Progress Towards Cryo FIB Lift-Out

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Why Cryo?

1st Rule of Sample Preparation: DO NO HARM

**Biological**

Chemical fixation can induce artifacts, such as the bacterial mesosome

**Ill-V Materials**

Room temperature promotes undesirable Ga+ reactions during and after ion milling
## GaN FIB Milling Temperature

<table>
<thead>
<tr>
<th>Temperature (C)</th>
<th>Exposure (nC/(\mu)m²)</th>
<th>Side Effect (Qualitative)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 C</td>
<td>5.5</td>
<td>Severe</td>
</tr>
<tr>
<td>-25 C</td>
<td>5.5</td>
<td>Severe</td>
</tr>
<tr>
<td>-50 C</td>
<td>5.5</td>
<td>Intermediate</td>
</tr>
<tr>
<td>-100 C</td>
<td>5.5</td>
<td>Minimized</td>
</tr>
<tr>
<td>-145 C</td>
<td>5.5</td>
<td>None Observed</td>
</tr>
</tbody>
</table>

-50 C

-100 C

-145 C
FIB Milling Temperature Effects
(Dose: 5.5 nC/µm²)

Room Temperature

-145° C

• GaN

Better

• Copper

No Benefit
GaN Warm-up After Cryo-FIB

- Samples cryo-milled (-145°C)
- Dose: $5.5 \text{nC/\mu m}^2$

Imaged at -145°C  Imaged at Room Temp
Cryo-FIB Benefits for Bio

Cryoultramicrotome sections may have severe (70%) compression

Bio Cryo FIB H-Bar Prep

Rigort A et al.
PNAS 2012;109:4449-4454

http://dx.doi.org/10.1016/j.jsb.2010.02.011
Cryo-TEM of Cryo-FIB Prepared Sample

Polio virus inside cell membrane
Why Use Cryo Lift-Out?

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 -138°C for all steps
• Education
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

Dig and Shovel

Platinum Attach

Force Friction

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

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

A Sehrbrock et al., 2011
MC2011 Poster
Cryo Manipulation - Harvard

Harvard University CNS

- Leica EM VCT100 Cryo Transfer
- Zeiss Nvision
- OmniProbe 300
Avoid Bent Grids

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

- Platinum precursor improves curtain artefacts

No Precursor

With Precursor

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

Early Work Used Pt

- Tip Attach

- Grid Attach

New Improvements

- Attach using ice condensation
- The tip is clean and ‘like new’ upon warming
- Simultaneously attach with ice & separate the tip
- Adding Pt (100nm) reduces curtains

Mitigates thermal drift
Amorphous Ice Confirmed

H-bar lamella for TEM diffraction

FACE OF GRID POST

GAS FLOW

4 um

10 um

Selected Area Diffraction Image

Line corresponds to 0.370 nm spacing

Line corresponds to 0.214 nm spacing

TEM bright field image
Cryo Lift-Out (CAMCOR)

Site of Interest

Probe Contact

Water Attach & Lift

Grid Attach

Simultaneously flow water, cut needle

Platinum Coat

Clean and Thin

Avoid e-beam during thinning!
Cryo Lift-Out Thinning

Lift-Out Sample Structure

2nd Pt

1st Pt

Ice (tip, grid attach)

Yeast

Final Thinning, Ion Beam View

Avoid e-beam imaging!
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
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