MCELLBI 205 | 3 units

Modern Optical Microscopy for the Modern Biologist

Instructors: Na Ji and Eric Betzig

Description: The last thirty years have seen an explosion of new forms of optical microscopy that offer unprecedented detail and allow us to understand the findings of biochemistry, molecular biology, neurobiology, and structural biology in spatially compartmentalized and highly dynamic living systems. This course is intended for graduate students in the early stages of their thesis research who are contemplating using modern microscopy tools as part of their work. It endeavors to cut through the confusion of the wide array of new imaging methods, with a practical description of the pros and cons of each. In addition to providing an intuitive physical understanding how these microscopes work, the course will offer hands on experience with cutting-edge microscopes where students will be able to see firsthand how different imaging modalities perform on their own samples, and where they will be able to access computational tools for the visualization and analysis of their data.

Instructional Format: The course meets twice a week for 90 minute lectures. Mandatory homework includes instructor demonstrations and student use of laboratory microscopes across campus, as well as readings and reports on the scientific literature.

Grading Policy: Letter grades are based on: class participation (20%); literature reviews (30%); lab project (30%); and final exam (20%).

Weekly Schedule:

Week 1a: Introduction and overview, the renaissance of optical microscopy, motivations for live cell imaging, application examples.

Week 1b: Optical microscopy concepts – lenses and image formation, ray optics, refraction, interference, point spread function, optical transfer function.

Week 2a: Optical microscopy practical basics – objective lenses, numerical aperture, working distance, field of view, immersion media, correction collars, cover slips, inverted vs upright.

Week 2b: General fluorescence microscopy – immunolabeling, fluorescent proteins, biosensors, organic fluorophores, attachment strategies, lasers, filters, detectors, photon budget.

Week 3a: Widefield fluorescence microscopy – depth of focus, missing cone, 3D deconvolution, structured light optical sectioning, total internal reflection fluorescence.

Week 3b: Confocal and spinning disk confocal microscopy – optical sectioning, resolution extension, pinhole size, crosstalk, photon reassignment, photostimulation.

Week 4a: Tour and initial instruction in Molecular Imaging Core (MIC) microscopes. Guest lecturer, Holly Aaron.

Week 4b: Tour and initial instruction in Advanced Bioimaging Center (ABC) microscopes. Guest lecturer, Gokul Upadhyayula. Goal of week 4 is to impart the knowledge necessary for students to prepare samples suitable for later imaging on MIC or ABC microscopes.

Week 5a: Multiphoton microscopy – scattering length, ballistic vs scattered, wavelength dependence, two photon, three photon, second harmonic, pulse splitter, ultrafast lasers.

Week 5b: Light sheet microscopy – Gaussian, scanned Gaussian, two photon, Bessel, lattice, multiview.

Week 6a: Guide star and sensor-based adaptive optics – System and sample induced aberrations, Shack-Hartmann sensor, deformable mirrors, spatial light modulators, de-scanned guide star, isoplanatic patch.

Week 6b: Sensorless adaptive optics – phase retrieval, phase diversity, genetic algorithms, serial Zernike, serial and parallel pupil segmentation.

Week 7a: Image processing – deconvolution, stitching, linear unmixing, denoising, flat-field correction, particle tracking, multi-channel registration. Guest lecturer, Gokul Upadhyayula?

Week 7b: Visualization software – Image J/ Fiji, Matlab, Imaris, Amira, Arivis. Guest lecturer, Gokul Upadhyayula?

Week 8a: Clearing and expansion microscopy – Clarity, iDISCO, CUBIC, SCALE, proExM, iterative ExM, ExPath, ExFISH, ExLLSM, caveats.

Week 8b: Single molecule localization microscopy – switchable fluorophores, 2D and 3D localization, sample prep and label issues, live imaging and sptPALM.

Week 9a: Stimulated emission depletion microscopy – concept, labels, 2D and 3D, bleaching mitigation, RESOLFT.

Week 9b: Structured illumination microscopy – 2D, 3D, optical sectioning, TIRF, GI-SIM, nonlinear SIM, lattice light sheet, comparisons to other SR.

Week 10a: Fast volumetric microscopy – light sheet, two-photon Bessel, light field, SCAPE, mesoscope, random access.

Week 10b: Label free microscopy – phase contrast, differential interference contrast, coherent anti-Stokes Raman, stimulated Raman scattering. Possible guest lecture by Laura Waller?

Week 11a: Correlative light and electron microscopy – resin embedded, sectioned vs serial block face, high pressure freeze, plunge freeze, cryo EM tomography, miniSOG, APEX.

Week 11b: Choosing the right technique – pros and cons of different imaging methods

Weeks 12& 13: Student presentations

Week 14a: Review

Week 14b: Written in-class final exam

Suggested Reading:

Fundamentals of Light Microscopy and Electronic Imaging, 2nd Edition, Douglas B. Murphy, Michael W. Davidson, ISBN: 978-0-471-69214-0, Wiley-Blackwell (2012).
https://micro.magnet.fsu.edu/primer/index.html
http://zeiss-campus.magnet.fsu.edu/tutorials/
https://www.microscopyu.com/
http://olympus.magnet.fsu.edu/index.html
https://www.ibiology.org/online-biology-courses/microscopy-series/

Homework: Homework is comprised of three components:
Weeks 1-4: reading/viewing suggested tutorials
Weeks 5-9: summaries/critiques of assigned research papers
Weeks 5-13: small group project to prepare, image, and analyze samples on advanced microscopes for oral and written presentation in weeks 12 and 13.