Course Syllabus: Imaging cell biology in living systems from Single Molecules to Animal Models :

In the last several years optical imaging using fluorescence imaging methods has become a central research tool in Cell Biology research. Its increased utility is founded on multiple fronts; the development of several new imaging modalities.; advances in highly sensitive quantitative array detectors, inexpensive high speed computing, advances in optical design; novel probe technologies and multiple model animal systems. The focus of this new course will be to study relevant problems in Cell Biology and how they have been solved using imaging approaches. For example at the cell level we will investigate how techniques such as TIRF and high speed confocal have addressed basic problems in endocytosis; at the organismal level we will use multiphoton, confocal, FRET, and other approaches to understand aspects of cell biology in cell polarity, respiration and organ development in c. elegans, drosophila, zebra fish and mice. In each case the application will focus on how imaging tools are used to study defined problems in living systems.

The course will follow a Lecture/Journal Club format. Lectures will be two part, the first 1/4 will be a description of the technology, how it was developed and how it works (10-15 minutes) followed by description of the scientific problem and how it was solved. Lectures will be interspersed with a journal club discussion of a relevant paper on each technology. Students will prepare the Journal Club presentations in an alternating fashion. Examination will be a combination of class participation, journal club and written exam. Grading will be 50% class participation in journal clubs and 25% in each exam.

Expectations of Faculty

1: Develop a lecture which highlights the use of imaging tools in your scientific question. This is not just giving a seminar, rather

- Describe the central hypothesis
- The methods other than using imaging that were used
- How you integrated the imaging approach
- What novel tools were used or developed
- What the pitfalls were
- How you quantified your results
- What the future of imaging in your science will be

2: Journal clubs. It is essential that each faculty attend each journal club for their lecture and provide material to be discussed. You will also be expected to grade the student performance in your journal club

Course Outline

The course is divided into two roughly equal parts. The first half deals with principals and confocal approaches, the second half with widefield microscopy and other methods. There will be two context based exams, these will be one hour in lecture exams and replace the journal club that week.

There will be no journal club the first week, rather there will be two "principals" lectures given by the course director. These lectures will introduce the important components of the modern fluorescence microscope, and provide critical technical information for the rest of the course

All Lectures start at 2pm and run up to two hours

(01/09/2024) Lecture 1a: Optical Principals (Watkins)
Subjects covered:
- History of microscopy (5 minutes)
- Principals of image formation, magnification, NA (20 minutes)
- Image Contrast (DIC, PHASE advantages and disadvantages) (15 minutes)
- How cameras work, cooled CCD, EM CCD and Scientific CMOS (20 minutes)

(01/11/2024) Lecture 1b: Fluorescence Principals (Watkins)
Subjects covered:
- How fluorophores work (10 minutes)
- Considerations in microscope design for fluorescence (10 minutes)
- Basic Live cell microscope design (10)
    - Automation
    - Environment control
- Probes (Basics)
    - Proteins (10 minutes)
    - Dyes (10 minutes)
    - Stains (10 minutes)

(01/16/24 and (JC 01/18/2024) Lecture 2a and 2b: (confocal microscopy)
(St Croix):
- Fundamentals of confocal, how confocal works. Limitations and advantages of each platform

(01/23/2024) Lecture 3: TIRF microscopy (Watkins)
Subjects covered
- Principals of TIRF microscopy (possibilities and limitations) (10 minutes)

(01/25/2024) Lecture 4: Probes (St. Croix

(01/30/2024 and (JC 02/01/2024) Lecture 5: Microscopic imaging of C.elegans and the use of Optogenetic approaches to manipulate neuronal activity in vivo (Lamitina)

Journal Club article: Will focus on how optogenes, such as Channel rhodopsin (ChR2), are used to define the cells and circuits that control complex behaviors. We will also perform a simple optogenetics demonstration experiments in class using C. elegans.

(02/06/2024 and (JC 02/08/2024) Lecture 6: (Confocal 3) High speed confocal solving problems in cell biology (Sorkin)

Subjects covered:

- High speed 3D methods (10 minutes)
    - Spinning disk/Slit confocals
    - What limits Speed
- Hypothesis and Aims
    - Signaling processes initiated by plasma membrane receptors are tightly regulated by intracellular trafficking of these receptors and subcellular distribution of downstream signaling components.
    - Defining the localization of receptors and downstream molecules during the signal transduction process is important in order to determine where in the cells signaling begins and where it is terminated.
- Spinning disk confocal imaging of signaling molecules
    - RNAi Knock-down- and-reconstitution (KDAR) approach to generate a physiological cell model for the 4D imaging analysis of signaling in time and space.
    - Spinning disk 4-D (time course of z-stacks of two-dimensional images) imaging is used to analyze the localization of signaling proteins. Example of activated EGF receptors and a key EGF-receptor-binding signaling adaptor Grb2.
    - Quantification of 4D images to determine concentrations of signaling proteins in different cellular compartments and the extent of co-localization of two components.
- Advantages of spinning disk microscopy
    - High sensitivity and resolution
    - High quality confocal images acquired at a high speed
    - Very low phototoxicity and photobleaching
- Pitfalls of imaging analysis
    - Full functionality of xFP-fusions of signaling proteins is not always achieved and can be easily examined

- - Technical difficulties in generating KDAR cells and especially KDAR cells with multiple xFP-fused proteins
- Methods other than using imaging that were used
  - Subcellular membrane fractionation as an alternative approach of defining subcellular localization of signaling molecules.
- Integrative analysis of signaling using imaging and biochemical approaches
  - Imaging kinetics of the dynamics of the EGF receptor and Grb2 is accompanied with the biochemical analysis of the outcomes of Grb2 binding to the EGF receptor – activation of Ras, MAPK/ERK and Akt.
- Future of the signal transduction imaging
  - Combining KDAR approach and photoswitchable xFPs
  - Fast live-cell super resolution imaging
  - Development of new methods of 4D multicolor image analysis.

(02/13/24 and JC 02/15/24) Lecture 7: light sheet imaging (Watkins)

- **Subjects covered**
- Principals of the various forms of light sheet microscopy (possibilities and limitations) J/C will be a three paper comparison of the major approaches.

02/20/2024 and 02/22/2024 Lecture 8 and Lecture 9 (Watson) Imaging Big and Imaging Deep and dealing with big data

02/27/2024 Lecture 10: Super-resolution Imaging (Watkins) You will also get the mid term EXAM take home

Subjects covered

- Theory of super-resolution microscopy, limits of widefield/confocal (review)
- Why not just use EM?
- SIM
  - Pros and cons
- STORM
  - Pros and cons
- STED
  - Pros and cons
- Limits of Super-Res microscopy
- Probes for super-Res microscopy

Futures for Super-Res microscopy

(02/29/2024)- Lecture 10: (Confocal 2) Confocal studies of Cell Polarity Single Molecule Imaging In Cell Biology (Hong)

(03/05/2024- Lecture 10: CONTINUED (Confocal 2) Confocal studies of Cell Polarity Single Molecule Imaging In Cell Biology (Hong)

Subjects covered:
- Principals of Point scanning confocal (10 minutes)

(03/07/24)

SPRING BREAK MARCH 12 – 14, 2023

(03/19/2024 and (JC 03/21/2024) Lecture 11: Multiphoton imaging of living systems (Camirand)

Subjects covered:

- Relevance of multiphoton imaging
    - Principles, advantages and limitations
    - Biological questions answered with the use of multiphoton imaging
- Applications in living systems (4D imaging)
    - Intravital imaging of immune cells
    - Intravital imaging of organ function
    - 4D data analysis

Journal club: Given the highly motile nature of immune cells, multiphoton imaging has been mainly applied to answer questions related to this field. A recent high-profile publication demonstrating novel applications of multiphoton imaging of immune cells will be selected for the Journal club presentation.

(03/26/24) FRET St Croix

(03/28/2024 and (JC 04/02/2024) Lecture 12: Membrane folding and microscopy (Aridor)
Subjects covered:
- Live cell imaging in complex environments

(04/04/2024 and (JC 04/09/2024), second harmonic imaging of collagen in health and disease (Anne Robertson)

(04/16/24 and 04/18/2024) Lecture 14: Imaging Zebra fish, model systems of neuronal disorders (Ed Burton)
Subjects covered:

- Motility and kinematics of whole animals and CNS cells in vivo
- Measuring and manipulating neuronal mitochondrial biology in vivo

(04/23/2024 and (JC 04/25/2024) Lecture 15: Single Molecule Imaging In Cell Biology (Hammond)

Subjects covered:

- Resolution: a refresher
- Principles of optical super-resolution microscopy
- Flavors and instruments
- Practical considerations for single molecule localization microscopy
    - Is my data worth reconstructing?
      How should I localize my single molecule data?
    - What's the best way to build the image?
    - What's the resolution of my image?

Journal club article

- **JC: is not a JC. We will work through reconstruction of real single molecule localization data acquired in CBI!**

The students will have their final exam given to them after Dr. Hammond's lectures. The question will be to design an experiment to test some aspect of imaging using Non- confocal approaches, we will come up with the question. They will be expected (in no more than 2 pages) to describe the approach to be used, probes, cells, imaging modality, readout, analysis and statistics. They will be graded on practicality, an understanding of what will and will not work using approaches other than confocal tools. This is a take home exam. There will be a one week break here before exam return and final lecture/JC (material not included in exam) Exams should be returned by Tuesday, 04/23/24 or Thursday, 04/25/24.