Exploring the Origin of Life and Consciousness by Ultra high-speed Microscopes

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Department of Physics and Astronomy
Why are we here?
The Origin of Universe and Particles

Time

0
1B years
2
3
4
5
6
7
8
9
10
11
12
13
14

Big Bang!
First Galaxy formed
Solar System formed
First life on the Earth
Plants, Fish...
Homo sapiens
We were born.

Origin of Universe
Origin of Particles
Origin of Life
Accelerators
Telescopes
Fossils
Unification of Forces

- strong force
- electromagnetic
- weak force
- electroweak
- gravity

Planck Epoch

- $100 \text{ GeV}$
- $10^{16} \text{ GeV}$
- $10^{19} \text{ GeV}$

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Physicists’ View of Early Universe

Fiat lux
Let there be light
Physicists’ View of Early Universe

Lorentz Invariance
Local Gauge Invariance
Structure of DNA
Symmetry Breaking

Simple

Symmetry Break Down

Complex

Time

0
1B years
2
3
4
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7
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13
14
First Event at LHC – Recreation of the Big Bang! (Nov 7, 2009)
The Four Largest Mysteries in Nature

- **Big Bang!**
- **First Galaxy formed**
- **Origin of Universe**
- **Origin of Particles**
- **Origin of Life**
- **Origin of Consciousness**
- **You were born**
- **Homo sapiens**
- **Plants, Fish…**
- **First life on the Earth**
- **Solar System formed**
- **Telescopes**
- **Accelerators**
- **Microscopes**

Time:
- 0
- 1B years
- 2
- 3
- 4
- 5
- 6
- 7
- 8
- 9
- 10
- 11
- 12
- 13
- 14

2/4/1010

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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.
Campus-wide Collaborations on High-Speed Bio-imaging

California Nano Systems Institute (CNSI, Laurent Bentolila)
Dept. of Physics & Astronomy (Dolores Bozovic, John Miao, 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, Andrew Charles)

Industrial Partners (Hamamatsu Photonics, Photron)
Origin of Life
Organic Polymers (4.5B → 4B years)

an amino acid

organic monomers from space

a protein

organic polymers

an amino acid

organic monomers

inorganic molecules from Earth

methane

water

carbon dioxide

hydrogen cyanide
RNA Word (4B → 3.5B years ago)


2. RNA molecules become self-replicating.

3. Membrane-enclosed pre-cells arise.

4. True cells with RNA genome appear.

5. Modern cells with DNA genome evolve.

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Eukaryote (~2B years ago)

Symmetry breaking

Cell made by proteins

Gene made by DNA

Cell dimensions:
- Up to ~2 m long
- 2 nm wide

Gene dimensions:
- 10 – 50 μm
What is Life?

- **Emergent Property**
  - Strongly-interacting, complex system
  - $\sim 10^4$ of different proteins in one cell
  - $\sim 10^{14}$ cells in one life

- **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
Replication of Double Helix
The H33D detector attaches to a standard fluorescence microscope. It will permit to track multicolor qdot-labeled proteins in live cells virtually background-free.

Particle Physics Detector

Nano Technology

Extracellular Medium

Cytoplasm

Nucleus

EGF-R

Sos-1

Ras

Grb2

QD

QD

QD

QD

Raf

MEK

ERK

Elk-1

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January 2006
Emission of Quantum Dot

Shimon Weiss (Chemistry)
Protein Folding by single pair Förster Resonant Energy Transfer (spFRET)

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

\[ G = \text{Energy} \]

\[ R = \text{Reaction coordinate} \]

Unfolded

Intermediate

Native

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

nm $\rightarrow$ nsec
Hamamatsu Hybrid APD

Single Channel HAPD

64 Channel HAPD + Readout

Motohiro Suyama (Hamamatsu)
Xavier Michalet
Shimon Weiss (Chemistry)
1, 2, 3 ... Photo-electron Distribution

True photon counting

Output Pulse Height

Frequency

1, 2, 3, 4, 5, 6 Photo-electrons
Decay Time Measurement by HAPD

- $FWHM_{IRF} = 210 \text{ ps}$
- $\tau_{FITC} = 3.94 \text{ ns}$

Pulse Shape

Decay Time

Time Resolution = 80 psec

FWHM = 1.5 ns

No after pulse
Principle of High-speed Bio Imaging

Wide Field

CCD + FADC (10 – 50 MHz)

CMOS [ FADC (50 MHz) * 100 ]

Confocal

PMT + FADC (10 – 50 MHz)

[ HAPD + FADC (1 GHz) ] * 64
Micron 1.3M-Pixel CMOS Sensor

- Controller
- ROW_ADDR
- ROW_START_N
- LD_SHIFT_N
- SYSCLK

2 μsec/row
2 msec/frame

Column Parallel 10-bit ADC 640 x 1
Even Columns
Odd Columns

(10 bits, 66MHz)
Gold nano particle (40nm) attached to Transferrin Receptor (TfR) on Cancer Cell

Manuel Penichet (Oncology), John Miao (Physics)

10,000 frame/sec

UCLA Fast Bio-Imaging Group

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Mean Square Displacement $<r^2>$ of TfR on a Human Multiple Myeloma Cell vs. Time

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

Type A

Type B

Brownian Motion
How to speed up microscopes

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

4D Nano Biophysics
Nikon Microscope TE200E with TIRF at CNSI

Laurent Bentolila (CNSI)

CMOS Camera
Photron SA-1

5k – 500k fps
**Principle of ICMOS**

**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**
MCP (Micro Channel Plate)

Gain = 100 - 1000

(5 – 10 μm φ)
High-speed Confocal Microscope with ICMOS at CNSI

(1,000 frame/s)

ICMOS Camera
(Photon SV200i)

EMCCD Camera
(Ando iXon 897)

Leica Microscope

Confocal Spinner
(Yokogawa CSU-X1)

Laurent Bentolila (CNSI)
Yokogawa CSU-X1

2,000 fps

Confocal Dual Spinning Disk

ICMOS Camera
Speeding up evolution of life by accelerating mutations.
Origin of Consciousness
Brain

Universe

100 Billions Neurons

100 Billions Galaxies

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

Andrew Charles (Neurobiology)

15 frame/sec
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 → Brain and spinal cord → INTEGRATION → Interneurons → Motor (efferent) Neurons

Peripheral nervous system (PNS) → MOTOR OUTPUT → Effector

Central nervous system (CNS)
Anatomy of Inner Ear

Dolores Bozovic (Physics)

Meredith LeMasurier and Peter G. Gillespie, Neuron, Vol. 48, 2005
Mystery of Hearing

- Extremely wide dynamic range in amplitude.
  - $10^6$ compressed to 100
  - Smallest amplitude is 0.3 nm

- Extremely wide frequency range.
  - 20 Hz – 20 kHz
    - Dynamic range of 1000
    - Corresponding to $10^6$ in $k$
  - Selectivity of 0.2%
    - up to 5 kHz
  - How can the brain handle up to 20 kHz?
    - mismatch to the speed of action potential of 1 kHz

\[ \omega = \sqrt{\frac{k}{m}} \]
CMOS Camera (Photron SA-1)

EMCCD Camera (Andor iXon 897)

Microscopes

Objective

Dolores Bozovic’s Lab (Physics)
Mechanical Motion of Hair Cells in Inner Ear

Dolores Bozovic, Lea Fredrickson (Physics)

1,000 frame/sec

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UCLA Fast Bio-Imaging Group
How can I recognize a woman so far away?

- Genetically encoded?
- Learning and memory?
Nature vs. Nurture

Nature

[Images of DNA, family tree, and neurons]

Nurture

[Images of eye, hand, and painting]
The Cerebral Cortex

Conscious

Unconscious

Thalamus

Subcortical areas
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

440 nm

500 – 600 nm

880 nm

500 – 600 nm

pinhole

dichroic mirror

scattering tissue

objective lens

DETECTOR
Spatio-Temporal Excitation-Emission Multiplexing (STEM) Microscope

- 4 Beams
- 240 frame/sec
- 80 MHz (100 fs pulse)
- Ultrafast laser
- Beamlet delay
- Relay lenses
- Closed-loop scanning mirror 1 kHz
- Resonant scanning mirror 16 kHz
- Emission dichroic mirror
- Oculars
- Excitation dichroic mirror
- Piezoelectric objective scanner
- Microelectrode micromanipulator
- Stage, sample, anesthesia and heating blanket

Adrian Cheng (Physics)

HAPD #1
HAPD #2

Katsushi Arisaka, UCLA
In vivo calcium imaging of neuronal activity

Katsushi Arisaka, UCLA
3D Structure of Barrel Cortex of Mouse

150 µm deep

Adrian Cheng (Physics)
Tiago Goncalves, Peyman Golshani, Carlos Portera-Cailliau (Neurology)

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In vivo calcium imaging with STEM

Barrel Cortex
Layer 2/3
150 µm deep

240 fps
Raw Data
(x3 faster than real)

Beam 1
(0 ns)
Beam 2
(+3 ns)
Beam 3
(+6 ns)
Beam 4
(+9 ns)
In vivo calcium imaging with STEM

Barrel Cortex
Layer 2/3
150 µm deep

After averaging
(x3 faster than real)

58 neurons
(~100 billons neurons in our brain)
In vivo calcium imaging of layer 2/3 neurons in barrel cortex with STEM

Adrian Cheng (Physics)
Adrian Cheng (Physics)

Tiago Goncalves, Peyman Golshani, Carlos Portera-Cailliau (Neurology)

3D Structure of Barrel Cortex of Mouse

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Simultaneous in vivo calcium imaging in 4 axial planes

Barrel Cortex Layer 2/3

60 fps
(x3 faster than real)

Beam 1 90 μm

Beam 2 120 μm

Beam 3 150 μm

Beam 4 180 μm
Simultaneous in vivo calcium imaging of neuronal activity in 4 axial planes with STEM

Adrian Cheng (Physics)

Katsushi Arisaka, UCLA
Future Directions
How can we recognize and memorize the space?

Mayank Mehta (Physics, Neurology)
Activity of (excitatory) pyramidal neurons in CA depends on rat’s position: place cells

Hippocampus has a cognitive map of space

Mayank Mehta (Physics, Neurology)
A mouse running in a Maze of Virtual Reality

David Tank (Princeton)
Virtual Reality Experiment on Awake Rats

Two Photon Excitation Microscope

Ti:Sa Laser

Spherical Screen for Virtual Vision

Olfactory Stimulator

Whisker Stimulator

Floating Ball

Tetrodes

Speakers

Optical Mice

Pressurized air

Daniel Aharoni
Bernard Willers
Mayank Mehta
(Physics)
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
Ca^{2+} Signal in cultivated Rat’s Brain by Confocal Microscope
LCOS (Liquid Crystal on Silicon) for SLM (Spatial Light Modulator)

Hamamatsu

X10468 Head and Controller

Optical Tweezers

LCOS chip inside the Head

Modulated only phase!
Voltage Sensing Dye by FRET

Tom Otis (Neurobiology)

DiO

DPA
Voltage Sensing Dye

Tom Otis (Neurobiology)
Ca$^{2+}$ Signal in cultivated Rat’s Brain by Confocal Microscope
Summary
The Four Largest Mysteries in Nature

- **Time**
  - 0: Big Bang!
  - 1B years: First Galaxy formed
  - 2
  - 3
  - 4
  - 5
  - 6
  - 7
  - 8
  - 9: Solar System formed
  - 10
  - 11: First life on the Earth
  - 12
  - 13: Plants, Fish...
  - 14: Homo sapiens, You were born.

- **Accelerators**
- **Telescopes**
- **Microscopes**

- **Origin of Universe**
- **Origin of Particles**
- **Origin of Life**
- **Origin of Consciousness**
Four Major Science

Origin of Particles
Particle Physics

Origin of Universe
Cosmology

Origin of Life
Molecular Biology

Origin of Consciousness
Neurophysics
Future of Ultra High-speed Bio Imaging

- **Origin of Life:**
  - Networks of molecules in/on a cell
  - Competition against Brownian: 10 – 100 nm / 1 msec

- **Origin of Consciousness:**
  - Neural networks
  - Action potentials: 1 msec

- > 1,000 frame/sec with nano second time stamp.
  - Gated Image Intensified CMOS
  - Super-PIAS (GHz Photon-counting Imager)
  - 6D Imaging by Streak-CMOS Camera
Concluding Remarks

- “Life” is a complex system in 4 dimensional space-time.
  - Emergent property
  - Strongly interacting

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

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

- “Ultra high-speed imaging” may reveal the fundamental principle of the complex life like ours.
  - How can life overcome thermal fluctuation?
  - Networks in a cell and between cells (neurons)
Acknowledgments

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  - Roberto Peccei: Vice Chancellor
  - Joe Rudnick: Dean
  - Ferd Coroniti: Chair
  - Leonard Rome: Formal Director of CNSI

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  - UCLA, CNSI
  - NSF MRI
  - NIH R01: Shimon Weiss
  - NIH Recovery Act: Carlos Portera-Cailliau
  - Industrial Partners: Photron, Hamamatsu
and thanks to wonderful collaborators!

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