

CRYOEM 001 : TOOLS OF THE TRADE — SIDE ENTRY SYSTEMS

NCCAT Embedded Training — Master Class series

October 21, 2020

NATIONAL CENTER FOR
CRYOEM ACCESS & TRAINING



New York Structural
Biology Center

SIMONS ELECTRON
MICROSCOPY CENTER



CRYOEM 001 : SINGLE PARTICLE MASTERCLASS

Introduction to cryoEM: SPA

Building a cryoEM toolkit

EM compatible samples

EM support films and grids

Sample preparation

Tools of the trade:

microscopes and detectors

Microscope operations

Data collection strategies

Data assessment & QC

Data processing:

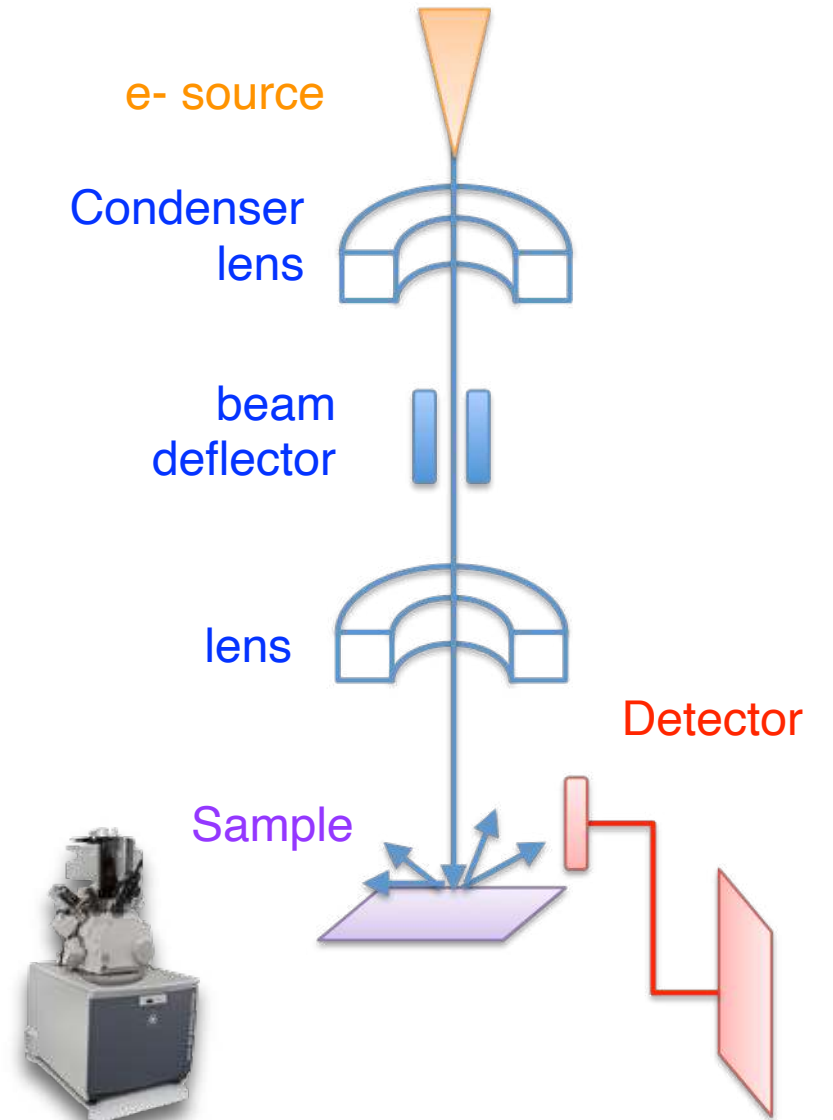
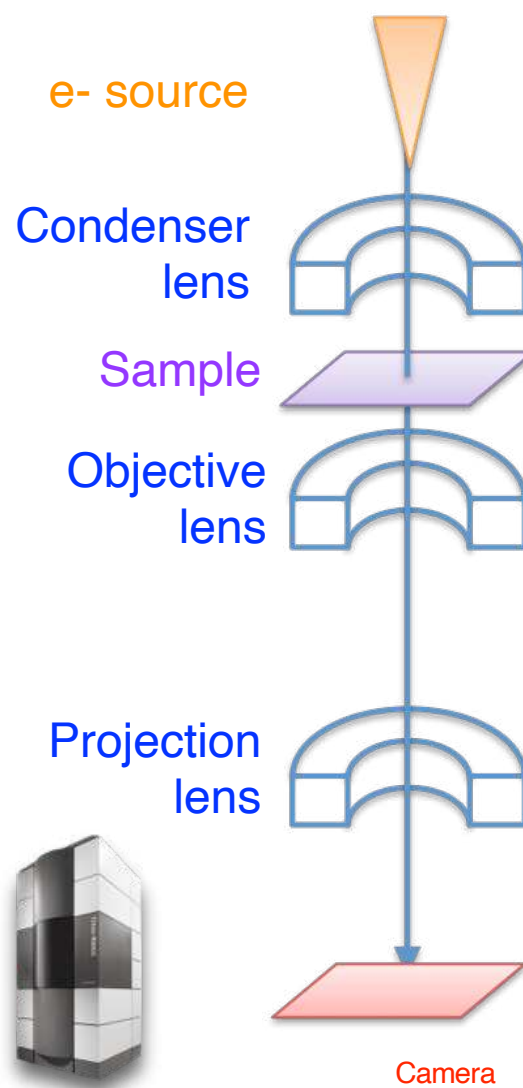
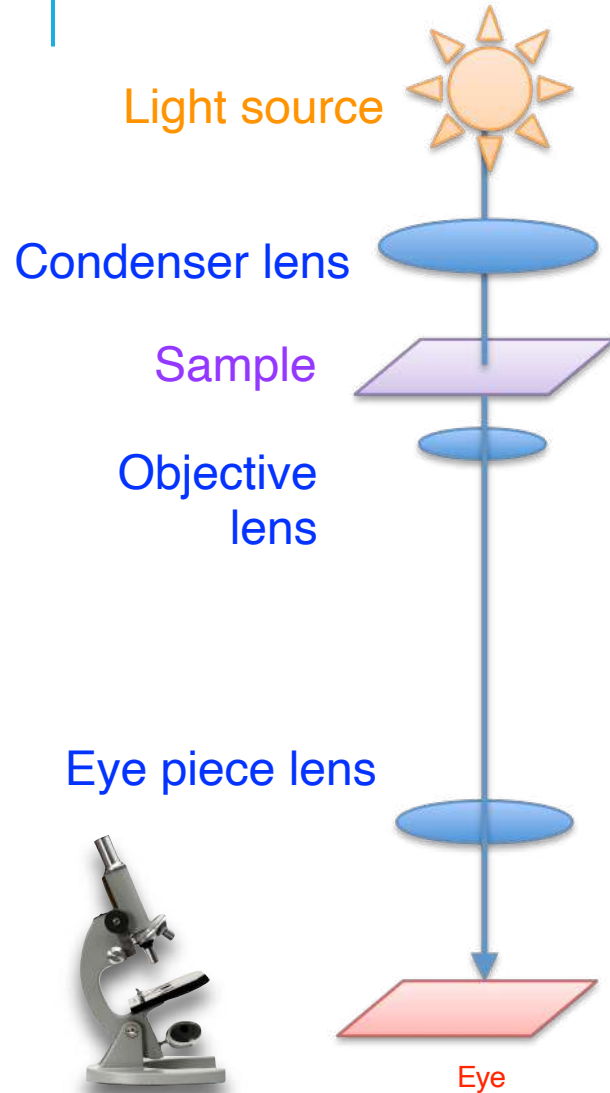
cryoEM IT infrastructure

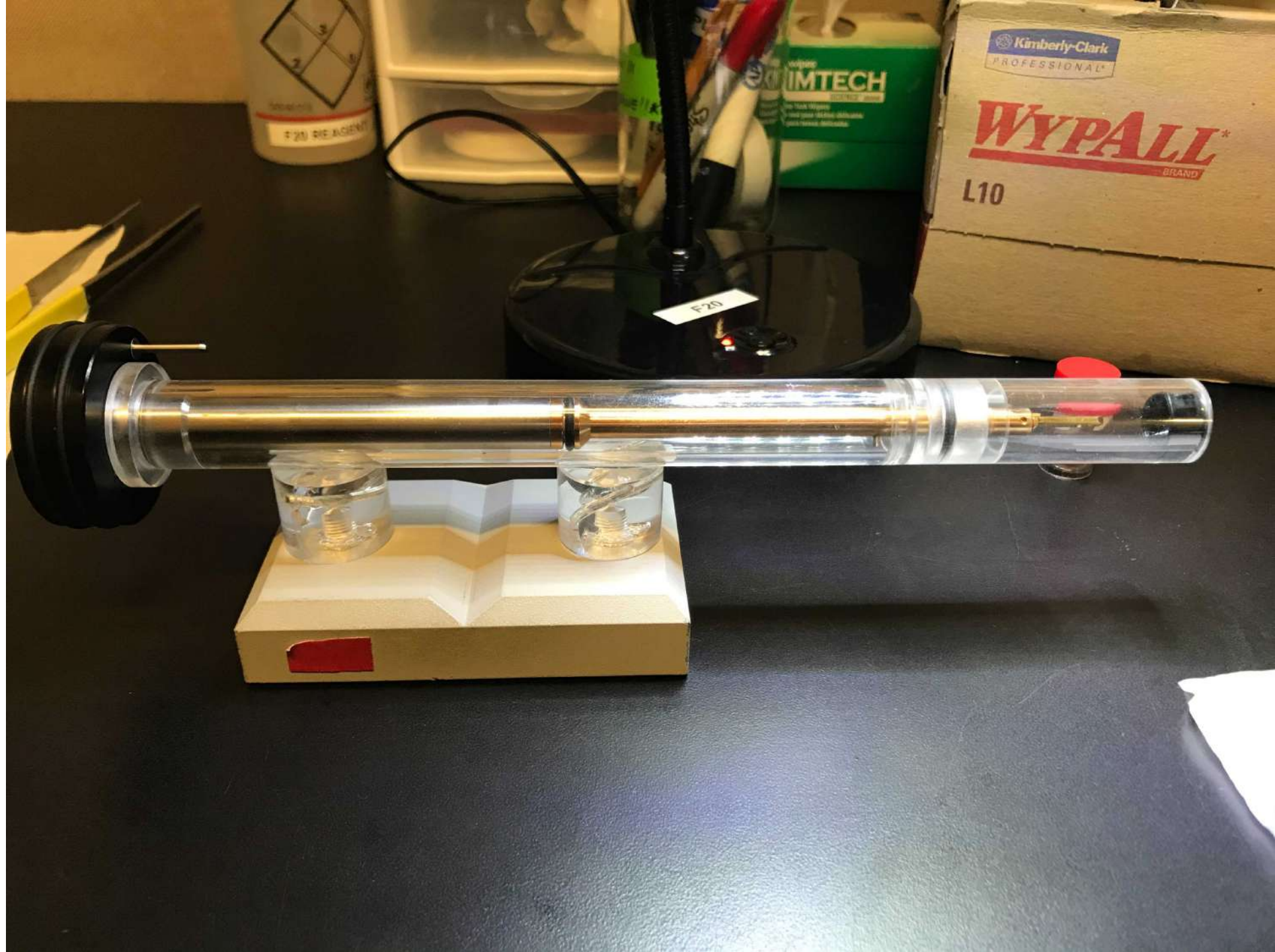
On-the-fly feedback

3D Reconstruction

Visualization and validation

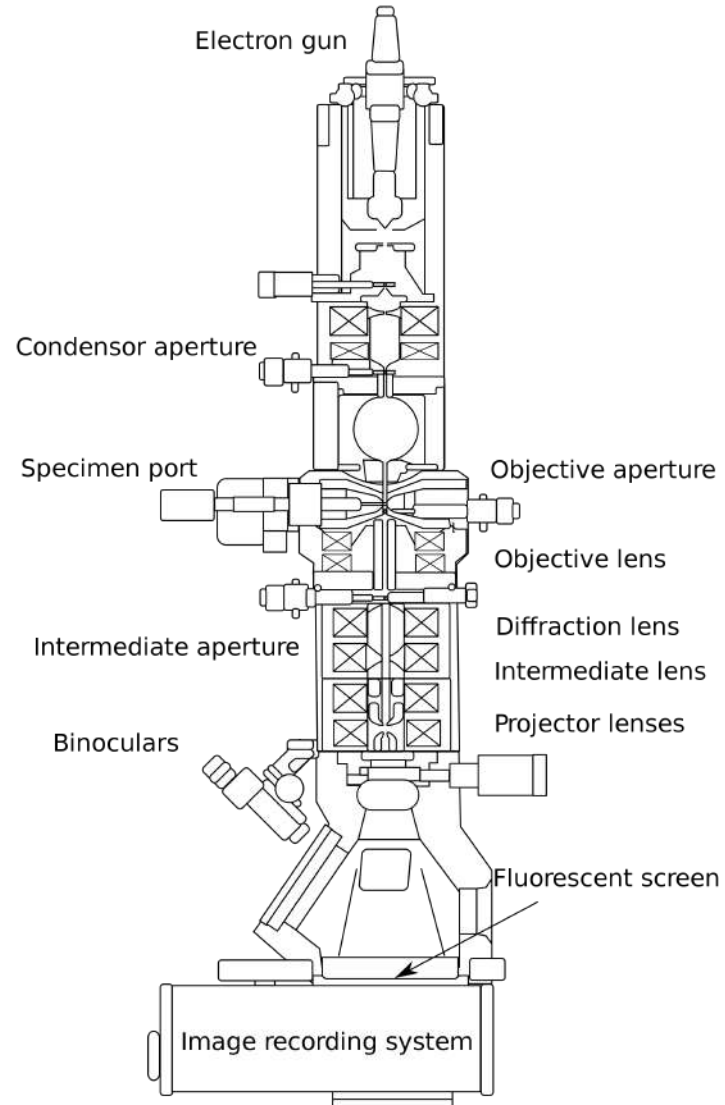
CRYOEM TOOLS







MAIN PARTS OF AN EM



Electron sources



Vacuum systems

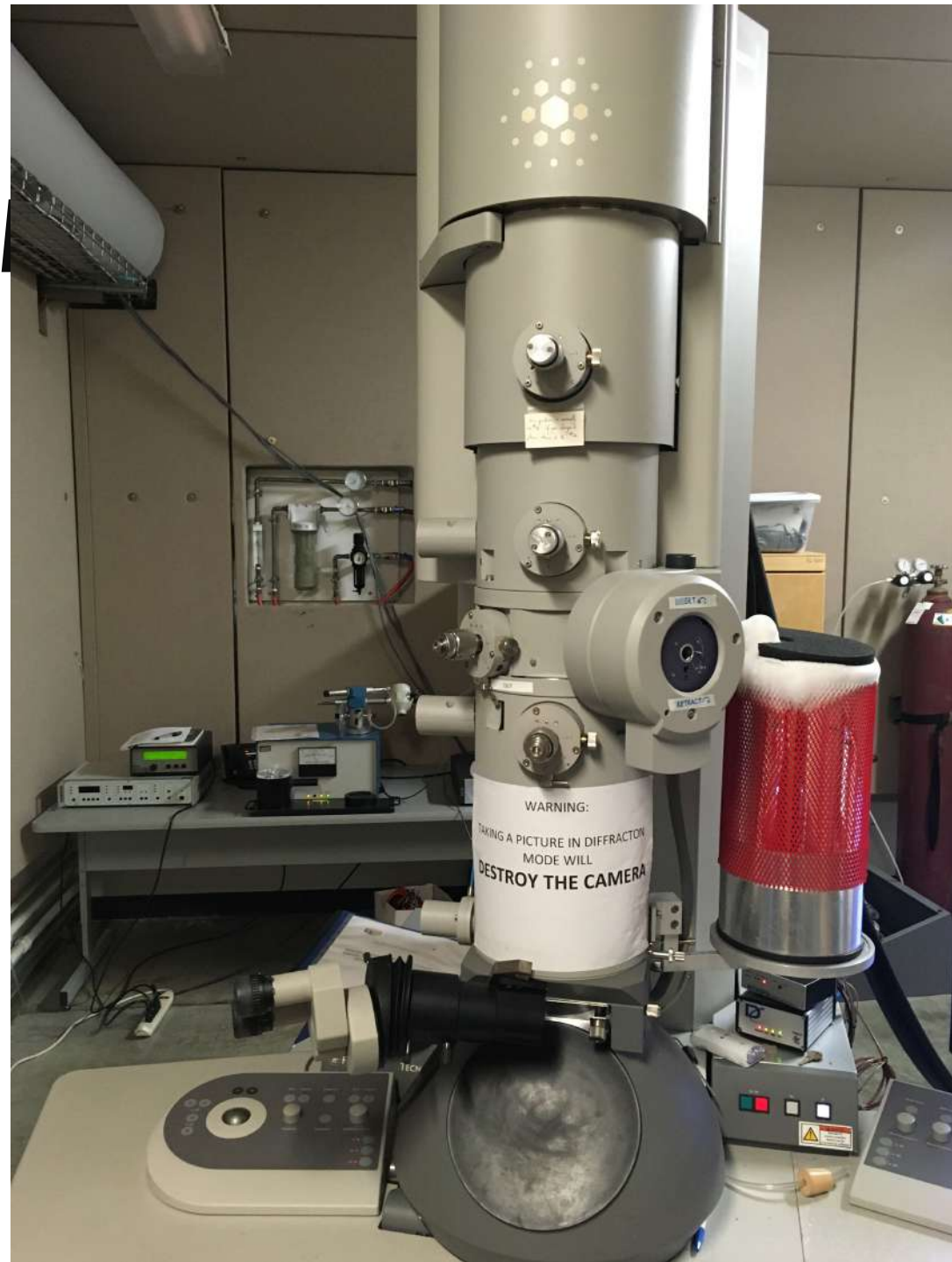
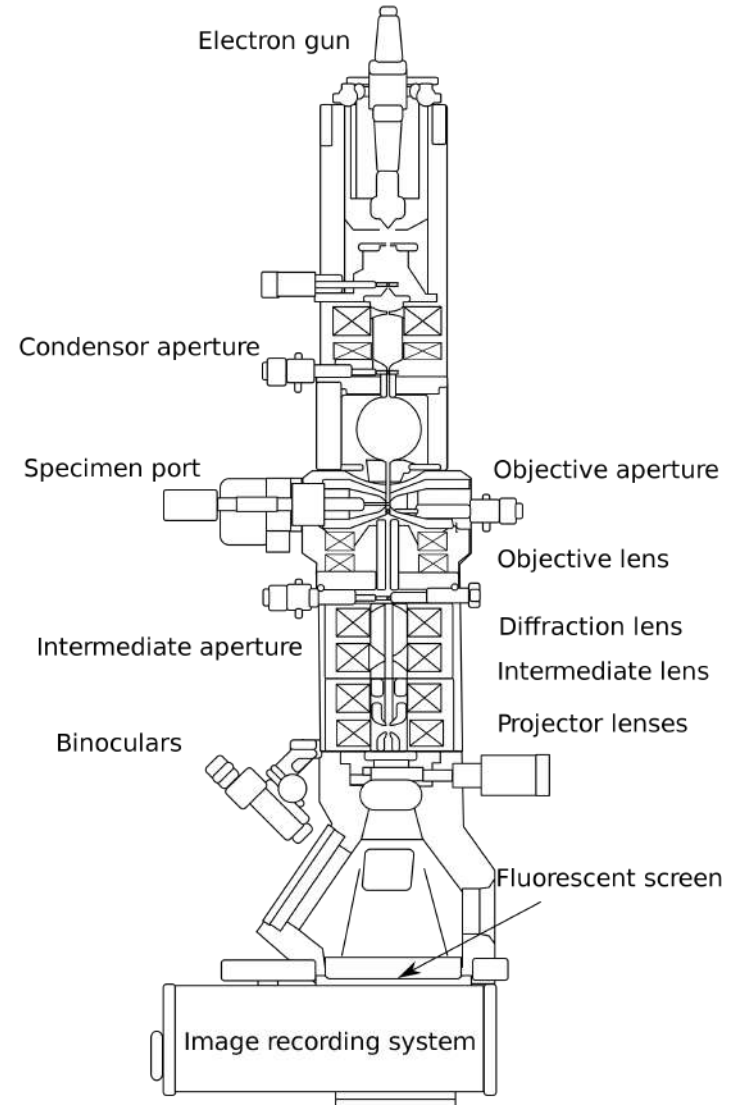


Lenses



Detectors

MAIN PARTS OF AN EM



SAMPLE INSERTION

Recording You are viewing Mahira Aragon's screen View Options 50:30 View

F20 Microscope PC - VNC Viewer

Meas exp XXX TEM Bright field
SA 55000 x C2 44.793 %
High tension: 200 kV Intensity zoom: OFF X -0.04 µm Obj Lenc: 89.4444 %
Defocus: -5.00 µm Spot size: XXX Y 0.08 µm Col IGP: 6
Focus step: 5 Near exp: XXX Z -17.82 µm A 0.03 deg

NYSBC

Eugene Chua

Press (⌘+A) to unmute or hold space bar to temporarily unmute.

Unmute Stop Video

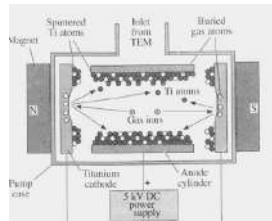
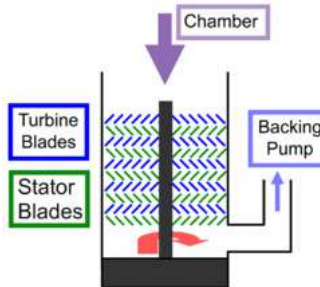
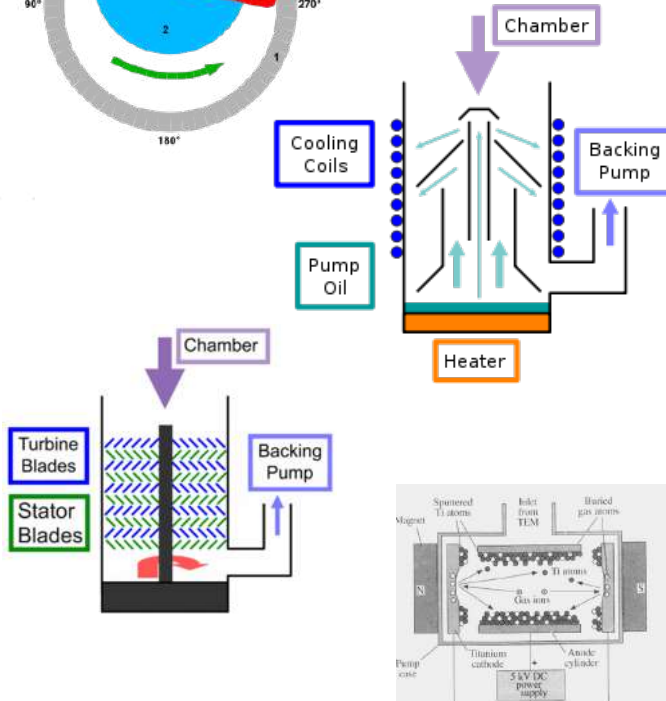
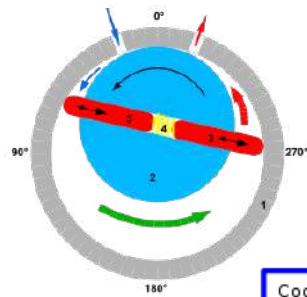
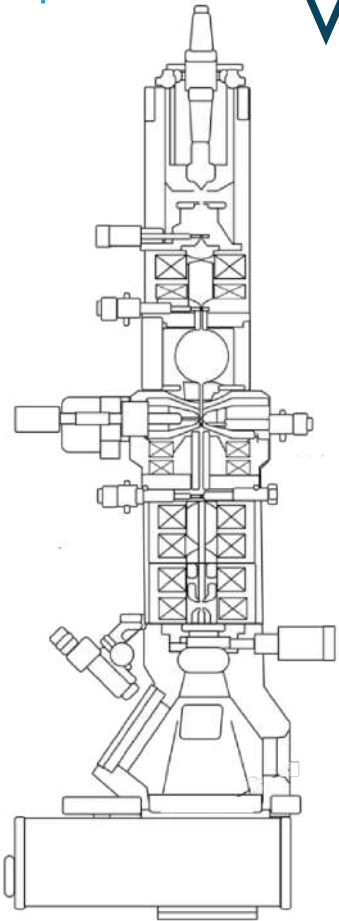
Participants 7 Chat Share Screen Record Reactions Leave

The image is a screenshot of a VNC viewer window. The main window displays the 'F20 Microscope PC' interface, which includes various control panels on the left and a large central area for the microscope feed. The status bar at the bottom of the VNC window shows technical data: 'Meas exp XXX TEM Bright field', 'SA 55000 x C2 44.793 %', 'High tension: 200 kV Intensity zoom: OFF X -0.04 µm Obj Lenc: 89.4444 %', 'Defocus: -5.00 µm Spot size: XXX Y 0.08 µm Col IGP: 6', and 'Focus step: 5 Near exp: XXX Z -17.82 µm A 0.03 deg'. To the right of the main window, there are two smaller video feeds. The top one shows a desk with various items, labeled 'NYSBC'. The bottom one shows a person working at a desk, labeled 'Eugene Chua'. At the bottom of the VNC viewer, there is a chat bar with a message 'Press (⌘+A) to unmute or hold space bar to temporarily unmute.' and buttons for 'Unmute', 'Stop Video', 'Participants', 'Chat', 'Share Screen', 'Record', 'Reactions', and 'Leave'.



VACUUM SYSTEMS

What types of pumps do we have?



$1 \text{ mm Hg} = 1 \text{ Torr} = 10^2 \text{ Pa}$
 $1 \text{ atm} = 760 \text{ Torr} = 7.5 \times 10^4 \text{ Pa}$

PVP / Rotary $1\text{-}10^{-3} \text{ Torr}$ | $>0.1 \text{ Pa}$

Diffusion $10^{-3}\text{-}10^{-6} \text{ Torr}$ | $0.1\text{-}10^{-4} \text{ Pa}$

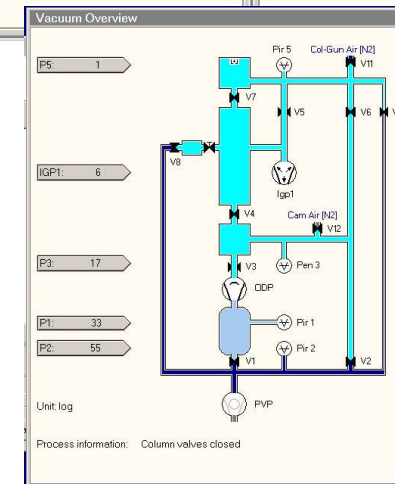
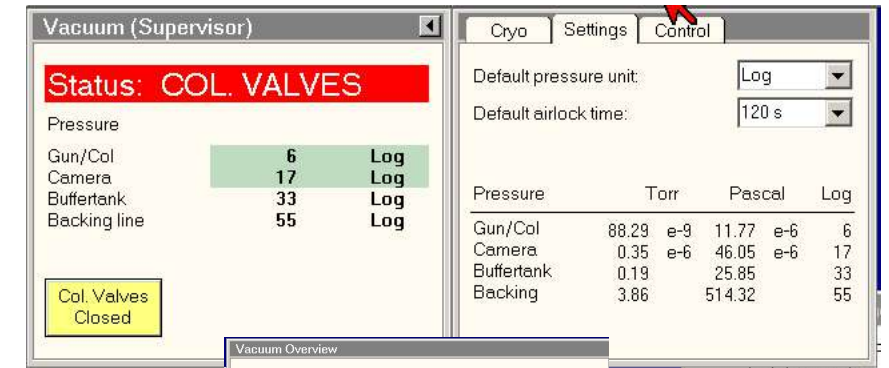
Turbo $10^{-6}\text{-}10^{-9} \text{ Torr}$ | $10^{-4}\text{-}10^{-7} \text{ Pa}$

IGP $10^{-9}\text{-}10^{-12} \text{ Torr}$ | $10^{-7}\text{-}10^{-9} \text{ Pa}$



A detailed technical line drawing of a microscope, viewed from the side. The drawing illustrates the internal mechanical components, including the objective lenses, eyepiece, stage, and base. The drawing is oriented vertically, with the eyepiece at the top and the base at the bottom. The drawing shows the complex arrangement of lenses, mirrors, and mechanical parts that make up the microscope's internal structure.

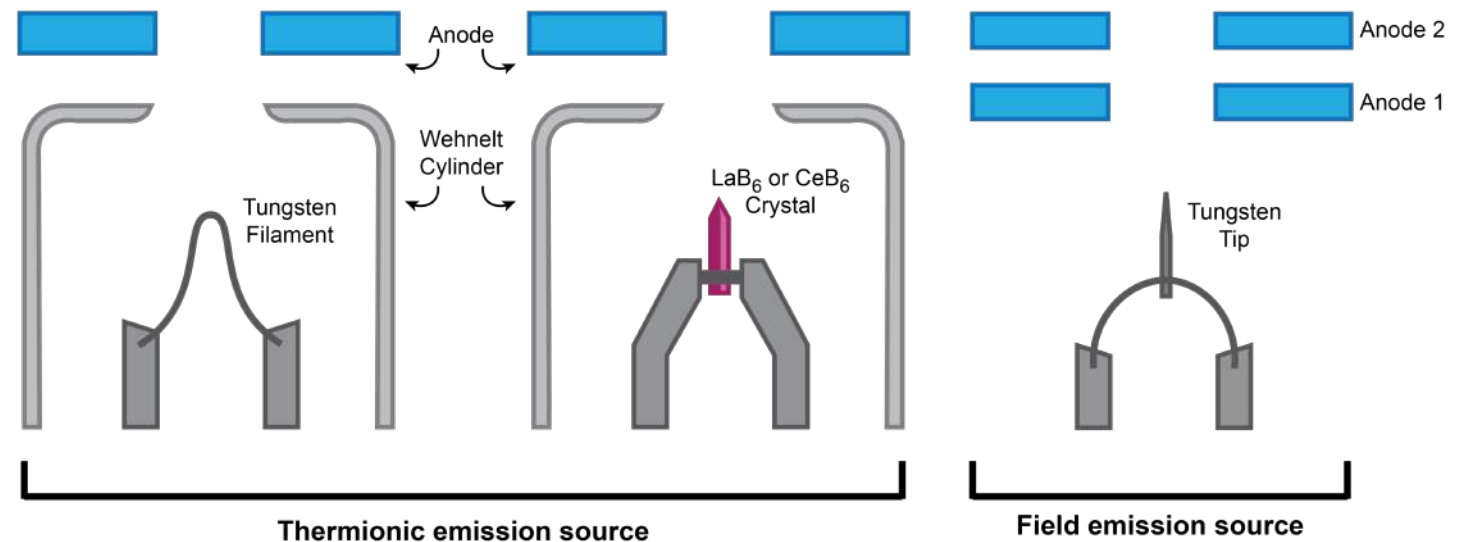
1 mm Hg = 1 Torr = 10^2 Pa
1 atm = 760 Torr = 7.5×10^4 Pa

 $10^{-5} - 10^{-6}$ Torr



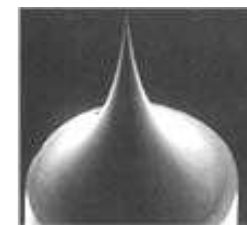
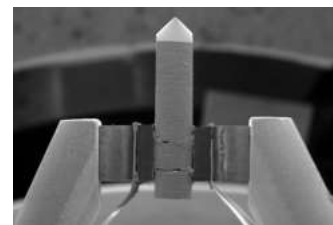
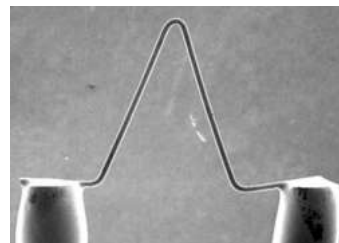
ELECTRON SOURCES

What are the 3 main kinds of electron sources?



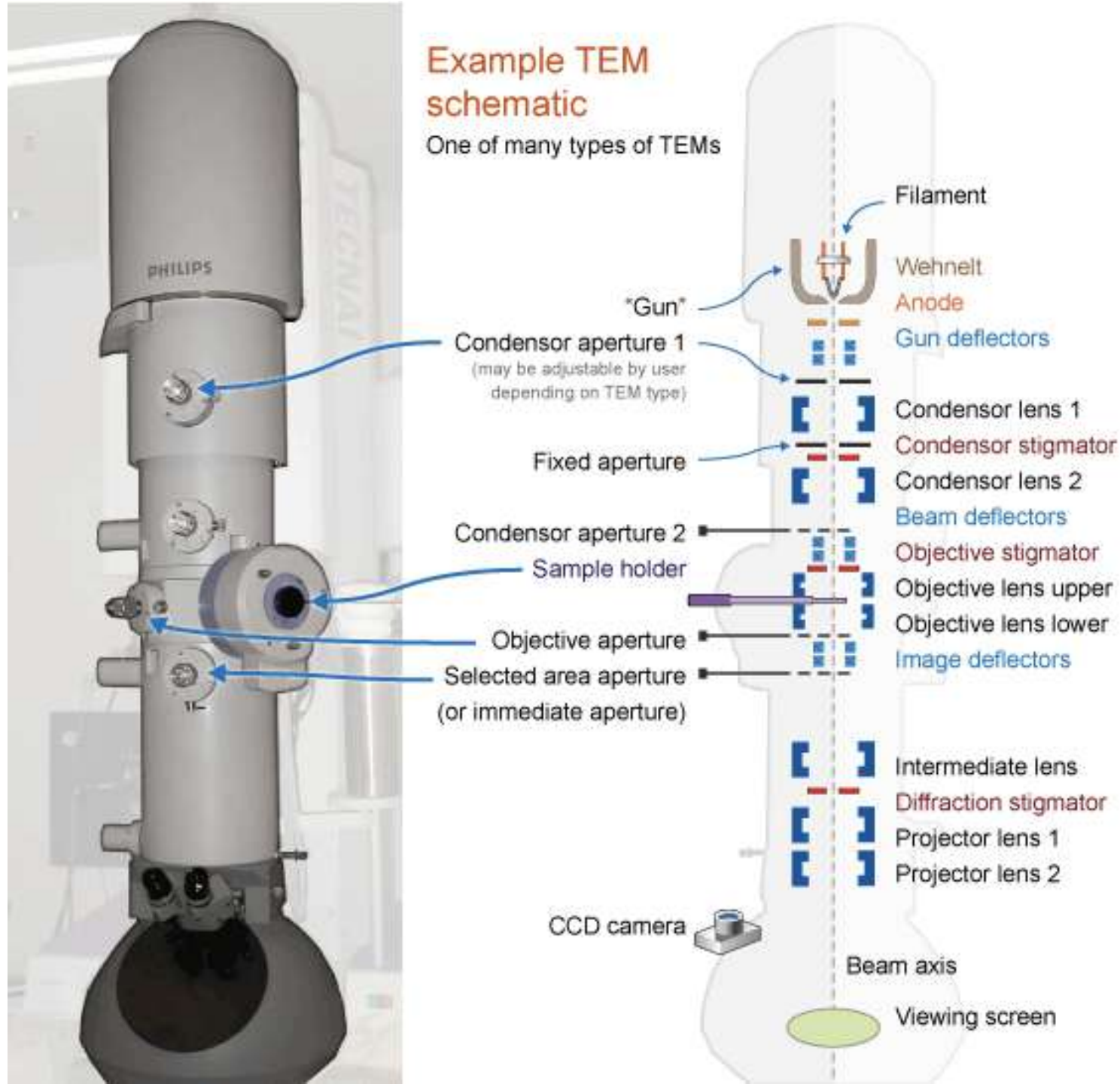
www.thermofisher.com

nanoscience.com

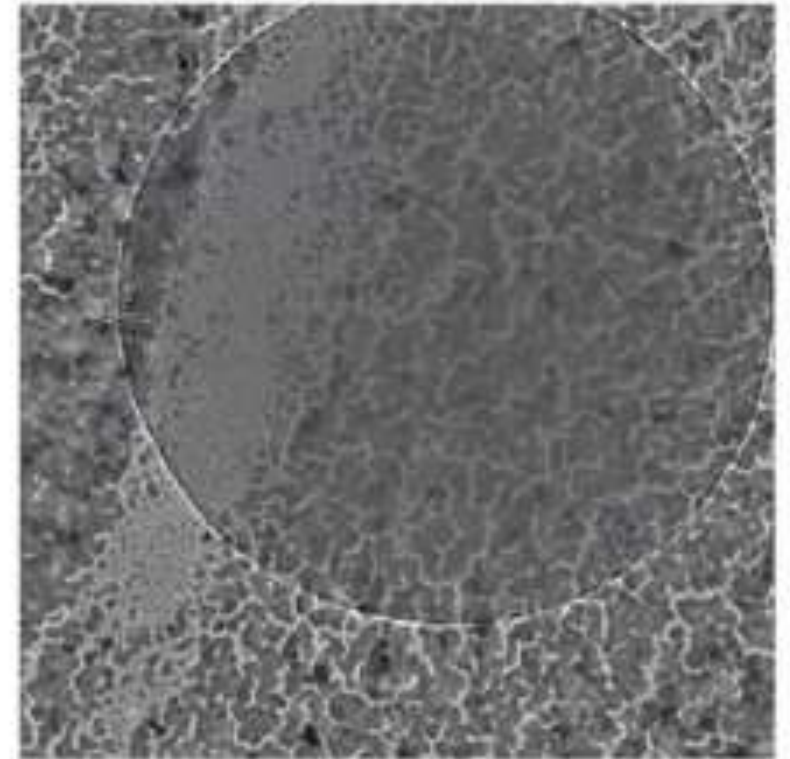
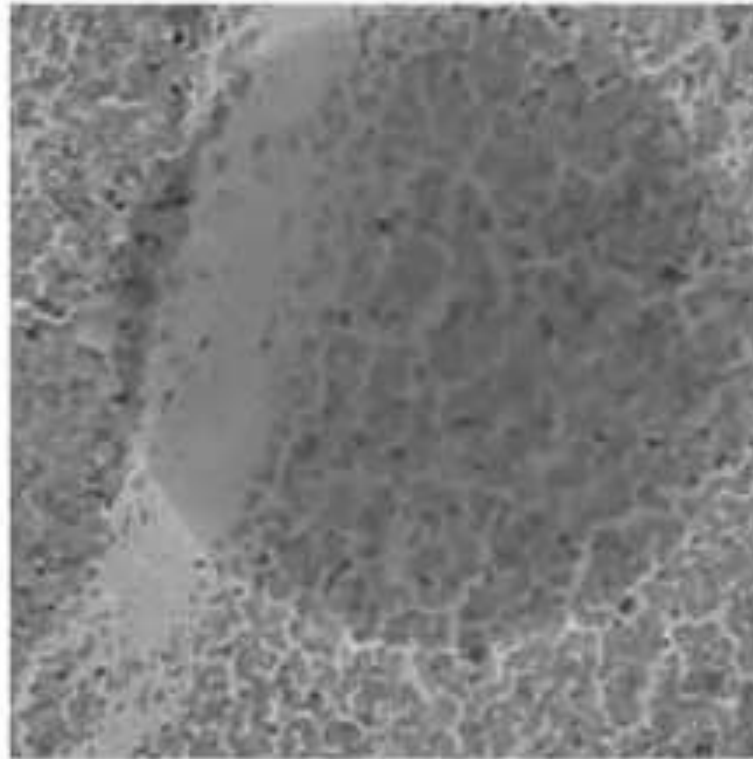
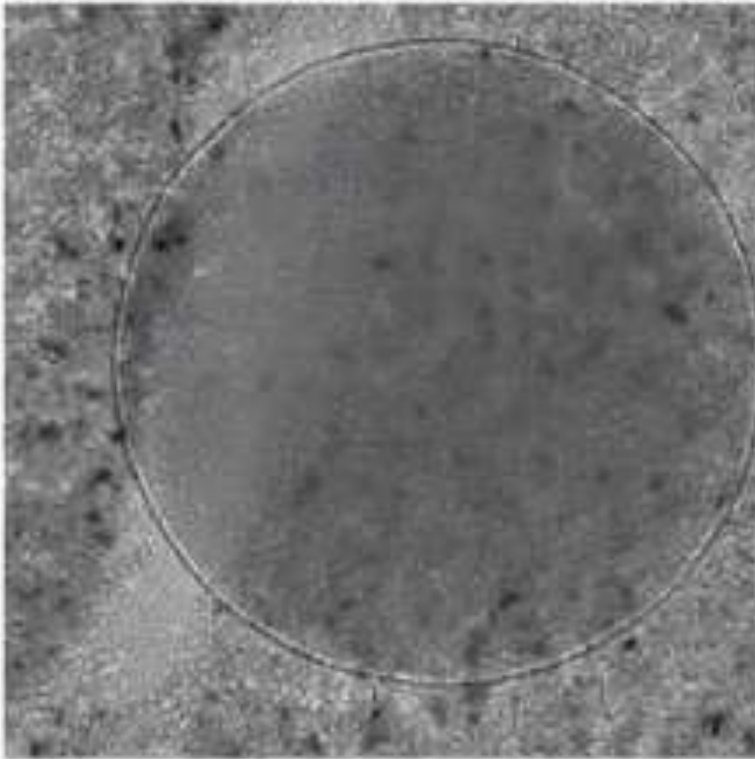
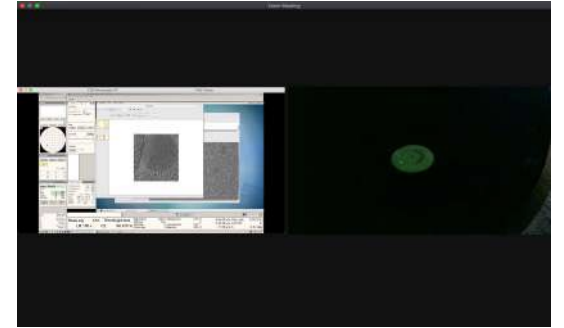


Example TEM schematic

One of many types of TEMs



FOCUS





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