SPPIN SAINTS-PERES Paris Institute for the Neurosciences

In vivo imaging in awake animals

Michael Graupner (PhD)

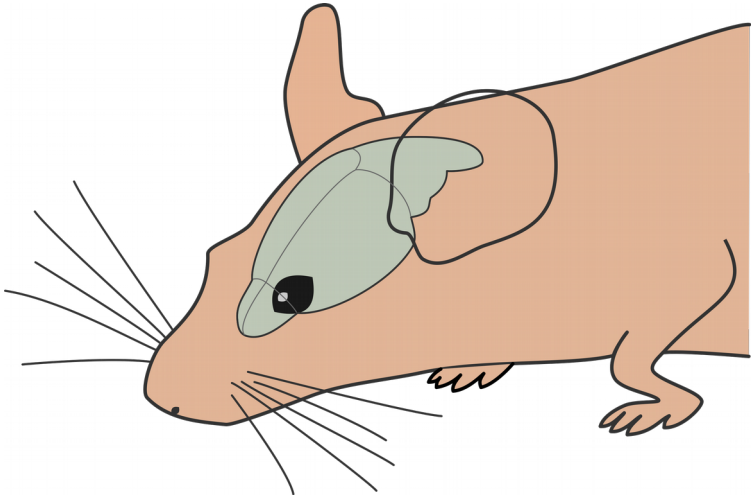
Saints-Pères Paris Institute for the Neurosciences

CNRS UMR 8003, Université Paris Descartes

slides on : https://www.biomedicale.parisdescartes.fr/~mgraupe/teaching.php

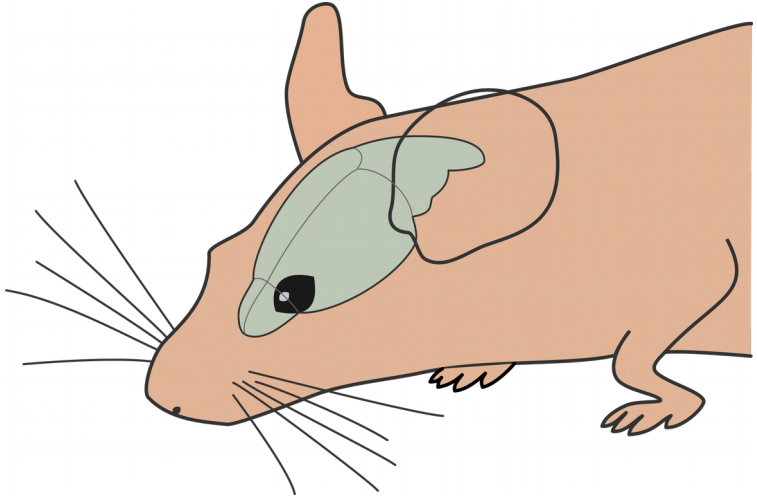
Aim

- study brain activity during relevant tasks – tasks which the brain has evolved and optimized to deal with
- explore brain function in its natural environment
- record (neural activity) from the brain of an *alive, awake* animal performing a task

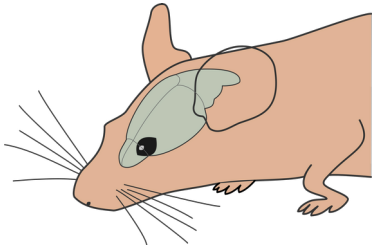


Challenges

- access region/neurons of interest
- assure animal's health and well-being
- make the animal perform a task
- perform stable recordings



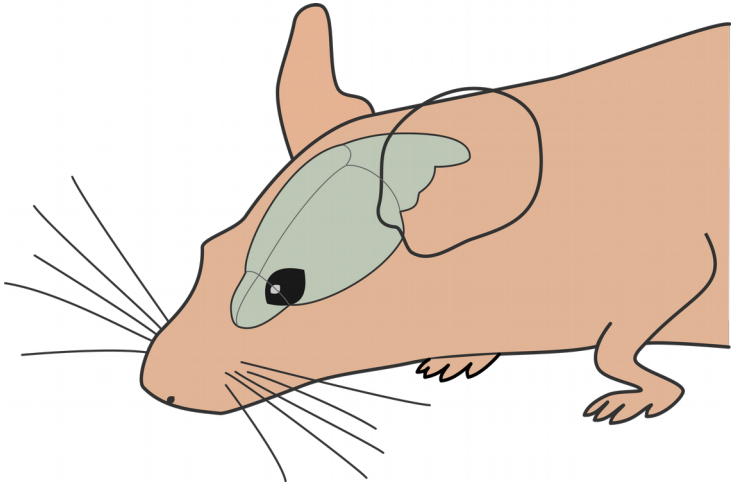
Outline of the talk



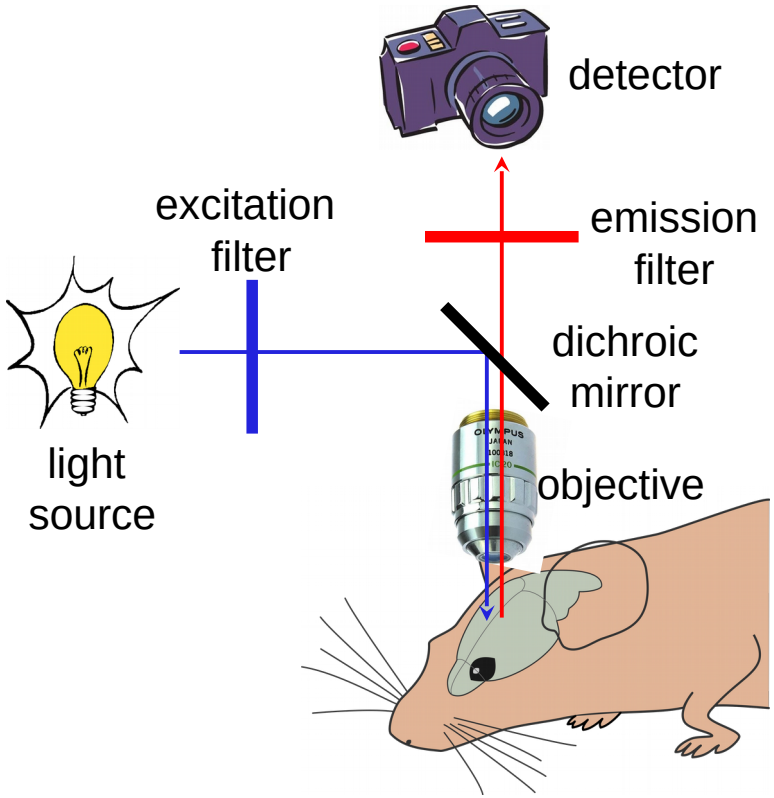
1. Basics of *in vivo* imaging
   - parts list for imaging experiment
   - 1- vs. 2-photon imaging
   - image reconstruction in 2-photon imaging
2. Considerations of *in vivo* imaging in awake animals
   - sensory modalities studied
   - practical implementation : optical access, head-fixed vs. 'freely' moving
   - virtual reality systems
   - movement artifacts
   - calcium vs. voltage imaging
3. Cerebellum and motor control

General parts list for *in vivo* imaging

Which general parts do we need if we want to record neural activity optically ?

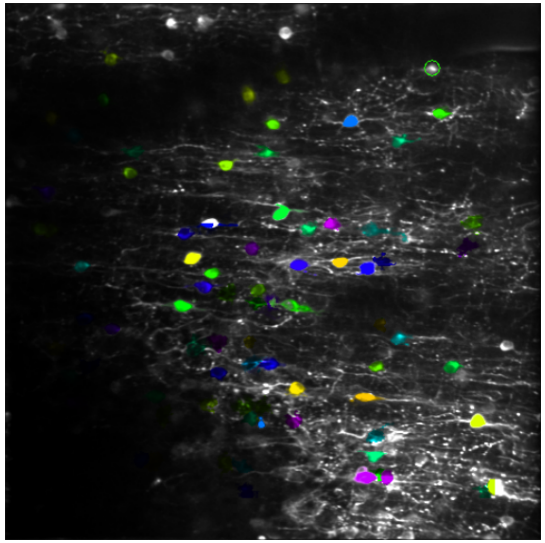


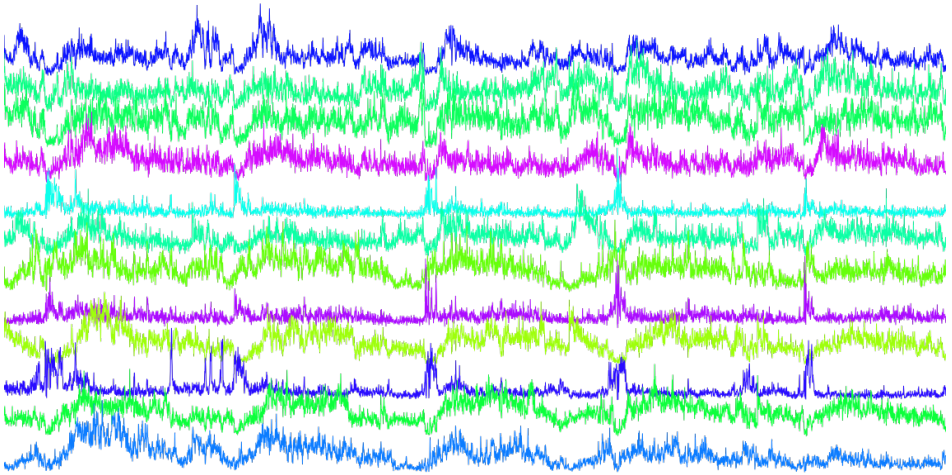
General parts list for *in vivo* imaging



- **Light source**: LED, laser, mercury vapor lamp,...
- **Excitation filter**: enables to select a specific excitation range.
- **Dichroic mirror**: reflects wavelengths that are under a cutoff value and transmit wavelengths above this value.
- **Objective** : focuses light on region of interest
- **Sample** : structure labeled with fluorophore
- **Emission filter**: enables to select fluorescent photons in a given range.
- **Detector**: camera, PMT, eye,...

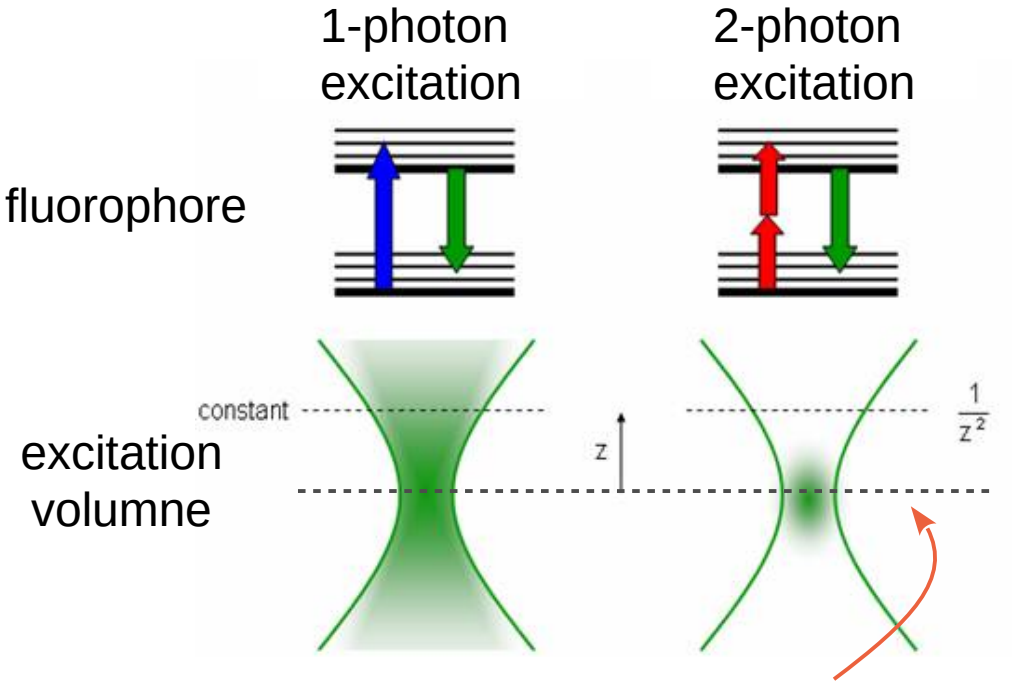
Current method of choice : Calcium imaging using GECIs





- Genetically encoded indicators (GECIs) can be targeted to specific neuron populations
- Non-invasive and repeatable means to measure neural activity from large populations of neurons

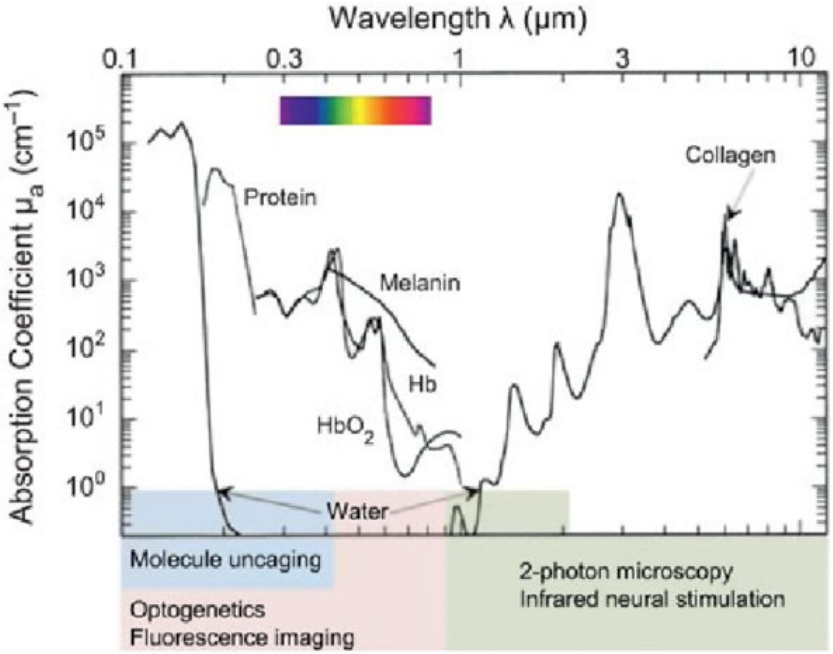
One photon vs. 2-photon fluorescence : resolution



[z is distance from focal plane]

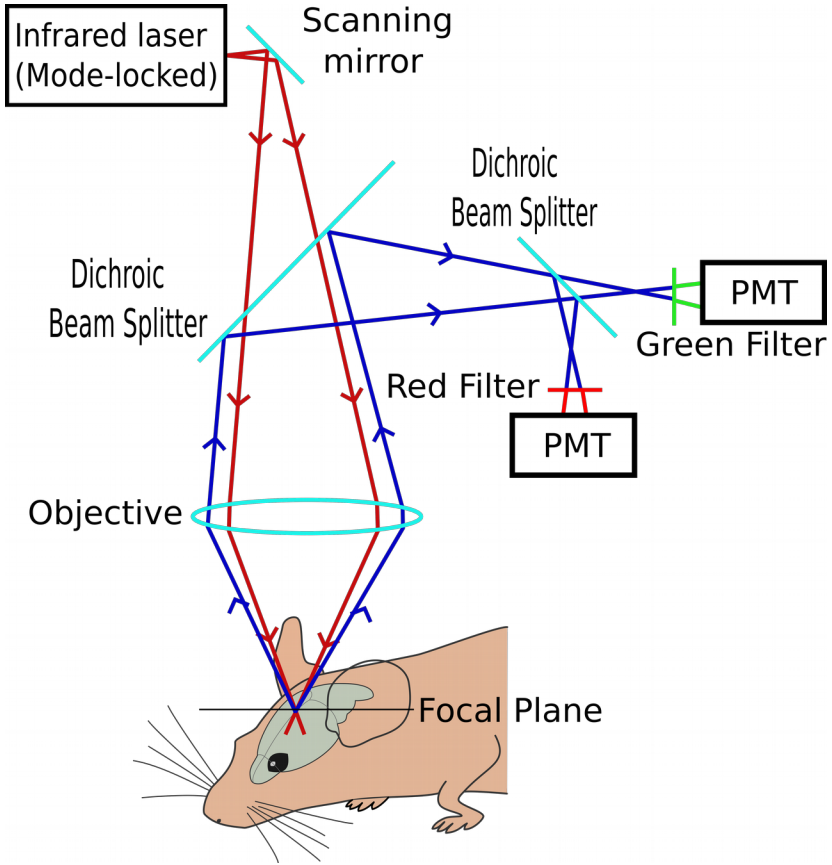
- excitation volume/fluorescence is confined to the focal center of the laser beam
- fluorescence falls off as ~$1/z^2$, while it falls off as $1/z$ with single photon excitation
  → 3D-imaging with out-of-focus background rejection similar to a confocal microscope
  → much higher spatial resolution can be achieved

One photon vs. 2-photon fluorescence : depth



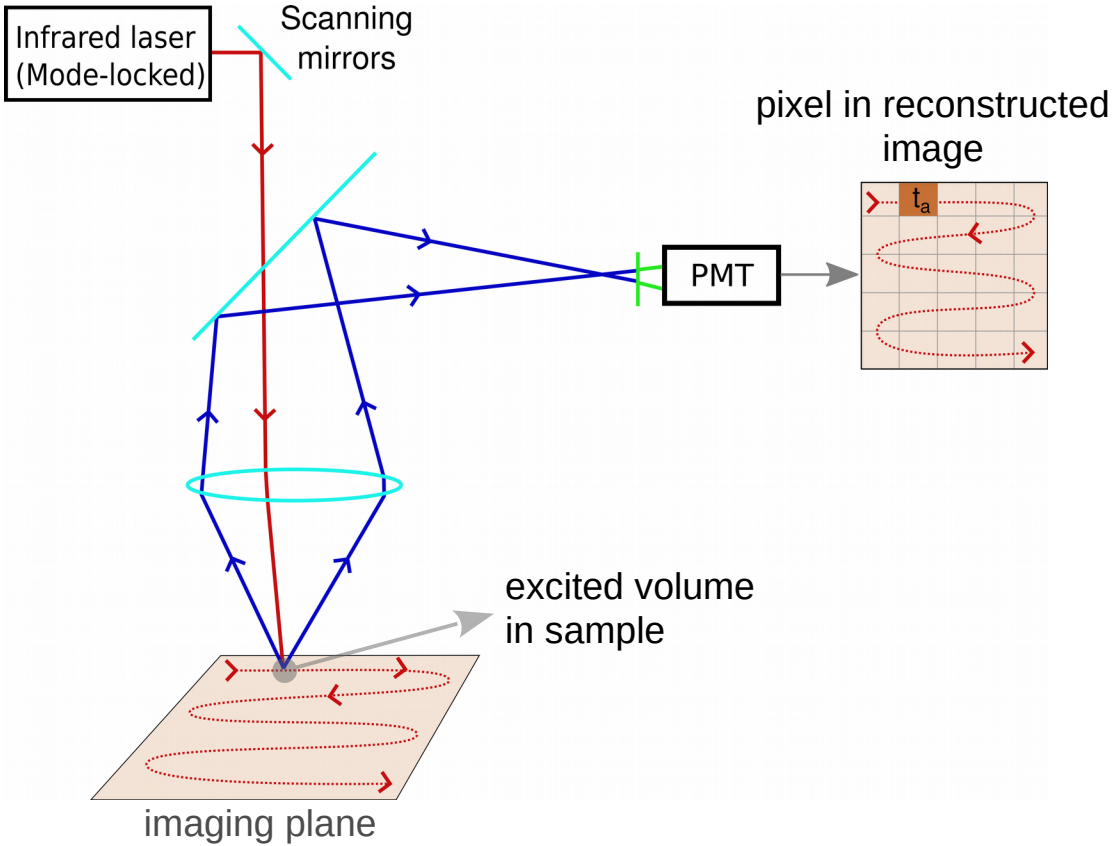
- infrared light can penetrate deeper in biological tissue due to little absorption
- commonly used: titanium-sapphire tunable laser of wavelength 650 nm-1100 nm

Modified parts list for 2-photon *in vivo* imaging



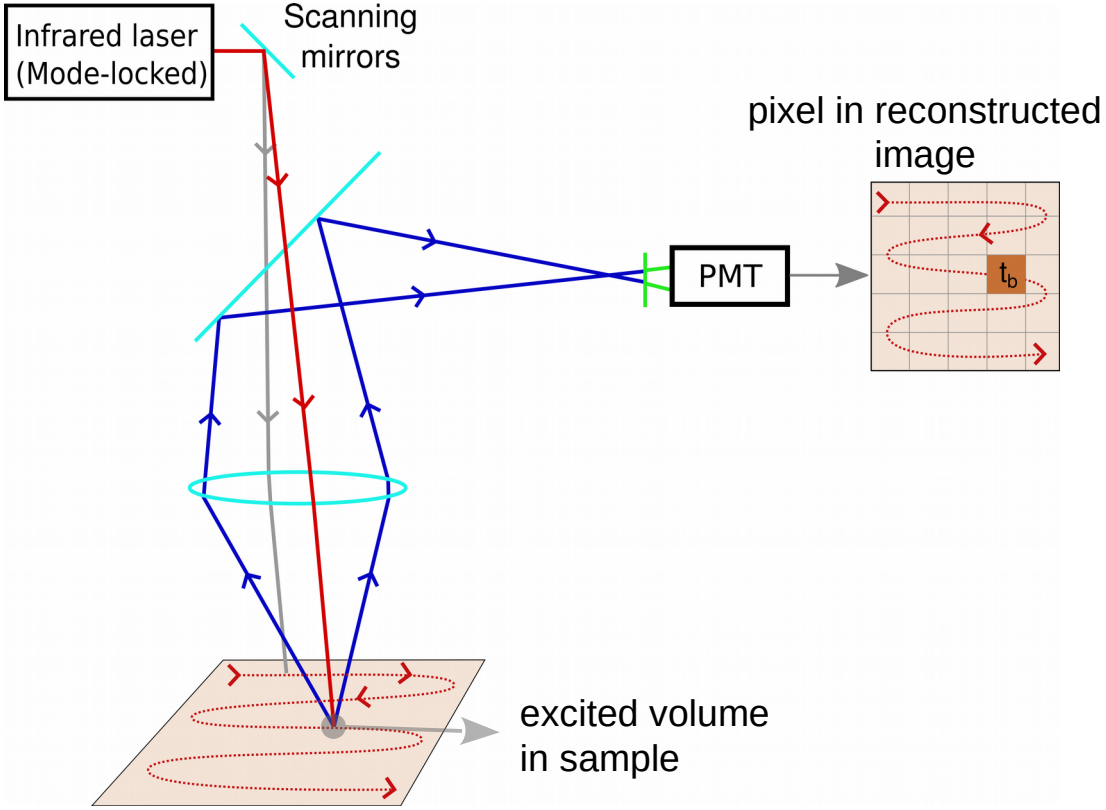
- **Light source**: laser producing light pulses on the order of femtoseconds (10^{-15} s)
- **Excitation filter**: not required since laser produces single wavelength
- **Scanning mirrors:** directs/scans the laser beam over the sample
- **Dichroic mirror**
- **Objective**: focuses light on region of interest
- **Sample**: structure labeled with fluorophore
- **Emission filter**: enables to select fluorescent photons in a given range.
- **Detector**: PMT

Image construction in 2-photon microscopy



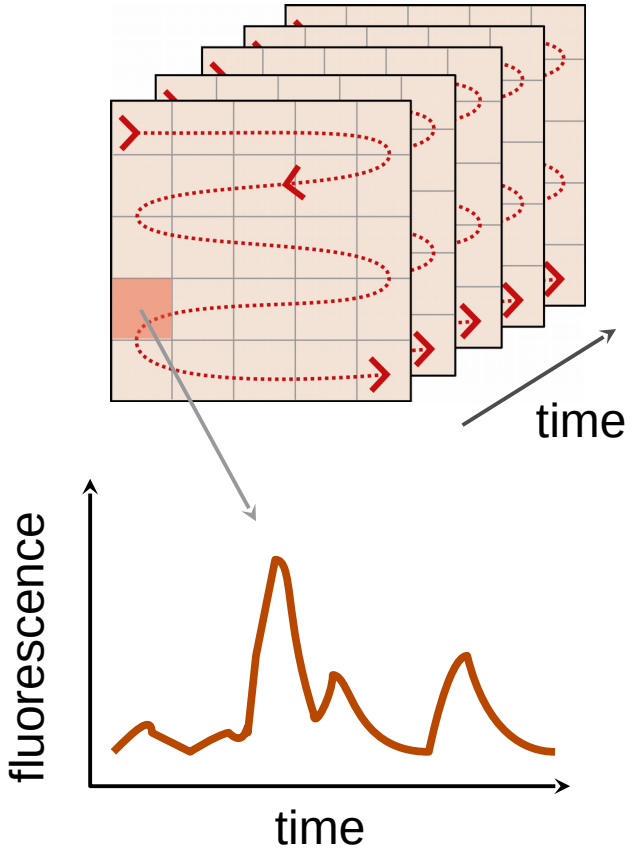
- there exists a spatial mapping between **mirror position, point of laser in the sample** and **image space**
- at time-point t_a, the laser-light excites a specific volume in the sample
- all fluorescent light at time-point t_a is mapped to the pixel linked to the location of the laser-light at the same moment in time

Image construction in 2-photon microscopy



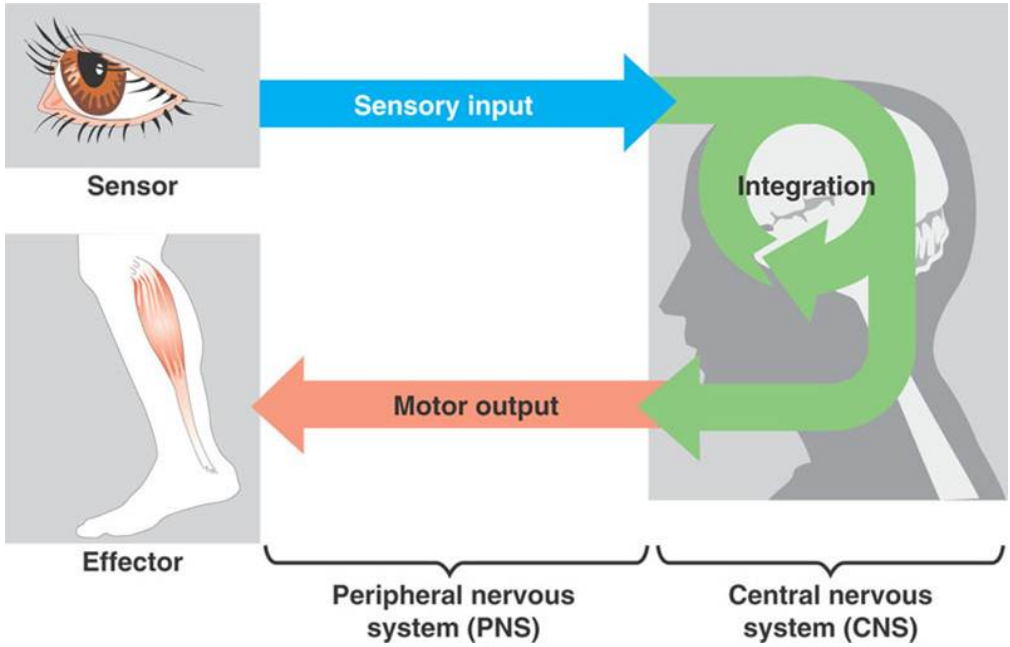
- at time-point t_b, the laser-light excites another volume in the sample
- all fluorescent light at time-point t_b is mapped to the corresponding pixel

Image construction in 2-photon microscopy



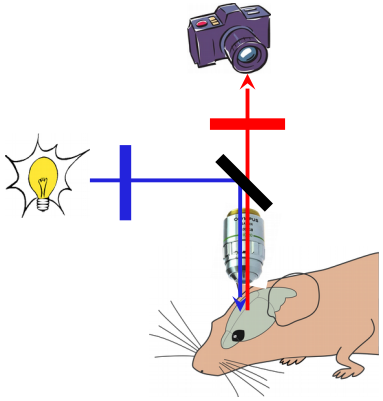
- the scan of the image plane is repeated many times which provides the temporal fluorescent trace for a given point in space
- repetition rate of the scan determines frame rate (typically 30 Hz)

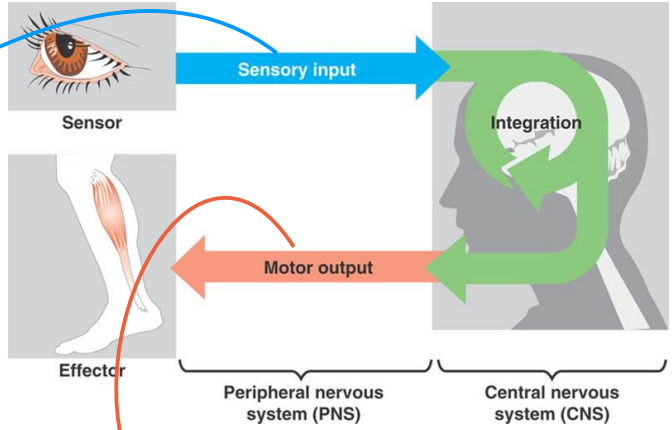
Rational behind *in vivo* experiments



- **goal** : naturalistic behaviors, where one's actions determine sensory stimulation
- **initially** : *in vivo* approaches focused on sensory perception (passive stimulation of single sensory modality)
- **however** : sensorimtor processing varies with behavioral state/output
- **interactive setting** : study sensoriomotor interactions with the outside world

Feasibility of *in vivo* imaging experiments

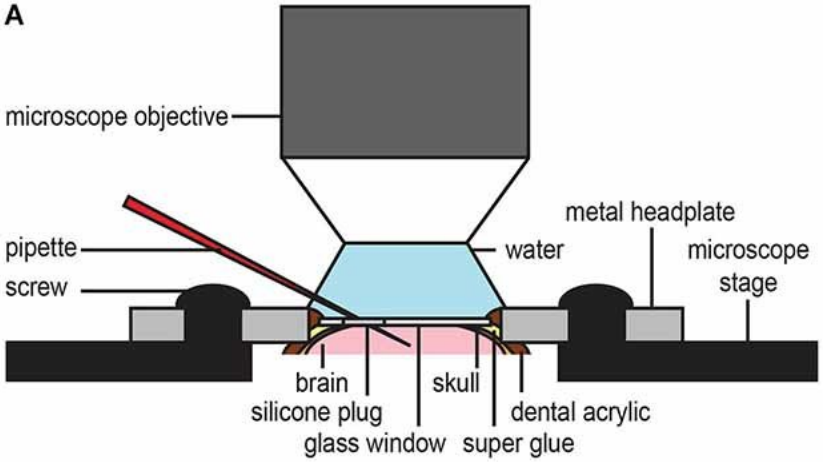


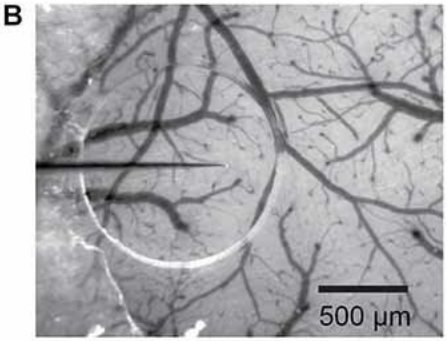


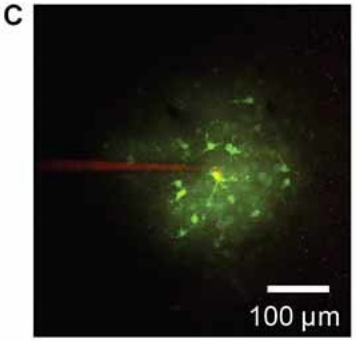
- **sensory stimuli :**
  - easy to implement : touch (whisker), vision, smell, taste, sound
  - difficult : vision, equilibrium (vestibular)

- **motor output** :
  - easy : licking, paw/arm movement, gaze, whisking
  - difficult : locomotion

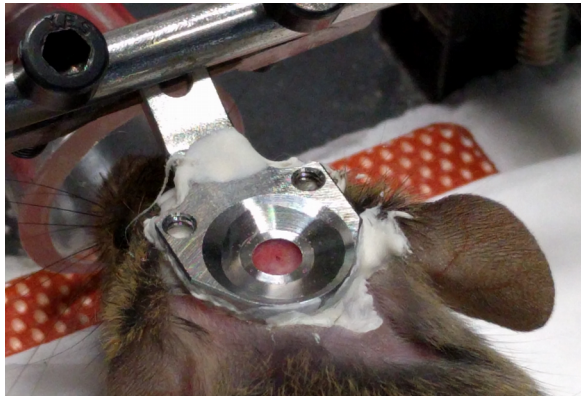
Optical access through chronic window



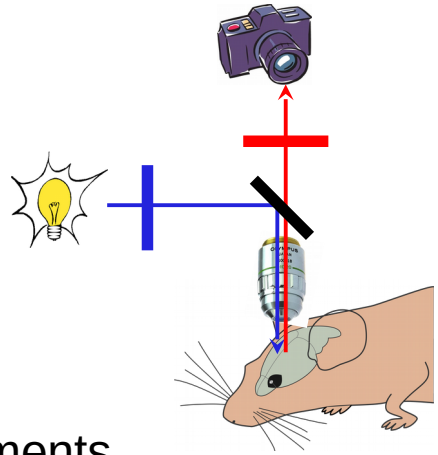




- Transparent window implanted in place of skull over region of interest
- bone thinning can provide sufficient visibility
- access port can allow for additional electrode access



Assure stability btw. imaging system and ROI



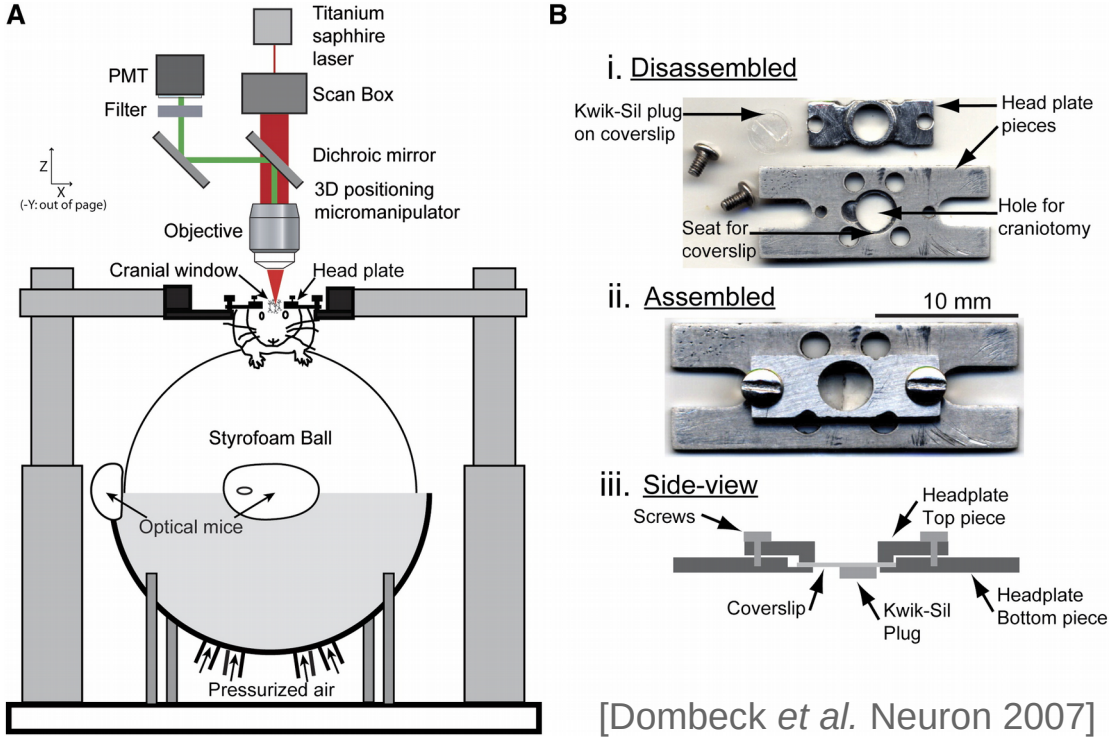
1) Minimize relative movements between animal to be imaged and the microscope

→ fix the animal head under the microscope

2) Place (parts of) microscope on the head of the animal, i.e., microscope moves with the animal

→ miniaturize imaging system

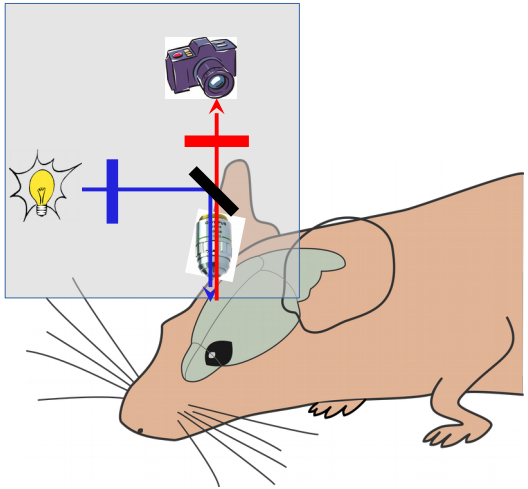
Most 2-p imaging experiments use head-fixation



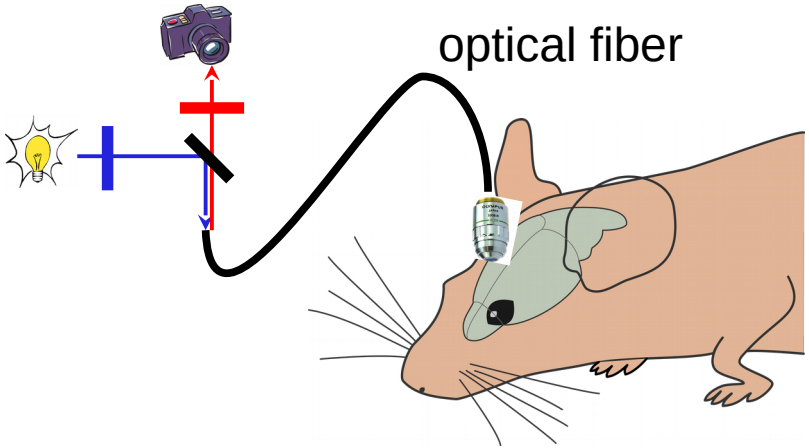
[Dombeck *et al.* Neuron 2007]

- Minimizes relative movements between animal – to be imaged – and the microscope
- adapter – headplate – is implanted on the animal's head to allow for solid and repeated fixation in the experimental setup
- allows to study sensorimotor integration for many sensorimotor modalities

'Freely' moving animal solutions



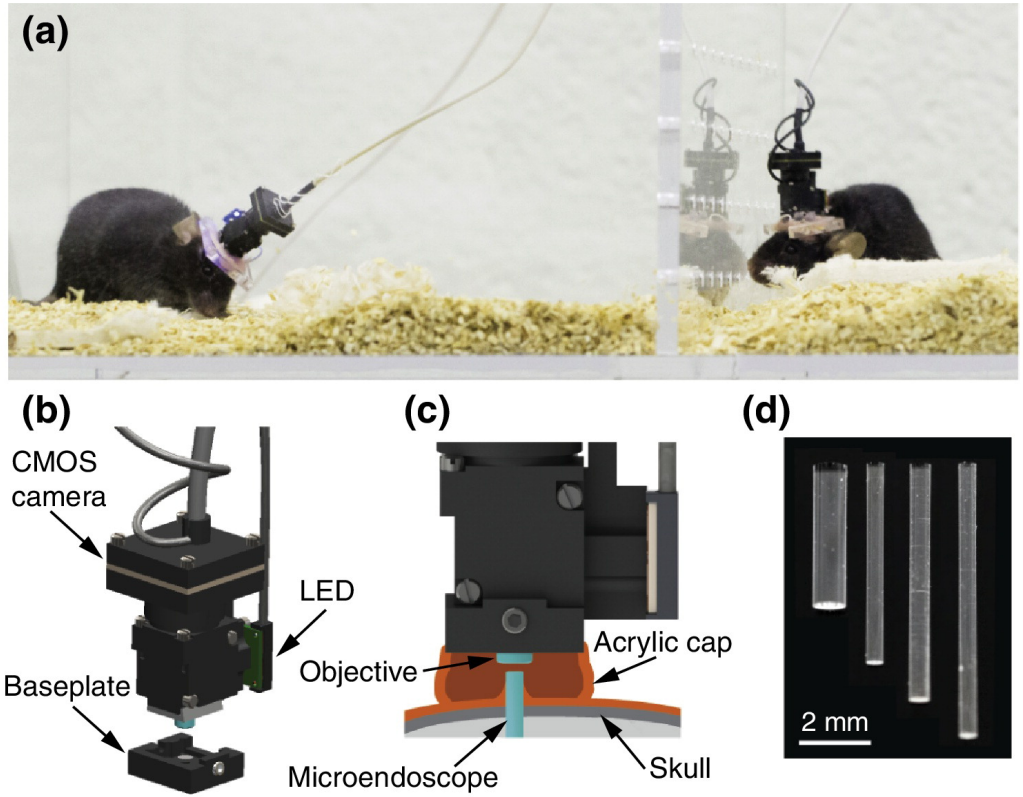
- miniaturized microscope mounted on animals head
- feasible for epifluorescence imaging



- flexible optical fiber connects light source/detector and animal-mounted optics
- allows for 2-photon imaging in 'freely' moving animals

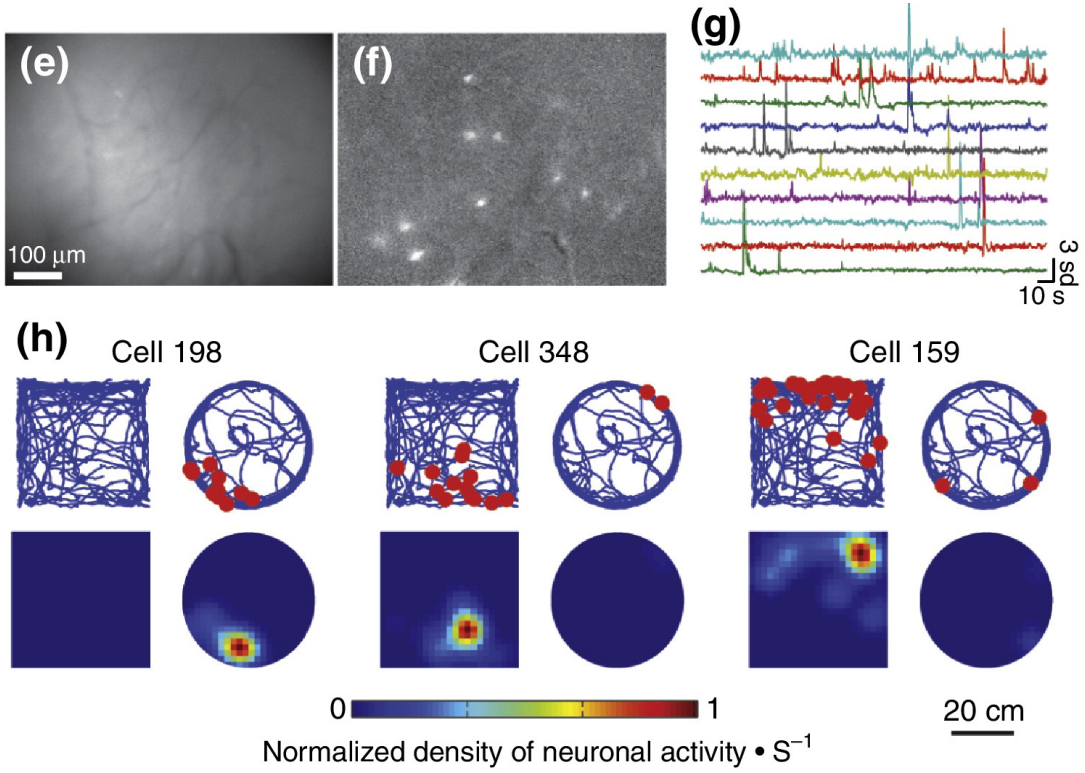
Head-mounted wide-filed epifluorescence

weight ~ 2g



[Ziv & Ghosh, *Current Opinion in Neurobiol* 2015]

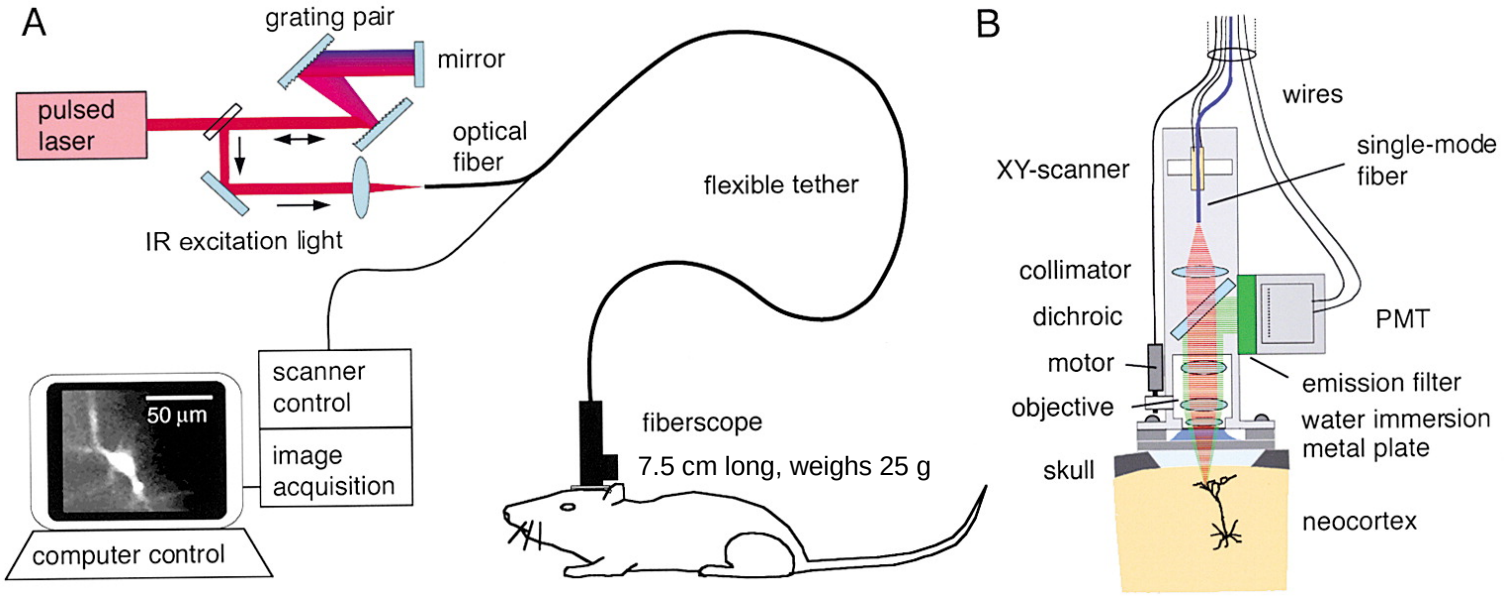
Hippocampal Ca dynamcis in behaving mice



[Ziv & Ghosh, *Current Opinion in Neurobiol* 2015]

- epifluorescence imaging of pyramidal cells in CA1 region of the hippocampus
- cells in this region feature place-cells : cell which fire when animal enter a particular place in environment

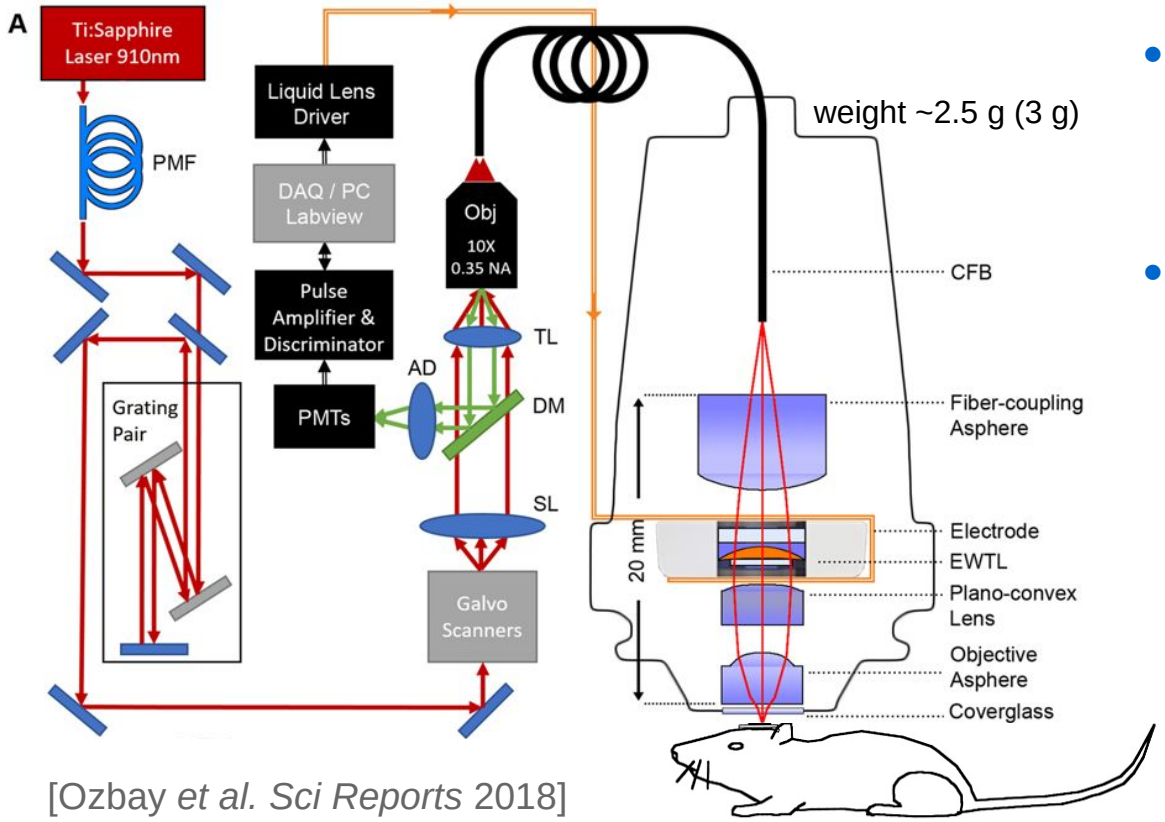
2p-laser scanning fiber-coupled microscope: 1



[Helmchen et al. *Neuron* 2001]

- light source at remote location from the animal
- scanning mirrors and detector in fiberscope on the animal's head
- too heavy and bulky for small animal applications

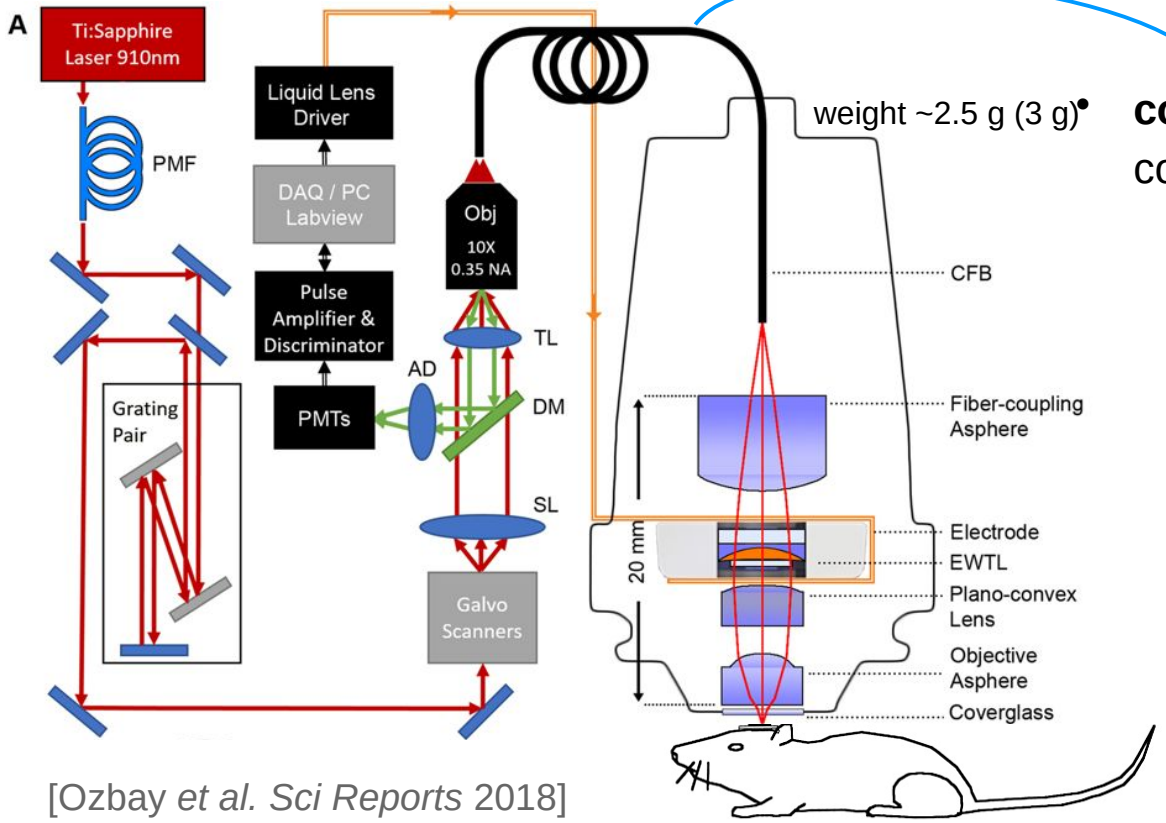
2p-laser scanning fiber-coupled microscope: 2



[Ozbay *et al. Sci Reports* 2018]

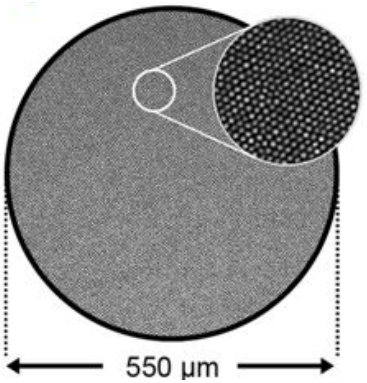
- light source, scanning mirrors and detector at remote location from the animal
- excitation and emission light transmitted through coherent fiber bundle: preserves spatial information of excitation

2p-laser scanning fiber-coupled microscope: 2



[Ozbay *et al. Sci Reports* 2018]

coherent fiber bundle : ~15,000 cores, core diameter ~2.9 µm, spacing ~4.5 µm

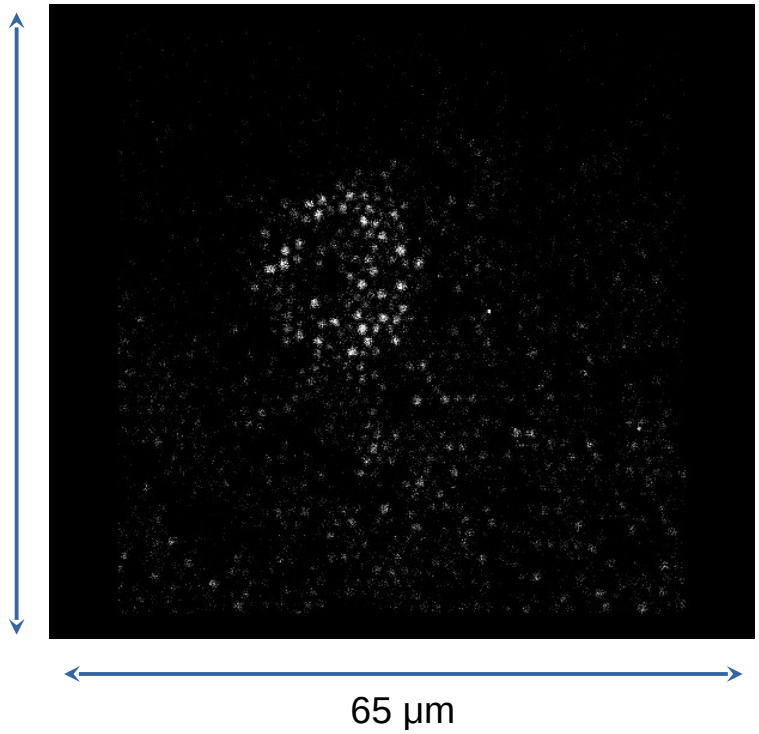


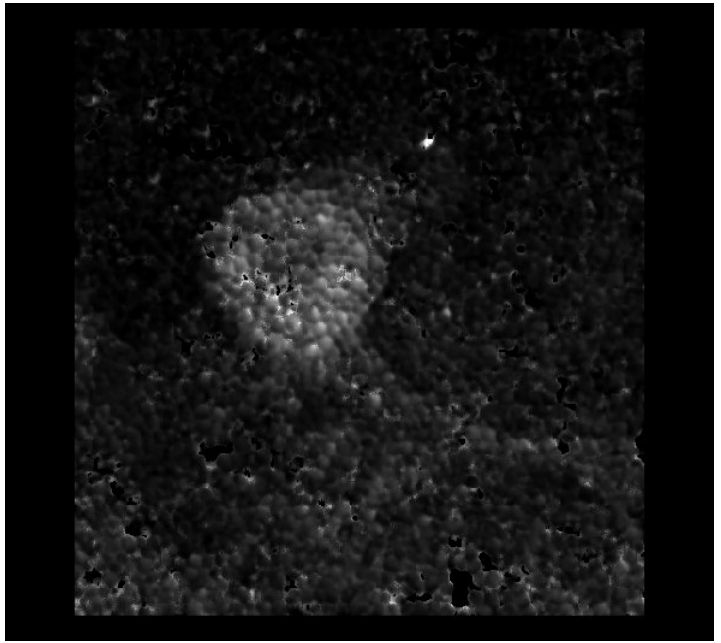
2p-laser scanning fiber-coupled microscope: 2

Video

[**ad** : see M2 internship project with Desdemona Fricker and myself]

Each fiber forms a pixel in the recorded image

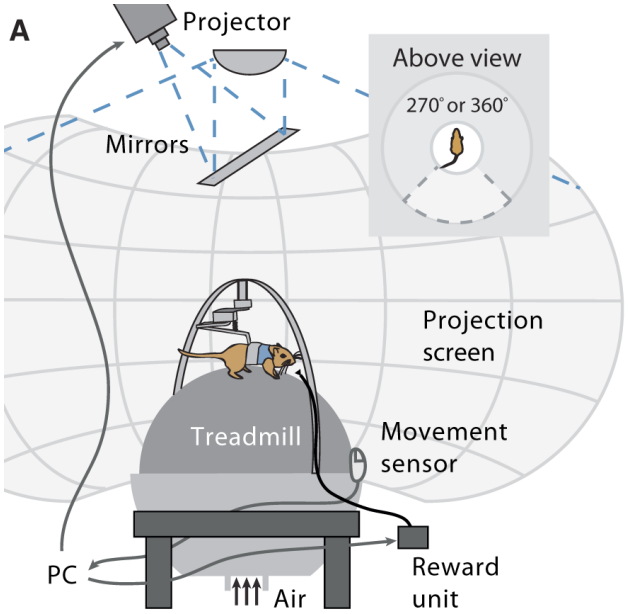


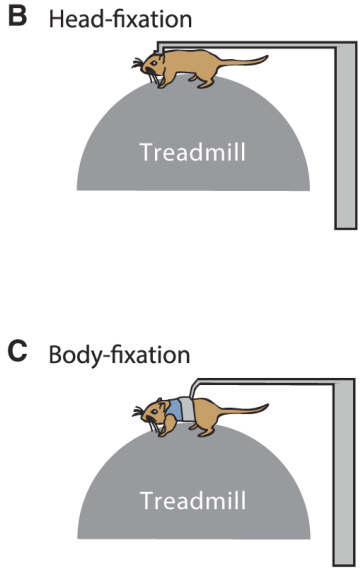


- image of a labelled Purkinje cells through the multi-core fiber

Virtual reality systems

1. Visual virtual reality



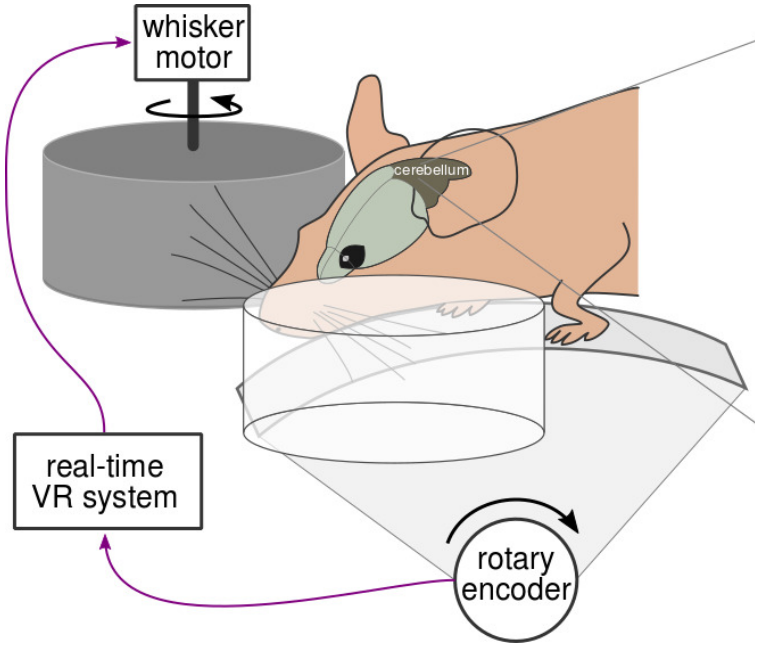


[Thurley & Ayaz, *Current Zoology* 2017]

- creating a sensorimotor loop between locomotion and visual feedback (i.e. optical flow linked to movement)
- animal is restrained, animals paw movement is recorded and controls sensory stimulation
- https://www.youtube.com/watch?v=1DJOTEDBA2c

Virtual reality systems

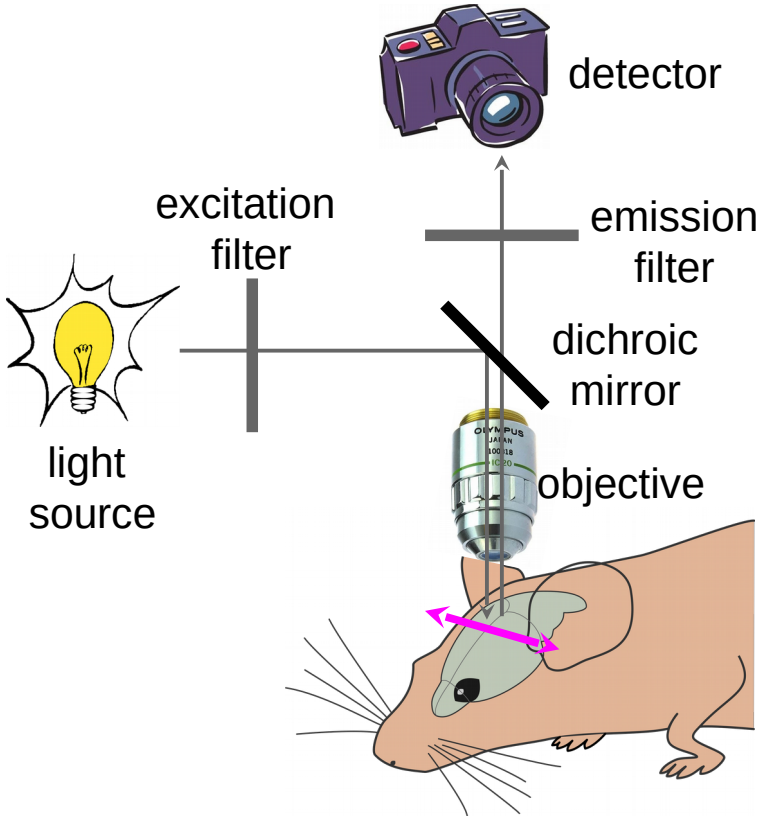
2. Tactile virtual reality



[Stell *unpublished* 2019]

- creating a sensorimotor loop between locomotion and tactile feedback (i.e. mechanic stimulation linked to movement)
- animal is restrained, animals paw movement is recorded and controls rotation of whisker wheels

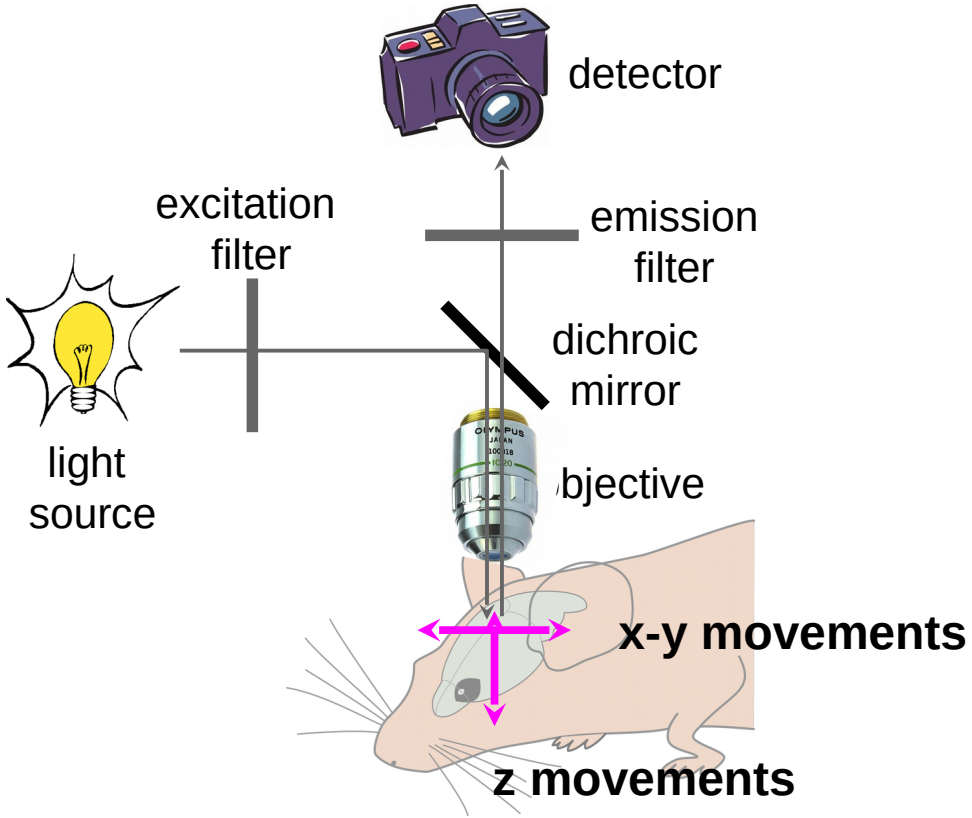
Challenge : movement artifacts



Reasons for relative movements

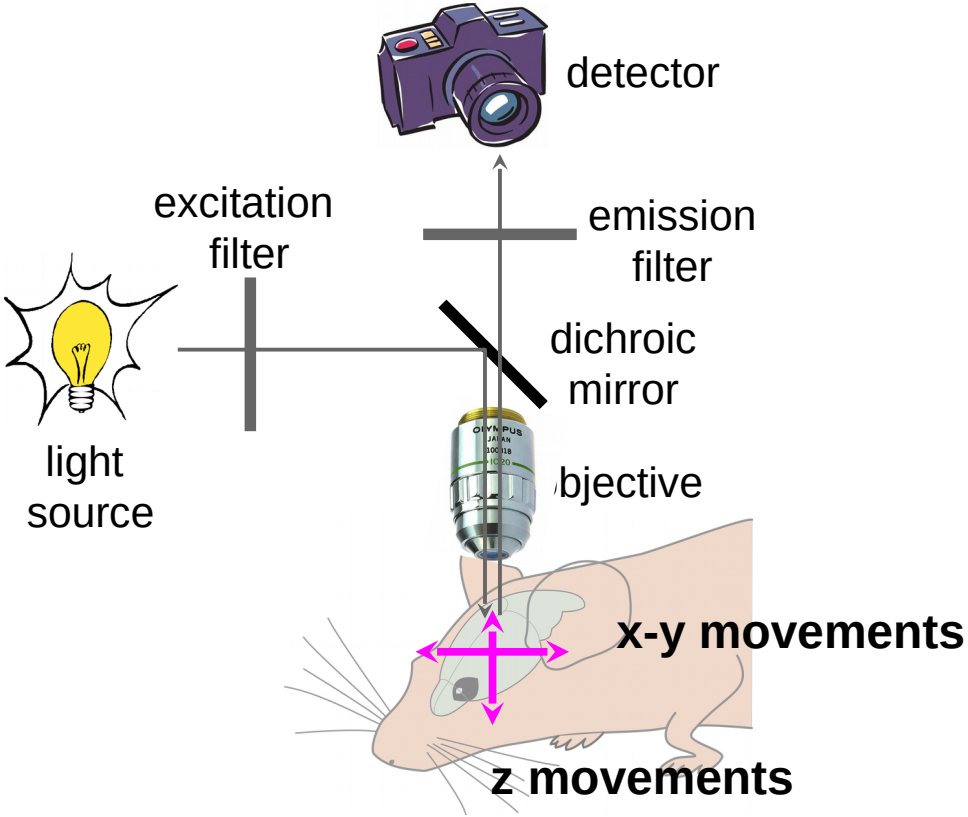
- imperfect head-fixation
- movement from respiration
- movement from heartbeat/blood flow
- animal movement translated to the brain

Image registration : tackle movement artifacts



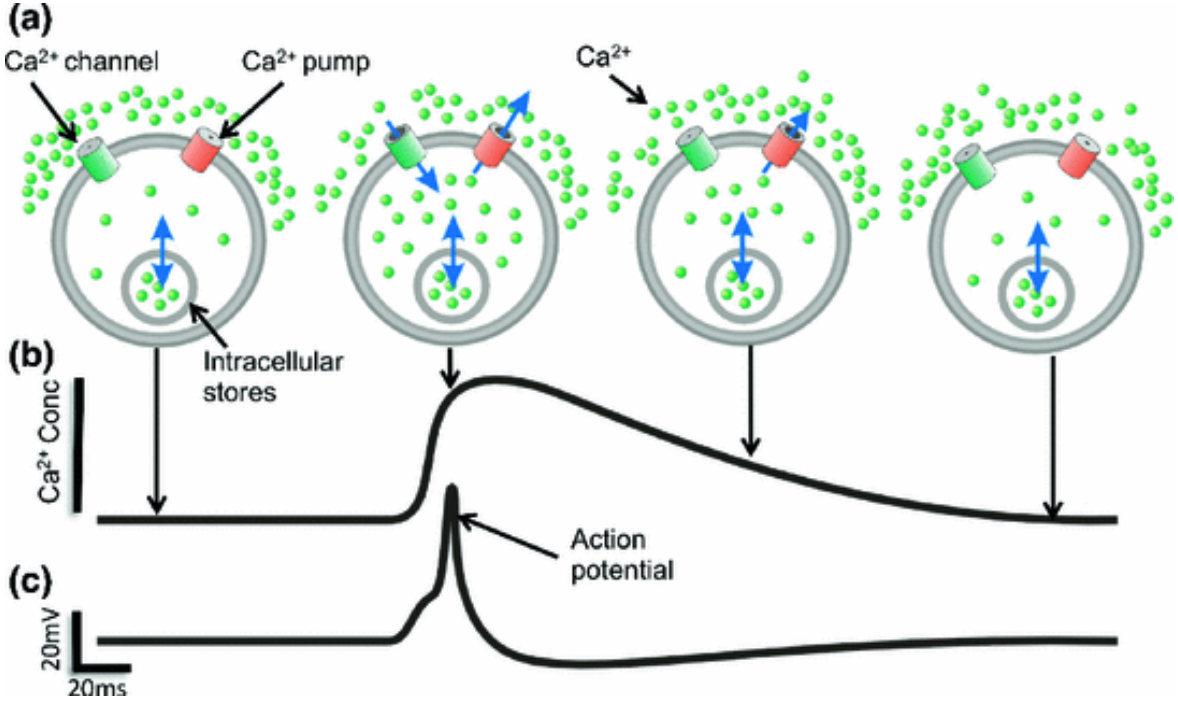
- x-y displacements – displacement within the focal plane – can be corrected in post-hoc analysis
- z-displacements cannot be corrected

Image registration : tackle movement artifacts



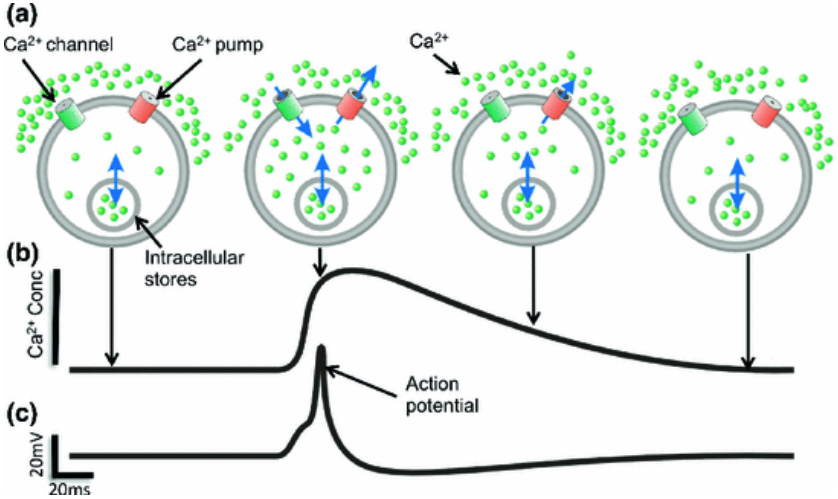
- x-y displacements – displacement within the focal plane – can be corrected in post-hoc analysis
- z-displacements cannot be corrected
- https://www.youtube.com/watch?v=tlDLn1SmuTY

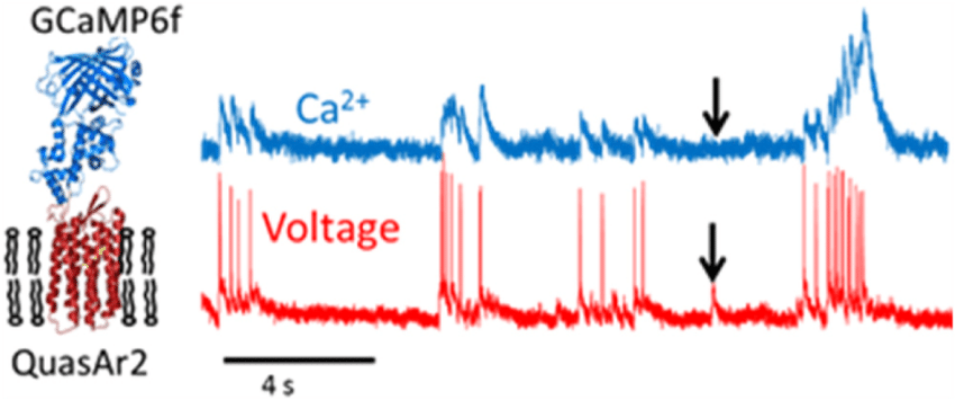
Calcium vs. voltage imaging



- membrane potential depolarizations induce calcium transients
- calcium is a proxy of neural activity
- calcium transients are much longer (~100 ms) than membrane potential depolarizations (~2 ms)

Calcium vs. voltage imaging





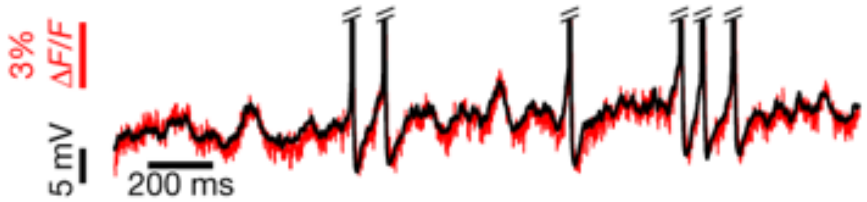
Calcium imaging

- GECIs report calcium trace
- Uses nuclear calcium signal as proxy for neuronal activity

Voltage imaging

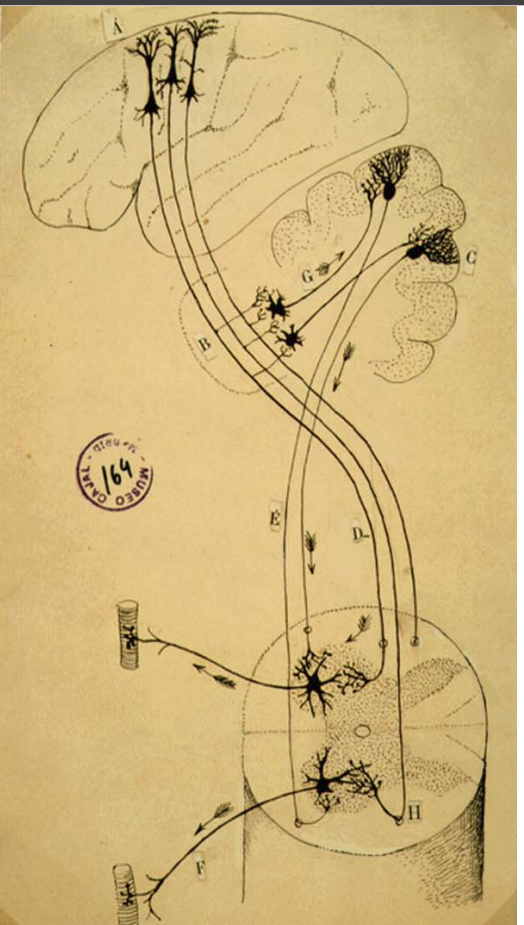
- GEVIs (e.g. QuasAr, ASAP) report directly transmembrane voltage
- located in cell membrane

Challenges of voltage imaging



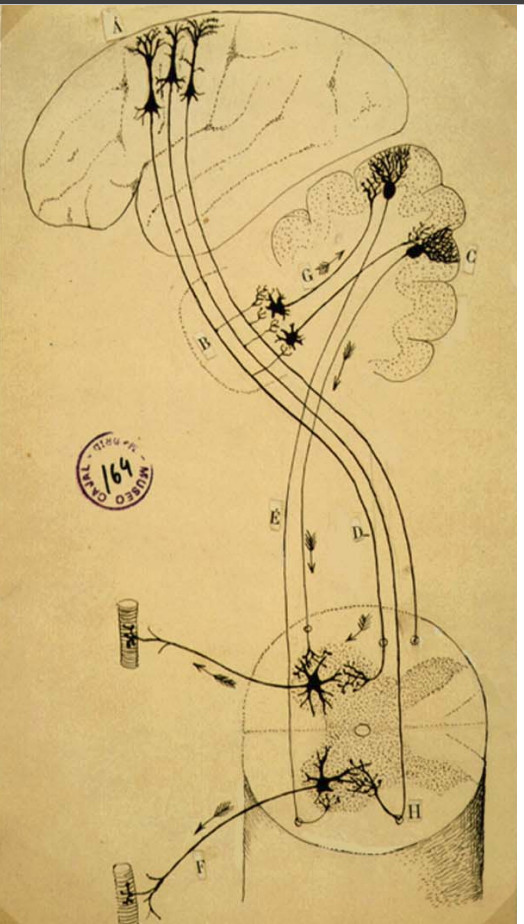
- Requires high-speed microscopes due to short duration of action potentials (~2 ms)
- Photobleaching due to constant illumination
- Requires good membrane trafficking of fluorophores
- Requires exceptionally bright fluorescence due to fewer fluorescent proteins in FOV (volumne vs. surface)

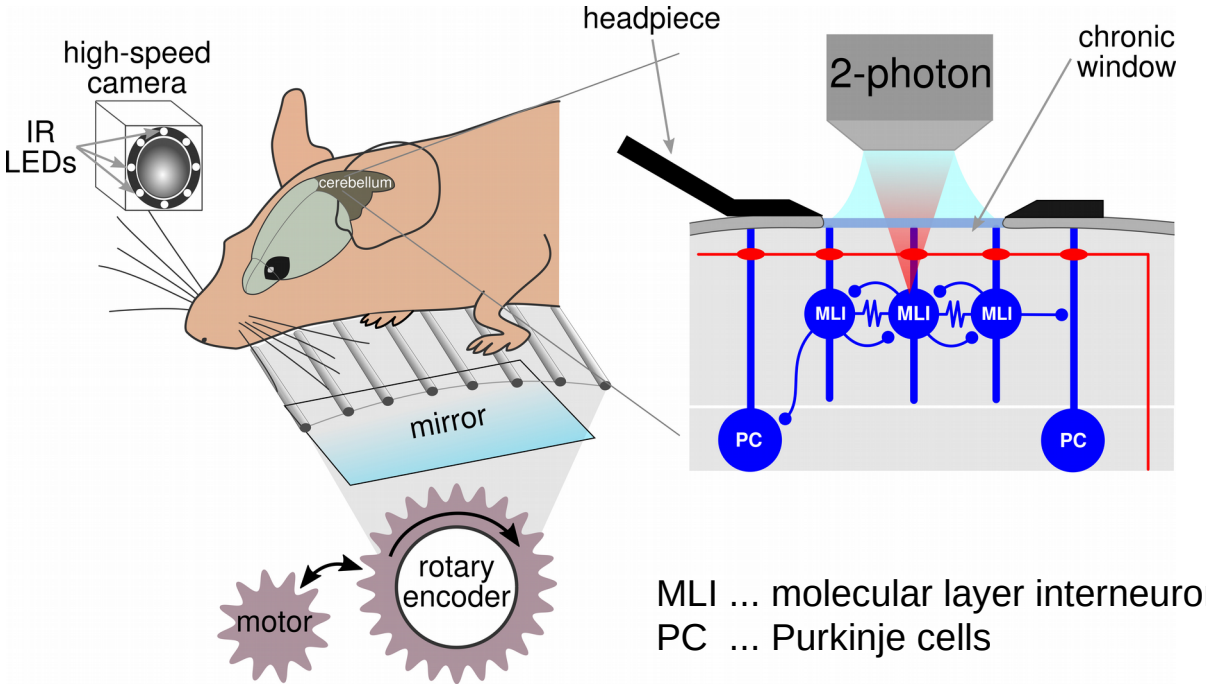
My project : Cerebellum and locomotion



- motor neurons in the spinal cord receive inputs from motor cortex and the cerebellum
- neurons in the cerebellum encode motor variables
- role of the cerebellum in motor control unclear

Role of cerebellar interneurons in complex motor task



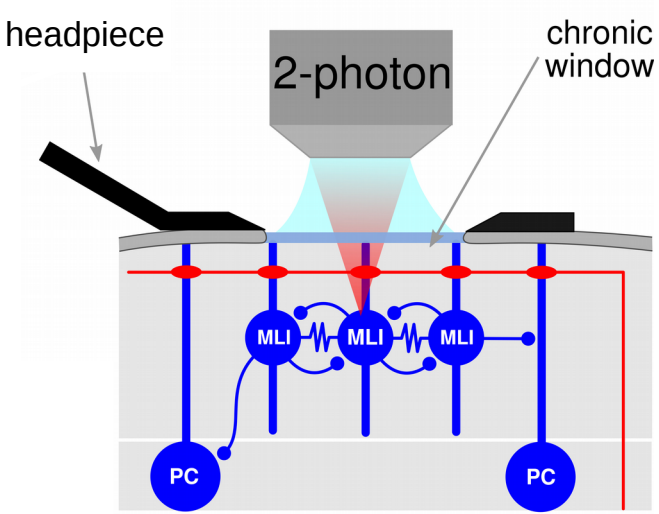


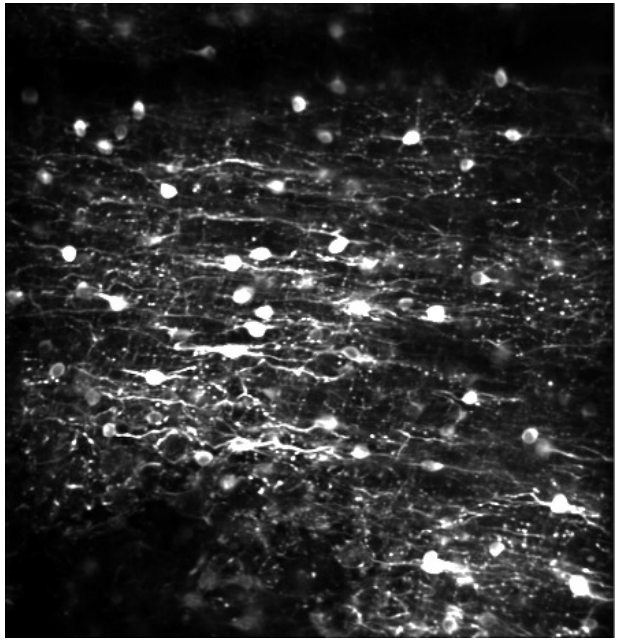
MLI ... molecular layer interneurons
PC ... Purkinje cells

Mouse walking on treadmill with bars (rungs)

Video

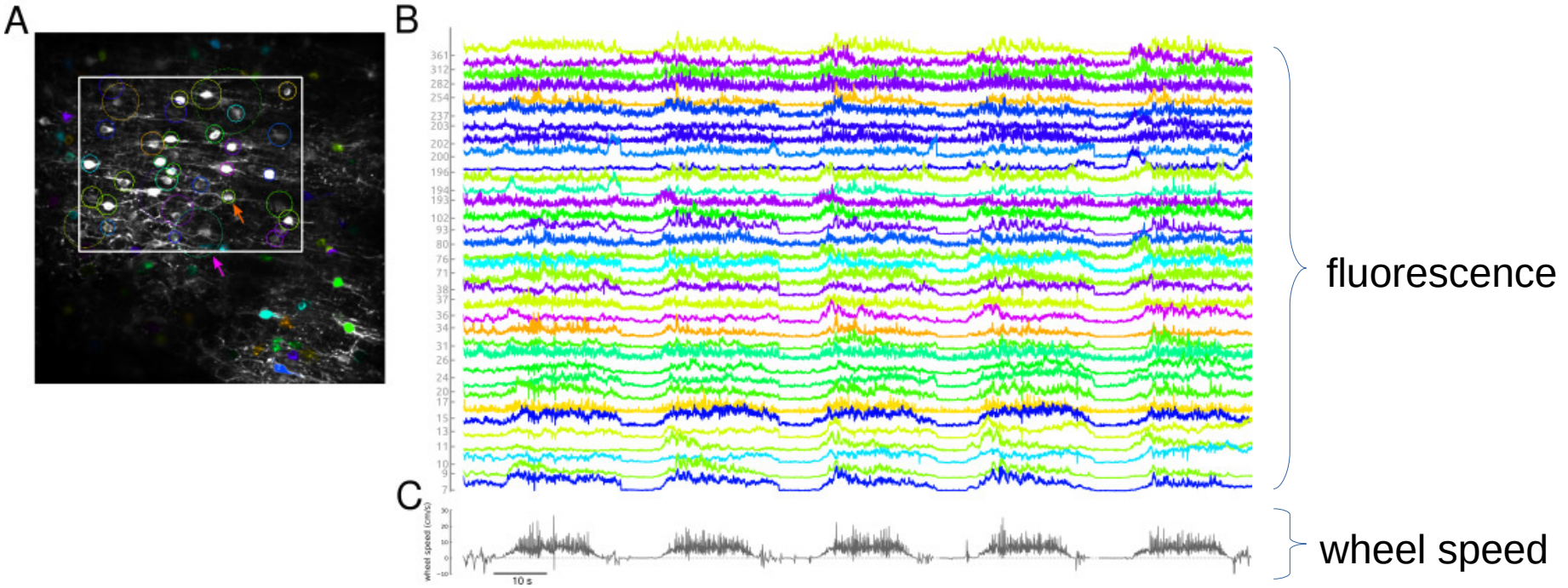
Interneurons are imaged during the task





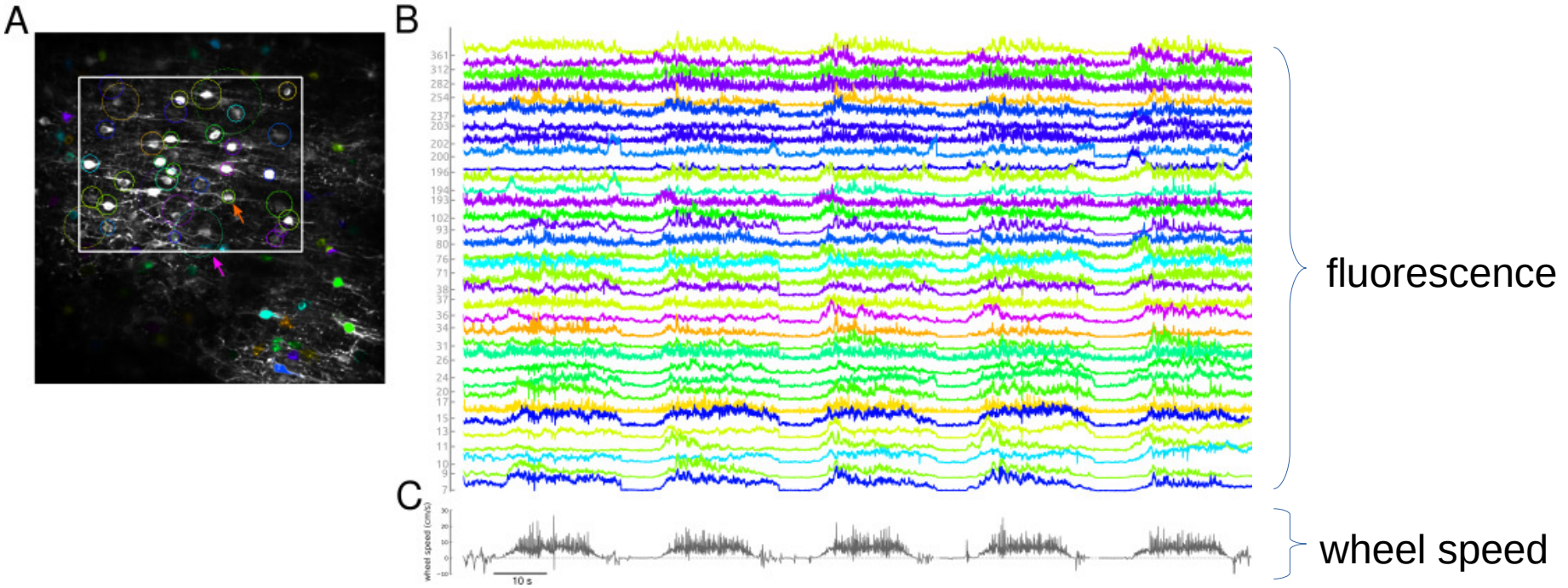
- molecular layer interneurons express GCaMP6f
- GCaMP6f is expressed through transgenic approach : PV-Cre x GCaMP6f-Tigre

Interneurons exhibit locomotion related activity



What is there contribution to learning, performing the task?

Interneurons exhibit locomotion related activity



[**ad** : see M2 internship project]

In vivo imaging as tool to study sensorimotor integration