Fundamentals of fluorescence microscopy (PGS 288K/NEU 285L)

- Lectures will be Wednesdays (12:00 noon to 1:00 pm) in PHR 3.114B.
- Demos will be in MBB 1.4126 (ICMB microscope facility). Demos will immediately follow lectures and be from 1:00 pm – 3:00 pm.
- Microscope usage fee: $200. Fees are to off-set costs of utilizing the ICMB core microscopes for demos and must be paid by an account provided by the student’s PI.
- There are no exams. Evaluation will be based on participation in class and during demos.

Description of lectures:

Part I: Optics

1. Optics in light microscopy I; Aug 31, 2016 (Lecture: Som; Demo: Julie).
   - Image creation by lenses.
   - Refractive index.
   - Optical train in a modern compound microscope.
   - Conjugate planes in a microscope.
   - Kohler illumination.
   - Demo of Kohler illumination.

2. Optics in light microscopy II; Sept. 7, 2016 (Lecture: Som; Demo: Julie).
   - Objectives.
   - Diffraction: single slit and Airy disk.
   - Resolution in XY and Z.
   - Nyquist theorem.
   - Numerical Aperture of a lens.
   - Contrast (phase, differential interference contrast etc.).
   - Demo of contrast methods.

Part II: Epifluorescence

   - Why fluorescence?
   - Principles of fluorescence emission.
   - Spectral properties of common fluorophores.
   - Light sources for epifluorescence.
   - Inverted and upright microscopes.
   - Demo using inverted and upright microscopes.
4. Epifluorescence microscopy II; Filters Sept 21, 2016 (Lecture: Som; Demo: Julie).
   • Why do we need filters?
   • Excitation and emission filters.
   • Beam splitters.
   • Band-pass filters.
   • Quad polychroic mirrors.
   • Autofluorescence.
   • Bleedthrough.
   • Demo.

6. Demo only Optics and Epifluorescence microscopy; September 28, 2016.


Part III: Confocals

8. Confocal microscopy I; Oct 12, 2016 (Lecture: Som; Demo: Julie).
   • Limitations of epifluorescence microscopy
   • Laser scanning confocals: how do they work and what can they be used for.
   • Limitations of laser scanning confocal.
   • Spinning disk confocals.
   • Swept field confocals.
   • Resolution limits of a confocal microscope.
   • Demo

9. Confocal microscopy II – Detectors; October 19, 2016 (Lecture: Som; Demo: Julie).
   • Detection by Photomultiplier tubes and camera technology

10. Review II: Review of Part III, followed by time for Demo, October 26, 2016. (Lecture: Som; Demo: Julie)

Part IV: Lectures by Industry Faculty


11. Guest Lecture II: Timothy Murphy-Stevens, Andor Technologies. November 9, 2016 (Detectors)

Part V: Immunostaining and Final Presentations
12. Immunofluorescence, November 16, 2016 (Lecture: Som; Demo: Julie).
   - Techniques
   - Pitfalls
   - Demo
