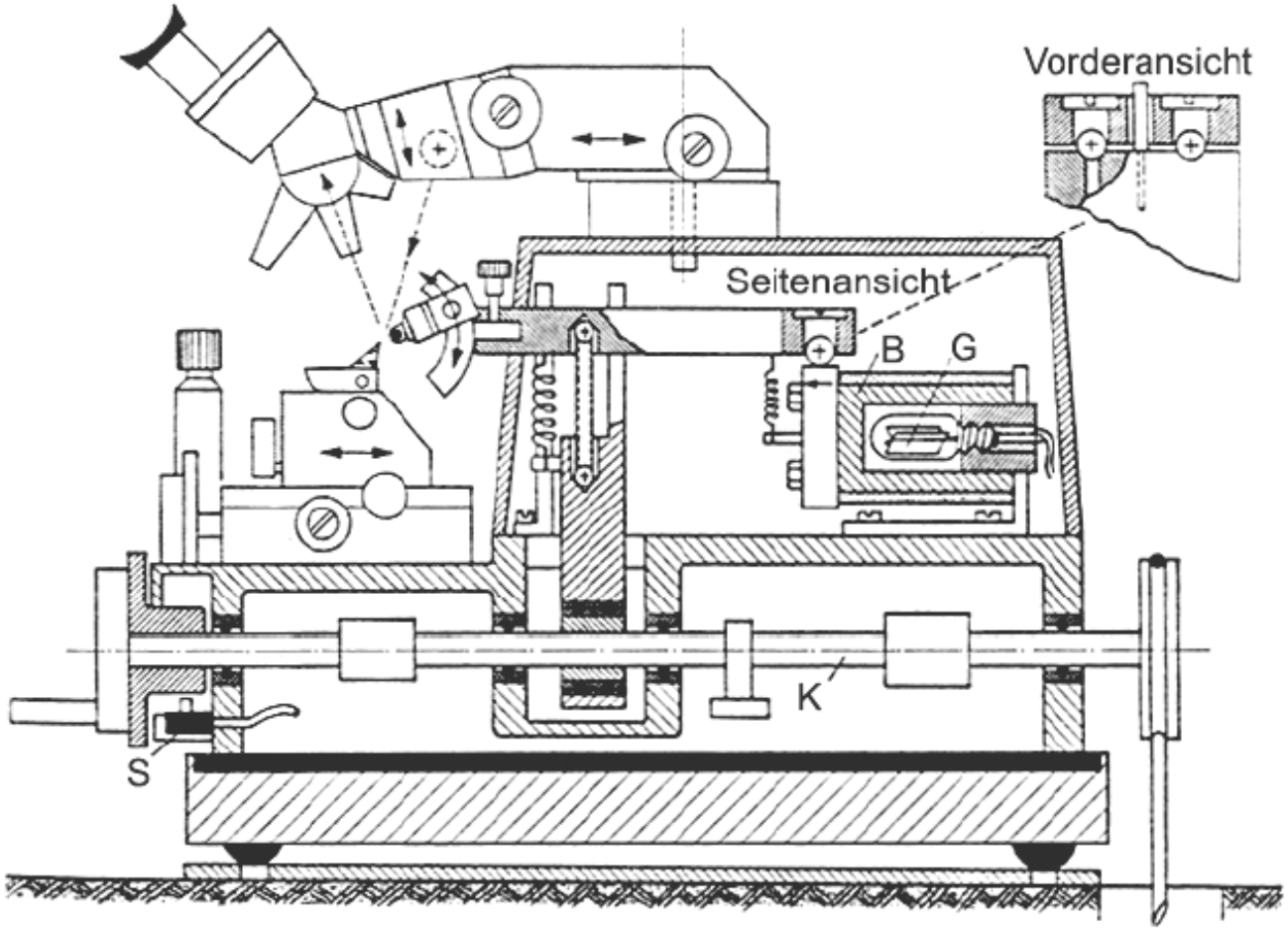
# Methods in Transmission Electron Microscopy and their application

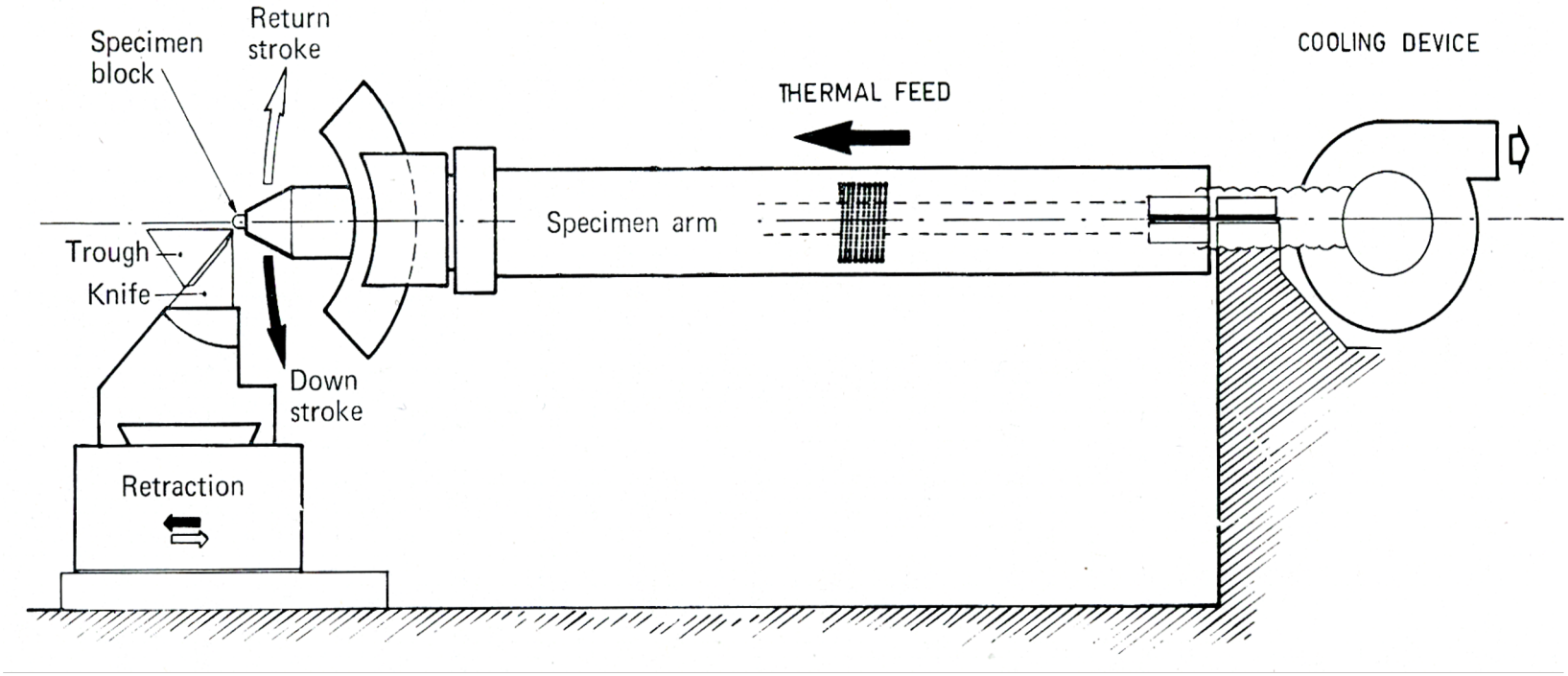
**Day 5**

Composition of a microtome

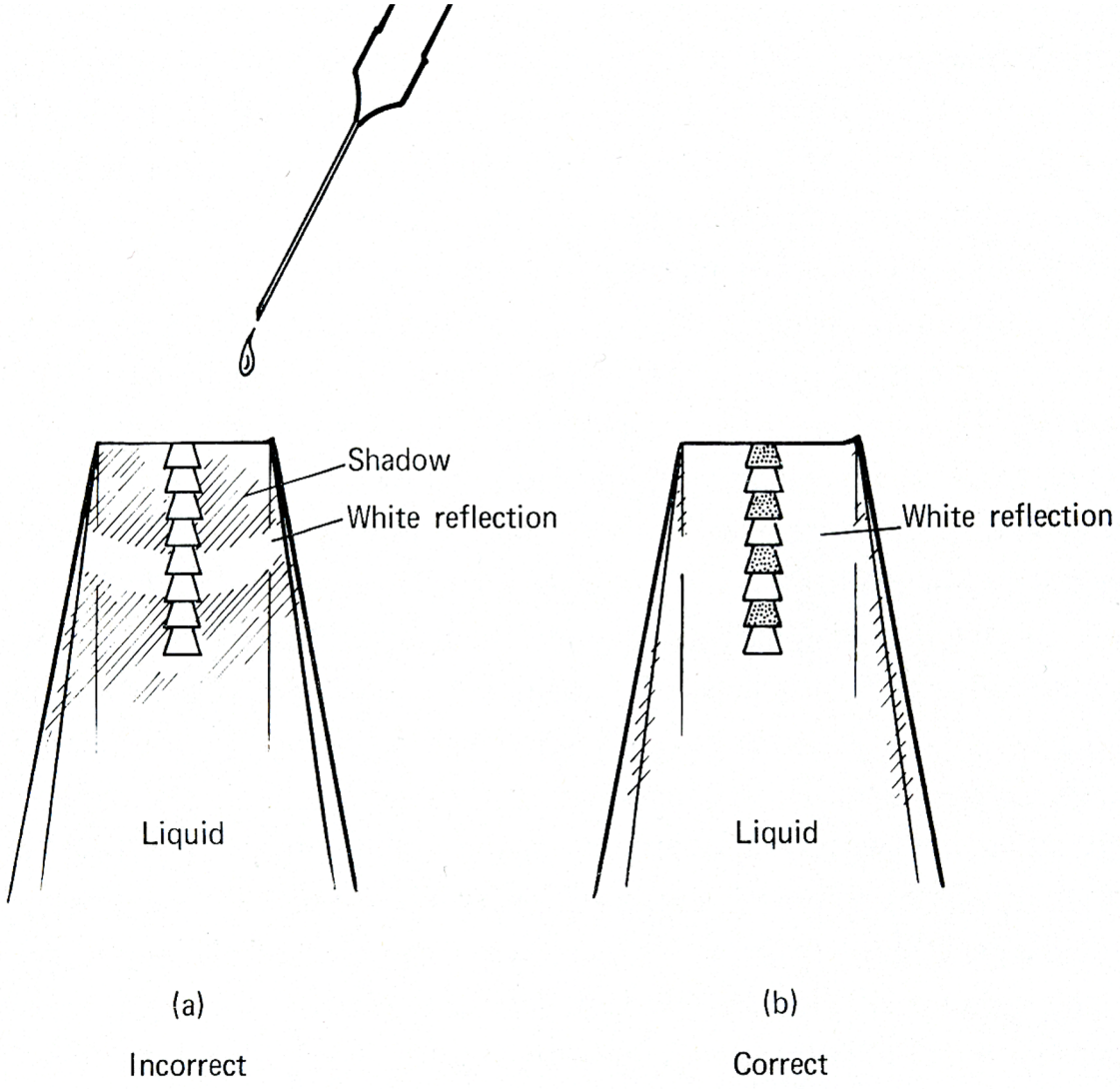



Schematische Darstellung eines Ultramikrotoms /Reimer67/

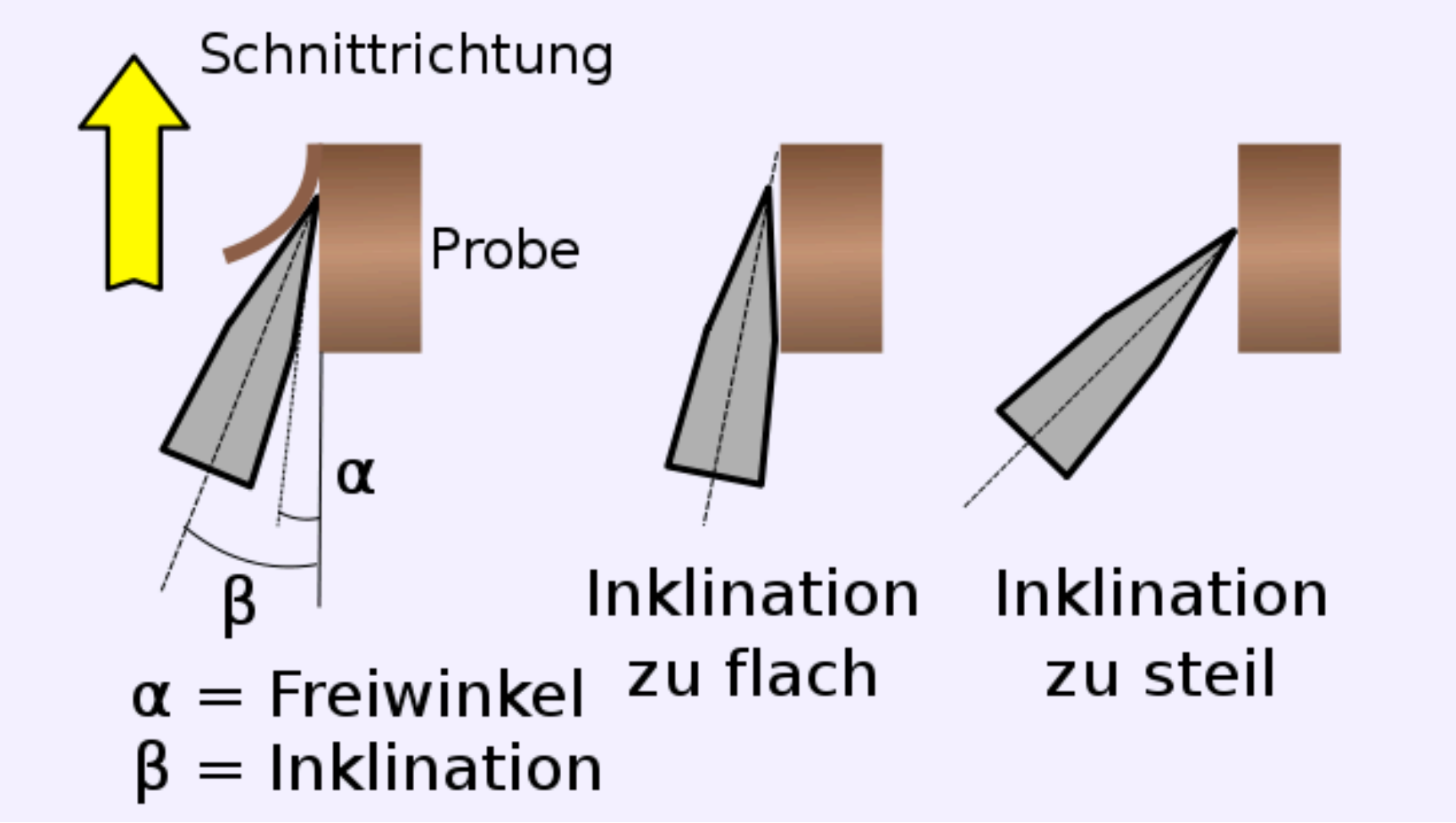
- periodic up and down movement of specimen arm
- thermal or mechanid feed



The water level has to be adjusted correctly.

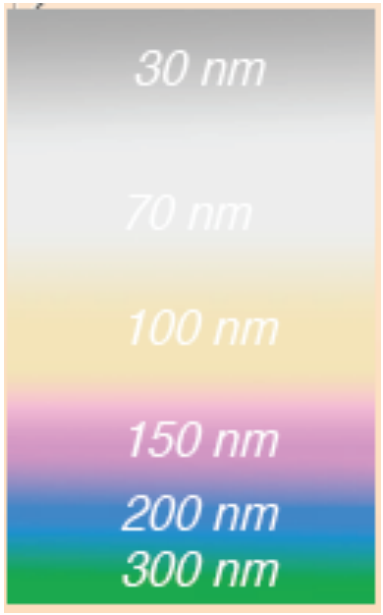


To perform sectioning the right way, the inclination (angle) is very important.

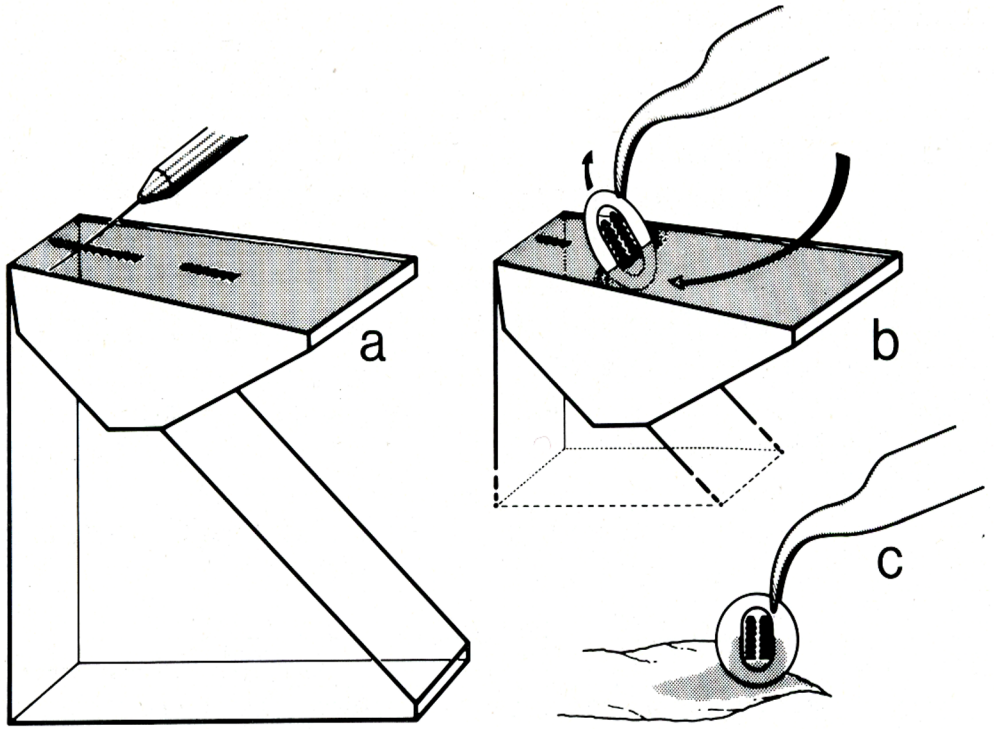


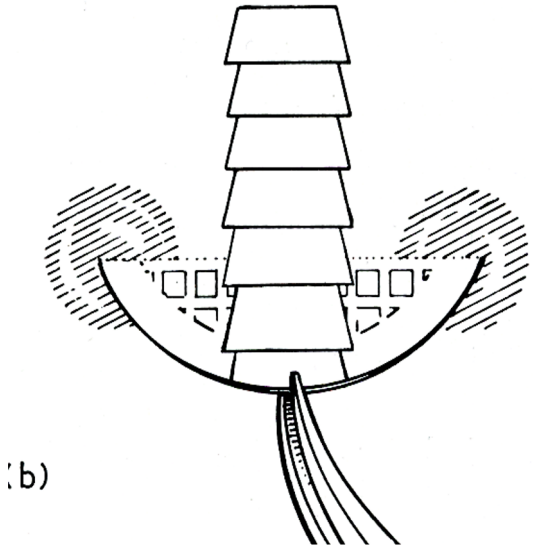
The section thickness can be determined by estimating the interference colour

| Interference colour | Section thickness in nm |
|---|---|
| grey | < 60 |
| silver | 60 - 90 |
| gold | 90 - 150 |
| purple | 150 - 190 |
| blue | 190 - 240 |



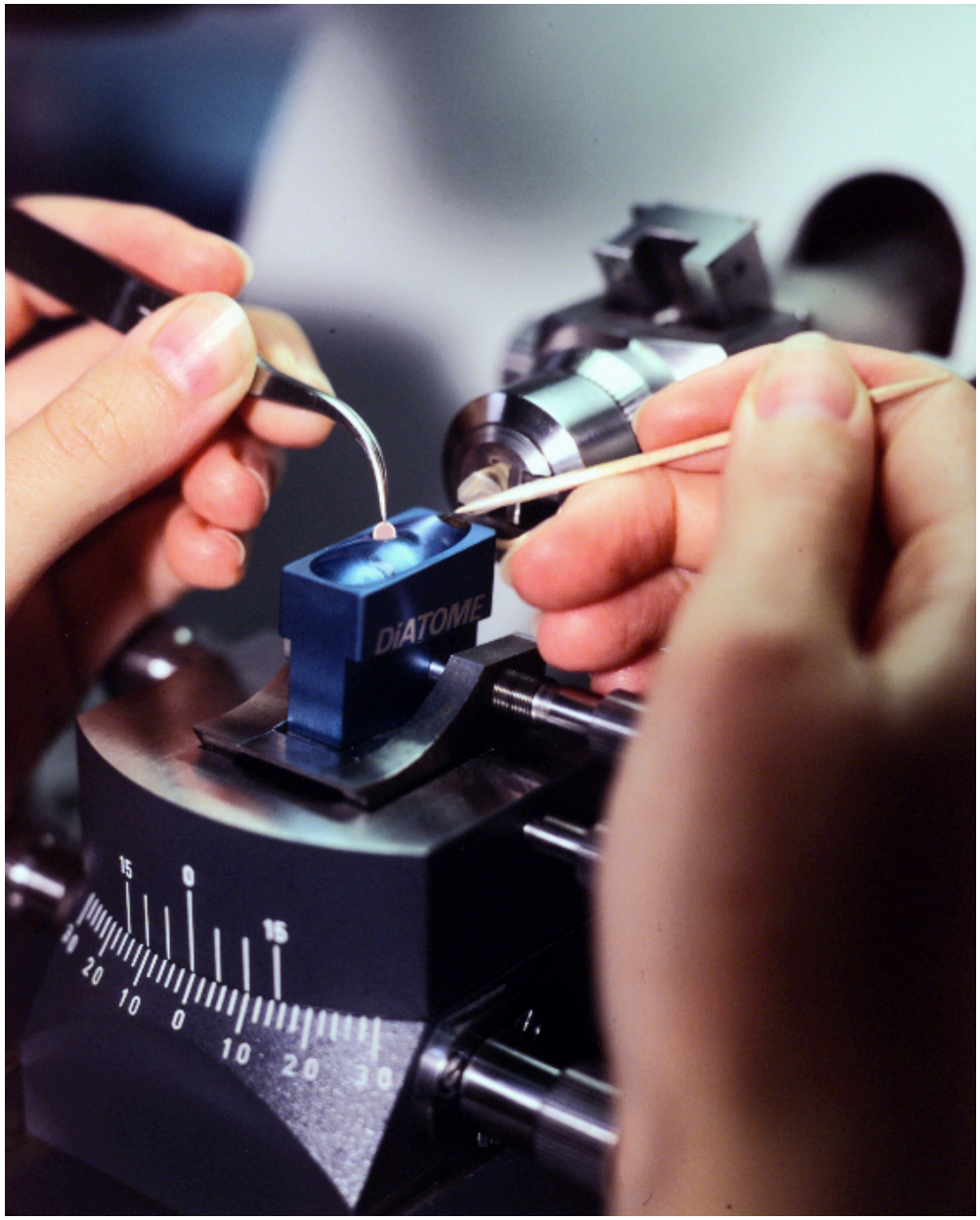
Removal of the sections from the knife edge using an eyelash

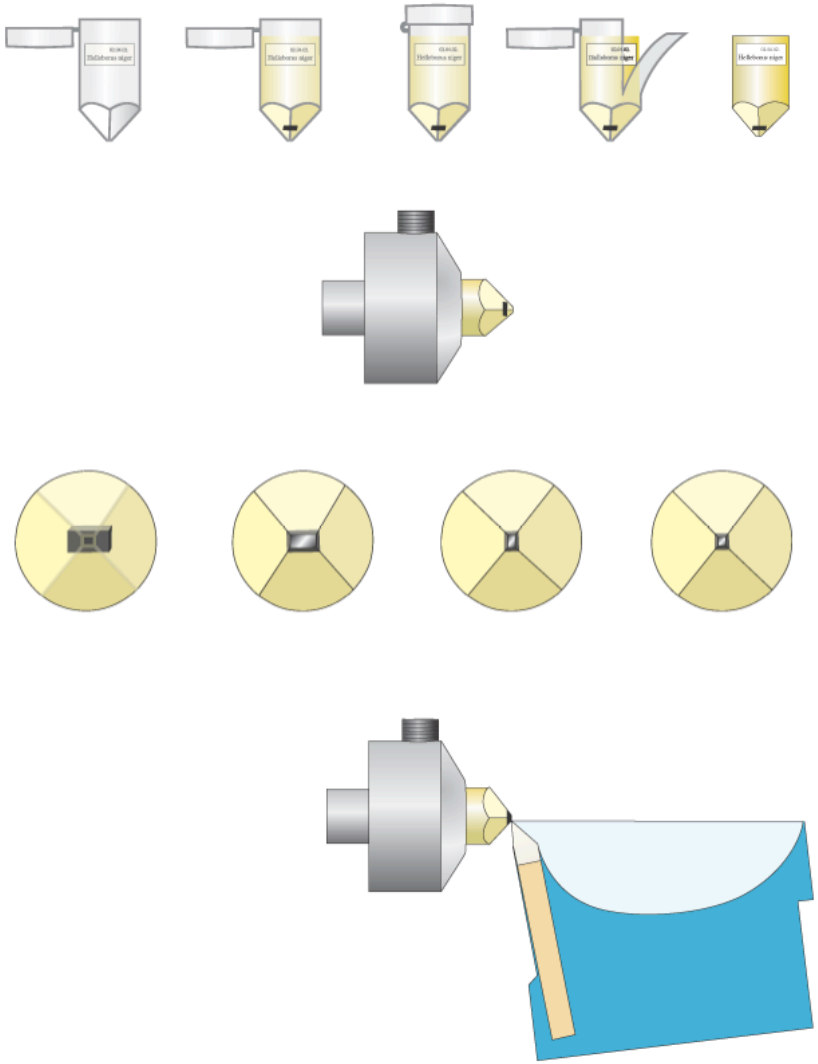



Fishing of the sections with a grid (attachment from top or tilted grid from underneath

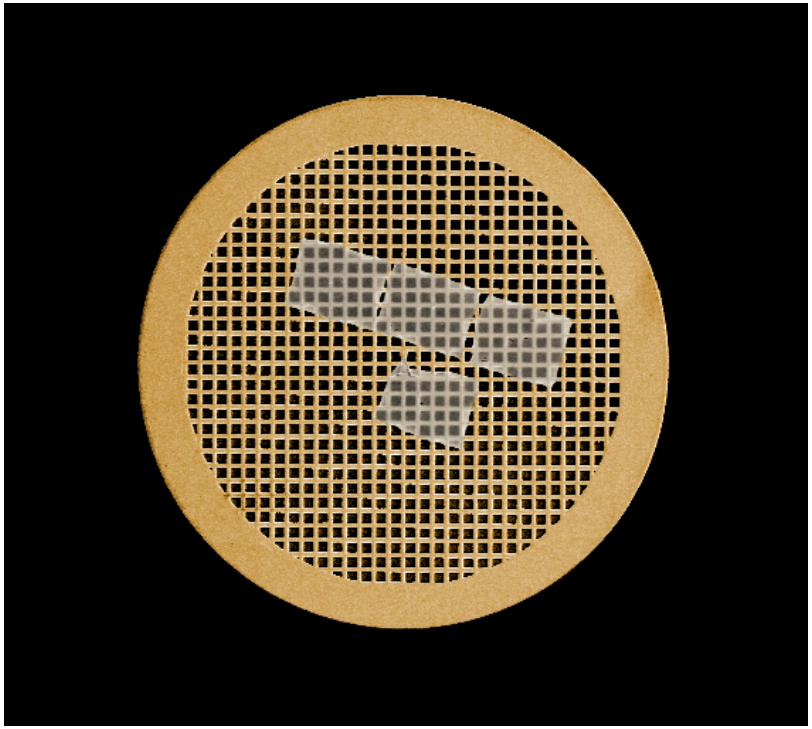
Manipulation of the sections on the water surface: eyelash, guinea pig hair (Meerschweinchenhaar) or whiskers glued to a toothpick using dental wax.

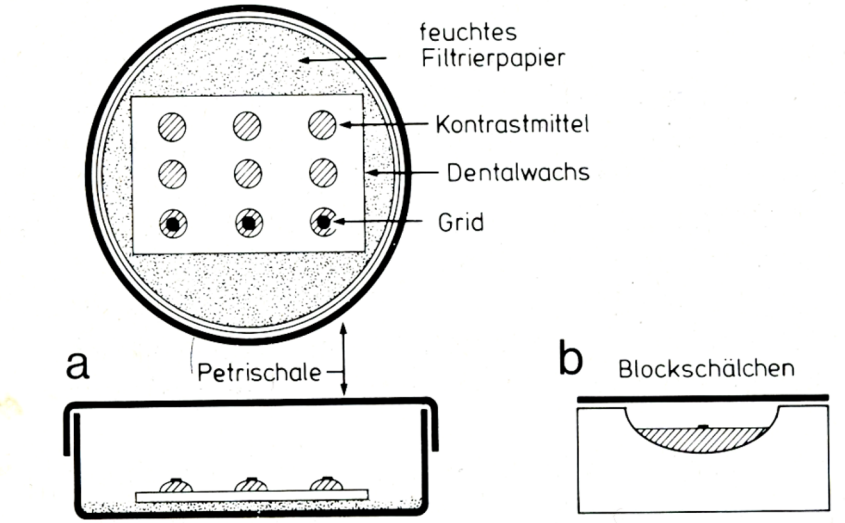


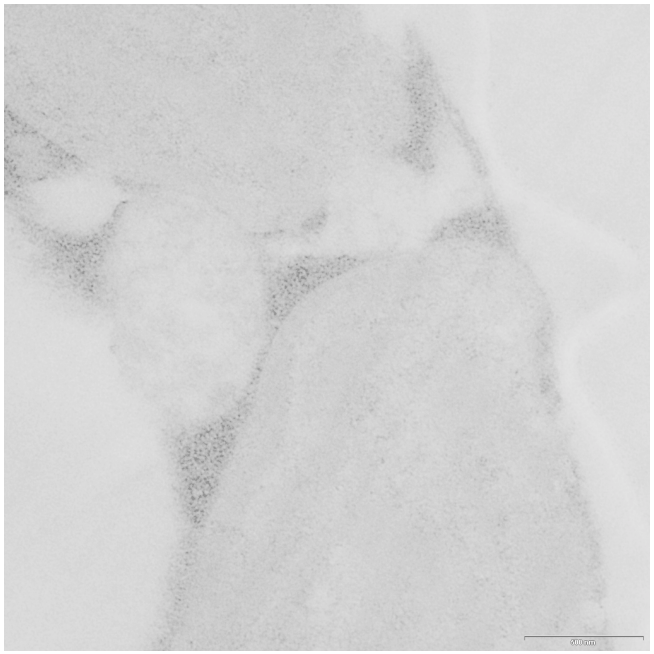


Ultramicrotomes used for EM represent rotation microtomes.

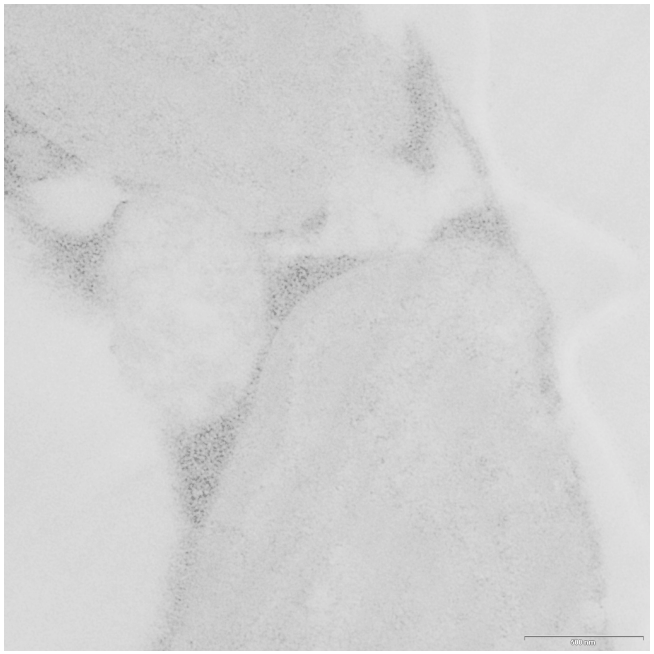
The major building blocks of cells consist of C, O, H and N. This is resulting in poor contrast, therefore we have to perform a post staining with lead citrate and uranyl acetate (heavy metal salts): double staining.



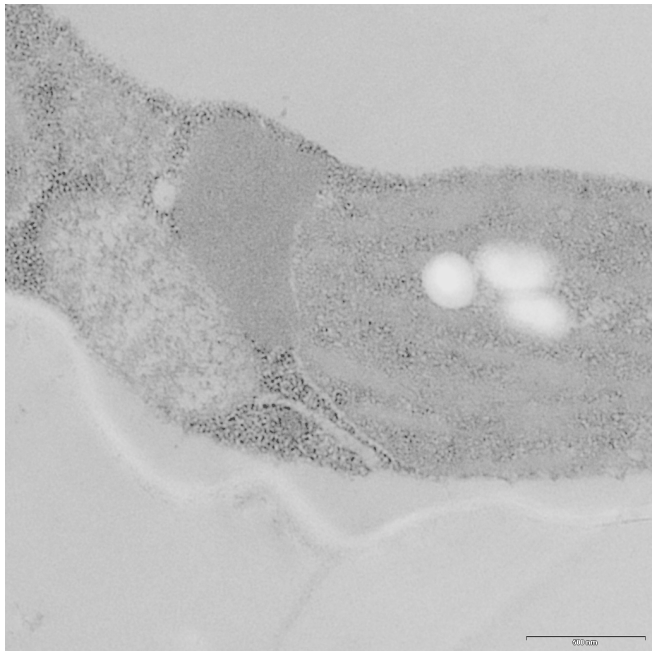




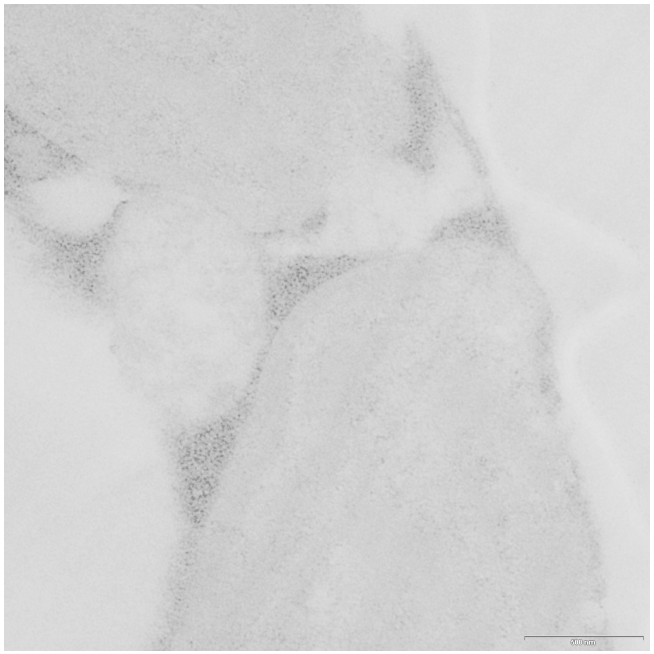
**Glutaraldehyde**



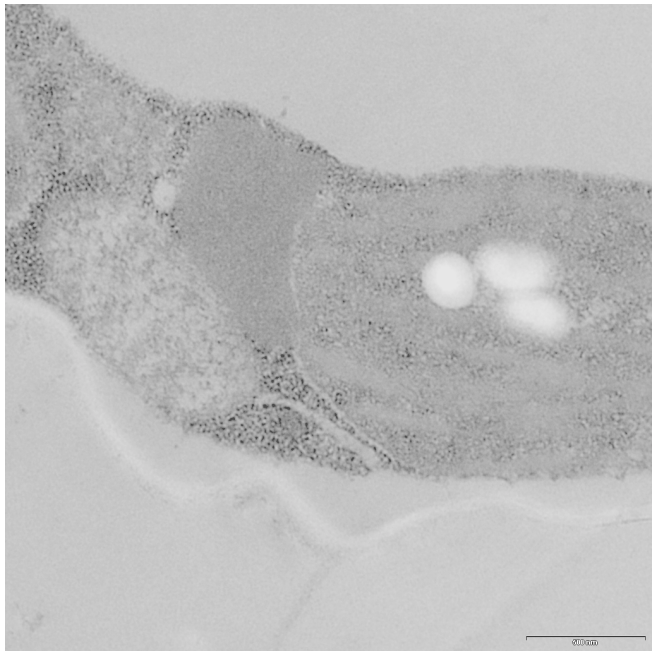
**Glutaraldehyde**



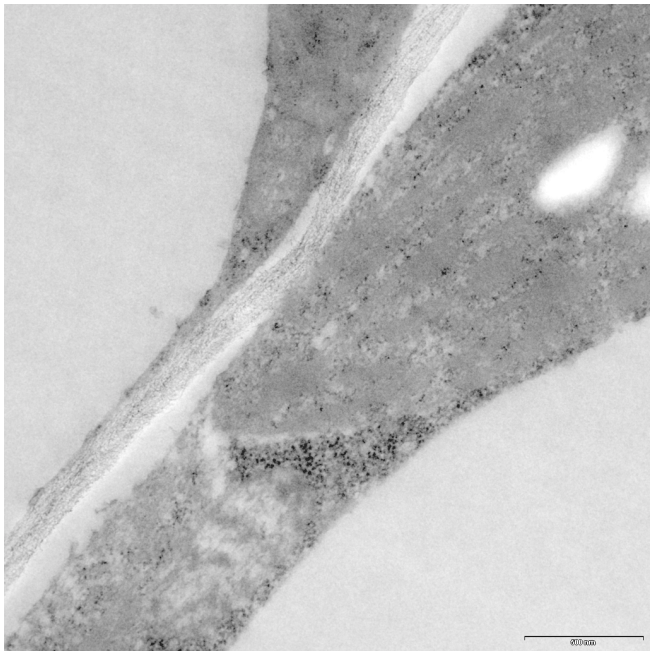
**Glutaraldehyde + lead citrate**



**Glutaraldehyde**



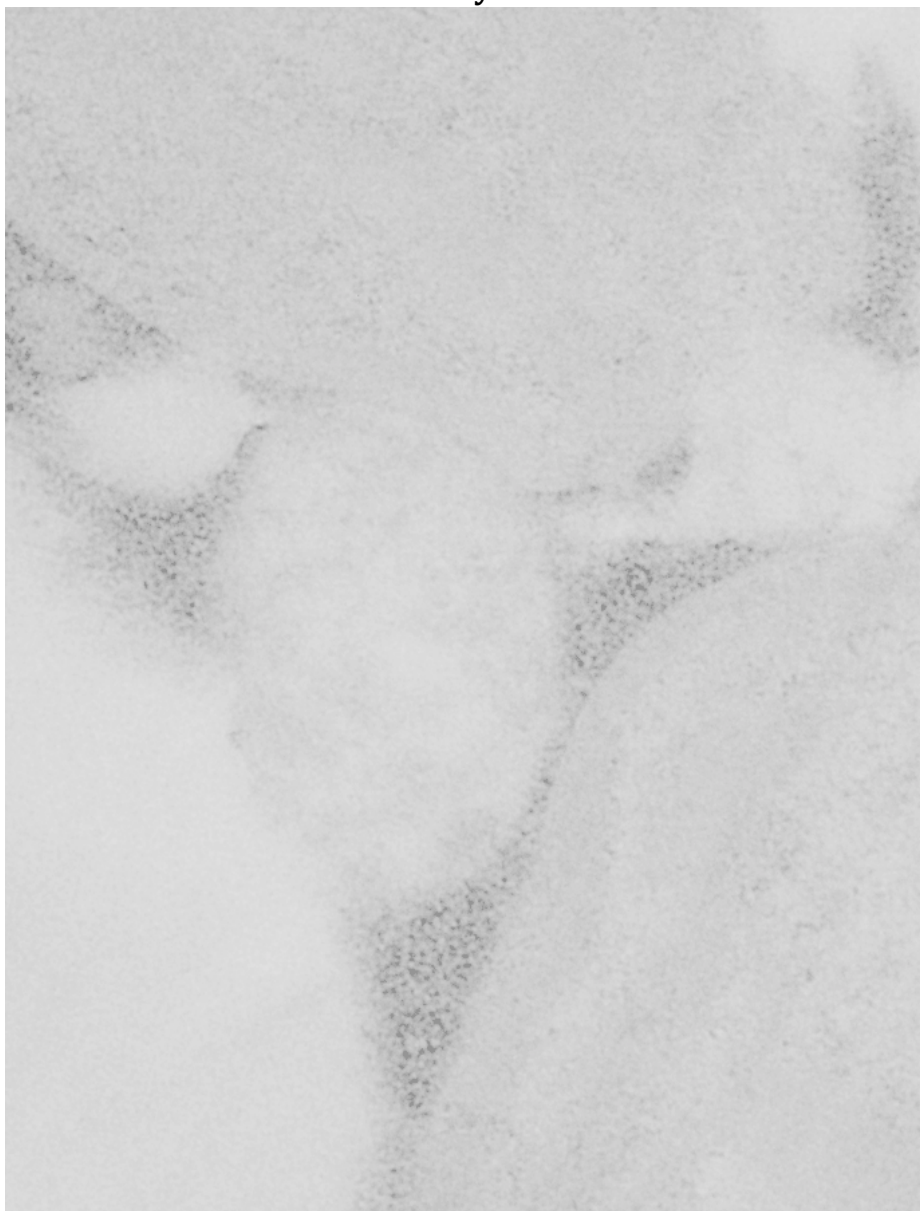
**Glutaraldehyde + lead citrate**



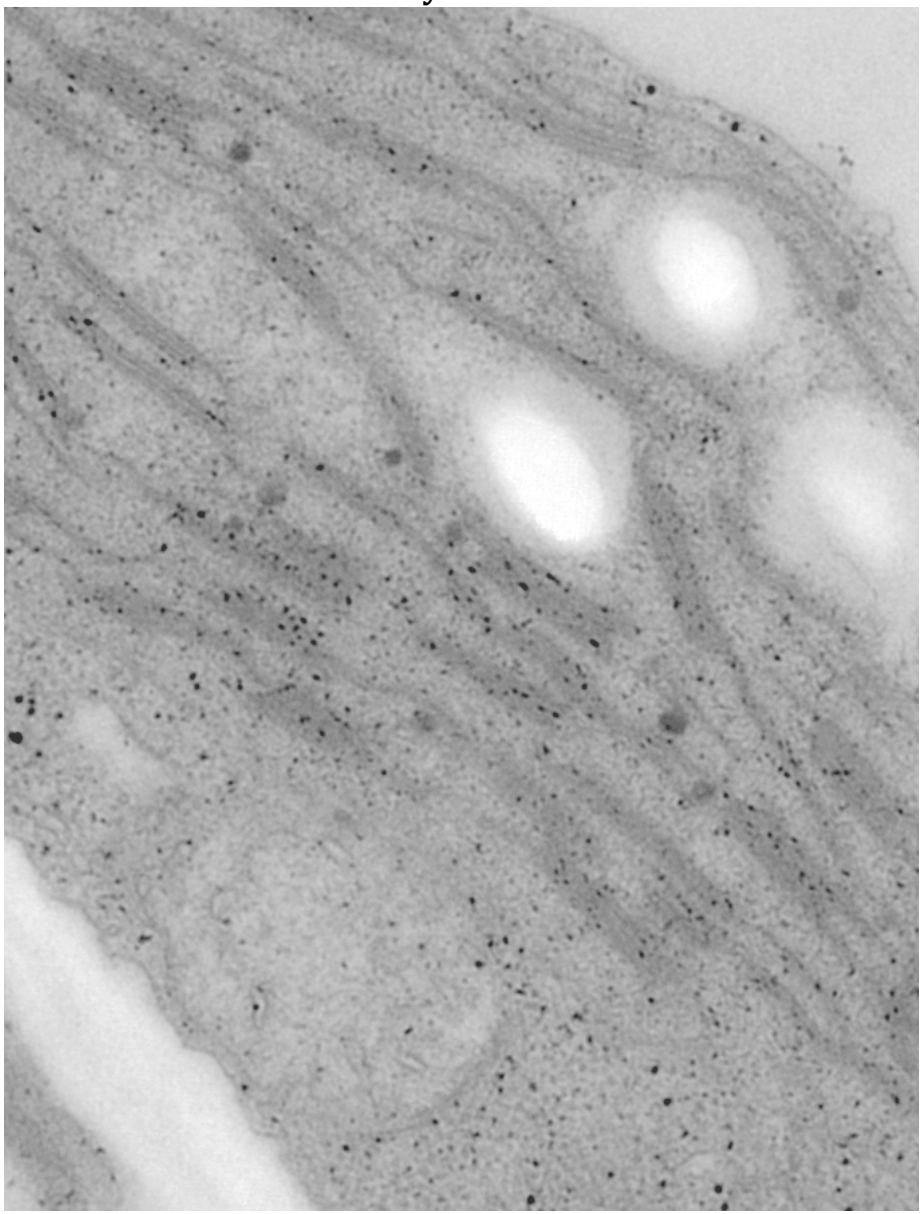
**Glutaraldehyde + uranyl acetate**

# Osmium is fixing and contrasting all membranes

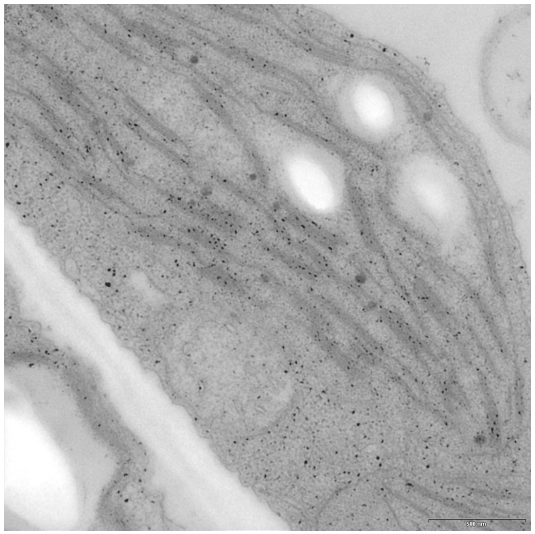
**Glutaraldehyde**



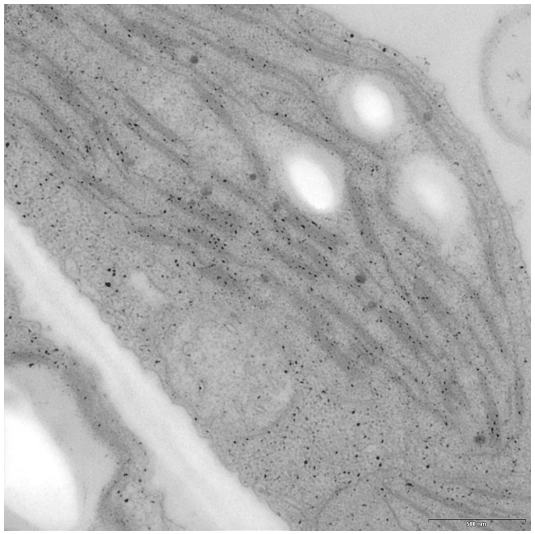
**Glutaraldehyde + osmium tetroxide**



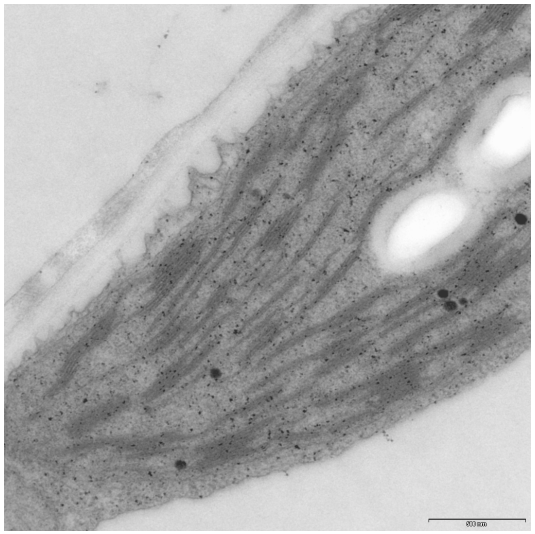
**Glutaraldehyde + osmium**



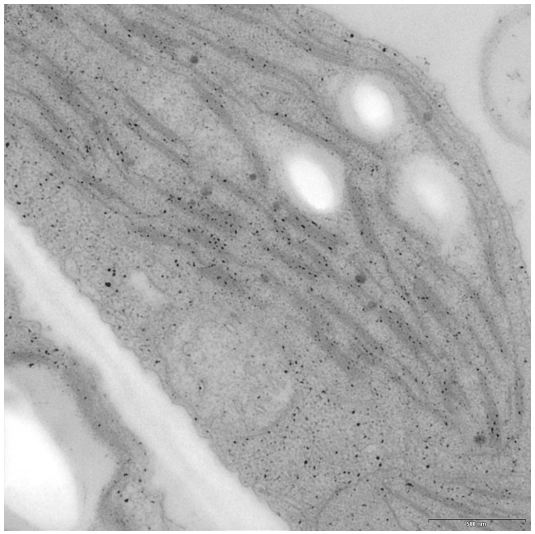
**Glutaraldehyde + osmium**



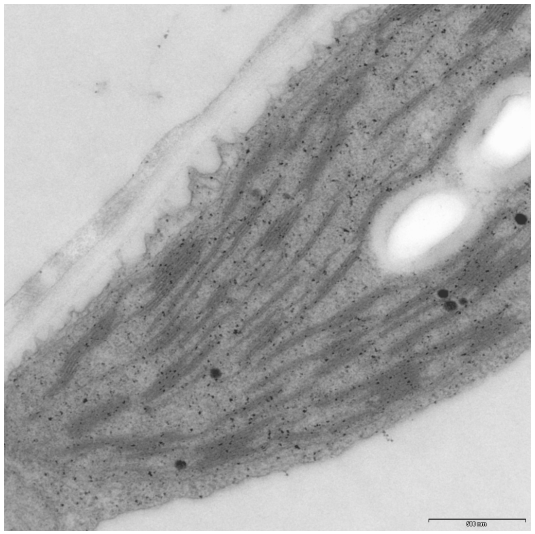
**Glutaraldehyde + osmium + UrAc**



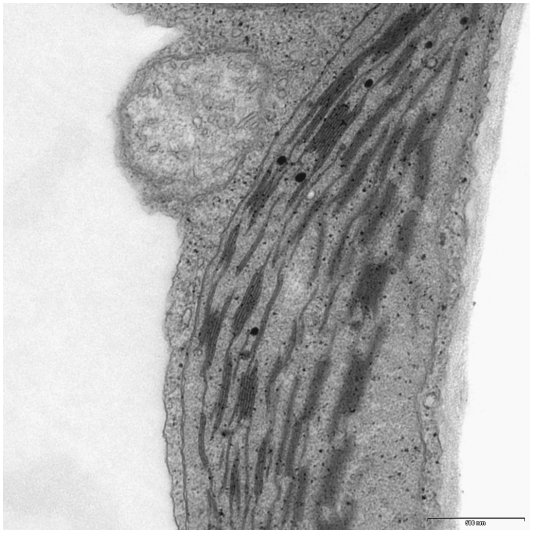
**Glutaraldehyde + osmium**



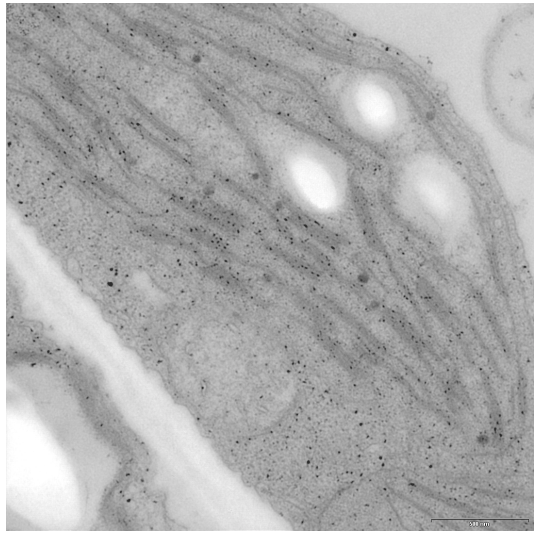
**Glutaraldehyde + osmium + UrAc**



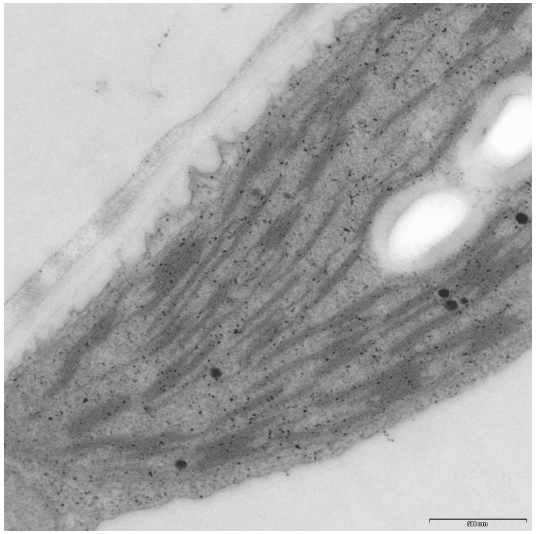
**Glutaraldehyde + osmium + Pb**



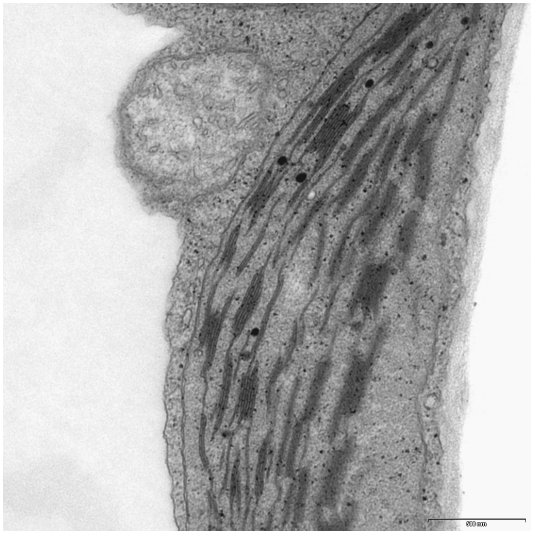
**Glutaraldehyde + osmium**



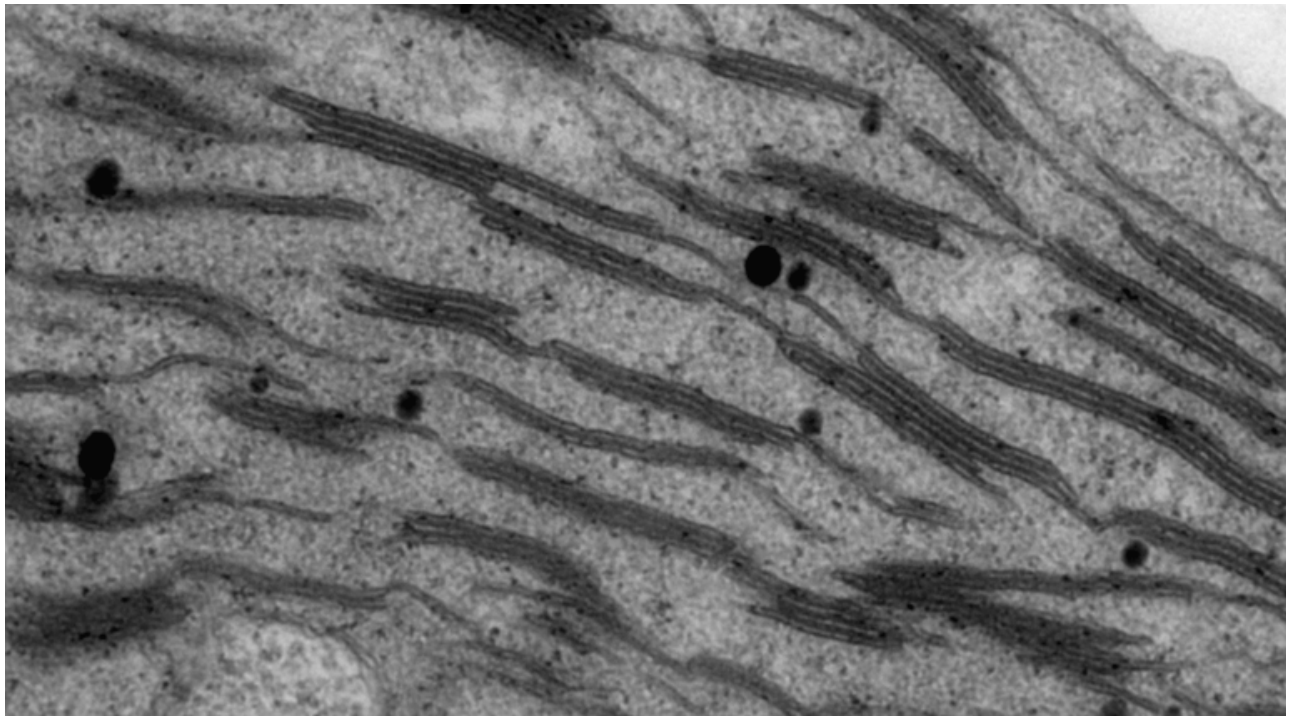
**Glutaraldehyde + osmium + UrAc**



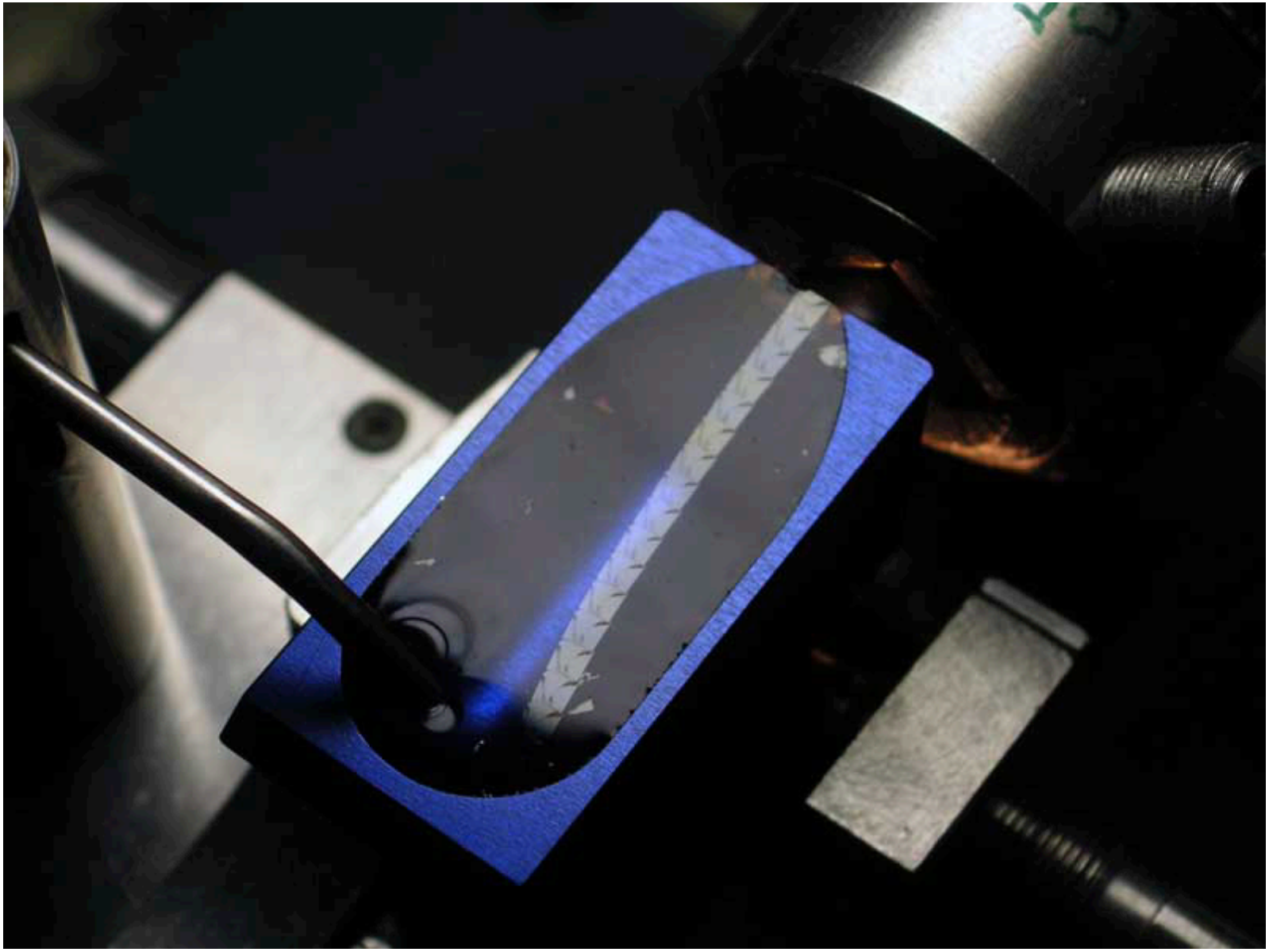
**Glutaraldehyde + osmium + Pb**



**Glutaraldehyde + osmium + UrAc + Pb**

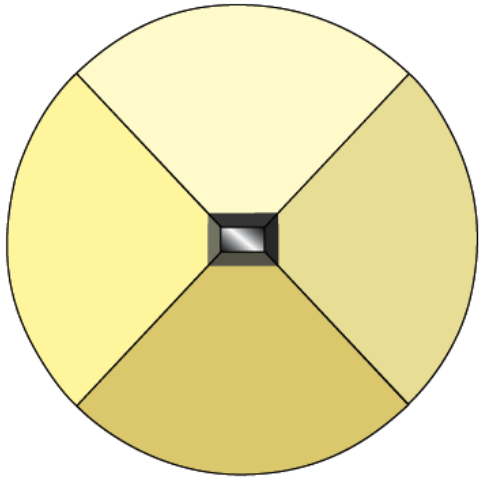


# Serial section



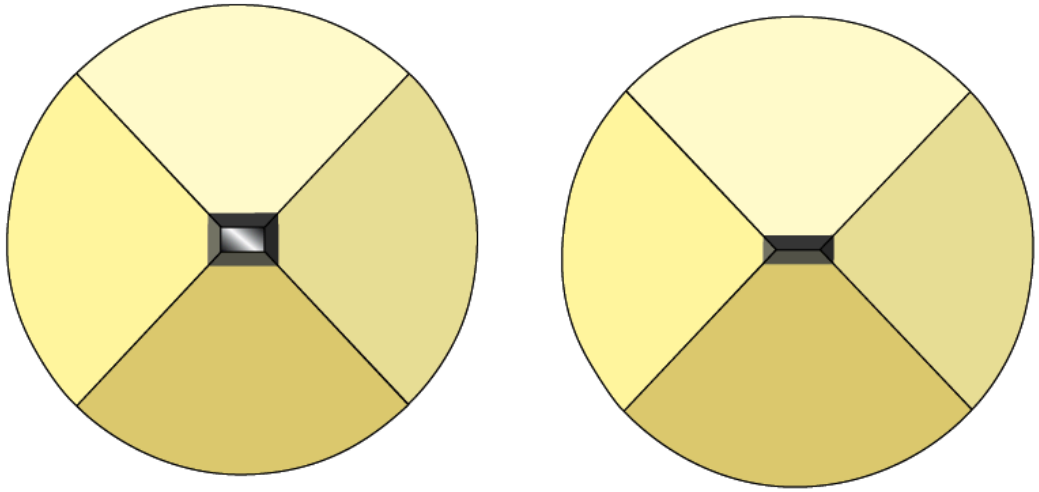
# Pyramid with narrow tip!

Investigating an ultrathin section in the TEM:
Looking for a suitable position.



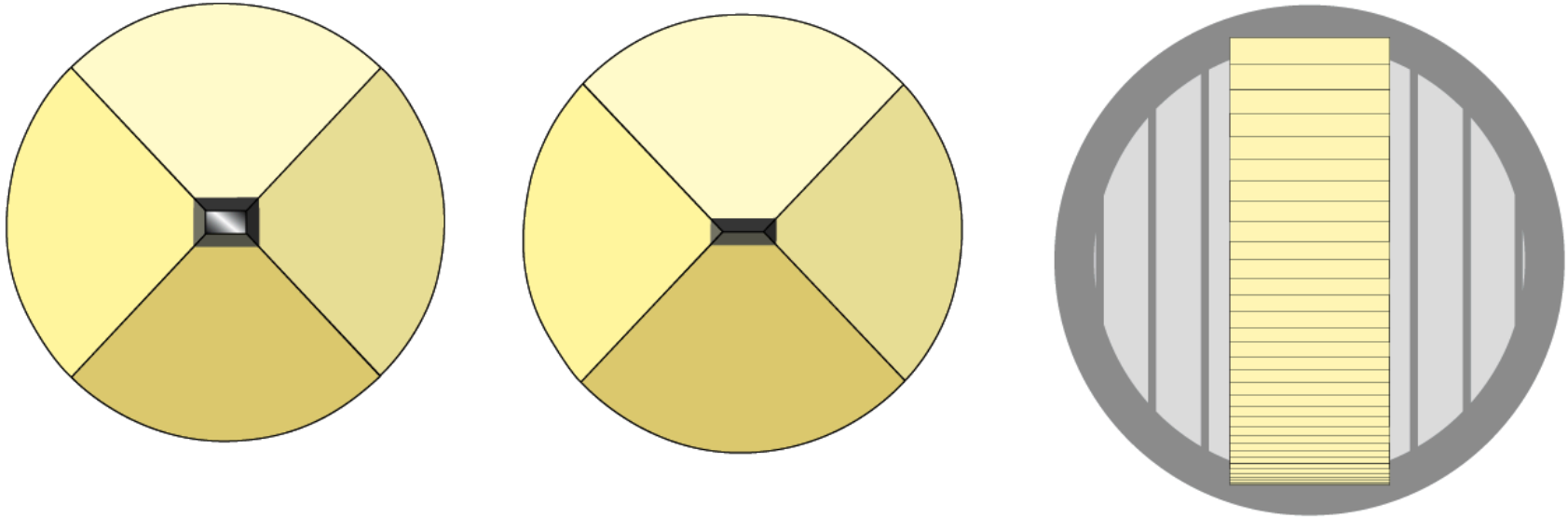
# Pyramid with narrow tip!

Trimming a prism at a suitable position.



**Pyramid with narrow tip!**

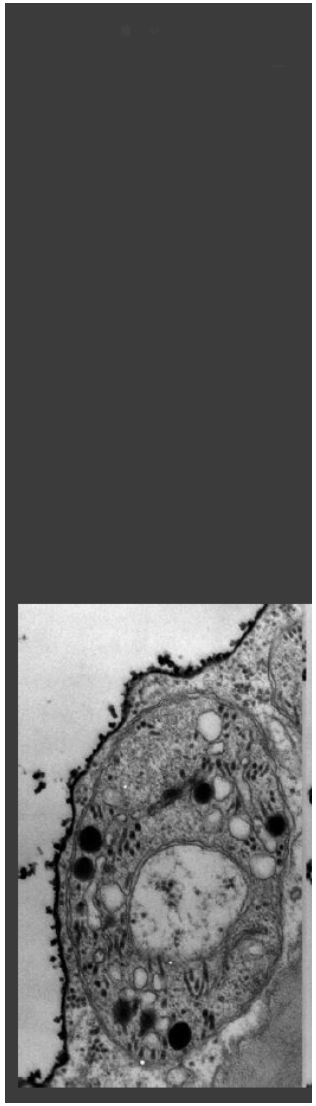
**50 – 100 sections on one grid!**



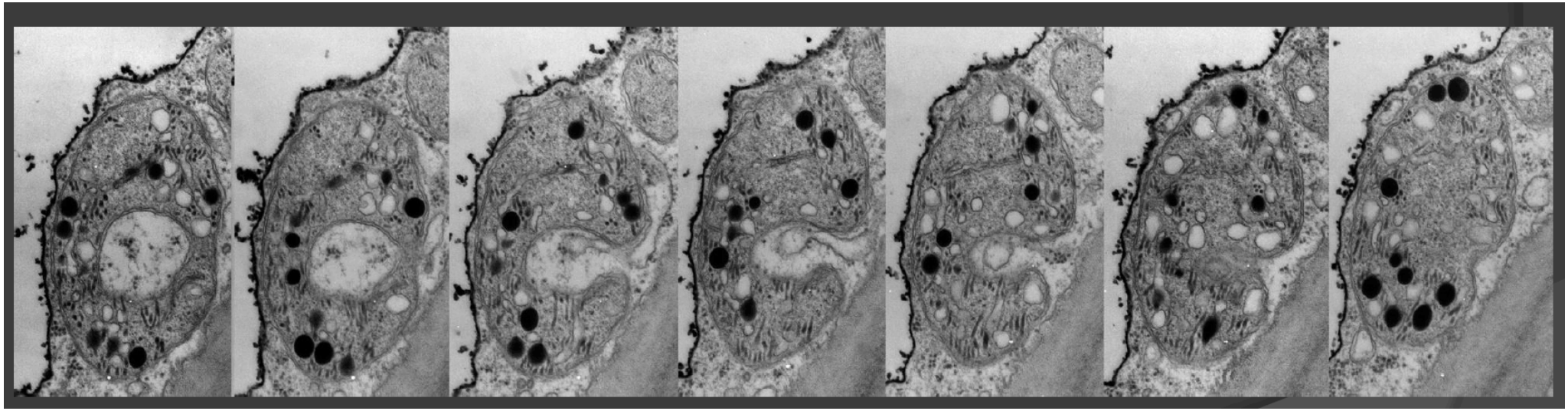
# Serial section

*Strelitzia reginae*

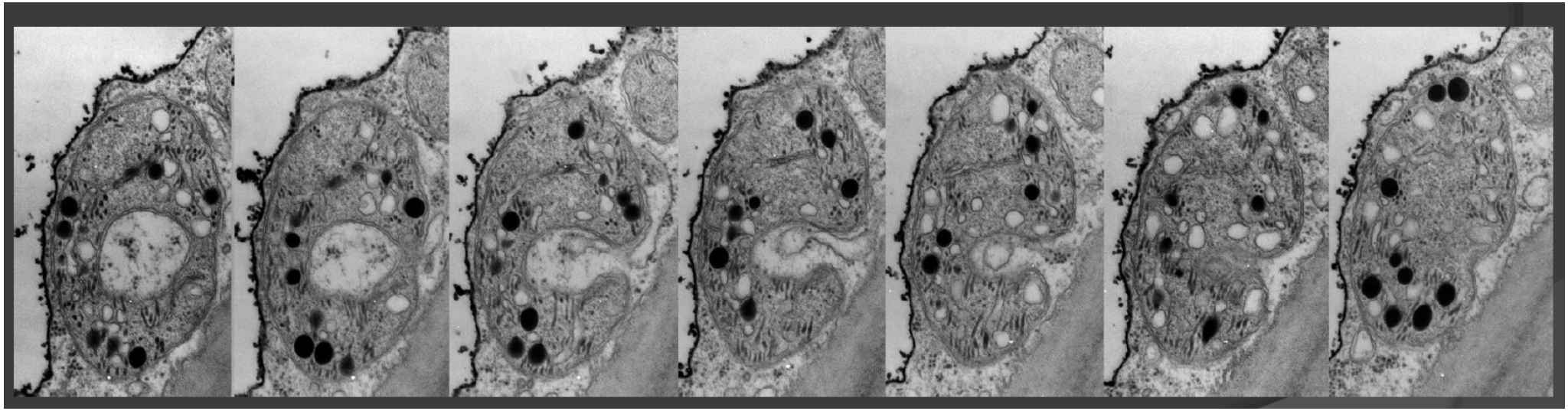
# Serial section



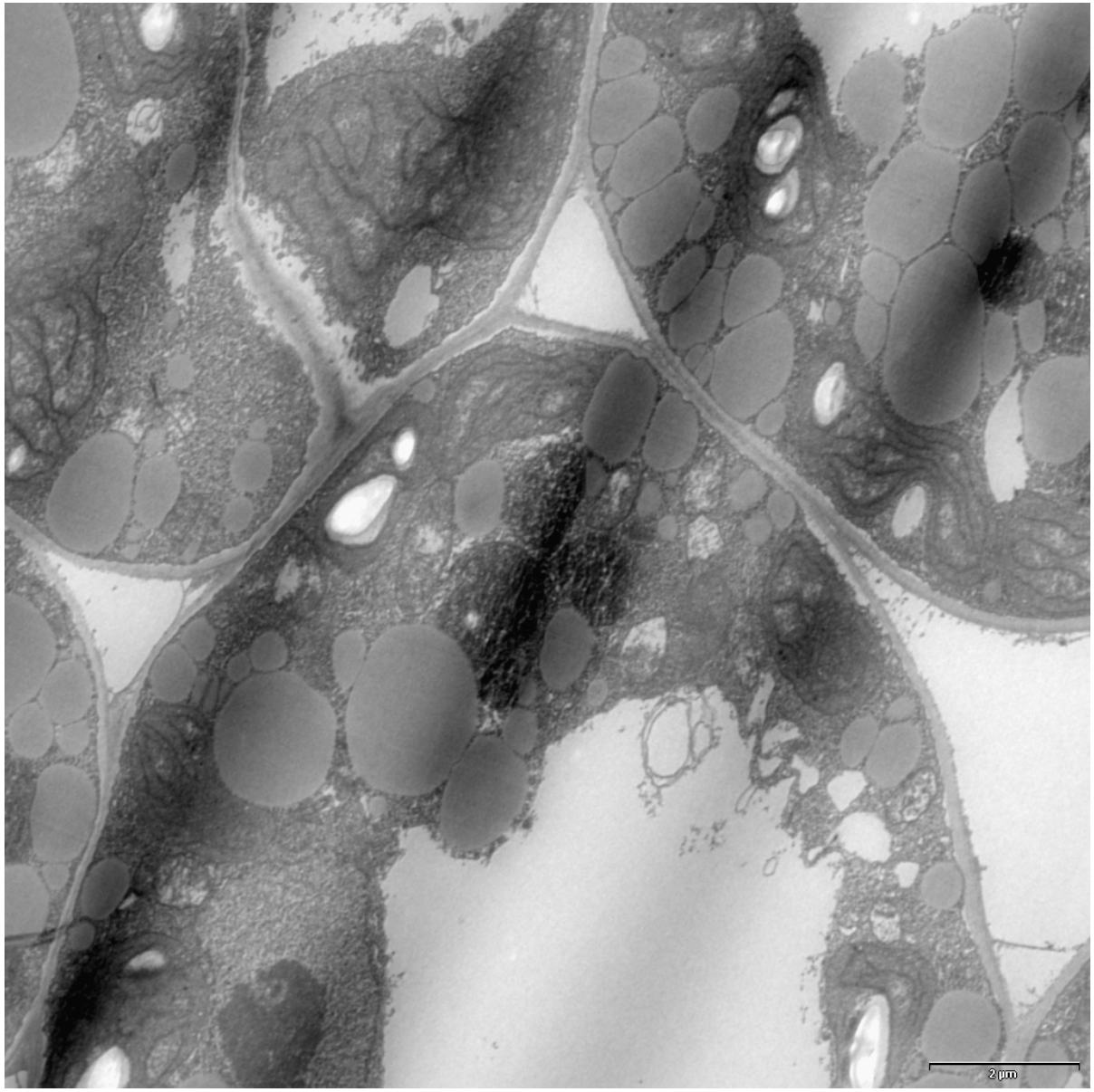
# Serial section



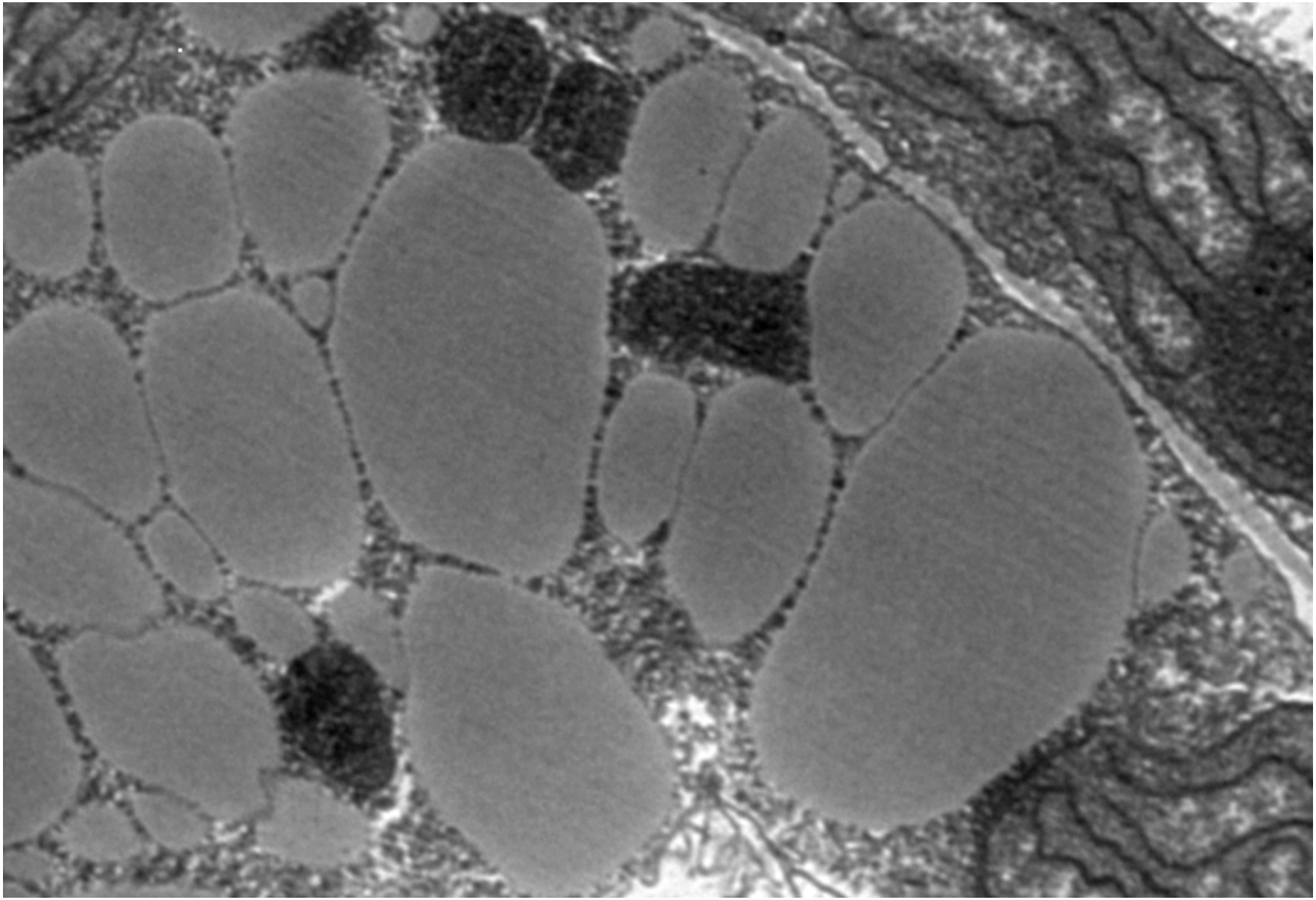
# Problems concerning sectioning



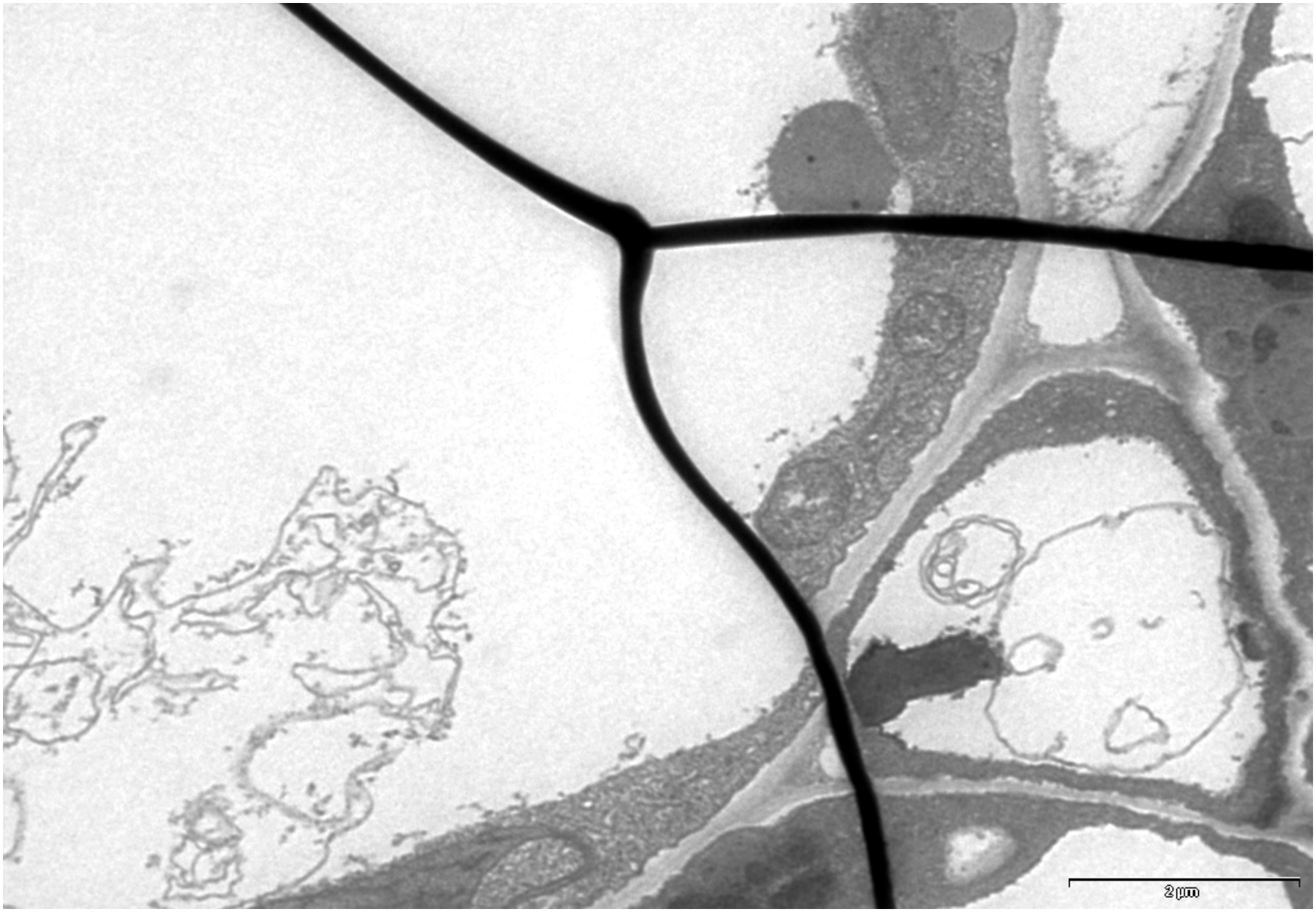
# „Chatter“ (vibration/judder)



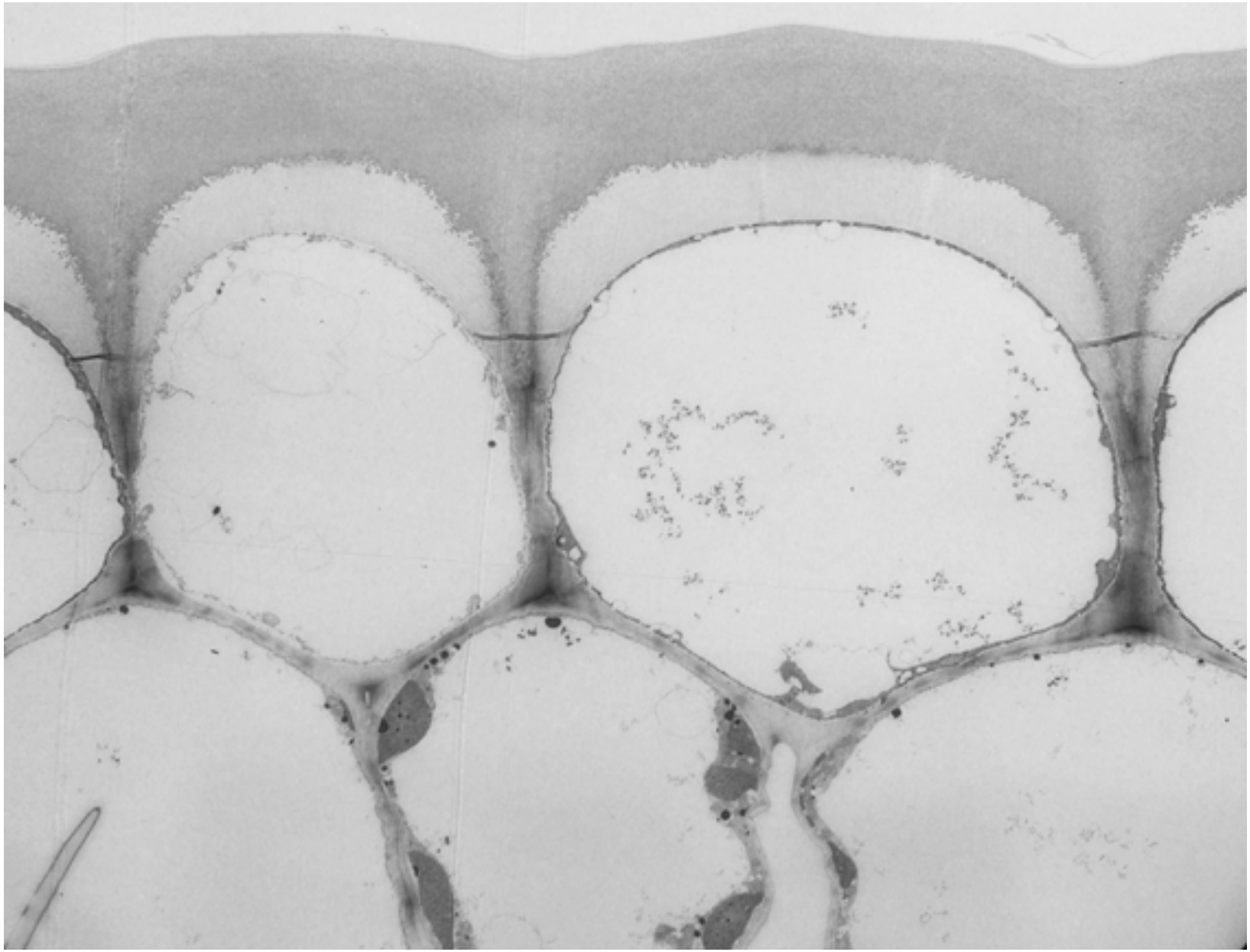
# Dirty knife edge



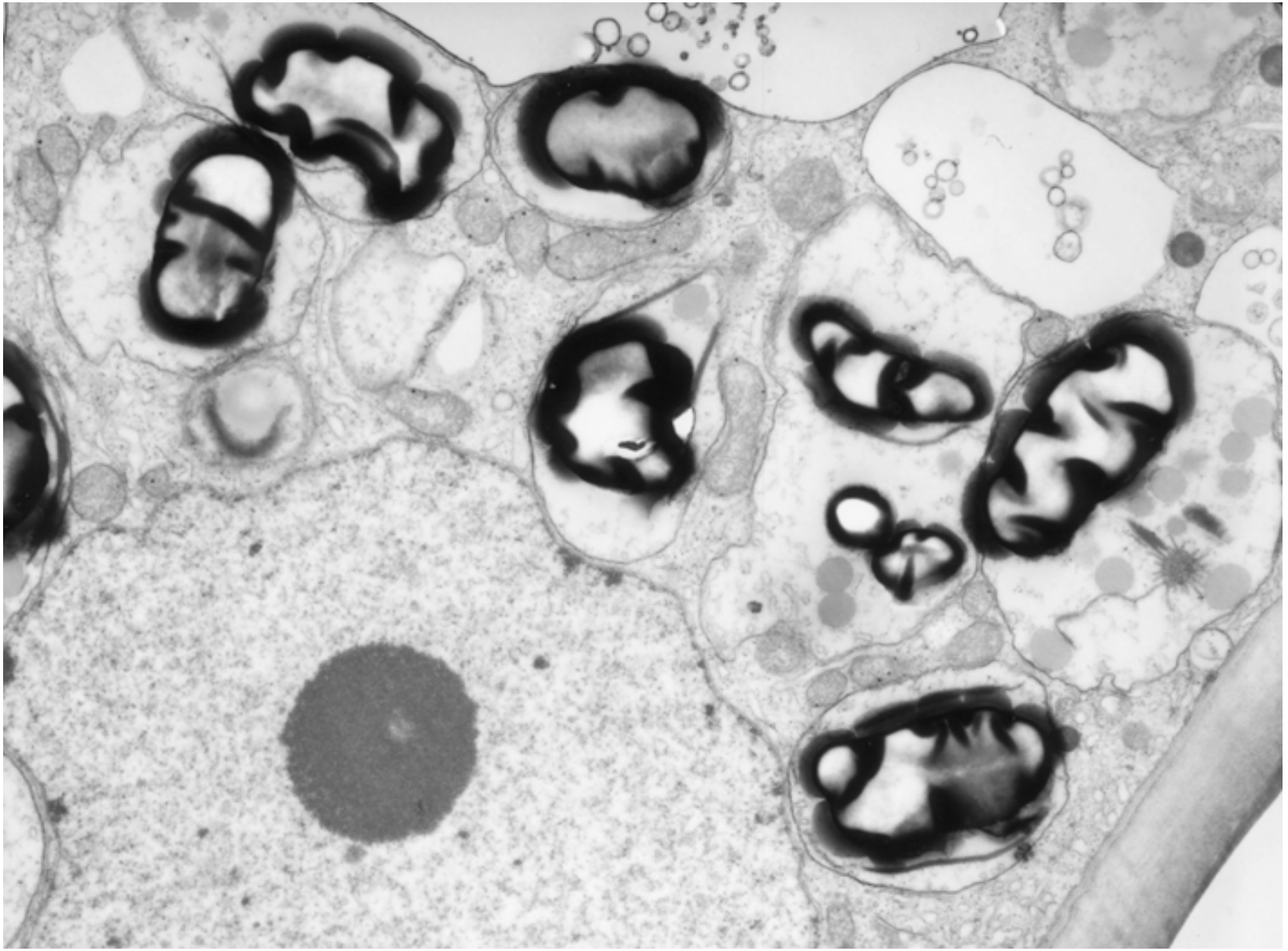
# Folds



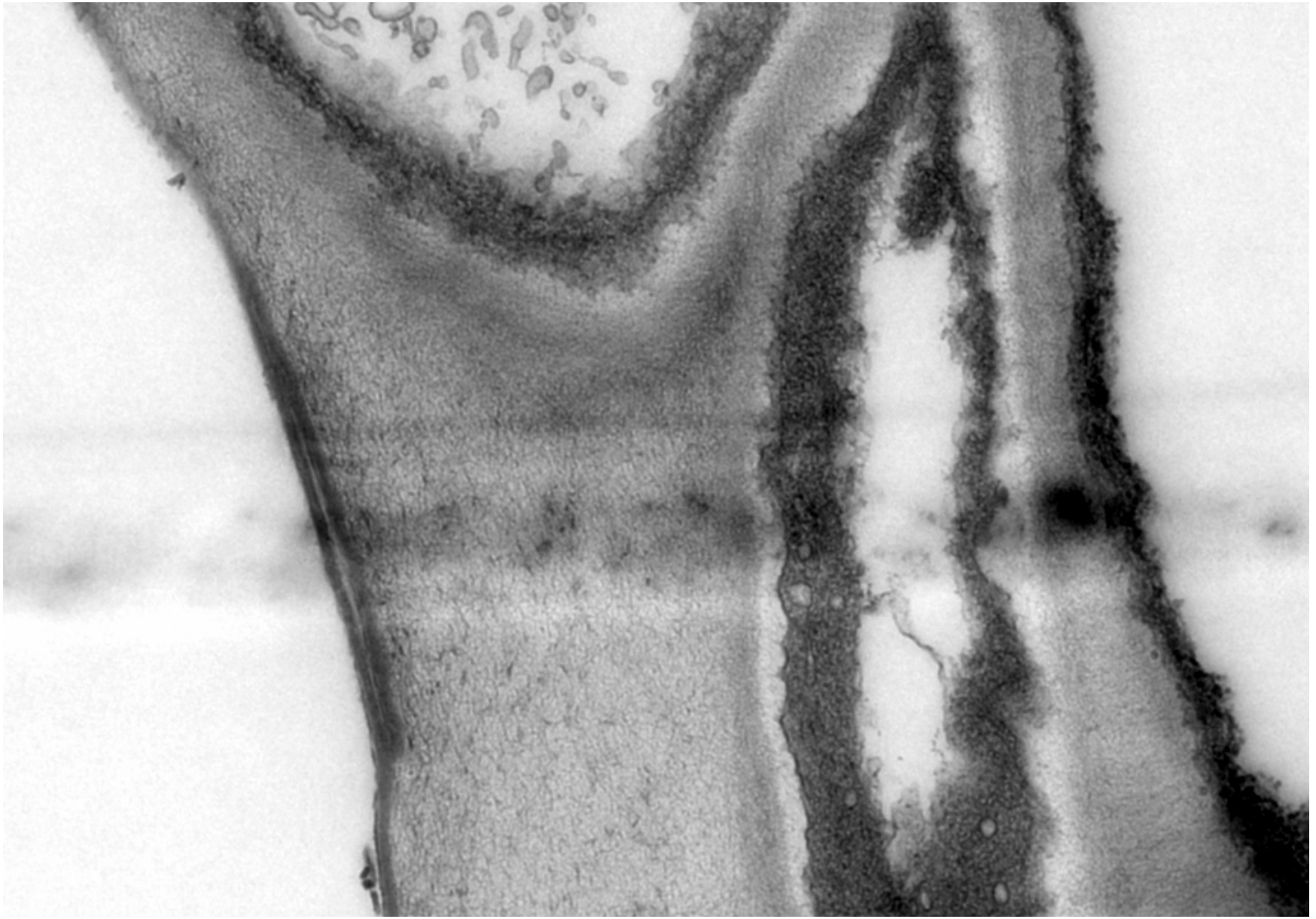
# Folds or pits?



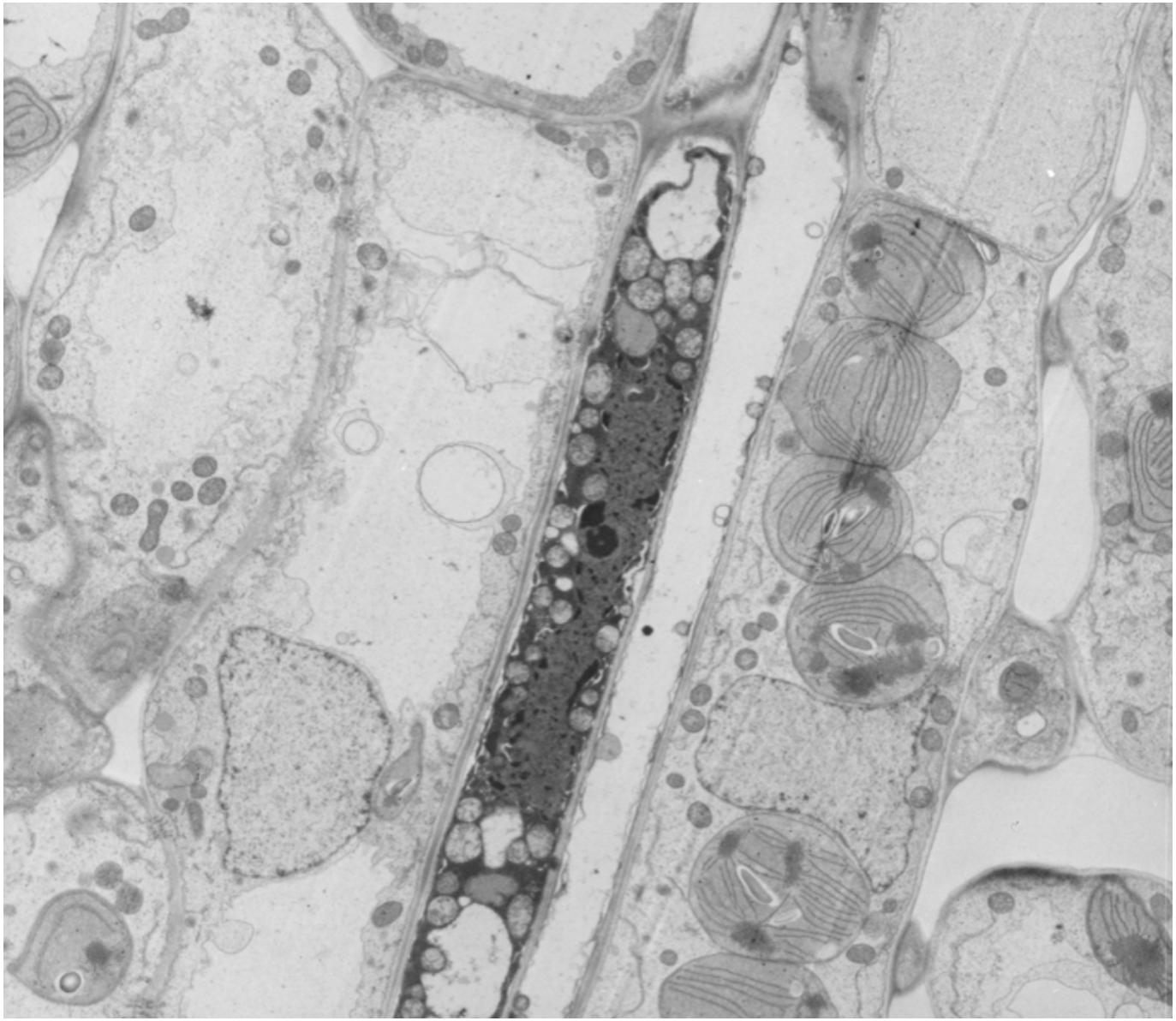
# Starch – always very poor imaging!



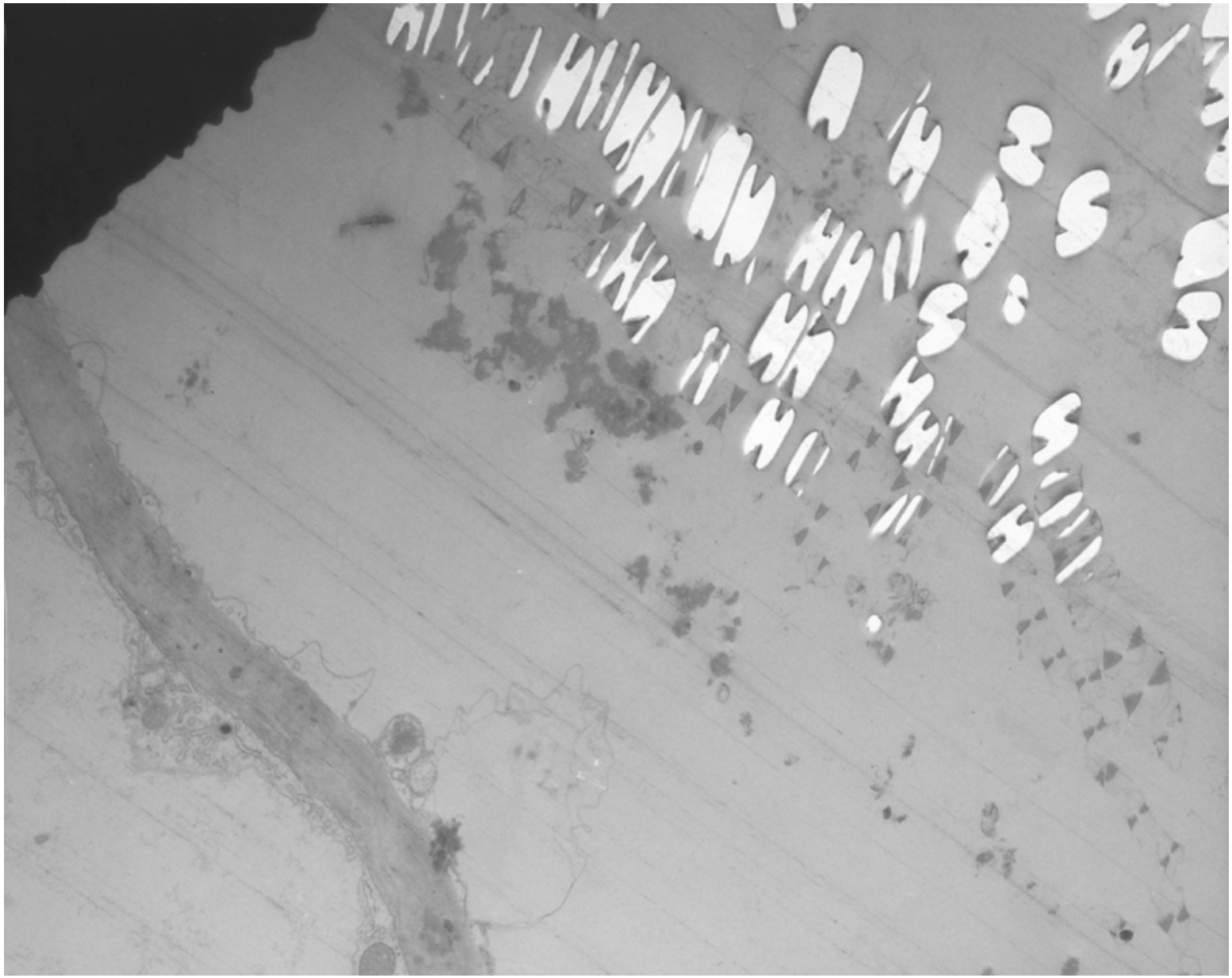
# Nick/notch of the knife



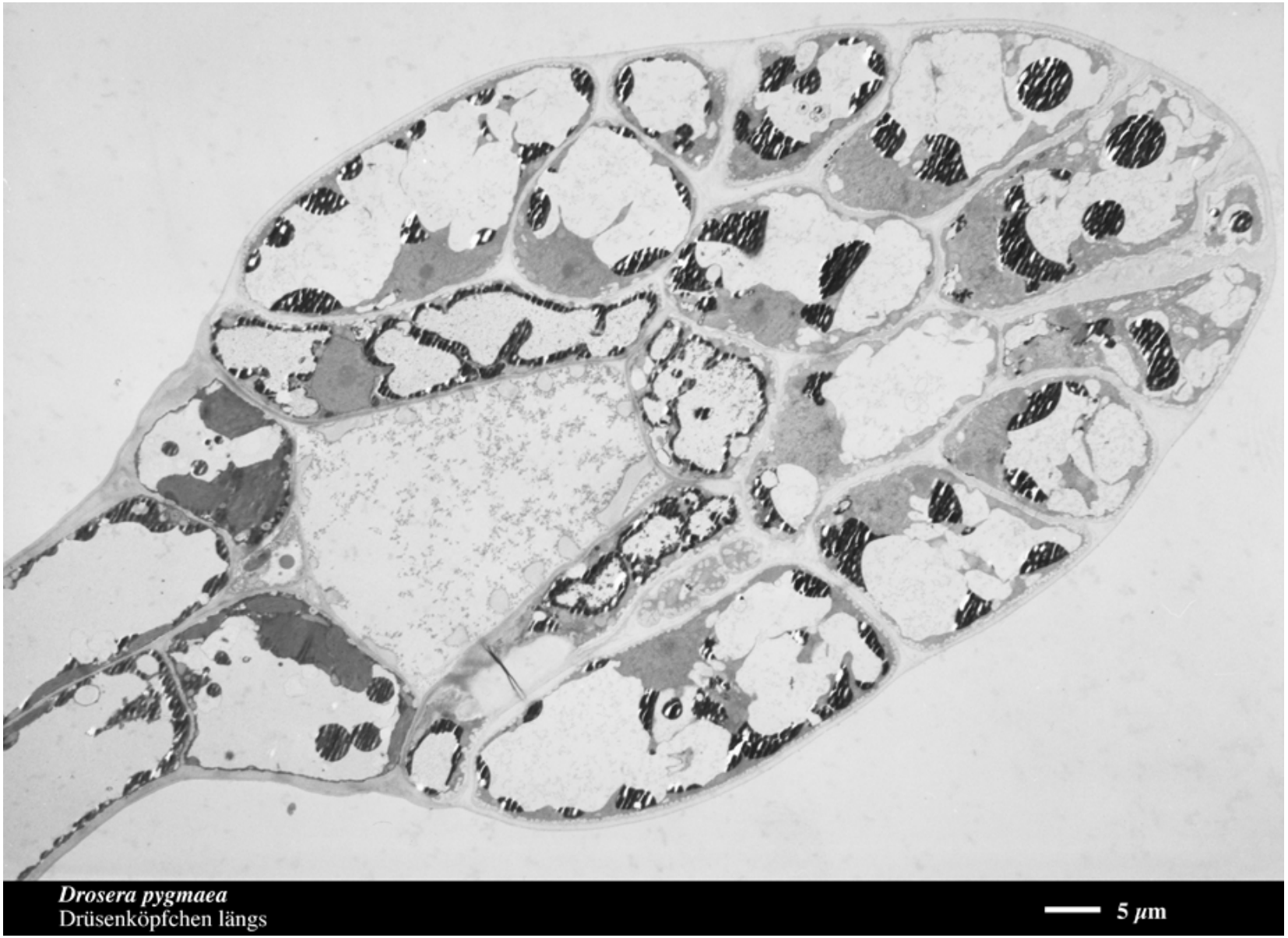
# Nick/notch of the knife



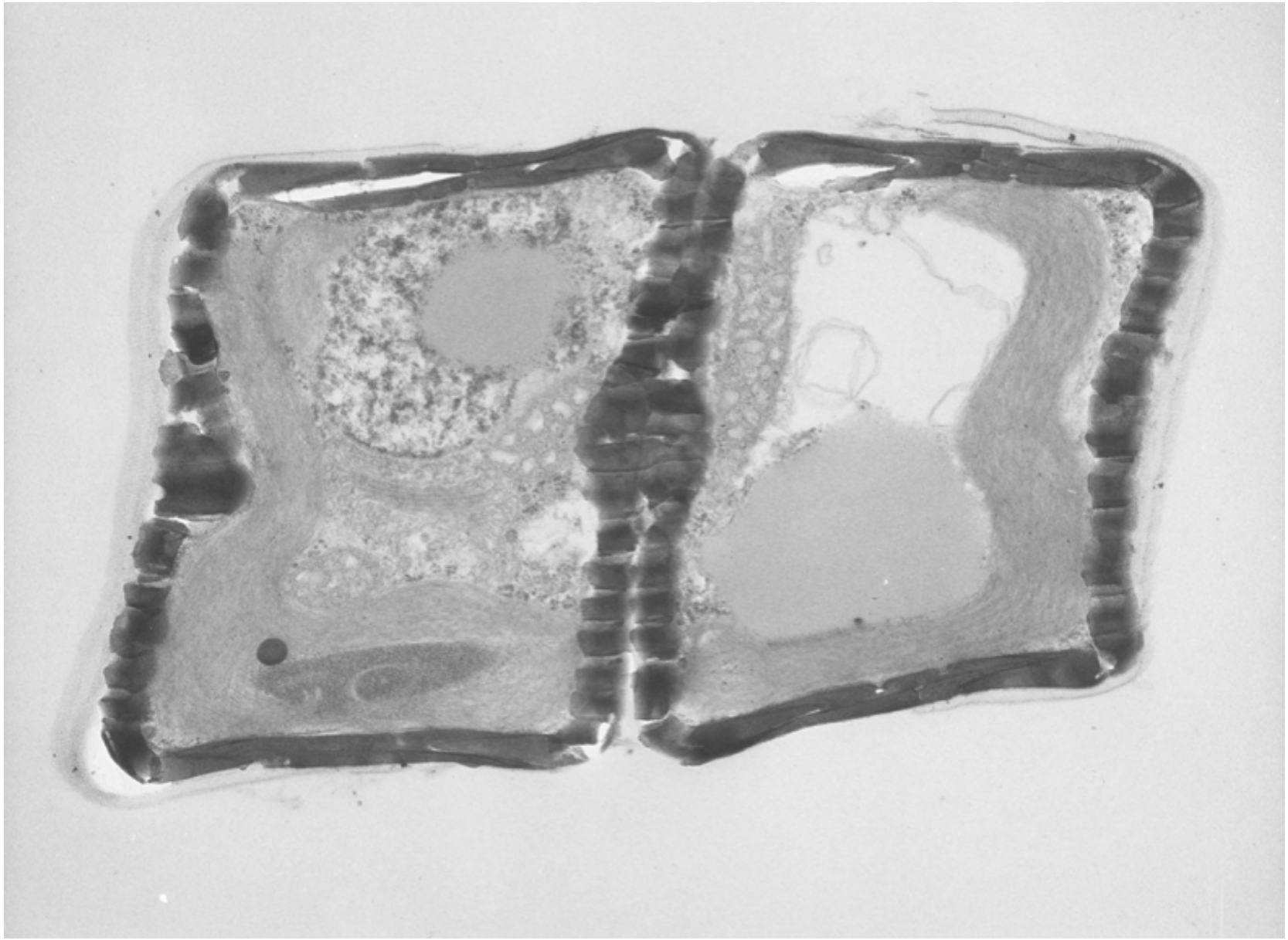
# Holes and knife marks/grooves



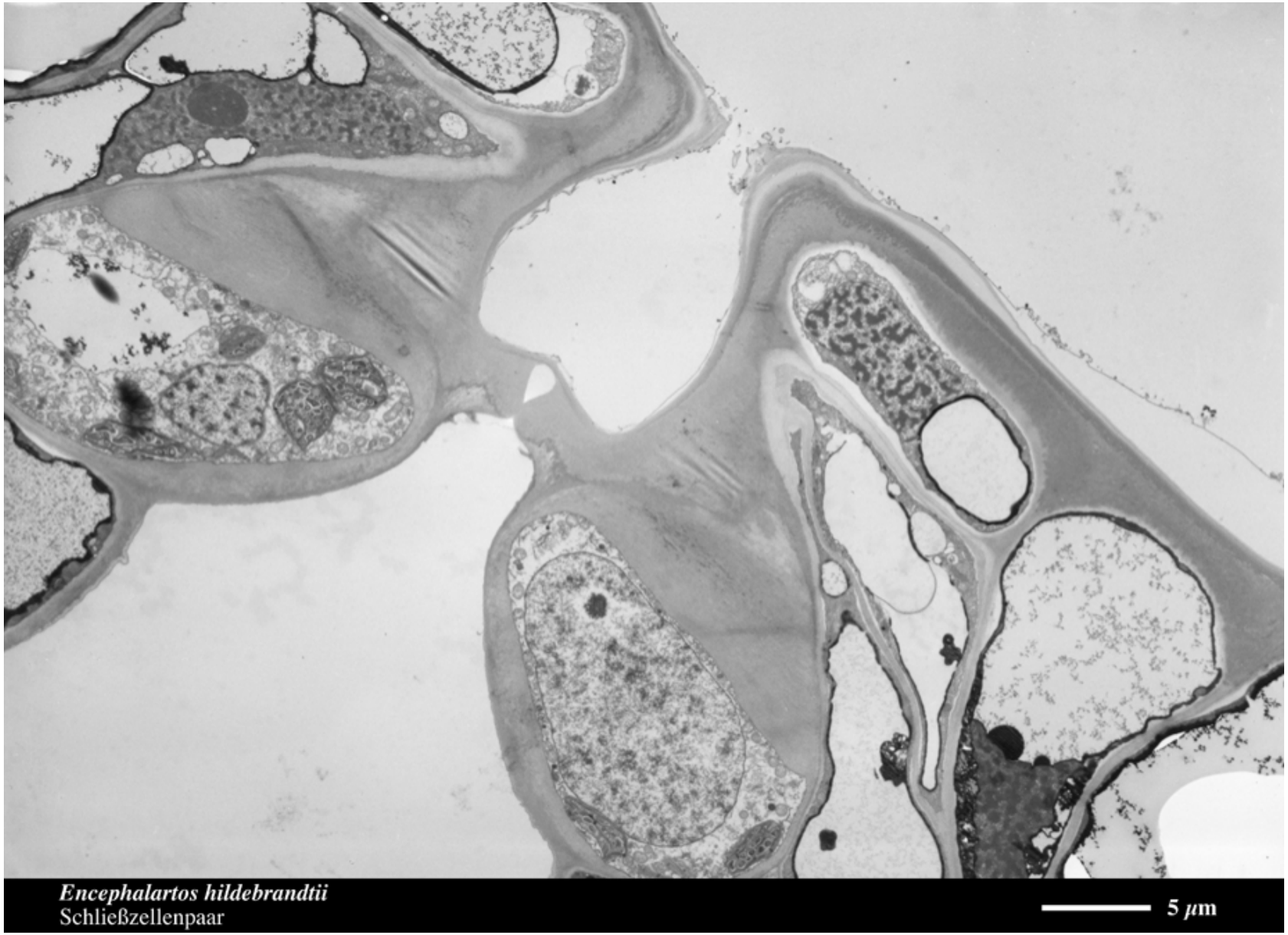
# Holes



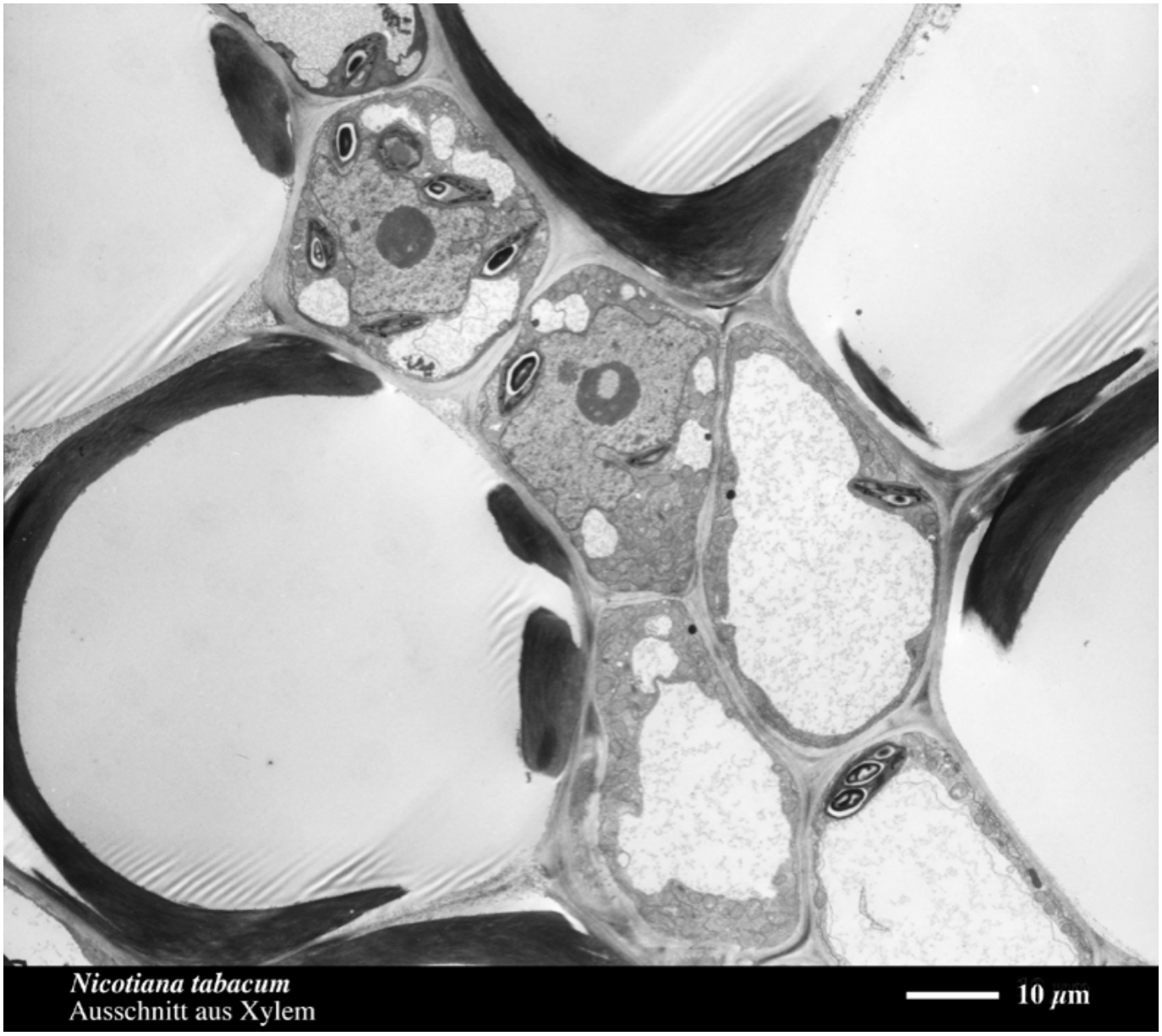
# Holes



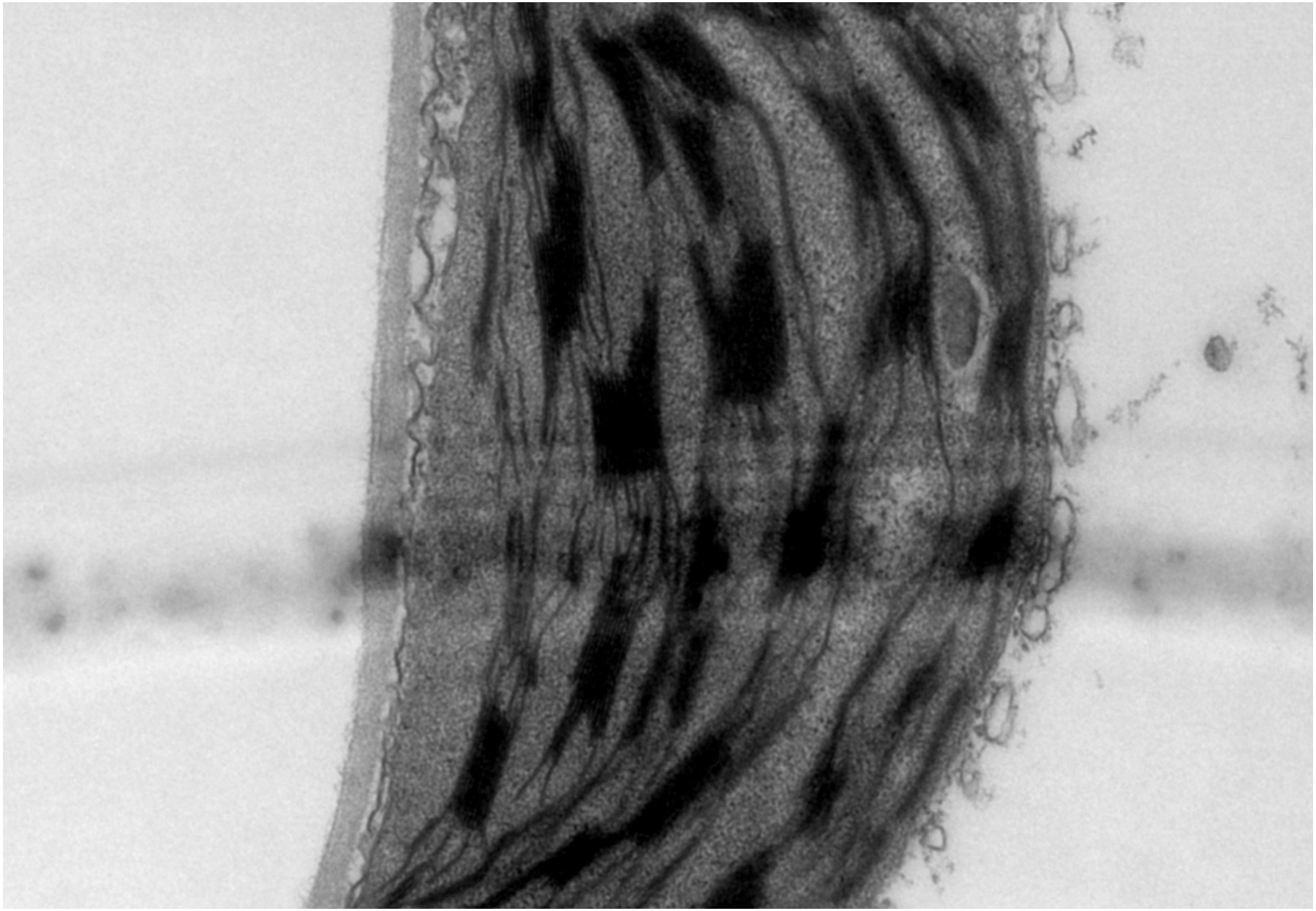
# Compression lines



# Compression lines



# Bad fixation & notch/nick of the knife



# Broken tissue after fixation