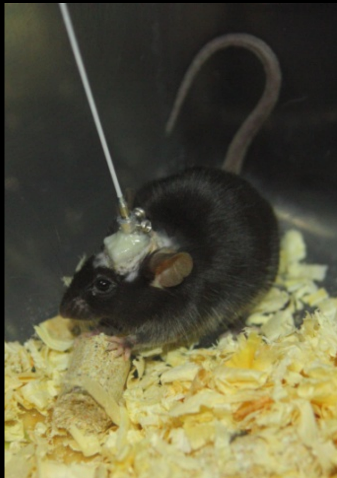

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Inserm

Functional fluorescence imaging and photoactivation in freely behaving rodents

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Cold Spring Harbor Laboratory Course
Imaging Structure & Function in the Nervous System
August 8, 2019

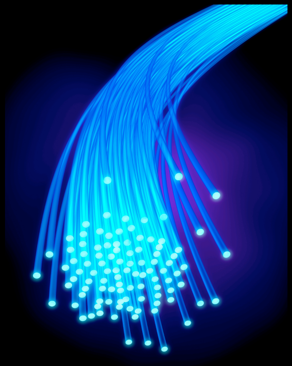
Outline

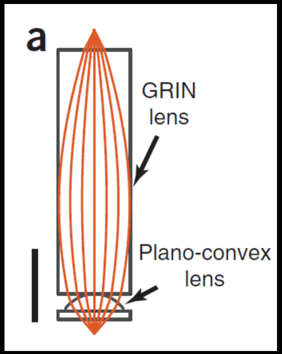
1- Introduction

1.1- Why do we need methods for fluorescence imaging and photoactivation in freely-behaving rodents ?

1.2- 2 configurations: miniature microscopes and fiberscopes

1.3- Necessary ingredients: optical fibers and miniature optics

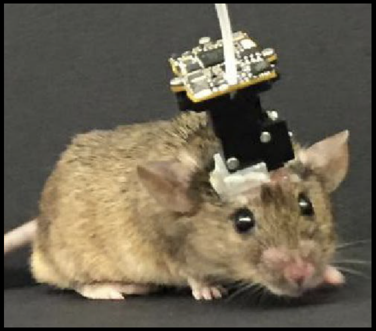


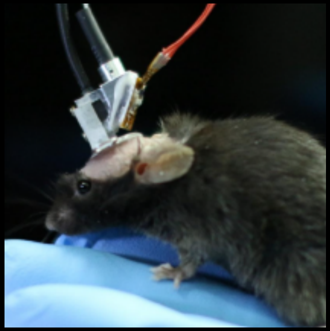


2- Miniature microscopes

2.1- Conventional widefield imaging

2.2- 2-photon imaging



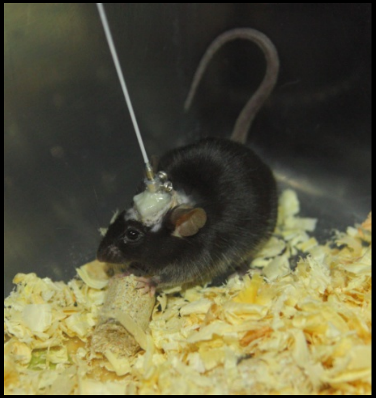


3- Fiberscopes

3.1- Conventional widefield imaging

3.2- 2-photon imaging

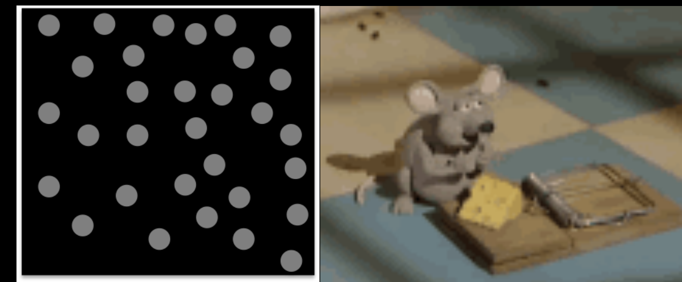
3.3- Fast confocal imaging and targeted photoactivation



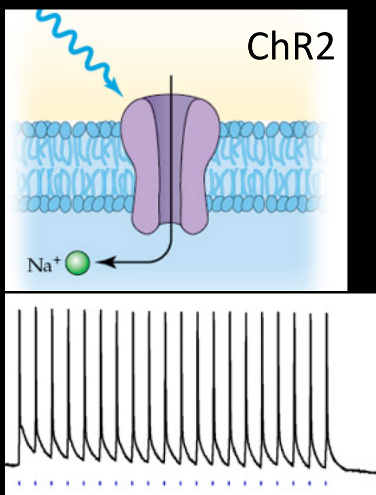
Framework: study of the links between neuronal activity and behaviors

Example: measuring endogenous activity patterns corresponding to specific behaviors and testing their causality

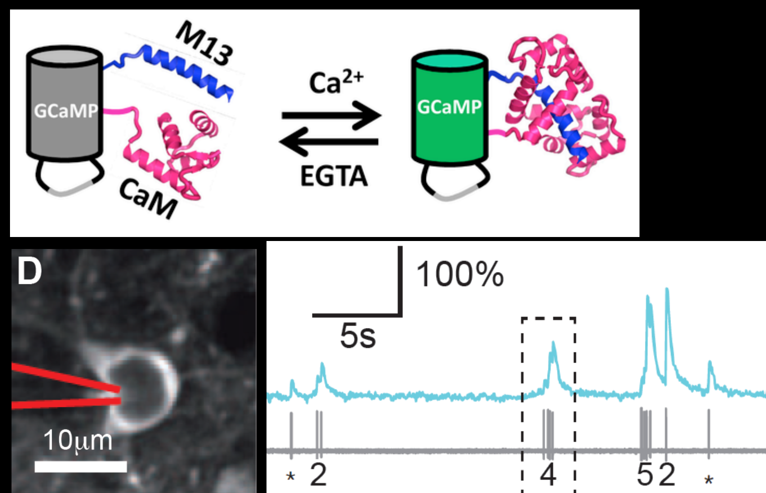
➡ Manipulating and recording neuronal activity with cellular precision during behavior



Can be done using optogenetics

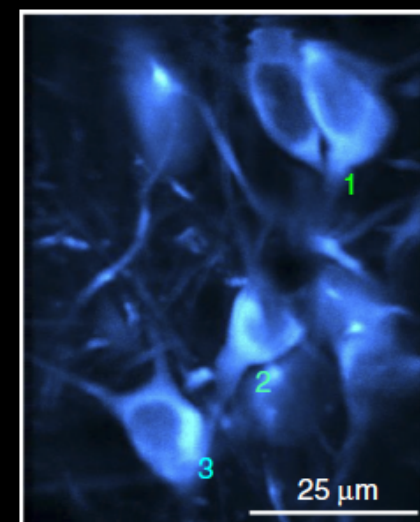


Aravanis et al (2007)



Chen et al, Nature 499, 295 (2013)

coupled with microscopy techniques

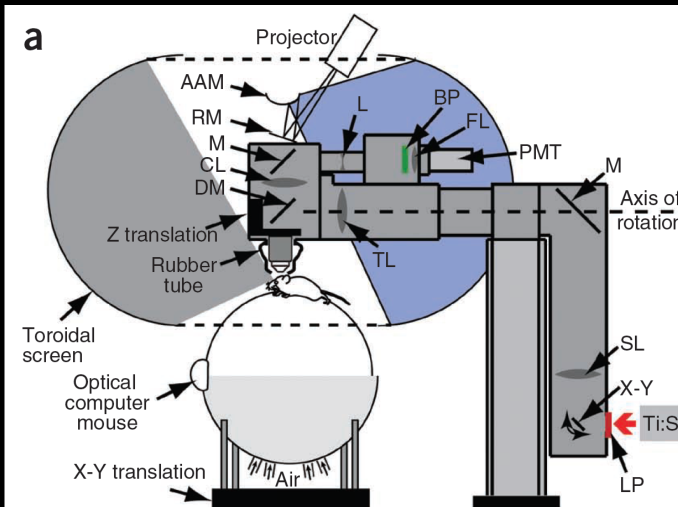


Main advantages of optical methods

- Access to many neurons simultaneously with cellular precision
- Longitudinal studies over weeks
- Information on the cell type or connectivity with pre- or post- synaptic cells

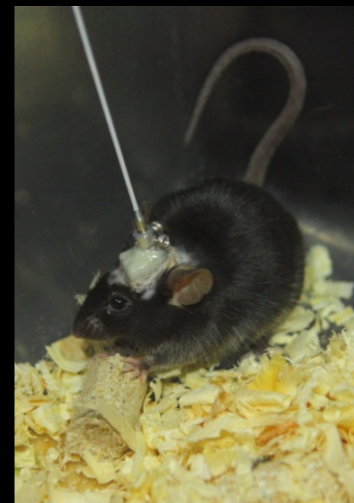
Optogenetics methods in behaving rodents

Awake, head-restrained rodents

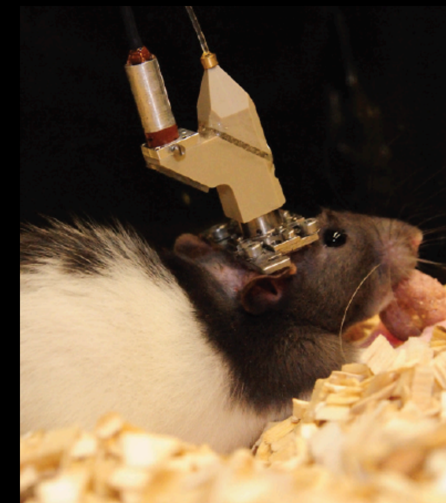


Dombeck et al. Nat Neuroscience **13**, 1433 (2010)

Awake, freely-behaving rodents



Szabo et al. Neuron **84**, 1157-1169 (2014)



Sawinski et al, PNAS **106**, 19557 (2009)

Advantages

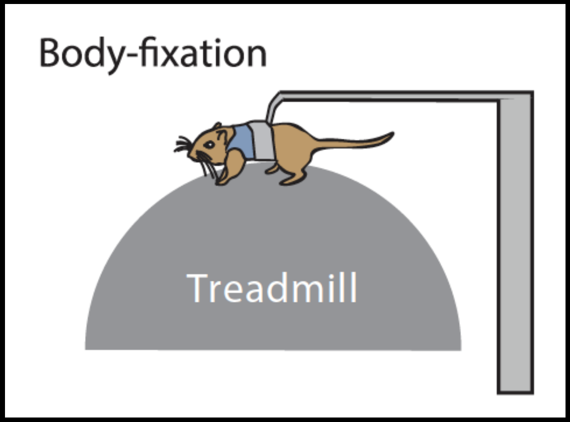
- Compatible with high performance imaging techniques
- Precise control of stimuli/sensory cues (coupling with virtual reality)

Advantages

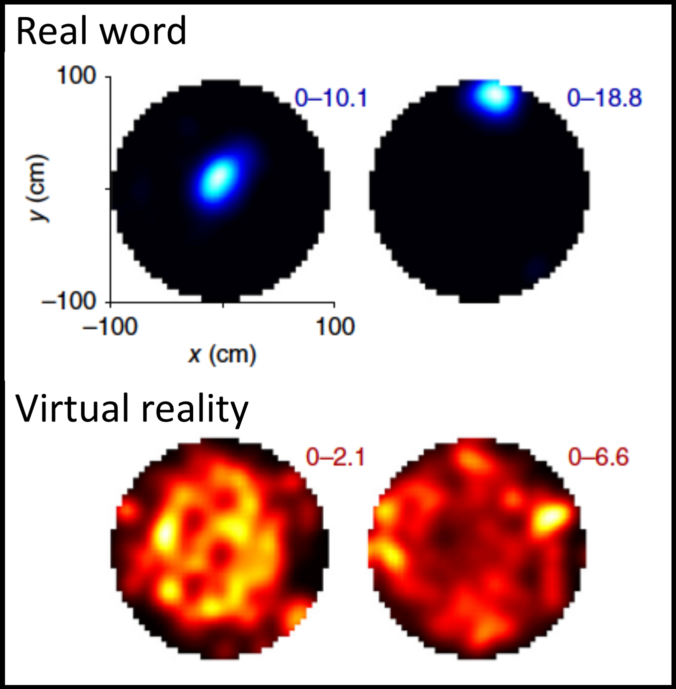
- Less stressful for the animal (can learn faster)
- Wide range of possible behavior experiments

- Closer to natural behaviors

Space coding in body-fixed vs freely-behaving animals



Thurley et al, Current Zoology, 2017



Aghajan et al. Nature Neurosci. (2015)

Two possible interpretations

- It is difficult to make a 2D virtual reality realistic for the rodent
- Vestibular input are necessary for precise space coding

➡ Head-fixation or body fixation is not the best paradigm to study navigation

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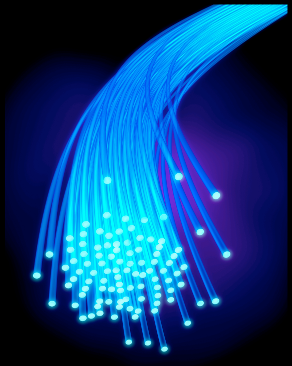

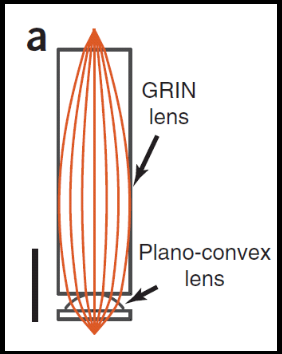

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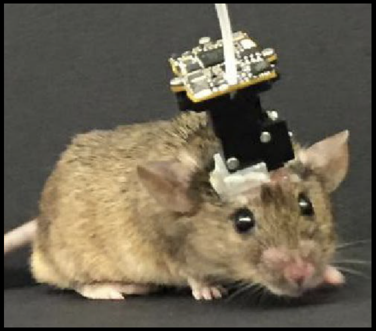

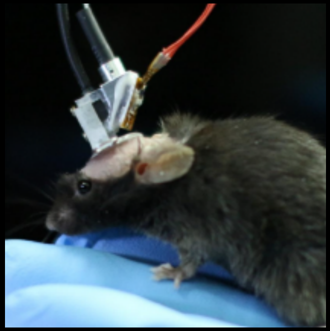

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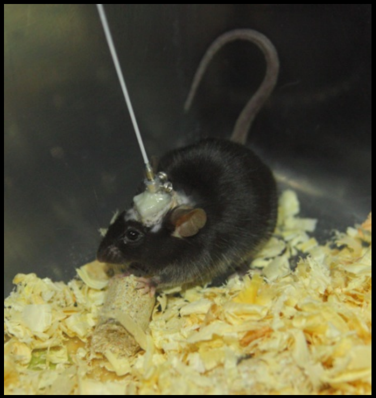
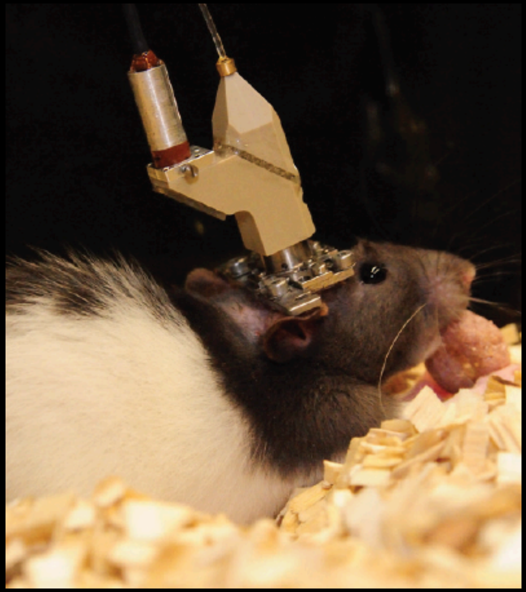


2 strategies: miniature microscopes and fiberscopes

Miniature microscope

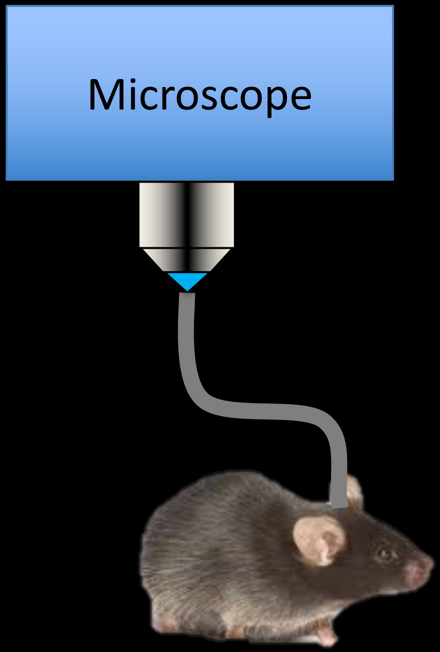
Most of the optical elements are miniaturized and directly placed on the head of the animal



Sawinski et al, PNAS **106**, 19557 (2009)

Fiberscope

Regular complex microscope coupled to the animal with an image guide



➡ Necessary ingredients: optical fibers and miniature optical elements, light sources and detectors

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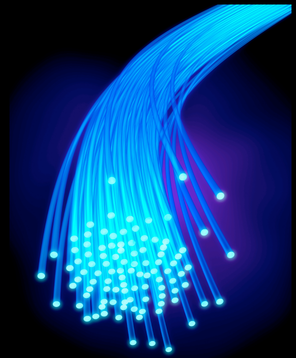

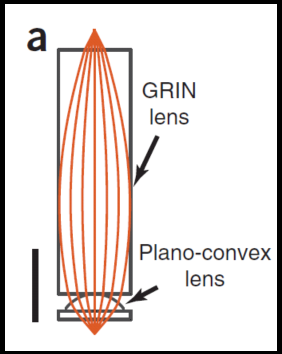

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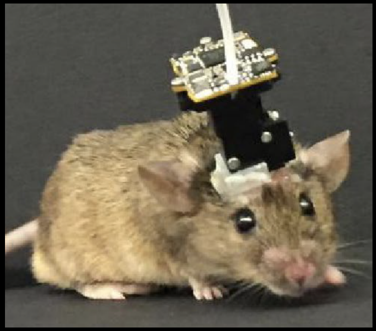

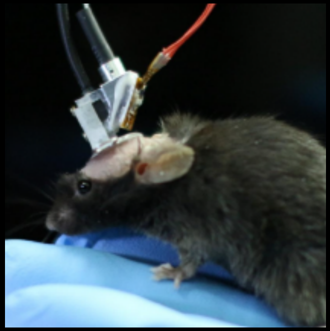

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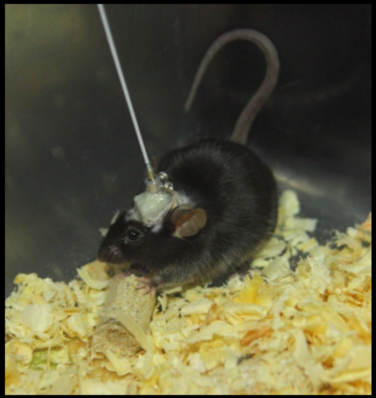
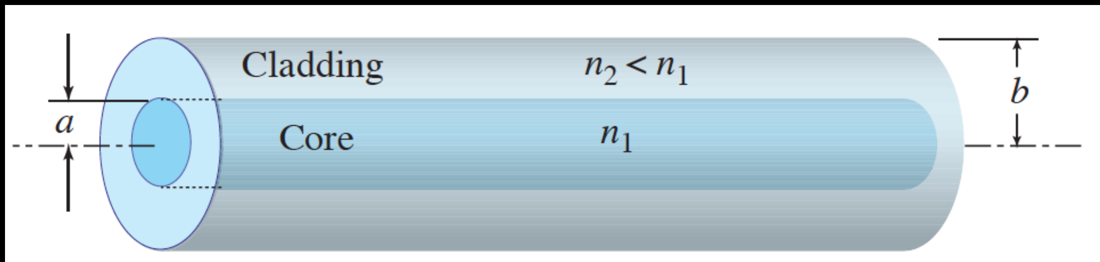


Step-index fibers: the most common type of optical fibers



Fundamentals of Photonics, Saleh, Wiley Publishing

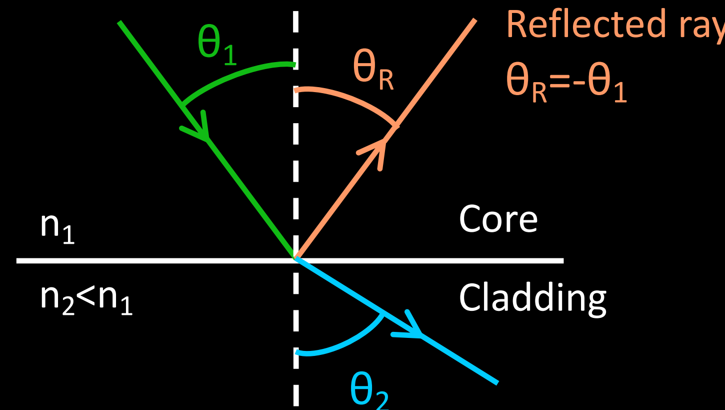
Mechanism to guide light: total internal reflection

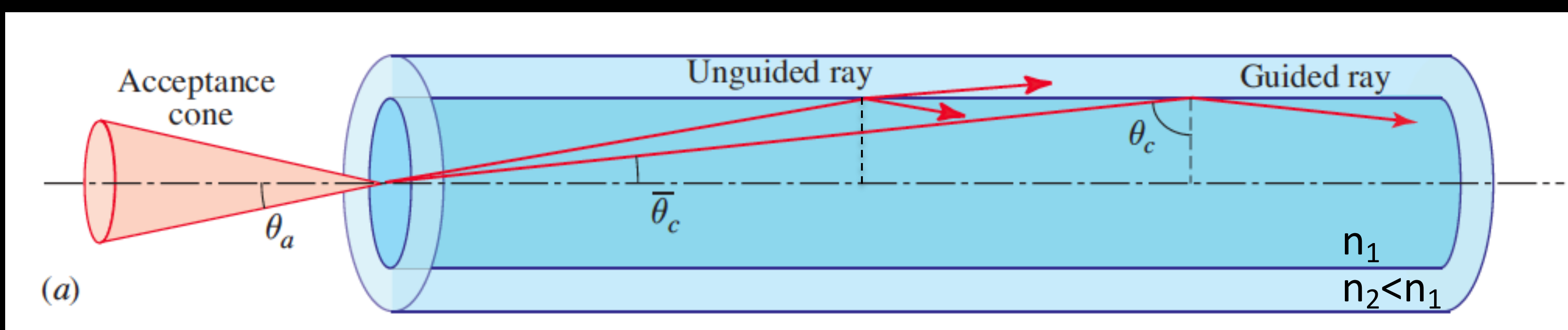
Critical angle: θ_c with $\sin\theta_c = n_2/n_1$

$\theta_1 < \theta_c$ Refracted ray (θ_2)

$n_2 \sin\theta_2 = n_1 \sin\theta_1$

$\theta_1 > \theta_c$ No refracted ray





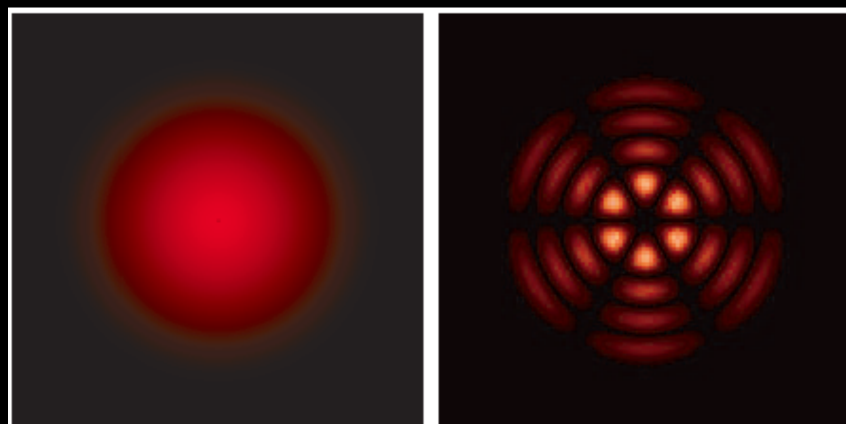
Numerical aperture

$$\sin\theta_a = n_1 \sin\overline{\theta_c} = n_1 \cos\theta_c = n_1\sqrt{1-\sin^2\theta_c} = n_1\sqrt{1-\left(\frac{n_2}{n_1}\right)^2} = \sqrt{(n_1)^2-(n_2)^2}$$

Step-index fibers, an electromagnetic-optics approach

Method: solving Maxwell's equations

Results: Spatial modes, characterized by a specific propagation constant and field distribution in the transverse plane



Fundamentals of Photonics, Saleh, Wiley Publishing

An important parameter: the V parameter

$$V = 2\pi \frac{a}{\lambda_o} \mathrm{NA}.$$

a = radius of the fiber

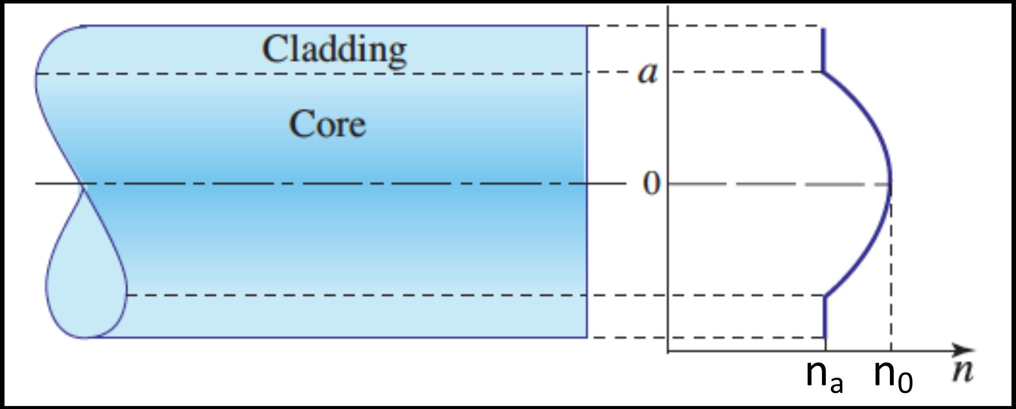
$NA = (n_1^2 - n_2^2)^{1/2}$

V determines the number of modes in the fiber

$V<2.405$: $M = 1$

$V>>1$: $M = (4/\pi^2)V^2$

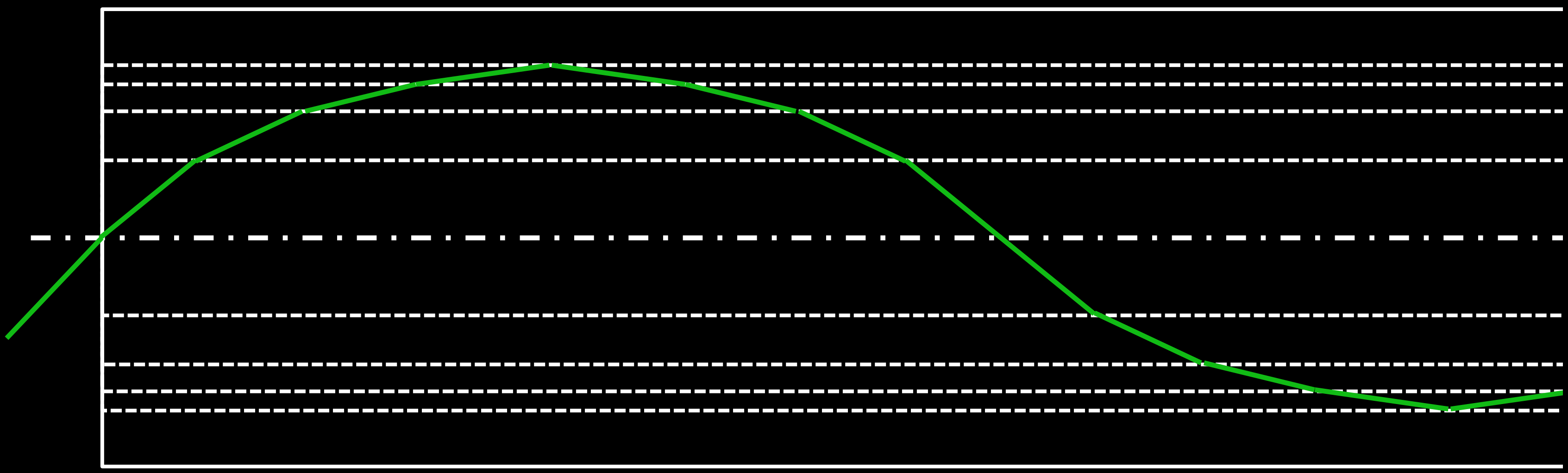
Gradient-index (GRIN) fibers



Index profile

$$n(r) = n_0(1 - g^2r^2/2)$$

Light propagation in GRIN fibers:

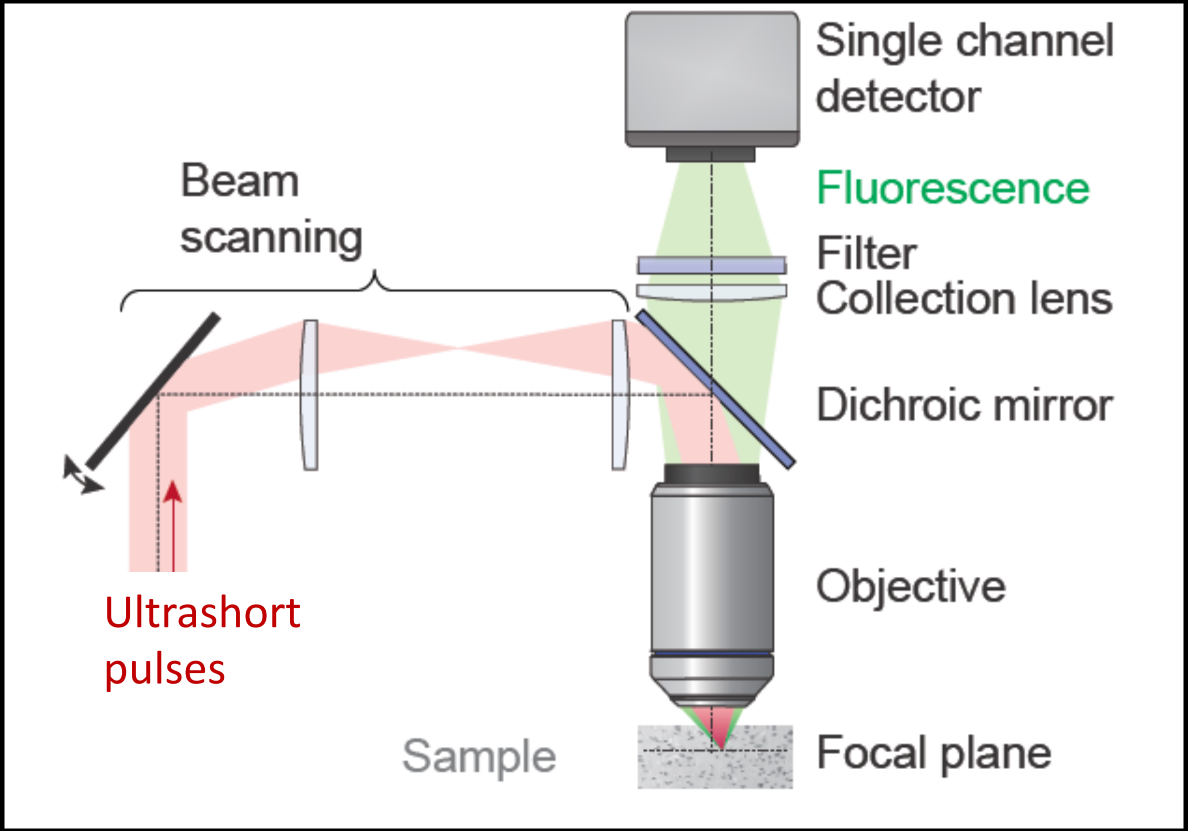


By discretizing the index profile and applying Snell Descartes at each step, we find that rays propagate along a sinusoid

Optical fibers for 2-photon microscopy

Ultrashort pulses are necessary for 2-photon microscopy

Duration: 100fs
Average power: >100mW



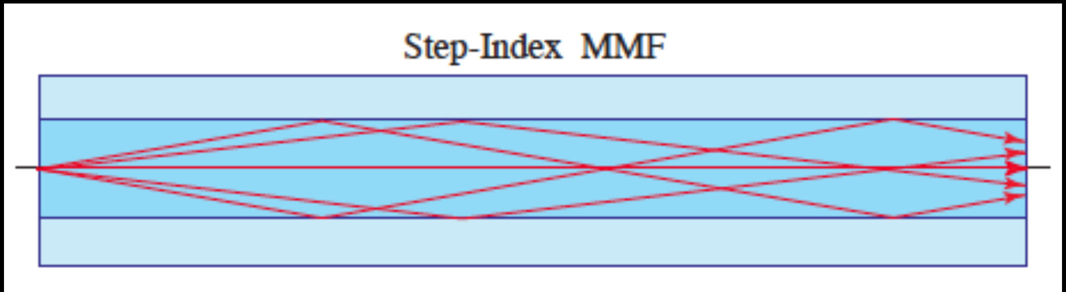
Sources delivering high-intensity ultrashort pulses cannot be miniaturized

Transport in optical fibers is mandatory !

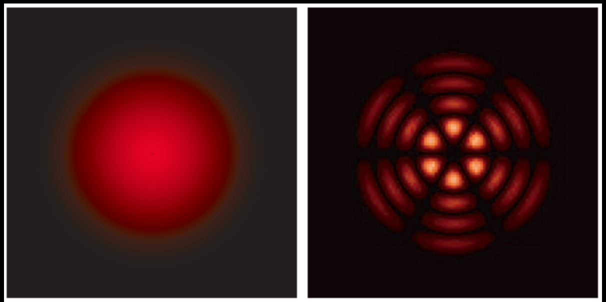
Propagation of ultrashort pulses in fibers ?

Pulse propagation in optical fibers

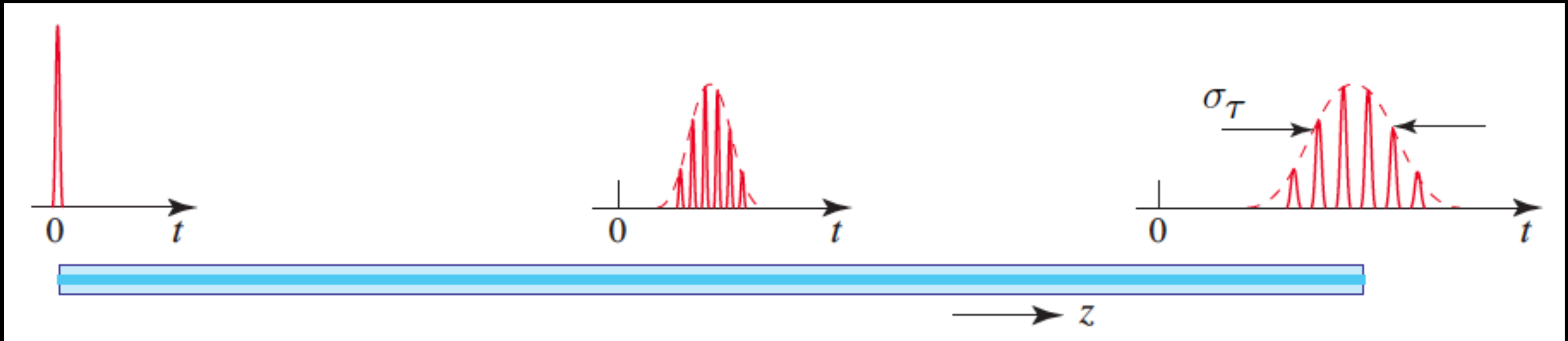
Step-index multimode fibers



Rays corresponding to larger incident angles have longer trajectories in the fiber



Each spatial mode has a specific group velocity



$\sigma_T \approx (n_1 - n_2)L/2c$ Example: $n_1=1.46$, $n_2=1.44$, $L=2m$ } $\sigma_T \approx$ 67ps

>2 orders of magnitude larger that what is typically used for 2-photon imaging

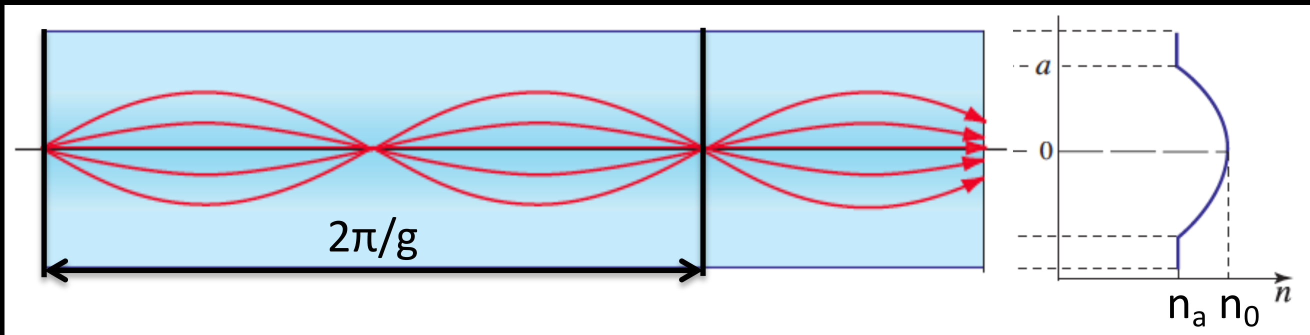
Fundamentals of Photonics, Saleh, Wiley Publishing

Pulse propagation in optical fibers

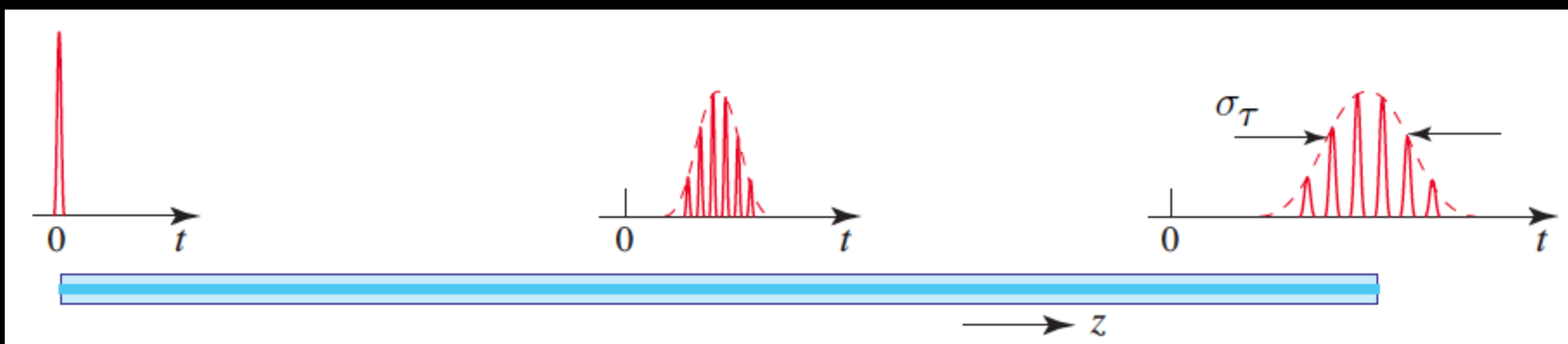
GRIN fibers with quadratic index profile

Index profile

$$n(r) = n_0(1 - g^2r^2/2)$$



Refraction index is reduced away from the optical axis, compensating for the longer trajectory



$\sigma_T \approx (n_0-n_a)^2 L/(4n_0c)$ much smaller than with a step index fiber of same index mismatch $\sigma_T \approx (n_1-n_2)L/2c$

Example: $n_0=1.46$, $n_a=1.44$, $L=2m$ } $\sigma_T \approx 0.5ps$

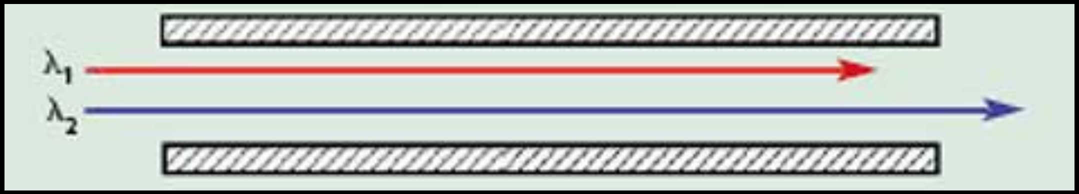
Fundamentals of Photonics, Saleh, Wiley Publishing

Pulse propagation in optical fibers

Single mode step-index fibers

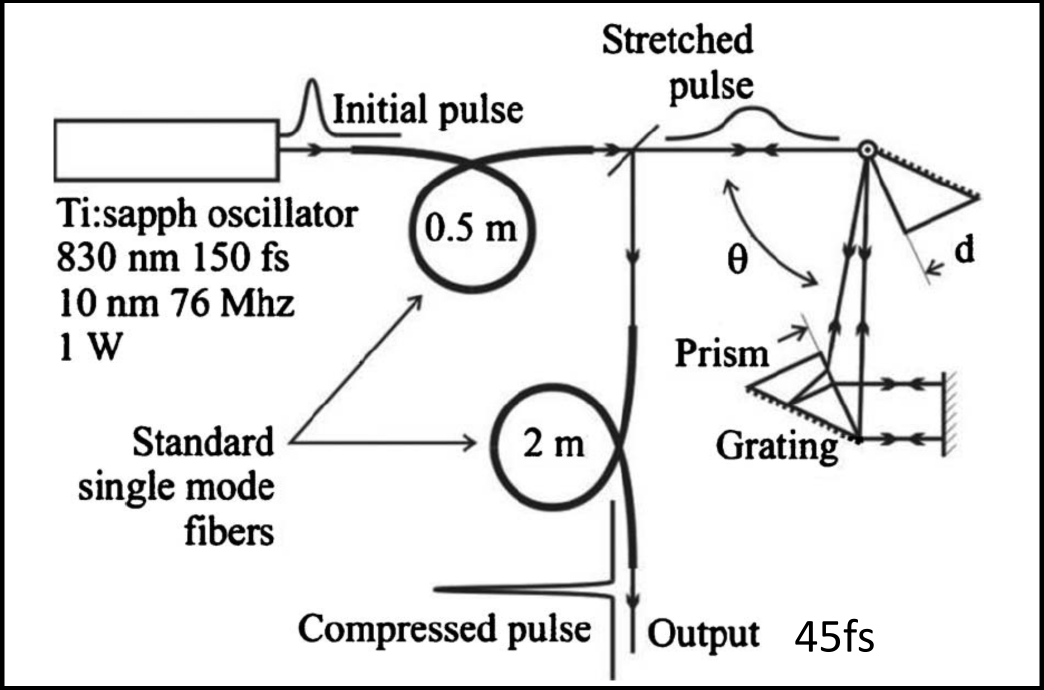
2 types of dispersion induce pulse distortion:

- Linear dispersion



- Non-linear dispersion (self phase modulation) $n(I)=n_0+n_{nl}I$

Can be compensated

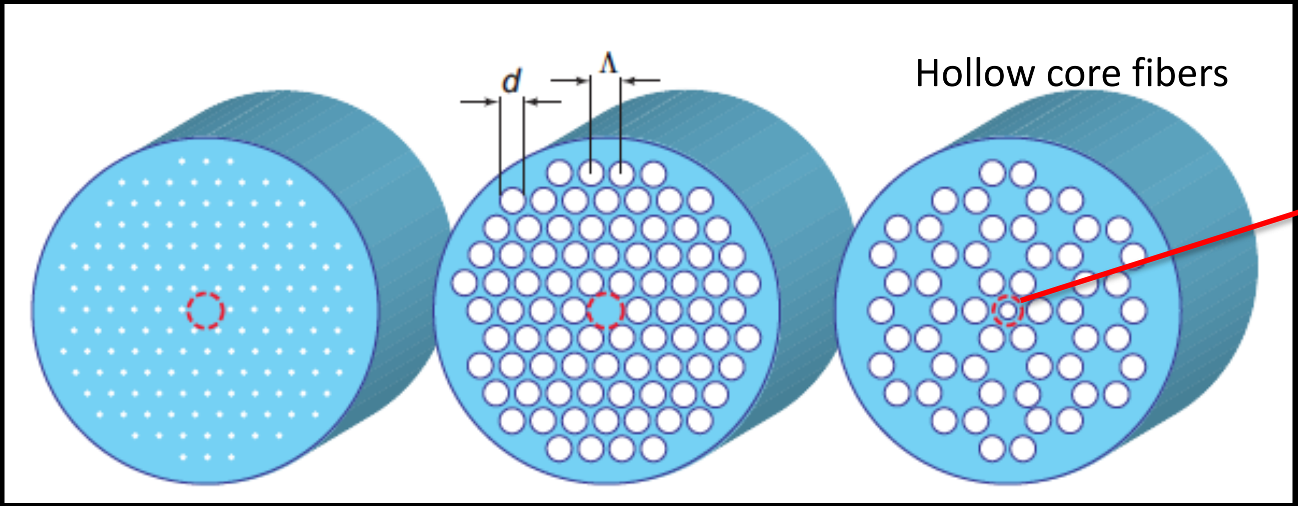


Lefort et al, Optics Letters 2011

Pulse propagation in optical fibers

Photonic crystal fibers

- Pure silica fiber containing multiple cylindrical air holes
- Periodic structures with dimensions close to λ
- The core is a obtained as a defect in this structure
- Propagation in the cladding is prohibited (photonic bandap, destructive interference of multiple reflections in the cladding)



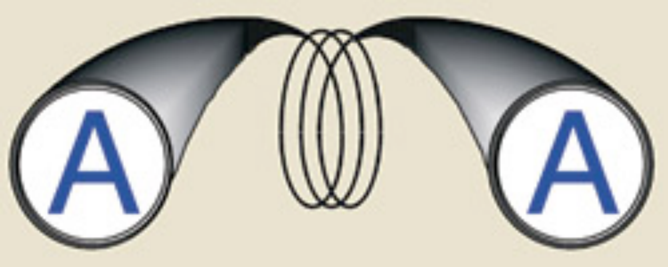
Air core

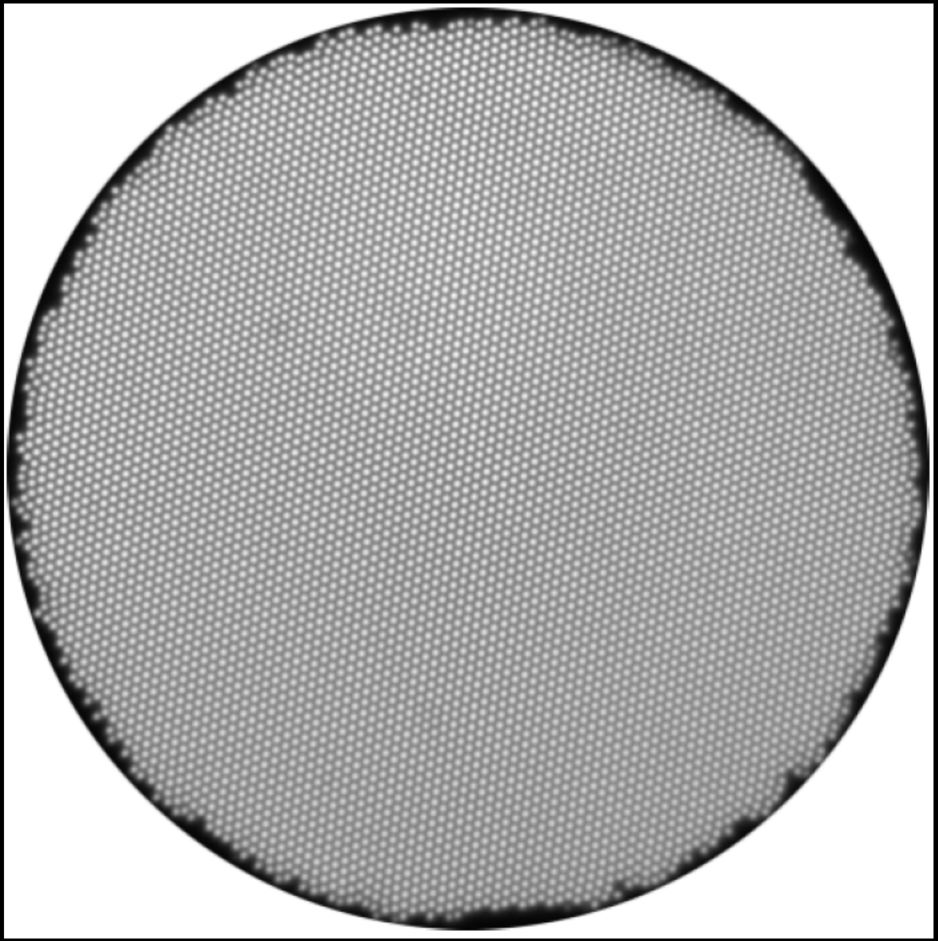
Linear and nonlinear dispersion are highly reduced !

Fundamentals of Photonics, Saleh, Wiley Publishing

Image guides (or fiber bundles)

Purpose: transporting images between remote locations

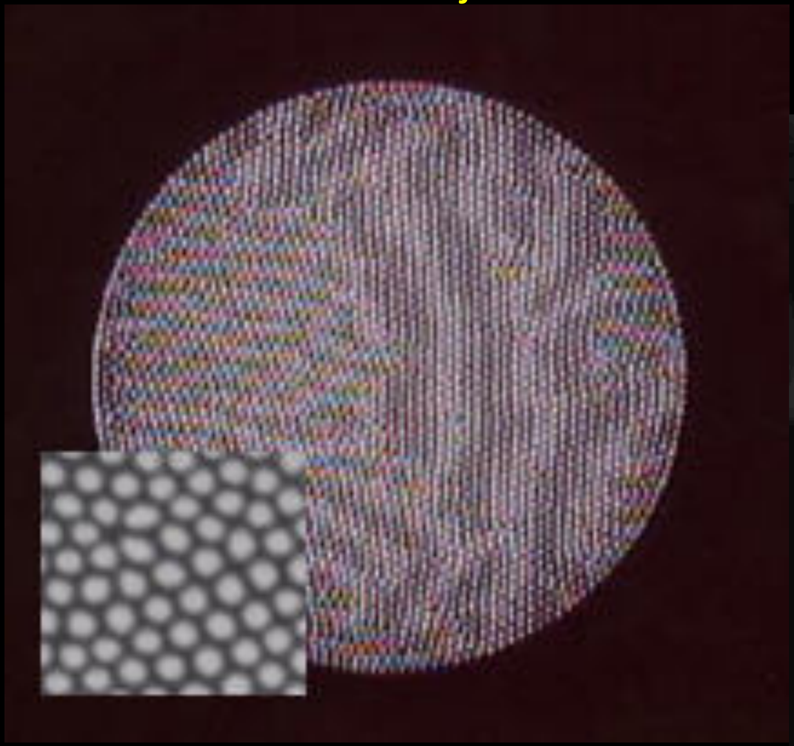




- Composed of many optical cores (>10,000)
- Light is guided in the cores
- Each core transmit one pixel of the image (intensity)

Image guides (or fiber bundles)

Fused fibers from Fujikura



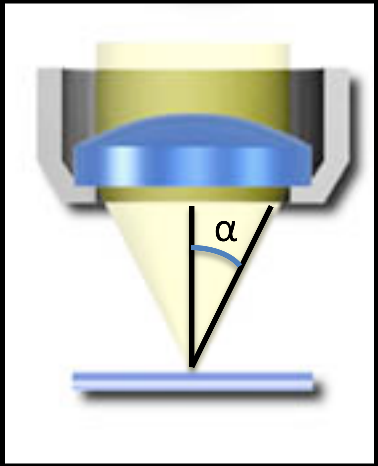
Flexibility

- Intercore distance ≈ 3.3 µm
- Large inhomogeneities in the shapes and sizes of individual core
- NA ≈ 0.3
- Pixel count: 30,000 commonly used (diameter: 600µm)

Miniature optics

Conventional microscope objective

An important parameter: the numerical aperture (NA)



$NA = n \sin \alpha$

http://micro.magnet.fsu.edu/primer/anatomy/numaperture.html

Lateral resolution of the microscope: $R \approx \lambda/2NA$

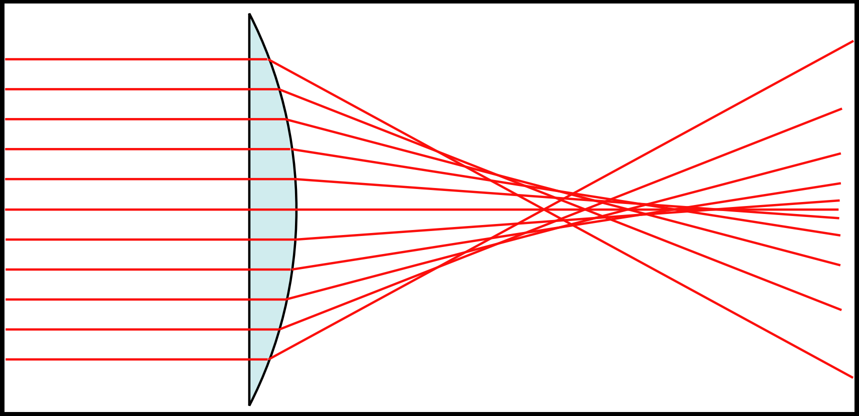
The amount of collected fluorescence photons scales with NA^2

➡ We need high-NA miniature objectives

Can we use a single spherical lens as a high-NA microscope objective ?

Single spherical lenses suffer from several aberrations:

Spherical aberration:

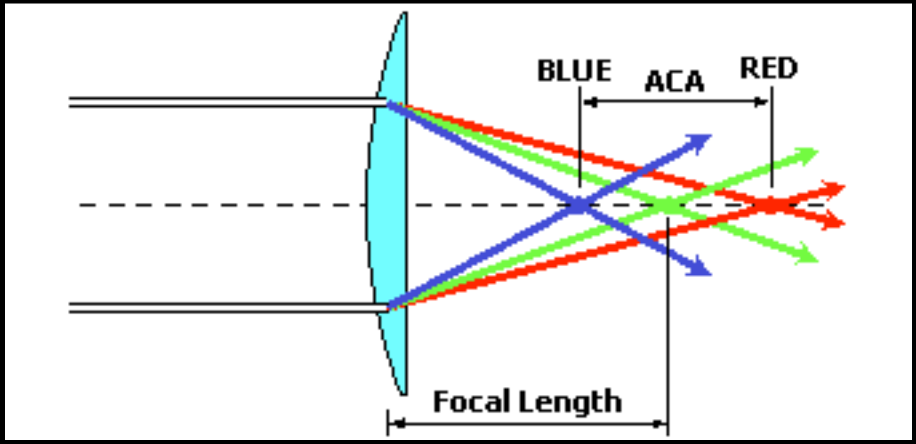


Wikipedia

Loss of resolution

The relation R ≈ λ/2NA is true when aberrations are small !

Chromatic aberration:



http://www.opticampus.com

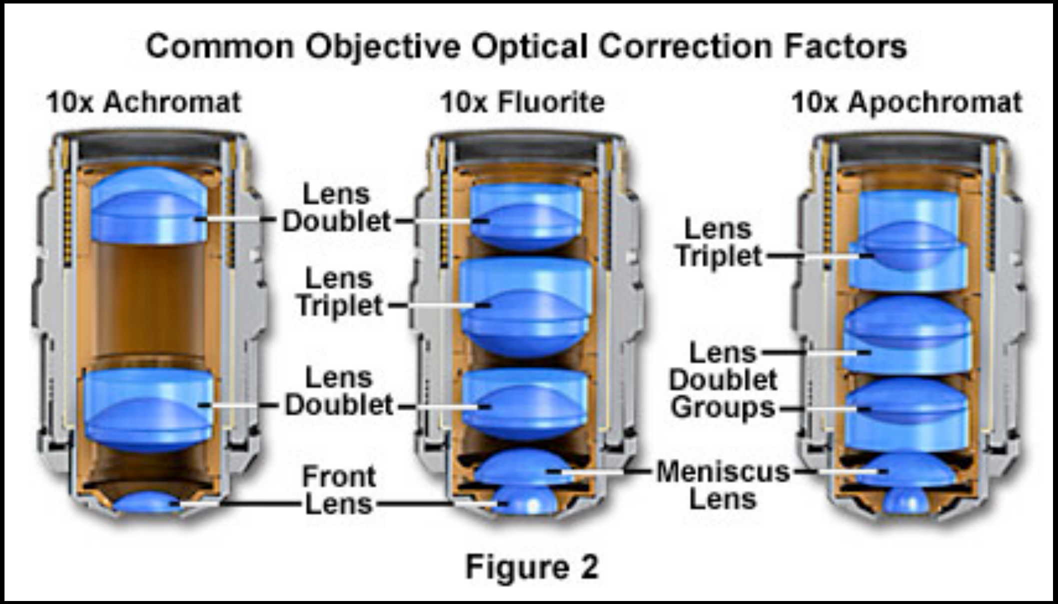
Imaged fluorophores are located at different planes

And many other geometric aberrations: coma, astigmatism…

What is inside a microscope objective?

Microscope objectives are composed of several lenses (some of them aspheric) for aberrations compensation

Objective Type	Spherical Aberration	Chromatic Aberration	Field Curvature
Achromat	1 Color	2 Colors	No
Plan Achromat	1 Color	2 Colors	Yes
Fluorite	2-3 Colors	2-3 Colors	No
Plan Fluorite	3-4 Colors	2-4 Colors	Yes
Plan Apochromat	3-4 Colors	4-5 Colors	Yes

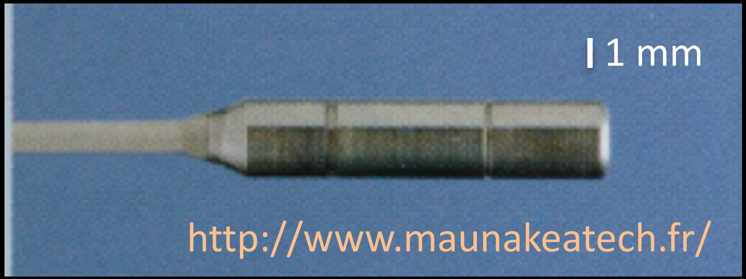


http://www.olympusmicro.com/primer/anatomy/objectives.html

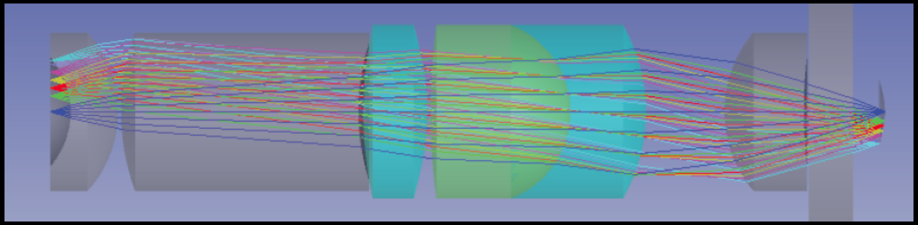
Miniature high-NA microscope objective

1st approach: Scaling the design of conventional microscope objectives

Maunakea Technologies



Our design



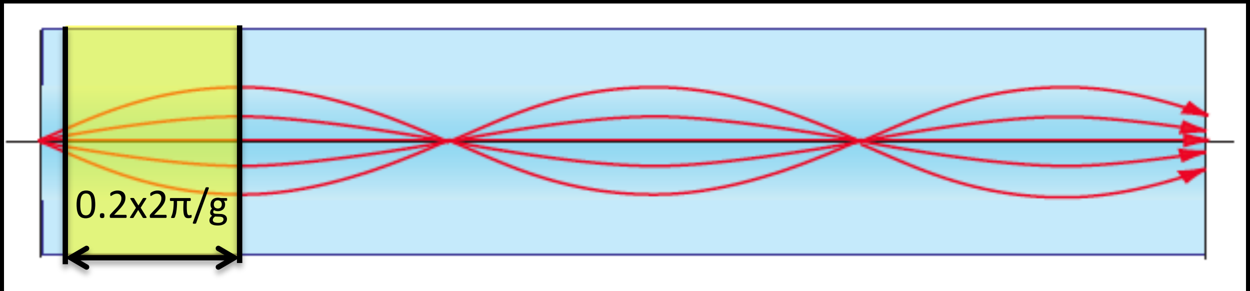
Tip diameter	2.6mm
Working distance	60µm
NA	0.7-0.8
Field-of-view	240µm
Geometric and chromatic aberrations	Limited

Current limitations:

- Not commercially available
- Need for custom-designed miniature lenses of small diameter -> High cost

Miniature high-NA microscope objective

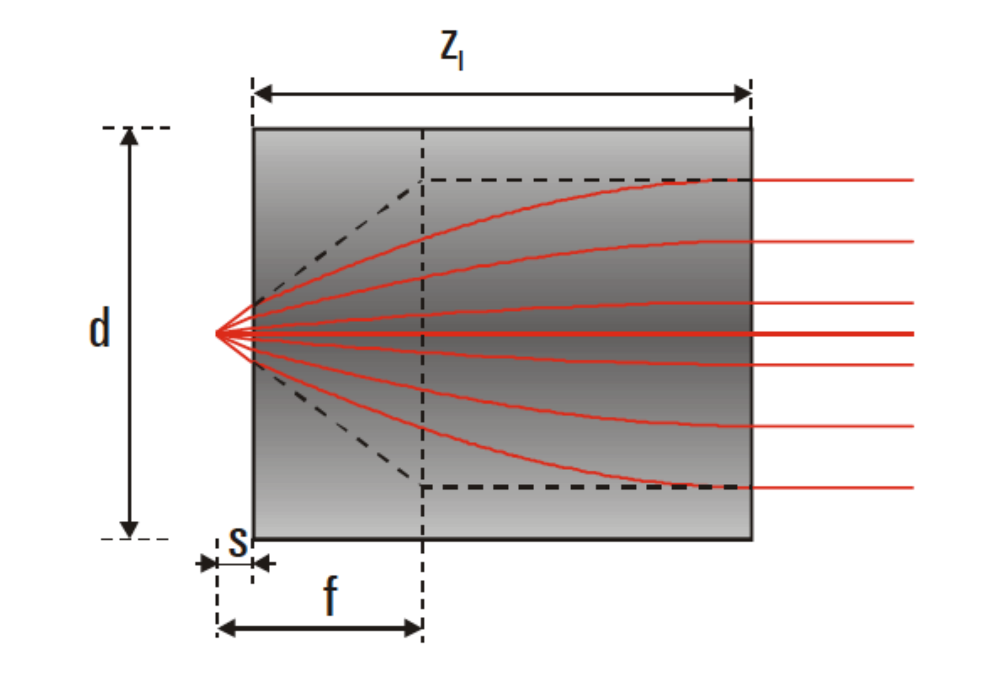
2^{nd} approach: Gradient index (GRIN) lenses



Index profile

$$n(r) = n_0(1 - g^2r^2/2)$$

Gradient-index (GRIN) lenses:



Focal length

$$f = \frac{1}{n_0 g \sin(g z_l)}$$

Working distance

$$s = \frac{1}{n_0 g \tan(g z_l)}$$

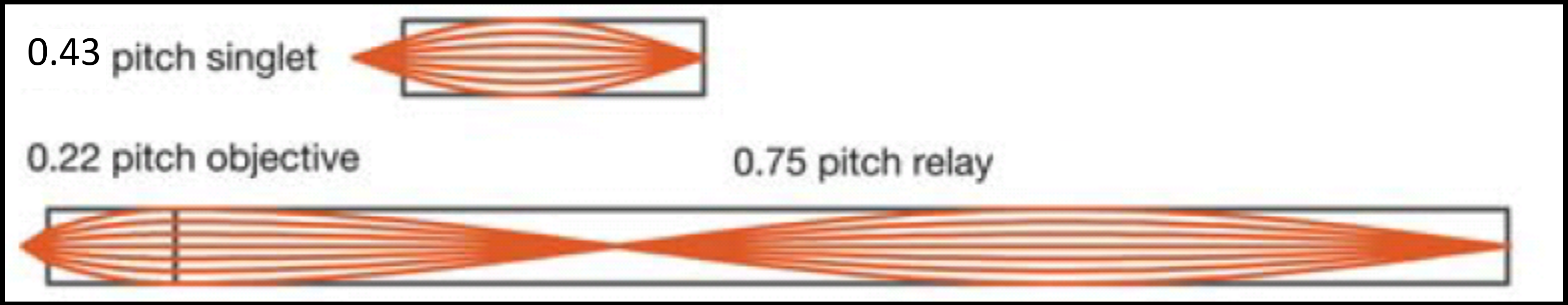
Numerical aperture

$$NA = \sqrt{n_0^2 - n_R^2}$$

http://www.grintech.de/

Miniature high-NA microscope objective

2nd approach: Gradient index (GRIN) lenses

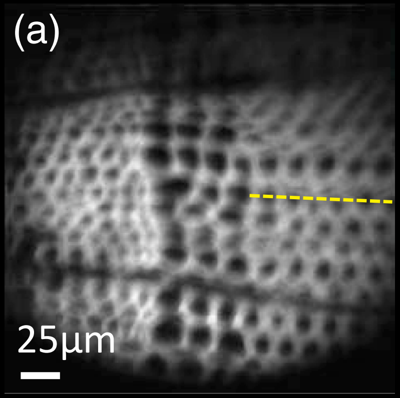


Barretto and Schnitzer, Cold Spring Harbor Protocol, 2012

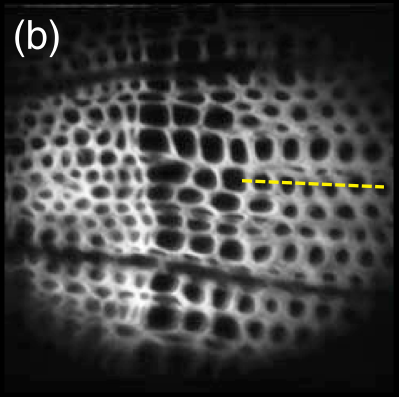
Advantages: Thin and low cost

Drawbacks:
- NA ≤ 0.5 (limited by index gradient amplitude)
- Suffers from geometric aberrations (spherical aberration, coma, astigmatism) and chromatic aberrations

Imaging of a test fluorescent sample using a grin lens



After aberration correction (mostly: spherical, coma, astigmatism)



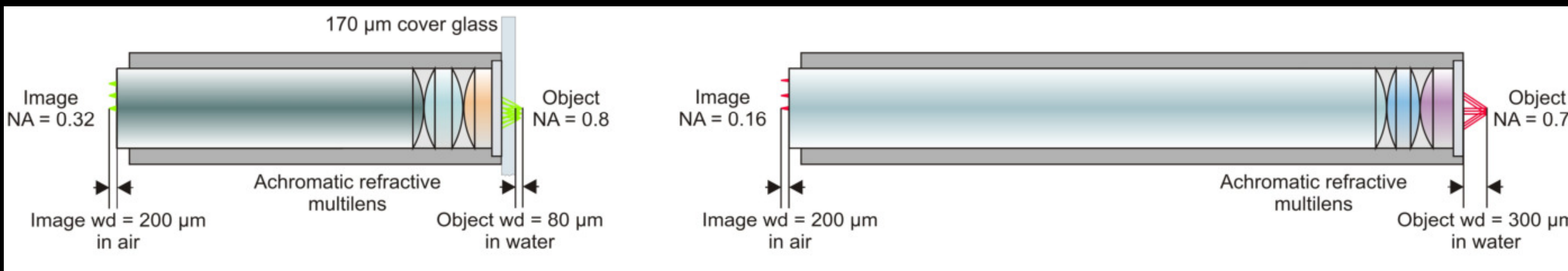
Lee at al. Optics Letters 2011

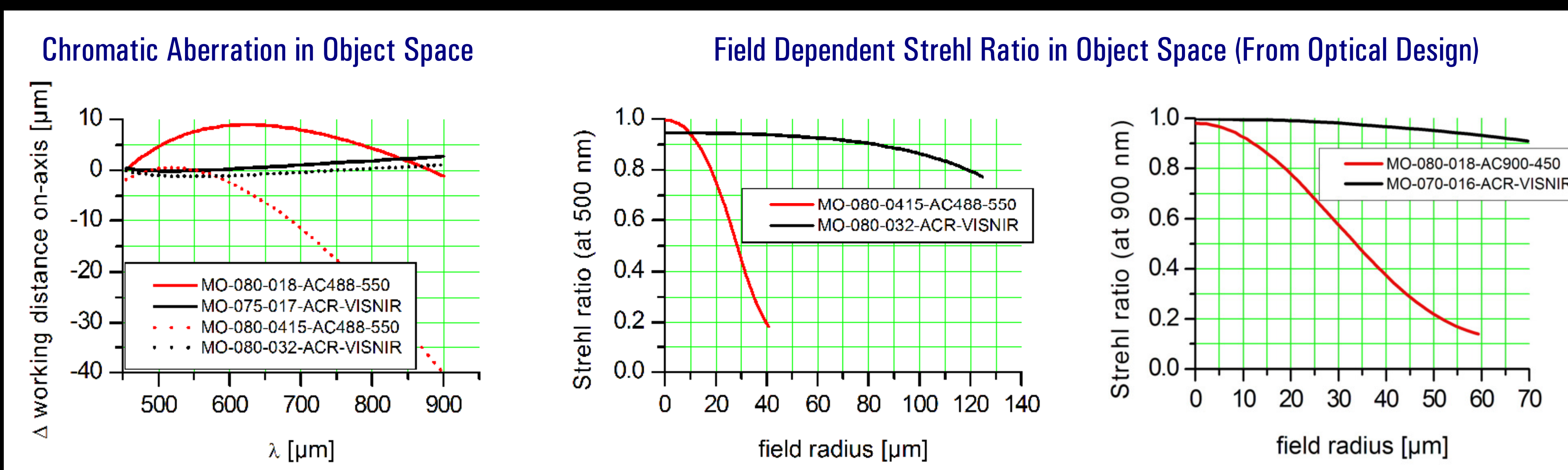
Miniature high-NA microscope objective

2nd approach: Gradient index (GRIN) lenses

Novel designs with chromatic and geometric aberration correction

GT-MO-080-0.32-ACR-VISNIR GT-MO-070-0.16-ACR-VISNIR



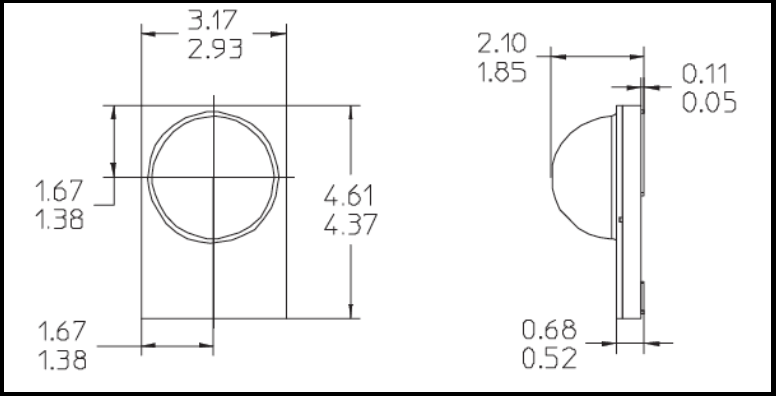


http://www.grintech.de/

Miniature light sources

Miniature LEDs for 1-photon microscopy

Dimensions (in mm)

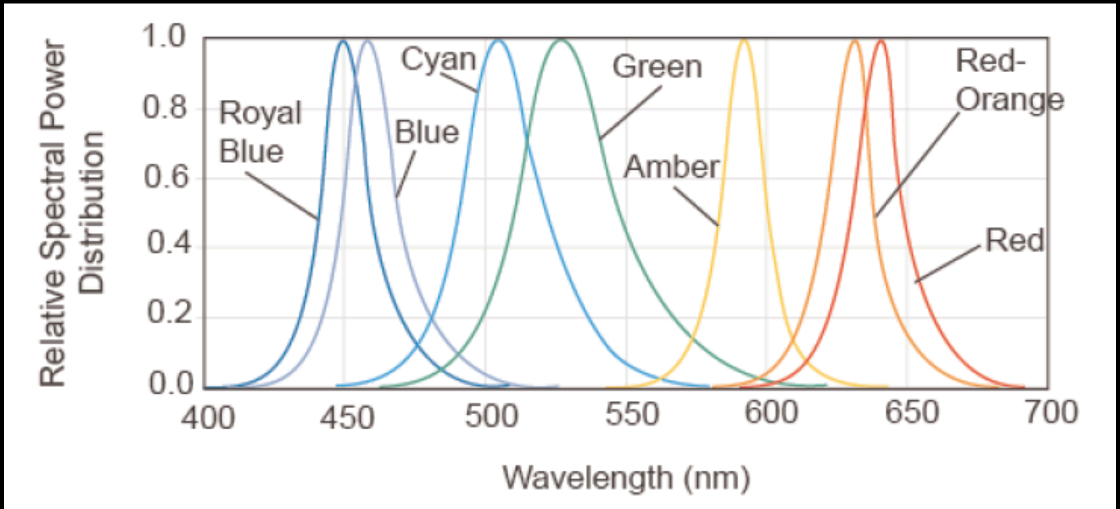


farnell.com

Output power >100mW

Multiple wavelengths available

LED must be installed
on a heat sink



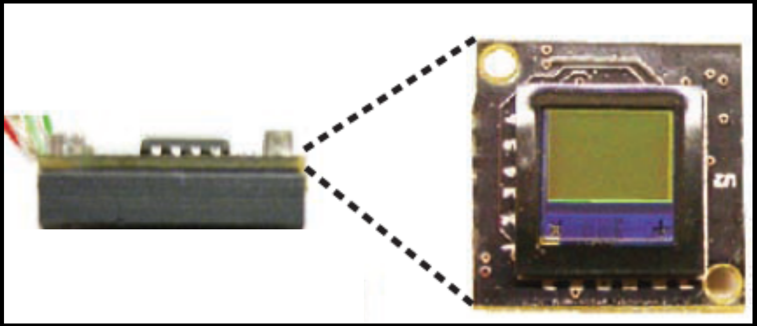
No miniature source for 2-photon imaging

Miniature cameras

sCMOS camera (Hamamatsu ORCA-Flash4.0)

2kg

Miniature camera (Aptina Imaging, MT9V021)



Ghosh et al, Nat Methods, 2011

8mm

≈1g

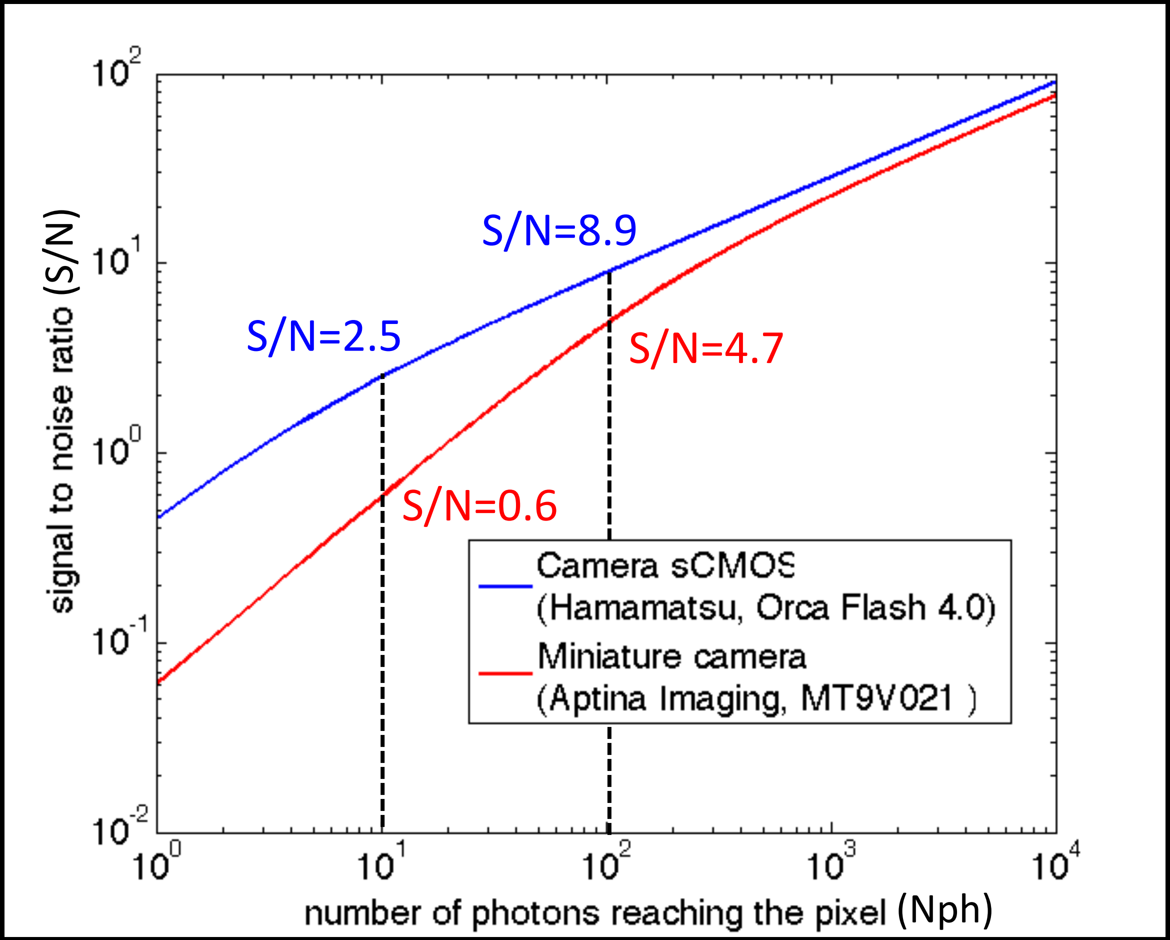
sCMOS camera	Miniature camera
2048x2048 pixels	640 x 480 pixels (now 1440 x 1080)
Quantum efficiency: QE = 82%	QE = 60%
Speed: 100 fps full frame (Cameralink)	30 fps (now 120 fps)
Readout noise: σ_{read} = 1.6e-	σ_{read} = 10e-

$$\frac{S}{N} = \frac{Nph.QE}{\sqrt{Nph.QE + (\sigma_{read})^2}}$$

Miniature cameras

A quick comparison of the signal to noise ratios

$$\frac{S}{N} = \frac{Nph.QE}{\sqrt{Nph.QE + (\sigma_{read})^2}}$$



➡ Characteristics are approaching that of high performance cameras except for the noise level

Outline

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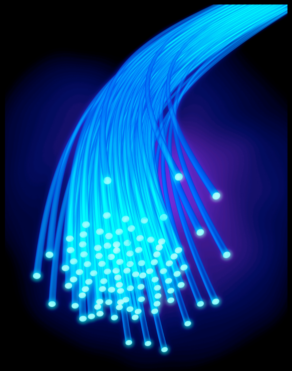
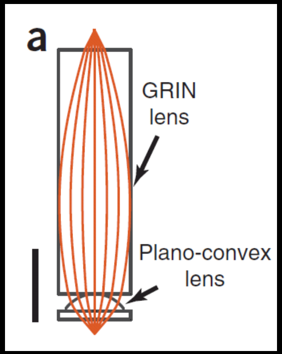
1.3- Necessary ingredients: optical fibers and miniature optics

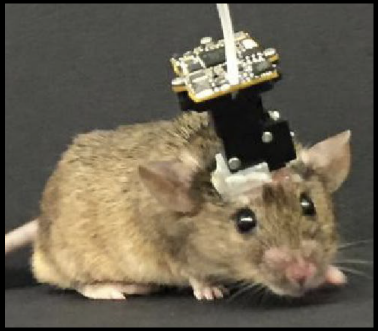


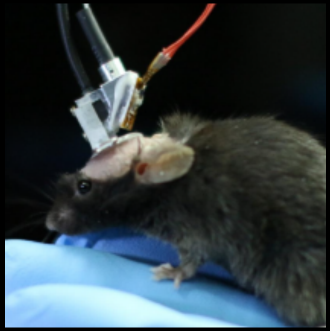


2- Miniature microscopes

2.1- Conventional widefield imaging

2.2- 2-photon imaging



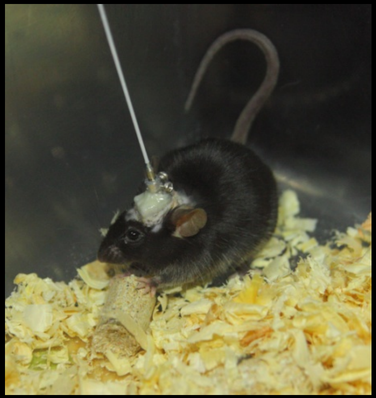


3- Fiberscopes

3.1- Conventional widefield imaging

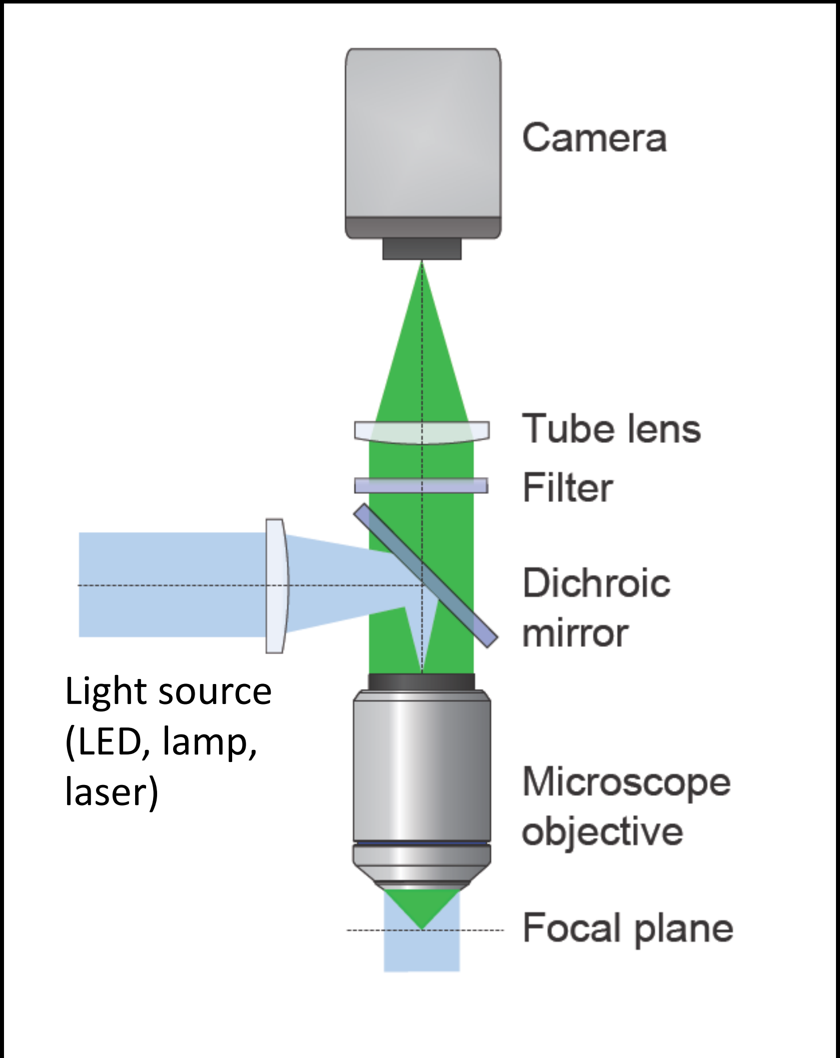
3.2- 2-photon imaging

3.3- Fast confocal imaging and targeted photoactivation



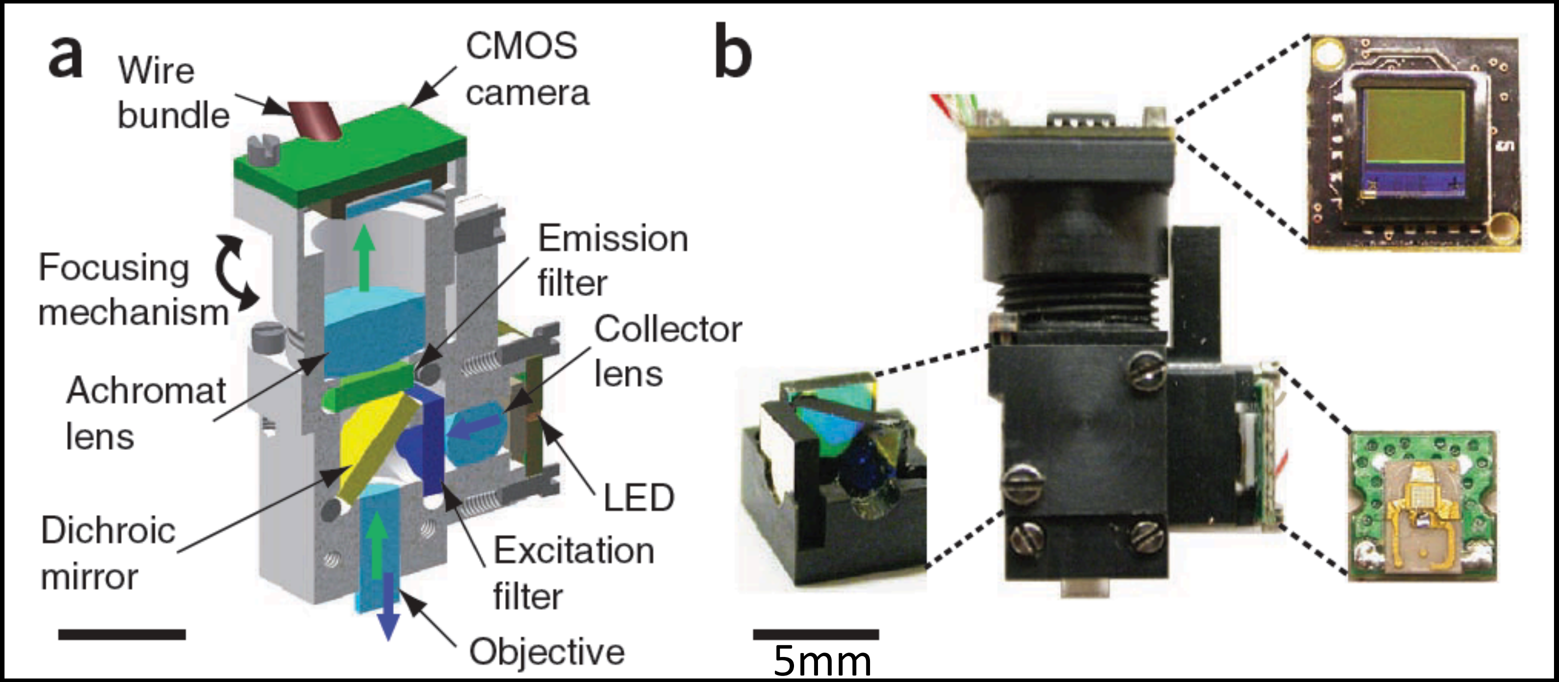
Conventional widefield imaging

Architecture of the microscope



All the elements can be miniaturized!

Miniature conventional widefield microscope

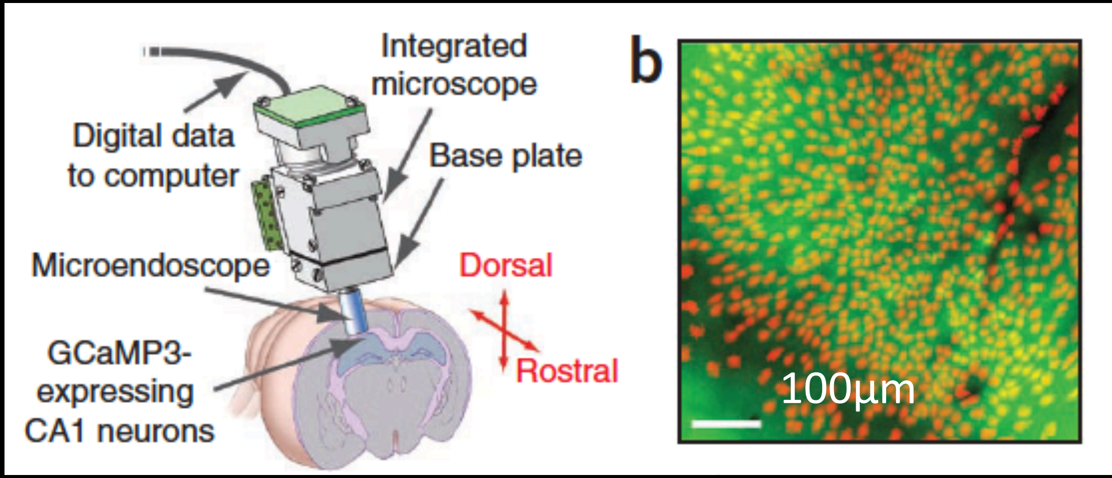


Ghosh et al., Nat Methods, 2011

- Weight: 1.9 g
- Focusing by adjusting the camera position
- Speed: 36Hz full frame (FOV: 600x800μm^2),
  100Hz with binning (FOV: 375x375μm^2)
- Lateral resolution: 2.5µm, limited by camera pixel size
- Objective: NA=0.45
- Motion artifact < 1µm

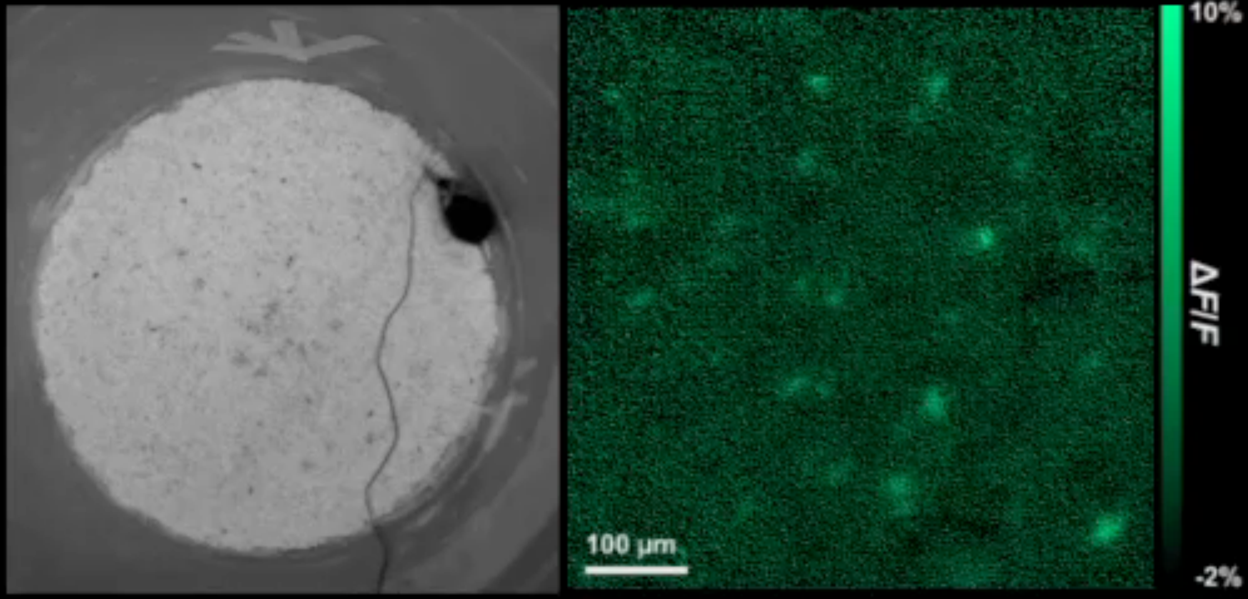
Miniature conventional widefield microscope

Application to the study of long term dynamics of CA1 place codes



- Imaging of GCaMP3 in the hippocampus (cortex is removed)
- Base plate implanted : imaging over 45 days in the same cells
- Up to 500-1000 cells per mouse

(Green: GCaMP3 fluorecence
Red: Identified cells)



Ziv, Burns, Cocker, O Hamel, Ghosh, Kitch, El Gamal, Schnitzer, Nat Neurosc, 2013

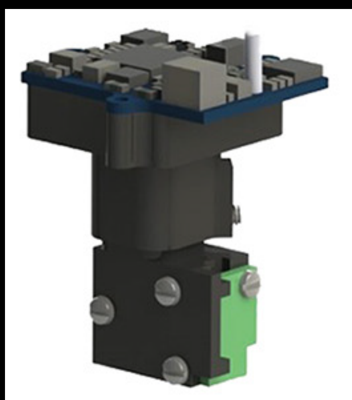
Miniature widefield microscopy, a successful story

Used in hundreds of labs

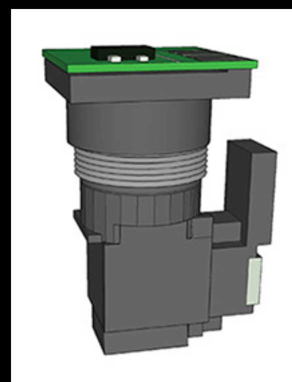
Commercial systems www.inscopix.com http://doriclenses.com/

Open source systems

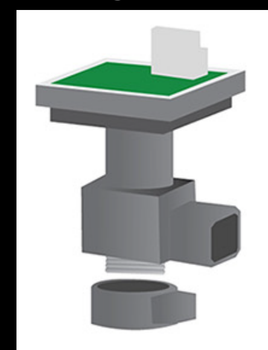
UCLA Miniscope (UCLA)



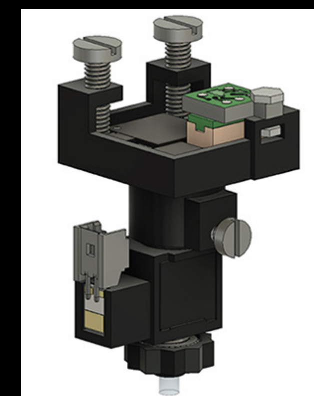
FinchScope (Boston University)



miniScope (National Institute on Drug Abuse)



CHEndoscope (Univ. of Toronto)



Novel developments

- Large field-of-view imaging in rats (Scott et al, Neuron, 2018)
- Volumetric imaging with light field microscopy (Scocek et al, Nature Methods, 2018)
- Wire-free recordings (battery powered) (Schuman et al, bioRxiv, 2018)`
- 2 color fluorescence / coupling with widefield photoactivation (Doric & open source systems)
- Coupling with electrophysiology

Review: Aharoni et Hoogland, Frontiers in Cellular Neuroscience, 2019

Miniature conventional widefield microscope: conclusion

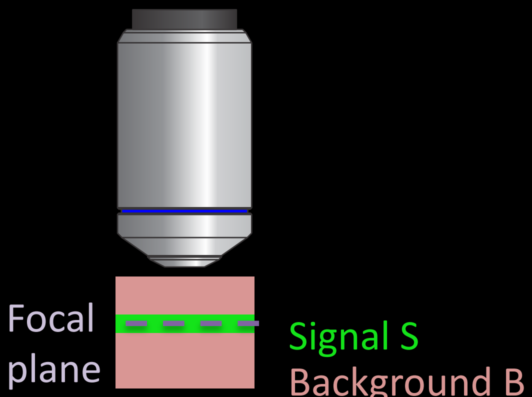
Advantages:

- Fast (up to 120 Hz for the next gen miniscope)
- Large fields of view (mm^2)
- Few motion artifacts (1-2µm)
- Light (and simple) microscope (<2g) that can be adapted to mice
- Flexible cable

Drawbacks:

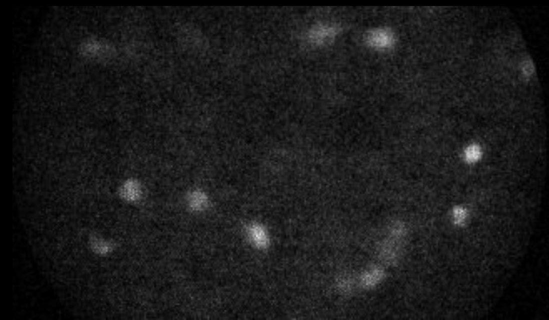
- Small working distance
- Targeted photoactivation has not been demonstrated
- Lack of optical sectioning

Optical sectioning, a key element



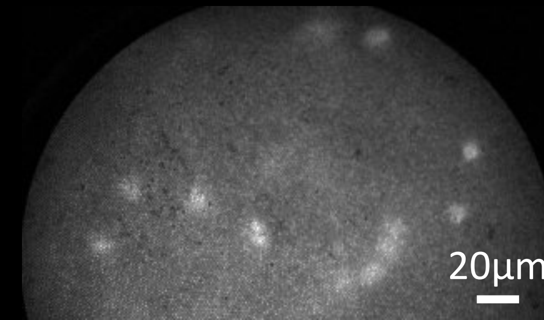
Sectioned image

S



Conventional widefield image

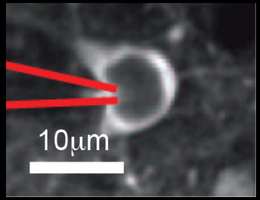
S + B



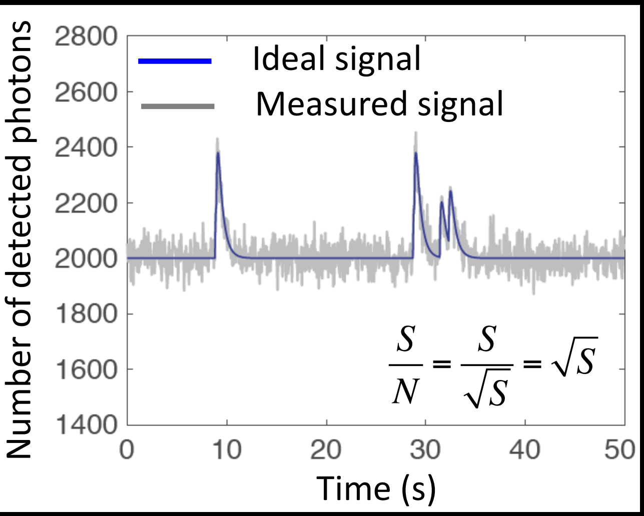
1- Loss of contrast

2- Decrease of signal to noise ratio for neuronal activity recording

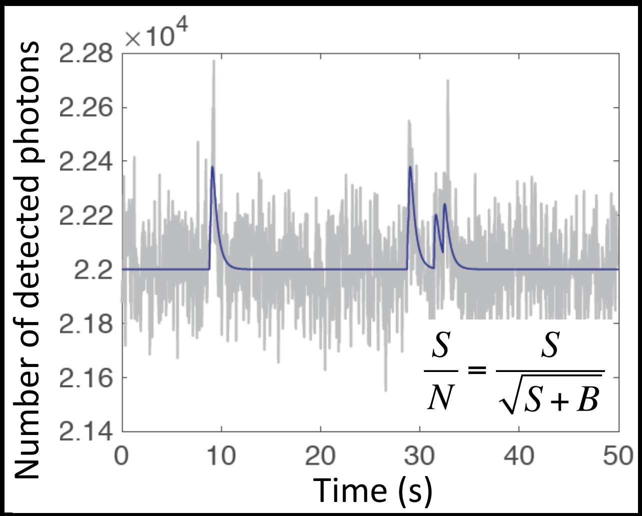
(Simulations, 1 neuron in the focal plane)



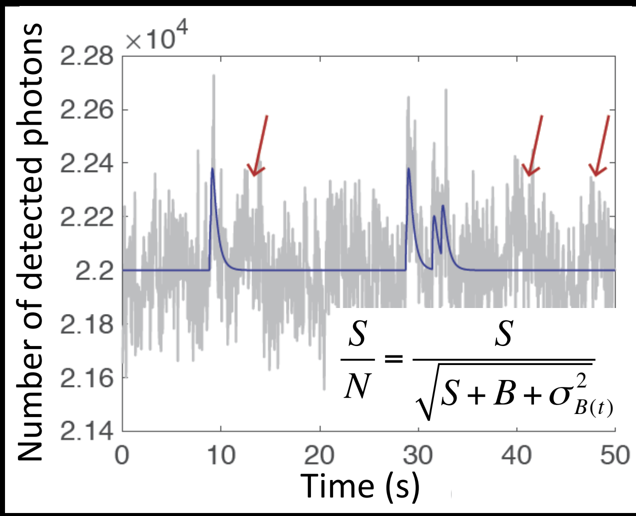
S



S + B

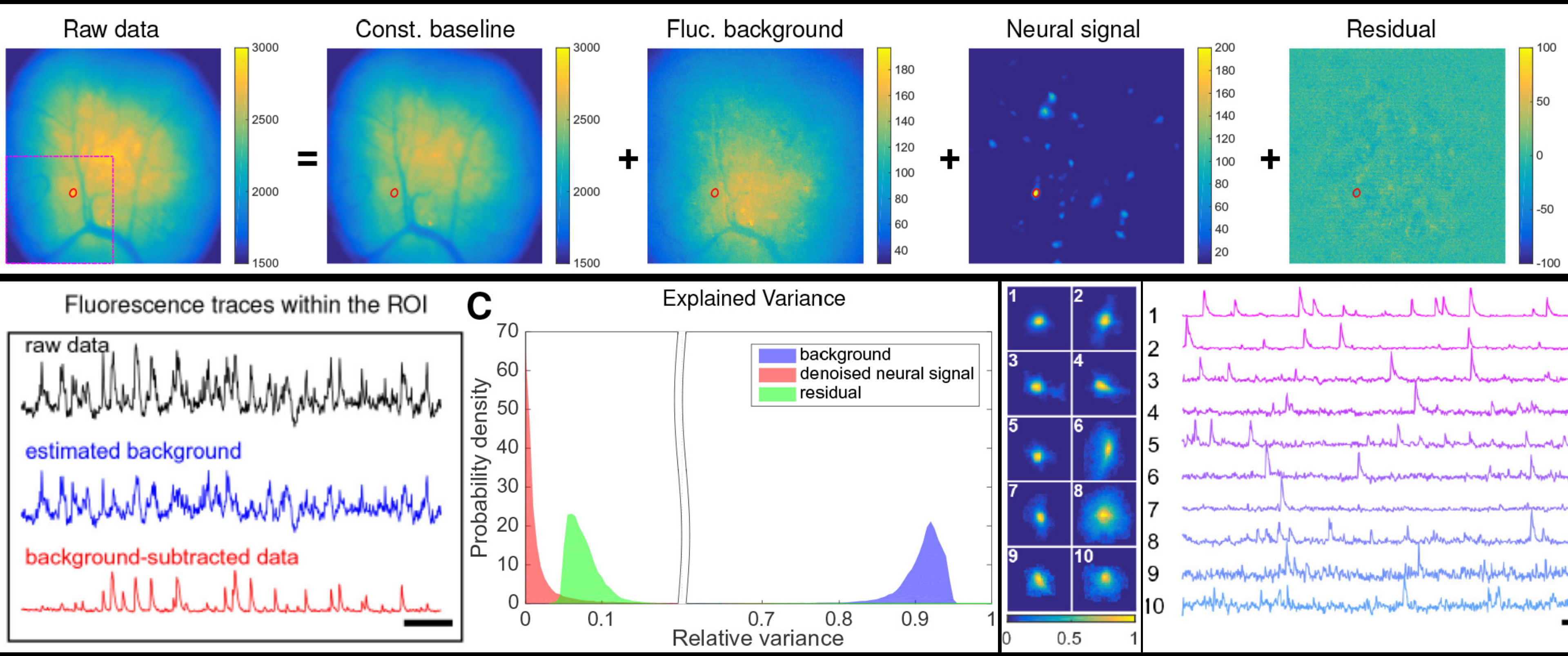


S + B(t)



Compensating the lack of optical sectioning with high-end algorithms

Algorithms to localize neurons and extract Ca2+ signals



Most of the raw data variance is due to background fluctuations

Reliability of these algorithms for experiments with a lot of background noise ?

Zhou et al, elife 2018

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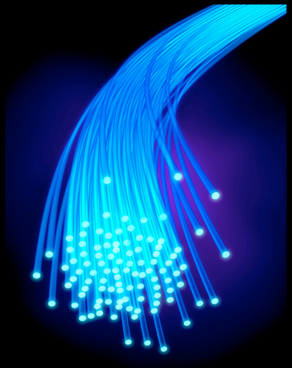
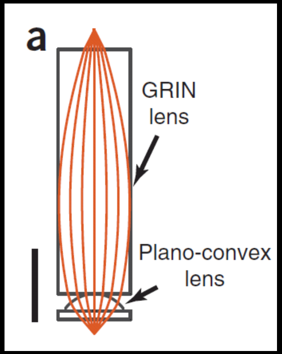
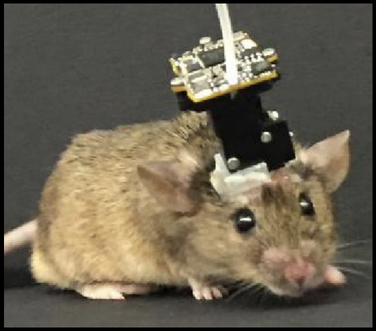


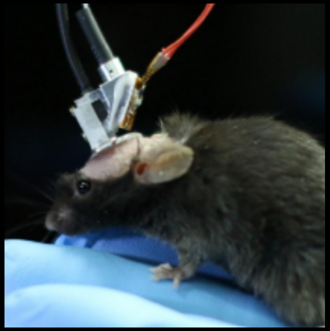


2- Miniature microscopes

2.1- Conventional widefield imaging

2.2- 2-photon imaging





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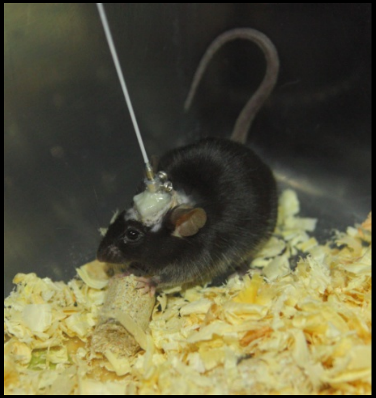
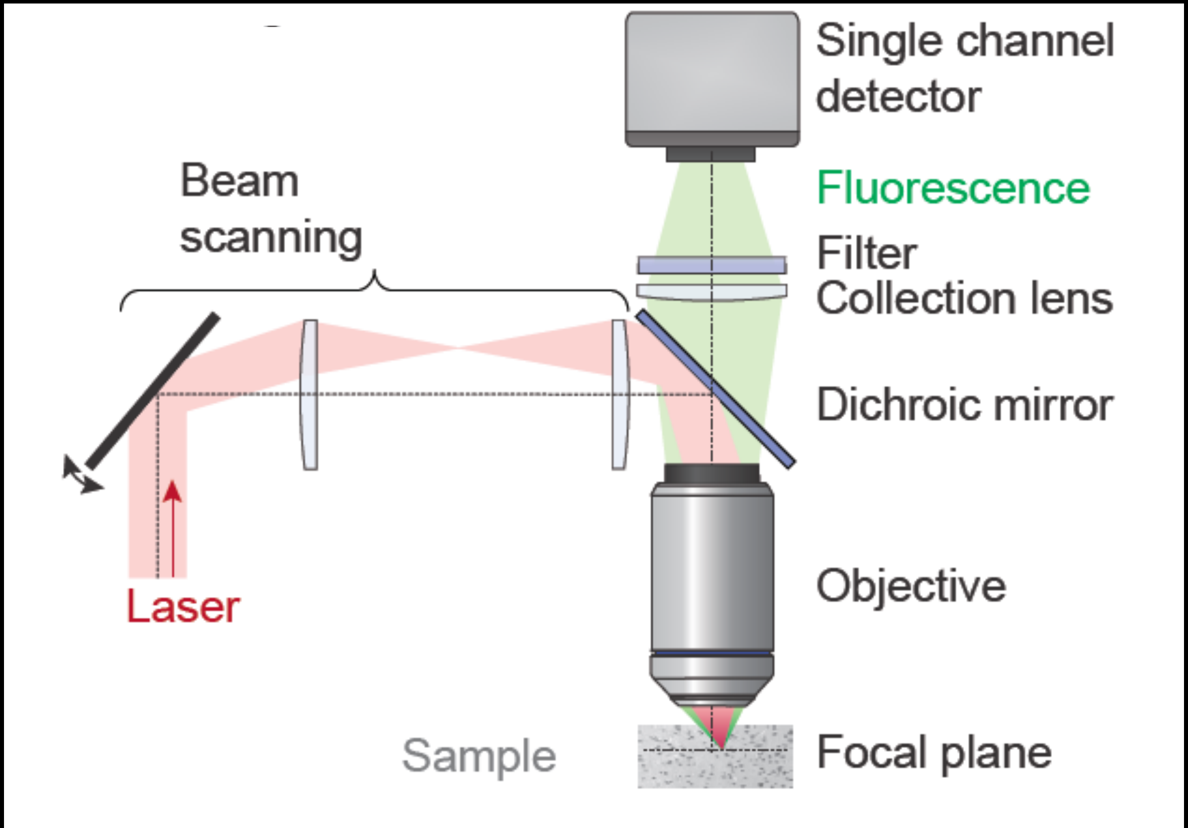


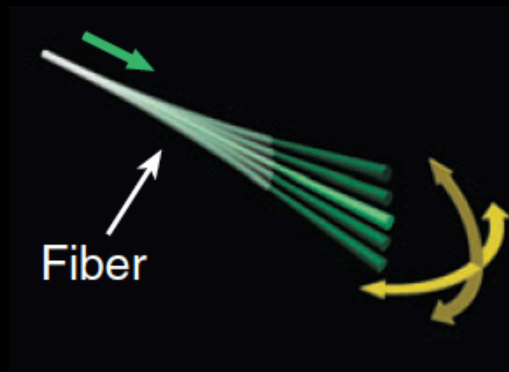
Miniaturization of 2-photon microscopy?

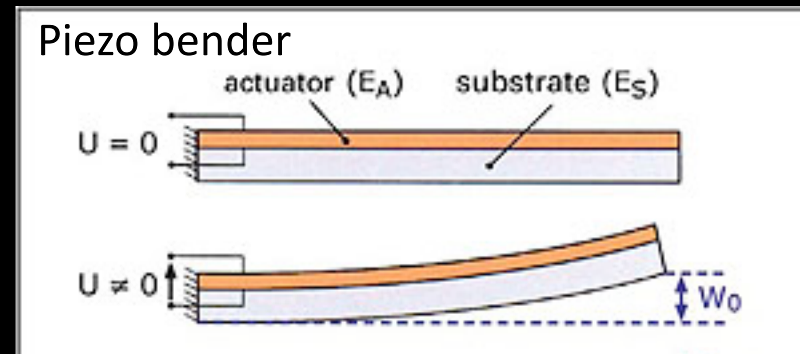


- Light source cannot be miniaturized
- Miniaturized detectors are not as performant as regular detectors

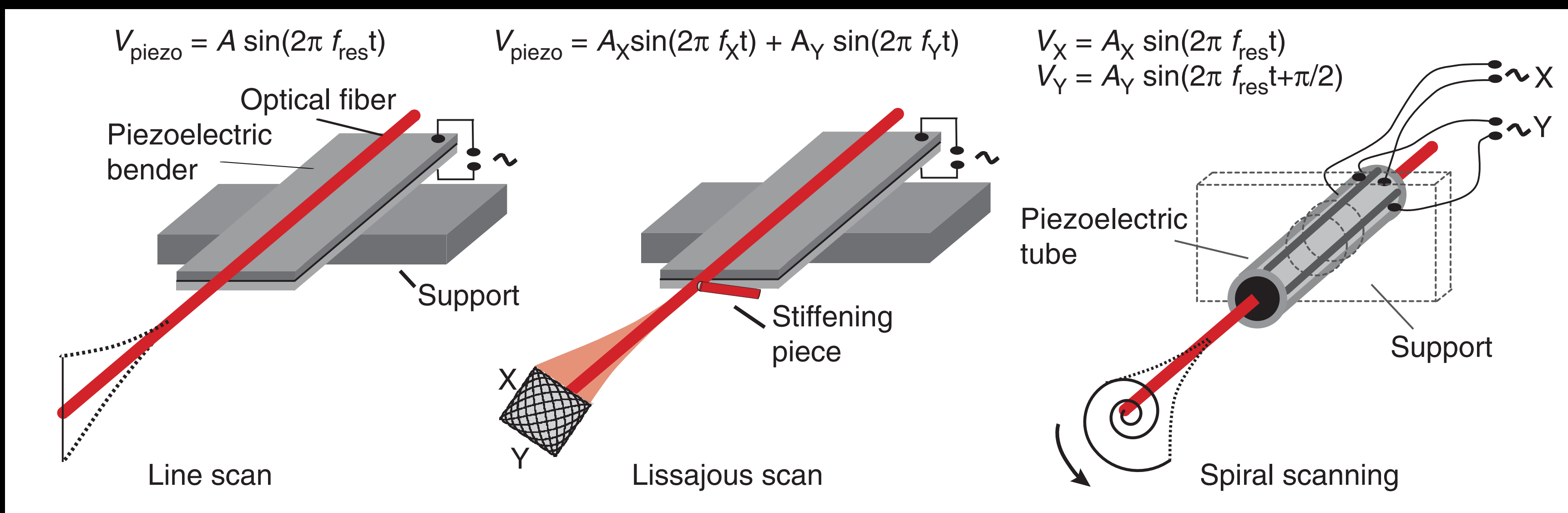
➡ Miniaturization of all the elements but the light source and detector
Use of optical fiber(s) to connect with regular light source and detector

Miniature beam-scanner #1





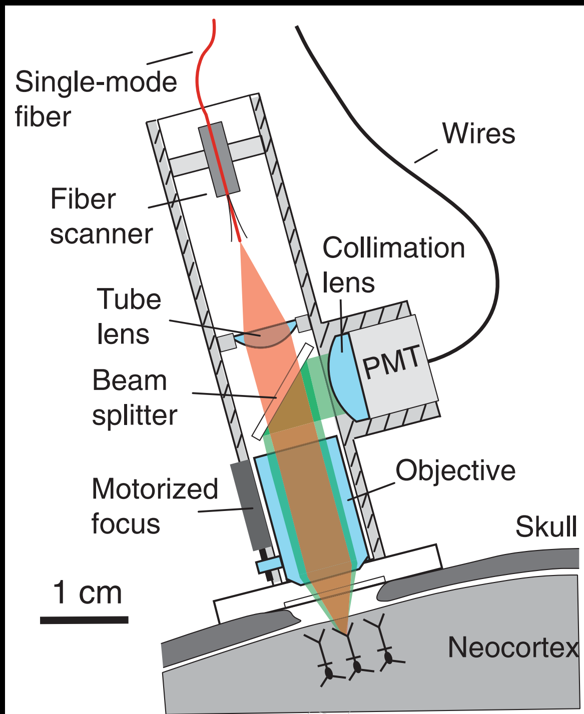
2D resonant beam scanner



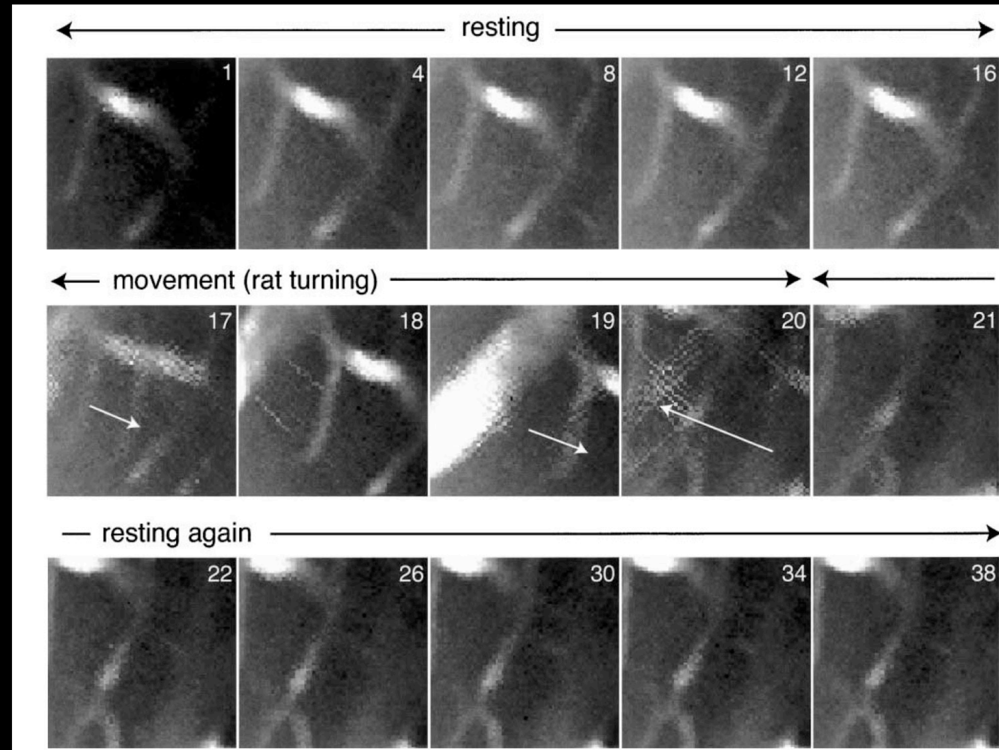
Helmchen et al. Cold Spring Harbor Protocols, 2013

Practical implementation #1

The pioneer experiment



Fluorescently labeled blood in capillaries



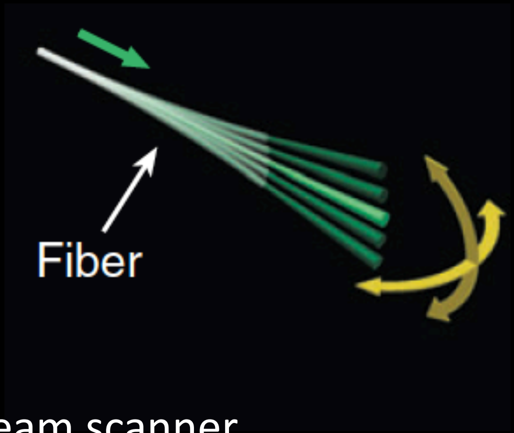
- Weight: 25 g
- Field of view : 65µm
- Max frame rate: 2Hz

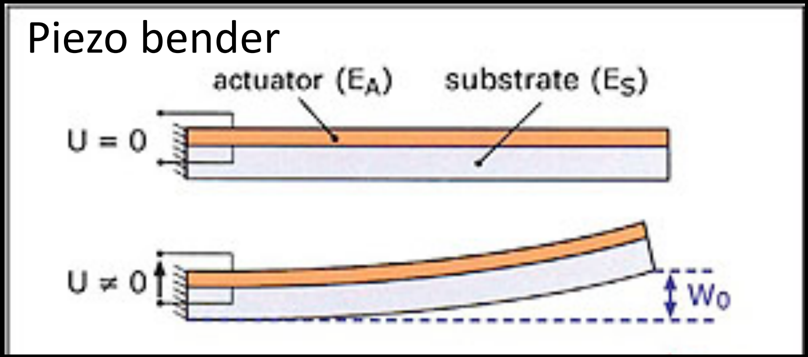
Unstable : "lateral shifts (few tens of µm) and changes in the focal plane position can occur"; "imaging is impossible during rapid movements"

F. Helmchen, M.S. Fee, D.W. Tank, & W. Denk, Neuron **31**, 903–912 (2001)

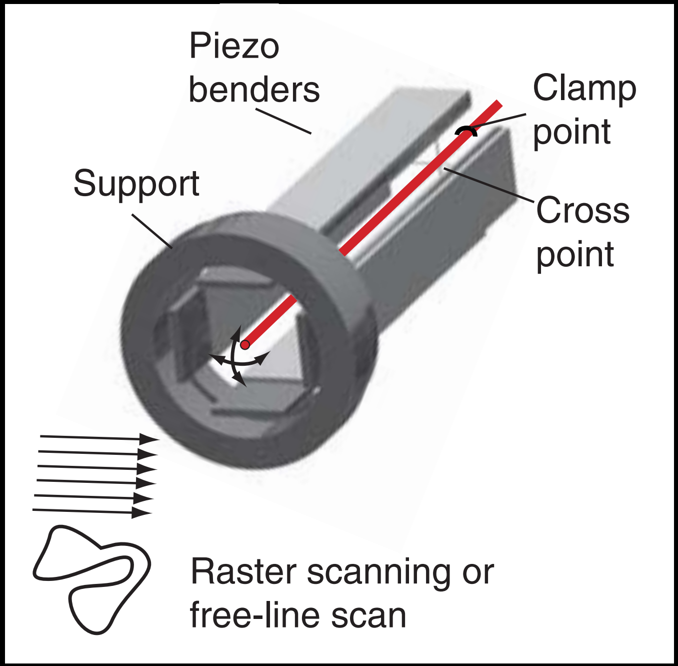
+ Other implementations never applied to freely-behaving rodents

Miniature beam scanner #2





2D nonresonant beam scanner

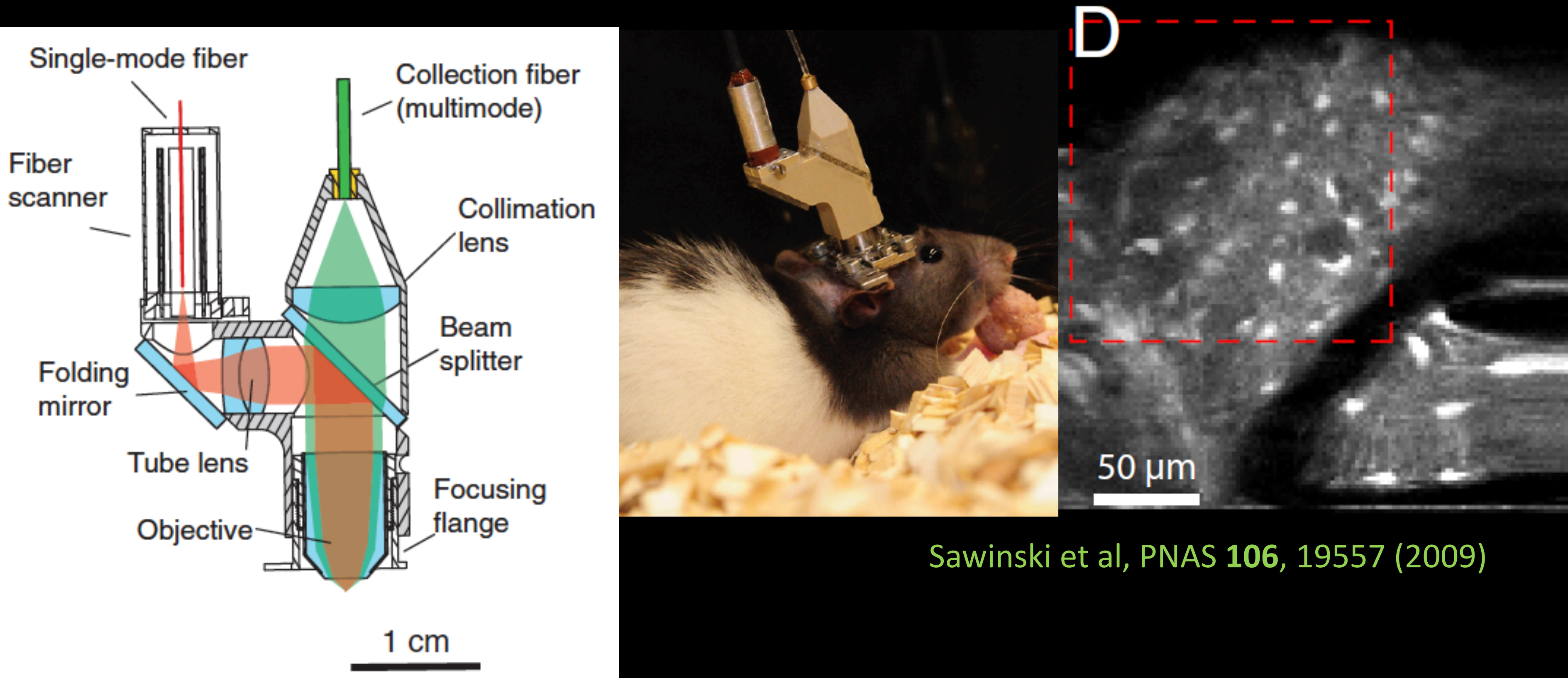


Cross point close to the clamp point : allows for large movements of the fiber tip

Raster scanning or random access scanning is possible

Helmchen et al. Cold Spring Harbor Protocols, 2013

Practical implementation #2: imaging in freely-behaving rats



Sawinski et al, PNAS **106**, 19557 (2009)

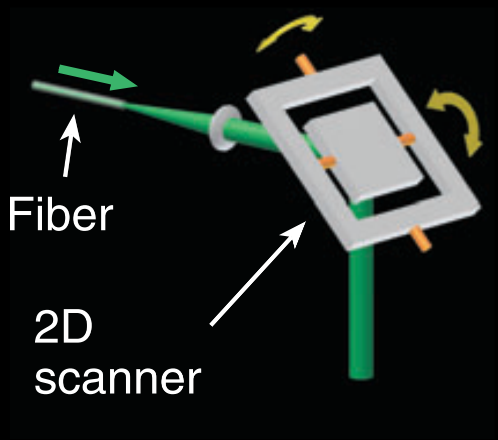
- 5.5g
- 10.9fps
- 64x64 pixels
- Custom objective (NA = 0.9, WD = 0.7mm)

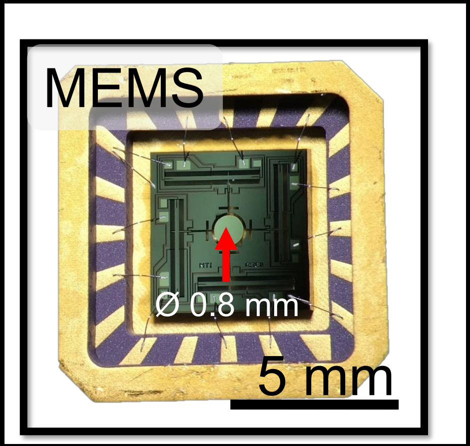
- Lateral resolution: 0.9 µm
- Imaging up to 250 µm depth
- Pulse duration: 2ps
- Multicolor imaging

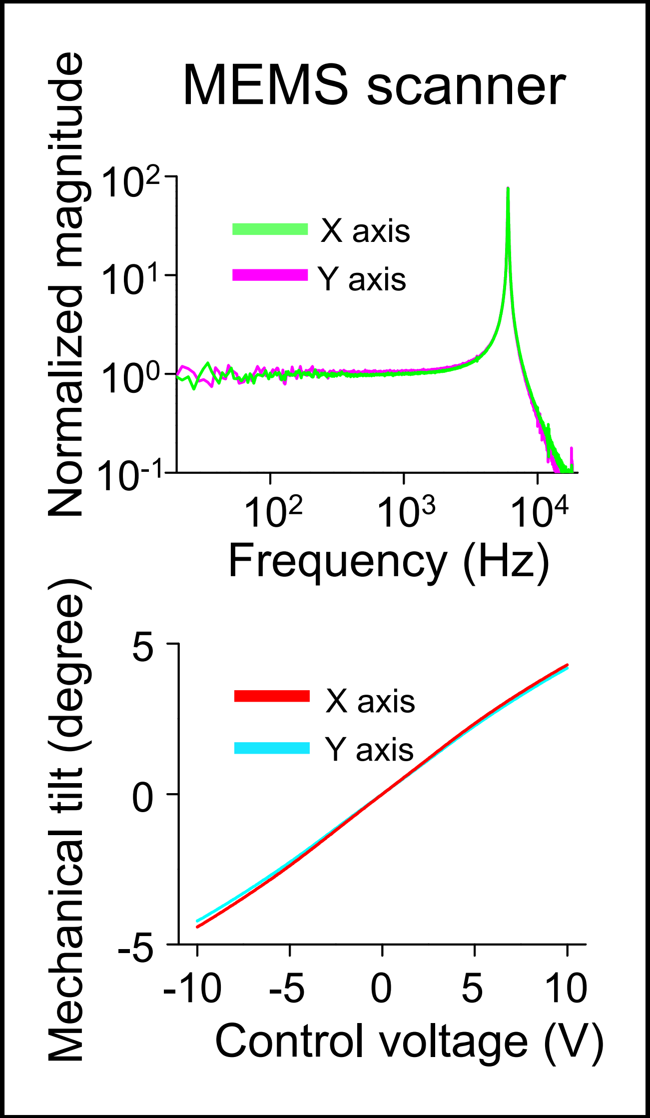
Too heavy for mice

Miniature beam scanner #3

MEMS (micro-electromechanical systems) scanner





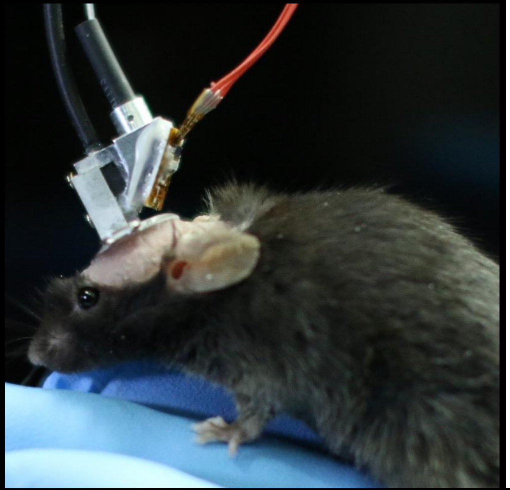


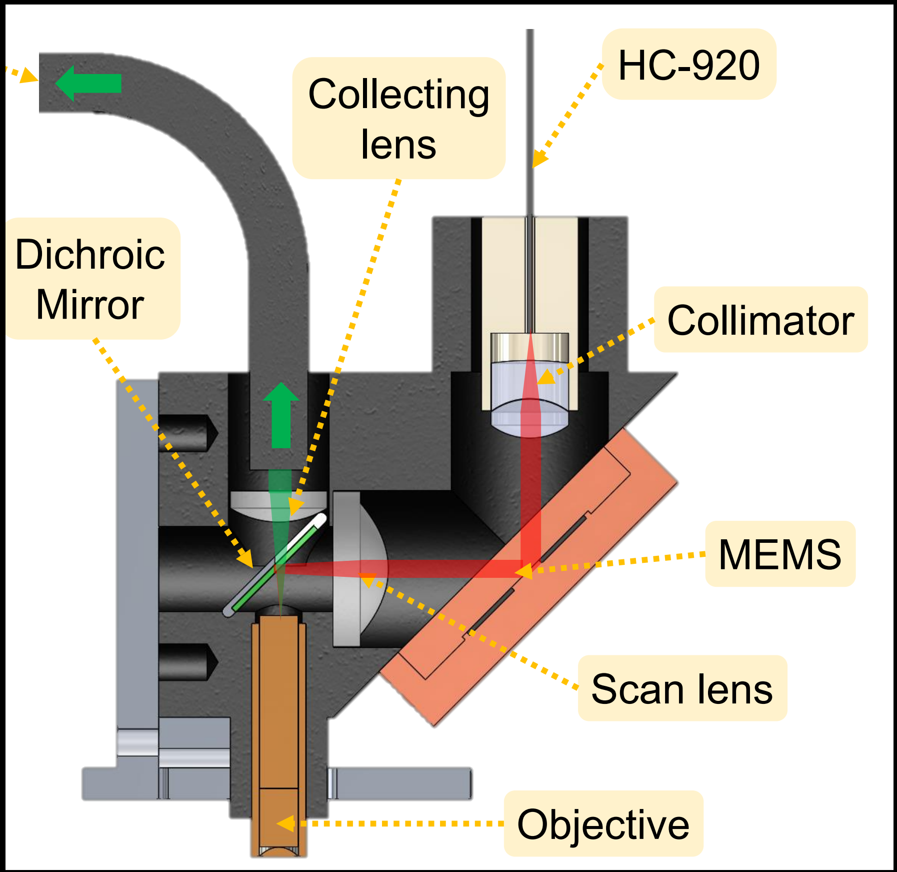
- 2D scanner
- resonant frequency at 6.2 kHz
- scanning angle ± 5.0° (± 10° optical deflection angle)
- diameter 0.8 mm

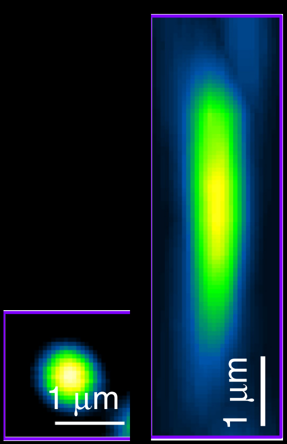
Zong et al. Nature Methods 2017

Practical implementation #3: imaging in freely-behaving mice

- Designed for GFP/GCaMP6 imaging (λ_{exc} = 920 nm)
  Custom hollow core optical fiber
- GRINTECH miniature objective (NA = 0.8)
- MEMS mirror for 2D scanning
- Lightweight (2.15g) and small ($1cm^3$)





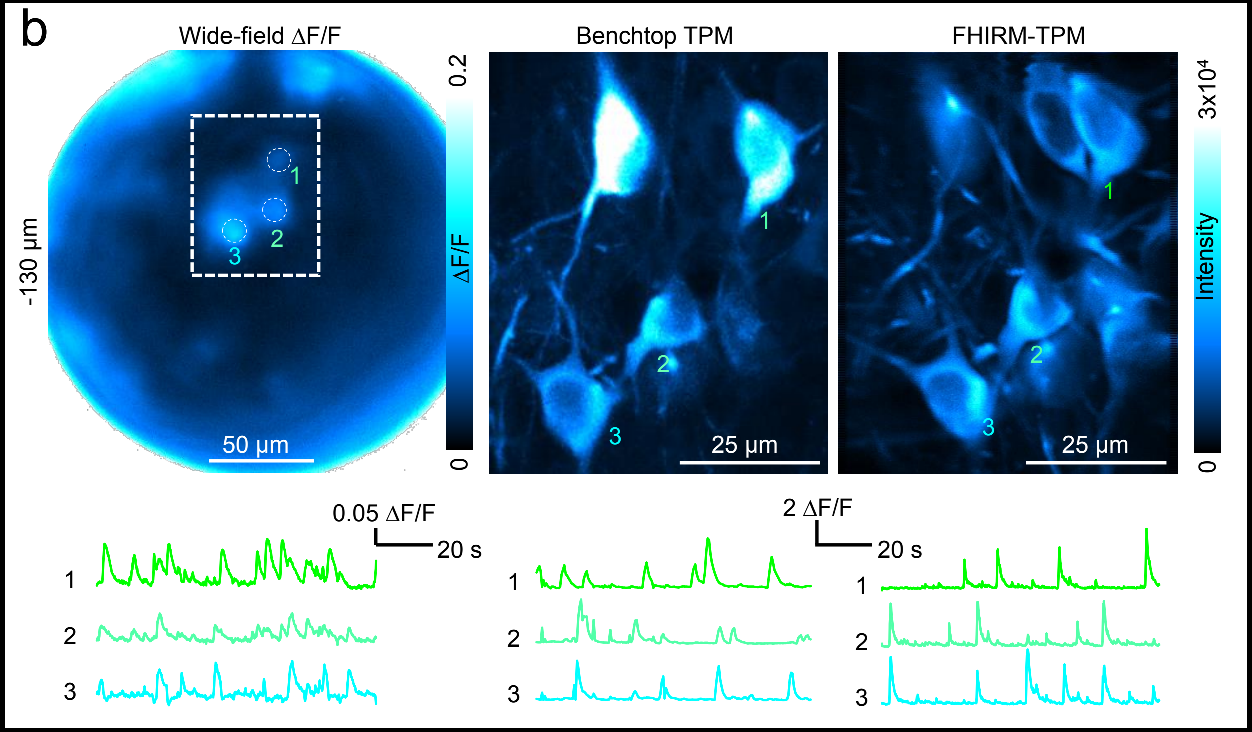


40Hz imaging, FOV $130x130\mu m^2$, 256x256 pixels
Lateral and axial resolutions of 0.5 µm and 3µm (FWHM)

Zong et al. Nature Methods 2017

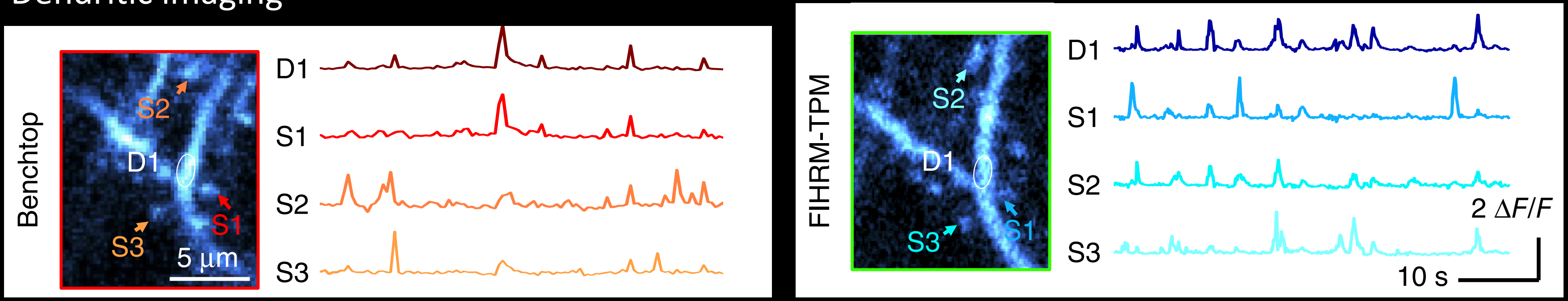
Comparison of 3 techniques for functional imaging

GCaMP6f imaging in the prefrontal cortex of head-fixed, awake mice (following AAV injections)



➡ Similar Ca2+ traces are measured with benchtop and miniature 2ph-microscopes

Dendritic imaging



Zong et al. Nature Methods 2017

Exploring various behaviors incompatible with head-fixation

Conclusion on 2-ph miniature microscopes

Achievements

- 2-photon imaging in freely behaving mice
- High resolution enabling dendritic imaging
- Fast imaging (40Hz)

Current limitations

- Fixed axial plane
- Small field of view

} Currently being improved

- Targeted photoactivation has not been demonstrated

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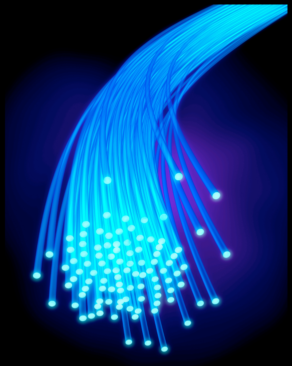
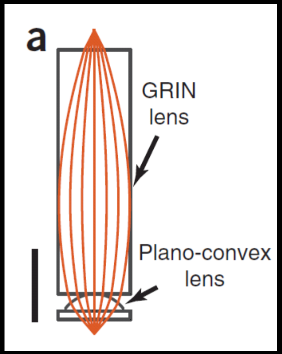
1.2- 2 strategies: miniature microscopes and fiberscopes

1.3- Necessary ingredients: optical fibers and miniature optics

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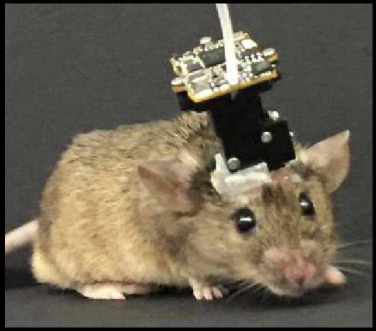
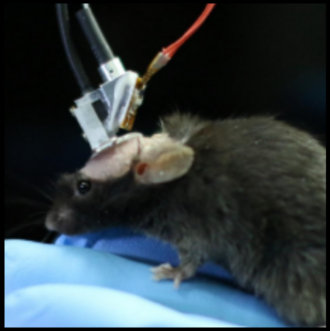

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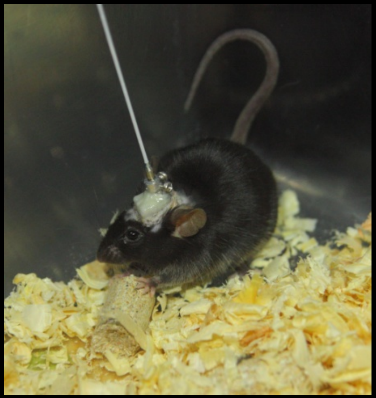
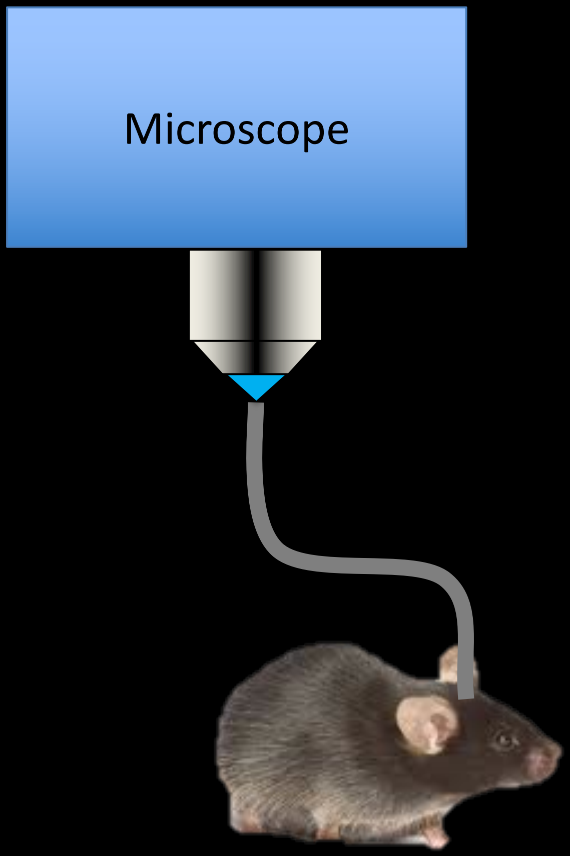
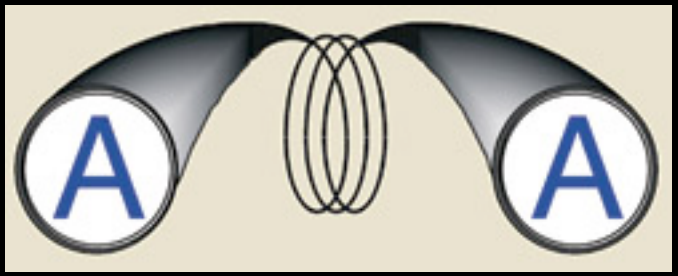
3.3- Fast confocal imaging and targeted photoactivation

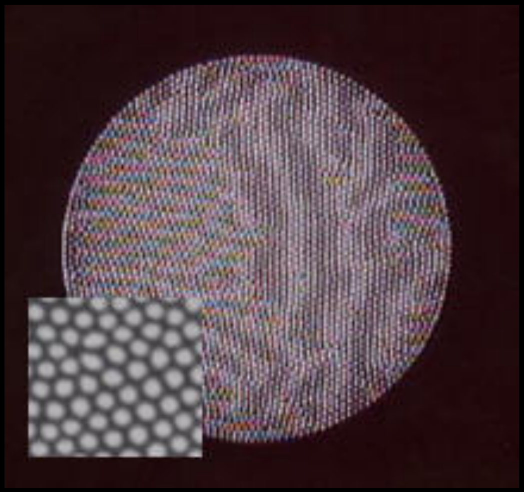


Principle of the fiberscope



A key element: the image guide





Enable implementing more sophisticated techniques or combining different techniques

Outline

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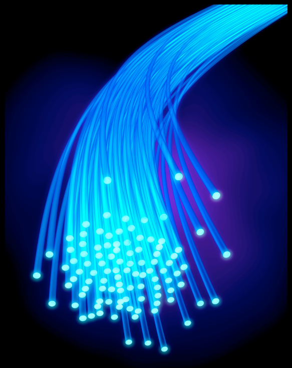

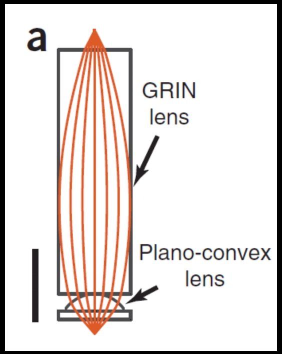

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2.1- Conventional widefield imaging

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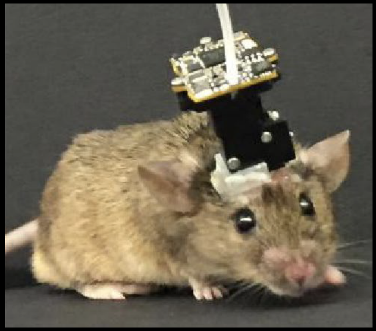

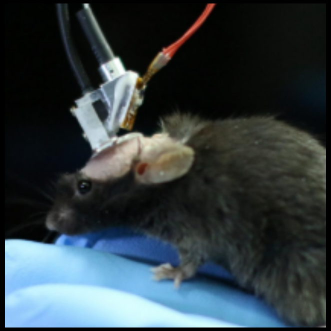

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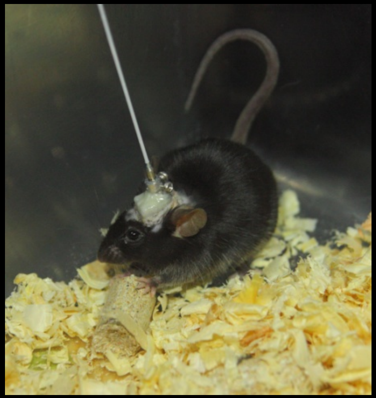
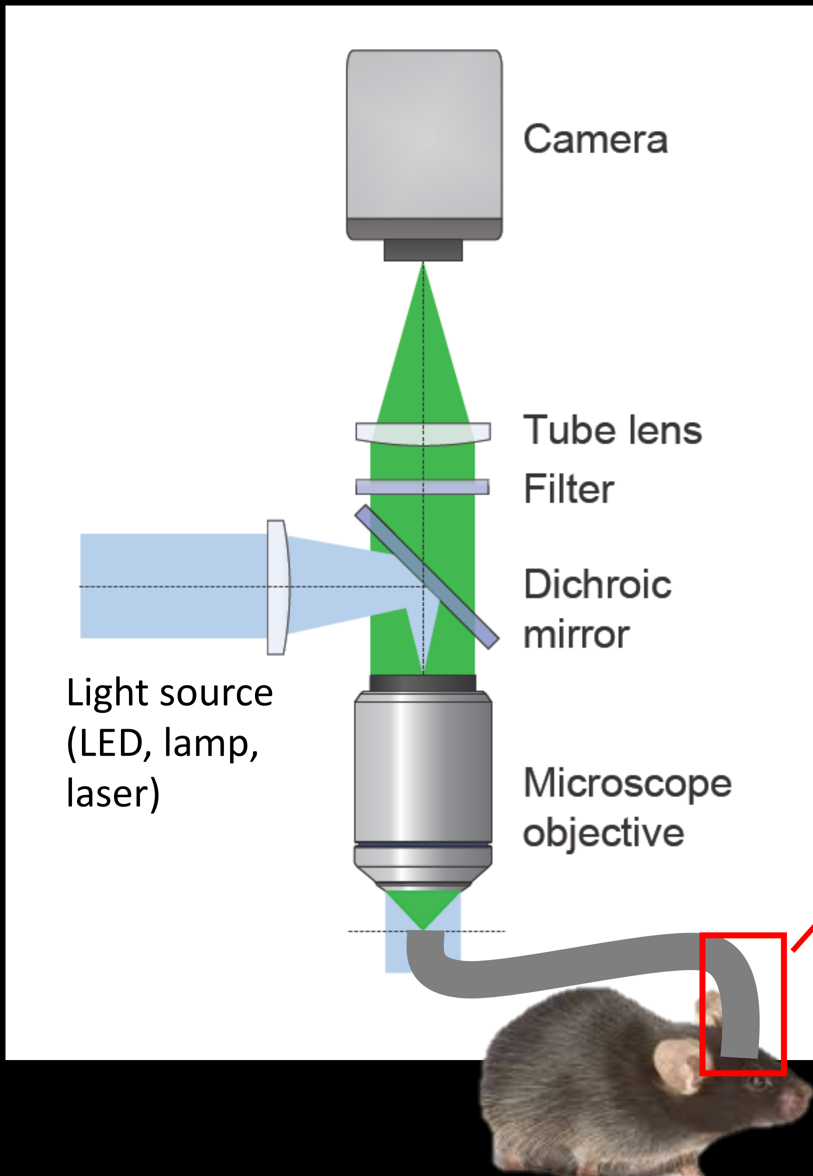
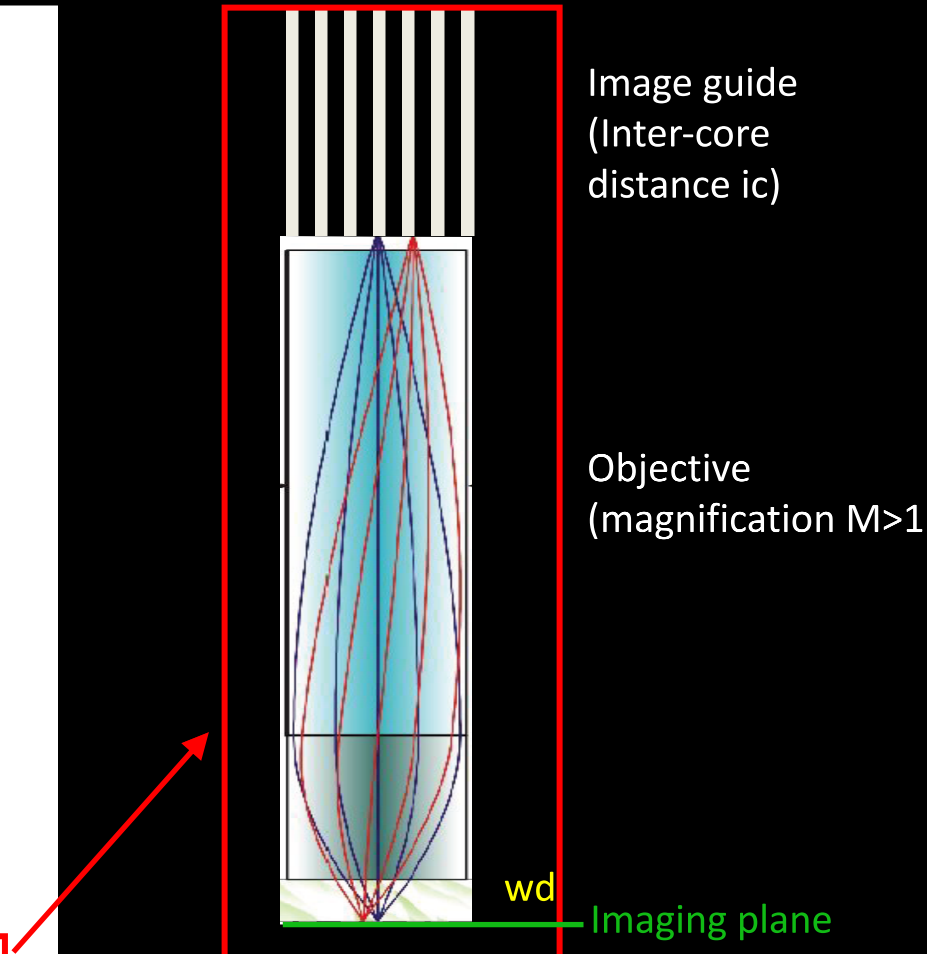


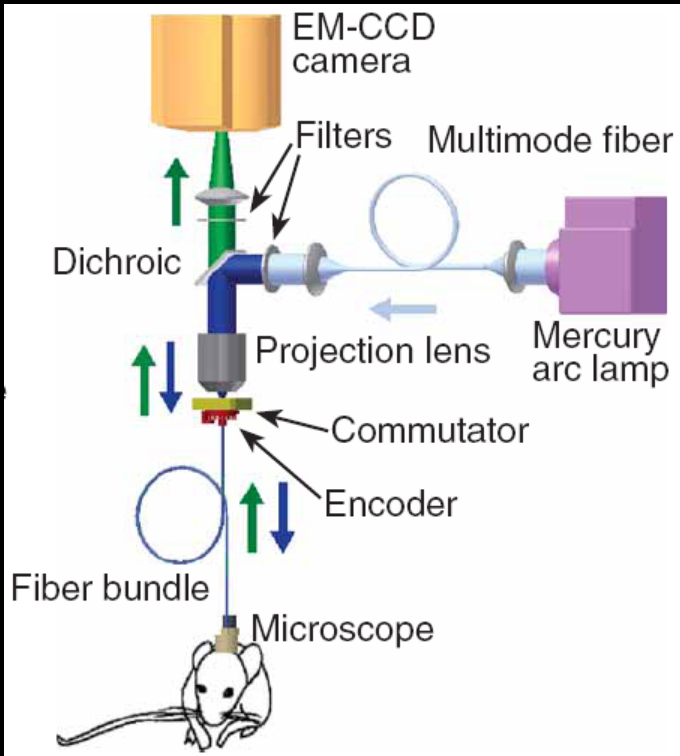
Principle of widefield imaging with a fiber bundle
Use of a micro-objective

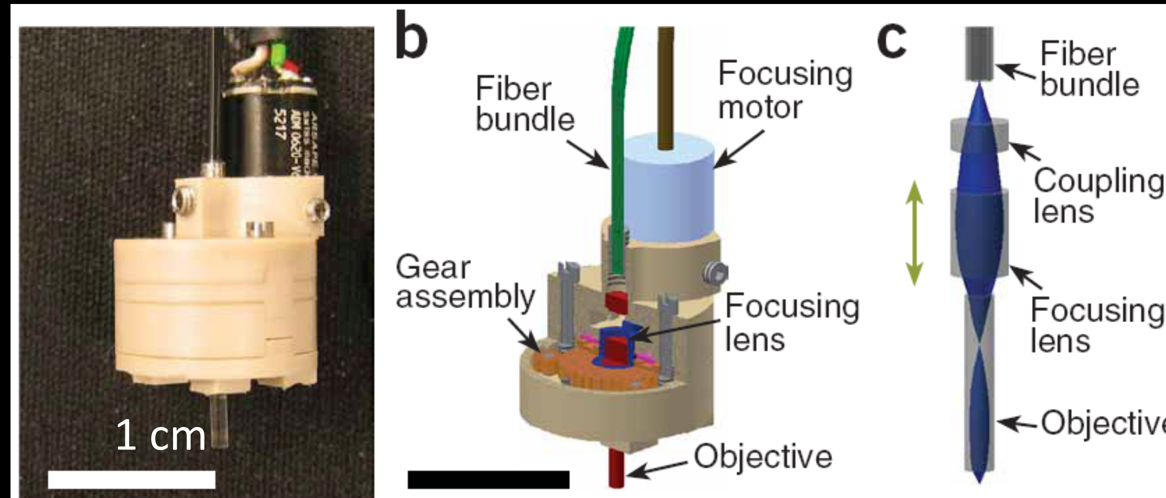




- Lateral resolution: 2 ic/M
- Imaging plane: at a working distance wd
- Increasing collection efficiency

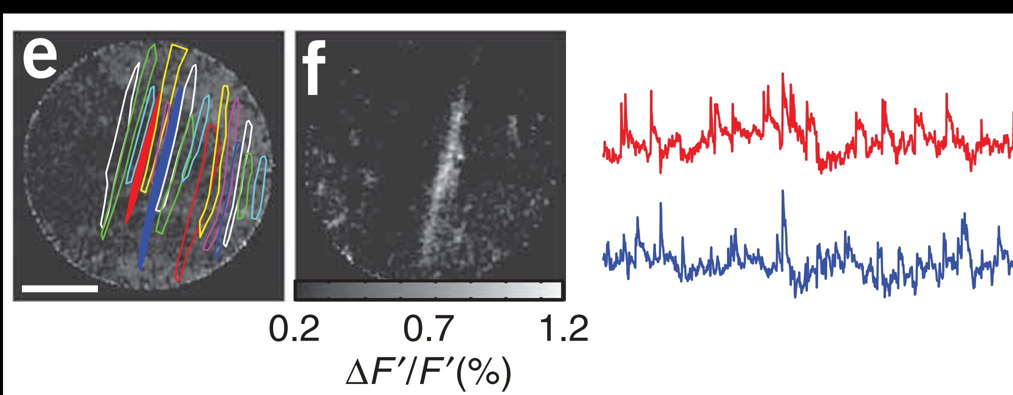
The first implementation providing single-cell resolution





- Fiber bundle: 1.5-m-long, 30,000 cores
- Weight: 1.1 -2.3 g
- Speed ≤ 100Hz
- Field of view: 240–370 μm diameter
- Lateral resolution: 2.8–3.9 μm
- Axial resolution: ~10 μm

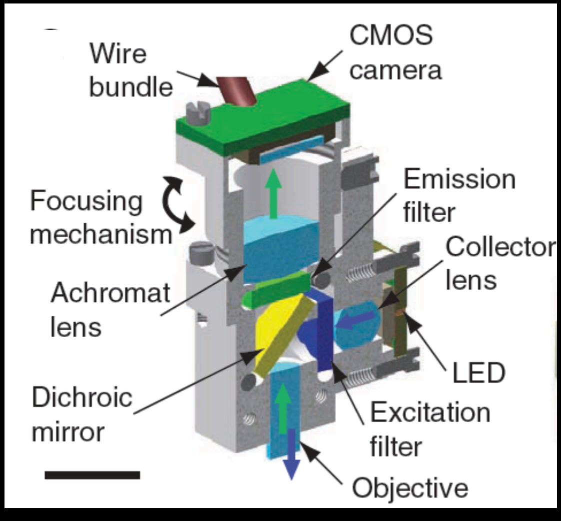
Ca imaging in purkinge cells dendrites



Flusberg et al, Nat Meth **5,** 935 (2008)

Comparison of the widefield microscope and fiberscope

Ghosh et al. Nat Methods, 2011



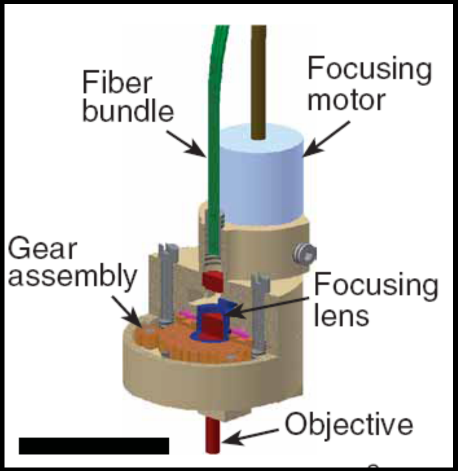


Table 1 | Comparison of integrated and fiber bundle–based fluorescence microscopes

	Integrated microscope	Fiber-bundle microscope[6]
Nyquist detection resolution	~2.5 μm	~2.8–3.9 μm
Optical resolution	~1.5 μm	~2.8–3.9 μm
Detection-limited field of view	0.48 mm^2 ← x7	0.07 mm^2
Optical field of view	1 mm^2	0.07 mm^2
Fluorescence throughput to sensor	~95%	~20%
Signal transmission	Digital image data	Fluorescence photons
Mechanical tether to mouse	Floppy	Deflection-dependent stress
Reference frame of optics	All optics move with mouse	Lamp and camera stay in lab frame
Illumination field at specimen	Does not rotate	Rotates with animal movement
Portability of optics	Fit in wallet or purse	Benchtop instrument on air table
Total system portability	Briefcase-compatible	Benchtop instrument on air table
Portability of optical function	No realignment needed	Optical realignment needed
Cost of optoelectronic parts	~$1–10 (Miniscope price: about 1000$)	~$25,000–50,000
Manufacturing scalability	Mass-producible	No batch fabrication for lamp and camera

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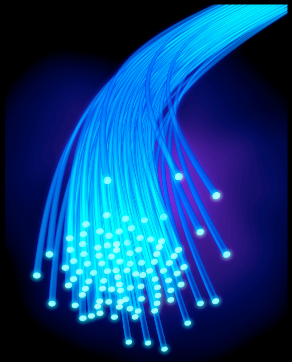

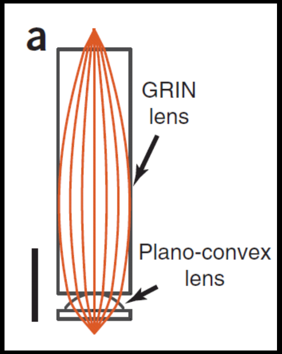

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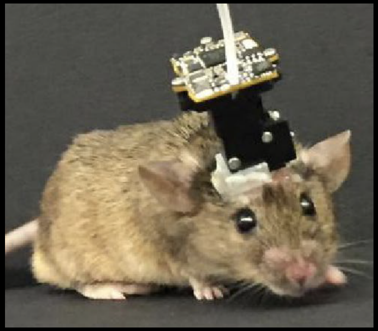

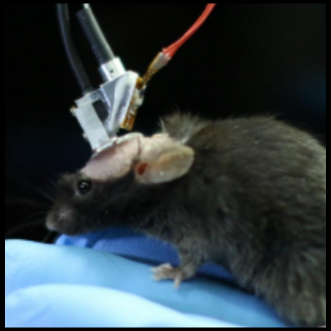

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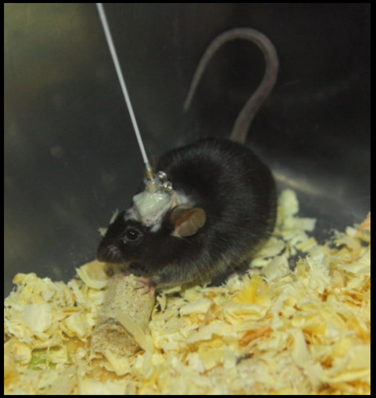
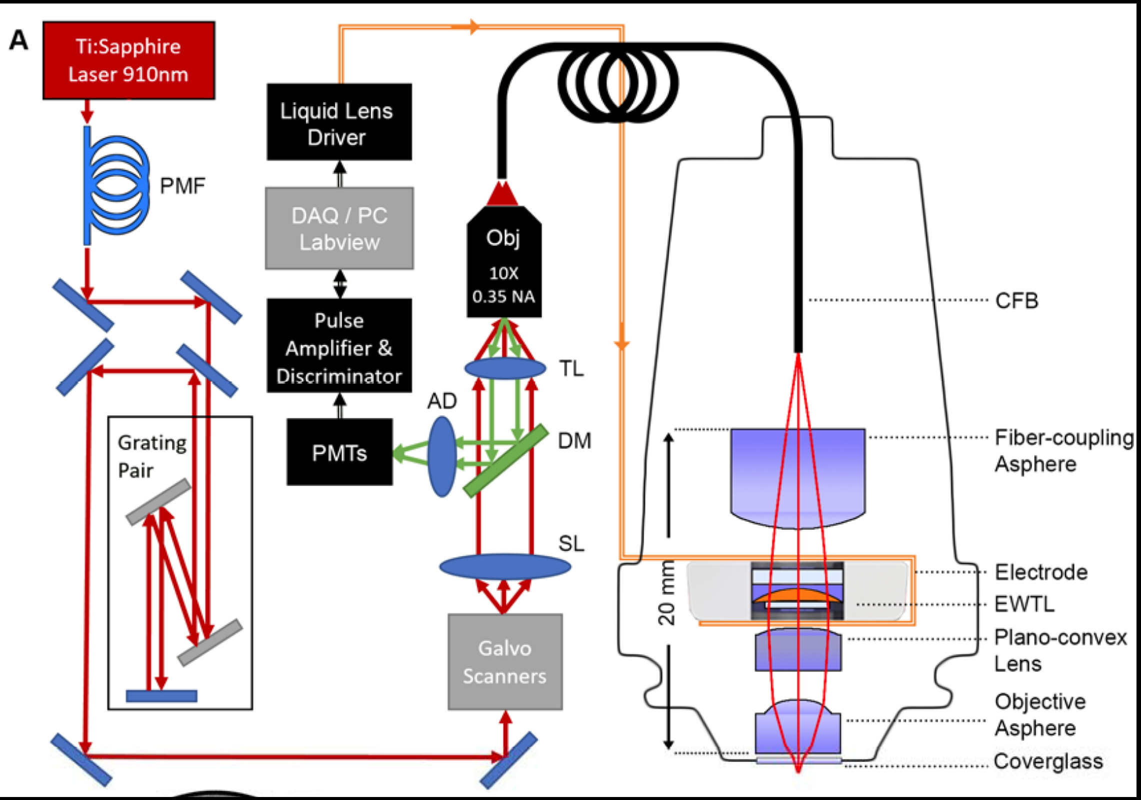
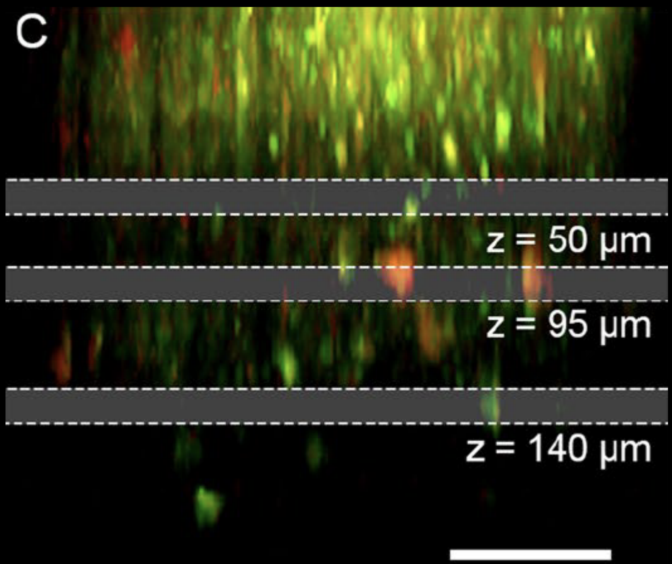


2-photon fiberscope

Ozbay et al, Scientific Reports 2018





Advantages

- 3D imaging
- 2 color imaging

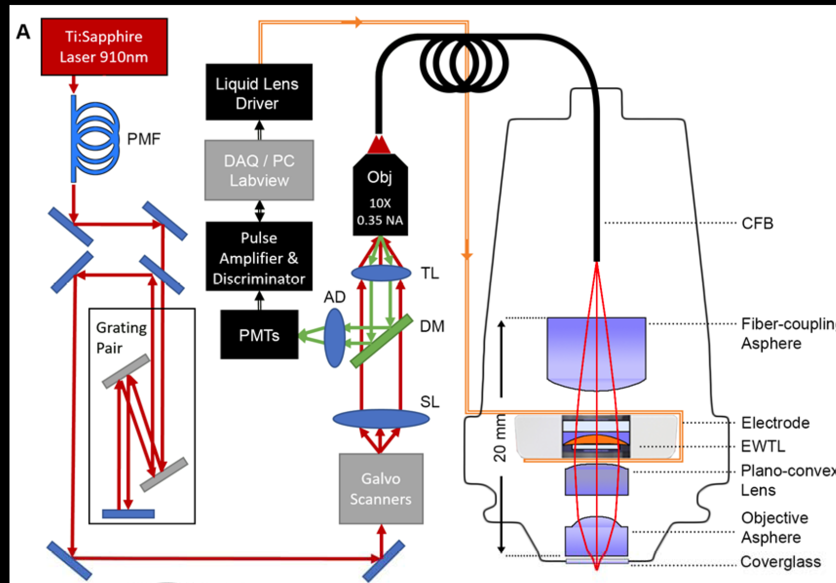
Main characteristics

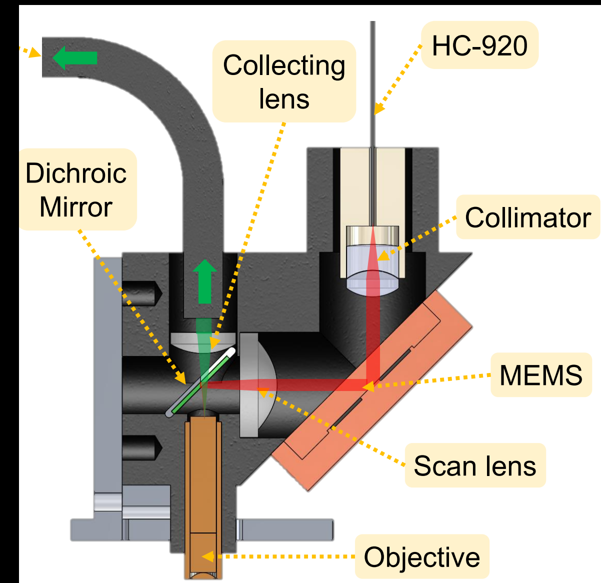
- Head mount weight: 3g
- Image guide with 15,000 cores
- Lateral resolution: 3.6 µm.
- 3D imaging with electrowetting tunable lens

Limitations

- Low signal (low excitation and collection efficiency, losses due to image guide and low NA (0.45) objective)
  - ➡ Low repetition rate (<2.5Hz)
    Low penetration depth (<200 µm)
- Low number of pixels (15000)
  - ➡ Low resolution (3.6 µm)

Comparison of 2-photon miniature microscope and fiberscope





Ozsbay et al. Sci Reports 2018

Zong et al. Nature Methods 2017

Lateral resolution	3.6 µm	0.6 µm
Number of pixels	15000	66000
FOV diameter	240 µm	170 µm (soon to be improved)
Frame rate	2.5Hz	40Hz
Signal to noise ratio for activity recording	Lower than benchtop microscopes	Similar to benchtop microscopes
Maximum imaging depth	about 150µm	200µm
3D imaging ?	Yes	Soon

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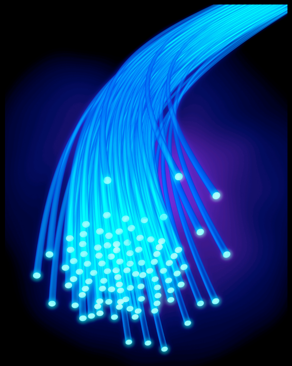

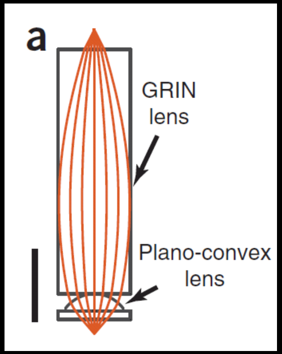

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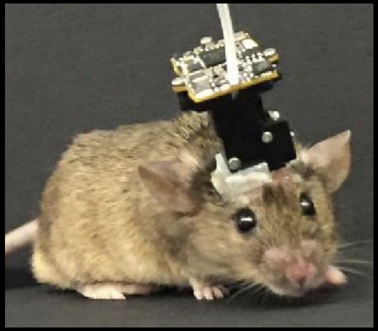

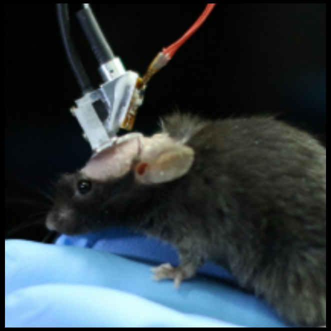

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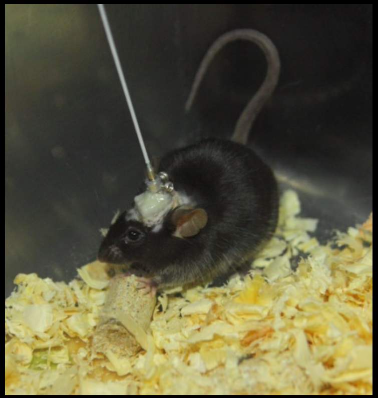
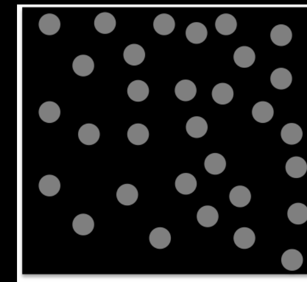


Specificities of the optical setup that we want to design



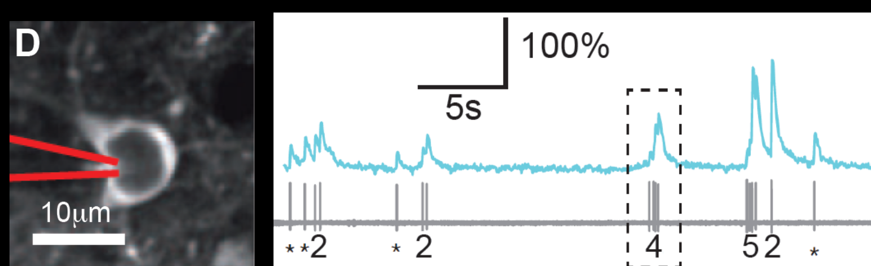
Goal Being able to measure endogenous activity patterns corresponding to specific behaviors and testing their causality

Fluorescence imaging in freely-behaving mice allowing for:

- High speed (>100Hz)
- High imaging contrast
- High signal to noise ratio

} Optical sectioning

In vivo recording of neuronal activity

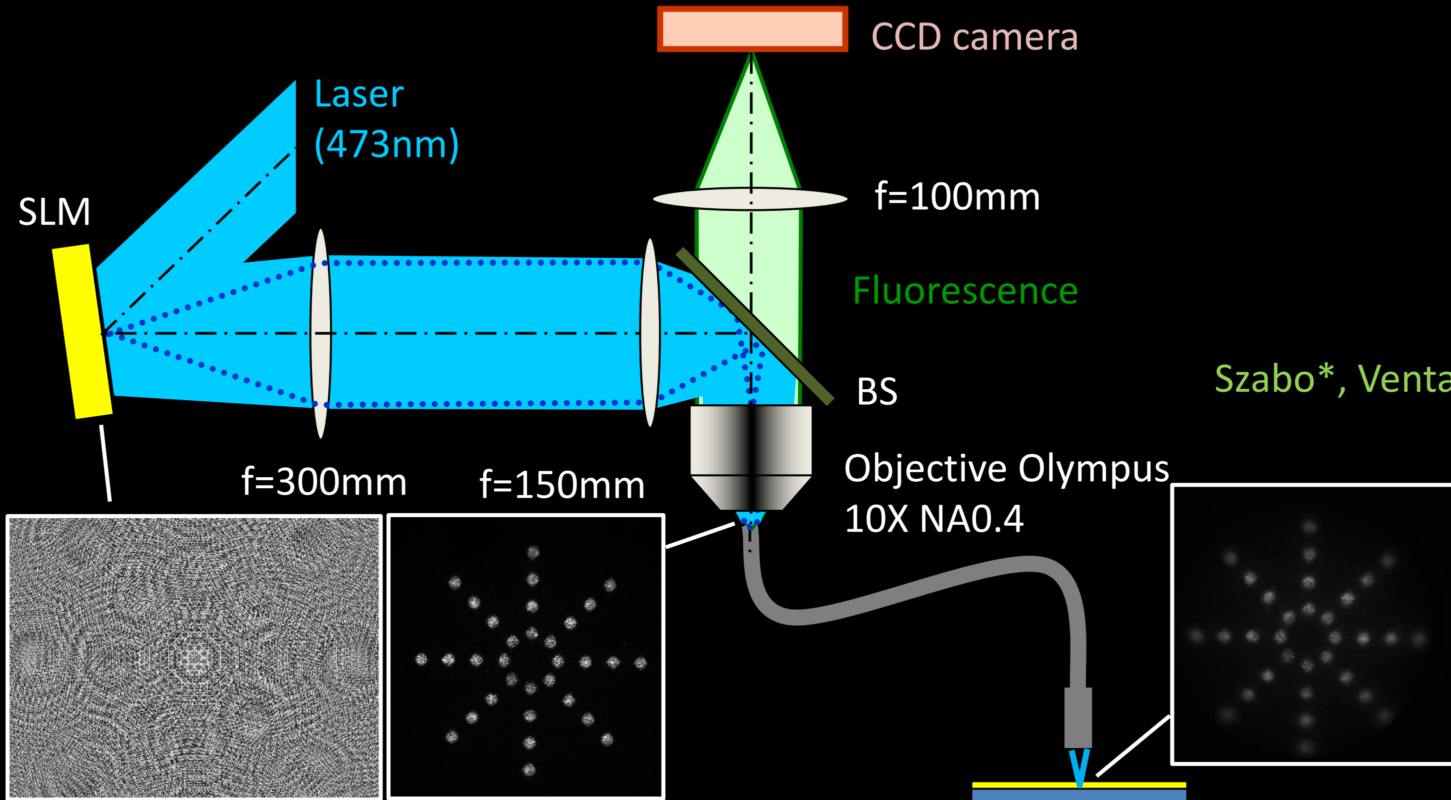


Chen et al, Nature 499, 295 (2013)

- Large field-of-view (a few 100μm) - to access a large number of cells

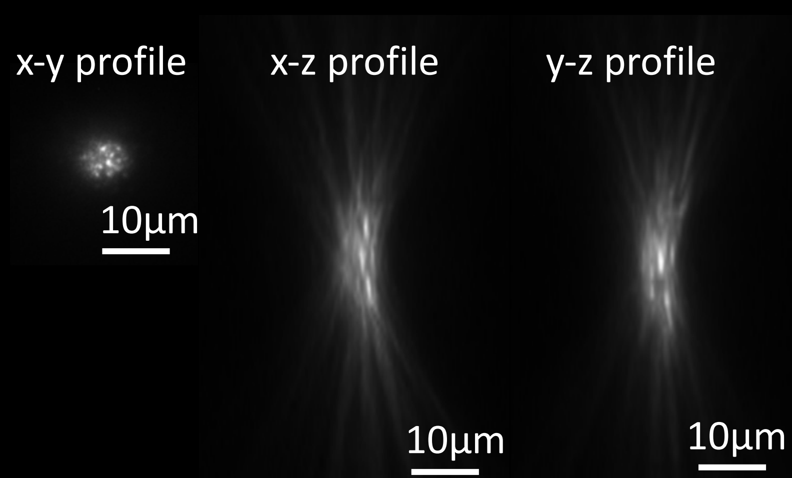
Coupling with targeted photoactivation

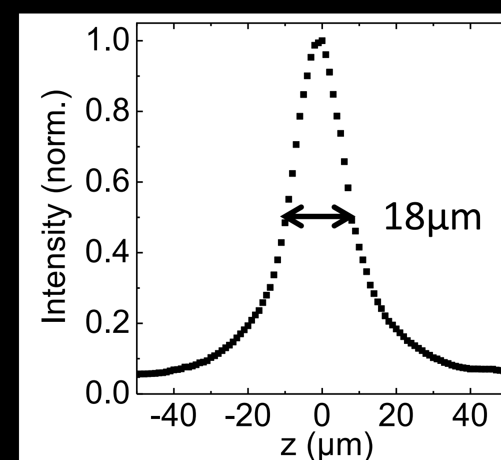
Targeted photoactivation with computer-generated holography



Szabo*, Ventalon* et al, Neuron 2014

Characterization
Spot of
diameter 5µm

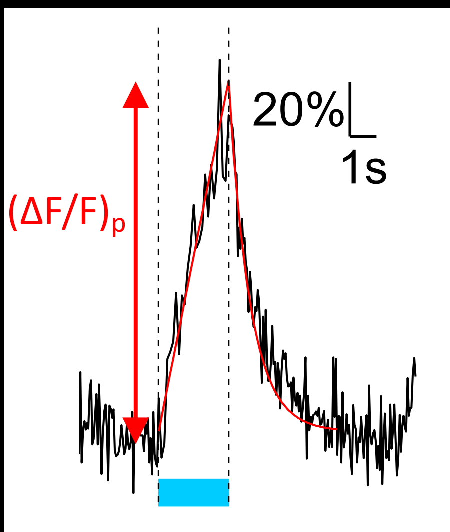


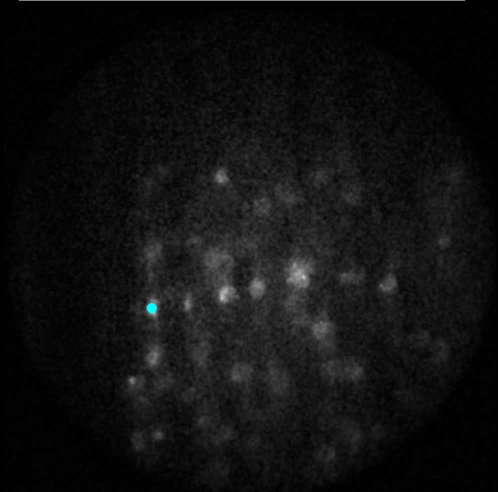


Targeted photoactivation with near-cellular resolution in freely-behaving mice

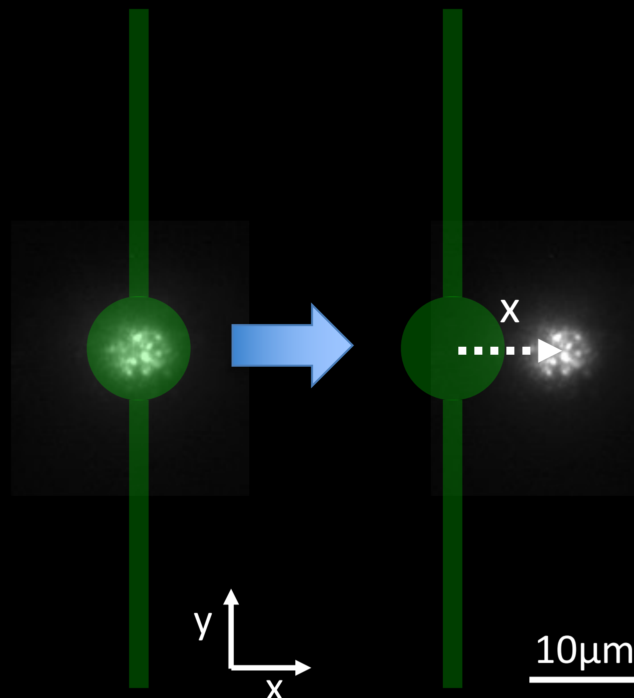
Interneurons of the cerebellar molecular layer co-expressing ChR2 and GCaMP5-G

Photoactivation

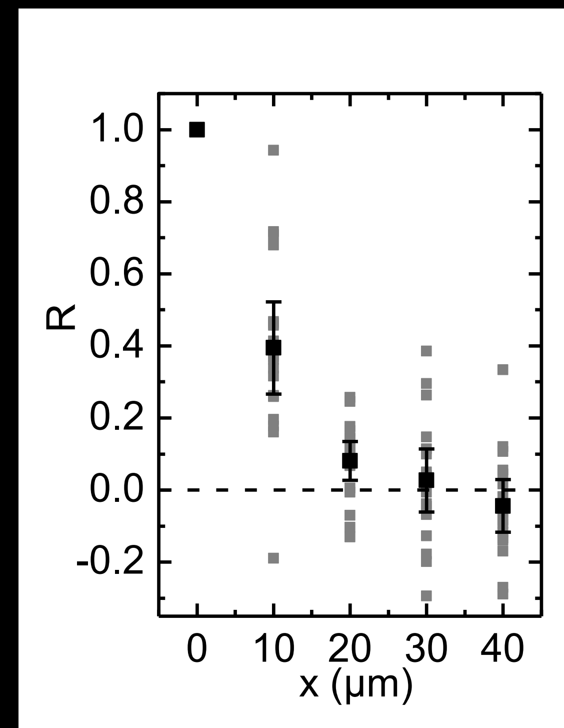




Lateral resolution



$R = (\Delta F/F)_{p,\text{ off cell}}/(\Delta F/F)_{p,\text{ on cell}}$



$\Delta x_{HWHM} < 10\mu m$
(n = 18 cells in 5 mice)

Szabo*, Ventalon* et al, Neuron 2014

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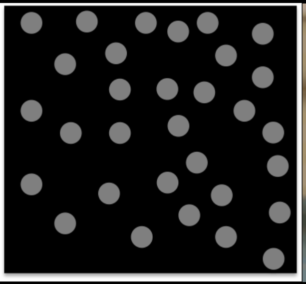

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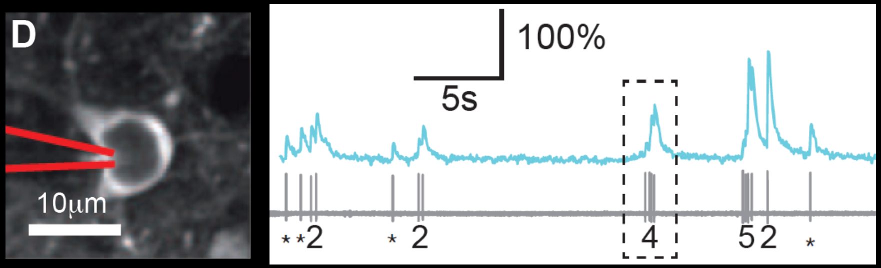
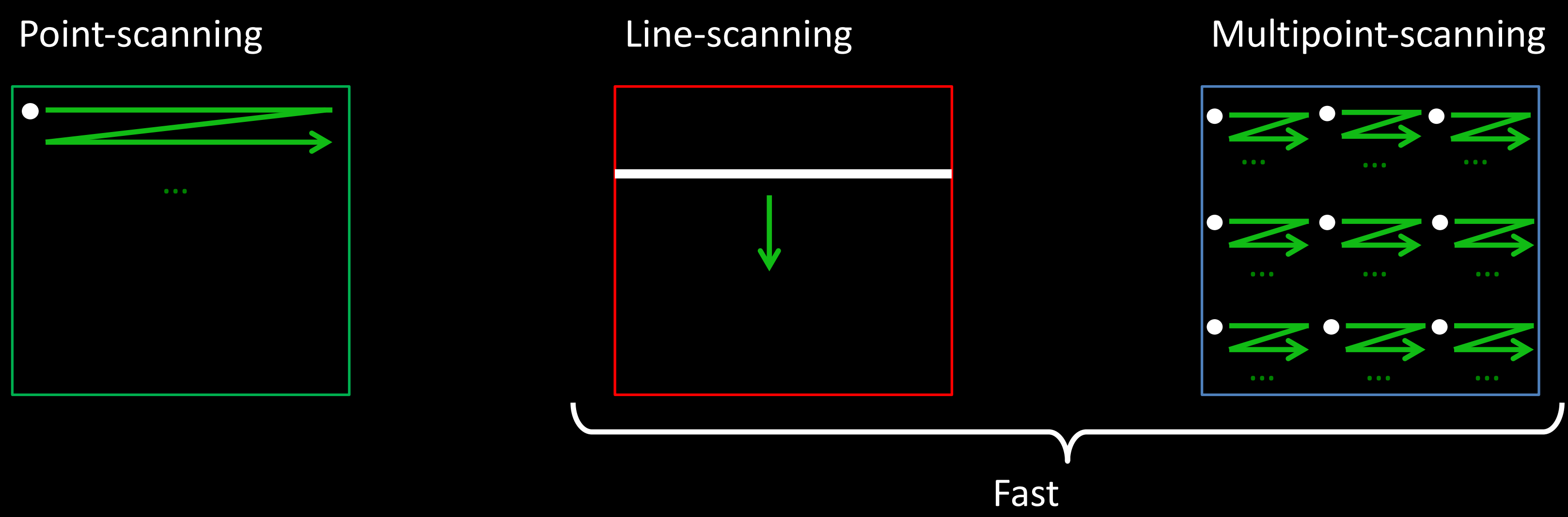


Chen et al, Nature 499, 295 (2013)

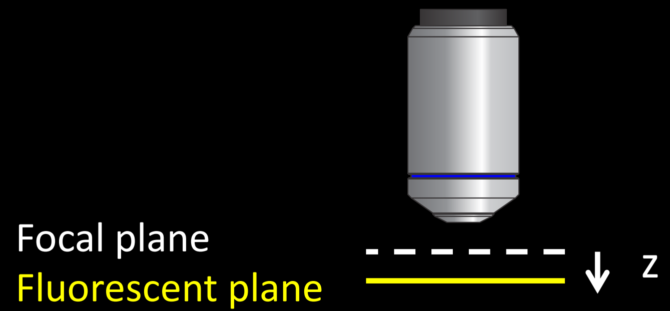
- Large field-of-view (a few 100μm) - to access a large number of cells

Coupling with targeted photoactivation

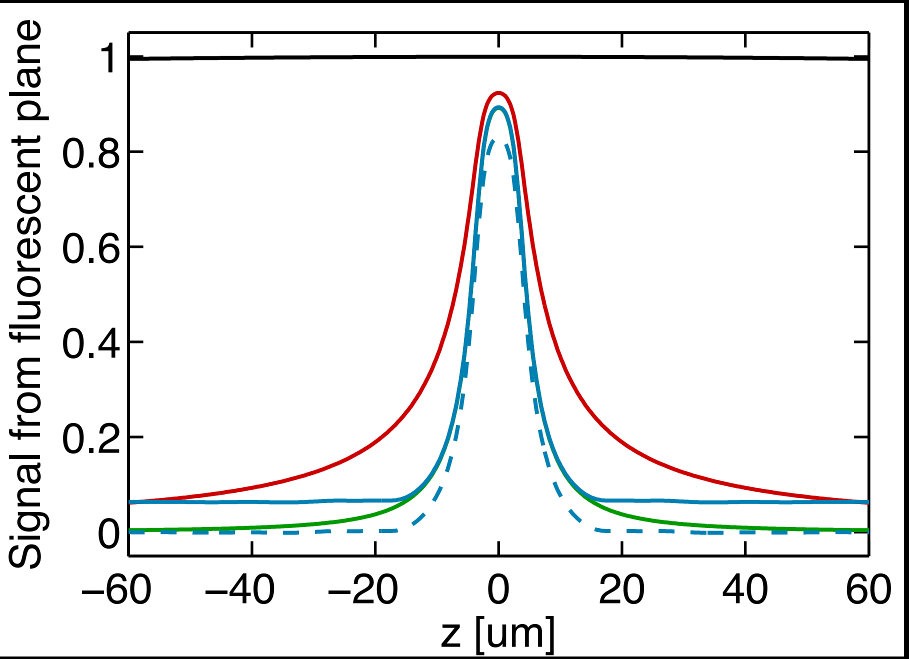
Combination with confocal imaging



Quantification of optical sectioning



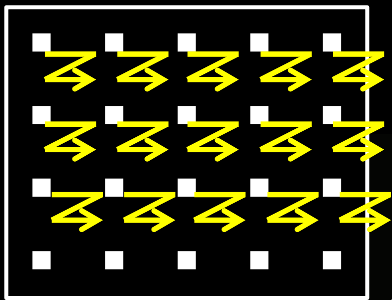
2 methods
- Differential multipoint-scanning confocal imaging
- Line-scanning confocal imaging



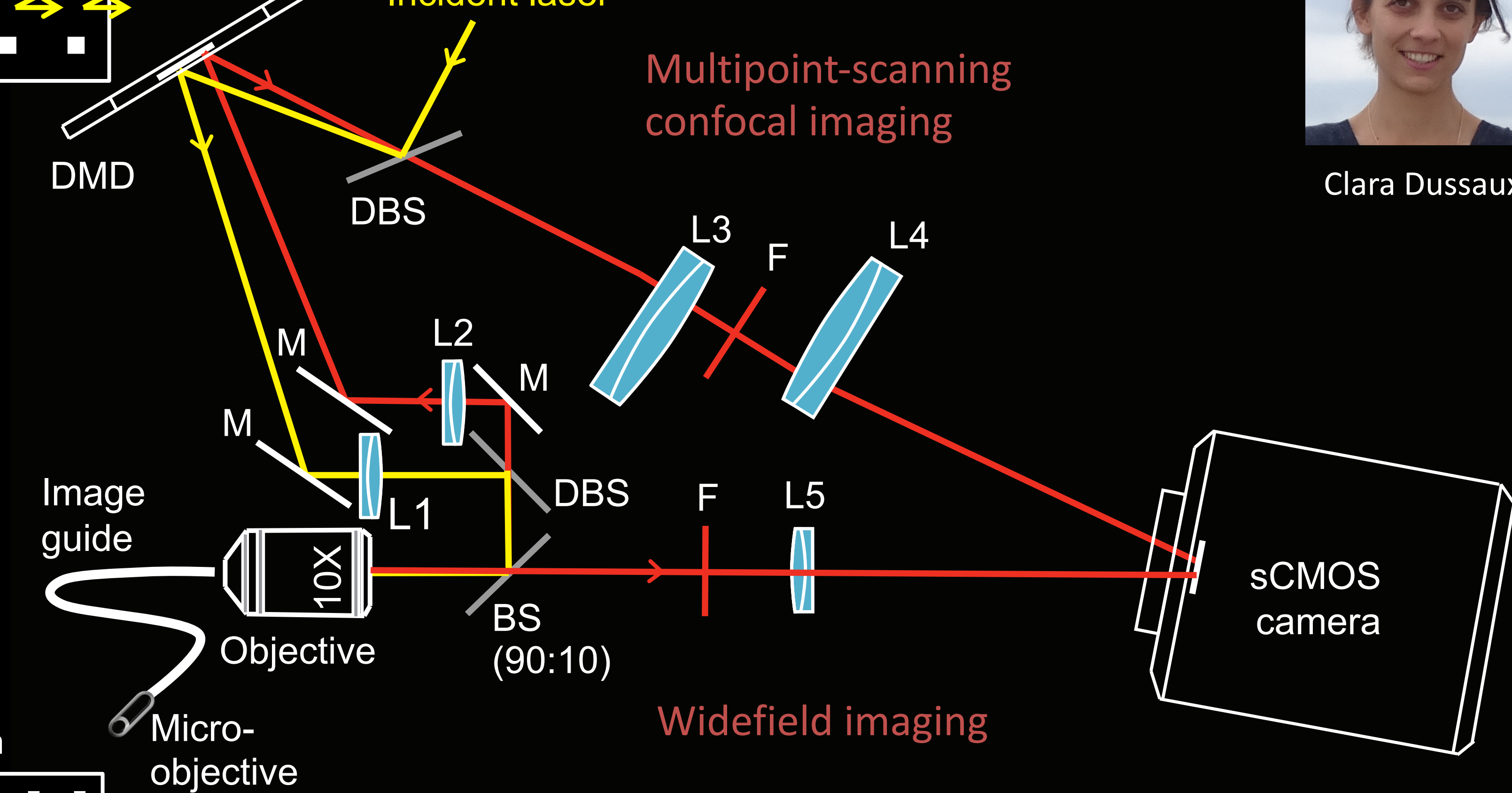
Verveer et al, J Microsc 1998 - PAM microscopy

Implementation of multipoint-scanning confocal imaging

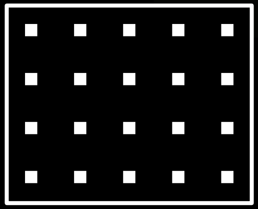
Dussaux et al, Scientific Reports, 2018



Clara Dussaux

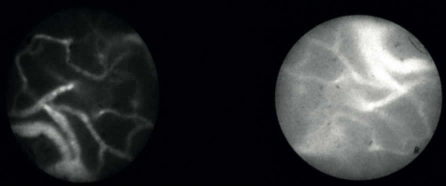


Sample illumination



Simultaneous acquisition of the multipoint confocal image and the widefield image

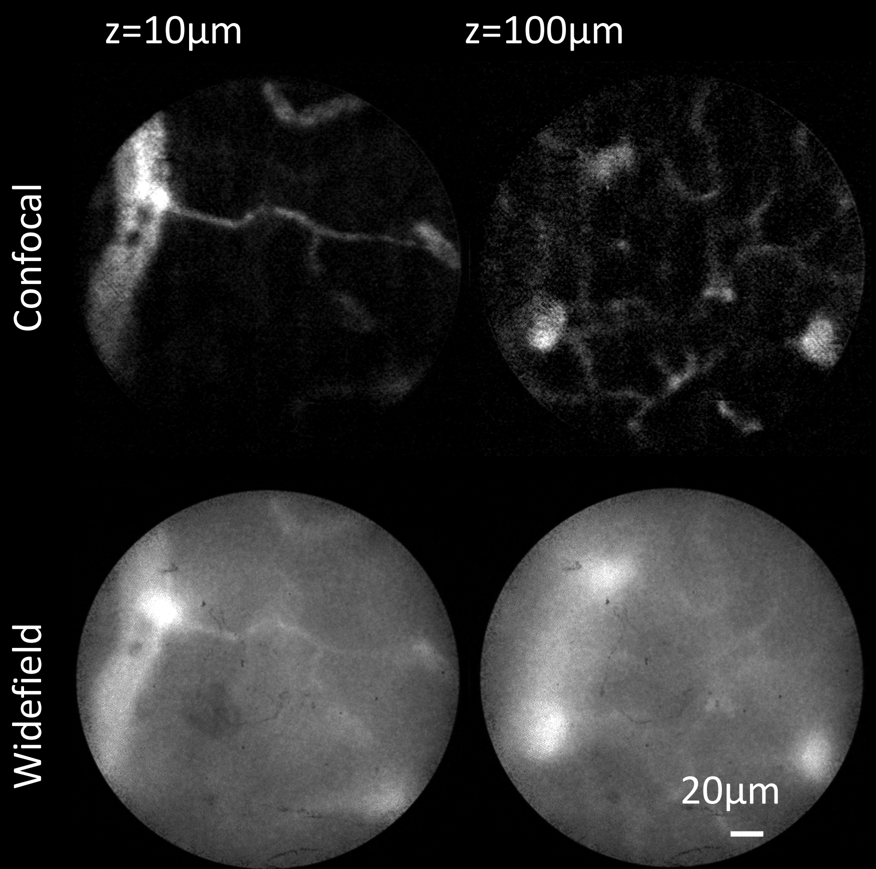
➡ Online subtraction of residual background



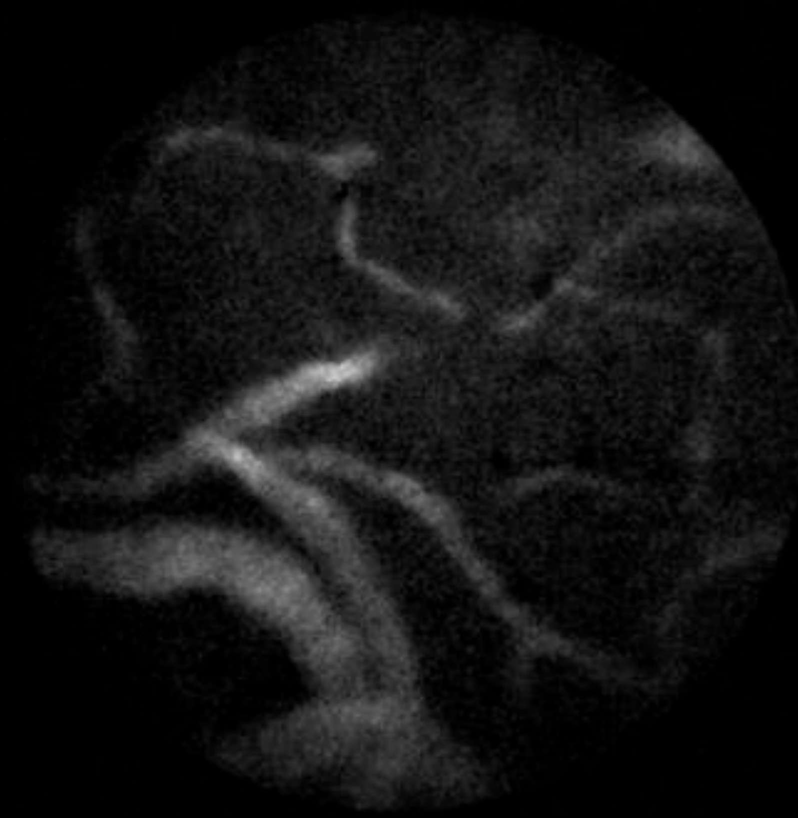
Differential multipoint-scanning confocal imaging of microvasculature

Imaging microvasculature in the cortex following intravenous injection of rhodamine

Anesthetized mouse, 100Hz imaging



Freely-moving mouse, 200Hz imaging

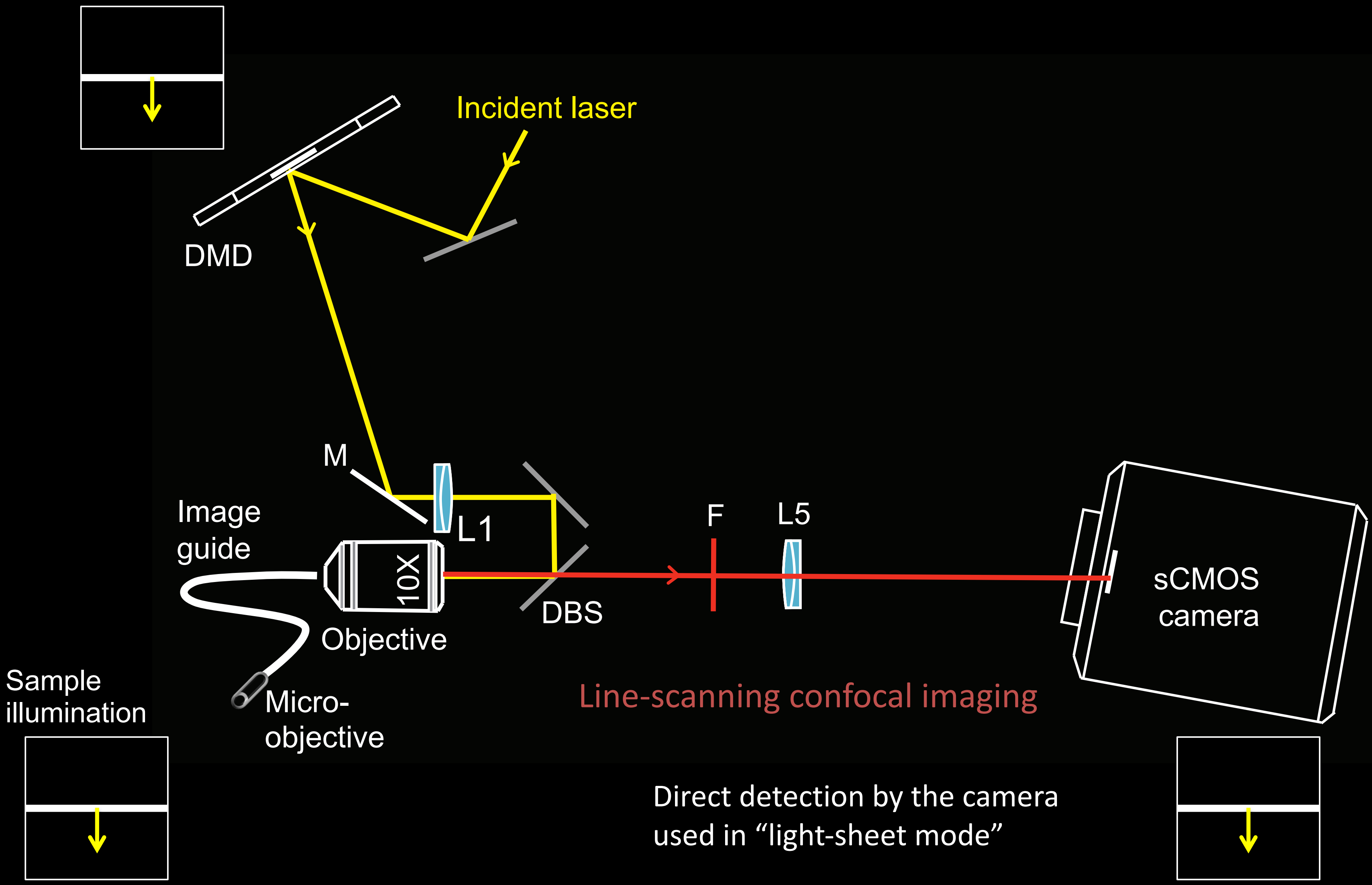


Background to signal was 1.3 10^{-2} times lower with differential multipoint scanning confocal imaging

slowed down 10x

Dussaux et al, Scientific Reports, 2018

Implementation of line-scanning confocal imaging



Direct detection by the camera used in "light-sheet mode"

Micro-objective characteristics determines imaging performances

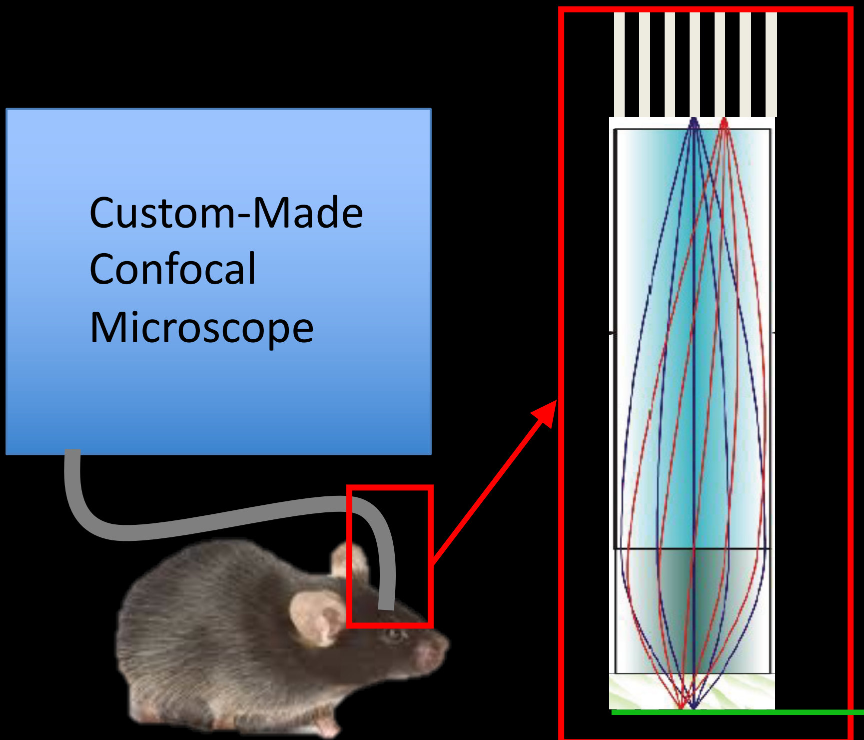


Image guide (Inter-core distance ic)

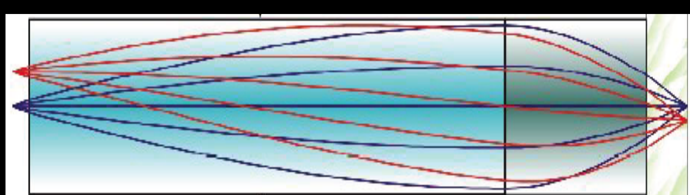
Objective (magnification M>1)

Imaging plane

Roles of micro-objective:

- Improving lateral resolution (2ic -> 2 ic/M)
- Increasing the working distance
- Increasing signal (α NA^2)

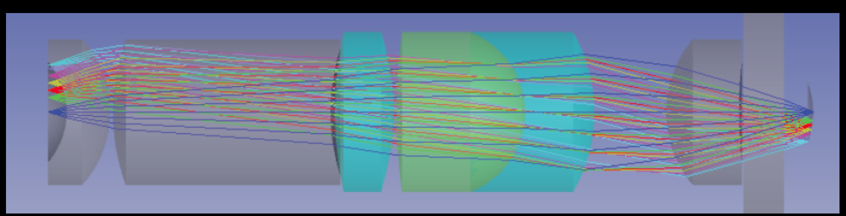
GRIN lenses objectives



- NA<0.5
- Large chromatic and geometric aberrations

➡ Limited confocal signal

Assembly of spherical and aspherical micro-lenses



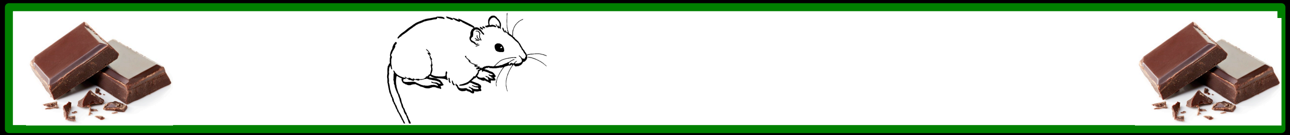
- NA >0.7 over a large field of view
- Low chromatic and geometric aberration

Not commercial yet

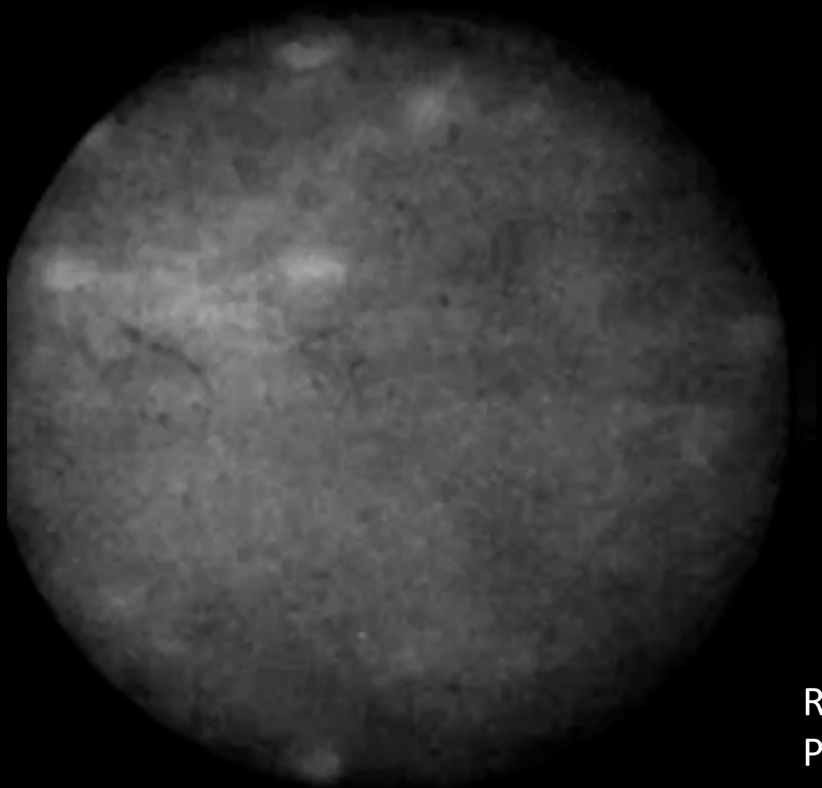
Fast confocal imaging of neuronal activity

GCaMP6 imaging @100Hz in the hippocampus of freely-behaving mice

Behavior task



Optical recordings



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Raw data after movement correction
Play-back speed: x4

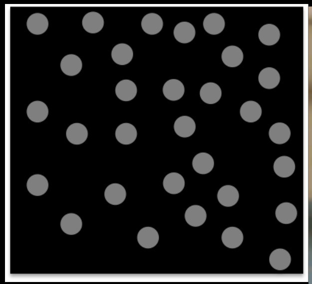
Fast confocal imaging and targeted photoactivation with a fiberscope

Advantages

- Versatile: various imaging modalities (line- and multipoint-scanning confocal imaging)
- Optical sectioning
- Fast imaging (300Hz)
- Lateral resolution : 2.6µm
- Light and simple microscope (<2g) that can be adapted to mice
- Coupling with fast targeted photoactivation (intensity modulation with a DMD)

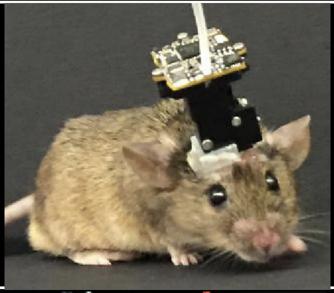
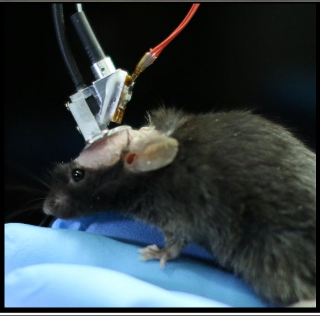
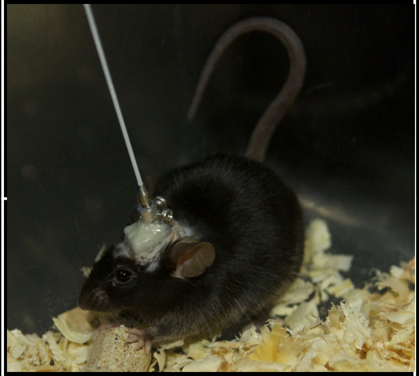
Drawbacks

- Number of pixels limited by the bundle (->limited FOV)
- Collection efficiency limited by the bundle (partly compensated by the camera high QE)
- Small working distance (<150µm)



We have all the ingredients!!

Conclusion

	Advantages	Limitations	
Miniature widefield microscope	• Quite fast (30Hz) • Large field of view (mm2)	• No optical sectioning • Small working distance • Targeted photoactivation not demonstrated	
2-photon miniature microscope	• Quite fast (40Hz) • High resolution (0.6µm) • Good optical sectioning • Large working distance	• Small field of view • Only 2D imaging • Targeted photoactivation not demonstrated	
Confocal fiberscope	• Cellular resolution • Optical sectioning • Fast (300Hz) • Compatible with targeted photoactivation	• Number of pixels limited by the bundle • Small working distance	
with 1-photon targeted photoactivation	• Near-cellular resolution	• Potential out-of-focus excitation	

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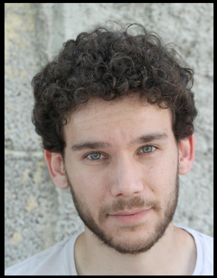

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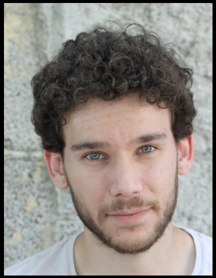

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