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

- **Course Coordinator & Instructor:** Dr. Somshuvra Mukhopadhyay. Office: BME 3.510E. Phone: 512-232-8200. E-mail: som@austin.utexas.edu. There are no other faculty instructors; please direct all communication to Dr. Mukhopadhyay.

- **Objectives:** This course is designed for advanced graduate students who use light microscopy (confocal or wide-field) for their experimental end-points. The goals are to give students an overview of the fundamental scientific and technological principles involved in light microscopy and in camera technology. The course also covers principles of image analyses and quantification as well as immunofluorescence microscopy. At the end of this course, a student should be able to understand what should or should not be done in imaging experiments, have a better grasp of how artefactual observations can be easily made in microscopy-based techniques, and be skilled in applying these techniques for their own experiments. Details of individual lectures are provided below.

- **Times:** 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.

- **Evaluation:** There are no exams. Evaluation will be based on participation in class and during demos.

- **Special notes:** This course charges a “Microscope usage fee” of ~$200. Fees are to offset costs of reserving time on the ICMB core microscopes for demos. This fee must be paid by the PI of the student using an account that is approved by our Office of Sponsored Projects (Student cannot pay this fee themselves).

**Description of lectures:**

**Part I: Optics**

1. **Optics in light microscopy I; Aug 29, 2018 (Lecture: Som; Demo: Anna).**
   - 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. 5, 2018 (Lecture: Som; Demo: Anna).**
   - 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.

3. Review I: Optics; Sept 12, 2018 (Lecture: Som; Demo: Anna).

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 September 26, 2018 (Lecture: Som; Demo: Anna).
   • Why do we need filters?
   • Excitation and emission filters.
   • Beam splitters.
   • Band-pass filters.
   • Quad polychroic mirrors.
   • Autofluorescence.
   • Bleedthrough.
   • Demo.

6. Review II: Review of epifluorescence, followed by time for Demo October 3, 2018

Part III: Confocals

7. Confocal microscopy I; October 10, 2018 (Lecture: Som; Demo: Anna).
   • 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. Guest lecture Mike Davis, Nikon, Inc October 17, 2018
   • Detection by Photomultiplier tubes and camera technology

10. Review III: Review of confocal and detectors, followed by time for Demo, October 31, 2018. (Lecture: Som; Demo: Anna)


Part IV: Immunostaining and Final Presentations

   • Techniques
   • Pitfalls
   • Demo


14. Image quantification and manipulation (Lecture: Som; Demo: Som). November 28, 2018

15. Student presentations December 5, 2018.