Emerging Approaches in biophysics and structural biology, Paris December 2013

Two photon optogenetics by wave front shaping of optical beams

Valentina Emiliani

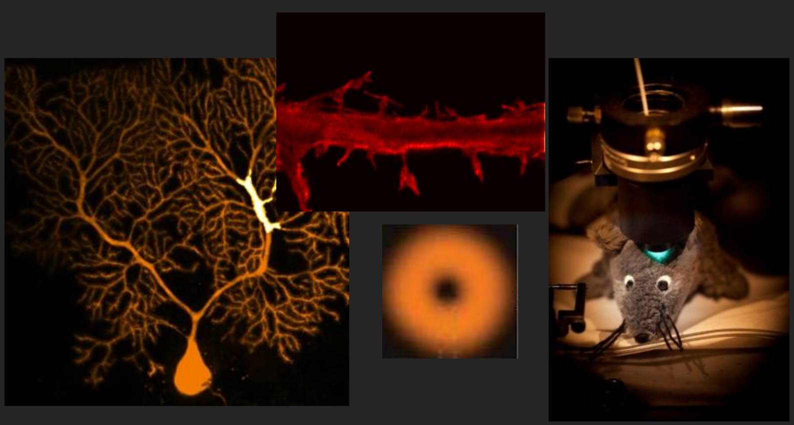
Wave-front engineering microscopy group

Neurophotonics Laboratory

http://www.biomedicale.parisdescartes.fr/neurophotonics/

Paris Descartes University,

Paris France



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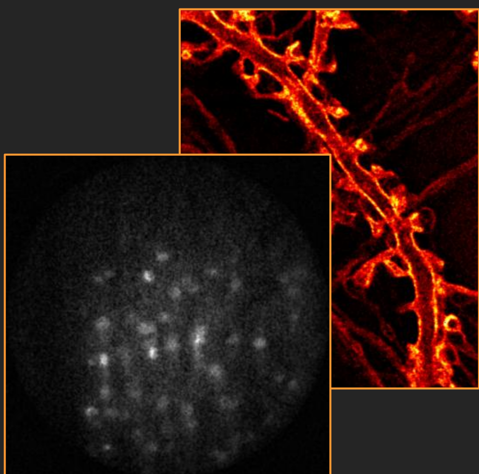
Development and use of advanced optical methods for Neurobiology

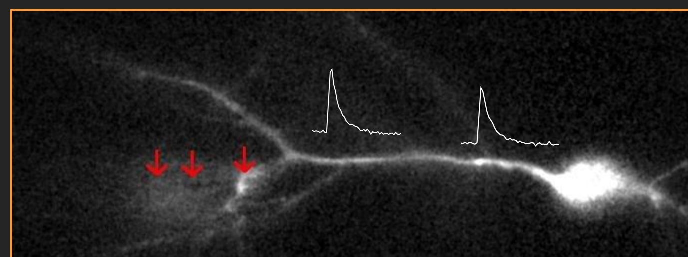
Imaging



- *STED*
- *Endoscopy*

Functional imaging

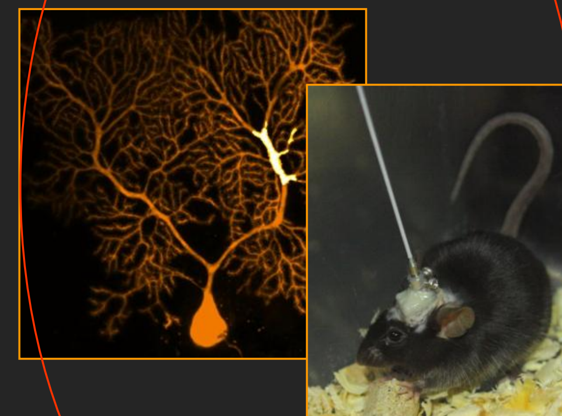
- Ca $^{2+}$ indicators
- Voltage sensitive dyes



- *Holographic imaging*
- *Remote focusing*

Optical stimulation

- Photolysis
- Optogenetics

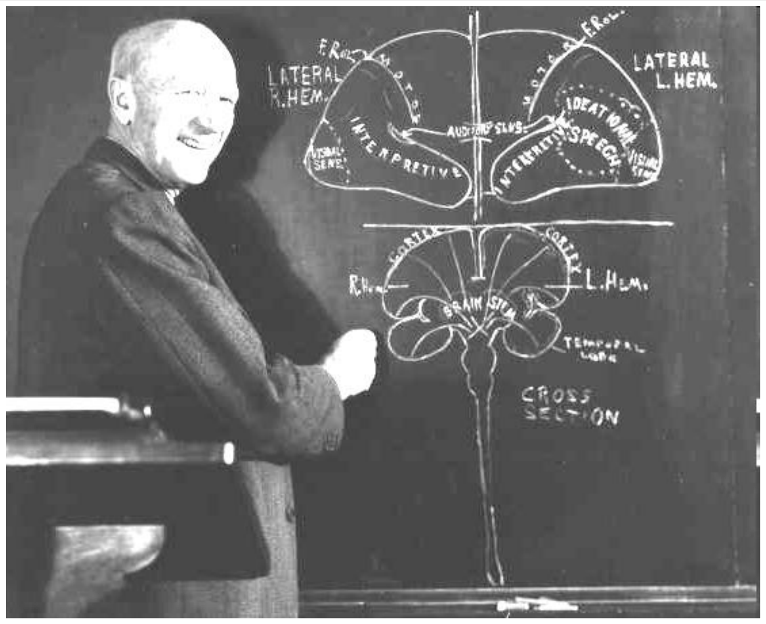


- *Holography*
- *Generalized phase contrast*
- *Temporal focusing*
- *Endoscopy*

Sub cellular (~30 nm)

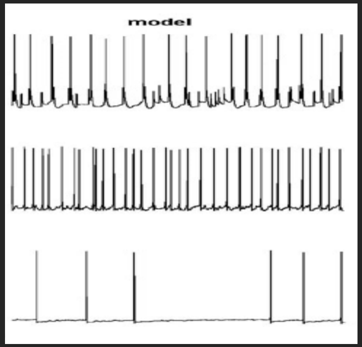
In vivo

INTRODUCTION:



Wilder Penfield

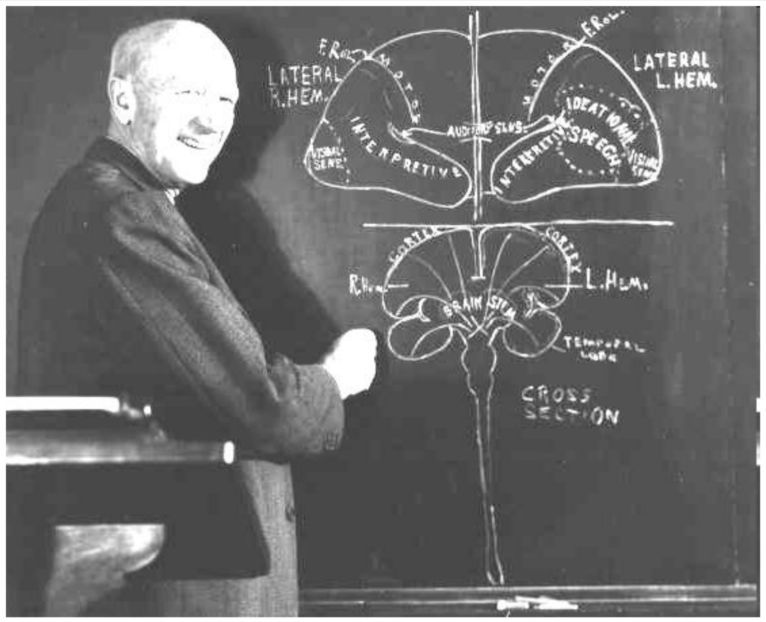
A fundamental task in neuroscience research is to establish a map of the neural connections within the brain



- Electrode stimulation

Some experimental challenges:
mechanical damages
limited spatial resolution
difficulty in inhibiting neurons

INTRODUCTION:



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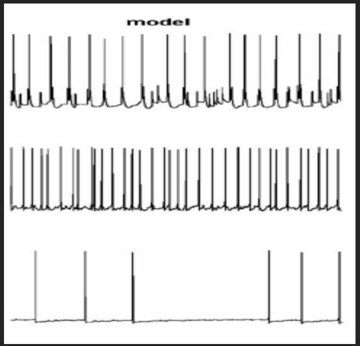


- <u>Electrode stimulation</u>

<u>Some experimental challenges:</u>

mechanical damages
limited spatial resolution
difficulty in inhibiting neurons

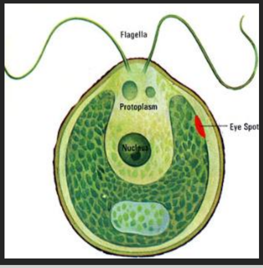
- <u>Light stimulation</u>

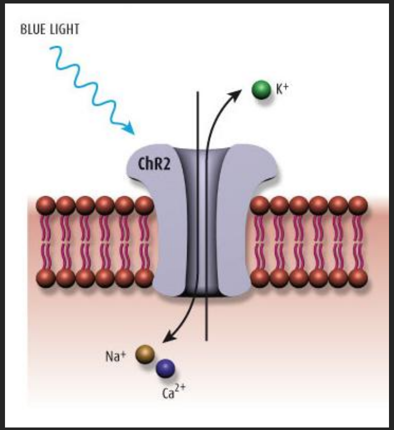
not invasive (reversible)
spatial and temporal resolution

Optogenetics: Light gated channels and pumps

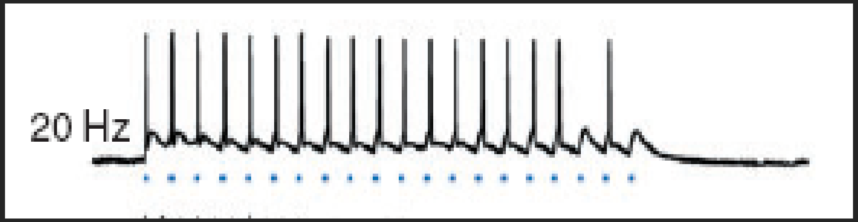
The demonstration of functional expression of Channelrhodopsin-2 in mammalian (Nagel et al. 2003) and neuronal cells (Boyden et al. 2005) marked the <u>beginning of optogenetics</u>

Channelrhodopsin ChR2: *excitation*



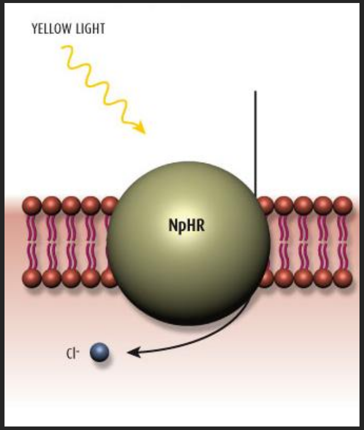


Chlamydomonas reinhardtii (algae)

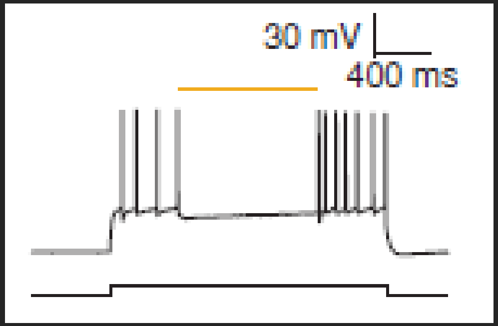


Zhang et al. (2006)

Halorhodopsin NpHR: *inhibition*



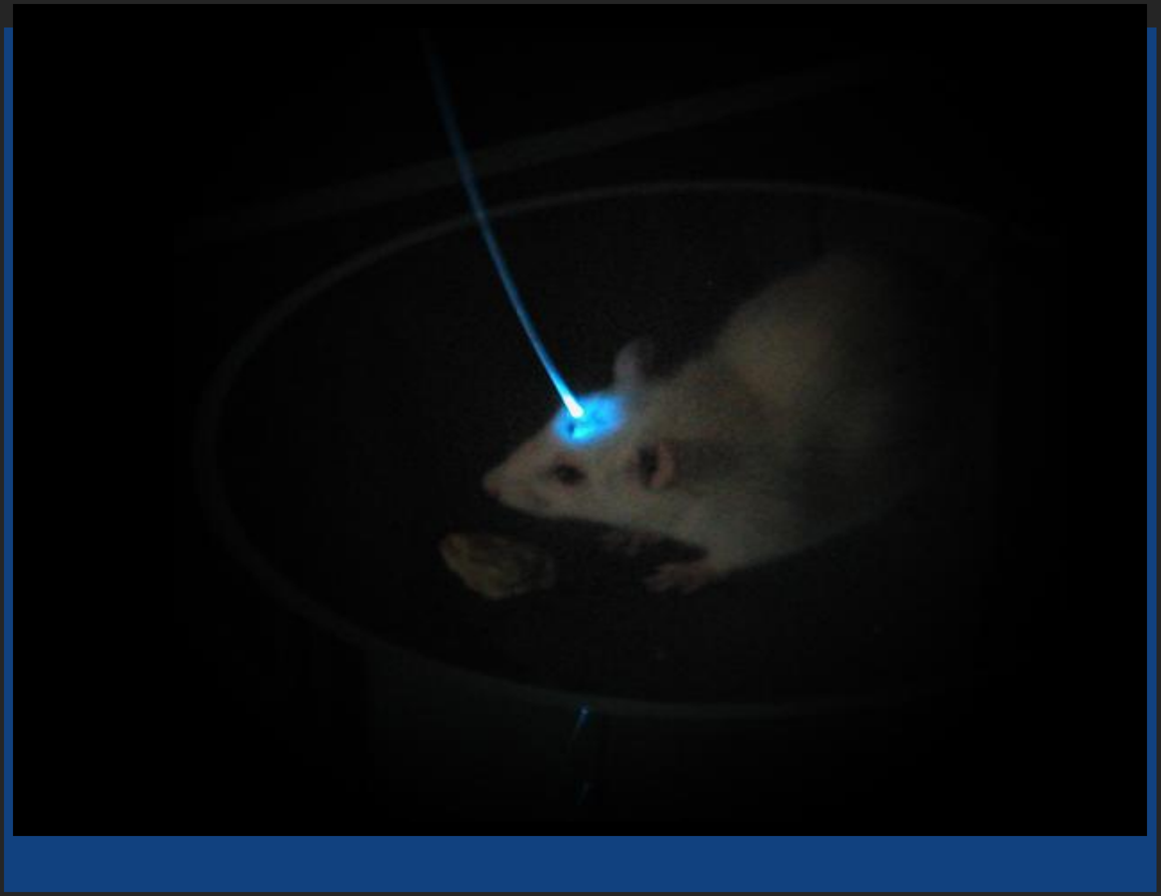
Natronomonas pharaonis (archaeobacteria)



Zhang et al. (2007)

Examples:

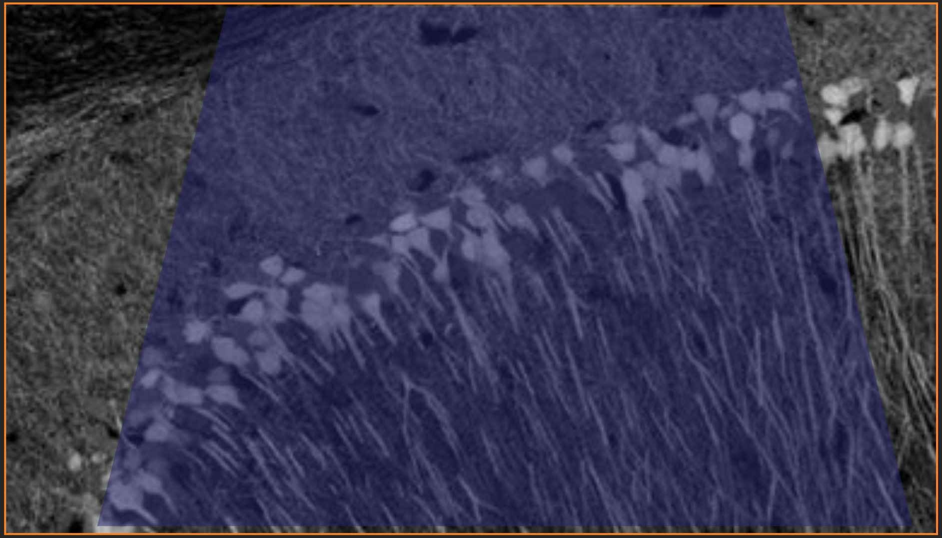
- Blue light stimulation of the right secondary motor cortex in transgenic mice expressing ChR2 (Thy1::ChR2-EYFP)



V. Gradinaru et al. J Neurosci. 2007

Optimal illumination method?

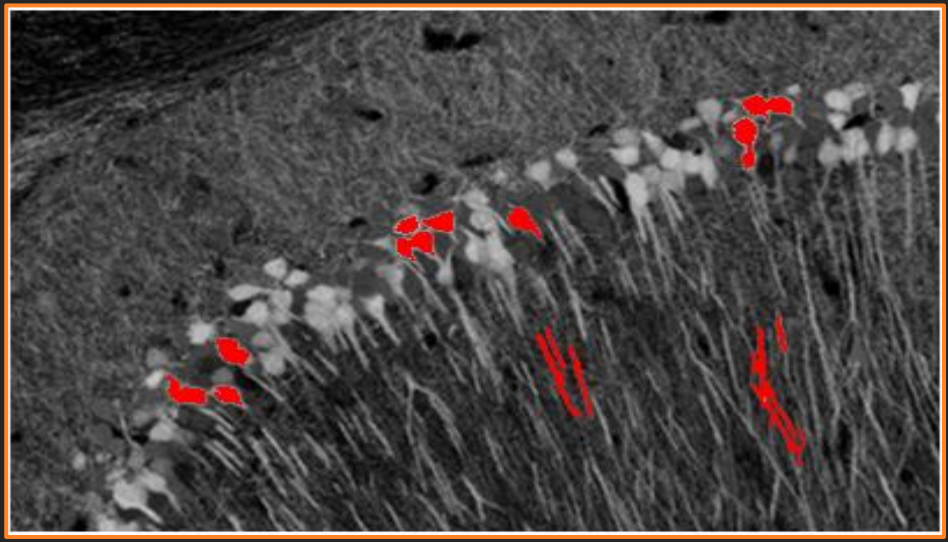
Key biological questions have been already addressed with simple illumination methods based on wide-field blue light illumination (lamps, optical fiber, LED)



Low excitation level (~1 mW/mm^2)
Genetic specificity

Optimal illumination method?

mimicking the c<u>omplex nature of brain circuits</u> *requires targeting subsets of genetically identical connected cells with single cell precision*



Optimal illumination method?

Challenges:

- *Flexibility: cover different excitation configuration such as a single cell process, a whole cell body or multiple cells*
- *Temporal resolution: sub-millisecond-millisecond time scale*
- *Axial resolution: select a single cell layer (~10μm) or a single cell process (1 - 3μm)*
- *Penetration depth: compensate scattering by disordered media (~1/λ^4)*

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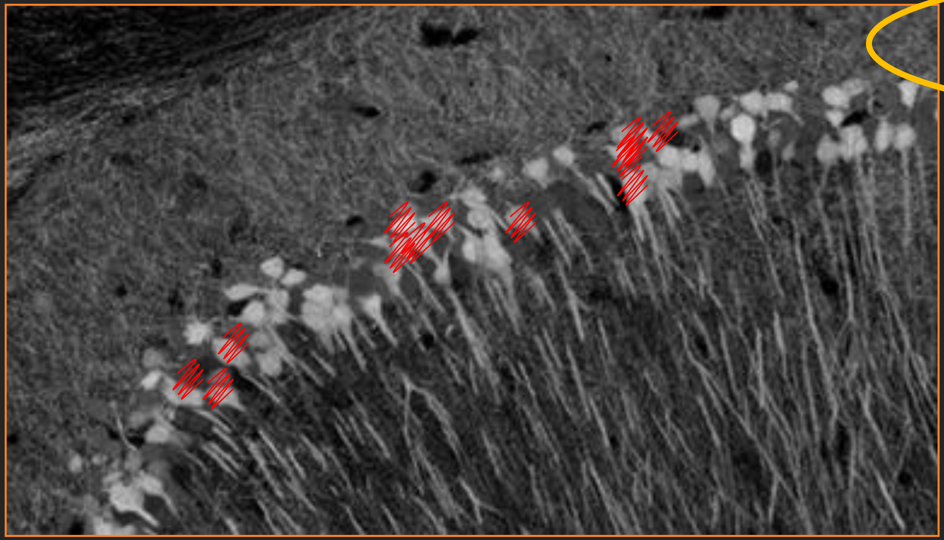
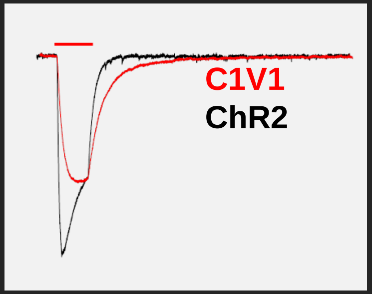
Two photon excitation (longer wavelength, confined excitation volume)

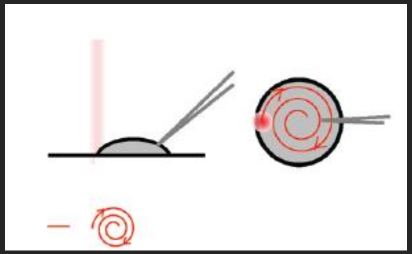
Optimal illumination method? Laser Scanning

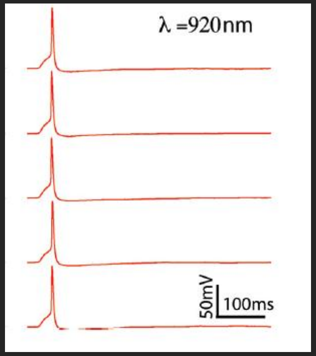


- Main limitation: temporal resolution (5-70ms)
- Low conductivity (~80 fS)
  Feldbauer et al PNAS, 2009
- Low density of ChR2 channels (~130 molecules/µm²)
  Nagel et al, FEBS Lett.,1995

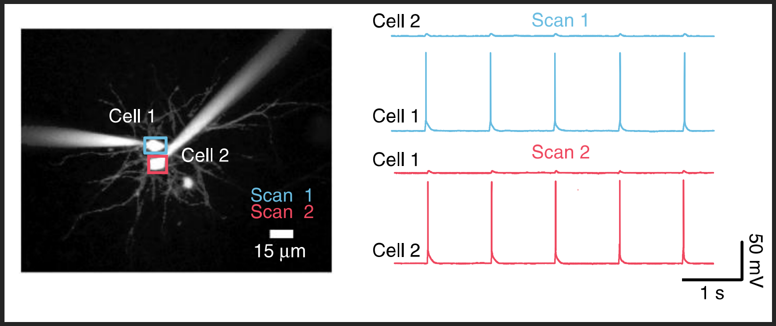
C1V1 (red variants of ChR2)







(Rickgauer and Tank 2009)



(Prakash et al. 2013)

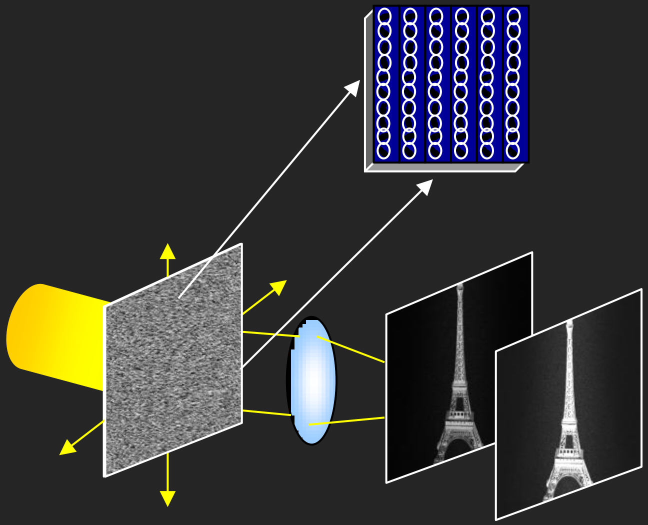
(Packer et al. 2013)

Flexibility: two approaches

Is it possible to control light propagation to excite simultaneously and selectively the targeted cells/cellular processes?

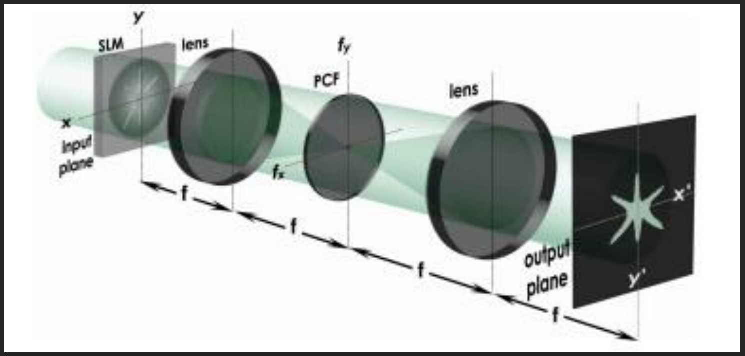
Computer generated holography

Generalized phase contrast



Reicherter , Opt Lett (1999)

Gerchberg and Saxton algorithm , Optik (1972)



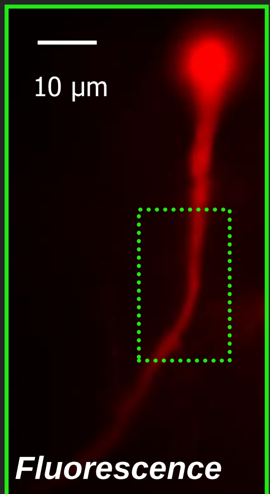
J. Glückstad, Optic. Comm (1996)

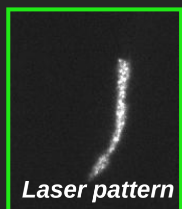
SLM plane = Fourier plane

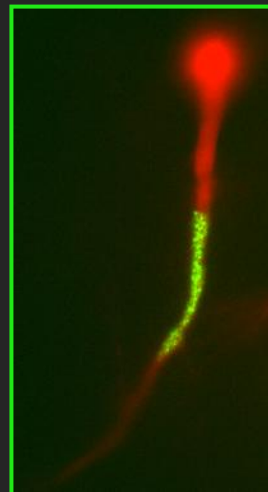
SLM plane = conjugate plane

Examples

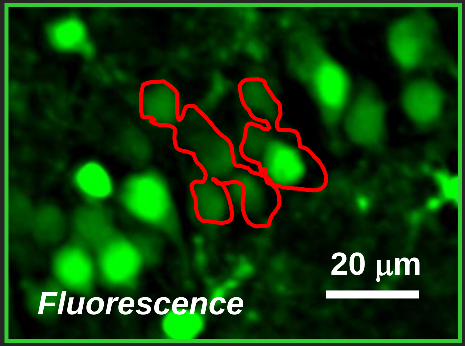
1P light patterning by CGH

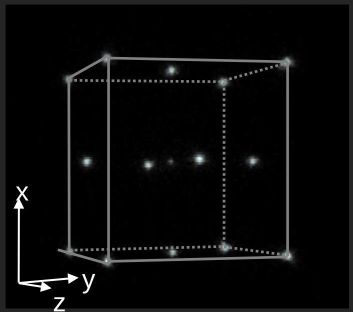


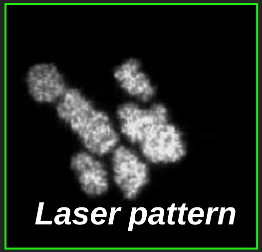




C. Lutz et al. Nature Methods (2008)



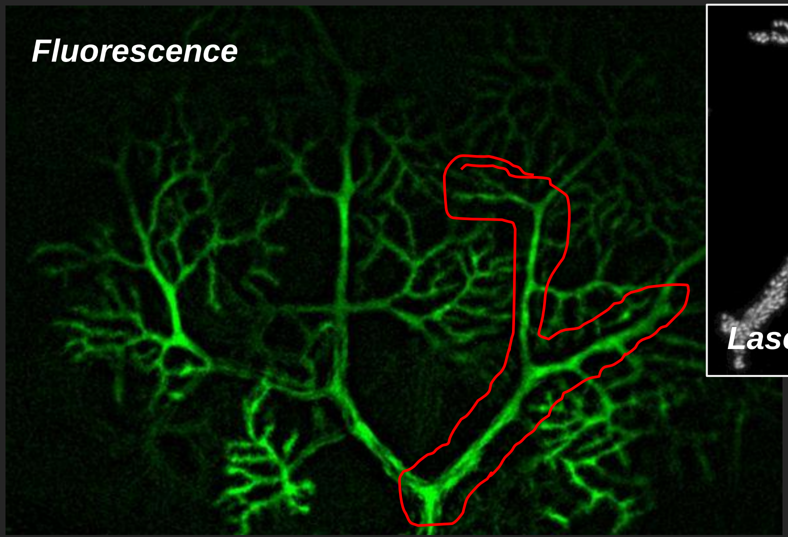


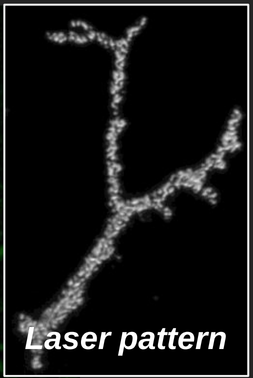


M. Zahid et al. PlosOne (2010)

S. Yang, et al., J Neuronal Engineering, (2011)

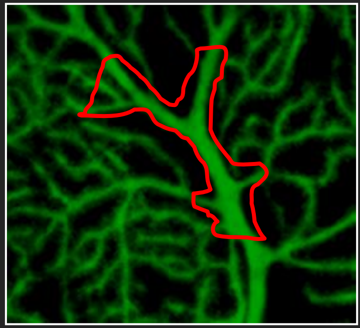
2P light patterning by CGH





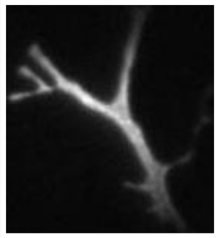
2P light patterning by GPC

Fluorescence



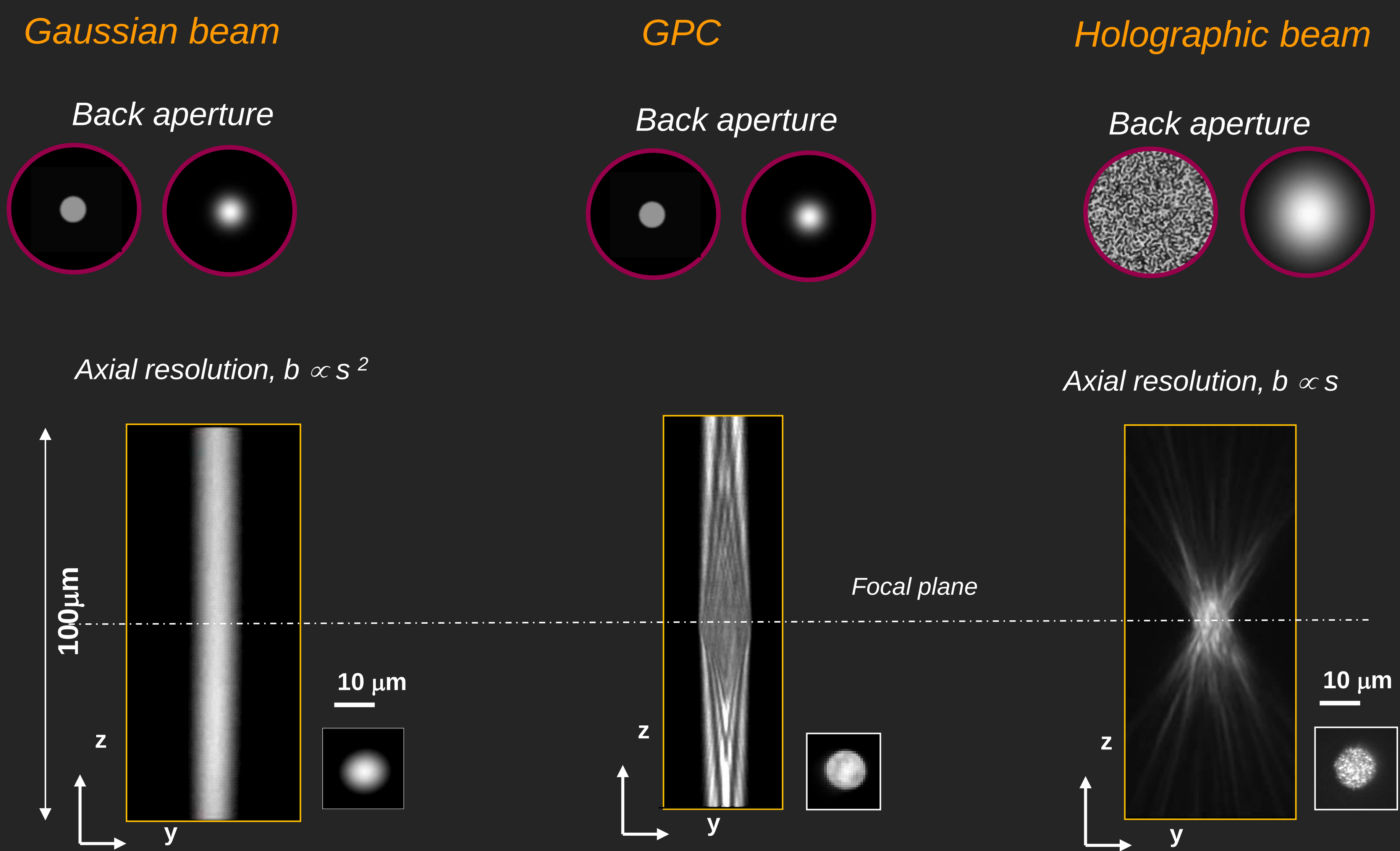
Phase mask

Laser pattern



E. Papagiakoumou, et al., Nature Methods (2010)

Axial propagation?

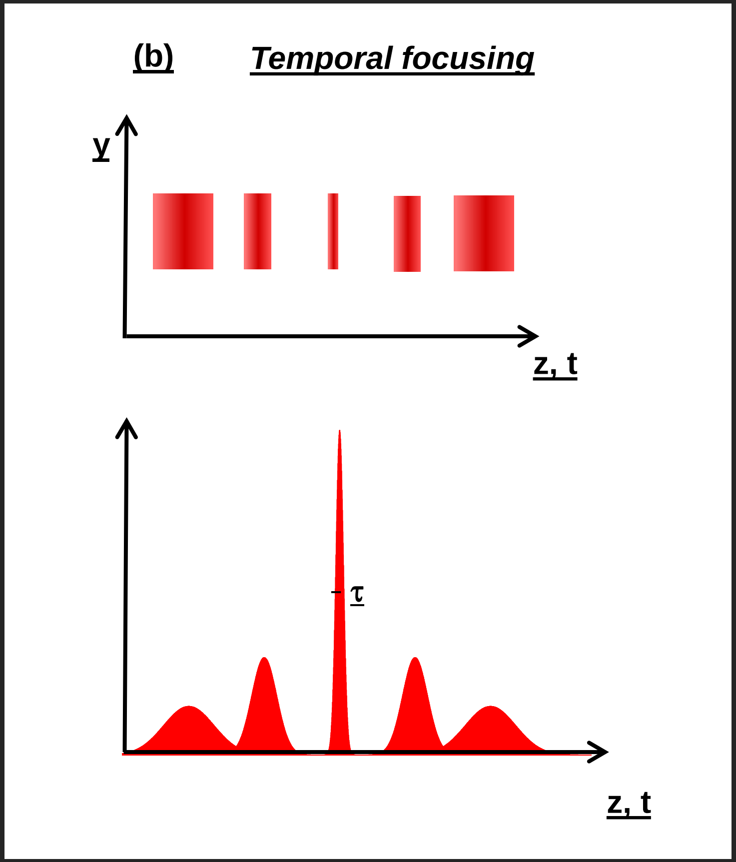


Temporal focusing

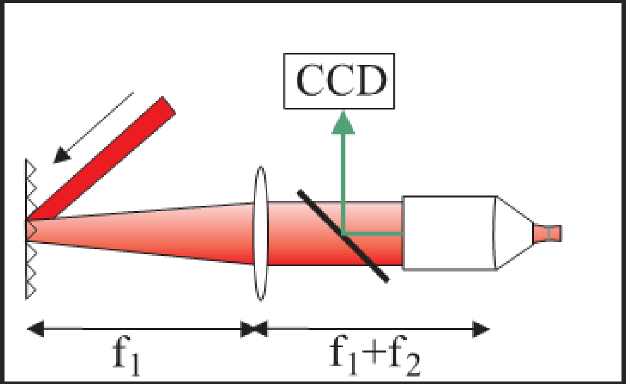
Originally proposed for wide field two-photon microscopy

(D. Oron, et al, Optics Express 2005)

$$S_{2PE} \propto \frac{I^2}{\tau \cdot f}$$



Vaziri&Emiliani, *Curr. Opinion in Neurobiology* (2012)



Different color of the laser pulse are diffracted through different angles: this introduces a difference in the optical paths with consequent pulse broadening

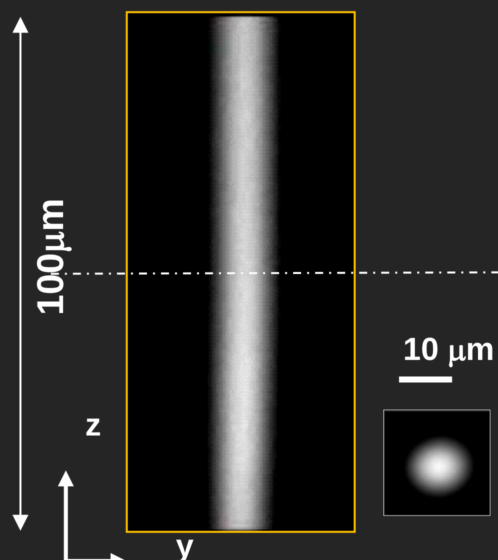
$$I_{line} \approx \left[1 + \left(\frac{z}{z_R}\right)^2\right]^{-0.5}$$

$$\Delta Z = \sqrt{3} z_R$$

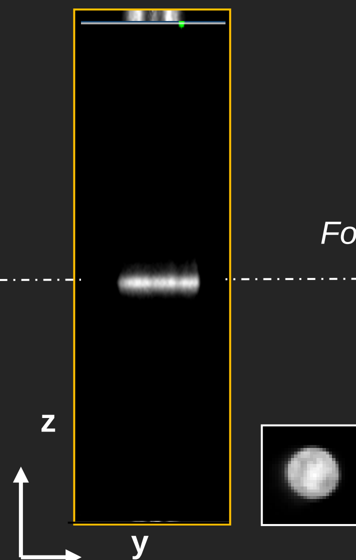
Axial propagation?

Gaussian beam

Axial resolution, $b \propto s^2$



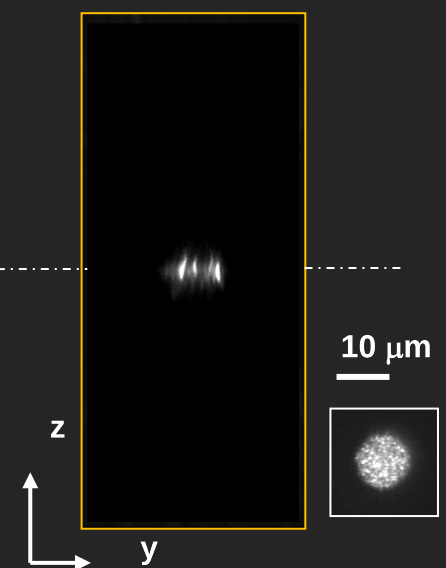
GPC



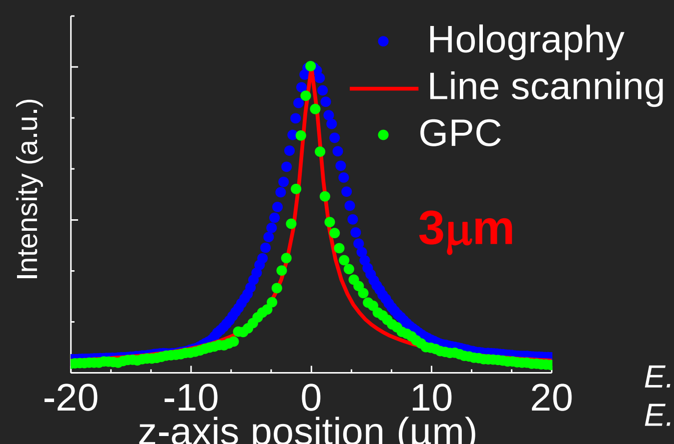
Holographic beam

Axial resolution, $b \propto s$

Focal plane



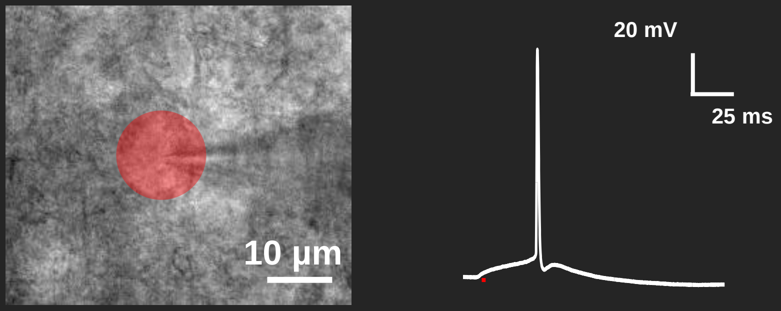
Grating=830 l/mm
Obj =60x NA 0.9
f=500mm

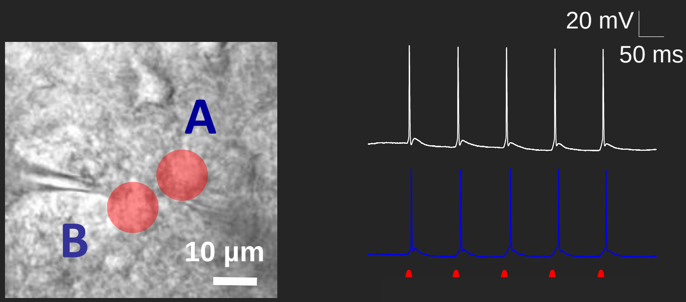


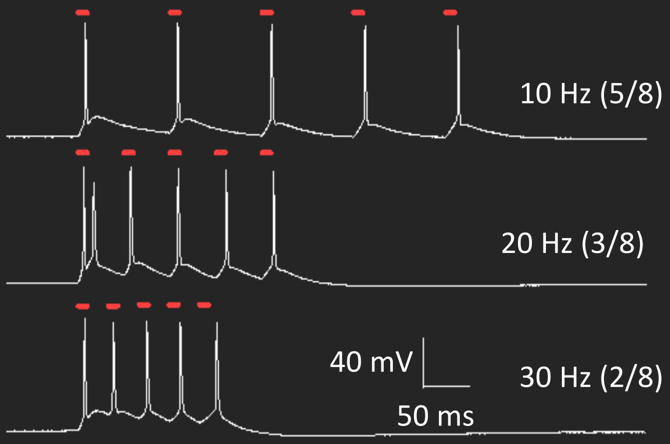
E. Papagiakoumou et al. Nature Methods (2010)
E. Papagiakoumou et al. Optics Express (2008)

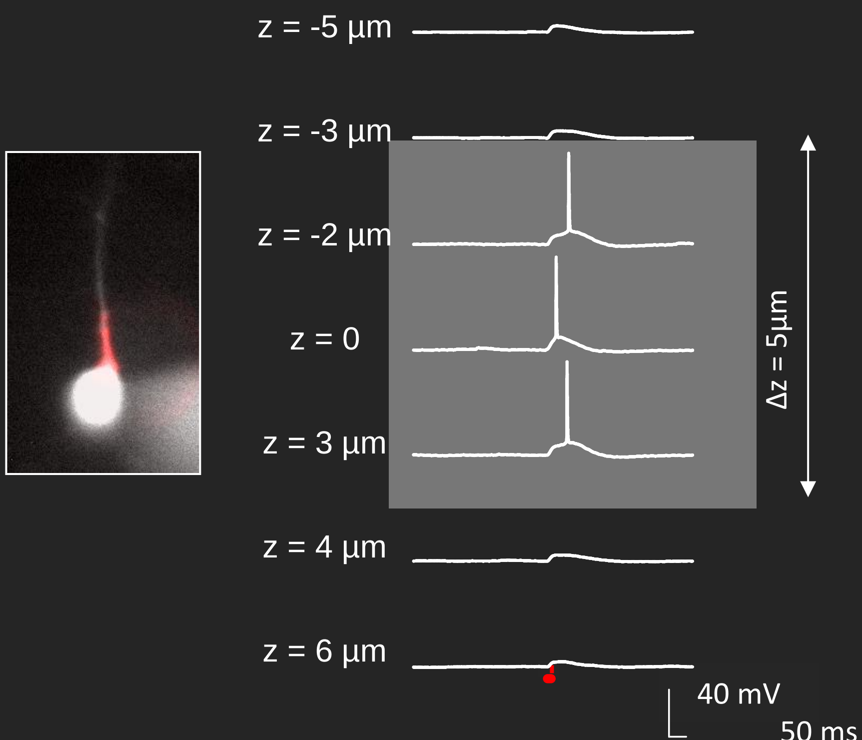
Examples: 2P activation of ChR2 in brain slices (GPC and TF)

Thy1-ChR2-YFP transgenic mice Excitation =0.3-0.5 mW /µm^2; depth 60-70µm , 1-10ms









E. Papagiakoumou et al., Nature Methods (2010)

Is it patterned photoactivation:
compatible with in vivo applications?

- Two photon microscopy head restrained mice:
Propagation in depth?

- Optical fiber: transmission through the fiber

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compatible with in vivo applications?

- Two photon microscopy head restrained mice:
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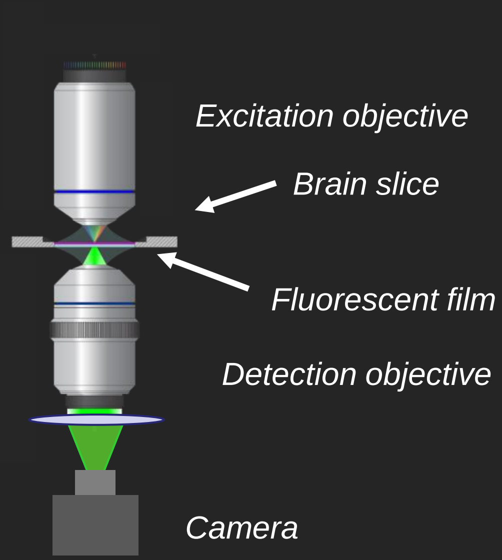
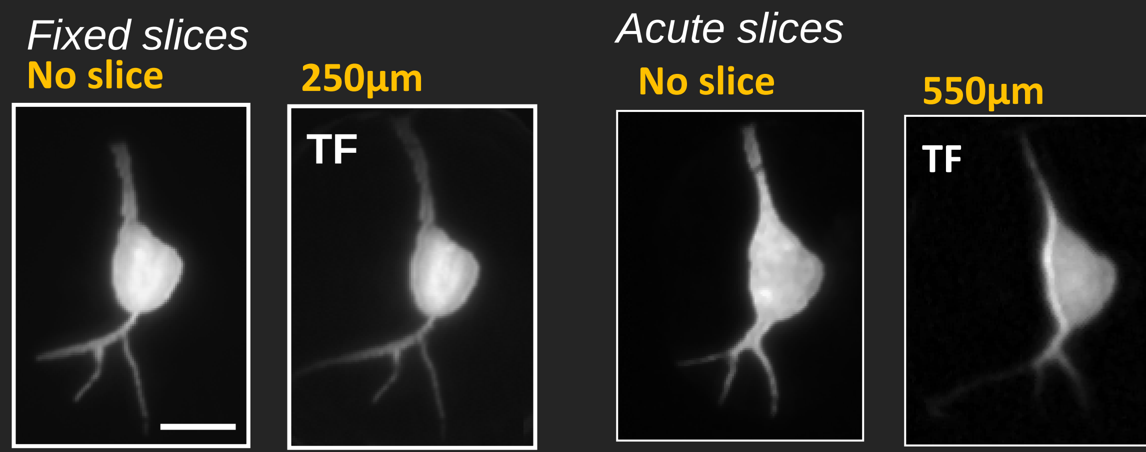
Propagation of shaped beams deep inside scattering tissue

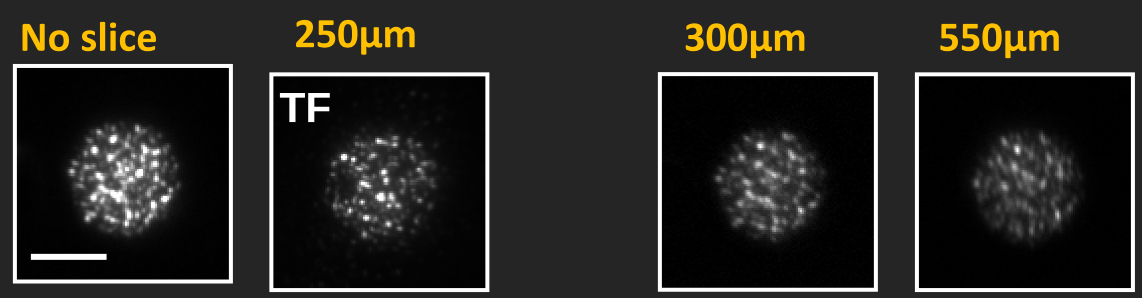


Generalized Phase contrast



E. Papagiakoumou et al ., Nature Photonics (2013)

Computer generated holography



A. Begue et al ., Biomedical Optics Express (2013)

Is it patterned photoactivation:
compatible with in vivo applications?

- *Two photon microscopy head restrained mice:*
  Propagation in depth?

- *Optical fiber: transmission through the fiber*

CONCLUSIONS:

- *3D shaping of excitation volume permits efficient 2P optogenetics*
- *Temporal focusing: allows controlling axial resolution*
  AND
  maintaining shaped patterns in scattering media paving the way for in vivo 2P optogenetics
- *Patterned fiberscope permits patterned photoactivation in freely behaving animals with single cell precision*
- *First commercial prototype (*Intelligent Imaging Innovations, Inc)

Wave-front engineering microscopy group

- Benoit Forget
- Marc Guillon
- Eirini Papagiakoumou

- Vincent De Sars
- Cristhophe Tourain
- Rossella Conti
- Valeria Zampini

- Marcel Lauterbach
- Emiliano Ronzitti
- Emmanuelle Chaigneau
- Amanda Joy Foust
- Dimitri Tanese

- Vivien Szabo
- Oscar Hernandez
- Osnath Assayag

Collaborations

D. Oron,
(Weizmann Institut)

J. Glueckstadt
(DTU Fotonik)

E. Isacoff
(Berkeley Univ.)

K. Deisseroth
(Stanford Univ.)

J Bradeley, B. Stell
(Paris Descartes)

Former members

- Cathie Ventalon (now @ ENS, Paris)
- Leona Enke (MPI, Frenkfurt)
- Aurélien Begue (post doc @Harvard Medical School)
- Francesca Anselmi (post doc @CSHL)
- Christoph Lutz
- Morad Zahid

FRM Equipe