

Open-Access TEM, SEM and LM at the BSIR

(Biological Science Imaging Resource)

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BSIR Introduction

The BSIR is an open access facility offering state of the art transmission and scanning electron microscopy (TEM & SEM) as well as light microscopy (LM). For TEM, we provide access to a Titan Krios™ with high throughput robotic capabilities, two CM120 microscopes. We also offer full service plastic embedding and sectioning or negative staining services. For SEM we provide the Nova SEM. For LM, we provide a Zeiss LSM 510 laser scanning confocal microscope and an Olympus BX61. BSIR staff provides training for all BSIR instruments as well as advice with sample preparation and image analysis.

Contact Information

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Training Offered

- TEM instrument operation & data collection
- SEM instrument operation & data collection
- Zeiss LSM 510 confocal instrument operation
- Olympus light microscope operation
- Support equipment operation (TEM holders, vacuum evaporator, Vitrobot, and more)

Titan Features

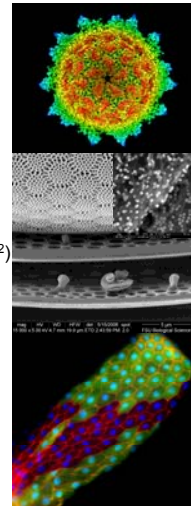
The FEI Titan Krios™ is a state-of-the-art dedicated imaging robot optimized for high-resolution structural biology.

- 120 or 300 kV
- 8k x 8k DE64 Direct Detector
- Energy Filter – Gatan GIF w/ 2k x 2k Tridem™ CCD
- Parallel illumination
- Dual axis tilt holder (+/- 70 degrees) enabling dual axis tomography
- Electron energy loss spectroscopy (EELS)
- Load up to 12 grids at once



Titan Imaging

- 2000+ images per day (125+ GB)
- 40+ tomograms per day (275+ GB)
- 1.2 Å resolution (raw data)
- 2.8 Å resolution (AAV virus reconstruction from Titan data¹)



Nova SEM Imaging

- FEG SEM
- Low voltage & vacuum
- 1nm resolution (*Diatom & Nano Gold*²)
- Multiple methods: SED, BSE, EDS, nanolithography, IR CCD

Zeiss LSM 510 Imaging

The Zeiss LSM 510 is a general Purpose upright confocal imaging system.

- Two photon
- 3D Imaging
- <0.4 µm resolution (*Drosophila ovary*³)

Imaging Techniques

TEM:

- Single particle, Tomography, RCT, OCT
- Thin sectioning and plastic embedding
- Cryogenic and room temperature samples
- Electron counting, energy filtering & EELS

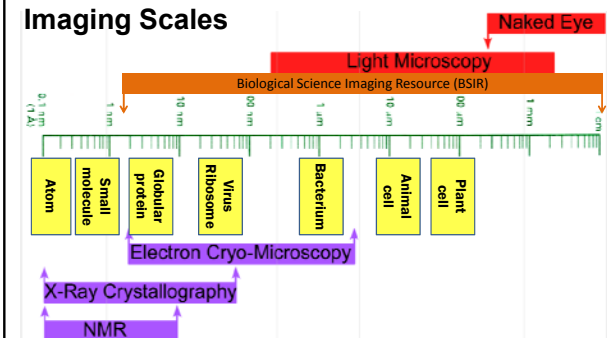
SEM:

- Imaging, EDS spectroscopy, and e-beam lithography
- Low voltage & low vacuum for soft materials
- In-lens SE (TLD-SE) and BSE (TLD-BSE) detectors
- X-ray detector, IR CCD

LM:

- Confocal imaging (458, 477, 488, 514, 543, 633 nm)
- Two photon (<400 nm)
- 3D imaging

Imaging Scales



References

1. Spear, et al. The influence of frame alignment with dose compensation on the quality of single particle reconstructions. *J Struct Biol.* 2015 192(2), 196-203.
2. 15,000x and 1,000,000x Nova SEM image generously provided by Dr. AKSK. Prasad & Tom Fellers.
3. 40x LSM 510 confocal image generously provided by Dr. Yoichiro Tamori from the Deng Lab.

6 Easy Steps to Open-Access Automated TEM



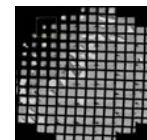
1. Schedule Data Collection



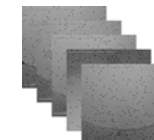
2. Ship Grids



3. Load Grids



4. Screen Grids

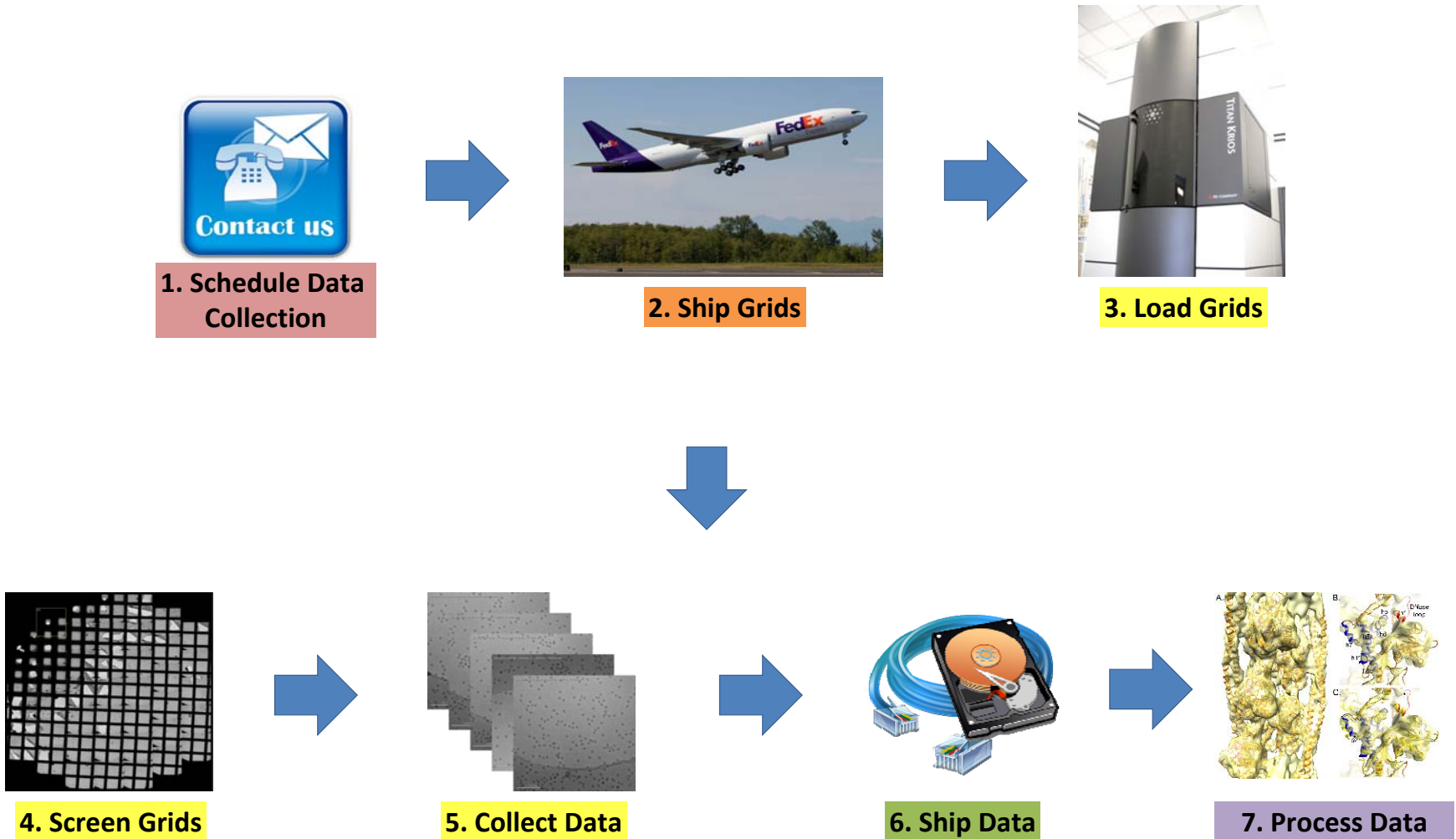


5. Collect Data



6. Ship Data

Titan Krios Pipeline Overview



DQE Comparison

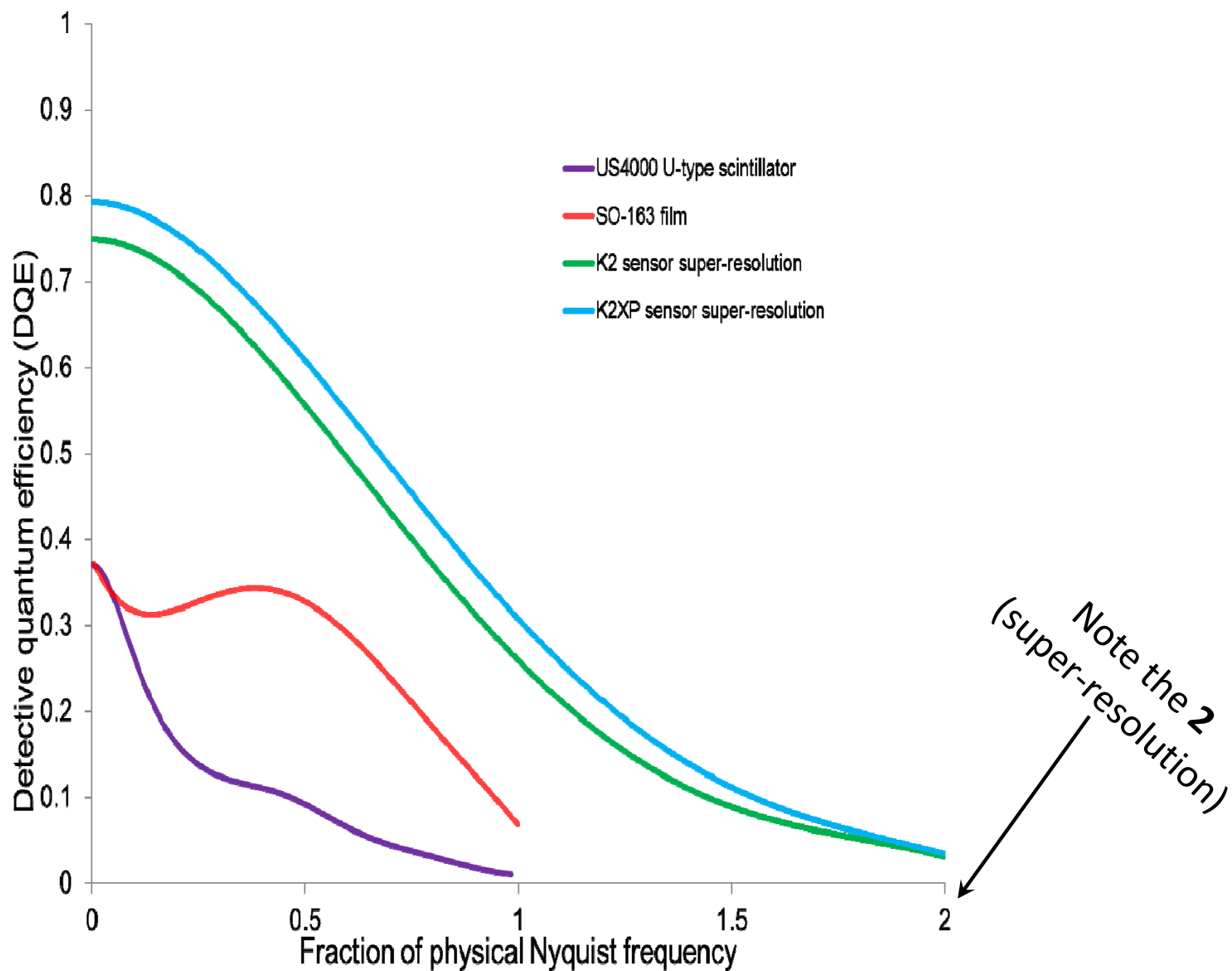
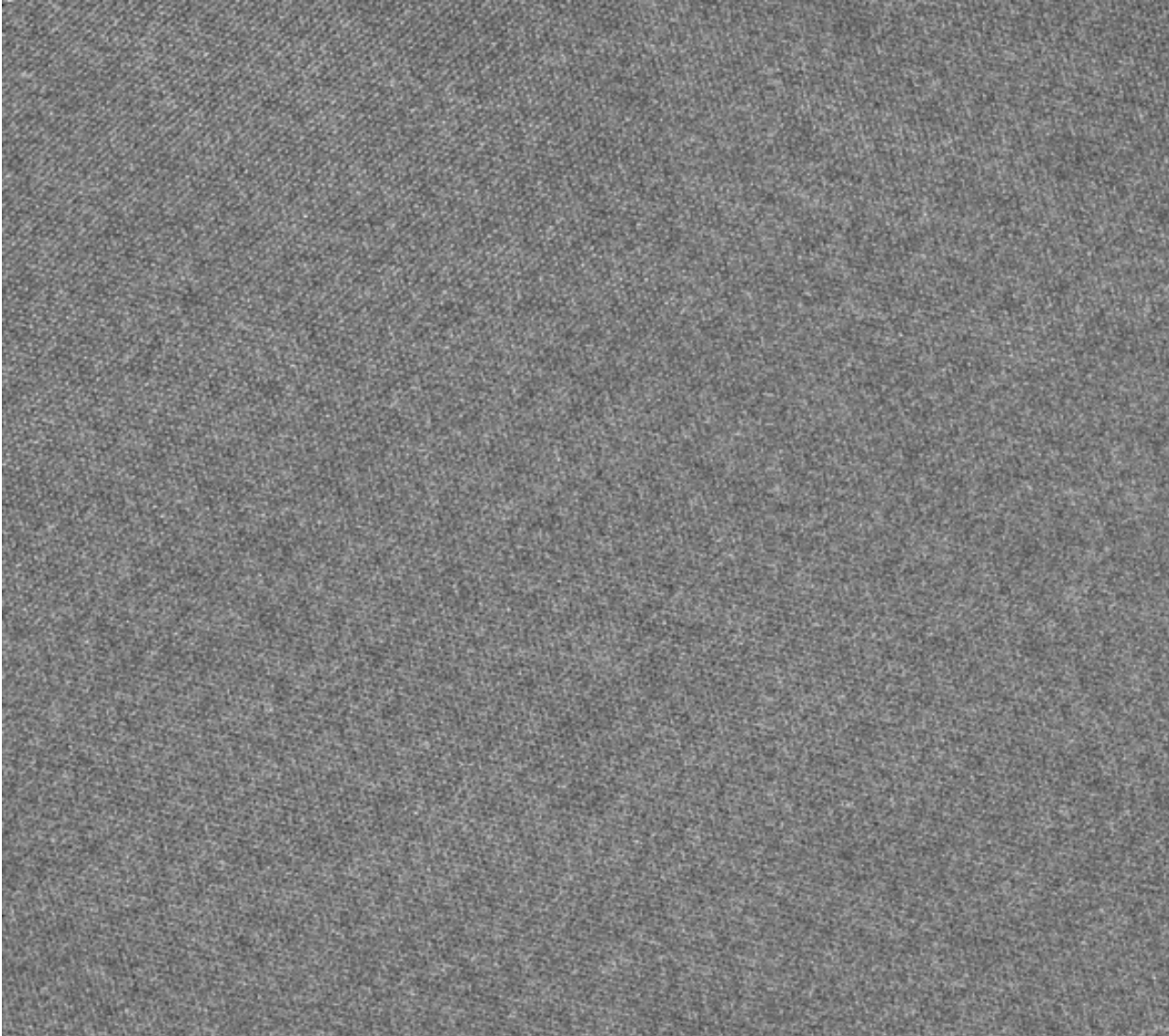


Image courtesy of GATAN(TM)

<http://www.gatan.com/products/tem-imaging-spectroscopy/k2-direct-detection-cameras>



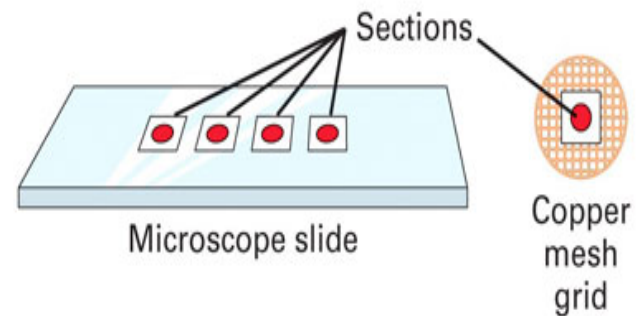
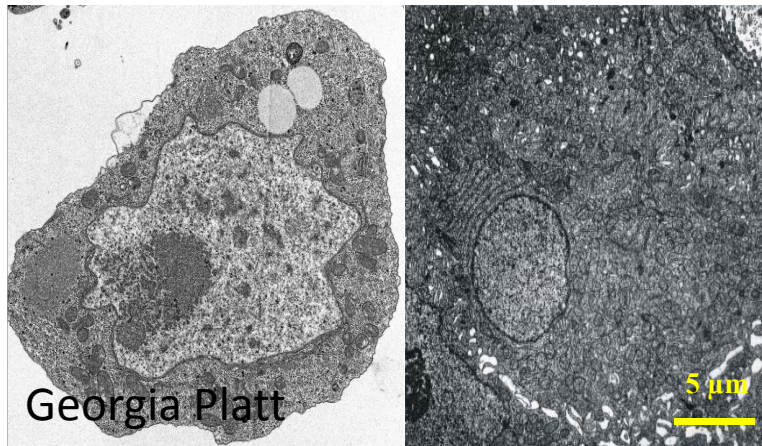
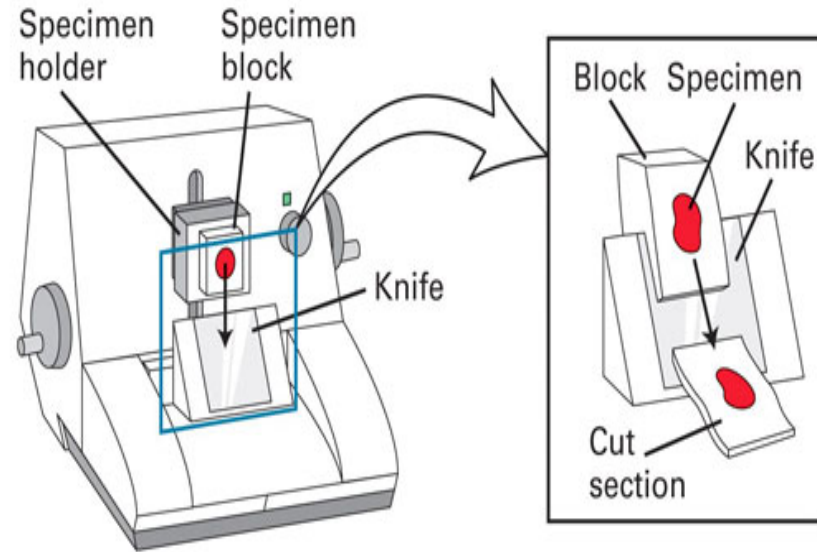
Hexagonal Ice Image

- 32 images per second
- 30+ MB per image
- 1-4 TB of data per day on the Titan Krios
- Detector
Quantum
Efficiency = 0.3
at nyquist

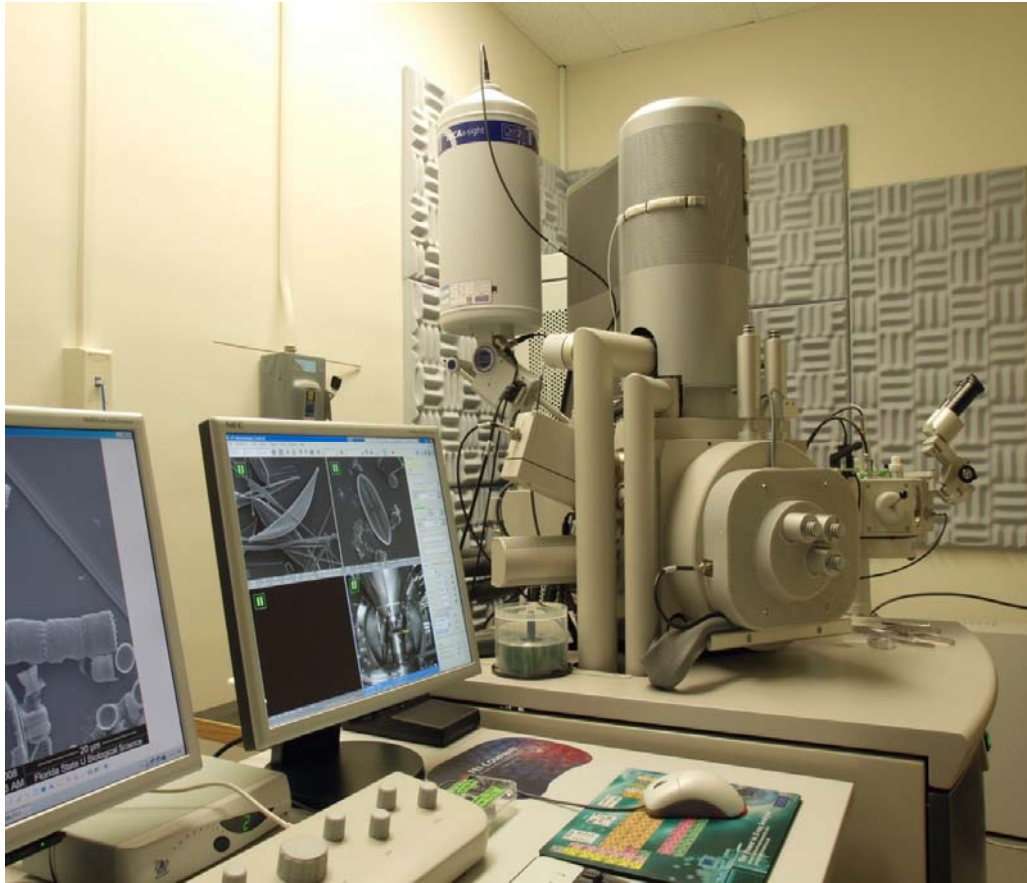
Conventional Sectioning



1 – 40 μm slice thicknesses



FEI Nova 400 NanoSEM



FEI Nova Nano SEM 400:

- 1 nm resolution raw data
- Low vacuum:
 - Low vacuum IR CCD
 - Helix SED detector
 - SED (LVD)
 - BSED (GAD)
- High vacuum:
 - In-lens SE detector (TLD-SE)
 - In-lens BSE detector (TLD-BSE)
 - Everhardt-Thornley SED
- X-ray detector (Oxford INCA X-sight)
- Cryo capable
- IR CCD
- Nano Lithography