

Neurophotonic Summer School 2009

	Monday 01	Tuesday 02	Wednesday 03	Thursday 04	Friday 05	Saturday 06
9:00-10:30	Daniel Côté: Lasers and imaging	Robert Campbell Engineered fluorescent proteins and genetically encoded biosensors	Lisa Topolnik: Two-photon microscopy in acute brain slices: practical considerations and functional applications	Denis Boudreau: Principles of Fluorescence	Francisco Bezanilla Fluorescence spectroscopy reveals conformational dynamics in ion channels.	Free time
10:30-11:00	Coffee break	Coffee break	Coffee break	Coffee break	Coffee break	
11:00-12:30	Richard Robitaille: Calcium imaging to study glial-neuron interactions	Thomas Kuner Clomeleon	Brian Pogue: Fluorescence Imaging of Brain Tumors combined with MR and CT	Yasunori Hayashi Fluorescence, FRET and Lifetime – Applications	Alan Fine Optical quantal analysis via two-photon microscopy of calcium transients	
12:30-13:30	LUNCH	LUNCH	LUNCH	LUNCH	LUNCH	
13:30-14:30	Experiment preview	Experiment preview	Experiment preview	Experiment preview	Experiment preview	
14:30-18 :30	Hands-on Lab experiments	Hands-on Lab experiments	Hands-on Lab experiments	Hands-on Lab experiments	Hands-on Lab experiments	
18:30-19:30	Dinner	Dinner	Group dinner	Dinner	Dinner	
19:30-22:00	Data analysis	Data analysis		Data analysis	Data analysis	

	Sunday 07	Monday 08	Tuesday 09	Wednesday 10
9:00-10:30	Gang Zheng Molecular beacons: smart optical probes and therapeutics	Paul De Koninck Tracking proteins on the move in neurons	Yasunori Hayashi Molecular mechanism of hippocampal synaptic plasticity	Trainees Presentations
10:30-11:00	Coffee break	Coffee break	Coffee break	
11:00-12:30	Maxime Dahan: Investigating cellular events one quantum dot a time	Daniel Côté: <i>In vivo</i> imaging	Projects	
12:30-13:30	LUNCH	LUNCH	LUNCH	Debriefing
13:30-18:30	Projects	Projects	Projects	
18:30-19:30	Dinner	Group dinner	Dinner	
19:30-22:00	Data analysis		Data analysis	

Hands-on sessions (Monday 01 to Friday 05):

Afternoons will be dedicated to hands-on experiments in the different labs (in teams of 3 students). Each team will perform a different experiment every day under the guidance of tutors. Topics covered include:

<p>#01 - FRET-FLIM:</p> <ul style="list-style-type: none">-Different approaches for FRET: spectral, acceptor photobleaching method and Fluorescence Lifetime Imaging Microscopy (FLIM)-Applied to the protein-protein interactions and ion fluctuations in different subcellular compartments of a neuron	<p># 04 - Linear and non-linear video-rate microscopy in live animals</p> <ul style="list-style-type: none">- Animal preparation, optical tool, video-rate hardware, movement correction <p>CARS Microscopy</p> <ul style="list-style-type: none">-Myelin imaging in nerves and spinal cord using Coherent Raman
<p>#02 - Cell tracking in live tissue:</p> <ul style="list-style-type: none">-Tracking live cells migration and differentiation in live tissue using different labeling strategies (retroviral infections, single cell electroporation, etc.) and imaging modalities	<p>#05 - Live imaging of GFP-tagged protein translocation in cultured neurons</p> <ul style="list-style-type: none">-Time lapse imaging of the dynamic translocation of GFP-tagged intracellular proteins in live neurons.-Measurements of movement dynamics with FRAP approaches and photo-stimulation of proteins tagged with photo-inducible GFP.
<p>#03 - Two-photon calcium imaging in neuron dendrites in acute hippocampal brain slices</p> <ul style="list-style-type: none">- Imaging Ca²⁺ transients evoked by action potentials and synaptic stimulation- Quantitative analysis of Ca²⁺ transients: intracellular concentration, fluctuations and endogenous buffering capacity, transients with dual indicators. <p>Optical mapping of synaptic connectivity between inhibitory interneurons in acute hippocampal slices:</p> <p>Two-photon photostimulation of hippocampal interneurons by glutamate uncaging; Mapping synaptic connectivity with two-photon photostimulation</p>	<p>Video-rate wide-field imaging of single molecule dynamics</p> <ul style="list-style-type: none">-Tracking of single membrane receptors on cultured neurons with Quantum dots as fluorescent tags.-Monitoring of synaptic receptor lateral movement on the membrane in and out of synapses.-Quantification of receptor mobility, area covered, dwell time inside synapse, etc

Daniel Côté: *Lasers and imaging*

Abstract

Richard Robitaille: *Calcium imaging to study glial-neuron interactions*

The main goal of the ongoing research in my laboratory is to study the involvement of glial cells in the regulation of synaptic activity and properties. We use various imaging techniques coupled to electrophysiological recordings of neurons, glial cells and synaptic activity. I will discuss recent data illustrating the regulation of synaptic activity by glial cells at the level of single CNS synapses and in heterosynaptic, complex neuronal network. Emphasis will be put on the usefulness and requirement of imaging techniques.

Robert Campbell: *New tools for visualizing biochemistry where and when it happens: engineered fluorescent proteins and genetically encoded biosensors*

The concept of exploiting the Aequorea jellyfish green fluorescent protein (GFP) for live cell fluorescence imaging made its dramatic debut in 1994 when an image of a nematode worm with a green fluorescent neuron appeared on the cover of Science magazine [1]. Ever since, the popularity and number of applications of fluorescent proteins has increased at a dramatic pace, with the most recent evidence of their impact on life science research being the awarding of the 2008 Nobel prize in Chemistry to Osamu Shimomura, Martin Chalfie, and Roger Y. Tsien for their contributions to the discovery and development of this now ubiquitous class of tools. Through the efforts of protein engineers, there is now a complete spectrum of fluorescent protein variants available, all of which are close structural homologues of the GFP archetype, with fluorescent hues ranging from blue to far red. This seminar will provide an introduction to fluorescent proteins and their applications with specific focus on the use of fluorescent proteins as the basis for biosensors that change their fluorescence hue or intensity in response to specific biological events.

Thomas Kuner: *Clomeleon*

The genetically encoded Cl indicator Clomeleon will be introduced and its basic properties and new variants described. Genetic strategies of expressing Clomeleon in subsets of neurons will be discussed and several applications and imaging strategies of Clomeleon will be presented. This will include measurements of resting Cl⁻, gradients of intracellular Cl⁻, dynamic Cl⁻ caused by synaptic stimulation, in vivo Cl⁻, multiphoton and FLIM-based Cl⁻ measurements using Clomeleon.

Lisa Topolnik: *Two-photon microscopy in acute brain slices: practical considerations and functional applications*

Two-photon excitation laser scanning microscopy allows high-resolution and high-sensitivity imaging of activity at the level of individual synapses, neurons and neuronal ensembles in intact brain tissue. I will review the major applications of this versatile technique (two-photon Ca²⁺ imaging and the glutamate-uncaging-based photostimulation), discuss some limitations and give practical advice and protocols for its successful use in acute brain slices.

Brian Pogue: *Fluorescence Imaging of Brain Tumors combined with MR and CT*

Fluorescence imaging has the potential for molecular imaging of tumors in vivo, and can be integrated synergistically with CT, MRI and ultrasound. In this talk, optical imaging will be reviewed, and the most productive ways to integrate this with standard imaging will be discussed. The computational and experimental methods will be reviewed, with primary focus on glioma imaging in vivo, and tracking response to therapeutic intervention.

Denis Boudreau: *Principles of Fluorescence*

Principles of photoluminescence will be reviewed, from the first recorded observations of what would be later be known as fluorescence, to the different energy transfer processes involved, the main factors influencing the intensity of fluorescence, and finally the different modalities (steady-state, time-resolved, FRET) in which fluorescence is used.

Yasunori Hayashi: *Fluorescence, FRET and Lifetime – Applications*

In this presentation, I will cover some basic information about FRET in the context of biological applications. Then I will move onto more research related topic that use FRET under two-photon microscope to detect biological phenomenon underlying synaptic transmission and plasticity.

Francisco Bezanilla: *Fluorescence spectroscopy reveals conformational dynamics in ion channels.*

In this lecture I will cover a series of applications of fluorescence that have given important information of structural changes in ion channels during their function. The applications will include site directed fluorescence spectroscopy using quenching, lifetime, FRET, LRET, local electric field detection and single molecule.

Alan Fine: *Imaging function and plasticity of individual synapses in brain slices: optical quantal analysis via two-photon microscopy of calcium transients*

Electrophysiological recording has been the key tool for analyzing function and plasticity of CNS synapses. Such recording may sample multiple synapses, however, complicating interpretation. This ambiguity can be resolved by fluorescence microscopy of activity-dependent signals from individual visualized synapses. We have developed methods to do this in acute and cultured brain slices, using fluorescent calcium sensors and 2-photon microscopy. I will describe how the clarity provided by this approach can help to resolve difficult and long-standing controversies regarding the expression of long-term synaptic plasticity, an underlying mechanism of memory.

Gang Zheng: *Molecular beacons: smart optical probes and therapeutics*

Molecular beacons are target-activatable optical probes that control their fluorescence emission in response to specific biological stimuli. By reducing the background and the selectivity dependence on cell surface receptors, they offer potentials for improving imaging sensitivity and specificity. Moreover, by extending the control of fluorescence emission to cytotoxic singlet oxygen generation, therapeutic beacons can be developed

Maxime Dahan: *Investigating cellular events, one quantum dot at a time*

Nanomaterials hold great promise for advanced biological imaging. In particular, the advent of functionalized quantum dots (QDs) now enables the tracking of individual molecules in live cells. I will present the properties of QDs as biological probes and discuss the optical, colloidal and biofunctional characteristics

that are essential for experiments at the single nanoparticle level in live cells. The interest of single QD tracking experiments in cell biology will be illustrated with several examples on the dynamics of membrane receptors, the transport properties of molecular motors and the release of presynaptic vesicles.

Paul De Koninck: *Tracking proteins on the move in neurons*

Neuronal signaling and remodeling involve a myriad of interactions between proteins, which occur in a timely fashion in the various subcompartments of highly ramified neuronal. To understand these signaling events, it is valuable to monitor the dynamics of proteins throughout the neuron, either with single or multiple molecule resolution. We will discuss various approaches to track the movement of cytoplasmic and membrane proteins in neurons and in and out of synapses.

Daniel Côté: *In vivo* imaging

Abstract

Yasunori Hayashi: *Molecular mechanism of hippocampal synaptic plasticity*

I will cover our recent work on the structural plasticity of dendritic spine, with emphasis on cytoskeleton and CaMKII.