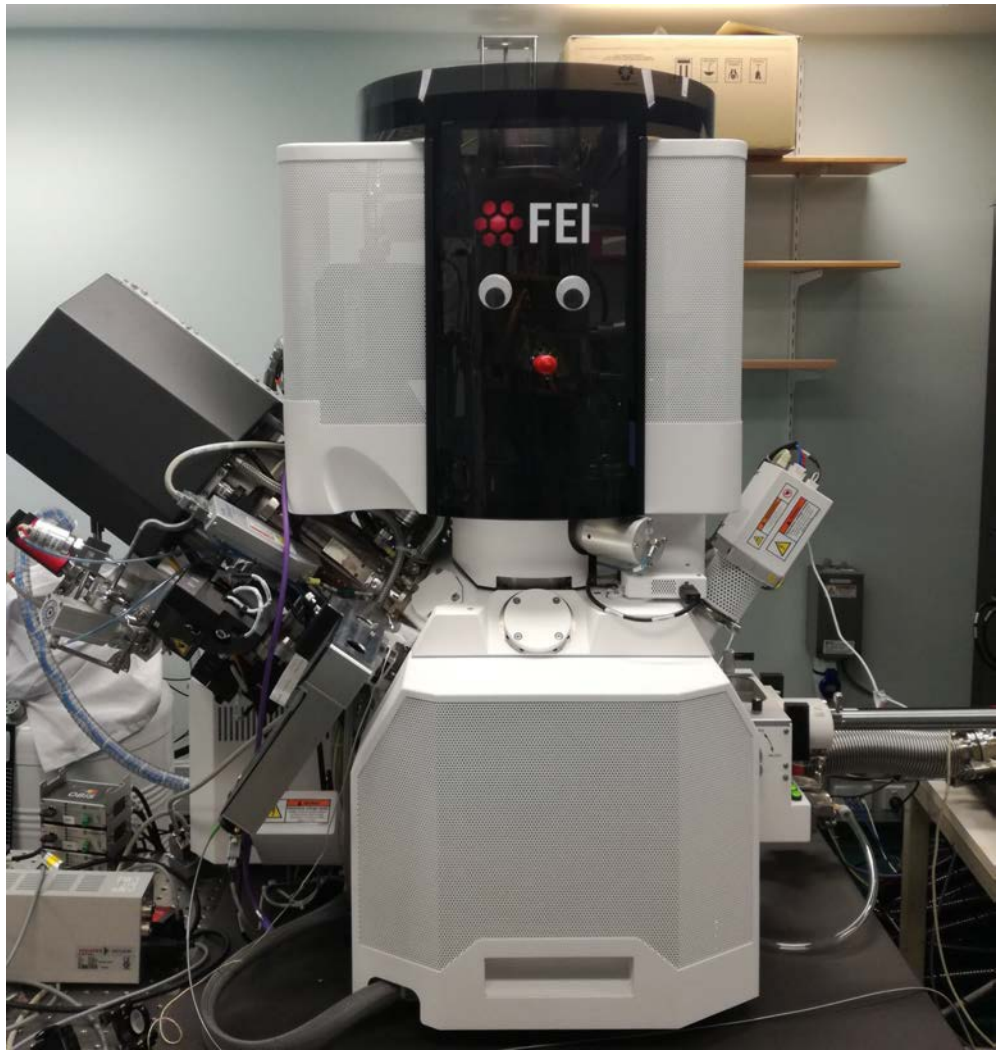
FIBs

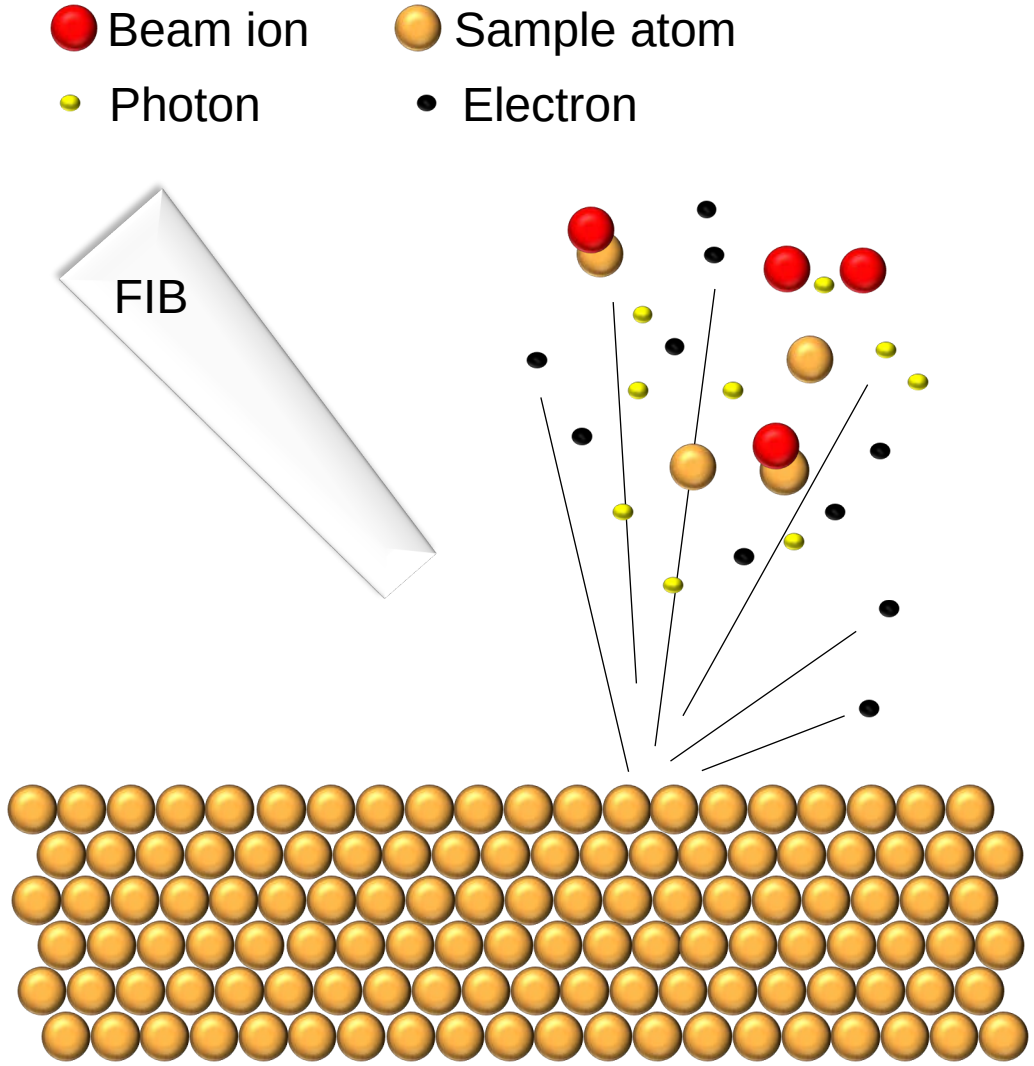
Alex de Marco

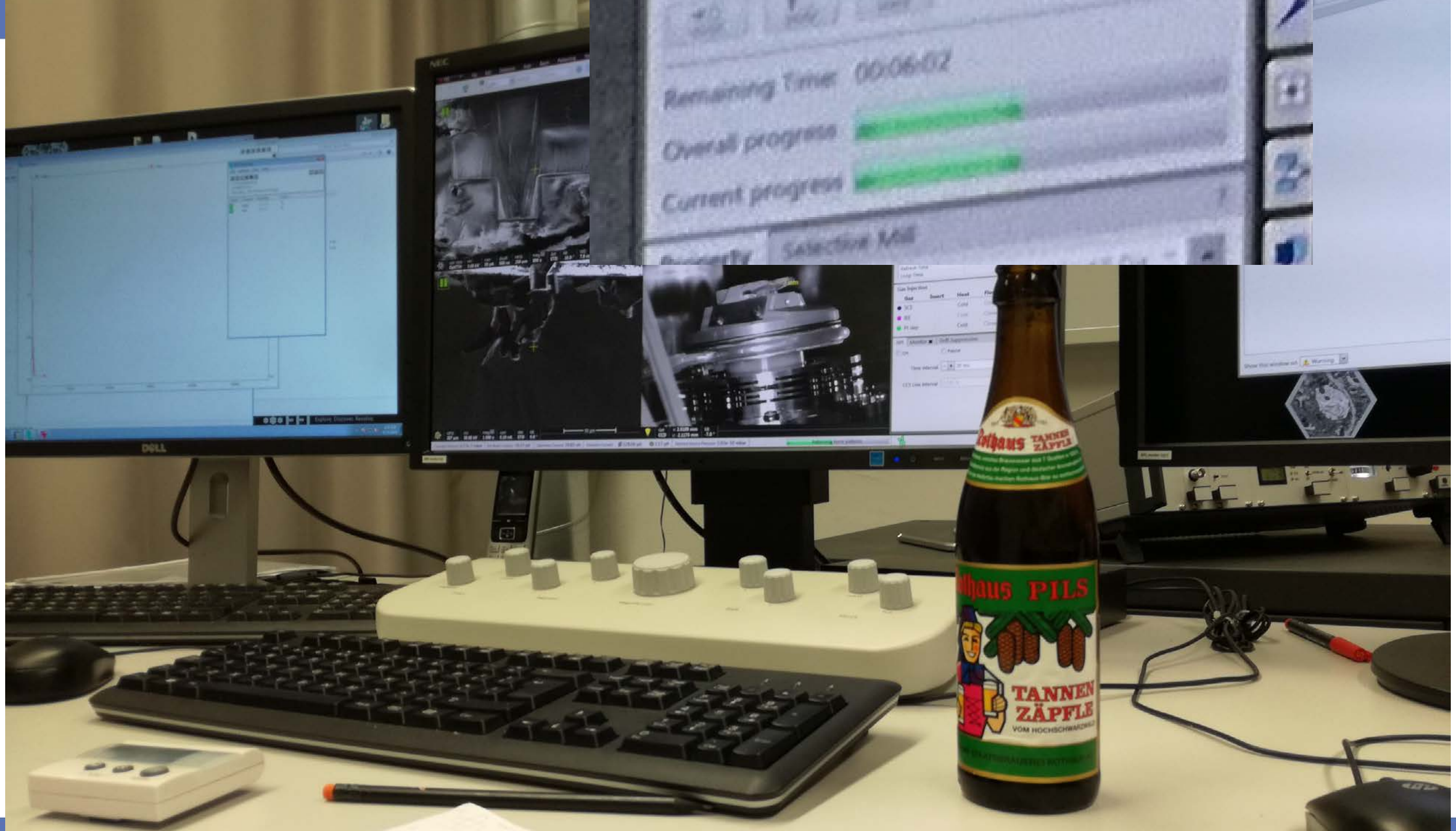
Tuesday, April 1, 2025

NYSBC SEMC

Focused ion beam microscopy

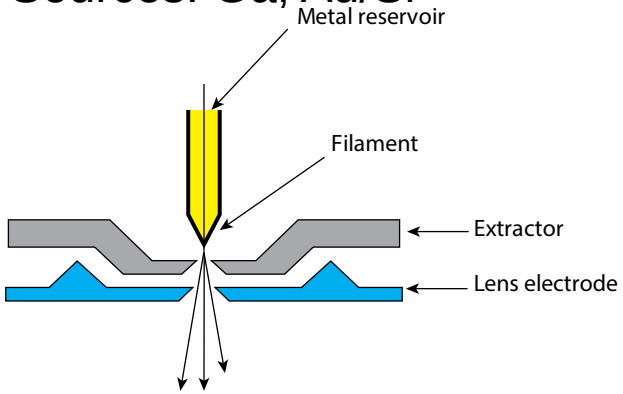




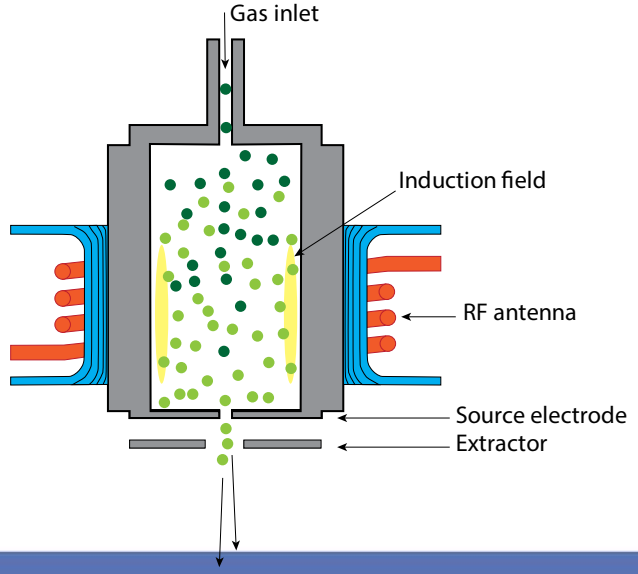

Remaining Time: 00:06:02
Overall progress
Current progress
Selective Mill
DELL
NEC
Rothaus PILS
TANNEN ZÄPFLE
VOM HOCHSCHWARZWALD

Plasma focused ion beam microscopy

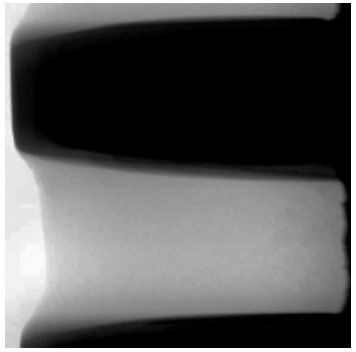
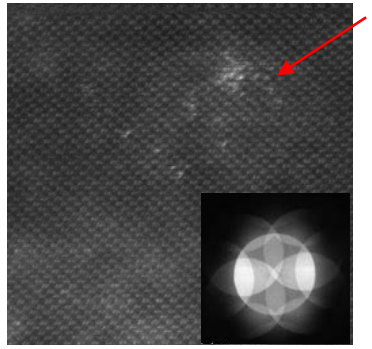
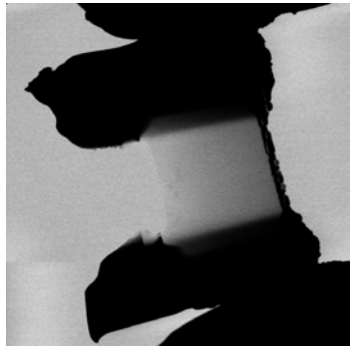
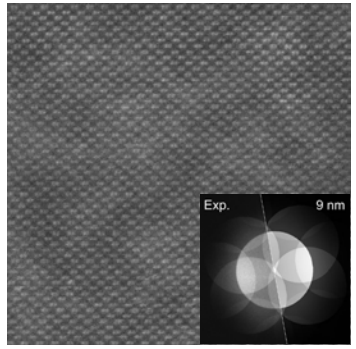
LMIS: 1pA to 1e5 pA
Sources: Ga, Au/Si



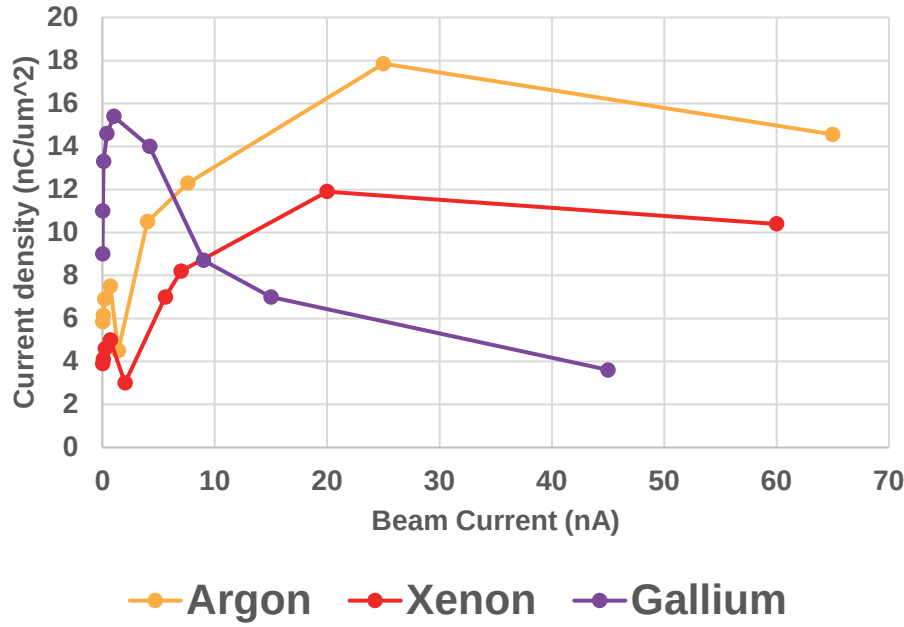
Plasma: 1pA to 2.5e6 pA
Sources: He, Ne, Ar, Kr, Xe, O, N



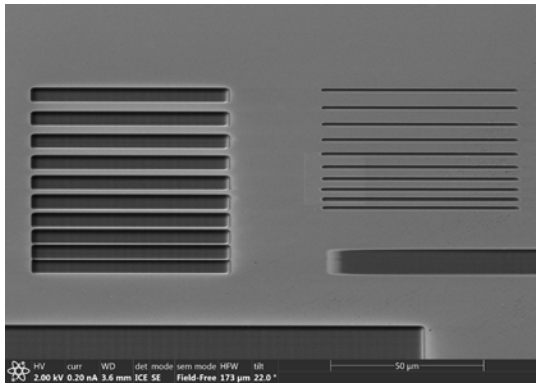
TEM sample preparation on C-diamond

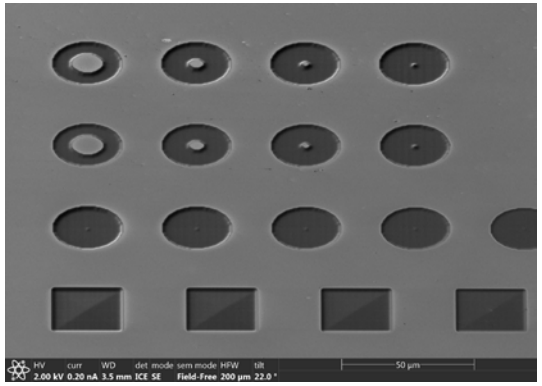
	LM	HR
Ga		
O2		

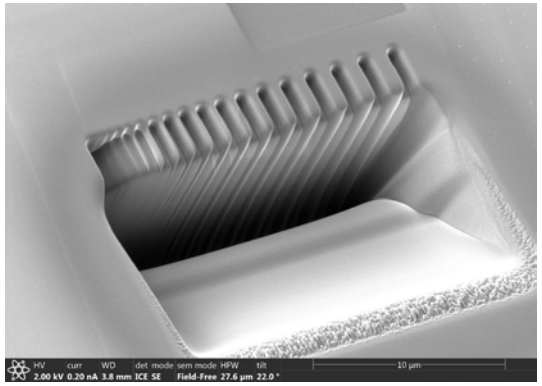
Metal implantation

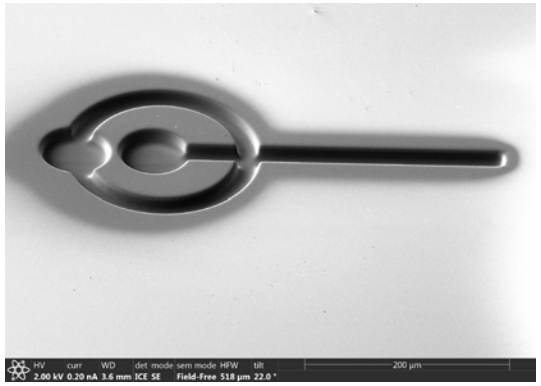


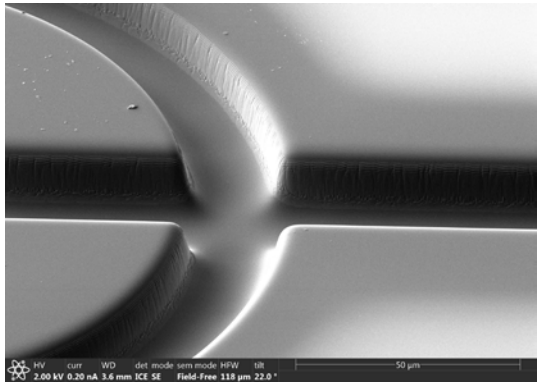
FIB uses

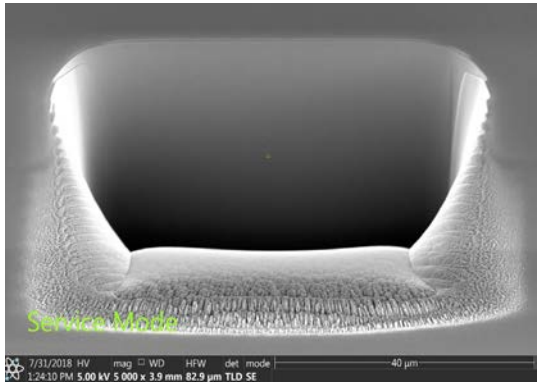






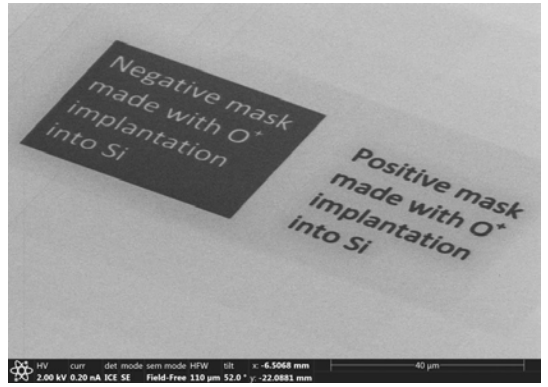




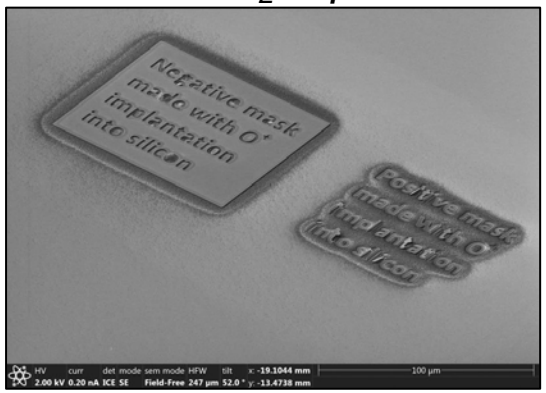


Direct mask writing

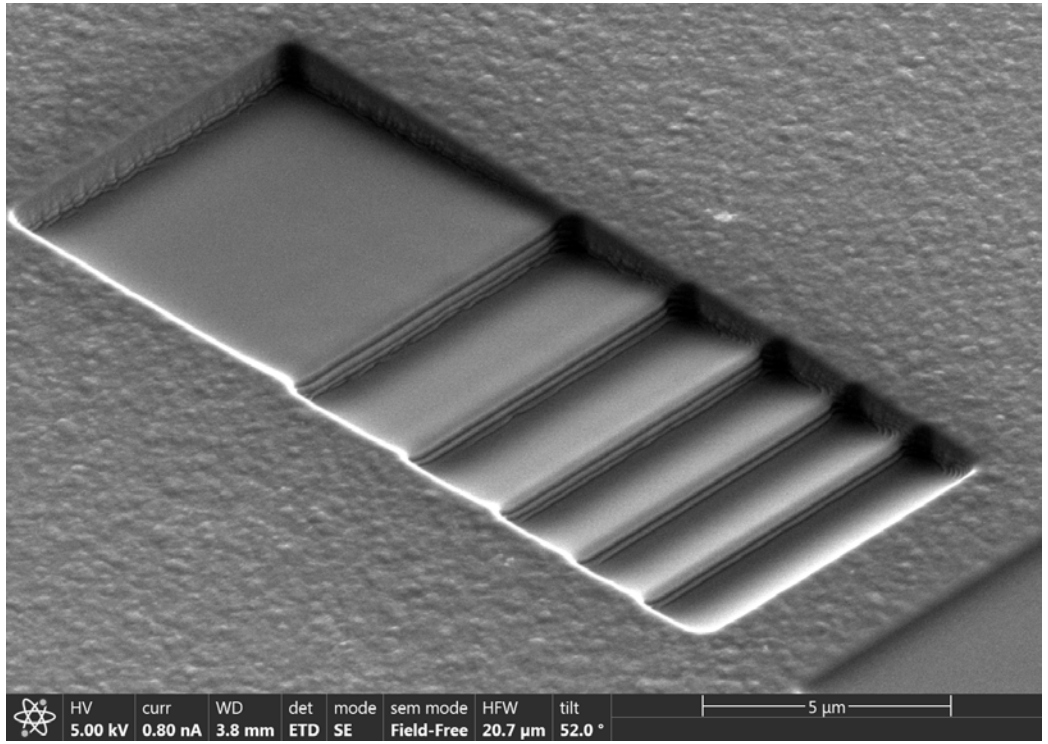
Si substrate irradiated with O^+

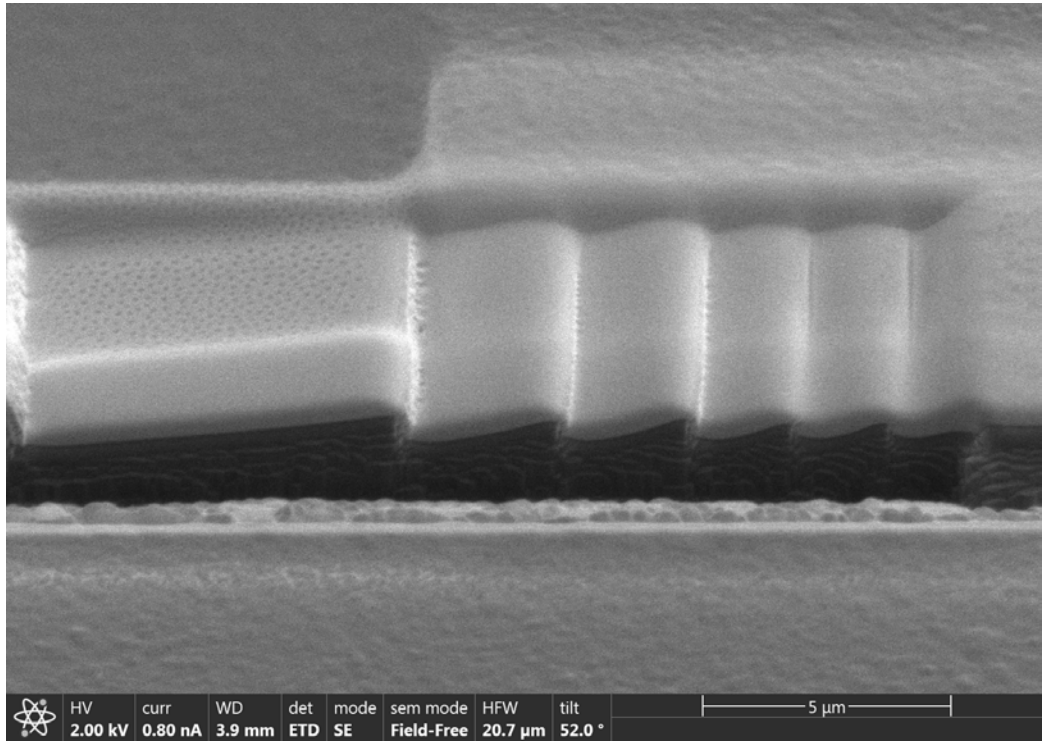


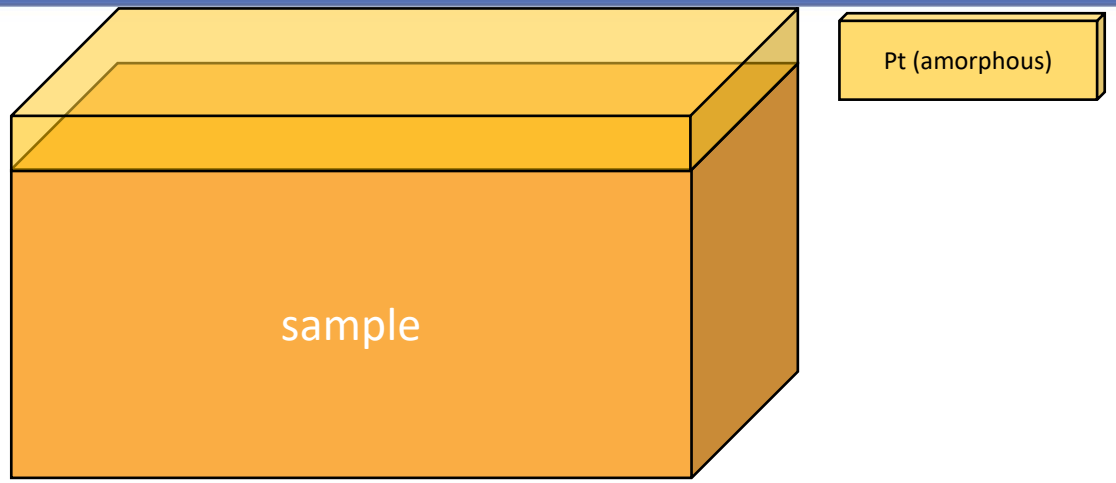
After XeF_2 exposure



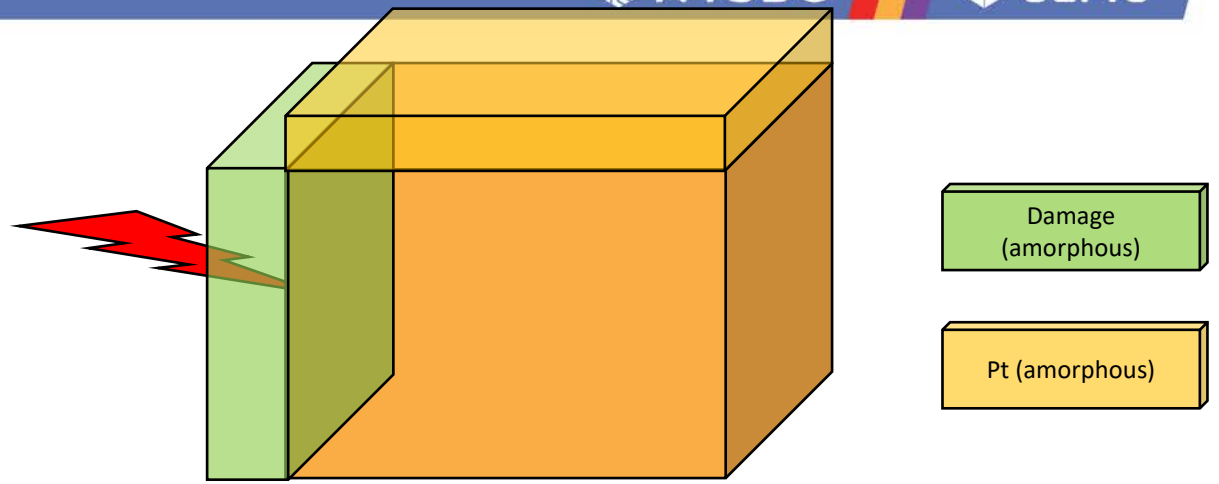
Ion dose can be tuned



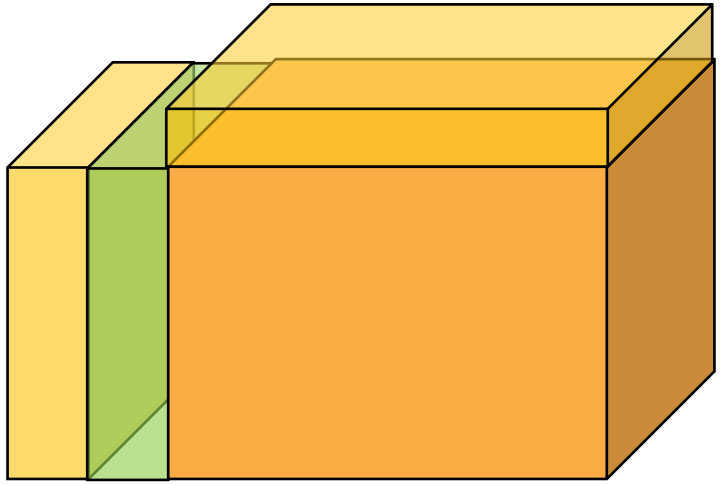




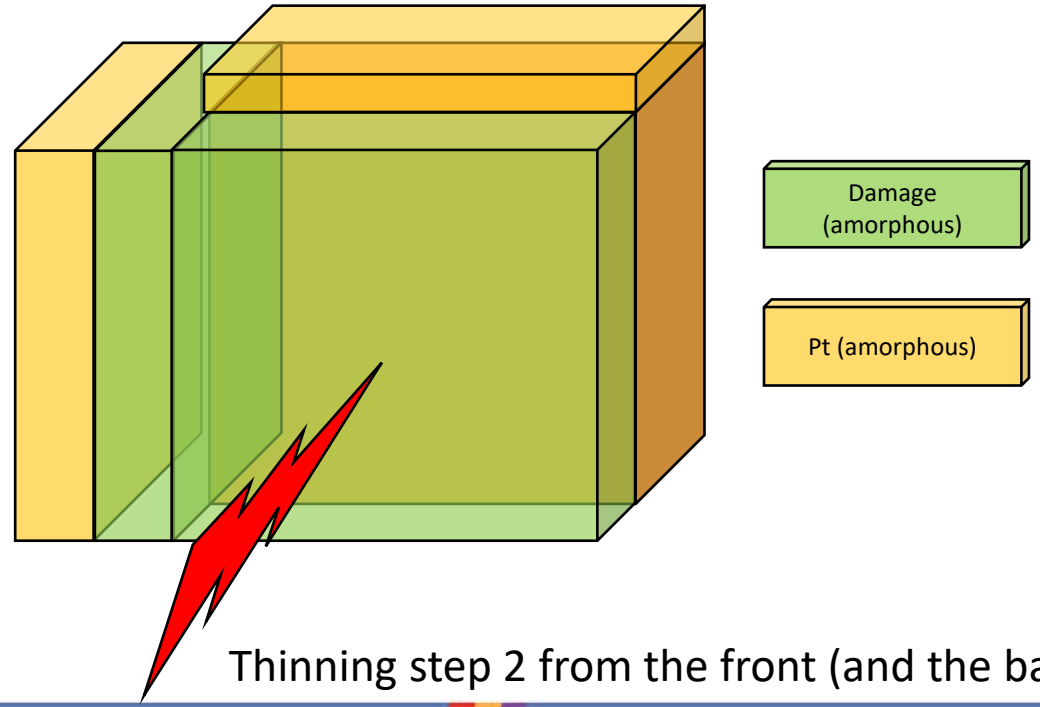
Original state



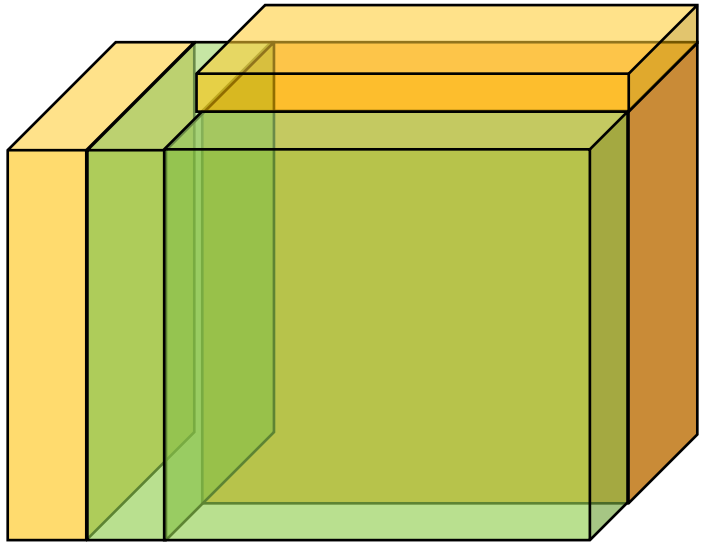
Thinning step 1 from the side



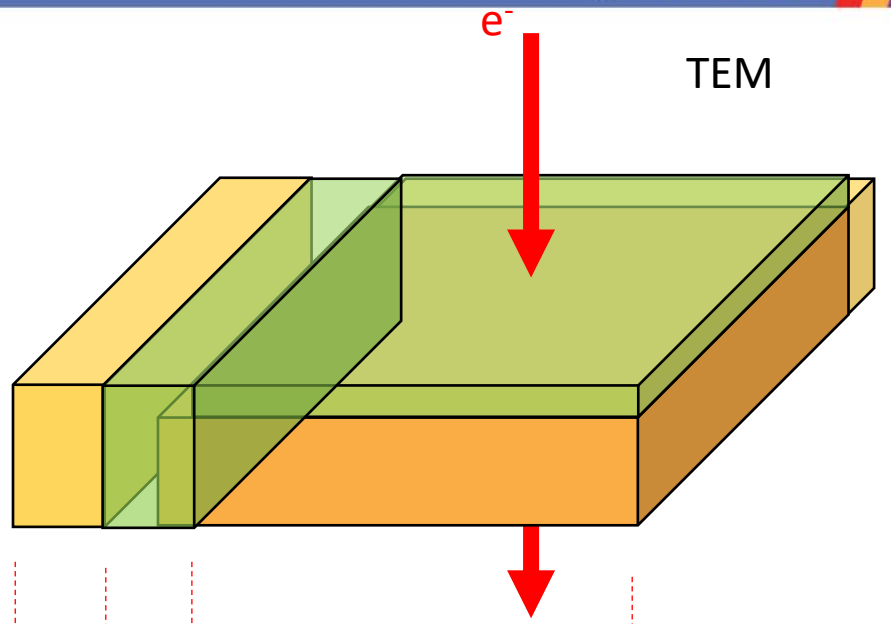
Add Pt cap on the side



Thinning step 2 from the front (and the back)

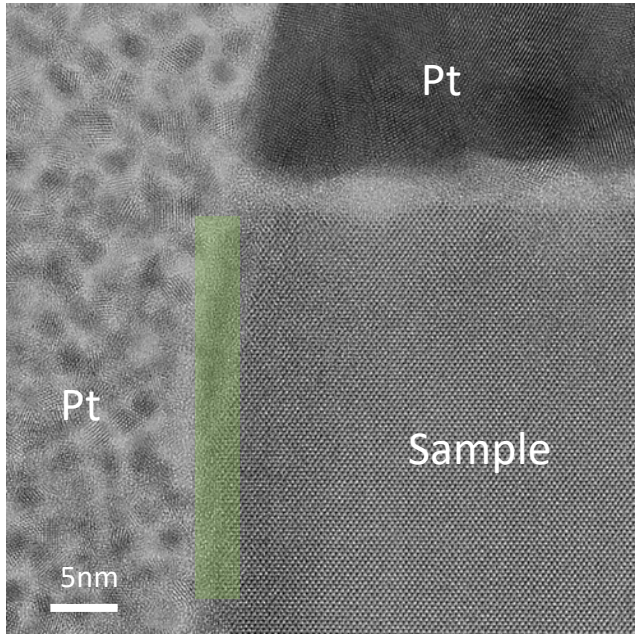


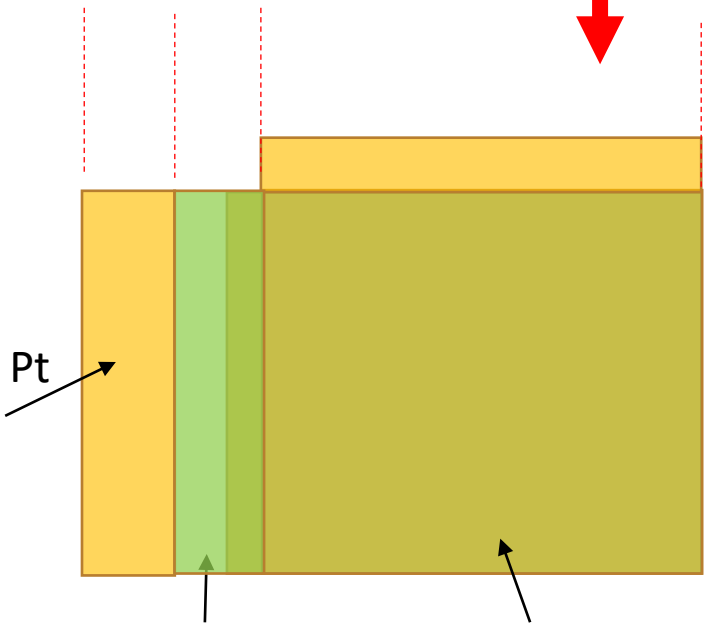
Flip the sample



Damage (amorphous)

Pt (amorphous)

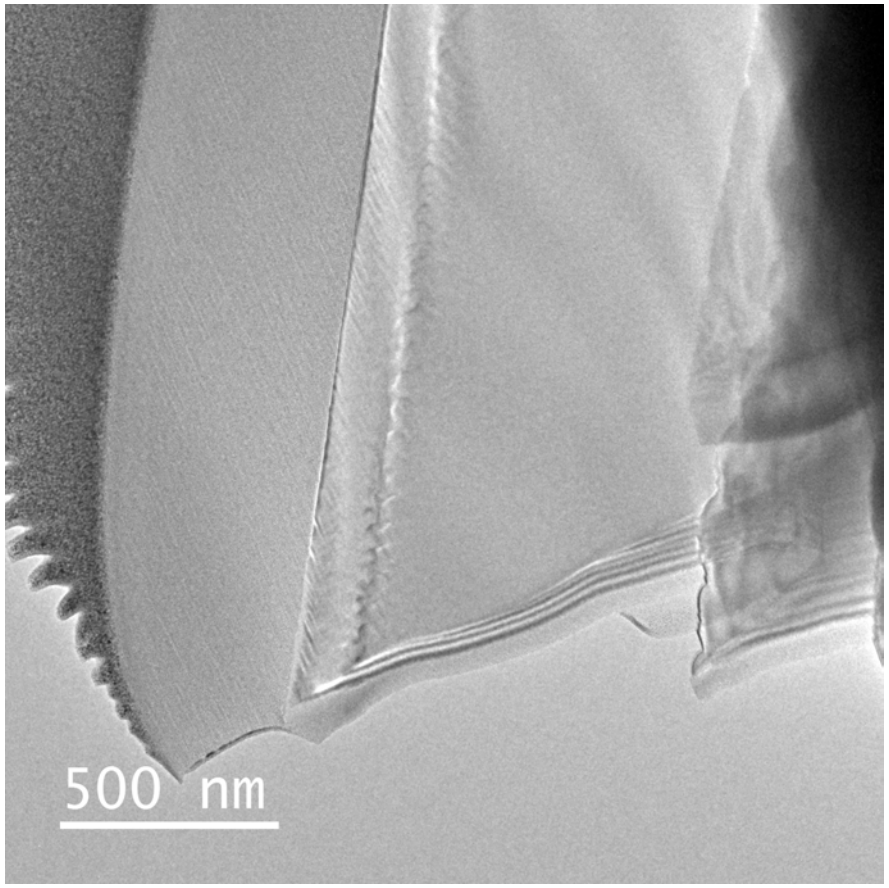




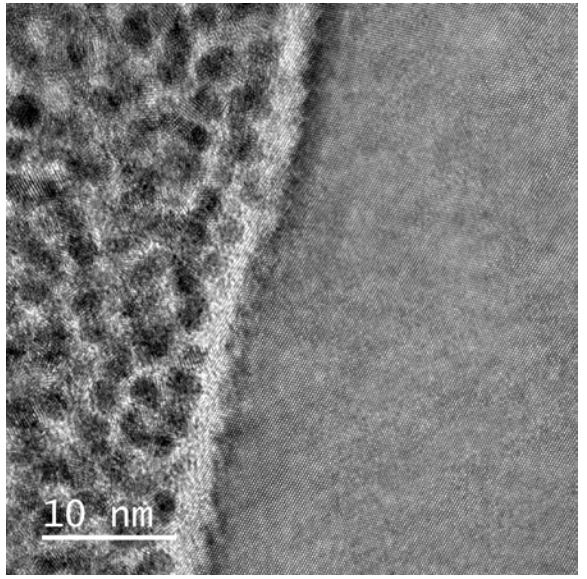
Damage layer From step 1

Damage layer + Crystal (mainly)

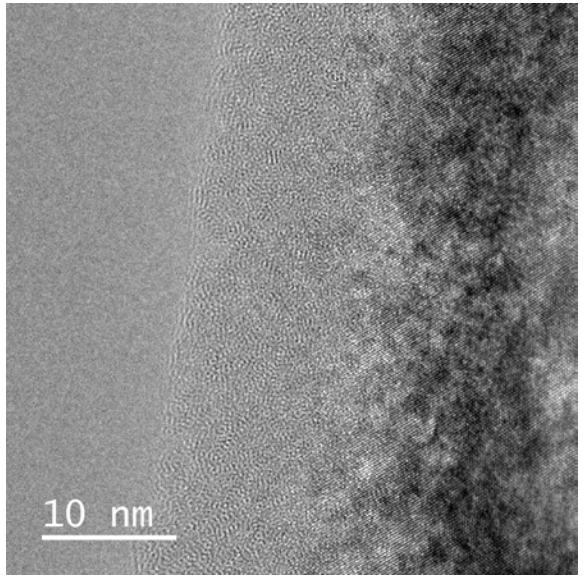
Damage on Diamond



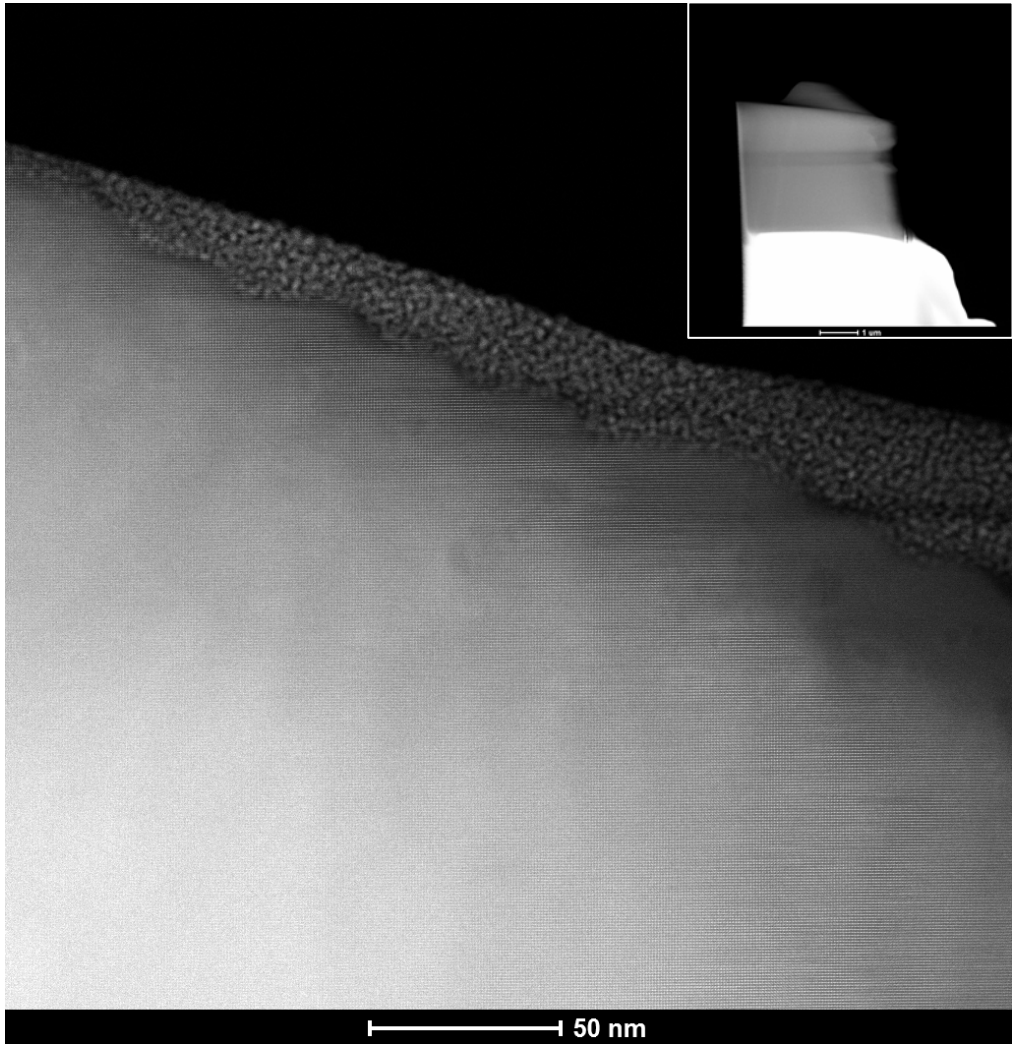
Ga

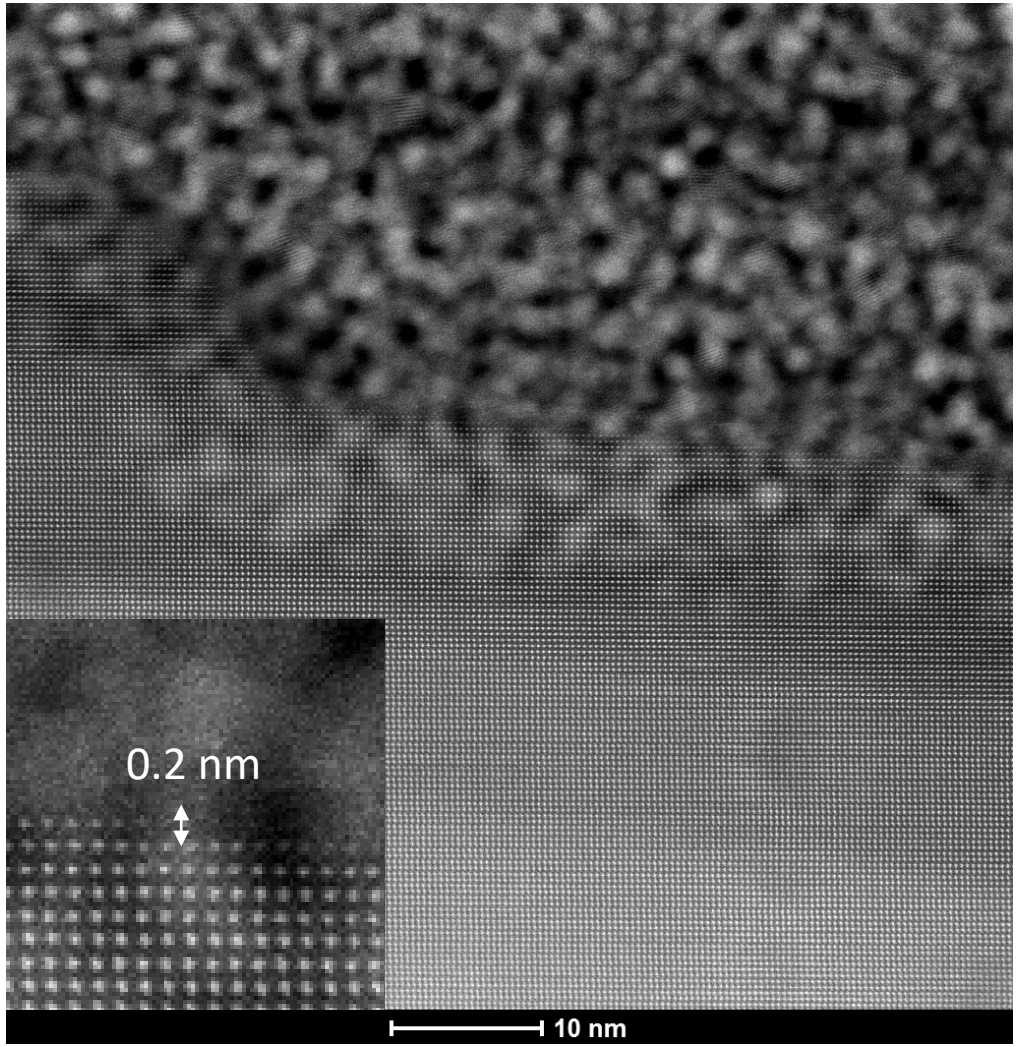


O2

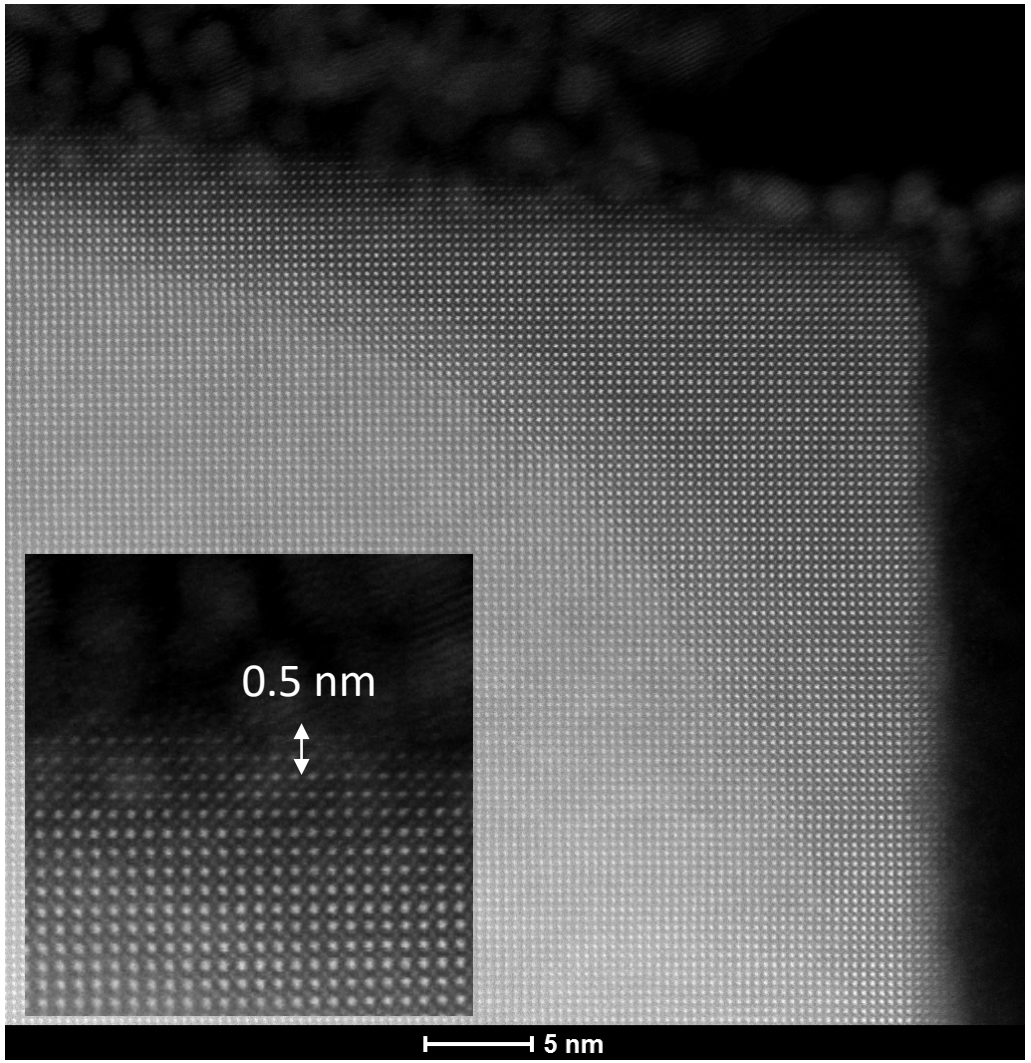


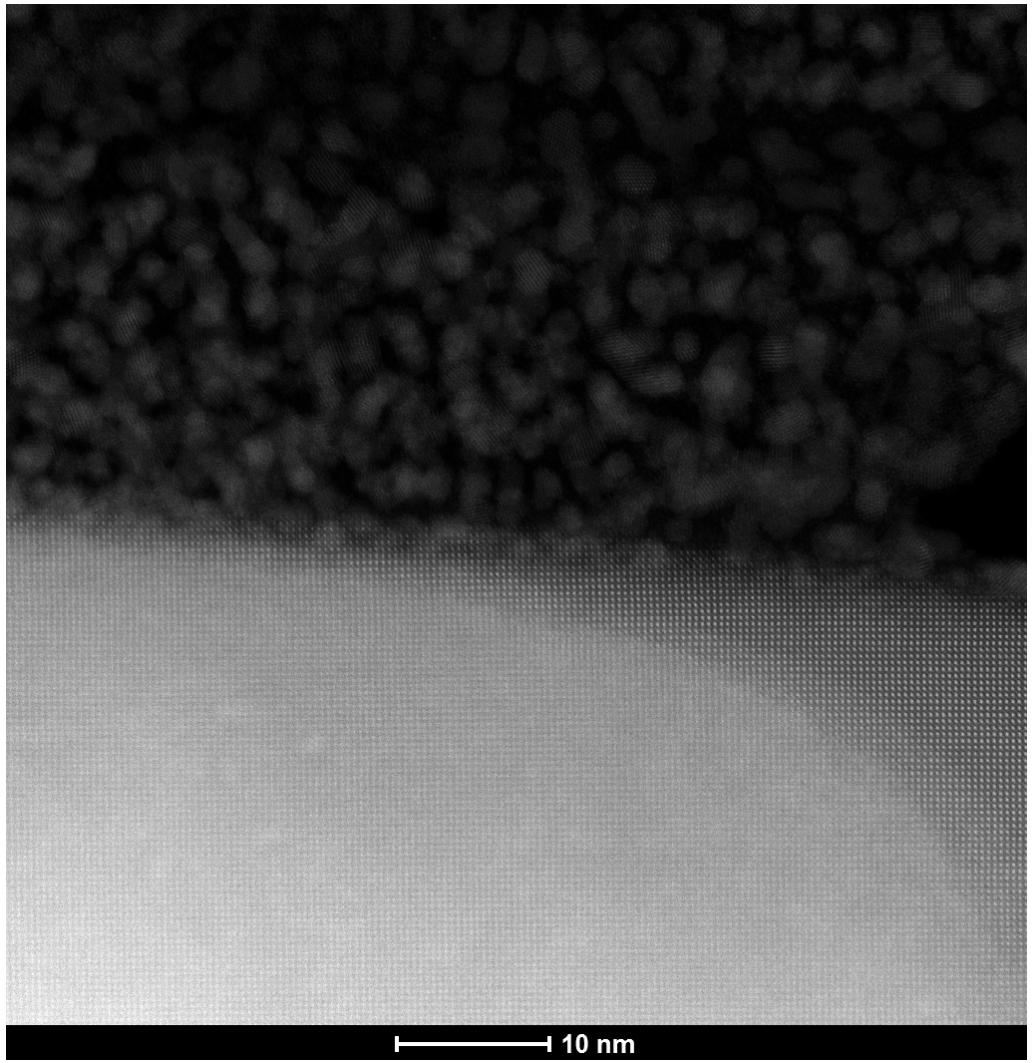
Damage on BFO using Xe



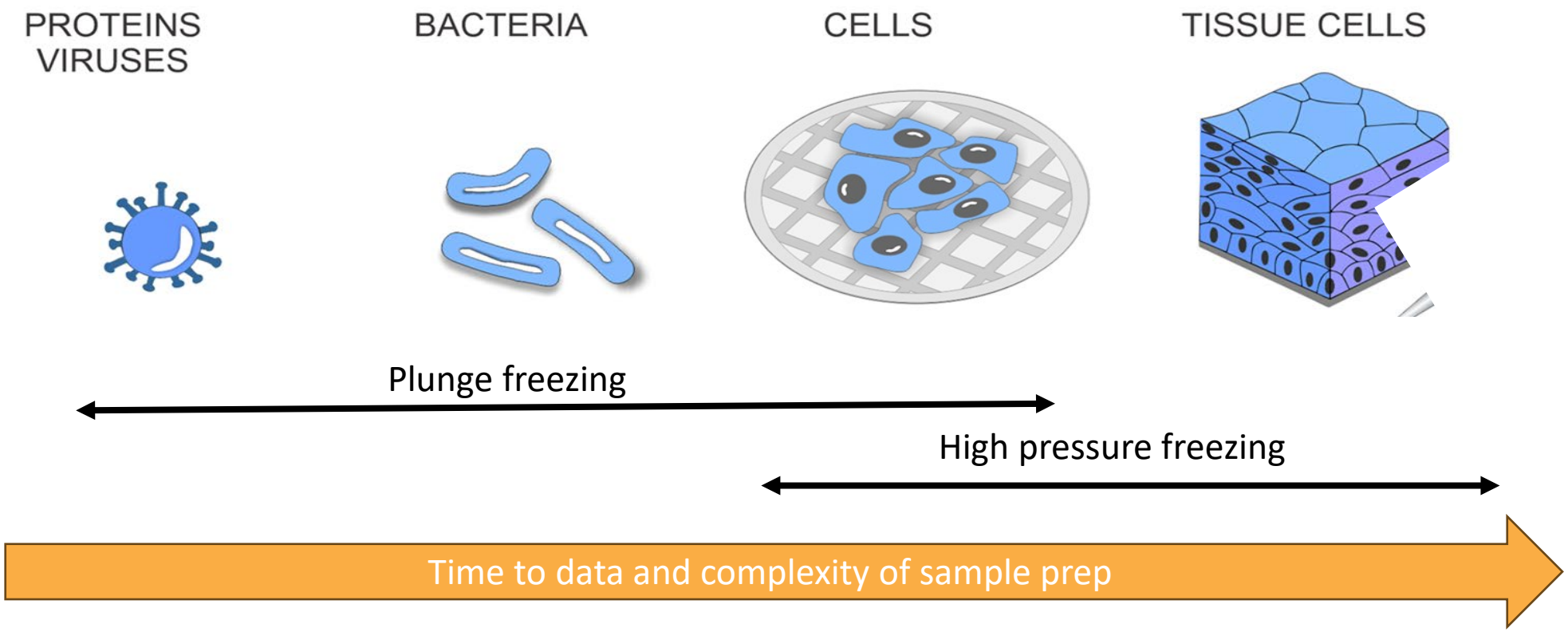


Damage on BFO using Ar





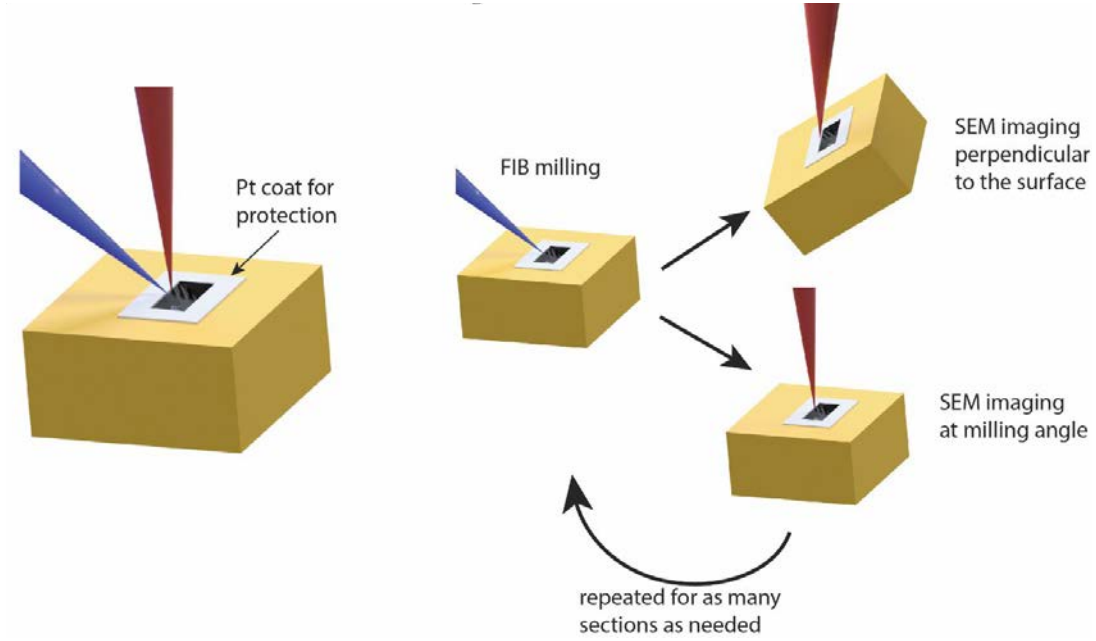
Samples



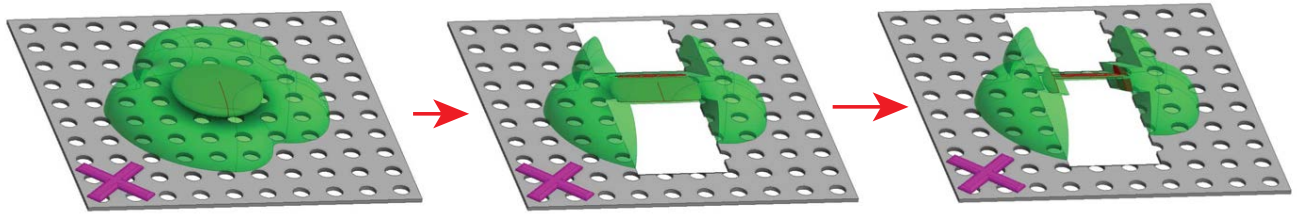
Adapted from Klumpe et al Mic today 2022

FIB in life sciences

FIB/SEM tomography



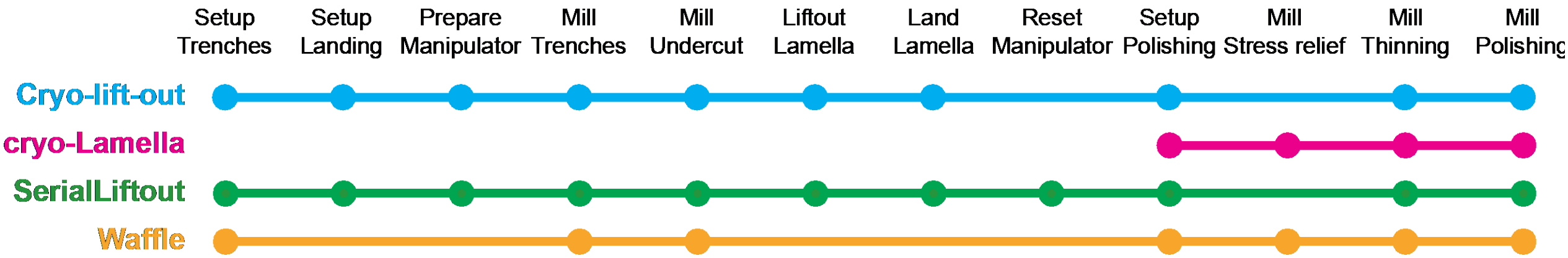
Cryo-lamella preparation



The sample preparation protocol is defined by the sample

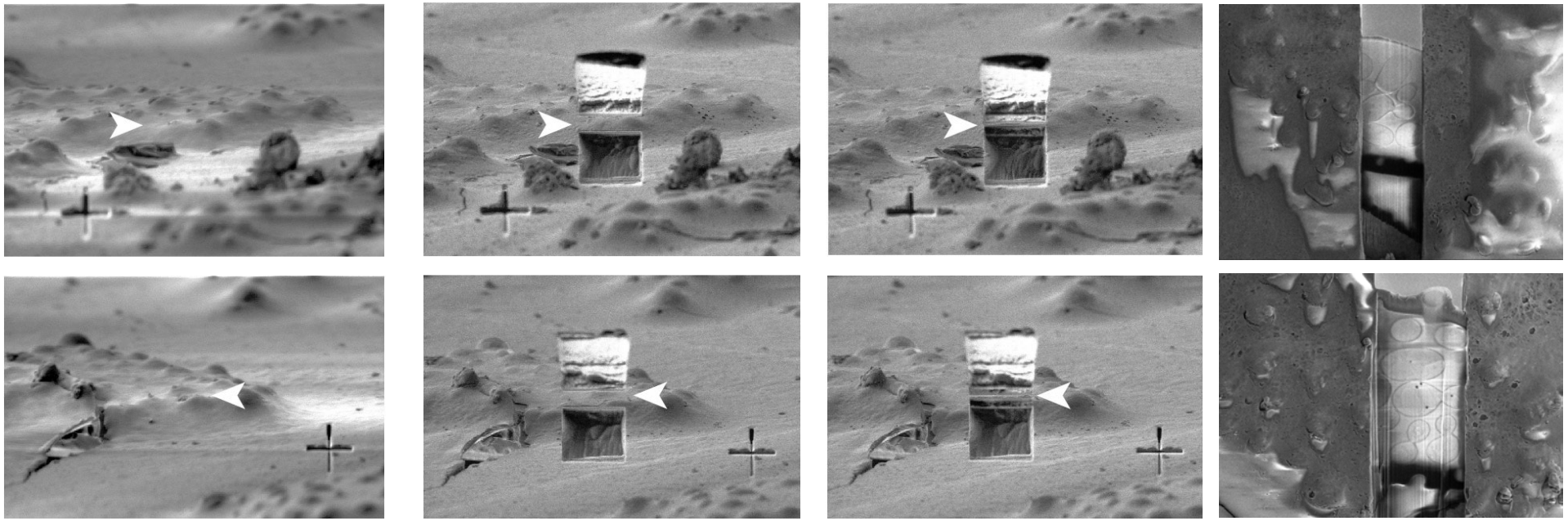
3D Imaging is typically performed over tens of microns at 5-20 nm resolution

FIB workflows are all the same

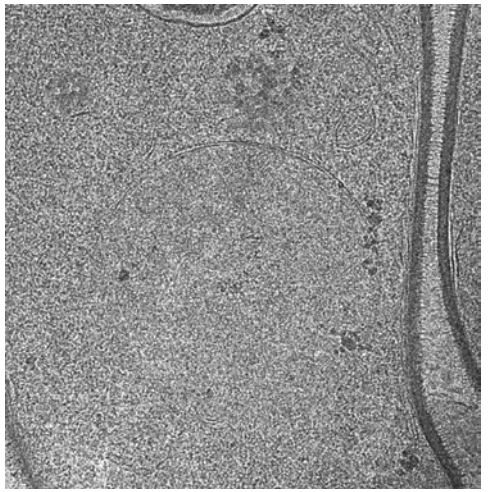


Automated lamella preparation

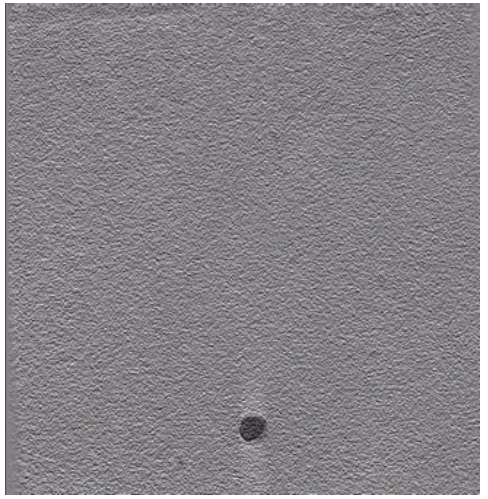
Select ROIs → Rough milling → Polish



Low mag cryo-TEM



Cryo-ET

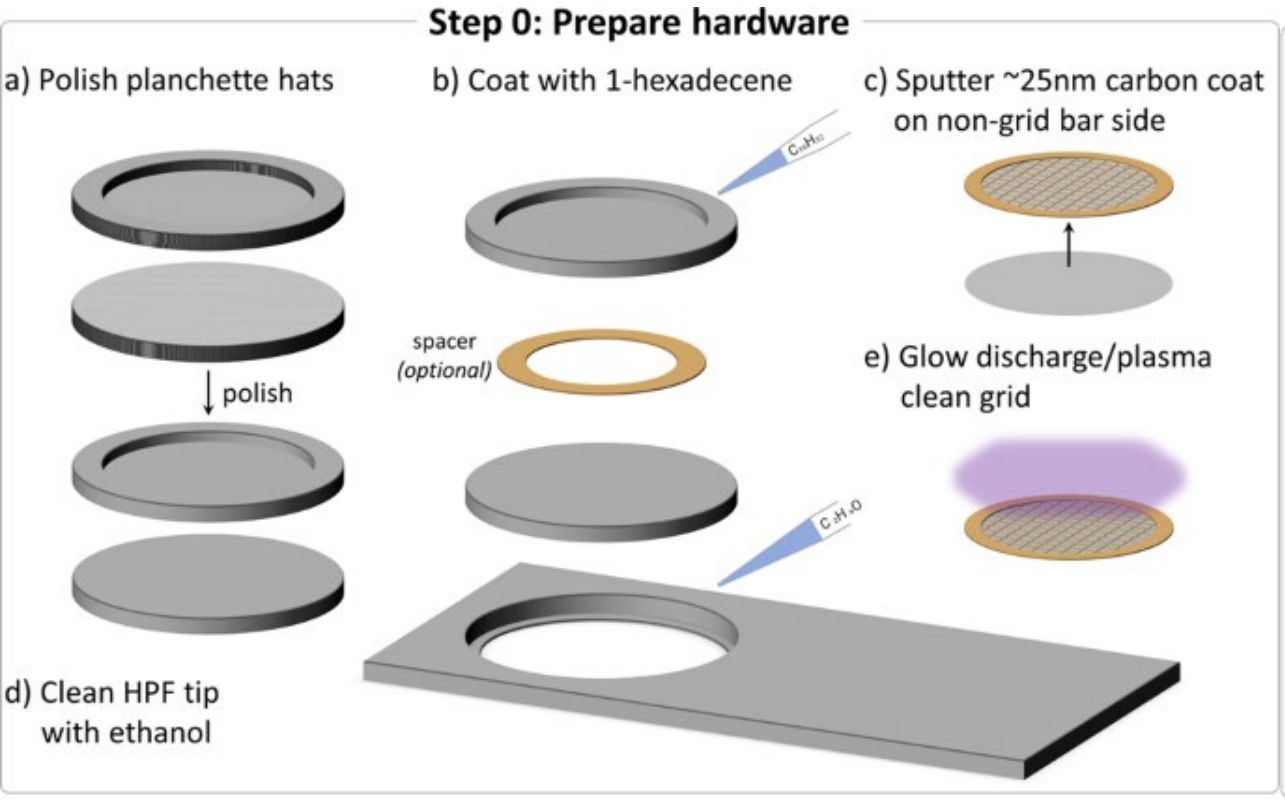


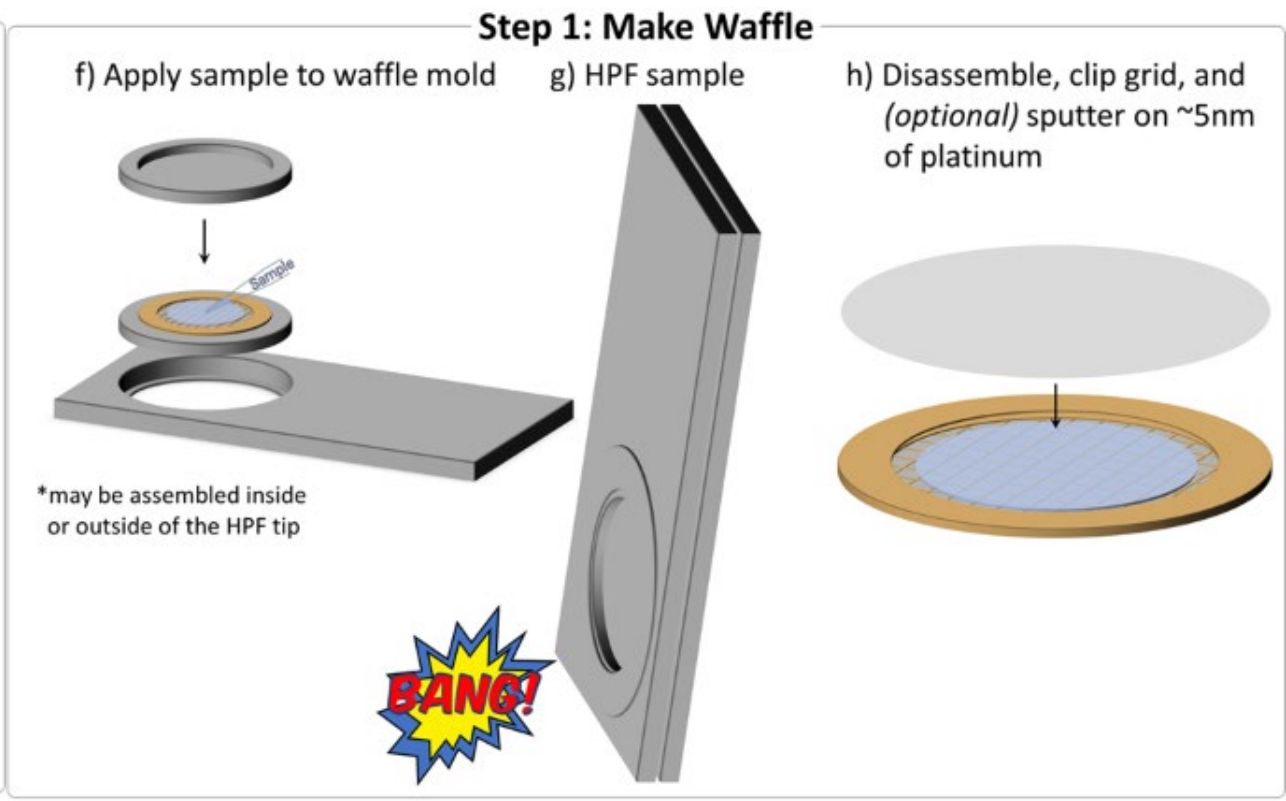
Batch lamellae preparation leads to up to 4-6 lamellae / hour

www.github.com/demarcolab/autolamella

Buckley et al. JSB 2020

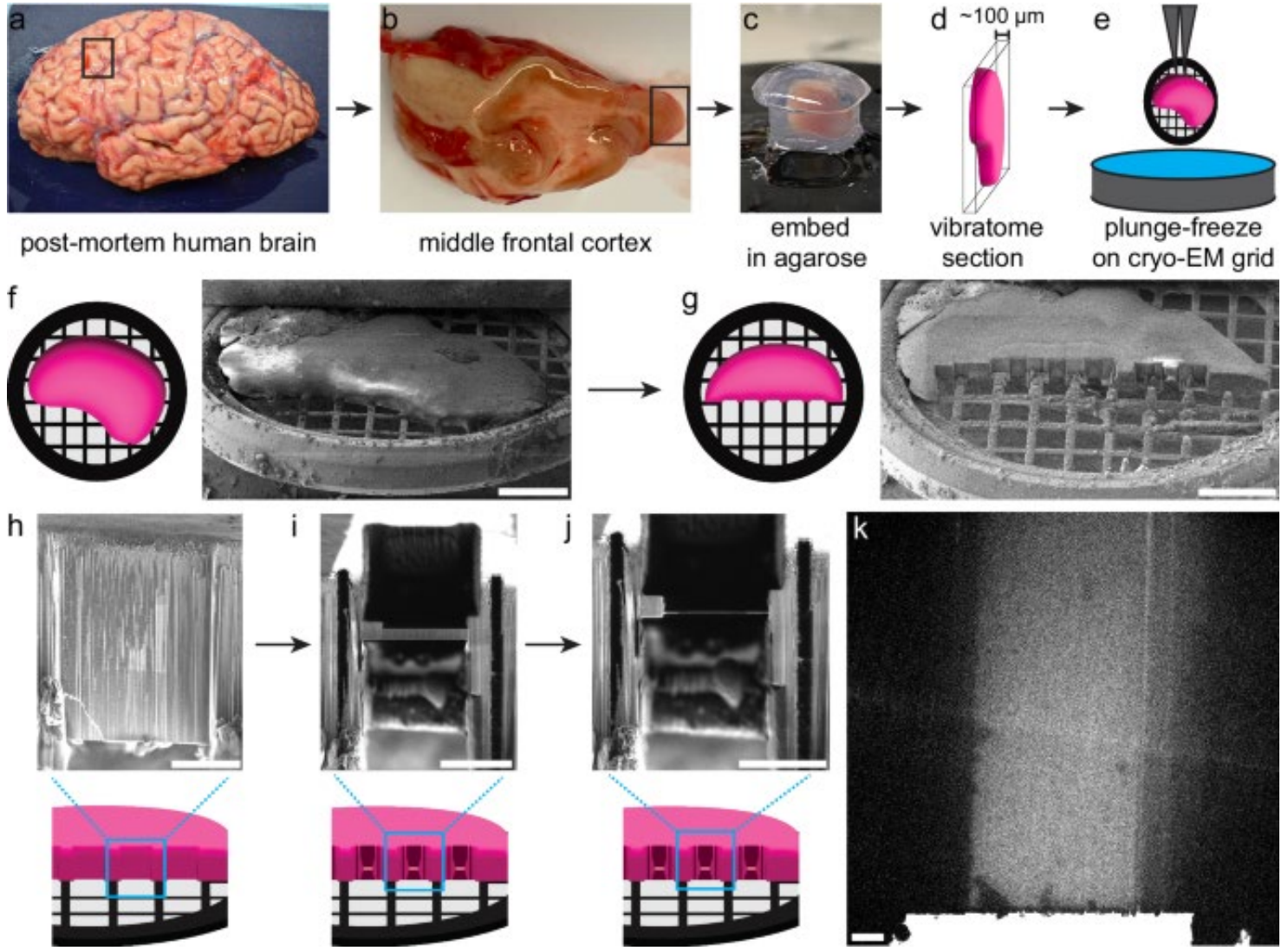
For thicker samples - waffle





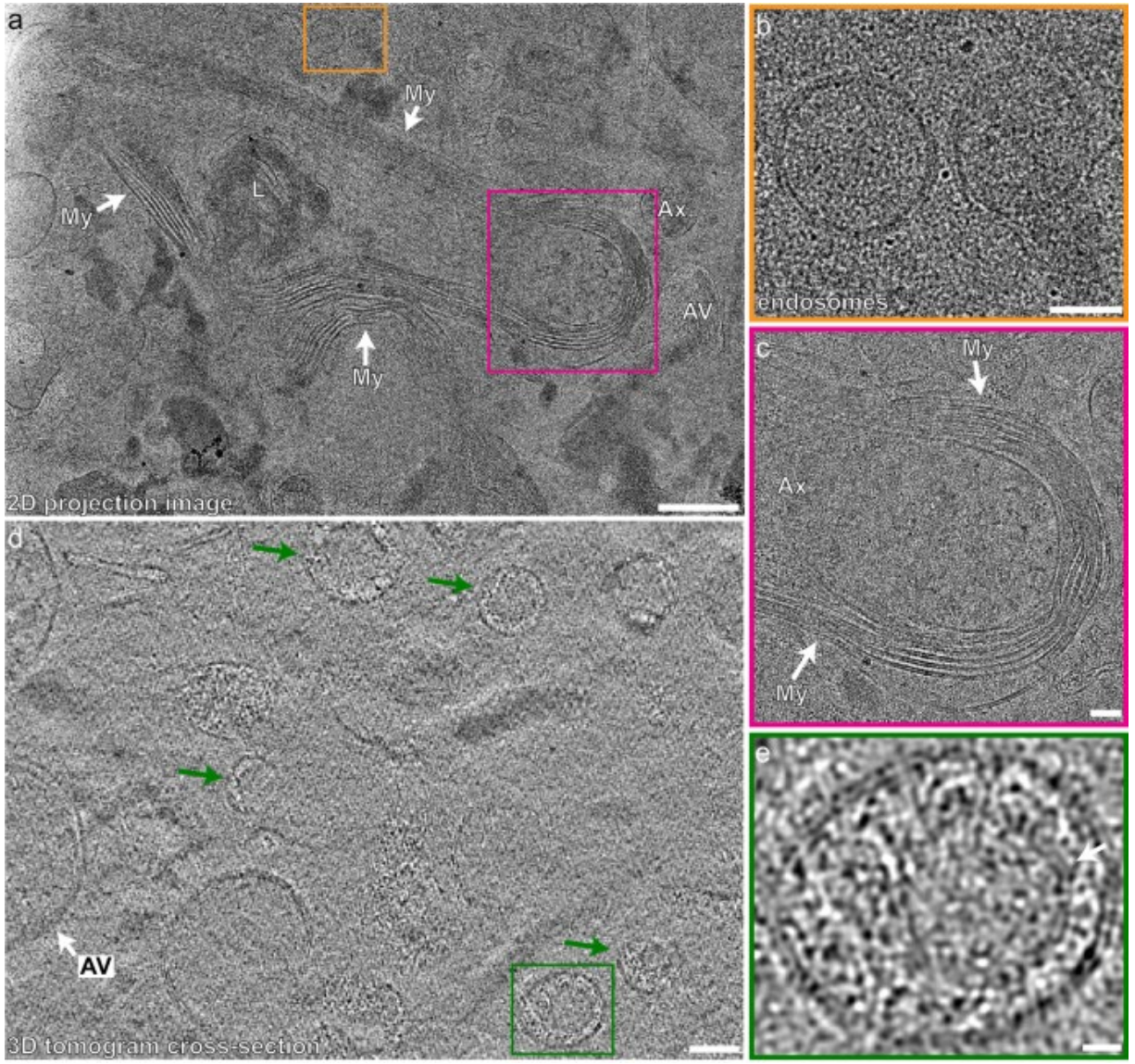
Kelley, K. *Nat Commun* **13**, 1857 (2022)

On-site lamellae in tissue sections

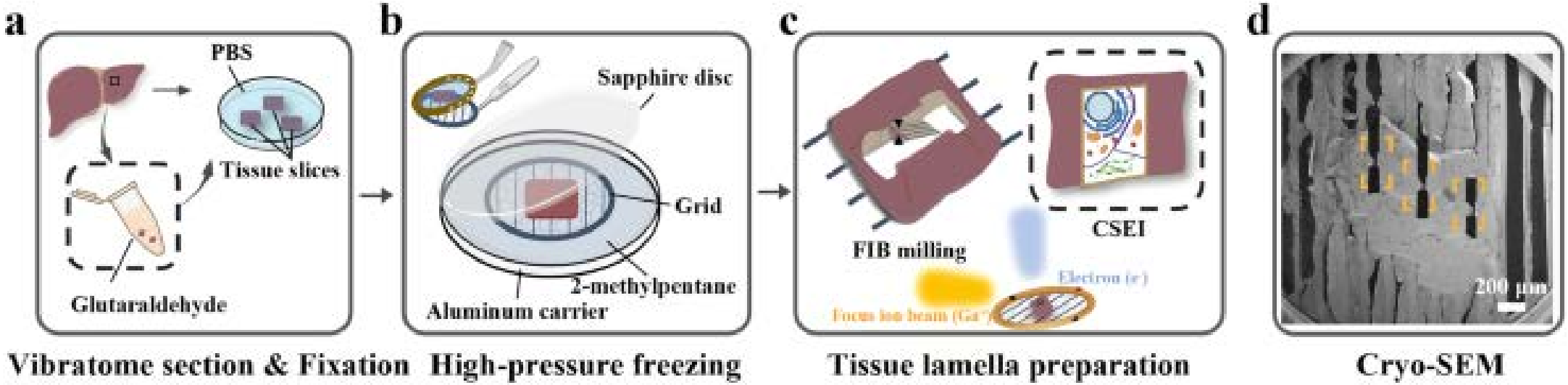


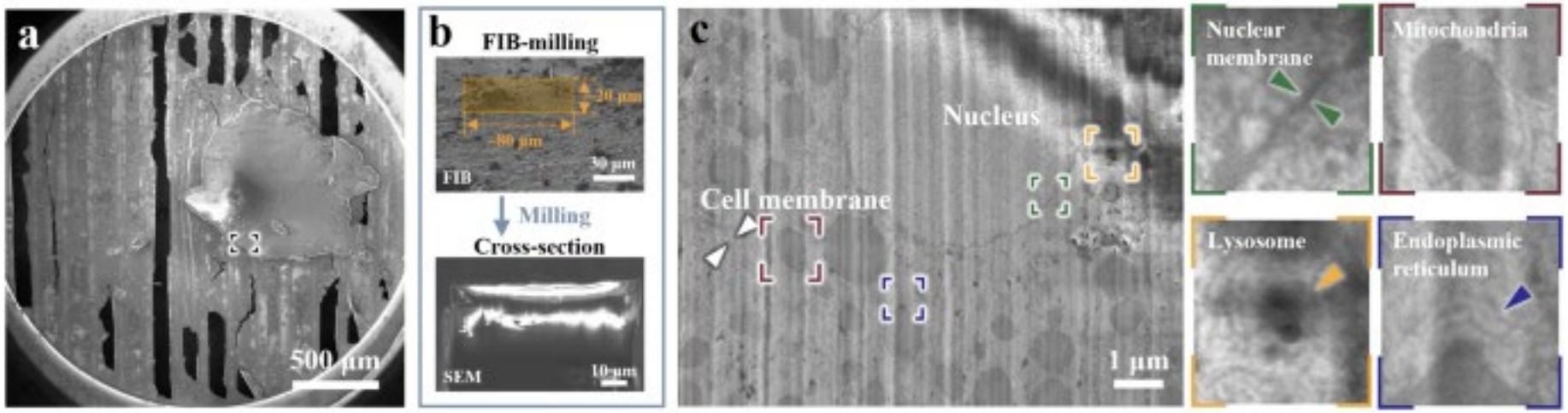
Creekmore et al 2024 Nat Comm

On-site lamellae in tissue sections



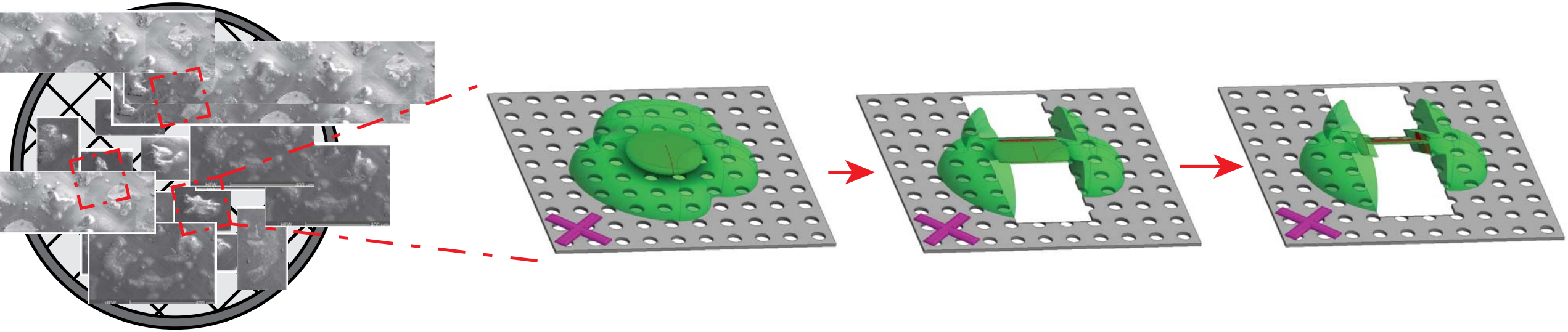
Creekmore et al 2024 Nat Comm

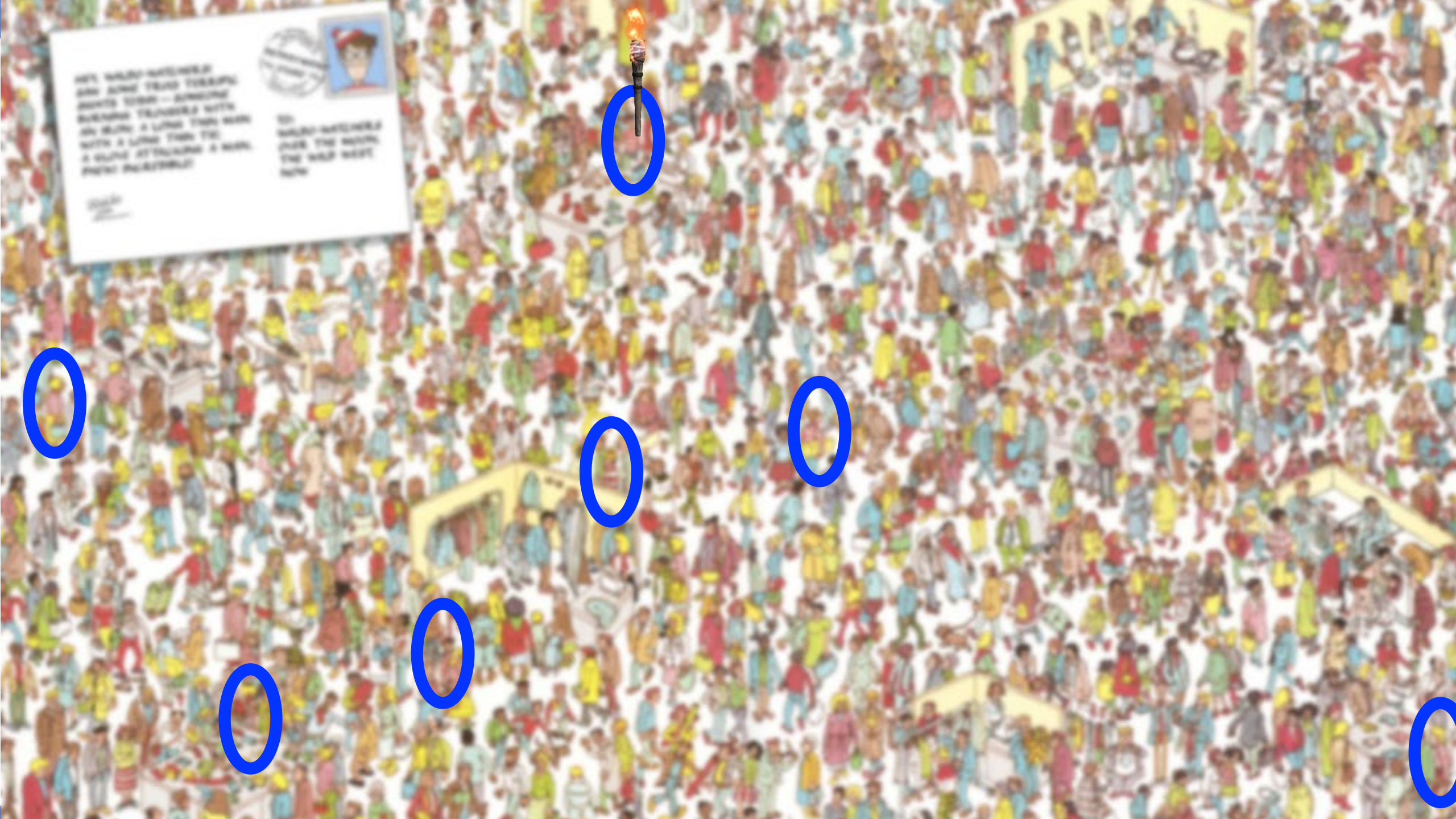




Wang, S. *Sci Rep* **13**, 5879 (2023)

Cryo lamella preparation

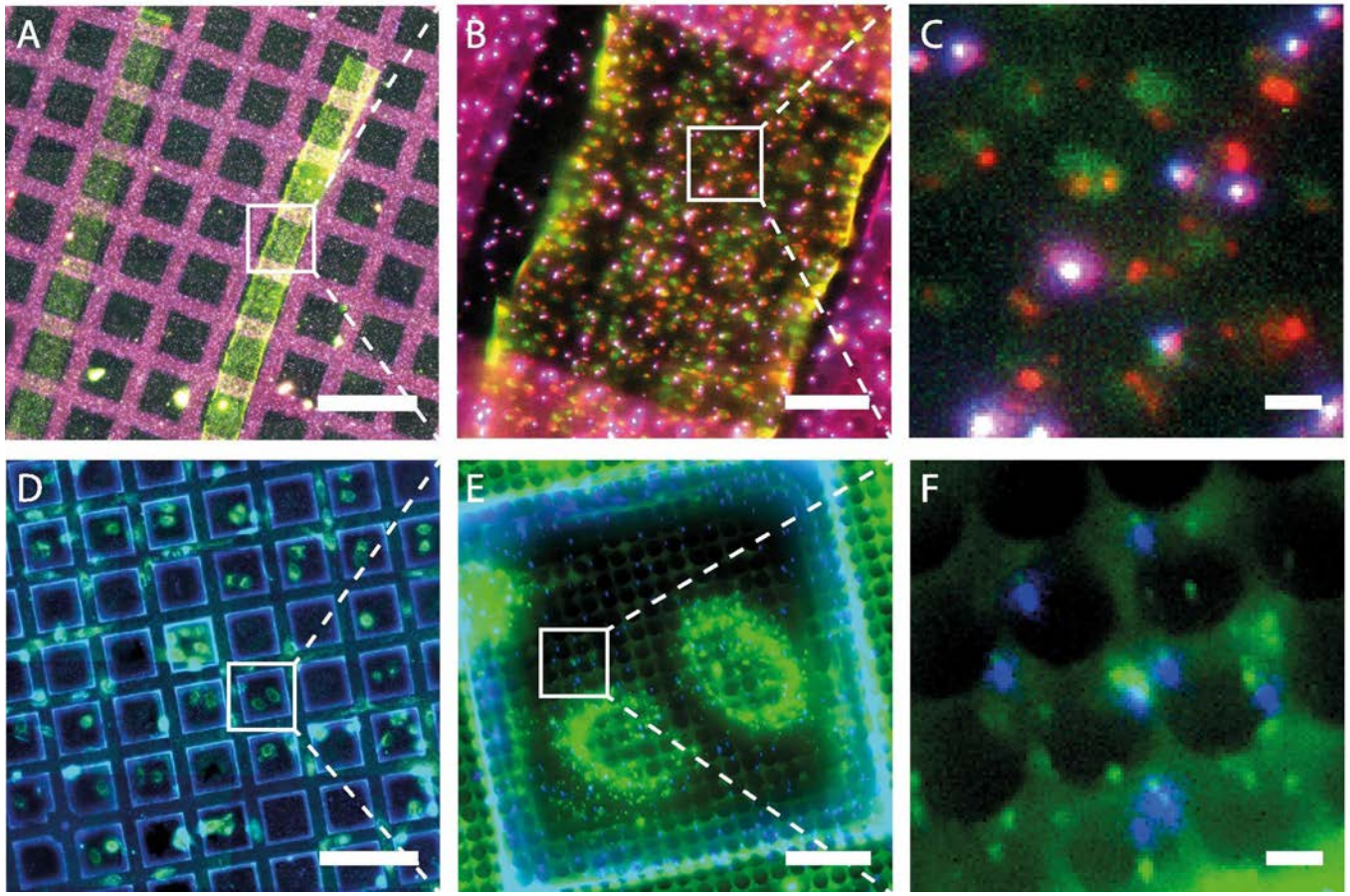




Cryo-Light Microscopy

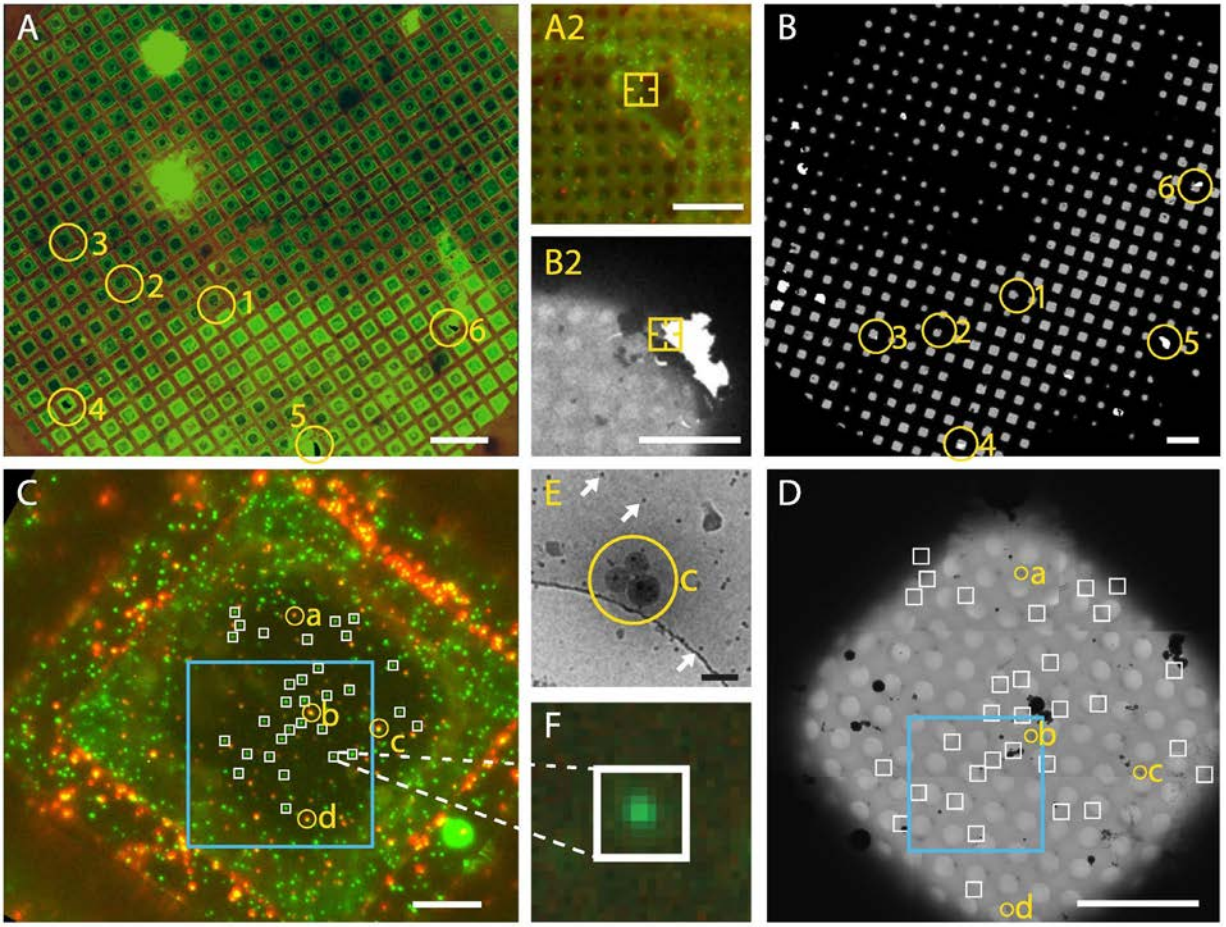
CEMOVIS

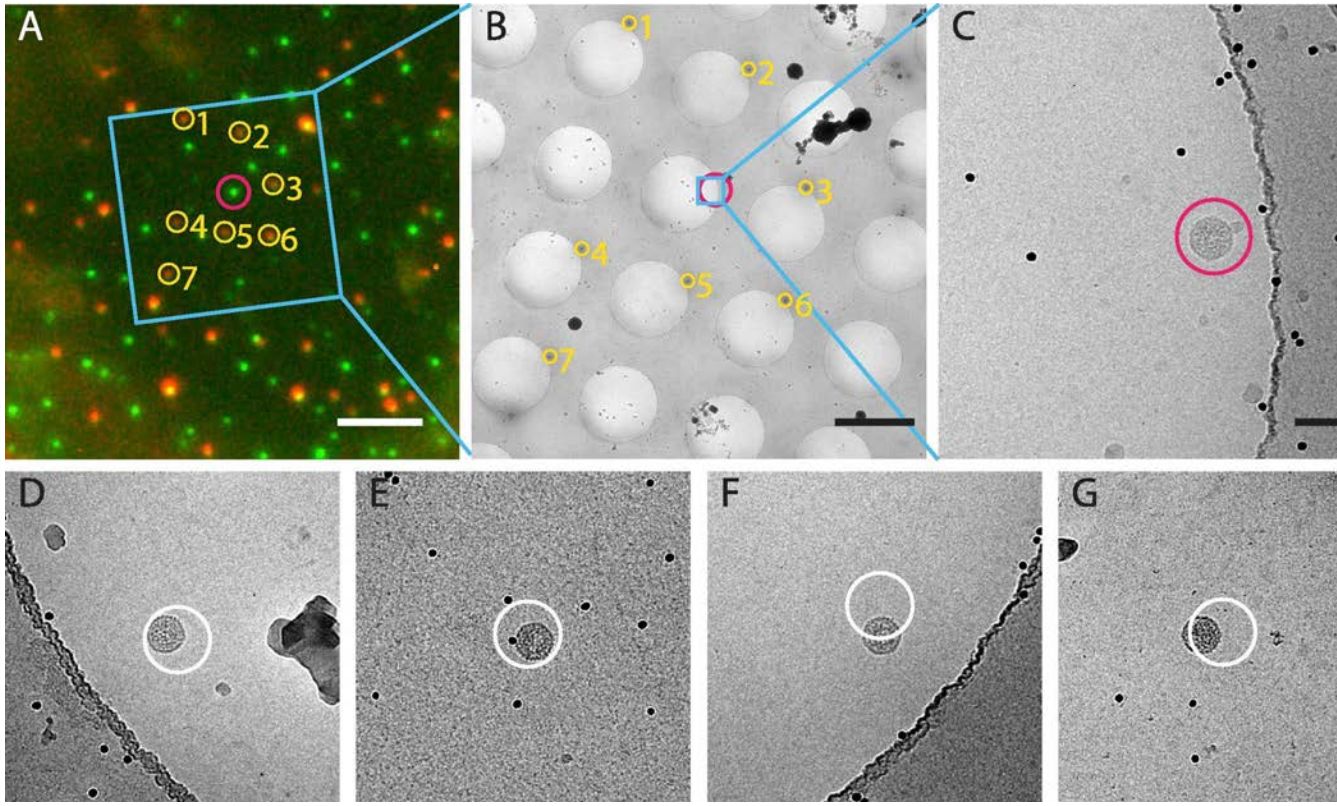
Culture on grid



From Schorb et JSB 2017

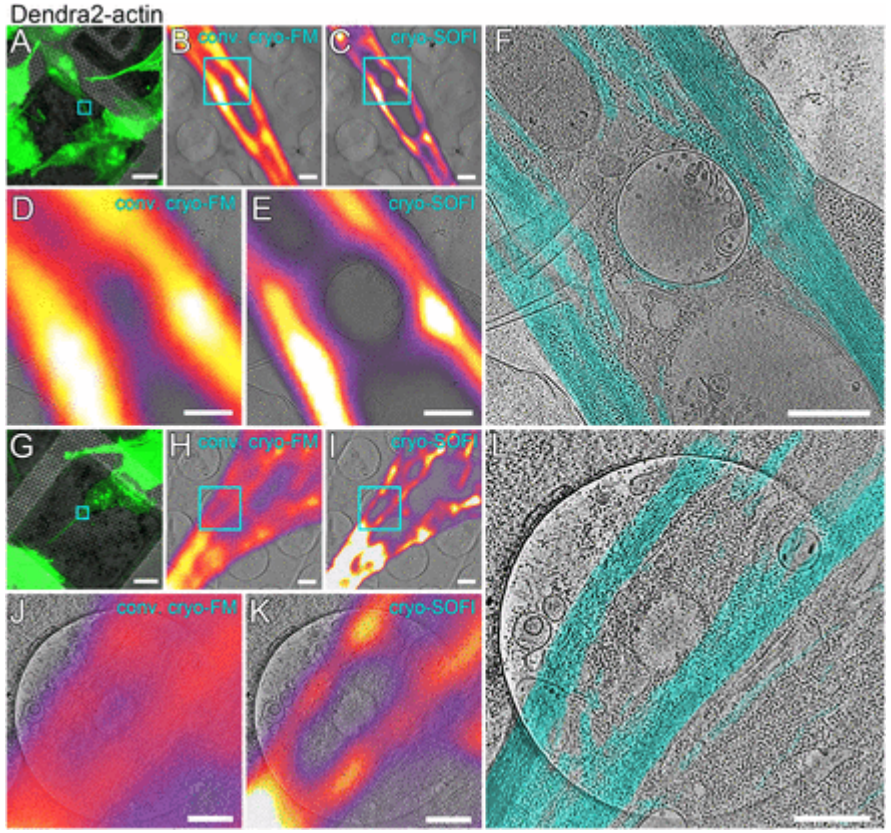
Cryo-CLEM

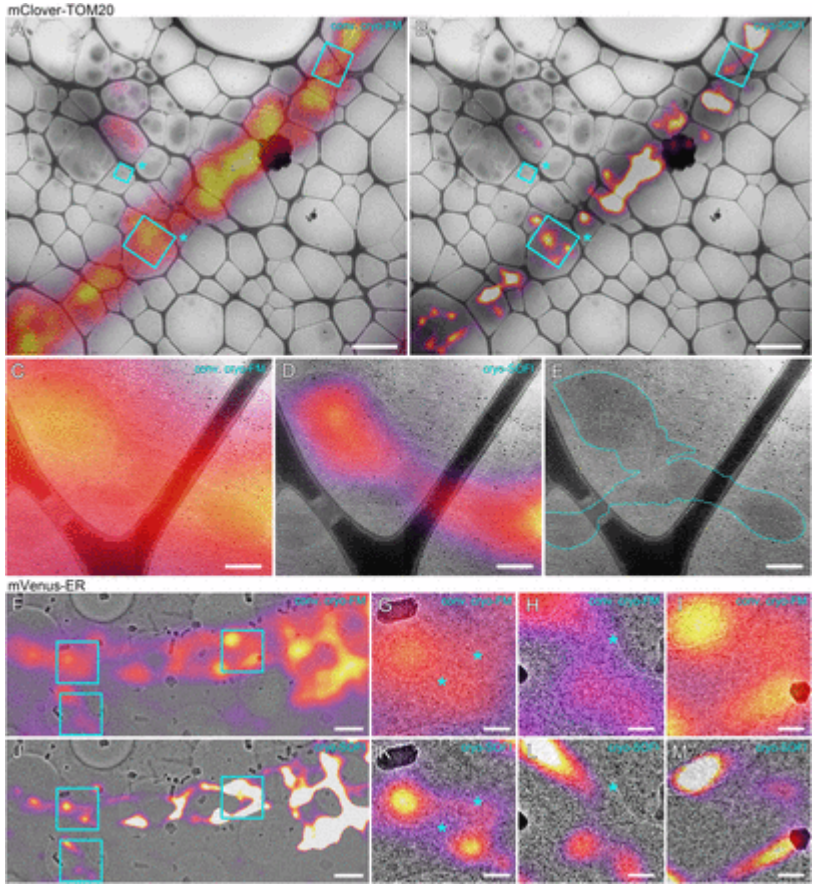




From Schorb et JSB 2017

Super resolution Cryo-Light Microscopy (SOFI)

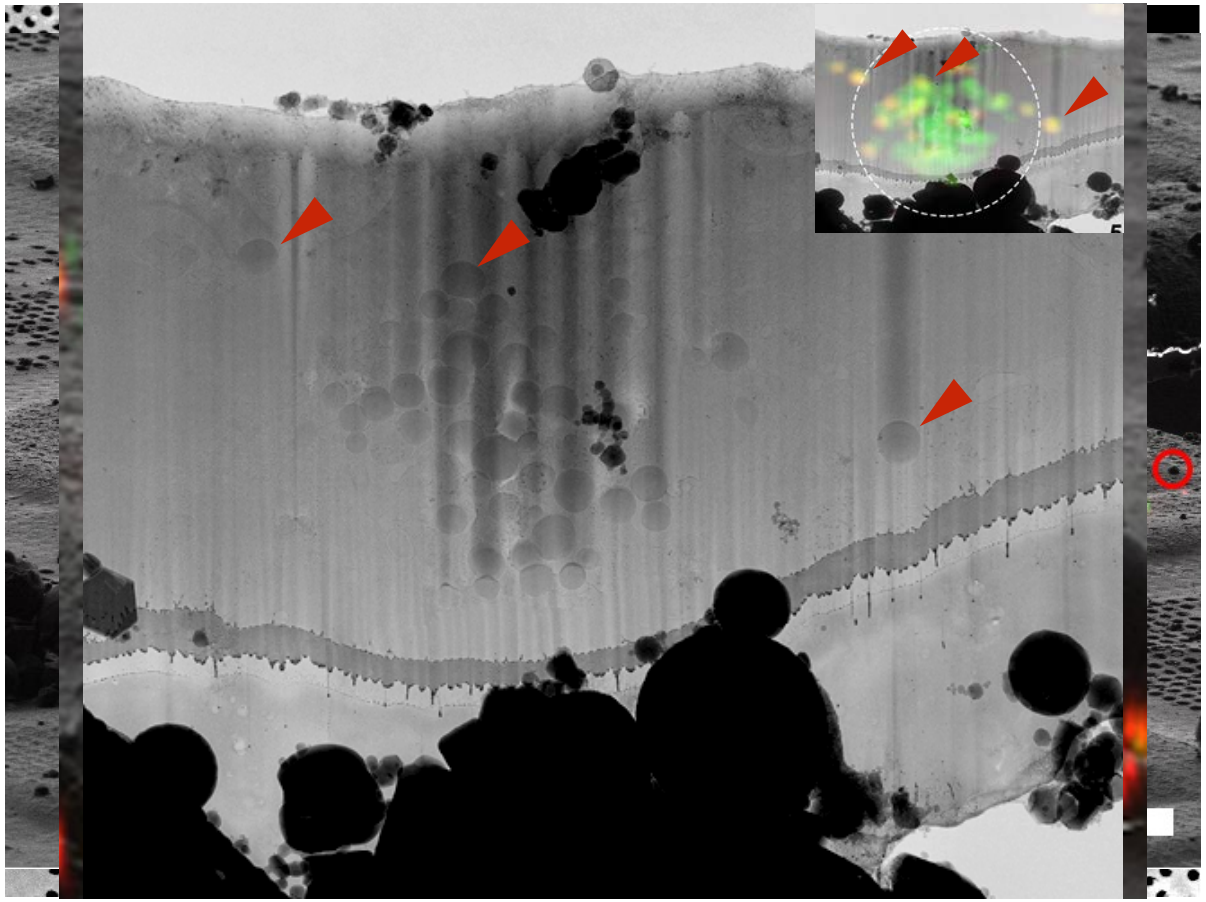




Moser et al PNAS 2019

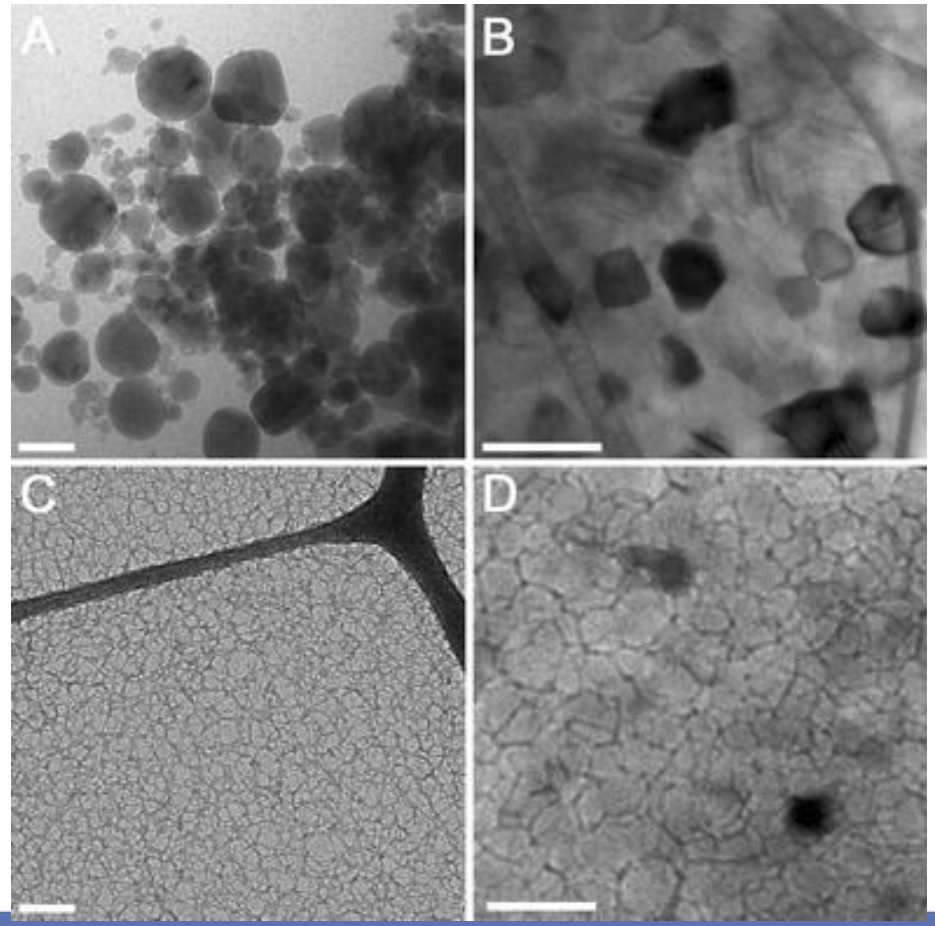
An example of 3D correlation:

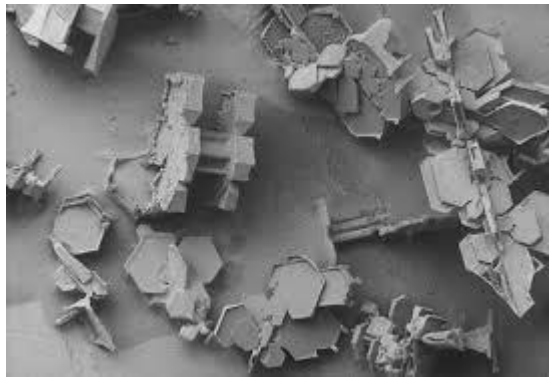
- Localise a cluster of lipid droplets in mammalian cells

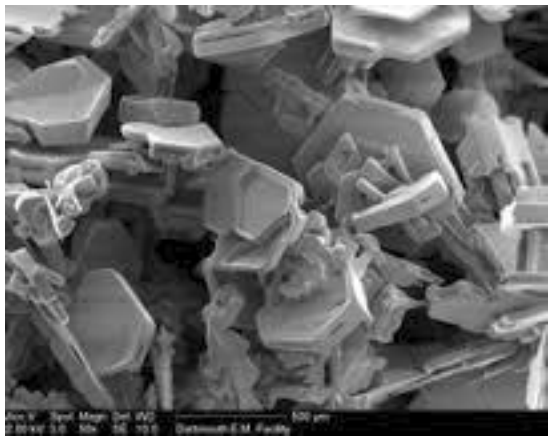


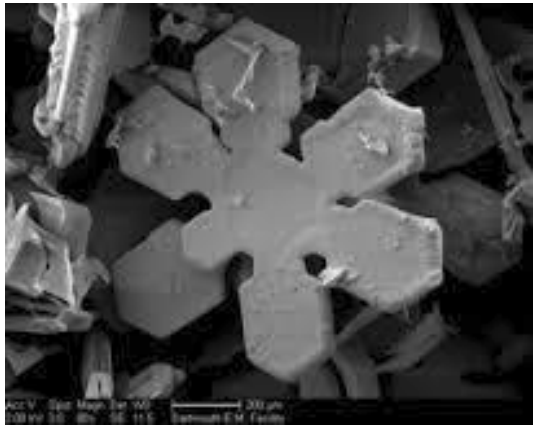
Arnold et. al. Bioph. J. 2016

Cryo-FIB milling and CLEM



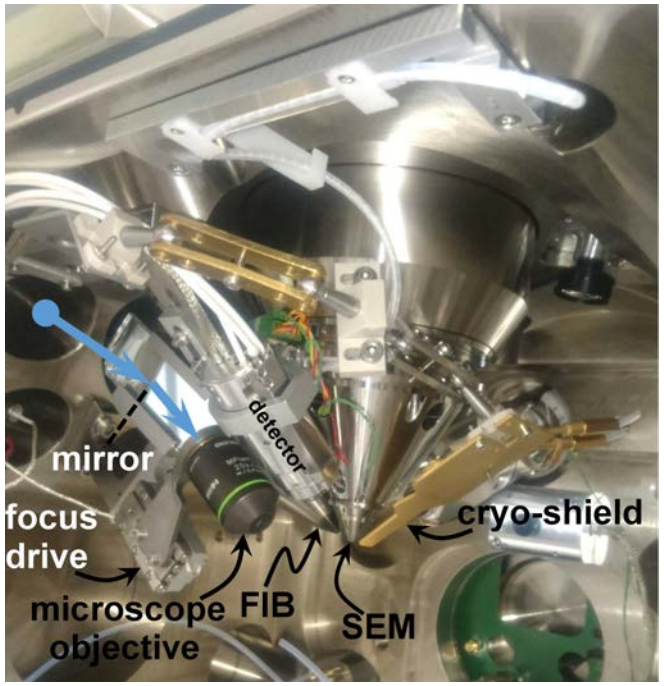




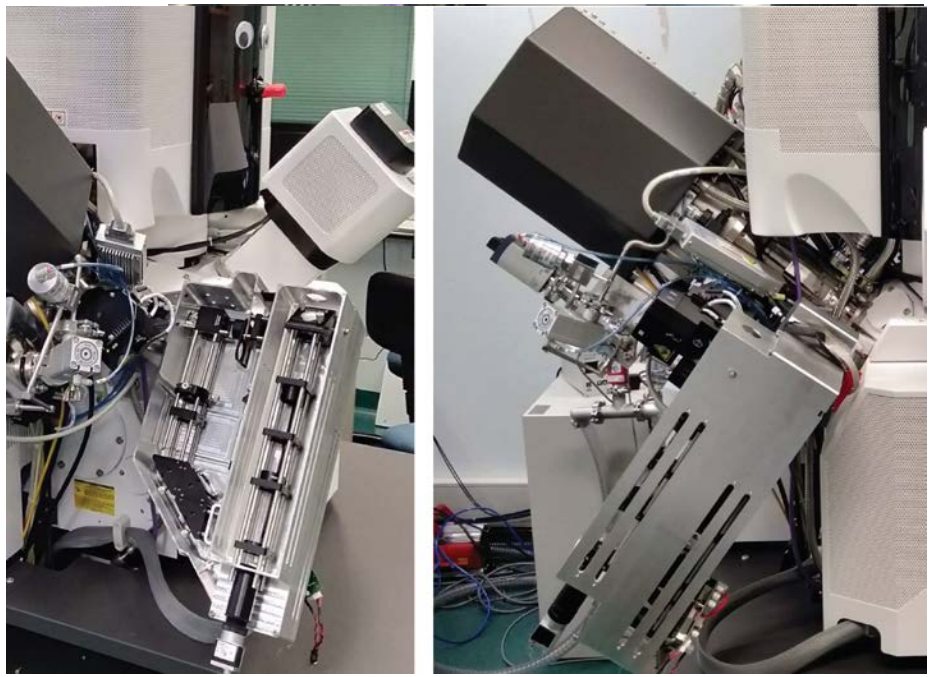


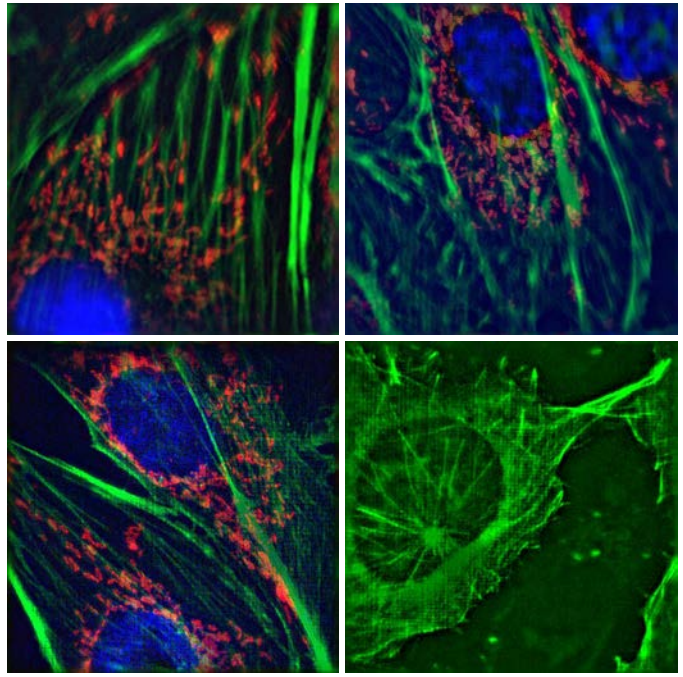
cryo-CLEM

LM → Freeze → cryoLM → cryoFIB → cryoLM → cryoTEM

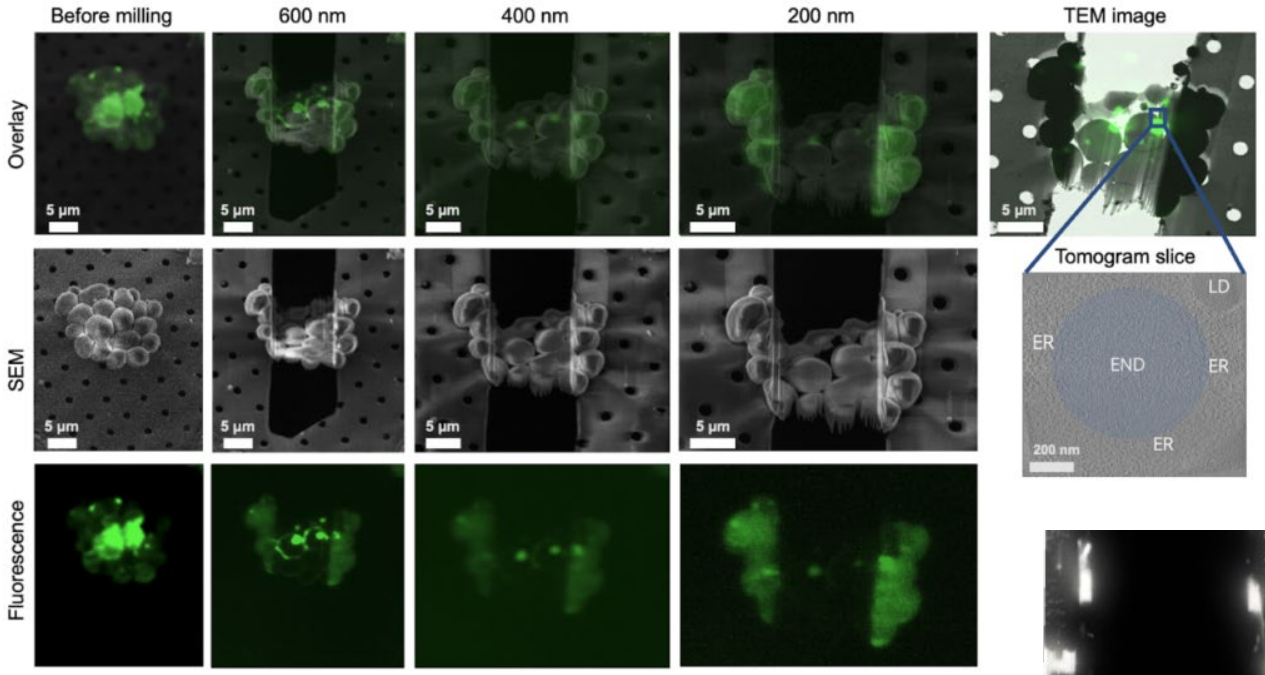


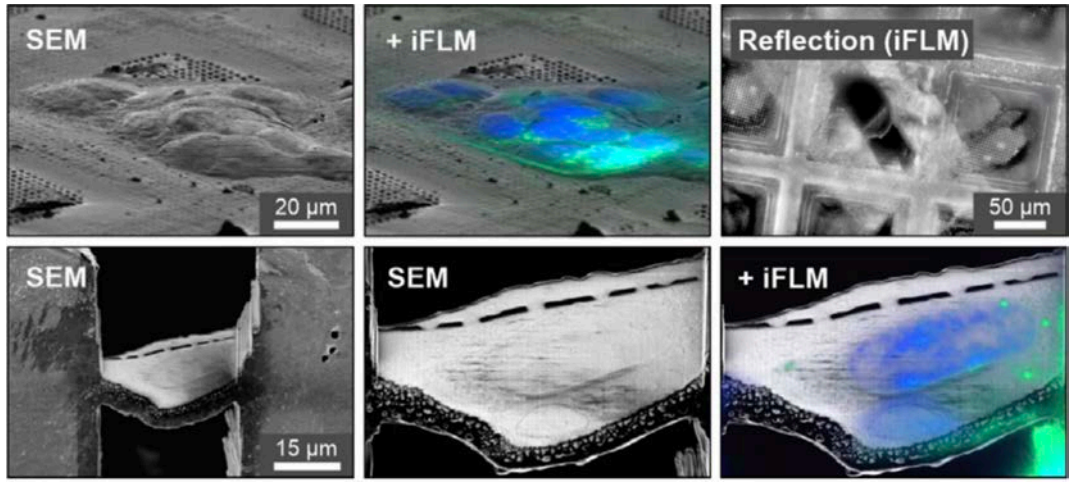
sr PIE-scope

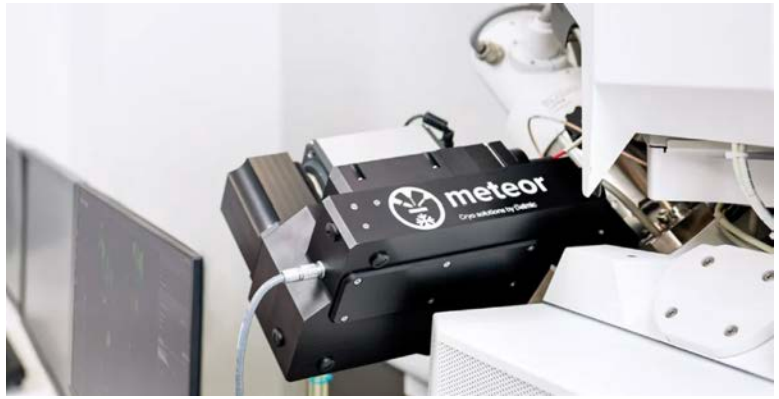


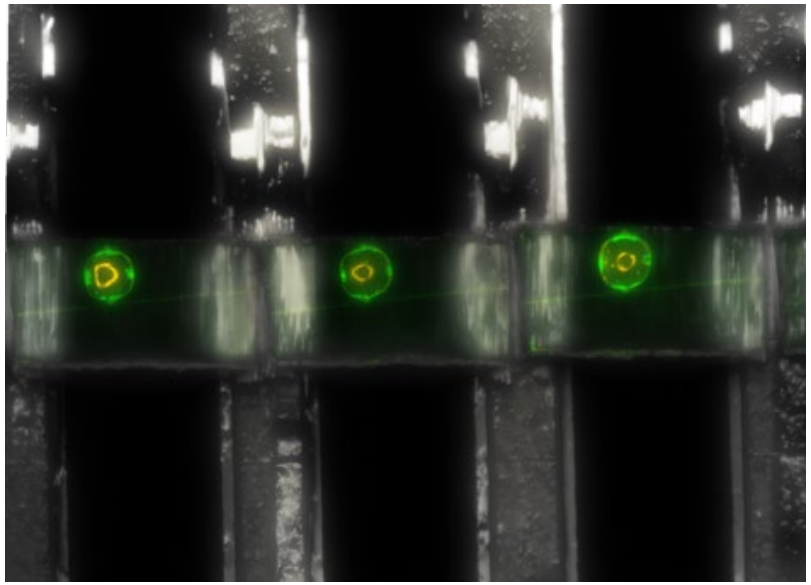


Uses of integrated FIB/LM



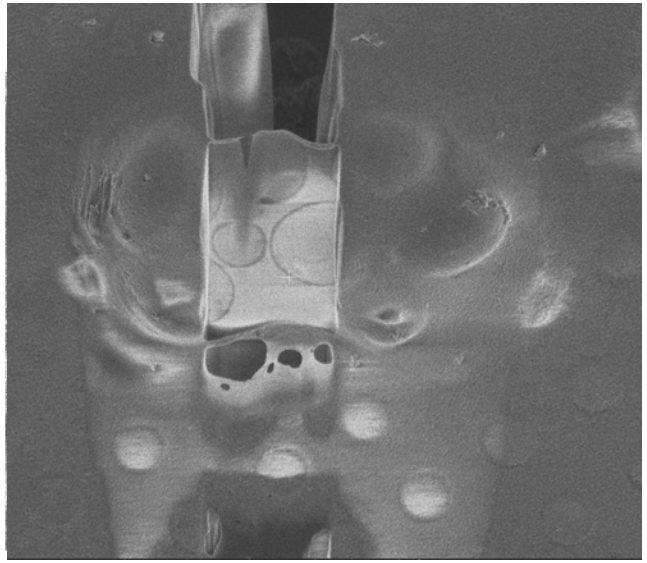




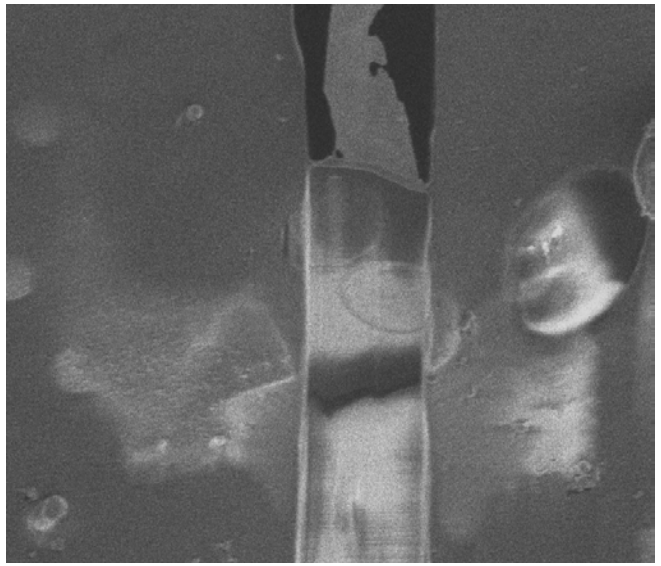


PFIB for cryo-lamella prep

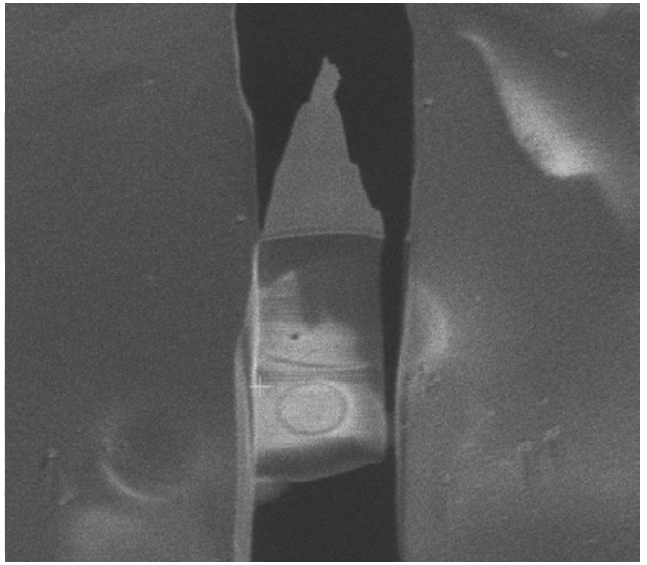
Ga



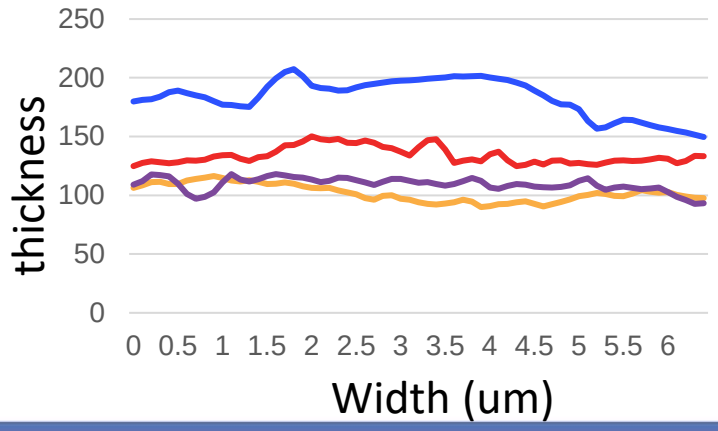
Xe



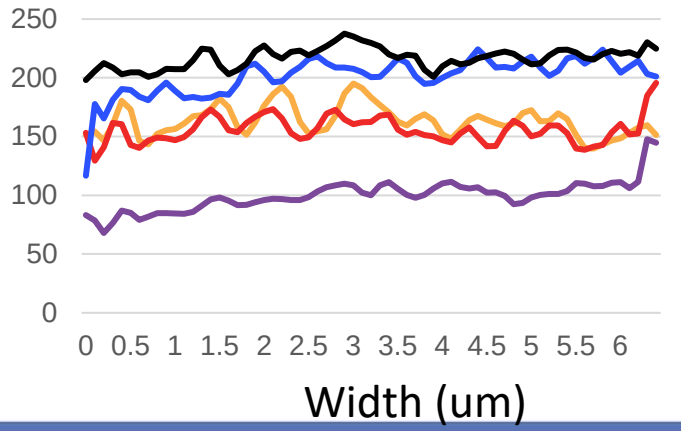
Ar



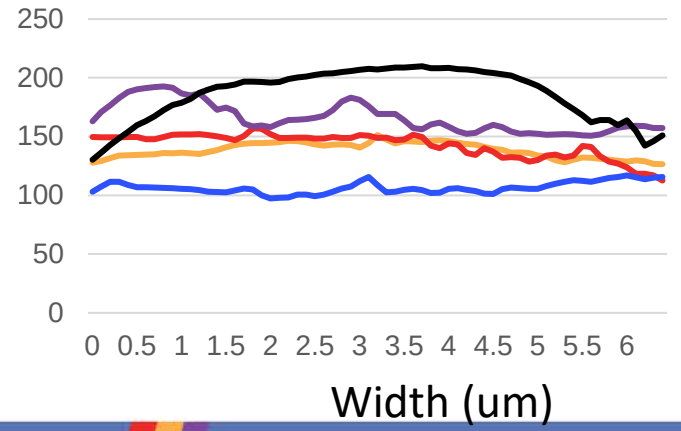
9 Min



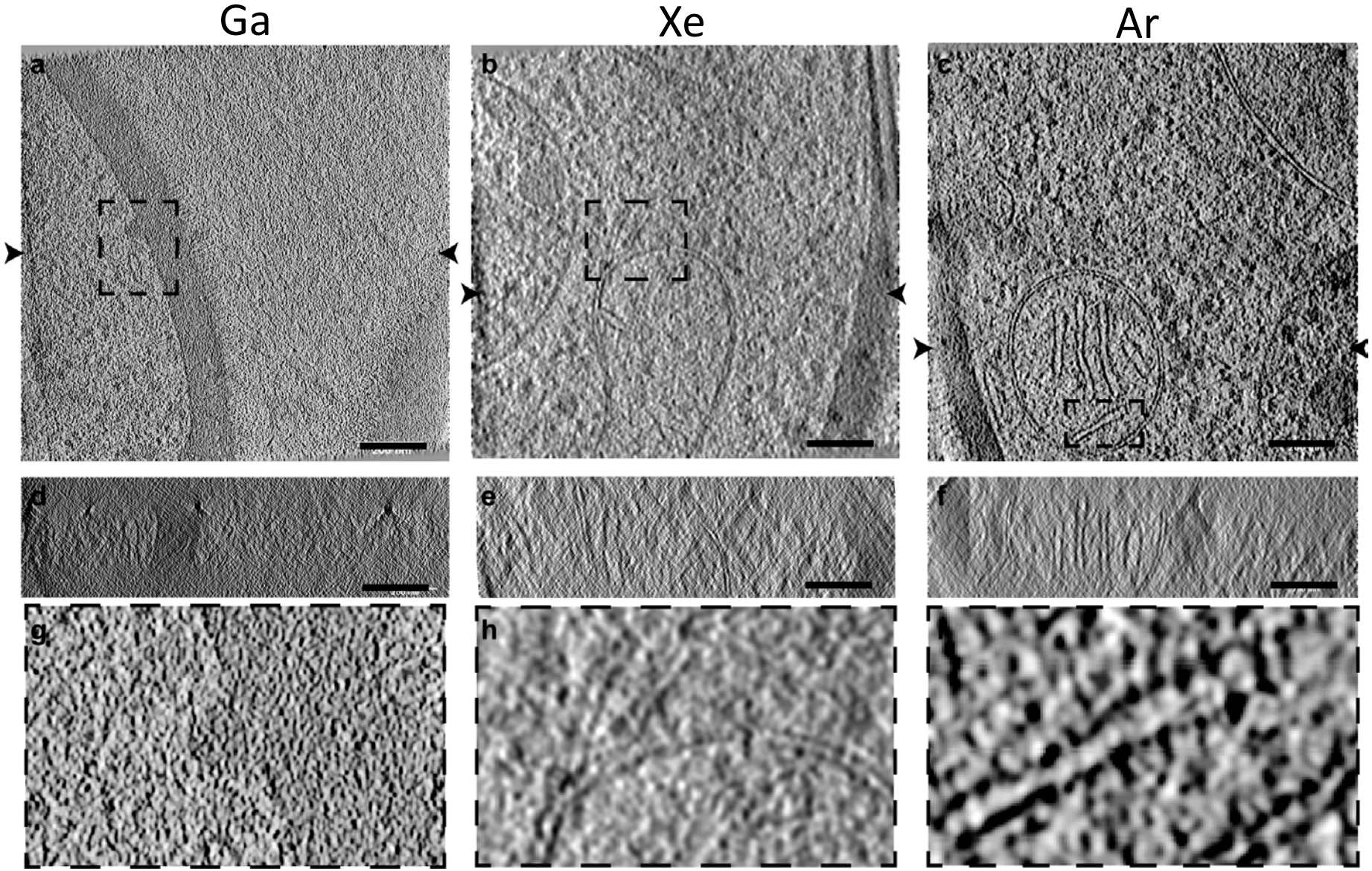
15 Min



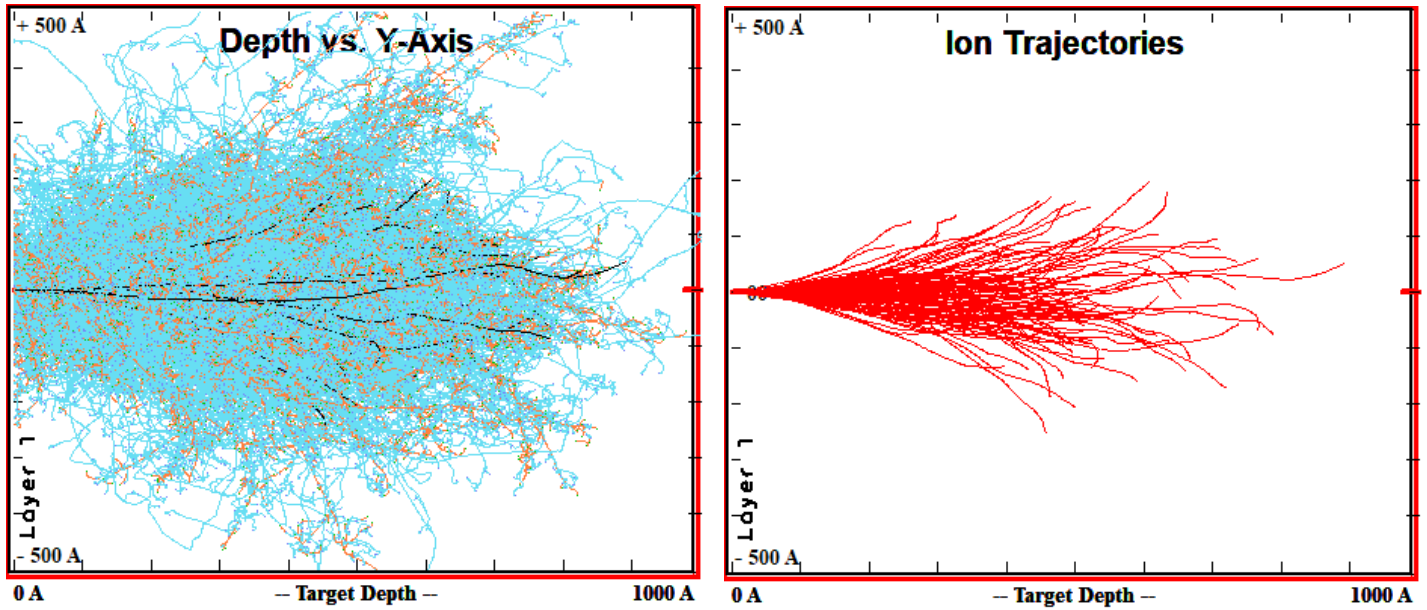
7 Min



PFIB for cryo-lamella prep

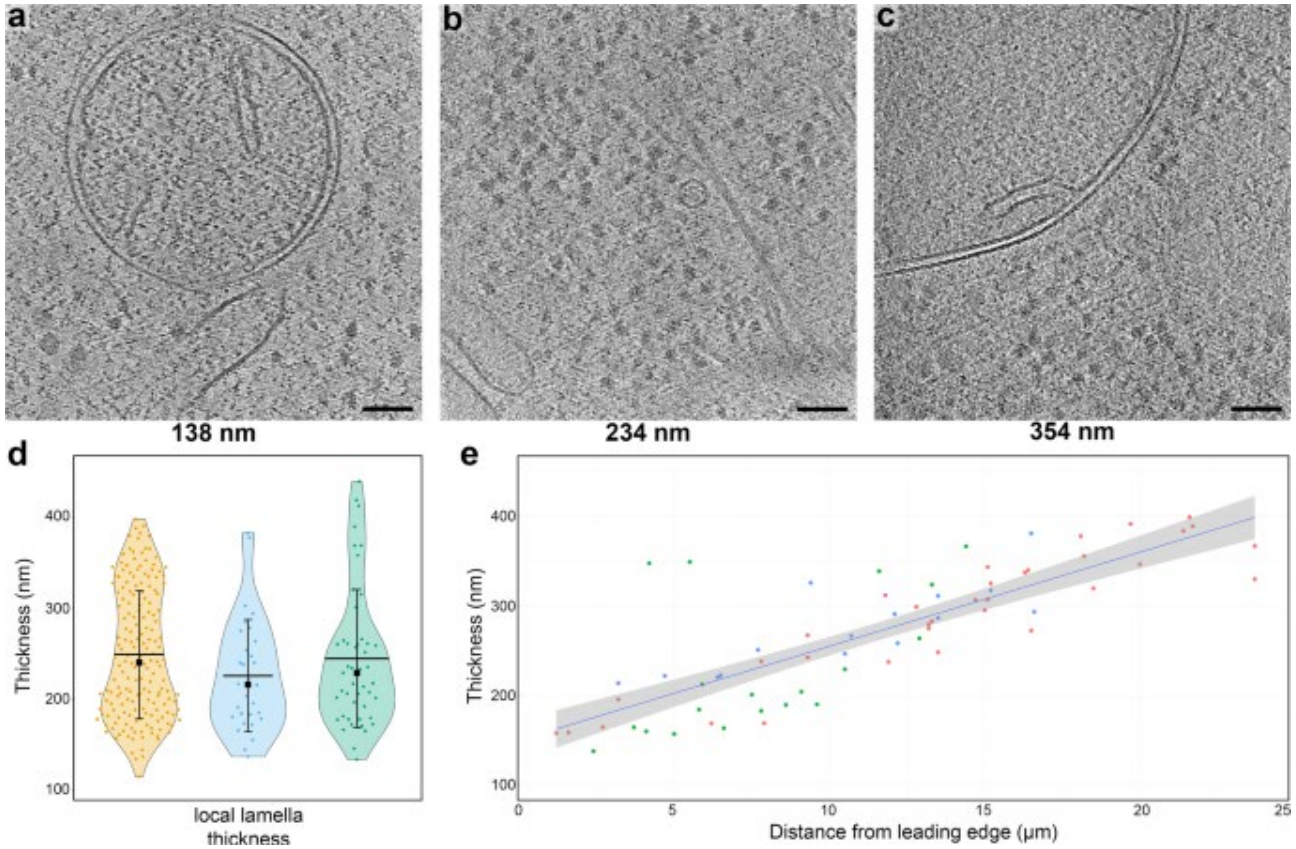


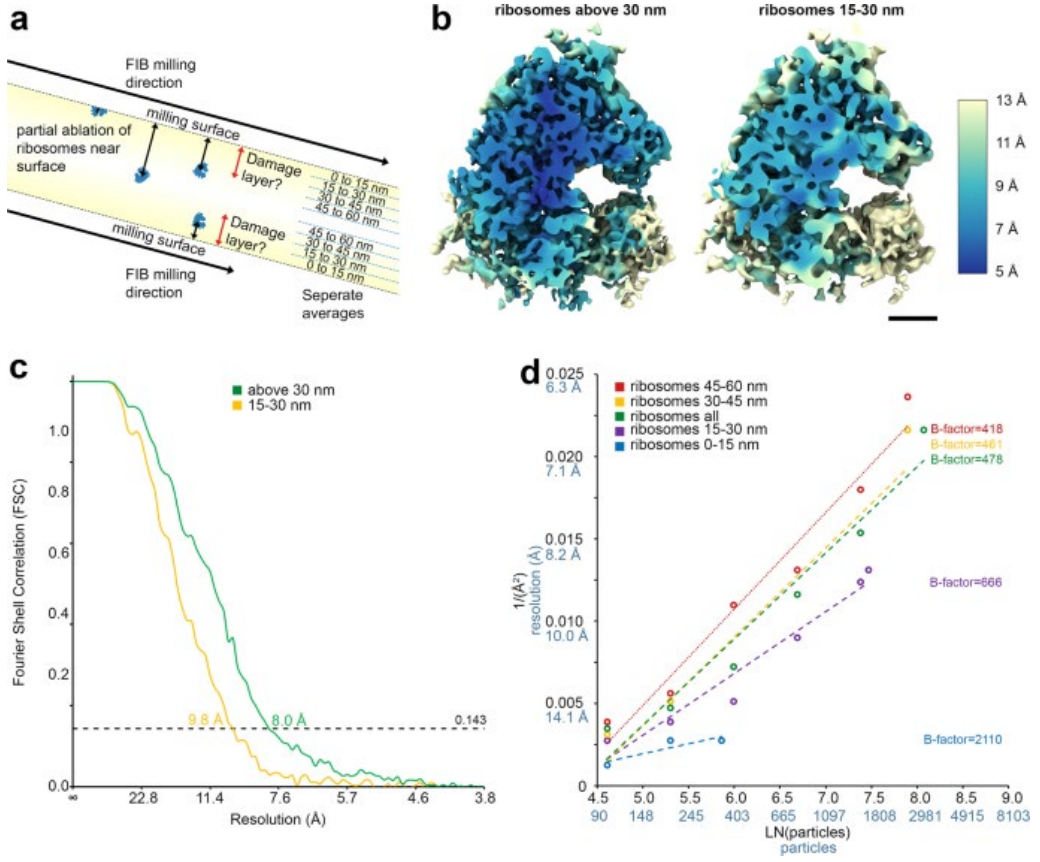
Cryo lamella preparation using plasma



Plunge-frozen yeast

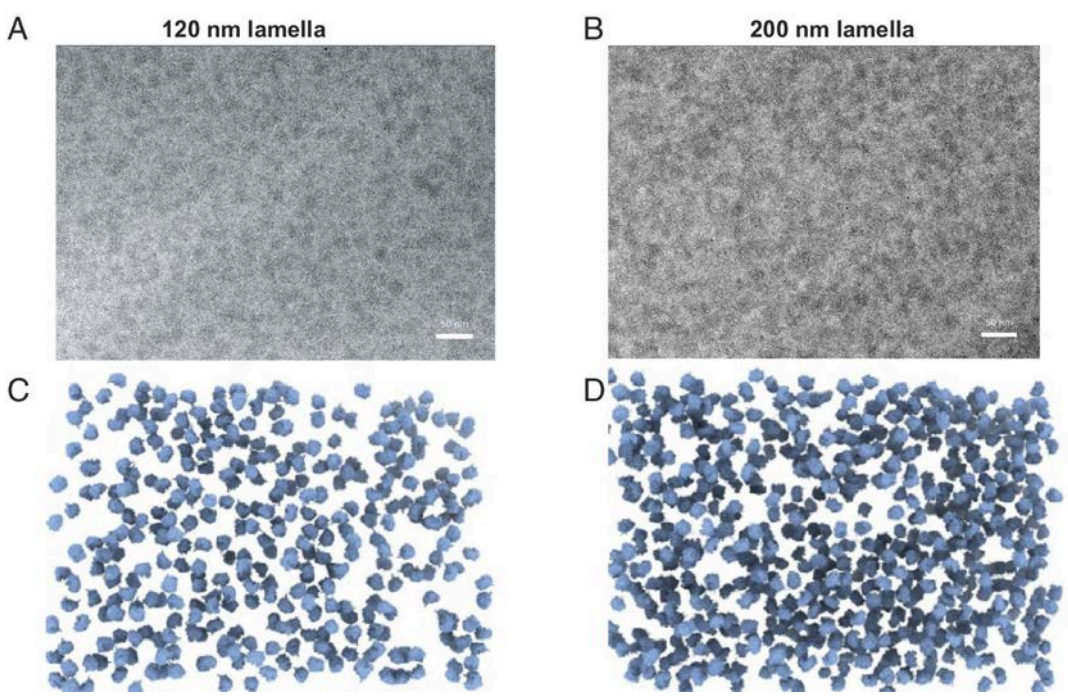
PFIB for cryo-lamella prep

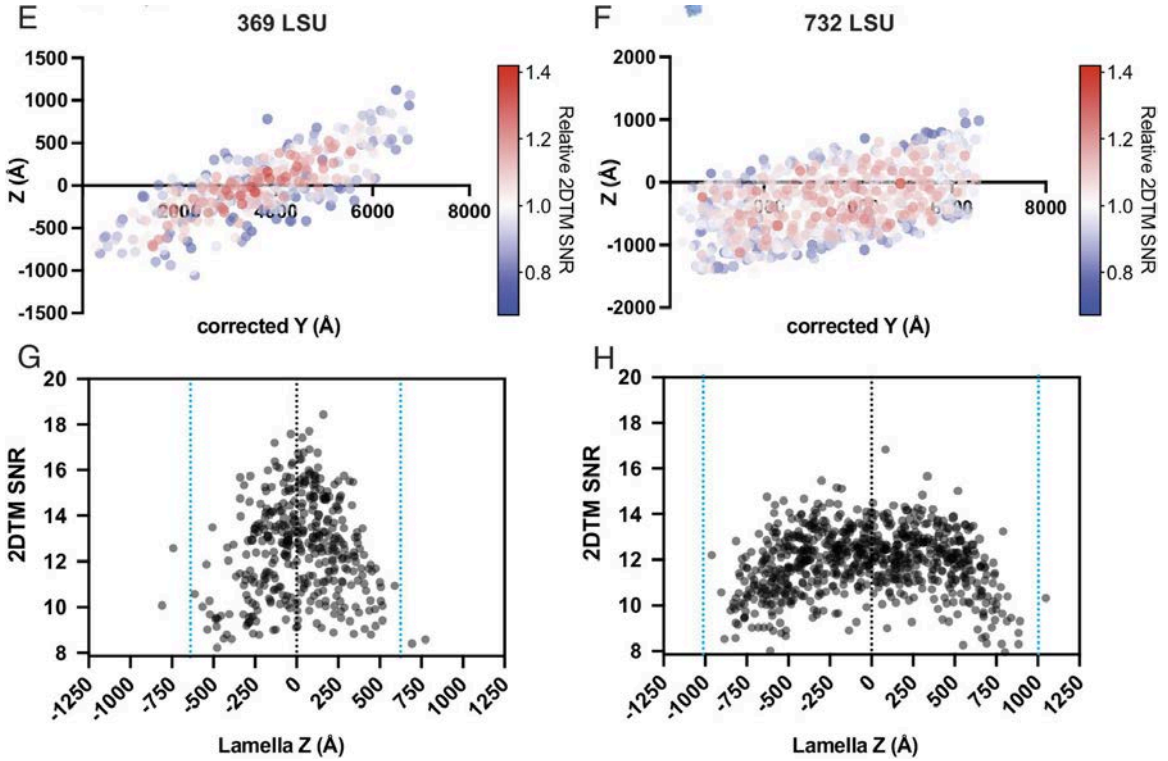




Berger et al Nat com 2022

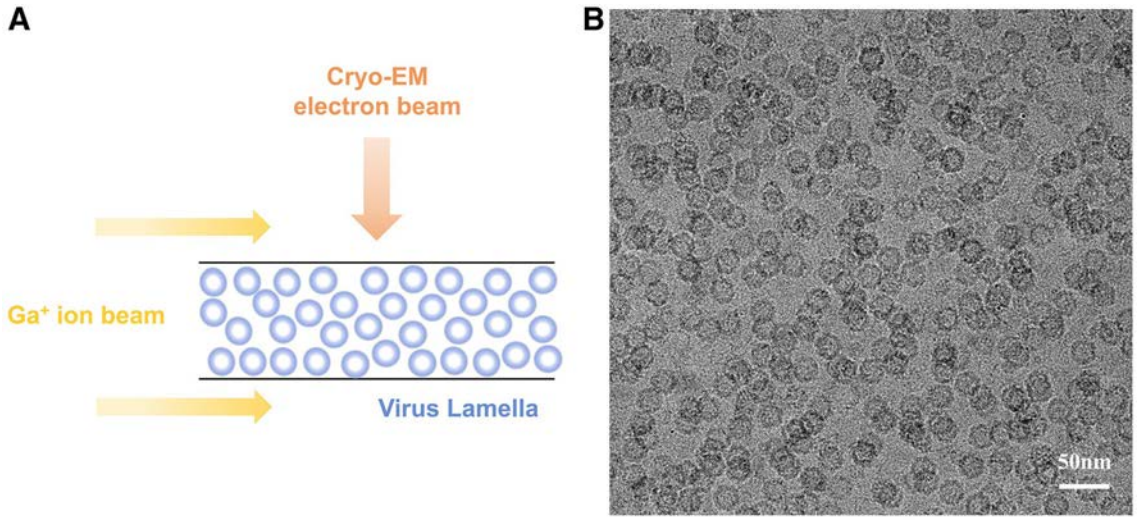
cryo-lamella prep: damage

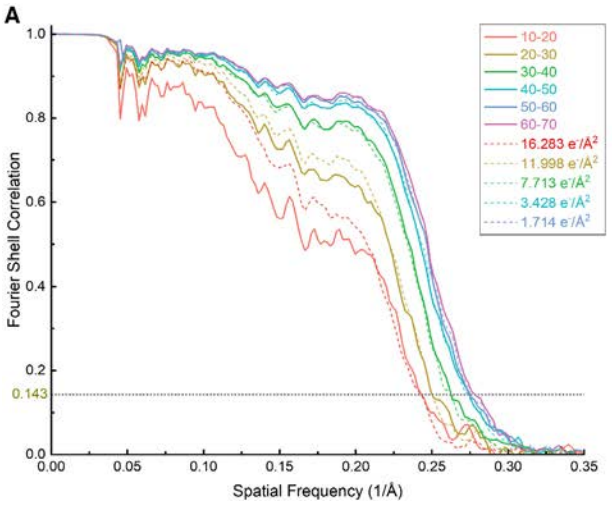


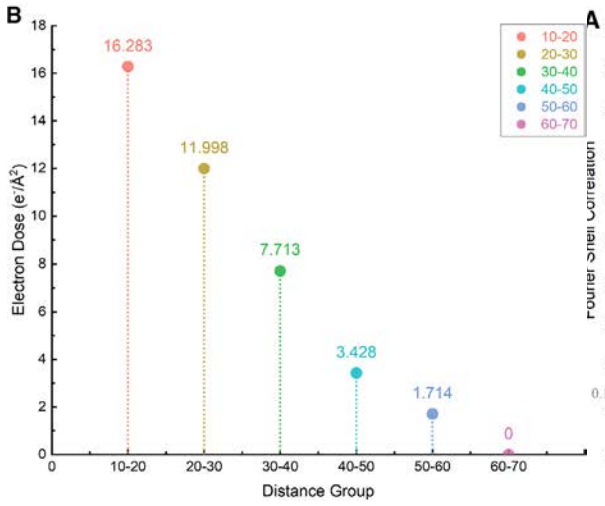


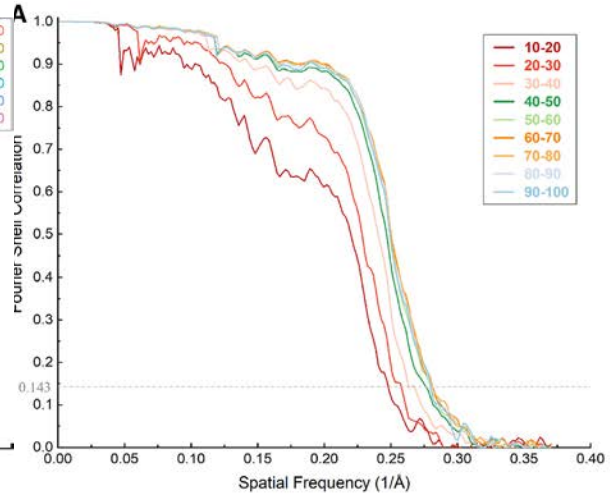
Lucas et al PNAS 2023

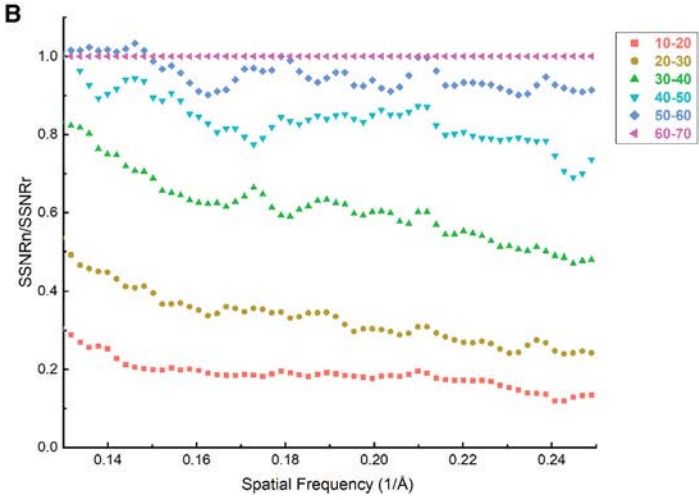
Cryo-lamella prep: damage







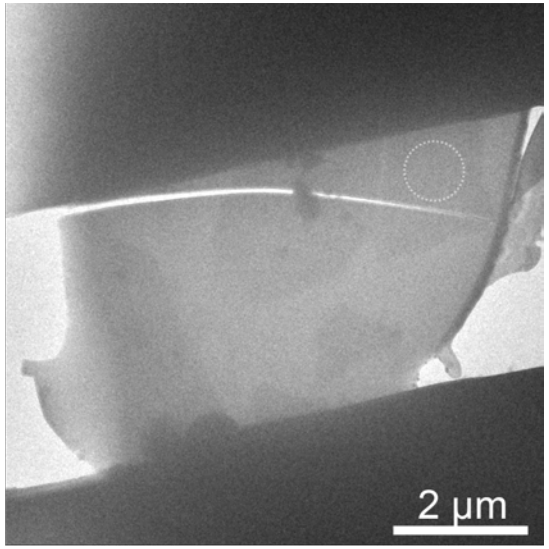


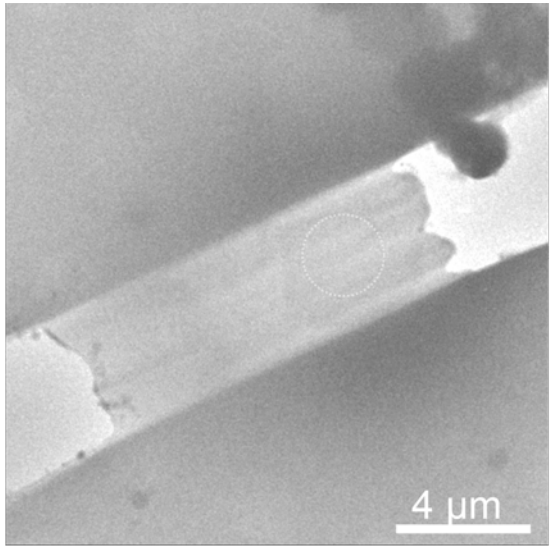


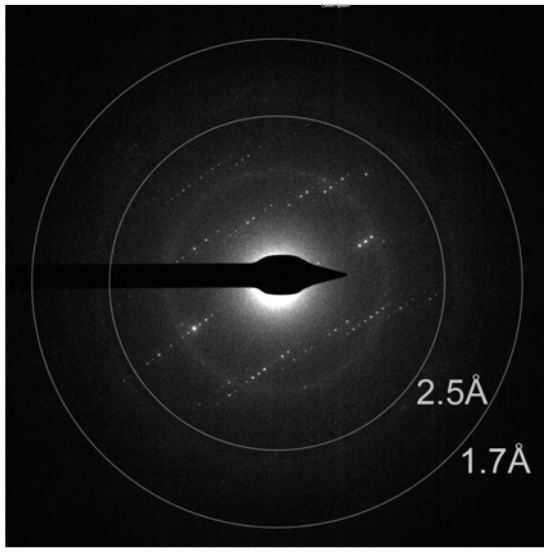
Yang Q,. Structure. 2023 Oct 5;31(10):1275-1281

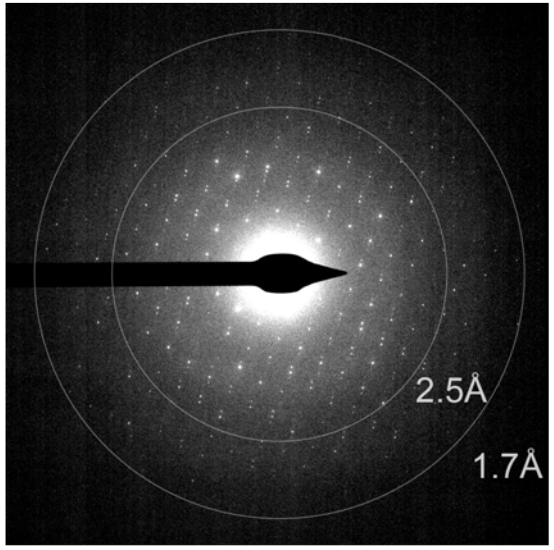
PFIB for cryo-lamella prep

Gallium (8 min) Argon (3 min)



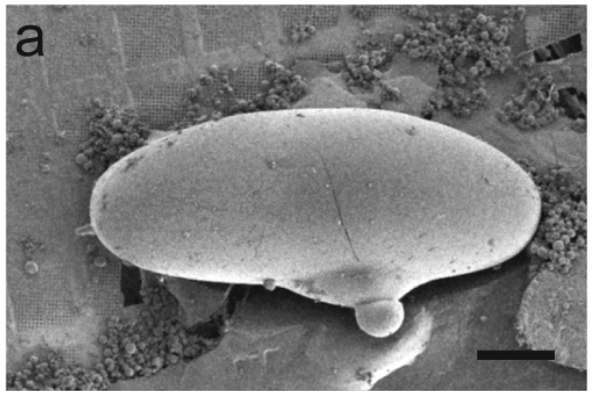






PFIB for cryo–bulk removal

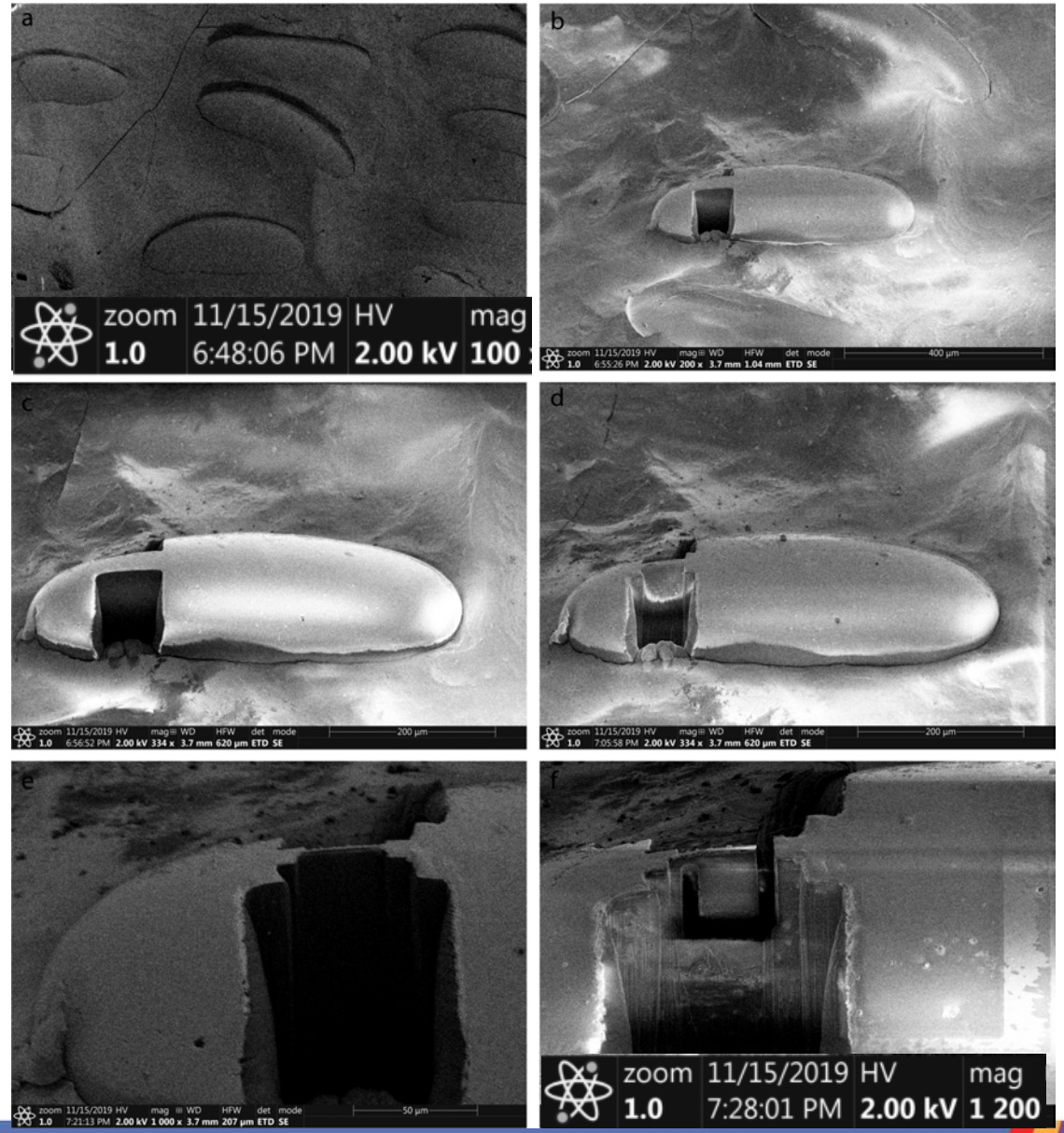
Ga



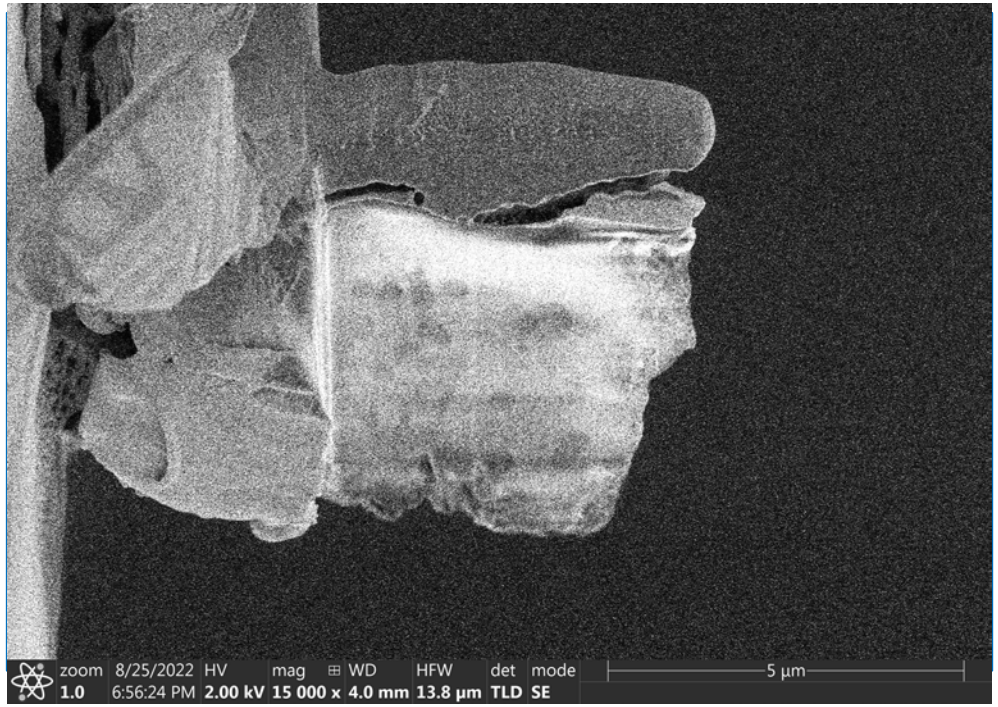
Heavy ions display increased re-deposition

Light ions display clean cut

PFIB preparing cryo-lift out



Liftout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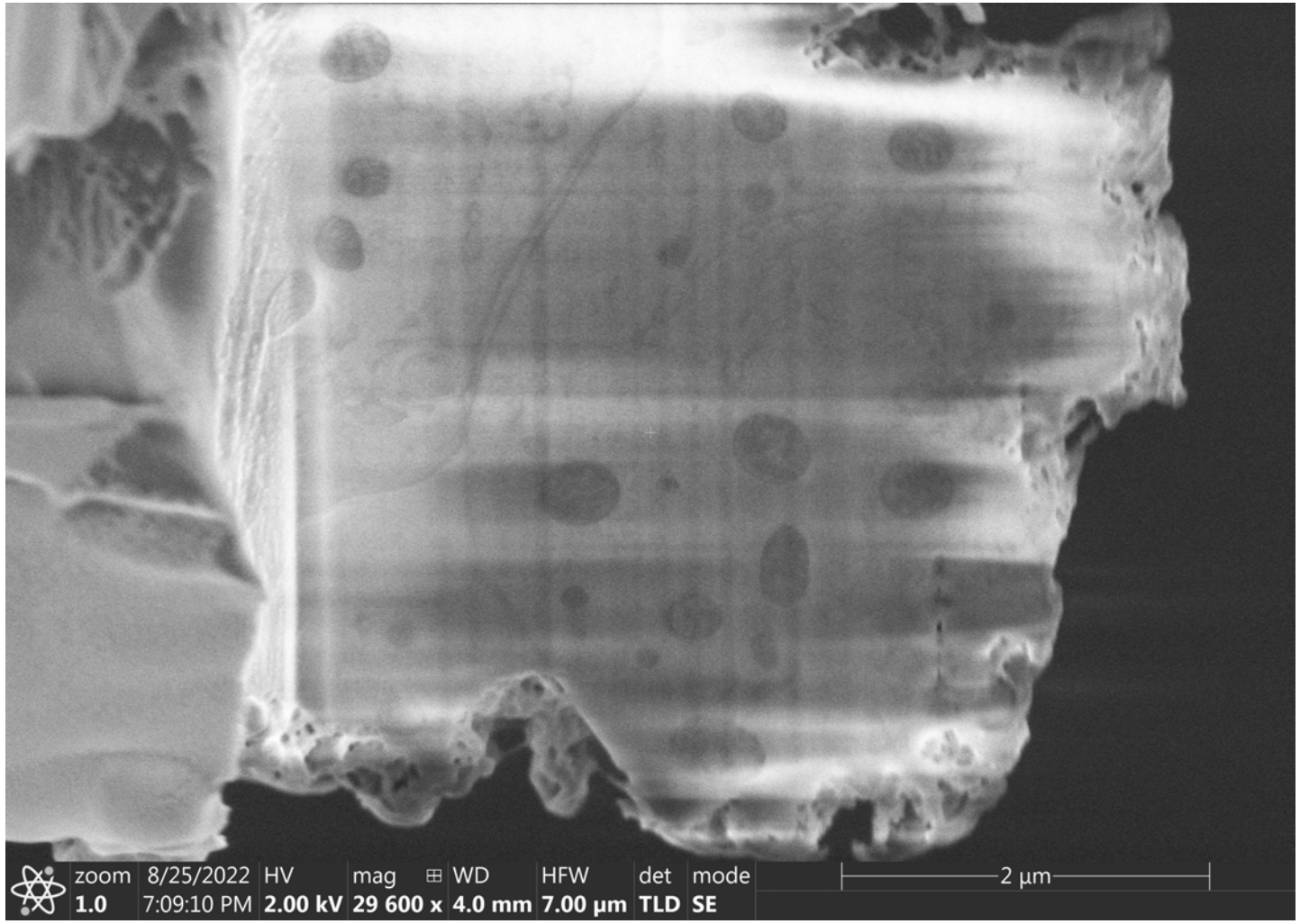
8/25/2022 6:56:24 PM
HV 2.00 kV
mag 15 000 x
WD 4.0 mm
HFW 13.8 µm
det TLD
mode SE
5 µm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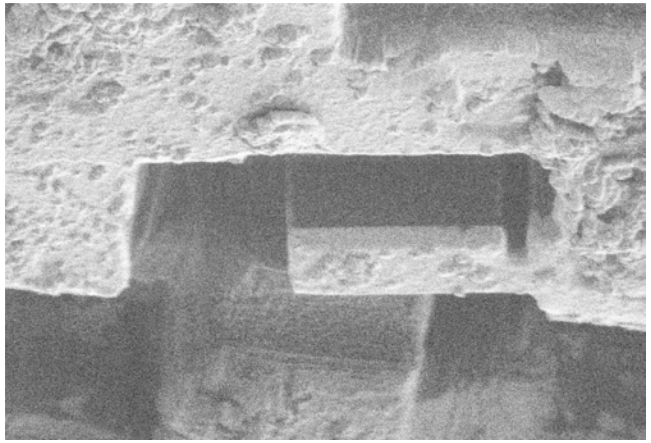
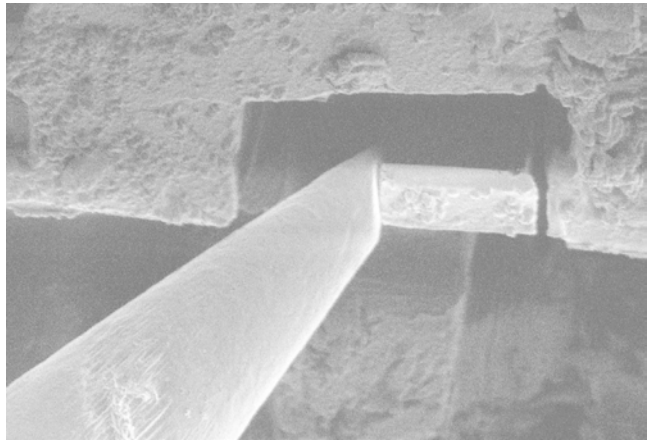
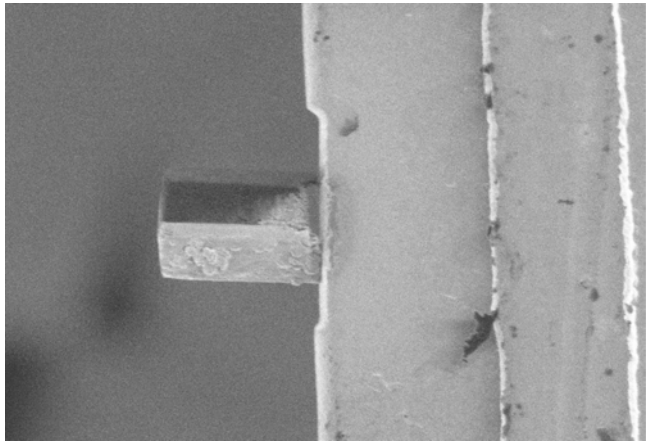
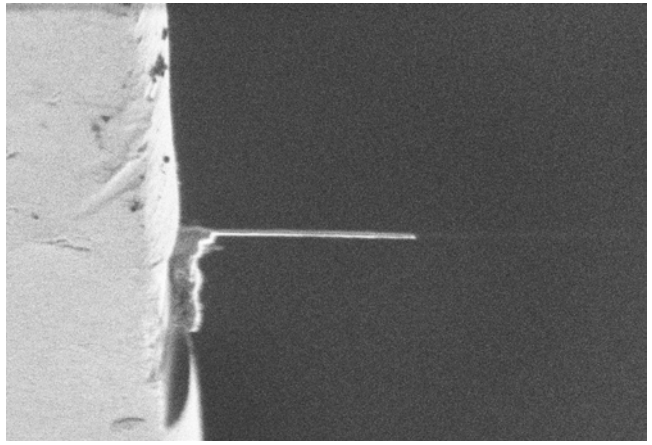
mag 29 600 x
WD 4.0 mm
HFW 7.00 µm
det TLD
mode SE
2 µm

Liftout

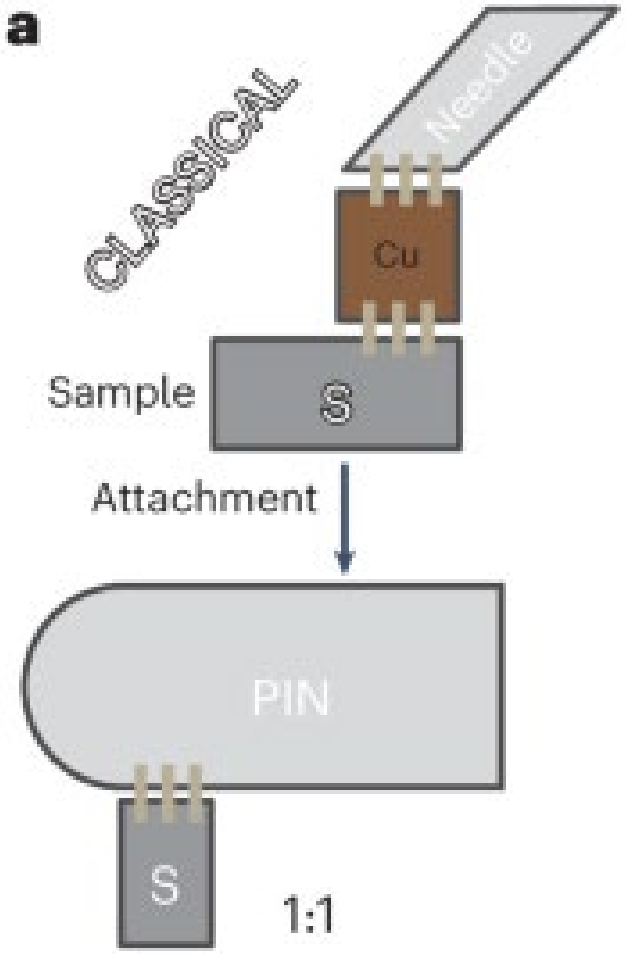








Liftout


a
CLASSICAL
Needle
Cu
Sample
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Attachment
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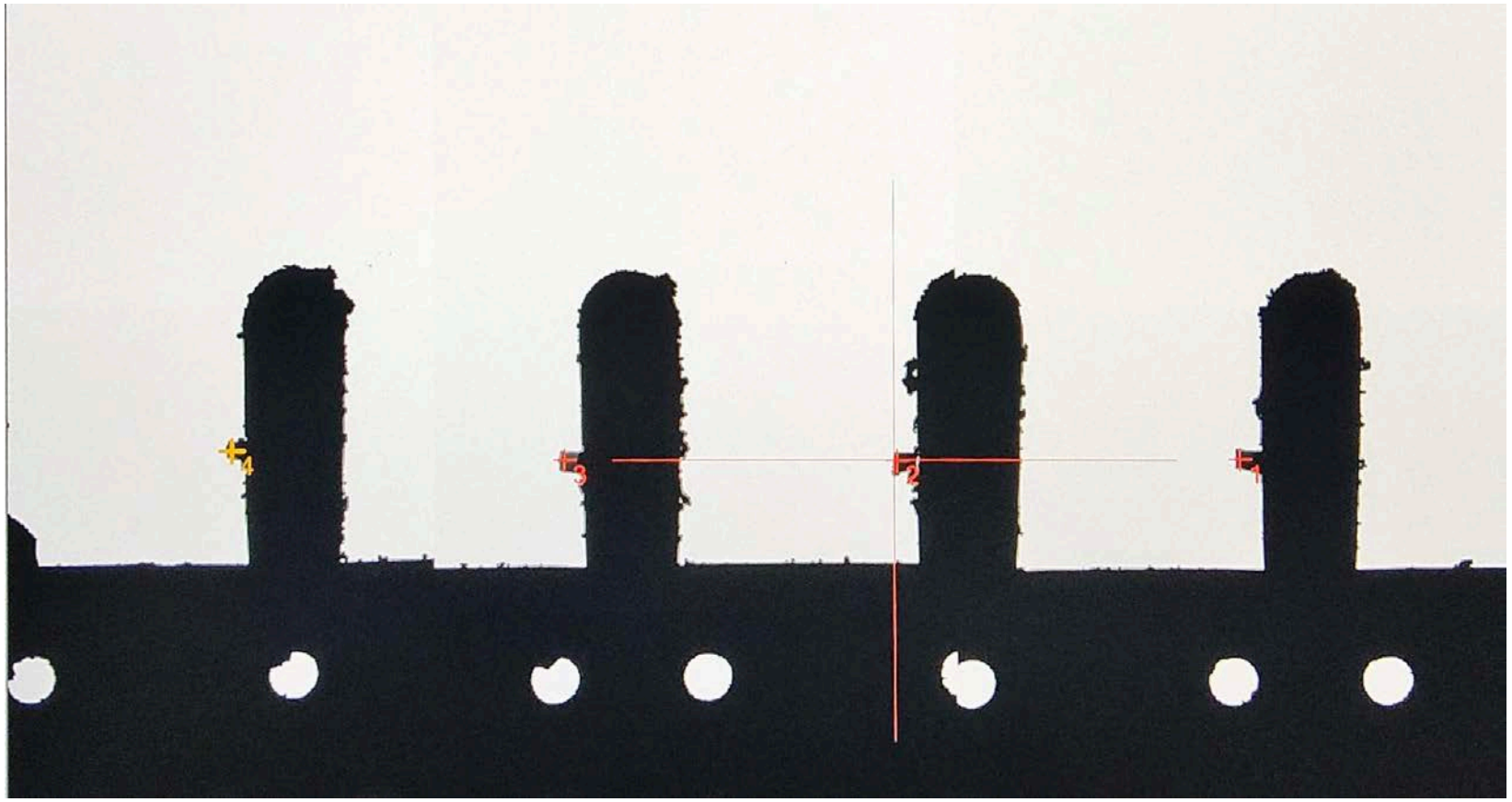
1:1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3
2
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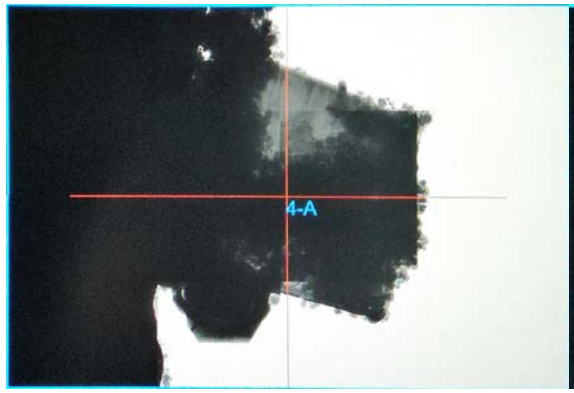
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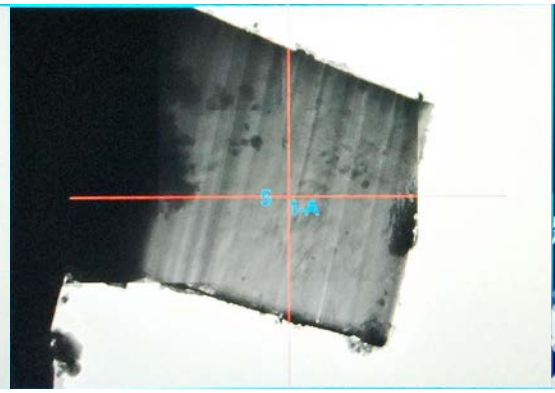
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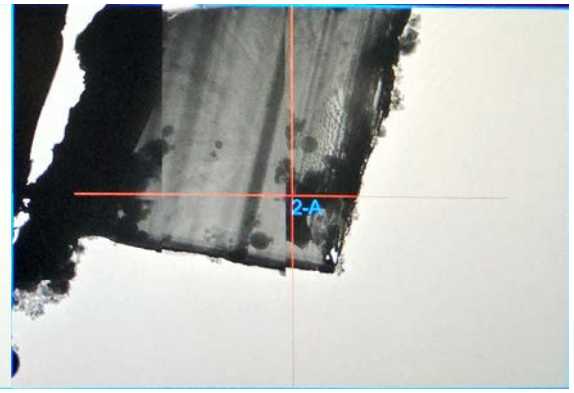
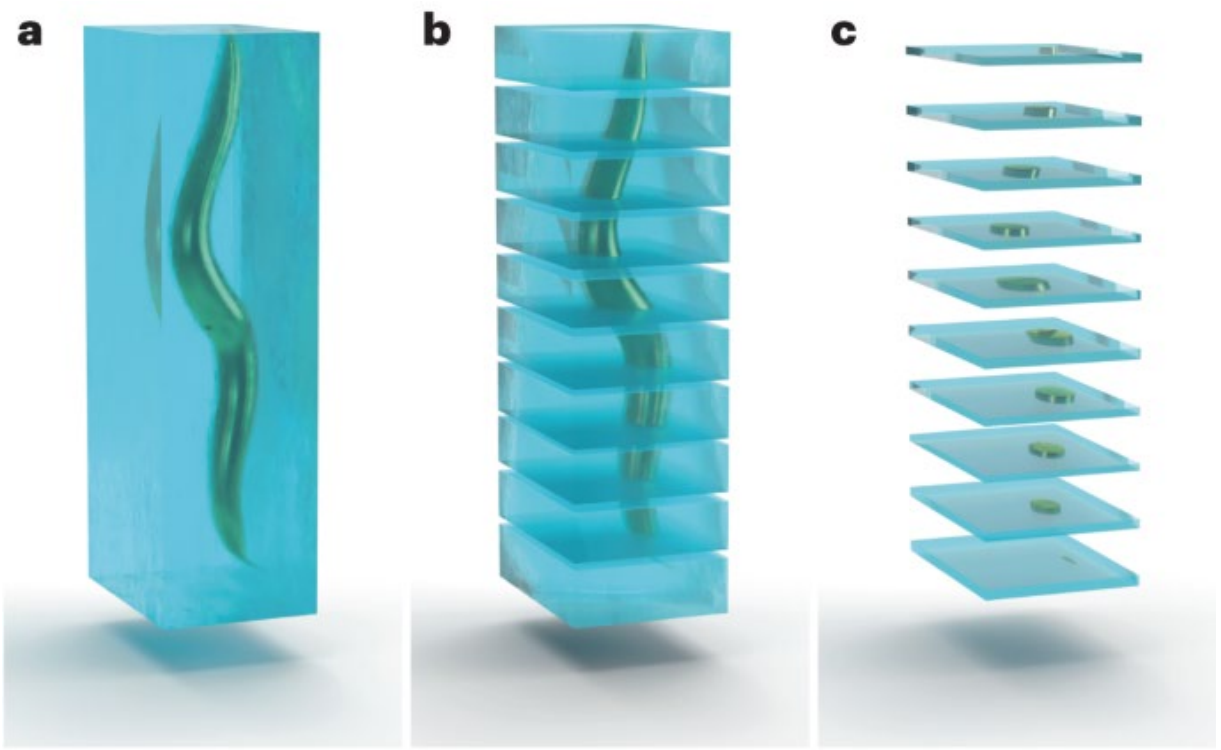
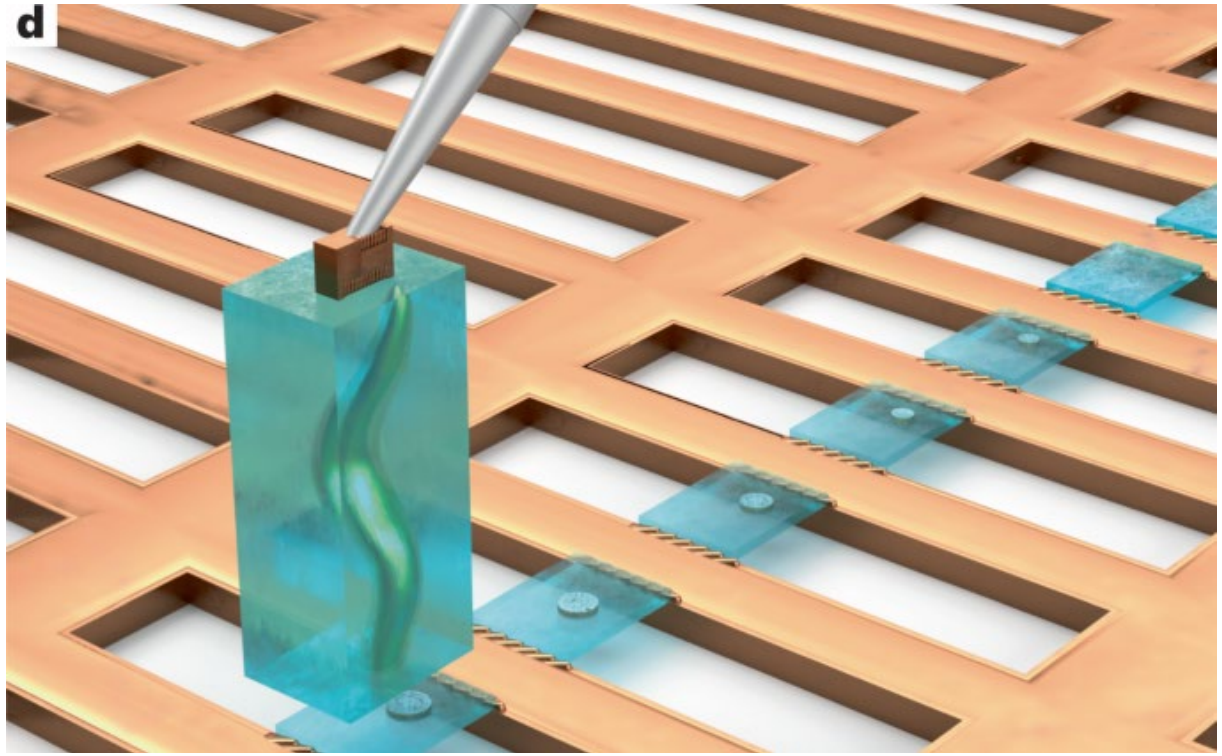

2-A

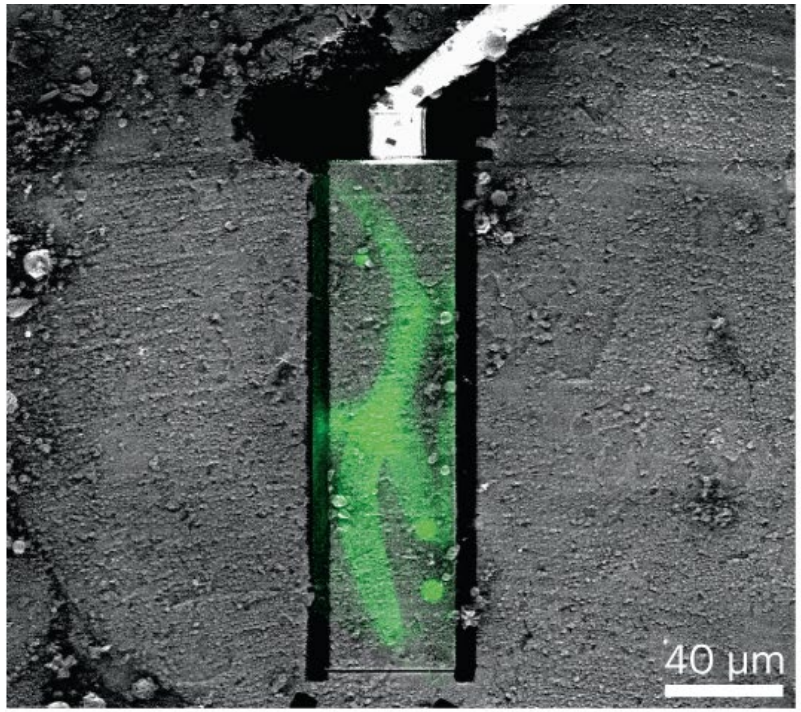
Serial Liftout

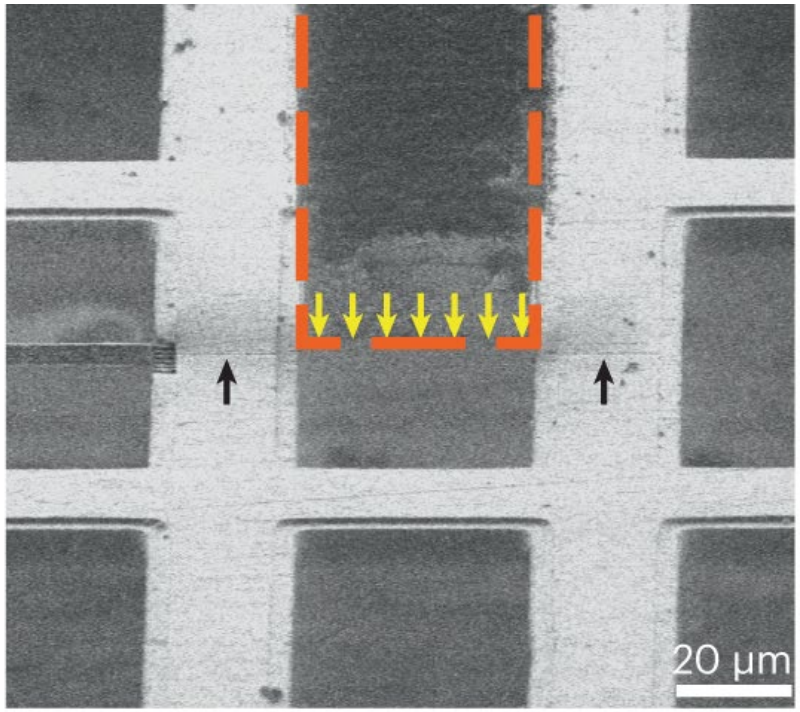


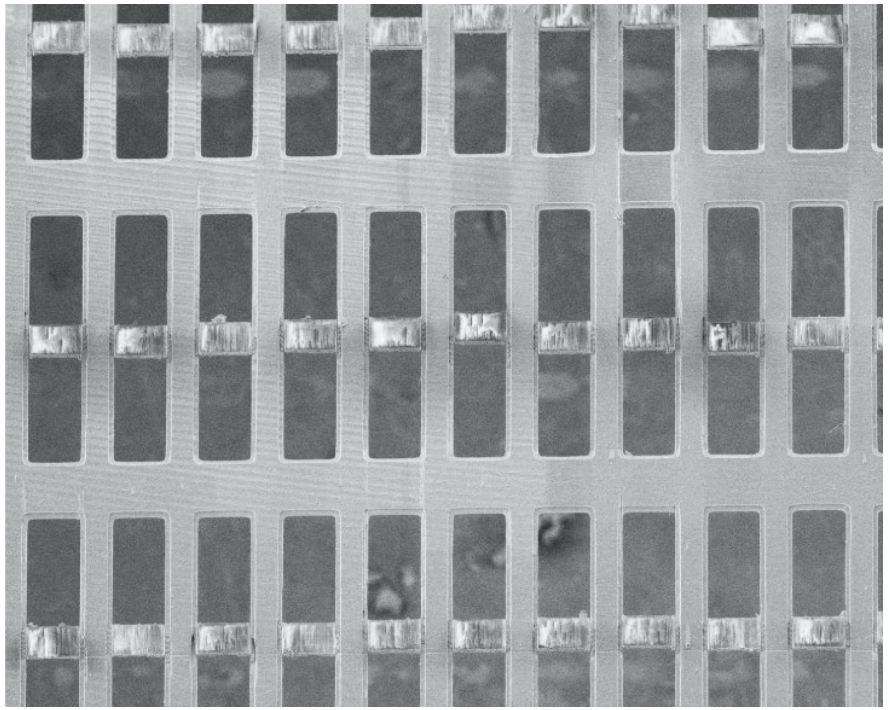


Schiøtz, O.H *Nat Methods* **21**, 1684–1692 (2024)

Serial Liftout

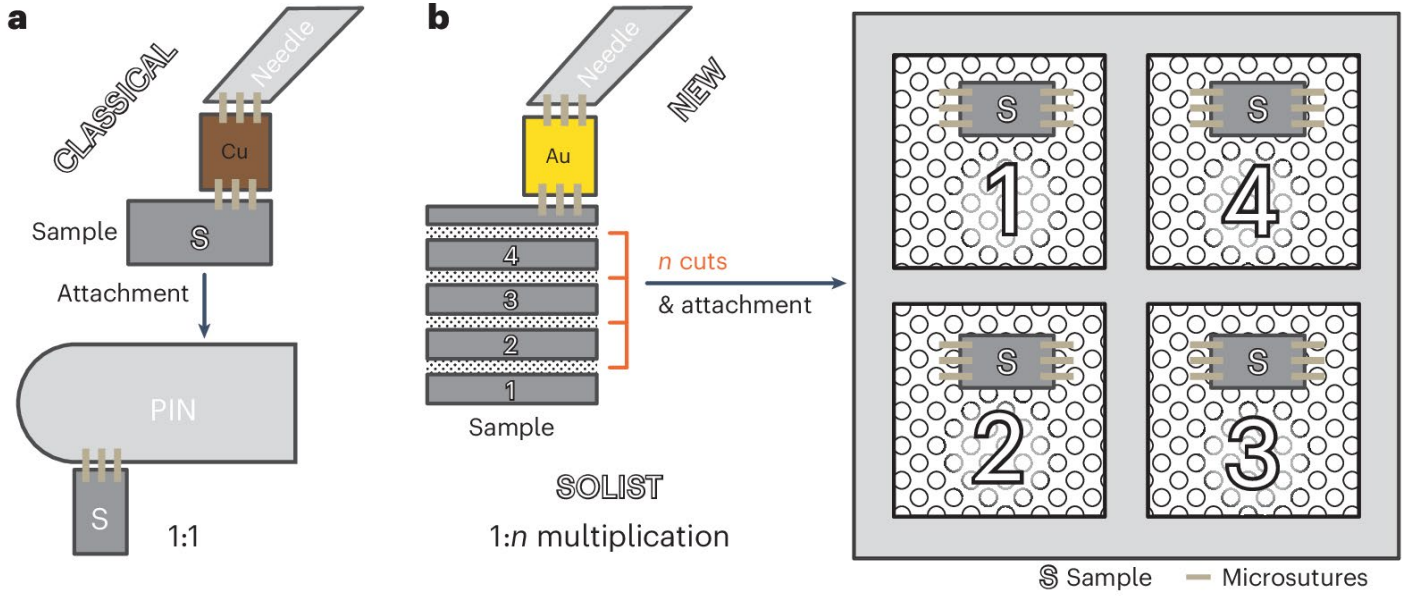


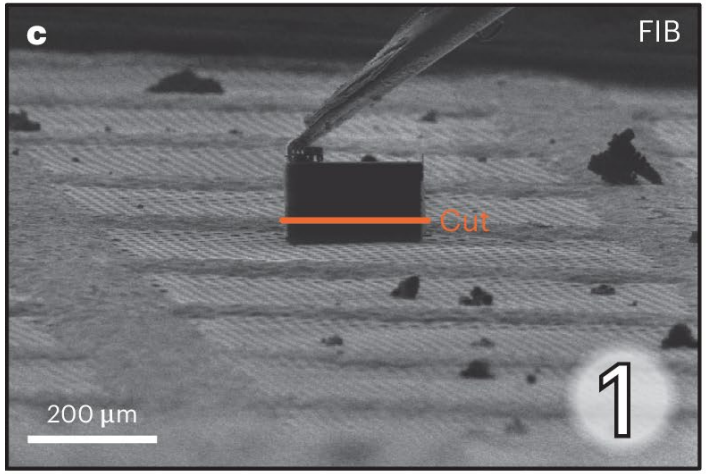


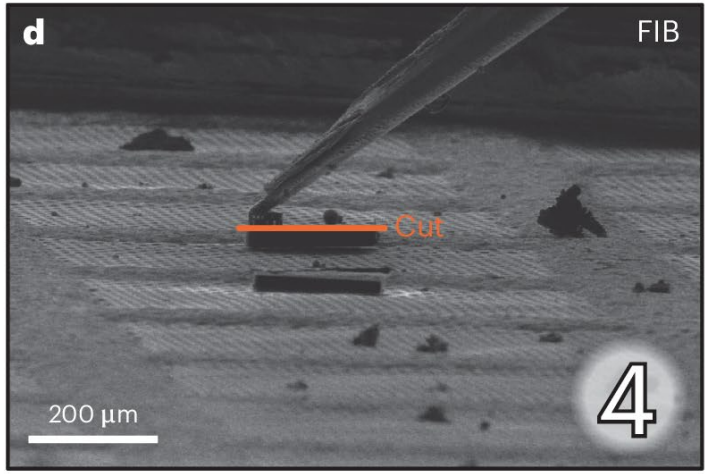


Schiøtz, O.H *Nat Methods* **21**, 1684–1692 (2024)

Serial liftout (SOLIST)

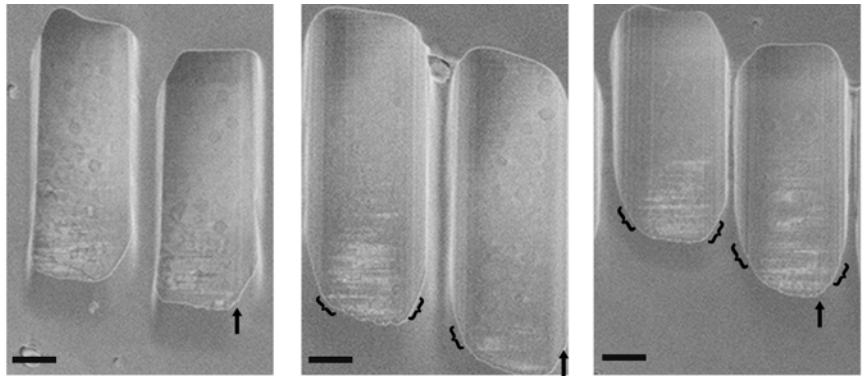


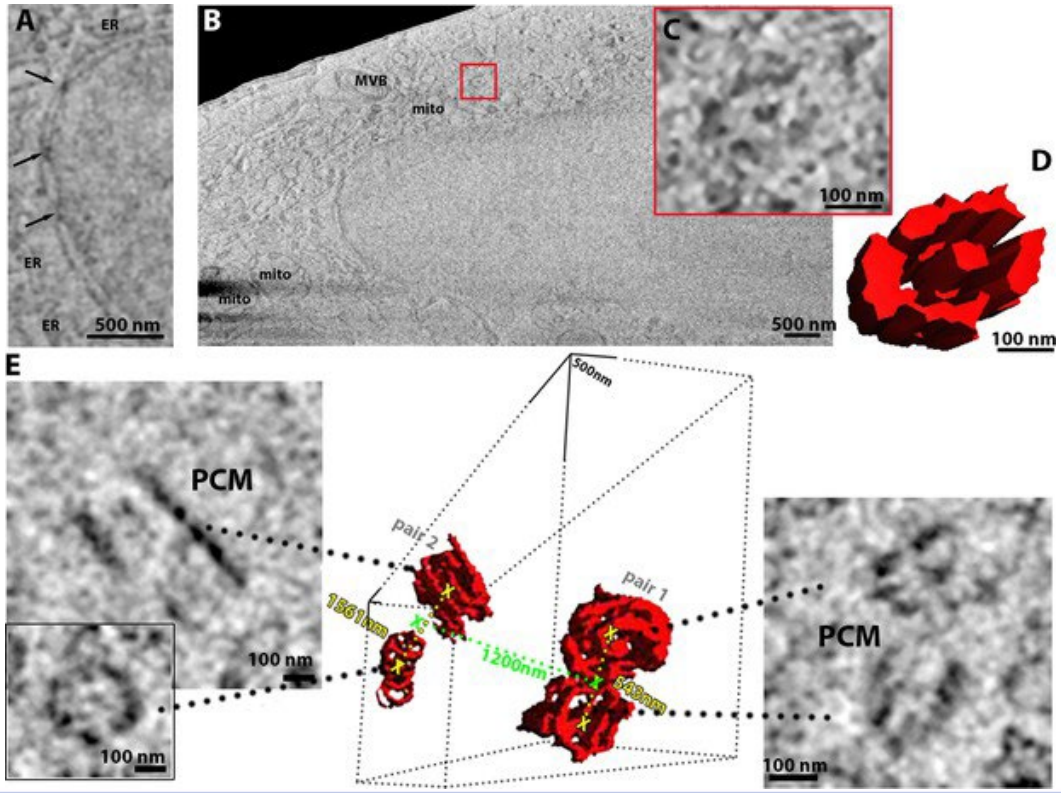


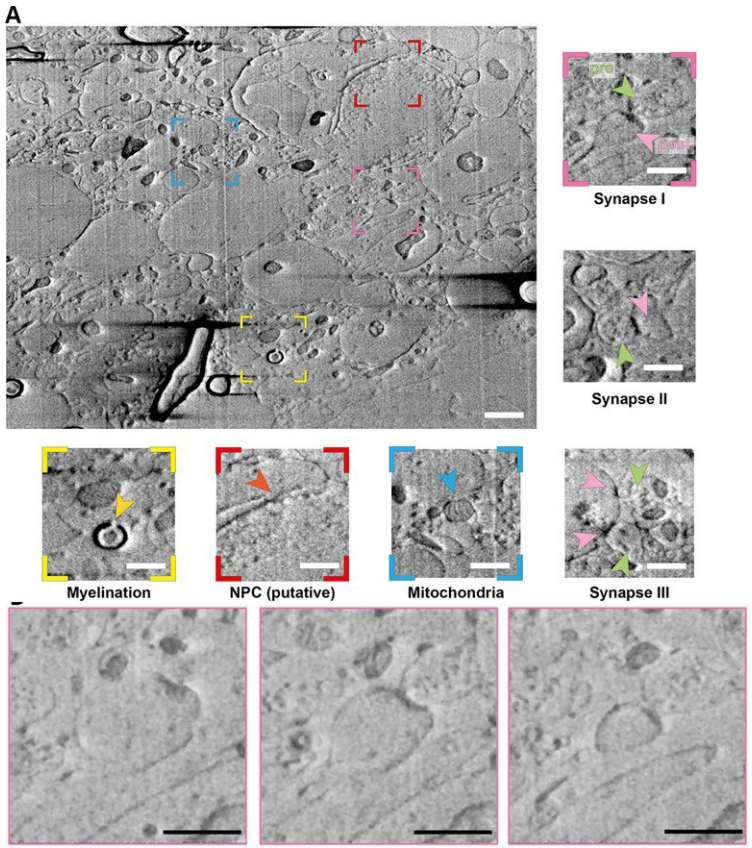


Nguyen, H.T.D., *Nat Methods* **21**, 1693–1701 (2024)

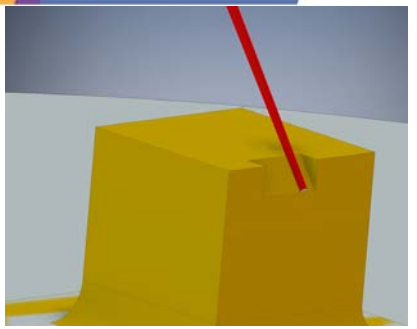
What if you need context?

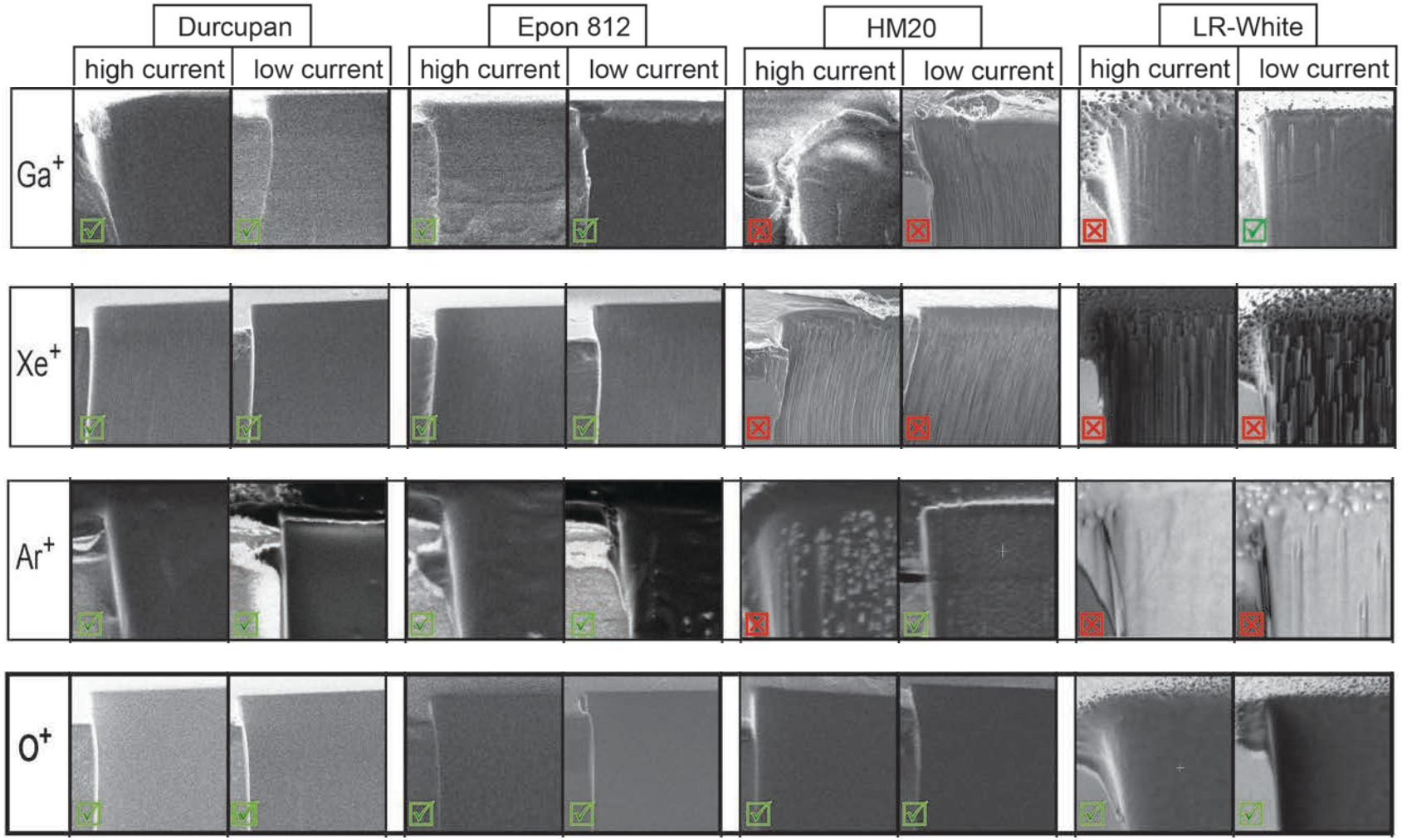






Resin/ion beam compatibility





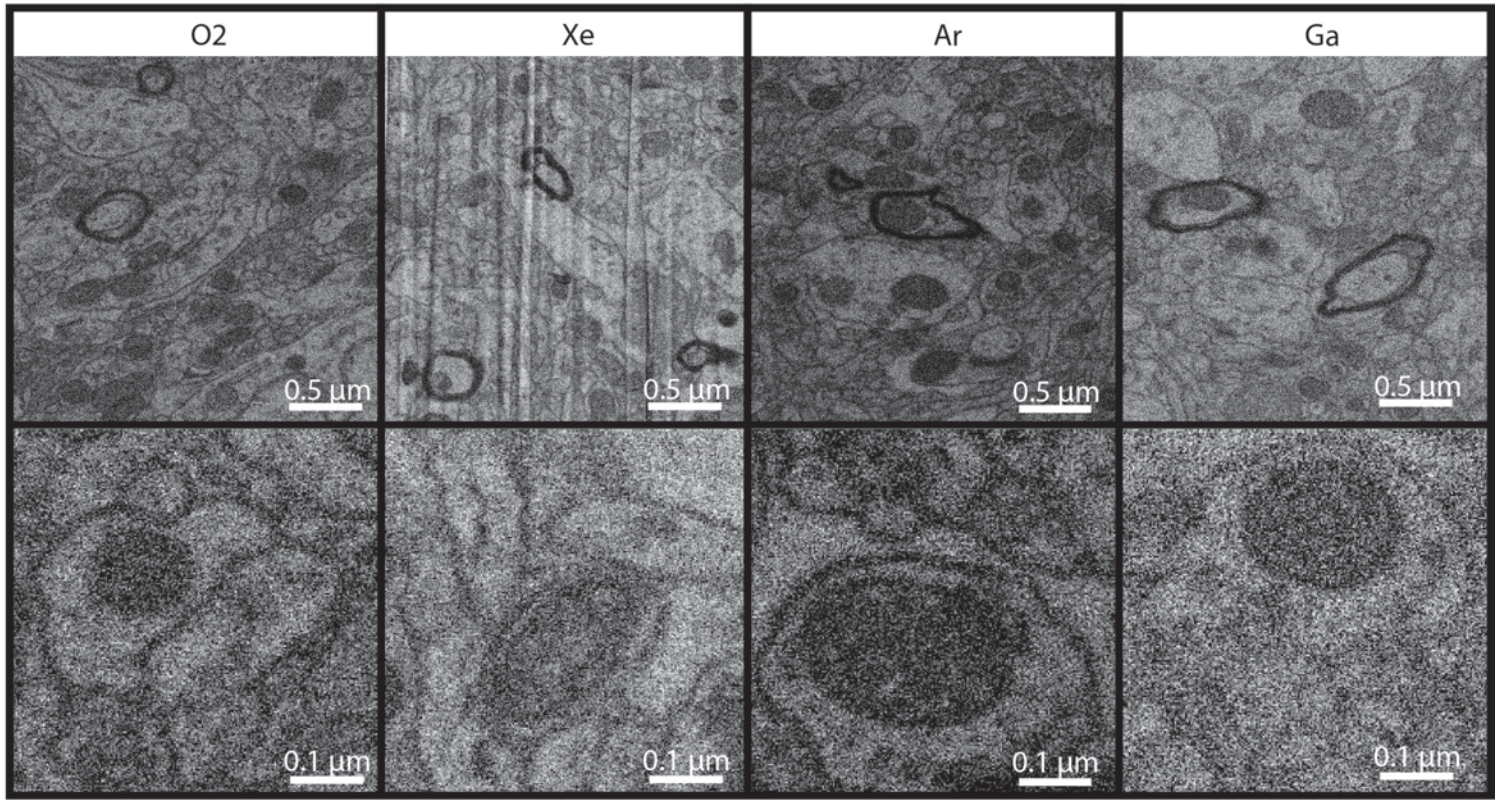
Works well on hard epoxy resins,
but acrylic based resins curtain or melt

Usable on hard epoxy resins,
but it always displays curtains

Usable on hard epoxy resins, but it
displays irregular milling efficiency

Performs equally on every
tested resin

Ga implantation reduces SNR when imaging



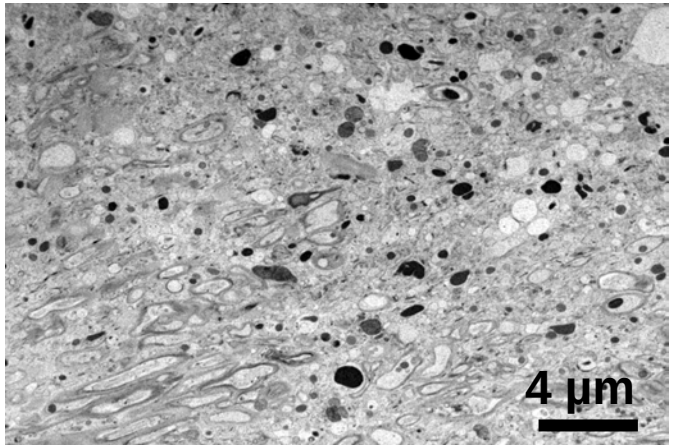
30% higher contrast when using non-metallic beams

Faster imaging or better image quality

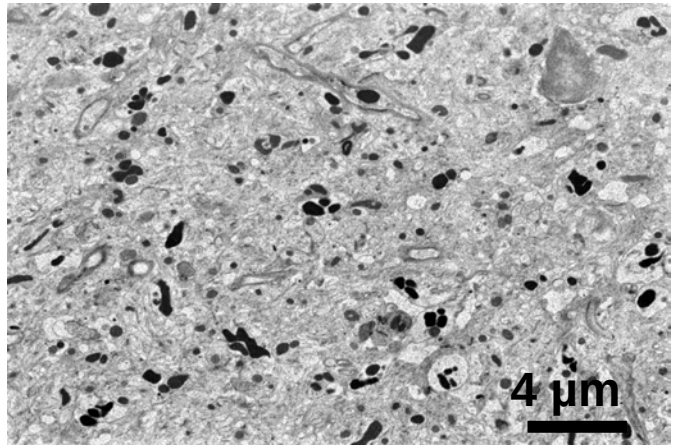
Mouse brain, embedded in epon

O2 PFIB increases throughput on S&V

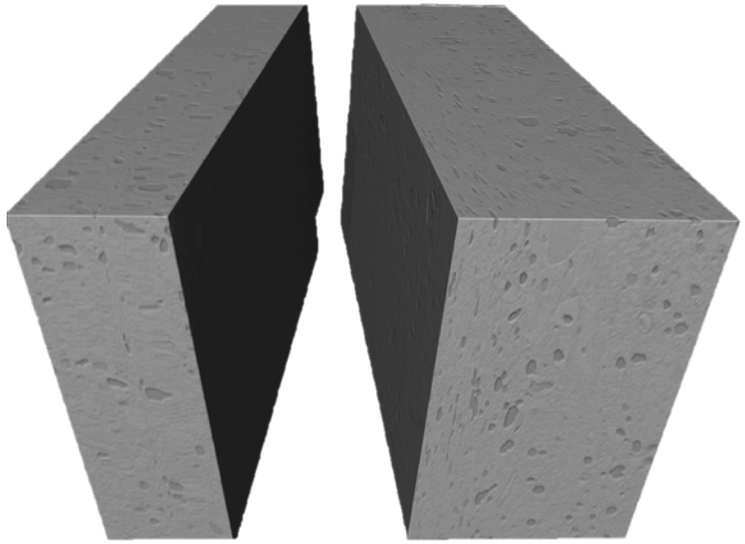
Mouse brain, embedded in epon acquired over 12h @5nm



Ga



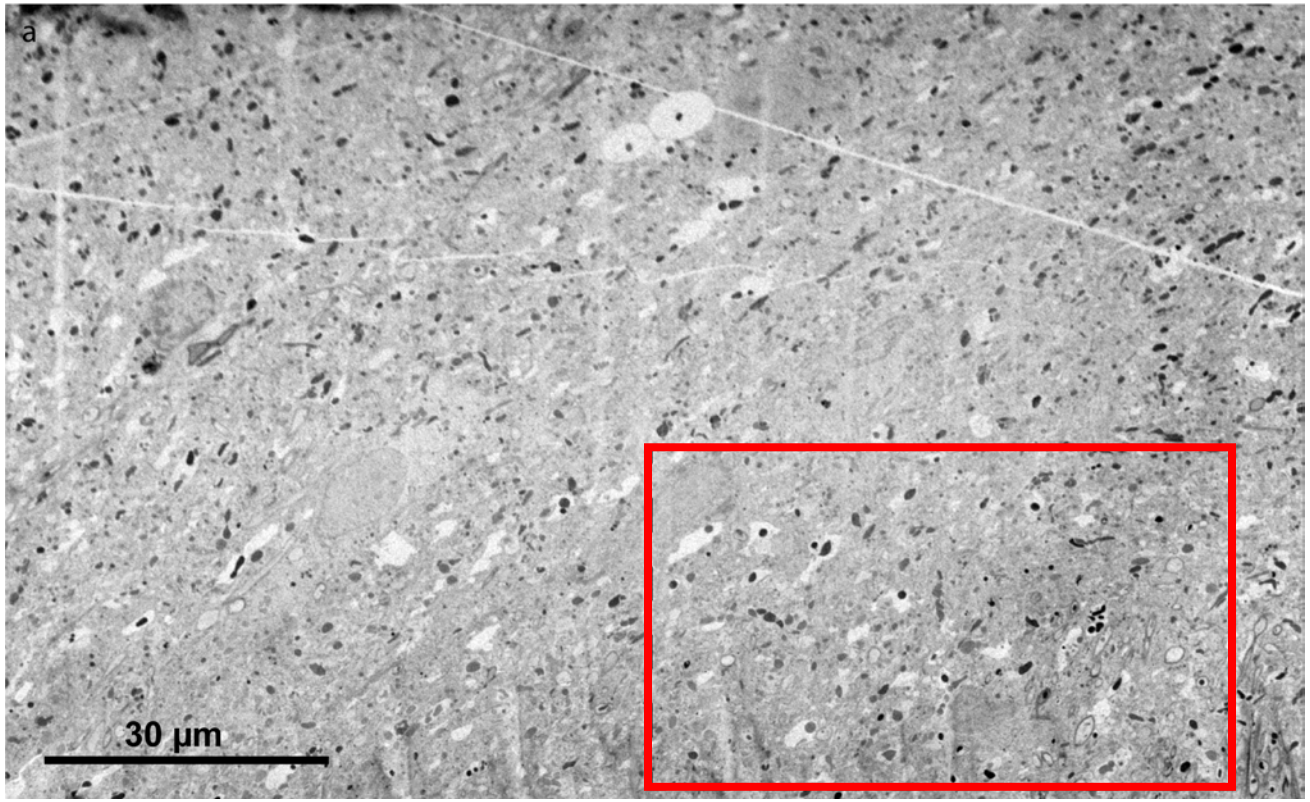
O2



5.5 µm

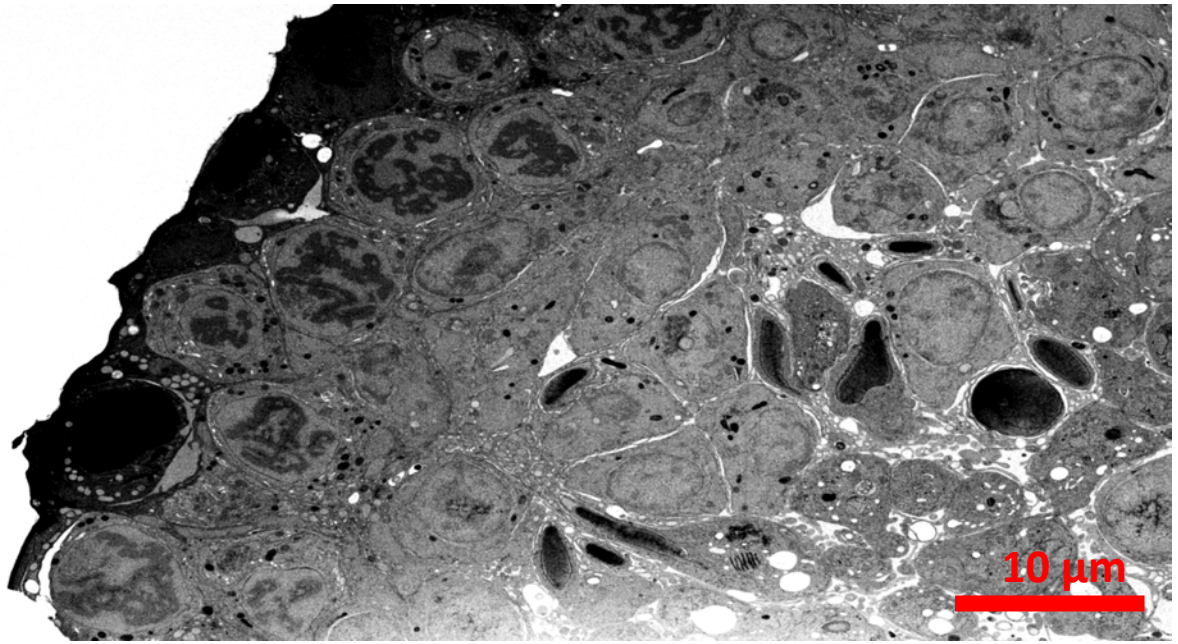
10.1 µm

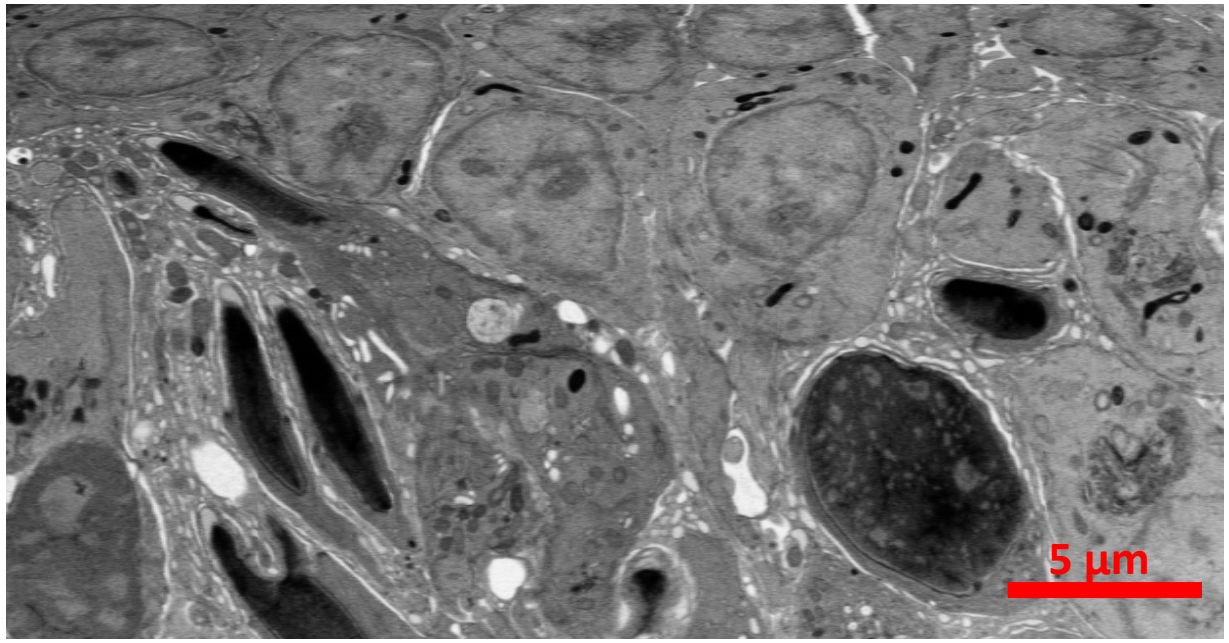
Large area on S&V (130x82 µm)



Increased throughput on S&V using O-PFIB

Mouse seminiferous tubule

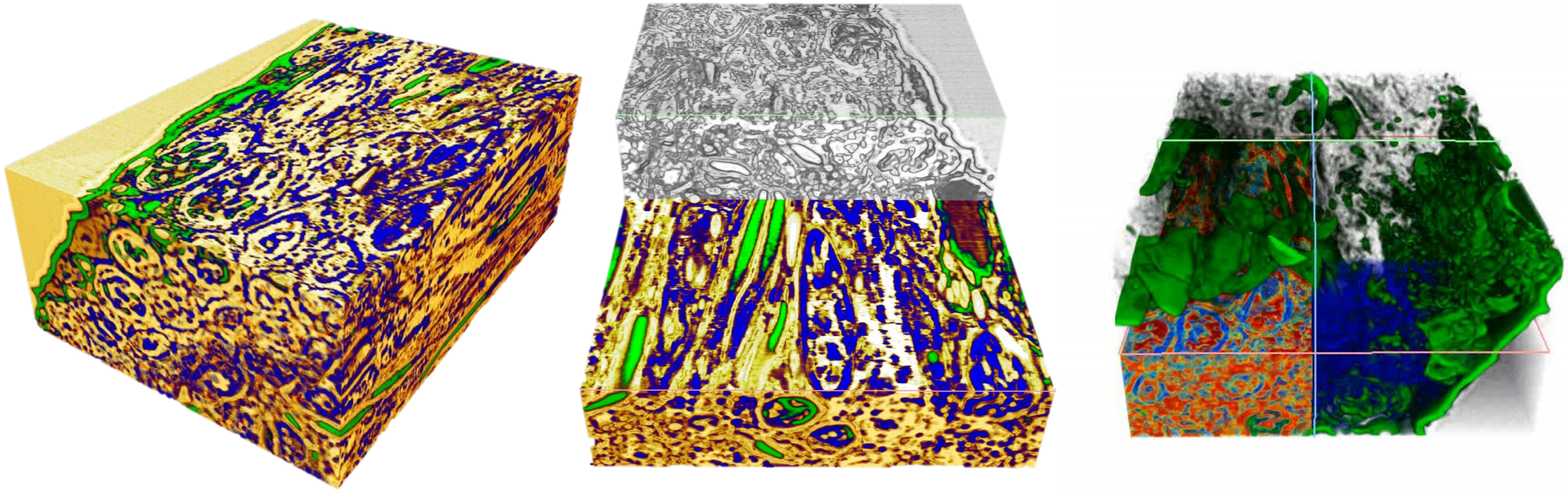




2000 slices 6144*4096 pixel, voxel size 10*10*10 nm (61.44 * 40.96 um scan area). 45 nA oxygen ion beam. 2keV, 200pA electron beam 2 min per slice (milling + imaging)

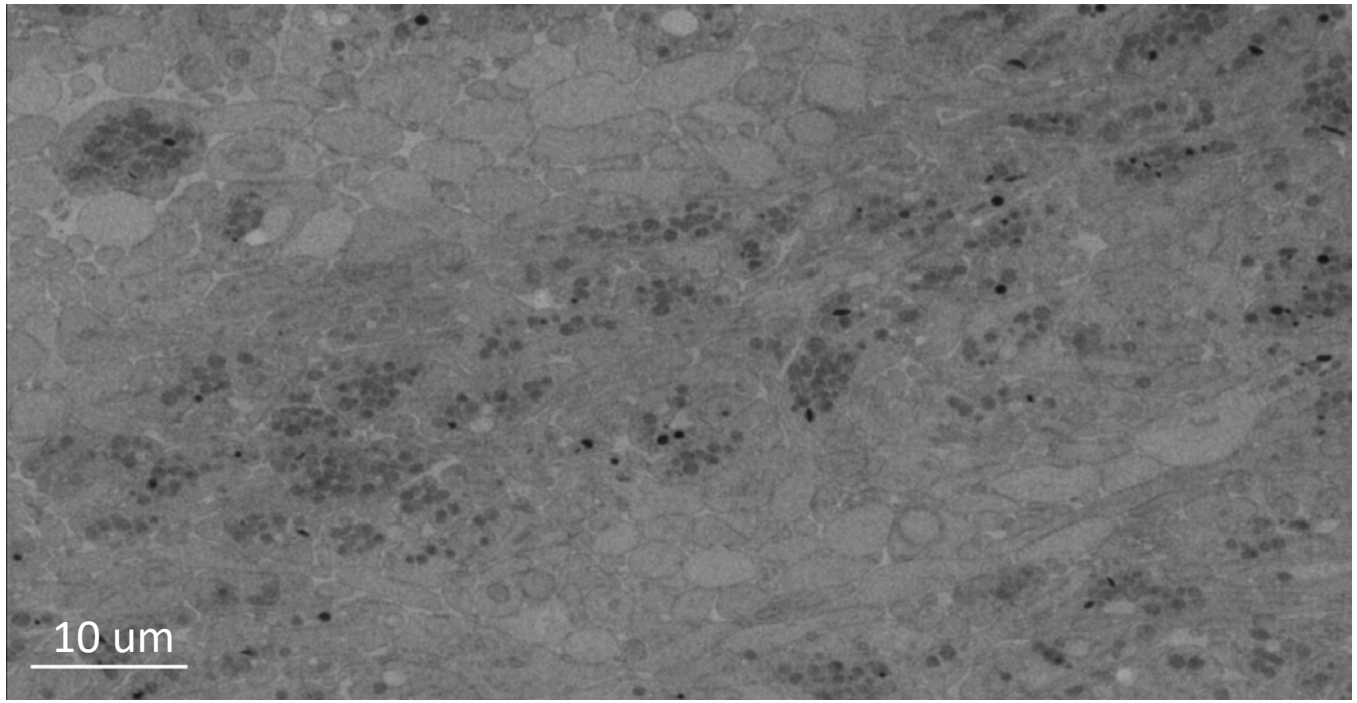
Increased throughput on S&V using O-PFIB

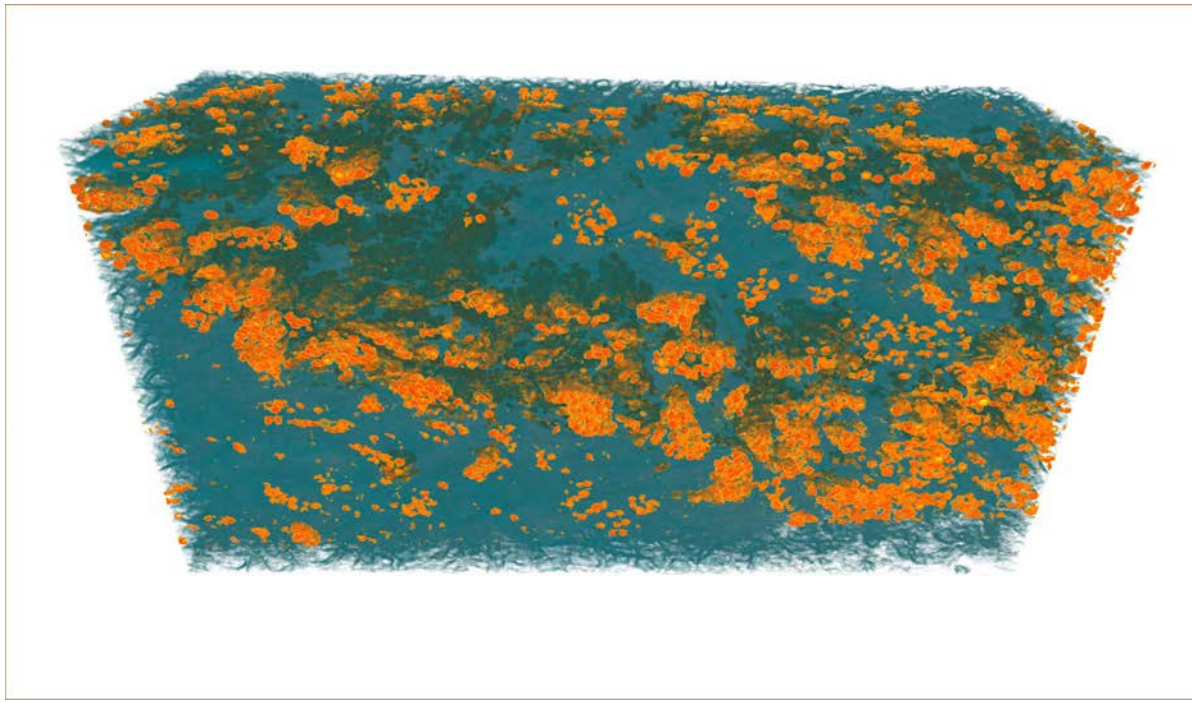
Mouse seminiferous tubule



2000 slices 6144*4096 pixel, voxel size 10*10*10 nm (61.44 * 40.96 um scan area). 45 nA oxygen ion beam. 2keV, 200pA electron beam 2 min per slice (milling + imaging)

Large volume FIB/SEM tomography





Blood clots imaged at 10 nm resolution
Trench size width is 100um
Imaged volume is 85x62x15 um over a weekend

Questions?