Title: Biological Microscopy

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Spring 2023

Rationale: This course will be open to graduate students and also to undergraduate students with approval. No specific prior coursework needed. The course will be an elective of 3 credits.

Imaging based approaches are common in almost all domains of scientific research, particularly in life sciences and related industry. This course is aimed at teaching students the fundamentals of science of imaging by in-depth discussion of the light-matter interaction, optics fundamentals, and concepts and anatomy of most commonly used advanced microscopy approaches, their pros and cons, and the best practices for optimal imaging results. Application examples will be taken from cell biology, development biology and neuroscience domains. The students will be exposed to fundamentals of wide-field, confocal, multi-photon, light-sheet, super-resolution, photometry and miniaturized microscopes, and their most common variants. Whenever possible, the students will also be given in-depth practical demos of some of these implementations. The course will conclude by a brief introduction to the fundamentals of image analysis using commonly used tool ImageJ/Fiji, and also a discussion of a few student-initiated topics.

Grade assessments will be based on class participation (10%), assignments (20%), mid-term exam (20%), final-term exam (30%) and team paper presentations (20%). Please note this may change depending on COVID related circumstances at the time.

Bulletin Description: This is an advanced microscopy course aimed at graduates and advanced undergraduate students, who are interested in learning about the foundational principles of microscopy approaches and their applications in life sciences. The course will introduce the fundamentals of optics, light-matter interaction and in-depth view of most commonly used advanced microscopy methods, explore important practical imaging parameters, and also introduce digital images and their analysis.

Full course description: During the course, we will start by understanding the fundamentals of wave and geometrical optics, various optical components, light-matter interaction and modern image sensors. Building upon these, we will have in-depth look at the foundational concepts and anatomy of commonly used advanced microscopy systems including wide-field imaging, Confocal, multi-photon, light-sheet, super-resolution, photometry and miniaturized microscopes. We will cover some of the most successful variants of these approaches. The main focus will be on the practical understanding of various parameters and the pros and cons of these imaging approaches in specific application domains, including cell biology, development biology and neurosciences. The students will also be introduced to fundamentals of digital images, sampling, and basic image analysis tools such as ImageJ.

Policy on academic integrity

The intellectual venture in which we are all engaged requires of faculty and students alike the highest level of personal and academic integrity. As members of an academic community, each one of us bears the responsibility to participate in scholarly discourse and research in a manner characterized by intellectual honesty and scholarly integrity.

Scholarship, by its very nature, is an iterative process, with ideas and insights building one upon the other. Collaborative scholarship requires the study of other scholars' work, the free discussion of such work, and the explicit acknowledgement of those ideas in any work that inform our own. This exchange of ideas relies upon a mutual trust that sources, opinions, facts, and insights will be properly noted and carefully credited.

In practical terms, this means that, as students, you must be responsible for the full citations of others' ideas in all of your research papers and projects; you must be scrupulously honest when taking your examinations; you must always submit your own work and not that of another student, scholar, or internet agent.

Any breach of this intellectual responsibility is a breach of faith with the rest of our academic community. It undermines our shared intellectual culture, and it cannot be tolerated. Students failing to meet these responsibilities should anticipate being asked to leave Columbia.

For more information on academic integrity at Columbia, students may refer to the *Columbia University Undergraduate Guide to Academic Integrity*: http://www.college.columbia.edu/academics/academicintegrity

Information for students with disabilities

In order to receive disability-related academic accommodations, students must first be registered with Disability Services (DS). More information on the DS registration process is available online at www.health.columbia.edu/ods. Faculty must be notified of registered students' accommodations before exam or other accommodations will be provided. Students who have, or think they may have, a disability are invited to contact Disability Services for a confidential discussion at (212) 854-2388 (Voice/TTY) or by email at disability@columbia.edu.

Text: Readings from literature.

Approximate organization of lectures (Topics and their distribution may be adjusted)

Section I : Optics fundamentals

Week 1-5:

Overview, application examples, Vision and Lenses

Wave optics

- Dual nature of light
- Polarization, Snell's law, and Refraction
- Interference

Geometric and physical optics
- Ray approximation and tracing
- Pont spread function
- Resolution and sampling
- Deconvolution

Properties of various optical components:
- Coherent and Non-coherent light sources: Lamps, LEDs, LASERs
- Mirror, Lenses, and Coatings
- Dichroic mirrors; Longpass, Shortpass and Bandpass filters.
- Objectives, Tube lens
- Basic anatomy of a microscope
- Detectors: PMTs, CCD and sCMOS Cameras. Dynamic range, Quantum efficiency.

Section II : Fundamentals of microscopy

Weeks 5-6:

Anatomy of Wide field microscope

Generating contrast
- Brightfiel
- Phase contrast
- DIC
- Fluorescence, GFP, Dyes, et al.
- Sample mounting, fixation and clarification; live sample preparations.

Section III : Fundamentals of Images

Weeks 7-8:

Digital Images & Analysis
- Image basics
- Discrete Sampling
- Pixel/Voxel size

ImageJ/Fiji
- Basics of image visualization
- Setting properties
- Measurements
- Automation by Macros

Section IV : Optical sectioning microscopy

Weeks 9-11:

Confocal Microscopy and variants
- Concept of Pinhole

- Spinning disk
- Airy scan concept
- Optical setup
- Application examples for 3D imaging

Multi-photon microscopy

- 2-photon non-linear phenomenon
- 3-photon non-linear phenomenon
- RAMAN scattering (maybe)
- Stimulation (maybe)

Light-sheet microscopy

- History, Static and Dynamic Sheets
- Bessel beams, Lattice sheets
- Application specific implementations: COLM, SPED, SCAPE etc.

<u>Section V : Special topics</u>

Super-resolution microscopy, Fiber photometry

- Abbe's diffraction limit
- STED, PALM, STORM
- Electron Microscopy (if time permits)

Fiber photometry, Miniaturized microscopes

- Fiber photometry
- Miniaturized microscopes