

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

3. Epifluorescence microscopy I: Why fluorescence and common fluorophores Sept 14, 2016 (Lecture: Som; Demo: Julie).

- 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.

7. Review I: Review of Parts I and II, followed by time for Demo, October 5, 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 I: Mike Davis, Nikon, Inc. November 2, 2016 (Super Resolution and new technologies).

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

13. November 23, 2016 No Class Thanksgiving.

14. Student Presentations, November 30, 2016