4th International Frontiers in Neurophotonics Symposium

FiNs

Meeting Program

UNIVERSITÉ LAVAL

université de BORDEAUX

frontiersneurophotonics.org

October 3 - 6, 2015

Musée de la civilisation

Québec | Canada

The 2015 Frontiers in Neurophotonics international symposium

The 2015 Frontiers in Neurophotonics international symposium (FiNS) is the fourth edition of a highly succesful series of conferences organized jointly by Université Laval and Université Bordeaux. FiNS 2015 will focus on leading edge photonics approaches and technologies to understand brain structure and function.

Advances in neuroscience critically hinge on the development of approaches that enable probing and controlling cellular and molecular events in live cells and within the intact brain. Optical approaches are rapidly becoming central to bridge molecular and systems neuroscience. This meeting brings together world leaders in the fields of photonics and neuroscience and showcases the latest technological breakthroughs to push further the limits of experimental, pre-clinical and clinical neuroscience. It is an exceptional opportunity to catch up with the latest discoveries and conceptual advances in this field, in a relaxed and pleasant atmosphere of open discussions.

Scientific Program Committee

Yves De Koninck | Université Laval
Paul De Koninck | Université Laval
Daniel Côté | Université Laval
Daniel Choquet | Université de Bordeaux
Laurent Cognet | Université de Bordeaux
Jean-Baptiste Sibarita | Université de Bordeaux
Valentin Nägerl | Université de Bordeaux
Rainer Friedrich | Friedrich Miescher Institute
Tim Murphy | University of British Columbia
Robert Campbell | University of Alberta
Pierre Marquet | Université Laval

Organizing Committee

Mario Méthot (Québec)
Annie Castonguay (Québec)
Cyril Bories (Québec)
Simon Labrecque (Québec)
Julie Poupart (Québec)
Cédric Lopez (Bordeaux)
Marie-Noëlle Gouineau (Bordeaux)

Scientific Program

Saturday, October 3 2015

13:30 – 17:00	Registration
17:00 – 17:15	Welcome remarks
17:30 – 19:30 Session 1	Session Chair: **Jean-Baptiste Sibarita** 17:30-18:00 **Jérôme Mertz**, Boston University, USA – *Adaptive optics without guide stars* 18:00-18:30 **Mathias Fink**, École supérieure de physique et de chimie industrielles de la ville de Paris, France – *Ultrasound and Neuroscience* 18:30-19:00 **David Perrais**, Université de Bordeaux, France – *Visualizing membrane trafficking in living cells with new pH sensitive fluorescent proteins* 19:00-19:30 **Istvan Katona**, Institute of Experimental Medicine, Budapest, Hungary – *Cell type-specific STORM superresolution imaging of synaptic endocannabinoid signaling*
19:30 –	Welcome cocktail + Museum visit (Musée de la civilisation)

Sunday, October 4 2015

9:00 - 11:00 Session 2a	Session Chair: **Valentin Nägerl** 9:00-9:30 **Sebastian Jähne**, University of Göttingen Medical Center, Germany – *Super-resolution investigation of synaptic function* 9:30-10:00 **Markus Sauer**, Biozentrum Universität Würzburg, Germany – *dSTORM Coming of Age: Applications in Neuroscience* 10:00-10:15 - Short talk **Paul De Koninck**, Centre de recherche de l'Institut universitaire en santé mentale de Québec, Canada – *Fluorescence lifetime nanoscopy for measuring FRET in dendritic spines* 10:15-10:30 - Short talk **Tamas Fuzesi,** Hotchkiss Brain Institute, Calgary, Canada – *Hypothalamic CRH neurons balance opposing stress behaviours* 10:30-11:00 ***Jean-Baptiste Sibarita,*** University of Bordeaux, France - *Single-objective Selective-Plane Illumination Microscopy for high- and super-resolution imaging of biological structures*
11:00 - 11:30	Coffee break at exhibit hall Sponsored by the **Centre d'Optique Photonique et Laser**
11:30 - 12:30 Session 2b	Session Chair: **Valentin Nägerl** 11:30-12:00 **Takeharu Nagai**, Institute of Scientific and Industrial Research, Osaka University, Japan – *Genetically-encoded fluorescent and chemiluminescent indicators for bioimaging* 12:00-12:30 **David Kleinfeld**, University of California in San Diego, USA – *Blood flow, brain vascular dynamics, and the basis of resting state connectivity*
12:30 - 14:00	Lunch Poster Session A
14:00-14:05	Special announcement

14:05 - 14.25	Industry talks **Tatsuo Nakata** – Olympus, Senior Manager, R&D/Engineering Olympus Scientific Solutions Americas – *New R&D Business Unit: Advancing research through engineering customization*
14:25 - 16:45 Session 3a	Session Chair: **Paul De Koninck** 14:25-14:55 **David Boas**, Martinos center for biomedical imaging, USA – *Optical Imaging of Oxygen Delivery and Consumption : Guiding Interpretation of BOLD fMRI –* 14:55-15:10 - Short talk **Benoit Gosselin** – Université Laval, Québec, Canada – *A Wireless Headstage for Combined Optogenetics and Multichannel Electrophysiological Recording in Freely Behaving Animals* 15:10-15:30 - Industry Talk **Sead Doric** – Doric Lenses – *The multimodal evolution of miniature fluorescent microscope* 15:30-15:45 – Short talk **Sarah Aufmkolk** – Julius-Maximilians University, Würzburg, Germany – *Super-resolution fluorescence microscopy of synaptic proteins* 15:45-16:15 **Tim Murphy,** University of British Columbia, Canada – *High throughput imaging of mesoscopic functional connectivity in mouse cortex* 16:15-16:45 **Kevin Briggman**, NIH-NINDS, USA – *Modern 3D electron microscopy for mapping neural circuits*
16:45 - 17:15	Coffee break at exhibit hall Sponsored by **Huron Digital Pathology**
17:15 - 18:45 Session 3b	Session chair: **Yves De Koninck** 17:15-17:45 **Paul Wiseman**, McGill University, Canada – *New strategies and pitfalls for measuring receptor oligomerization with fluorescence fluctuation methods* 17:45-18:00 - Short Talk **Pieter Vanden Berghe**, University of Leuven, Belgium – *A correlative second harmonic (SH) – electron microscopy (EM) approach to investigate microtubules and intracellular transport phenomena*

18:00-18:15 – Short talk
Flavie Lavoie-Cardinal -Centre de recherche de l'Institut universitaire en santé mentale de Québec, Canada – *Gold nanoparticle-assisted all optical localized stimulation and monitoring of Ca^{2+} signaling in neurons*

18:15-18:45
Nelson Spruston, Janelia Farm, USA – *Assessing cell type-specific connectivity in the mouse brain using large-scale array tomography and two-photon microscopy*

Monday, October 5 2015

9:00 - 10:30 Session 4a	Session chair: **Pierre Marquet** 9:00-9:30 **Yves De Koninck**, Centre de recherche de l'Institut universitaire en santé mentale de Québec, Canada – *Fibre-optics for in vivo optogenetics; from single cells to hard-to-get-to areas of the nervous system* 9:30-10:00 **Casper C. Hoogenraad**, Utrecht University, Netherlands – *Monitoring and manipulating intracellular transport* 10:00-10:15 – Short talk **Benjamin Rappaz** – EPFL, Lausanne, Switzerland – *Image-based label-free screening of GABA agonists, antagonists and modulators* 10:15-10:30 - Short talk **Antoine Godin**, Université de Bordeaux - Single-molecule imaging of carbon nanotubes in live neuronal samples and 3D super-resolution microscopy using phase imaging
10:30 - 11:00	Coffee break at exhibit hall Sponsored by **Doric Lenses**
11:00 - 12:00 Session 4b	Session chair: **Pierre Marquet** 11:00-11:30 **Robert Campbell**, University of Alberta, Canada – *Red fluorescent protein-based neurophotonic probes for visualization of neuronal activity* 11:30-12:00 **Santiago Costantino**, Université de Montréal, Centre de recherche de l'hôpital Maisonneuve-Rosemont, Canada – *Laser-assisted single-cell tagging and membrane functionalization*
12:00 - 14:00	Lunch - Poster Session B
14:00-14:30	14:00-14:30 – Industry talk **Patrick Myles** – Huron Digital Pathology – Advances in Whole Mount Brain Scanning

14:30 – 17:00 Session 5a	Session chair: **Rainer Friedrich** 14:30 – 15:00 **Arthur Konnerth**, Technische Universität München, Germany – Deep*two-photon imaging of neuronal networks in vivo with a red-shifted indicator* 15:00 – 15:30 **Georg Keller**, FMI – Friedrich Miescher Institute, Germany – *Visuomotor learning in mouse primary visual cortex*
15:30 - 16:00	Coffee break in exhibit hall Sponsored by **Olympus**
16:00 - 17:00 Session 5a continued	16:00-16:30 **Thomas Oertner**, Institute for Synaptic Physiology, Germany – *Optogenetic investigation of long-term plasticity at individual synapses* – 16:30-17:00 **Jason Kerr**, Max Planck Institute, Germany – Turning calcium transients into spikes and watching the animal in action
17:15	Departure in front of the Musée towards le Manoir Montmorency (banquet dinner)

Tuesday, October 6

9:00 – 11:00 Session 6a	Session chair: **Tim Murphy** 9:00-9:30 **Rainer Friedrich,** Friedrich Miescher Institute for Biomedical Research, Switzerland – *Deconstructing and reconstructing neuronal circuits for olfaction* 9:30-10:00 **Minoru Koyama**, Janelia farm, USA – *Synaptic mechanisms underlying integration of early-born and late-born motor circuits* 10:00-10:15 – Short talk **Pierre Bon** – Université de Bordeaux, France – *Quantitative Phase Imaging for label-free live-cell cytoskeleton, organelles trafficking and for 3D fluorescence super-resolution* 10:15-10:30 – Short talk **Masha Prager-Khoutorsky** – McGill University, Montreal, Canada – *Superresolution imaging reveals unique microtubule structure mediating mechanotransduction in neurons* 10:30-11:00 **Claire Wyart**, Institut du Cerveau et de la Moelle Épiniere (ICM), France – *Investigation of a novel sensory interface relaying information from the cerebrospinal fluid to motor circuits*
11.00 - 11:30	Coffee break at exhibit hall Sponsored by **Zeiss**
11:30 - 12:30 Session 6b	Session chair: **Tim Murphy** *11:30-12:30* **Karl Deisseroth**, Stanford University, USA – *Recent development in optogenetics*
12:30 - 12:45	Closing remarks
12:45 - 14:00	Lunch

Keynote speaker

Recent developments in optogenetics

Public Lecture Tuesday October 6 - 11:30

sponsored by the Quebec city SfN chapter

Karl Deisseroth, MD, PhD
Stanford University, USA

D.H. Chen Professor of Bioengineering and of Psychiatry and Behavioral Sciences
Stanford University
Howard Hughes Medical Institute

Amongst his many accomplishments, Karl Deisseroth is known as one of the inventors and a pioneer of the groundbreaking field of optogenetics. Optogenetics allow researchers to use light to control the activity of neurons in living organisms, and has led to a better understanding of the circuitry inside the brain. Dr. Deisseroth's team has also introduced a method to make fixed brains transparent, called CLARITY, which allows researchers to view large networks of neurons with unprecedented ease and accuracy. This method has allowed researchers to identify specific structures and networks of neurons in human brain tissue. The methods and approaches introduced by Dr. Deisseroth and his team are considered as some of the most promising to further our understanding of the brain and nervous system, and have revolutionized neuroscience.

"*Developing and employing novel molecular tools, Dr. Deisseroth has brilliantly demonstrated a new way of understanding how the brain functions and provided neuroscientists, along with other medical researchers, new tools for exploring function and connectivity at the cellular level, ultimately shedding light on disease development and treatment*," said Thomas R. Insel, M.D., director of the National Institute of Mental Health, USA, quoted in a recent press release by the Albany Medical Center.

Dr. Deisseroth is also a practising psychiatrist, with a great interest in using new technologies to help patients.

This public lecture is sponsored by the Quebec city SfN Chapter

Social events

Saturday, October 3 - 19:30

Welcome cocktail and Museum visit

The venue chosen for the meeting and welcome cocktail is Quebec City's Musée de la civilisation whose current exhibit takes you on a fascinating journey into the legendary world of ancient Egyptian Magic. After the last talks on Saturday evening you are invited to a welcome cocktail and tour of the exhibition.

Ancient Egyptians had an unshakeable faith in the power of magic. For them it was both a source of supernatural wisdom and a way to influence their destiny. According to an Egyptian myth, the gods used magic to create the world and give humans powers that would help them survive. Egyptian Magic is a chance to discover the world of magic in Ancient Egypt through its practitioners, its use in daily life and sacred worship, and its ties to the beyond.

Monday, October 5 - 17:15

Banquet dinner at Manoir Montmorency

The Manoir Montmorency is a magnificent historical site providing splendid views of the Montmorency Falls, St. Lawrence River and Île d'Orleans.

The banquet dinner will take place at the Manoir Montmorency on Monday evening. Buses will be taking us there, leaving at 5:15 pm (sharp) in front of the Museum. Buses will take you back directly to the Clarendon Hotel after dinner.

frontiersneurophotonics.org

Many thanks to our sponsors and supporters

Platinum sponsor

HURON Digital Pathology

Gold sponsors

OLYMPUS

ZEISS

doric

Silver sponsors

SPIE.

COHERENT.

nature methods

Scientifica

axion BioSystems

Nikon

Spectra-Physics.

COPL Centre d'optique, photonique et laser

Leica MICROSYSTEMS

Bronze sponsor

Laserglow Technologies

Institutional sponsors

UNIVERSITÉ LAVAL

université de BORDEAUX

NSERC CRSNG

Brain Canada

Institut universitaire en santé mentale de Québec

IINS

cnrs

CIHR IRSC

Abstracts

Invited speakers

Adaptive optics without guide stars

Invited Talk

Jérôme Mertz
Boston University, USA

Sub-surface microscopy is often limited by poor image quality due to sample-induced aberrations. Adaptive optics (AO) can counter such aberrations, though generally over limited fields of view. In most applications, AO is either slow or requires a "guide star" in the sample to serve as a localized reference target. We describe a fast closed-loop feedback implementation of AO that requires no guide stars, where the sample itself serves as the reference. Several features of our implementation are new. First, it is based on a high resolution, single-shot wavefront sensor that is compatible with extended samples. Second, it is applied to widefield (i.e. non-scanning) microscopy in a conjugate AO configuration that increases field of view. Third, it makes use of a fast algorithm to identify sample-induced aberrations using illumination from an arbitrarily shaped source. We present the principle of our technique and proof-of-concept experimental demonstrations.

Ultrasound and Neuroscience

Invited Talk

Mathias Fink

École supérieure de physique et de chimie industrielles de la ville de Paris, France.

Recent developments in the field of ultrasound (time-reversal mirrors and ultrafast and ultrasensitive Doppler imaging) are opening new avenues for brain exploration, therapy and brain machine. Ultrasound can be used not only to cure by thermal ablation, but also to image brain vascularization with unprecedented spatio-temporal resolution, to remotely palpate tissues as well as to neurostimulate local brain regions or to open the blood brain barrier.

We will introduce the concept of fUltrasound (by analogy to fMRI). fUltrasound (fUS) that is able to provide unique whole brain images on rodents and preterm infants with unprecedented resolutions (100 µm and 200 ms). Examples such as the functional imaging of cerebral blood volume during epileptic seizures will be presented and ill emphasize the potential of this new imaging modality. We will emphasize how fUS combined with ultrasonic neurostimulation could lead to first extracorporeal brain machine interfaces.

Visualizing membrane trafficking in living cells with new pH sensitive fluorescent proteins

Invited Talk

David Perrais
Université de Bordeaux, France

Receptor endocytosis and recycling is crucial for many aspects of cellular physiology. In neurons, it has a central role in receptor signalling, in the establishment of neuronal polarity and in synaptic plasticity. Fluorescent proteins (FPs) with pH-sensitive fluorescence are valuable tools for the imaging of exocytosis and endocytosis. We have followed receptors tagged with superecliptic phluorin (SEP), a fluorescent marker which is visible at neutral pH and not at the acidic pH of endosomes. To reveal the dynamics of endocytic zones in neuronal dendrites we have adapted the pulsed pH protocol, developed in non-neuronal cell lines (Merrifield et al. Cell 2005), by blocking acid sensing ion channels or finding a non-acidic substitute to quench the fluorescence of plasma membrane receptors. We show that endocytic zones have a similar endocytic activity, regardless of their location relative to glutamatergic synapses. In addition we have characterized pHuji, a red FP with a pH-sensitivity that approaches that of SEP, making it amenable for detection of single exocytosis and endocytosis events. We perform simultaneous two-color imaging of clathrin-mediated internalization of both the transferrin receptor (labelled with pHuji) and the β2-adrenergic receptor (labelled with SEP). These experiments reveal that the two receptors are differentially sorted at the time of endocytic vesicle formation.

Cell type-specific STORM superresolution imaging of synaptic endocannabinoid signaling

Invited Talk

Istvan Katona, Barna Dudok, Laszlo Barna, Vivien Miczan, Marco Ledri
Institute of Experimental Medicine, Budapest, Hungary

A central task in neuroscience is to decipher the qualitative and quantitative molecular properties shaping normal or abnormal synaptic communication in the brain. However, our knowledge on several significant molecular parameters still remains rather limited due to several technical obstacles. For example, although retrograde endocannabinoid signaling is a key regulator of several forms of synaptic plasticity and its pathophysiological activity is associated with several brain disorders, but the specific biological principles, which determine its signaling efficiency under physiological or pathophysiological conditions have remained unknown. This is important, because quantitative molecular changes in the density and nanoscale location of key endocannabinoid signaling molecules at chemical synapses have been described in temporal lobe epilepsy and in Fragile X Syndrome. To facilitate monitoring of molecular alterations at the nanoscale in the intact brain tissue, we introduce here a new imaging approach based on the combination of whole-cell patch-clamp electrophysiology with correlated confocal and Stochastic Optical Reconstruction Microscopy (STORM) super-resolution imaging. This approach makes cell-type specific molecular investigations readily feasible at the nanoscale level and in a high throughput manner together with the direct measurements of related physiological and anatomical parameters from the very same neurons. To support these investigations, we also present a new open-source software called VividSTORM, which enables the efficient visualization and measurement of the nanoscale density of any target protein in identified subcellular compartments by the correlation of pixel-based fluorescent images with the 3D molecular coordinates obtained by single-molecule localization-microscopy. This new approach allowed us to determine some key molecular parameters, which contribute to the cell-type specific differences in the strength of synaptic endocannabinoid signaling under physiological conditions in hippocampal microcircuits. Moreover, this methodology could visualize robust, but reversible downregulation of presynaptic CB1 cannabinoid receptors on GABAergic axon terminals after chronic high-dose treatment with the psychoactive compound of marijuana demonstrating its usefulness to monitor molecular changes in a cell-type-specific manner.

Super-resolution investigation of synaptic function

Invited Talk

Sebastian Jähne (Silvio Rizzoli)
University of Göttingen Medical Center, Germany

Super-resolution microscopy has enabled imaging beyond the diffraction barrier in the last decade, allowing many laboratories, including ours, to obtain information on the localization and on the organization of different cellular elements, from membrane arrangements to synaptic vesicles. However, despite the advances in imaging and cell biology that have been brought about by this type of technology, there has been little progress in imaging the longer-term temporal changes in biological samples. This type of changes, typically termed turnover, is beyond the abilities of optical imaging. We can image the movement of proteins or organelles, but neither super-resolution fluorescence studies, nor electron microscopy, nor X-ray microscopy can image the turnover of cells and organelles. We need to combine optical imaging with an instrument of similar resolution, but different principle. We have recently correlated super-resolution fluorescence microscopy with secondary ion mass spectrometry, which can be easily adapted to turnover investigations, thus creating correlated optical and isotopic nanoscopy (COIN). Using this approach, we were able to obtain information on the turnover of different organelles in cultured hippocampal neurons. COIN can now be applied to any cell or organism, and therefore should enable the investigation of the turnover of virtually any organelle or sub-cellular structure.

dSTORM Coming of Age: Applications in Neuroscience

Invited Talk

Markus Sauer

Department of Biotechnology and Biophysics, Wuerzburg University

Super-resolution microscopy by single-molecule photoactivation or photoswitching and position determination (localization microscopy) has the potential to fundamentally revolutionize our understanding of how cellular function is encoded at the molecular level. Among all powerful high-resolution imaging techniques introduced in recent years localization microscopy excels at it delivers single-molecule information about the distribution and, adequate controls presupposed, even absolute numbers of proteins present in subcellular compartments. This provides insights into biological systems at a level we are used to think about and model biological interactions. We briefly introduce basic requirements of localization microscopy, its potential use for quantitative molecular imaging, and discuss present obstacles and ways to bypass them. Finally, we demonstrate the use of dSTORM for imaging of synaptic proteins and discuss how quantitative information on the organization of synaptic proteins can be obtained.

Single-objective Selective-Plane Illumination Microscopy for high- and super-resolution imaging of biological structures

Invited Talk

Jean-Baptiste Sibarita
University of Bordeaux, France

The recent advent of optical super-resolution techniques represents a new and fundamental step toward understanding biological mechanisms at the molecular level in single cells. Single molecule based techniques offer the capabilities to count, locate and track the movement of bio-molecules in their cellular environment. Several illumination schemes of photoswitchable organic (STORM) or photoactivable genetically encoded (PALM) dyes have been developed to perform single molecule localisation based super resolution. However, these illumination schemes confine the super-resolution region to a single plane close to the coverslip interface and failed therefore to study live dynamical processes in whole 3D cells. On the other hand, selective plane illumination microscopy (SPIM) proved to be highly effective in providing information in whole organisms by virtue of fast, low light and low background illumination of millimeter size organisms. This technique uses at least two objectives to create a static or scanned light sheet illumination orthogonal to the direction of detection. Experimental constraints however hamper the use of this approach with high numerical aperture (NA) objectives required to work at the single cell level. Combining both approaches would be an efficient way to enable in depth optical sectioning single molecule based imaging at the single cell level in order to study live cellular dynamical processes at the nanoscale. We here demonstrate how a single high numerical aperture objective can be used to produce an excitation light sheet perpendicular to the optical axis for optimal sectioning and high background rejection [1]. We named this technique soSPIM for single objective Selective Plane Illumination Microscopy. Compatible with standard inverted microscopes and single-molecule imaging, soSPIM relies on the unique combination of 45° arrayed micro-mirrors directly fabricated on a disposable coverslip with a specific beam steering add-on unit. Reflexion on those 45° mirrors enable to achieved micrometre sectioning capabilities and high photon collection through a unique high numerical aperture objective. Thanks to this method we demonstrated 3D high resolution comparable to spinning disk approaches and 3D single-molecule based super-resolution imaging up to 30 µm deep inside suspended cells and nuclei. Lastly we demonstrated the capability of the soSPIM architecture to also image thick sample such a whole drosophila embryos by scaling up the mirror size and using lower magnification objective. In conclusion, soSPIM approach provides the capability to image from whole drosophila embryos down to the single cell level with single molecule detection sensitivity up to 30 µm inside the sample.

Genetically-encoded tools to optically control and image neuronal activity

Invited Talk

Takeharu Nagai
Institute of Scientific and Industrial Research, Osaka University, Japan

In living organism, Ca2+ is one of the most versatile second messenger to control biological processes such as muscle contraction, hormonal secretion and apoptosis induction. Its spatial and temporal dynamics has key roles to regulate these physiological phenomena. To reveal such dynamics, variety of Ca2+ indicators had been developed. They enabled noninvasive visualization of Ca2+ dynamics, provided meaningful information for research in wide range of biological field. However, for deeper understanding of relationship between the spatiotemporal Ca2+ dynamics and the following response, development of tools to manipulate intracellular Ca2+ level have been desired. In current methods, Ca2+ concentration can be controlled by light through Ca2+ binding compounds with photocleavable moieties. However, they require irradiation of toxic ultraviolet wavelength light and/or cell loading associated with disruption of the cell membrane. These properties which have possibility to impair cells become big problem especially in the case of in vivo measurement. In addition to this, Ca2+ release from such compounds is irreversible. To overcome this, we developed a genetically-encoded photoactivatable Ca2+ releaser called PACR (PhotoActivatable Ca2+ Releaser). That is composed of Ca2+ binding protein and light-sensitive protein. Affinity of PACR for Ca2+ was decreased during irradiation of blue light. Thus reversible and repeatable increasing of Ca2+ concentration in cell is possible without damage to living specimens. By using PACR, we succeeded nucleus specific temporal Ca2+ increase in HeLa cells and excitation of specific neuron in freely moving C. elegans by blue light irradiation. This useful tool is expected to contribute on researches to reveal the role of Ca2+ dynamics in complex biological phenomena. In addition to this manipulation tool, I would like to introduce not only color variants of super-duper luminescent protein but also a voltage indicator, which can be used to monitor neuronal activity operated by multiple optogenetic actuators.

Blood flow, brain vascular dynamics, and the basis of resting state connectivity

Invited Talk

Celine Mateo, Per M. Knutsen, Philbert S. Tsai, Andy Y. Shih, **David Kleinfeld**
University of California in San Diego, USA

Functional magnetic resonance imaging (fMRI) is a powerful tool to probe the extent of activity in the human brain. In fact, the blood-oxygenation-level-dependent (BOLD) fMRI signal, discovered by Ogawa and colleagues [1], forms the central technology of modern cognitive neuroscience. An intriguing issue, discovered by Hyde and colleagues [2], is that ultra-slow variations (~ 0.1 Hz) in the oxygenation of brain tissue appear to be mirrored across conjugate brain areas across the two hemispheres. This is referred to as "resting-state" BOLD fMRI [3] and this finding has been inverted in many studies of human cognition, so that ultra-slow co-fluctuations are interpreted as "function connections" [4]. Yet the mechanism to support the relation between co-activation of neuronal activity and the ultra-slow oscillations in blood oxygenation is lacking. We address this relation using ultra-large field two-photon laser scanning microscopy [5], along with conventional techniques, in awake mice, through microscopic measurements of neuronal activity, vascular dynamics, and tissue oxygenation. We provide evidence for a biophysical basis that links co-activation of ongoing neuronal activity with ultra-slow oscillations in blood oxygenation. This result may justify inferring neuronal connections from synchronous ultra-slow vasodynamics across different brain areas.

1. Ogawa, S, Lee, T-M, Nayak, AS, and Glynn, P (1990) Oxygenation-sensitive contrast in magnetic resonance image of rodent brain at high fields. Magnetic Resonance in Medicine 14:68-78.

2. Biswal, B, Yetkin, FZ, Haughton, VM, and Hyde, JS (1995) Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. Magnetic Resonance in Medicine 34:537-541.

3. Fox, MD and Raichle, ME (2007) Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. Nature Reviews of Neuroscience 8:700-711.

4. Sporns, O, Tononi, G, and Kötter, R (2005) The human connectome: A structural description of the human brain. Public Library of Science Computational Biology 1:e42.

5. Tsai, PS, Mateo, C, Field, JJ, Schaffer, CB, Anderson, ME, and Kleinfeld, D (2015) Ultra–large field-of-view two-photon laser scanning microscopy. Optics Express 23:13833-13847.

Optical Imaging of Oxygen Delivery and Consumption : Guiding Interpretation of BOLD fMRI

Invited Talk

David Boas
Martinos Center, Massachusetts General Hospital, Harvard Medical School

BOLD fMRI is used extensively to map out brain activity patterns elicited by varied stimuli. BOLD fMRI measures the vascular response to neuronal activity and is thus not a direct measure of the underlying neuronal response to stimulus. A detailed understanding of neurovascular coupling is required to understand this relationship. Further, BOLD fMRI is an uncalibrated measure of the changes in deoxygenated hemoglobin during brain activation. BOLD is usually calibrated with a hypercapnic procedure and a model of the BOLD signal that itself is not well validated. I will review our efforts to understand the vascular response to neuronal activity at a macroscopic level and our procedures to cross-validate the BOLD calibration procedure. To gain a more microscopic validation of the BOLD signal model, we have performed numerous microscopic studies of the microvascular blood flow and oxygenation response to neuronal activity. I will review these microscopic methods and our validated bottom up model of the BOLD signal and the predictions it made that we have subsequently verified.

High throughput imaging of mesoscopic functional connectivity in mouse cortex

Invited Talk

Timothy Murphy, Jeff Ledue, Greg Silasi, Jamie Boyd, Raghu Katreddi, Dirk Haupt, Dongsheng Xiao, Matthieu Vanni, Yicheng Xie
University of British Columbia,

Spontaneous activity carries information about mesoscopic brain mapping. We describe multiple recombinant sensors for imaging mouse cortical resting state activity (GCAMP6 and iGluSNfr). Single unit recording was used to examine relations between local firing (within cortex or sub-cortical areas) and cortical maps under anesthesia. Wide-field imaging of intracellular calcium in behaving mice revealed the functional parcellation of the mouse cortex. When mice are trained on a head-fixed forelimb lever pulling task we find wide scale changes in the functional parcellation of cortex that extend outside of the forelimb area. However, conventional imaging approaches for linking rodent behavior to neurocircuits necessitates the use of specialized equipment and considerable investigator intervention for both training and assessment. We employ a new strategy of mouse home cage wide field imaging. The work extends previous 2-photon imaging work (Scott et al. 2013) to multiple mice per cage and mesoscopic scales 24 h/day. Home cage training and imaging assessment supports group housing of 5 mice at a time and requires minimal investigator intervention. The platform is cost-effective and can yield over 50, 30 s sessions of autonomous imaging per mouse per cage each day. Using this procedure in genetically-encoded calcium indicator GCAMP6 fast and slow (Ai-93 and Ai-94) transgenic mice, we longitudinally monitor cortical mesoscopic functional circuits 24 h/day by assessing spontaneous and sensation-evoked activity.

Modern 3D electron microscopy for mapping neural circuits

Invited Talk

Kevin Briggman
NIH-NINDS, USA

A fundamental limitation to understanding the function of neuronal circuits is the lack of complete wiring diagrams. Any computational model that proposes to explain a particular computation must be consistent with the underlying synaptic wiring of a circuit. Therefore, connectomes allow many classes of models to be ruled out while simultaneously providing crucial data to develop anatomically realistic models. A current open question is to what level of detail do circuits need to be reconstructed to develop such realistic models?

We explored the importance of detailed connectivity mapping in the direction selectivity circuit of the mouse retina. Starburst amacrine cell (SAC) dendrites are selective for radial (centrifugal) motion in the retina. We previously found that this radial motion preference is transformed into a rectilinear coordinate system through highly selective connections between SAC dendrites and direction selective ganglion cells (DSGCs). A remaining mystery in the circuit is the mechanism by which SAC dendrites become radially selective. We therefore collected a large 3D serial block-face scanning electron microscopy (SBEM) dataset to explore the network connectivity of SACs.

By densely reconstructing the synaptic inputs to SACs, we identified a previously undescribed asymmetry in the distributions of excitatory and inhibitory inputs along SAC dendrites. This distribution of synapses is inconsistent with numerous existing computational models of SAC DS. We then developed an anatomically realistic network model of SAC connectivity and studied the role of inter-SAC inhibition in generating SAC DS. We found that the particular placement of excitatory synapses onto SACs and inhibitory synapses between neighboring SACs significantly extends the range of visual contrast over which the cells remain DS. We then performed two-photon dendritic calcium imaging to test the predictions of the model.

New strategies and pitfalls for measuring receptor oligomerization with fluorescence fluctuation methods

Invited Talk

Paul Wiseman
McGill University, Canada

The ability to measure protein interactions in subcellular compartments is key to understanding cell signalling mechanisms, but quantitative analysis of these interactions in situ has remained a major challenge. We present a fluorescence imaging based analysis technique called spatial intensity distribution analysis (SpIDA) (Godin et al., PNAS 2011). SpIDA allows for quantitative measurement of fluorescent particle densities and oligomerization states within individual images acquired with conventional confocal laser-scanning microscopy. The method, an analog imaging extension of the photon counting histogram approach, is based on fitting intensity histograms calculated from images with super-Poissonian distributions to obtain density maps of fluorescent molecules and their quantal brightness values. Importantly, because distributions are acquired in space, SpIDA can be applied, not only to analysis of live cells, but also to that of chemically fixed tissue.

An important technical challenge faced by fluorescence microscopy-based measurement of oligomerization is the fidelity of receptor labeling. In practice, imperfect labeling biases the distribution of oligomeric states measured within an aggregated system. We show the extension of SpIDA to enable analysis of high-order oligomers from fluorescence microscopy images, by including a probability weighted correction algorithm for non-emitting labels. We demonstrate that this fraction of non-emitting probes could be estimated in single cells using SpIDA measurements on model systems with known oligomerization state. Previously, this artefact was measured using single-step photobleaching. We show validation of the new approach using computer-simulated data and, the imperfect labeling was quantified in cells with ion channels of known oligomer subunit count. We then show an application to quantify the oligomerization states in different cell compartments of the proteolipid protein (PLP) expressed in COS-7 cells. Expression of a mutant PLP linked to impaired trafficking resulted in the detection of PLP tetramers that persist in the endoplasmic reticulum, while no difference was measured at the membrane between the distributions of wild type and mutated PLPs.

Assessing cell type-specific connectivity in the mouse brain using large-scale array tomography and two-photon microscopy

Invited Talk

Nelson Spruston
Janelia Farm, USA

A major goal of neuroscience is to understand animal behavior in terms of neural circuit function. A necessary step to this end is to determine the connections between different brain regions and different types of neurons. With the mouse brain consisting of thousands of different cell types comprising a total of ~70 million neurons and billions of synapses, this is a challenging task. New methodologies are now available to tackle this problem, including array tomography and a two-photon microscopy. I will describe efforts in my lab to use array tomography to understand the local connections between different types of inhibitory interneurons contacting pyramidal neuron dendrites in the hippocampus. In addition, I will describe a nascent team project at Janelia directed at studying long-range axon projections in the mouse brain using a newly developed resonant-scanning two-photon microscope with an integrated vibratome, which allows axon arborizations to be imaged and reconstructed in the whole mouse brain.

Fibre-optics for in vivo optogenetics; from single cells to hard-to-get-to areas of the nervous system

Invited Talk

Yves De Koninck
Centre de recherche de l'Institut universitaire en santé mentale de Québec, Canada

Fiber-optics-based probes are becoming increasingly instrumental for minimally-invasive, targeted optical measurements and manipulations *in vivo* in behaving animals. Yet the scale of probing sought and geometry of the system present different design challenges. We present development of probes to address challenges at both ends of the spatial scale. At one end, we developed an aluminum-coated, fibre optic-based glass microprobe with multiple electrical and optical detection capabilities while retaining tip dimensions that enable single cell measurements (diameter <10 µm). The probe enables optical separation from individual cells expressing different fluorescent proteins within the same brain nucleus, functional optical measurements and optogenetics activation from single cells, and color conversion of photoswitchable fluorescent proteins. The combination of metal on the outside of the probe and of a hollow core within the fiber yields a microelectrode enabling simultaneous single unit and population field potential recordings. We also designed an electro-conducting glass which transmits visible light with >80% efficiently, yielding a microstructurable, electrolyte-free micro-optrode for long-term recording. At the other end of the spatial scale, broad delivery of light to certain portions of the central nervous system present challenges due to the geometry of the tissue. For example, difficulties in delivering sufficient light to the spinal cord of freely-behaving animals has hampered the use of spinal optogenetics for analgesia. Here we show the use of an epidural optic fiber with a diffusive tip to successfully produce spinal analgesia in freely moving animals. These fiber-optics probes expand possibilities for *in vivo* optogenetics from single cell multimodal capabilities to access to previously out of reach areas of the CNS.

Monitoring and manipulating intracellular transport

Invited Talk

Casper C. Hoogenraad
Cell Biology, Faculty of Science, Utrecht University

Controlling protein-protein interactions in live cells represents a powerful tool in modern biology and has opened up new avenues for manipulating cellular processes. Chemical and light induced dimerization systems allow spatially and temporally control of transcriptional activation, signal transduction pathways, subcellular protein translocations and other cellular processes. We have currently developed inducible cargo transport assays to study the basic trafficking rules in neurons. By recruiting specific motor proteins (kinesin, dynein or myosin) to selected organelles (e.g. synaptic vesicles, mitochondria or RNA particles), these organelles will be forced to move anterogradely, retrogradely or become immobilized. Because these approaches allow spatiotemporally controlled removal and positioning of selected organelles, they will be invaluable tools to unravel their local functions in developing and mature neurons. Here we will discuss recent advances in engineering inducible tools and discusses future directions to monitor and manipulate intracellular transport processes in living neurons.

Red fluorescent protein-based neurophotonic probes for visualization of neuronal activity

Invited Talk

Robert Campbell
Department of Chemistry, University of Alberta

The Campbell research group is focused on the use of protein engineering for the development of fluorescent proteins and fluorescent protein-based reporters for live cell imaging. These efforts require a combination of rational protein design and directed protein evolution. Over the last decade, this approach has proven to be both effective and versatile and has led to the development of a variety of new genetically encoded fluorescent probes. Indeed, by exploiting iterative cycles of high-throughput fluorescence image-based screening of bacterial colonies, and low throughput testing of promising variants in mammalian cells, the Campbell group has developed a growing selection of fluorescent protein-based tools with improved brightness, photostability, and biosensing or photoconversion properties. In this seminar I will present some of our most recent efforts to engineer an improved generation of reporters. Specifically, I will provide an update on the expanding palette of calcium ion reporters our lab has been developing, and describe how we are using similar engineering efforts to make reporters for membrane potential and neurotransmitters. In addition, I will introduce a new optogenetic manipulation strategy that promises to open up a wide array of new opportunities for controlling biological activities with light.

Laser-assisted single-cell tagging and membrane functionalization

Invited Talk

Santiago Costantino

Université de Montréal, Centre de recherche de l'hôpital Maisonneuve-Rosemont, Canada

Laser Assisted Protein Adsorption by Photobleaching was originally introduced as a method to engineer cell culture substrates by creating protein patterns of optical resolution and a high dynamic range of concentrations. A laser is used to photobleach and bind fluorescently tagged molecules to a surface via generation of reactive species. The modulation of laser power and scanning velocity across the surface allows creating arbitrary patterns of substrate bound macromolecules. Here we show that laser illumination can be used to adsorb fluorescent molecules to the plasma membrane of living cells, as a way to identify arbitrary individual cells and groups of cells in microscopy images. Selected cells can be sorted by flow cytometry and tagged for ultra-microscopy. Furthermore, it is possible to induce transient cell adhesions to the substrate only on irradiated cells for micro-engineering co-cultures with spatially patterned cell types.

Deep two-photon imaging of neuronal networks in vivo with a red-shifted indicator

Invited Talk

Arthur Konnerth
Technische Universität München, Germany

In vivo Ca2+ imaging of neuronal populations in deep cortical layers has remained a major challenge, as the recording depth of two-photon microscopy is limited due to scattering and absorption of photons in brain tissue. A possible strategy to increase the imaging depth is the use of red-shifted fluorescent dyes, as scattering of photons is reduced at long wavelengths. Here, we tested the red-shifted fluorescent Ca2+ indicator Cal-590 for deep tissue and dual-color two-photon imaging experiments in vivo. Cal-590 has a maximum for two-photon excitation at a wavelength around 1050 nm and a maximum emission wavelength at 590 nm. To explore the potential of Cal-590 for measurements of neuronal activity in deep cortical layers of the mouse brain, we used bulk loading of the acetoxymethyl (AM) ester version of Cal-590 to label populations of neurons in layers 5 and 6, respectively. Combined two-photon imaging and cell-attached recordings revealed that, despite the relatively low affinity of Cal-590 for Ca2+ (Kd = 561 nM), single action potential-evoked Ca2+ transients were discernable with a good signal-to-noise ratio in most bulk loaded neurons. We were able to record spontaneous Ca2+ transients with rapid kinetics in the six layers of the cortex at depths of up to -900 µm below the pial surface. Similar results were recorded for individual neurons electroporated with Cal-590, with dendritic rise and decay times sufficient to distinguish the peaks of individual Ca2+ transients even for high-frequency trains (100 Hz) of action potentials. In addition to the deep imaging experiments, we used Cal-590 for multi-color functional imaging experiments in combination with other Ca2+ indicators. Ca2+ transients in the dendrites of an individual OGB-1-labeled neuron and the surrounding population of Cal-590-labeled cells were recorded simultaneously on two spectrally separated detection channels. We conclude that the red-shifted Ca2+ indicator Cal-590 allows acute in vivo two-photon Ca2+ imaging experiments in all layers of the mouse cortex as well as multi-color functional imaging experiments.

Visuomotor learning in mouse primary visual cortex

Invited Talk

Alexander Attinger, Bo Wang, **Georg Keller**
FMI - Friedrich Miescher Institute, Germany

Neurons in primary visual cortex are driven both by visual stimuli and by motor-related signals. Based on this coexistence of sensory and motor signals, processing in visual cortex has been speculated to underlie sensorimotor integration and sensorimotor mismatch detection. Visual responses are known to be shaped in an experience-dependent manner; how experience affects sensorimotor integration in visual cortex, however, is unknown. Here we show that the development of normal activity in primary visual cortex of the mouse is critically dependent on experience of visual feedback that is coupled to movement. Using virtual reality environments, we raised mice either in a condition of normal visuomotor coupling, in which forward locomotion was coupled to backward visual flow, or in a yoked control condition of random visuomotor coupling in which locomotion had no influence on visual flow. Even though mice in both conditions experienced the same visual input and on average exhibited the same locomotion behavior, we found profound difference in sensorimotor processing between the two groups. The development of selective feedback mismatch signals was strongly dependent on coupled visuomotor experience. Moreover, by recording from genetically identified inhibitory subtypes we could show that visuomotor coupling establishes a finely tuned balance between excitation and inhibition. This balance may underlie sensorimotor integration and the comparison between actual and expected visual feedback.

Synaptic plasticity controls synaptic lifetime

Invited Talk

Thomas Oertner
Institute for Synaptic Physiology, Germany

Long-term potentiation (LTP) and long-term depression (LTD) change synaptic transmission in an activity-dependent manner. On the level of entire pathways, averaging over many synapses, both LTP and LTD seem to be stable over days. It is less clear, however, how plasticity affects individual synapses over time. We showed previously that LTD preferably leads to elimination of low release probability synapses, suggesting that weight adjustments affect the lifetime of synapses. Thus, reversible changes in the functional connectivity of neuronal networks induced by classical long-term plasticity (LTP/LTD) could be made permanent through synapse elimination and stabilization. During normal experience, synapses may be exposed to multiple plasticity-inducing events both increasing and decreasing synaptic weights. Thus, the persistence of synapses may depend directly on the precise sequence of potentiation and depression. We do not know, however, how potentiation and depression interact at individual synapses to regulate their persistence.
In slice cultures of rat hippocampus we combined optogenetic stimulation of identified Schaffer collateral axons with two-photon imaging of the genetically encoded calcium indicator GCaMP6 in active spines. All-optical induction of LTD and LTP allowed us to measure the strength of individual synapses and to follow their fate after depression or potentiation over 7 days. We found that LTP induction, using a presynaptic theta-frequency stimulation protocol, resulted in potentiation of postsynaptic calcium responses. Interestingly, successful potentiation was dependent on dendritic calcium spikes during the induction protocol. Synaptic stimulation after LTP induction often triggered dendritic calcium spikes invading neighboring, previously non-responding spines. Although concomitant spine volume increase was not sustained for > 24 h, spine survival was enhanced during the week following LTP induction. Thus, analogous to LTD, functional adjustments induced by LTP are stored in the network by stabilization of synapses. Interestingly, LTP induction 24 h after LTD induction completely reversed the reduction in synaptic lifetime, indicating that LTD did not trigger irreversible degradation of synapses. Our results indicate that individual synapses keep track of multiple potentiation and depression events distributed over many hours and that their probability of survival is adjusted accordingly.

Turning calcium transients into spikes and watching the animal in action

Invited Talk

Jason Kerr
Max Planck Institute, Germany

Multiphoton-imaging allows unambiguous access to neuronal populations and neuronal substructures located well below the cortical surface. In combination with genetically encoded activity indicators this approach can be used to infer spiking activity from neuronal populations in the awake animal, with single cell and single action-potential accuracy. I will present recent imaging and analysis tools that are necessary to accurately record activity from genetically encoded calcium indicators in neuronal populations using the multi-photon excitation principle. I will also outline strategies that have allowed access to neuronal activity in the freely moving animal using multiphoton excitation and recent advances to simultaneously track the precise head and eye positions of these freely behaving animals.

Deconstructing and reconstructing neuronal circuits for olfaction

Invited Talk

Adrian Wanner, Tafheem Masudi, Christel Genoud, **Rainer Friedrich**
Friedrich Miescher Institute for Biomedical Research, Switzerland

We use the olfactory system of zebrafish as a model to understand neuronal computations involved in pattern classification. Exploiting the small size of the zebrafish brain, we measure neuronal activity patterns using multiphoton calcium imaging, manipulate neuronal activity using optogenetics, and reconstruct neuronal circuits using serial block face scanning electron microscopy (SBEM). In the olfactory bulb, we identified and characterized multiple computations that stabilize odor-evoked activity patterns against variations in stimulus intensity and facilitate their classification. Recently, we densely reconstructed the neurons in the olfactory bulb of a zebrafish larva using SBEM. This work includes the development of new methods and will be the focus of the presentation. Topological analyses of circuit structure revealed a specific organization of long-range connectivity that is governed by the identity of olfactory glomeruli. These results provide insights into the structure and function of neuronal circuits that are likely to be of fundamental importance for olfactory pattern classification.

Synaptic mechanisms underlying integration of early-born and late-born motor circuits

Invited Talk

Minoru Koyama
Janelia farm, USA

Brain is a complex biological network composed of large number of components interacting each other. Yet it grows dramatically after birth as an animal acquires increasingly sophisticated behaviors. During this process, brain somehow stays functional while individual neurons go through dramatic developmental changes to acquire new connections. We are aiming to understand the network architectures underlying the extensibility of the brain.
Here we examined how nervous systems organize synaptic inputs from neurons born at different time points using hindbrain descending neurons in larval zebrafish as a model system. We characterized their birthtime, recruitment during behavior and synaptic connectivity to spinal cord and found that they have distinct synaptic targets with synaptic properties consistent with the motor patterns they are involved in. This suggests one mechanism nervous systems can use to acquire increasingly sophisticated circuit mechanisms while maintaining pre-existing circuits. I will also touch on our effort to examine the development of functional circuits at a larger scale taking advantage of optical access in zebrafish.

Investigation of a novel sensory interface relaying information from the cerebrospinal fluid to motor circuits

Invited Talk

Urs Bohm, Kevin Fidelin, Lydia Djenoune, Jeffrey Hubbard, Andrew Prendergast, Filippo Del Bene, **Claire Wyart**
Institut du Cerveau et de la Moelle Épiniere (ICM), France

The cerebrospinal fluid (CSF) is a complex solution flowing around the brain and spinal cord. Behavior has long been known to be influenced by the content and flow of the CSF, but the underlying mechanisms are completely unknown. CSF-contacting neurons by their location at the interface with the CSF are in ideal position to sense CSF cues and to relay information to the nervous system. By combining electrophysiology, optogenetics, bioluminescence monitoring with calcium imaging in vivo, we demonstrate that neurons contacting the CSF in the spinal cord detect local bending and in turn feed back GABAergic inhibition to multiple interneurons driving locomotion in the ventral spinal cord. Behaviour analysis of animals deprived of this mechano-sensory pathway reveals its contribution in modulating frequency of locomotion. Altogether our approach developed in a transparent animal model shed light on a novel pathway enabling sensory motor integration between the CSF and motor circuits confined to the spinal cord.

Abstracts

Short talks

Fluorescence lifetime nanoscopy for measuring FRET in dendritic spines

Short talk

Christian Tardif, Gabriel Nadeau, Daniel Côté, **Paul De Koninck**
Centre de Recherche de l'Institut Universitaire en Santé Mental de Québec. (CRIUSMQ)

Localization of protein interactions in the synaptic area can impact on the signaling cascade implicated in learning and memory. Knowing the nanometric position of those interactions inside and around the post-synaptic density could provide insights on the role of their partnership. Fluorescence Lifetime Imaging (FLIM) to quantify Foster Resonant Energy Transfer (FRET) is useful to study protein interactions. FRET-FLIM approach provides limited spatial resolution due to the diffraction of light (~250nm), particularly for studying interactions in synaptic domains. Super resolution methods have been developed to beat this resolution limit. We combined STimulated Emission Depletion (STED) with FRET-FLIM technique to study molecular interactions within spines at nanoscale. We built a STED microscope that achieves an x/y resolution of ~50 nm. In order to incorporate FLIM with STED, we determined with simulation the optimal parameters of data acquisition and analysis, considering the limited amount of photons available. To measure protein interactions, we used an immuno-FRET approach, labelling the donor and acceptor putative binding partners with Atto dye-labelled antibodies. Following a series of positive and negative control to demonstrate the reliability of the approach, we examined the interaction of receptors and signaling proteins inside nanodomains of dendritic spines. Thus this approach should help the investigation of cell signaling at the nanometer scale.

Hypothalamic CRH neurons balance opposing stress behaviours

Short Talk

Tamas Fuzesi, Nuria Daviu, Jaideep S. Bains
Hotchkiss Brain Institute

When we are undecided about a course of action in response to a given stimulus, we exhibit specific displacement behaviors that are repetitive and stereotyped. These displacement behaviors appear to be out of context, and in some individuals become even more pronounced when the stimulus is aversive or stressful. How the brain chooses between displacement behavior and behavior that is purposeful and intentional in response to a particular stimulus is not known. In rodent models, grooming is a widely recognized displacement behavior that is easily distinguished from context-dependent, purposeful behaviors such as freezing or rearing. Here using cell-type specific optogenetic manipulations, we show that changing the environmental context after exposure to a stressor alters the ratio between displacement and purposeful behavior. We demonstrate that this behavioral shift is driven by changes in the activity of hypothalamic CRH neurons. Silencing CRH neuron activity after stress favors purposeful behavior at the expense of displacement behavior. Furthermore, we demonstrate that in the absence of stress, CRH neurons drive a local circuit to cause graded shifts in behavior from purposeful to displacement even in situations that strongly favor purposeful behavior. These findings indicate that displacement and purposeful activities are mutually exclusive, environmentally sensitive behaviors that are balanced by hypothalamic CRH neurons.

A Wireless Headstage for Combined Optogenetics and Multichannel Electrophysiological Recording in Freely Behaving Animals

Short Talk

Benoit Gosselin
Université Laval

We present a wireless optogenetic headstage combining neural recording and optical stimulation capabilities, while enabling spike detection and data compression in-situ. The proposed headstage, which is intended to perform photo-stimulation and electrophysiological recordings simultaneously in freely moving transgenic rodents, is entirely built using commercial off-the-shelf components, and includes 32 recording channels and 32 photo-stimulation channels at 465 nm. It can detect, compress and transmit full action potential waveforms collected from 32 channels in parallel, and in real, time using an embedded digital signal processor performing spike detection and data compression in-situ. Simultaneous optical stimulation and recording have been performed in-vivo in the somatosensory cortex and the Hippocampus of a transgenic mouse expressing ChannelRhodospin (Thy1::ChR2-YFP line4) under anesthetized conditions. Experimental results show that the proposed headstage can collect, detect and compress microvolt amplitude neuronal activity evoked by photo-stimulation from multiple channels in parallel while providing a high signal-to-noise ratio (SNR) and achieving overall compression ratios above 500. This is the first reported wireless optogenetic device combining multichannel photo-stimulation and multichannel recording. This headstage presents a lifetime of 105 minutes, and uses a rigid-flex printed circuit board, which makes it lightweight (2.8 g) and compact (17x18x10 mm^3).

Super-resolution fluorescence microscopy of synaptic proteins

Short Talk

Sarah Aufmkolk[a], Markus Sauer[a], Thomas Andreska[b], Robert Blum[b]
a -Department of Biotechnology & Biophysics, Biozentrum, Julius-Maximilians-University Würzburg, Germany. b -Institute for Clinical Neurobiology, University Hospital, Julius-Maximilians-University Würzburg, Germany.

The neuron is a specialized cell with a dynamic network of molecules that interact to restrict or propagate signals at defined contact areas called synapses.

Neurotransmitters at neuronal synapses are released by the regulated fusion of synaptic vesicles (SVs) with the plasma membrane (PM). The interface between the SV-triggering and fusion machinery and the PM is occupied by a set of regulatory proteins that mostly reside at active zone (AZ) fusion sites. These regulatory proteins control the speed, fidelity, and short-term plasticity (STP) of the transmitter-release process, and thereby determine key brain functions, such as cortical gain control, sensory adaptation, sound localization, and motor control. However, the spatial organization of these AZ proteins, and how they interact with each other and with other PM, cytosolic and SV components of the transmitter-release machinery, are not completely known.

Determining quantitative information about the molecular distribution and densities of synaptic proteins calls for a method that offers a precision which resolves the spatial relationship between different single synaptic proteins.

Here, we used super-resolution microscopy by direct stochastic optical reconstruction microscopy (dSTORM) (1) to visualize different synaptic proteins with virtually molecular resolution.

Furthermore, we developed efficient sample preparation and staining protocols for multicolor dSTORM of synaptic proteins and actin filaments. These methods can be used advantageously to establish comprehensive blueprints of synaptic protein architecture that reflect relevant functional states. We demonstrate how nanomaps of endogenous synaptic proteins (2) such as BDNF, Trk receptors, homer, bassoon, and mRNAs in neuronal cells can be generated.

In addition, we demonstrate dSTORM imaging of synaptic proteins in brain tissues enabling the visualization of protein organization in the context of intact neural circuits. This helps to identify structural variations in different brain areas.

References
(1) van de Linde, S., Aufmkolk, S., Franke, C., Holm, T., Klein, T., Löschberger, A., Proppert, S., Wolter, S., Sauer, M.: Investigating Cellular Structures at the Nanoscale with Organic Fluorophores. (2013), Chem Biol, 20, 8 – 18
(2) Andreska, T., Aufmkolk, S., Sauer, M., Blum, R.: High abundance of BDNF within glutamatergic presynapses of cultured hippocampal neurons. Frontiers in Cellular Neuroscience. 8, (2014)

A correlative second harmonic (SH) – electron microscopy (EM) approach to investigate microtubules and intracellular transport phenomena

Short Talk

Michiel Martens[a], Valérie Van Steenbergen[a], Koen Clays[b], Marcel Ameloot[c], Katlijn Vints[d], Peter Baatsen[d], **Pieter Vanden Berghe[a]**

a- Laboratory for Enteric NeuroScience, TARGID, University of Leuven, b- Molecular visualization and photonics, University of Leuven, c -BIOMED, University of Hasselt, d- VIB Center for the Biology of Disease, University of Leuven

Second harmonic generation is a nonlinear optical effect that arises when non-centrosymmetric organized biomolecules like for instance microtubules (MT) are exposed to pulsed laser light. SH imaging microscopy (SHIM) is as a label-free technique a valuable addition to classic fluorescence microscopy especially when it comes to studying MT. These cytoskeleton components are critical for healthy cell functioning as they are involved in many cellular processes such as transport, motility and mitosis. Because the correct optical and biomolecular conditions have to be met for the SH signal to be generated, SHIM provides an extra level of detail about the organisation, orientation and dynamical properties of MT.

In this study we used primary neuron cultures and in vitro polymerized microtubules to first investigate the main determinants of SH generation and explore the possibilities and limitations of SHIM in biomedical research. We provide proof of the microtubular origin of the SH signal by imaging living cells transfected with GFP-tubulin, and indicate several limitations when using SHIM in biological samples. We show that in vitro polymerized microtubules are incapable of SHG, contrary to what is observed in living cells, where SH was detected from individual fibrilar structures.

We also found that SH cannot be maintained after fixation as several fixatives, based on drying or crosslinking, lead to disappearance of the signal. To investigate this further, we used electron microscopy (EM) to on the one hand confirm that MT were not disrupted after fixation with glutaraldehyde but also to quantify whether the number of MT correlated with the SH intensity in certain cell areas and processes. SHIM-EM reveals that individual MT within a cell are not sufficient, but that bundles of MT are required to efficiently generate SH. Our EM data show that MT can form small bundles so compact that they cannot be distinguished from one another using light microscopy, even superresolution techniques would not be sufficient. The specific biomolecular and structural requirements, combined with the fact that in fixed cells the SH signal disappears, prove that SHIM reports on a dynamic microtubule state, which makes it a unique live imaging tool.

In conclusion, we provide evidence that SHIM, with the right precautions for live cell imaging, is a powerful tool to gain insight into the dynamics of the microtubule network and demonstrate its potential in neuroscience research.

Gold nanoparticle-assisted all optical localized stimulation and monitoring of Ca^{2+} signaling in neurons

Short Talk

Flavie Lavoie-Cardinal[a], Charleen Salesse[a], Éric Bergeron[b], Michel Meunier[b], Paul De Koninck

[a]Université Laval, [b]École Polytechnique de Montréal.

Light-induced stimulation of whole neurons or neuronal networks with optogenetic tools is a well-established and powerful approach to study circuit function. On the other hand, local membrane stimulation at the nm to µm scale, which would be useful to study the cellular rules governing neuronal and dendritic functions, remains a challenge. We introduce an all optical method for the stimulation and the monitoring of localized Ca2+ signaling in neurons that takes advantage of plasmonic excitation of gold nanoparticles (AuNPs). While non-specific binding of bare AuNPs onto the neurons was possible through passive sedimentation, we show that AuNP functionalization with monoclonal antibodies can be used to specifically target the AuNPs on neuronal membranes expressing the targeted antigen. To combine optical stimulation and optical read-out of neuronal activity, we used the off-resonance plasmonic excitation of AuNPs with near-infrared light at 800 nm as a source for localized neural stimulation, and optical imaging of Ca2+ oscillations, using GCaMP6s. Our results indicated that we were able to induce localized or widespread Ca2+ elevation inside the neurons, depending on i) laser intensity, ii) surface area of illumination, or iii) number of functionalized NPs on targeted cells. We combined optical imaging and whole-cell patch clamp recording to demonstrate that this NP-Assisted Localized Optical Stimulation (NALOS) on large ROIs on the cell body can be used to drive APs. However, NALOS on dendritic compartment drives local and small currents capable of inducing localized Ca2+ responses in presence of TTX. Finally, we show that this NALOS can be used to study local signaling downstream of Ca2+, by monitoring the spatial and temporal dynamics of the Ca2+/calmodulin-dependent kinase (CaMKII) tagged with GFP. Our results suggest that localized, controlled light-based stimulation of a single functionalized NP on neurons can be used to characterize the impact of local or widespread Ca2+ transients on downstream signaling and that NALOS provides a new complement to several light-dependent methods to control neuronal activity and cell signaling.

Image-based label-free screening of GABA agonists, antagonists and modulators

Short talk

Benjamin Rappaz[ab], Pascal Jourdain[ac], Damiano Banfi[ab], Pierre Marquet[acd], Gerardo Turcatti[ab]
a-EPFL, b- BSF, c-LNDC, d- CHUV

Chloride channels represent a group of targets for major clinical indications. However, molecular screening for chloride channel modulators have proven to be difficult and time-consuming approaches as essentially rely on the use of fluorescent dyes or invasive patch-clamp techniques not compatible with the screening of large sets of compounds.

To address this problem, we have developed a label-free optical method, Digital Holographic Imaging (DHI). The DHI signal is linked to ion fluxes across the plasma membrane (and the associated water fluxes) and therefore allows non-invasive monitoring of the ion channel activity without using any electrode or fluorescent dye.

The quality of the assay measured with a control solution of 10 µM GABA yielded a Z'-factor of 0.8. A selection of known agonists, antagonists and modulators were screened with DHI and validated with dose-response curves and electrophysiological recordings. Following a miniaturization to 384 well-plates, a larger collection was screened thus demonstrating High-Throughput-Screening capabilities.

This image-based screening approach allows to rapidly characterizing a large number of compounds under physiologically conditions using a non-invasive optical approach.

Single-molecule imaging of carbon nanotubes in live neuronal samples and 3D super-resolution microscopy using phase imaging

Short talk

Antoine Godin, Laurent Cognet
Laboratoire Photonique Numérique et Nanosciences – Institut d'Optique, Université de Bordeaux & CNRS

The optical microscopy of single molecules has recently been beneficial for many applications, in particular in neurosciences. It allows a sub-wavelength localization of an isolated nano-object and a subtle probing of its spatio temporal nano-environment including in living cells.

For many bio-applications, near infrared nanoprobes and/or non-spherical nanoprobes could be advantageous. In this context, the use of single walled carbon nanotube tubes is expected to be favorable. Here we demonstrate that individual nanotubes can be imaged using fluorescence microscopy and tracked in deep live brain tissue (up to 60 µm deep). Long trajectories (>10 min) of single nanotubes diffusing in the extracellular space were recorded and, by analysis their movements we could unravel live brain structuration ranging from micrometers down to ~80 nm. Finally, significant increase in the SWNTs diffusion was found upon biochemical modulations of the brain extracellular matrix.

I will also present our recent developments in super-resolution imaging using a generalization of quantitative phase imaging (QPI) technique to super-resolution microscopy. I will show that QPI allows retrieving the 3D spatial distribution of single fluorescent molecules in a single measurement by the simultaneous use of intensity and phase imaging. 3D super-resolution imaging of cytoskeleton in mammalian cells and NMDA receptors in neurons will be shown.

Quantitative Phase Imaging for label-free live-cell cytoskeleton, organelles trafficking and for 3D fluorescence super-resolution

Short Talk

Pierre Bon, Matthieu Palayret, Brahim Lounis, Laurent Cognet
Institut d'optique – CNRS – Université de Bordeaux

Quantitative phase imaging (QPI) is a powerful method to obtain quantitative information about a biological sample. It relies on the capability to measure not only the intensity of the light but also its phase. This provides several types of information depending on the illumination scheme or imaging mode (trans-illumination, fluorescence…).

QPI was initially developed to image large structures inside semi-transparent samples (ex. nucleus), without labelling/staining and using coherent transmitted light -such as a laser. We will show recent breakthrough using our QPI technique based on quadriwave lateral shearing interferometry [1] coupled with incoherent halogen trans-illumination. It allows label-free cytoskeleton and organelle trafficking imaging at up to 50 Hz and for any duration [2].

We will then present for the first time, a generalization of our QPI technique to fluorescence imaging. This allows retrieving the 3D spatial distribution of fluorescent molecules by the simultaneous use of intensity and phase imaging. We will show results of 3D super-resolution imaging of both cytoskeleton in mammalian cells and receptors in neurons.

[1] Bon, Maucort, Wattellier, and Monneret, "Quadriwave lateral shearing interferometry for quantitative phase microscopy of living cells," Opt. Express 17, 13080-13094 (2009)

[2] Bon, Lécart, Fort, Lévêque-Fort, "Fast Label-Free Cytoskeletal Network Imaging in Living Mammalian Cells", Biophysical J., 106, 1588 – 1595 (2014)

Superresolution imaging reveals unique microtubule structure mediating mechanotransduction in neurons

Short Talk

Masha Prager-Khoutorsky, Arkady Khoutorsky, and Charles W. Bourque
McGill University

The electrical activity of mammalian osmosensory neurons is increased by plasma hypertonicity to command thirst, antidiuretic hormone release, and increased sympathetic tone during dehydration. Osmosensory transduction is a mechanical process whereby decreases in cell volume cause the activation of transient receptor potential vanilloid type-1 (TRPV1) channels to induce depolarization and increase spiking activity in osmosensory neurons. However, it is not known how cell shrinking is mechanically coupled to channel activation. Using superresolution imaging, we found that osmosensory neurons are endowed with a uniquely interweaved scaffold of microtubules throughout their somata. Microtubules physically interact with the C terminus of TRPV1 at the cell surface and provide a pushing force that drives channels activation during shrinking. Moreover, we found that changes in the density of these interactions can bidirectionally modulate osmosensory gain. Microtubules are thus an essential component of the vital neuronal mechanotransduction apparatus that allows the brain to monitor and correct body hydration.

Abtracts

Posters

An axon-based lightsheet microscope for large scale and high resolution brain imaging

Poster

Cléophace Akitegetse, Véronique Rioux, Yves De Koninck, Daniel Côté, Martin Lévesque

Centre de recherche de l'Institut universitaire en santé Mentale de Québec, Université Laval

Laser scanning microscopy allows visualizing the very complex circuitry of the brain with high resolution. However, imaging the entire brain is challenging because of its relative opacity to light. In addition, laser scanning microscopy systems are time-consuming because the image points are obtained one at a time.

We present an approach combining optical clearing techniques and light sheet microscopy to obtain whole brain images in record time with a resolution comparable to that of laser scanning microscopy systems.

Here we present the use of the axicon in a two-photon fluorescence light sheet microscope. A light sheet is obtained by a quick scan of the thin line formed by the axicon while a camera placed perpendicular to the scanning plane capture the emitted fluorescence.

In a previous study, we were the first to use a conical lens (axicon) to extend the depth of field of a two-photon microscope. Using an axicon, we are able to generate two-photon fluorescence over a thin long line with constant thickness. More importantly, we are able to decouple the length and thickness of the line; thereby increasing the depth of field without resolution reduction.

This property is very useful in our light sheet microscope since, we are able to have a large field of view (defined by the line length) without compromising the axial resolution (defined by the line thickness). Producing light lines with sufficient energy remains challenging since the emission of two-photon fluorescence is a function of the square power of the excitation laser. That why we use of a regenerative-amplifier laser, which produces femtosecond pulses with 320 times more photons compared to standard pulsed laser used in two-photon microscopy.

The combination of this technology with optical clearing promises to transform our ability to understand the neuro-circuitry of the brain and thus significantly advance understanding of neurological and psychiatric diseases which involve remodelling of brain connections.

Investigating the role of CaMKII in the activity dependant trafficking of AMPA receptors

Poster

Benoit Audet, Simon Labrecque, Paul De Koninck

CRIUSMQ, Université Laval

The potentiation of synaptic efficacy in the hippocampus involves pre and post-synaptic molecular modifications. On the post-synaptic side, glutamate receptors of the AMPA subtypes (AMPARs), NMDA subtypes (NMDARs) and the Ca2+/calmodulin-dependent protein kinase II (CaMKII) play an important role in this potentiation. Strong synaptic transmission triggers Ca2+ influx through NMDARs, activating CaMKII, leading to an increase in the amount of AMPARs at excitatory synapses. The underlying molecular mechanisms that mediate the action of CaMKII on the recruitment of AMPARs to the synapse are not fully understood. The synaptic delivery of AMPARs in the postsynaptic sites should result from interplay between their rates of exocytosis and endocytosis, as well as diffusional trapping of the receptors at synapses. In this study, we are investigating the role of CaMKII in the exocytosis process of AMPARs in cultured hippocampal neurons, using gene transfer of recombinant SEP-GluA1 (AMPA receptor subunit fused to pH-sensitive GFP) and TIRF microscopy to monitor the fusion of single vesicles of AMPAR in the plasma membrane. We developed an analysis routine for the automated detection of single vesicle fusion events. We evaluated the impact of CaMKII on the frequency and amplitudes of the events using a combination of pharmacological agents, shRNA-based knock-downs, and overexpression of dominant negative and positive CaMKII mutants. Our data suggests that CaMKII activity modulates the rate of AMPAR delivery to the plasma membrane, suggesting that CaMKII-dependent synaptic potentiation involves an increase in AMPAR delivery to the plasma membrane.

Motion-free endoscopic system for brain imaging at variable focal depth using liquid crystal lenses

Poster

Arthur Bagramyan, Tigran Galstian, Armen Saghatelyan
University Laval

We present a motion-free system for microendoscopic imaging of biological tissues at variable focal depths. Fixed gradient index and electrically tunable liquid crystal lenses (TLCL) were used to build the imaging optical probe. The design of the TLCL enables polarization-independent relatively low-voltage operation, significantly improving the energy efficiency of the system. A focal shift of approximately 53 ± 2μm could be achieved by electrically controlling the TLCL using the driving frequency at a constant voltage. The potential of the system was demonstrated by imaging neurons and spines in thick adult mouse brain sections and in vivo, in the adult mouse brain at different focal planes. The results confirmed that the developed system may enable 3D imaging of the morpho-functional properties of neural circuitries in freely moving animals and can thus be used to investigate the functioning of these circuitries under normal and pathological conditions.

Application of Stochastic Optical Reconstruction Microscopy (STORM) in brain tissue: considerations for imaging fixed sections

Poster

László Barna, Barna Dudok, István Katona
Momentum Laboratory of Molecular Neurobiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary,

Recent developments in single molecule localization microscopy enable the optical mapping of the distribution of molecules within cells with unprecedented precision. Most of the studies employing the novel super-resolution methods focus on cell cultures. While cell cultures are very efficient model systems for a wide number of questions, the understanding of many biological phenomena require the analysis of anatomically intact tissue, especially in the case of highly complex organs like the mammalian brain. However, acquiring single-molecule images from biological tissue with sufficient signal-to-noise ratio is difficult, due to issues from light diffraction and spherical aberration.

To be able to visualize nanoscale molecular distribution within identified subcellular compartments in fixed brain tissue, we have developed a correlative imaging approach based on consecutive confocal- and STORM imaging on the same microscope setup. In the present study, we describe a tissue processing and immunostaining protocol optimized for STORM, which can yield high precision single-molecule localizations of immunostained endogenous proteins in brain, heart and kidney sections. Furthermore, we present a detailed guide for optimizing several aspects of STORM and dSTORM imaging of tissue samples, such as calibration for astigmatic 3-dimensional STORM, imaging depth, labelling density, and background subtraction.

Development of an intravital multi-plane multiphoton microscopy platform for functional cellular imaging in living mice

Poster

Erik Bélanger, Feng Wang, Sylvain Côté, Daniel Côté, Yves De Koninck
CRIUSMQ, Université Laval

Pain sensation is propagated from the periphery to the central nervous system by dorsal root ganglion (DRG) neurons. These neurons detect different stimuli and convey information to the dorsal horn where they form synapses. The project focuses on the development of an intravital multi-plane multiphoton microscopy platform for functional cellular imaging in living mice, to study calcium dynamics of DRG neurons stimulated peripherally. We image DRG neurons labelled with the Ca2+ indicator GCaMP6s directly through a laminectomy and stimulate these with feedback-controlled thermal and mechanical stimulators applied to the paw. Subsequent Ca2+ responses are identified using a home-made software to find responsive neurons in real-time. The platform we developed allows online adjustments of the digital zoom level, image size, spatial sampling, acquisition speed and to trigger or be triggered by external devices; all essential capabilities for live animals physiological experiments. The sensory-evoked activity from DRG neurons stimulated peripherally is recorded at video-rate, and multi-plane capability is ensured by mounting the objective on a piezoelectric actuator allowing nearly whole-DRG functional microscopy. Because animals' movement can seriously degrade acquisitions, we register the resulting movies offline using graphic card acceleration. Also, we developed an automated pipeline for data processing and visualization in addition to a database to organize and filter experimental results, all of these in the context of Big Data. The system we built is therefore tailored to fit the specific needs of in vivo whole-organ functional microscopy.

Single live-cell biotinylation: laser-assisted membrane functionalization

Poster

Loic Binan, Javier Mazzaferri, Santiago Costantino
Université de montréal

The ability to fluorescently tag individual cells based on an arbitrary criteria allows the study of cells chosen based on specific interactions with their environment. Existing labeling techniques typically rely on biochemical properties that are common to a population of cells and are not specific to the cells of interest. We here propose a method based on Laser Assisted Protein Adsorption by Photobleaching in which fluorescently tagged biotin is photobleached using focused illumination to create a reactive species that binds to the cell membrane. Labeled cells can be arbitrary chosen as groups of cells or as individual cells in a microscopy field. A subsequent incubation with fluorescently tagged streptavidin, a molecule that specifically binds biotin, makes biotinylated cells become fluorescent

Migrating cells can therefore be stained and followed during their movement for several days, or neurons can be studied based on their anatomy and synaptic connections rather than on the proteins they express.

The technique we developed allows to visually identify and target a specific cell, by tethering molecules using a biotin-streptavidin pair. This has several promising applications such as imaging, tracking specific live cells during several days without toxicity, sorting them using flow cytometry, and fabricating cells patterns.

Modulation of sensory processing by epidural optogenetics

Poster

Robert Bonin[a], Mireille Desrochers-Couture[b], Alicja Gasecka[b], Marie-Eve Boulanger[b], Daniel Cote[b], Yves De Koninck[b]
[a]University of Toronto, [b]Université Laval.

Optogenetic tools enable cell-selective and temporally-precise control of neuronal activity; yet, difficulties in delivering light to the spinal cord of freely-behaving animals has hampered spinal optogenetic research. Here, we describe an effective epidural optic fiber designed for chronic spinal optogenetics. We demonstrate the utility of this fiber through the optogenetic control of sensory afferent and dorsal horn interneuron activity to modulate nociception and mechanical sensitization.

Distinct roles for P/Q- and N-type voltage-gated calcium channels in synchronous glutamate release

Poster

Simon Chamberland, Alesya Evstratova, Katalin Tóth
Université Laval

In presynaptic terminals, calcium elevations are shaped by several key parameters, including the properties, density, combination and the spatial location of VGCCs. These features allow presynaptic terminals to translate complex firing frequencies to postsynaptic signals by regulating the amount of neurotransmitter released. For example, the number of vesicles fusing to the membrane can be amplified through mechanisms such as synchronization of multivesicular release or recruitment of additional release sites. While synchronous release relies on both P/Q- and N-type VGCCs at hippocampal MF-CA3 synapses, the contribution of individual types of VGCCs to the mechanisms controlling neurotransmitter release remains unknown.

To dissect the roles of P/Q and N-type VGCCs, we used random-access two-photon calcium imaging and electrophysiology in combination with electron microscopy.

Our results show that calcium influx through P/Q- and N-type VGCCs differently influence glutamate release through specialized calcium dynamics. First, two-photon calcium imaging in giant mossy fiber terminals revealed that P/Q-type VGCCs mediated a larger fraction of calcium elevations than N-type VGCCs for a single action potential. Consistent with calcium imaging data, P/Q-type VGCCs showed a larger contribution to glutamate release than N-type VGCCs. However, this difference was dependent on the external calcium concentration, as decreasing the aCSF calcium concentration to 1.2 mM revealed a similar effect for both toxins. To investigate how calcium entry through N-type VGCCs can mediate a larger fraction of EPSCs in conditions of low release probability without changes in presynaptic calcium entry, we used a coefficient of variation (CV) analysis of single and train of stimuli. While blocking N-type VGCCs decreased the quantal size of EPSCs, blocking P/Q-type VGCCs reduced EPSC amplitude by reducing the number of active release sites. Furthermore, CV analysis revealed that application of ω-Agatoxin IVA or EGTA-AM had similar effects on short-term facilitation by eliminating the recruitment of additional release sites.

Altogether, our results demonstrate the highly specialized roles of P/Q- and N-type VGCCs in neurotransmitter release. While N-type VGCCs are tightly coupled to calcium sensors and provide local calcium elevations, P/Q-type VGCCs are strategically involved to support global calcium elevations and recruit additional release sites during trains of activity.

Ultrafast measurement of membrane potential in multiple neuronal compartments using a second-generation genetically encoded fluorescent voltage indicator

Poster

Simon Chamberland[a], François St-Pierre[b], Michael Z Lin[b], Katalin Tóth[a]

[a]Quebec Mental Health Institute, Department of Psychiatry and Neuroscience, Laval University, Quebec, Canada, [b]Department of Bioengineering, Stanford University, Stanford, California, USA,

Patch-clamp recordings are the gold standard for measurements of electrical physiological activity. In neurons, whole-cell recordings provide more than necessary signal to noise ratio and sampling rate to measure signals across the full regime of activity experienced by the cell. However, whole-cell patch-clamp recordings are less amenable to smaller neuronal compartments. These are physically hard to access, limited in number of recording sites and can rapidly deteriorate due to wash-out. Therefore, an optical method allowing multisite subcellular measurement of voltage in thick preparations would be desirable.

To monitor voltage in multiple neuronal compartments, we combined random-access two-photon microscopy with the second-generation Accelerated Sensor of Action Potentials (ASAP2). ASAP2 was expressed in organotypic hippocampal slices by bulk electroporation. For measurements in acute (300 µm thick) hippocampal slices, ASAP2 was virally-delivered to neurons by stereotaxic injections 1 to 2 weeks before experiments. Whole-cell patch-clamp recordings were obtained from ASAP2-expressing neurons. Two-photon excitation was performed at 900 nm.

First, ASAP2 could report action potentials (APs) under two-photon excitation with a $\Delta F/F$ of 14.1 ± 0.6% (n = 16 neurons). For a single trial at a scanning frequency of 925 Hz, ASAP2 reported APs with a signal to noise ratio twice larger than ASAP1 (ASAP2: 7.9 ± 0.5, n = 14; ASAP1: 3.5 ± 0.2, n = 0.2, $P < 0.001$). Furthermore, single-voxel detection of action potentials in single trial with ASAP2 was possible in almost all neurons tested (n = 15/16 neurons). Using two-photon excitation of ASAP2, APs could be detected at the cell body, in dendrites, dendritic spines, axons and putative axonal terminals. Finally, simultaneous multisite recording (28 to 41 voxels) at high-frequency (452 to 662 Hz) in dendrites could track the strong attenuation of backpropagating APs with distance from the soma (n = 7). Furthermore, ultrafast (3 700 Hz) scanning revealed that the delayed initiation and peak of action potential compared to the soma could be resolved in distal dendrites.

Together, these results demonstrate an optical method allowing ultrafast simultaneous recording of electrical activity at multiple sites. In addition, this method allows measurement of electrical activity in small subcellular compartments in thick preparations.

frontiersneurophotonics.org

Fluorescence correlation spectroscopy and its utilization in the analysis of NFAT synapse-to-nucleus signaling

Poster

Kevin C. Crosby, Mark L. Dell'Acqua
Dept. of Pharmacology, University of Colorado School of Medicine

Long-term memory and learning have been shown to have a dependence upon gene-transcription triggered by synaptic activity. One of the transcription factors that may be involved in this process is the nuclear factor of activated T-cells (NFAT). Translocation of NFAT to the nucleus is mediated by the Ca2+-dependent phosphatase calcineurin (CaN), which binds to NFAT and dephosphorylates multiple residues on this transcription factor, resulting in the exposure of its nuclear localization sequence and subsequent translocation to the nucleus. Ca2+ influx through voltage-gated L-type calcium channels (L-VGCCs) plays a required and exclusive role in initiating this cascade. This is facilitated by the A-kinase anchoring protein (AKAP) 79/150, which serves to recruit both CaN and the regulatory protein kinase A (PKA) to the channel. Preliminary evidence from our group suggests that NFAT is also tethered to this assembly via an association with the AKAP. This scenario suggests a model of excitation-transcription coupling wherein NFAT signaling is initiated by local synaptic activity and the actuated transcription factor is then conveyed to the nucleus via physical transport over long intracellular distances. However, the molecular details and mechanisms of this process have yet to be elucidated.

One approach we are utilizing to study this phenomena is fluorescence correlation spectroscopy (FCS). FCS is a technique that has the capability of discerning quantitative details on the characteristics of molecular mobility in living cells by measuring intensity fluctuations produced by fluorescence molecules (or fluorescently-tagged molecules) diffusing through a confocally confined detection volume. However, in order to fully exploit the quantitative potential of FCS, certain considerations have to be made for its application in neurons. These include accounting for dendritic morphology and the photophysical behavior of fluorescence proteins. Here we will present data from our efforts along with a discussion of the challenges and prospects of FCS studies in neuronal cell biology.

Optical Guidance for Deep Brain Stimulation Electrode Implantation

Poster

Damon DePaoli[a], Laurent Goetz[a], Martin Parent[a], Leo Cantin[b], Michel Prudhomme[c], Tigran Galstian[c], Younes Messadeq[c], Daniel Côté[a]

[a]CRIUSMQ, [b]Hôpital de l'Enfant-Jésus, Hôpital de l'Enfant-Jésus, [c]COPL.

Deep Brain Stimulation (DBS) surgery is a very effective way to treat the motor symptoms of Parkinson's disease after standard medications, such as Levodopa, no longer have a positive effect. The surgery's effectiveness relies on the placing of a stimulating electrode inside the brain with its tip placed very precisely within a millimeter-sized region called subthalamic nucleus (STN). Currently there is no onboard guidance for the stimulating electrode during the surgery, with only an x-ray projection as a means for navigation after the STN is located. Onboard guidance would lead to decreased surgery time, and increased accuracy.

With this in mind, we have designed a cheap, optical fiber-based device that is small enough to be placed within commercially available DBS stimulating electrodes' hollow cores and is capable of sensing biological information from the surrounding tissue using diffuse reflectance spectroscopy. Using our device, we have shown the ability to sense the difference between white and grey matter both in vitro, in human and primate brain tissue, as well as in vivo, in rats. With the current probe, we can already theoretically provide increased accuracy and efficiency during neurosurgery; however, there is a high ceiling for increased utility using optical enhancements. We are currently designing probes with micro-optical components that can drastically increase the axial resolution of our device and thus, will give it the ability to identify even smaller structures within the brain, with increased precision.

Network inference from experimental data

Poster

Patrick Desrosiers, Simon Labrecque, Maxime Tremblay, Mathieu Bélanger, Bertrand de Dorlodot, Daniel Côté
Centre de recherche de l'Institut universitaire en santé mentale de Québec

Functional connectivity maps of neuronal networks are critical tools to understand how neurons interact to form circuits, how information is encoded and processed by neurons, how memory is shaped and how these basic processes are altered under pathological conditions. Current light microscopy allows to record calcium or electrical activity of thousands of neurons simultaneously, yet assessing accurate and comprehensive connectivity maps directly from such data remains a non-trivial analytical task. There exist simple statistical methods, such as cross-correlations and Granger causality, but they only detect linear interactions between neurons. Other more involved inference methods inspired by information theory, such as mutual information and transfer entropy, identify more accurately connections between neurons but that also require more computational ressources.

We carried out a comparative study of several connectivity inference methods used in neuroscience. In order to determine the relative accuracy and computational cost of each inference method, we used simulated fluorescence traces generated with realistic computational models of interacting neurons in networks of different types (clustered and non-clustered topologies) and sizes (from 10 to 1000 neurons). Also to bridge computational and experimental work, we recorded intracellular calcium activity of live hippocampal neuronal cultures that were infected with GCaMP6f a fluorescent calcium marker. The spontaneous activity of the network was recorded from 20 to 50 Hz consisting of 50-100 neurons per field of view on a microscope controlled by a homemade software. We implemented all connectivity inference methods in the software that rapidly loads a calcium fluorescences movie, segments the images, extracts the fluorescence traces, and assesses the functional connection strength and direction between each pair of neurons. We used this software to assess, in real time, the functional connectivity from real calcium imaging data in basal conditions, under plasticity protocols and epileptic conditions.

Nanoscale molecular organization of cannabinoid signalling at GABAergic synapses revealed by cell type-specific STORM

Poster

Barna Dudok[a], László Barna[a], Szilárd I. Szabó[a], Eszter Szabadits[a], Balázs Pintér[a], Stephen G. Woodhams[a], Christopher M. Henstridge[a], Gyula Y. Balla[a], Rita Nyilas[a], Csaba Varga[a], Sang-Hun Lee[a], Máté Matolcsi[b], Judit Cervenak[c], Imre Kacskovics[c], Masahiko Watanabe[d], Miriam Melis[e], Marco Pistis[e], Ivan Soltesz[a], István Katona[a]

[a]Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary, [b]Alfred Renyi Institute of Mathematics, Hungarian Academy of Sciences, Budapest, Hungary, [c]ImmunoGenes Kft, Budakeszi, Hungary, [d]Department of Anatomy, Hokkaido University School of Medicine, Sapporo, Japan, [e]Department of Biomedical Sciences, University of Cagliari, Cittadella Universitaria di Monserrato, Italy.

Understanding synaptic function requires the integrated analysis of molecular, anatomical, and the arising electrophysiological properties of neuronal signaling, however, such efforts are hindered by the lack of appropriate methods. Single-molecule localization microscopy (SMLM) is a family of superresolution imaging approaches that allow the precise nanoscale characterization of molecular distribution. A major challenge for SMLM methods is to position the resulting data in the context of the anatomically complex neuronal tissue. To overcome this limitation, we have developed a method, based on the combination of patch-clamp recording, single-cell labeling, immunostaining, confocal imaging and superresolution Stochastic Optical Reconstruction Microscopy (STORM), which reveals the nanoscale localization of immunolabeled molecules in axon terminals of identified, electrophysiologically and anatomically characterized neurons.

The CB1 cannabinoid receptors are some of the most important regulators of presynaptic neurotransmitter release in the CNS. Yet, the organizing principles underlying the precise subcellular targeting of the receptors are poorly understood. In this study, we have used correlated confocal and STORM microscopy, to study how the localization and amount of CB1 cannabinoid receptors on given axon terminal types determine the efficacy of cannabinoid signalling. We have compared the axon terminals of two major subtypes of hippocampal CB1-expressing GABAergic interneurons exhibiting different sensitivity to cannabinoids. Using our correlative imaging approach, we have found that the nanoscale distribution of CB1 was uniform, and the density of CB1 was similar over the extrasynaptic plasma membrane in both cell types. In contrast, bassoon, an integral component of the presynaptic release machinery, was organized into clusters, and exhibited different degree of fragmentation between cell types. The resulting relative arrangement of CB1 and bassoon led to an estimated twofold higher nanodomain receptor-effector ratio at the synapses of perisomatically targeting

interneurons, which may underlie their increased sensitivity to cannabinoids. Furthermore, chronic Δ9-tetrahydrocannabinol treatment, which is known to reduce cannabinoid efficacy on GABA release, resulted in a robust decrease in surface CB1 levels at perisomatic synapses. Together, these findings suggest that the number of available CB1 receptors in the vicinity of release sites controls the efficacy of cannabinoid inhibition of neurotransmitter release.

Our results highlight that the correlated imaging and analysis with conventional microscopy and SMLM can be utilized to link molecular distribution data (with resolution previously exclusive to electron microscopy) to the electrophysiological and anatomical parameters of the same neuron and synapse.

Development of adaptive intelligent methods for bio-imaging techniques

Poster

Audrey Durand, Flavie Lavoie-Cardinal, Christian Gagné, Paul De Koninck
Université Laval

Recent advances in the resolution of optical microscopy are contributing to our increased understanding of cellular function, plasticity, and dysfunction. When using techniques such as super-resolution live-cell microscopy, the need for parameter optimization is critical in order to achieve high precision in the localization of structures of interest. Furthermore, the temporal and biological variability calls for distinct sets of optimally determined imaging parameters and for the capacity to adapt during the measurements. Thus, we aim to develop adaptive intelligent methods for super-resolution imaging. We present a simulation platform that produces realistic STED and confocal images based on the microscope, fluorescence and imaging parameters. By calculating the photon dose on the sample, it can simulate the bleaching effect of changing imaging parameters. The simulation platform will assist in the development of parameter optimization schemes for coordinate targeted super-resolution methods such as RESOLFT and STED nanoscopy. Those parameters include depletion intensity, scanning speed and scanning precision prior to measurements.

Optimization on typical imaging routines using simulated images was performed by combining computer vision with machine learning algorithms. Acquisition time of a STED image was reduced without decreasing the spatial resolution on simulated microtubule filaments by using solely the information contained in its corresponding confocal image. Moreover, the simulations show an increase in imaging efficiency and an improvement in the signal to noise ratio without increasing the photon dose on the imaged structures. Therefore for a given resolution, a higher number of scanning steps would be possible with reduced photobleaching effect during a live-cell super-resolution imaging routine.The effect of optimized parameters on photostability and number of possible scanned frames was compared. For example, applying a foreground extraction algorithm reduced the imaging time for simulated microtubules images by a factor of 63% and simultaneously reduced photobleaching by a factor of 25%. Combining the foreground extraction method with machine learning algorithms lead to better adaptation to structure variability and dynamic changes during live-cell imaging. The results from our simulations will next be implemented on a super-resolution microscope as an adaptive intelligent method for bio-imaging.

Target-specific neuromodulation of descending prefrontal cortical inputs to the dorsal raphe nucleus by cannabinoids

Poster

Sean Geddes[a], Saleha Assadzada[a], David Lemelin[a], Alexandra Sokolovski[a], Richard Bergeron[a], Samir Haj-Dahmane[b], Jean-Claude Béïque[a]
a- University of Ottawa, b- University at Buffalo.

The serotonin (5-HT) system has long been implicated in mood regulation. The coding features of 5-HT neurons per se are however complex and multifaceted and it has historically been difficult to capture them in a simple and unifying framework. At least part of this complexity likely arises from the sole nature of the synaptic network in which 5-HT neurons are embedded: indeed the dorsal raphe nucleus (DRN), where the majority of 5-HT neurons reside, receives strong innervation from a vast array of subcortical and cortical regions. It has further been historically difficult to study in isolation these descending inputs in order to identify with precision how they modulate the excitability of the DRN subnetwork. For instance, the medial prefrontal cortex (mPFC) send long-range axons to the DRN but the basic processing features of this input to the DRN is still elusive and somewhat controversial. The details of this top-down control from the mPFC to the DRN are of particular interest, in part because of its role in stress processing and in mediating antidepressant-like effects. Here, using a combination of immunohistochemistry, optogenetics and electrophysiological whole-cell recordings we dissect out the functional properties of the mPFC-DRN projections. We found that the mPFC inputs to the DRN: 1) are glutamatergic; 2) mono-synaptically activate both 5-HT neurons and local GABA neurons located primarily in the lateral wings of the DRN; 3) permissive to strong feedforward inhibition; 4) are modulated by endocannabinoids. We further identify a target-specificity in the CB1R-mediated neuromodulation of mPFC inputs to the DRN that results in a powerful gating of PFC information flow in the DRN by favoring the direct excitatory drive to 5-HT neurons at the expense of the feedforward inhibition. The precise elucidation of how information flow is dynamically regulated by neuromodulators in this mood-related network may lead to the development of informed pharmacological strategies for the treatment of affective disorders.

Unraveling live brain tissue nano-structure using single fluorescent carbon nanotubes

Poster

Antoine G. Godin, Juan Varela, Zhenghong Gao, Brahim Lounis, Laurent Groc, Laurent Cognet
Université de Bordeaux

The complex biological network composing a functional organ, like the brain, is a highly dynamic structure largely driven by active processes. The extracellular space (ECS) occupies an essential part of the network, but its role in modulating organ functions has been mostly unexplored. This is a direct consequence of the lack of adequate approaches capable of providing quantitative information in intact tissue.

To investigate the complex dynamic ECS (re)organization and its impact on brain functions, we developed innovative optical nano-imaging methods based on single carbon nanotubes (SWNTs) tracking. SWNTs have intriguing intrinsic fluorescence properties which make them promising probes for biological studies. They can be more than few microns long with extremely small diameters (min).

Here we demonstrate that individual SWNTs can be imaged using fluorescence microscopy and tracked in deep live brain tissue (up to 60 µm deep). Long trajectories (>10 min) of single SWNT diffusing in the ECS were recorded and, by analysis their movements along and perpendicular to their principal axis, we could unravel live brain structures ranging from micrometers down to ~80 nm. Finally, significant increase in the SWNTs diffusion was found upon biochemical modulations of the brain extracellular matrix.

Heterogeneity of intracellular pH in dendritic spines; potential impact on synaptic signalling and plasticity

Poster

Tushare Jinadasa
Laval University

Neuronal plasticity is believed to involve activity-dependent changes of proteins at the synapse. Calcium entry and downstream signalling are critical for the induction of plasticity yet other ions such as protons, transiently increase and have the potential to modify the structure and function of the synapse. It has previously been proposed that microdomains of pH fluctuation, induced by membrane depolarization and calcium entry, could influence neuronal function. At the synapse, voltage gated channels as well as CaMKII, which is critically involved in spine potentiation, are known to be pH sensitive. Furthermore, pH has long been known to influence intracellular trafficking and signalling such as the Erk signalling pathway. The influences protons have on synapses have been principally examined extracellularly, while less attention has been given to proton fluctuations intracellularly. We are testing the hypothesis that changes in intracellular pH modulate plasticity, by combining optical imaging of pH and Ca2+ fluctuations simultaneously in cultured rat hippocampal neurons.

With the use of a genetically-encoded ratiometric pH indicator (pHred), we have estimated the intracellular pH in various compartments of the neurons. Surprisingly, spines demonstrate a significantly more alkaline environment compared to dendrites. To further validate this observation, we repeated these measurements with a different fluorescent pH reporter (pHluroin2). We also used another method to quantify the pH, fluorescence lifetime imaging, since the fluorescence lifetime of pHred is also sensitive to pH. These experiments confirmed that several spines exhibit higher pH than their parent dendrite. This heterogeneity between spines might contribute to differential modulation of synaptic proteins. We are interested in understanding the impact of this heterogeneity in spine pH on synaptic plasticity.

We have observed that chemical stimulation used to induce long-term potentiation in cultured hippocampal neurons (cLTP) results in an acidification of the soma, dendrites and spines. In our experiments recovery from this acidification is primarily dependent on sodium transport but is also influenced by bicarbonate-dependant transport. In order to determine how pH fluctuation in spines can influence synaptic function we are combining optical, genetic and pharmacological approaches. We found that disruption of the acidification associated with synaptic stimulation results in a reduction of CaMKII clustering. These data suggest a regulatory role for protons on synaptic signalling and remodelling in dendritic spines.

Hyperspectral imaging to monitor simultaneously multiple proteins subtypes and track their spatial dynamics on live neurons

Poster

Simon Labrecque[a], Jean-Philippe Sylvestre[b],Stephane Marcet [b], Francesca Mangiarini[b], Marc Verhaegen[b], Sébastien Blais-Ouellette[b], Paul De Koninck[a]

[a]CRIUSMQ, Universite Laval, [b]Photon etc

The efficacy of existing therapies and the discovery of innovative treatments for Central Nervous System (CNS) diseases have been limited by the lack of appropriate methods to investigate complex molecular processes at the synaptic level. In order to better understand the fundamental mechanisms that regulate diseases of the CNS, we designed a fast fluorescence hyperspectral imaging platform to track simultaneously various neurotransmitter receptors trafficking in and out of synapses. This new imaging platform allows fast simultaneous acquisitions of at least five fluorescent markers in living neurons with a high spatial resolution. We used quantum dots of different emission wavelengths and we then tracked single receptors on the membrane of living neurons and access their mobility under normal and ischemic conditions. With this hyperspectral imaging platform, it was possible to image simultaneously five different synaptic proteins, including subtypes of glutamate receptors (mGluR, NMDAR, AMPAR), postsynaptic density proteins, and signaling proteins. This technique provides an effective method to observe several synaptic proteins at the same time, thus study how drugs for CNS impact the spatial dynamics of these proteins.

Roles of βCaMKII in synaptic trapping of AMPA receptors

Poster

Simon Labrecque, Christian Tardif, Paul De Koninck
CRIUSMQ, Universite Laval

The α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) trafficking at synapses is a key molecular element in learning and memory. Previous work using single particle tracking of AMPARs have shown that they are highly mobile in the synaptic membrane and can exchange between synaptic and extrasynaptic domains by lateral diffusion. Anchoring of AMPARs in synapses is dependent on presynaptic activity and on post-synaptic αCaMKII activation. A current model for long-term potentiation is the enhanced recruitment of AMPARs at synapses, following strong synaptic activity, through the trapping of AMPARs. This would be mediated by NMDAR-dependent activation of αCaMKII which in turn accumulates into spines and phosphorylates stargazin. This molecular cascade would thus play a critical role in LTP induction. The involvement of the α subunit of CaMKII has been recognized for a long time; less is known about the mechanistic involvement of theβ subunit in LTP, despite its high abundance in the brain.

Here, using quantum dots tracking on synaptic AMPAR and methods for gene replacement by combining shRNA and overexpression of CaMKII subunits, we show that the decrease in αCaMKII or βCaMKII reduces the trapping of AMPAR at synapses. Moreover, after cLTP protocols in cells knocked down for βCaMKII, we observed an unexpected increase in AMPAR synaptic diffusion. These results might be the consequences of changes in the trafficking of CaMKII. To study this we used spt-PALM approach to characterizeαCaMKII-mEos2 intracellular diffusion and thus evaluate the impact of βCaMKII on αCaMKII trafficking. To do this, we built a homemade TIRF microscope that stabilizes the sample in 3D with a nanometer range precision using image correlation without the uses of fiduciary markers or additional lasers. We monitored the immobilization of αCaMKII-mEos2 under cLTP protocols inside spines and dendrites and we show that this immobilization is compromised when βCaMKII is knocked down. These studies suggest a critical role for the βCaMKII in synaptic plasticity in different levels of synaptic signaling.

Optimal illumination and collection with micro-optical fiber-based devices

Poster

Nicolas Lapointe, Damon Depaoli, Rob Bonin, Steve Begin, Joël Crepeau, Yves De Koninck, Daniel Côté
CRIUSMQ

Deep brain stimulation's effectiveness relies on the ability of the stimulating electrode to be properly placed within a small target area of the brain. Optical guidance techniques that can increase the accuracy of the procedure, without causing any additional harm, are therefore of great interest.

We have designed a cheap optical fiber-based device that is small enough to be placed within commercially available DBS stimulating electrodes' hollow cores and that is capable of sensing biological information from the surrounding tissue, using low power white light. With this probe we have shown the ability to distinguish white and grey matter as well as blood vessels, in vitro, in human brain samples and in vivo, in rats. We have also repeated the in vitro procedure with the probe inserted in a DBS stimulating electrode and found the same results.

We are currently designing a second state-of-the-art fibre optic device with precise micro-optical components that will result in label free, molecular level sensing capabilities, using coherent anti-Stokes Raman scattering. The end objective will be to use this data in real time, during DBS neurosurgery, to increase the safety and accuracy of the procedure.

NMDA receptor-dependent intracellular calcium release instructs spatially-clustered synapse development

Poster

Kevin F.H. Lee, Cary Soares, Jean-Philippe Thivierge, Jean-Claude Béïque
University of Ottawa

Neurons undergo robust dendritic growth and synapse formation during early postnatal development, marking a key period in neural circuit assembly. Despite the eminent role of calcium in synapse regulation, remarkably little is known about calcium dynamics during synaptogenesis. Using whole-cell electrophysiology, two-photon calcium imaging and glutamate uncaging in rodent hippocampal slices, we found that synaptic NMDA receptor activation triggered intracellular calcium release at CA1 pyramidal cell dendrites during a narrow developmental epoch, driving local propagation of calcium signals to nearby spines. This functional coupling of NMDARs to calcium release machinery enabled dendrites to biochemically encode spatiotemporal features of synaptic input, and could thus serve to locally regulate the activity-dependent development of co-active ensembles of neighboring synapses. Consistent with this hypothesis, we describe a novel local cooperative plasticity rule that requires ryanodine receptor activation and, in addition, we provide evidence for spatially-clustered synapse development. These results reveal striking developmental features of NMDAR-dependent calcium dynamics that are suited to instruct the patterning of synaptic connectivity to support non-linear dendritic computation.

Postsynaptic GABA(A) receptor subtype switch in the dorsal horn of neuropathic animals

Poster

Louis-Etienne Lorenzo[a], Antoine Godin[b], Karine Bachand[a], Alfredo Ribeiro-da-Silva[c], Yves de Koninck[a]

[a]Institut Universitaire de Santé Mentale de Québec, [b]Université de Bordeaux , [c]McGill University.

A few decades ago, it had clearly been shown that intrathecal injections of GABAA receptor (GABAAR) or glycine receptor (GlyR) antagonists could spontaneously generate neuropathic pain symptoms. While both GABAAR and GlyR play a role in the control of dorsal horn neuron excitability, their relative contribution to inhibition of primary afferent terminals remains controversial. To address this, we designed an approach for quantitative analyses of the distribution of GABAAR-subunits, GlyRα1-subunit and their anchoring protein, gephyrin on terminals of rat spinal sensory afferents. The approach was conceived for confocal microscopy, and an algorithm was designed to recognize structures with dimensions similar to those of the microscope resolution. To avoid detecting false colocalization, the latter was considered significant only if the degree of pixel overlap exceeded that expected from randomly overlapping pixels given a hypergeometric distribution. We found that primary afferents were devoid of GlyR, gephyrin and α1GABAAR whereas the α2/α3/α5 and β3 GABAAR were significantly expressed in primary terminal, as other GABAAR-associated-proteins. TEM and RT-qPCR confirmed these data. These results indicate that dorsal horn inhibitory synapses follow different rules of organization at pre vs. postsynaptic sites and that the absence of gephyrin clusters from primary afferents suggests a more diffuse mode of GABAA-mediated transmission at pre- than at postsynaptic sites. Since the intrathecal pharmacological blockade of the spinal synaptic inhibition had consequences at the periphery, a large number of studies have reversely focused on the consequences of Peripheral Nerve Injury (PNI) pain models on the spinal inhibitory transmission. According to the studies and the techniques used, results have been so far highly controversial, but as we have shown here that gephyrin was restricted to the dorsal horn neurons, we used this inhibitory postsynaptic marker as a tool to generate a binary-mask to study variations in GABAAR subtype intensities at postsynaptic sites in PNI: we quantified a clear reduction in the number of inhibitory pre- and postsynaptic contacts of the dorsal horn. Paradoxically, at the remaining inhibitory synapses, we measured an over-expression of specific GABAARs but not of the α1-GlyR. In nerve induced allodynia, we conclude that the GABAergic analgesic effect would essentially be postsynaptically mediated and GABAAR subtype specific.

Superresolution in confocal microscopy using beam shaping

Poster

Louis Thibon, Harold Dehez, Louis-Étienne Lorenzo, Yves De Koninck, Michel Piché
Université Laval

Laser scanning microscopy like confocal microscopy is limited in lateral resolution by the diffraction of light. This resolution limit is for the classically used Gaussian beam roughly half the wavelength of the light, which gives a minimum of 200 nm if we stay in the visible spectrum.

Superresolution techniques have been developed since the 90s to overcome this limitation set by the diffraction of light. In this ocean of techniques we can cite the well known STED, PALM and STORM that give very high resolution images (that can go down to 10 nm). But this always comes at the expense of great complexity in the setup (high power lasers, very long acquisition, specific fluorophores) and limitation of the observable samples. Other techniques like structured illumination give more modest improvement in the resolution but with a reduced complexity and fewer limitations on the applications of the techniques.

We propose a method to improve the resolution of confocal microscopy using different laser modes and deconvolution. Two images of the same field with different laser modes are acquired with the confocal microscope and are given to the decovolution algorithm. The two laser modes used are the classic vertically polarized Gaussian mode (TEM00) and an azimutally polarized donut mode (TE01).The two laser modes have different Point Spread Function and thus give complementary information to the deconvolution algorithm. By changing the laser modes, and doing so the PSF, we are able to improve the efficiency of the deconvolution algorithm. We obtain images with a better resolution than with a simple deconvolution of the confocal image with the same algorithm. We also show that with more complex laser modes containing smaller structures, like the Hermie Gaussian modes, the improvement is even better.

The proposed technique is based on laser scanning microscopy and requires only a few add-ons to a classic confocal microsope (to be able to change the laser mode).

VividSTORM: a new software for the correlated visualization and analysis of pixel intensity-based and localization microscopy data

Poster

Vivien Miczán[a], Barna Dudok[a], László Barna[a], András Horváth[b], István Katona[a]
[a]Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary, [b]Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, Budapest, Hungary.

Determining the nanoscale targeting and distribution of proteins within cells or cellular compartments is necessary to delineate the molecular mechanisms underlying physiological and pathophysiological processes of the nervous system. Single-Molecule Localization Microscopy (SMLM) offers a flexible and efficient way to obtain precise molecule localization data. However, its capability to visualize the respective cellular and subcellular context of molecular information is rather limited. To circumvent this obstacle, we recently developed a new approach, which combines SMLM with confocal microscopy for cell-type- and subcellular-compartment-specific nanoscale molecular imaging, and thereby facilitates functional interpretation of quantitative molecular observations. The correlated use of these two imaging modalities also require adequate software tools for the simultaneous visualization and handling of the different image types, e.g. for the filtering of respective SMLM coordinates belonging to the selected cell and subcellular compartment on the confocal image. Although the localization-based data are amenable for a wide variety of analysis approaches that are impossible to perform on conventional images, but to date, these features have not been included in available software packages. Therefore, we developed VividSTORM, a unique tool for the correlated visualization and analysis of pixel intensity-based and localization microscopy data. By using VividSTORM, the custom subpopulations of SMLM localization points can be selected based on the cellular and subcellular environment with manual selection or by an automatic image segmentation method called Morphological Active Contours Without Edges (MACWE). As the next step, one can rapidly measure molecular abundance, clustering, internalization, surface density, and inter-molecular distances on the selected localization points. The software is freely accessible with an easy-to-handle graphical user interface and the source code written in Python is also available for custom modifications. To demonstrate its usefulness, we employed VividSTORM and determined the nanoscale localization and abundance of molecular components of the endocannabinoid system at subcellular domains of specific cell types.

Simultaneous high-speed imaging and optogenetic inhibition in the intact mouse brain

Poster

Claudio Moretti[a], Serena Bovetti[a], Stefano Zucca[a], Marco Dal Maschio[a], Paolo Bonifazi[b], Tommaso Fellin[a]

[a]Istituto Italiano di Tecnologia, [b]School of Physics and Astronomy, Tel Aviv University.

Genetically encoded calcium indicators and optogenetic actuators can report and manipulate the activity of specific neuronal populations. However, applying imaging and optogenetics simultaneously has been difficult to establish in the mammalian brain, even though combining the techniques would provide a powerful approach to reveal functional organization of neural circuits. Here, we developed a technique based on patterned two-photon illumination to allow fast scanless imaging of GCaMP6 signals in the intact mouse brain at the same time as single-photon optogenetic inhibition of Archaerhodopsin. Using combined imaging and electrophysiological recording, we demonstrate that single and short bursts of action potentials in pyramidal neurons can be detected with millisecond precision and improved signal-to-noise ratio in the scanless configuration compared to the raster scanning approach. Moreover, we demonstrate that our system removes the artifacts in the fluorescence detection that are induced by single-photon optogenetic illumination and that prevent simultaneous imaging and optogenetic manipulation in laser scanning microscopes. Finally, we apply our technique to study the role of parvalbumin-positive (PV) interneurons in the control of spontaneous cortical dynamics. We find that optogenetic inhibition of PV-positive cells significantly affects ongoing sparse activity of GCaMP6-expressing layer II/III neurons, resulting in increased correlation among calcium signals in the layer II/III network. By allowing the readout of the effect of the optogenetic manipulation on neuronal network with unprecedented spatial and temporal resolution in vivo, simultaneous high-speed imaging and optogenetics can dramatically advance our knowledge of the functional organization of mammalian neural circuits.

Optical Imaging of miniature synaptic Ca2+-transients to monitor synaptic potentiation

Poster

Gabriel Nadeau, Theresa Wiesner, Mado Lemieux, Paul De Koninck
Laval University

Classical measurements of synaptic plasticity have involved electrophysiological methods which provide high sensitivity for detecting small changes in synaptic strength. However, this approach does not provide much information about the location of the synapses that undergo plastic changes. Because synaptic plasticity can be synapse-specific, having the ability to monitor changes in synaptic strength at individual synapses is important in order to enable simultaneously monitoring of local molecular mechanisms associated with the plasticity. New fluorescent tools developed in the last decades allow to directly visualize synaptic activity, signaling, and remodeling at individual synapses. In this study, we are using optical imaging of a genetically-encoded Ca2+ sensor, GCaMP6f, to record miniature synaptic Ca2+-transients (MSCTs) in cultured rat hippocampal neurons. For these experiments, we perform video-microscopy on neurons perfused with external solution lacking Mg2+ and containing Tetrodotoxin (TTX). We have observed highly localized and transient increases of intracellular Ca2+ in dendritic compartments and spines. To test whether these MSCTs can be potentiated, we have measured them before and after a 5 min stimulation known to induce plasticity in cultured neurons (0Mg2+/Glycine, cLTP). A lasting increase in the frequency and amplitude of MSCTs, for at least an hour, arose from this stimulation protocol. We are thus investigating the molecular mechanisms of this plasticity. The MSCTs are mostly mediated by NMDA receptors, since they are almost totally blocked by the selective antagonist to the receptor, AP5. Moreover, addition of AP5 only during the cLTP stimulation blocks the MSCT plasticity. It thus appears that both the MSCTs and their plasticity are NMDA receptor-dependent. Interestingly, the MSCTs and their plasticity are not blocked by the AMPA receptor antagonists NBQX, pointing to possible changes in NMDA receptor content, post-synaptic Ca2+ signaling, or neurotransmitter release. To test theses hypotheses, we are combining Ca2+ imaging with imaging of other pre and postsynaptic components, and various pharmacological treatments to identify the molecular mechanisms responsible for the MSCT plasticity. Our approach might provide new knowledge on the diversity of molecular processes that support synaptic potentiation.

Regulation of doublecortin during neuronal polarization

Poster

Cecilia Rocha, Gary Brouhard
McGill University

Microtubules are essential components of the cytoskeleton that play a crucial role in the establishment of neuronal polarization. Doublecortin (DCX) is a microtubule-associated protein that is essential for neuronal migration and differentiation during brain development, and promotes tubulin polymerization and stabilization in neurons. DCX is enriched in the growth cones of elongating neurons and Mutations in the DCX gene cause a human cerebral cortical malformation known as Lissencephaly that shows abnormal cortical lamination as a result of a breakdown in neuronal migration. Our group demonstrated that DCX binds to polymerization intermediates at growing microtubule ends and observed that patient mutations impair microtubule binding and assembly. DCX activity and regulation is not fully understood. DCX is likely to be regulated by subcellular localization, interaction with regulatory proteins, and phosphorylation. The goal of our project is to determine how microtubules control neuronal morphology. We have examined the expression pattern and localization of DCX during neuronal polarization by immunofluorescence. We use super-resolution microscopy to study dense microtubules networks in the growth cones and the leading processes of developing cortical neurons at different stages of development. The study of DCX regulation and distribution will provide novel insights into neuronal development and non-centrosomal microtubule nucleation.

Homeostatic influence on Hebbian plasticity rules at central synapses

Poster

Cary Soares, Jean-Claude Béïque
University of Ottawa

Alterations at several levels of circuit architecture are thought to contribute to information storage in the brain. Such malleability of neural circuits in part relies on the ability of synapses to express rapid structural and functional modifications in response to incoming activity through mechanisms collectively referred to as Hebbian synaptic plasticity (i.e., LTP and LTD). Homeostatic mechanisms, acting on a slower timescale, operate in concert with Hebbian mechanisms to regulate synaptic strength and are believed to contribute to network stability. Interestingly, homeostatic and Hebbian synaptic plasticity act through at least partially overlapping mechanisms, as both have been shown to regulate the function of excitatory glutamate receptors of the AMPA and NMDA subtypes. Thus, homeostatic adaptions are well poised to alter Hebbian plasticity rules, although this possibility has received little direct attention. Using whole-cell electrophysiology, two-photon imaging and glutamate uncaging, we investigated whether and how homeostatic plasticity influences Hebbian plasticity rules at CA1 synapses. We show that prolonged network silencing, a homeostatic plasticity paradigm, prevented the expression of LTP induced by either a pairing or an STDP protocol, suggesting a potential occlusion of postsynaptic AMPAR incorporation. Contrary to this prediction, however, we found that both spine structural enlargement and incorporation of surface GluA1-containing AMPARs in dendritic spines induced by two-photon glutamate uncaging, two hallmarks of postsynaptic LTP expression, were unaltered by this homeostatic paradigm. Rather, we found that network silencing drastically altered presynaptic release dynamics in ways to profoundly alter the induction of plasticity. Collectively, these results begin to elucidate the mechanistic intricacies by which Hebbian plasticity rules are regulated by perpetually adapting neural networks.

Quantitative high-resolution imaging reveals Src and cortactin function in neuronal growth cone motility

Poster

Yingpei He, Yuan Ren, Bingbing Wu, Boris Decourt, Aih Cheun Lee, Aaron Taylor, Daniel M. Suter
Purdue University

Src tyrosine kinases have been implicated in axonal growth and guidance; however, the underlying cellular mechanisms are not well understood. Specifically, it is unclear which aspects of actin organization and dynamics are regulated by Src in neuronal growth cones. Here, we used a combination of various high-resolution imaging techniques to investigate the function of Src2 and one of its substrates, cortactin, in lamellipodia and filopodia of Aplysia growth cones. We used stochastic optical reconstruction microscopy (STORM) to study the localization of Src2, cortactin, and Arp3. Furthermore, differential interference contrast (DIC) time-lapse imaging and fluorescent speckle microscopy (FSM) was employed to quantify protrusion and actin dynamics, respectively. Lastly, we used scanning electron microscopy (SEM) to analyze the ultrastructure of the actin cytoskeleton in lamellipodia and filopodia. STORM imaging revealed a repetitive pattern of cortactin clusters along filopodia, partially colocalizing with activated Src2. We found that up-regulation of Src2 activation state or cortactin increased lamellipodial length, protrusion time, and actin network density, whereas down-regulation had opposite effects. Furthermore, Src2 or cortactin up-regulation increased filopodial density, length, and protrusion time, whereas down-regulation promoted lateral movements of filopodia. FSM revealed that rates of actin assembly and retrograde flow were not affected in either case. In summary, our results support a model whereby Src and cortactin regulate growth cone motility by increasing actin network density and protrusion persistence of lamellipodia by controlling the state of actin-driven protrusion versus retraction. In addition, both proteins promote the formation and stability of actin bundles in filopodia.

Trpv1-positive neurons are sensitive to both noxious thermal and mechanical stimuli

Poster

Feng Wang, Erik Bélanger, Sylvain Côté, Daniel Côté, Yves De Koninck
Centre de recherche de l'Institut universitaire en santé mentale de Québec

Dorsal root ganglion (DRG) neurons are the primary sensory neurons in the somatosensory pathways. The properties of individual DRG neuron have been thoroughly studied and many types of DRG neurons with distinct sensitivity have been identified. However, it is still unclear how the DRG neurons encode different types of ambient stimuli. The current dominating theory is specificity theory, which holds that specific population of DRG neurons are uniquely activated by certain type of stimulus, and the activation of these neurons ultimately gives rise to the sensation corresponding to the stimulus. The theory has received supports from large amounts of studies, mainly using various types of transgenic mice. TRPV1 is a heat-sensitive cation channel and mainly expressed in subpopulation of DRG neurons. Behavior test, by ablating TRPV1-containing afferent fibers, showed that TRPV1-postive neurons were only involved in the thermal but not mechanical nociception, which strongly supported the specificity theory. However, in vivo, the sensitivity of TRPV1-positive neurons is still unclear. Here, we used AAV9 virus to specifically label Trpv1-positive DRG neurons with GCaMP6s, a genetically encoded calcium indicator, in Trpv1-Cre mice. Then we conducted in vivo calcium imaging from these DRG neurons using video rate laser scanning two-photon microscopy. The responsiveness of the neurons to various thermal and mechanical stimuli were recorded and analyzed. Our results showed that Trpv1-postive neurons are sensitive to both noxious hot and noxious mechanical stimulation. Thus, our studies provide new insight into the physiological function of Trpv1-positive neurons and call for a revisit for the specificity theory of somatosensation.

Contacts

FINS2015 sponsors

Sponsor	Contact	email
SPIE (Neurophotonics)	Karolyn Labes	karolyn@spie.org
Scientifica	Louisa Mafeld	louisa.mafeld@scientifica.uk.com
Olympus	Gabriel Lapointe	gabriel.lapointe@olympus.com
Zeiss	Éric Trudel	eric.trudel@zeiss.com; chris.souwand@zeiss.com
Coherent	Mike Boehlke	Michael.Boehlke@coherent.com
Spectra Physics	Paul Presunka	paul.presunka@spectra-physics.com
Nikon	Sam Lacoche	slacoche@nikon.ca
Laserglow	Priyanka Mehrotra	pmehrotra@laserglow.com
Huron Digital Pathology	Patrick Myles	patrick@hurondigitalpathology.com
Leica microsystems	Lianne Dale	lianne.dale@leica-microsystems.com
Nature Methods	Nina Vogt	nina.vogt@us.nature.com
Doric Lenses	Sead Doric	sead@doriclenses.ca
Axion Biosystems	Emily Matheu	ematheu@axion-biosystems.com
COPL	Diane Déziel	diane.deziel@copl.ulaval.ca

FINS2015 Registered attendees

Agboola, Adebayo
hagbobayor@yahoo.com
Nigeria

Akitegetse, Cleophace
cleophace.akitegetse.1@ulaval.ca
Canada

arrigoni, marco
marco.arrigoni@coherent.com
USA

Audet, Benoit
benoit.audet.3@gmail.com
Canada

Aufmkolk, Sarah
Sarah.Aufmkolk@physik.uni-wuerzburg.de
Germany

Bagramyan, Arthur
arutyun.bagramyan.1@ulaval.ca
Canada

Barna, Laszlo
barna.laszlo@koki.mta.hu
Hungary

Beique, Jean Claude
jbeique@uottawa.ca
Canada

Bélanger, Erik
erik.belanger@crulrg.ulaval.ca
Canada

Binan, Loic
loic.binan@polytechnique.org
Canada

Bisht, Kanchan
bishtkanchan8@gmail.com
Canada

Bito, Haruhiko
hbito@m.u-tokyo.ac.jp
Japan

Boas, David
dboas@nmr.mgh.harvard.edu
USA

Bon, Pierre
pierre.bon@institutoptique.fr
France

Bonin, Robert
rob.bonin@utoronto.ca
Canada

Bories, Cyril
cyril.bories@gmail.ca
Canada

Bretzner, Frederic
Frederic.Bretzner.1@ulaval.ca
Canada

Briggman, Kevin
briggmankl@mail.nih.gov
USA

CALLADO PÉREZ, AMALIA
amalia.callado.perez@gmail.com
Canadá

Campbell, Robert
robert.e.campbell@ualberta.ca
Canada

Castonguay, Annie
annie.castonguay@crulrg.ulaval.ca
Canada

Chamberland, Simon
simon.chamberland.2@ulaval.ca
Canada

Chetoui, Nizar
nizar.chetoui@crulrg.ulaval.ca
Canada

Cognet, Laurent
laurent.cognet@u-bordeaux.fr
France

COLLINGRIDGE, Graham
collingridge@lunenfeld.ca
Canada

Costantino, Santiago
santiago.costantino@umontreal.ca
Canada

Cote, Daniel
daniel.cote@crulrg.ulaval.ca
Canada

Crosby, Kevin
kevin.crosby@ucdenver.edu
United States

Culver, Joseph
culverj@wustl.edu
United States

Deisseroth, Karl
deissero@stanford.edu
USA

De Koninck, Yves
yves.dekoninck@neuro.ulaval.ca
Canada

De Koninck, Paul
paul.dekoninck@neuro.ulaval.ca
Canada

Desrosiers, Patrick
patrick.desrosiers.1@ulaval.ca
Canada

Dovonou, Axelle
axelledov@gmail.com
Canada

Dudok, Barna
dudok@koki.hu
Hungary

Dudok, Barna
dudok@koki.hu
Hungary

Durand, Audrey
audrey.durand.gif@gmail.com
Canada

Ferrini, Francesco
francesco.ferrini@unito.it
Italy

Fink, Mathias
mathias.fink@espci.fr
France

Fréchette, Julie
jfrechet@outlook.com
Canada

Friedrich, Rainer
Rainer.Friedrich@fmi.ch
Switzerland

Fuzesi, Tamas
tfuzesi@ucalgary.ca
Canada

Gasecka, Alicja
elmo.ag@gmail.com
Canada

Geddes, Sean
sgeddes@uottawa.ca
Canada

Geddes, Sean
geddes.sean@gmail.com
Canada

GEORGIOU, John
georgiou@lunenfeld.ca
Canada

Godin, Antoine
antoine.godin@institutoptique.fr
France

Gosselin, Benoit
benoit.gosselin@gel.ulaval.ca
Canada

Hamel, Edith
edith.hamel@mcgill.ca
Canada

Hoogenraad, Casper
c.hoogenraad@uu.nl
Netherlands

Hovius, Ruud
ruud.hovius@epfl.ch
Switzerland

Jähne, Sebastian
sebastian.jahne@ucdconnect.ie
Germany

Jinadasa, Tushare
tushare.jinadasa@gmail.com
Canada

Jinadasa,
Tushare
tushare.jinadasa@gmail.com
Canada

Katona, Istvan
katona@koki.hu
Hungary

Kerr, Jason
jason.kerr@caesar.de
Germany

Khoutorsky, Arkady
arkady.khoutorsky@mcgill.ca
Canada

Kleinfeld, David
dk@physics.ucsd.edu
USA

Konnerth, Arthur
arthur.konnerth@tum.de
Germany

Labonté, Isabelle
isabelle.labonte@crulrg.ulaval.ca
Canada

Labrecque, Simon
simon.labrecque.1@ulaval.ca
Canada

Labrie-Dion, Étienne
etienne.labrie-dion@douglas.mcgill.ca
Canada

Lavoie-Cardinal, Flavie
flavie.lavoie@googlemail.com
Canada

Lecrux, Clotilde
clotilde.lecrux@mcgill.ca
Canada

Lee, Kevin
kevin.fh.lee@gmail.com
Canada

Lévesque, Martin
martin.levesque@fmed.ulaval.ca
Canada

Lopez, Cedric
cedric.lopez@u-bordeaux.fr
FRANCE

Lorenzo, Louis Etienne
louis-etienne.lorenzo@crulrg.ulaval.ca
CANADA

Marquet, Pierre
Pierre.Marquet@neuro.ulaval.ca
Canada

Mertz, Jerome
jmertz@bu.edu
USA

Méthot, Mario
mario.methot@crulrg.ulaval.ca
Canada

Miczan, Vivien
miczan.vivien@koki.mta.hu
Hungary

MORETTI, CLAUDIO
claudio.moretti@iit.it
Italia

Murphy, Tim
thmurphy@mail.ubc.ca
Canada

Nadeau, Gabriel
gabriel.nadeau.2@ulaval.ca
Canada

Nagai, Takeharu
ng1@sanken.osaka-u.ac.jp
Japan

Nägerl, Valentin
valentin.nagerl@u-bordeaux.fr
France

Oertner, Thomas
thomas.oertner@zmnh.uni-hamburg.de
Germany

Pagés Puertas, Pau Aleix
paualeixpages@gmail.com
Canada

Paquet, Marie-Eve
mep@crulrg.ulaval.ca
Canada

Perez-Sanchez, Jimena
jimena.perez-sanchez.1@ulaval.ca
Canada

Perrais, David
david.perrais@u-bordeaux.fr
France

Plasencia Fernandez, Isabel
isabelplasencia89@gmail.com
Canada

Poupart, Julie
julie@scienceweb.ca
Canada

Prager-Khoutorsky, Masha
maria.khoutorsky@mail.mcgill.ca
Canada

Rappaz, Benjamin
benjamin.rappaz@epfl.ch
Switzerland

Rocha, Cecilia
cecilia.rocha@mcgill.ca
Canada

Ruthazer, Edward
edward.ruthazer@mcgill.ca
Canada

Saghatelyan, Armen
armen.saghatelyan@fmed.ulaval.ca
Canada

Salesse, Charleen
charleen.salesse.1@ulaval.ca
Canada

Sauer, Markus
m.sauer@uni-wuerzburg.de
Germany

Savage, Julie
julie.savage@gmail.com
Canada

sehgal, kapil
kapilseh@gmail.com
Canada

Sharma, Kaushik
sharmakaushikp@gmail.com
Canada

Sibarita, Jean Baptiste
jean-baptiste.sibarita@u-bordeaux2.fr
France

Soares, Cary
csoar047@gmail.com
Canada

Spruston,
Nelson
sprustonn@janelia.hhmi.org
USA

Suter, Daniel
dsuter@purdue.edu
USA

Thibon, Louis
louis.thibon.1@ulaval.ca
Canada

Toth, Katalin
katalin.toth@fmed.ulaval.ca
Canada

Vanden Berghe, Pieter
pieter.vandenberghe@med.kuleuven.be
Belgium

Wang, Feng
Feng.Wang@crulrg.ulaval.ca
Canada

Wiesner, Theresa
theresa.wiesner@gmail.com
Canada

Wiseman, Paul
paul.wiseman@mcgill.ca
Canada

Wyart, Claire
claire.wyart@gmail.com
France

4th International Frontiers in Neurophotonics Symposium

Many thanks to our sponsors and supporters!

Platinum sponsor

HURON Digital Pathology

Gold sponsors

OLYMPUS

ZEISS

doric

Silver sponsors

SPIE.

COHERENT

nature methods

Scientifica
Experts in Electrophysiology & Imaging

axion BioSystems

Nikon

Spectra-Physics
A Newport Company

COPL
Centre d'optique, photonique et laser

Leica MICROSYSTEMS

Bronze sponsor

Laserglow Technologies

Institutional sponsors

UNIVERSITÉ LAVAL

université de BORDEAUX

NSERC CRSNG

Brain Canada

Institut universitaire en santé mentale de Québec

IINS
Interdisciplinary Institute for NeuroScience

cnrs

CIHR IRSC
Canadian Institutes of Health Research
Instituts de recherche en santé du Canada