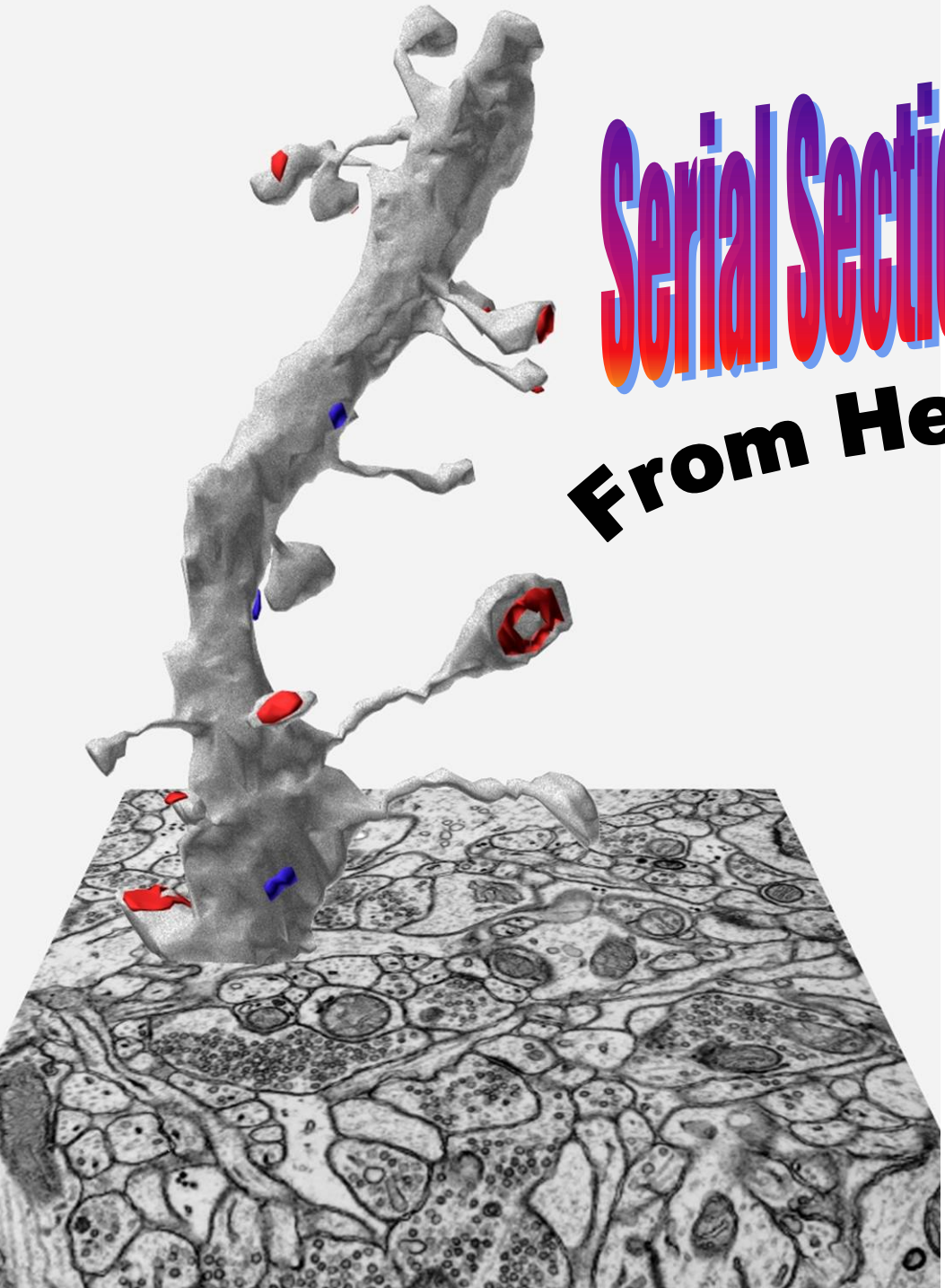
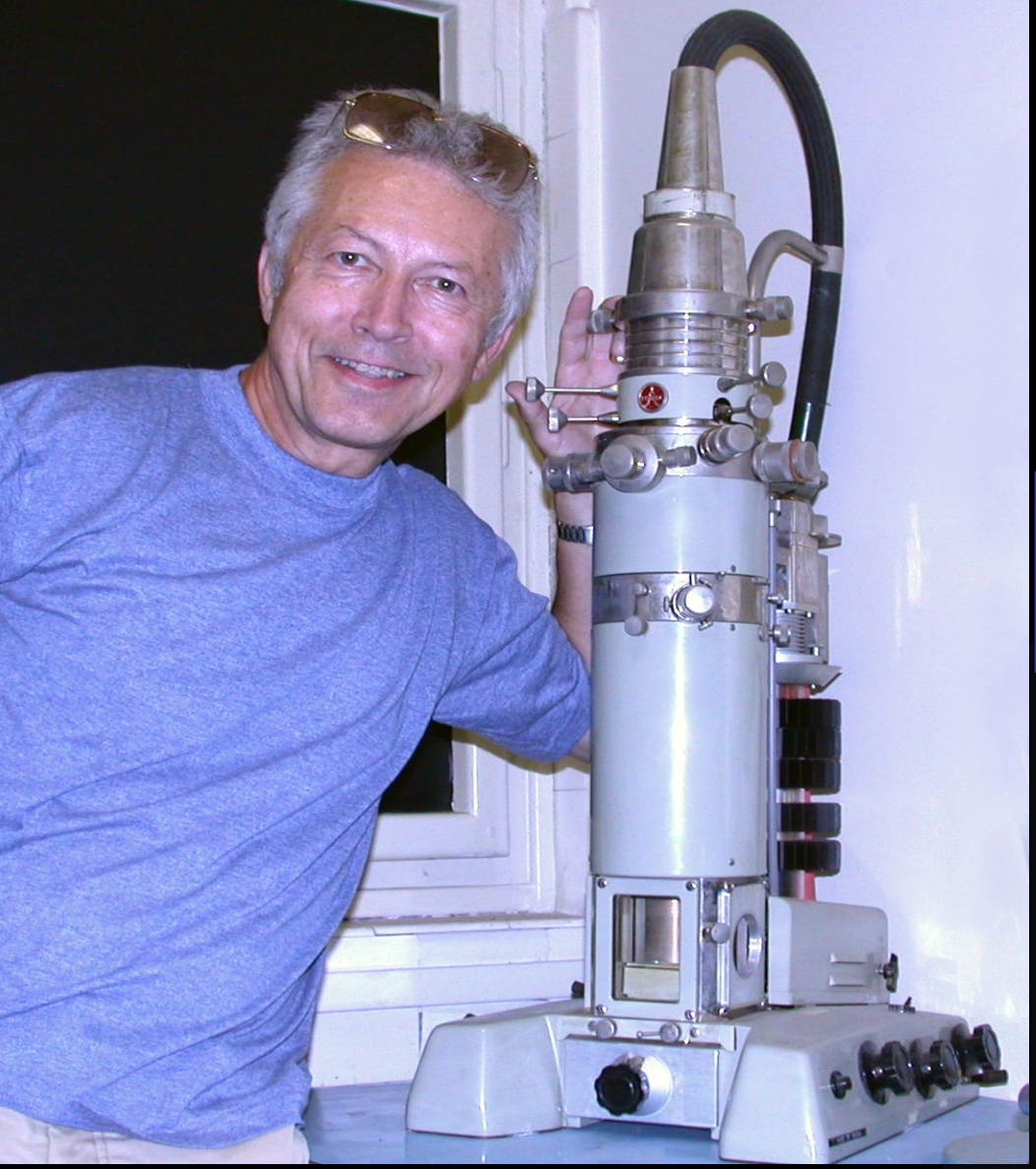


Serial Section Electron Microscopy

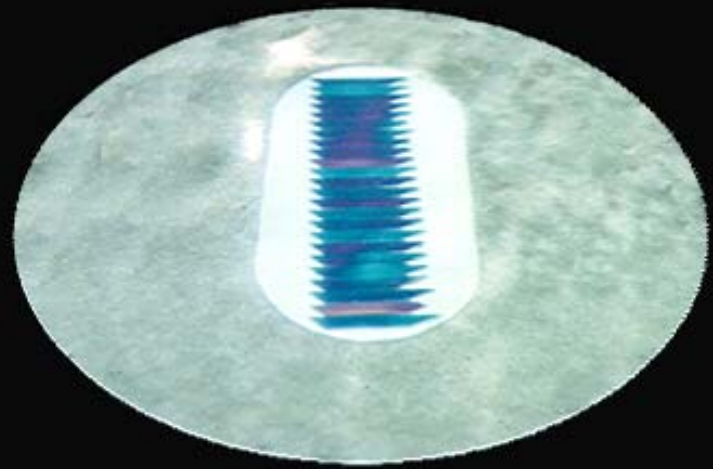
From Heroic to Routine

Contributions From:
Kristen Harris
Josef Spacek
John Fiala

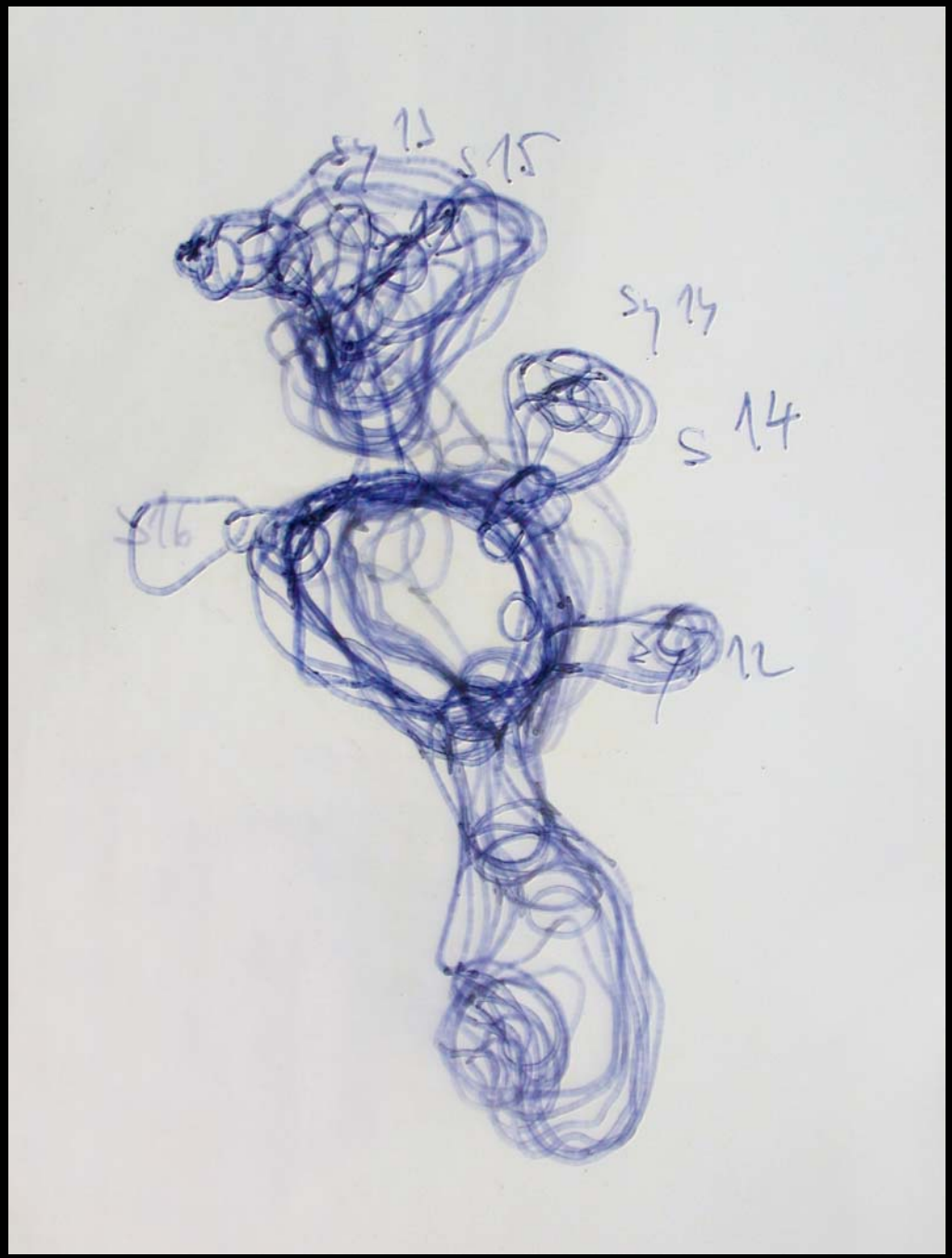
Special thanks to
Pasko Rakic
Bitao Shi
Elizabeth Perry
Robert Smith



Some Heroes



Josef Spacek



Aligned Outlines

Solid Models

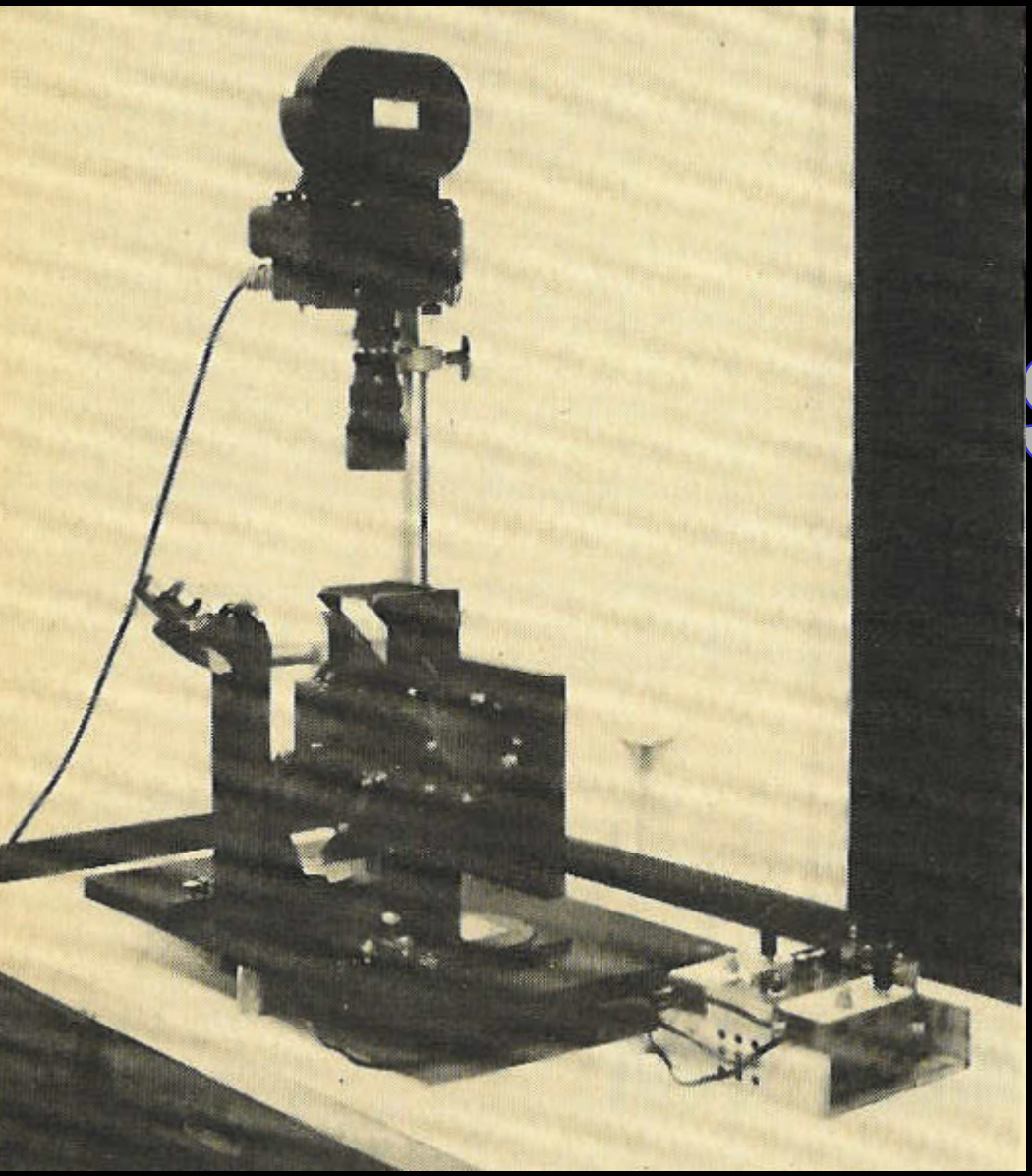
Schwann Cell Process

Synapse on Dendritic Spine

Photomicrographs Laid End to End!

Kip Riley on the roof of Children's Hospital, Boston
Mid 1970's – Radial Glia Reconstructions

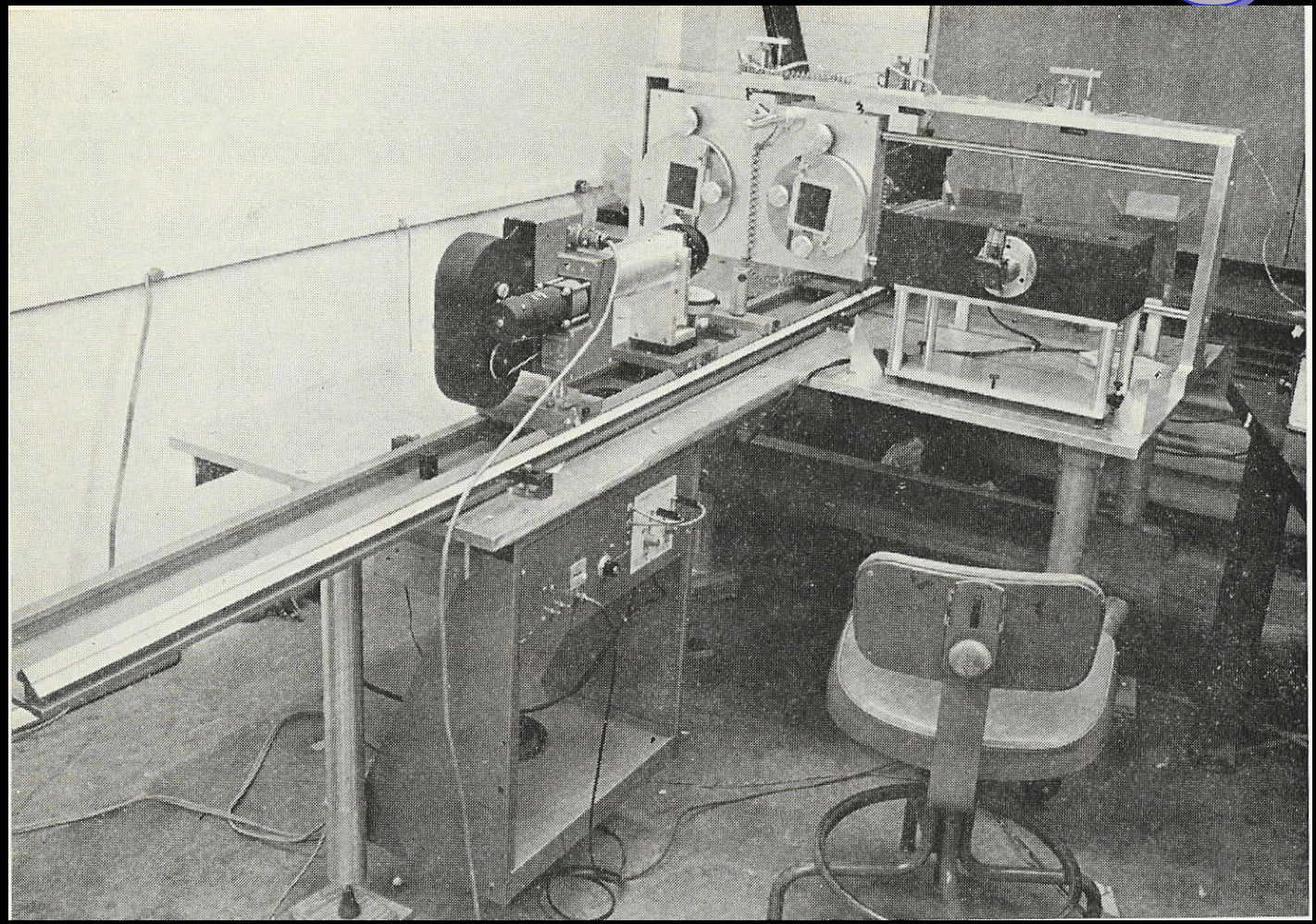
Courtesy Pasko Rakic



Cine Alignment of Serial Images Using Strobe Lights

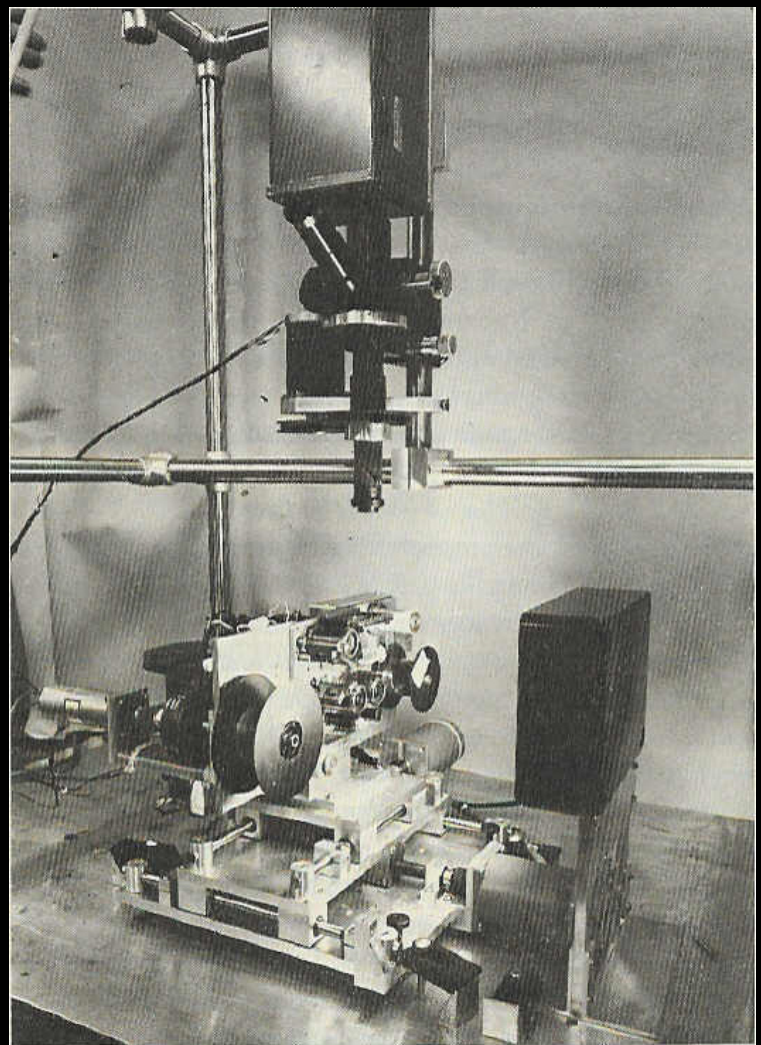
1972 Levinthal and Ware,– Nature 236: 207-210)

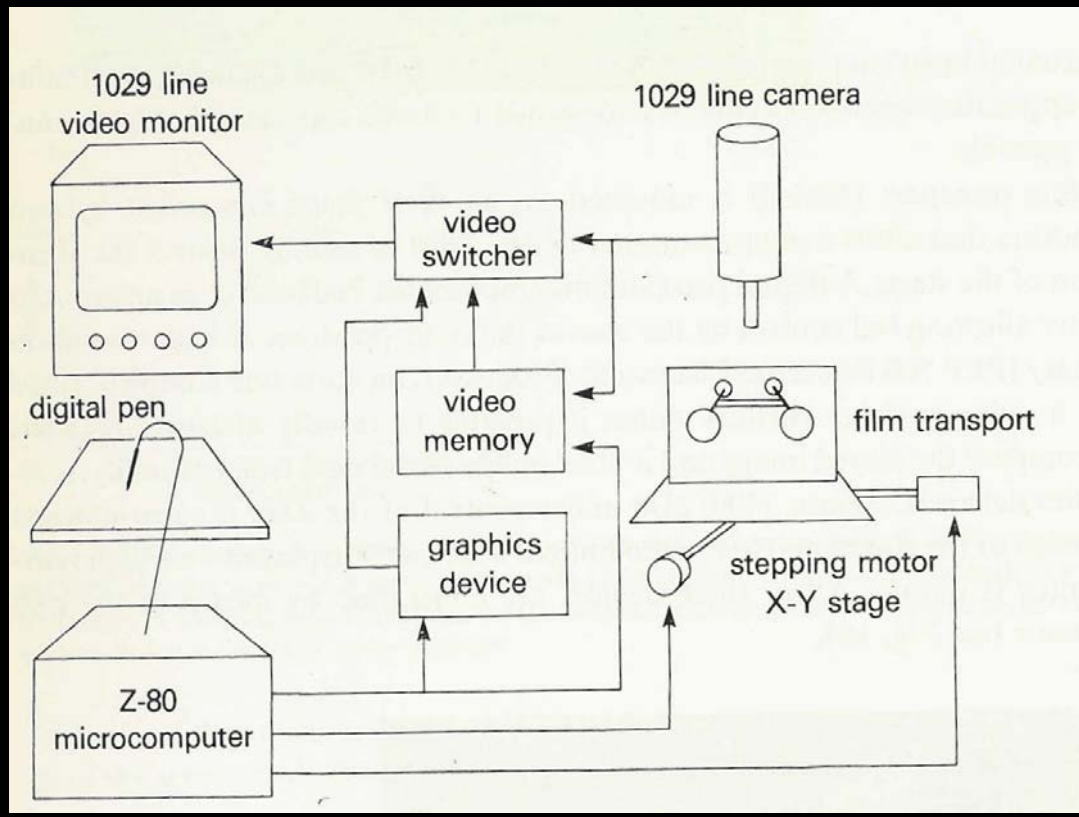
Movie-making



1980 Stevens et al., Brain Res. Rev. 2:265

Movie Transport and Computer-based Alignment

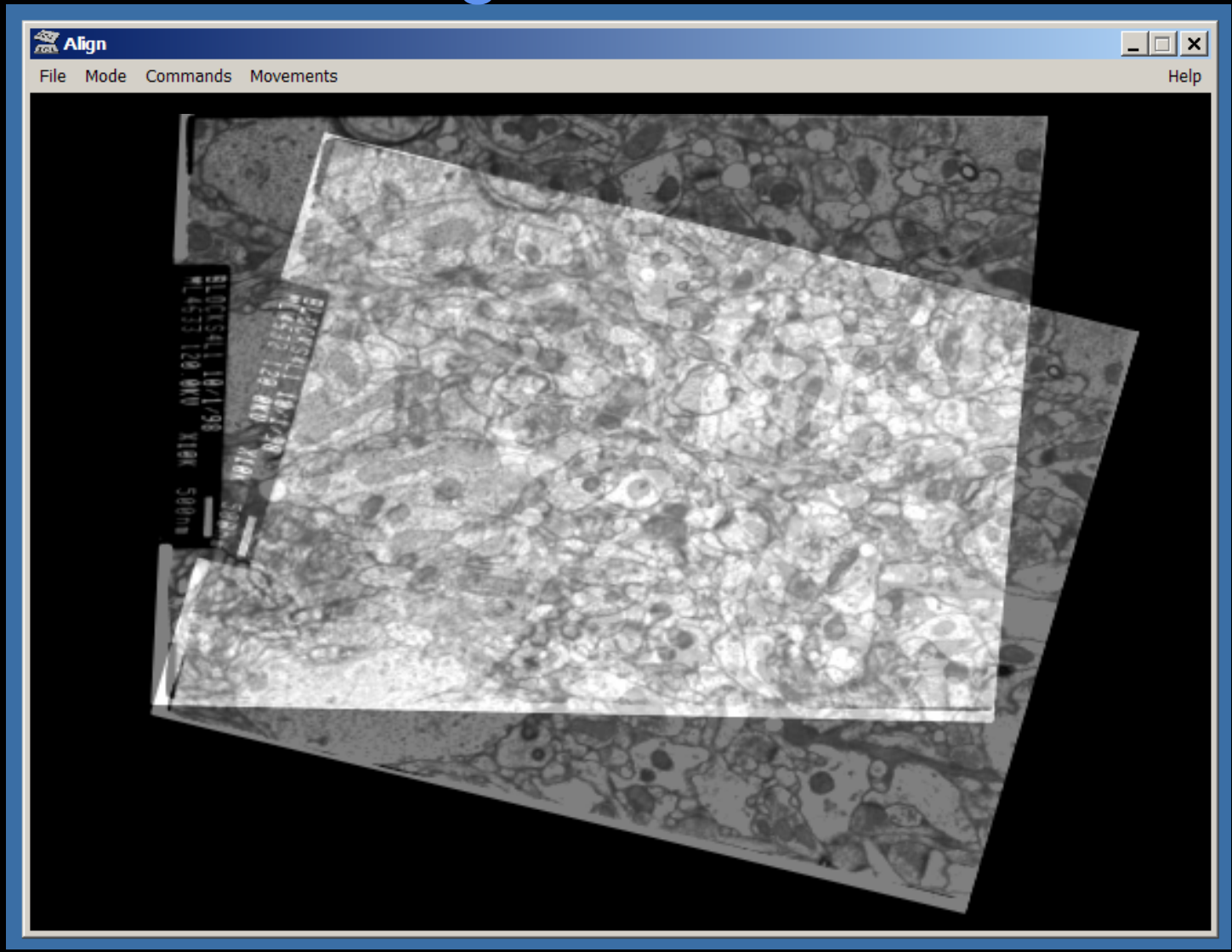




1980 - Stevens et al.,
Brain Res. Rev. 2:265

Sharing the Excitement

IGL Align (1997) Alignment by Image Movement



John Fiala

Routine Methods for Serial Section Electron Microscopy and 3D Reconstruction

Kristen M. Harris

Are POSTED AT:

SynapseWeb: synapses.mcg.edu

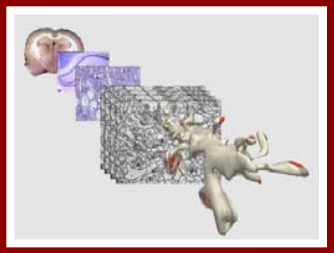
Acknowledgements:

Libby Perry – “NEVER CUT CORNERS”!

Robert Smith

Marcia Feinberg

Jamie Hurlburt

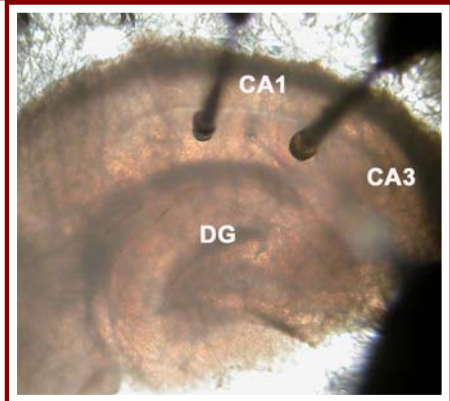


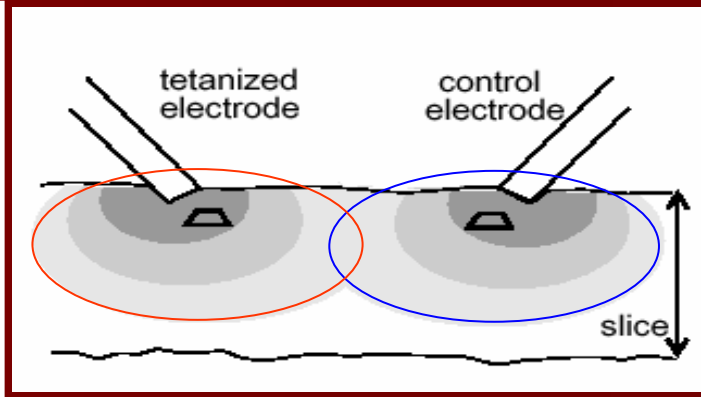
Laboratory of Synapse Structure and Function

Synapses and Cognitive Neuroscience Center

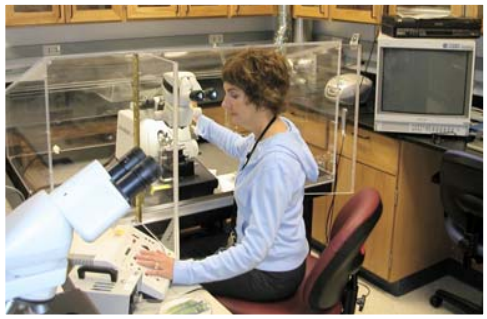
Kristen M. Harris, PhD, Principal Investigator

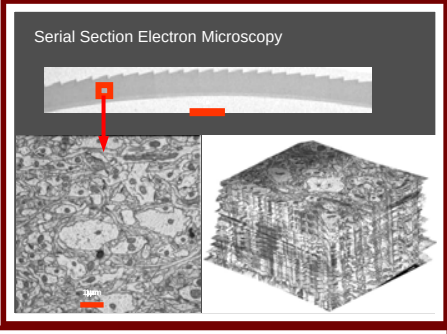
Physiology and Processing



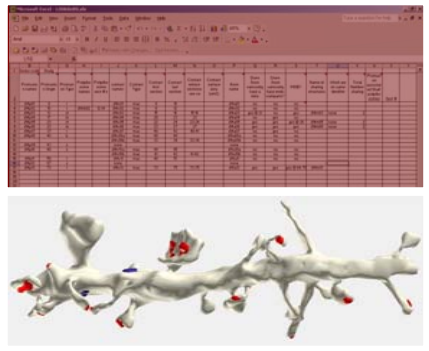


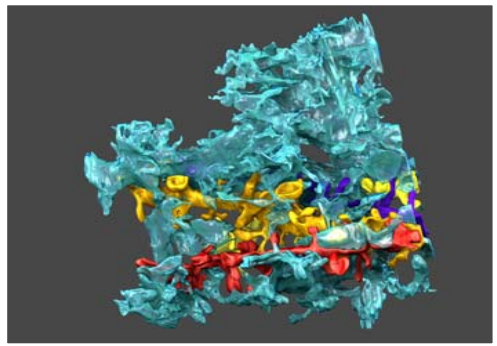
Sectioning and Microscopy





Tracing, Data Analysis & 3D Reconstruction



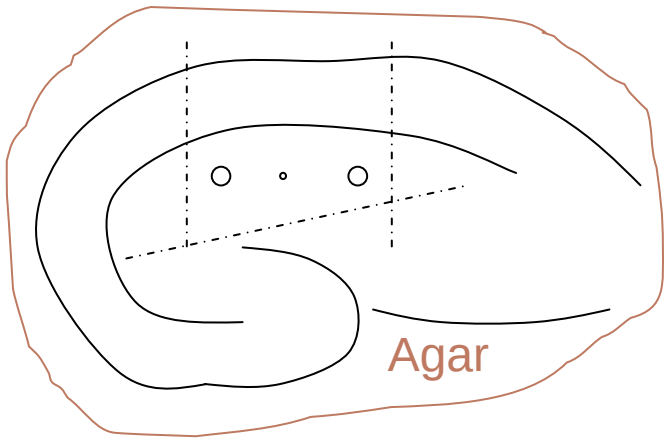


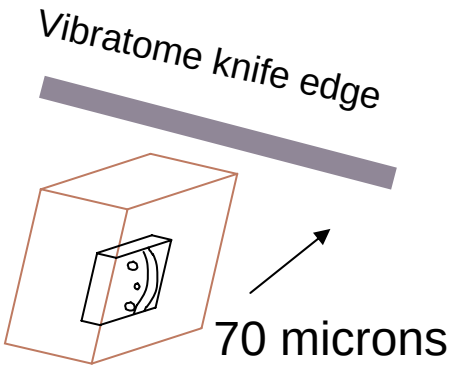
PN15 rat hippocampal Slice

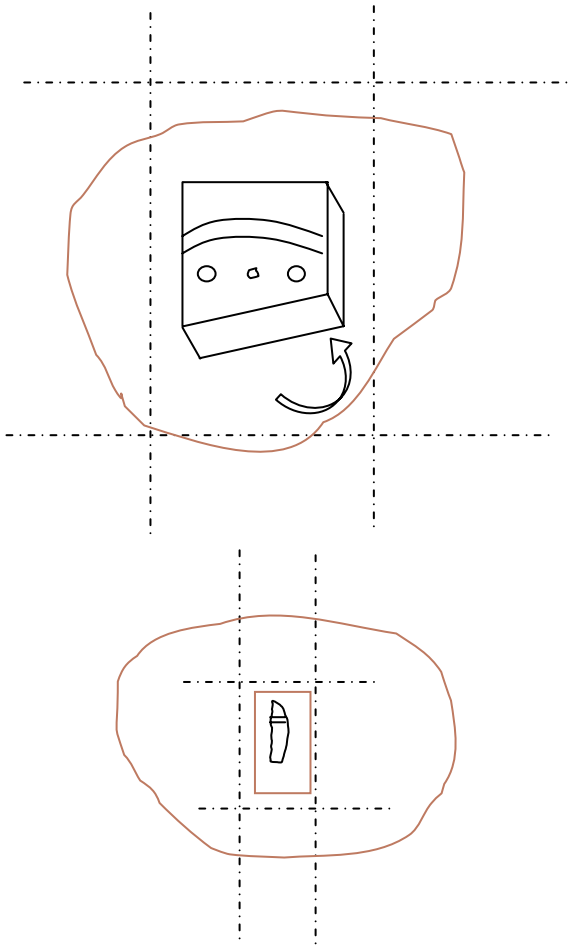
At end of the experiment

- Remove electrodes
- Place slice on net, upside down onto a glass ring in fixative in a MW-oven
  - 6% glut, 2% para, in 0.1 M Cacodylate buffer with 2mM Ca^{2+} and 4mM Mg^{2+}
- MW for about 8-20 seconds (700 Watts full power)
  - final temp ~37^{o}C, not > 45^{o}C
- Fix overnight
- Vibratome and process next day!

Trimming a region of interest





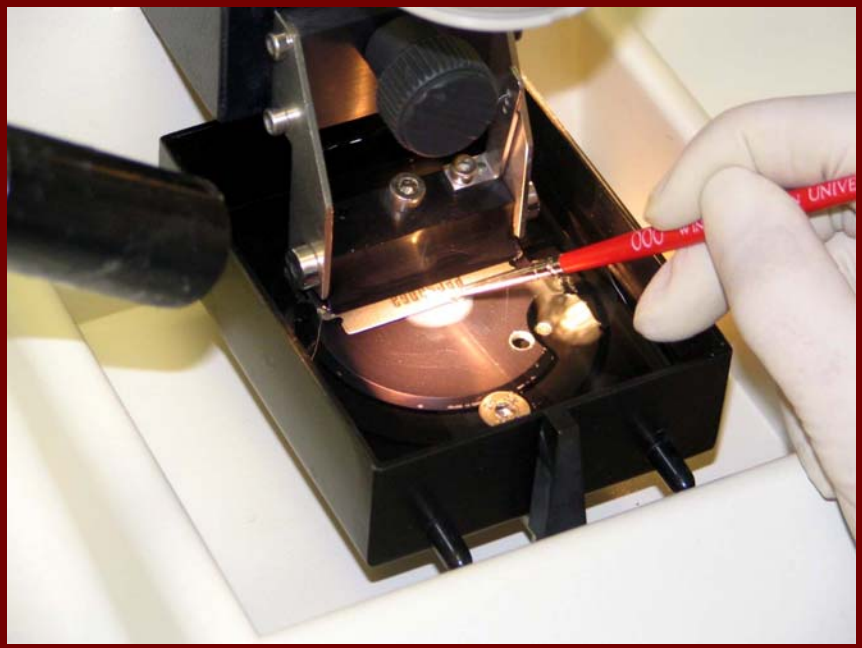


Re-embed to support and orient

Vibratome at 70 microns

Ensures uniform penetration of osmium into the tissue

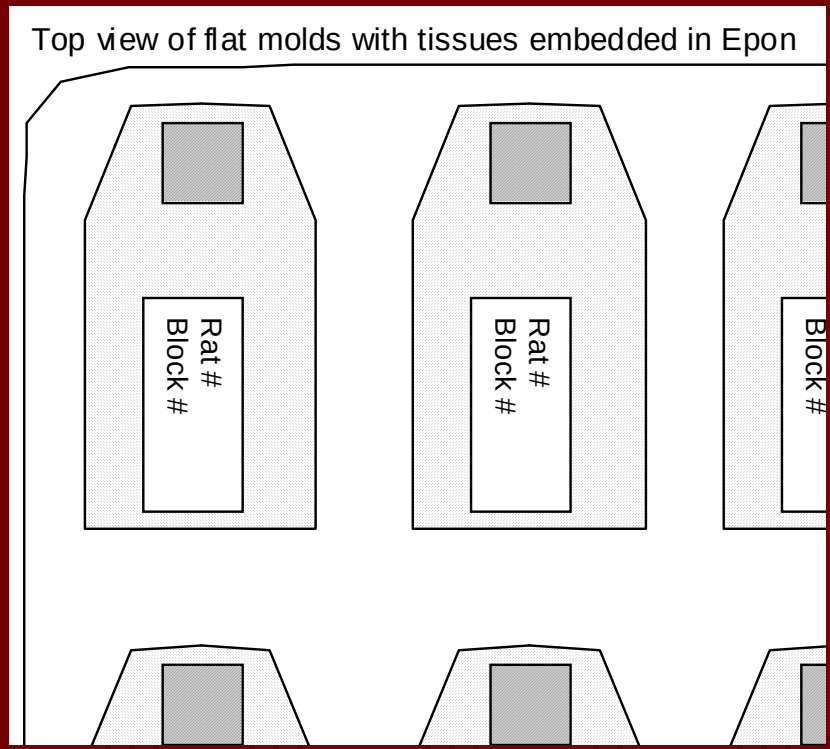
Transfer 70 micron slices sequentially to 24 well TC dishes



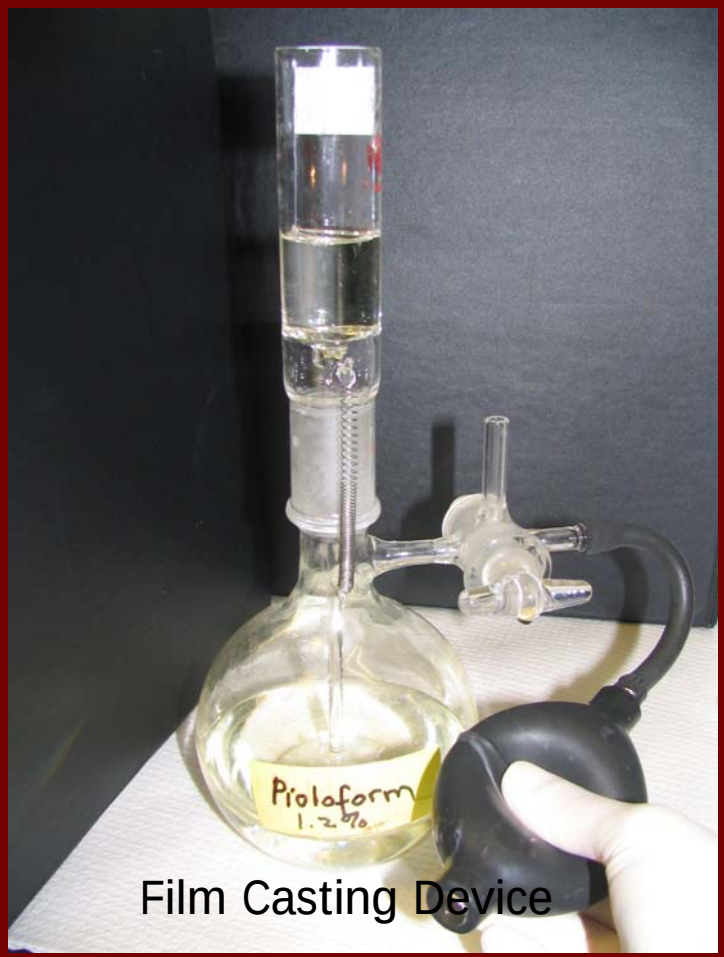
Collect sections with brush on corner of excess Agar

Example Processing

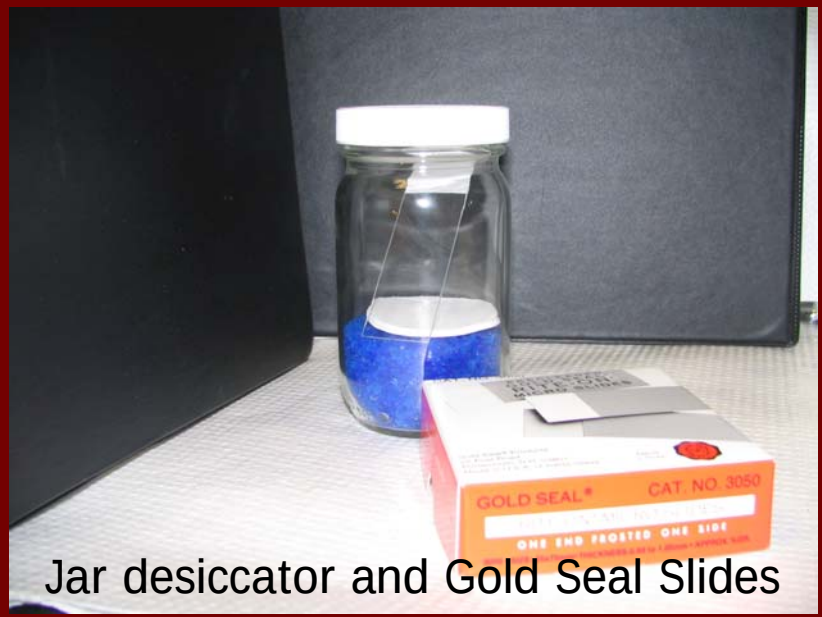
- Osmium - K-Fe reduced then 1%
  - Optimizes membranes
- UA during dehydration in ethanols
  - Optimizes polyribosomes
- Acetone (or Propylene oxide?)
  - Acetone - translucent -measure
  - PO – black tissue
- Epoxy Resin (LX112) in Coffin Mold
- **Cure at 60 ºC - 48 hrs!**
- Resurface with Epon/Aclar
  - To see through block in LM
- Details and “others” at:
  - synapses.mcg.edu - lab protocols



Make Pioloform-coated Slot Grids within 24 hours prior to serial sectioning



Film Casting Device



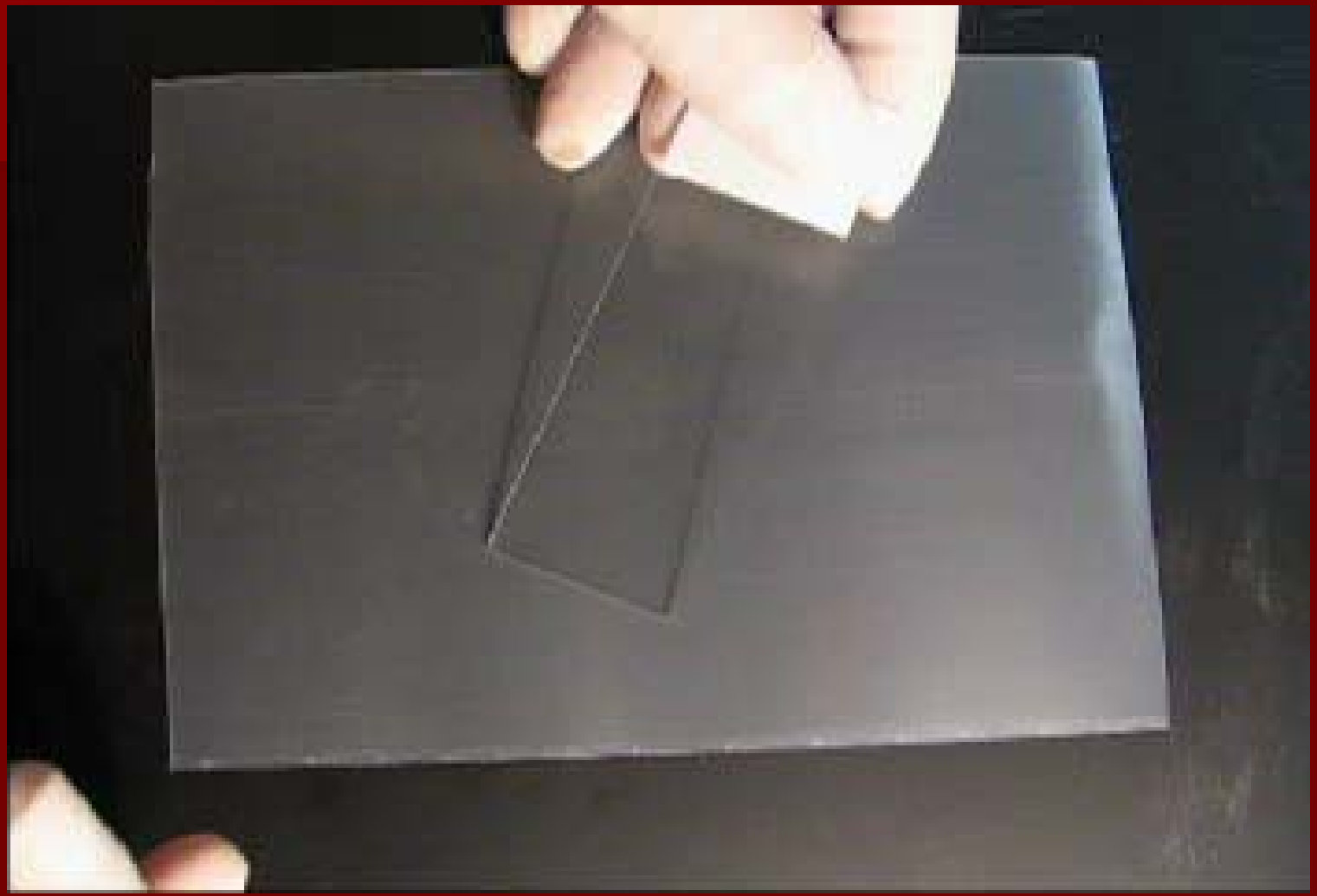
Jar desiccator and Gold Seal Slides

People who make this happen

Robert Smith

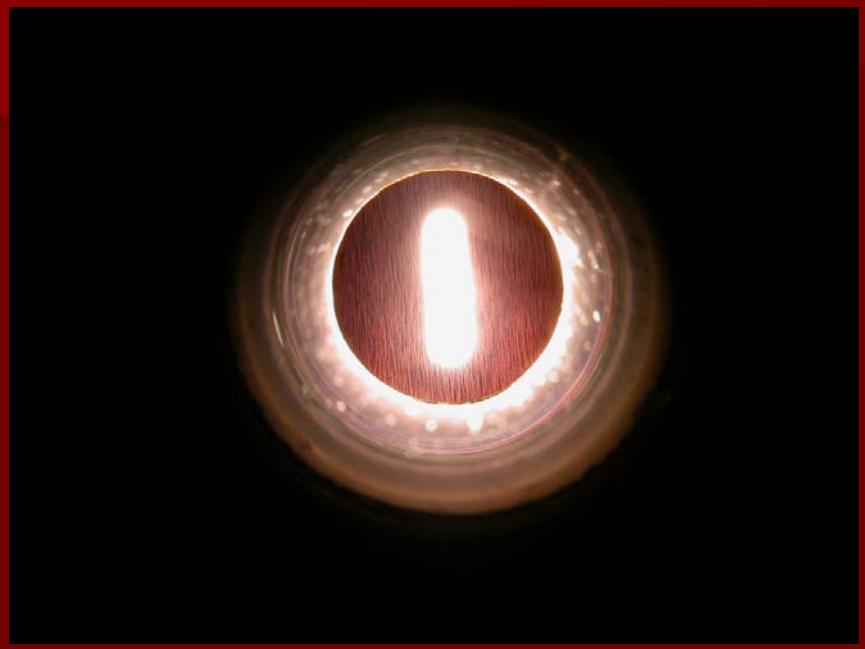
Libby Perry

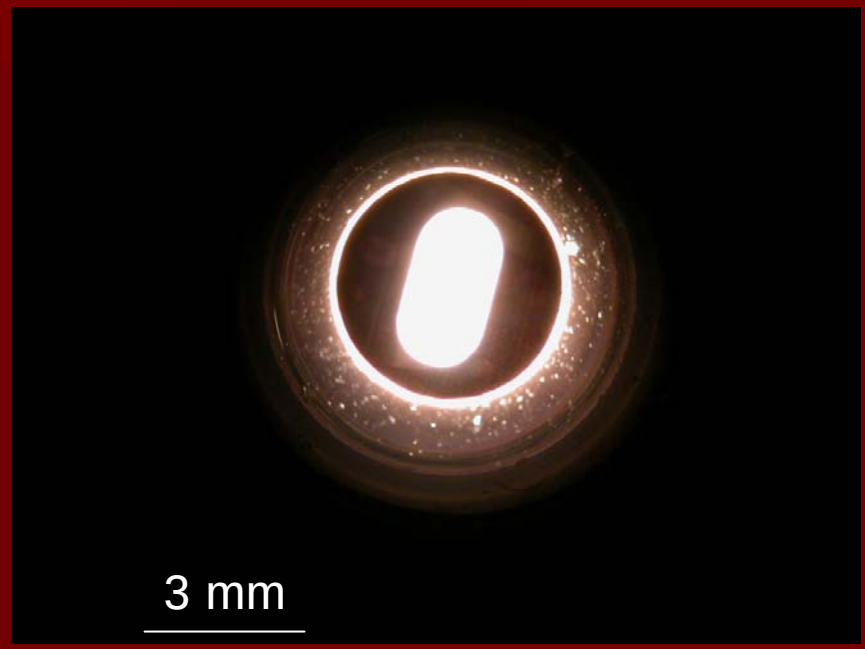
Grid coating



score coated slide and float off pioloform film

Synaptek™ Slot Grids





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

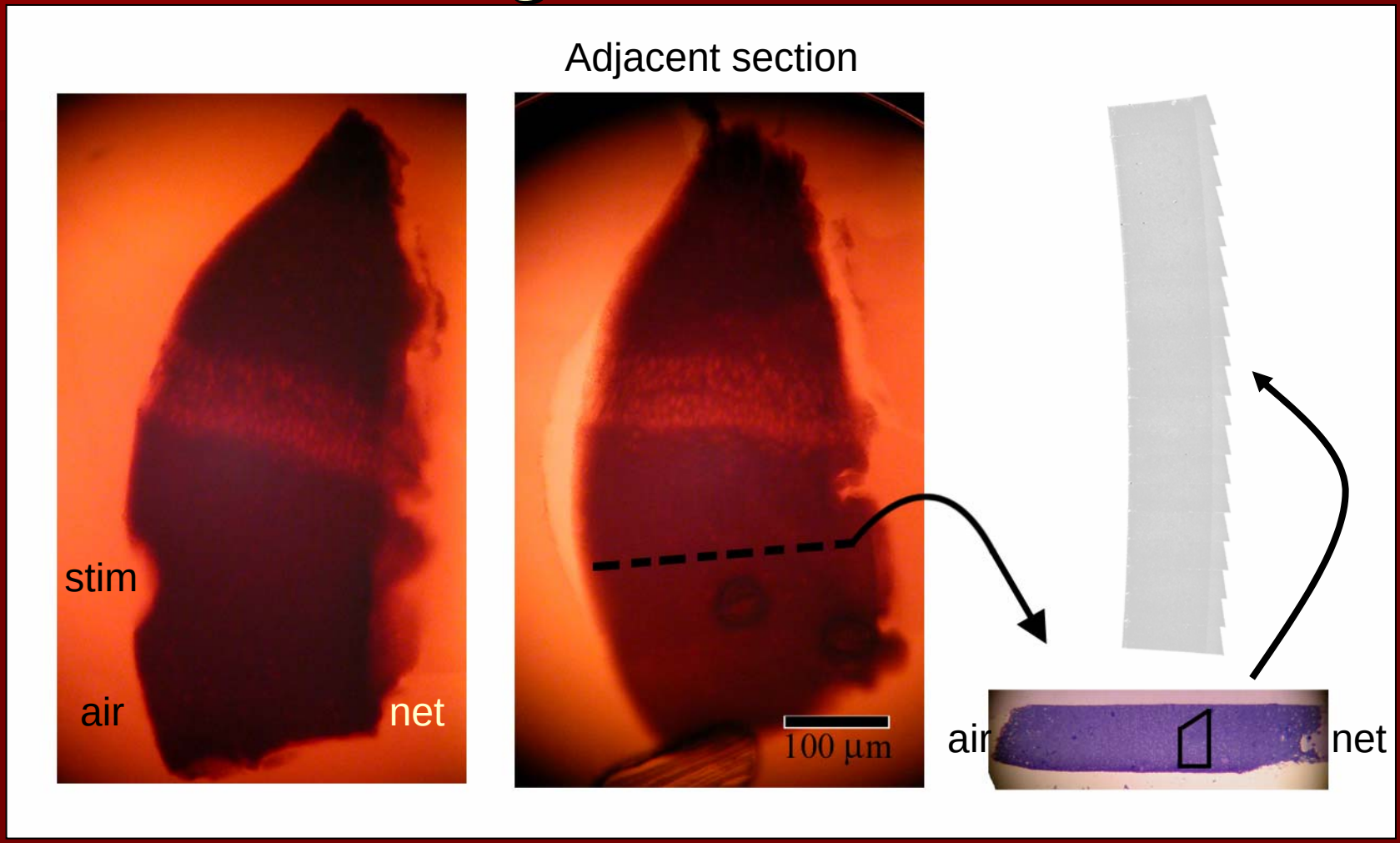
1.0 mm width
Less stable, easier to center a long ribbon

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

Place grids notch side up on silver colored film

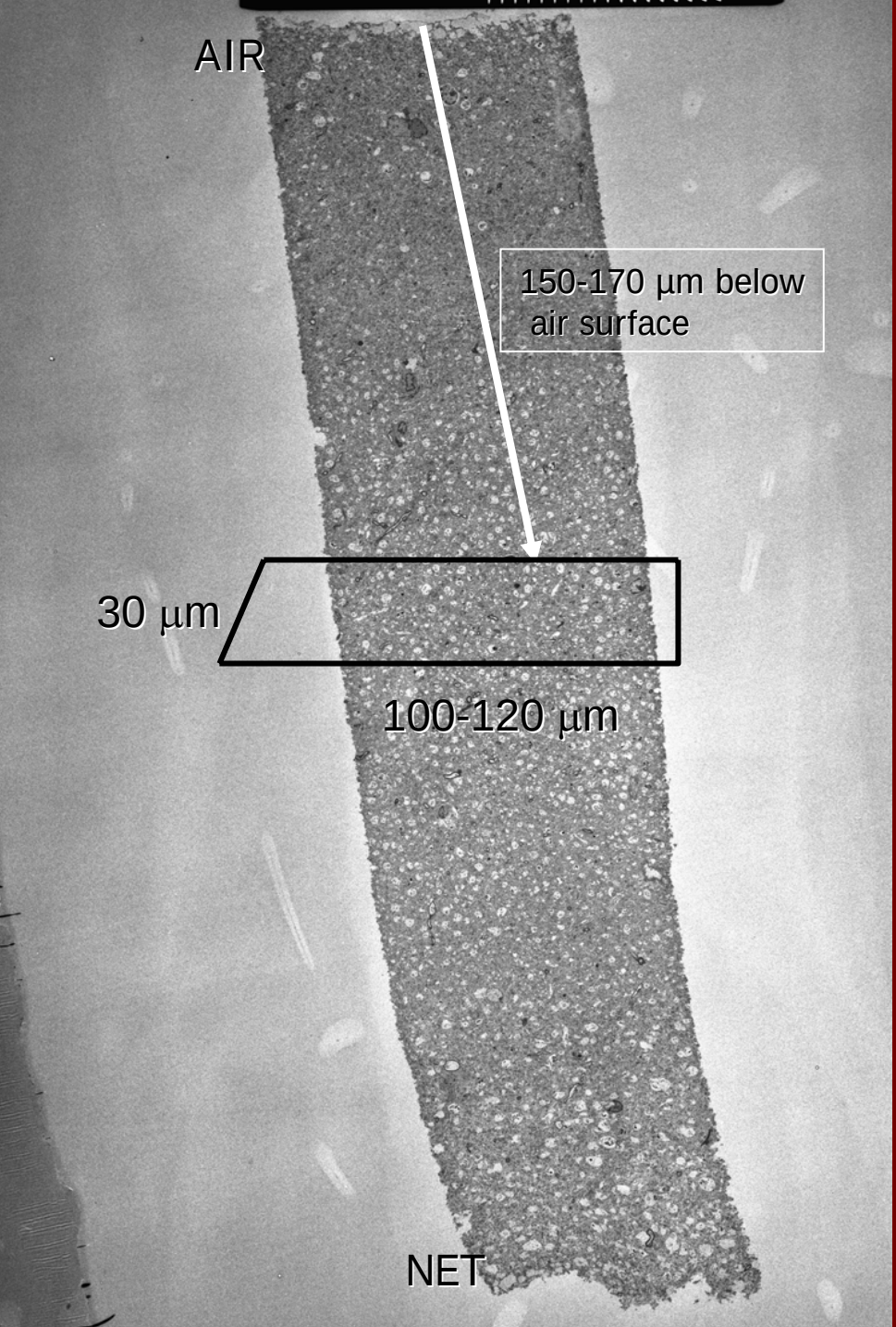
Stretch and secure film on grids

Goal: Uniformly thin serial sections in Region of Interest



70 micron sections
viewed through epoxy resin

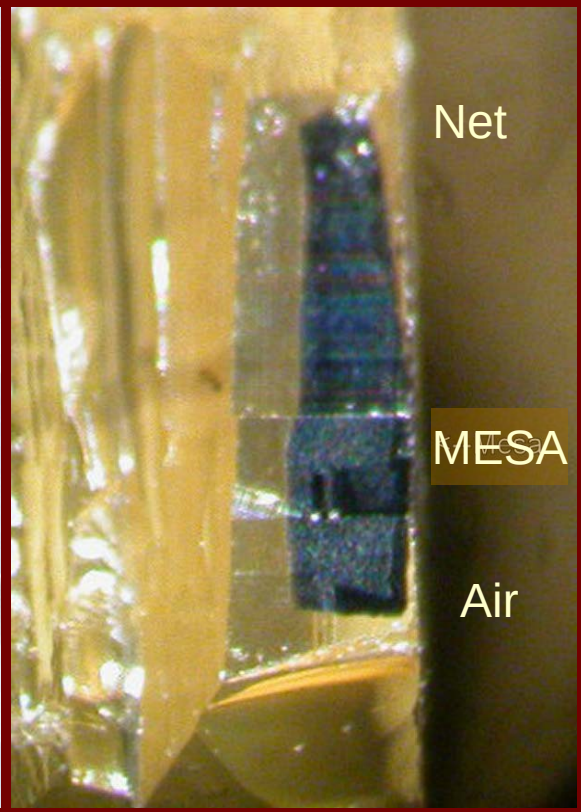
Next Slides
show steps

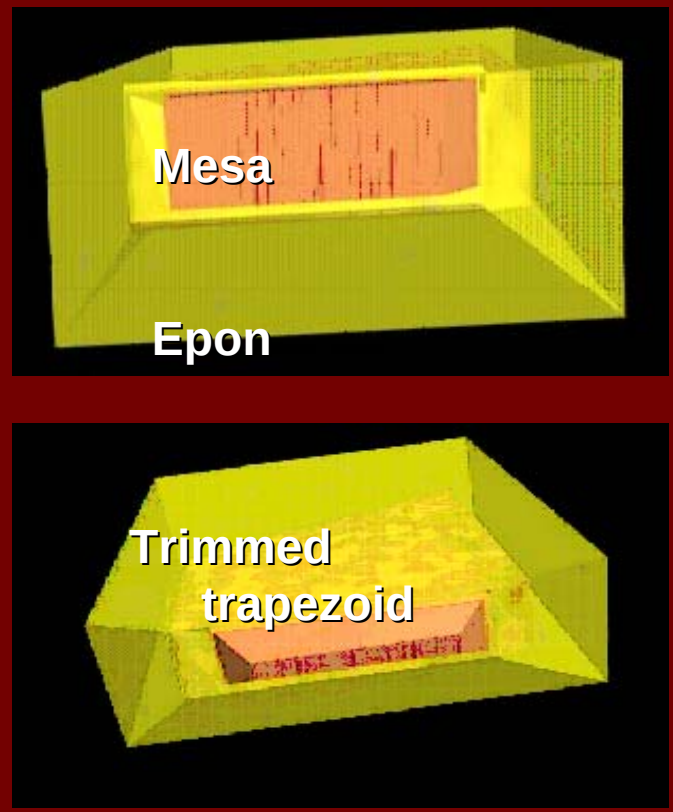


Test thin across slice

- Cut and visualize test thin across depth of slice
- Evaluate tissue for good ultrastructure
- Identify where to place trapezoid for series
- Blind as to condition of course!

Trim Mesa and Trapezoid





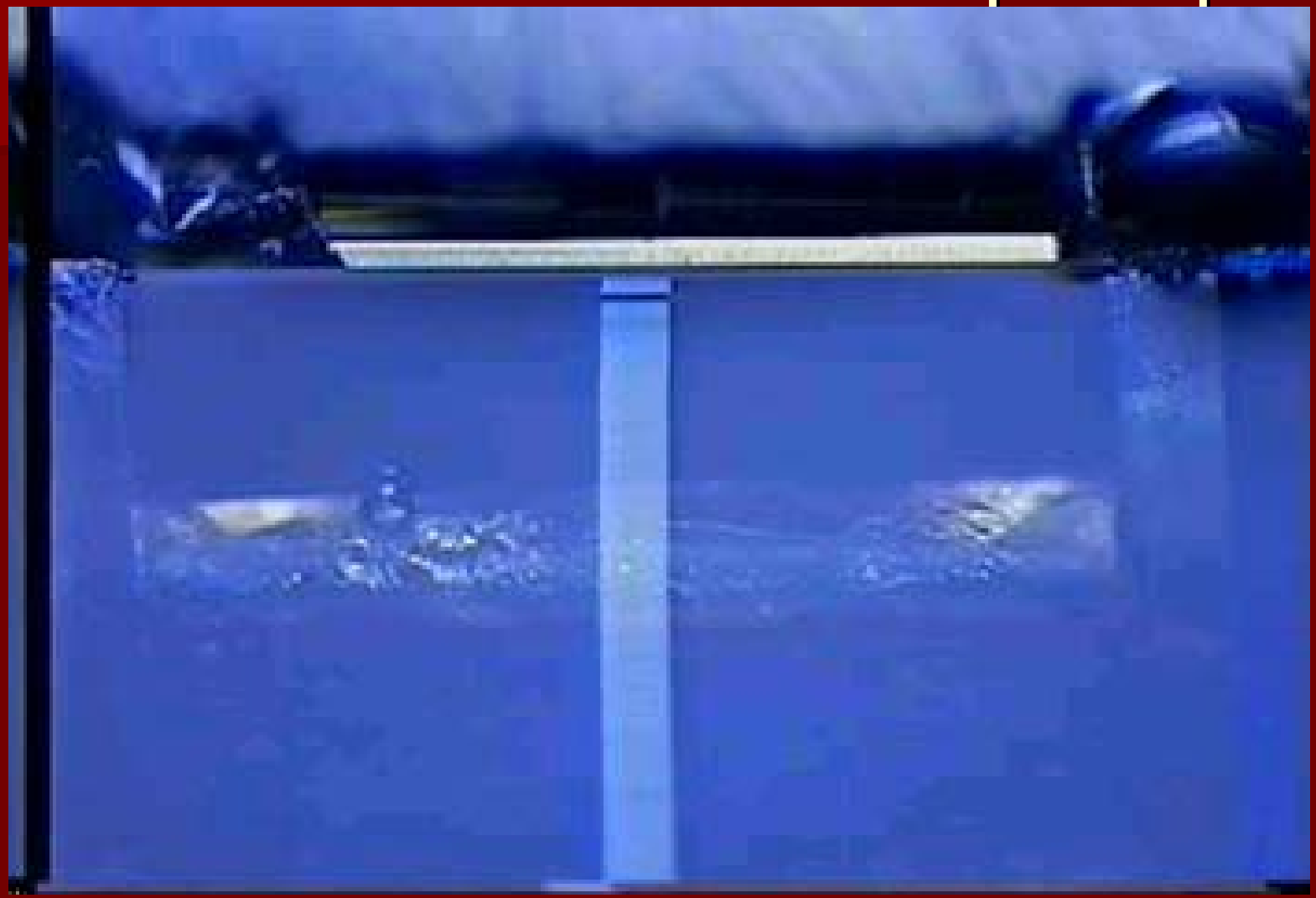
Diatome CryoTrim 45^{o} Tool for Precise trimming of the Trapezoid

Position diamond knife for serial sectioning

Ribbon coming off of Knife

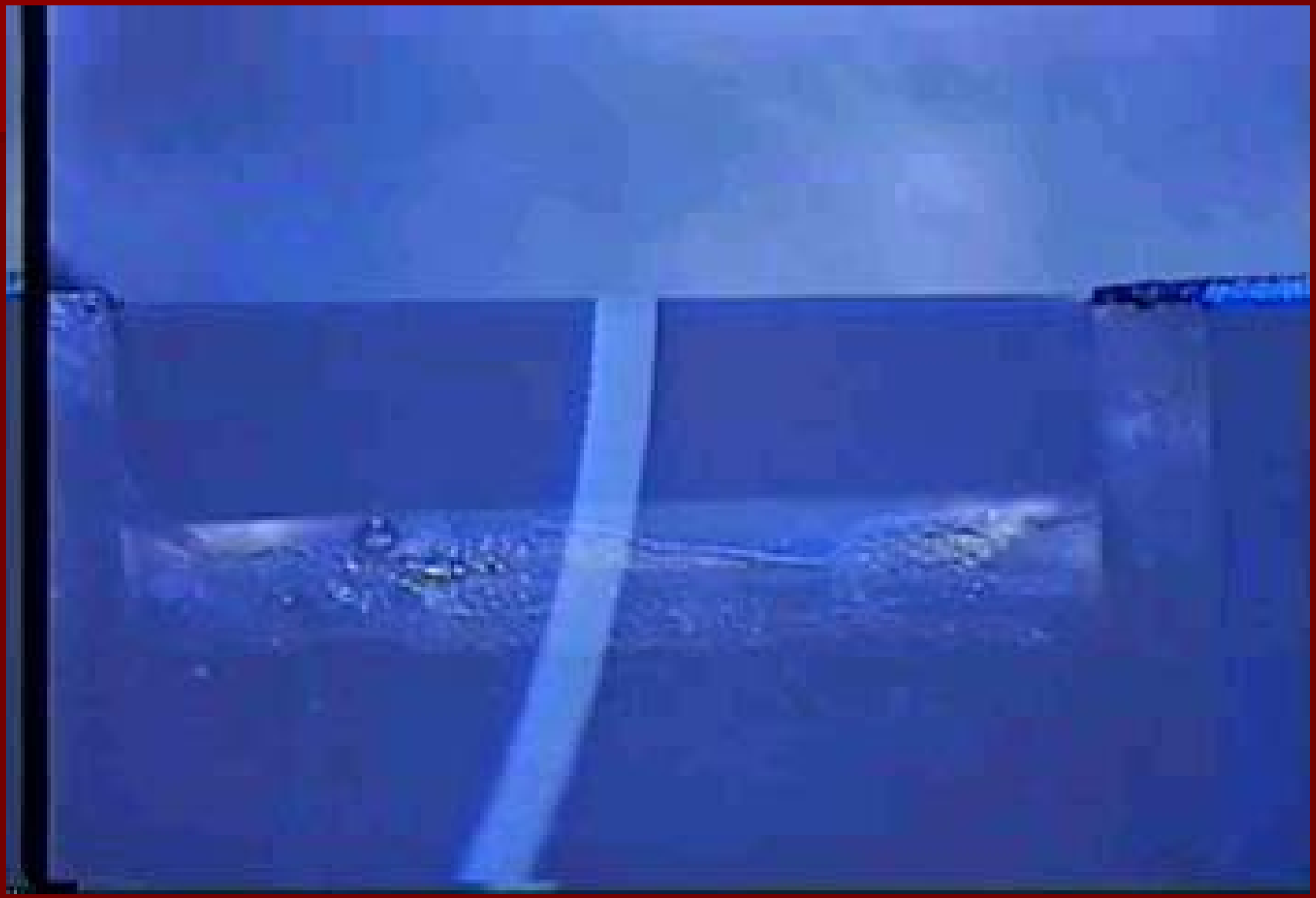
Sectioning rate: 1 mm / second; 40 – 50 nm thickness

420 section series ribbon/pick up



The Perfect Eyelash Set available from EMS, Cat. #70616-10

Broken Series pick up



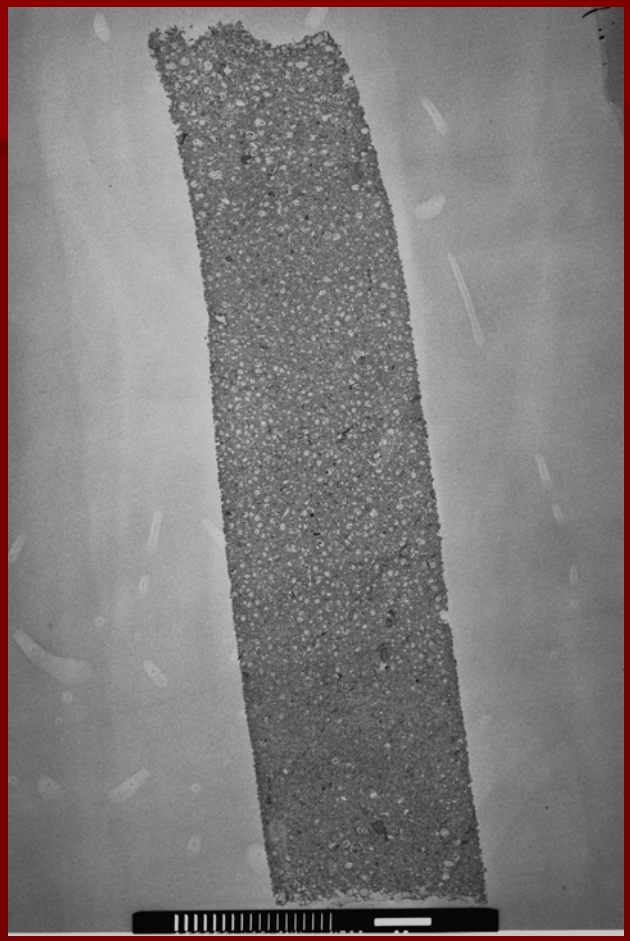
Grid staining, Washing, WICKING DRY!

Loading grid to Gimbol

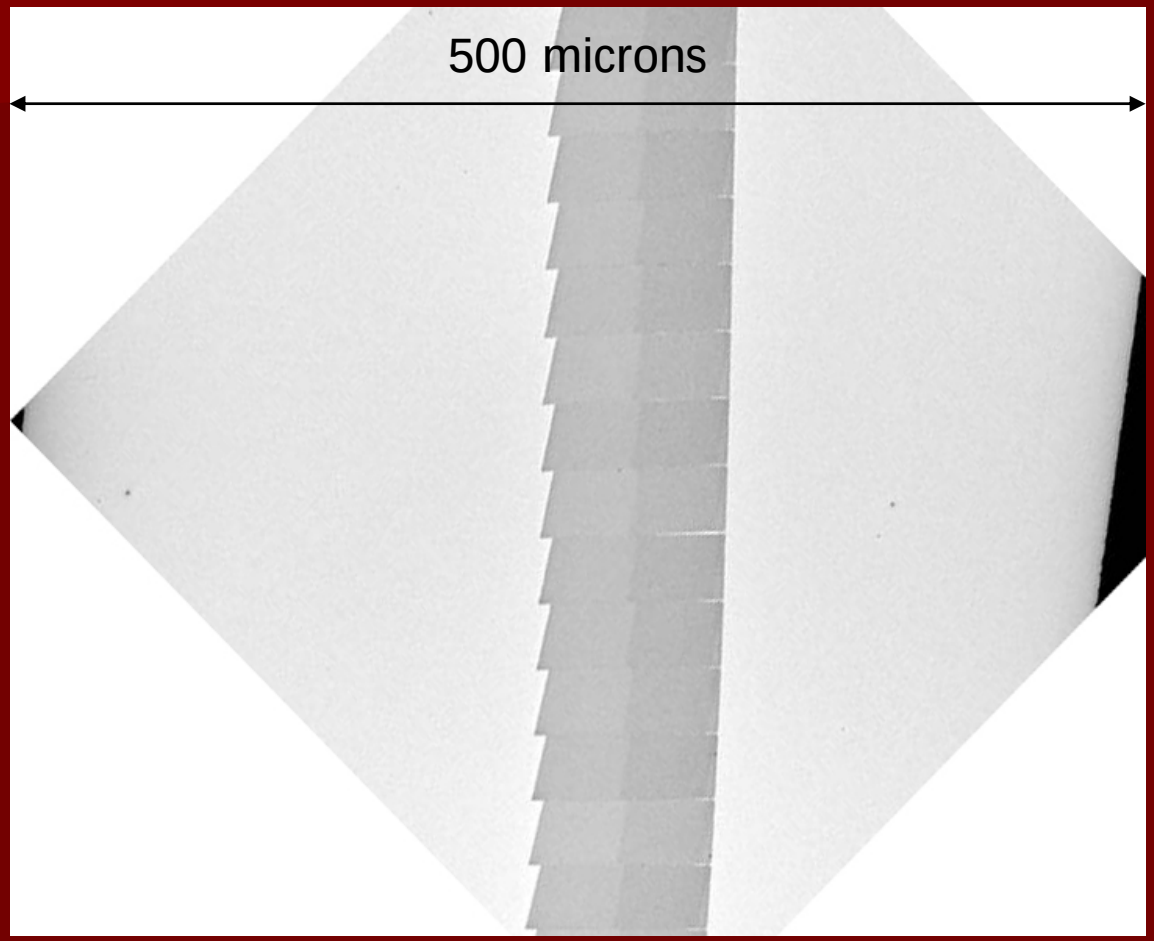
Loading to a Rotational Holder

Digital photography
JEOL 1230 with 4K Gatan

Fold-free, clean, uniform sections



Test Thins

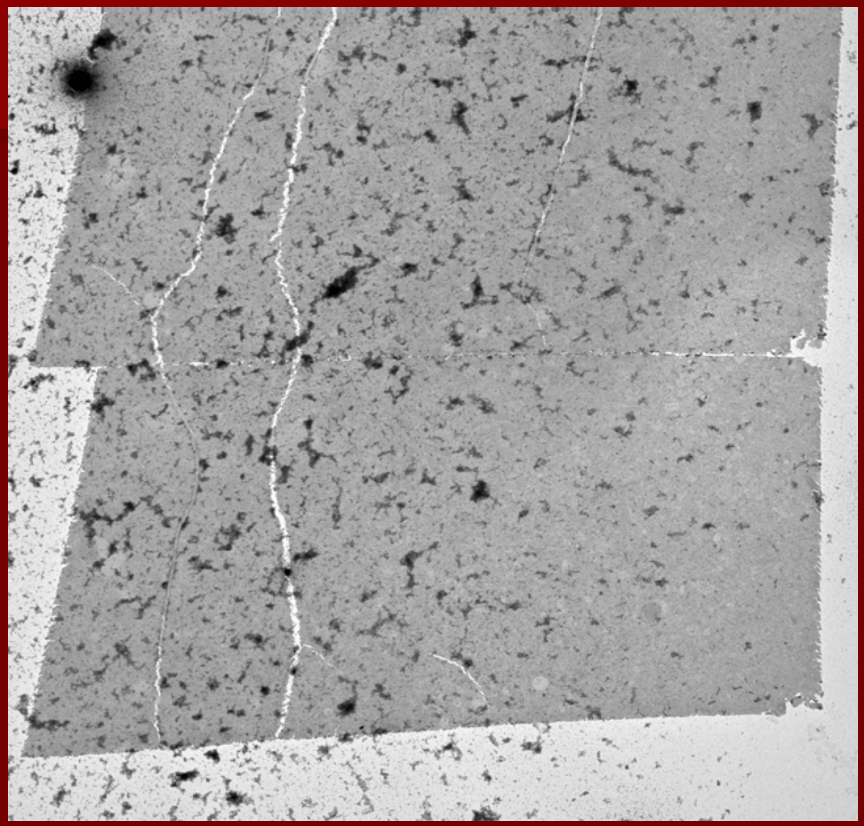


Serial Sections

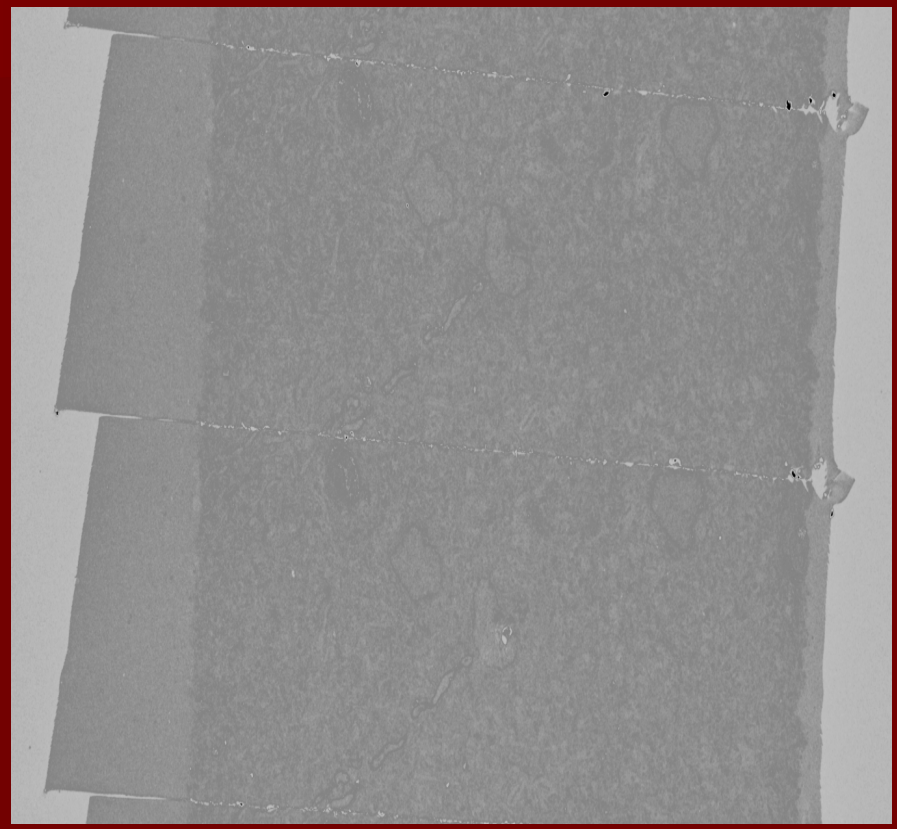
PITFALLS!

And solutions... ☺

Dirty old stains, or improperly dried

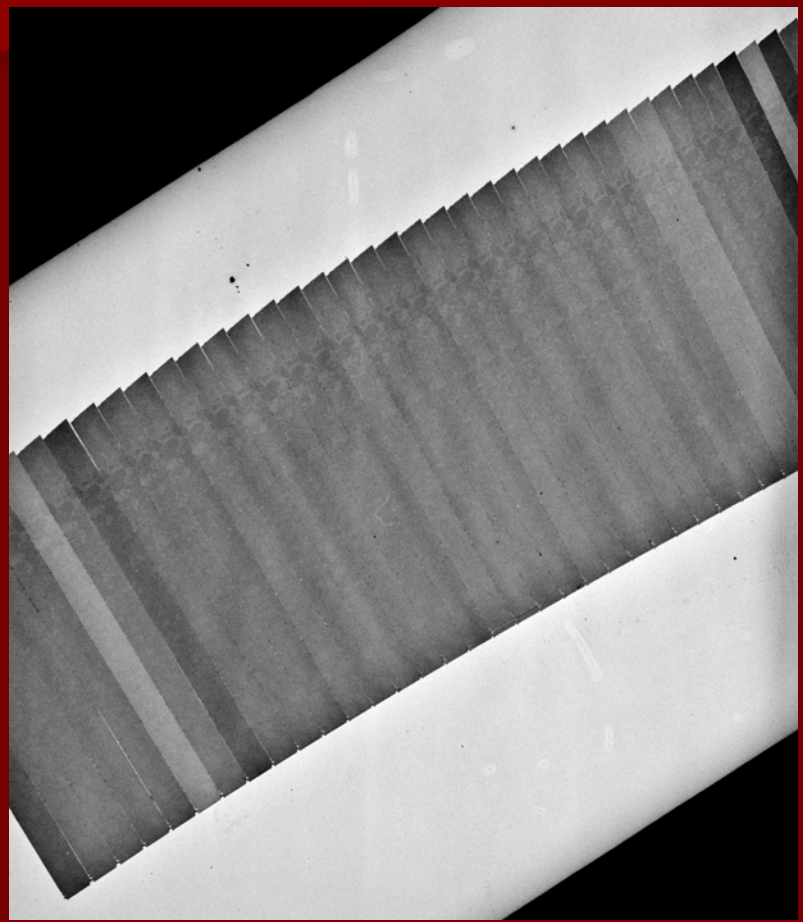


Lead Citrate Stain Precipitate



Make fresh stain often
to have clean sections

Uneven section thickness

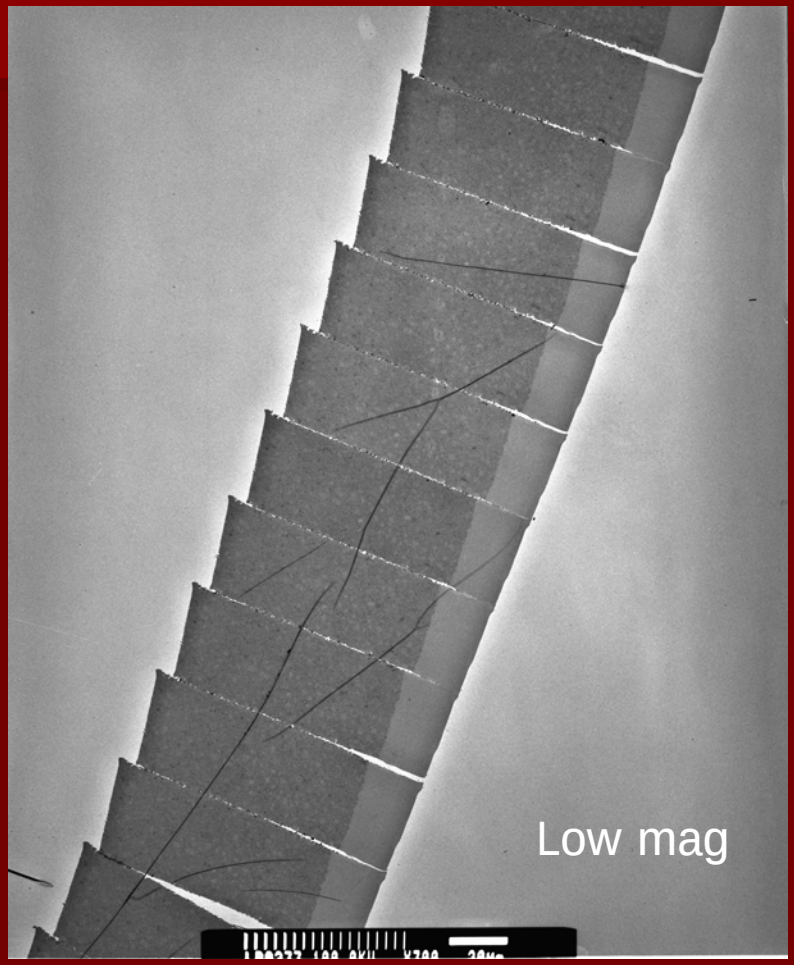


- Eliminate Airdrafts!
- Eliminate Heat variation
  - Body temperature
    - Get out of the room!
- Ensure parallel top and bottom trimming
- Perfect Epon curing
- Make knife angle parallel to block face
- Etc...

Graphite plexiglas enclosure fixes most uneven sections problems

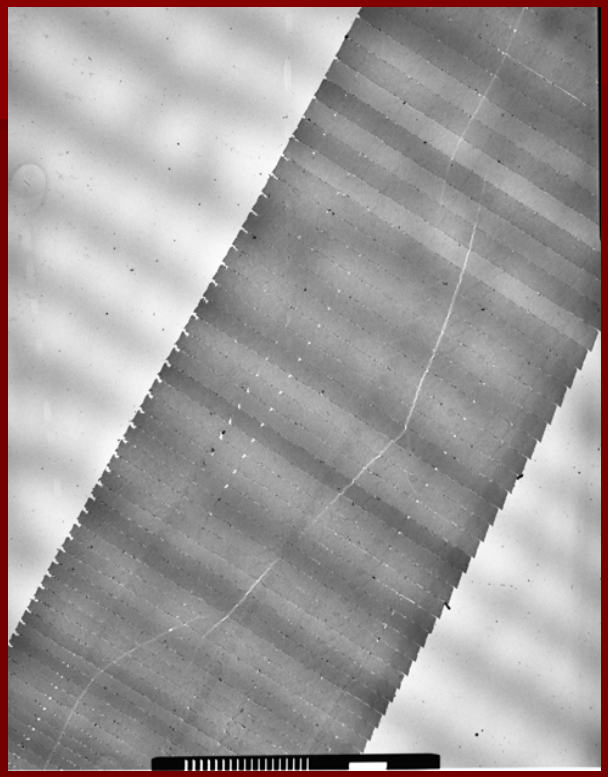
Here enclosing the Leica NEW UC6 ultramicrotome

Folds, Ugh! – >1 day
Old Pioloform Slot grids

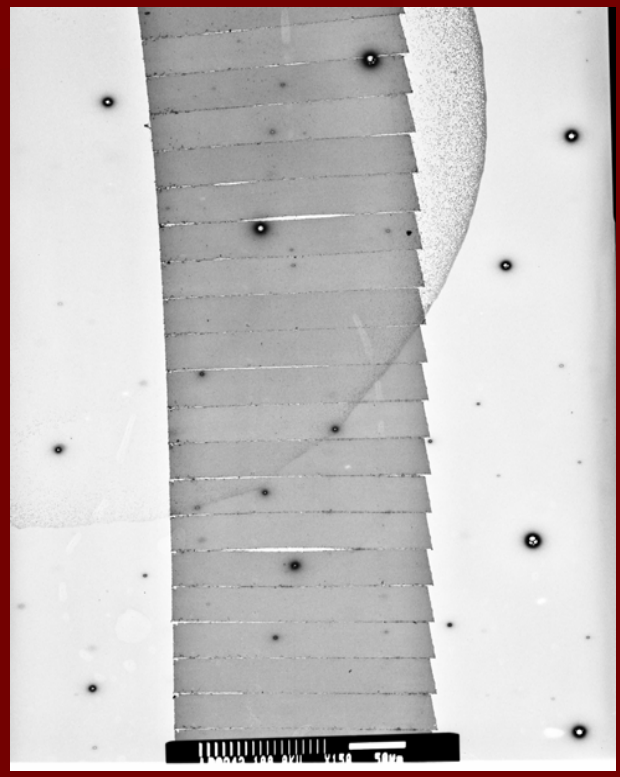


Folds caused by section drying down on a saggy Pioloform film.

Other Grid Coating Problems

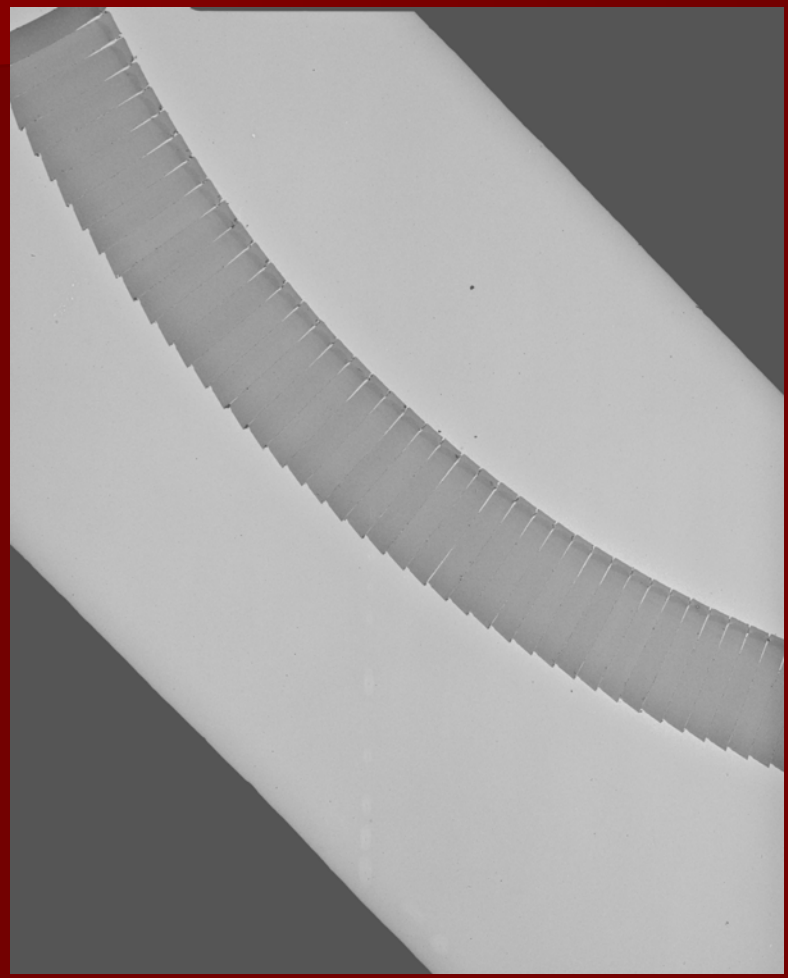


Uneven Pioloform
Too Thick coats unevenly
(Gold not Sliver
Interference Color)



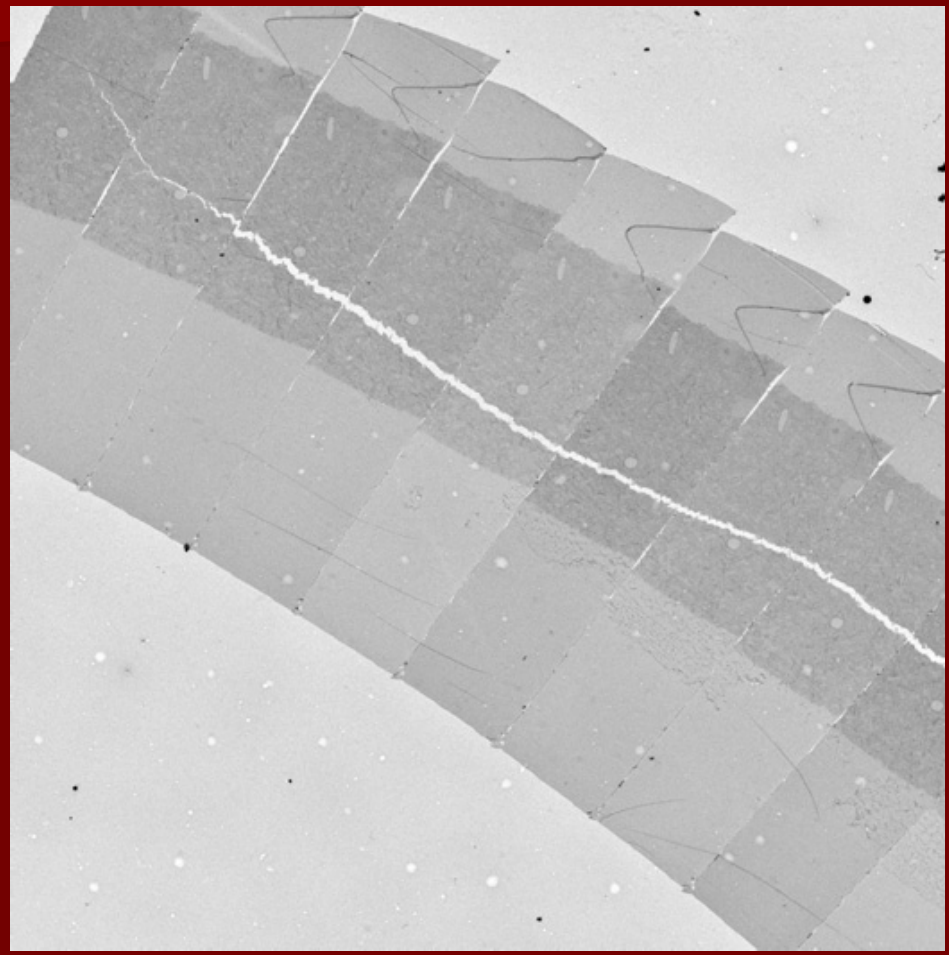
Pioloform
Holes Caused by
moisture

Curved Ribbon – hard to center on the narrow slot grids



Non-parallel top and bottom

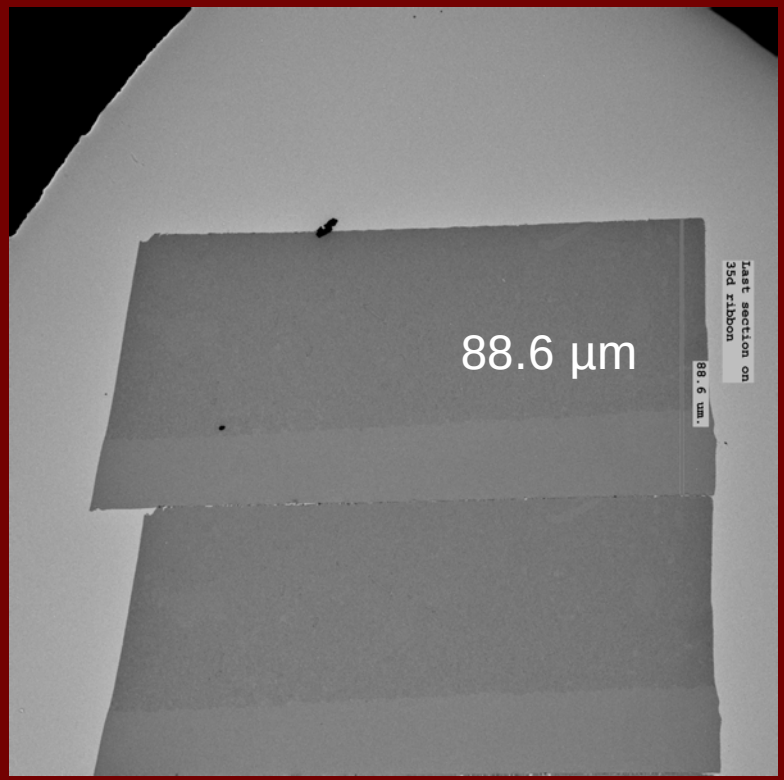
Tissue cracks



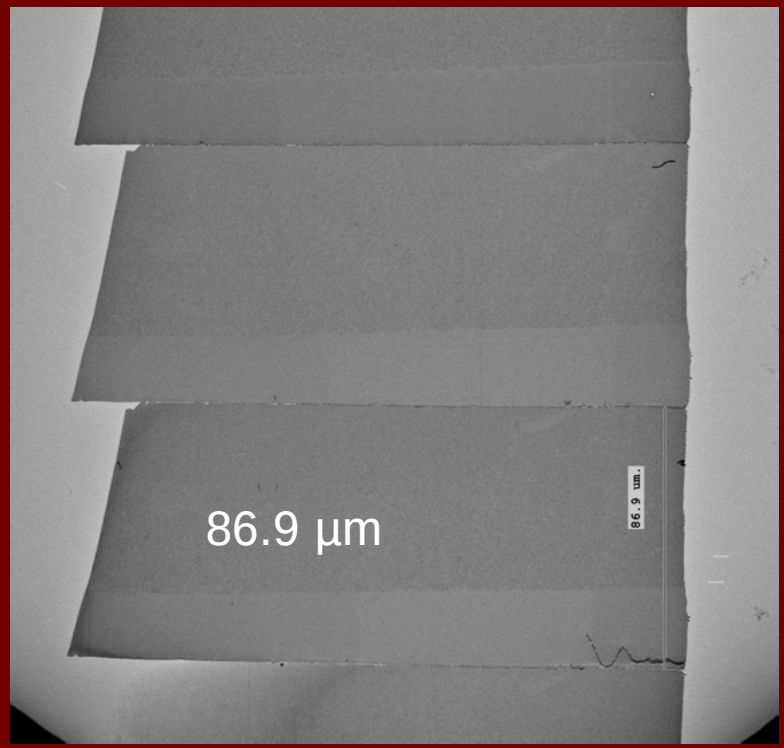
Poor Resin Infiltration

35° diamond knife for serial sectioning gives least compression

Acute knife angles cause more compression



Section 200 cut with 35º Knife

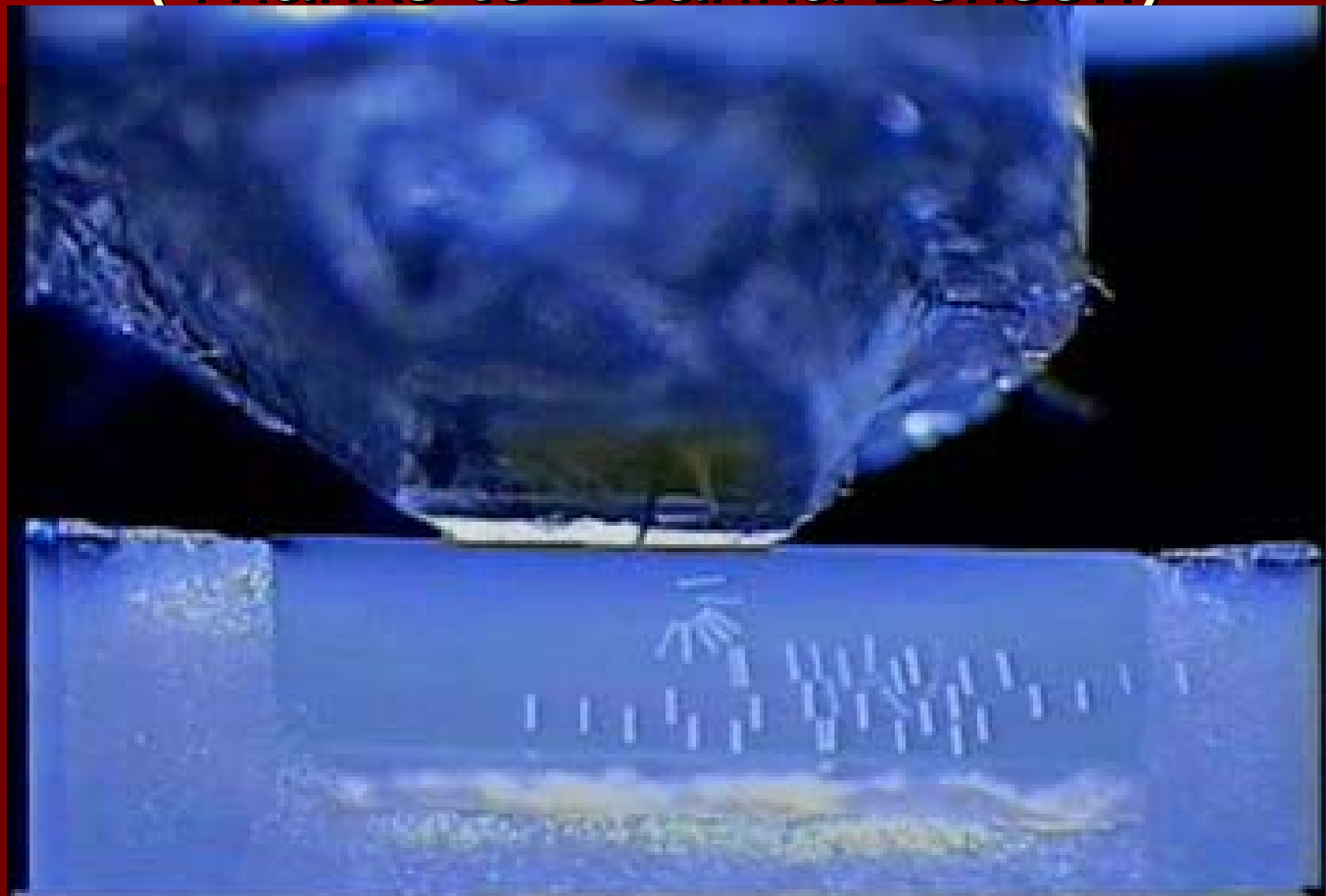


Section 201 in same series cut with 45º Knife
Has 10% more compression

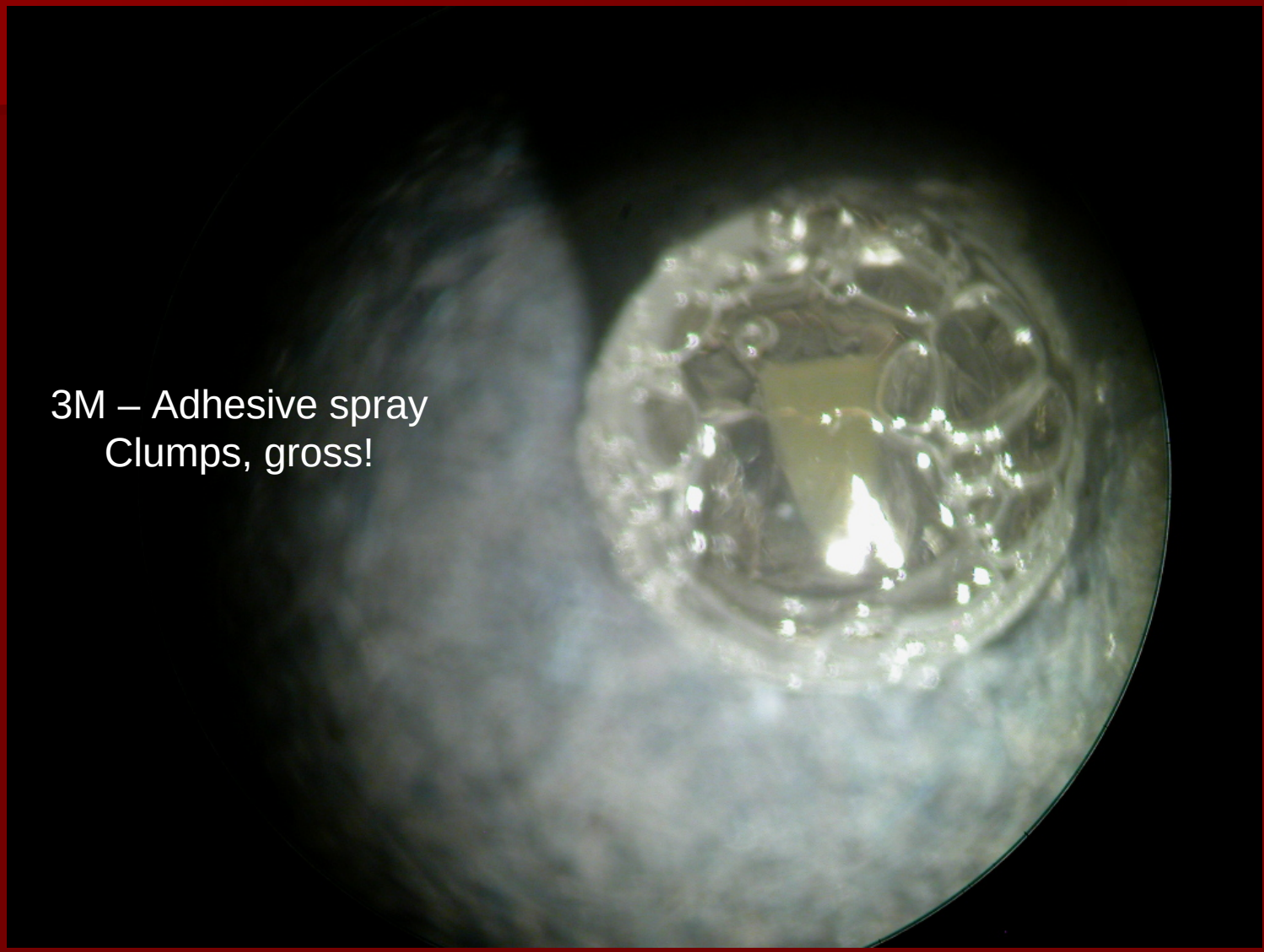
How ‘bout series and other Resins

- Lowicryl for post-embedding Immunogold labelling...
- Huge problems for 3D series – solved last week!

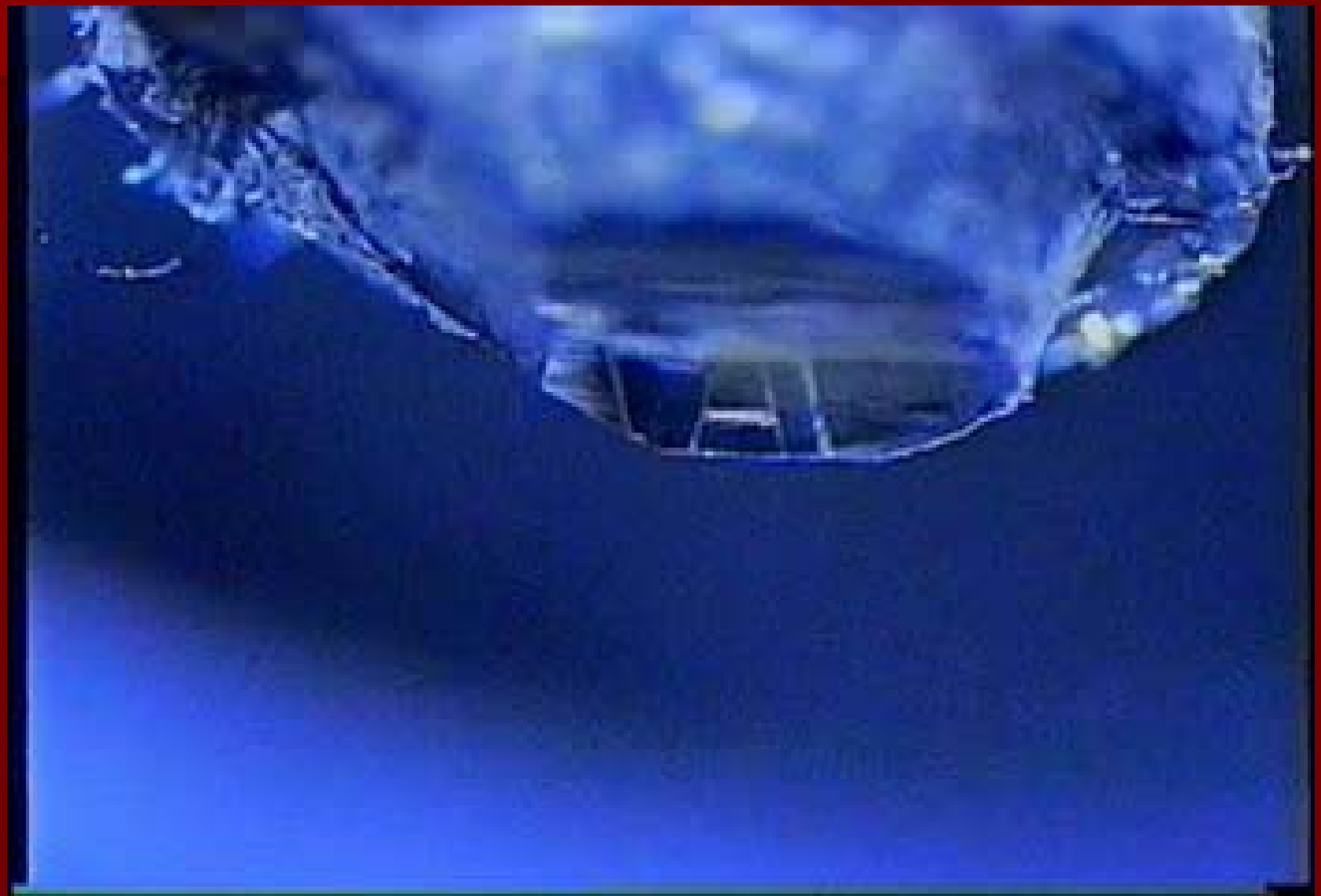
Lowicryl
(Thanks to Deanna Benson)



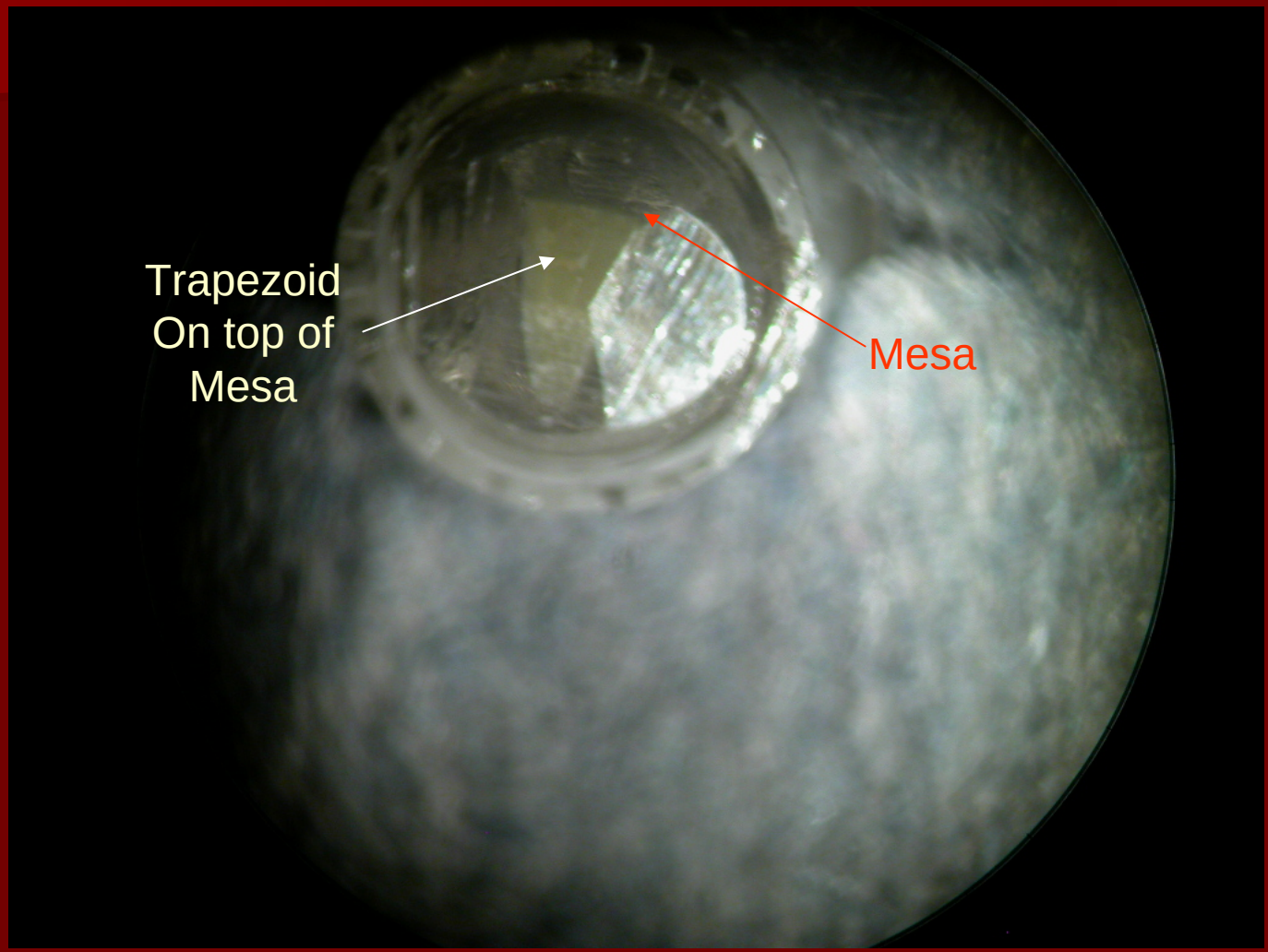
Not Just Any Glue will do

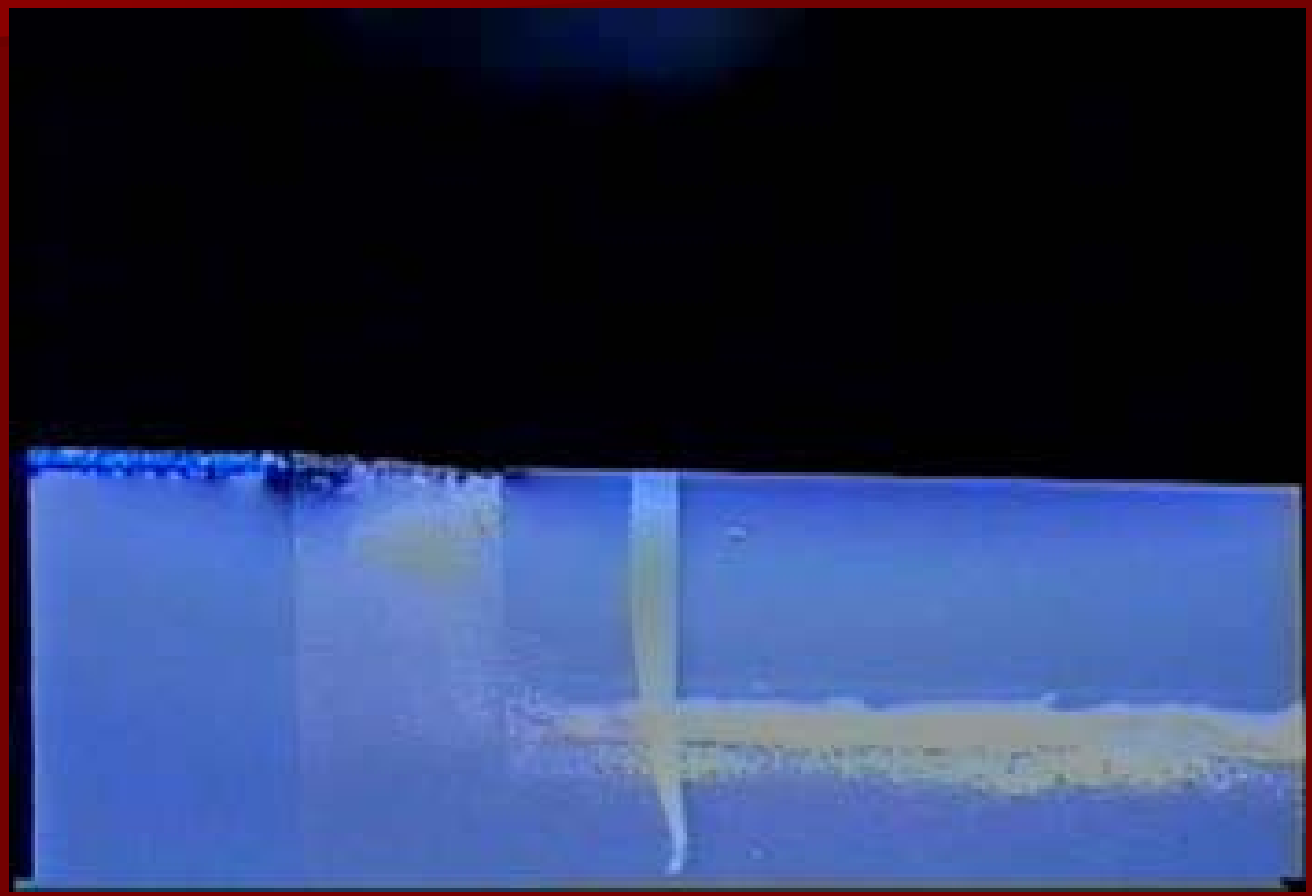


Hair Salon Hair Spray



Clean dry hair spray

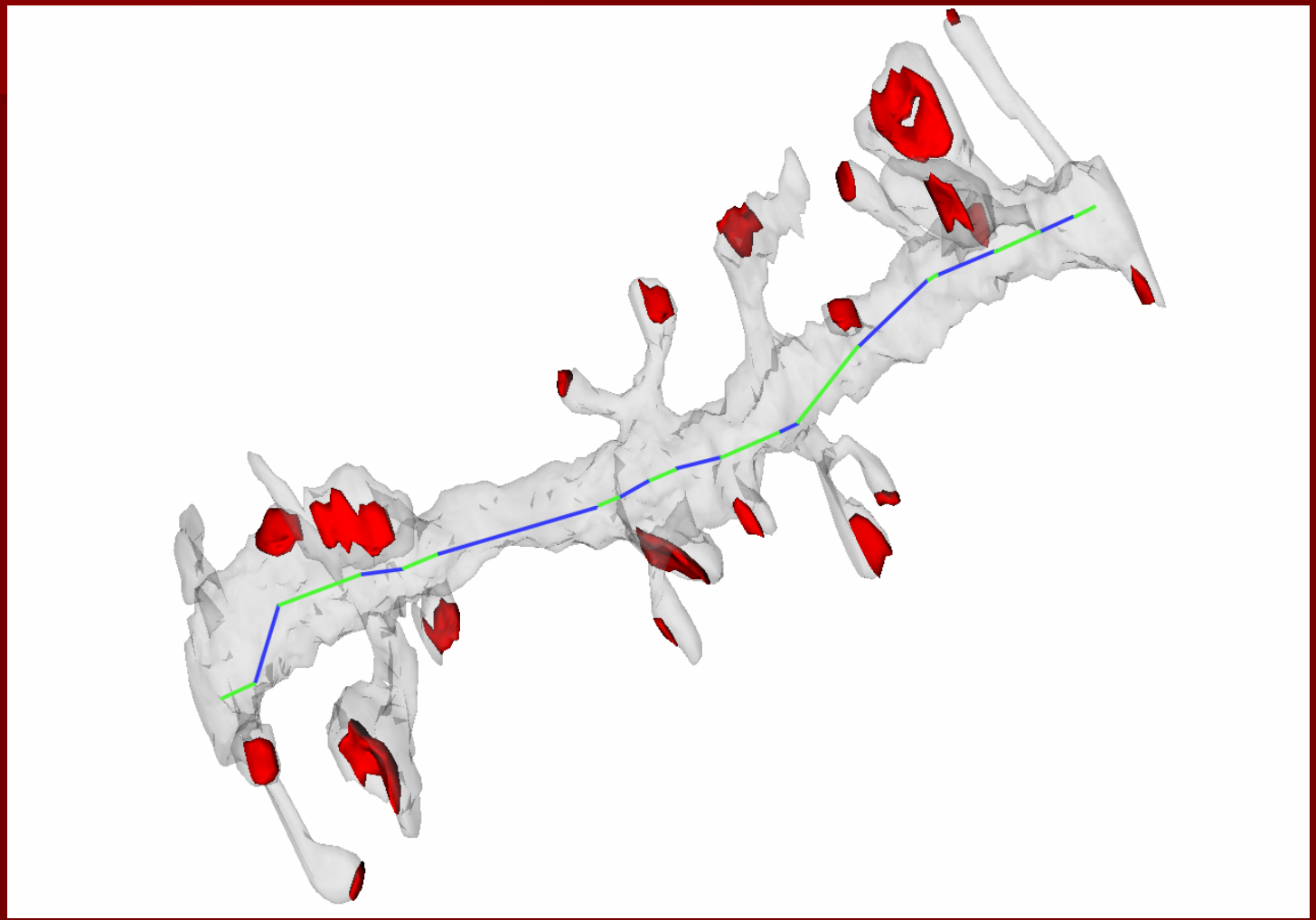




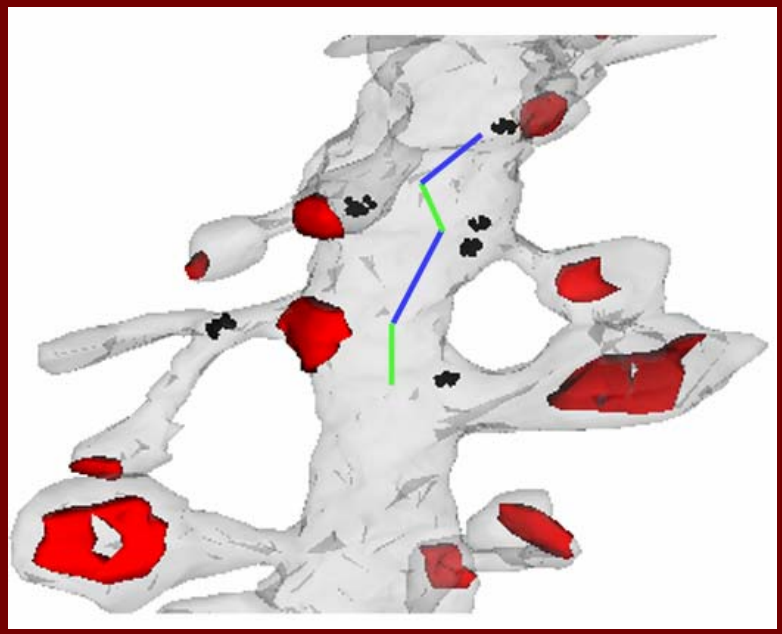
Posters with new data - Harris Lab

- 1:00 Sat. #43.18 (Mishra et al)
  - Dense core vesicles as substrate for synaptogenesis in hippocampus
- 8:00 Mon. #385.1(Witcher et al)
  - Plasticity of Perisynaptic astroglial process at mature hippocampal synapses
- 1:00 Mon. #501.2 (Bourne and Harris)
  - Old title: Spinules and LTP at mature CA3->CA1 synapses
  - New Title: Structural Plasticity of Mature CA3-> CA1 synapses during LTP
- 1:00 Mon. #570.5 (Shi et al.)
  - 3d Editor – new software development

Linear Next Neighbor Analysis:
A Powerful Measure of Spine Density



Detect Polyribosome clustering
Most PR closer together in LTP



So how do we do this?

RECONSTRUCT
John Fiala
Download from: synapses.bu.edu

Reconstruction through Serial EM

- Example from LO 114c
  - Start section 55
  - Go up – stop at 67 to illustrate PR at a synapse
  - Go up to second synapse
  - Do 3D sects 55-86
  - D01 then d01c04a3D and d01c04b3d
  - Change to spheres and add the PR in this protrusion d01rh04a

LO114 test sections

- Demonstrate image quality correction
  - Domain – adjust contrast
  - Domain attributes – see new values for brightness and constrast (B/C)
  - Apply these B/C values to all Sections
- Demonstrate alignment
  - Images are digital from a 4K X 4K Gatan camera on the JEOL 1230 EM
  - Hence, pretty well-aligned from the scope
  - RECONSTRUCT – bring into perfect alignment.
    - Sect 99 → 100 to start.
- Demonstrate calibration

RECONSTRUCT – NQDBP sect 28
Demonstrate tracing and 3D – see also nice MSB.