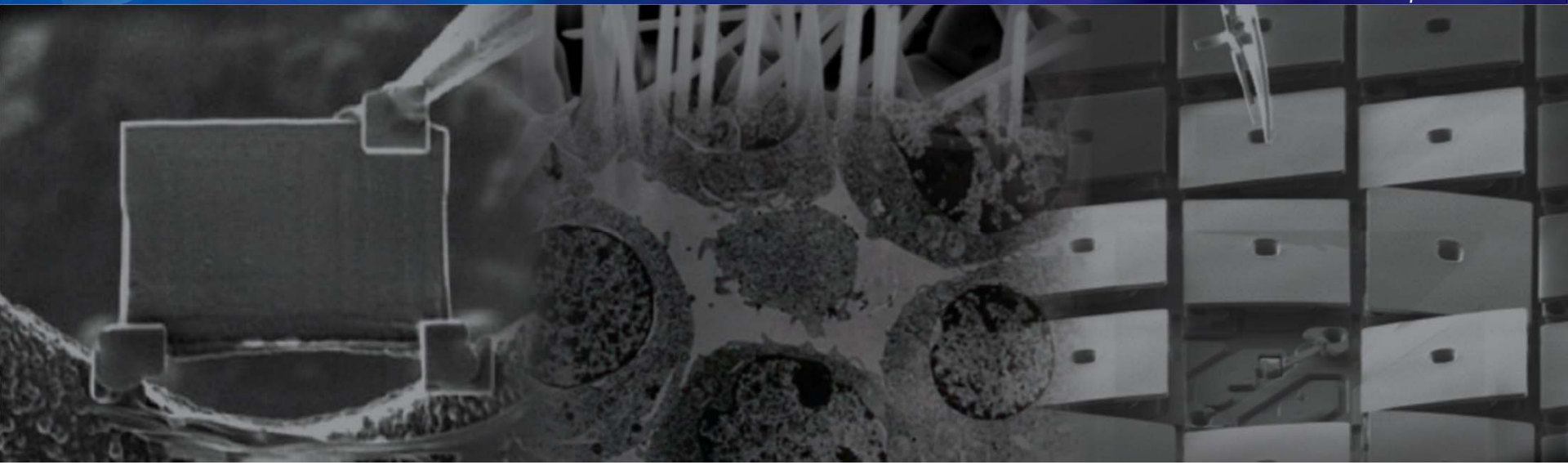
OXFORD INSTRUMENTS

The Business of Science®



Progress Towards Cryo FIB Lift-Out

Cheryl Hartfield, Oxford Instruments
Nicholas Antoniou, Harvard University
Jeff Ditto, University of Oregon, CAMCOR

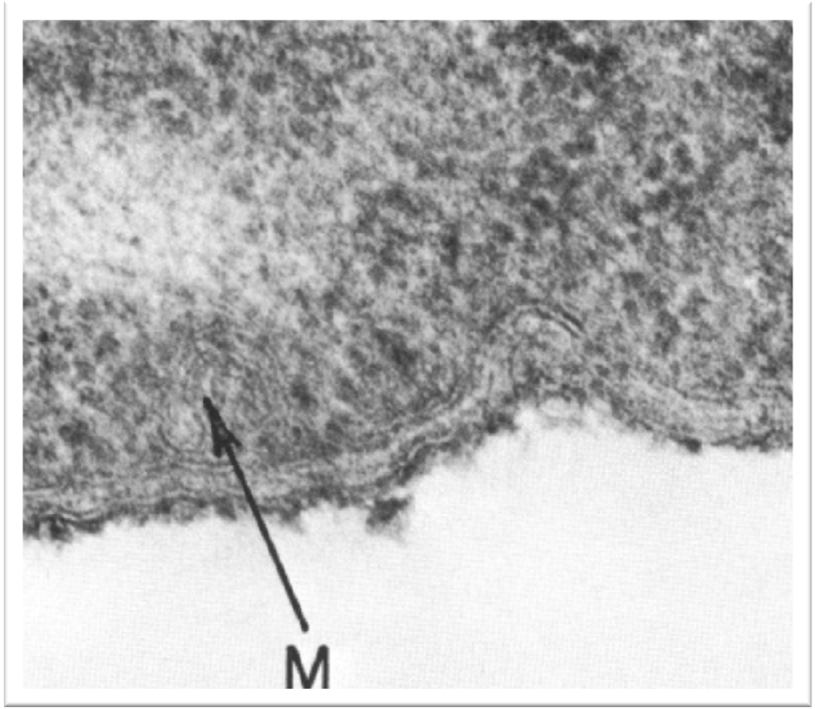
6th North American FIB-SEM Workshop, March 1, 2013, Cambridge, MA

Why Cryo?

OXFORD INSTRUMENTS
The Business of Science®

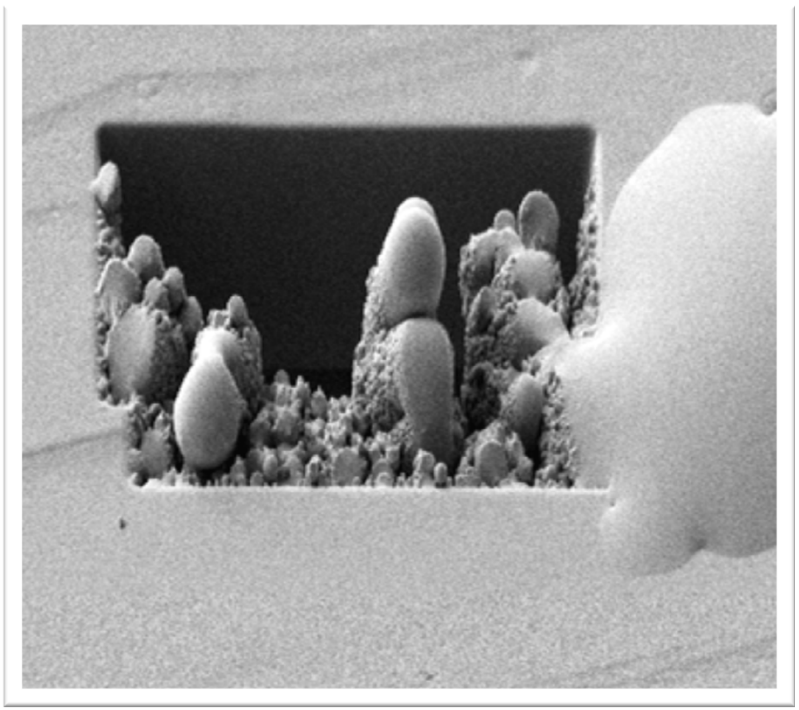
1st Rule of Sample Preparation: DO NO HARM

Biological



Chemical fixation can induce artifacts, such as the bacterial mesosome

III-V Materials

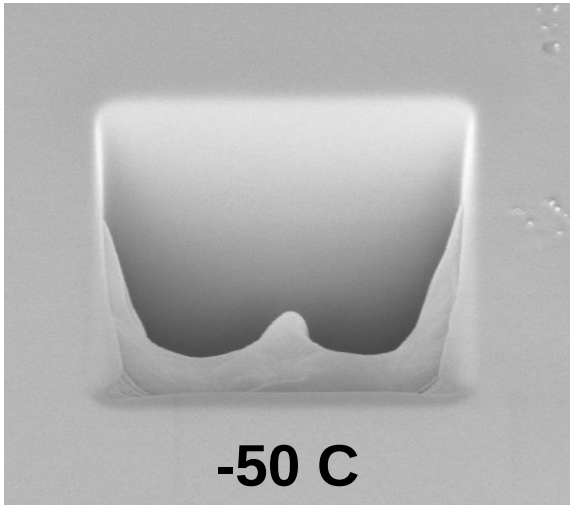


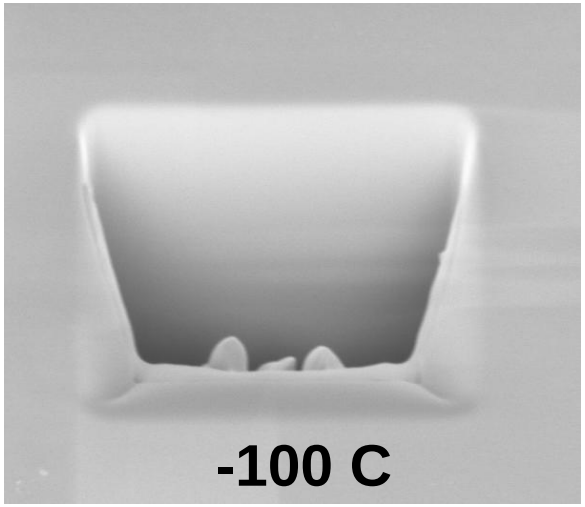
Room temperature promotes undesirable Ga+ reactions during and after ion milling

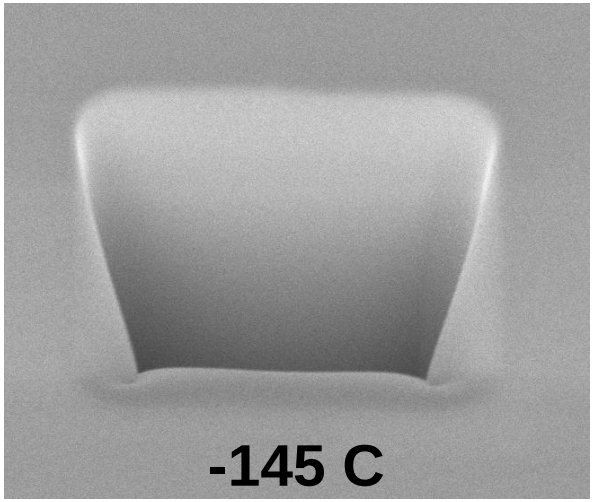
GaN FIB Milling Temperature

OXFORD INSTRUMENTS
The Business of Science®

Temperature (C)	Exposure (nC/μm²)	Side Effect (Qualitative)
0 C	5.5	Severe
-25 C	5.5	Severe
-50 C	5.5	Intermediate
-100 C	5.5	Minimized
-145 C	5.5	None Observed

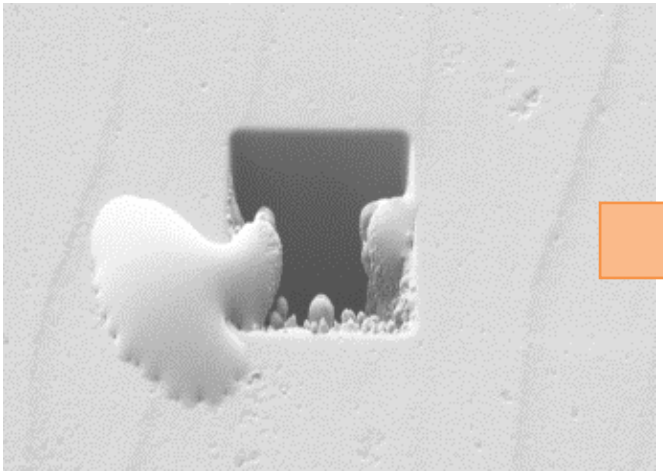
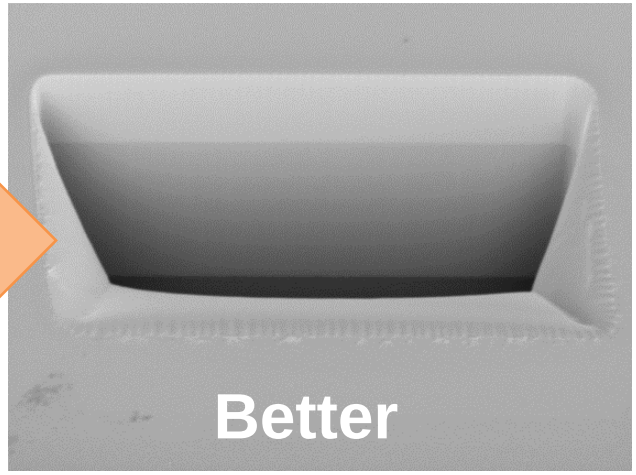
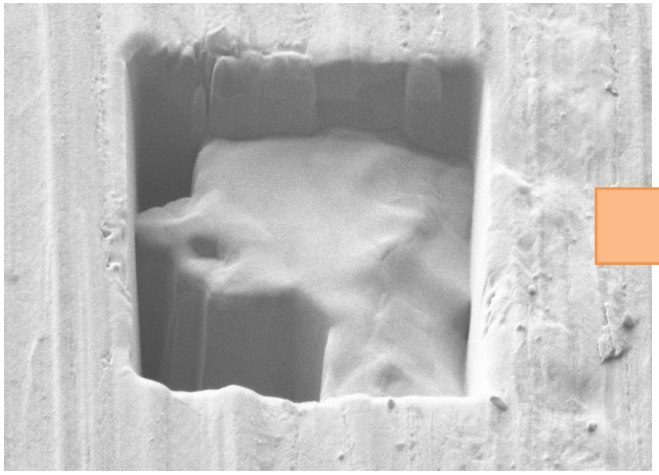
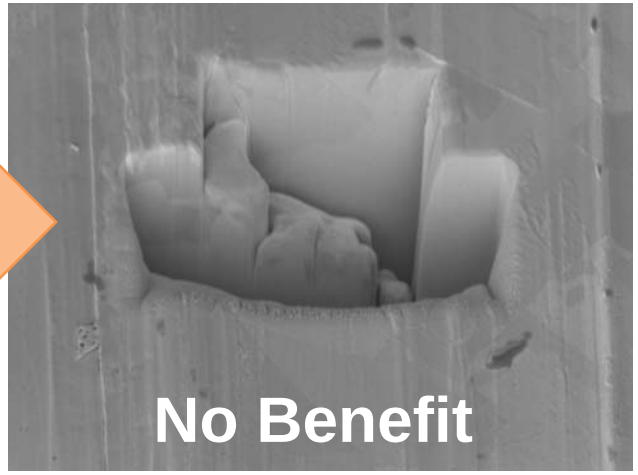






FIB Milling Temperature Effects (Dose: *5.5 nC/μm²*)

OXFORD INSTRUMENTS
The Business of Science®

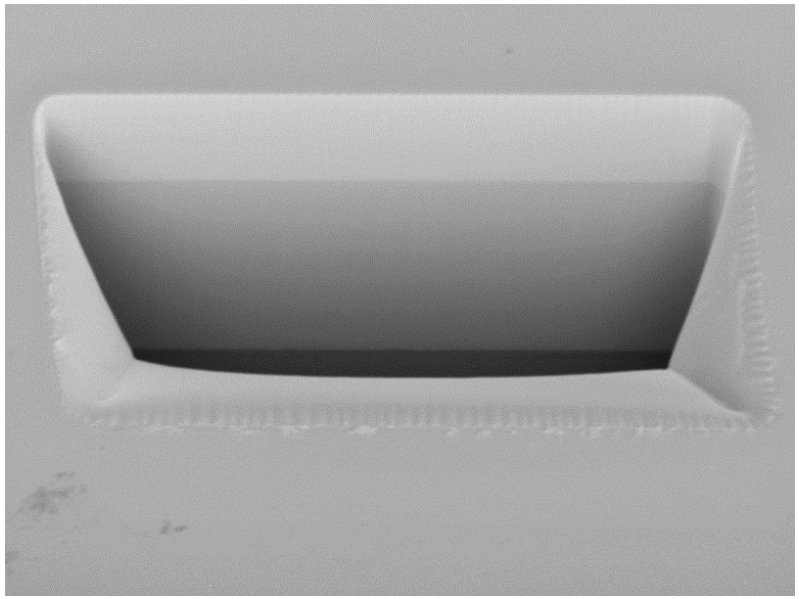
	Room Temperature	-145° C
• GaN		
• Copper		

GaN Warm-up After Cryo-FIB

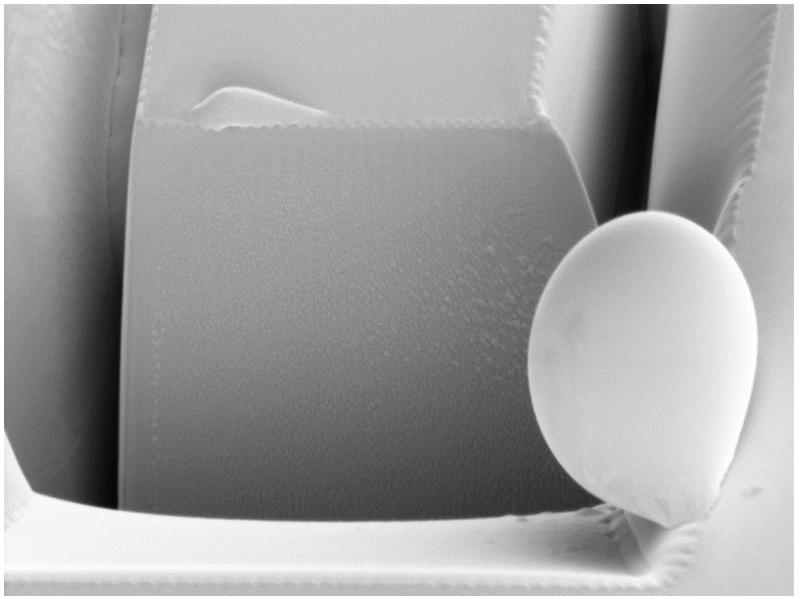
OXFORD INSTRUMENTS
The Business of Science®

- **Samples cryo-milled (-145ºC)**
- **Dose: *5.5 nC/μm^2***

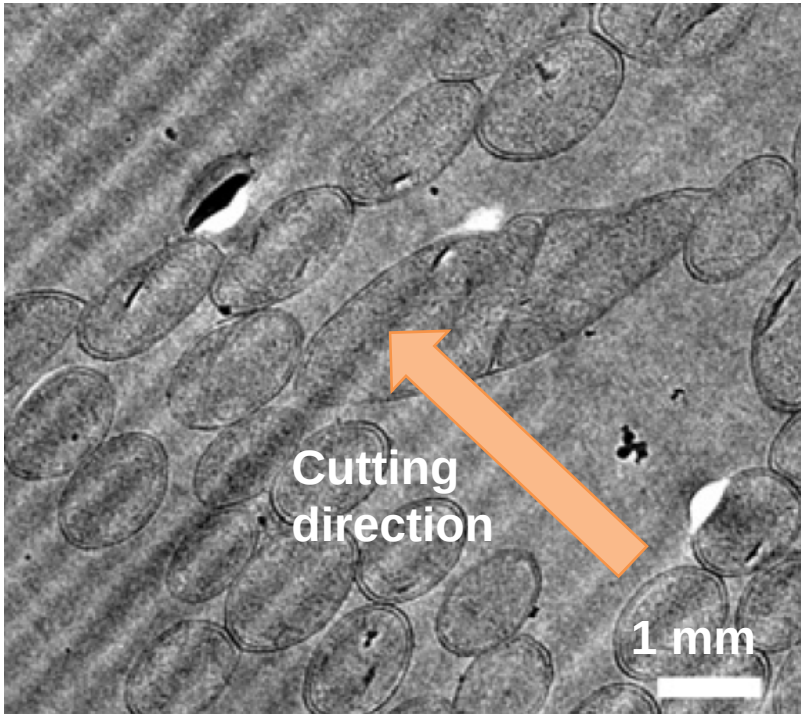
Imaged at -145ºC



Imaged at Room Temp



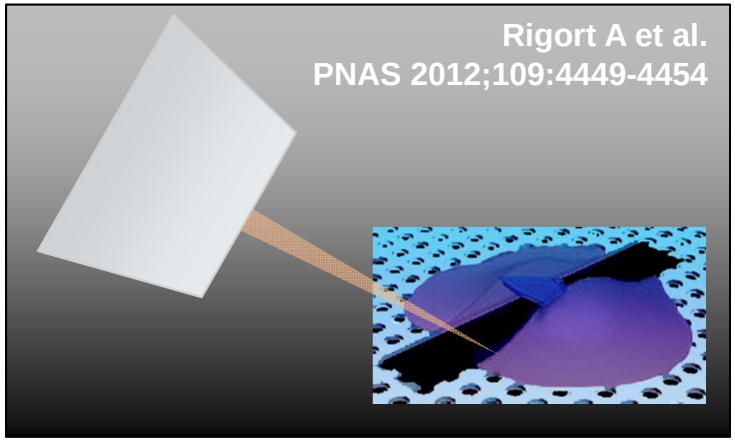
Cryo-FIB Benefits for Bio

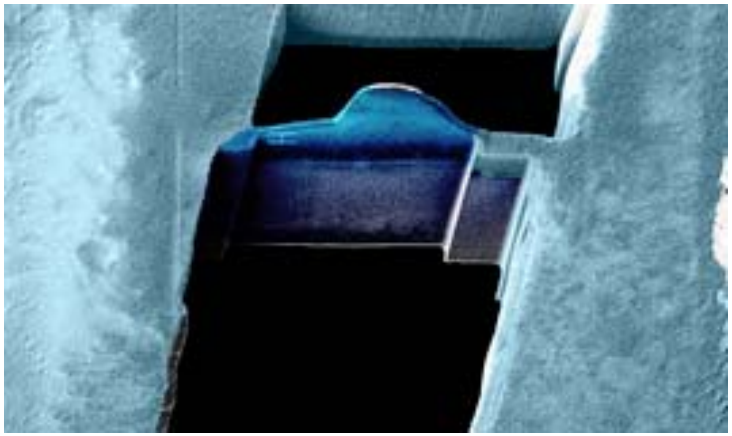


http://dx.doi.org/10.1016/j.jsb.2010.02.011

Cryoultramicrotome sections may have severe (70%) compression

Bio Cryo FIB H-Bar Prep

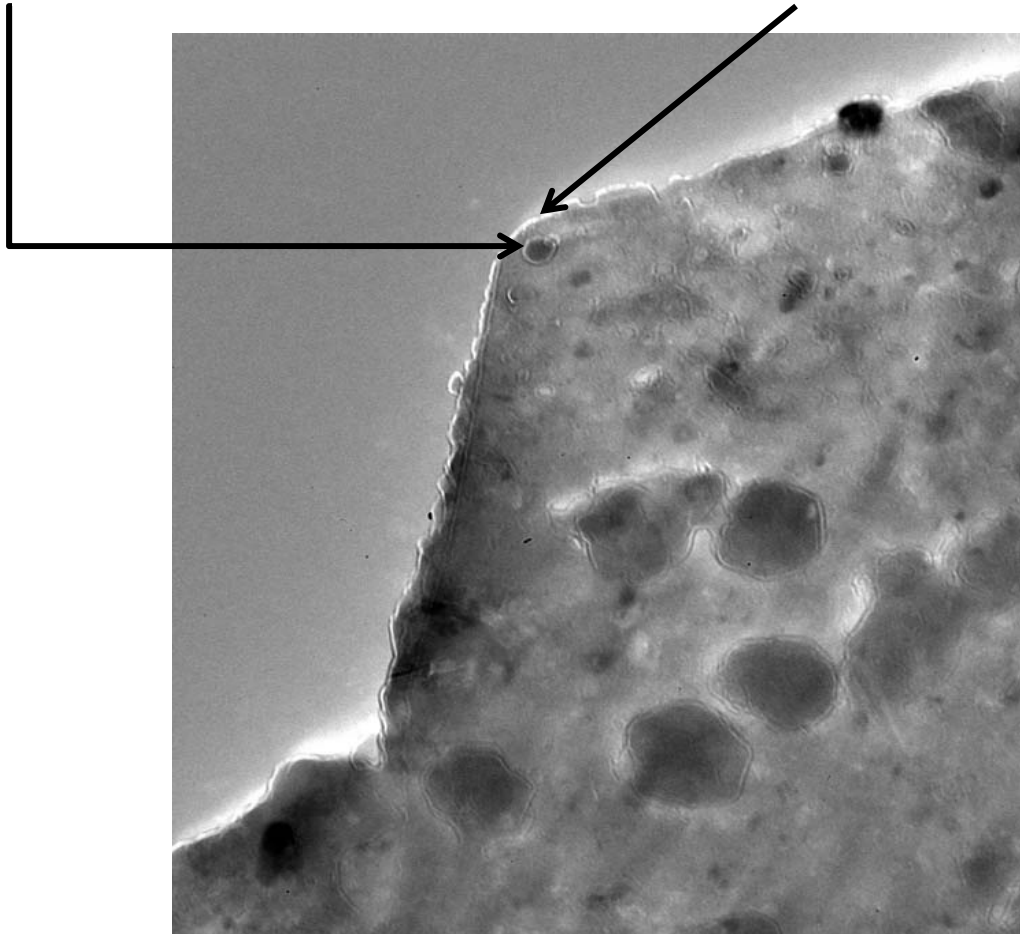




Cryo-TEM of Cryo-FIB Prepared Sample

OXFORD INSTRUMENTS
The Business of Science®

Polio virus inside cell membrane



Why Use Cryo Lift-Out?

OXFORD INSTRUMENTS
The Business of Science®

Benefits

- One protocol for many samples (all sizes, types and industries)
- Site-specific and can position for SIMS, EDS, EBSD etc.
- Custom shaping: micropillar tomography specimens to avoid missing wedge

Challenges

- Ga+ implantation and curtaining
- Condensing behavior of standard gases
- Keeping sample below -138ºC for all steps
- Education

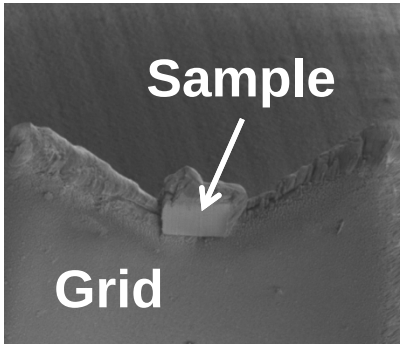
Biological Sample Beam Control

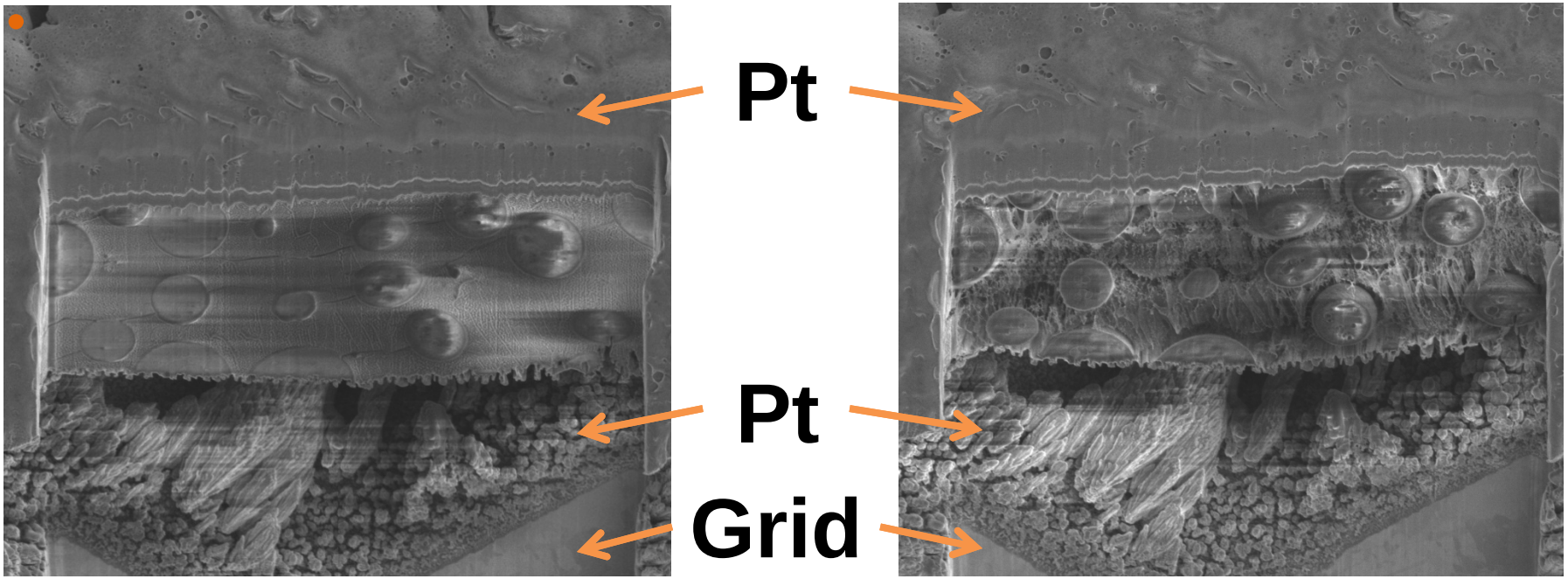
OXFORD INSTRUMENTS
The Business of Science®

Baker's yeast in aqueous suspension

9.3nA trench milling too aggressive

- Use low dose rate ~2 ions/nm²/s
- For a 20µm² area of E. coli suspension:
  - Two minutes to thin 1100nm to 500nm

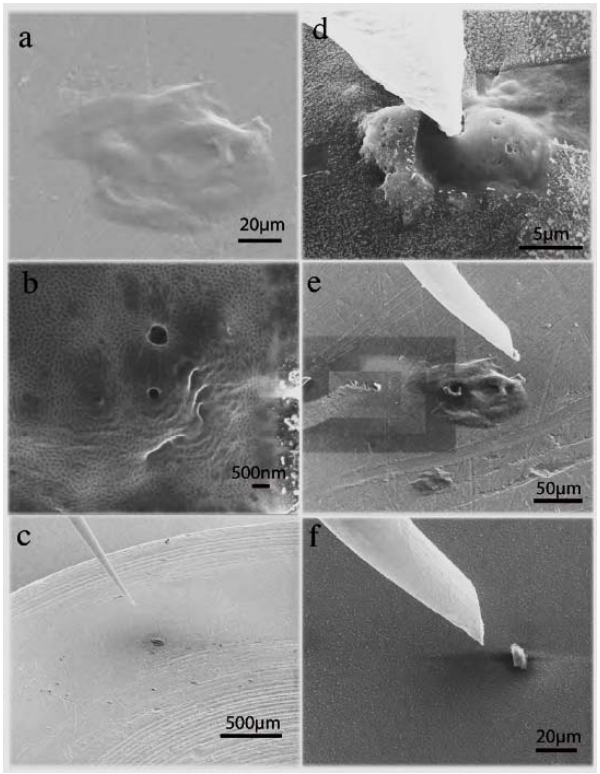




Cryo Lift-Out Approaches

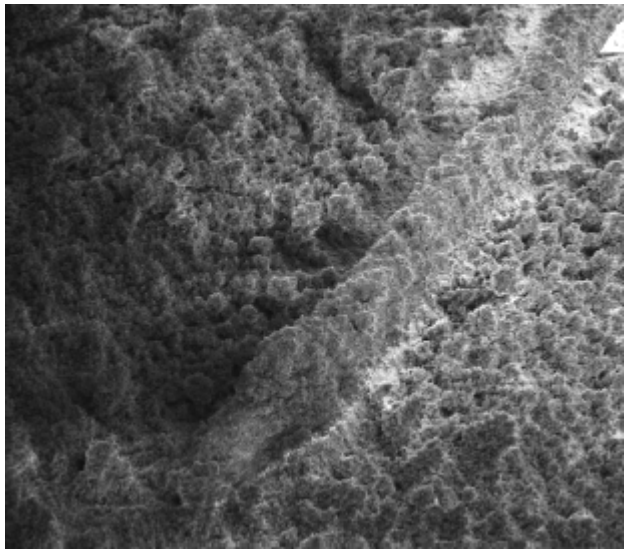
The Business of Science®

Dig and Shovel



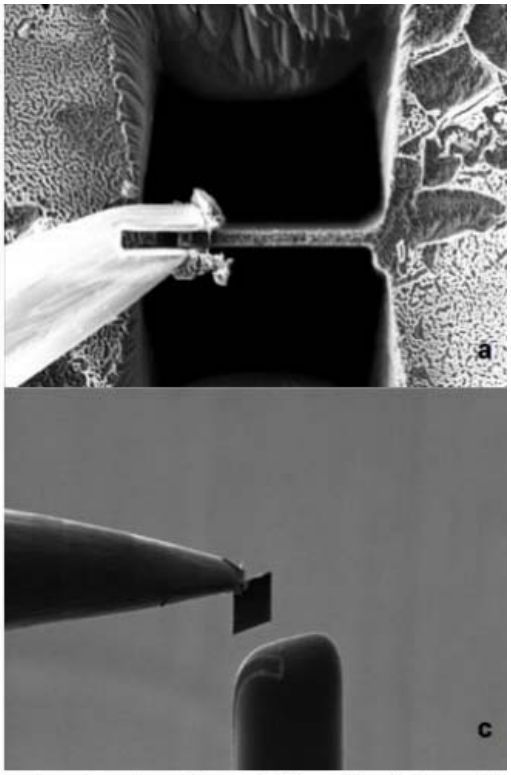
J. McGeoch, 2007
10.1111/j.1365-2818.2007.01798.x

Platinum Attach



MF Hayles et al., 2007
10.1111/j.1365-2818.2007.01775.x

Force Friction



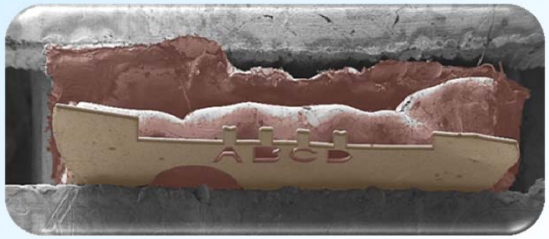
A Sehrbrock et al., 2011
MC2011 Poster

Cryo Manipulation - Harvard

OXFORD INSTRUMENTS
The Business of Science®

Harvard University CNS

- Leica EM VCT100 Cryo Transfer
- Zeiss Nvision
- OmniProbe 300

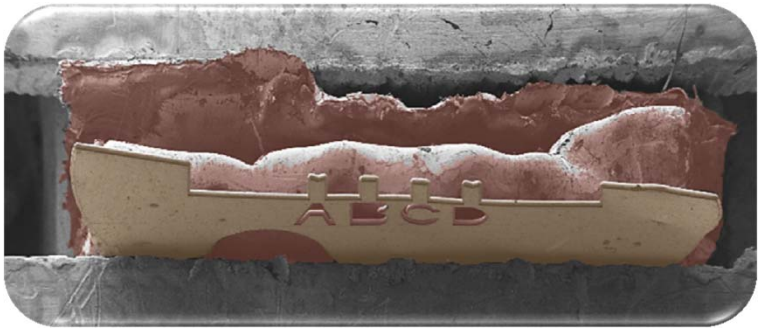


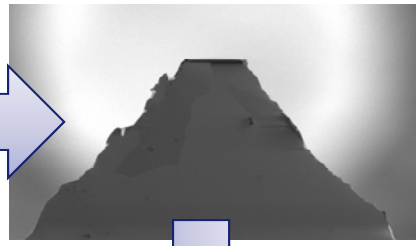
Avoid Bent Grids

The Business of Science®

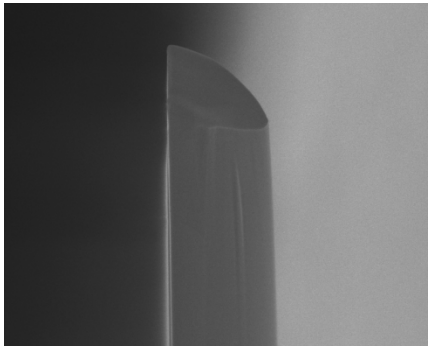
Silicon Half Grids: Rigid Substrates for Cryo Lift-Out

Include photo of new shape for easier TEM holder loading





FIB modification (<10 mins)

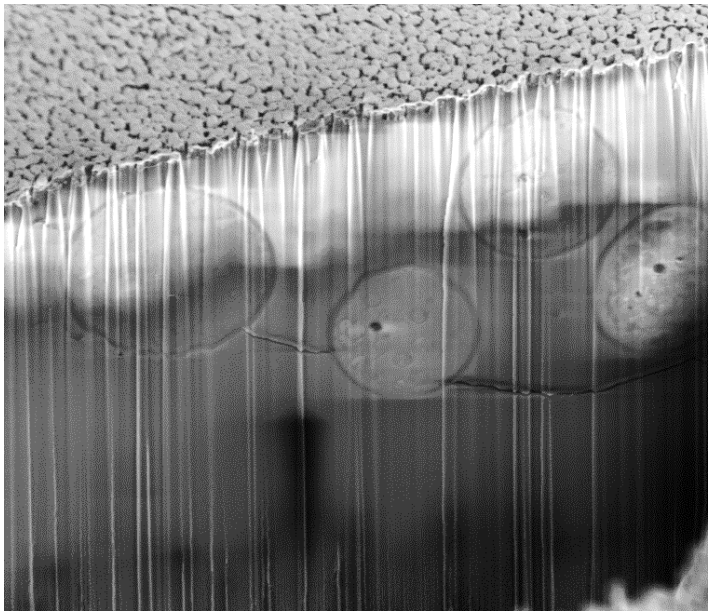


Minimize Curtaining

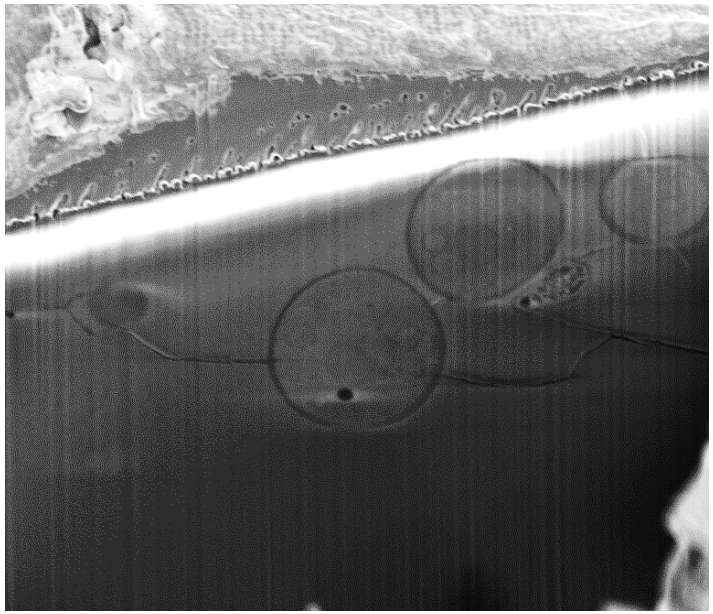
OXFORD INSTRUMENTS
The Business of Science®

- **Platinum precursor improves curtain artefacts**

No Precursor



With Precursor



- **Global condensation is controlled by:**
  - **Temperature (use Pt at 27C)**
  - **Distance (gas nozzle 3mm from sample)**
  - **Time (a few seconds)**

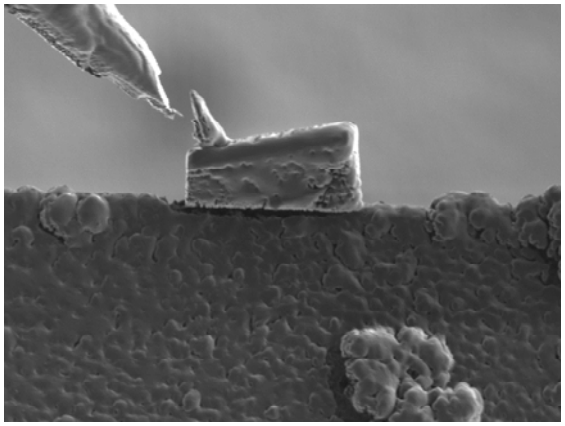
Attaching: CAMCOR Best Practices

OXFORD INSTRUMENTS
The Business of Science®

Early Work Used Pt

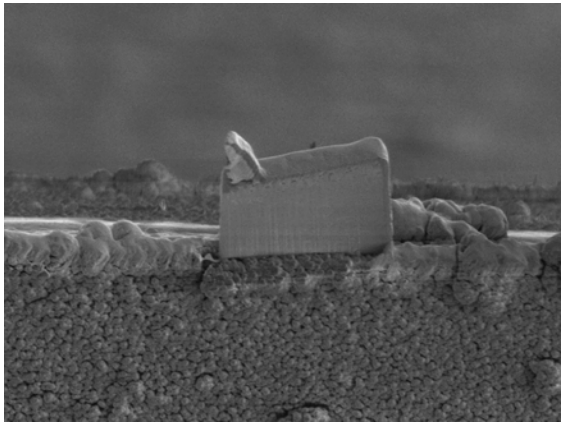
New Improvements

- **Tip Attach**



- **Attach using ice condensation**
- **The tip is clean and 'like new" upon warming**

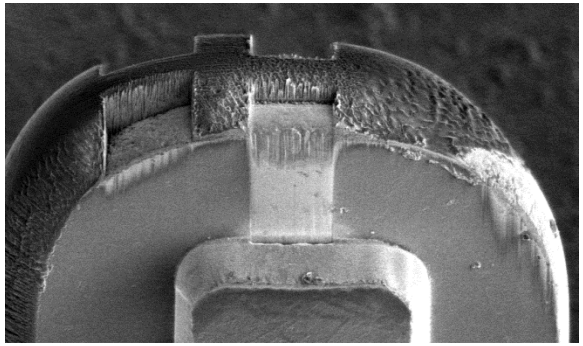
- **Grid Attach**



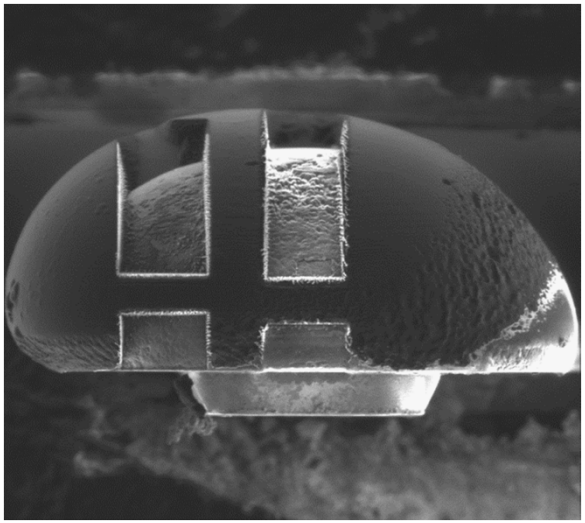
- **Simultaneously attach with ice & separate the tip**
- **Adding Pt (100nm) reduces curtains**

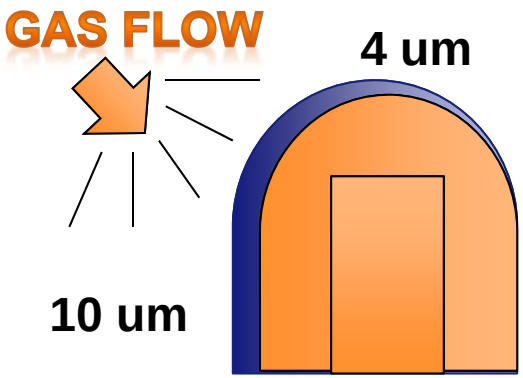
Mitigates thermal drift

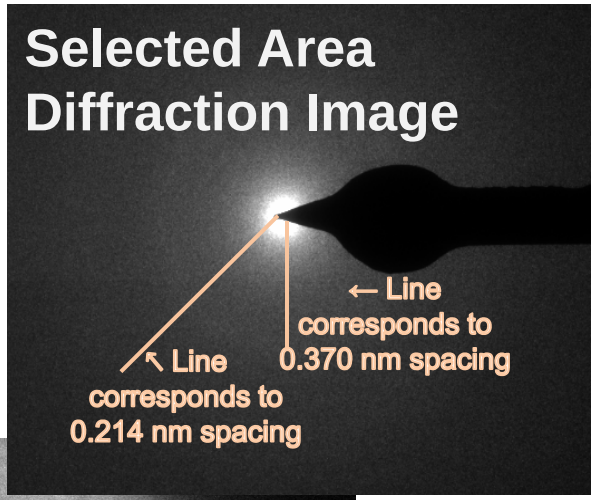
Amorphous Ice Confirmed

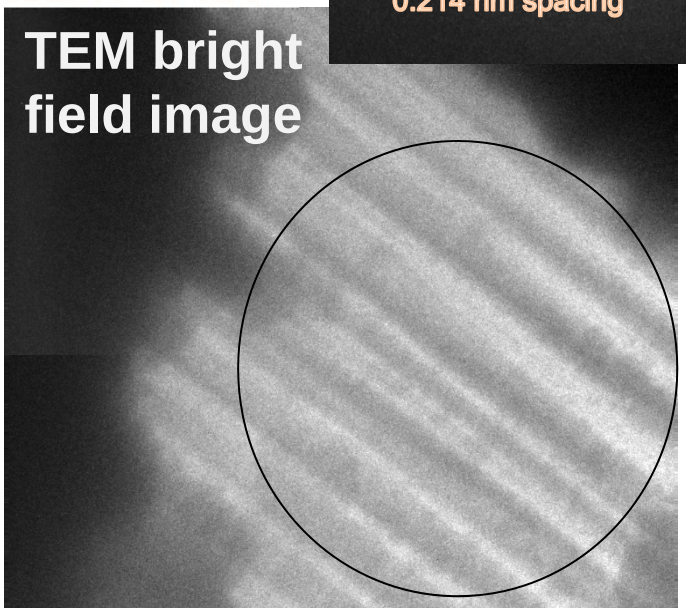


H-bar lamella for TEM diffraction





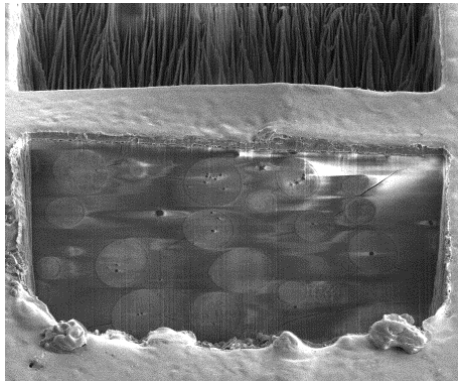




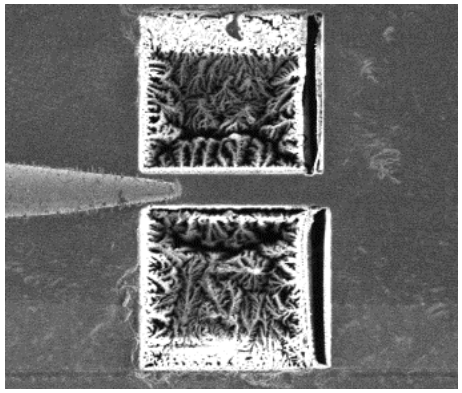
Cryo Lift-Out (CAMCOR)

OXFORD INSTRUMENTS
The Business of Science®

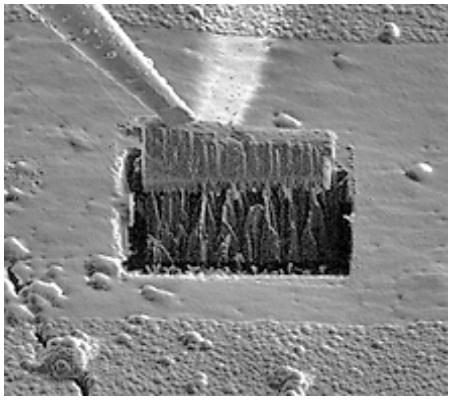
Site of Interest



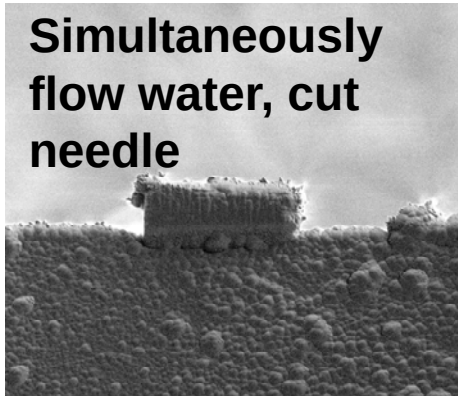
Probe Contact



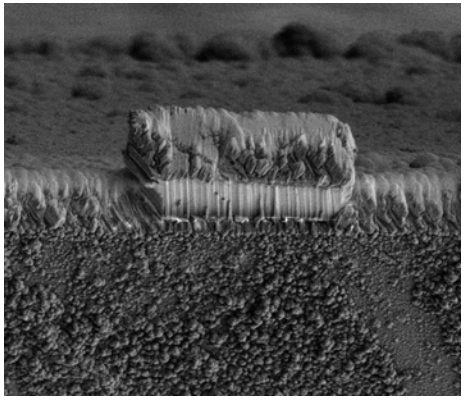
Water Attach & Lift



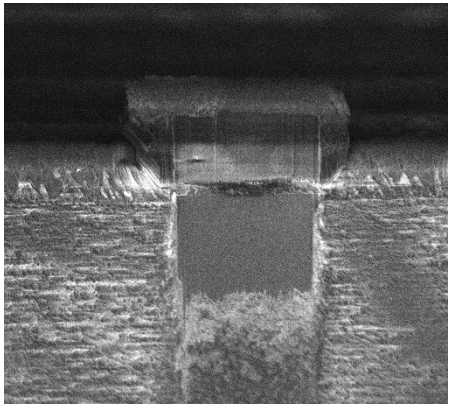
Grid Attach



Platinum Coat



Clean and Thin

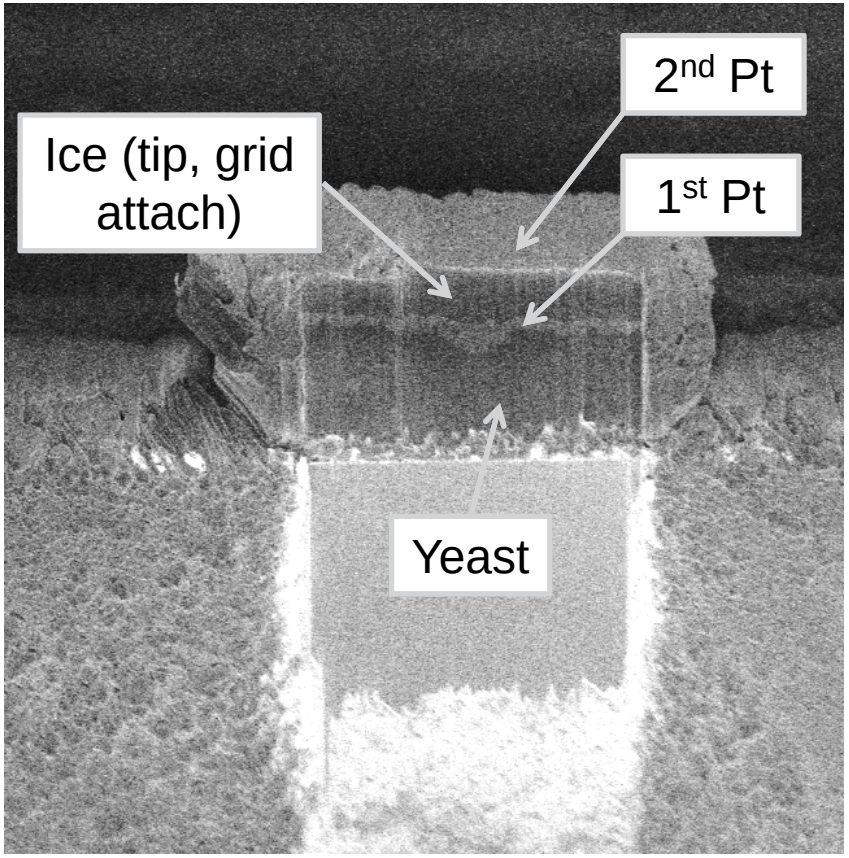


Avoid e-beam during thinning!

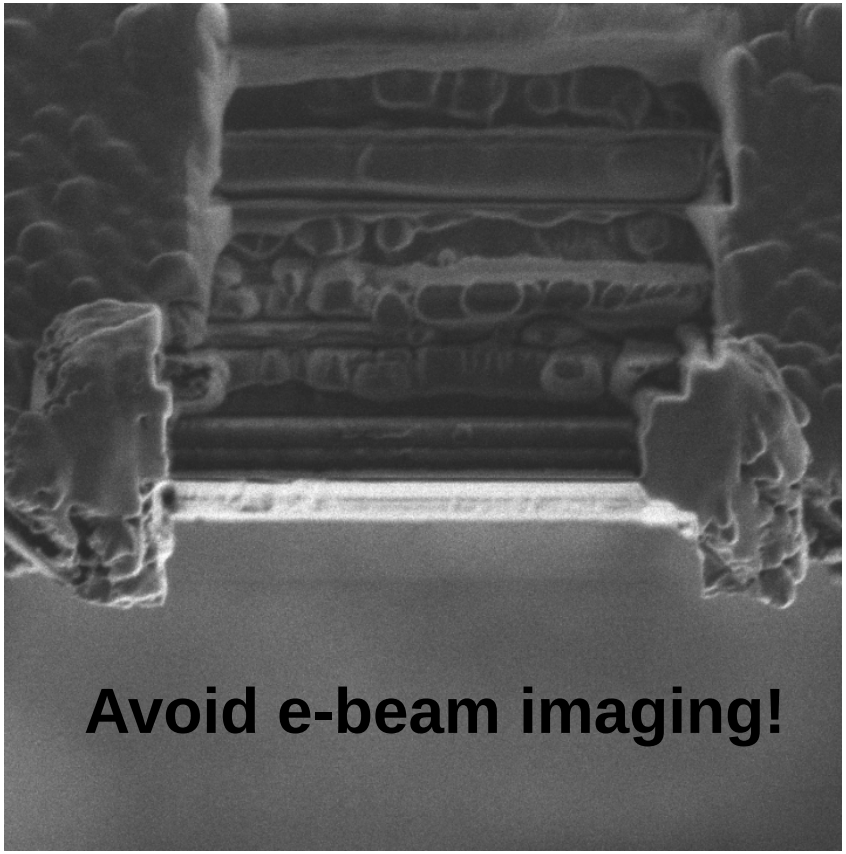
Cryo Lift-Out Thinning

The Business of Science®

Lift-Out Sample Structure



Final Thinning, Ion Beam View



Summary

- **Cryo FIB is useful for both materials and biological samples**
- **Progress has been made in cryo lift-out methods and hardware**
  - **Cooled manipulator tip assembly**
  - **Robust lift-out grids**
  - **“Ice welding”**
  - **Managing the beam**
- **Method refinements continue**

Acknowledgements

- Dr. Kamal Baloch (Harvard) for the GaN samples
- Dr. Kim Rensing (Leica) for sharing his cryo-EM experience
- Ms. Christalyn Sims (Harvard) for the cryo-TEM image
- Gonzalo Amador (Oxford Instruments) for the cryo tip assembly
- Funding Entities
  - CAMCOR is supported by grants from the W.M Keck Foundation, the M.J. Murdock Charitable Trust, ONAMI, the Air Force Research Laboratory (agreement number FA8650-05-1-5041), NSF (award numbers 0923577, 0421086) and the University of Oregon.
  - Harvard's Center for Nanoscale Systems (CNS) is a member of the National Nanotechnology Infrastructure Network (NNIN), which is supported by the NSF (under award no. ECS–0335765).