# Optical Instruments and Imaging

BRAIN Initiative Investigators Pre-meeting:

Large Scale Recording and Modulation

| Presentation Order | PI Name(s) All | Title | Project Number |
|---|---|---|---|
| 1 | FREEMAN, DANIEL KENNETH | Neural Monitoring with Magnetically-Focused Electrical Impedance Tomography (mf-EIT) | 1 R21 EY026360-01 |
| 2 | MERTZ, JEROME C | Ultra-miniaturized single fiber probe for functional brain imaging in freely moving animals | 1 R21 EY026310-01 |
| 3 | PARK, BORIS HYLE (contact); BAZHENOV, MAKSIM V; BINDER, DEVIN K | Label-free 4D optical detection of neural activity | 1 R21 EY026441-01 |
| 4 | PIESTUN, RAFAEL | High-speed Deep Brain Imaging and Modulation with Ultrathin Minimally Invasive Probes | 1 R21 EY026436-01 |
| 5 | RAMACHANDRAN, SIDDHARTH (contact); HAN, XUE | Multiplexed Multiphoton Interrogation of Brain Connectomics | 1 R21 EY026410-01 |
| 6 | ZHOU, CHAO (contact); BERDICHEVSKY, YEVGENY | Space-division multiplexing optical coherence tomography for large-scale, millisecond resolution imaging of neural activity | 1 R21 EY026380-01 |
| 7 | CUI, MENG (contact); GAN, WENBIAO | High resolution deep tissue calcium imaging with large field of view wavefront correction | 1 U01 NS094341-01 |
| 8 | DEVOR, ANNA (contact); FAINMAN, YESHAIAHU L | Non-degenerate multiphoton microscopy for deep brain imaging | 1 U01 NS094232-01 |
| 9 | GOLSHANI, PEYMAN (contact); KHAKH, BALJIT ; MARKOVIC, DEJAN ; SILVA, ALCINO J. | Building and sharing next generation open-source, wireless, multichannel miniaturized microscopes for imaging activity in freely behaving mice | 1 U01 NS094286-01 |
| 10 | HELMCHEN, FRITJOF | Multi-area two-photon microscopy for revealing long-distance communication between multiple local brain circuits | 5 U01 NS090475-02 |
| 11 | HILLMAN, ELIZABETH M | SCAPE microscopy for high-speed in-vivo volumetric microscopy in behaving organisms | 1 U01 NS094296-01 |
| 12 | NEDIVI, ELLY (contact); SO, PETER T. | Next generation high-throughput random access imaging, in vivo | 5 U01 NS090438-02 |
| 13 | PICAUD, SERGE (contact); EMILIANI, VALENTINA | Three Dimensional Holography for Parallel Multi-target Optogenetic Circuit Manipulation | 5 U01 NS090501-02 |
| 14 | ROUKES, MICHAEL L (contact); SHEPARD, KENNETH L; SIAPAS, ATHANASSIOS ; TOLIAS, ANDREAS | Modular nanophotonic probes for dense neural recording at single-cell resolution | 5 U01 NS090596-02 |
| 15 | VAZIRI, ALIPASHA | High-speed volumetric imaging of neuronal network activity at depth using Multiplexed Scanned Temporal Focusing (MuST) | 1 U01 NS094263-01 |
| 16 | WANG, LIHONG | FAST HIGH-RESOLUTION DEEP PHOTOACOUSTIC TOMOGRAPHY OF ACTION POTENTIALS IN BRAINS | 5 U01 NS090579-02 |
| 17 | XU, CHRIS | Optimization of 3-photon microscopy for Large Scale Recording in Mouse Brain | 5 U01 NS090530-02 |
| 18 | YANG, CHANGHUEI (contact); GRADINARU, VIVIANA | Time-Reversal Optical Focusing for Noninvasive Optogenetics | 5 U01 NS090577-02 |
| *10 min presentation* | *RAZANSKY, DANIEL* | *Five-dimensional optoacoustic tomography for large-scale electrophysiology in scattering brains* | *1 R21 EY026382-01* |
| *10 min presentation* | *XU, CHRIS* | *Optimization of 3-photon microscopy for Large Scale Recording in Mouse Brain* | *1 R21 EY026391-01* |

# Magnetically-Focused Electrical Impedance Tomography (mf-EIT)

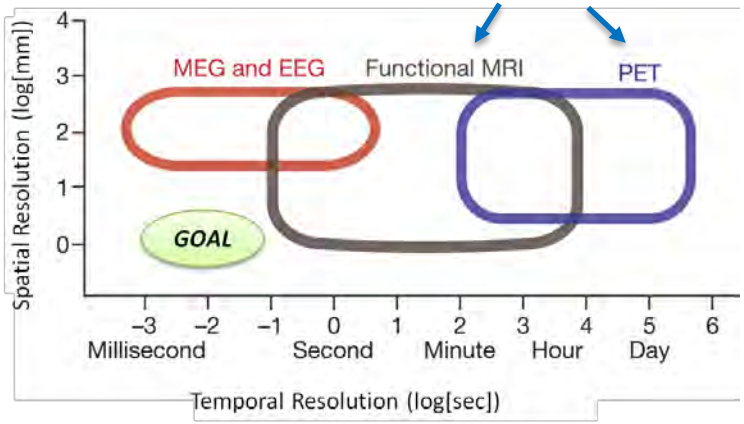
DRAPER

Daniel Freeman, Draper Laboratories

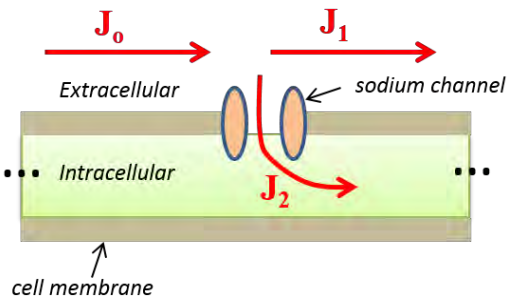
**Problem Statement**: There is a need for a non-invasive technique to measure electrical activity in the deep brain

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

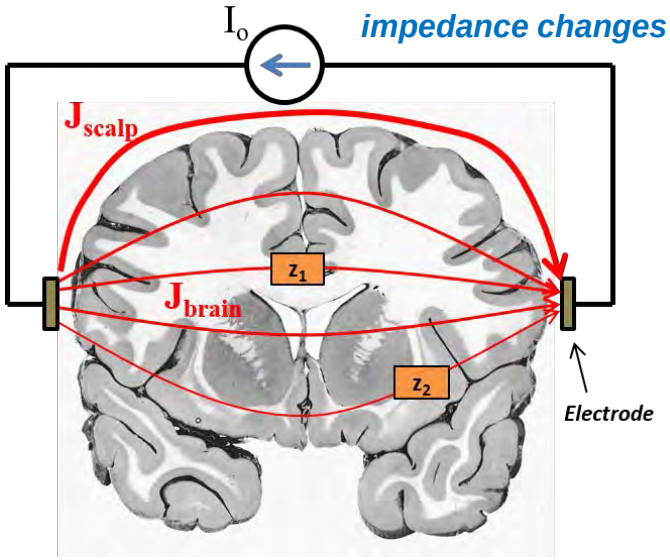
*Not capable of sensing electrical activity*




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




*Inject current through scalp electrodes and measure impedance changes*



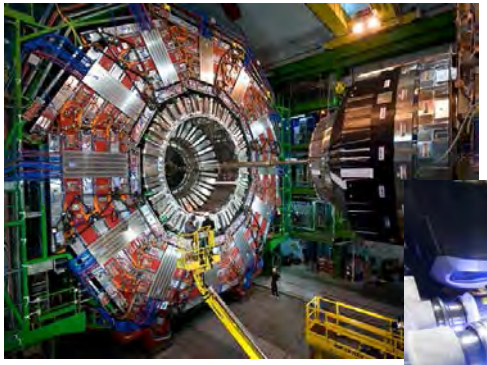
# Magnetically-Focused Electrical Impedance Tomography (mf-EIT)

DRAPER

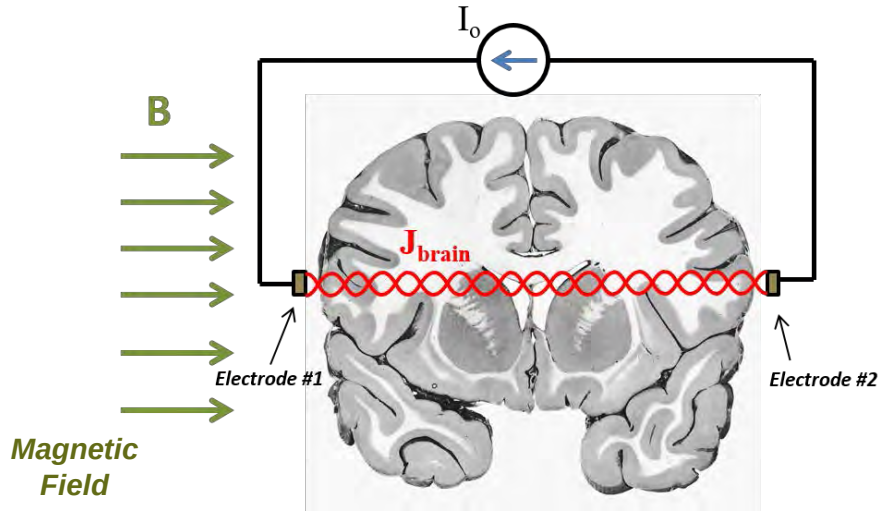
Daniel Freeman, Draper Laboratories

**Hypothesis**: A magnetic field can be used to confine the current to a collimated beam

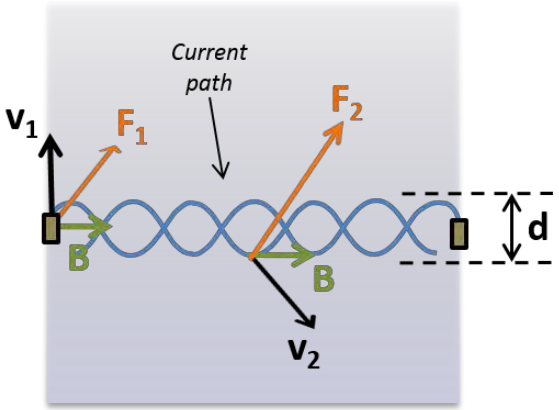
**Experimental Plan**: Theoretical and computational work, followed by benchtop testing in saline



*Large Hadron Collider uses magnetic fields to steer charged particles*




***Lorentz Force:*** $F = q\,(E + v \times B)$

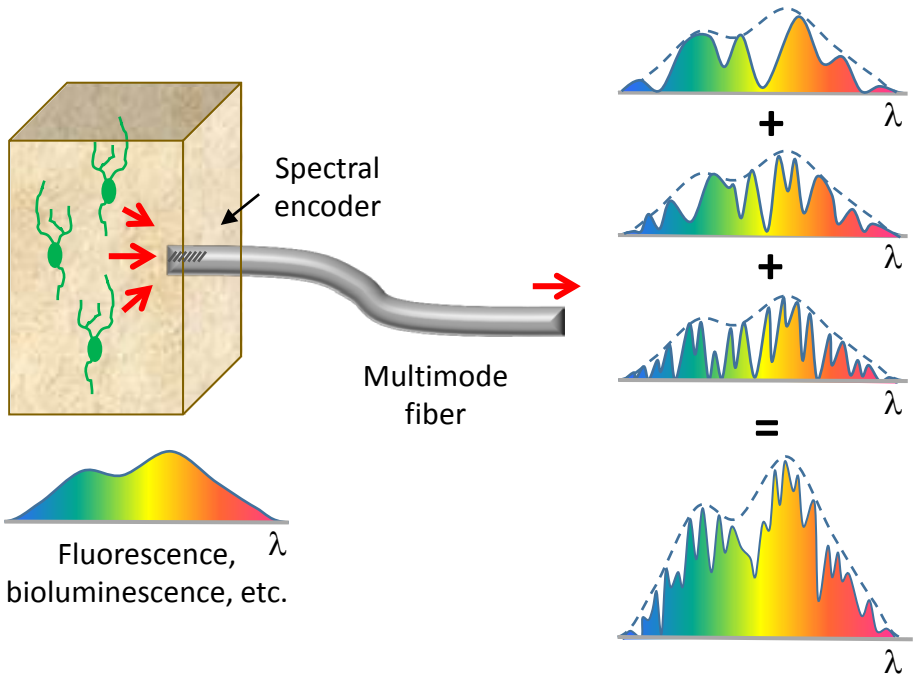


# Ultra-miniaturized single fiber probe for functional brain imaging in freely moving animals

**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.



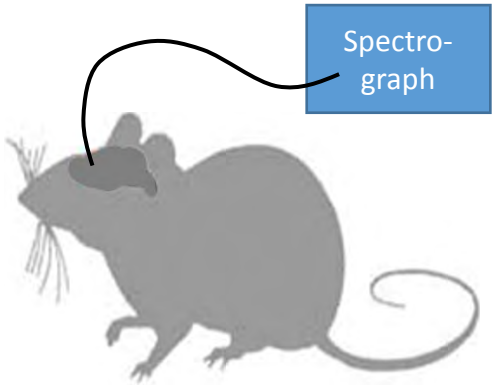
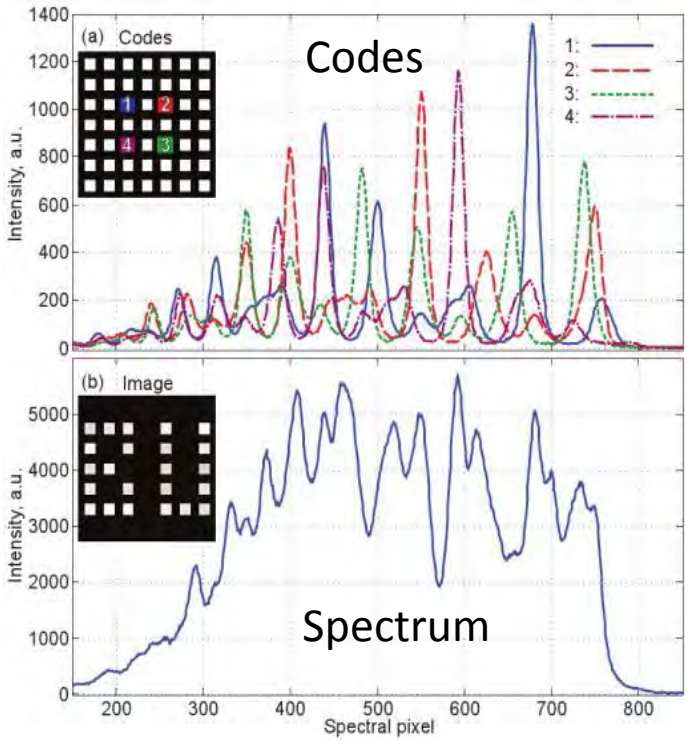




Image reconstruction performed by numerical decoding of detected spectrum.

# How does the system “learn” the spectral codes?

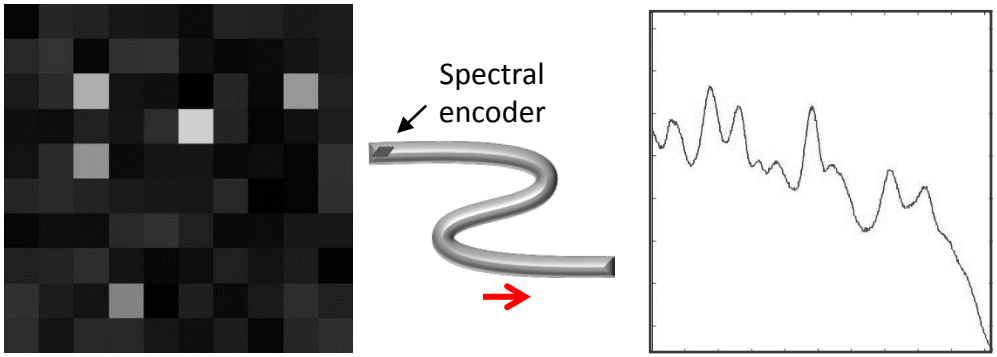
**Supervised learning**

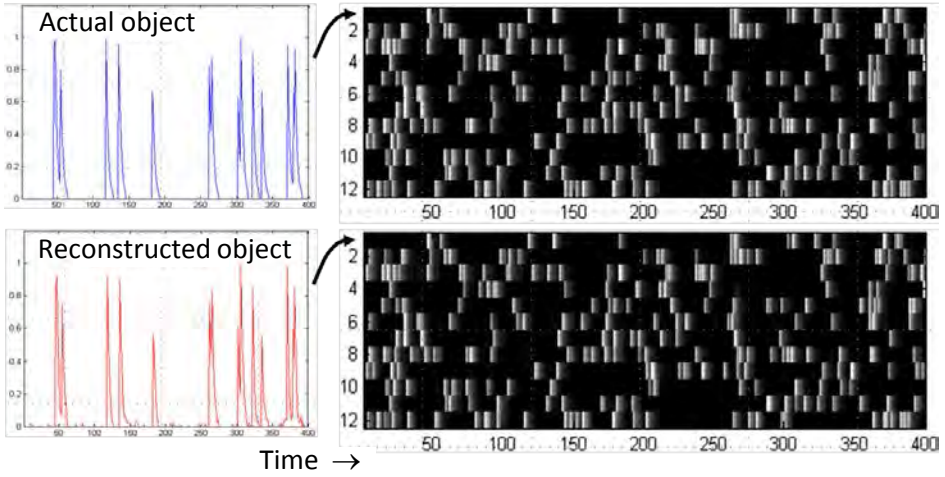
The system is taught the codes in a calibration procedure prior to imaging




**Unsupervised learning**

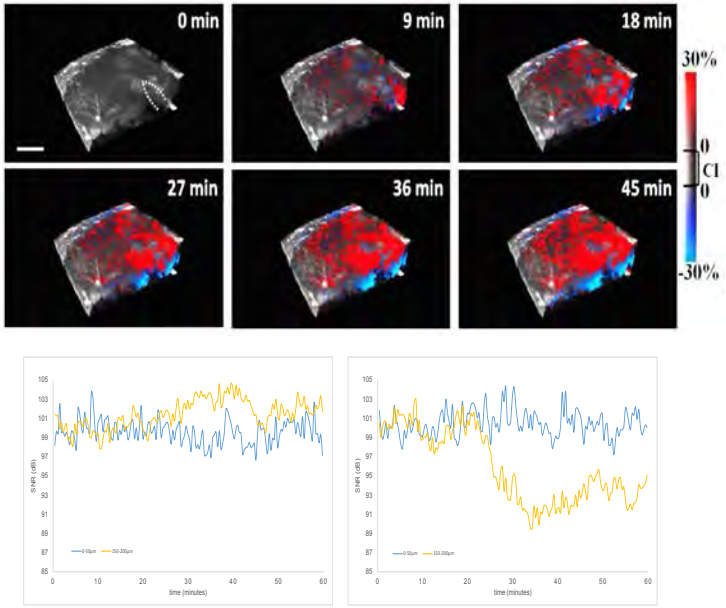
The system is learns the codes on the fly based on temporal diversity of signals



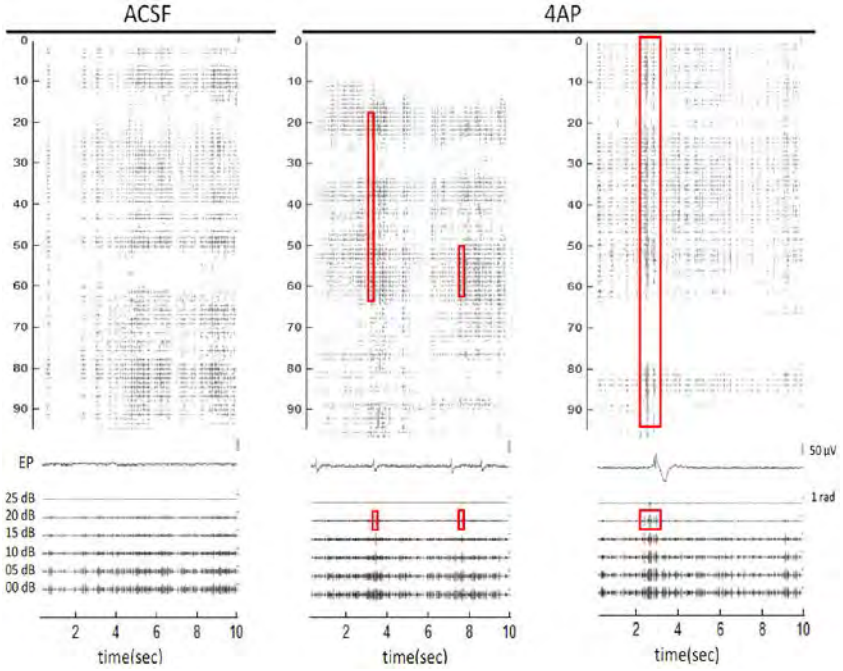



# OCT-based detection of neural activity

- Changes in intensity / attenuation

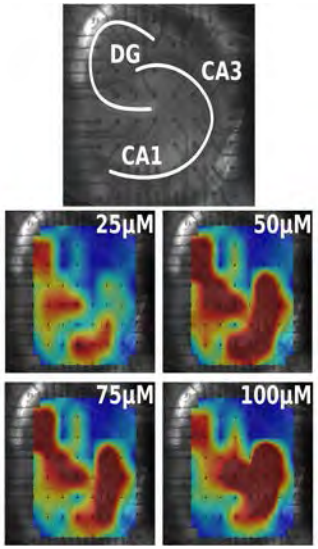


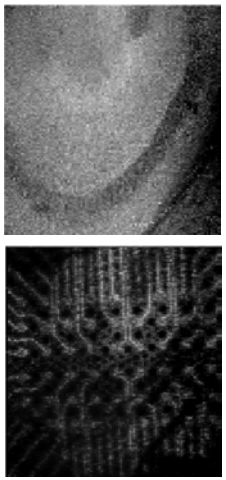

- Changes in optical phase

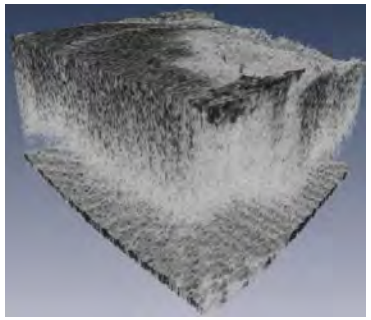


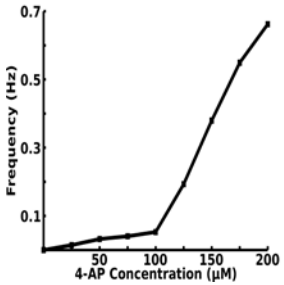
# Specific aims

- Seizure → multi-unit activity

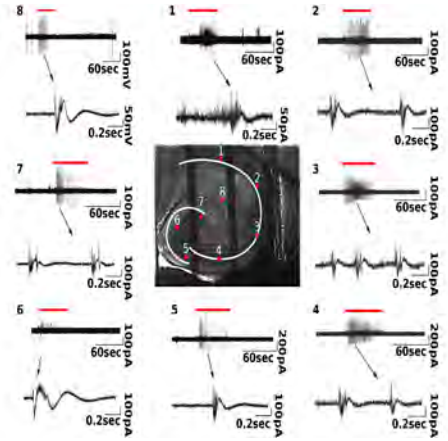







- Localization of activity



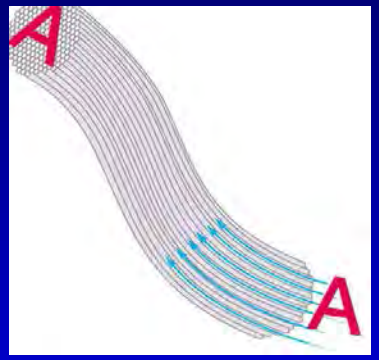
# Single Multi-Mode Fiber Fluorescence Micro-Endoscope

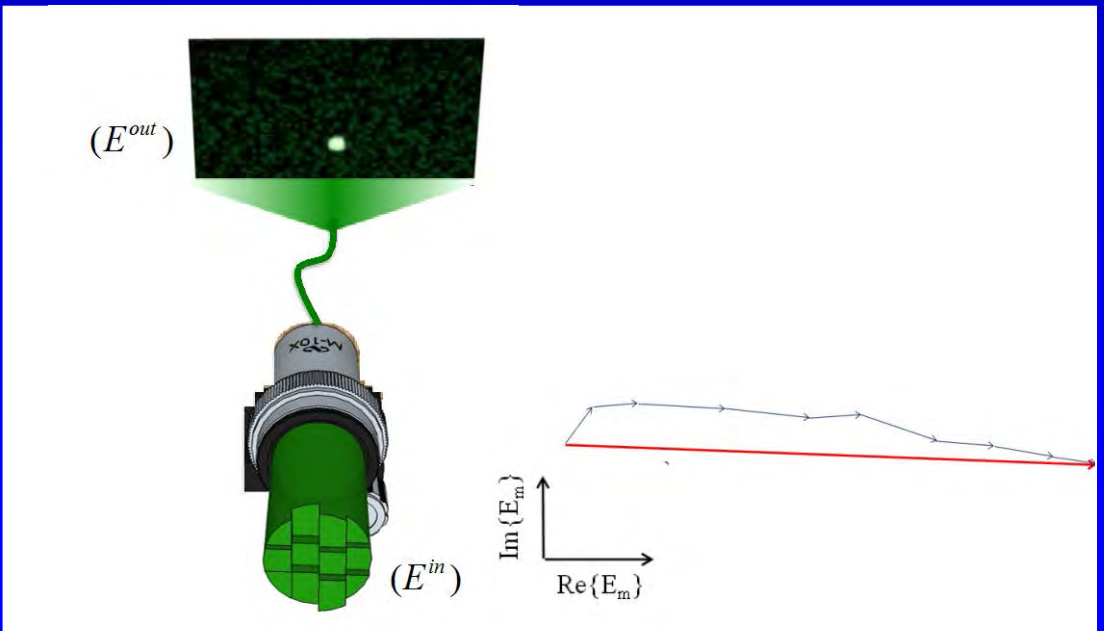
**Antonio Caravaca Aguirre and Rafael Piestun**

University of Colorado at Boulder

*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



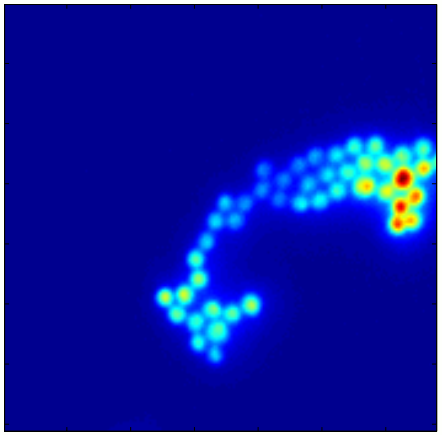

- Fiber is pre-calibrated
- Shape and temperature changes modify the spatial configuration of optical modes

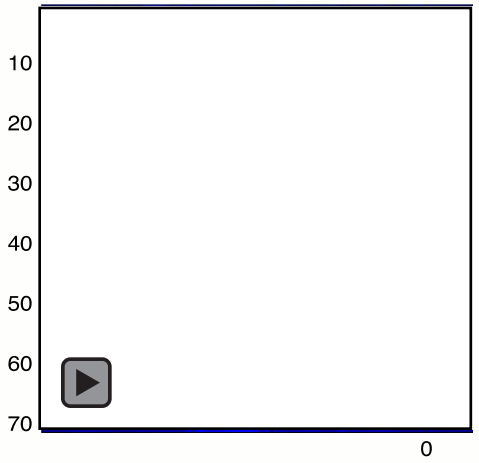
**Approach**

- Fast re-focusing system
- Explore robust fibers + fast calibration

# Fluorescence image reconstruction

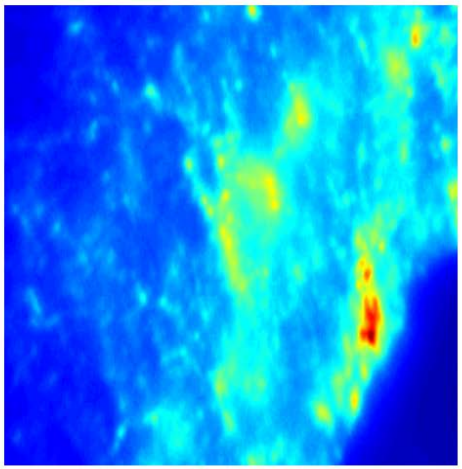
4μm fluorescent beads

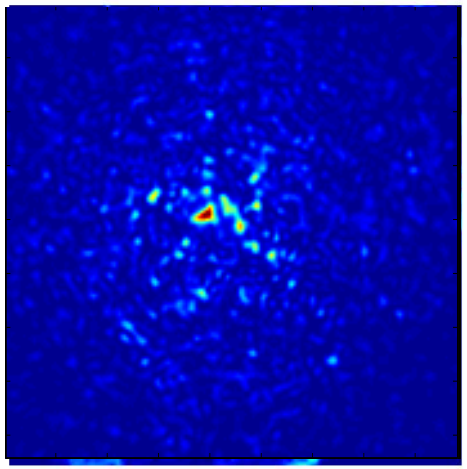





Mouse brain slice
labeled with Alexa 532

Fluorescence image





*Thanks to Dr. Shay Ohayon and Dr. DiCarlo from MIT for the preparation of the samples*

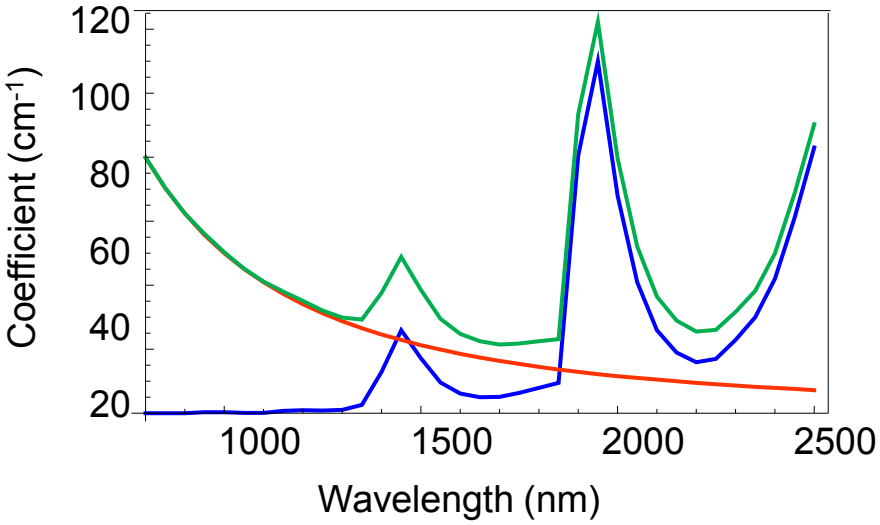
# Multiplexed Multiphoton Interrogation of Brain Connectomics

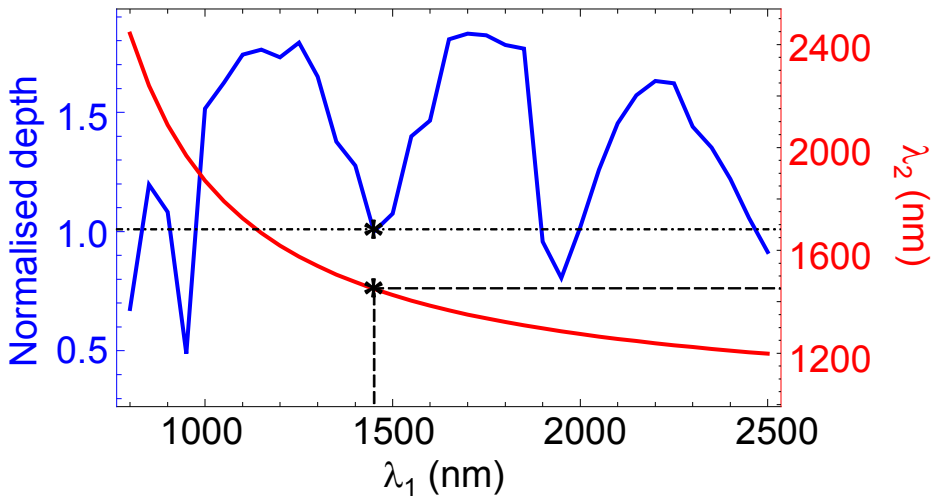
PI: Ramachandran; Xue; Mertz; 1R21EY026410-01; Program Start: Oct. 2015

**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.**

**Aim 3:Proof of principle of non-degenerate 3-photon microscopy in labelled mouse brain.**




**Degenerate Excitation**

**1300 & 1600-1700nm**

**Non-Degenerate Excitation**

- *Many more possibilities:*
  - *(3 $\lambda_1$) or (2 $\lambda_1$ + 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**

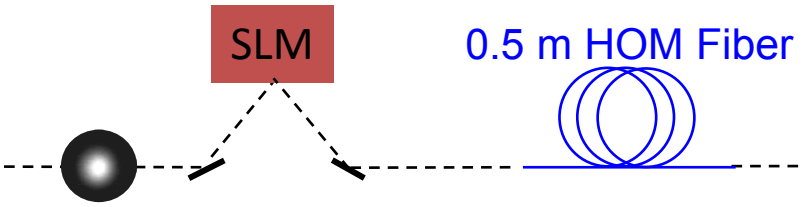
**Fiber sources… turn key, ↓ $, ↑ efficiency, ↑ power (rep. rate), flexible**

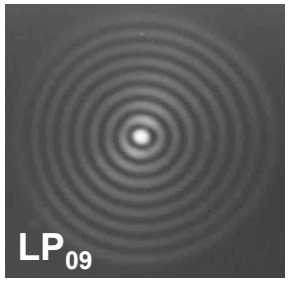
**but cannot scale energy at 1300 nm today.**

BOSTON UNIVERSITY

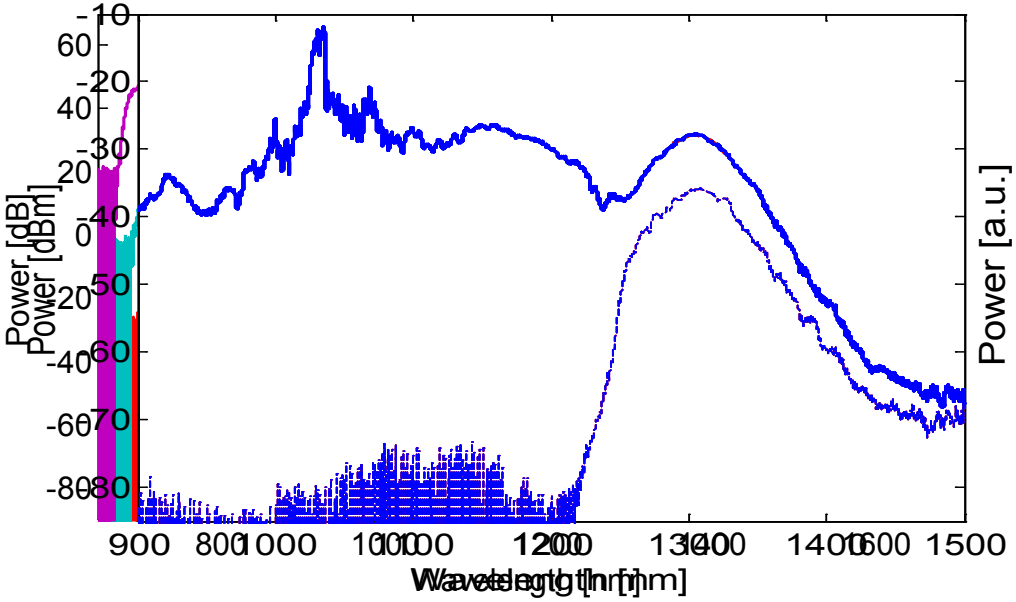
# High Energy Raman Soliton Shifting

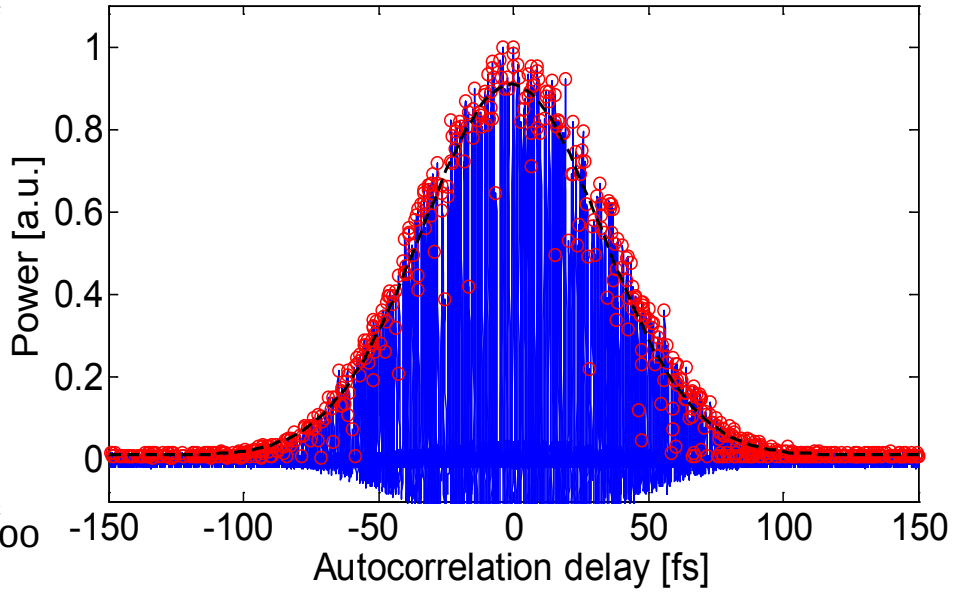





Calmar Cazadero (370 fs; 1030 nm)




$\lambda$ ~ 1308 nm; $\tau_{FWHM}$ ~ 50 fs;

$E_{pulse}$ ~ 30 nJ; $P_{peak}$ ~ 0.6 MW

Rep. rate = 120 kHz; $P_{av}$ ~ 3.6 mW

**Next steps:**

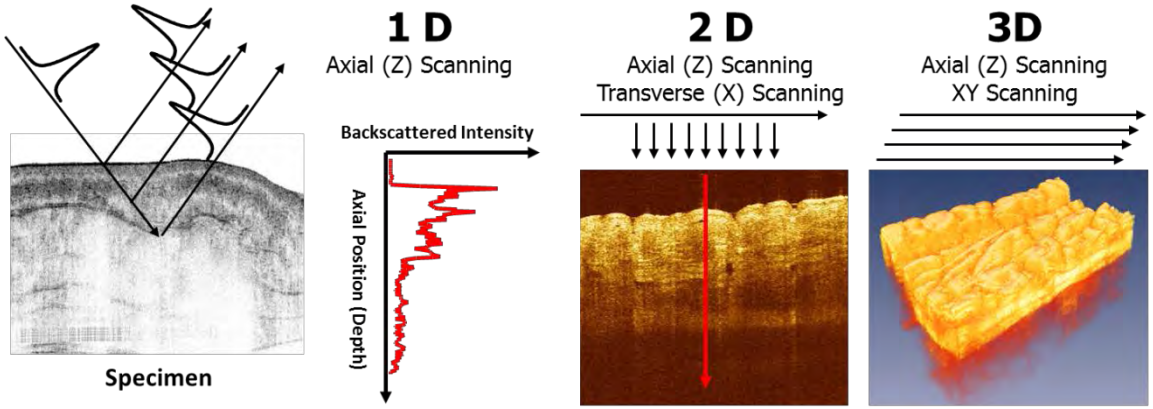
**Shift to next window (1700 nm)**

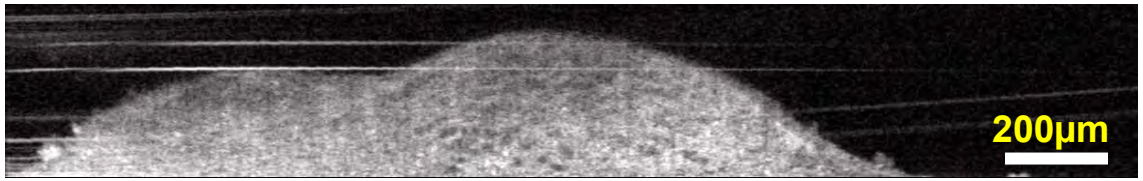
**Scale energy by 2-3x**

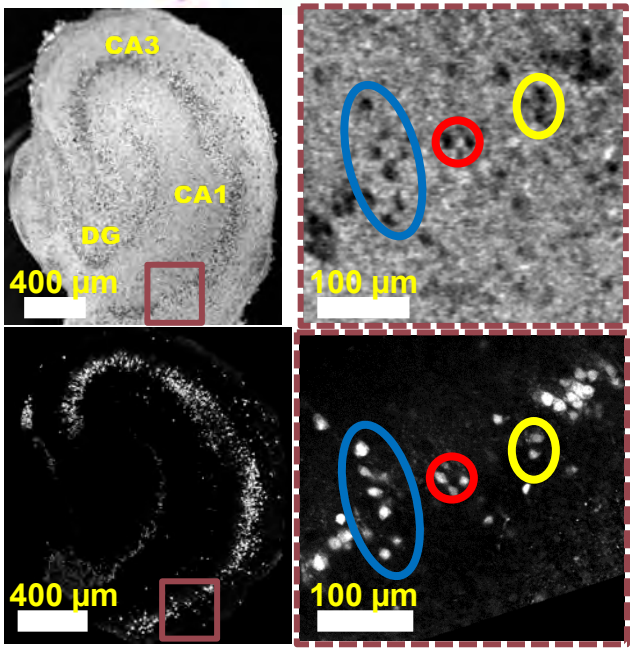
# Space-division multiplexing optical coherence tomography for large-scale, millisecond resolution imaging of neural activity (1R21EY026380-01)

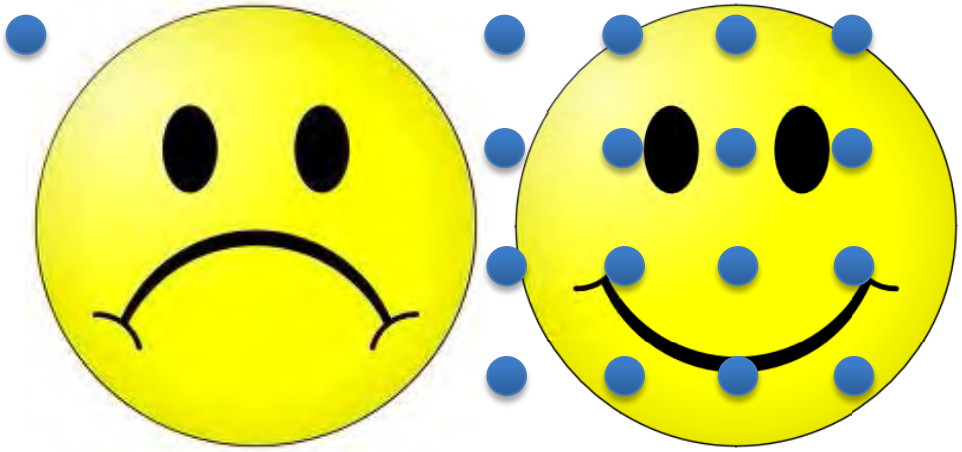
**Co-PIs: Chao Zhou, Yevgeny Berdichevsky**

LEHIGH UNIVERSITY.




- ✓ 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.

Zhou, *et al*, *Optics Express*, 21(16), 19219-19227, 2013
Li, *et al. Neurophotonics*, 1(2), 025002, 2014

# Space-division multiplexing optical coherence tomography for large-scale, millisecond resolution imaging of neural activity (1R21EY026380-01)

**Co-PIs: Chao Zhou, Yevgeny Berdichevsky**

LEHIGH UNIVERSITY.

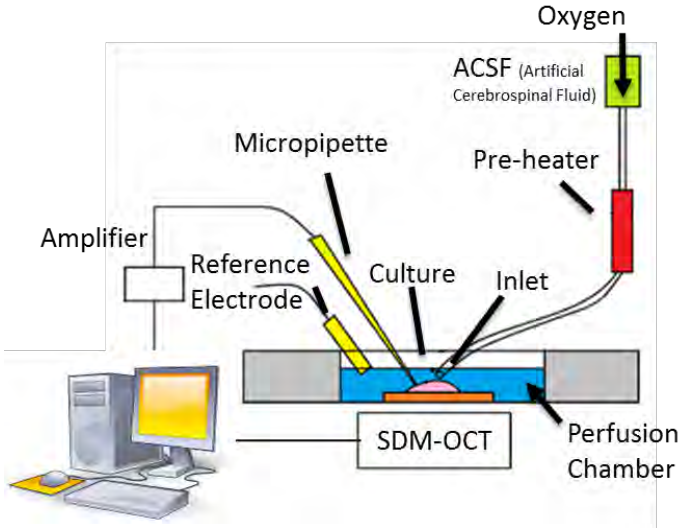
## Specific Aims:

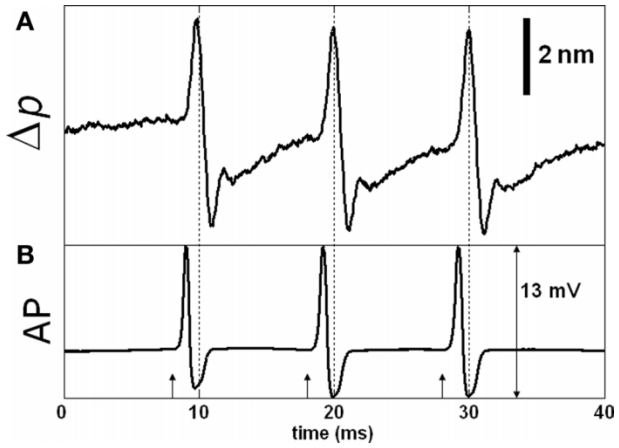
**Aim 1:** Develop an integrated electrophysiology and ultrahigh speed SDM-OCT imaging system to record **fast intrinsic optical signals** associated with neural activity.

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.

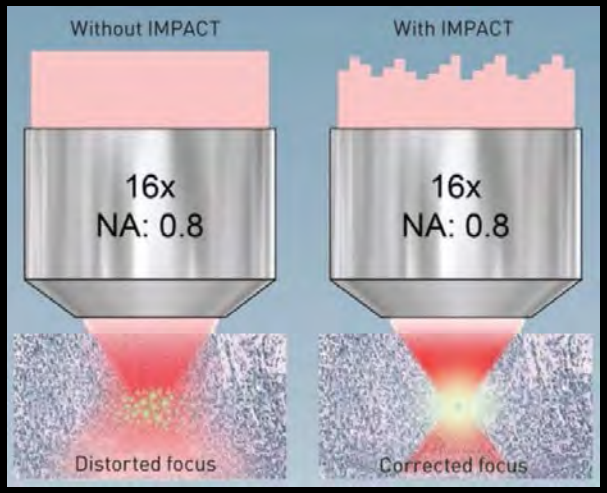


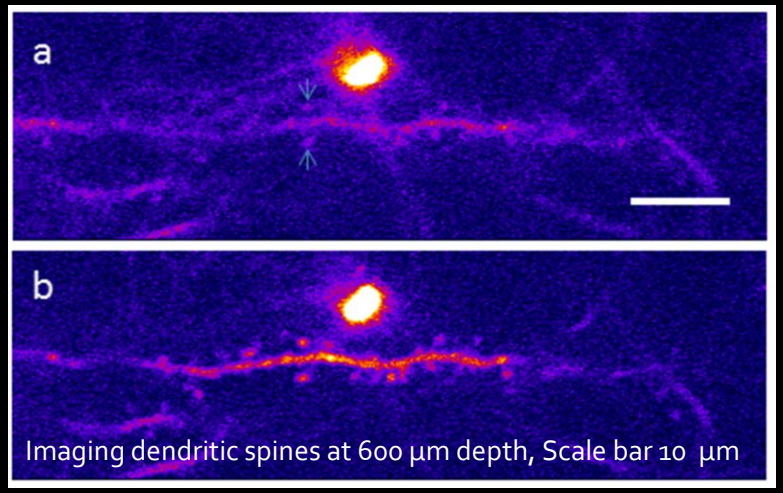




Akkin, *et al*, *Frontiers in Neuroenergetics*, 2: 22, 2010

# Wavefront engineering for high resolution deep tissue calcium imaging

## Cui lab at Purdue University



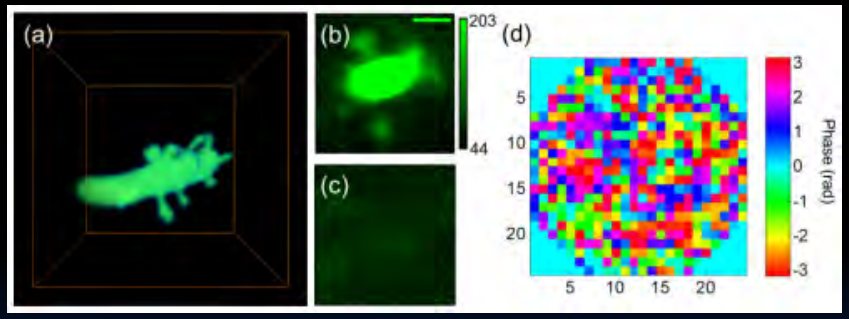

Iterative multiphoton adaptive compensation technique (IMPACT)

Opt Lett 36 (6), 870-872 (2011)

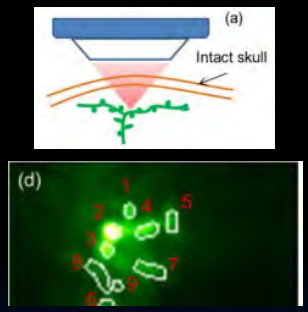
PNAS 109(22):8434 (2012)

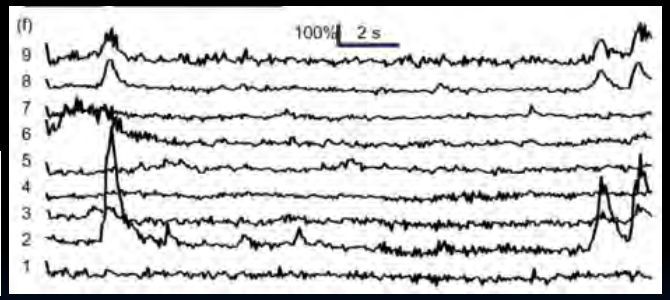
Opt Express 20(15):16532 (2012)

## High resolution imaging through intact skull

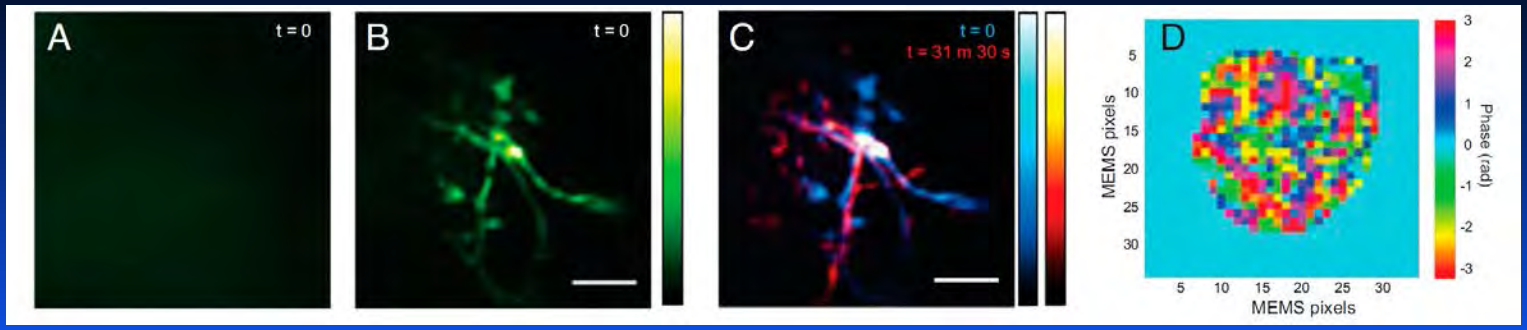



Opt Express 22(20):23786 (2014)



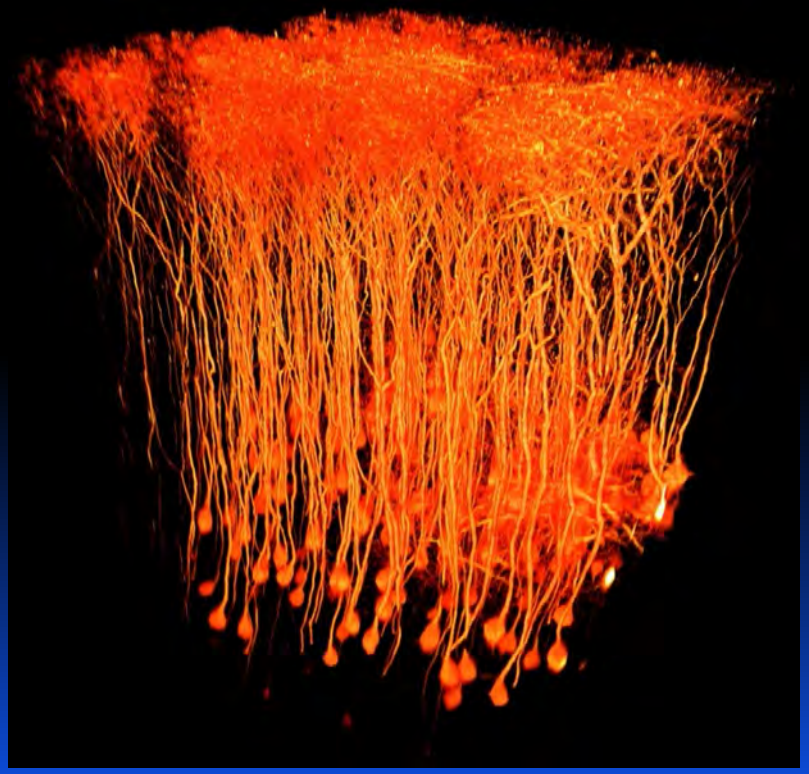

Opt Express 23(5):6145 (2015)

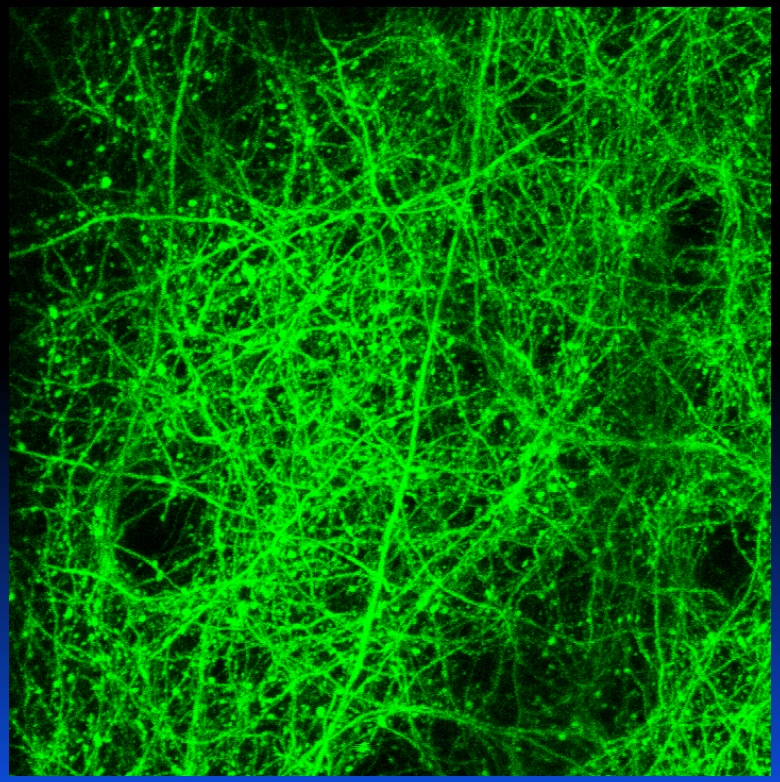



PNAS 112(30) 9236-9241, (2015); Opt Express 23(6):7463 (2015); Scale bar: 5 µm

# 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





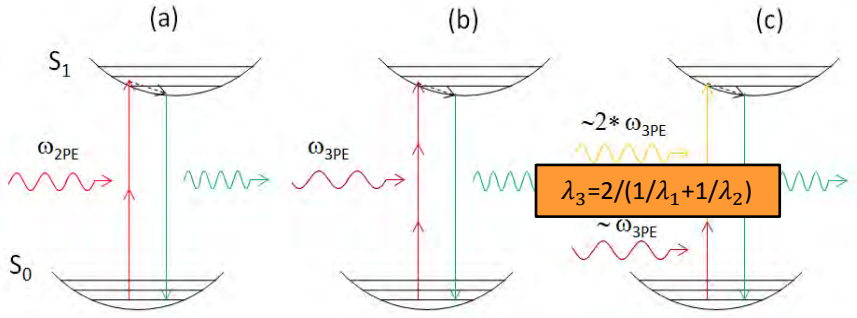
Acknowledgment: NIH, Purdue University, HHMI.

# Non-Degenerate Multiphoton Microscopy for Deep Brain Imaging

## U01 NS094232

## MPI: Devor A., Fainman Y.

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

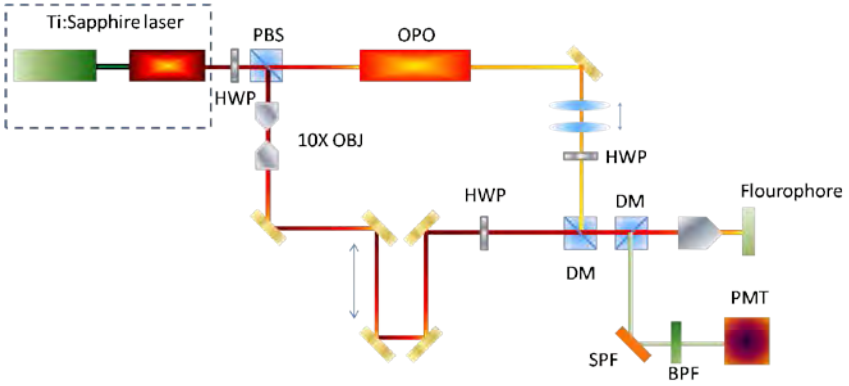


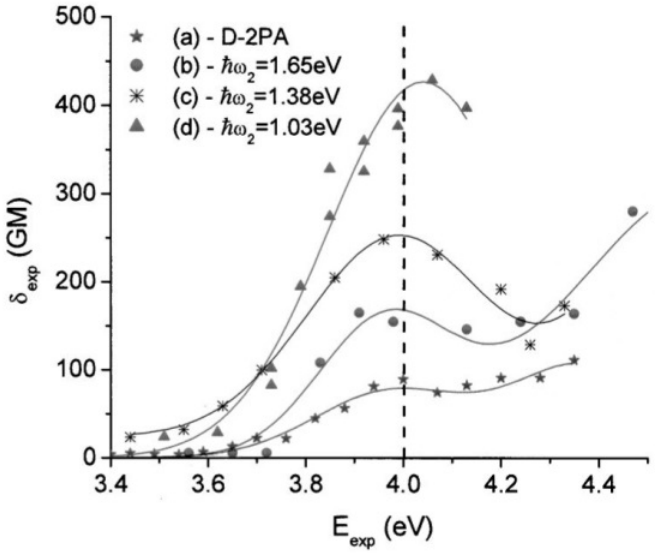

$F \propto I_{NIR}{}^2 \sigma_{D2PE}$ "conventional"

$F \propto I_{NIR}{}^3 \sigma_{3PE}$ Horton.. Xu 2013

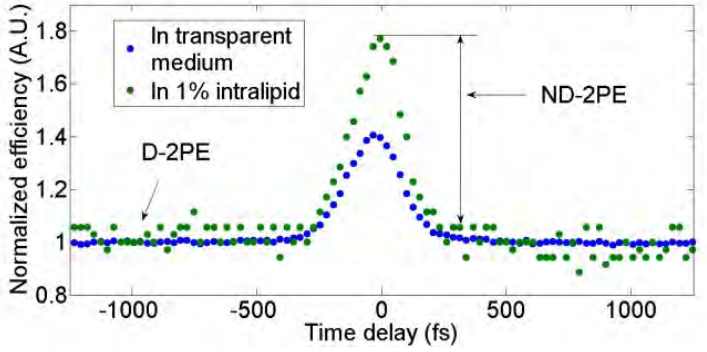
$F \propto I_{NIR} I_{SWIR} \sigma_{ND2PE}$ Mahou.. Beaurepaire 2012

Mu-Han Yang Shaya Fainman Roger Tsien Sergei Vinogradov




Hales.. Brédas 2004



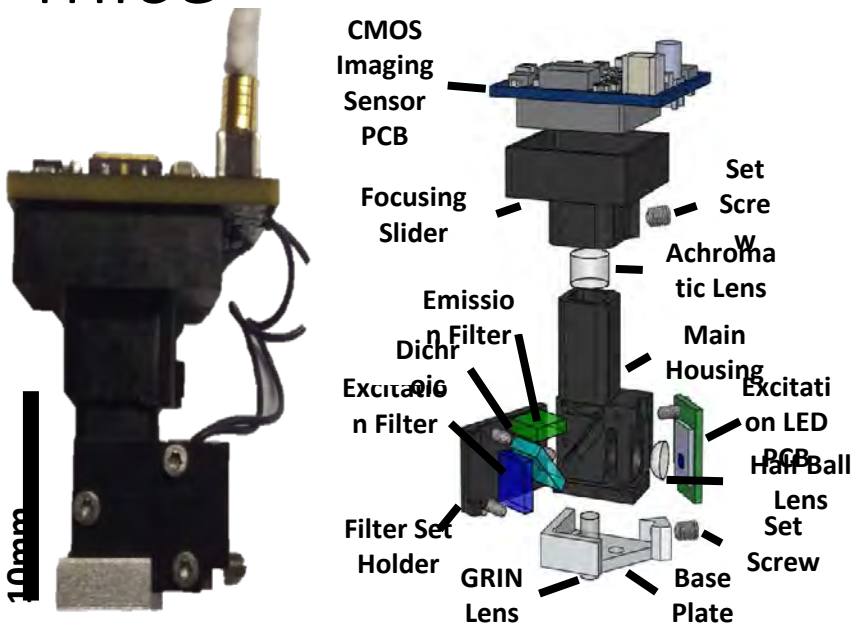

- Create a supercontinuum to allow efficient search for the optimal combination of NIR and SWIR wavelengths
- Implement AO to correct the phase distortions experienced by the NIR beam
- Strategically displace NIR and SWIR beams to avoiding surface excitation

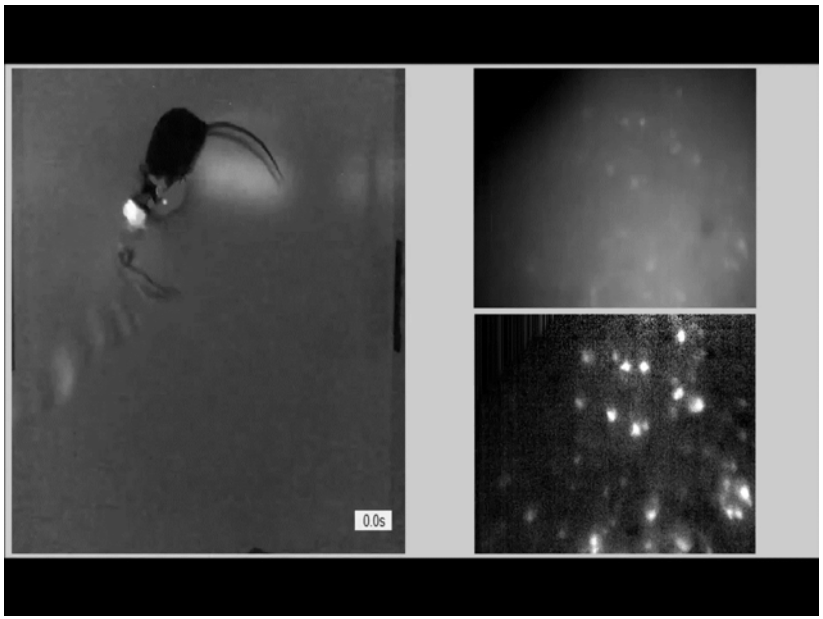
# Miniscope BRAIN Initiative

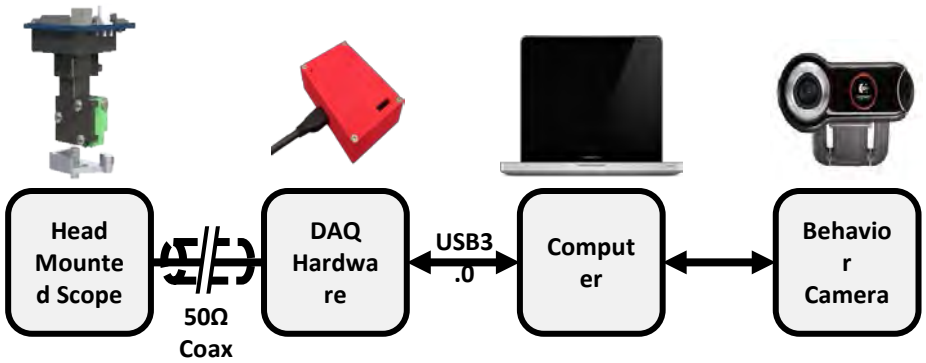
UCLA

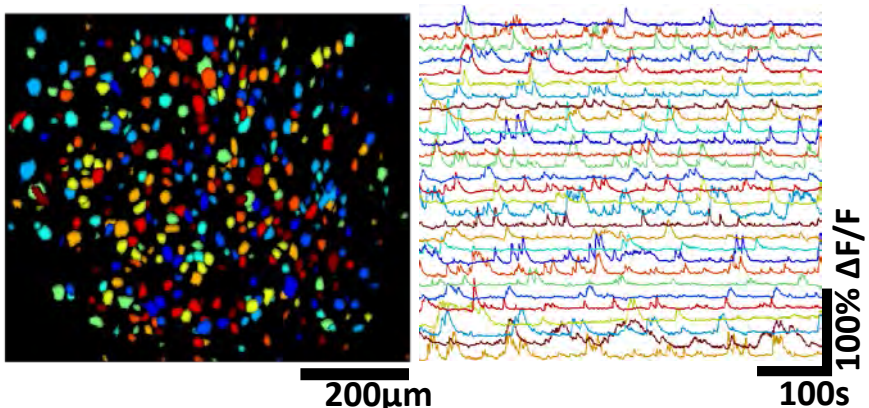
Peyman Golshani (Communicating PI)
Alcino Silva
Baljit Khakh
Dejan Markovic
Daniel Aharoni

# Building and sharing next generation open-source miniaturized microscopes for imaging activity in freely behaving mice

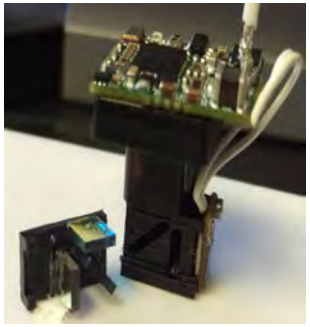


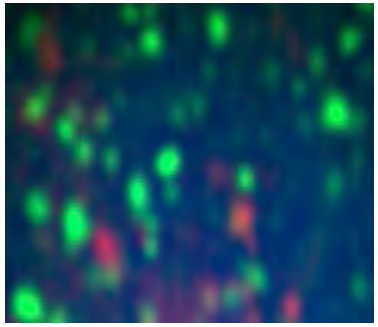



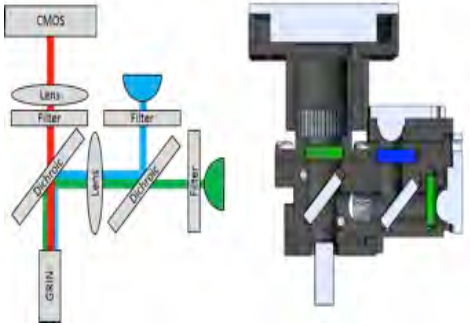



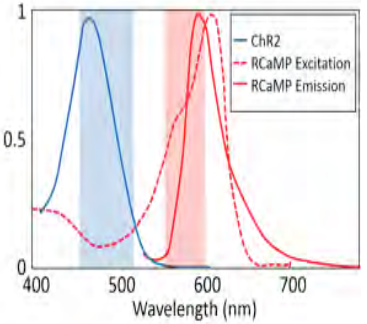
- Two-channel wearable miniaturized microscope



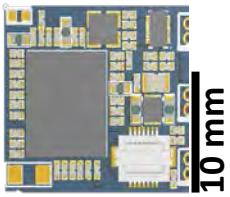


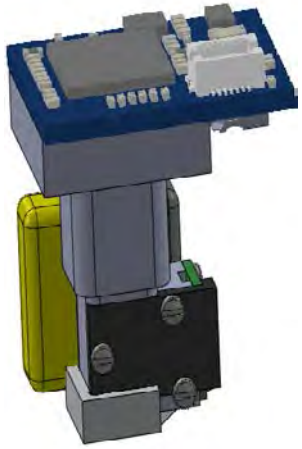
- Optogenetics-capable wearable miniaturized microscope



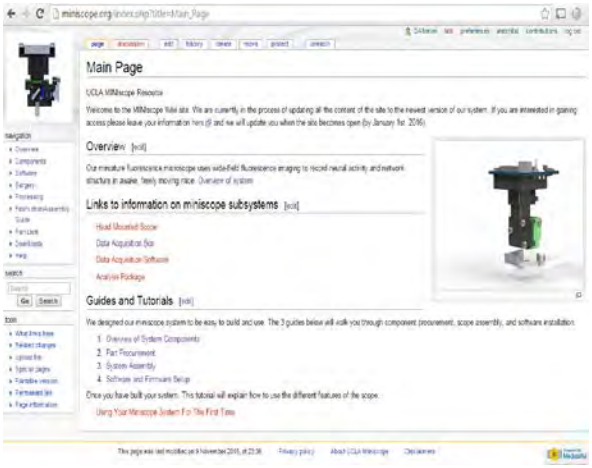




- Wireless miniaturized wearable miniaturized microscope



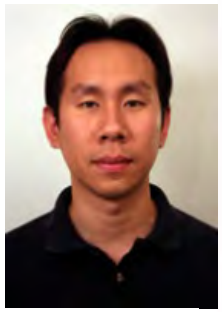
- Create an open-source platform for freely sharing this technology



# Multi-area two-photon microscopy

Group Fritjof Helmchen
Brain Research Institute
University of Zurich
Switzerland

Fritjof Helmchen



Jerry Chen

Philipp Bethge

Fabian Voigt

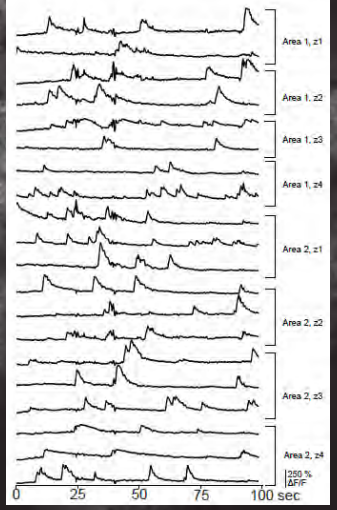
University of Zurich[UZH]

YCX2.60 in L2/3 of transgenic mouse neocortex

100 µm

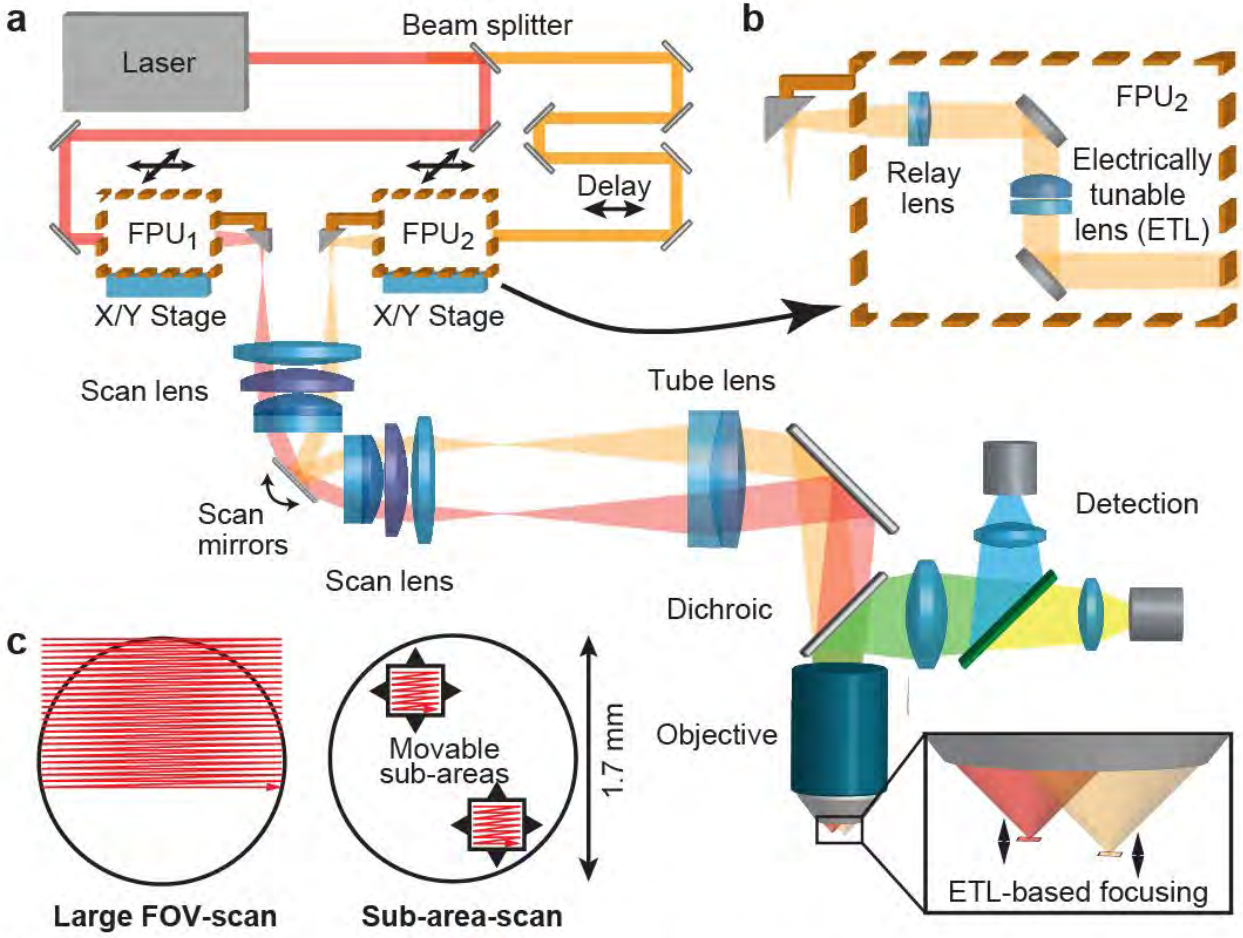
100 µm

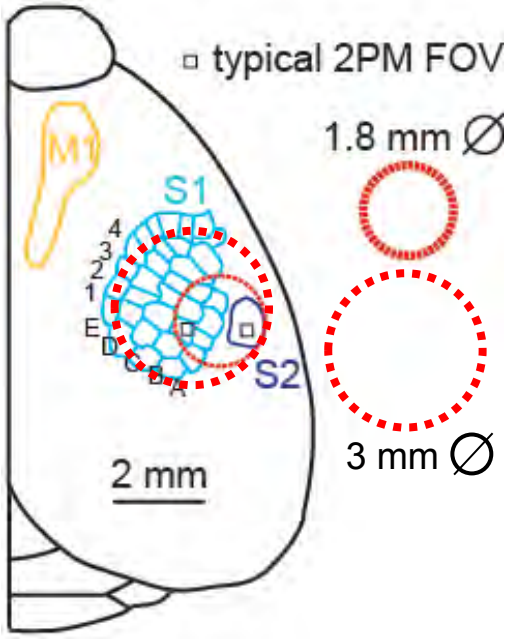
GCaMP6m signals



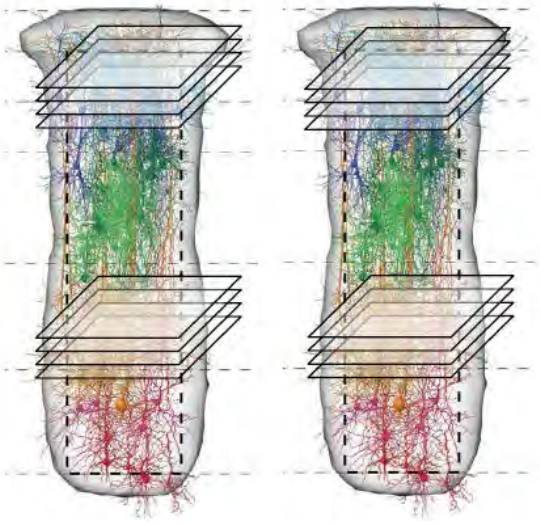

500 µm

# Multi-area two-photon microscopy




Next goal:



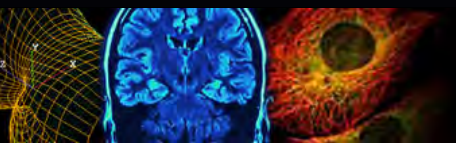
# SCAPE microscopy for high-speed in-vivo volumetric microscopy in behaving organisms

**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. Mann[2,6], Wesley B. Grueber[3,6], Randy M. Bruno[4,5,6] and David Schoppi[k7]**
**Matthew B. Bouchard[1], Venkatakaushik Voleti[1], Wenze Li[1], Cesar Mendes[2], Clay Lacefield[4], Marie Greaney[7], Evan Schaffer[8]**

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

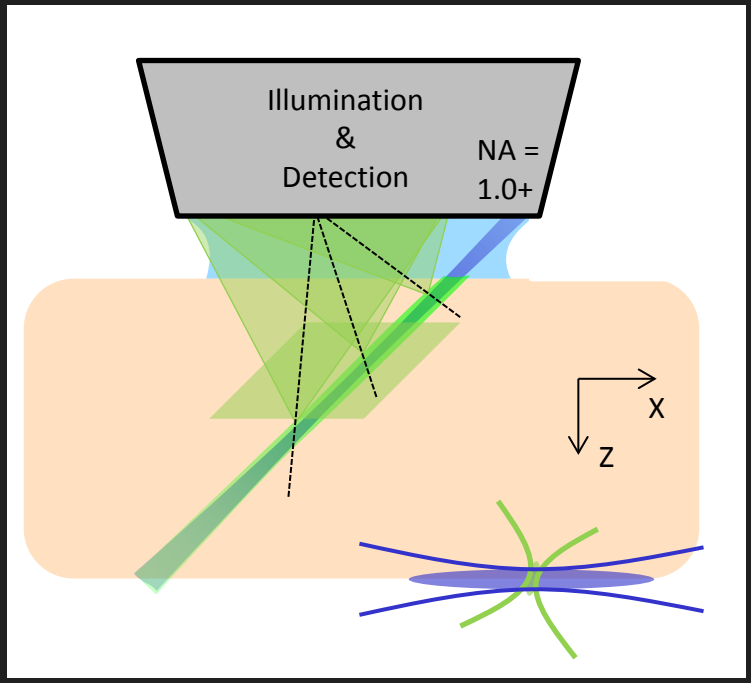
BIOMEDICAL ENGINEERING

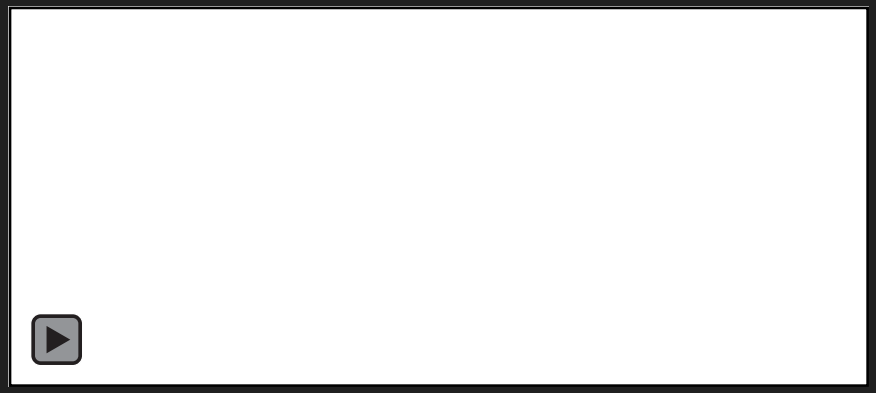


COLUMBIA UNIVERSITY
IN THE CITY OF NEW YORK

# **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.

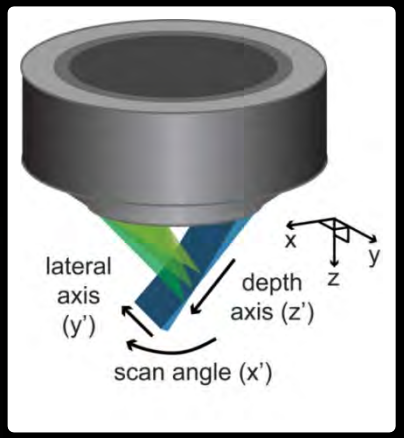


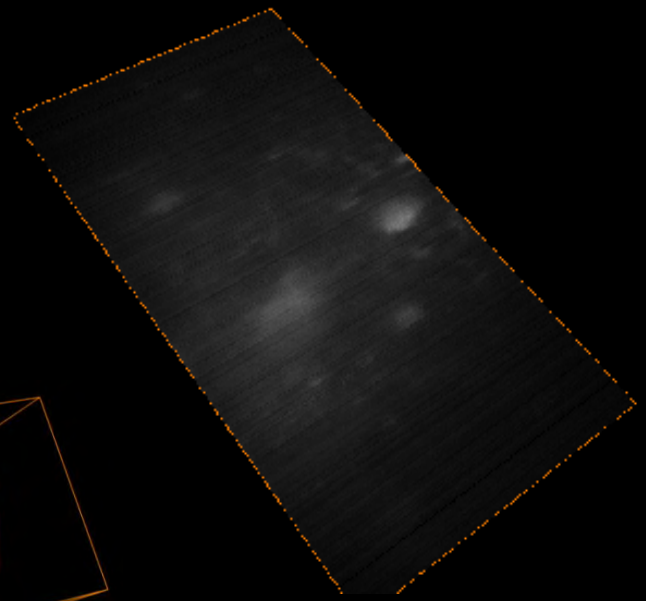
Columbia University
Laboratory for Functional Optical Imaging

Bouchard et al, Nature Photonics, 2015

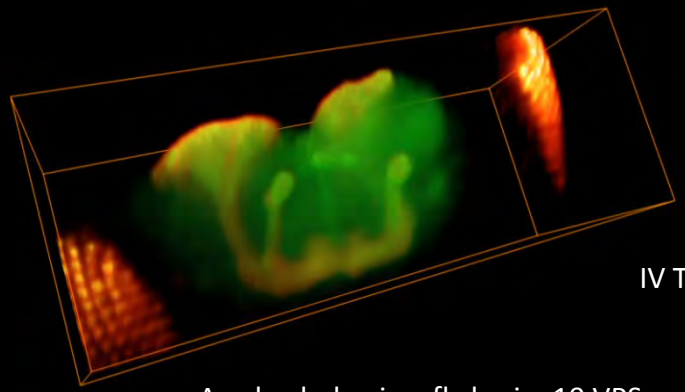
Elizabeth M C Hillman, BRAIN NIH (2015)

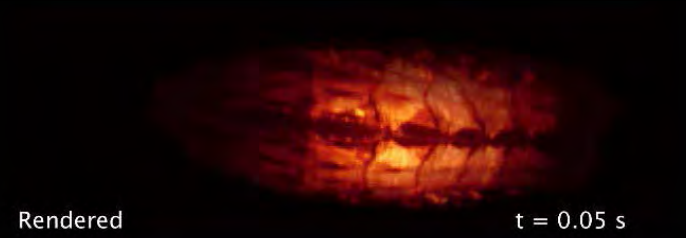

Bouchard et al, Nature Photonics, 2015

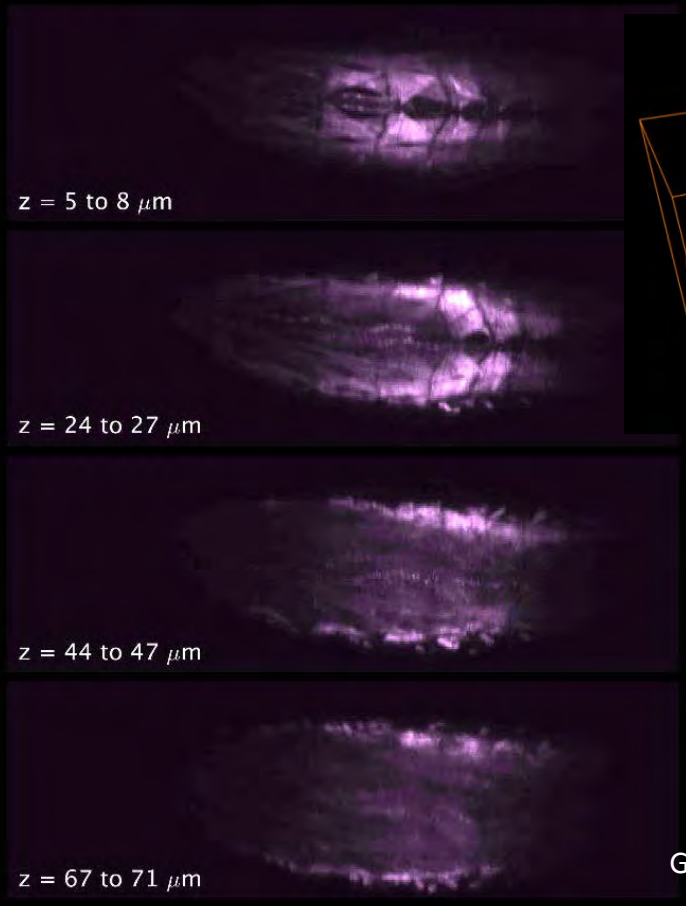


IV Texas red-dex + GCaMP6F in layer 5 neuron apicals, awake behaving mouse 10 VPS

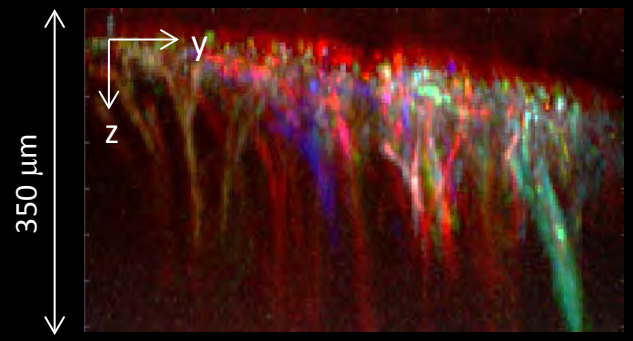


Awake, behaving fly brain, 10 VPS

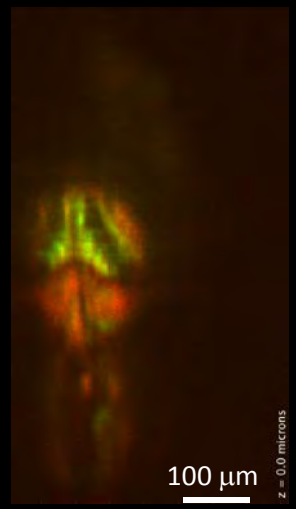



mhc-GFP Drosophila larva 20 VPS




GCaMP6F layer 5 neuron apicals, awake behaving mouse 10 VPS



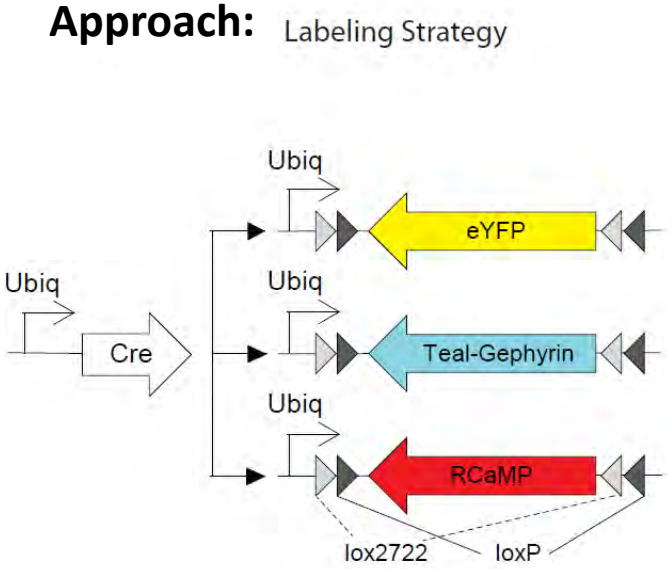

Zebrafish brain 10 VPS

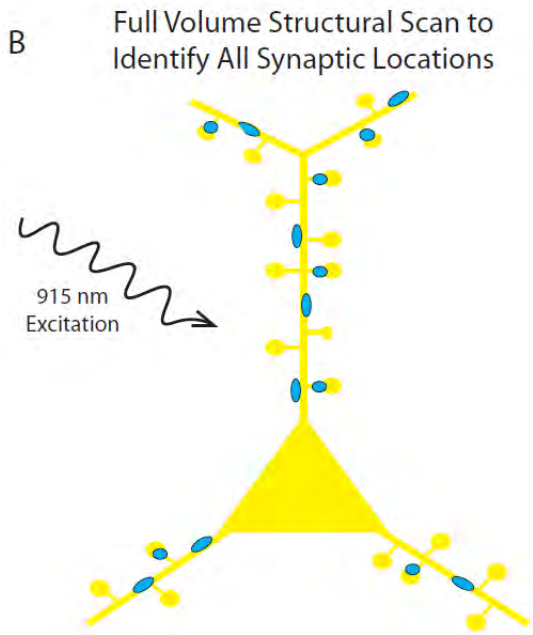
# Next generation high-throughput targeted excitation imaging in vivo

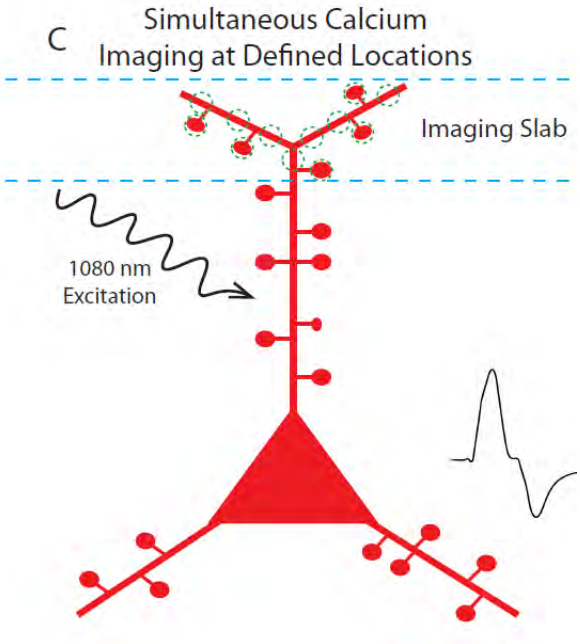
*Chris Rowlands, Kalen Berry, Jaichandar Subramanian, Yi Xue, Yu Takiguchi, Peter So, Elly Nedivi*
Massachusetts Institute of Technology

**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:**




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

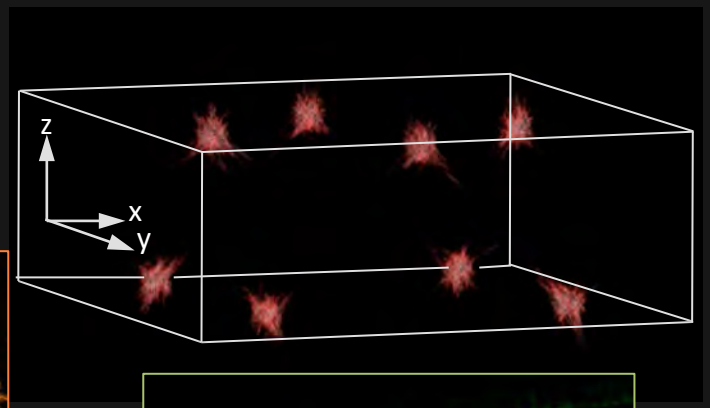
**Serge Picaud & Valentina Emiliani**
**Key investigators: Simon Schultz, Claire Wyart, Amanda Foust, Jens Duebel, Olivier Marre**

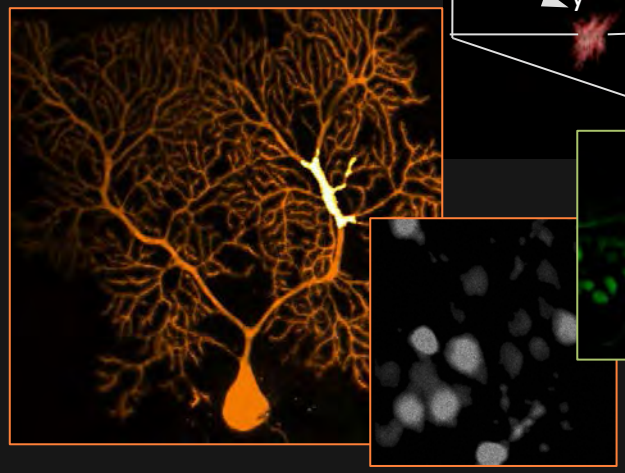
✓✓ Control of brain signaling through holographic light shaping and optogenetics

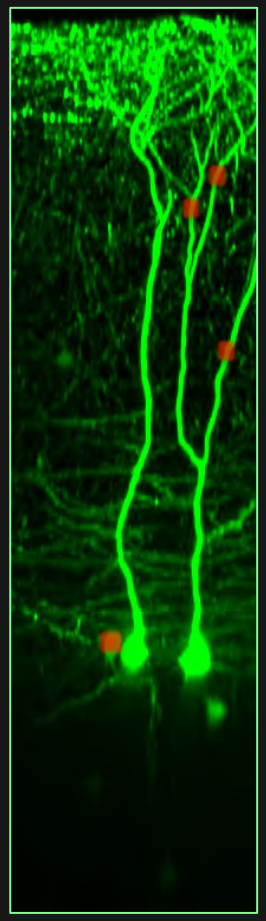
1. *Development of a holographic op+cal system for in vivo, in depth neuronal circuit manipula+on*

- *Precise sculpting of the excitation volume by spatial and temporal shaping of optical wave fronts*
- Simultaneous, Muli-Location, Three-Dimensional

•Independent control of Position, Shape, and Intensity

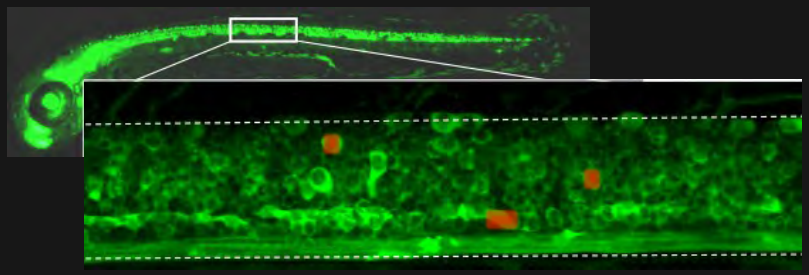


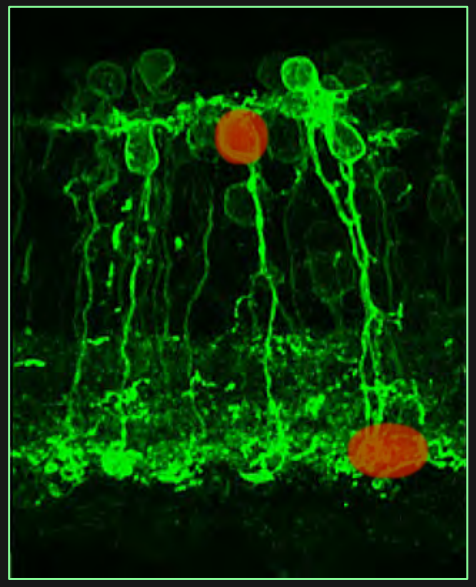





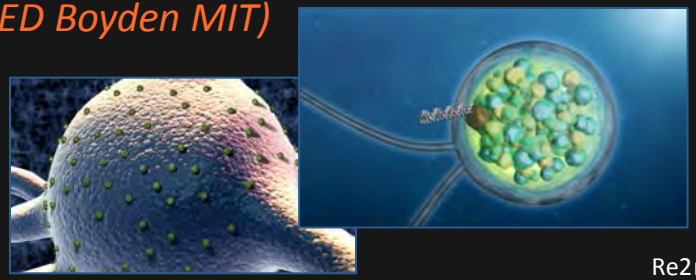
*2. Iterative optimization of the system in different model systems*

- 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 Simula2on, Oscar Hernandez; Zebrafish, Claire Wyart; Re2nal 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

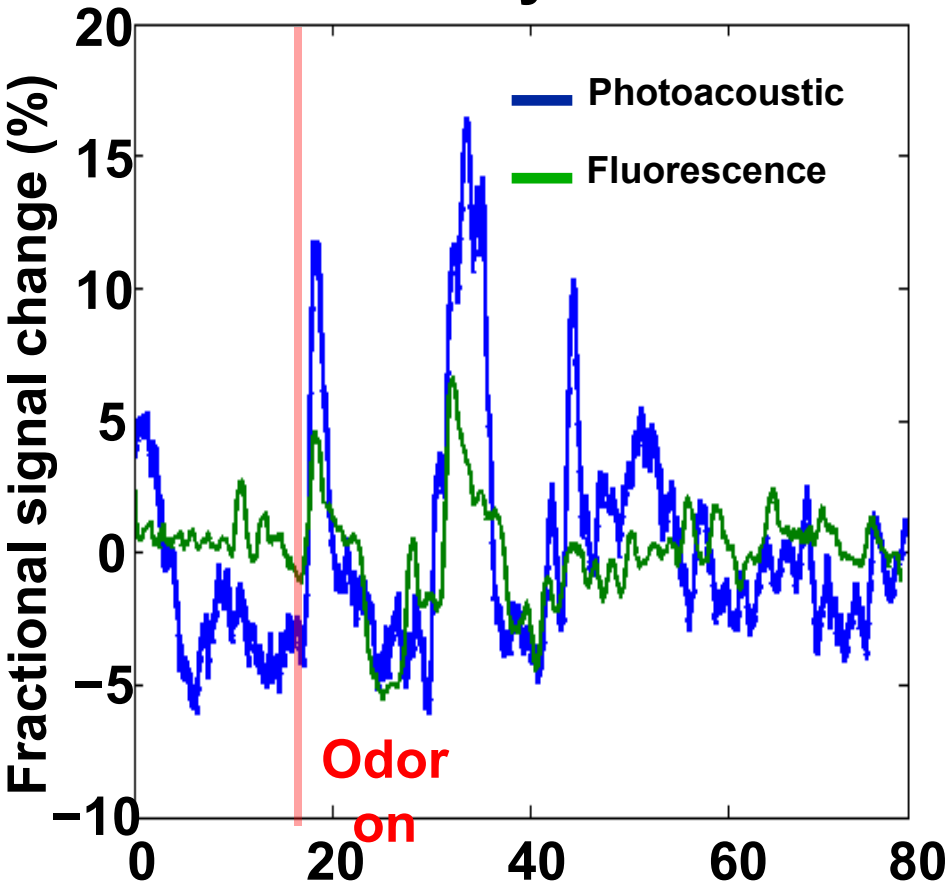
Investigators: ROUKES, MICHAEL L (contact); SHEPARD, KENNETH L; SIAPAS, ATHANASSIOS ; TOLIAS, ANDREAS

# High-speed volumetric imaging of neuronal network activity at depth using Multiplexed Scanned Temporal Focusing (MuST)

Investigator: VAZIRI, ALIPASHA

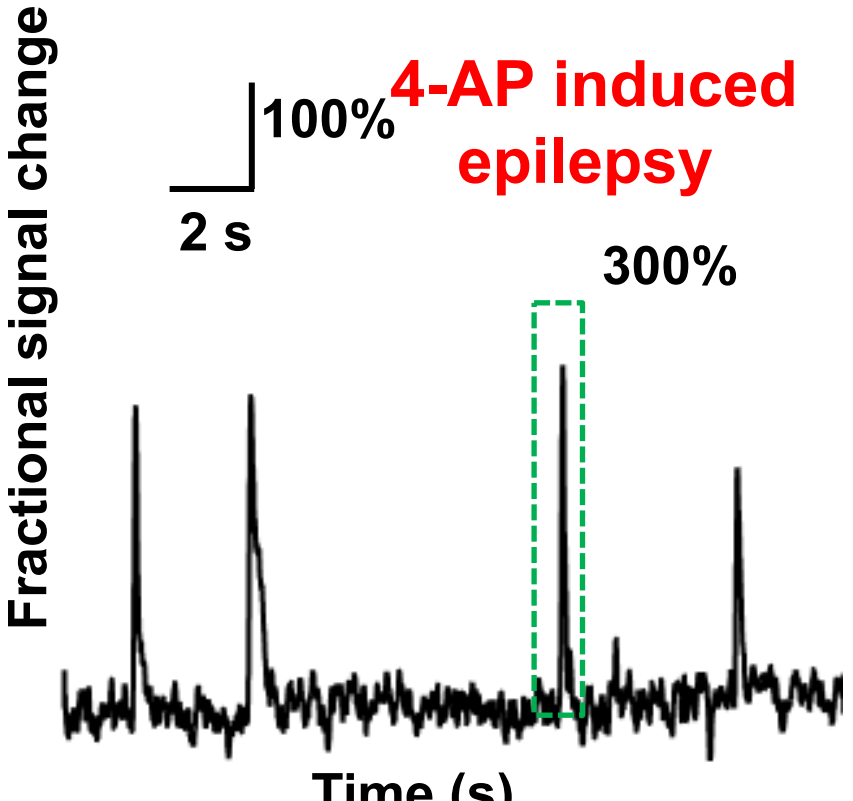
# Photoacoustic Calcium/Voltage Indicators

## Calcium-sensitive protein GCaMP5G in fruit fly brain



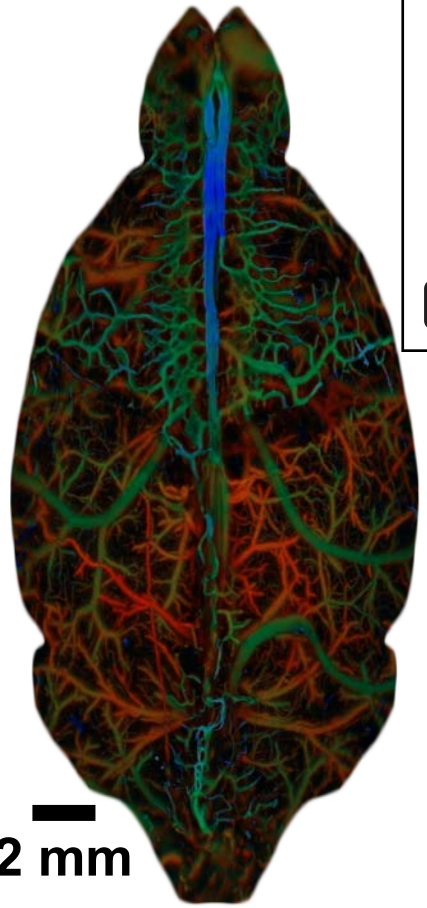

RY Zhang, B Rao, HY Rong, B Raman@WUSTL, LV Wang, *unpublished*

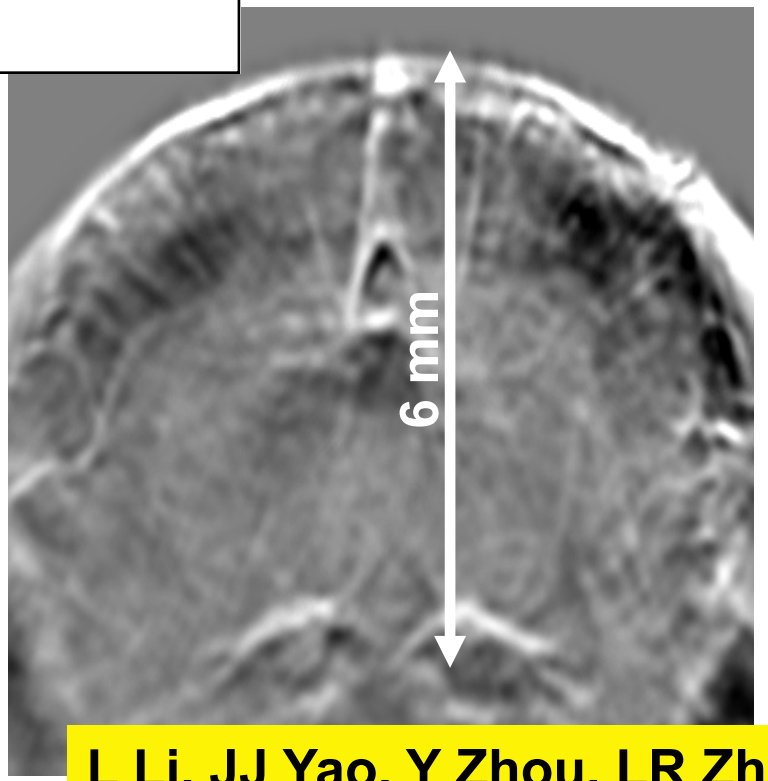
## Voltage dye Dipicrylamine (DPA) in mouse brain

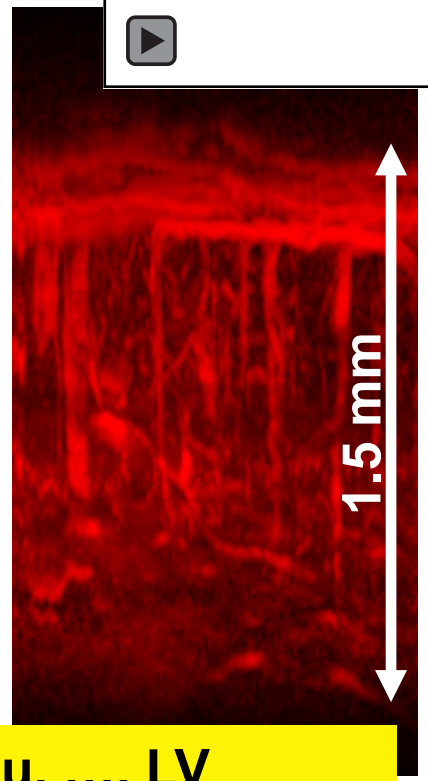



RY Zhang, B Rao, LV Wang, *unpublished*

# Photoacoustic Tomography of Mouse Brain *In Vivo*

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

# Optimization of 3-photon microscopy for Large Scale Recording in Mouse Brain

**PI: Chris Xu, Cornell University**

**Co-I: David Tank, Princeton University**

## 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.

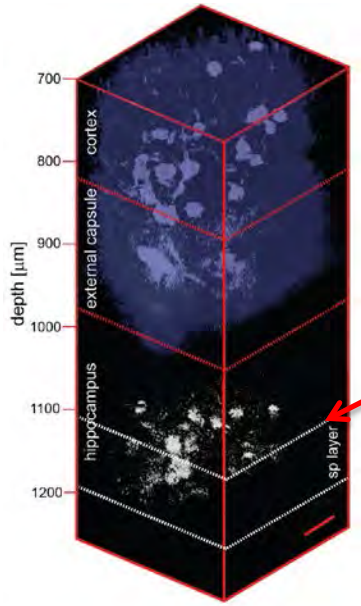
## Progress

- 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.

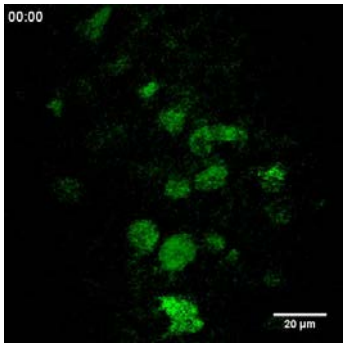
## On-going and near term future work

- 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.

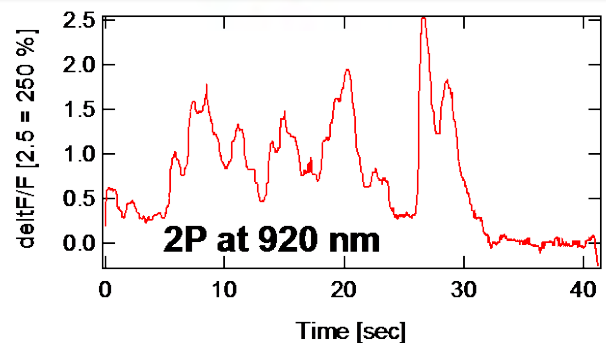
# 3P-imaging of GCaMP6s neurons at 1.35 µm

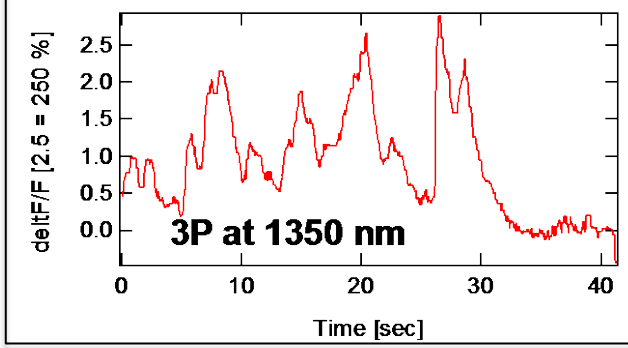



**Imaging depth at 1.13 mm**



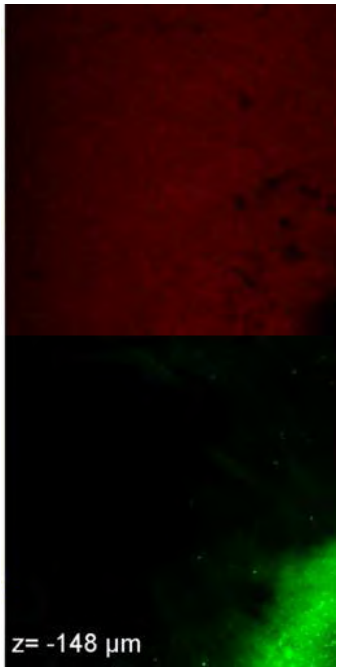

**5 min movie, sped up 10x**



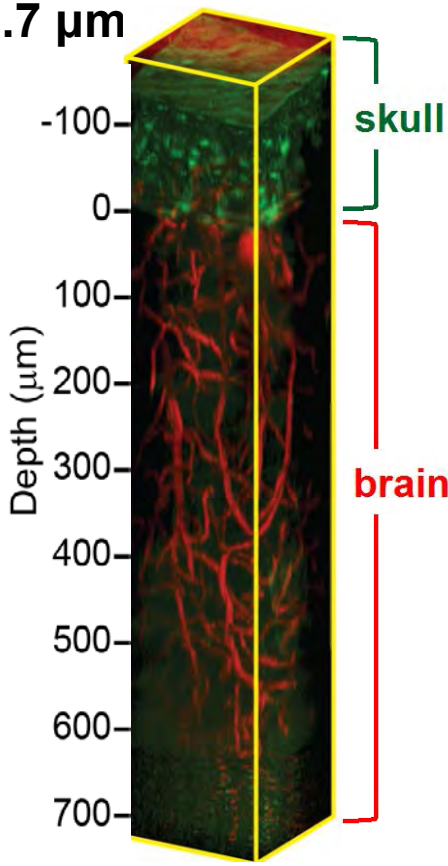

Simultaneous 2-photon (top) and 3-photon (bottom) imaging of the same GCaMP6-labeled neuron at ~ 200 µm depth. The same time trace is obtained.

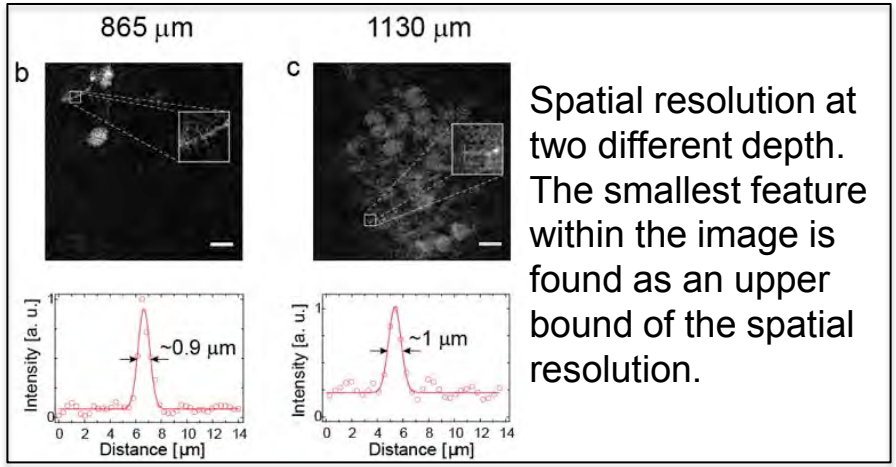
# 3P-imaging through unthinned mouse skull at 1.7 µm




Green: THG

Red: Texas-red fluorescence



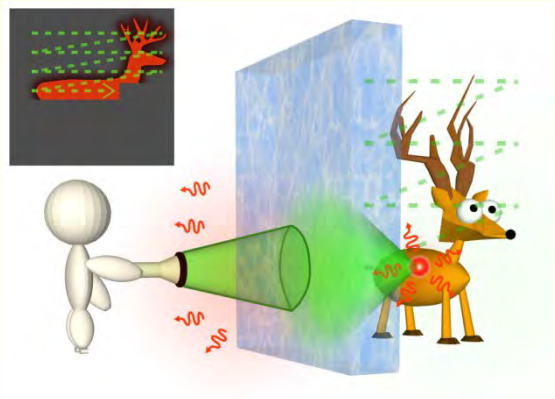



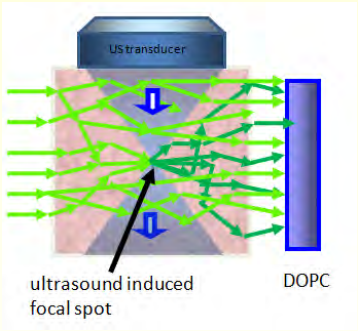

Spatial resolution at two different depth. The smallest feature within the image is found as an upper bound of the spatial resolution.

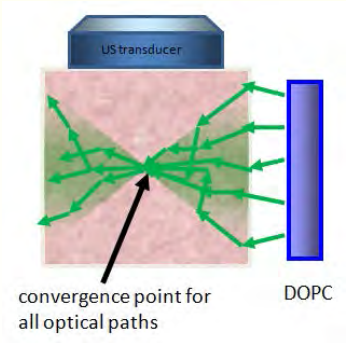
# Time-reversal optical focusing for non-invasive optogenetics

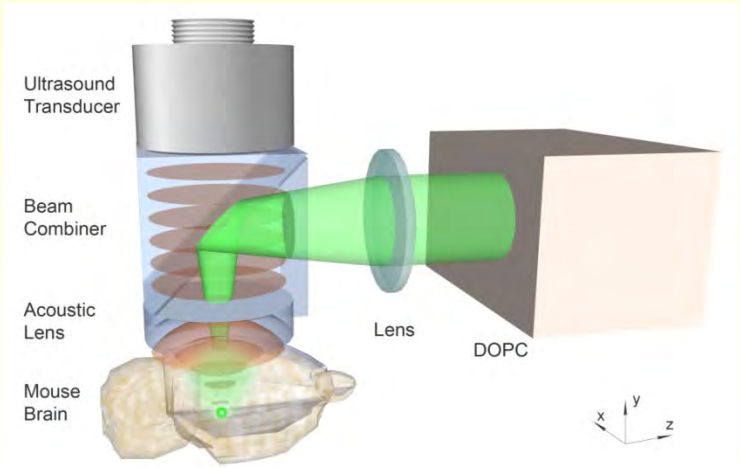
Changhuei Yang, Viviana Gradinaru
California Institute of Technology

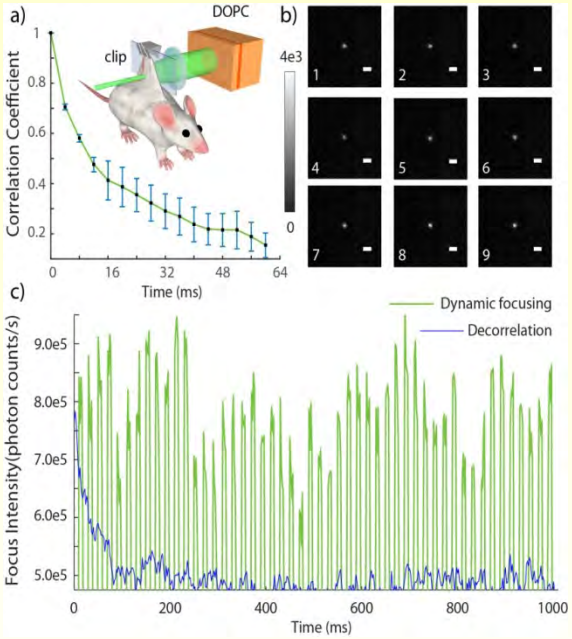
**We are developing a high speed time-reversal ultrasound encoded optical focusing technology for optogenetic use.**



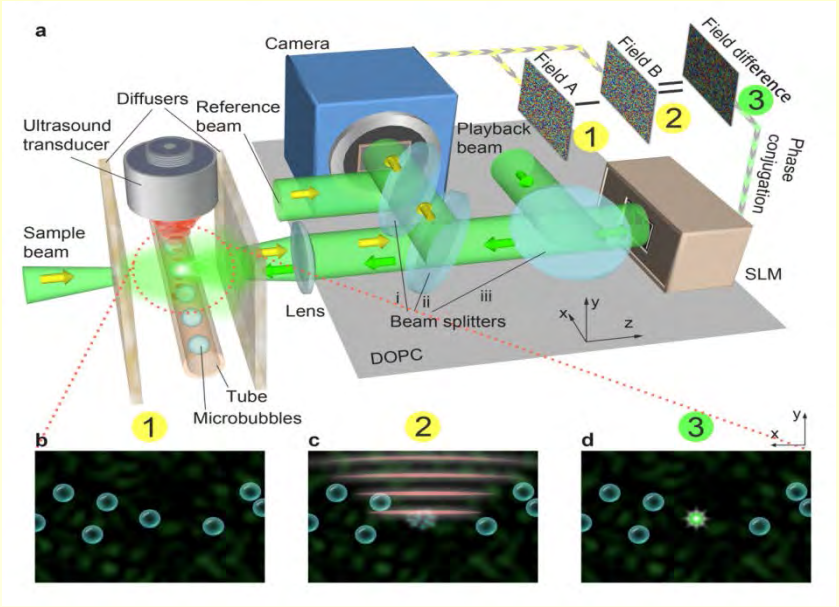


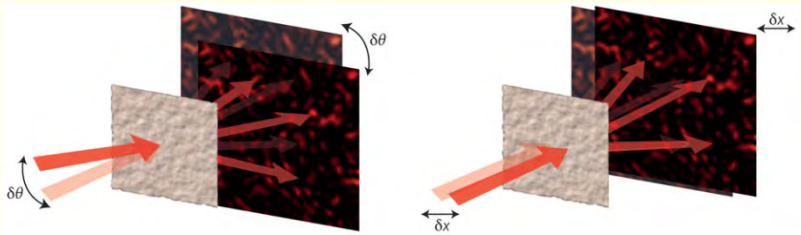






Demonstration of dynamic focusing through living animal.




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.


D Wang, E Zhou, J Brake, H Ruan, M Jang, C Yang, Optica 728 (2015).
M Jang , H Ruan, C Yang, Nature Comm (accepted)
B Judkewitz, R Horstmeyer, I Vellekoop, I Papadopoulos & C Yang, Nature Physics, 684 (2015)