Advanced Optical Microscopes and their Applications to Neuroscience

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(with Laurent Bentolila)

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Department of Physics and Astronomy
Outline

Introduction
- Observations of Nature
- Principle of Microscopes

Advanced Microscopes
- Super resolution
- High-speed

Applications to Neuroscience
- High-speed motion and action potentials
- Neural networks

Future directions
- Voltage sensing dyes
- Optical Excitation
Introduction
Brain

100 Billions Neurons

Universe

100 Billions Galaxies

Observation of Natures
Seven Phases of Cosmic Evolution

14 billion years ago

Origin of Particles
Origin of Structure
Origin of Life
Origin of Consciousness
Andromeda

~100 Billions Stars in a Galaxy
Hubble Telescope
Hubble Deep Field

~100 Billion Galaxies
CERN and LHC in Geneva

27km Circumference

7+7=14 TeV
LHC Tunnel with Magnets
Particle detectors constructed at Westwood, now at LHC, CERN
First Event at LHC – Recreation of the Big Bang! (Nov 7, 2009)
What is Life?

- **Emergent Property**
  - Strongly-interacting, complex system
  - $\approx 10^4$ of different proteins in one cell
  - $\approx 10^{14}$ cells in one life
  - $\approx 10^{11}$ neurons in our brain, $\approx 10^{14}$ connections

- **Continuous, countless “symmetry breaking” towards coherent states**
  - Origin of life
  - Evolution of life
  - Growth from a single cell to a multi-cell body
  - Learning and memory
How to observe the Life and Consciousness?

- We must look for “Live Life”
- Exactly the same way as we look for the “Origin of Universe”

Telescope ↔ Microscope

- Take advantages of the state of art “Photon Detectors” in particle physics.
Seven steps of cosmic evolution

Spontaneous Symmetry Breaking

14B years ago

Accelerator <-> Telescope <-> Microscope
Principle of Optical Microscopes
Origin of a Light Wave: When the valence electrons of atoms absorb energy, they are lifted from a ground level to more energetic states. After a very short time, the electrons drop back to ground level, releasing the energy in the form of a photon, an energy quantum associated with an electromagnetic wave.

Properties of a Light Wave:
- has an electric vector \( E \) and a magnetic vector \( B \).
- has a wave length \( \lambda \), an amplitude, and polarity (\( E \)).

High energy photon = shorter \( \lambda \) = Bluer color
Low energy photon = longer \( \lambda \) = redder color

Color Vision of the Human Eye:
- Violet: 410 nm
- Blue: 475 nm
- Green: 530 nm
- Yellow: 580 nm
- Red: 670 nm

Direction of Propagation
- 1 cycle = “wave length”

= natural white light
Prisms Used to Re-Direct Light In Imaging Path While Mirrors Are Used in Illumination Path
# Light Microscopy Timeline

<table>
<thead>
<tr>
<th>Year</th>
<th>Milestone</th>
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<tr>
<td>1595</td>
<td>Invention of the microscope (Milestone 1)</td>
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<td>1858</td>
<td>First histological stain (Milestone 2)</td>
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<td>1871</td>
<td>Synthesis of fluorescein (Milestone 2)</td>
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<td>1873</td>
<td>Diffraction limit theory (Milestone 3)</td>
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<td>1911</td>
<td>First fluorescence microscopy (Milestone 4)</td>
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<td>1929</td>
<td>First epifluorescence microscope (Milestone 4)</td>
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<td>1935</td>
<td>Phase contrast microscopy (Milestone 5)</td>
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<tr>
<td>1939</td>
<td>Polarization microscopy (Milestone 6)</td>
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<tr>
<td>1942</td>
<td>Immunofluorescence (Milestone 7)</td>
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<td>1955</td>
<td>Differential interference contrast (Milestone 8)</td>
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<td>1961</td>
<td>Concept of confocal microscopy (Milestone 9)</td>
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<td>1967</td>
<td>The dichroic mirror (Milestone 4)</td>
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<td>1972</td>
<td>Fluorescence correlation spectroscopy (Milestone 10)</td>
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<td>1976</td>
<td>FRAP (Milestone 10)</td>
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<td>FRET (Milestone 11)</td>
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<td>Calcium probes (Milestone 12)</td>
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<td>1981</td>
<td>Video-enhancement differential interference contrast (Milestone 8)</td>
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<td>1983</td>
<td>TIRF microscopy (Milestone 13)</td>
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<td>1987</td>
<td>Deconvolution microscopy (Milestone 14)</td>
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<td>1990</td>
<td>Realization of confocal microscopy (Milestone 9)</td>
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<td>Two-photon microscopy (Milestone 15)</td>
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<td>1993</td>
<td>Light sheet microscopy (Milestone 16)</td>
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<td>Single molecule microscopy (Milestone 17)</td>
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<td>1994</td>
<td>GFP (Milestone 18)</td>
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<td>1997</td>
<td>Fluorescent protein-based biosensors (Milestone 19)</td>
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<tr>
<td>1999</td>
<td>Red fluorescent proteins (Milestone 20)</td>
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<td>2000</td>
<td>Breaking the diffraction limit: STED (Milestone 21)</td>
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<tr>
<td>2002</td>
<td>Photoactivatable fluorescent proteins (Milestone 20)</td>
</tr>
<tr>
<td>2006</td>
<td>Breaking the diffraction limit: PALM/STORM (Milestone 21)</td>
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</tbody>
</table>

http://www.nature.com/milestones/light-microscopy
Some Properties of Lenses

**Refraction:** When a beam of light passes between substances with different **refractive indices**, such as air (n=1) into water (n=1.333), it is bent in a characteristic fashion (wavelength dependant).

**Focal Length**

**Lens Aberrations & Corrections:**

**Curvature of Field:** Plan

**Chromatic:** Apo

**Spherical:** Fluorite
Objective Design Criteria Most Critical for Fluorescence:

- Numerical Aperture
- Immersion choices
- Magnification
- Transmission UV/Vis/IR
- Chromatic Correction
- Coverslip vs Chamber
- Optical Correction
- Working Distance

**Achromats:** corrected for chromatic aberration for red, blue

**Fluorites:** chromatically corrected for red, blue; spherically corrected for 2 colors

**Apochromats:** chromatically corrected for red, green & blue; spherically corrected for 2 colors

**Plan-** further corrected to provide flat field
Some Functional Parameters of the Microscope Objectives

**Numerical Aperture (NA):** Light-gathering ability of the objective \((NA=n \cdot \sin\theta)\). Thus, the NA is directly related to the resolving power of the lens.

\[
NA = n \sin(\theta)
\]

\[
0.41 = 1.0 \sin 24.5^\circ
\]

\[
0.83 = 1.0 \sin 56.4^\circ
\]

**Resolution:** Defined as the smallest distance between two points on a specimen that can still be distinguished as two separate entities.

\[
R = \frac{0.61\lambda}{NA}
\]

**Magnification:** Product of the magnifications of objective and ocular.
Specialized Types of Light Microscopes (Unstained Specimens)

- Darkfield Illumination
- Differential Interference Contrast (DIC)

Diagram showing the components of a light microscope for darkfield illumination and DIC.
Common Light Sources

Arc lamp

Common Laser System Configurations

Anatomy of the Helium-Neon Laser

Figure 7

Laser Illumination Source Emission Spectra

Mercury Arc Lamp UV and Visible Emission Spectrum

Xenon Arc Lamp Emission Spectrum

Figure 9

Mercury Arc Lamp (HBO)

Argon Emission Spectrum

Krypton Emission Spectrum

XBO - Xenon Burner

Relative Spectral Radiation

Wavelength (Nanometers)

300 400 500 600 700 800 900 1000 1100 1200 1300

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In **fluorescence**, a molecule is excited to higher energy states by radiation of specific wavelengths.
Emission of Quantum Dot

Shimon Weiss (Chemistry)

Wavelength (nm)

460 560 730 1030 1770

2.1nm CdSe 4.6nm InP InAs

CdSe

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Electronic Transitions: Jablonski Diagram

**S\textsubscript{0}** (ground singlet state)

**S\textsubscript{1}** (first excited singlet state)

**S\textsubscript{2}** (second excited singlet state)

**T\textsubscript{1}** (first excited triplet state)

**ABSORPTION** (10\textsuperscript{-15} sec)

**FLUORESCENCE** (10\textsuperscript{-8} sec)

**NON-RADIATIVE DECAY**

**PHOSPHORESCENCE** (>10\textsuperscript{-6} sec)

**INTERNAL CONVERSION** (10\textsuperscript{-12} sec)

**INTERSYSTEM CROSSING**

**Vibrational sublevels**

$h_{\nu_a1}$  $h_{\nu_a2}$  $h_{\nu_F1}$  $h_{\nu_F2}$
Principle of Confocal Microscopy

- An aperture (detection pinhole) allows 3D optical sectioning and suppression of out-of-focus light.
- Sample is scanned with point laser beam in the x- and y-directions.
- The sample is moved along the optical axis (z-direction).

Focused + out-of-focus light
Non-confocal
Confocal
Out-of-focus light suppressed

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Advanced Microscopes
Super Resolution
Optical Diffraction Limit

\[ \Delta x = \frac{\lambda}{2n \sin \alpha} \]

Abbe (1873), Arch. Mikroskop. Anat. 9, 413

Problem:

\[ \text{PSF} > d \]
Microscopy at the Nanoscale: Stimulated Emission Depletion (STED)

1st physical concept to break the diffraction barrier in *far-field* fluorescence microscopy (Stefan Hell)

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Summary: STED Resolution

- Not limited any more by the wavelength of light!
- Just depends on the level of fluorescence depletion.
- Practically, diffraction unlimited resolution (lateral resolution < 90 nm)

A threefold improvement in resolution along the x/y focal plan
→ 9 effective scanning spots created out of 1!

- 1st physical concept to break the diffraction barrier in far-field fluorescence microscopy with visible light and regular lenses!
- Light microscopy at the nanoscale = nanoscopy!
- New law; a modification of Abbe’s law:
- First STED microscope in the USA!

Application of STED


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Saturated Structured-Illumination Microscopy (SSIM)

Use Moiré pattern effect to detect diffraction limited objects. A resolution of 100 nm in the lateral direction and 300 nm in the axial direction has been achieved.
Photo-activated Localization Microscopy (PALM)

Same concept as STORM but different Dyes (FPs: EoS and Dronpa)

1) Activate Eos with 405
2) Image activated Eos with 488
3) Activate Eos
4) Continue to image all Eos
5) Deactivate all Dronpa with strong 488
6) Activate Dronpa with 405
7) Image activated Dronpa
8) Continue to image all Dronpa

Cytoskeletal α-actinin and adhesion complex localized vinculin

Stochastic Optical Reconstruction Microscopy (STORM)

Target structure

Localizing activated subset of probes

Super-resolution image

B-SC-1 cell, Microtubules stained with anti-β tubulin Cy3 / Alexa 647 secondary antibody

Cy3 / Alexa 647: Clathrin, Cy2 / Alexa 647: Microtubule

FLIM
(Fluorescence Life time Imaging)
Protein Folding by single pair Förster Resonant Energy Transfer (spFRET)

$G = \text{Energy}$

$[\text{GdCl}] = \text{Conformational Coordinate}$

Unfolded

Intermediate

Native

$R = \text{Reaction coordinate}$

Shimon Weiss (Chemistry)
Förster Resonant Energy Transfer (FRET)

nm $\rightarrow$ nsec
Fluorescence Lifetime Imaging (FLIM)

Principle
• Each fluorescent dye has its own lifetime in the excited state
• In live cells, fluorescence lifetime is affected by the local environment
• FLIM is insensitive to fluctuations in fluorochrome concentration and excitation light intensity
• It is based on time-correlated single photon counting technology

Applications
• Mapping of local environment such as pH, oxygen, ion concentrations, intracellular signal transduction, FRET, and membrane potential

![Confocal image](image1.png) ![FLIM image](image2.png)
High-speed Microscopes
The H33D detector attaches to a standard fluorescence microscope. Laser
It will permit to track multicolor qdot-labeled proteins in live cells virtually background-free.

Single Molecule Imaging

January 2006

Particle Physics Detector

Shimon Weiss (Chemistry)

Nano Technology

Extracellular Medium

Cytoplasm

Nucleus

EGF-R

Sos-1

Ras

Grb2

QD

EGF

QD

Raf

MEK

ERK

Elk-1

QD

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Principle of High-speed Bio Imaging

Wide Field

Sample

CMOS [ FADC (50 MHz) * 100 ]

Confocal

Sample

PMT + FADC (10 – 50 MHz)

[ HAPD + FADC (1 GHz) ] * 64

CMOS [ FADC (50 MHz) * 100 ]
All the existing microscopes are limited by the narrow bandwidth of readout.

- Just one channel of FADC (Flash Analog to Digital Converter) running at 10 – 50 MHz
- So-called Video Rate (30 frame/sec)

The first step is to adopt multiple channels of FADC for massive parallel processing.

- Like high energy experiments (such as LHC)

In addition, we need Single Photon Sensitivity with high Quantum Efficiency.
Micron 1.3M-Pixel CMOS Sensor

Controller
ROW_ADDR
ROW_STRT_N
LD_SHFT_N
SYSCLK

ADC Controls

Column Parallel 10-bit
ADC 640 x 1

Even Columns
Odd Columns

Control Logic/Decoders

ROW 4

PIXEL ARRAY

Column Parallel 10-bit
ADC 640 x 1

2 μsec/row
2 msec/frame

(10 bits, 66MHz)
**PRELIMINARY SPECIFICATIONS:**

- 5,000 full mega pixel resolution fps (frames per second)
- Two microsecond global shuttering
- Two memory configurations available:
  - 8GB (6 sec. @ 1,000 full fps)
  - 16GB (12 sec. @ 1,000 full fps)
- Genuine twelve-bit resolution
- Maximum speed at reduced resolution is 150,000 fps
- Full control via Gigabit Ethernet or remote keypad with built in LCD display
- Superior light sensitivity
- IRIG/GPS
- Light weight and compact
- DC operation
- SDI and RS-170 video outputs

5000 frame per sec  
(200 nsec/row)
Gold nano particle (40nm) attached to Transferrin Receptor (TfR) on Cancer Cell

Manuel Penichet (Oncology), John Miao (Physics)
Mean Squared Displacement $<r^2>$ of Tfr on a Human Multiple Myeloma Cell vs. Time

Type A

Type B

Brownian Motion

$<r^2>$ (nm$^2$)

$T$ (msec)
Arisaka’s Campus-wide Collaborations on High-Speed Bio-imaging

Industrial Partners (Hamamatsu Photonics, Photron, Leica)

- Dept. of Physics & Astronomy (Dolores Bozovic, Mayank Mehta)
- Dept. of Electrical Engineering (Bahram Jalali)
- Dept. of Chemistry & Biochemistry (Shimon Weiss)
- California Nano Systems Institute (CNSI, Laurent Bentolila)
- Dept. of Surgical Oncology (Manuel Penichet)
- Dept. of Neurology & Neurobiology (Carlos Portera-Cailliau, Jack Feldman, Tom Otis)

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User-shared Core Facility of High-speed Microscopes at CNSI

CNSI (California Nano Systems Institute)
Feb 17 (Thu)

9:00am  Breakfast and Registration  
        CNSI Lobby

9:30-11:30am  Core Lab Presentations  
              CNSI Auditorium

Welcoming Remarks
Paul Weiss, CNSI Director and Fred Kavli  
Chair in NanoSytems Science

Global Bio Lab  
Room 6310  
Hilary Godwin, Faculty Director

Integrated Systems Nanofabrication  
Cleanroom  
A Level  
Brian Matthews, Research Engineer

Integrated NanoMaterials Lab  
Rooms 2133, 2139  
Baolai Liang, Technical Director

Electron Imaging Center for NanoMachines  
Rooms B120, B140, B146  
Xing Zhang, Technical Director

Molecular Screening Shared Resource  
Room 2145  
Robert Damoiseaux, Scientific Director

Nano and Pico Characterization Lab  
Room B133  
Adam Stieg, Technical Director

Advanced Light Microscopy/Spectroscopy  
(with Macro-Scale Imaging Facility)  
Room B145  
Laurent Bentolila, Scientific Director

11:30-3:00pm  Core Lab Tours & Demonstrations  
              Levels A, B, 2, 6  

All labs will be open to view equipment and learn more about each lab’s specialty.  

Demonstrations of the Equipment Reservation System will be available throughout the building.

1:00pm  Lunch  
        CNSI Lobby

Guests who participate during the presentations or visit a core lab will receive lunch.
Nikon Microscope TE200E with TIRF at CNSI

Laurent Bentolila (CNSI)

Low refractive index (specimen) Evanescent wave at the coverglass-specimen interface, typically less than 100 nm

High refraction index (coverglass)

Reflected light Range of incident angles greater than the critical angle \( \theta \)

Incident light

Specimen Coverglass

Objective Laser illumination

5k – 500k fps

CMOS Camera Photron SA-1

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Image Intensified CMOS Camera

Photron

GaAsP Photocathode (50% QE)

MCP (Micro Channel Plate)

CMOS Camera (> 1,000 frame/sec)

Multi-channel High-speed Digitization

Window

High Voltage Power Supply

Fluorescent Screen

Fiber Optic Tapered Bundle
High-speed Confocal Microscope with ICMOS at CNSI

(1,000 frame/s)

ICMOS Camera
(Photron SV200i)

Leica Microscope

EMCCD Camera
(Ando iXon 897)

Confocal Spinner
(Yokogawa CSU-X1)
Yokogawa CSU-X1

2,000 fps

Confocal Dual Spinning Disk
Frame Rate vs. Resolution

- CMOS (SA-1)
- ICMOS (SV200i)
- New Systems
- 64-beam MMM
- Confocal Scanner (Yokogawa CSU-X1)
- Video Rate (Response of human eyes)

Frame Rate (frame/sec) vs. No. of Pixels (in X-Y)

- SA-1
- 1024PCI
- iXon 897
- Neuro-SMQ
- ORCA-R2
- 4-beam MMM
- EMCCD (iXon 897)
- 4-beam MMM
- 64-beam MMM
- CCD (ORCA)
- CCD (Neuro-SMQ)
- Conventional

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6D Bio-Imaging

- Ideally observation of Live cells requires the detection of photon intensity ($I$) in 6 dimension: $I(x, y, z, \lambda, \tau, T)$
  
  - $x$: 
  - $y$: 
  - $z$: Location in depth 
  - $\lambda$: Wave length of emitted photons 
  - $\tau$: Time delay of emitted photons after excitation 
  - $T$: Frame rate.

- Photo-detectors have lower dimension:
  
  - PMT: $I(T)$ at 50 MHz 
  - CCD: $I(x, y, T)$ at 30 Hz 
  - CMOS: $I(x, y, T)$ at 5 kHz 

- Therefore, “compactification of extra dimension” is required.
Applications to Neuroscience
100 Billions Neurons
100 Billions Galaxies

Brain

Universe

Ca^{2+} Signal in cultivated Rat’s Brain by Confocal Microscope

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# Comparison of Imaging

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<th>Electrophysiology</th>
<th>Optical Microscope</th>
<th>fMRI</th>
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<td><strong>Sensitive to</strong></td>
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<tr>
<td>Electric potential</td>
<td>Electric potential</td>
<td>Calcium iron</td>
<td>Metabolism</td>
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<tr>
<td>Position resolution</td>
<td>10 µm</td>
<td>1 µm</td>
<td>200 µm</td>
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<tr>
<td>Temporal Resolution</td>
<td>0.1 msec</td>
<td>10 msec</td>
<td>1 sec</td>
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<tr>
<td>Coverage</td>
<td>~200 µm cube</td>
<td>≤500 µm cube</td>
<td>Whole brain</td>
</tr>
<tr>
<td>Penetration depth</td>
<td>Any depth</td>
<td>up to 500 µm</td>
<td>Whole brain</td>
</tr>
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<td>Advantages</td>
<td>Single spike resolution</td>
<td>Anatomical identification of neurons</td>
<td>Whole brain imaging, non-invasive</td>
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<td></td>
<td>Invasive, can’t identify active neurons</td>
<td>Shallow penetration, poor time resolution</td>
<td>Poor spatial and temporal resolution</td>
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<td>Limitations</td>
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</table>
High-speed observations
Neural Networks for Breathing

~300 neurons in rat’s brain (pre-Botzinger Cells) responsible for breathing

Nature by Naohiro Koshiya (1999)

Jack Feldman (Neurobiology)
High-speed Ca$^{2+}$ Imaging of pre-Botzinger Cells of Rats

Jack Feldman (Neurobiology)

1,000 frame/sec
Sensory Input and Decision Making in Brain

- Sensory (afferent) Neurons
- Sensory input
- Integration
- Interneurons
- Motor (efferent) Neurons
- Motor output
- Brain and spinal cord
- Peripheral nervous system (PNS)
- Central nervous system (CNS)
Human Eyes

~100M photo receptors

540M years ago (Cambria explosion)
Human Ears

~10,000 Hair Cells

~50M years ago
Anatomy of Inner Ear

Human auditory system

Cross-section of the cochlear

Hair bundle

Molecular mechano-transduction machinery

Meredith LeMasurier and Peter G. Gillespie, Neuron, Vol. 48, 2005
Hair cell, bullfrog sacculus

A. J. Hudspeth, Rockefeller University

CMOS Camera
(Photon SA-1)

EMCCD Camera
(Andor iXon 897)

Microscopes

Objective

Dolores Bozovic’s Lab (Physics)
Neural Networks
Conscious

Unconscious

The Cerebral Cortex

Thalamus

Subcortical areas

Conscious

Unconscious

Thalamus

Subcortical areas

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Assembly of rat’s cortical circuits during development

How/when do neurons establish networks? → Symmetry Breaking

Carlos Portera-Cailliau (Neurology)
Mutiphoton Microscope

Conventional Confocal

Two Photon Excitation

500 – 600 nm

440 nm

880 nm

500 – 600 nm
Absorption of light
Neural Network in Vivo
3D image by two photon microscope

Layer I
Layer II/III
Layer IV
Layer V

0.1 mm

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Neural Network
3D image by two photon microscope

Cortical neurons in a fixed, 100 um-thick brain slice from a transgenic mouse with sparse neuronal GFP expression. Left: Mosaic of 81 high-resolution image stacks acquired on a 2-photon laser-scanning microscope, part of a larger data set spanning several consecutive brain slices. Right: Individual stack from this data set, containing a GFP-expressing cortical layer 2/3 pyramidal neuron. Its dendritic (cyan) and axonal (magenta) compartments were identified and traced within the 3D data containing many other neuronal structures, some of which appear to make contact with the highlighted cell. Images: Miguel Vaz Afonso.
Spatio-Temporal Excitation-Emission Multiplexing (STEM) Microscope

4 Beams
240 frame/sec

Ultrafast laser

relay lenses

Closed-loop scanning mirror

1 kHz

Resonant scanning mirror

16 kHz

HAPD #1

HAPD #2

Piezoelectric objective scanner

Excitation dichroic mirror

Oculars

Stage, sample, anesthesia and heating blanket

100 fs pulse

Spatio-temporally multiplexed MMM

Adrian Cheng (Physics)
Surgery to Open a Cranial window
Prof. Carlos Portera-Cailliau

*Final cranial window from the actual surgery, as seen under the imaging microscope.*
SPATIOTEMPORAL MULTIPLEXING 2-PHOTON MICROSCOPE

University of California, Los Angeles, Depts. Physics and Neurology

New UCLA-designed microscope records firing of thousands of individual neurons in 3-D

Imaging system could help reveal 'miscommunications' in autism, schizophrenia

By Mark Wheeler | January 11, 2011

Some disorders of the brain are obvious — the massive death of brain cells after a stroke, the explosion in the growth of cells that marks a tumor. Other disorders, such as autism, schizophrenia and mental retardation show no physical signs of damage and are believed to be caused by problems in how brain cells communicate with one another.

To understand the root of the problem of these latter diseases, visualizing brain activity is key. But even the best imaging devices available — fMRIs and PET scans — can only give a "coarse" picture of brain activity.

UCLA neuroscientists have now collaborated with physicists to develop a non-invasive, ultra-high-speed microscope that can record in real time the firing of thousands of individual neurons in the brain as they communicate, or miscommunicate, with each other.

"In our view, this is the world's fastest two-photon excitation microscope for three-dimensional imaging in vivo," said UCLA physics professor Katsushi Arisaka, who designed the new optical imaging system with UCLA assistant professor of neurology and neurobiology Dr. Carlos Portera-Cailliau and colleagues.

Their research appears in the Jan. 9 edition of the journal Nature Methods.

Because neuropsychiatric diseases like autism and mental retardation often display no physical brain damage, it's thought they are caused by conductivity problems — neurons not firing properly. Normal cells have patterns of electrical activity, said Portera-Cailliau, but abnormal cell activity as a whole doesn't generate relevant information for the brain's function.
Future Directions
Virtual Reality Experiment on Awake Rats

Two Photon Excitation Microscope

Ti:Sa Laser

Spherical Screen for Virtual Vision

Tetrodes

Speakers

Olfactory Stimulator

Whisker Stimulator

Floating Ball

Optical Mice

Pressurized air

Mayank Mehta
Daniel Aharoni
Bernard Willers
(Physics)
GRIN 2PE Endoscope

Mark Schnitzer (Stanford)

Diagram:
- High NA multimode fiber
- Coated micro-prism
- Relay lens
- Objective lens
- Stiffening piece
- Piezo scanner
- DC micromotor
- Photonic bandgap fiber

Images:
- Laser spot is Lissajous scanned in this plane
- Day 239
- Day 241
- Merge

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Optogenetic Excitation of Neurons

Excitation by Channelrhodopsin-2 (ChR2)

Inhabitation by Halorhodopsin (NpHR)

Karl Deisseroth (Stanford)
Outer world vs. Inner word

- **Outer world**: Five senses → Manipulate by Virtual Reality
  - Vision
  - Sound
  - Touch
  - Smell
  - Taste

- **Inner world** → Manipulate by Photo Excitation of single neurons
  - Neural network in brain

- Establish direct link between **Inner world** & **Outer world**
  - Control outer world – Virtual reality
  - Control inner world – Neural reality
Voltage Sensing Dye by FRET

DPA

DiO

Tom Otis (Neurobiology)
Voltage Sensing Dye

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Ca\textsuperscript{2+} Signal in cultivated Rat’s Brain by Confocal Microscope

Photo Excitation

SLM + Voltage Sensing
Summary
Seven Phases of Cosmic Evolution

14 billion years ago:
- Origin of Particles
- Origin of Structure
- Origin of Life
- Origin of Consciousness
Seven steps of cosmic evolution

Spontaneous Symmetry Breaking

14B years ago

Simple  →  Coherent Complex System
Seven steps of cosmic evolution

Spontaneous Symmetry Breaking

14B years ago

Accelerator ← Telescope → Microscope

2/15/2011

Katsushi Arisaka, UCLA
Concluding Remarks

- Life, in particular, our brain is a complex system in 4 dimensional space-time.
  - Emergent property
  - Strongly interacting

- Countless “spontaneous symmetry breakings” during the evolutorial and developing process of life

- Fully controlled experiments by “Virtual Reality” under way.
  - Outer world (environment) vs. Inner world (brain)

- “Super-resolution high-speed 4D imaging” may reveal the fundamental principle of the most complex system in nature – our brain.
  - How can life overcome thermal fluctuation?
  - Networks in a cell and between cells (neurons)