TEM Sample preparation

Sample has to be electron transparent
(also stable at e-beam and vacuum..)

Max. Diameter 3 mm

Strong interaction → very thin samples

~ 10 nm for HRTEM
~ 10 – 50 nm for EELS, quantitative EDS
(thicker samples have multiple scattering..)

Soft materials: we typically use approximately 50-150 nm

- Higher the voltage – the thicker the sample can be

- But thick samples have lower resolution (chromatic aberration) and EELS-spectroscopy is impossible with thick samples (multiple scattering)

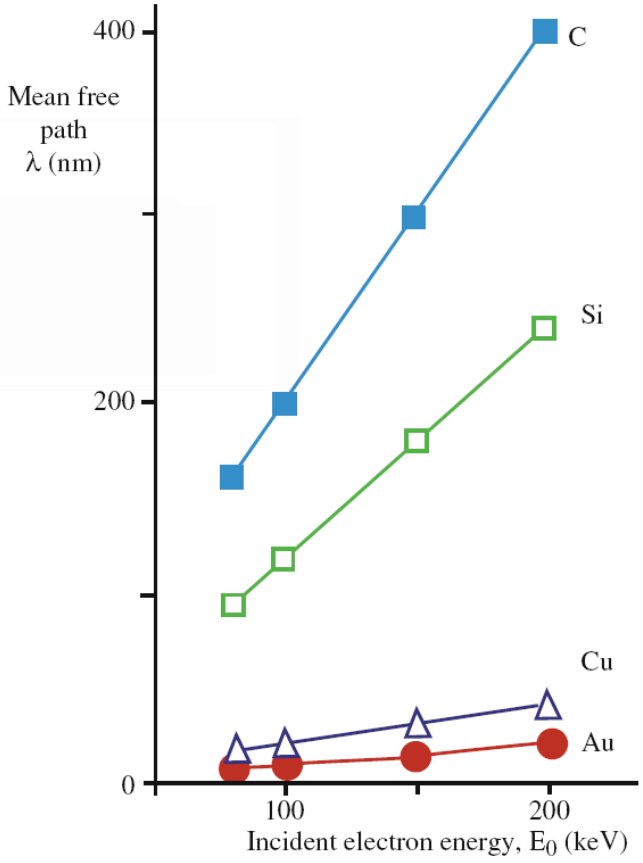


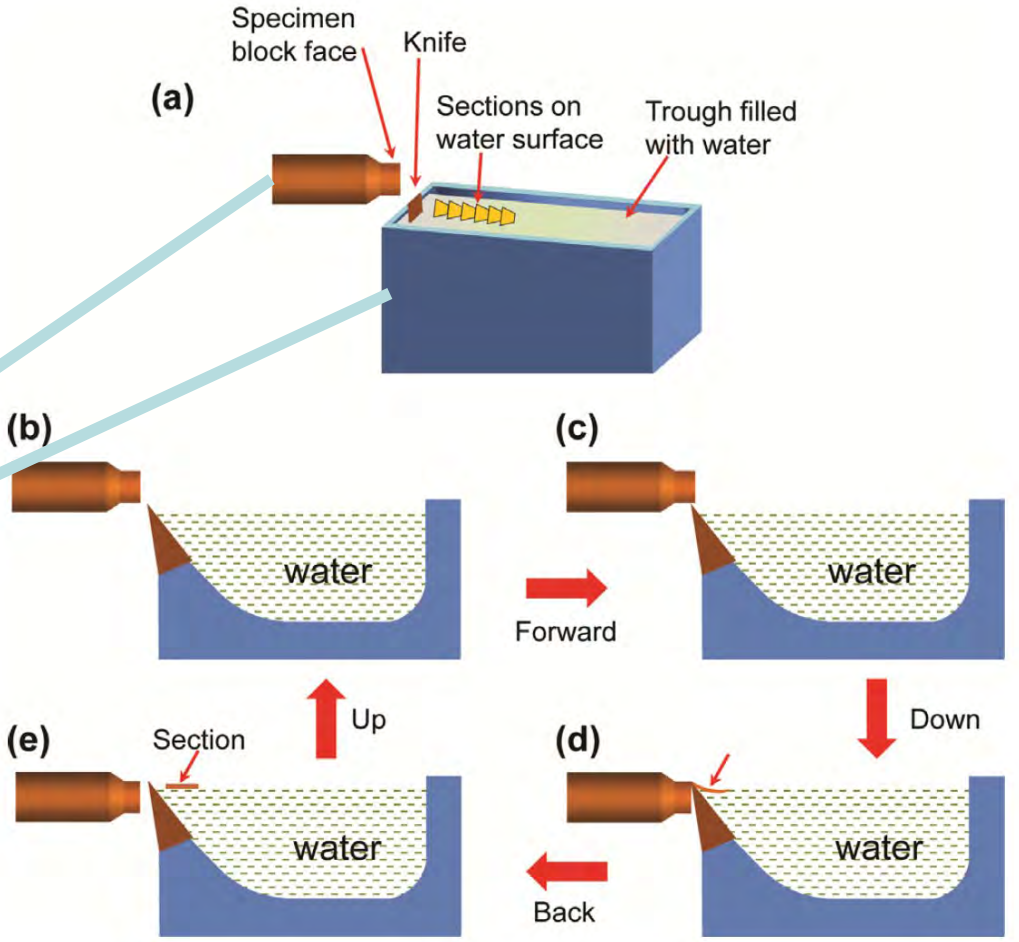
FIGURE 3.4. The variation of the mean free paths of elastic scattering for four different elements as a function of the beam energy, calculated assuming a screened, relativistic Rutherford cross section.

Sample preparation

- 1. Ultramicrotomy (RT and Cryo) – Soft materials
- 2. Free standing thin films, solution cast films, Dispersions
- 3. Replica, Freeze fracture, Etching
- 4. Cleaving etc..
- 5. Polishing and ion milling (Hard materials, metals etc.)
- 6. Focused ion beam (FIB) (Lide FIB lecture)
- 7. Cryo vitrification... (Cryo lecture)

Sample preparation 1.

Ultramicrotome



A diamond knife is attached to a trough, which is filled with deionized (DI) water (a). On the microtome instrument, the knife is at the fixed position, whereas the sample moves up and down, and also forth and back to produce thin sections. The procedure details are shown in Fig. (b–e). Staring from the position in Fig. (b), the block moves forward (the thickness of the thin section), so that it reaches above the blade (Fig. c). It moves down so that a section is made, and the section is floated on the water surface (Fig. d). Then the block moves backward to reach the position in Fig. (e). When it moves up, it reaches the position in Fig. (b) for the next cycle.

Sample Preparations: making thin films – ultramicrotomy

1. Embedding

For easier handling small samples are typically embedded inside the epoxy resin. (Large samples can be cut without epoxy..) Also cryo cutting is quite often done without embedding.

Also Porous samples needs extra support, and water containing samples – water can be replaced by epoxy (→ethanol → aceton → Epoxy ...)

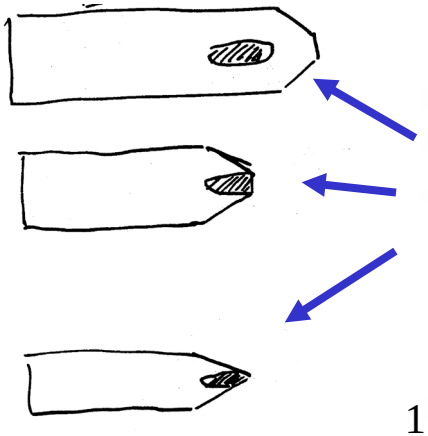
2. Trimming

3. Cutting: Diamond knife or (Glass knife)

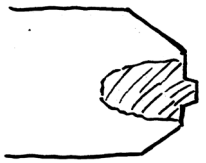
Room temperature or Cryo

Trimming

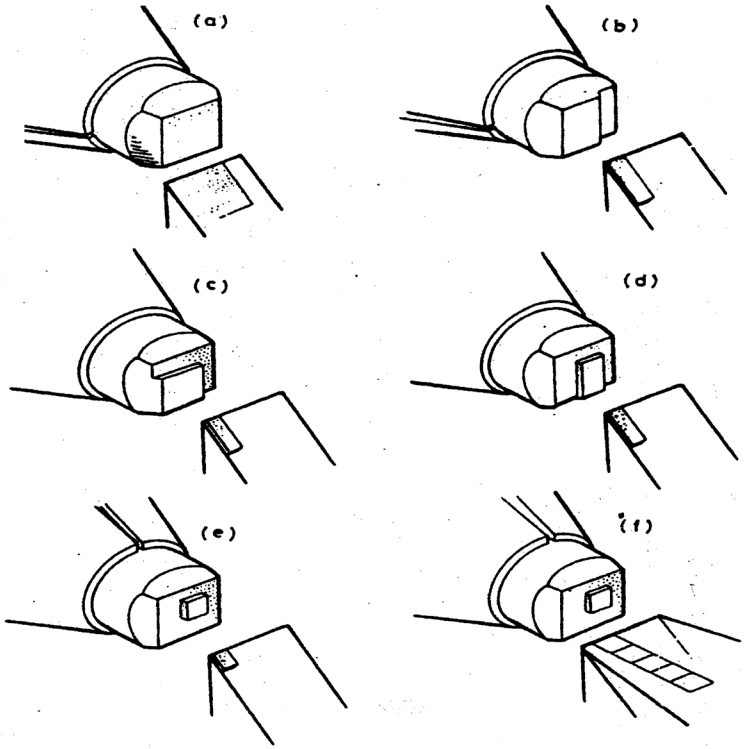
Epoxy block from
Side view



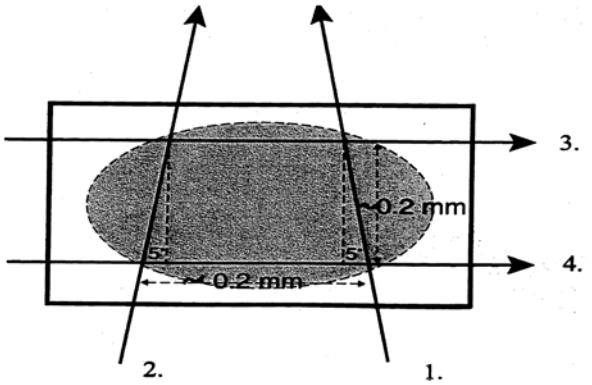
1. Milling machine trimming



2. By using
trimming knife

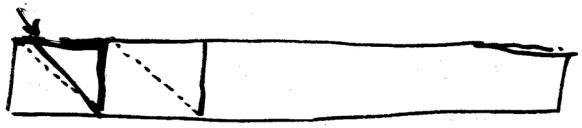


In room temperature: block is trimmed usually in the shape of trapezoid (5 degrees). Typical size approx. 0.1 - 0.2 mm.
From small trapezoid it is easier to cut thin sections.



Diamond vs. Glass knife

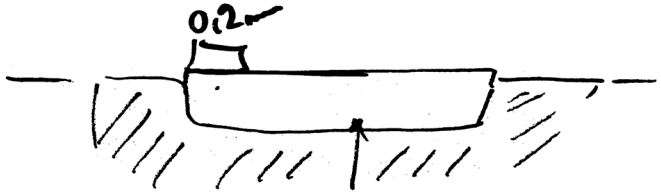
Glass knife:



Is made but splitting glass bar…
Inexpensive, sharp (edge radius < 2 nm
can be almost atomistic sharp)
Wears easily (5-15 cuts)

Room Temperature knife

Diamond knife:



Diamond typically 2mm wide

Hardest material
Relatively expensive (2 mm ~2000 €)
but if well handled can be used years

Cryo (dry) knife

Cryo (wet) knife

Table 10.1. Comparison of dry and wet techniques for section collection

	Dry sectioning	Wet sectioning
Pro	– Specimen and knife have identical temperatures – No effect of floating liquid	– Easy collection of sections – Less section compression – Low electrostatic charging – Knife-friendly
Contra	– Electrostatic charging – Difficulty in collecting sections – High compression	– Reaction of floating liquid with sample is possible – Sample and knife temperature may vary during cryosectioning

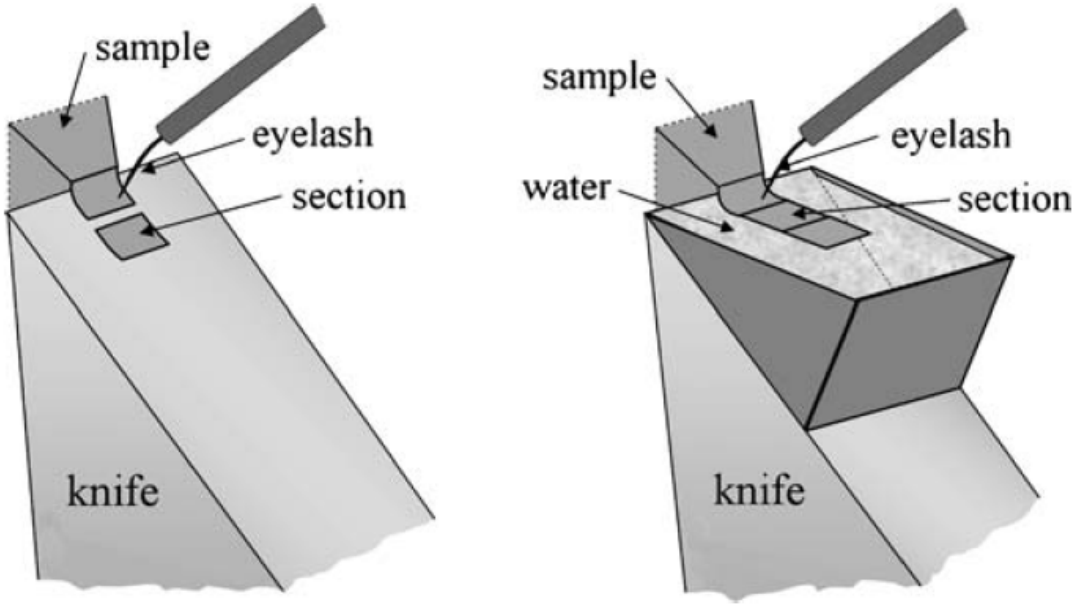
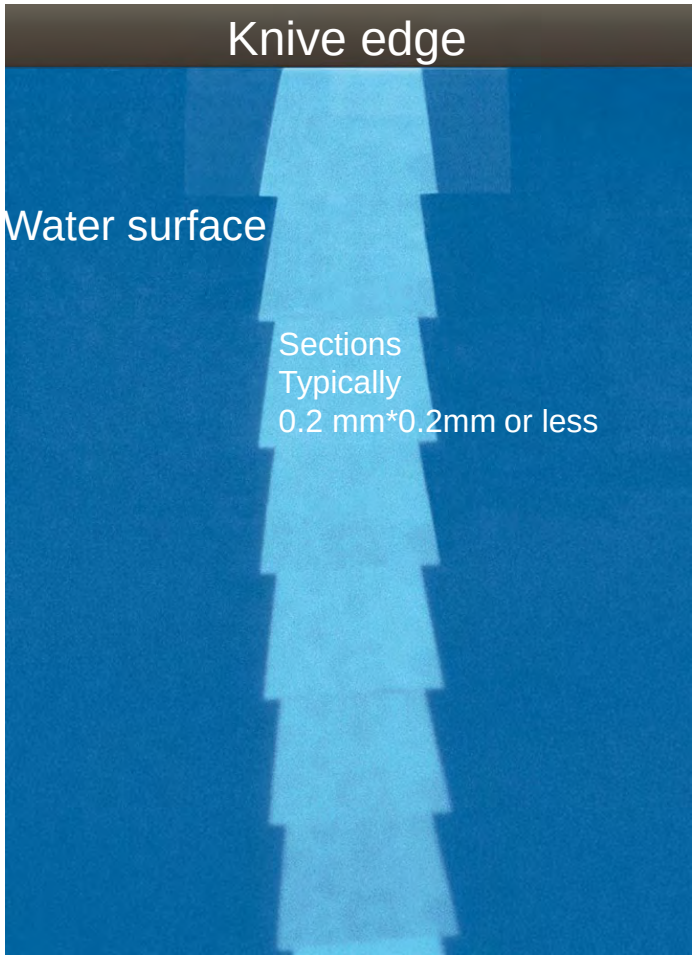


Fig. 10.8. Controlling the paths of the sections with the aid of an eyelash during dry and wet sectioning

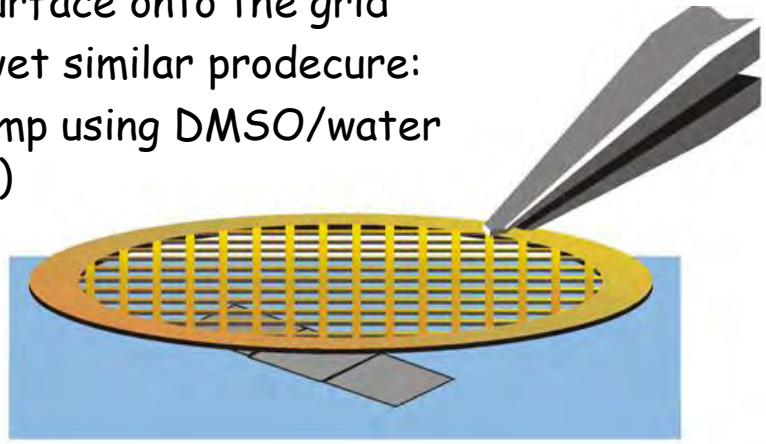
Room temperature cutting materials embedded in epoxy, section will float top of the water surface

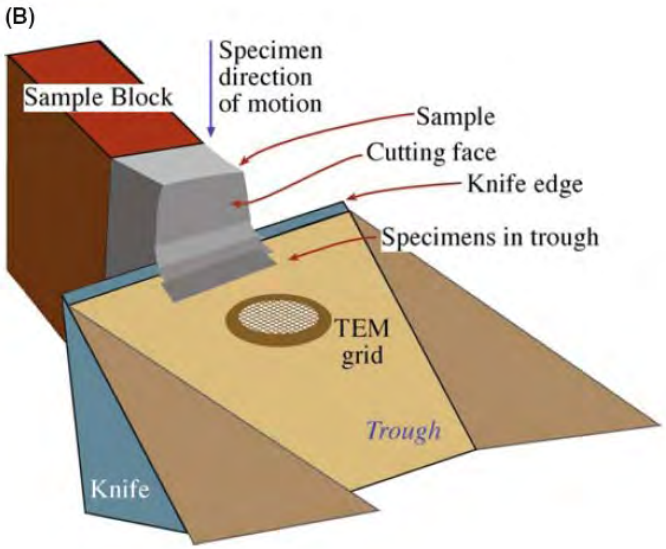


section collection directly from the water surface onto the grid

(also Cryo-wet similar prodecure:

Minimum temp using DMSO/water ~ -45 °C)





Cryo ultramicrotomy

Many polymers are too soft for room temperature cutting... (glass transition temperature below the room temperature)

Epoxy embedding is not normally needed (→ benefit: no possibility to get side reaction between the epoxy and sample - which is quite usual when embedding soft materials)

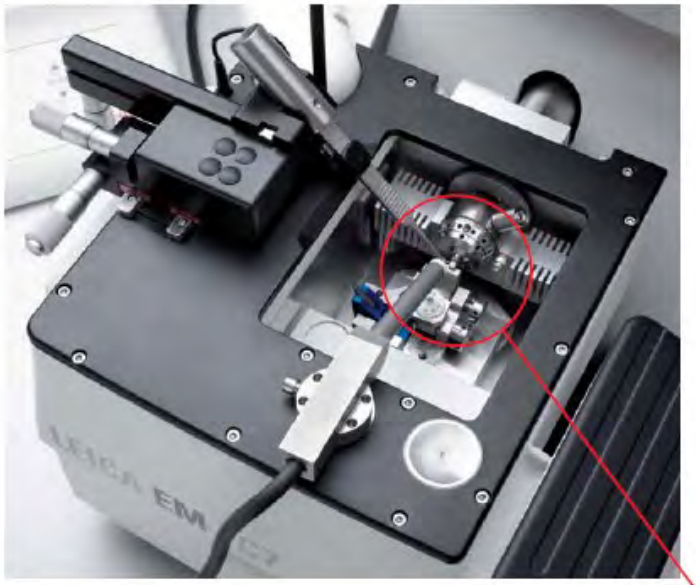
Cryo wet at -20 - -50^{o}C "easy" DMSO/Water mixture can be used as a liquid

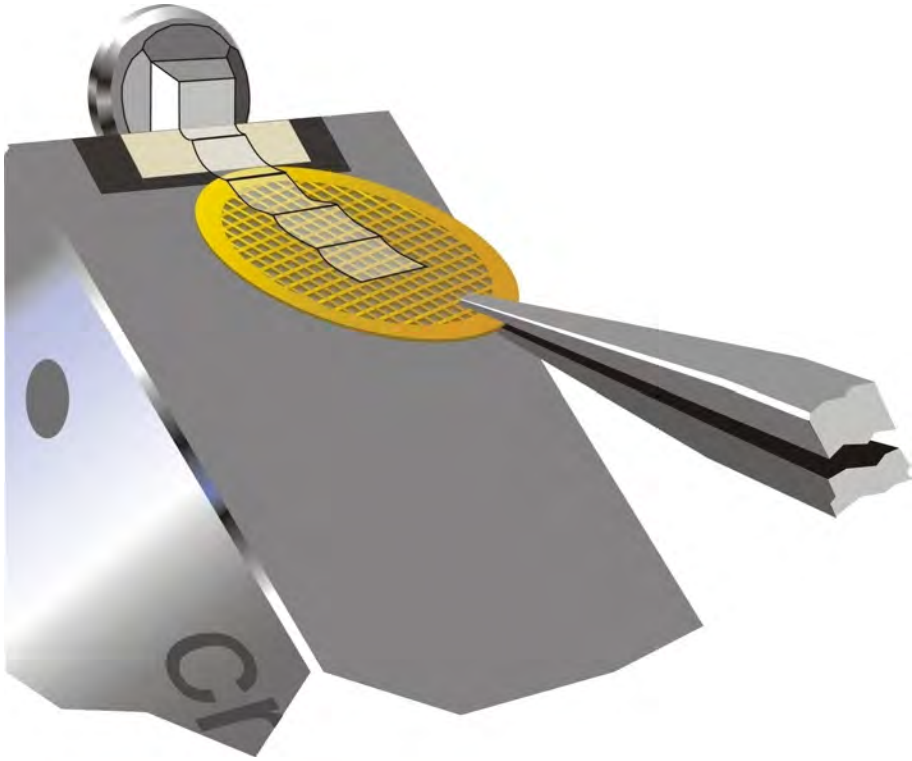
With glass knife – also other solvents possible (example. propanol -90 ^{o}C)

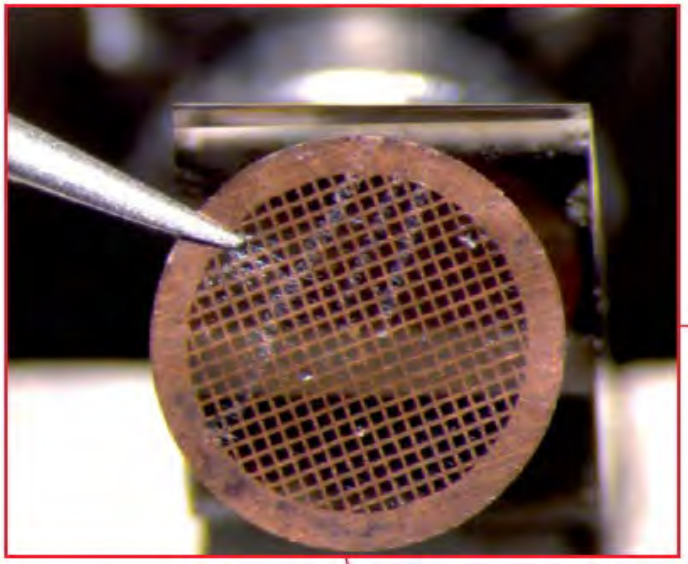
•Diamond knife: other than DMSO/water mixture is not recommend (epoxy seal which is holding the diamond may be destroyed...)

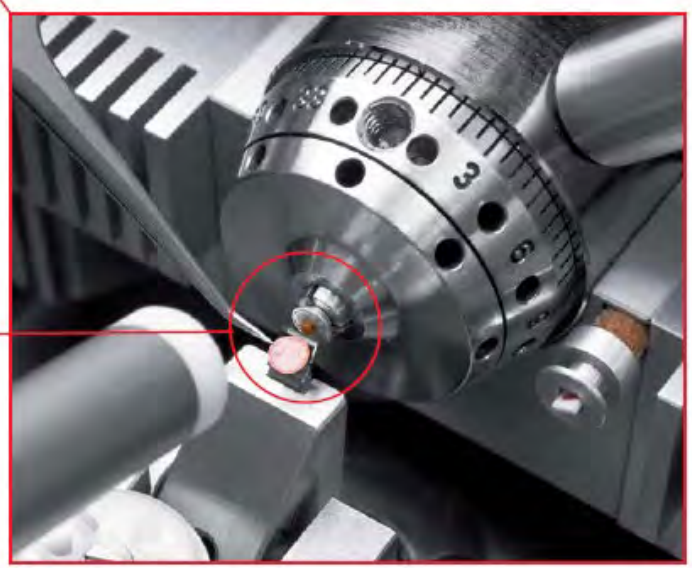
•**Cryo dry** (typically -40 ^{o}C to -150 ^{o}C)

Dry or cryo sectioning and section pick-up and direct mounting on grid









Cryo-section pick-up: two methods

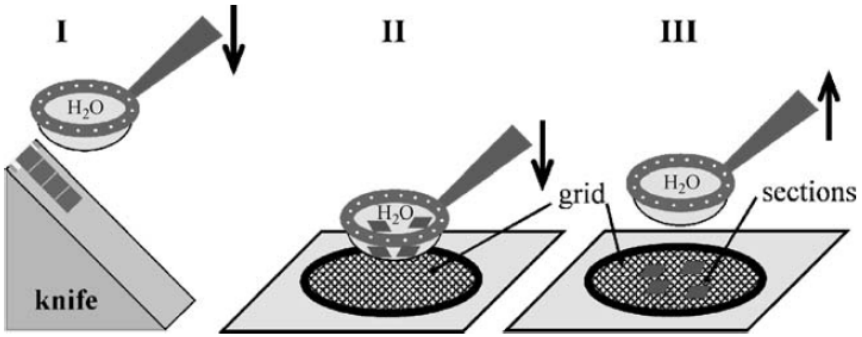


Fig. 10.12. Collecting sections during dry sectioning using a drop of water in a loop

Table 10.3. Advantages and disadvantages of using distilled water or sugar solution for to fish out sections during dry sectioning

	Sugar solution	Distilled water
Pro	– Can be used while sectioning at both room temperature and cryotemperatures	– Can be used during room-temperature sectioning
Contra	– Rapid working is essential, otherwise the drops become frozen – Sugar solution should be removed. The grids containing the sections are washed carefully with distilled water and then dried	– Cannot be used at cryotemperatures

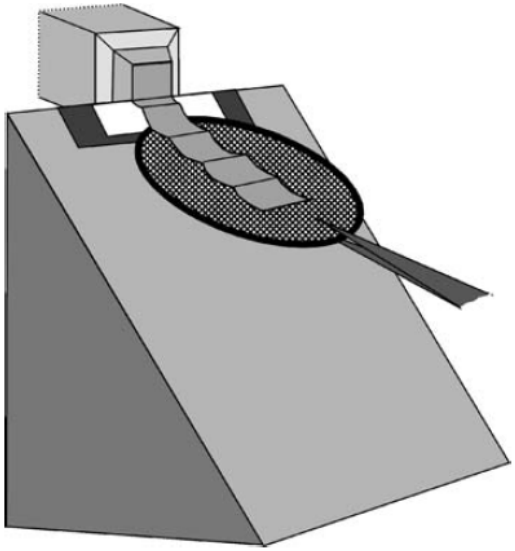


Fig. 10.13. Direct transfer of a series of sections onto a grid

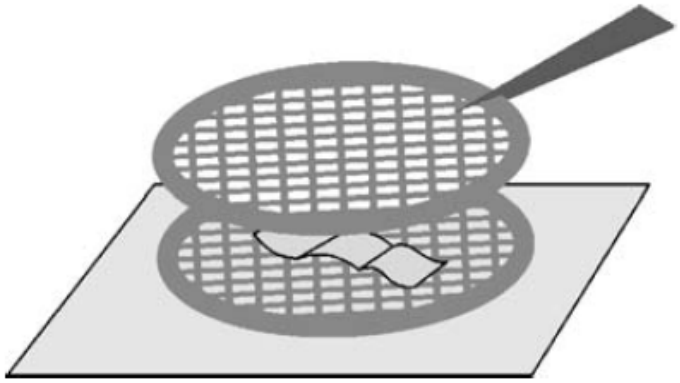


Fig. 10.14. Fixation of sections with the aid of a second grid

General microtoming – RT and Cryo

- https://youtu.be/es1sOxMPELo

Cutting soft metals (Aluminum)

- https://youtu.be/p9vaiXq8T7Y

Grids....

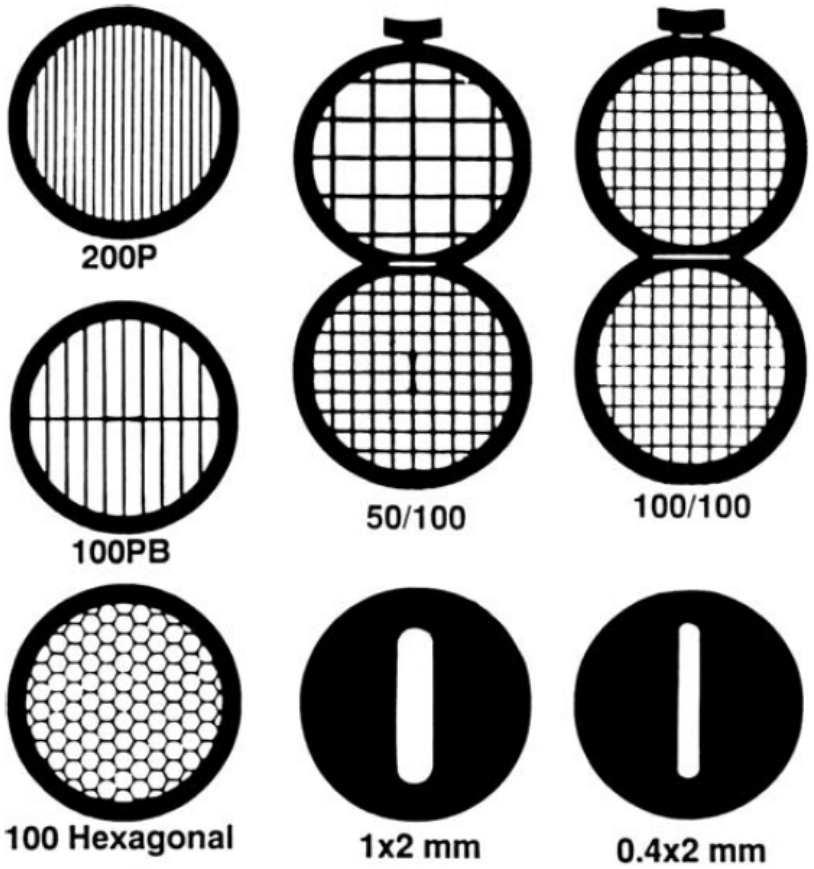
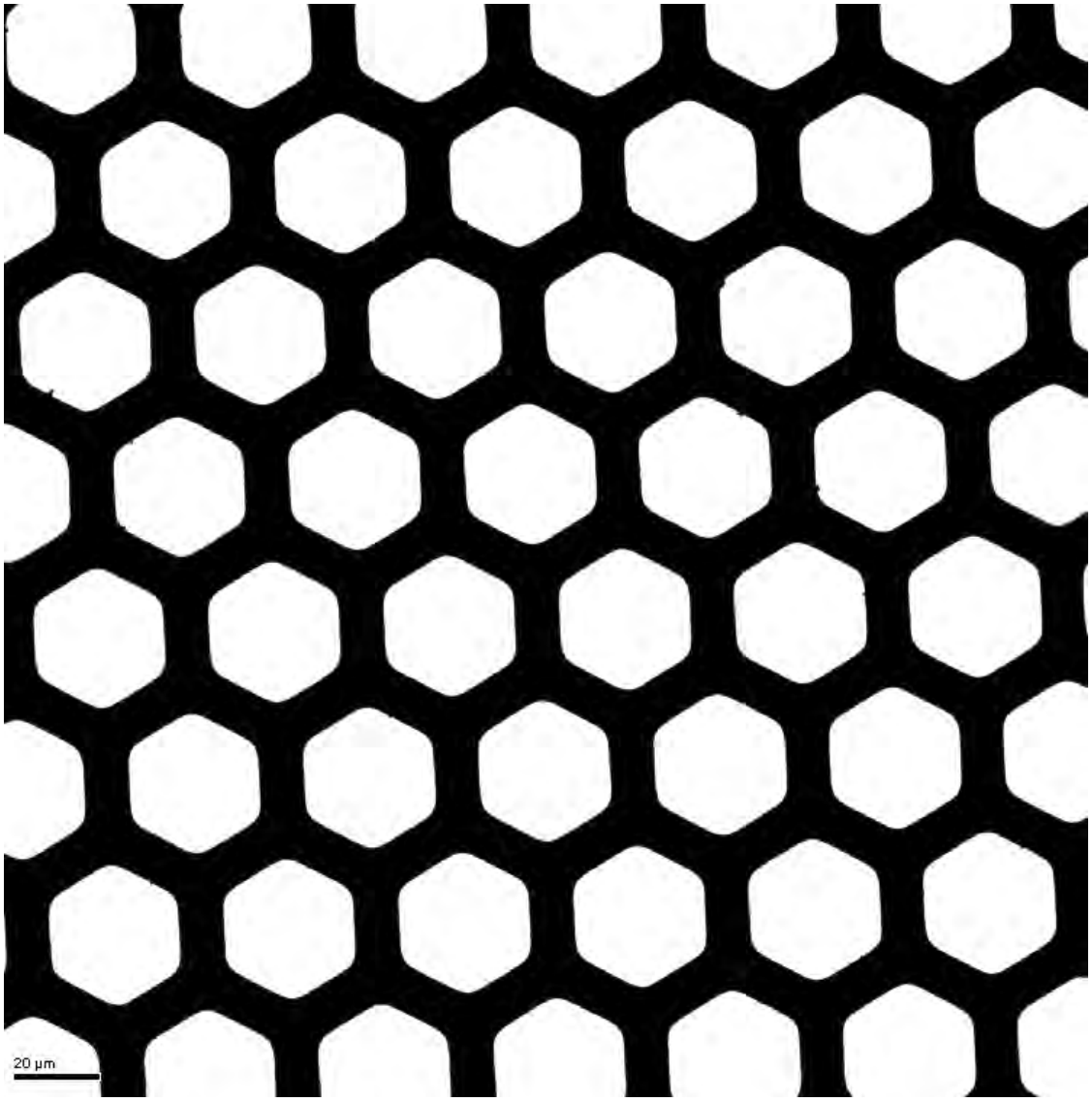


FIGURE 10.3. A variety of specimen support grids of different mesh size and shape. At top right is the oyster grid, useful for sandwiching small slivers of thin material.

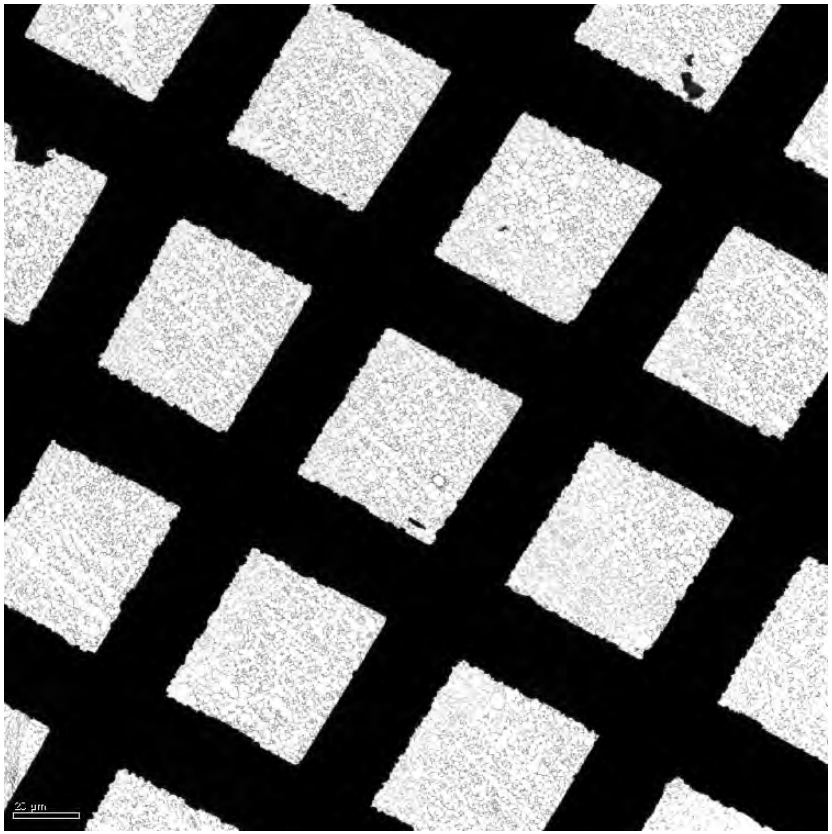
Also support film grids: Carbon coated, holey carbon, lacey carbon, polymer support film,
Other materials than Copper: Gold, berillium...

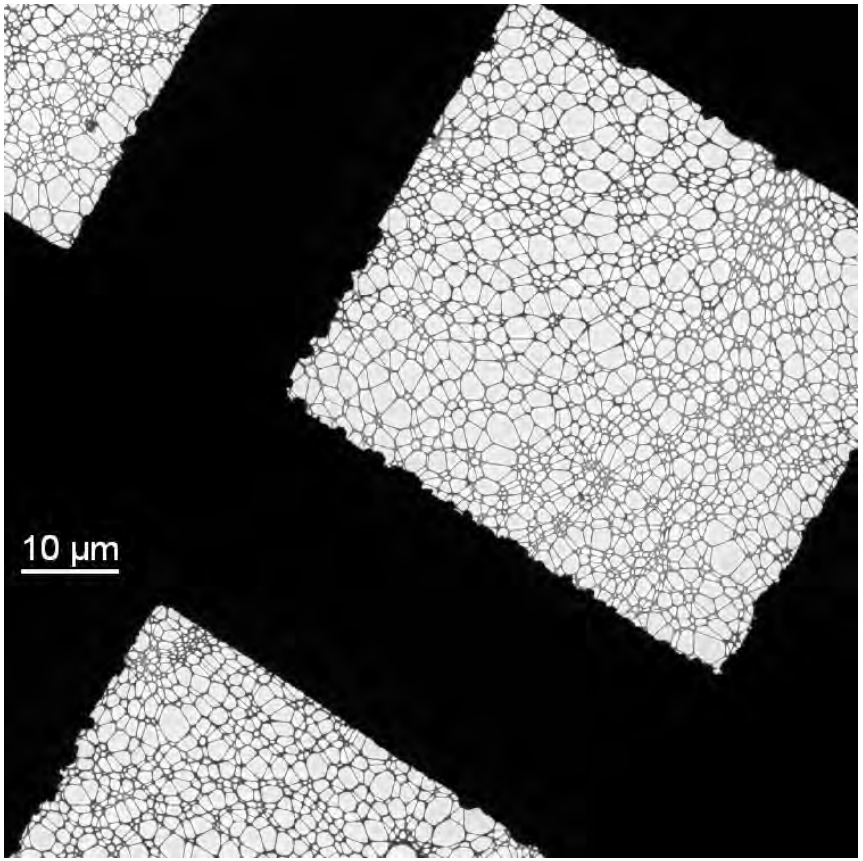


Example: Hexagonal thinbar grid 600 mesh

Normally we use ultrathinbar Gilder Grids T601H-Cu from EMS, hole 37 micron bar 5 micron)
These are good for room temperature microtomed sections

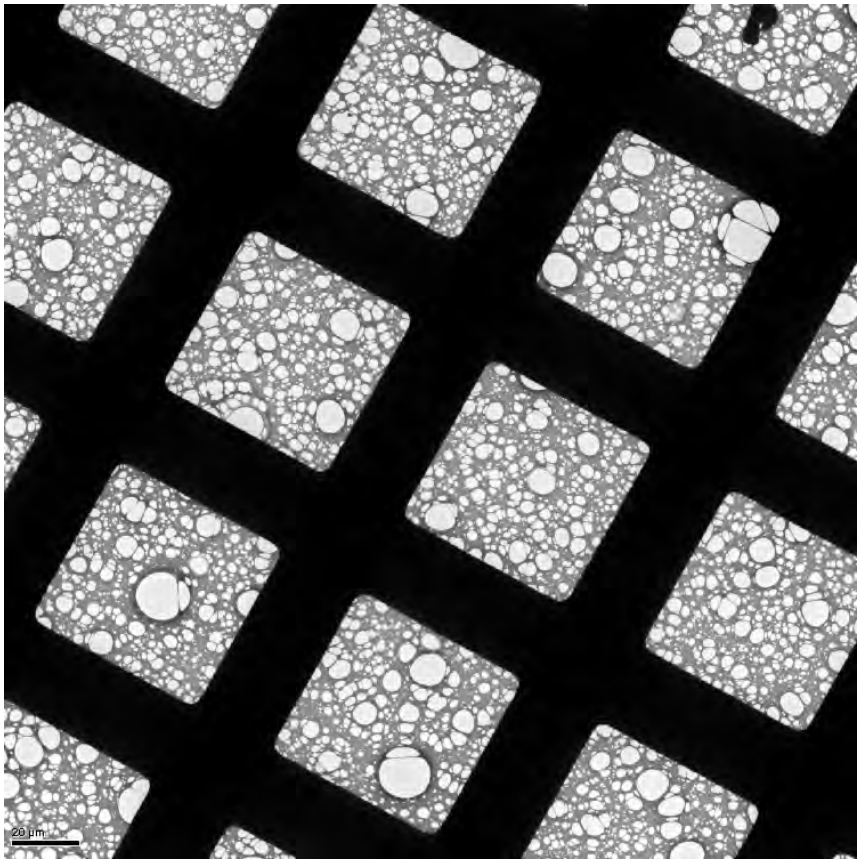
Lacey carbon grids

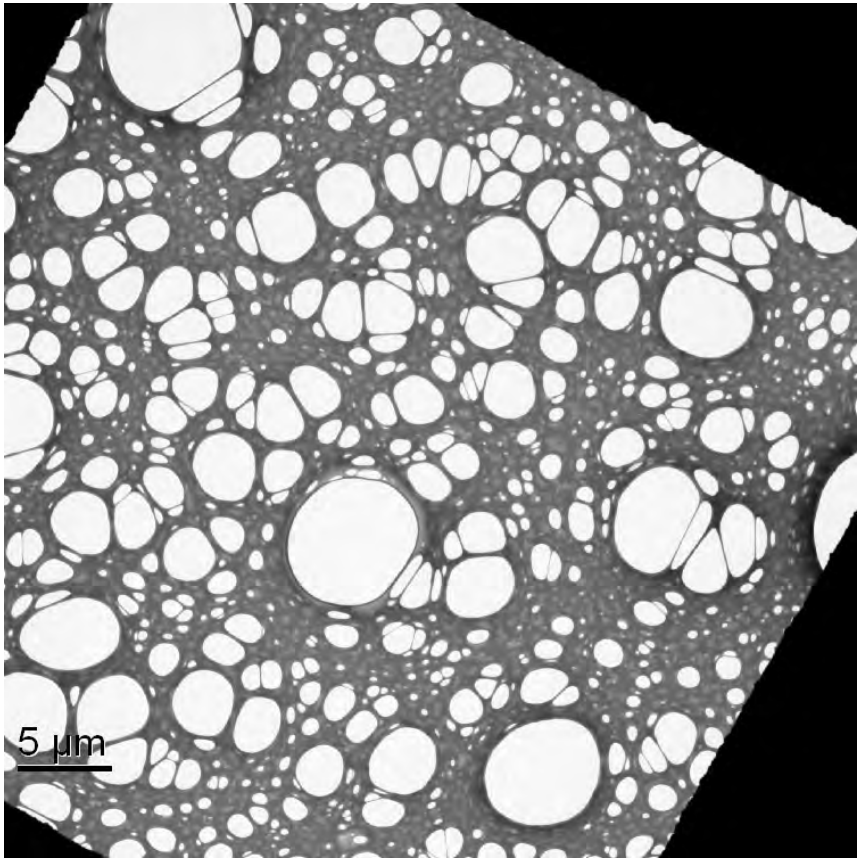




This is good for cryo sections (and sometimes also for cryo vitrification)

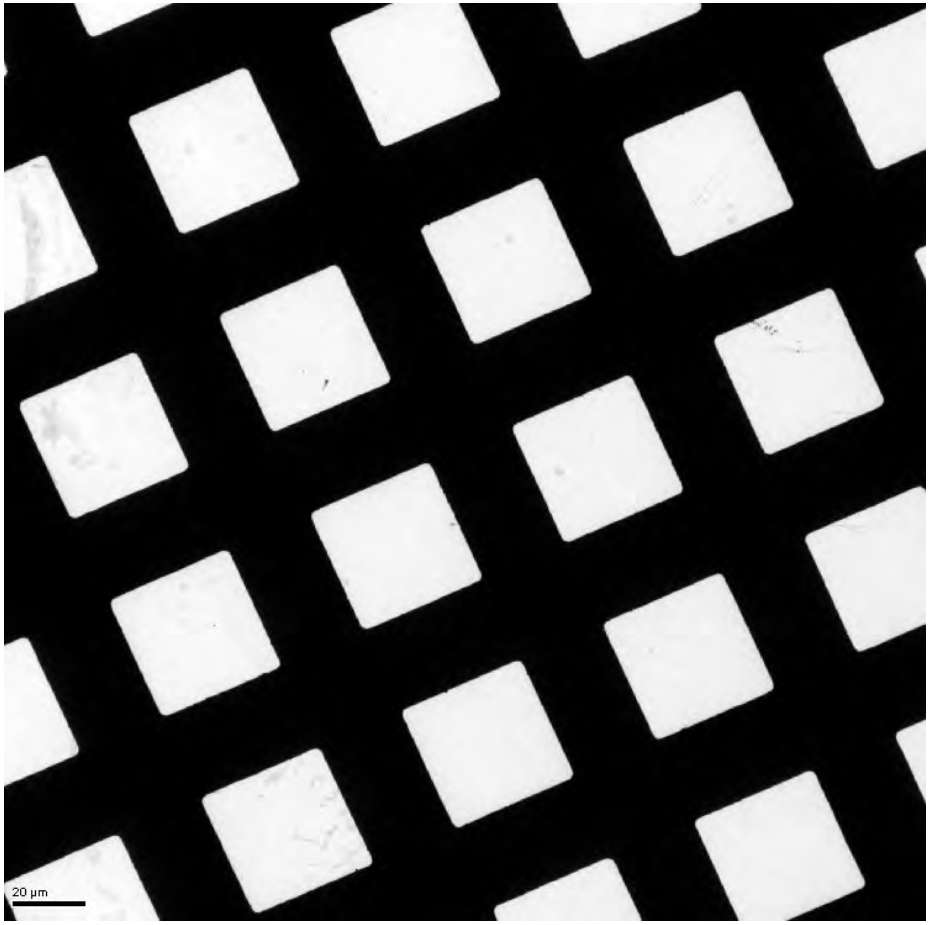
Holey carbon or Holey formvar carbon

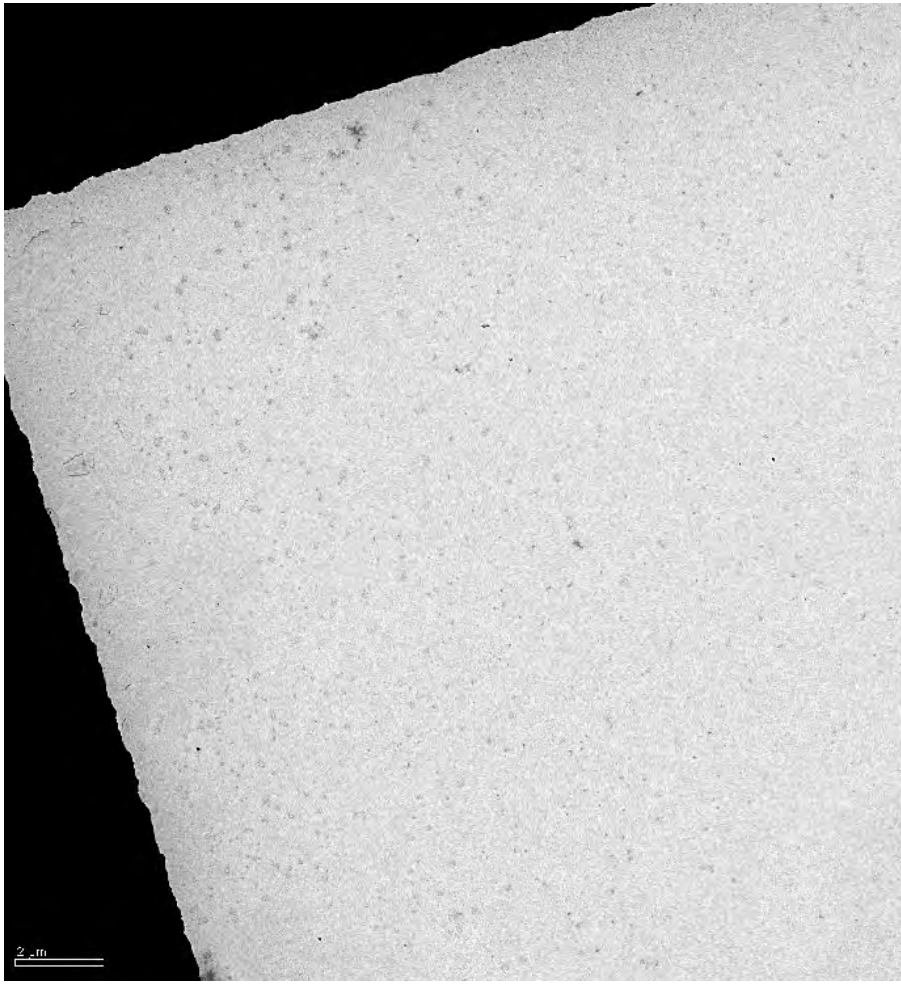




Good for dispersions, nanoparticles etc.

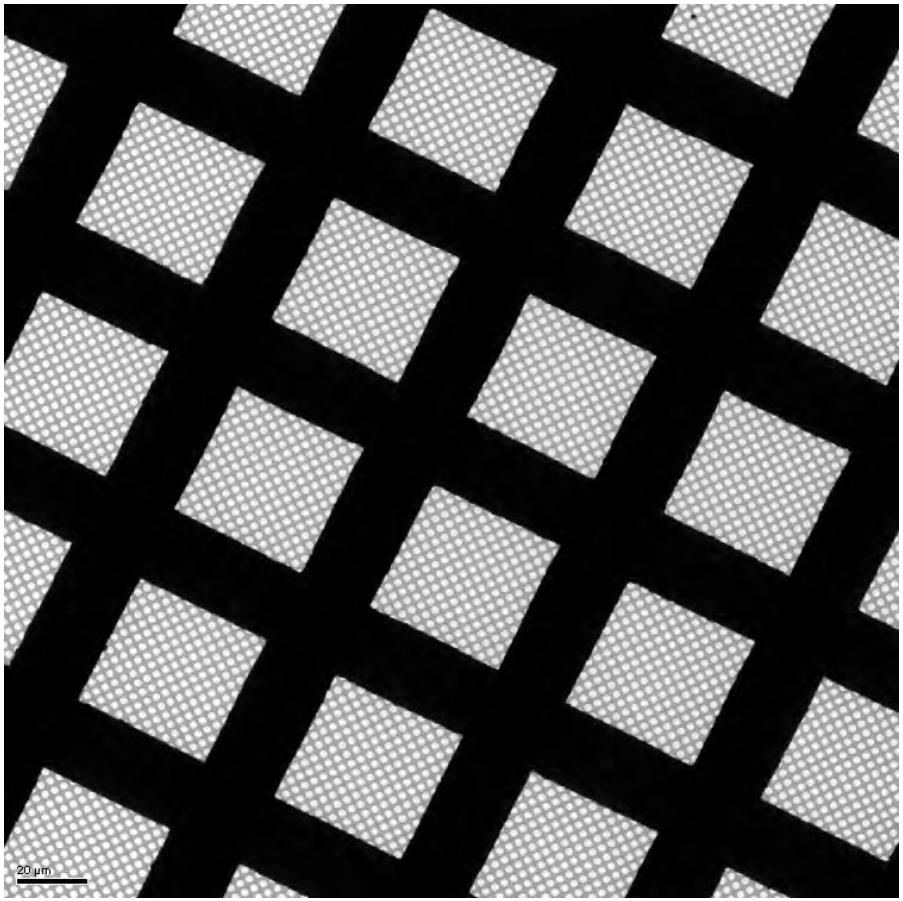
Carbon only or formvar-carbon only

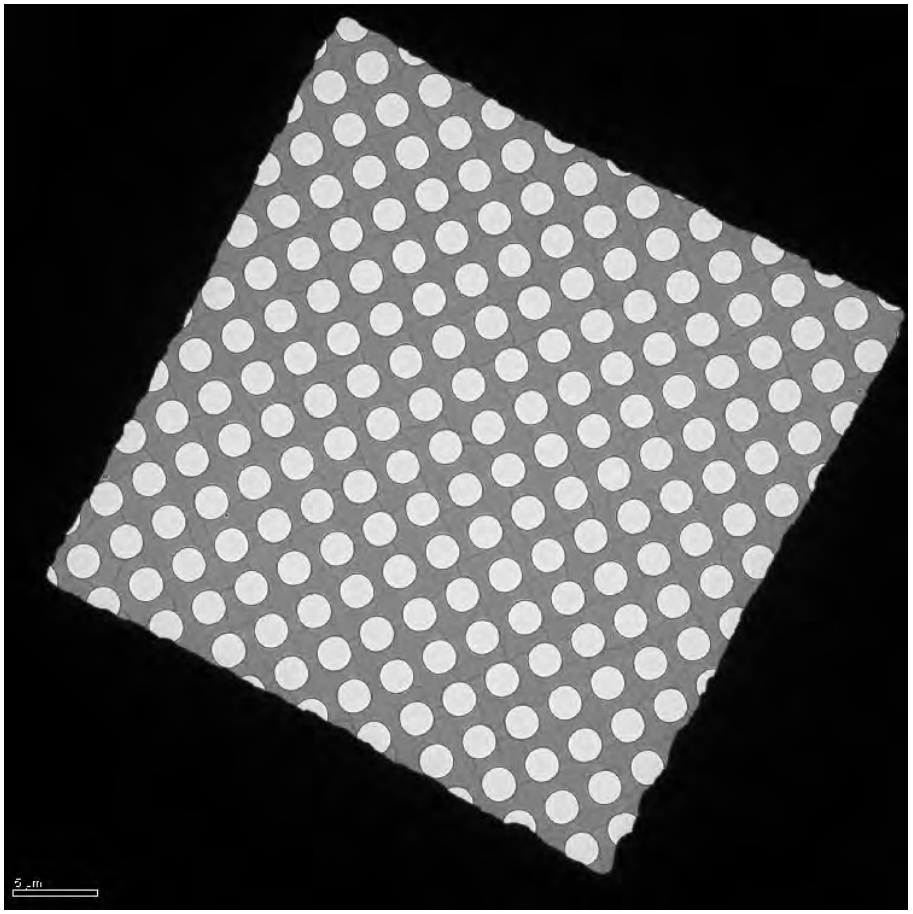




Good for dispersions, nanoparticles etc. (formvar-carbon not good for cryo imaging)

Quantifoil or C-flat (cryo)



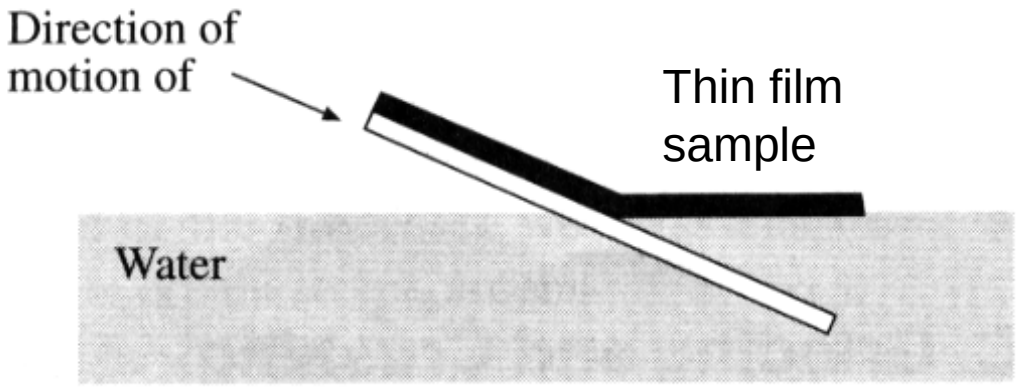


Example Quatifoil R2/1 = 2 micron holes with 1 micron separation..
Good for Cryo vitrification

Sample preparation 2. a) Free standing thin films

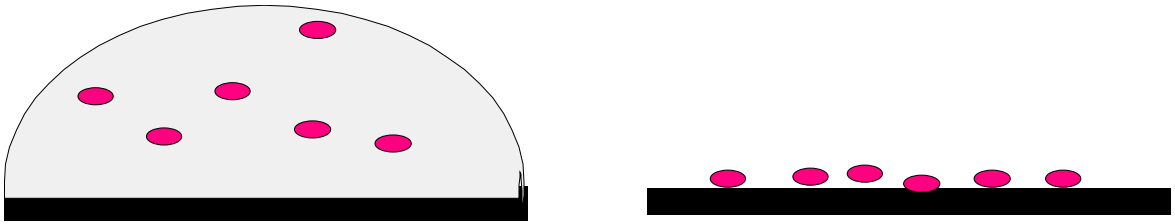
Thin films can be spin coated on top of ***NaCl crystals*** and floated onto the water surface

→ Picked up directly onto the TEM grids



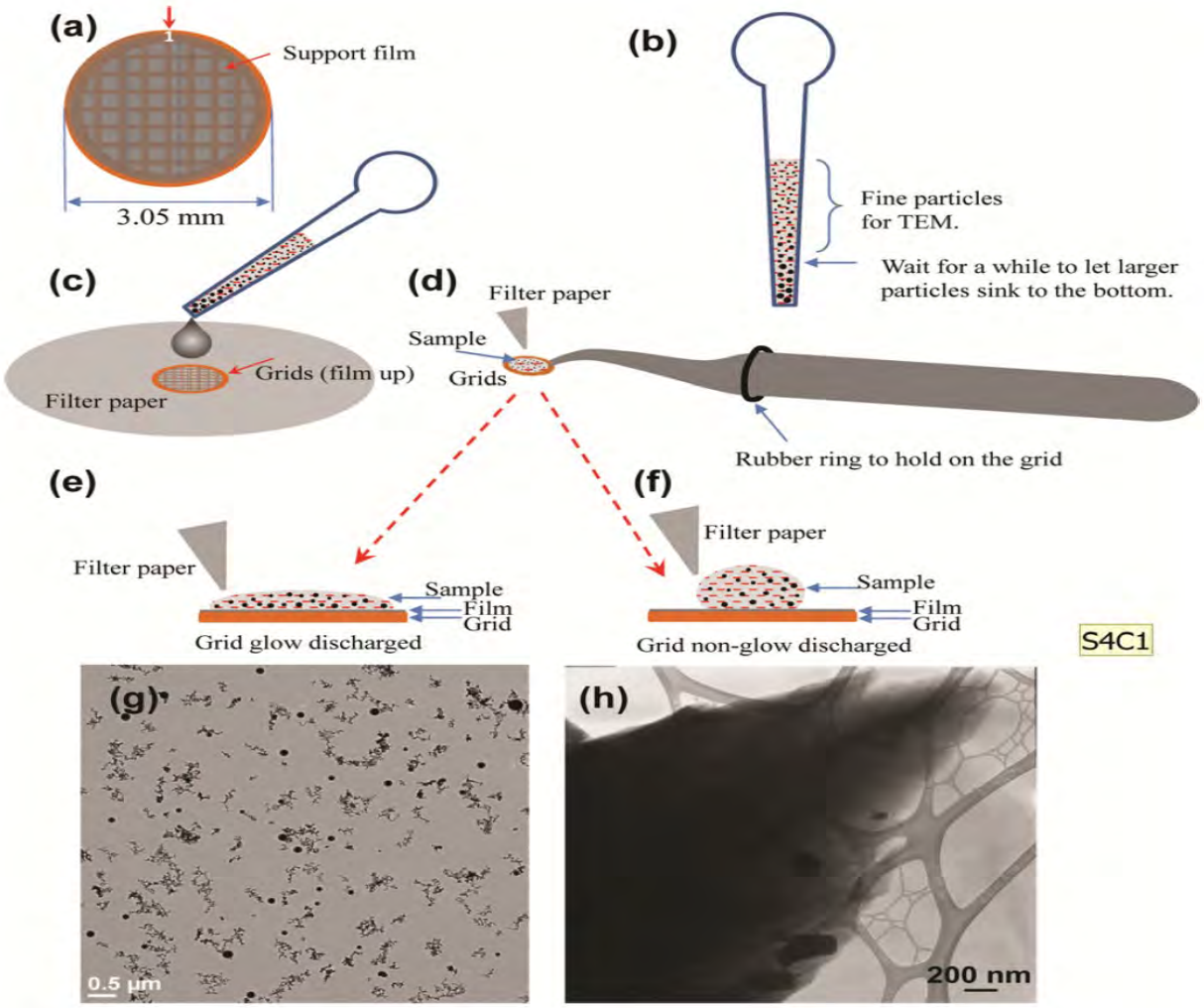
2. Sample preparation: b) *Dispersions*

- Nanoparticles, carbon nanotubes, nanofibers, viruses…. *Small particles & objects*



TEM grid

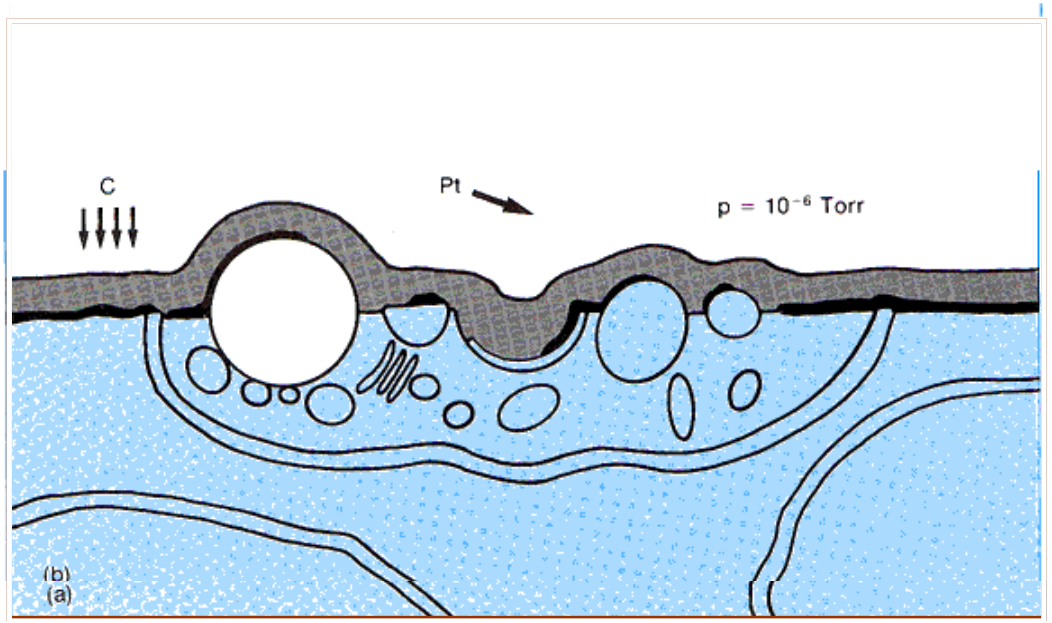
Carbon coated,
Formval/carbon,
Holey Carbon …



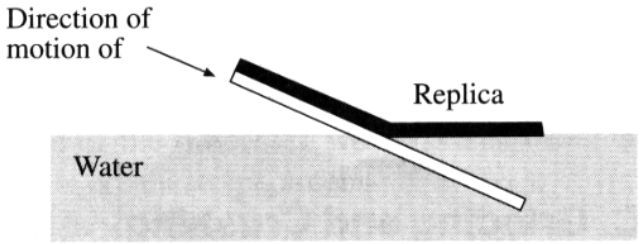
TEM grid preparation. (a) A TEM grid with a support film coated on the top side; (b) a dropper to transfer the solution; (c) a droplet of solution is dropped onto a piece of grid on a piece of filter paper; (d) a pair of tweezers to hold a piece of upward grid and a small droplet of solution is deposited on its surface. A small piece of filter paper is used to remove any extra solution; (e) glow discharged grid with hydrophilic surface so that the droplet solution spreads on the surface; (f) non-glow discharged grid with hydrophobic surface, where the droplet does not spread on the surface; (g) TEM image of well-dispersed alumina particles on pure carbon support film; (h) a fragment of carbon fiber powders deposited on holey mesh support film.

Book: A Practical Guide to Transmission Electron Microscopy

Sample preparation 3. The freeze fracture technique and replication



modified after: Friedrich Kopp, Electron Microscopy



Double replica..

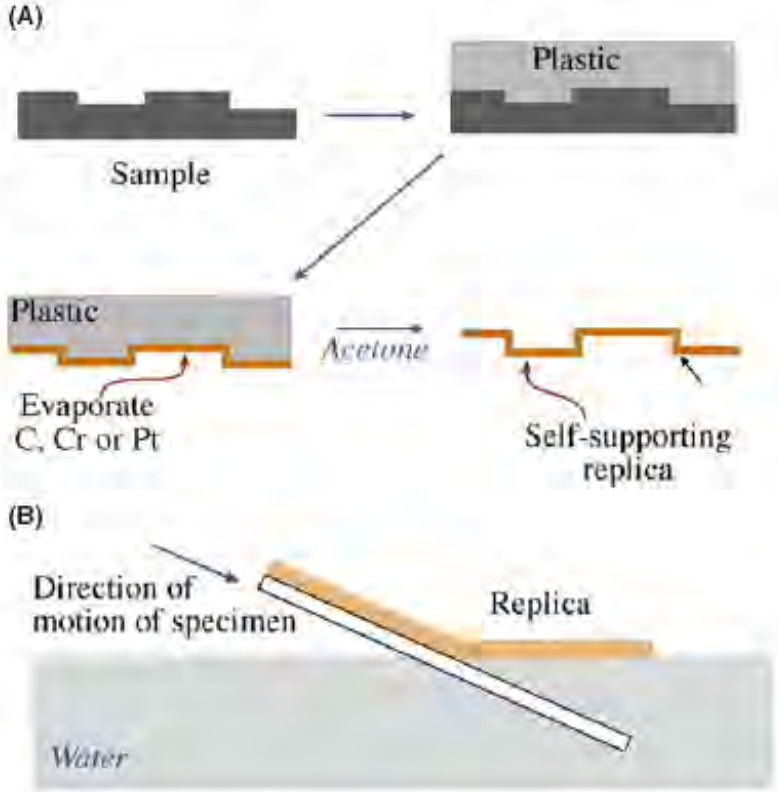
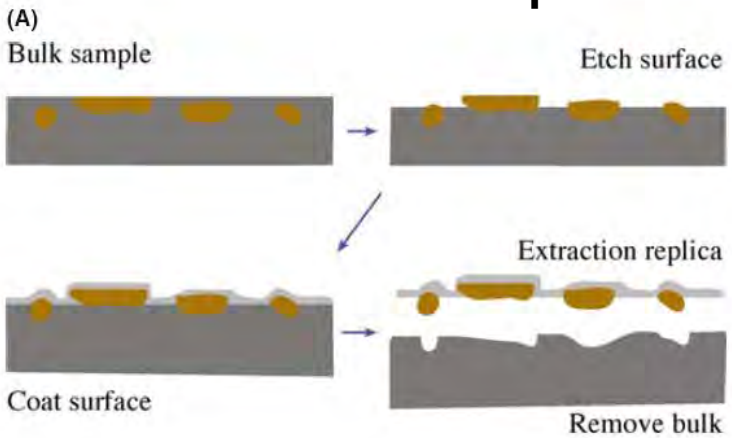


FIGURE 10.16. (A) Replication of a surface by the two-step method: spray acetone on the surface to be replicated before pressing a plastic (usually cellulose acetate) onto the surface which softens in contact with the acetone; the plastic is removed from the surface when it has hardened and a C, Cr, or Pt film is evaporated onto the replicated plastic surface; the plastic is then dissolved with acetone and the evaporated film retains the original topography. (B) Alternatively, the direct carbon replica of a metal surface may be floated off on distilled water after scratching the carbon and etching to free the film, which may subsequently be shadowed obliquely to enhance the topography.

Extraction replica..



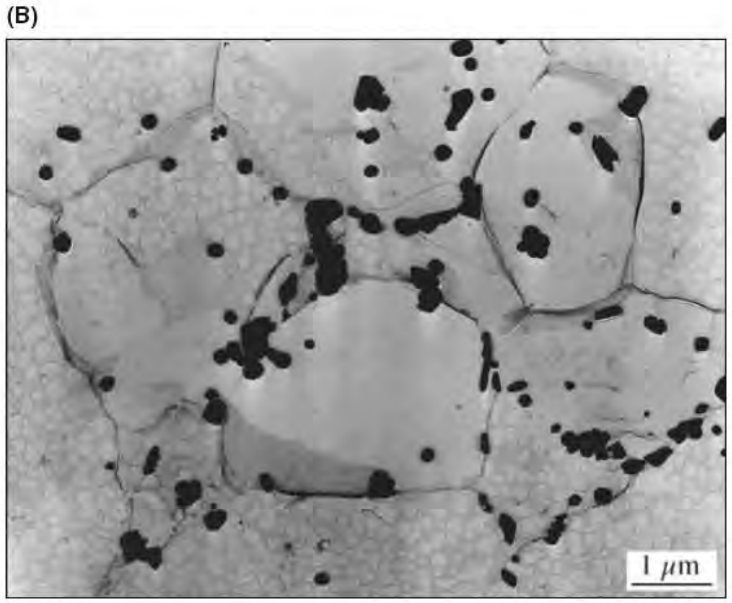


FIGURE 10.17. (A) Making the extraction replication: particles embedded in a matrix are revealed by etching the matrix, which leaves the particles standing proud of the surface; a thin amorphous carbon film is evaporated over the particles, then the rest of the matrix is etched away leaving the particles adhering to the carbon film. (B) Example from a γ/γ' alloy showing not only that the particles are mainly located at the grain boundaries but also the different contrast from γ' grains and two-phase γ/γ' grains.

Sample preparation 4. Cleaving..

90 degree wedge specimen

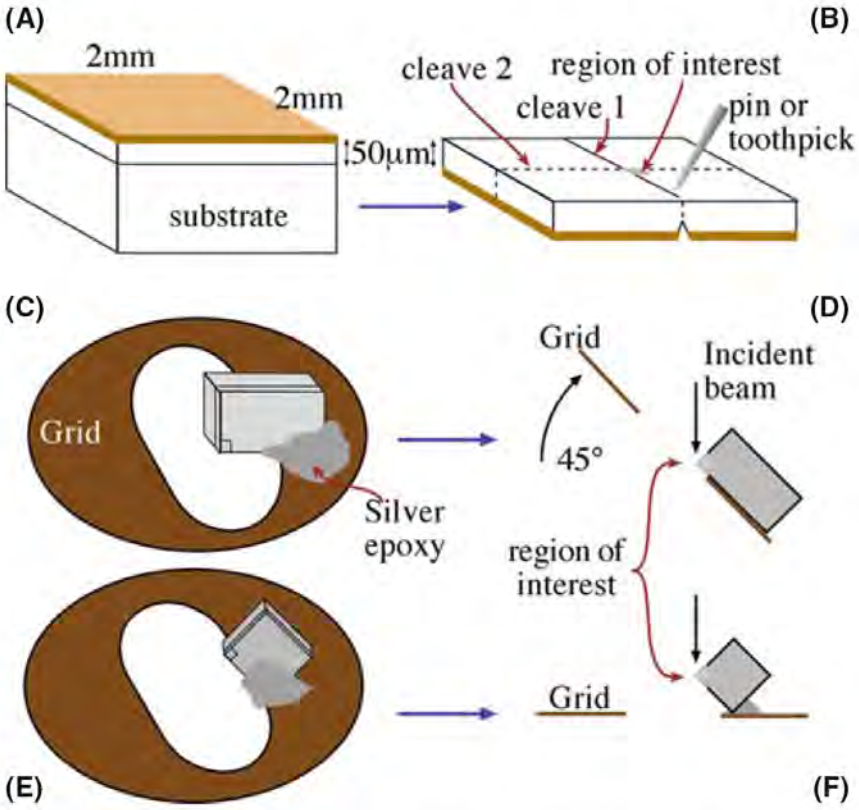


FIGURE 10.20. The 90°-wedge specimen: (A) prethin to create a 2-mm square of the multilayers on a Si substrate; (B) scribe the Si through the surface layers, turn over, and cleave; inspect to make sure the cleavage is clean, giving a sharp 90° edge; reject if not; (C, E) mount the 90° corner over the edge of a hole in a Cu grid; (D, F) then insert in the TEM; note that two different orientations are available from a single cleavage operation.

Small Angle Cleaving Technique SACT

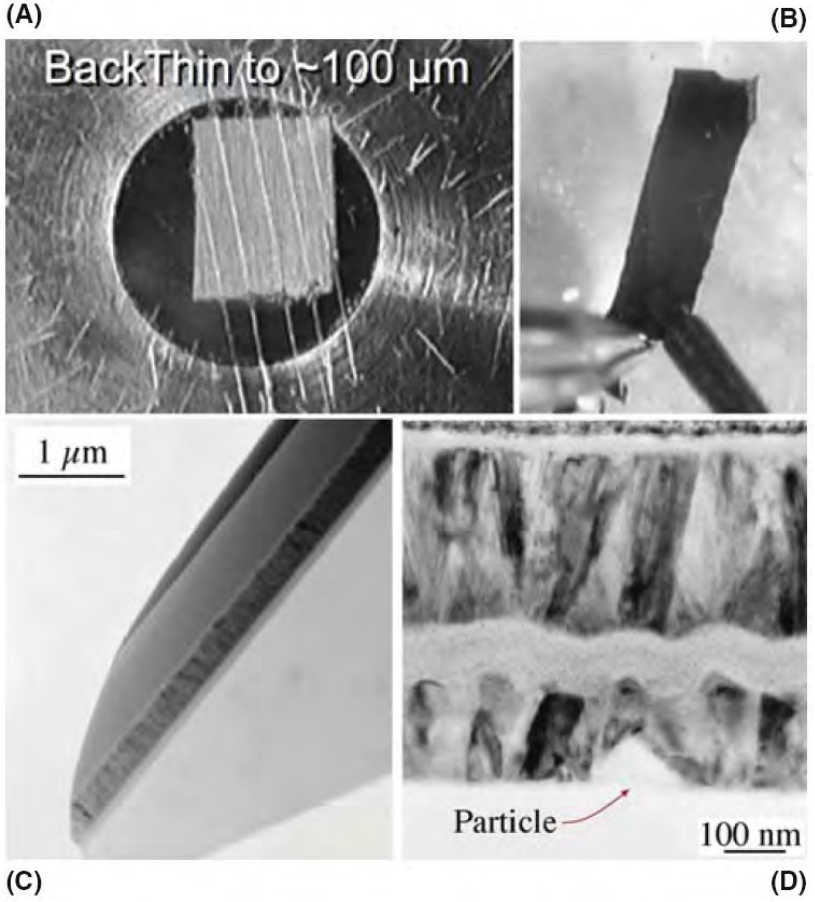


FIGURE 10.19. SACT of a coating on glass. (A) Scratch the sample; (B) cleaving along the scratch; (C, D) TEM images.

Sample preparation. Lithography..

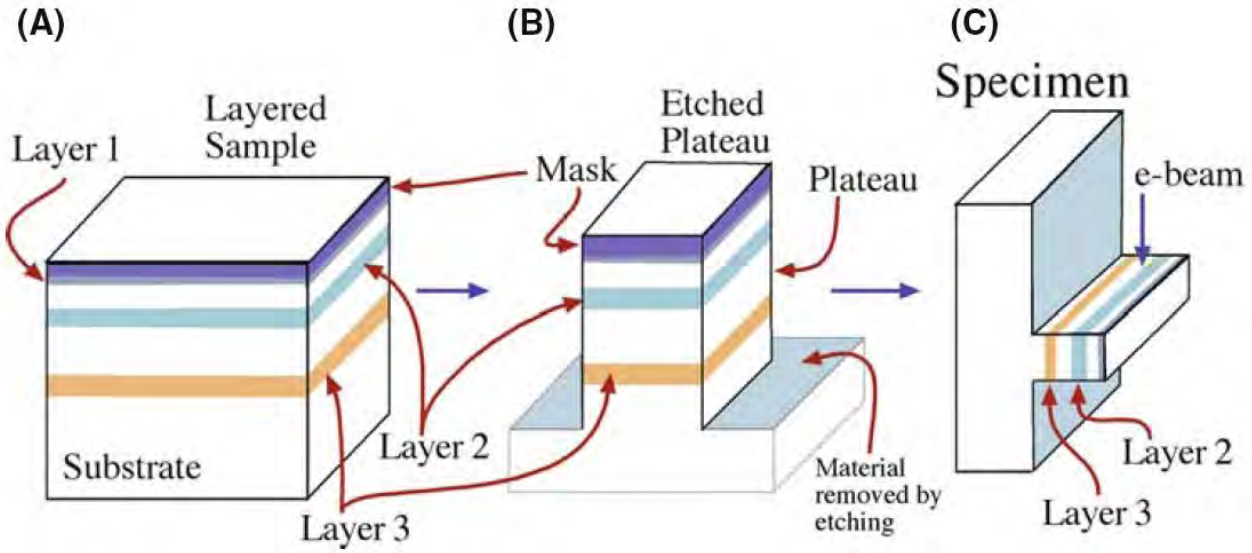


FIGURE 10.21. Etching of a multilayer sample (A). Etch away most of the sample, leaving a small etched plateau (B); mask a region <50 nm across and etch away the majority of the surrounding plateau. If this thin region is turned 90° and mounted in a specimen holder (C), the interfaces are now parallel to the electron beam.

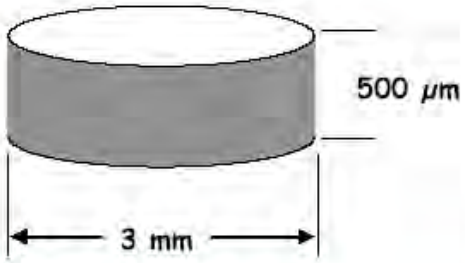
Sample preparation 5: Polishing and ion milling

gatan

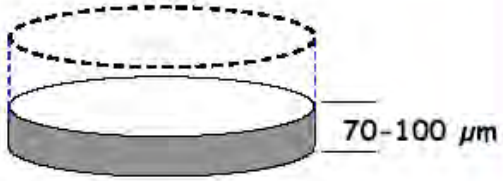
TEM SPECIMEN PREPARATION

Basic Preparation Steps

Step 1 Disc Cutting



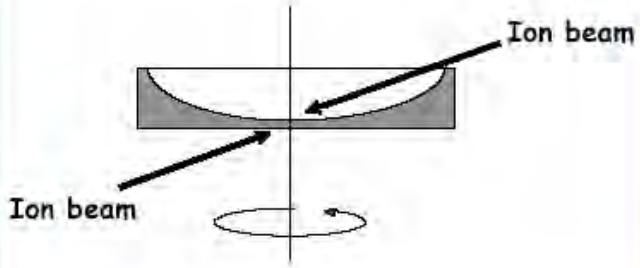
Step 2 Disc Grinding



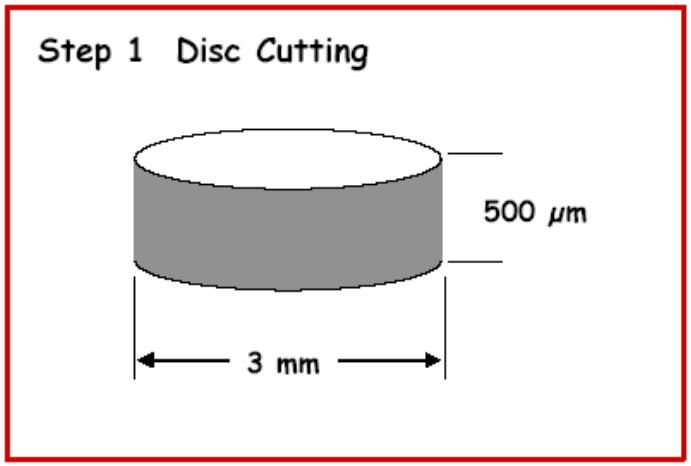
Step 3 Dimple Grinding

Drawings Not to Scale

Step 4 Ion Milling



Disk cutting (coring) tools..



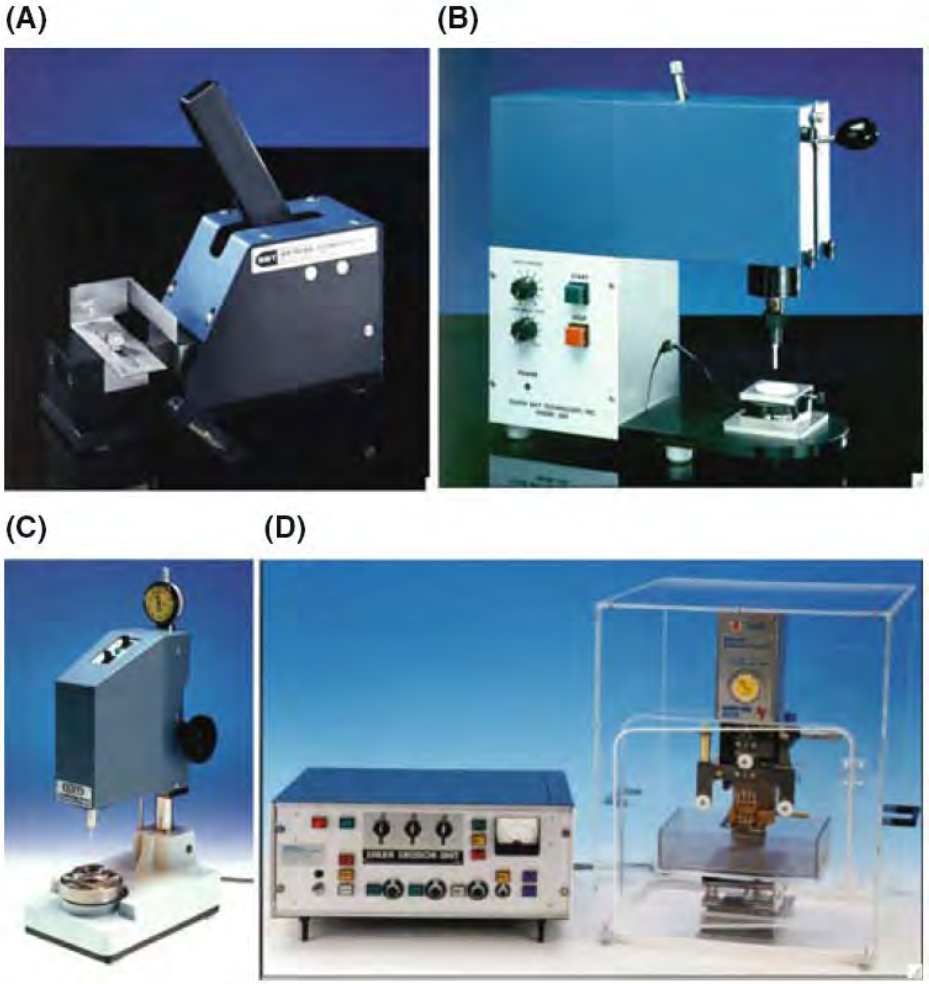


FIGURE 10.4. Four different coring tools from South Bay Technology. (A) A mechanical punch for stamping disks from thin sheets of ductile materials. A sheet sample is placed in the punch and the handle on the right is pushed down, ejecting a 3-mm-diameter disk suitable for thinning. (B) An abrasive-slurry disc cutter uses a rotary motion of the coring tube to drill round the disk. (C) An ultrasonic cutter. (D) A spark-erosion cutter; the erosion takes place under a solvent and behind a safety shield.

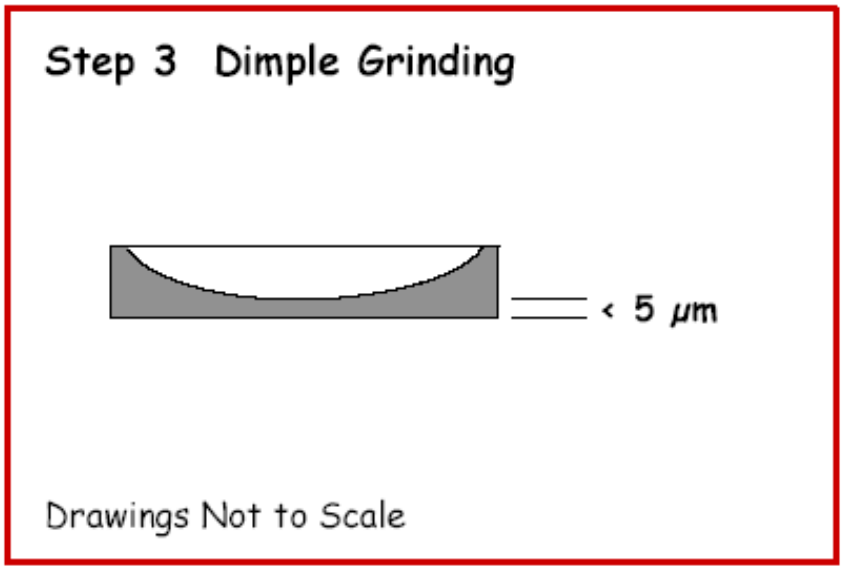


Fig. 2.5 *(a) Dimpling process; (b) geometry.*

Mechanical dimpling

(B)

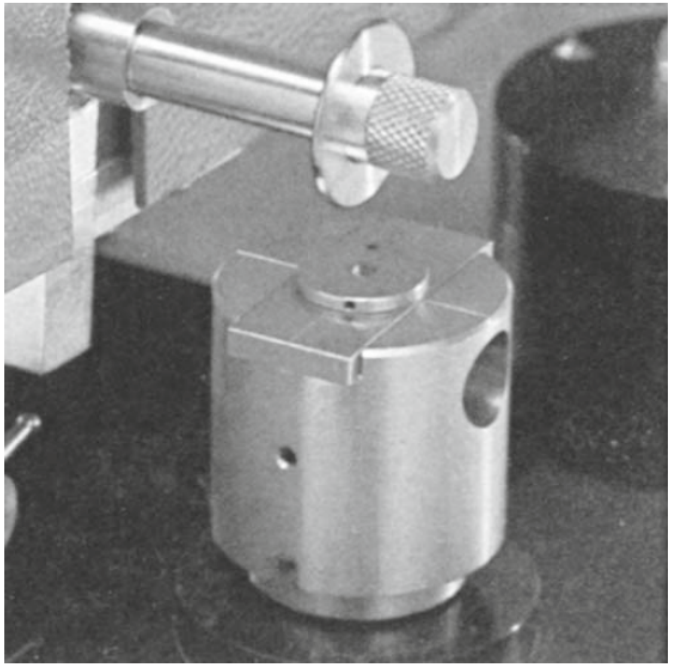
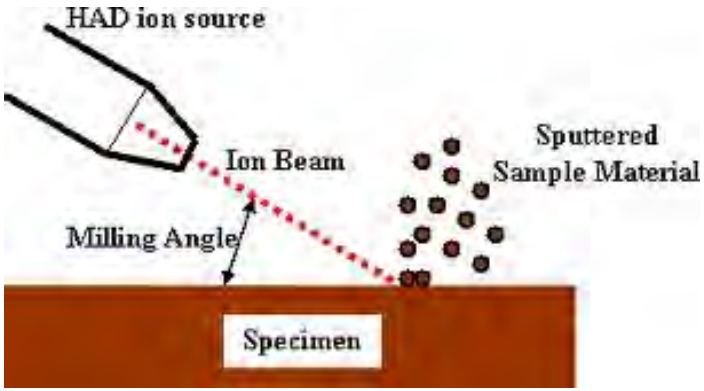
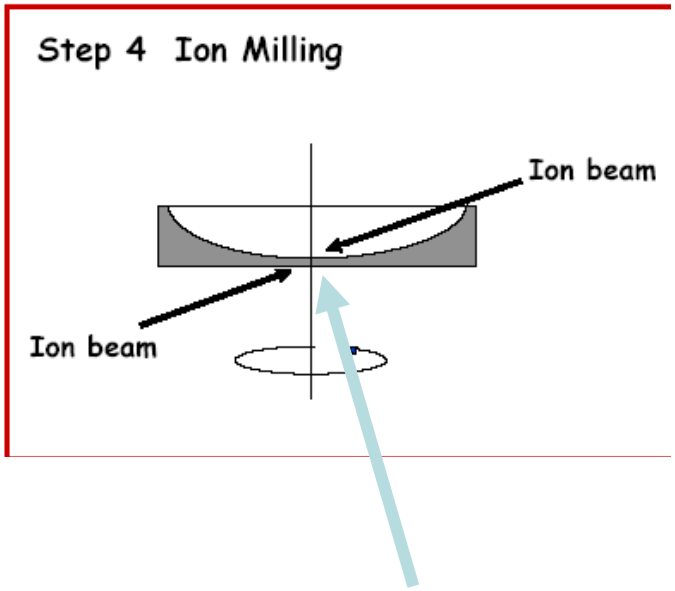
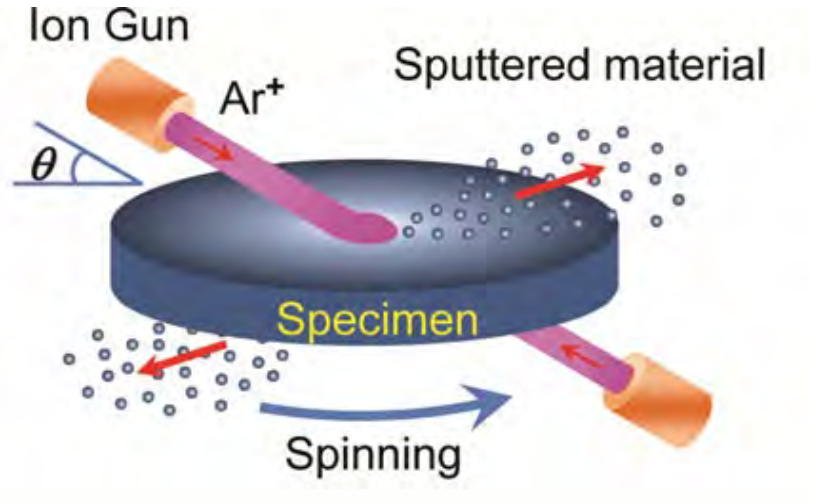


FIGURE 10.5. (A) Dimpling apparatus; (B) the grinding tool and specimen support block.

Ion milling Gatan Solarus 9500







Finally, after the milling – hole will appear in the middle and thin TEM transparent areas are around the hole..

Table 2-2 Typical milling rates

MATERIAL	MILLING RATE (µM/HR/GUN PAIR)
Copper	22
Silicon	24
Silicon carbide	16
Stainless steel 316	14
Tantalum	8

2.11.4.2 PIPS Milling Parameters

As mentioned earlier, higher beam energies (keV) and milling angles lead to higher milling rates. However, they also lead to relatively more damage (on the order of a few nanometers) to the surface of the sample. Lower energies and milling angles produce not just a lower amount of surface damage but also a larger amount of thin area. Therefore, it is essential to arrive at a reasonable compromise between the amount of damage and the milling time.

While setting the milling angles, one must be careful that the rim around the dimpled sample does not cast a shadow over the central region. Table 2-3 shows the minimum milling angle that can be chosen, depending on rim/initial bulk thickness.

NOTE: Values are for samples dimpled down to 5 µm with a 15 mm wheel.

Table 2-3 Bulk/rim thickness vs. minimum milling angle

BULK/RIM THICKNESS (µM)	MINIMUM MILLING ANGLE
40	3°
50-70	4°
70-100	5°
100-150	6°
150-200	7°

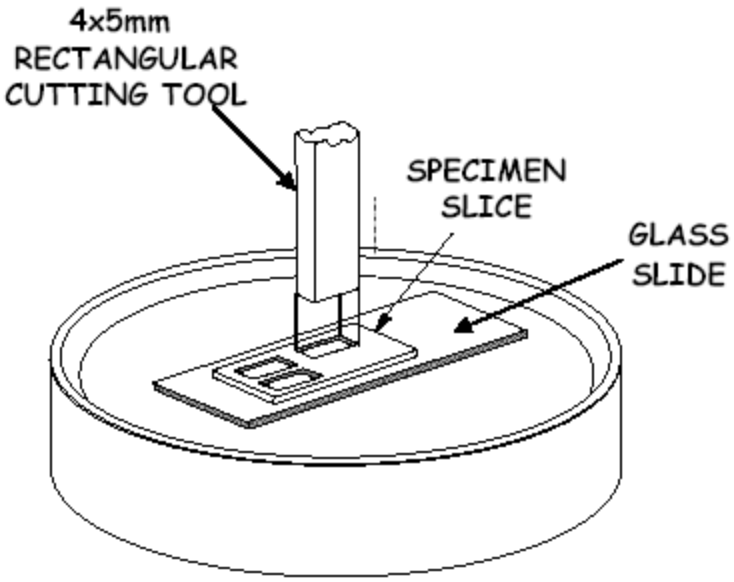
Use the sample recipe below as a starting guide to choosing PIPS milling parameters. This recipe works best for a silicon-based sample of bulk thickness 60 µm dimpled to 5 µm with a 15 mm wheel.

Table 2-4 PIPS milling parameters

GUN KEV	GUN ANGLE	BEAM MODULATION	ROTATION	TIME
4.0	5° Top 3° Bottom	Dual Beam	3 rpm	Until perforation reaches area of interest
2.5	4° Top 2° Bottom	Dual Beam	3 rpm	About 5 min
0.5	4° Top 2° Bottom	Dual Beam	3 rpm	About 2 min for clean-up

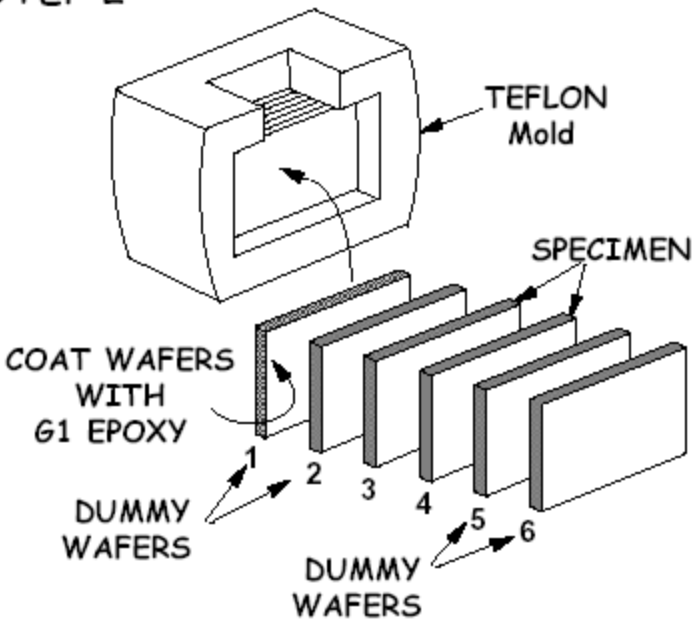
Preparing Cross-Sectional Specimens

STEP 1



Cut rectangular wafers from bulk material using ultrasonic disc cutter

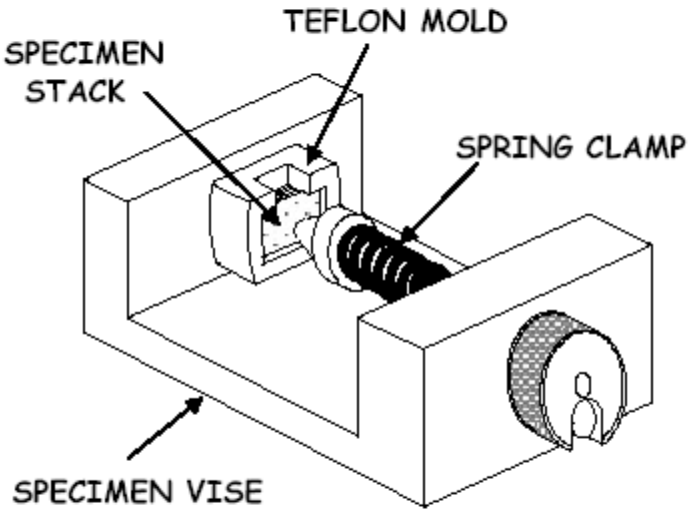
STEP 2



Coat all wafers with a thin layer of G-1 & load into Teflon™ mold

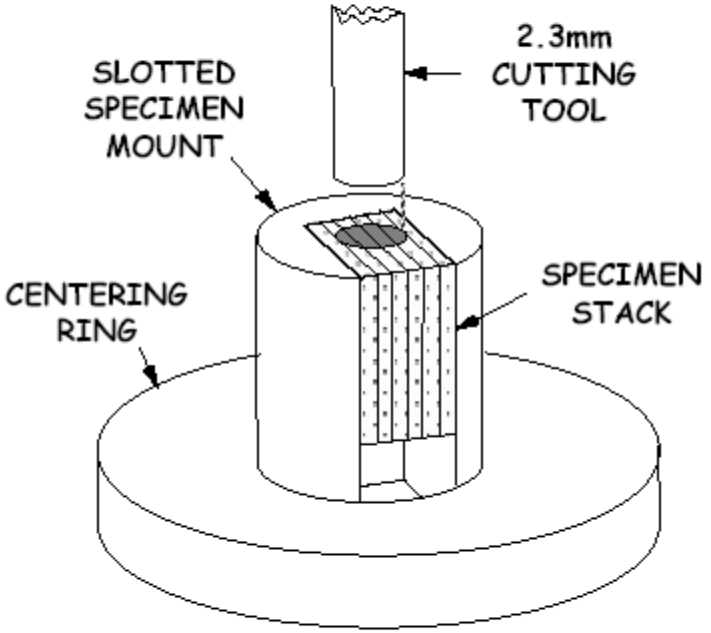
Preparing Cross-Sectional Specimens

STEP 3



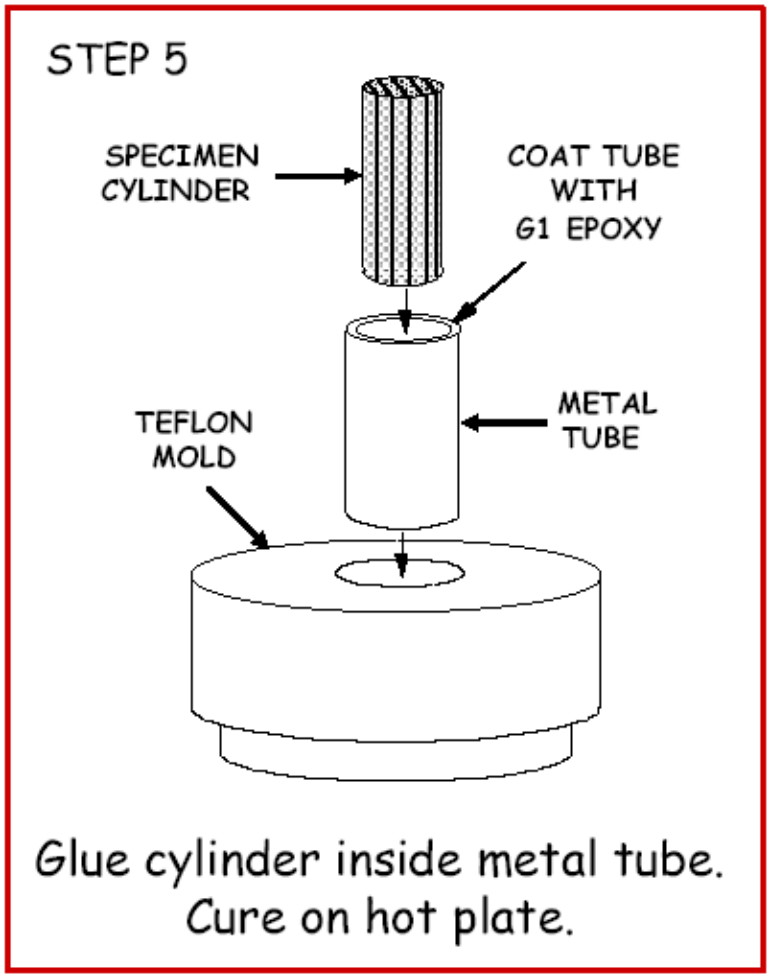
Cure glued stack under pressure to form a strong bond between wafers.

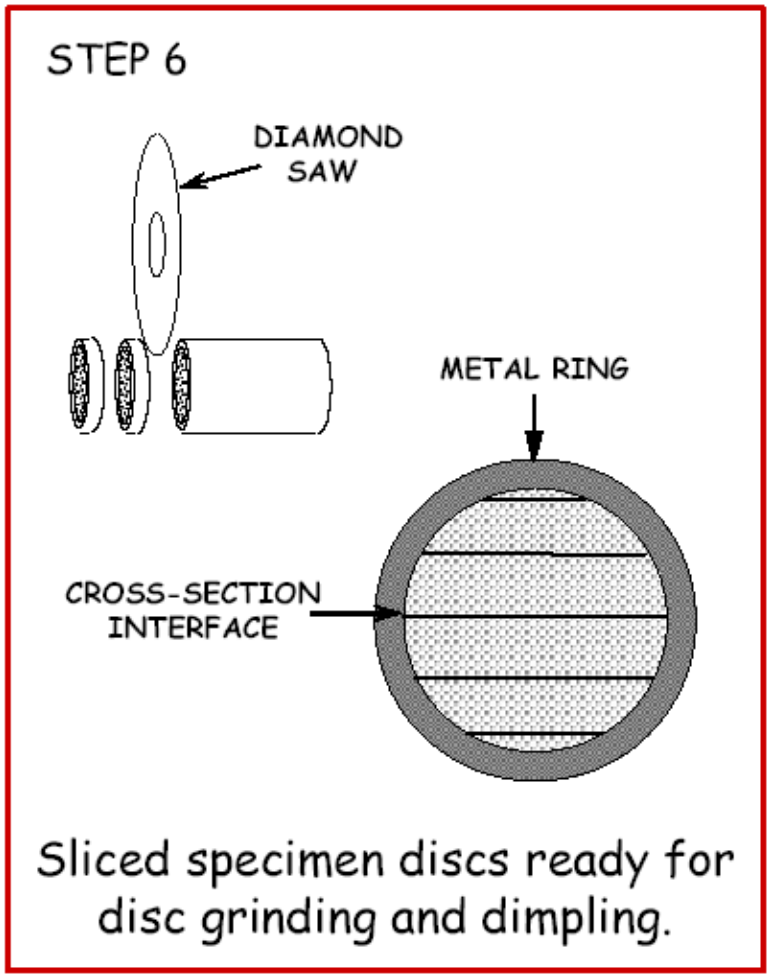
STEP 4



Cut cylinder from stack using ultrasonic disc cutter

Preparing Cross-Sectional TEM Discs



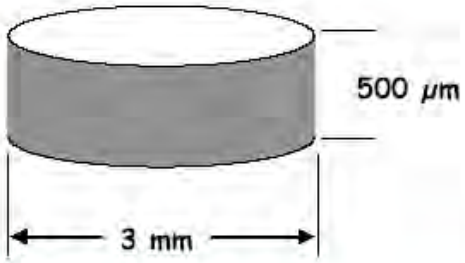


After that you do the basic preparation steps 1-4

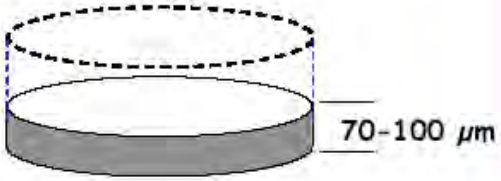
Basic Preparation Steps

TEM SPECIMEN PREPARATION

Step 1 Disc Cutting



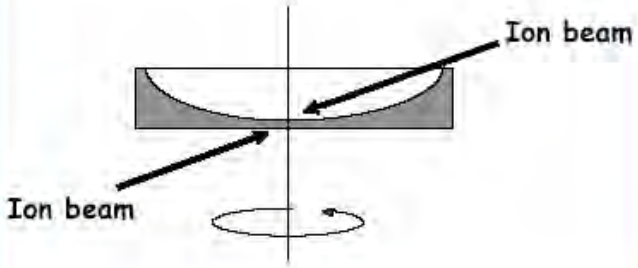
Step 2 Disc Grinding

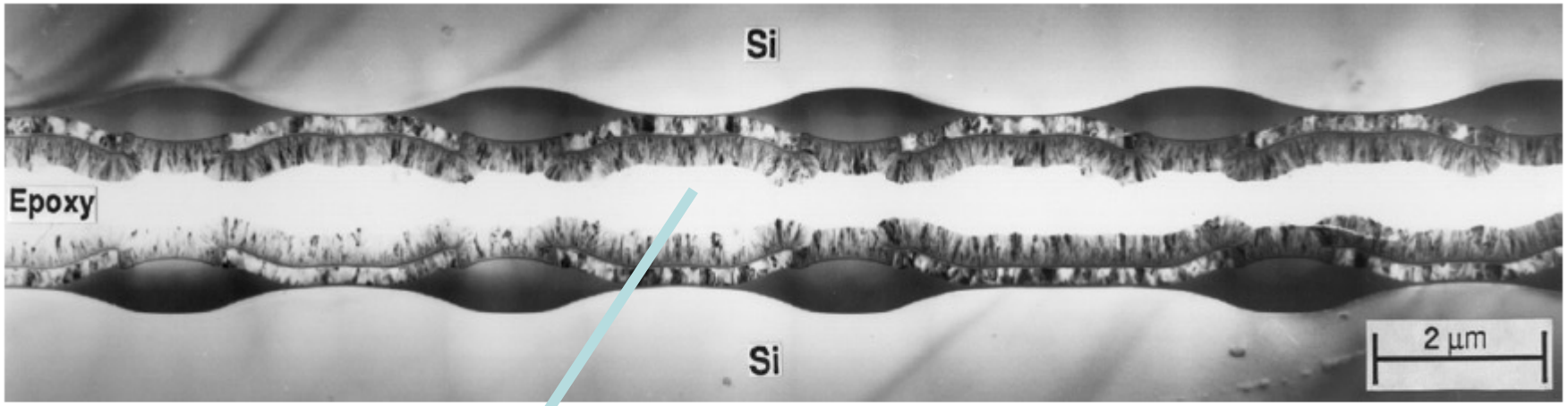


Step 3 Dimple Grinding

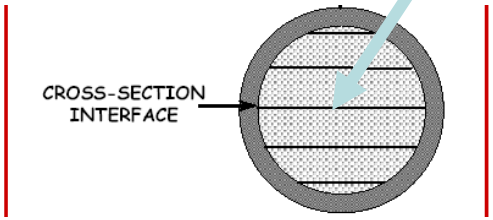
Drawings Not to Scale

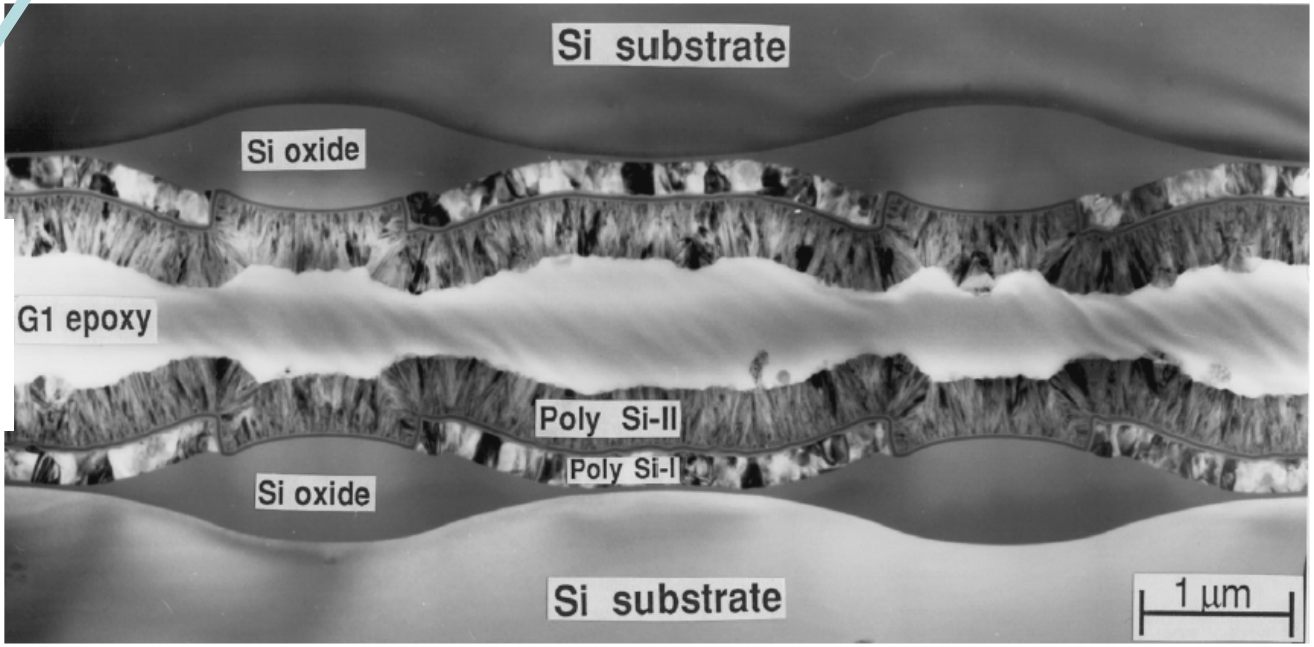
Step 4 Ion Milling





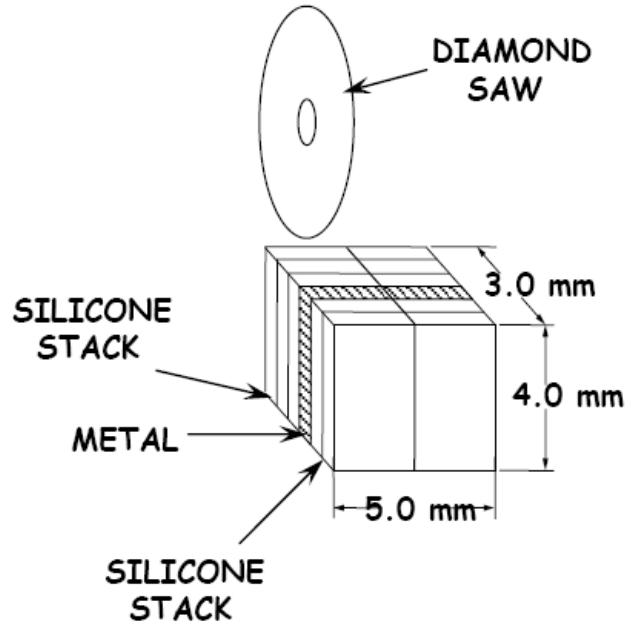
Cross-Section of a semi-processed IC device.





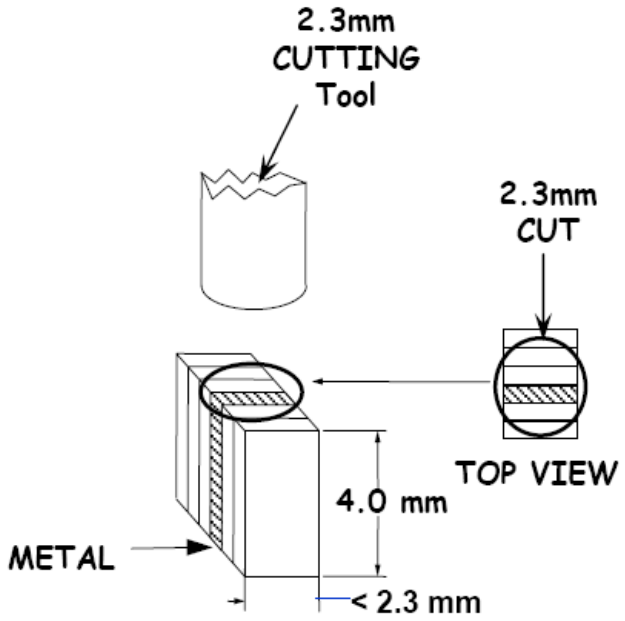
Metals/Hard Materials Cross-Sections

STEP 1



Cut stack using diamond saw to form 2 stacks 2.3mm wide

STEP 2

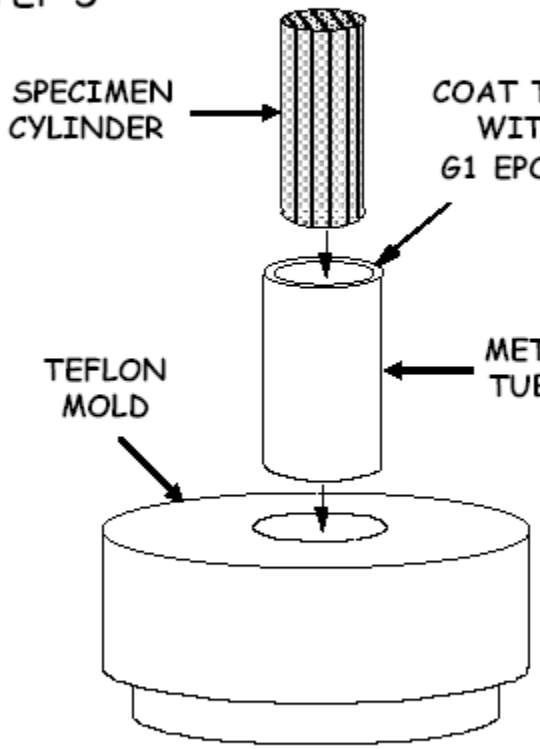


Cut cylinder from stack using ultrasonic disc cutter

Repeat Steps 5 & 6 from previous slide

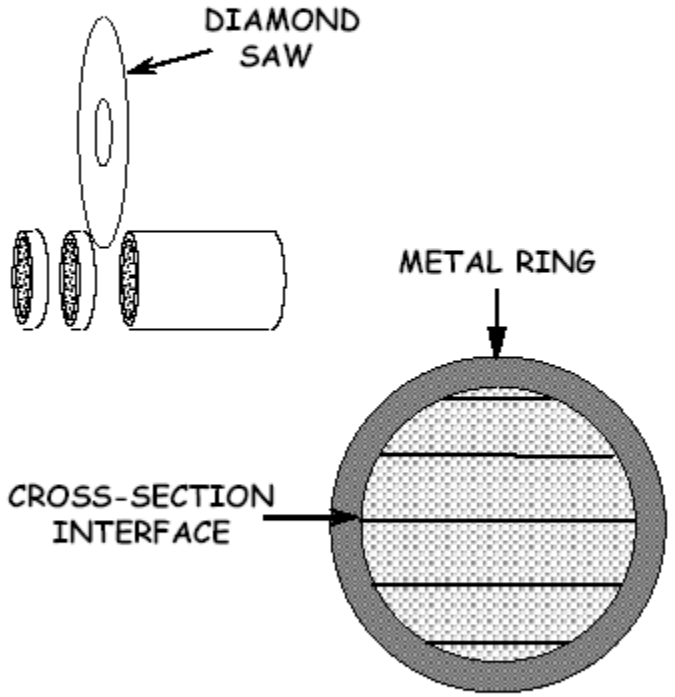
Preparing Cross-Sectional TEM Discs

STEP 5



Glue cylinder inside metal tube. Cure on hot plate.

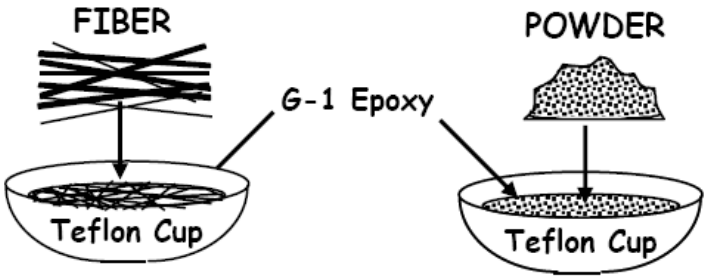
STEP 6



Sliced specimen discs ready for disc grinding and dimpling.

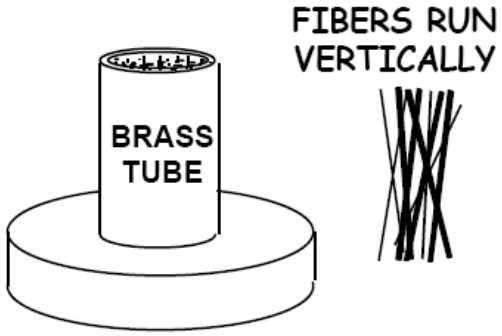
Preparing Powders and Fibers

Step 1



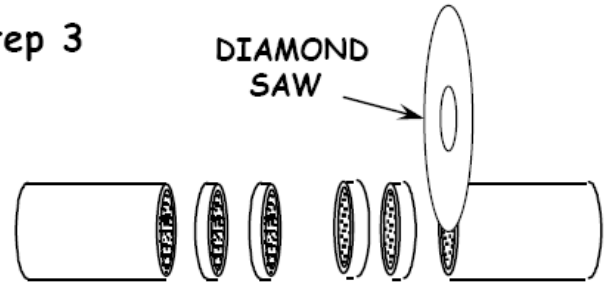
Mix G-1 epoxy with fibers or powder transfer mixture to a brass tube

Step 2



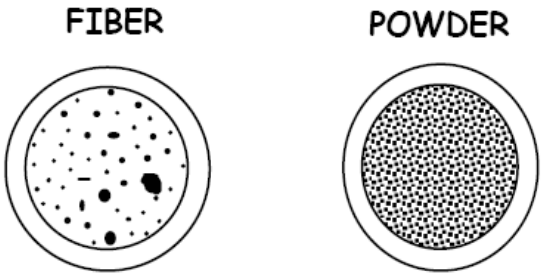
Cure epoxy on hot plate for 10 minutes at 130°C

Step 3



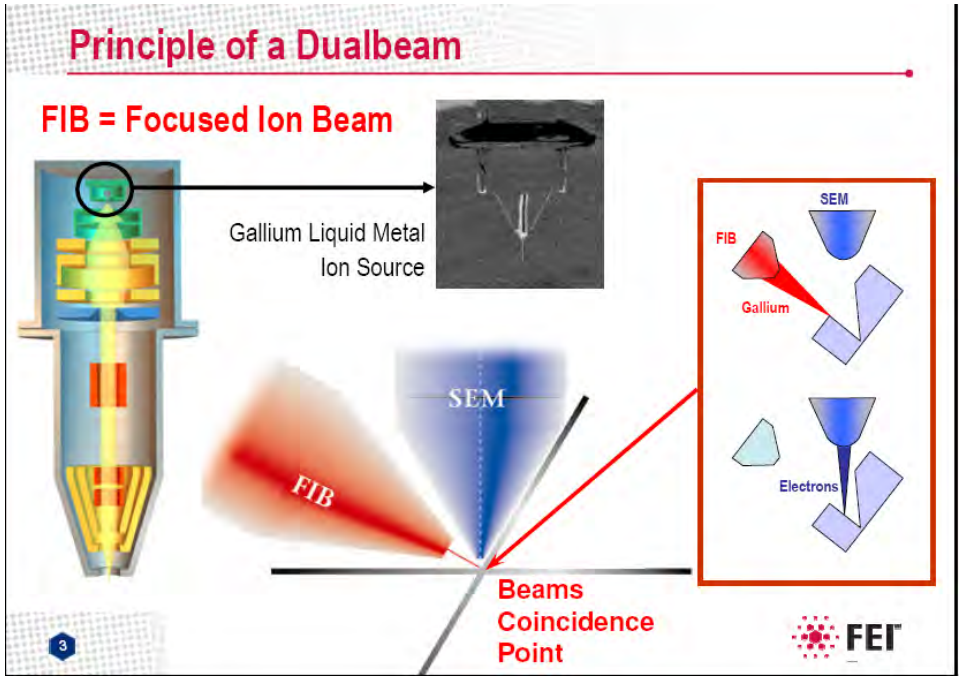
Cut brass tube into disc's for grinding to required thickness

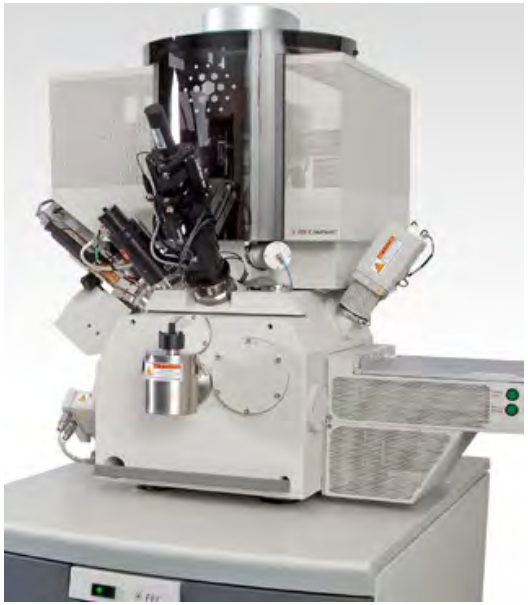
Step 4

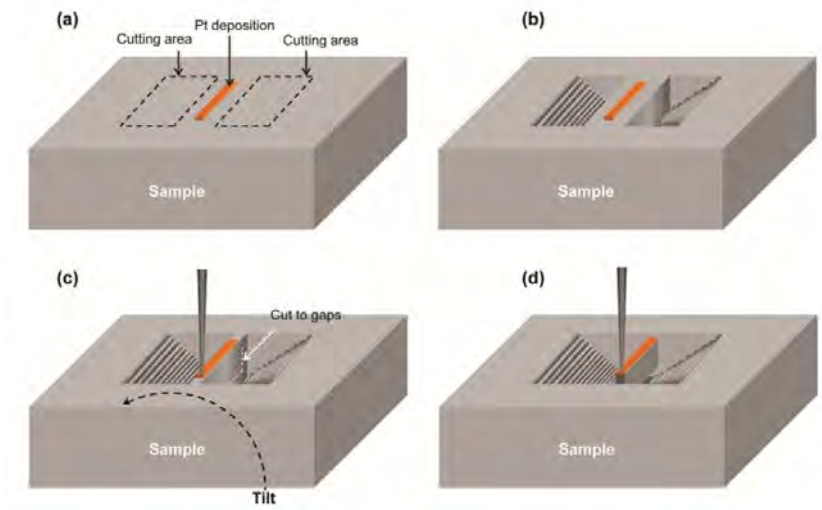


Disc grind, dimple grind and ion mill to perforation

TEM specimen preparation 5: Focused Ion Beam





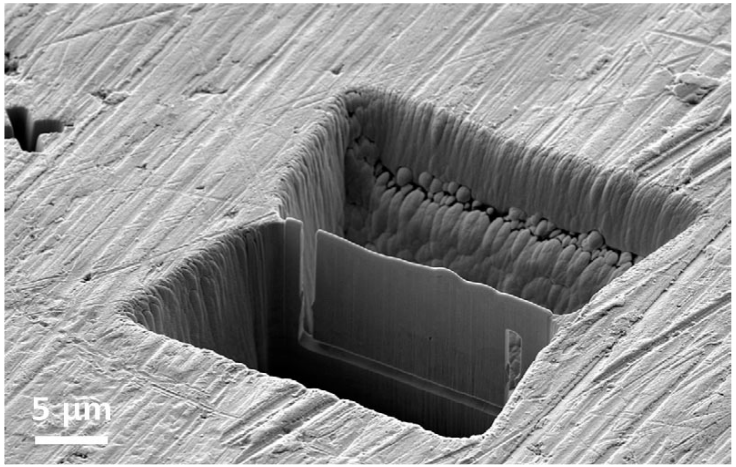


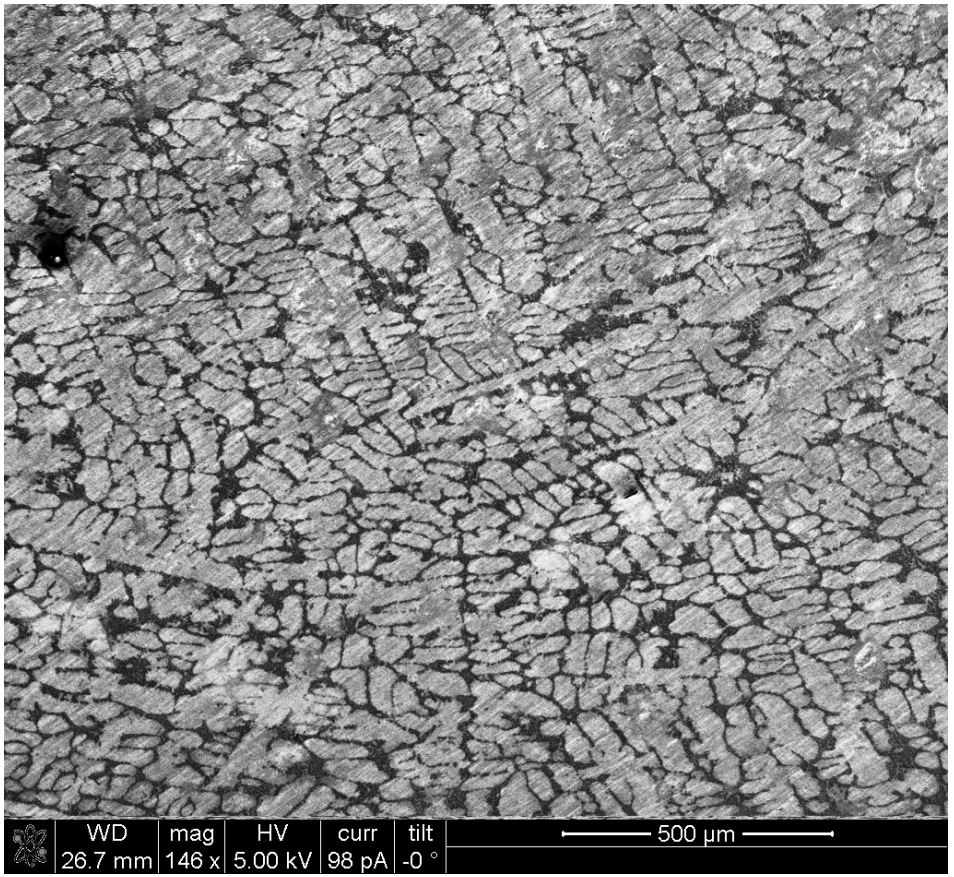
FIB sample preparation was shown in Lide's FIB lecture

https://youtu.be/vNOpzDViAhE

Example 1: TEM specimen preparation- Using a Focused Ion Beam

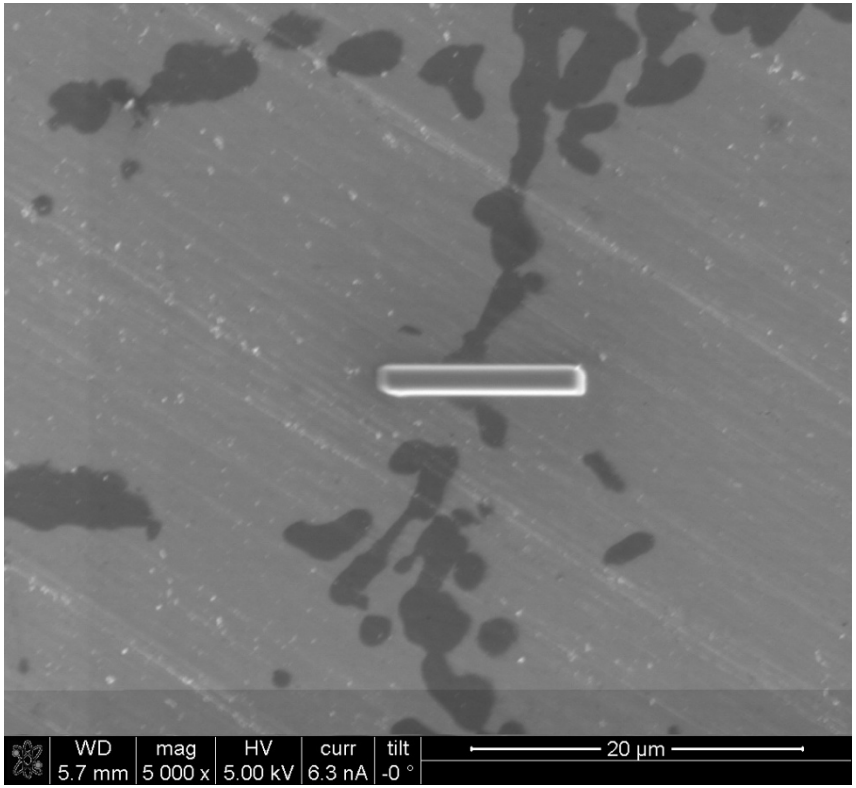
- The alloy microstructure is shown alongside. It has a two-phase microstructure.
- Sample composition: Magnetic cobalt alloy with Fe and Ni additions.

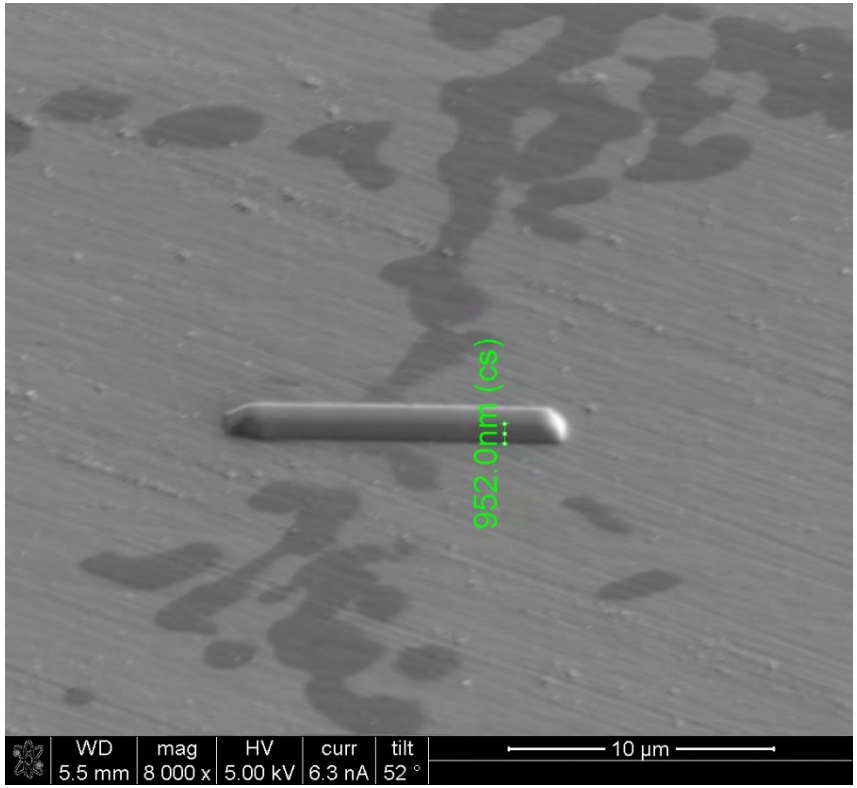




• After selecting the area to investigate, a micron thick strip of Platinum is electro-deposited to protect the area beneath from being contaminated by the Gallium ions.

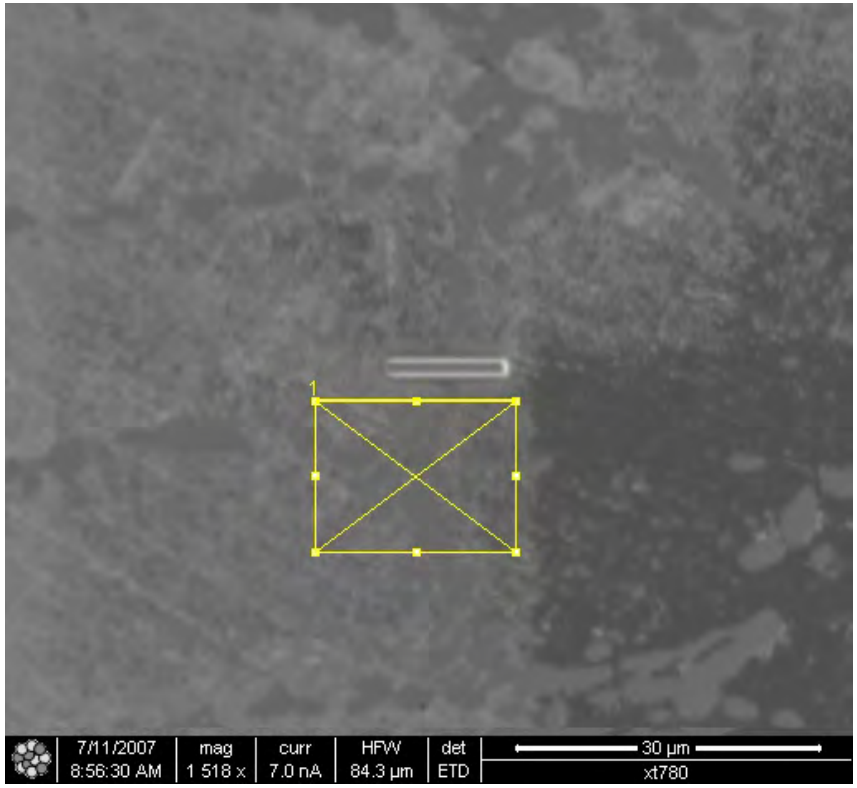
•The electron beam is maintained at 5 kV and a current of 6.3nA.



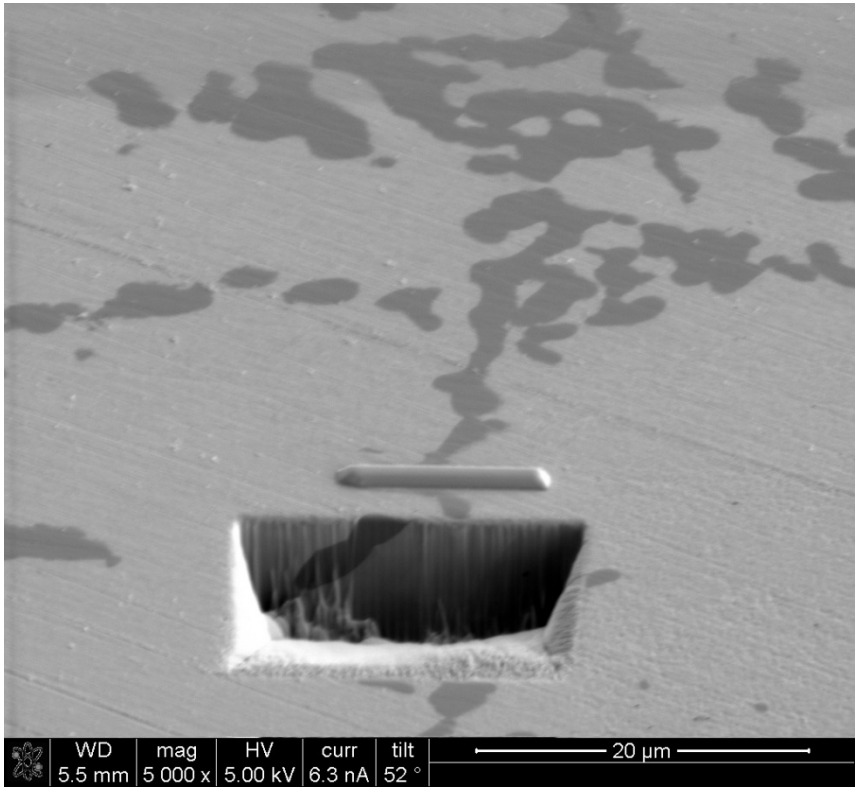


• The selection is made to mill away the specimen on both sides of the Pt strip using the Gallium ion beam maintained at 30 kV and 7 nA.

ION BEAM VIEW

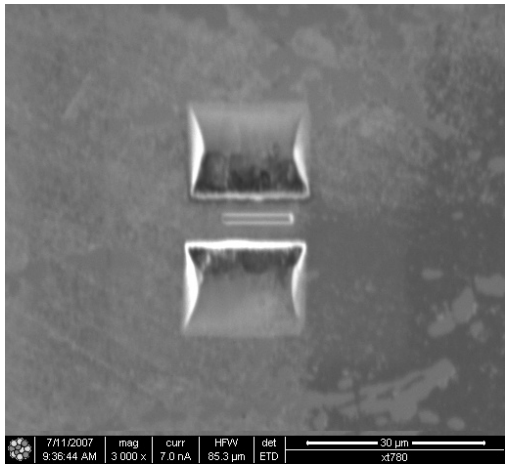


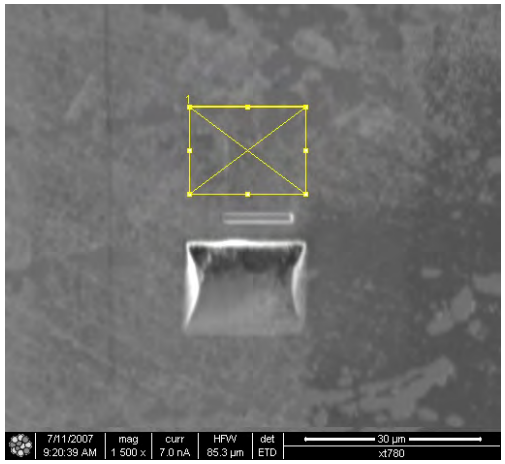
ELECTRON BEAM VIEW

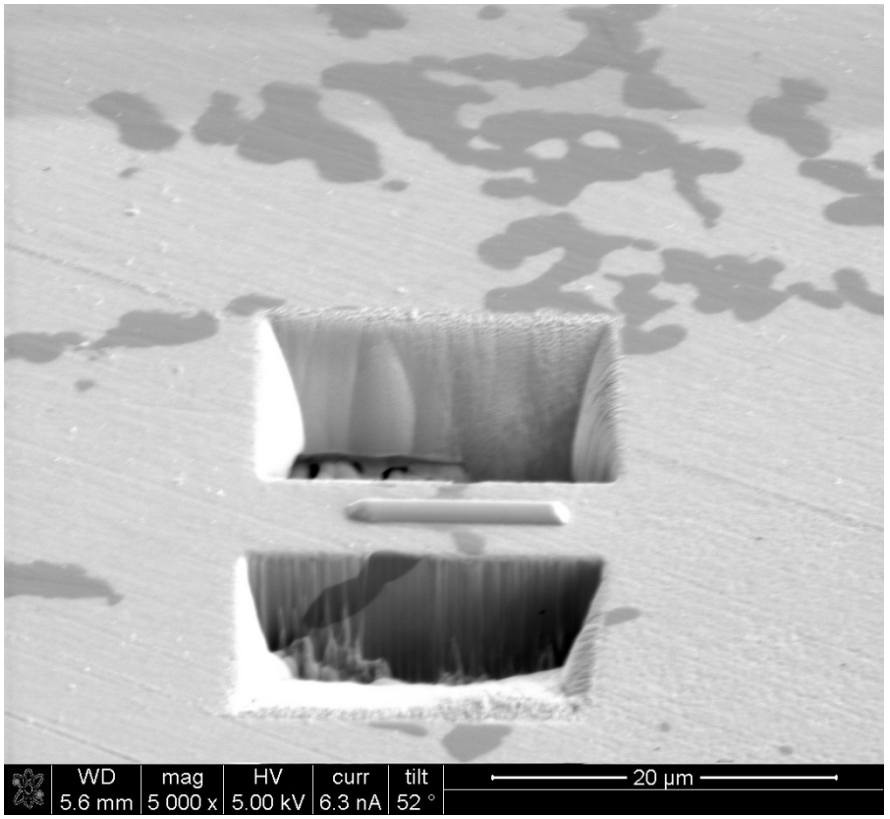


• The other side of the Pt bar suffers the same fate.

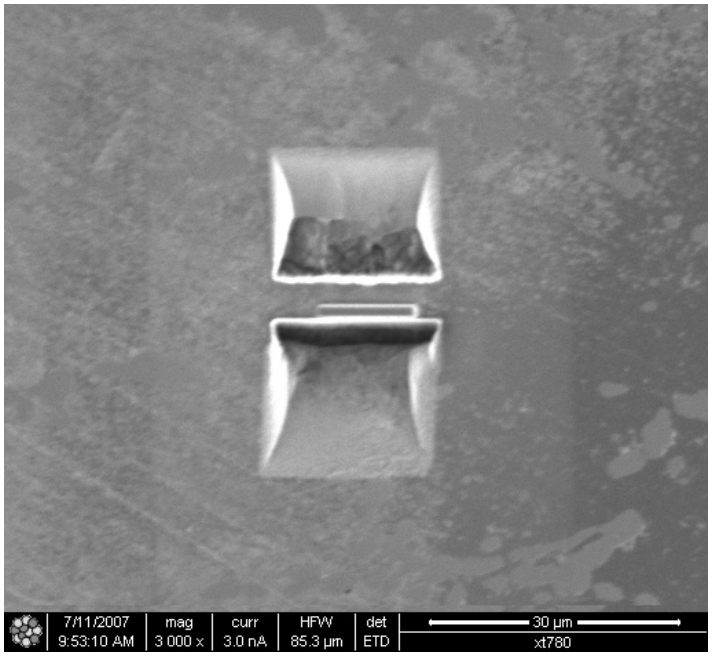
• Further thinning of the specimen on either side of the Pt strip is carried out at a reduced ion current of 3 nA. (see next page)



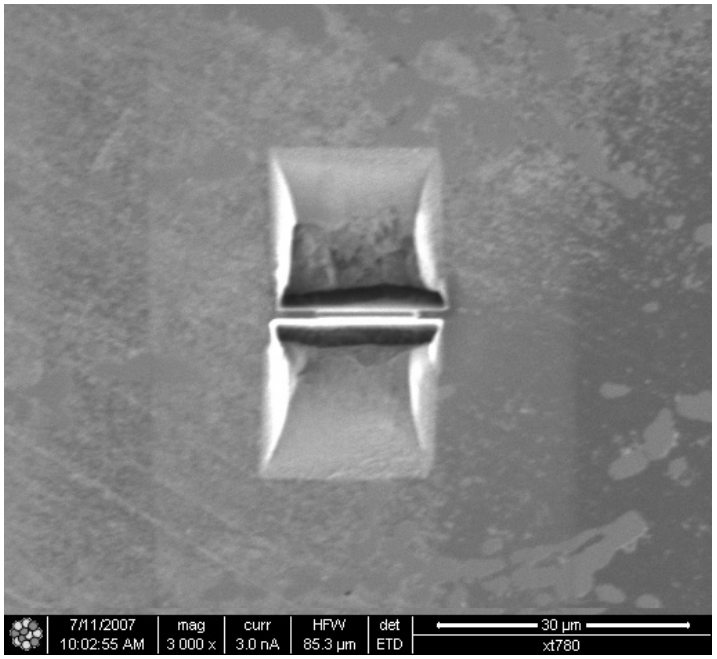




ION VIEW →

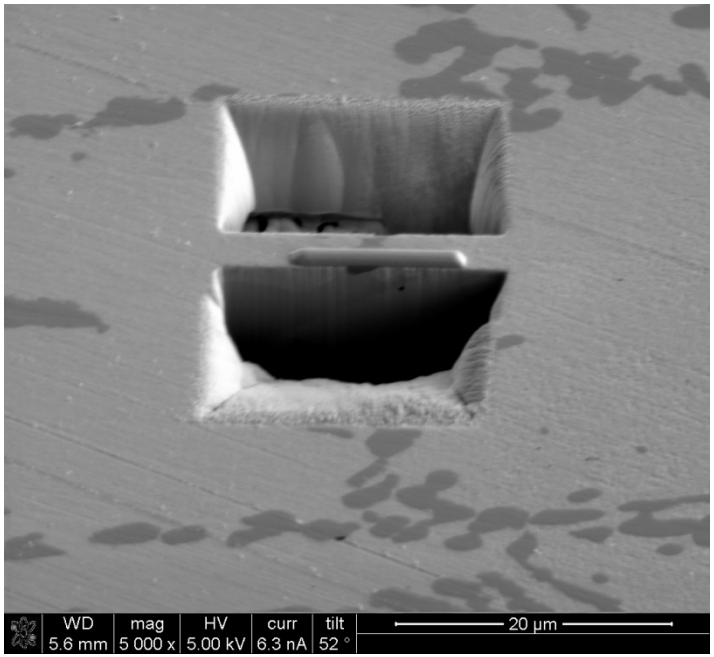


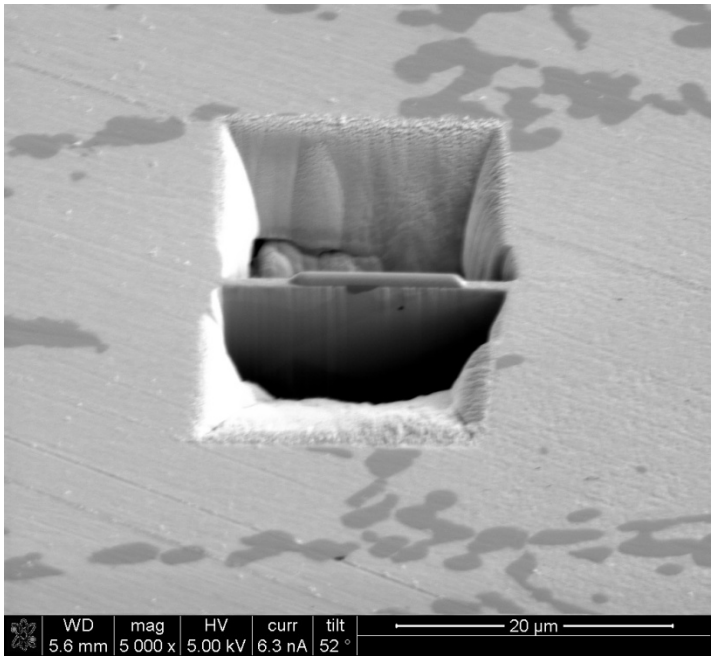
FURTHER THINNING



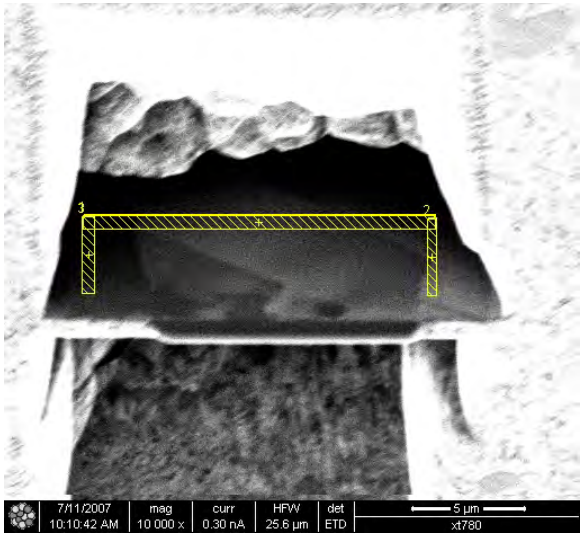
← ION VIEW

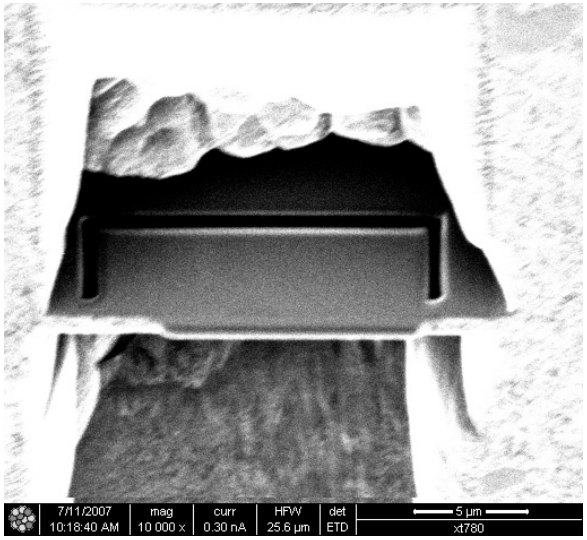
ELECTRON VIEW →



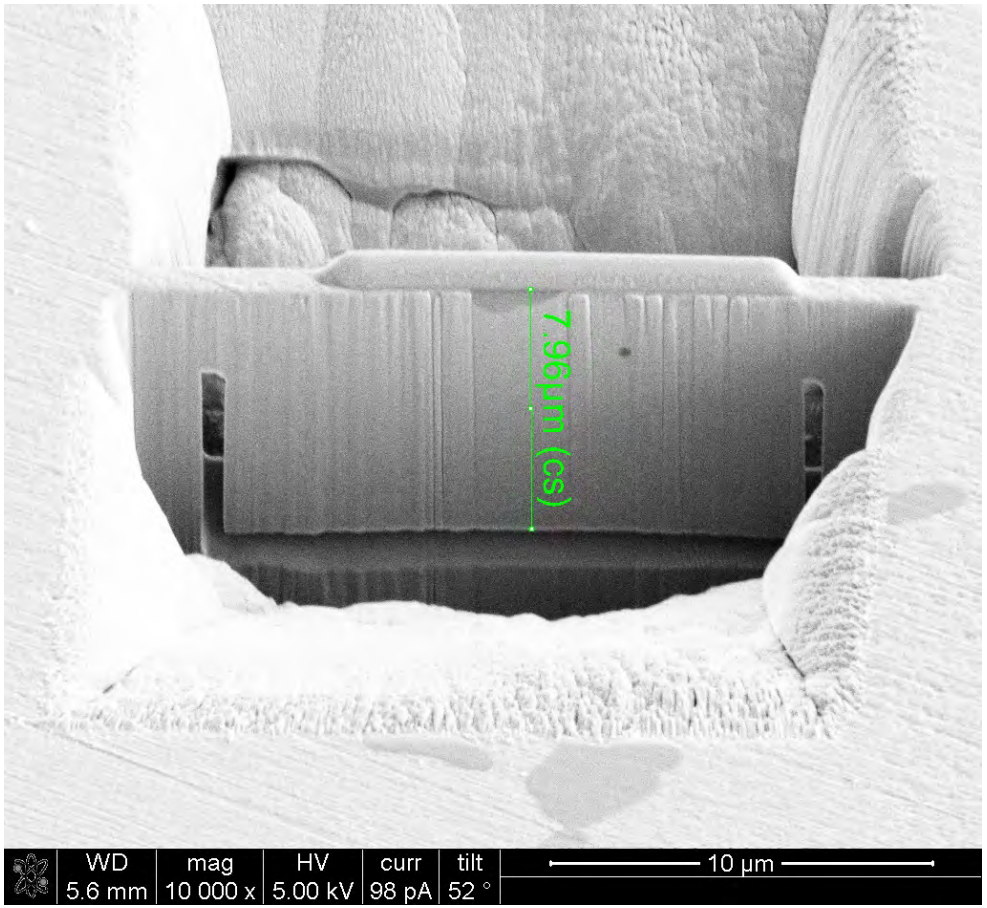


← ELECTRON VIEW



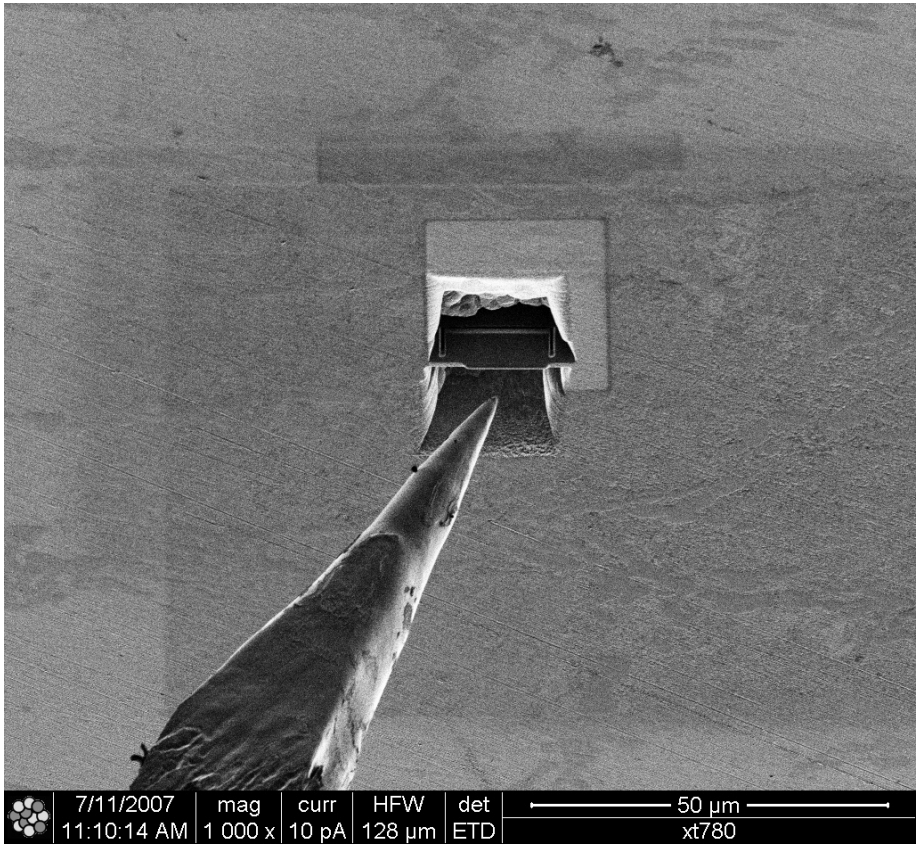


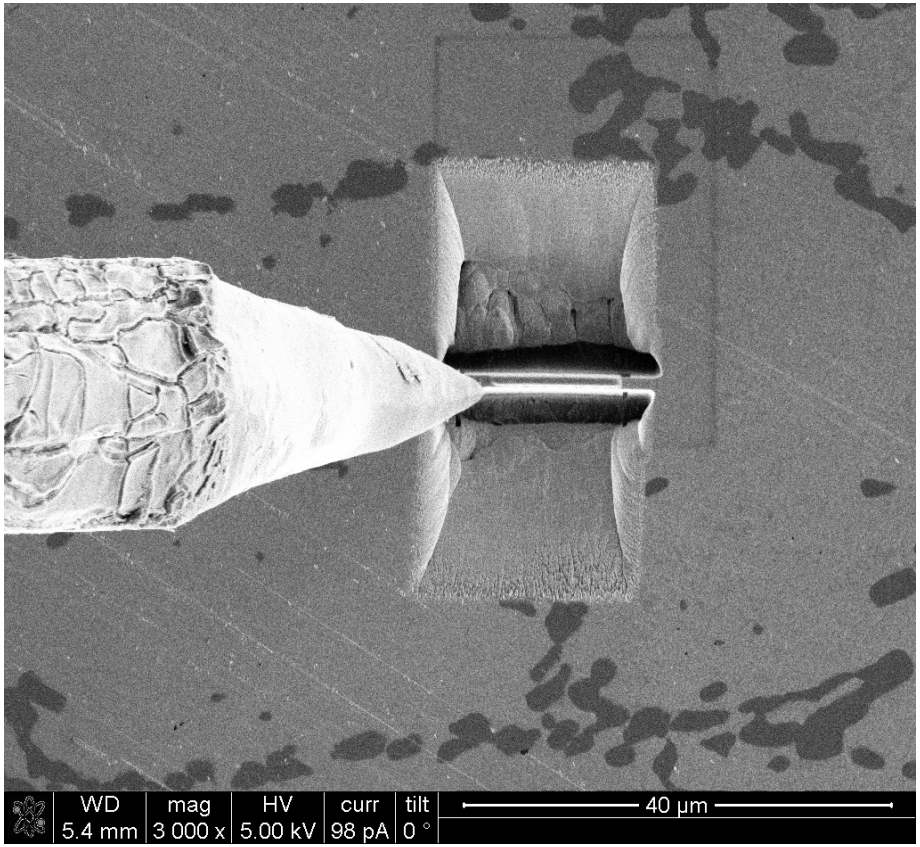
The sample is tilted to 7° (from horizontal) and areas for the release cuts are marked, and the approximate z-depth (on the order of the thinned slab) is entered and the selected areas are cut…



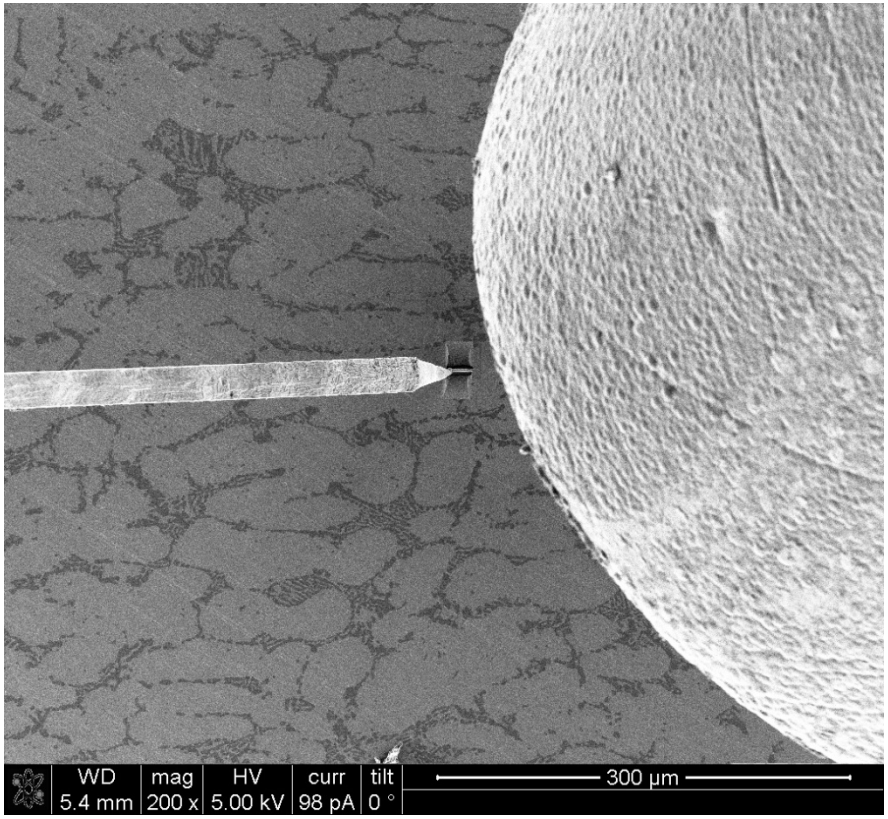
The ion current is decreased further to 0.3 nA.

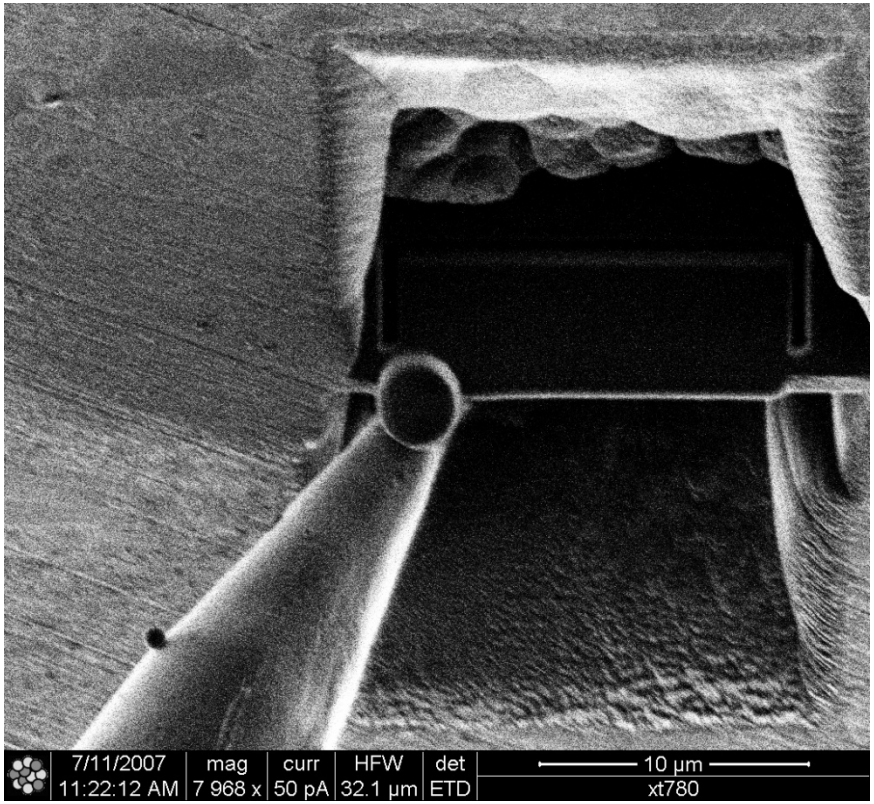
- A probe is now affixed to the side of the specimen.





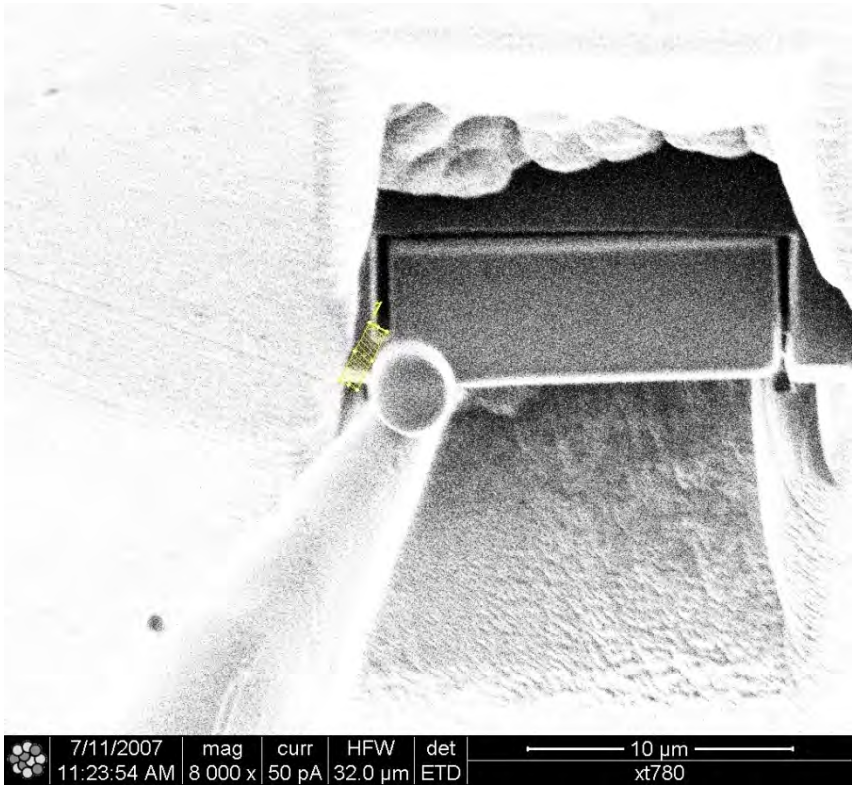
- Pt deposition at the probe tip binds it tightly to the strip

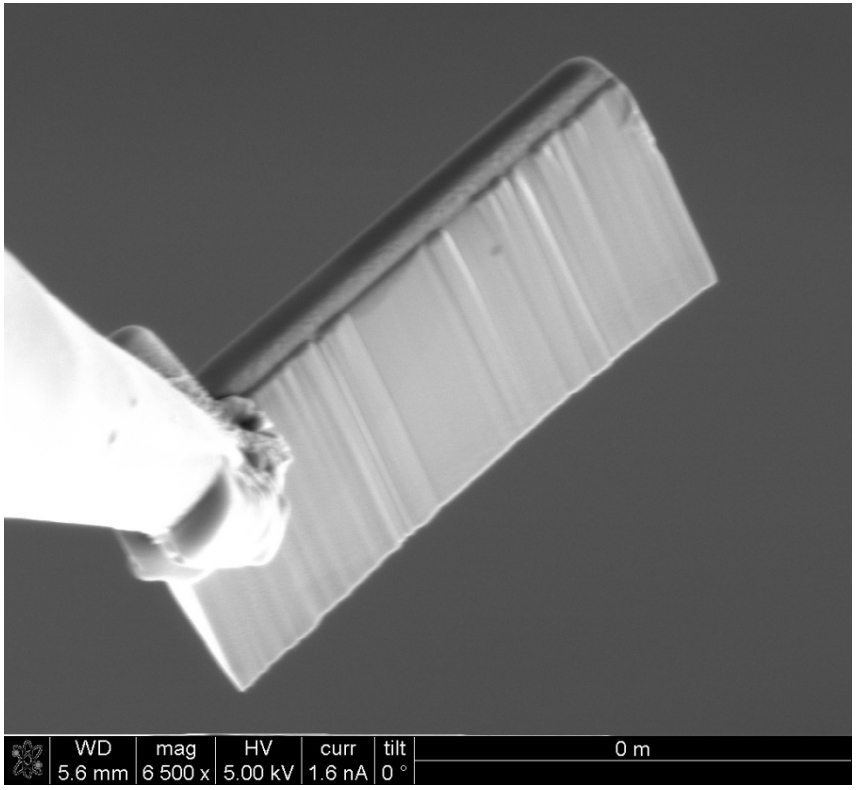


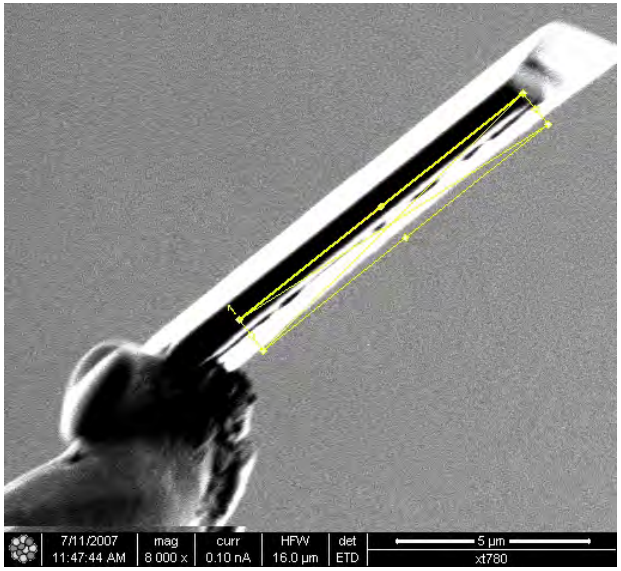


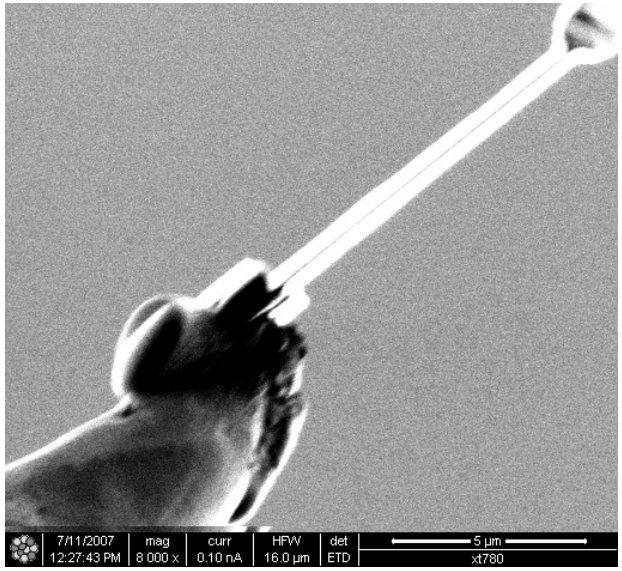
• The final release cuts are now made to free the specimen from the underlying bulk. These cuts are made at very low current of 10 pA.

• The final polishing (see next slide) is made to thin the specimen even further with an ion beam current of 100 pA.

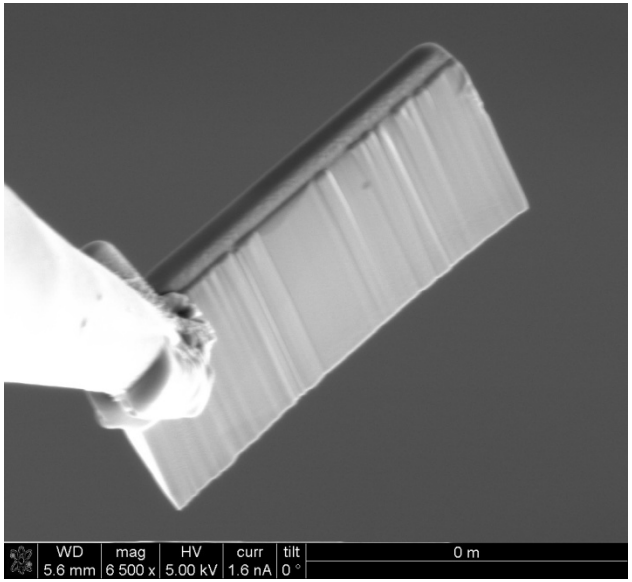


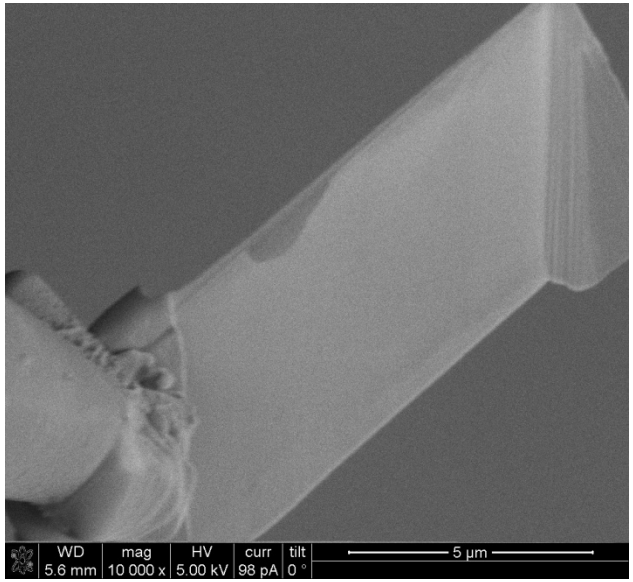






Finally Thinned





- Rest of the slides are just supporting information…. (not lectured in the Course this Spring)

Staining:

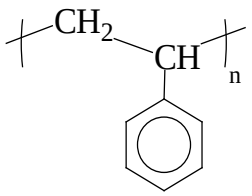
Polymers contain typically only light elements: such as *Carbon C, Hydrogen H, Oxygen O and Nitrogen N* → Electron density difference is very small between the different polymer domains. →Contrast is very weak.

Solution: *Selective staining with haevy elements*

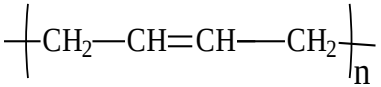
Example:

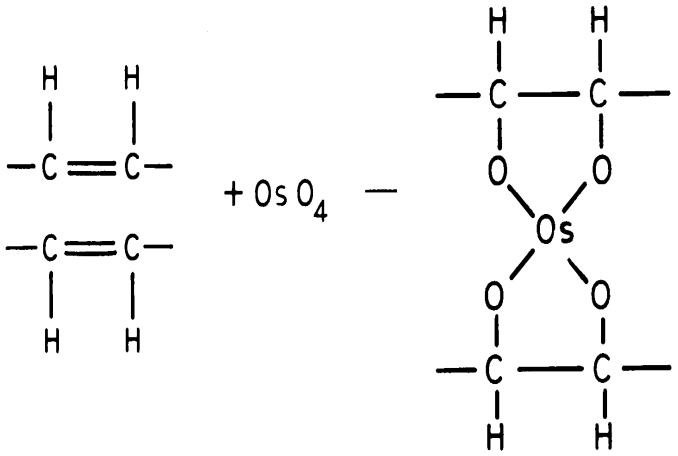
Osmium tetroxide OsO_4 staining

Polystyrene
(glass)



Polybutadiene
(Rubber)





Staining

Staining: involves the incorporation of ***electron dense*** atoms ***selectively*** into the material in order to increase electron density and therefore contrast.

Either pre-staining before the ultramicrotomy or ***post-staining the thin films***.

Negative or **positive** staining

Positive staining: Sample is stained (this is commonly used in polymer/sorf materials staining)

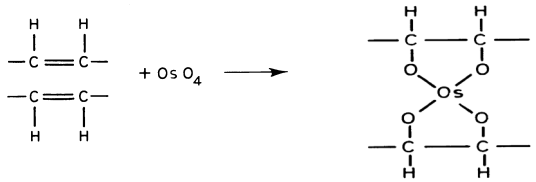
Negative staining: Outside are from the sample is stained (sample shape …e.g latex particles, viruses…)

Typical staining agents:

- Osmium tetroxide
- Ruthenium tetroxide
- Iodine
- Phosphotungsten acid
- Uranium salts
- Chlorosulfonic acids

Osmium tetroxide OsO_4 (Toxic)

Most common use: to stain multiphase polymers which contains double bond.



Stains and hardens (cross-links)

Cross-links also eye mucous membranes !!!

Available in solid crystals or water solution.

Staining: Sample sections in **osmium vapor** or inside water solution

Staining time approx. 5 min – 2h for thin sections

Typical polymers which are stained with osmium:

Polybutadiene block copolymers Example. Thermoplastic elestomers SBS

Polybutadiene blends

Polychloroprene (stains and hardens).

PET (stain amorfous domains/Crystalline domains are not stained)

HIPS (high impact polystyrene) (stains the rubber phase)

Polyuretanes

Ruthenium tetroxide RuO_4

More reactive than OsO_4 – makes reaction also with aromatic rings (example. Polystyrene)

Examples: Polystyrene blends

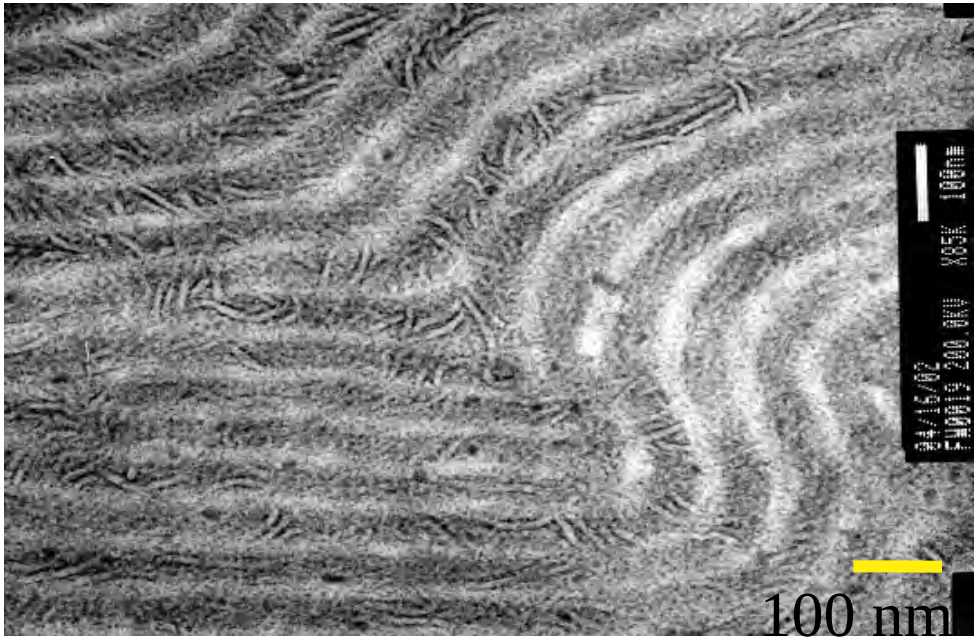
Polyethylene PE and polypropylene PP polymers (stains only amorphopus domains)

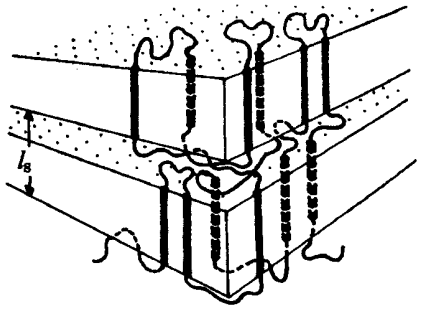
Stains polymers which contains following chemical goups:

- Ethers
- Alcohols
- Aromatics
- Amines

Example RuO_4 pre-staining – polyethylene block copolymers

sPP-PE diblock copolymers





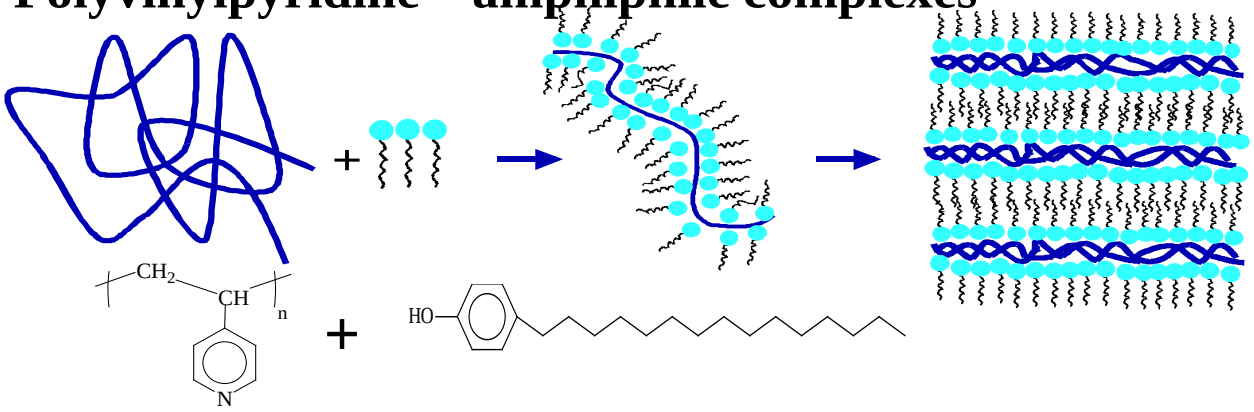
Crystalline domains are not stained (appear white in the image)
Amrophous domains are stained and dark in the image

Iodine I_2

Example: Nylons, Polyvinylalcohol, PET,Cellulose, Polyvinylpyridine

Does not make as strong interaction as Os, Ru, → will evaporate more easily in vacuum

Example: Polyvinylpyridine – amphiphile complexes



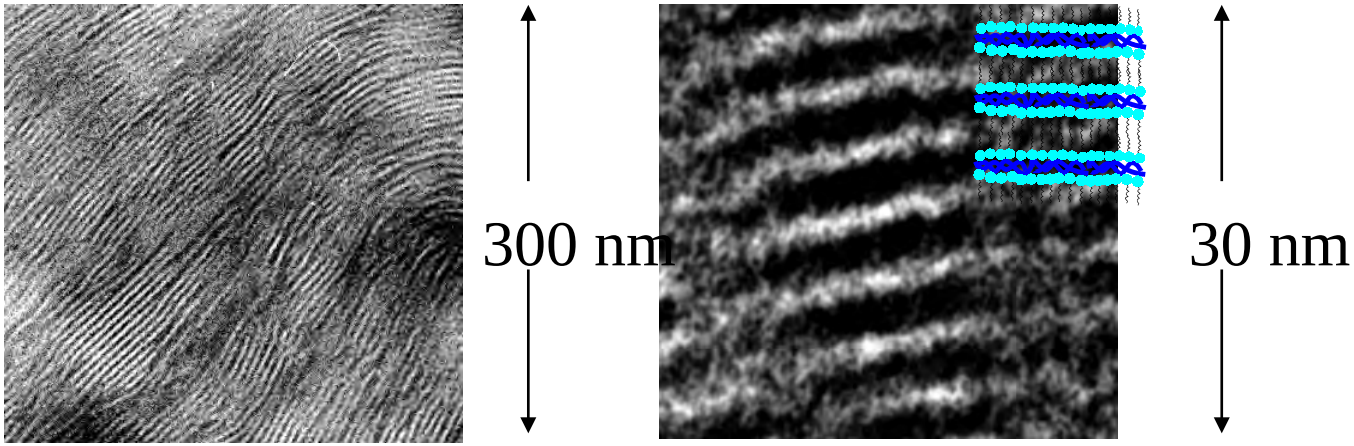


Table 4.1 Polymer functional groups and stains

Polymers	*Stains*
Unsaturated hydrocarbons, alcohols, ethers, amines	Osmium tetroxide
Acids or esters	(a) Hydrazine (b) Osmium tetroxide
Unsaturated rubber (resorcinol–formaldehyde–latex)	Ebonite
Saturated hydrocarbons (PE and PP)	Chlorosulfonic acid/ uranyl acetate
Amides, esters and PP	Phosphotungstic acid
Ethers, alcohols, aromatics, amines, rubber, bisphenol A and styrene	Ruthenium tetroxide
Esters, aromatic polyamides	Silver sulfide
Acids, esters	Uranyl acetate

Table 4.3 Osmium tetroxide staining

(a) Multiphase polymers stained by OsO_4
Acrylonitrile–butadiene–styrene Acrylonitrile–styrene–acrylate Styrene–butadiene–styrene High impact polystyrene Impact poly(vinyl chloride)
Copolymers of 1,4-polybutadiene and *cis*-1,4-polyisoprene
Blends with unsaturated rubber, isoprene, or isoprene included in fibers, e.g. PET or nylon
Polyoxyethylene allyl included in membranes

(b) Polymers requiring pretreatment prior to OsO_4	
Polymer	*Pretreatment*
Acids, esters	Hydrazine
Epoxy thermosets	Tetrahydrofuran
Ethylene–vinyl acetate copolymers	Alkaline saponification
Chlorinated PE	Bicyclic amine
Polyesters	Allyl amine

Table 4.2 Specific functional groups, examples and stains

Functional group	*Examples*	*Stains*
–CH–CH–	Saturated hydrocarbons (PE, PP) (HDPE)	Chlorosulfonic acid Phosphotungstic acid Ruthenium tetroxide
–C=C–	Unsaturated hydrocarbons (Polybutadiene, rubber	Osmium tetroxide Ebonite Ruthenium tetroxide
–OH, –COH	Alcohols, aldehydes (Polyvinyl alcohol)	Osmium tetroxide Ruthenium tetroxide Silver sulfide
–O–	Ethers	Osmium tetroxide Ruthenium tetroxide
$-NH_2$	Amines	Osmium tetroxide Ruthenium tetroxide
–COOH	Acids	Hydrazine, then Osmium tetroxide
–COOR	Esters (butyl acrylate) (polyesters) (ethylene–vinyl acetate)	Hydrazine, then Osmium tetroxide Phosphotungstic acid Silver sulfide Methanolic NaOH
$-CONH_2$ –CONH–	Amides (nylon)	Phosphotungstic acid Tin chloride
Aromatics	Aromatics Aromatic polyamides Polyphenylene oxide	Ruthenium tetroxide Silver sulfide Mercury trifluoroacetate
Bisphenol A based epoxies	Epoxy resin	Ruthenium tetroxide

Sample and knife aligment ...

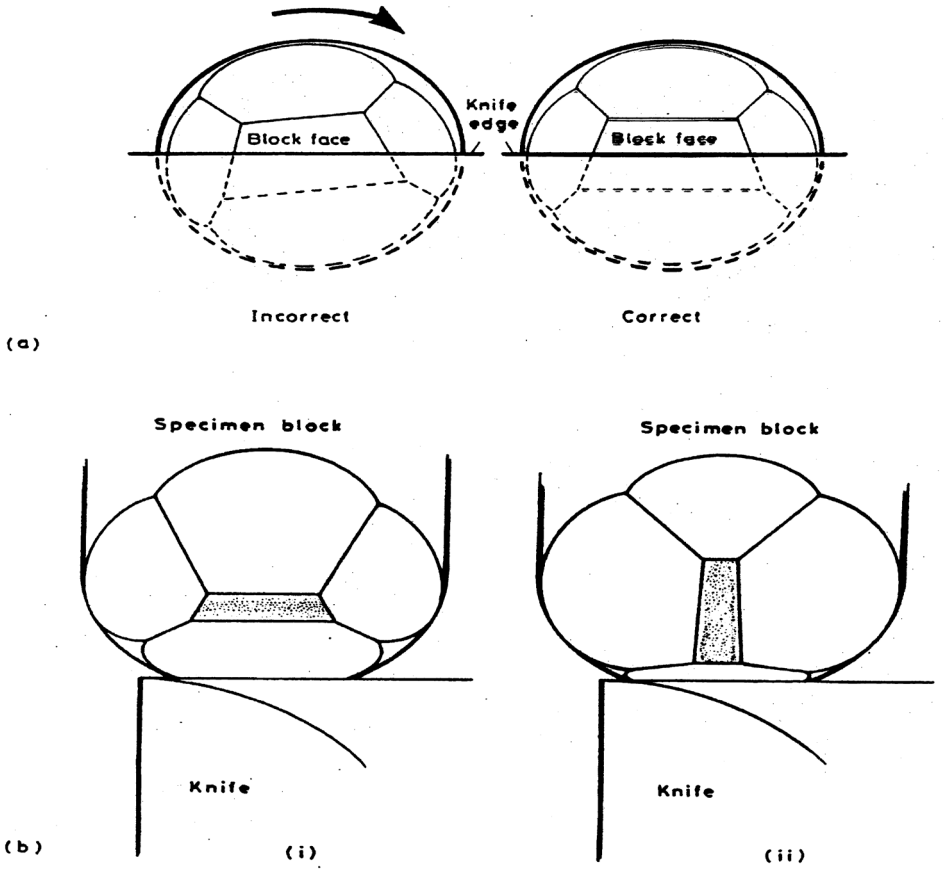


Fig. 6.3. (a) The specimen block is orientated so that the upper and lower edges of the block face are parallel to the knife edge. The broken lines indicate the part of the face that is behind the knife when viewed from the front. (b) When the block face has a large area and (i) the long edges of the block face are parallel to the knife edge, vibrations may occur and result in banding or chatter. (ii) Orientation of the block with the short edges of the block face parallel with the knife edge will reduce this effect.

Sample and knife aligment ...

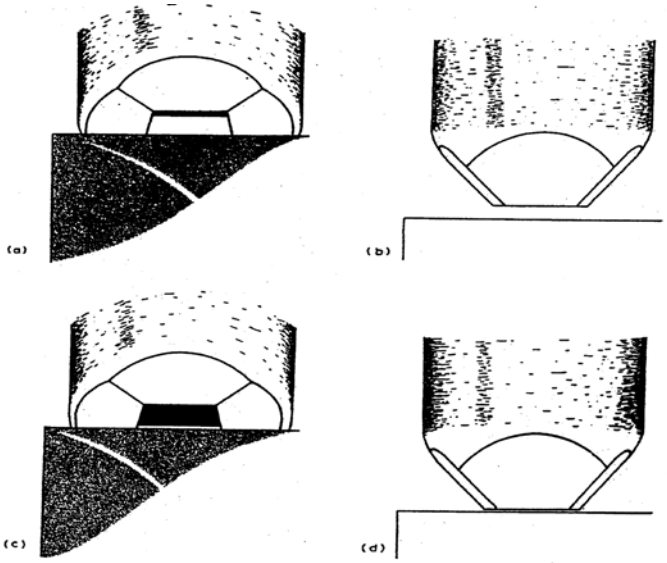


Fig. 6.6. (a) The gap between the knife and block with the knife at a distance away from the block face, as seen (a) by the reflection method, and (b) viewed from above. The appearance when the knife is almost touching the block, as seen (c) by the reflection method, and (d) viewed from above.

How to determine *the distance between the sample block and knife*

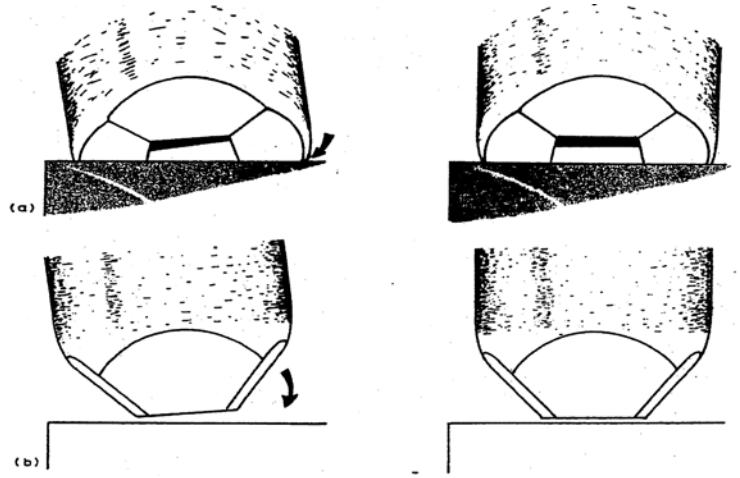


Fig. 6.4. (a) To ensure that the whole of the block face is cut, the specimen holder is adjusted until the knife edge is parallel to its reflection seen in the mirror-like block face. Either the block holder or the knife stage is turned, whichever is easier. (Viewed at a small angle from the vertical.) (b) When viewed from the vertical position, the upper edge of the block face should be parallel to the knife edge.

How to see that sample and knife are parallel ... (horisontal)

Sample and knife aligment ...

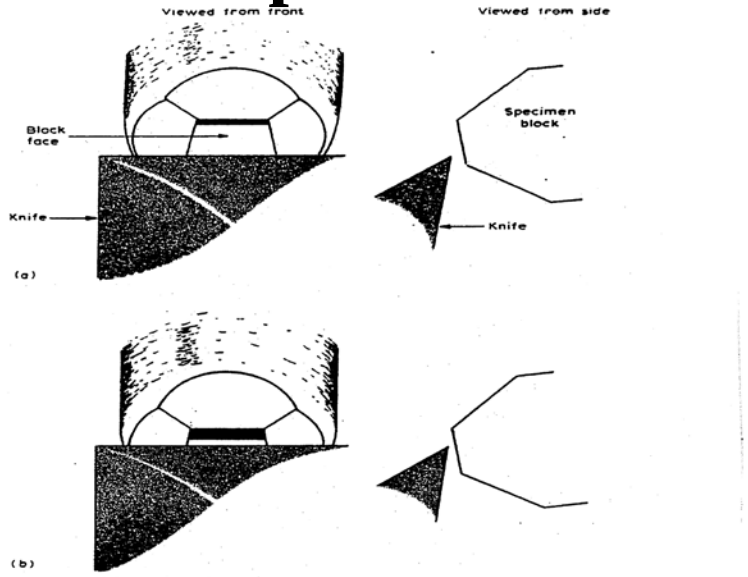


Fig. 6.5. Vertical alignment of the specimen block. If the gap, between the block face and the knife (shown as a white area), is not the same when the specimen arm is at the top of its stroke (a) and at the bottom of its stroke (b), the specimen block should be adjusted vertically until the gap remains constant (c,d) (see page 166).

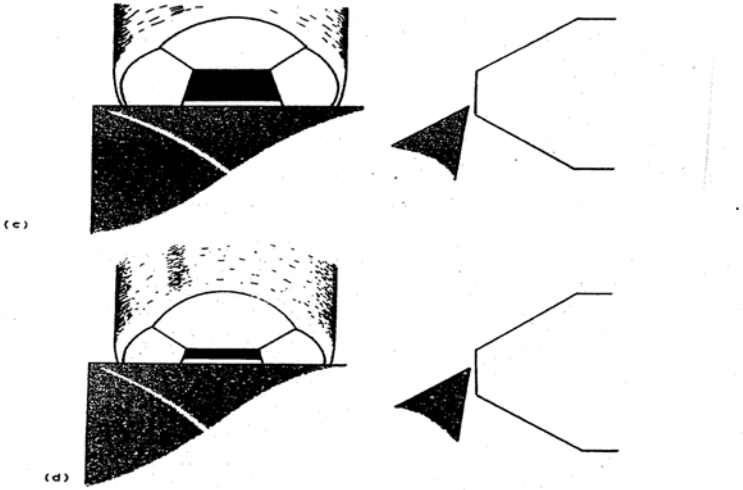


Fig. 6.5 (continued). The specimen block has been adjusted vertically so that the gap between the block face and the knife (shown as a white area) remains constant (c,d).

How to see that sample and knife are parallel ... (vertical)

Determination of the section thickness

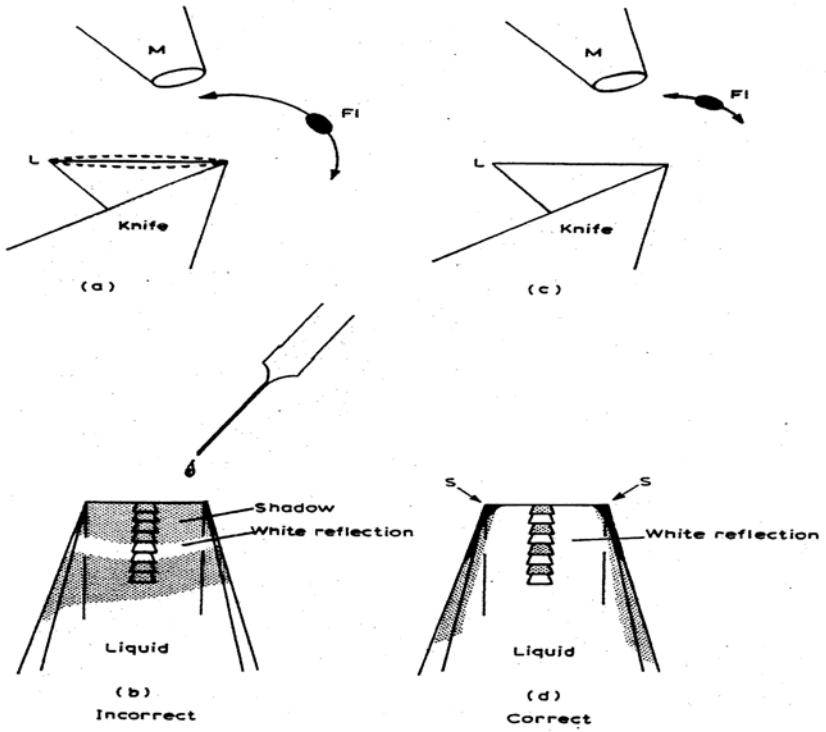


Fig. 6.7. Judging the thickness of sections by viewing them in reflected light. The reflection depends on the position of the binocular microscope (M), the fluorescent light (Fl), and the liquid level (L) in the knife trough. (a) If the water level is too high, or too low (indicated by the broken lines), or the light is in the wrong position, the reflection will appear as in (b) where only a small area of the liquid surface has the correct reflection and the thicknesses of the sections are not easily judged. (c) The correct positions with the water level approximately horizontal and a small reflected angle between the light and the microscope. (d) The correct reflection reveals the true colours of the sections and any variation in thickness, and therefore of colour is easily seen. There are usually small shadows (S) in the corners where the trough joins the knife edge and these are useful in checking for the presence of external vibrations

Determination of the section thickness

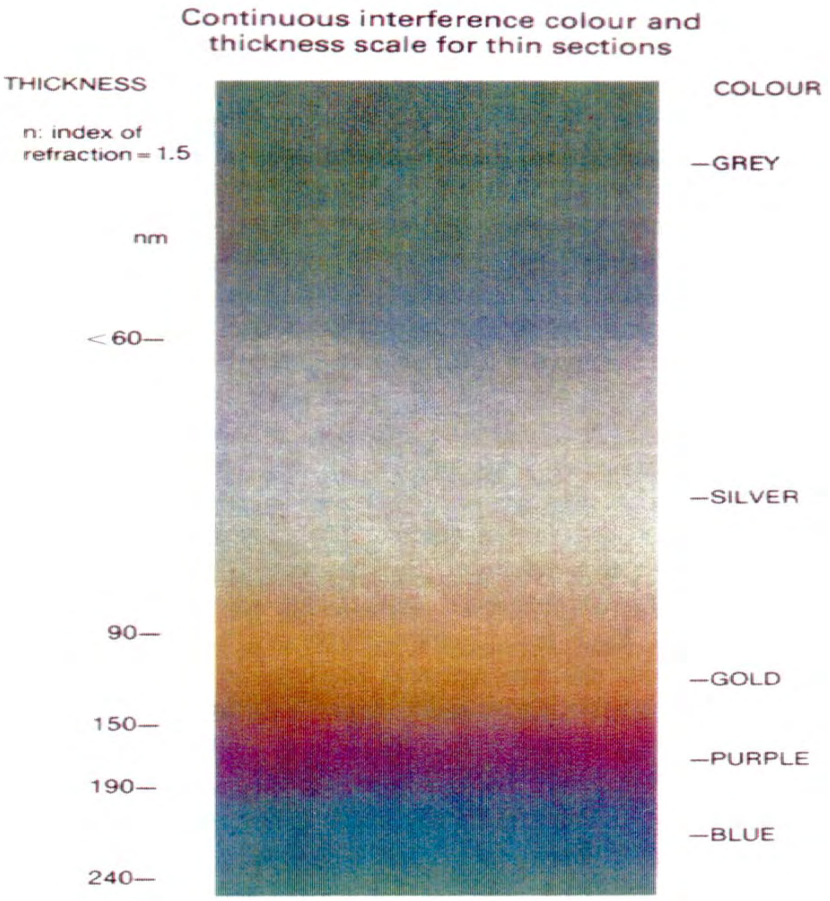
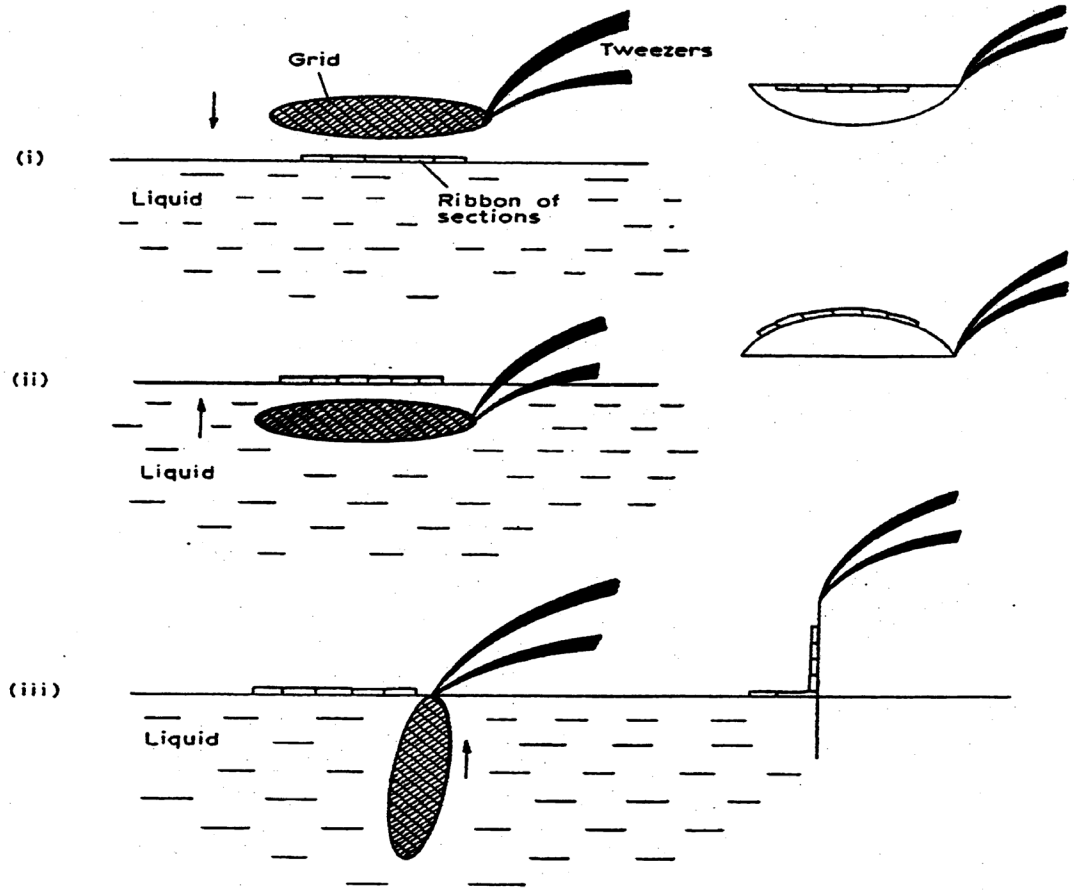


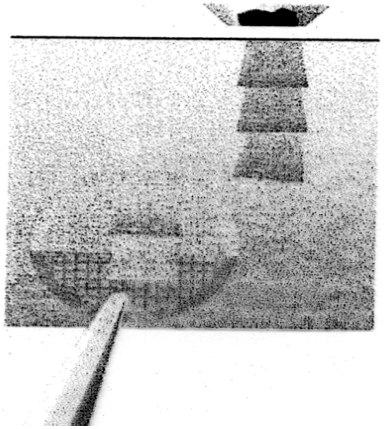
TABLE 9.2
Interference colour and section thickness as determined by measurement of re-sectioned sections (data from Yang and Shea 1975)

Original section Interference colour	*Re-sectioned section Mean thickness (nm)*
Darkest grey	15.7
	16.5
Dark grey	20.3
Grey*	30.1
Grey	41.1
Silver	48.9
Silver*	52.2
Silver	55.7
Silver*	60.2
Yellowish silver	66.5
Yellowish silver	67.5
Pale gold*	77.8
Pale gold	78.4
Pale gold	81.8
Pale gold	89.0
Gold	94.7
Dark gold	105.1
Dark gold	109.0

*chloroform-stretched section

Collectin sections onto the specimen grid (RT)





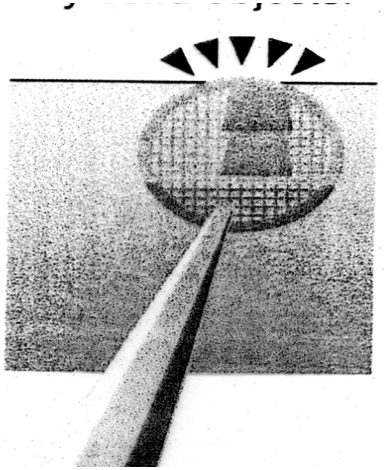


Fig. 8.6. Methods for collecting sections from the trough. (i) The grid is held in a horizontal position and is lowered over the sections. After removal from the trough the sections are covered with a droplet of liquid. This is carefully drained away with filter paper. (ii) The grid is lowered into the liquid behind the sections and is brought into a horizontal position beneath them. The grid is then lifted directly upwards. After collection the sections are floating on the surface of a droplet of liquid and this is carefully blotted away with filter paper. (iii) The grid is submerged and held vertically just behind the ribbon of sections. As the grid is withdrawn from the trough the sections are brought into contact with it, either by moving the ribbon with an eyelash probe or by a slight forward movement of the grid. The grid is then lifted vertically upwards and the sections are drawn up with it.

Ultramictoming Troubleshooting

1. Problem: Difficulty Wetting the Knife Edge
Solution:
•Clean the knife edge with alcohol (100%) and a cleaning rod.
•Or Fill the boat with distilled water until the water level is a little too high, wait a few minutes, and then carefully remove the excess water.
•Or A cleaning rod is passed over the cutting edge (the boat being full and mounted in position) using the same motion as the cleaning method.
•Or use eyelash to drawn over the tongue and then passed over the cutting edge of the knife while the boat is full and clamped in place.
If the cementing material is damaged and you suspect that this is the reason you can not wet the knife edge, please contact knife manufacturer so they may arrange to get your knife back and recement it for you.

2. Problem: Block Face is Getting Wet
Causes:
For epoxy resins: Block faces may get wet due to electrostatic charging (low room humidity and or transportation).
For methacrylates: Some of these embedding materials are hydrophilic and tend to wet the block surface because they attract water (Lowicryl, LR White, etc.).
Solution:
For epoxy resins:
•Lower the water level ever so slightly.
•Dry the block face with filter paper.
•Eliminate electrostatic charging with an antistatic device.
For methacrylates:
•Lower the water level to a concave shape.
Two problems will become pronounced by lowering the water level.
•You will have difficulties wetting the edge.
>> To combat this just follow the steps outlined above in edge wetting.
•You will have difficulties with reflection.
>> To alleviate your reflection problem you should adjust the light source to the appropriate angle.
If your ultramicrotome does not allow for adjustment of the light source proceed as follows:
•Tape a small piece of aluminum foil to the light source.
•Slowly bend the foil until you reach your desired reflection.

Problem 3: Poor ribbon formation

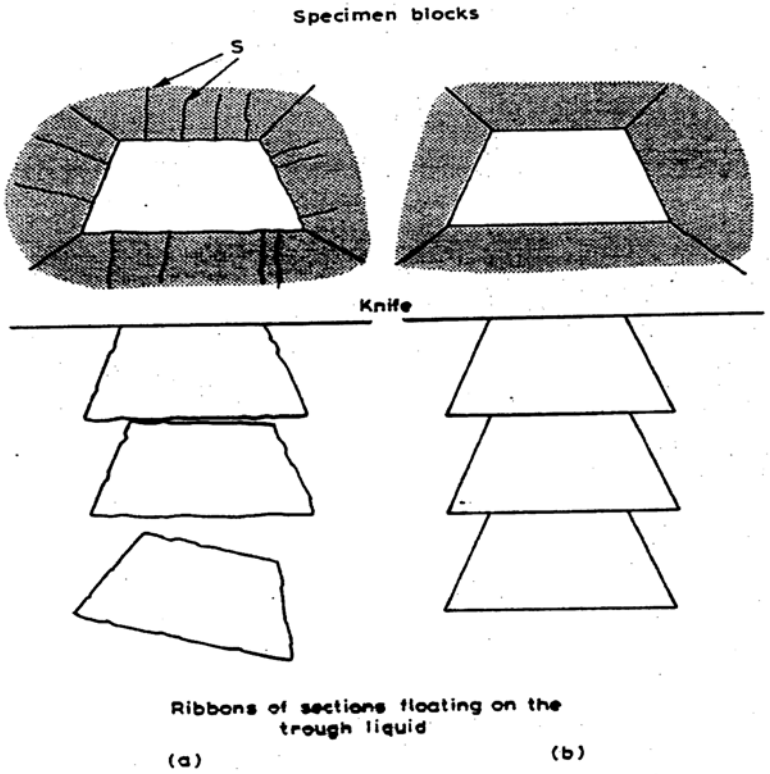


Fig. 7.5. Failure to form a ribbon of sections. (a) When a damaged blade is used for trimming the specimen block, score marks (S) are made in the sides of the block and cause the edges of the block to be uneven. The sections are then only in contact at a few points and easily separate. (b) When the edges of the block face are smooth, good contact between the sections is ensured and a stable ribbon is formed.

Block is not well trimmed or trimming knife is damaged?

Problem 3: Poor ribbon formation

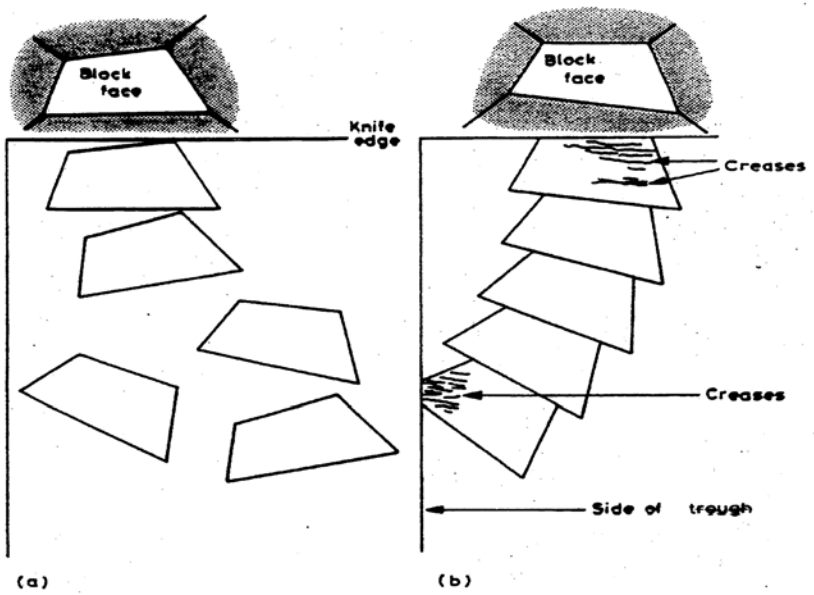


Fig. 7.6. (a) When the upper and lower edges of the block face are not parallel to each other and only the lower edge is parallel to the knife edge, the sections only have point contact and quickly separate. (b) When only the upper edge of the block face is parallel to the knife edge, the sections form a ribbon but it curves to one side of the trough. There is then a danger that the movement of the ribbon will become obstructed by the side of the trough causing creases to appear in the sections. A curved ribbon also forms when part of the section is compressed by a blunt knife.

- ylä- ja alasivut eivät ole yhdensuuntaisia.

Muita syitä:

- terän toinen reuna tylsempi
- nestepinnan väärä korkeus
- liian nopea leikkaus
- ilmavirtaukset

Problem 4: Compression

Causes:

•The block is too soft.
•The knife angle is too big.
•The knife is dull.
•The clearance angle is too big and or the cutting speed is too high.

Solution:

•Make the blocks harder.
•Switch from the 45 to the 35 degree angle knife (or 25 degree or ultrasonic).
•Send the knife back to us for evaluation and possible resharpening.
•Reduce the clearance angle by 1-2 degrees and reduce the cutting speed from 1mm/sec to 0.5mm/sec.

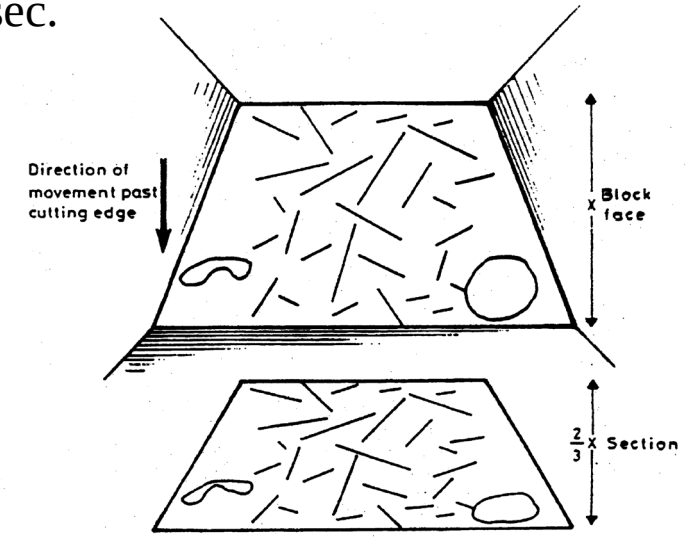


Fig. 7.10. Compression during cutting has resulted in an altered orientation of the structures within a section in the direction of cutting.

Problem 5: Striations

Causes:

- Very fine imperfections in the cutting edge.
- Poorly polymerized blocks.
- Inhomogenous blocks.

Solution:

- Make sure the blocks are fully polymerized.
- Change the block.
- Try another portion of the cutting edge.

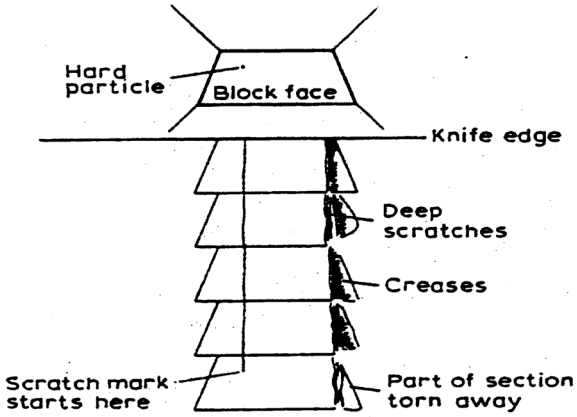


Fig. 7.3. A variation in section thickness on either side of a demarcation line which is perpendicular to the knife edge indicates that a part of the knife edge is damaged. The right-hand side of the knife edge was damaged before sectioning commenced and consequently the scratches appear from the start of the first section in the ribbon. The sections are creased and part of one section has been torn away. The scratch on the left is not seen in the first part of the leading section, indicating that the damage to the knife edge was caused by contact with a particle within the specimen.

Problem 6: Chatter

Causes:

Chatter manifests itself in many ways and is caused by different reasons.

- External vibrations.
- A faulty microtome.
- Screws are not fully tightened(block, block holder, and knife).
- Cutting pressure is too big.
- Clearance angle is too small (may cause friction between the block face and the diamond face).

Solution:

- Change the location of the microtome.
- Have the microtome checked by a service engineer.
- Make sure all of the screws are tightened.
- Reduce the block width.
- Increase the clearance angle by 1-2 degrees.

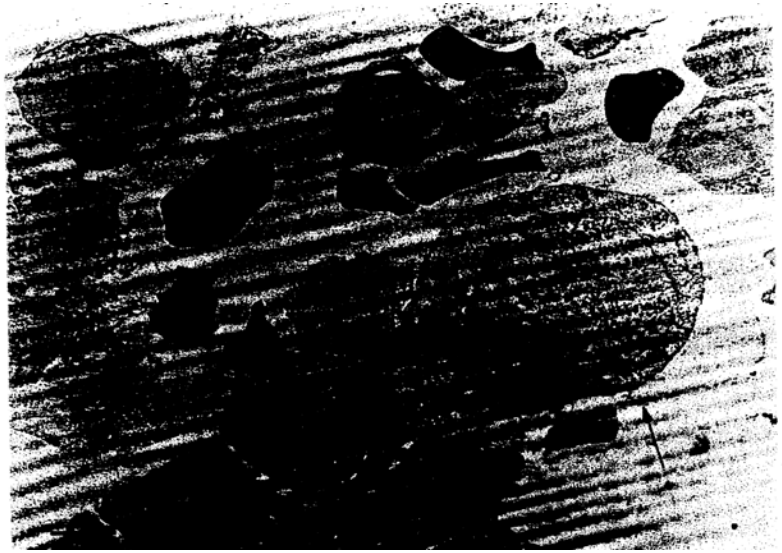


Fig. 7.9. An ultrathin section of bone marrow embedded in Araldite. Chatter appears as alternating light and dark bands resulting from closely-spaced variations in section thickness. The bands are perpendicular to the direction of cut which can be identified by the scratch marks (arrow). The bar indicates 2 μm.

10.6 Problems, Errors and Solutions

10.6.1 Overview

Despite careful operation, errors can still occur during sample preparation and due to the wrong choice of sectioning technique [13,19]. Instrumental errors seldom take place. The most common errors encountered during sectioning are the following:

- Inappropriate embedding is used
- Insufficient fixation or hardening is employed
- The surface area is too large to be sectioned
- The specimen and/or knife is loose
- There are defects or damage on the knife edge
- An unsuitable section thickness is chosen
- The wrong sectioning parameters (speed, temperature, etc.) are used
- An improper method of sectioning is applied
- There is a lack of skill in transferring the sections onto the grid
- Impure solvents and dirty apparatus are used
- Inhomogeneous materials with hard and soft inclusions (such as polymers with glass fibres or inorganic fillers) are being investigated

Typical errors and their origins are listed in Table 10.4.

10.6.2 Typical Errors and Possible Solutions

In the following, typical errors encountered during the different working steps and solutions to them are discussed in more detail.

Embedding

- Check whether other fixation methods without embedding are possible
- Choose the correct embedding agent, especially the final hardness
- Check whether the composition and homogeneity of the embedding agent is correct (the deviation from the standard composition may significantly affect the section consistency).

Staining (See also Chap. 13)

- Select the most suitable agent (usually RuO_4 and OsO_4)
- Check the staining procedure (e.g. whether the block material or the section is stained)
- Optimise the time and temperature of the staining.

Trimming

- Trim the surface to be cut properly (a pyramid-like surface is suitable)
- Sectioning surface area should be 0.1 mm × 0.1 mm or smaller depending on the sample material

Table 10.4. Typical errors and their origins

Characteristics of the errors	Possible origin
Periodic waves, different thicknesses along the section	– Damaged or blunt knife edge – Improperly or loosely placed knife – Needle-like trimmed sample – Elastic sample – Insufficiently fixed sample or sample holder – Building vibrations or contact with the ultramicrotome during sectioning – Sample is too soft
Single or several nonperiodic waves in a section	– Extreme building vibrations – Unstably positioned ultramicrotome
Changing thicknesses along an ultrathin section	– Inhomogeneities (i.e. hard and soft phases) in the sectioning area – Wrong trimming
Variation of thickness from section to section	– Air movement and temperature fluctuations in the laboratory – Section thickness attempted is too low – Material is too soft
Strongly compressed sections	– Wrong knife angle – Wrongly adjusted free angle – Sample material is too soft
Folds in ultrathin sections	– Use of a bad knife (damaged edge) – Sectioning velocity is too high – Very large sectioning area or sections are too thick
Impurities in sections	– Impure water used as floating liquid – Insufficiently cleaned apparatus (such as glass envelope, tweezers, needles, etc.) – Impure staining solution, excessive staining
Folds in sections on the grid	– Wrong floating and transfer of the sections onto the grid – Sections are too large