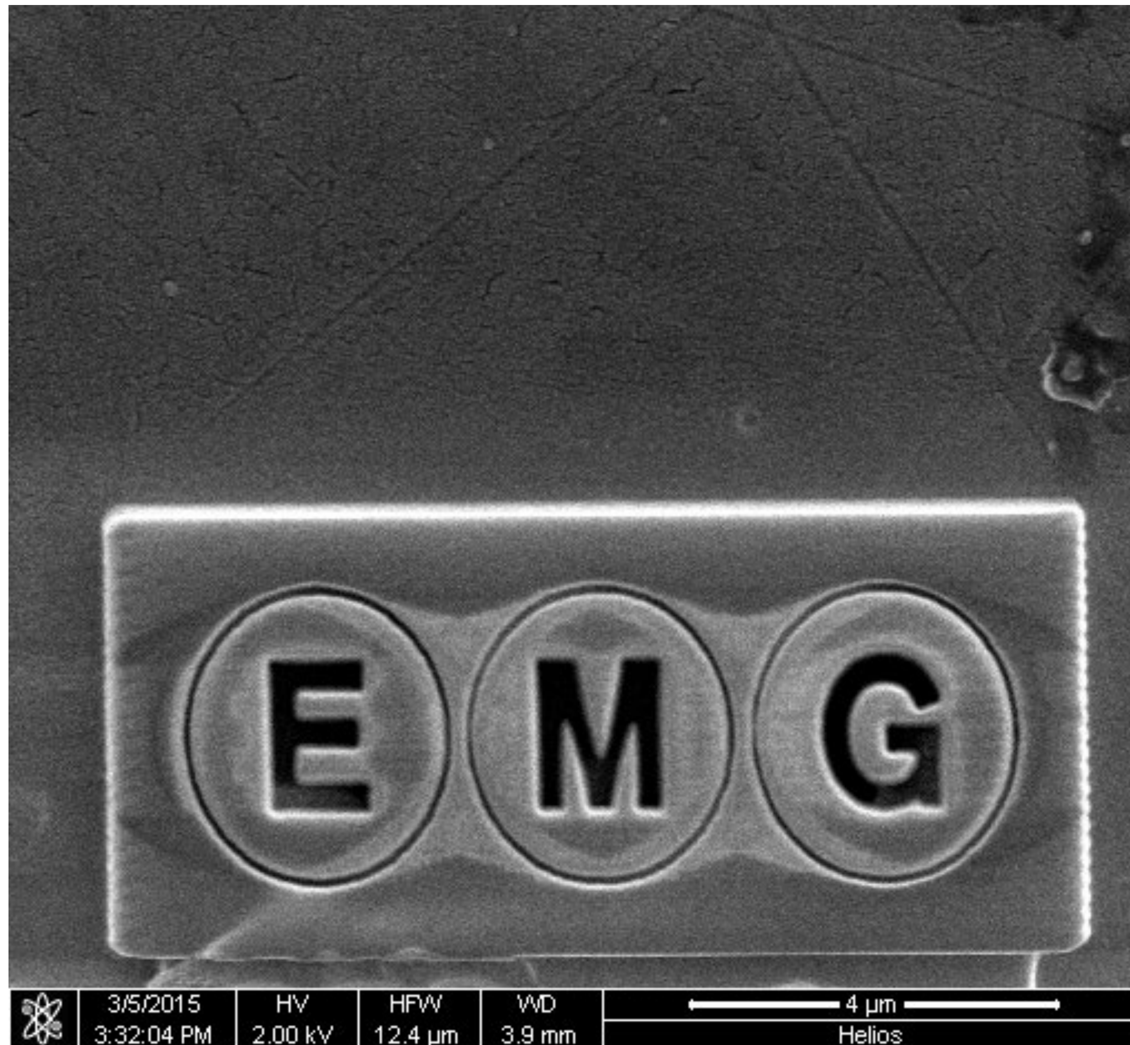
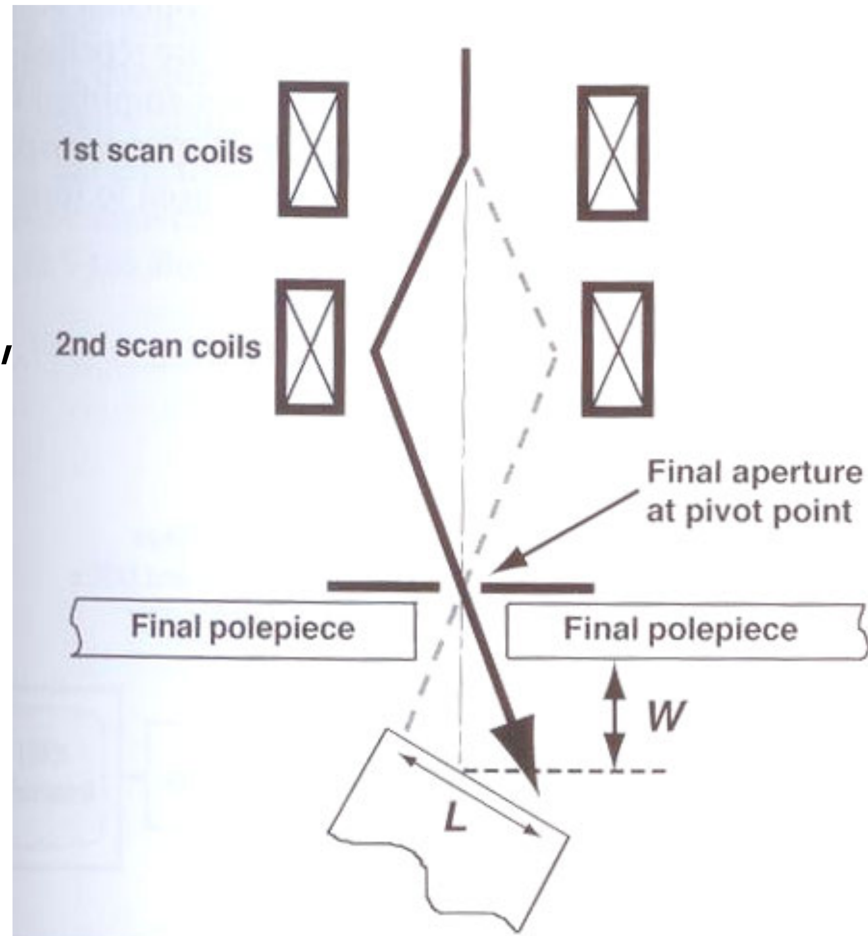
FIB/SEM
Feb. 29, 2016



SEM Basics

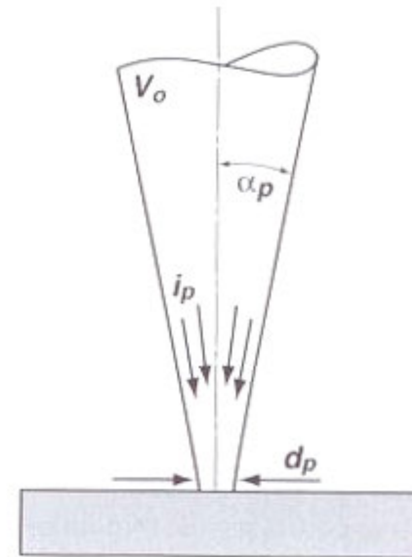
- Electron probe is focused to a sharp point
- The probe is scanned across the specimen point by point, with each point producing signal
- Scan coils deflect beam to move across sample



Goldstein et al, 2003

SEM Beam: probe size

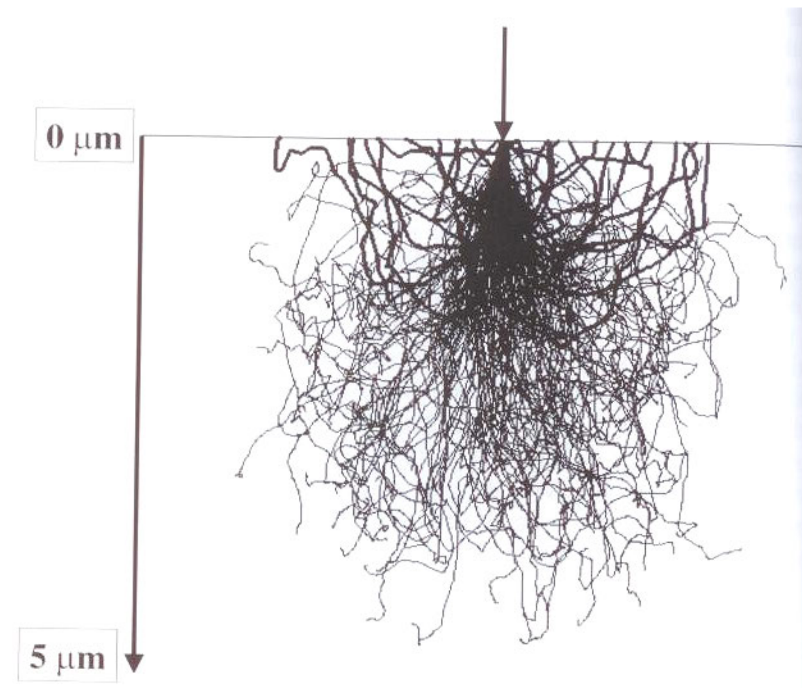
- Ideally want as small a probe as possible, relative to pixel size
- Probe size is determined by voltage, current, divergence angle
- Lens distortions
  - Spherical aberration (focus different at center and edge of lens) – instrument dependent
  - Aperture diffraction
  - Astigmatism (user correctable)
  - Chromatic aberration – voltage dependent



Goldstein et al, 2003

Beam-Specimen Interaction

- Monte Carlo simulation of a 20 keV beam in Si
  - Dark traces: electrons which left the sample (BSE's)
- Electrons may be scattered elastically or inelastically
- Probability of elastic scattering ~ Z^2
- Inelastic scattering:
  - Secondary electrons
  - X-rays

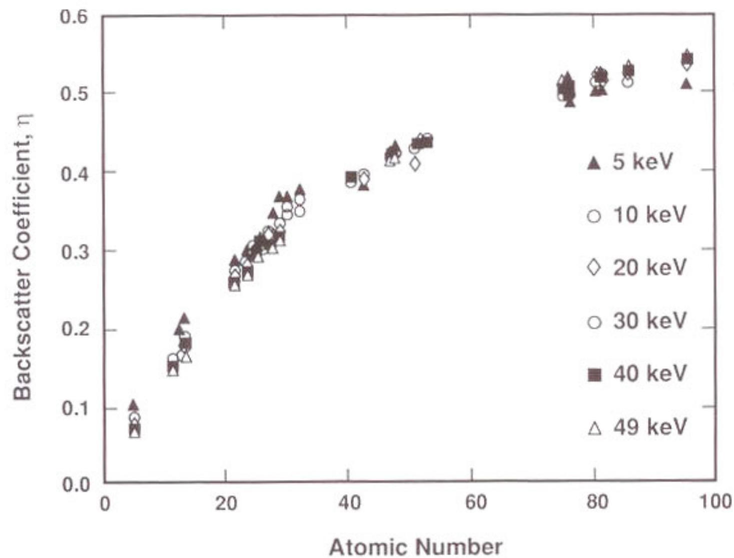


Goldstein et al, 2003

BSE’s and SE’s

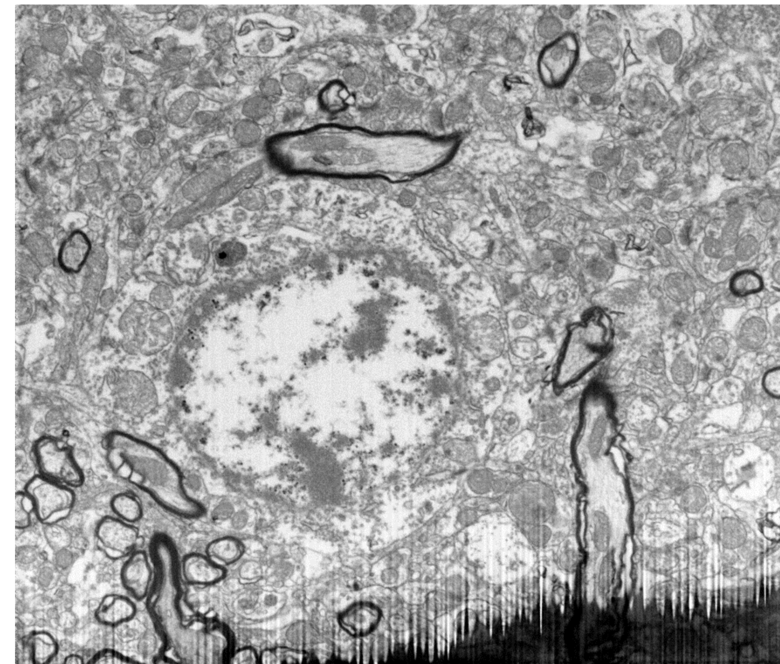
BSE efficiency is material dependent, voltage independent

Fraction of
e's that backscatter

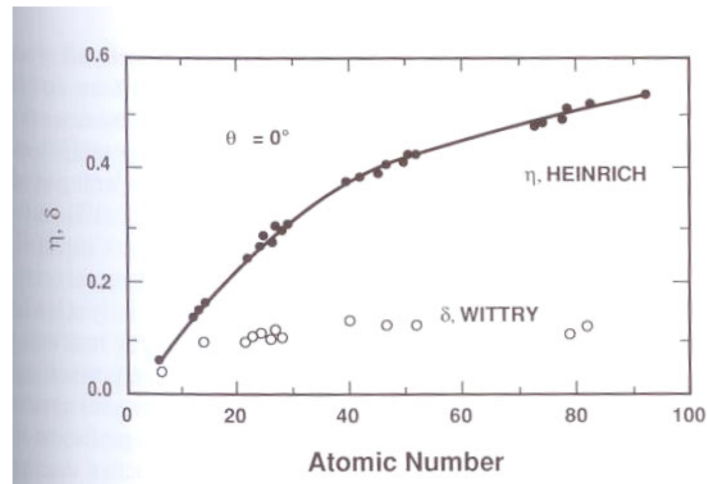


Goldstein et al, 2003

BSE's give contrast between
light and heavy elements



Specimen Dependence of BSE, SE

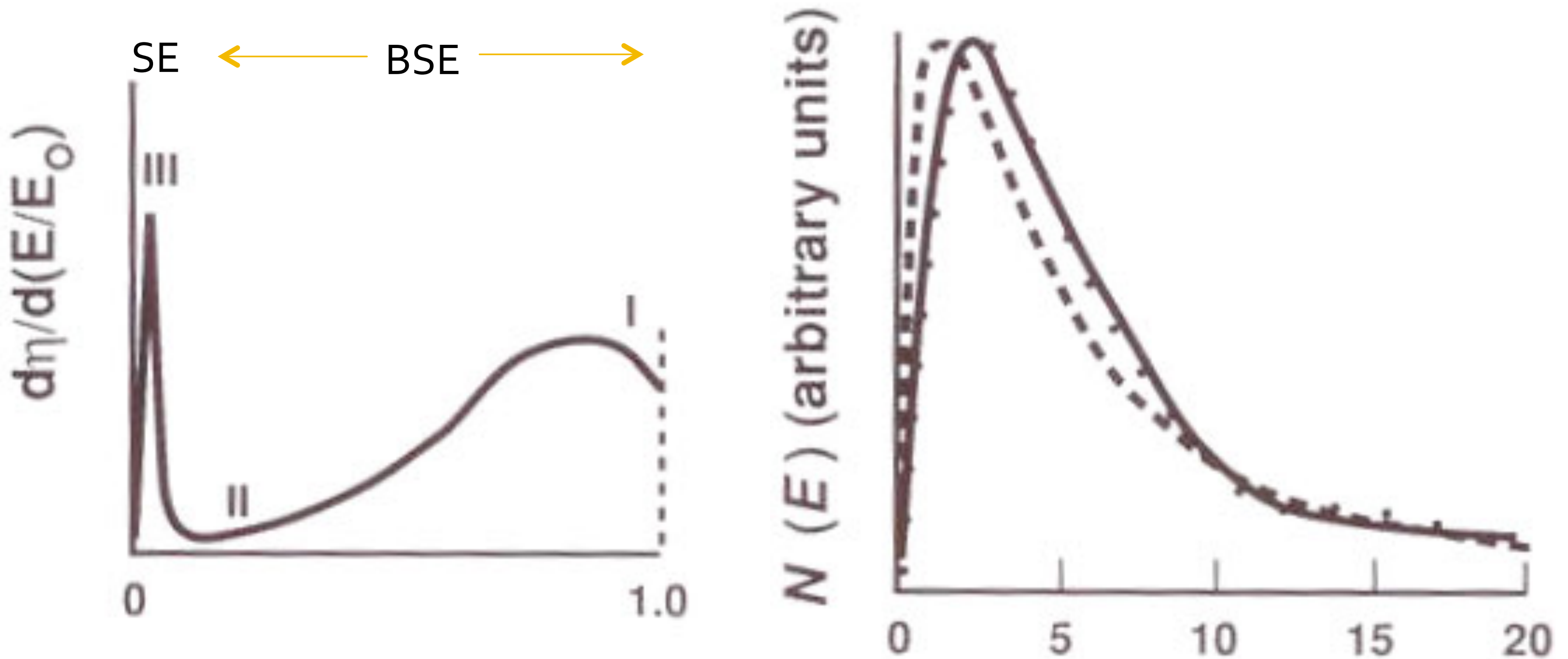


30 keV

SE's are less sensitive to atomic number than BSE's
(may be more sensitive at lower beam energies)

Goldstein et al, 2003

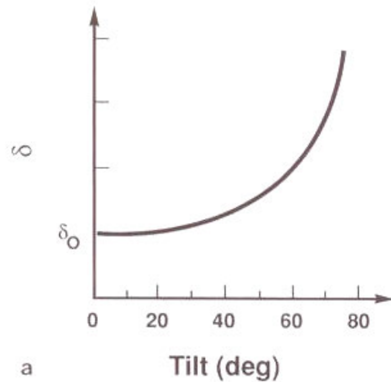
Energies of SE and BSE



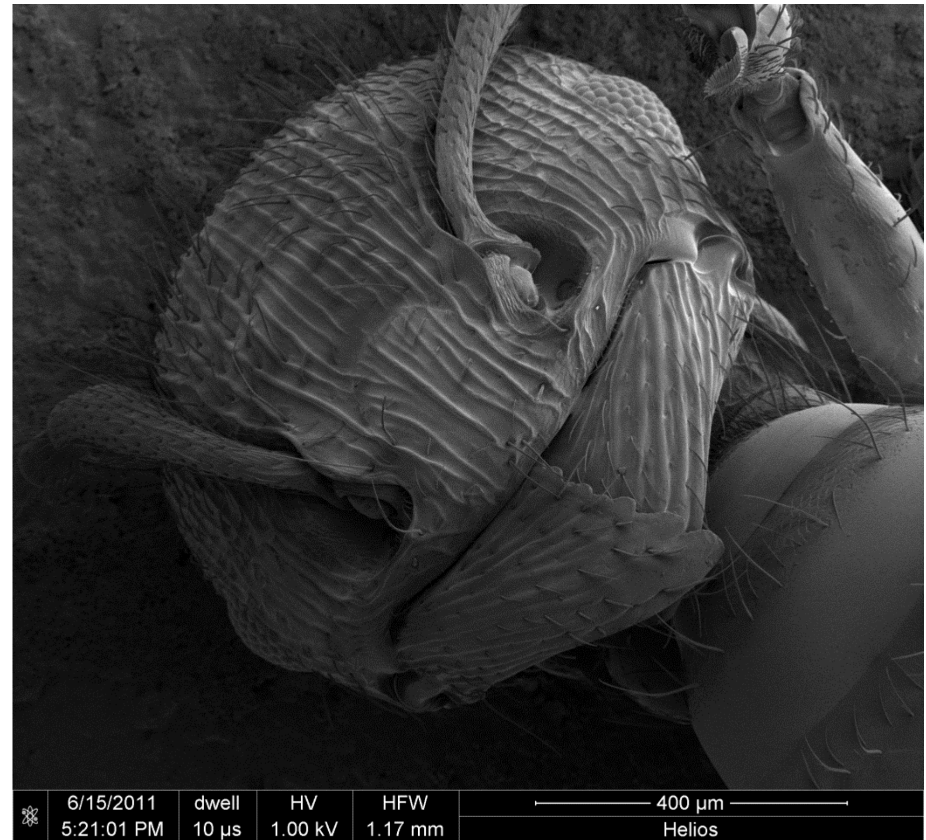
SE's have much lower energy (majority < 20 eV)

Goldstein et al, 2003

Angular Dependence of SE's



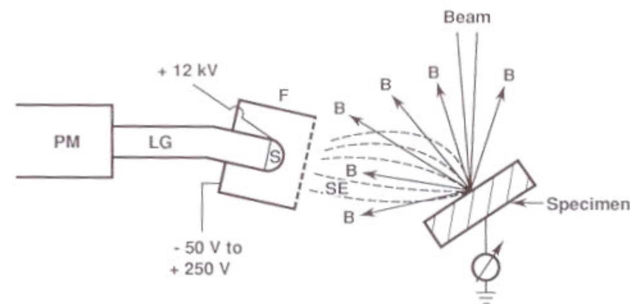
Goldstein et al, 2003



SE's also give topographic information

Detection of BSE’s, SE’s

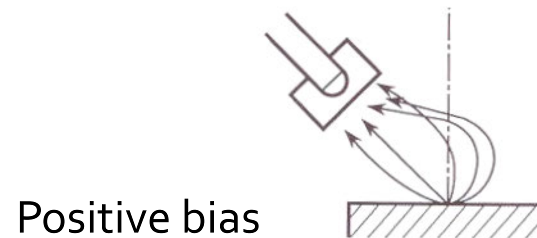
Everhart-Thornley (ET) Detector



Electrons strike scintillator, releasing photons
Photons travel to photomultiplier tube
Eventually converted to electric signal, storing intensity values
Combined SE/BSE detector

Apply bias

- Bias can be applied to detector, directing electrons toward or away from it
  - Negative bias (< -50 V): detect only BSE's
  - Positive bias: collect more SE's, indirect BSE's : greater total signal



Goldstein et al, 2003

SEM summary

- Images formed by scanning points across sample
- For higher resolution, want to minimize both probe size and interaction volume
  - Low voltage operation
  - But still need enough signal for detection
- Use Through-lens detector for higher resolution imaging
- Apply negative bias to detect mainly BSE's

FIB Operation

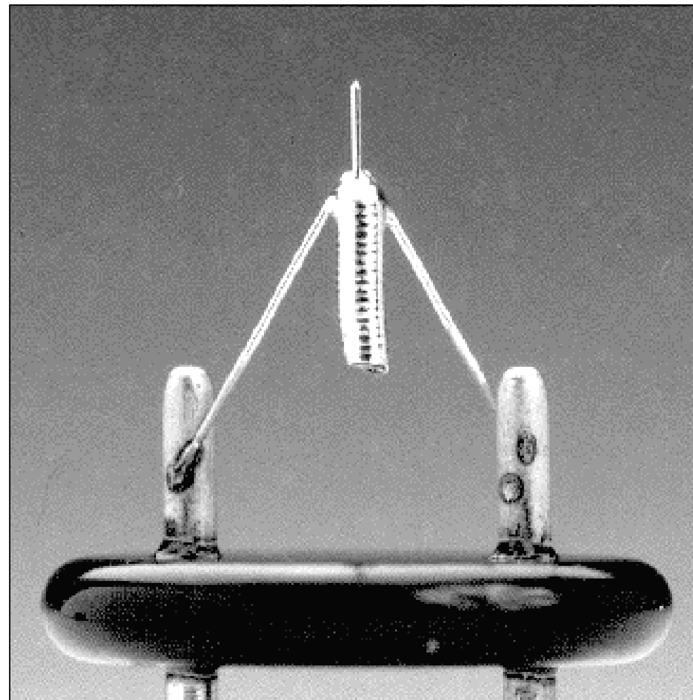
Basic Mechanism

- Liquid Flow from Reservoir
- Ion Formation
- External Beam Interactions

FEI™

Gallium is the Most Popular LMIS

- A liquid metal
- Room temperature operation
- Long lived (500-1500 hr sources)
- High vacuum compatible
- Large ion for sputtering



FEI™

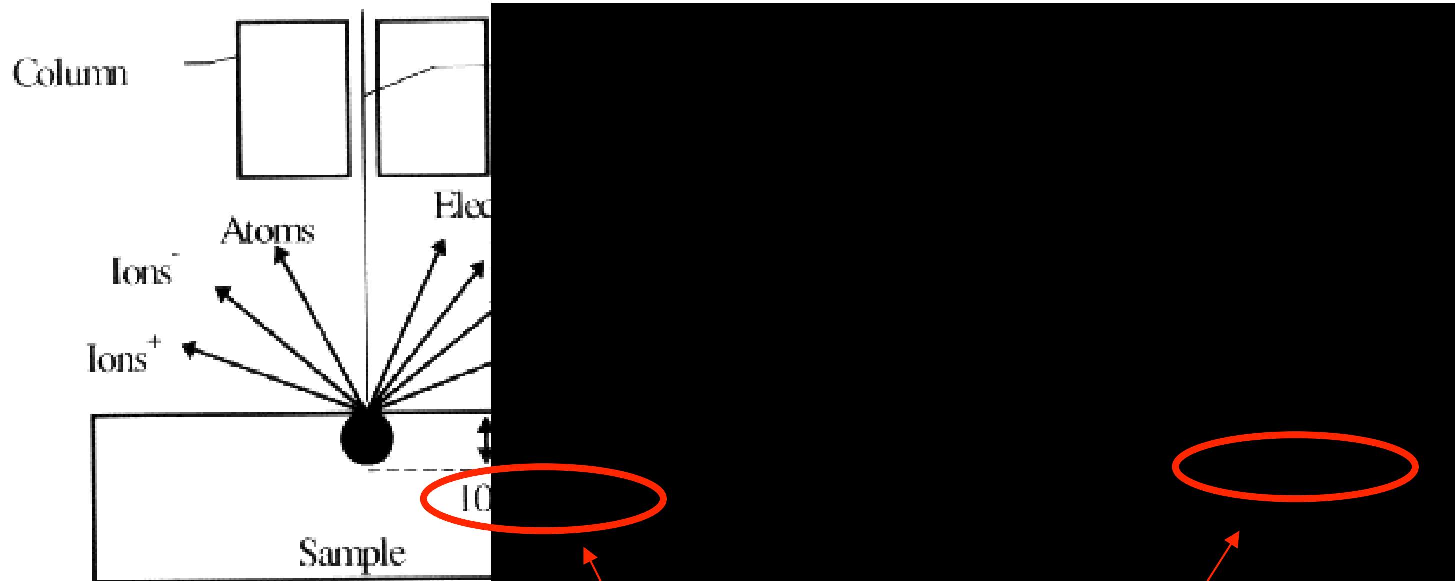
Ion Column

- Source - LMIS at top
- Focusing Optics
  - Use Electrostatic lenses since ions are heavier than electrons.
- Deflection Electronics/Pattern Board
- High-speed Blanking
  - Need to prevent milling while blanking

FEI™

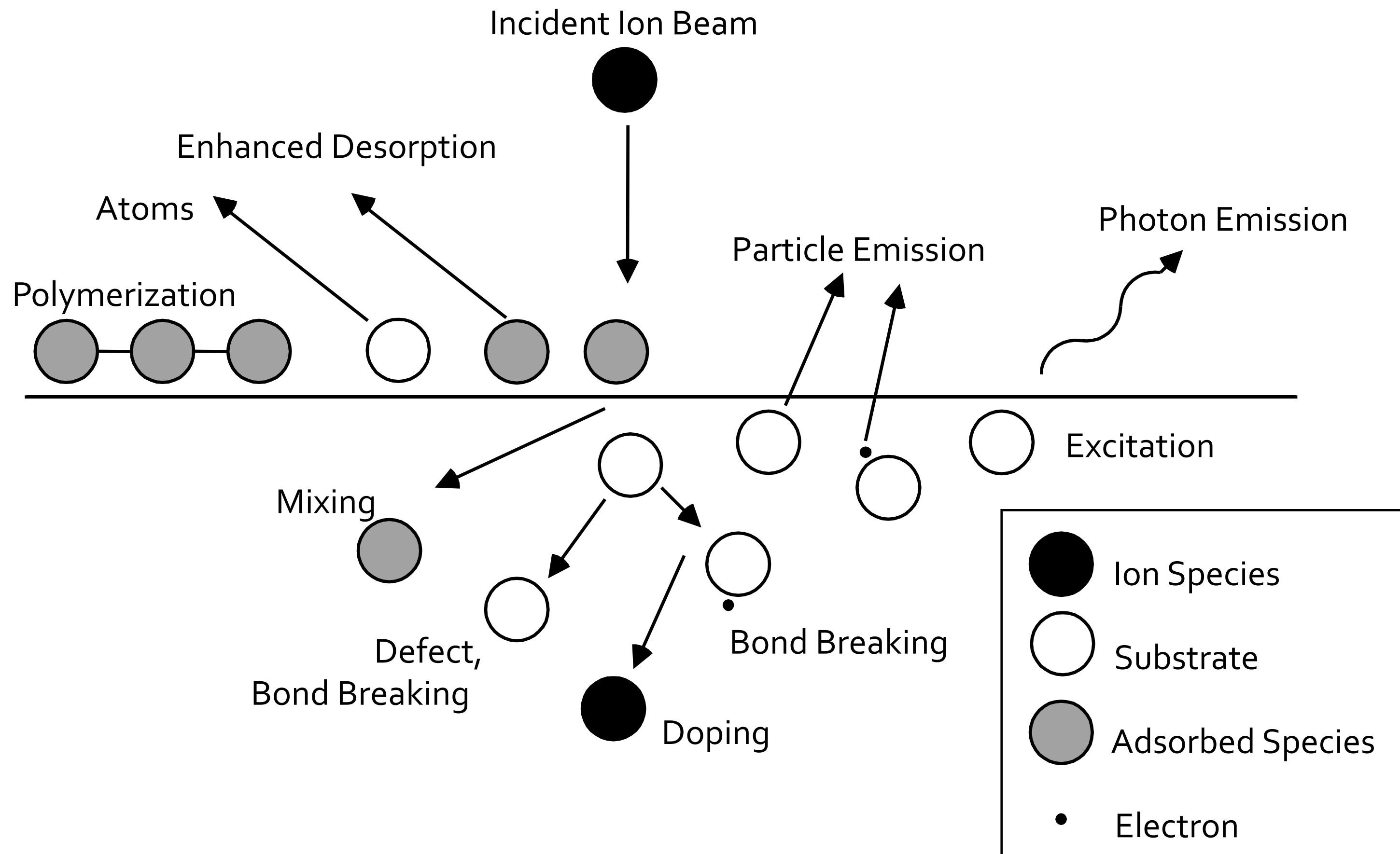
Using the System

- Beam Interactions



Note difference in interaction volume

Ion Beam to Sample Interactions



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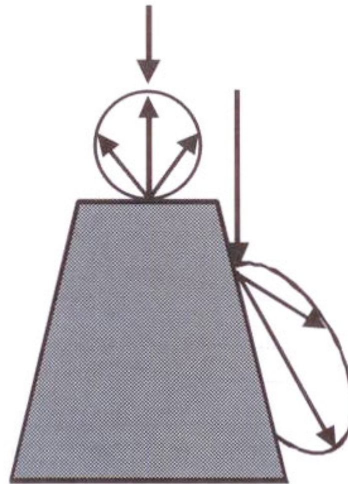
Sputtered Particles

Sputtered Particle Ejection Behavior



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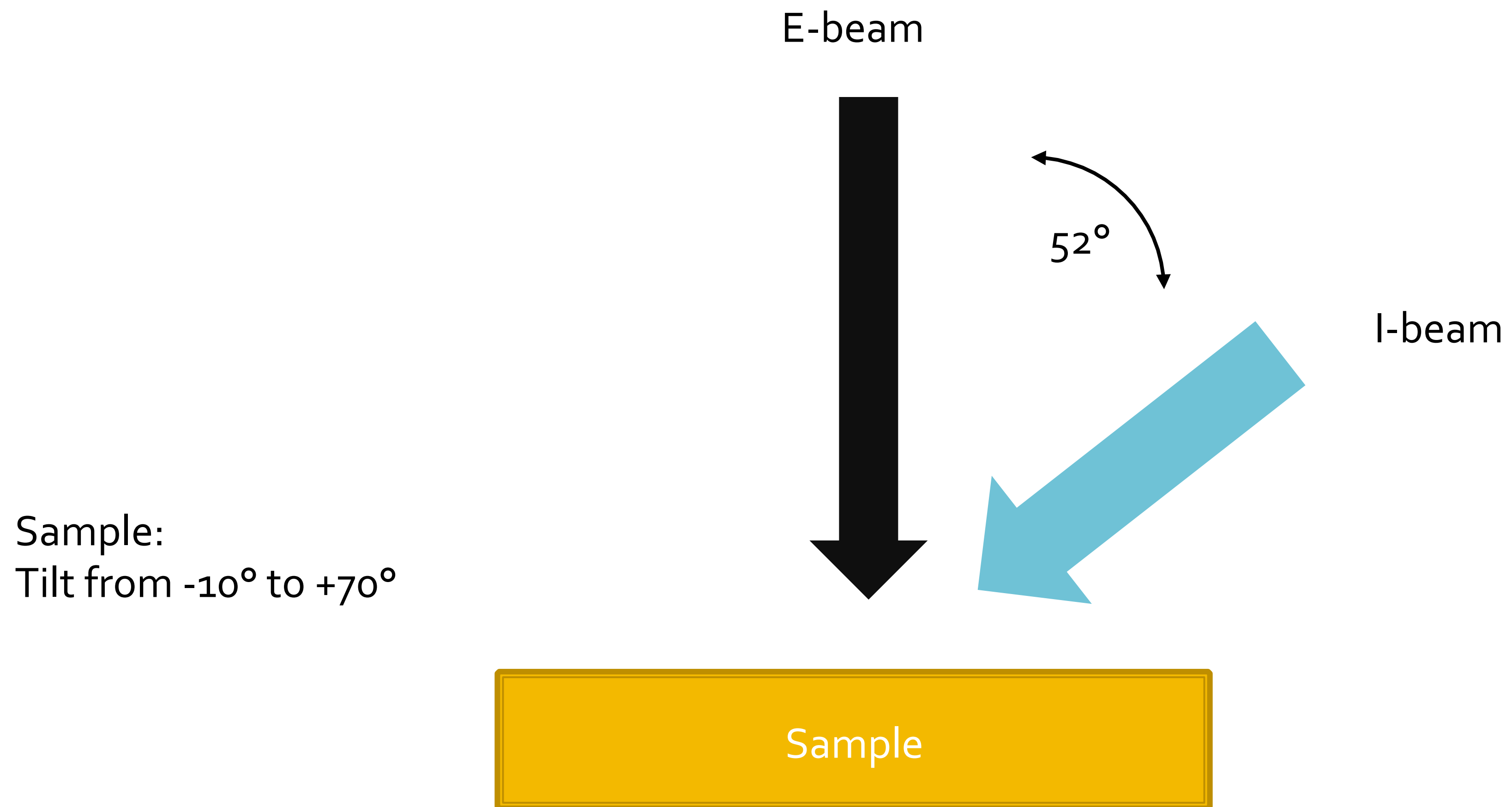
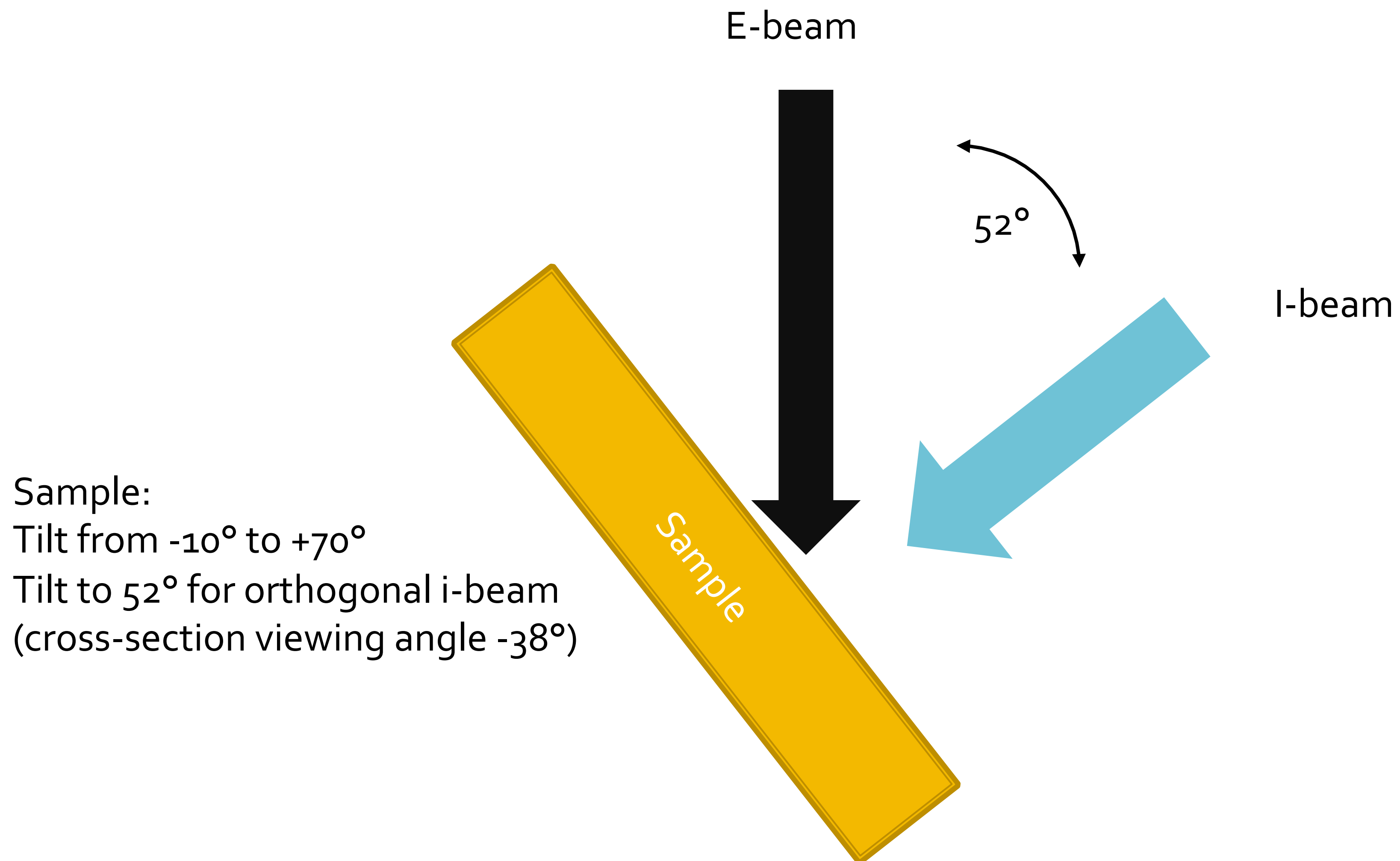
Geometry



Geometry



Applications to Resin-embedded tissue

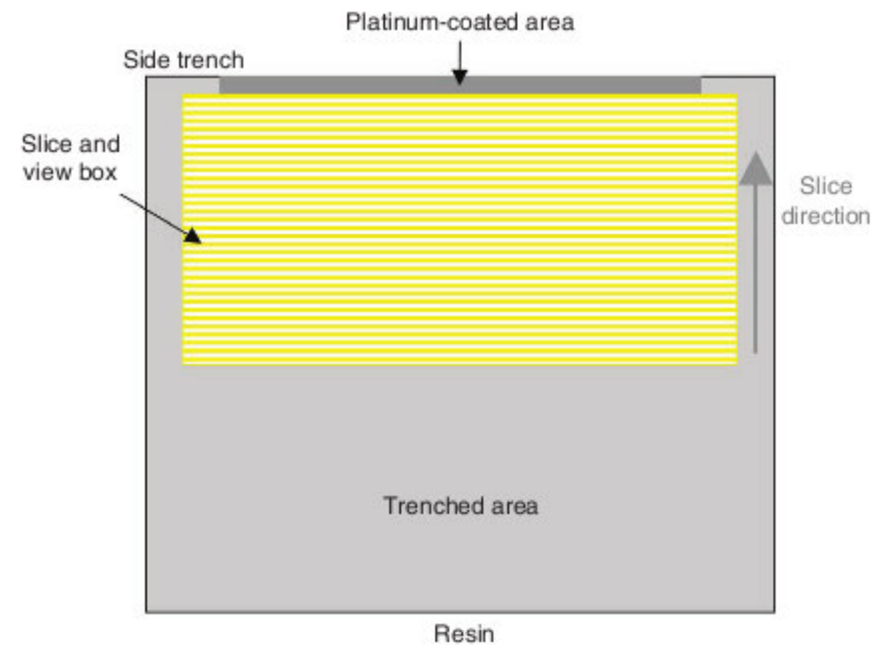
Tissue or Cells

- Sample Prep
  - High Pressure frozen (optional)
  - Chemically fixed, freeze substituted
  - Resin embedded
  - En bloc staining
    - OsO_4, U Ac, Pb citrate
    - Osmium impregnation (OTO)
    - Want to make samples more conductive, more heavily stained
  - Thin conductive layer (C, Pt, Au-Pd) coated just before insertion

Imaging conditions

- Low voltage (2 keV or less)
  - Want to image only the surface
  - Minimal depth penetration (slice as thin as 10 nm)
  - No topographic information
  - Elemental contrast (C vs Os)
  - Through-lens detector for highest resolution
  - BSE mode (positive bias)
  - Stained parts will show up as bright on dark

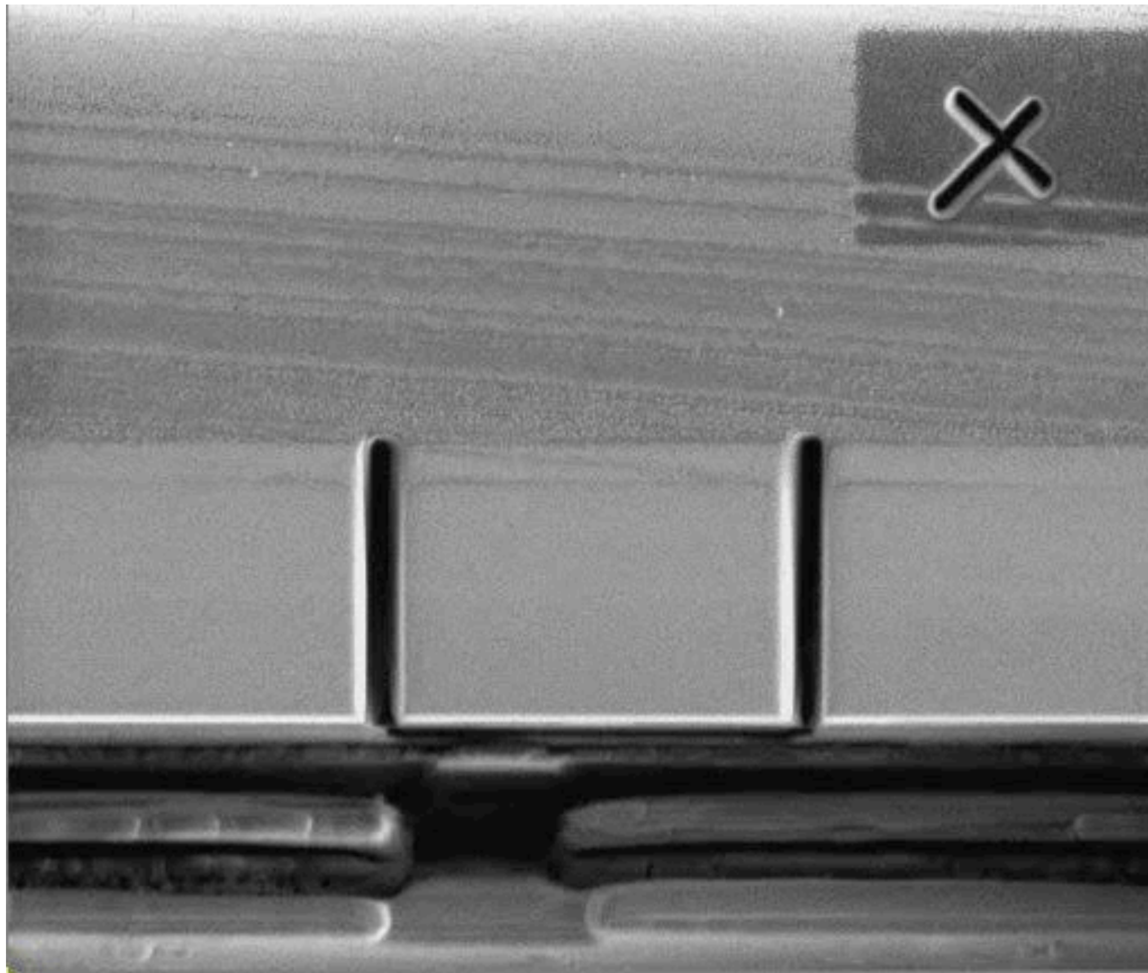
Milling Samples



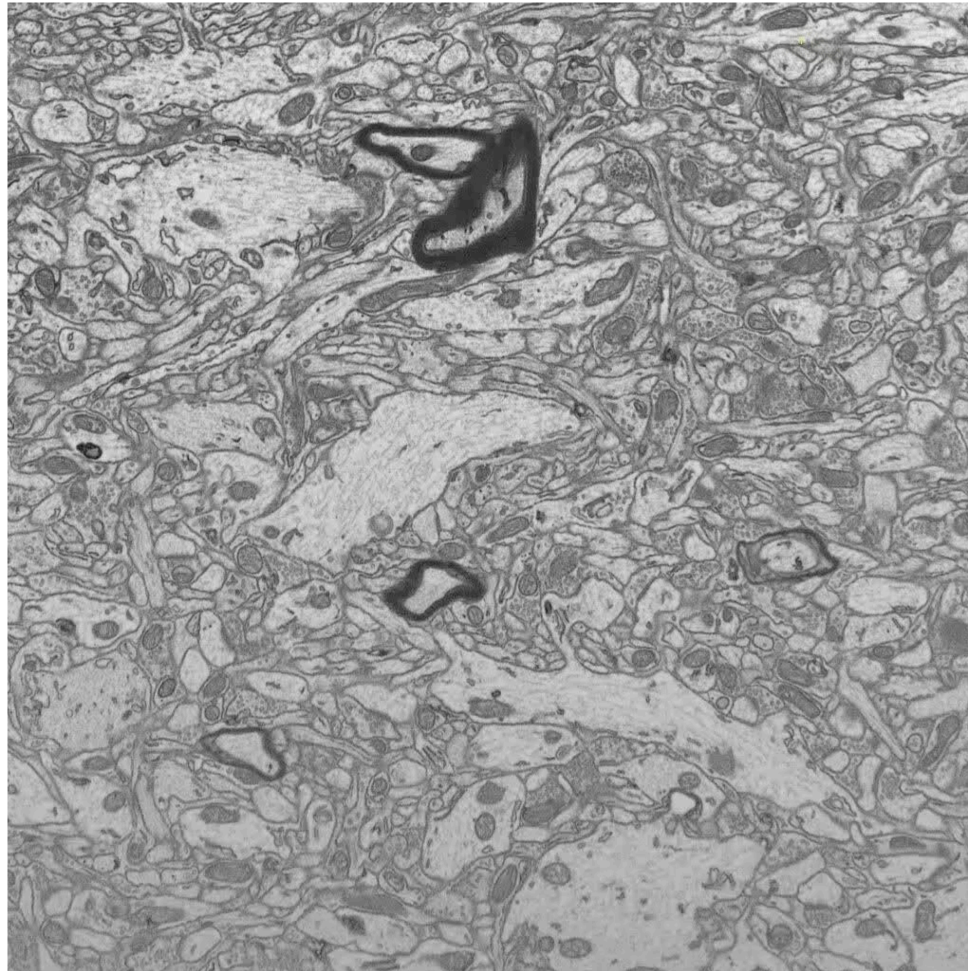
Bushby et al, 2011

Set up for Slice and View

Milling: i-beam view



Example Movie: Neural Tissue



Ideal workflow

- Samples stained and embedded
- Thin slice for overall map – make easier to find features (LM or ultrathin EM)
- Face of block polished
- Set up for slice and view (1 day)
- Collect slices (1-5 days)
- Align, process (IMOD, Amira) (1 day)
- Segmentation (IMOD, Amira) (weeks-months)