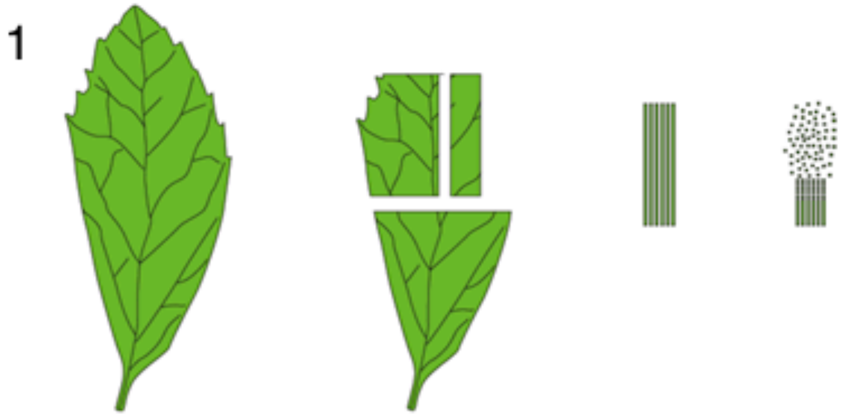
Methods in Transmission Electron Microscopy and their application

Day 4/5

SEM: sample preparation

TEM: thin sectiong

SEM
Sample preparation



Sample material, e.g. plant leaves, need to be cut into small pieces (edge length 1 mm)

Fixation with glutaraldehyde

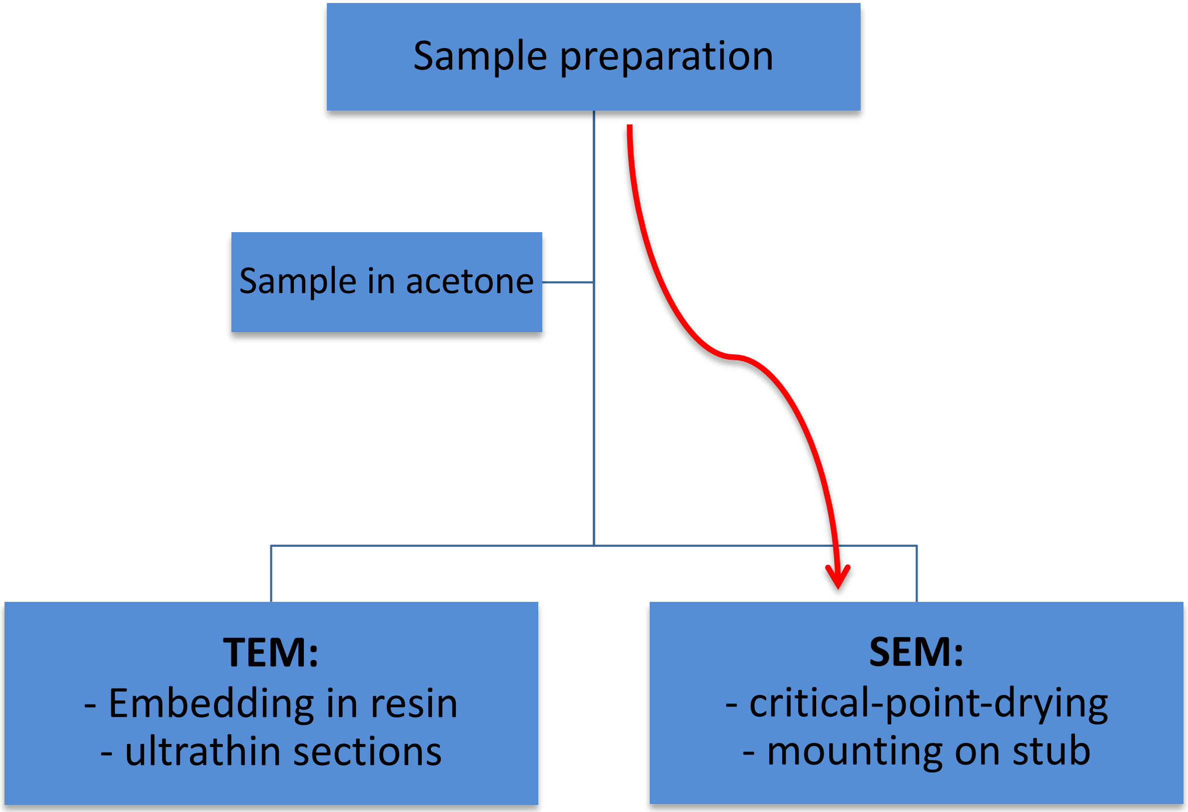
Withdrawal of fixing agent

Washing with buffer

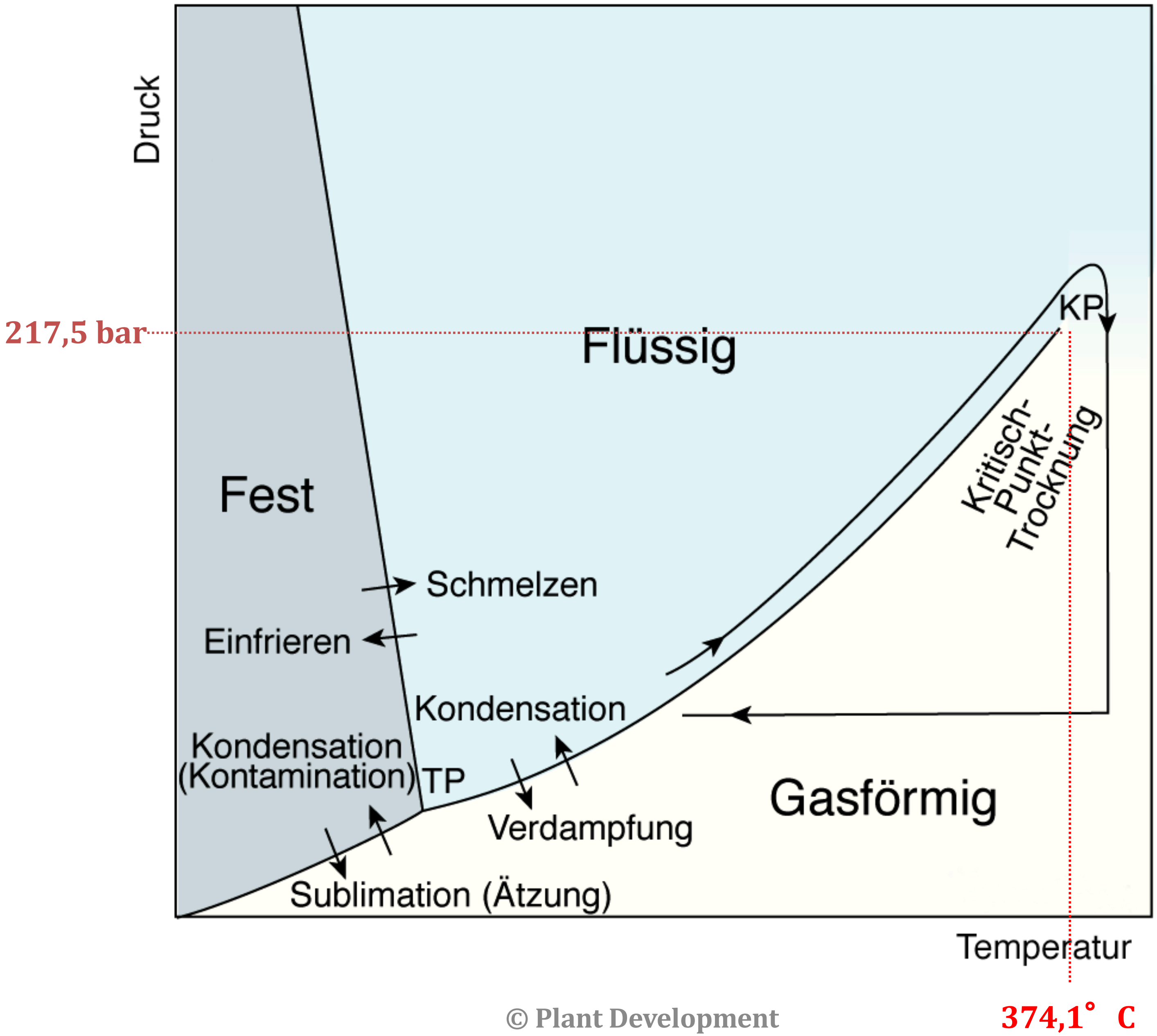
Post-fixation with OsO_4

Dehydration with acetone

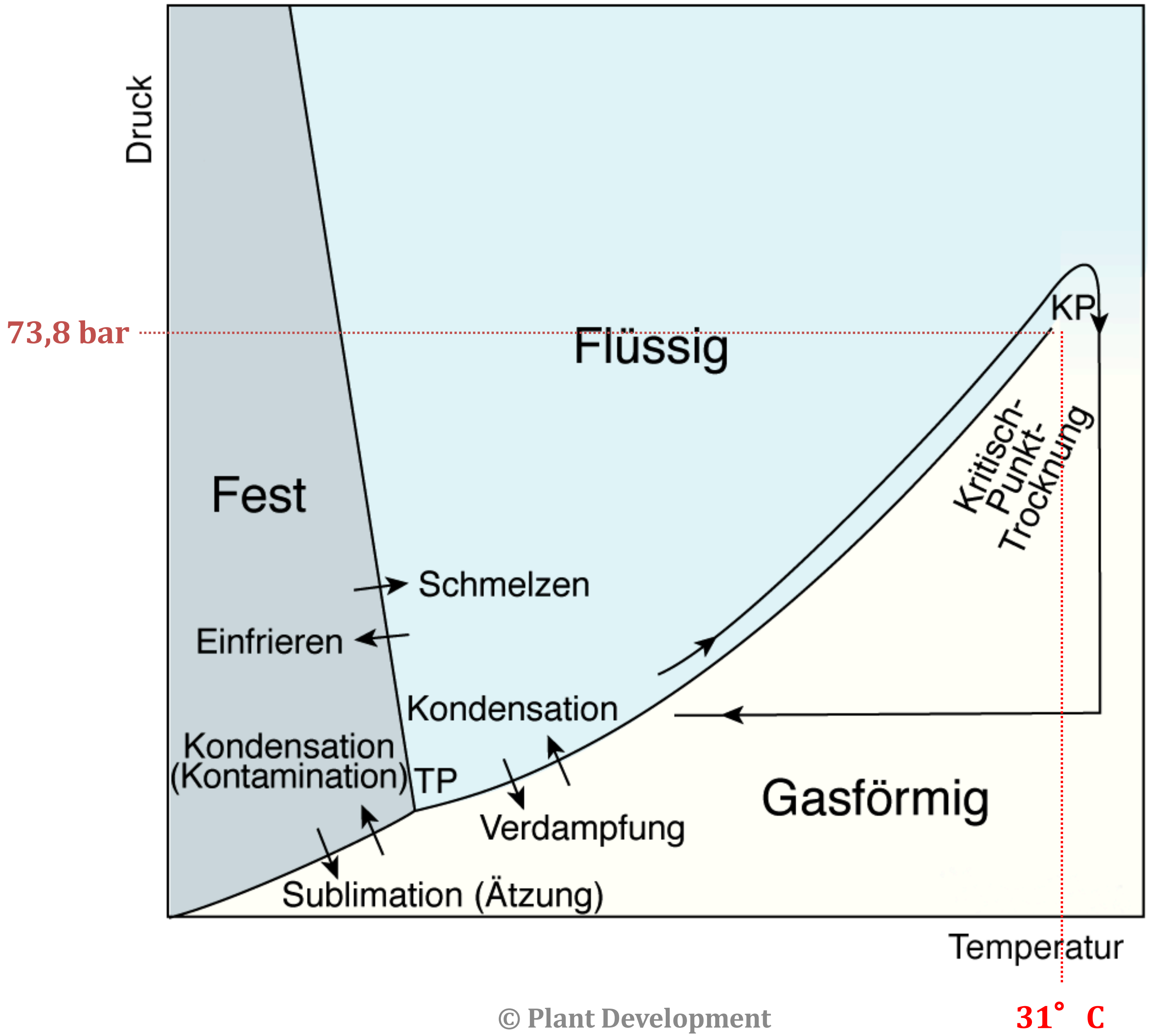
Sample preparation



Phase diagram water



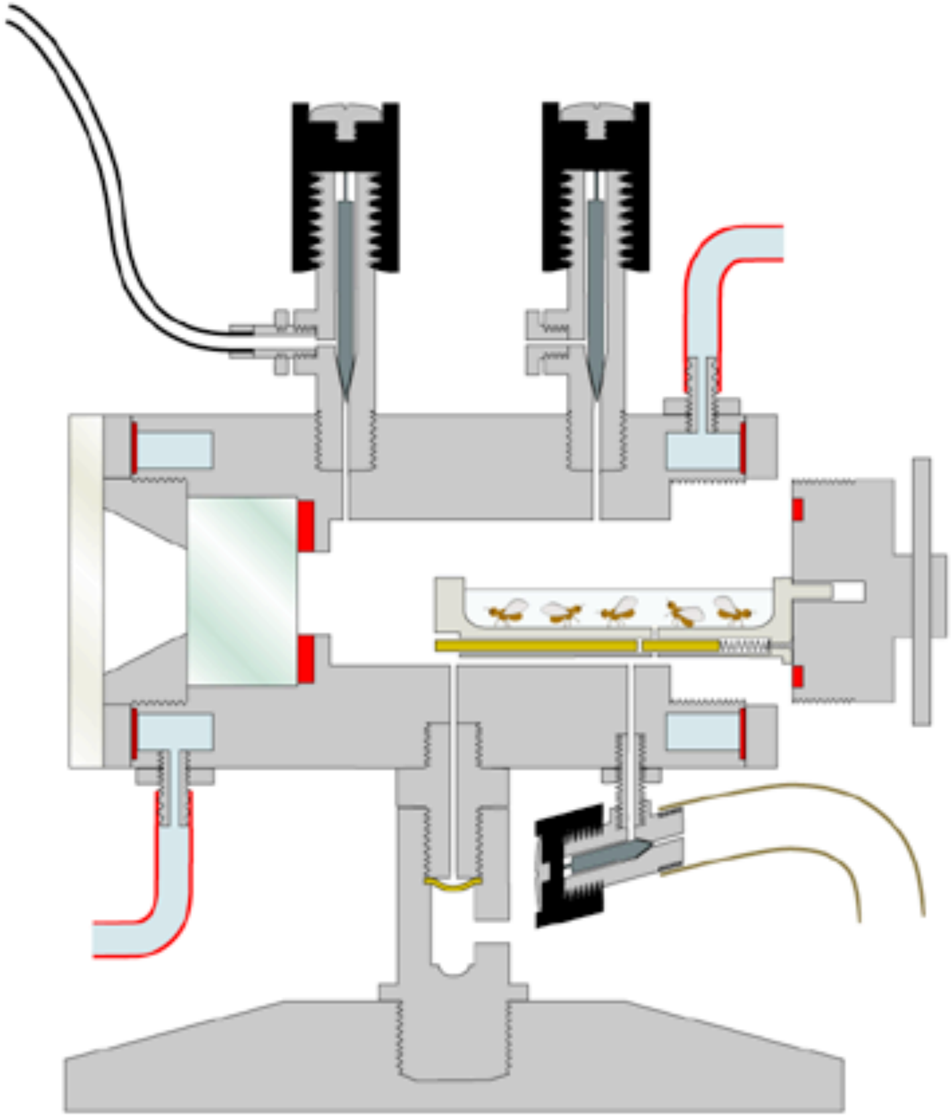
Phase diagram water CO_2



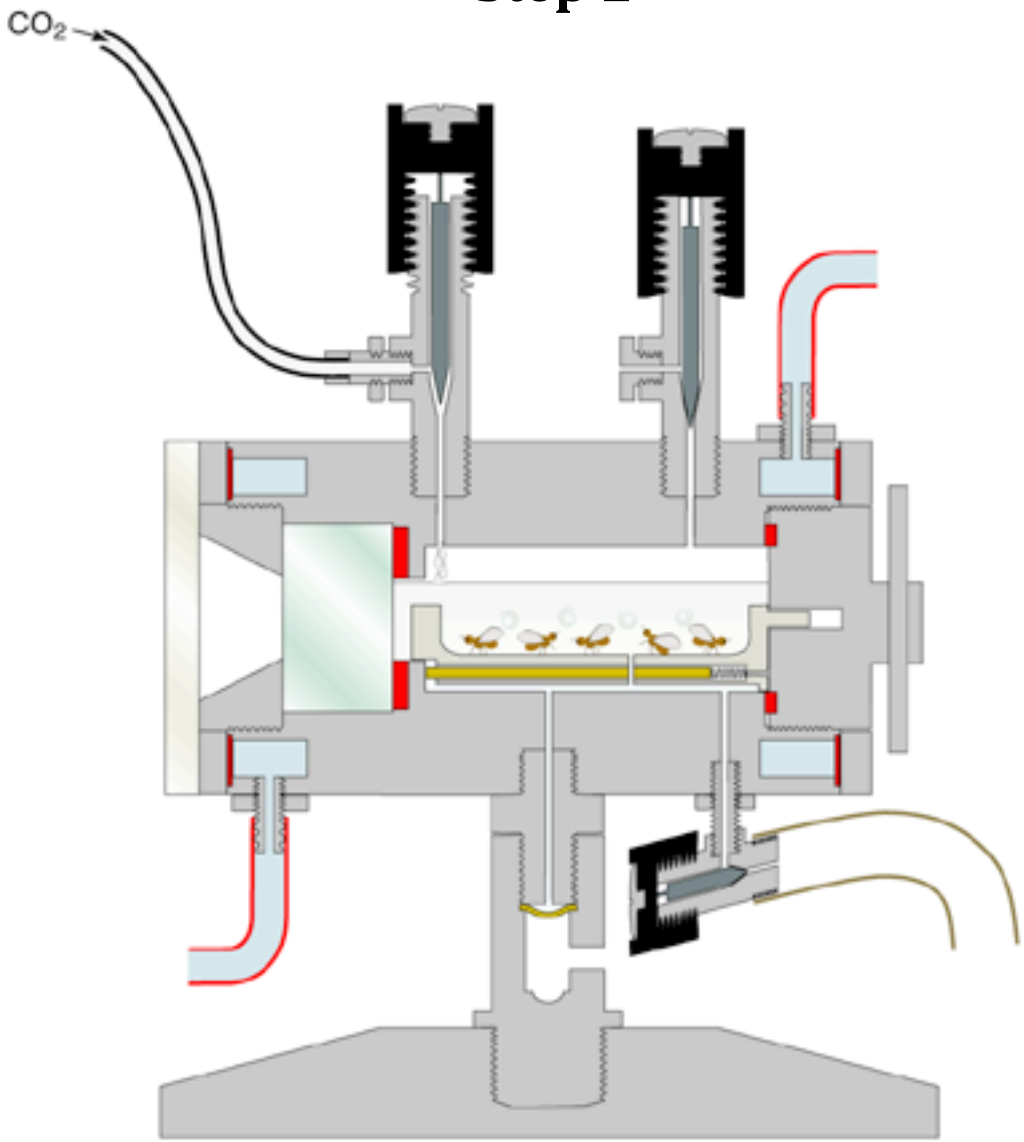
Critical-point-drying (CPT)

Sample preparation

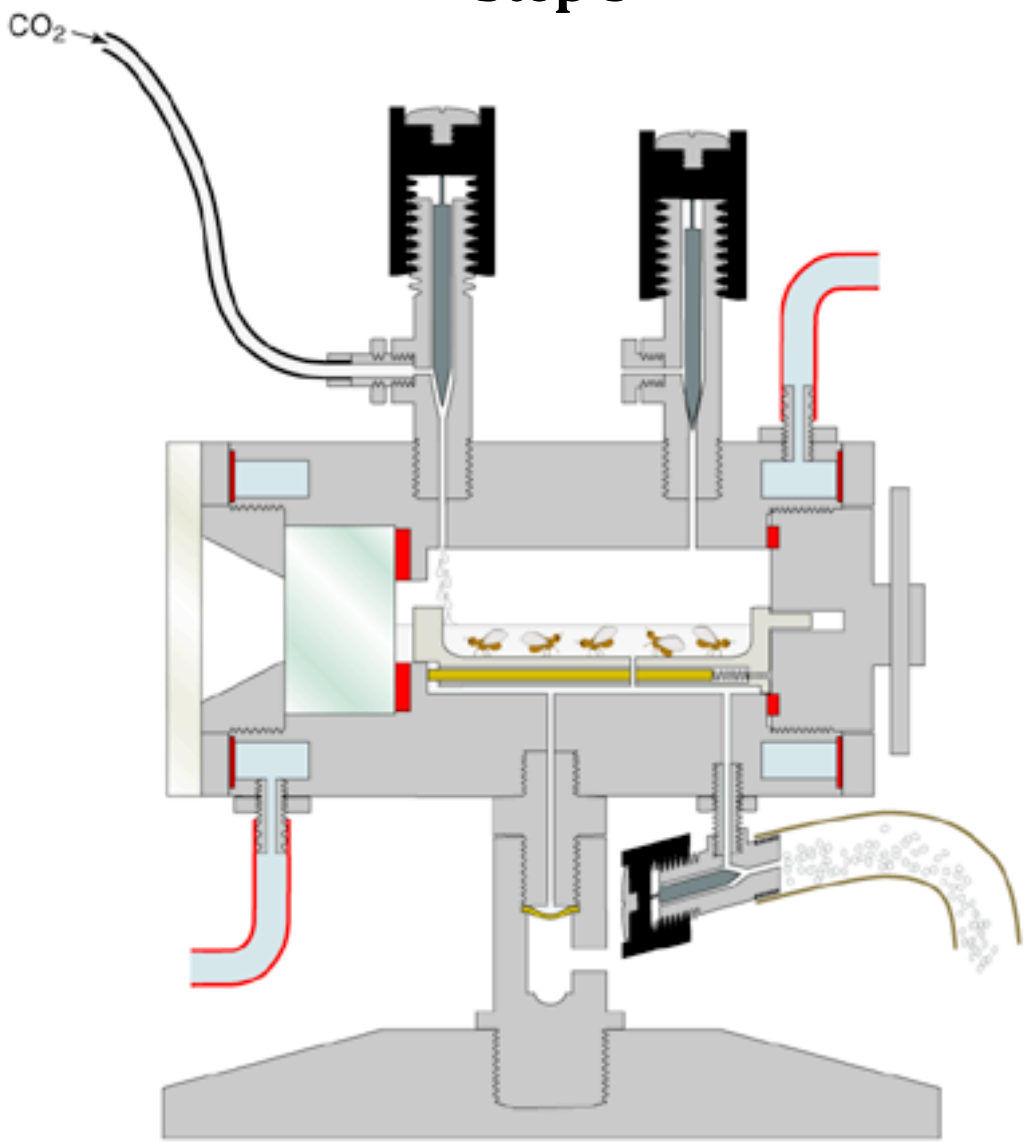
Step 1



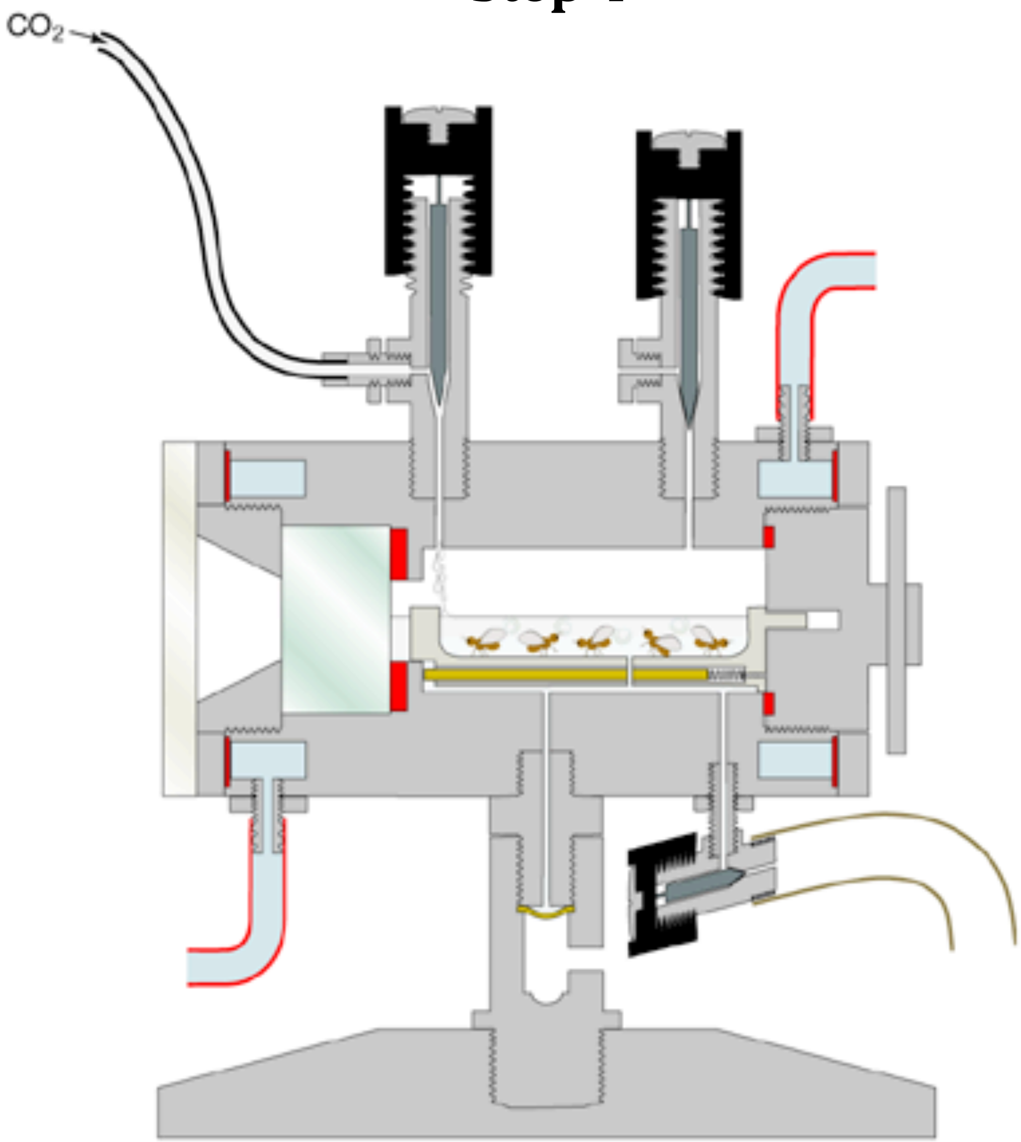
Step 2



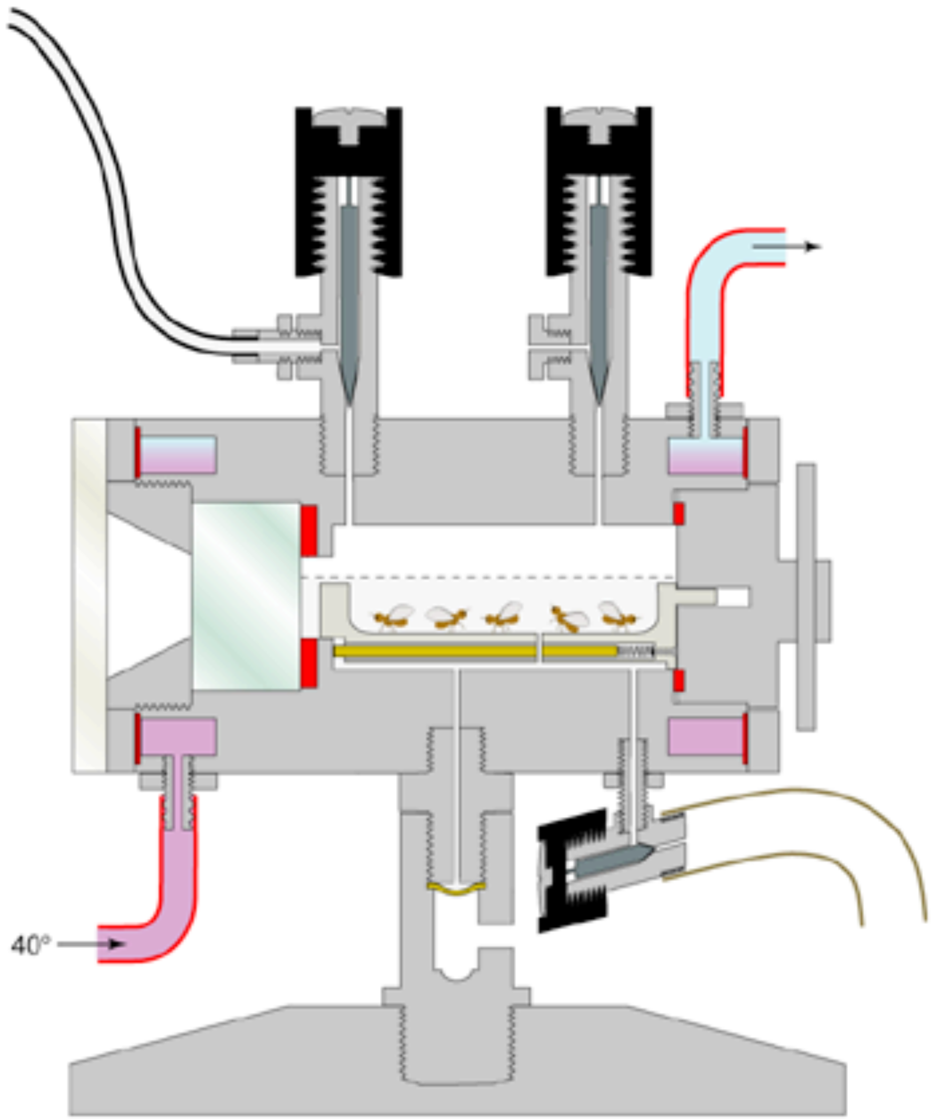
Step 3



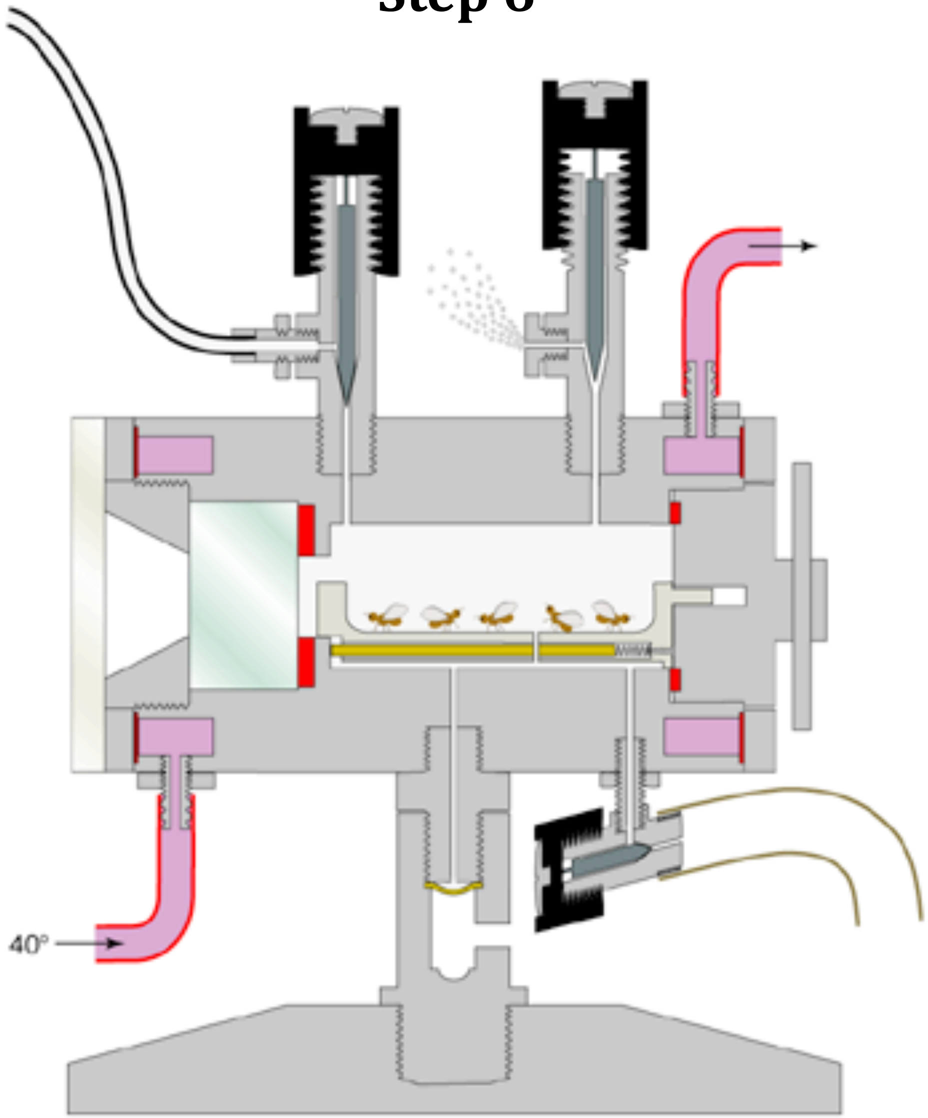
Step 4



Step 5



Step 6



Sample mounting

Vacuum metal evaporation – sputter coating

Leitsilber – the classic

Composition:
Silver colloid in eucalyptus oil

Pros:
fast
Very high conductivity

Cons:
Organic solvent
Gets sucked into sample
Moderate adhesion
Bright background

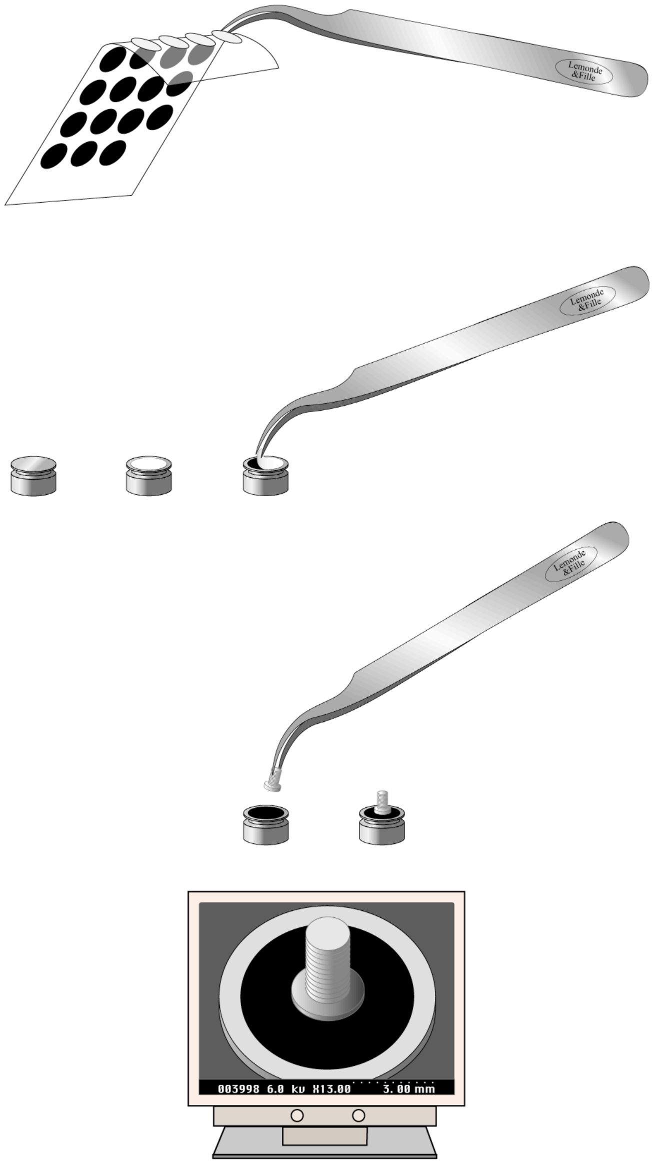
Leit-C (organic or aqueous)

Composition:
Carbon black/grime in butyl acetate
Carbon black/grime in aqueous suspension

Pros:
Dark background
Good conductivity
texture ± adjustable

Cons:
Organic solvent
Gets sucked into sample
Moderate adhesion
Aqueous solvent:
Hydration

Leittabs

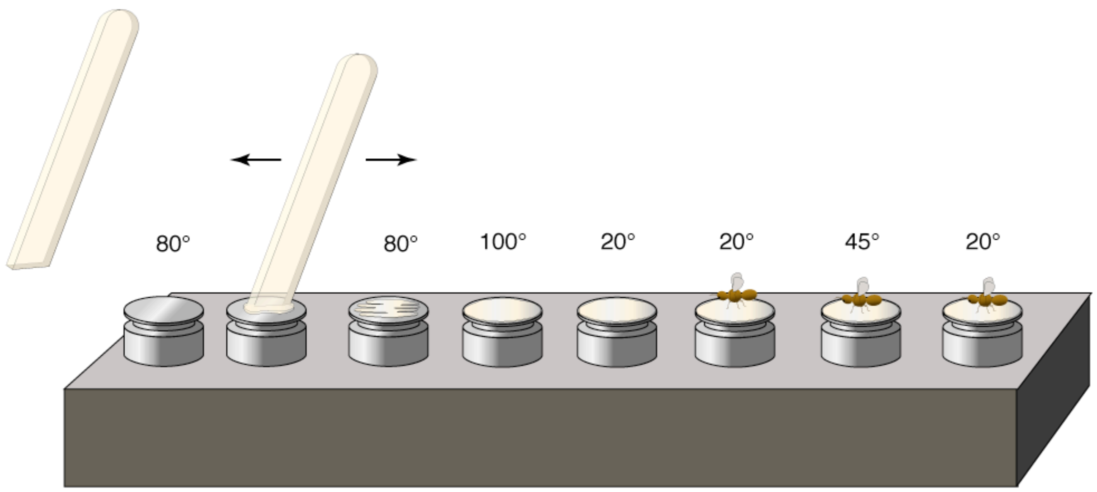


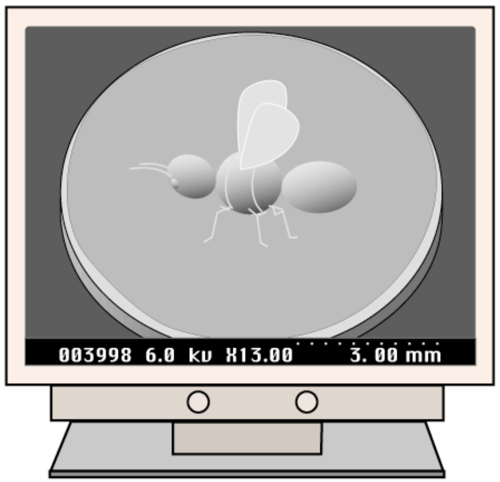
Composition:
Double-sided adhesive foil containing carbon black/grime

Pros:
Very fast
Good adhesion
Dark background

Cons:
Crack formation
Thermal drift
Moderate conductivity

Tempfix



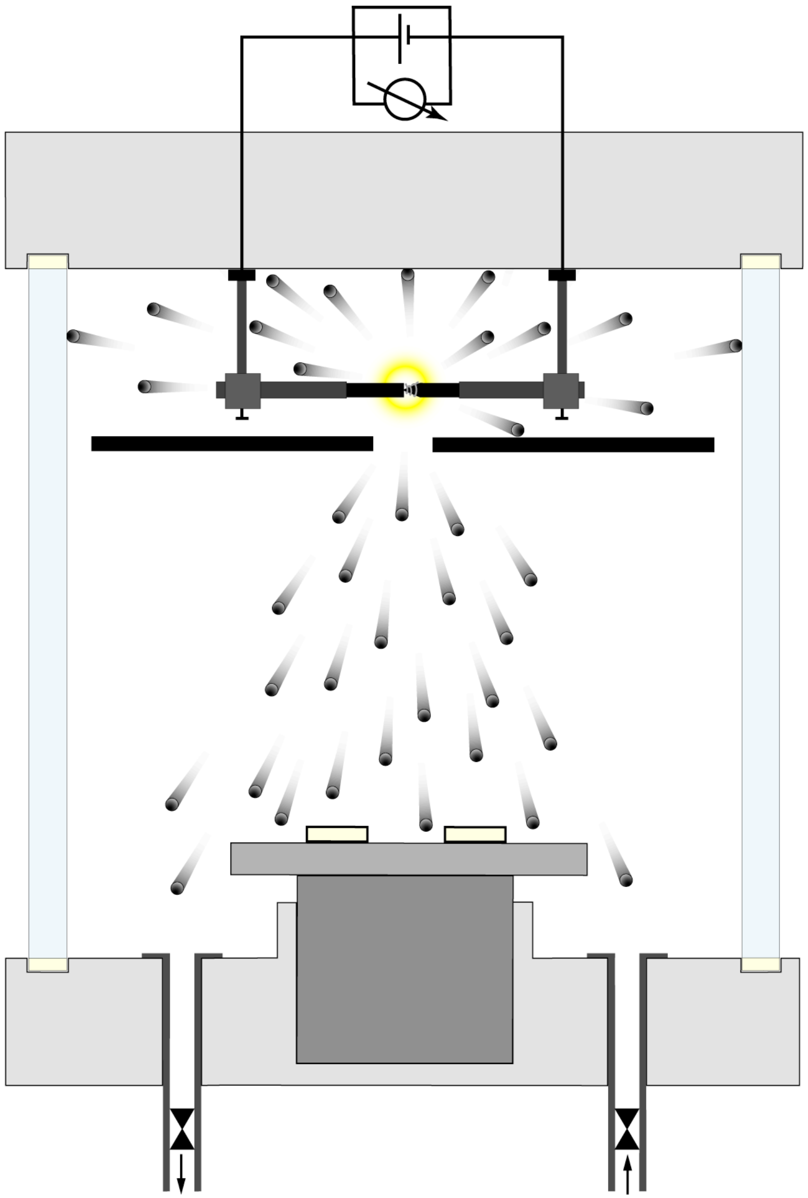


Composition:
Thermoplastic polymer

Pros:
Best adhesion
Very smooth background
Dark background
No crack formation
Sinking- depth of sample „adjustable“
Almost no thermical drift

Cons:
Often time consuming
Not conductive
Application demands great skill

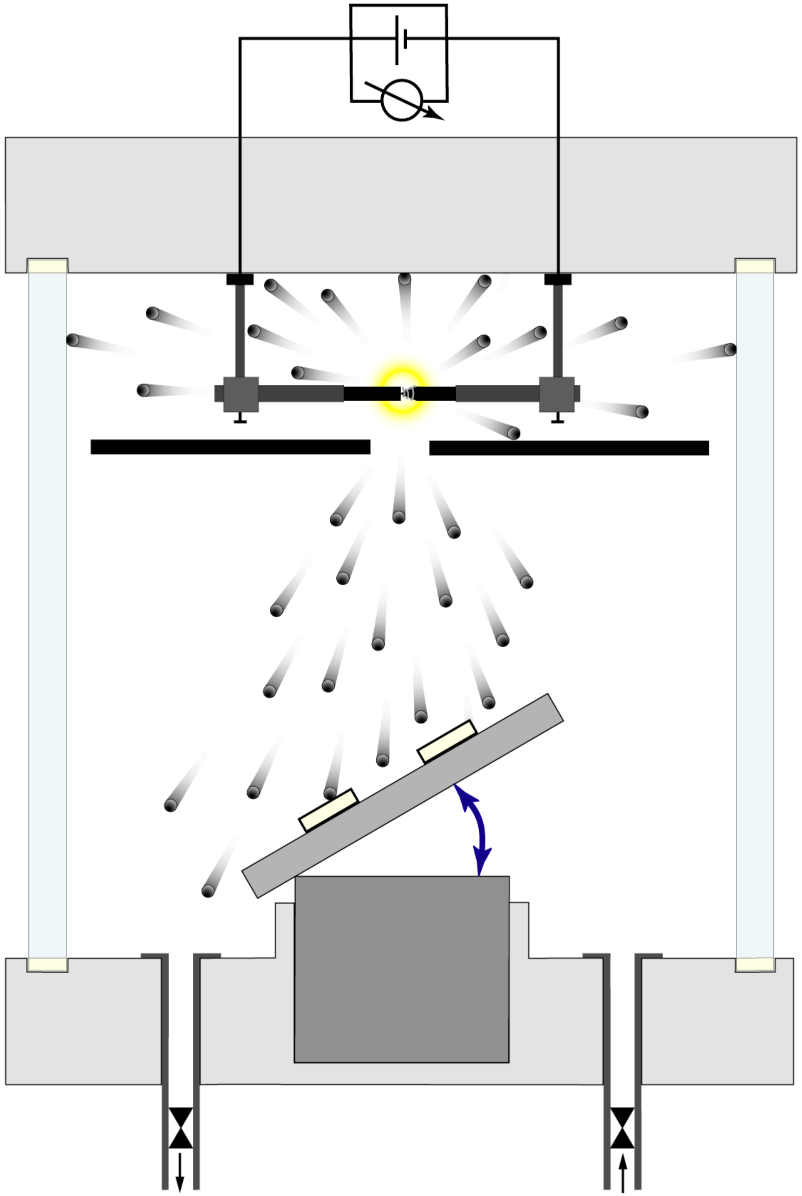
Vacuum metal evaporation



Properties
High vacuum
Hot particles
„Light and shadow“
Oblique evaporation

Parameters:
distance: sample - metal
Quality of vacuum
Amount of metal

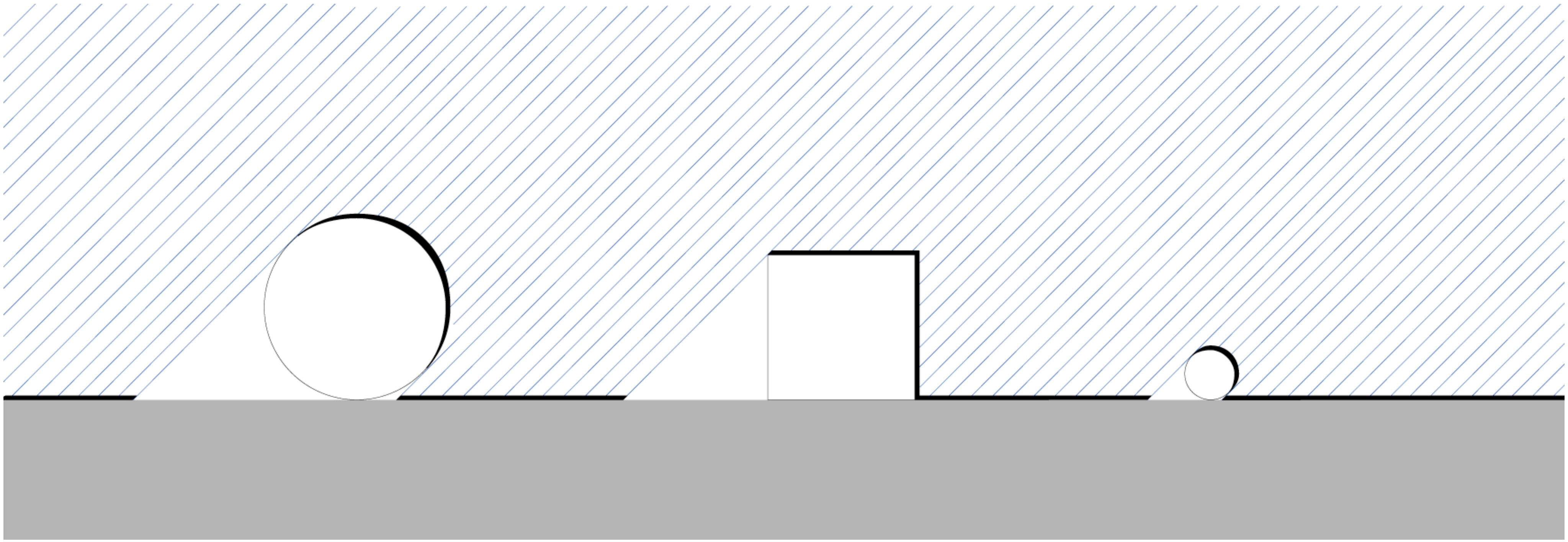
Vacuum metal evaporation



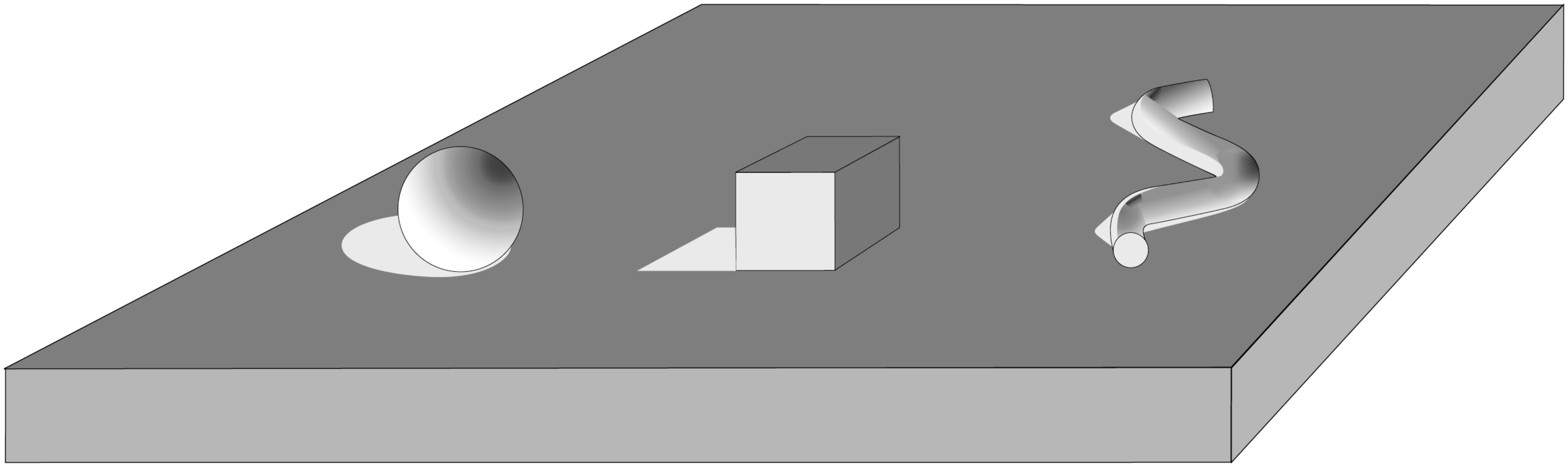
Properties
High vacuum
Hot particles
„Light and shadow“
Oblique evaporation

Parameters:
distance: sample - metal
Quality of vacuum
Amount of metal

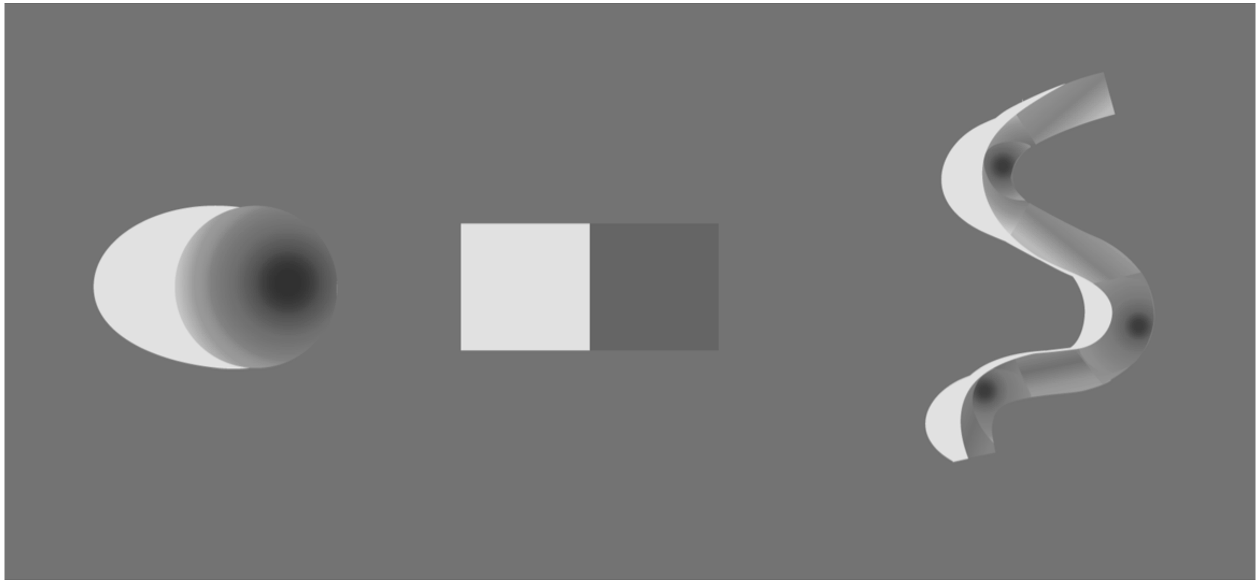
Oblique evaporation: „Light and shadow"

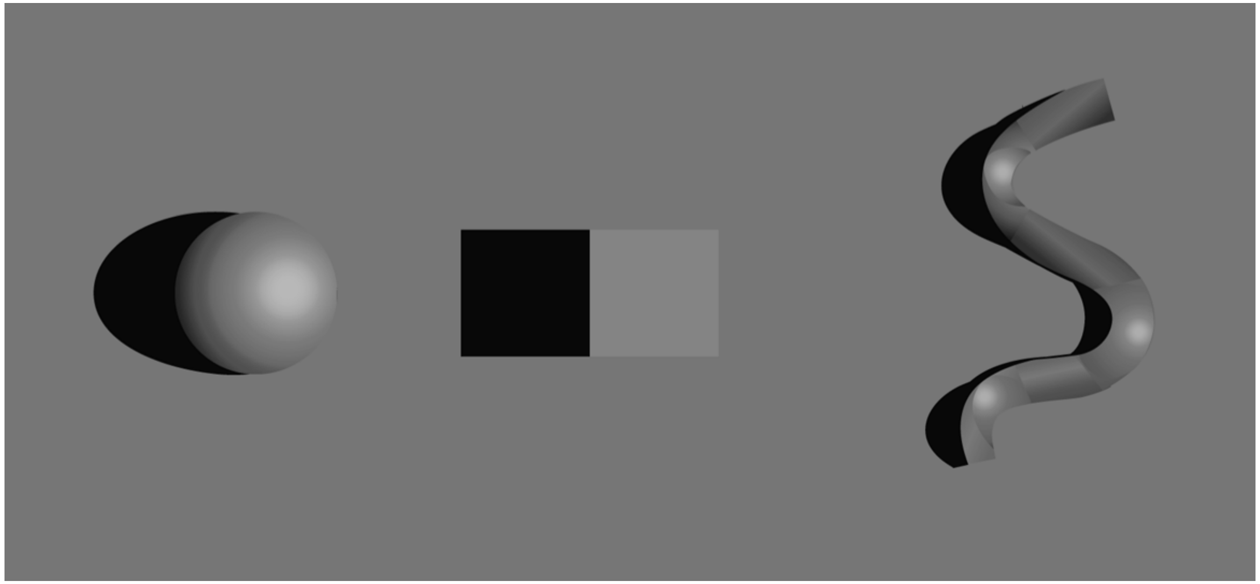


Oblique evaporation: „Light and shadow“ (TEM)

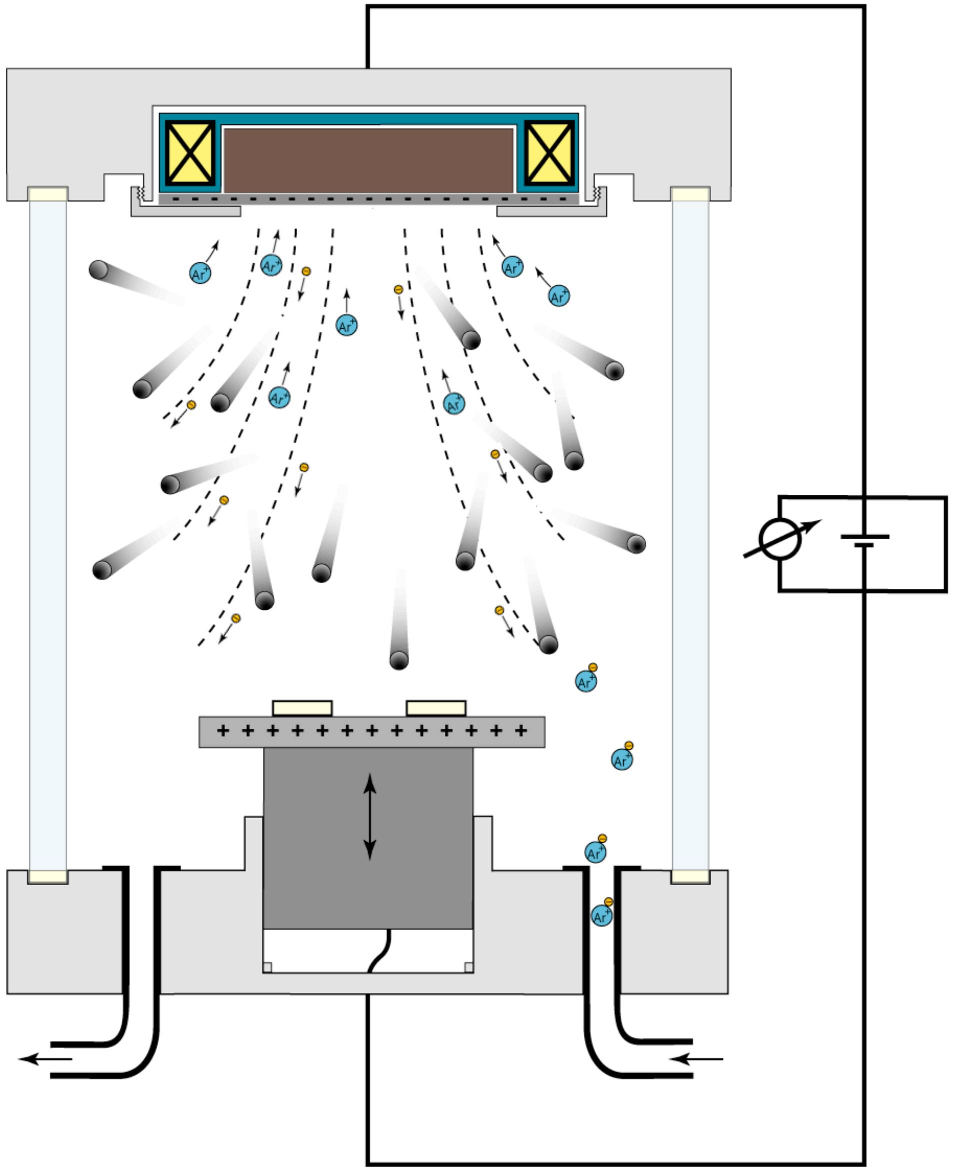


Vacuum metal evaporation





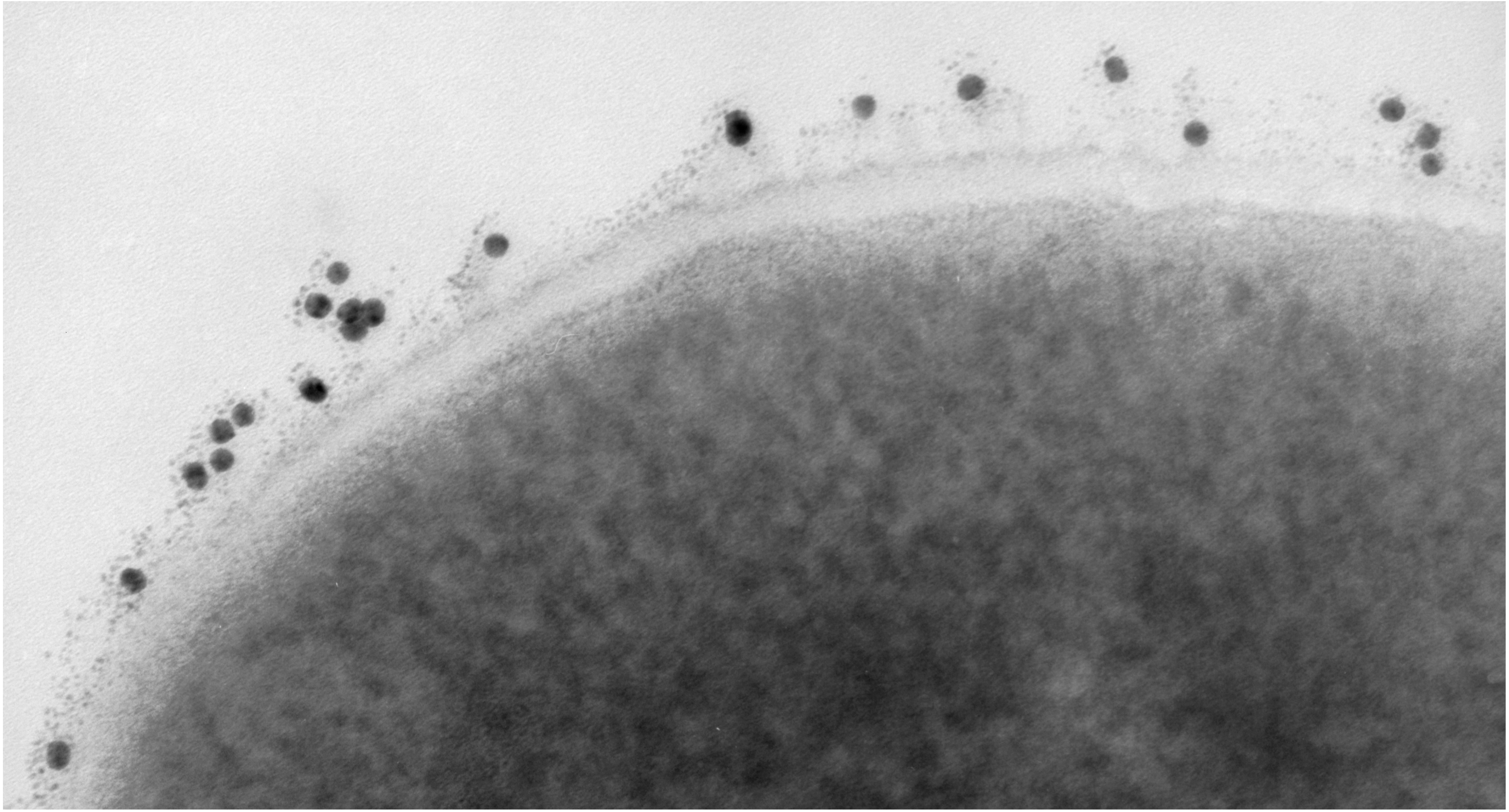
Sputter coating



Properties
Moderate vacuum
Cold particles
± even layer

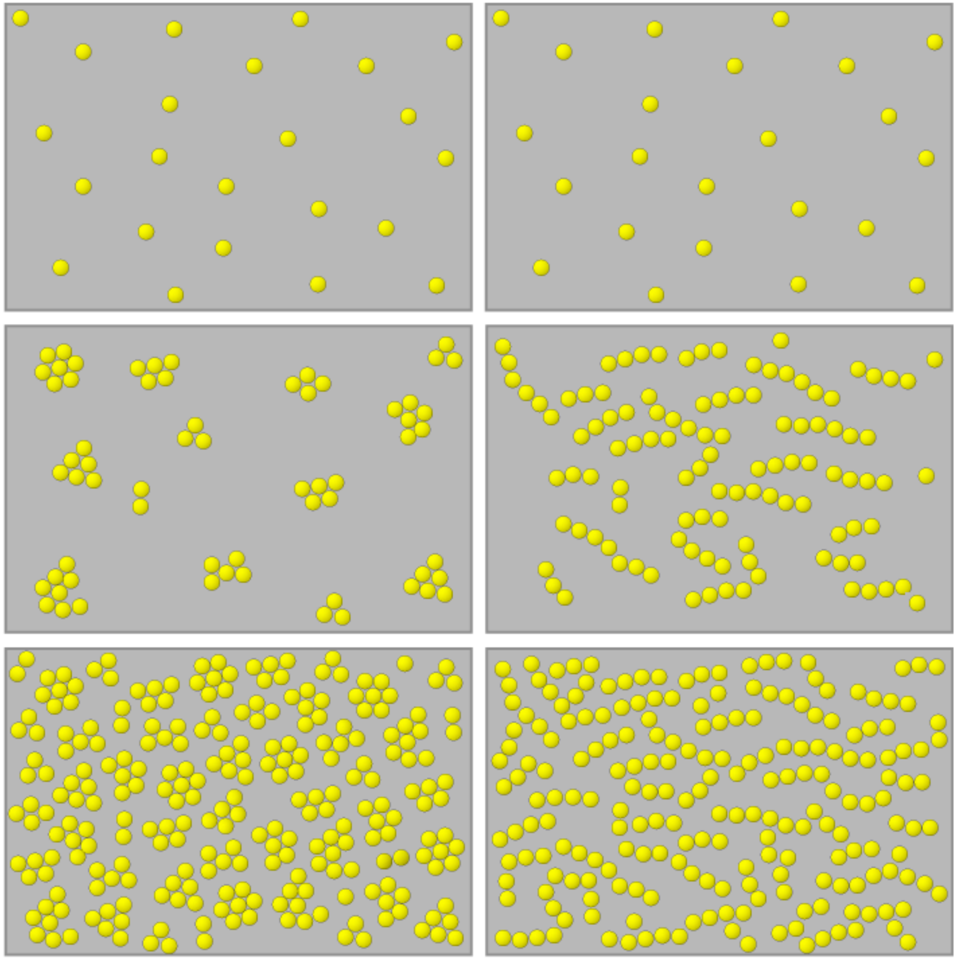
Parameters:
Distance: sample-target
Quality of vacuum
High tension
Current flow (argon ions)
Duration

Sputter coating



E. faecalis **anti-AG/anti-Kaninchen-10 nm Gold + 2 nm Au/Pd**

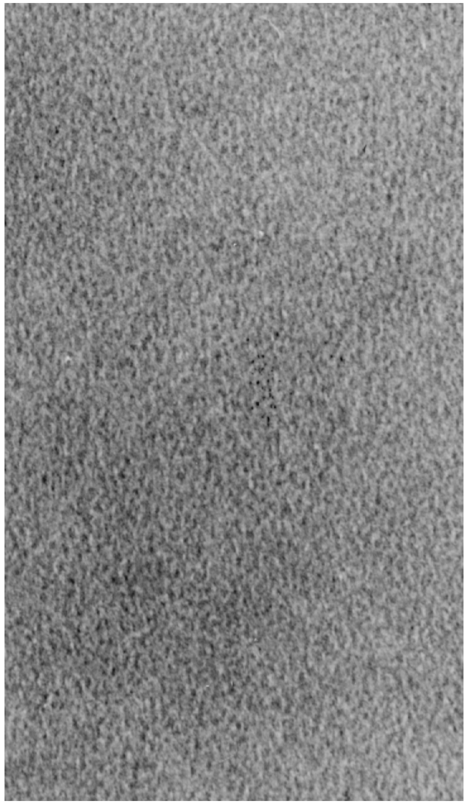
Formation of clusters



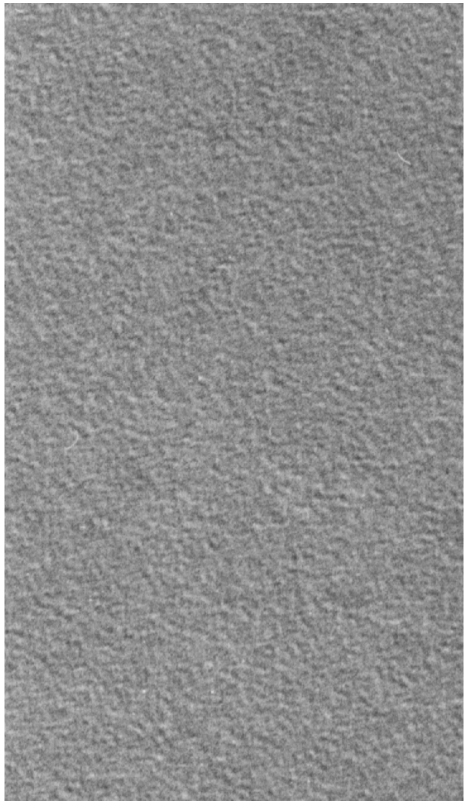
Gold:	strong
Gold/palladium (80/20):	weak
Platinum:	no

Comparison of Sputter coating materials (20 nm layer thickness each)

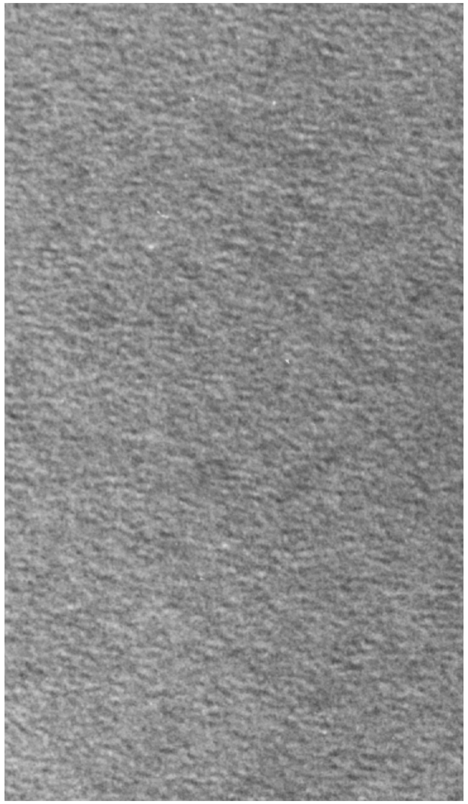
. = 1 nm



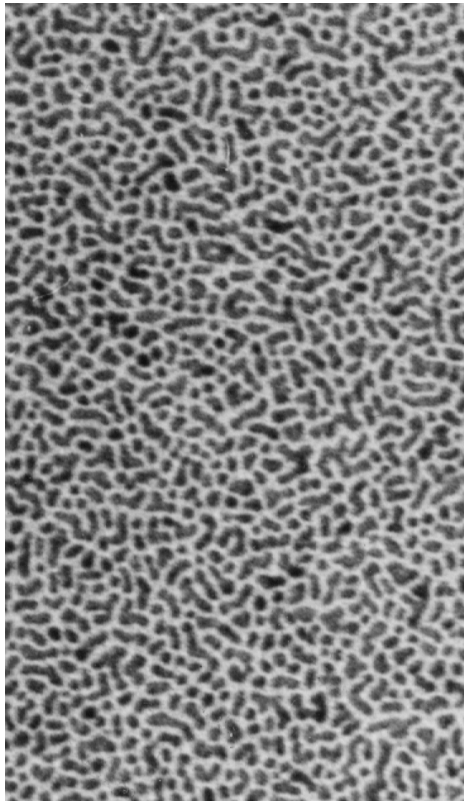
Cr
Chromium
Atomic number: 24
Atomic mass: 53
Density: 7,2



Ta
Tantalum
Atomic number: 73
Atomic mass: 181
Density: 16,6

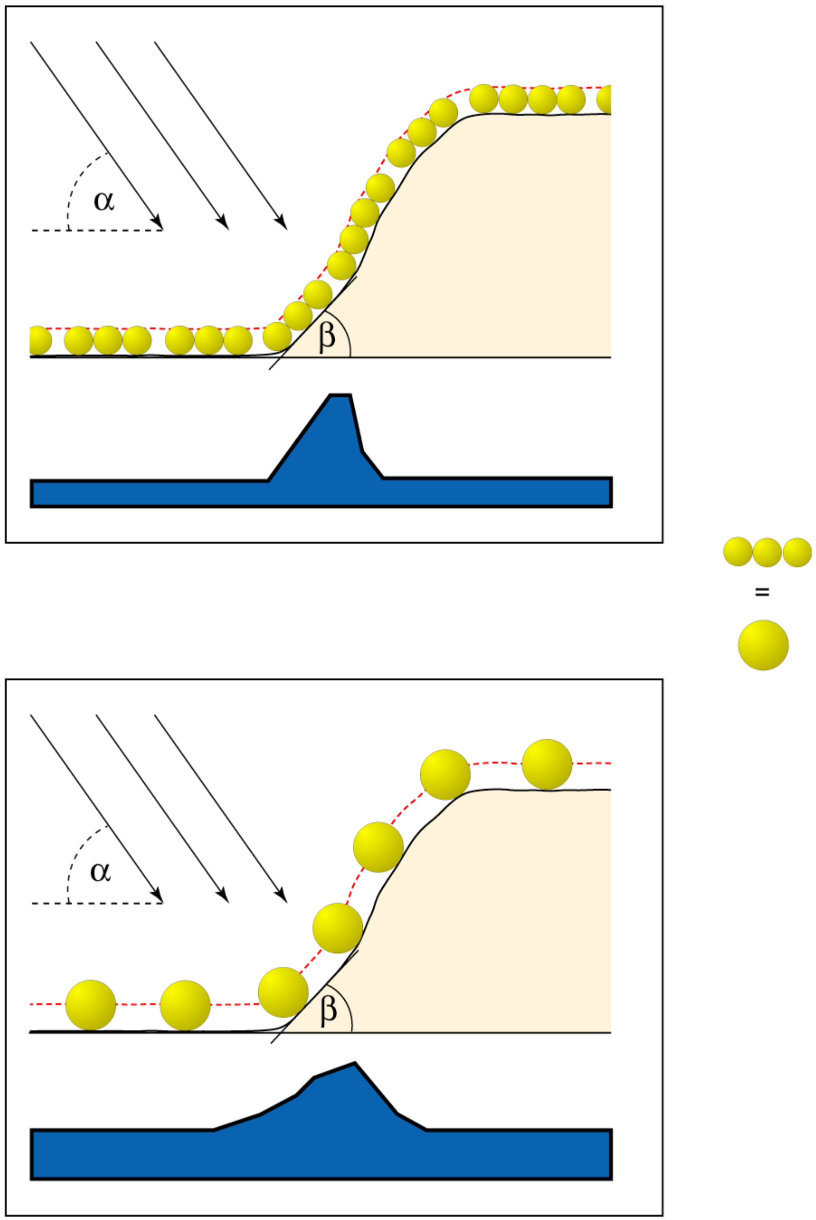


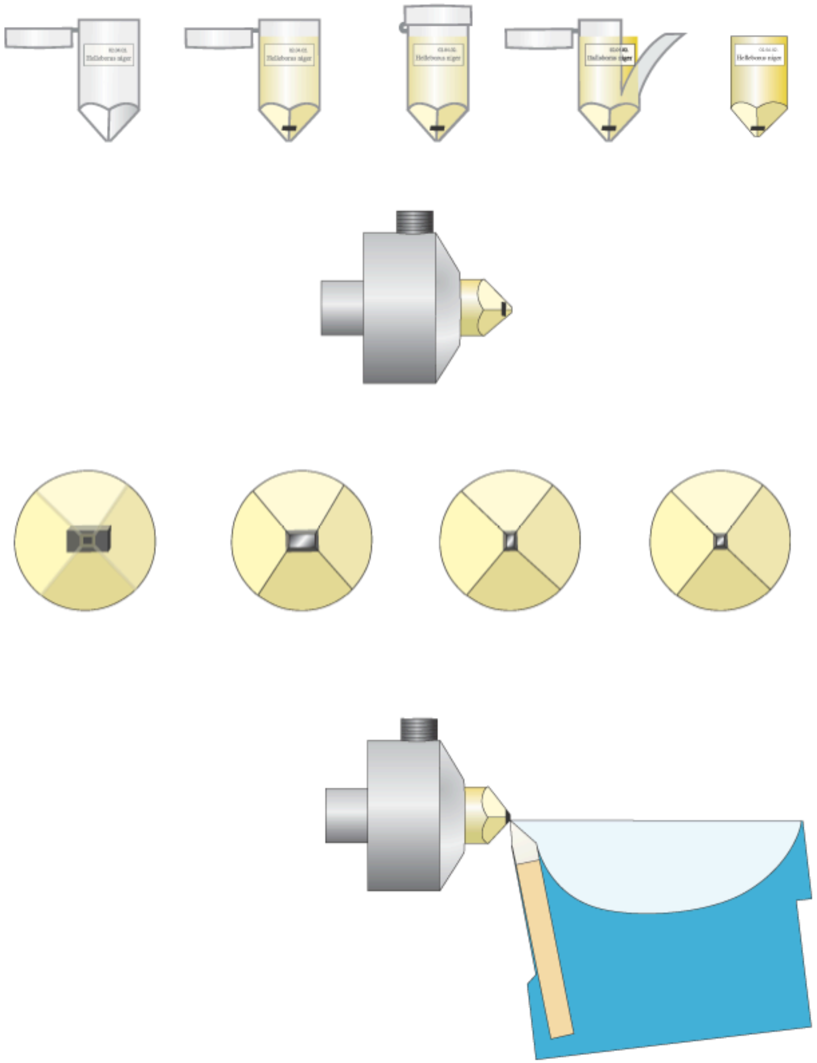
W
Tungsten
Atomic number: 74
Atomic mass: 184
Density: 19,3



Au
Gold
Atomic number: 79
Atomic mass: 197
Density: 19,3

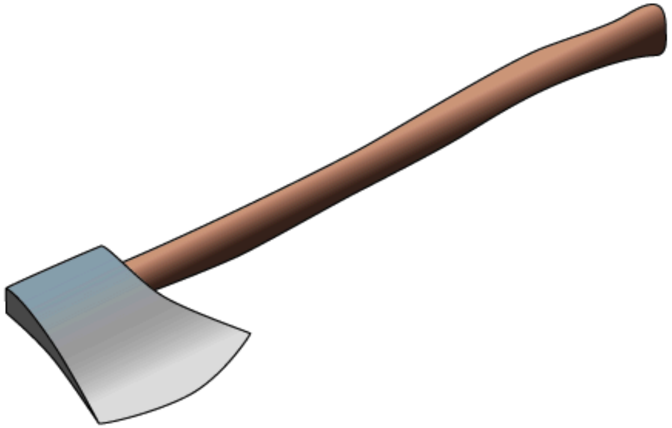
Layer thickness – signal – resolution

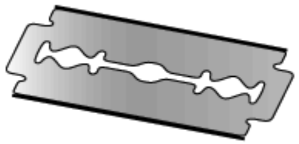




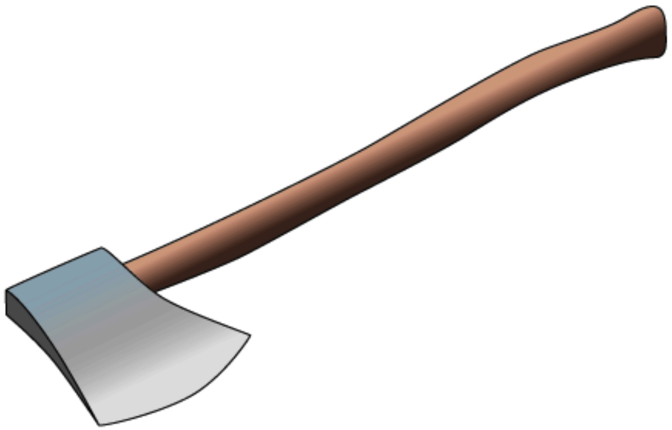
Ultramicrotomes used for EM represent rotation microtomes.

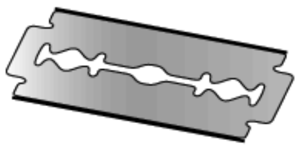
What is sharpness?

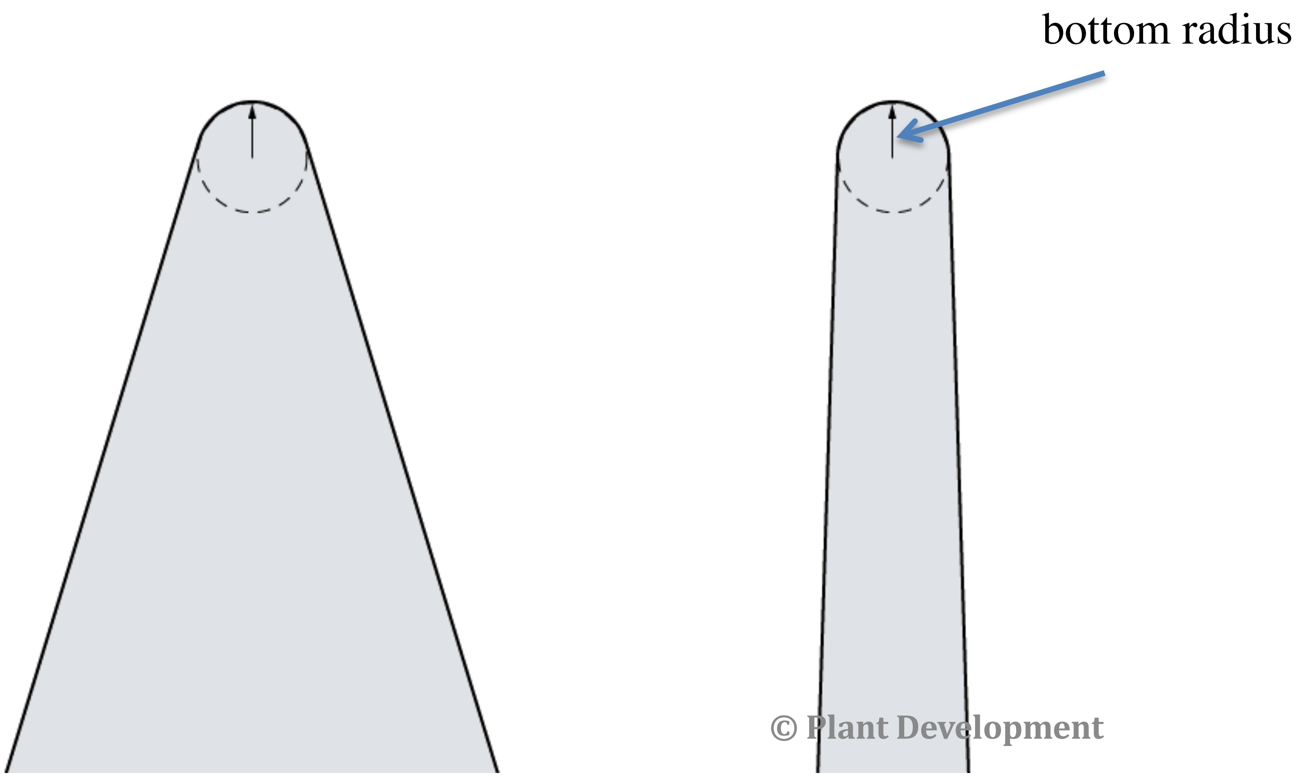




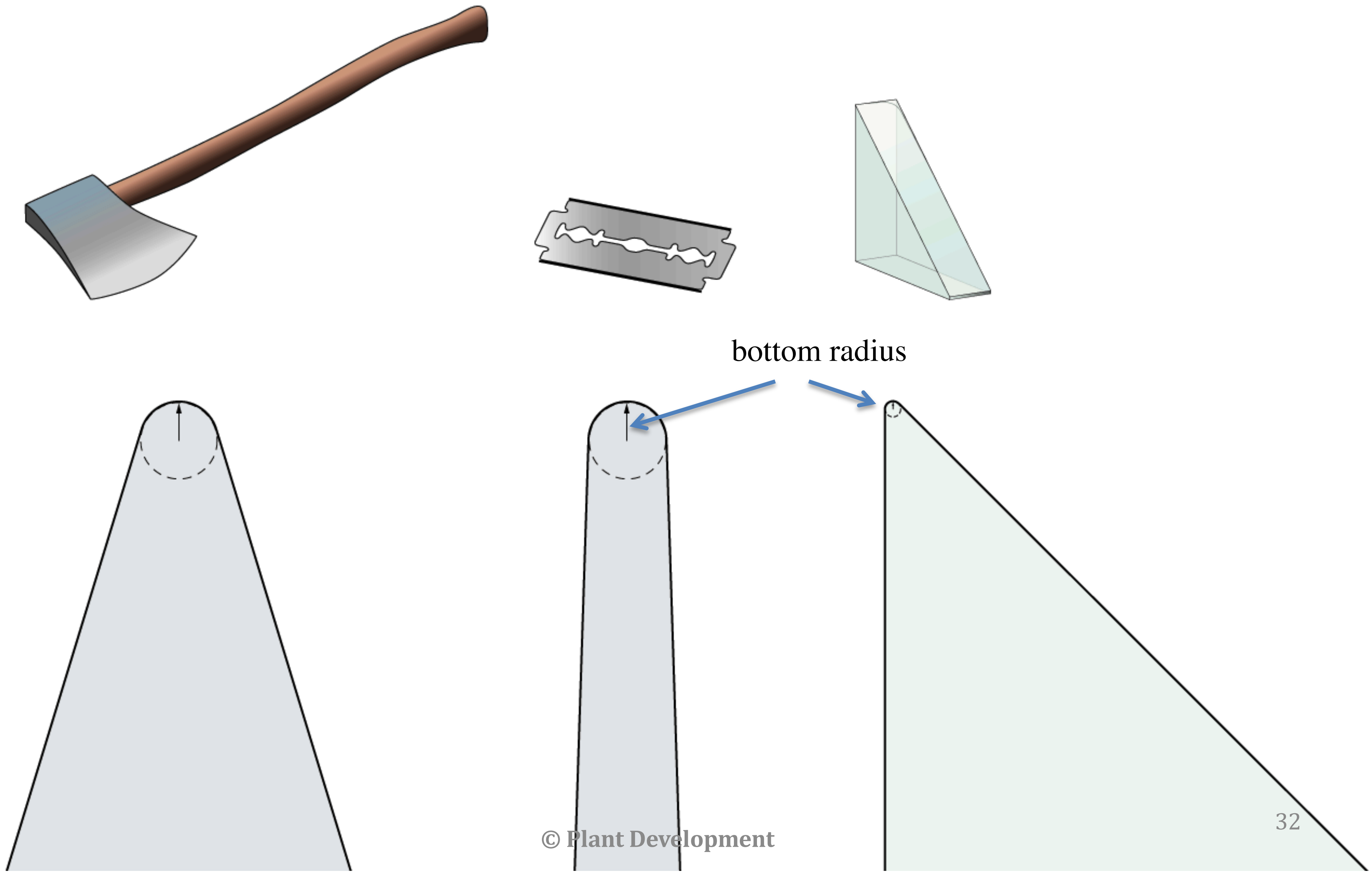
What is sharpness?







What is sharpness?

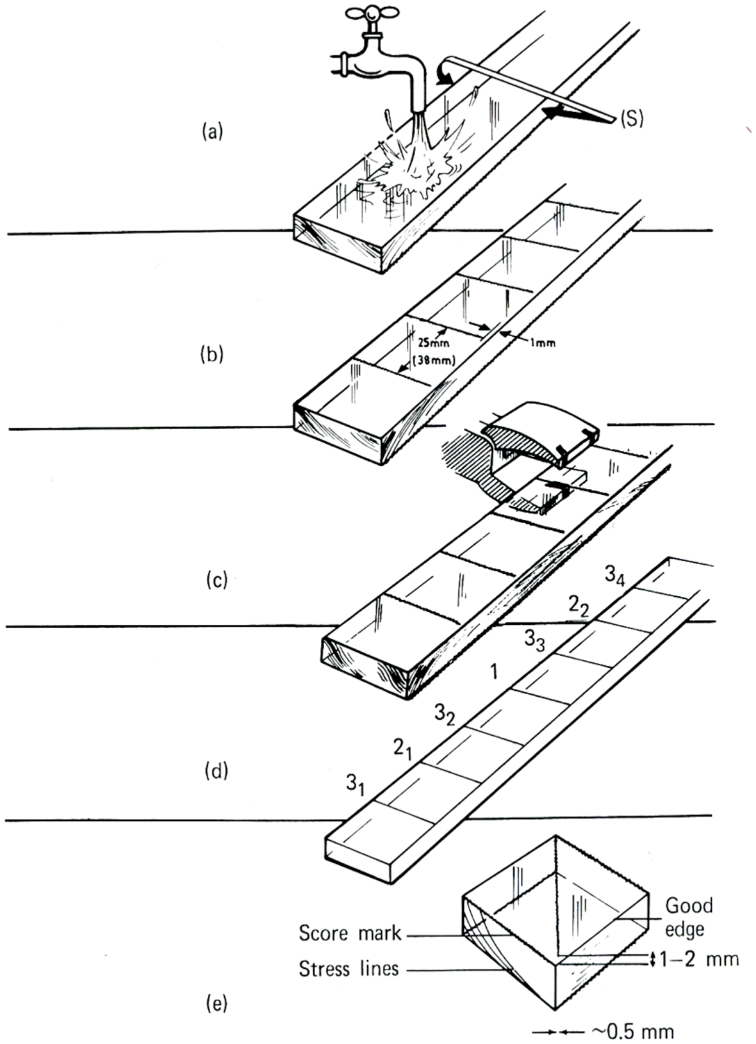


Production of glass knives

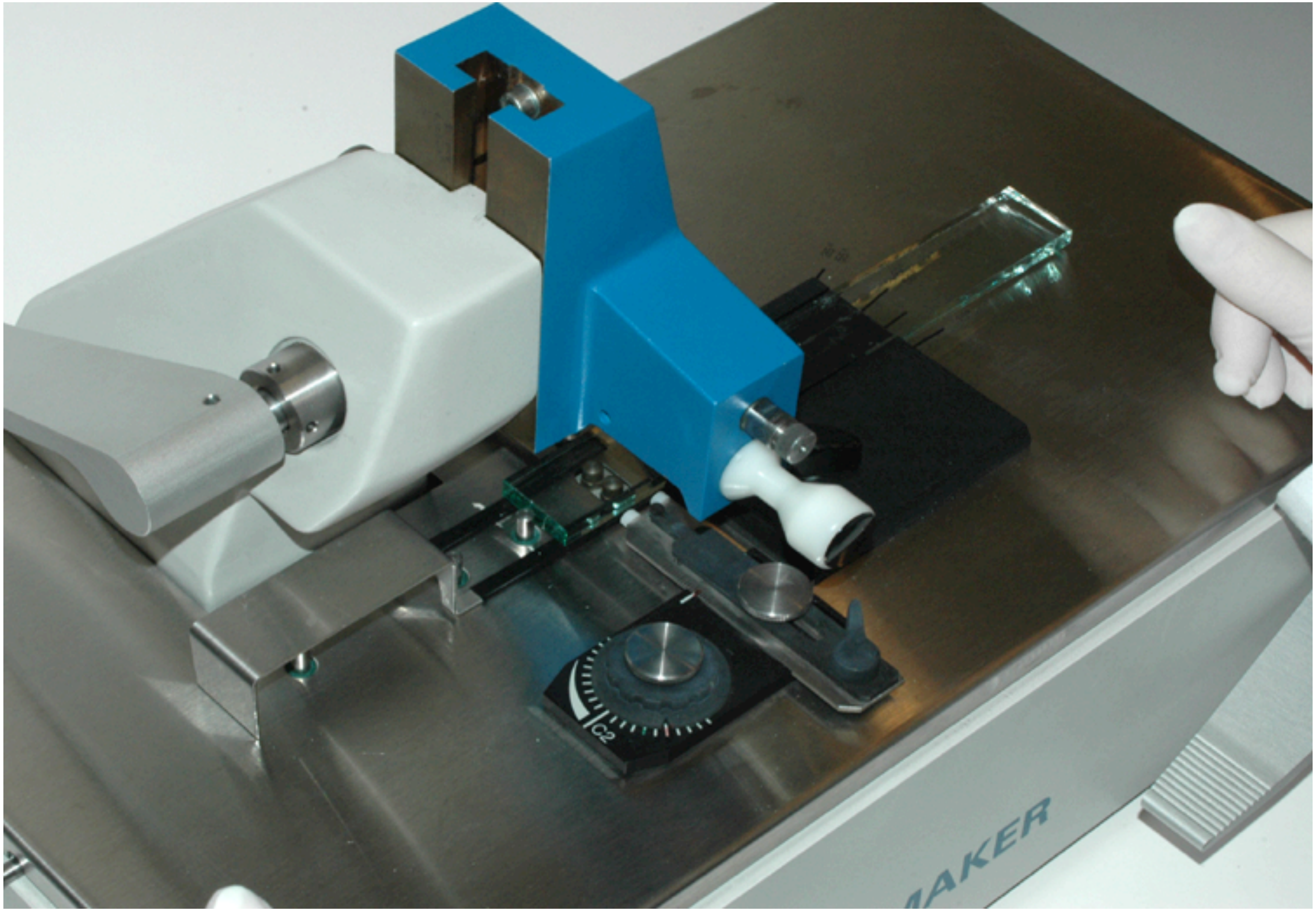
By using the Knifemaker, small glass squares are made from long glass bars (scratching and breaking)

The glass squares are mounted into the Knifemaker. Afterwards they are scratched diagonally and finally broken.

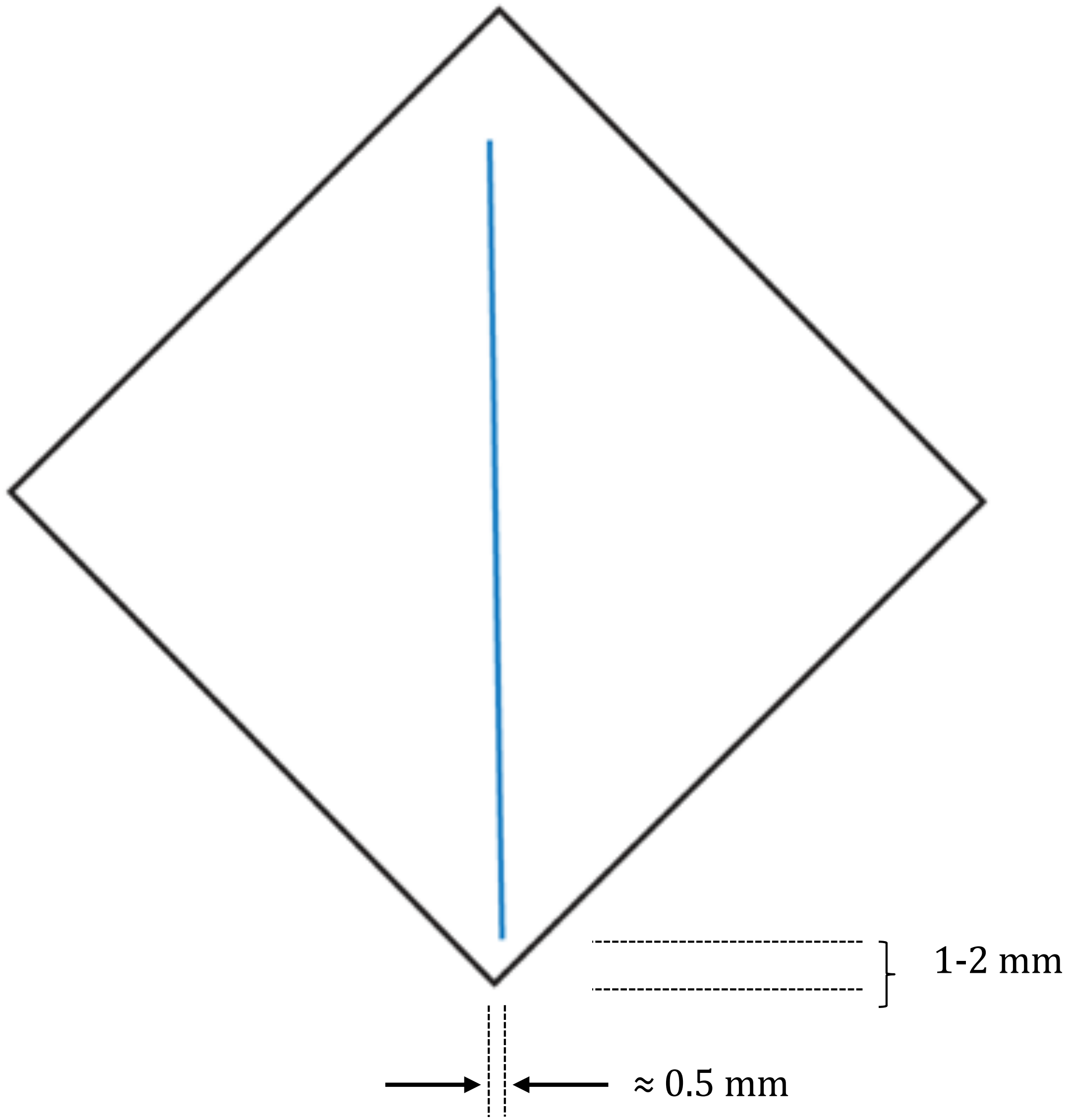
This leads to triangular glass knives.



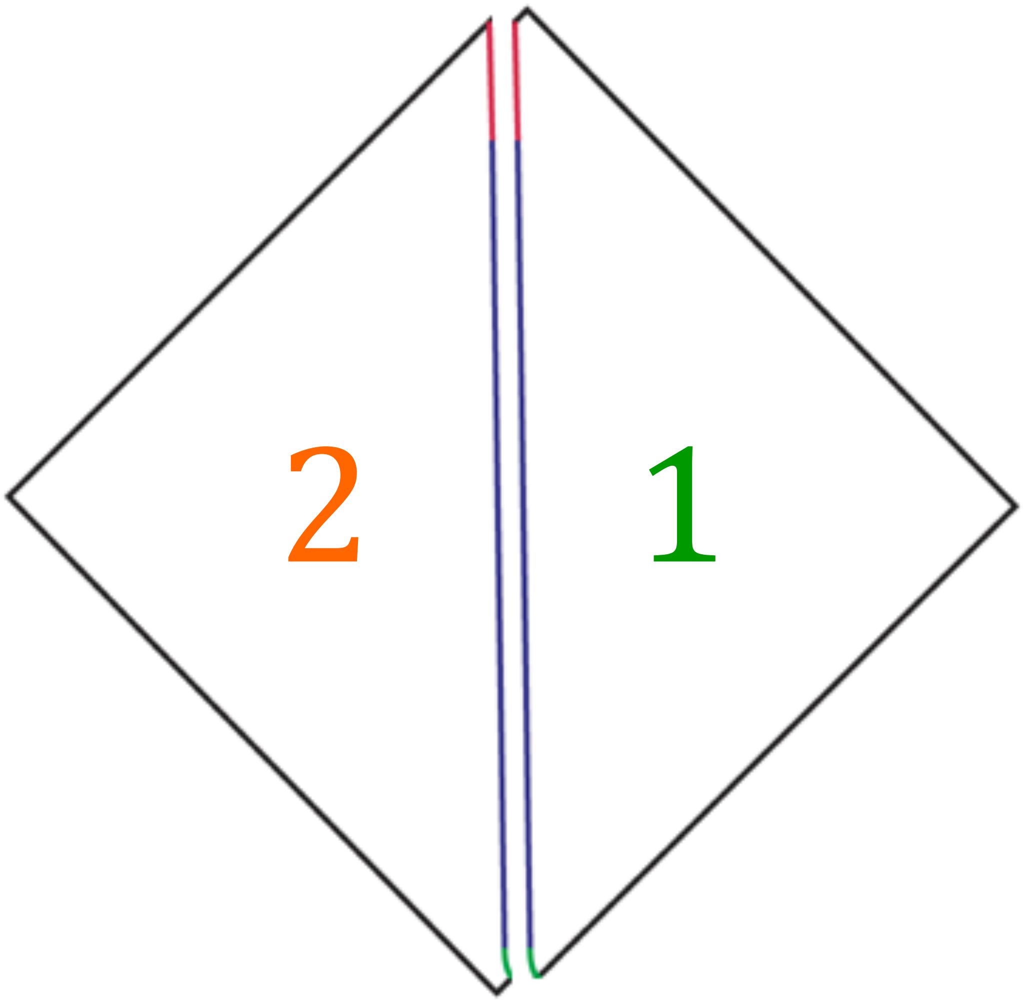
Breaking glass squares



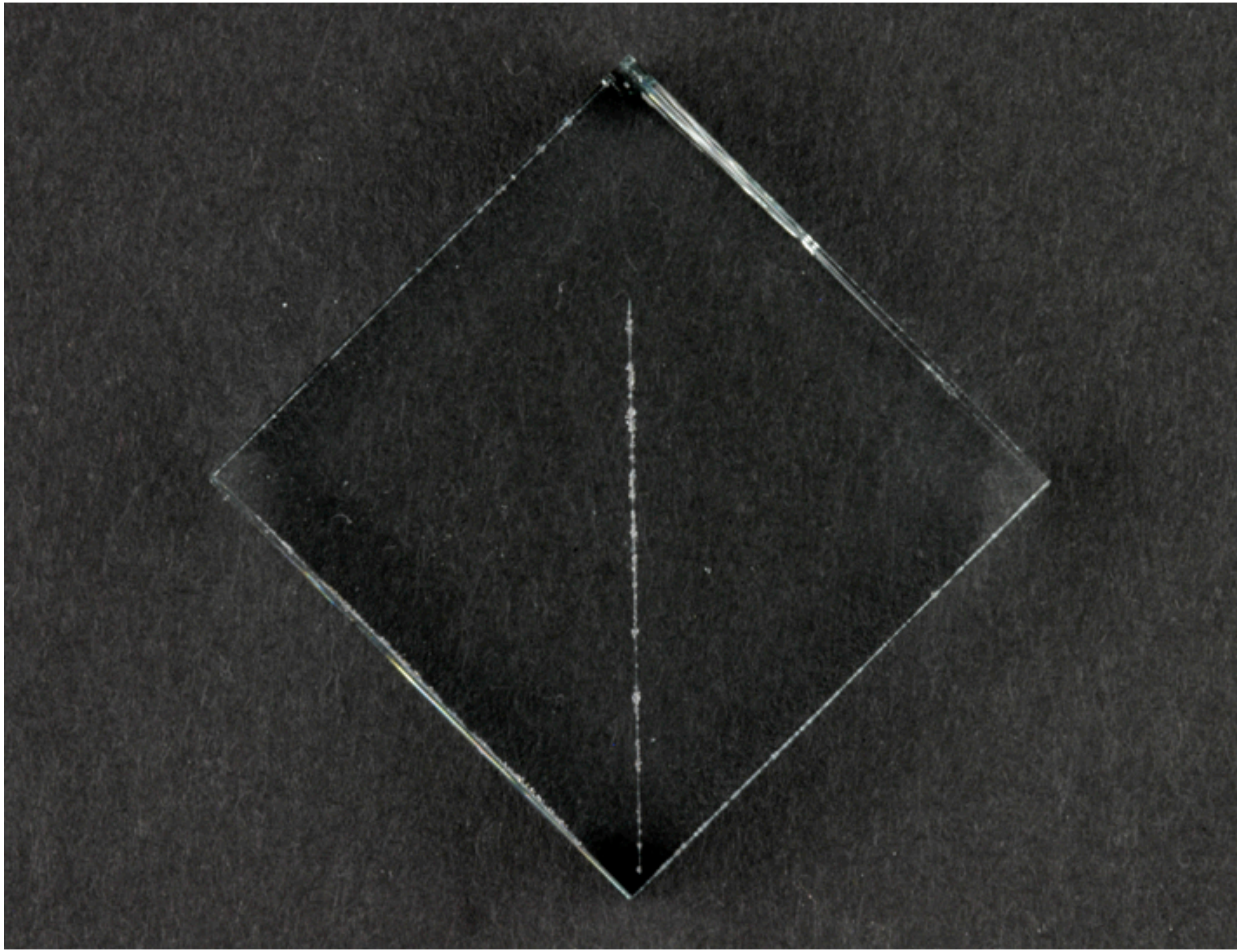
Ideal scratch line



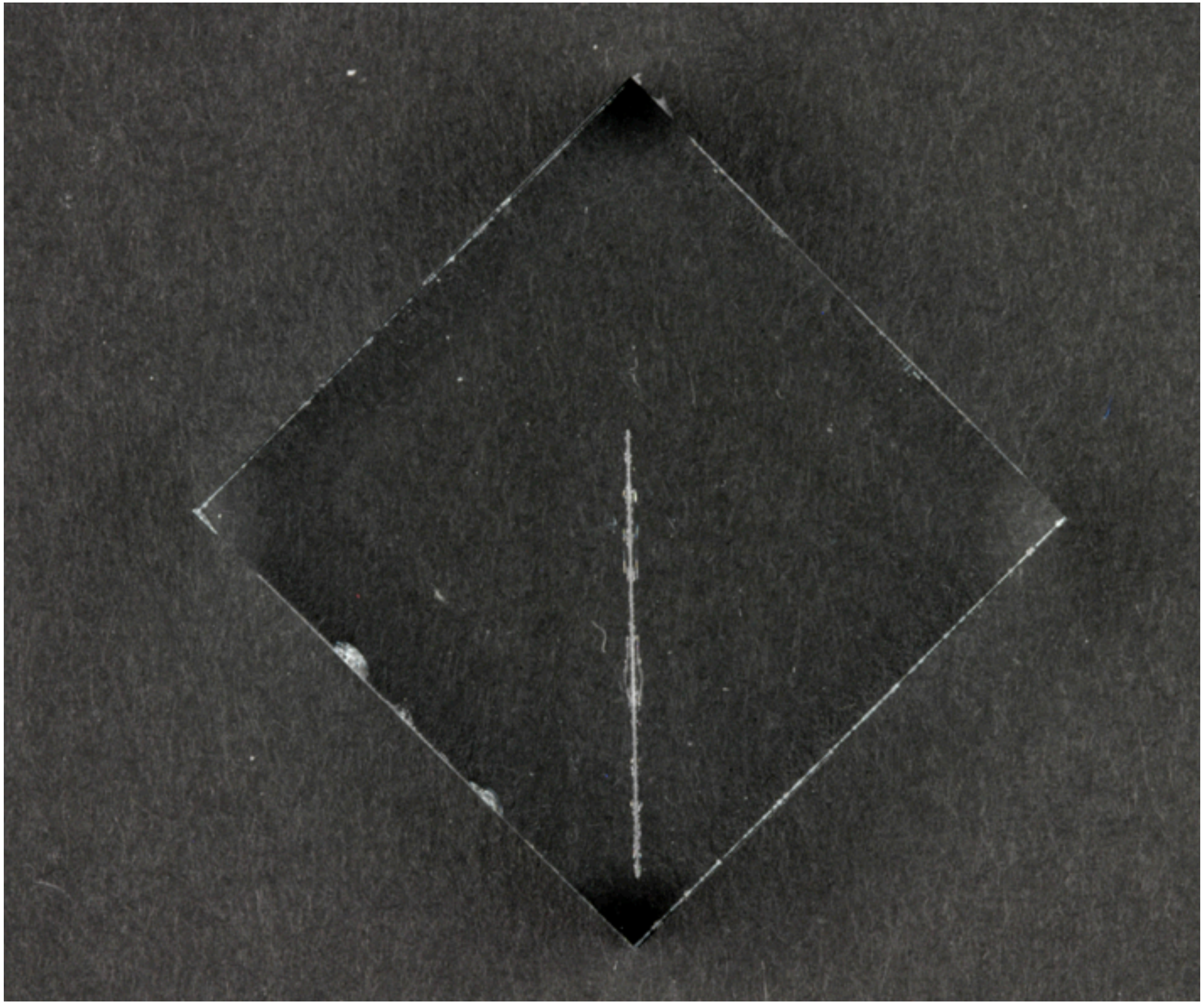
A good and a „bad“ knife



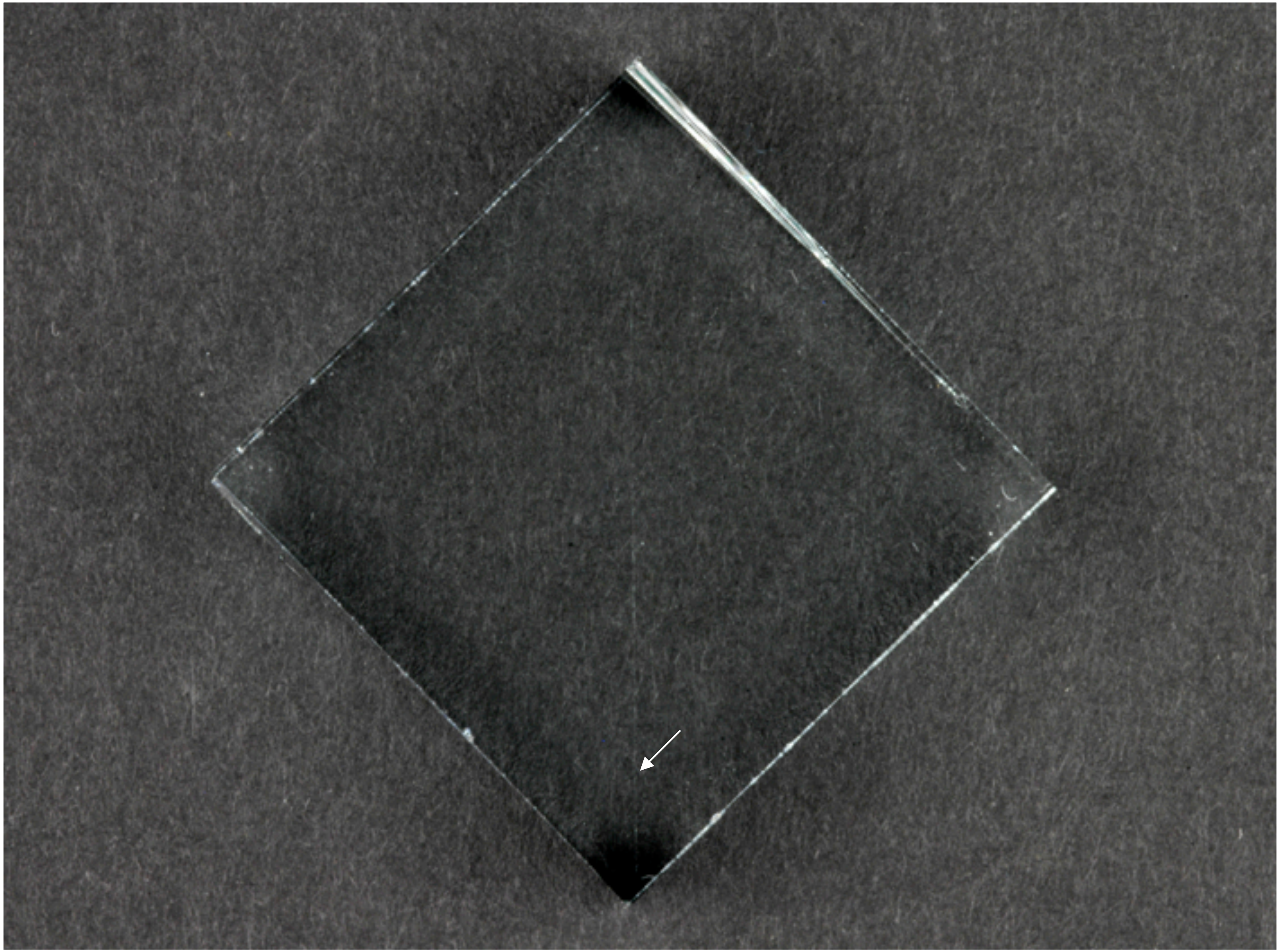
Scratch line too strong



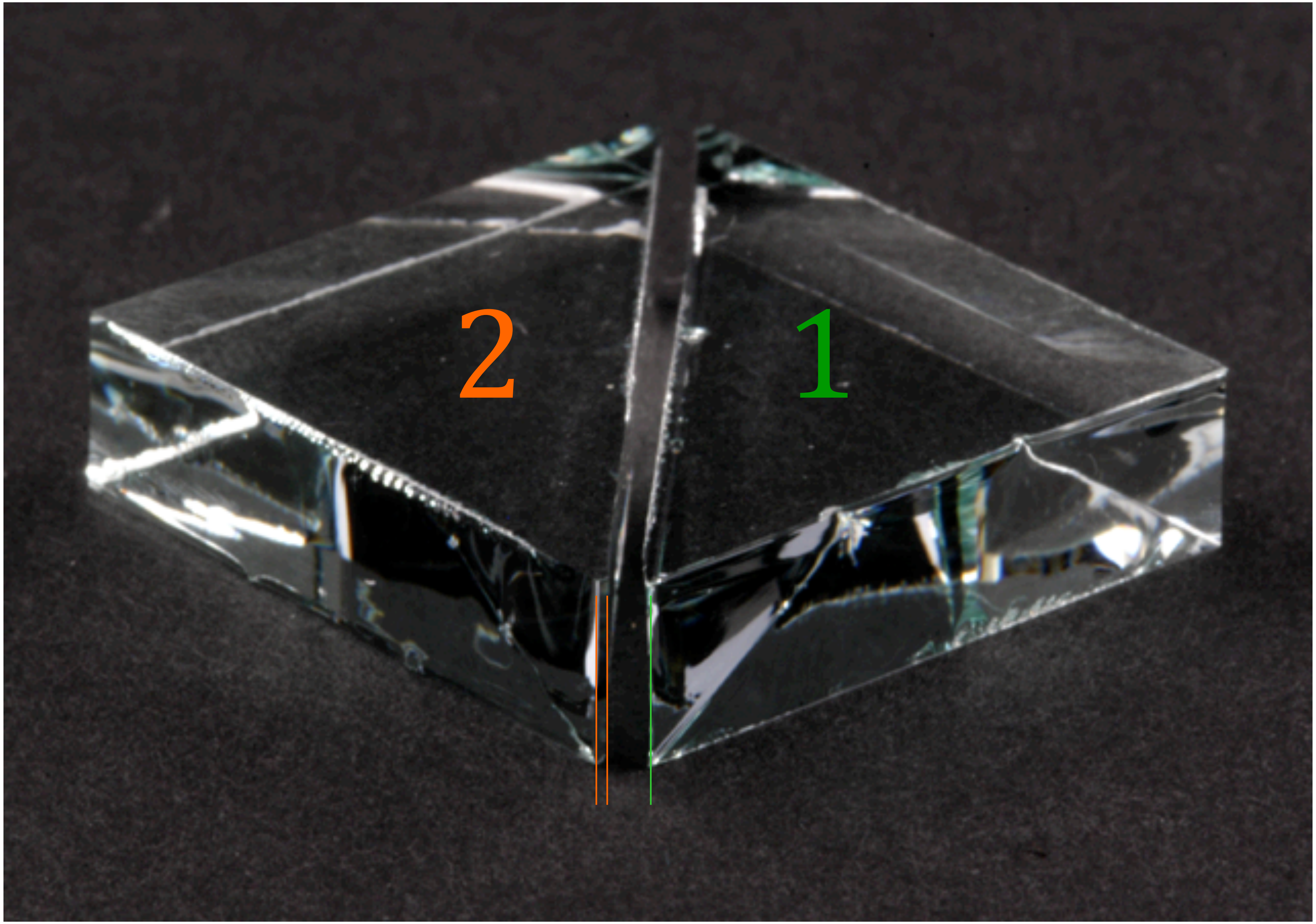
Scratch line too short



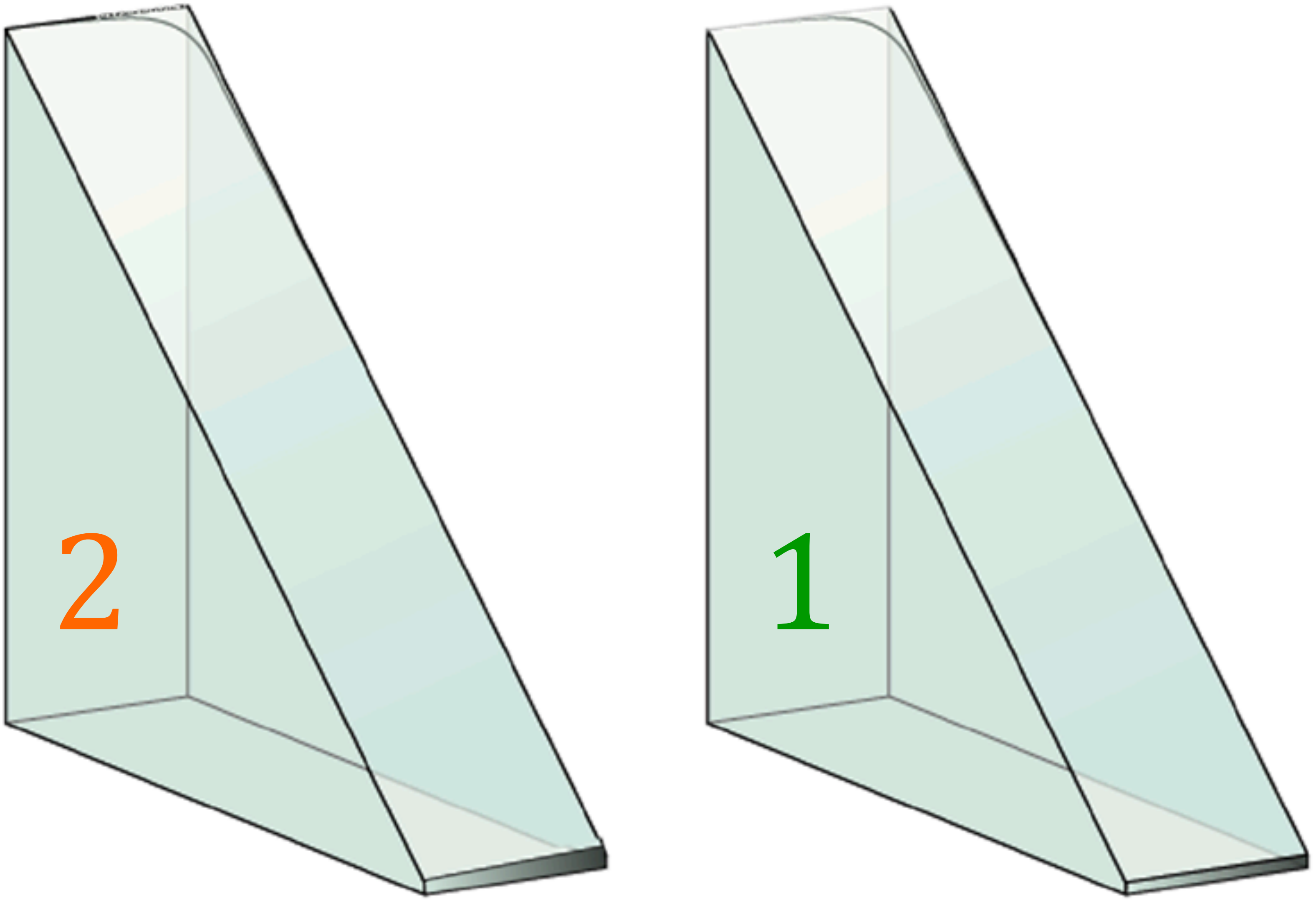
Ideal scratch line



2 knives: the good & the bad one



2 knives: the good & the bad one



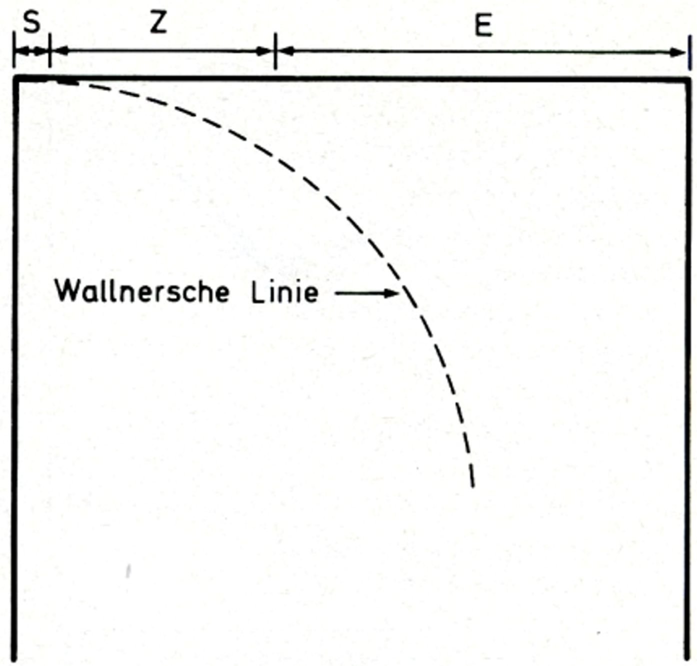
Progression of break line: Scale for quality

<u>Cutting edge of a glass knife:</u>

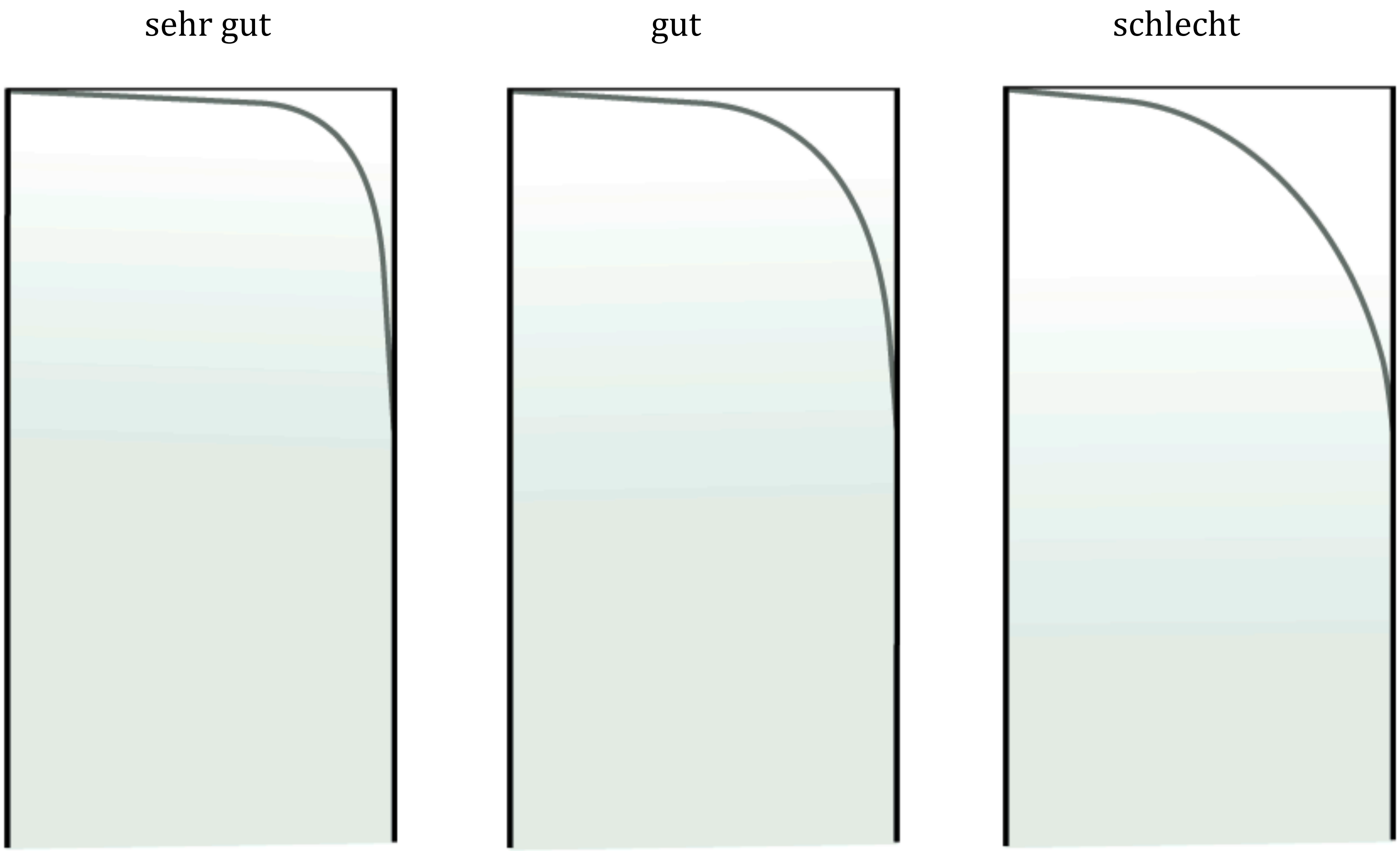
S: Corner, cannot be used for sectioning.

Z: Sectioning zone.

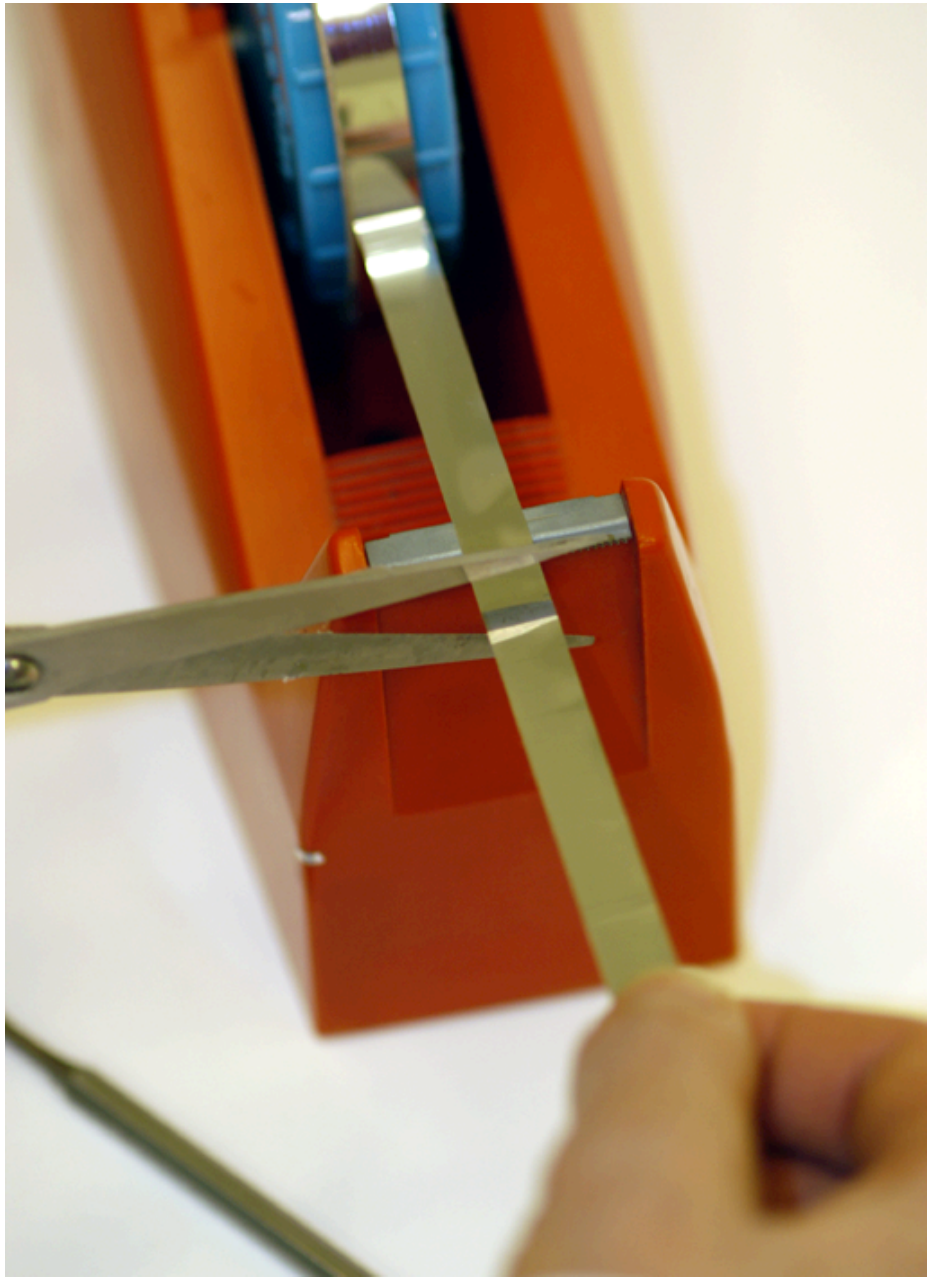
E: Depending on quality, this part can sometimes be used for sectioning.

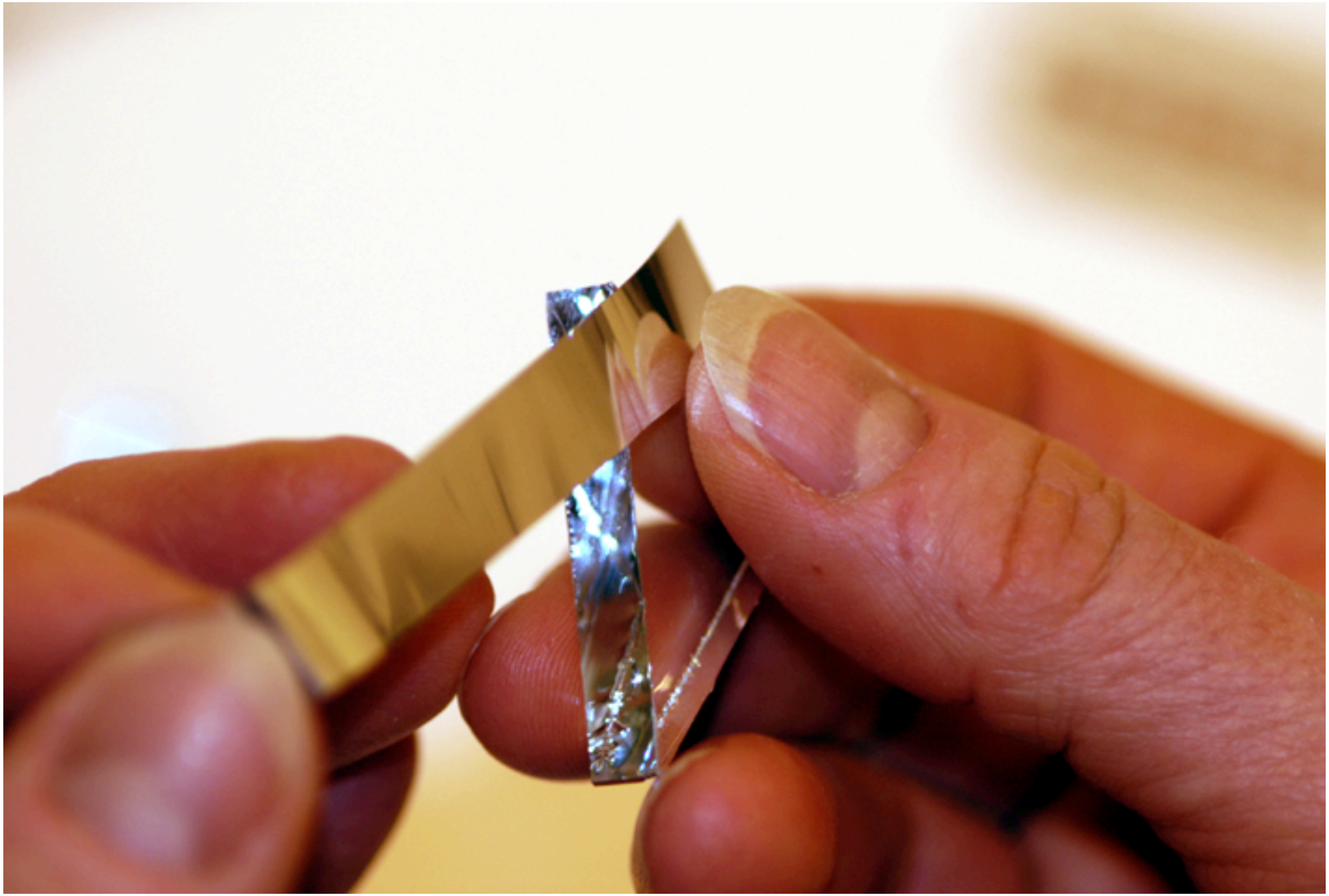


Progression of break line: Scale for quality



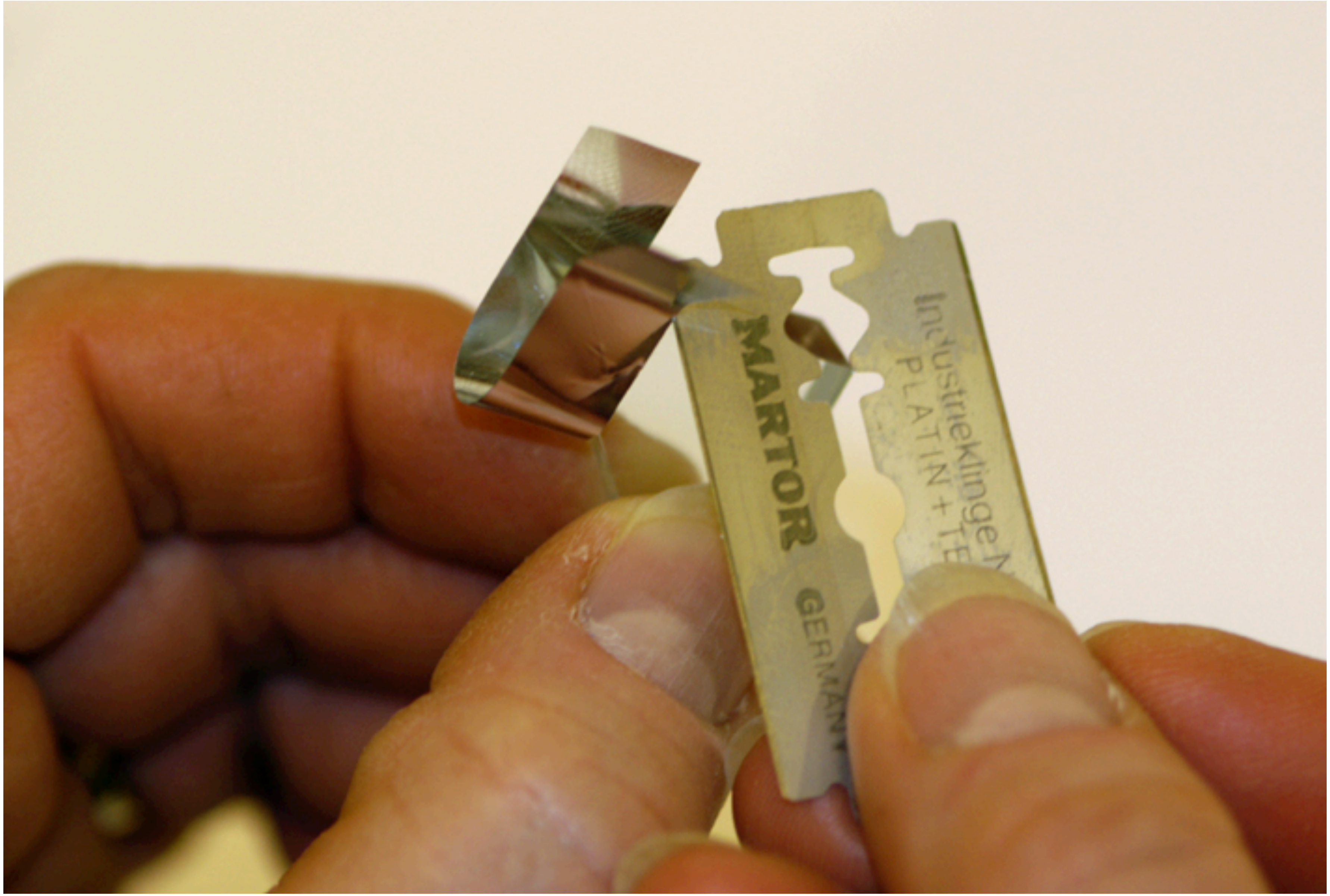
Implements to assemble the through

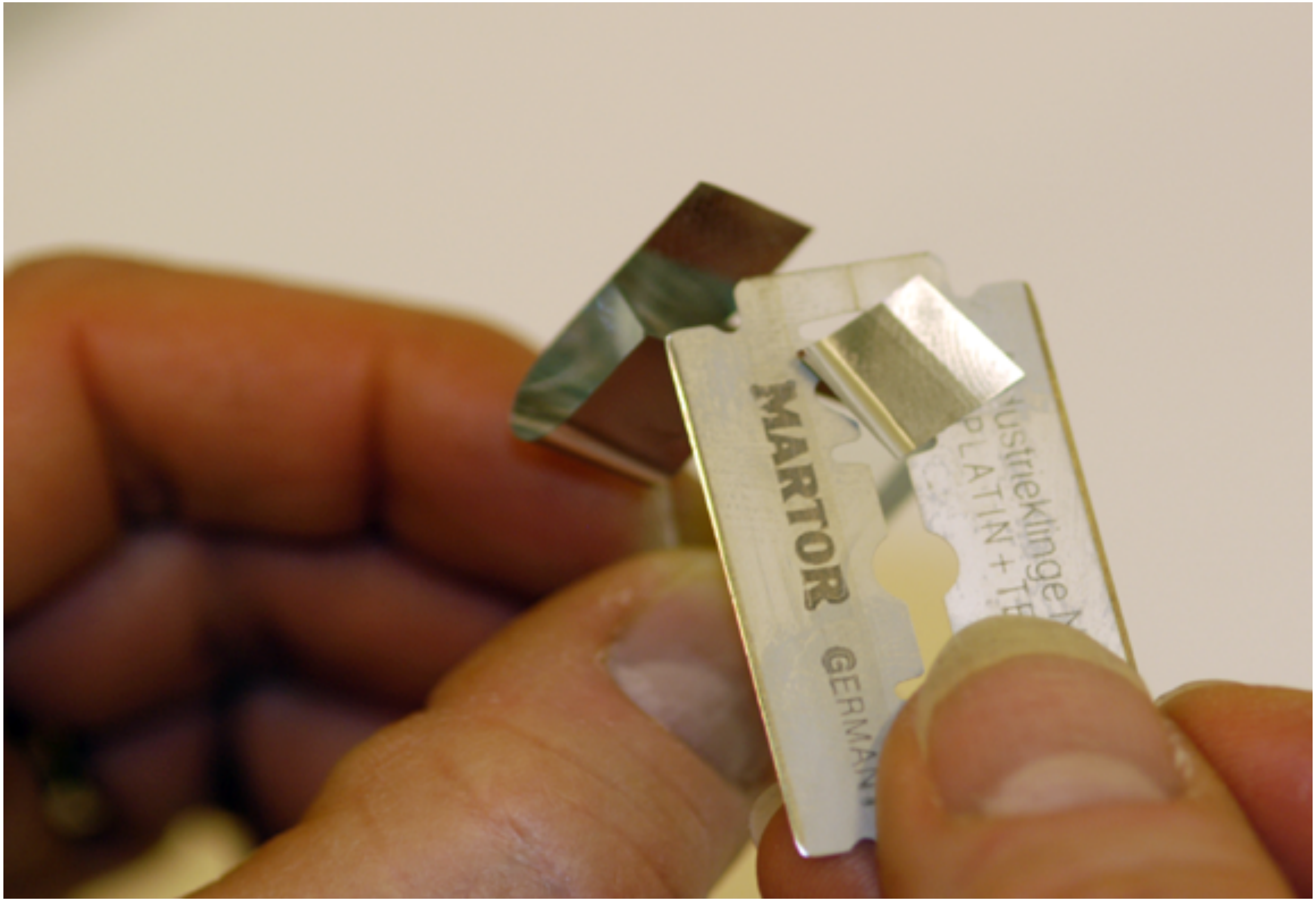




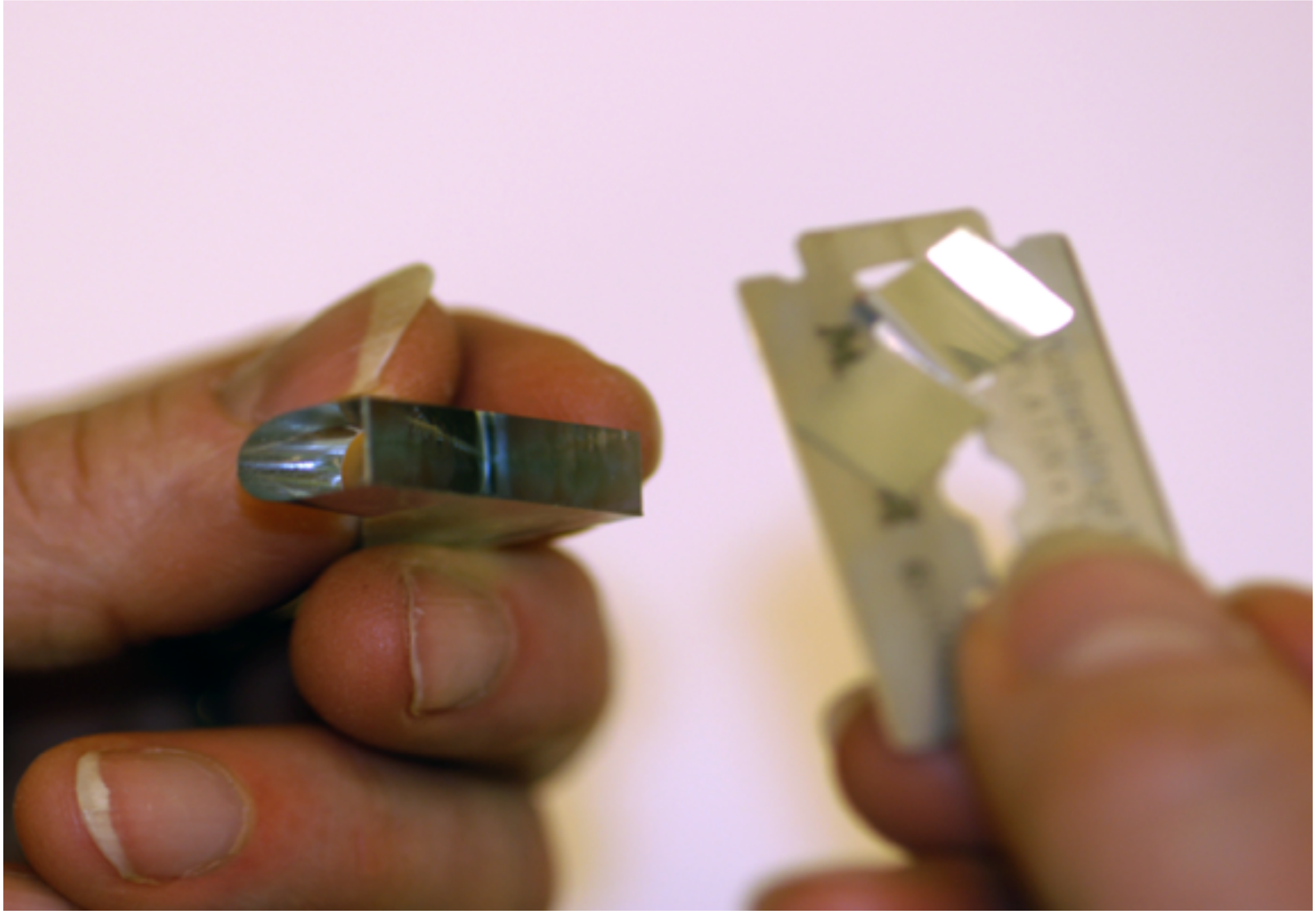
Keep the angle in mind: -6˚

Keep the angle in mind: -6˚

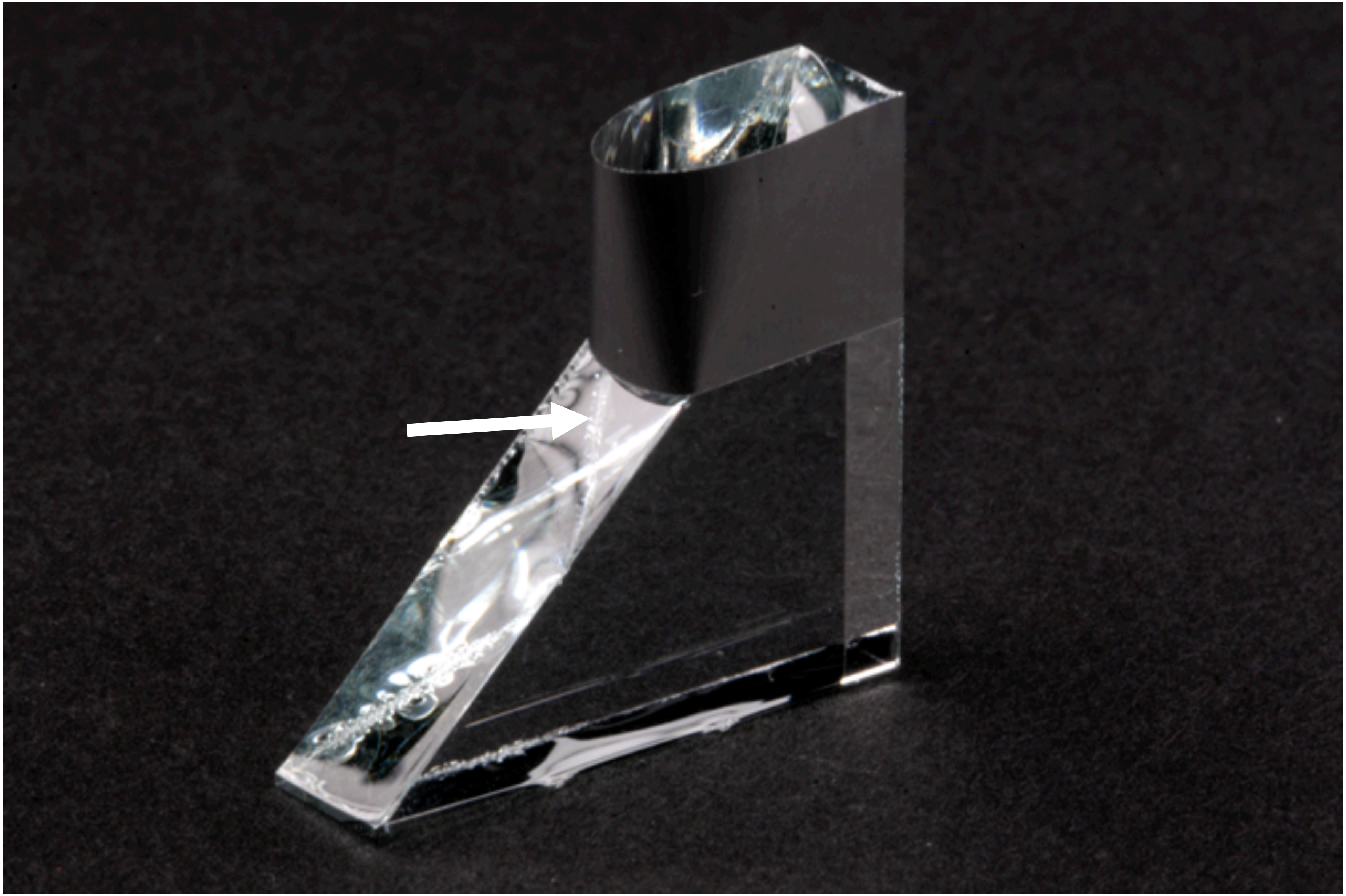

MARTOR


MARTOR

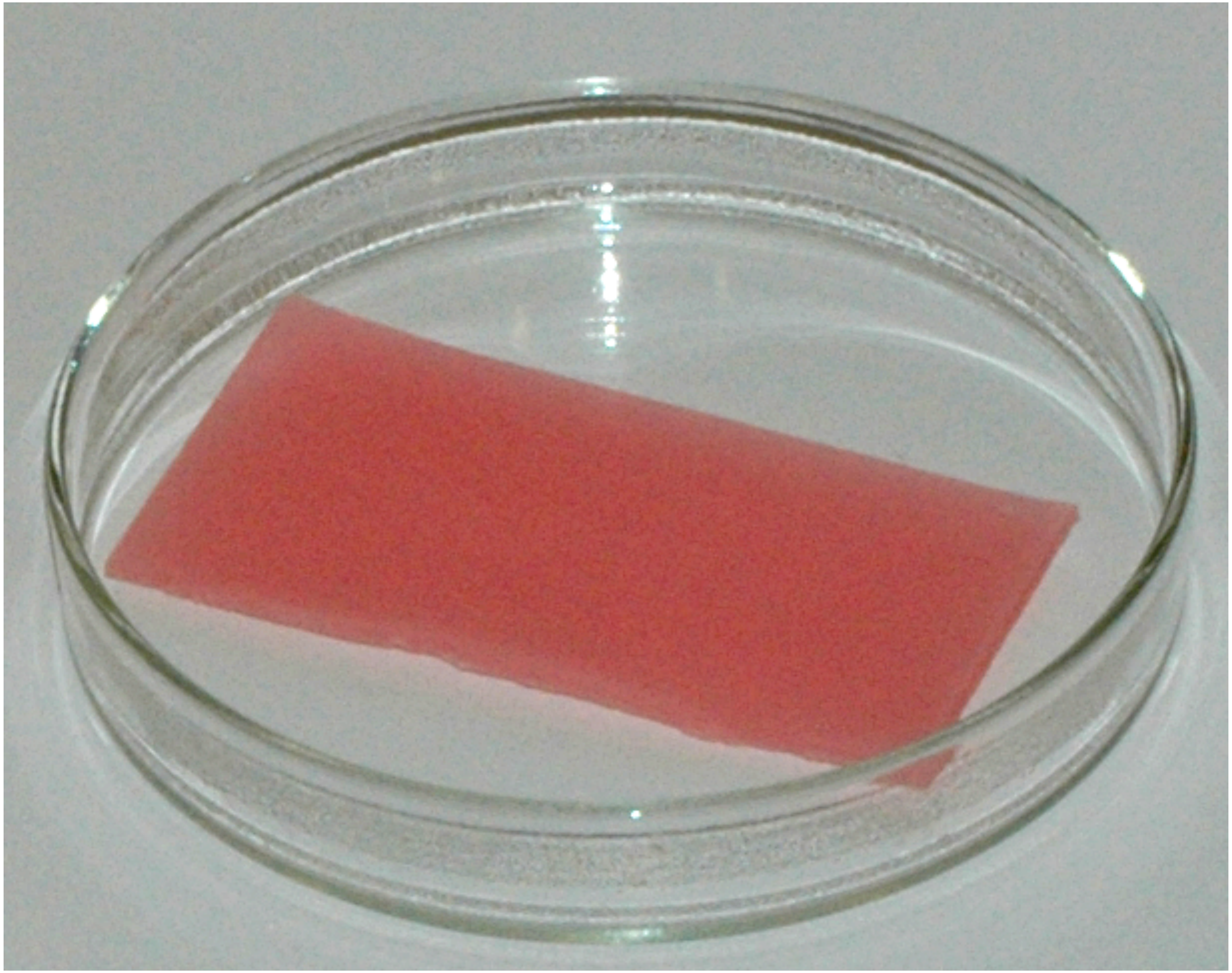

MARTOR



Through is finished

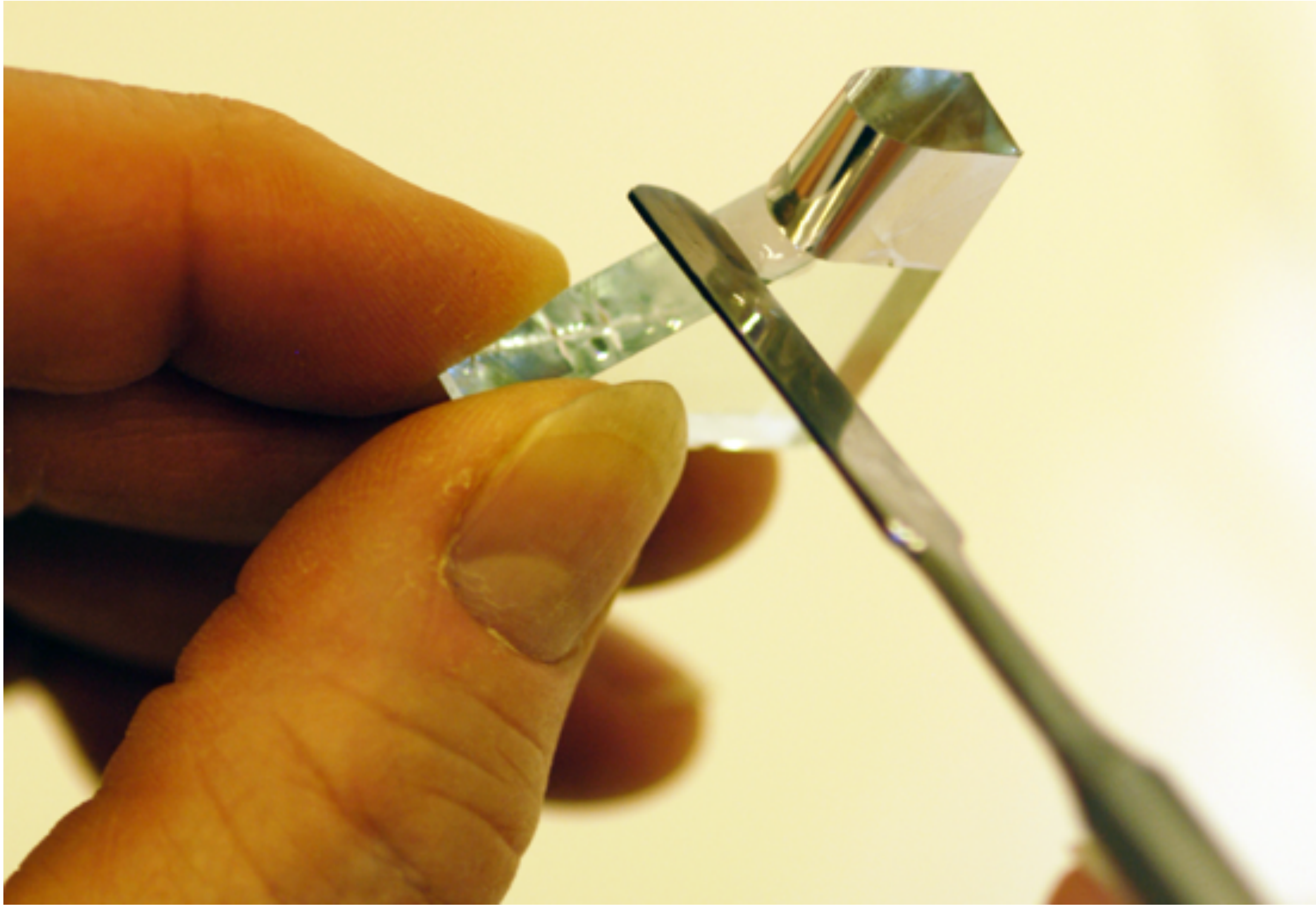


Dental wax for sealing

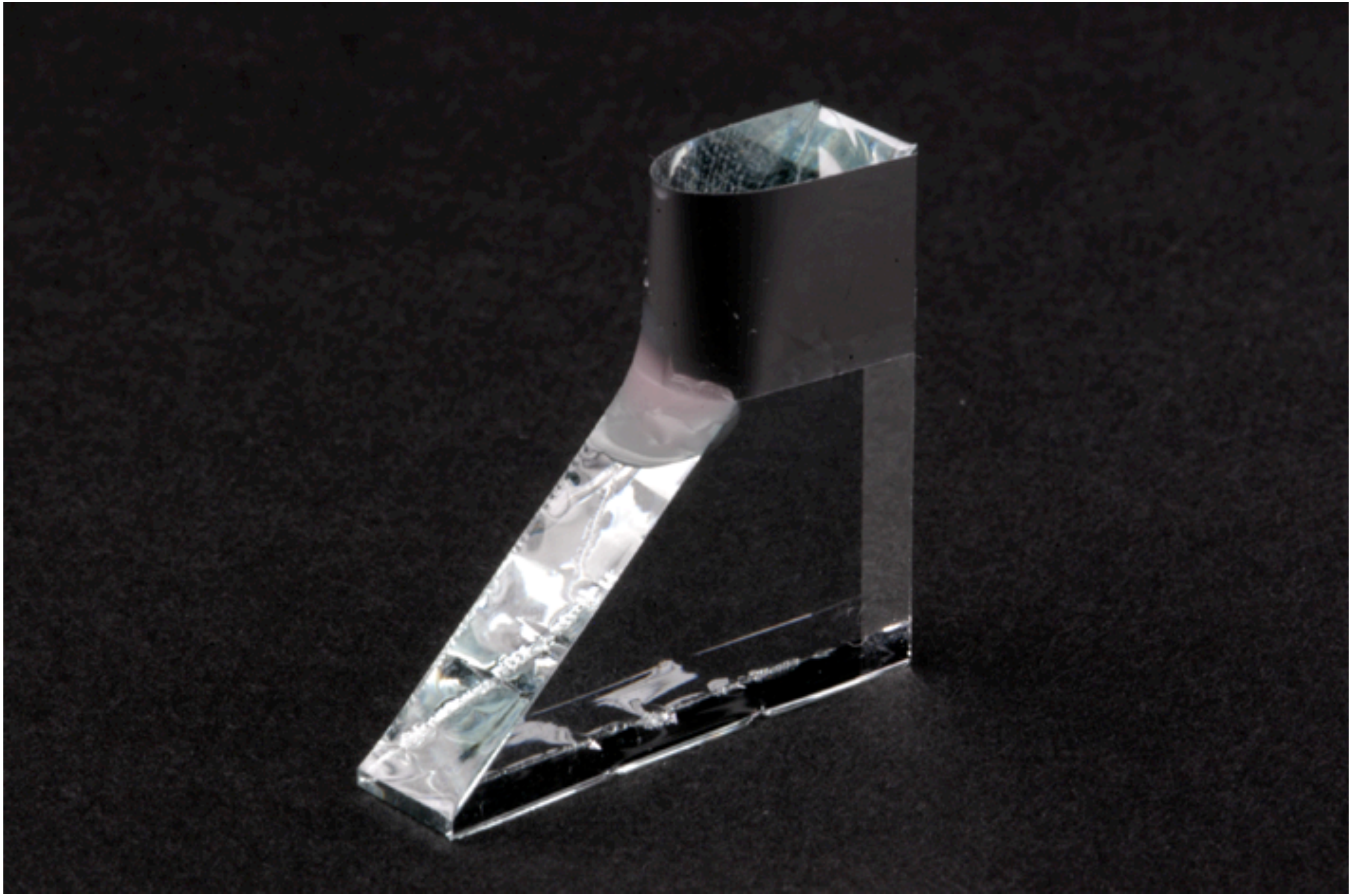


Melting wax

Seal the backside



Sealing of the backside



Sealing the wings – glass knife is ready!