Fundamentals of fluorescence microscopy (PGS 288K/NEU 285L Unique: 58425)

- 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; Sept 6, 2017 (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. 13, 2017 (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.

5. Epifluorescence microscopy II; Filters October 4, 2017 (Lecture: Som; Demo: Julie).
  • Why do we need filters?
  • Excitation and emission filters.
  • Beam splitters.
  • Band-pass filters.
  • Quad polychroic mirrors.
  • Autofluorescence.
  • Bleedthrough.
  • Demo.


Part III: Confocals

7. Confocal microscopy I; October 18, 2017 (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

8. Confocal microscopy II – Detectors; October 25, 2017 (Lecture: Som; Demo: Julie).
  • Detection by Photomultiplier tubes and camera technology

9. Review III: Review of Part III, followed by time for Demo, November 1, 2017. (Lecture: Som; Demo: Julie)

Part IV: Lectures by Industry Faculty


Part V: Immunostaining and Final Presentations

13. Immunofluorescence & Student presentations November 29, 2017 (Lecture: Som; Demo: Julie).

- Techniques
- Pitfalls
- Demo