



# CRYO WORK

## BIOCRYO FACILITY

Charlene Wilke

# Welcome to 2020

Charlene Wilke

- Northwestern for 12 years in July.
- BioCryo Facility: A satellite facility, we are in the Silverman | Hogan | Tech buildings.
- What I do... Specimen preparation, imaging, training, equipment maintenance –my soldering skills leave a lot to be desired.
- While I do have a background in both biological and materials work applying to both TEM and SEM, I have had more experience in biological + TEM applications.

NUANCE  
Tech  
Talks  
...explore with us

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

**Northwestern**  
RESEARCH

**SHYNE**  
Soft and Hybrid Nanotechnology  
Experimental Resource



# This presentation will be about

- Short Intro | Tech Talk Format
- What makes it cryo?
- A few pieces of cryo equipment.
- Time for questions or discussions.

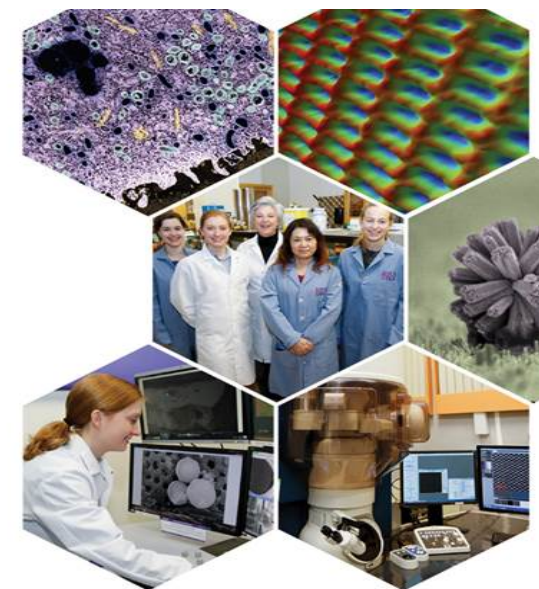
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Bring your questions and suggestions!



January 9, 2020

Charlene Wilke, BioCryo Electron Microscopy Imaging Specialist

MSE Conference Room, Cook Hall #2058  
12 - 1 p.m.

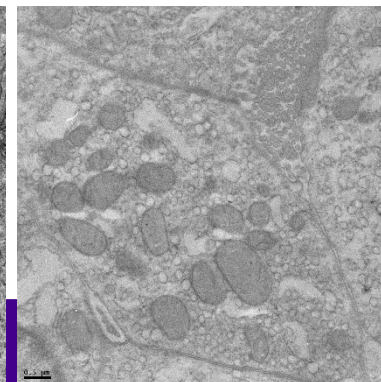
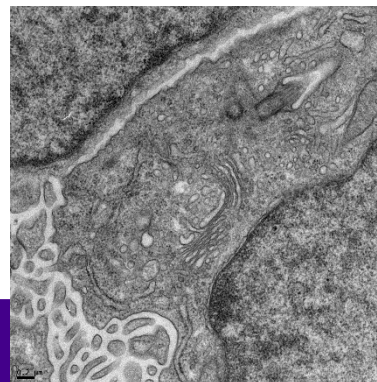
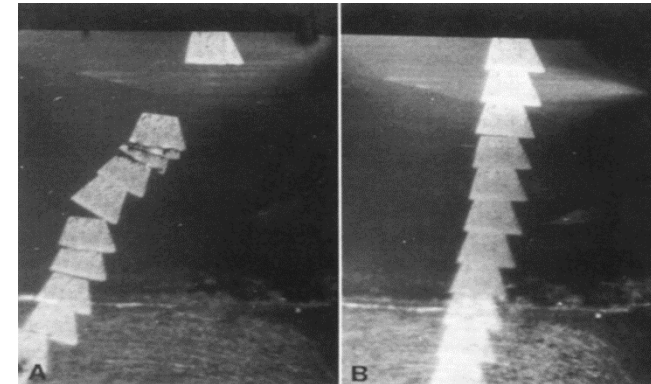
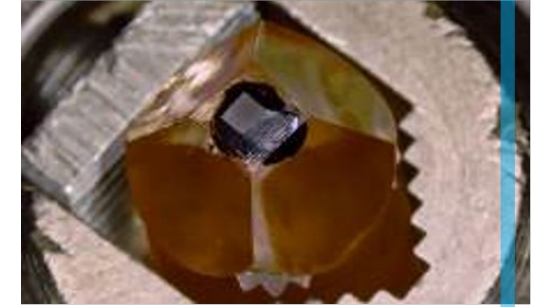
### Cryo Work

Bio-Cryo EM Specialist Charlene Wilke's talk entitled "Cryo Work" will cover several freezing techniques as well as the support equipment Bio-Cryo currently has available. The talk will be followed by plenty of time for open discussion, so bring your questions and queries!

*Brown bag. Light refreshments will be served.*

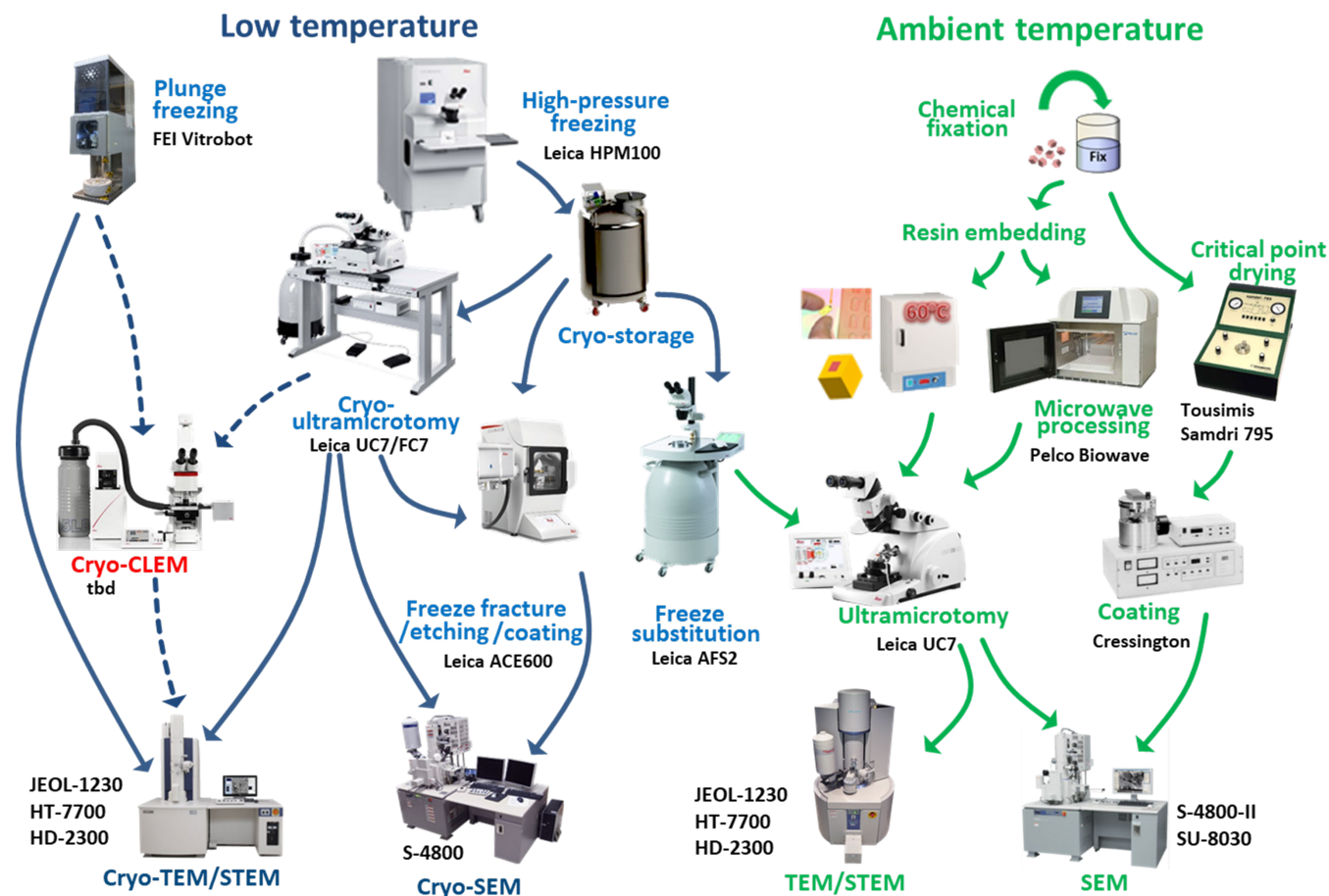
# Cryo vs. What?

- EM is microscopy and spectroscopy that is utilized for both materials and biological samples.
- Biological sample preparation involves fixing the tissue or cells, dehydrating, embedding and sectioning. There are several opportunities to introduce contrasting agents into the sample during this preparation.
- Soft materials sample preparation usually involves stabilizing the sample for sectioning, which can include embedding, but may benefit from lowering the temperature of the sample in order to stabilize it for ultra thin sectioning. Allowing it to return to room temperature for imaging.



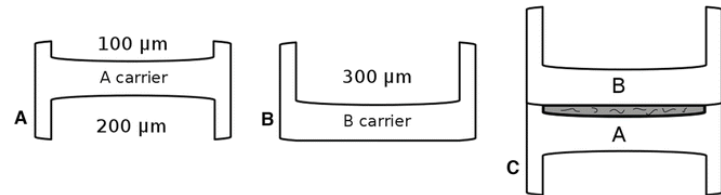
# What Makes It Cryo?

- Work flow comparison between the conventional treatment of biological samples and cryo work.
- The objective of cryo is to immobilize active or transient hydrated samples while preventing the development of hexagonal ice.
- Freezing is preferred over chemical fixation because it stops biological processes in milliseconds, rather than minutes. Maintains the system in a more natural state.
- Speed, pressure, volume and cryo protectants are the guiding principles of freezing.



# High Pressure Freezing Leica HPM100

- Increased depth of freezing
- Increased freezing volume
- Assortment of samples carriers = assortment of experiments. Plancettes, tubes, fracture, sapphire disks.
- Easy to use
- What else... Light or electrical stimulation for dynamic freezing, CLEM

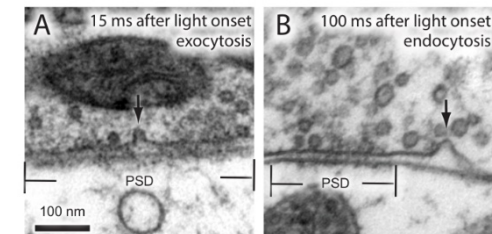
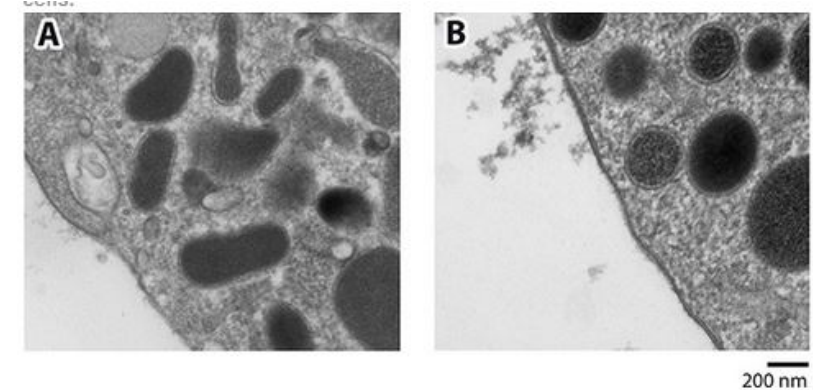
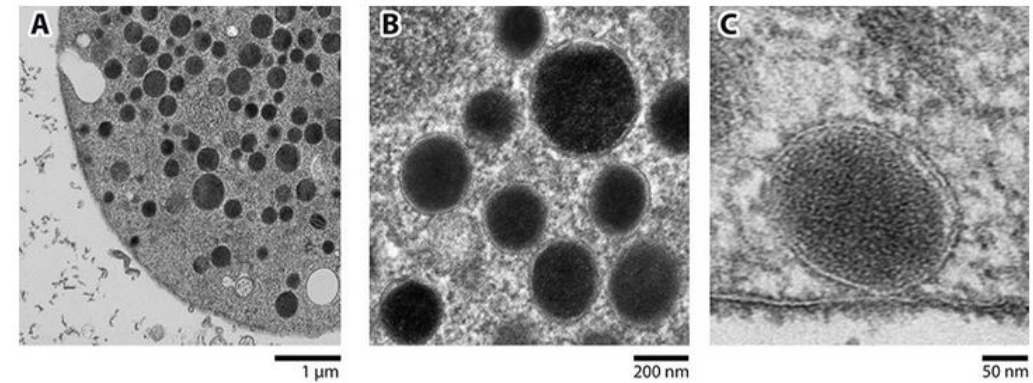


# Freeze Substitution

## Leica EM AFS2

- Usually carried out on HPF samples, also slam frozen. PLT
- Better specimen preservation.
- A preferred method of processing for immuno-gold labeling.
- Easy to use but, protocols can be tedious to develop.
- Very flexible. Has an automated system.

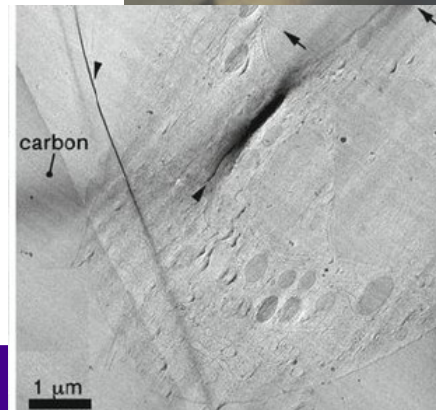
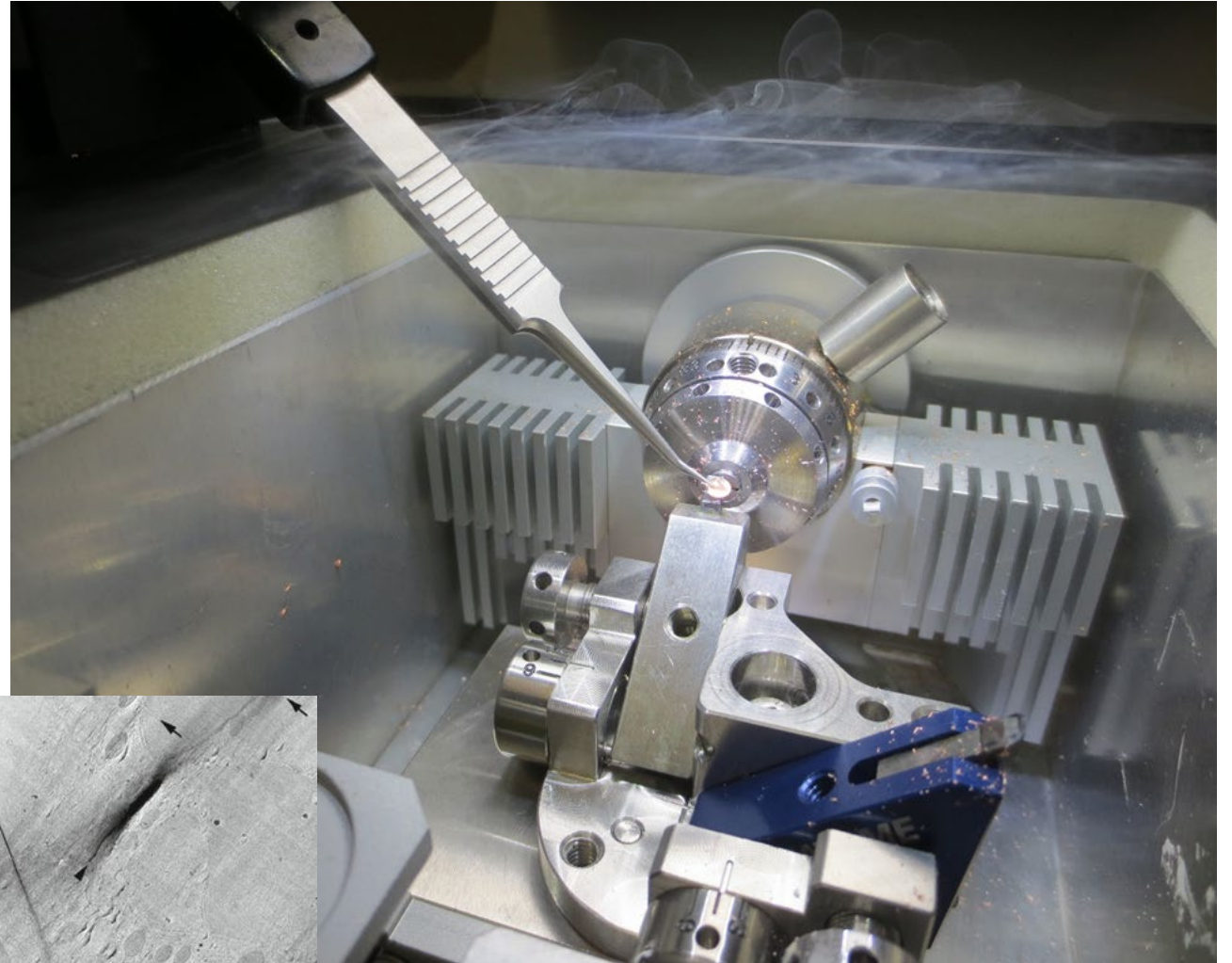
Image from the Leica website: Ultrastructural Preservation and Improved Visualization of Membranes in Primary Bovine Chromaffin Cells: Capturing Dynamic Processes with High Pressure Freezing  
Gianvito Arpino , MSc, Ling-Gang Wu , MD, PhD. Christopher Bleck , PhD



# Cryo Sectioning & Low Temperature Manipulation

## Leica EM UC7/FC7 Cryo-Ultramicrotome

- Cryo planing of soft materials; Use the Glass Transition temperature of a material to optimize for sectioning or planing the material's surface.
- Immuno-gold labeling | Tokuyasu sectioned at -80.
- Cryo sectioning followed by cryo TEM imaging.
- Sectioning requires skill and patience. Challenging to master.
- Sectioned biological material is low contrast.
- Morphological artifacts develop from compression.

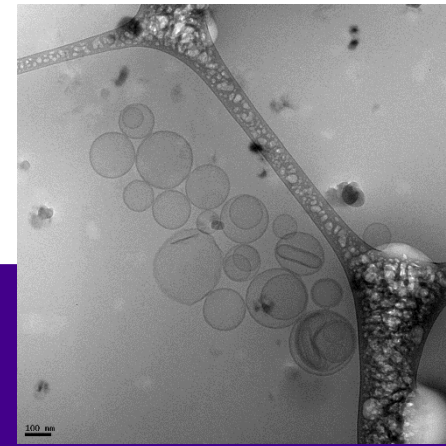
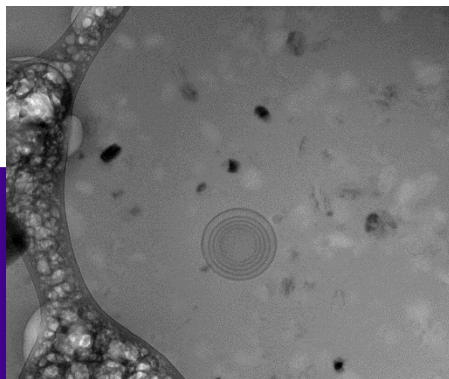
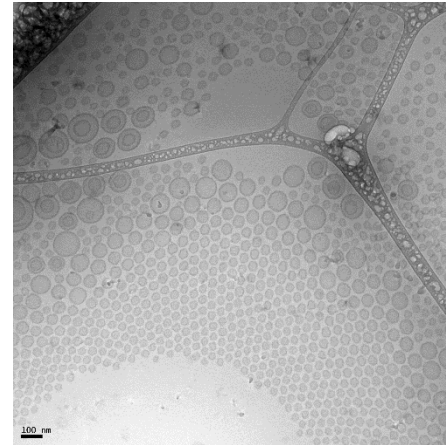
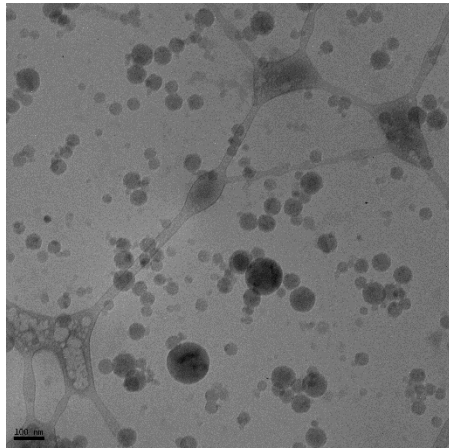
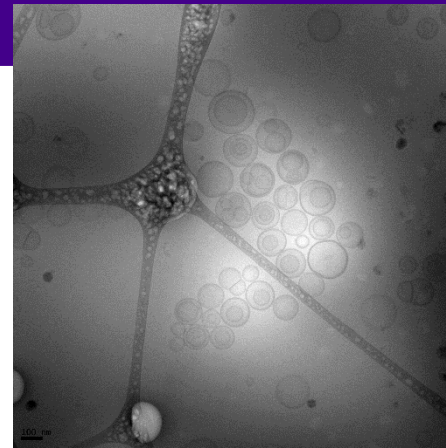




# Plunge Freezing

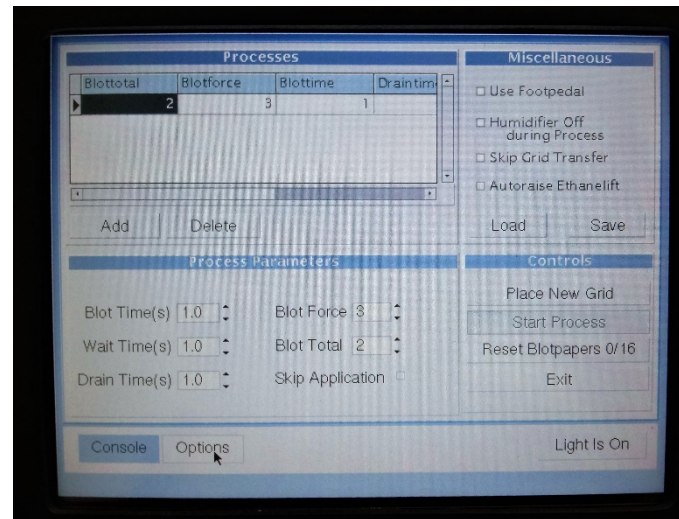
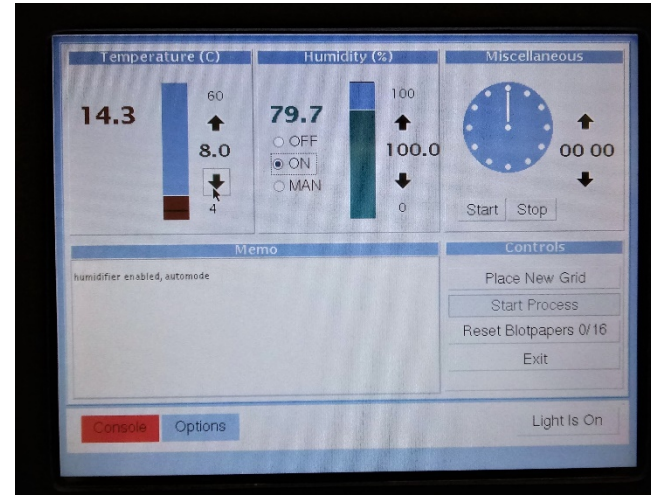
## FEI Mark III and Mark IV Vitrobot

- Suspensions & emulsions freeze as a thin film, then TEM imaged using a cryo holder (CTH).
- Most commonly used cryo technique at BioCryo Facility.
- Must have strong TEM skills.
- Easy to operate and adapt blotting conditions.



# Vitrobot vs Hand Blotting

- Reproducibility
- Predictability
- Temperature and humidity controls

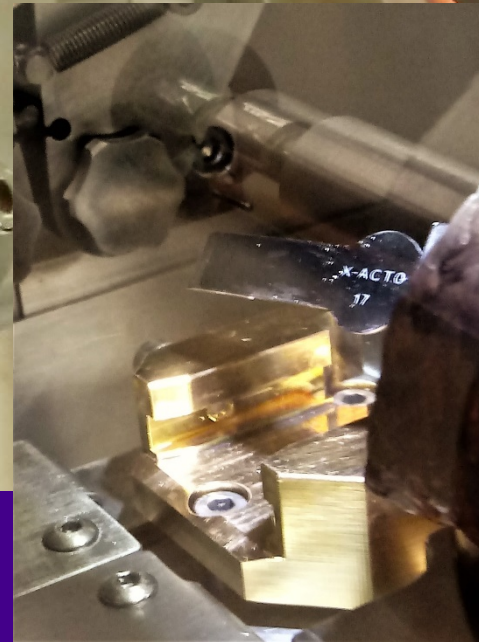
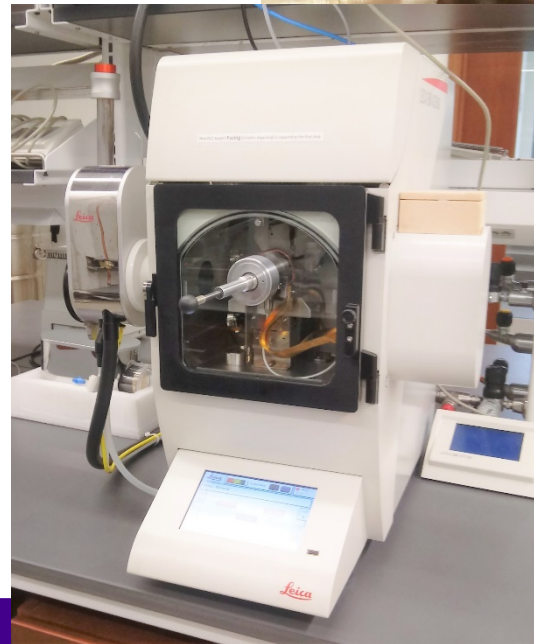
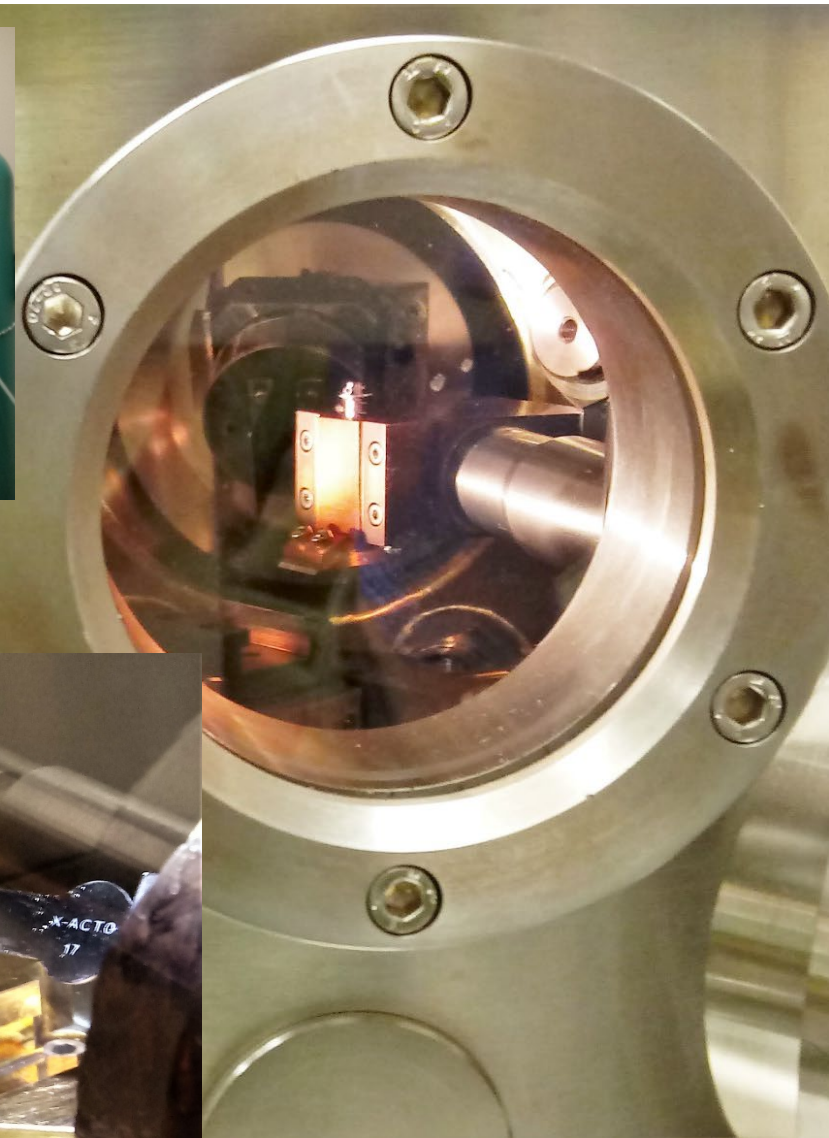
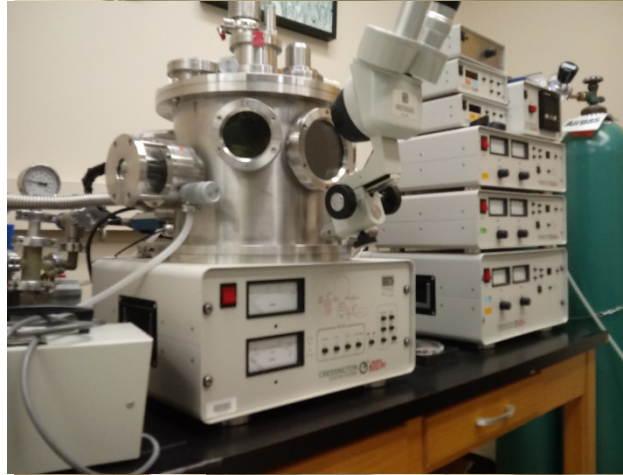
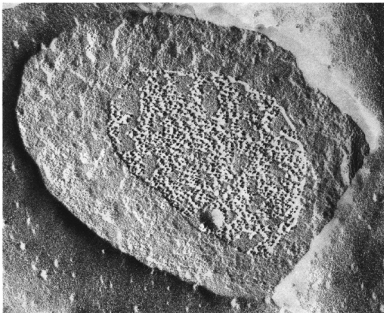


# QFDE & Replicas

## Leica ACE600 & Cressington

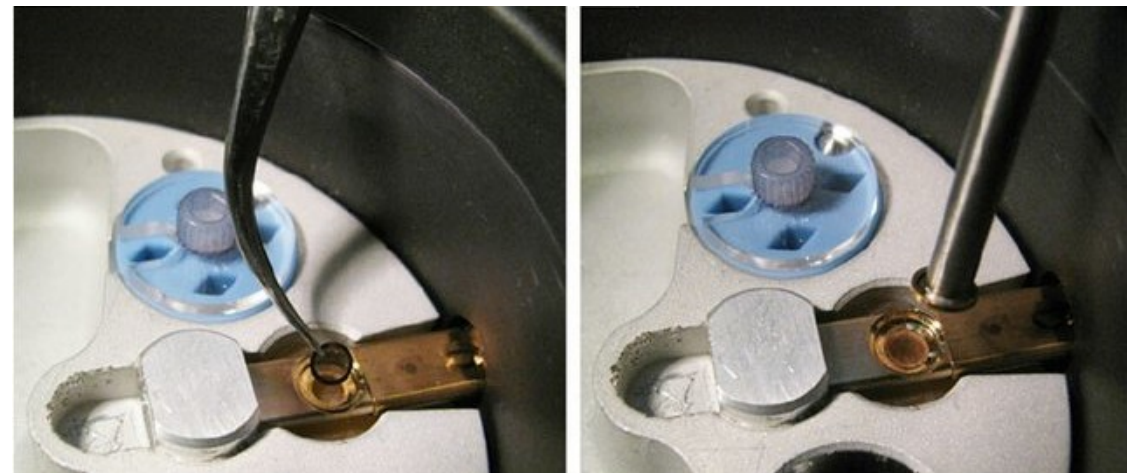
Carried out at low temperatures and high vacuum, frozen samples are skillfully fractured to expose a desired area of interest. The fractured surface is sublimated to expose more ultrastructure.

A thin metal film is deposited by sputter coating, followed by a layer of carbon. The sample is brought to ambient temperatures and the replica is detached by digesting the sample with a bleach solution. Replica is picked up on a TEM grid for TEM imaging.



# Challenges: Cryo Holders & Workstations

- Requires strong TEM skills. What can go wrong, will go wrong.
- Delicate.
- Very Expensive.
- Variety of capabilities.



Thank you, any questions?

