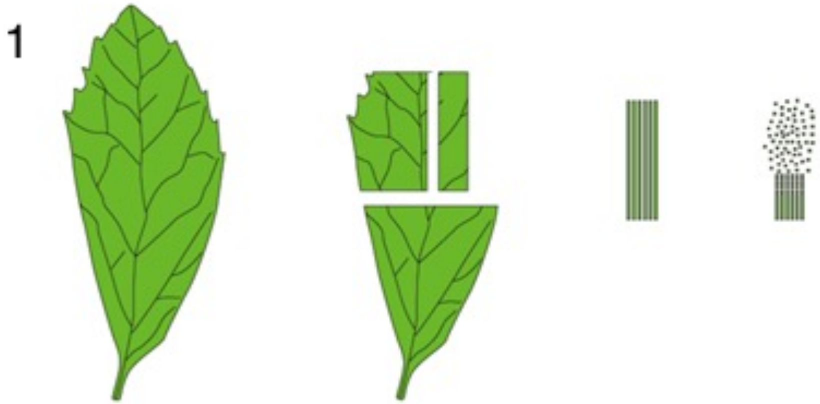
SEM - Scanning electron microscopy

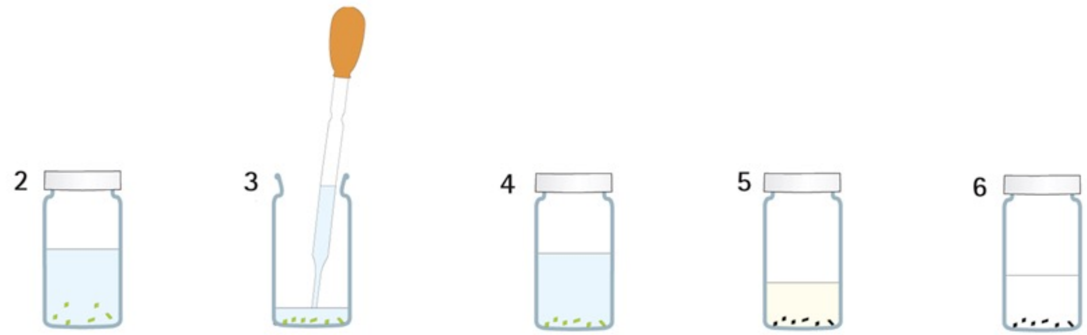
Sample preparation



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



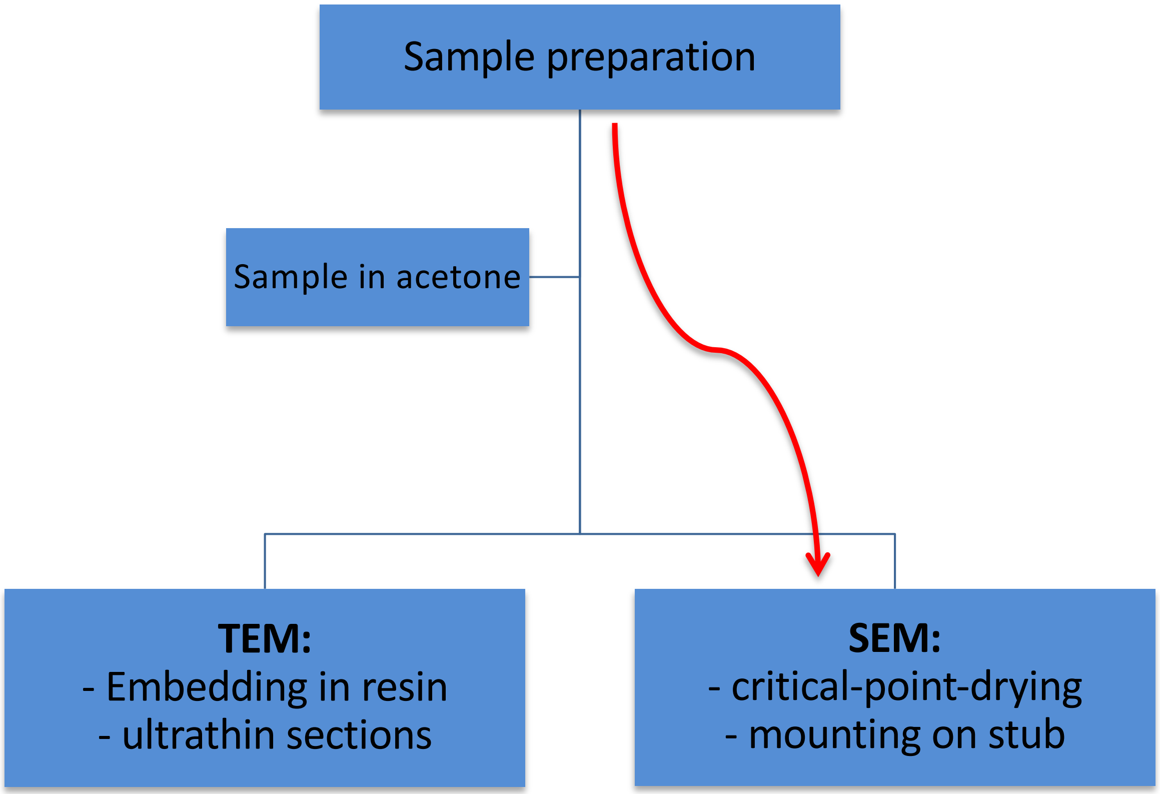
Fixation with glutaraldehyde

Withdrawal of fixing agent

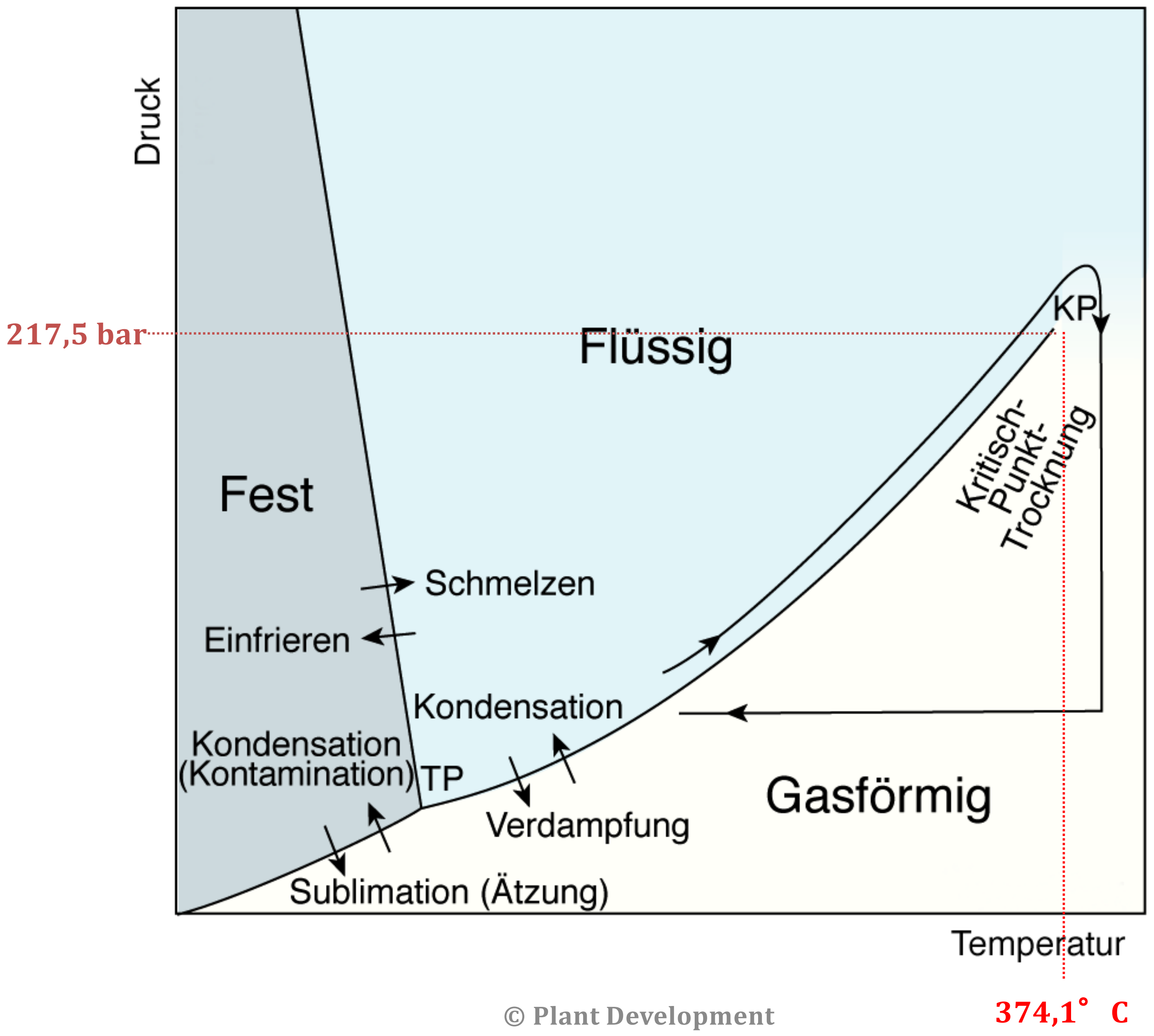
Washing with buffer

Post-fixation with OsO_4

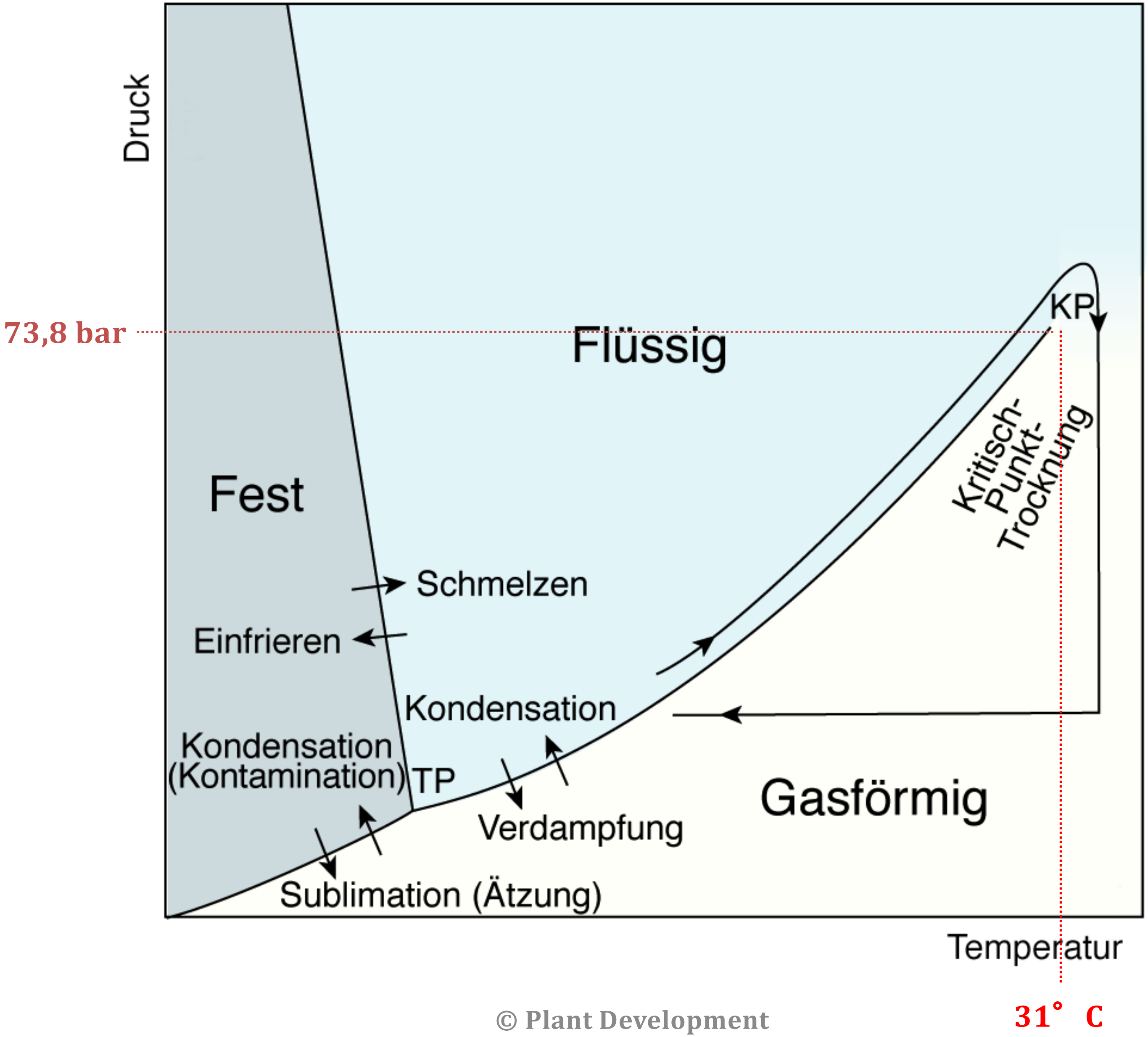
Dehydration with acetone



Phase diagram water



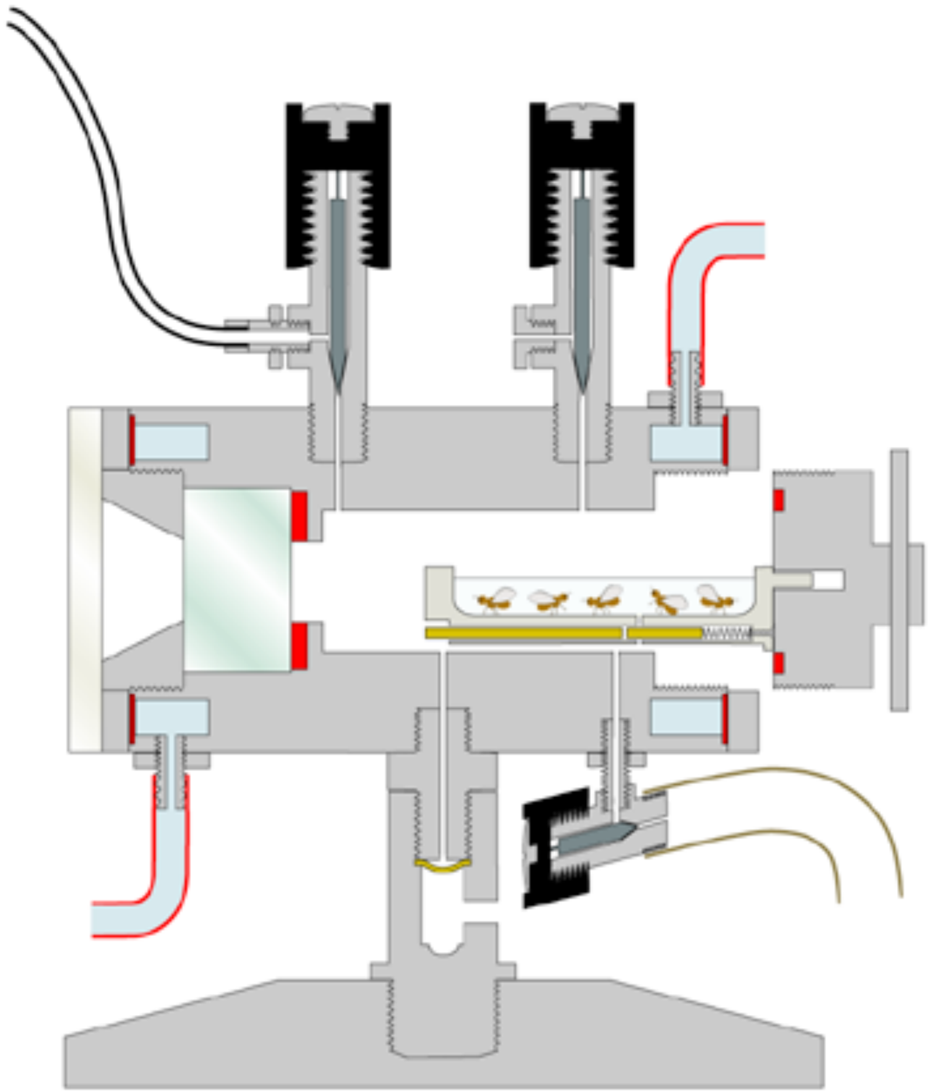
Phase diagram CO_2



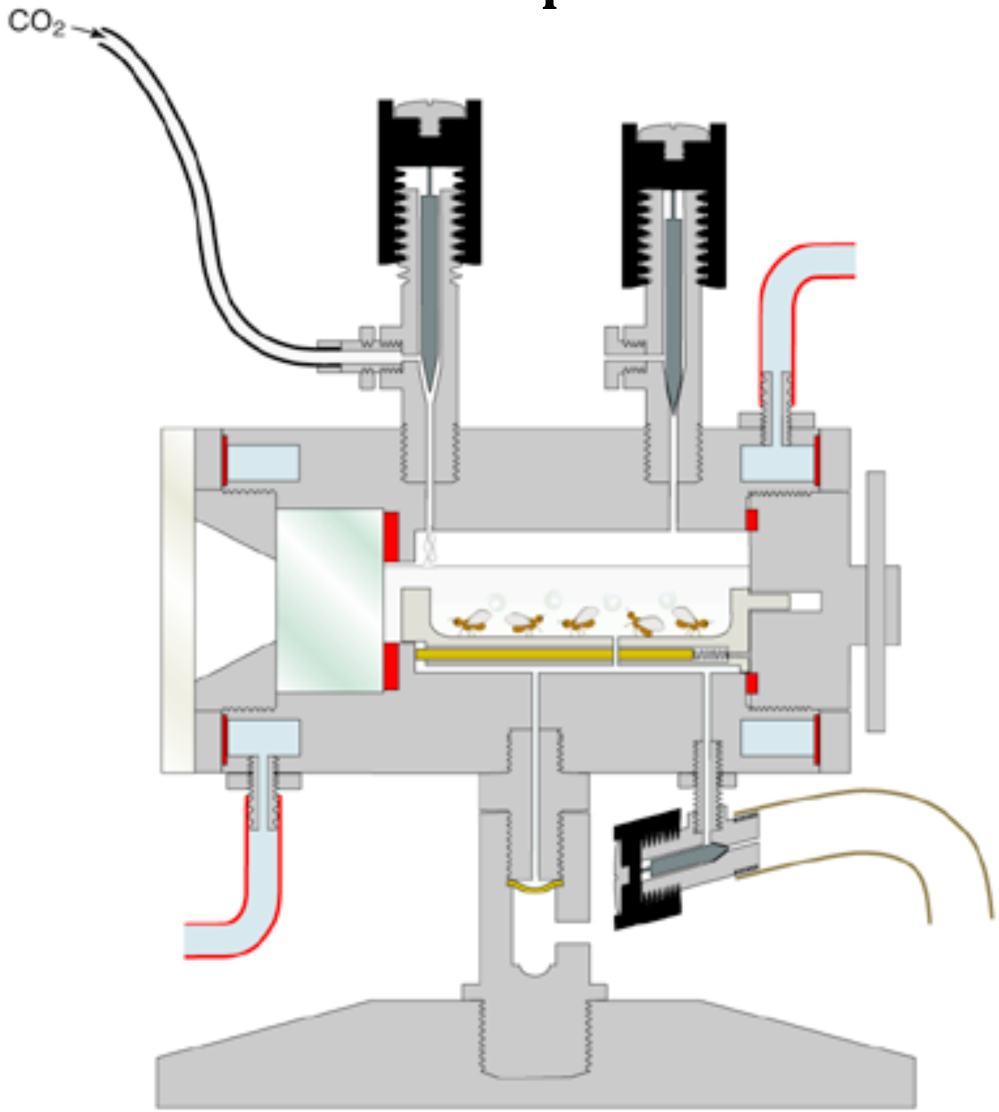
Critical-point-drying (CPT)

Sample preparation

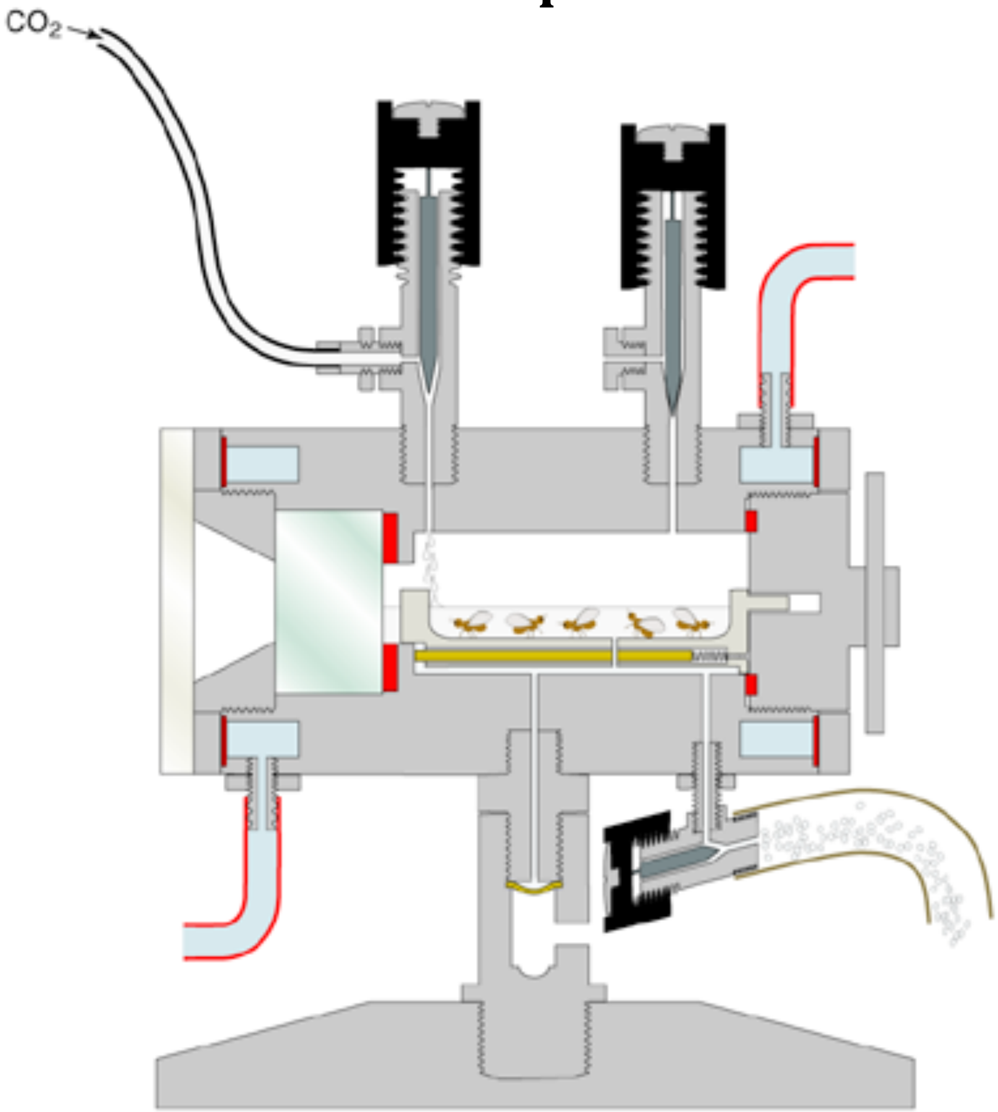
Step 1



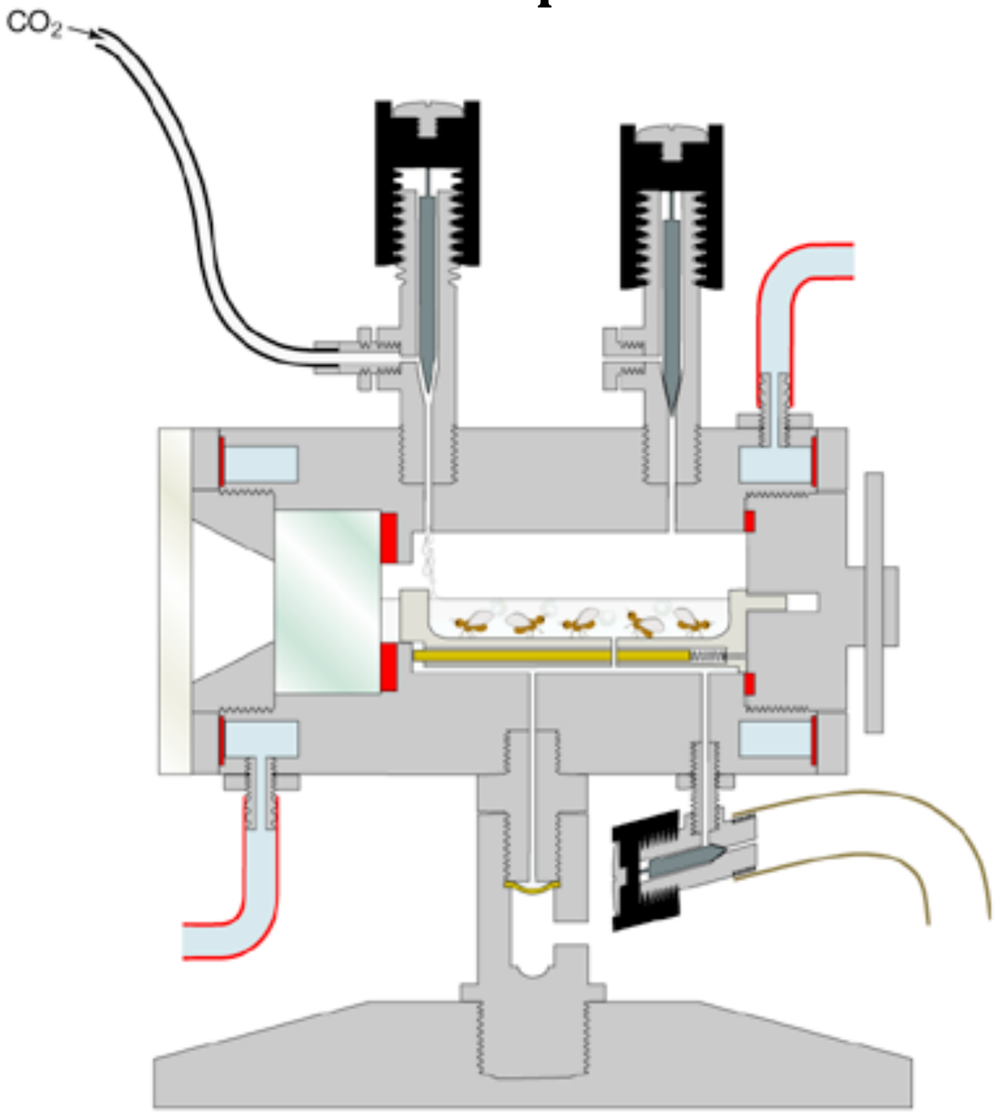
Step 2



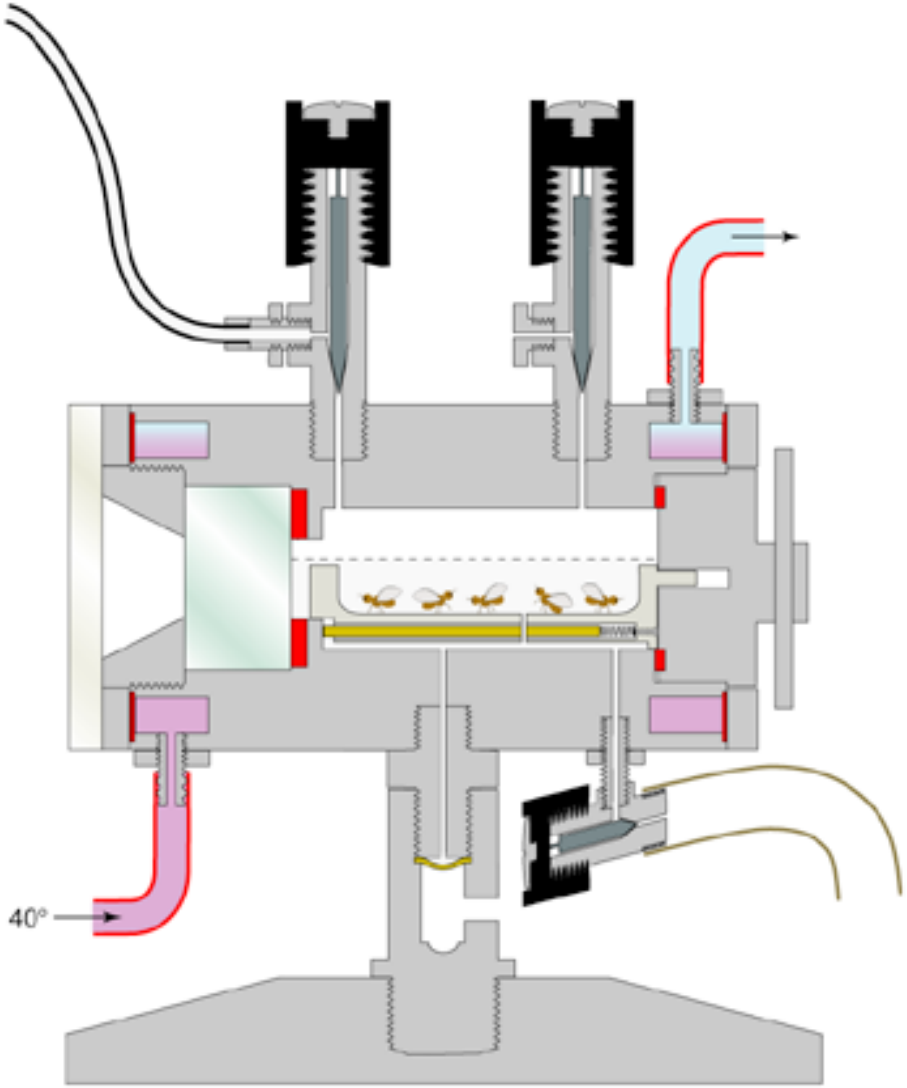
Step 3



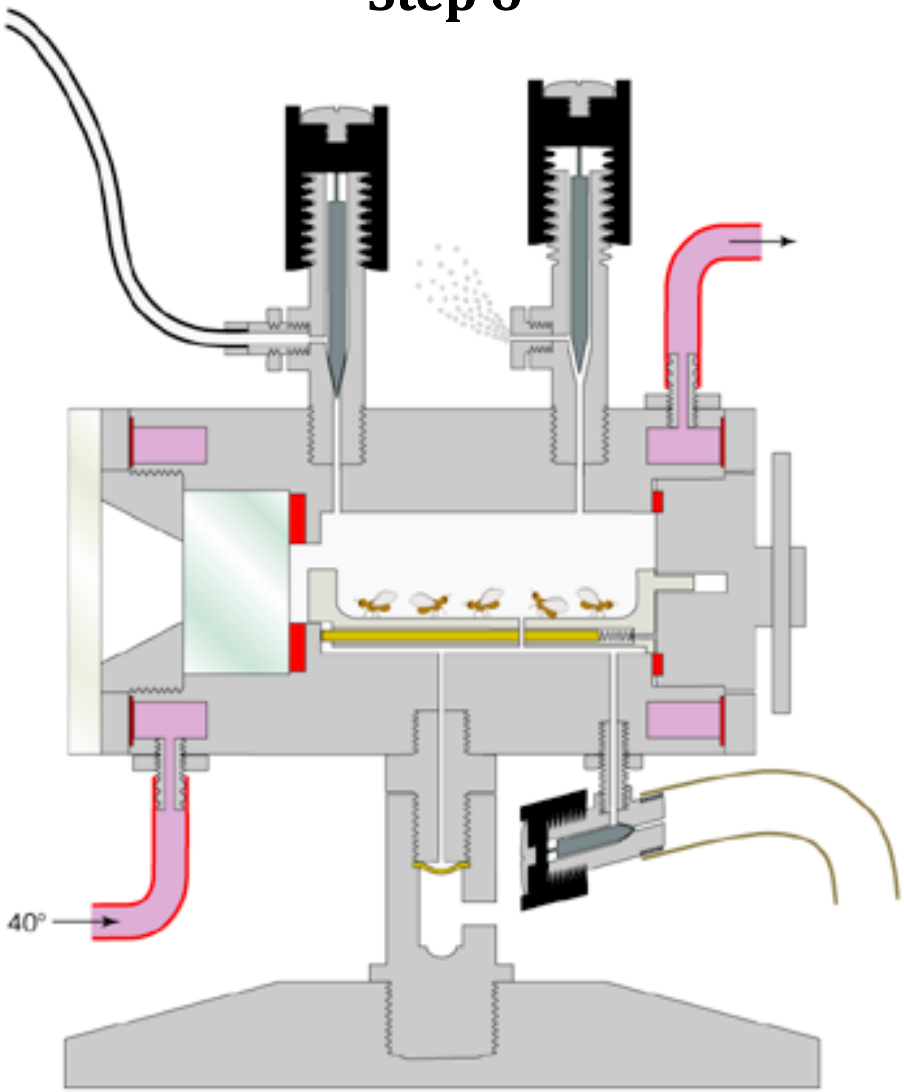
Step 4



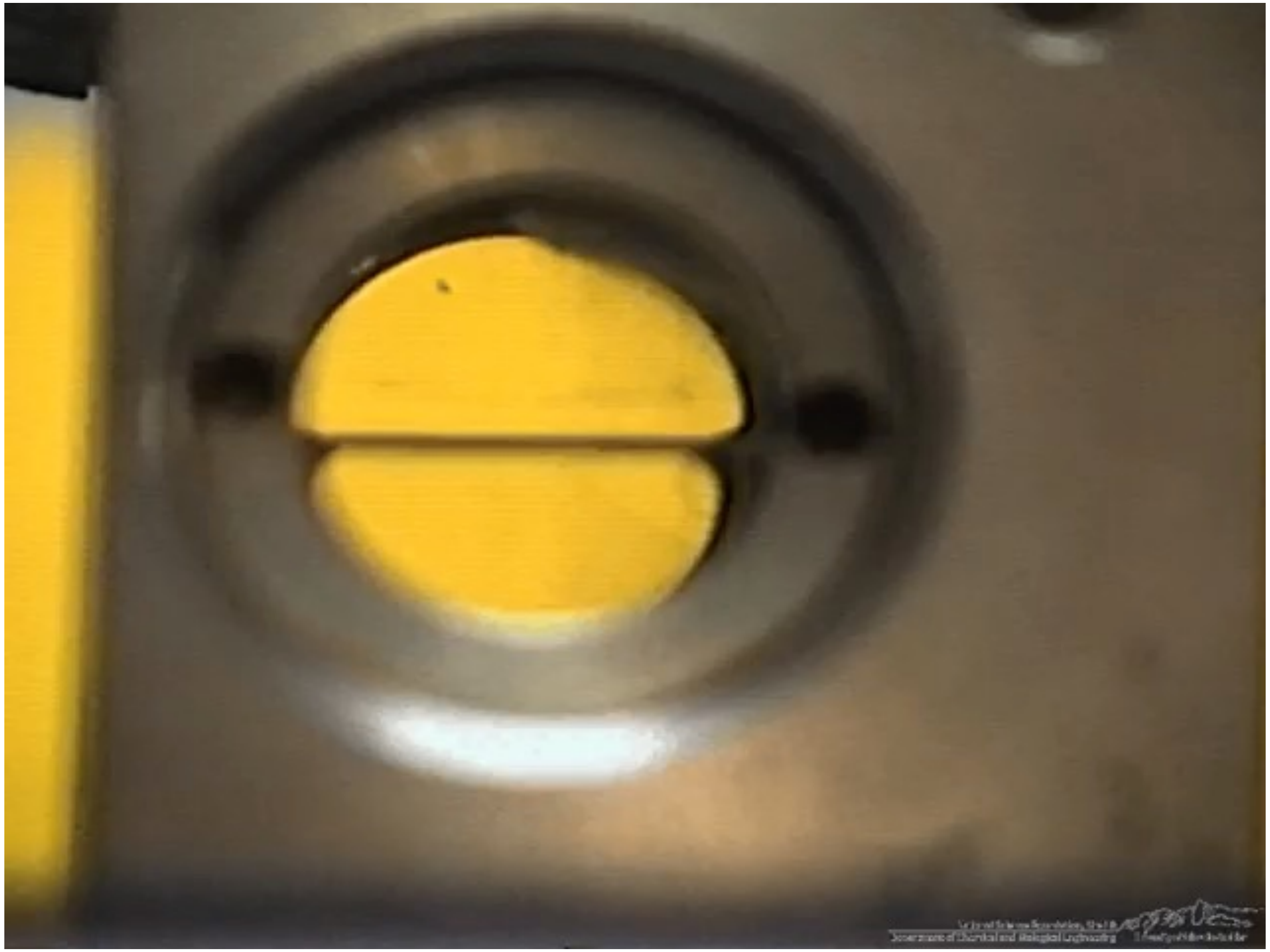
Step 5



Step 6



Critical-point-drying (CPT)



https://learncheme.com/

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

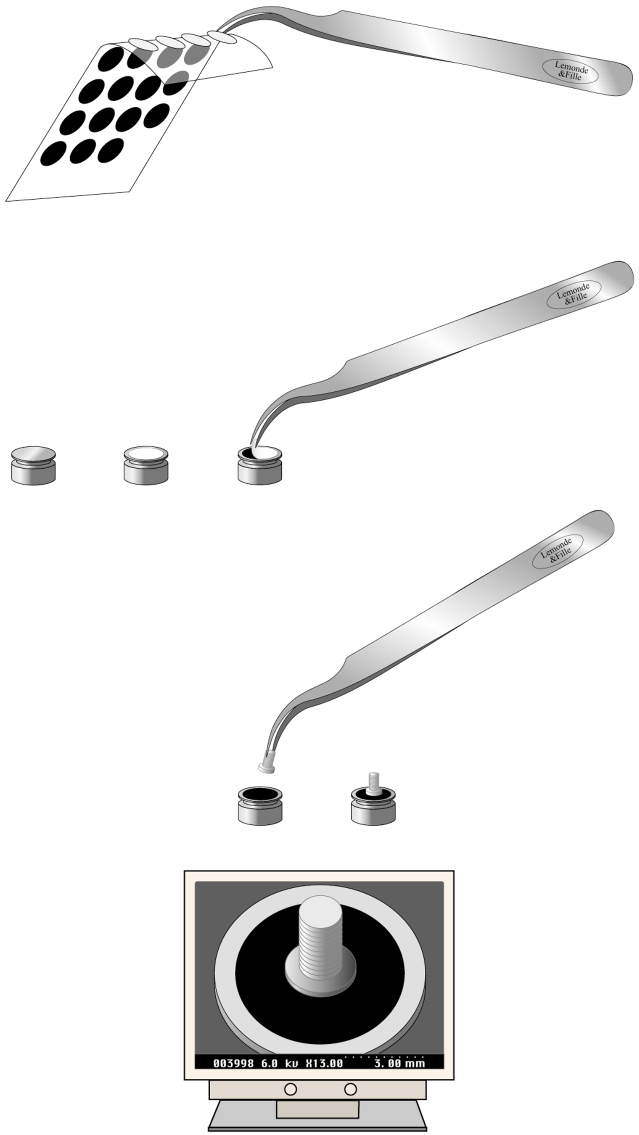
Leit-C (organic or aqueous)

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

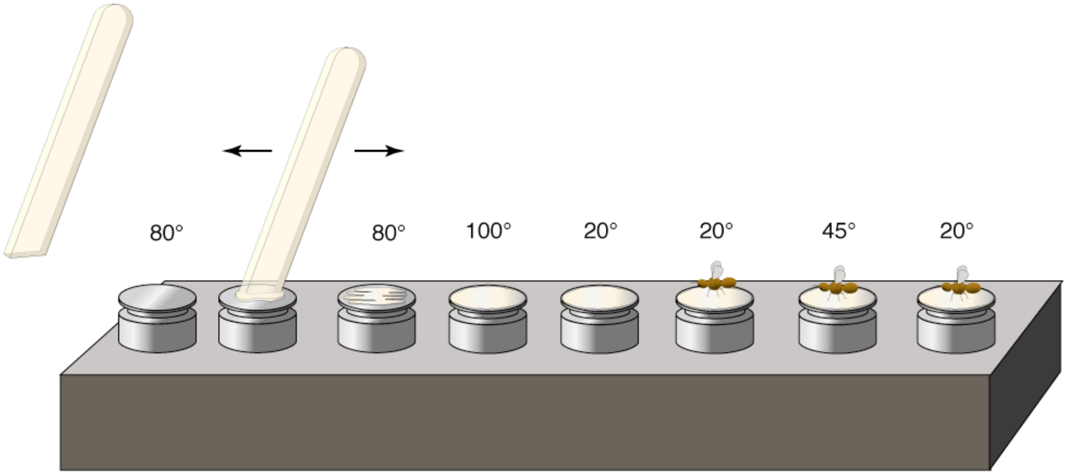


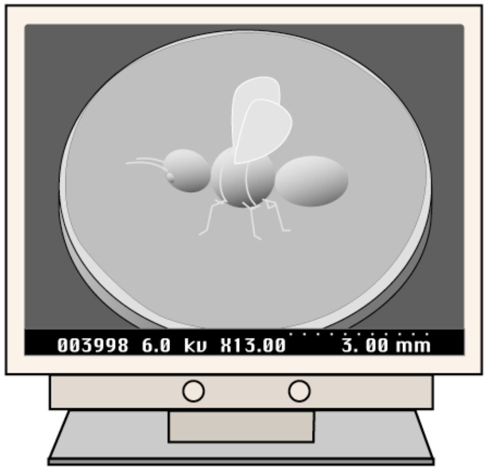
Composition:
Double-sided adhesive foil containing carbon black/grime

Pros:
Very fast
Good adhesion
Dark background

Cons:
Crack formation
Thermal drift
Moderate conductivity

Tempfix



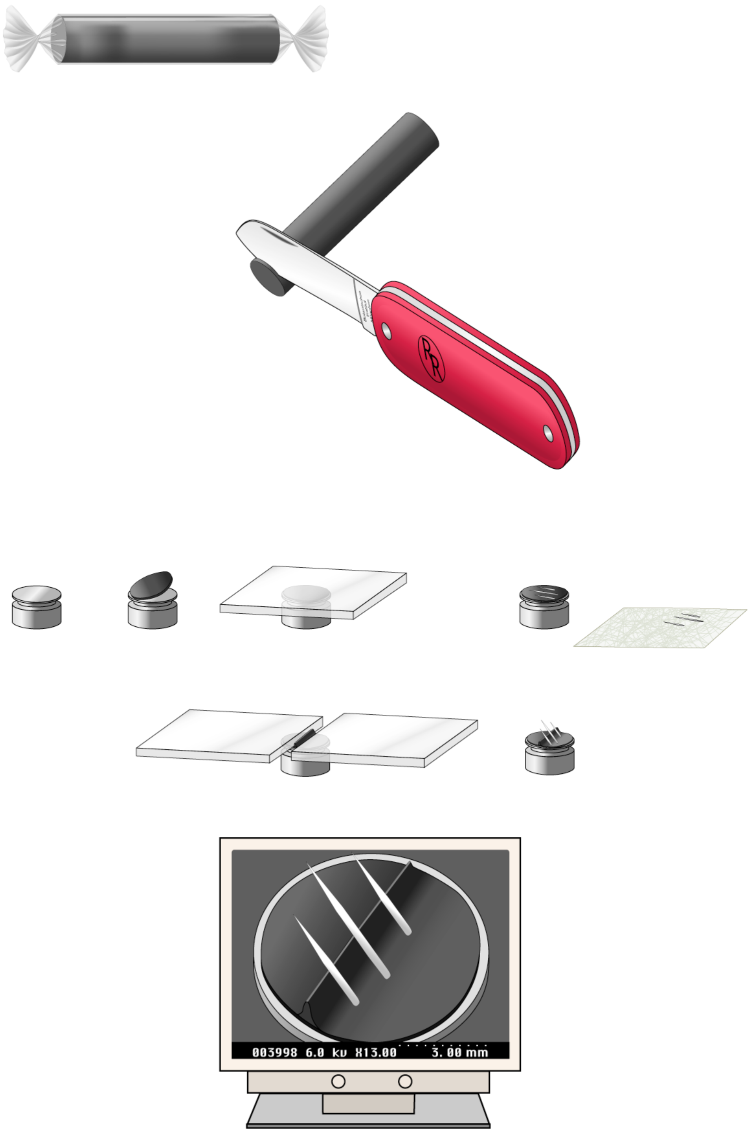


Composition:
Thermoplastic polymer

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

Leitplast



Composition:
Noble plasticine with carbon black/grime

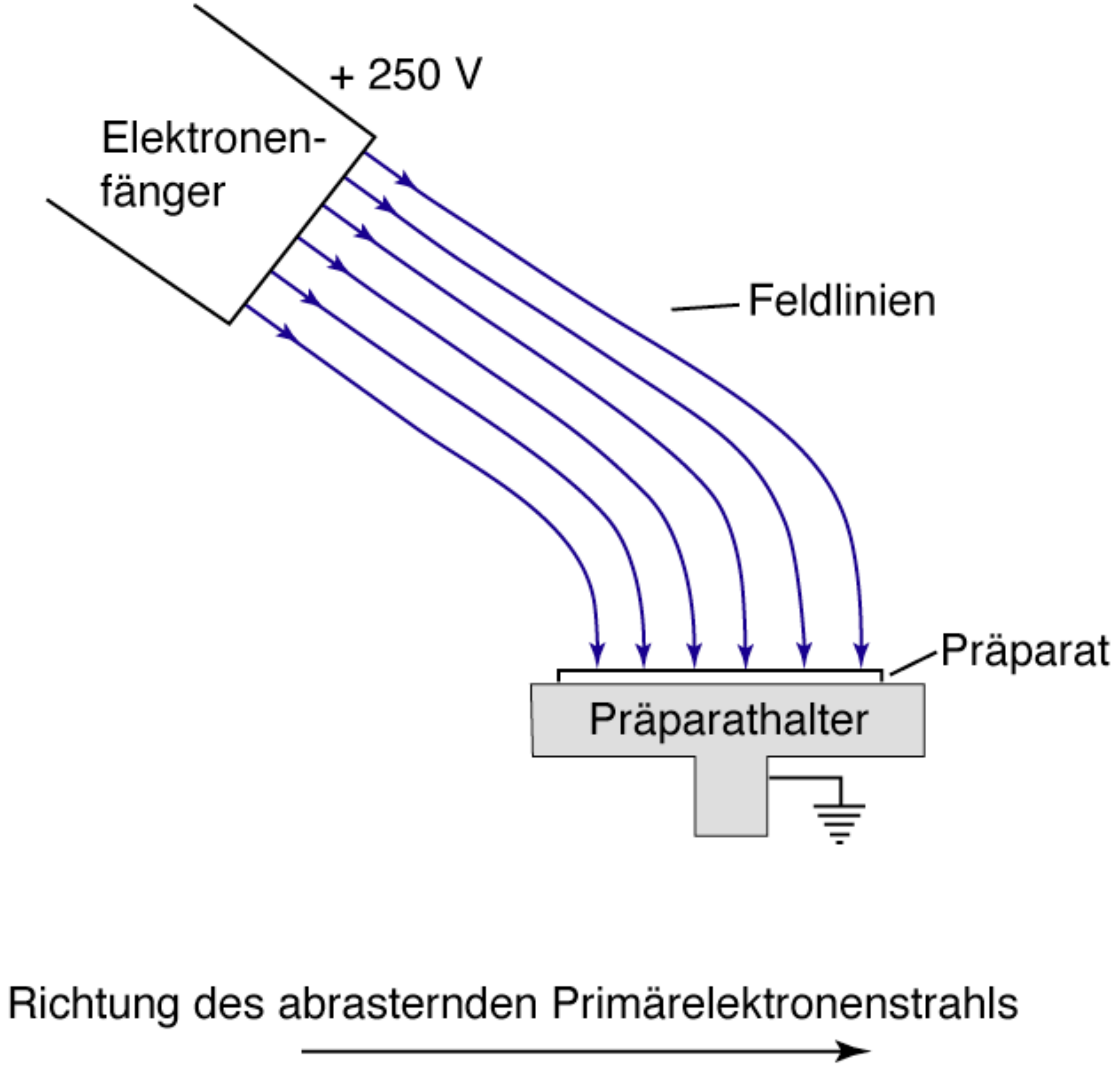
Pros:
Best orientation of sample
Good for larger samples
Object-orientation changeable

Cons:
Poor adhesion properties
Often time consuming
Application demands great skill

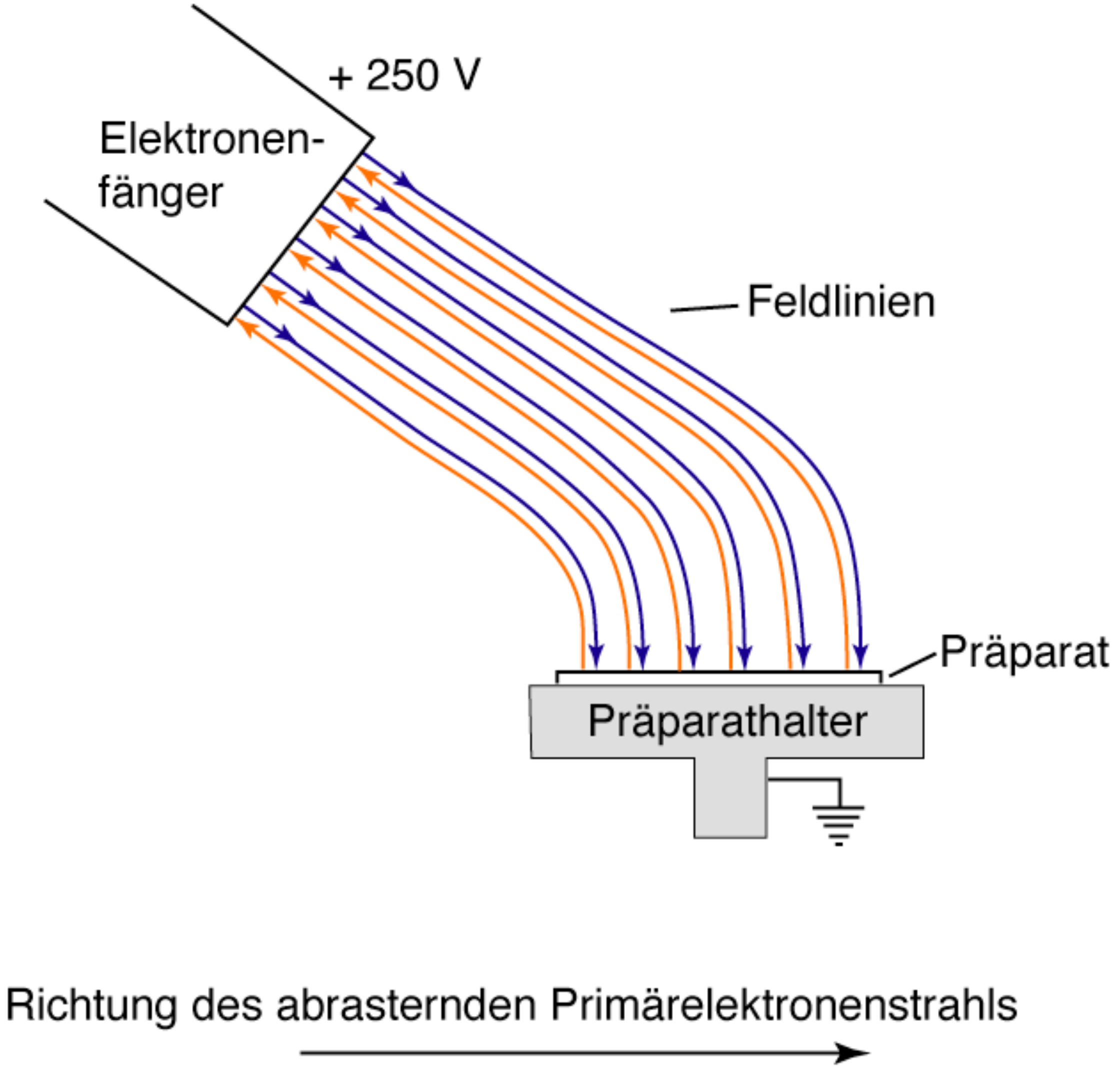
Charging in SEM

A routine problem

Electric flux lines: sample ↔ SE-detector

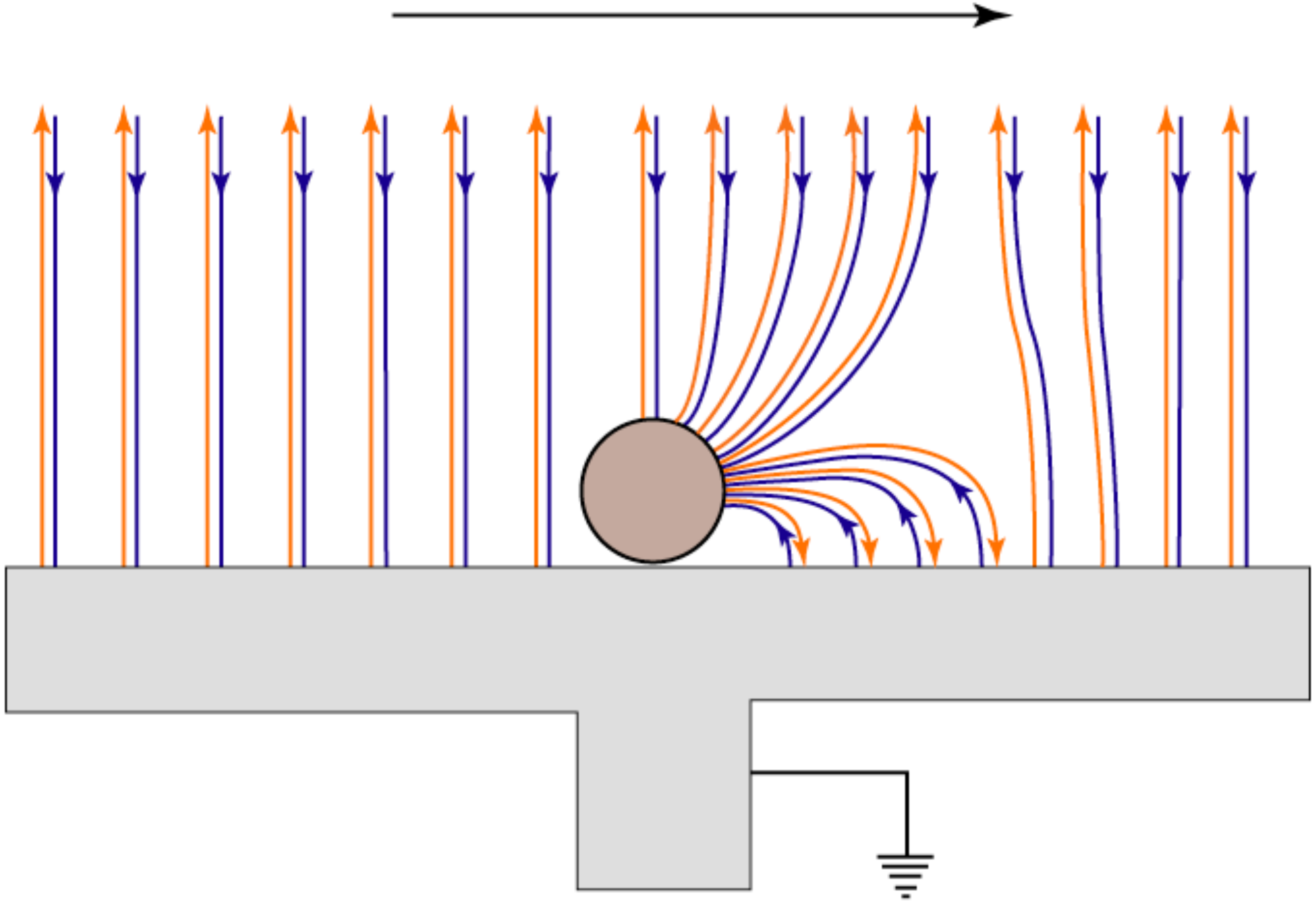


Path of SE-electrons to the detector

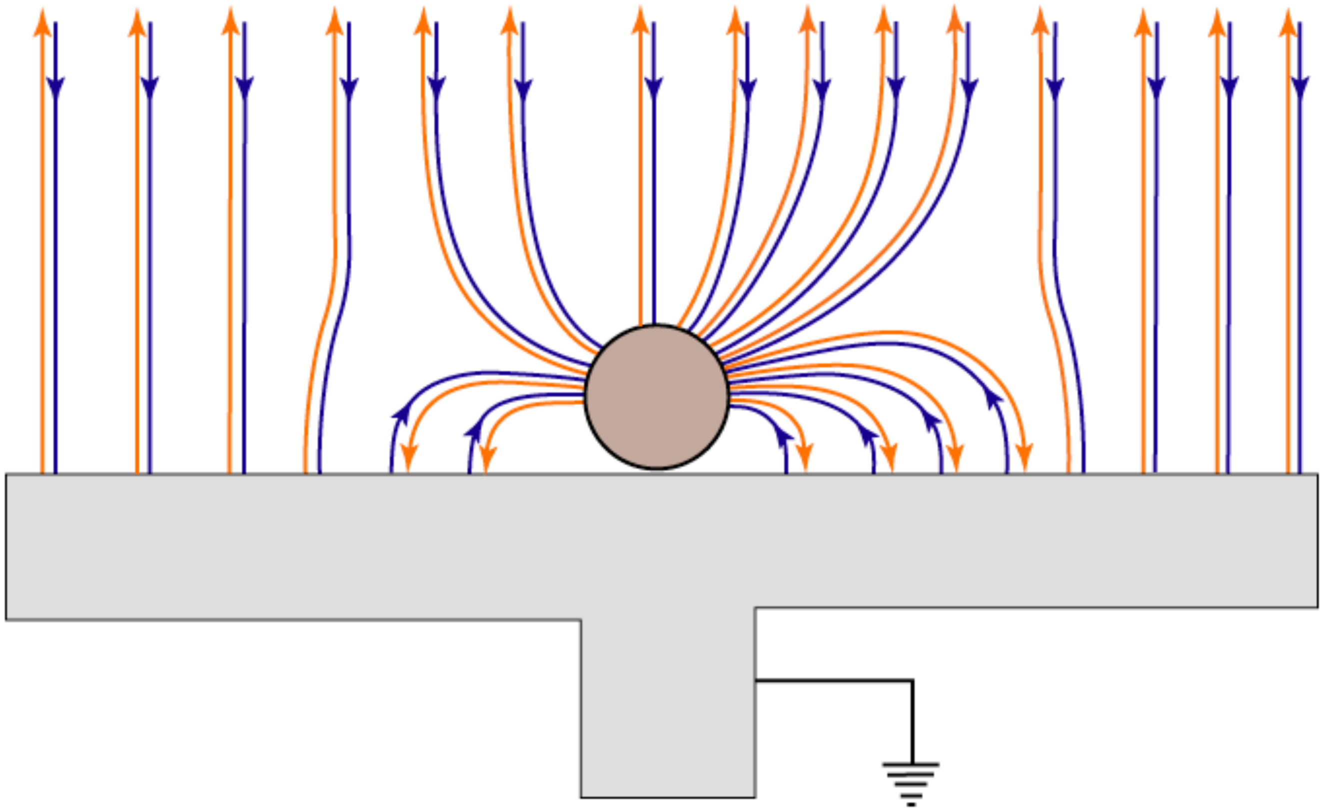


Charging: 1st scan

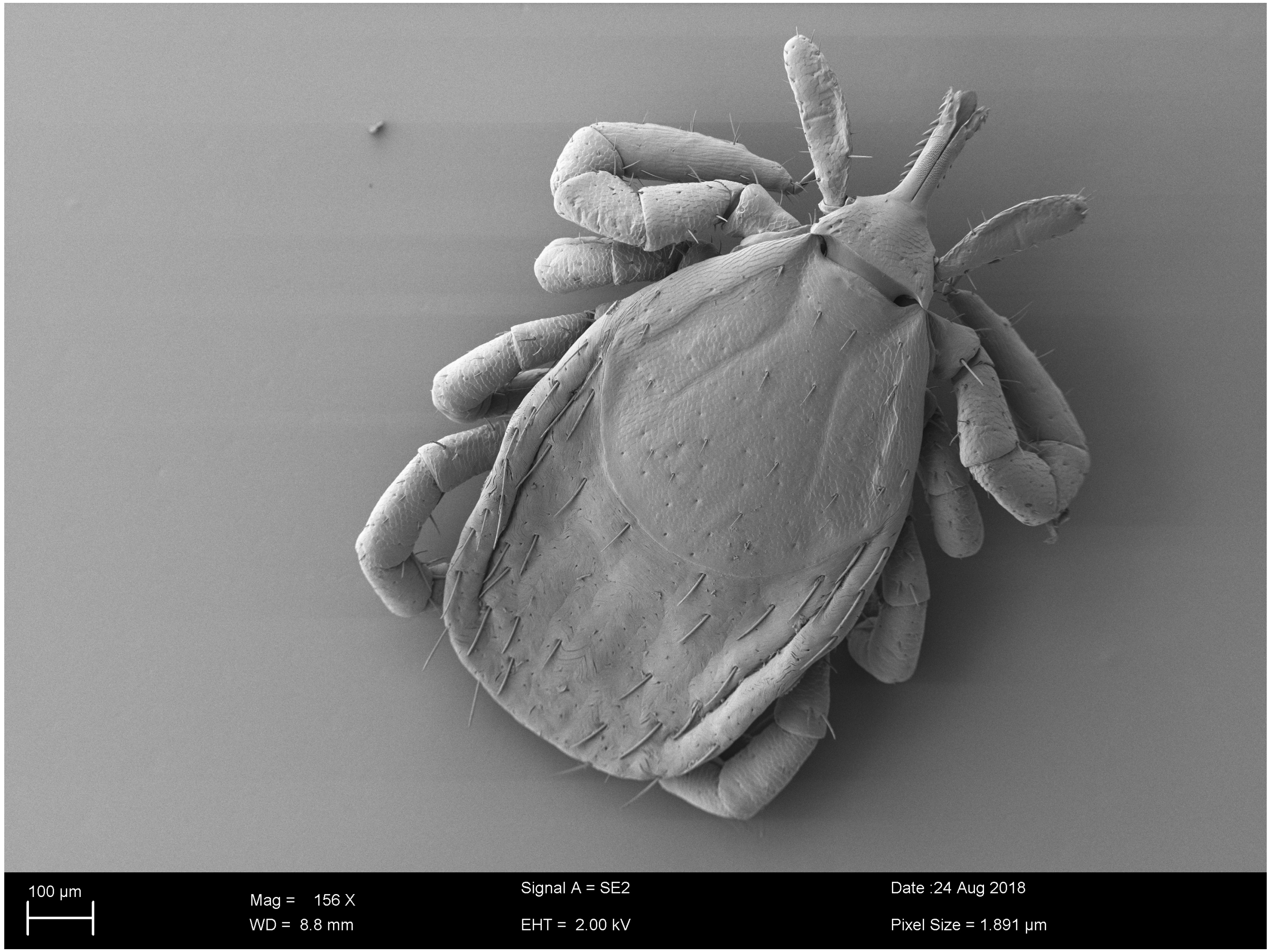
Richtung des abrasternden Primärelektronenstrahls



Charging: following scans

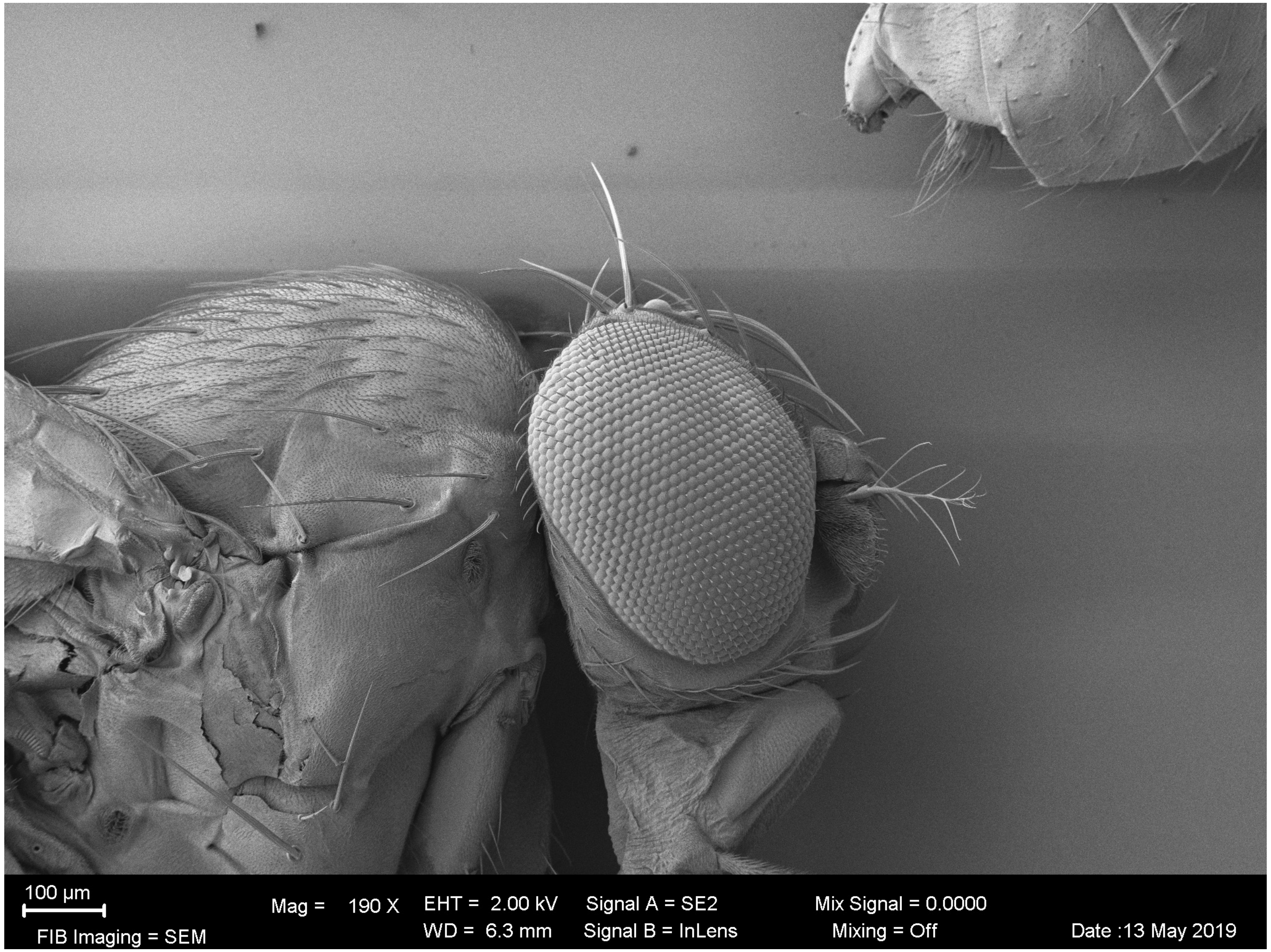


Charging: examples



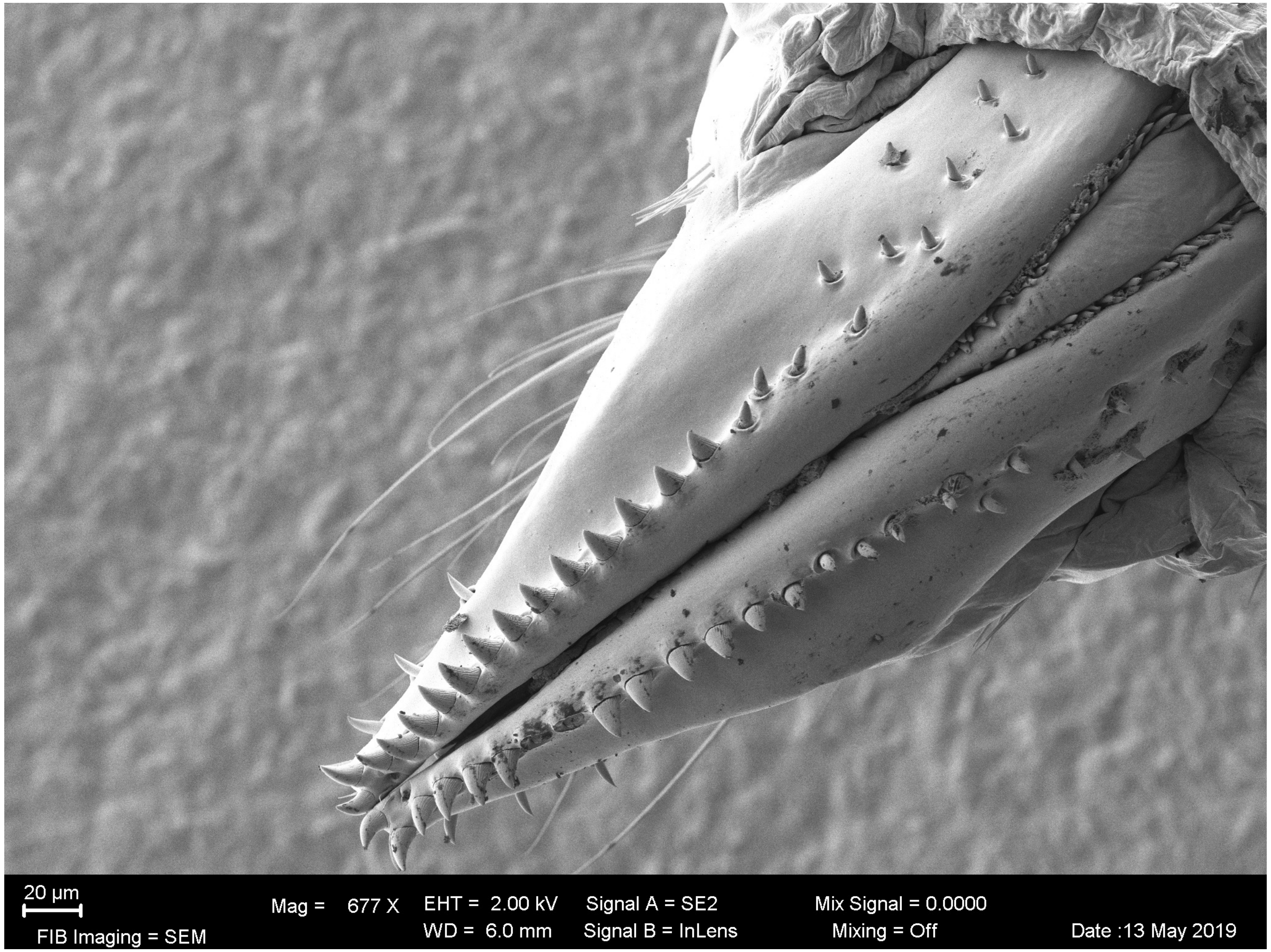
© A. Klingl

Charging: examples



© A. Klingl

Charging: examples



© A. Klingl

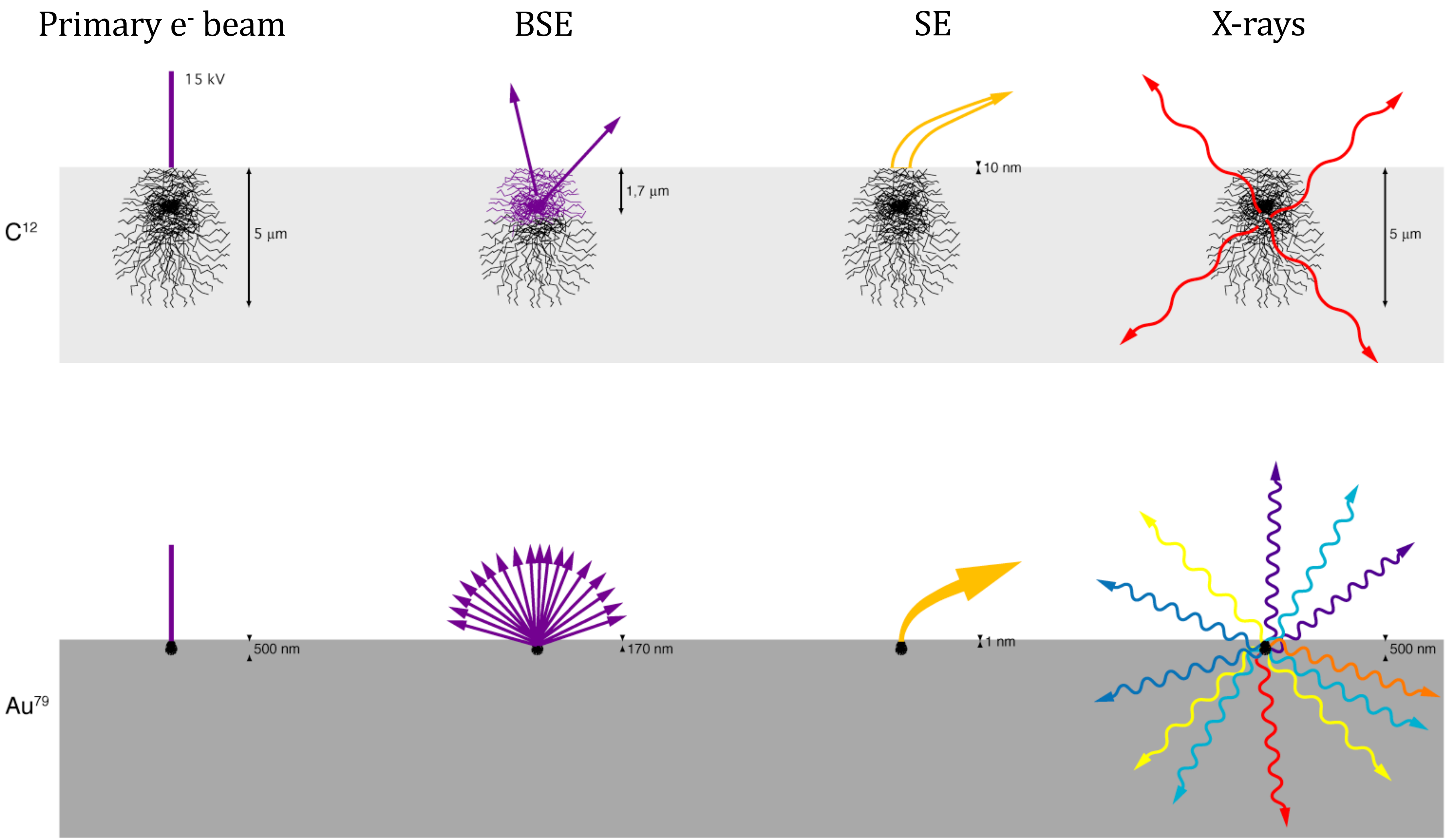
Metal coating of sample:

1. Conductivity (reduction of charging)
2. Enhancement of SE yield
3. SE from near-surface layers (1nm)
4. Increasing signal/noise ratio
5. Reduction of beam damage

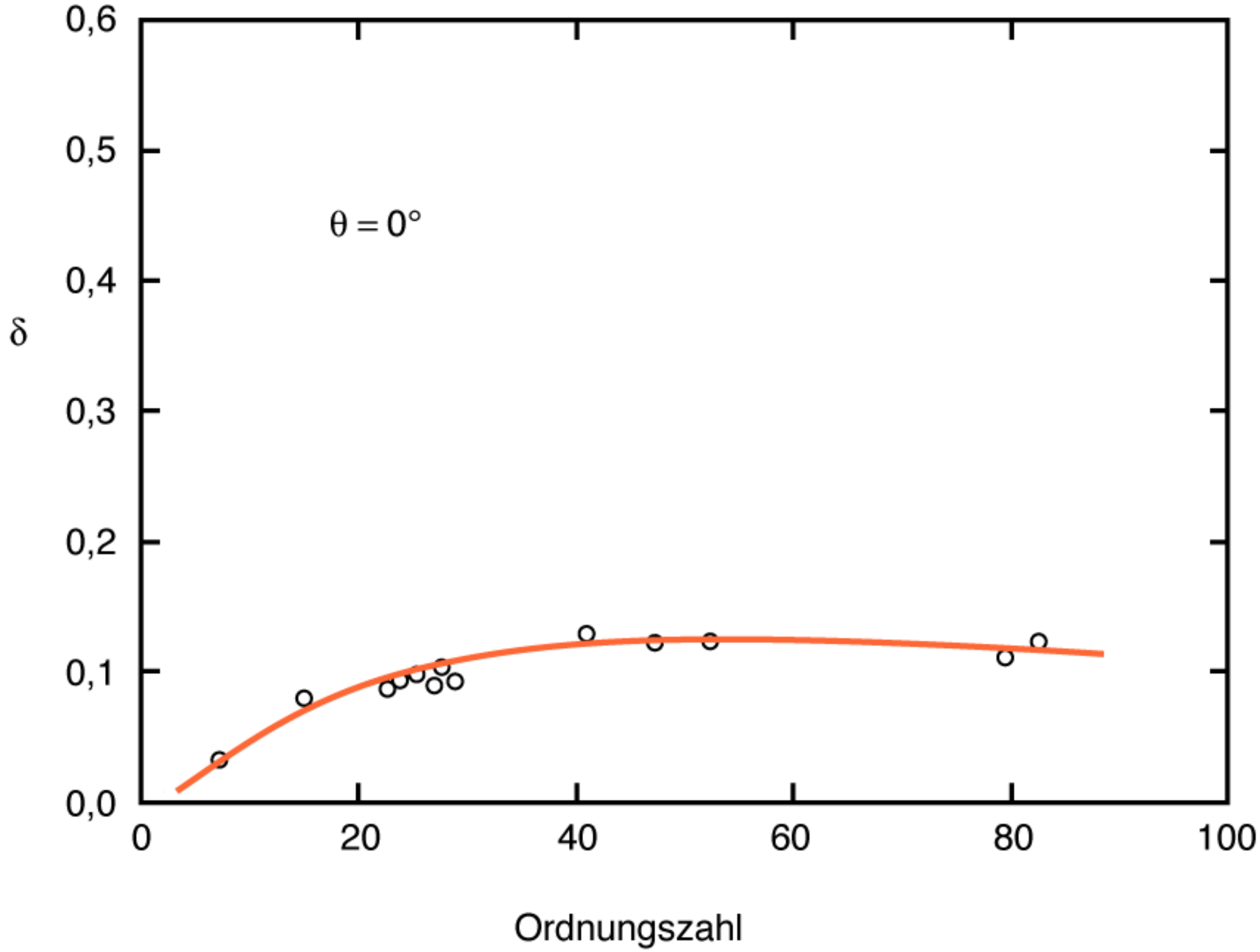
but:

- Impairment of resolution concerning object details
- Decoration effects

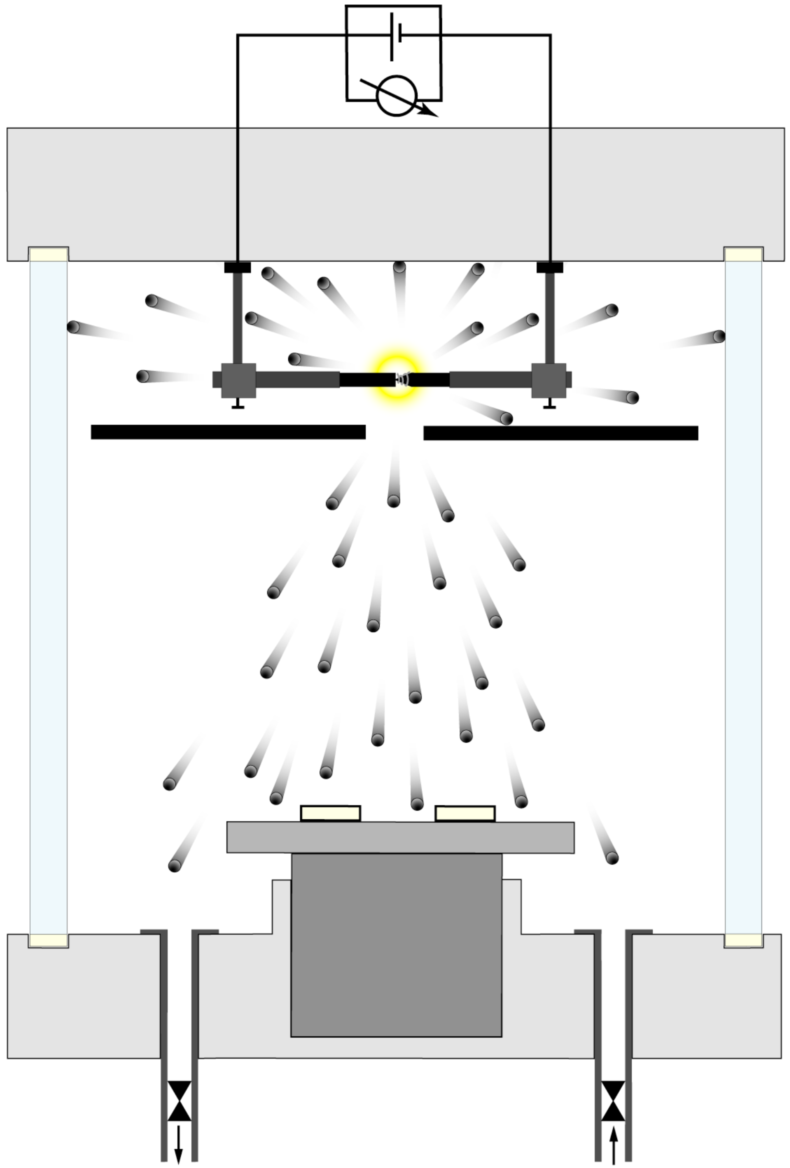
Comparison: emerging depths of signals



The dependence of high-contrast images from atomic number: (SE-coefficient)



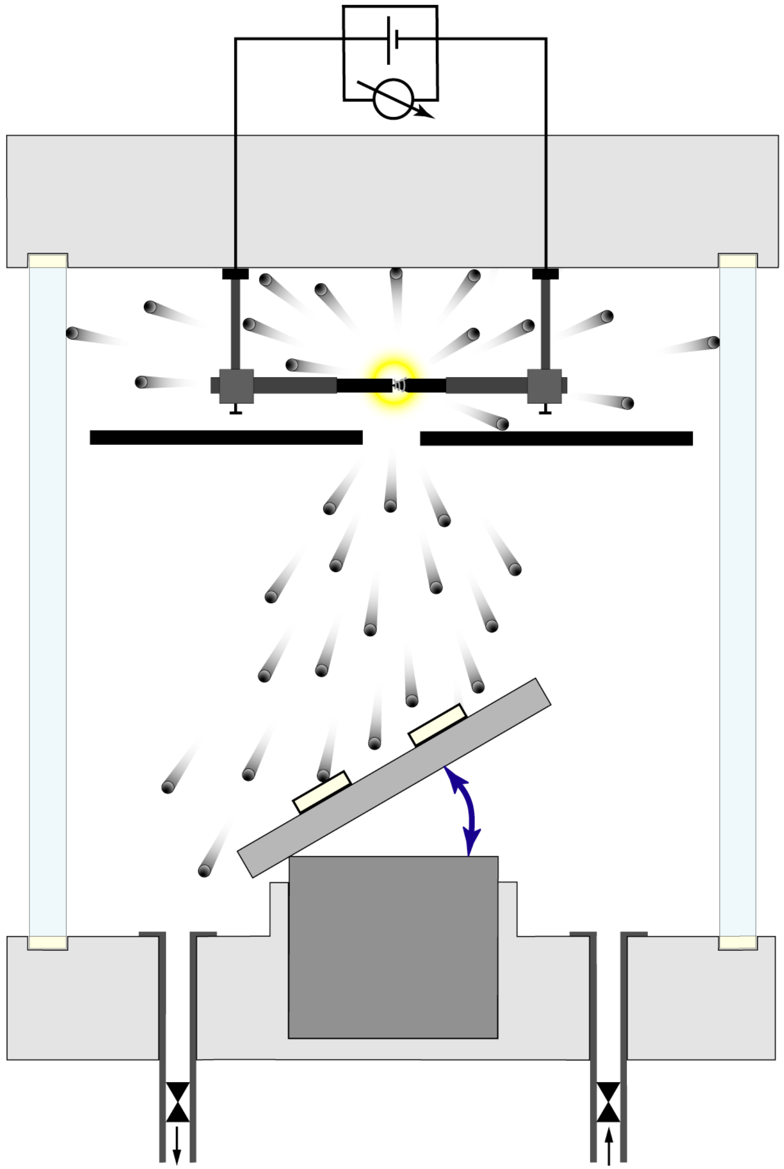
Vacuum metal evaporation



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

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

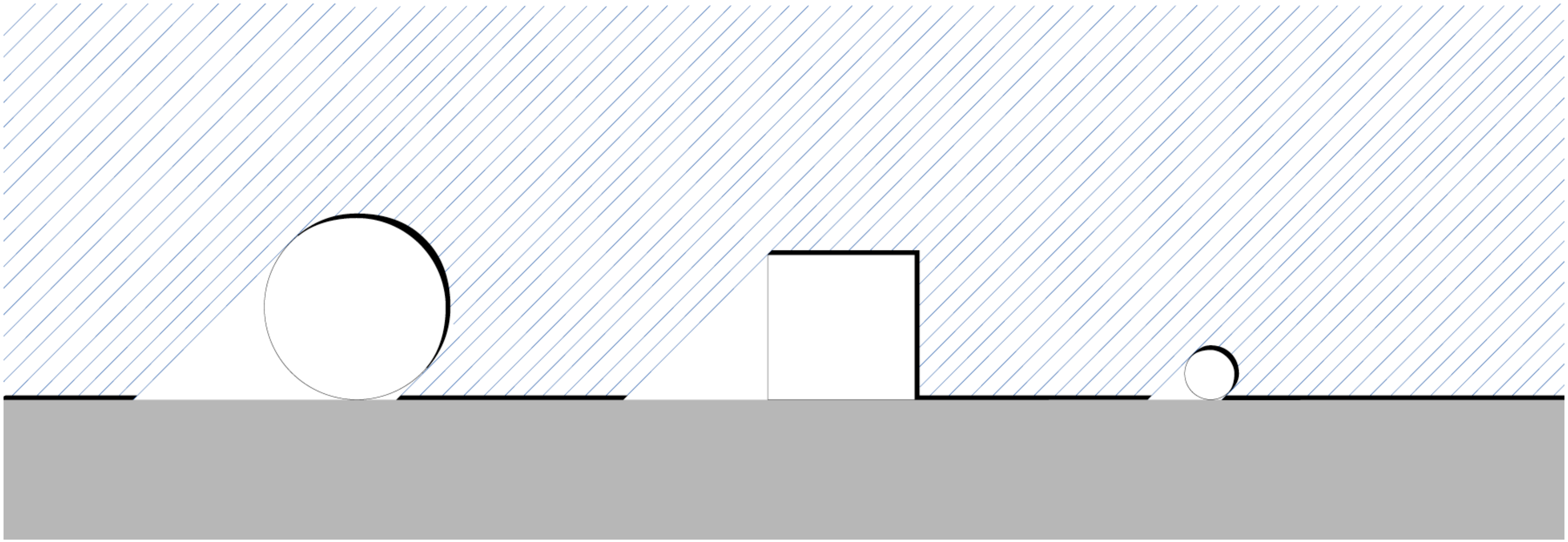
Vacuum metal evaporation



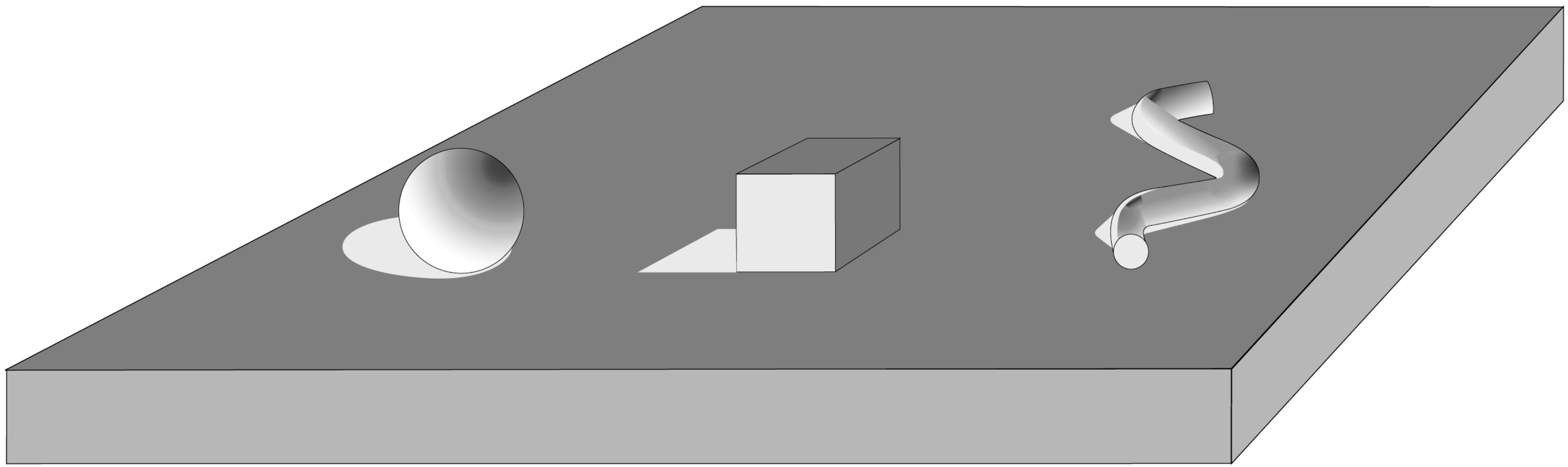
Properties
High vacuum
Hot particles
„Light and shadow“
Oblique evaporation

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

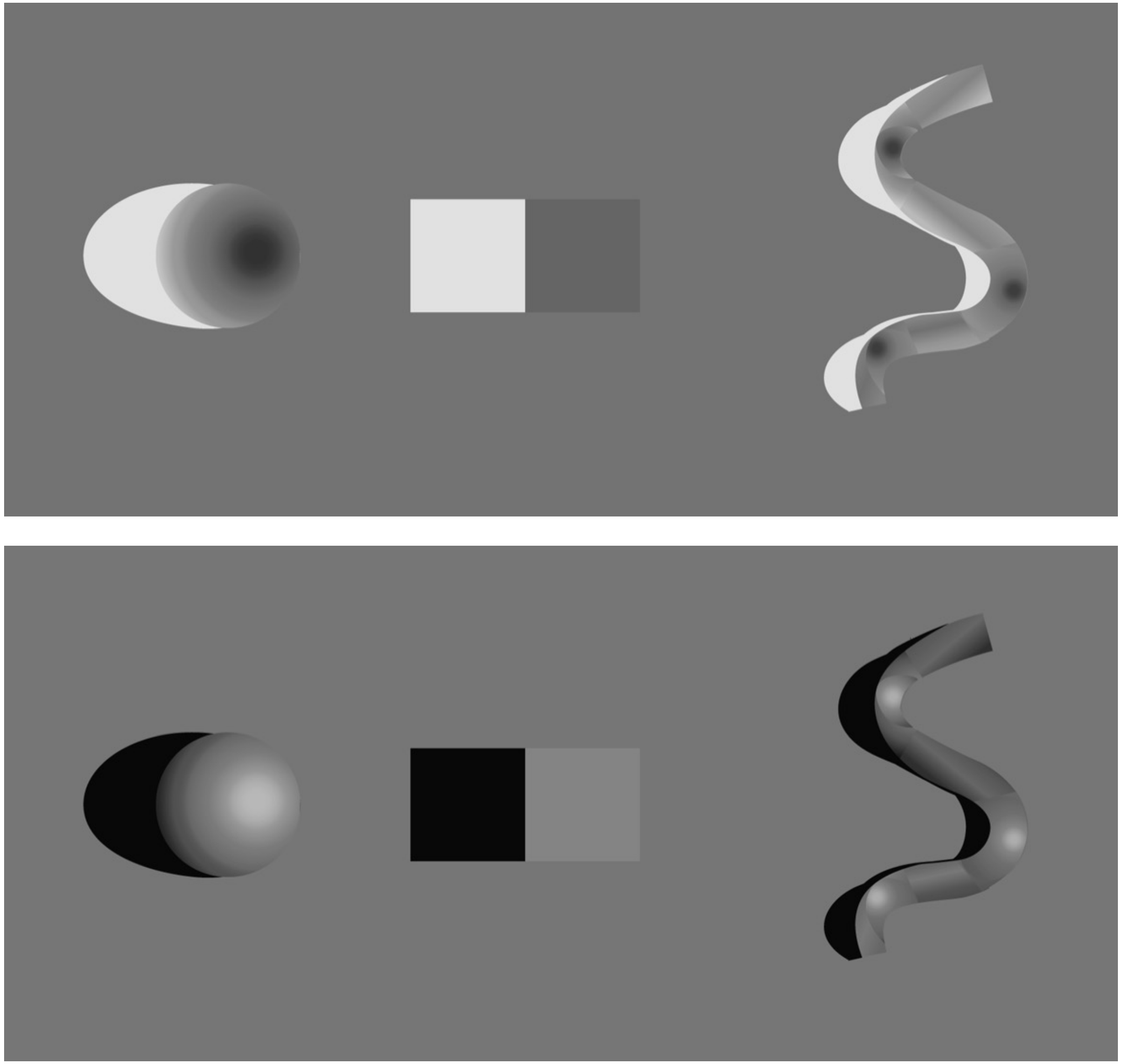
Oblique evaporation: „Light and shadow“



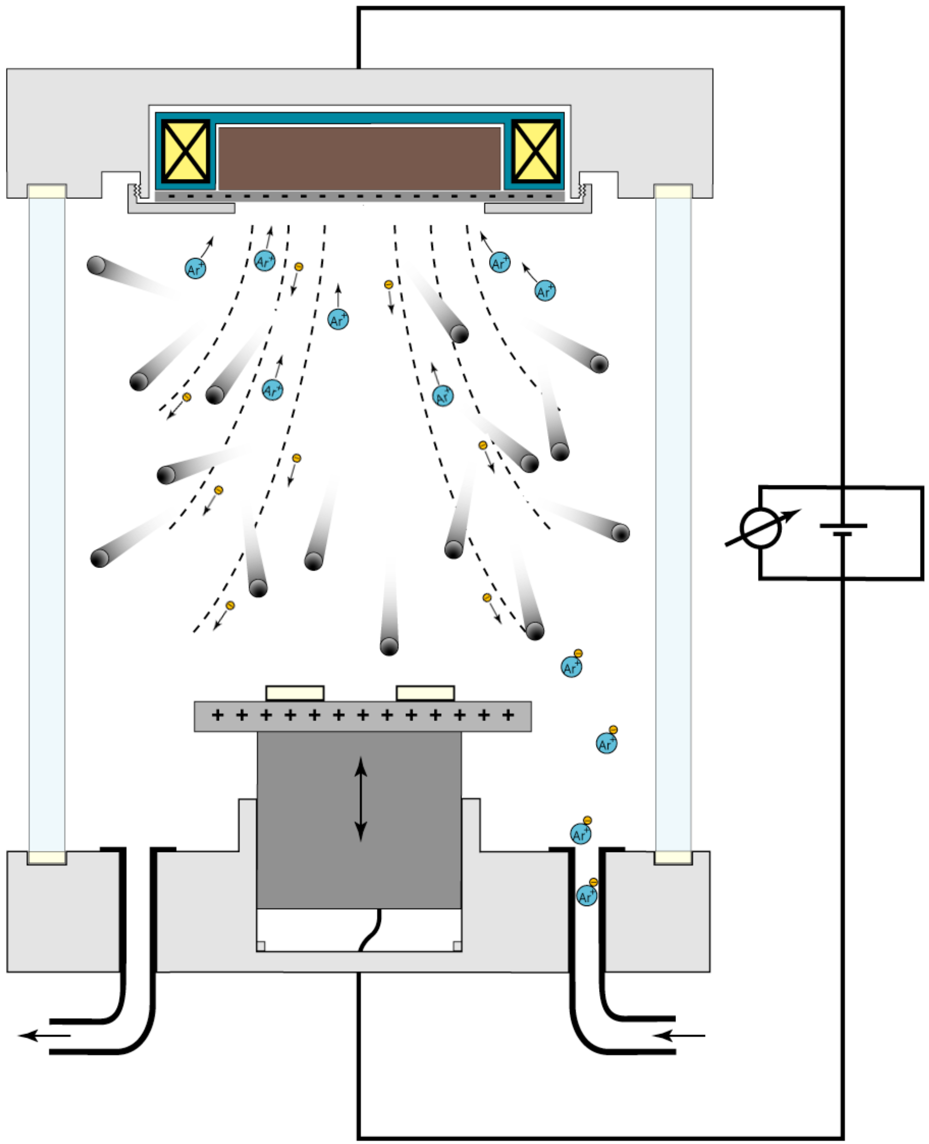
Oblique evaporation: „Light and shadow“ (TEM)



Vacuum metal evaporation



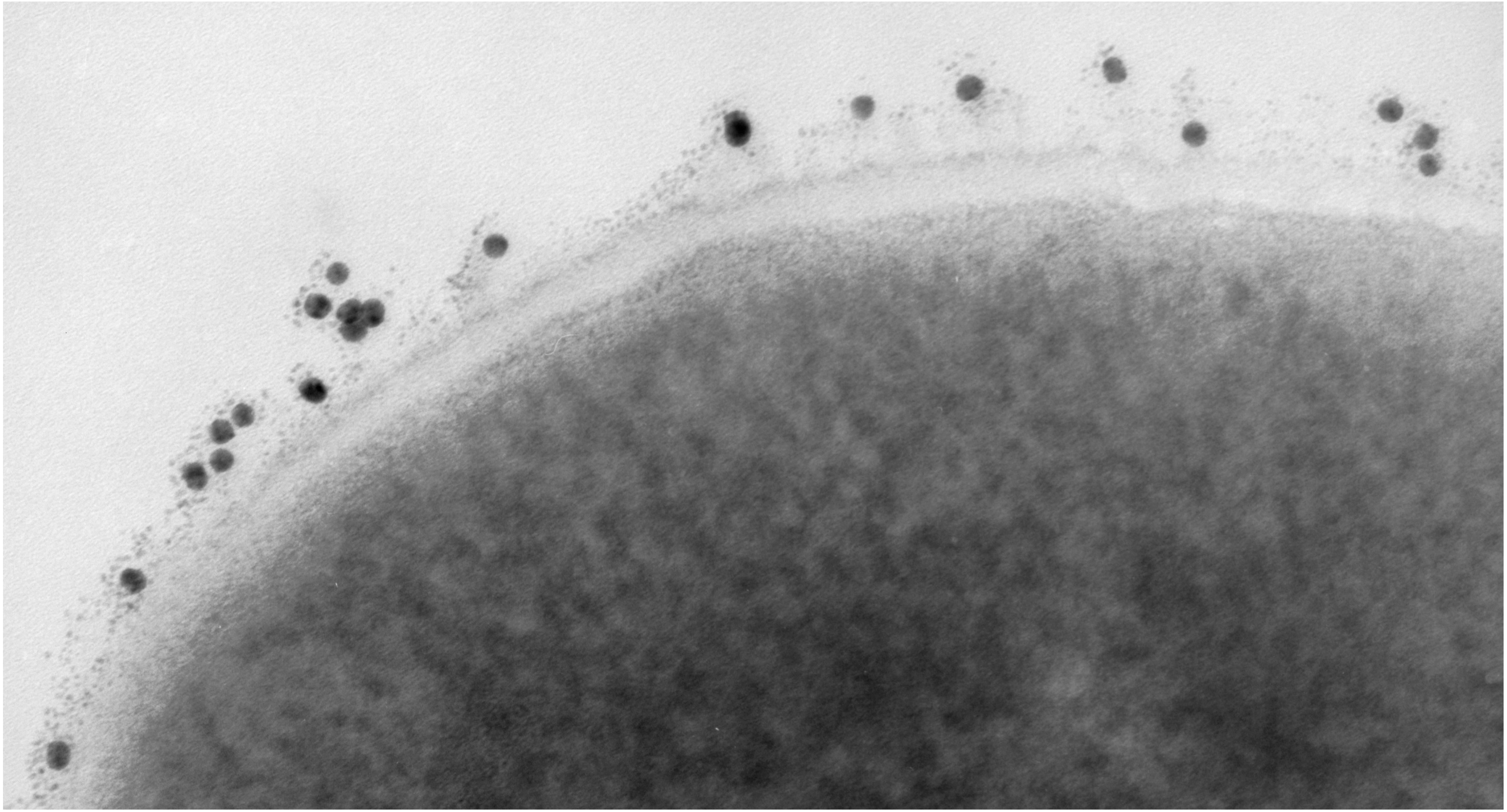
Sputter coating



Properties
Moderate vacuum
Cold particles
± even layer

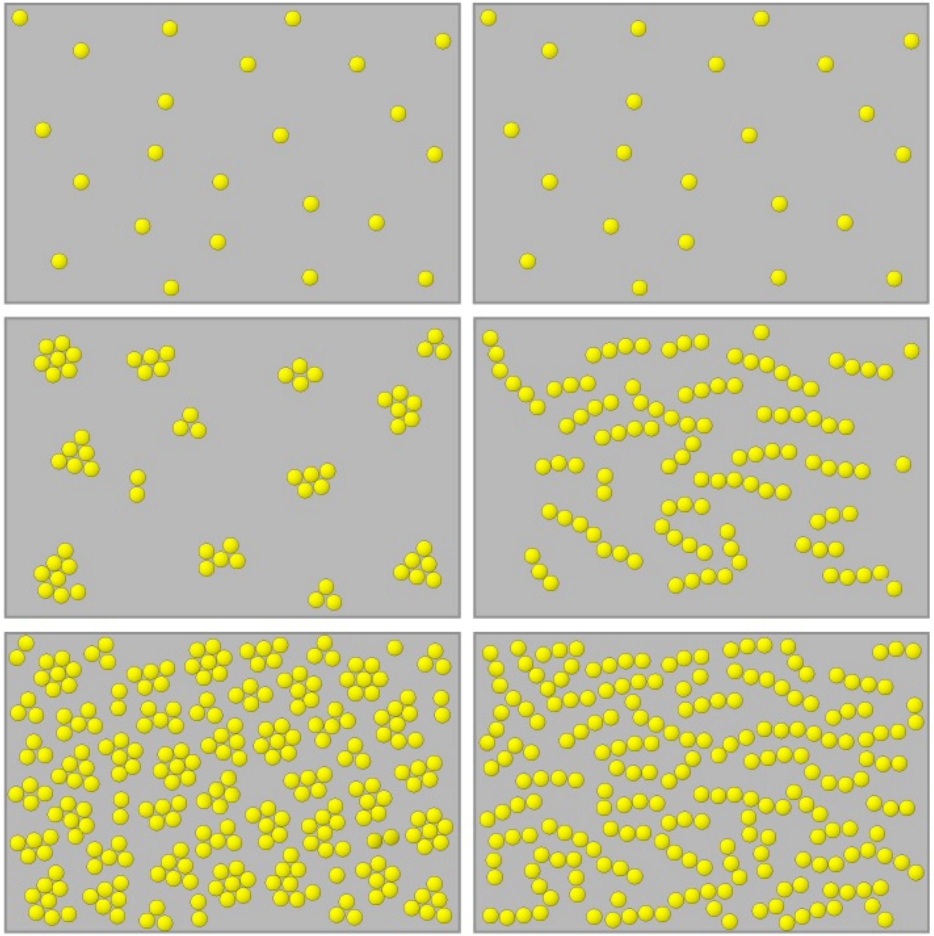
Parameters:
Distance: sample-target
Quality of vacuum
High tension
Current flow (argon ions)
Duration

Sputter coating



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

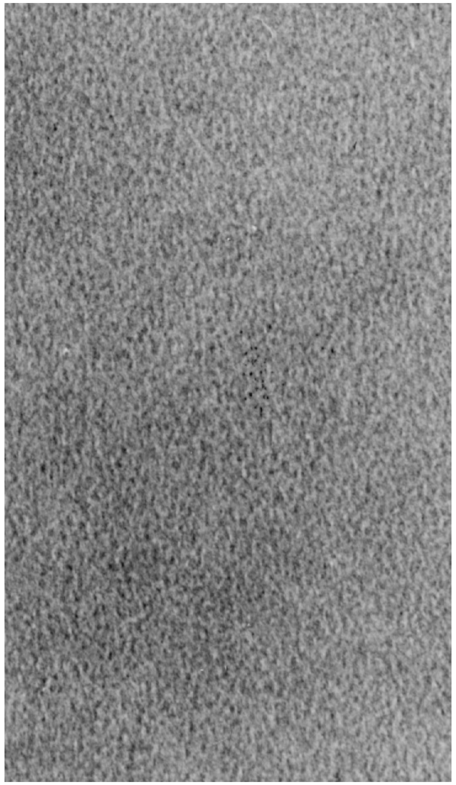
Formation of clusters



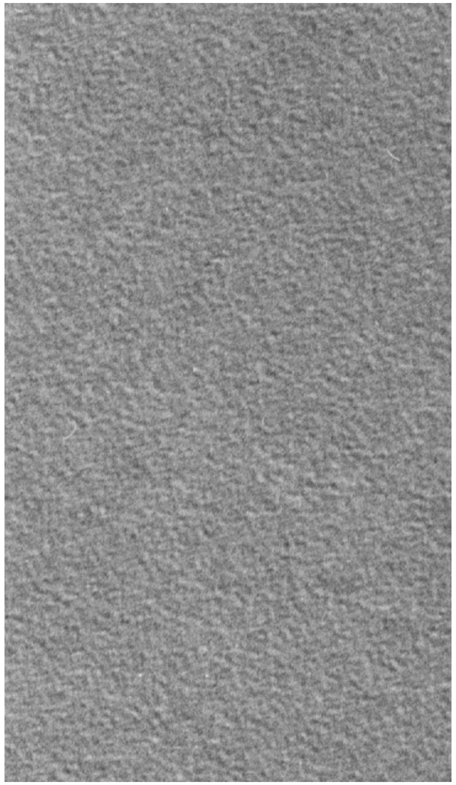
Gold:	strong
Gold/palladium (80/20):	weak
Platinum:	no

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

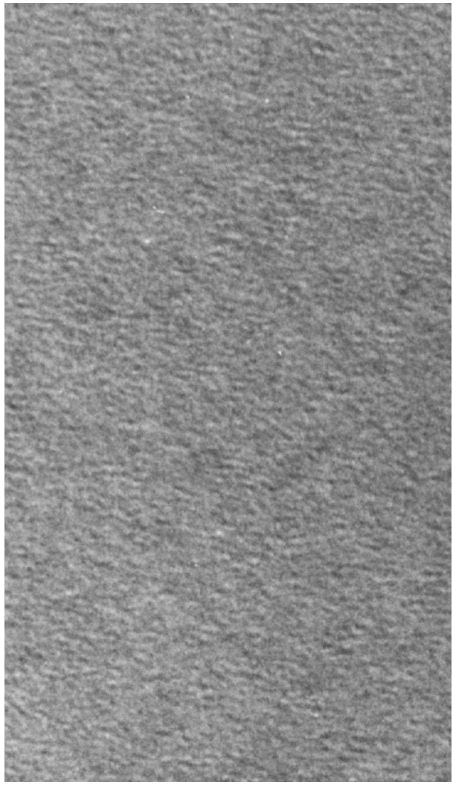
. = 1 nm



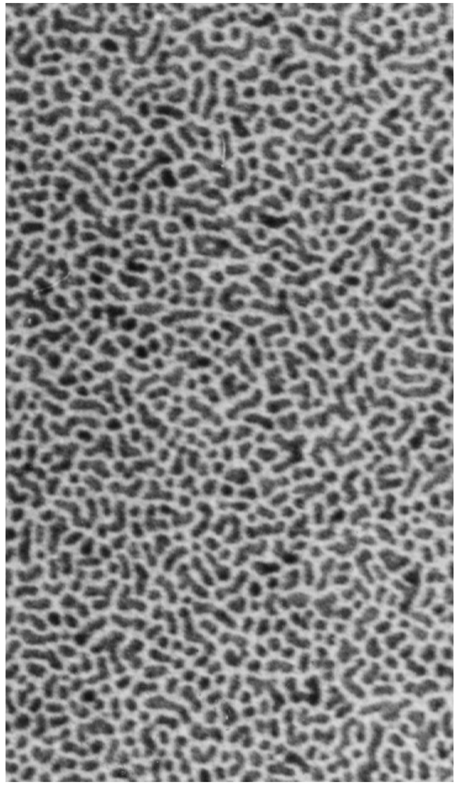
Cr
Chromium
Atomic number: 24
Atomic mass: 53
Density: 7,2



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

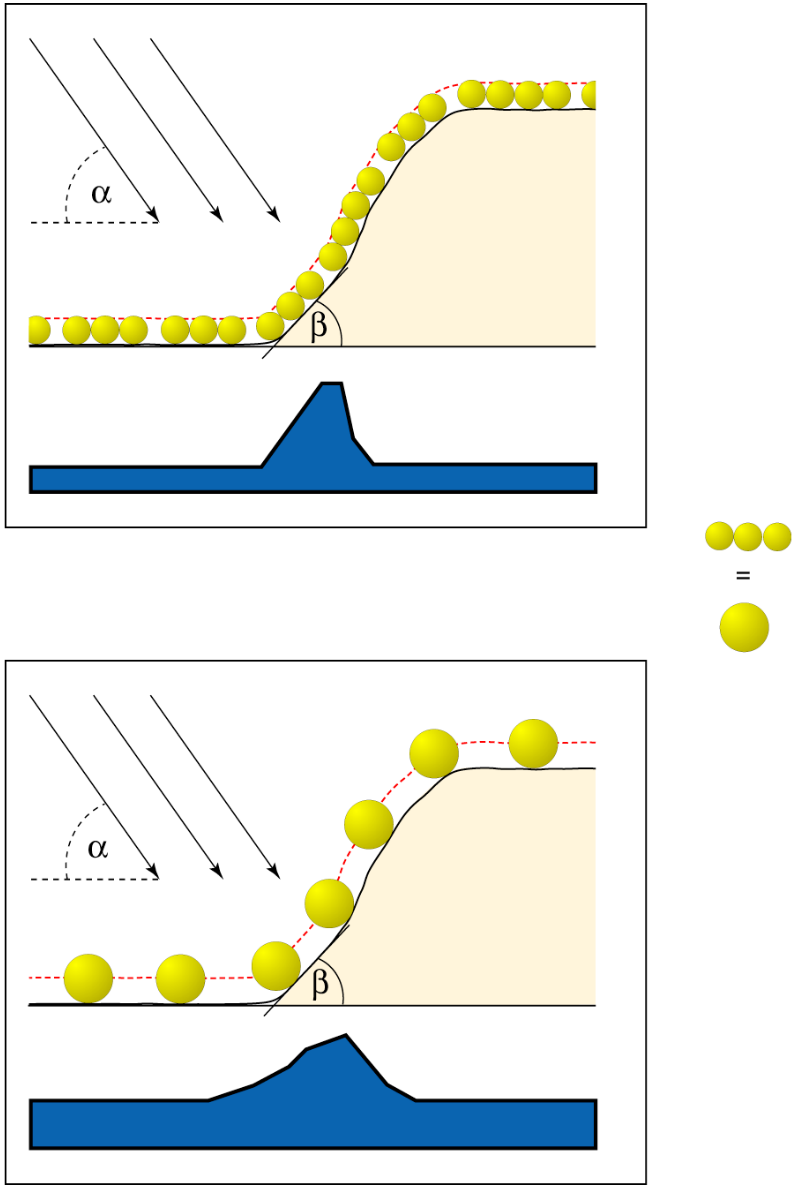


W
Tungsten
Atomic number: 74
Atomic mass: 184
Density: 19,3

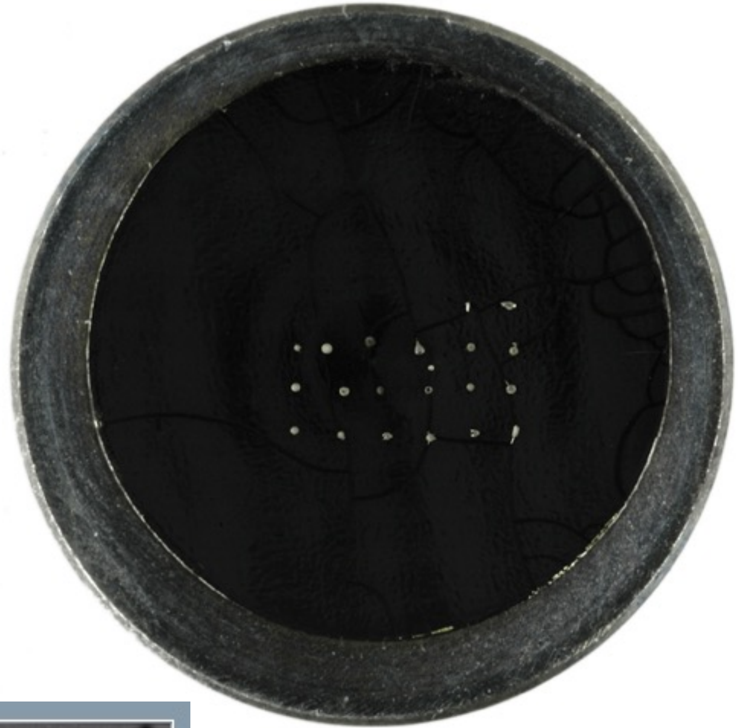


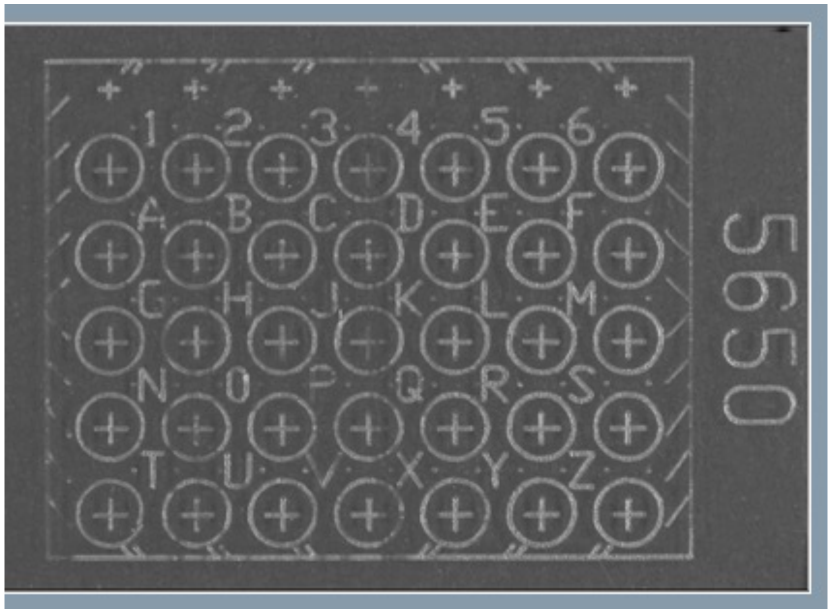
Au
Gold
Atomic number: 79
Atomic mass: 197
Density: 19,3

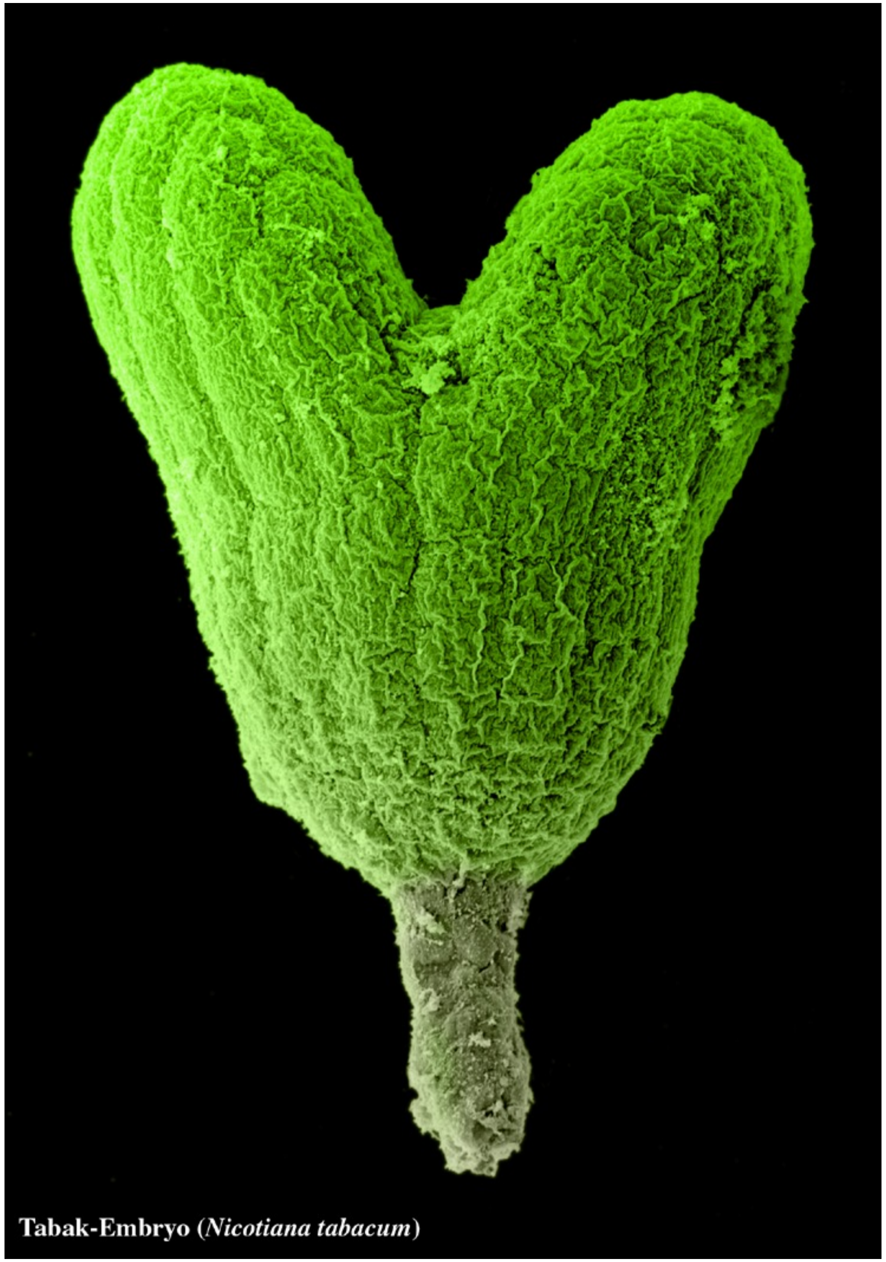
Layer thickness – signal – resolution



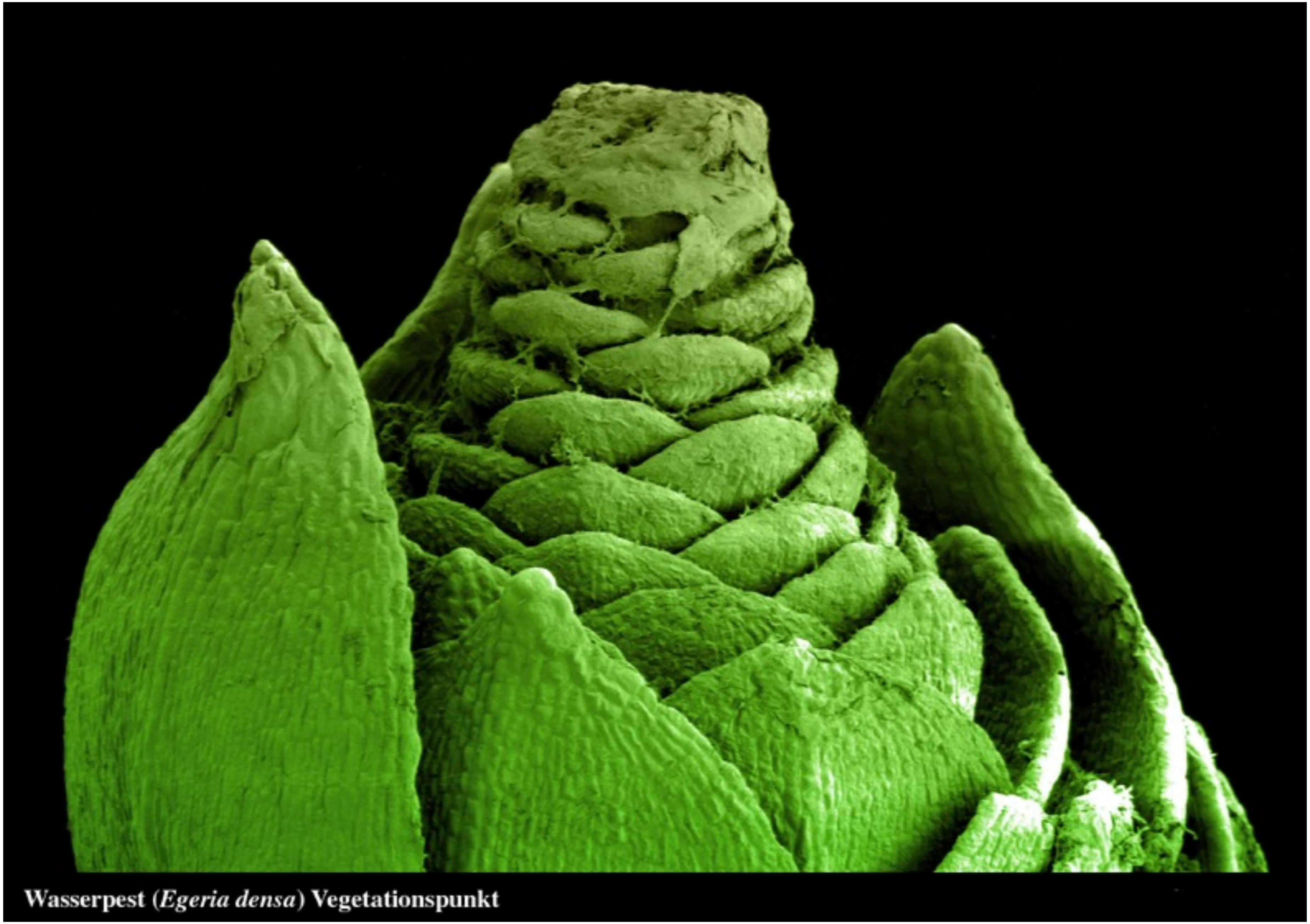
Samples



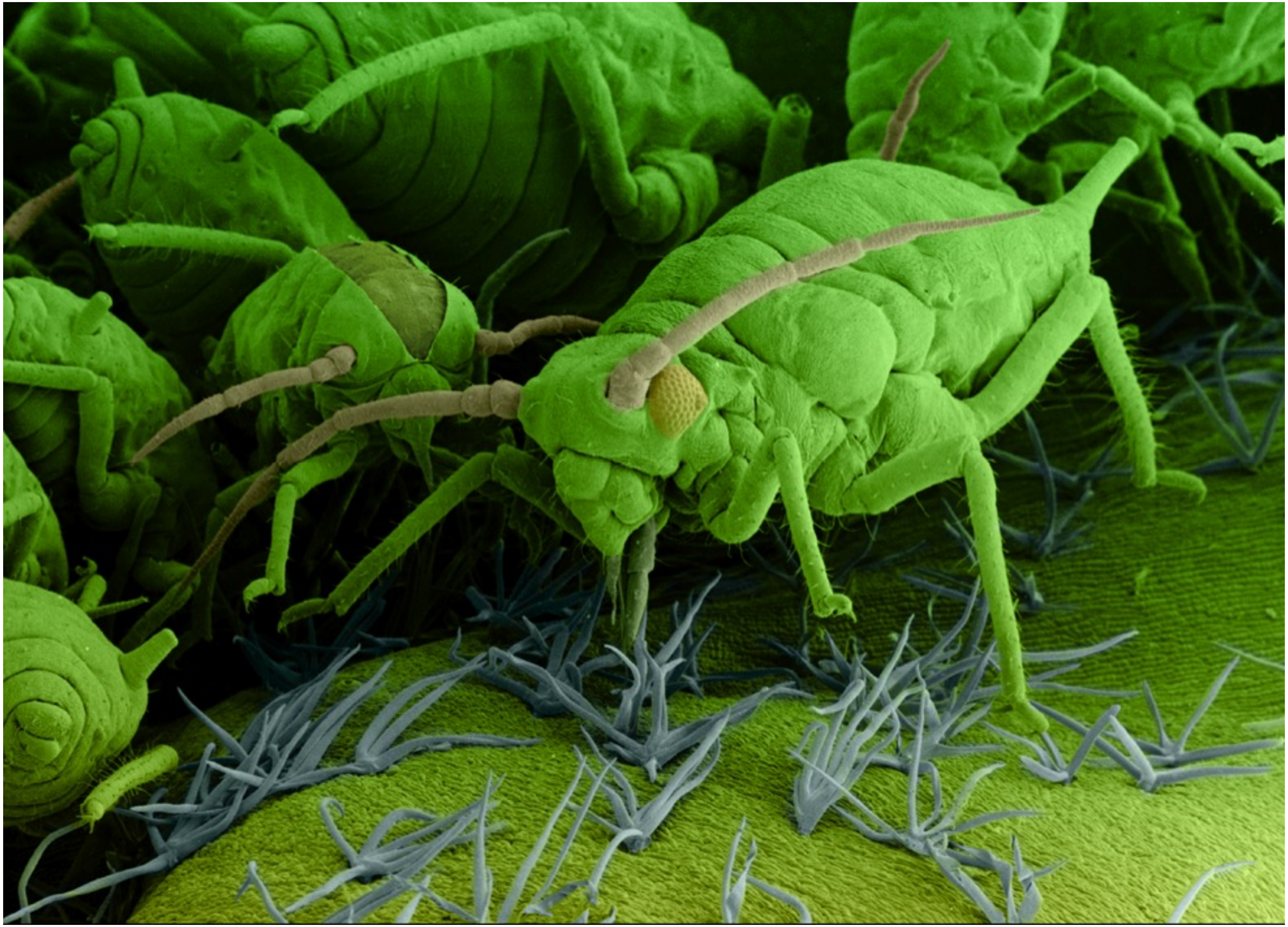




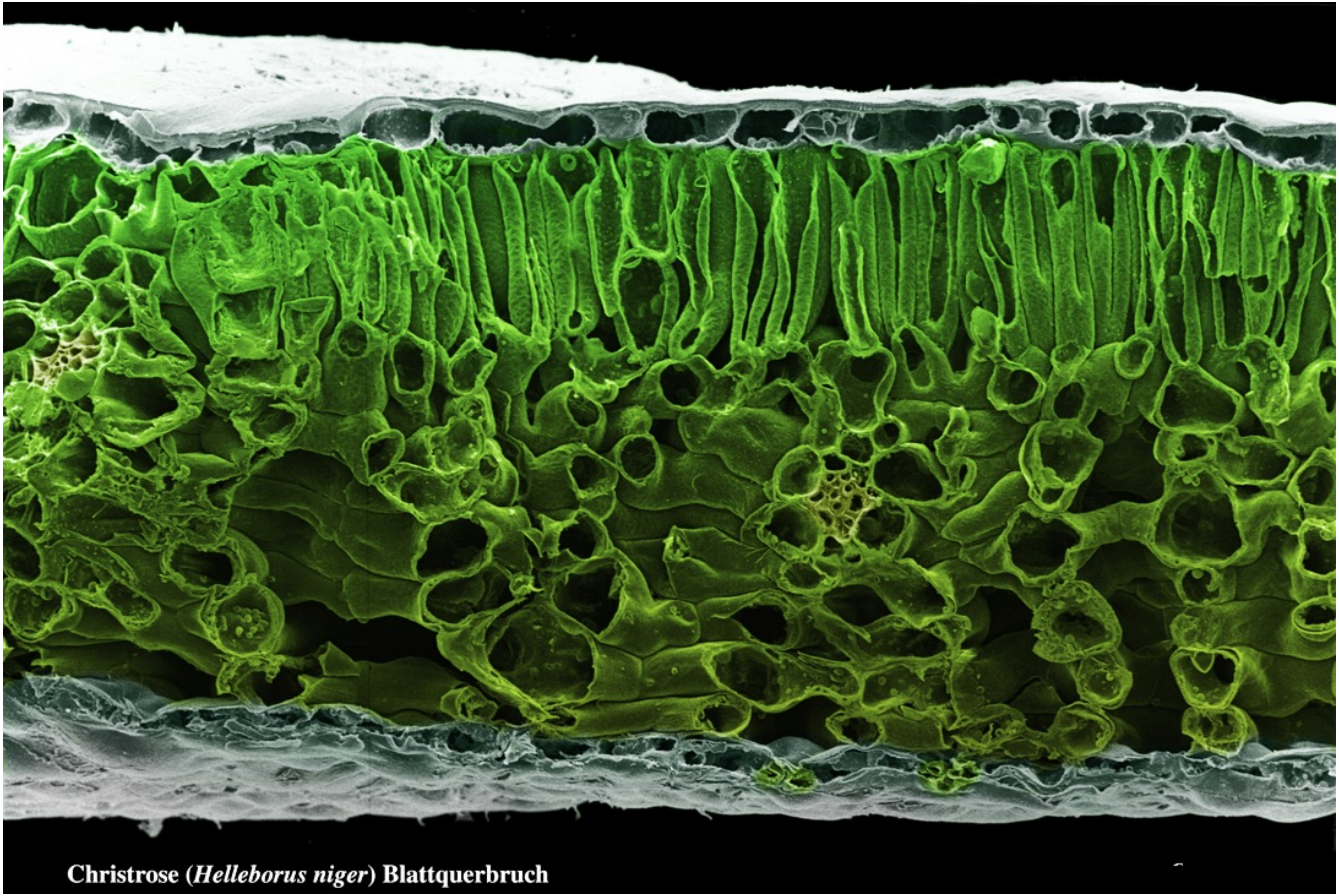
© Gerhard Wanner



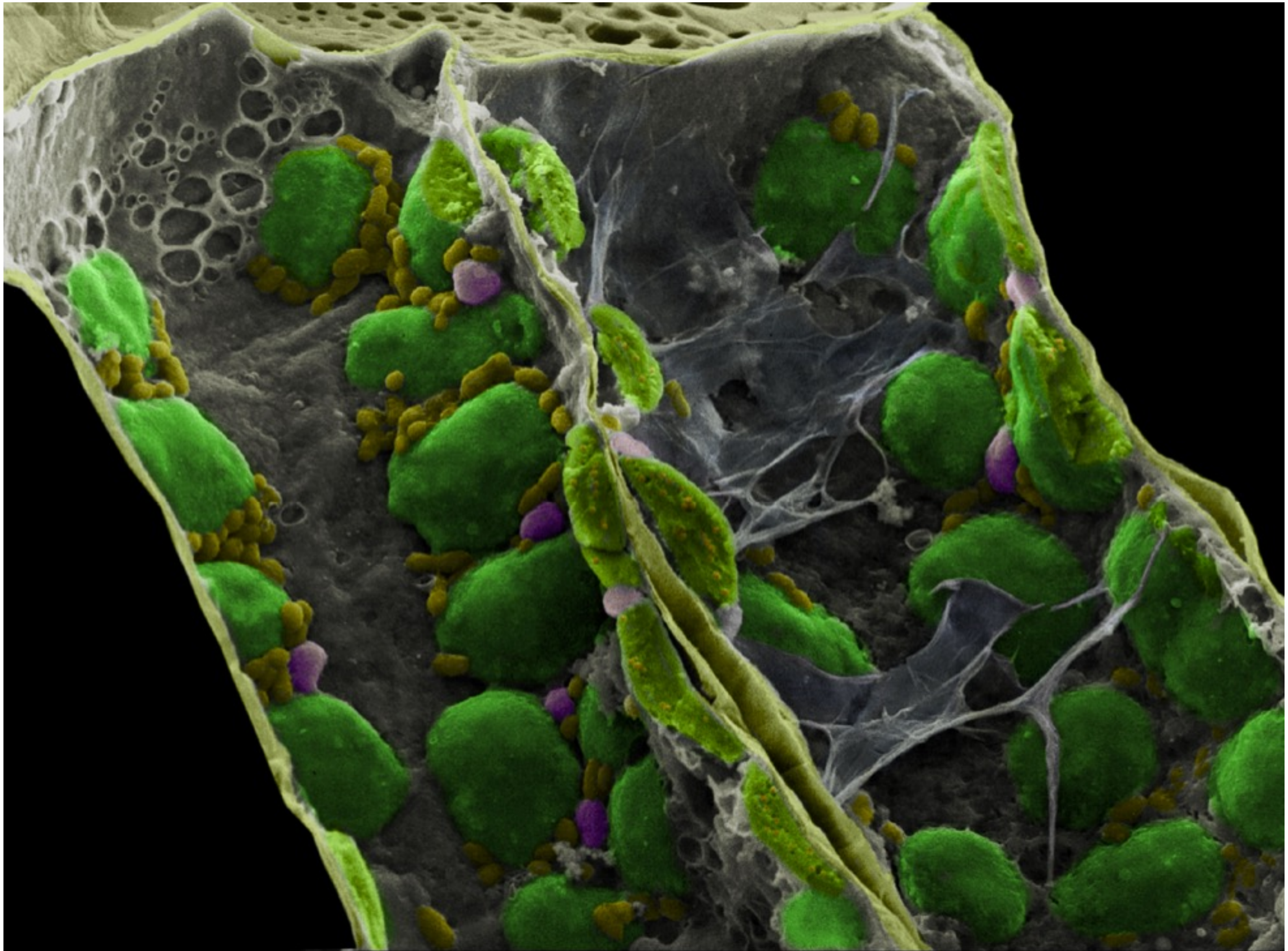
Wasserpest (*Egeria densa*) Vegetationspunkt



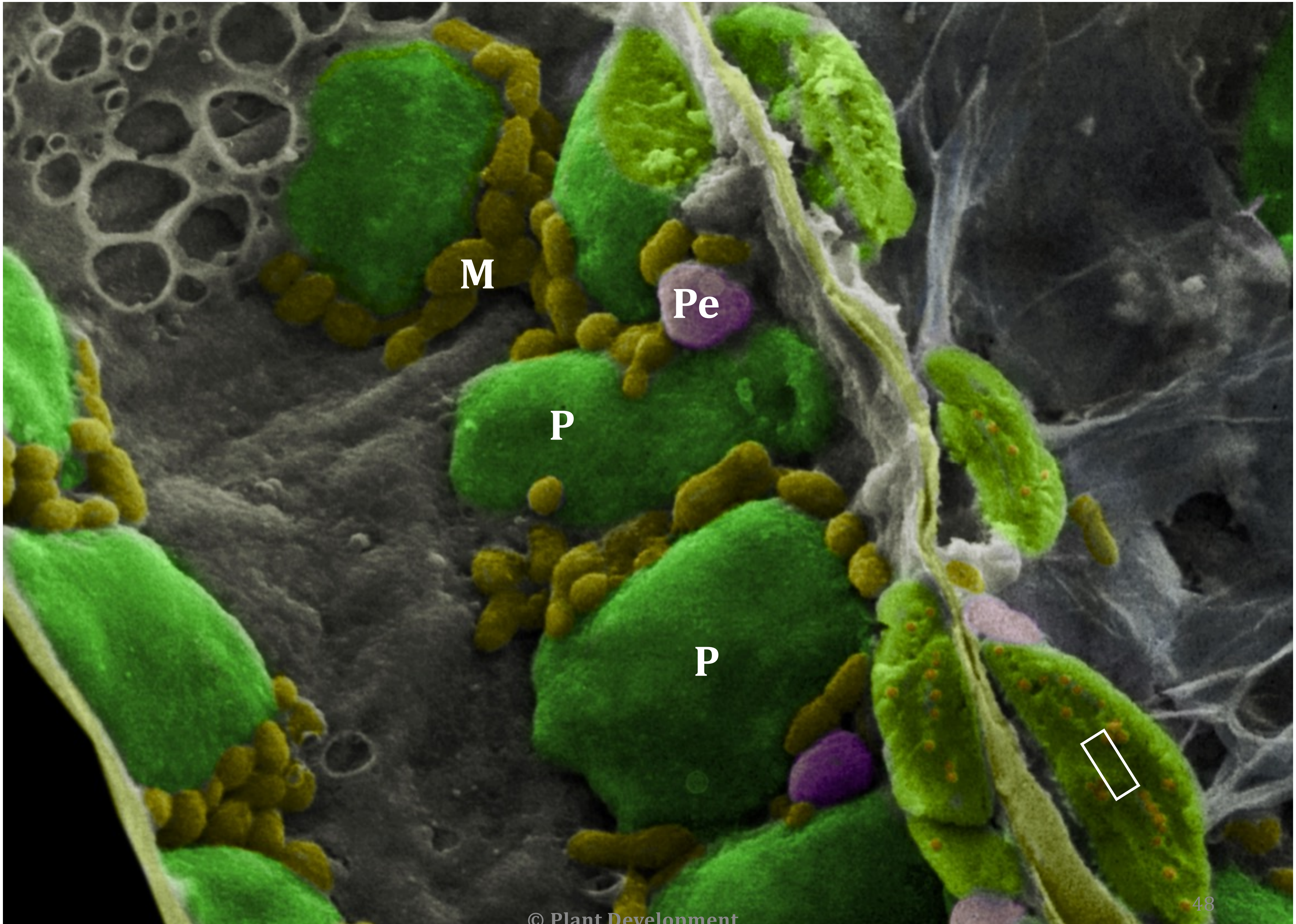
© Gerhard Wanner

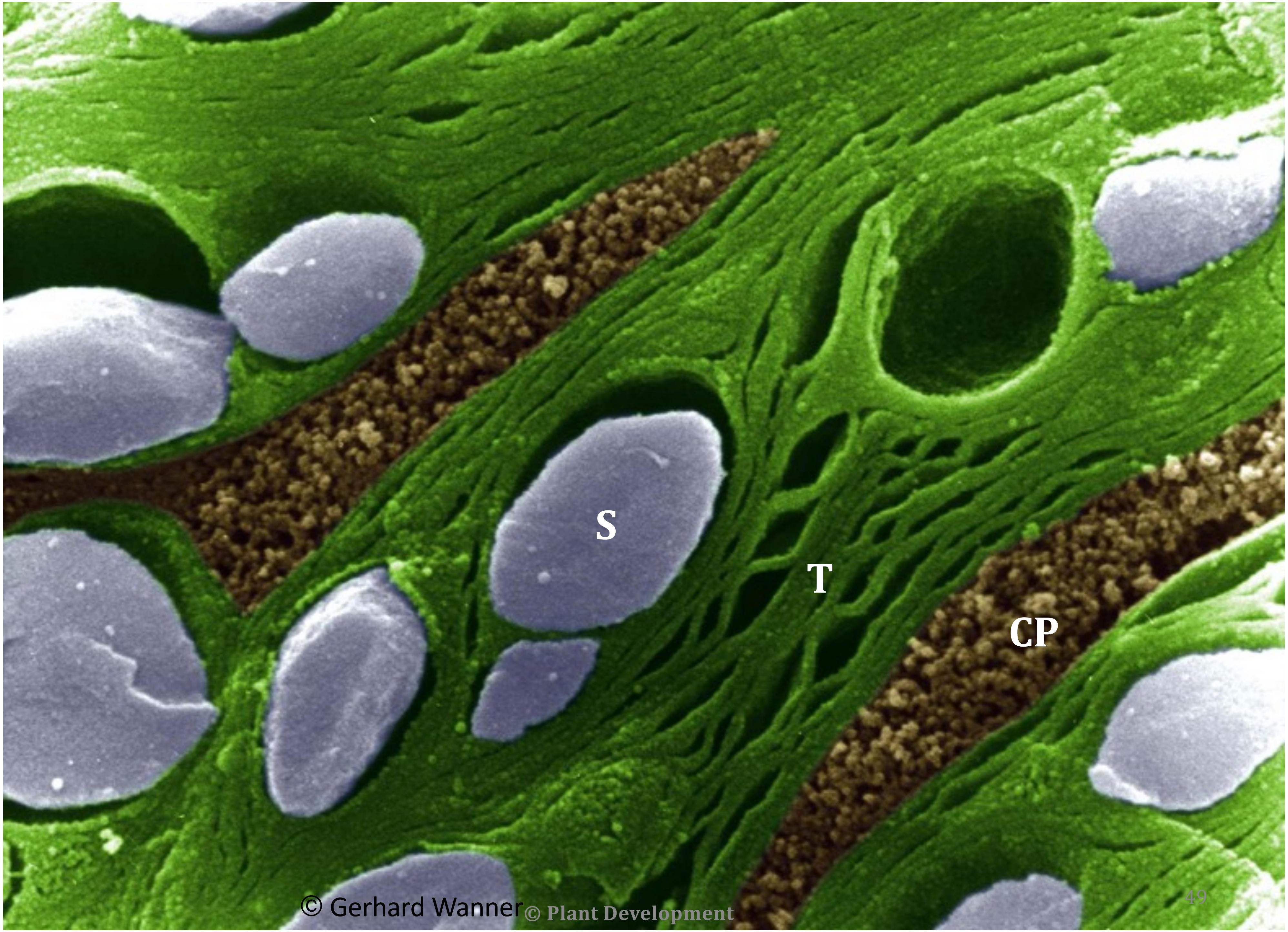


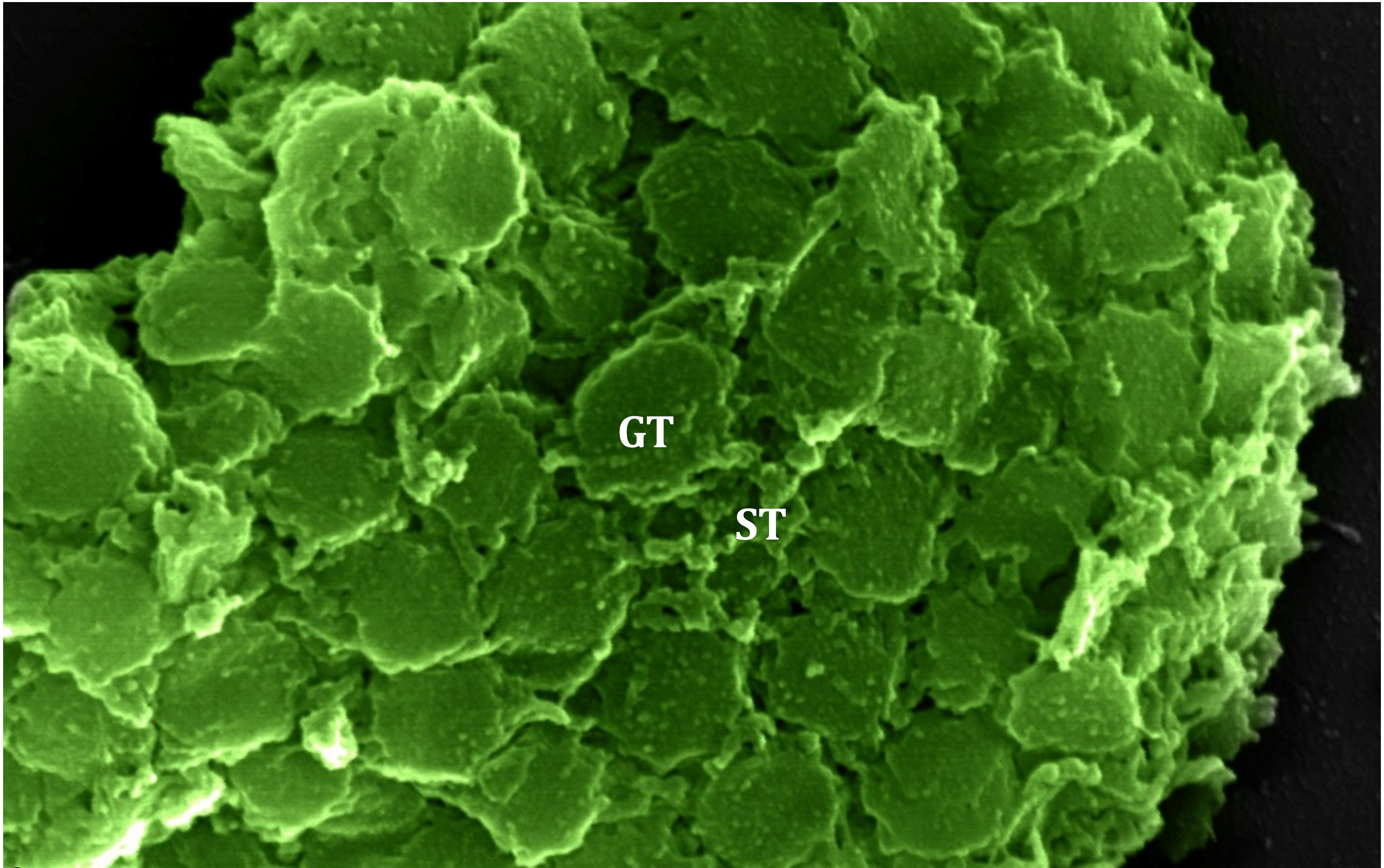
Christrose (*Helleborus niger*) Blattquerbruch



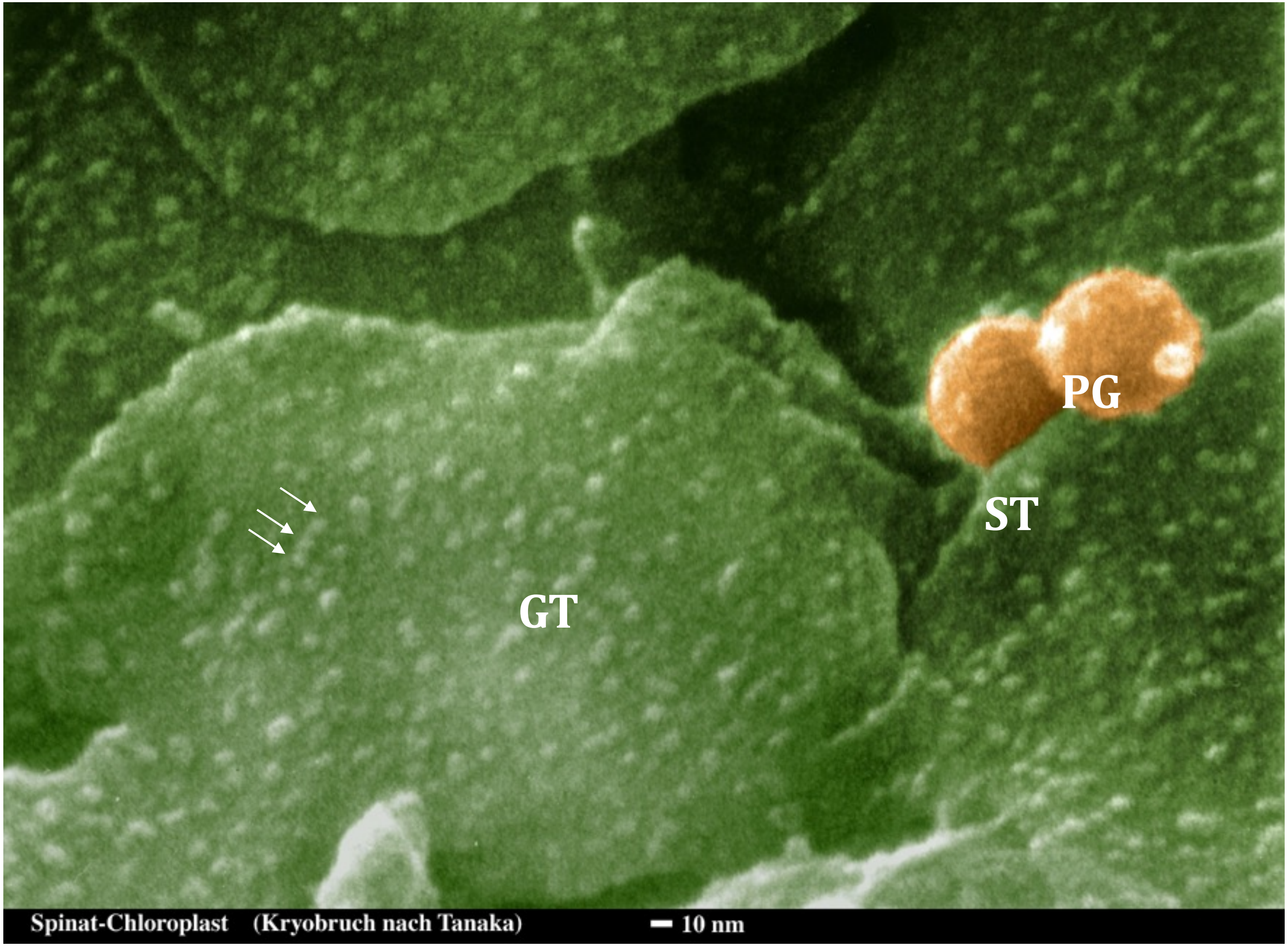
Christrose (*Helleborus niger*) Blattquerbruch mit Epidermis und Palisadenparenchym

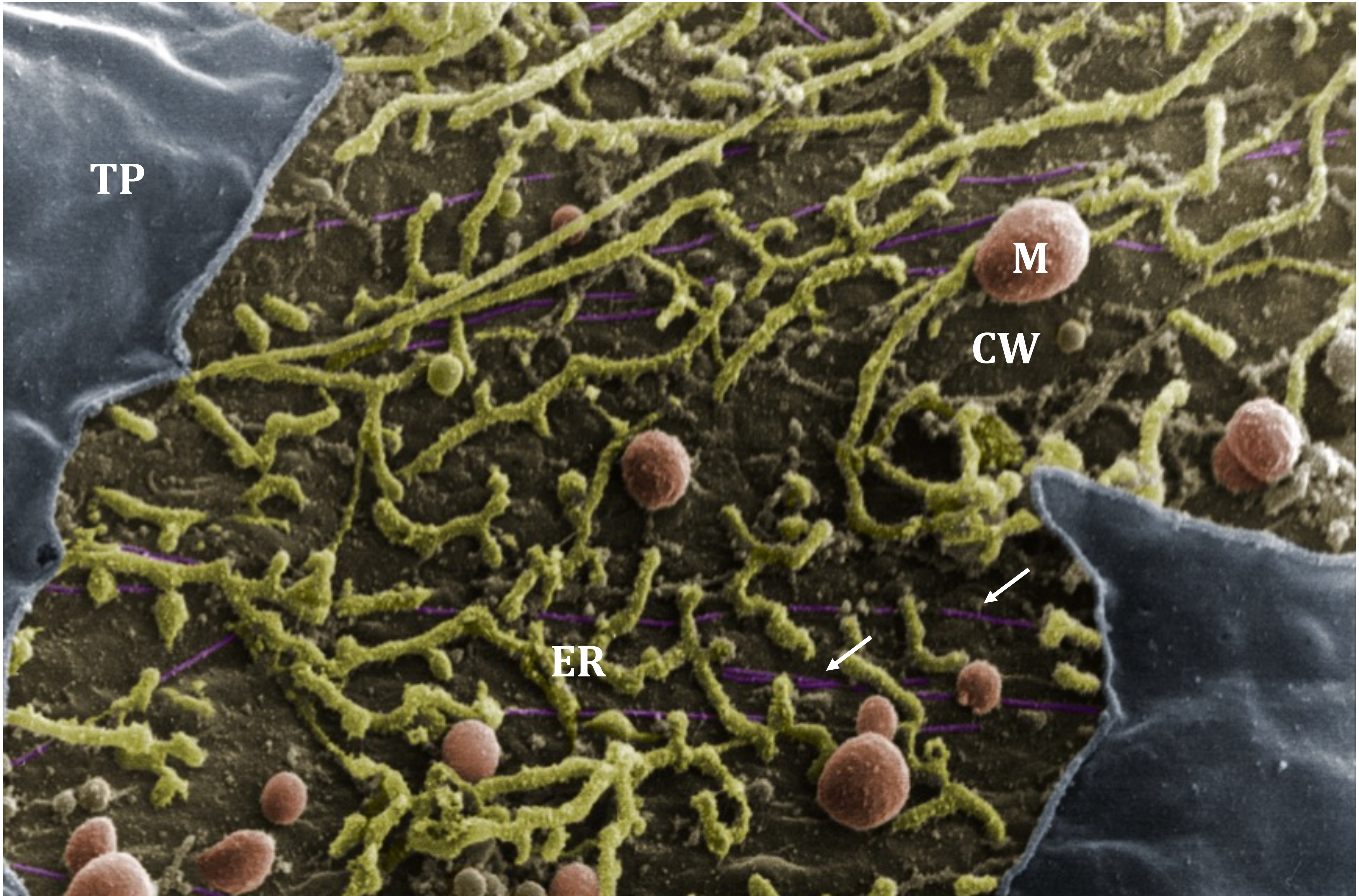

M
Pe
P
P
48

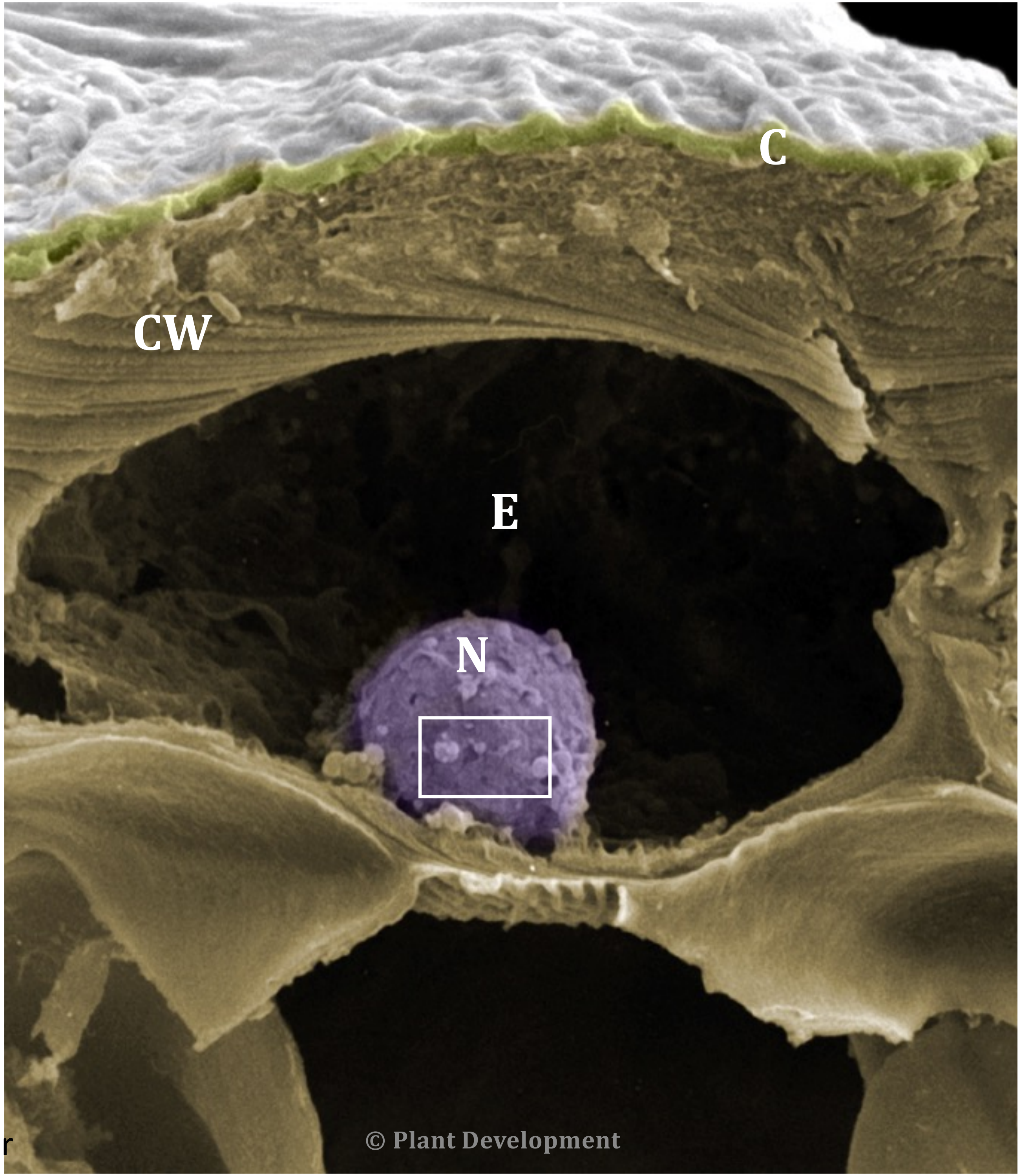

S
T
CP

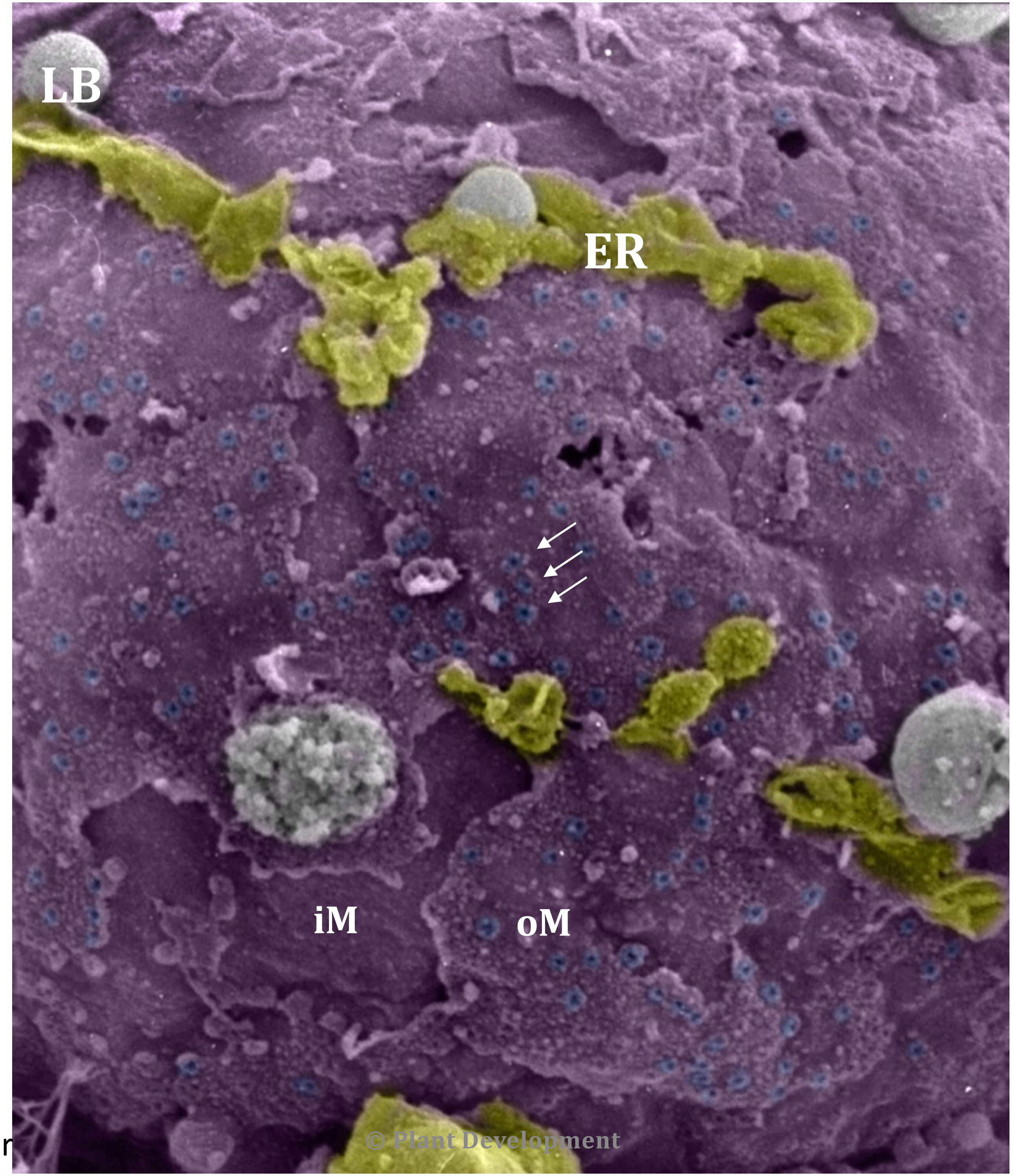


© Gerhard Wanner


PG
ST
GT
Spinat-Chloroplast (Kryobruch nach Tanaka)
10 nm


TP
M
CW
ER


C
CW
E
N
© Plant Development


LB
ER
iM
oM