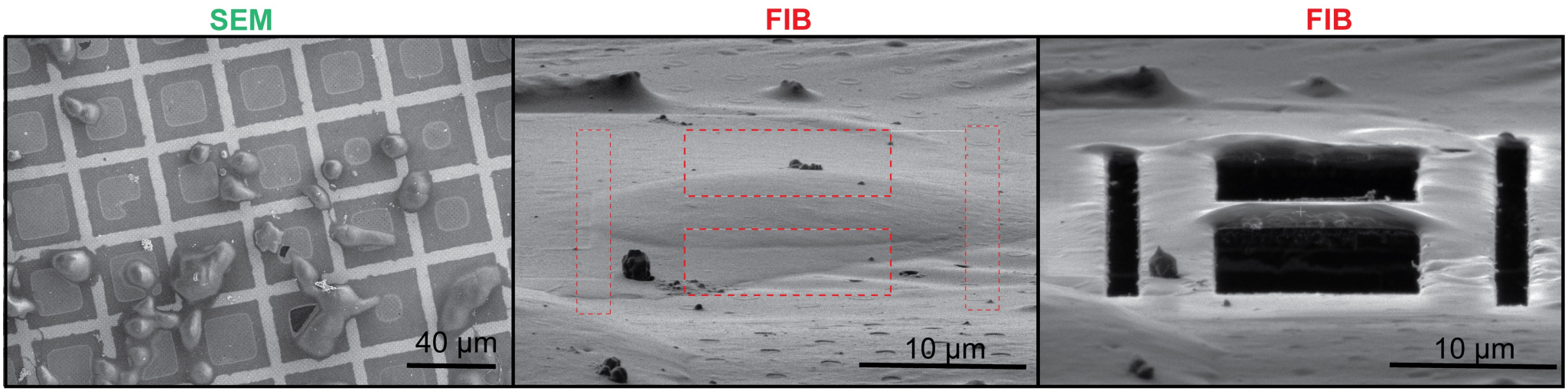






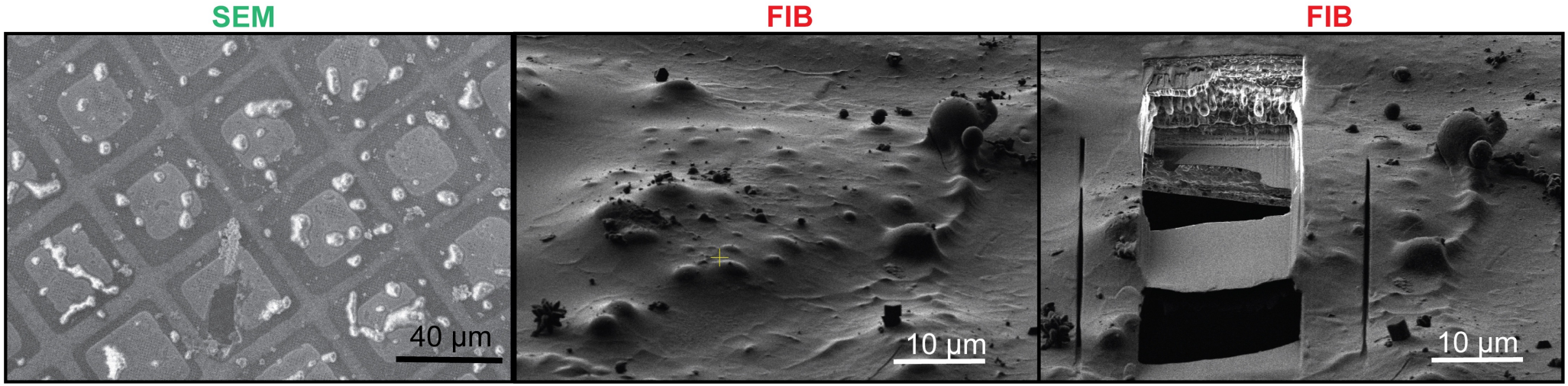


Ideal mammalian cell samples for cryo-FIB



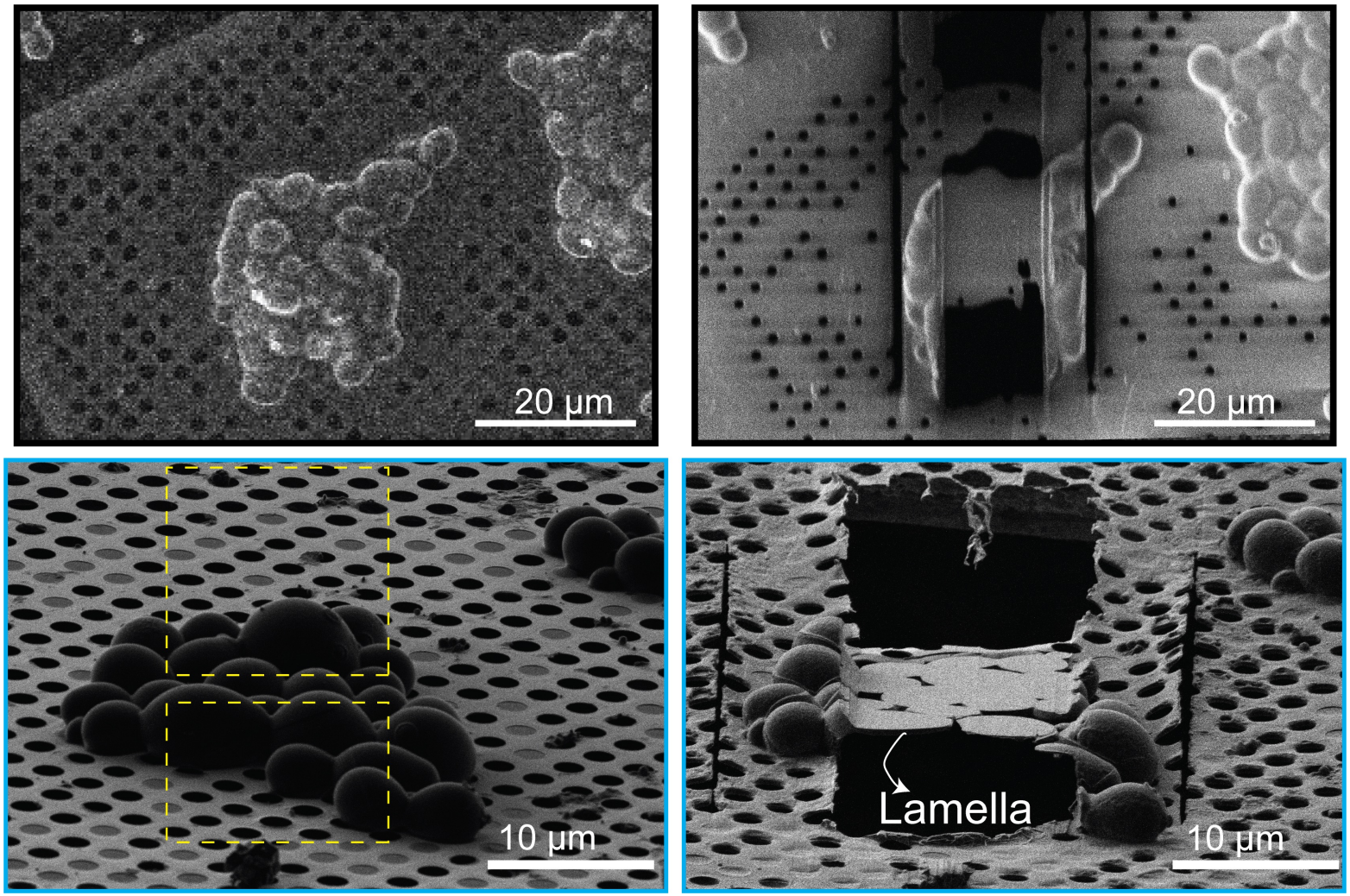
- Ideal density ~1 cell in middle of grid squares.
- Cells near grid bars can't be milled.
- Balance between good vitrification & hydration.
- Bigger clump of cells (>1) likely to have bad vitrification deep-down.

Ideal yeast samples for cryo-FIB

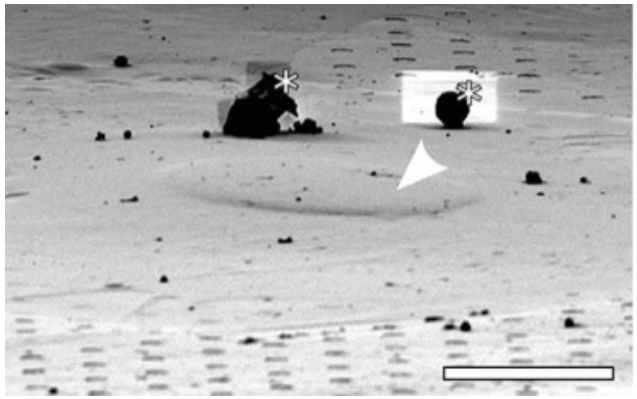


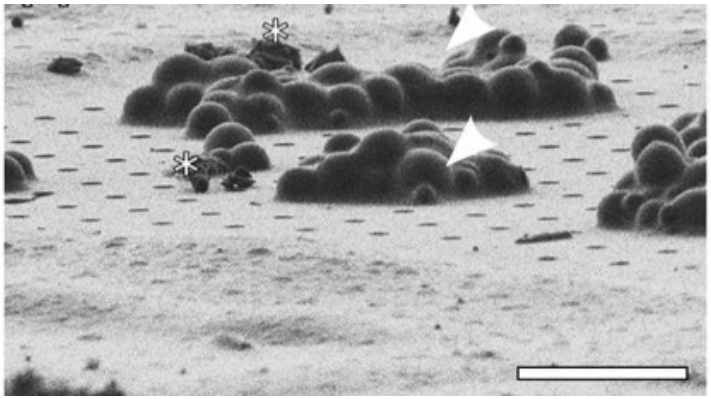
- Ideal density: clumps of 7-20 cells in middle of grid squares.
- Yeast clumps ’protrude’ out of the grid surface, making their identification in FIB view easier.
- Certain cells with cell-wall can withstand low-hydration.

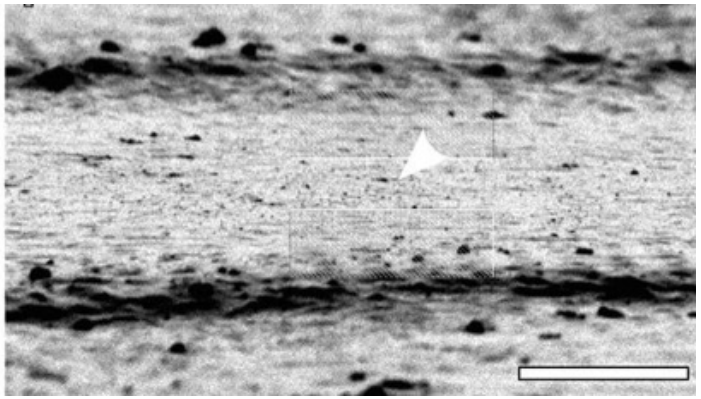
Cells with cell-wall provide wider blotting range



Mammalian vs. Yeast vs. Bacteria



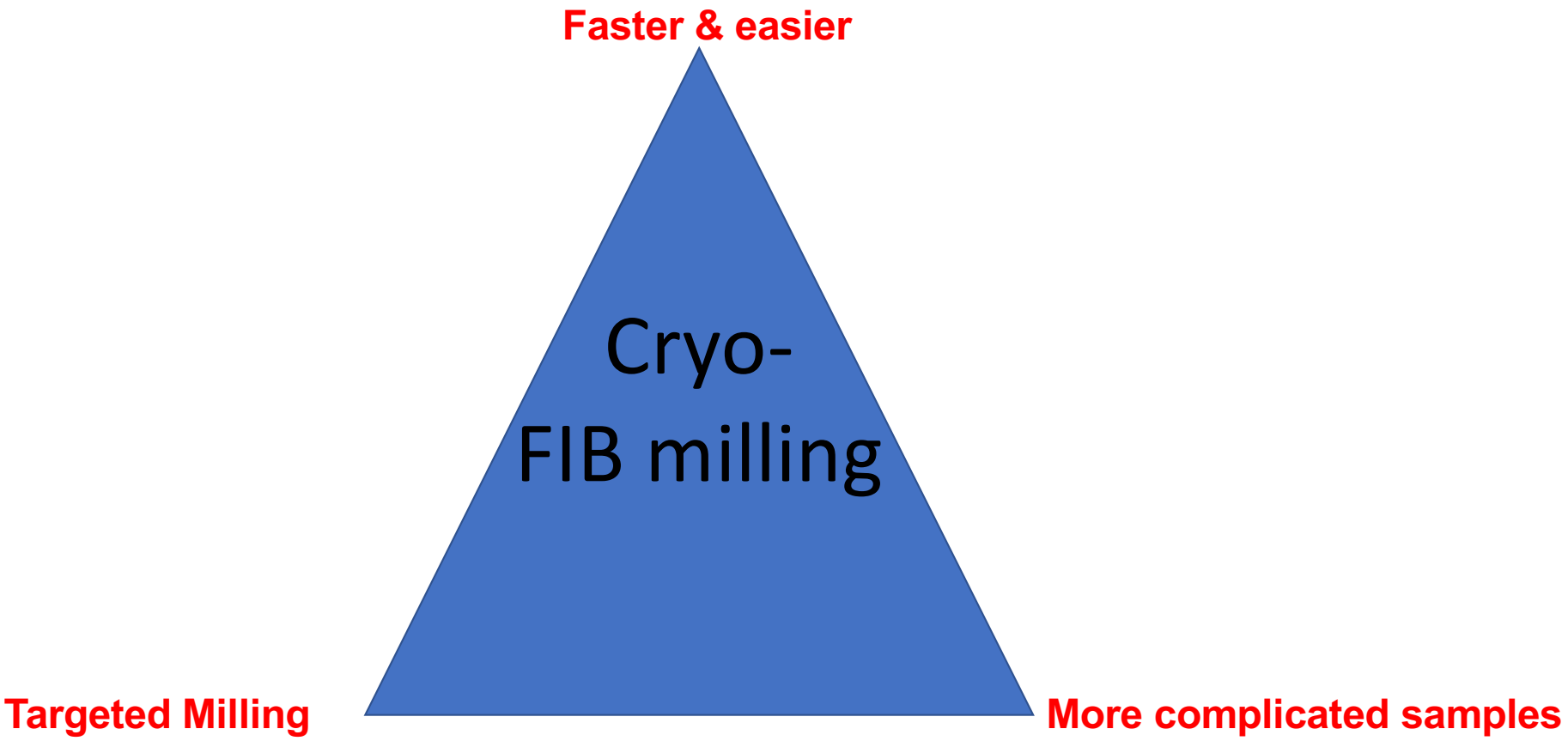




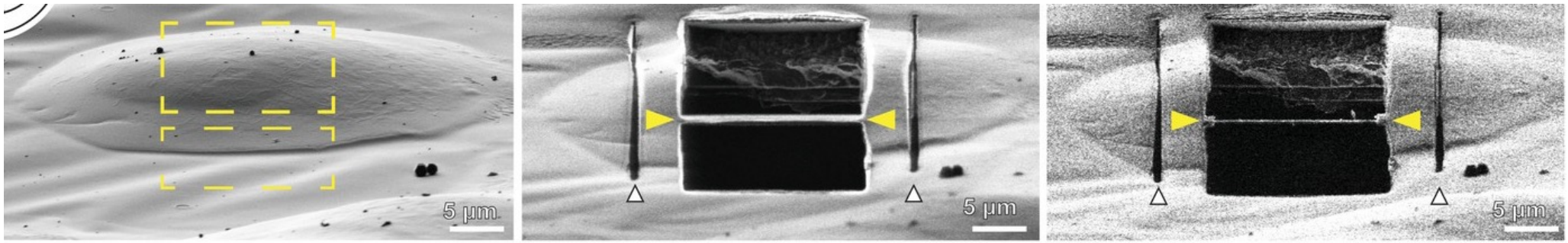
- Ideal density for bacteria: Monolayer of bacterial cells covered in vitreous ice.
- Difficult identification in FIB views.

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

Expanding the scope & ease of cryo-FIB milling

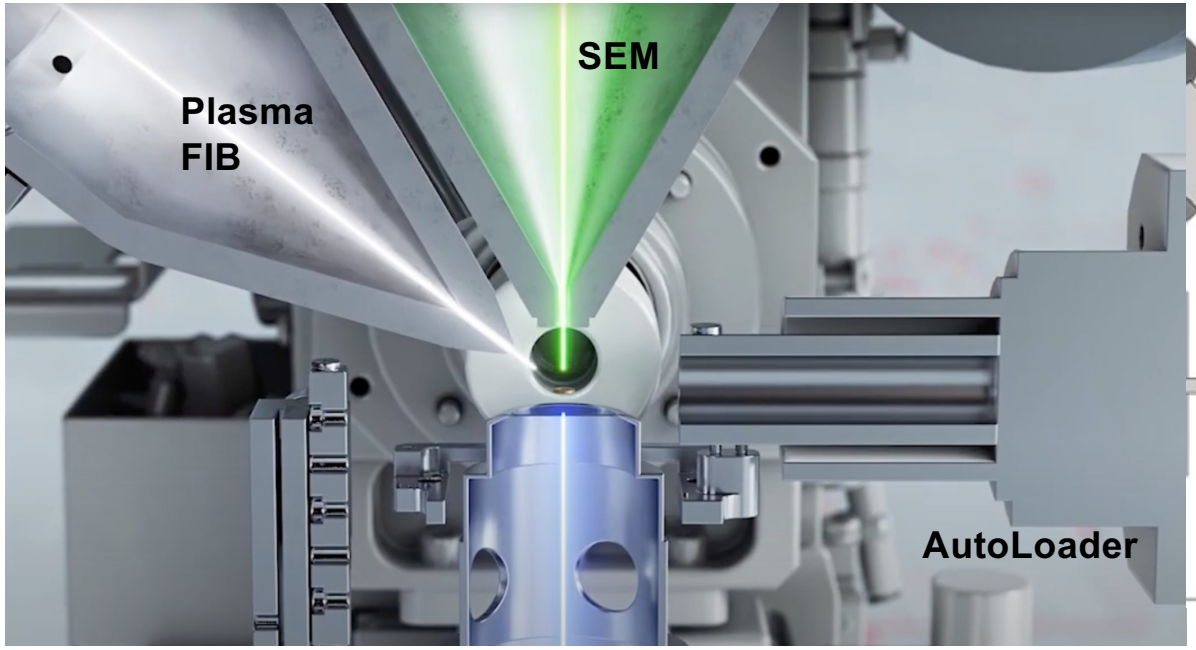


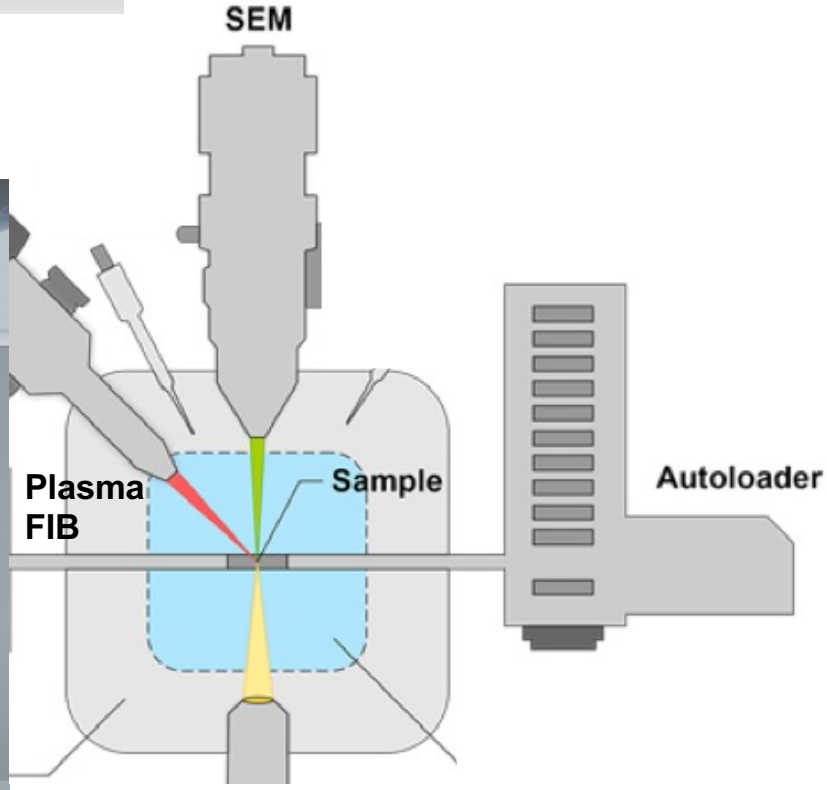
Faster & Easier:
Automated milling



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

Faster :
Plasma FIB



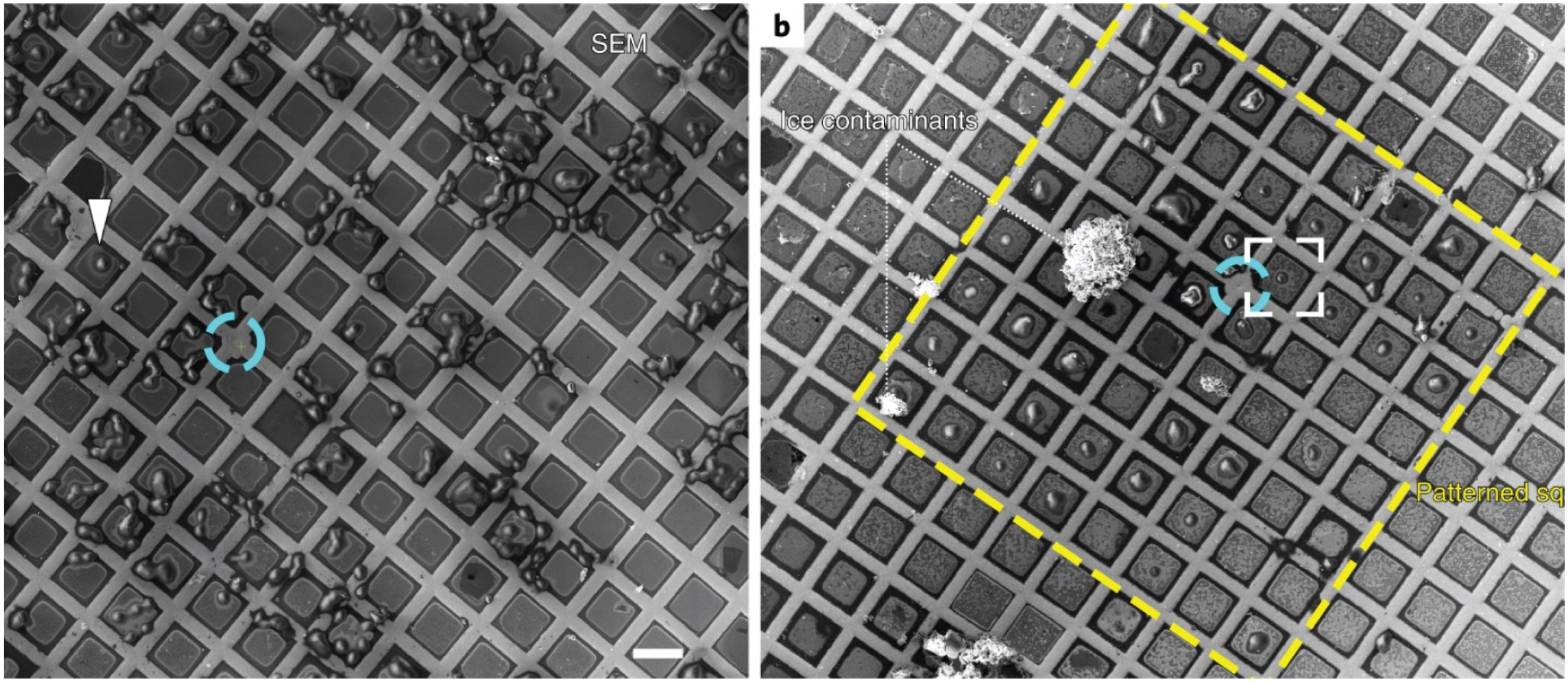


Berger *et al.* (2023)

Thermo Fisher

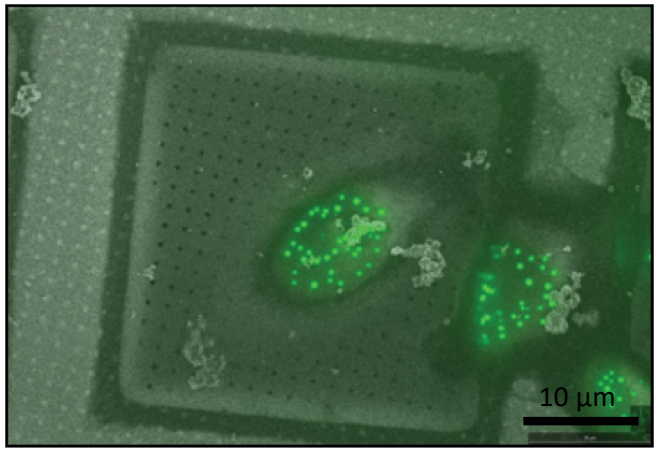
Easier :

Surface patterning for desired placement of cells on the grid



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

Targeted Milling :
Fluorescence for identifying targets

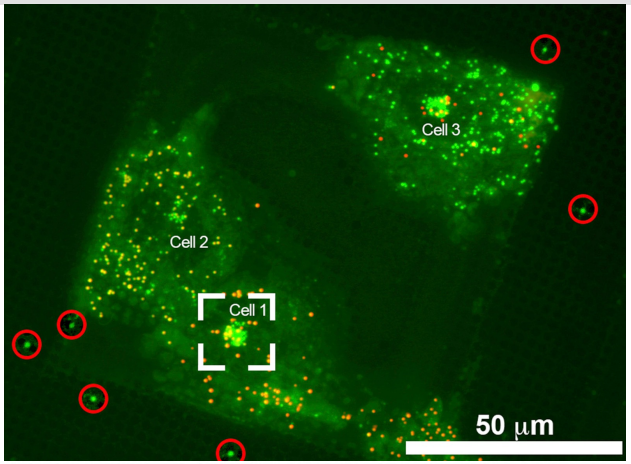


Yu *et al*. Science (2021)

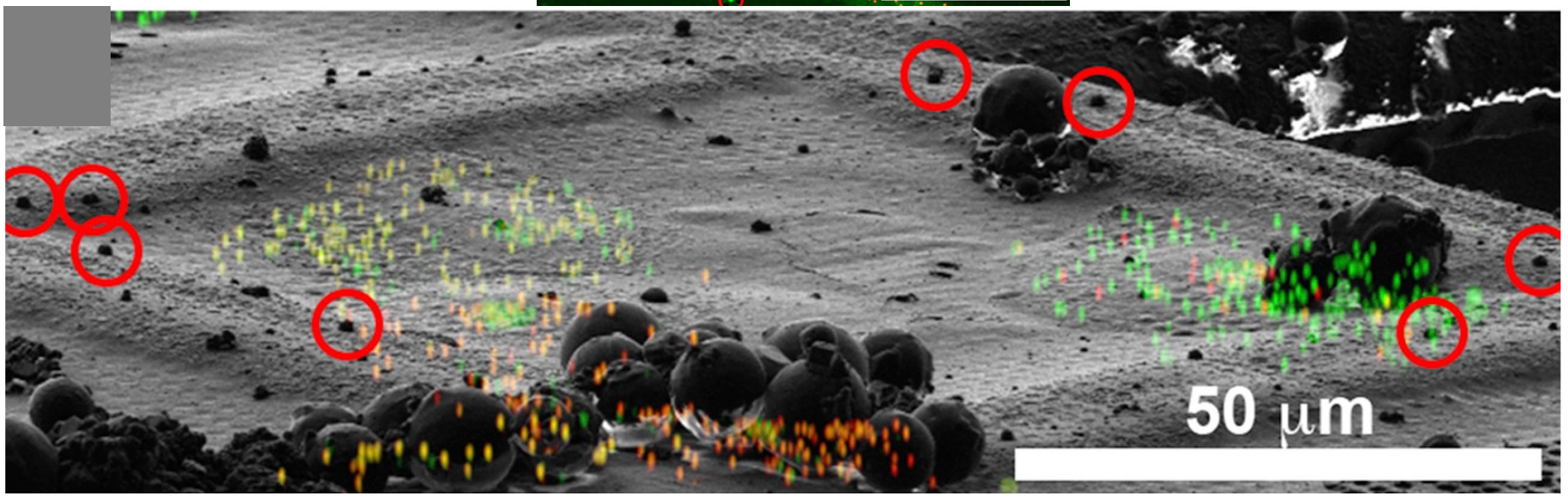
Leica

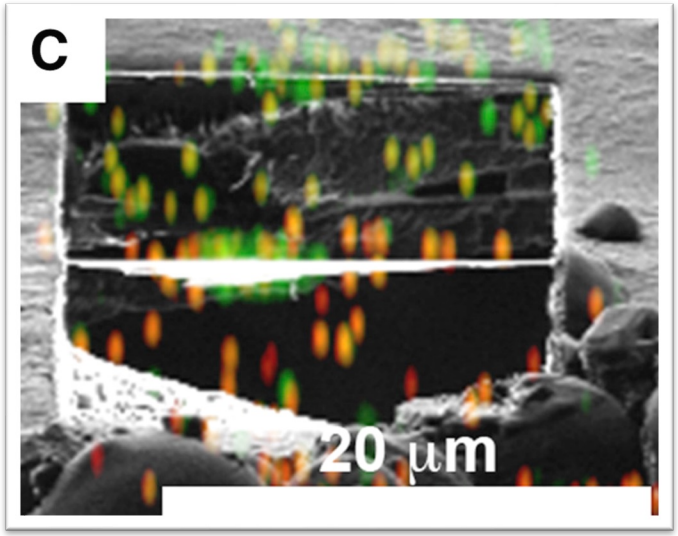
No conflicts of interest with them

Targeted Milling :
Getting the targets within the lamellae

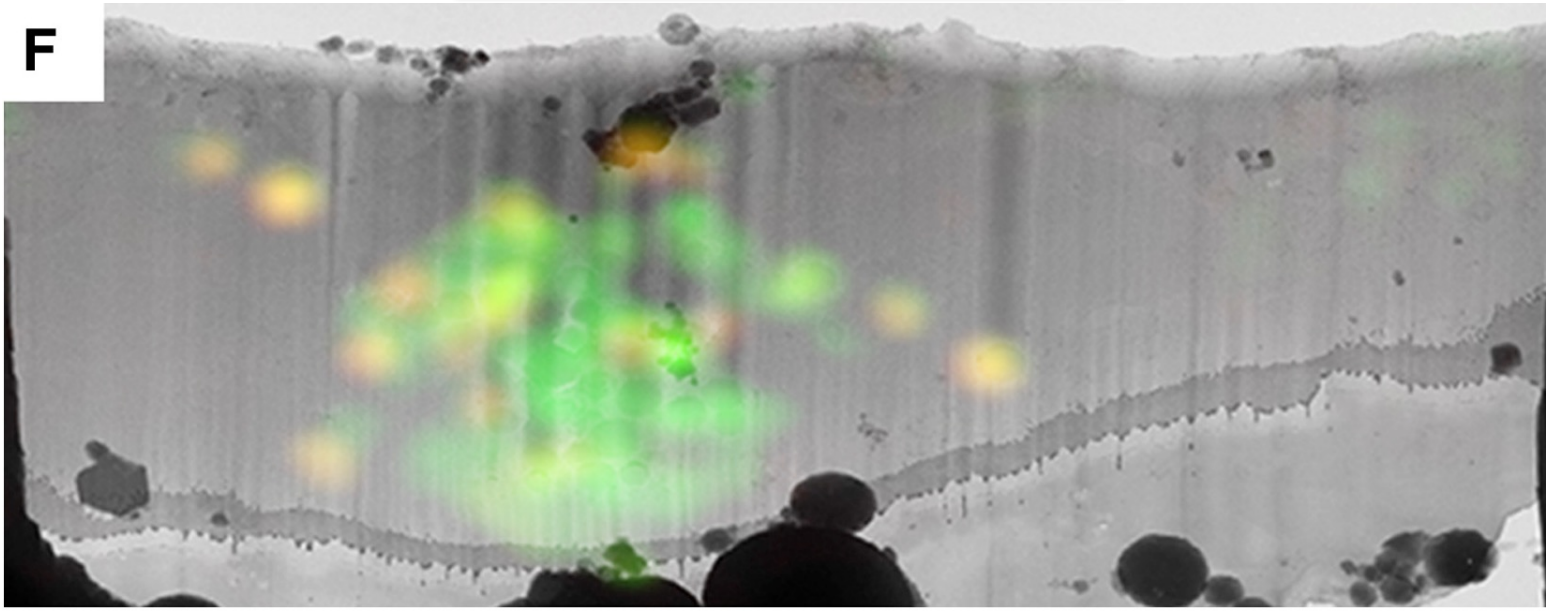


Arnold *et al.* (2016)

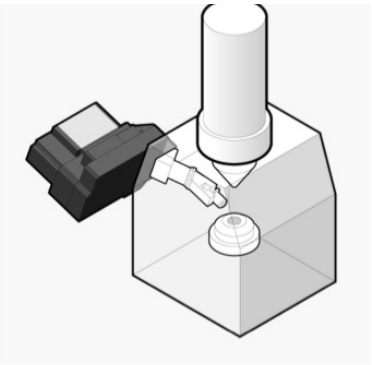


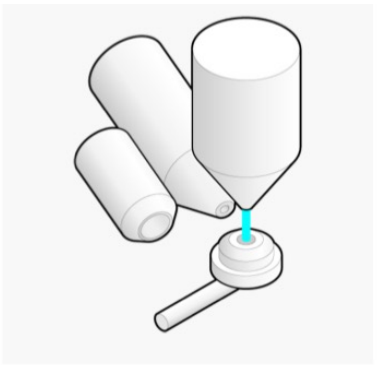


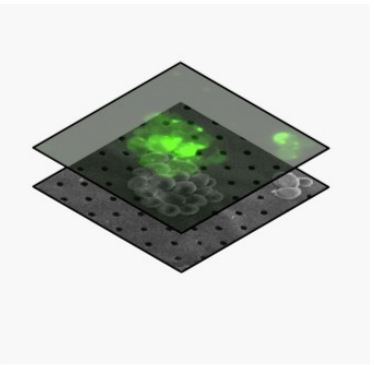
Arnold *et al.* (2016)

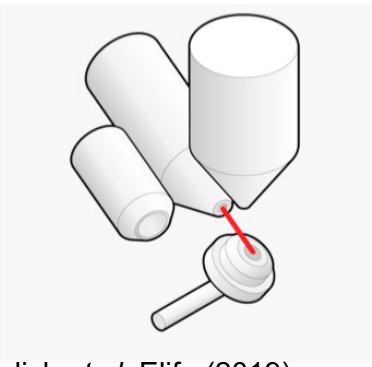


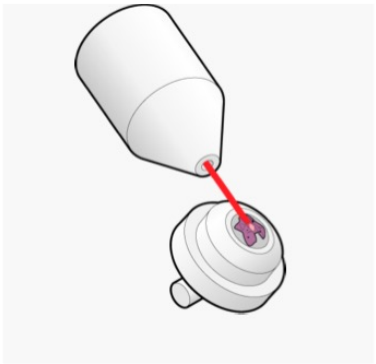
Targeted Milling :
Fluorescence inside the dual-beam

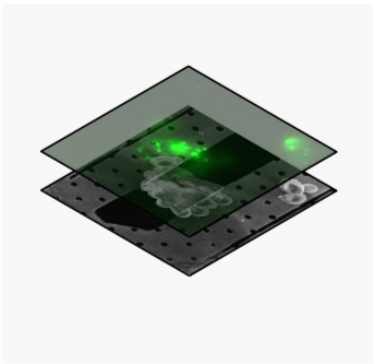












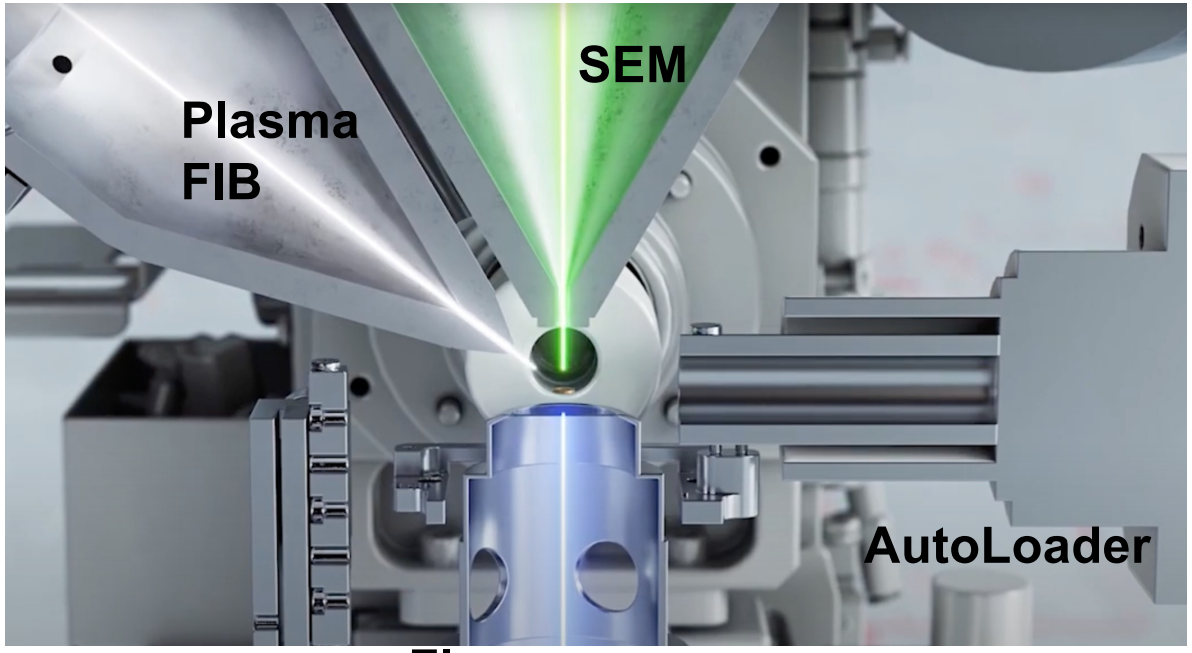
Thermo Fisher

delmic

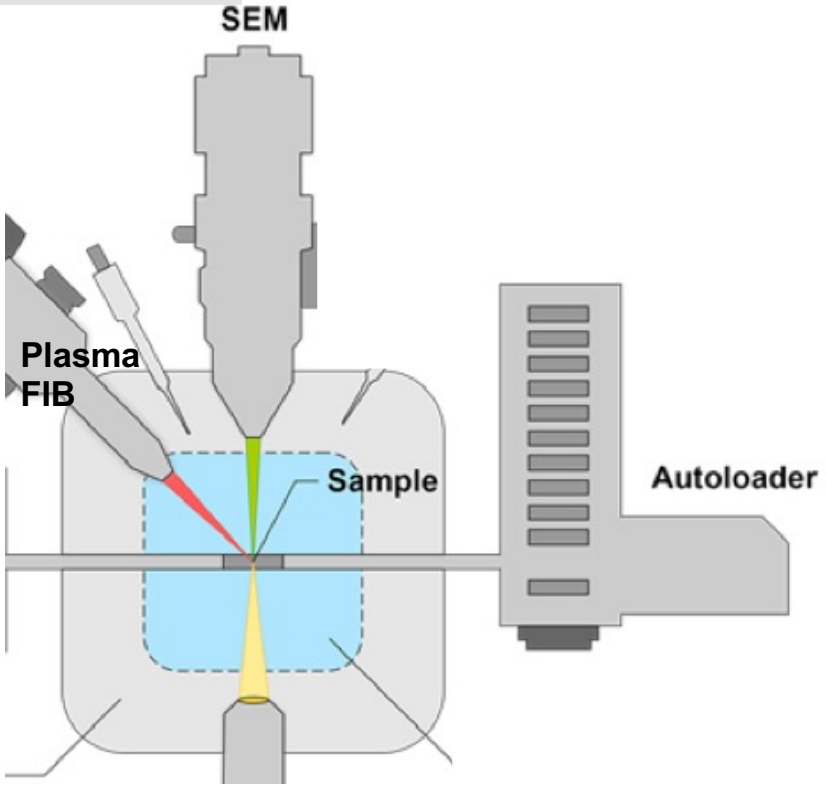
Gorelick *et al.* Elife (2019)

No conflicts of interest

Targeted Milling :
Fluorescence while milling



Fluorescence

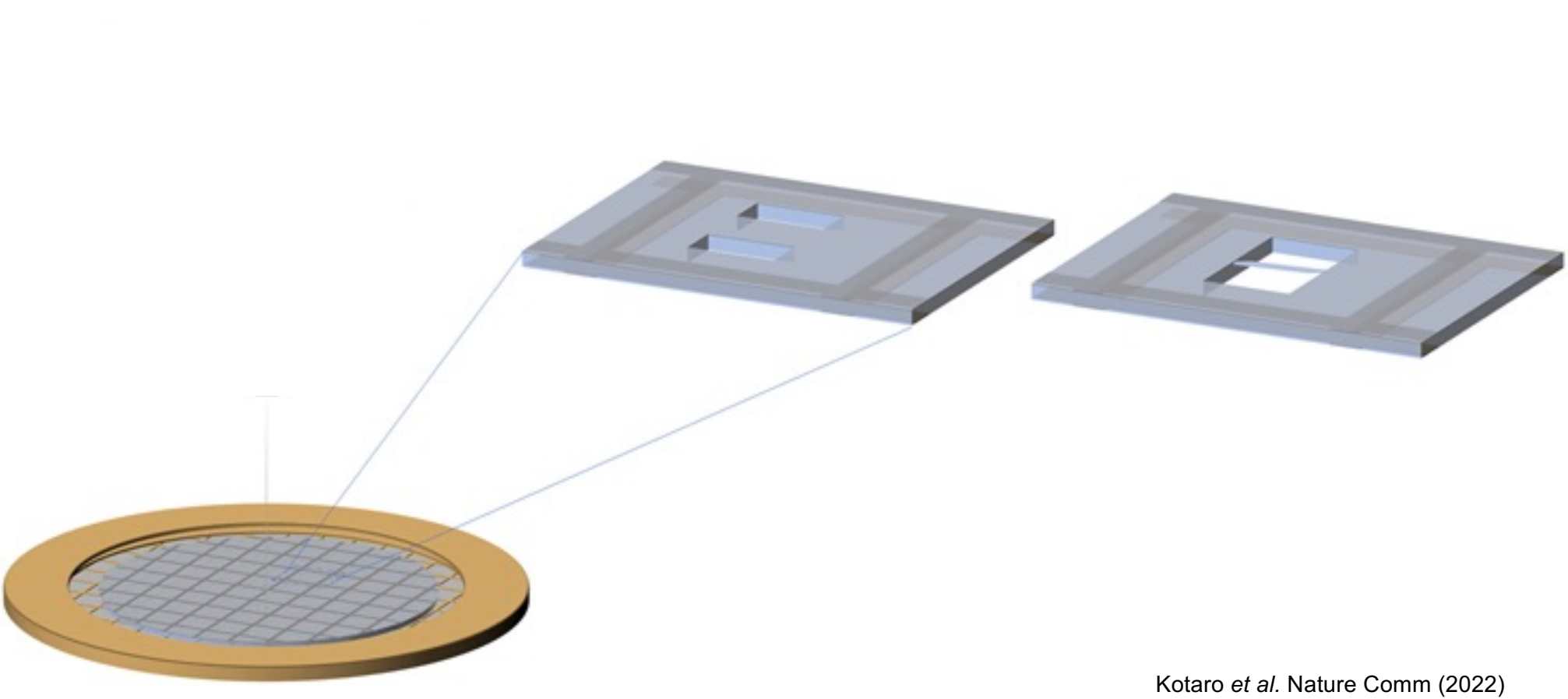


Fluorescence

Berger *et al.* (2023)

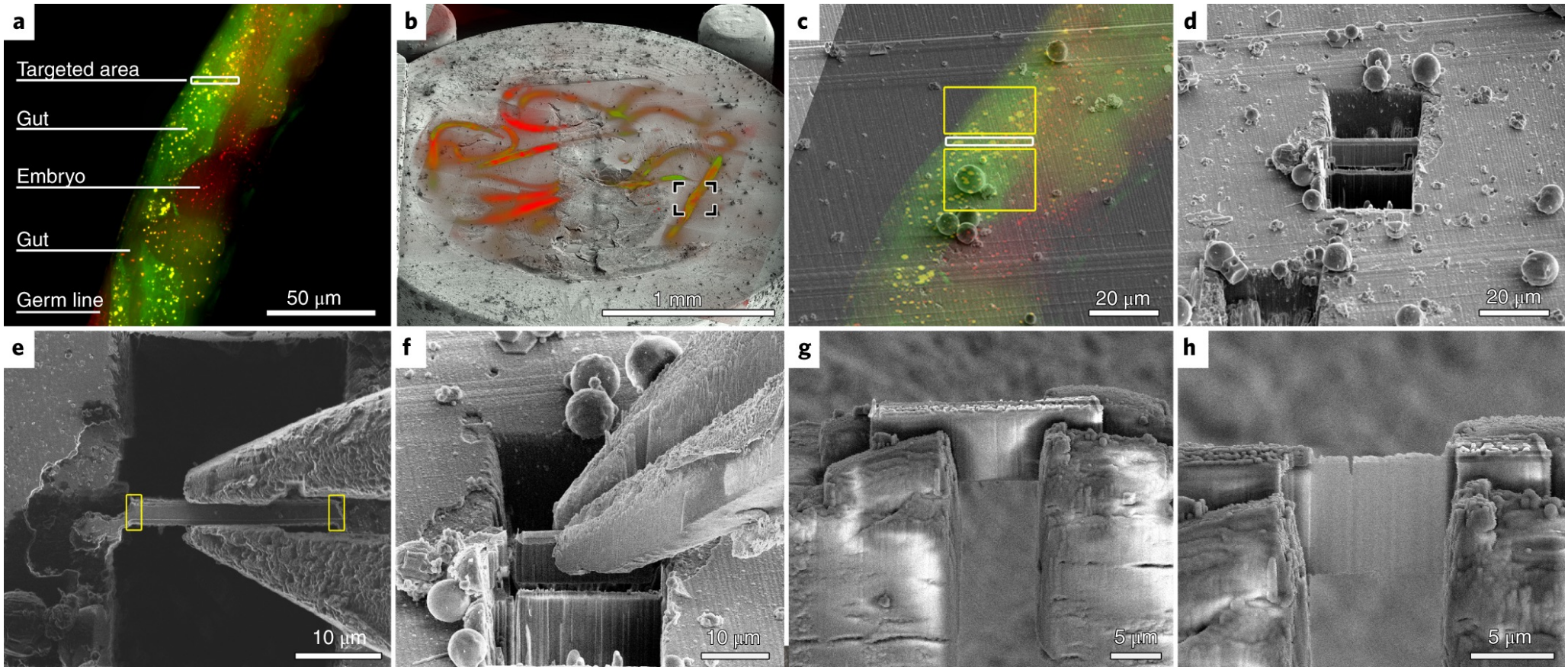
Thermo Fisher

More complicated samples :
Waffle method for high-pressure frozen and thicker samples

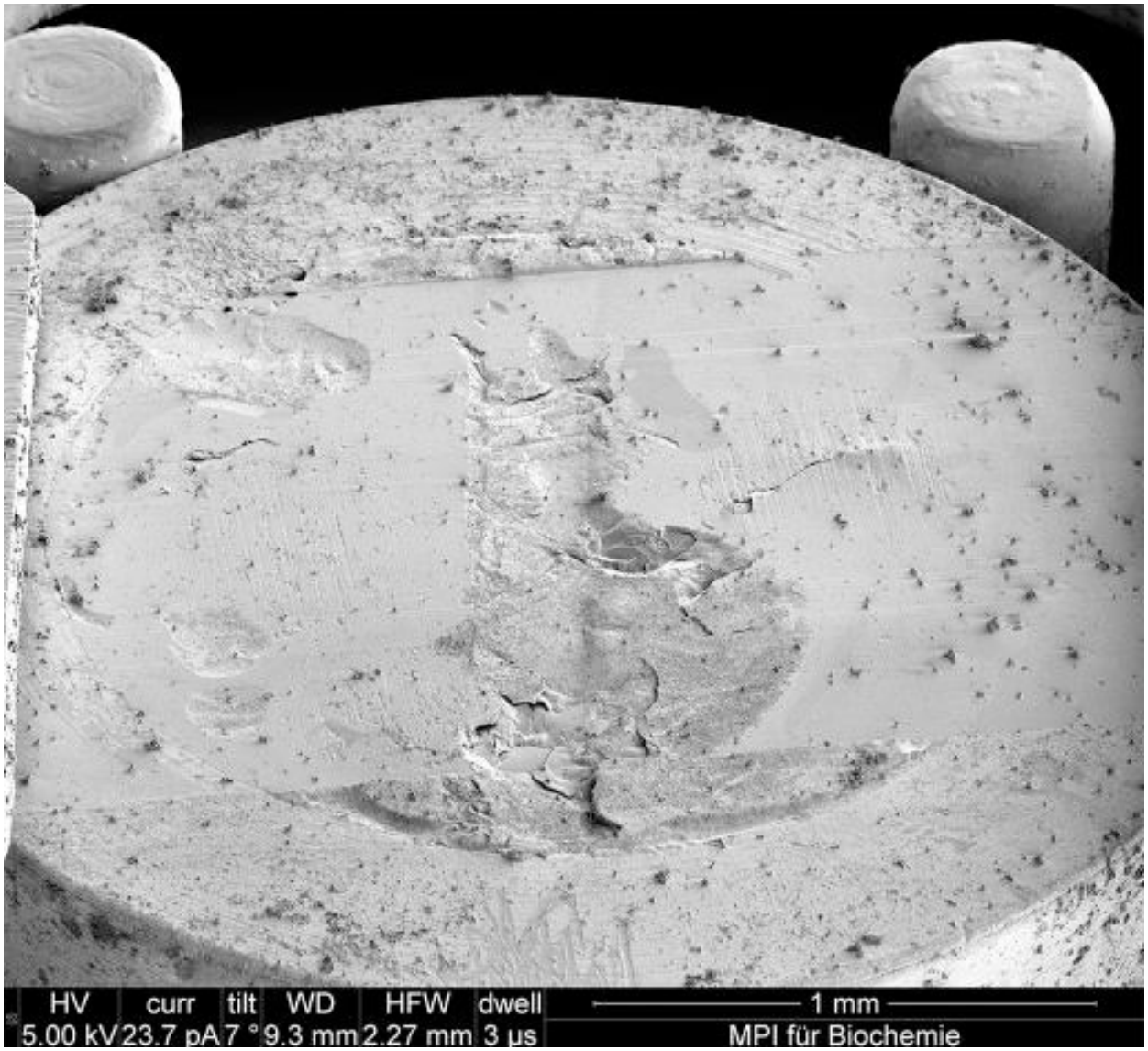


Kotaro *et al.* Nature Comm (2022)

More complicated samples :
Bringing tissues and other thick samples within reach

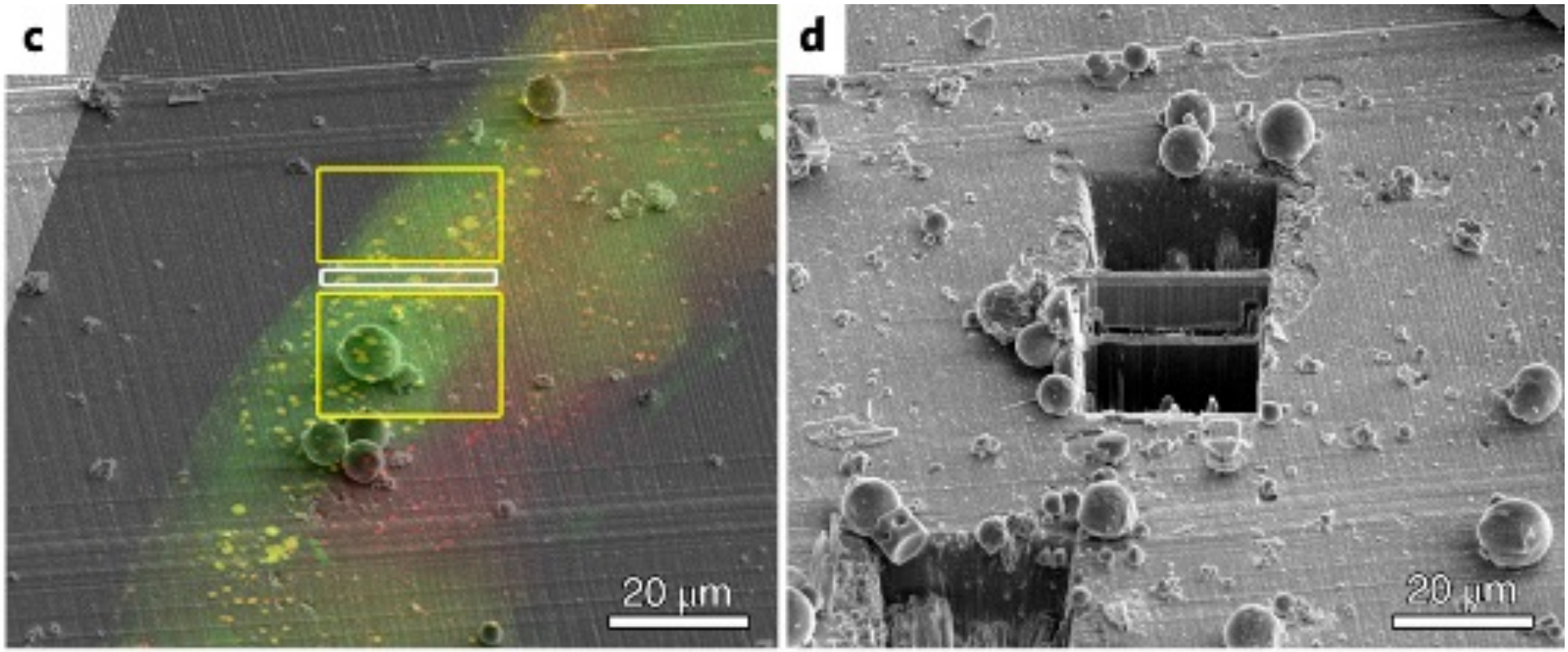


Schaffer et al. Nature Methods (2019)



Schaffer et al. (2019)

Lift out to circumvent poor Z- resolution



Schaffer *et al.* (2019)

Thank you !