

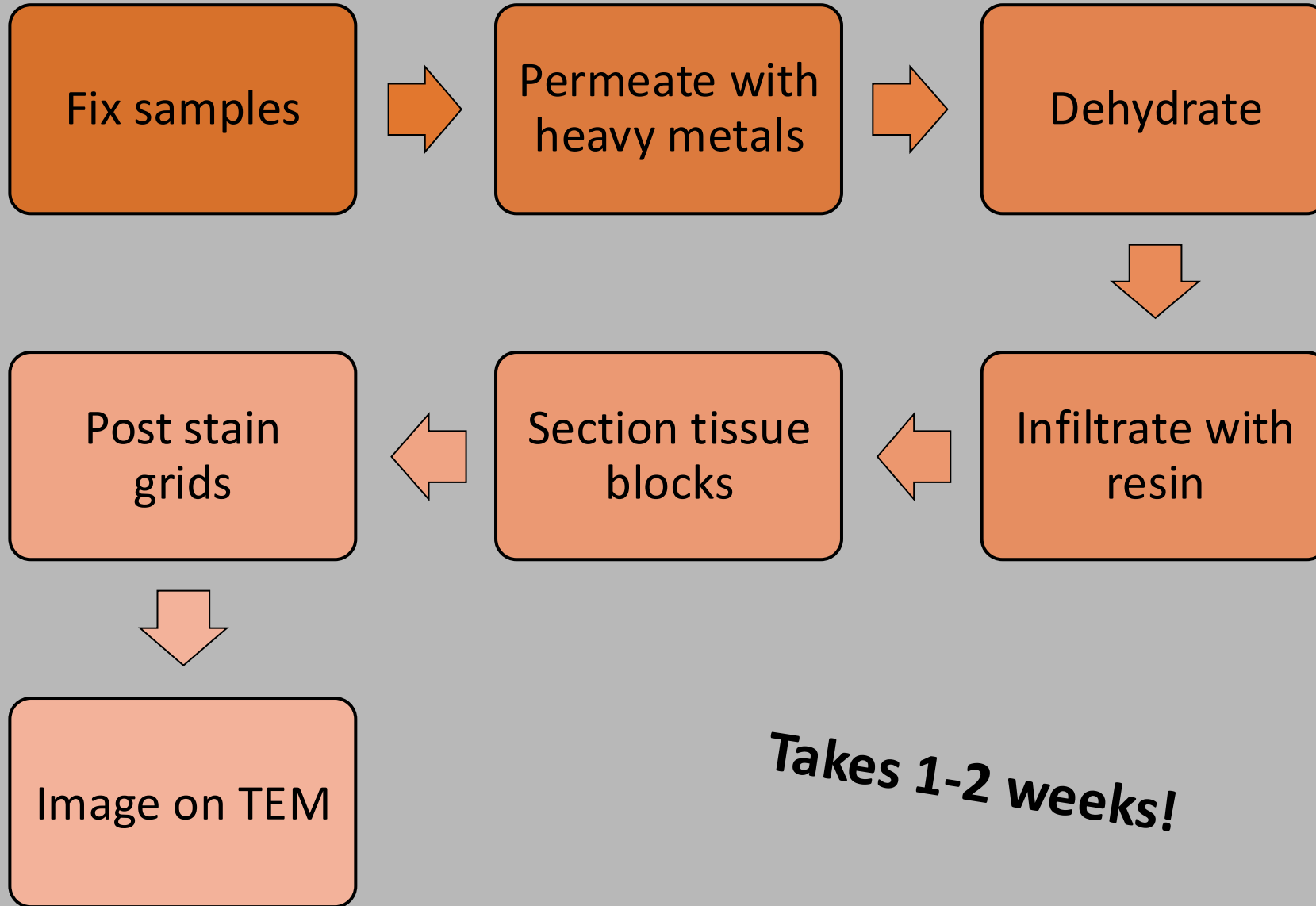
A transmission electron micrograph (TEM) showing a cross-section of biological tissue. The image displays various cellular structures, including membranes, organelles, and small vesicles, all rendered in grayscale. The background is a complex network of these structures, with some larger, more prominent cells and smaller, more numerous vesicles scattered throughout.

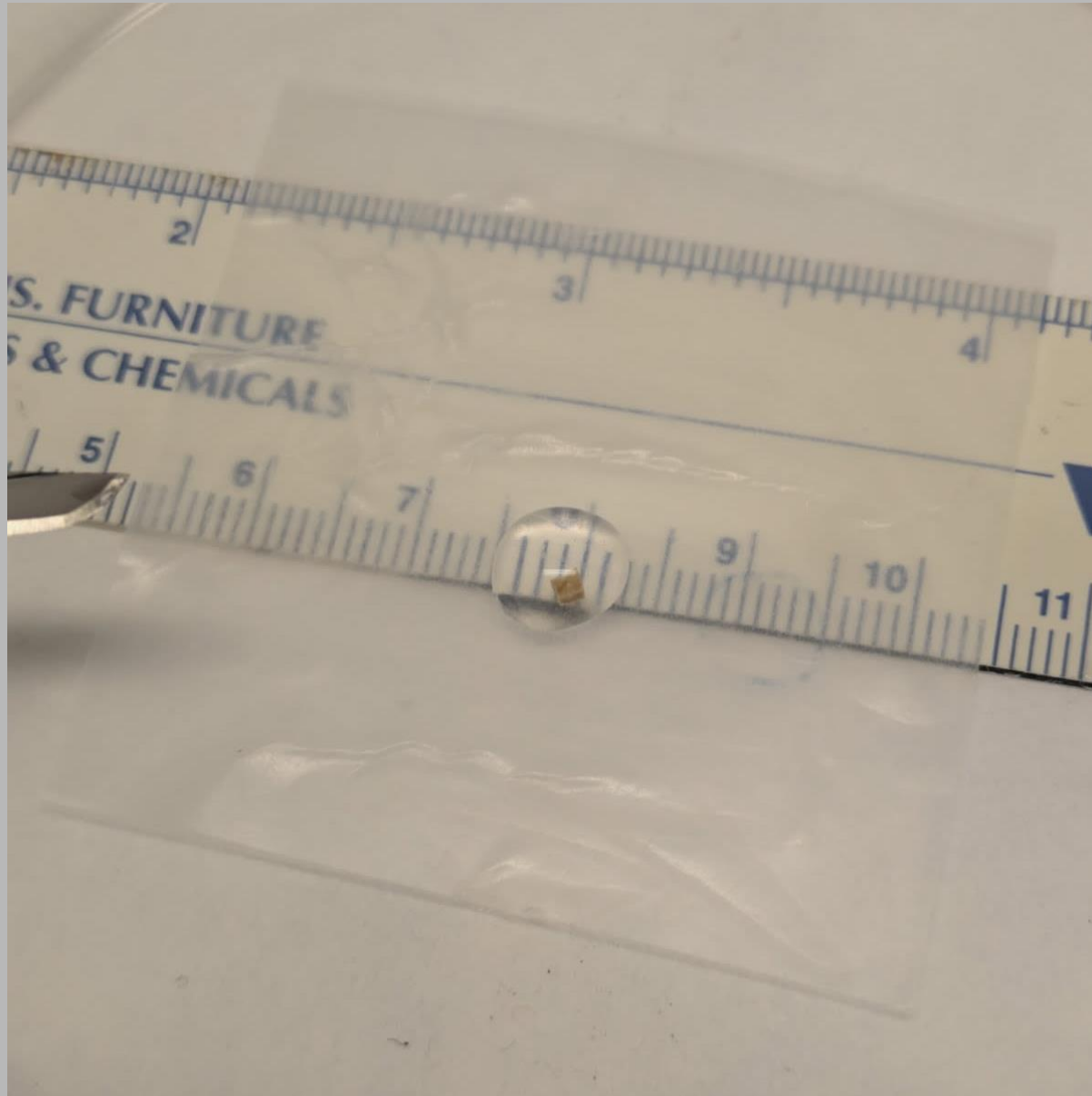
# Conventional Biological Tissue Preparation for Transmission Electron Microscopy

Rebecca Jackson

April 2026

# Work flow - overview





## More in depth- prep tissue for good fixation

Tissue is cut into pieces no larger than  $1\text{mm}^3$

Tissue is always submerged in the fixative – never let it dry out!

Initial tissue handling and fixation very important for good ultrastructure preservation

# Safety

Chemical exchanges are done under the hood – very toxic chemicals!

Gloves, lab coat, and goggles worn

Separate hood for radioactive heavy metals



# Embedding tissue in resin

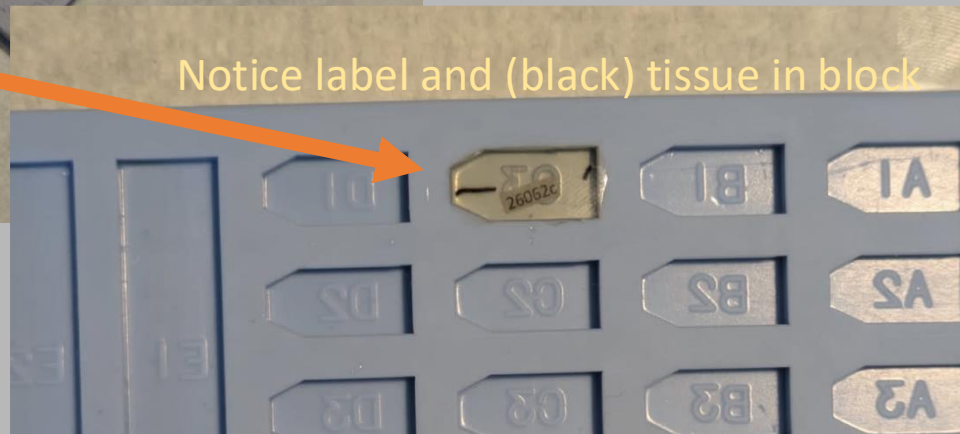
Choose appropriate molds for tissue orientation

Add label – printed or pencil written

60°C oven 24+ hrs – make sure resin polymerizes

Various embedding molds

Notice label and (black) tissue in block



# Sectioning

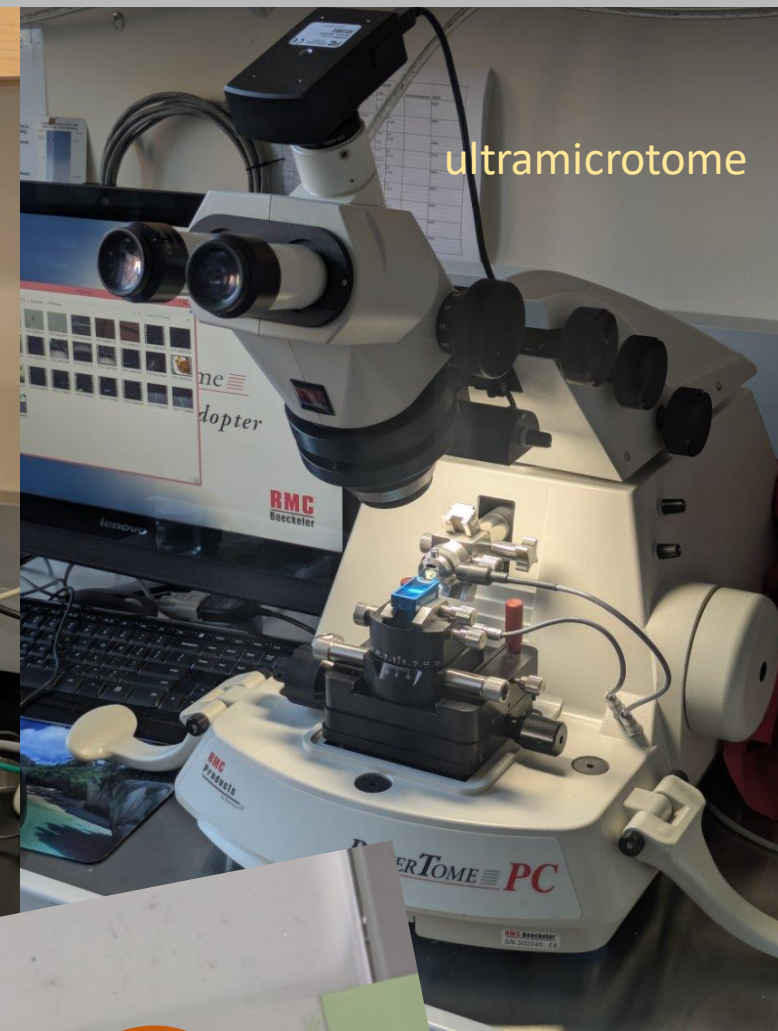
Ultramicrotome used to section **semi-thin** sections first to verify region of interest and tissue quality - ~200-300nm thick

Stained with toluidine blue

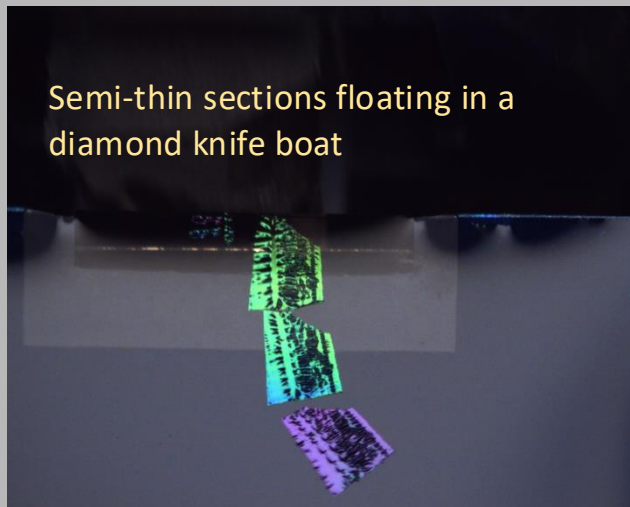
Use of light microscope to observe tissue sections



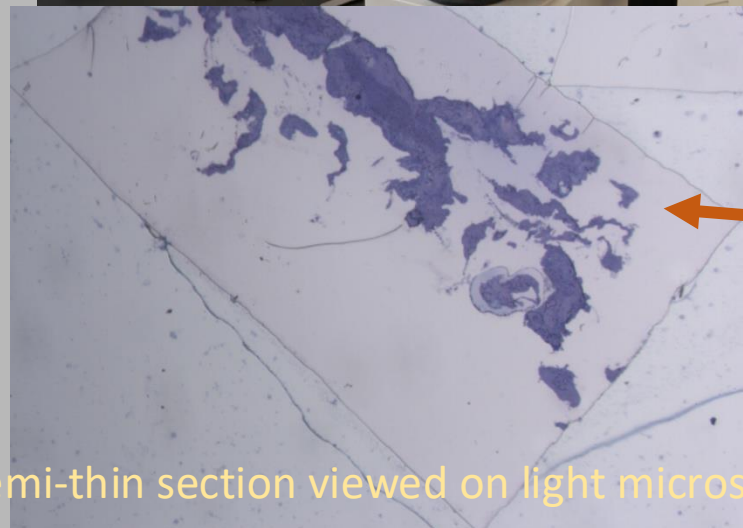
Light microscope



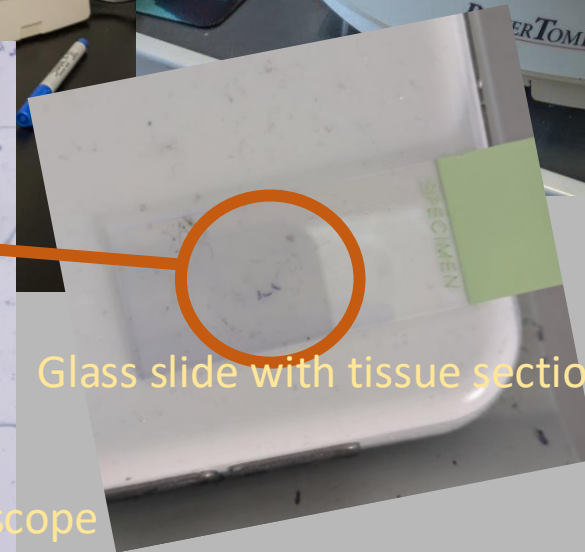
ultramicrotome



Semi-thin sections floating in a diamond knife boat



Semi-thin section viewed on light microscope

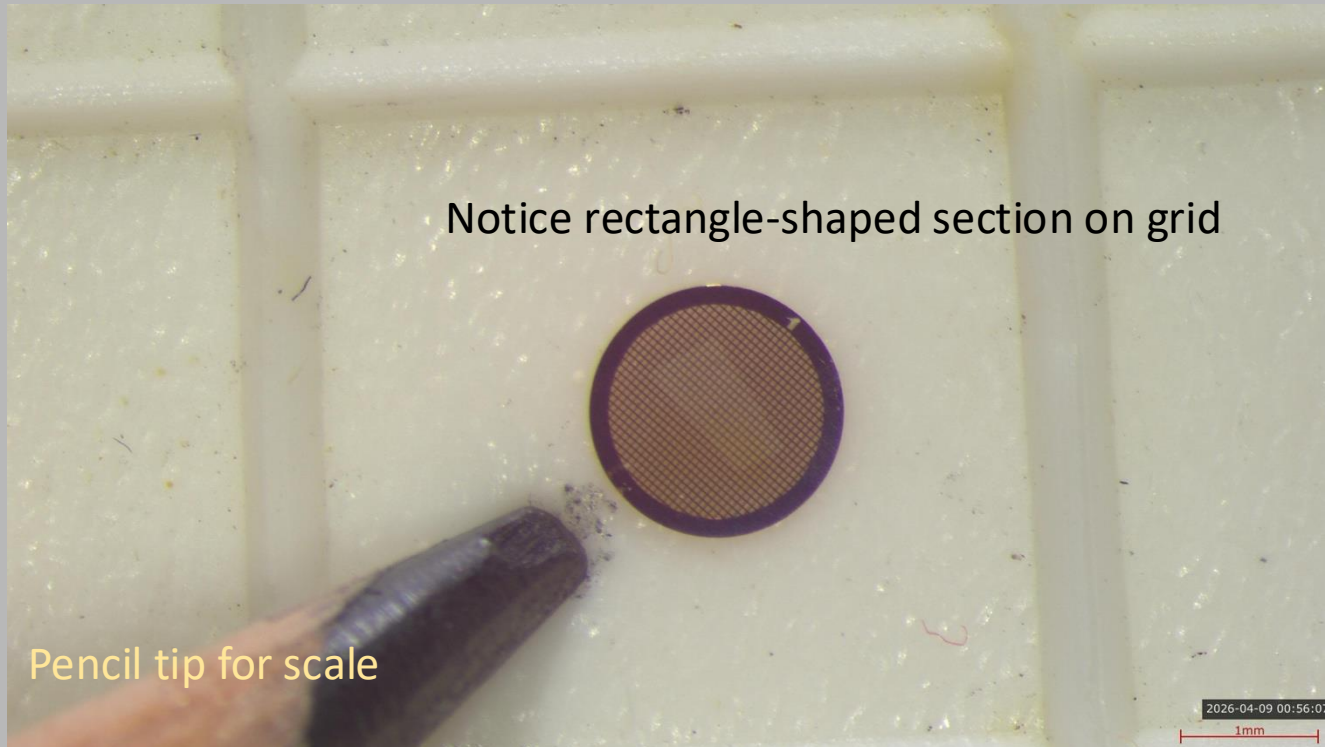


Glass slide with tissue sections

## Sectioning cont'd

Ultramicrotome used to section **thin** sections  
~90nm thick

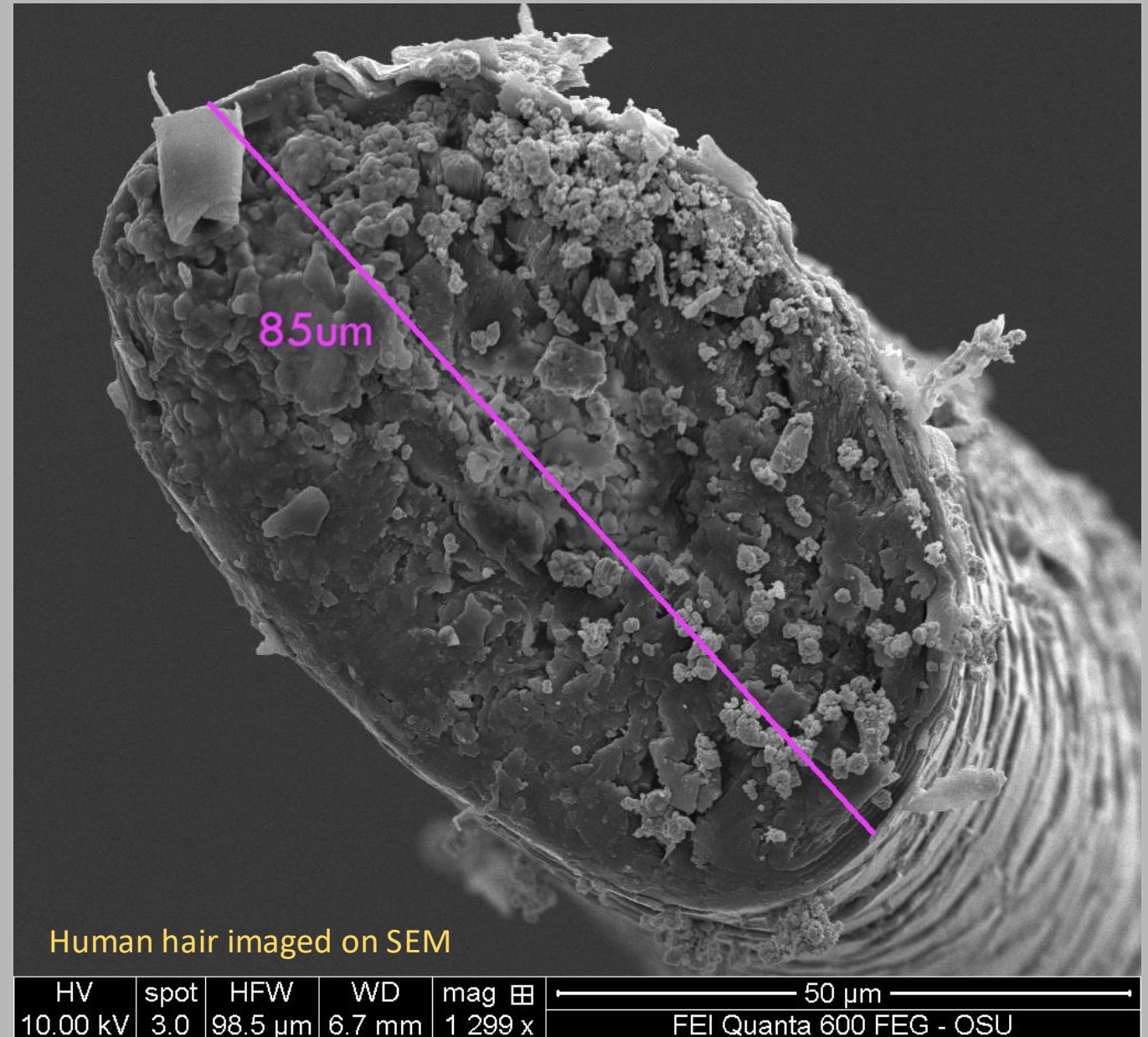
Collected onto grids and post stained

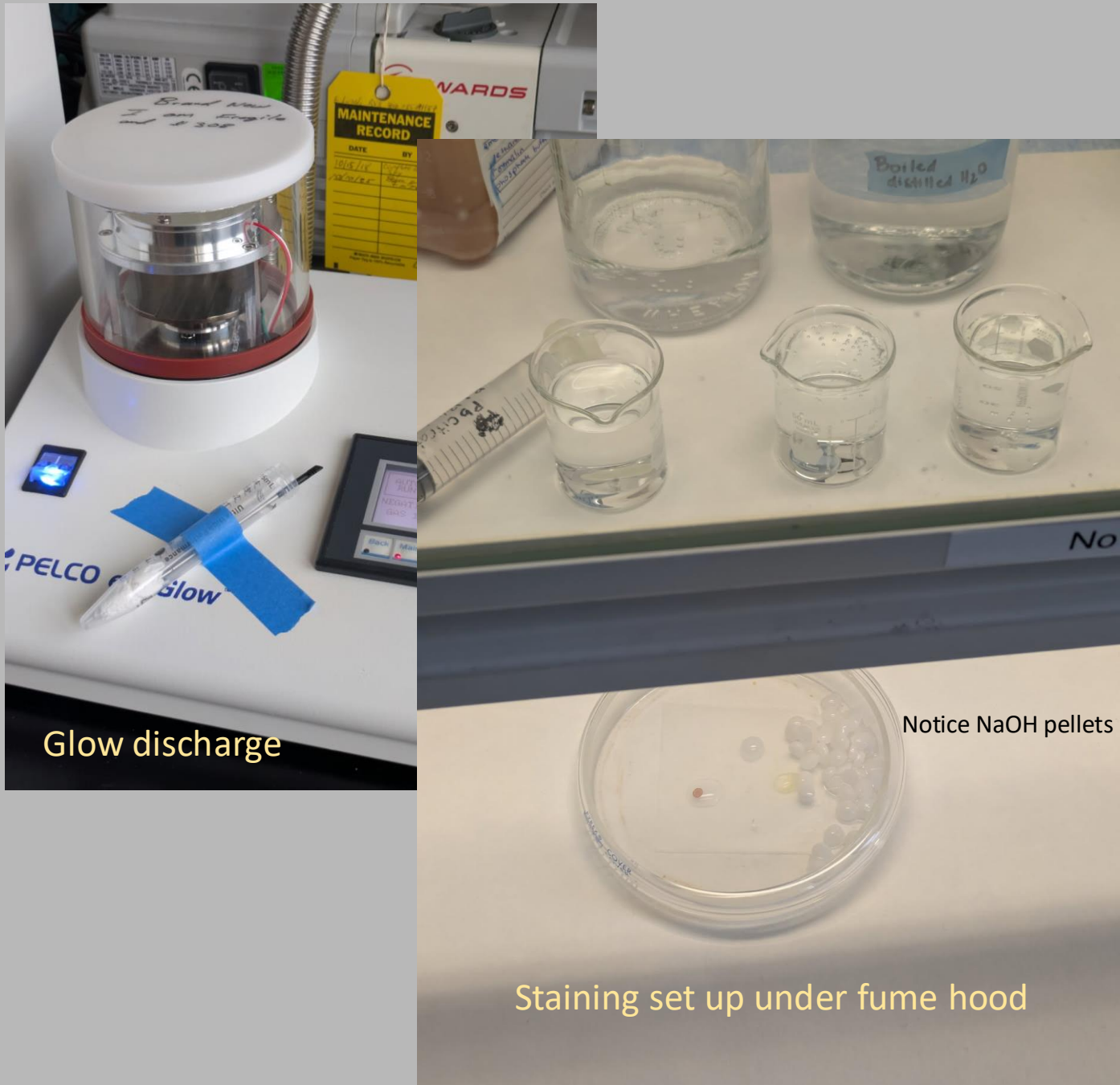


# Perspective

This hair is 85um, or 85,000nm thick

Over 1,000 sections can be cut through the thickness of this hair!





Glow discharge

Notice NaOH pellets

Staining set up under fume hood

## Post staining grids

Grids are glow discharged then post stained with uranyl acetate and lead citrate

Add even more contrast to tissue so it can be seen easier in the TEM

UA done in radioactive hood

Pb requires sodium hydroxide pellets to prevent precipitation

Finally time to image  
on the TEM!

TEM set to 80keV for better contrast

Don't forget that objective aperture for even  
more contrast!

