Course proposal: 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

3: Try to attend any other lectures that you feel you can contribute to or learn from.

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

(8/30/2011)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)

(9/1/2011) 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)

(9/6/2011 and (JC 9/8/2011)Lecture 2: (Confocal 1) Confocal imaging of development in Zebra Fish (Traub)

Subjects covered:

- Principals of Point scanning confocal (10 minutes)

(9/13/2011 and (JC 9/15/2011)Lecture 3: (Confocal 2) Confocal studies of Cell Polarity (Hong)

Subjects covered:

- Inefficiencies in confocal microscopy (5 minutes)
- Reconstruction and image processing in 3D (5 minutes)

(9/20/2011 and (JC 9/22/2011)Lecture 4: (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.

(9/27/2011 and (EXAM 9/29/2011)Lecture 5: Probes and FRET methods and NO (St. Croix)

Subjects covered

- Probe choices (5 minutes)
- FRET optimization, application and controls (5 minutes)
- FRET realities (5 minutes)
- Comparative FRET methodologies (10 minutes)
    - Donor sensitization
    - Acceptor photobleaching
    - Spectral methods
- Other Technologies (5 minutes)
    - BIFC
    - Duolink
- Imaging Zinc in living cells and animals (10 minutes)
    - The importance of Zinc
    - The role of NO on Zinc and Metallothionein
    - The hypothesis
- FRET measurements of Metallothionein function in living cells (10 minutes)
    - The Experiments
    - Endothelial cell responses to Zinc
- FRET measurements of Metallothionein function in living animals (10 minutes)
    - The experiments
    - Problems and Pitfalls
- Future Studies.

There is no journal club this week. The students will have an exam. The question will be to design an experiment to test some aspect of imaging using confocal approaches (either fast or slow). 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 confocal approaches.

(10/4/2011 and (JC 10/6/2011)Lecture 6: TIRF microscopy and endocytosis (Traub)
Subjects covered

- Principals of TIRF microscopy (possibilities and limitations) (10 minutes)

(10/11/2011 and (JC 10/13/2011)Lecture 7: High speed imaging in Cell biology (Watkins)
Subjects covered:

- What limits speed in cell biology
    - New camera design (10 minutes)
    - Photon starvation and microscope design (10 minutes)
    - Phototoxicity (5 minutes)
- How to build a fast microscope (7 minutes)
- Historic comparisons of methods for high speed imaging of flow (15 minutes)
    - Doppler
    - Streak imaging
    - Multicamera methods

- Imaging blood flow in Zebra fish (20 minutes)
    - The model
    - The method
    - The assay
    - The results
    - Future potential of the assay
- Improving on this technology (5 minutes)
    - 3D methods

Journal club article: This will be an article which studies high speed imaging in 3D probably a recent article on mouse development and blood flow/heart beat by either Mary Dickinson or Scott Frazier.

(10/18/2011 and (JC 10/20/2011)Lecture 8: Microscopic imaging of C.elegans and Cell fate regulation (Silverman/Luke)

Subjects covered:

- *C. elegans* as a model organism: Pros and Cons (5 mins)
- Amalgamation of 3 biological disciplines using *C. elegans*: Genetics, Biochemistry and Cell Biology (15 mins)
    - Genetics of C. elegans
    - Biochemical techniques using *C. elegans*
    - Cell Biological techniques
- General considerations for Imaging *C. elegans* (15 minutes)
    - Labeling Techniques
        - Transgenic approaches
        - Traditional Labeling
    - Immobilization Methods
        - Chemical
        - Physical
    - Special concerns
        - Autofluoresence
        - Optic Limitations and specimen thickness
        - Phototoxicity 2 (abberent results from too much light)
- How to image living animals in 3D at speed. (15 min)
    - Imaging lysosomal movement and function
        - The hypothesis
        - Methodology
        - Results
        - Conclusions and future directions
- Future Improvements and technologies

Journal Club article: Will focus on high speed 3D imaging technologies in *C. elegans* of either protein trafficking or organelle movement probably of embryo protein movement during cell division.

(10/27/2011 and (JC 10/27/2011)Lecture 9: Multiphoton imaging of living systems (Watkins)

Subjects covered:

- Principals of multiphoton imaging (10 minutes)
- Where and when to use multiphoton imaging (10 minutes)
- Principals of second harmonic generation (5 minutes)
- Imaging the vascular system in 3D (5 minutes)
- Extracting 3D quantitative data from complex 3D imaging (10 minutes)

Journal club, this article will focus on different material, specifically on 3D imaging in the immune system, probably one of the recent articles by Ellen Robey on neutrophil swarms in the lymph node or an equivalent paper by Ron Germaine.

(11/1/2011 and (JC 11/3/2011)Lecture 10: Quantitative imaging: ion and free radical imaging (Watkins St.Croix)

Subjects covered:

- How Ratiometric Imaging works (20 minutes)
    - Dipoles
    - Advantages of ratiometric imaging over non-ratiometric imaging
    - Excitation ratiometric dyes and emission ratiometric dyes
    - Instrumentation considerations
    - Other ways of measuring ion concentrations using FRET and FAP approaches
- Imaging calcium flux in the immune system (10 minutes)
    - Nanotubes, what they are and how they were found
    - Calcium flux in nanotubes
    - The potential significance of nanotubes
- Imaging free radicals (25 minutes)
    - How to do it
    - Limitations and possibilities
    - Mitochondria and free radicals
    - Measuring free radicals in endothelials cells
    - Measuring free radicals in living animals

Journal club, A discussion of one of multiple papers where free radical imaging was wrong…

(11/8/2011 and (JC 11/10/2011)Lecture 11: Membrane folding and microscopy (Aridor)

Subjects covered:

- Live cell imaging in complex environments

(11/15/2011 and (Exam 11/17/2011)Lecture 12: super-resolution and single particle imaging (Bruchez)

Subjects covered

- Comparison of super-resolution methods (15 minutes)
    - SIM
    - STED

- STORM/PALM

<u>There is no journal club this week. The students will have their final exam. 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.</u>