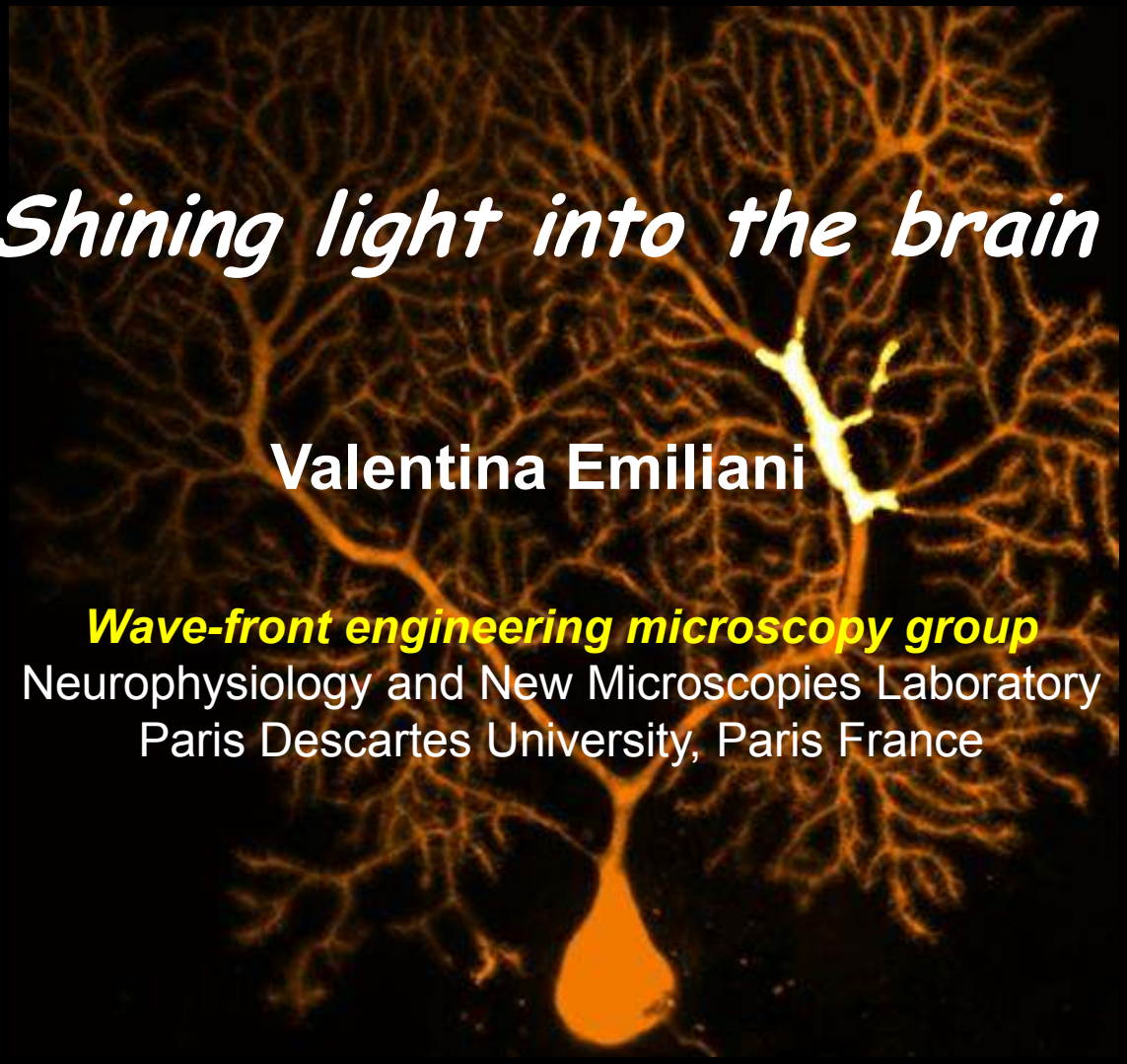
Shining light into the brain



Valentina Emiliani

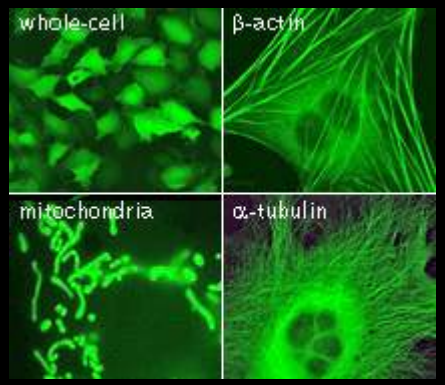
Wave-front engineering microscopy group
Neurophysiology and New Microscopies Laboratory
Paris Descartes University, Paris France

Optics and biology: imaging and functional imaging

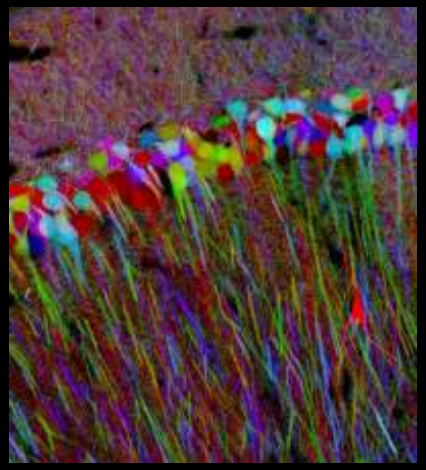
Imaging

1995 - Green fluorescent protein **(Nobel price in 2008): a revolution in the imaging of living cells**





http://www.biocat.com

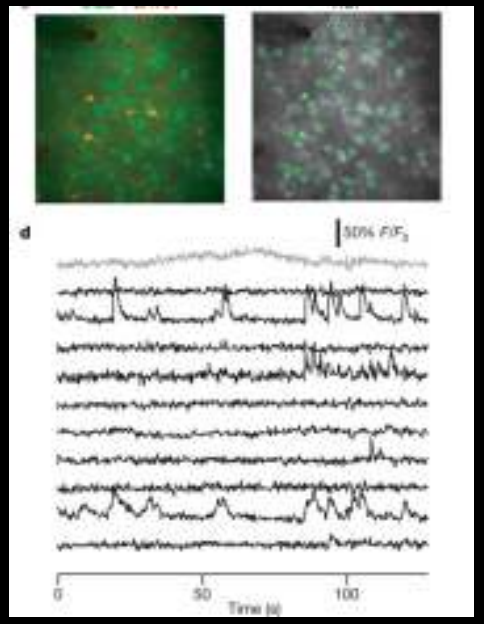


J. Livet et al. *Nature* (2007)

Functional imaging

A physiological signal is converted into a fluorescent response

- **Ca2+ indicators,**
- **Voltage sensitive dyes**

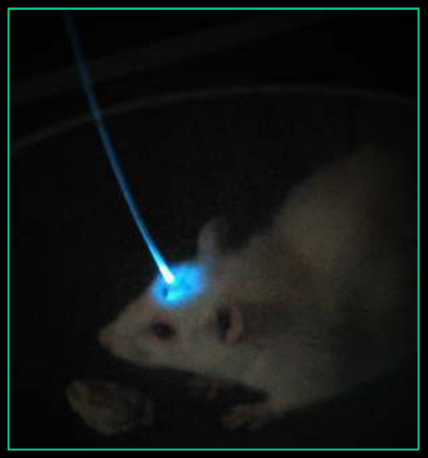


T. Komiyama et al, *Nature* (2010)

Optics and biology: optogenetics

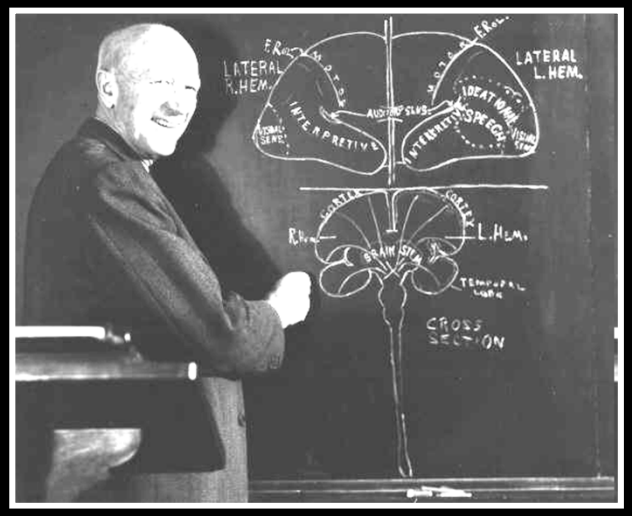
Optogenetics

2003 - Light gated channels and pumps
a revolution in neuroscience: light as an active tools for the stimulation of the brain

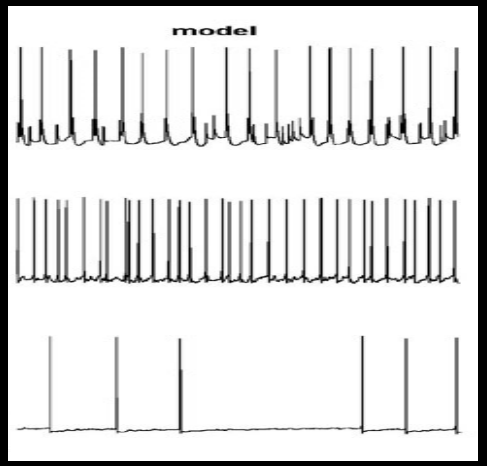


V. Gradinaru et al. *J Neurosci*. (2007)

Optogenetics: introduction



A fundamental task in neuroscience research is to establish a map of the neural connections within the brain, the 'connectome".



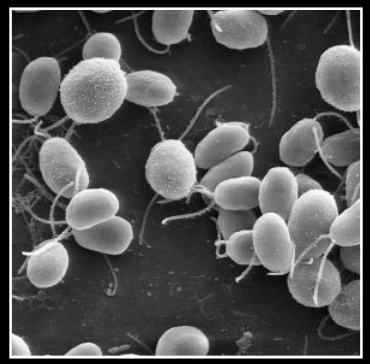
- Electrode stimulation

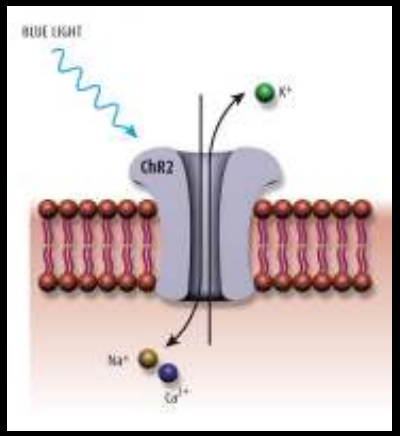
Some experimental challenges:
mechanical damages
limited spatial resolution
difficulty in inhibiting neurons

A promising alternative: Optical stimulation

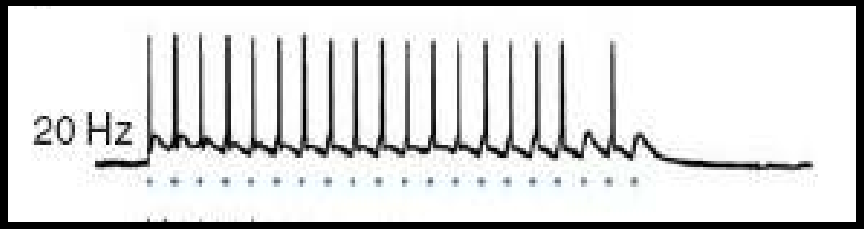
Optogenetics: *Light gated channels and pumps*

Channelrhodopsin ChR2: *excitation*



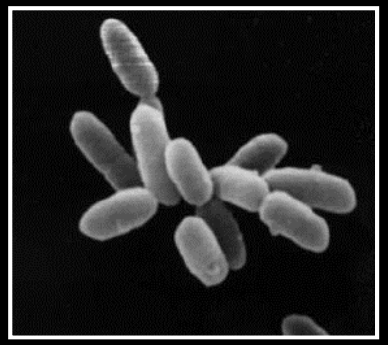


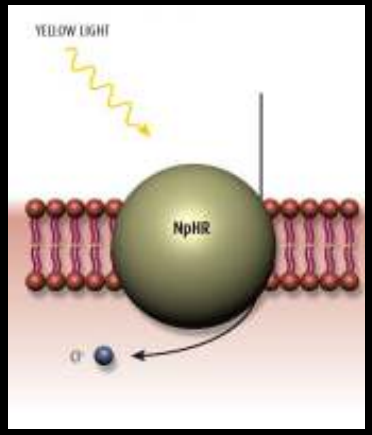
Chlamydomonas reinhardtii (algae)



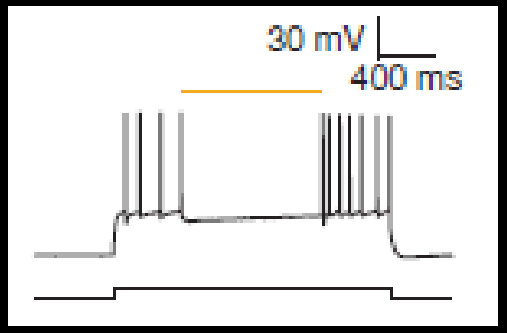
Zhang et al. (2006)

Halorhodopsin NpHR: *inhibition*





Natronomonas pharaonis (archaeobacteria)

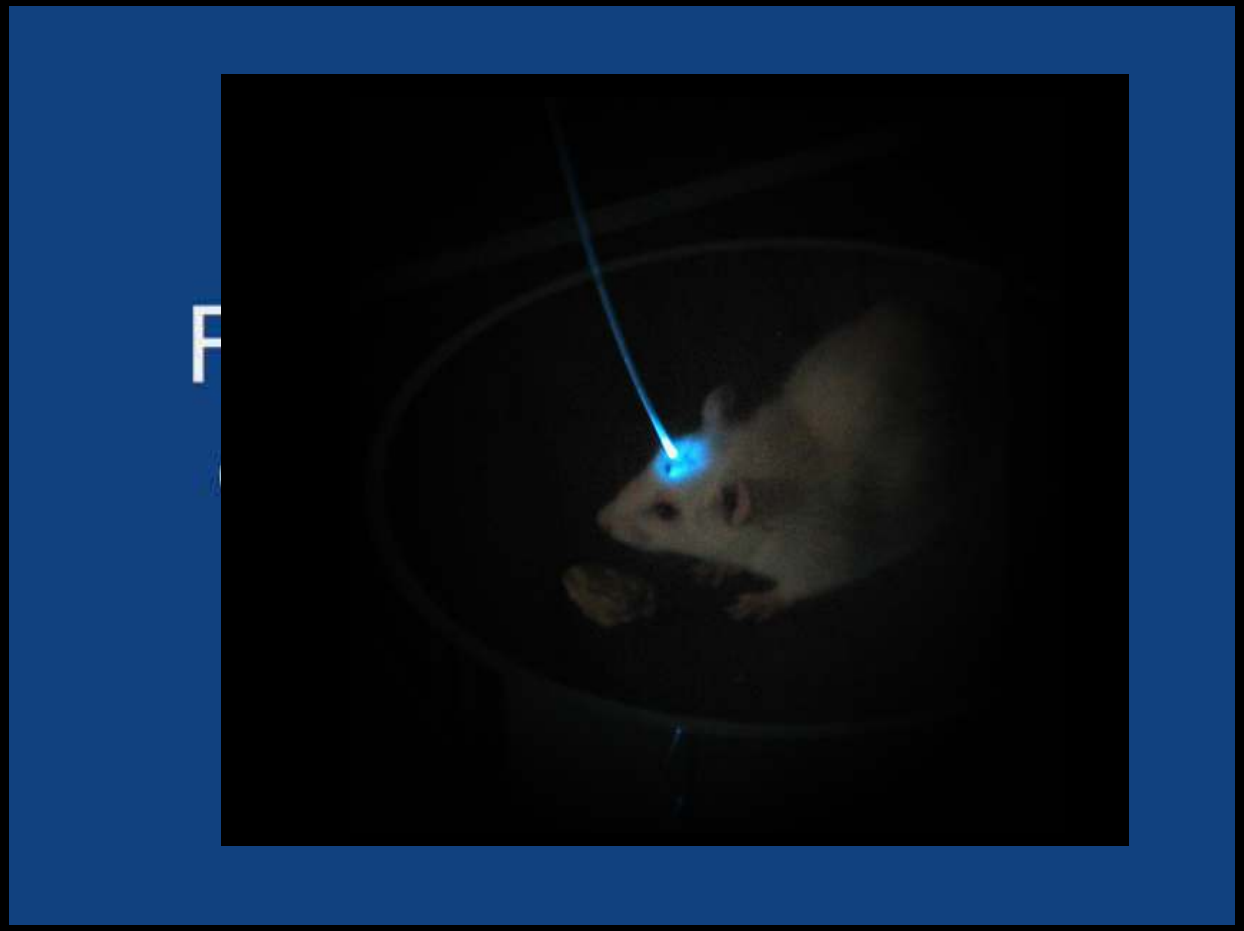


Zhang et al. (2007)

Valentina Emiliani
L'optique à Paris

Examples: Excitation

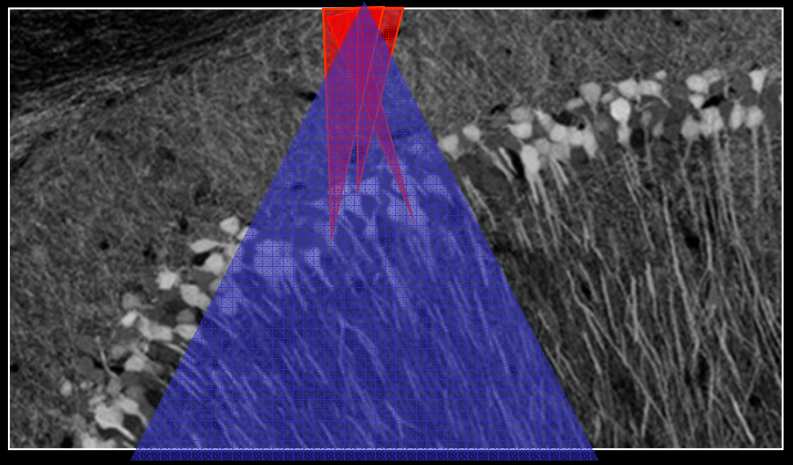
- Blue light stimulation of the right secondary motor cortex in transgenic mice expressing ChR2 (Thy1::ChR2-EYFP)



V. Gradinaru et al. J Neurosci. 2007

Optimum illumination method?

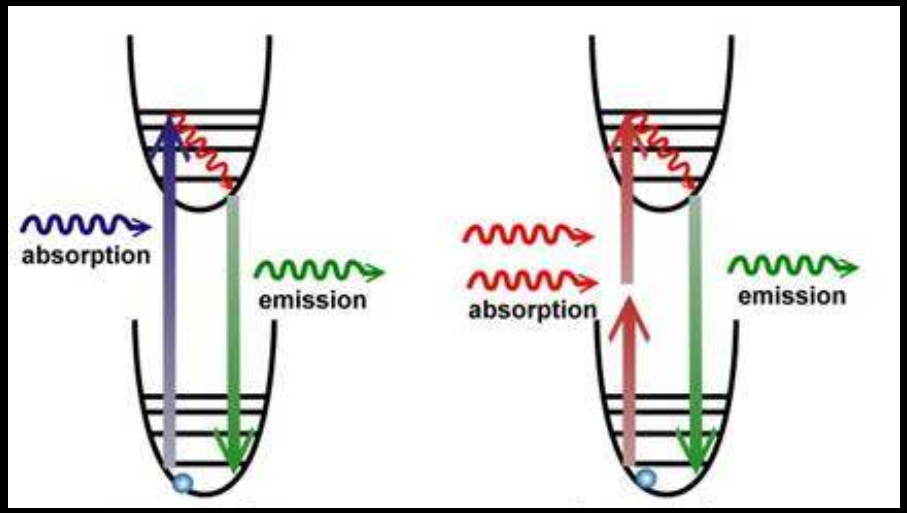
[..the major challenge facing neuroscience is the need to control one type of cell in the brain while leaving others unaltered]
Francis Crick Nobel price in medicine 1962



Challenges:

- **Precise control of the excitation volume in a scattering medium**

Two-photon excitation



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



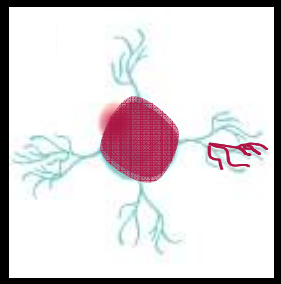
Single-photon ——————————————————→

Two-photon ——————→



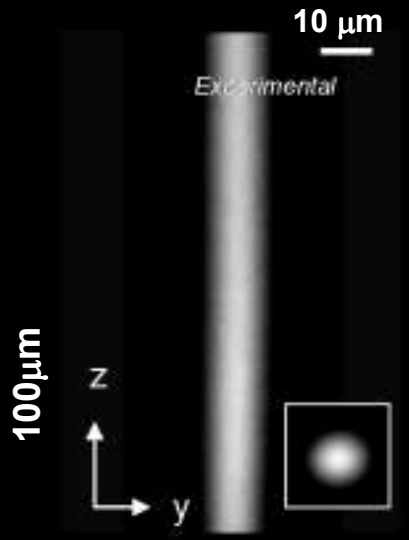
Optimum illumination method?

Two-photon optogenetics?



- **Low conductivity**
  (Feldbauer et al PNAS, 2009)
- **Low density of channels**
  (Nagel et al, FEBS Lett.,1995)

Two-photon excitation in one spot can not activate enough channels to evoke an Action Potential

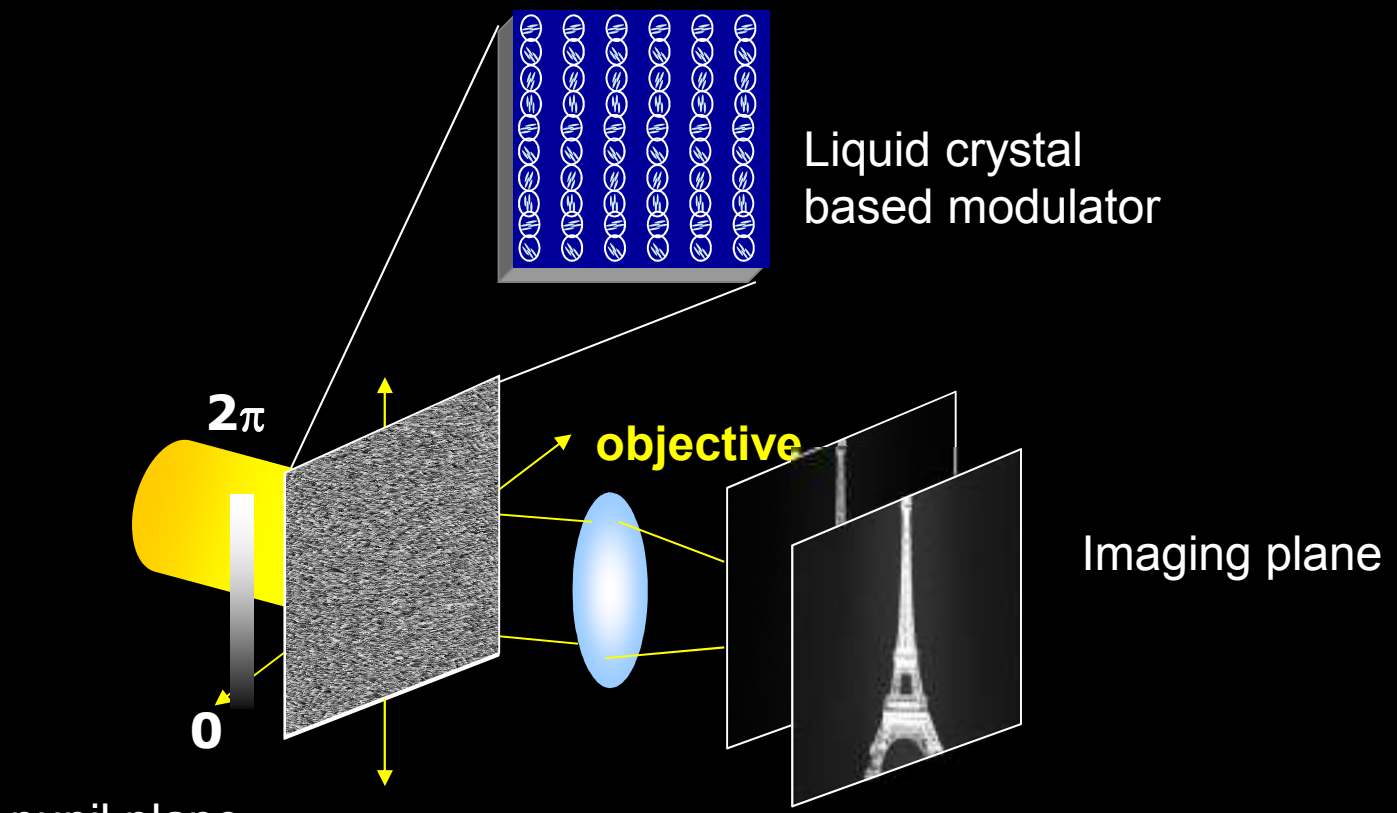


axial resolution $\propto s^2$

We need an optical technique which permits:

- **Large excitation area axially confined**
- **Flexibility**
- **Temporal resolution (ms)**

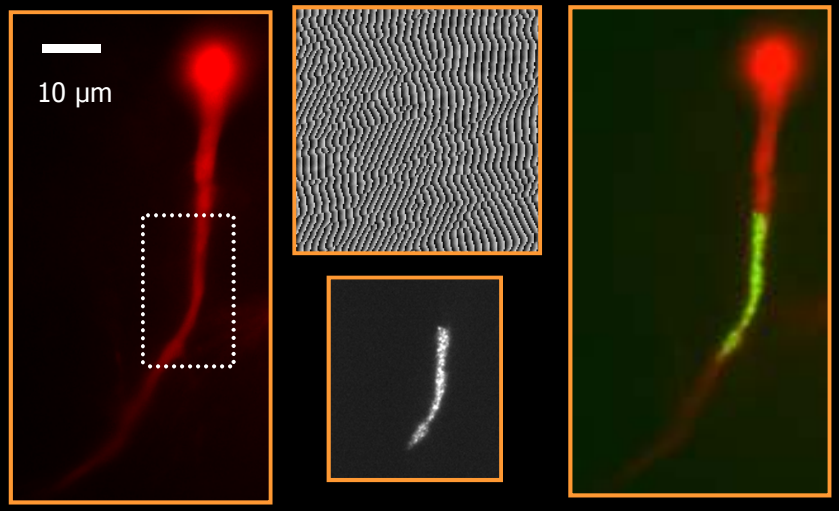
Flexibility: *Digital holography*



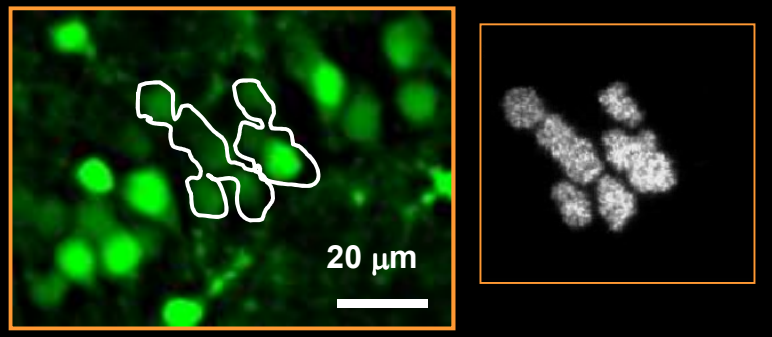
Gerchberg and Saxton algorithm [Optik (1972)]

Digital holography: Results

Single-photon

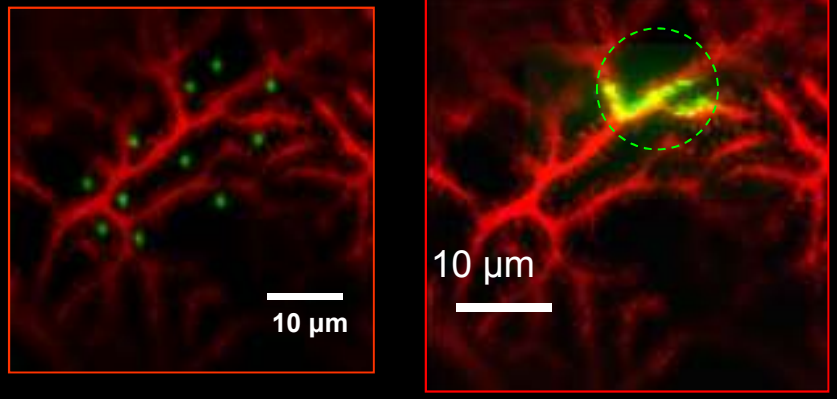


C. Lutz, et al., Nature Methods (2008)



M. Zhaid, et al., PlosONE (2010)

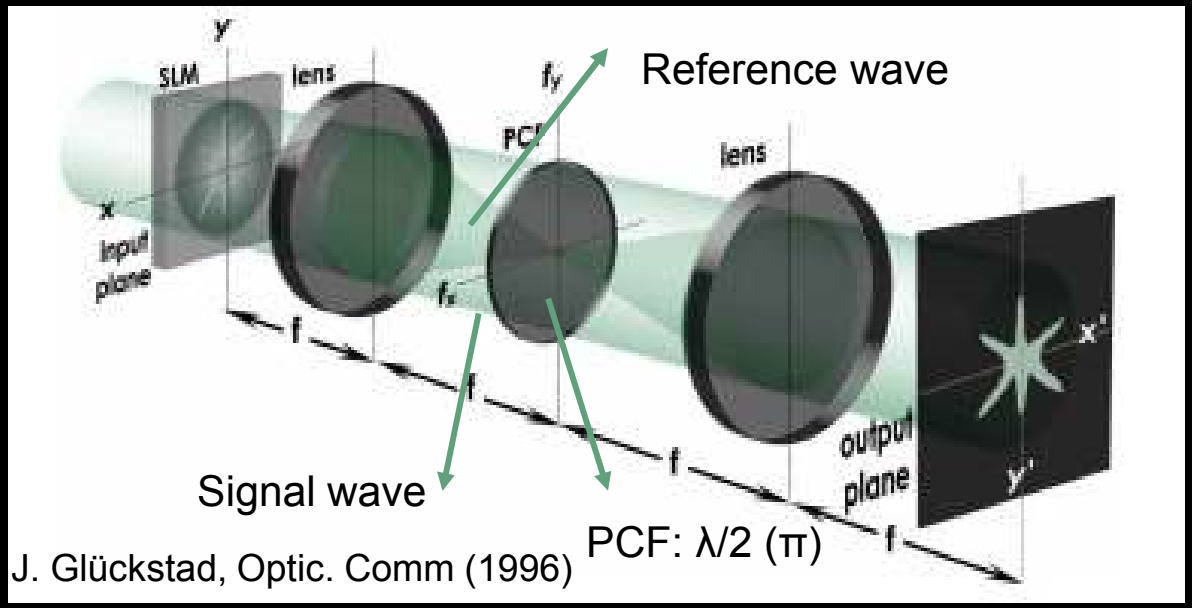
Two-photon



E. Papagiakoumou, et al., Optics Express (2008), E. Papagiakoumou, et al., Optics Express (2009)

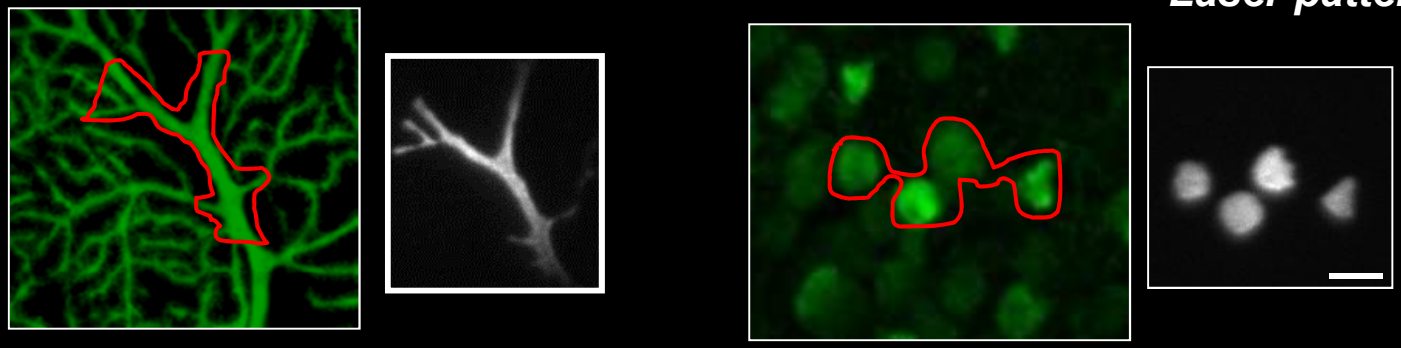
Flexibility: *Generalized phase contrast method*

Extension of phase contrast method (Frederik Zernike 1930)



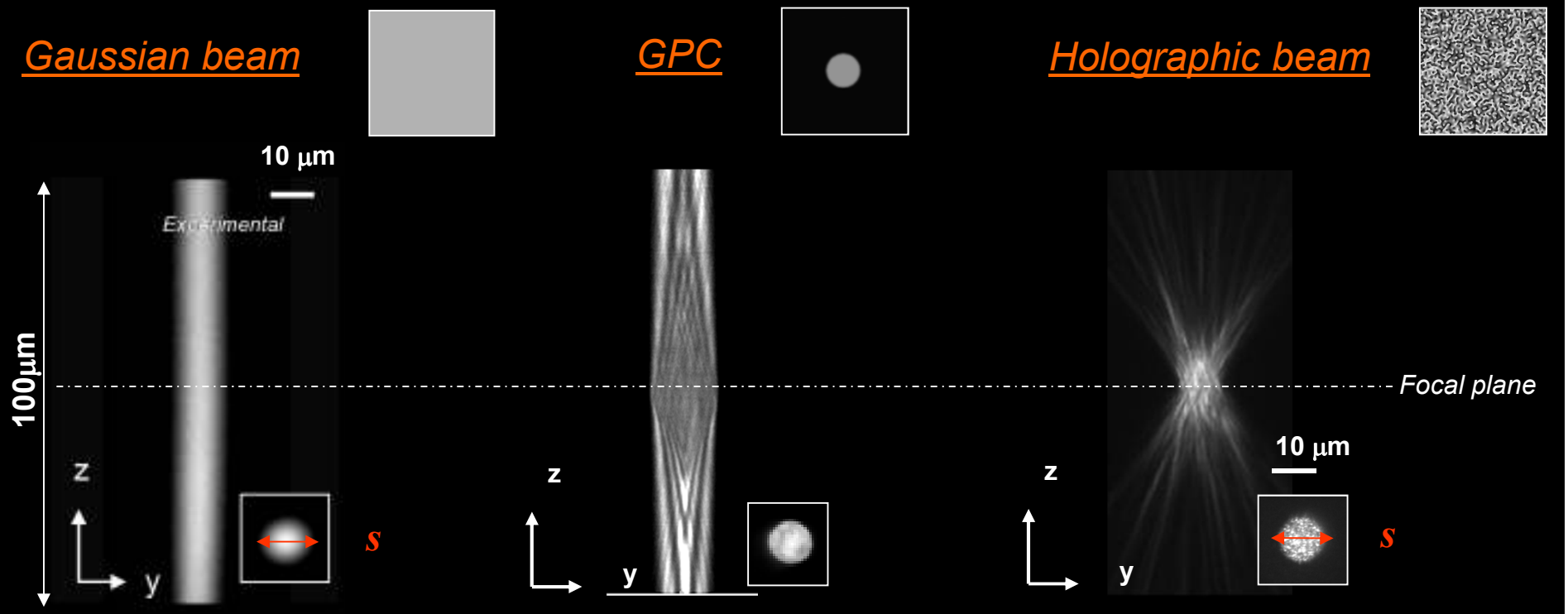
J. Glückstad, Optic. Comm (1996)

Fluorescence ***Laser pattern*** ***Fluorescence*** ***Laser pattern***



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

Axial resolution?



axial resolution $\propto s^2$

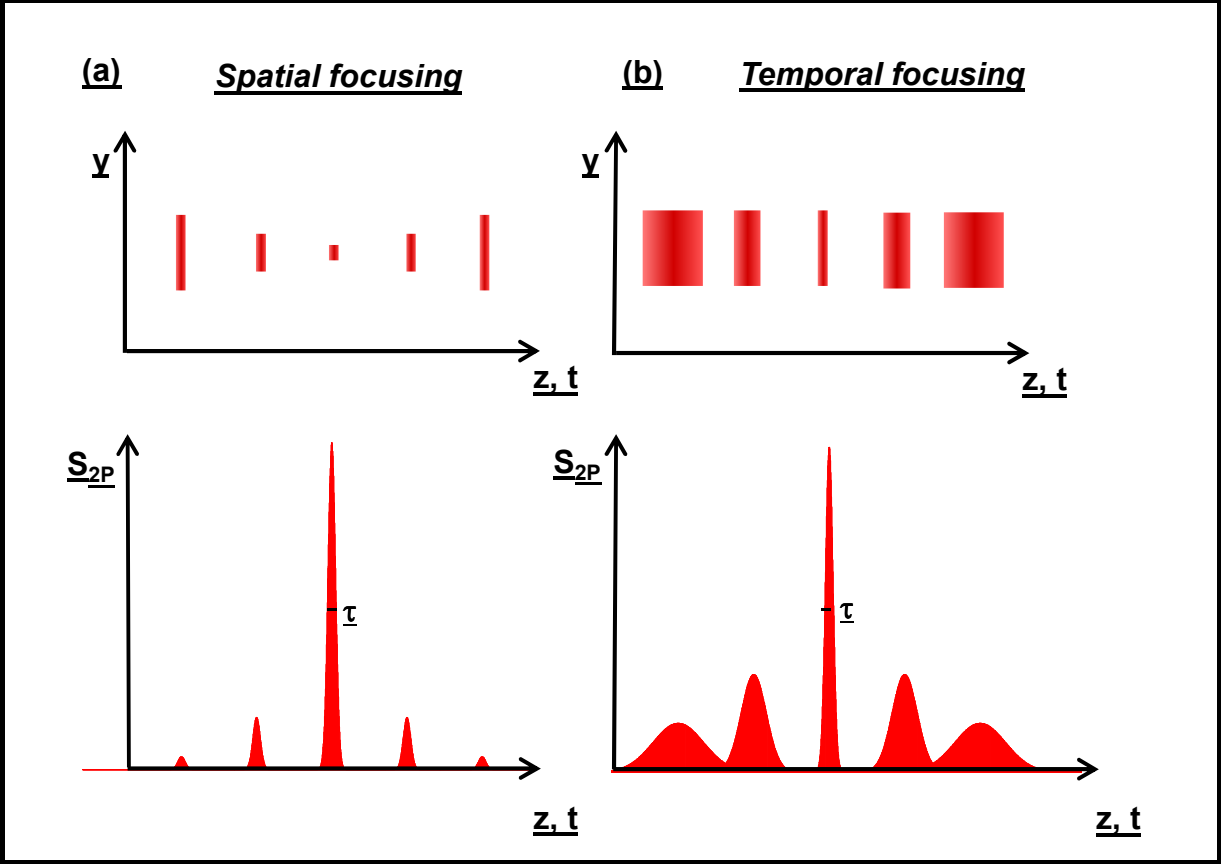
axial resolution $\propto s$

E. Papagiakoumou, et al., Optics Express (2008),
E. Papagiakoumou, et al., Optics Express (2009)

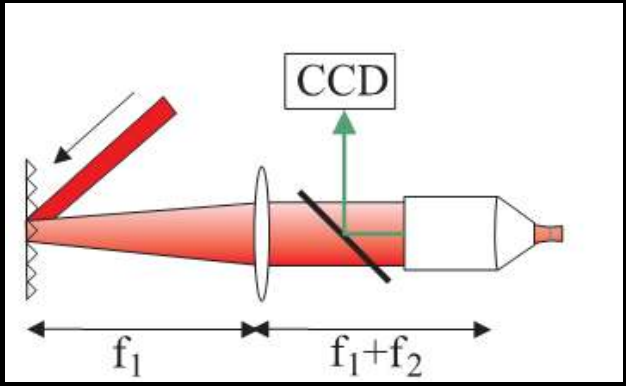
Axial resolution: Temporal focusing

Originally used for wide field two-photon microscopy

D. Oron, E. Tal, Y. Silberberg, Optics Express (2005)

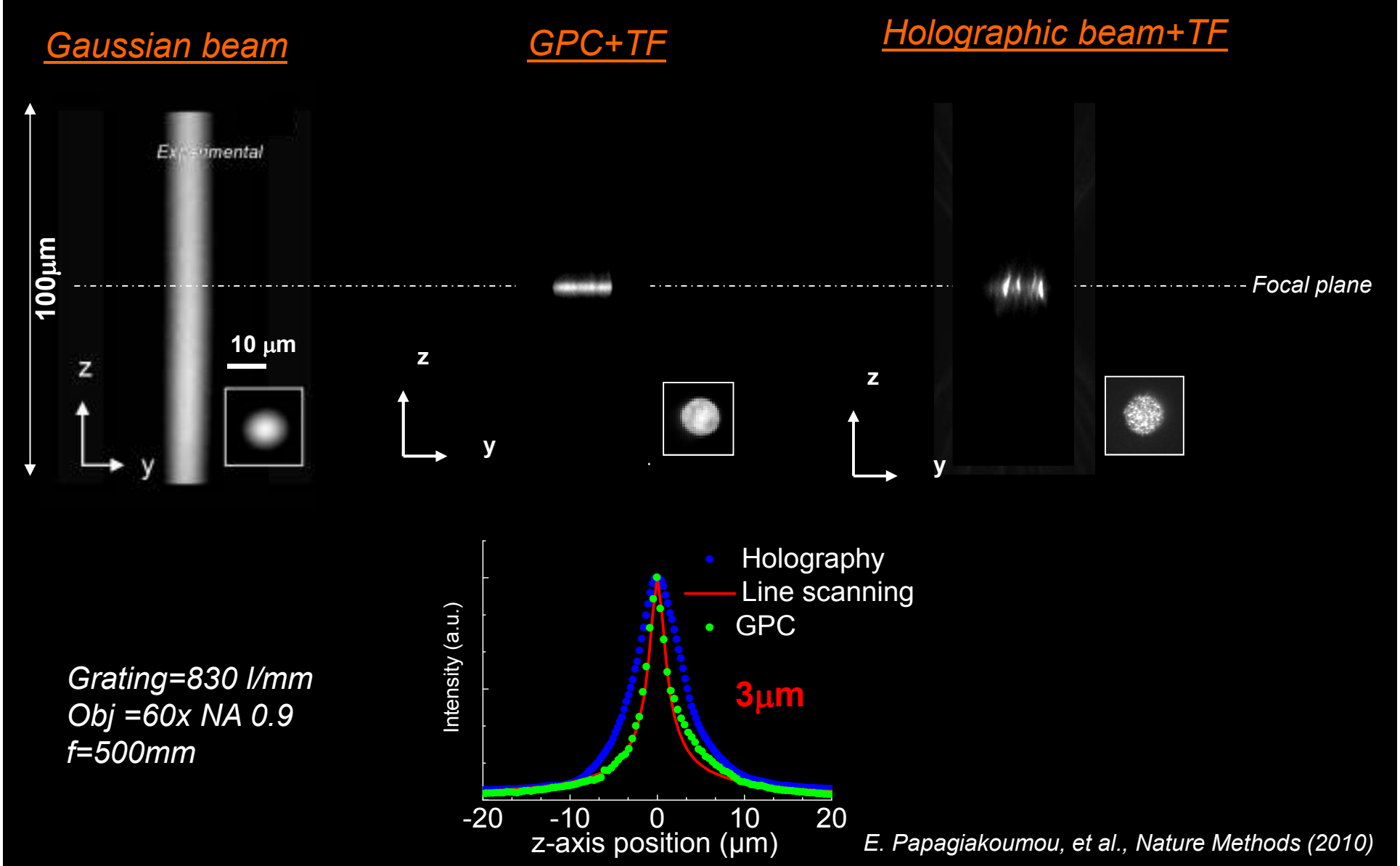


$$S_{2PE} \propto \frac{I^2}{\tau \cdot f}$$



Vaziri&Emiliani. in press (2011)

Axial resolution with Temporal focusing: Results

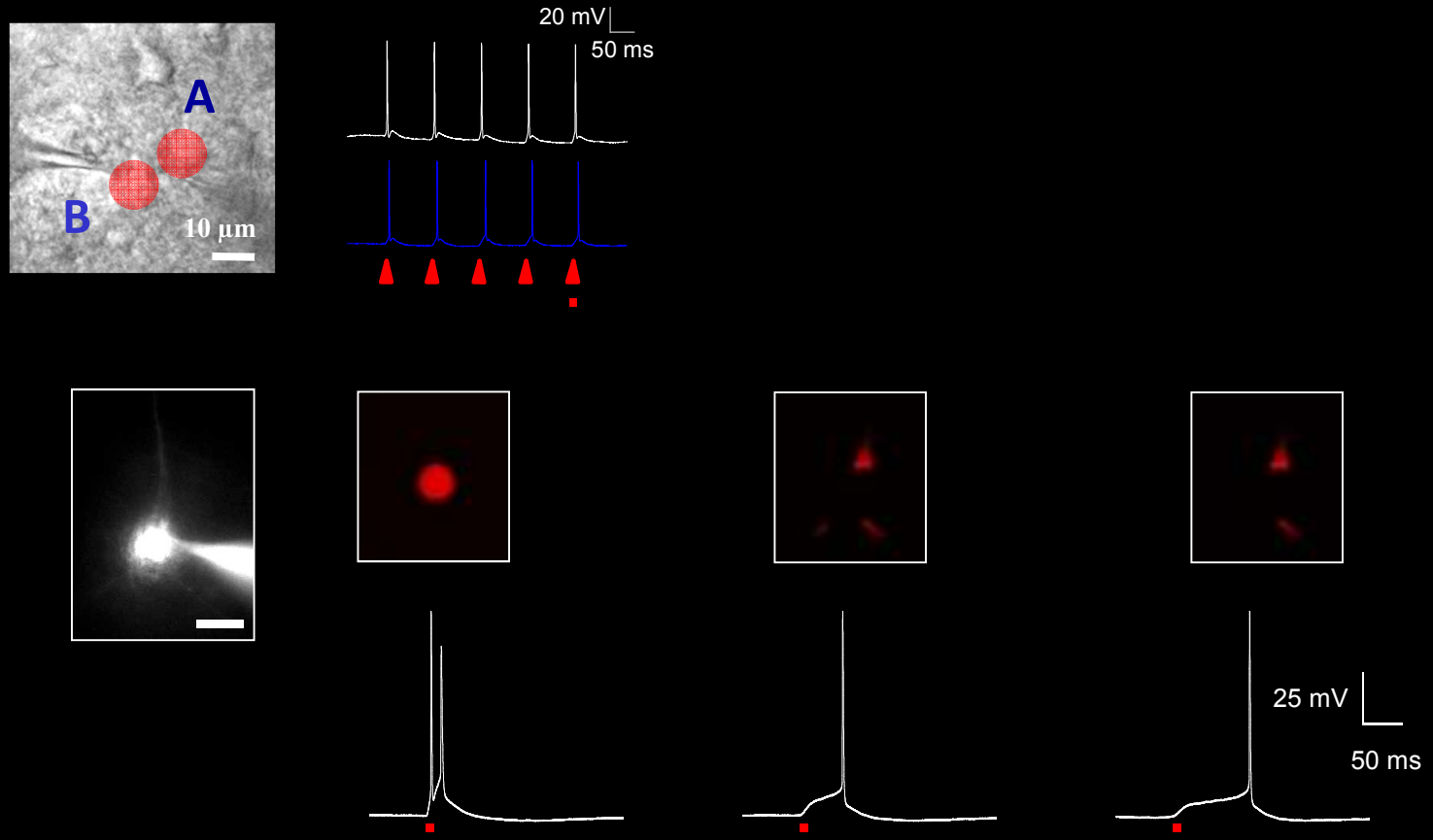


Grating=830 l/mm
Obj =60x NA 0.9
f=500mm

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

Two-photon ChR2 activation in brain slices

Thy1-ChR2-YFP transgenic mice
Excitation =0.3-0.5 mW /μm^2; depth 40-50μm



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

INSIGHT REVIEW NATURE|Vol 461|15 October|doi:10.1038/nature08540

Electrophysiology in the age of light

Massimo Scanziani[1] & Michael Häusser[2]

Electrophysiology, the 'gold standard' for investigating neuronal signalling, is being challenged by a new generation of optical probes. Together with new forms of microscopy, these probes allow us to measure and control neuronal signals with spatial resolution and genetic specificity that already greatly surpass those of electrophysiology. We predict that the photon will progressively replace the electron for probing neuronal function, particularly for targeted stimulation and silencing of neuronal populations. Although electrophysiological characterization of channels, cells and neural circuits will remain necessary, new combinations of electrophysiology and imaging should lead to transformational discoveries in neuroscience.

THANK YOU!!

Wave front engineering microscopy group

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