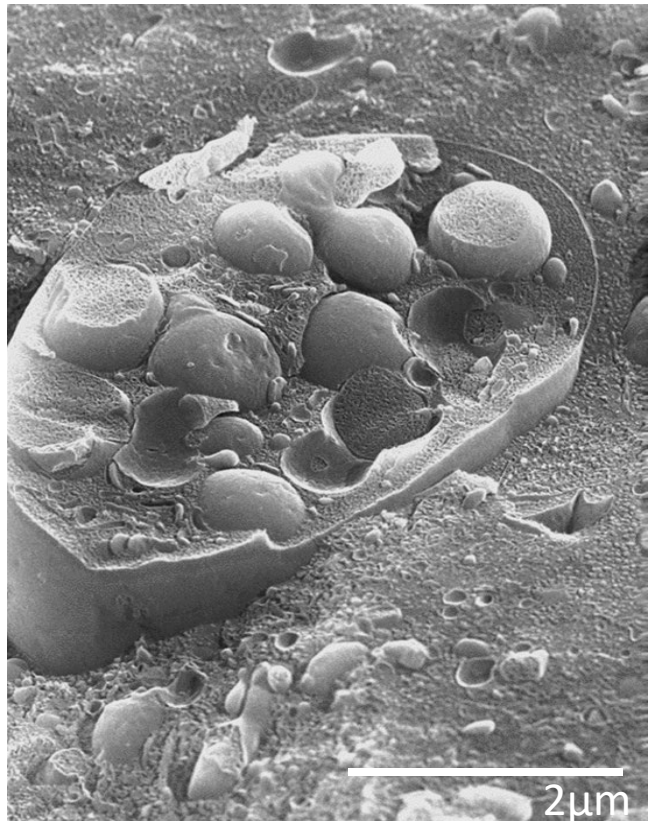
"Cryo-SEM at the BioCryo Facility: Workflow, Instrumentation and Examples"



July 21, 2022
11:00 AM CST

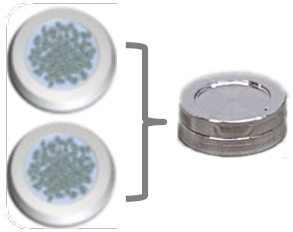
Dr. Reiner Bleher, BioCryo Facility Manager, and Assistant Research Professor

"Cryo-SEM at the BioCryo Facility: Workflow, Instrumentation, and Examples"

Cryo-SEM Workflow

1 2 3 4 5 6 7

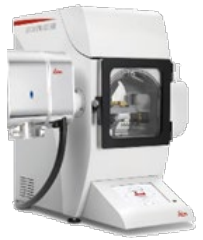
Mounting the sample → Freezing the sample → Storage in LN2 → Loading the sample → Freeze fracture → Freeze etching → Cryo coating → Cryo-SEM Imaging



HPM100
or liquid ethane
w/ cryo-protectant)

Loading dock

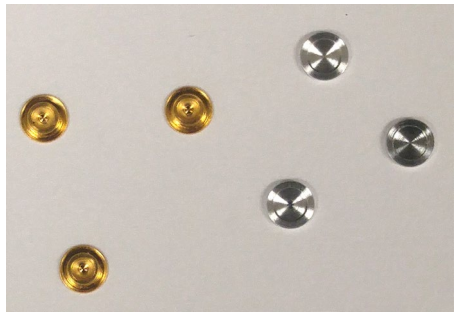
Cryo-shuttle

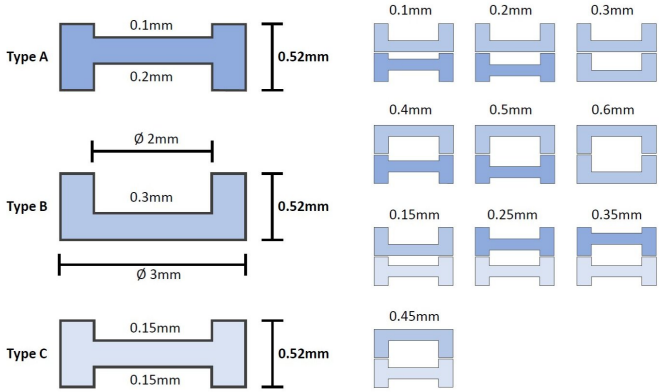


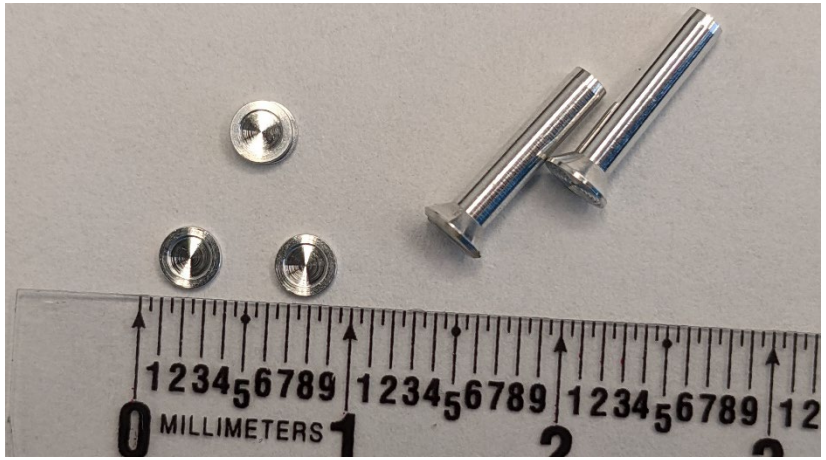
ACE600

S-4800

Cryo-SEM Workflow – Sample carriers



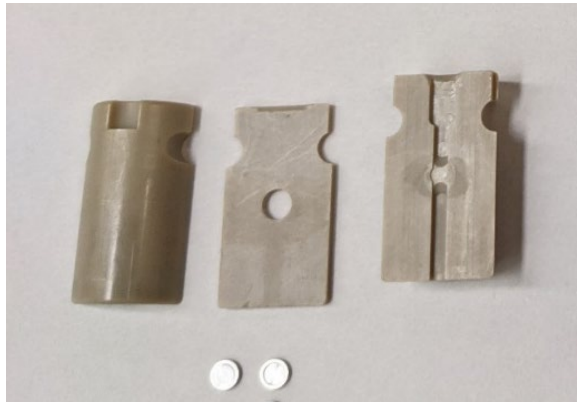




Slices of wet samples with exact thickness for the carriers

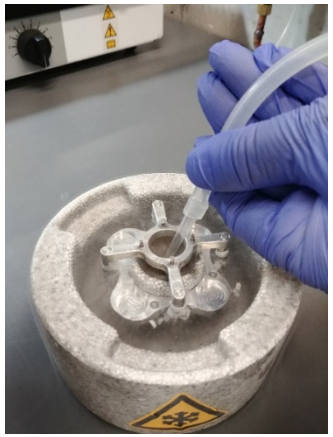
Cryo-SEM Workflow – Sample Freezing

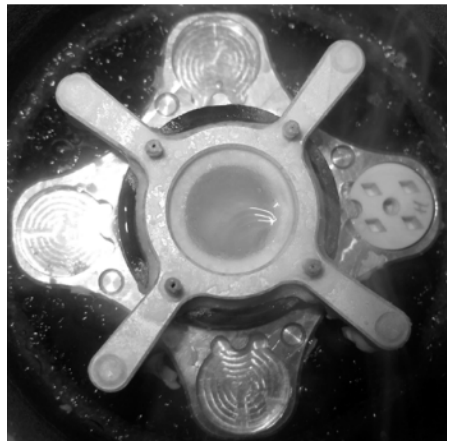
HPF
(high-pressure freezing with HPM100)



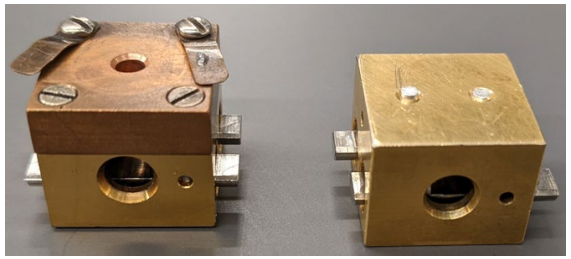
“Plunge” Freezing
In liquid ethane

Requires cryo-protectant: glycerol or ethanol

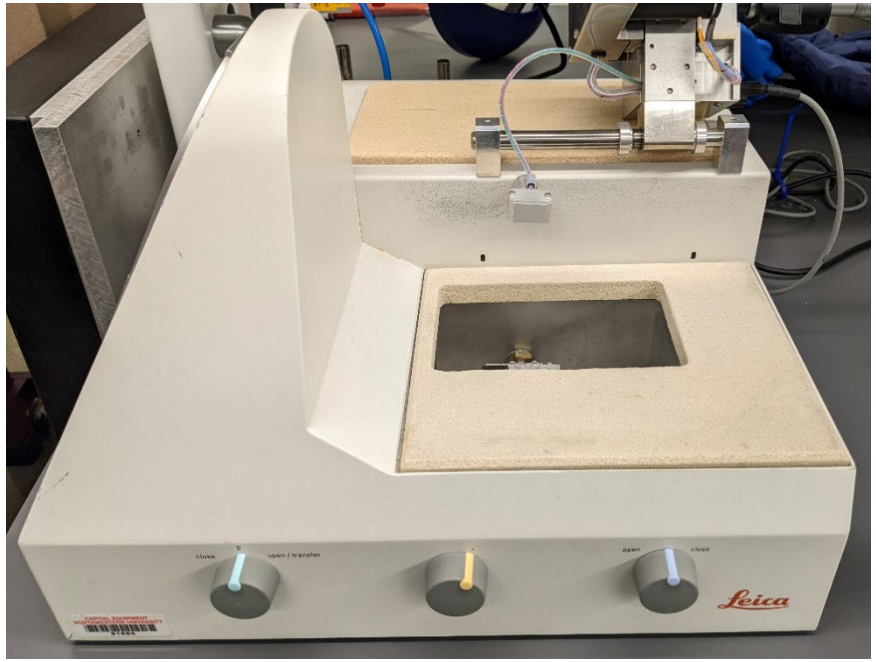




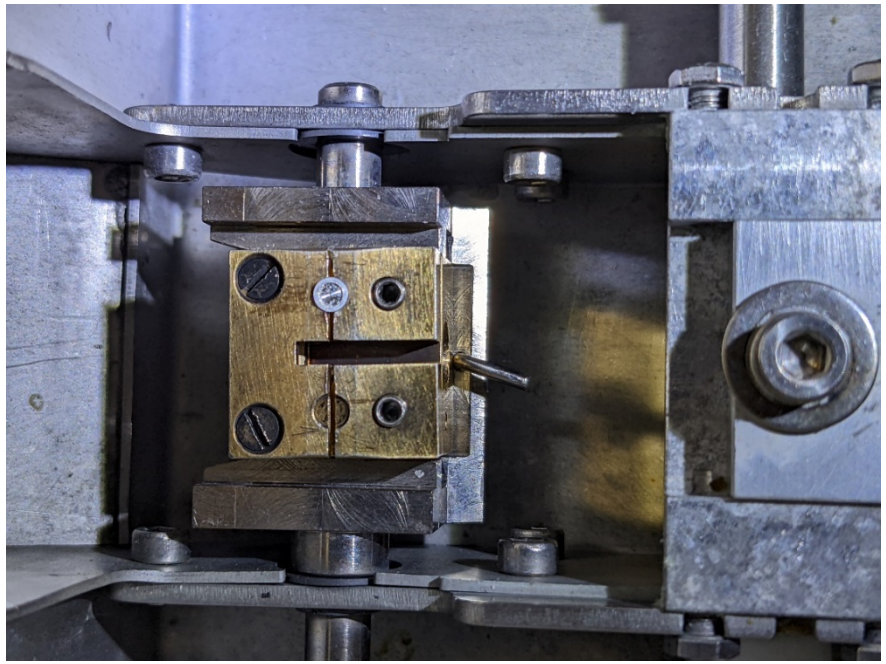
Cryo-SEM Workflow – Sample Loading



Loading station



Loading station



Sample holder

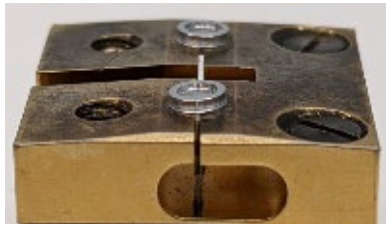
Cryo-SEM Workflow – Sample Loading

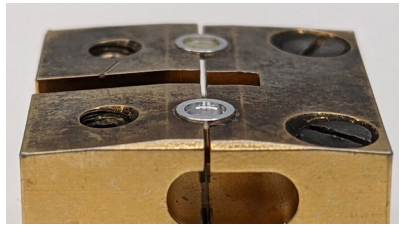
Loading station

Cryo-shuttle

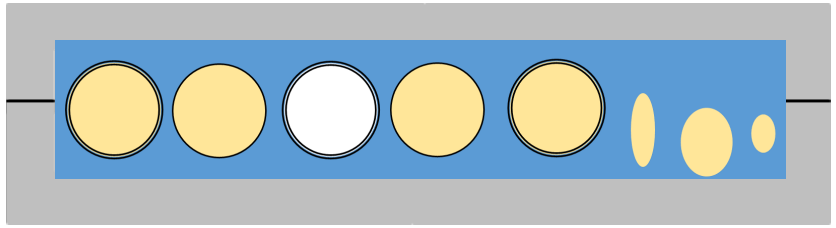
1. EM ACE600
2. Dewar
3. VCT - dock
4. Cryo - stage

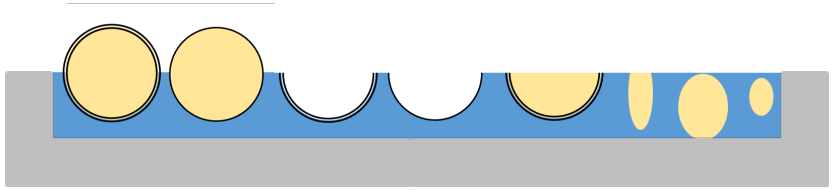
Cryo-SEM Workflow – Freeze Fracturing, Etching, cryo-Coating in the ACE600





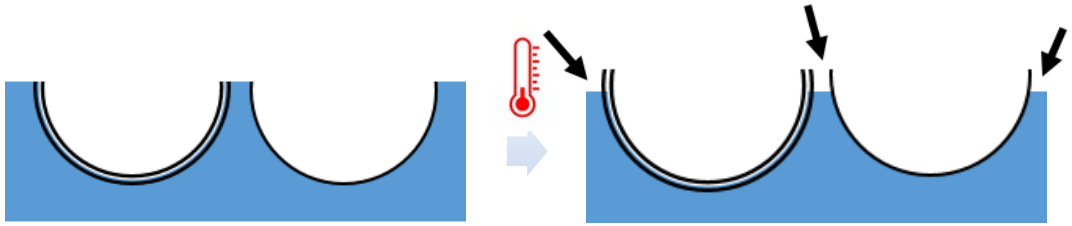
Freeze fracture





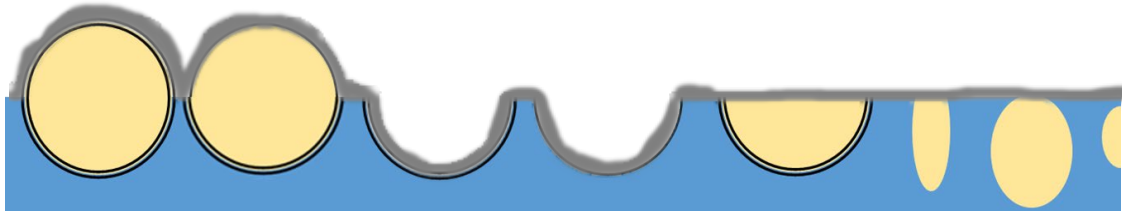
Different fracture planes

Freeze etching



Superficial ice is sublimated, more details from the fracture-face are revealed

Cryo coating



The surface is coated with a layer of Pt and C to increase emission of SE and the conductivity

Cryo-SEM Workflow – Freeze Etching in the ACE600

Saturation Vapor Pressure

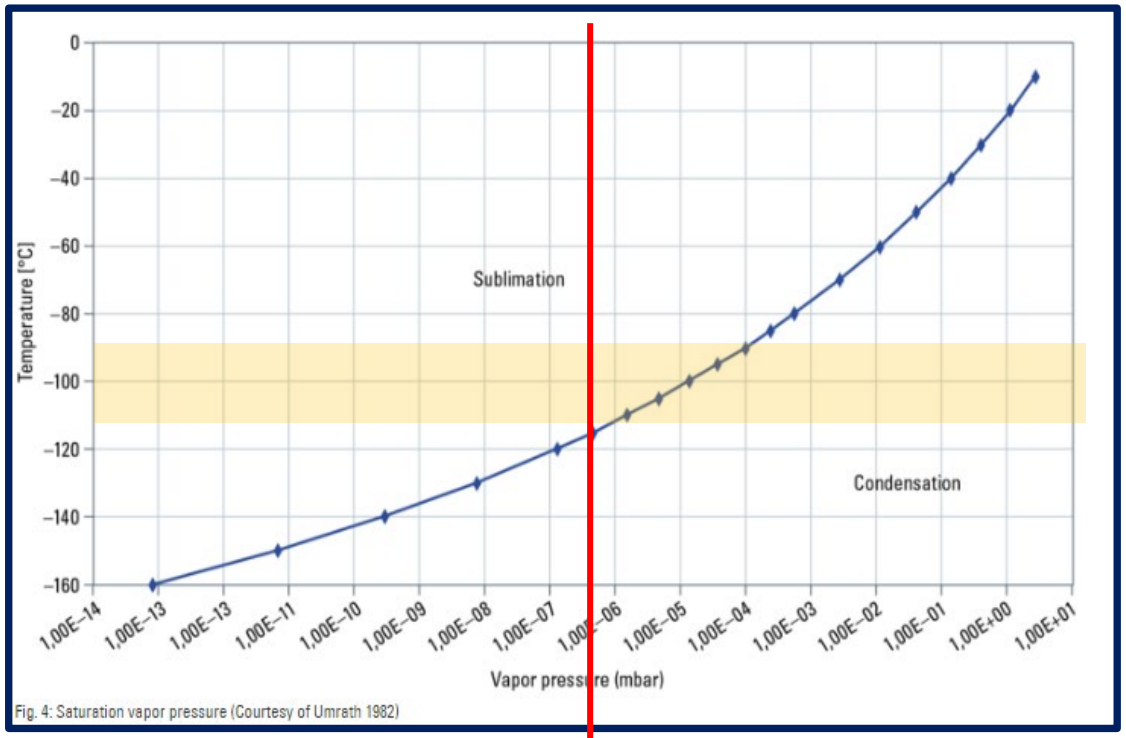


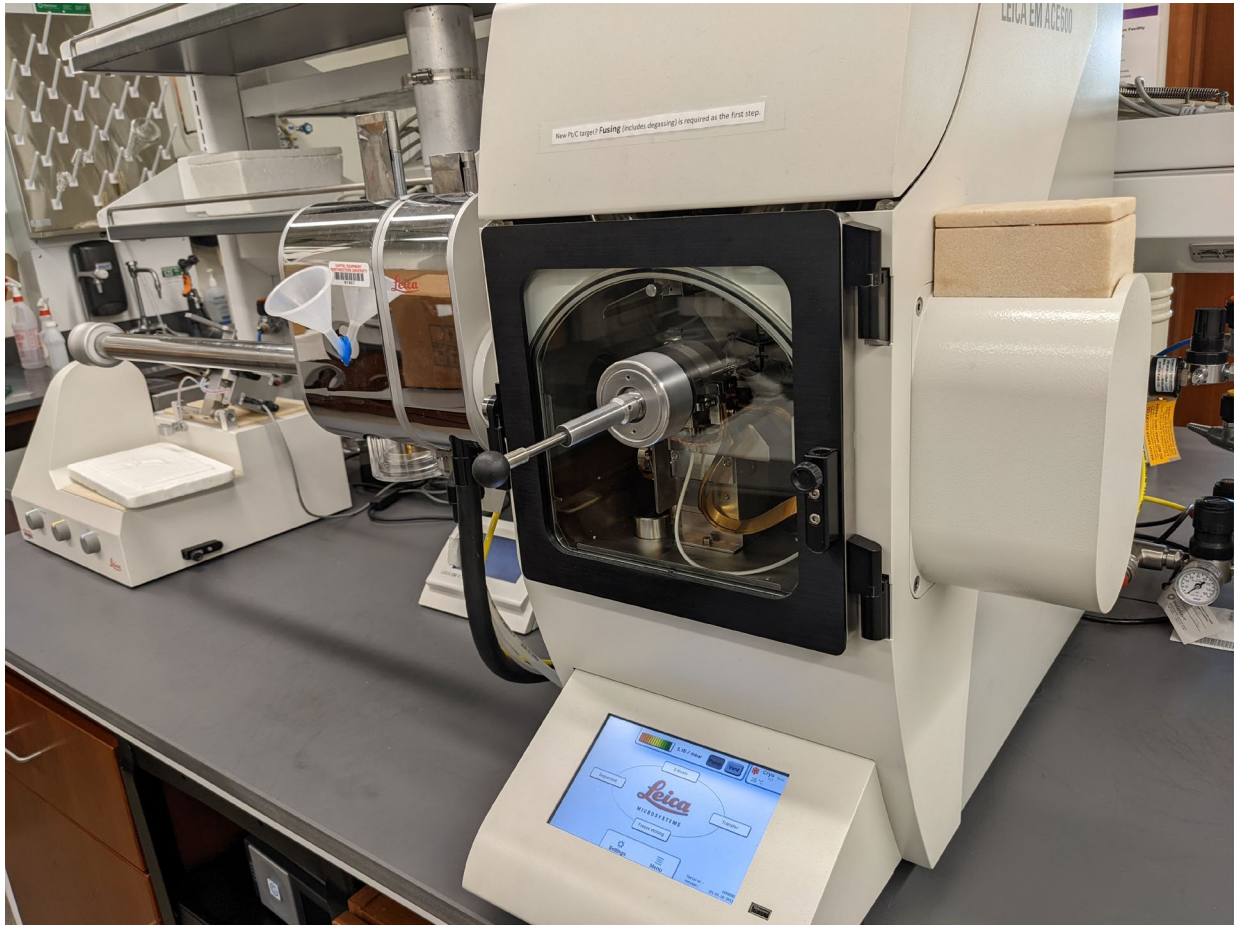
Fig. 4: Saturation vapor pressure (Courtesy of Umrath 1982)

ACE600 vacuum

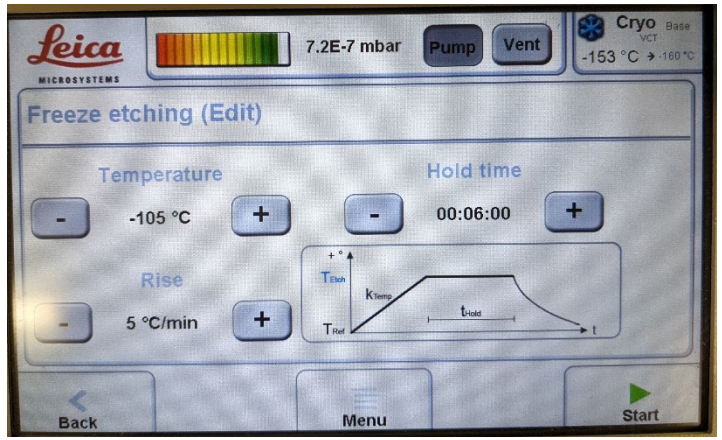
Sublimation Rates

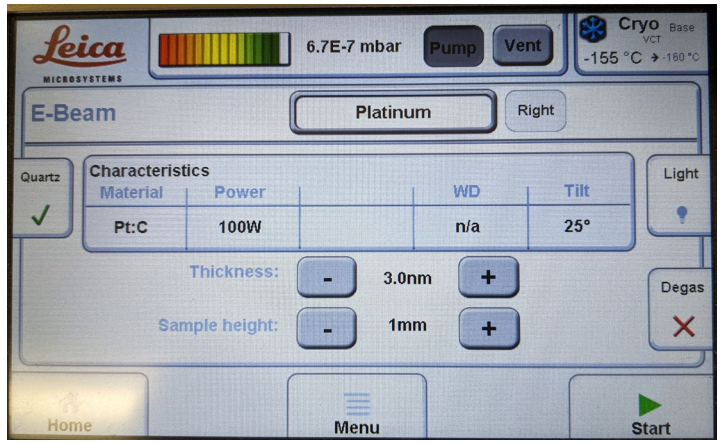
Temperature (°C)	Saturation pressure (mbar)	Sublimation rate (g/cm^2*s)	Increase of dry layer = Etch rate (1/s)
-10	2,60	$2,98 \times 10^{-2}$	324 µm
-20	1,03	$1,21 \times 10^{-2}$	131 µm
-30	$3,81 \times 10^{-1}$	$4,54 \times 10^{-3}$	49,3 µm
-40	$1,29 \times 10^{-1}$	$1,57 \times 10^{-3}$	17,0 µm
-50	$3,93 \times 10^{-2}$	$4,90 \times 10^{-4}$	5,30 µm
-60	$1,08 \times 10^{-2}$	$1,37 \times 10^{-4}$	1,48 µm
-70	$2,59 \times 10^{-3}$	$3,37 \times 10^{-5}$	364 nm
-80	$5,36 \times 10^{-4}$	$7,16 \times 10^{-6}$	77,0 nm
-85	$2,29 \times 10^{-4}$	$3,10 \times 10^{-6}$	33,3 nm
-90	*$9,35 \times 10^{-5}$*	*$1,28 \times 10^{-6}$*	*13,7 nm*
-95	*$3,61 \times 10^{-5}$*	*$5,02 \times 10^{-7}$*	*5,39 nm*
-100	*$1,32 \times 10^{-5}$*	*$1,87 \times 10^{-7}$*	*2,00 nm*
-105	*$4,57 \times 10^{-6}$*	*$6,55 \times 10^{-8}$*	*0,70 nm*
-110	*$1,48 \times 10^{-6}$*	*$2,15 \times 10^{-8}$*	*0,23 nm*
-115	$4,45 \times 10^{-7}$	$6,58 \times 10^{-9}$	70,4 pm
-120	$1,24 \times 10^{-7}$	$1,86 \times 10^{-9}$	19,9 pm
-130	$7,38 \times 10^{-9}$	$1,15 \times 10^{-10}$	1,22 pm
-140	$2,88 \times 10^{-10}$	$4,64 \times 10^{-12}$	49,5 fm
-150	$6,68 \times 10^{-12}$	$1,12 \times 10^{-13}$	1,19 fm
-160	$8,02 \times 10^{-14}$	$1,40 \times 10^{-15}$	0,01 fm

Cryo-SEM Workflow – Freeze Fracturing, Etching, cryo-Coating in the ACE600



ACE600 with cryo-shuttle attached





Cryo-SEM Workflow

High-pressure freezing or freezing in liquid ethane

Cryo-storage

Loading Station

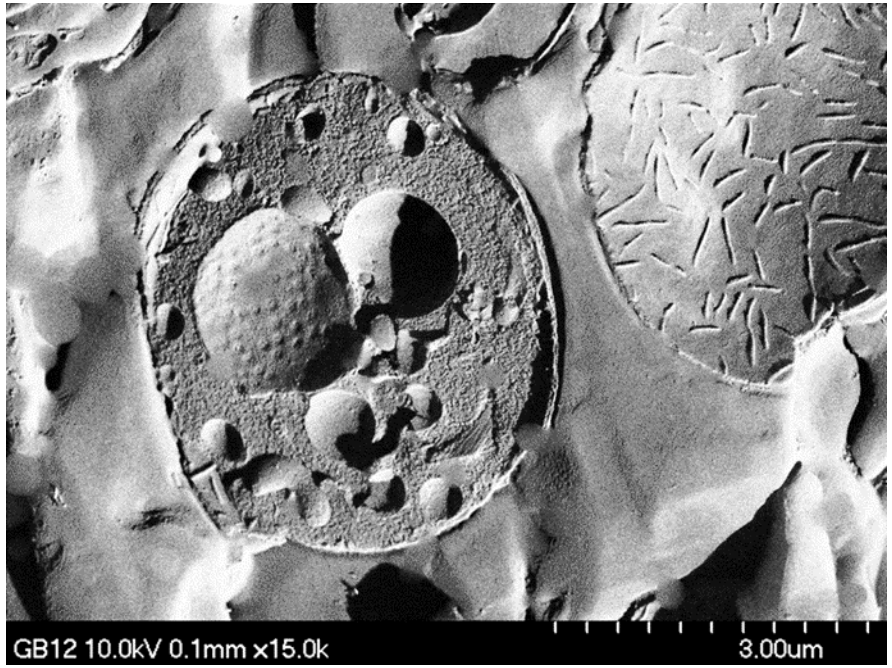
Cryo-shuttle

ACE600
Freeze fracture
/etching
/coating

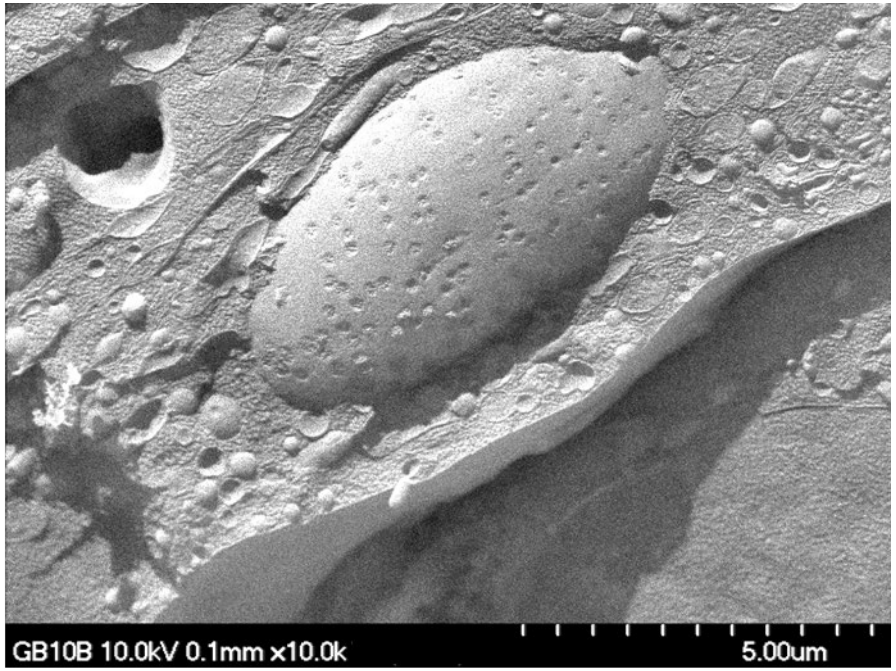
Cryo-SEM
S-4800

Cryo-SEM examples: yeast and epithelial cell

Saccharomyces cerevisiae

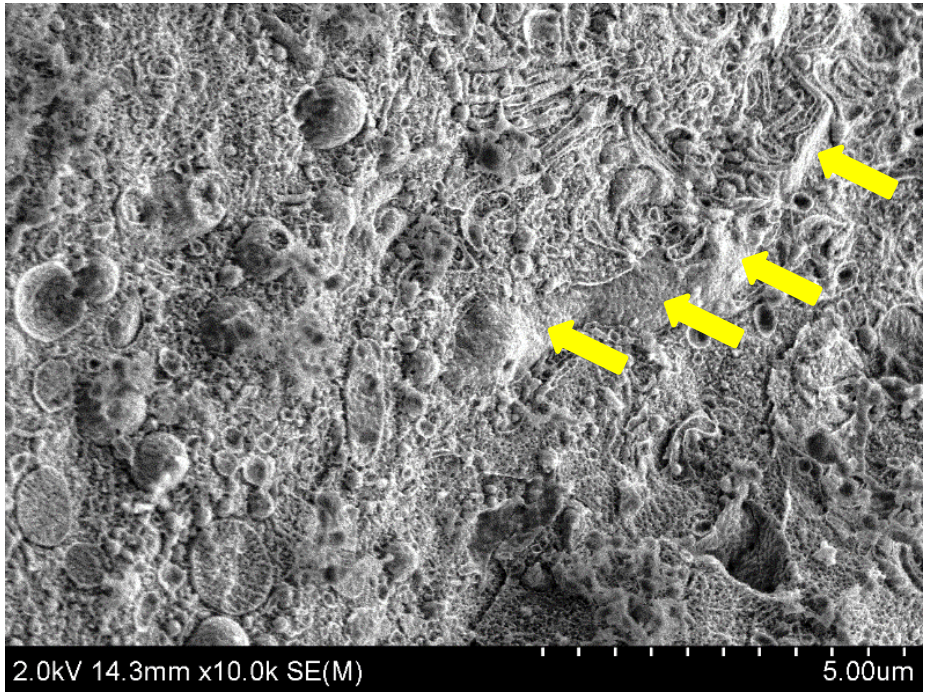


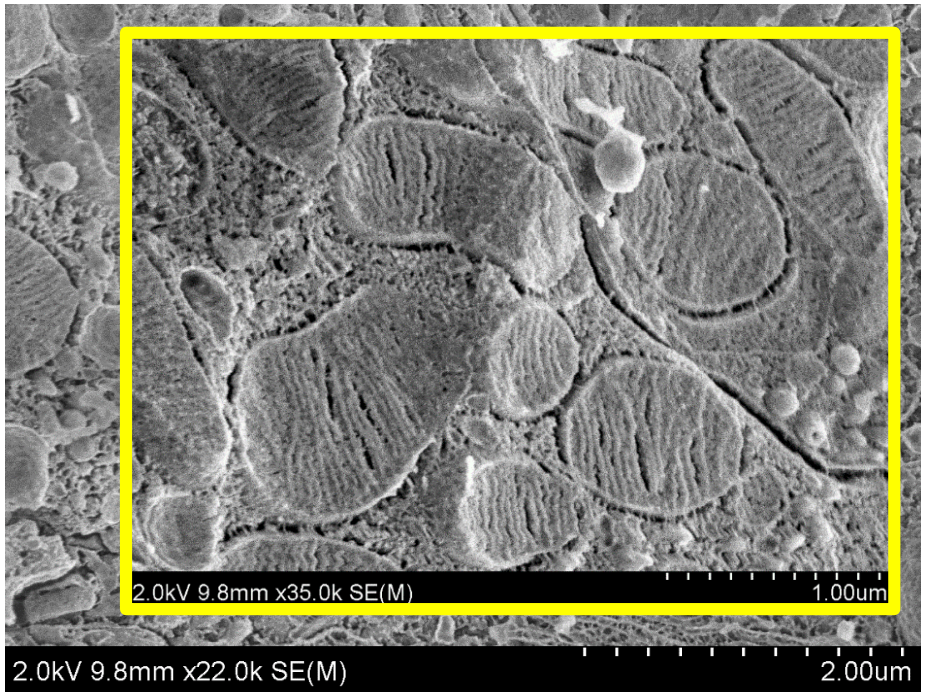
Outer mantle epithelium of A. cygnea



- High pressure freezing
- Freeze fracturing
- Freeze etching
- Pt and C coating
- Observation of frozen sample in the cryo-SEM

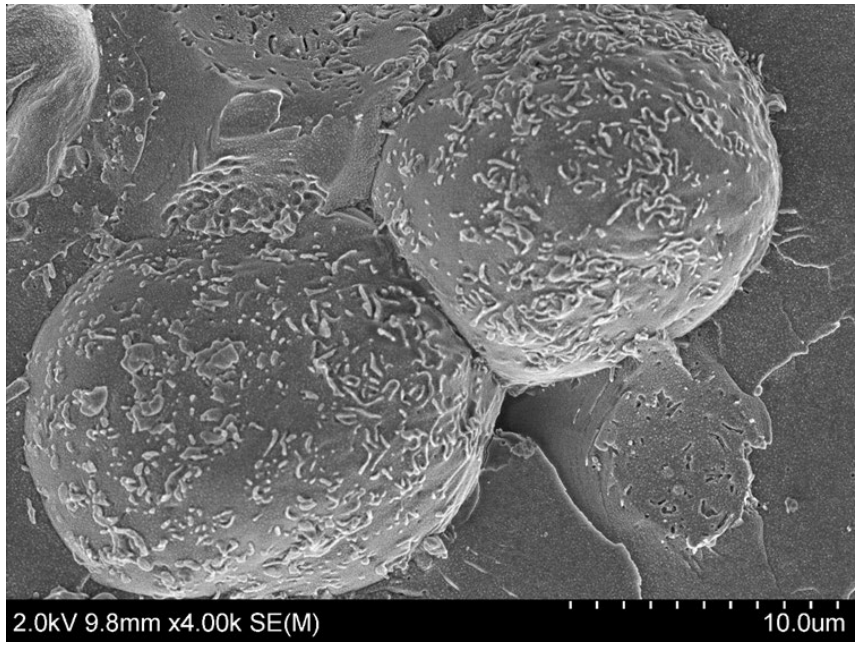
Cryo-SEM example: mouse kidney tissue cell

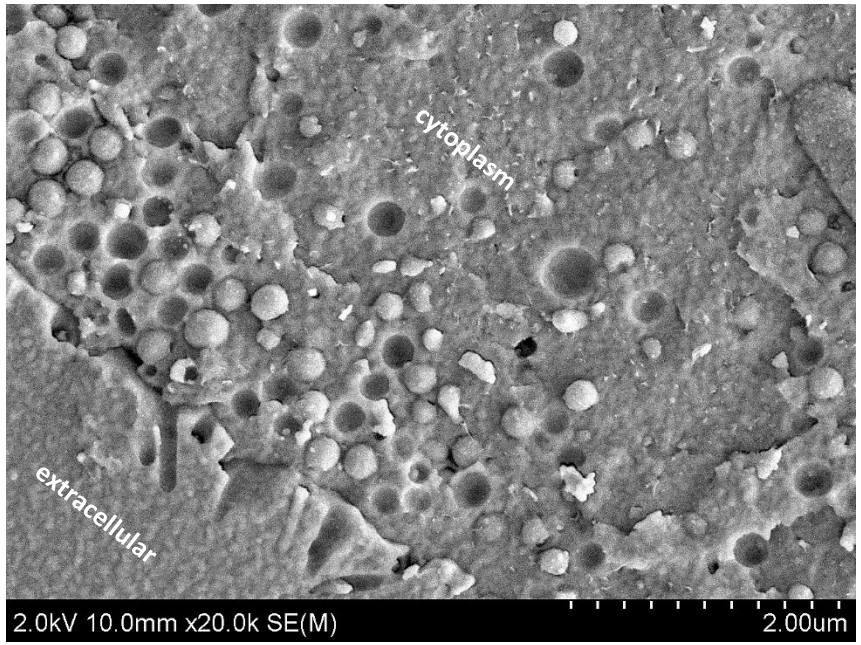




- High pressure freezing
- Freeze fracturing
- Freeze etching (deep etch)
- Pt and C coating
- Observation of frozen sample in the cryo-SEM

Cryo-SEM example: HT29 cells





- High pressure freezing
- Freeze fracturing
- freeze etching
- Pt and C coating
- Observation of frozen sample in the cryo-SEM

Wang, Xin, Reiner Bleher, Vadim Backman, Gajendra S. Shekhawat, and Vinayak P. Dravid. "Comparison of Sample Preparation Methods for Analysis of Mucus-Secreting Colon Cancer Cells by Scanning Electron Microscopy." *Microscopy and Microanalysis* 21, no. S3 (2015): 185-186.

Cryo-SEM example: enamel crystal nucleation

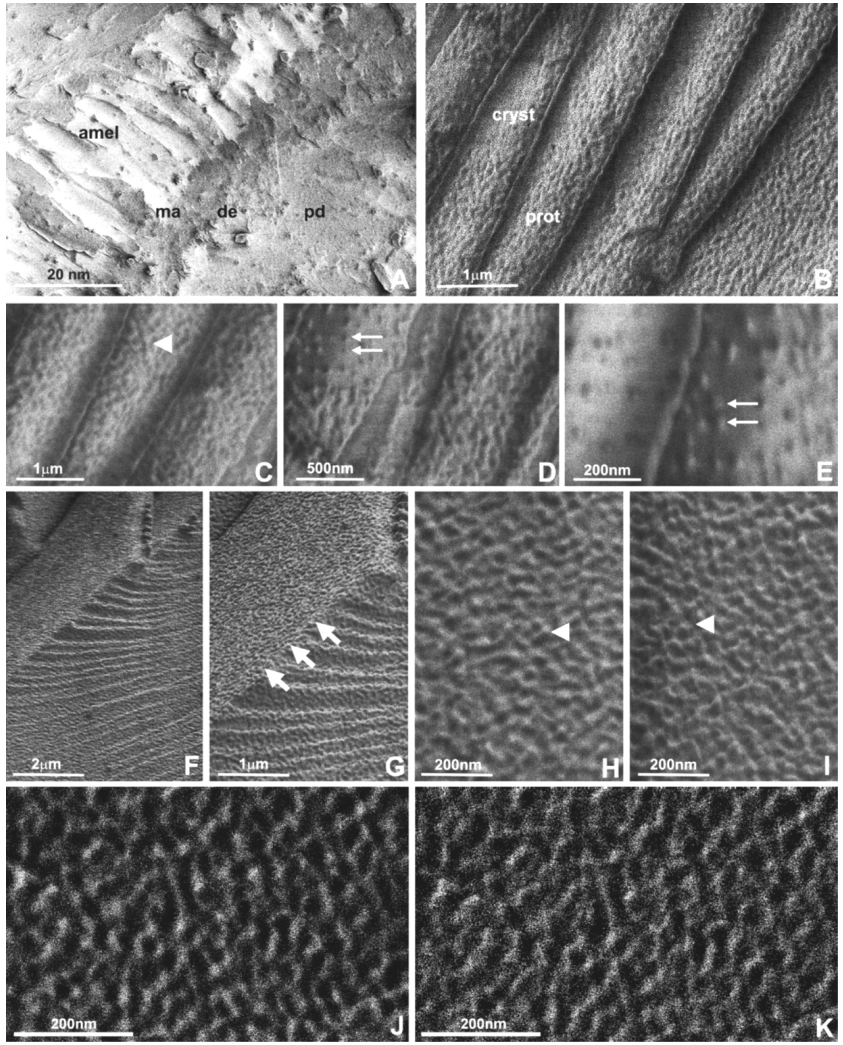


Figure 4. Cryo-EM analysis of freeze-fractured 3 days postnatal developing tooth enamel and enamel organs. **(A) Overview** micrograph illustrating the freeze-fracture topography at the interface between ameloblasts (amel), (dentin) (de), and predentin (pd). There was a thin layer of protein matrix (ma) between the ameloblast cell layer (amel) and the dentin layer (de). **(B–K) Freeze-fracture cryo-electron micrographs from the early enamel layer positioned between ameloblasts (amel) and dentin (de). (B) Freeze-fracture topography of the organic matrix (prot) immediately associated with the inorganic crystal surface (cryst).** (C,D) Identification of 50−100 nm annular protein matrix assembly rings (arrowheads) on crystal surfaces. (D,E) 20 nm spherical matrix subunit position on the crystal surface (double arrows). (F−I) Protein matrix at the ameloblast face of developing enamel crystals (arrows, G). The arrowheads in (H,I) point to annular subunit compartments measuring approximately 50−100 nm in diameter. (J,K) Image processing technologies were applied to either enhance the contrast (J) or to emboss the 3D surface relief (K) of the micrograph in (H) to further define the structural basis of enamel protein assemblies on crystal surfaces.

Jokisaari, Jacob R., Canhui Wang, Qiao Qiao, Xuan Hu, David A. Reed, Reiner Bleher, Xianghong Luan, Robert F. Klie, and Thomas GH Diekwisch. "Particle Attachment-Mediated and Matrix/Lattice-Guided Enamel Apatite Crystal Growth." ACS nano (2019).

Cryo-SEM Example: mouse fetal liver erythroblasts

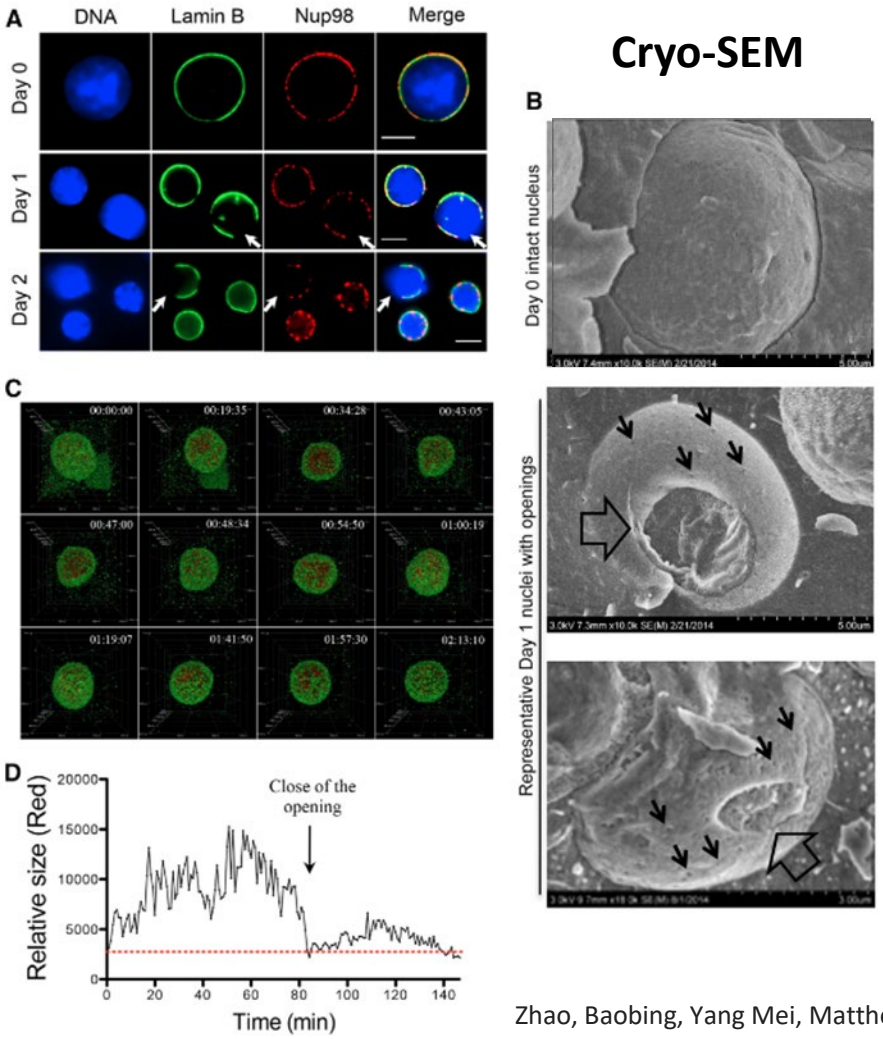
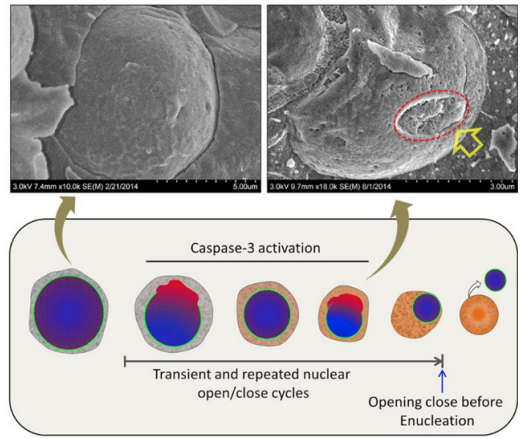
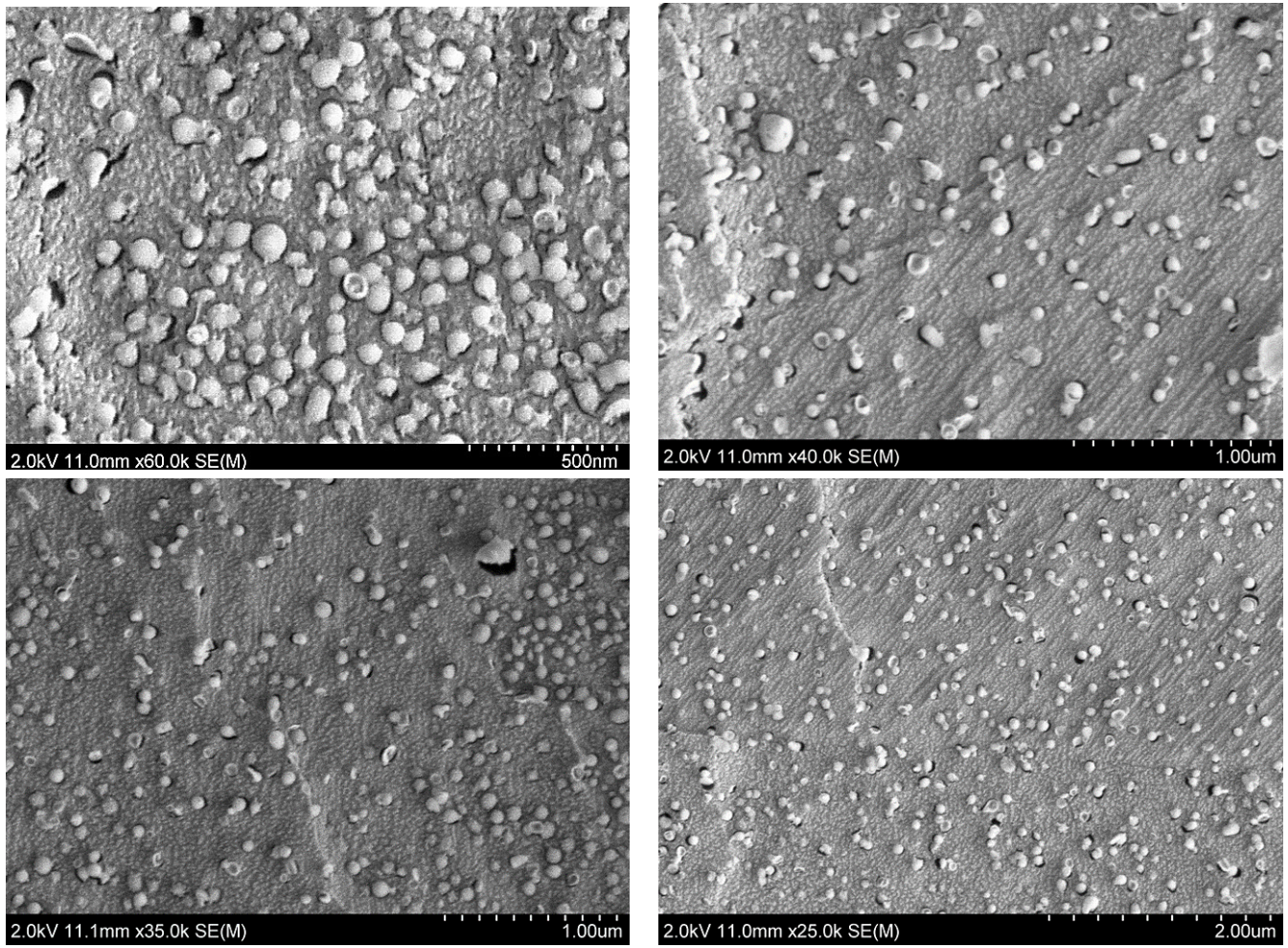


Figure 2.
Nuclear Opening Formation Is Dynamic
(A) Ter119-negative E13.5 mouse fetal liver erythroblasts were purified and cultured in vitro in erythropoietin-containing medium. Immunofluorescence stains for lamin B, Nup98, and DNA (DAPI) from erythroblasts cultured on different days were performed. Arrows indicate nuclear openings. Scale bars, 5 μm.
(B) Cryo-scanning electron microscopy analysis of the cultured mouse fetal liver erythroblasts on day 0 and day 1 (two representative nuclei). Large open arrows point to the nuclear openings. Small black arrows point to several nuclear pore complexes for comparison. The images are representative of >40 cells with the same openings in two independent experiments. Approximately 20% of the exposed nuclei on day 1 showed the illustrated openings.
(C) G1ER cells were transduced with MSCV-GFP-lamin B1 (green) and MSCV-mCherry-H2A (red). The cells were induced by estradiol and analyzed by time-lapse fluorescence microscopy. 3D reconstructions of confocal images of a representative cell at the indicated time points (hr: min: s) are illustrated.
(D) The size of the opening over time was quantified by analyzing the area of mCherry-H2A signals (red) in (C) using ImageJ. Data are representative of three independent experiments.



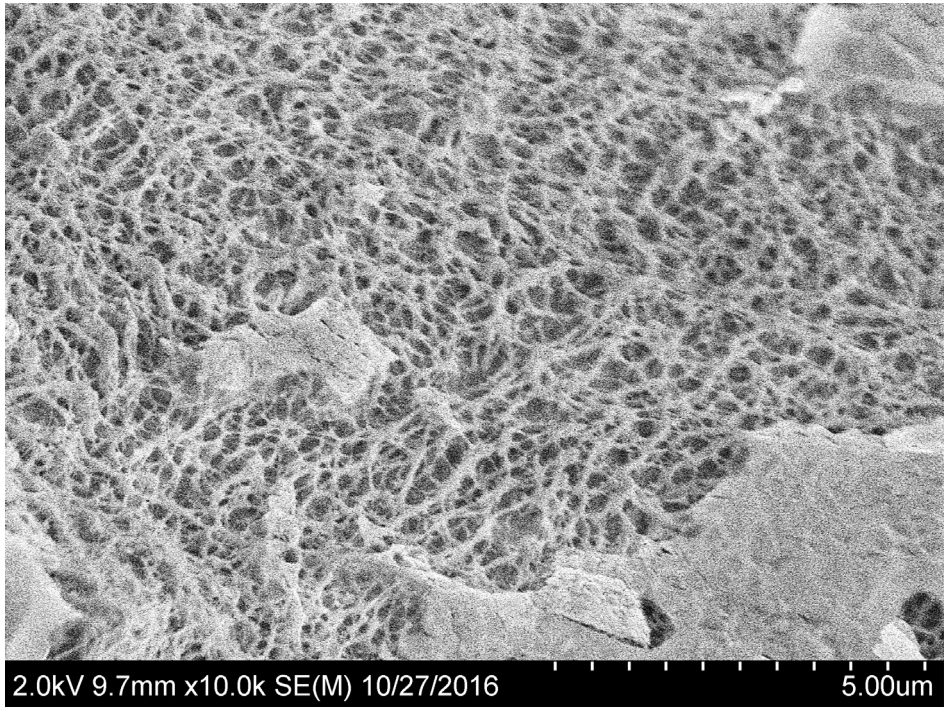
Zhao, Baobing, Yang Mei, Matthew J. Schipma, Eric Wayne Roth, Reiner Bleher, Joshua Z. Rappoport, Amittha Wickrema, Jing Yang, and Peng Ji. "Nuclear Condensation during Mouse Erythropoiesis Requires Caspase-3-Mediated Nuclear Opening." *Developmental cell* 36, no. 5 (2016): 498-510.

Cryo-SEM example: liposomes



- High pressure freezing
- Freeze fracturing
- Freeze etching
- Pt and C coating
- Observation of frozen sample in the cryo-SEM

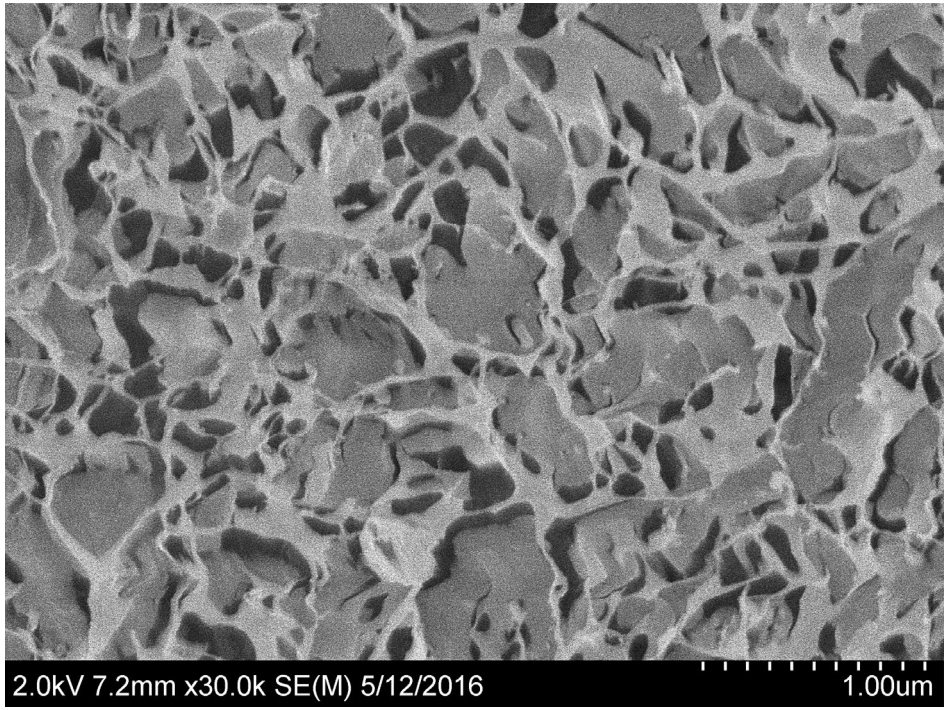
Cryo-SEM examples: hydrogels



Hydrogel scaffold

Cryo-SEM reveals the influence of surface functionalization on the underlying scaffold microstructure.

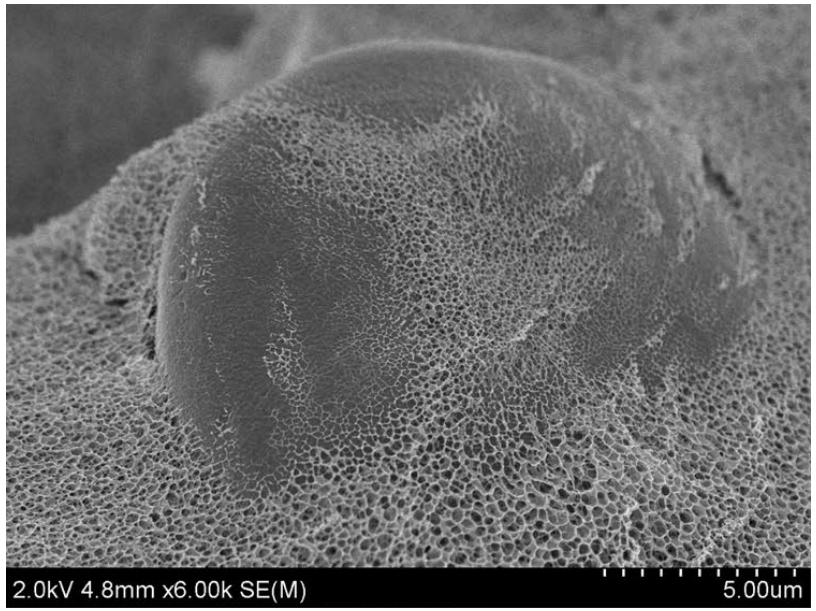
Nick Karabine, PhD; SQI Center; Scott Group, NU

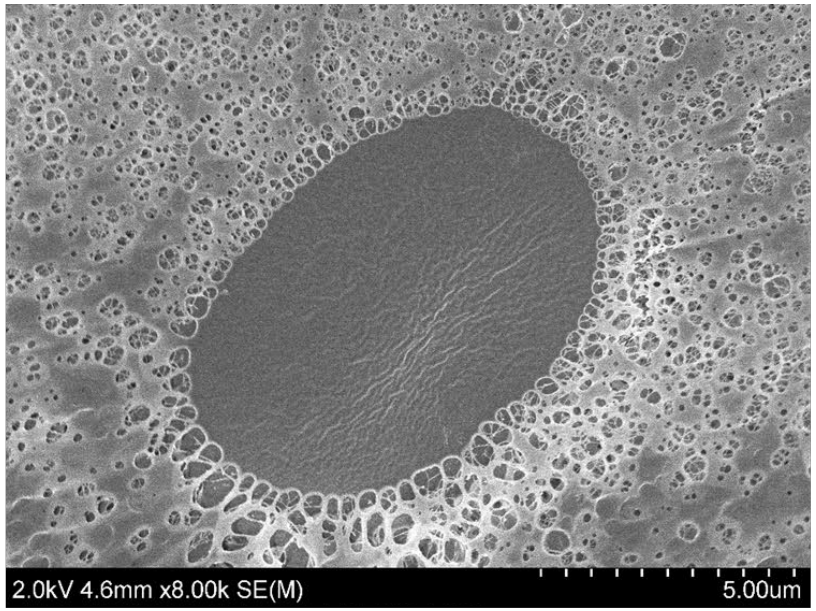


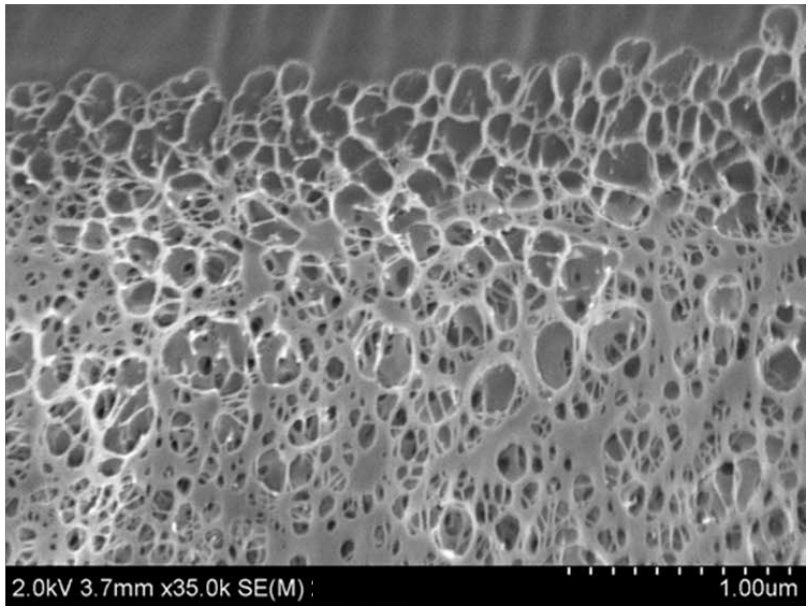
Hydrogel that will function as a catalyst scaffold.

Ben Jones, PhD; MSE; Stupp Group, NU

Cryo-SEM example: hydrogel







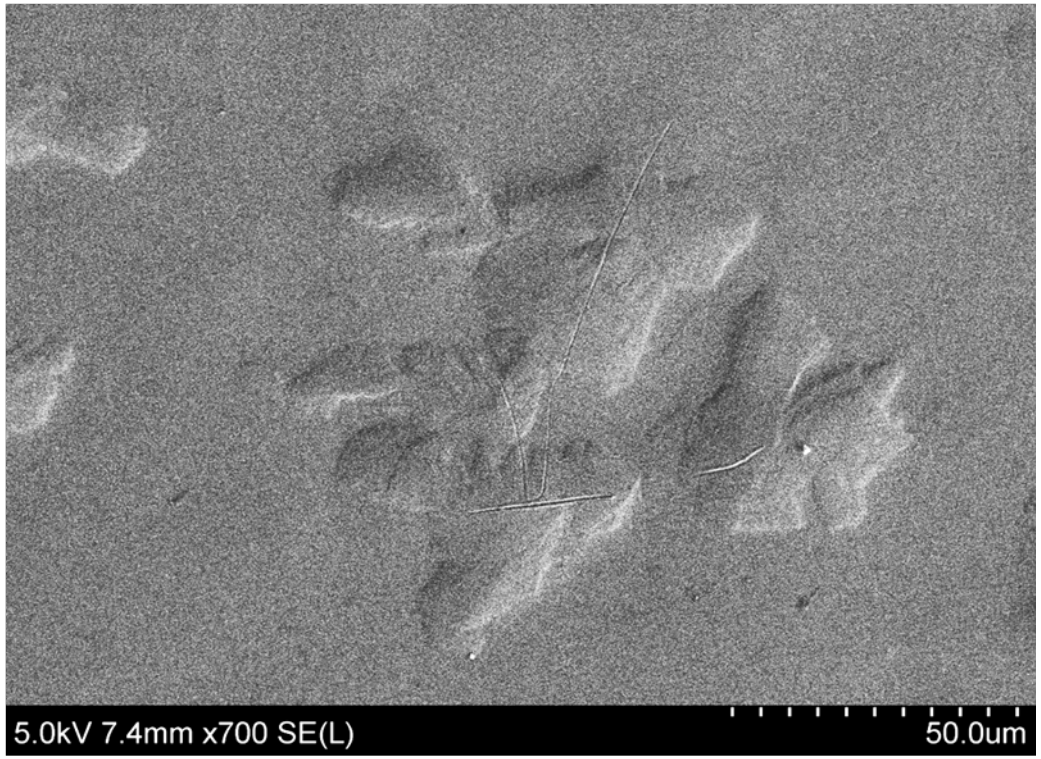
Cryo-SEM was used to demonstrate the interface between starch grains and the hydrogel matrix

Fang, Yin, Endao Han, Xin-Xing Zhang, Yuanwen Jiang, Yiliang Lin, Jiuyun Shi, Jiangbo Wu et al. "Dynamic and programmable cellular-scale granules enable tissue-like materials." Matter 2, no. 4 (2020): 948-964.

Cryo-SEM example: niobium hydrate formation

Cryo-SEM experiment showing formation of niobium hydrides at cryogenic temperatures. The image was taken at -136° C.

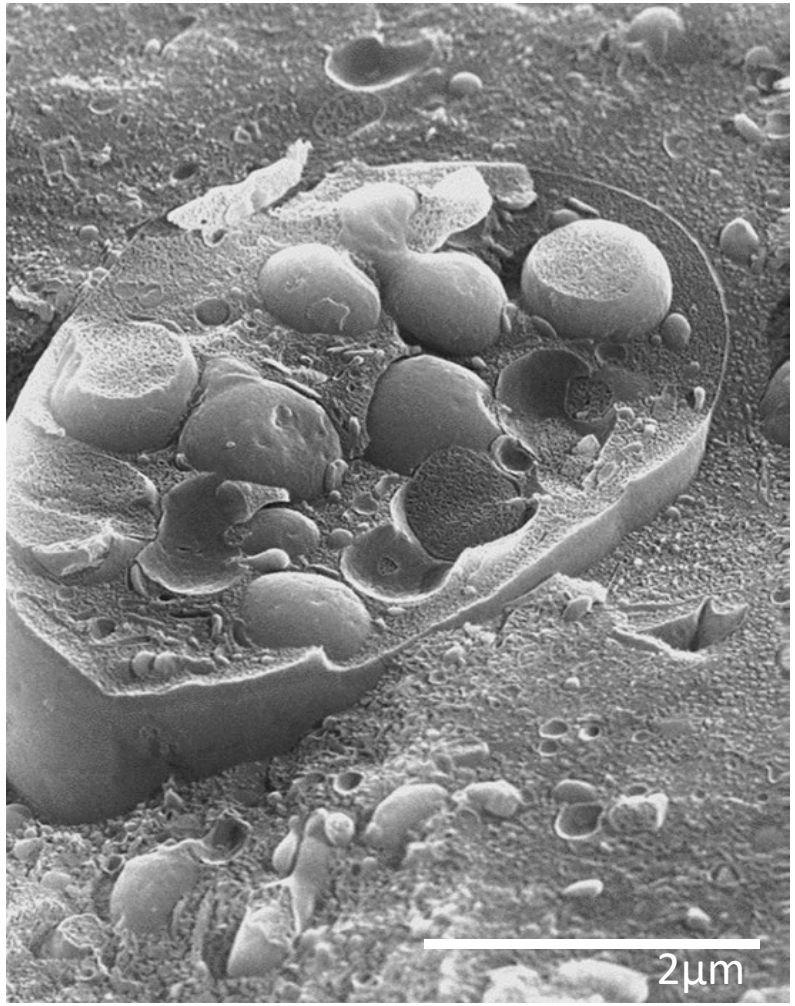
Formation of niobium hydrides on the polished surface was induced by lowering the temperature of the cryo sample stage inside the SEM.



Project with Dr. Yulia Trenikhina, Fermilab

Cryo-SEM at NU*ANCE*-BioCryo

- User is trained on RT operation of the S-4800
- The cryo stage is mounted in the S-4800 SEM in the morning (by BioCryo Staff)
- The cryo stage in the SEM is cooled down at around 11:30am (lowest temp is -150°C)
- User can use the cryo-SEM (including loading dock, shuttle and ACE600 all day (the fee is per run, not per hour)
- Next morning: the cryo stage is removed from the S-4800 (by BioCryo Staff)



Thank you for your Attention!

Questions?

Contact: r-bleher@northwestern.edu