

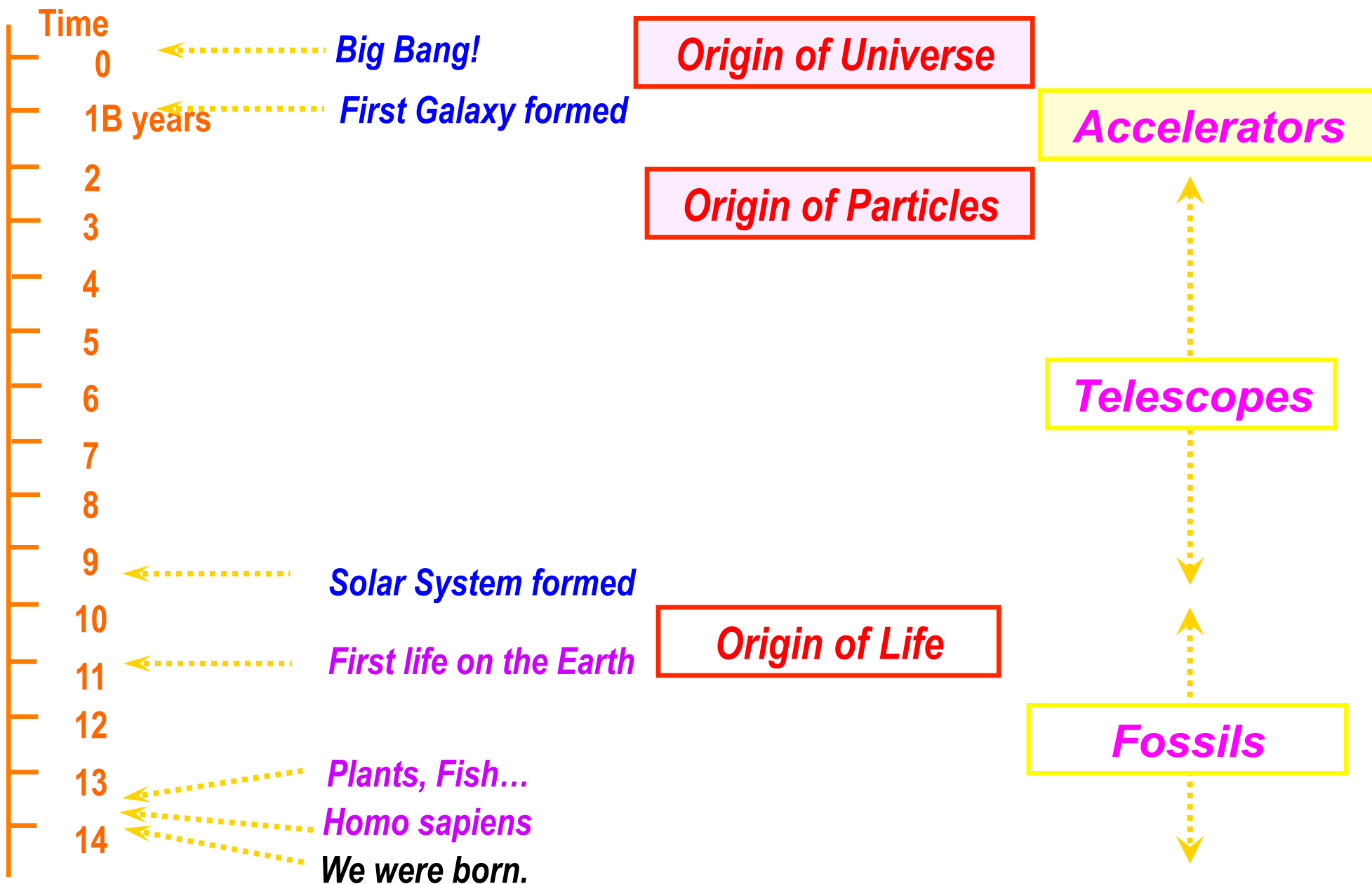
Exploring the Origin of Life and Consciousness by Ultra high-speed Microscopes

Katsushi Arisaka

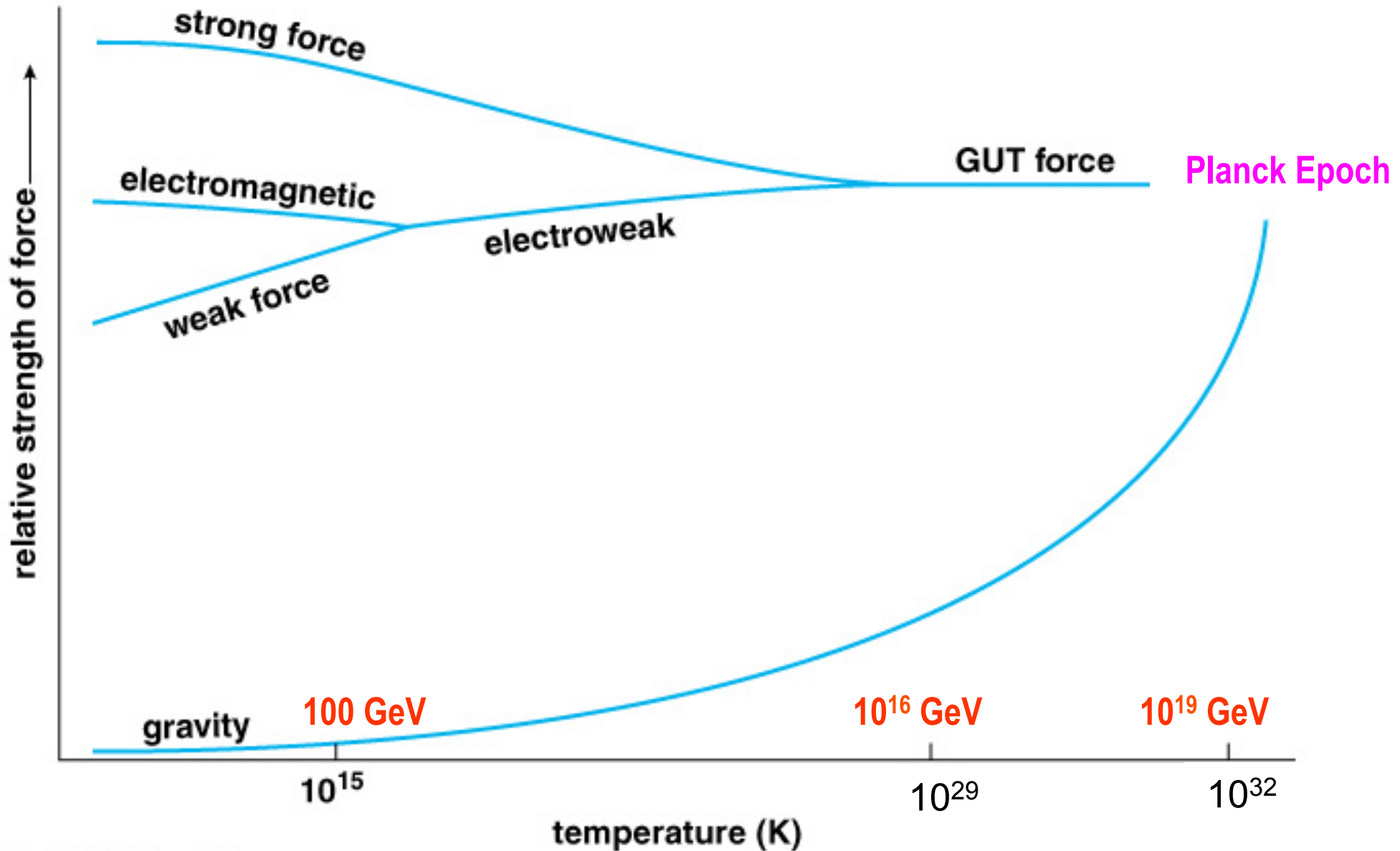
***University of California, Los Angeles
Department of Physics and Astronomy***

Why are we here?

The Origin of Universe and Particles



Unification of Forces

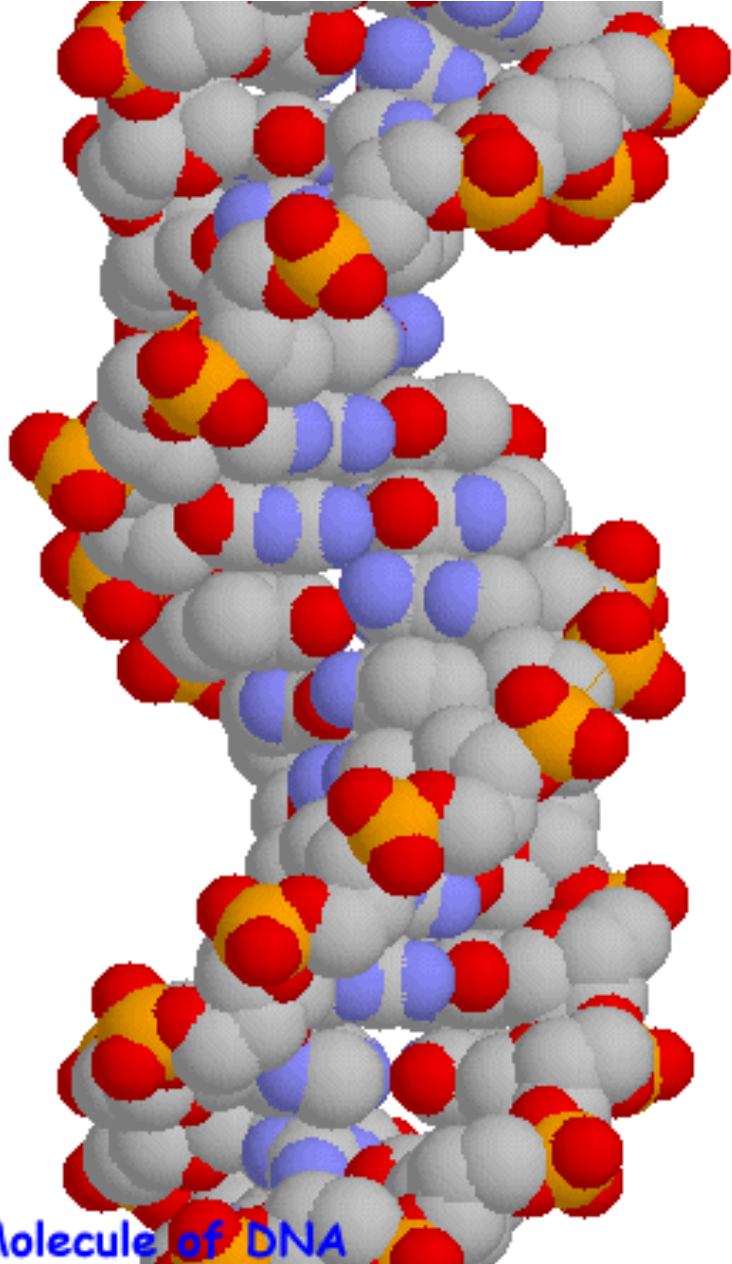


Copyright © Addison Wesley.

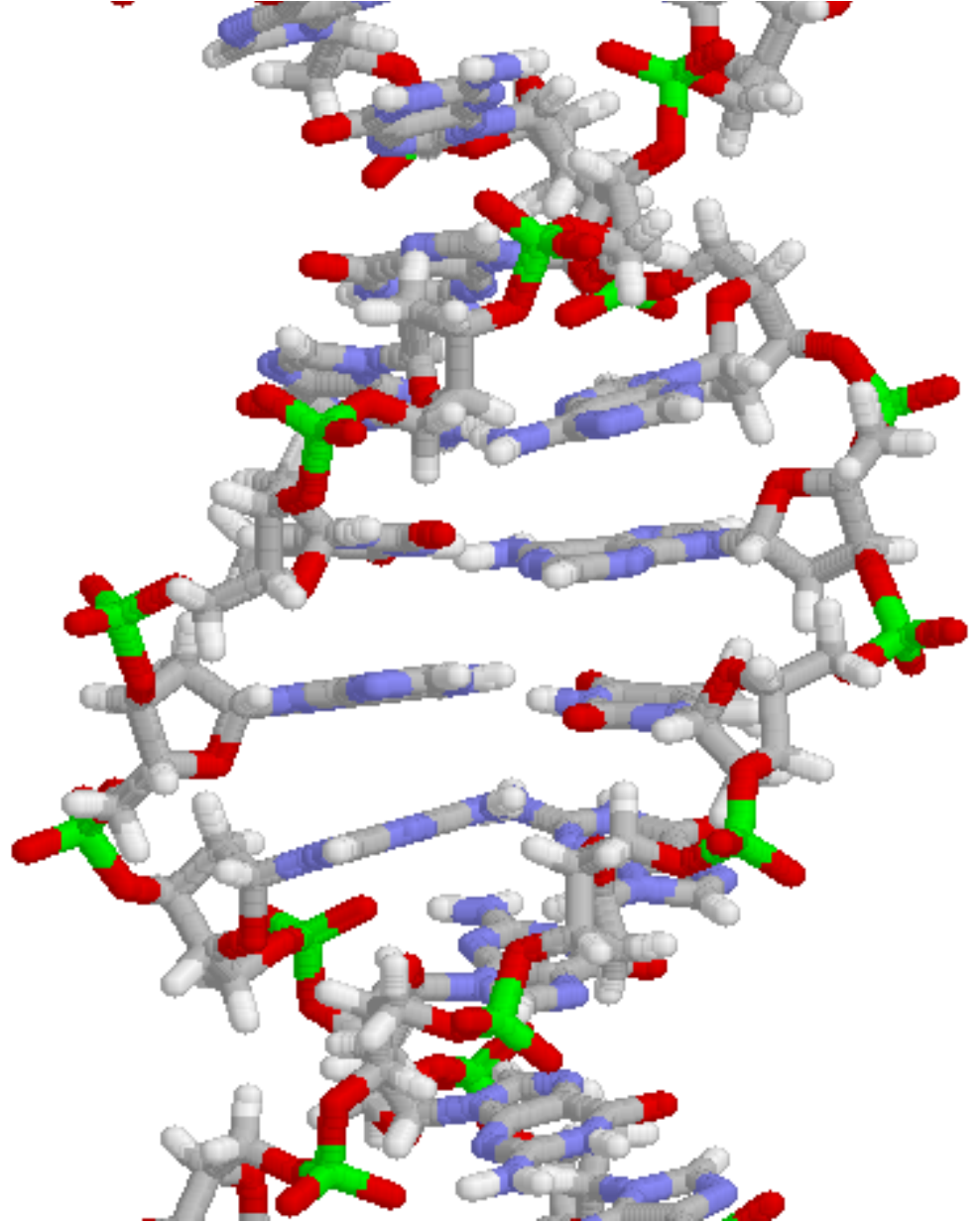
Physicists' View of Early Universe

Lorentz Invariance
Local Gauge Invariance

Structure of DNA

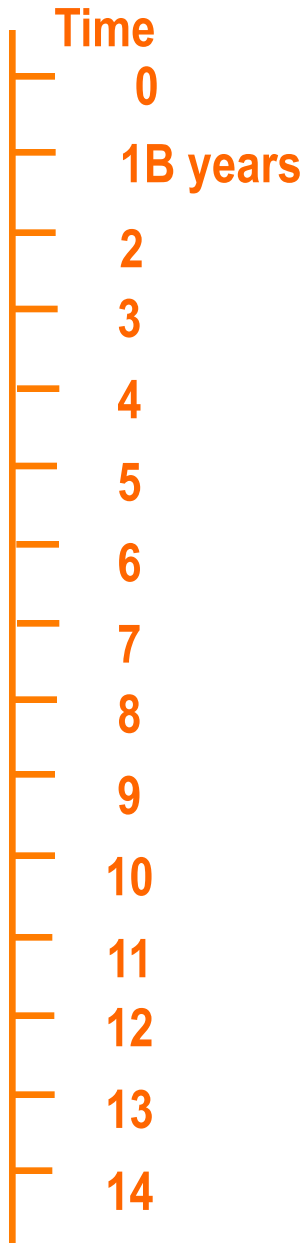


©Rothamsted Experimental Station, 1997, 1998



Molecule of DNA

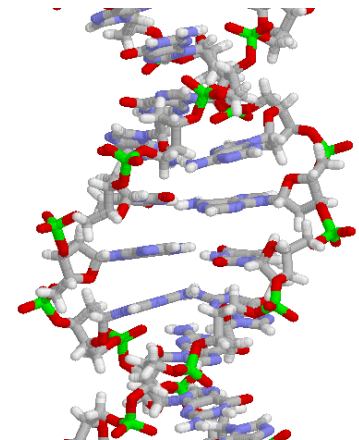
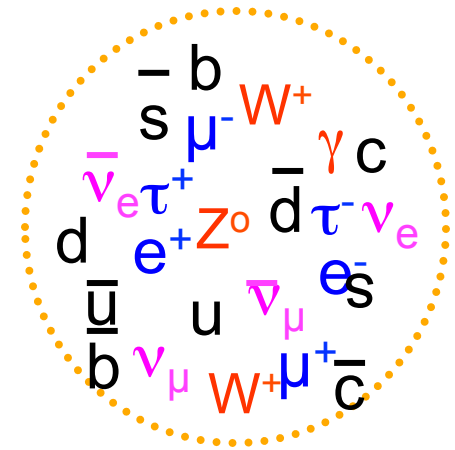
Symmetry Breaking



Simple

*Symmetry
Break Down*

Complex



CMS Detector

Data_taken 2009-Nov-07 19:12:36.880368 GMT

Run_no 120015

Event_no 8

Lumi_sec 1

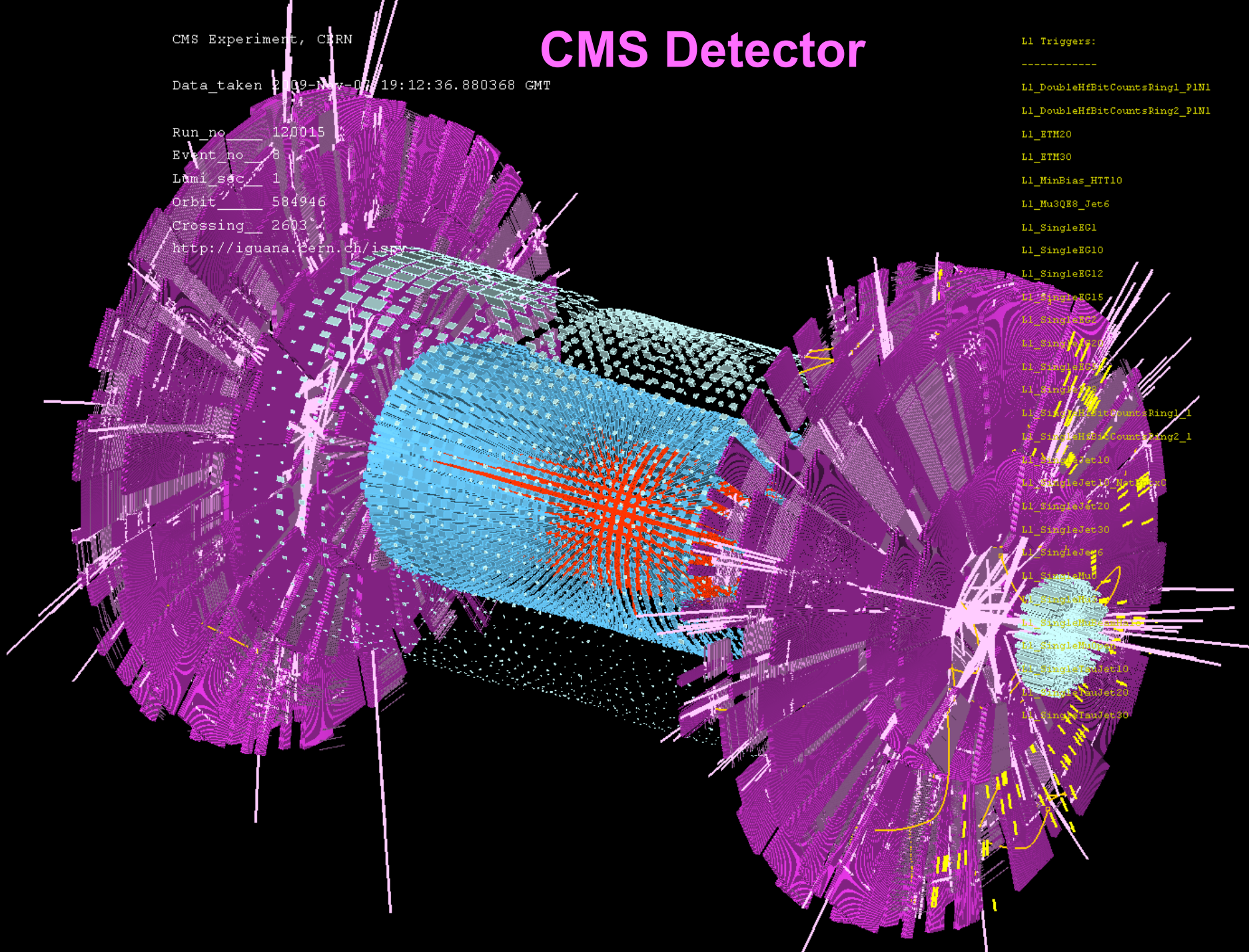
Orbit 584946

Crossing 2603

<http://iguana.cern.ch/isy/>

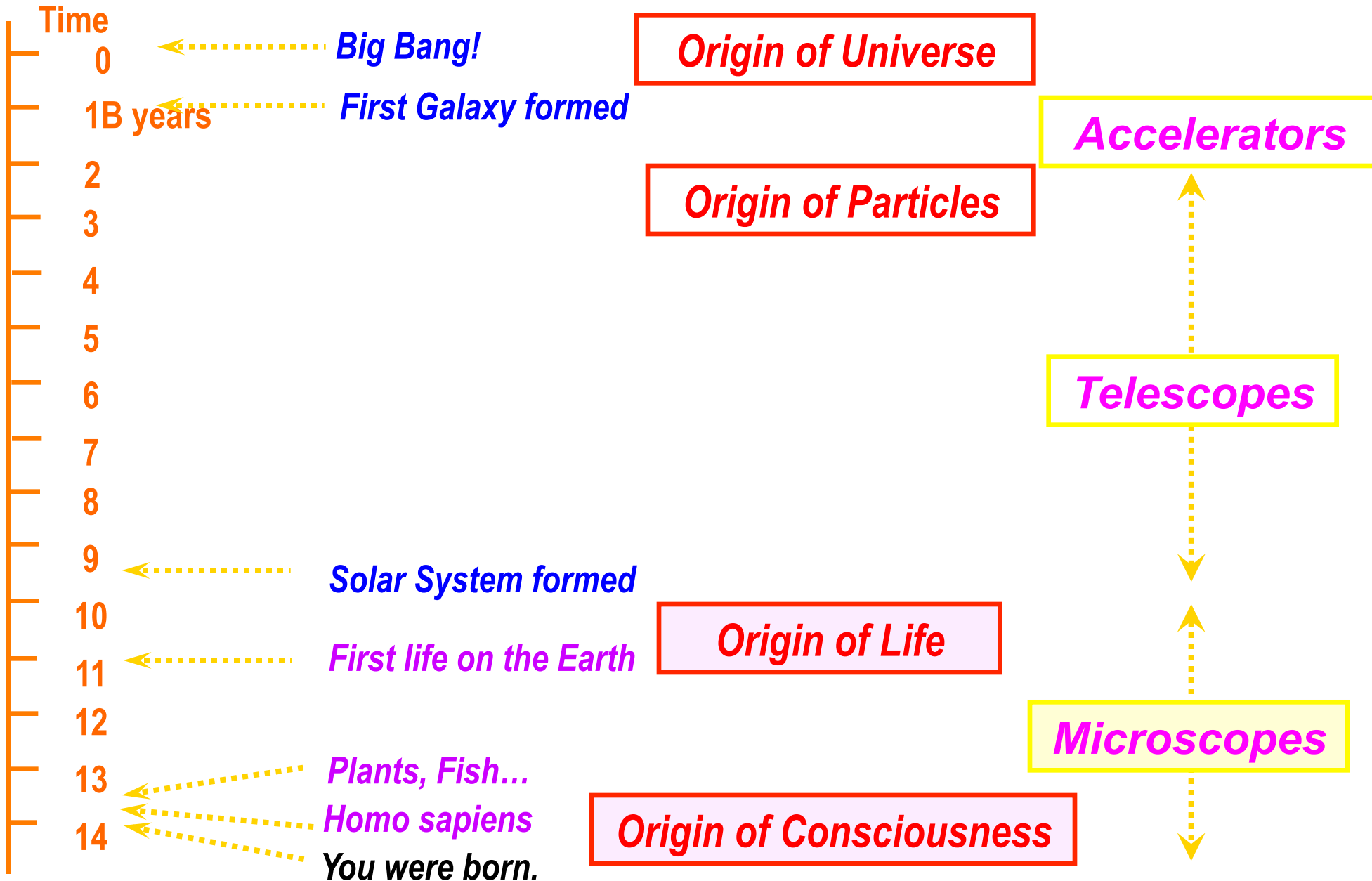
L1 Triggers:

-
- L1_DoubleHfBitCountsRing1_P1N1
- L1_DoubleHfBitCountsRing2_P1N1
- L1_ETM20
- L1_ETM30
- L1_MinBias_HTT10
- L1_Mu3QE8_Jet6
- L1_SingleEG1
- L1_SingleEG10
- L1_SingleEG12
- L1_SingleEG15
- L1_SingleEG20
- L1_SingleEG25
- L1_SingleEG30
- L1_SingleEG35
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- L1_SingleEG980
- L1_SingleEG985
- L1_SingleEG990
- L1_SingleEG995



First Event at LHC – Recreation of the Big Bang! (Nov 7, 2009)

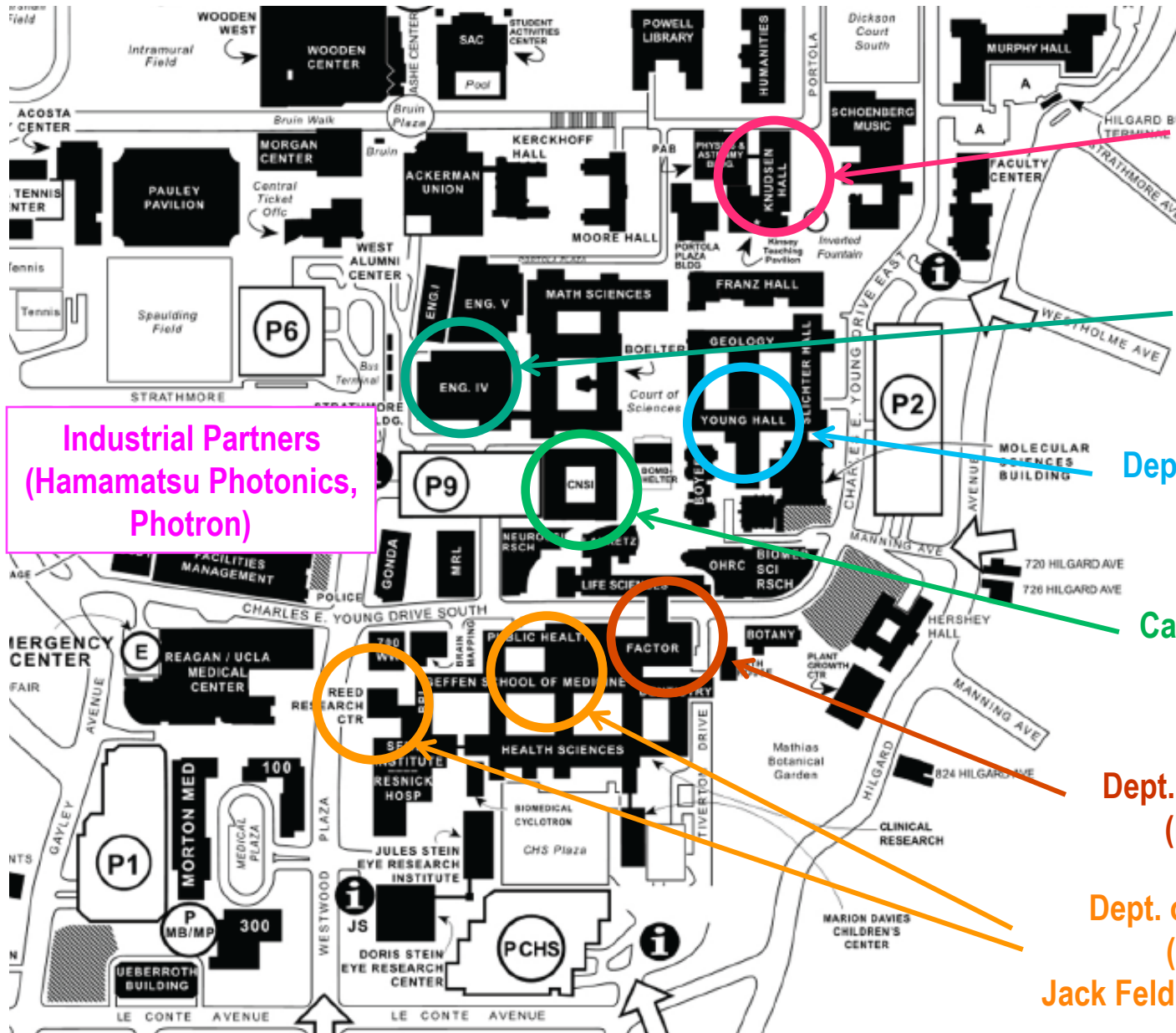
The Four Largest Mysteries in Nature



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



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)

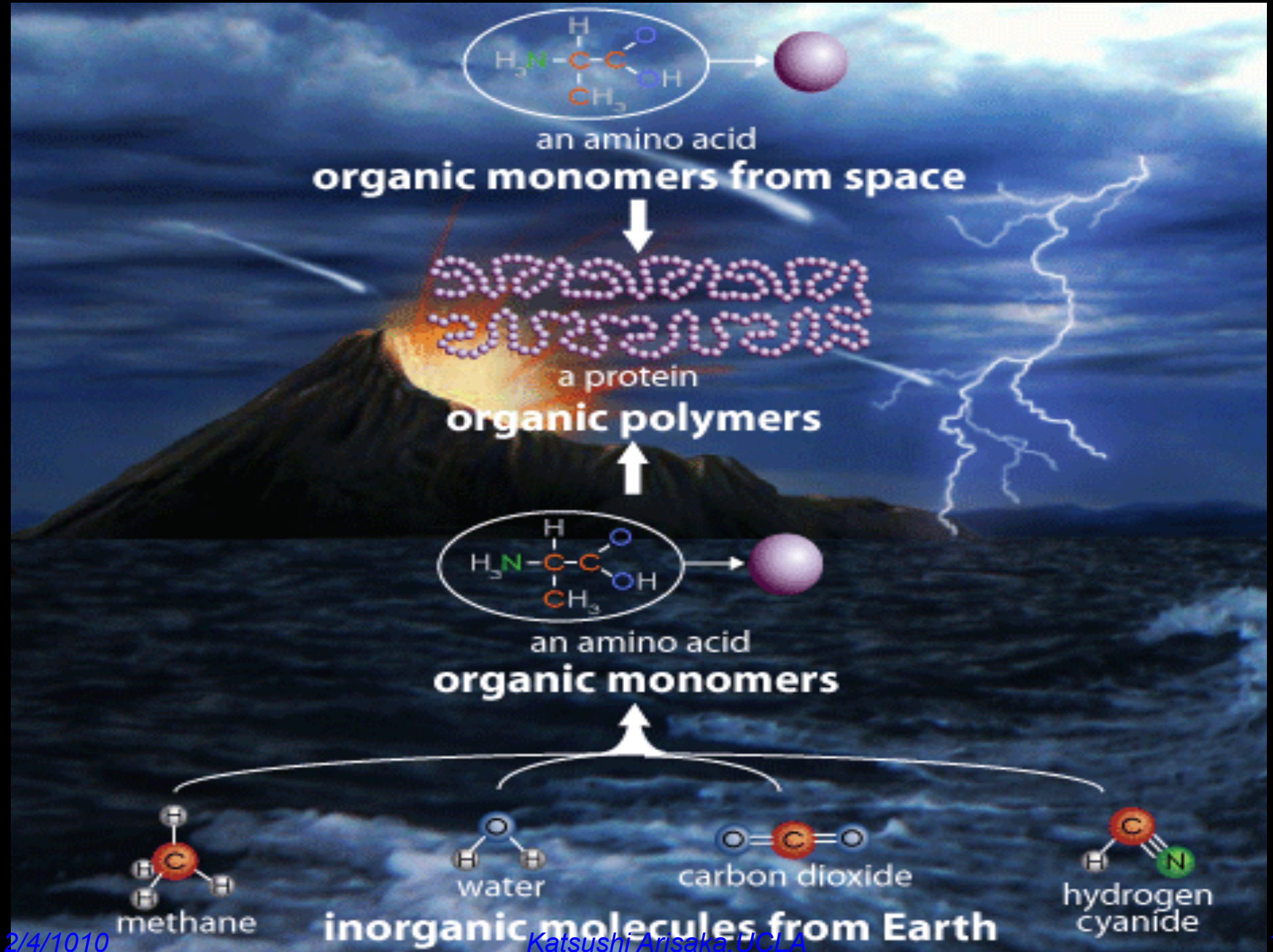
Dept. of Surgical Oncology
(Manuel Penichet)

Dept. of Neurology & Neurobiology
(Carlos Portera-Cailliau,
Jack Feldman, Tom Otis, Andrew Charles)

Industrial Partners
(Hamamatsu Photonics,
Photron)

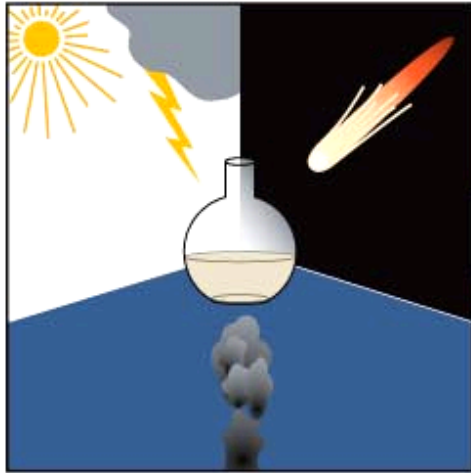
Origin of Life

Organic Polymers (4.5B → 4B years)

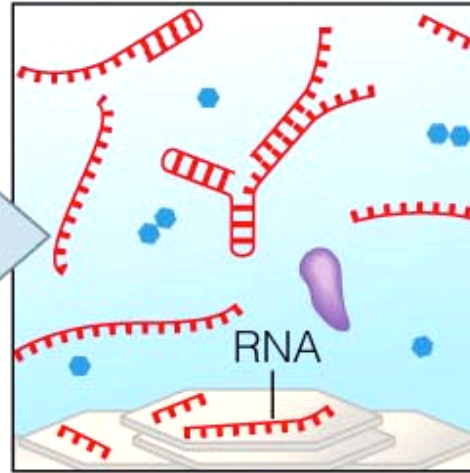


RNA World (4B → 3.5B years ago)

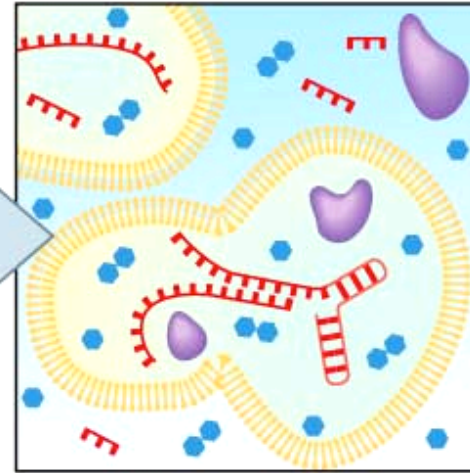
1. Organic precursor molecules appear.



2. RNA molecules become self-replicating.

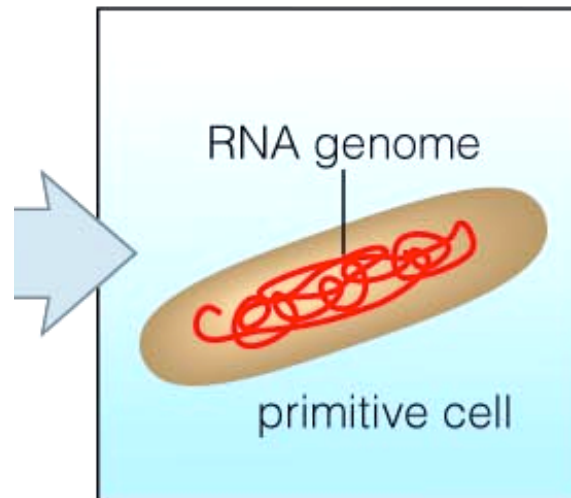


3. Membrane-enclosed pre-cells arise.

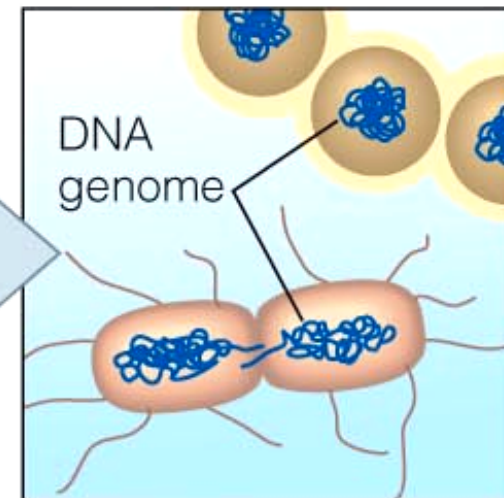


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4. True cells with RNA genome appear.



5. Modern cells with DNA genome evolve.



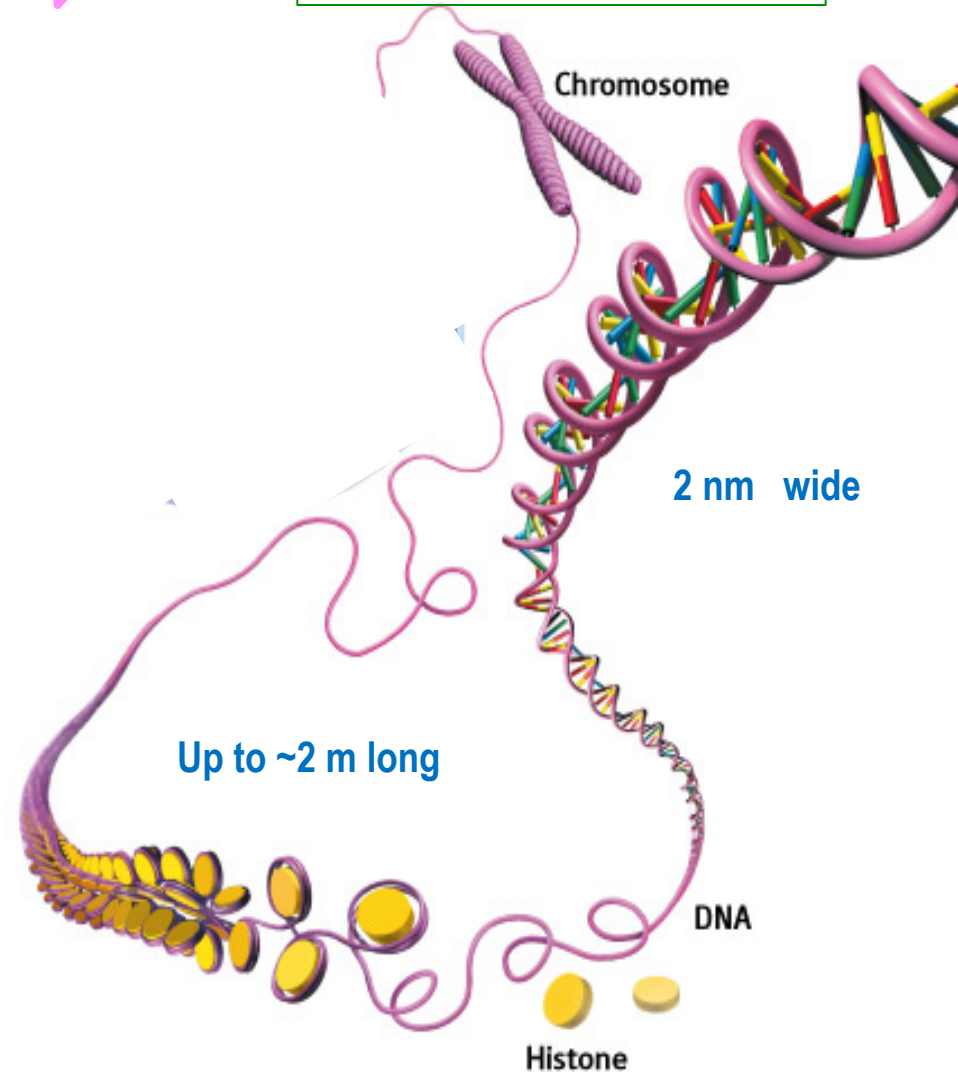
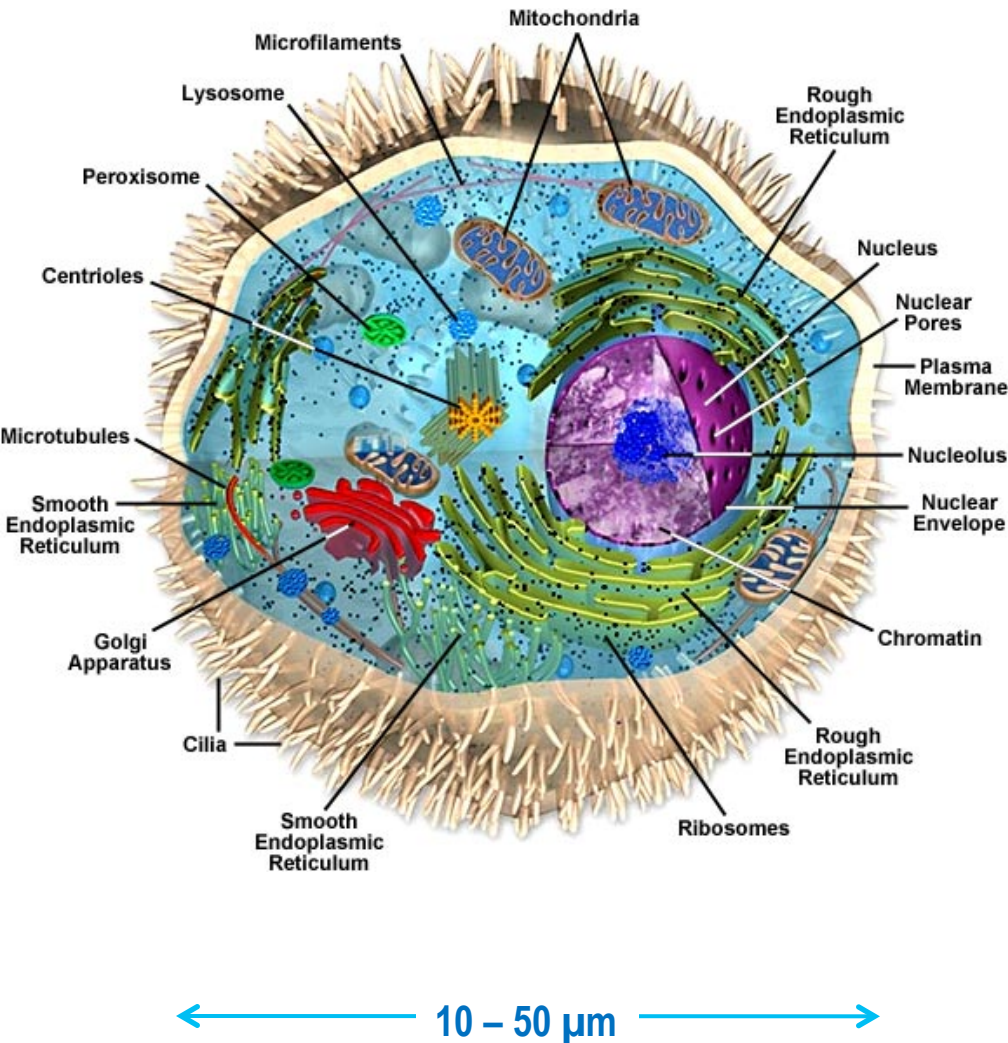
Eukaryote (~2B years ago)

Symmetry breaking

Cell made by proteins



Gene made by DNA



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

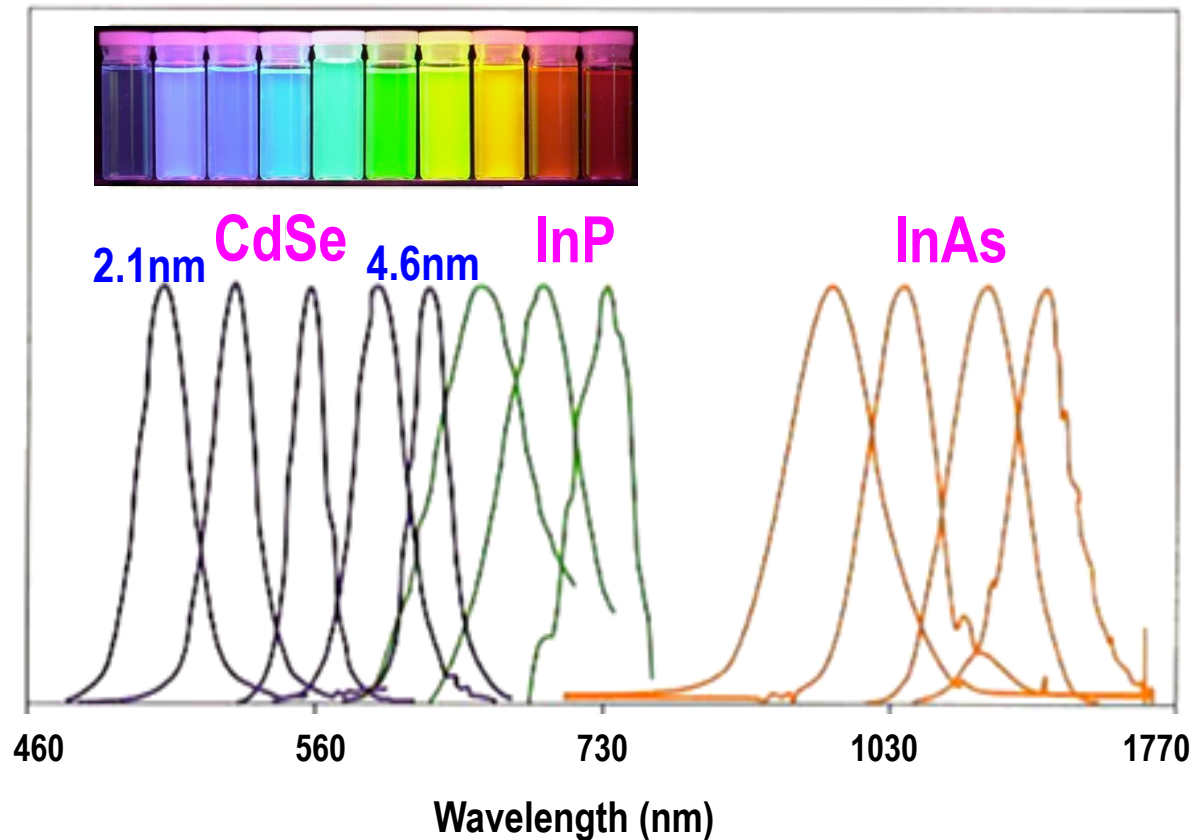
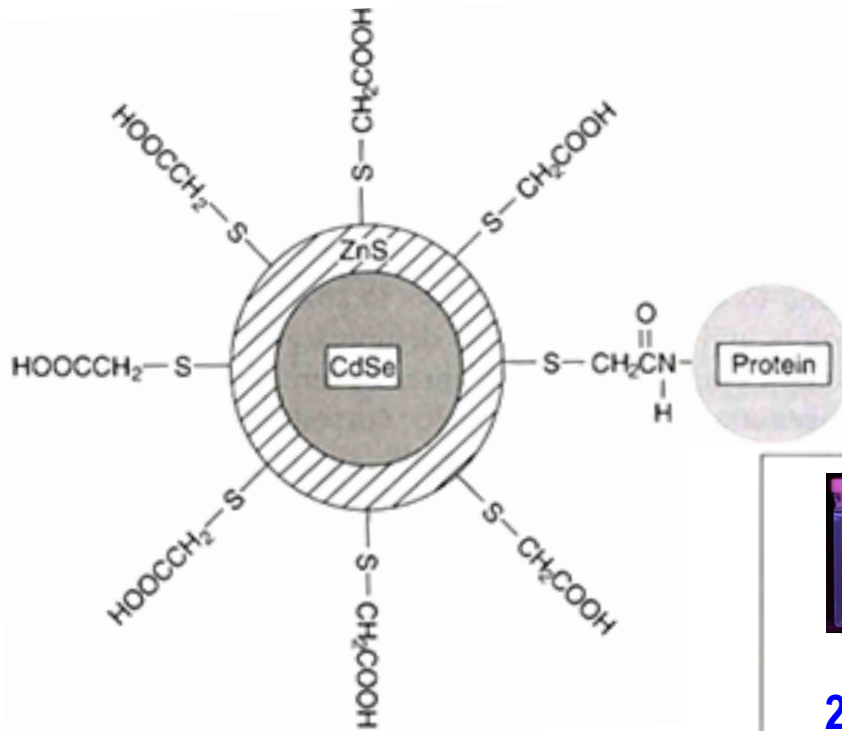
➤ Continuous, countless “symmetry breaking” towards coherent states

- Origin of life
- Evolution of life
- Growth from a single cell to a multi-cell body
- Learning and memory

Replication of Double Helix

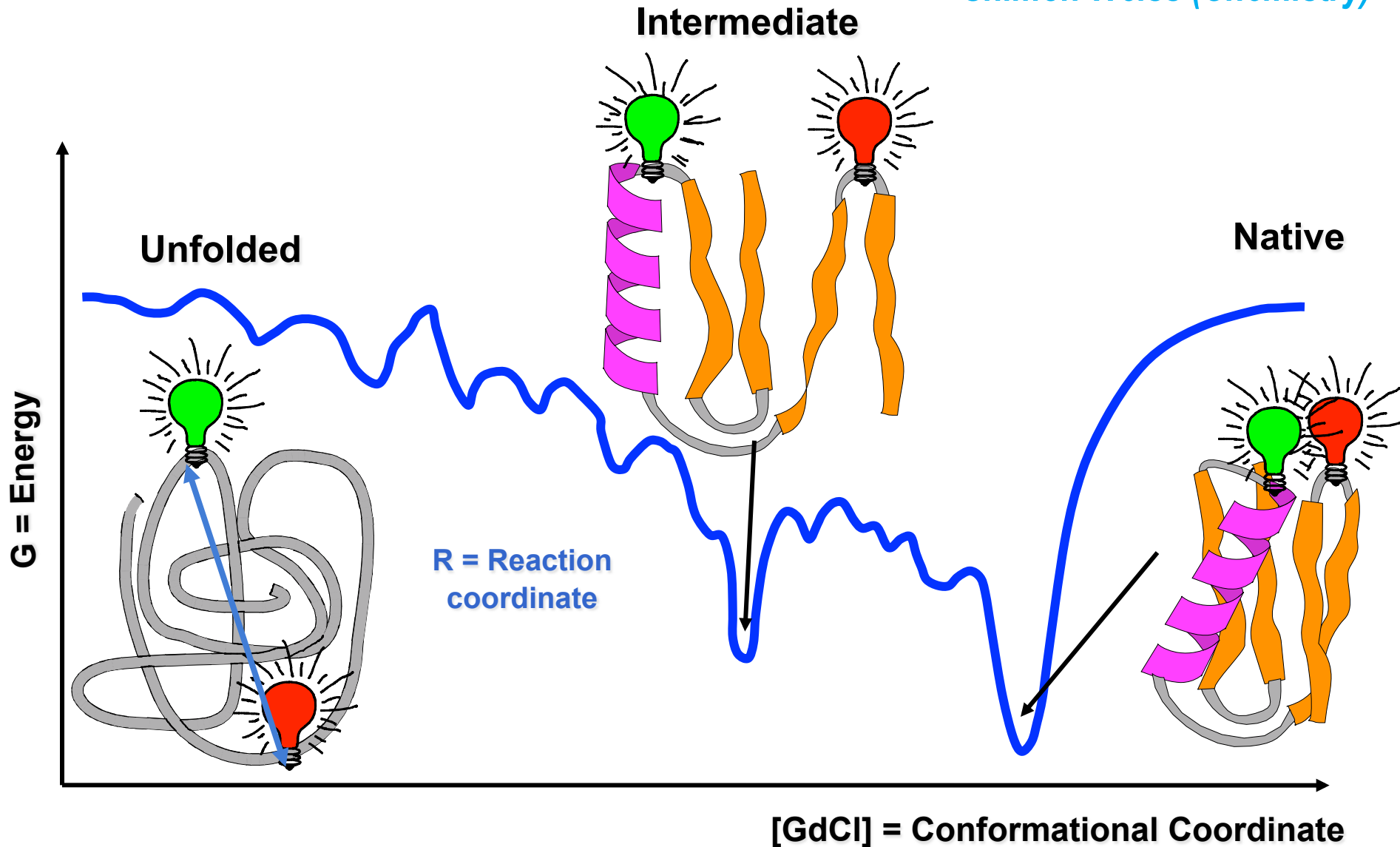
Emission of Quantum Dot

Shimon Weiss (Chemistry)

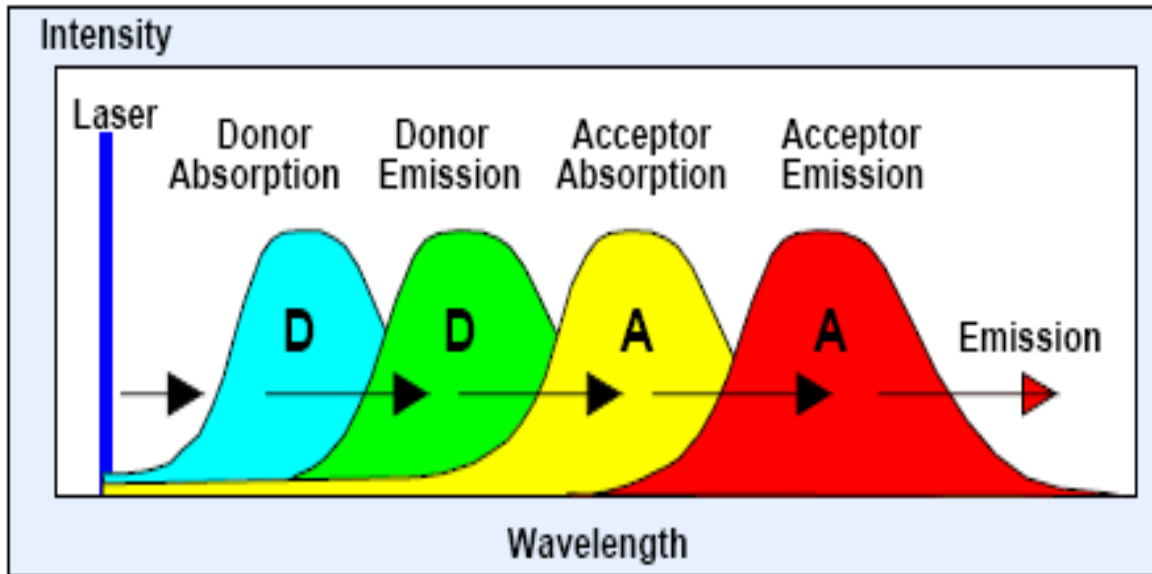


Protein Folding by single pair Förster Resonant Energy Transfer (spFRET)

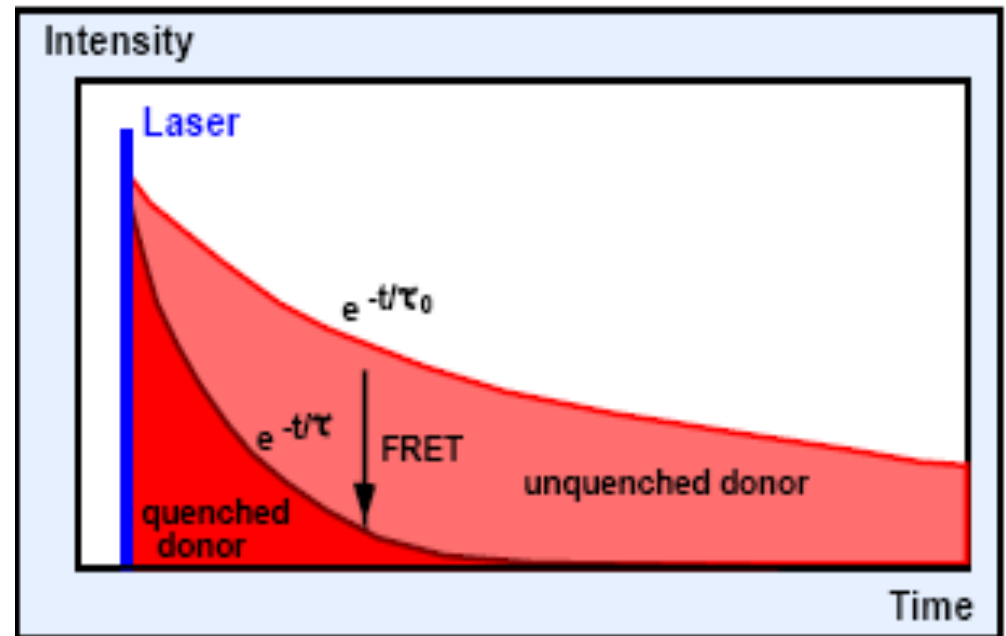
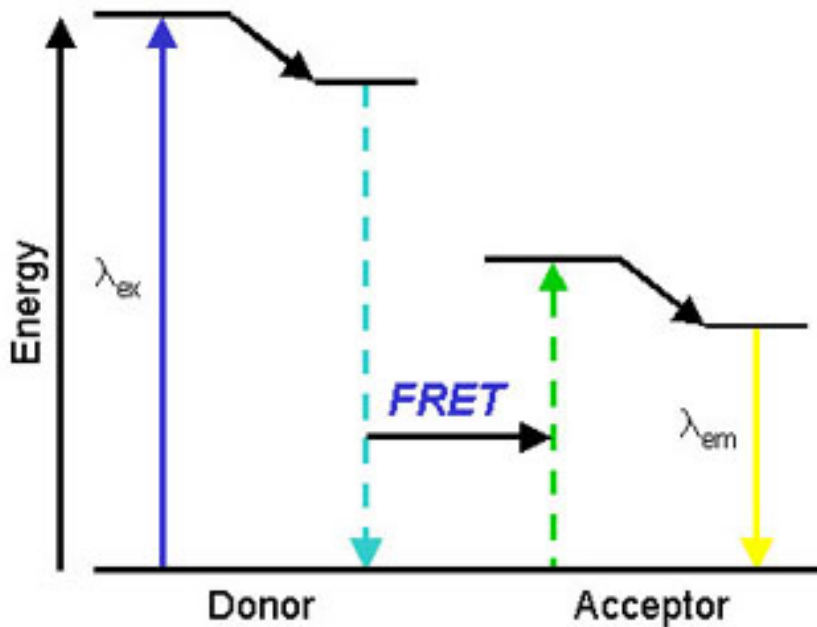
Shimon Weiss (Chemistry)



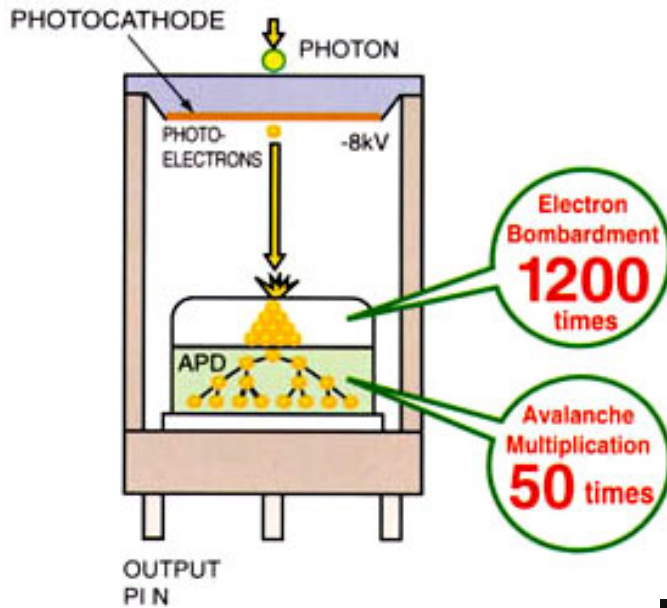
Förster Resonant Energy Transfer (FRET)



nm \rightarrow *nsec*



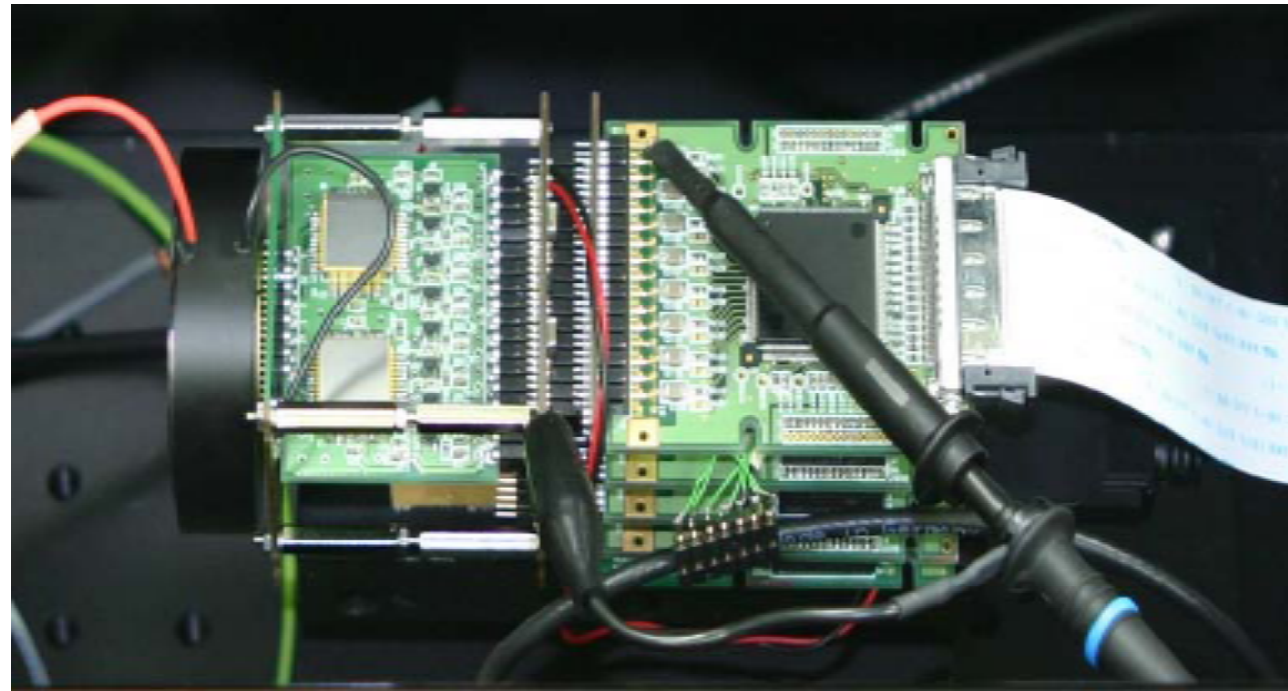
Hamamatsu Hybrid APD



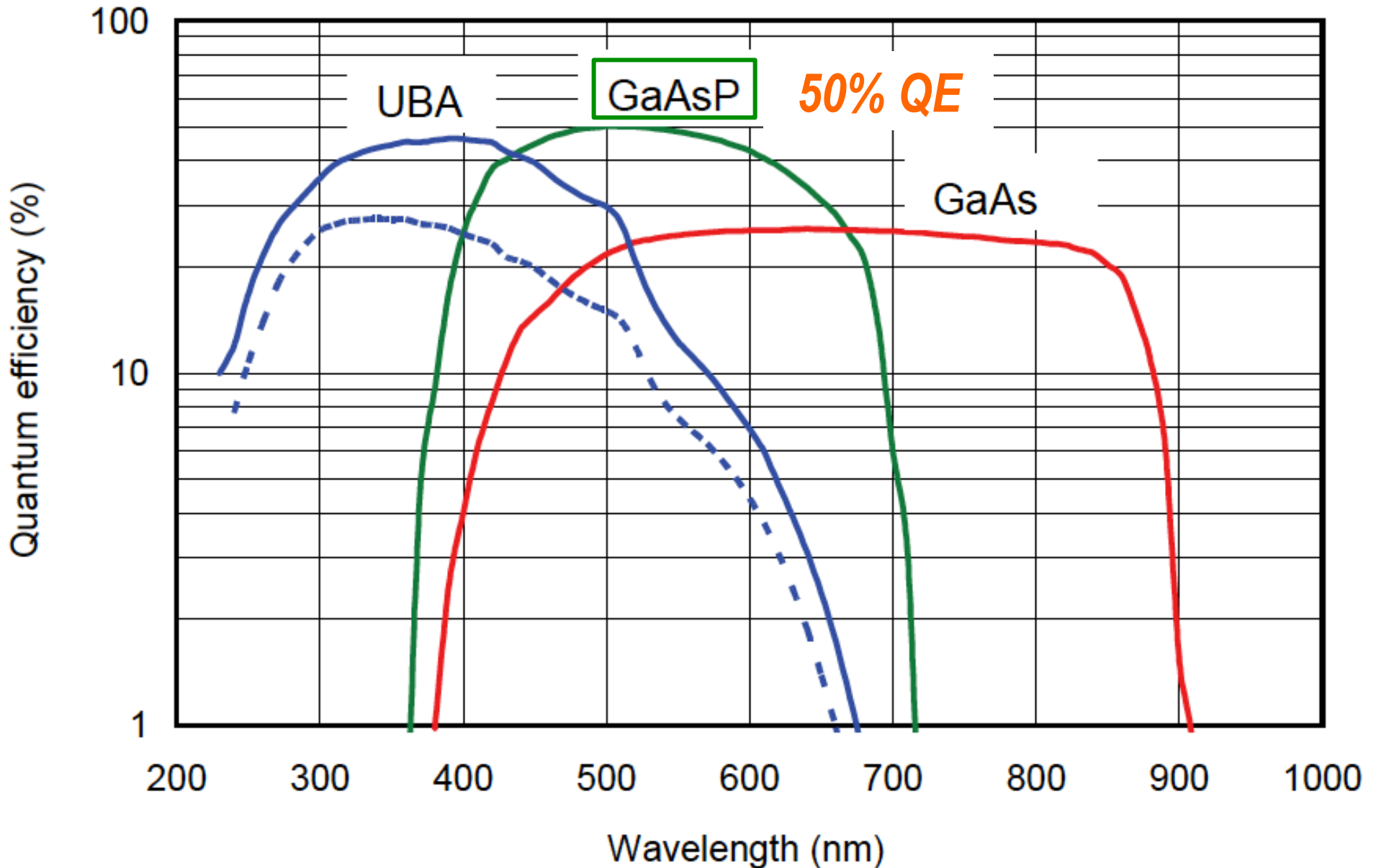
Single Channel
HAPD

64 Channel HAPD
+ Readout

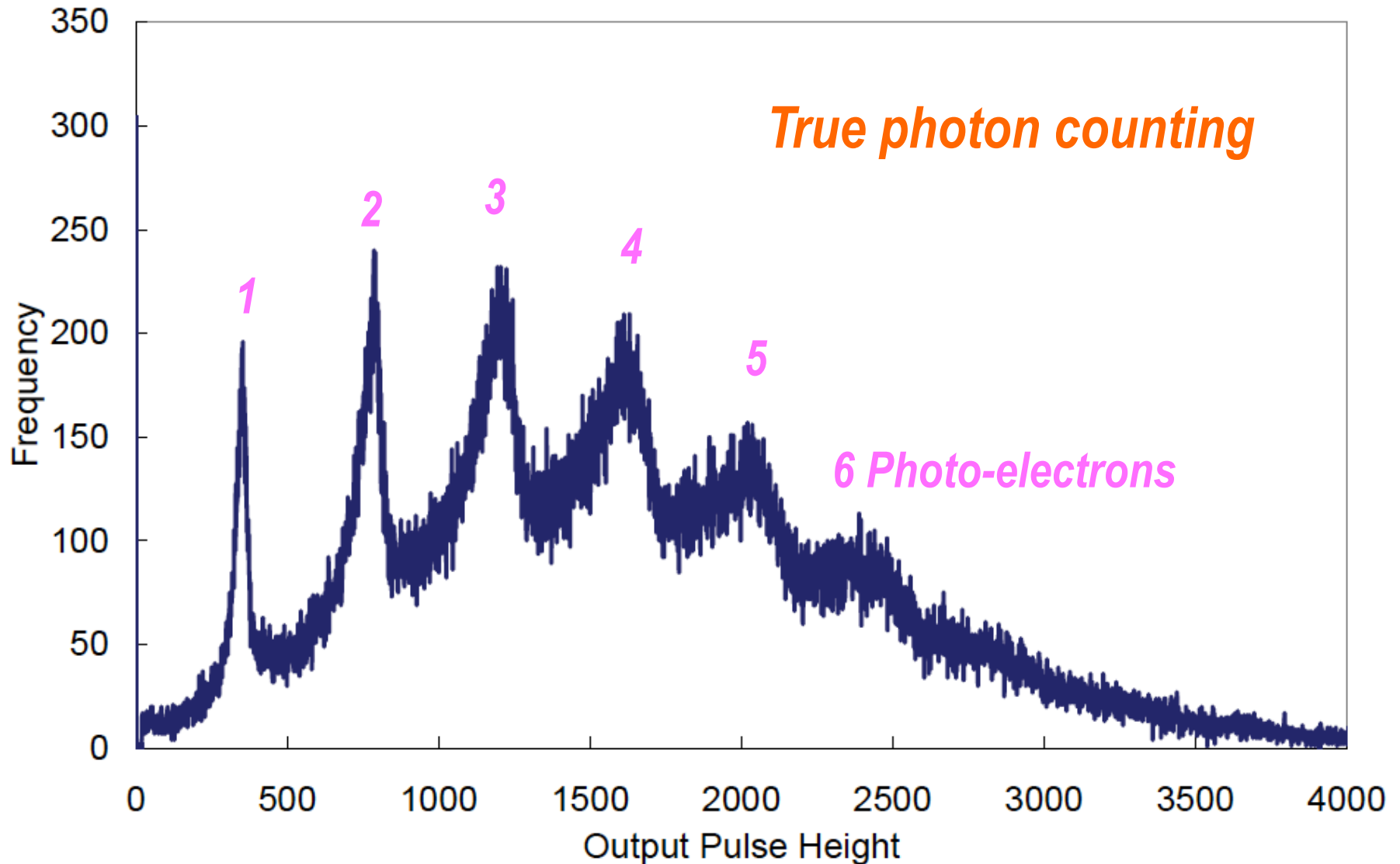
Motohiro Suyama
(Hamamatsu)
Xavier Michalet
Shimon Weiss
(Chemistry)



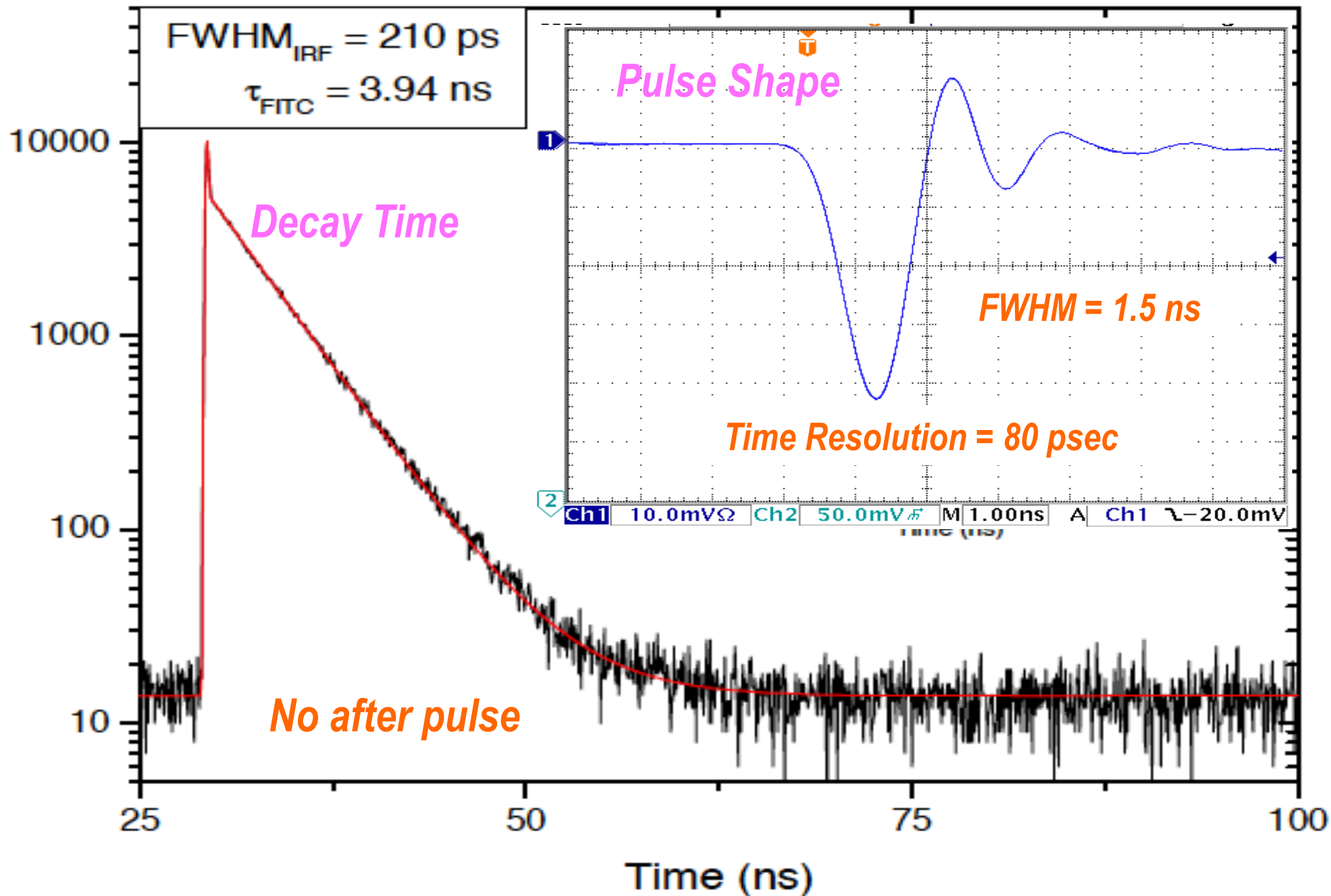
Quantum Efficiency of UBA, GaAsP and GaAs



1, 2, 3 ... Photo-electron Distribution

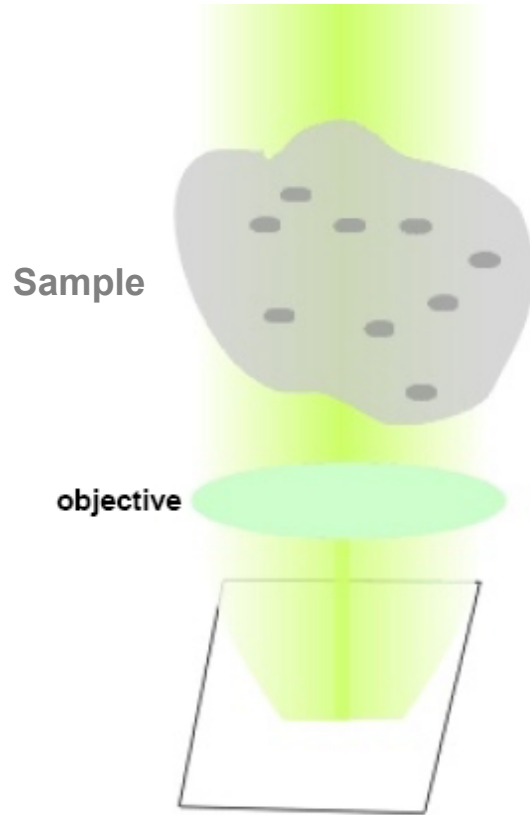


Decay Time Measurement by HAPD



Principle of High-speed Bio Imaging

Wide Field

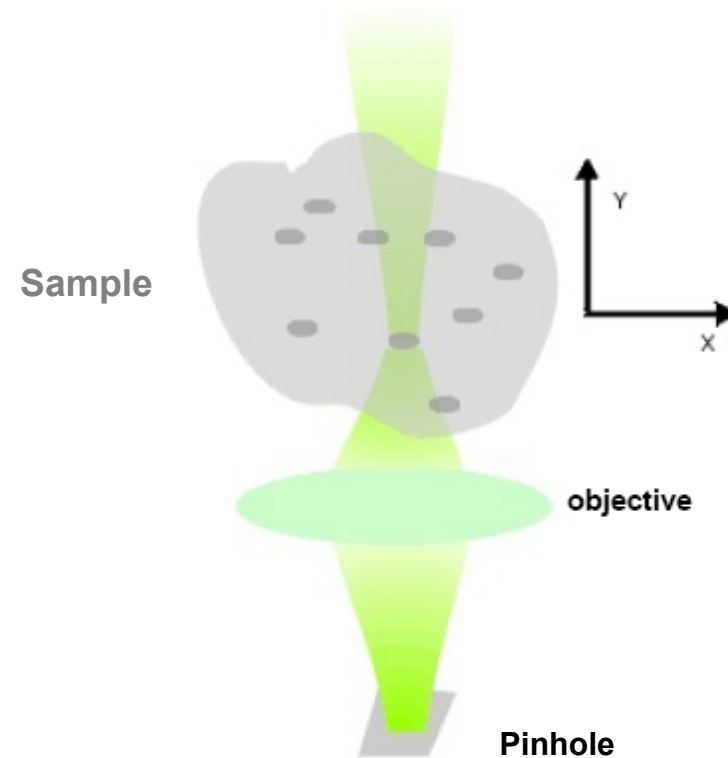


CCD + FADC (10 – 50 MHz)



CMOS [FADC (50 MHz) * 100]

Confocal

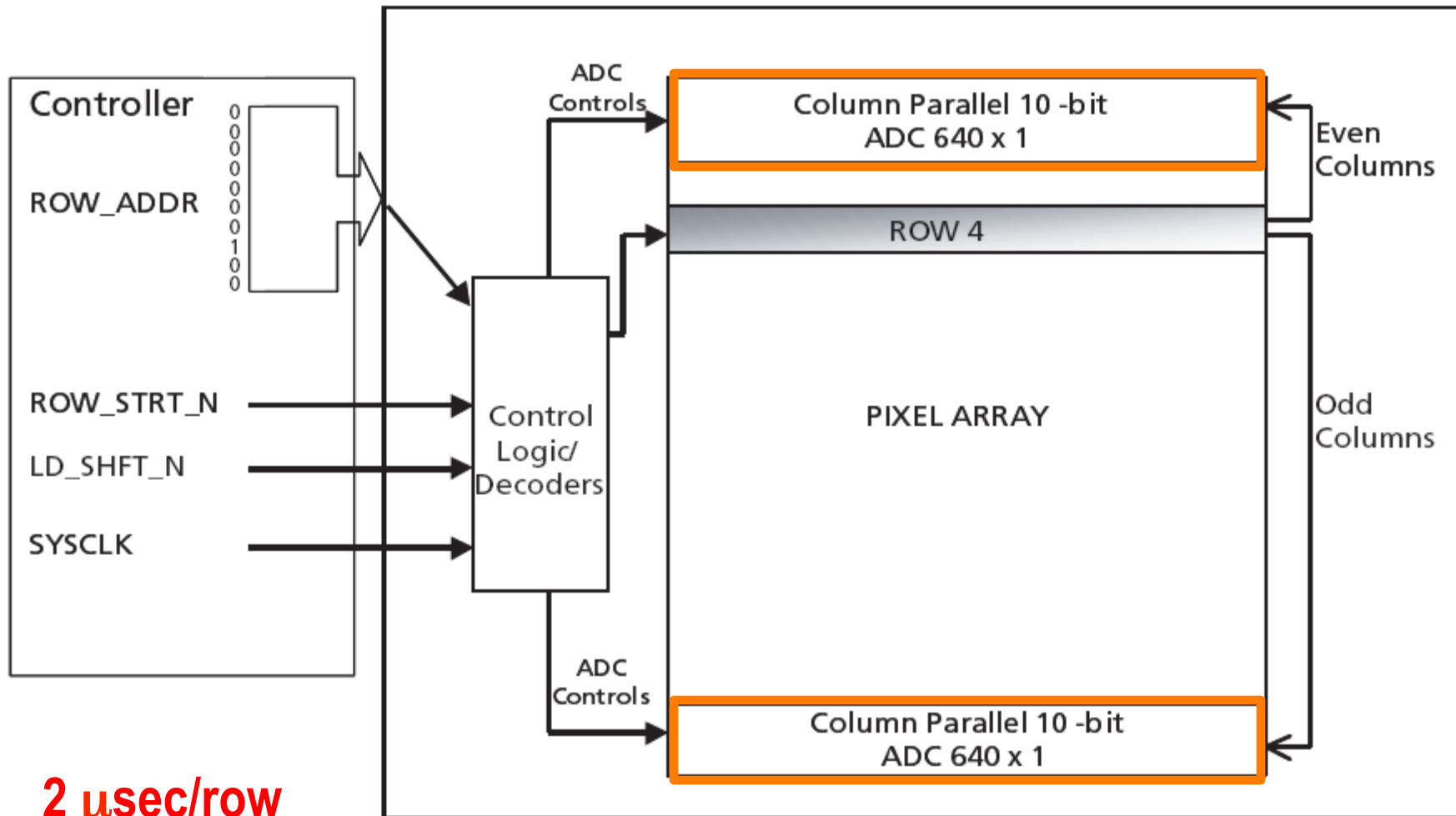


PMT + FADC (10 – 50 MHz)



[HAPD + FADC (1 GHz)] * 64

Micron 1.3M-Pixel CMOS Sensor

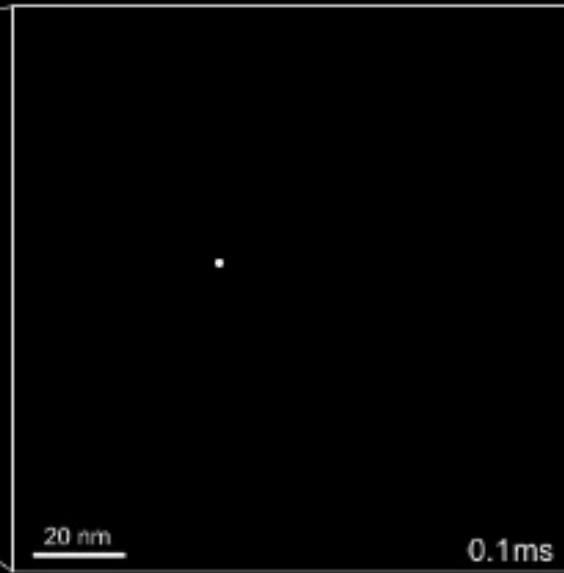
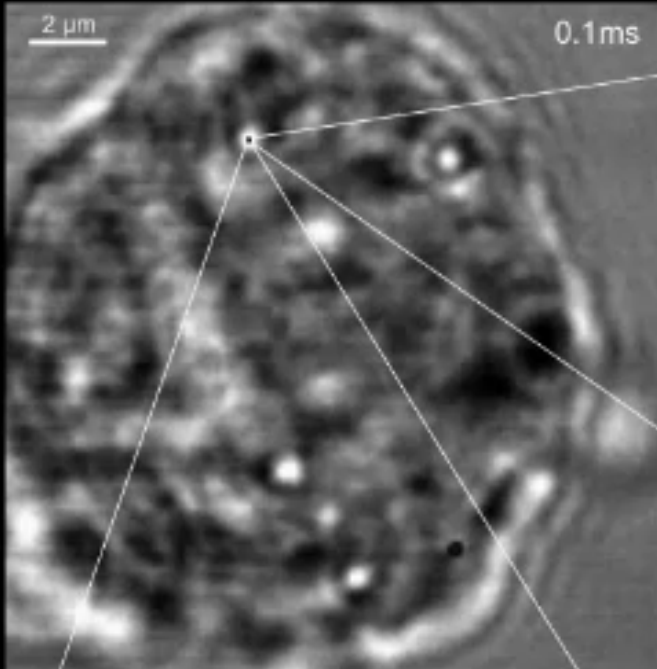


2 μ sec/row
2 msec/frame

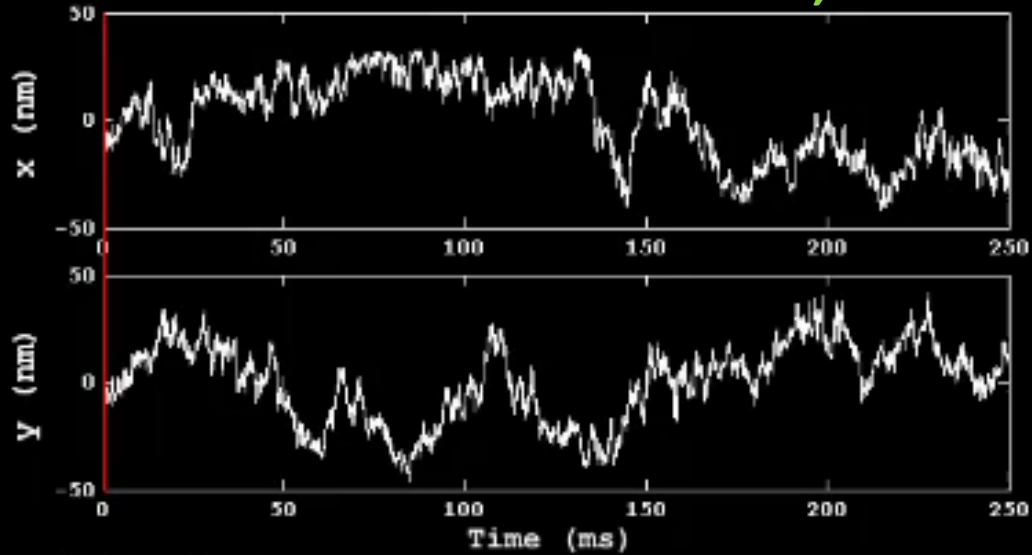
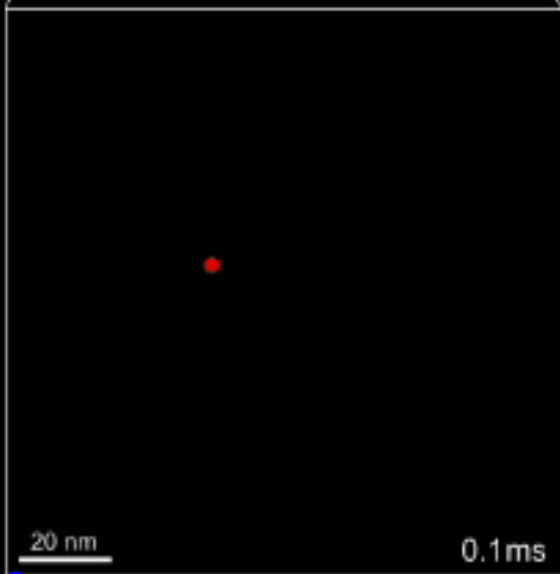
(10 bits, 66MHz)

Gold nano particle (40nm) attached to Transferrin Receptor (TfR) on Cancer Cell

Manuel Penichet (Oncology), John Miao (Physics)



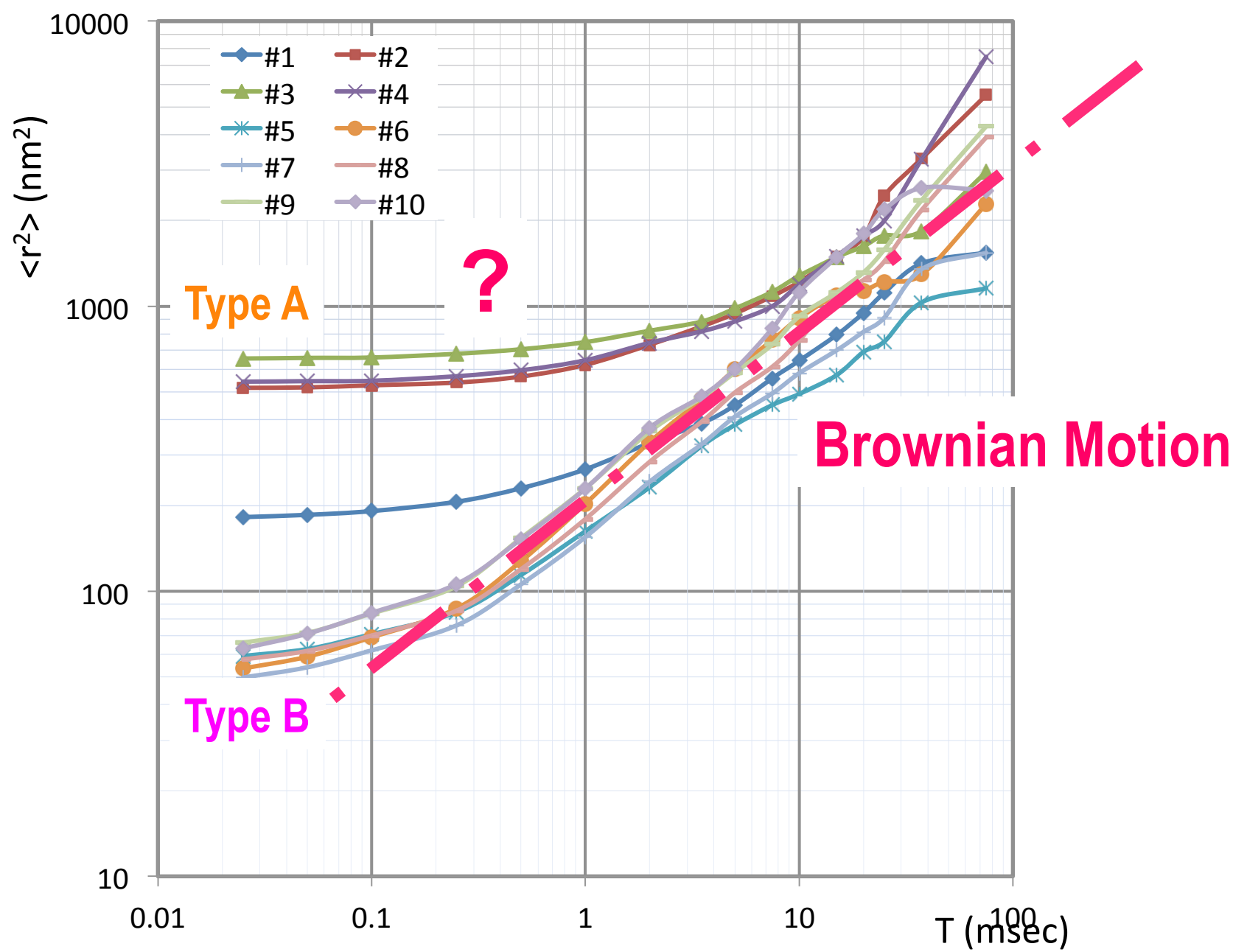
10,000 frame/sec



UCLA Fast Bio-Imaging Group

L. Fredrikson, A. Rodriguez, C. A. Cheng, K. Jewhurst, J. Miao, K. Arisaka

Mean Squire Displacement $\langle r^2 \rangle$ of TfR on a Human Multiple Myeloma Cell vs. Time



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.

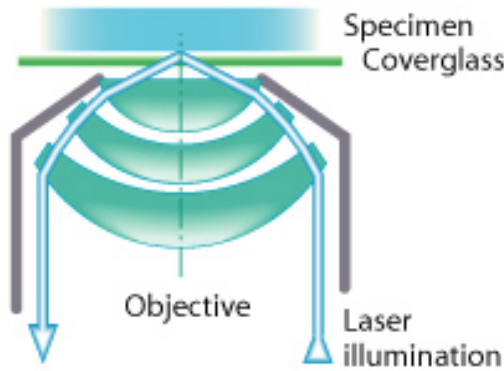
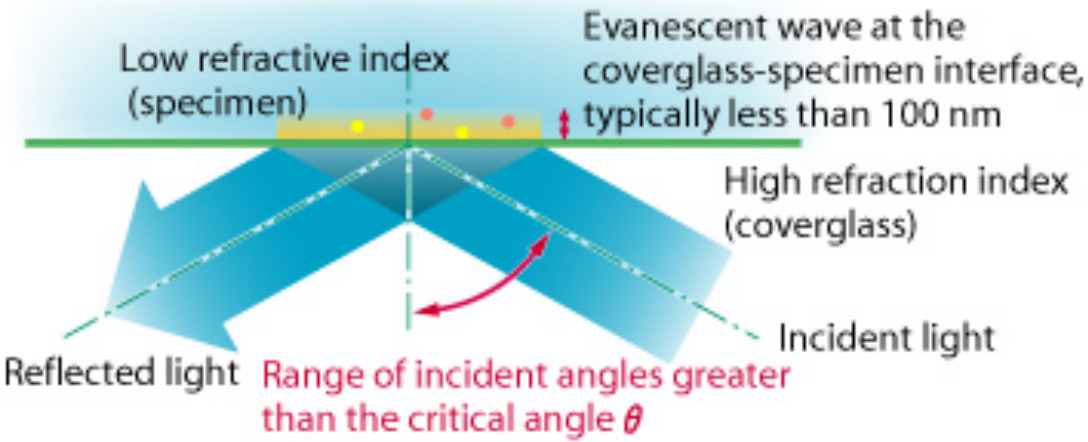
User-shared Core Facility of High-speed Microscopes at CNSI



4D Nano Biophysics

Nikon Microscope TE200E with TIRF at CNSI

Laurent Bentolila (CNSI)



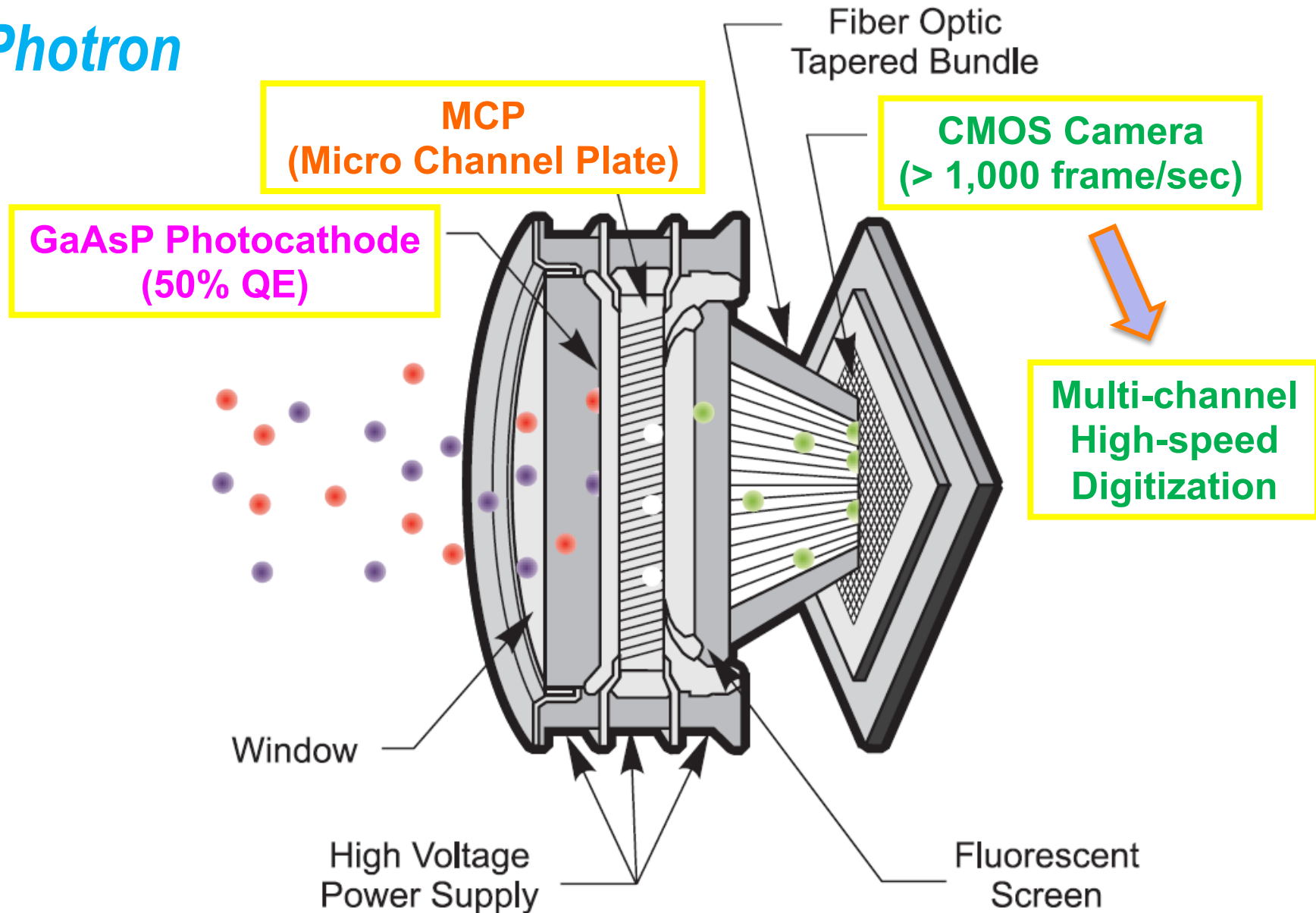
5k – 500k fps



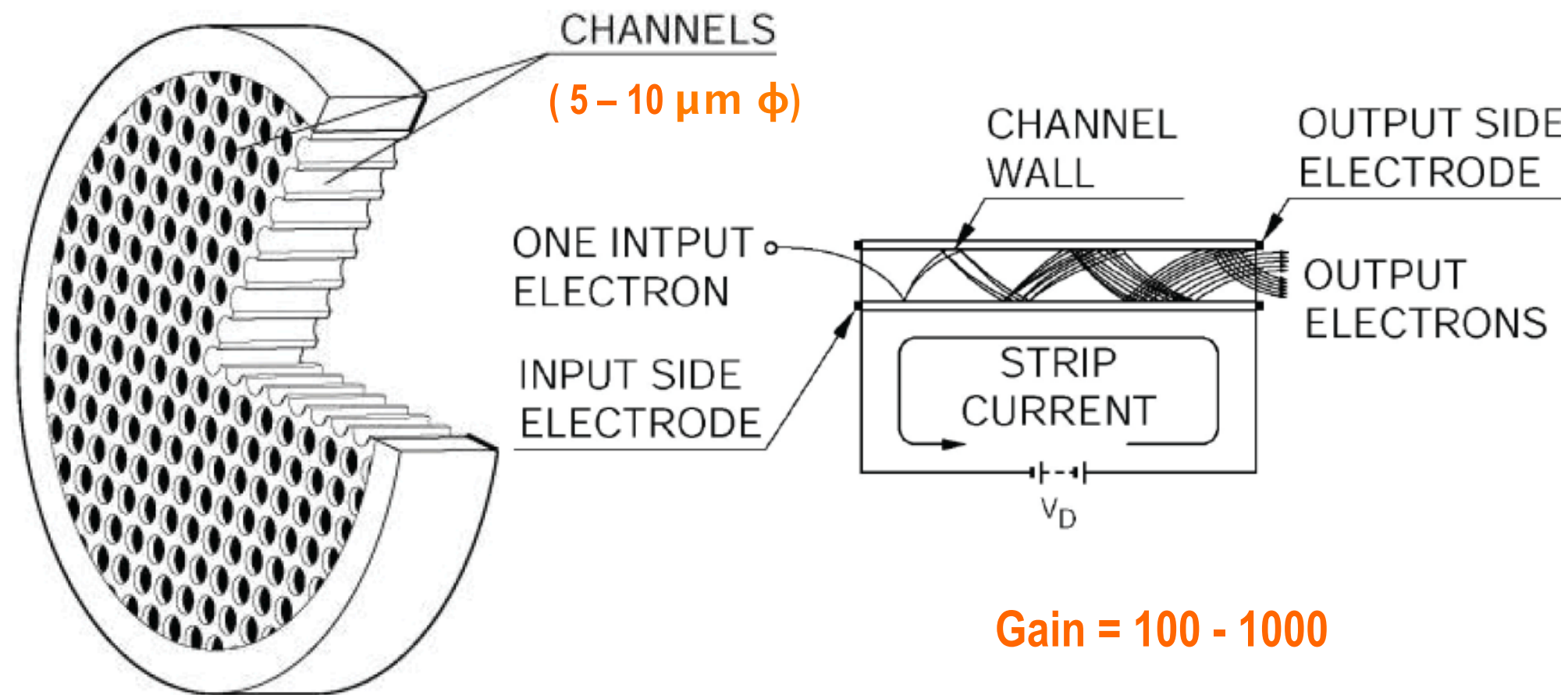
CMOS Camera
Photron SA-1

Principle of ICMOS

Photron



MCP (Micro Channel Plate)



Gain = 100 - 1000

High-speed Confocal Microscope with ICMOS at CNSI

(1,000 frame/s)

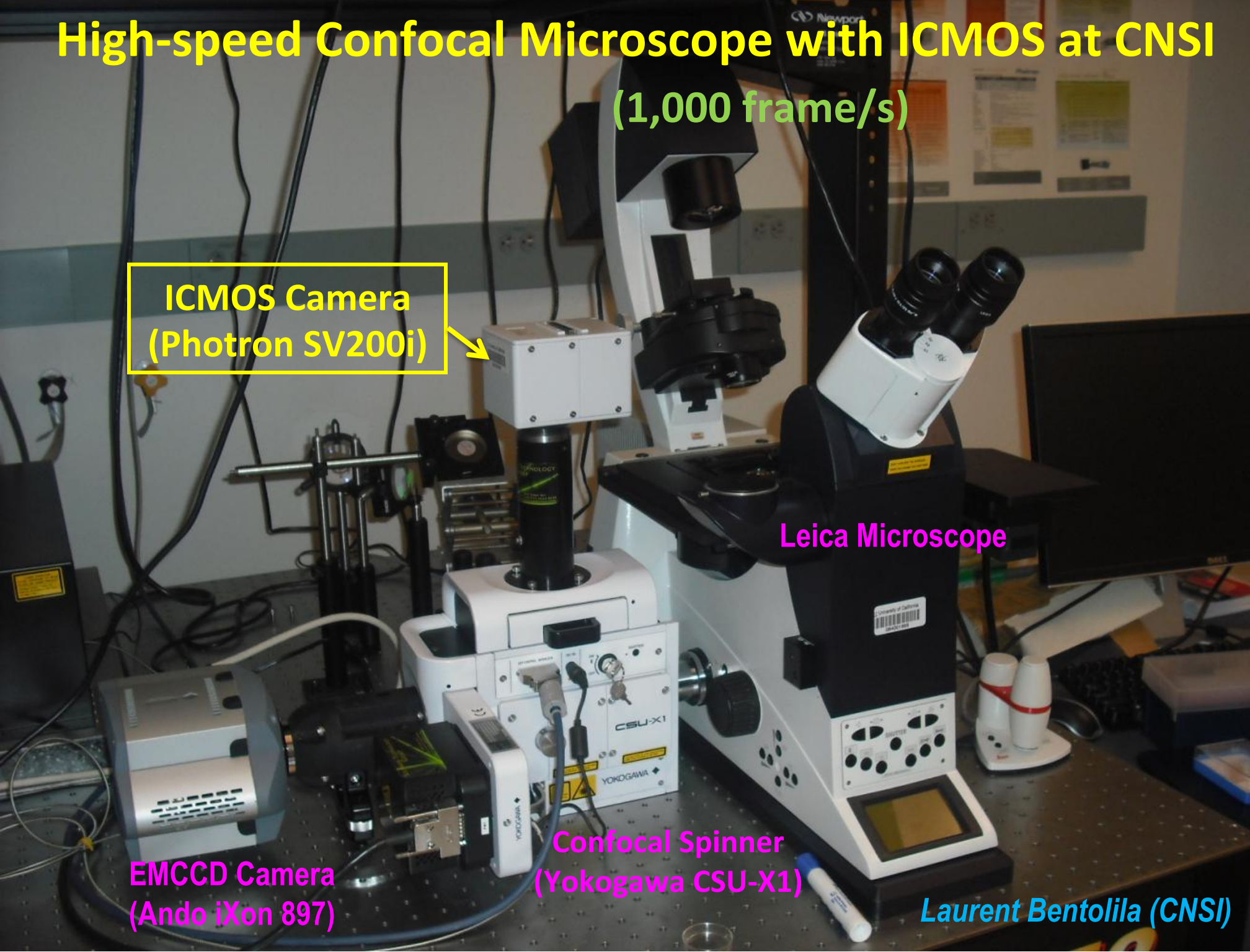
ICMOS Camera
(Photron SV200i)

Leica Microscope

EMCCD Camera
(Ando iXon 897)

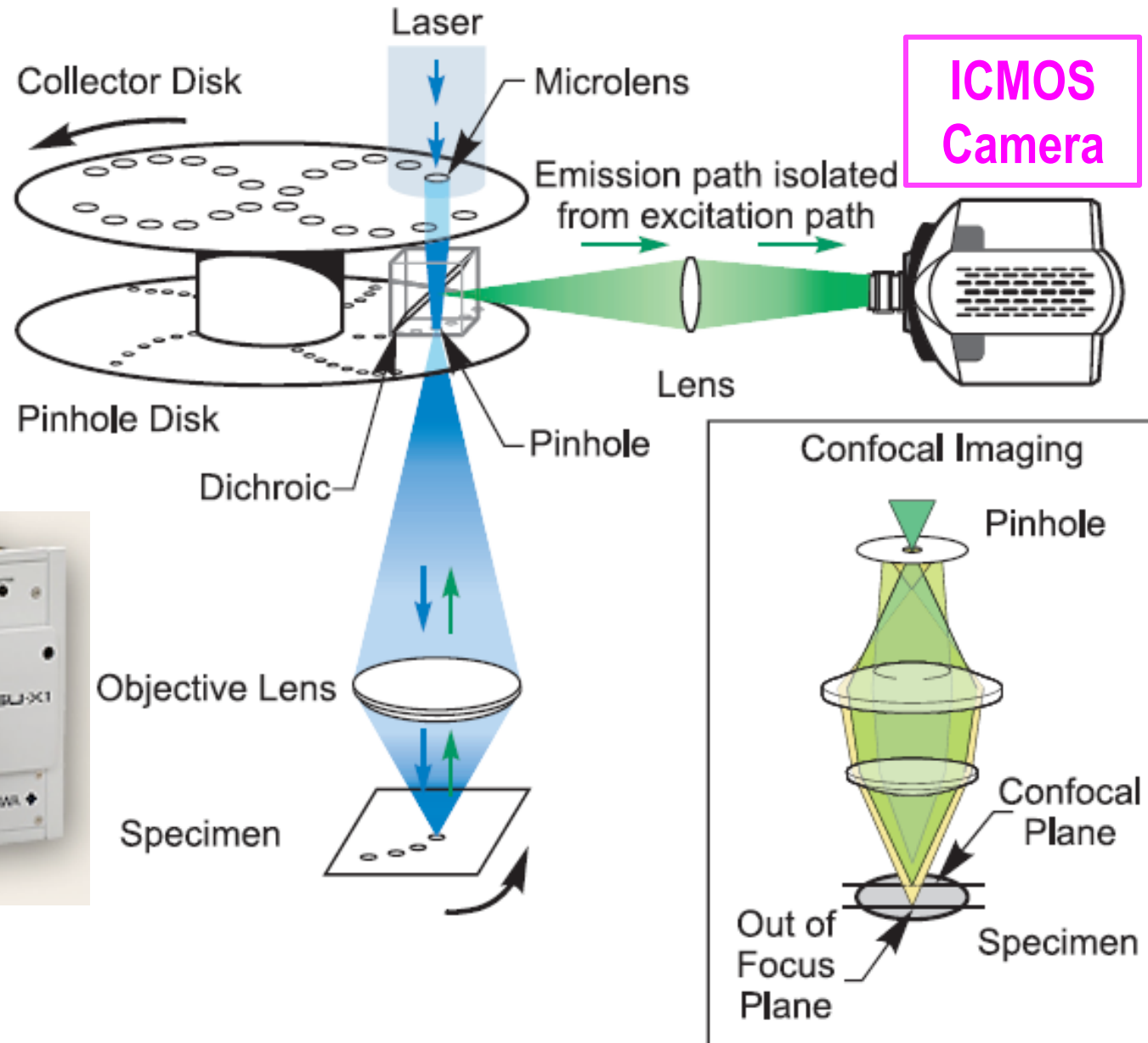
Confocal Spinner
(Yokogawa CSU-X1)

Laurent Bentolila (CNSI)



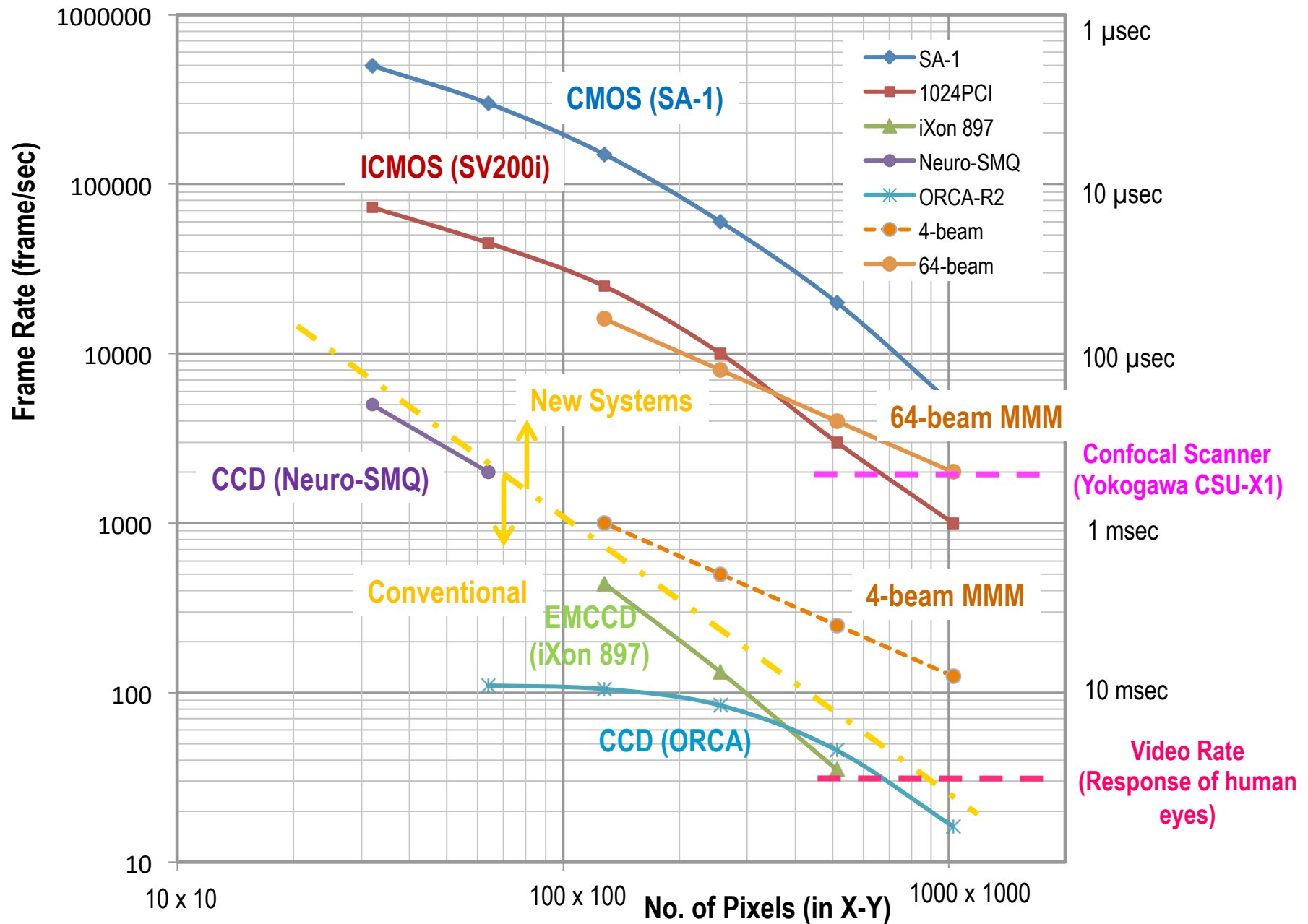
Yokogawa CSU-X1

2,000 fps



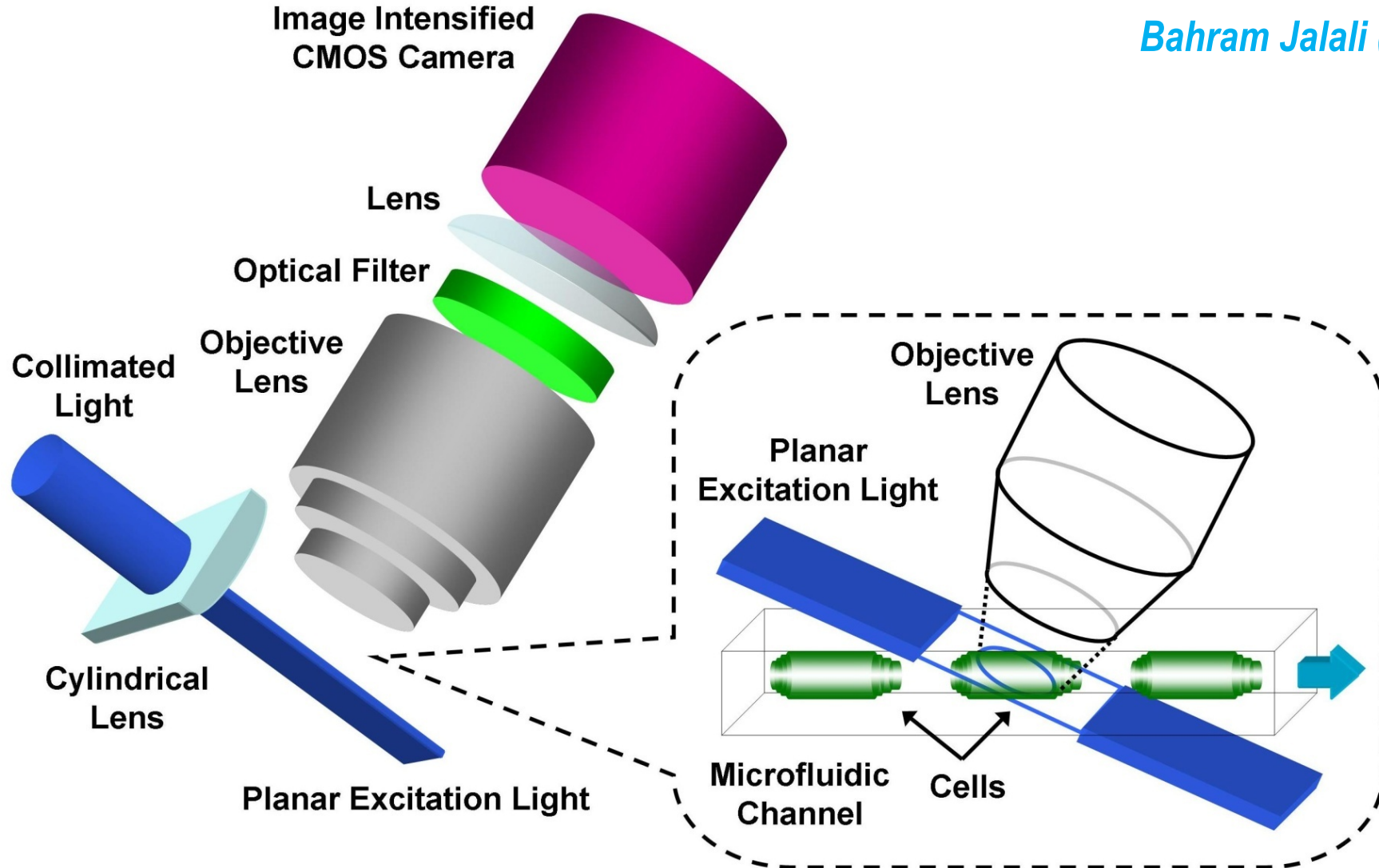
Confocal Dual Spinning Disk

Frame Rate vs. Resolution



Imaging Flow Cytometer by Planar Illumination

Bahram Jalali (EE)

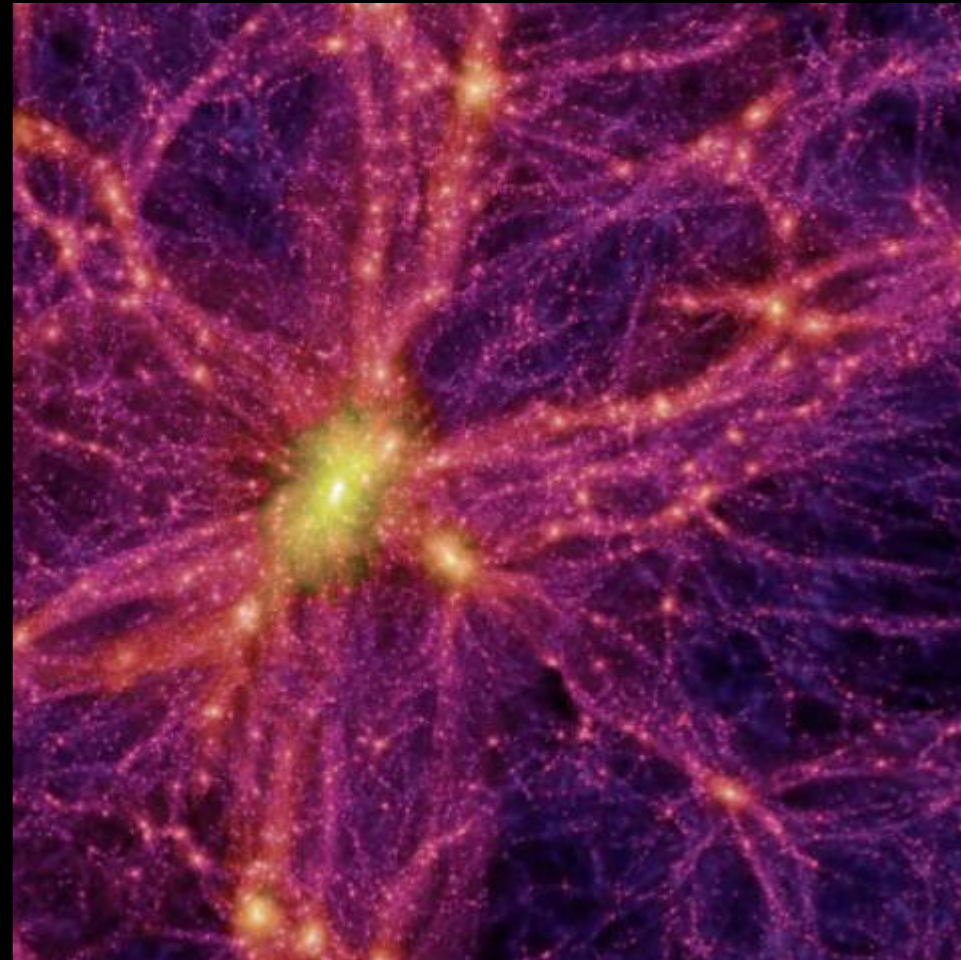
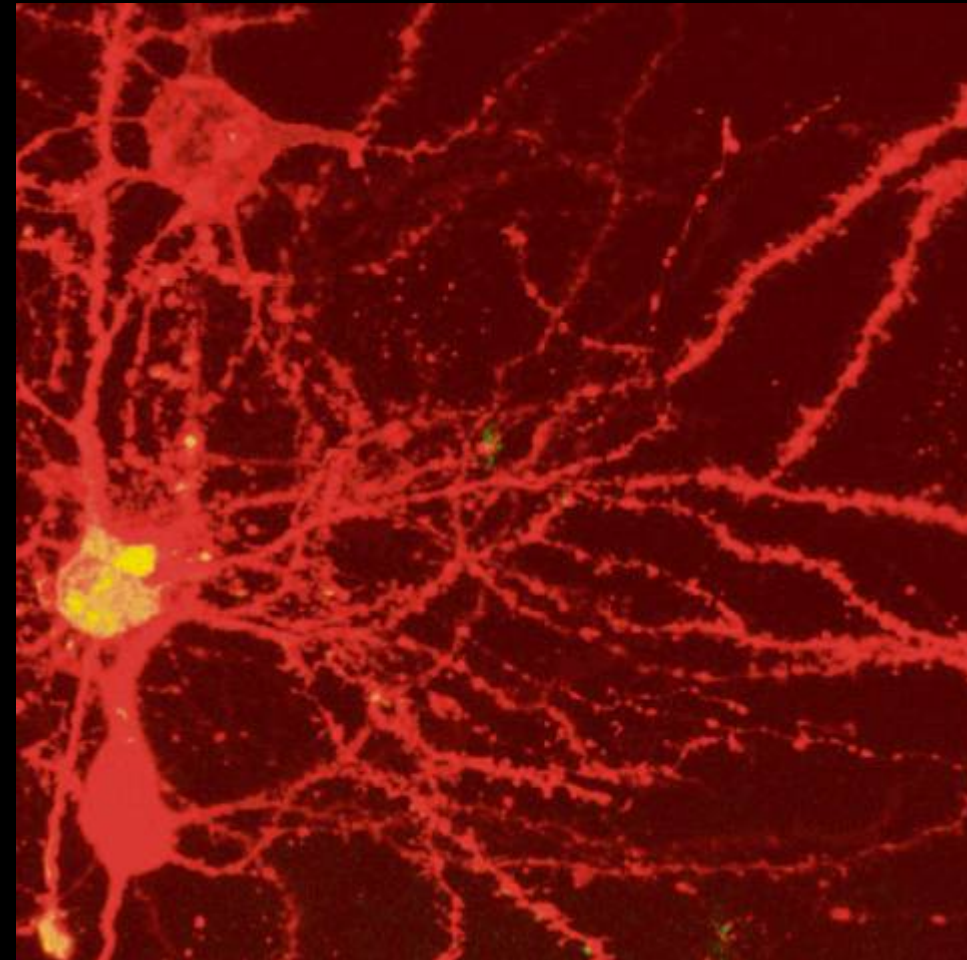


Speeding up evolution of life by accelerating mutations.

Origin of Consciousness

Brain

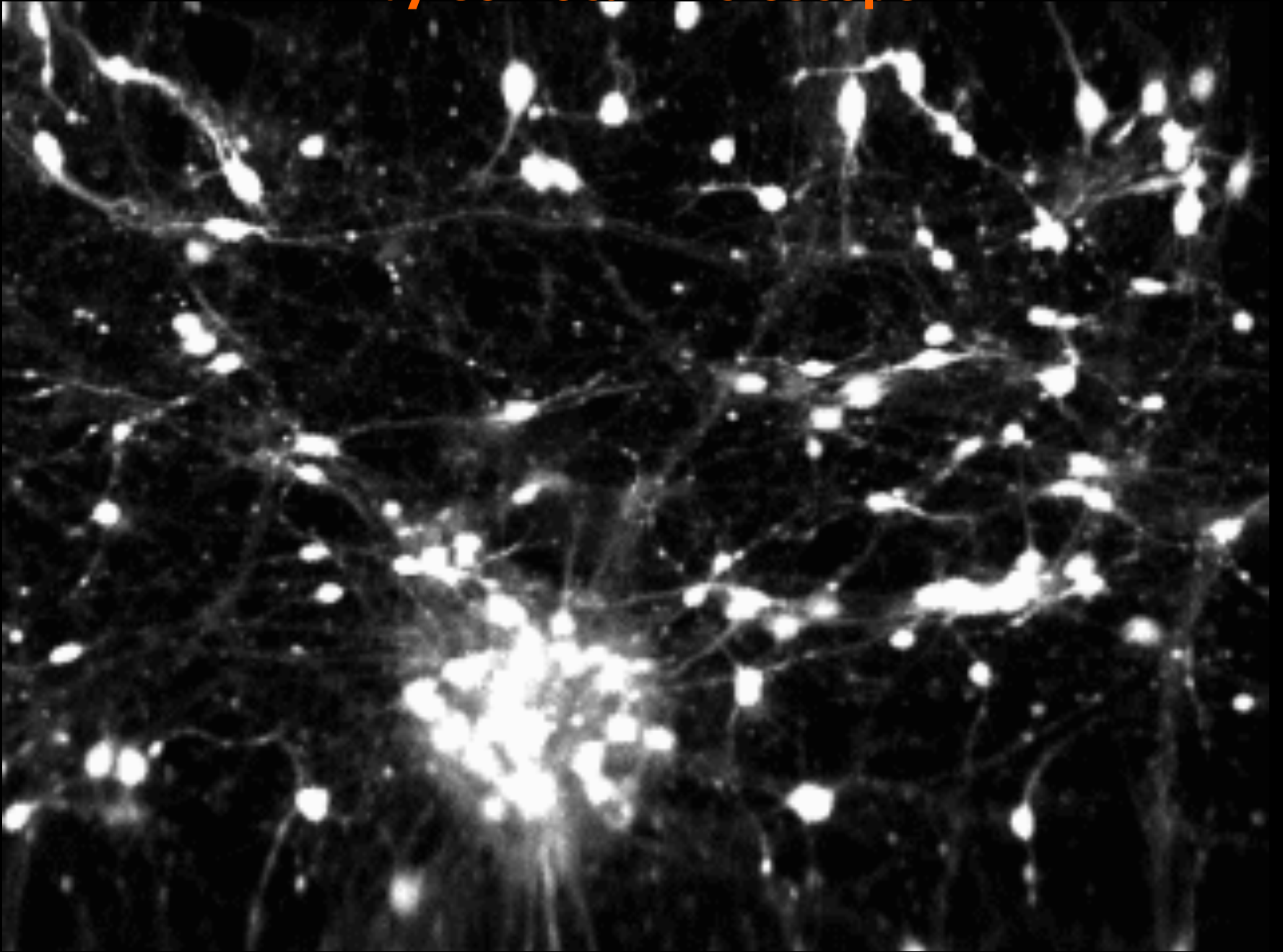
Universe



100 Billions Neurons

100 Billions Galaxies

Ca²⁺ Signal in cultivated Rat's Brain by Confocal Microscope

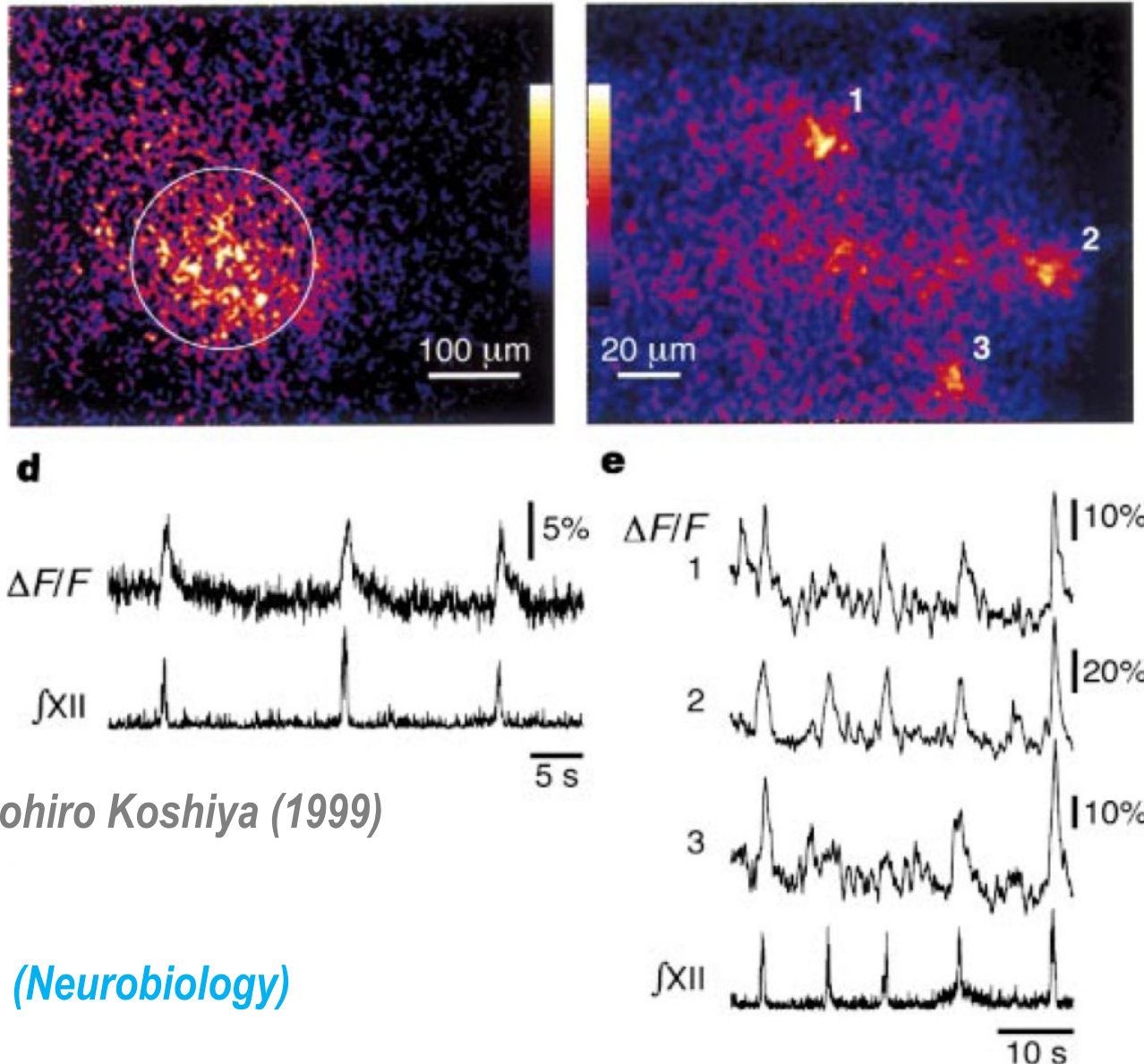


Andrew Charles (Neurobiology)

15 frame/sec

Neural Networks for Breathing

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

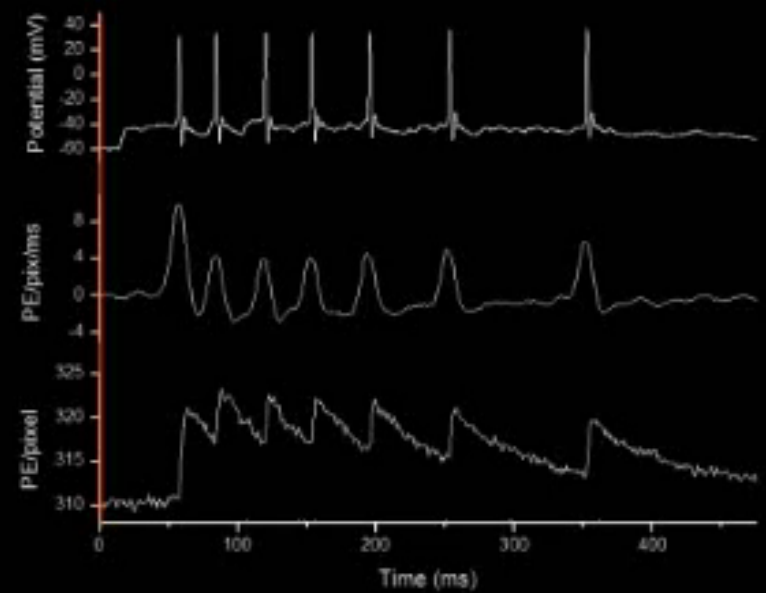
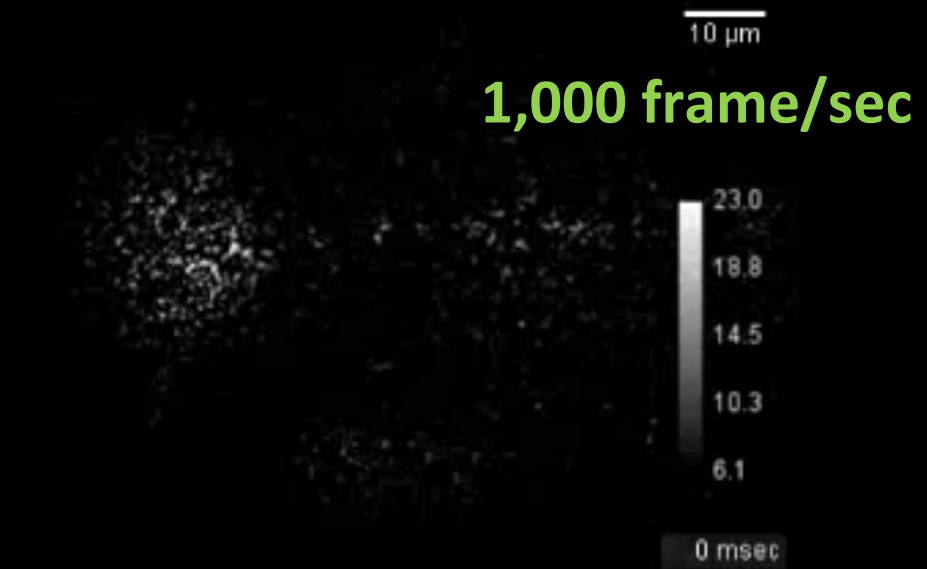


Nature by Naohiro Koshiya (1999)

Jack Feldman (Neurobiology)

High-speed Ca^{2+} Imaging of pre-Botzinger Cells of Rats

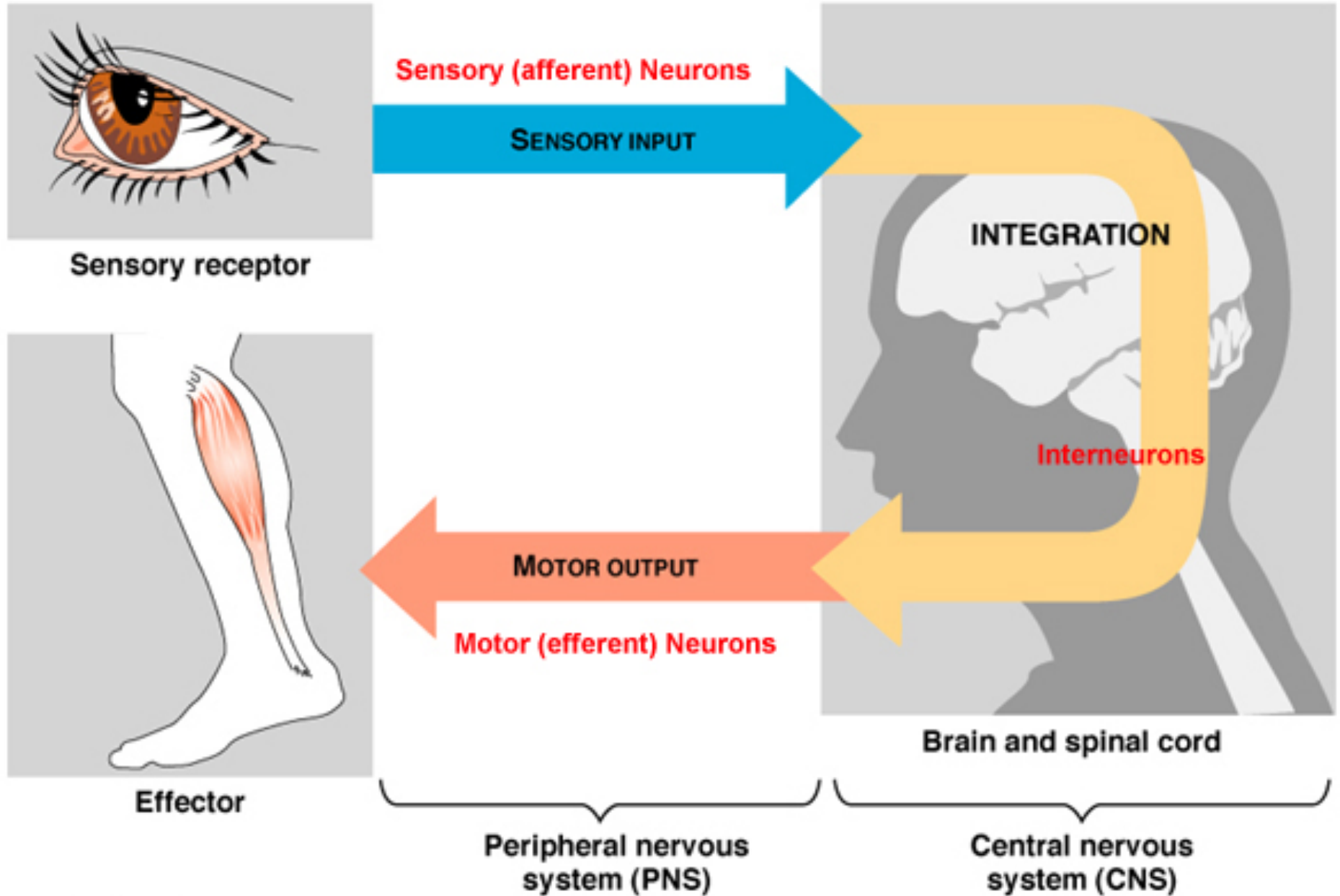
Jack Feldman (Neurobiology)



UCLA Ultra-fast Bio-imaging Group

C. Morgado, A. Cheng, L. Frederickson, K. Arisaka, J. Feldman

Sensory Input and Decision Making in Brain



Anatomy of Inner Ear

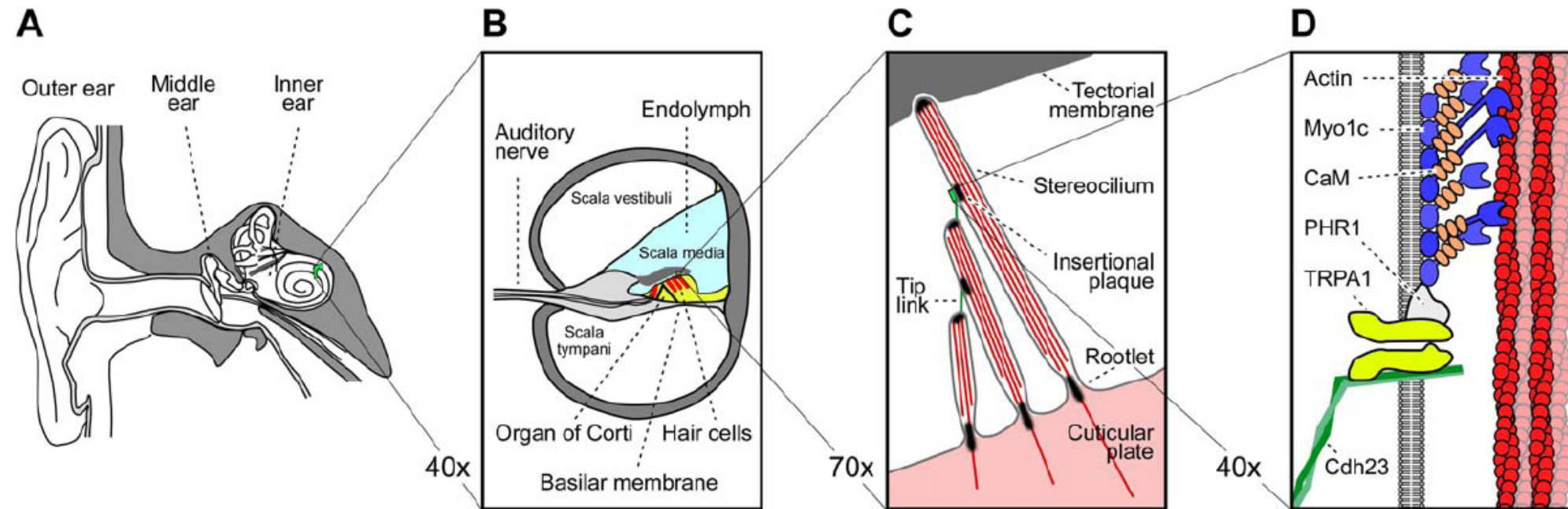
Dolores Bozovic (Physics)

Human auditory system

Cross-section of the cochlear

Hair bundle

Molecular mechano-transduction machinery



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?**
 - miss match to the speed of action potential of 1 kHz

$$\omega = \sqrt{\frac{k}{m}}$$

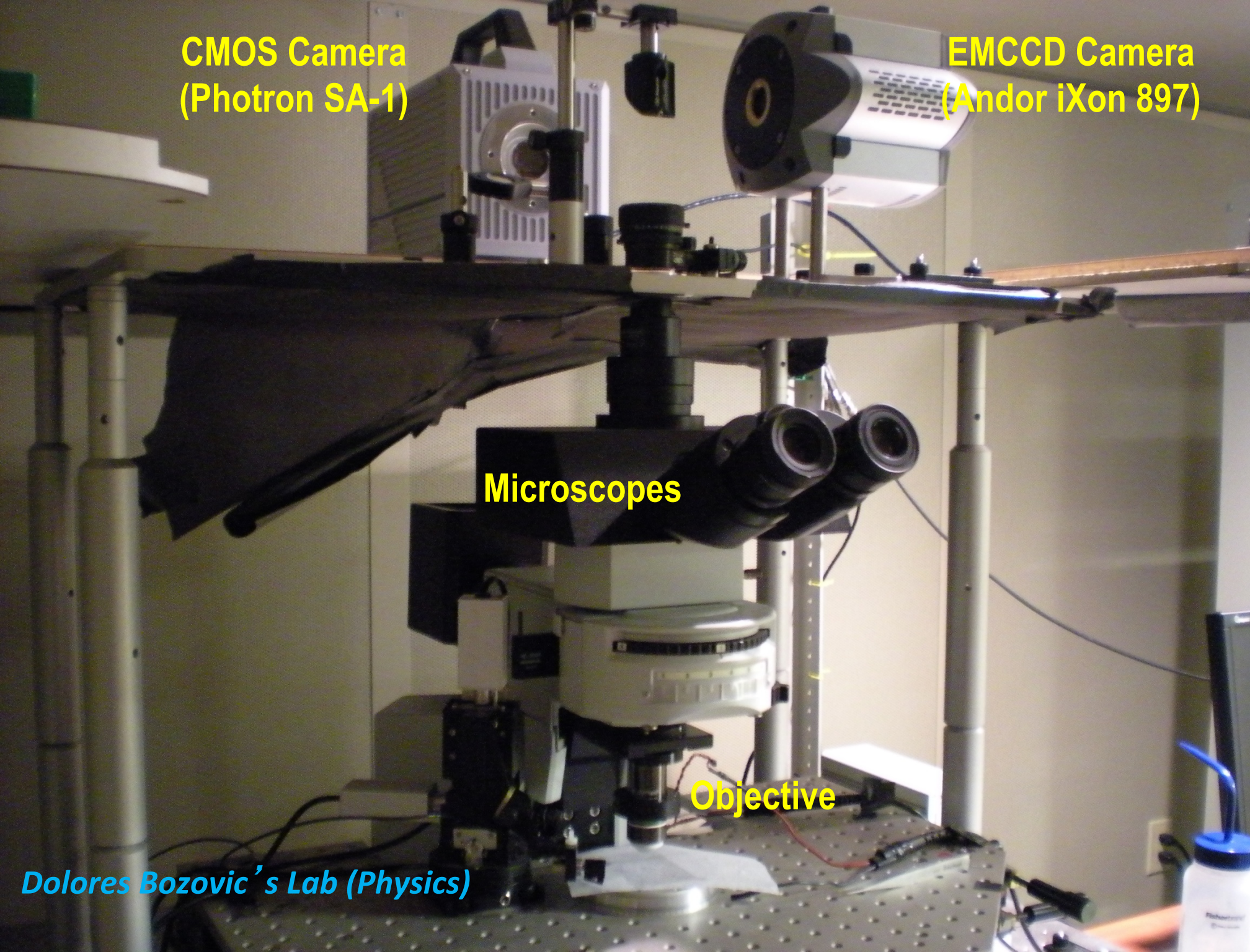
**CMOS Camera
(Photron SA-1)**

**EMCCD Camera
(Andor iXon 897)**

Microscopes

Objective

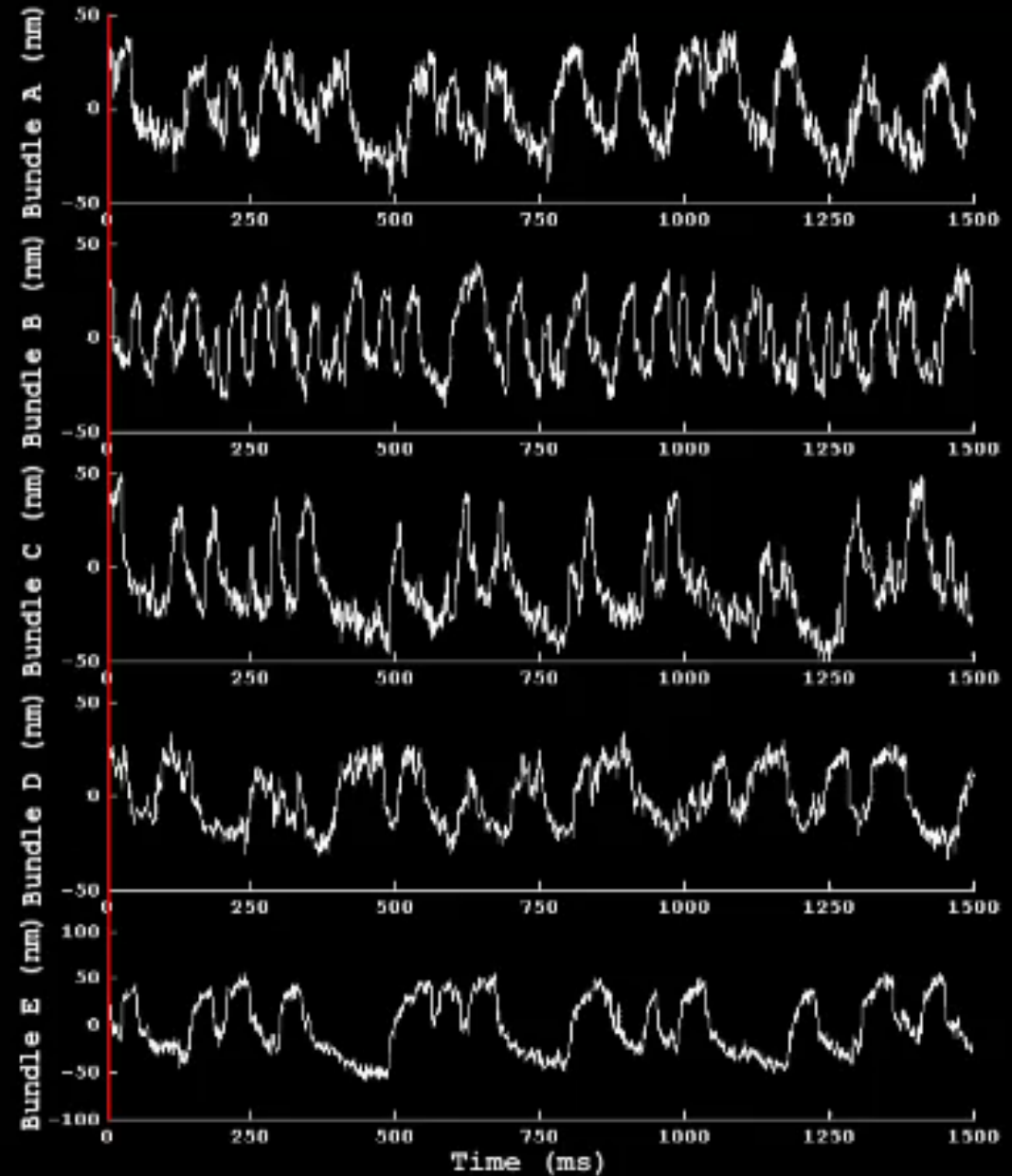
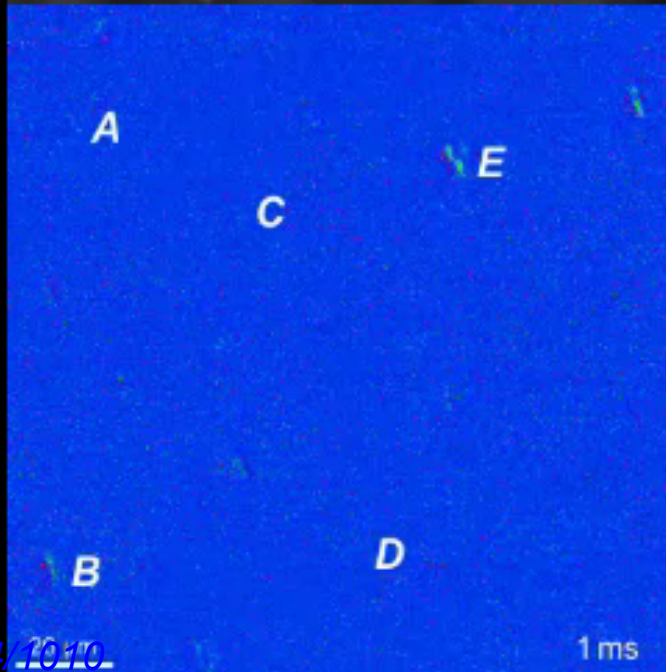
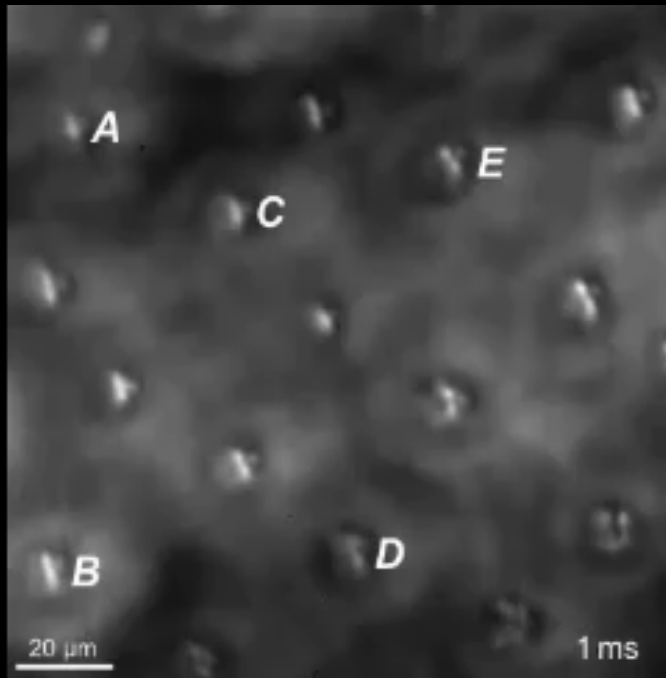
Dolores Bozovic's Lab (Physics)



Mechanical Motion of Hair Cells in Inner Ear

Dolores Bozovic, Lea Fredrickson (Physics)

1,000 frame/sec



UCLA Fast Bio-Imaging Group

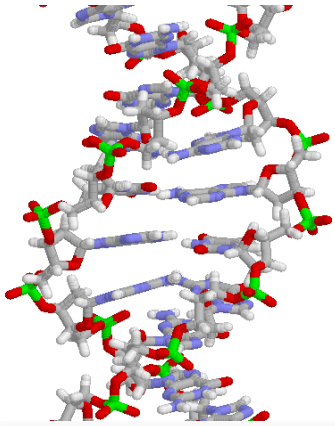
L. Fredrickson, A. Cheng, J. C. Jewhurst, C. E. Stribu, D. Bozovic, K. Arisaka

How can I recognize a woman so far away?

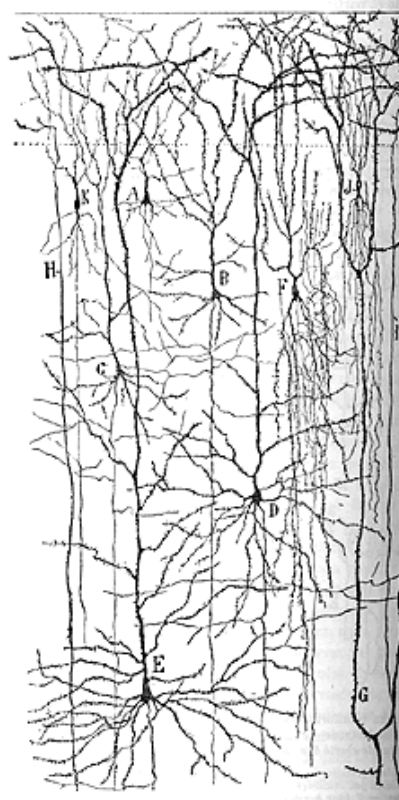


- Genetically encoded?
- Learning and memory?

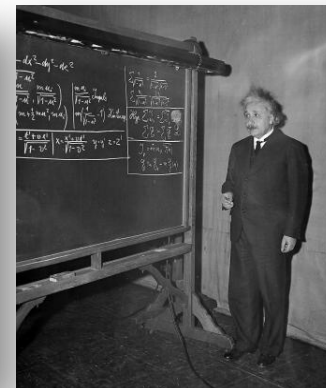
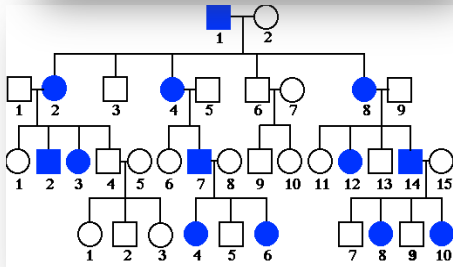
Nature vs. Nurture



Nature

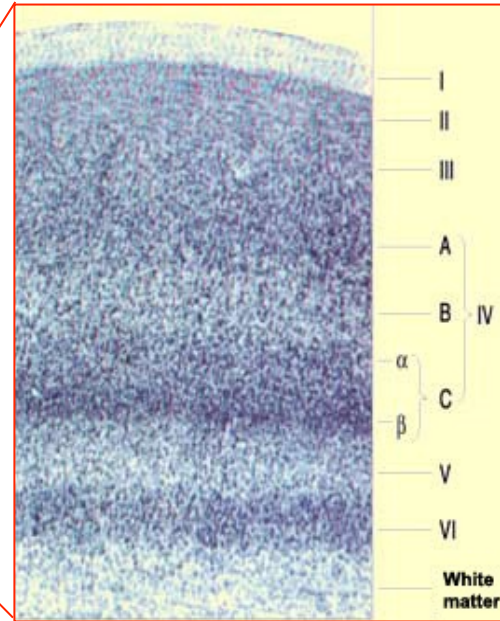
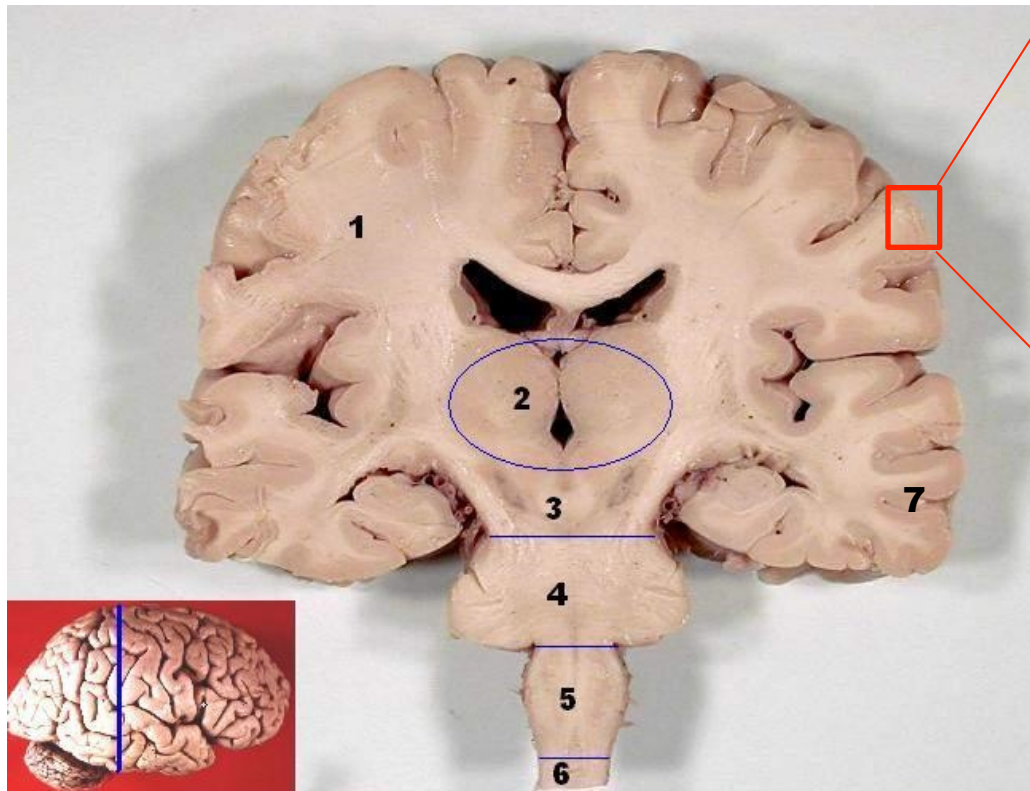


Nurture

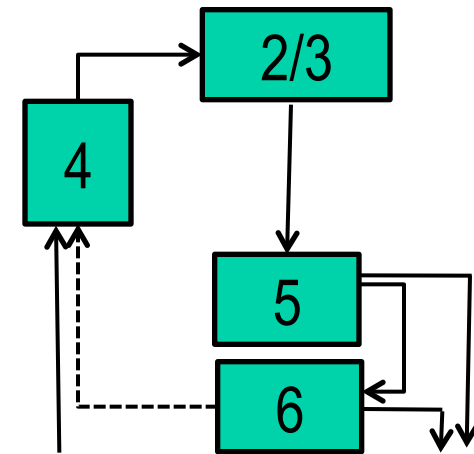


The Cerebral Cortex

Conscious
↑



↓
Unconscious

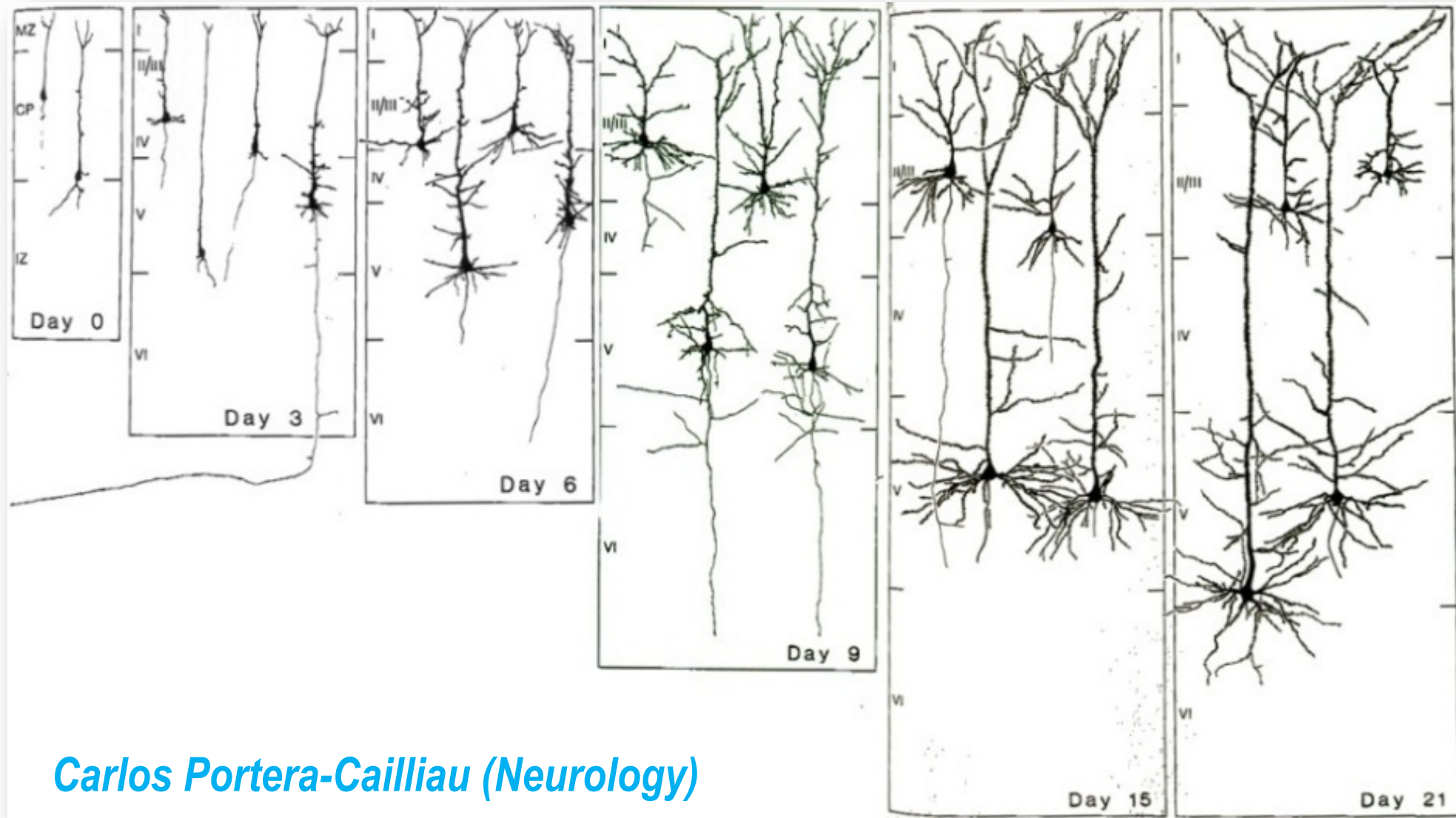


Thalamus

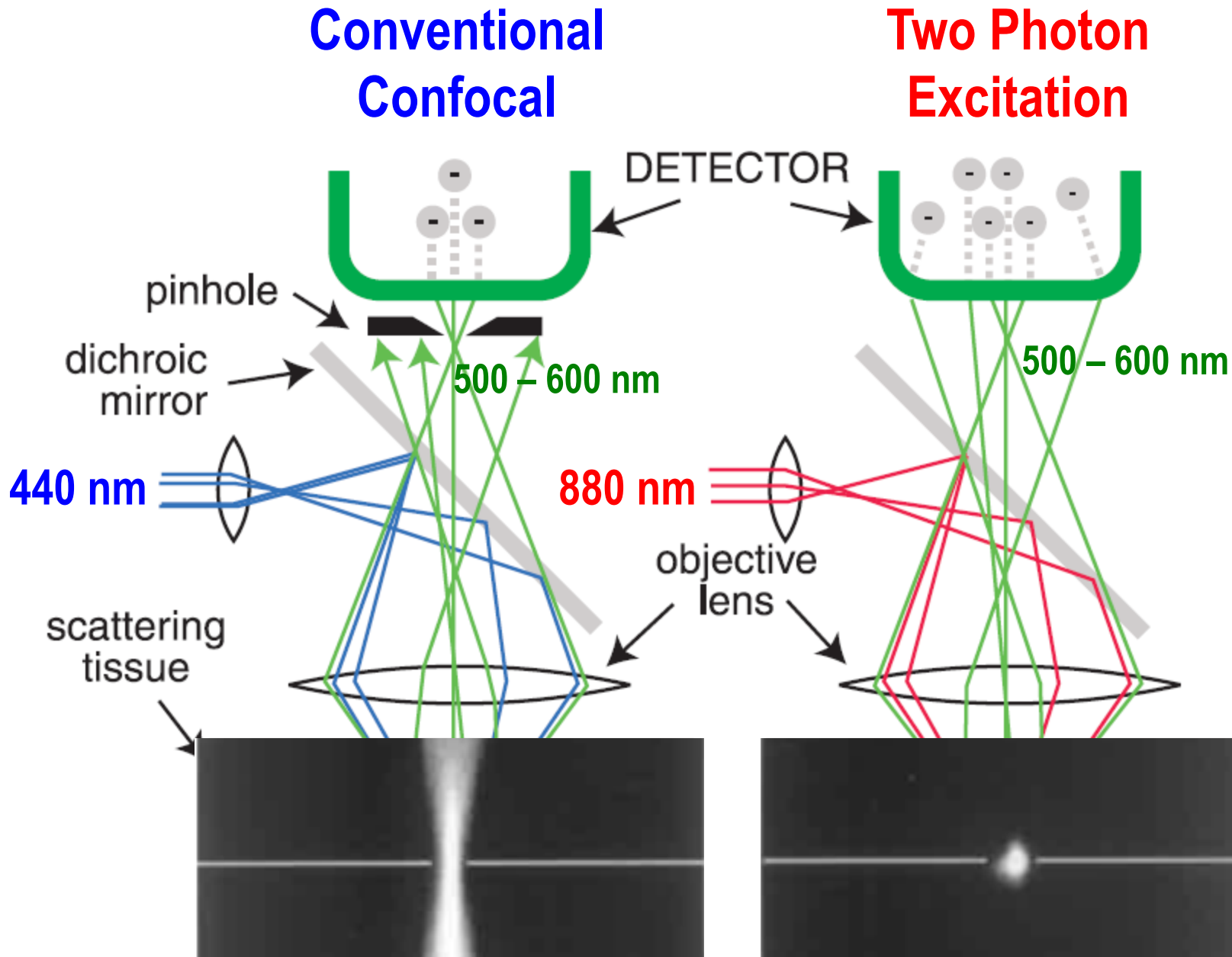
Subcortical areas

Assembly of rat's cortical circuits during development

How/when do neurons establish networks? → *Symmetry Breaking*

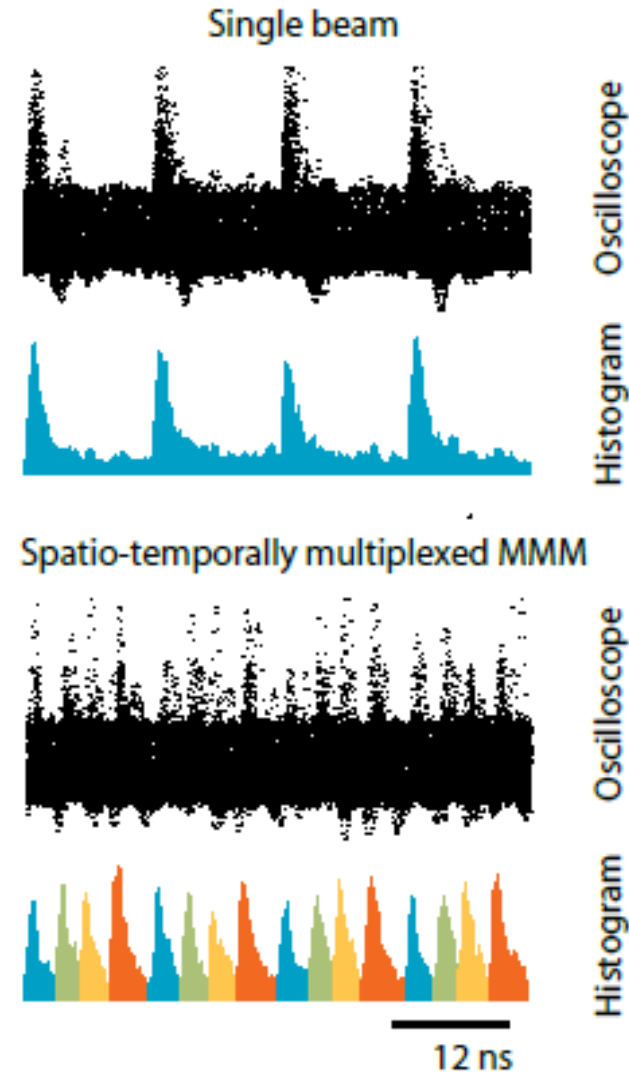
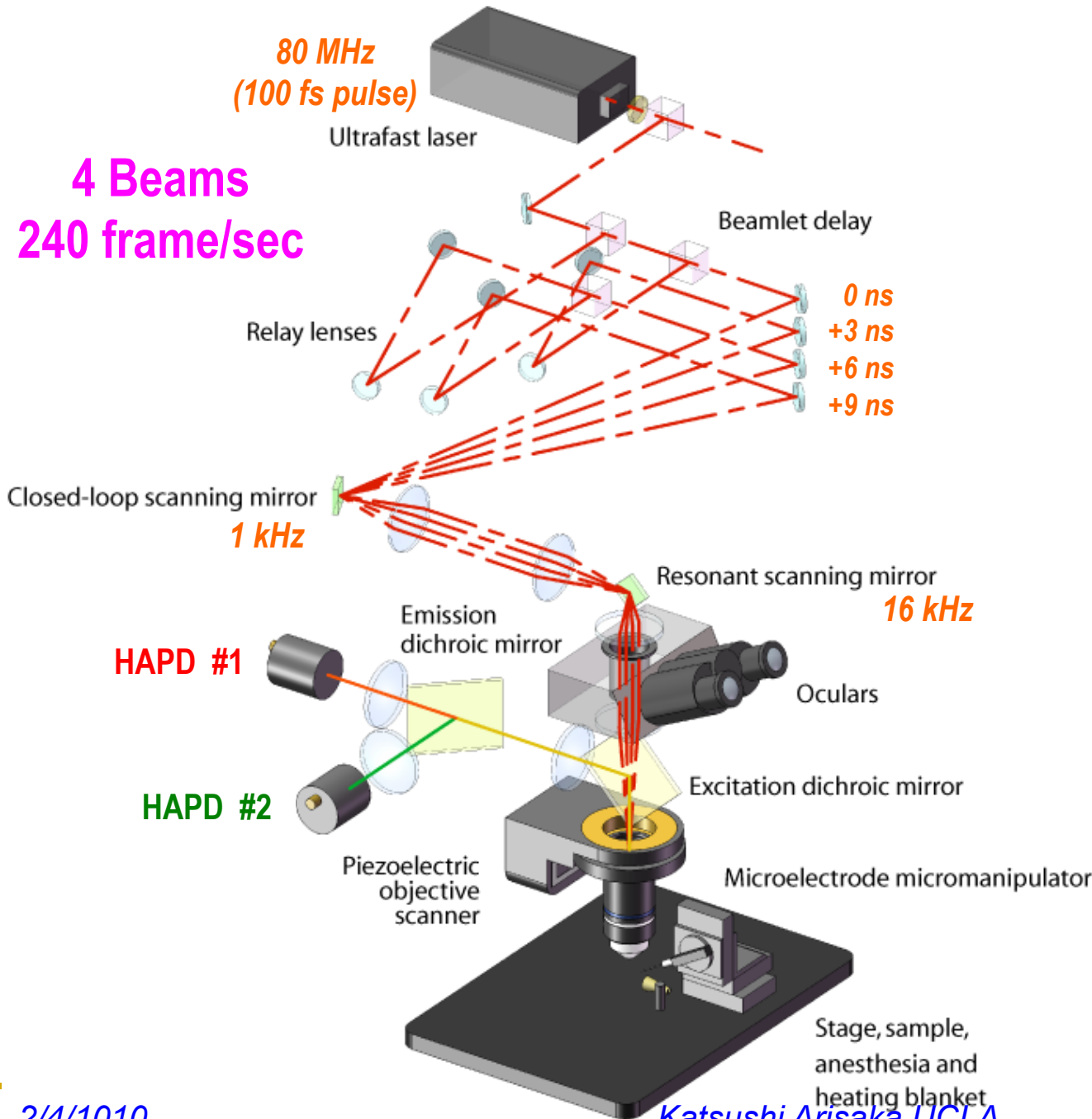


Multiphoton Microscope

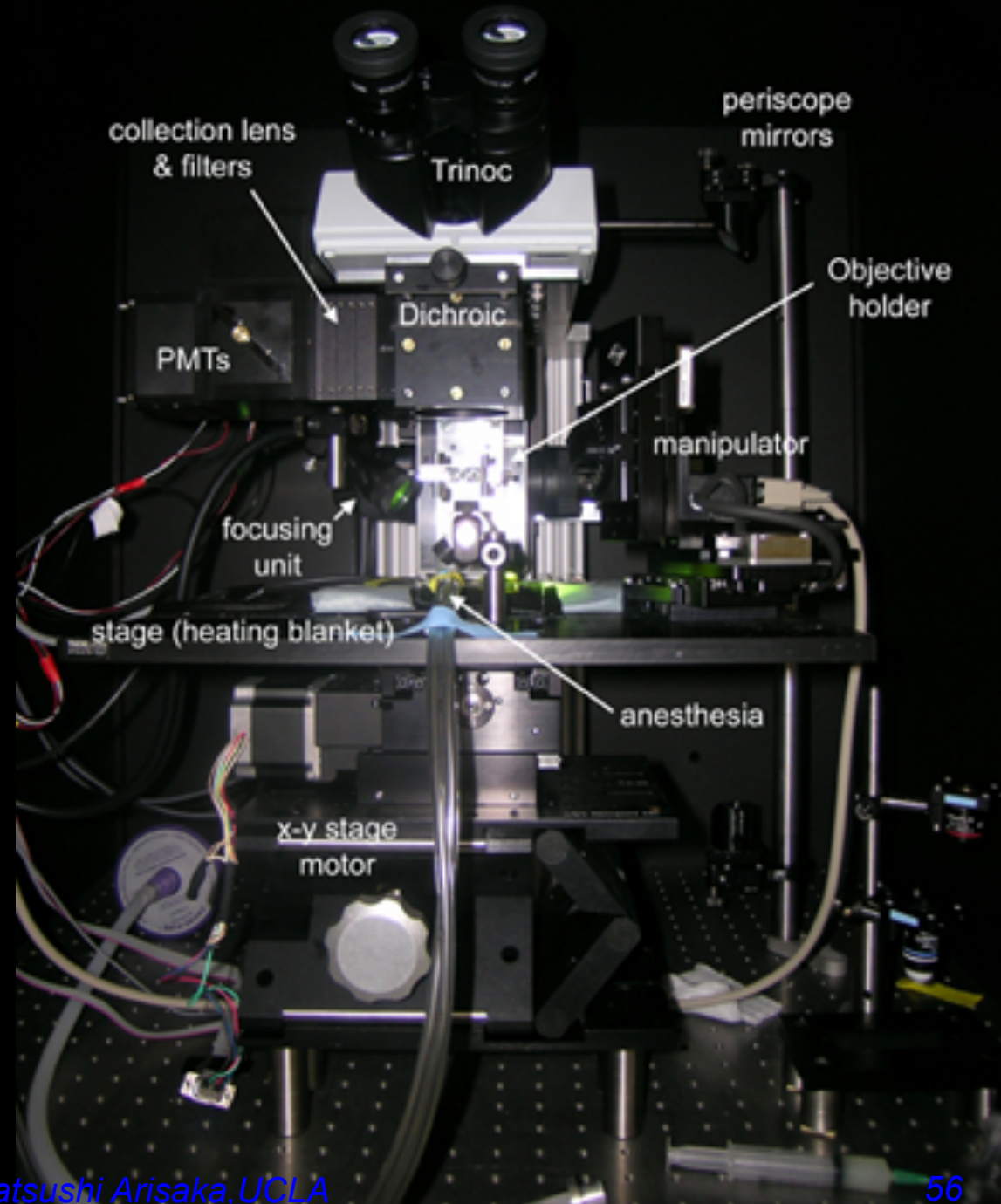
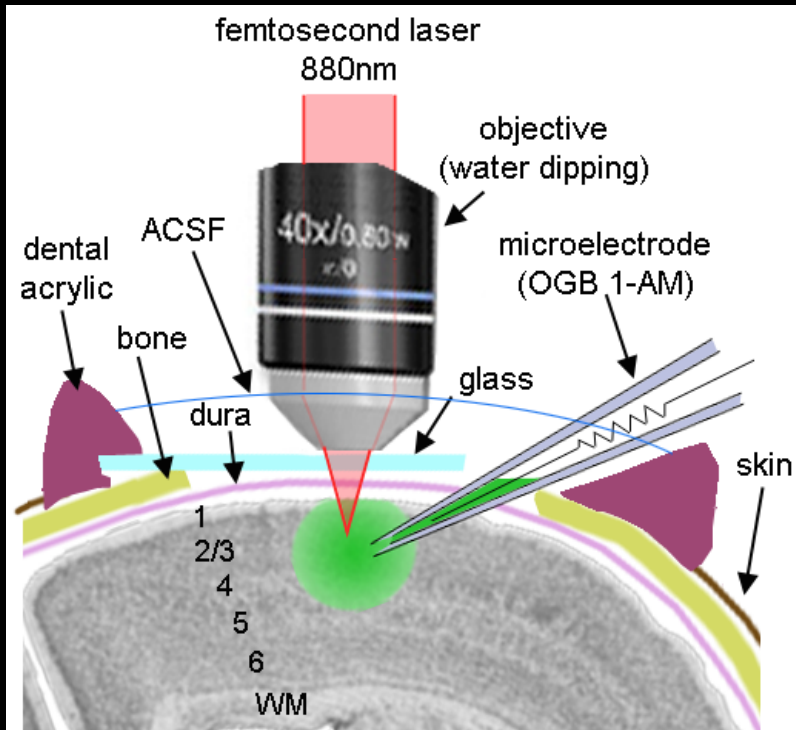


Spatio-Temporal Excitation-Emission Multiplexing (STEM) Microscope

Adrian Cheng (Physics)



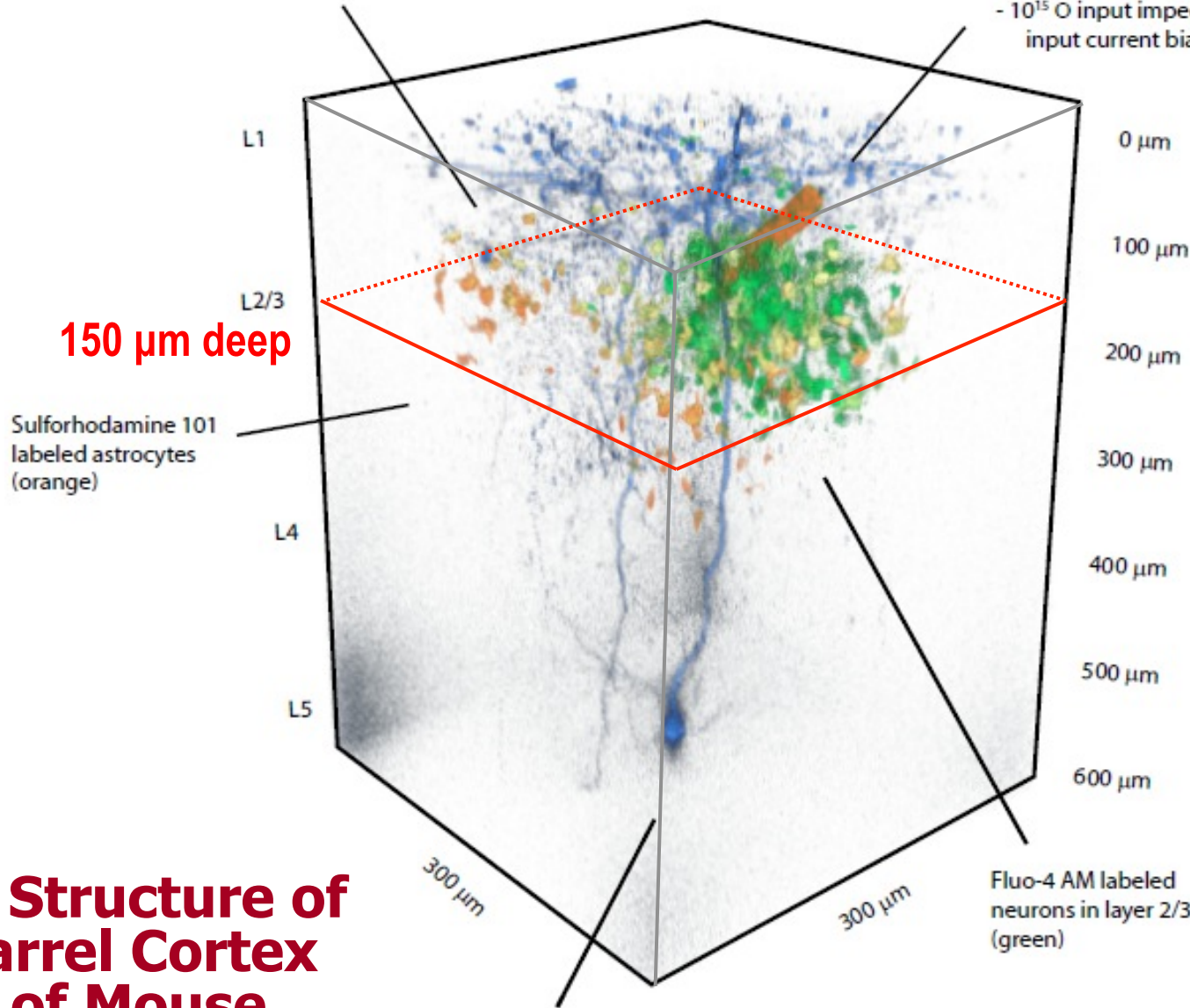
In vivo calcium imaging of neuronal activity



Fluo-4 AM labeled astrocytes are colabeled with sulforhodamine 101 to eliminate background (yellow)

Glass microelectrode for dye injection and electrophysiology

- cell-attached voltage follower
- whole-cell voltage/current clamp
- $10^{15} \Omega$ input impedance, $< 150 \text{ fA}$ input current bias



*Adrian Cheng
(Physics)*

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

3D Structure of Barrel Cortex of Mouse

Layer 5 pyramidal neuron soma and apical dendrite from a transgenic animal demonstrates imaging depth (blue)

Katsushi Arisaka, UCLA

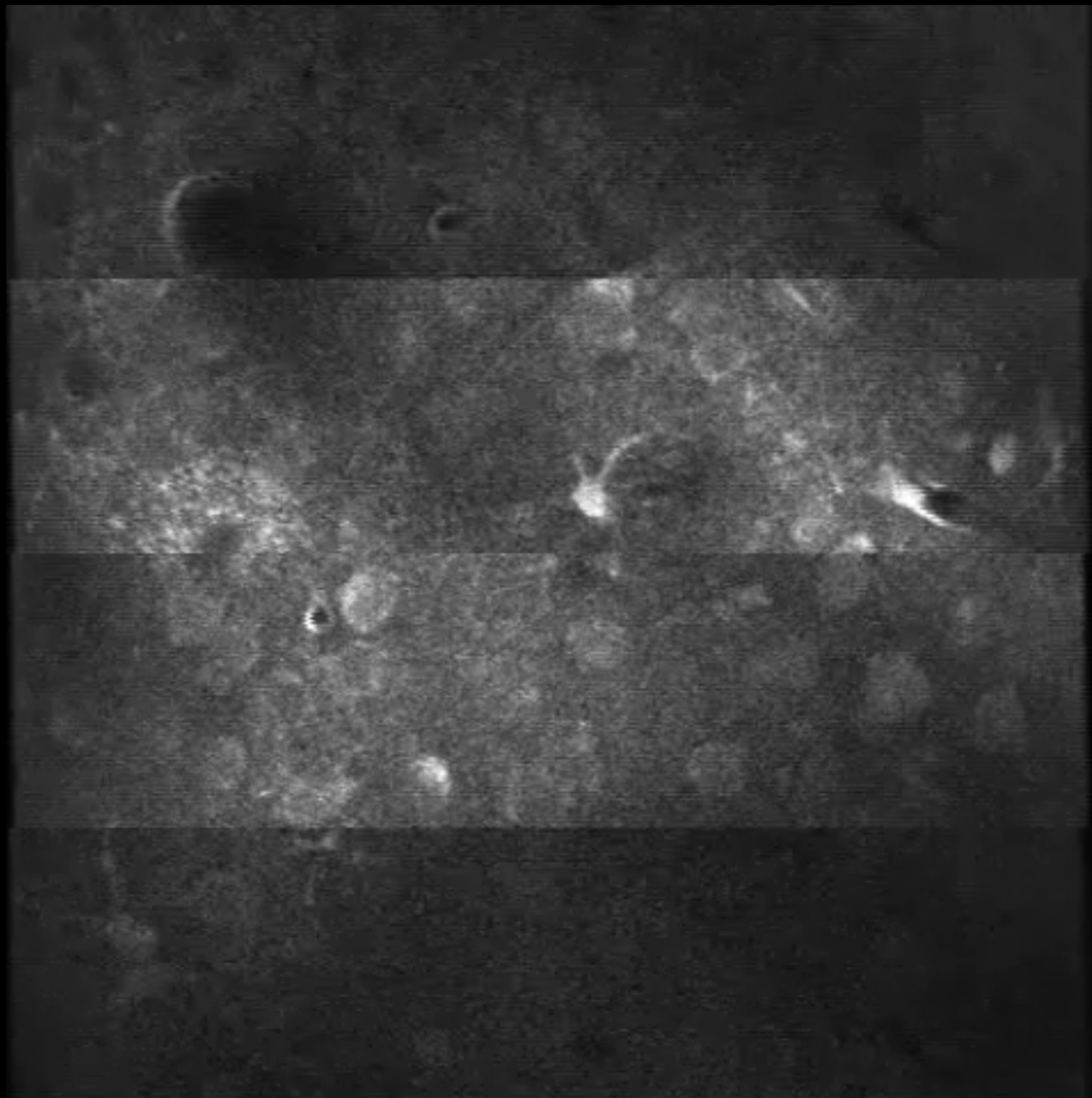
In vivo calcium imaging with STEM

**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)**

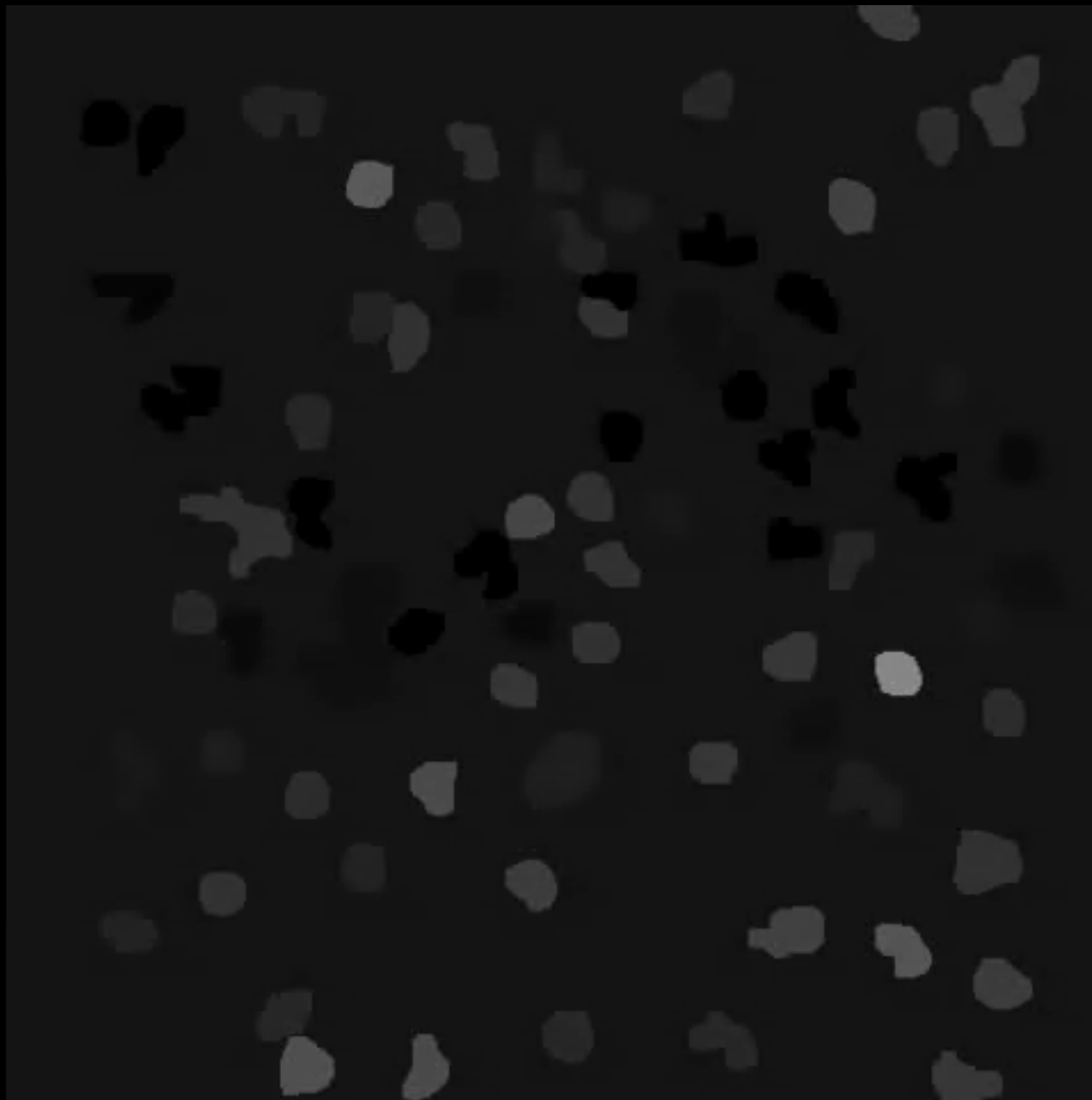
In vivo calcium imaging with STEM

**Barrel Cortex
Layer 2/3**

150 μm deep

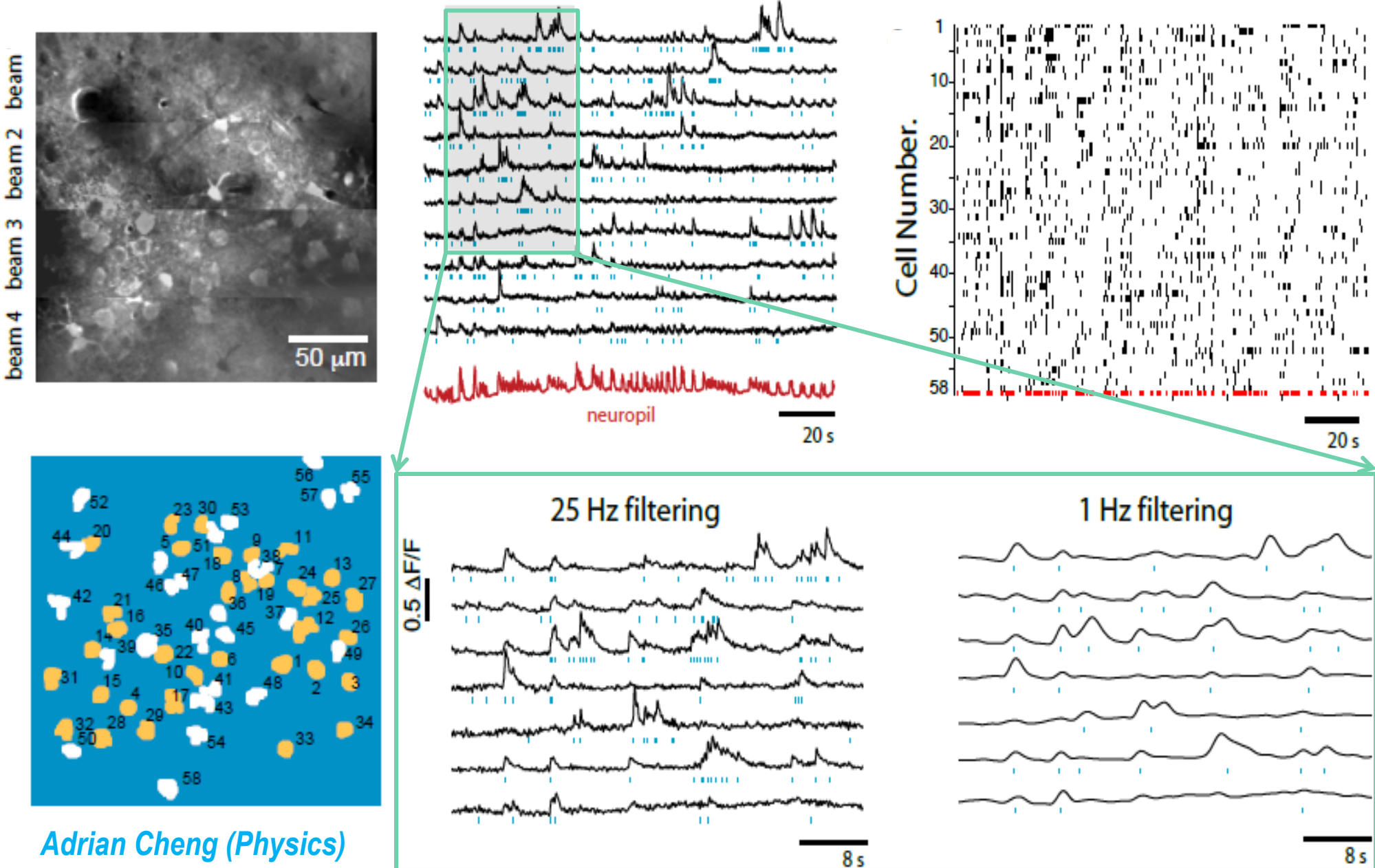
After averaging

**(x3 faster
than real)**



58 neurons
**(~100 billions
neurons
in our brain)**

In vivo calcium imaging of layer 2/3 neurons in barrel cortex with STEM

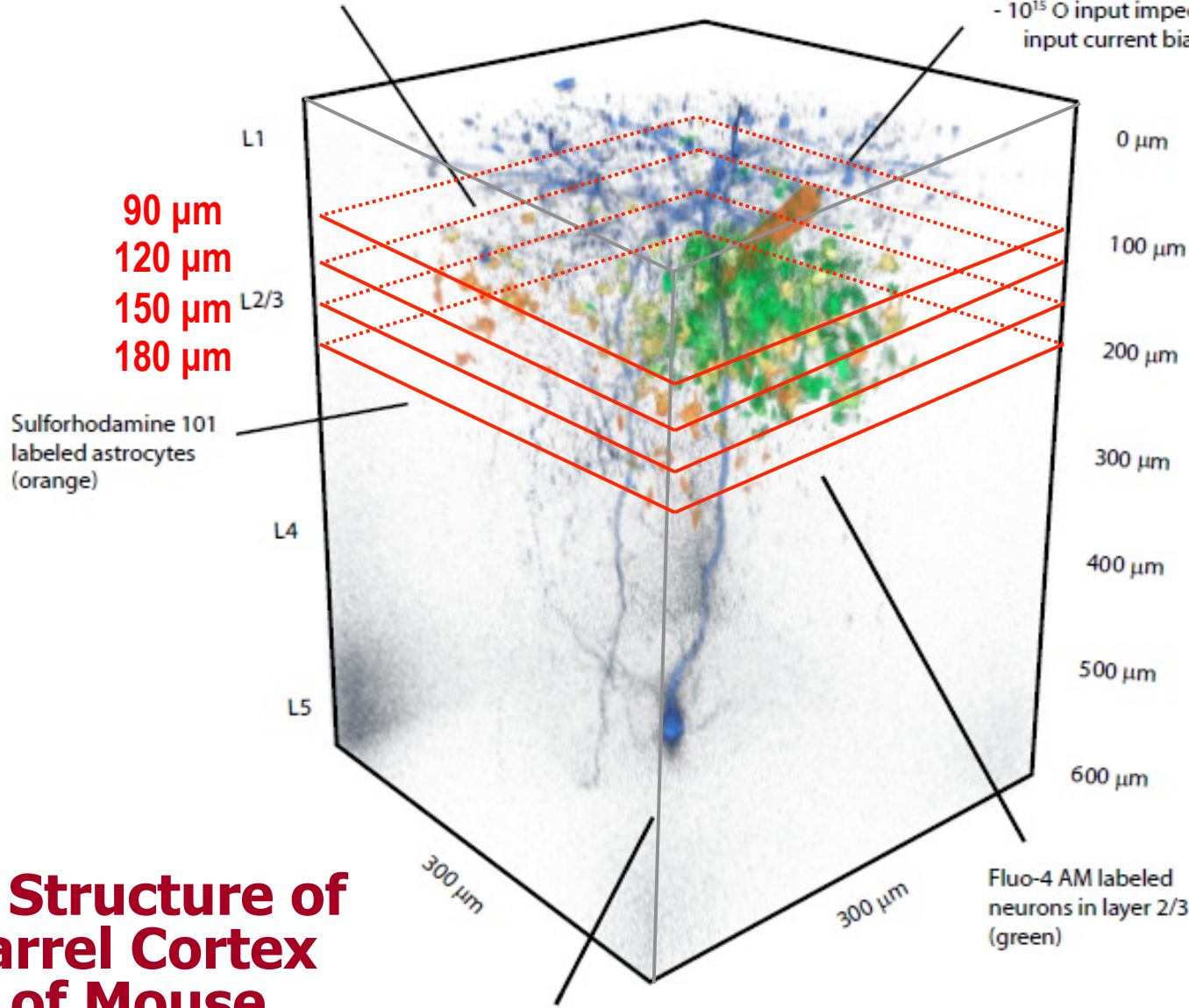


Adrian Cheng (Physics)

Fluo-4 AM labeled astrocytes are colabeled with sulforhodamine 101 to eliminate background (yellow)

Glass microelectrode for dye injection and electrophysiology

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3D Structure of Barrel Cortex of Mouse

Adrian Cheng
(Physics)

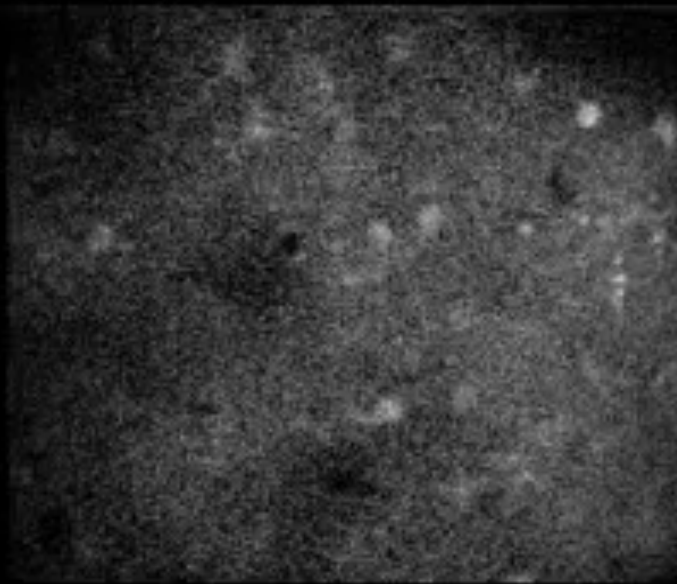
Tiago Goncalves,
Peyman Golshani,
Carlos Portera-Cailliau
(Neurology)

Layer 5 pyramidal neuron soma and apical dendrite from a transgenic animal demonstrates imaging depth (blue)

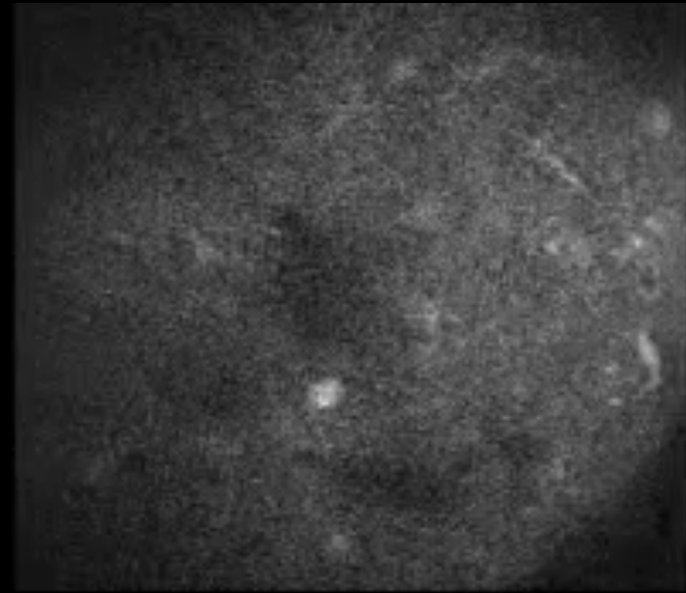
Katsushi Arisaka, UCLA

Simultaneous in vivo calcium imaging in 4 axial planes

Barrel
Cortex
Layer 2/3



Beam 1 90 μm



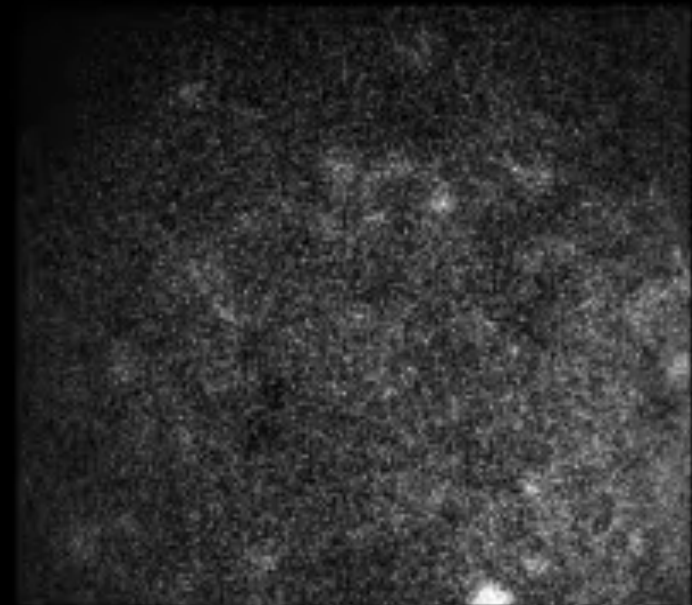
Beam 2 120 μm

60 fps

(x3 faster
than real)

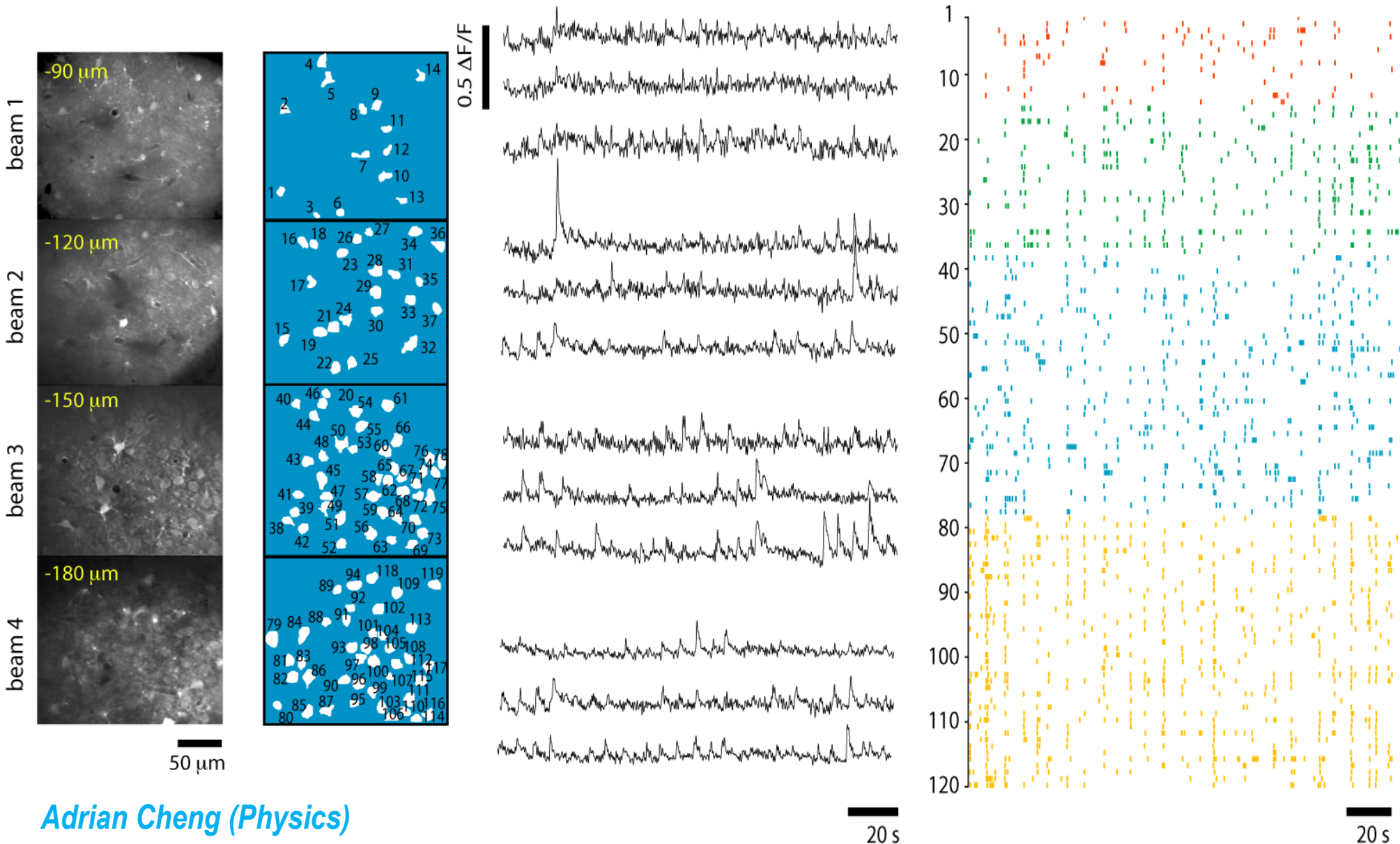


Beam 3 150 μm



Beam 4 180 μm

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



Adrian Cheng (Physics)

Future Directions

How can we recognize and memorize the space?

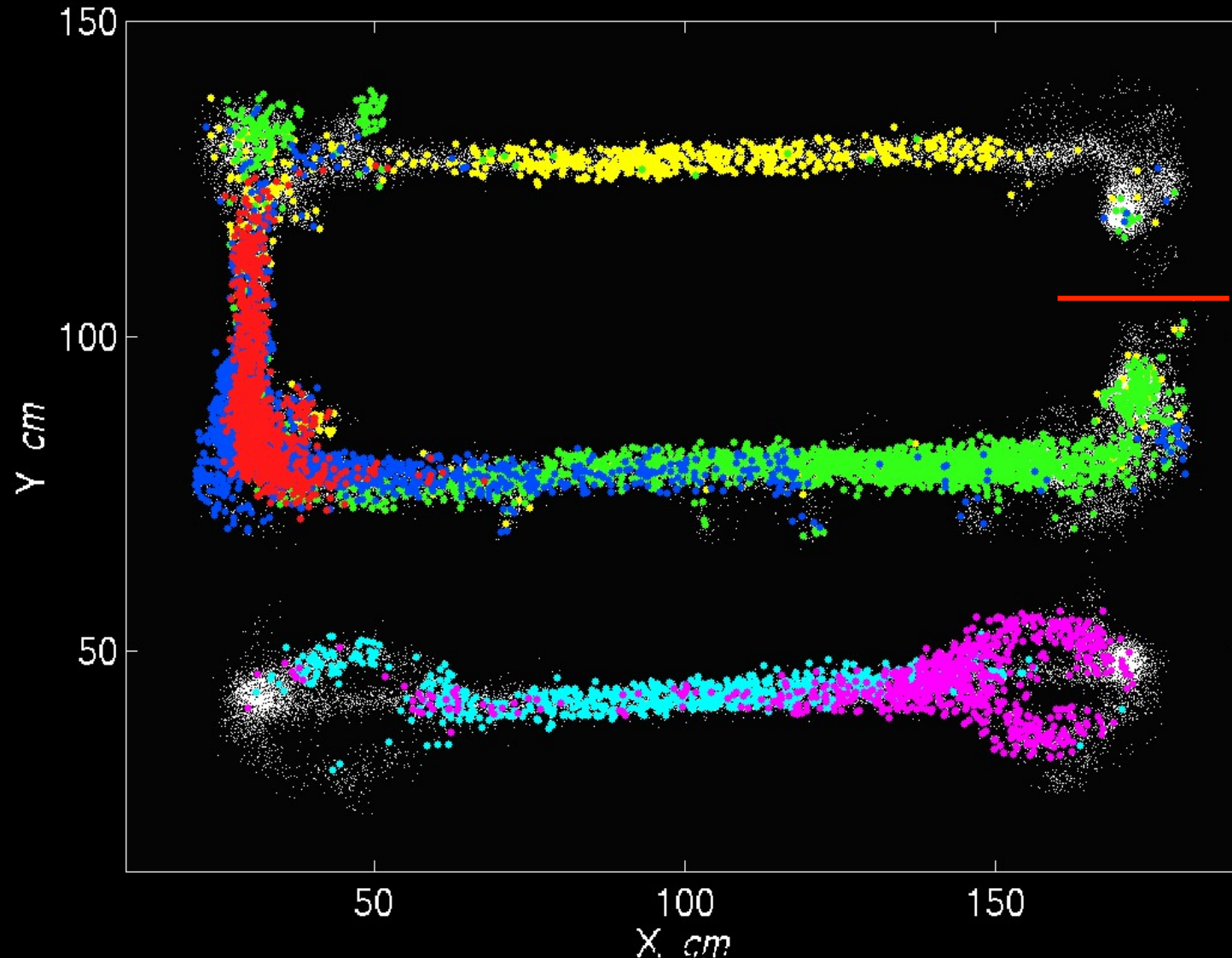
Mayank Mehta (Physics, Neurology)



Tetrodes

Activity of (excitatory) pyramidal neurons in CA depends on rat's position: place cells

Mayank Mehta (Physics, Neurology)

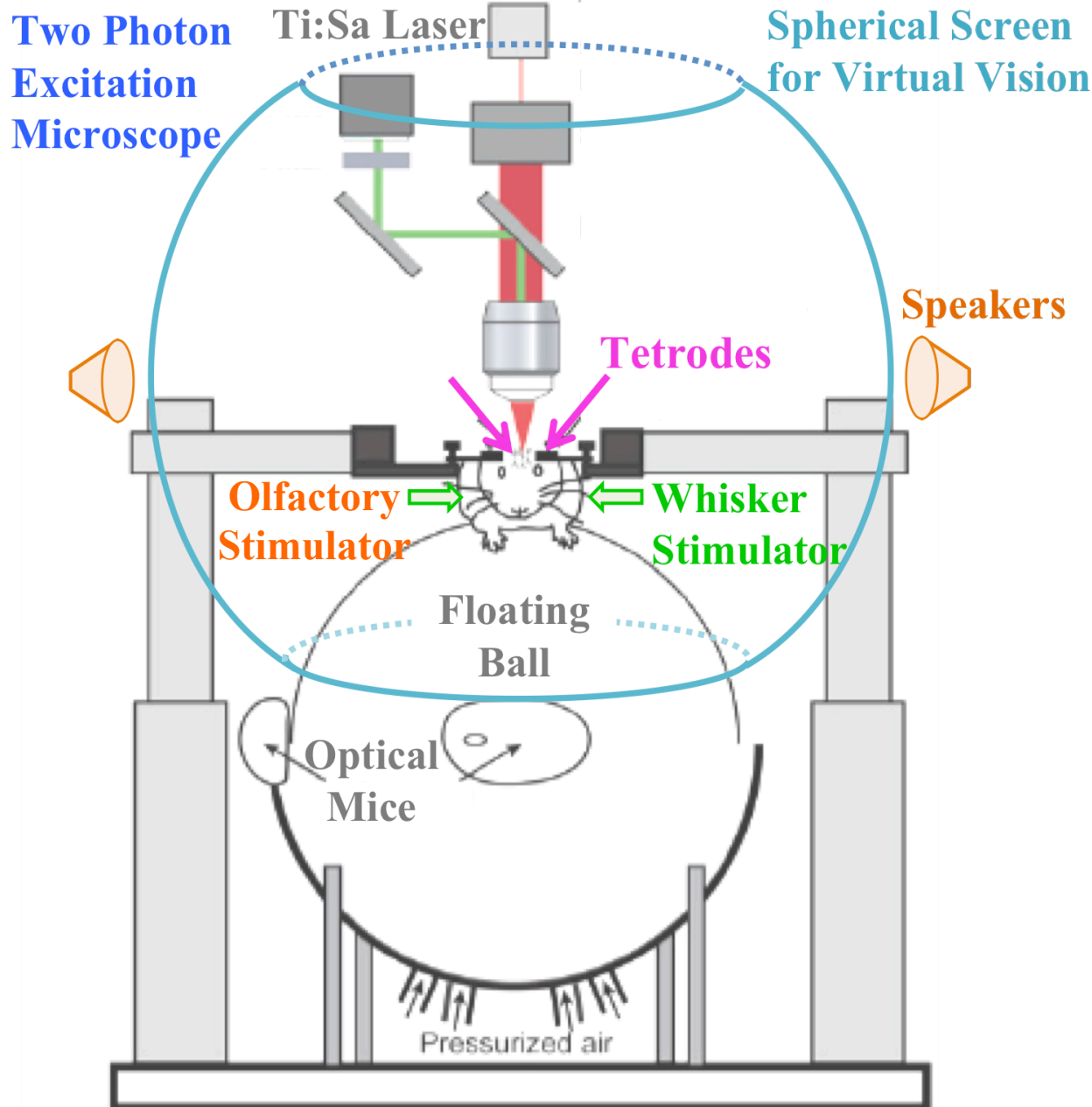


Hippocampus has a cognitive map of space

A mouse running in a Maze of Virtual Reality



Virtual Reality Experiment on Awake Rats



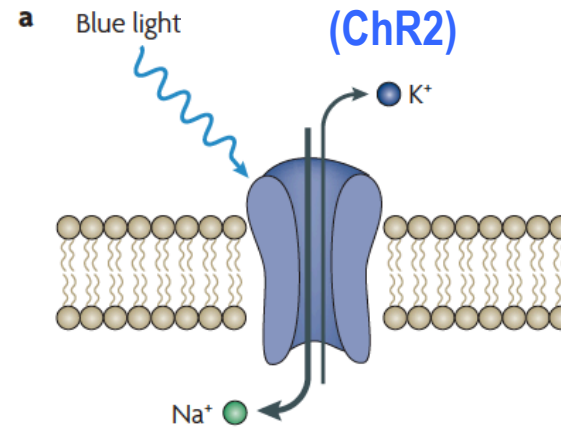
*Daniel Aharoni
Bernard Willers
Mayank Mehta
(Physics)*

Optogenetic Excitation of Neurons

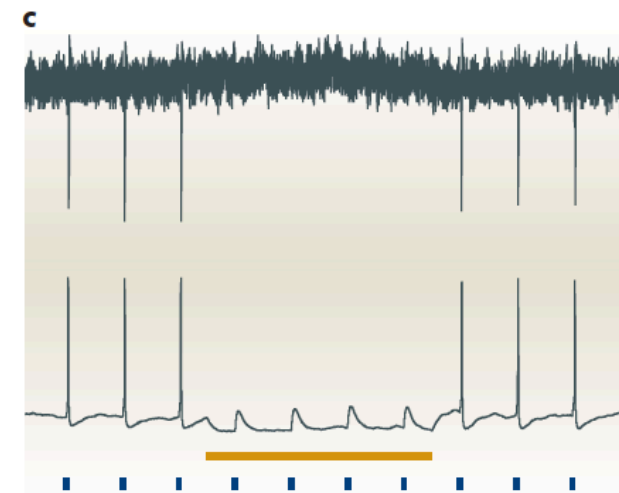
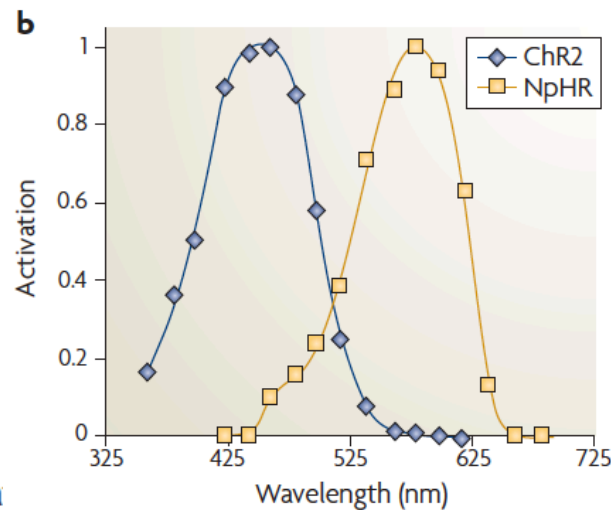
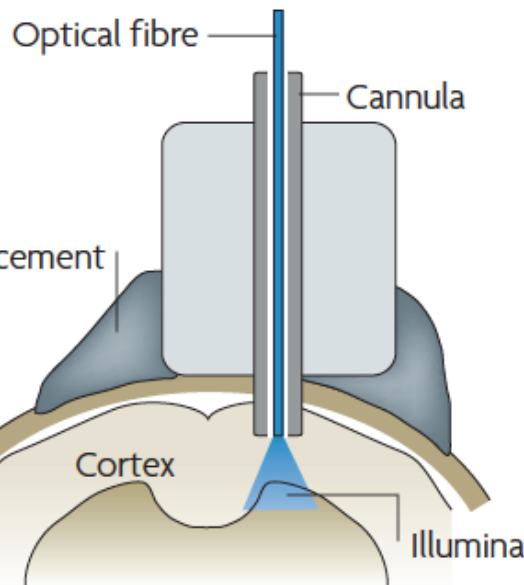
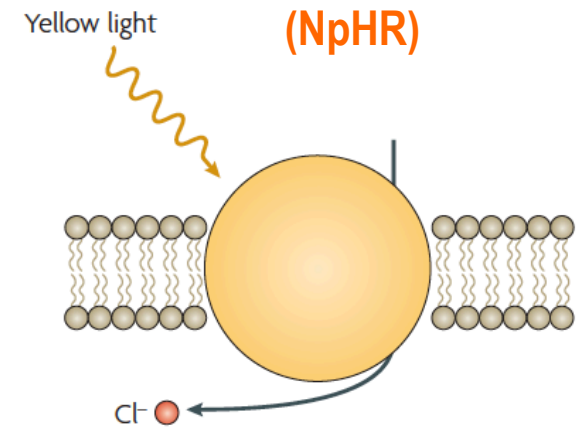
Karl Deisseroth (Stanford)



Excitation by
Channelrhodopsin-2
(ChR2)



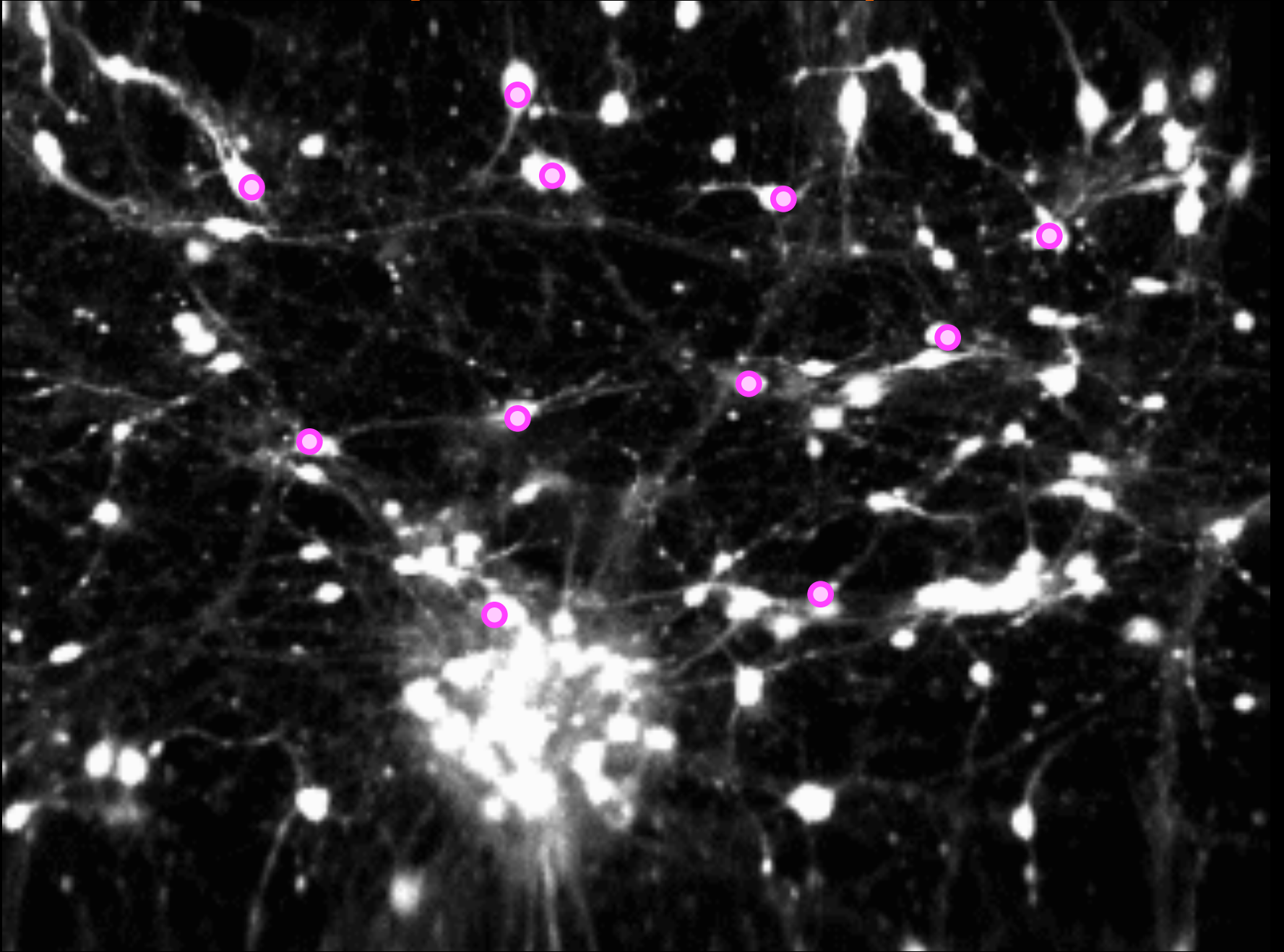
Inhibition by
Halorhodopsin
(NpHR)



Outer world vs. Inner world

- 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

Ca²⁺ Signal in cultivated Rat's Brain by Confocal Microscope

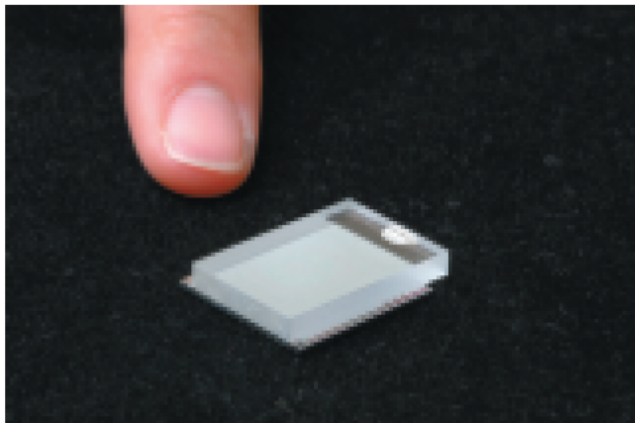


LCOS (Liquid Crystal on Silicon) for SLM (Spatial Light Modulator)

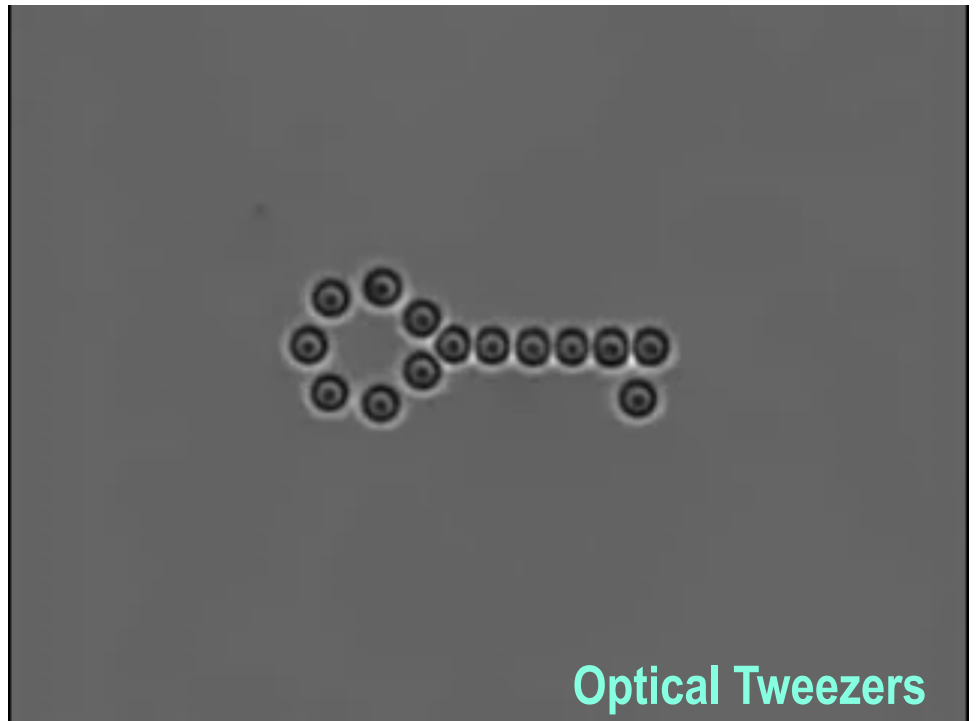
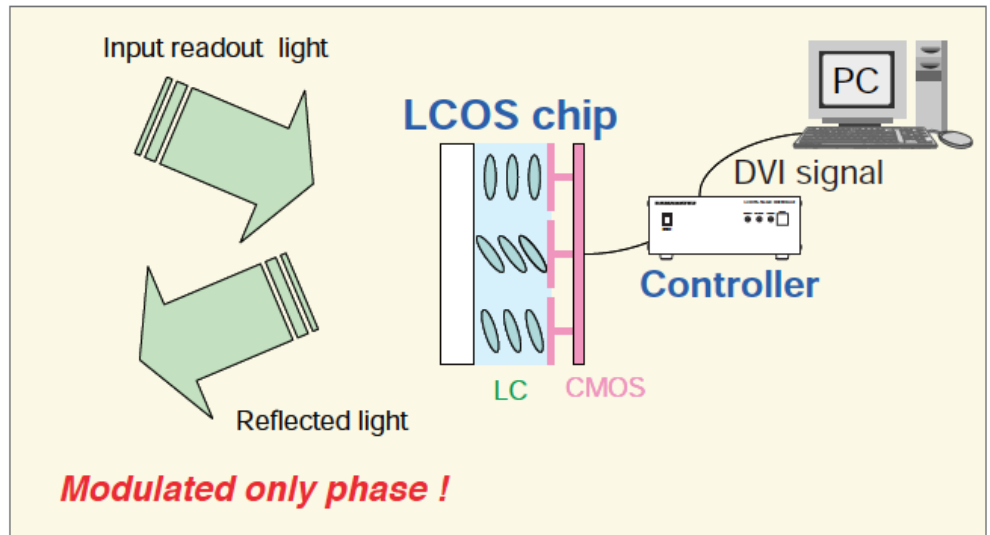
Hamamatsu



X10468 Head and Controller

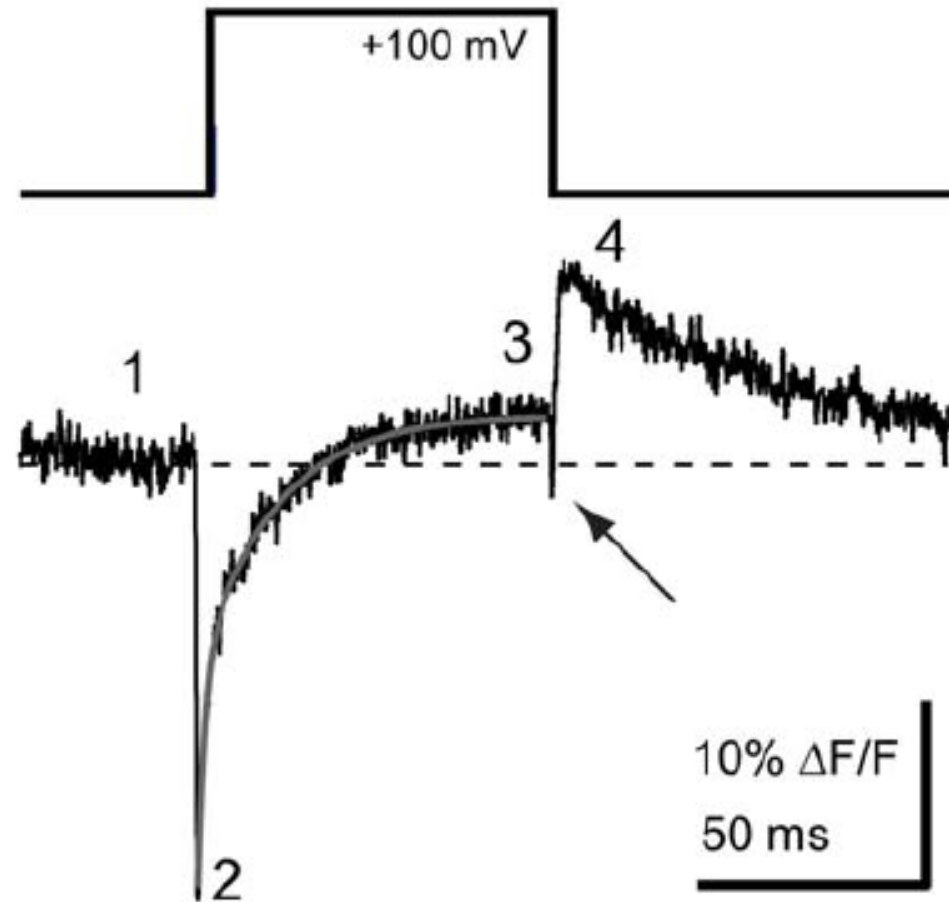
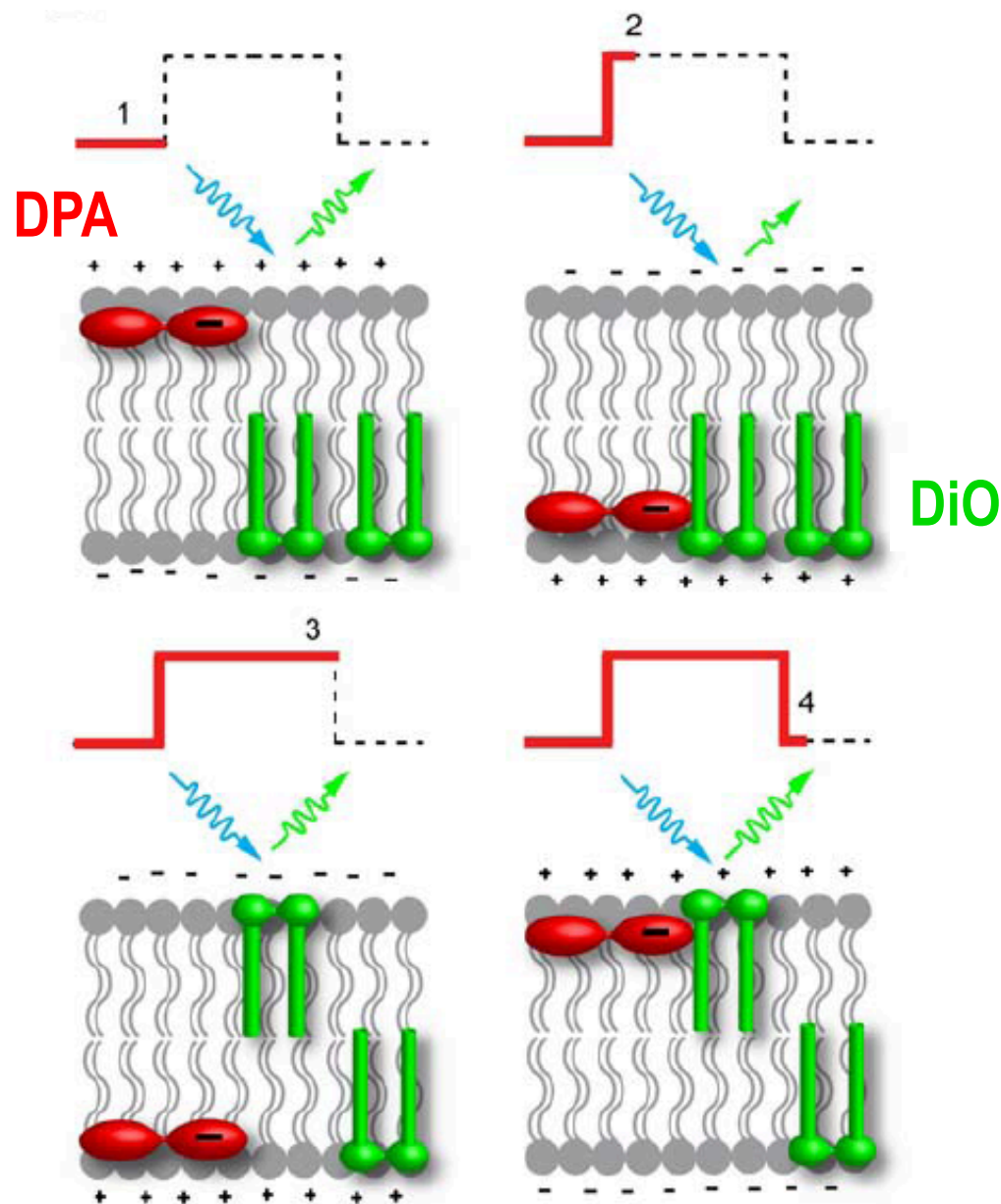


LCOS chip inside the Head



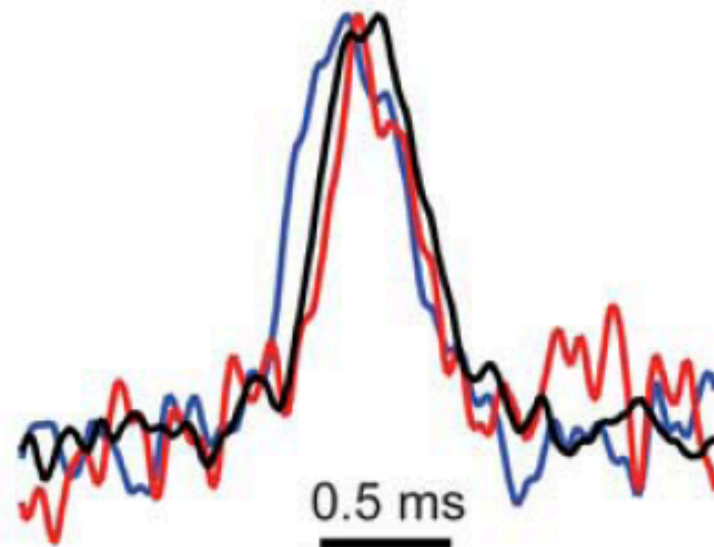
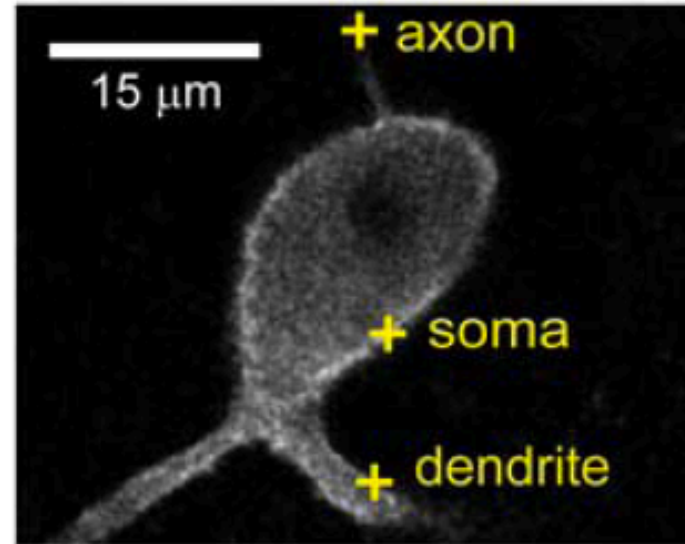
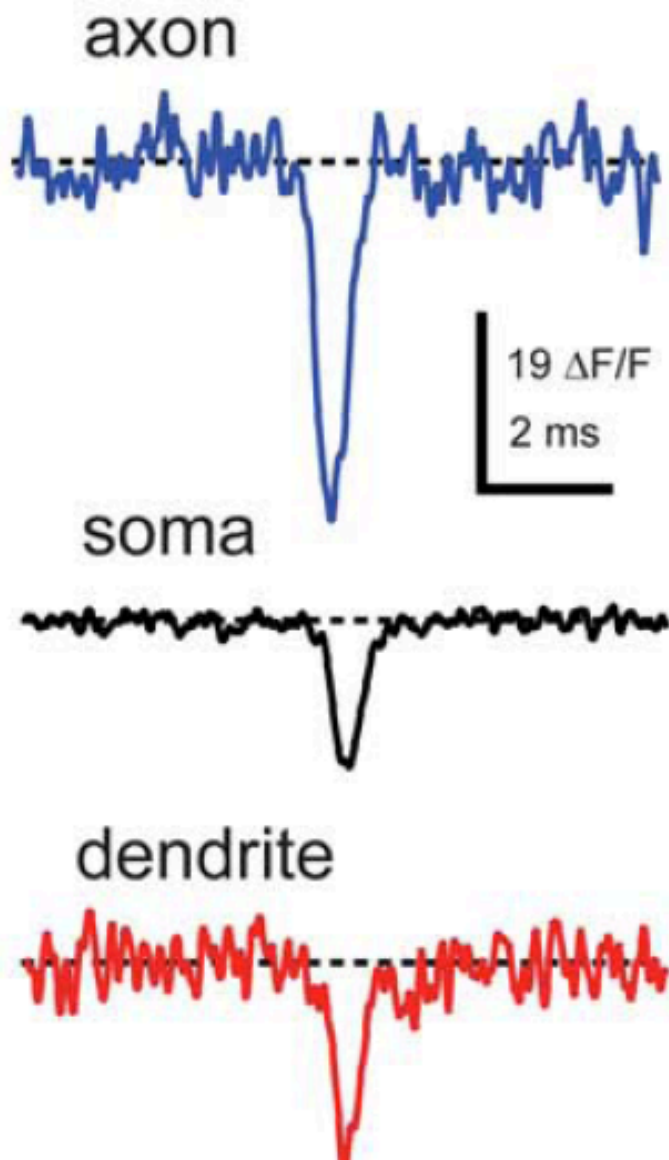
Voltage Sensing Dye by FRET

Tom Otis (Neurobiology)

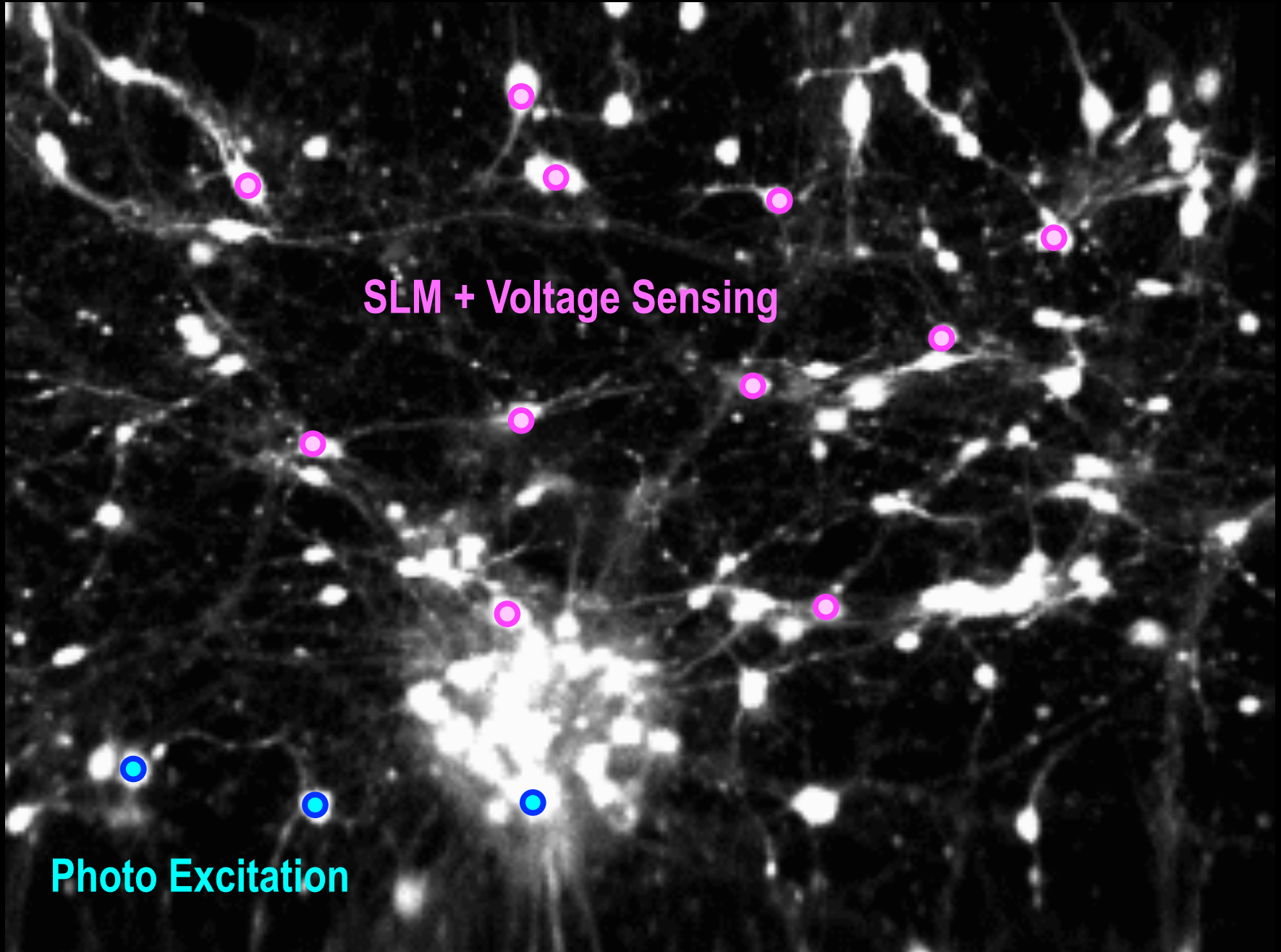


Voltage Sensing Dye

Tom Otis (Neurobiology)

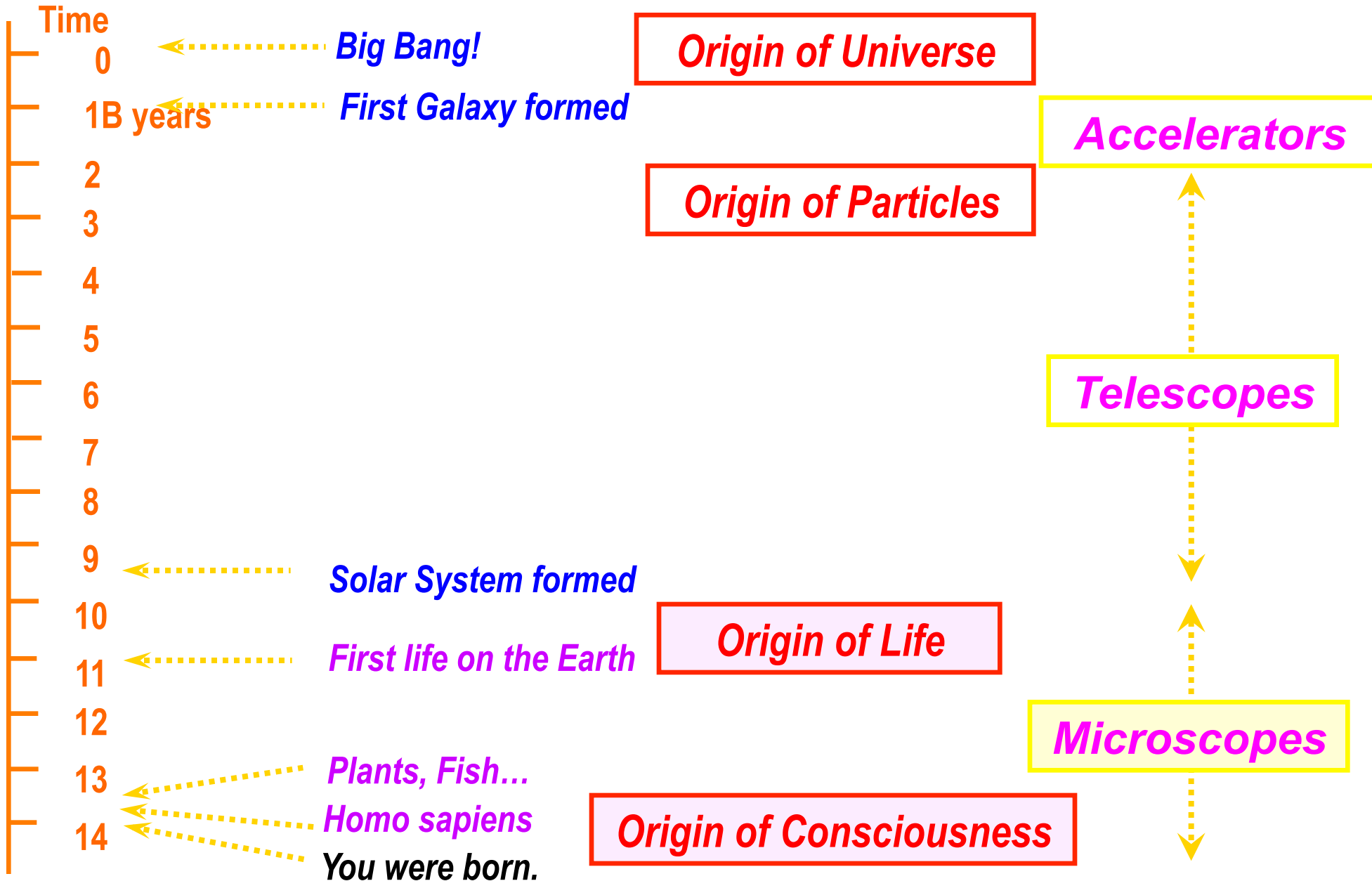


Ca²⁺ Signal in cultivated Rat's Brain by Confocal Microscope



Summary

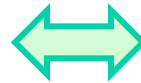
The Four Largest Mysteries in Nature



Four Major Science

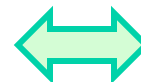
Origin of Particles
Particle Physics

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 evolutionary 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

➤ Special thanks go to the UCLA VIP Team:

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

➤ Financial Supports:

- UCLA, CNSI
- NSF MRI
- NIH R01 *PI: Shimon Weiss*
- NIH Recovery Act *PI: Carlos Portera-Cailliau*
- Industrial Partners *Photron, Hamamatsu*

and thanks to wonderful collaborators!

Scientific Objectives	Department	Prime PI	Other Senior Person	Grad Students	Sample	High-speed Microscopes	Funding	Activities
Neurophysics (Virtual Reality)	Physics & Astronomy, Neurology	Mayank Mehta		Daniel Aharoni, Bernard Willers	Rat	2PE, Tetrode	Keck Foundation pending	2009 - Now
Hair cell motion	Physics & Astronomy	Dolores Bozovic		Lea Fredrickson	Frog	CMOS	NSF MRI	2007 - Now
Neutral Networks	Physics & Astronomy	Katsushi Arisaka	Luis Beltran-Parrazal	Adrian Cheng	Zebrafish	2PE, Conforcal	NSF MRI	2008 - 2009
Single Molecule	Chemistry	Shimon Weiss	Xavier Michalet, Ryan Colyer	Daniel Aharoni	Cells	64ch FCS, ALEX	NIH R01	2006 - Now
TfR tracking on Cancer cells	Oncology, Physics & Astronomy	Manuel Penichet, John Miao	Gustavo Helguera, Jose Rodriguez		Cells	CMOS, ICMOS	(NSF MRI shared)	2007 - Now
Neural networks for breathing	Neurobiology	Jack Feldman	Consuelo Morgado	Adrian Cheng	Rat	ICMOS, Confocal	(NSF MRI shared)	2007 - 2008
Neural networks (Voltage sensing dyes)	Neurobiology	Tom Otis		Bernard Willers	Sliced Brain	ICMOS, 2PE	NIH Rec. Act	2009 - Now
Development of neural networks	Neurology	Carlos Portera-Cailliau	Peyman Golshani, Tiago Goncalves	Adrian Cheng	Mouse	2PE	NIH Rec. Act pending	2008 - Now
High-speed Imaging Flow Cytometer	Electrical Engineering	Bahram Jalali			Cells	ICMOS	NIH R21 pending	2009 - Now
Core facility of High-speed Bio-imaging	CNSI	Shimon Weiss	Laurent Bentolila		Anything	CMOS, ICMOS, EMCCD	CNSI internal	2008 - Now
Multi-channel HAPD, LCOS	Hamamatsu	Suyama Motohiro				2PE, EMCCD	EMCCD, LCOS donated	2006 - Now
CMOS Camera	Photron	Tak Takimizu				CMOS, ICMOS	3 CMOS donated	2007 - Now
High-speed Microscope	Nikon	Jeff Huber				CMOS, ICMOS, 2PE	Microscope discounted	2009 - Now