SUPPLEMENTAL FIGURES (SF) & SUPPLEMENTAL MOVIES (SM)
Harris et al., 2006
Uniform Serial Sectioning For Transmission Electron Microscopy

Double click on the Movies to view them.

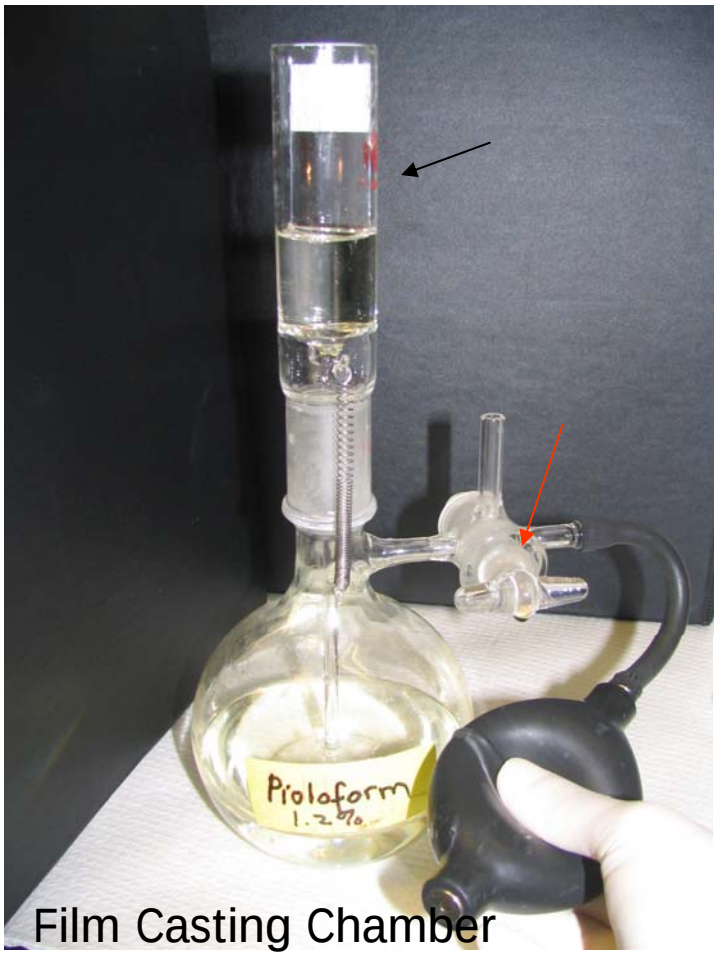
Some movies have music, which can be muted by muting the sound on your computer.

Movie Set 1

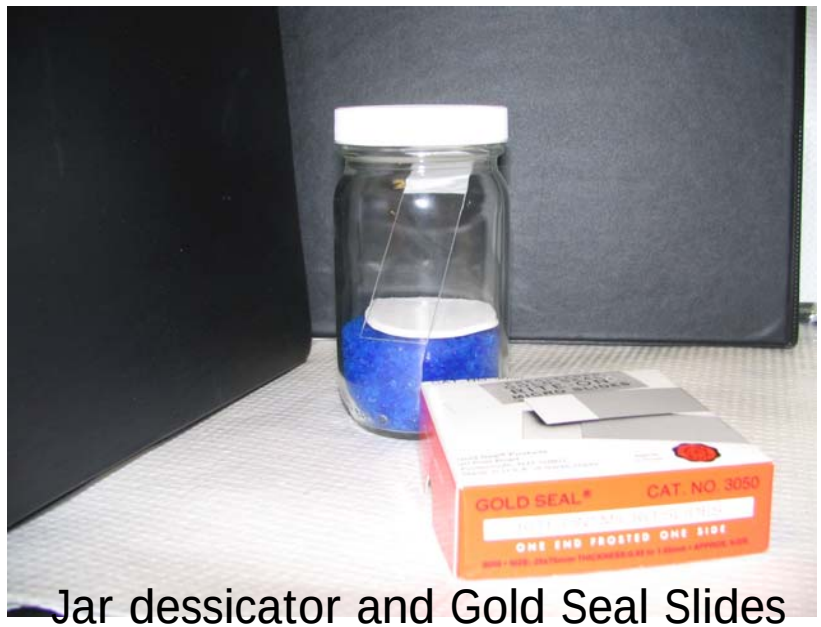
Preparing Slot Grids

Supplemental Figure 1
Pioloform-coating Slot Grids

A



Film Casting Chamber



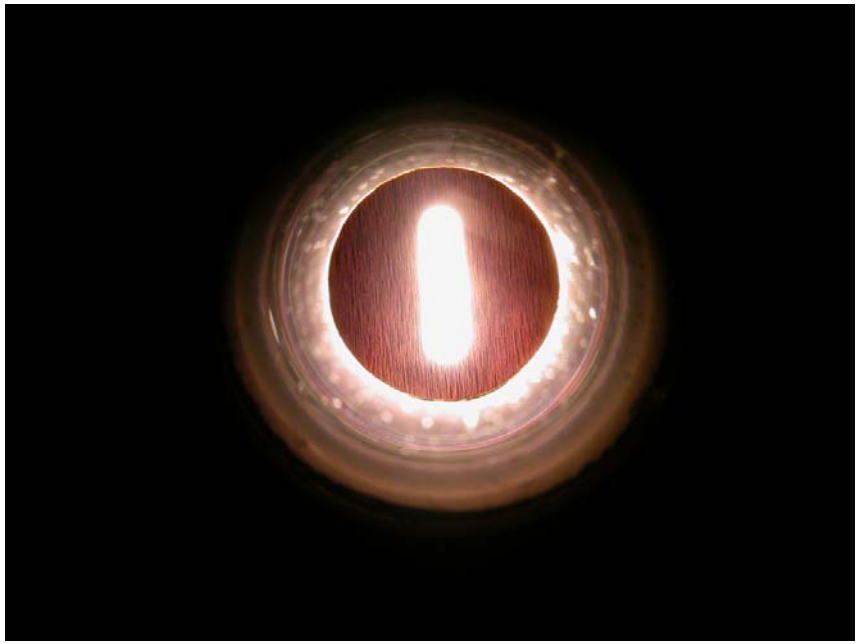
Jar dessicator and Gold Seal Slides

Make within 24 hours prior to serial sectioning!

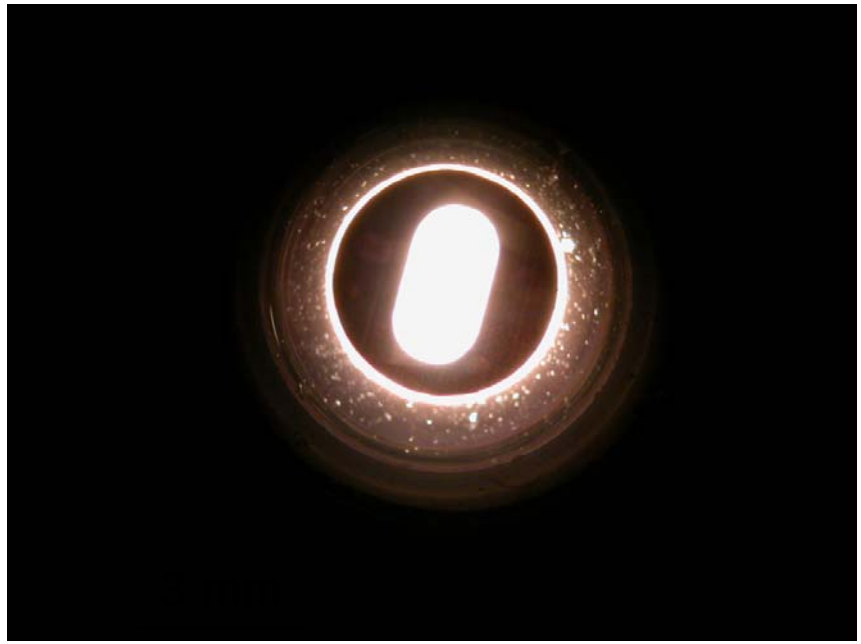
Pioloform-coating Slot Grids Procedure

- Prepare 250 ml of 1.2% Pioloform in Chloroform solution within 24 hours prior to coating grids.
- Pour Pioloform solution into the clean dry film casting chamber.
- Open stopcock (red arrow).
- Squeeze bulb to push Pioloform solution into the top cylindrical portion of the chamber (black arrow).
- Close the stopcock.
- Place a Gold Seal slide pre-cleaned slide into the full chamber.
  - If the slides are dirty, open a new box rather than trying to clean yourself.
- Slightly open the stop cock and slowly drain the chamber allowing the solution to cling to the slide as the fluid level drops.
  - The rate of flow is crucial to a uniform film.
  - If the flow rate is too rapid, you will have a thicker film.
  - If the flow rate is too slow, the film will be thinner.
- Immediately place slide into a dessicator jar lined with silica gel and filter paper.
  - This prevents moisture from collecting on the slide.
- Dry for one minute.

Supplemental Figure 1:
Synaptek™ Slot Grids



0.5 mm width
More stable, more difficult to center a long ribbon



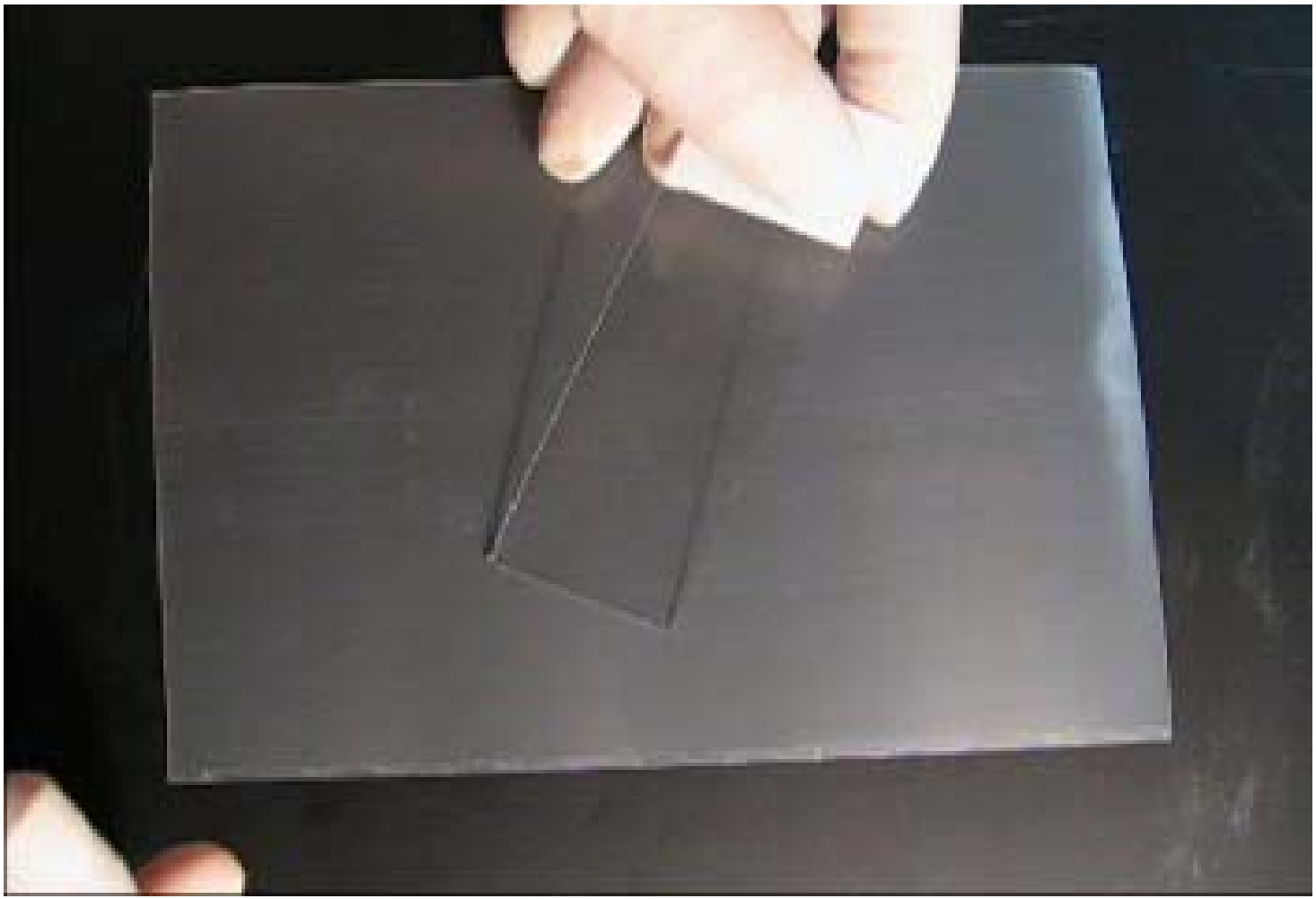
1.0 mm width
Less stable, easier to center a long ribbon

(stiff - beryllium-copper, 4 mil (100µm), from EMS or Ted Pella)

Slot Grid Preparation Procedure

- Use clean Synaptek™ beryllium-copper slot grids – notch style is best.
- (4 mil thick (100µ) and 3.05mm in diameter available at EMS and Pella).
- Narrow 0.5x2.mm slots shown on the left decreases the surface area of the film and are more stable, but more difficult to pick up a long ribbon in the center of the slot.
- Wider 1x2mm slots are somewhat less stable and more susceptible to folding under the weight of the ribbons, but will accommodate larger sections and provide for easier pickup.
- Grid cleaning:
  - Sonicate in absolute ethanol (5 min)
  - Sonicate in acetone (5 min)
  - Air dry in covered petri dish (dust free environment).
  - Can store clean un-coated grids indefinitely in clean dust free Petri dishes.
- Use Parafilm or tape to seal the edges so that you don’t accidentally spill them!

Supplemental Movie1
Scoring and Floating Pioloform Films



Scoring and Floating Pioloform Films Setup

- Obtain a rectangular Plexiglass shield to protect the floating film from room dust.
- Suitable dimensions are 2 feet wide, 16 inches deep, and 16 inches high with a closed back and base
- Fill a Glass Dish covered with black paper with fresh Dionized water and place in the center of the shield towards the front.
- Place a lamp inside the shield at a back corner to illuminate the surface of the water.
- Place a Petri dish containing clean Synaptek Grids at the back of the chamber
- Place a curved forceps on filter paper near the Petri dish.
- Place a clean box of gold Seal slides in the back of the shield.
- Cut 2 strips of Parafilm each about 3 inches square and place in the shield.
- Place one clean large (150 mm by 15 mm) covered polystyrene dish in the shield for storing the coated grids at the end.
- Place two acetone-cleaned and water rinsed single-edge razor blades to one side inside the shield.

Scoring and Floating Pioloform Films Procedure

- Darken the room to optimize viewing the reflection surface.
- Score the film with a razor blade on both sides of the slide to enable the film to release from both sides of the slide as it is floated onto the surface of water.
- Quickly dip the slide vertically into the water one time and slowly pull it out (first film clip of this movie).
  - Nothing should float off at this time.
- Still in a vertical position, slowly lower the slide into the water a second time to release the film from both sides of the slide (second film clip).
  - At the camera angle of this film clip it is not possible to see the films – it merely illustrates the speed used.
- If you have difficulty releasing the film simultaneously from both sides, slant the slide, and lower at an angle (third film clip).
- Films with silver to platinum interference colors provide the best stability with the least background graininess in the scope.
- Gold to purple films are discarded as too thick.
- Gray to clear films are discarded as too thin.
- Uniformly colored silver/platinum films with no wrinkles, folds, tears or other imperfections are used.

Supplemental Movie 2:
Placing grids notch side up on silver Pioloform film.

Placing grids notch side up on silver Pioloform film.

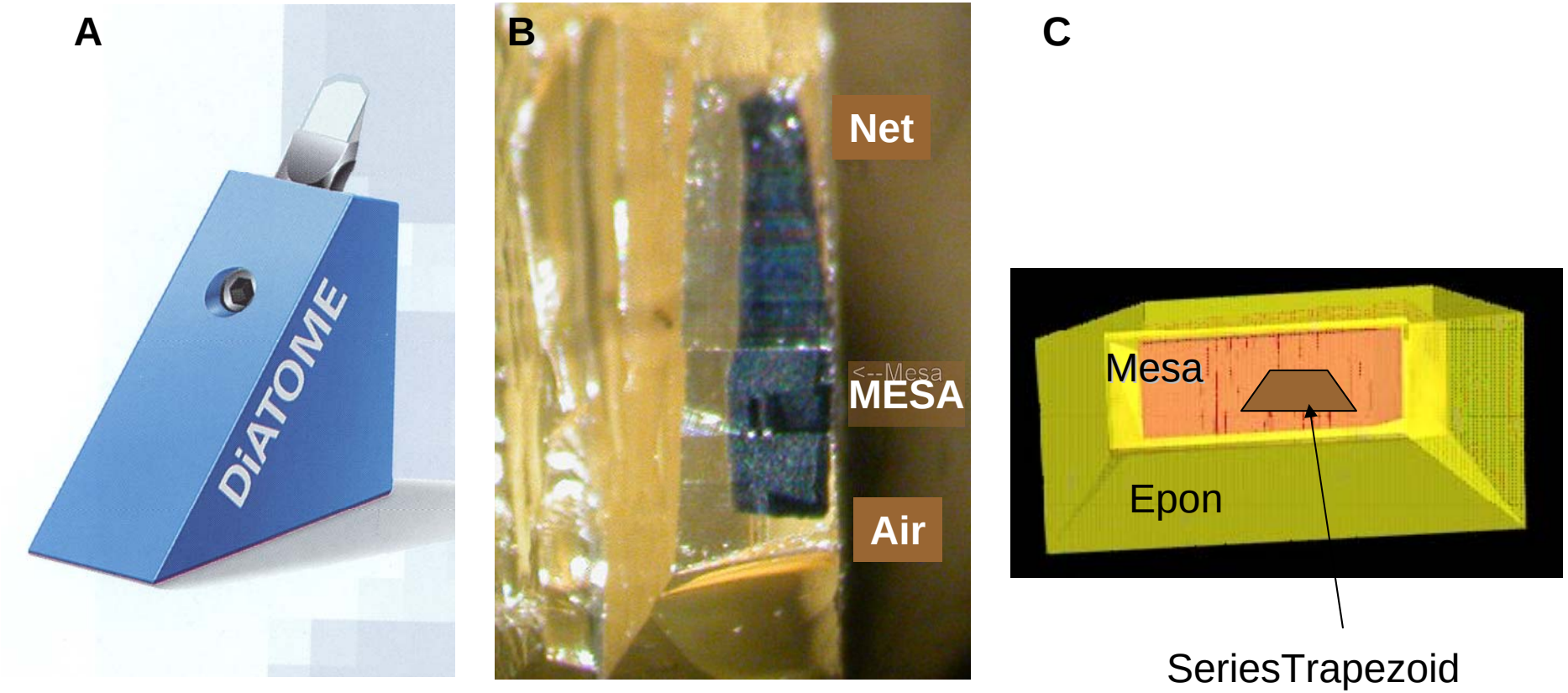
- Move Petri dish containing the clean Synaptek Grids towards the front of the shield for easy access.
- Use curved forceps to pick up a grid and gently drop it, notch side up, onto the floating Pioloform film.
- Gently tap down on the edges of the grid to stretch and adhere the Pioloform film across the matte, non-notch side of the grid.
  - See also supplemental Movie 9
- Wrap a clean, dry Gold-seal glass slide with the Parafilm strip.
- Position the Parafilm-coated slide lengthwise over the floating film.
- Push the top of the coated slide down along the upper edge of the floating film
- Use a continuous smooth motion to scoop up and collect the grids onto the Parafilm with the Pioloform film side up.
- Set the slide inside the covered polystyrene petri dish and allow the grids to dry overnight before using.

Supplemental Movie 3:
Stretch and secure film on grids

Movie Set 2

Preparing Trapezoid

Supplemental Figure 3
Trimming Mesa and Trapezoid



Supplemental Figure 3 Legends

- A: Use the Diatome CryoTrim 45^{o} Trim Tool for precise Series Trapezoid Trimming

- B: First trim a Mesa near to the final region of interest to provide a platform for preparing the Series Trapezoid. The height of the Mesa should be about 50-100 microns to provide a stable base without removing all of the underlying tissue, which might be returned to later.

- C: The Series Trapezoid is trimmed at about 20 microns height on top of the Mesa – as illustrated in SM1 and 2.

Supplemental Movie 4:
Series Trapezoid E-W side Trimming

North

West

East

South

Series Trapezoid E-W side Trimming Procedure Part A

- Load the specimen block into the chuck.
- Set the knife stage, cutting arc, block and chuck angles in the 0 position.
- Set cutting arm to 1 micron per turn and aspeed of 1mm per second.
- Center the trim tool in front of the block face.
- Advance the trim tool to a safe distance about 1mm from the block face using the shadow cast by the trim tool knife edge.
- Slide the left edge of the trim tool to the east edge of the series trapezoid.
- Use the left “corner” of the trim tool to remove 20-30 microns of Epon, creating a 45 degree slope.
- Back the knife stage away.

Series Trapezoid E-W side Trimming Procedure Part B

- Remove Epon shavings with a small piece of tape (or brush) – being careful not to touch the blade itself.
  - The static attraction between tape and Epoxy Resin will usually be sufficient to remove the shavings.
- Rotate the block 10° northeast so that the west side of the trapezoid will have a slanted orientation angle when done.
  - This orientation angle will help to determine the order of your sections as your pick them up on coated slot grids.)
- Move the knife stage to the middle.
- Re-advance using the shadow of the small trapezoid (not the mesa) to a safe distance of about 1 mm.
- Slide to the west and position the right edge of the trim tool about 150-200 microns from the east edge estimating this distance by using the line micrometer in the eyepiece.
  - At the highest magnification the distance between the long lines of a Leica UC6 line micrometer is 250 microns
  - Maximum width of trapezoid should not exceed slot grid width, either 500 or 1000 microns, see below.)
- Remove the same depth (~20-30 microns) from the west side of the trapezoid at the same trimming speed.

Supplemental Movie 5:
Series Trapezoid N-S Trimming

East

North

(air side)

South

(Net side)

West

Rotate 90 degrees

Series Trapezoid N-S Trimming Procedure

- Leave settings at 1 micron per turn and speed of 1mm per second
- Remove Epon shavings with tape (or small brush) avoid touching of the knife edge of the trim tool.
- Rotate the block 90 degrees to trim the north and south edges.
- The north and south edges are trimmed to a depth of 15-20 microns to provide a more stable base for sectioning.
  - This depth is slightly less than the east/west sides which can produce a more stable block
- Position the left edge of trim tool on the south side of the trapezoid
- Determine the exact region of interest while shining a fiber optic light source to visualize the block face.
  - In this example, the best tissue quality is usually located between 100 to 150 microns below the air surface of a hippocampal slice.
- Remove 15-20 microns.
  - The movie doesn't show all 15-20 strokes to save time.
- Back the trim tool away about 1 mm.
- Slide trim tool about 20-60 microns horizontally to the left to prepare the North edge.
- DO NOT ROTATE ANY OF THE SETTINGS AS THE GOAL IS TO HAVE PERFECTLY PARALLEL NORTH AND SOUTH EDGES TO OBTAIN A RIBBON THAT HANGS TOGETHER!!
- Use the right "corner" of the trim tool to remove 15-20 microns from the south slope.
- Use small brush (or tape) to brush away Epon shavings.
- ROTATE block back to original position with North at the top.

Movie Set 3

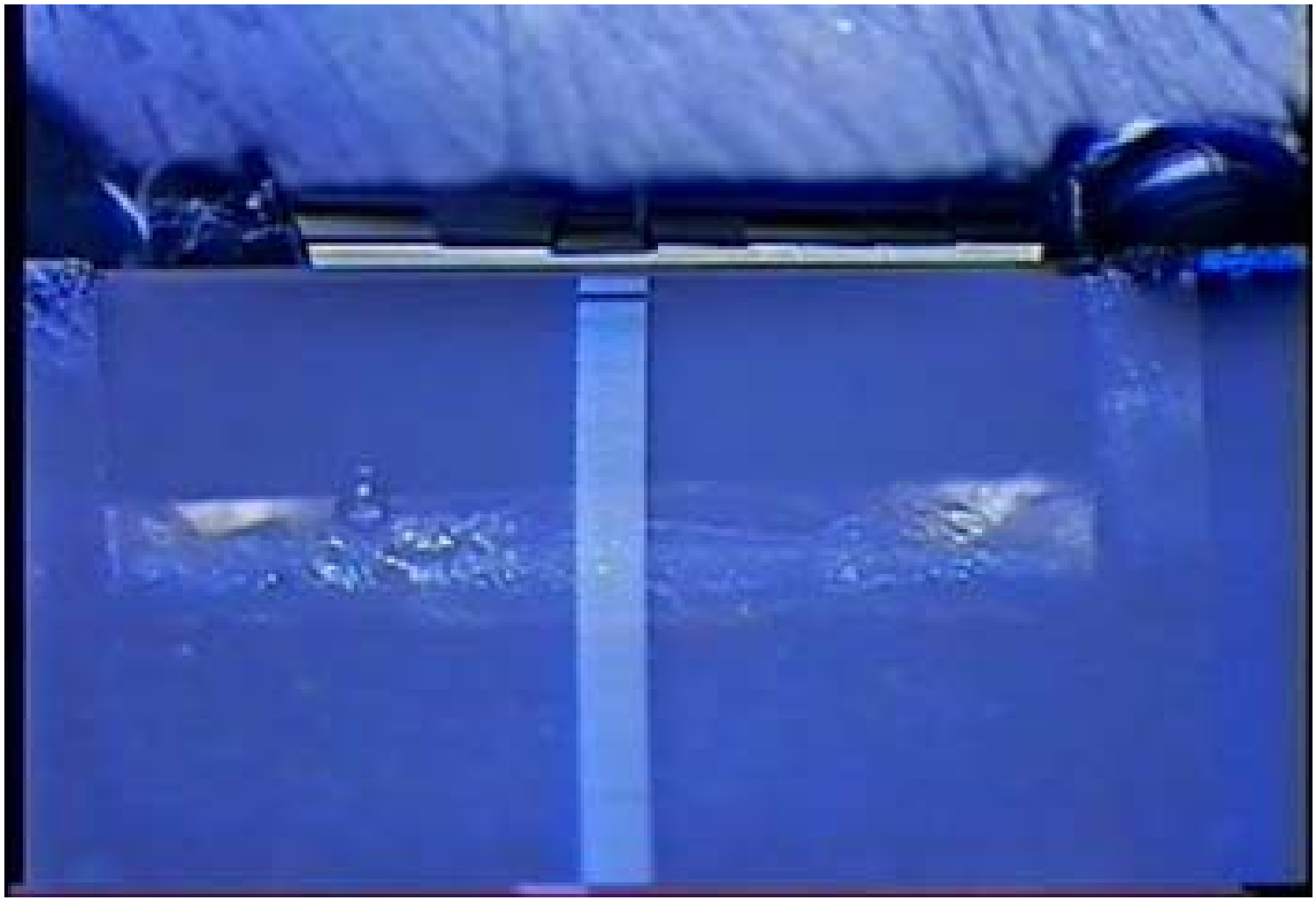
Cutting & Retrieving Ribbons of Serial Thin Sections

Supplemental Movie 6
Section Ribboning on Diamond Knife

Section Ribboning on Diamond Knife Procedure

- Place a high quality diamond knife with no nicks along the edge in the holder.
- Advance to within about 25 microns of the tiny trapezoid face.
- Adjust settings to 45 nm thickness to advance rapidly until the first section is cut.
- Immediately adjust settings to 1 mm / second, leaving the 45 nm thickness setting untouched.
- Sections starting to come off the knife edge should form a continuous ribbon of serial sections.
  - If not, back away from the edge and assess whether the north and south edges are parallel, recheck settings)
- If microtome is properly calibrated, the absolute section thickness will range from 40-50 nm
- This section thickness is later measured from the digital images across serial sections by the cylindrical diameters method:
  - Fiala JC, Harris KM (2001) Cylindrical diameters method for calibrating section thickness in serial electron microscopy. J of Microscopy. 202(Pt 3):468-72.

Supplemental Movie 7
Picking Ribbons up on Pioloform Coated Grids



Picking Ribbons up on Pioloform Coated Grids

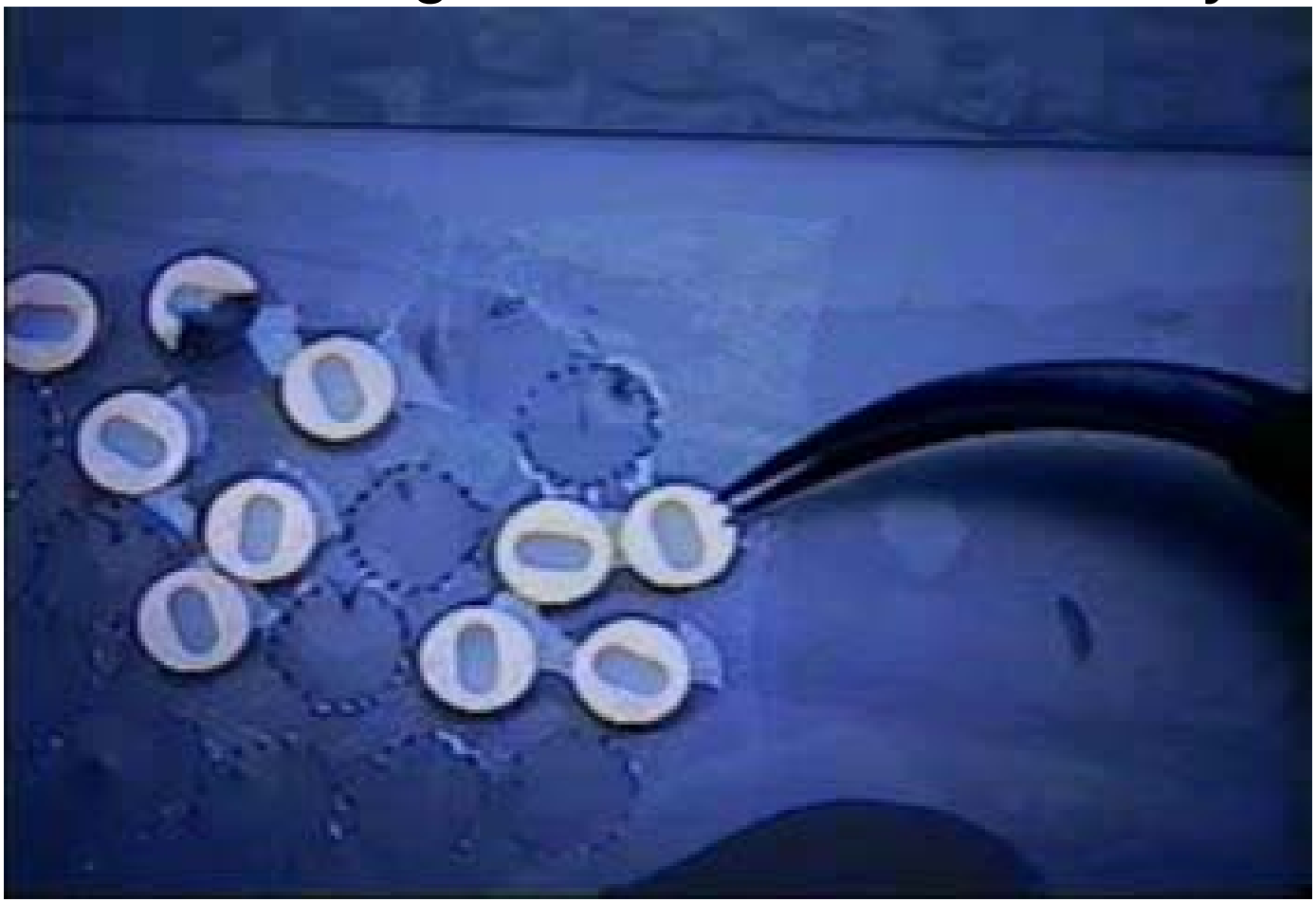
- Gently tap portions of the ribbon into segment lengths that will fit on each slot grid.
- A clean eyelash attached to a stick is used to perform this operation
  - The Perfect Eyelash Set used in our lab is available from EMS, Cat. #70616-10
- If a dust particle falls into the boat, use the eyelash to remove it.
- Use 70% alcohol and double-distilled water and dust-free lens paper to clean the eyelash before re-using it to continue further tapping and section separation.
- NB: At the beginning of this movie, sectioning was briefly re-started after ~400 serial sections of uniform thickness were obtained.
- This illustrate that section thickness can change when the block movement is interrupted
- or when the operator's body heat is introduced into the vicinity of sections that are being cut.
- Once the sections have been cut, section thickness is not affected by operator body temperature or other factors.

Supplemental Movie 8
Retrieving grids method 1: poke edges and lift.

Retrieving grids method 1: poke edges and lift.

- Retrieving the grids to pick up serial section ribbons.
- Obtain the covered Petri dish that contains the Pioloform coated slot grids (which had been prepared previously within 24 hours).
- The film is covering all the grids uniformly, hence care must be used to lift just one grid and its associated film without accidentally tearing the film off of neighboring grids.
- Method one is to perforate the edge surrounding a grid with the tips of a pair of curved number 7 forceps just prior to lifting it off the Parafilm surface.
- Clean the forceps with a piece of lens paper or get a second forceps prior to picking up the grid.
- Occasionally, there may be a few tiny "shards" of Pioloform hanging off the side of the grid
  - Gently tap the side of the grid on the slide or some other clean surface and the shard will adhere to the side of the grid
  - This “shard” will not be visible and will not interfere with section pick up or subsequent scoping.

Supplemental Movie 9
Retrieving Grid method 2 - Shimmy



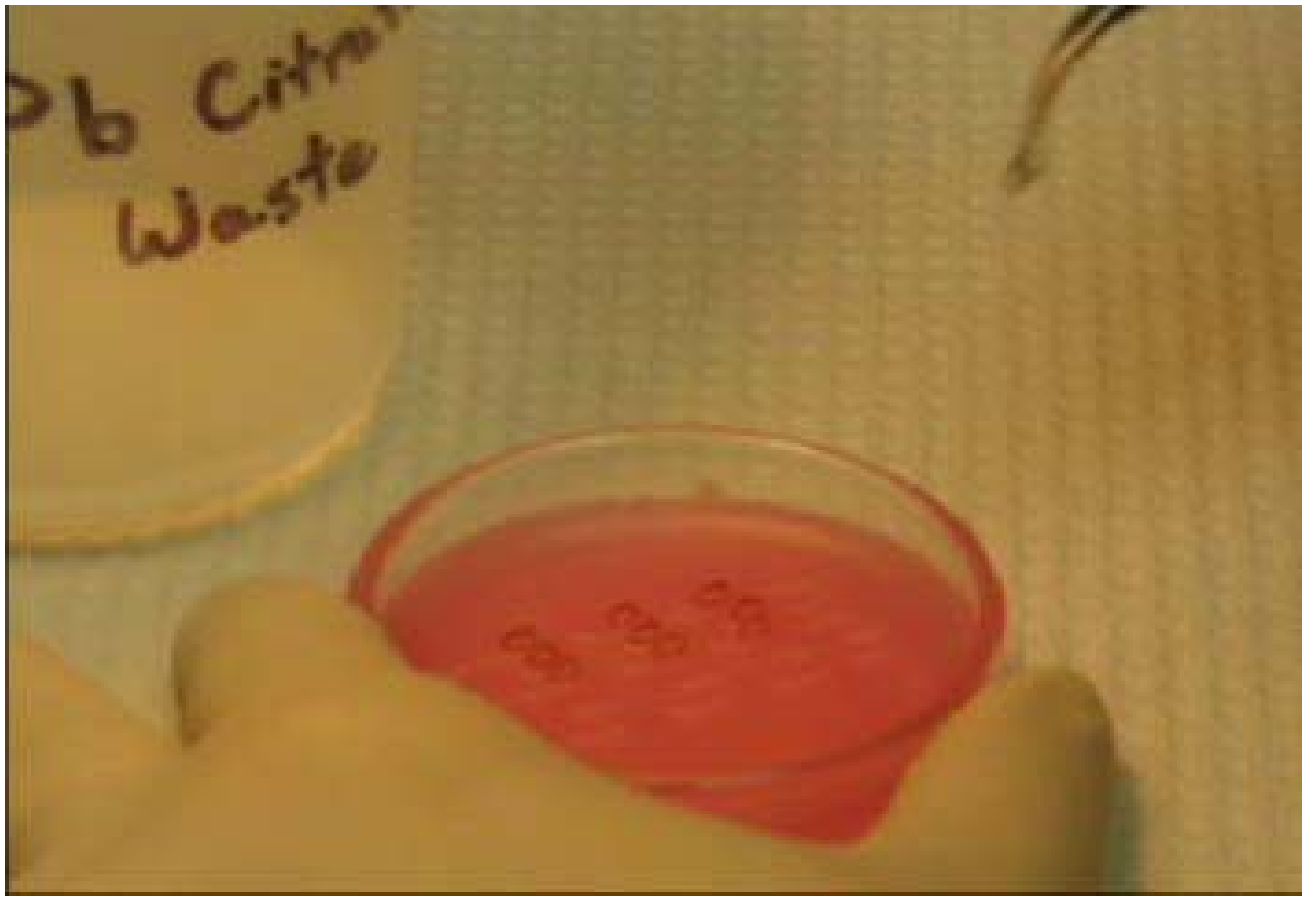
Retrieving Grid method 2 - Shimmy

- Method two is quicker which is good because less time means less risk of dust landing on the floating sections while getting the grids.
- However this method has more risk of removing film from neighboring grids.
- Use the #7 curved forceps to grab the grid at the notch.
- Then gently shimmy the grid side-for-side while picking it up.
- Angling the grid a bit away from neighboring grids will usually break the film at that edge without lifting it from the neighboring grid.
- This method can become preferred with practice because it minimizes the time required to pickup the serial sections – which are at risk of dust while they are floating in the diamond boat.

Movie Set 4

Staining sections and loading grids for photography

Supplemental Movie 10
Grid staining, Washing, WICKING DRY!



Grid staining, Washing, WICKING DRY!

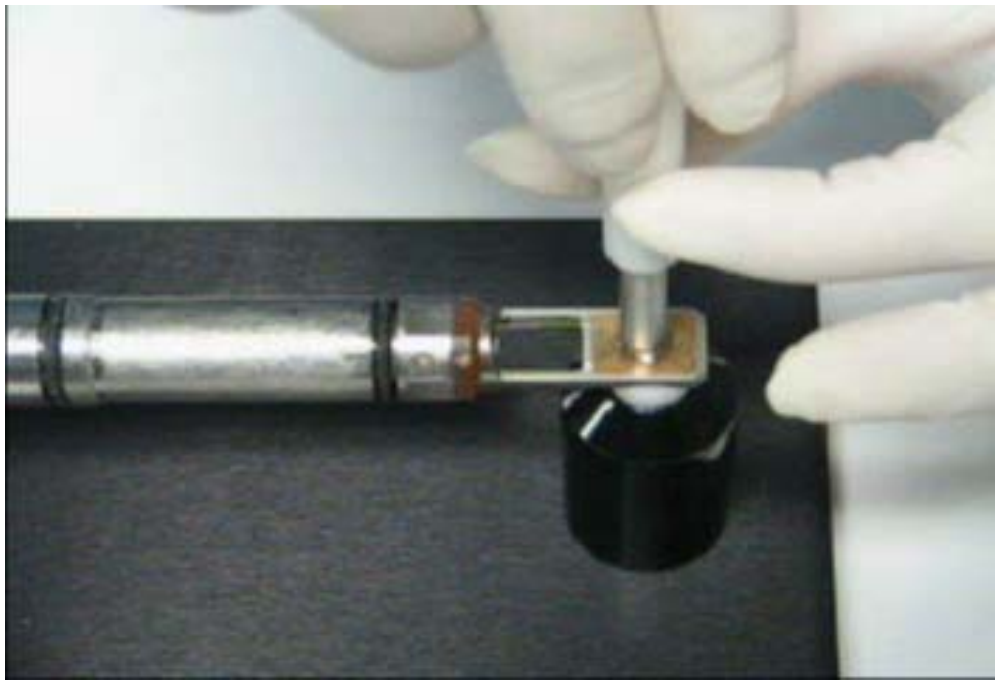
- Place grids in slots in dental wax that has been melted to the bottom of a Petri dish.
- not shown in movie but it helps to turn the forceps over after placing the grid and gently tap it into place using the back side of the curve
- Add drop of grid stain to the section side of each grid, first UA then Reynold's Lead Citrate.
  - Note that a syringe holds this solution – be sure all of the air is out of the syringe.
- Cover – top of the cover is filter paper moistened with 0.02 N NaOH to create a carbon-dioxide-free environment.
- Rinse thoroughly with double distilled and filtered water after the requisite time (we use 5 min each for UA and Lead)
- Wick the remaining water droplet away from the grid until it is completely dry.
- Store grids allow grids to dry overnight before loading in grid cassettes.

Supplemental Movie 11
Loading Grid into a Cassette

Loading Grid into a Cassette

- The grid is loaded in the cassette and then transferred to a labeled gelatin capsule and stored in a dry area.
- We use a dessicator with shelves and Beem capsule holding trays from Pella or EMS.

Supplemental Movie 12
Loading Cassette into Rotational Holder

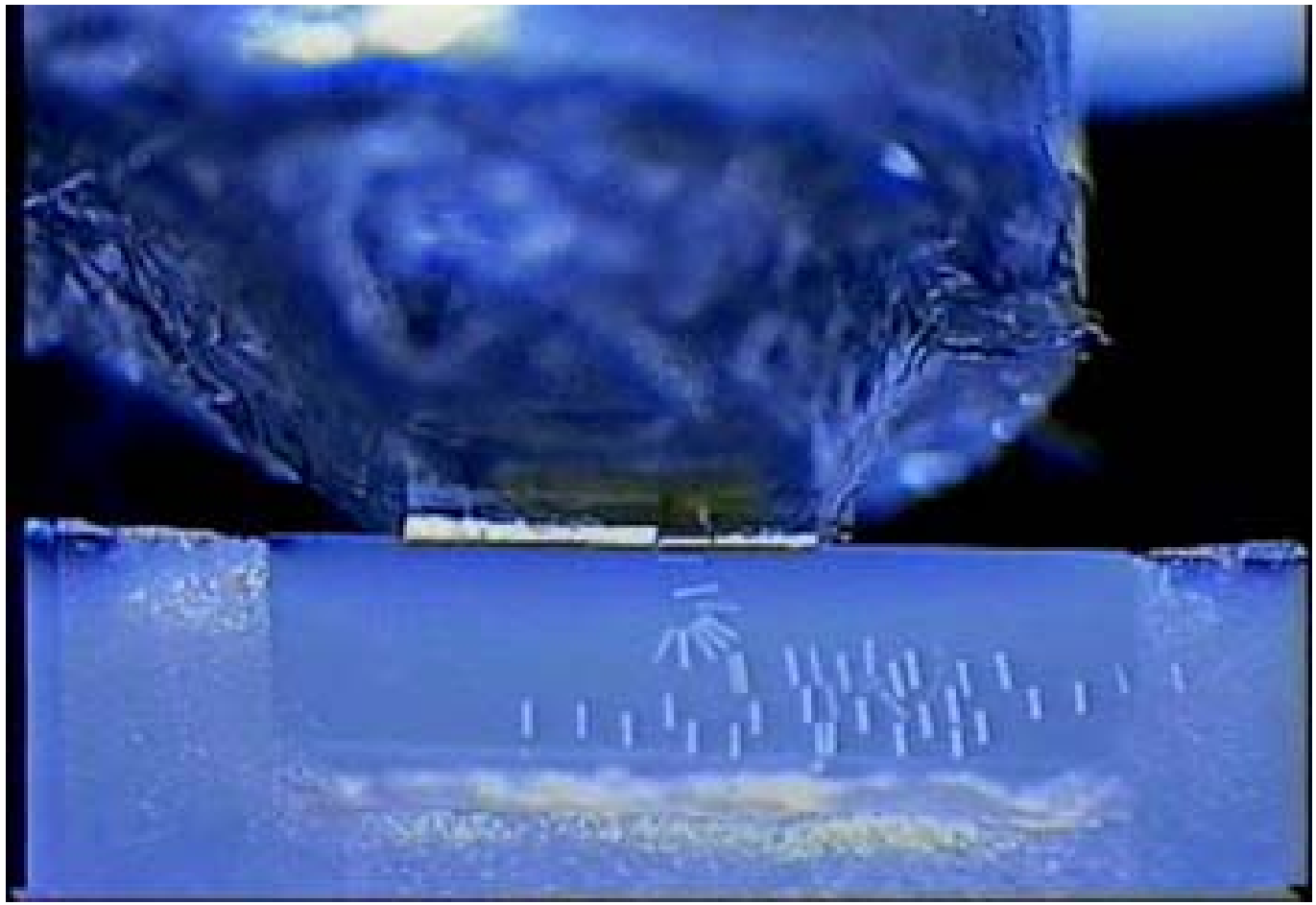


Movie Set 5

Ribboning of hydrophilic sections after application of hair spray

e.g. Lowicryl

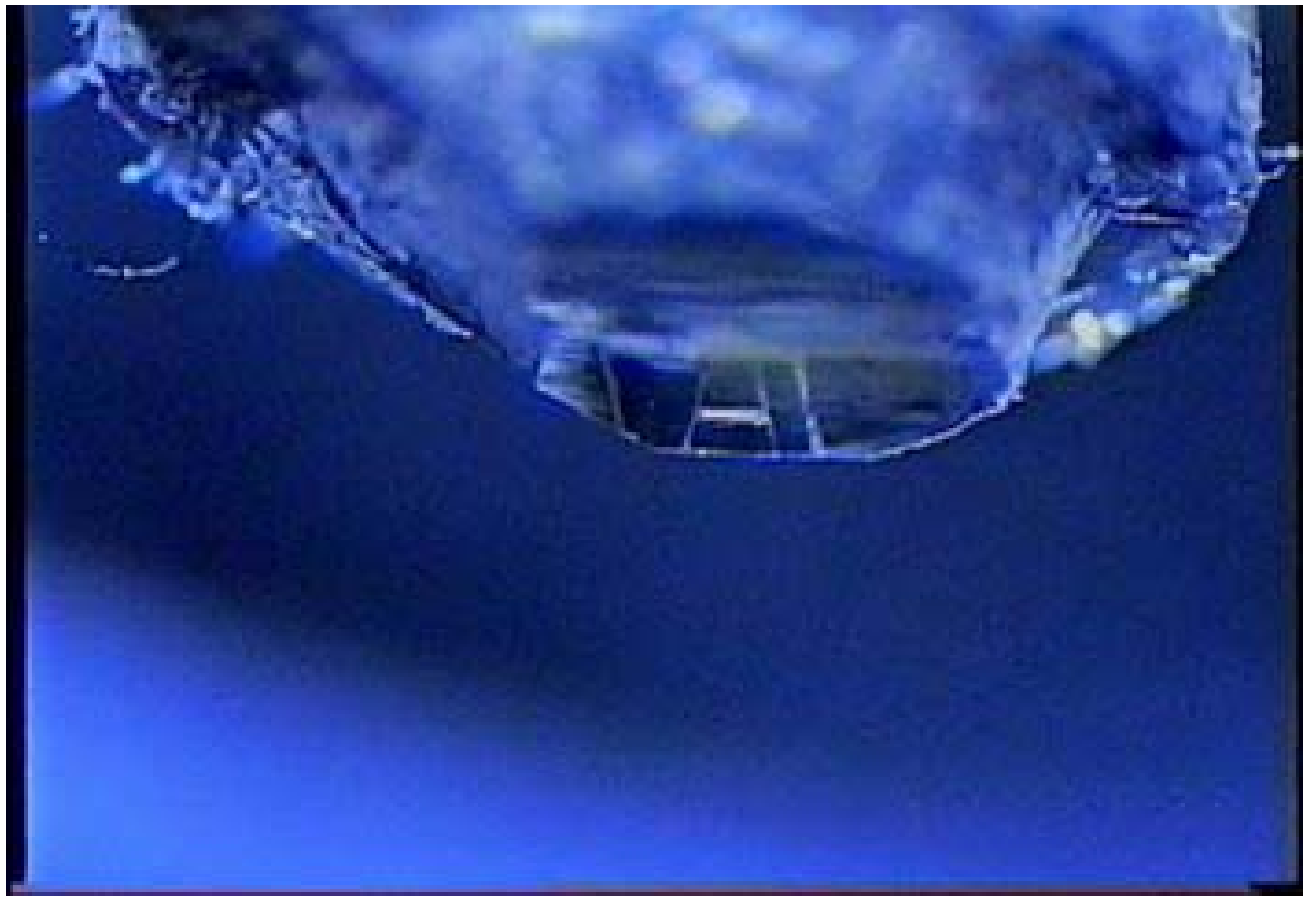
Supplemental Movie 13
Lowicryl block does not ribbon properly



Lowicryl block does not ribbon properly

- Even with superb technique, some special purpose embedding materials do not readily ribbon.
- This example shows how sections burst apart when the hydrophilic resin Lowicryl is used.

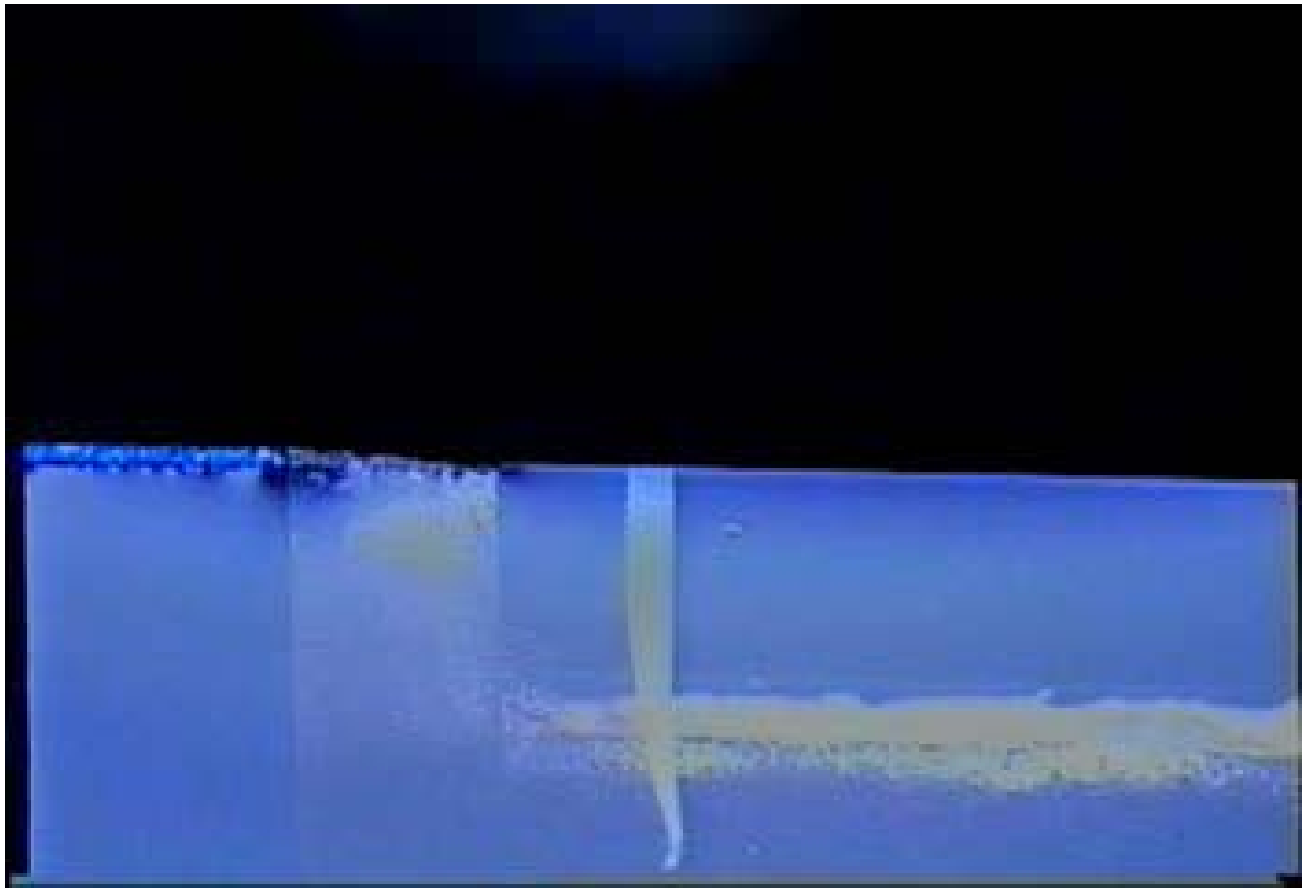
Supplemental Movie 14
Four bursts of hair spray, dry overnight



To solve this problem: Hair Spray!

- First trim the block face to the desired dimensions using the standard protocols described in Supplemental Movies 1 and 2.
- Place filter paper around the back of the block to protect the chuck and microtome from becoming sticky.
- Mist the trapezoid with salon-quality hairspray that has a thin viscosity.
- Give 4 bursts of the spray – allowing a few seconds between each burst.
- Allow to dry overnight.
- If possible, leave the chuck properly positioned in the microtome so that you won't have to reset the orientation.

Supplemental Movie 15
Hairspray coated Lowicryl-embedded series



Same block as in Supplemental Movie 13 now ribbons perfectly.