User Group Meeting

About the meeting

Every fall, we organize a meeting to introduce our microscopes and services to new members of the Dept and also to discuss any topics our users bring up e.g., sample preparation technique, imaging protocol, facility policy, etc. You can find below topics that we have covered in the past.

User Group Meeting, 2016 Nov 18

- introduction of the confocals, EM, prep lab, and other services provides by the Imaging Facility
- highlighted resources for microscopy and other imaging facilities available on campus
- discussed our needs for better EM equipment and pointed out the problems with our current SEM and TEM
- Cheryl encouraged us to reach out to other UW campuses for potential users

User Group Meeting, 2015 Nov 18

- introduction of the confocals, EM, prep lab, and other services provides by the Imaging Facility
- highlighted the spectral detectors on the SP5
- Aaron and Tyler from the Vaughan Lab talked about expansion microscopy
- Kelly and Lauren from the Bosma Lab talked about using Clarity
- introduced the Olympus Morada 11 MP camera that has replaced the Gatan Slow Scan Camera on our TEM

User Group Meeting, 2014 Oct 24

- Leslie talked about the microtechnique equipment for paraffin-embedded sectioning in KIN 026
- introduction of the confocals, EM, prep lab, and other services provides by the Imaging Facility

User Group Meeting, 2013 Oct 28

- introduction of the confocals, EM, prep lab, and other services provides by the Imaging Facility

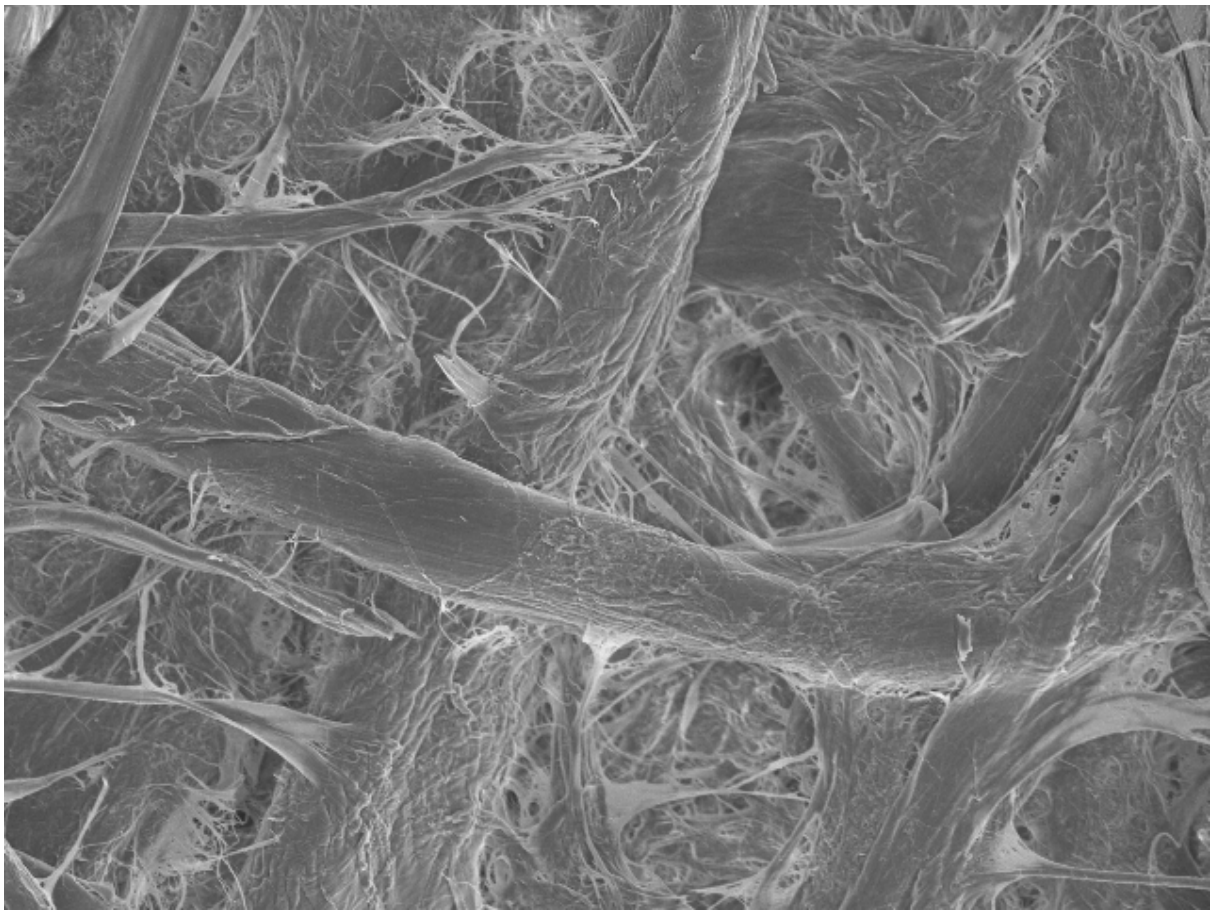
- quick tour of the imaging facility for new users

The first User Group Meeting, 2011 Nov 22

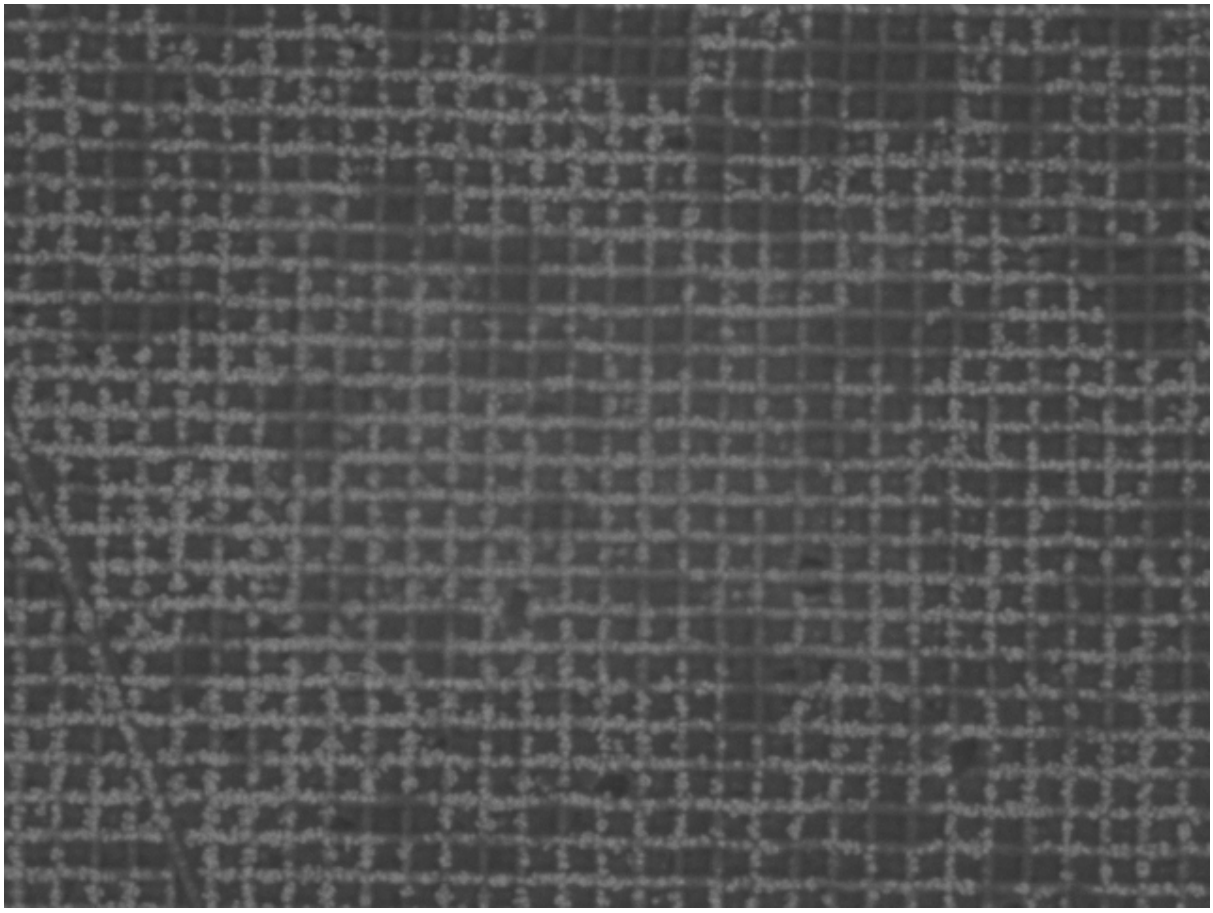
Fun stuff

For the first meeting, we went into details of our microscopes and provided sample images to illustrate the preformance , or lack of it...

- Imaging Facility website: provided a brief description of the lab layout and equipment
- EM: SEM and TEM
  - examples of SEM image

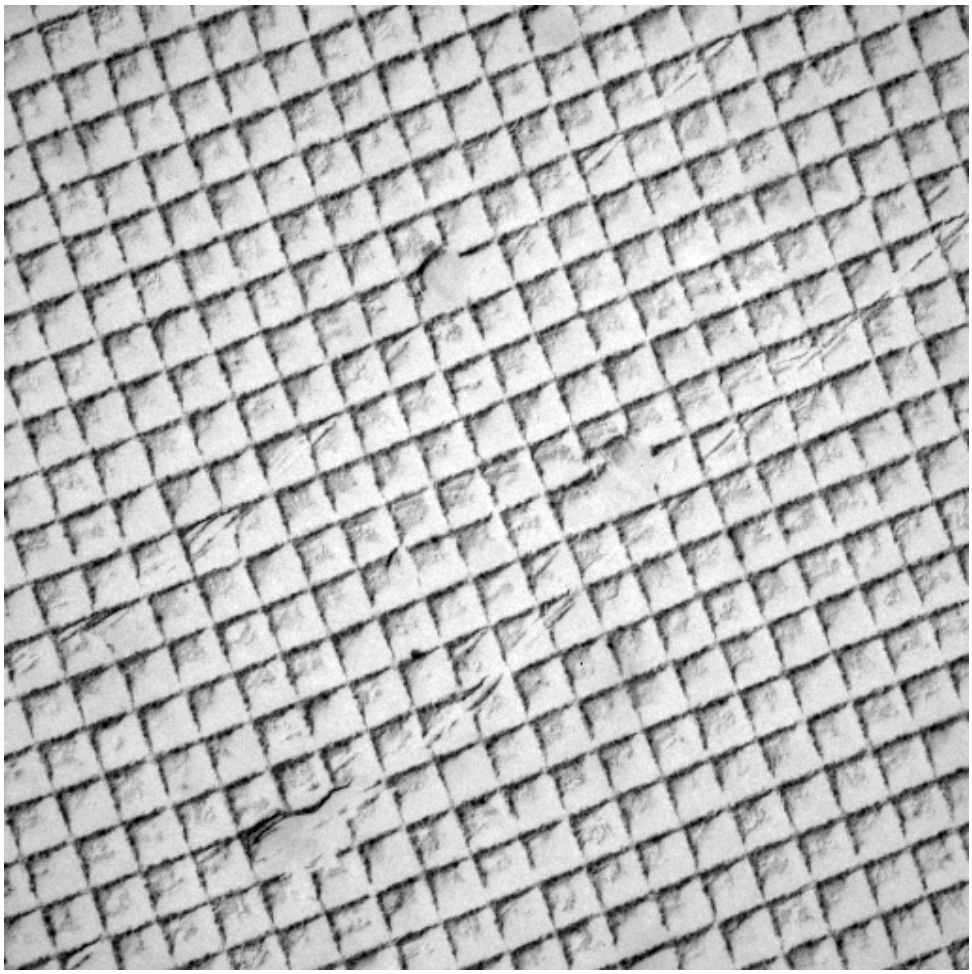


Filter paper imaged at 1000× and 226 nm/pixel.

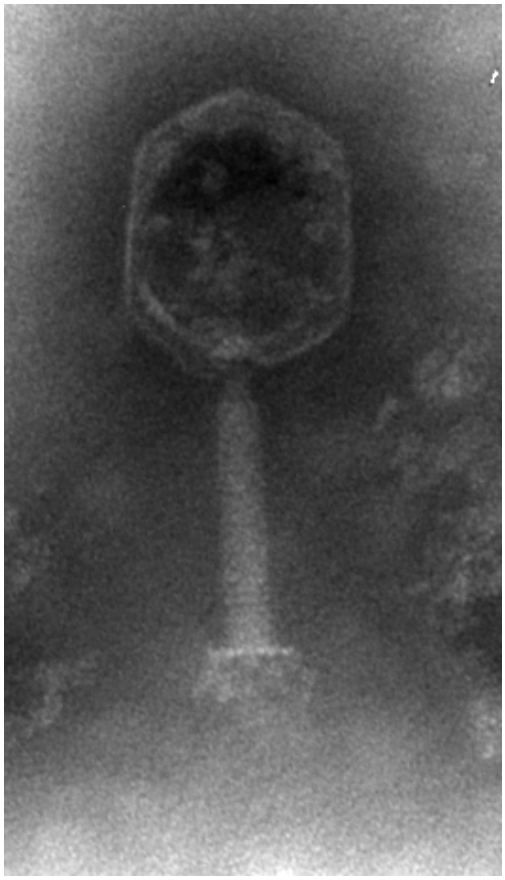


A grating replica with a "d" spacing of 0.4629 µm imaged at 10k× and 23 nm/pixel. Images at high magnification may be hard to get because the focus may drift during the long 80 S exposure time.

- resolution of our TEM

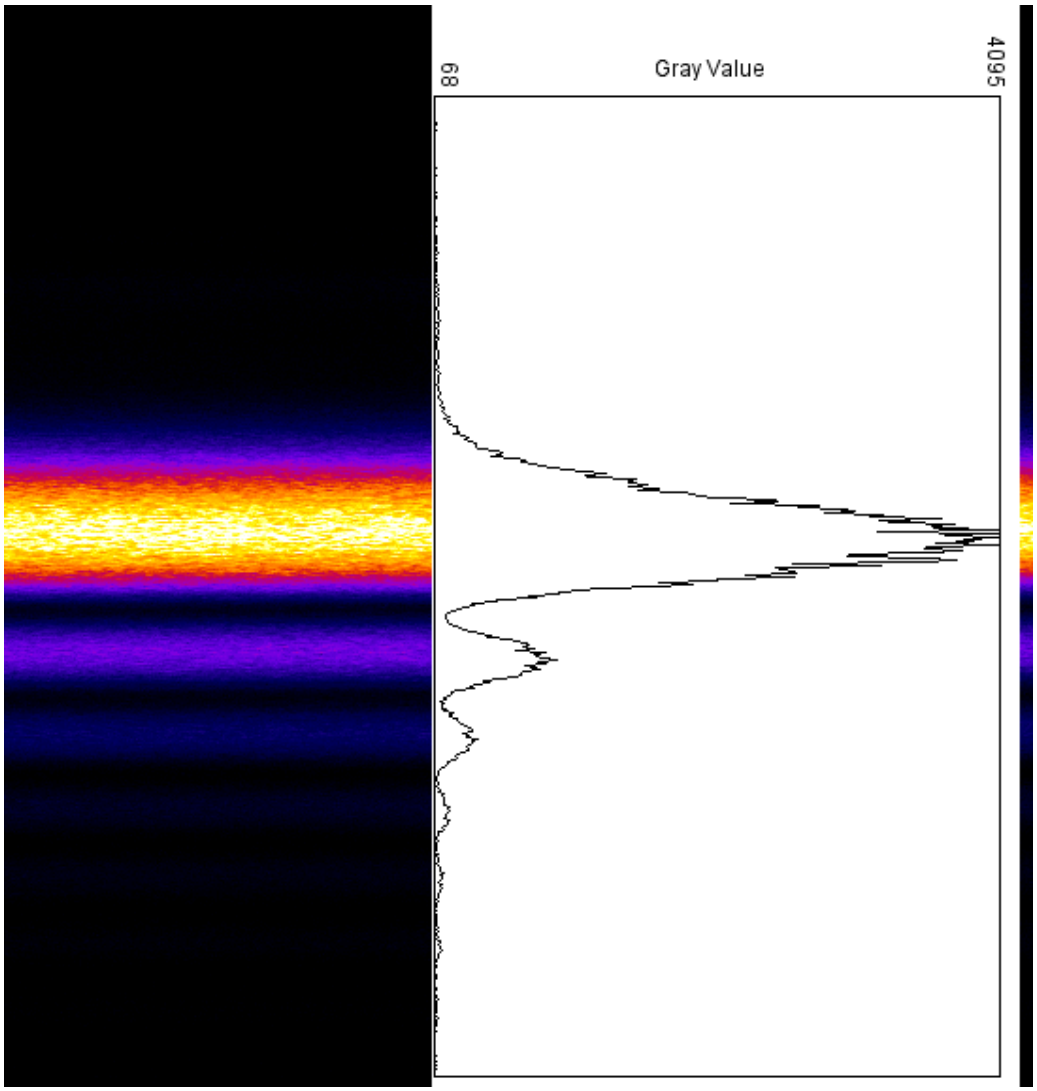


A grating replica with a "d" spacing of 0.4629 μm imaged at 10 500× and 16.8 nm/pixel.

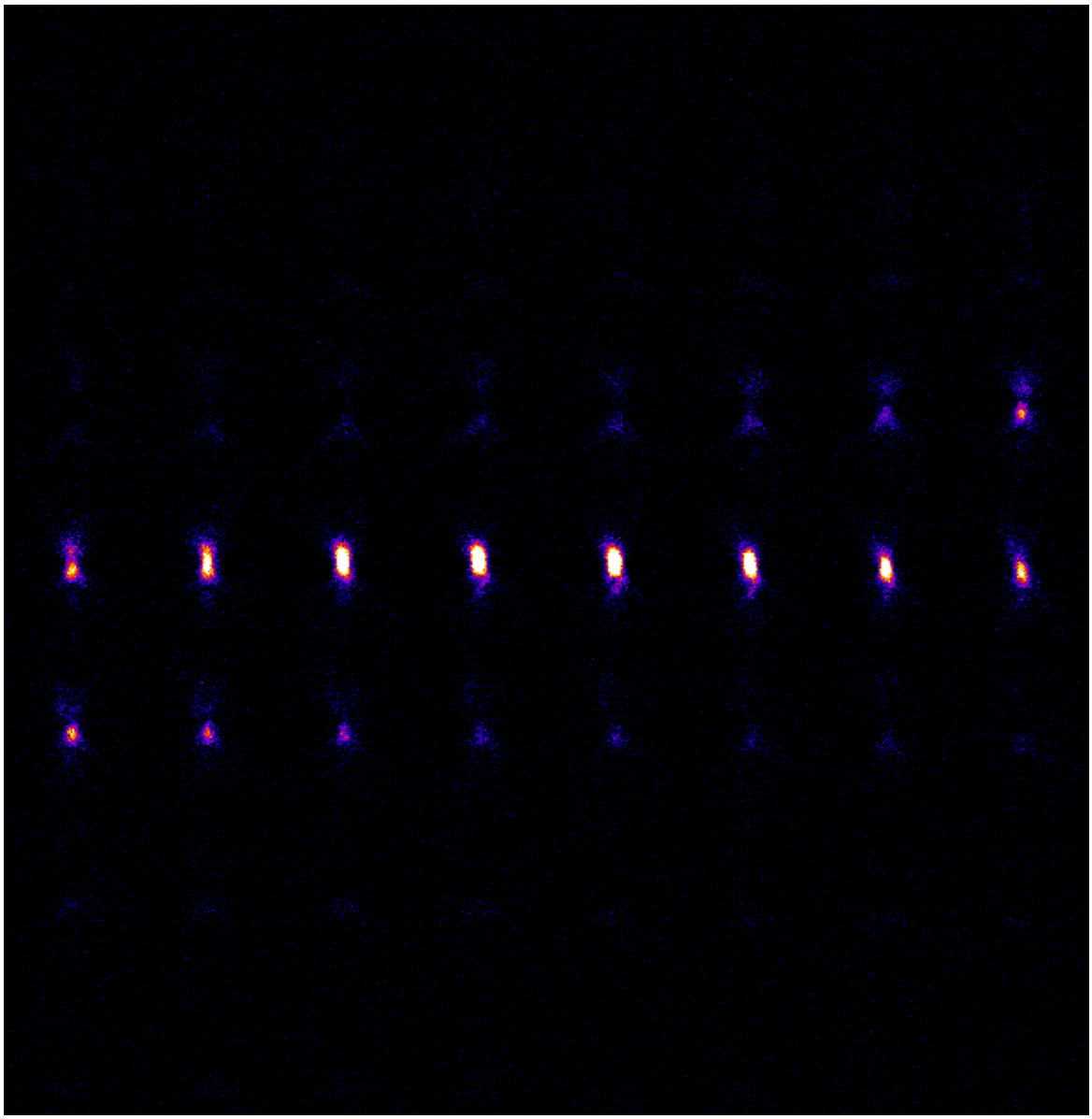


Negative staining of T4 phage imaged at 130k× and 0.7 nm/pixel; the gp18 subunit of the tail sheath is about 4 nm tall with 1.5 nm spacing in between which our TEM struggles to resolve

- Confocal: compared the 2 confocals we have and discussed the performance of the SP5 63× oil immersion, 1.4 NA objective lens
  - resolution

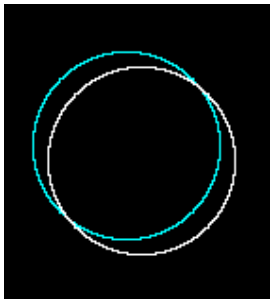


XZ profile plot of a mirror in reflection mode produced with 488 nm illumination. The FWHM of the major peak provides an indication of the axial resolution. Our lens registered at 348 nm.

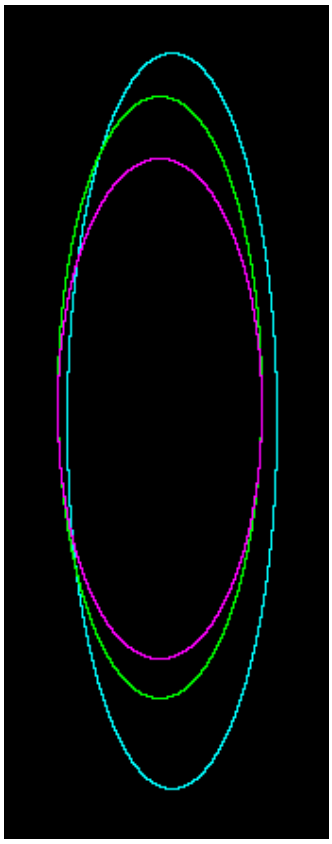


Gallery view of a XZY series of a 175 nm fluorescent microsphere excited at 488 nm (515 nm emission). The axial and lateral resolution were indicated by the FWHM of the image in the respective axes: 472 nm (z), 222 nm (x), and 192 (y)

- color registration for excitation in UV (405 nm) vs visible wavelength (488 nm, 561 nm, 633 nm): outline profile plots of the corresponding emission pattern were represented by cyan, green, red, and magenta below



XY profile plot of a 4 µm tetraspeck fluorescent microsphere showed the image from UV excitation deviated from those taken with visible excitation by about 500 nm. This misalignment can be corrected by Leica's service engineer.



XZ profile plot of a 4 µm tetraspeck fluorescent microsphere showed the image from UV excitation deviated from those taken with visible excitation by over 2 µm. This misalignment is a function of the lens optics and UV correction module, and cannot be corrected manually. There are some lesser deviations among images from the three visible excitation wavelength.

  - additional applications available on the SP5: tile-scan, mark and find, bleaching/photoactivation, FRAP and FRET, linear unmixing
  - optimization: discussed about limitation and aberration of optical systems
- postprocessing of images: provided general guidelines about image manipulation as described in this JCB article
- software packages for analyses: talked about ImageJ/Fiji which is the most common one to use, and others such as BioimageXD and FARSight

Policy

current policy

Open discussion/user suggestion

There were some questions about how to set up an appointment and a suggestion to require users to update the schedule when finish early or cannot make the appointment. Our policy has been updated to included this requirement.