Conventional Biological Tissue Preparation for Transmission Electron Microscopy

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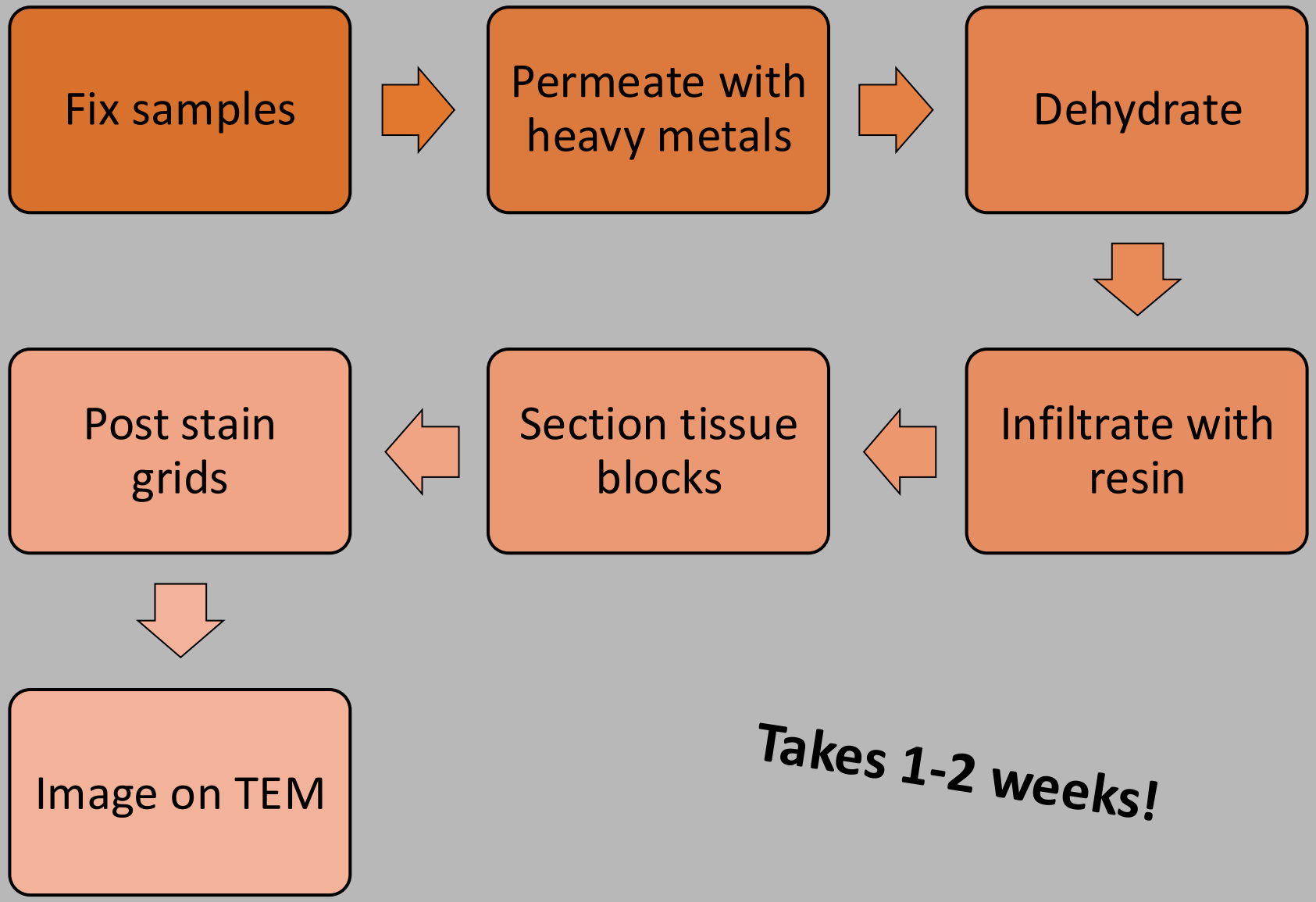
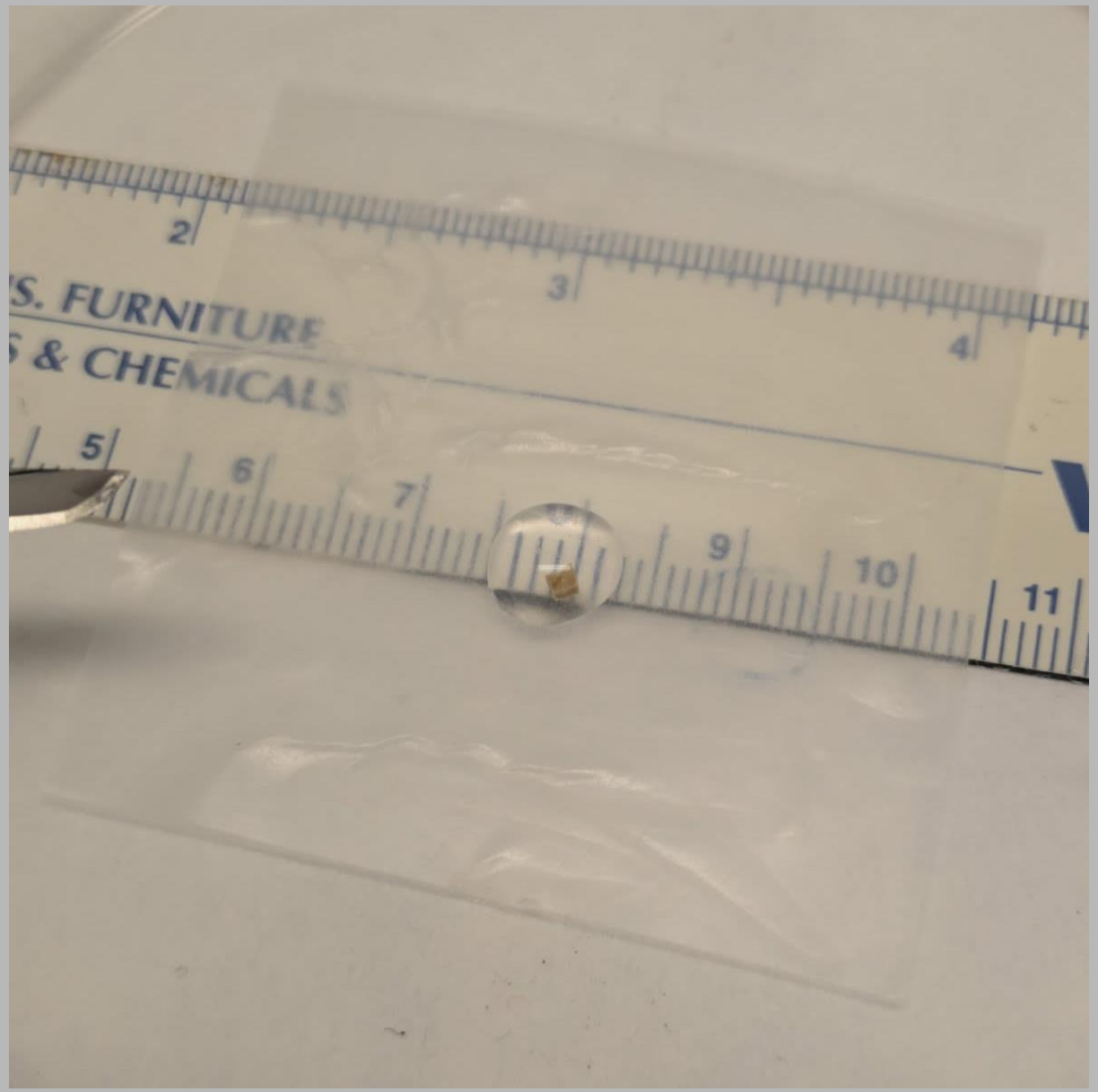
Work flow - overview



Takes 1-2 weeks!



More in depth- prep tissue for good fixation

Tissue is cut into pieces no larger than $1mm^3$

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

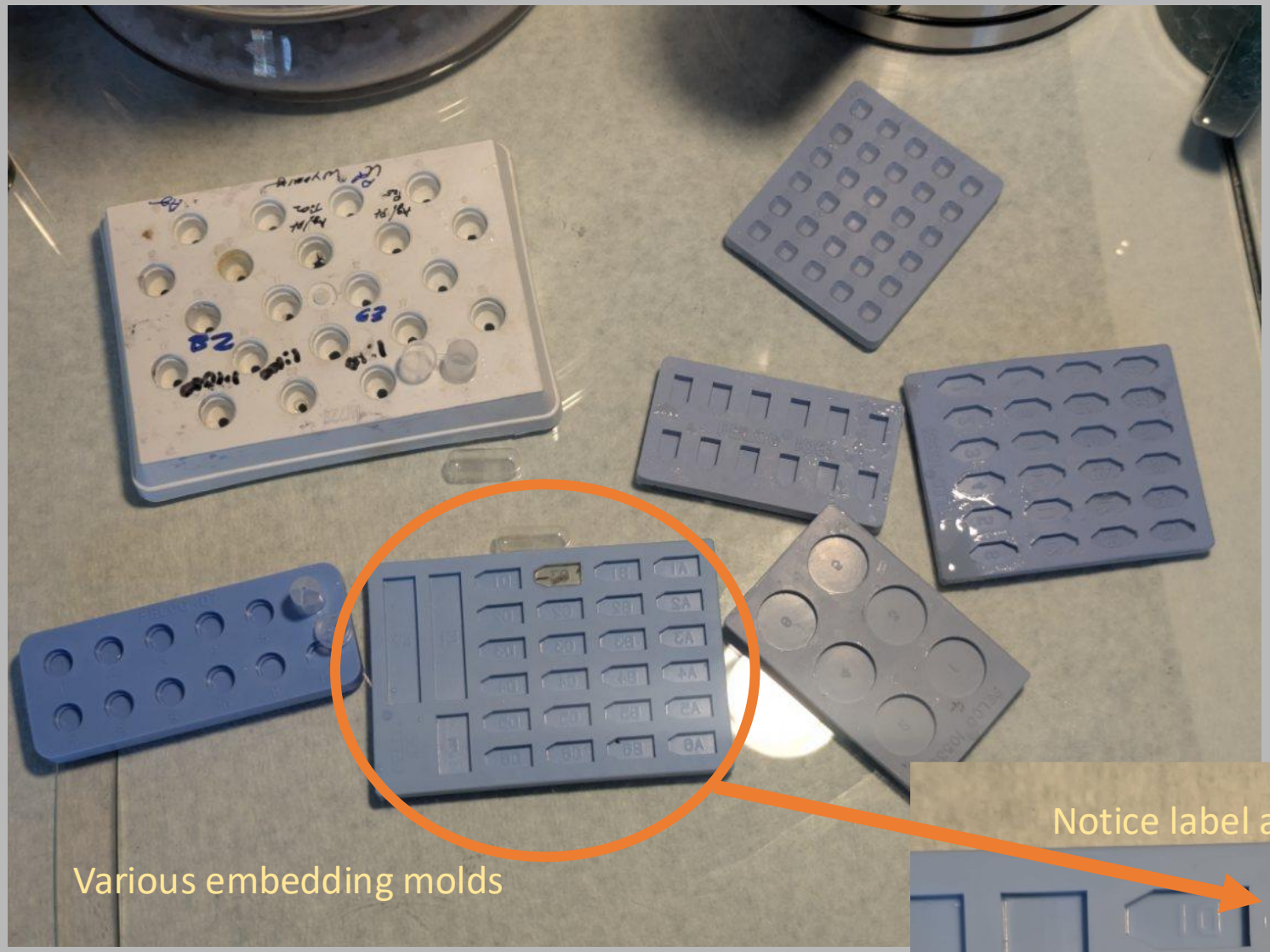
Separate hood for radioactive heavy metals

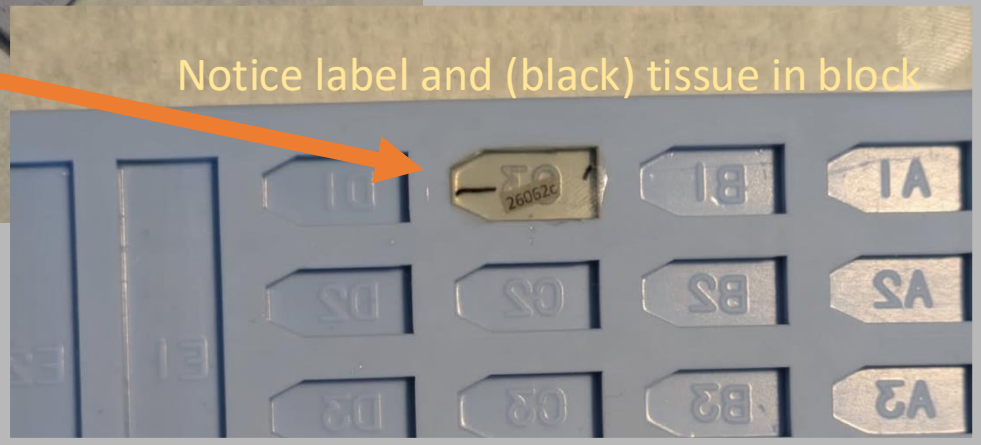
Embedding tissue in resin

Choose appropriate molds for tissue orientation

Add label – printed or pencil written

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



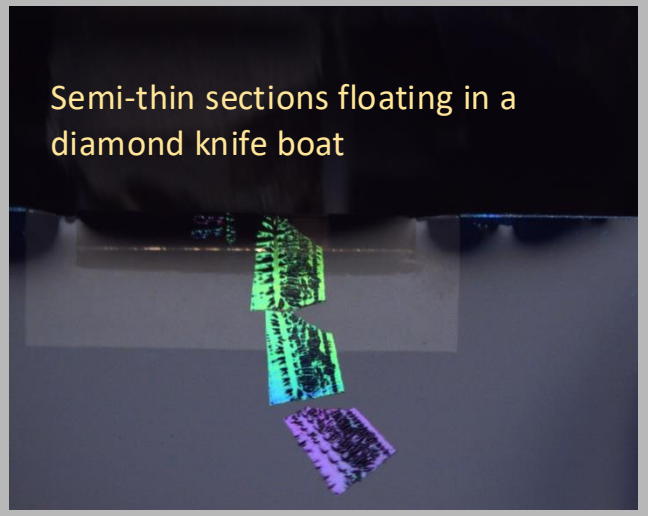


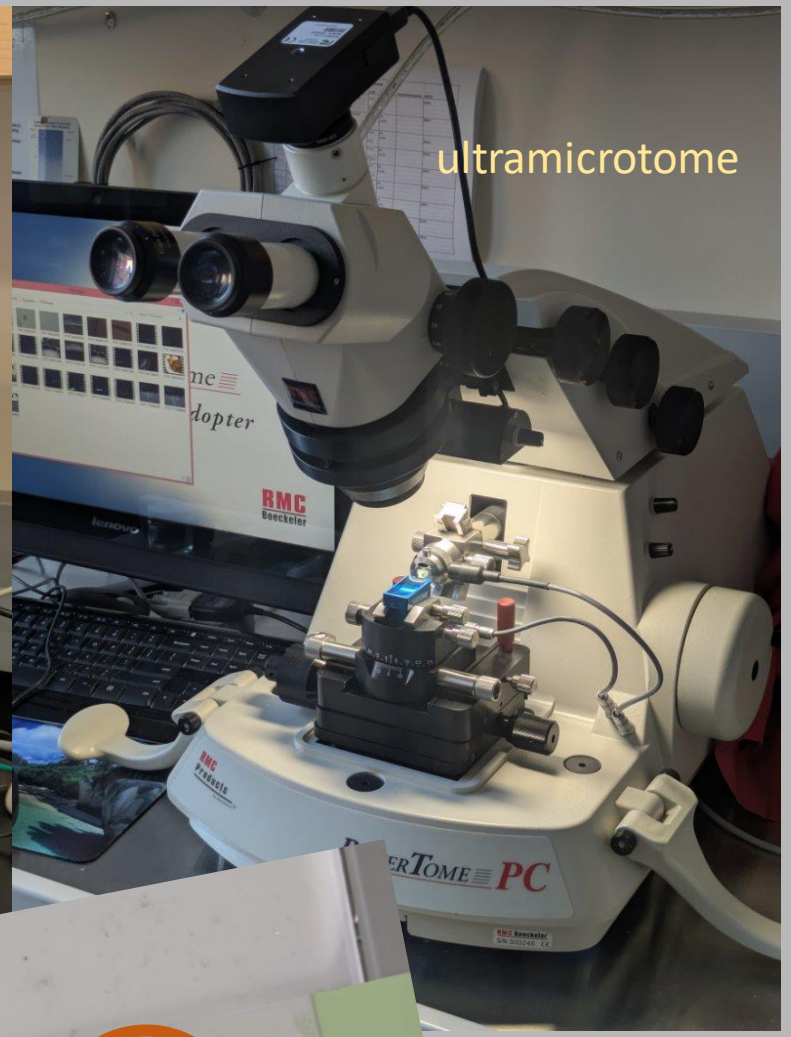
Sectioning

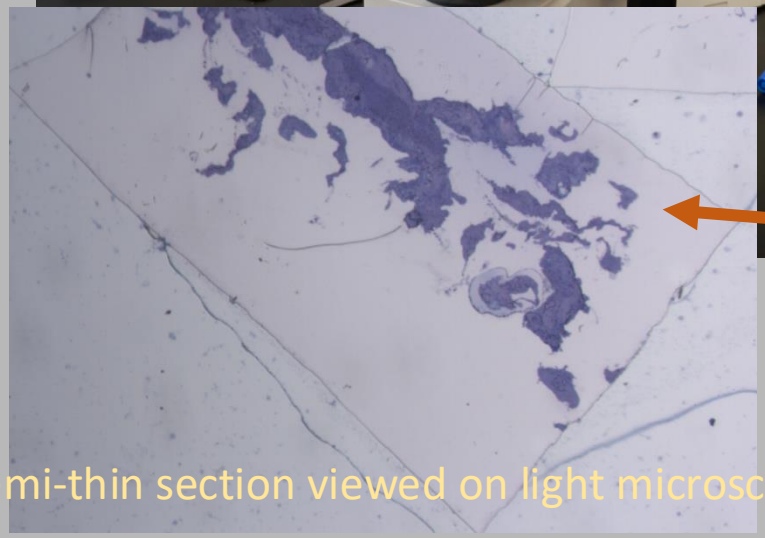
Ultramicrotome used to section **semi-thin** sections first to verify region of interest and tissue quality - ~200-300nm thick

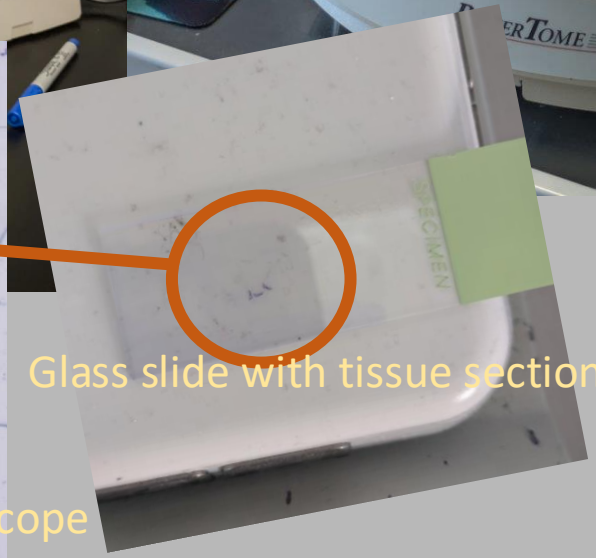
Stained with toluidine blue

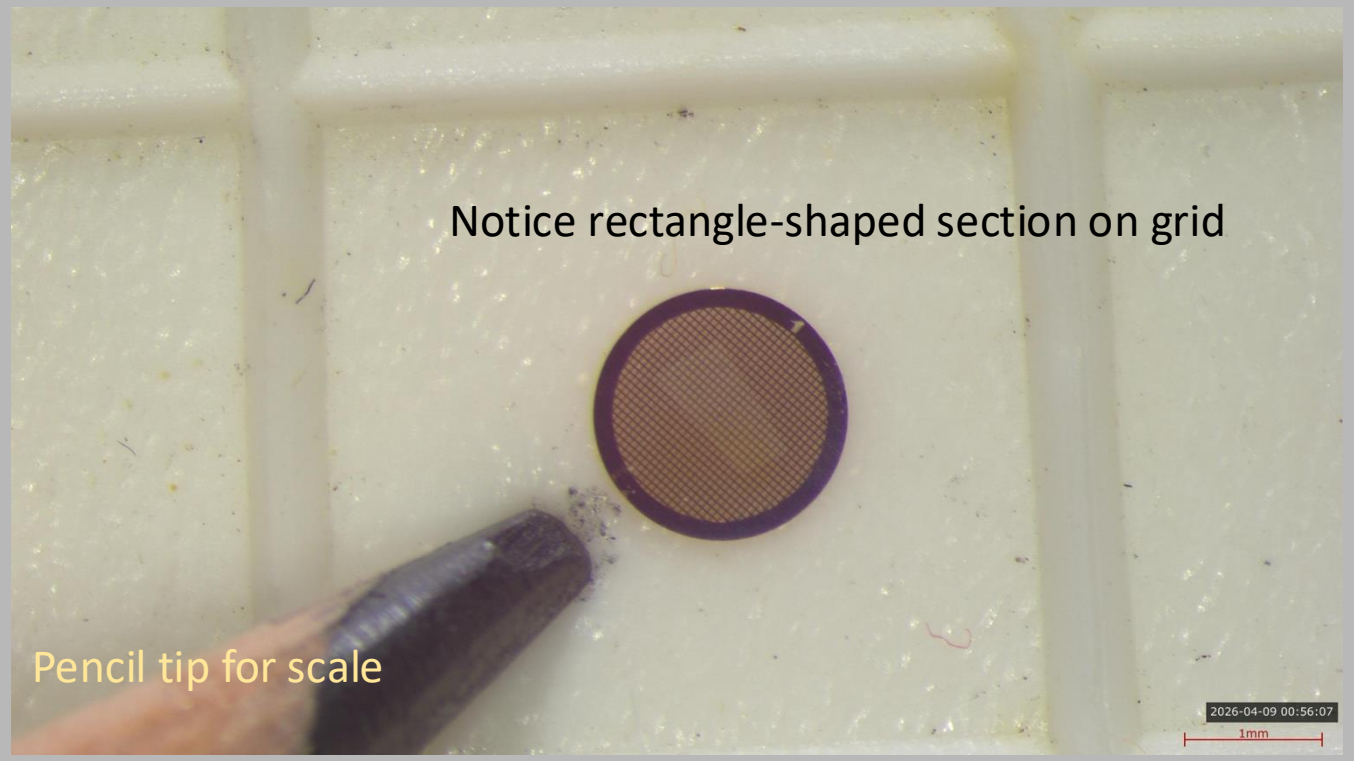
Use of light microscope to observe tissue sections











Sectioning cont’d

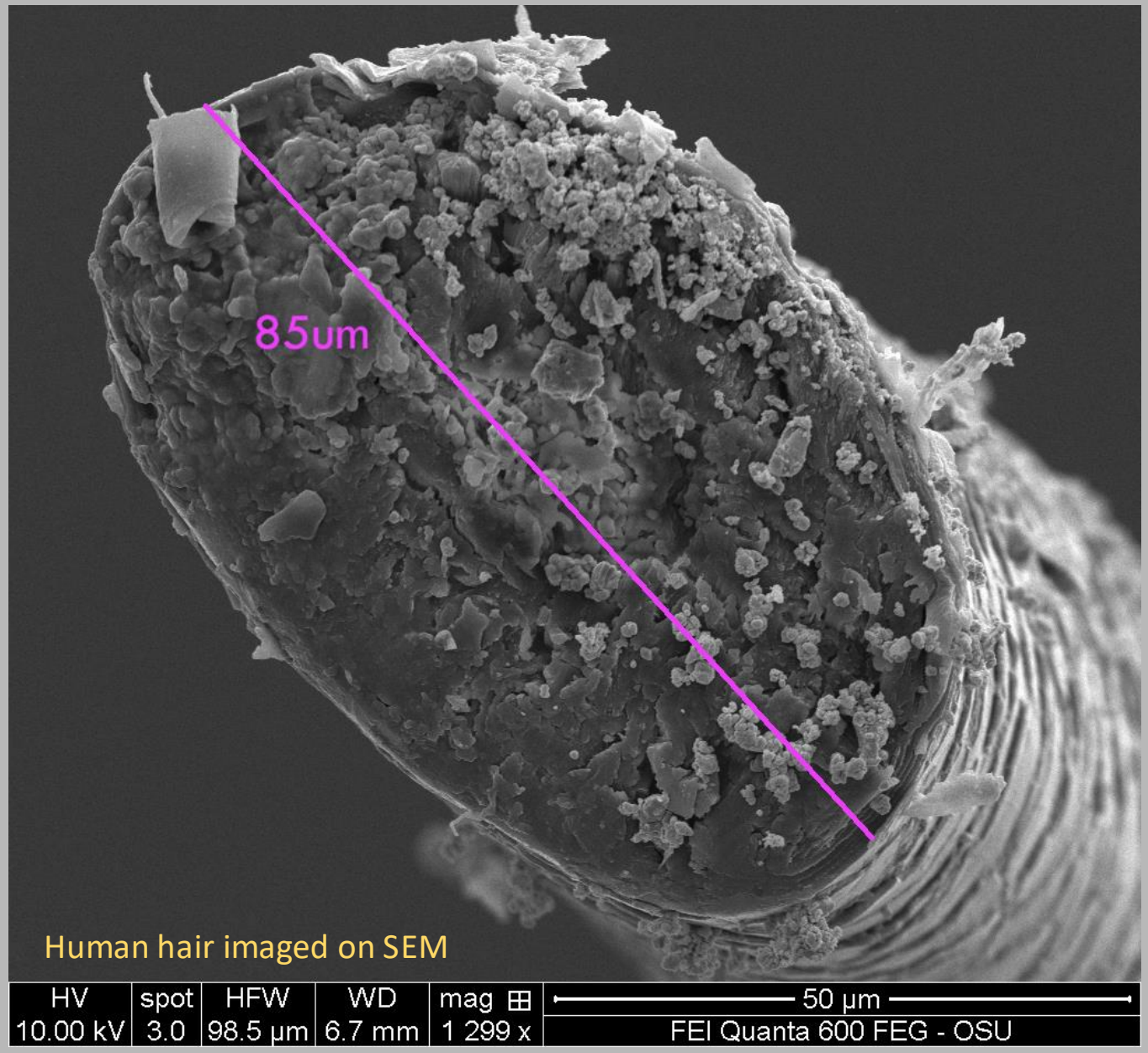
Ultramicrotome used to section **thin** sections ~90nm thick

Collected onto grids and post stained

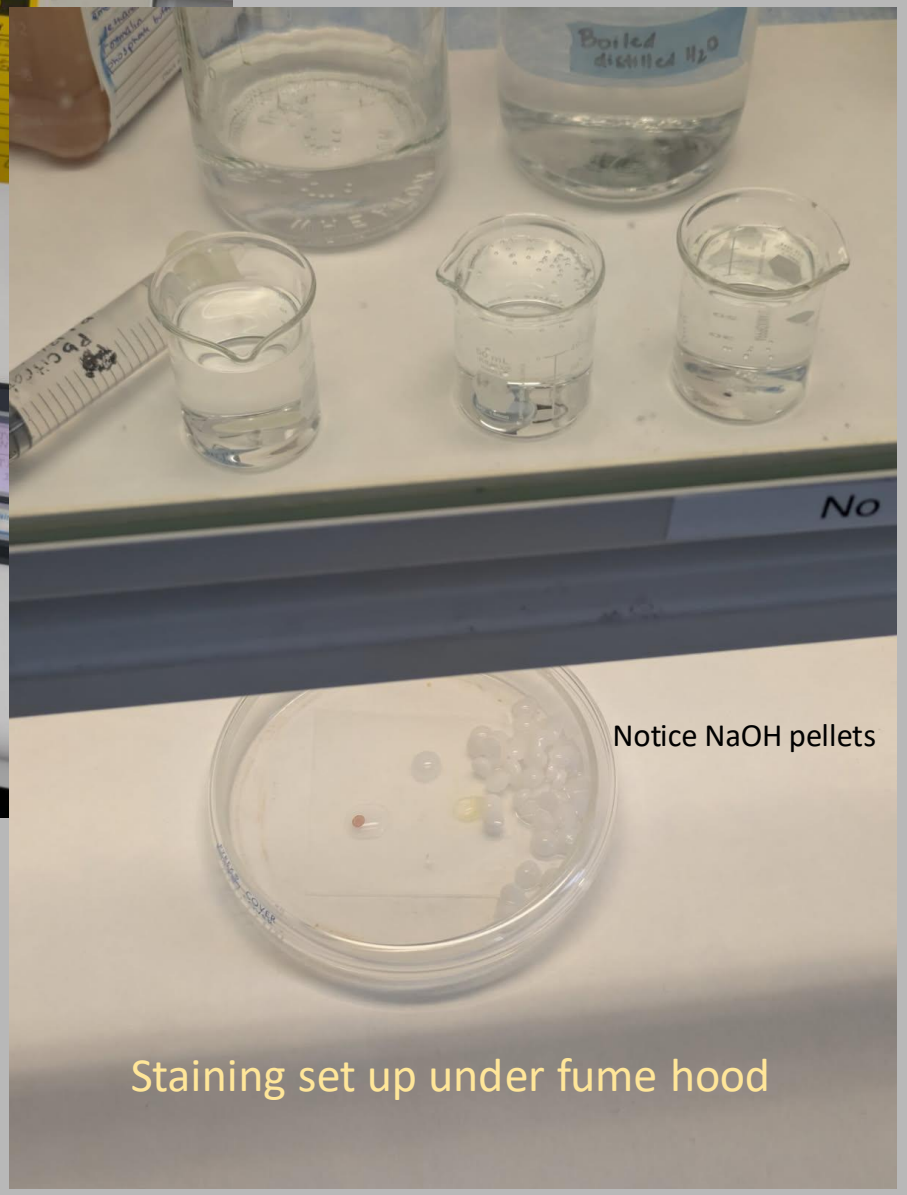
Perspective

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

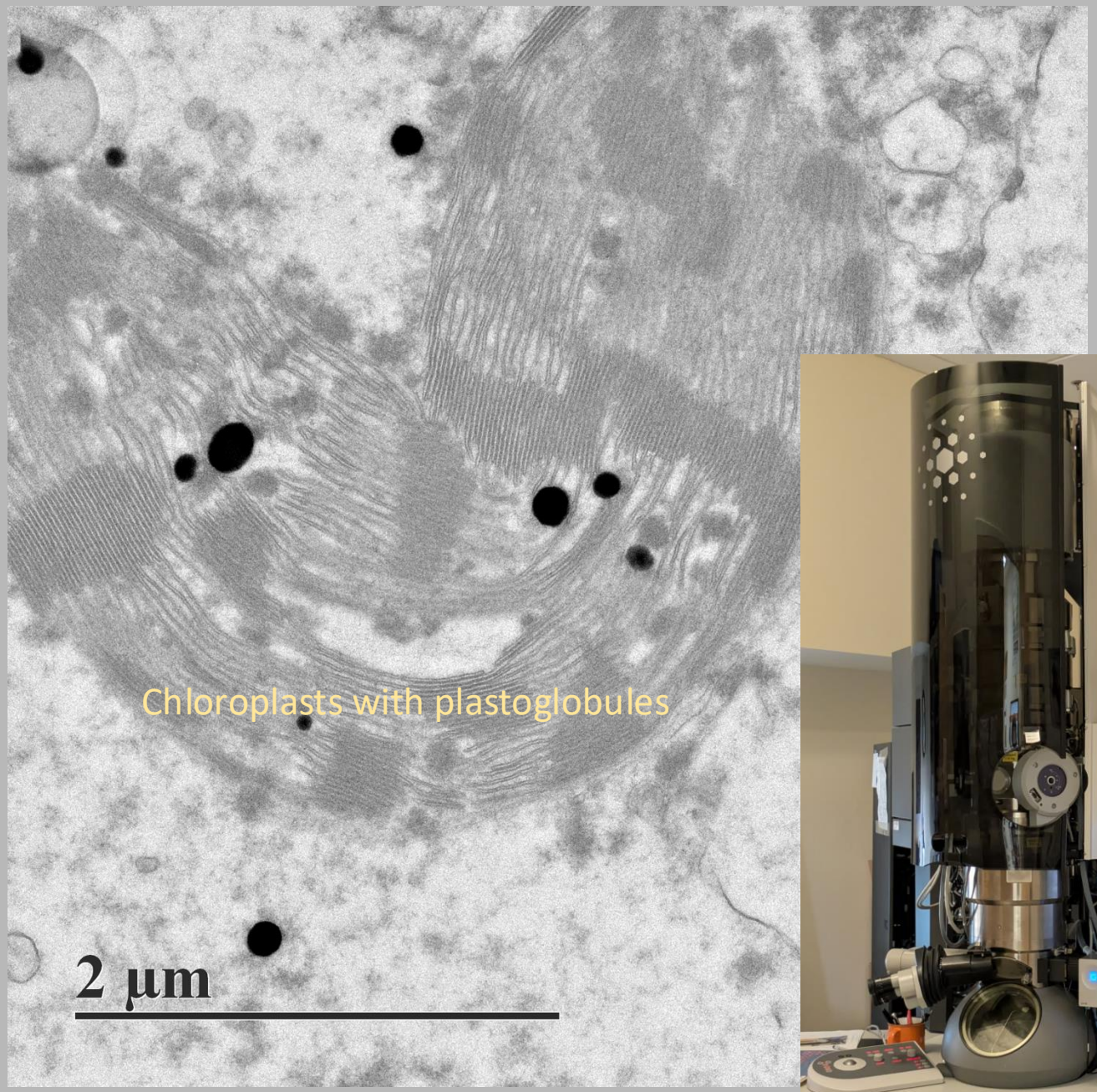
UA done in radioactive hood

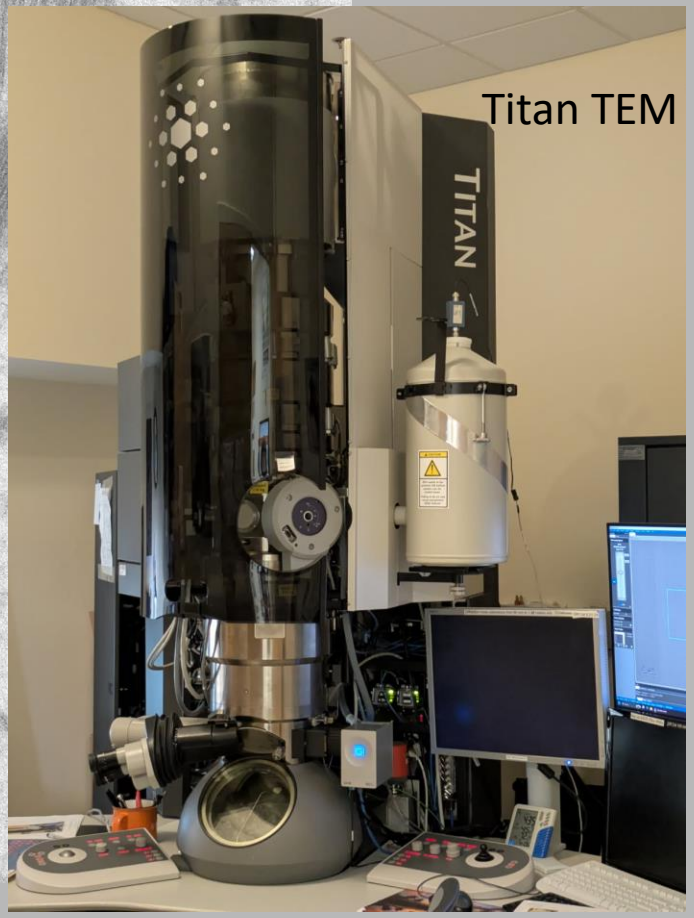
Pb requires sodium hydroxide pellets to prevent precipitation

Finally time to image on the TEM!

TEM set to 80keV for better contrast

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





Titan TEM