

## EM Training Session

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Email: gina.funicelli@smu.ca

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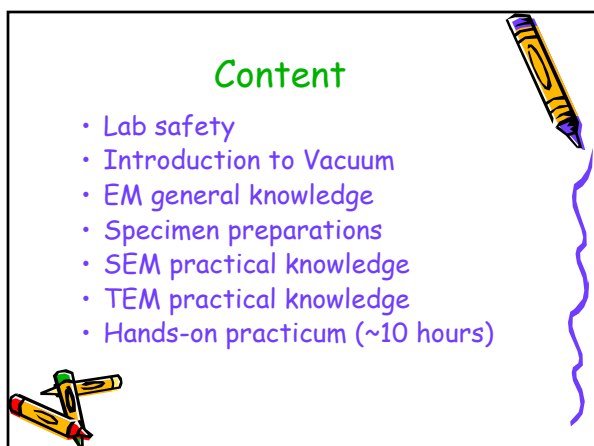
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## Content

- Lab safety
- Introduction to Vacuum
- EM general knowledge
- Specimen preparations
- SEM practical knowledge
- TEM practical knowledge
- Hands-on practicum (~10 hours)

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## Lab Safety

- Why lab safety is important
- General rules
- LN2 safety operation
- What to do in case of emergency?
- Lab layout and emergency evacuation plan
- Penalties



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## Why Safety ?

- High voltage: TEM - 100 kV  
SEM - 30 kV
- Dangerous Chemicals
- Sharp tools
- High Pressure Gas Cylinders
- High/Low Temperature
- Radiation



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## ARGON, CO<sub>2</sub> AND NITROGEN

- These gases are inert, colorless, odorless, and tasteless but can cause asphyxiation and death in confined, poorly ventilated areas. Do not lean into or place your head into a freezer.
- In addition these gases can cause severe frostbite to the eyes or skin.
- Some carbon dioxide cylinders contain an eductor tube and are intended for liquid withdrawal. These cylinders are specially marked; be sure you are using equipment appropriate to the application.



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## Large leaks of nitrogen

- N<sub>2</sub> High Pressure Cylinder
- Liquid Nitrogen
- Example:
- Cold Stage -- Liquid Nitrogen



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## Liquid nitrogen

- Oxygen depletion is the main danger from the use of liquid nitrogen.
- Eye protection and gloves must be worn whenever handling cryogenic liquids
- When handling liquid nitrogen remember to open any curtains and doors to avoid oxygen depletion.



No open toed shoes in the lab



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## Large leaks of nitrogen

Oxygen Content (vol. %)	Effects and symptoms (at atmospheric pressure)
20 - 14	Diminution of physical and intellectual performance without person's knowledge.
14 - 10	Judgement becomes faulty. Severe injuries may cause no pain. ill temper easily aroused. Rapid fatigue on exertion.
10 - 6	Nausea and vomiting may appear. Loss of ability to move vigorously or at all. Inability to walk, stand or crawl is often first warning and it comes too late. Person may realise they are dying but does not care. Resuscitation possible if carried out immediately.
	Fainting almost immediate, painless death ensues, brain damage even if rescued.



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## Dangerous Chemicals

- Osmium Tetroxide
- Aldehydes
- Buffer solutions
- Propylene Oxide
- Embedding Resins

Treat all fixatives with respect;

they will fix your tissue too



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## Osmium Tetroxide

- Osmium tetroxide is a volatile chemical with disagreeable chlorine-like odor.
- The toxic vapors cause lacrimation, eye and respiratory irritation and coughing, blurred vision and headache, following acute exposure.
- It should be used only in a functioning fume hood and stored in tightly sealed containers.
- The TLV is 0.0002 ppm (TWA) and 0.0006 ppm (STEL).

Threshold Limit Values (TLV)



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### Short Term Exposure Limit (STEL)

a 15 minute time-weighted average exposure which should not be exceeded at any time even if the eight-hour time-weighted average is within the TLV

### Time Weighted Averages (TWA)

the average airborne concentration of substances to which it is believed nearly all workers may be repeatedly exposed during a normal 8-hour workday and 40-hour week, day after day without adverse effect.



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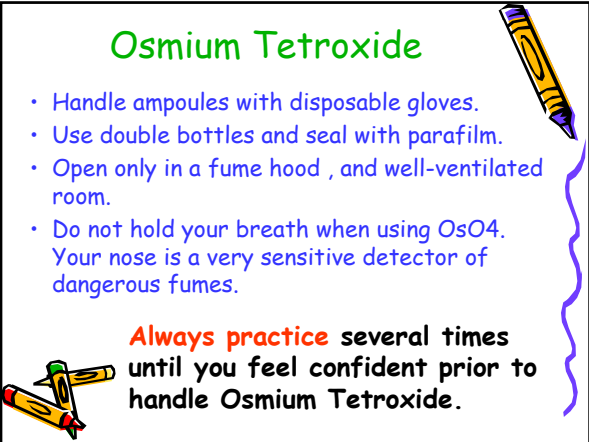
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## Osmium Tetroxide

- Handle ampoules with disposable gloves.
- Use double bottles and seal with parafilm.
- Open only in a fume hood, and well-ventilated room.
- Do not hold your breath when using  $OsO_4$ . Your nose is a very sensitive detector of dangerous fumes.

**Always practice several times until you feel confident prior to handle Osmium Tetroxide.**




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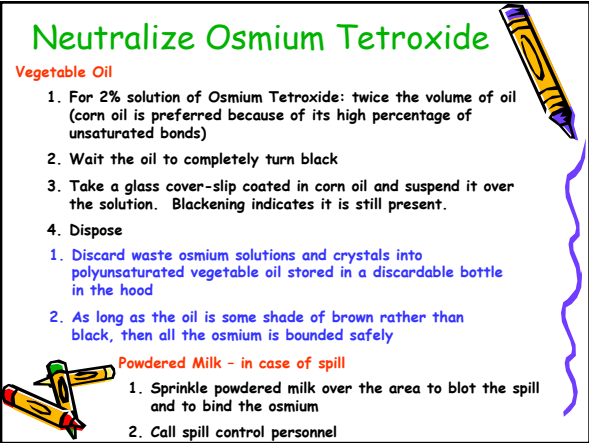
## Neutralize Osmium Tetroxide

**Vegetable Oil**

1. For 2% solution of Osmium Tetroxide: twice the volume of oil (corn oil is preferred because of its high percentage of unsaturated bonds)
2. Wait the oil to completely turn black
3. Take a glass cover-slip coated in corn oil and suspend it over the solution. Blackening indicates it is still present.
4. Dispose
  1. Discard waste osmium solutions and crystals into polyunsaturated vegetable oil stored in a discardable bottle in the hood
  2. As long as the oil is some shade of brown rather than black, then all the osmium is bounded safely

**Powdered Milk - in case of spill**

1. Sprinkle powdered milk over the area to blot the spill and to bind the osmium
2. Call spill control personnel




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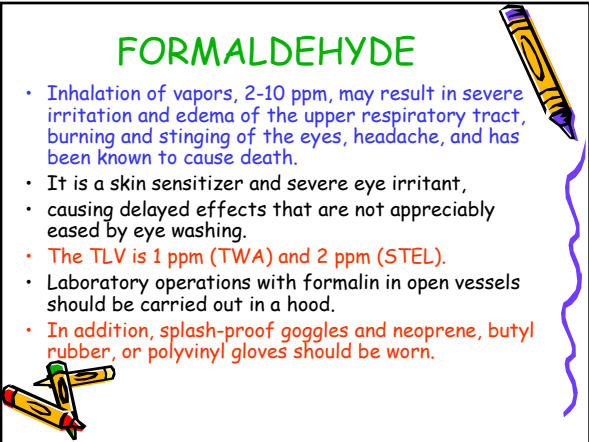
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## FORMALDEHYDE

- Inhalation of vapors, 2-10 ppm, may result in severe irritation and edema of the upper respiratory tract, burning and stinging of the eyes, headache, and has been known to cause death.
- It is a skin sensitizer and severe eye irritant, causing delayed effects that are not appreciably eased by eye washing.
- The TLV is 1 ppm (TWA) and 2 ppm (STEL).
- Laboratory operations with formalin in open vessels should be carried out in a hood.
- In addition, splash-proof goggles and neoprene, butyl rubber, or polyvinyl gloves should be worn.




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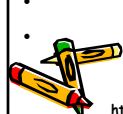
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## Safety Kits for your safe

- Formaldehyde Spill Response™ Kit



- Glutaraldehyde Clean-up™ Kit
- Glutaraldehyde Spill Control



Ampoule Breaker

[http://www.emsdiasum.com/microscopy/products/safety/lab\\_spills.aspx](http://www.emsdiasum.com/microscopy/products/safety/lab_spills.aspx)

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## What should you do?

- Make sure you have done everything to make your lab activity safe.
- Make sure you have well prepared to react in case of accident such as chemical spills. (this includes the spill control packages)
- Bring WHIMS training record prior to using the lab instruments



**Do not bring your own chemicals into EM lab** because the lab is not designed for handling chemicals.

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## EM Lab Safety References

**ELECTRON MICROSCOPY SAFETY HANDBOOK**; Vernon C. Barber and Deborah L. Clayton, Editors. San Francisco Press Inc., 1985 ISBN 0-911302-56-5

**ELECTRON MICROSCOPY PRINCIPLES AND TECHNIQUES FOR BIOLOGISTS**; John J. Bozzala and Lonnie D. Russell, Jones and Bartlett Publishers., 1992 pp. 498-519. ISBN 0-86720-126-6

**SAFETY IN THE SCANNING ELECTRON MICROSCOPY LAB**; J. Bastacky and T.L. Hayes, Scanning 7:255-72., 1985

**Cautions, Common Sense, and Rationale for the Electron Microscopy Lab**; E. B. Smithwick, Journal of Electron Microscopy Technique, 2(3): 193-200 (1985)



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## General Safety Rules

1. Listen to or read **instructions** carefully before attempting to do anything. If you're not sure what to do, **ask for help**.
2. Wear safety goggles to protect your **eyes** from **chemicals**, **heated materials**, or things that might be able to **shatter**.
3. Notify lab tech if ANY **accidents occur**.



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## General Safety Rules

4. Know the **location** of the fire extinguisher, eyewash station and first aid kit.
5. Keep your work area **uncluttered**. Take to the lab station only what is **necessary**.
6. Never play **practical jokes** in the lab.
7. **Clean** up your lab area after finishing your research



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## Electrical Safety

1. Be sure your hands and your lab area are **dry** before using electrical equipment.
2. Never poke anything into electrical **outlets**.
3. Unplug cords by pulling the **plug** and not the cord.



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**NEVER** attempt to operate ANY equipments without prior instruction by qualified people in the lab or without reading the operation instructions **CAREFULLY**.



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**NEVER** assume that the gloves available are suitable protection for every chemical; check the manufacturer's recommendations.



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### EM emergency shut down

- **Personal safety** always remains a priority at all times and by no means should you put yourself at risk.
- Press the **OFF** button on the Emergency Control Panel, The microscope will shut down and minimize any hazards to itself or anyone nearby.



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## EM emergency shut down

- Immediately leave the microscope room and follow the evacuation procedures.
- If there is a risk of electrocution, shut off the main power switches for each microscope



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## First Aid---Burns

To do: Immediately flush with **cold** water until burning sensation is lessened.



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## First Aid -- Cuts, bruises

To do: Do not touch an open wound without safety gloves. Pressing directly on minor cuts will stop bleeding in a few minutes. Apply cold compress to bruises to reduce swelling.



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### First Aid -- Fainting

To do:

Provide fresh air and have the person recline so that their head is lower than the rest of their body.



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### First Aid -- The eyes

To do: Flush eyes immediately with plenty of water for several minutes. If a foreign object is lodged in the eye, do not allow the eye to be rubbed.



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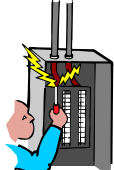
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### First Aid -- Electrical shock

To do:

Shut off the current at the source.  
Remove wire with rubber gloves.  
Alert the Lab Tech immediately.



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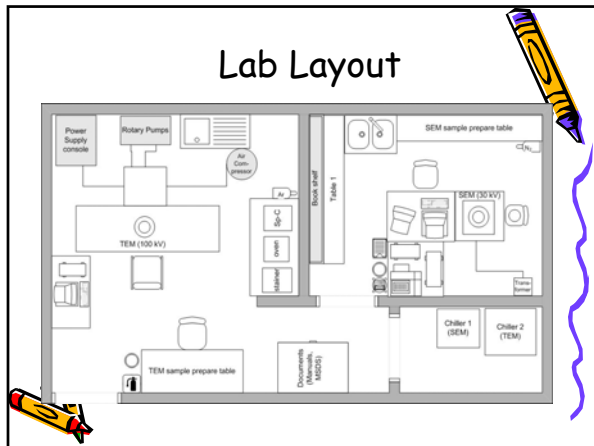
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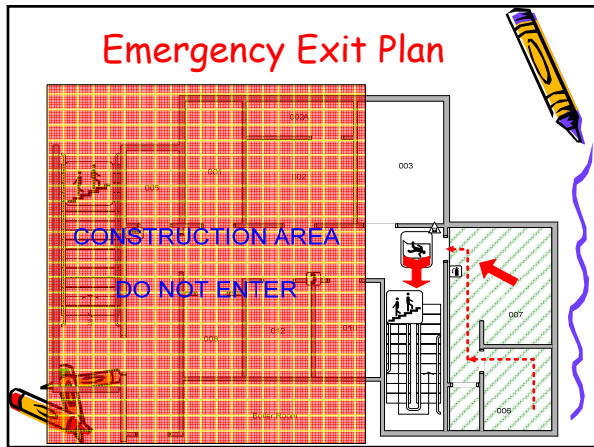
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- ### Violation of Safety Rules
- **Loss of Privilege** in Using EM facilities
  - **First offense:** Oral warning
  - **Second offense:** Written warning send to student as well as supervisor
  - **Third offense:** Termination of using EM facilities
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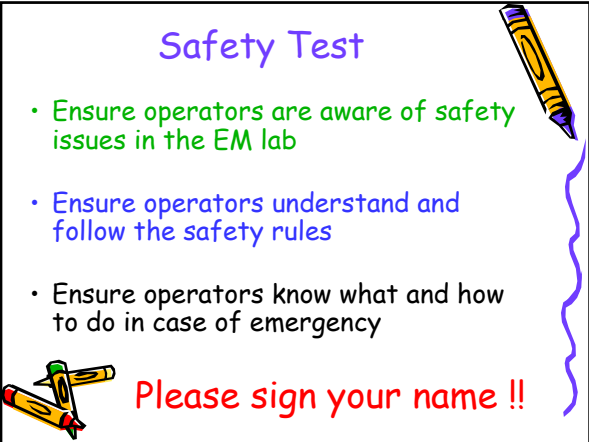
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## Safety Test

- Ensure operators are aware of safety issues in the EM lab
- Ensure operators understand and follow the safety rules
- Ensure operators know what and how to do in case of emergency

**Please sign your name !!**



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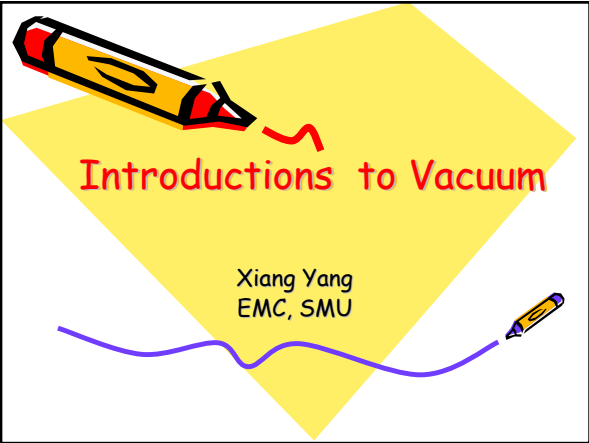
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## Introductions to Vacuum

Xiang Yang  
EMC, SMU



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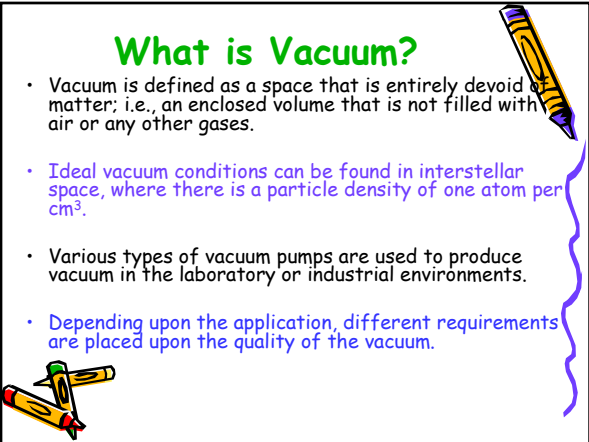
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## What is Vacuum?

- Vacuum is defined as a space that is entirely devoid of matter; i.e., an enclosed volume that is not filled with air or any other gases.
- Ideal vacuum conditions can be found in interstellar space, where there is a particle density of one atom per  $\text{cm}^3$ .
- Various types of vacuum pumps are used to produce vacuum in the laboratory or industrial environments.
- Depending upon the application, different requirements are placed upon the quality of the vacuum.



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## Degrees of Vacuum

- **Low vacuum**  
from  $10^3$  to 100 mbar; i.e., for vacuum packaging
- **Medium vacuum**  
from 100 to  $10^{-3}$  mbar; i.e., for manufacturing incandescent lamps
- **High vacuum**  
from  $10^{-3}$  to  $10^{-7}$  mbar; i.e., for high vacuum furnace and casting
- **Ultrahigh vacuum (UHV)**  
from  $10^{-7}$  to  $10^{-12}$  mbar; i.e., for space simulation or scientific research


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## Some Numbers

1	atm (atmosphere pressure)
1.013	Bar (1 bar = 1000 mbar)
1.033	Kg/cm <sup>2</sup>
14.7	psi (lb/in <sup>2</sup> , pound/square inch)
760	mmHg (millimeter of mercury)
760	torr
101,325	Pa (Pascal, 1Pa = 1N/m <sup>2</sup> )


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## Atmosphere to Low Vacuum

- Atm. to  $10^{-3}$  torr
- Wet air (75%-85%), Water vapor



Rotary Pump


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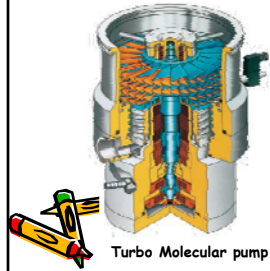
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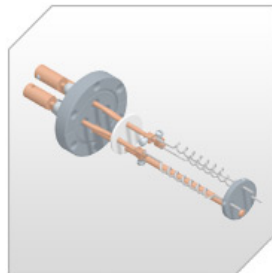
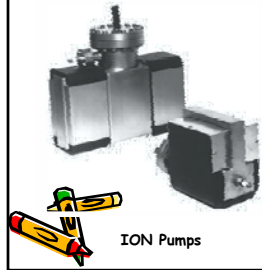
## From Low to High Vacuum

- $10^{-3}$  to  $10^{-8}$  Torr
- Water (80%), CO



## Ultrahigh Vacuum (UHV)

- $<10^{-8}$  torr
- $H_2$



## How to keep a vacuum System Clean ?

- Store in enclosed cabinet to keep off dust
- Cover each item in the cabinet for added protection
- Wipe with Methanol with special clean wipes
- **Oil free:** please wear gloves when load and unload samples.
- **Dust free:** blow the surface before loading.

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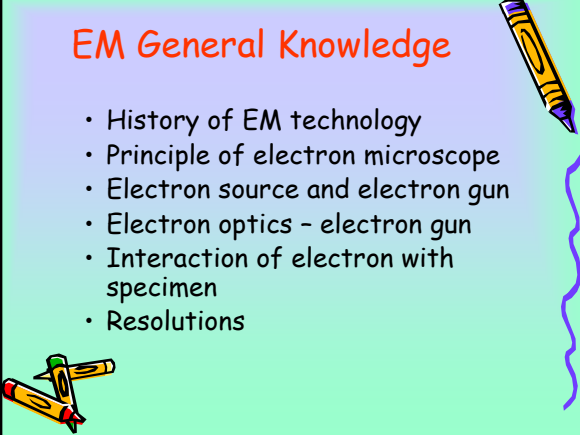
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## EM General Knowledge

- History of EM technology
- Principle of electron microscope
- Electron source and electron gun
- Electron optics - electron gun
- Interaction of electron with specimen
- Resolutions




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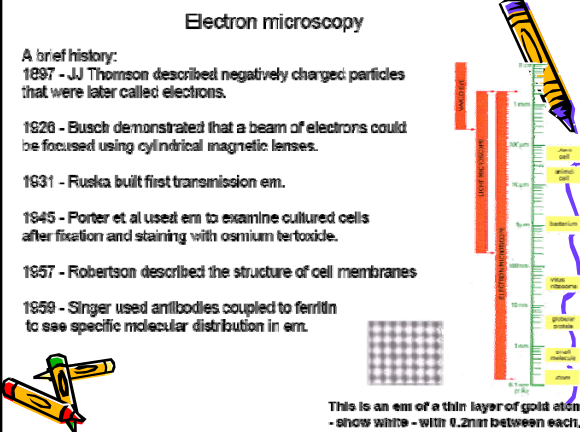
### Electron microscopy

**A brief history:**

- 1897 - JJ Thomson described negatively charged particles that were later called electrons.
- 1926 - Busch demonstrated that a beam of electrons could be focused using cylindrical magnetic lenses.
- 1931 - Ruska built first transmission em.
- 1945 - Porter et al used em to examine cultured cells after fixation and staining with osmium tetroxide.
- 1957 - Robertson described the structure of cell membranes
- 1959 - Singer used antibodies coupled to ferritin to see specific molecular distribution in em.



This is an em of a thin layer of gold atoms  
- show white - with 0.2nm between each.




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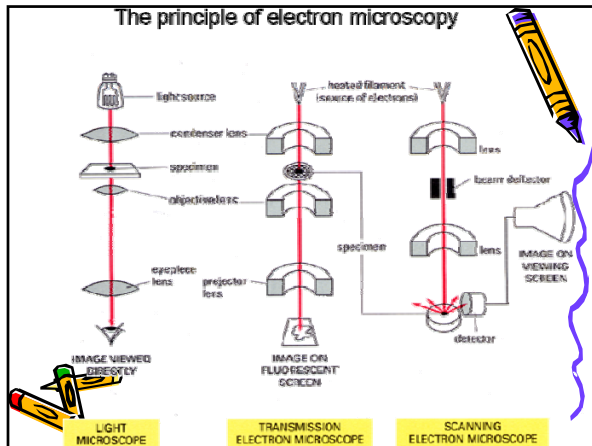
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**Transmission Electron Microscope**  
 Optical instrument in that it uses a lens to form an image

**Scanning Electron Microscope**  
 Not an optical instrument (no image forming lens) but uses electron optics. Probe forming-Signal detecting device.

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**Electron Sources**

**Tungsten emitters**  
 Wire bent into a loop of various dimensions.  
 W (m.t. 3410 degrees C.)

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## ELECTRON GUN

- **The Filament**
  - Thermionic Emission
  - Tungsten, Lanthanum Boride
  - Field Emission Gun

Tungsten Wire      LaB<sub>6</sub> Crystal

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## ELECTRON GUN

- **Wehnelt Cap**
  - negative bias
- **Anode**
  - positive bias
- **Vacuum**

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## Electron Optics

### Electrostatic lens

0-V

Must have very clean and high vacuum environment to avoid arcing across plates

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### Electromagnetic Lenses

Electromagnetic lenses are comprised of windings of wire through which electric current is applied. This creates a strong magnetic field through which negatively charged electrons must pass.

Labels in diagram: Source of electrons, Axis, Electron trajectory, Copper windings, Iron shroud, Magnetic lens field, Focal point.

Due to the magnetic field, the electrons follow a helical trajectory which converges at a fine focal point after it emerges from the lens. (DC-powered magnets behave similar to converging glass lenses)

Field Strength determines the focal length which varies with:

$$(\text{focal length}) f = K (V / i^2)$$

K = constant based on the number of turns of lens coil wire and the geometry of the lens.  
 V = accelerating voltage  
 i = milliamperes of current put through the coil

Potentiometer controls which vary the current to the various lenses are the means by which focus and magnification of the electron beam are achieved.

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### Interaction of Electron with Thin Specimen

Labels in diagram: Primary Electrons, Specimen Atom, Inelastically Scattered Electron (Low Angle), Elastically Scattered Electron (High Angle), Unscattered (Transmitted) Electron.

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Labels in diagram: Condenser lens, Aperture, Specimen grid with sections, Objective lens, Objective aperture, Transmitted electrons (image forming), Deflected electrons (lost).

Some of the scattered electrons will only be partially scattered and thus will reach the screen in an inappropriate position giving a false signal and thus contributing to a degradation of the image. These forward scattered electrons can be eliminated by placing an aperture beneath the specimen.

### This is TEM Case

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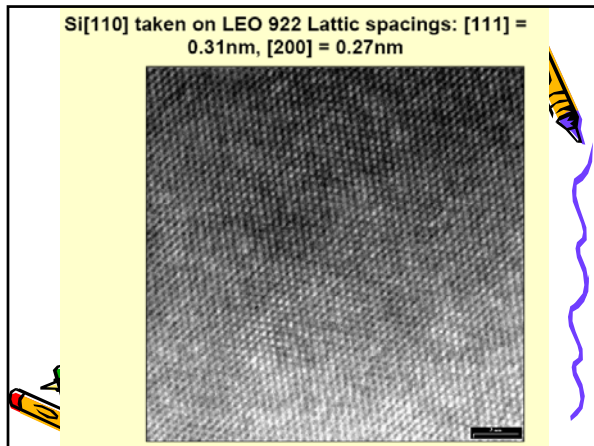
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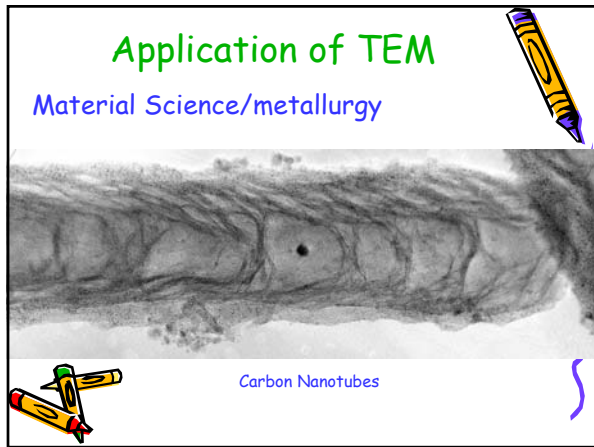
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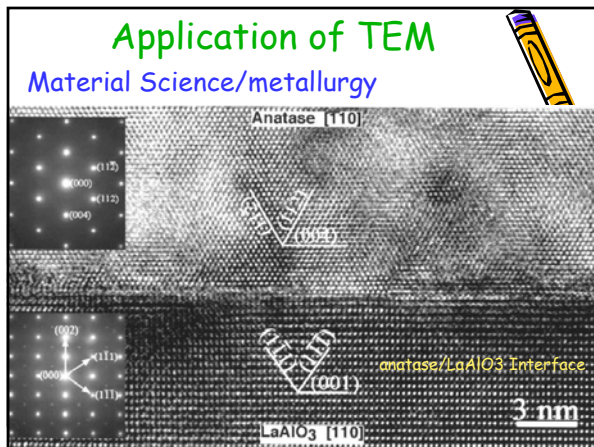
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**Application of TEM**  
Geology - texture



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**Application of TEM**  
Biological Science -- Sea urchin



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**Application of TEM**  
Biological Science - human blood cells



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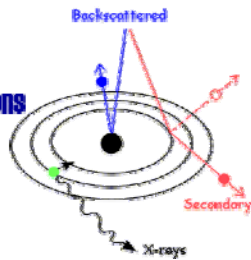
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## Interaction of Electron with Bulk materials

- Secondary electrons
  - topography
- Back scatter electrons
  - compositional
- X-rays
  - chemistry




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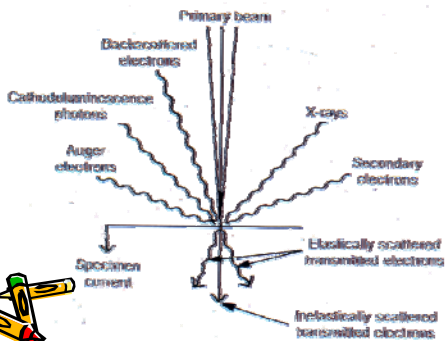
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## Interaction of Electron with Bulk materials




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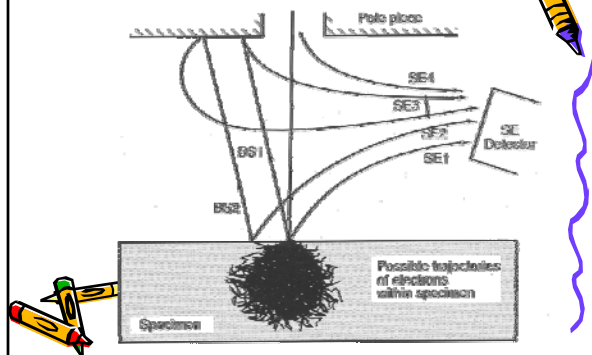
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## This is how SEM works !!




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## SEM Images -- 1

**Toner x2,500**      **Gold particles x36,000**      **Integrated Circuit x720**

**Eye of a fly x100**      **Kosher Salt x75**      **Toilet Paper x500**

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## SEM Images --2

**Black widow spider x500**      **Cucumber skin x350**      **Staple in paper x35**

**Big Radiolarian x500 and x2,000**      **Ceropia moth x350 and 15,000**

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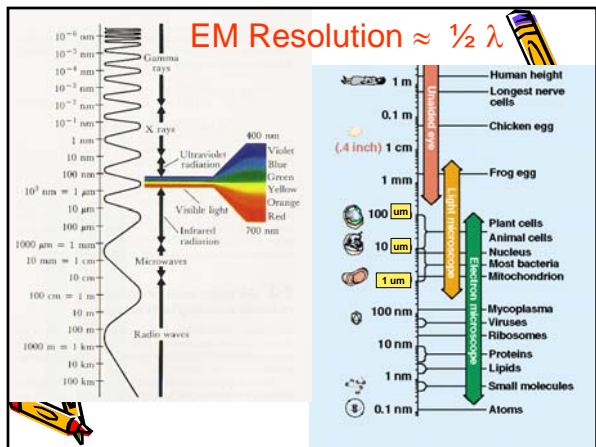
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## What could you expect from the SEM in the lab?

- Surface morphology (SE and VP detectors)
- Element Information (EDS detector)
- Textures analysis (mini CL detector)
- Atom number info. (BSD detector)
- Heated or frozen samples (cold stage)
- Size of samples up to 200mm in diameter and 30mm thick



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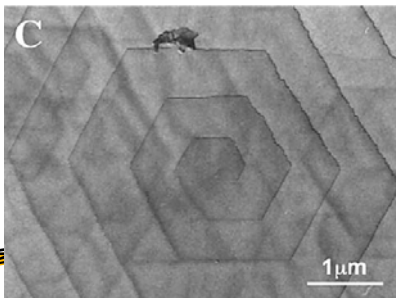
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## Applications of SEM

- Crystal growth pattern



From: RIKEN Review No. 42 (December, 2001): Focused on Ecomolecular Science Research



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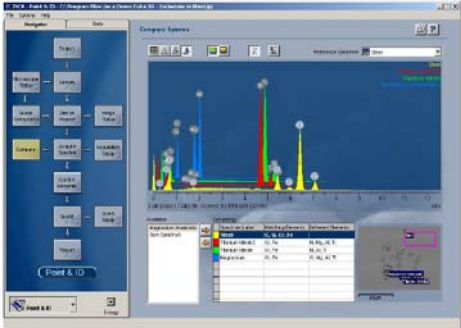
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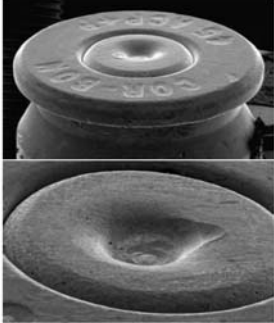
## Applications of SEM

- Element information



## Applications of SEM

- Forensic Investigations



*Low magnification, image of a .45 cartridge. Image courtesy Terry McAdam Washington State Patrol Crime Laboratory Seattle USA*

**Bullet comparison**

*Firing pin impression in centre of a .45 cartridge.*

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## Applications of SEM

- Life Science



*Low magnification image at a long working distance. This image illustrates the excellent high signal level in VP mode at 20Pa without any shadowing on this highly topographic sample.*

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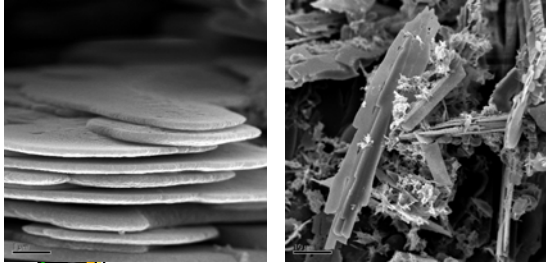
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## Applications of SEM

- Materials Sciences



**C60 crystals.**

<http://biology.berkeley.edu/EML/sem.html>

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## Application of SEM

- Material Science -Iron nanoparticles (UW)



Width = 2.702 μm  
The Name = e-beam100k.tif  
100nm  
Mag = 100,000 X  
WD = 8 mm  
Date = 12 Apr 2006  
Time = 13:24:01  
EHT = 1.00 kV  
Signal A = InLens System Vacuum = 7.5E-007 mBar

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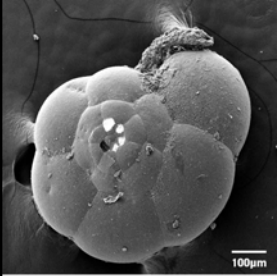
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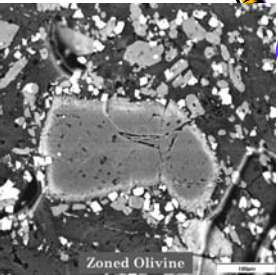
## Applications of SEM

- Earth Science

Recent shell of a foraminifera (a single-celled animal) that lived in salt-water marsh in the Boston area.



*Trochammina inflata*



Zoned Olivine

Zoned olivine in sand grain from pottery, Megido, Israel. Brighter areas in this backscatter image contain elements of higher atomic number.

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## Applications of SEM

- Textures info. - CL image of Zircon



[http://gsc.nrcan.gc.ca/ebeam/sem\\_gallery\\_e.php](http://gsc.nrcan.gc.ca/ebeam/sem_gallery_e.php)

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## Applications of SEM

- Textures info. - BSD image of Zircon



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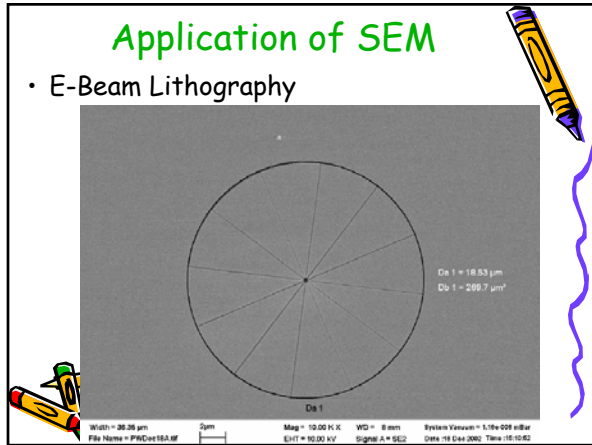
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## Application of SEM

- E-Beam Lithography



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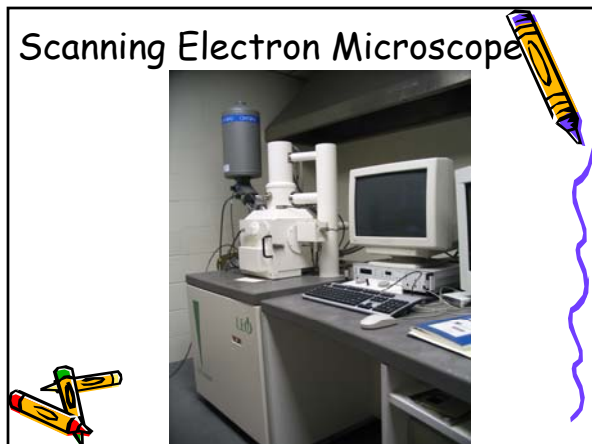
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## Scanning Electron Microscope



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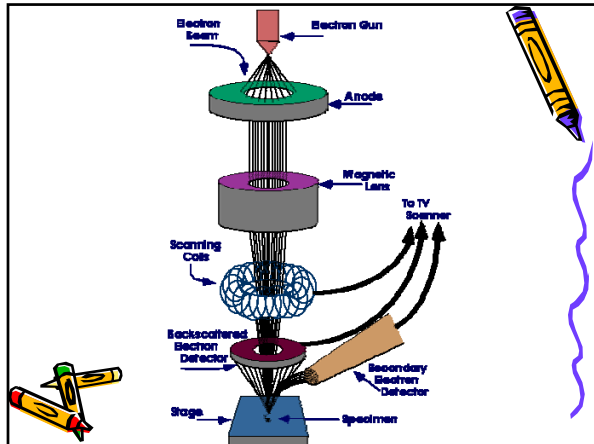
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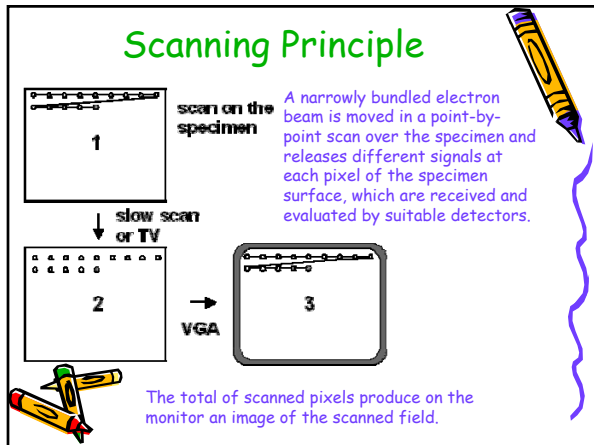
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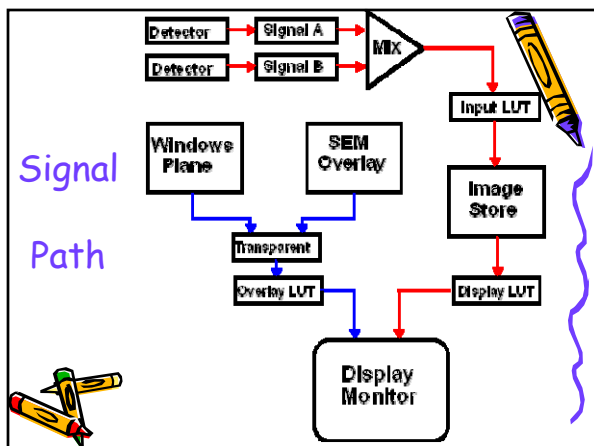
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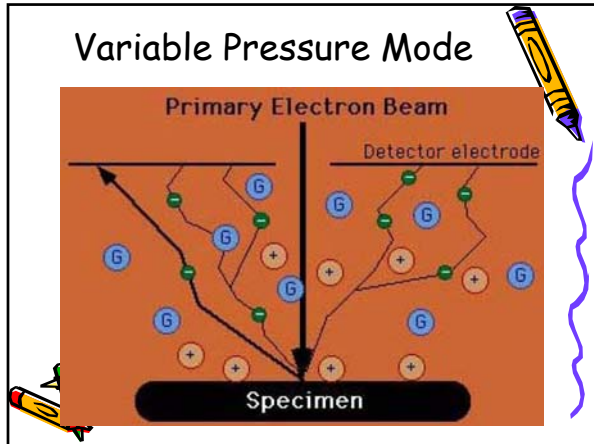
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# Variable Pressure Mode



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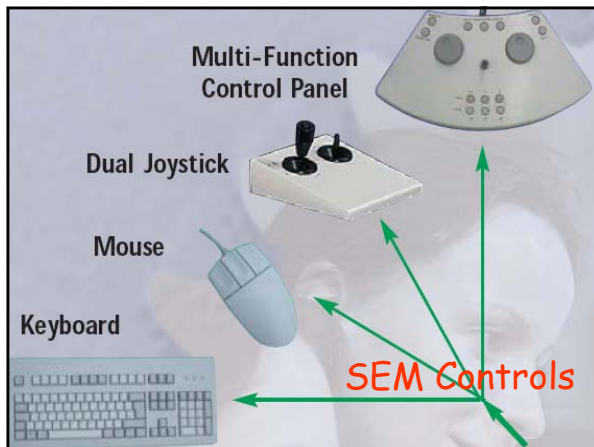
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## To be Remembered

- Brightness and Contrast
- Working Distance
- Scan Speed
- Voltage Setting
- Specimens Requirements
- Good Laboratory Practices
- Final Words




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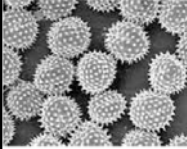
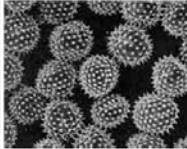
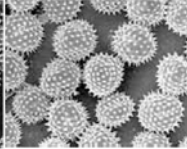
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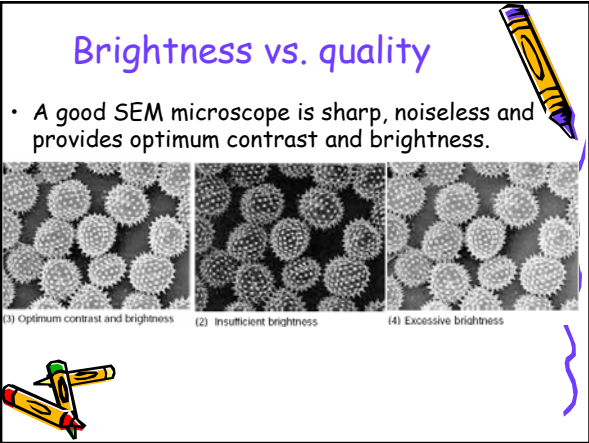
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## Brightness vs. quality

- A good SEM microscope is sharp, noiseless and provides optimum contrast and brightness.

(3) Optimum contrast and brightness
(2) Insufficient brightness
(4) Excessive brightness




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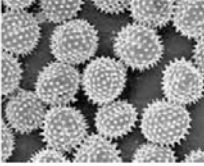
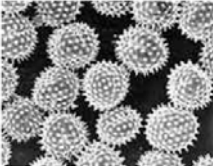
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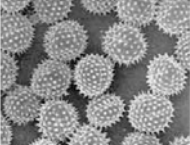
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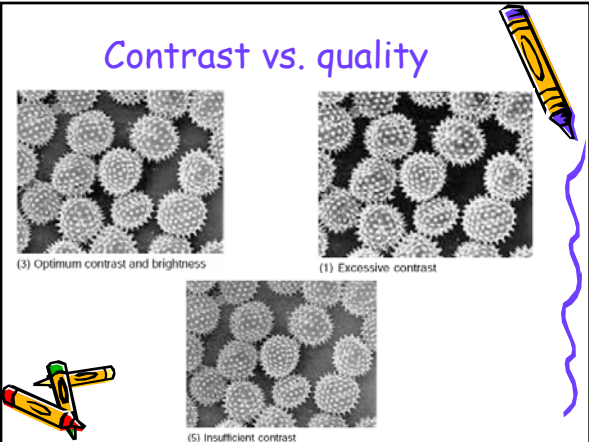
## Contrast vs. quality

(3) Optimum contrast and brightness
(1) Excessive contrast



(5) Insufficient contrast




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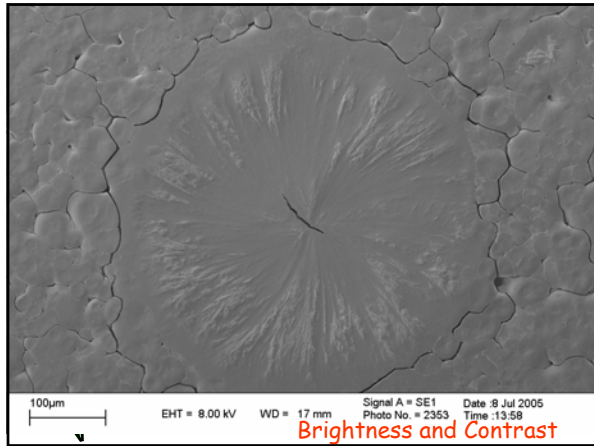
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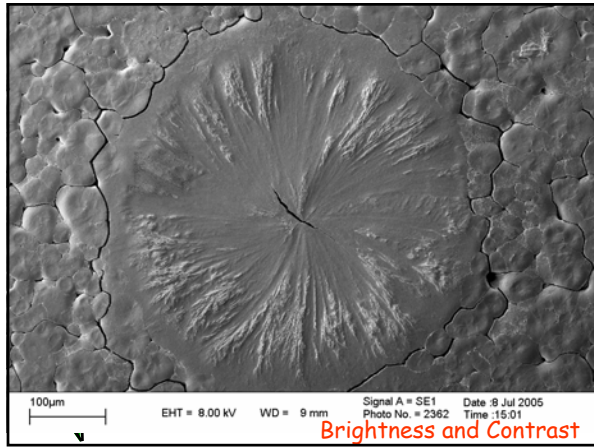
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### Brightness/contrast control

Auto B/C

Manual B/C

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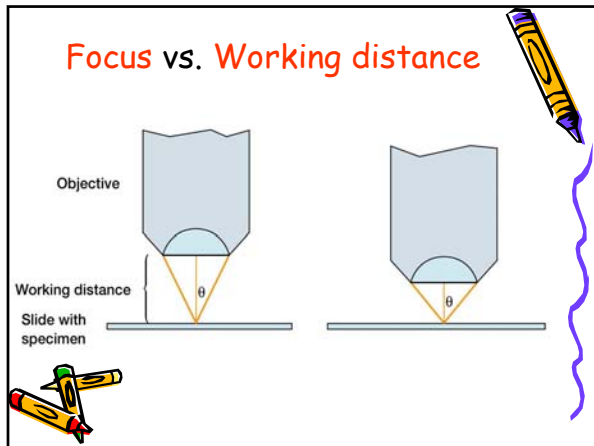
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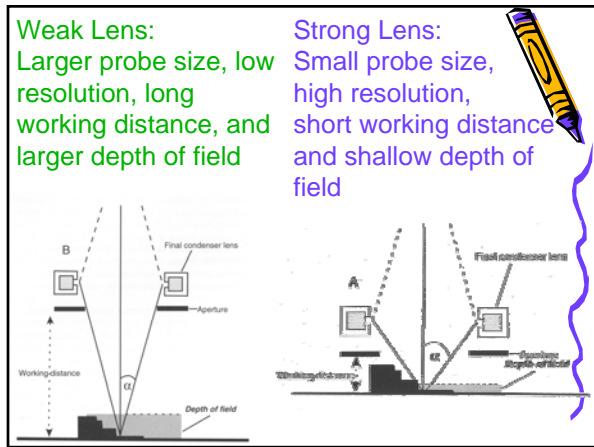
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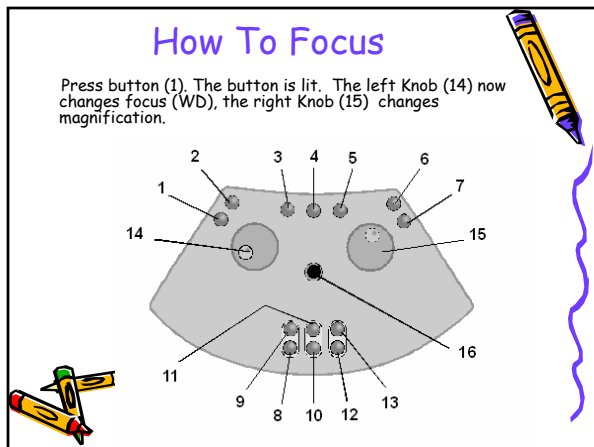
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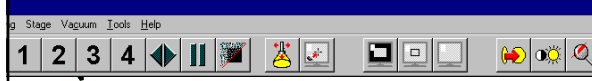
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## Scan Speed vs. Image Quality

- **Fastest scan speed** and **low magnification** are best settings to get your bearings on sample stage.
- Faster scan:** better Signal/Noises ratio with less detailed surface info.
- Slower scan:** better detailed surface info. but poor S/N ratio



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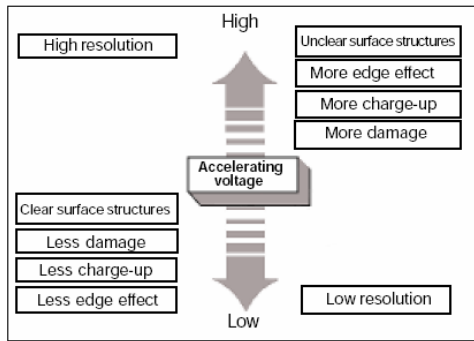
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## How to Set Voltage



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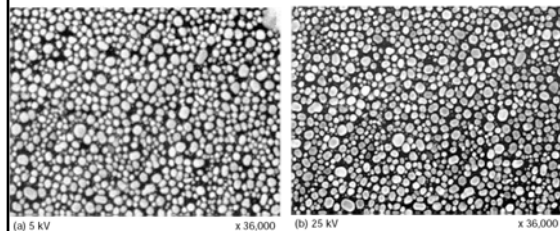
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## Voltage vs. Resolution



Higher Voltage ===== Higher Resolution

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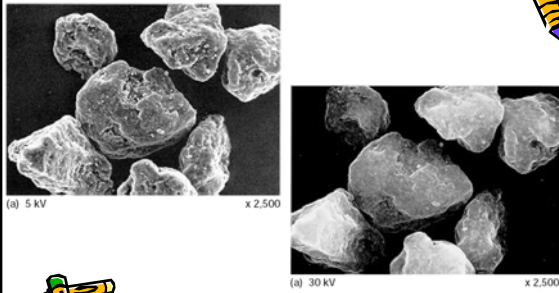
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## Voltage vs. Surface Info.



Higher Voltage == Deeper surface Info

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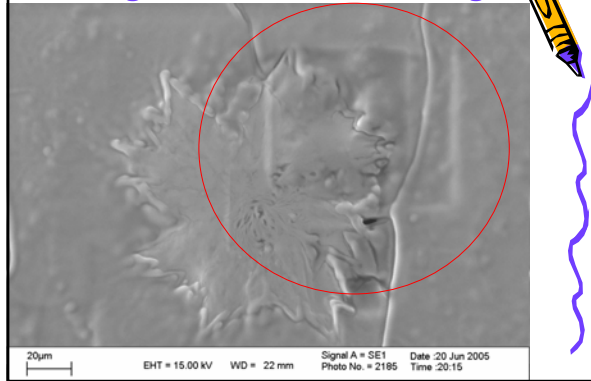
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## Voltage vs. Surface Damage



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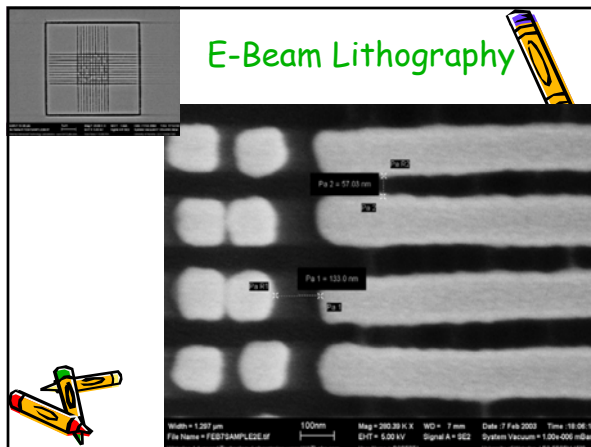
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## E-Beam Lithography



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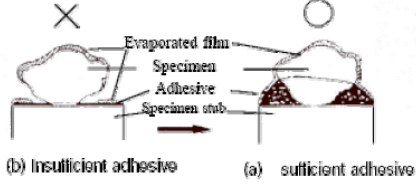
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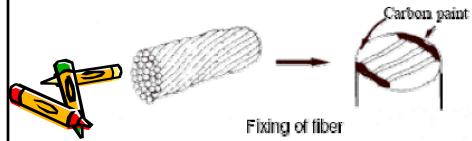
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## Specimens Req. vs. Charging



Fixing of a bulk specimen



Fixing of fiber

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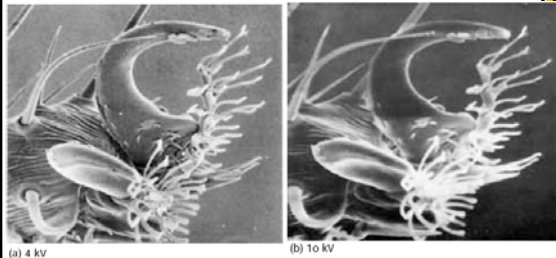
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## Voltage vs. Charging



(a) 4 kV

(b) 10 kV

*Charge-up can be reduced by using low accelerating voltage.*

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## Powder Materials



Remove excessive particles

Example: Pollens

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## Spot Size vs. Resolution

High Resolution: 120 to 230

Backscattered electron imaging: 380 to 450

X-Ray analysis: 460 to 500

Charge/beam sensitive samples: 160 to 220

In most cases, a value of 333 gives a good video signal without over exposing the sample to electron bombardment.



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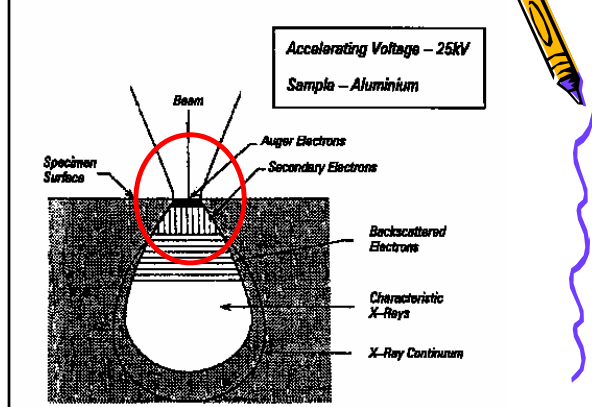
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## Beam-specimen interaction volume Diagram



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## Astigmatism ?

### What is astigmatism?

The aberration caused by the machining accuracy and material of the polepiece is called "astigmatism."

### How to correct astigmatism?

This astigmatism can be removed by adjusting the two knobs, X and Y, of the stigmator.



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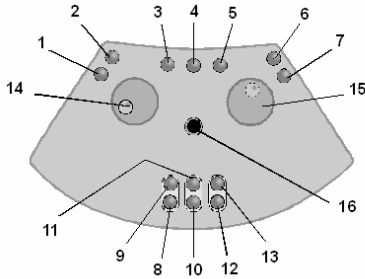
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## How To correct astigmatism

Press button (2). The button is lit. The left Knob (14) now corrects X direction astigmatism, the right Knob (15) corrects Y direction astigmatism.




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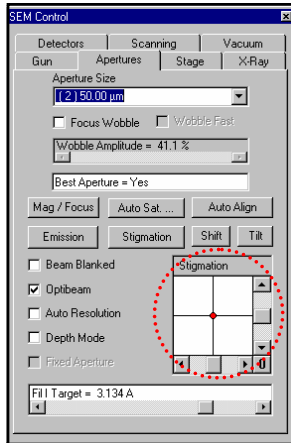
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How To Reduce Astigmatism -- 2

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## Image Defect Causes

DEFECT	CAUSE
(1) Low contrast, lack of video signal (if spot size too small), possible specimen damage (if too high)	Incorrect Spot Size
(2) Lack of image sharpness, image shift when focusing	Incorrect final aperture alignment
(3) Less image sharpness in one direction, poor resolution	Insufficient astigmatism correction
(4) Noisy image, beam deflection on charging sample, specimen damage	Wrong scanning period
(5) Poor image quality	Wrong brightness level selected
(6) Poor image quality	Wrong contrast level selected
(7) Blurring on penetration and charging	Wrong accelerating voltage

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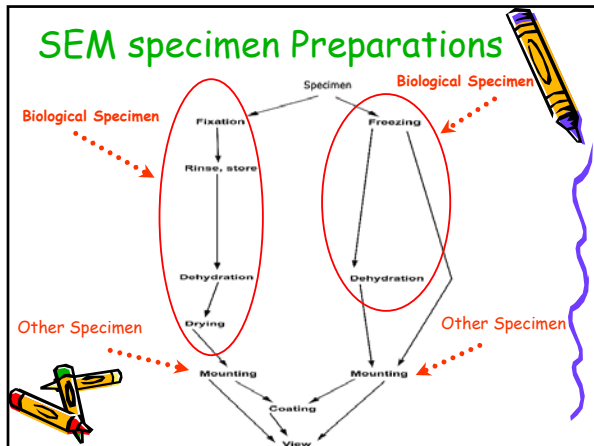
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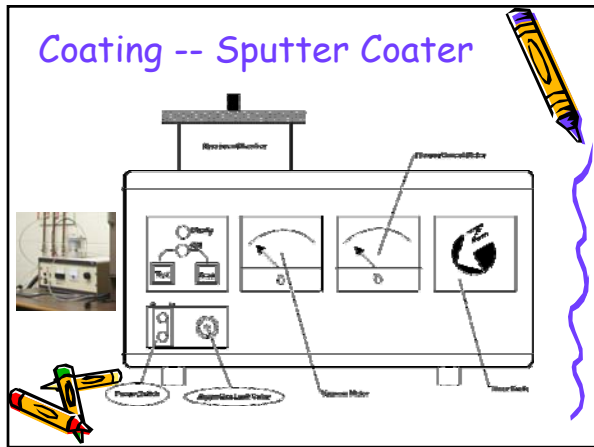
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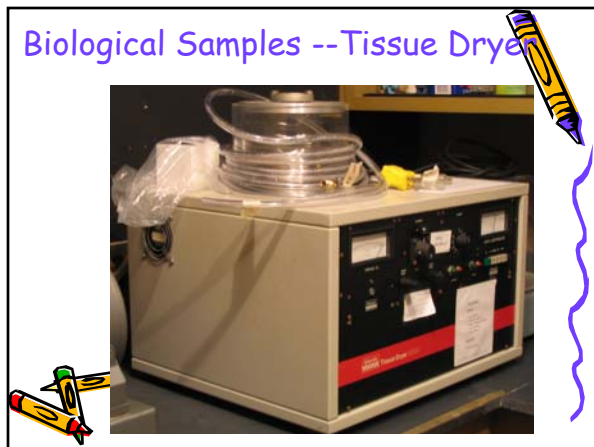
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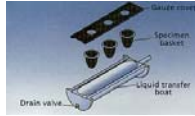
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## Biological Samples -- Critical Point Dryer (CPD)



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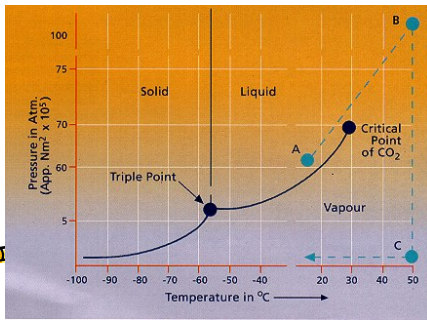
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## CPD: critical point of $CO_2$

Purpose: To completely dry specimen for mounting while maintaining morphological details.



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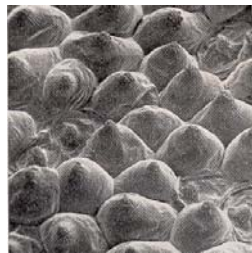
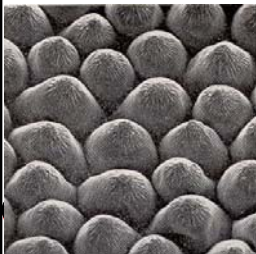
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## CPD v.s. Air Dry

SEM image (850X) of rose petal surface, CPD



SEM image (850X) of rose petal surface, fixed and air dried

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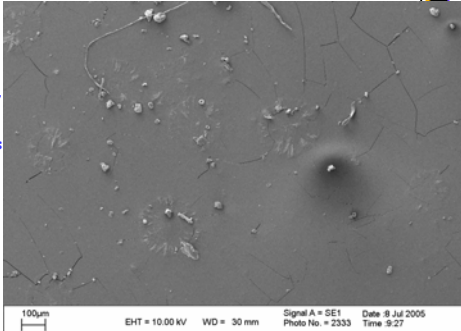
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## Specimen Preparations

NEVER let your specimens, tools, grids, glassware become contaminated with dust or fingerprints.

In even the most immaculate lab, dust is falling like rain, so keep your grids and specimens covered.



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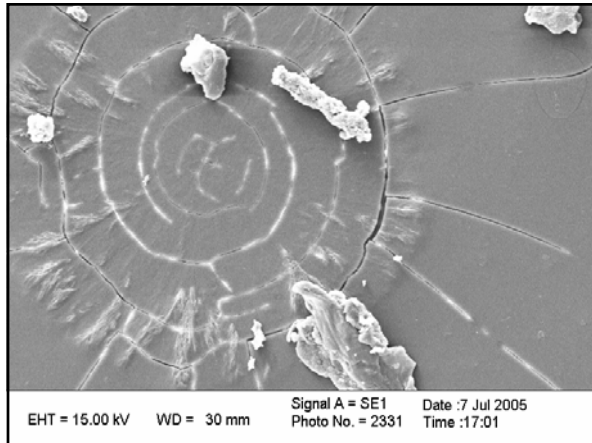
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## How to Load Specimens

- Make Sure specimens are dry
- Using conductive tape/glue
- Coat with Gold if specimens are insulator
- Vent the chamber
- Load the specimen (wearing gloves)
- Pump



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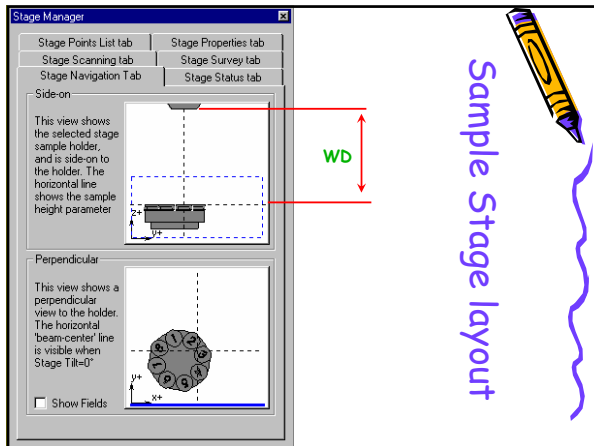
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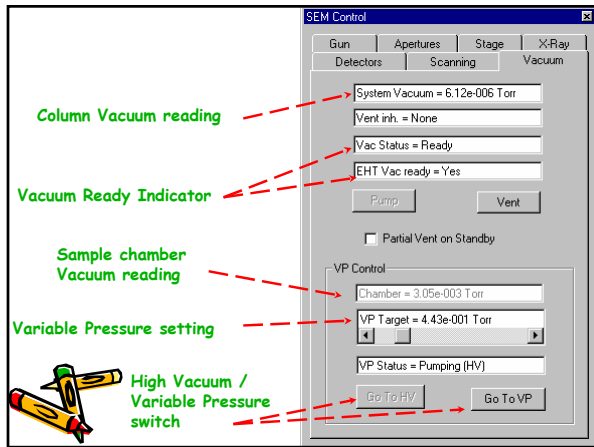
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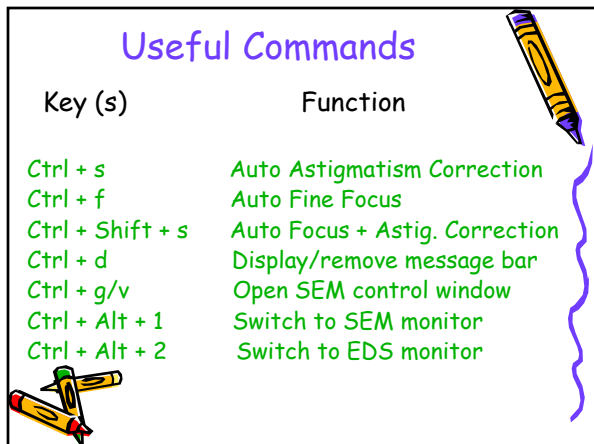
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## Good EM Laboratory Practice

- Always wear gloves when unload or load EM specimens.
- Don't wear open toe shoes in the lab
- Always make sure you know what you are doing before next step
- Always ask for help if not sure
- Report any accidents
- Fill the log sheet



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## How to start SEM operation

- Username Created
- Logon to operate SEM
- Logoff to quite operation

User Name	Image Directory	User level
C:\Program Fil...032\User\Dave R	C:\Program Fil...E0VL032\Images	NoVICE
C:\Program Fil...32\User\default	C:\Program Fil...E0VL032\Images	Any
C:\Program Fil...2\User\dnoreau	C:\Program Fil...Images\dnoreau	Expert
C:\Program Fil...1E032\User\dnoug	C:\Program Fil...032\Images\dnoug	NoVICE
C:\Program Fil...32\User\service	C:\Program Fil...E0VL032\Images	Any
C:\Program Fil...E032\User\guest	C:\Program Fil...E0VL032\Images	Expert
C:\Program Fil...2\User\jenifer	C:\Program Fil...Images\jenifer	NoVICE
C:\Program Fil...E032\User\jroch	C:\Program Fil...32\Images\jroch	NoVICE
C:\Program Fil...32\User\karinne	C:\Program Fil...Images\karinne	NoVICE
C:\Program Fil...E032\User\kewin	C:\Program Fil...E0VL032\Images	NoVICE
C:\Program Fil...1E032\User\marc	C:\Program Fil...032\Images\marc	Expert
C:\Program Fil...32\User\default	C:\Program Fil...E0VL032\Images	NoVICE
C:\Program Fil...32\User\service	C:\Program Fil...E0VL032\Images	Any
C:\Program Fil...2\User\default	C:\Program Fil...E0VL032\Images	Any
C:\Program Fil...E032\User\vliong	C:\Program Fil...32\Images\vliong	Full
C:\Program Fil...32\User\vliong	C:\Program Fil...32\Images\vliong	NoVICE



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## To New Users

- Apply to EM training
- Get and fill the NEW USERS FORM
- Get EM training and demonstrate ability to operate the instrument
- Be able to follow the EM lab safety and booking rules
- Fill the Sample request form before use



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## What you have to do?

1. Book your desire time in advance
2. Show up in the lab on time
3. Operating instruments
4. Fill the log book and indicate the beam usage
5. Report any accident/errors to lab tech (it may not be your fault)

Failed to report Errors = Loss of privilege in using EM facilities.

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## SEM Log file contains everything

### Example 1

03:33 26-06-2003 :\*\*\*\*\* : Logged On Successfully  
04:18 26-06-2003 :Error Number :501 : Stage Touching  
21:12 09-09-2003 :\*\*\*\*\* : Logged On Successfully  
03:02 10-09-2003 :Error Number :501 : Stage Touching  
22:01 01-10-2003 :\*\*\*\*\* : Logged On Successfully  
22:16 01-10-2003 :Error Number :501 : Stage Touching  
09:53 27-02-2004 :\*\*\*\*\* : Logged On Successfully  
11:32 27-02-2004 :Error Number :501 : Stage Touching

### Example 2

10:31 12-06-2003 :\*\*\*\*\* : Logged On Successfully  
10:37 12-06-2003 :Error Number :582 : Stage Command Overrun Error

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## Reports make different -1

### Example 1 failed to action

14:07 21-08-2001 :Error Number :593 : Water flow has failed  
14:41 21-08-2001 :Error Number :593 : Water flow has failed

↓  
Stage overheated

↓  
Stage inactivated by system

↓  
Electronics overheated

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## Reports make different -1

Example 2

Error Number :593 : Water flow has failed

Shut off SEM power to prevent overheat

- Check chiller working properly
- Check water line is not blocked
- Check the water switch functioning

SEM back to work second day

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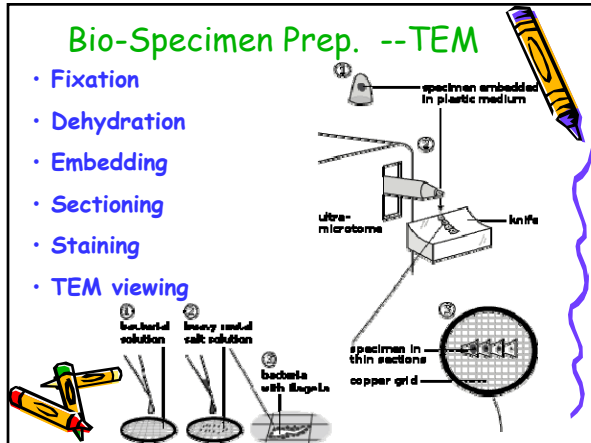
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## Bio-Specimen Prep. --TEM

- Fixation
- Dehydration
- Embedding
- Sectioning
- Staining
- TEM viewing



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