Preparation Sample for analysis by Scanning Electron Microscope

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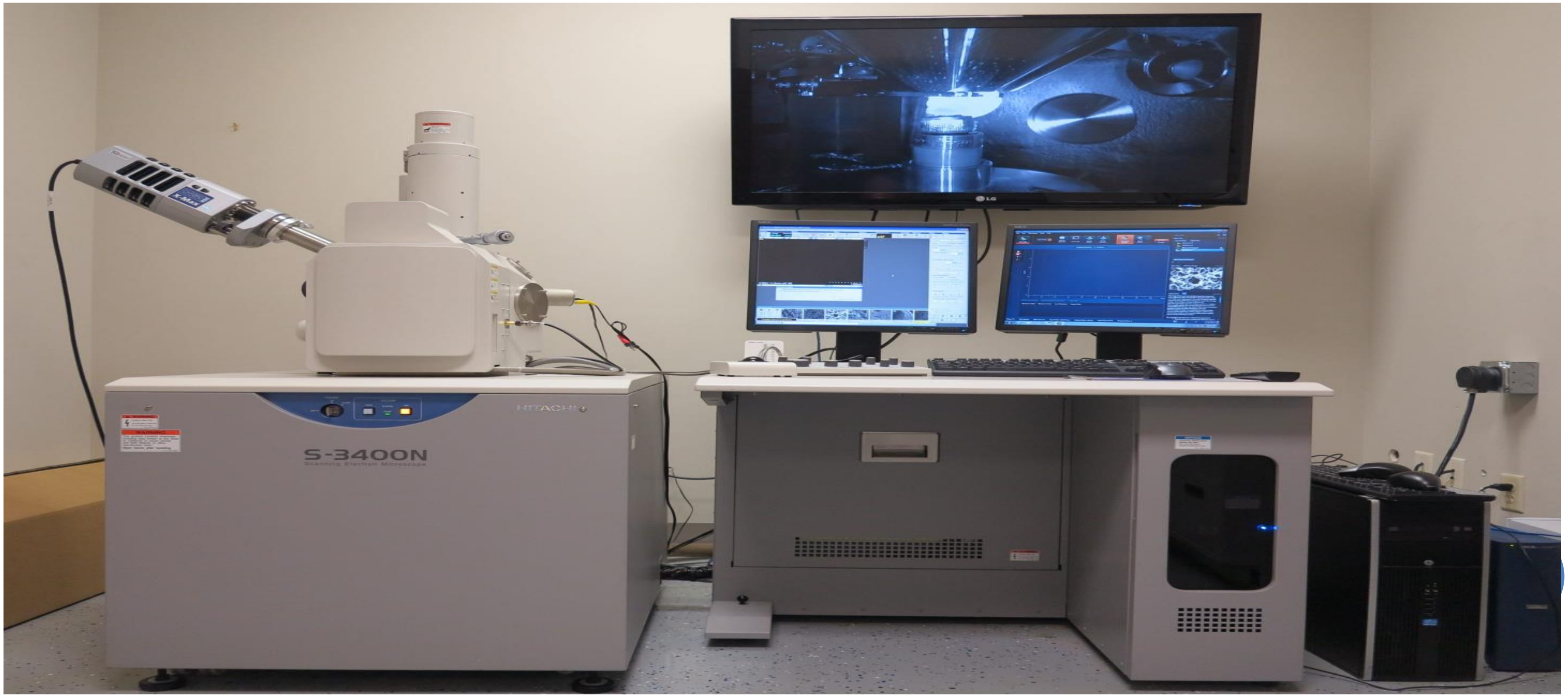
Scanning Electron Microscope (SEM)

- ❖ SEM is a subtype of electron microscope that scans the sample analysis using an electron beam.
- ❖ is an effective tool to obtain information about a specimen's structure and other nanoscale properties.



Light Microscope Vs Electron Microscope

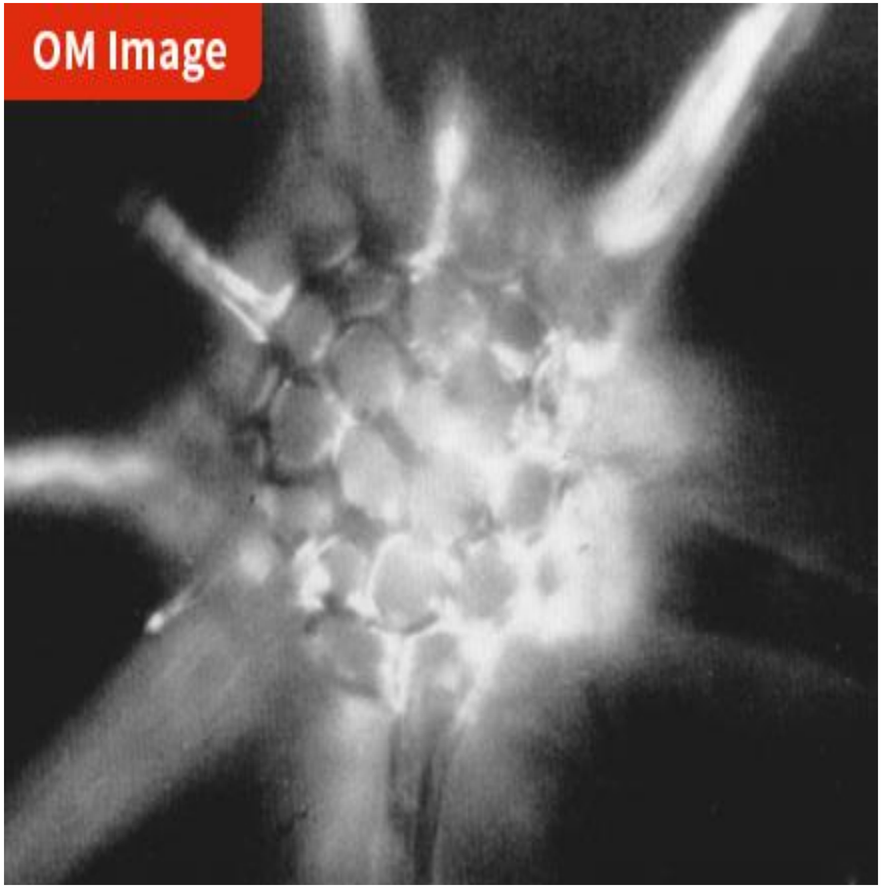
Light Microscope	Electron Microscope
Optical microscopes are easy to use, where samples can be analyzed in air or water and the resulting images are in natural color	SEMs are typically larger and operate in a vacuum, which can increase the time to image a sample. Plus, the resulting image is grey-scaled
Specimen preparation takes about a few minutes or an hour	Specimen preparation takes several days
Both live and dead specimens can be seen	Only dead and the dried specimen can be seen
The image formation depends upon the light absorption from the different zones of the specimen	The image formation depends upon the electron scattering
The image is seen through the ocular lens. No screen needed	The image is received on a zinc sulfate fluorescent screen
Useful magnification of 500x to 1500x	Direct magnification as high as 16000x and photographic magnification as high as 1000000x
Low resolution	High resolution
Inexpensive and requires low maintenance cost	Expensive and high maintenance

Optical Microscope

- Low resolving power, Low depth of focus

Scanning Electron Microscope

- High resolving power, High depth of focus



SEM Components

Components of the SEM

1-Electron gun:

An electron beam is thermionically emitted from an electron gun fitted with a tungsten filament cathode. Tungsten has the highest melting point and lowest vapour pressure of all metals, thereby allowing it to be heated for electron emission, and because of its low cost

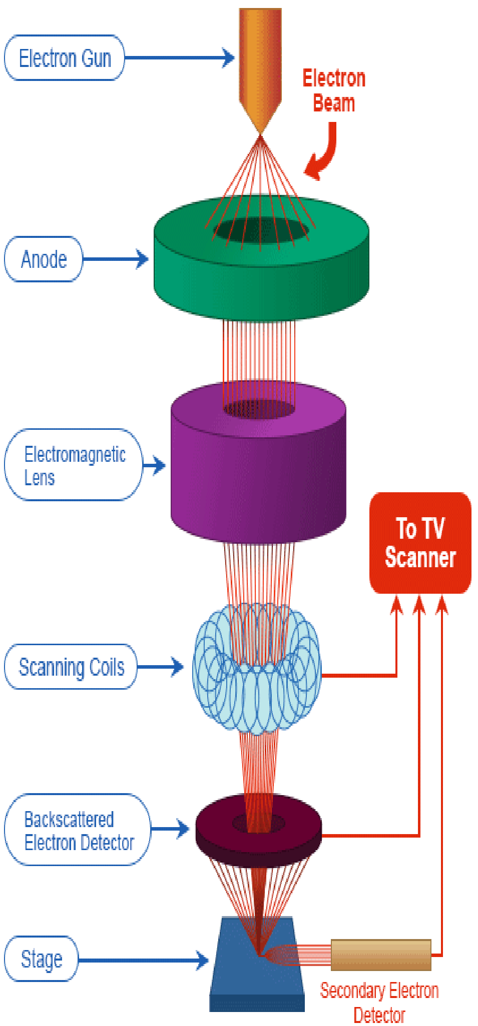
2- Lens:

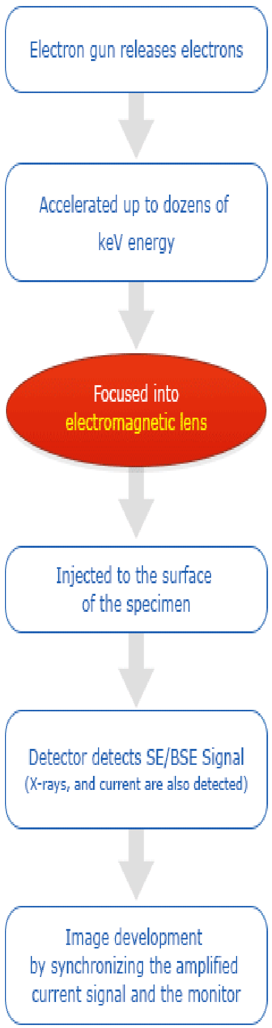
a- Condenser lens: It is focusing the electron beam to the objective lens.

b- Objective lens: It is responsible for size of electron beam that hit sample surface.

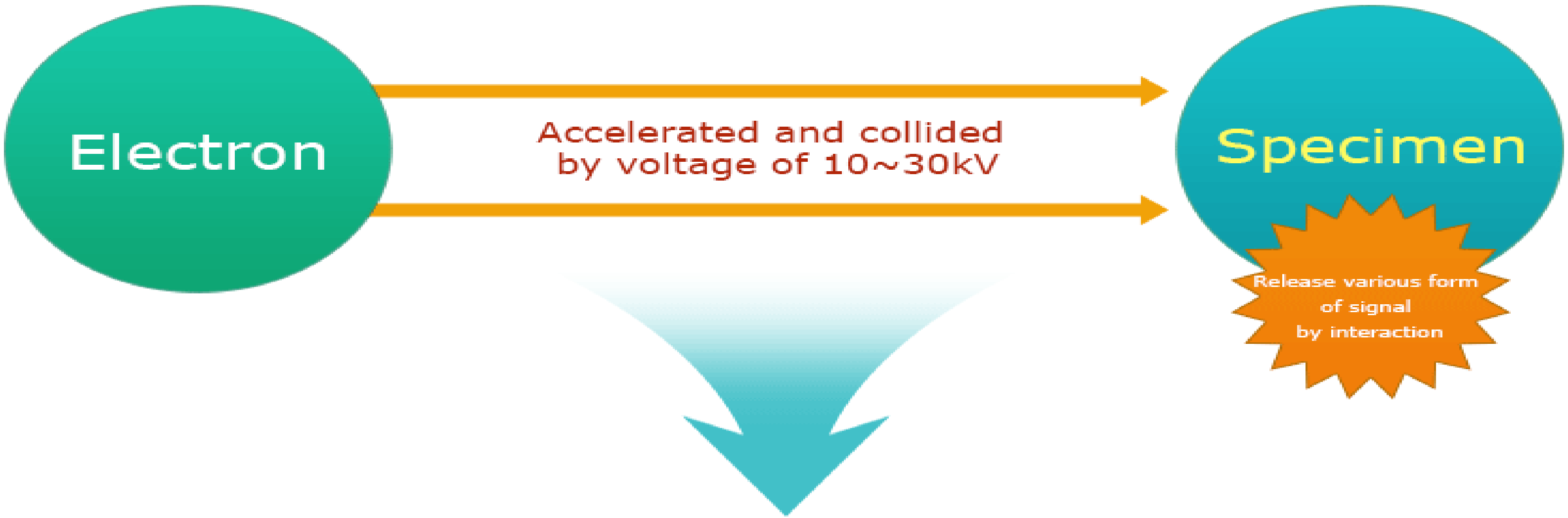
3-Detectors:

Collect the signal generated from interaction of beam with specimen. Electronic detectors convert the signal into digital images





Interaction volume and signal



1. Penetrate several μm into specimen
depending on electron speed • density of specimen

2. After exposed, electrons interact with atoms in specimen
no matter how small the diameter of electron beam exposed to surface of specimen

3. Dispersed by being scattered to random direction
until energy of electron is exhausted

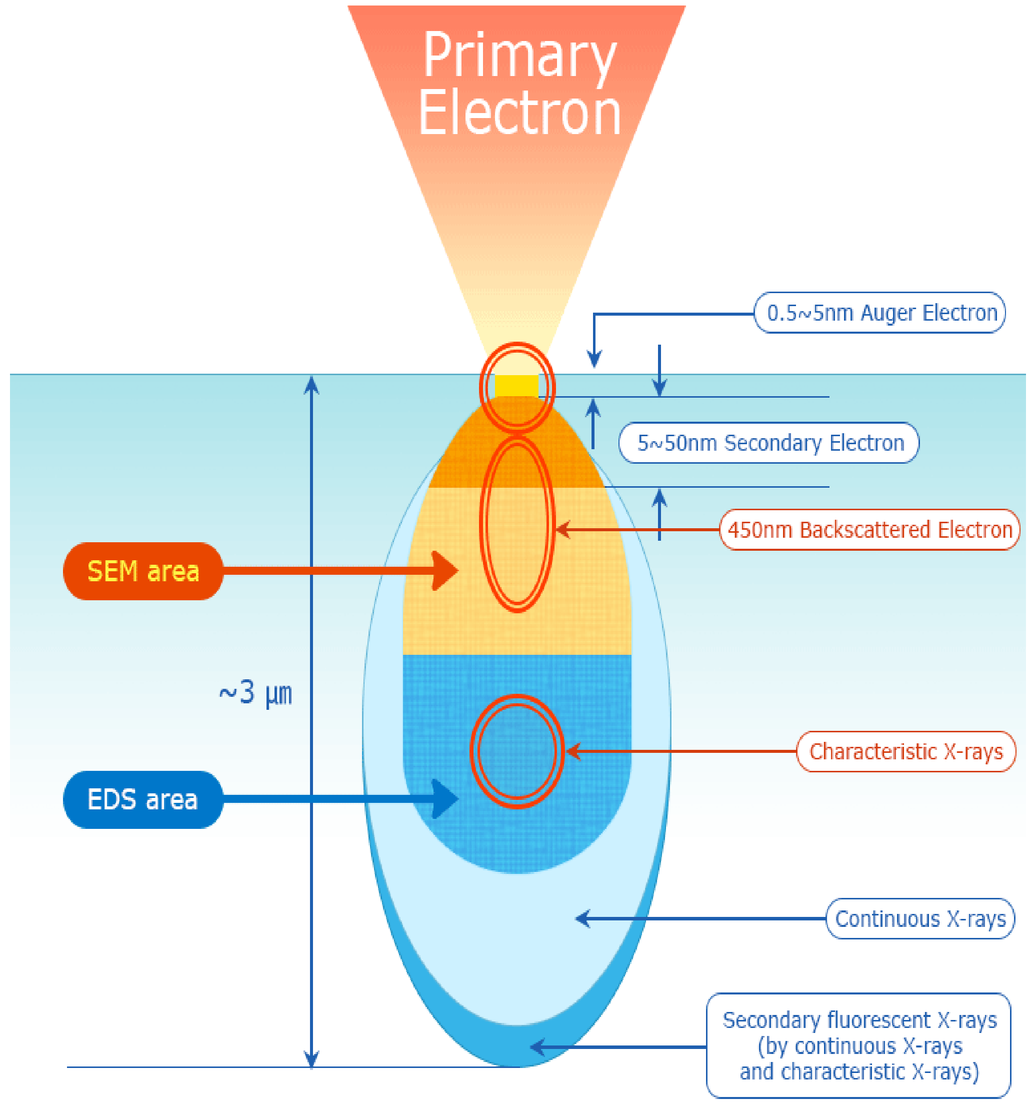
Interaction of Electron Beam with Specimen

Interaction of Electron Beam with Specimen

- When the primary electron beam interacts with the sample, the electrons lose energy by repeated random scattering and absorption within a teardrop-shaped volume of the specimen known as the interaction volume

- The energy exchange between the electron beam and the sample results in the reflection of high-energy back scattered electrons by elastic scattering, emission of low energy secondary, auger electrons by inelastic scattering and the emission of electromagnetic radiation (X-rays) which can be detected by respective detectors.

- Electronic detectors convert the signals into digital images and displayed on a computer monitor.



SEM Sample Preparing

Why Should SEM Sample be Prepared?

- SEM samples should be dry and conductive. Samples containing water cause problems when vacuuming the SEM chamber,
- Also energetic electrons incidence on the sample may evaporate the residual water and reduce image quality.
- The conductivity of the sample surface is vital to prevent charge build-up on its surface. Accordingly, some specimens require a preparation step before SEM imaging.

SEM Sample Preparation Process

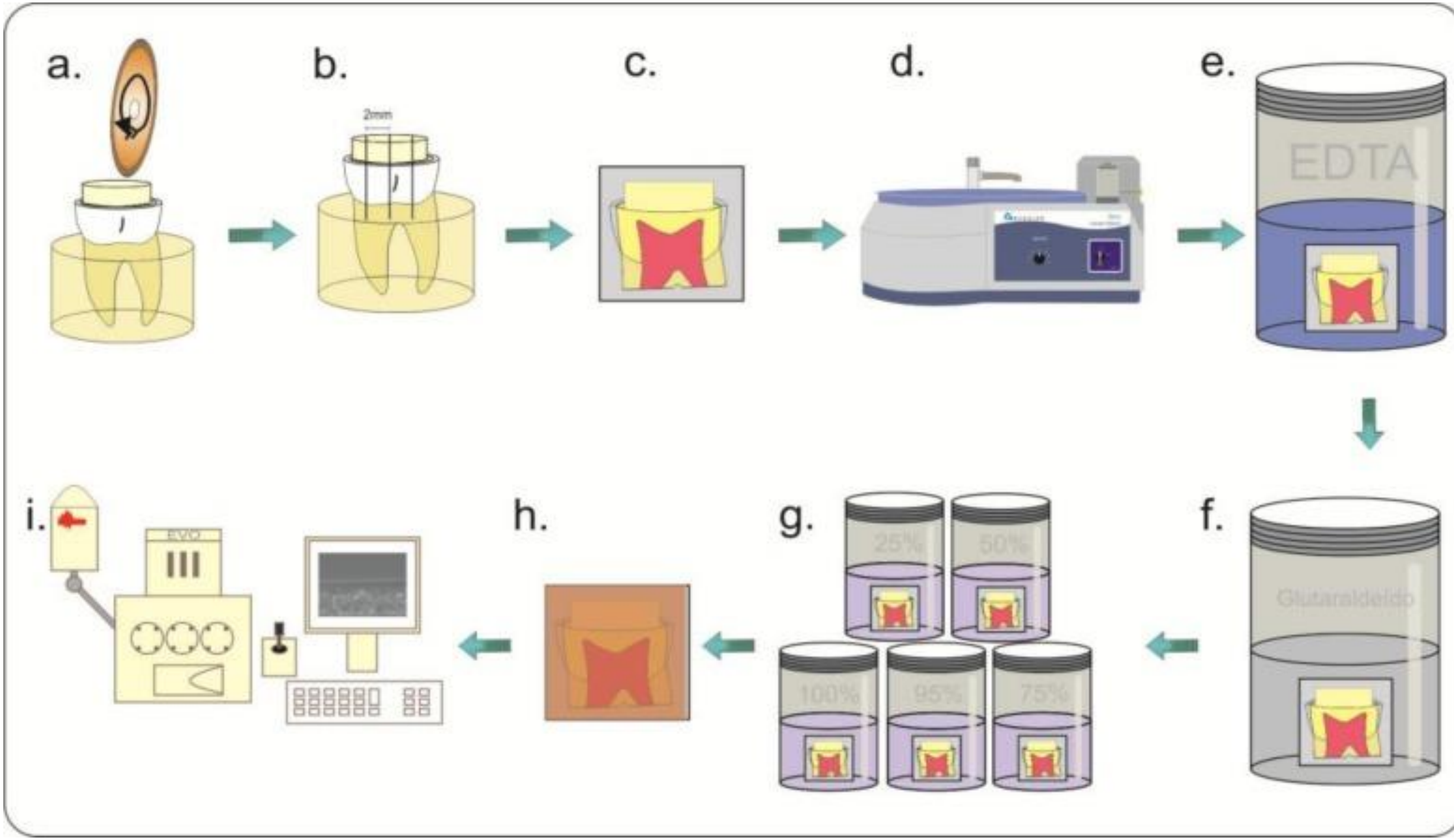
The SEM sample preparation mainly depends on the sample characteristics, it may need some or all of the following steps

1. Cleaning and cutting
2. Fixation and stabilization
3. Dehydration
4. Drying
5. Coating with a conductive material

Non-Conductive Samples

- Non-conducative samples (including biological ones) can trap incident beam electrons on the surface and result in shinning spots in SEM images.
- Also this built-up charge can cause overheat and deterioration of the sample. Coating a **thin film** of a conducting material on the sample surface can prevent this phenomena
- The coating thickness must be enough to prevent charging, while preserving specimen surface details (around 10 nm).

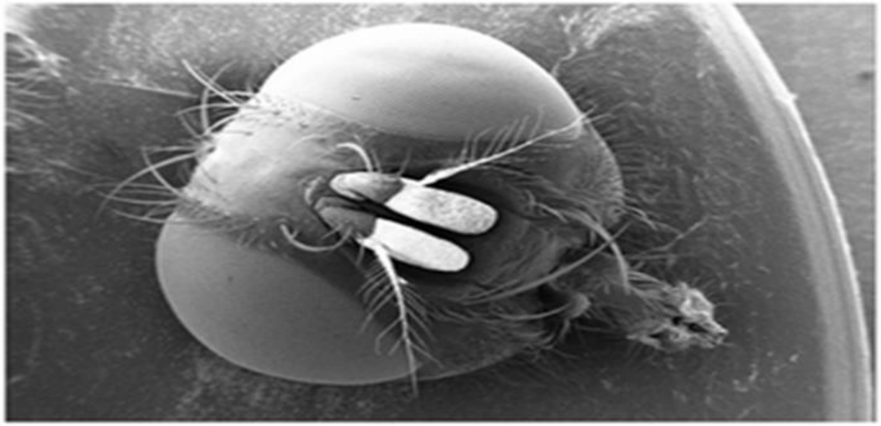
Deposition of a layer of gold, silver, platinum,



Schematic of SEM preparation. (a) and (b): 2 mm cut; (c) embedded in a polyester resin; (d) surface polishing; (e) EDTA cleaning; (f) fixation with glutaraldehyde; (g) alcohol dehydration; (h) gold sputter-coated; (i) SEM analysis

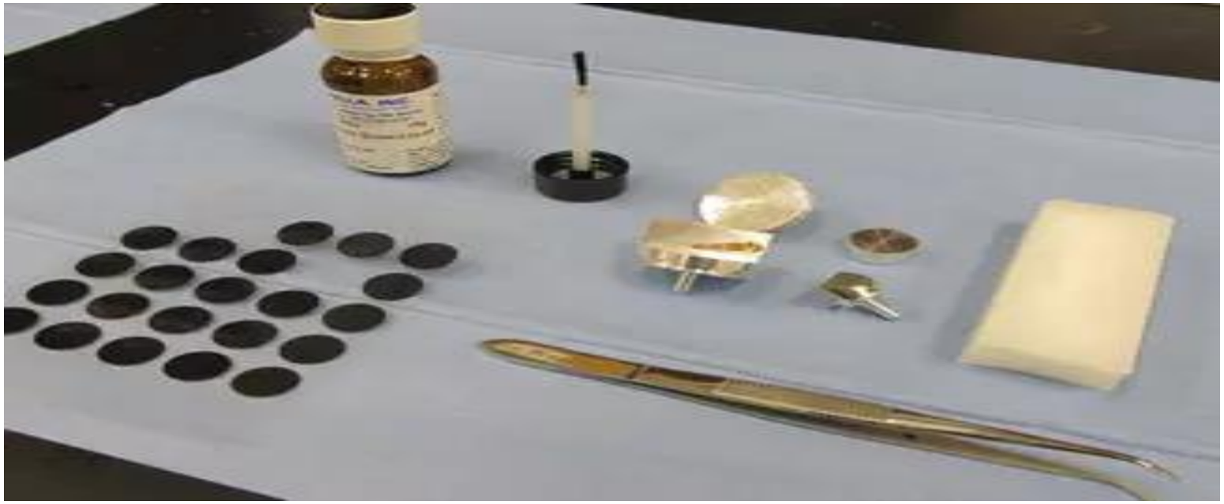
Biological Samples

- Biological samples should undergoes fixation and dehydration processes, and then dried to remove the water inside them. This will allow higher vacuum SEM imaging and clearer images.



Powder Samples

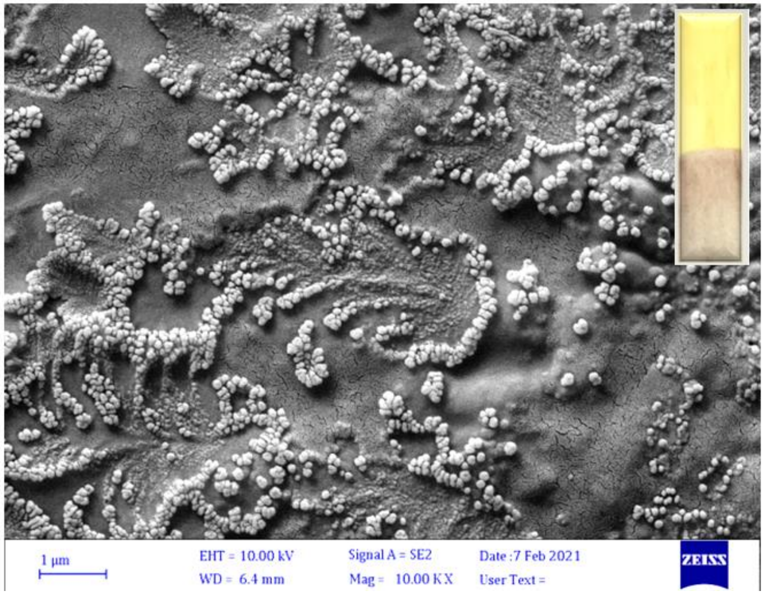
- Powder samples may fly off under **vacuum condition** inside the SEM chamber, so they must be processed.
- Large amount of powder can be compressed to a pill and in small amount they should be dispersed in a volatile solvent and dropped on a suitable substrate.



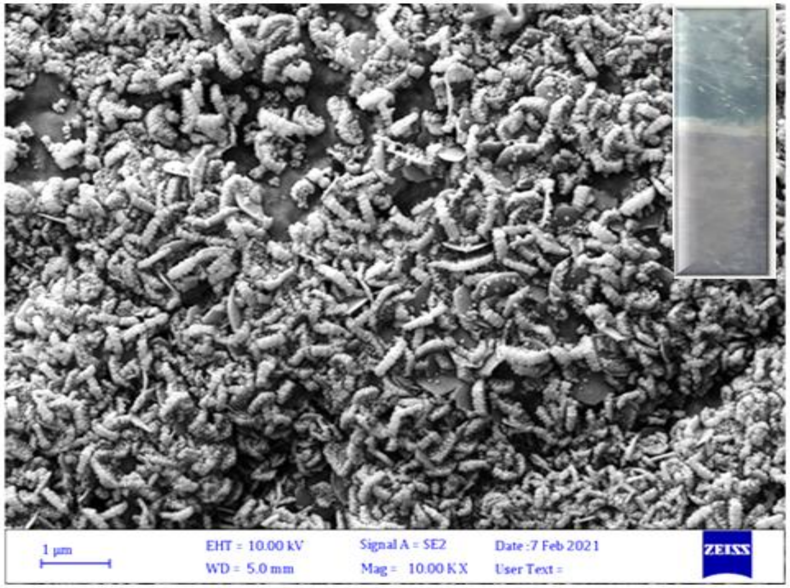
Applications of Scanning Electron Microscopy

1- **Topography**:

The surface features of an object or "how it looks",



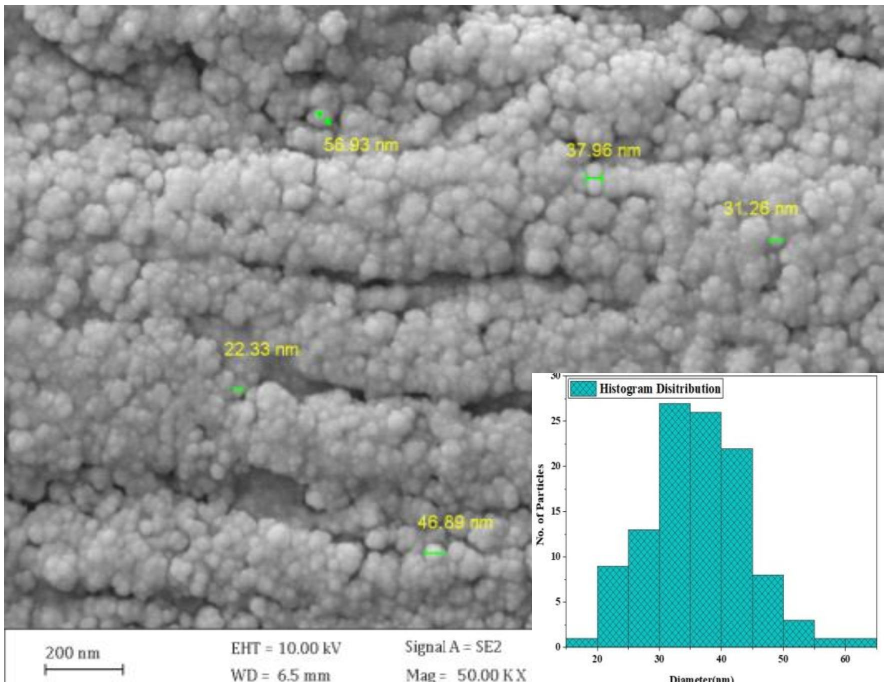
The growth of Pd nanoparticles (PdNPs) on brass therefore obey a Volmer-Weber (VW: island formation)



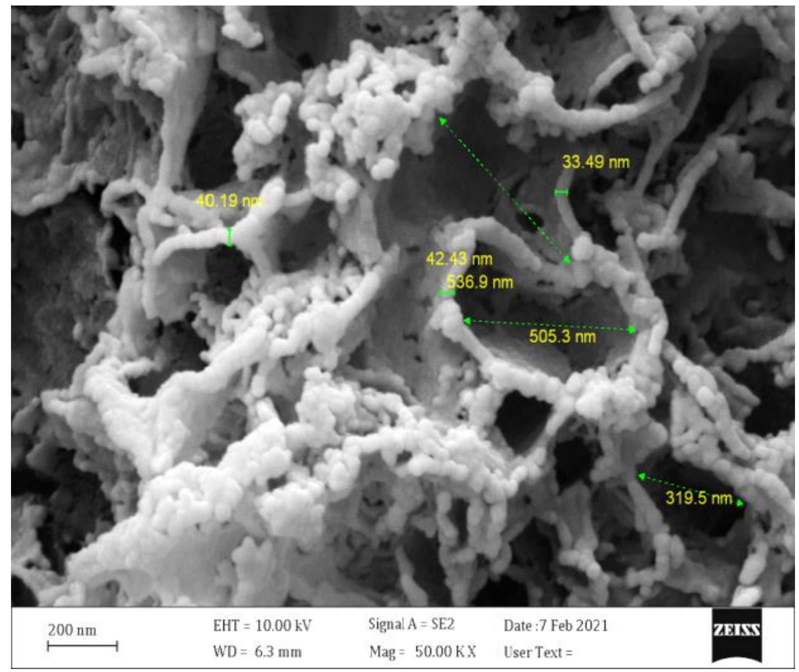
Uniform Pd nano particles accumulation as wax worms assembled shape

2-Morphology

The shape and size of the particles making up the object; direct relation between these structures and materials properties (ductility, strength, reactivity…etc.)



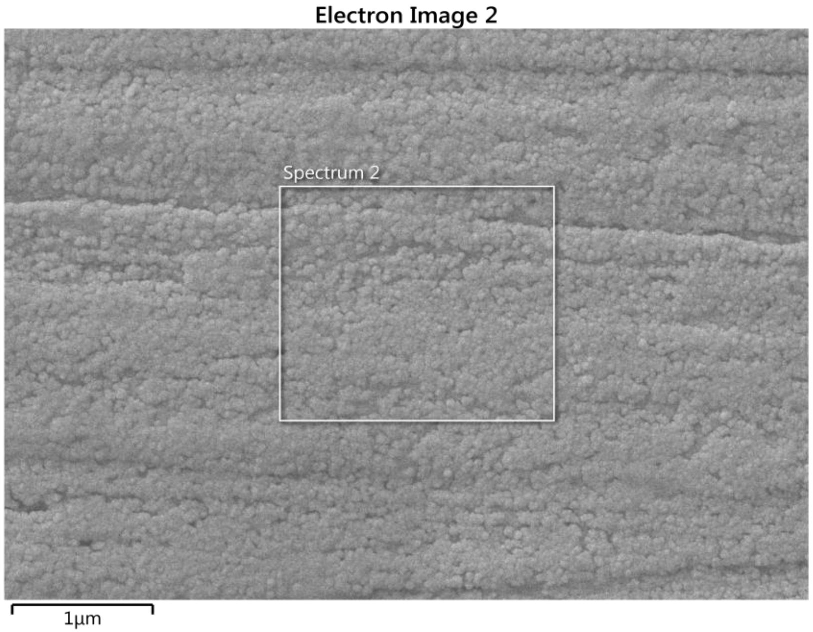
nanoparticles organized in compact grains

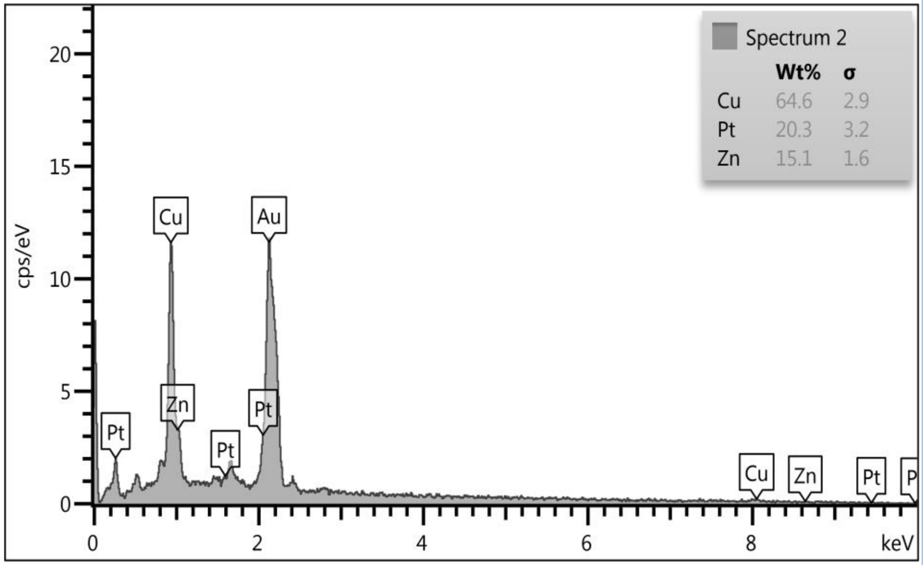


Form of nested sponge holes

3- Composition

- : The elements and compounds that the object is composed of and the relative amounts of them; direct relationship between materials properties (melting point, reactivity, hardness…etc.)





Advantages of SEM

- It gives detailed 3D and topographical imaging and the versatile information garnered from different detectors.
- This instrument works very fast.
- Modern SEMs allow for the generation of data in digital form.

Disadvantages of SEM

- SEMs are expensive and large.
- Special training is required to operate an SEM.
- SEMs carry a small risk of radiation exposure associated with the electrons that scatter from beneath the sample surface.

THANKS