Optical Instruments and Imaging

BRAIN Initiative Investigators Pre-meeting: Large Scale Recording and Modulation
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**10 min presentation RAZANSKY, DANIEL**

- **Five-dimensional optoacoustic tomography for large-scale electrophysiology in scattering brains**
  - Project Number: 1 R21 EY026382-01

**10 min presentation XU, CHRIS**

- **Optimization of 3-photon microscopy for Large Scale Recording in Mouse Brain**
  - Project Number: 1 R21 EY026391-01
**Problem Statement:** There is a need for a non-invasive technique to measure electrical activity in the deep brain.

**Potential Solution:** Electrical impedance tomography (EIT) has potential if we can focus the current.

**EIT Basics:** Channel opening decreases tissue impedance.

Inject current through scalp electrodes and measure impedance changes. Not capable of sensing electrical activity.
**Hypothesis:** A magnetic field can be used to confine the current to a collimated beam

**Experimental Plan:** Theoretical and computational work, followed by benchtop testing in saline

**Lorentz Force:** \( F = q (E + \mathbf{v} \times \mathbf{B}) \)
Ultra-miniaturized single fiber probe for functional brain imaging in freely moving animals

PI: Jerome Mertz, Boston University

**Goal:** to develop a fluorescence microendoscope that provides imaging at arbitrary depth in brain while causing minimal surgical damage.

**Strategy:** light directions are converted into distinct spectral codes using a spread-spectral encoder that is embedded inside a lensless optical fiber.

Fluorescence, bioluminescence, etc.

Spectral encoder

Multimode fiber

Image reconstruction performed by numerical decoding of detected spectrum.
How does the system “learn” the spectral codes?

**Supervised learning**
The system is taught the codes in a calibration procedure prior to imaging.

**Unsupervised learning**
The system learns the codes on the fly based on temporal diversity of signals.

![Diagram showing supervised and unsupervised learning processes.](image-url)
OCT-based detection of neural activity

- Changes in intensity / attenuation
- Changes in optical phase
Specific aims

- Seizure → multi-unit activity
- Localization of activity
Goal: Transmit an image from inaccessible regions without a lens

- In biomedical applications, single mode fiber bundles used for imaging and energy delivery applications
- However, single fibers are desirable
  - Smaller cross section
  - Can bend over smaller radii of curvature
  - Low cost

Approach
- Fiber is pre-calibrated
- Shape and temperature changes modify the spatial configuration of optical modes
Fluorescence image reconstruction

4µm fluorescent beads

Mouse brain slice labeled with Alexa 532

Thanks to Dr. Shay Ohayon and Dr. DiCarlo from MIT for the preparation of the samples
Multiplexed Multiphoton Interrogation of Brain Connectomics
PI: Ramachandran; Xue; Mertz; 1R21EY026410-01; Program Start: Oct. 2015

Aim 1: Build an all-fiber energetic tunable two-color source (also enables endoscopy).
Aim 2: Build non-degenerate 3-photon microscope with raster scan capability.

**Degenerate Excitation**
- 1300 & 1600-1700nm

**Non-Degenerate Excitation**
- Many more possibilities:
  - (3 \(\lambda_1\)) or (2 \(\lambda_1 + \lambda_2\)) or (1 \(\lambda_1 + 2 \lambda_2\)) or (3 \(\lambda_2\))
  - 1.8x depth penetration (2 mm) possible

**High rep. rate (high power) and high energy ultrashort pulse sources needed.**
Bulk OPOs…… alignment sensitive, $$$, ↓ efficiency, ↓ beam quality
Fiber sources… turn key, ↓ $, ↑ efficiency, ↑ power (rep. rate), flexible but cannot scale energy at 1300 nm today.
High Energy Raman Soliton Shifting

Calmar Cazadero (370 fs; 1030 nm)

$\lambda \sim 1308 \text{ nm}; \quad \tau_{\text{FWHM}} \sim 50 \text{ fs};$

$E_{\text{pulse}} \sim 30 \text{ nJ}; \quad P_{\text{peak}} \sim 0.6 \text{ MW}$

Rep. rate = 120 kHz; $P_{\text{av}} \sim 3.6 \text{ mW}$

Next steps:
Shift to next window (1700 nm)
Scale energy by 2-3x
Space-division multiplexing optical coherence tomography for large-scale, millisecond resolution imaging of neural activity (1R21EY026380-01)

Co-PIs: Chao Zhou, Yevgeny Berdichevsky

- OCT can see individual neurons clearly in 3D based on intrinsic contrast.
- SDM-OCT allows parallel imaging of thousands of neurons with millisecond temporal resolution.

Li, et al. Neurophotonics, 1(2), 025002, 2014
Specific Aims:

**Aim 1:** Develop an integrated electrophysiology and ultrahigh speed SDM-OCT imaging system to record fast intrinsic optical signals associated with neural activity.

Fast intrinsic optical signals (e.g. changes in light scattering and phase occurred at millisecond timescale) are presumably related to alteration in the complex refractive index and small volume changes near the membrane, in response to the rapid osmotic changes associated with ion fluxes during action potentials.

**Aim 2:** Perform *in vitro* imaging and electrophysiological recording in 2D neural cultures.

**Aim 3:** Perform *in vitro* imaging and electrophysiological recording in 3D organotypic brain cultures.

Wavefront engineering for high resolution deep tissue calcium imaging
Cui lab at Purdue University

Iterative multiphoton adaptive compensation technique (IMPACT)

Opt Lett 36 (6), 870-872 (2011)
PNAS 109(22):8434 (2012)

High resolution imaging through intact skull

PNAS 112(30) 9236-9241, (2015); Opt Express 23(6):7463 (2015); Scale bar: 5 µm
For practical high resolution multiphoton calcium imaging

1. Flexible in wavelength 0.93, 1.04, 1.35 μm, ...
2. Automated operation (Biologists without optical physics background need to be able to use it on a daily basis)
3. Large volume simultaneous correction (throughput is very important in calcium imaging)!!!
4. Requires no additional labeling besides calcium indicators
5. Must be able to tolerate sample motion

Acknowledgment: NIH, Purdue University, HHMI.
Non-Degenerate Multiphoton Microscopy for Deep Brain Imaging
U01 NS094232
MPI: Devor A., Fainman Y.

\[ F = I_{exc}^2 \sigma \phi \beta[D] \]

- Create a supercontinuum to allow efficient search for the optimal combination of NIR and SWIR wavelengths
- Implement AO to correct the phase distortions experienced by the NIR beam
- Strategically displace NIR and SWIR beams to avoid surface excitation

Hales.. Brédas 2004

Horton.. Xu 2013

Mahou.. Beaurepaire 2012

Materials and Methods

Sergei Vinogradov
Roger Tsien
Shaya Fainman
Mu-Han Yang
Miniscope BRAIN Initiative

UCLA

Peyman Golshani (Communicating PI)
Alcino Silva
Baljit Khakh
Dejan Markovic
Daniel Aharoni
Building and sharing next generation open-source miniaturized microscopes for imaging activity in freely behaving mice
- Two-channel wearable miniaturized microscope
- Optogenetics-capable wearable miniaturized microscope
- Wireless miniaturized wearable miniaturized microscope
- Create an open-source platform for freely sharing this technology
Multi-area two-photon microscopy

Group Fritjof Helmchen
Brain Research Institute
University of Zurich
Switzerland

YCX2.60 in L2/3 of transgenic mouse neocortex

GCaMP6m signals
Multi-area two-photon microscopy

Next goal:
SCAPE microscopy for high-speed in-vivo volumetric microscopy in behaving organisms

Elizabeth M. C. Hillman Ph.D.
Associate Professor of Biomedical Engineering & Radiology
Mortimer B. Zuckerman Mind Brain Behavior Institute
Kavli Institute for Brain Science at Columbia University

Richard S. Mann2,6, Wesley B. Grueber3,6, Randy M. Bruno4,5,6 and David Schoppi7
Matthew B. Bouchard1, Venkatakaushik Voleti1, Wenze Li1, Cesar Mendes2, Clay Lacefield4, Marie Greaney7, Evan Schaffer8

1Departments of Biomedical Engineering and Radiology,
2Department of Biochemistry and Molecular Biophysics,
3Department of Physiology and Cellular Biophysics,
4Bruno Lab, 8Axel Lab, Department of Neuroscience,
5Kavli Institute for Brain Science,
6Mortimer B. Zuckerman Mind Brain Behavior Institute, Columbia University,
7Departments of Otolaryngology and Neuroscience & Physiology, Neuroscience Institute, NYU Langone School of Medicine.
SCAPE: Swept Confocally-Aligned Planar Excitation microscopy

- SCAPE is a high-speed 3D microscopy technique that combines light-sheet sectioning with confocal descanning to image at >40 volumes per second.
- Unlike conventional light-sheet, SCAPE uses a single, stationary objective lens allowing diverse, un-mounted samples to be imaged.
- SCAPE maintains alignment of the light sheet and detection plane using a single scanning mirror, making SCAPE surprisingly simple and inexpensive.
- SCAPE is compatible with single-photon and multi-photon excitation, multi-color detection and combined patterned photoactivation and 3D imaging.
- SCAPE has already been demonstrated on the awake, behaving mouse brain, adult fly brain, zebrafish brain and heart, crawling Drosophila larvae and the mouse olfactory epithelium.
confocal

SCAPE

Bouchard et al, Nature Photonics, 2015

IV Texas red-dex + GCaMP6F in layer 5 neuron apicals, awake behaving mouse 10 VPS

Awake, behaving fly brain, 10 VPS

mhc-GFP Drosophila larva 20 VPS

Zebrafish brain 10 VPS
**Goal:** Monitoring activity across all synapses of a given neuron *in vivo*

**First target:** Ca$^{2+}$ signals at ~ 10,000 locations with 100 ms temporal resolution

**Approach:**

**Labeling Strategy**

- Triple labeling: Ca$^{2+}$ indicator (R)
- Cell fill – spines (Y)
- Inhibitory synapses (B)

**Two photon structural scan**

- Full volume
- Two color
- Micron resolution = Coordinate map of all synaptic sites

**Selective holographic Ca$^{2+}$ excitation**

- 1,000 locations
- 15 µm Z slab; 300 x 300 µm XY plane
- 10 slabs
Year 1 progress

• Synaptic coordinate map does not interfere with RCaMP detection in dendrites (and converse).

• Holographic patterning can be used to precisely target up to 400 1µm-sized excitation spots in a 100 x 100 x 25 µm volume.

• A Gaussian-Laguerre element can be used to detect and decode emissions from multiple axial locations within a 10 µm thick volume.
Three Dimensional Computer-Generated Holography for Neural Circuit Reverse Engineering

Serge Picaud & Valentina Emiliani
Key Investigators: Simon Schultz, Claire Wyart, Amanda Foust, Jens Duebel, Olivier Marre

✓ Control of brain signaling through holographic light shaping and optogenetics

1. Development of a holographic optical system for in vivo, in depth neuronal circuit manipulation

- Precise sculpting of the excitation volume by spatial and temporal shaping of optical wave fronts
- Simultaneous, Multi-Location, Three-Dimensional
- Independent control of Position, Shape, and Intensity
2. Iterative optimization of the system in different model systems
   - Retinal,
   - Cortical
   - Zebrafish Motion Circuits.

3. Engineering of new opsins (ED Boyden MIT)

4. Wide dissemination of the technology
   - Commercial system development and optimization with industrial partner 3i
     (Denver, CO)

Photocredits: 3D Holography Simulation, Oscar Hernandez; Zebrafish, Claire Wyart; Retinal Bipolars, Olivier Marre; In-vivo mouse cortex, Simon Schultz; Engineering of opsins: Ed Boyden
Modular nanophotonic probes for dense neural recording at single-cell resolution

Investigators: ROUKES, MICHAEL L (contact); SHEPARD, KENNETH L; SIAPAS, ATHANASSIOS; TOLIAS, ANDREAS
High-speed volumetric imaging of neuronal network activity at depth using Multiplexed Scanned Temporal Focusing (MuST)

Investigator: VAZIRI, ALIPASHA
Photoacoustic Calcium/Voltage Indicators

Calcium-sensitive protein GCaMP5G
in fruit fly brain

Voltage dye Dipicrylamine (DPA)
in mouse brain

Fractional signal change (%)
Time (sec)

RY Zhang, B Rao, HY Rong, B Raman@WUSTL, LV Wang, unpublished

4-AP induced epilepsy

Fractional signal change
Time (s)

RY Zhang, B Rao, LV Wang, unpublished
Photoacoustic Tomography of Mouse Brain In Vivo

L Li, JJ Yao, Y Zhou, LR Zhu, …, LV Wang, unpublished

Oxygen saturation

SPECIFIC AIMS

- **Aim 1.** Develop an energetic fiber-based excitation source at 1300 nm for 3PM.
- **Aim 2.** Fabricate a new objective lens to collect the signal efficiently at depth and provide convenient integration with adaptive optics (AO).
- **Aim 3.** Implement AO for 3PM at 1300 nm.
- **Aim 4.** Test and validate 3PM at 1300 nm for imaging brain activity in awake and behaving mouse.

Progress

- We have successfully demonstrated recording of the mouse hippocampal activity using 3-photon microscopy.
- We have shown that the neuronal functions recorded by 2- and 3-photon fluorescence imaging are the same.
- We have incorporated a robust, and user-friendly source for 3-photon imaging at 1300 nm.
- We have developed an adaptive optics (AO) system for 3-photon fluorescence imaging, and compared the impact of AO for 2-, 3- and 4-photon excited fluorescence.

On-going and near term future work

- Improve and quantify Ca-imaging in the mouse hippocampus.
- Applying AO to in vivo imaging, and improving the signal collection efficiency.
- Applying 3-photon Ca-imaging in awake and behaving animal.
Simultaneous 2-photon (top) and 3-photon (bottom) imaging of the same GCaMP6-labeled neuron at ~200 μm depth. The same time trace is obtained.

Spatial resolution at two different depth. The smallest feature within the image is found as an upper bound of the spatial resolution.
Time-reversal optical focusing for non-invasive optogenetics
Changhuei Yang, Viviana Gradinaru
California Institute of Technology

We are developing a high speed time-reversal ultrasound encoded optical focusing technology for optogenetic use.
Demonstration of dynamic focusing through living animal.

Enhancing guidestar contrast through the use of microbubble destruction with ultrasound.

Discovery of optical memory effect that works for extended scattering medium = fast focused spot scanning feasible.

M Jang, H Ruan, C Yang, Nature Comm (accepted)