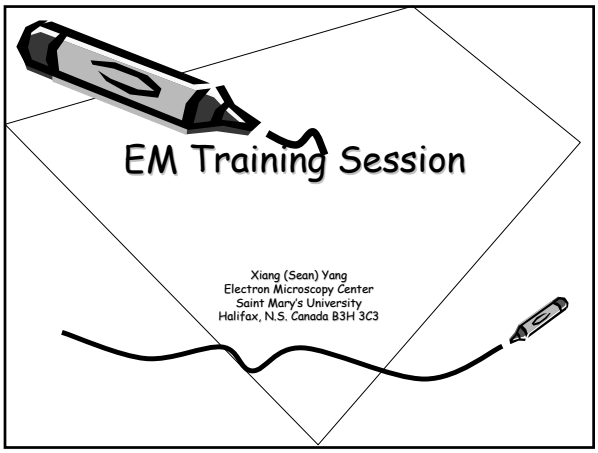

EM Training Session

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Complaints? -- Gina Funicelli

Phone: 902-491-6297
Email: gina.funicelli@smu.ca

Content

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

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

Why Safety ?

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

ARGON, CO_2 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.

Large leaks of nitrogen

- N_2 High Pressure Cylinder
- Liquid Nitrogen

- Example:
- Cold Stage –- Liquid Nitrogen

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

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.

Dangerous Chemicals

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

Treat all fixatives with respect;

they will fix your tissue too

Osmium Tetroxide

- Osmium tetroxide is a volatile chemical with a 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)

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.

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.

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**

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.

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

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.

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)

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.**

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

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.**

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

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

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.

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

First Aid---**Burns**

To do: Immediately flush with <u>cold</u> water until burning sensation is lessened.

First Aid -- **Cuts, bruises**

To do: Do not <u>touch</u> 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 <u>swelling</u>.

First Aid -- **Fainting**

To do:

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

First Aid -- **The eyes**

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

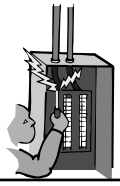
First Aid -- **Electrical shock**

To do:

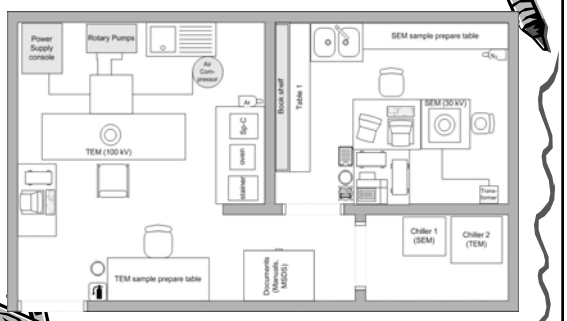
Shut off the current at the <u>source</u>.

Remove wire with rubber gloves.

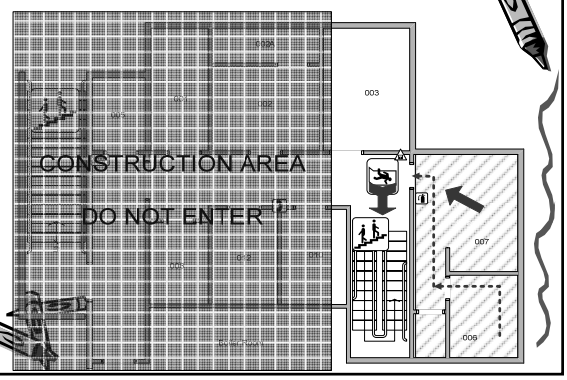
Alert the <u>Lab Tech</u> immediately.



Lab Layout



Emergency Exit Plan



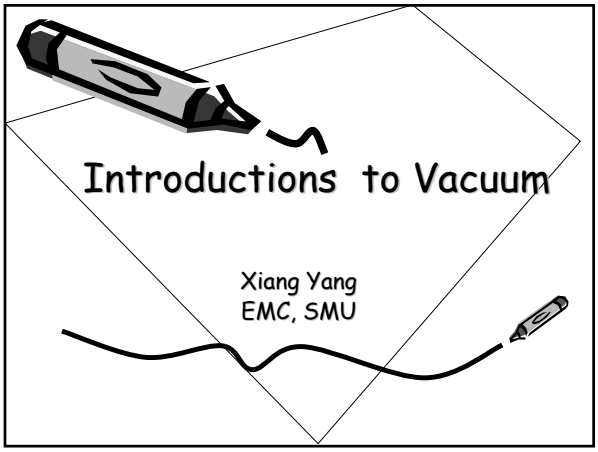
Violation of Safety Rules

- **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

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 !!



Introductions to Vacuum

Xiang Yang
EMC, SMU

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 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.

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

Some Numbers

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

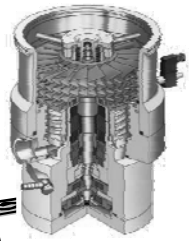
Atmosphere to Low Vacuum

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

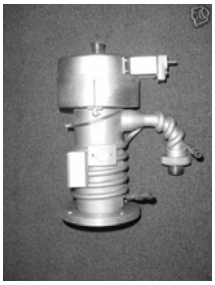
Rotary Pump

From Low to High Vacuum

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



Turbo Molecular pump

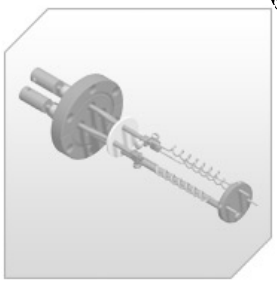


Oil Vapor Diffusion pump

Ultrahigh Vacuum (UHV)

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

ION Pumps



Titanium Sublimation Pump

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.

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

Electron microscopy

A brief history:

1897 - JJ Thomson described negatively charged particles that were later called electrons.

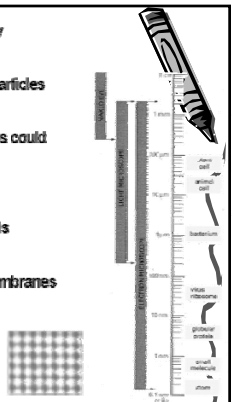
1926 - Busch demonstrated that a beam of electrons could be focused using cylindrical magnetic lenses.

1931 - Ruska built first transmission em.

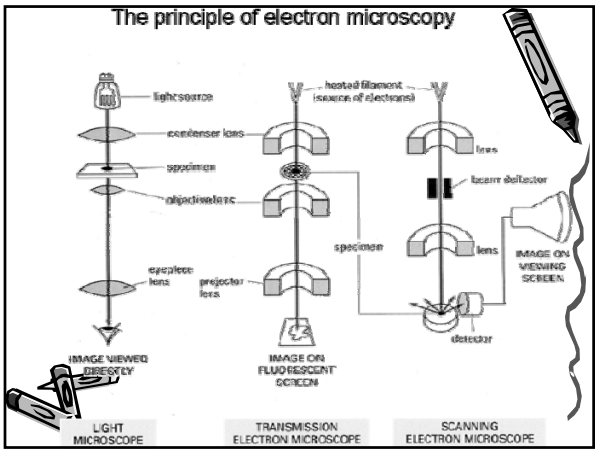
1945 - Porter et al used em to examine cultured cells after fixation and staining with osmium tertoxide.

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.



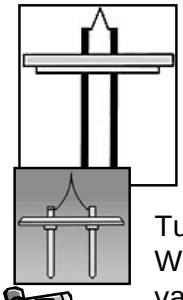
Transmission Electron Microscope

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.

Electron Sources



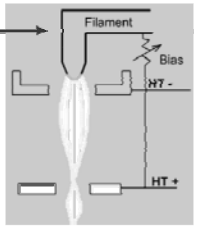
Tungsten emitters
Wire bent into a loop of various dimensions.
W (m.t. 3410 degrees C.)

ELECTRON GUN

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

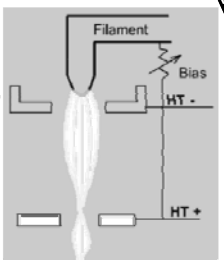
Tungsten Wire

LaB_6 Crystal



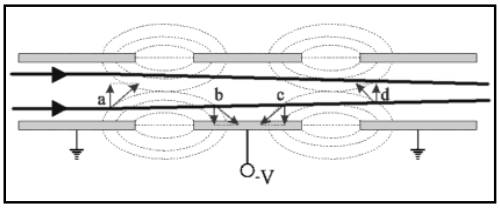
ELECTRON GUN

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



Electron Optics

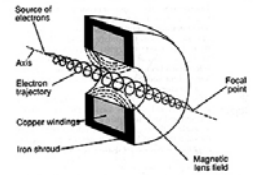
Electrostatic lens



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

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.



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:

(focal length) $f = K (V / i^2)$

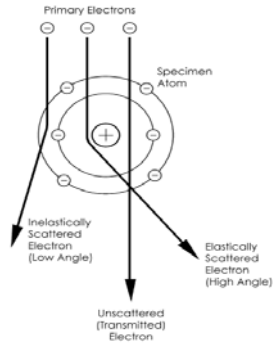
K = constant based on the number of turns of lens coil wire and the geometry of the lens.

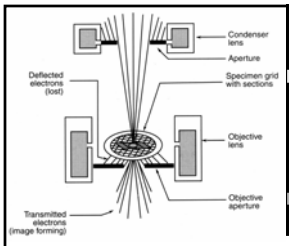
V = accelerating voltage

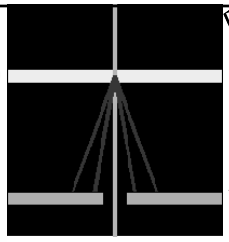
i = milliamps of current put through the coil

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

Interaction of Electron with Thin Specimen

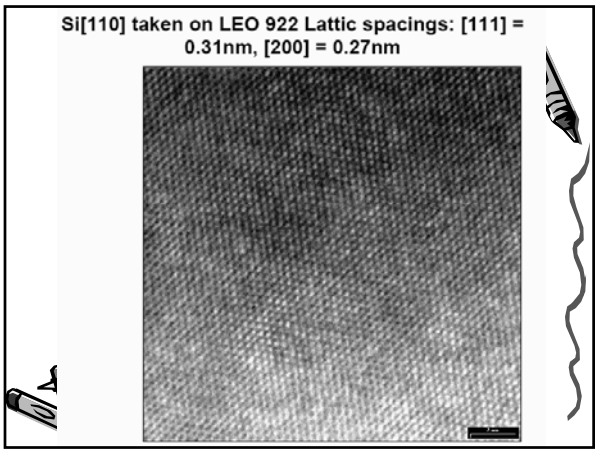






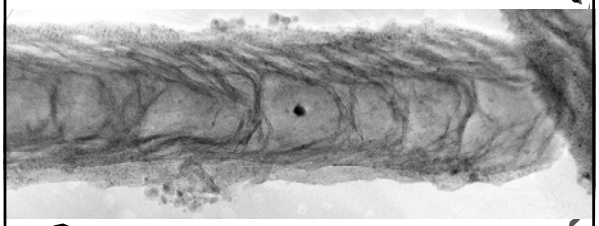
Some of the scattered electrons will only be partially scattered and thus will reach the screen in an inappropriate position giving a false signal and thus contributing to a degradation of the image. These forward scattered electrons can be eliminated by placing an aperture beneath the specimen.

This is TEM Case



Application of TEM

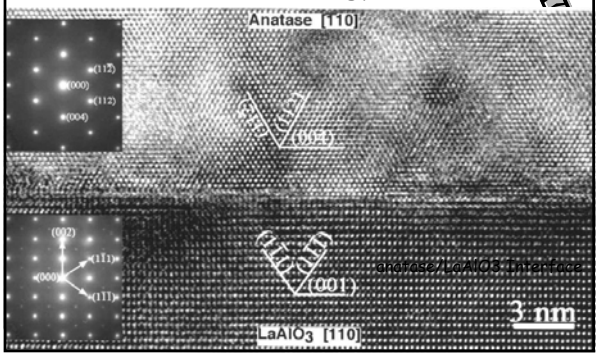
Material Science/metallurgy



Carbon Nanotubes

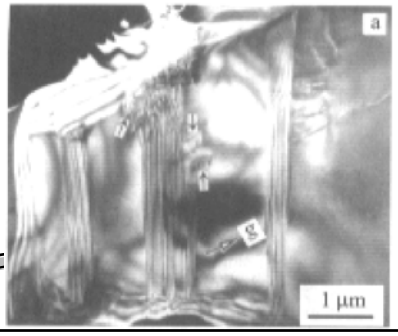
Application of TEM

Material Science/metallurgy



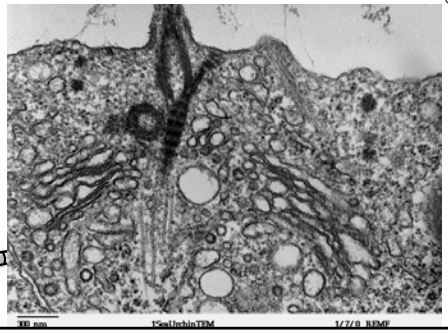
Application of TEM

Geology - texture



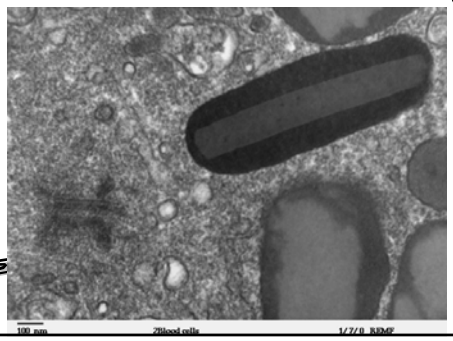
Application of TEM

Biological Science -- Sea urchin



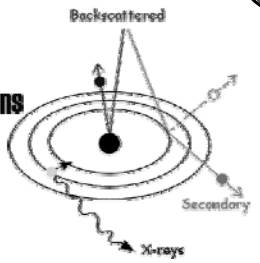
Application of TEM

Biological Science – human blood cells

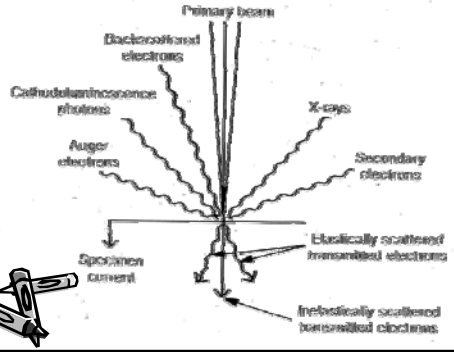


Interaction of Electron with Bulk materials

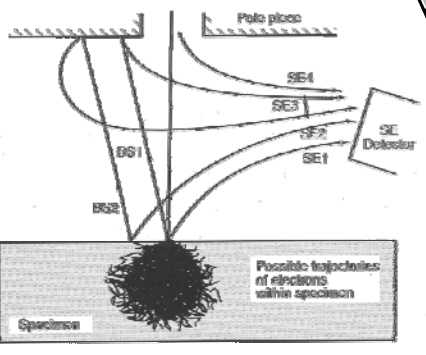
- **Secondary electrons**
  - **topography**
- **Back scatter electrons**
  - **compositional**
- **X-rays**
  - **chemistry**



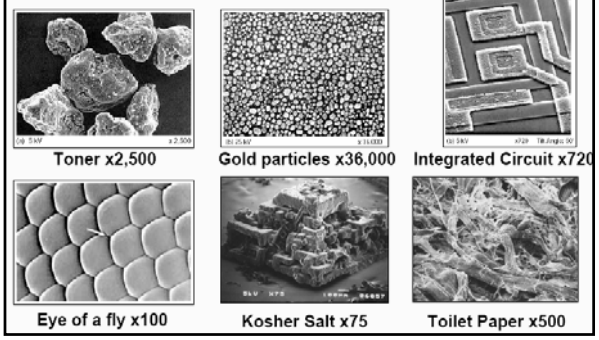
Interaction of Electron with Bulk materials



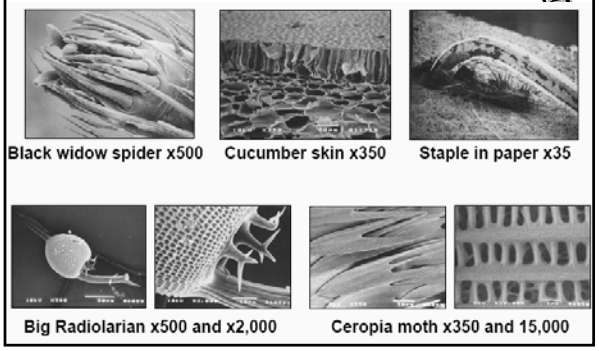
This is how SEM works !!



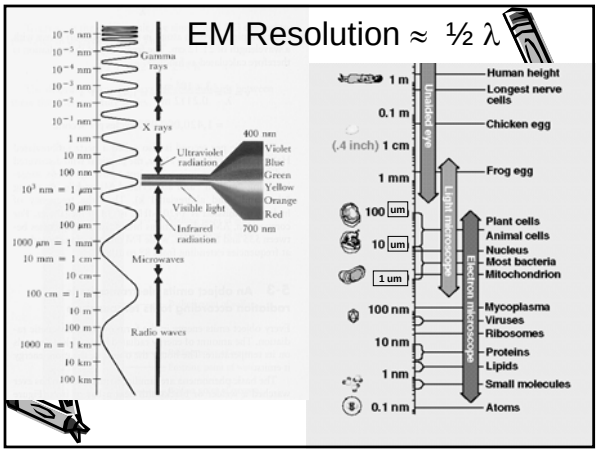
SEM Images -- 1



SEM Images --2



EM Resolution ≈ ½ λ

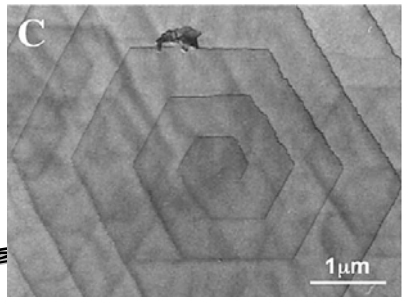


What could you expect from the SEM in the lab?

- 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

Applications of SEM

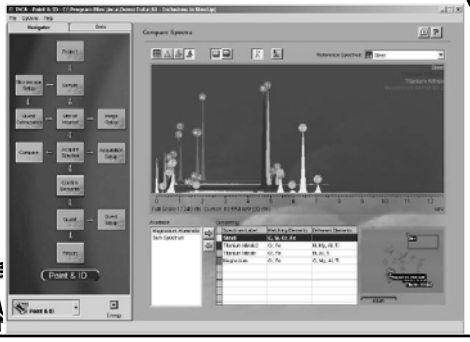
- Crystal growth pattern



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

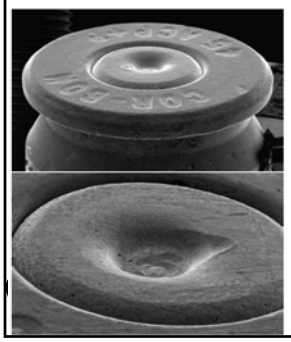
Applications of SEM

- Element information



Applications of SEM

- **Forensic Investigations**



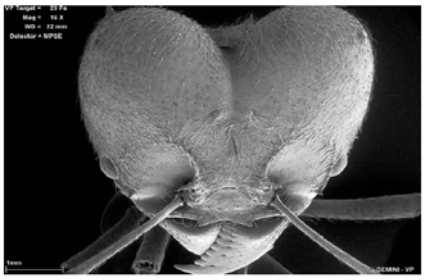
Low magnification, image of a .45 cartridge. Image courtesy Terry McAdam Washington State Patrol Crime Laboratory Seattle USA

Bullet comparison

Firing pin impression in centre of a .45 cartridge.

Applications of SEM

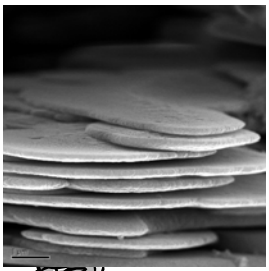
- Life Science

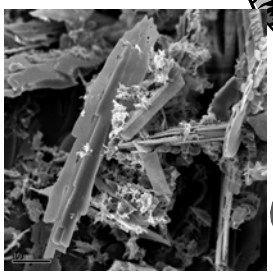


Low magnification image at a long working distance. This image illustrates the excellent high signal level in VP mode at 20Pa without any shadowing on this highly topographic sample.

Applications of SEM

- Materials Sciences



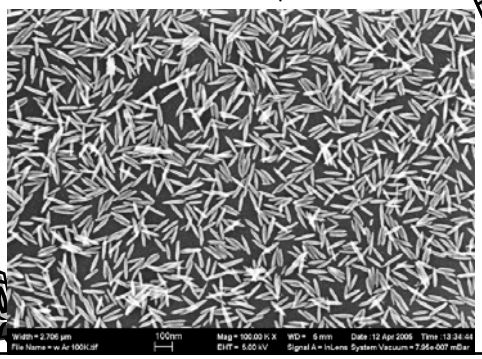


C60 crystals.

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

Application of SEM

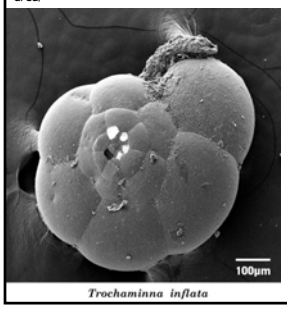
- Material Science –Iron nanoparticles (UW)

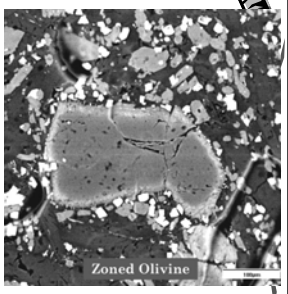


Applications of SEM

- Earth Science

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

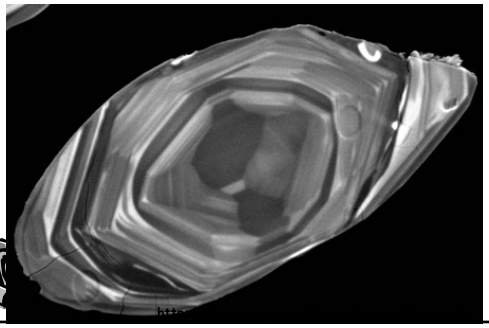




Zoned olivine in sand grain from pottery. Megido, Israel. Brighter areas in this backscatter image contain elements of higher atomic number

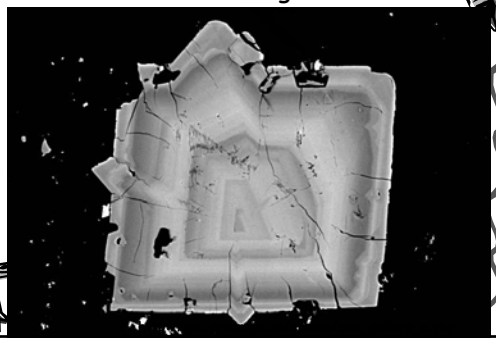
Applications of SEM

- Textures info. – CL image of Zircon



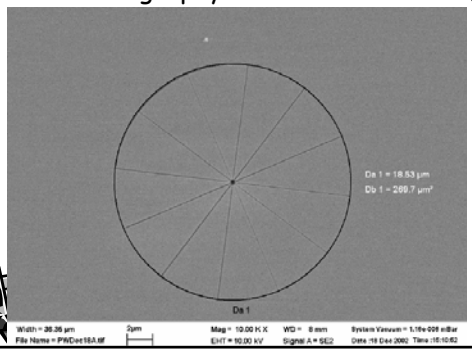
Applications of SEM

- Textures info. – BSD image of Zircon

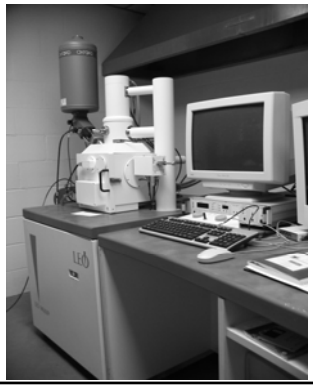


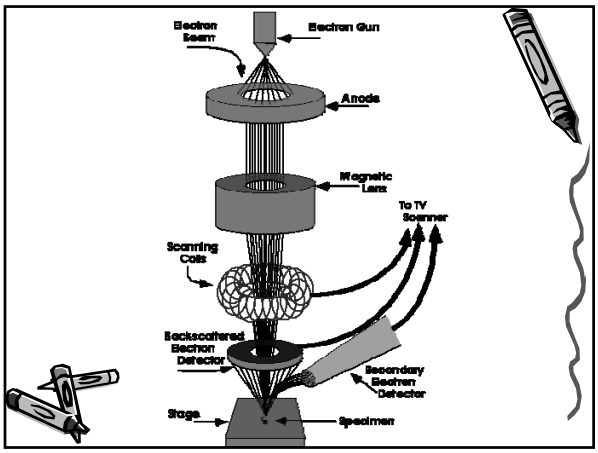
Application of SEM

- E-Beam Lithography

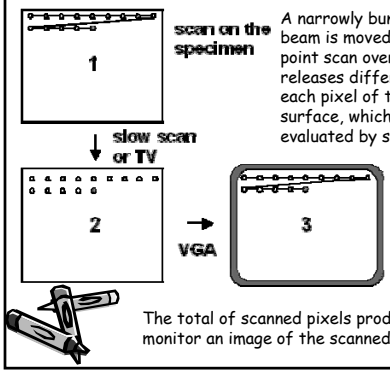


Scanning Electron Microscope



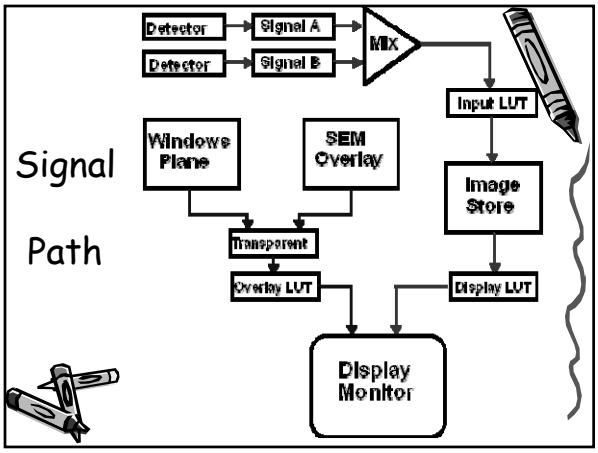


Scanning Principle

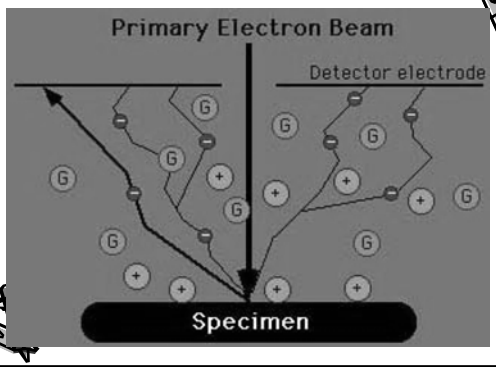


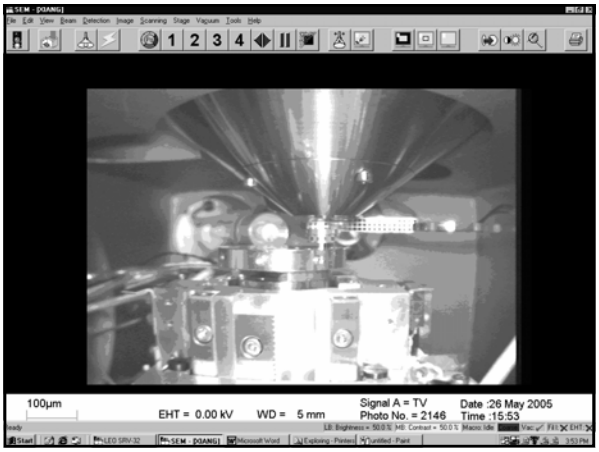
A narrowly bundled electron beam is moved in a point-by-point scan over the specimen and releases different signals at each pixel of the specimen surface, which are received and evaluated by suitable detectors.

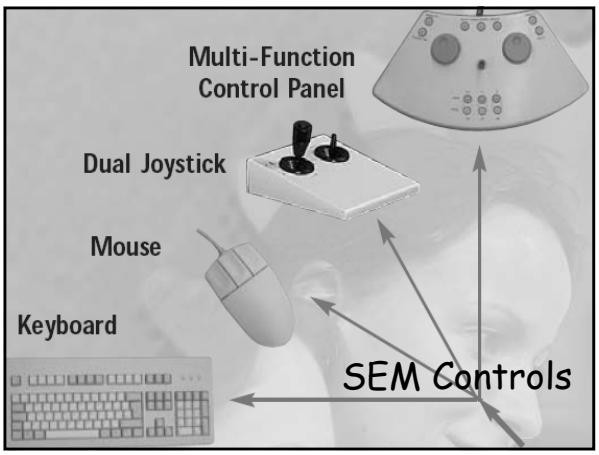
The total of scanned pixels produce on the monitor an image of the scanned field.



Variable Pressure Mode





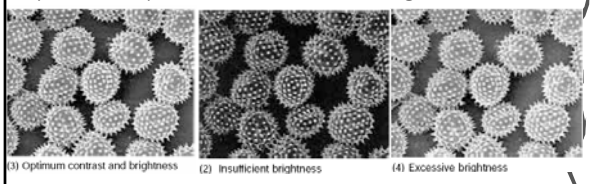


To be Remembered

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

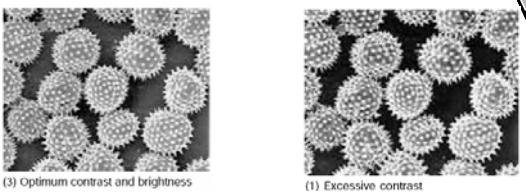
Brightness vs. quality

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



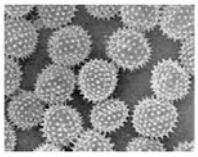
(3) Optimum contrast and brightness (2) Insufficient brightness (4) Excessive brightness

Contrast vs. quality

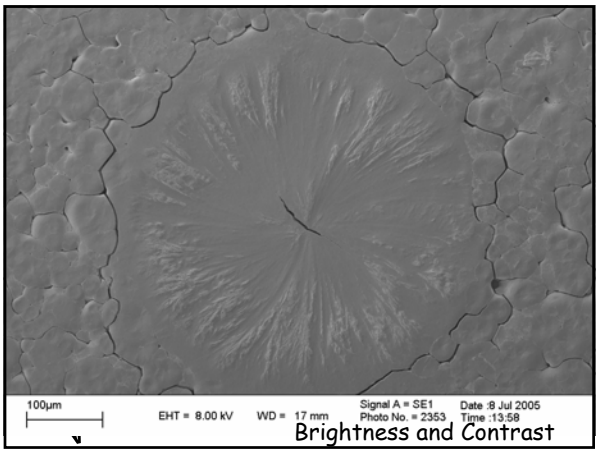


(3) Optimum contrast and brightness

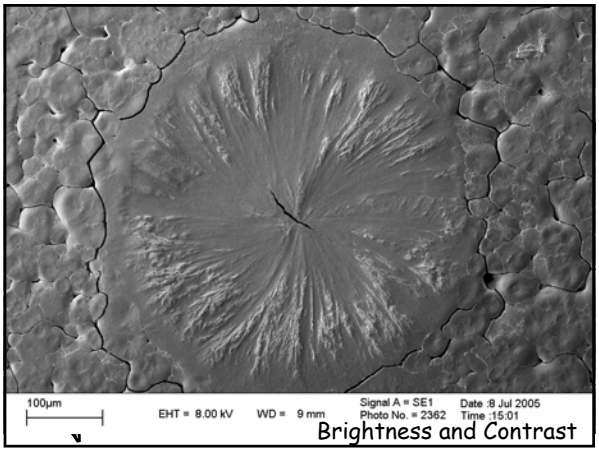
(1) Excessive contrast



(5) Insufficient contrast

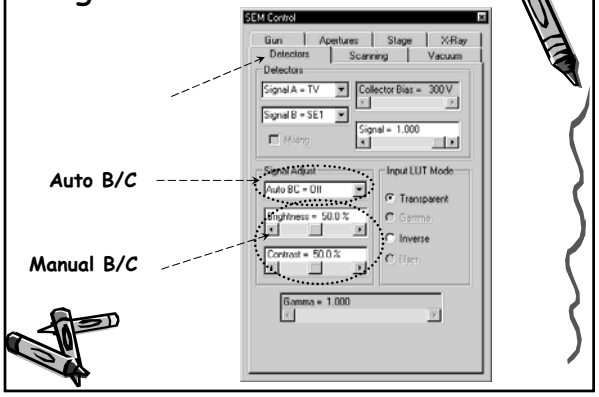


Brightness and Contrast

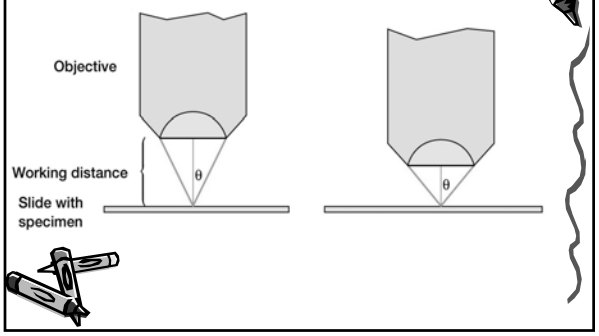


Brightness and Contrast

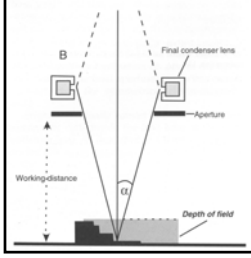
Brightness/contrast control



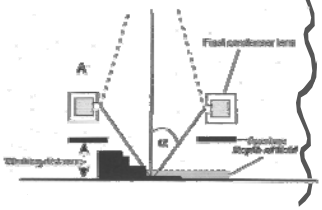
Focus vs. Working distance



Weak Lens:
Larger probe size, low resolution, long working distance, and larger depth of field

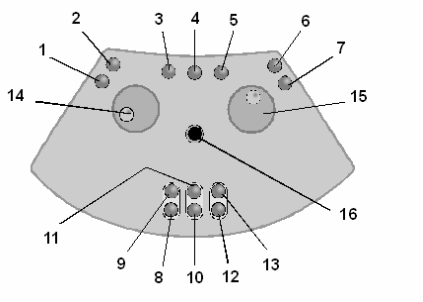


Strong Lens:
Small probe size, high resolution, short working distance and shallow depth of field



How To Focus

Press button (1). The button is lit. The left Knob (14) now changes focus (WD), the right Knob (15) changes magnification.



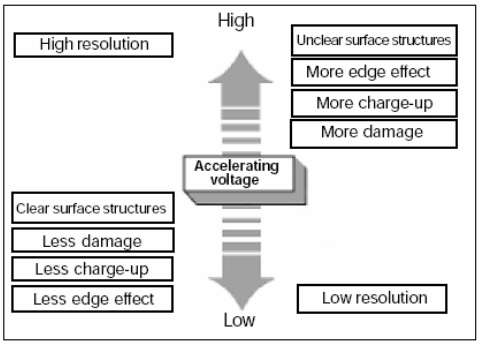
Scan Speed vs. Image Quality

- Fastest scan speed and low magnification are best settings to get your bearings on sample stage.

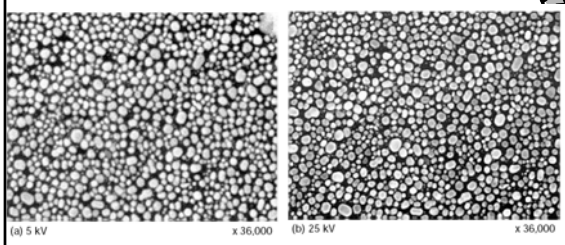
Faster scan: better Signal/Noises ratio with less detailed surface info.

Slower scan: better detailed surface info. but poor S/N ratio

How to Set Voltage

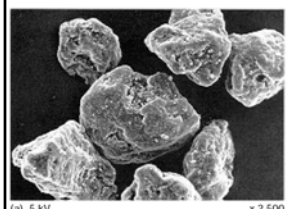


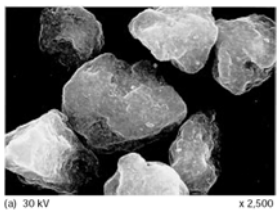
Voltage vs. Resolution



Higher Voltage ===== Higher Resolution

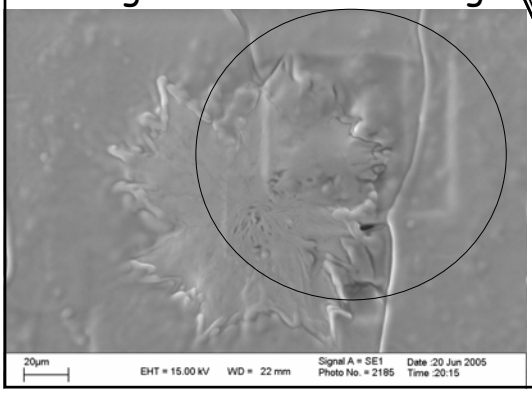
Voltage vs. Surface Info.



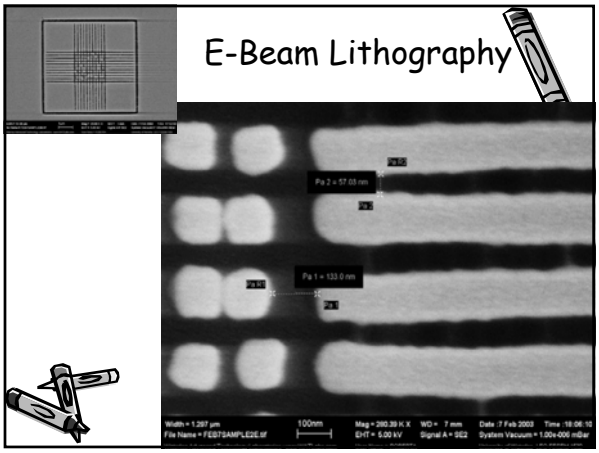


Higher Voltage === Deeper surface Info.

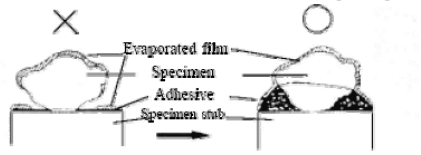
Voltage vs. Surface Damage



E-Beam Lithography

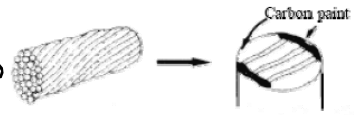


Specimens Req. vs. Charging



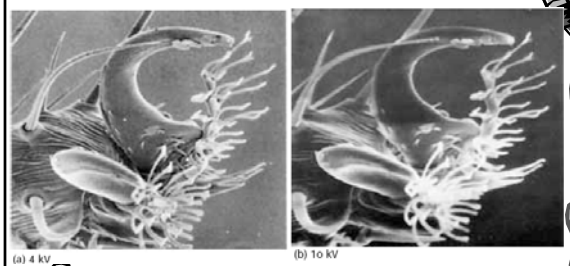
(b) Insufficient adhesive (a) Sufficient adhesive

Fixing of a bulk specimen



Fixing of fiber

Voltage vs. Charging



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

Powder Materials

Remove excessive particles

Example: Pollens

Spot Size vs. Resolution

High Resolution: 120 to 230

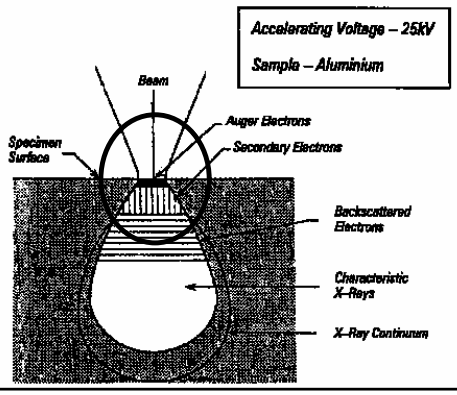
Backscattered electron imaging: 380 to 450

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.

Beam-specimen interaction volume Diagram



Astigmatism ?

What is astigmatism?

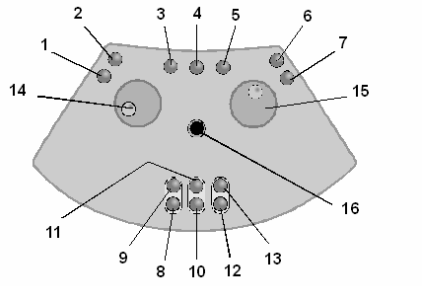
The aberration caused by the machining accuracy and material of the polepiece is called "astigmatism."

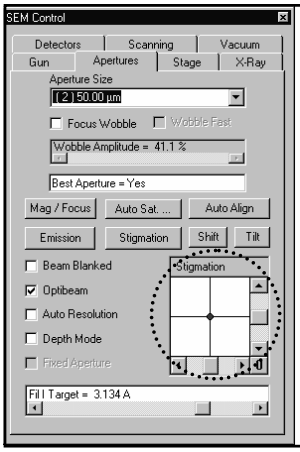
How to correct astigmatism?

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

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.



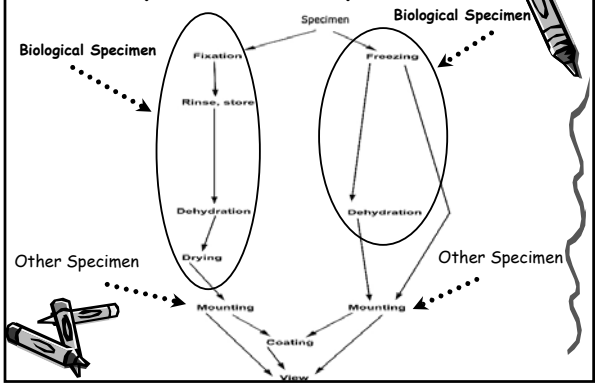


How To Reduce Astigmatism -- 2

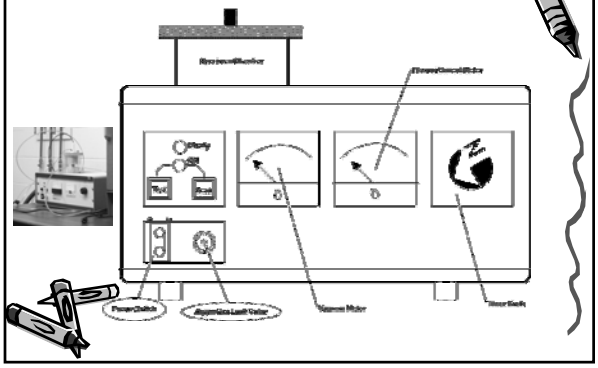
Image Defect Causes

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) Influence on penetration and charging	Wrong accelerating voltage

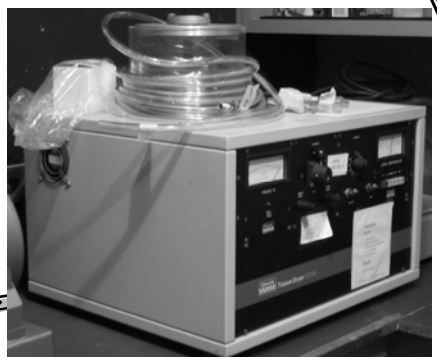
SEM specimen Preparations



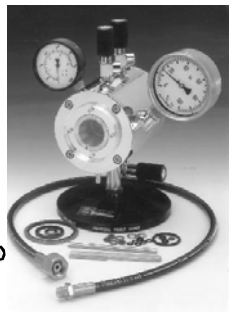
Coating -- Sputter Coater

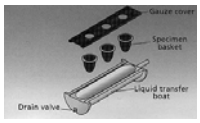


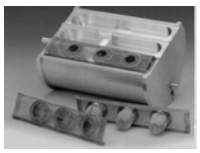
Biological Samples --Tissue Dryer



Biological Samples -- Critical Point Dryer (CPD)

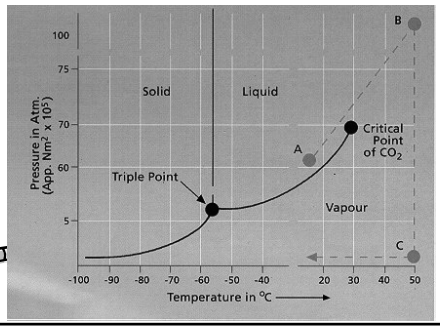






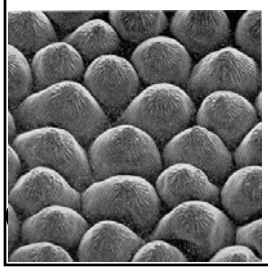
CPD: critical point of CO_2

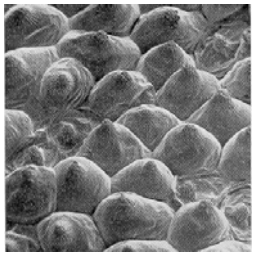
Purpose: To completely dry specimen for mounting while maintaining morphological details.



CPD v.s. Air Dry

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



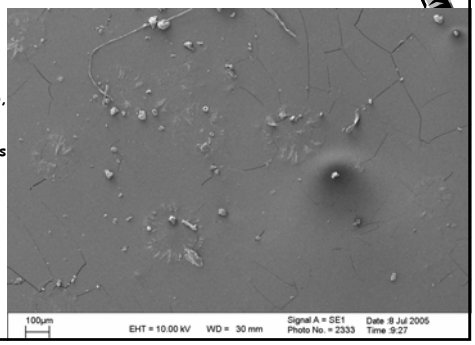


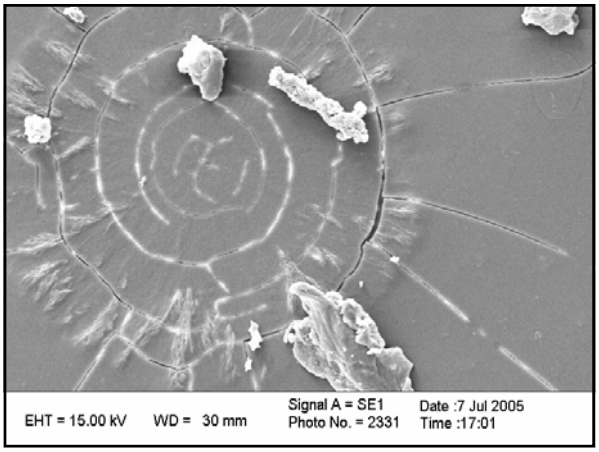
SEM image (850X) of rose petal surface, fixed and air dried

Specimen Preparations

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

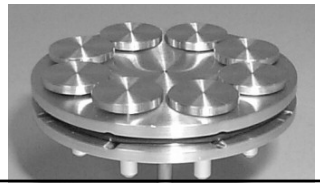
In even the most immaculate lab, dust is falling like rain, so keep your grids and specimens covered.

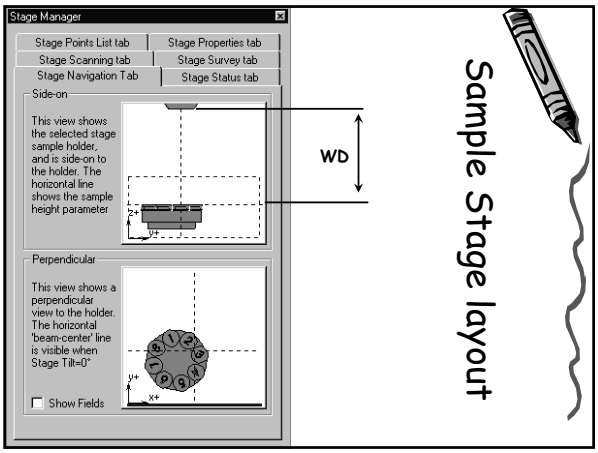


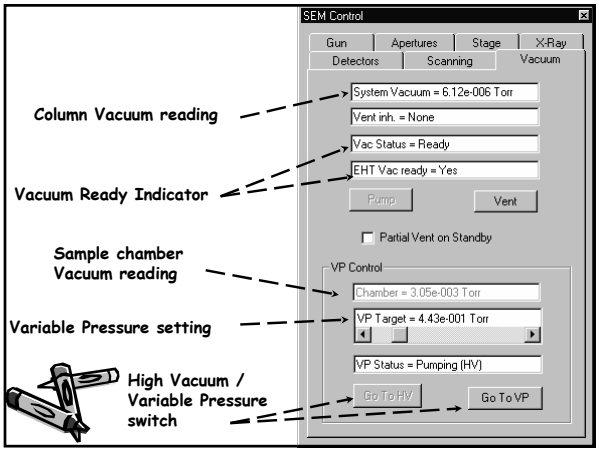


How to Load Specimens

- 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







Useful Commands

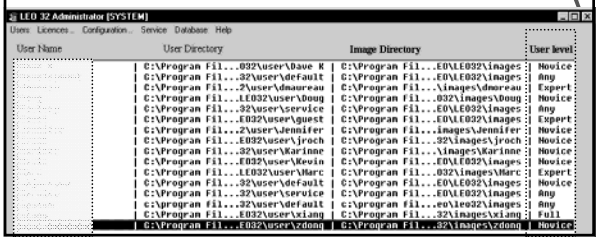
Key (s)	Function
Ctrl + s	Auto Astigmatism Correction
Ctrl + f	Auto Fine Focus
Ctrl + Shift + s	Auto Focus + Astig. Correction
Ctrl + d	Display/remove message bar
Ctrl + g/v	Open SEM control window
Ctrl + Alt + 1	Switch to SEM monitor
Ctrl + Alt + 2	Switch to EDS monitor

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

How to start SEM operation

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



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

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.

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

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

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

Bio-Specimen Prep. --TEM

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