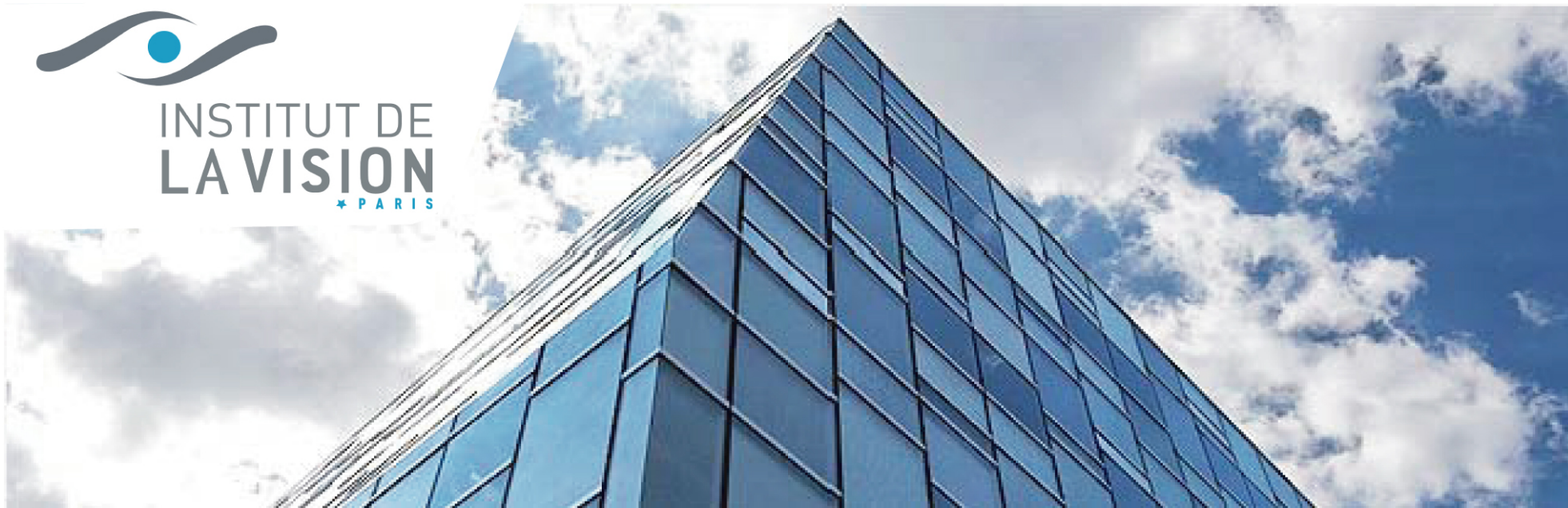




INSTITUT DE
LA VISION
★ PARIS



Circuits optogenetics and wave-front shaping

VALENTINA EMILIANI

Wave-front engineering microscopy group

Photonics Department

Vision Institute,

CNRS and UPMC

Paris France



LIFESENSES



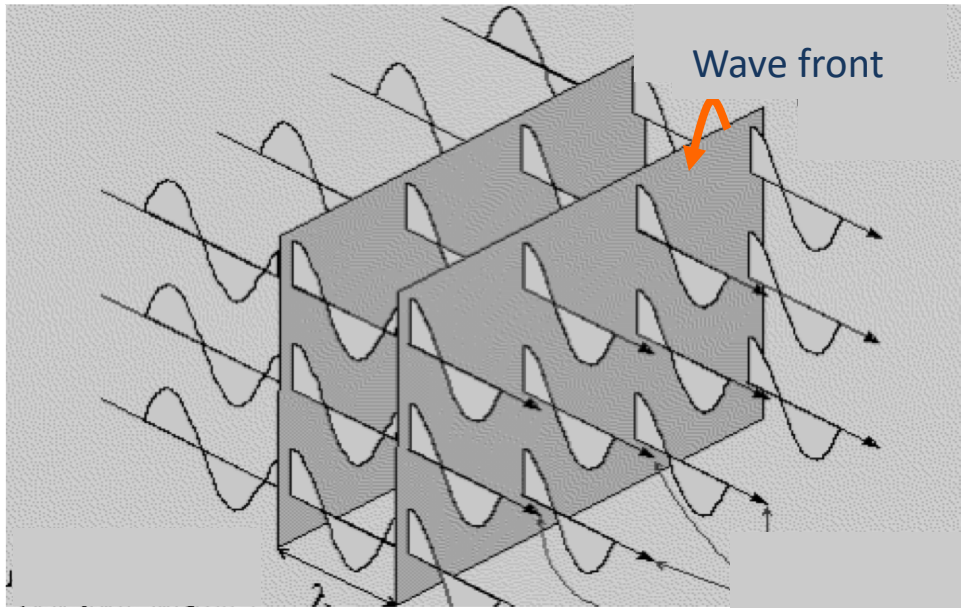
FONDATION
VOIR & ENTENDRE



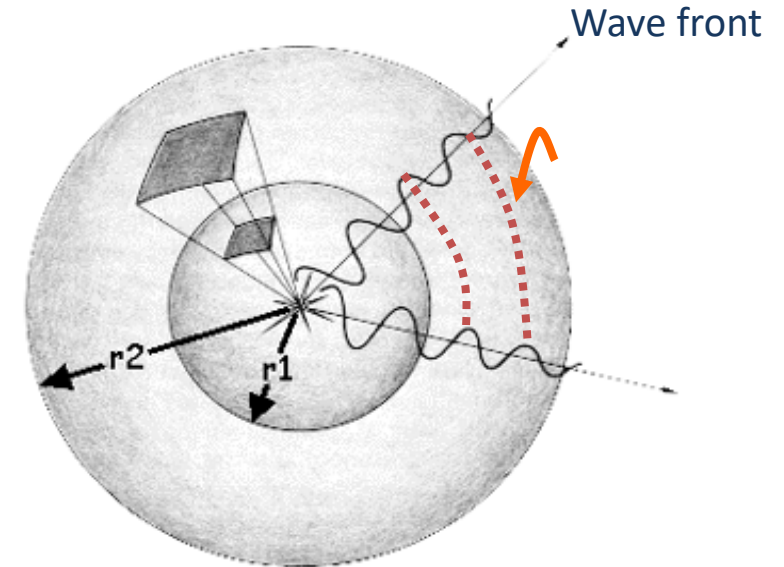
Wave-front

Wave fronts: parallel surfaces connecting similar or equivalent points on adjacent waves

Planar wave



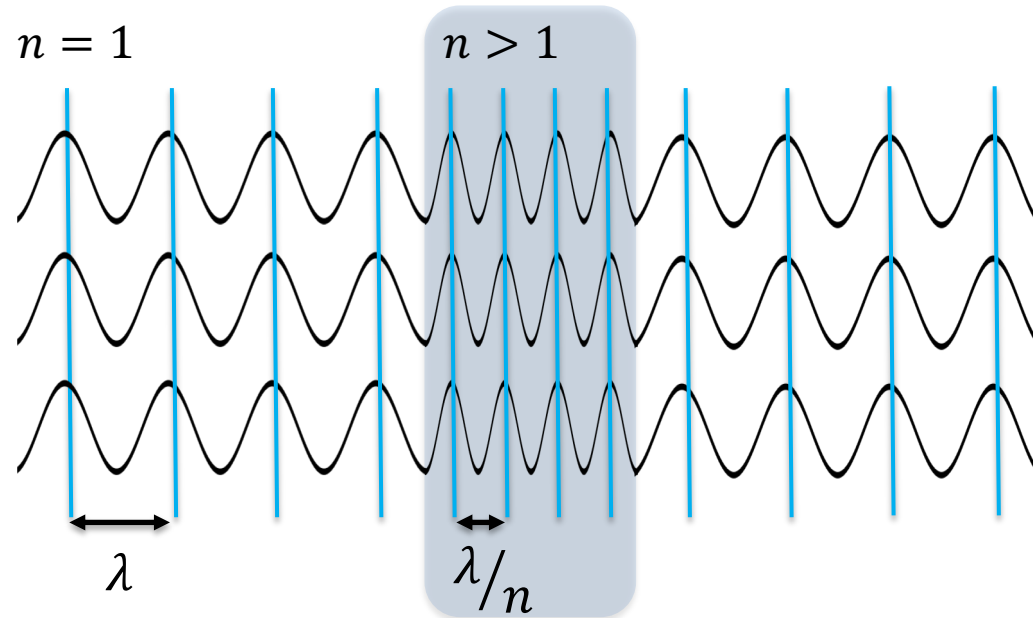
Spherical wave



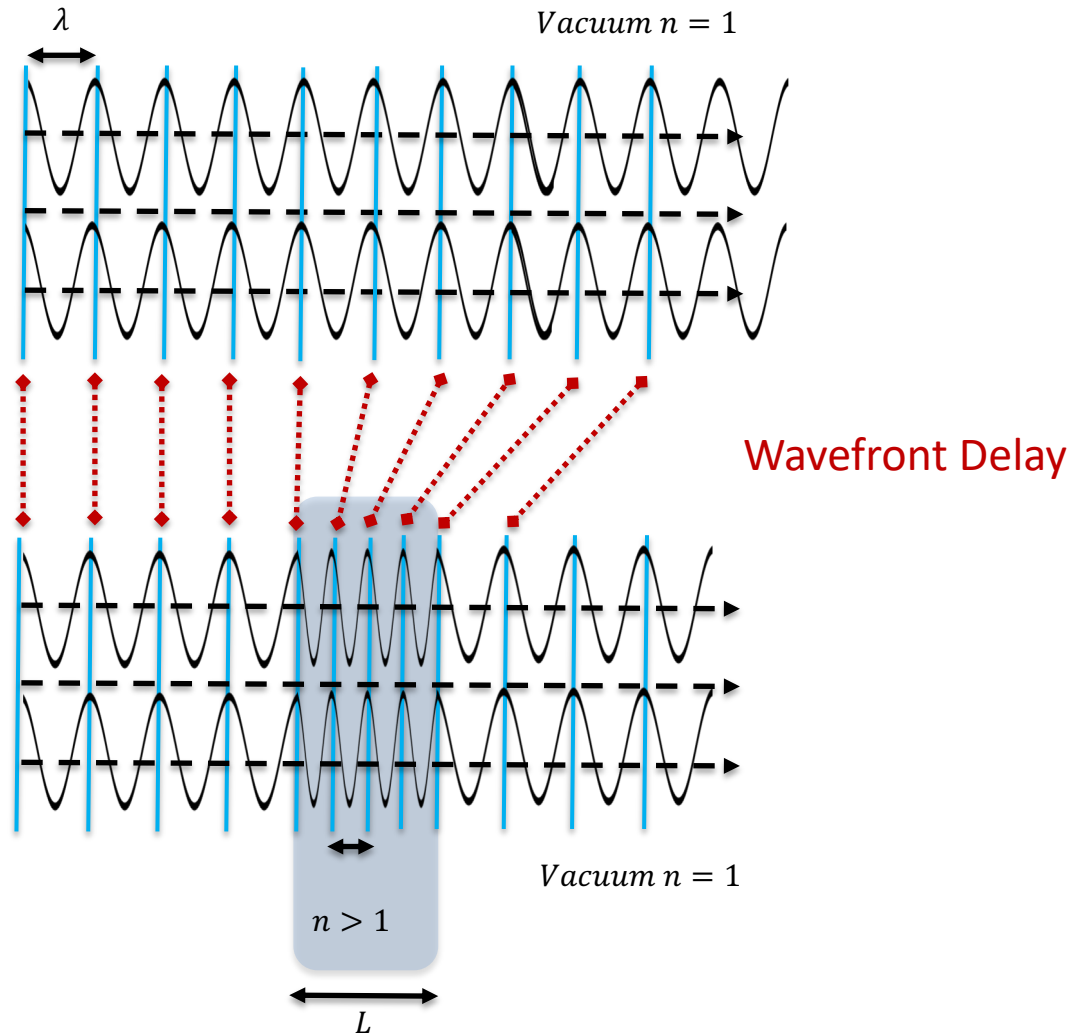
Medium phase shift

Light speed depends on the refractive index of the traveled medium

Speed of light in medium: $v = \frac{c}{n}$

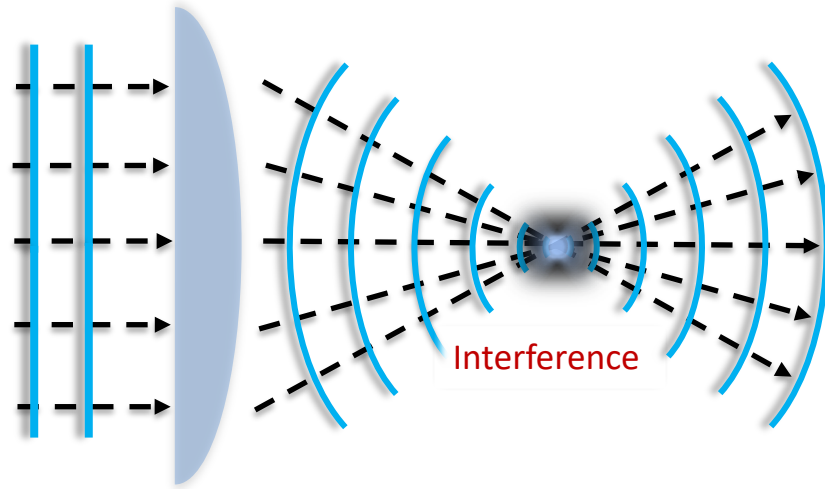


Wavefront Shaping



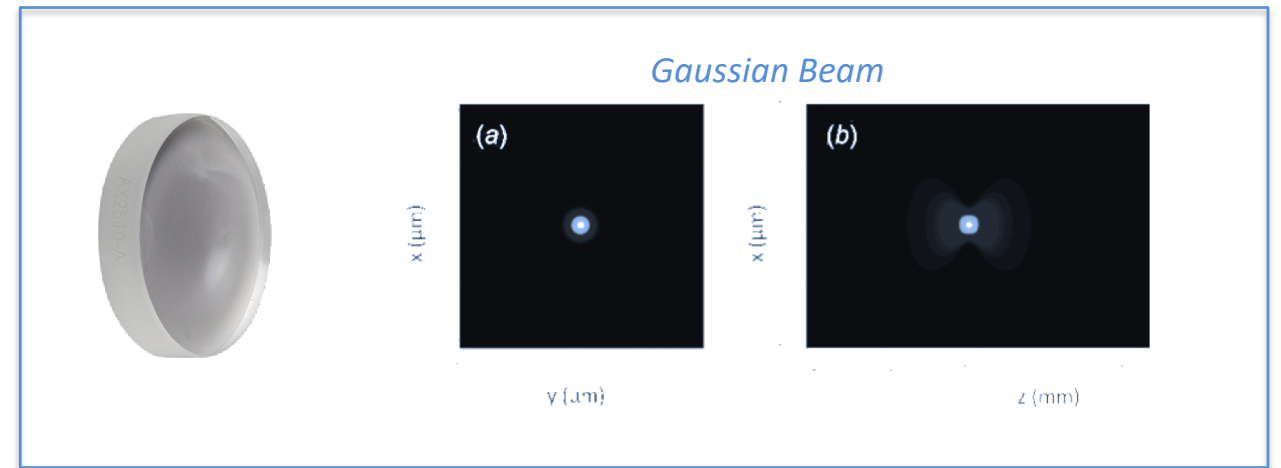
Wavefront Shaping

Beams traveling different thicknesses of material will result in different outgoing phases: the incoming wave front is deformed

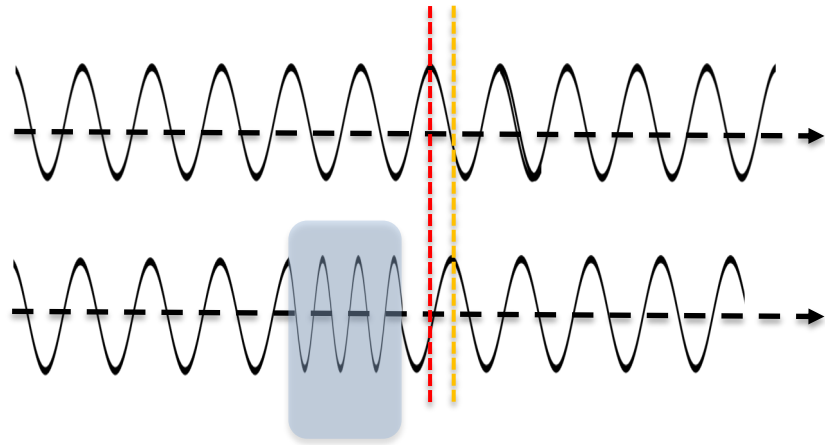


A thin lens transforms a **plane wave**
into a **paraboloidal wave**

Spherical Lens



Wavefront Shaping



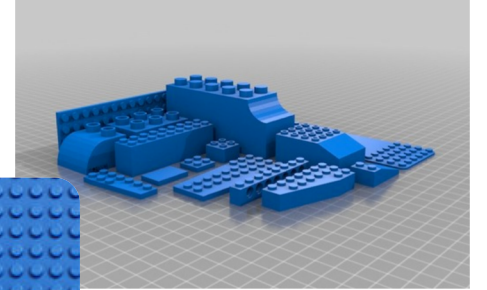
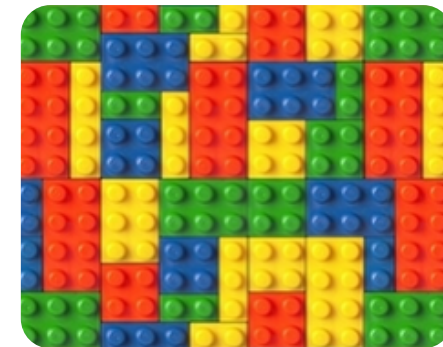
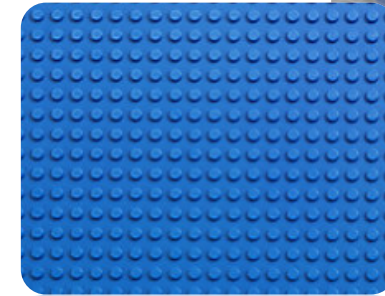
$$\Delta\varphi = \frac{2\pi}{\lambda} nL$$

Phase pattern controlled by
spatially controlling medium
thickness

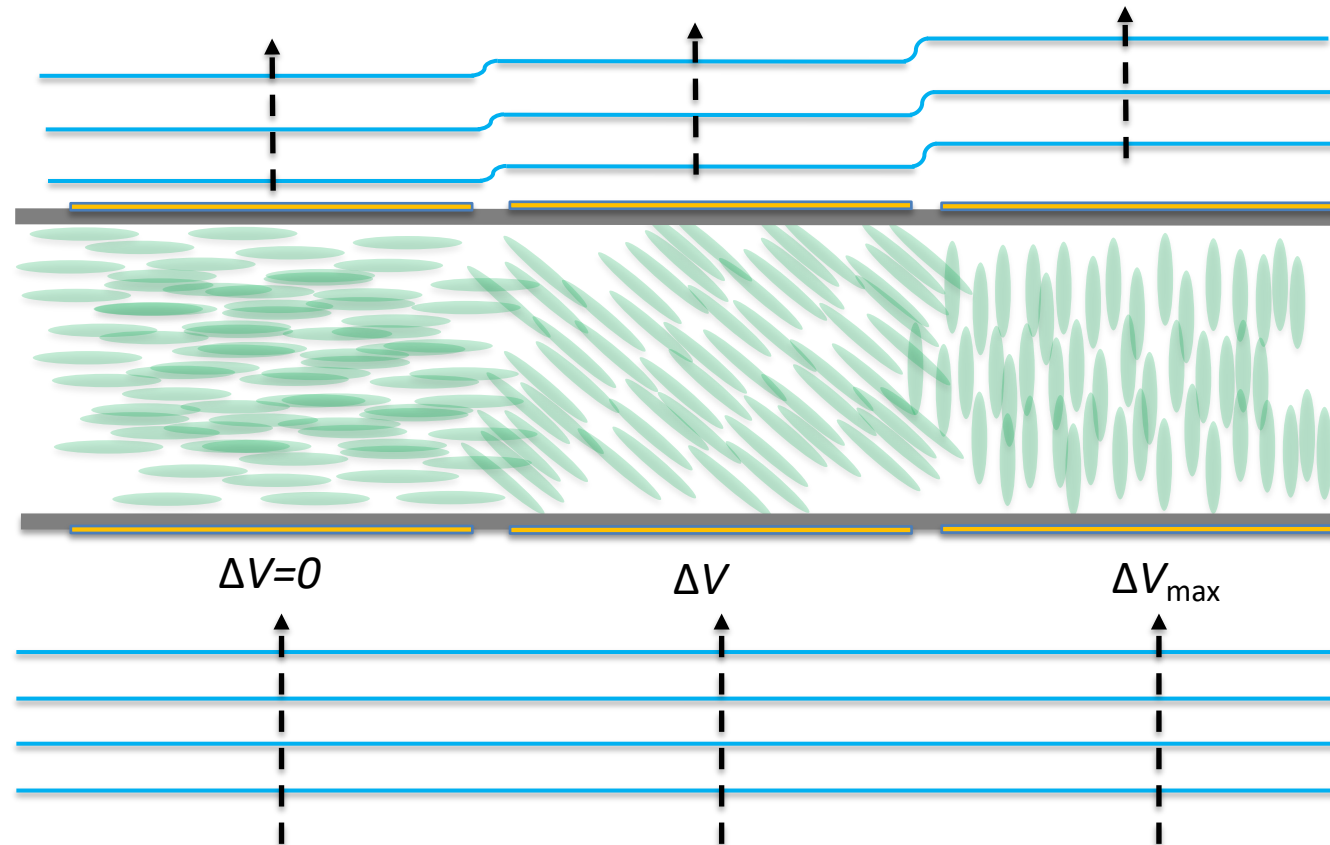
$$\Delta\varphi(x, y) = \frac{2\pi}{\lambda} n \cdot L(x, y)$$

Phase pattern controlled by
spatially controlling medium
refractive index

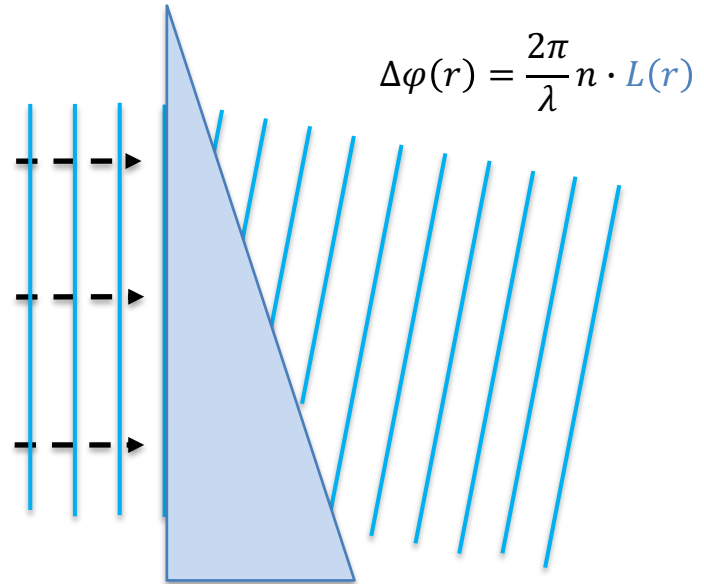
$$\Delta\varphi(x, y) = \frac{2\pi}{\lambda} L \cdot n(x, y)$$



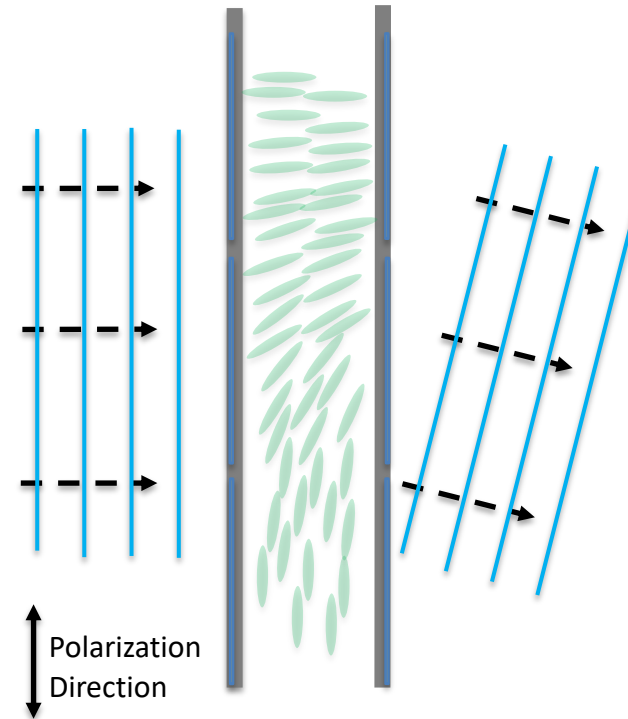
Liquid Crystals Matrix



Liquid Crystals as a Prism

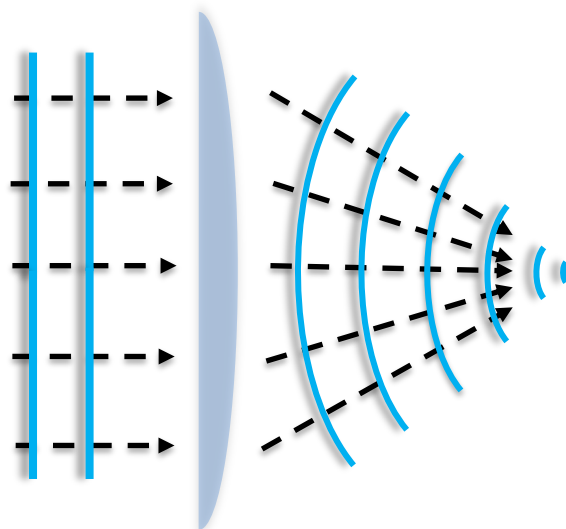


$$\Delta\varphi(r) = \frac{2\pi}{\lambda} L \cdot n(r, V)$$

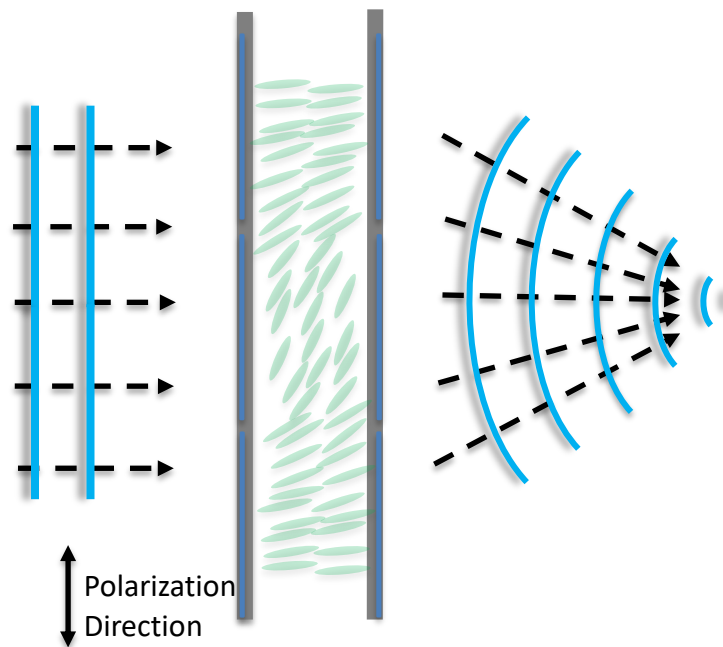


Liquid Crystals as a Lens

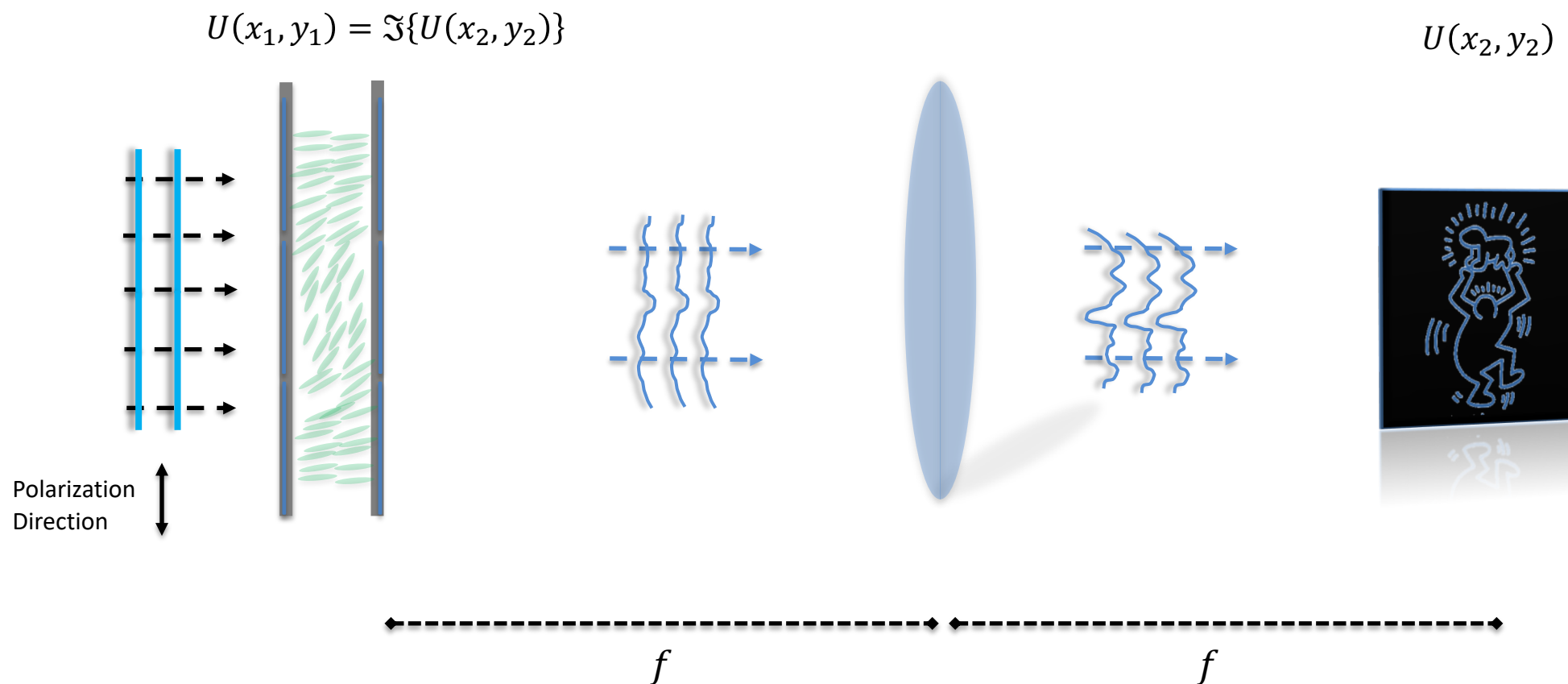
$$\Delta\varphi(r) = \frac{2\pi}{\lambda} n \cdot L(r)$$



$$\Delta\varphi(r) = \frac{2\pi}{\lambda} L \cdot n(r, V)$$

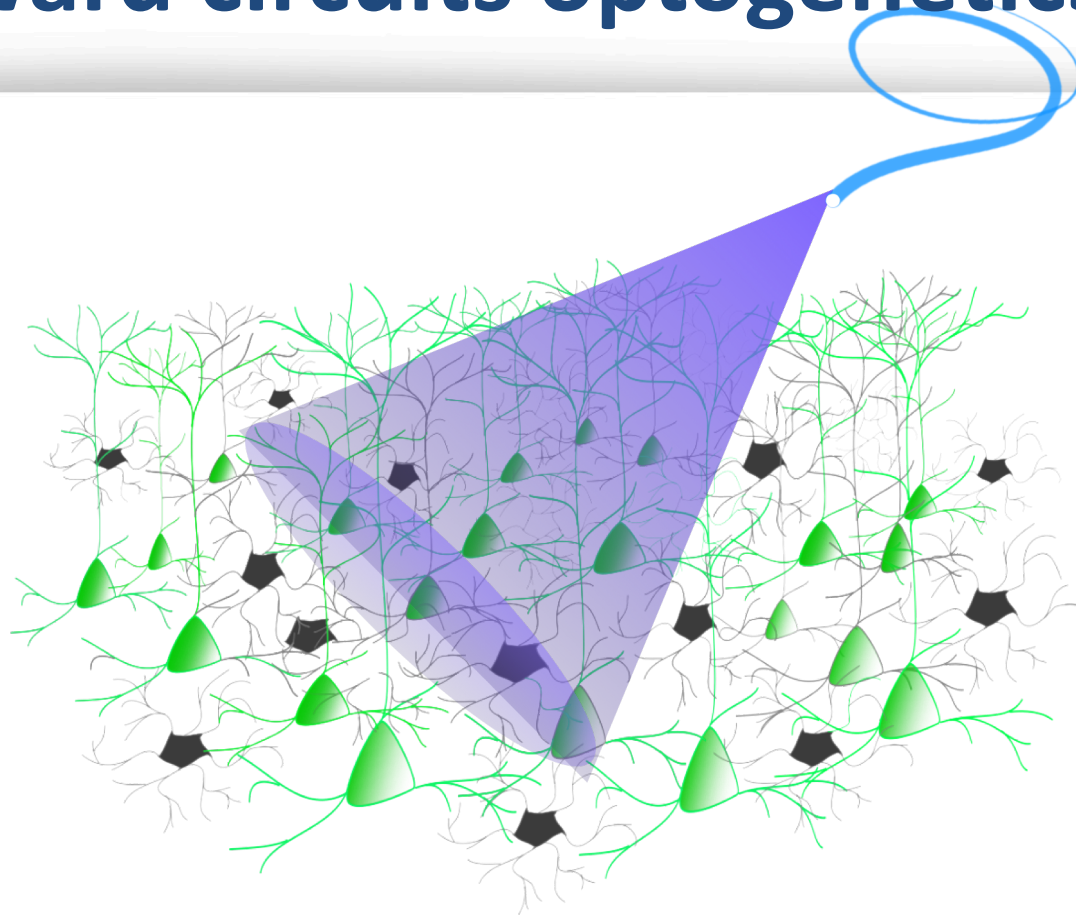


Pattern Shaping by Liquid Crystals

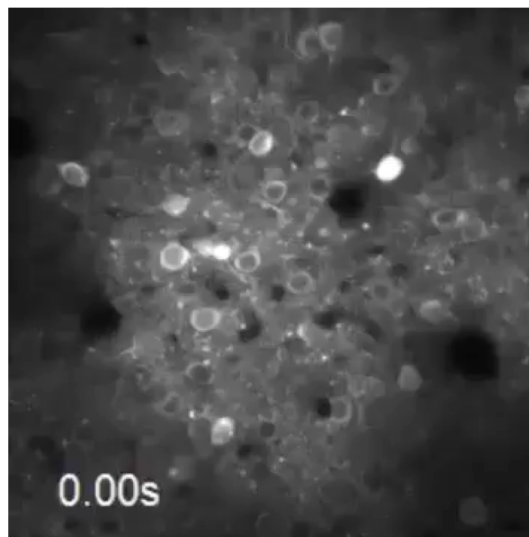
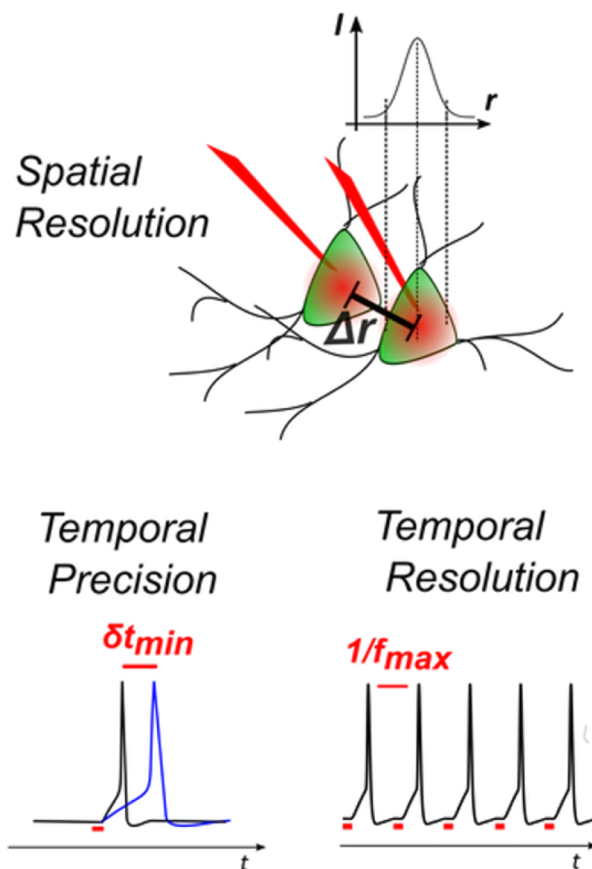


Introduction: toward circuits optogenetics

Optogenetics: Key questions already solved by using visible light wide- field illumination methods combined with genetic targeting

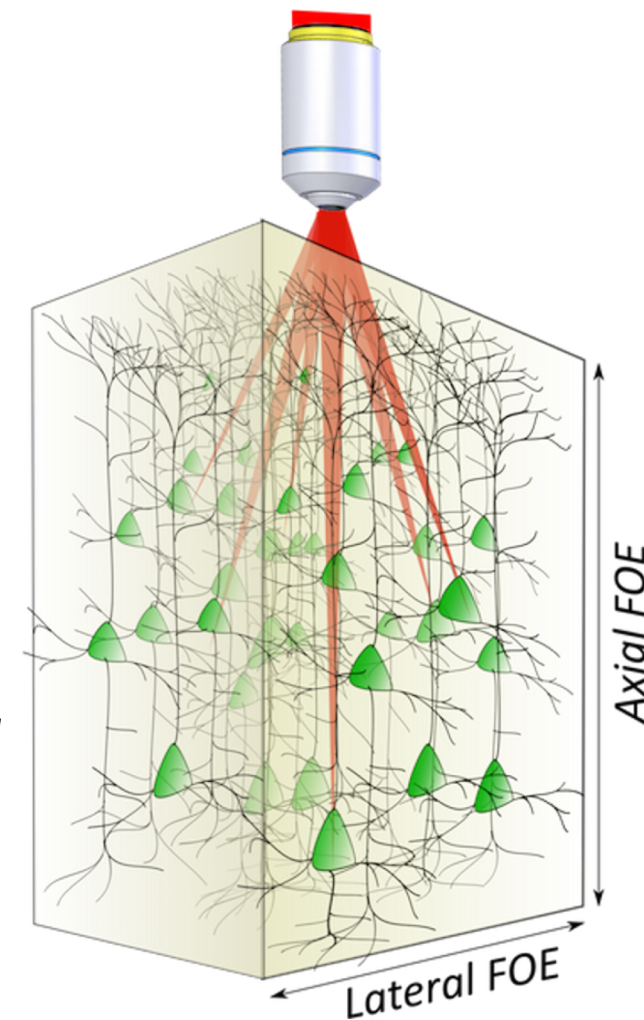


Introduction: toward circuits optogenetics



Visual cortex V1
(TW-Chen & GENIE prj Janelie)

- 3D, multiscale light patterning
- In depth



Introduction: toward circuits optogenetics

New phase, circuits optogenetics (precise optical manipulation of neuronal circuits)

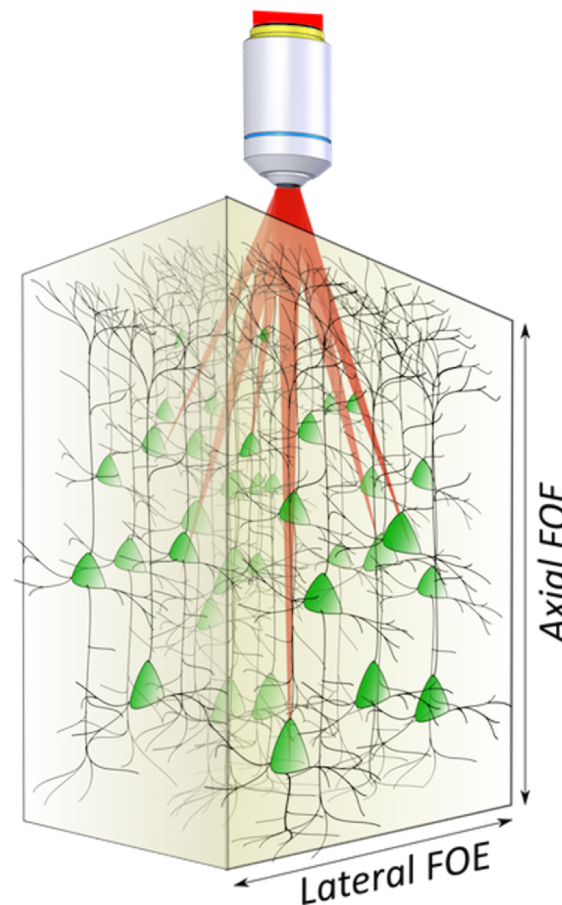
Recent joint progresses

Light delivering approaches

Opsin engineering

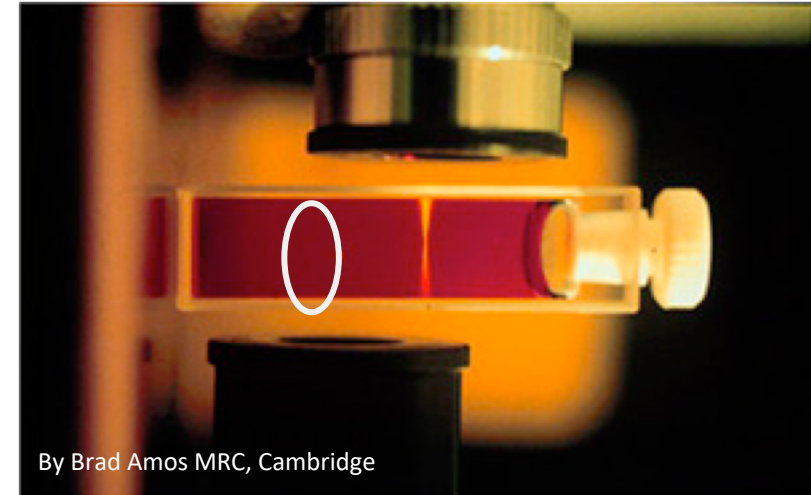
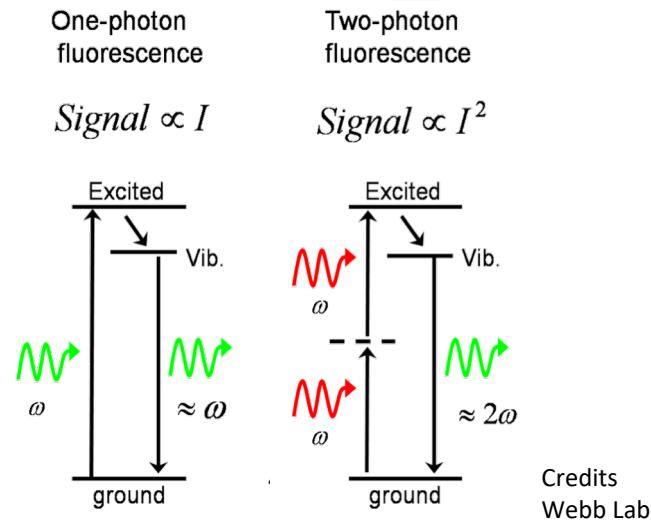
Laser development

Modeling

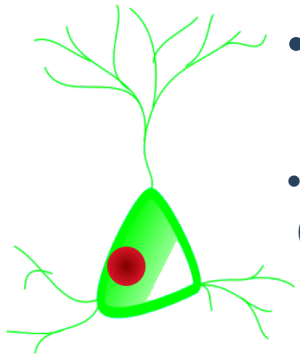


2P- optogenetics: single cell resolution

Two-photon excitation



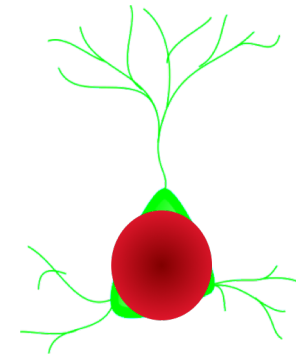
Within a 2P excitation volume, not enough channels to evoke an action potential



- Low conductance (~ 80 fS)
Feldbauer et al PNAS, 2009
- Low density of ChR2 channels
(~ 130 molecules/ μm^2)
Nagel et al, FEBS Lett., 1995



The cell membrane is covered by
rapid scanning the laser beam

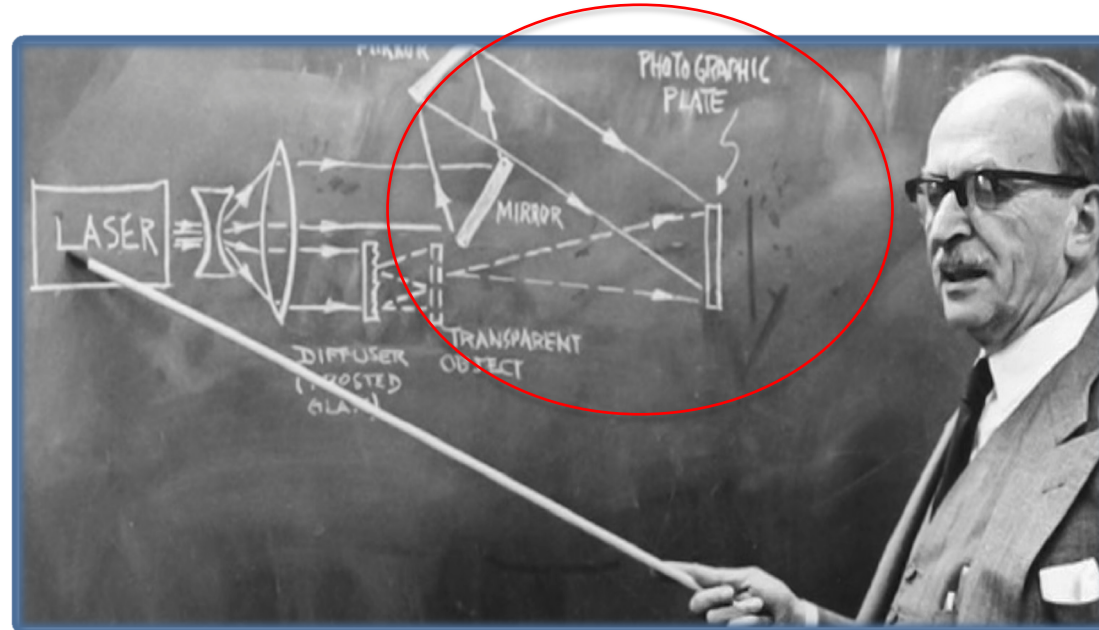


The cell membrane is covered by
generating shaped light

TEMPORAL RESOLUTION: $T_R = (t_s + t_{\text{well}}) \times n \times N$

$T_R = t_{\text{well}}$

Parallel (holographic) illumination



Dennis Gabor Prix Nobel en physique 1971

Parallel (holographic) illumination

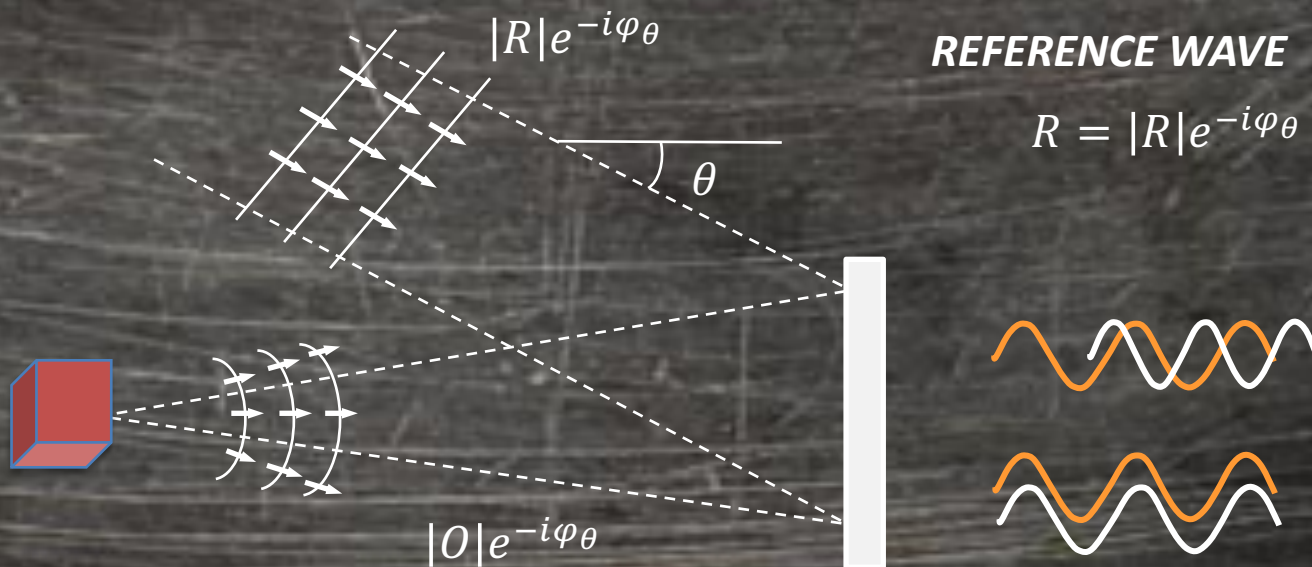
1) Writing the hologram

SIGNAL WAVE

$$O = |O|e^{-i\varphi_o}$$

REFERENCE WAVE

$$R = |R|e^{-i\varphi_r}$$



$$\Sigma = Re^{-i\varphi_r} + Oe^{-i\varphi_o}$$

$$I_h \propto \Sigma \Sigma^* = (Re^{-i\varphi_r} + Oe^{-i\varphi_o})(Re^{-i\varphi_r} + Oe^{-i\varphi_o})^*$$

$$= R^2 + O^2 + ROe^{-i\varphi_r+i\varphi_o} + ROe^{i\varphi_r-i\varphi_o} = R^2 + O^2 + 2RO \cos(\varphi_r - \varphi_o)$$

Parallel (holographic) illumination

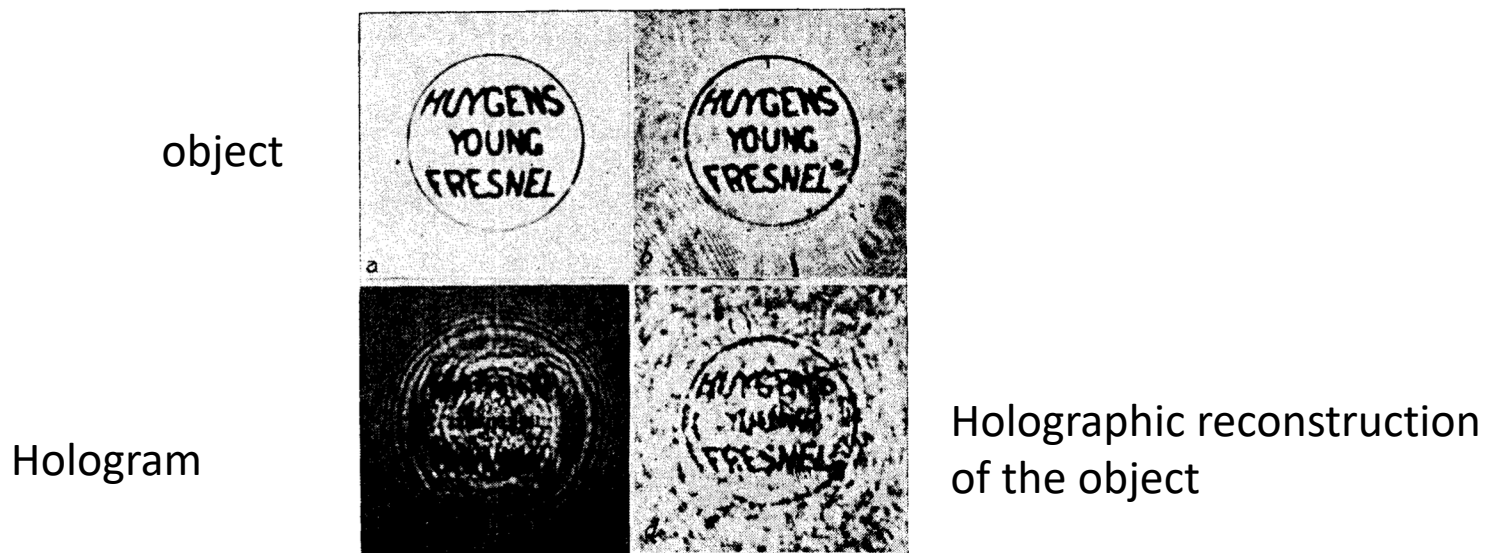


Fig. 2. (a) ORIGINAL MICROGRAPH, 1.4 MM. DIAMETER. (b) MICROGRAPH, DIRECTLY PHOTOGRAPHED THROUGH THE SAME OPTICAL SYSTEM WHICH IS USED FOR THE RECONSTRUCTION (d). AP. 0.04. (c) INTERFERENCE DIAGRAM, OBTAINED BY PROJECTING THE MICROGRAPH ON A PHOTOGRAPHIC PLATE WITH A BEAM DIVERGING FROM A POINT FOCUS. THE LETTERS HAVE BECOME ILLEGIBLE BY DIFFRACTION. (d) RECONSTRUCTION OF THE ORIGINAL BY OPTICAL SYNTHESIS FROM THE DIAGRAM AT THE LEFT. TO BE COMPARED WITH (b). THE LETTERS HAVE AGAIN BECOME LEGIBLE

Dennis Gabor, Nature, 161, 1948

Parallel (holographic) illumination

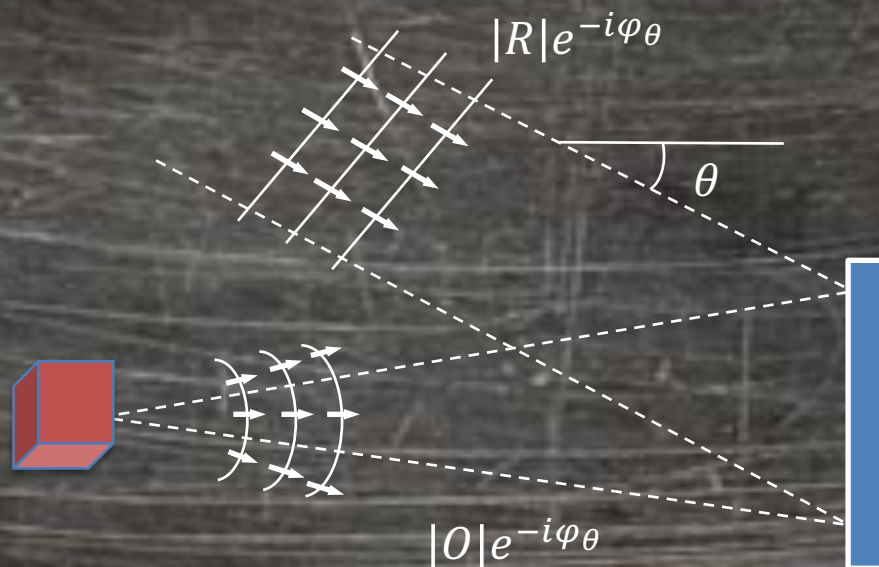
1) Writing the hologram

SIGNAL WAVE

$$O = |O|e^{-i\varphi\theta}$$

REFERENCE WAVE

$$R = |R|e^{-i\varphi\theta}$$

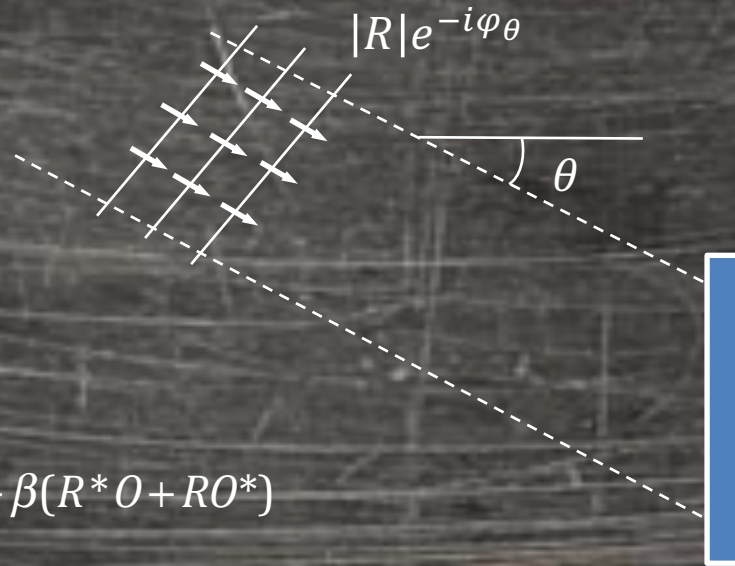


$$W = \int_{t_1}^{t_1+\Delta t} H(x, y) dt = \Delta t |O|^2 + \Delta t |R|^2 + \Delta t R^* O + \Delta t R O^*$$

$$T = T_0 - \beta(R^* O + R O^*)$$

Parallel (holographic) illumination

2) Reading the hologram



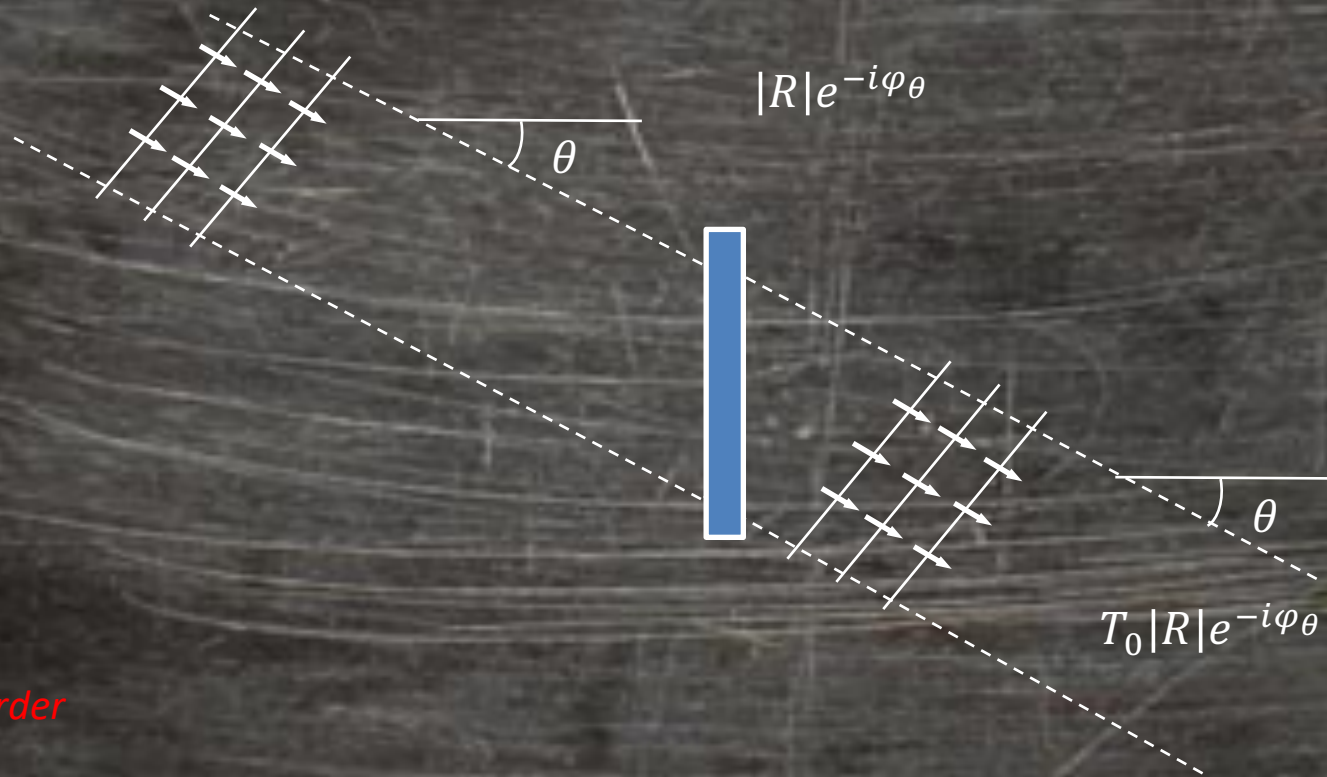
$$\Sigma_T = TR = T \times |R|e^{-i\varphi\theta} \quad T = T_0 - \beta(R^*O + RO^*)$$

$$= [1 - \beta(R^2 + O^2 + ROe^{i\varphi\theta - i\varphi_0} + ROe^{-i\varphi\theta + i\varphi_0})] \times Re^{-i\varphi\theta}$$

$$\Sigma_T = T_0R + \beta R^2O + \beta R^2O^*$$

Parallel (holographic) illumination

2) Reading the hologram

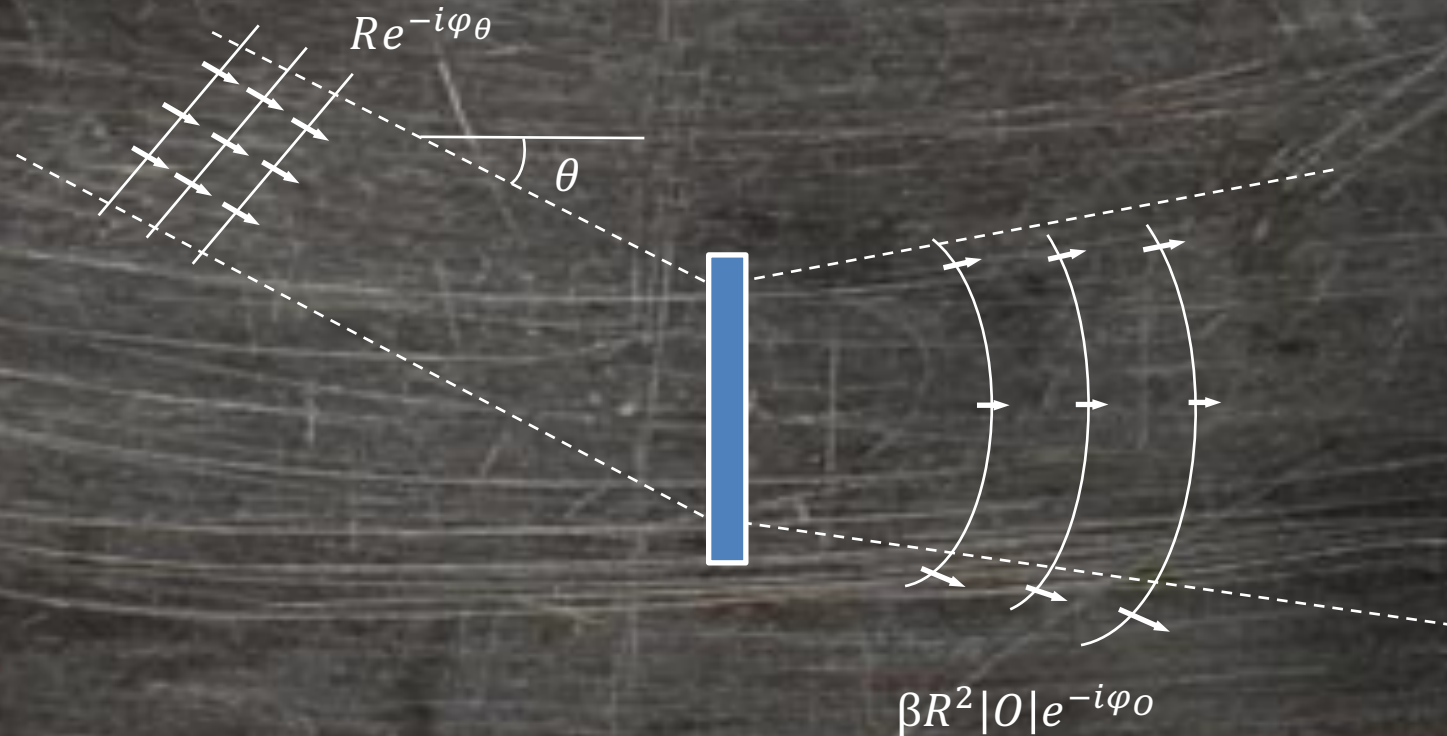


Zero order

$$\Sigma_T = T_0 R + \beta R^2 O + \beta R^2 O^*$$

Parallel (holographic) illumination

2) Reading the hologram

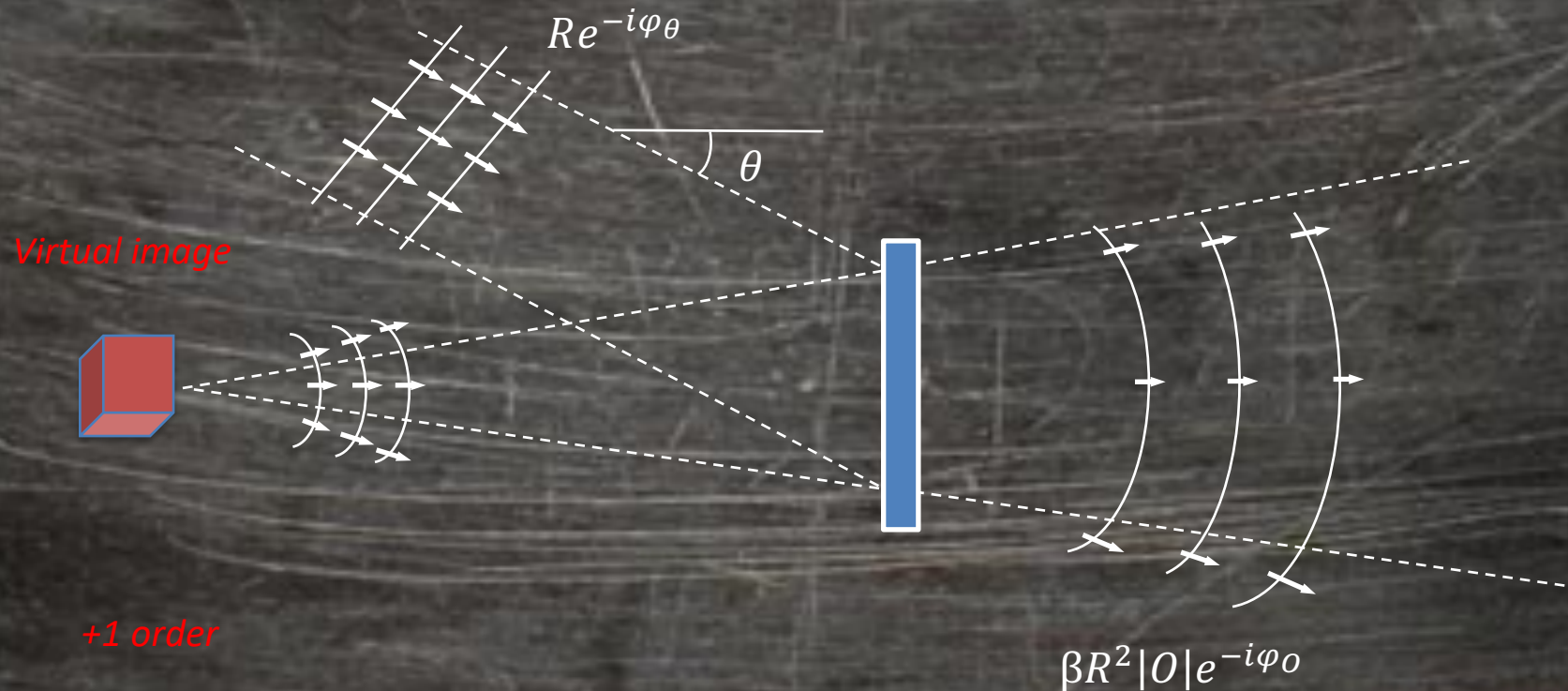


+1 order

$$\Sigma_T = T_0 R + \beta R^2 O + \beta R^2 O^*$$

Parallel (holographic) illumination

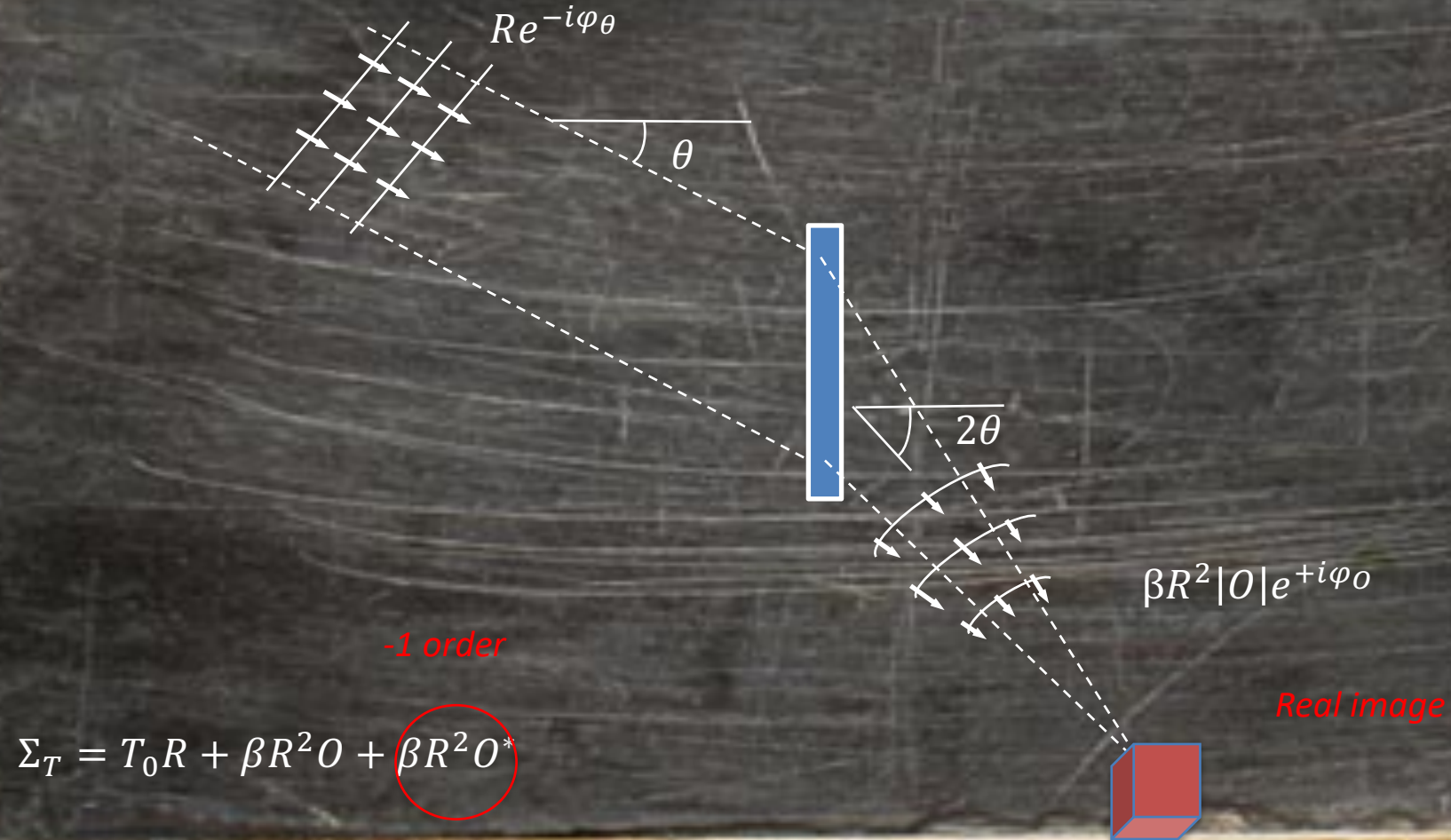
2) Reading the hologram



$$\Sigma_T = T_0 R + \beta R^2 O + \beta R^2 O^*$$

Parallel (holographic) illumination

2) Reading the hologram



Parallel (holographic) illumination

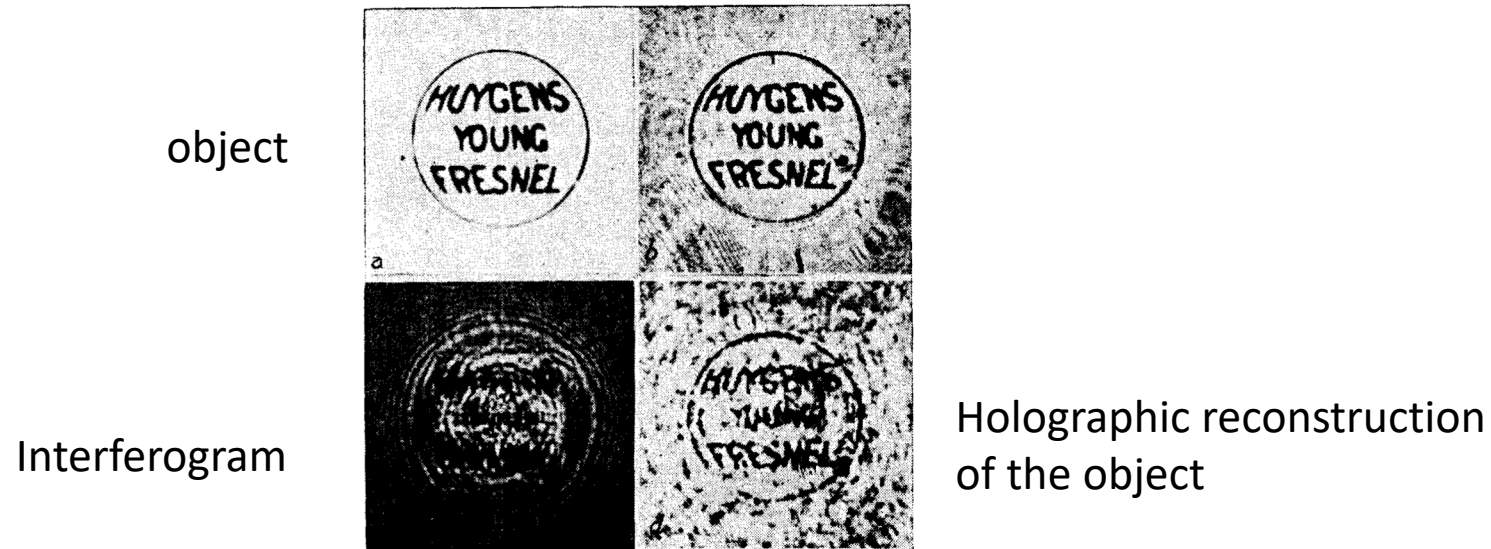


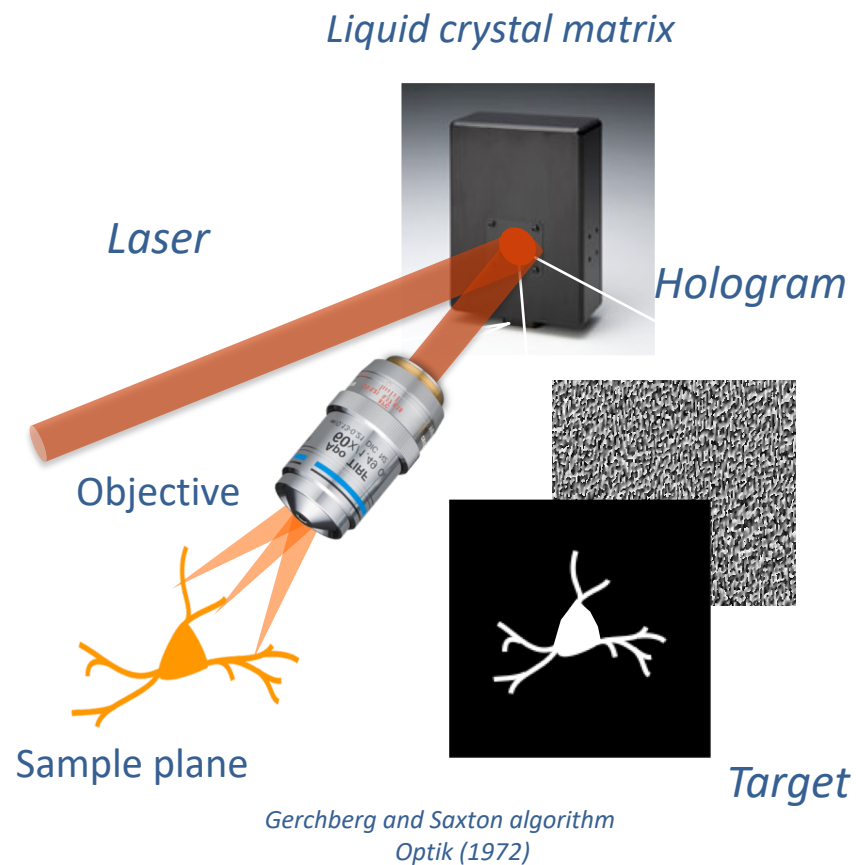
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Dennis Gabor, Nature, 161, 1948

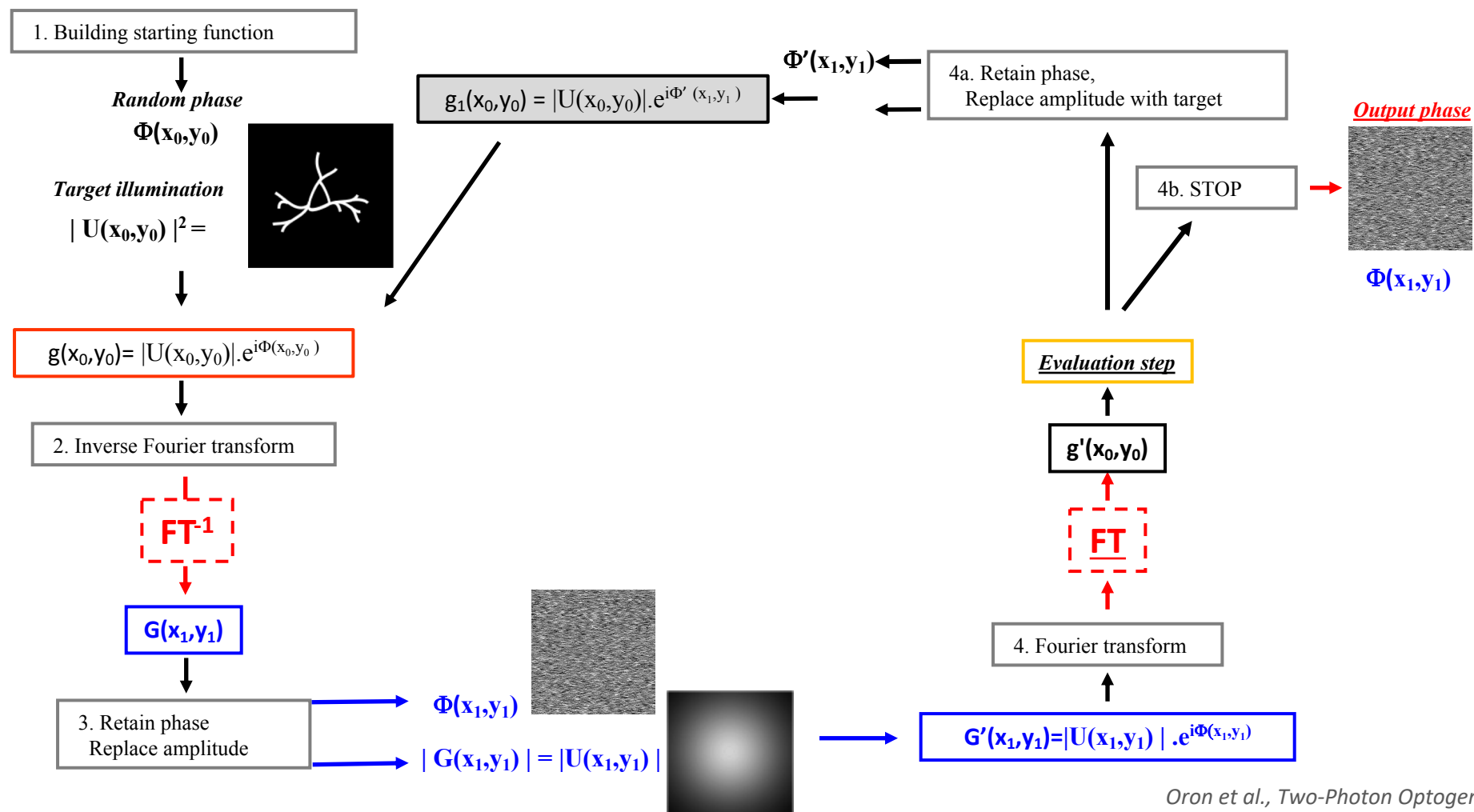
Parallel (holographic) illumination

Do we need the object?

Computer generated Holography



Computer Generated Holography (CGH)

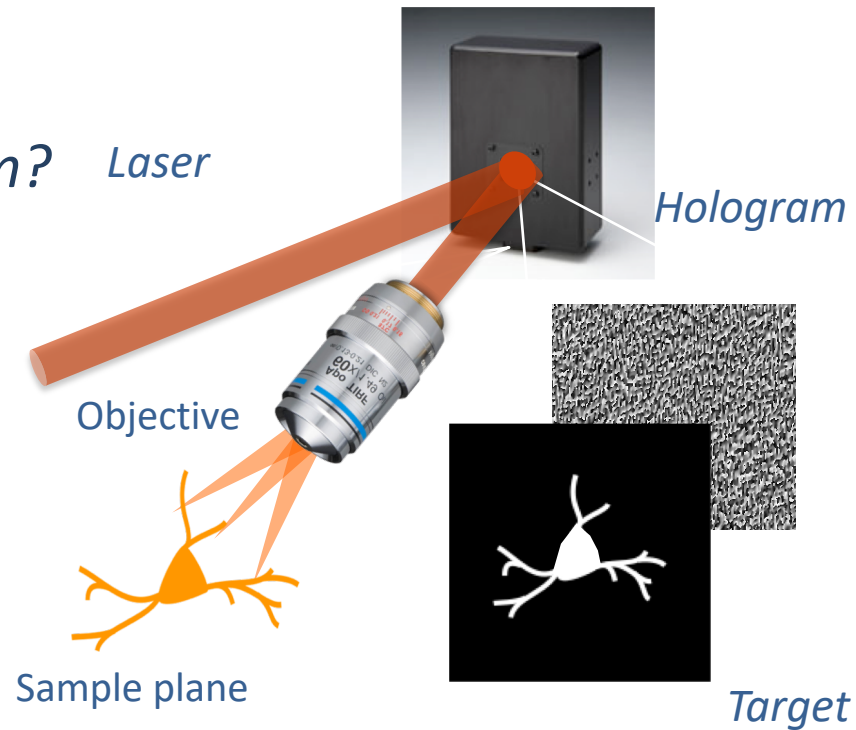


Parallel (holographic) illumination

Computer generated Holography

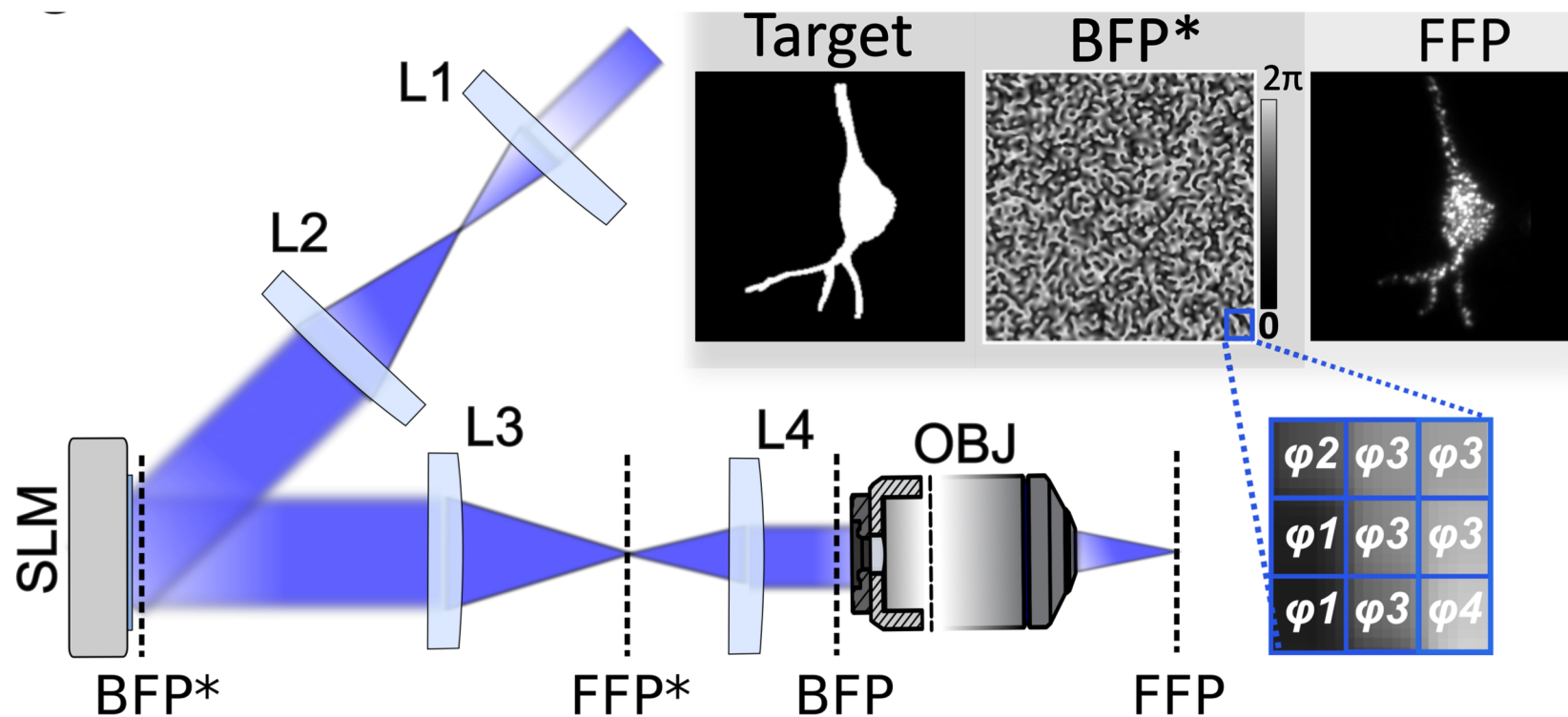
Liquid crystal matrix

Do we need to record the hologram? *Laser*

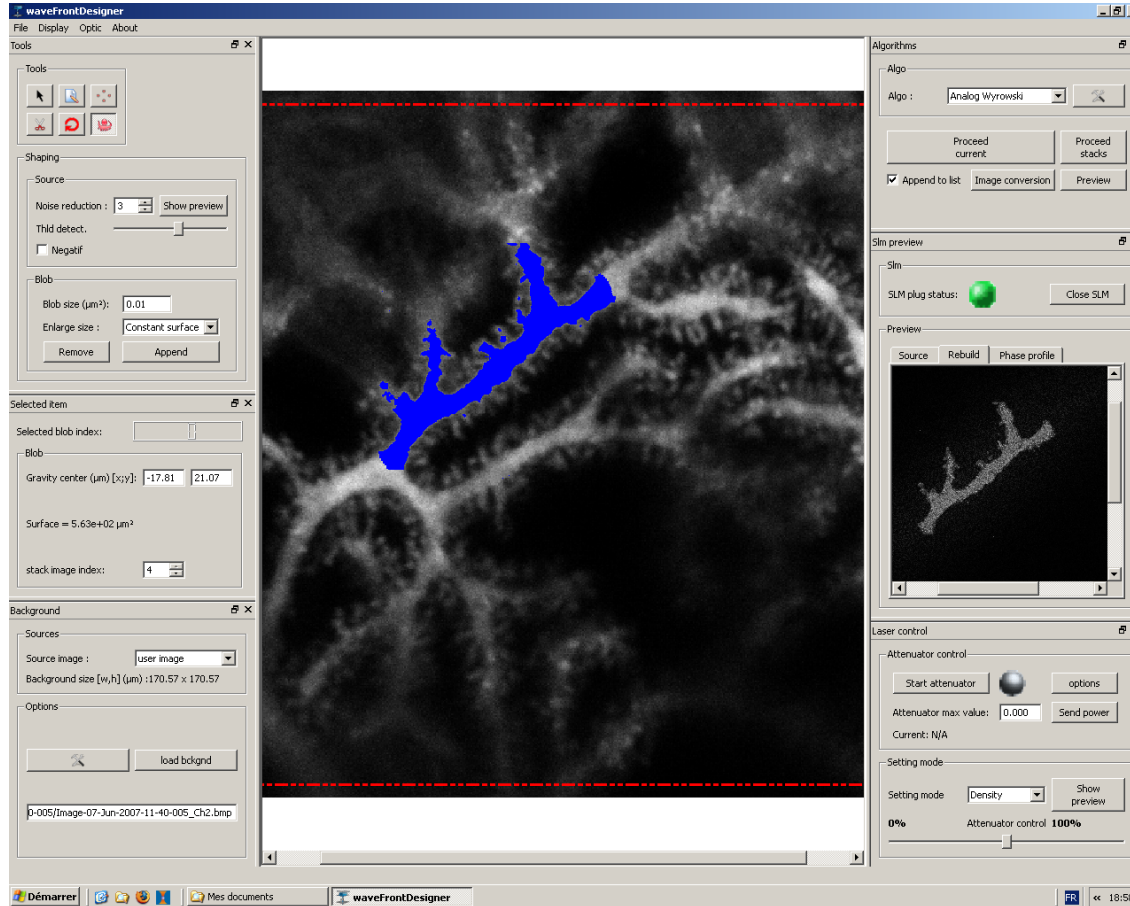


Gerchberg and Saxton algorithm
Optik (1972)

Light Sculpting by CGH



Light Shaping Software



Recording of fluorescence image

$T_1 \sim 100-200$ ms

Generation of phase hologram

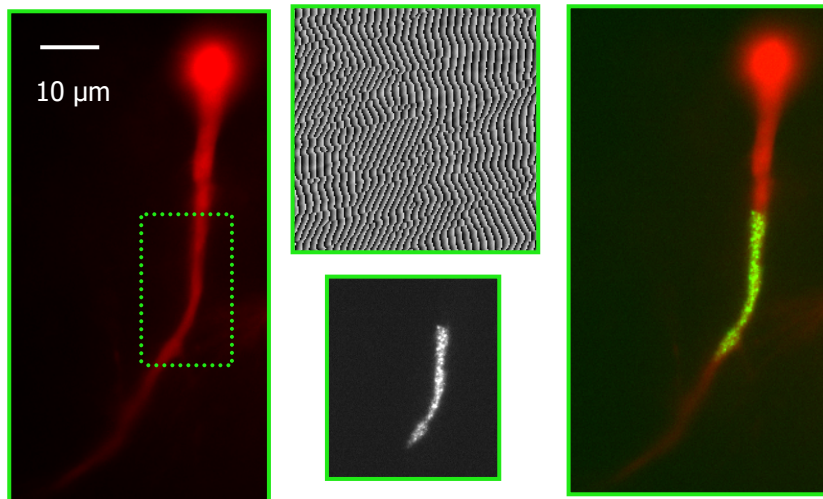
$T_2 \sim 50-100$ it/s $\rightarrow 200$ ms

SLM addressing

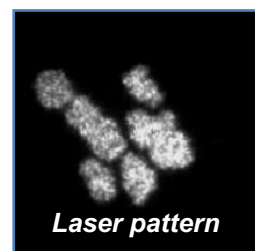
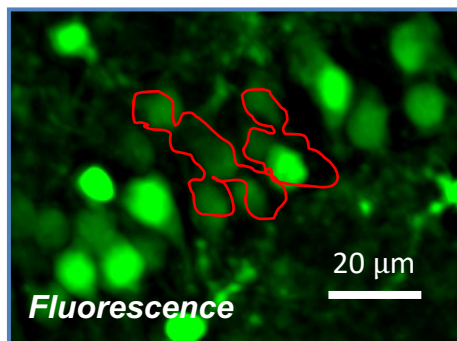
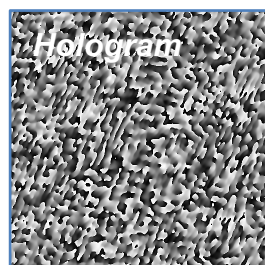
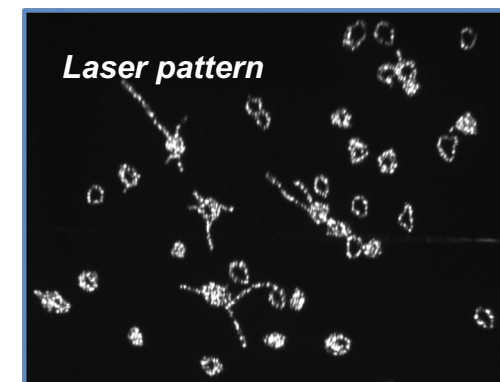
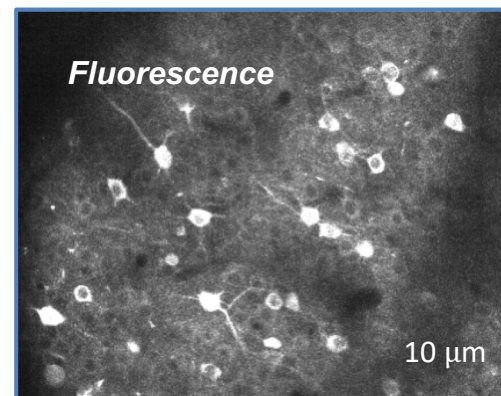
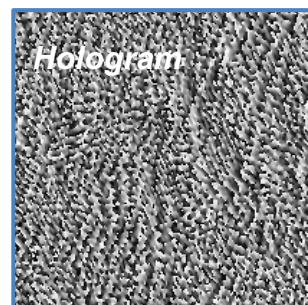
$T_3 \sim 15$ ms

*For sequential photoactivation,
 $\rightarrow$ limiting factor SLM refreshing rate
(60-200Hz)*

The holographic illuminations: examples

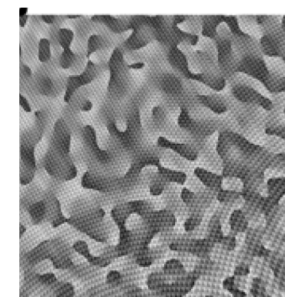


C. Lutz et al. Nature Methods (2008)

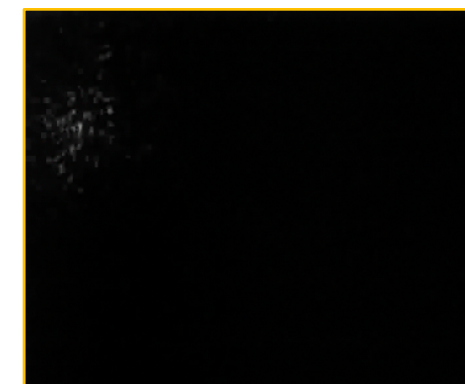


M. Zahid et al. PlosOne (2010)

3D-GS



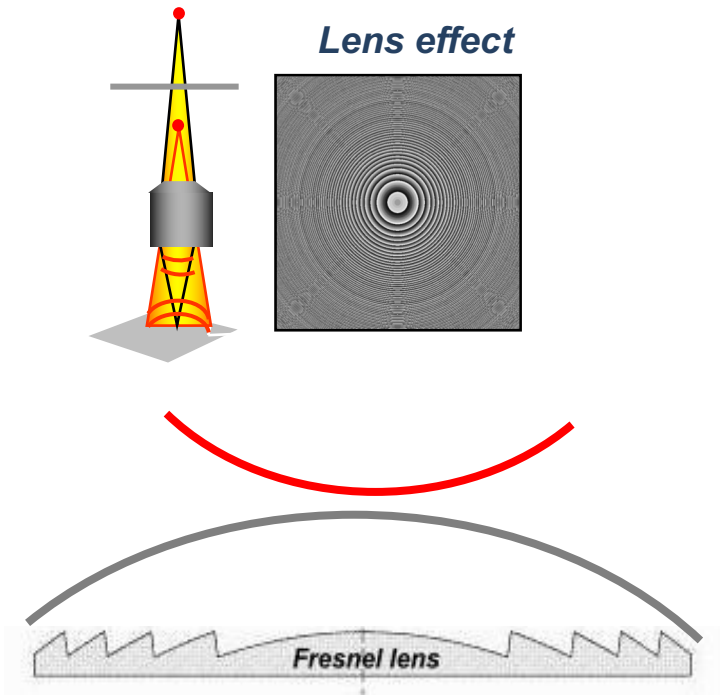
Haist et al. (1997)



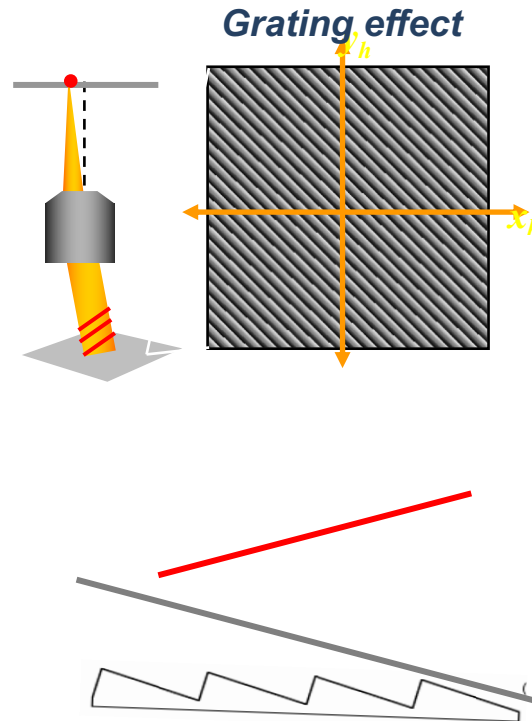
Hernandez et al. Nature communications (2016)

Dal Maschio et al. Neuron (2017)

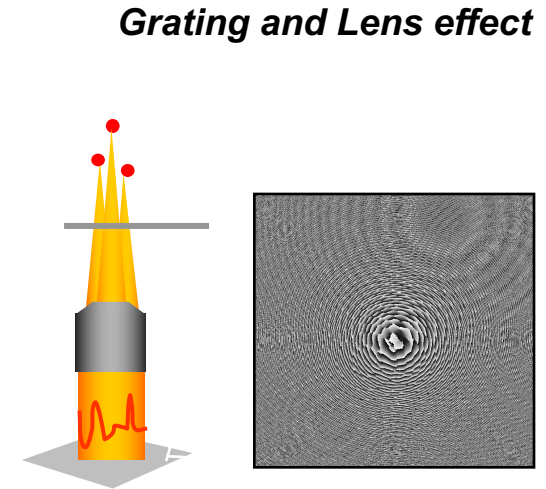
Prisms and Lenses



$$\phi_{lens}(x_h, y_h) = a' \cdot \Delta z (x_h^2 + y_h^2) \bmod 2\pi$$

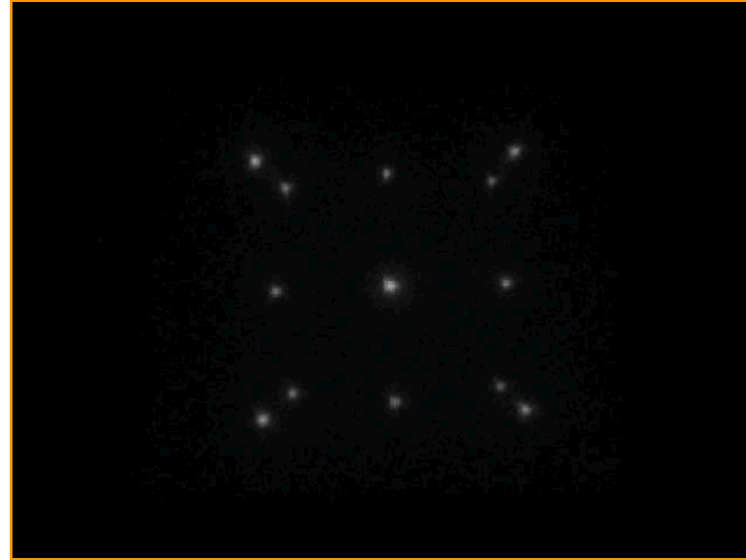


$$\phi_{grating}(x_h, y_h) = a(x_h \cdot \Delta x + y_h \cdot \Delta y) \bmod 2\pi$$



$$\phi_{tot} = \arg\left(\sum_{n=1}^N u_n e^{i(\phi_{grating} + \phi_{lens})_n}\right) \bmod 2\pi$$

Prisms and Lenses

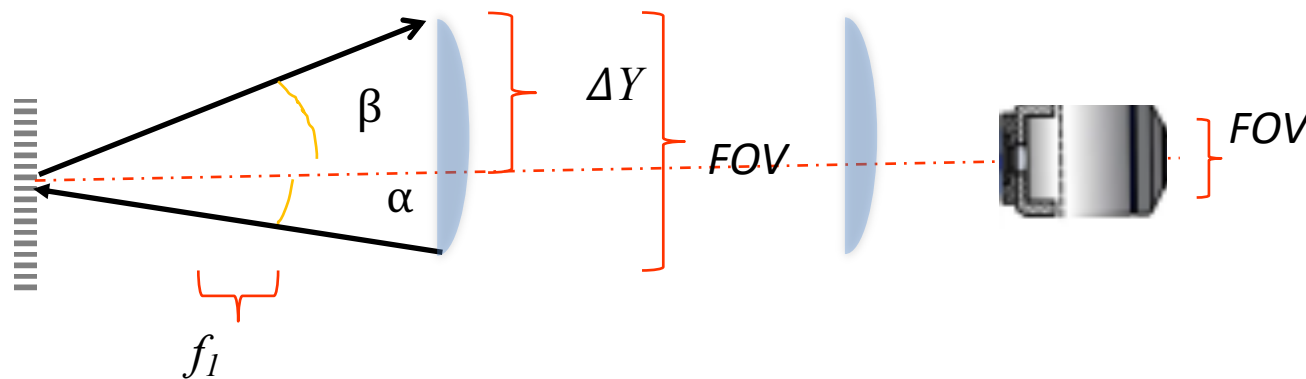


*For improved uniformity:
weighted Gerchberg and Saxton algorithm*

Di Leonardo et al. Optics Express 2006

Field of Excitation

- Field of excitation; FOV



$$\sin \alpha + \sin \beta = \frac{m\lambda}{d} = m\lambda f_0$$

$$\Delta Y \cong f_1 \cdot \lambda \cdot f_0$$

$$\Delta Y_{sample} \cong f_1 \cdot \lambda \cdot f_0 \frac{f_{obj}}{f_2}$$

$$f_{0max} = 1/2d_{SLM}$$

d_{SLM}



f_{0max}

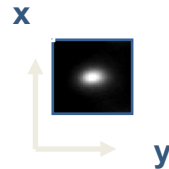
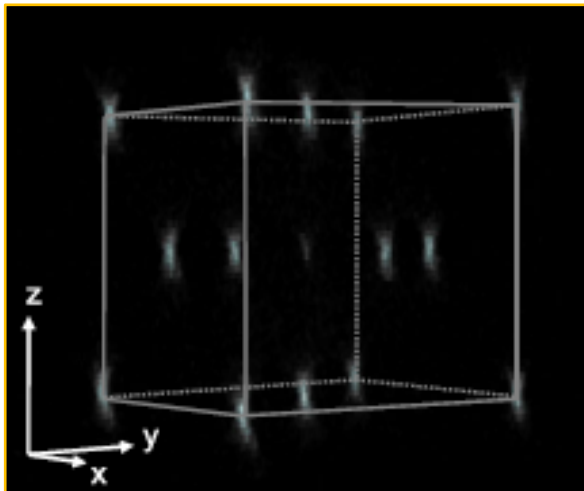
$$\Delta Y_{sample} \cong \frac{f_1 \cdot \lambda}{2d_{SLM}} \cdot \frac{f_{obj}}{f_2}$$

$$FOV = \left[2 \cdot \left(\frac{\lambda \cdot f_1}{2d_{SLM}} \right) \cdot \left(\frac{f_{obj}}{f_2} \right) \right] \times \left[2 \cdot \left(\frac{\lambda \cdot f_1}{2d_{SLM}} \right) \cdot \left(\frac{f_{obj}}{f_2} \right) \right] \sim 300 \times 300 \times 500 \mu m^3$$

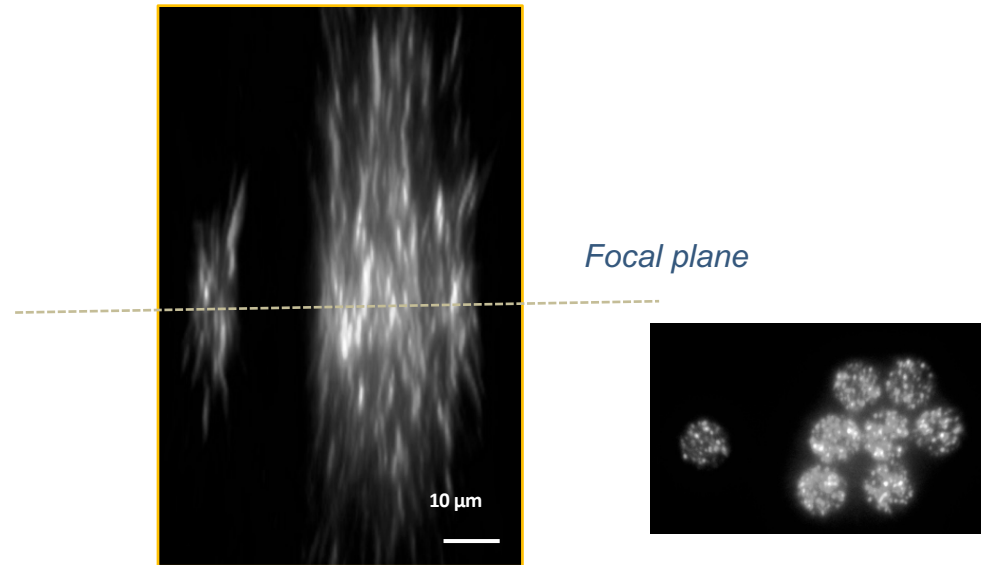
Axial Resolution

$$\Delta z(s) \propto \lambda / NA^2 \propto s^2 / \lambda$$

s = spot radius



$$z_R(s)_{2P} = \frac{2z_R\sigma}{\sqrt{(s^2 + \sigma^2)}} \propto \frac{s}{\lambda}$$



C. Lutz et al. Nature Methods (2018)

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

Axial resolution scales linearly with lateral spot size

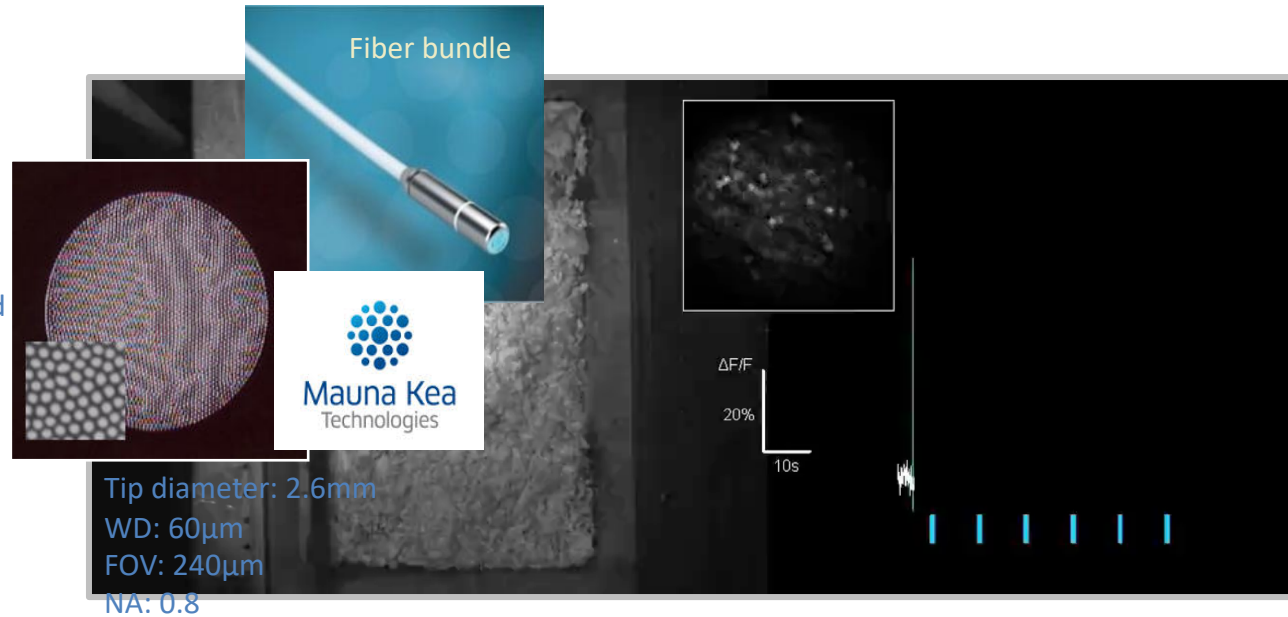
Holographic illumination in freely moving mice

2014

Neuron
NeuroResource

ChR2-GCaMP5 (souris éveillés)

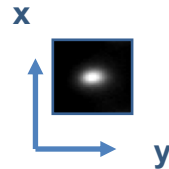
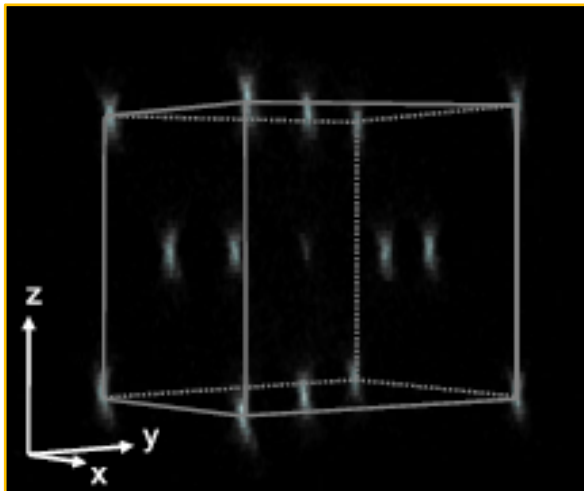
Photoactivation protocol repeated
every 30s for 5min ($P = 50 \text{ mW} / \text{mm}^2$, $P_i = 0.28 \text{ mW} / \text{mm}^2$)



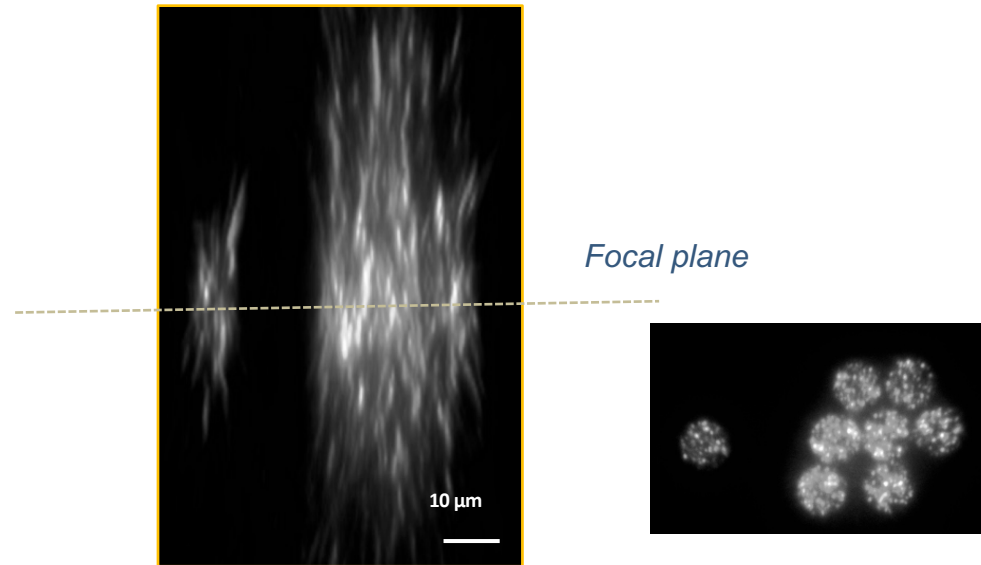
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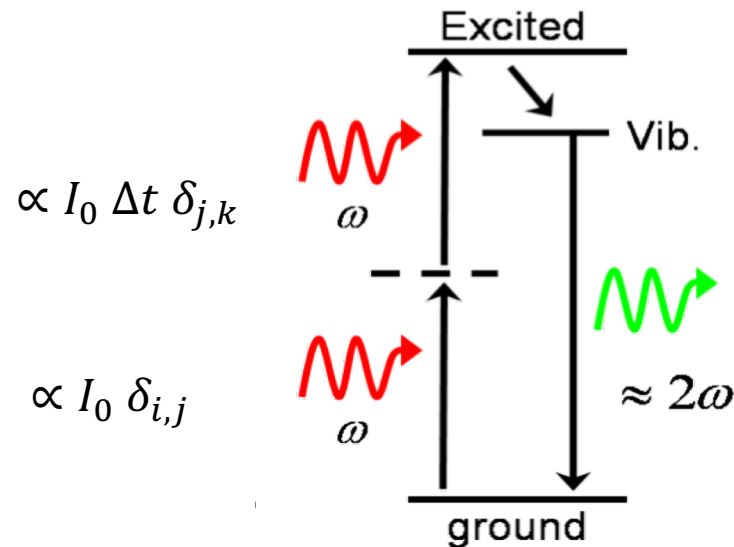
C. Lutz et al. Nature Methods (2018)

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

Axial resolution scales linearly with lateral spot size

Temporal focusing

Two-photon fluorescence



Two-photon absorption, is the process that promotes an atom or molecule from a ground (n) to an excited state (m) through the “quasi” simultaneous absorption of two photons, of frequency ω , and ω' through an intermediate very short-lived ($\Delta t \sim 10^{-16}$ s) “virtual” state .

$$2P \text{ absorption} \propto I(r, z, t)_0^2 \Delta t \delta_{i,k}^2$$

How do we get optical sectioning?

Spatial focusing

Power per surface unit

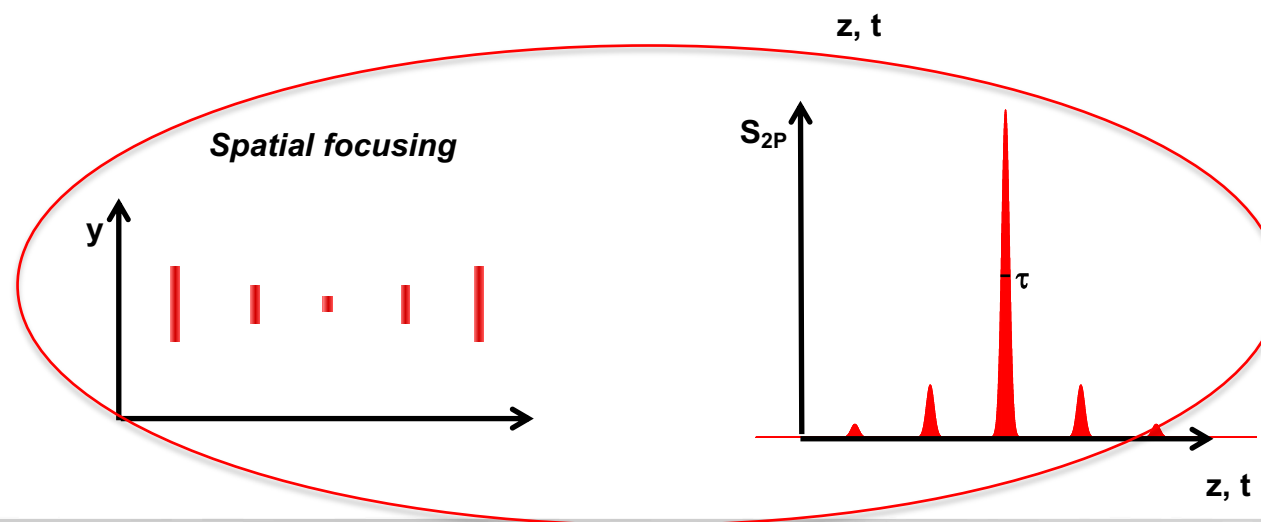
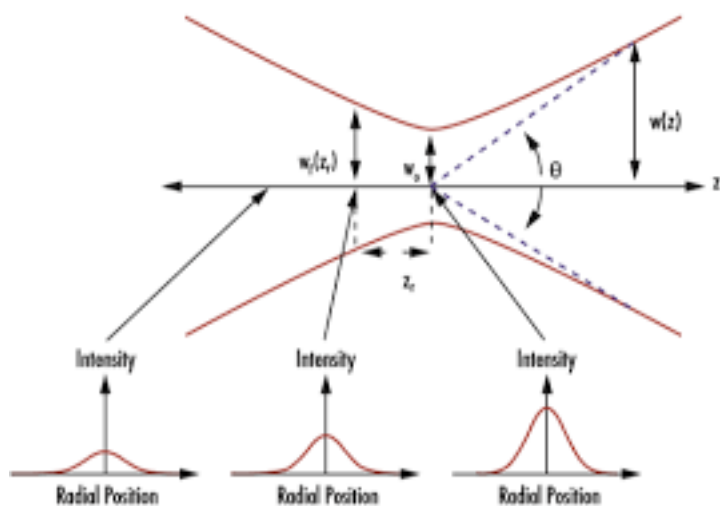
$$I_0(r, z, t) = 2 \frac{P(t)}{\pi w^2(z)} e^{-\frac{2r^2}{w(z)^2}}$$

$$\propto I(r, z, t)_0^2 \Delta t \delta_{i,k}^2 = I(r, z, t)_0^2 \sigma_{2P}$$

$$z_R = \text{Rayleigh range} = \pi w_0^2 / \lambda \propto \frac{\lambda}{NA^2}$$

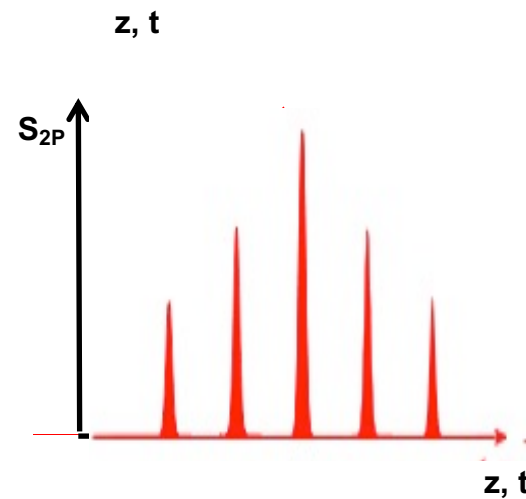
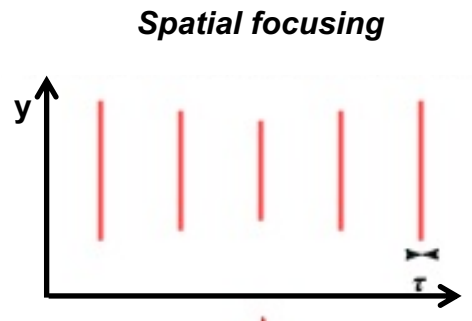
$$w(z) = w_0 \sqrt{1 + \left(\frac{z}{z_R}\right)^2}$$

$$S_{2P}|_S \propto \langle P(t)^2 \rangle \left(\frac{1}{w_0 \sqrt{1 + \left(\frac{z}{z_R}\right)^2}} \right)^2$$



Spatial focusing

$$S_{2P}|_S \propto \langle P(t)^2 \rangle \left(\frac{1}{w_0 \sqrt{1 + \left(\frac{z}{z_R} \right)^2}} \right)^2 \cong \text{const}$$



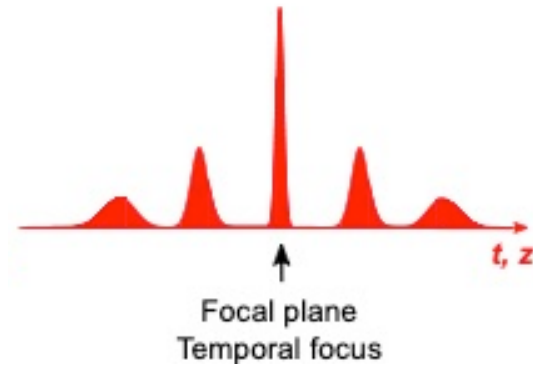
Weakly confined beams -> weak optical sectioning

Temporal focusing

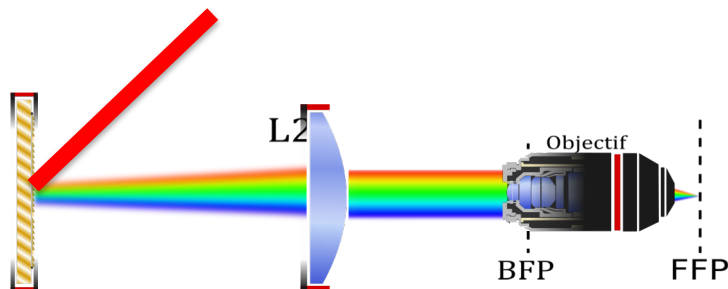
$$S_{2P}|_S \propto \langle P(t)^2 \rangle \left(\frac{1}{w_0 \sqrt{1 + \left(\frac{z}{z_R} \right)^2}} \right)^2 \cong \text{const}$$

$$S_{2P}|_S \propto \frac{\langle P(t) \rangle^2}{(\tau f)}$$

c Temporal Focusing



Temporal focusing



(D. Oron, et al, 2005; G. H. J. Zhu et al. 2005)

A dispersive grating placed at a plane conjugated with the objective focal plane generates a position dependent pulse broadening

Spatial focusing

$$S_{2P}|_S \propto \langle P(t)^2 \rangle \left(\frac{1}{w_0 \sqrt{1 + \left(\frac{z}{z_R} \right)^2}} \right)^2$$

Temporal focusing

$$S_{2P}|_S \propto \frac{\langle P(t) \rangle^2}{(\tau f)} \frac{1}{(\tau_0 f)} \left(\frac{1}{w_0^2 \sqrt{1 + \left(\frac{z}{z_R} \right)^2}} \right)$$

$$\tau(z) \approx \frac{2\sqrt{2\ln 2}}{\Omega} \left[1 + \frac{z^2}{z_R^2} \right]^{\frac{1}{2}}$$

(Durst et al, 2008)

$$z_R = \frac{2f_{ob}^2}{k_0(s^2 + \alpha^2 \Omega^2)}$$

Optical section of line scanning microscope

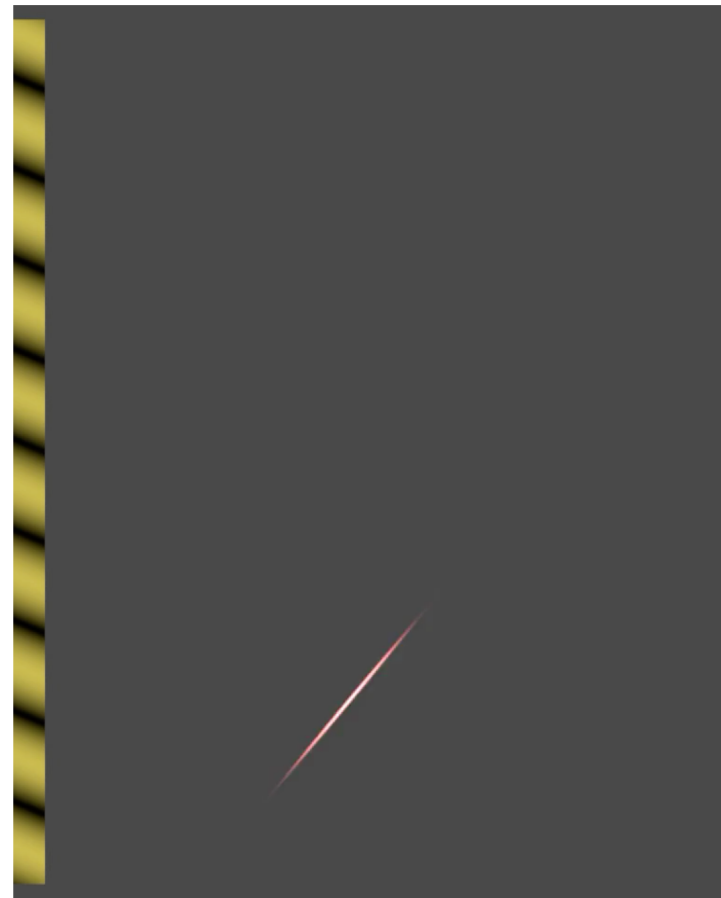
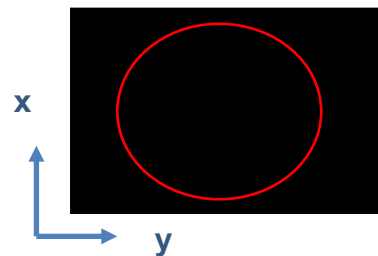
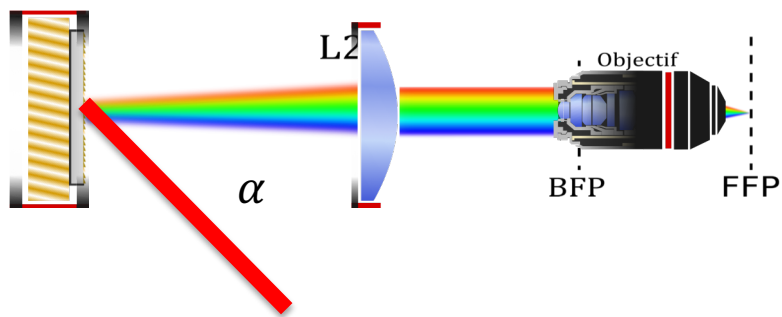
$$\Delta z = 2\sqrt{3}z_R$$

Temporal focusing

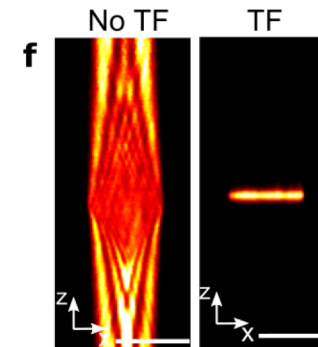
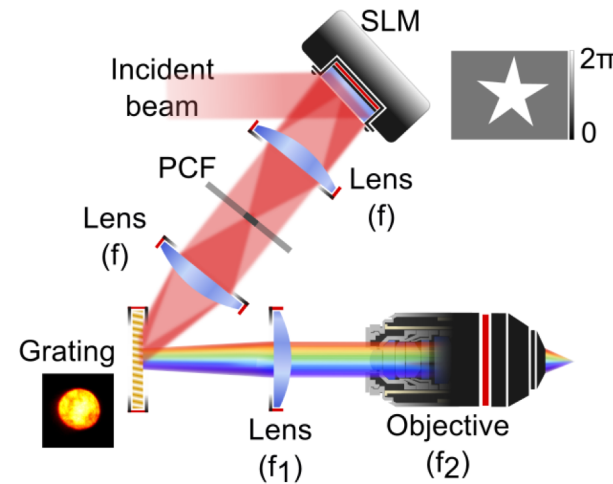
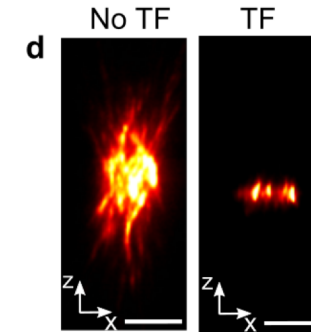
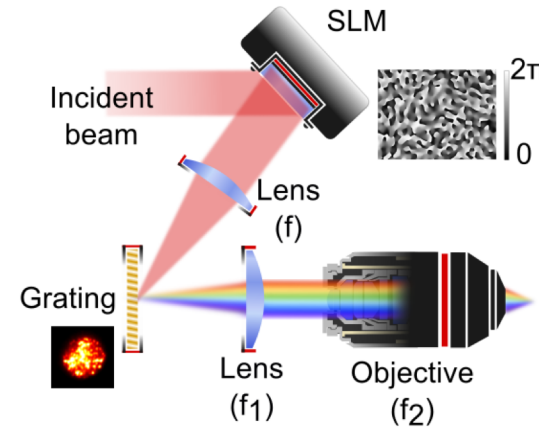
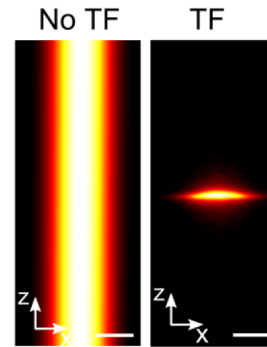
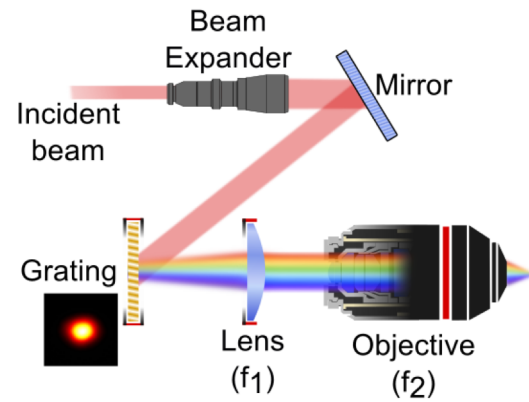
$$I_{line} \approx \left[1 + \left(\frac{z}{z_R} \right)^2 \right]^{-0.5}$$

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

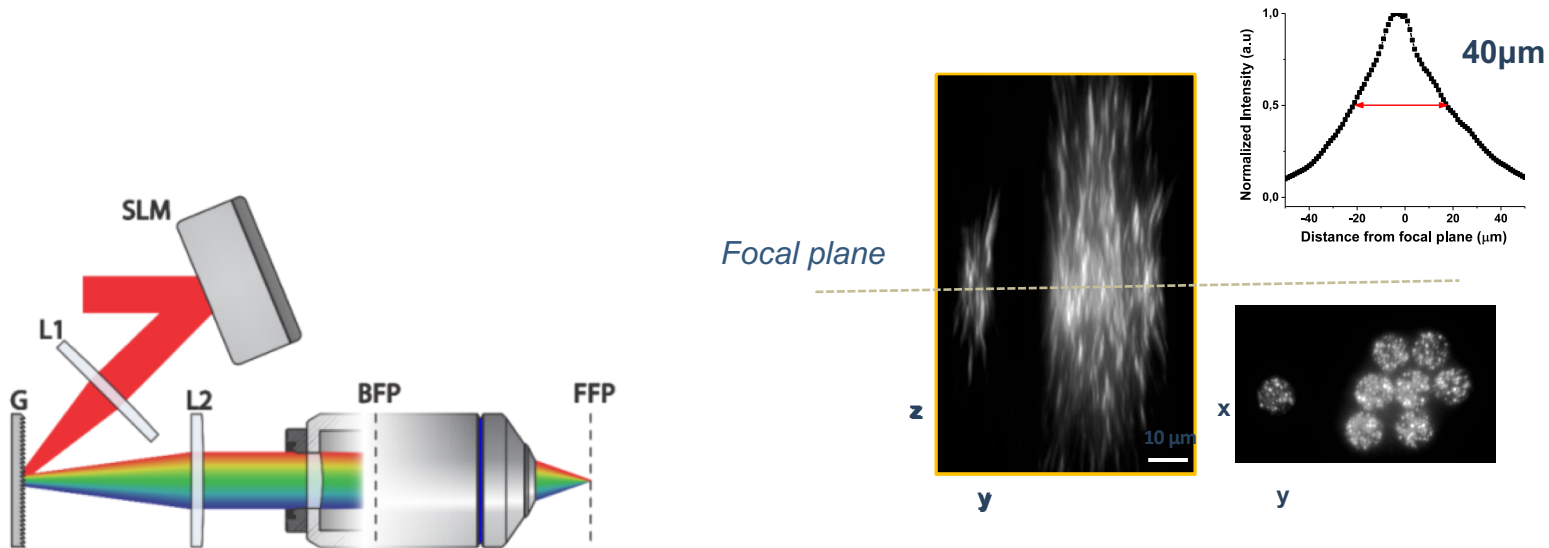
$$v_{speed} = \frac{c}{\sin \alpha} M \quad (5ps \text{ for } 20\mu m)$$



Light delivering approaches



Temporal focusing and holographic illumination



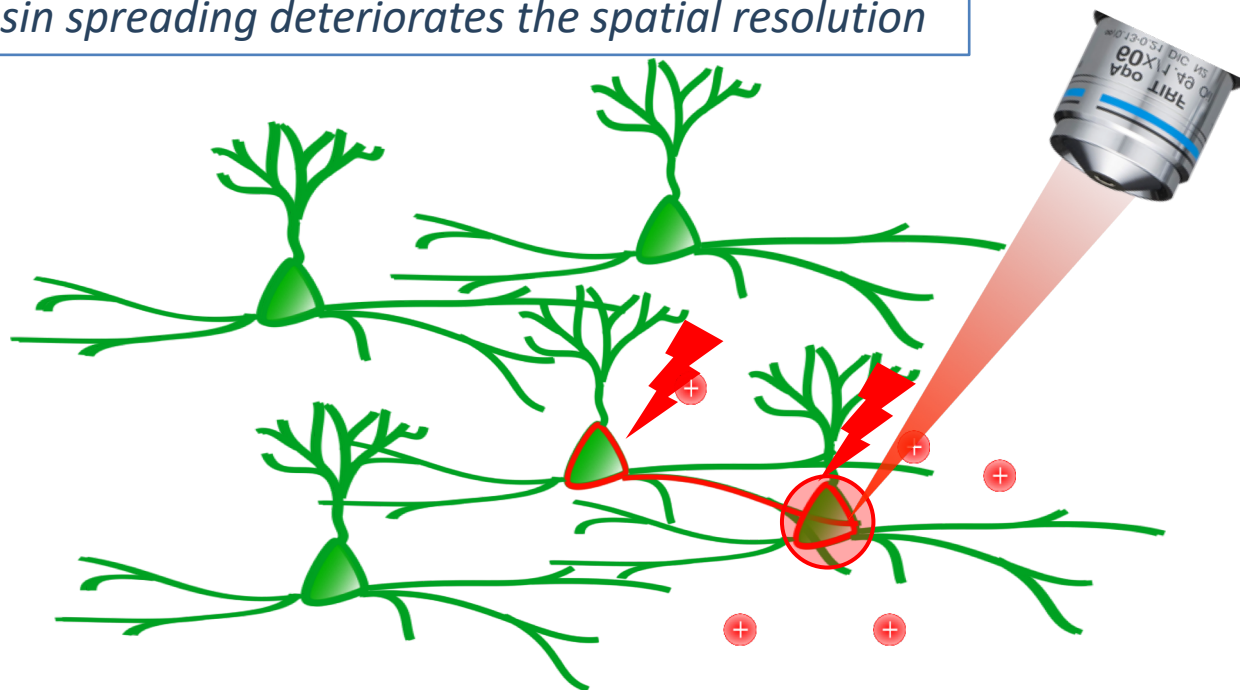
Axial resolution independent on lateral spot size

Take-home message

- *Wave front shaping using liquid crystal spatial light modulators enables precise lateral control of light patterning*
- *Existing algorithms, enable generation of 2D and 3D arbitrary light patterning*
- *Temporal focusing can be achieved using a dispersive grating conjugated with the sample plane, enables the control of axial resolution independently on lateral light shaping; axial resolution of a line scanning microscope*

Cellular resolution

Opsin spreading deteriorates the spatial resolution



Need for molecular focusing: soma-targeted opsins

Baker et al. *elife* 2016

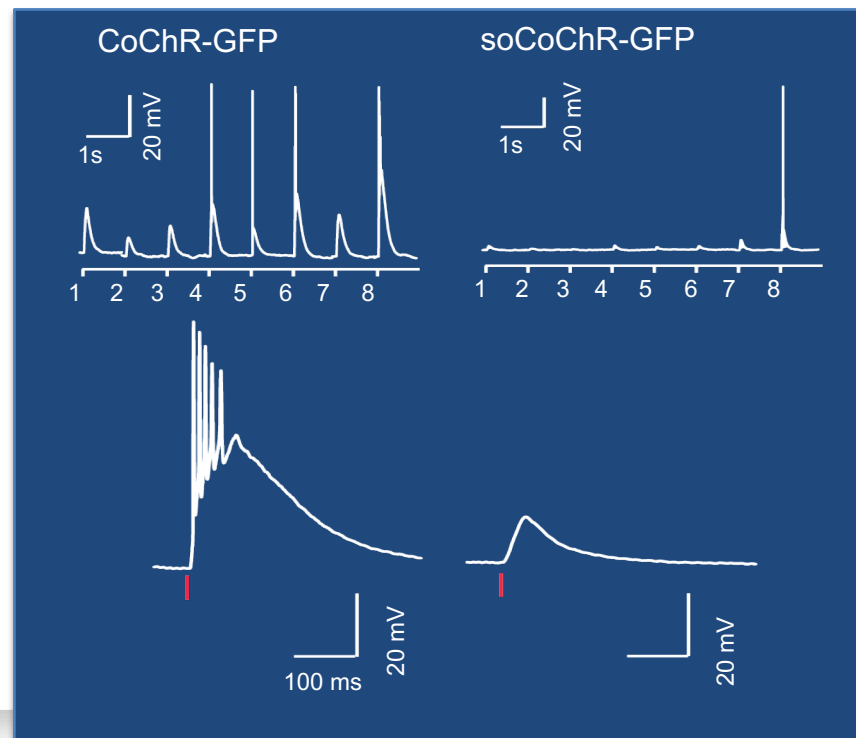
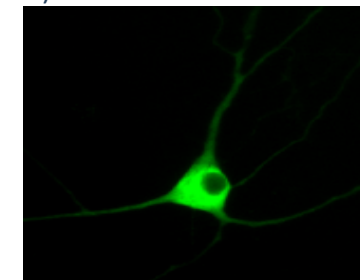
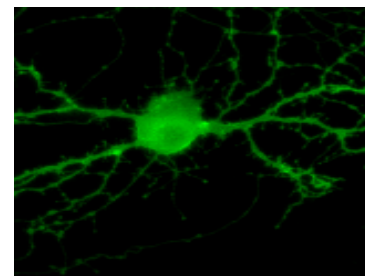
Shemesh, Tanese, Zampini et al. *Nature Neuroscience*, 2017

Mardinly et al. *Nature Neuroscience*, 2018

Forli et al. *Cell Report*, 2018

Collaboration with Ed. Boyden, MIT

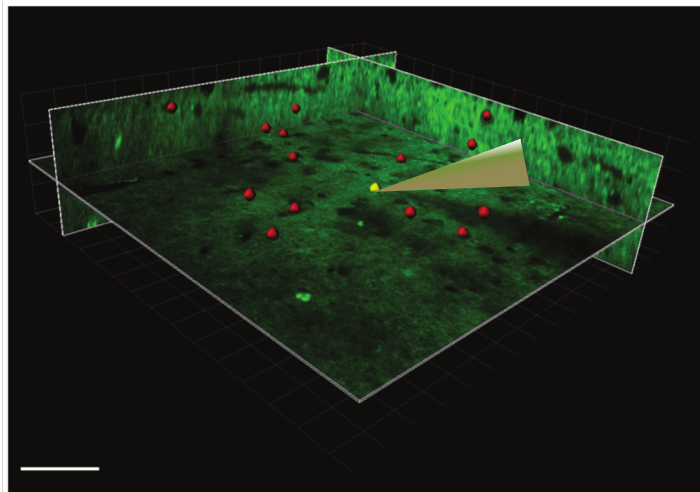
soCoChR-GFP
(N of KA2(1–150) to C GFP-CoChR)



Cellular resolution

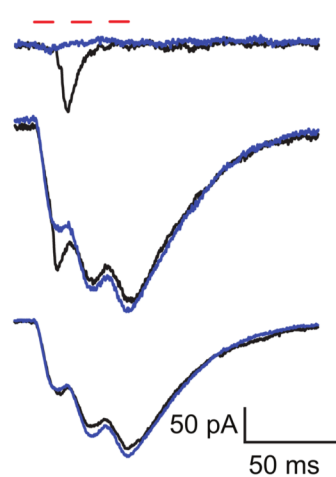
SEQUENTIAL EXCITATION

CoChR-GFP



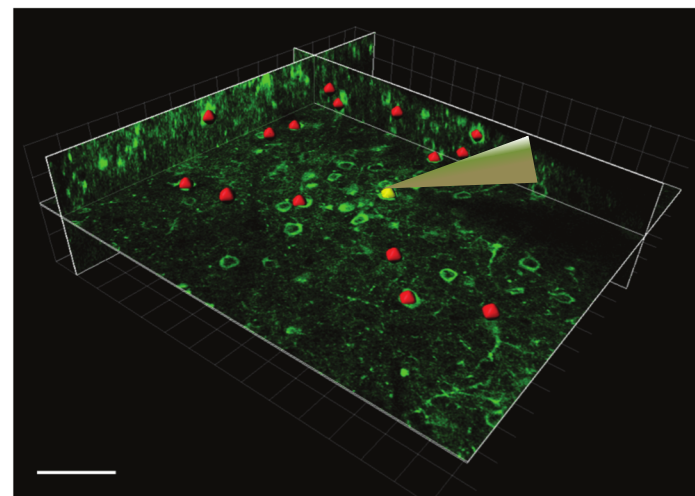
Before synaptic receptors blockers
In synaptic receptors blockers

CoChR-GFP

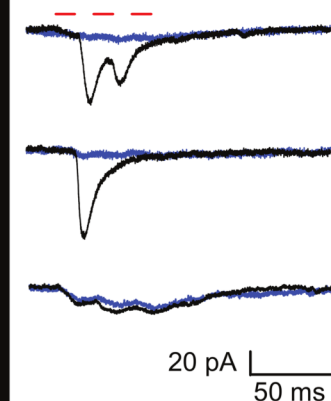


10 ms; 50 Hz
130-180 $\mu\text{W}/\mu\text{m}^2$

soCoChR-GFP



soCoChR-GFP

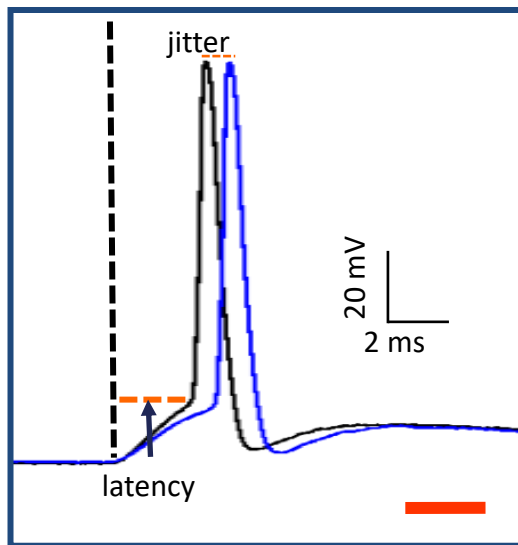


$71 \pm 14 \%$

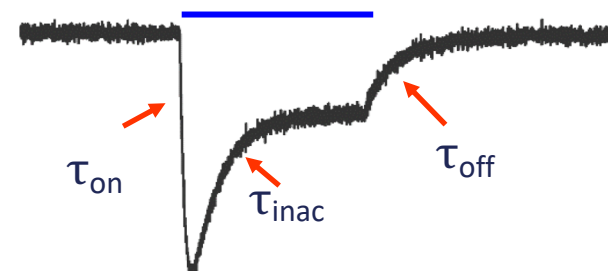
Temporal resolution and precision

Temporal resolution: time interval to get two subsequent spikes

Temporal precision: standard deviation of latency: jitter



$$\tau_{on} \approx \frac{1}{(\sigma I^2 + \frac{1}{\tau_{off}})}$$



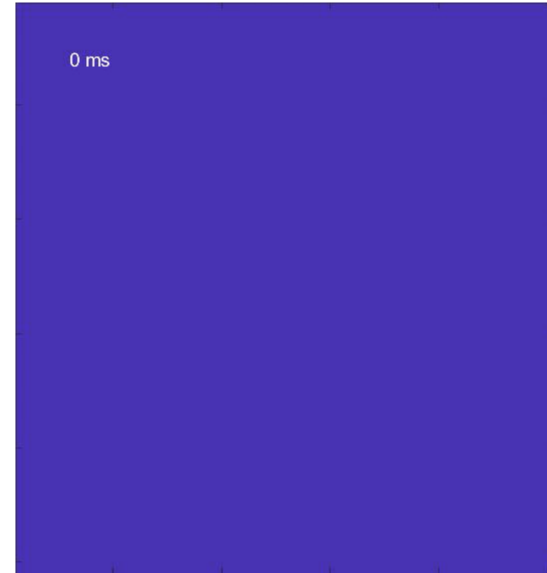
	Chrimsonf	ChR2	ChrimsonR	C1VI
	ChroMe			
	Chronos		CoChR	ReaChR
τ_{on} (ms)	0.73		2.4	8
τ_{inact} (ms)	9.3		200	443
τ_{off} (ms)	4.2		31	150

- Optimizing temporal resolution and precisions requires reducing the illumination time and knowing the opsin photocycle

Slow or fast opsins?

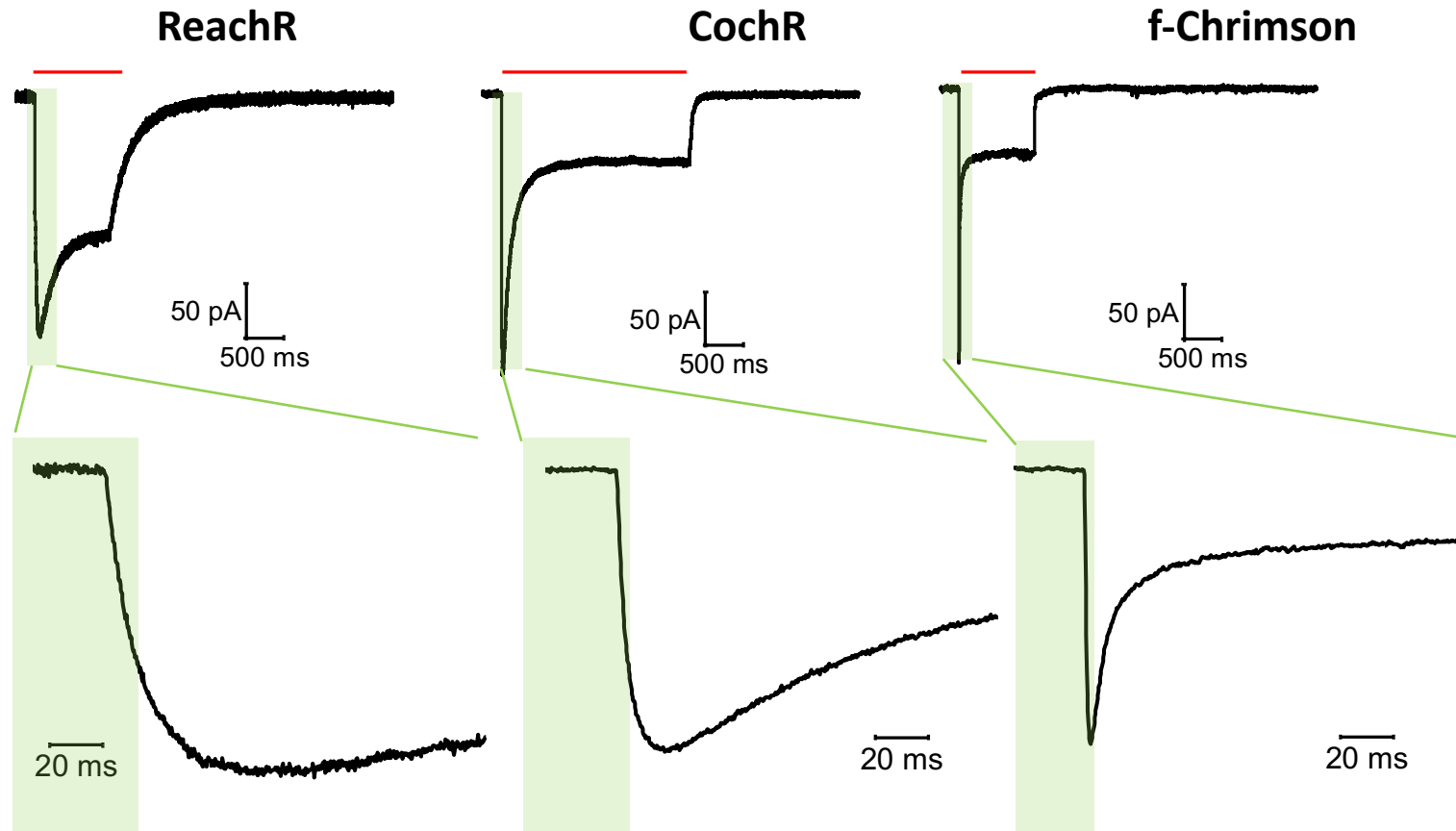


f-Chrimson ($\tau_{\text{off}} \approx 6 \text{ ms}$)



ReaChR ($\tau_{\text{off}} \approx 150 \text{ ms}$)

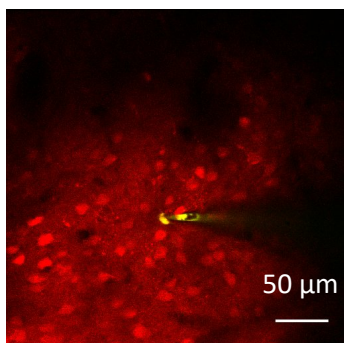
Slow or fast opsins?



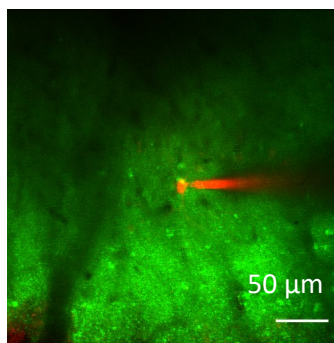
@saturation ($I \geq 90\% I_{max}$)

Temporal resolution and on-kinetics

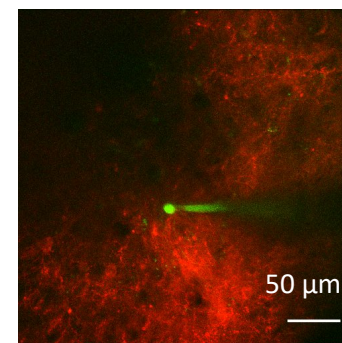
ReaChR ($\tau_{\text{off}} \approx 90$ ms)



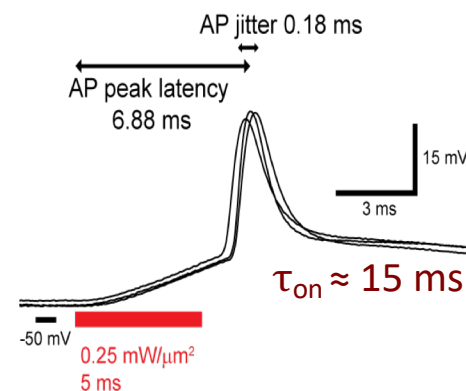
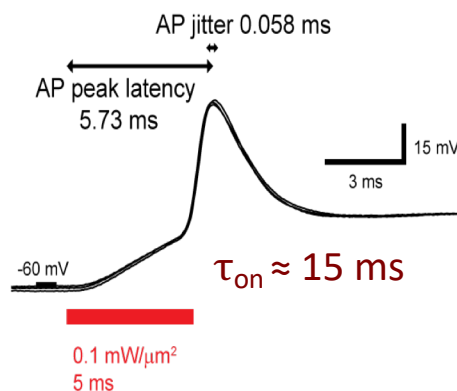
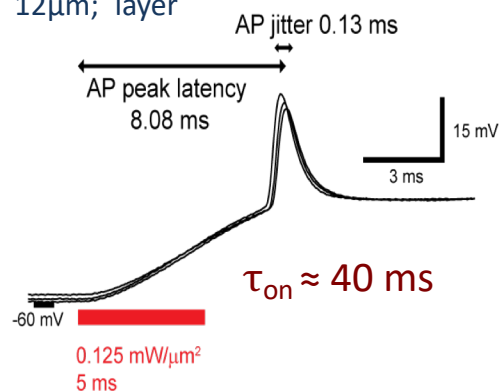
CoChR ($\tau_{\text{off}} \approx 30$ ms)



ChrimsonR ($\tau_{\text{off}} \approx 19$ ms)

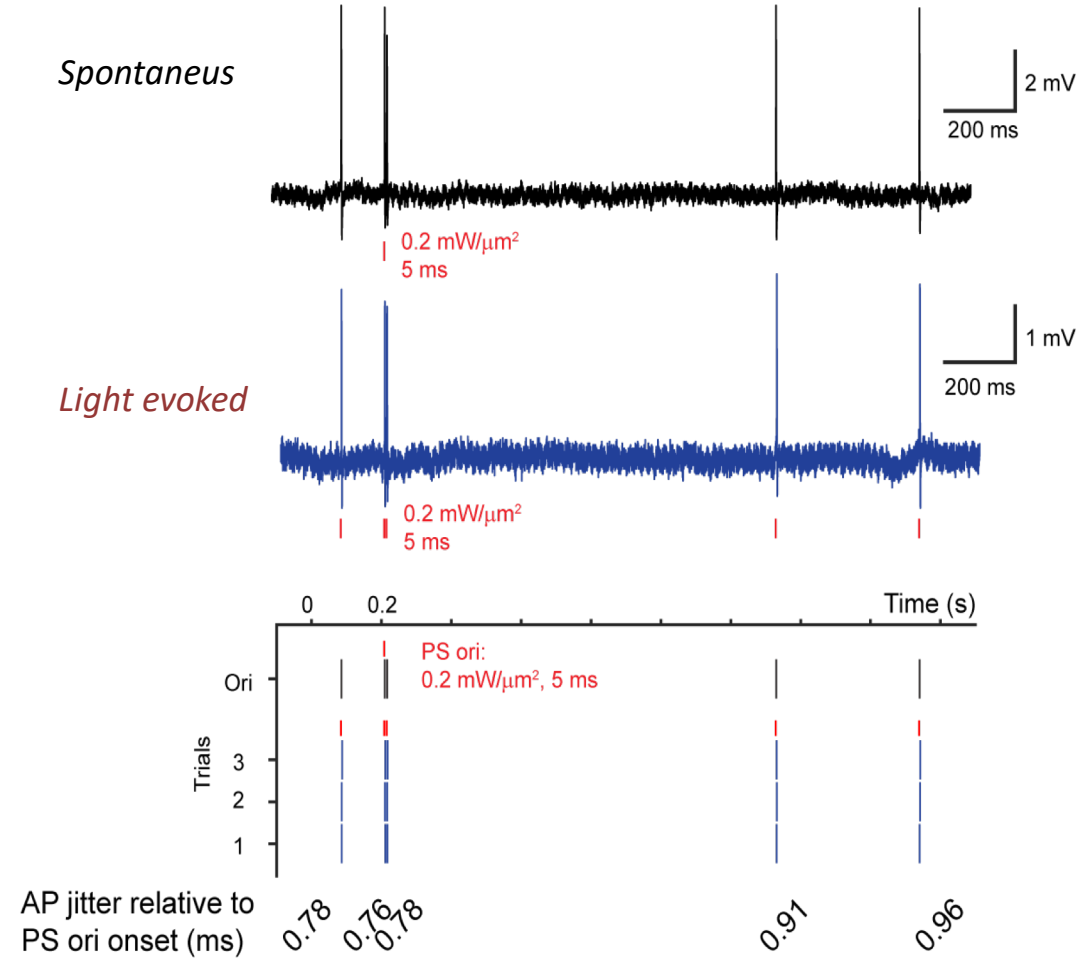
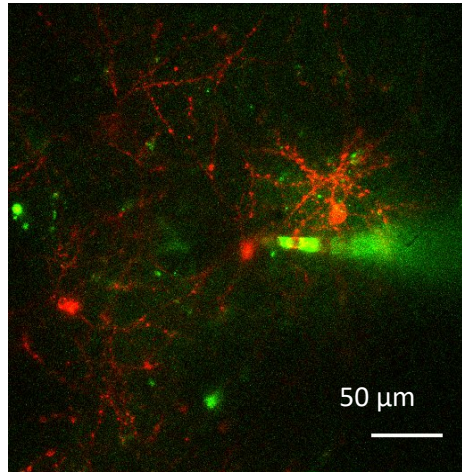


$\lambda=1030\text{nm}$; 500kHz; spot size $12\mu\text{m}$; layer
2/3 visual cortex

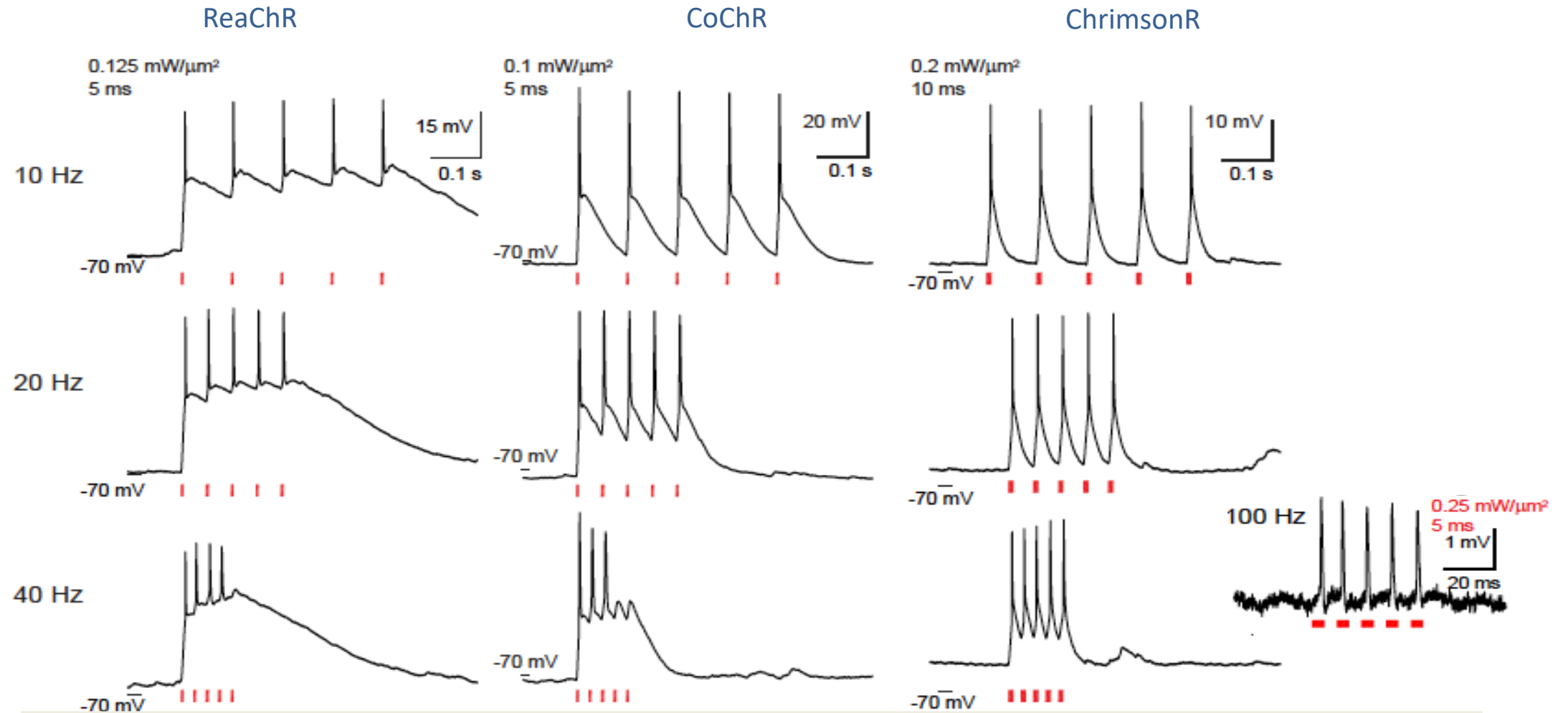


Sub-millisecond jitter independently on opsin- 'on-kinetics'

Temporal resolution and on-kinetics

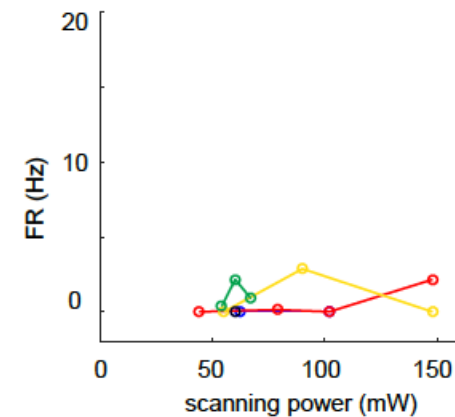
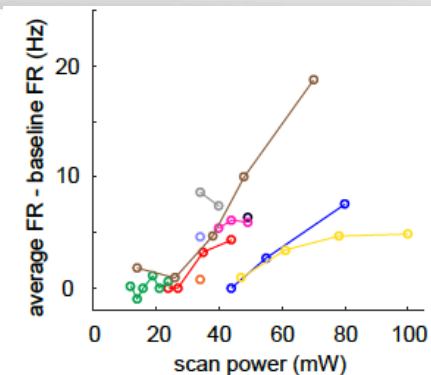
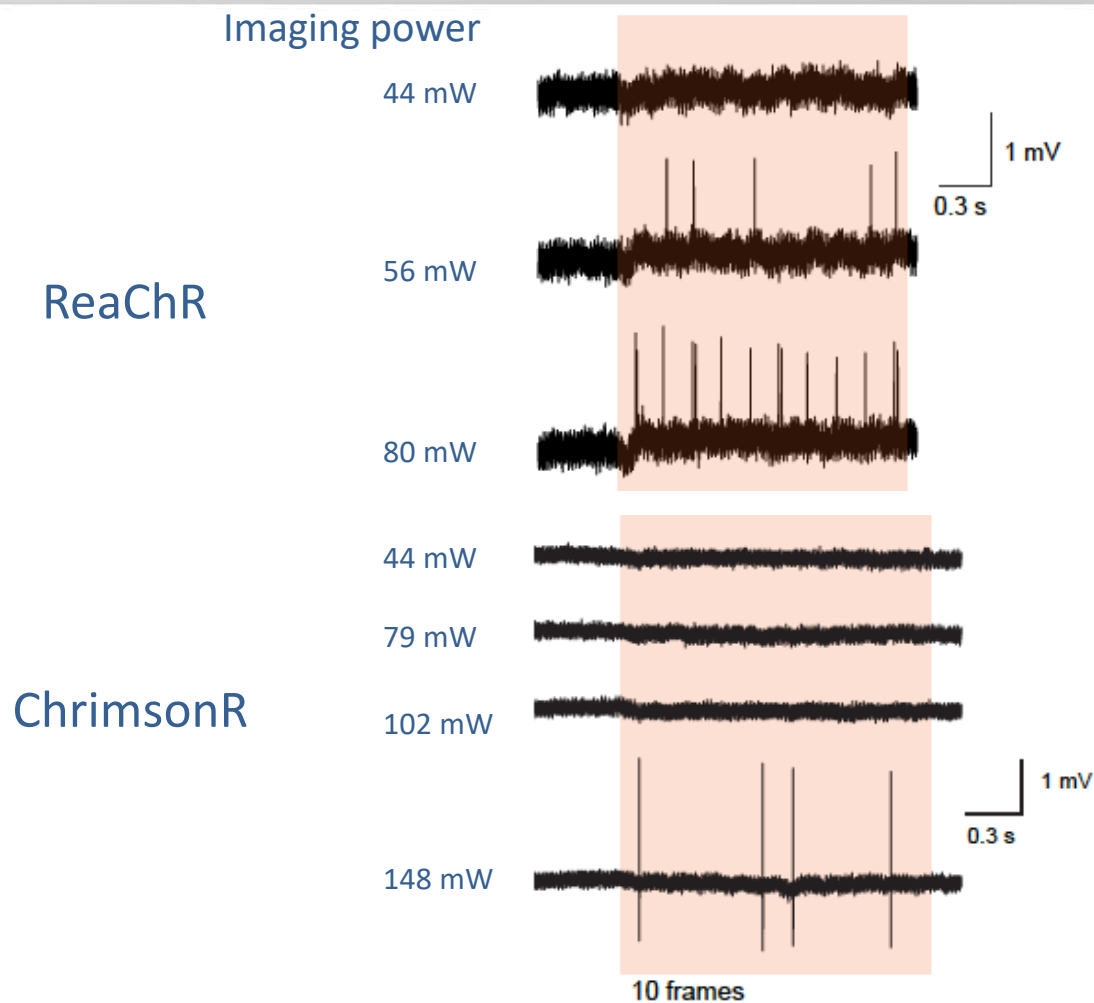


Temporal resolution and off-kinetics



The opsin-'off-kinetics' affects the maximum achievable spiking rate

Slow or fast opsins?

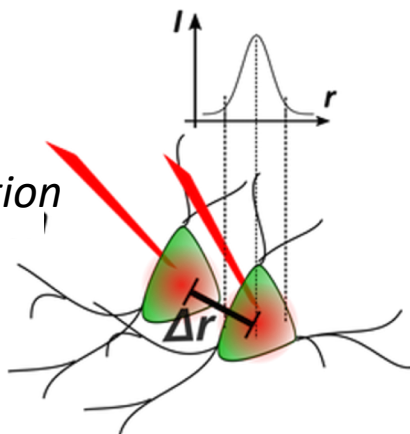


The opsin-'off-kinetics' limits the imaging power threshold

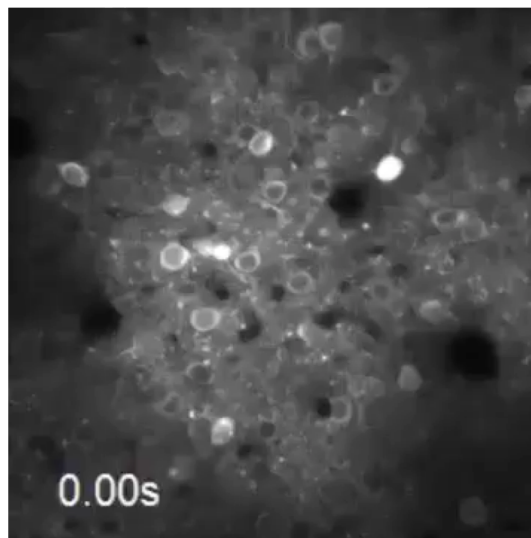
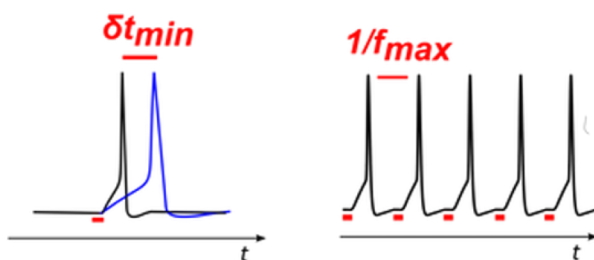
Take-home message

- *Light and molecular focusing enable neuronal control with single cell precision*
- *2P-parallel illumination enables illumination time shorter than the current rise time thus millisecond temporal resolution and sub-millisecond temporal precision can be achieved independently on the opsin kinetics*
- *2P-parallel illumination combined with fast opsins (Chronos, ChrimsonR, f Chrimson) enables sub-millisecond temporal precision also at high spiking rate (up to 100 Hz)*
- *Fast opsins: enable reducing cross talk and optimize axial resolution*

✓ *Spatial resolution*



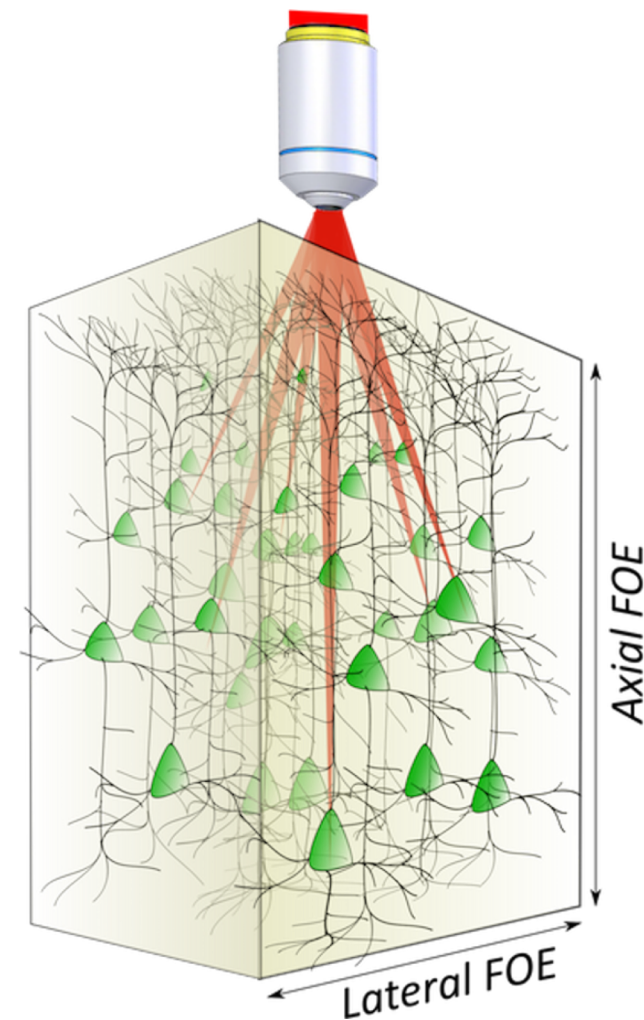
✓ *Temporal resolution, precision resolution*



Visual cortex V1
(TW-Chen & GENIE prj Janelie)

3D, multiscale light patterning

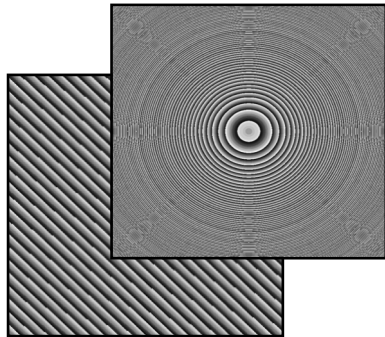
In depth



3D manipulation on large volumes

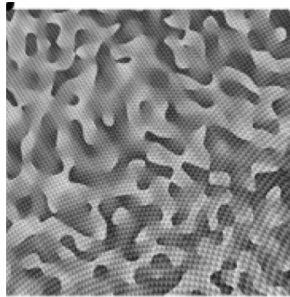
Temporal resolution independent of target number, requires parallel illumination

Prisms and lenses)

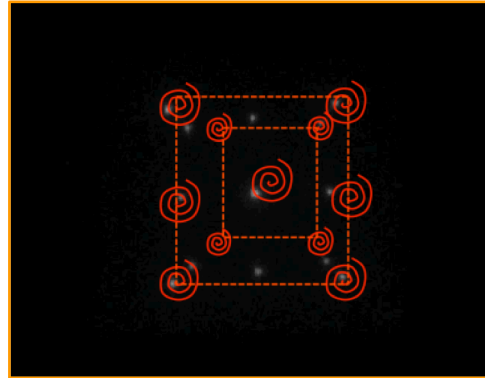


Leach et al. (2006)

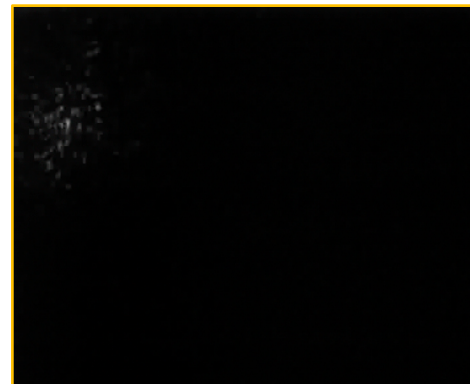
3D-GS



Haist et al. (1997)



Daria et al. Appl. Phys. Lett (2009)
S. Yang, et al., J Neuronal Engineering, (2011)

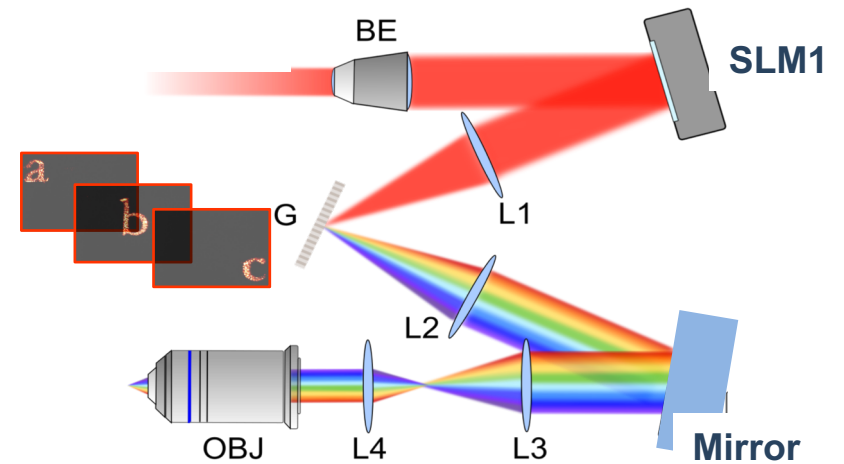


Hernandez et al. Nature communications (2016)

Dal Maschio et al. Neuron (2017)

Packer et al. Nat. methods (2012)

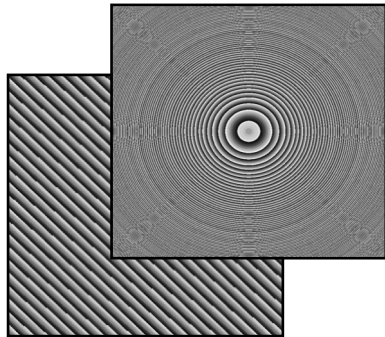
Yang et al. Elife (2018)



3D manipulation on large volumes

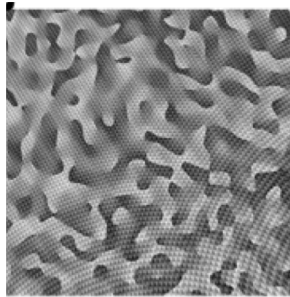
Temporal resolution independent of target number, requires parallel illumination

Prisms and lenses)

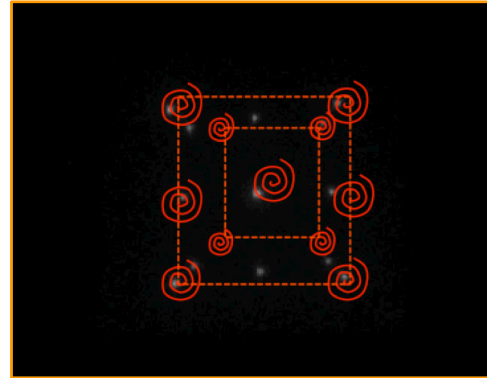


Leach et al. (2006)

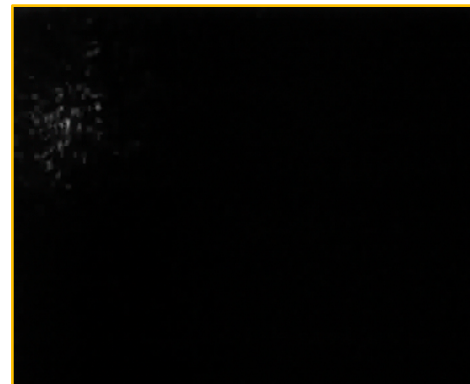
3D-GS



Haist et al. (1997)



Daria et al. Appl. Phys. Lett (2009)
S. Yang, et al., J Neuronal Engineering, (2011)

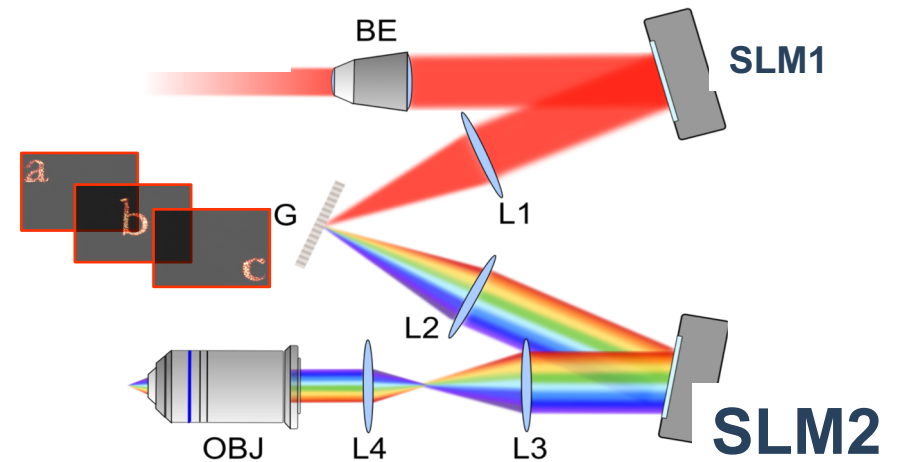


Hernandez et al. Nature communications (2016)

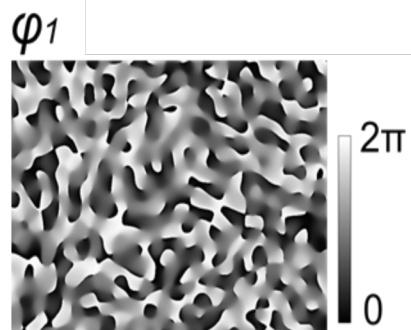
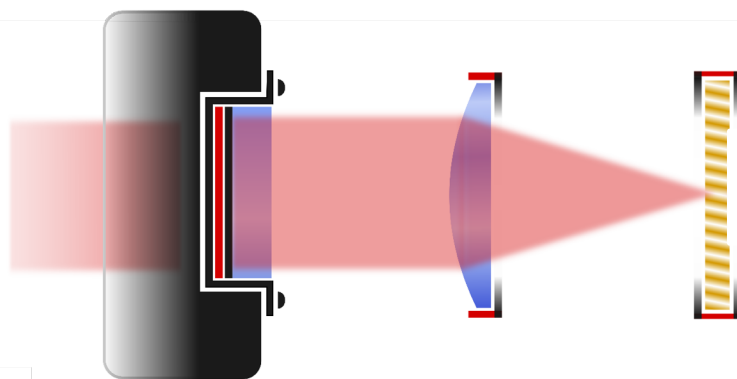
Dal Maschio et al. Neuron (2017)

Packer et al. Nat. methods (2012)

Yang et al. Elife (2018)

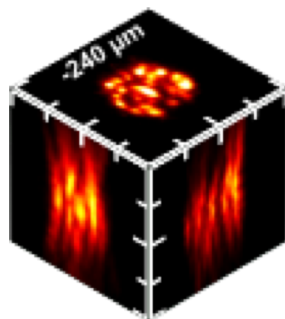
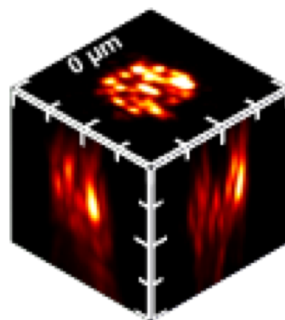
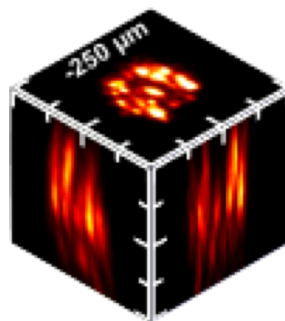
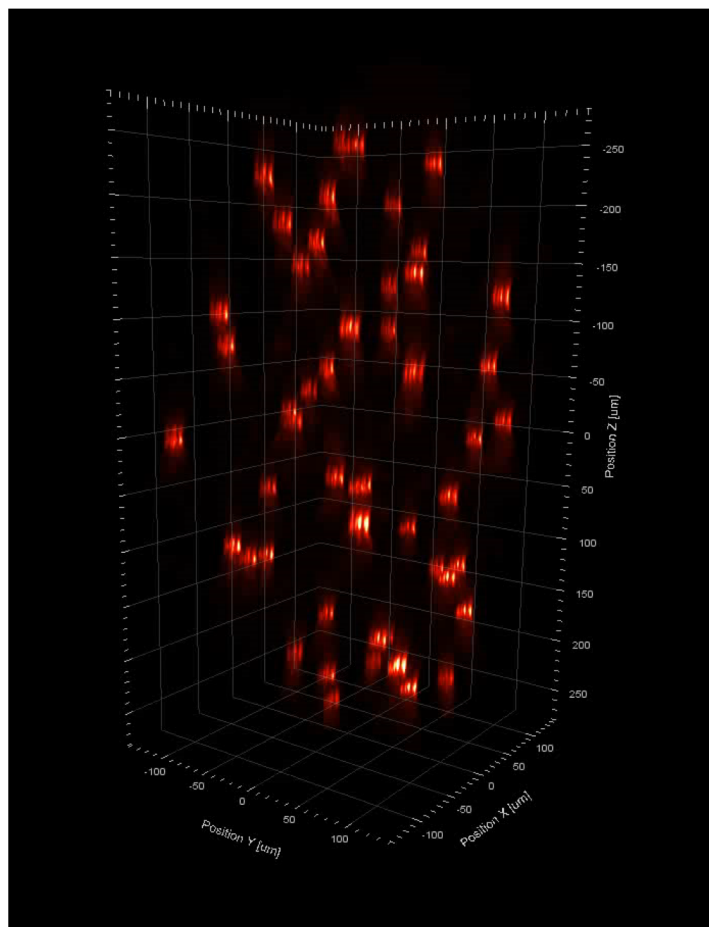


3D manipulation on large volumes



3D manipulation on large volumes

50 Spots of 15 μm



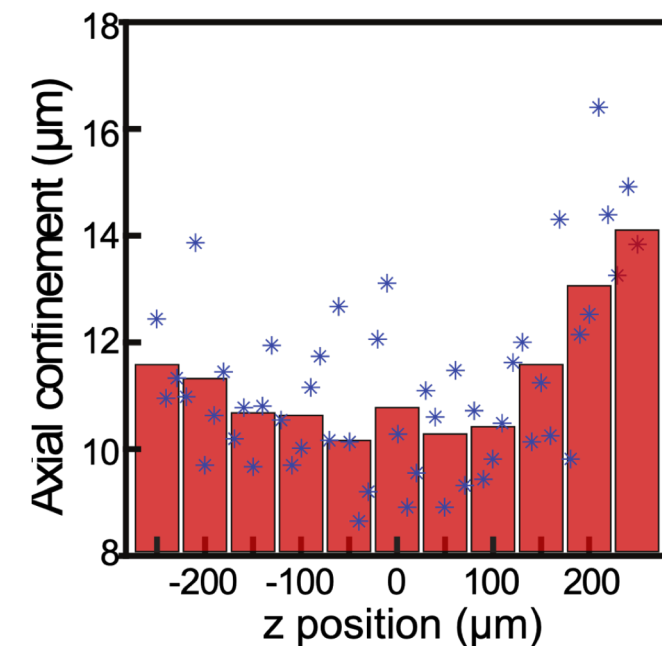
Field of View :

300 x 300 x 500 μm

Th: 750 x 750 x 950 μm

Axial Resolution :

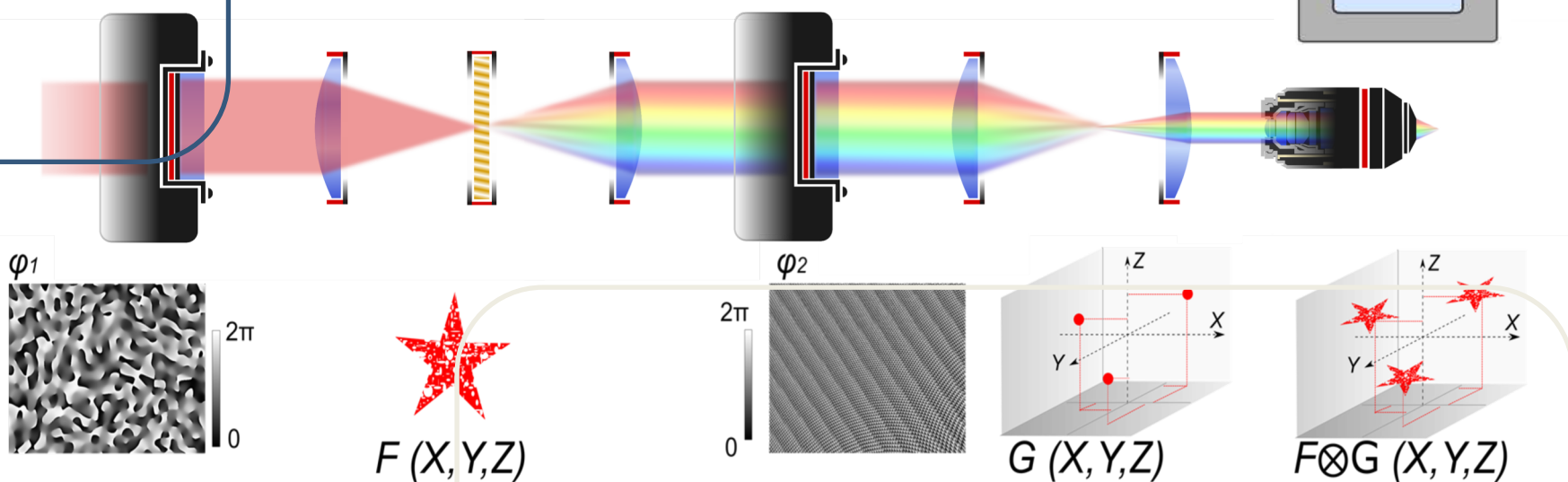
11 μm Average value



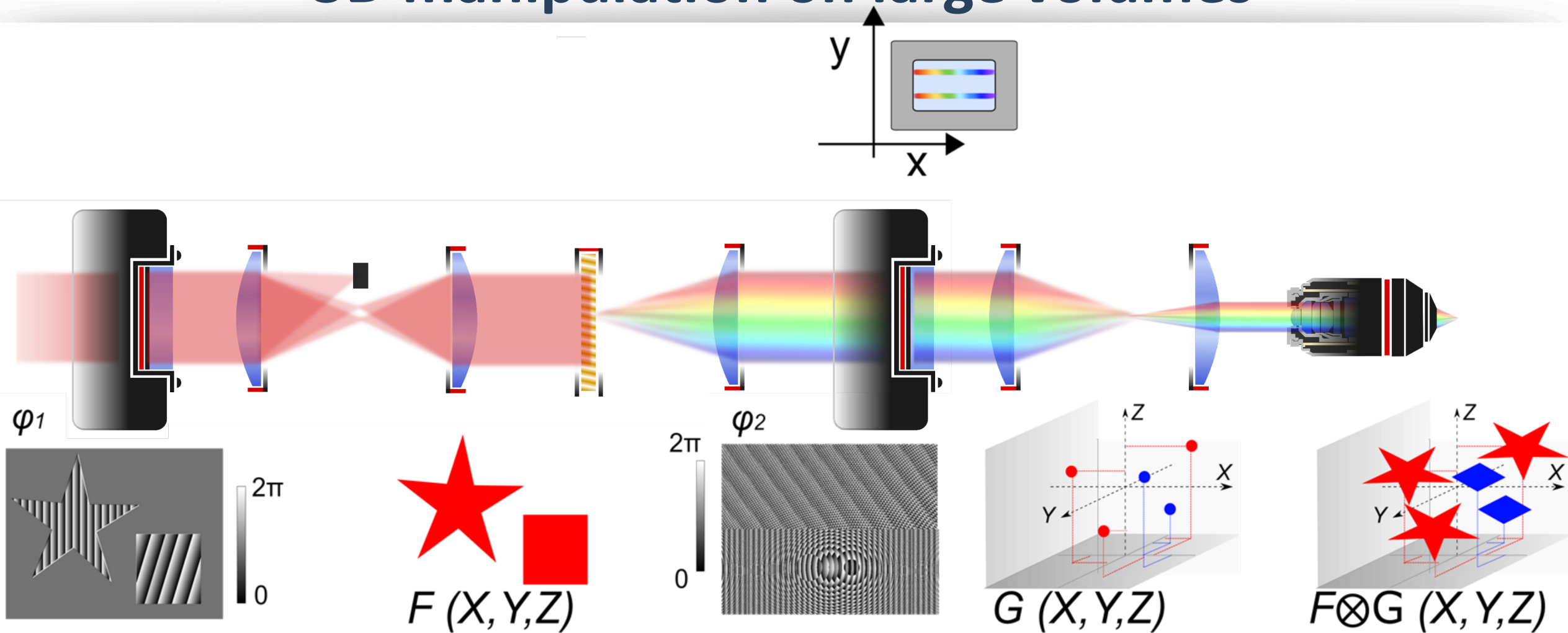
3D manipulation on large volumes

Replaced by

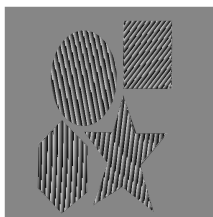
- Fixed phase mask
- Generalized phase contrast
- Gaussian beam (*Pegard et al. 2017; Sun et al. 2017*)
- Phase/Amplitude



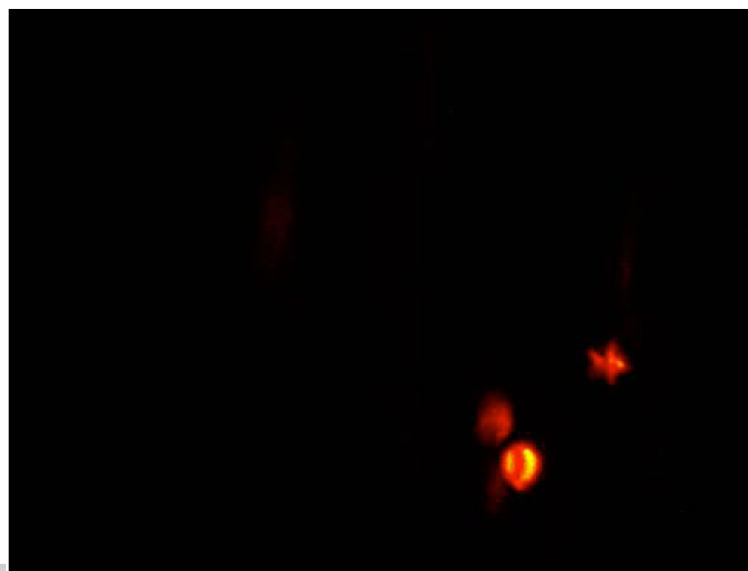
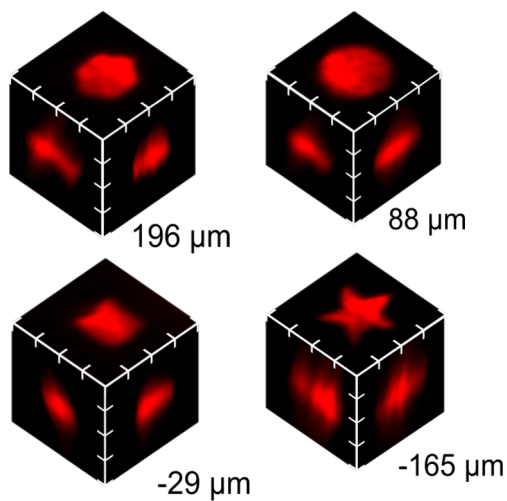
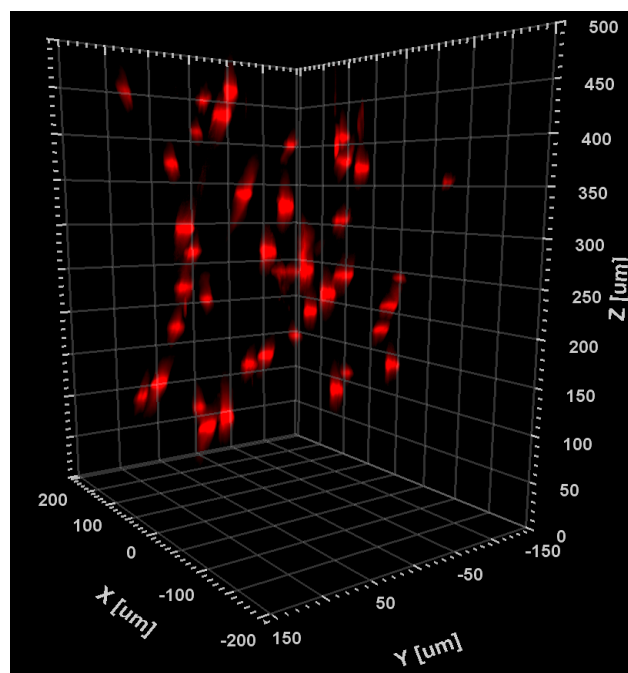
3D manipulation on large volumes



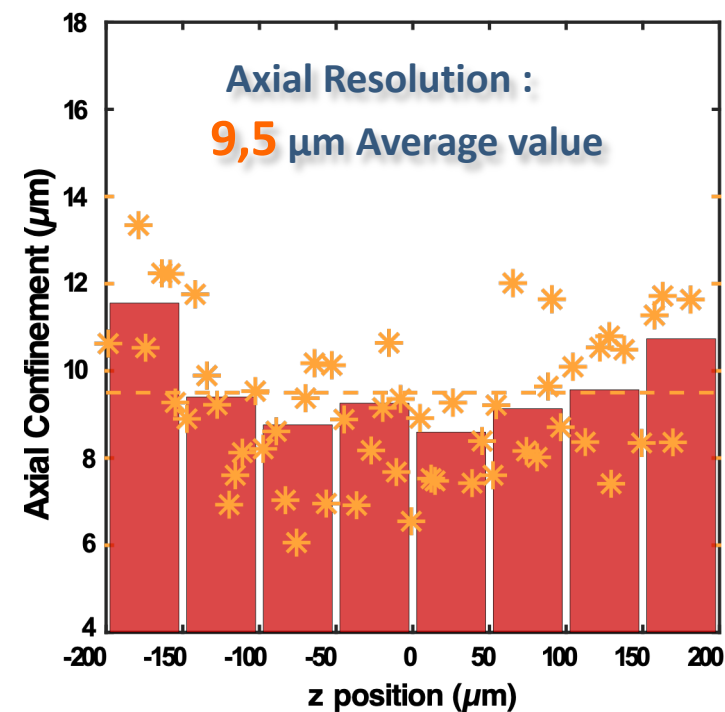
3D manipulation on large volumes



40 Spots of 15 μm



Field of View :
300 x 300 x 400 μm



3D manipulation on large volumes

TECHNIQUES CRITERIA		MTF - CGH	MTF - CGH Phase Mask	MTF - GPC	MTF - MS	MTF Gaussian
Complexity of the System						
	Number of SLMs	2	1	2	2	1
	Phase Mask	0	1	1	0	0
	Alignment	●	●	●	●	●
Flexibility						
	Change spot easily	YES	NO	YES	YES	NO
	MultiShape	NO	NO	NO	YES	NO
SLM2 Illumination						
	Surface in %	100%	100%	12%	48%	12%
Power Available						
	Total Transmission	40%	50%	40%	25%	40%
	Method limitation	None	None	< 0.2 W	< 0.8 W	< 0.2 W
Spot Quality						
	Speckle	YES	YES	NO	NO	NO
	Sharpness	●	●	●	●	●
Axial Resolution						
	Average Value	11 μ m	11 μ m	6 μ m	9.5 μ m	6 μ m

Take-home message

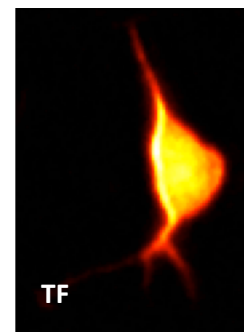
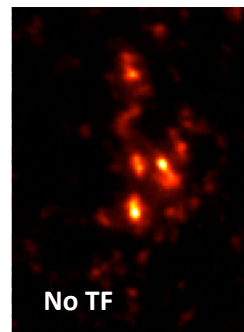
- *Multiplexing temporal focusing light shaping enables spot generation at multiple plane within $\sim 300 \times 300 \times 500 \mu\text{m}^3$ Volume*
- *Compatible with multiple variants*

How deep?

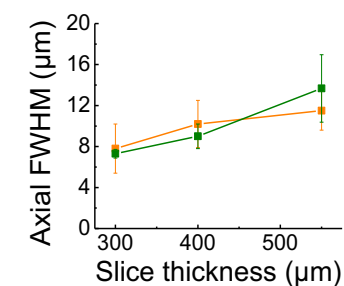
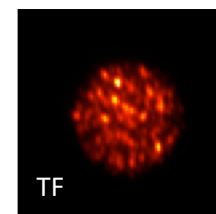
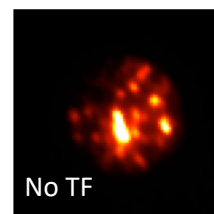
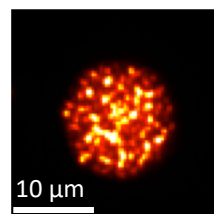
No tissue



After 550 μm of tissue

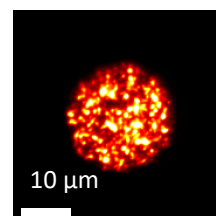


Coronal cortical acute slices of P21 rat

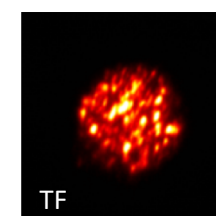
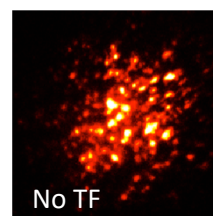


Zebrafish brain

No tissue

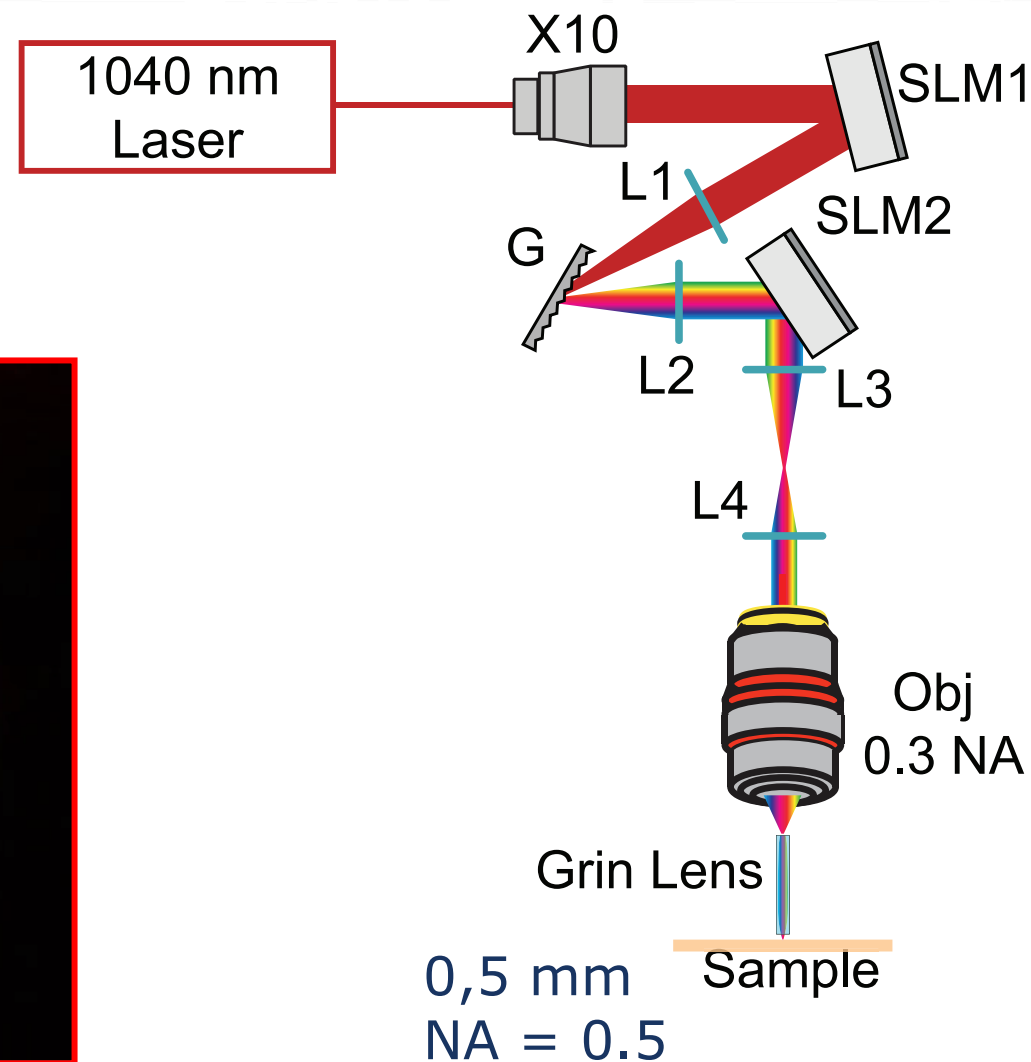


After all brain

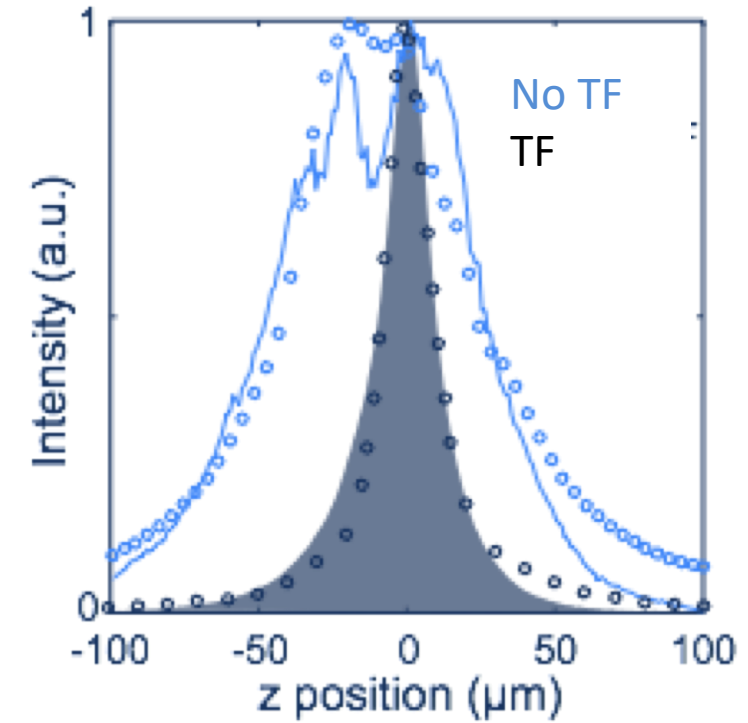
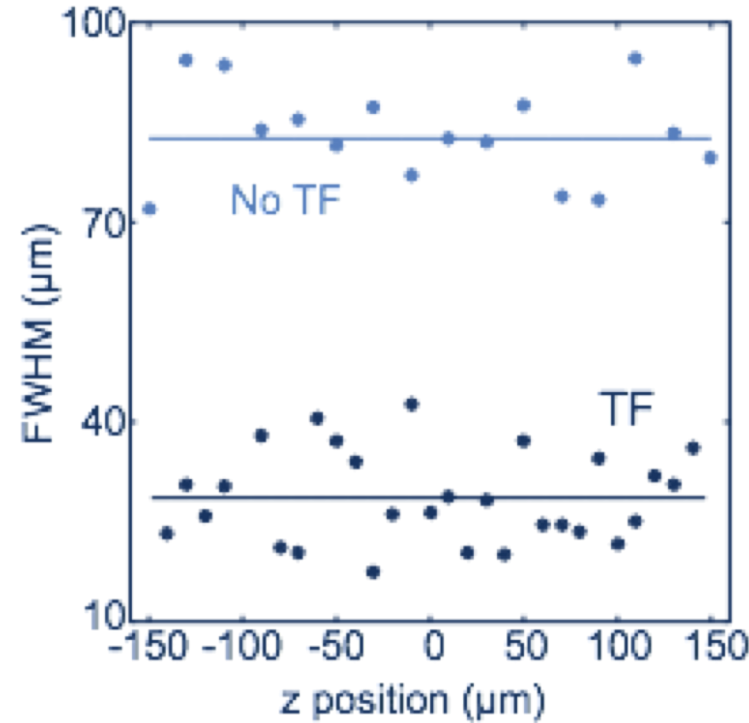
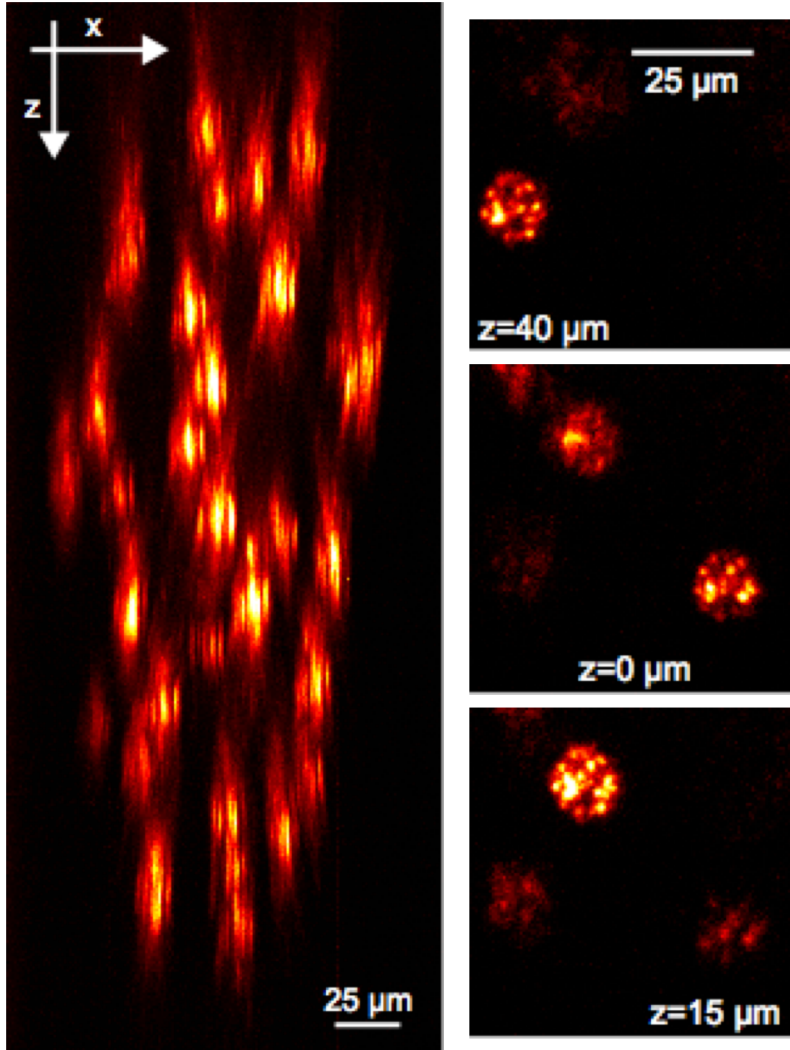


Temporal focusing enables robust light propagation through scattering tissue

Endoscopic 3D patterned illumination



Endoscopic 3D patterned illumination



30 spots in a Volume of $100 \times 150 \times 300 \mu\text{m}^3$

FWHM with TF $< 30 \mu\text{m}$,
limited by NA & aberrations

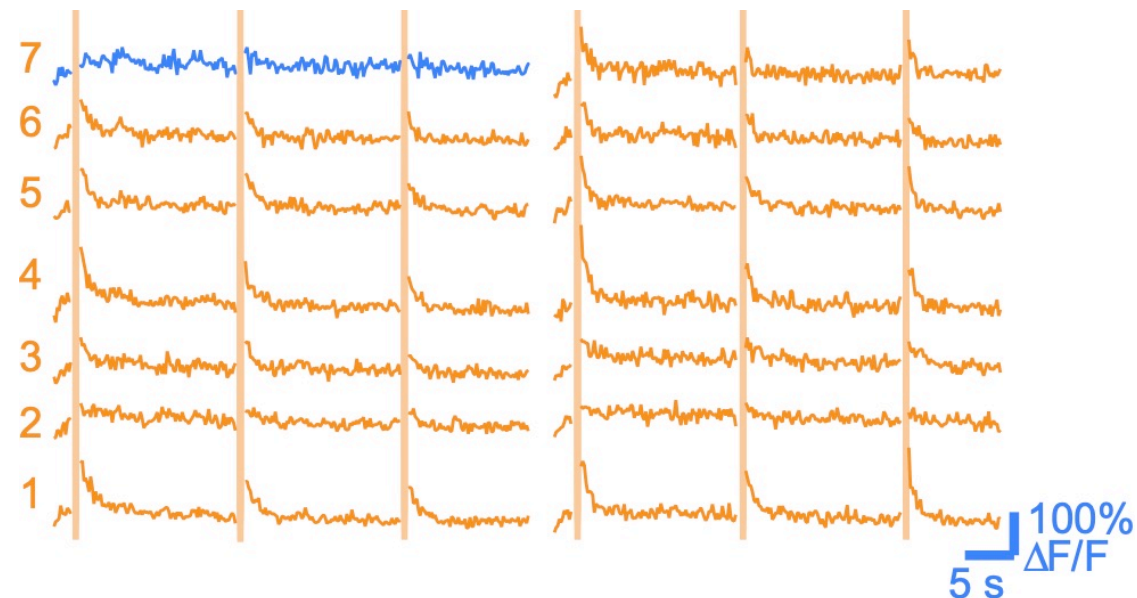
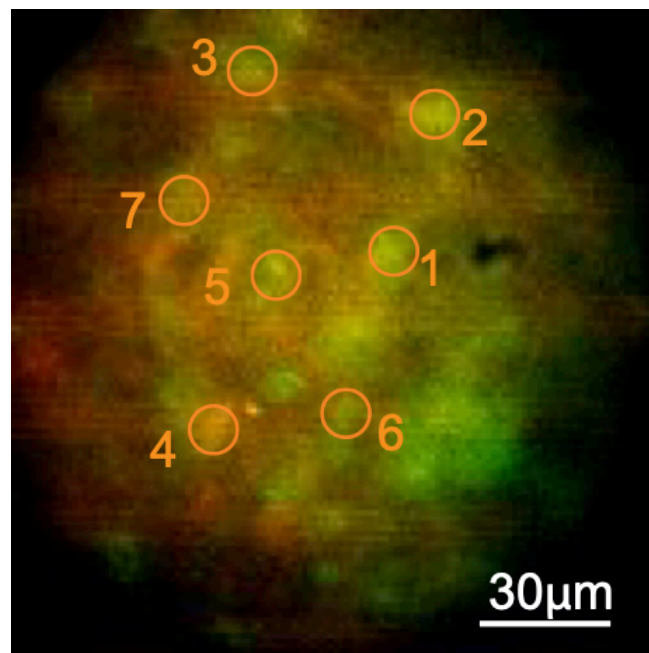
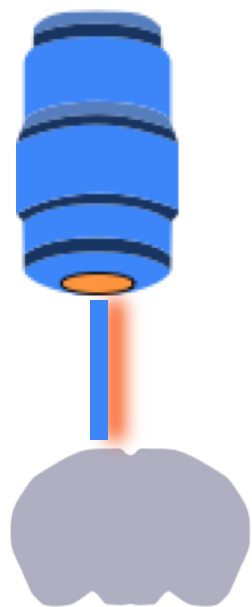
All-optical endoscopic neuronal control

Stimulation: $0.4 \text{ mW}/\mu\text{m}^2$, 10X 10-ms pulses at 11.24 Hz

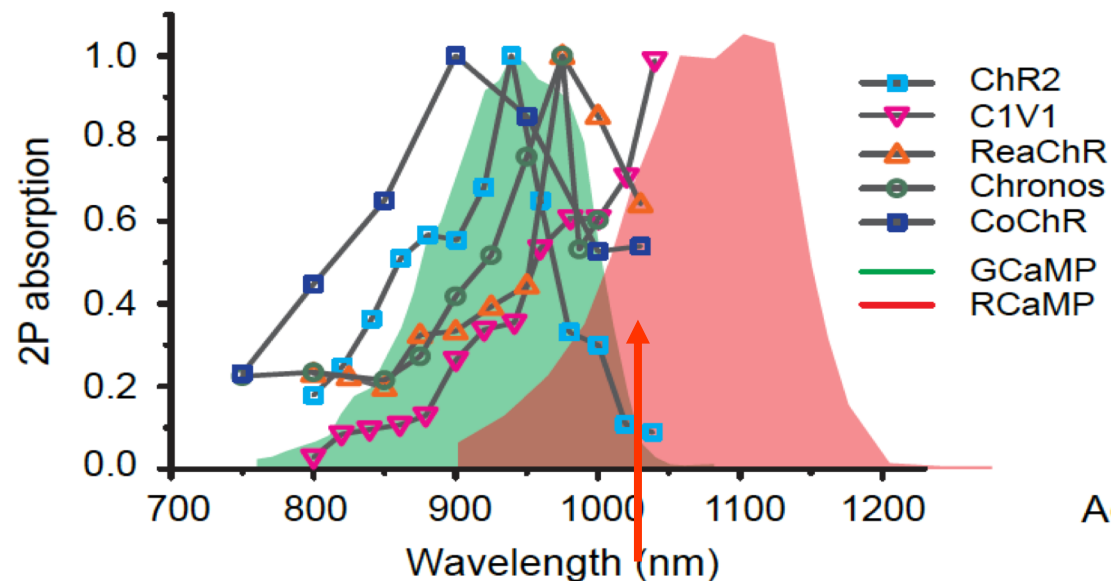
Imaging: 920 nm, 90 mW, 5.92 Hz,

Depth: $250\mu\text{m}$

Injected ReaChR-dTomato in GCaMP6s transgenic mice GP4.3



How many targets?



- *Tunable Ti:saff* (~300mW @900-950 nm; 80 MHz), in vitro, 10 - 40 mW/cell

10-20 cells

- *Fiber lasers* (up to 30 W @ 1030nm, 100kHz - 80MHz), in vitro, 1-10 mW, in vivo 10-20 mW

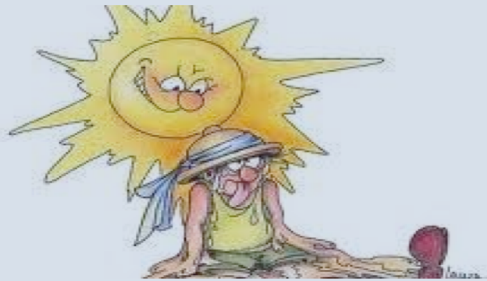
hundreds of cells

$$S_{2PE} \propto \frac{(I/w)^2}{\tau \cdot f}$$

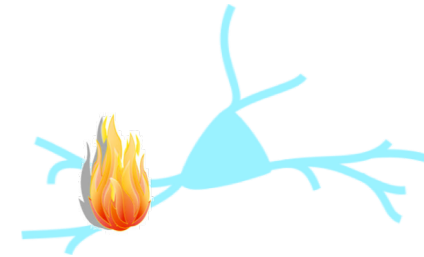
How many targets?

Photo-damage

Linear: Thermal $\propto$ Average power



Nonlinear $\propto$ Peak power

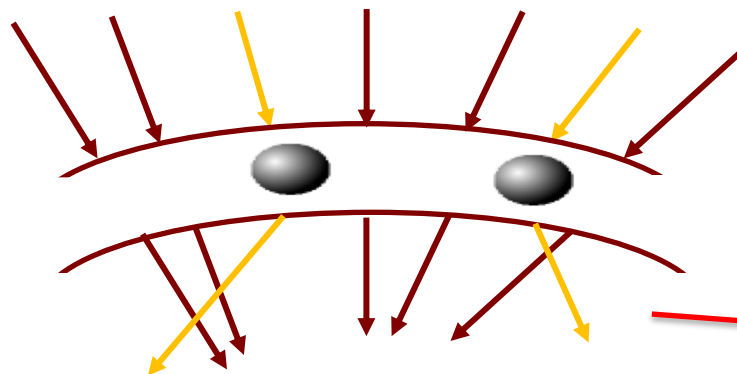


Average power = 10 – 20 mW/cell $\Rightarrow$ Excitation density at the sample $\sim 0.07 - 0.1 \text{ mW}/\mu\text{m}^2$ $\Rightarrow$ fluence mJ/cm^2

Light and heat spreading

Light spreading

Angular spectrum
of waves



Heat diffusion

Fourier's heat diffusion equation

$$\frac{\partial T}{\partial t} = D \nabla^2 T + \frac{q}{\rho C}$$

$$\Delta T = S(x, y, z, t) * G(x, y, z, t)$$

$$G(x, y, z, t) = \frac{1}{(4\pi\Delta t)^{3/2}} e^{-\frac{\sqrt{x^2+y^2+z^2}}{4D_T}}$$

Green's function formalism

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

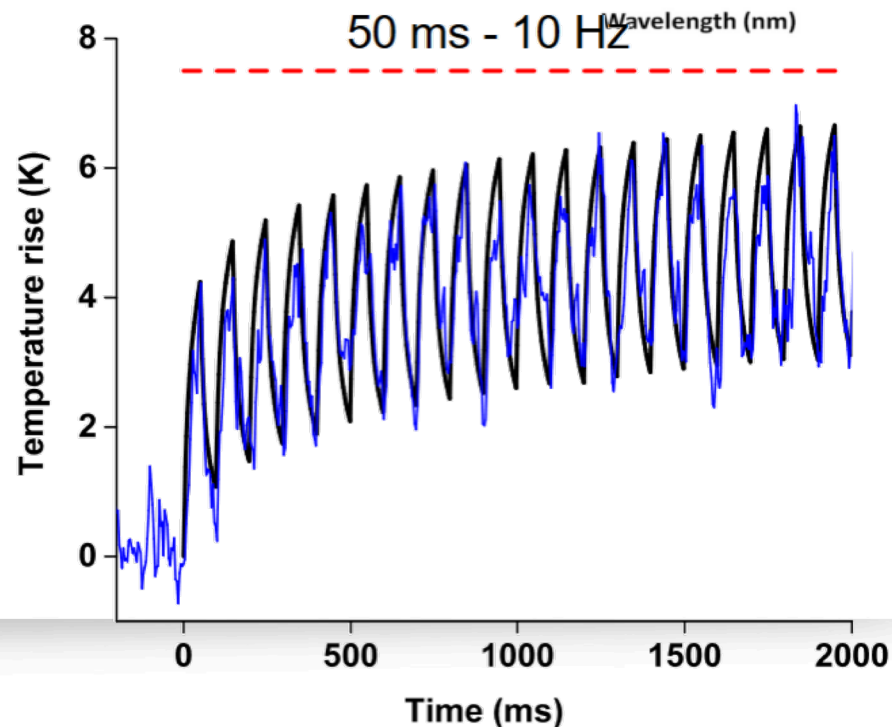
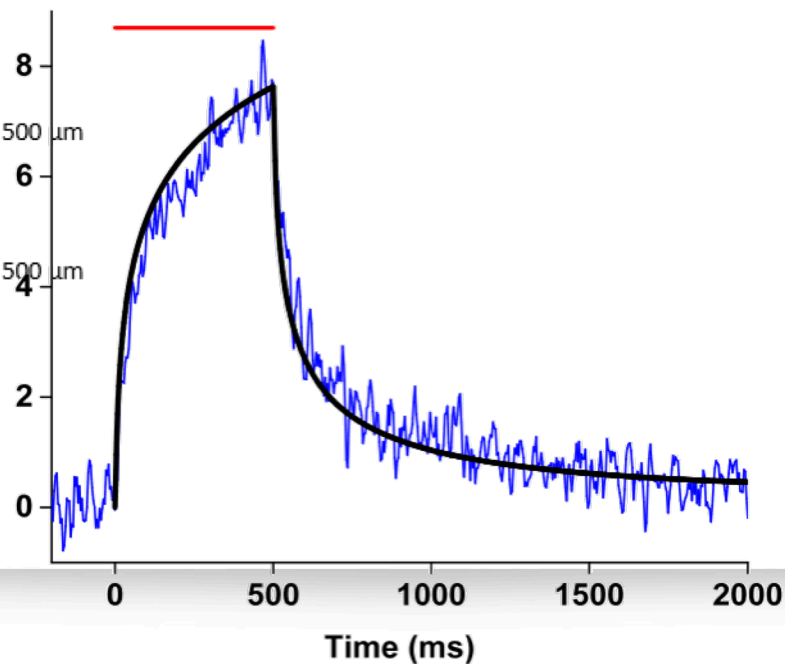
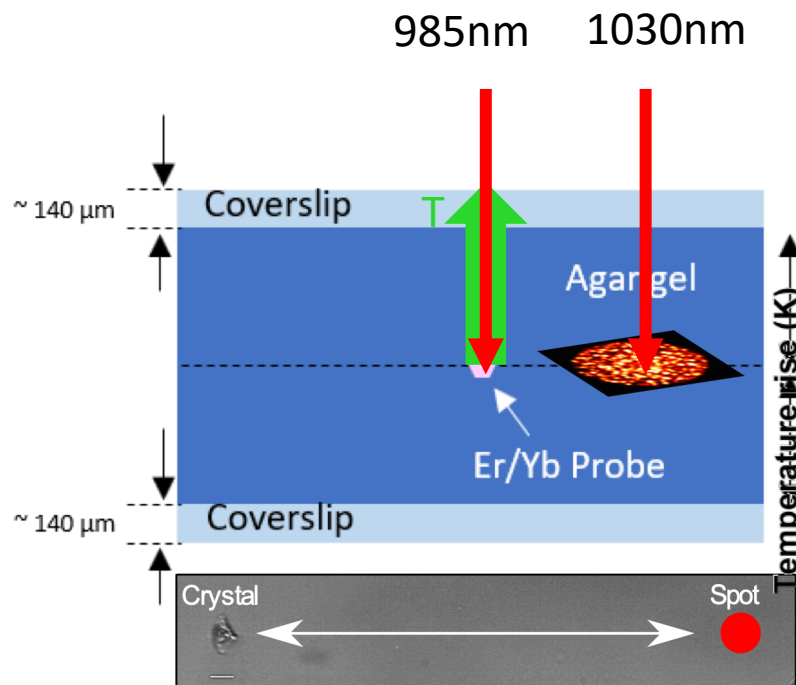
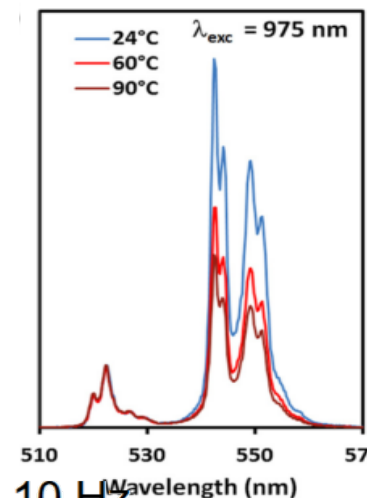
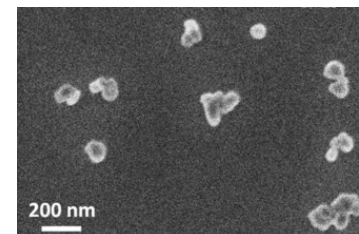
Experimental validation

Requirements :

- Few micrometers resolution
- Few milliseconds resolution
- For future : *in vivo* implementation

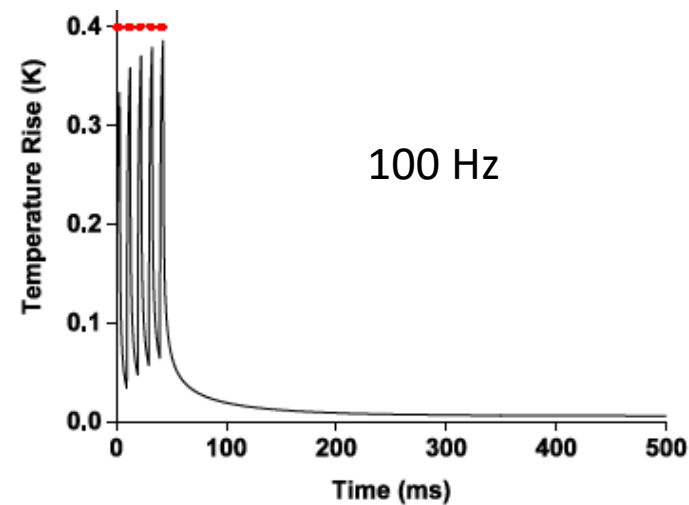
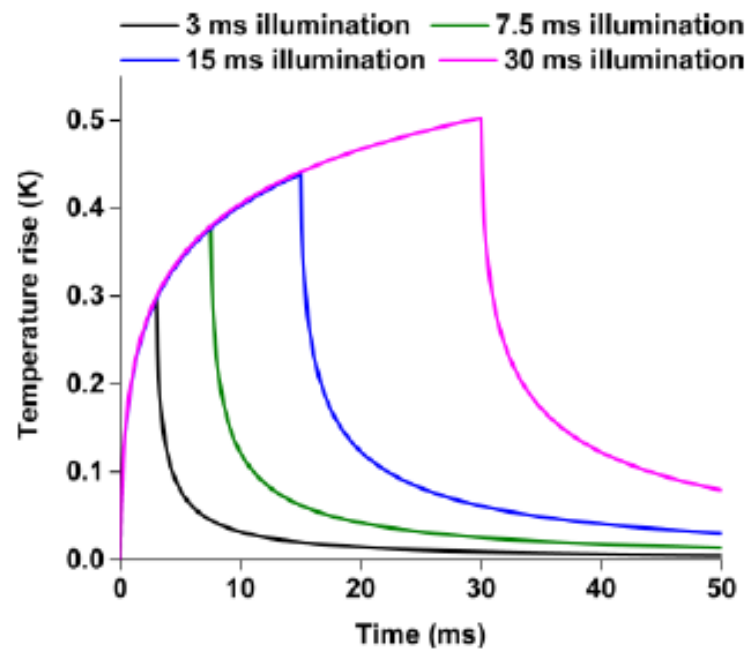
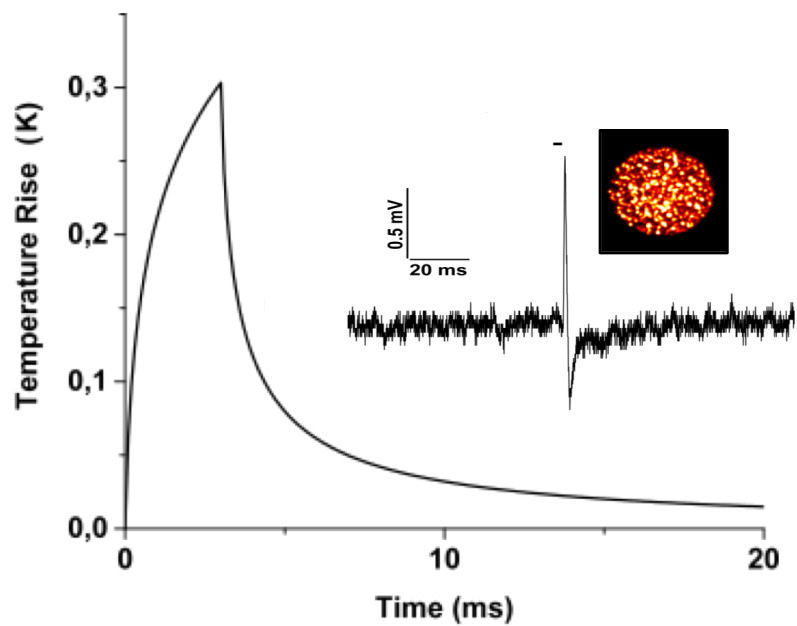
Er-Yb co-doped glass particles
(M. Mortier group, Chimie Paris)

Temperature dependent luminescence



Single spot

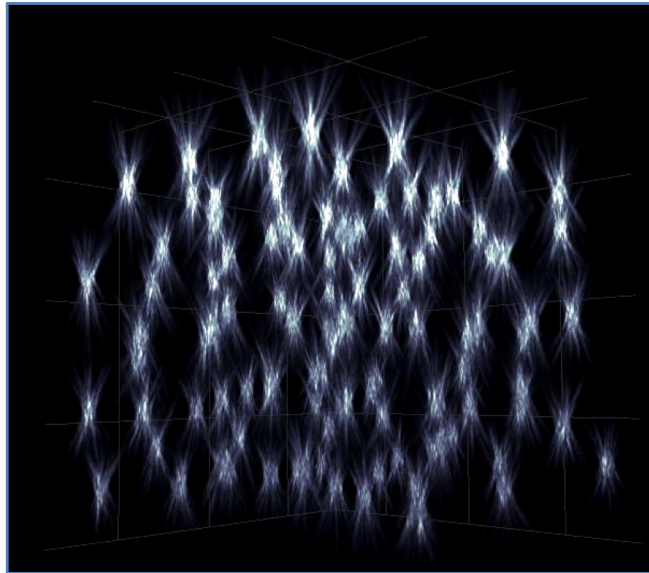
1 holographic spot
12 μm diameter
0.1 $\text{mW}/\mu\text{m}^2$ – 3ms
 $\lambda = 1030$ nm



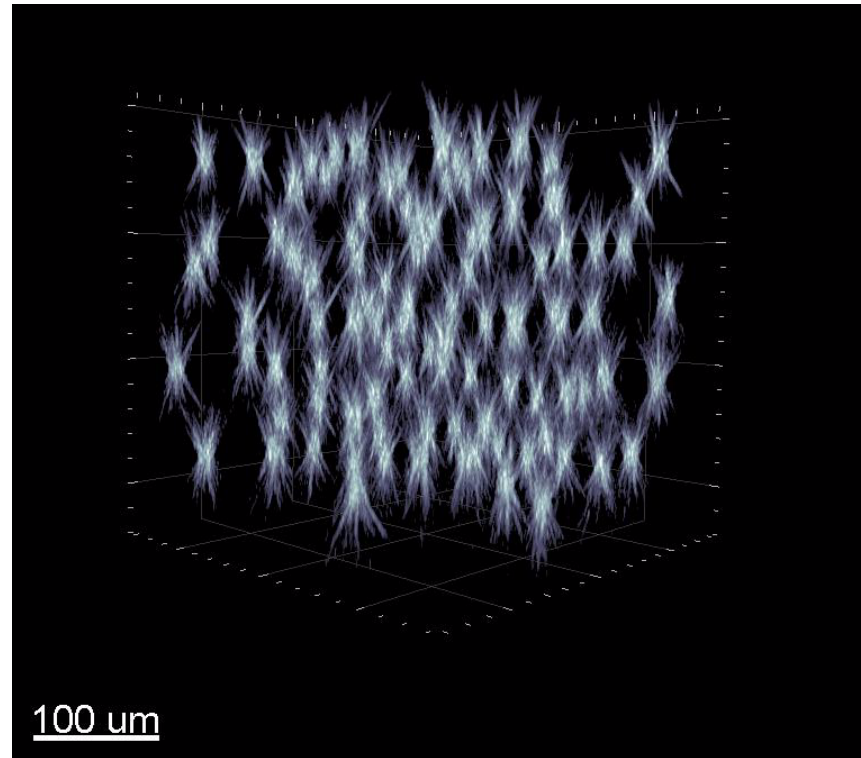
Multi spot

1) Building up the Source term taking into account the scattering

100 Holographic spots
300x300x300 μm



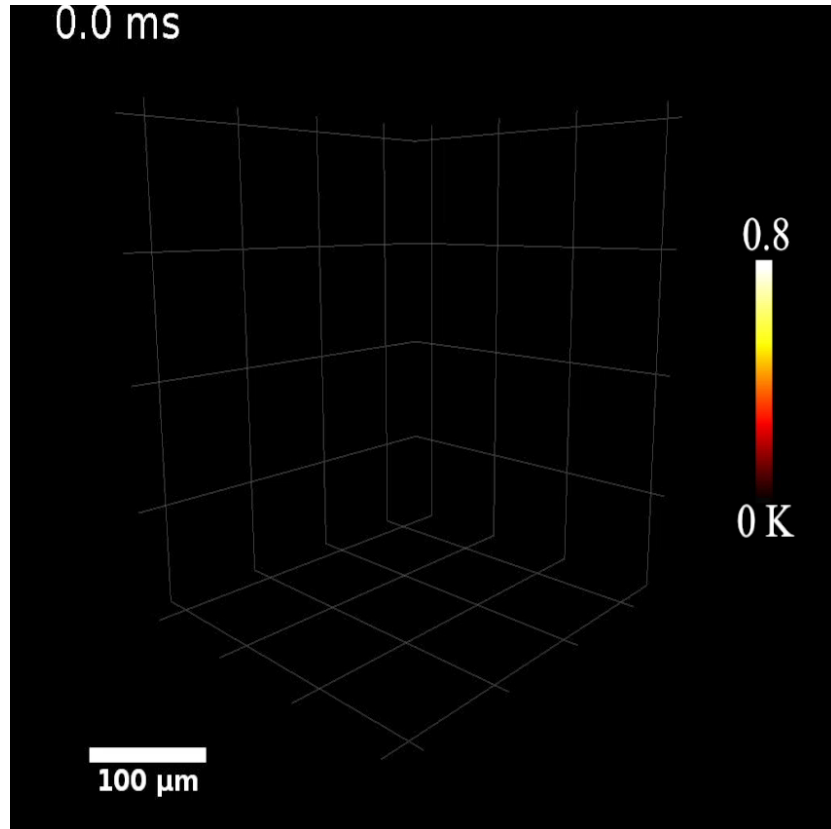
With graded phase mask for scattering compensation



0.1 mW/ μm^2

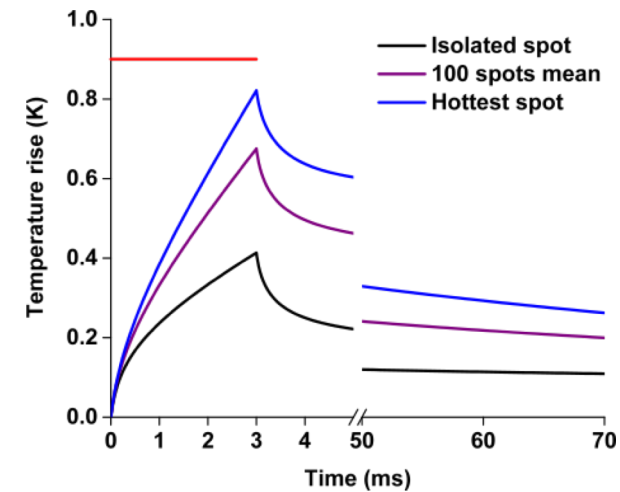
Multi spot

2) Calculation of the 3D spatiotemporal heat distribution

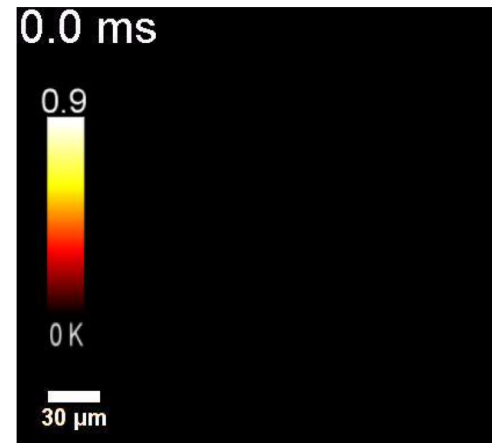
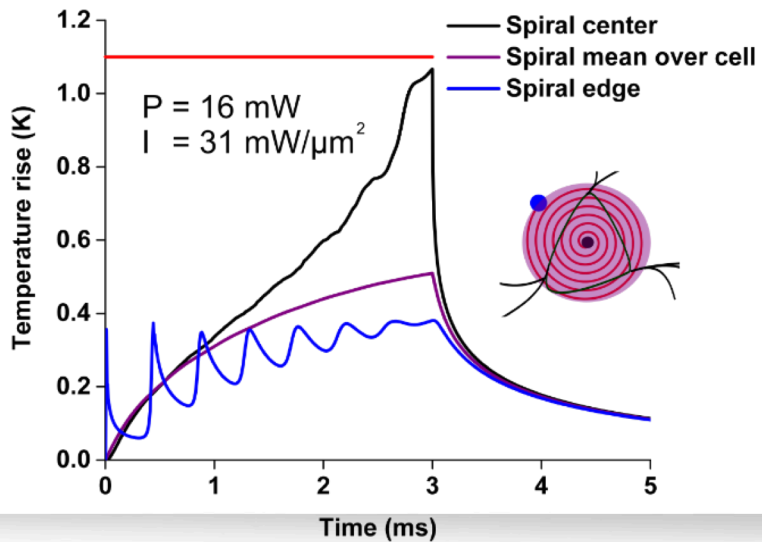
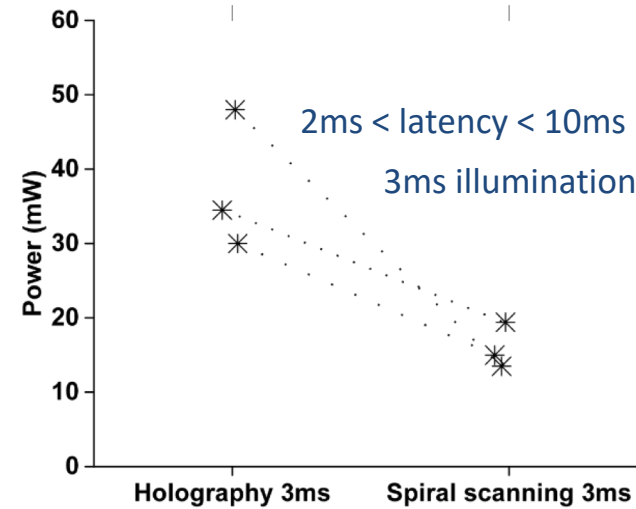
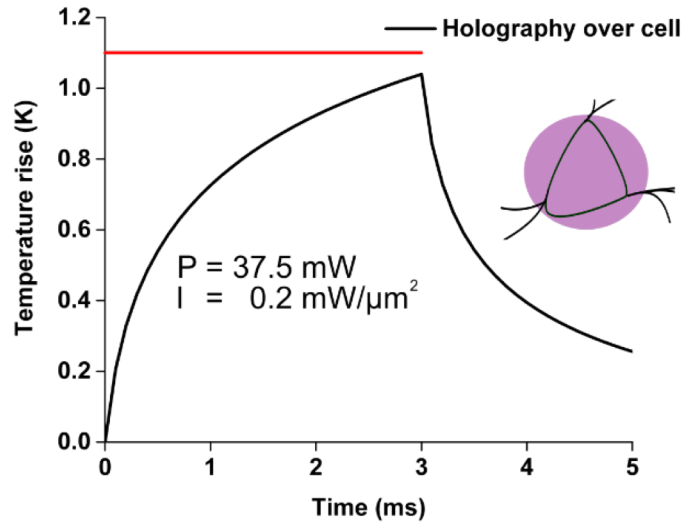


For multi-spot activation, **3D thermal diffusion length**, gives an indication of the average inter-spot distance that minimize heat accumulation

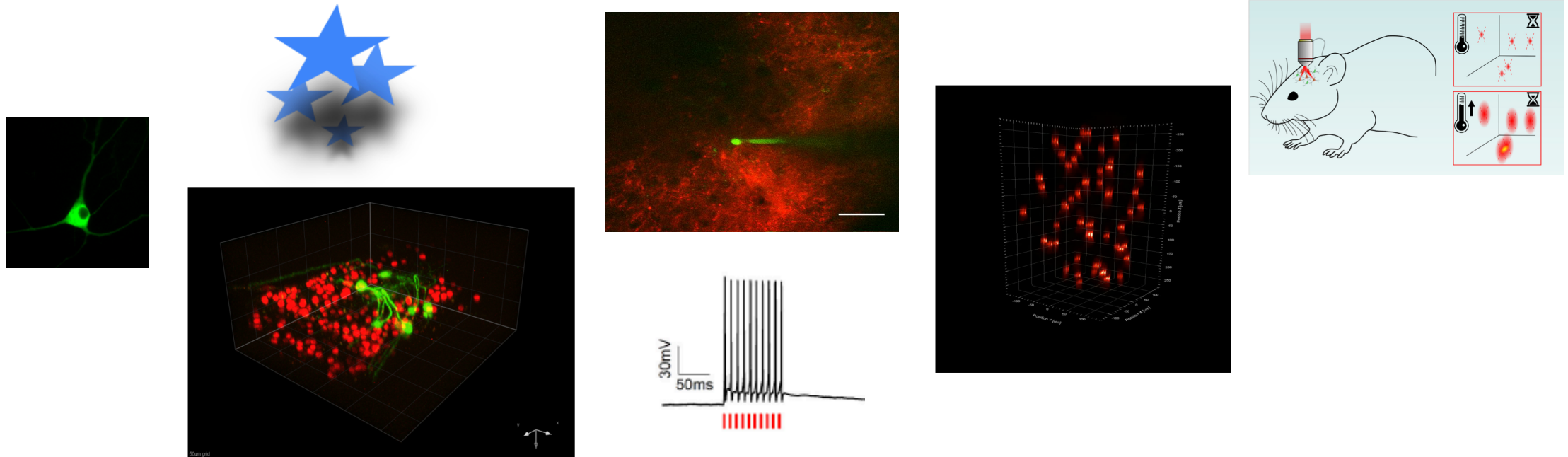
$$\ell_{th} = \sqrt{6Dt} \cong 50 \mu m$$



Scanning, holography



Conclusions

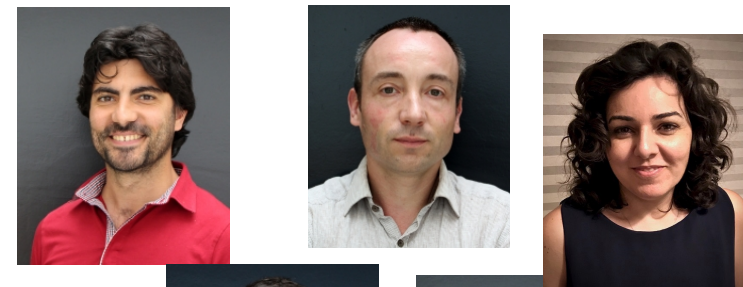


Light sculpting technology (illumination approaches, opsin and reporters engineering, modeling and laser source development) enables optical driving and reading of 3D neuronal microcircuits with cellular precision and millisecond temporal resolution: toward circuit optogenetics

Thank you!!

Ed BOYDEN, MIT
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Gilles TESSIER, Vision Institute
Dan Oron, Weizmann Institute

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Florence BUI **Alexis PICOT**
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• References

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Journal of Optics 19 , 113001
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Two-photon optogenetics by computer-generated holography,
In: Stroh A (eds) Optogenetics: A Roadmap,
Neuromethods 133, (2018) Humana Press, New York, NY, 175-197
- I-Wen Chen, Eirini Papagiakoumou, and V. Emiliani
Towards circuit optogenetics
Current Opinion in Neurobiology 50, 179-189 , (2018)
- Emiliano Ronzitti, Valentina Emiliani and Eirini Papagiakoumou
Methods for three-dimensional all-optical manipulation of neural circuits.
Front. Cell. Neurosci 12, 468 (2018),

Optogenetics school in Paris



AXE 10 - Microscopie et imagerie Microscopie photonique

Scientific and technical environment of the training course



INSTITUT DE LA VISION
PARIS

Institut de la vision
<http://www.institut-vision.org/fr>

COURSE DIRECTORS

Valentina EMILIANI
Senior researcher
UMR 7210

Erini PAPAGIAKOU MOU
Researcher
UMR 7210

LOCATION
PARIS (75)

ORGANISATION
5 days
From 09:00 am to 06:00 pm
Training course in english
From 7 to 20 attendees

TRAINING FEES
2500 Euros

AT THE END OF THE TRAINING COURSE
Satisfaction survey from trainees
A certificate of training is delivered.

COURSE DATE
Ref. 19 146 : from Monday 07/10/19 to Friday 11/10/19

January	February	March	April
May	June	July	August
Sept.	Oct. 19 146	Nov.	Dec.

Optical control of brain functioning with optogenetics and wave front shaping

OBJECTIVES

- Be able to choose the most suitable combination of actuator, illumination methods and targeting strategy for each specific application
- Acquire the skills and technologies required to design and build up an optical system for optical control of neuronal circuits

AUDIENCE
Neurophysiologists, physicists (researchers, PhD students or engineers)

PRE-REQUIREMENT
Attendees should have a strong background in neurosciences, in cell biology or in optical microscopy. To adapt the programme to trainees' expectations, we invite the attendees to download and fill out the survey from our web site.

TRAINING PROGRAMME

Lectures

- Introduction to optogenetics
- Opsin photo-cycle modeling
- Wave front shaping and liquid crystal matrix
- Computer generated holography
- Generalized phase contrast
- Temporal focusing
- Three dimensional light patterning and temporal focusing
- Two-photon optogenetics: scanning, spiral scanning, parallel illumination: examples and comparison among the different approaches
- How to build up an holographic optical set-up
- All-optical interrogation of brain circuits
- Patterned voltage and calcium imaging
- Viral vector design
- In depth patterned optogenetics

Practical courses (in small rotating groups of 7 participants max with 2 trainers by group)

- How to build up an holographic optical set up (optical design and software)
- In vitro and in vivo all-optical manipulation of neuronal circuits
- Projects from applicants (practical): proposition of projects by the participants and discussion on their feasibility

Lectures (14h), workshops (14h) and presentations of projects (8h)
Detailed programme available from ou web site

SPEAKERS

V. Emiliani, E. Papagiakoumou, N. Accanto, E. Ronzitti, D. Dalkara, D. Tanese (researchers, IDV, Paris), C. Wyart (researcher, ICM, Paris), D. Oron, O. Yizhar (researchers, Weizmann Institute) and P. Hegemann (PR, Institut für experimentelle Biophysik, Berlin)

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Peter Hegemann



Claire Wyart



Dan Oron



Ofer Yizhar



Deniz Dalkara

