

Two-photon microscopy for in vivo functional imaging

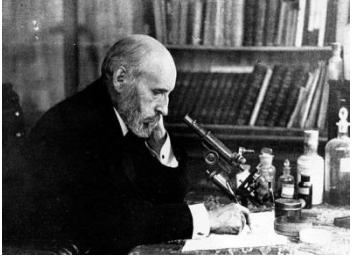
Serena Bovetti



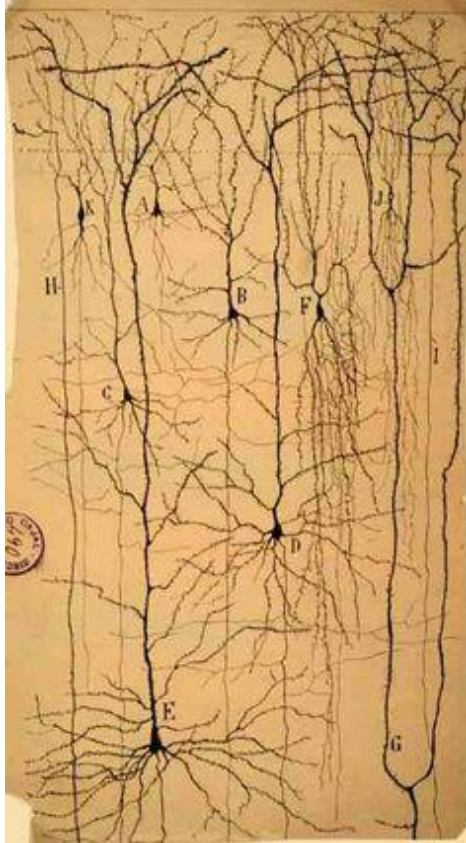
**Department of Neuroscience and
Brain Technologies
Italian Institute of Technology
Genova, Italy**

Analyzing structure and function

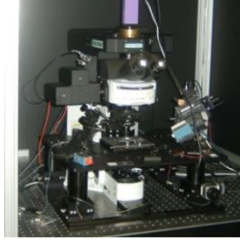
1900



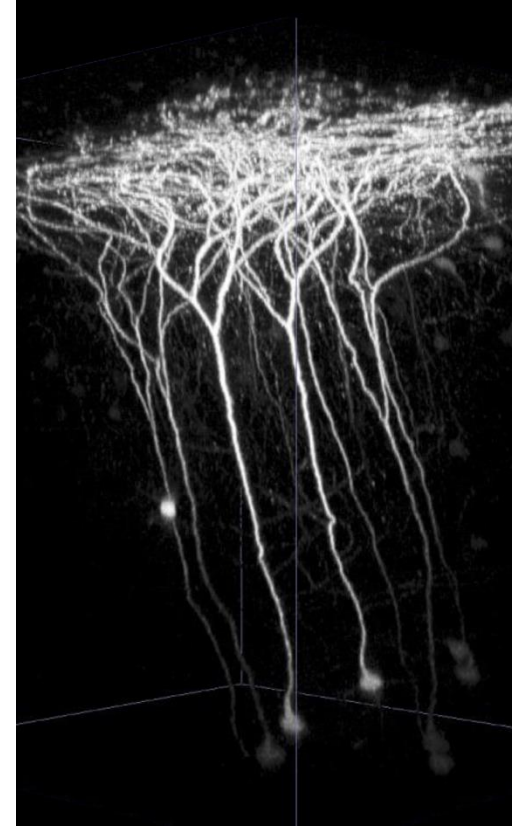
Ramon y Cajal
(1852-1934)



2016



2P microscopy
(1990-present)



Analyzing structure and function

Living cells

Small elements

High number

14 millions glutamatergic cells just into the mouse neocortex

3D

and the overall complexity of NS

Light scattering tissue

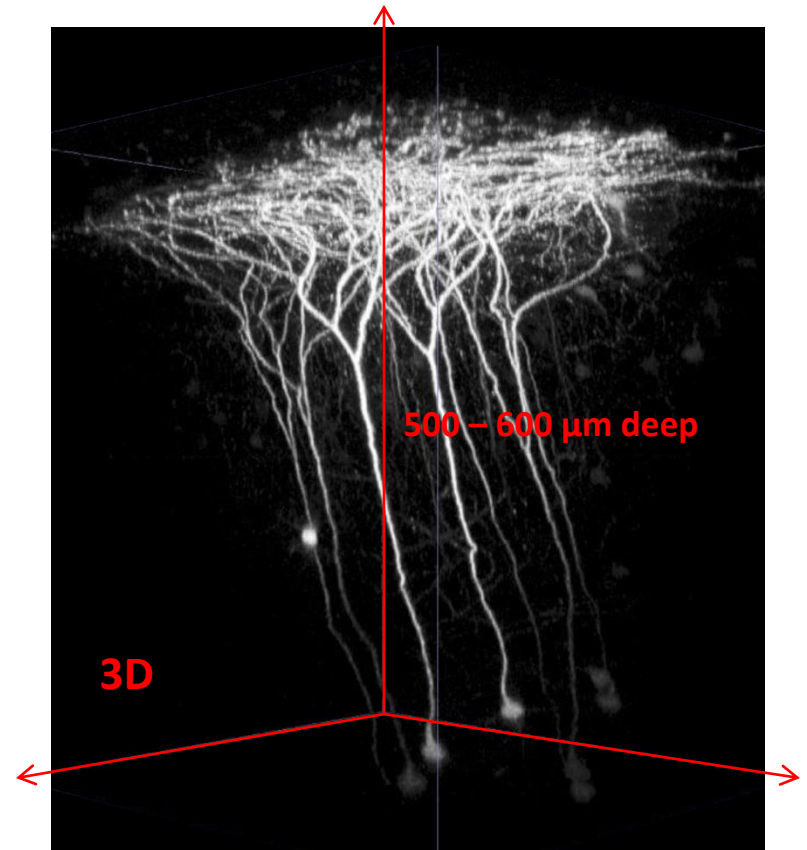
deflection of a light «ray» from its original direction and depend on refractive index of the structures that interact with the ray

Fast signalling

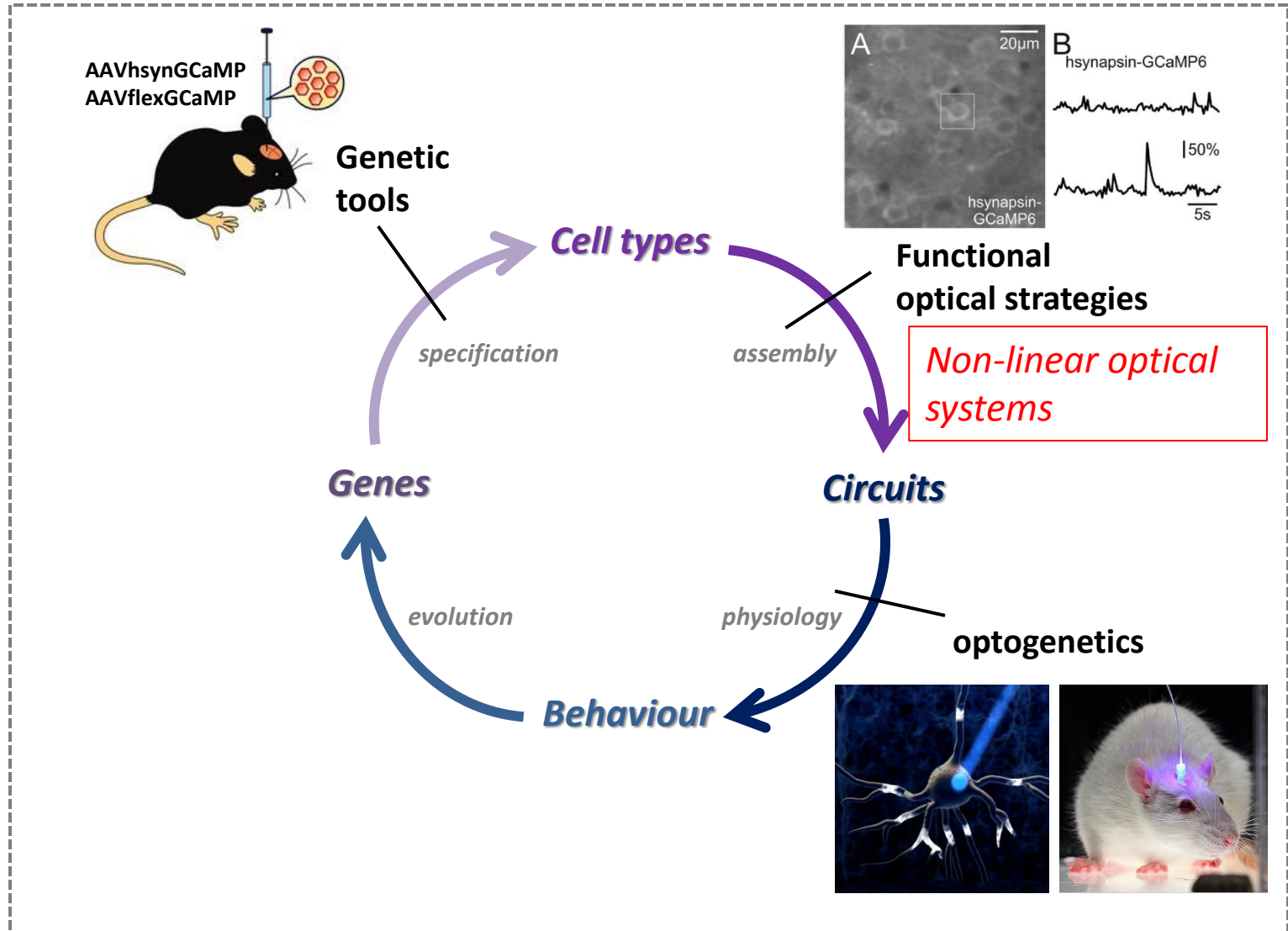
ms

Non-stationary

2016



Analyzing structure and function

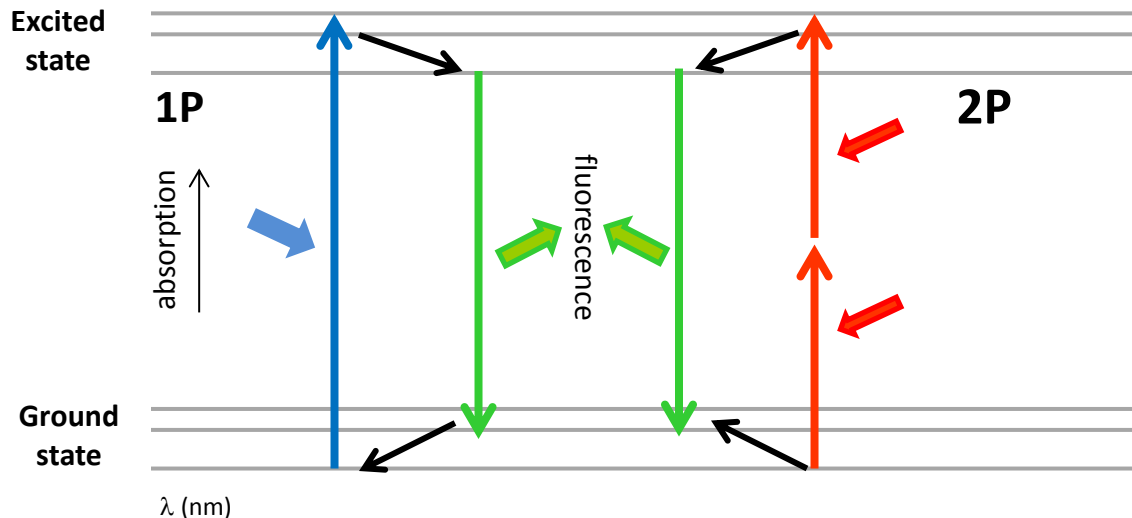


A simple introduction to multiphoton microscopy

Multiphoton microscopy is a powerful technique based on *non-linear* interactions between photons and matter. The most commonly used multiphoton imaging procedure is the *two-photon* excitation microscopy.

Linear microscopy: one photon is adsorbed by a fluorescence molecule and one single fluorescent photon is emitted

Non-Linear microscopy: uses «higher order» light-matter interactions involving multiple photons



2P: rare event in which 2 photons interact with the same molecule at the same time (interval less than 10^{-18} s)



Maria Goppert-Mayer
(1906-1972)
Nobel prize 1963

$$E = hc/\lambda$$

h = Planck constant

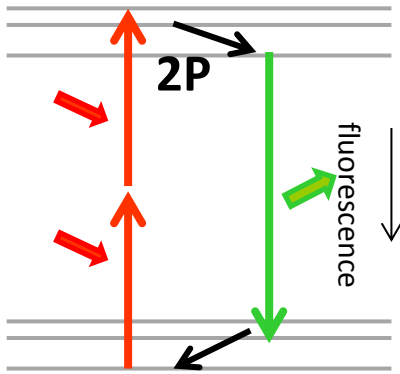
c = speed of light = 3×10^8 m/sec

λ = wavelength

A simple introduction to multiphoton microscopy



Multiphoton absorption: rare event...how do we get around this problem?



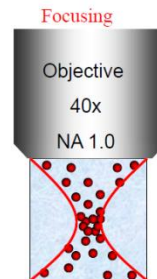
The efficiency of multiphoton absorption depends on:

- 1) The physical properties of the molecule (so called **«cross-section»**: the likelihood that an absorption event will occur)
- 2) The **temporal** and **spatial** distribution of the excitation light: an high number of photons (10^{20} - 10^{30} photons/ (cm²s) have to be concentrated in time (0.5 fs) and space.

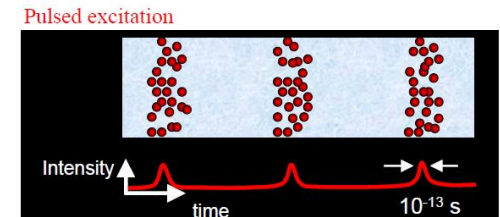


Winfried Denk
1990

concentration in *space*
(focusing): high NA obj that concentrates the light in a diffraction limited focal volume

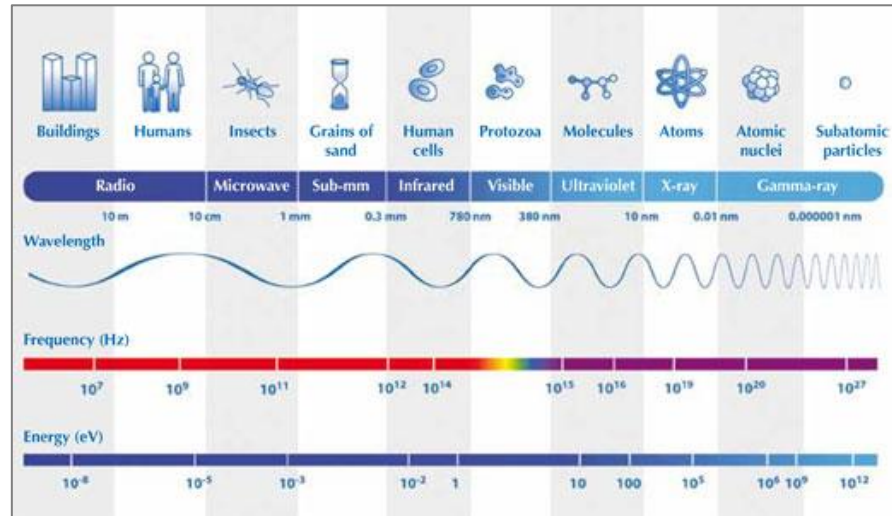
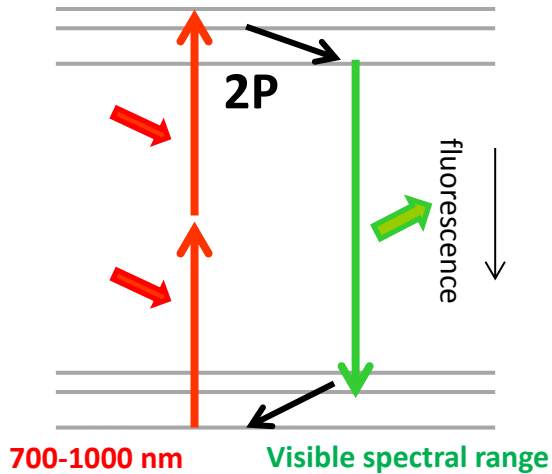


concentration in *time*
(pulsed excitation): ultrashort light pulses (~ 100 fs) with a repetition rate of ~ 100MHz (every 10 ns)



A simple introduction to multiphoton microscopy

Why non-linear in more than linear?



Longer wavelengths = less energy
Longer wavelengths = less subjected to scattering
Longer wavelengths = deeper penetration

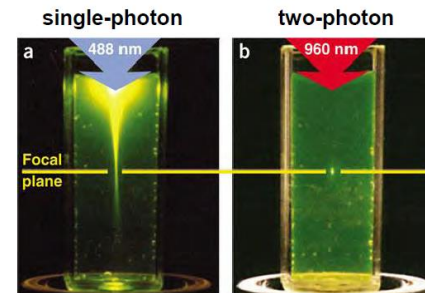
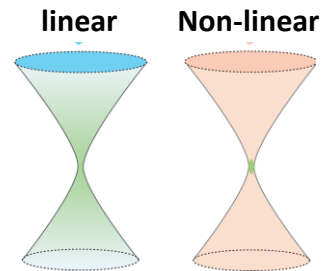
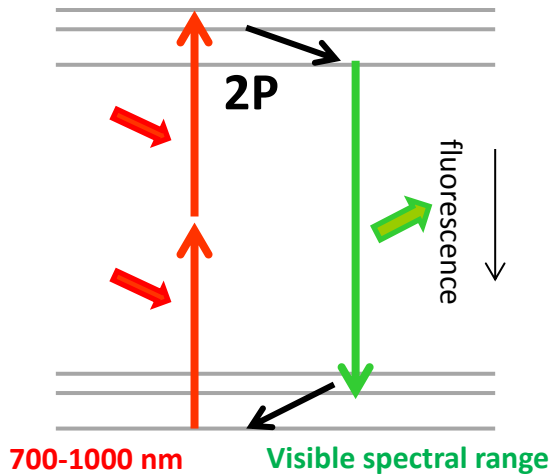
Near-infrared light penetrates deeper into scattering tissue and is generally less phototoxic

A simple introduction to multiphoton microscopy

Why non-linear is more than linear?

Because a 2P excitation event requires two photons to interact on the same molecule at the same time, the probability of an absorption event has a supralinear dependency on the density of photons and is proportional to the square of the instantaneous laser intensity:

$$P \propto I^2$$



Zipfel et al. 2003



- *Absence of multiphoton absorption in out-of-focus planes*
- *Since excitation is confined there is no need of a pinhole in detection*

A simple introduction to multiphoton microscopy

Light scattering in biological tissue

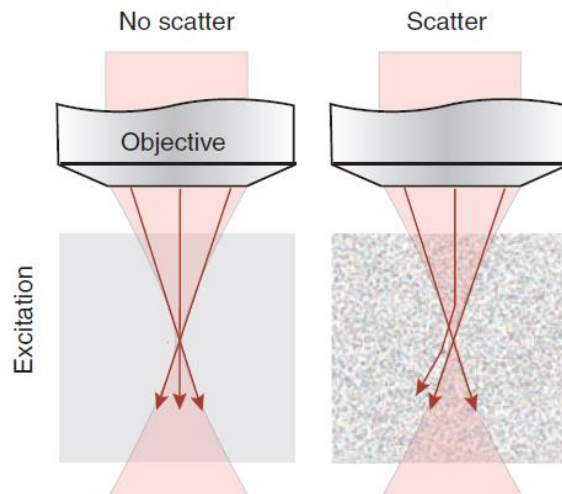
Scattering: deflection of a light «ray» from its original direction; it depends on refractive indexes of the structures that interact with the ray . Both excitation and emitted light is scattered BUT:

Excitation (infrared light)

Infrared light is less susceptible to scattering:

The density of scattered excitation photons is generally too low to generate a significant out-of-focus background signal.

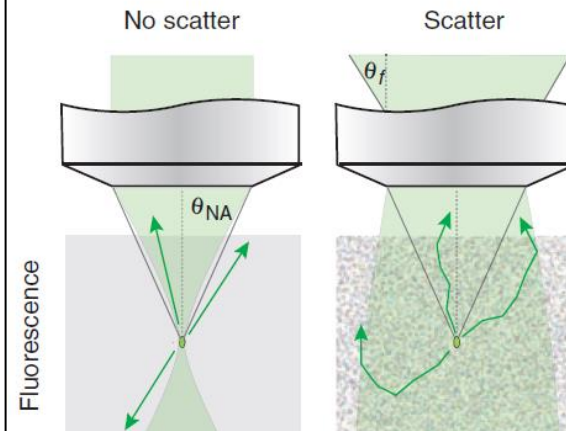
DIFFERENT from 1P excitation light that is highly scattered also in excitation



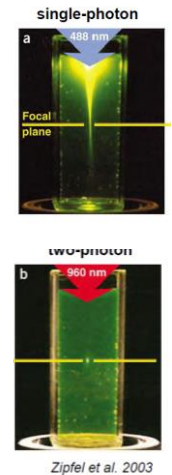
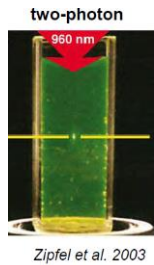
Detection (visible light)

Fluorescent emitted photons are in the visible range (shorter λ and higher energy) and are susceptible to scattering. BUT we know where the fluorescence light is coming from.

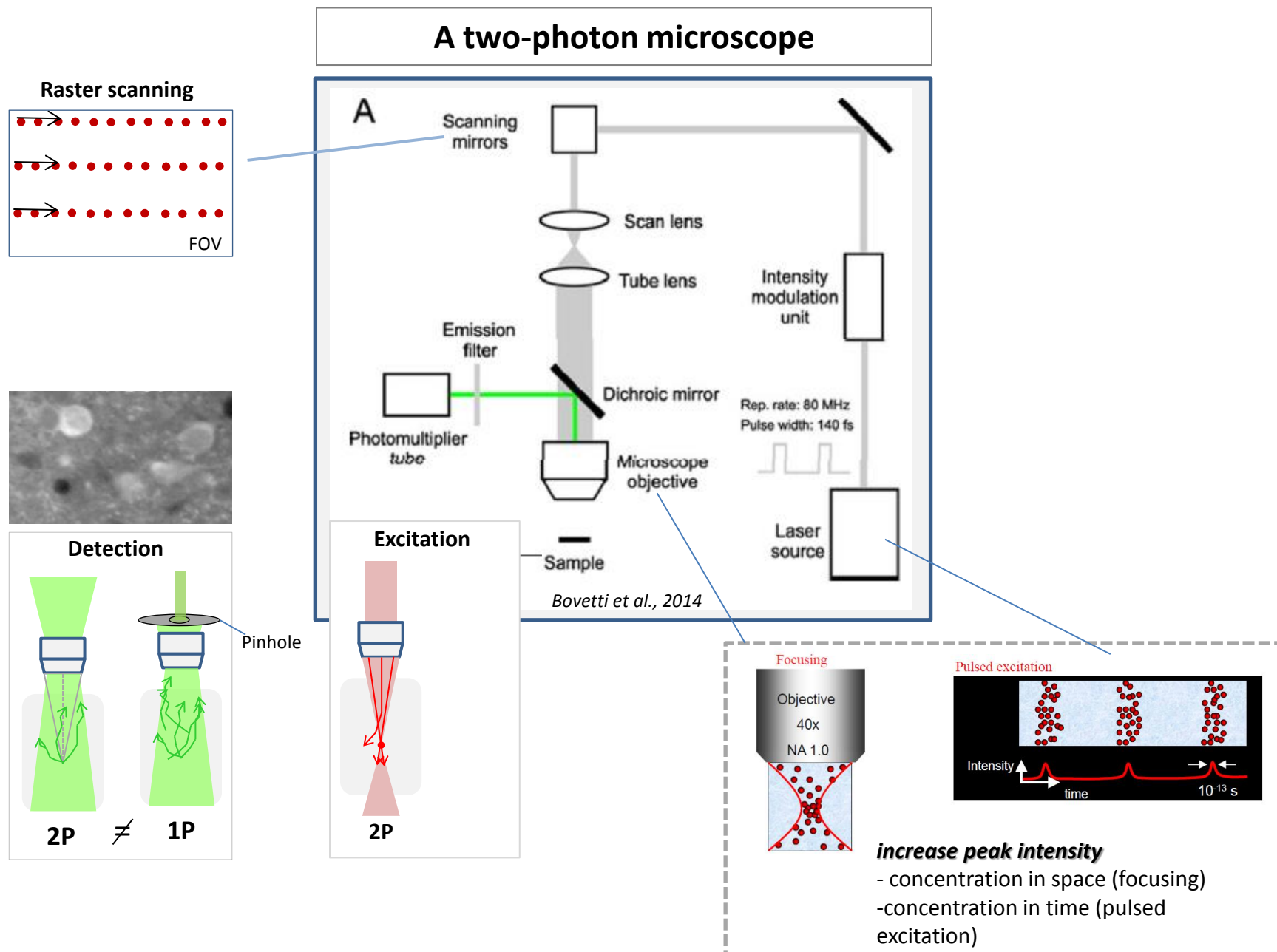
DIFFERENT from 1P emitted light that you don't know where is coming from



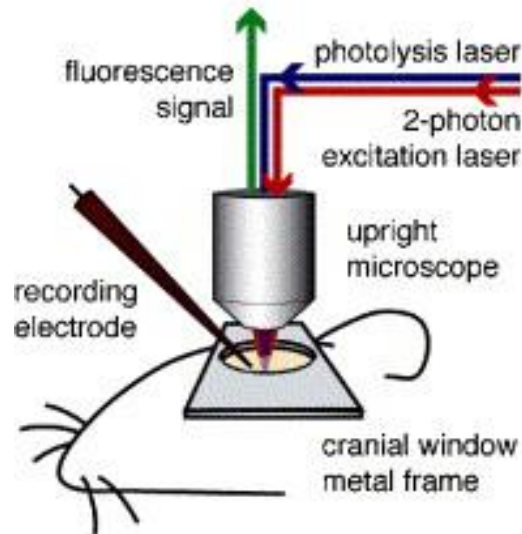
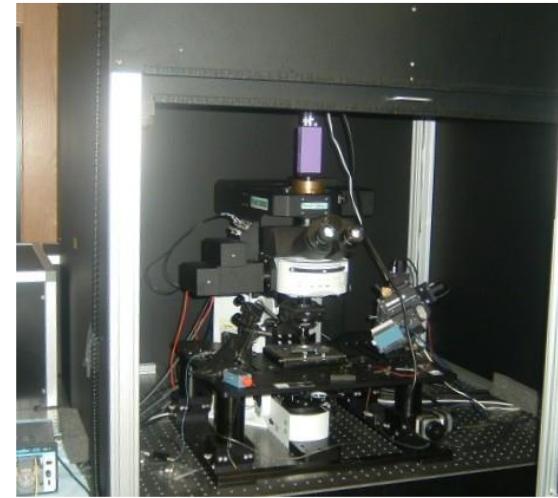
collect as much as you can!



A simple introduction to multiphoton microscopy

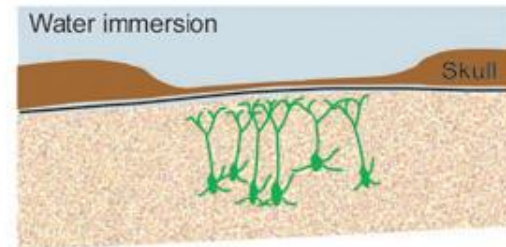


Two-photon fluorescence microscopy

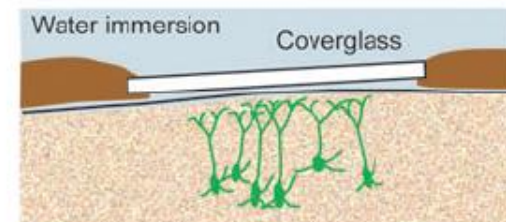


Wang et al. 2006

Thinned skull



Chronic window



In vivo functional two-photon imaging

A first necessary step toward elucidating the basic principles underlying brain function is to *precisely map the activity* of individual cellular elements in space and time *in vivo*



Electrophysiology has long been the preferred method for studying the central nervous system, however the final goal is to record from large networks at cellular resolution



In the last 20 years, the development of *nonlinear microscopy* in combination with *fluorescent activity reporters* has provided a valuable tool to reach this goal

Voltage indicators
Calcium indicators

Detect supra- and sub- threshold activity

Fast kinetics

BUT

Dim

Small signal-to-noise ratio: $\Delta F / \text{stdev of baseline noise}$

Detect supra-threshold activity

Slower kinetics

BUT

Higher brightness

Higher SNR

The Calcium Ion as an Indirect Reporter of Neuronal Activity

Both neurons and glia display increase calcium concentration in response to neuronal activity

[Ca²⁺] at rest
30-200 nM

VGCC, calcium-permeable
receptor-operated channels

[Ca²⁺] x 10 – x 100



One way to measure free cytosolic calcium variations optically is using molecules that change their fluorescence or absorbance properties upon calcium binding



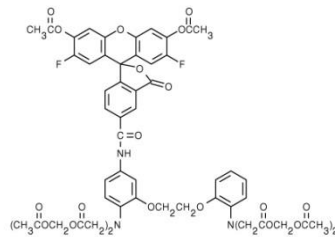
Synthetic dyes:

OGB

Fluo-2

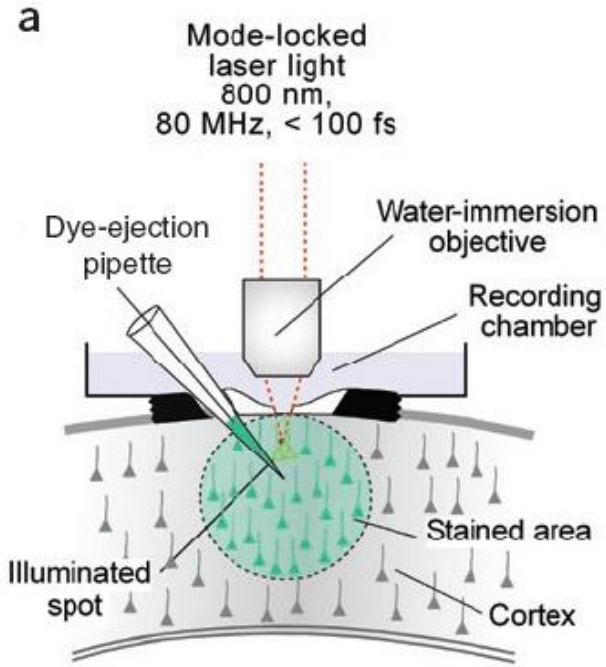
Fluo-4

...

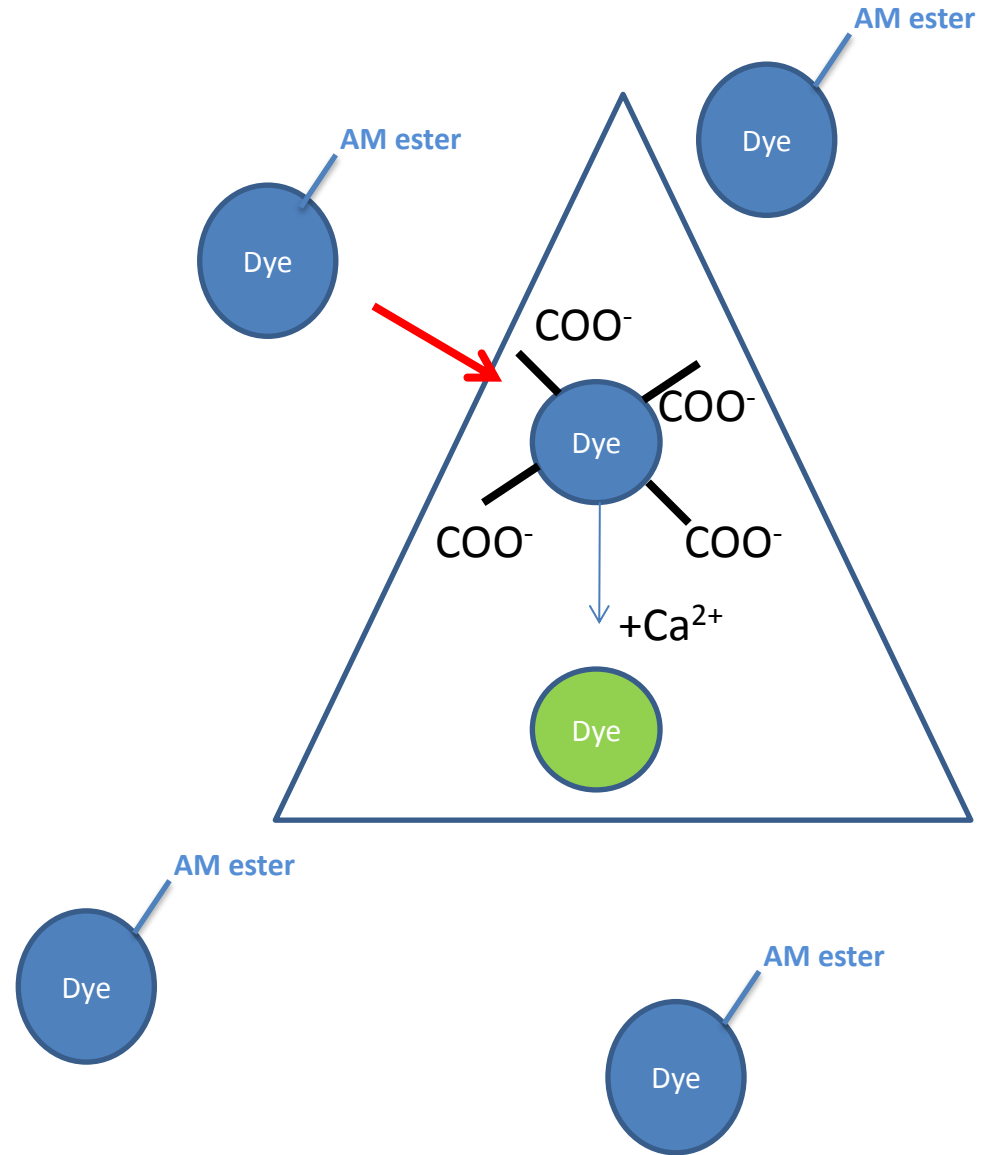


No labelling of specific cell population
Acute loading
Short life time

Bulk loading



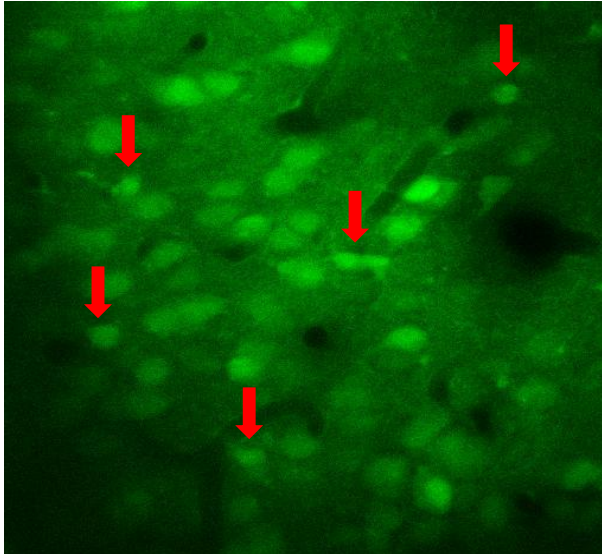
Garaschuk et al. 2006



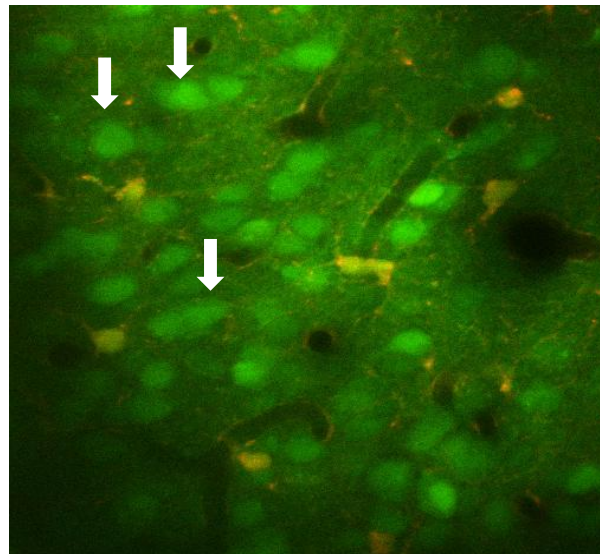
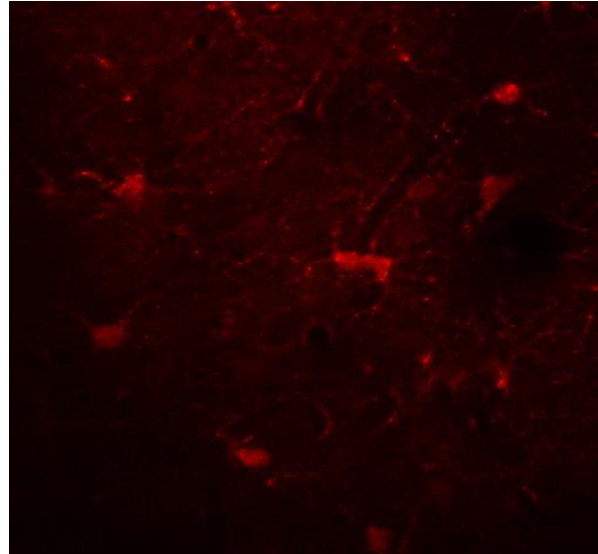
AM ester: Acetoxymethyl (AM) esters

Bulk loading

Oregon Green BAPTA



Sulforhodamine 101



The Calcium Ion as an Indirect Reporter of Neuronal Activity

Both neurons and glia display increase calcium concentration in response to neuronal activity



One way to measure free cytosolic calcium variations optically is using molecules that change their fluorescence or absorbance properties upon calcium binding

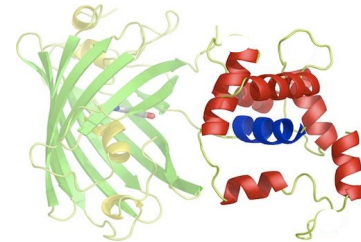


Genetically encoded indicators:

Camgoroo

Pericams

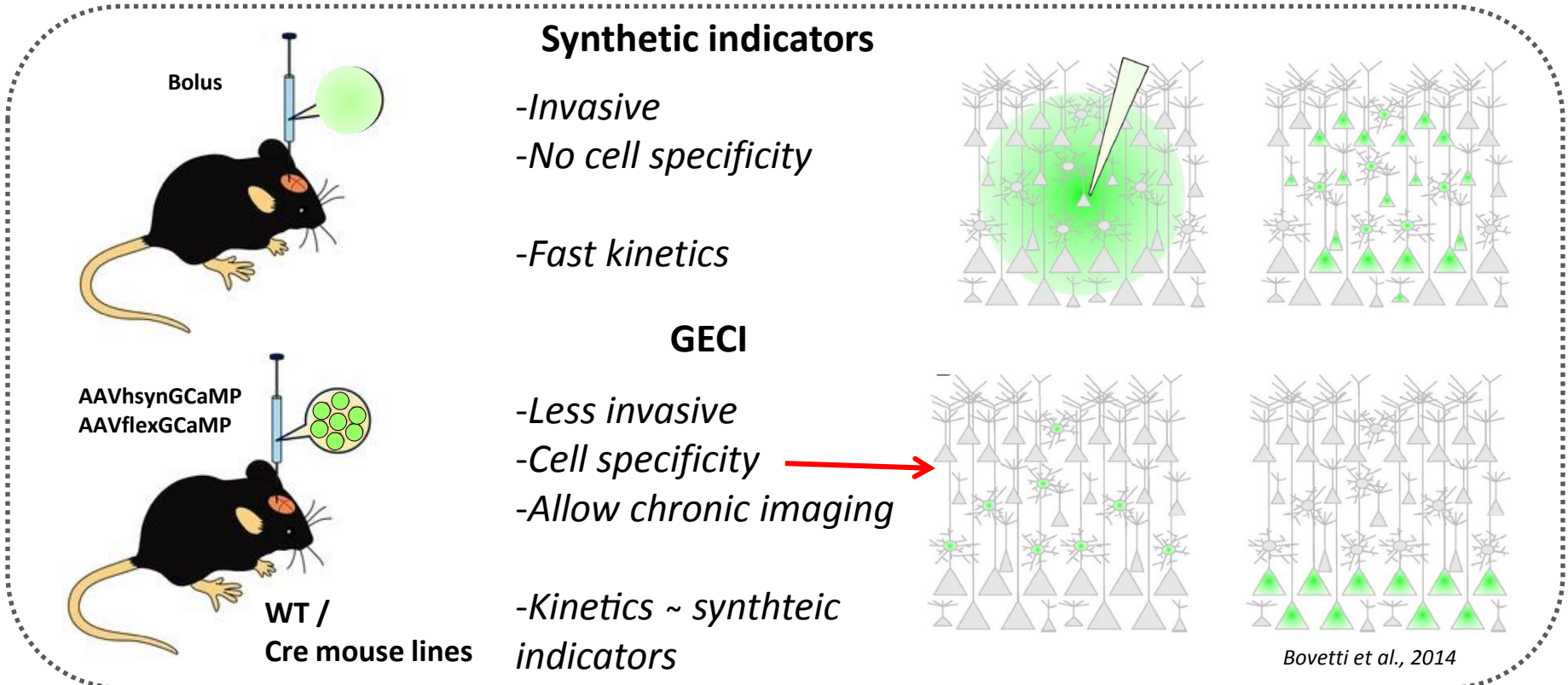
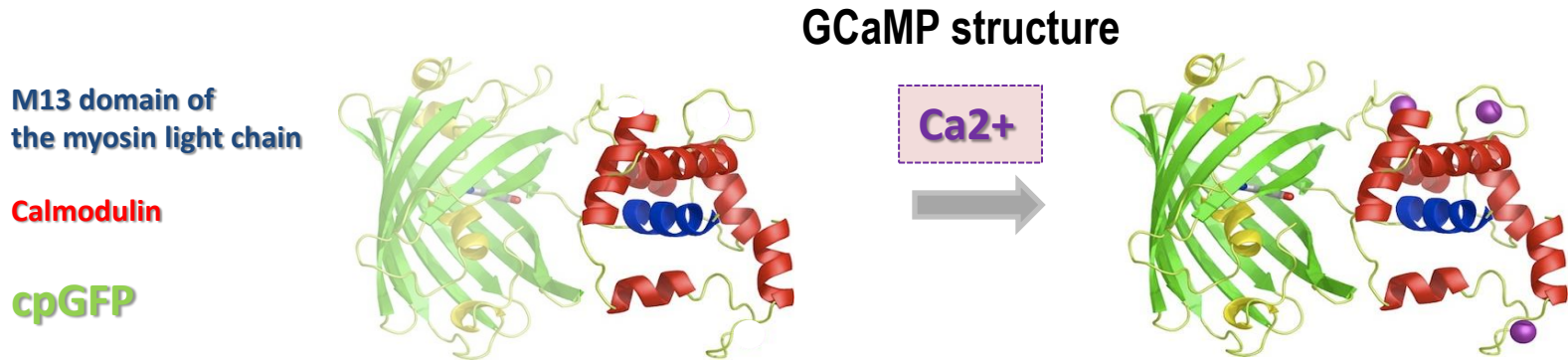
GCaMPs



Expressed in cell type specific manner

Allow chronic imaging

The Calcium Ion as an Indirect Reporter of Neuronal Activity

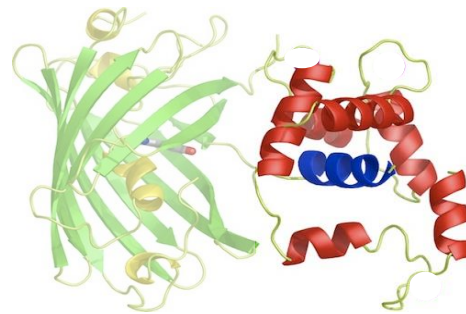


The Calcium Ion as an Indirect Reporter of Neuronal Activity

M13 domain of
the myosin light chain

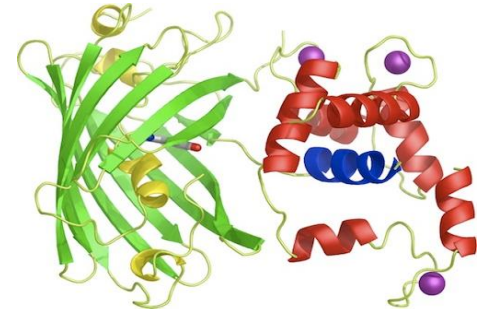
Calmodulin

cpGFP



GCaMP structure

Ca²⁺



Transgenic mouse lines

Promoter specific expression

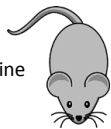
Thy1-GCaMP6
CAMKII-GCaMP6



Cre-dependent expression (cre-lox technology)

Promoter-lox-STOP-lox-GCaMP6
(i.e. CAG)

Cre-mouse line
(i.e. PV-cre)



X



Flex-
GCaMP6



Viral vector delivery

Each virus has characteristic **tropism** (targeting of cells) and **spread** from injection sites, in some cases via **retrograde or anterograde** transport of viral particles, which are important to consider when designing experiments.

Adeno-associated virus (AAVs)

Small (25 nm), Single-stranded DNA

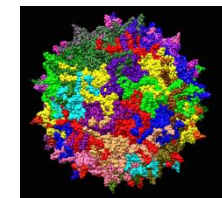
Different serotypes that influence virus tropism

Now mix serotypes are available

Small capacity for effective packaging (4.7 kb)

Both anterograde and retrograde (depending on serotypes) transport

Do not integrate into the host genome, remain as an episome (extragenomic circular DNA)

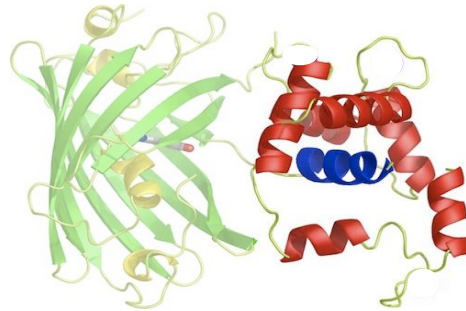


The Calcium Ion as an Indirect Reporter of Neuronal Activity

M13 domain of
the myosin light chain

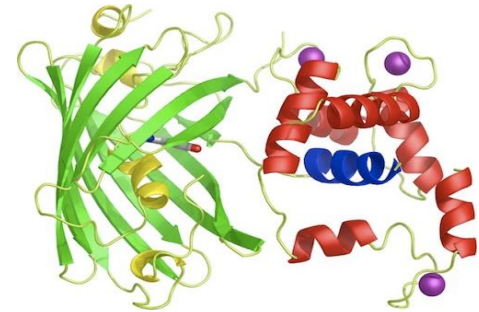
Calmodulin

cpGFP



GCaMP structure

Ca²⁺



Transgenic mouse lines

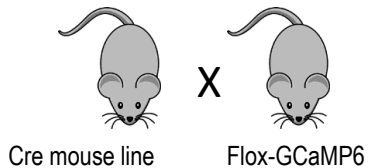
Promoter specific expression

Thy1-GCaMP6
CAMKII-GCaMP6



Cre-dependent expression (cre-lox technology)

Promoter-lox-STOP-lox-GCaMP6
(i.e. CAG)



Viral vector delivery

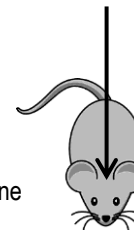
Promoter specific expression

AAV1.Syn.GCaMP6f.WPRE.SV40
serotype promoter indicator

Cre-dependent expression (cre-lox technology)

AAV1.Syn.flex.GCaMP6f.WPRE.SV40

WT or
Cre mouse line



~10¹³ GC/ml (tirate!!!)

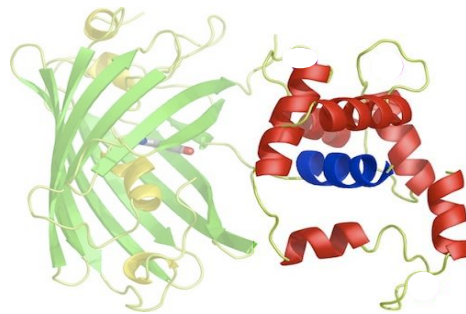
very low inj rate (20-50 nl/min)

The Calcium Ion as an Indirect Reporter of Neuronal Activity

M13 domain of
the myosin light chain

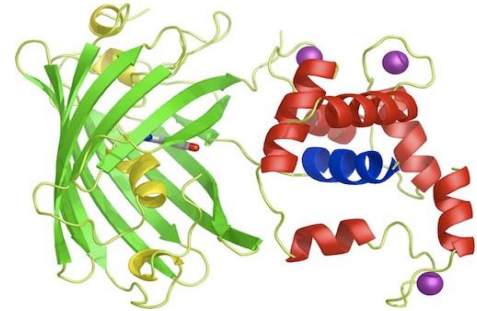
Calmodulin

cpGFP



GCaMP structure

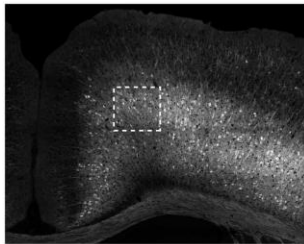
Ca²⁺



Transgenic mouse lines

Advantages:

More homogeneous expression
across brain regions



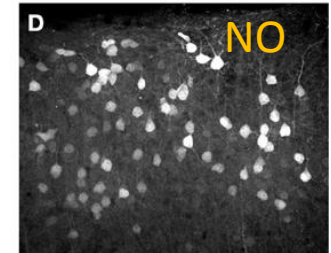
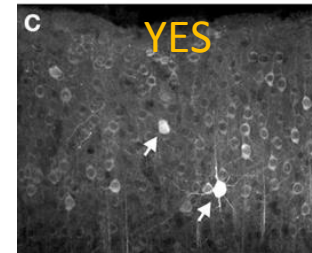
Disadvantages:

Low expression

Viral vector delivery

Advantages:

Higher expression



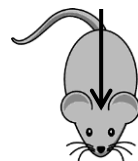
Disadvantages:

Less homogeneous expression across brain regions

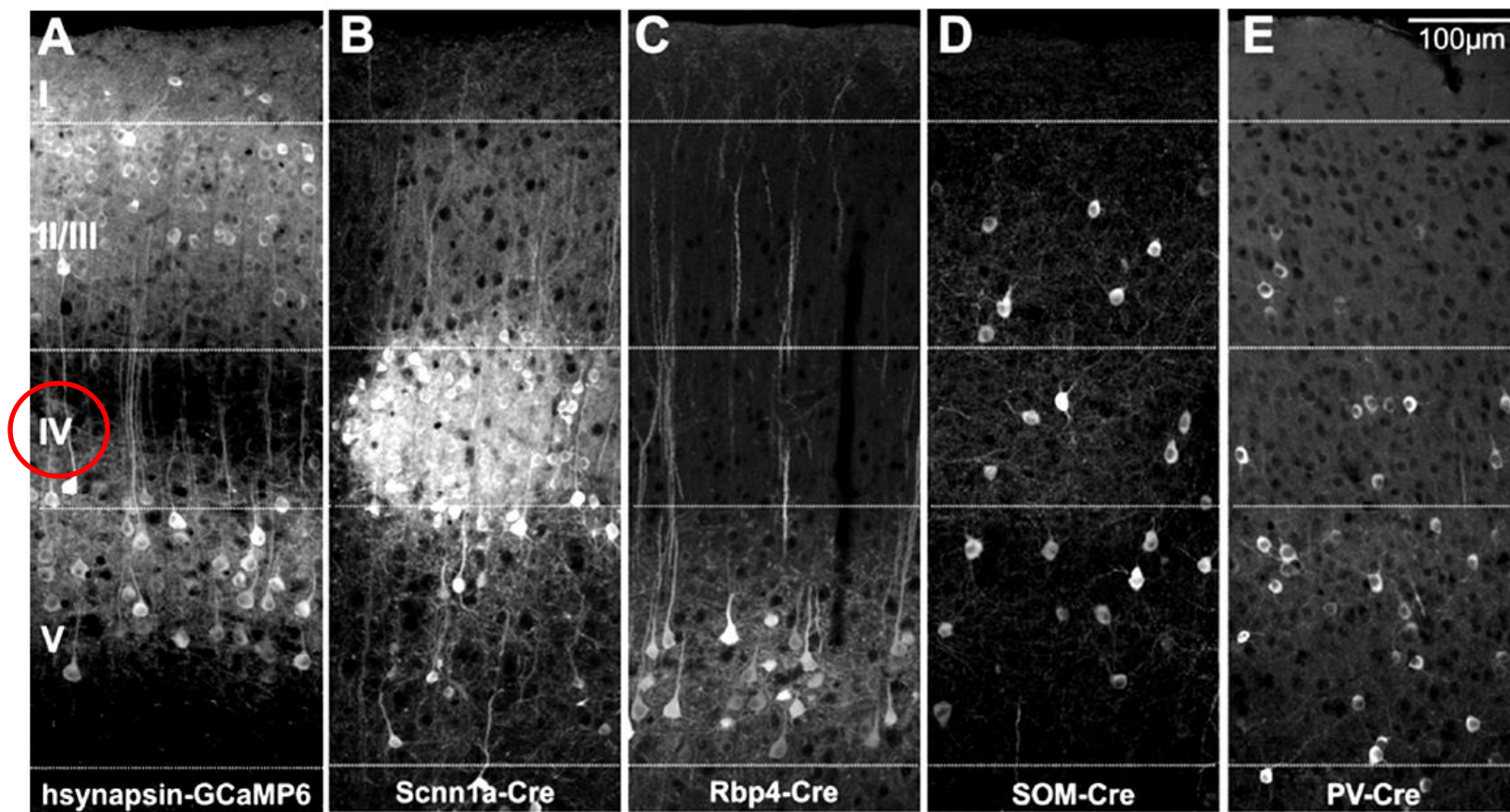
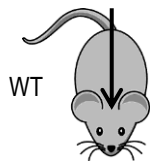
The Calcium Ion as an Indirect Reporter of Neuronal Activity

AAV1.Syn.GCaMP6f.WPRE.SV40

AAV1.Syn.flexGCaMP6f.WPRE.SV40



Cre-expressing mouse lines



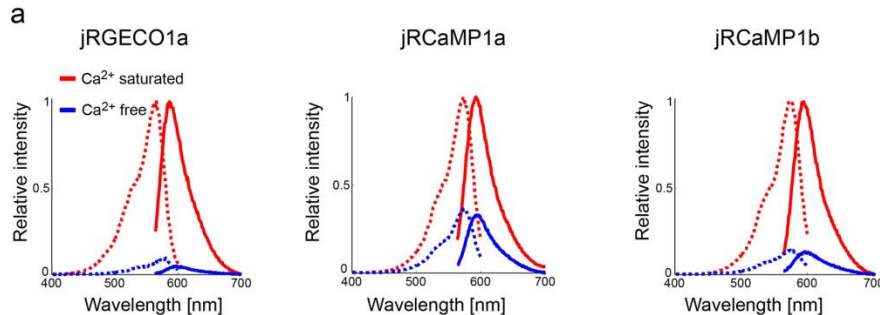
Bovetti et al., 2014

How to choose a calcium indicator

Fluorescence properties

Spectral properties (i.e. absorption and emission wavelengths):

1P Absorption and emission spectra



Dana et al., 2016

Quantum yield: Number of fluorescence photons emitted per excitation photon absorbed.

Photobleaching: Destruction of the excited fluorophore. Not reversible

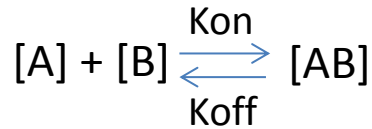
OTHERS: Quenching: Loss of fluorescence signal due to short-range interactions between the fluorophore and the local molecular environment, including other fluorophores. Reversible

Lifetime: The excited state exists for a finite time (typically 1–10 nanoseconds).

In vivo functional two-photon imaging

Biophysical properties

Affinity



at equilibrium (when [] do not change)

$$[A] \times [B] \times \text{Kon} = [AB] \times \text{Koff}$$

$$K_d = \frac{\text{Koff}}{\text{Kon}} = \frac{[A] \times [B]}{[AB]}$$

K_d: concentration of Ca²⁺ at which half the indicator molecules are bound with Ca²⁺ at equilibrium

High affinity indicators:

- K_d < 1 μM
- Saturate easier
- ex. fura-2, OGB-1, GCaMP

Low affinity indicators:

- K_d > 1 μM
- linear range
- ex. Mag-fura,

Dynamic range

$$R = \frac{F_{\max}}{F_{\min}}$$

Relative fluorescence change

$$\Delta F/F = (F_t - F_0)/F_0$$

Signal-to-noise ratio

$$\Delta F/SD_{\text{baseline noise}}$$

Kinetics: strongly depend on indicator affinity

Koff: influence the shape of the calcium transient

Kon: scaling factor for indicator response

Selectivity: [Ca²⁺] = nM

[Mg²⁺] = μM - mM



Competition on calcium binding sites

The Calcium Ion as an Indirect Reporter of Neuronal Activity

Comparison of biophysical properties between different calcium indicators

	λ for 2P excitation (nm)	λ_{em} (nm)	Rise time (ms)	Decay time (ms)	Dynamic range	Kd (nM)
OGB-1	810–850 ^{a,b}	520 ^{a,b}	8 ^b	$\tau_1 = 56^b; \tau_1 = 777^b$		
GCaMP3	920 ^c	510–520 ^c	83 ^c	610 ^c		
GCaMP6s	940 ^d	510–520 ^d	179 ^d	550 ^d	GCaMP6s 63	144
GCaMP6m	940 ^d	510–520 ^d	80 ^d	270 ^d		
GCaMP6f	940 ^d	510–520 ^d	45 ^d	142 ^d	GCaMP6f 52	375

^aYasuda et al. (2004).

^bGrewe et al. (2010).

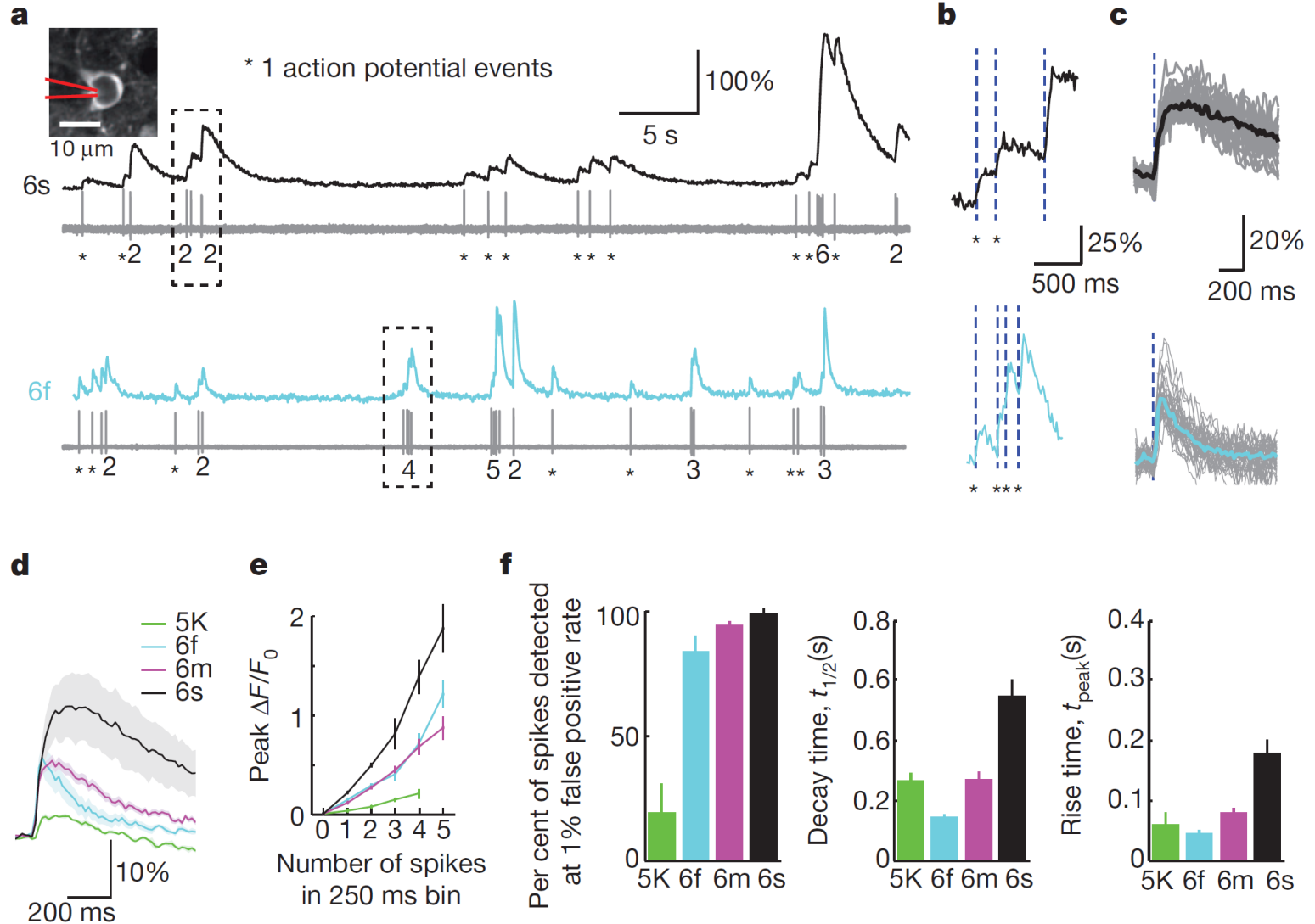
^cTian et al. (2009).

^dChen et al. (2013).

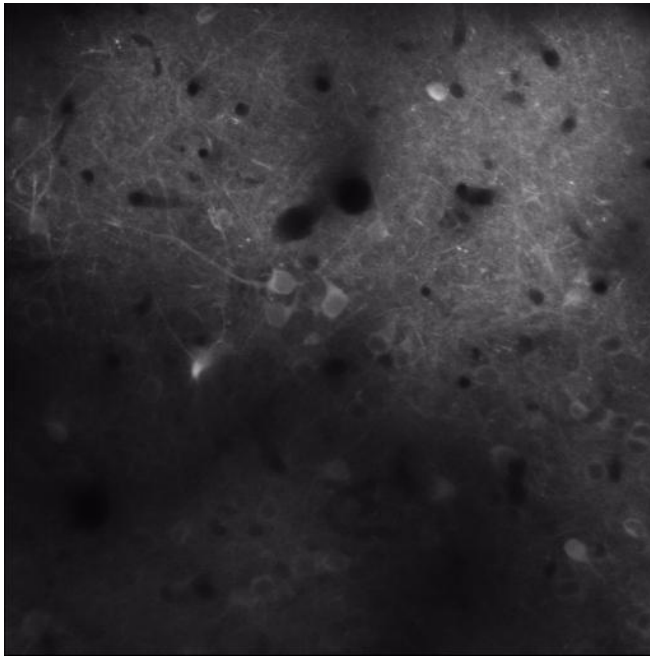
← Lower affinity, faster kinetics

Bovetti et al., 2013

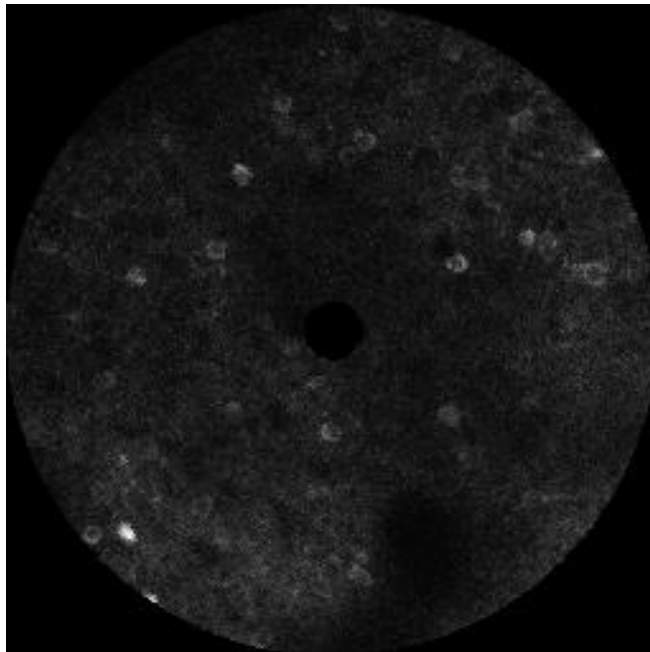
Detecting single cell action potential from calcium recording *in vivo*



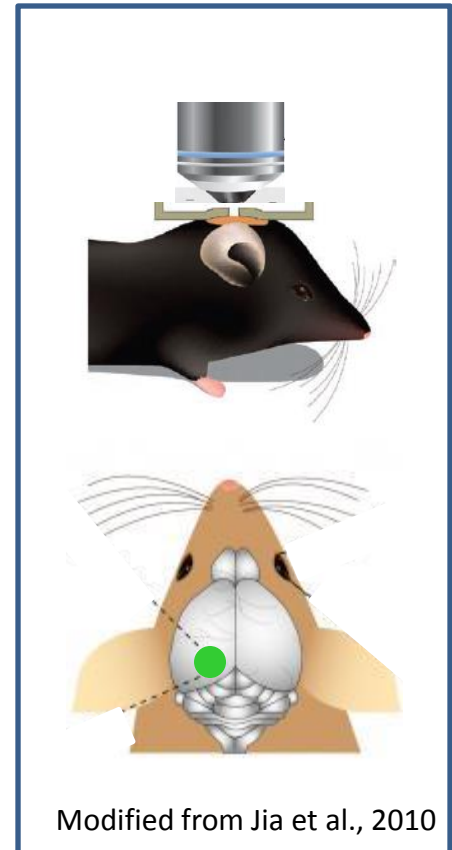
In vivo two-photon calcium imaging



Layer 2/3 cortical neurons expressing GCaMP6s
(somatosensory cortex)



Layer 4 cortical neurons expressing
GCaMP6s.
Selective expression has been
obtained injecting the AAV carrying
the flex GCaMP construct in the
Scnn1a-cre mouse line
(somatosensory cortex)



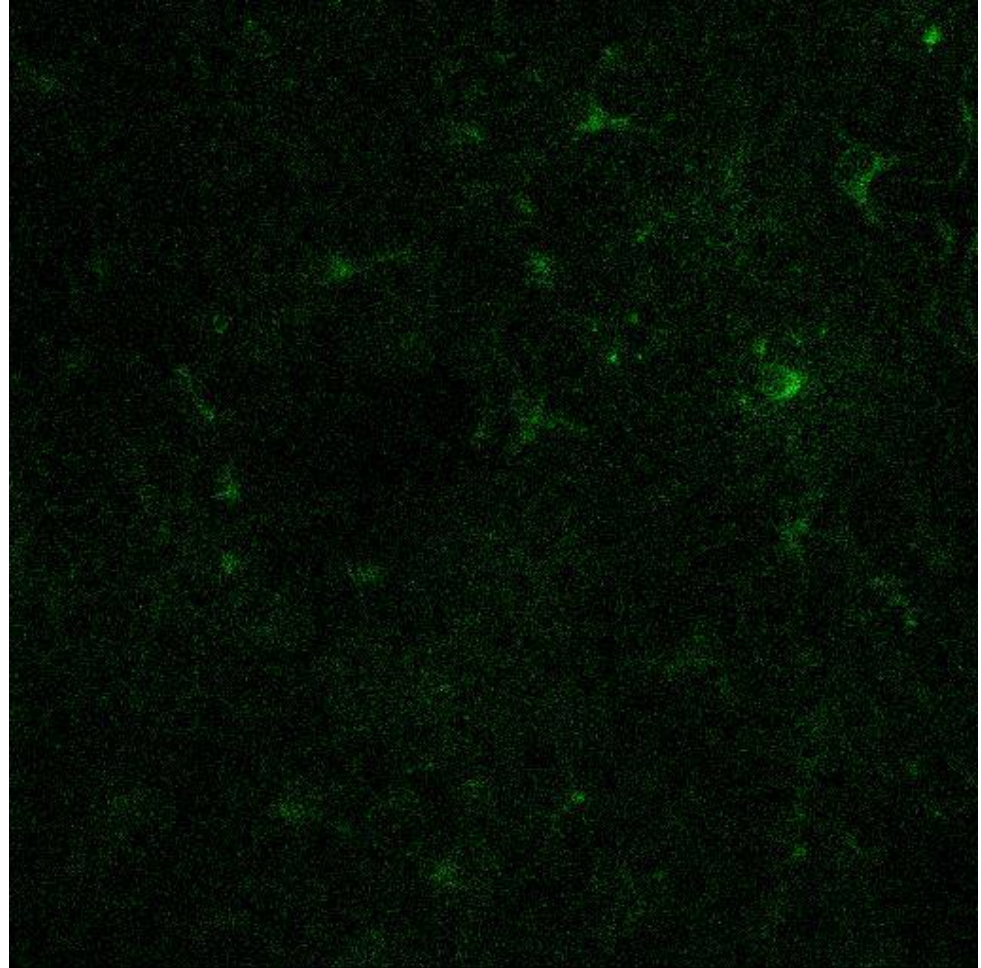
Modified from Jia et al., 2010

Astrocytes show calcium dynamics in vivo

AAV1.CAG.flexGCaMP6f.WPRE.SV40

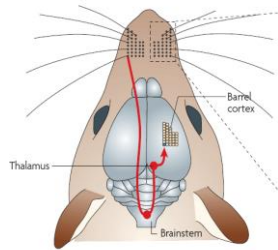


GLAST-CreER
mouse line



From functional imaging to 2P-circuit manipulation

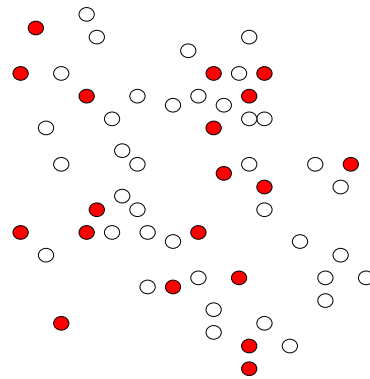
- ✓ In vivo two-photon functional imaging allows to study the complex spatial and temporal structure of neuronal activation that is fundamental for information processing within neuronal networks



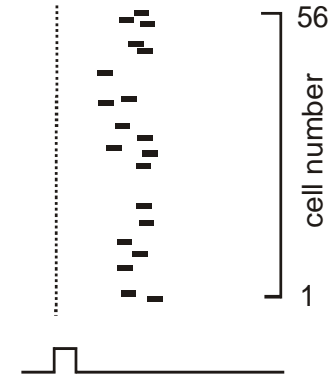
Diamond et al *Nat Rev Neurosc* 2008



SPATIAL



TEMPORAL



Modified from Grewe et al *Nat Methods* 2010

To causally test the role of specific circuitry we need a method that allows us to artificially manipulate cellular activity

Optogenetics!!!!

A simple introduction to optogenetics

Francis Crick, 1979

“The major challenge facing neuroscience is the need to control one type of cell in the brain while leaving others unaltered [...] **light** might have these properties” *in Scientific American, 1979*

2005 Optogenetics: combination of genetic and optical methods to cause or inhibit well defined events in specific cells or living tissue and behaving animals

Deisseroth, 2015

A simple introduction to optogenetics

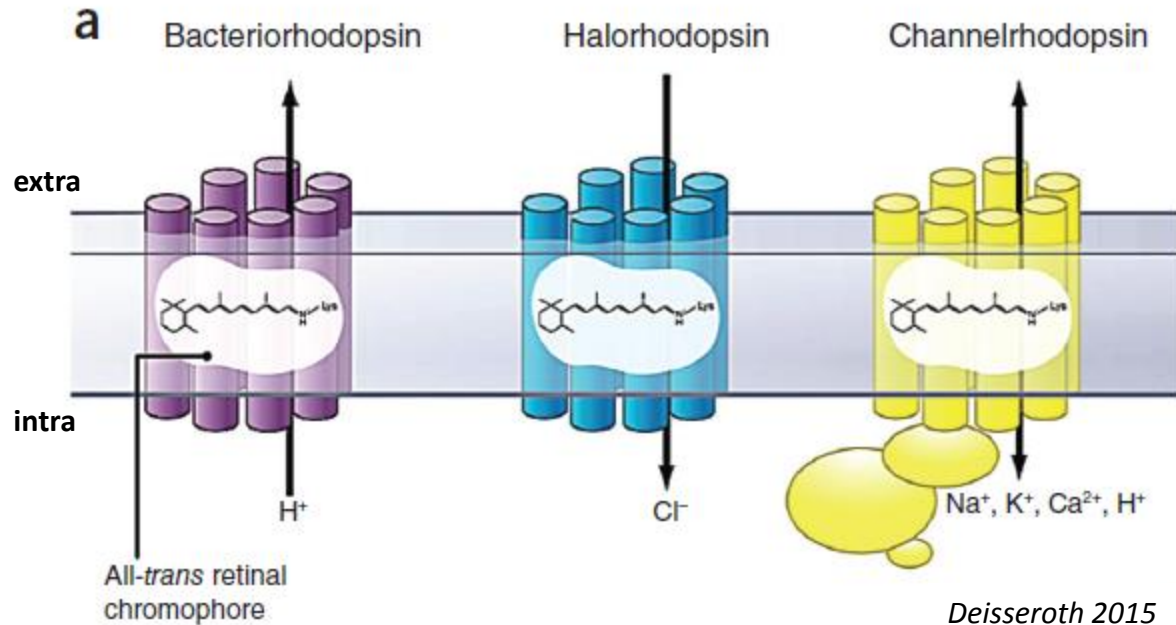
Optogenetics needs:

- 1) *Microbial opsins*: proteins that directly elicits electrical current across cellular membranes in response to light
- 2) *Methods for targeting* sufficiently strong and specific opsin gene expression to well-defined cellular elements in the brain
- 3) *Methods for guiding* sufficiently strong and precisely timed *light* to specific brain regions, cells or parts of cells

3 technologically distinct branches that are still evolving independently and that must be combined for optogenetics experiments

A simple introduction to optogenetics

Microbial opsins (type I): transduce photons into electrical current



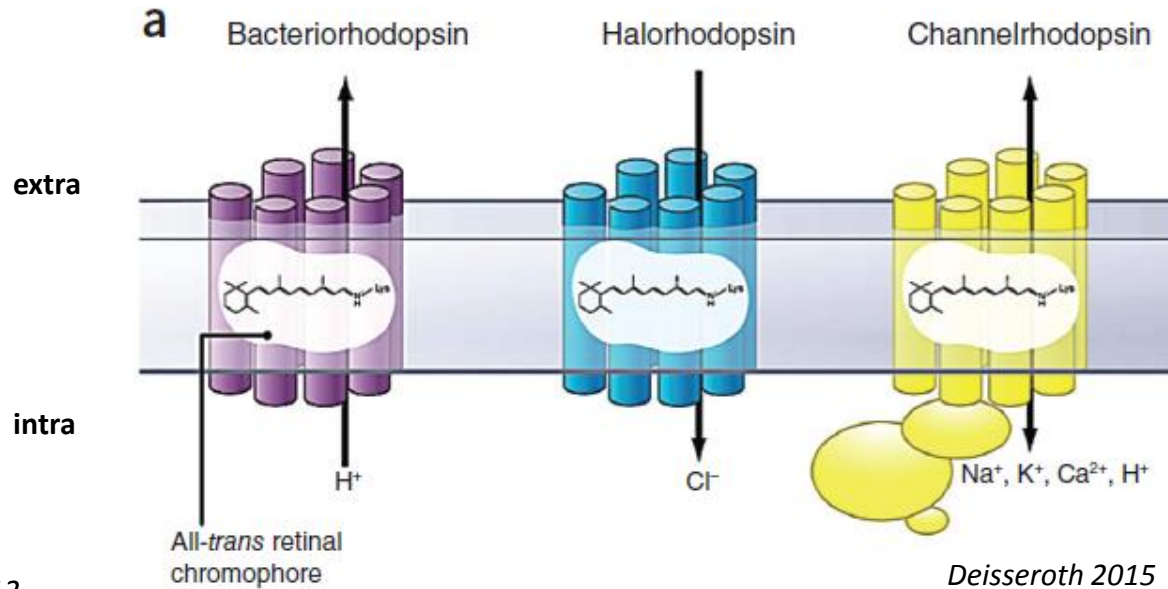
Each opsin protein requires the incorporation of a *retinal* to enable light sensitivity
opsin + retinal : rhodopsin

light

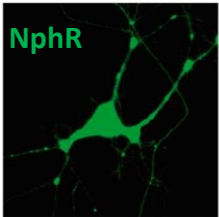
All-trans → 13-cis configuration: translocation of proton/cl-/cations

A simple introduction to optogenetics

Microbial opsins (type I): transduce photons into electrical current



Chow et al. 2012



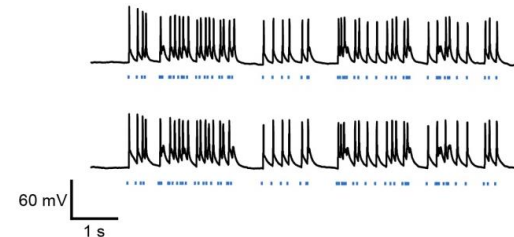
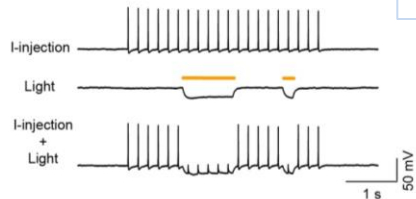
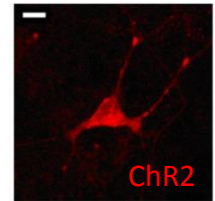
Hyperpolarizing current

**Arch, eArch,
eArch3.0, ArchT**

**NphR,
NphR3.0**

Depolarizing current

ChR2, ChETA, C1V1



A simple introduction to optogenetics

Microbial opsins (type I): are known since decades because their role in energy generation, flagellar beating and rotation, phototaxis, maintenance of membrane potential etc.

Why it took so long to apply these molecules in neuroscience?

1) *Biophysical properties* that influences the performance of opsins at the single-molecule level:

- **Efficiency of light absorption** (cross-section) defined in term of *extinction coefficient* (ϵ_{\max} : how strongly a substance absorbs light at a given wavelength) and *quantum efficiency* (Φ : the fraction of absorbed photons that are efficacious in driving the relevant conformational change)

- **Kinetics** defined in term of turnover time of the photocycle.
For inhibitory pumps 10-20 ms but it depends on membrane voltage
For ChR2 current is coupled to occupancy of the conducting state that depends on many factors

Conductance and photocurrent

See Yiazar et al, 2011

Mutation at different residues change the biophysical properties of opsins

[DB_Catalog_082715.pdf](#)

A simple introduction to optogenetics

Microbial opsins (type I): are known since decades because their role in energy generation, flagellar beating and rotation, phototaxis, maintenance of membrane potential etc.

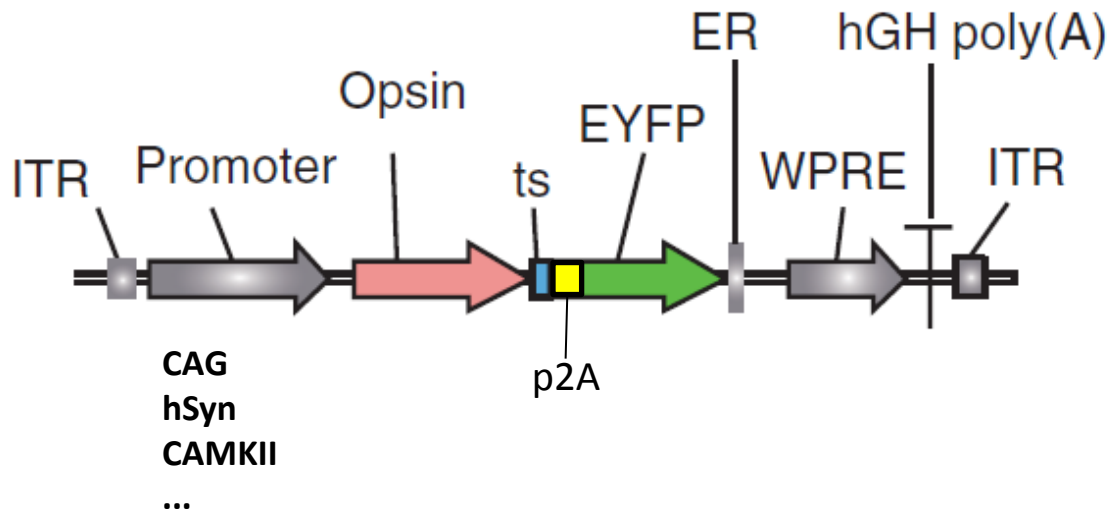
Why it took so long to apply these molecules in neuroscience?

2) *Cell biological properties* (effective transcription, translation, folding, membrane trafficking, targeting) and *opsin construct characteristics*

Methods for targeting sufficiently strong and specific opsin gene expression to well-defined cellular elements in the brain

Vector:

AAV: different serotypes
Lentivirus
Transgenic mouse lines

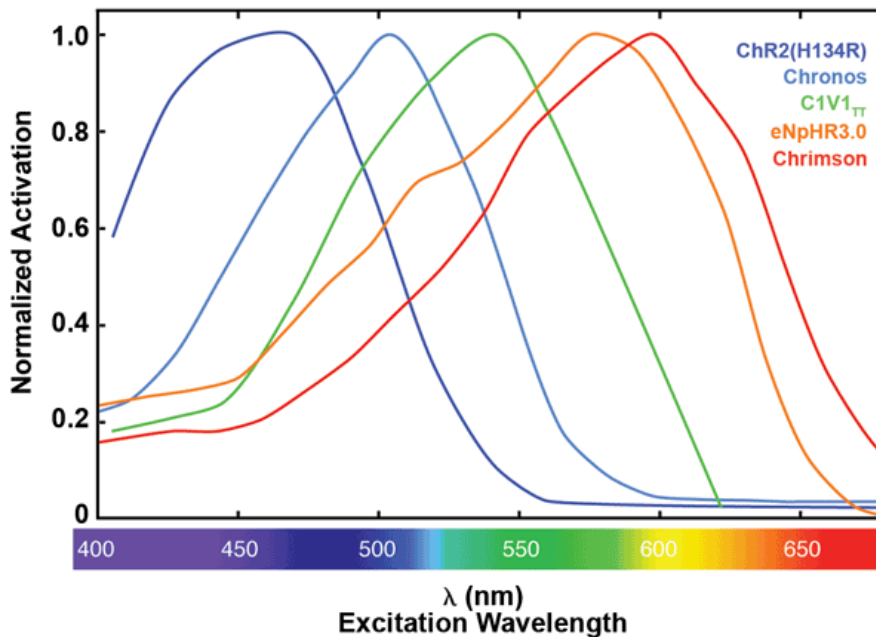


A simple introduction to optogenetics

Microbial opsins (type I): are known since decades because their role in energy generation, flagellar beating and rotation, phototaxis, maintenance of membrane potential etc.

Why it took so long to apply these molecules in neuroscience?

3) *Methods for guiding* sufficiently strong and precisely timed *light* to specific brain regions, cells or parts of cells



To consider for light delivery:

- Wavelength
(most common 473, 532, 561, 594, 638 nm)
- sufficient, adjustable and stable output power
(1 to 10 mW/mm² is need at the target)
- No photodamage
- rise/fall times and modulation of the light pulse

A simple introduction to optogenetics

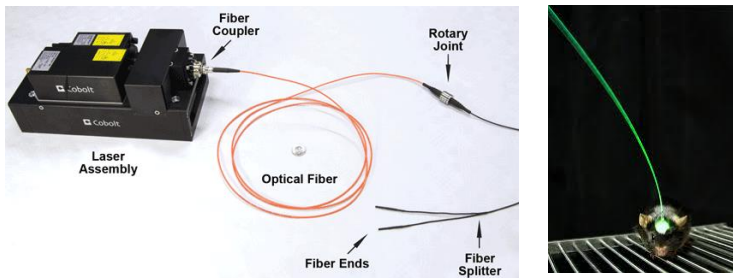
Microbial opsins (type I): are known since decades because their role in energy generation, flagellar beating and rotation, phototaxis, maintenance of membrane potential etc.

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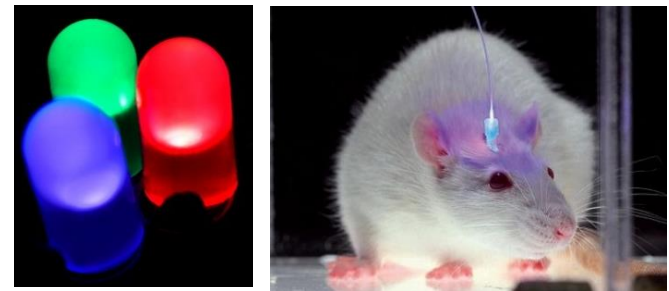
1P light delivery

Fiber from laser source



Expensive but stable, higher power, spatially precise, can collect emitted fluorescence

LEDs



Cheap but warm up the tissue, often not enough power is delivered, less precise in space, no back collection

A simple introduction to optogenetics

In vivo application of 1P optogenetics

Too many application to be listed:
See Deisseroth, 2015

Optogenetics: 10 years of microbial opsins in neuroscience

Karl Deisseroth

Stimulation of the right anterior motor cortex in a Thy1::ChR2-EYFP transgenic mouse with 20-Hz blue light flashes elicits contralateral circling.

[torinodic15\mn.4091-sv1.mov](#)

Cortex commands the performance of skilled movement

Jian-Zhong Guo, Adam W Hantman
Elife 2015

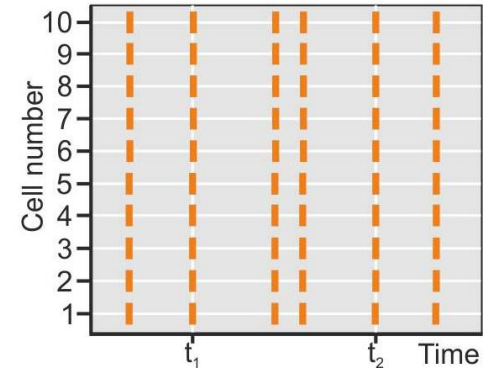
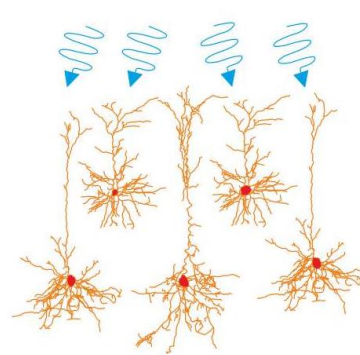
What is the role of cortex in skilled voluntary movements?
Testes by optogenetics inhibiting cortical activity trough the expression of ChR2 in all inhibitory interneurons

[torinodic15\elife_poa_e10774_Video_1.mov](#)

[torinodic15\elife_poa_e10774_Video_9.mov](#)

In vivo 2P-optogenetics

Wide – field 1P optogenetic manipulation



In vivo 2P-optogenetics can be used to selectively activate specific cells with high temporal and spatial resolution

In vivo 2P-optogenetics

Why is it difficult to perform 2P optogenetics in vivo?

1-Op sin biophysical properties :

ChR2 (920 nm) **C1V1** (1040 nm)

2P cross-section ++ ++

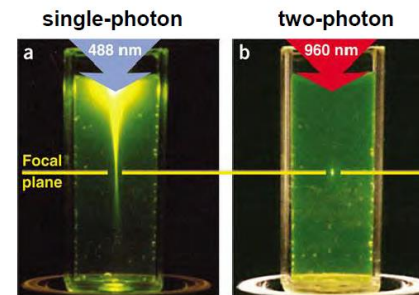
Conductance + ++

Many ops ins (thus a large portion of the cell)
must be activated near simultaneously to induce a cell to fire

Kinetics + ++

Longer deactivation time is better

The 2P cross section of many ops ins has been evaluated and many new ops ins have been developed to make them more suitable for near infrared λ excitation



Zipfel et al. 2003

2P allows to be very precise in the stimulation volume, however this restricts the number of molecules that we are activating

2- Op sin molecular properties

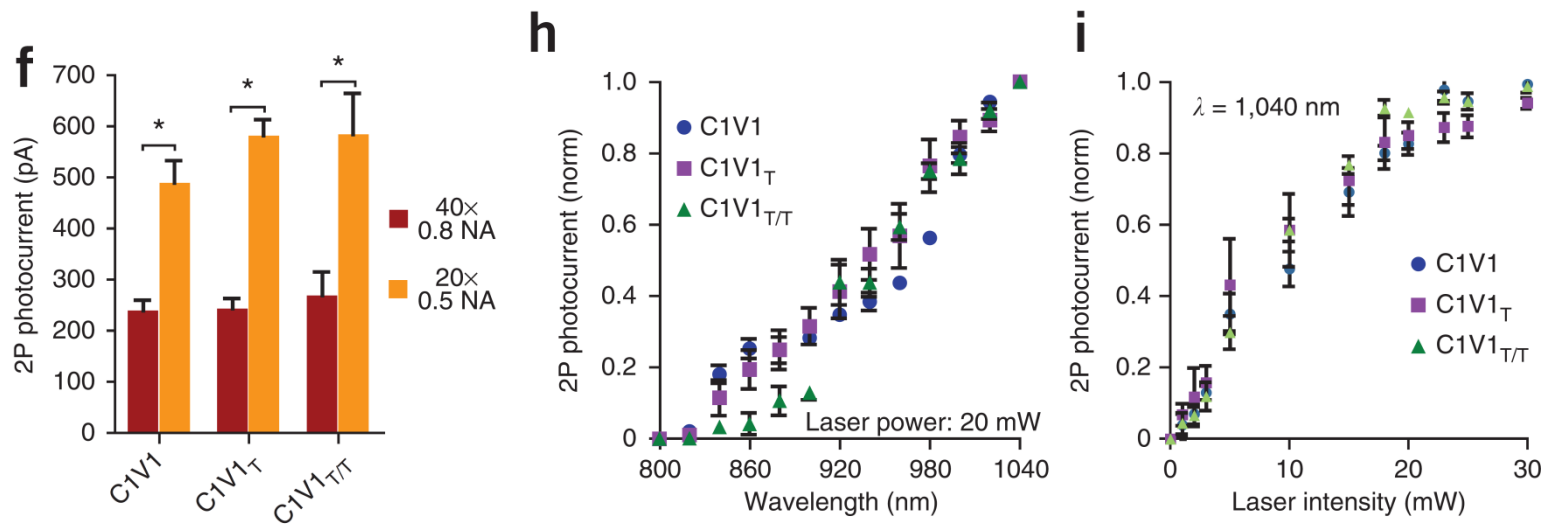
expression level, membrane trafficking...

3- Optical tool and light delivery:

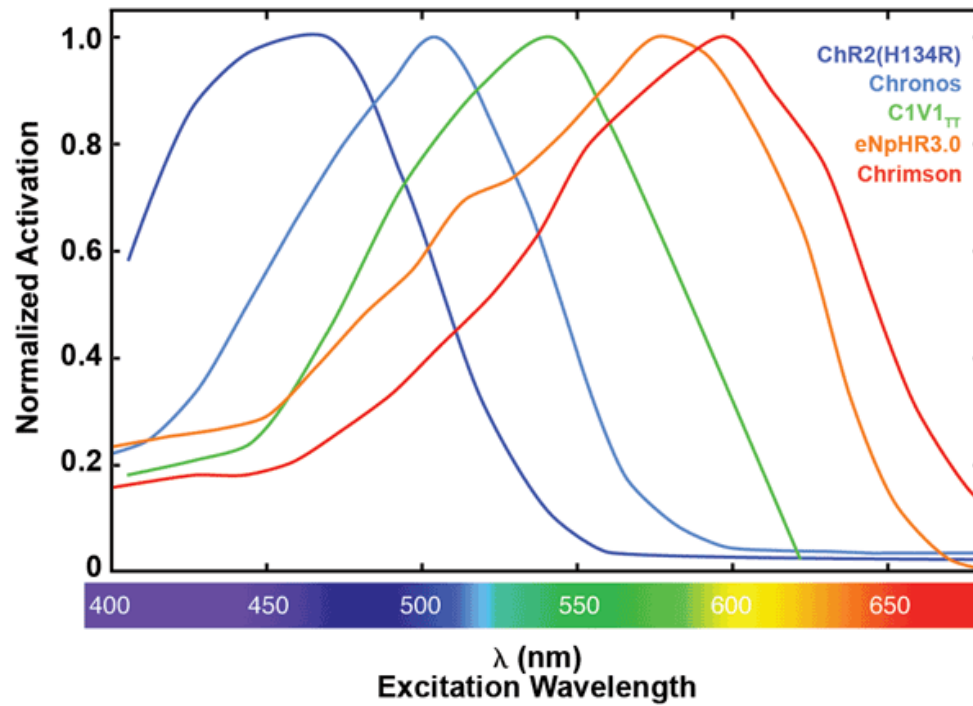
Selectively activate a group of cells with high spatial precision (and, eventually, simultaneously record the activity of the same/other cells)

Two-photon optogenetic toolbox for fast inhibition, excitation and bistable modulation

Rohit Prakash¹, Ofer Yizhar¹, Benjamin Grewe^{2,3}, Charu Ramakrishnan¹, Nancy Wang¹, Inbal Goshen¹, Adam M Packer⁴, Darcy S Peterka⁴, Rafael Yuste⁴, Mark J Schnitzer^{2,3,5,6} & Karl Deisseroth^{1,5-7}



In vivo 2P-optogenetics



Two-photon optogenetic manipulation



Two-photon excitation of channelrhodopsin-2 at saturation

John Peter Rickgauer^{a,b,c} and David W. Tank^{a,b,c,d,1}

^aDepartment of Molecular Biology, Princeton University, Princeton, NJ 08544; ^bThe Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ 08544; ^cPrinceton Neuroscience Institute, Carl Icahn Laboratory, Princeton University, Princeton, NJ 08544; and ^dDepartment of Physics, Princeton University, Princeton, NJ 08544

2P optogenetics

TECHNICAL REPORTS

nature
neuroscience

Simultaneous cellular-resolution optical per and imaging of place cell firing fields

John Peter Rickgauer¹⁻⁴, Karl Deisseroth⁵⁻⁸ & David W Tank¹⁻⁴

BRAIN MICROCIRCUITS

Imprinting and recalling cortical ensembles

Luis Carrillo-Reid,* Weijian Yang, Yuki Bando, Darcy S. Peterka, Rafael Yuste

In vivo 2P optogenetics and 2P imaging

Simultaneous all-optical neural circuit activity with cellular resolution *in vivo*

Adam M Packer^{1,2}, Lloyd E Russell^{1,2}, Henry W P Dalglish^{1,2} & Michael Häusser^{1,2}

Two-photon optogenetic manipulation

BRAIN MICROCIRCUITS

Imprinting and recalling cortical ensembles

2016

Luis Carrillo-Reid,* Weijian Yang, Yuki Bando, Darcy S. Peterka, Rafael Yuste

C1V1

Activate
this neuron
at 1050 nm

Focusing optics

GCaMP6

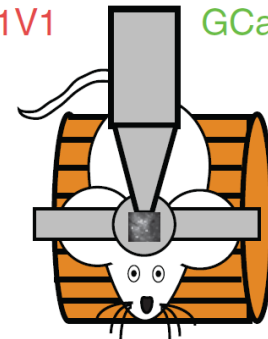
Detect signal
in this neuron
at 940 nm



A *in vivo* two-photon

photostim
1,064 nm
C1V1

imaging
940 nm
GCaMP6s



head fixed
free movement

Two-photon optogenetic manipulation

BRAIN MICROCIRCUITS

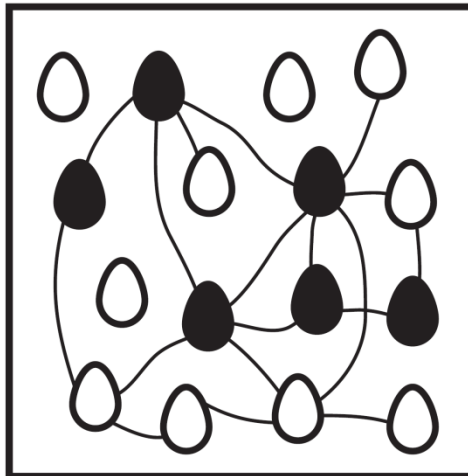
Imprinting and recalling cortical ensembles

Luis Carrillo-Reid,* Weijian Yang, Yuki Bando, Darcy S. Peterka, Rafael Yuste

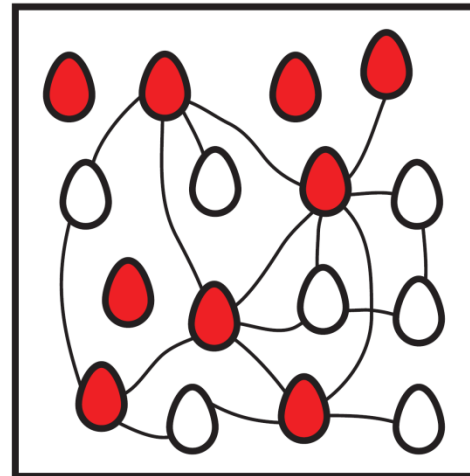
More than 60 years ago, Hebb proposed that repeated coactivation of a group of neurons might create a memory trace through enhancement of synaptic connections (12). Because of technical limitations, this hypothesis has been difficult to test with single-cell resolution in awake animals. By combining novel imaging and photostimulation techniques (14, 15) and analytical tools (19), our work can be interpreted as a confirmation of the Hebbian postulate and as a demonstration that cortical microcircuits can perform pattern completion.

F

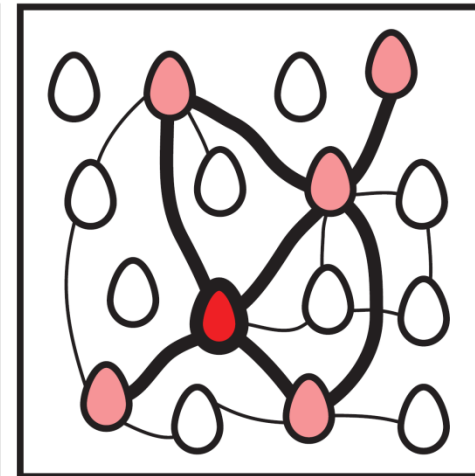
natural
ensemble



photostim
training



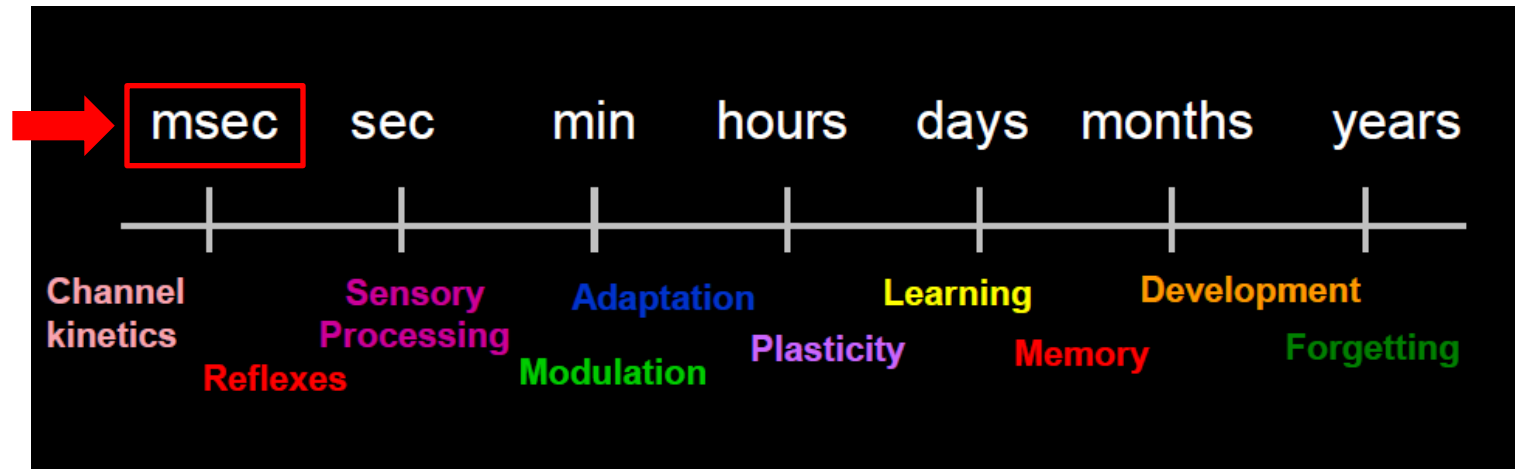
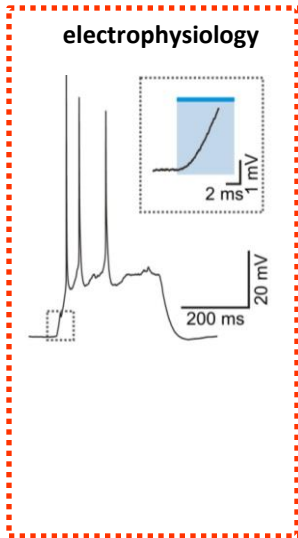
imprinted
ensemble



References

- Helmchen and Denk. 2005. Deep tissue two-photon microscopy
- Ustione and Pston 2011. A simple introduction to multiphoton microscopy
- Bovetti et al., 2014. Mapping brain circuit function in vivo using two-photon fluorescence imaging
- Zhang et al., 2011. The microbial opsin family of optogenetic tools
- Deisseroth 2015. Optogenetics: 10 years of microbial opsins in neuroscience
- Yizhar et al., 2011. Microbial opsins. A family of single-component tools for optical control of neural activity
- Guo et al., 2015 Cortex commands the performance of skilled movement
- Bovetti and Fellin 2015. Optical dissection of brain circuits with patterned illumination through the phase modulation of light
- Gradinaru et al., 2010. Molecular and Cellular Approaches for Diversifying and Extending Optogenetics

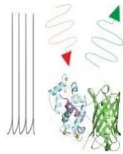
Temporal scale of neuronal network dynamics



Spatial and temporal complexity of neuronal network dynamics

Laser scanning two-photon microscopy

- imaging large field of view
- attaining high spatial resolution
- discriminating between different cell types

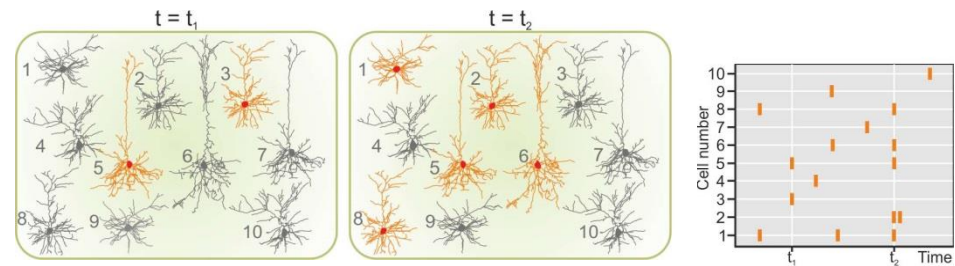


Genetically-encoded calcium indicators

- **Limited temporal resolution**

Laser scanning imaging of GCaMP6-expressing layer 2/3 neurons in the somatosensory cortex in vivo

Sensory information is encoded in spatiotemporal patterns of neuronal activation

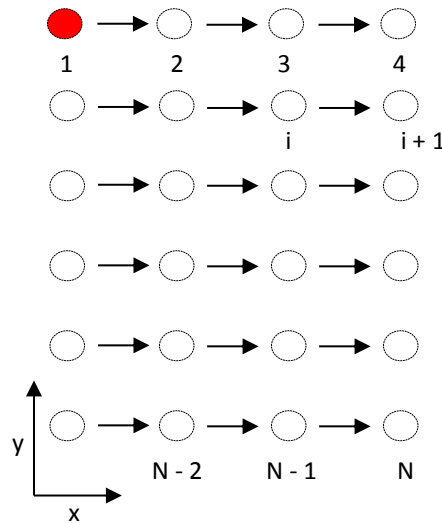
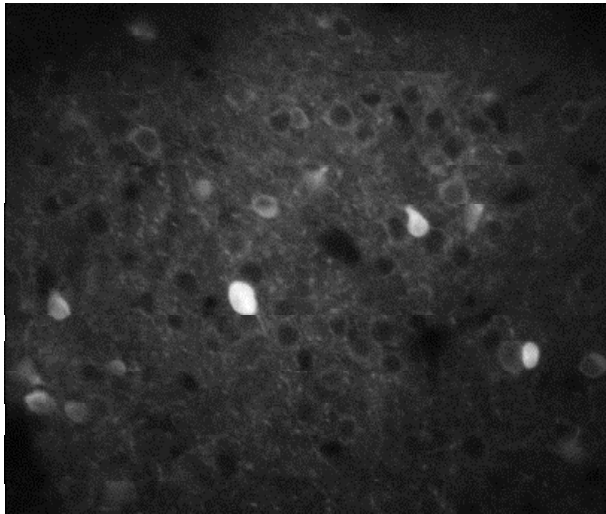


Which code does the mammalian brain use to drive perception?

Mapping and manipulating the activity of neuronal networks in space and time *in vivo* is crucial for understanding the role of spatiotemporal codes in brain functions

How to improve to improve the acquisition speed

Raster scanning



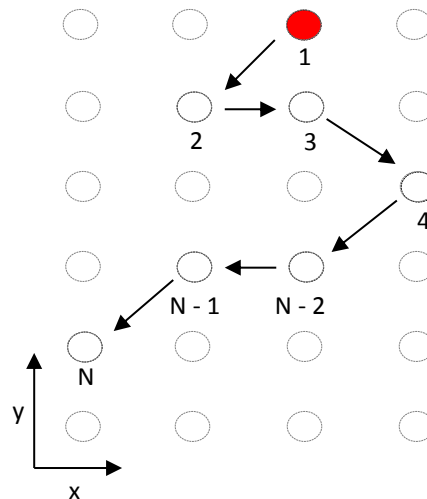
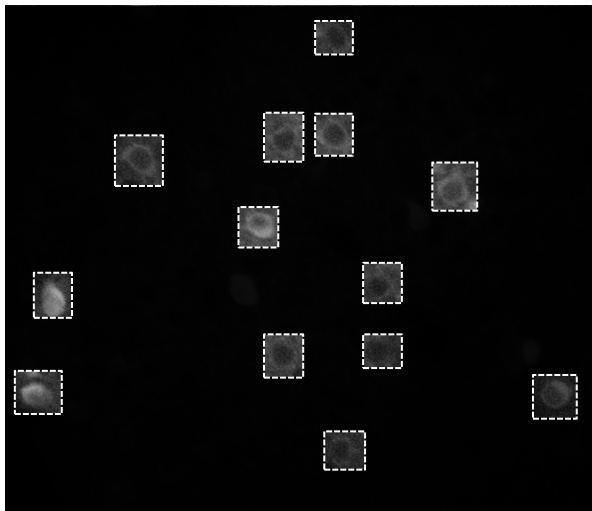
$$\text{Max acquisition frequency} = \frac{1}{(t_d * N + t_m * (N-1))}$$

t_d : dwell time

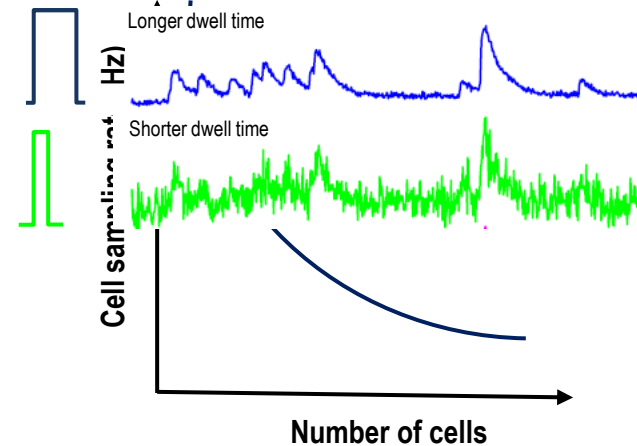
t_m : time to move from point i to $i+1$

N : total number of points

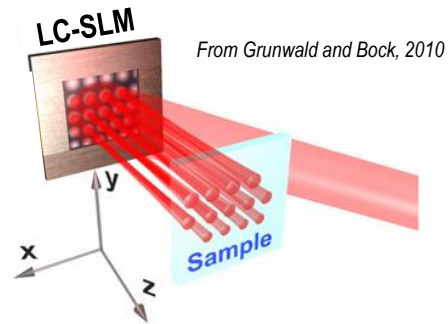
Random access scanning



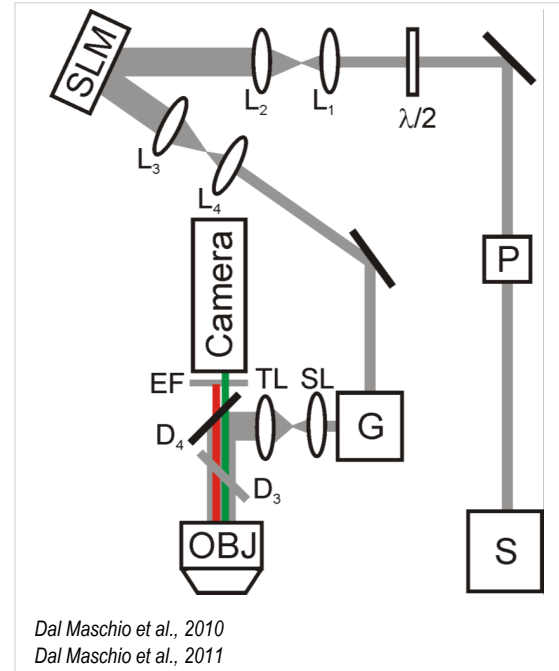
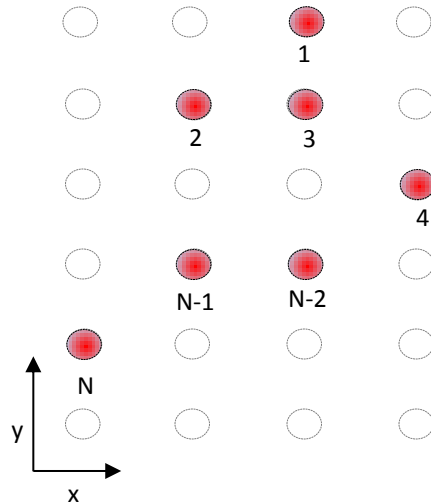
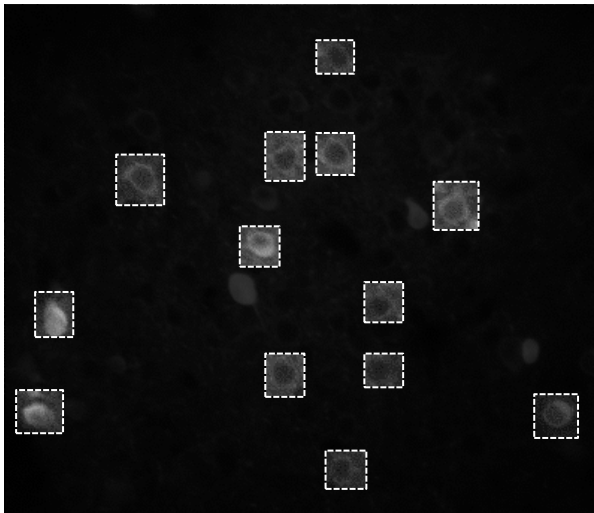
Sequential



Development of a structured light microscope for fast imaging *in vivo*



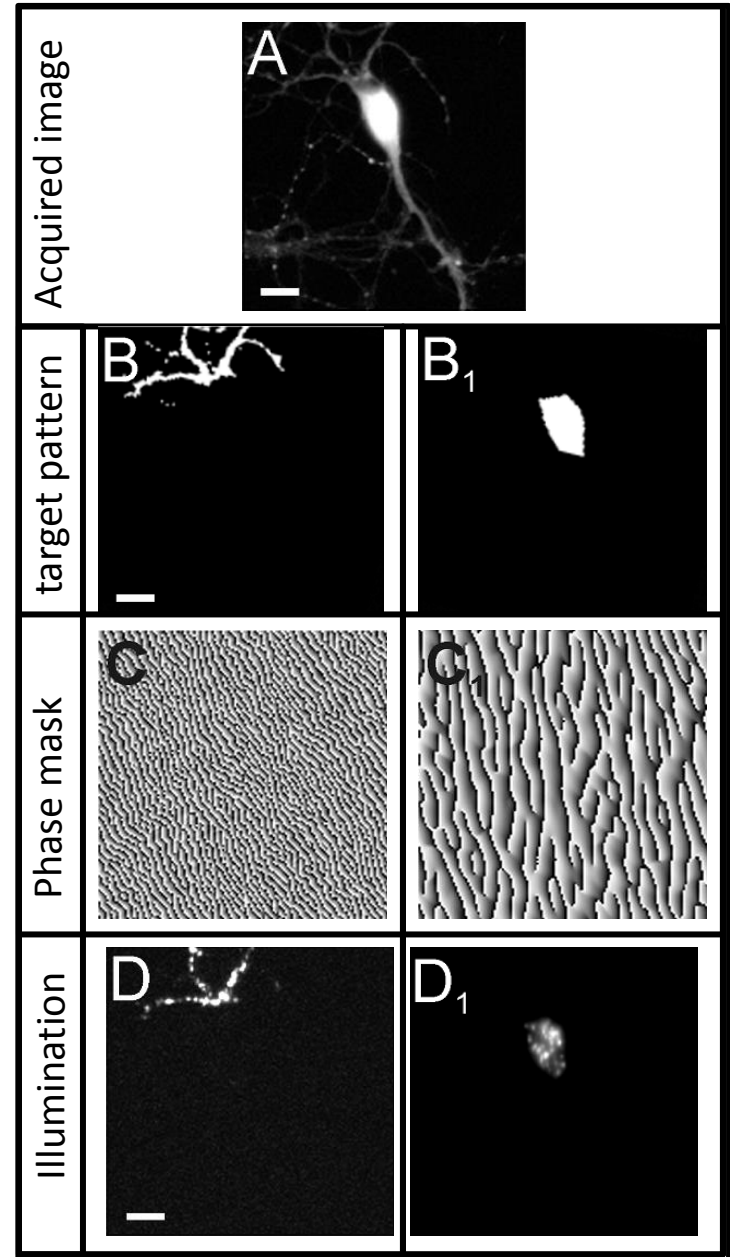
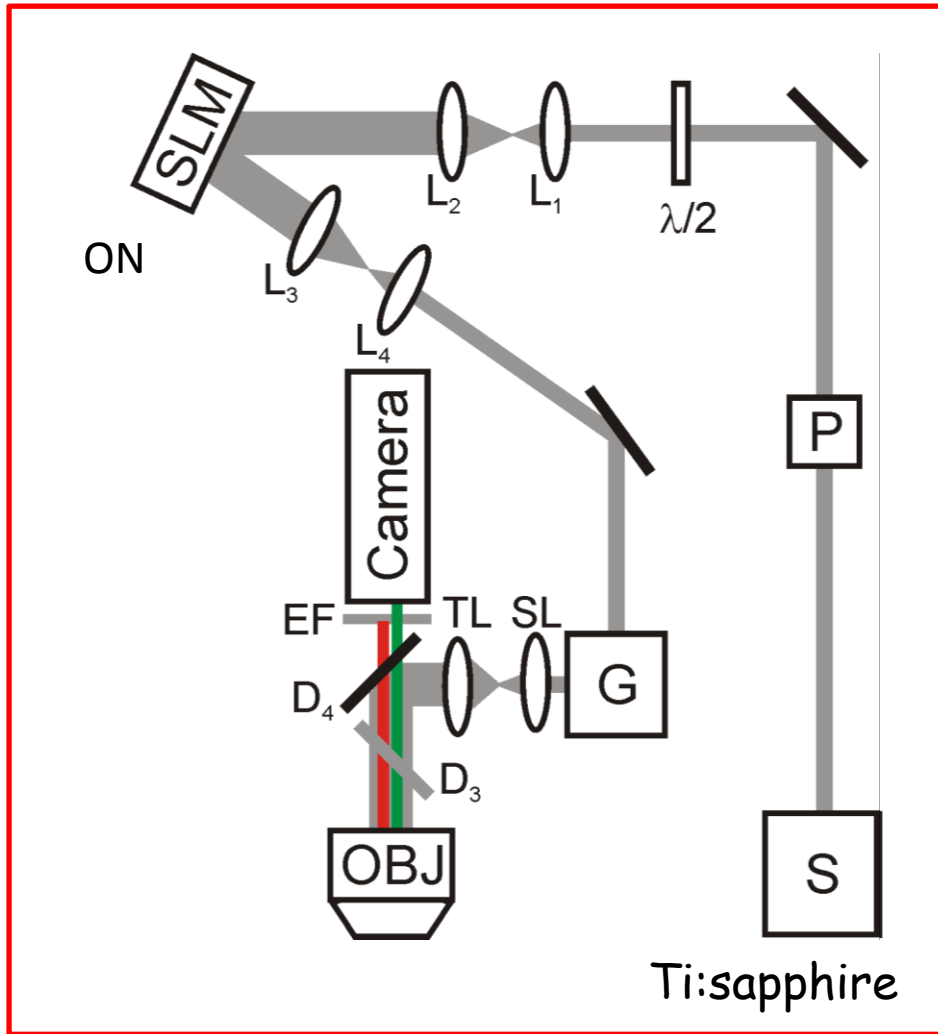
Scanless or parallel illumination



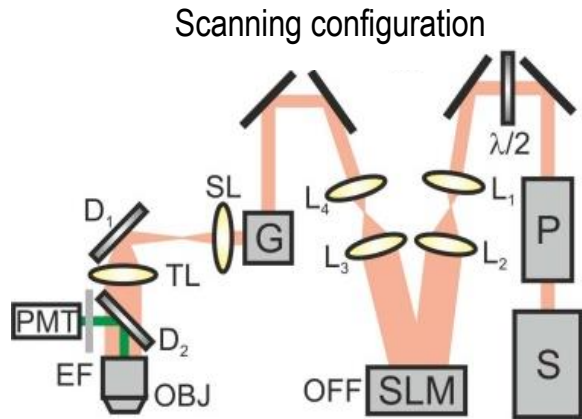
Dwell time = Exposure time

Max. acquisition frequency =
Camera max. frame rate

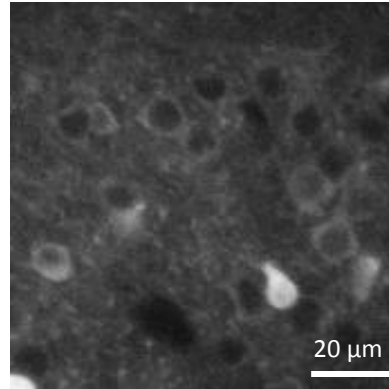
Building a structured light microscope



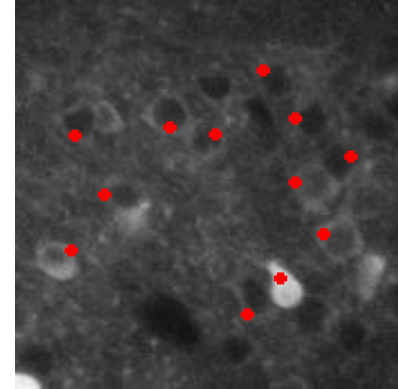
Scanless imaging of neuronal networks with structured light



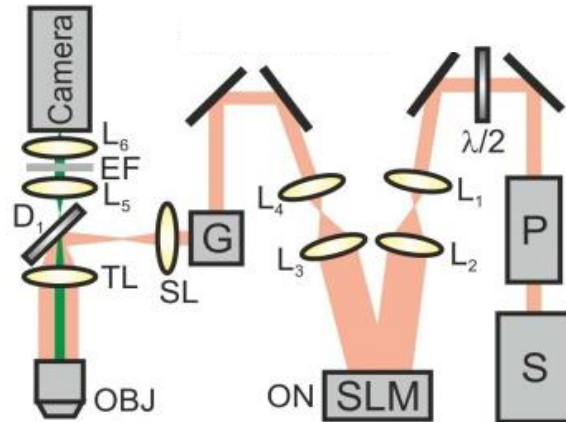
1- Acquire image



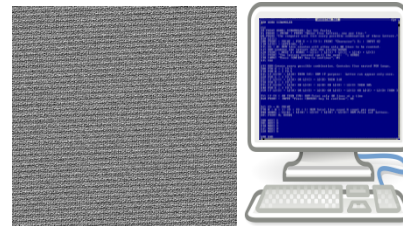
2- Target pattern



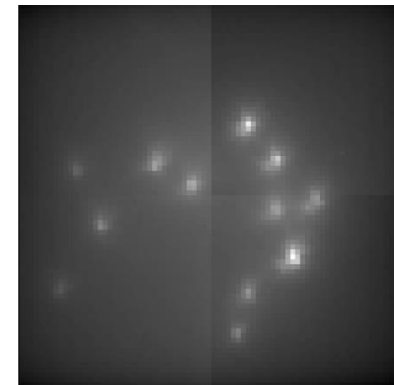
Scannless configuration



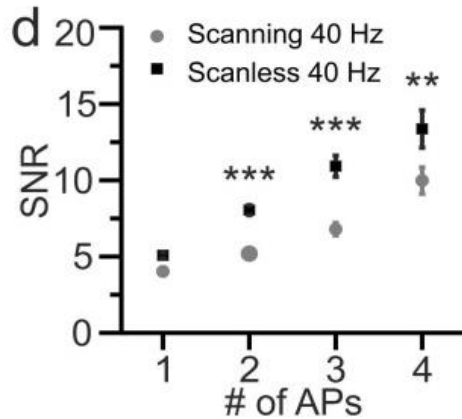
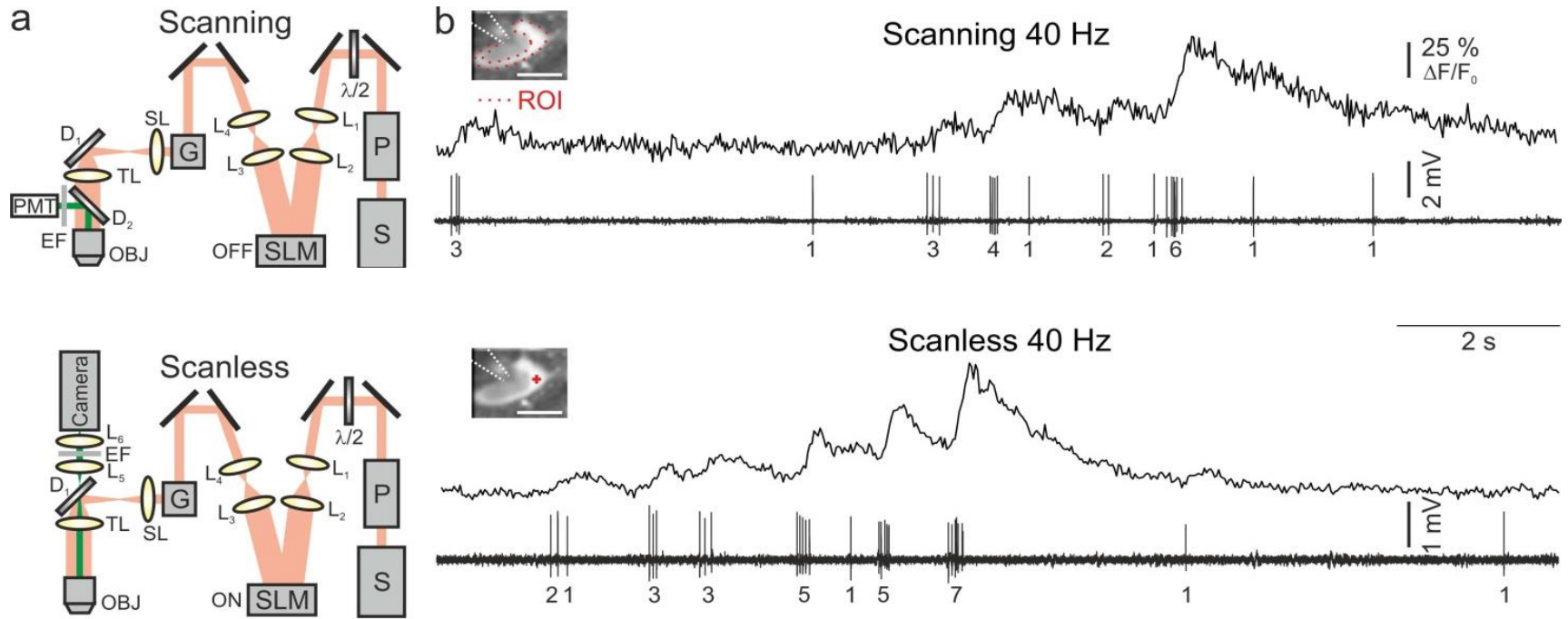
3- Phase mask



4- Illumination



Higher SNR of GCaMP signals in scanless than scanning imaging

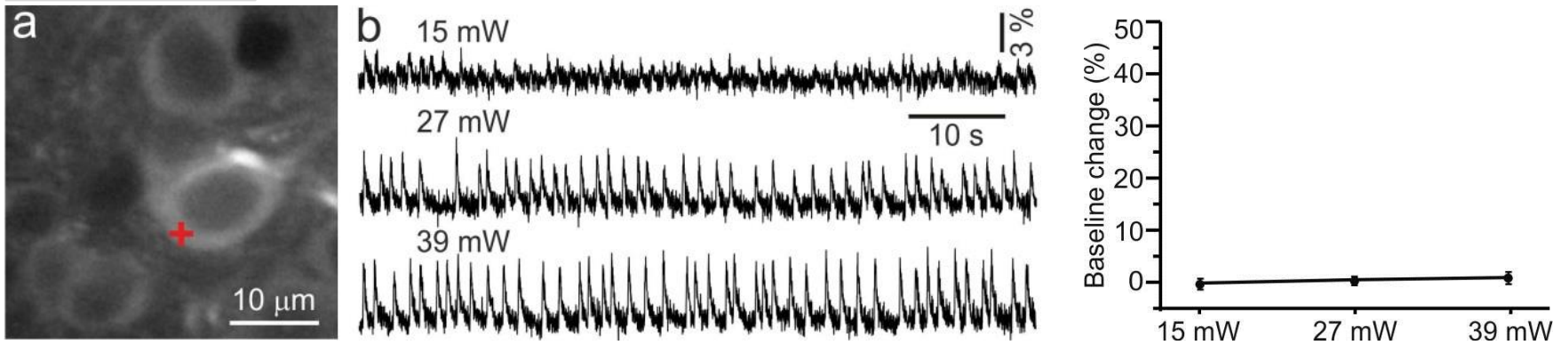


Validation of the structured light microscope

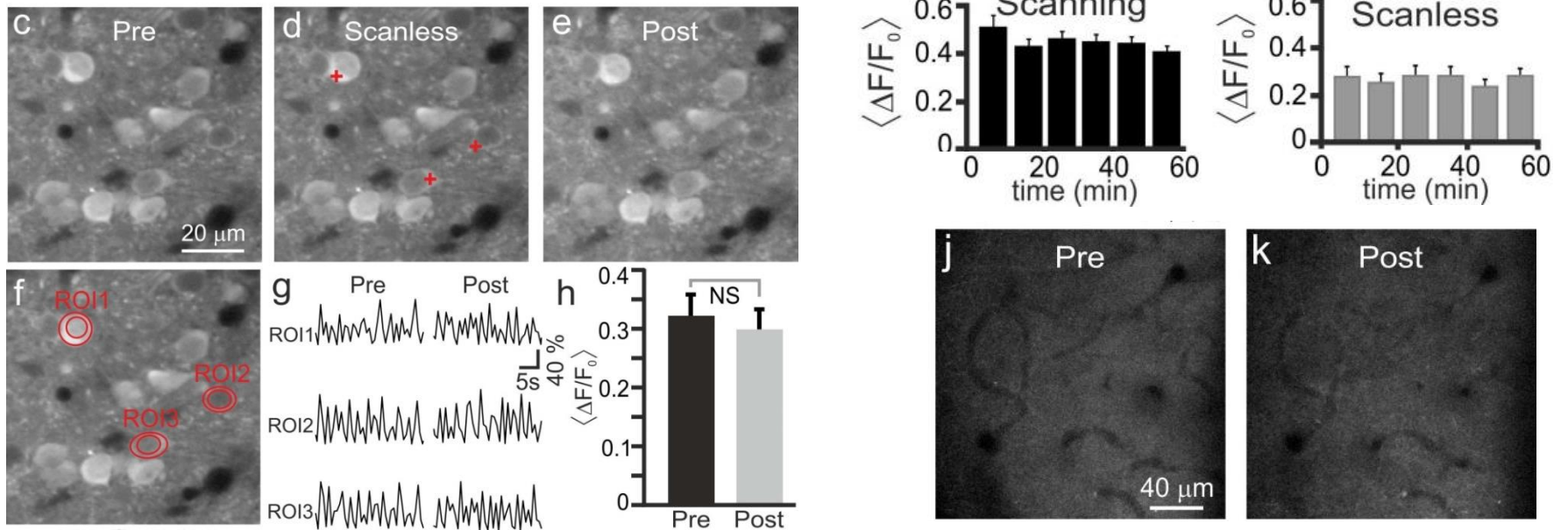
- Photobleaching
- Photodamage
- Spatial resolution
- Temporal resolution

No significant photobleaching and photodamage in scanless imaging

Photobleaching

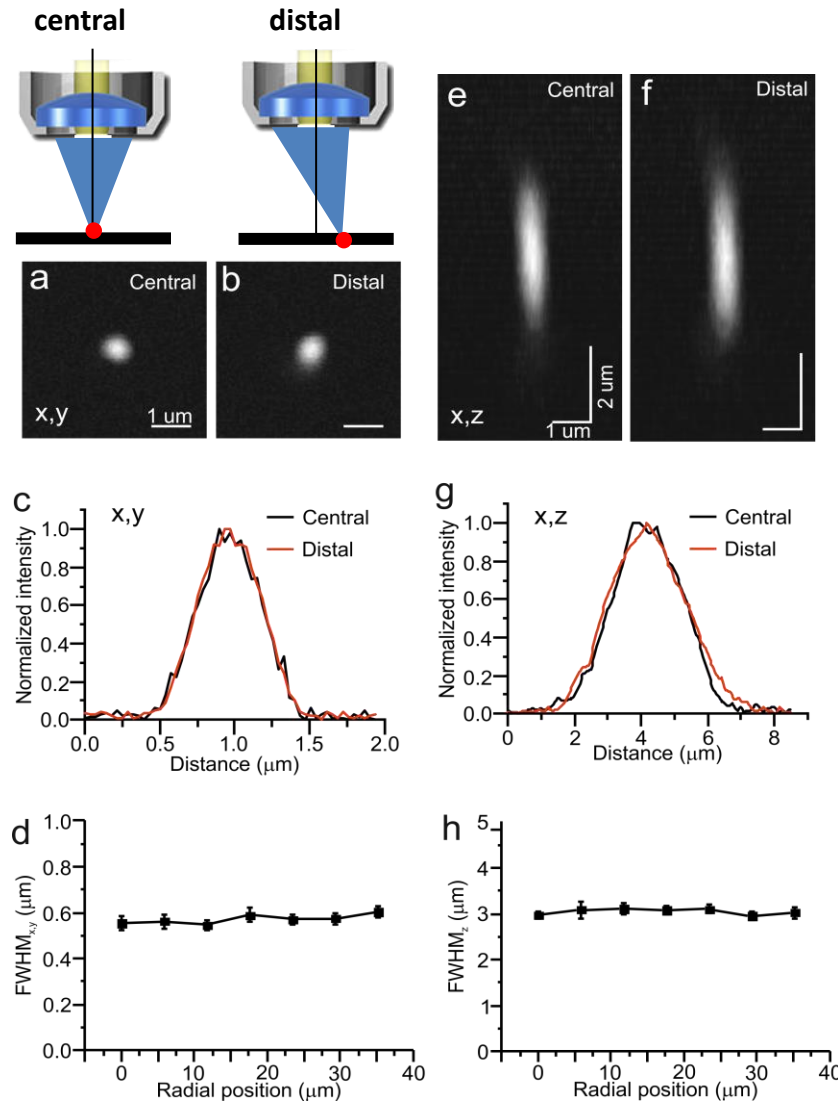


Photodamage



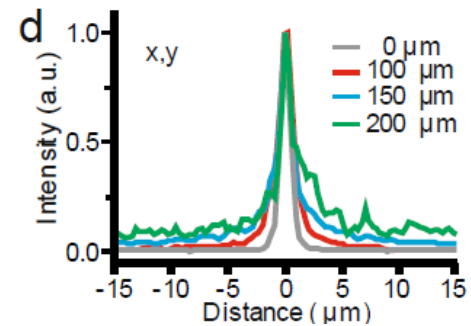
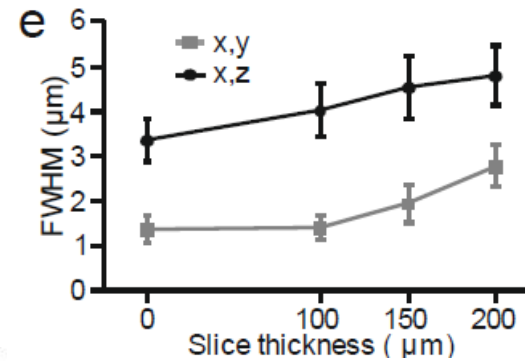
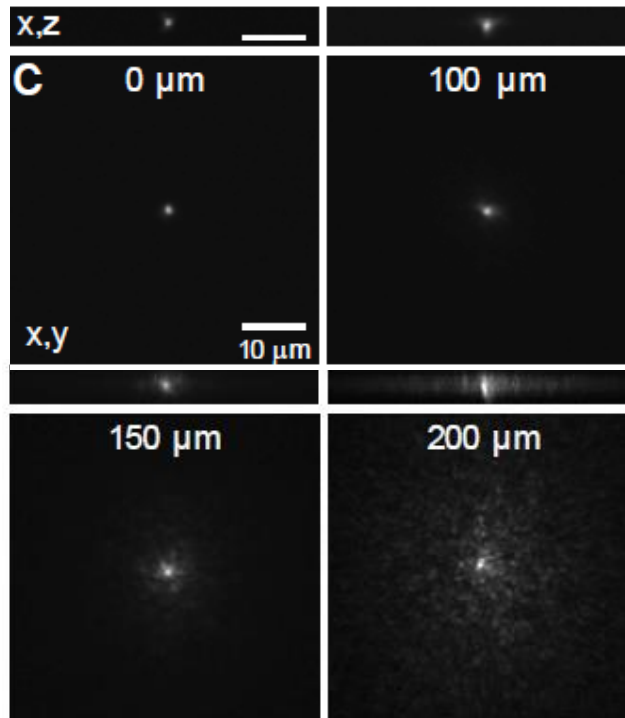
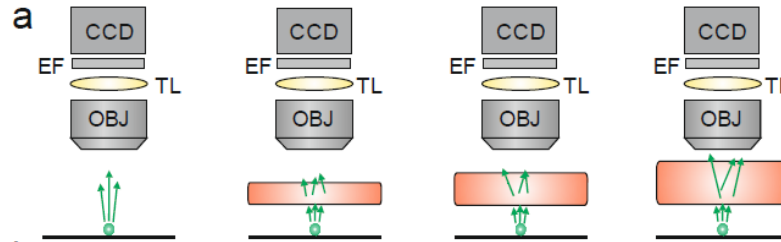
Spatial resolution

Excitation Point-spread-function

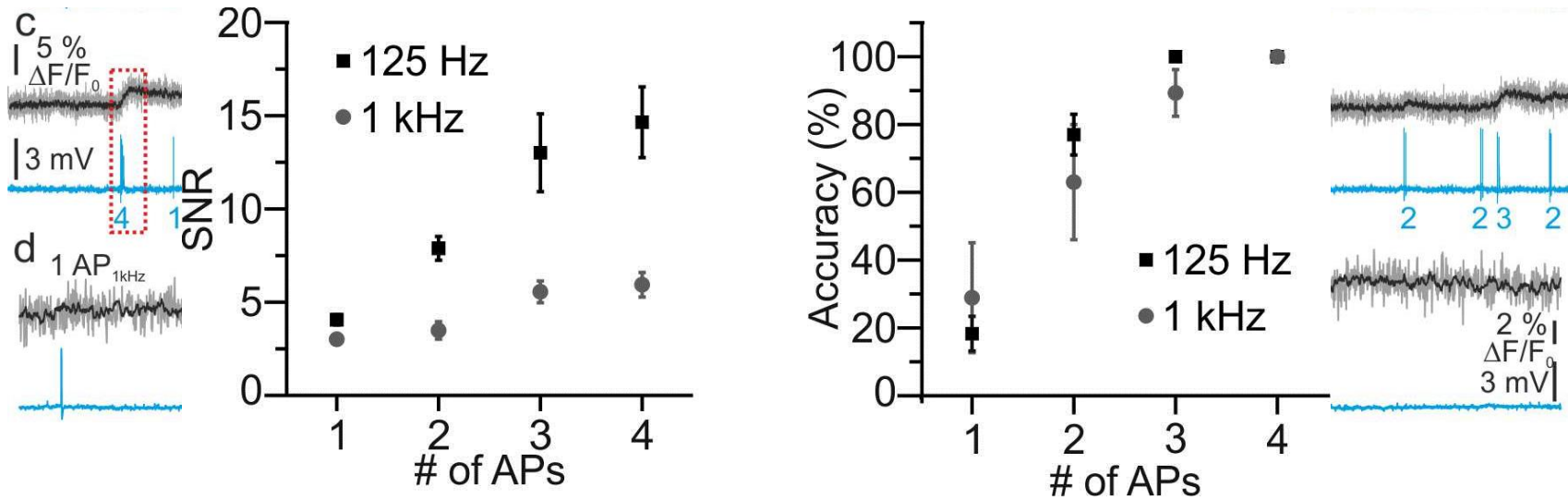
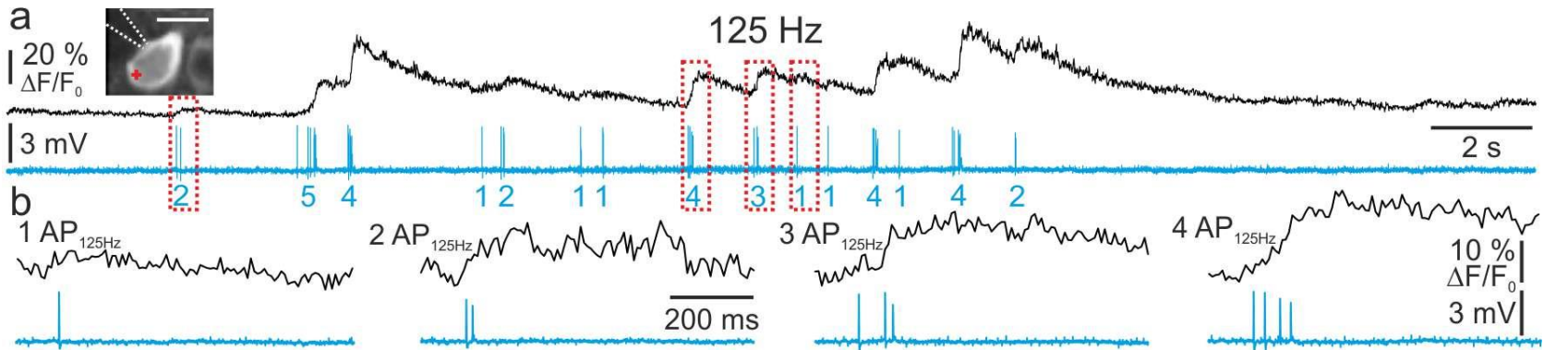


Spatial resolution

Effect of scattering on detection

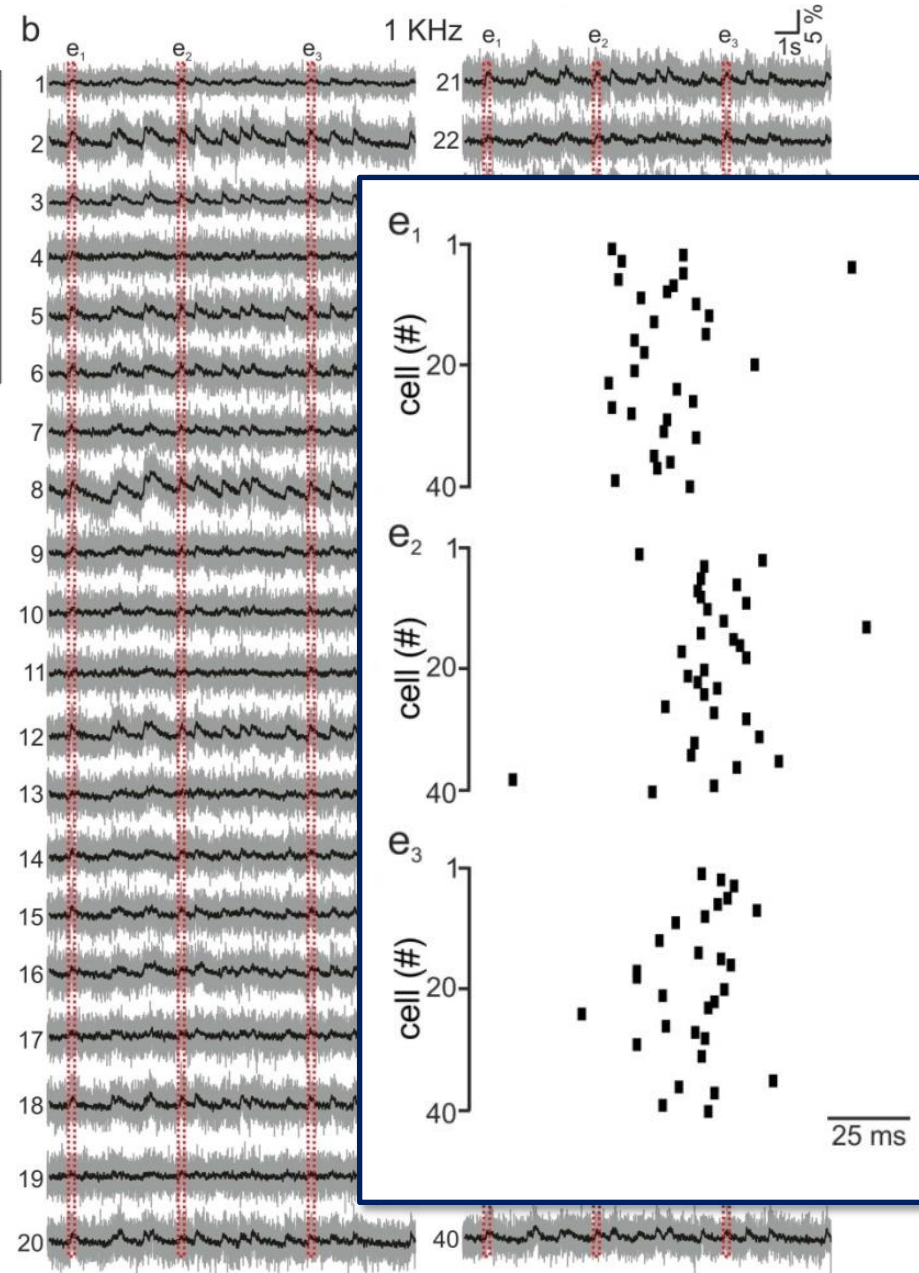
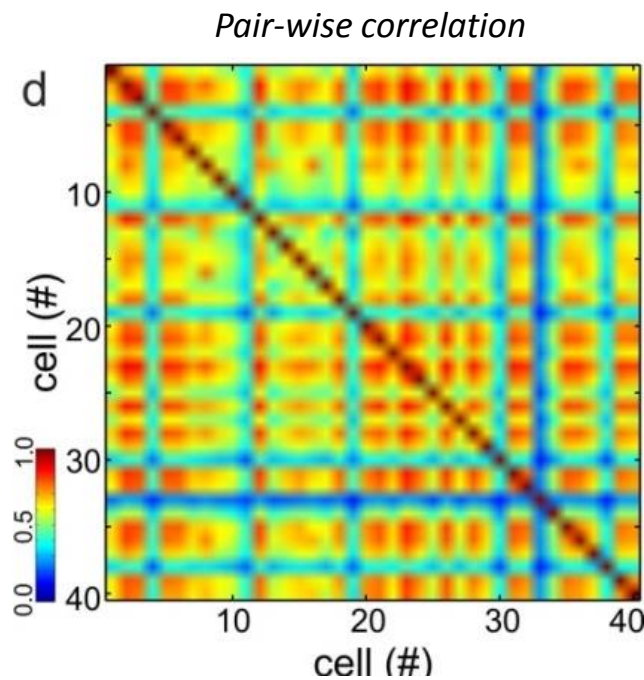
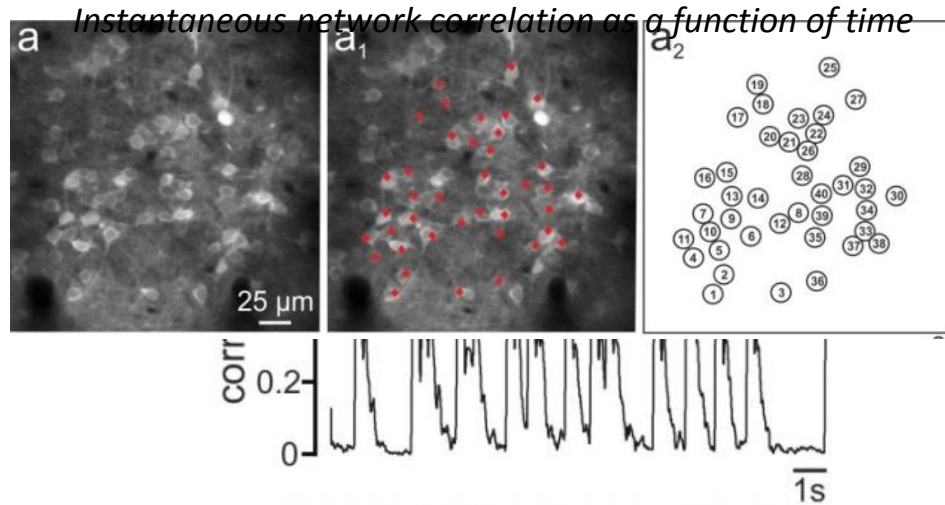


Temporal resolution and sensitivity



Estimate of first action potential: 8.4 ± 5.3 ms

Fast functional mapping of synchronous cortical dynamics *in vivo*



Acknowledgements



ISTITUTO ITALIANO
DI TECNOLOGIA



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