

NEGATIVE STAINING

José María Valpuesta

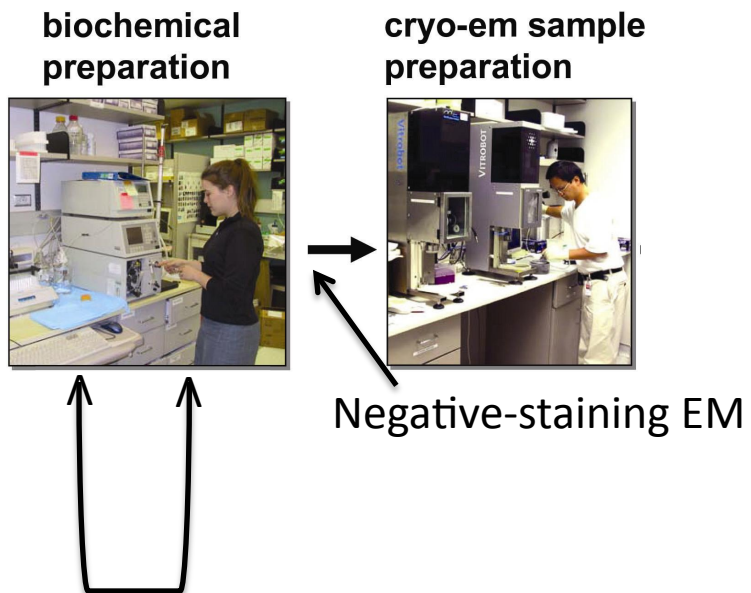


José M. Valpuesta
Centro Nacional de Biotecnología



Negative staining

From sample to structure



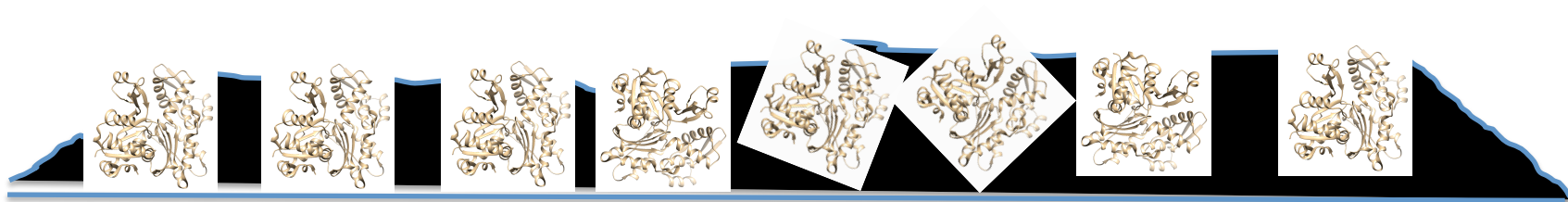
Biochemical characterisation:

- Electrophoresis
- Analytical ultracentrifugation
- Dynamic light scattering
- Calorimetric techniques
- Negative-staining EM

Negative staining



Aqueous solution



Negative staining

Staining agents

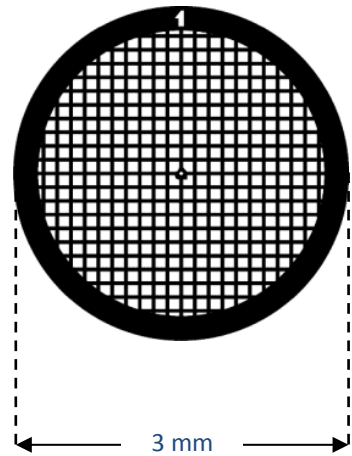
Properties:

- Minimal interaction with the sample
- High solubility in water
- High density (contrast)
- Small grain size
- Stable to interaction with electrons

More used:

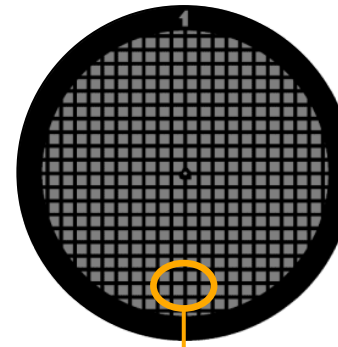
- Ammonium Molybdate
- Sodium phosphotungstate
- **Uranium salts (acetate and formate)**

Negative staining

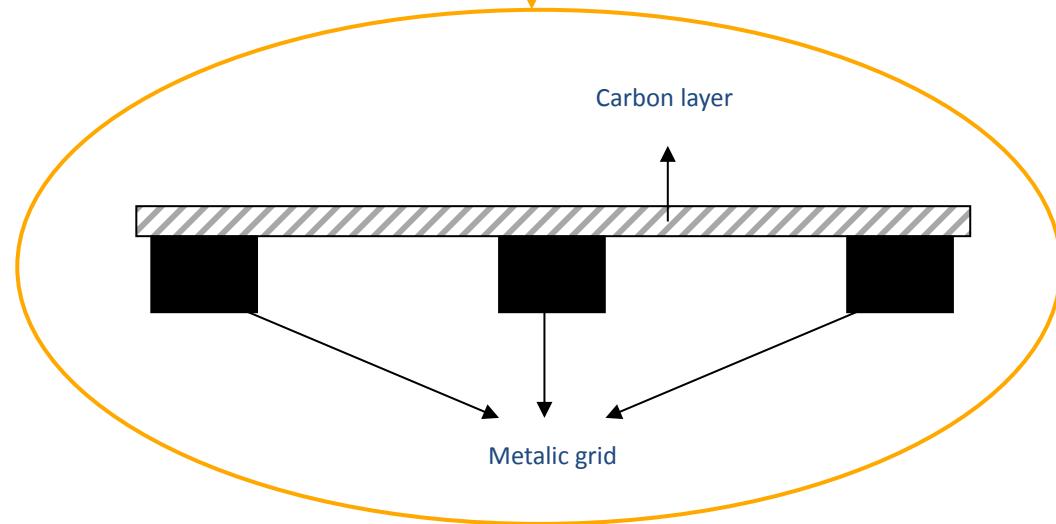


Microscopy grid (Cu, Au, Ni, Pd, Rh...)

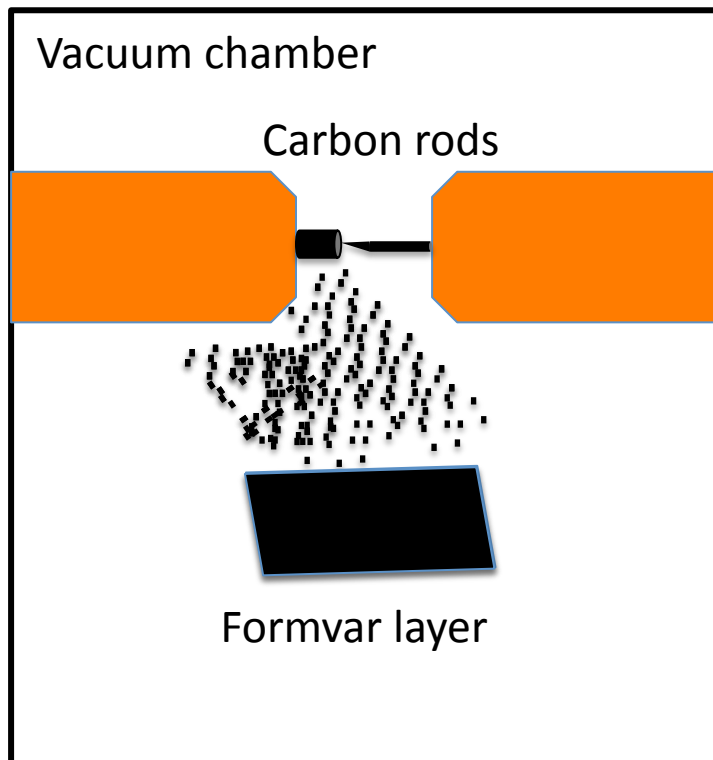
A thin carbon layer is evaporated on top of the grid



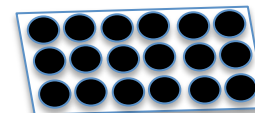
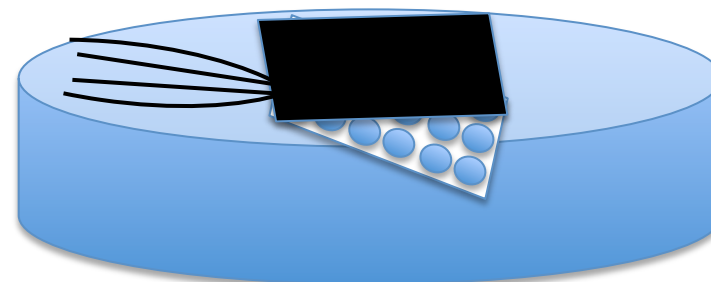
Section of the grid



Carbon coating



Beaker

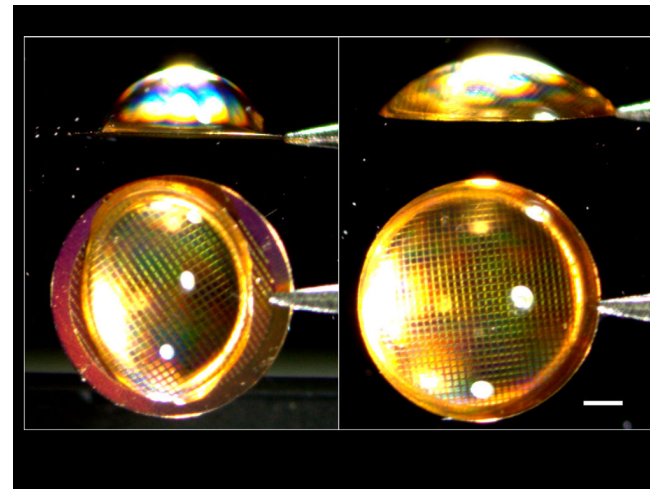
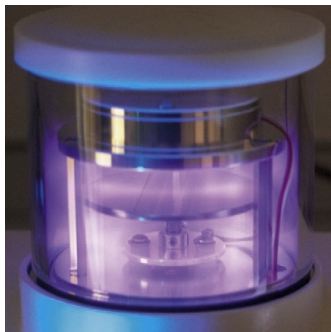


Negative staining

Grid ionisation by glow discharge

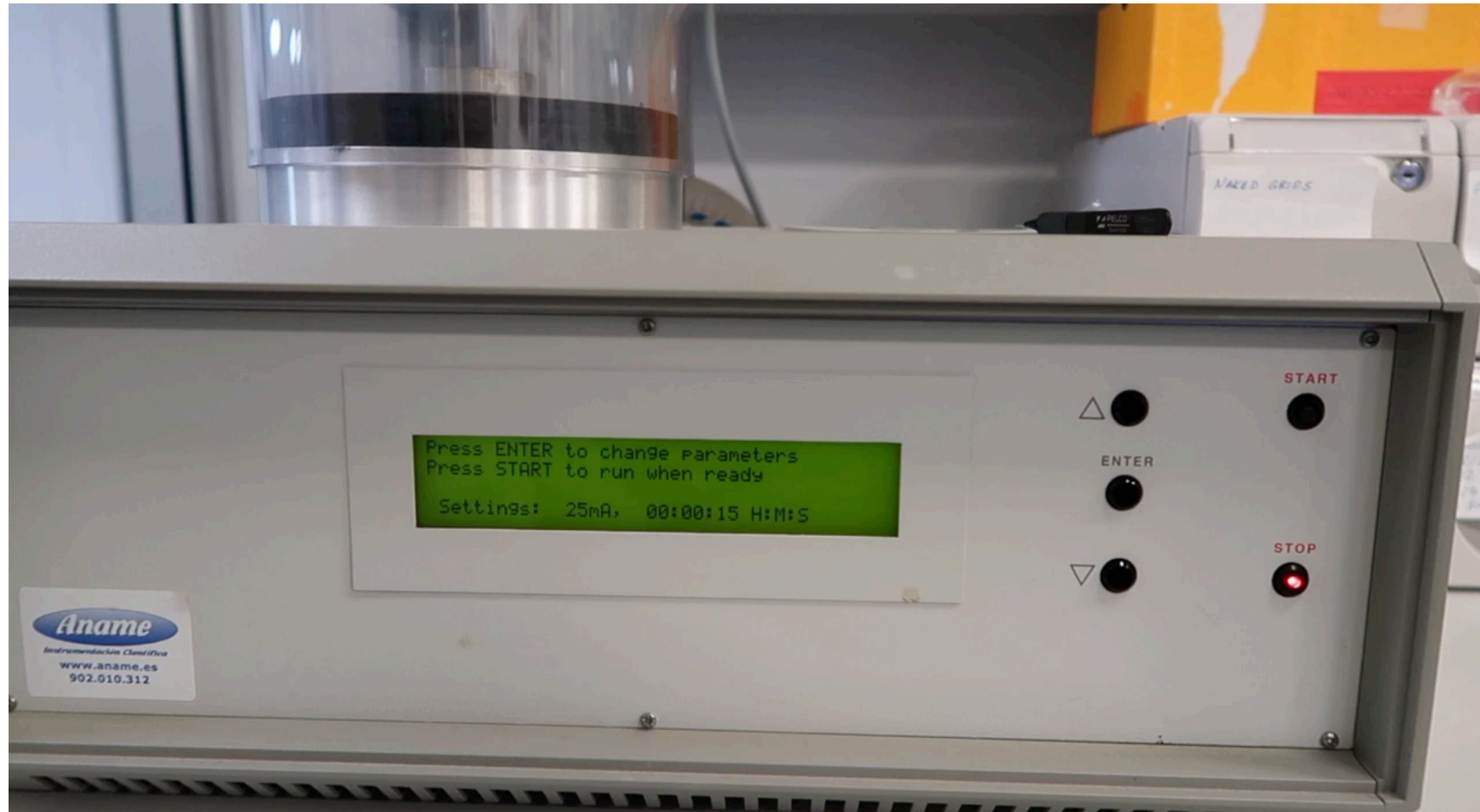
Glow discharge : It is a plasma formed by the passage of electric current through a gas. It is often created by applying a voltage between two electrodes in a glass tube containing a low pressure gas

- The plasma generated by low-pressure gas ionization causes the surface of the grid to ionize
- Ions interact with the surface to remove organic contamination and create a hydrophilic surface

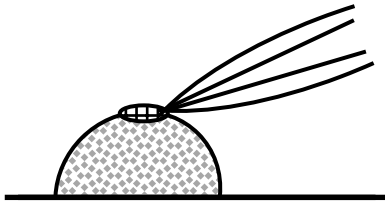


Negative staining

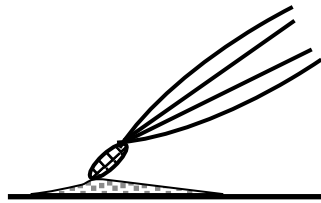
Grid ionisation by glow discharge



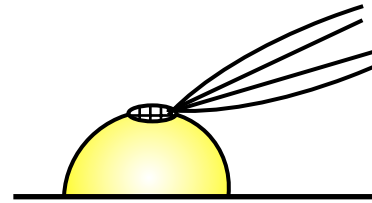
Negative staining



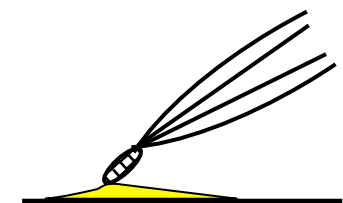
The specimen is adsorbed
on the grid



The excess of sample is
blotted

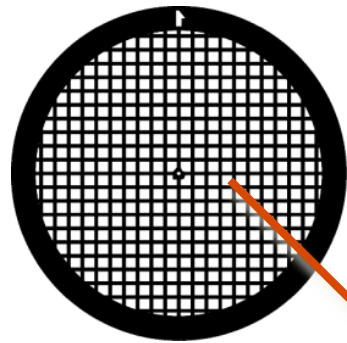


The sample is stained with a
heavy metal salt

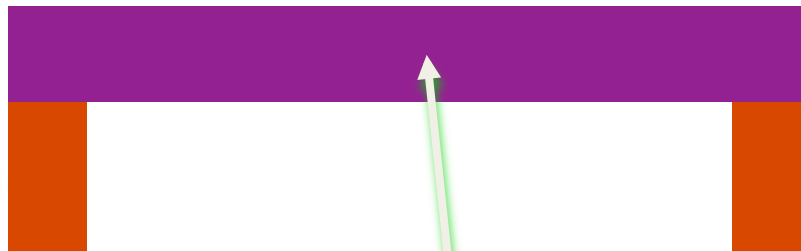


The excess of heavy metal is
blotted

Negative staining

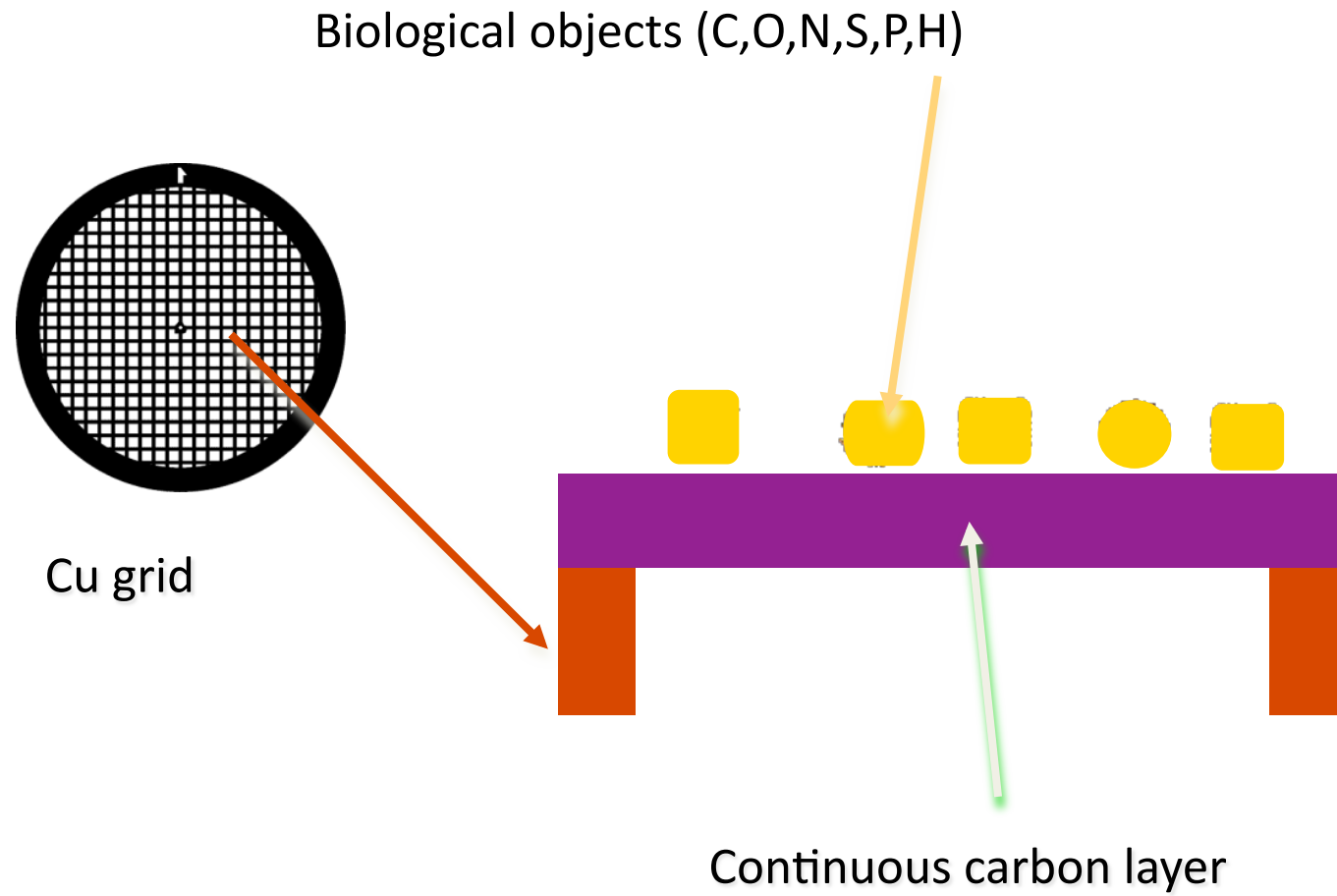


Cu grid

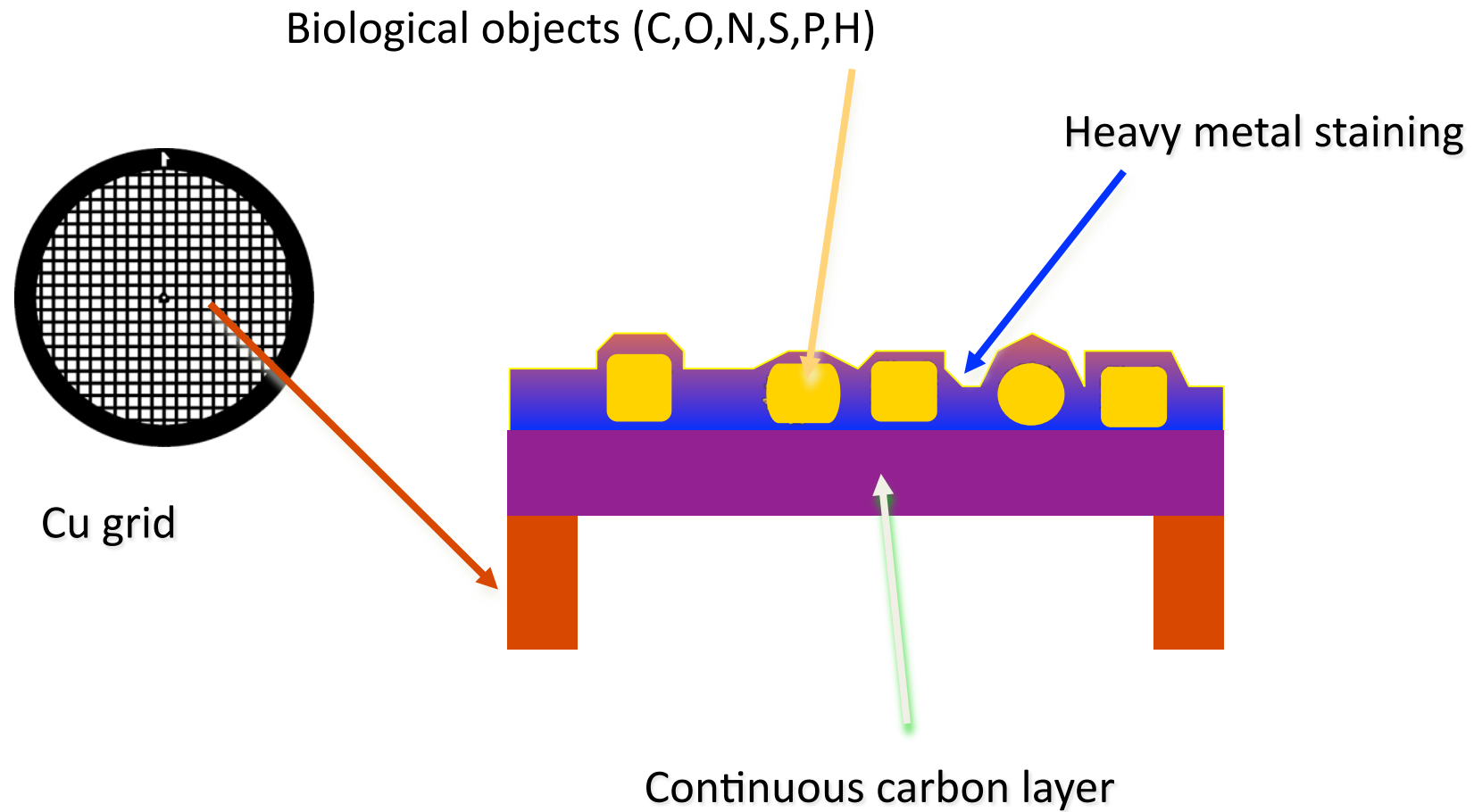


Continuous carbon layer

Negative staining



Negative staining



Negative staining



Negative staining

