Basic Components of All Microscopes That Use Lenses

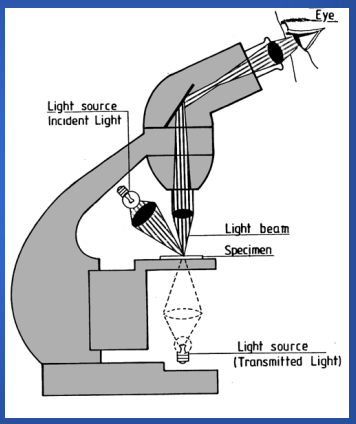
Illumination Source

Illumination Lens

Specimen

Magnifying Lenses

Detector/Viewer



Specimen Interactions, Signals & Detectors are intimately related, we will discuss these topics in parallel

1

Specimen Preparation

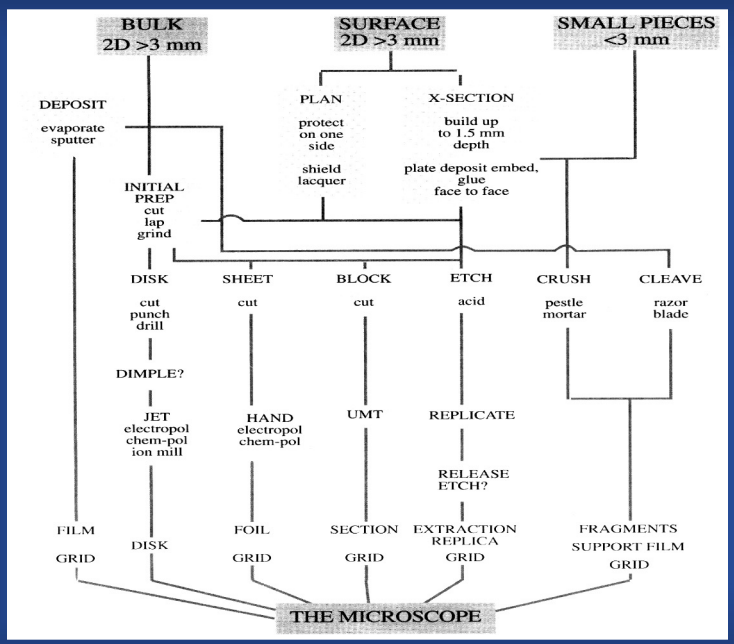
1. The TEM specimen must be electron transparent and representative of the material you want to study. In most cases you would like your specimen to be uniformly thin, stable under the electron beam and in the laboratory environment, conducting, and nonmagnetic.
2. In general we can divide specimens into two groups: self-supporting specimens and specimens resting on a support grid or thin washer; the grid is usually Cu but could be Au, Ni, Be.
3. Some specimens can be prepared by just using mortar and pestle to crush the specimen into tiny pieces and then suspend the small particles in a nonaqueous solvent, and then catch the particles on a carbon film TEM grid.
4. Some specimens have to be prepared by cutting the sample into thin slices using a diamond saw, then cutting 3-mm-diameter disks from the slice, thinning the disk on a grinding wheel, dimpling the thinned disk, then ion milling it to electron transparency.

2

Technology of specimen preparation

- Coarse preparation of samples:
  - Small objects (mounted on grids):
    - Strew
    - Spray
    - Cleave
    - Crush
  - Disc cutter (optionally mounted on grids)
  - Grinding device
- Intermediate preparation:
  - Dimple grinder
- Fine preparation:
  - Chemical polisher
  - Electropolisher
  - Ion thinning mill
    - PIMS: precision milling (using SEM on very small areas (1 X 1 μm^2)
    - PIPS: precision ion polishing (at 4° angle) removes surface roughness with minimum surface damage
    - Beam blockers may be needed to mask epoxy or easily etched areas
  - Focussed Ion Beam
- Each technique has its own disadvantages and potential artifacts

3

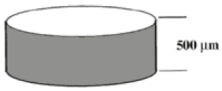


Williams & Carter, 1996, Fig. 10-3

4

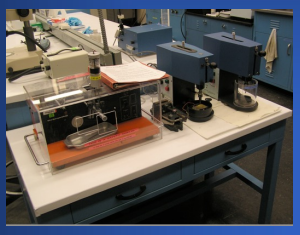
General Steps in TEM Spec. Prep

1. Disk Cutting



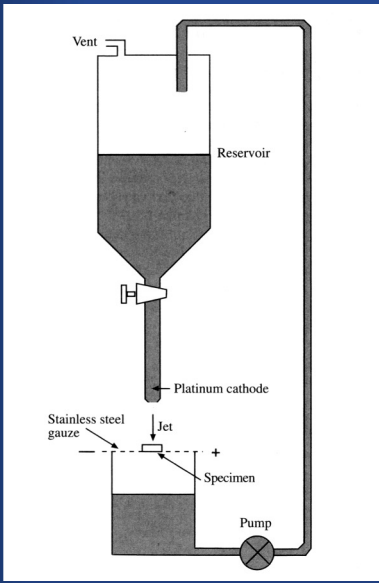
2. Disk Grinding

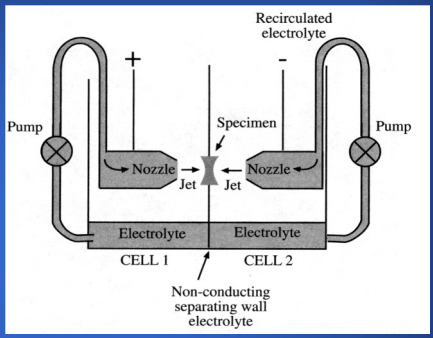
5



6

Gravity-fed & twin-jet electropolishing





Gravity-fed one surface electropolisher (left), which uses reservoir as cathode.

Twin-jet electropolisher uses specimen as conductor (above).

Williams & Carter, 1996, Fig. 10-7

7

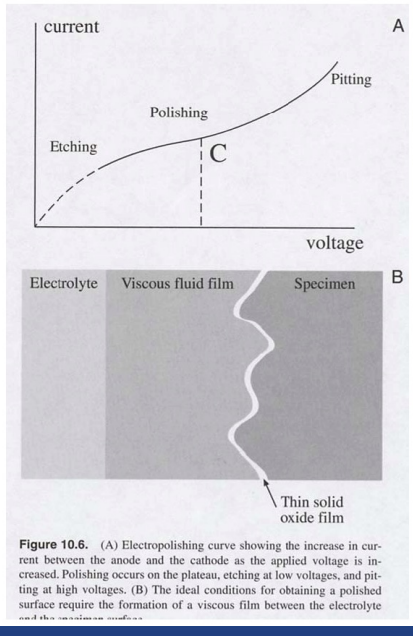
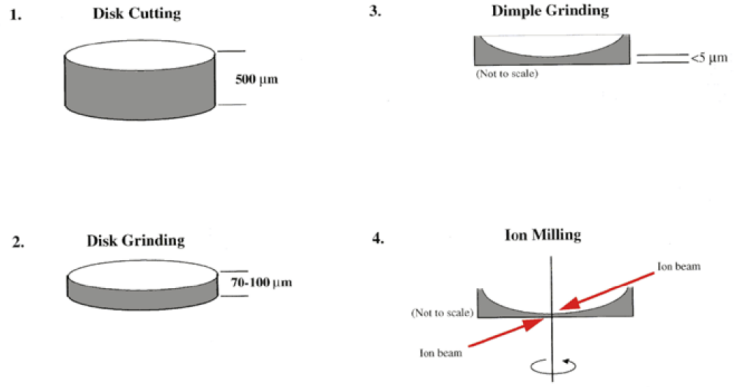


Figure 10.6. (A) Electropolishing curve showing the increase in current between the anode and the cathode as the applied voltage is increased. Polishing occurs on the plateau, etching at low voltages, and pitting at high voltages. (B) The ideal conditions for obtaining a polished surface require the formation of a viscous film between the electrolyte and the specimen surface.

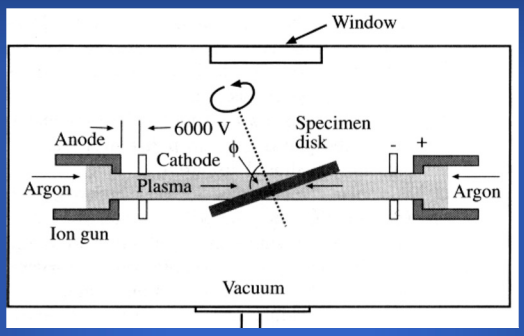
8

General Steps in TEM Spec. Prep



9

Ion mill schematic



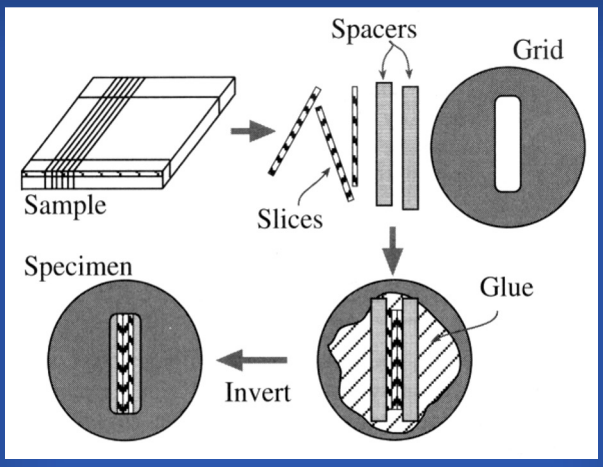
Schematic diagram of an ion-beam thinning device:

- Ar gas bleeds into the partial vacuum of ionization chamber
- 6 keV potential creates beam of Ar ions on rotating specimen
- Either one or both guns may be selected
- Rotation speed and angle may be altered
- Progress in thinning is viewed using a monocular microscope & back lighting.
- Specimen may be cooled to LN_2 temperatures.
- Perforation is detected by penetration of ions through specimen.

Williams & Carter, 1996, Fig. 10-8

10

Cross sectional views



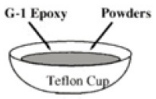
Cross sectional views of reasonably thin sliceable materials:

- Sheet sample is cut into slices and stacked with spacers placed to the outside
- Sandwiched materials are mounted in slot and glued together for support
- Material is observed in TEM

Williams & Carter, 1996, Fig. 10-12

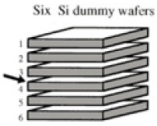
11

Cross-sectional technique



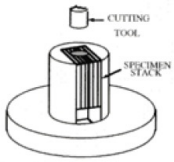
Mix G1 epoxy with powders and transfer mixture to gap between wafers 3 and 4

Step 1



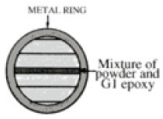
Coat other wafers with epoxy and cure glued stack under pressure

Step 2



Cut cylinder from stack and glue inside metal tube. Cure on hot plate

Step 3

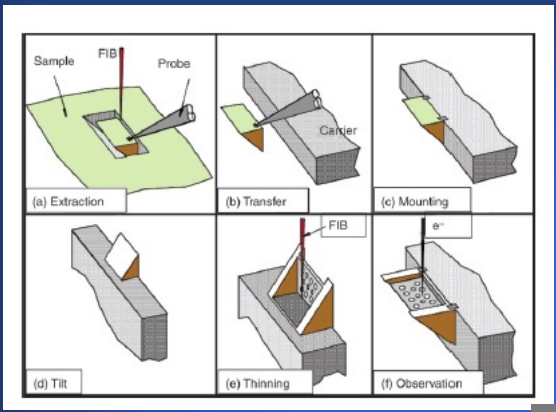


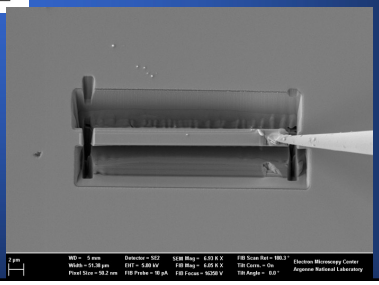
Cut reinforced specimen disks for disk grinding and dimpling

Step 4

12

FIB TEM Prep





13

Overview of Biological Specimen Preparation

Killing & Fixation
- Death; Molecular stabilization

Dehydration
- Chemical removal of H_2O

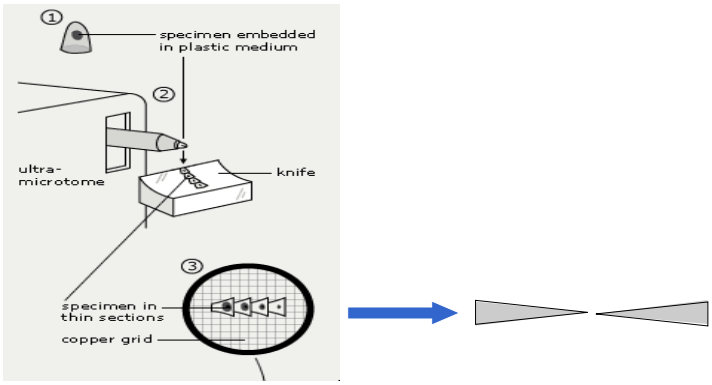
Infiltration
- Replace liquid phase with resin

Embedding & Polymerization
- Make solid, sectionable block

Sectioning
- Ultramicrotome, mount, stain

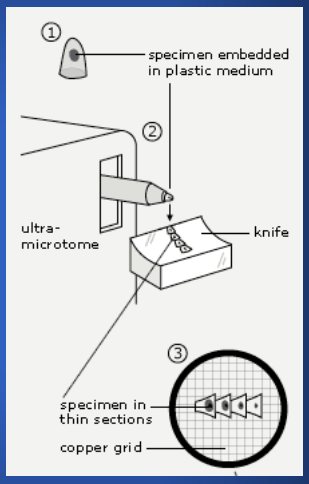
14

General Steps in TEM Spec. Prep



15

Ultramicrotome



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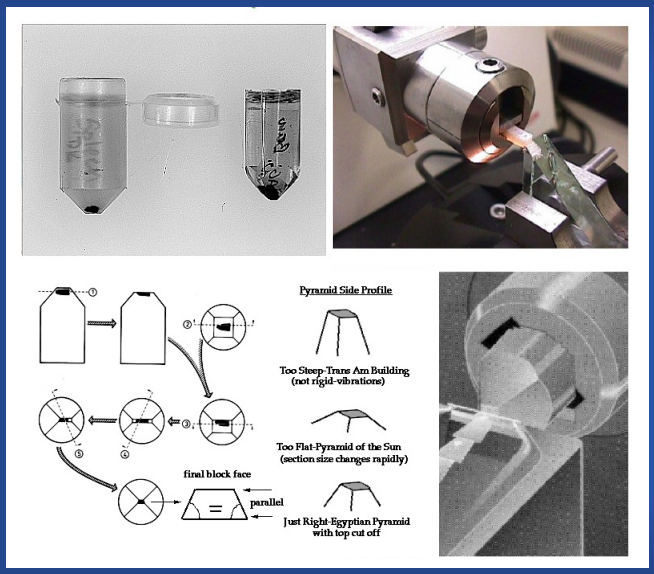
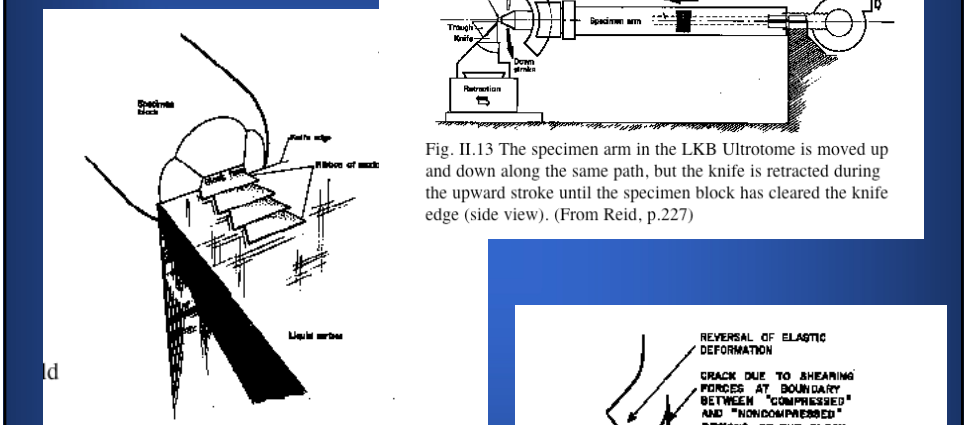

Pyramid Side Profile
Too Steep-Trans Am Building
(not rigid-vibrations)
Too Flat-Pyramid of the Sun
(section size changes rapidly)
final block face
parallel
Just Right-Egyptian Pyramid
with top cut off

17


Specimen block
Return stroke
Trough
Knife
Down stroke
Retraction
Specimen arm
Fig. II.13 The specimen arm in the LKB Ultrotome is moved up and down along the same path, but the knife is retracted during the upward stroke until the specimen block has cleared the knife edge (side view). (From Reid, p.227)
Fig. II.27 Sections adhering together in ribbon formation while floating on a liquid surface. (From Reid, p.222)
REVERSAL OF ELASTIC DEFORMATION
CRACK DUE TO SHEARING FORCES AT BOUNDARY BETWEEN "COMPRESSED" AND "NONCOMPRESSED" REGIONS OF THE BLOCK

18

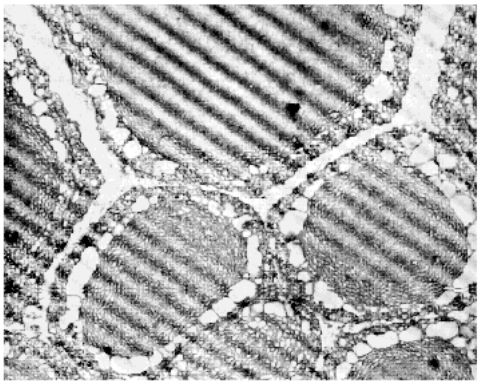
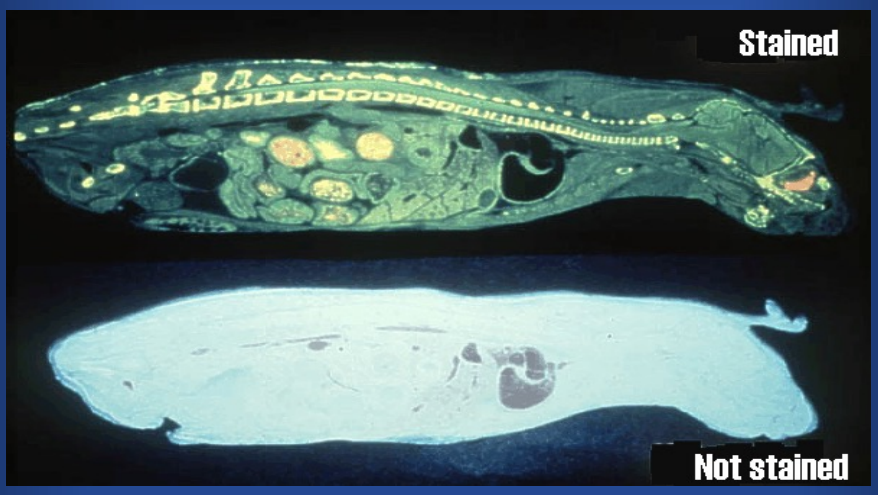


Fig. II.38. Periodic variation of section thickness - "chatter" - due to vibrations of the block tip caused by the impact of the knife on the block tip. (From Sjostrand, p.235)

19



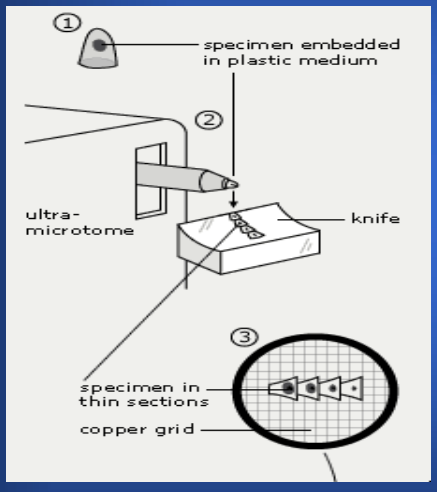
20

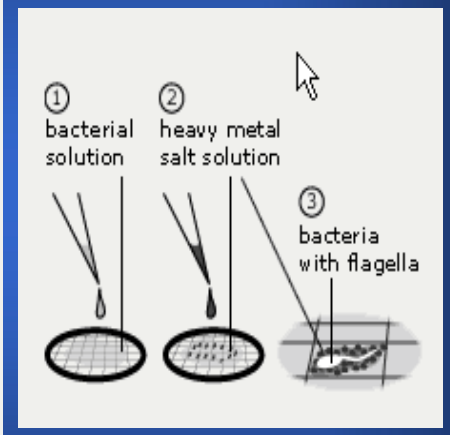
Biological Specimen Prep Staining

Positive staining involves treatment of the specimen with a chemical that increases the weight density.

Contrast enhancement by positive staining involves a direct interaction of a stain material with the protein

21





22

Biological Specimen Prep
Staining

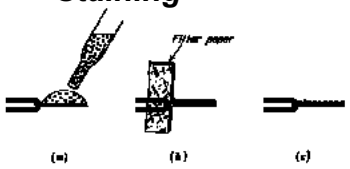
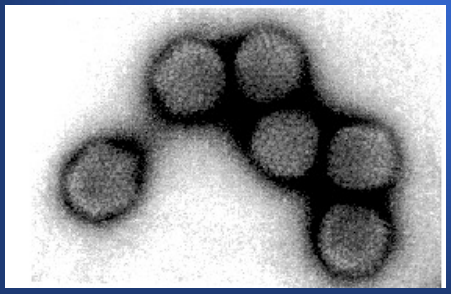


Fig. II.42. Preparation of a specimen from particles in aqueous suspension. (From Hall, p.290)

Fig. II.43. Washing a specimen. (From Hall, p.290)



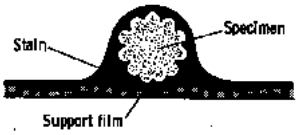


Fig. II.39. Schematic representation of a specimen particle completely embedded in a negative stain. (From Hayat and Miller, p.2)

23

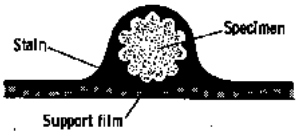


Fig. II.39. Schematic representation of a specimen particle completely embedded in a negative stain. (From Hayat and Miller, p.2)

The main purpose of negative-staining is to surround or embed the biological object in a suitable electron dense material which provides high contrast and good preservation (Fig. II.39). This method is capable of providing information about structural details often finer than those visible in thin sections, replicas, or shadowed specimens. In addition to the possibility of obtaining a spectacular enhancement of contrast, negative-staining has the advantage of speed and simplicity.

The technique has mainly been used to examine particulate (purified) specimens - e.g.. ribosomes, enzyme molecules, viruses, bacteriophages, microtubules, actin filaments, etc. at a resolution of 1.5-2.5 nm. This technique generally allows the shape, size, and the surface structure of the object to be studied as well as provide information about subunit stoichiometries and symmetry in oligomeric complexes. Any surface of the specimen accessible to water can potentially be stained, and thus, that part of the specimen will be imaged at high contrast.

24

1

Reactive Gas Plasma Specimen Processing for Use in Microanalysis and Imaging in Analytical Electron Microscopy

25

2

Introduction

- *Microstructural observations are not sufficient to characterize all the features which are encountered during characterization of materials. Using a combination of analytical spectroscopies such as XEDS, and EELS we can gain additional insight into the factors controlling or affecting materials properties beyond that which can be determined using standard imaging tools.*

- *During these analytical studies focussed probes are frequently employed to determine local compositions, however, subtle processes which involve the specimen, the electron beam and any mobile species on the sample surface frequently cause the build up of hydrocarbon contamination layers.*

26

3

Background

- *While serving to indicate the location of the electron probe, the contamination obliterates the area of the specimen being analyzed and adversely affects all quantitative microanalysis methodologies.*
- *A variety of methods including: UV, electron beam flooding, heating and/or cooling can decrease the rate of contamination, however, none of these methods directly attack the source of specimen borne contamination. (see reference 1)*
- *Research has shown that reactive gas plasmas may be used to clean both the specimen and stage for AEM, in this study we report on quantitative measurements of the reduction in contamination rates in an AEM as a function of operating conditions and plasma gases. (reference 2)*

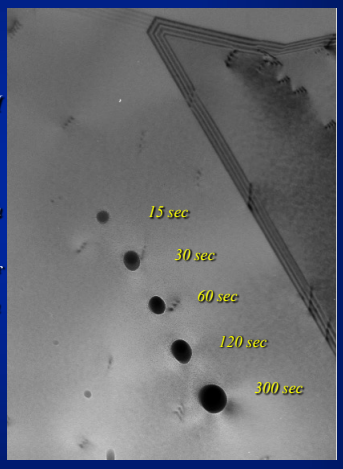
27

4

Reactive Gas Plasma Processing Applications to Analytical Electron Microscopy

Example:

- *The figure at the right shows the results of contamination formed when a 300 kV probe is focussed on the surface of a freshly electropolished 304 SS TEM specimen.*
- *The dark deposits mainly consist of hydrocarbons which diffuse across the surface of the specimen to the immediate vicinity of the electron probe. The amount of the contamination is a function of the time spent at each location. Here the time was varied from 15 - 300 seconds.*



28

5

Experimental

•*TEM specimens*

Electropolished 304 Stainless Steel
Chemically polished Silicon
Crushed $CaZrTiO_3$ on Holey Carbon Film
Si/Cr/Au Multilayer Ion-Milled

•*Microscopy*

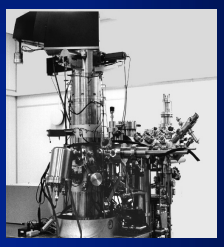
Philips CM30T at ANL Materials Science Div.
300 kV, LaB6 Gun, 20 nm/0.7 nA probe
RT DT Be Stage, LN_2 Cold Trap Used
EDAX PowerMX - XEDS System
Gatan 666 PEELS System

ANL-VG HB603Z AAEM
300 kV, CFEG, 1nm/1nA probe
RT DT Be Stage, No LN_2 Cold Traps
Oxford/Link XEDS System
VG EELS system

•*Plasma Cleaning System*

Model : PC-150 South Bay Technology
Power: 10 W, Gas Pressure 200 mT.
Gases: nominally pure Argon & Oxygen
mixed as needed in Model 150
Pumping: Conventional mechanical
roughing pump

Philips CM30T



VG HB 603Z

SBT PC 150

29

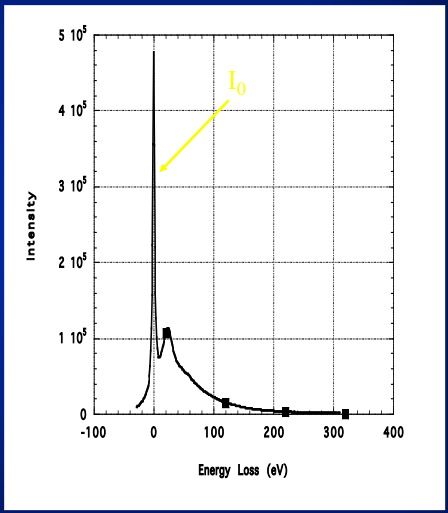
6

Experimental

- To measure the rate of contamination we employed electron energy loss spectroscopy (EELS) and monitored the rate of change of the intensity of the zero loss (I_0) to the total integrated intensity in the spectrum (I_T).
- This ratio is directly proportional to the local thickness of the specimen.

$$t = \lambda * \ln (I_0 / I_T)$$

λ = mean free path

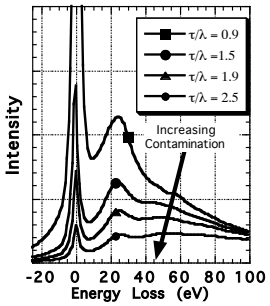


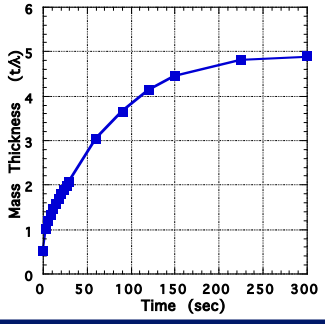
30

7

Data Analysis

- Individual Electron Energy Loss Spectra are measured as a function of time
- Spectra are then individually analyzed and the value of t/λ is determined.
- The instantaneous contamination rate is given by $\delta (t/\lambda)/\delta T$



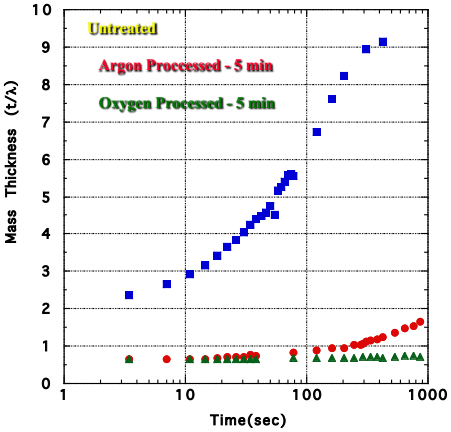


31

8

Results from Electropolished 304 SS

- Untreated Specimens exhibit severe contamination
- Argon gas processing for 5 minutes @ 10 W/200 mT reduces the contamination rate to less than 1/50 th of the untreated sample.
- Additional treatment of sample with pure Oxygen (5 minutes) reduces the contamination rate further to less than 1/ 500 th of the untreated sample.



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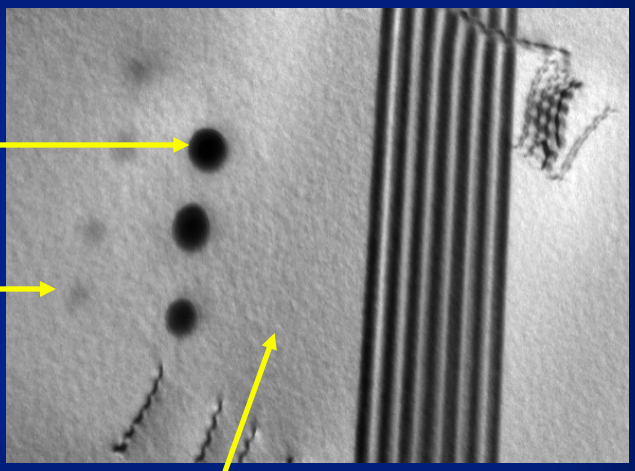
9

Comparision Results on Electropolished 304 SS

•Untreated Specimen

•After 5 minutes Argon Processing

•After 5 minutes of additional Oxygen Processing



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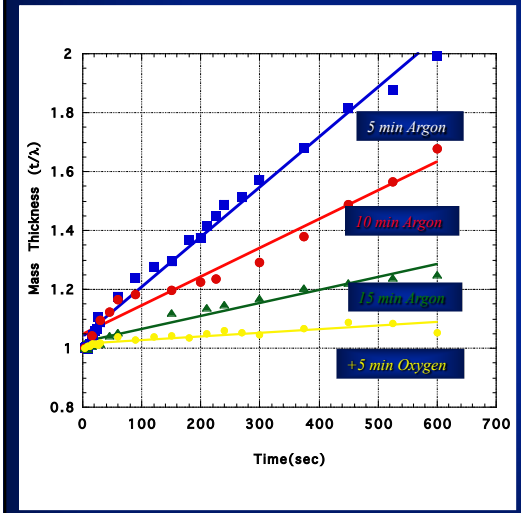
10

Results from Electropolished 304 SS



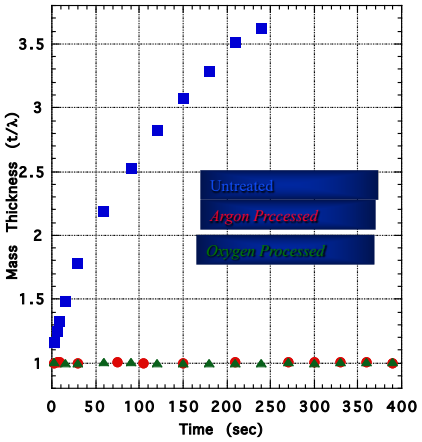
- Successive 5 minute processing of the same specimen with Argon continuously reduces the contamination rate but does not completely eliminate the problem
- A final 5 minute treatement in pure Oxygen always reduced the rate to lower levels. Regardless of the length of time of Argon processing

34

11

Results from Chemically Polished Silicon

- Initial Contamination rates of Silicon are less than 304SS
- Argon alone is very efficient in Silicon
- Oxygen has a small but measurable effect and always reduces the contamination rate, however, the difference is much less than in 304 SS



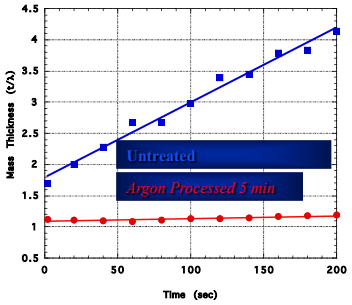
35

12

Results from Crushed Zirconolite on Holey Carbon

- Contamination of the Zirconolite is due to suspension of crushed mineral in solvents. A "drop" of the crushed mineral is then deposited on the H.C. film to make the sample. This leave organic residue on the sample and the Holey Carbon film.
- Argon treatment greatly reduces the contamination rate, a final treatment in pure Oxygen further decreases the problem.

Crushed Zirconolite on Holey Carbon

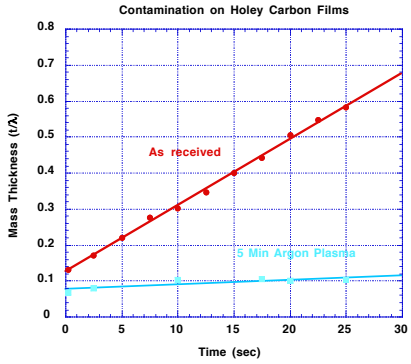


36

13

Results from Holey Carbon Films

- Contamination of theHoley Carbon is due to suspension of crushed mineral in solvents. A "drop" of the crushed mineral is then deposited on the H.C. film to make the sample. This leave organic residue on the sample and the Holey Carbon film.
- Long processing (~ 15minutes) can effect the Holey Carbon support film and should be avoided.



37

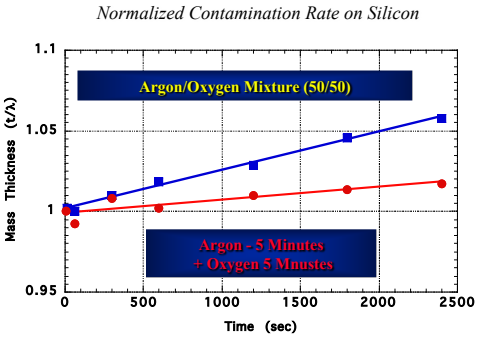
14

Gas Mixing Results

- In all cases tested the most effective cleaning occured when a two step process was carried out.

  5 Min pure Argon followed by
  5 Min pure Oxygen

- This was more effective and reduced the contamination rate more than using a Ar/O_2 mixture (50/50)

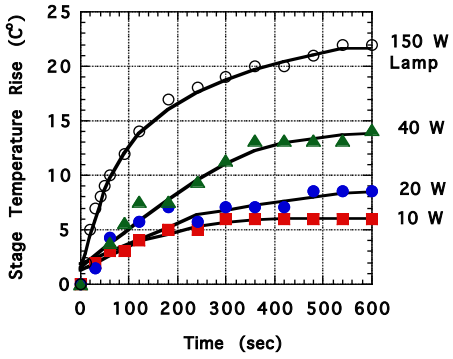


38

15

Heating Effects of the Plasma

- Using a conventional thermocouple in an AEM stage, the temperature rise of a SS sample and stage was measured as a function of input power to the plasma.
- Compared to a 150W flood lamp the increase in temperature is insignificant ~ 5-6 C° for the typical conditions used for cleaning (10 W @ 5 min).

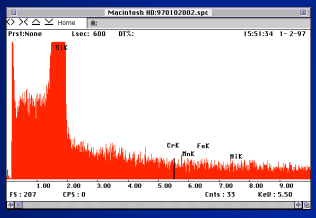


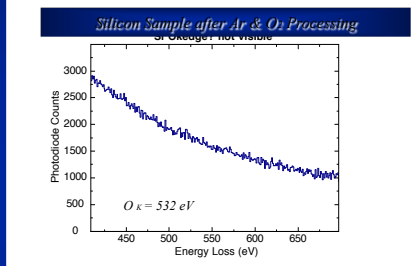
39

16

Analytical Results

Silicon Sample after Ar & O₂ Processing





•Using XEDS & EELS in the AEM no measurable redeposition of plasma chamber materials or oxide formation was observed on the Silicon or SS samples.

•**Improperly setting DC bias will sputter material off the r.f. antenna. (reference 3)**

40