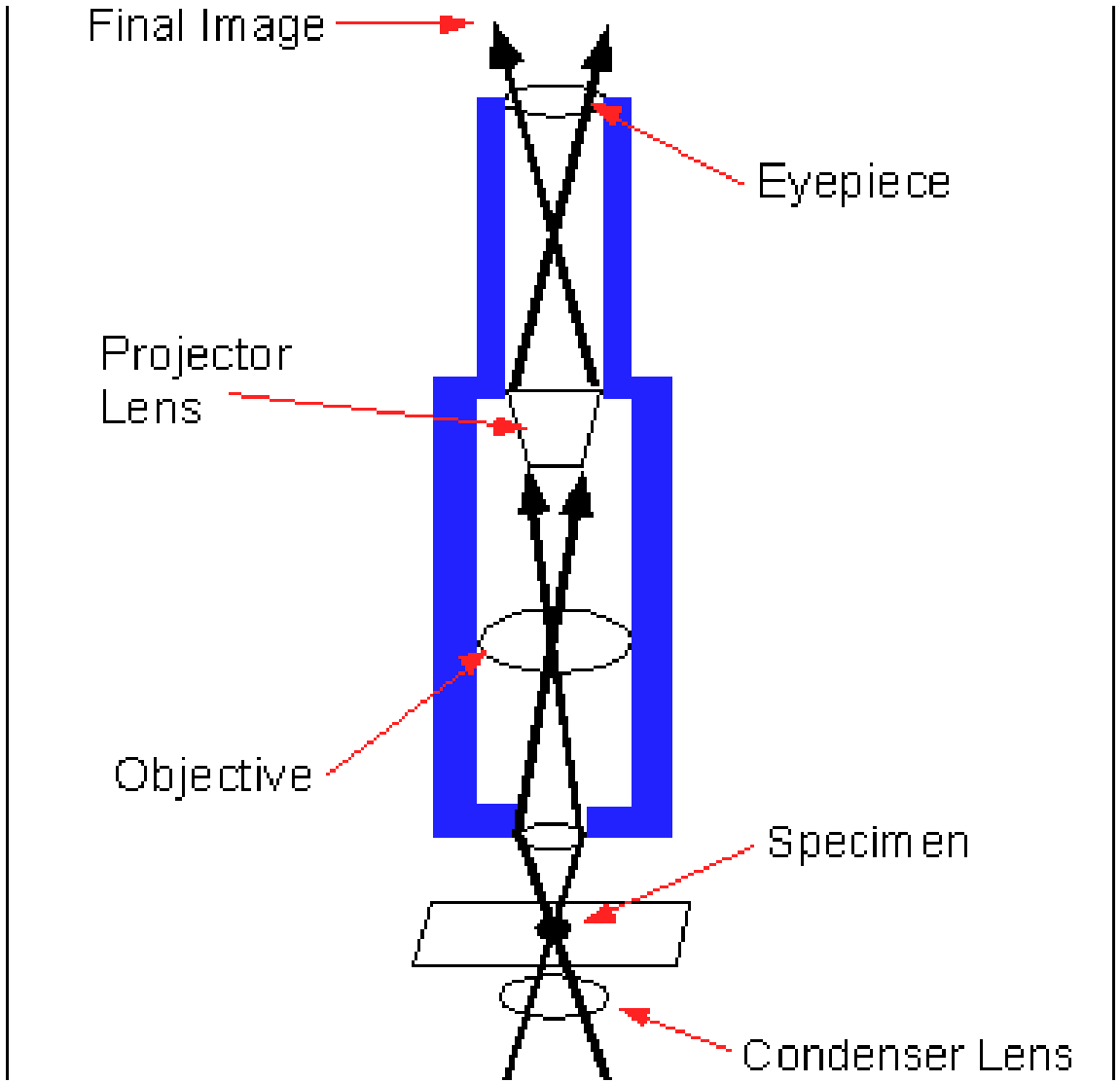
Microscopy

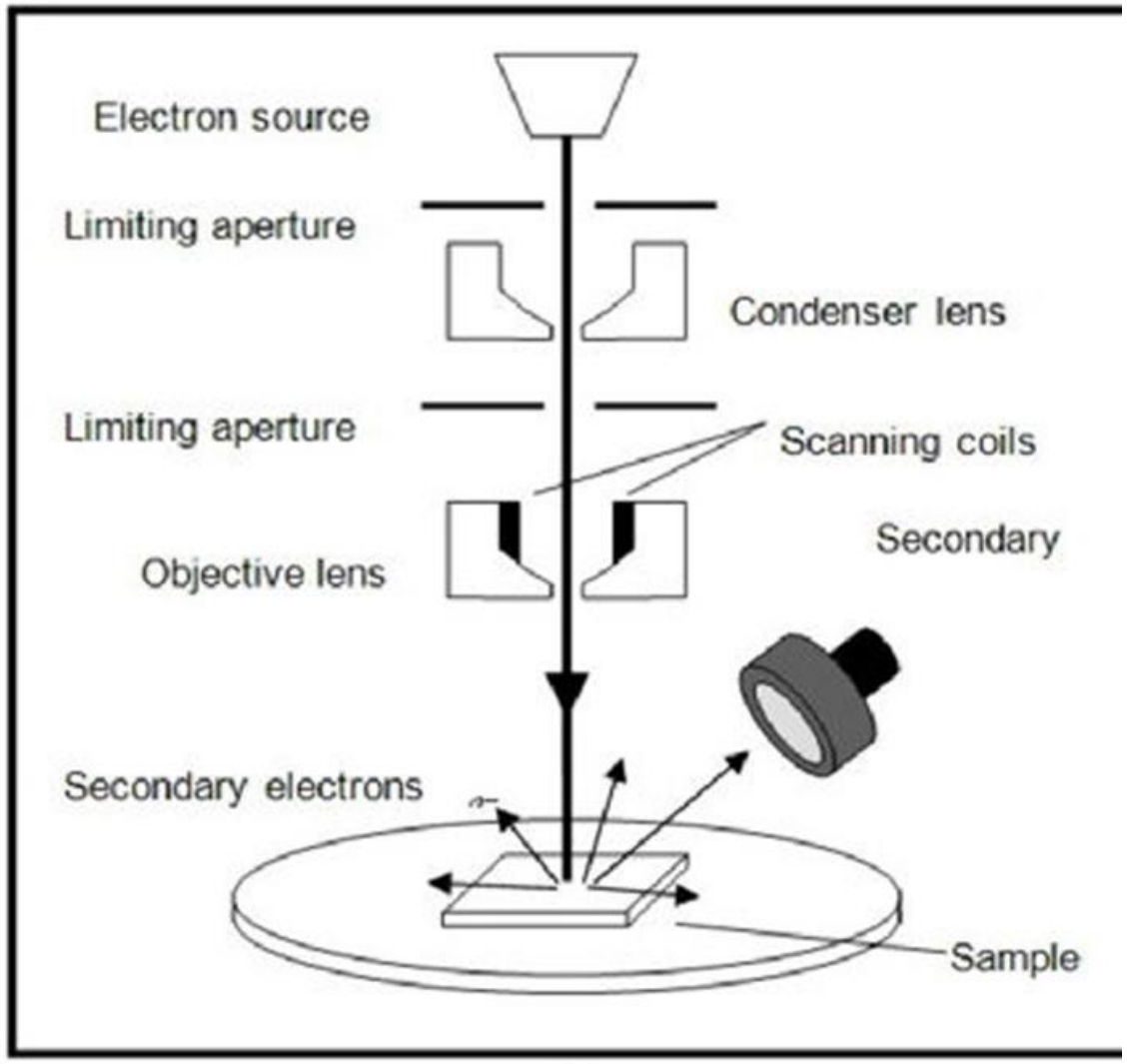
Light Microscopy

- “Basic” microscope
- Uses light focused through ground glass lenses to view samples
- View cells, tissues, bacteria
- Strength of microscope varies between models
  - Range from 450-2000x max. zoom
  - Best possible resolution is 0.2 microns or 200 nanometers
  - Most common viewing scale is in the micrometer scale

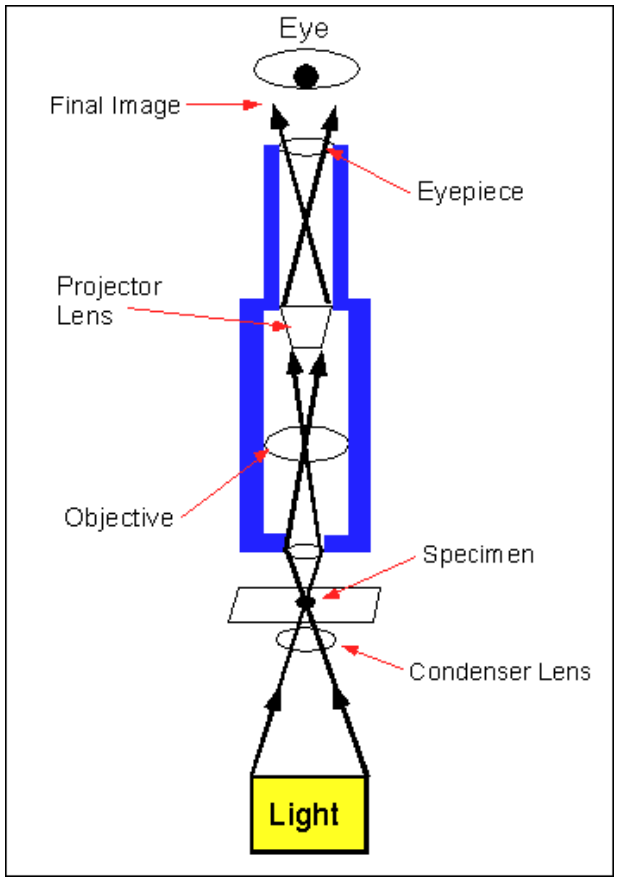


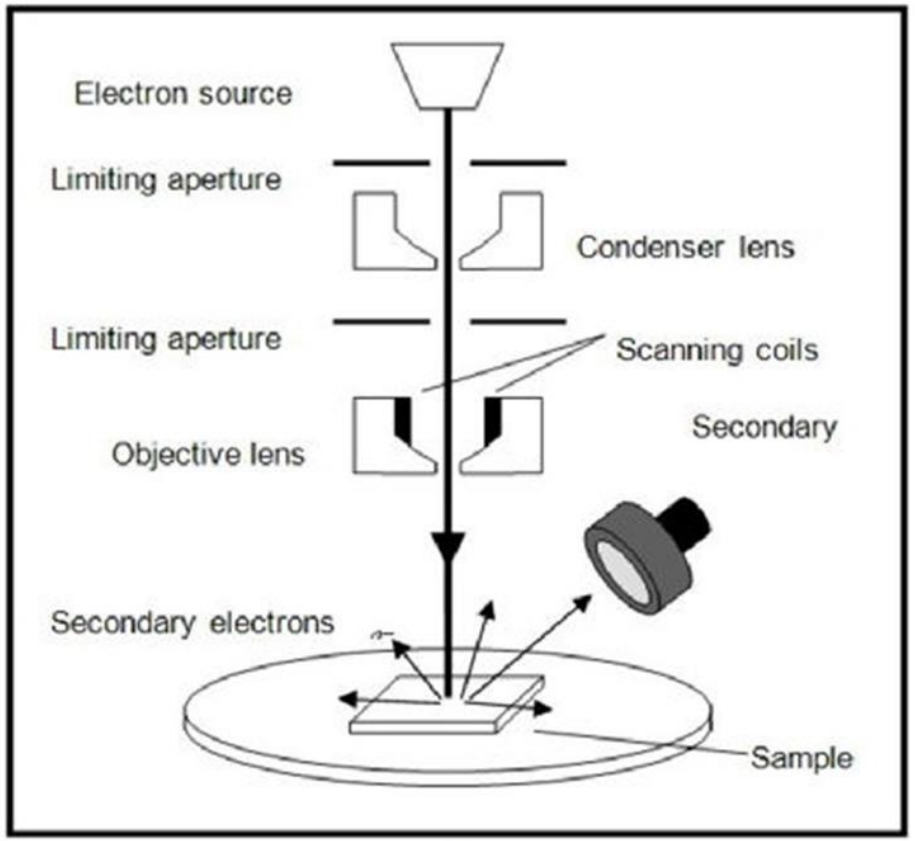
Scanning Electron Microscopy

- Uses a high energy electron beam that is focused using electromagnetic lenses
- Instrument is run under high vacuum which limits types of samples that can be analyzed
- Strength of microscope ranges from 25x up to 1,000,000x magnification
  - Can view samples down to the nanometer level resolution



LM vs SEM

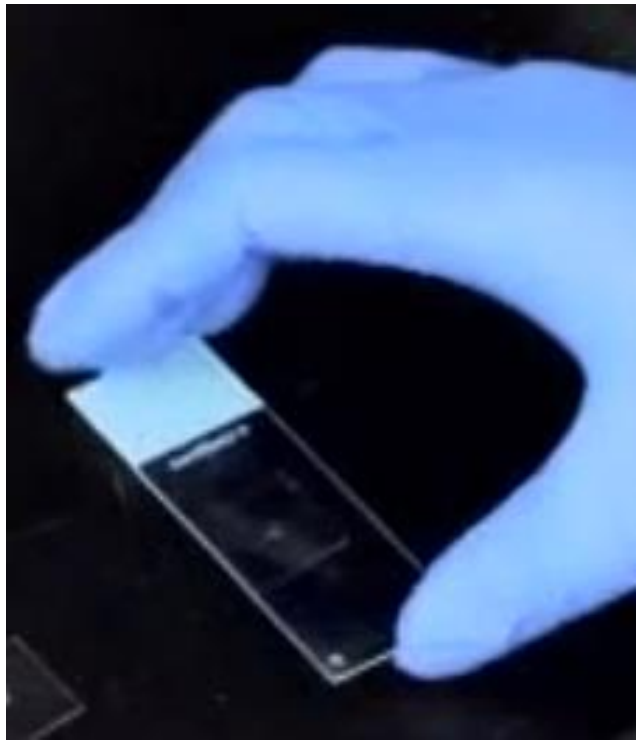

Eye
Final Image
Eyepiece
Projector Lens
Objective
Specimen
Condenser Lens
Light


Electron source
Limiting aperture
Condenser lens
Limiting aperture
Scanning coils
Secondary
Objective lens
Secondary electrons
Sample


3μm

Light Microscope vs. SEM Samples

Light Microscope

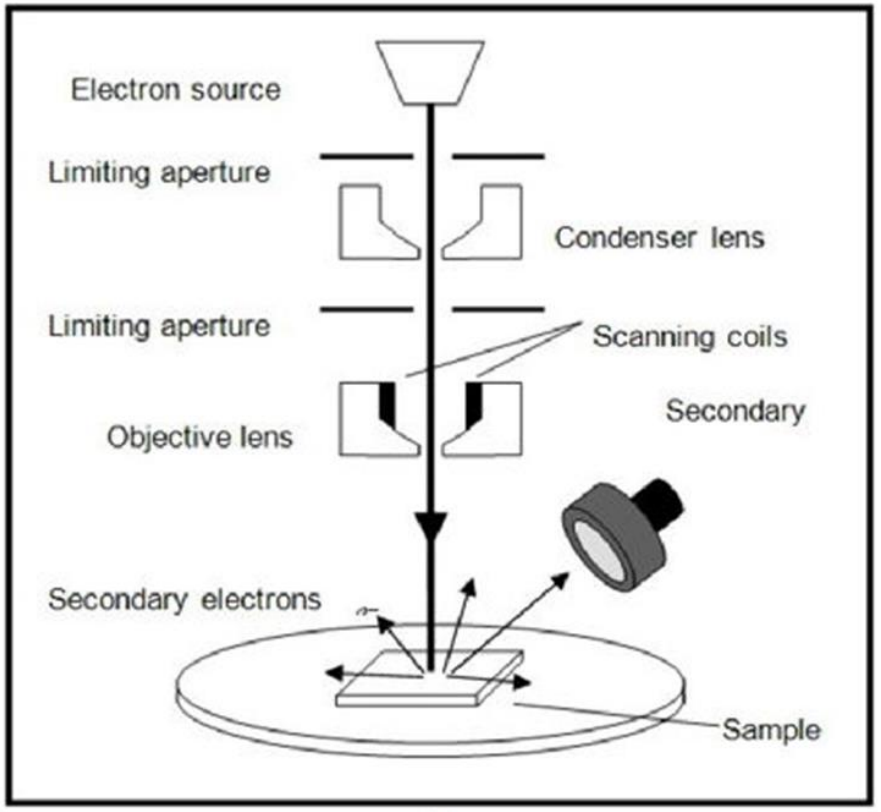


- Samples must be thin enough for light to shine through
- Uses clear glass slides to mount samples
- View organelles, internal structures

Scanning Electron Microscope

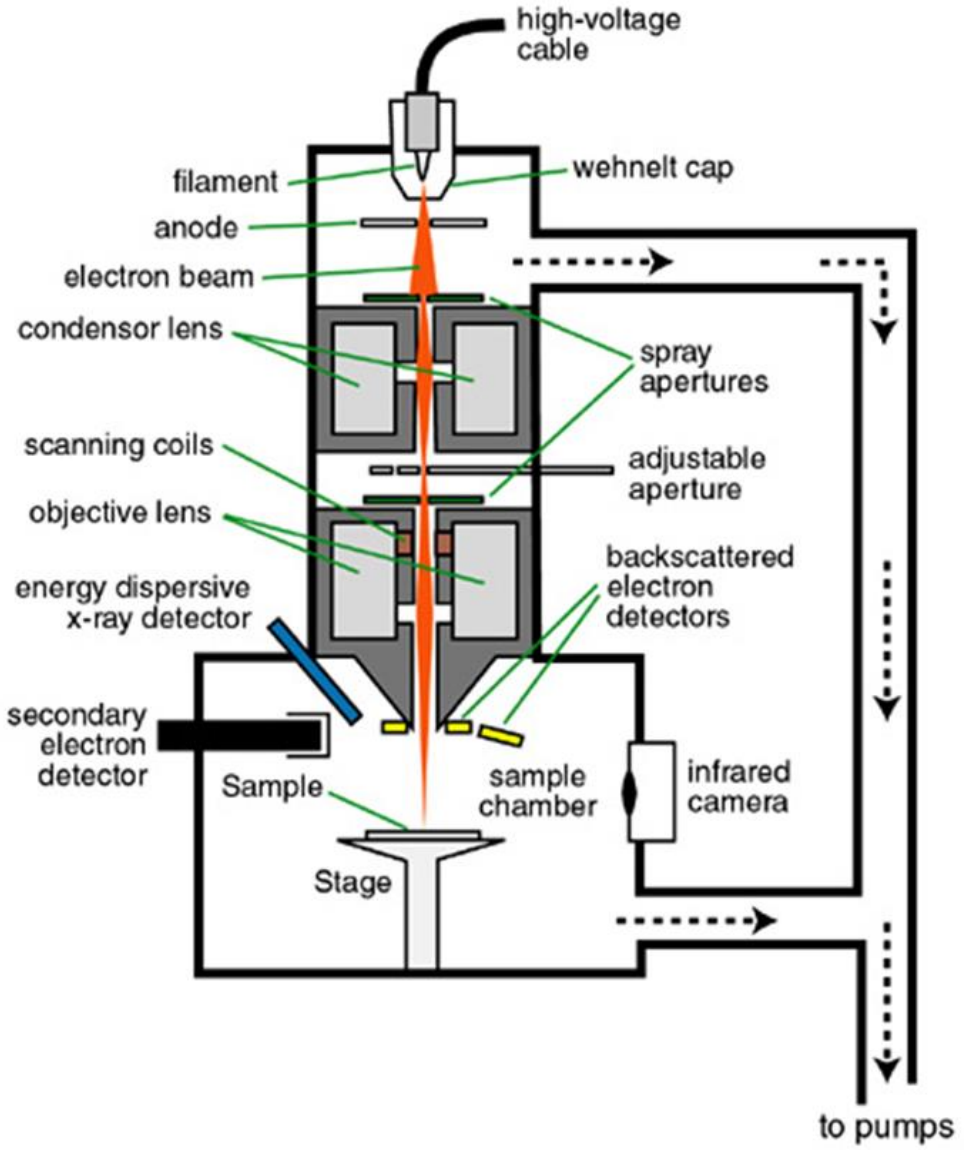
- Samples must fit in the sample exchange chamber
- Samples are mounted on aluminum stubs using carbon adhesive
- View morphology, surface

SEM

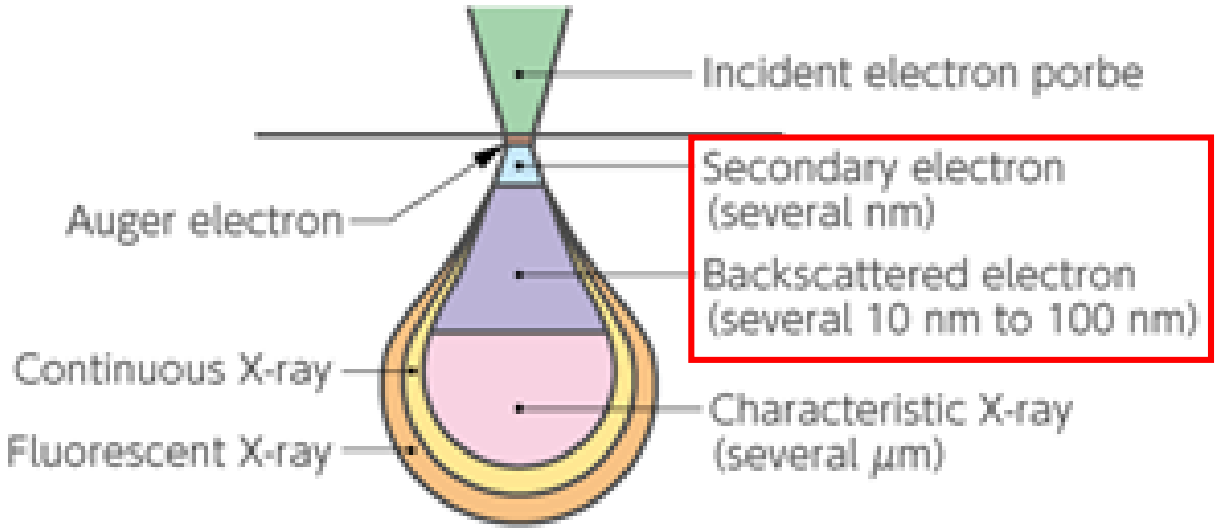

Electron source
Limiting aperture
Condenser lens
Limiting aperture
Scanning coils
Secondary
Objective lens
Secondary electrons
Sample

How does an SEM work?

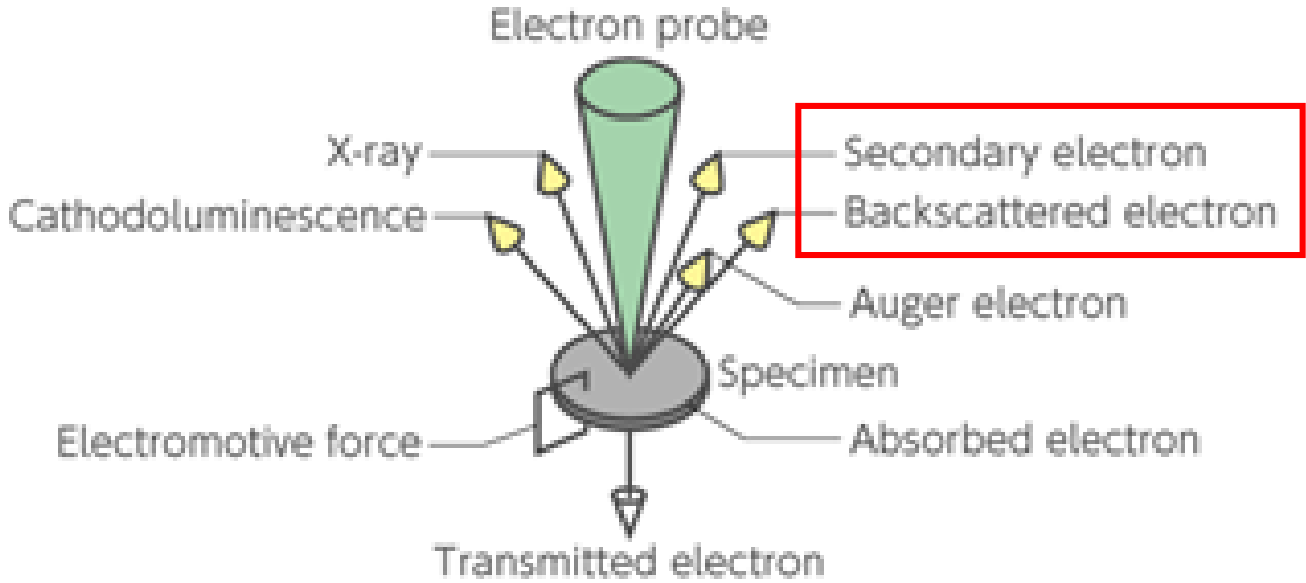
- A beam of electrons is produced at the top of the microscope by an electron gun.
- The beam is directed vertically through the microscope, which is held in a vacuum.
- Electromagnetic fields and lenses focus the beam down to the sample.
- When the beam hits the sample, electrons and X-rays are ejected.
- Detectors collect the secondary electrons, backscattered electrons and X-rays and convert them into a signal.
- The signal is sent to a monitor, which produces the final image.



How does the electron beam generate an image?



Depth of quantum emission and spatial resolution



Information obtained from specimen

Preparing the Samples

Biological Samples

- Samples such as plants, tissues, and some insects must undergo special treatment before going into the SEM chamber.
- Water and solvent must be removed from the samples because the SEM utilizes a vacuum.
- Putting a sample with water or solvent in it can damage both the vacuum and the sample, as the liquid will vaporize out of the sample.

Metals

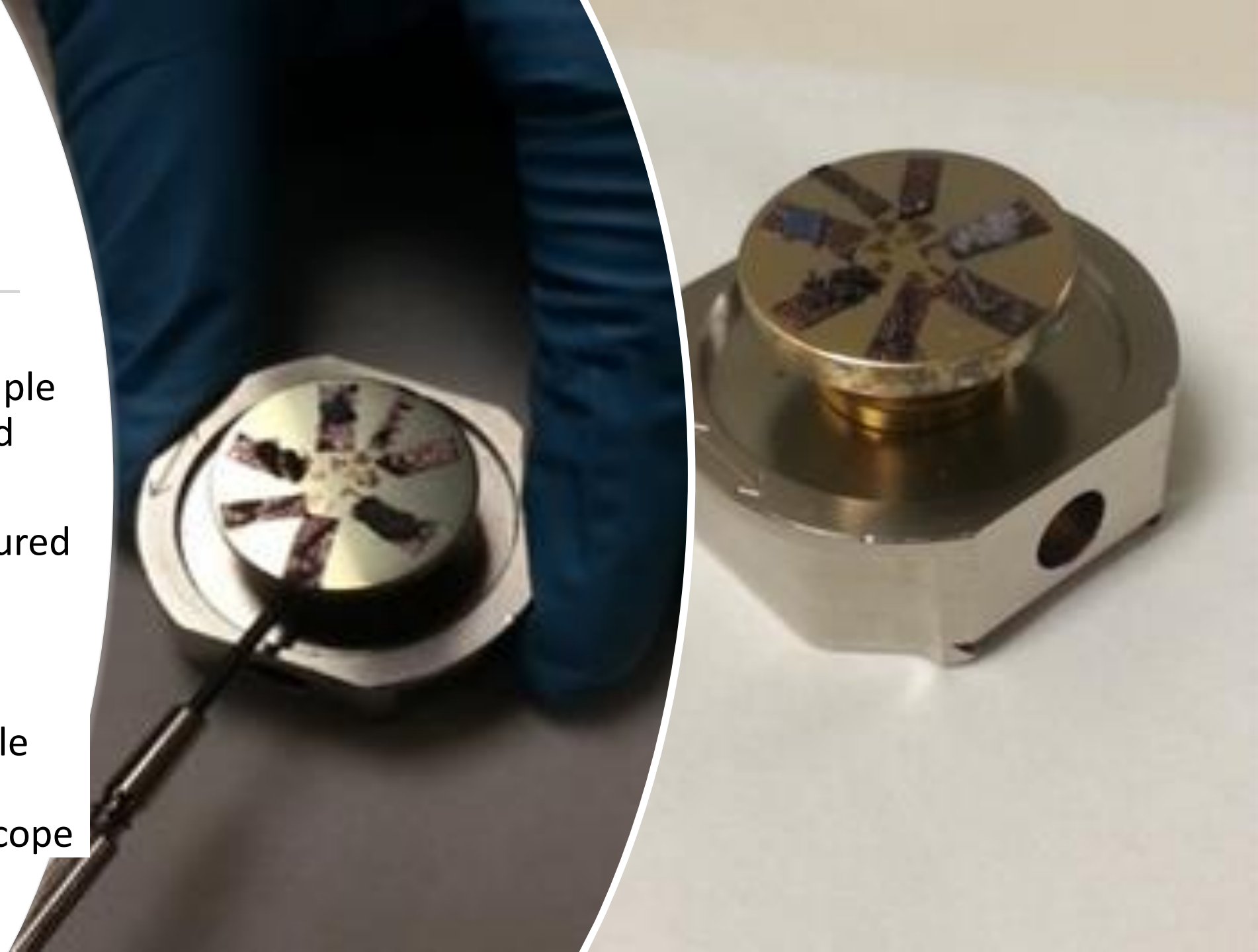
- Metals can be placed into the microscope without any type of extra preparation. This is because they are already conductive.

Non-metals

- Non-metals need to be made conductive. This is done using a sputter coater and a carbon evaporator. The sample is coated in a thin layer of gold, platinum, palladium, or carbon.

Mounting the Samples

- Prepared samples are mounted onto the sample stub using double sided carbon tape
- The sample stub is secured into the sample mount holder
- With the sample stub secured the sample is now ready to be placed into the microscope



What are the pros and cons of using the SEM?

Pros

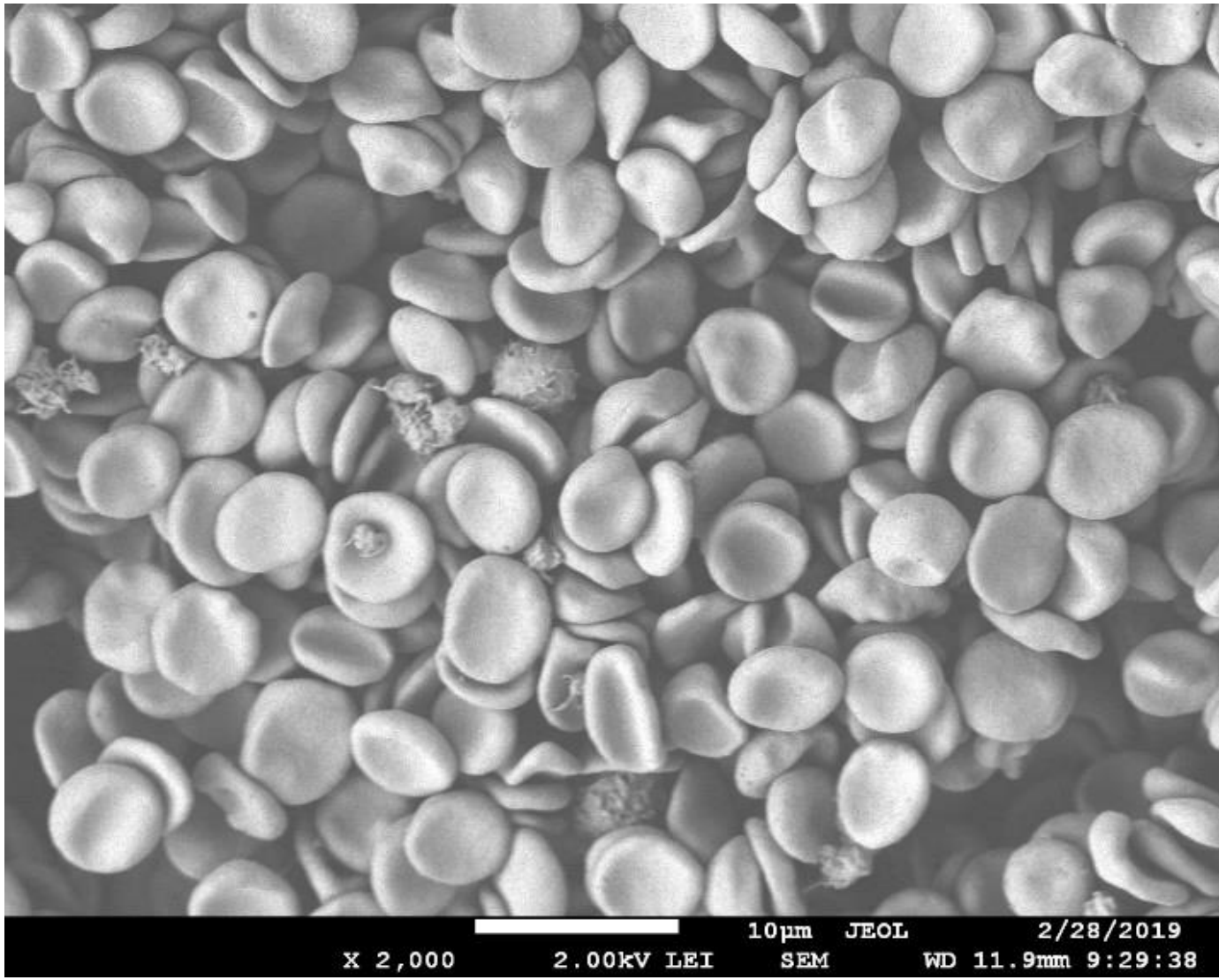
- Broad spectrum of applications in the study of solid materials
- Easy to operate
- User-friendly interfaces
- Minimal sample preparation
- Rapid data acquisition
- Data generated in digital forms – highly portable

Cons

- Samples must be solid
- Samples must be small enough to fit into the chamber
- Samples must be in a stable vacuum
- “Wet” samples are unsuitable
- Electrically conductive coating must be used on electrically insulating samples

Biological samples in the SEM

- Most animal samples are too large for SEM chamber, must be cut down
- Study small sections instead of entire animal
- Cells such as red blood cells require special treatment for visualization (chemical drying)
- SEM images are used for determination of morphology and size of samples



Biological research

- Image shows E. coli cells that have been treated with an antibiotic, colistin
- Colistin works by disrupting cell membranes in gram negative bacteria
- In this sample you can see undisturbed cell, red arrow, and a dead cell, yellow arrow.