Please join us for monthly user meetings!

Tech staff will:

- showcase our state-of-the-art capabilities,
- provide updates on the latest innovations,
- discuss any topics you find interesting.

Bring your questions and suggestions!

March 20, 2019 — Dr. Reiner Bleher, Assistant Research Professor
Materials Science & Engineering Conference Room, Cook #2036
12 - 1 p.m.

Successful Electron Microscopy of Biological and Soft Matter Samples

Adequate sample preparation is a pre-requisite for optimal results in electron microscopy. The BioCryo Facility of NUANCE offers a comprehensive array of methods and techniques for processing and preparing samples before they can be observed and analyzed in the electron microscope. The choice of the most suitable technique depends on the data we want to extract from a given sample. The intention of this tech talk is to give the users an idea of the capabilities of the BioCryo Facility and the rationale behind different workflows available.
The NUANCE Center
www.nuance.northwestern.edu

(Cook, Tech, Silverman, Hogan – Northwestern University)

EPIC
Electron Probe Instrumentation Center
SEM, TEM, FIB, EDS, EELS, eBeam-Litho, Sample Prep

BioCryo
Cryo- and Conventional Soft-Matter EM, Microanalysis, and Sample Prep
cryo/SEM, TEM, STEM, EDS...

Keck-II
Keck Interdisciplinary Surface Science
XPS, ToF-SIMS, FTIR, Confocal Raman ...

SPID
Scanned Probe Imaging & Development
AFM, DPN, NSOM, f-s Laser, Nano-Indenter

NUFAB
Micro/Nano Fabrication
Deposition, Photolithography, Thermal processing, Wet processing, Etching-Ashing
N. Basit, J. Ciraldo, A. Dhote, Y. Jia, S. Lu, +PD

SHyNE Director
Ben Myers

Business Office
Chad Goeser

Outreach Coordinator
Kathryn Dean

Program Administrator
Amy Morgan

ANL-APS
ANL-CNBM
ANL-EMC

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BioCryo Facility

- High-pressure freezing
- Plunge freezing
- Freeze fracture
- Cryo Ultramicrotomy
- Freeze Substitution
- Resin Embedding
- Ultramicrotomy
- Critical Point Drying

Sample Prep.

- Biological & Soft Matter
- Macromolecules
- Liposomes
- Cells, Tissues
- Hydrogels
- Polymers
- Katalysts
- MOFs
- Hybrid Materials

Samples

- Cryo S/TEM
- Cryo SEM
- TEM/SEM
- 3D reconstruction
- EDS
- EELS
- CLEM

Microscopy & Microanalysis

- Materials Science
- Life Sciences
- Interdisciplinary
- Industry

Users

- Training
- Collaboration
- Service
- Consulting
- Outreach (Tours, Workshops)

Sample Prep.

- Cryo S/TEM
- Cryo SEM
- 3D Reconstr.
- STEM-EDS

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Sample Prep.

- Training
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- Outreach (Tours, Workshops)
Basic workflows

Cryogenic

- Plunge freezing FeI Vitrobot
- High-pressure freezing Leica HPM100
- Cryo-ultramicrotomy Leica UC7/FC7
- Cryo-storage
- Freeze fracture/etching/coating Leica ACS600
- Cryo-CLEM tbd
- JEOL-1230 HT-7700 HD-2300
- Cryo-TEM/STEM
- S-4800 Cryo-SEM
- XRF
- AFM
- Tousimis Samdri 795
- Coating Cressington 208 HR
- FEI Vitrobot Plunge freezing
- FEI Vitrobot Freeze substitution Leica AF52
- FEI Vitrobot Freeze substitution
- FEI Vitrobot Cryo-substitution
- FEI Vitrobot Freeze-substitution
- FEI Vitrobot Vibrating blade microtomy Leica VT1200 S
- FEI Vitrobot Resin embedding
- FEI Vitrobot Chemical fixation and dehydration
- FEI Vitrobot Critical point drying
- FEI Vitrobot Microwave processing Pelco Biowave
- FEI Vitrobot Ultramicrotomy
- FEI Vitrobot TEM/STEM
- FEI Vitrobot XRF

Ambient temperature

- JEOL-1230 HT-7700 HD-2300
- JEOL-1230 HT-7700 HD-2300
- TEM/STEM
- S-4800-II SU-8030
- SEM
- LEICA ACE600
- LEICA AFS2
- LEICA UC7
- Tousimis Samdri 795
- Cressington 208 HR
- JEOL-1230 HT-7700 HD-2300
- AFM
- Leica VT1200 S
- JEOL-1230 HT-7700 HD-2300
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TE, HAADF, Z-contrast, SE, BSE, Diffraction, EDS, EELS, WDS, LM, LA...
Conventional sample preparation for TEM

Fixation:
- crosslinking of proteins with glutaraldehyde and/or formaldehyde in buffer
- preservation of antigenicity for immunolabeling

Postfixation
- OsO4 to stabilize and stain lipids (membranes)
- staining of charged sites with UA

Dehydration
- replacement of water with an ascending series of ethanol, acetone, or acetonitrile

Resin (epoxy or methacrylates):
- minimal shrinkage
- stability in the vacuum
- stable when exposed to the electron beam
- hardness/softness for ultramicrotomy
- stainability (LM and EM)
- preservation of antigenicity for immunolabeling
AUTOMATED conventional sample preparation for TEM

ASP-1000: Automated chemical fixation – dehydration – infiltration

- Time consuming
- Involves many steps
- Repeatability
- Reproducibility
Uptake of nanoparticles by cancer cells

Samples were chemically fixed, dehydrated, and resin embedded. Sections of ca. 80 nm thickness were used. (Cells were cultured by Naoyuki Shimazu, Mirkin Lab)
Detection and EDS analysis of nanoparticles inside of cancer cells with STEM

Project with DTC.
Serial resin sections for 3D-reconstruction of the nucleoid of E. coli

Images were processed with TrackEM2 ImageJ plugin

Exploring Inner Space

Biocryo Electron Microscopy

Imaging of a thick section of a resin-embedded breast tumor sample

Reiner Bleher, Project with Wenan Qiang, CLP
TEM of an ultrathin (~40nm) section of a resin embedded MOF sample

Sample: Xinyi Gong (Farha Group)
TEM: Roberto dos Reis (VPD Group)
Imaging/analysis of NPs and QDs

Reiner Bleher, Project with T. Duncan, FDA
Negative staining

- Tripod kind of structure
- Each protein monomer in the structure is coming from antibody and then connected by a linker

Sample from Justin Modica (Milan group)
Exploring Inner Space

Negative staining

Sample: Justin Modica (Milan group)
TEM: Sonali Dhindval (VPD Group)
Basic workflows

Cryogenic

- Plunge freezing, FEI Vitrobot
- High-pressure freezing, Leica HPM100
- Cryo-ultramicrotomy, Leica UC7/FC7
- Cryo-storage
- Cryo-TEM/STEM
- Cryo-CLEM, tbd
- JEOL-1230, HT-7700, HD-2300
- S-4800
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- Tousimis Samdri 795
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- Cryo-ultramicrotomy
- Cryo-ultramicrotomy
- Freeze fracture/etching/coating, Leica ACS600
- Freeze substitution, Leica AF52
- Fixing
- JEOL-1230, HT-7700, HD-2300
- S-4800-II, SU-8030
- COATING
- TEM/STEM
- SEM

- High-pressure freezing, FEI Vitrobot
- Vibrating blade microtomy, Leica VT1200 S
- AFM
- TEM/STEM
- JEOL-1230, HT-7700, HD-2300
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- SEM

TE, HAADF, Z-contrast, SE, BSE, Diffraction, EDS, EELS, WDS, LM, LA...
Cryo immobilization vs conventional processing

- Macromolecules
- Viral Particles
- Bacteria
- Cells
- Tissues
- Liposomes
- Micelles
- Hydrogels
- Microgels
- Nanofibers
- Nanotubes...

permeability changes/redistribution/loss

shrinkage/distortion/collapse
Conventional sample processing vs. Cryogenic sample processing

Chemical Fixation
- Slow process
- Osmotic effects
- Change of membrane permeability
- Loss/re-distribution of diffusible ions and small molecules
- Conformational changes of proteins
- Masking of antigens

Postfixation
- OsO₄: Depolymerization of proteins

Dehydration (or CPD for SEM)
- Shrinkage
- Conformational changes of proteins
- Extraction of lipids
- Collapse of structures (e.g. hydrogels)

Resin Embedding
- Extraction of lipids
- Shrinkage during polymerization

S/TEM, SEM, EDS, EELS, XRF, LM...

Cryo fixation
- Rapid process
- Vitrified sample w/o artifacts

Processing for Observation
- Cryo-ultramicrotomy for cryo-TEM
- Freeze fracture for cryo-SEM

Cryo-S/TEM, cryo-SEM, cryo-XRF, cryo-LM, FS...
Cryofixation

Realizable Cooling Rates

I Range of vitrified pure water
II Range of vitrified animal cells and tissues
III Range of specimens vitrified with high pressure

Which cryogens are suitable?

<table>
<thead>
<tr>
<th>Cryogen</th>
<th>Melting Pt. [°C]</th>
<th>Boiling Pt [°C]</th>
<th>Freezing Rate [°C/s]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freon 13</td>
<td>-181</td>
<td>-81</td>
<td>98000</td>
</tr>
<tr>
<td>Propane</td>
<td>-189</td>
<td>-42</td>
<td>98000</td>
</tr>
<tr>
<td>Ethane</td>
<td>-183</td>
<td>-89</td>
<td>97000</td>
</tr>
<tr>
<td>Isopentane</td>
<td>-160</td>
<td>28</td>
<td>45000</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>-209</td>
<td>-196</td>
<td>16000</td>
</tr>
</tbody>
</table>

### Achievable vitrified sample thickness

<table>
<thead>
<tr>
<th>Device</th>
<th>Freezing depth (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plunge freezer</td>
<td>10-20</td>
</tr>
<tr>
<td>Spray freezer</td>
<td>10-20</td>
</tr>
<tr>
<td>Slam freezer</td>
<td>20-40</td>
</tr>
<tr>
<td>Propane jet</td>
<td>40</td>
</tr>
<tr>
<td>High-Pressure freezer</td>
<td>50-400</td>
</tr>
</tbody>
</table>

Plunge freezing
Peptide amphiphile nanofibers

1: Peptide amphiphile molecules self-assemble into nanofibers in water. Used as an artificial extracellular matrix to promote cell growth.

2: The same peptide amphiphile molecules form shorter nanofibers through a different self-assembly process.

3: Peptide amphiphile molecule that self-assembles into twisted nanoribbons in water. This material facilitates neural regeneration.

Plunge Freezing

200 nm

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High-Pressure Freezing

When water freezes, its volume increases (Le Chatelier)

High pressure (~2050 bar) inhibits this expansion and reduces the critical freeze rate to a range between 100 and 500 °/s

How?
1) Lowering of the freezing point
2) Lowering the supercooling temp. limit
3) Reduction in the rate of ice crystal nucleation
4) Slowing the growth of ice crystals

High-pressure freezing

EMPACT2

HPM100

HPF Compact02

Ice

HPM100 Temperature and Pressure during a freeze on 11/15/16.
High-pressure freezing

• Keep processing time as short as possible.
• Avoid air bubbles!
• Use space fillers, e.g.: hexadecene, dextrane, BSA, yeast paste.
• Suspensions (bacteria, cells, liposomes, micelles...)
• Tissues, hydrogels
• Can be used in combination with cellulose capillaries

Cu Capillaries
0.65 mm o.D. and 0.3 mm i.D.

Sample Holders for HPM100
3 mm o.D. and 0.5 mm height
SEM and cryo-SEM of Hydrogels

SEM image of critical point dried hydrogel

Cryo-SEM image of a high-pressure frozen and freeze fractured hydrogel
Cryo-STEM of cryosections of E. coli ΔcusR

Cells were high-pressure frozen and sectioned at -170° C. Images were recorded at -165 ° C. The nominal thickness of the section was 100 nm.

Please note:
Samples that are too soft at RT can be cooled down and sectioned with the cryo-ultramicrotome, e.g. polymers, rubbers, chewing gum, etc..

Reiner Bleher, 2015
Exploring Inner Space

**Biocryo**

**Electron Microscopy**

**High-pressure freezing and freeze substitution**

**Glycogen Particles are well retained**

HPF – FS:

- Dissection and mounting
- High pressure freezing
- Transfer into freeze substitution medium (e.g. Acetone/OsO4) at low temp.
- Freeze substitution (-90 C to RT)
- Infiltration with resin
- Polymerization (can be at low temp. with UV)
- Ultramicrotomy
- (Immunolabeling)
- Contrasting
- Imaging/Analysis

High-pressure frozen and freeze substituted mouse kidney


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CLEM: Correlative Light and Electron Microscopy

For molecules/particle suspensions, monolayers of bacteria or small cells

Needs particular FS medium and resin that retains fluorescence.

Mainly for cell monolayers, thin tissue slices

Exploring Inner Space

Biocryo

Electron Microscopy

CLEM: Correlative Light and Electron Microscopy

Cryo- microscopy and microanalysis

Microscopy and microanalysis

Plunge freezing

High-pressure freezing

Freeze substitution

Ultramicrotomy

Light Microscopy

Conventional processing

Chemical fixation

Dehydration

Infiltration

Light Microscopy

High-pressure freezing

Freeze substitution

Ultramicrotomy

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Correlative fluorescence microscopy and STEM of structural details of actin filaments.

Exploring Inner Space

**Biocryo**

Cryo fluorescence and bright field image of ZincBY-1-labelled sperm cells.

-180°C

5 µm

**STEM image of a freeze dried sperm cell**

Reiner Bleher, Project with Tom O’Halloran Lab

(Cryo) CLEM: Correlative Light and Electron Microscopy

r-bleher@northwestern.edu
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Biocryo

Electron Microscopy

Thank you for your Attention!

Questions?

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