In vivo 2-photon microscopy for dissection of neuronal circuits

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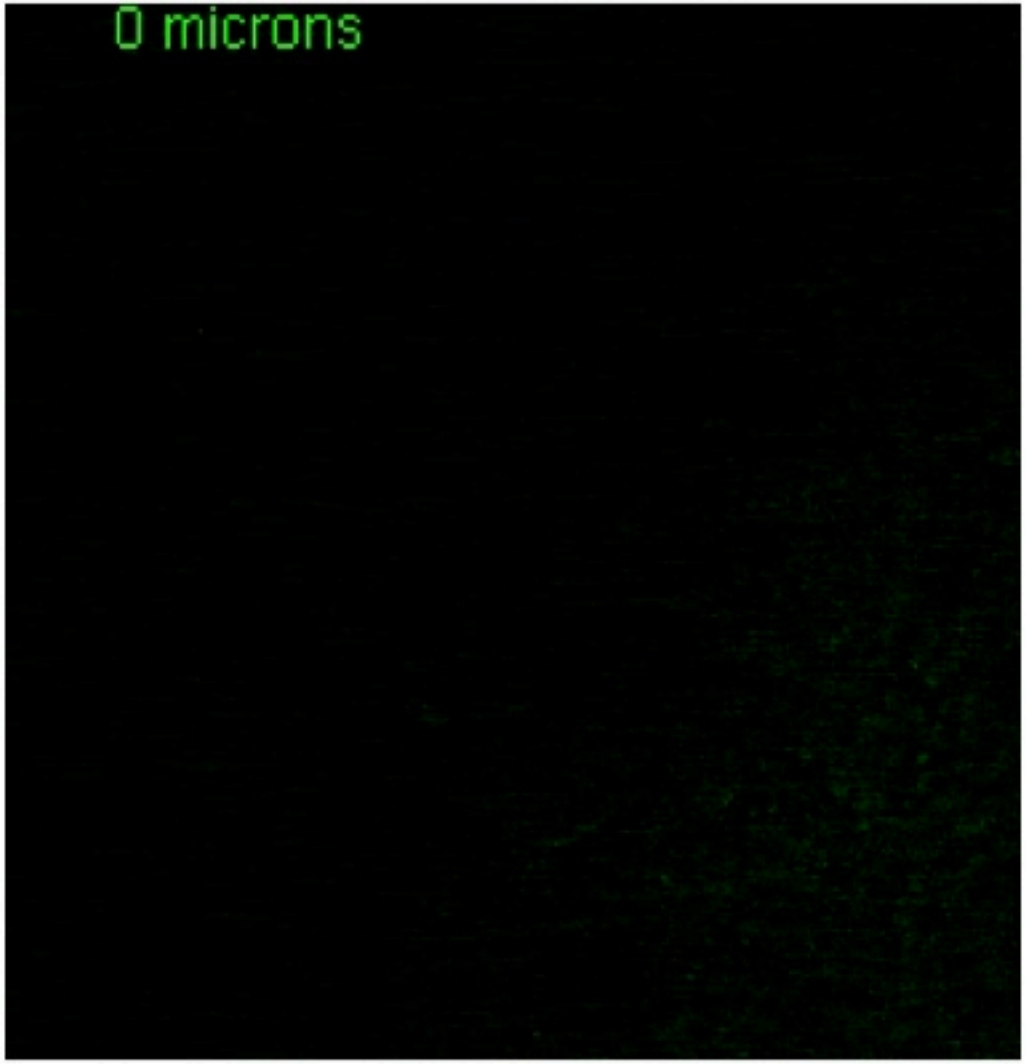
2PM: Imaging deep in the living brain



Mittmann *et al.*, 2011

Outline

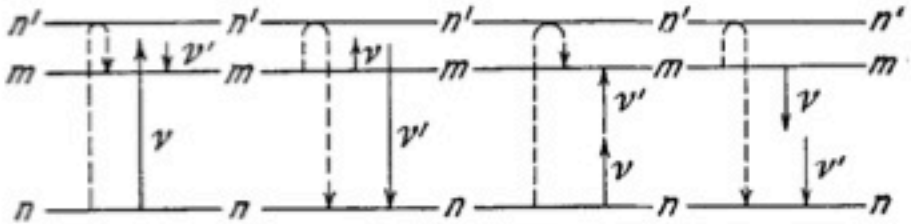
- Basics of 2-photon microscopy
- Experimental implementation for *in vivo* experiments
- Applications using the key strengths of 2pm

Principle of 2-photon excitation

Über Elementarakte mit zwei Quantensprüngen

Von Maria Göppert-Mayer

(Göttinger Dissertation)

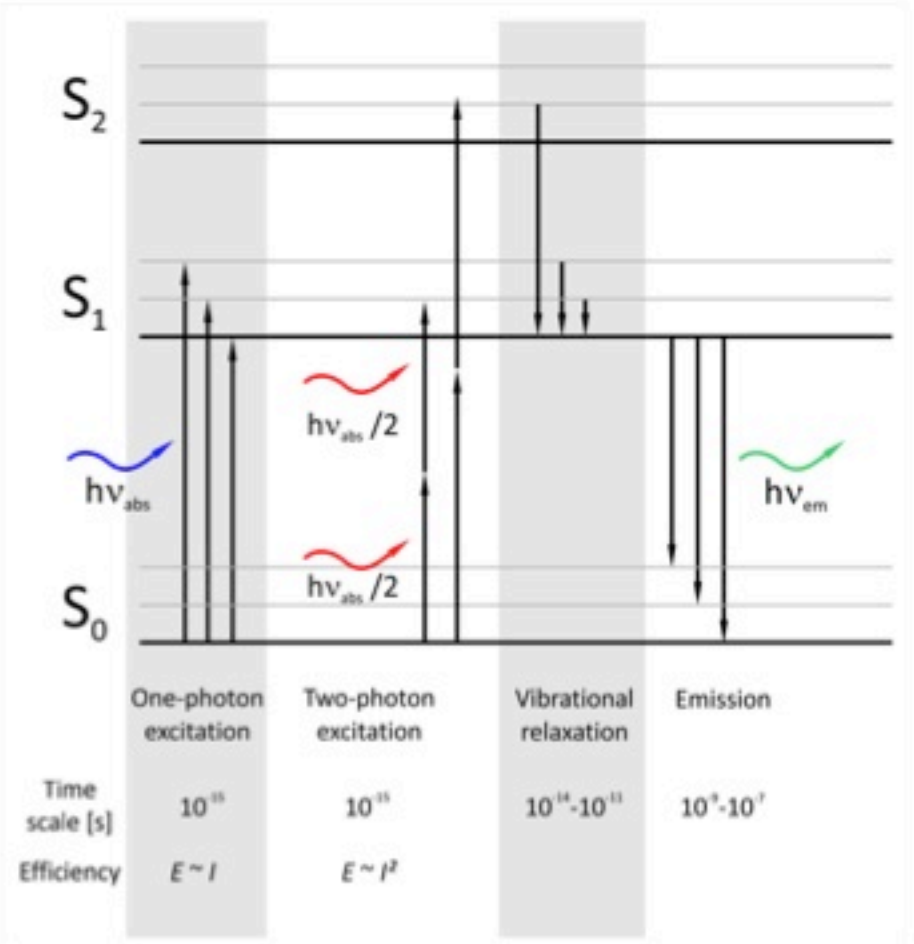


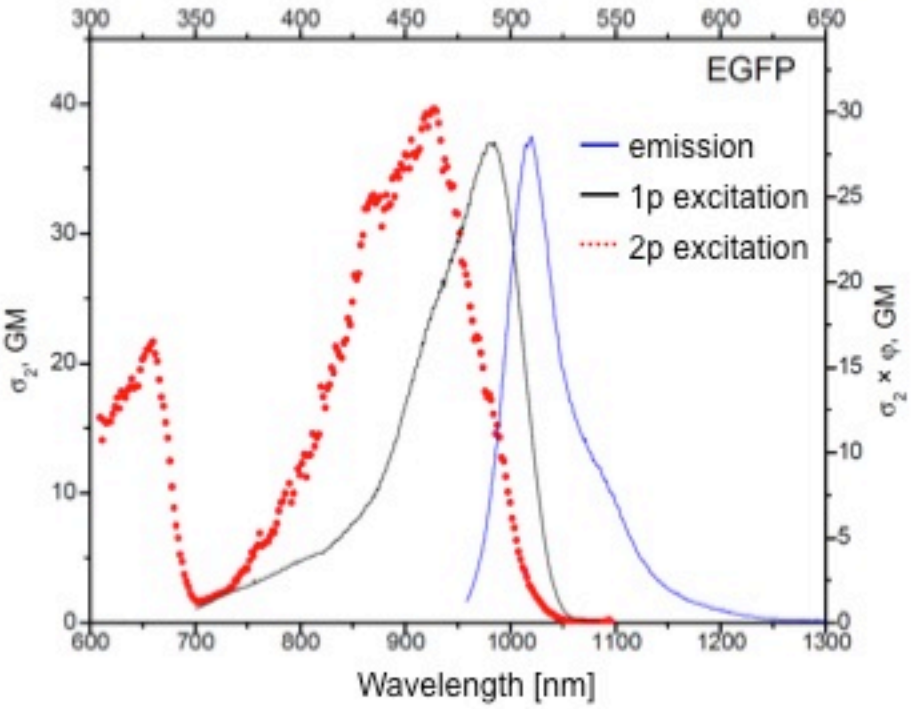
Die punktierten Linien bedeuten das Verhalten des Atoms, aufwärtsgerichtete Pfeile absorbierte, abwärtsgerichtete emittierte Lichtquanten

Figg. 1—4

Theoretical prediction: 1931 (M. Göppert-Mayer, Dissertation)
Experimental verification: 1961 (Kaiser and Barrett, *Physical Review Letters*)

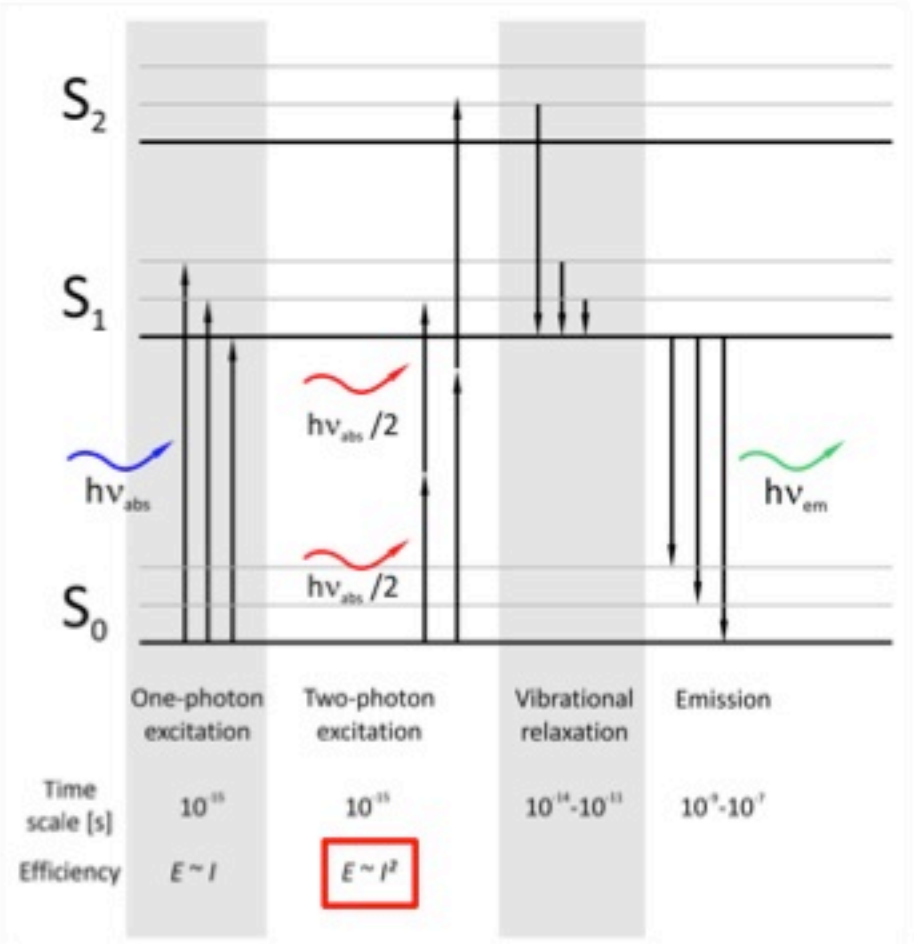
Principle of 2-photon excitation

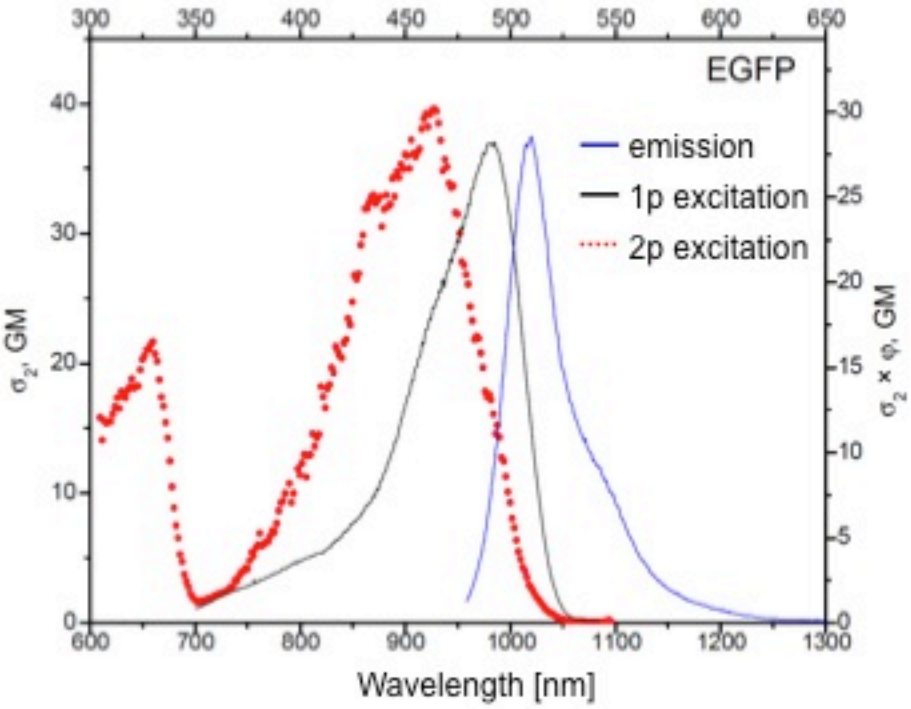




Drobizhev et al Nat Methods 2011

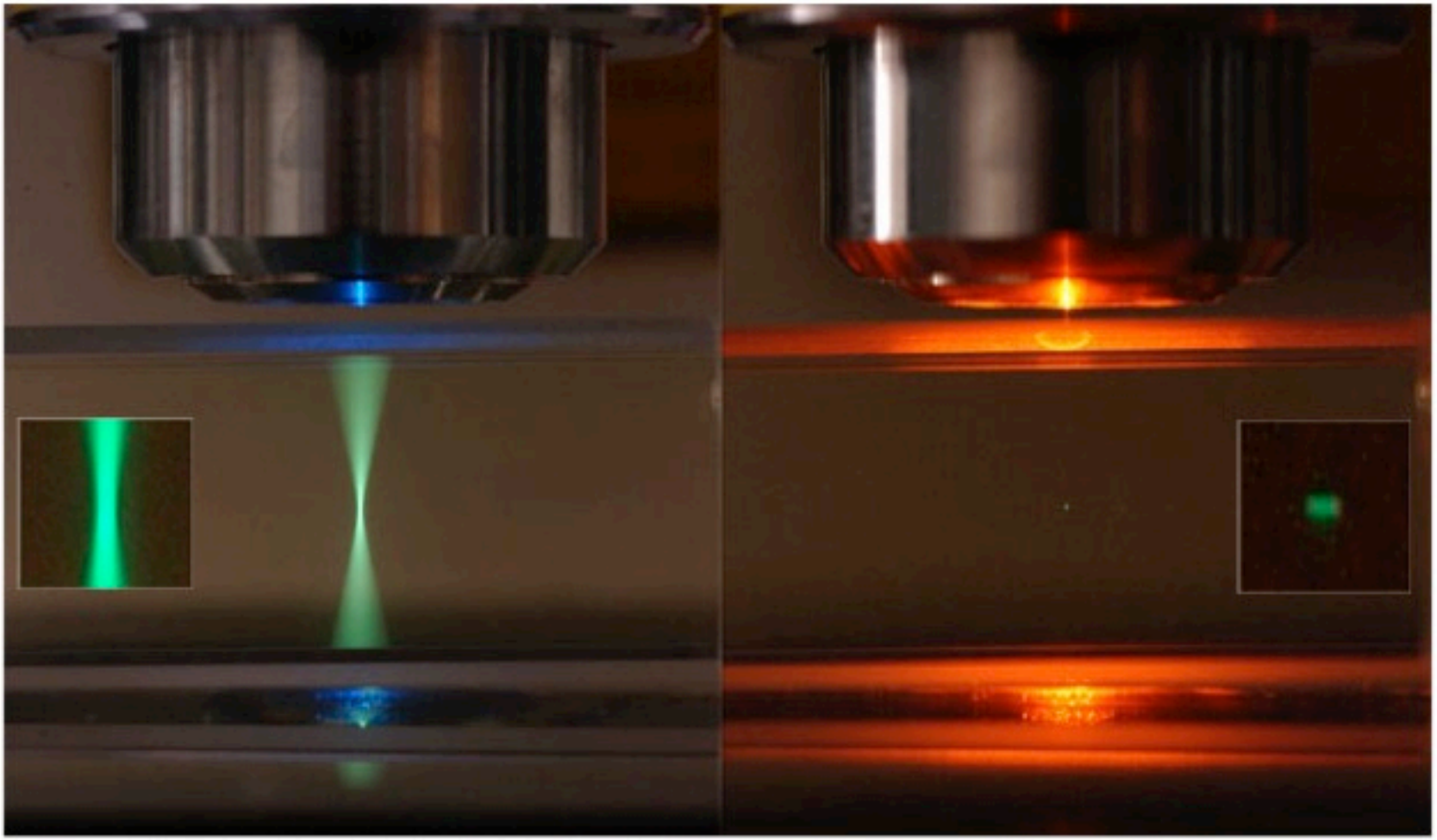
Principle of 2-photon excitation





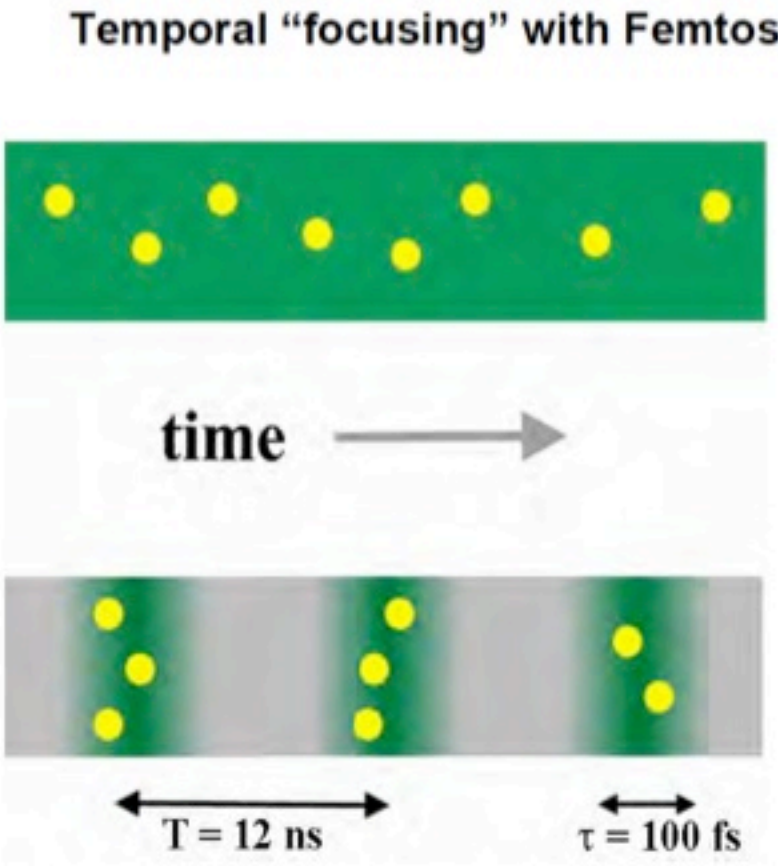
Drobizhev et al Nat Methods 2011

Advantage 1: Only the relevant volume is excited



Localized excitation instead of localized emission in a confocal microscope
Photo-bleaching restricted to imaged volume

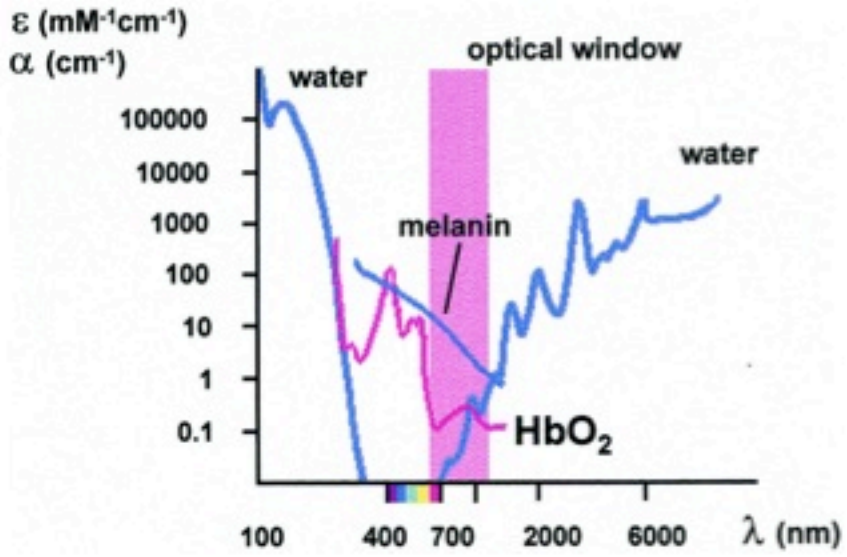
Only pulsed lasers can efficiently cause 2-photon excitation



$T : \tau \approx 120{,}000 : 1$ (!)

Advantage 2: Excitation light penetrates deeper into tissue

Absorption

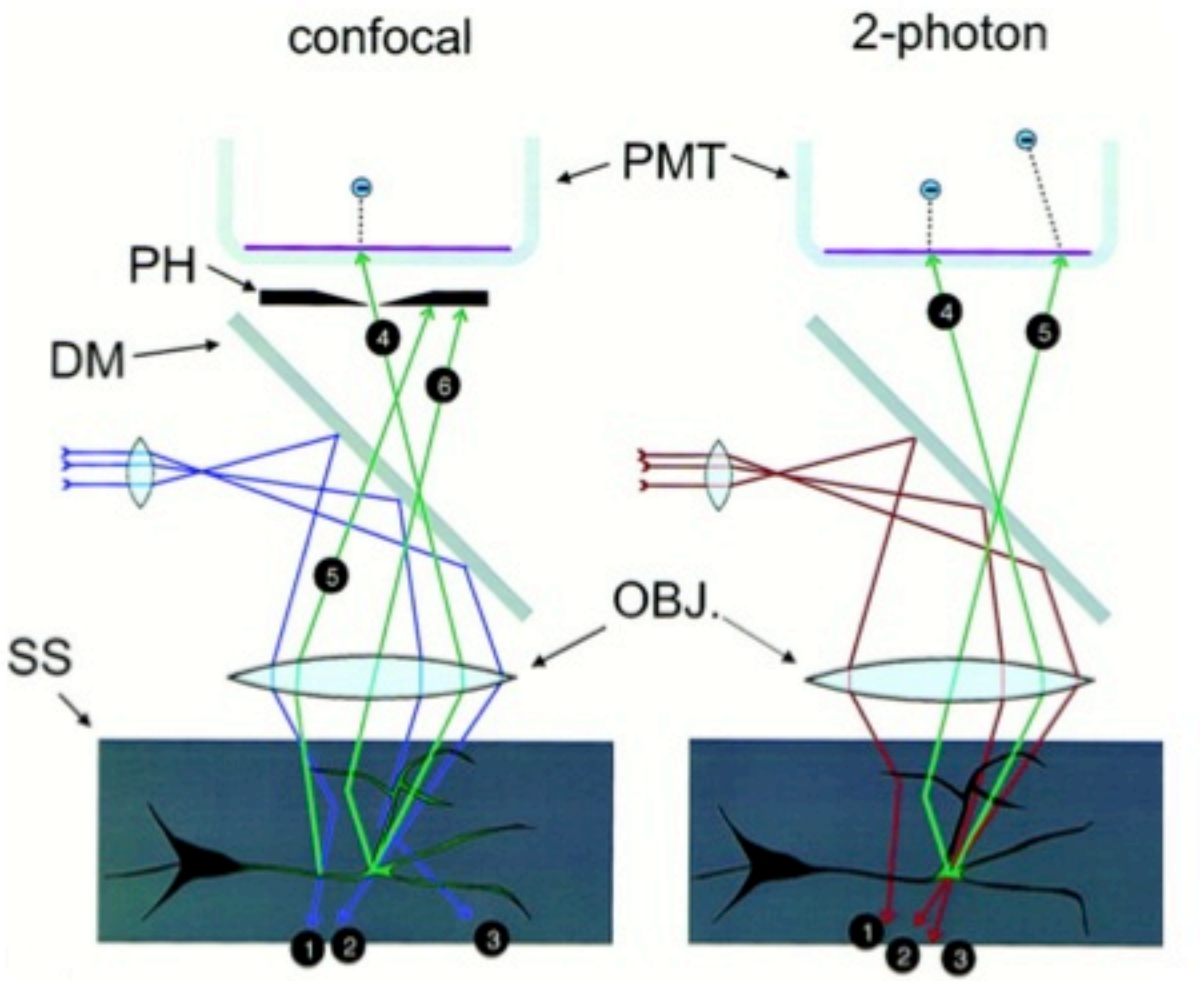


K. König, J Microscopy 2000

Scattering

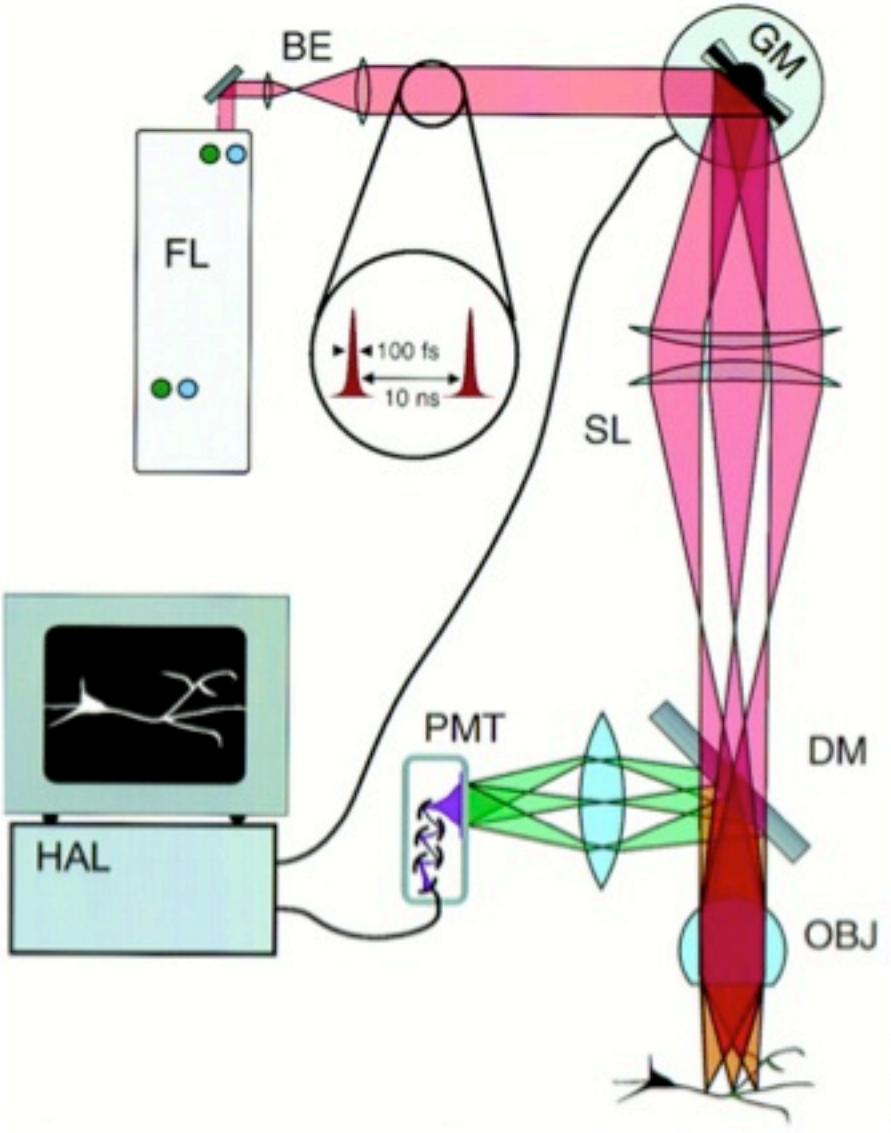
Rayleigh scattering (~$1/\lambda^4$) > biological scattering > Mie scattering(~$1/\lambda^{0.5}$)

Advantage 3: More efficient emission detection deep in scattering tissue



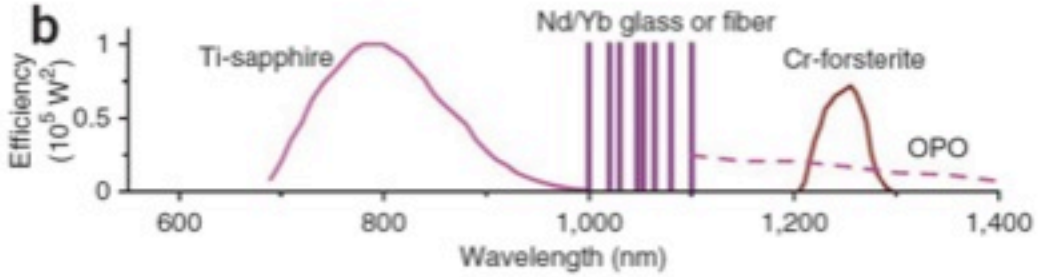
Denk & Svoboda 1997

Implementation of 2-photon imaging



Denk & Svoboda 1997
c.f. Denk *et al.*, 1990

Lasers: power & wavelength range



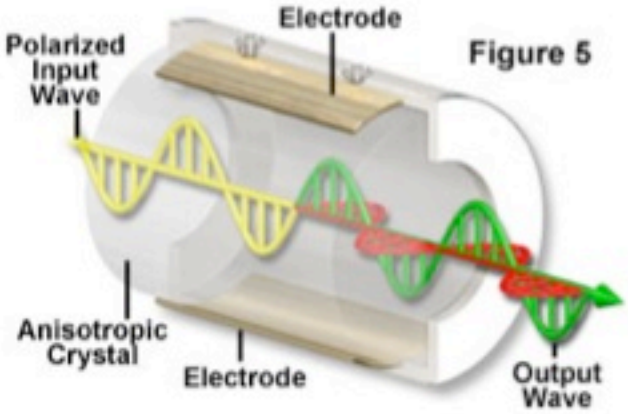
Drobizhev et al Nat Methods 2011

- **Ti-sapphire – output range approximately 700 to 1040nm**
- **Ti-sapphire with OPO (optical parametric oscillator)– output range approximately 700 to > 1300nm**
- **Fiber lasers – fixed wavelength, but cheaper**

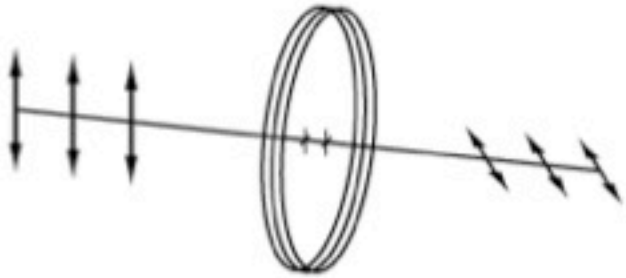
Lasers attenuation

Pockels cell: + fast
- expensive & sensitive

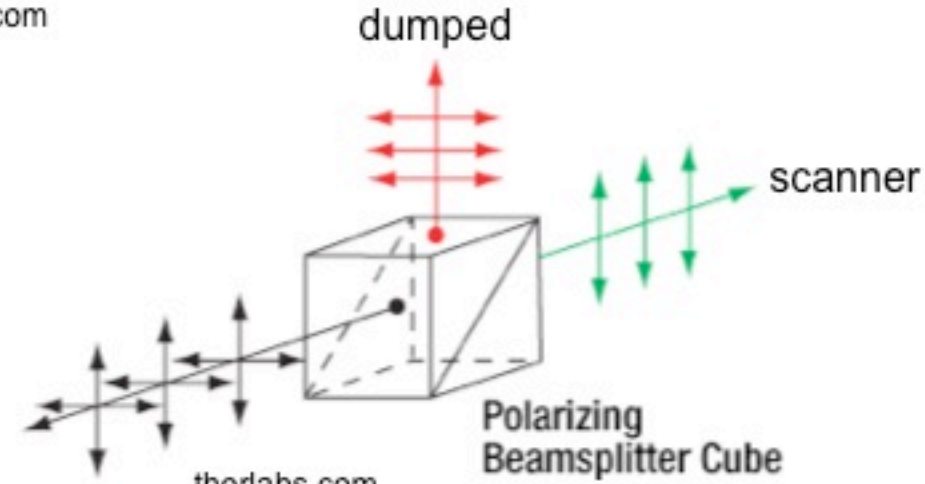
waveplate: + cheap & reliable
- slow (manual)



olympus.com

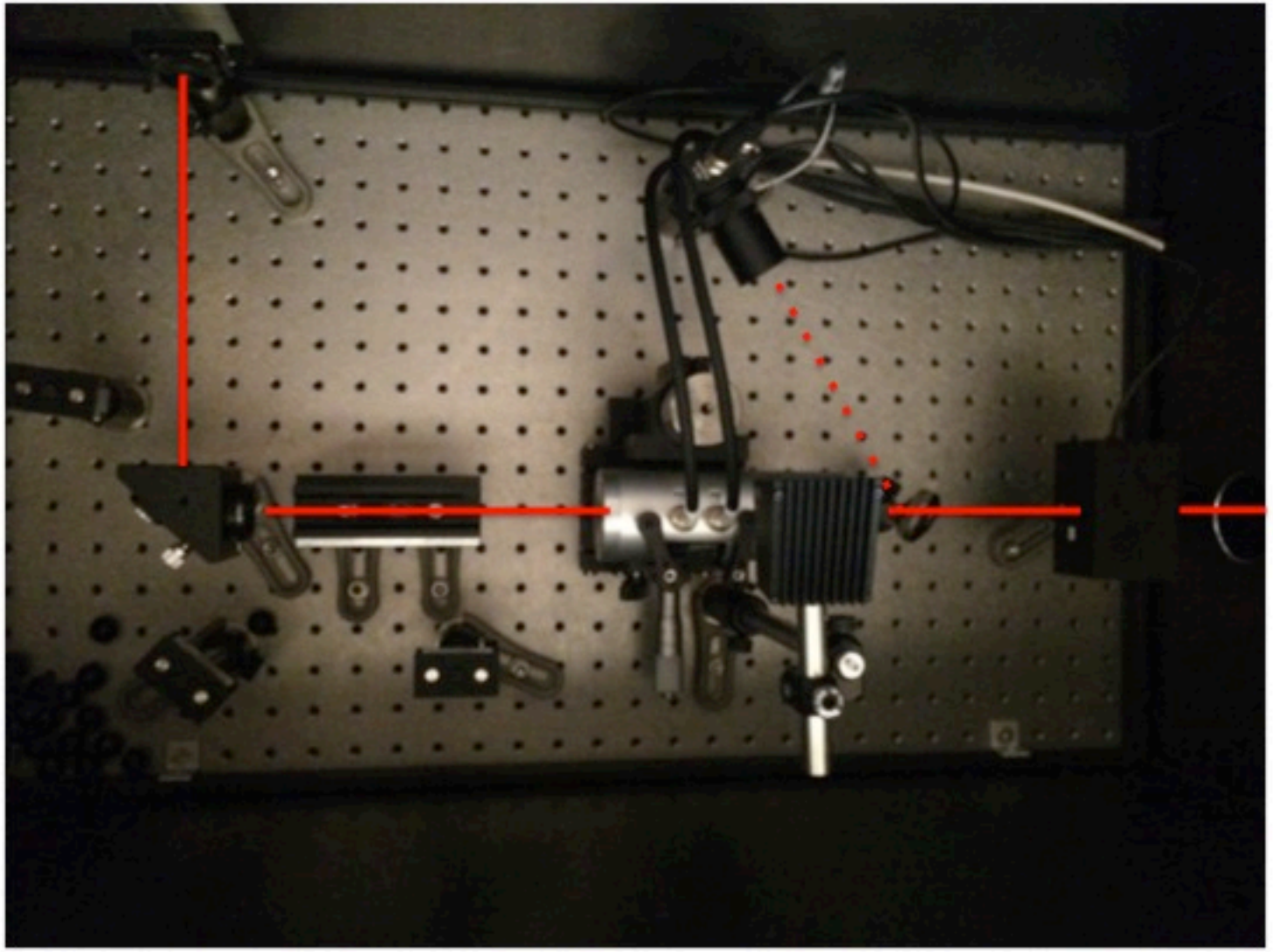


thorlabs.com

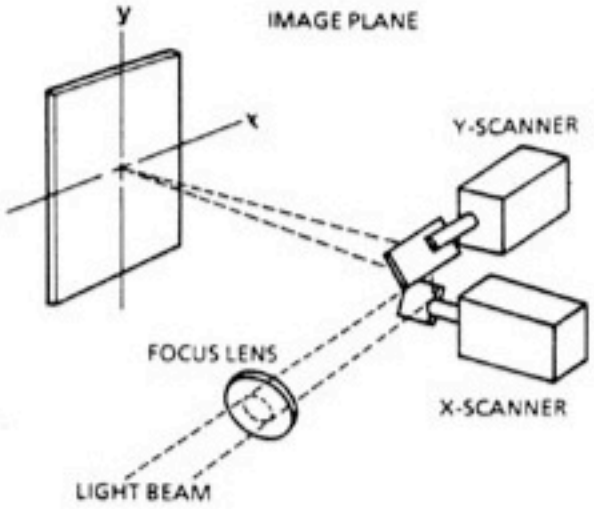


thorlabs.com

Lightpath implementation

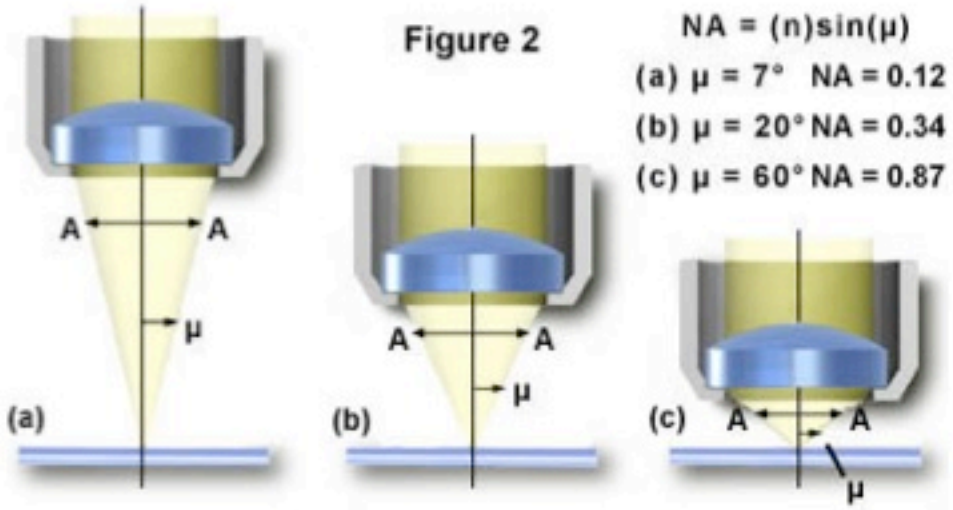


Scanners: speed & flexibility



- **Galvo-galvo system: variable scan speed; typical max. frame rate < 10Hz**
- **Resonant-galvo system: fixed scan speed on fast mirror; typical frame rate < 50Hz**
- **Acousto-optical deflectors: variable scan speed; frame rates > 1kHz; random access**

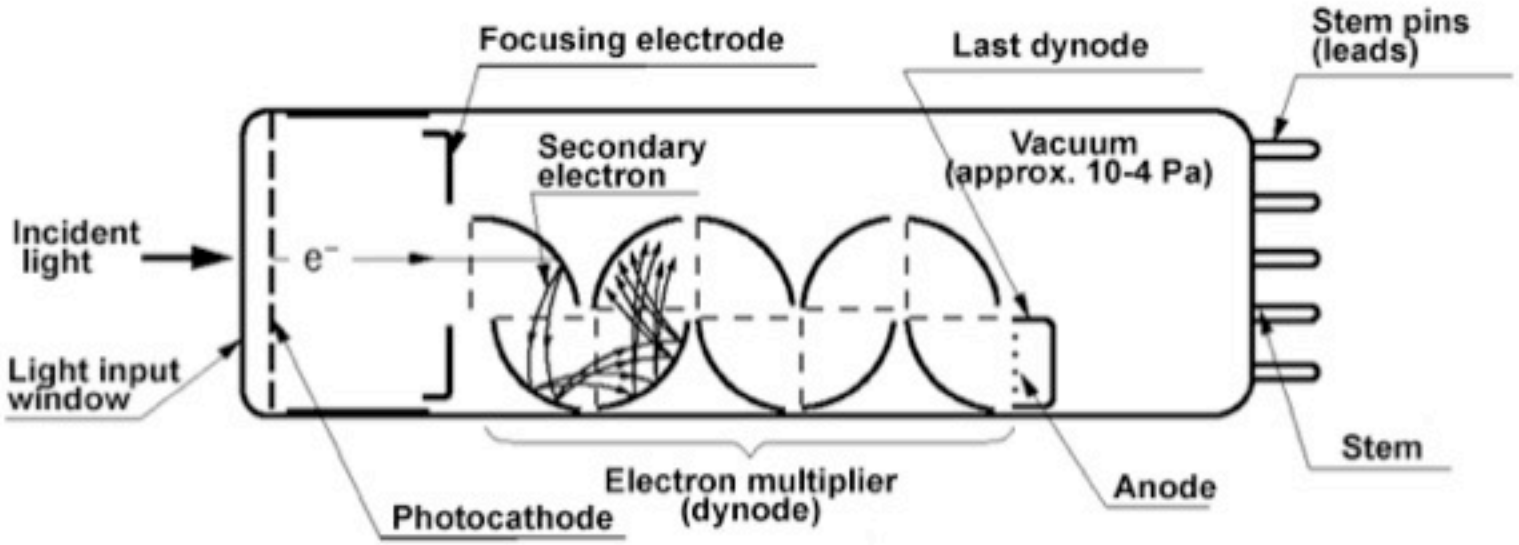
Objectives: low power & high NA



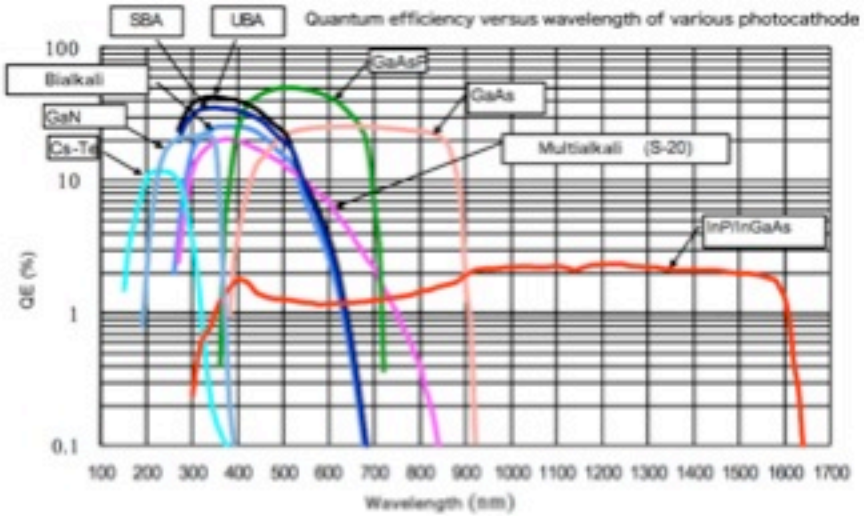
Olympus

- **The higher the numerical aperture, the more localized is excitation**
- **The higher the numerical aperture, the more scattered emission photons are collected**
- **The lower the magnification, the more scattered emission photons are collected**

Detectors: Photomultiplier tubes

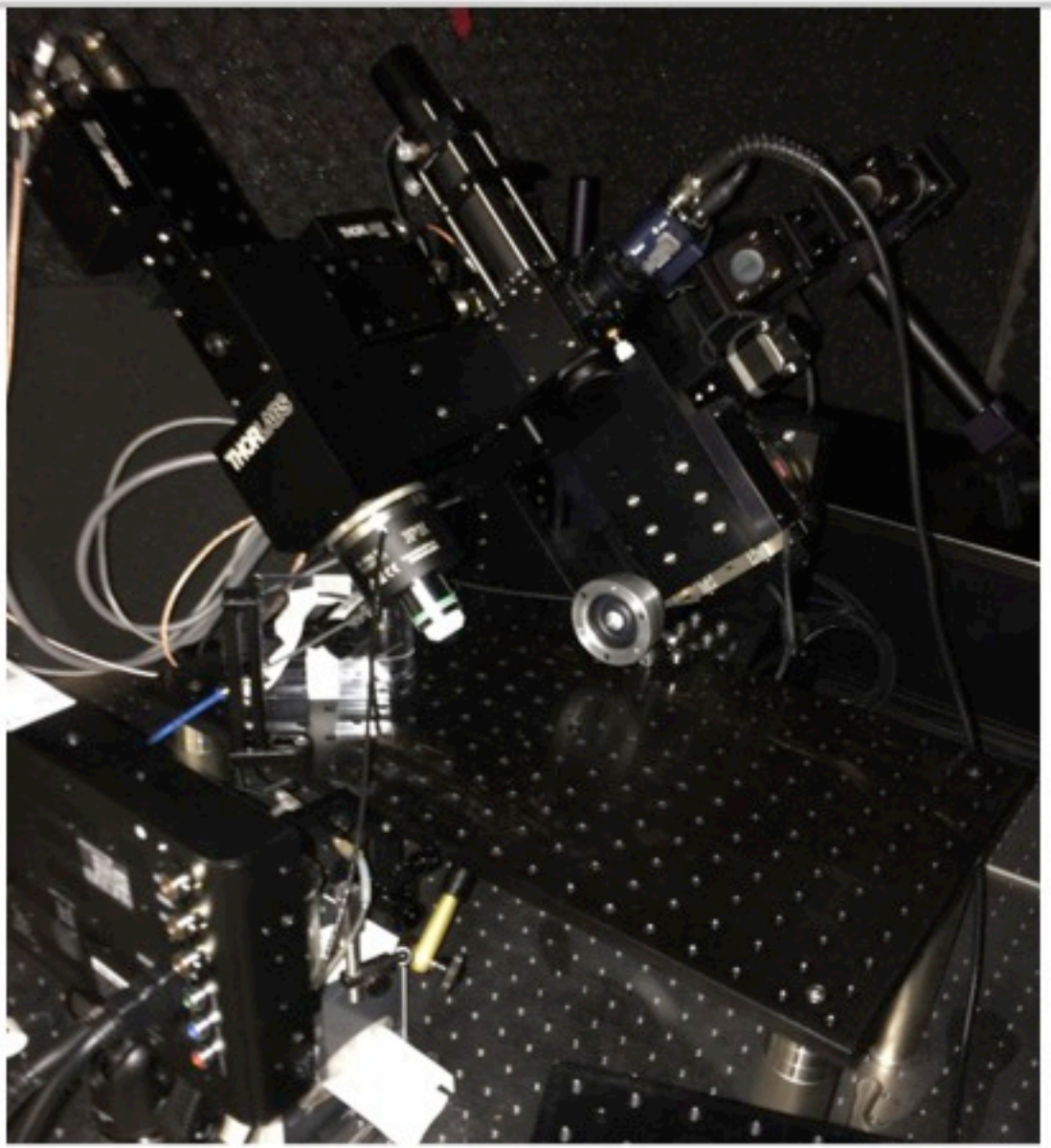


Gain 10^6 to 10^7

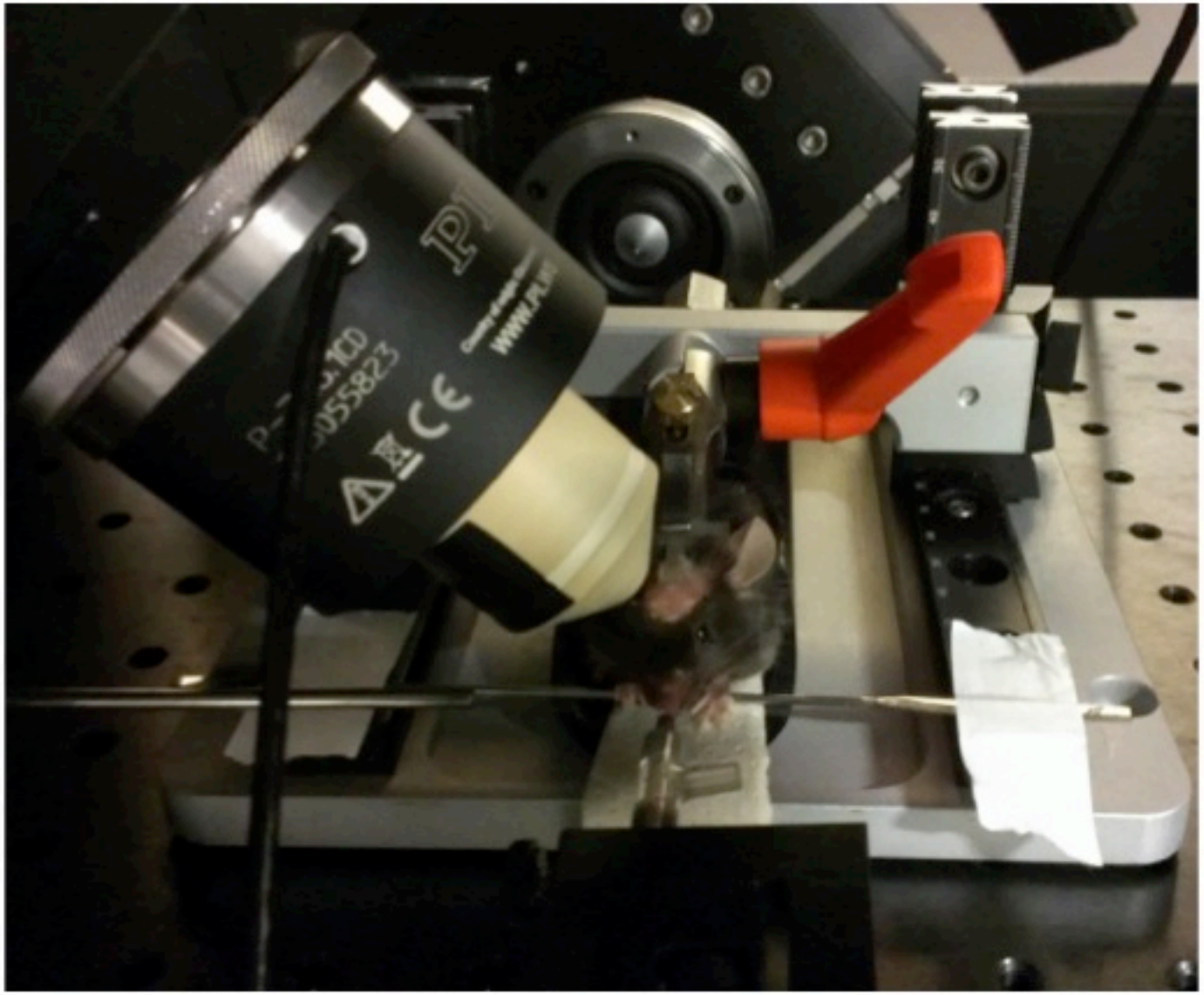


Hamamatsu

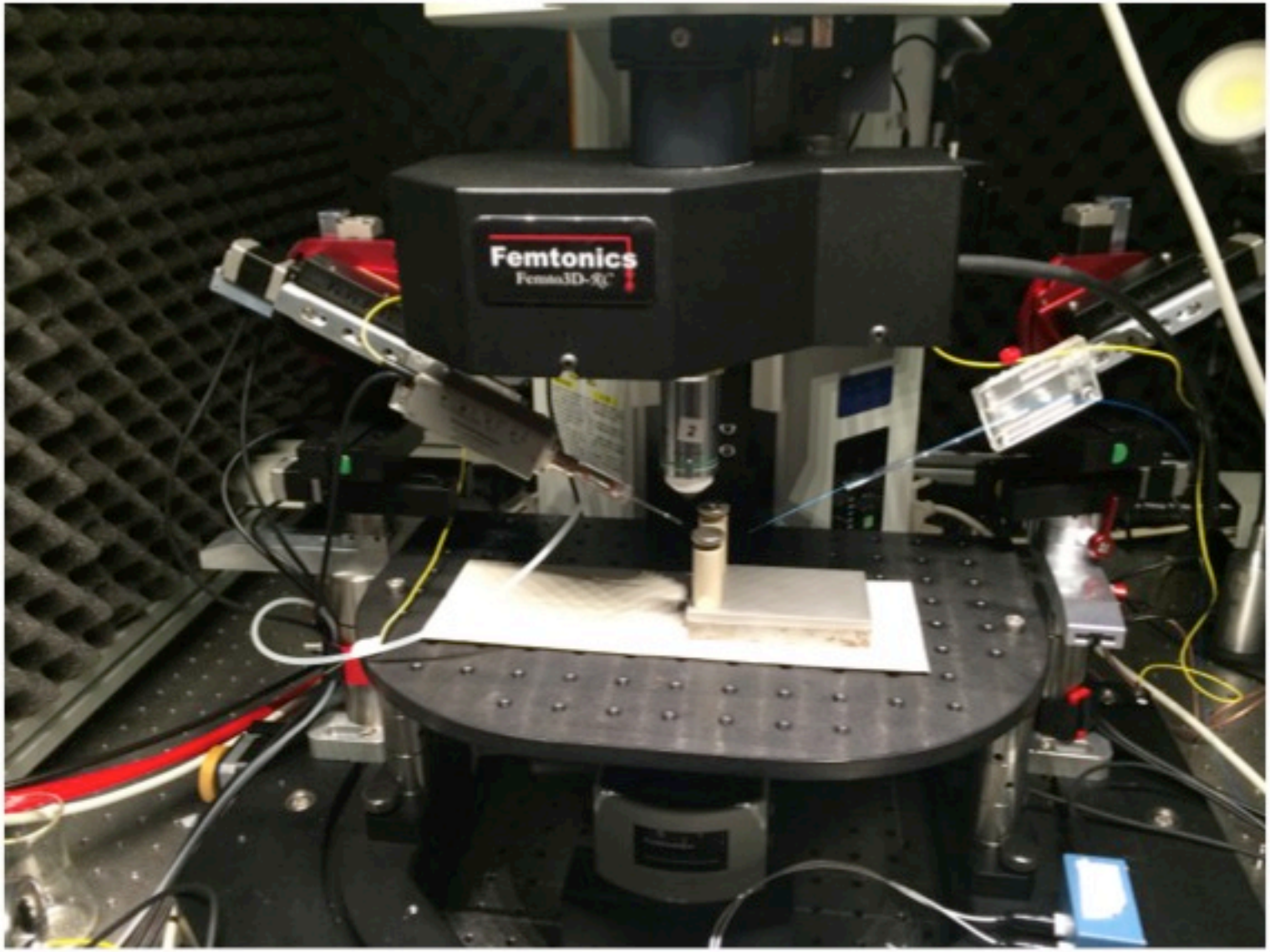
Microscope implementation for behaving mice



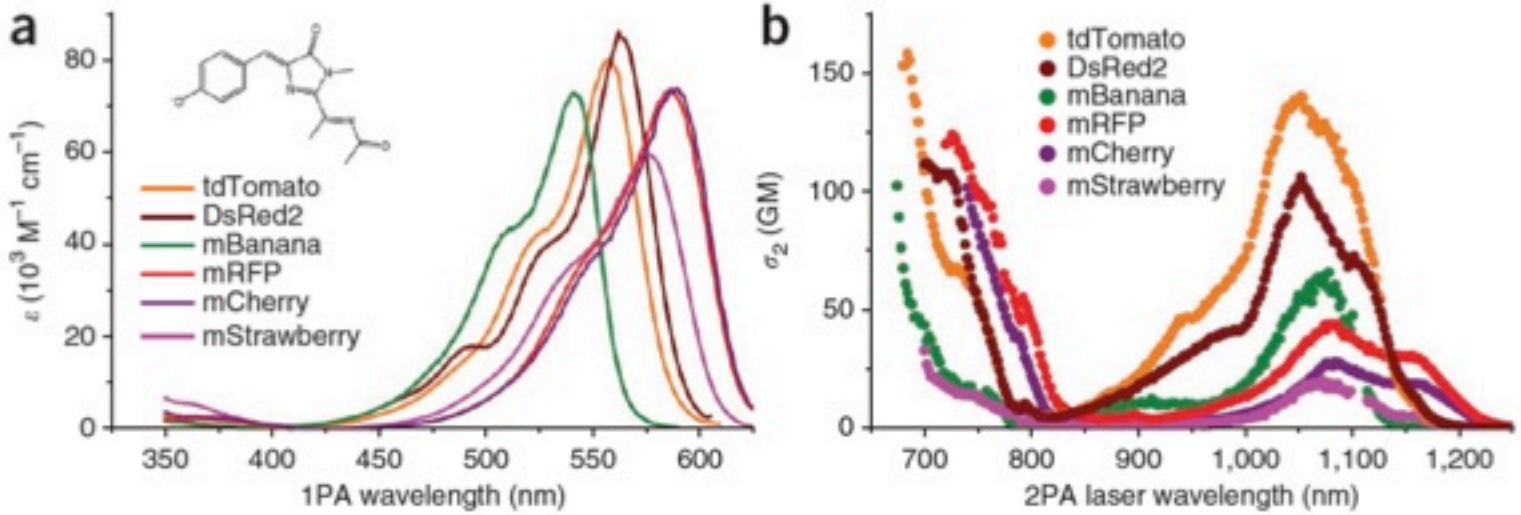
Microscope implementation for behaving mice



Microscope implementation for guided patch-clamp



Dyes for 2PM



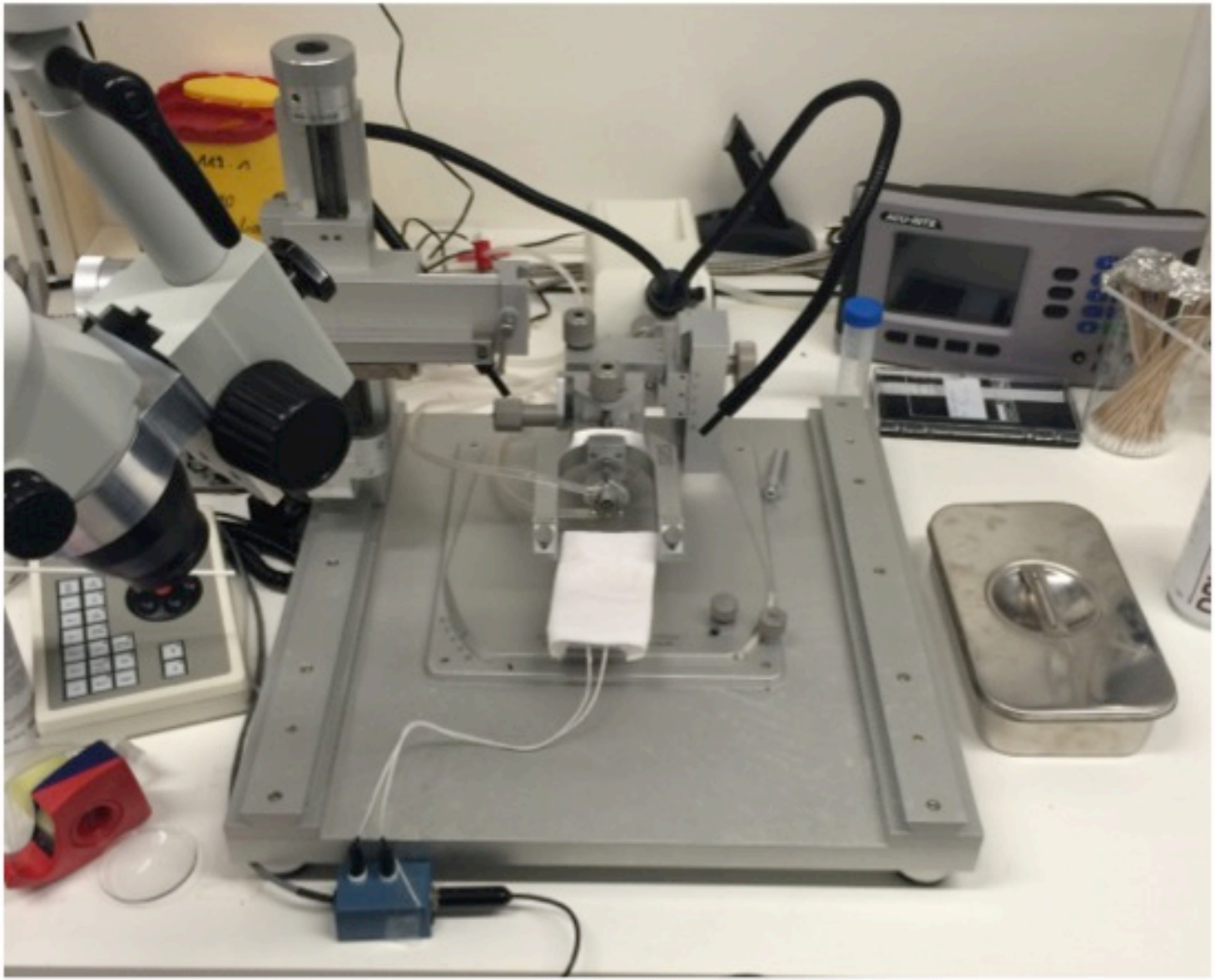
Drobizhev *et al.*, 2011

- **2p cross-section cannot be predicted from 1p excitation (both in shape and amplitude)**
- **Typically much broader then 1p spectra**

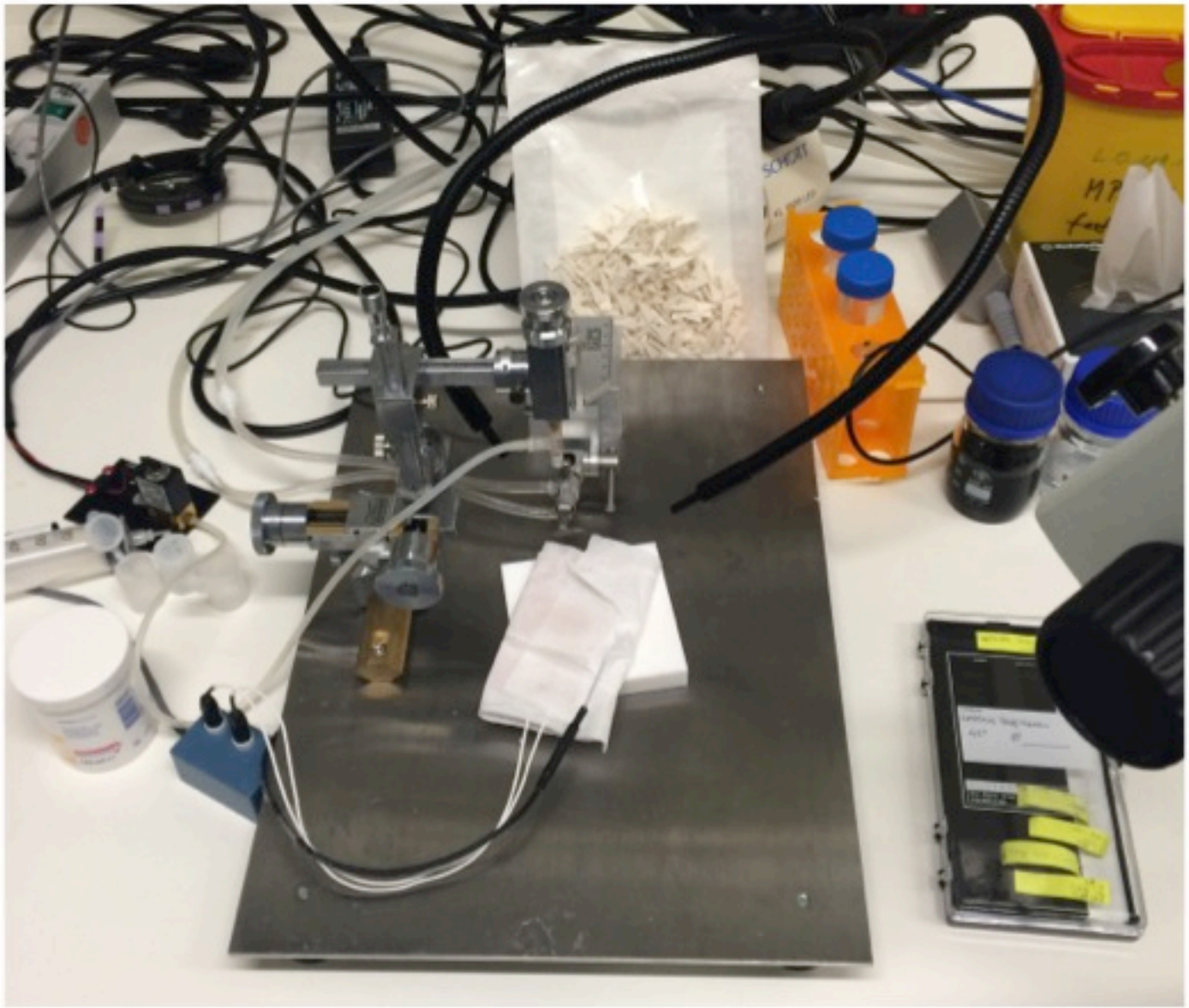
Experimental implementation of *in vivo* 2-photon imaging

- Surgery
- Anesthesia or awake
- Motion prevention / correction

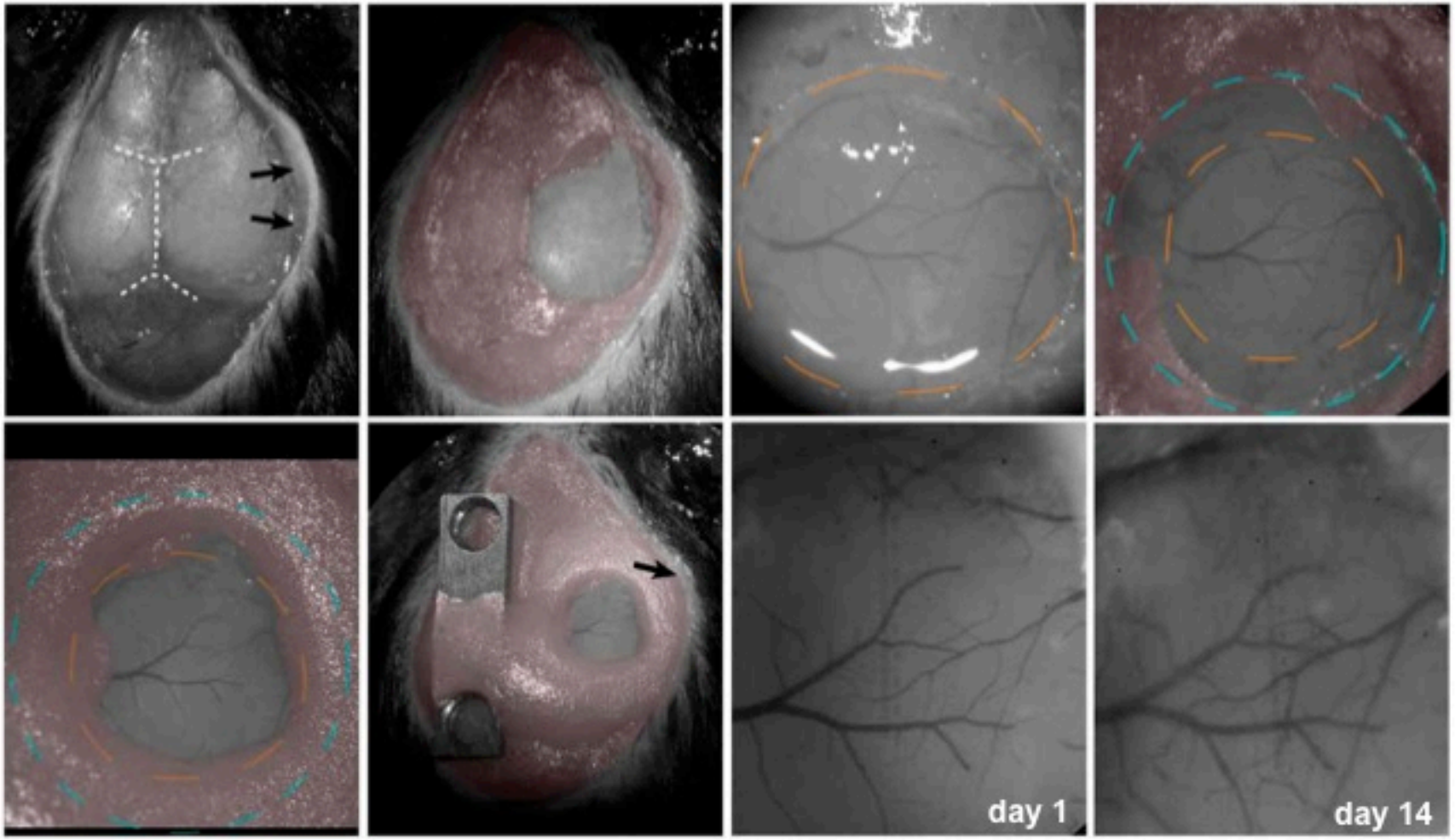
Stereotactic surgery



Stereotactic surgery

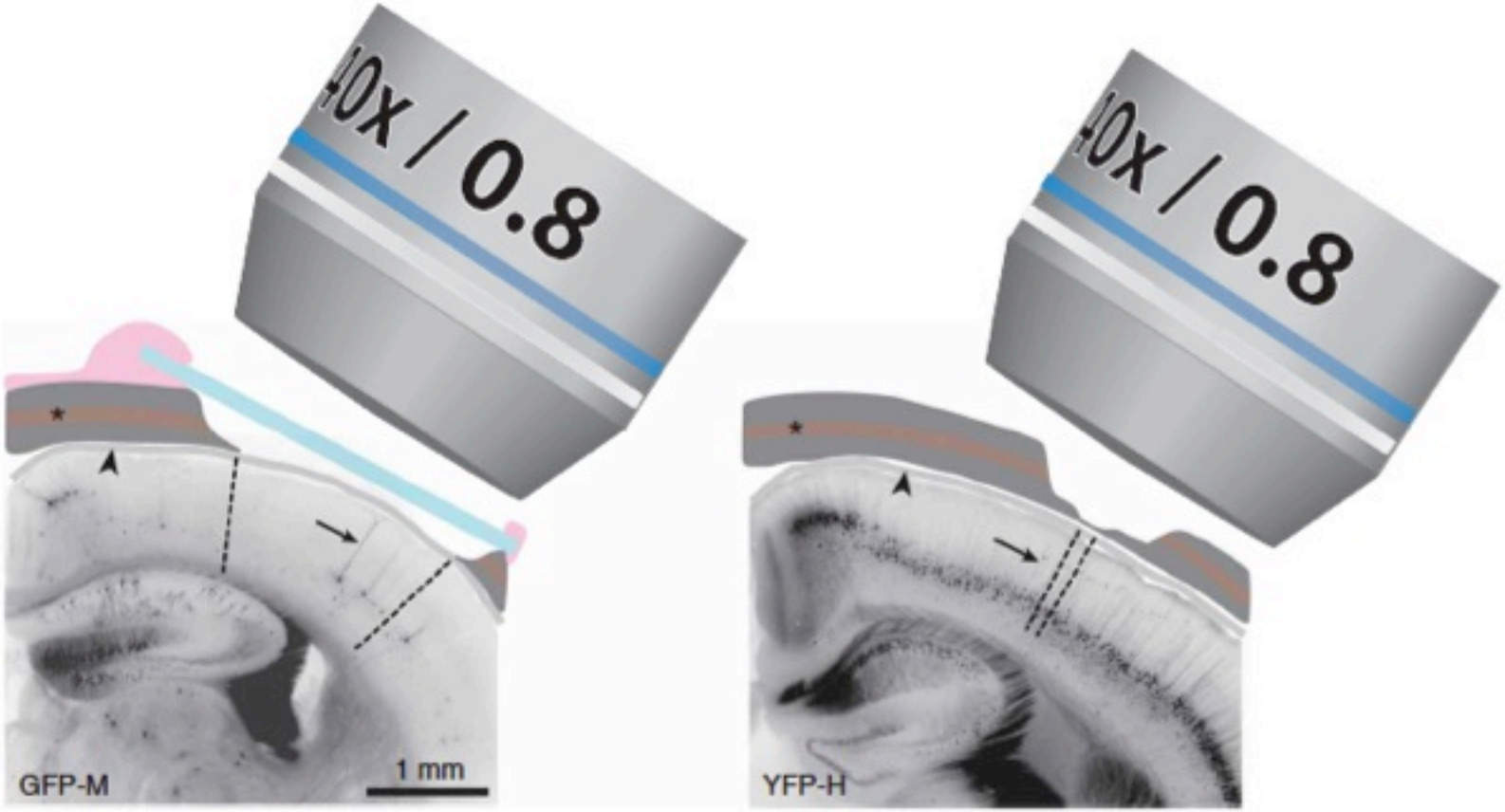


Craniotomy



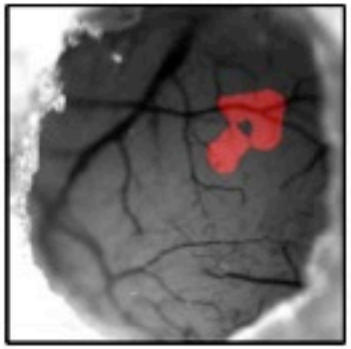
Holtmaat *et al.*, 2009

Craniotomy

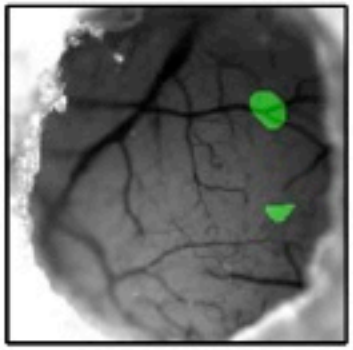


Holtmaat *et al.*, 2009

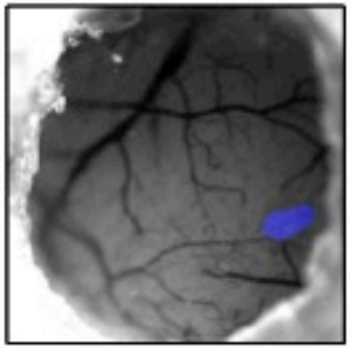
Finding the target area: intrinsic imaging



5kHz

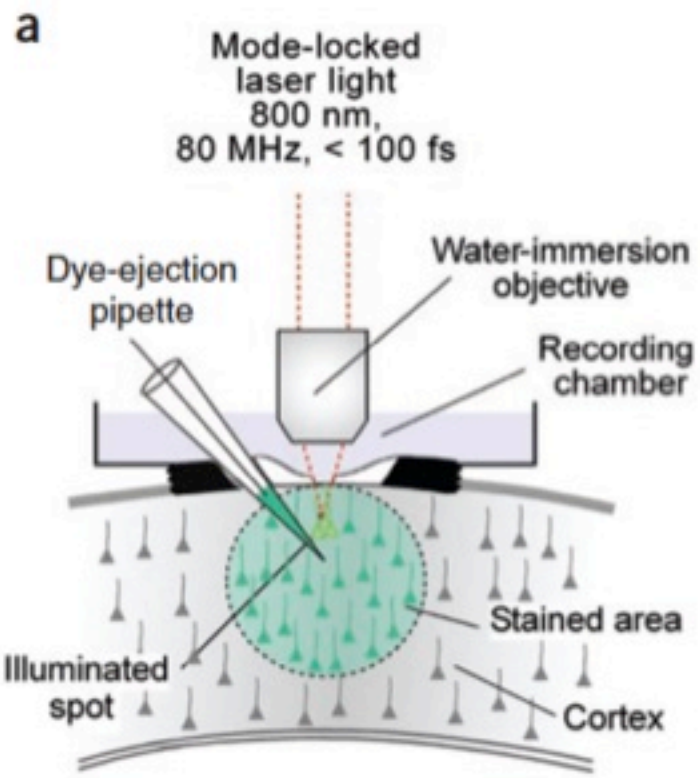


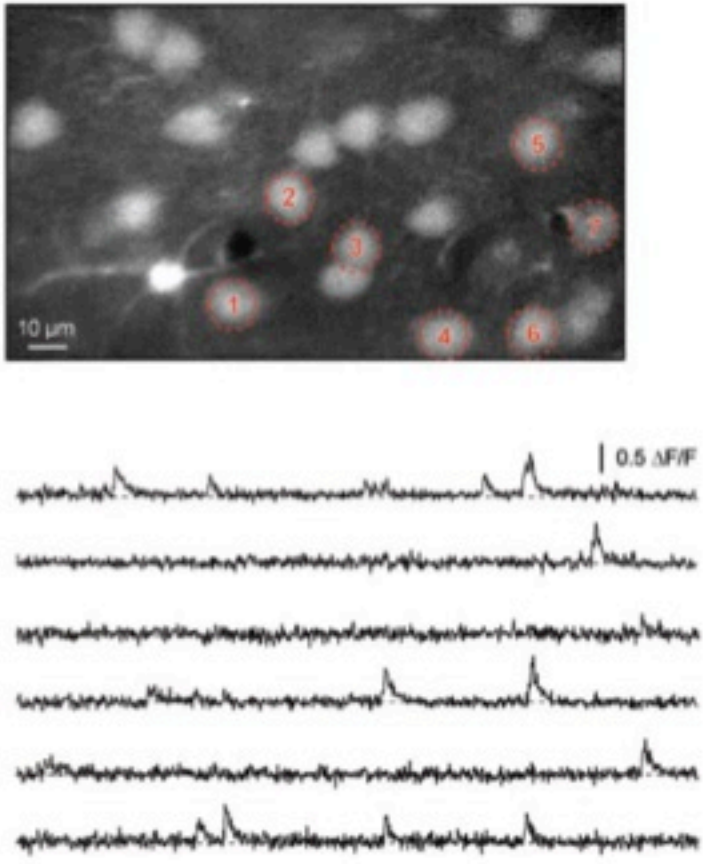
10kHz



15kHz

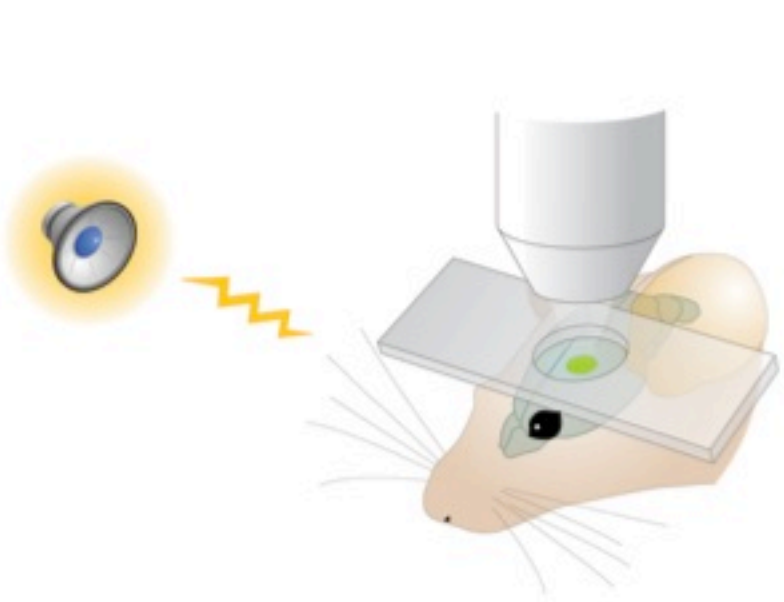
Acute Ca^{2+} imaging: OGB-1 AM

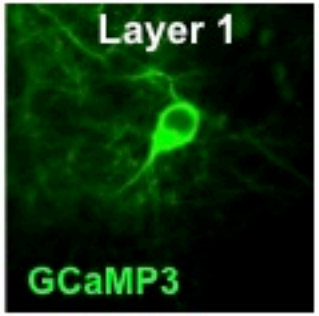


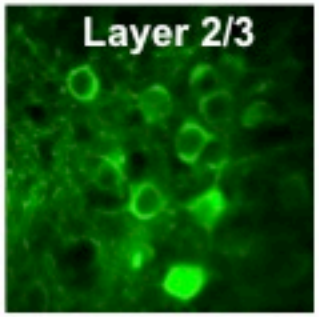


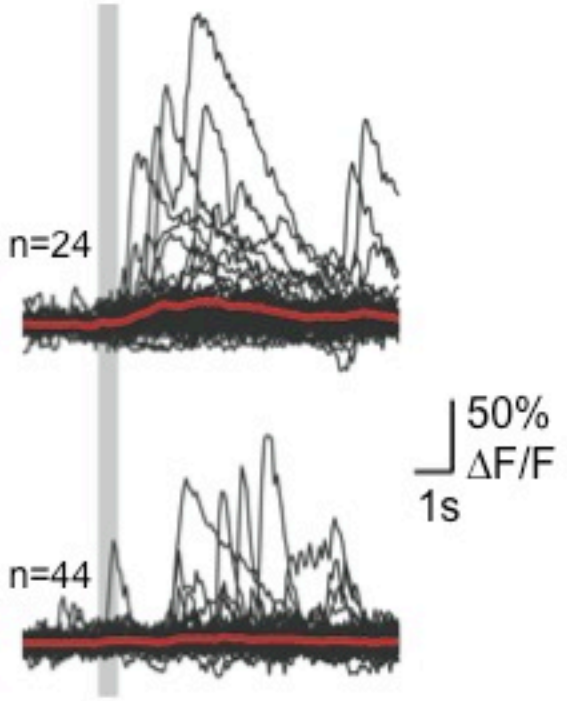
Garaschuk *et al.*, 2006

Chronic Ca^{2+} imaging: Viral vector mediated transduction



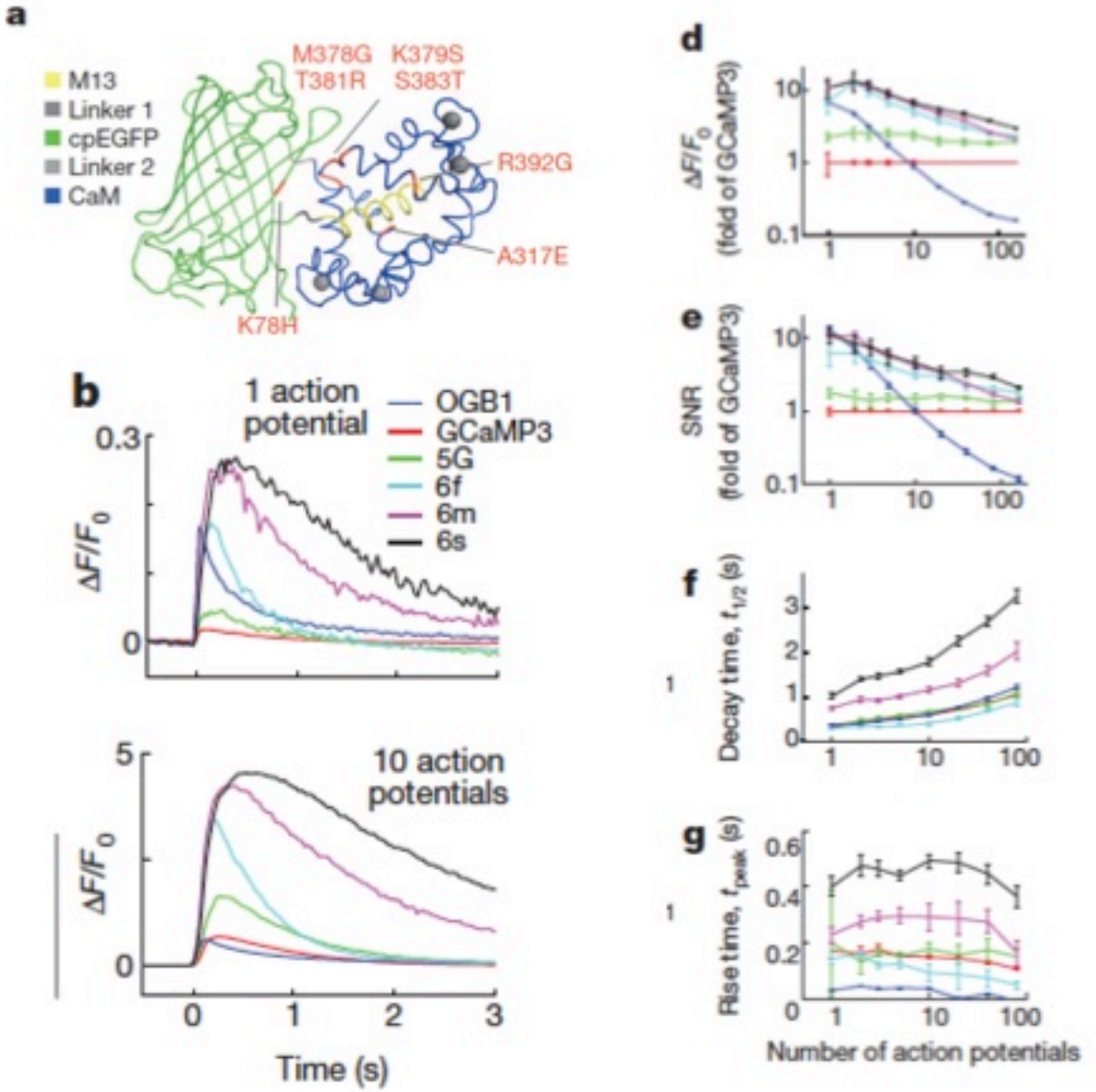






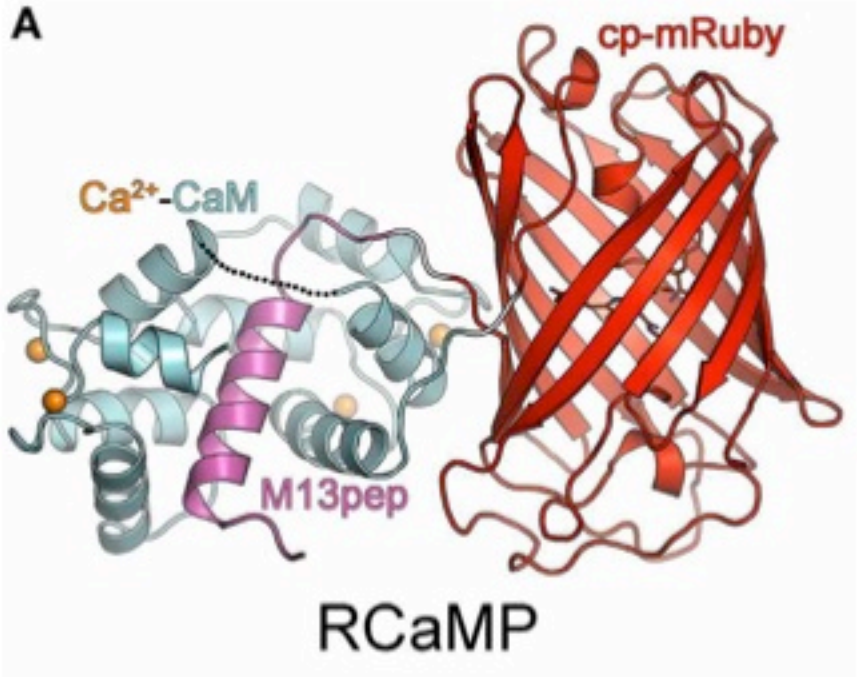
c.f. Chen *et al.*, 2013: Different GCaMP6 variants

GCaMP6: the most sensitive genetically-encoded Ca^{2+} indicator so far



Chen *et al.*, 2013

RCaMP: a promising red genetically-encoded Ca^{2+} indicator



Akerboom *et al.*, 2013

Anesthetized or awake?

Anesthetized or awake?

Anesthetized:

+ allows to keep the animal's internal state constant over long time periods
+ typically more time available for mechanistic dissections

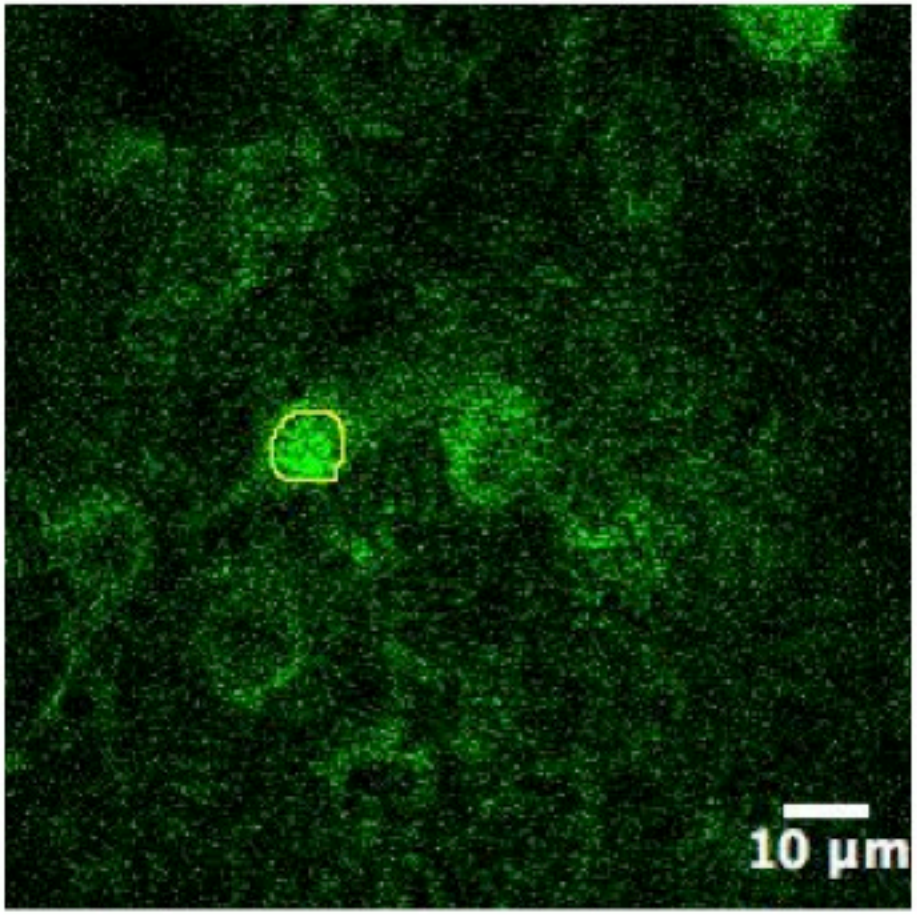
- unphysiological
- choice of anesthetic can strongly influence results
- difficult to keep mice at constant anesthesia level

Awake:

+ allows recordings during behavior
+ physiological (but head-fixed)

- animal's internal state will drift over time (> record behavioral performance)
- limited time for experiment
- motion artifacts

Motion artifacts



Preventing and dealing with motion artifacts

Prevention:

- **Habituate animals to head fixation well, and limit their traction on the table (e.g. spring-mounted treadmill)**

- **Design compact head-fixation, and tighten the screws!**

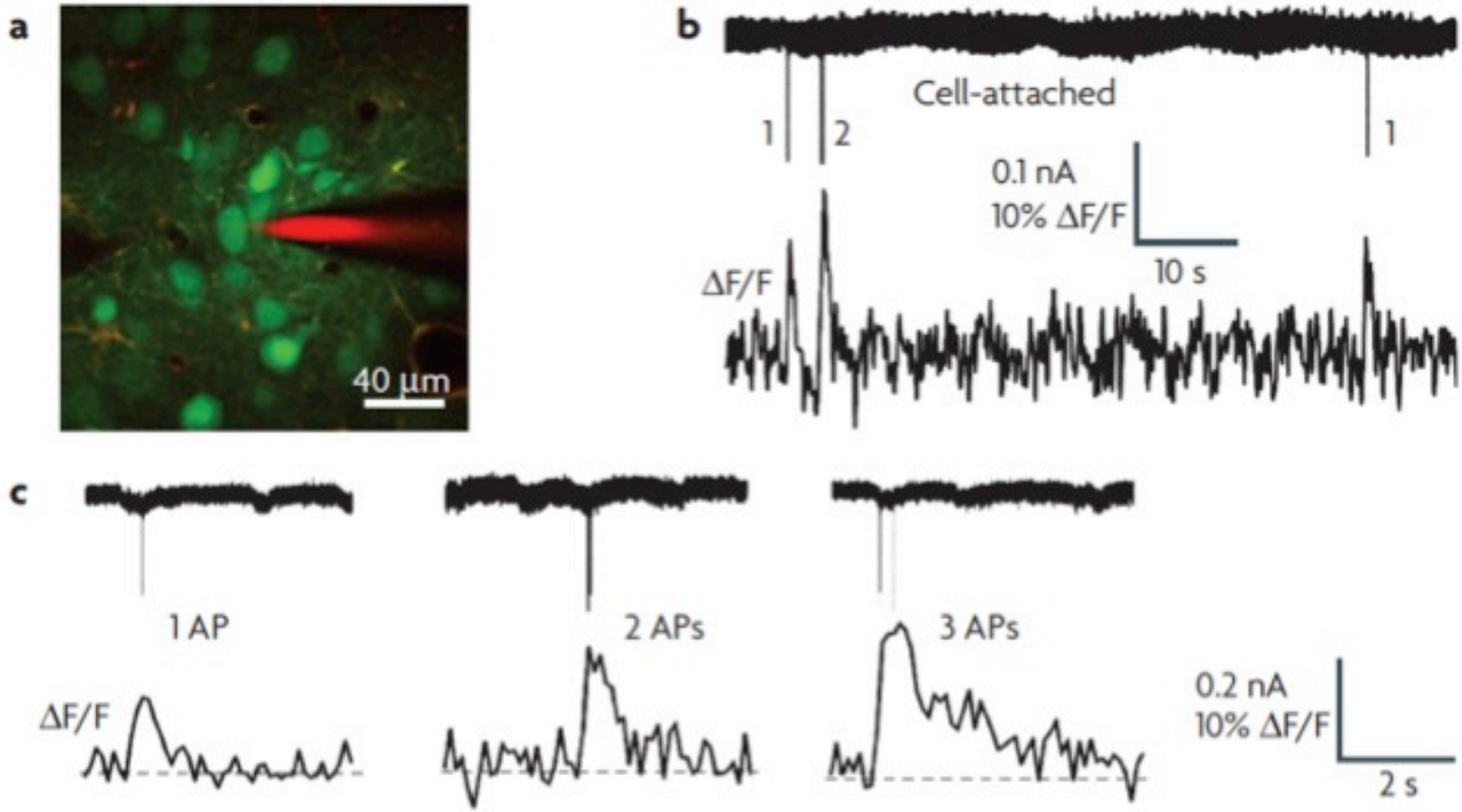
Dealing:

- **Do frame (not line) scans with a high frame rate**

- **Express a second (red) dye that is Ca^{2+}-insensitive and thus reports just motion**

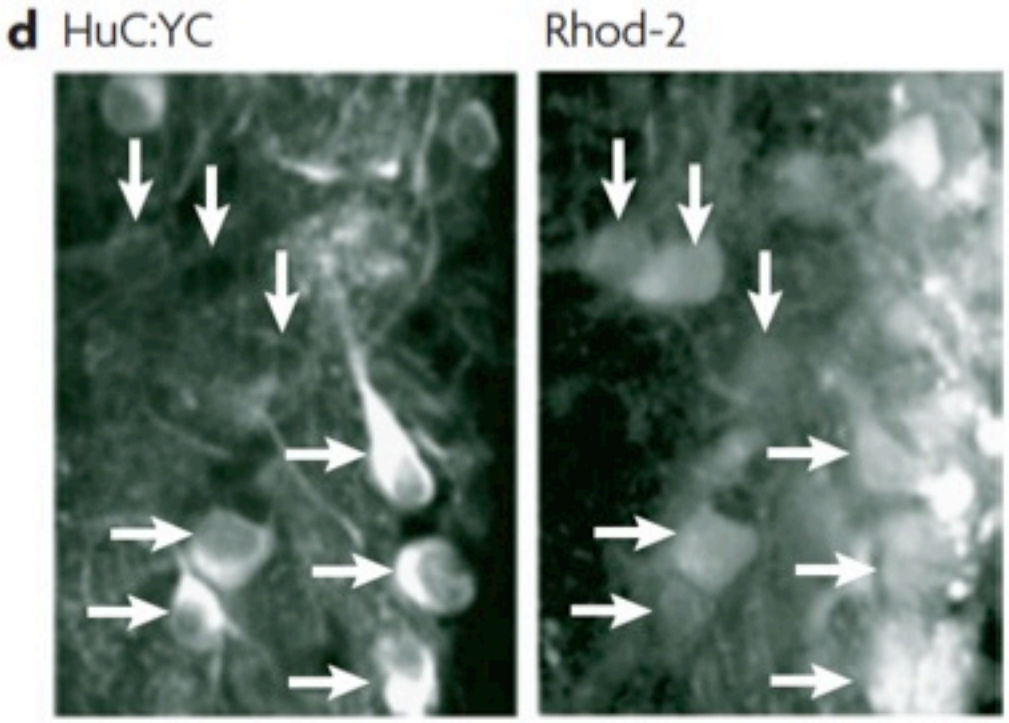
- **Several published motion correction algorithms work well (ImageJ stack reg; Greenberg & Kerr, 2009; Kaifosh *et al.*, 2014) > but only in 2D!**

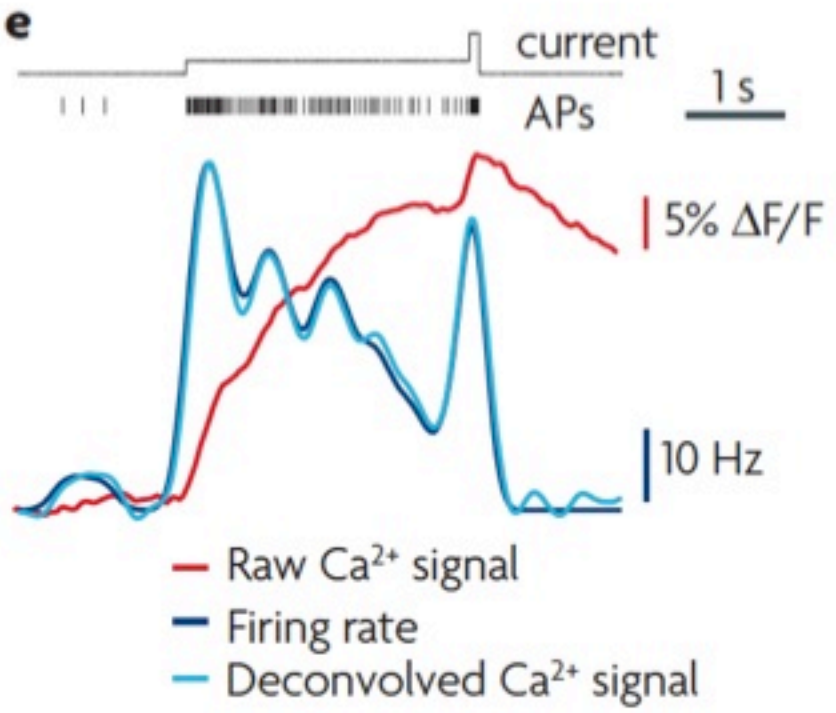
What does calcium imaging report?



Kerr & Denk, 2008

Firing rate extraction by deconvolution





Yaksi & Friedrich, 2006

Applications of *in vivo* 2PM

What are the unique strengths of *in vivo* 2-photon microscopy?

What are the unique strengths of *in vivo* 2-photon microscopy?

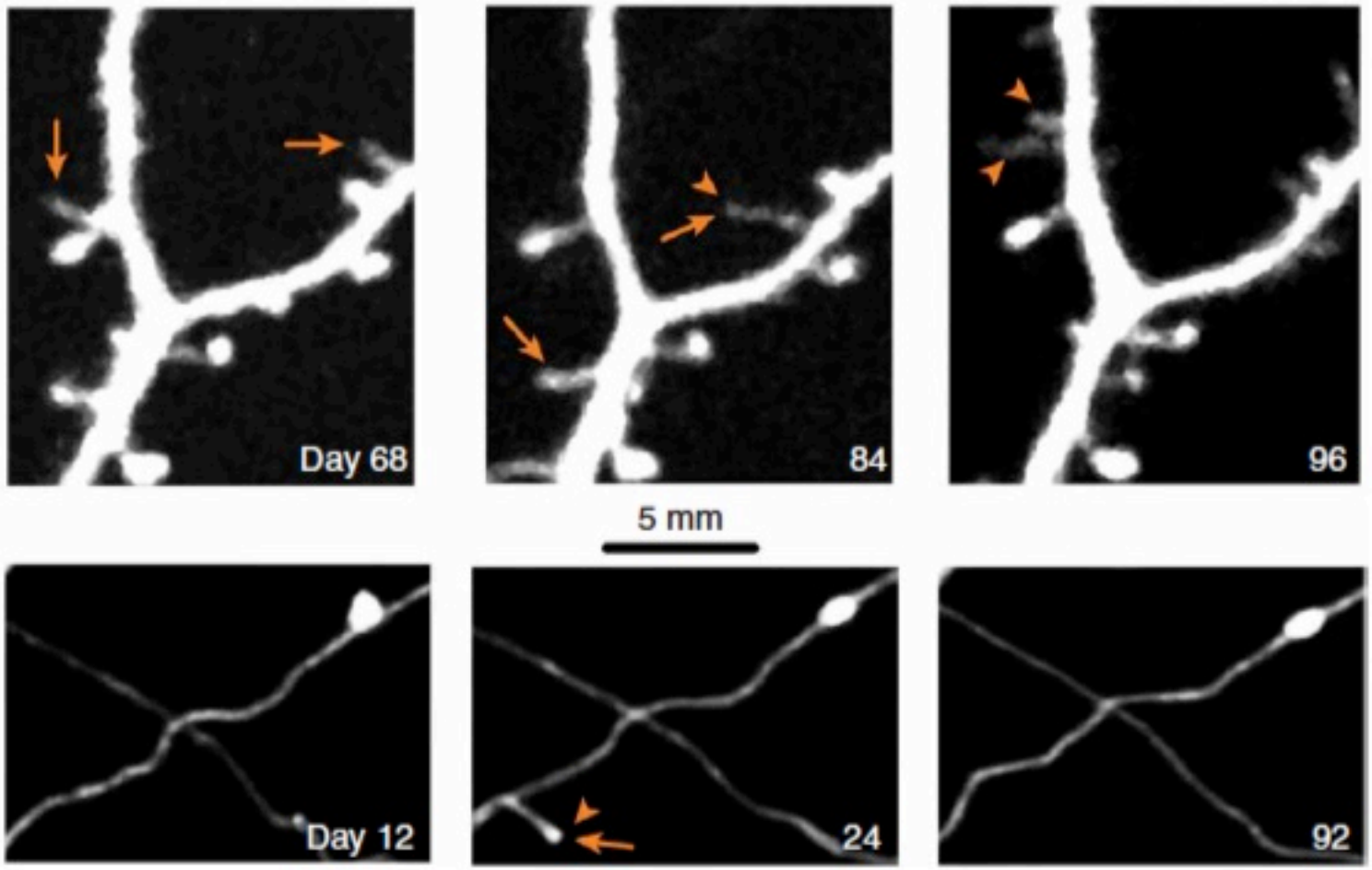
- recording of both structure and activity
- recording from subcellular compartments (dendrites, axons)
- recording from identified neuron types
- compatibility with electrophysiology
- compatibility with behavior
- long-term recordings of the same neurons

What are the inherent weaknesses of *in vivo* 2-photon microscopy?

What are the inherent weaknesses of *in vivo* 2-photon microscopy?

- limited depth of conventional 2pm
- calcium imaging typically has a much worse temporal resolution than electrophysiology
- calcium imaging is an indirect readout of activity, not easy to compare between different cell types or compartments
- only head-fixed behavior for conventional 2pm

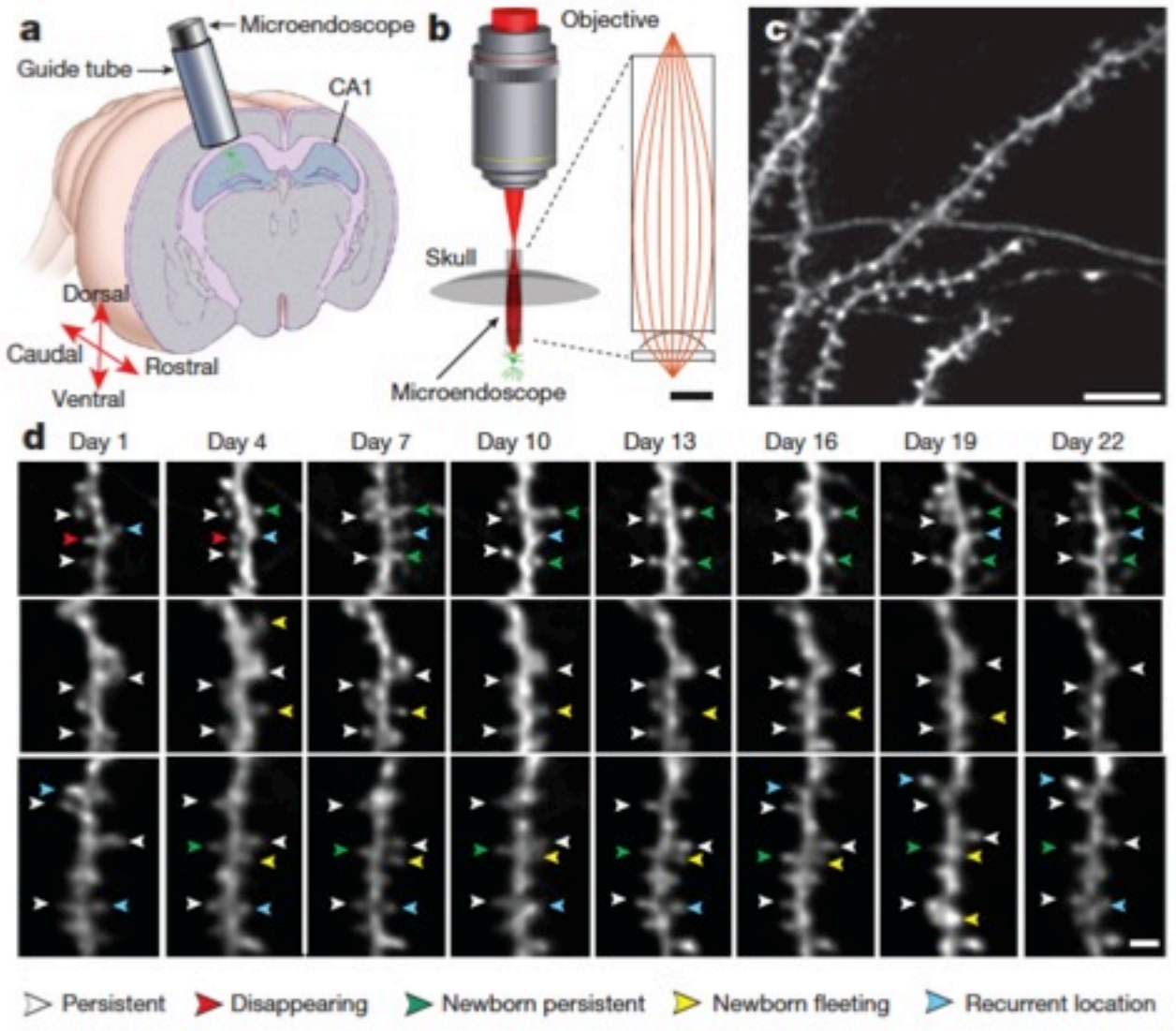
Structural plasticity



c.f. Svoboda *et al.;* Bonhoeffer *et al.;* Gan *et al.*

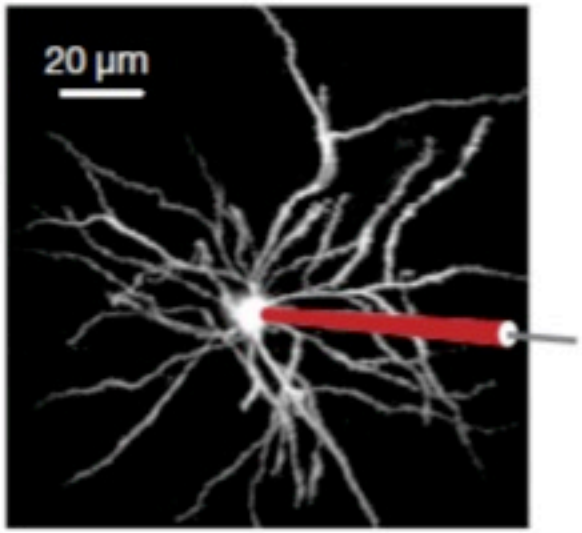
Holtmaat *et al.*, 2009

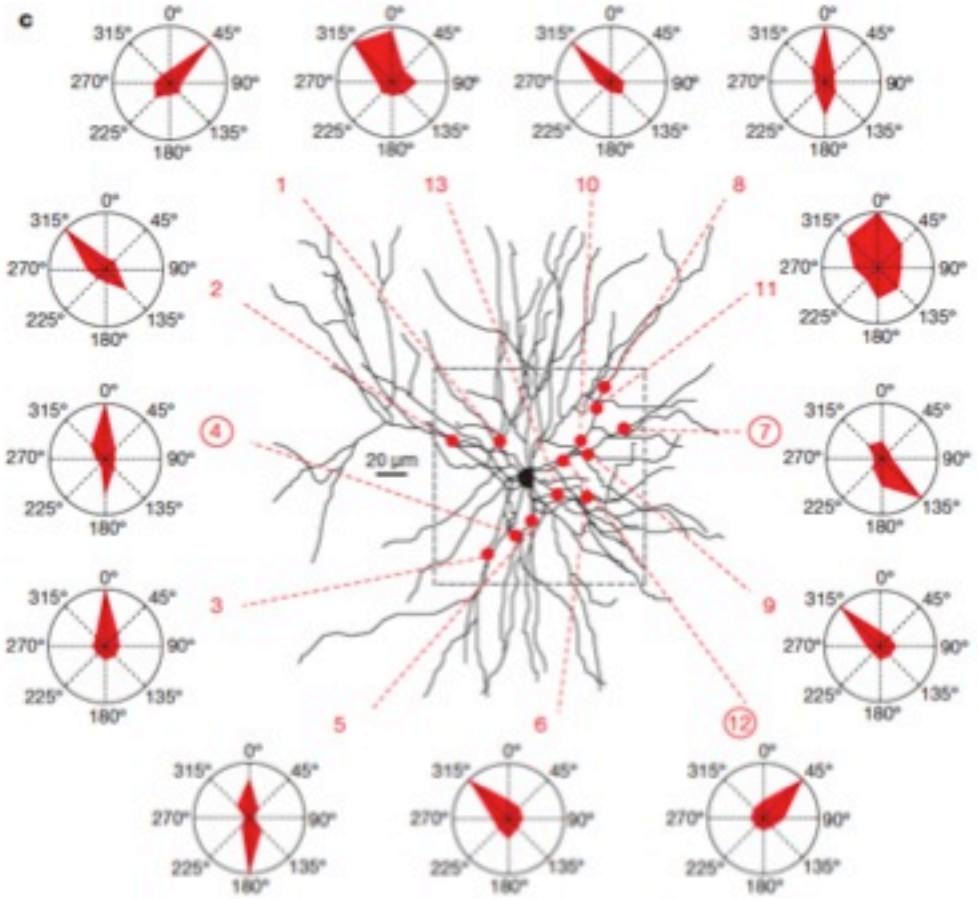
Structural plasticity



Attardo *et al.*, 2015

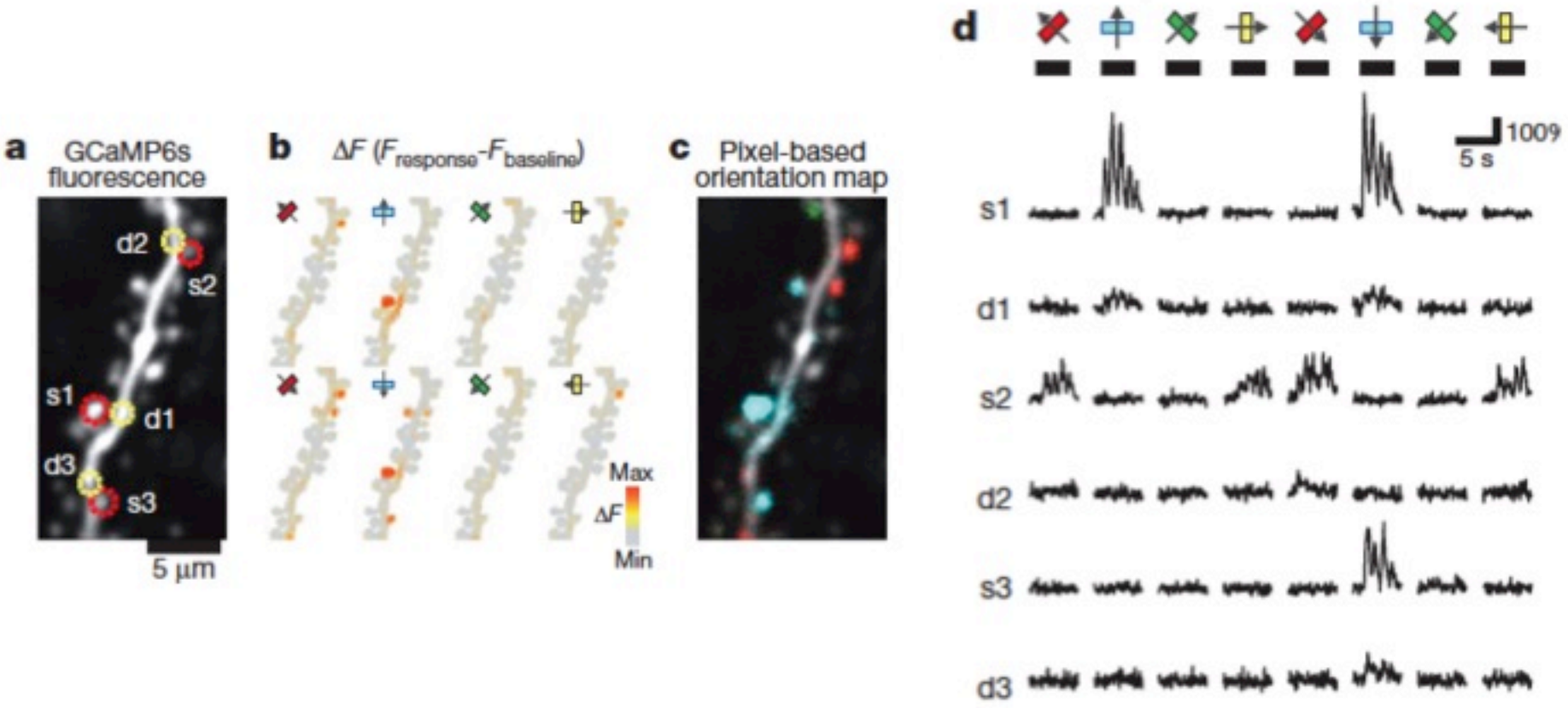
Spine Ca^{2+} imaging with a synthetic dye





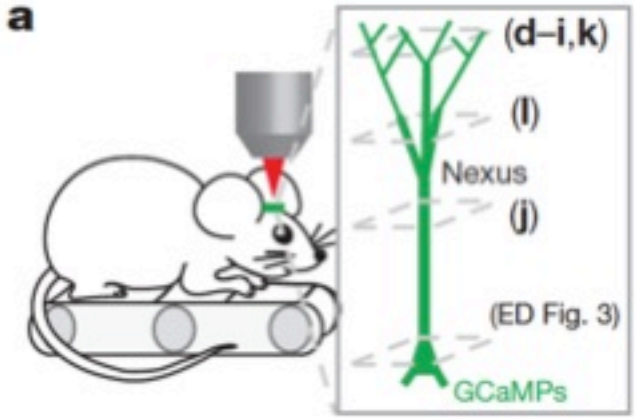
Jia *et al.*, 2010

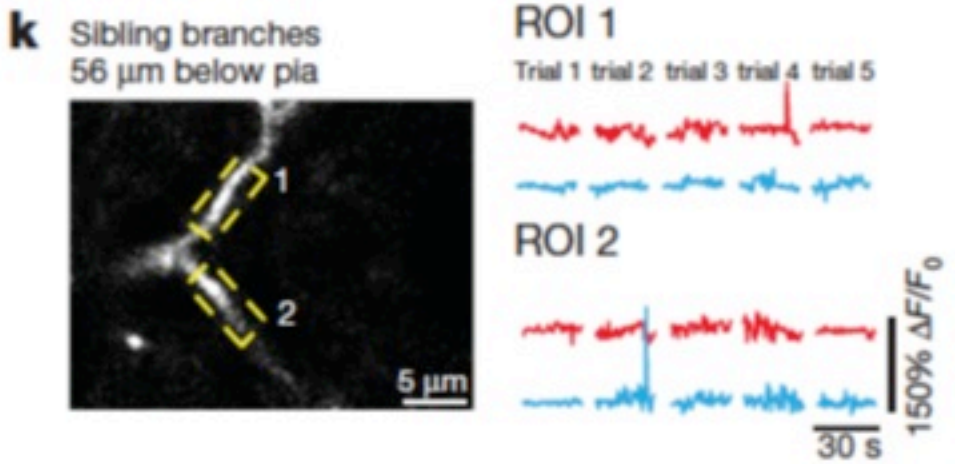
Spine Ca^{2+} imaging with GCaMP6s



Chen *et al.*, 2013

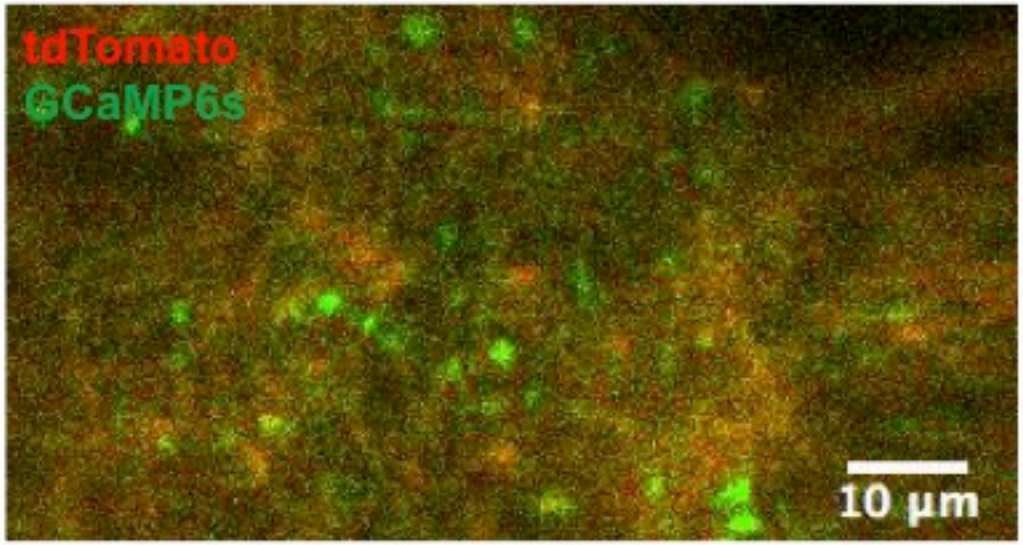
Dendritic Ca^{2+} imaging with GCaMP6s



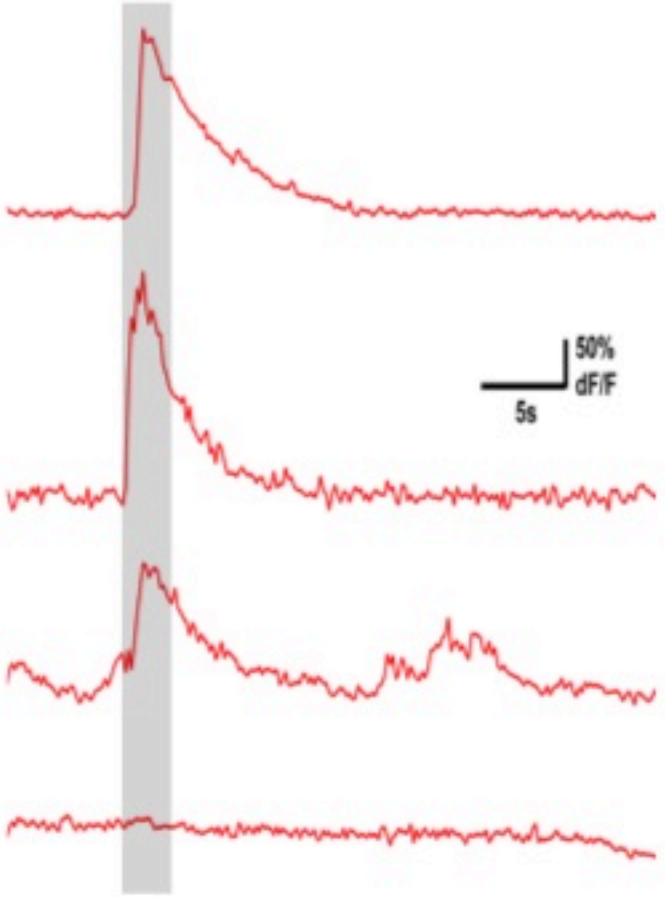


Cichon & Gan, 2015

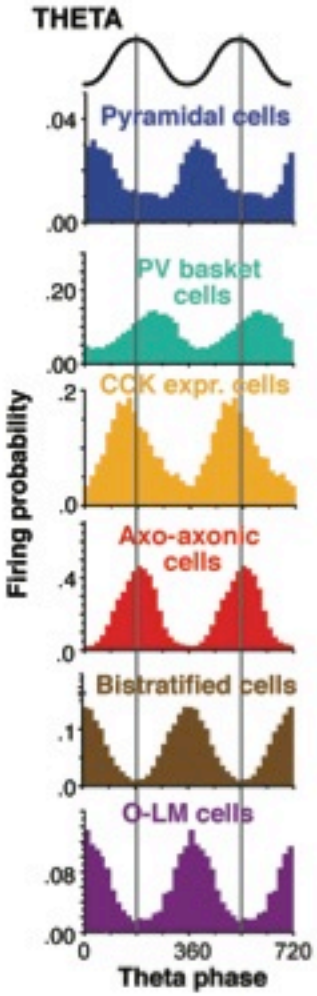
Axonal Ca^{2+} imaging with GCaMP6s

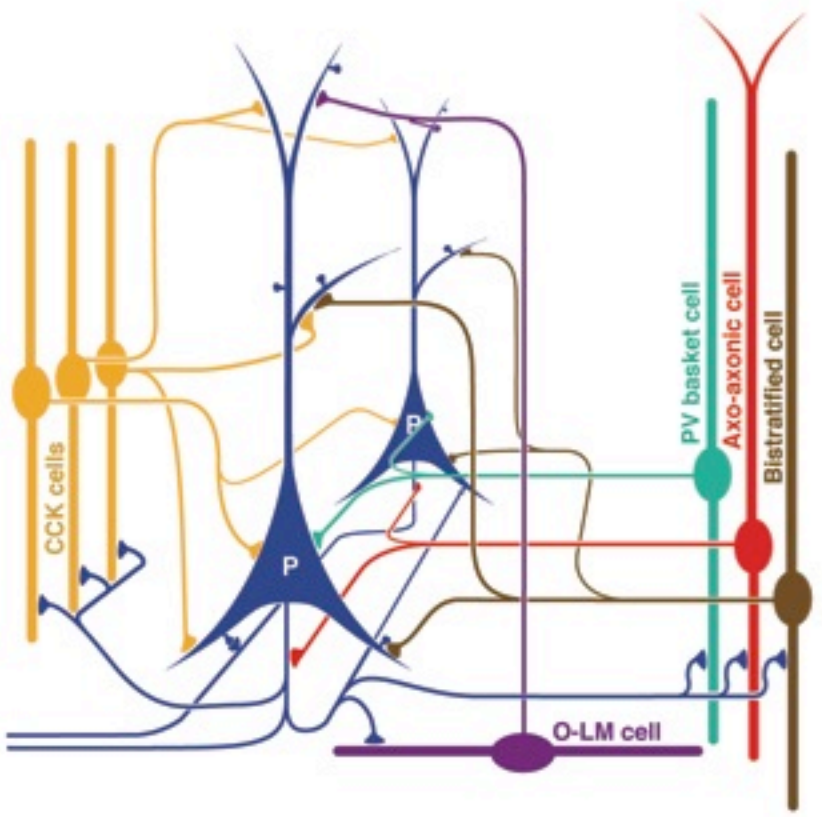


2x speed, 40s



Excursion: Neuronal identity determines computational function

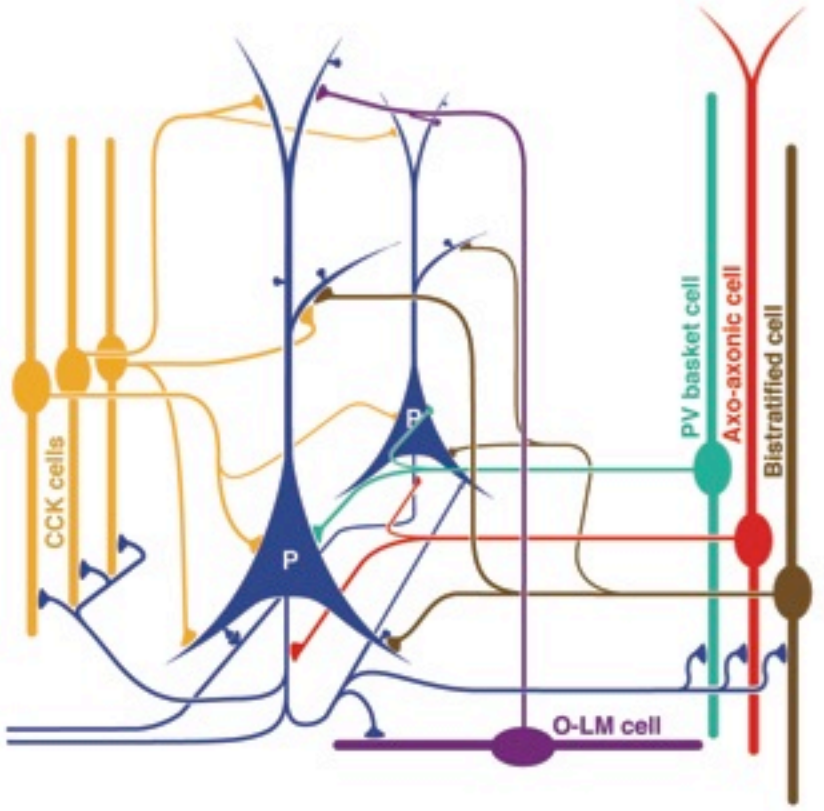




modified from: Klausberger & Somogyi, 2008

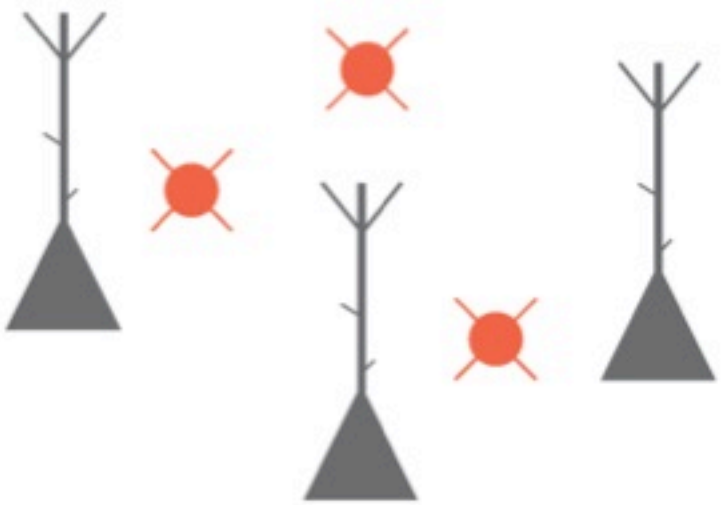
Neuronal and electronic circuits are composed of distinct elements

⇋

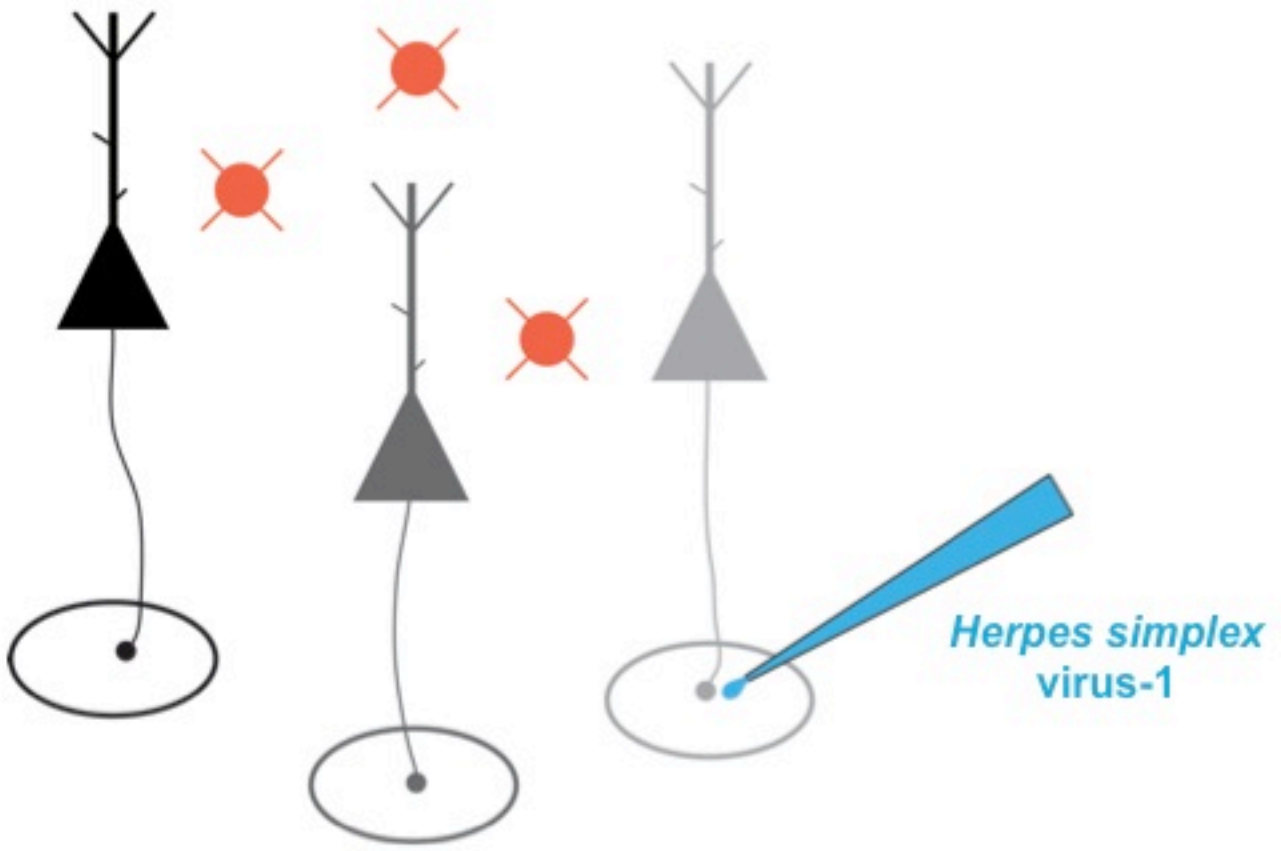


modified from: Klausberger & Somogyi, *Science* (2008)

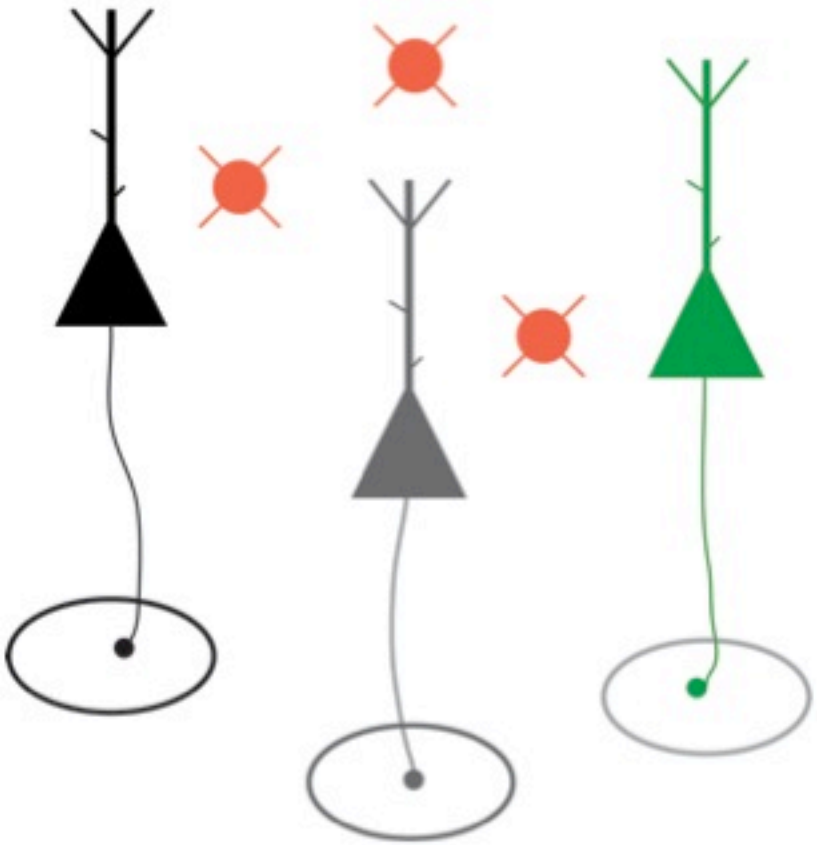
Neuronal identity in neocortical circuits



Projection neuron identification based on target areas

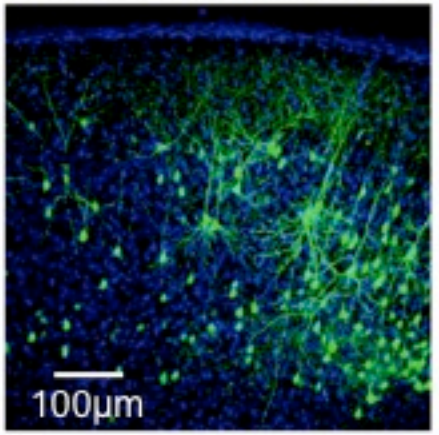


Projection neuron identification based on target areas

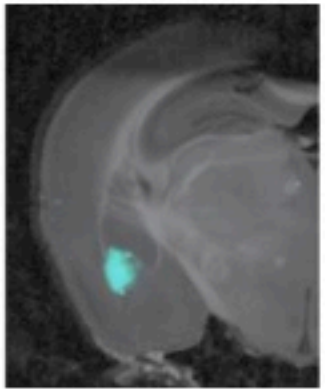


Target areas

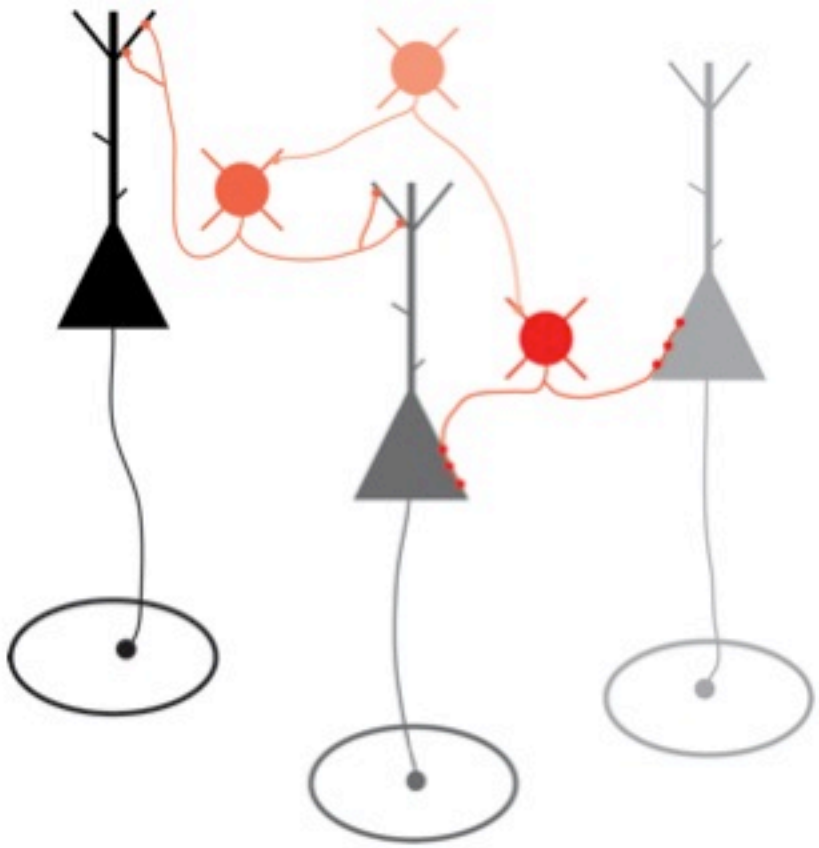
Amygdala-projecting auditory cortex neurons



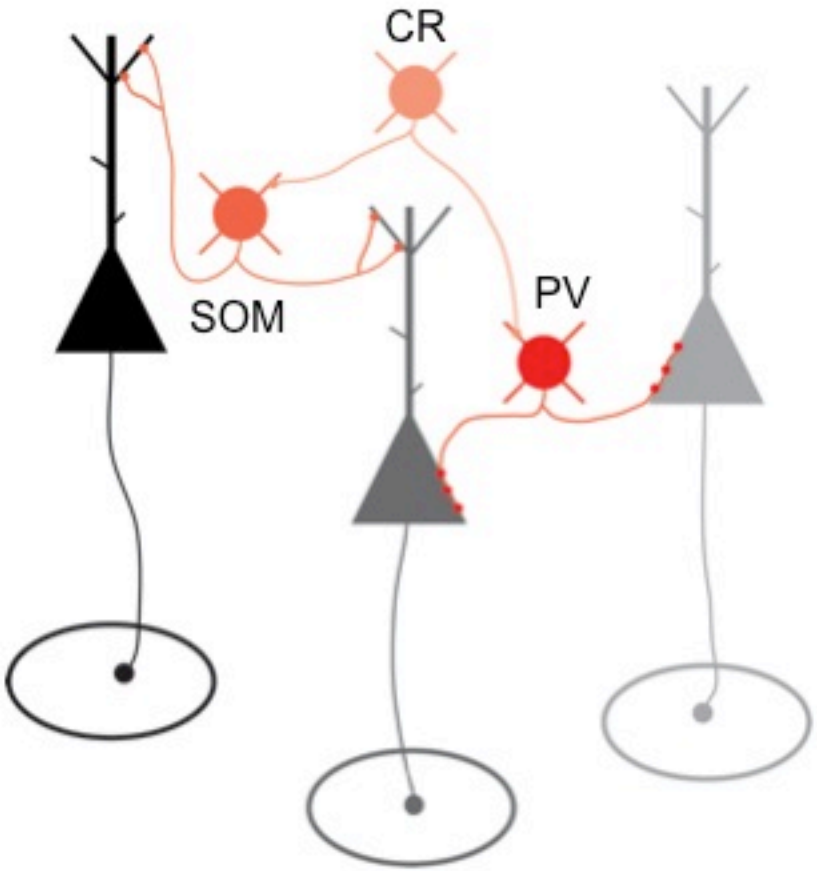
HSV-1 injection into amygdala



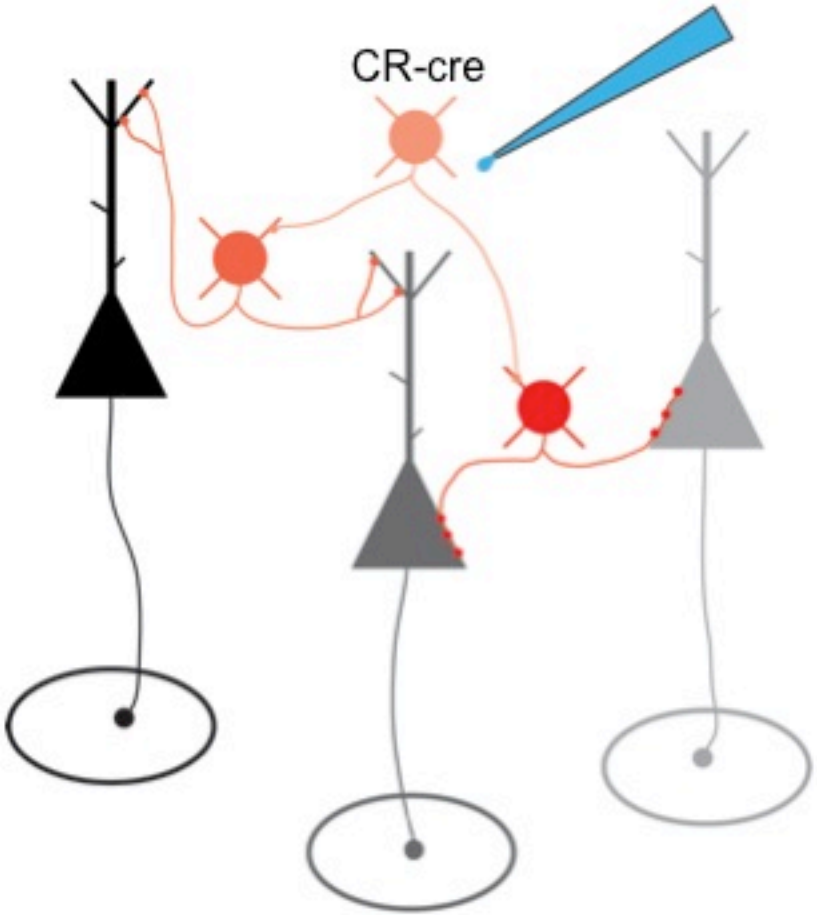
Interneuron identification based on marker expression

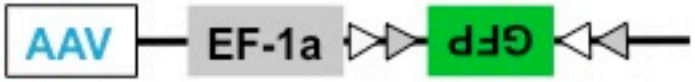


Interneuron identification based on marker expression

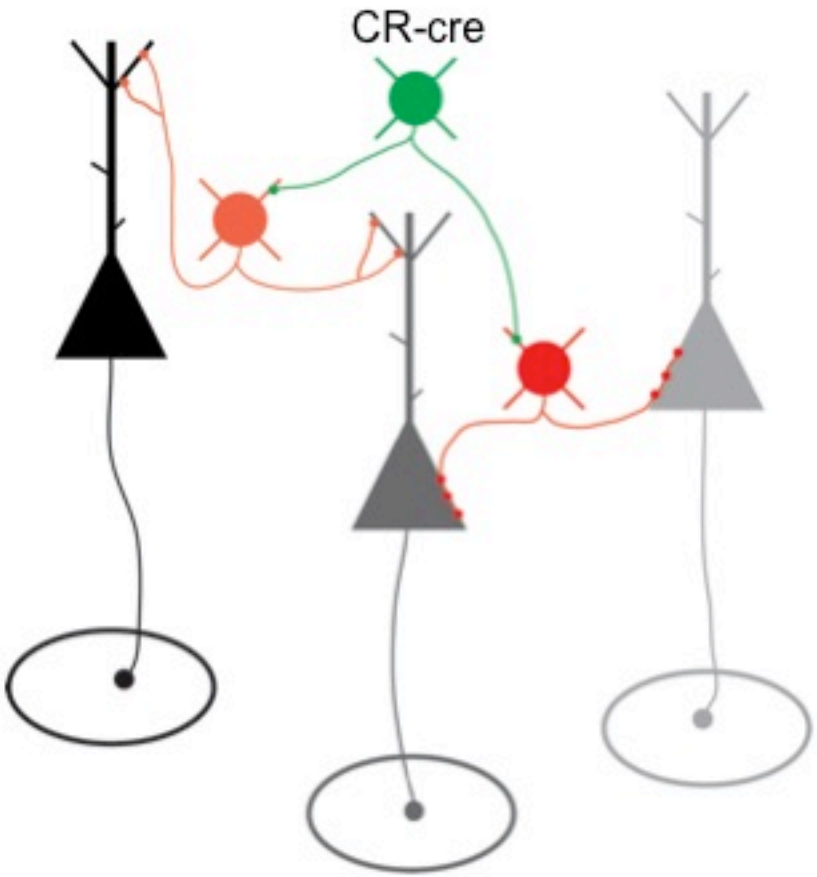


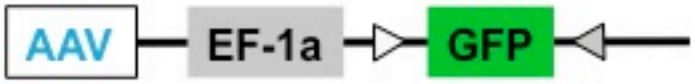
Interneuron identification based on marker expression



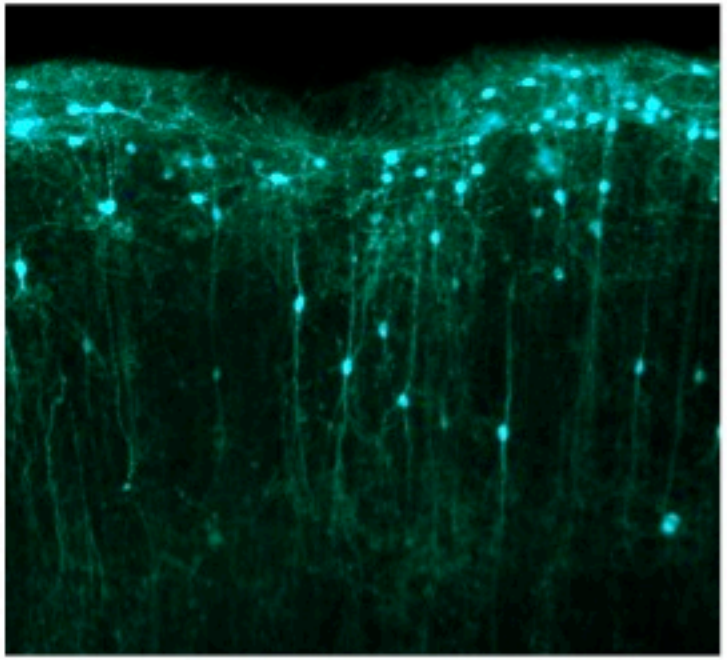


Interneuron identification based on marker expression

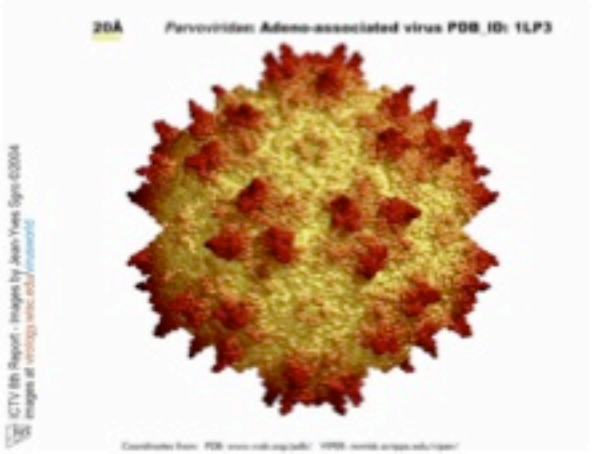




CR-cre / AAV-DIO-GFP

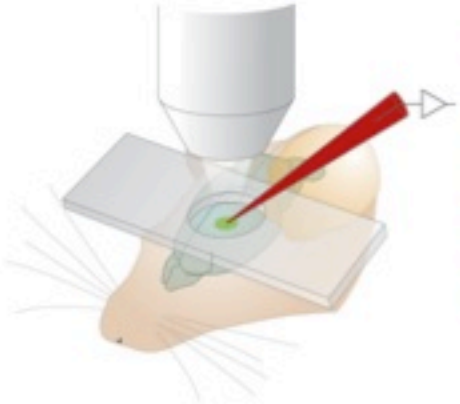


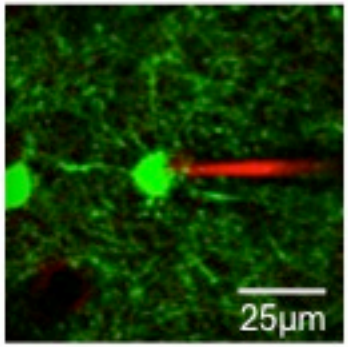
Chronic Ca^{2+} imaging: Adeno-associated vector transduction

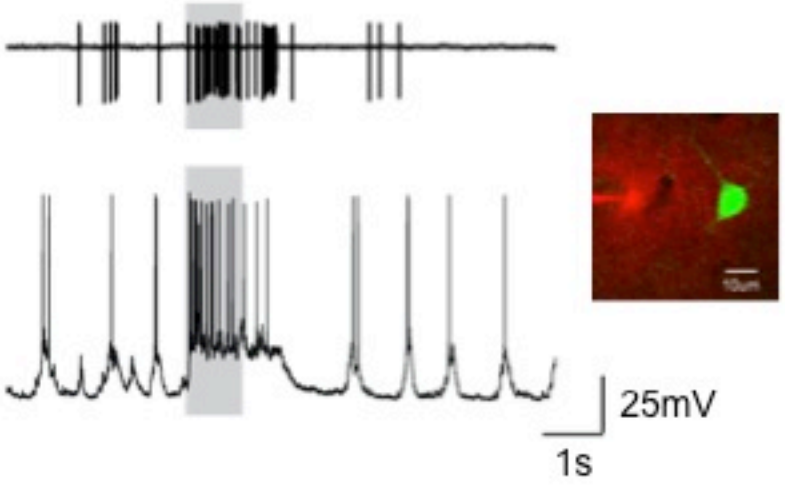


- **single-stranded DNA virus, relatively small capacity (~5kb)**
- **no pathogen, so very mild immune response and very little cytotoxicity**
- **non-enveloped > capsid mediates transduction**
- **relatively slow expression, but stable for years in postmitotic cells**
- **very little integration into host genome, i.e. little mutagenesis**
- **standardly produced with helper cell-lines, i.e. no contamination from helper viruses**

Using 2PM to targeted recordings to identified neurons

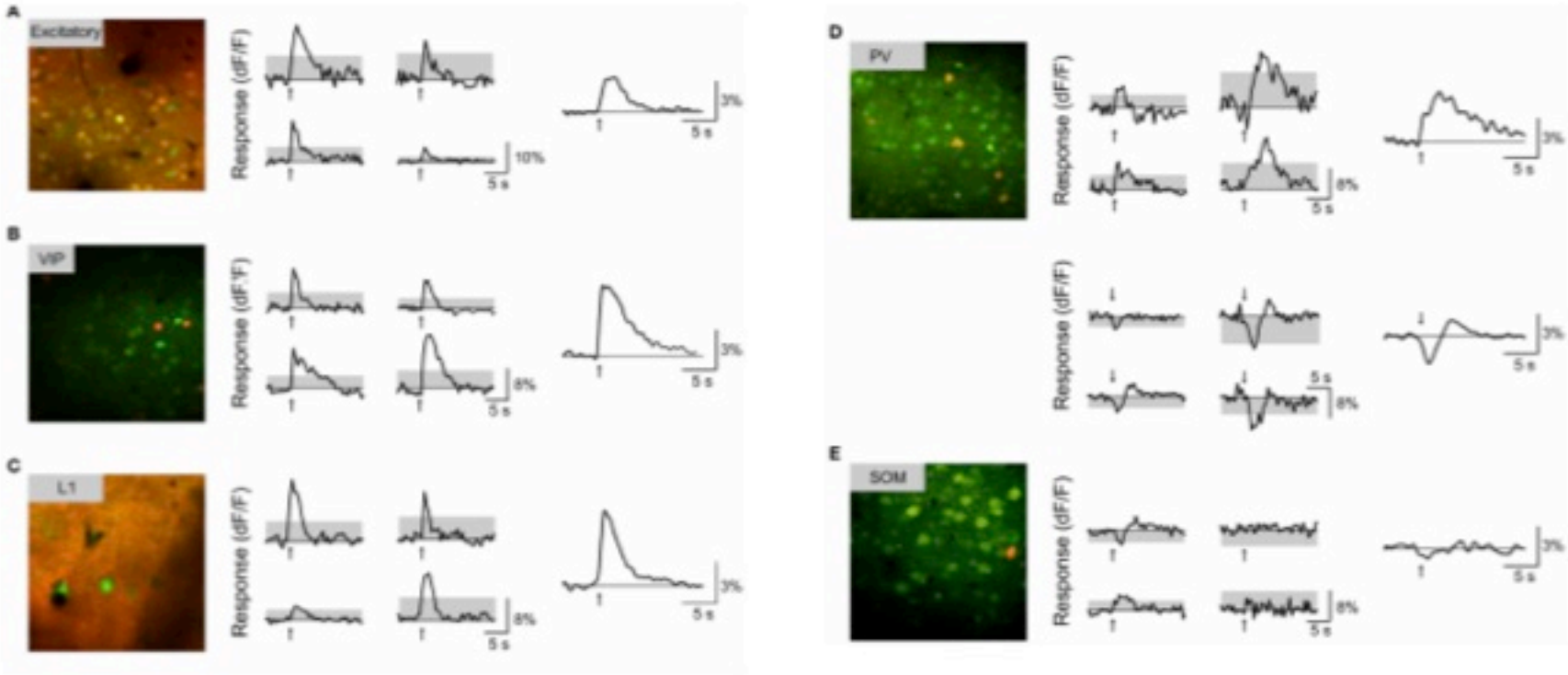






c.f. Komai *et al.*, 2006

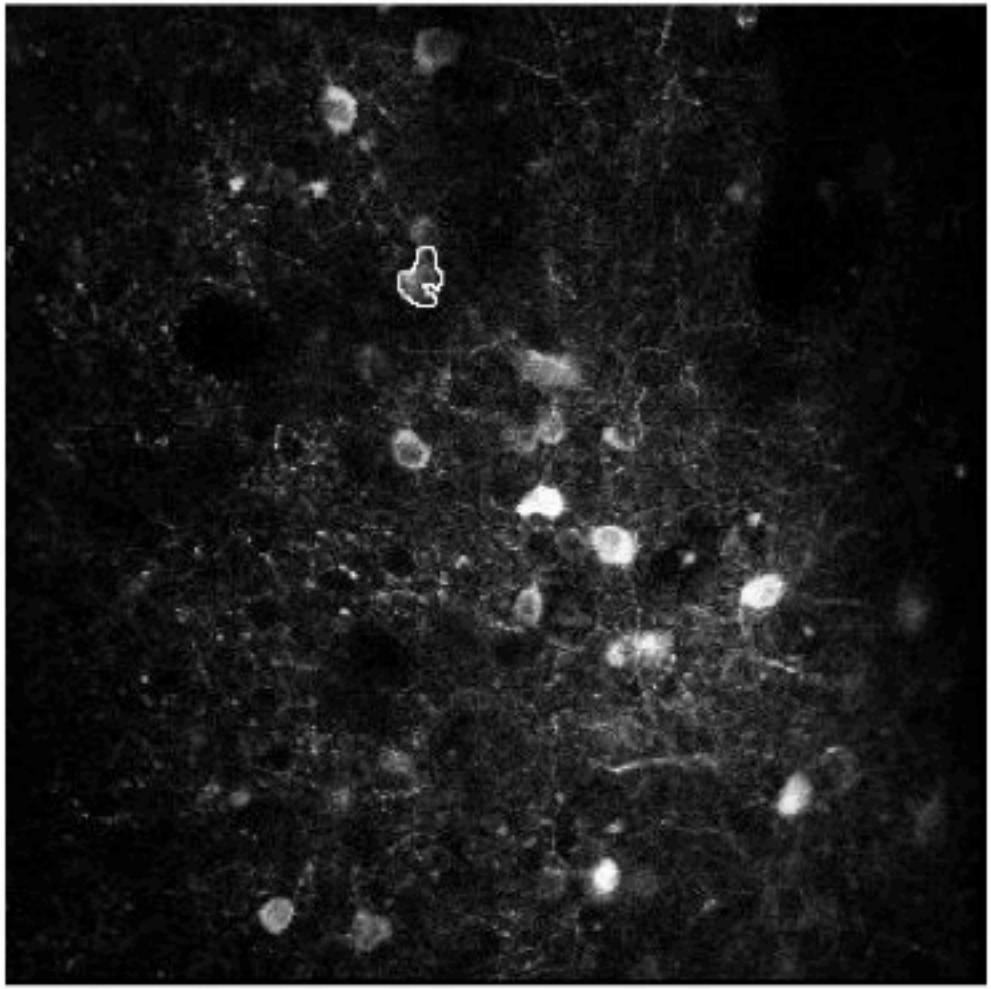
Ca^{2+} imaging of identified neurons with synthetic dye



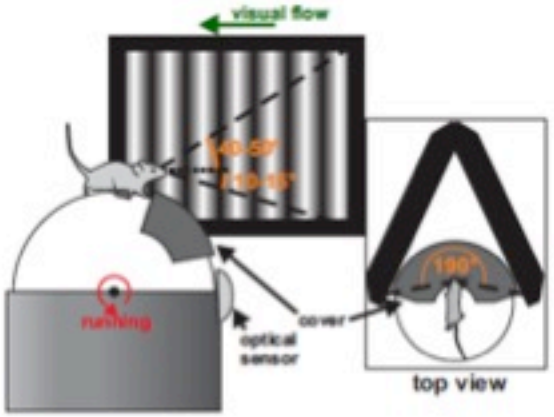
Alitto & Dan, 2013

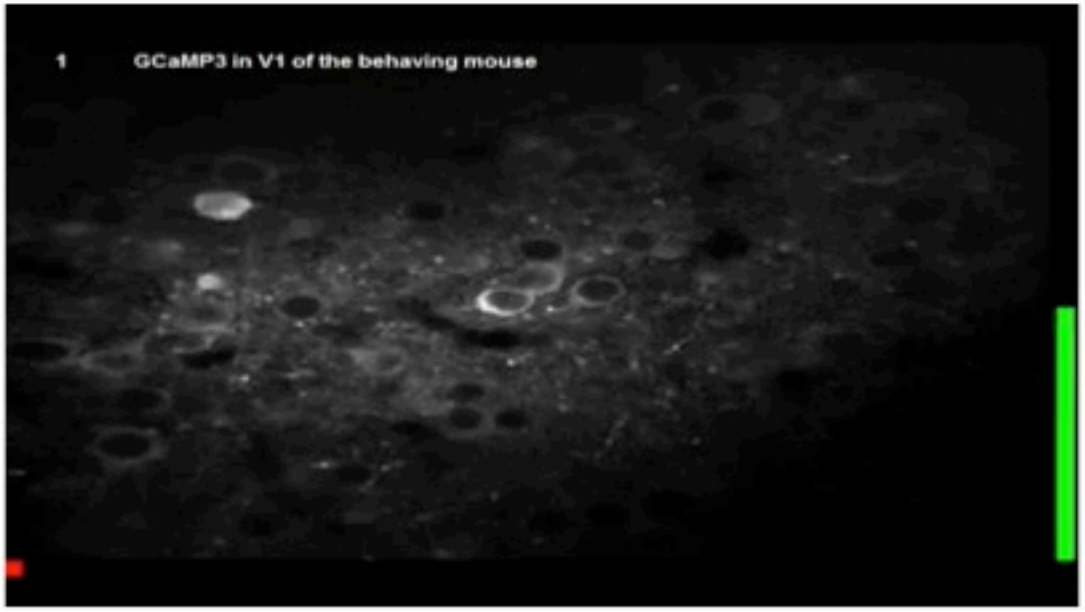
Ca^{2+} imaging of identified neurons in behaving mice with GCaMP6

GAD2-cre / AAV-DIO-GCaMP6s



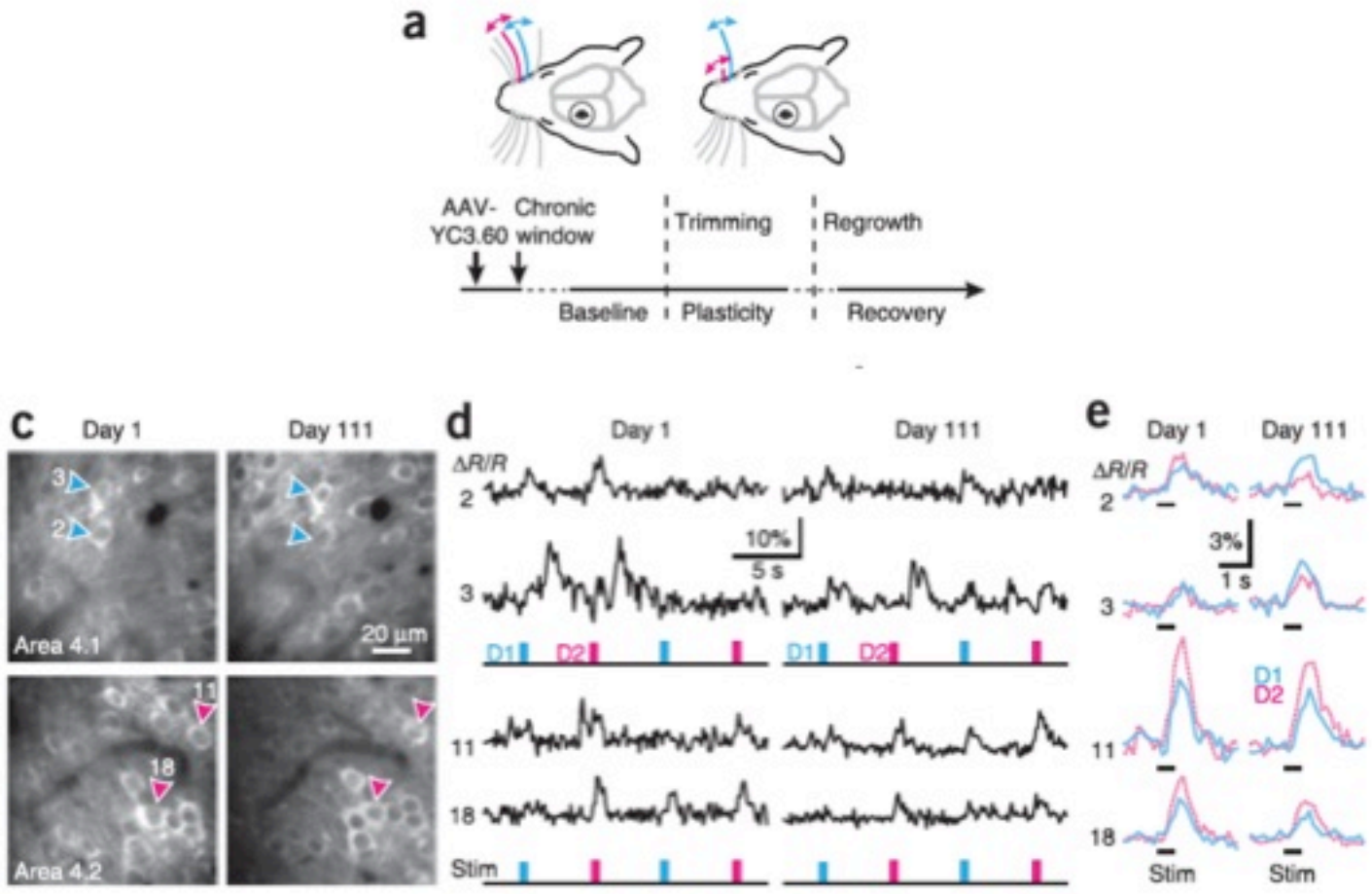
Ca^{2+} imaging of large populations independent of their activity





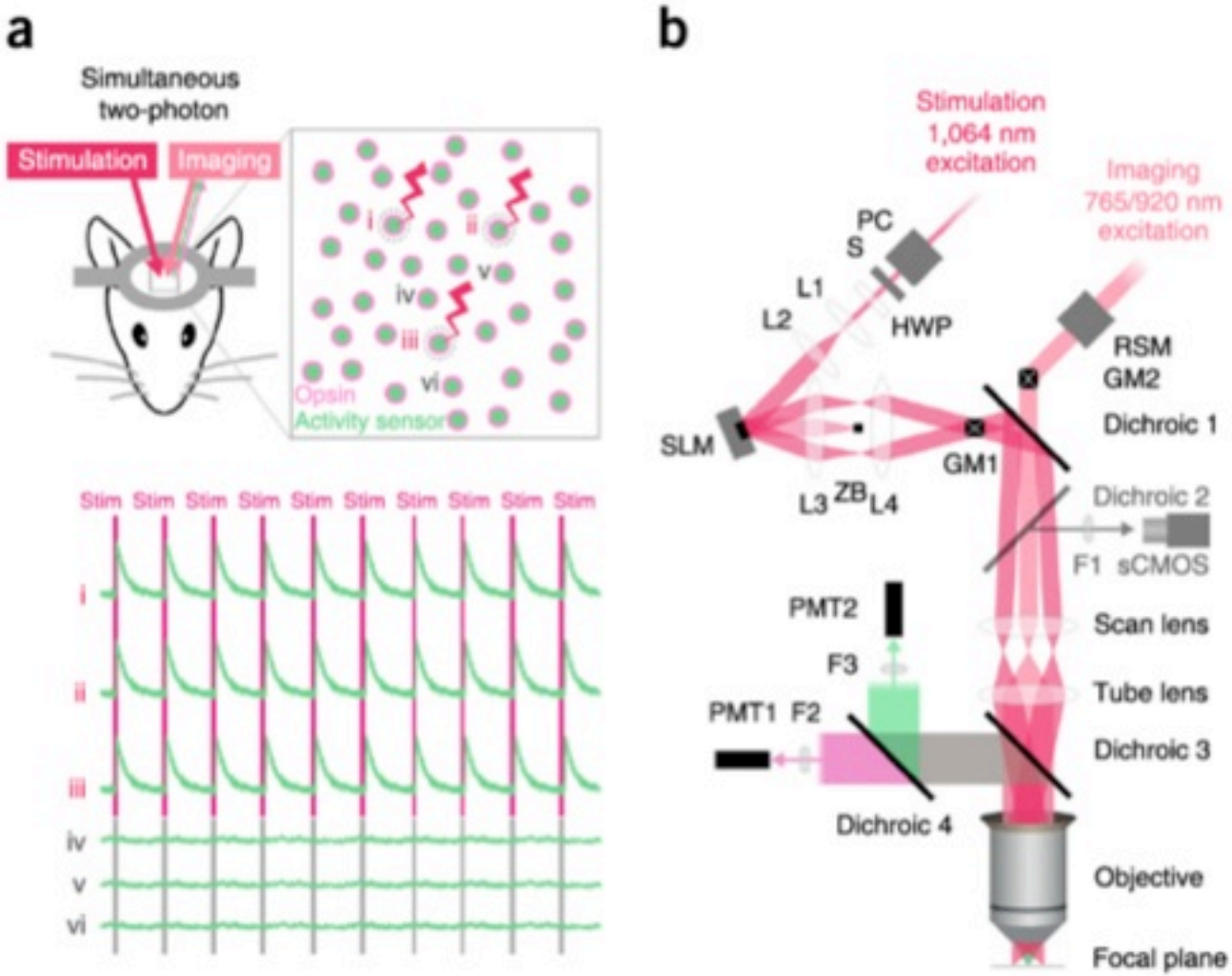
Keller *et al.*, 2012

Long-term Ca^{2+} imaging of large populations



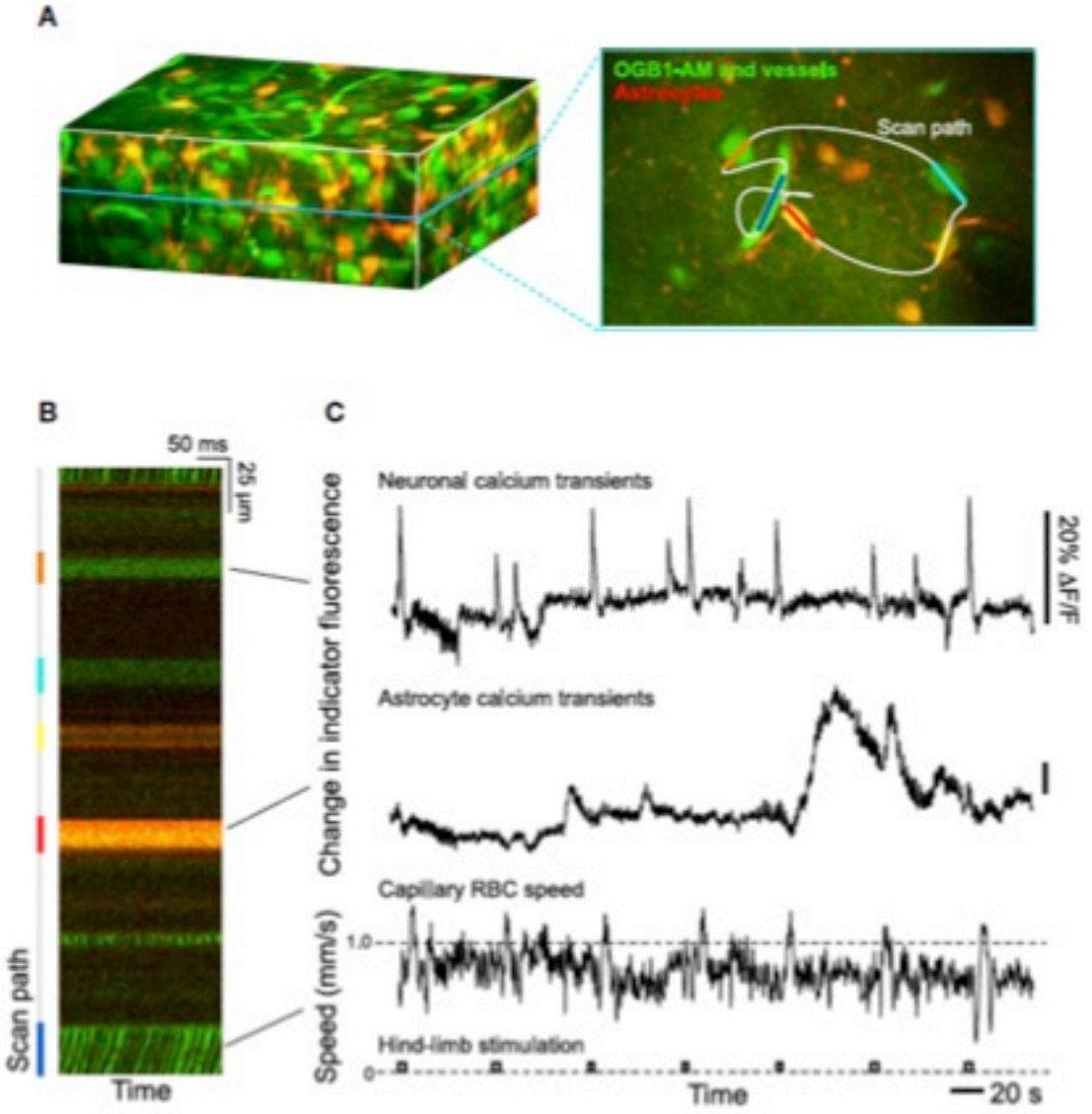
Margolis *et al.*, 2012

Combination with single-cell optogenetics



Packer *et al.*, 2015; c.f. Vaziri and Emiliani labs

Beyond the neuron: Imaging the neurovascular system



Kleinfeld *et al.*, 2011