CBPH710: Advanced Light Microscopy (cross listed NBIO710) (3 Units)

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Office Hours: as needed by appointment

Prerequisites: Graduate student in good standing at UNC typically in a biomedical, biological, or materials science. In the unlikely event that space is limited, preference will be given to Neuroscience and Cell Biology & Physiology graduate students.

Course Goals: To become "hands on" proficient in cutting edge microscope techniques and gain comfort using advanced microscopes available to researchers at UNC.

Course Description:

An intensive and comprehensive hands-on laboratory-oriented course in light microscopy for researchers in biology, medicine, and materials science. This course will focus on advanced quantitative fluorescence microscopy techniques used for imaging a range of biological specimens, from whole organisms, to tissues, to cells, and to single molecules. This course emphasizes the quantitative issues that are critical to the proper interpretation of images obtained with light microscopes.

Course Outline:

1. Fundamentals of Optics: An outline of the principles underlying the field of microscopy – from the properties of light to basic imaging light paths.
2. Building a Microscope: Ray optics, building an imaging path, imaging onto a sensor.
3. Imaging Transparent Specimens: Phase Contrast, Darkfield, Polarization and DIC imaging
4. Introduction to Fluorescent Probes and Widefield Microscopy: Discussion on the fundamentals of fluorescence excitation and emission, common dyes and fluorescent proteins, and the fluorescent filters and components that comprise a widefield fluorescence microscope.
5. Confocal Microscopy: Discussion of how this imaging method is able to collect serial optical sections from thick specimens.
6. Multiphoton Microscopy: An optical sectioning technique which relies upon the simultaneous absorption of multiple photons in a single quantized event and allows for optical sectioning without excitation above and below the plane of focus.
7. Detectors, Digital Imaging and 3D Image Deconvolution: In-depth discussion on the detectors used in fluorescence microscopy and the post-processing method of 3D Deconvolution to enhance spatial resolution.
8. Live Cell Imaging Techniques: Considerations for detector sensitivity, speed of image acquisition and photobleaching to consider for live cell imaging experiments.
9. Advanced Fluorescence Methods: light sheet microscopy, Forster resonance energy transfer (FRET), fluorescence lifetime imaging (FLIM), fluorescence recovery after photobleaching (FRAP), total internal reflection fluorescence (TIRF)

10. Introduction to Super-Resolution Microscopy: A discussion of a number of novel approaches that have been employed to circumvent the diffraction limit to improve the lateral (x-y) resolution down to tens of nanometers and their limitations. Including: SIM, STED, STORM, PALM methods
11. Quantitative Image Analysis Methods: Fundamental introduction to methods and software to support quantitative image analysis including segmentation, co-localization, tracking and presentation of scientific image data.

Target audience: Graduate students actively conducting research requiring microscopy usage, particularly in biomedical/biological/health fields or other experimental researchers with an interest in advance fluorescence or light microscopy.

Grading: The major expectation of students is that they perform all hands-on laboratory work, complete outside reading assignments, and contribute actively to discussion in class. Active and constructive critique of fellow students and their presentations are also required. The lecture hour will also include a post-lab review of the prior week's lab. The post lab review will have a rotating student assigned discussion leader.

50% Student formal presentation at end of semester (one per student per semester)/based on a single advanced microscopic technique as applied in a peer reviewed publication; 25% assigned post-lab discussion leader (1 session); 25% weekly discussion and critique during lecture hour/post-lab review

Course resources: Outside readings/videos will be provided most likely by web links or electronic documents via Sakai or direct email. Students are encouraged to independently seek out information based on their specific needs/weaknesses and interests.

Discussions: Class discussion is a grading component (25%) and helps students benefit from each other's knowledge and critiques and identify areas for further improvement.

Time Table: There is flexibility for the instructors in the frequency of class meetings, however, typically the class will meet two days a week for a 1 hour lecture followed by a 3-4 hour lab.

Honor Code: Students should follow the UNC honor code, however, students are welcome and encouraged to discuss reading materials outside of class to stimulate discussion of ideas and scenarios. Working in groups outside of class is permitted. All assignments are open internet/open book and can be done in groups.

Accommodations: The University of North Carolina at Chapel Hill facilitates the implementation of reasonable accommodations, including resources and services, for students with disabilities, chronic medical conditions, a temporary disability or pregnancy complications resulting in difficulties with accessing learning opportunities. Students must document their need for accommodations with Accessibility Resources & Service before any accommodations can be implemented.

Expectations & Attendance: Attendance and participation are major determinants of class grades (up to 25%). Therefore, missing class for many students will impact the grade a student can receive. Active helpful critique of fellow students' presentations is also a key grade determinant.