Физические принципа комбинирование Intrinsic- и Extrinsic- optical imaging:
- однофотонная и мультифотонная микроскопия *in vivo*
- оптическая когерентная томография
- ангулярно-флюоресцентная ламинарная томография

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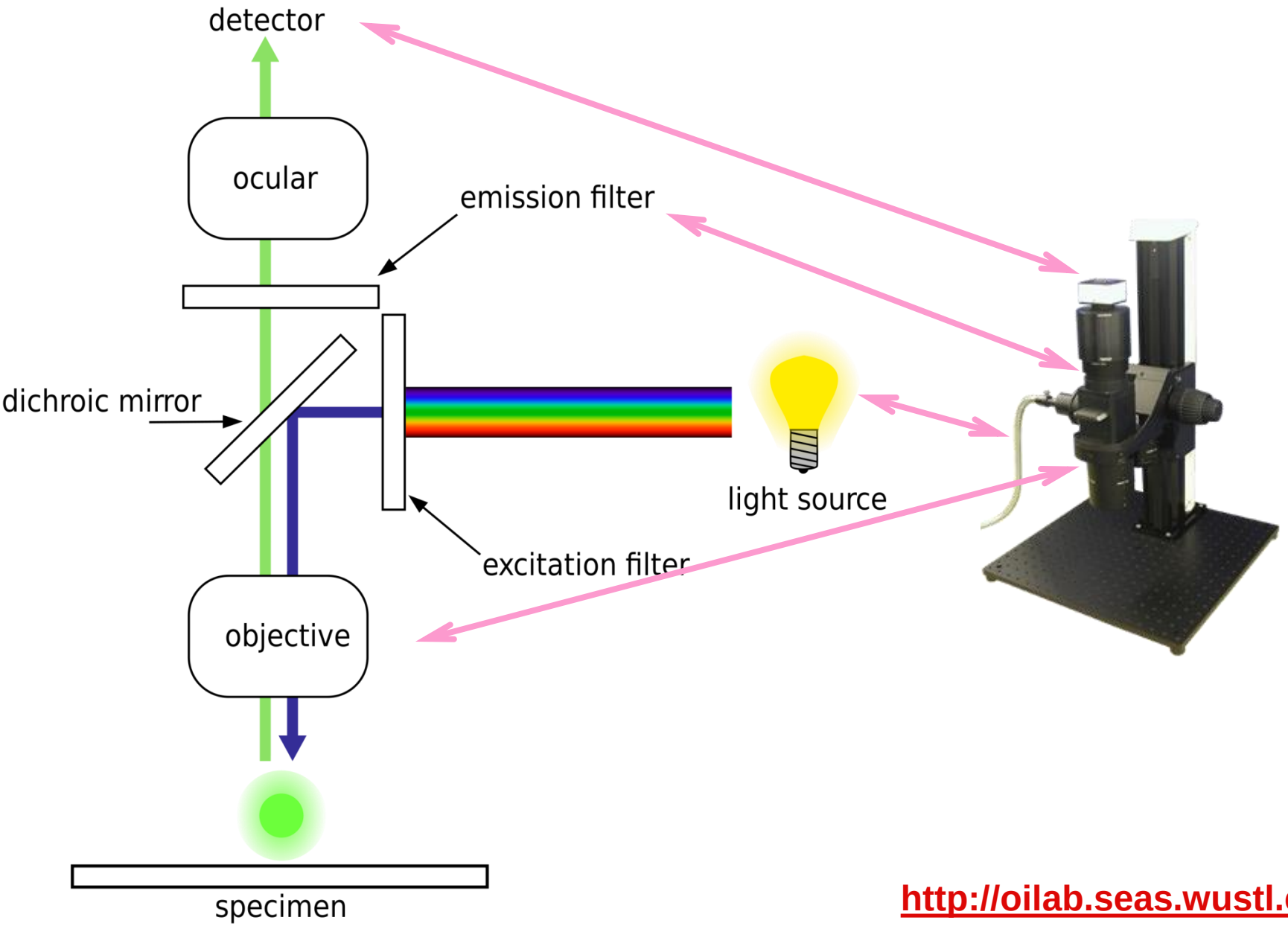
Fluorescence Microscopy *In Vivo* and *In Vitro*:



2-photon Microscopy

2-photon excitation has a number of advantages:

- **The longer wavelength excitation penetrates further into the sample**
- **Sometimes this means of excitation is less damaging**
- **Photobleaching or uncaging is possible with fine z-axis resolution**
- **Some fluorophores are only efficiently excited with 2 photon**

Fluorescence microscopy: 2-photon *versus* confocal

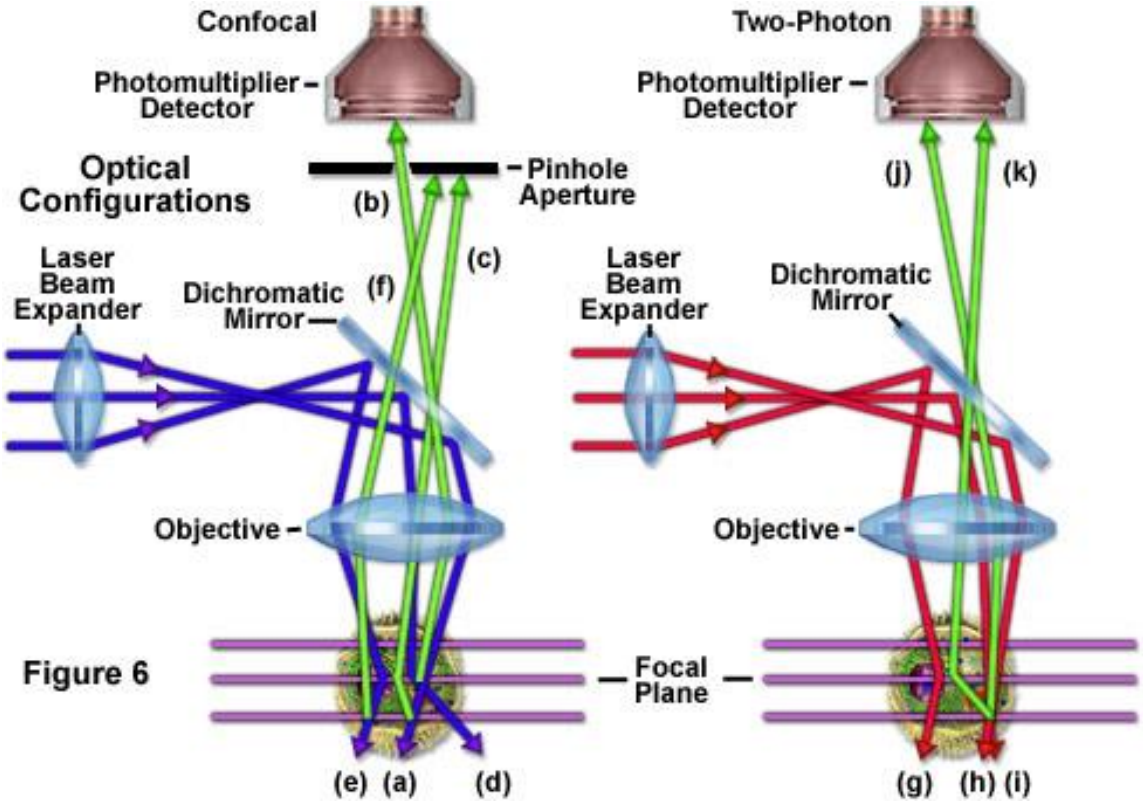
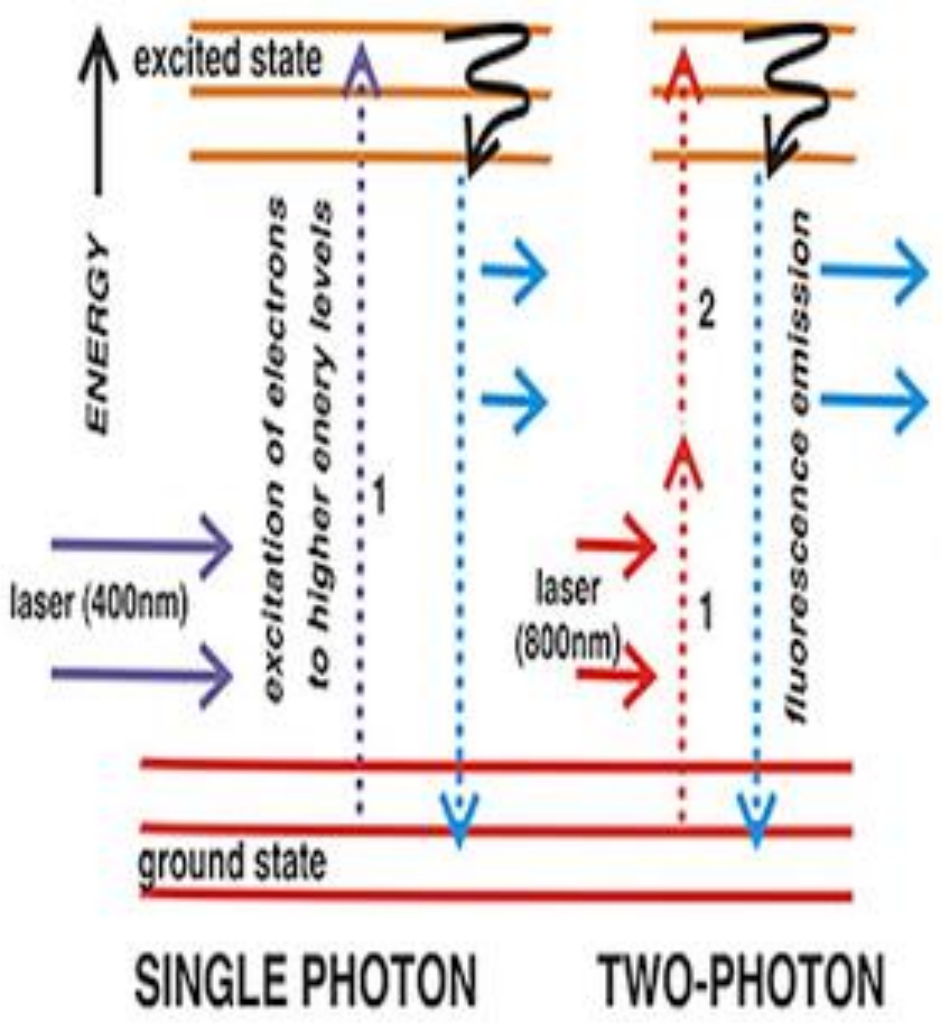
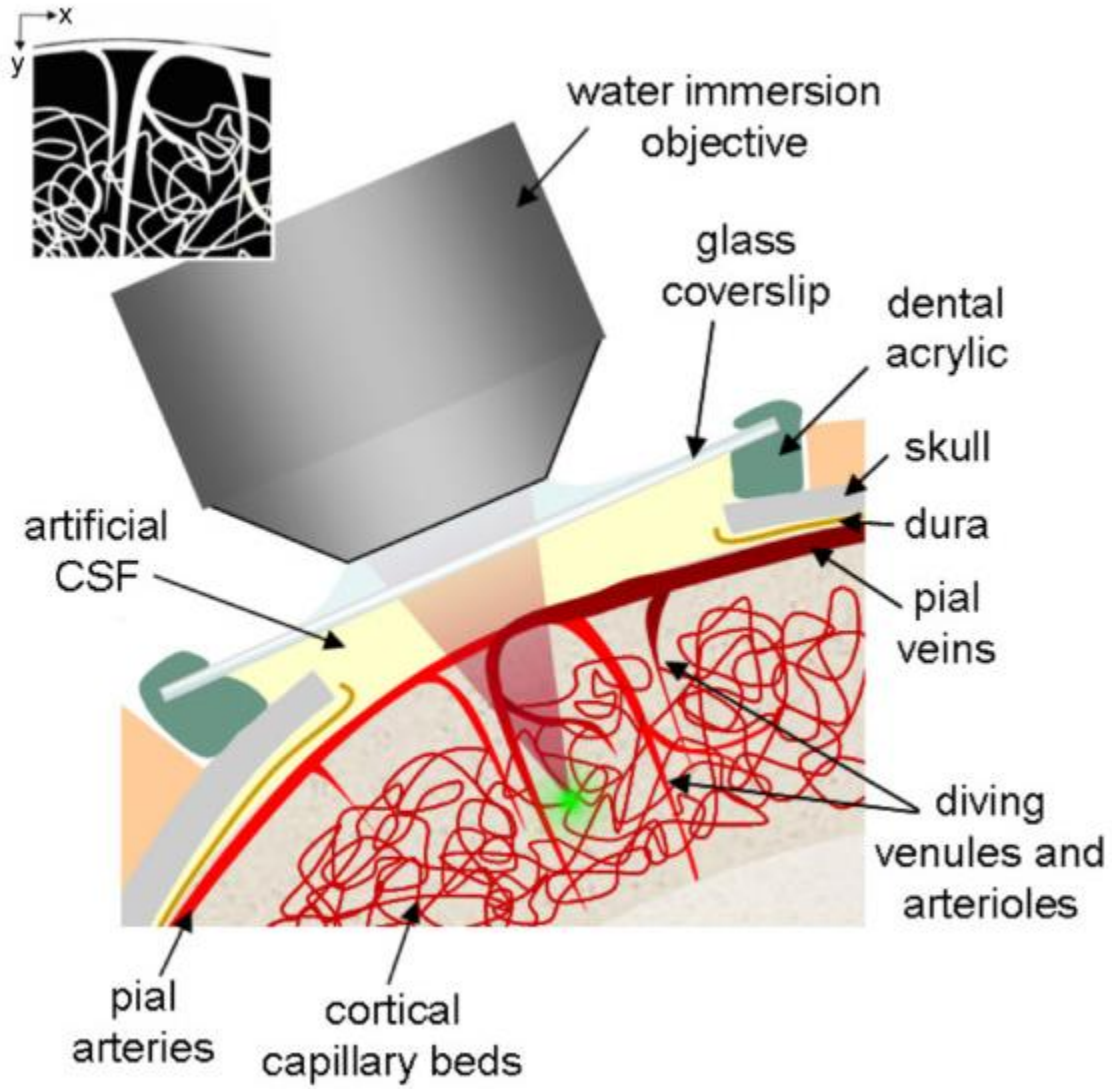


Figure 6

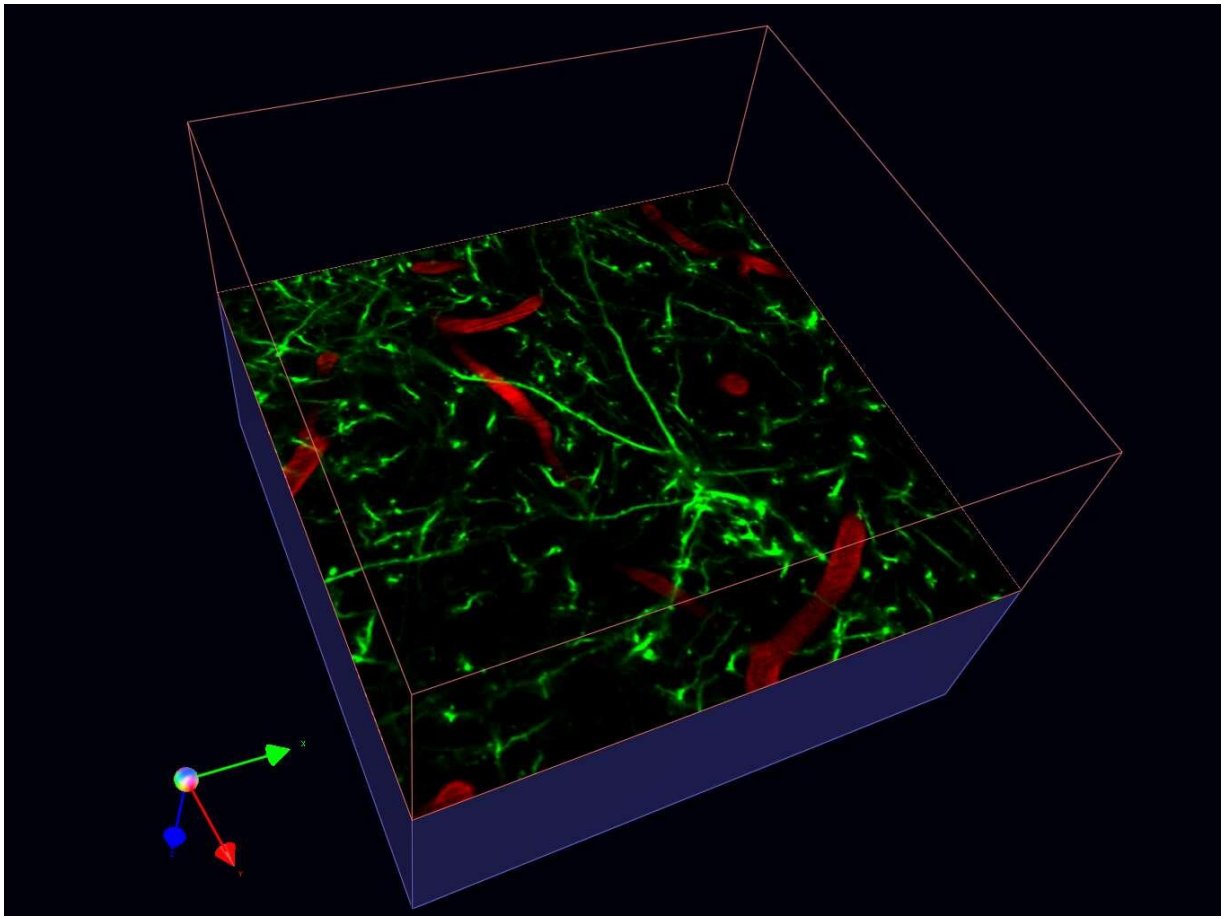
2-photon and 1-photon excitation

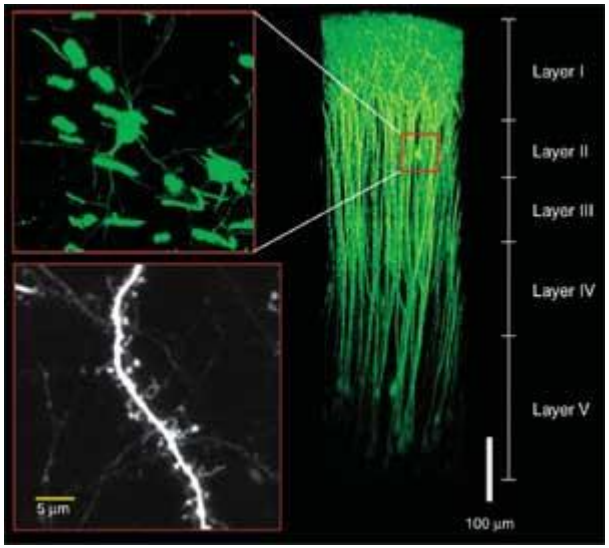


In Vivo 2-photon imaging



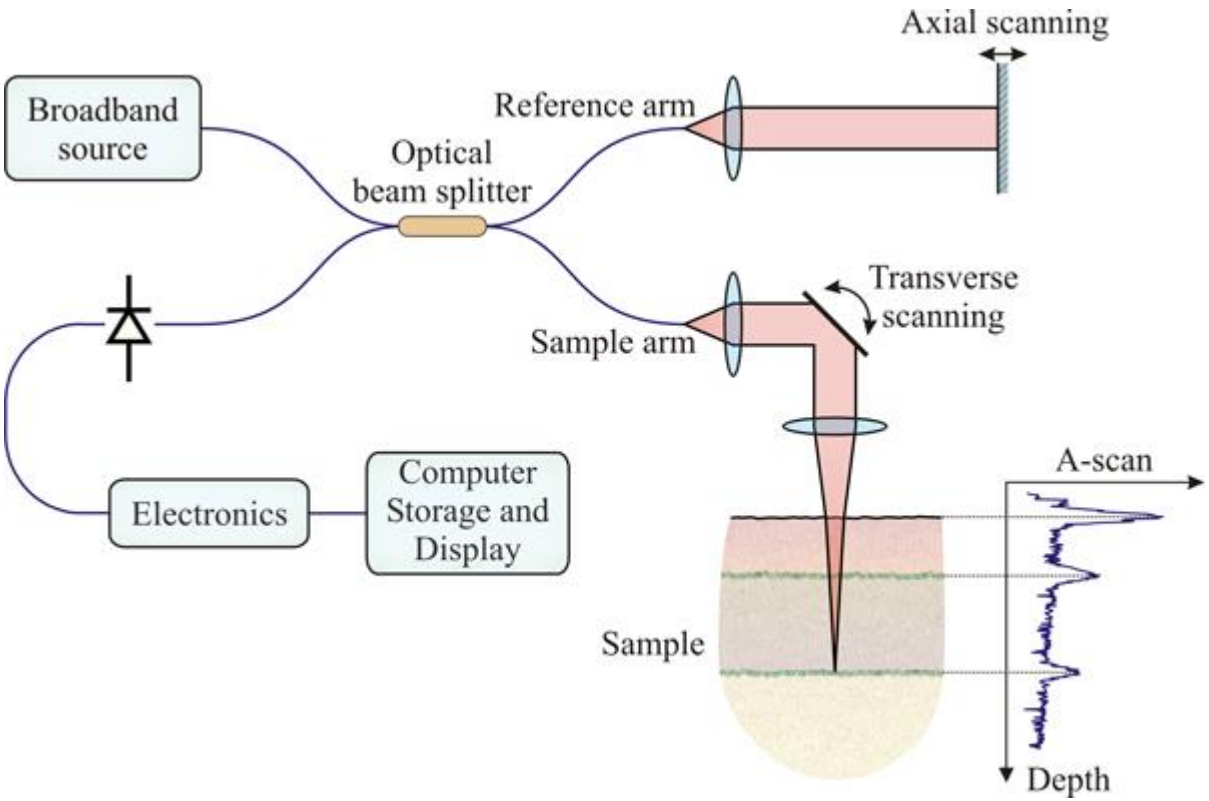
2-photon Imaging: 3D reconstruction



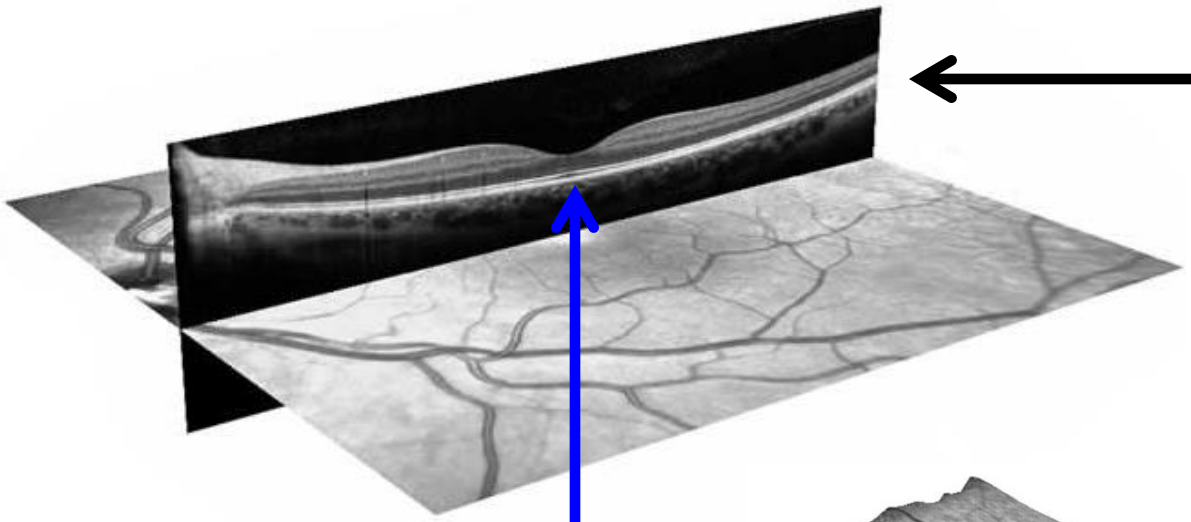


Eisenstein, M; Getting inside their minds; Nature Methods 6, 773 - 781 (2009)

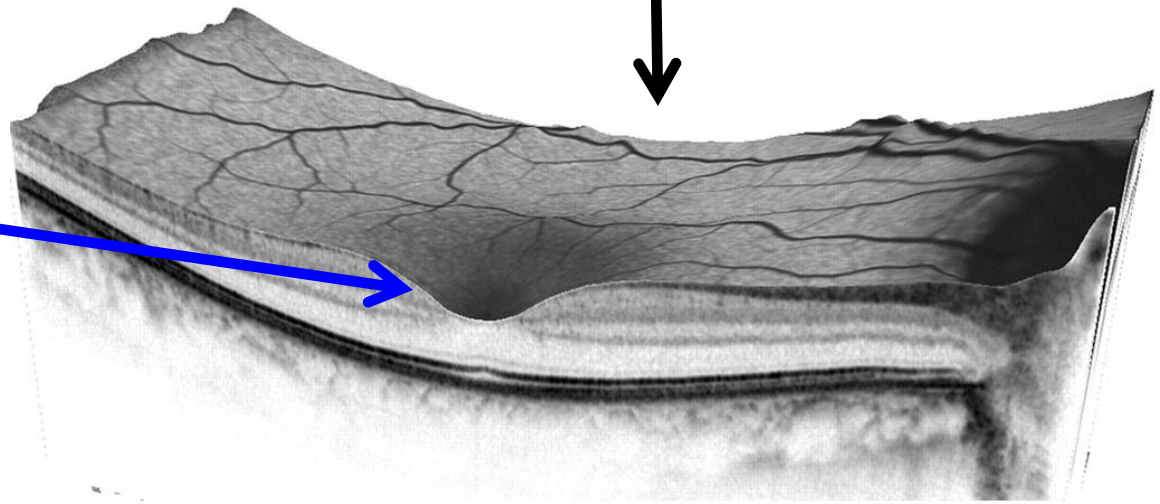
Optical Coherence Tomography



OCT: Eye Imaging

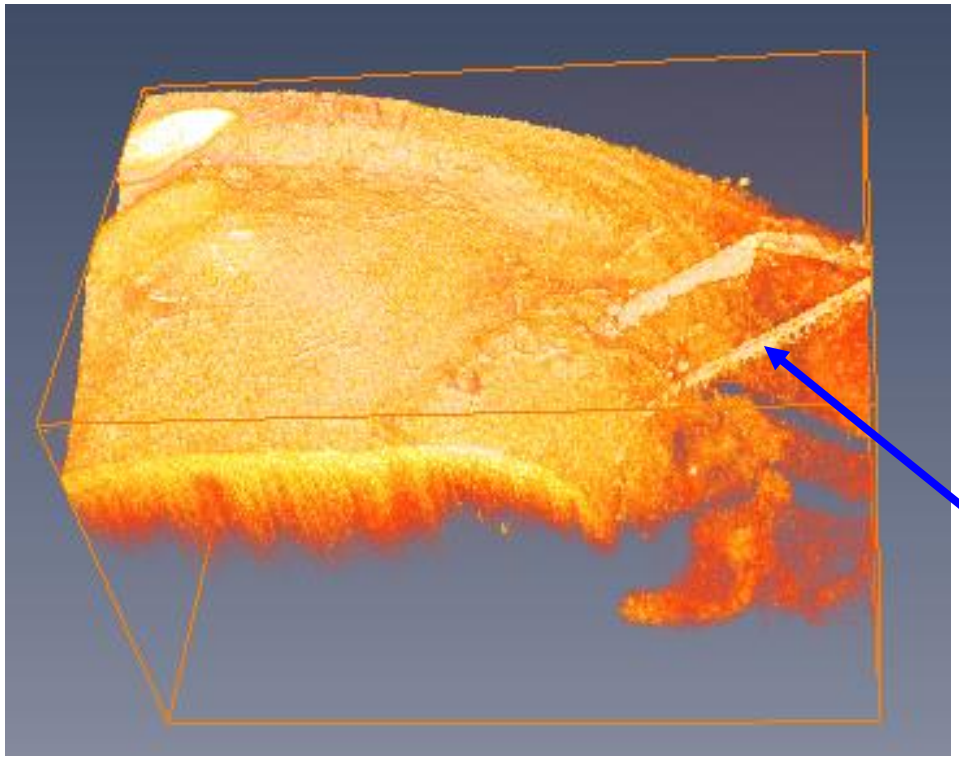


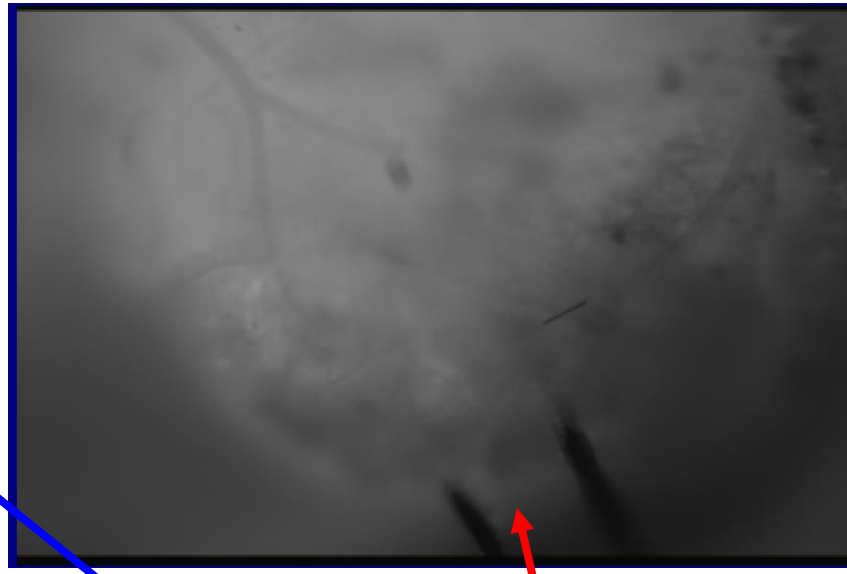
Retina



Blind spot

OCT Imaging of the Mice Neocortex

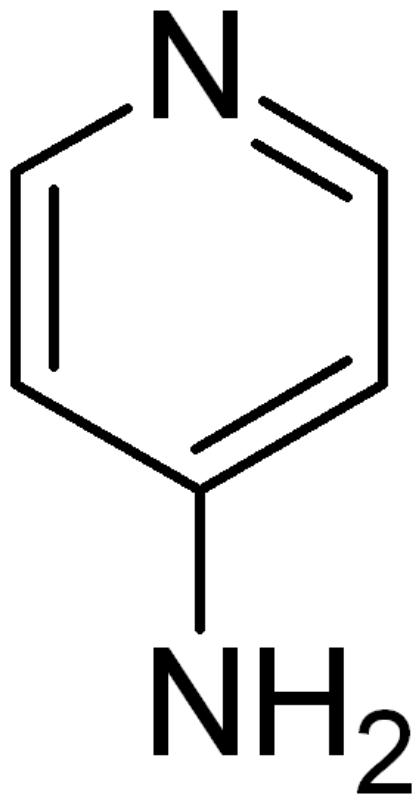




Electrode

4-AP Model of the Cortical Epileptic Seizures

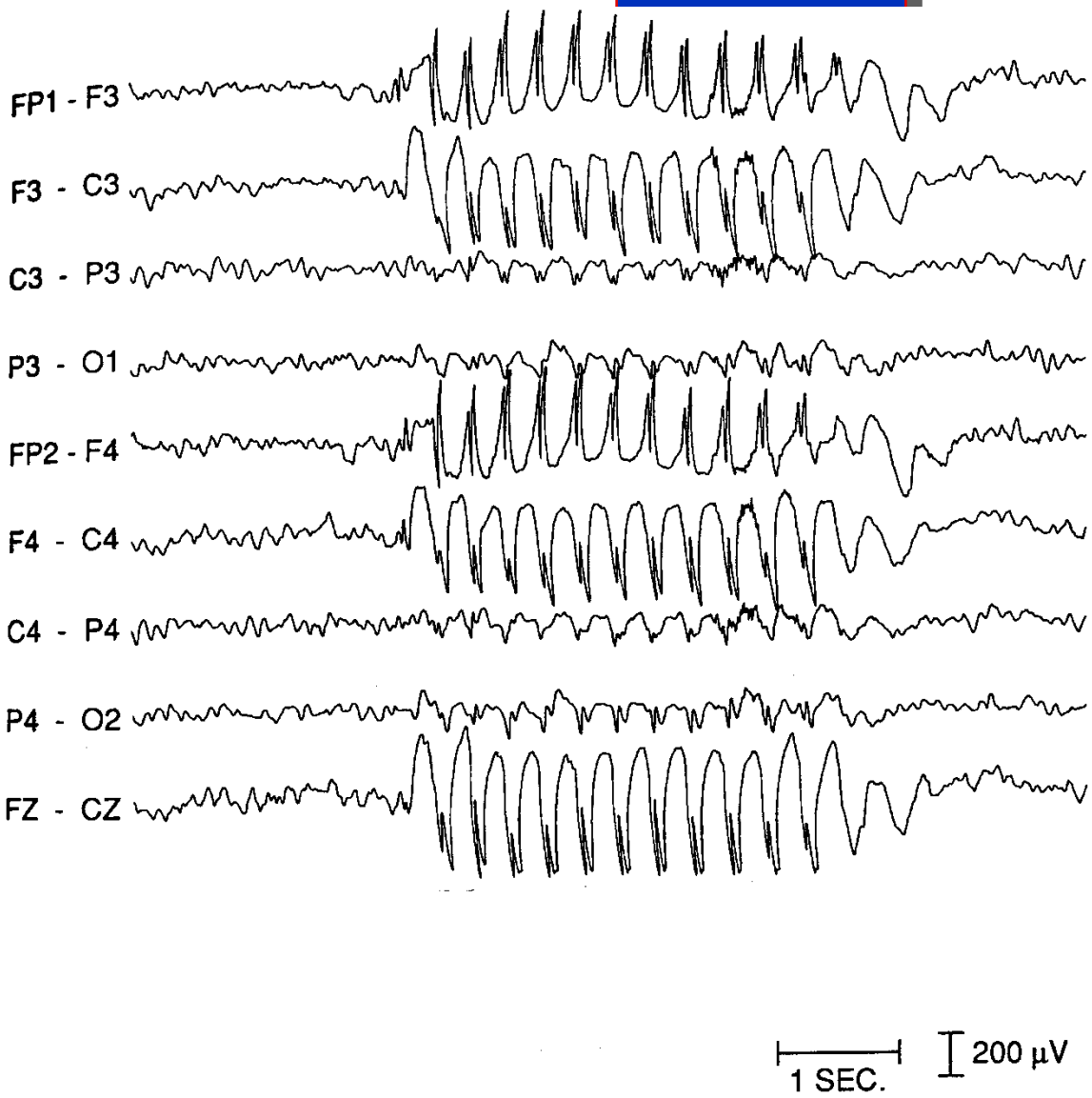
4-aminopyridine blocks a potassium current and in consequence enhances both EPSPs and IPSPs



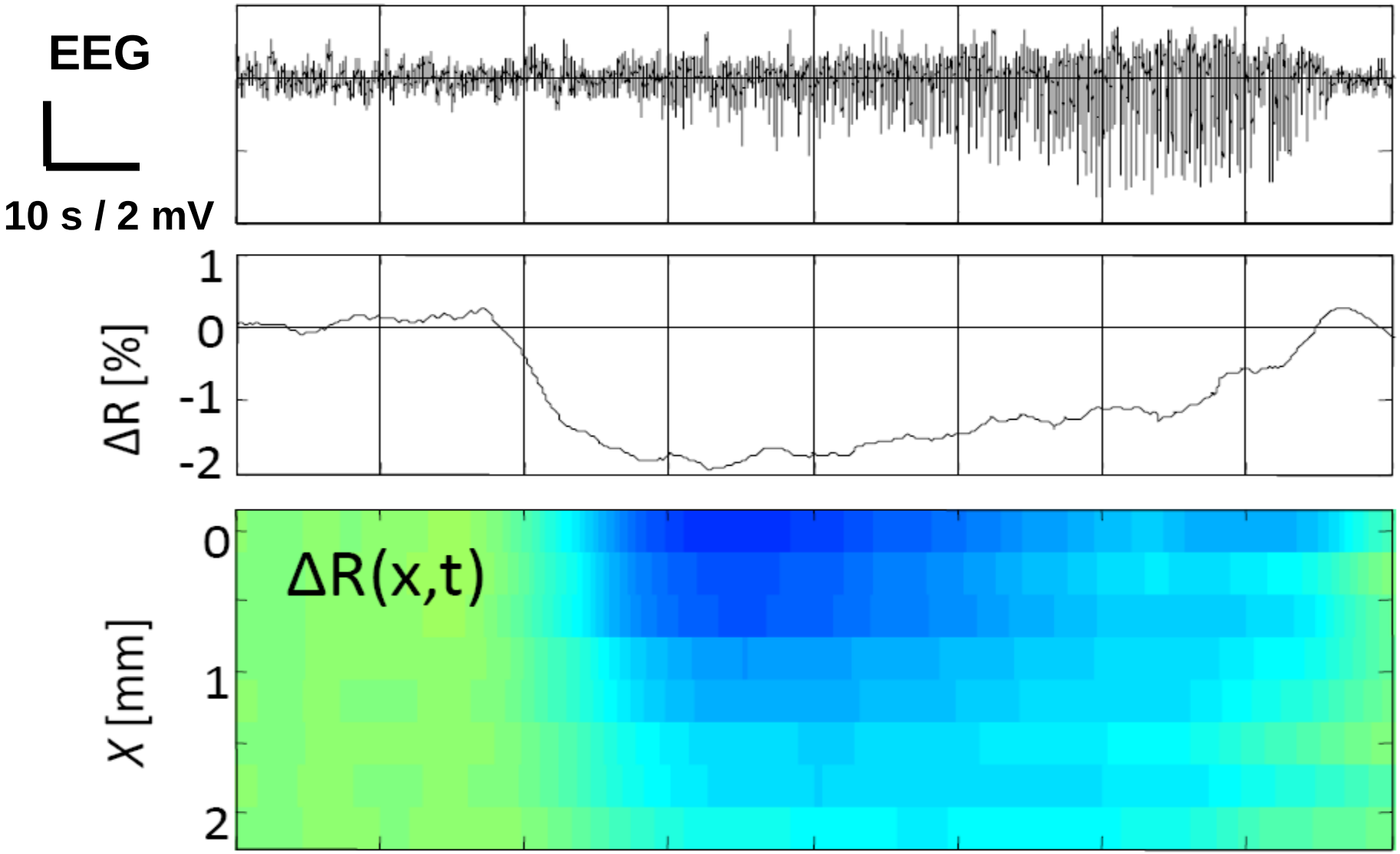
4-AP at doses less than 10 μM, blocks potassium currents and enhances the release of synaptic neurotransmitters =➔

Intracortical injection of 4-AP causes epileptic activity within few hours

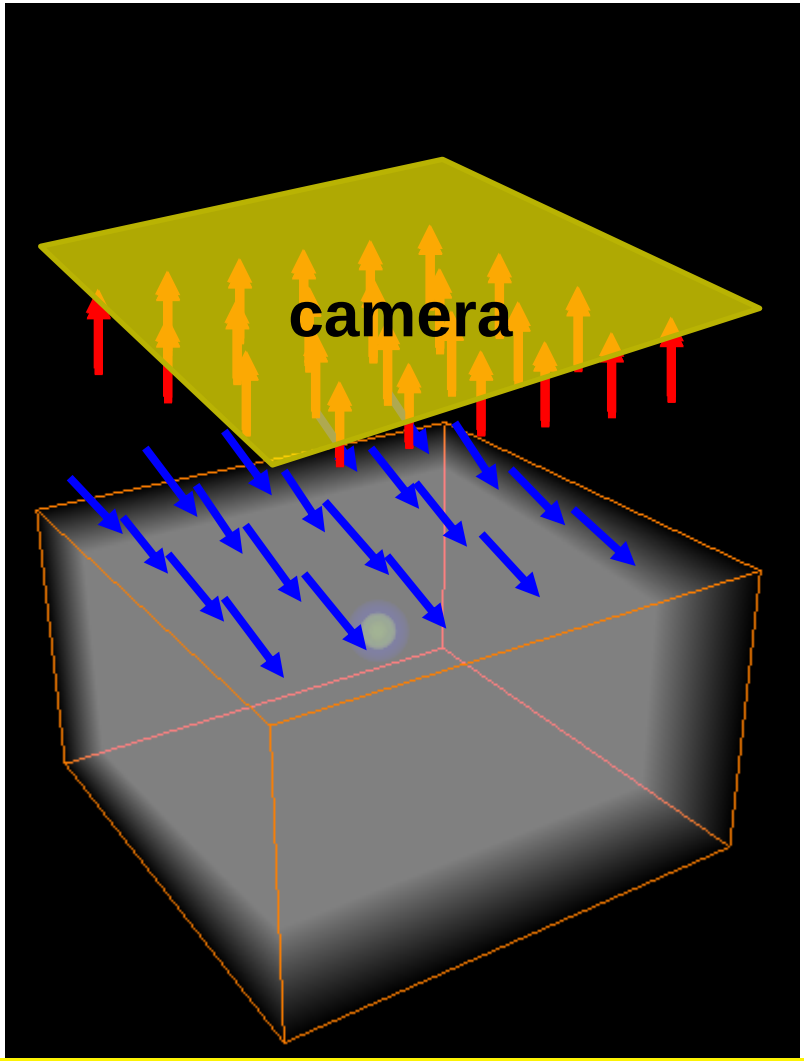
Epilepsy



Optical Coherent Tomography of the Epileptic Seizures

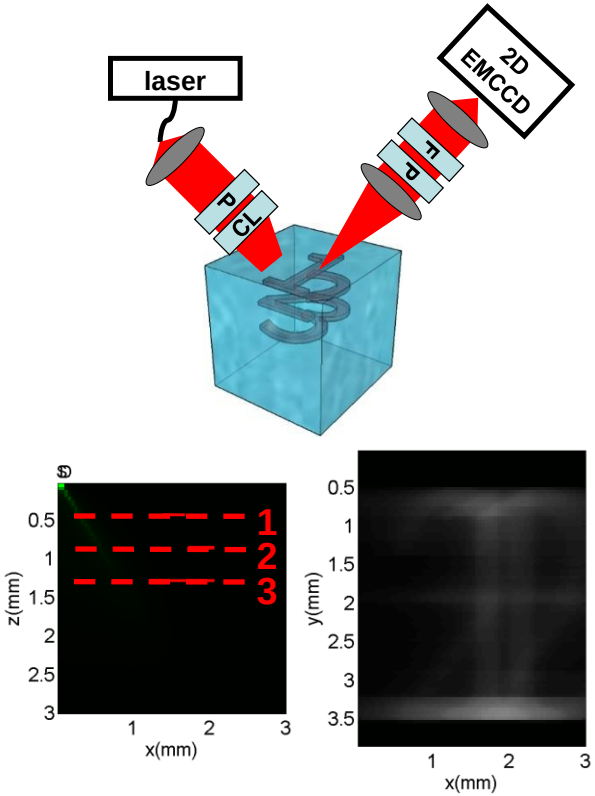


How the measurement is taken?
Line scan angled FLOT

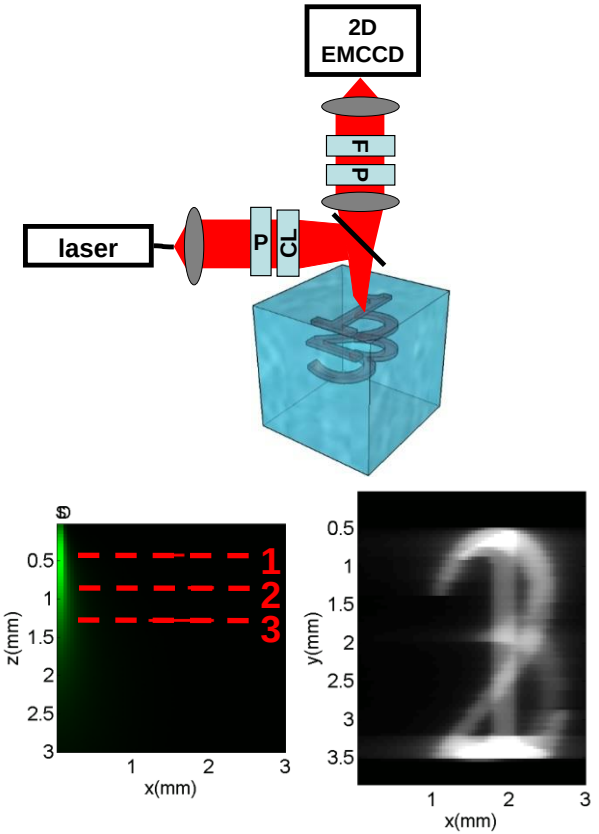


angled illumination changes photon distribution

Comparing aFLOT and FLOT



Different depth info appears



Different depth info appears

1. Oblique illumination/detection enhanced depth selectivity.
2. Discriminating the depths of fluorescing origins at the acquisition phase .

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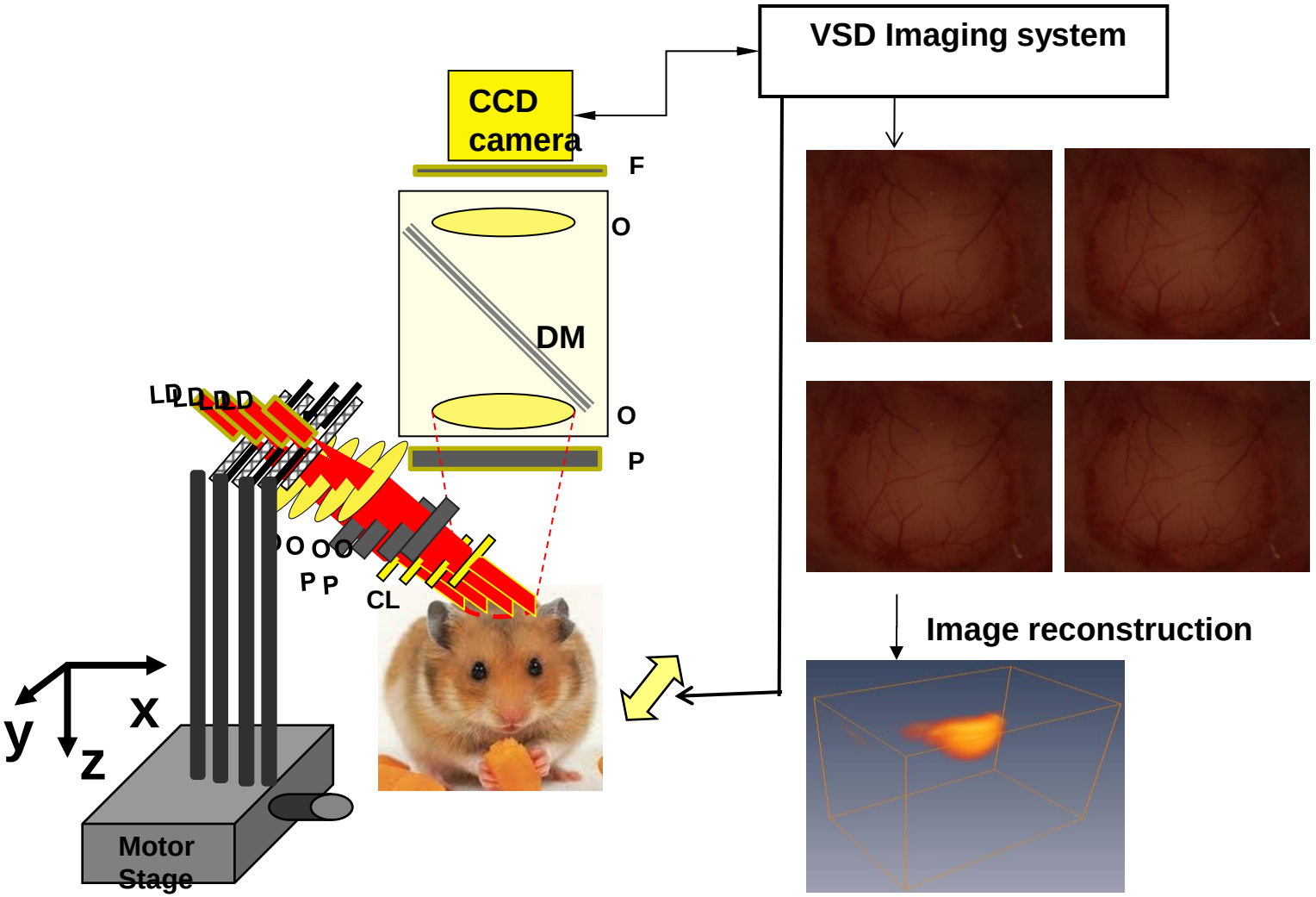
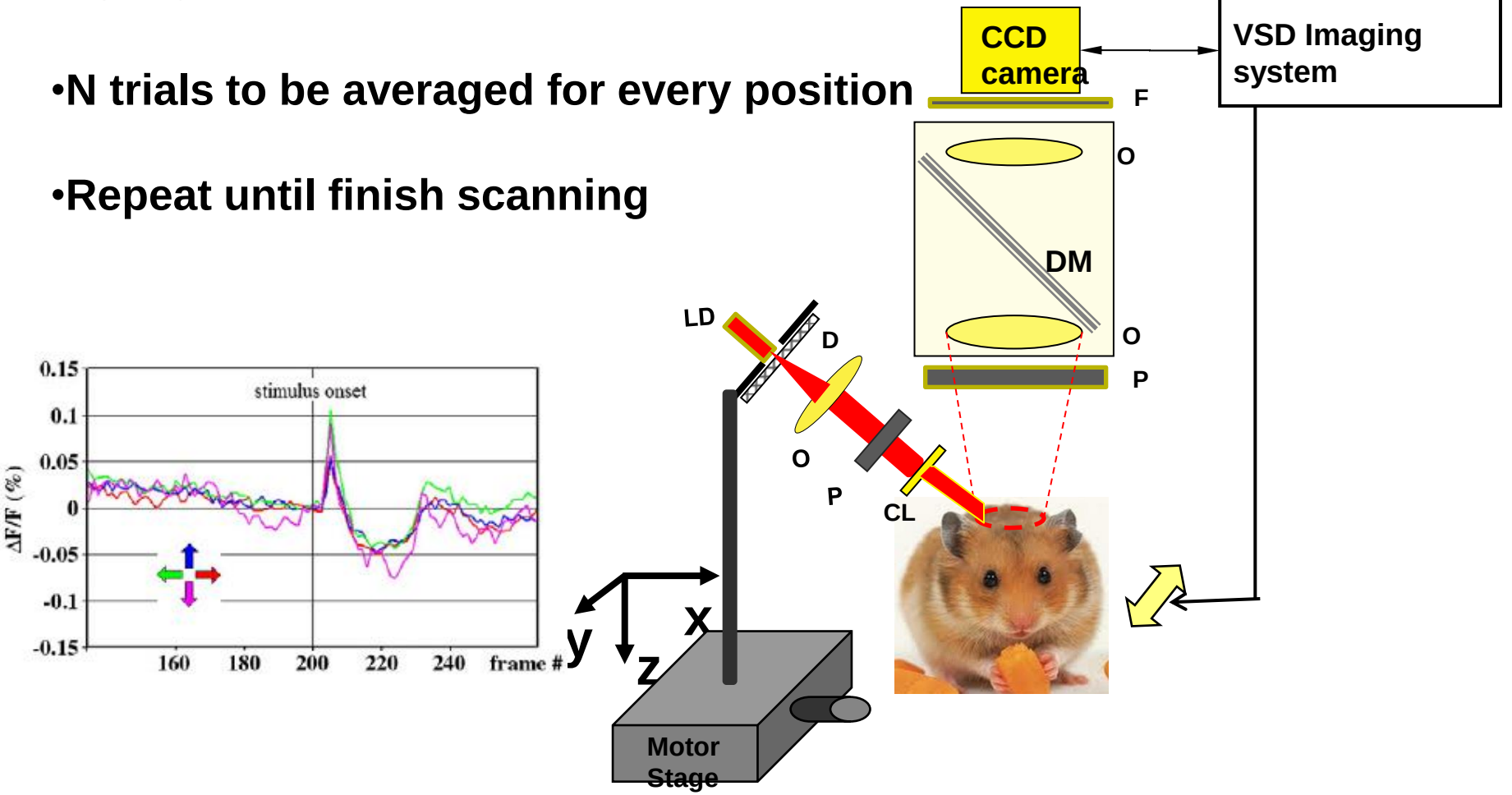
aFLOT based VSDi for subcortex 3-D noninvasive neuronal function imaging



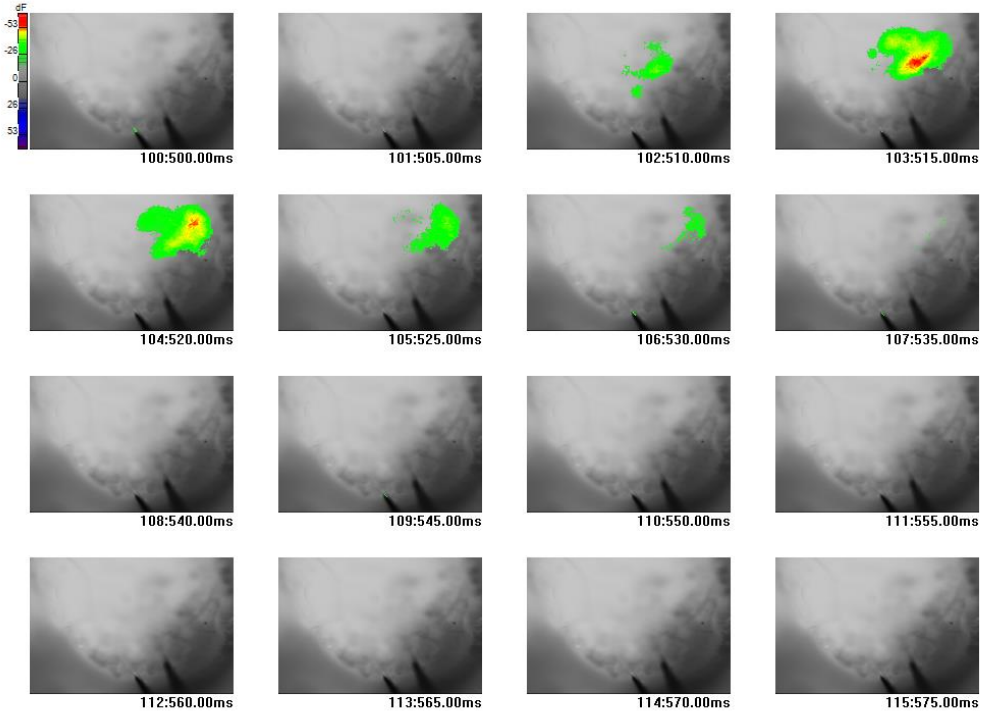
Schematic of the aFLOT based VSDi system. LD: laser diode; D: diffuser; O: objective lens; P: polarizer; CL: cylindrical lens; F: filter; DM: dichroic mirror .

Data acquisition

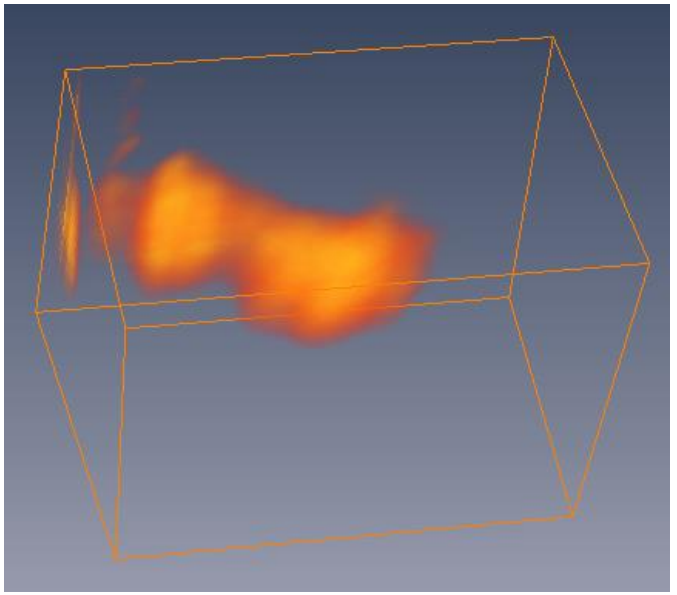
- Every line illumination position—frame all the trial
- N trials to be averaged for every position
- Repeat until finish scanning



Angular Fluorescence Laminar Tomography: Imaging of the Neural Activity

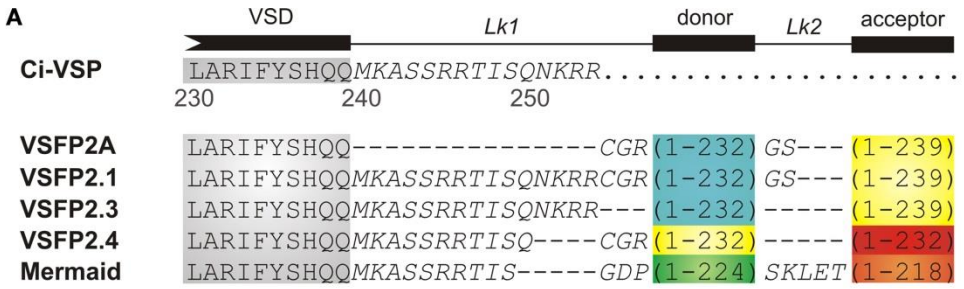


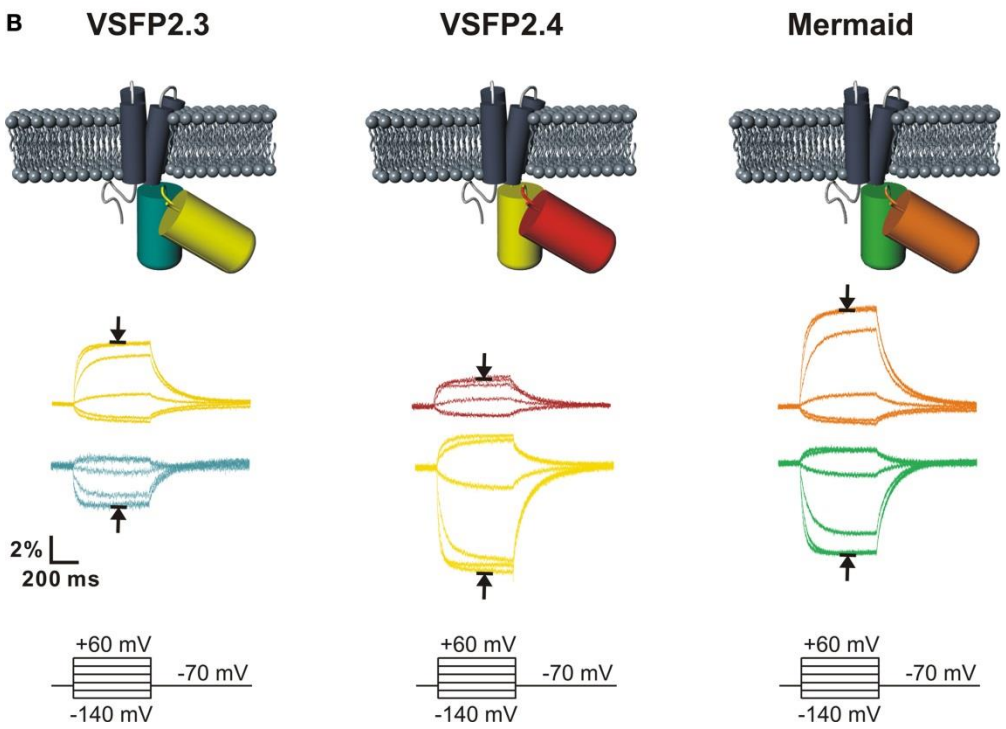
Conventional Voltage sensitive Dye Imaging



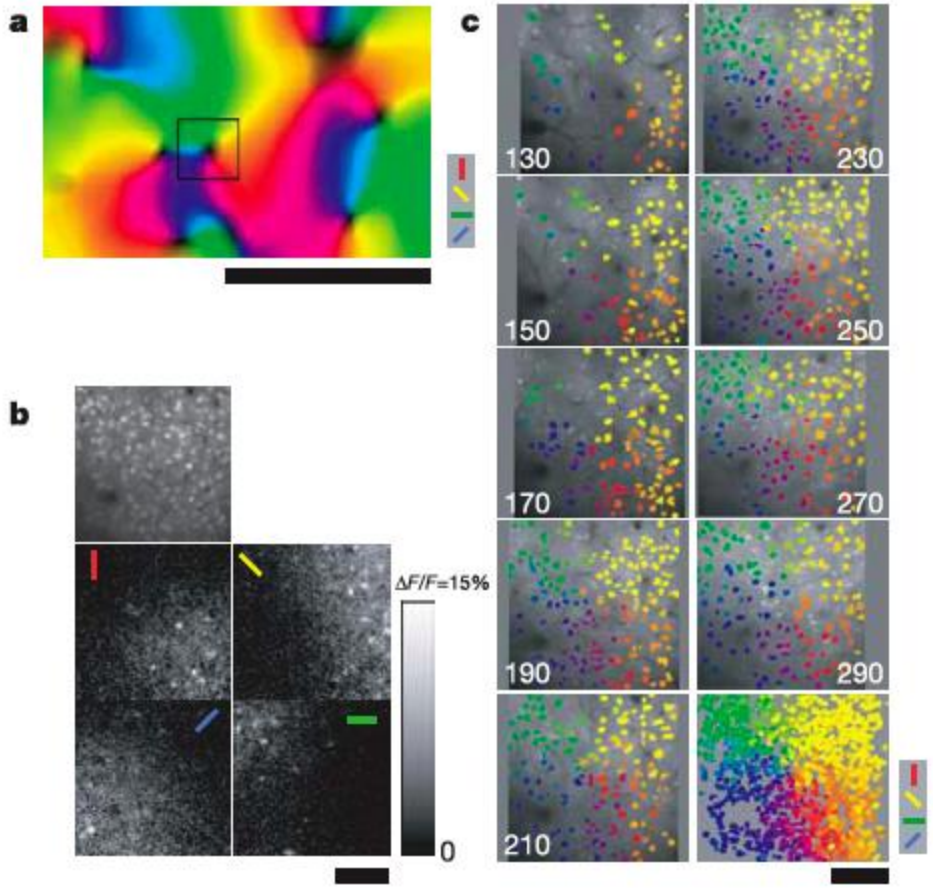
3D image reconstruction

Voltage-sensitive Fluorescent Proteins



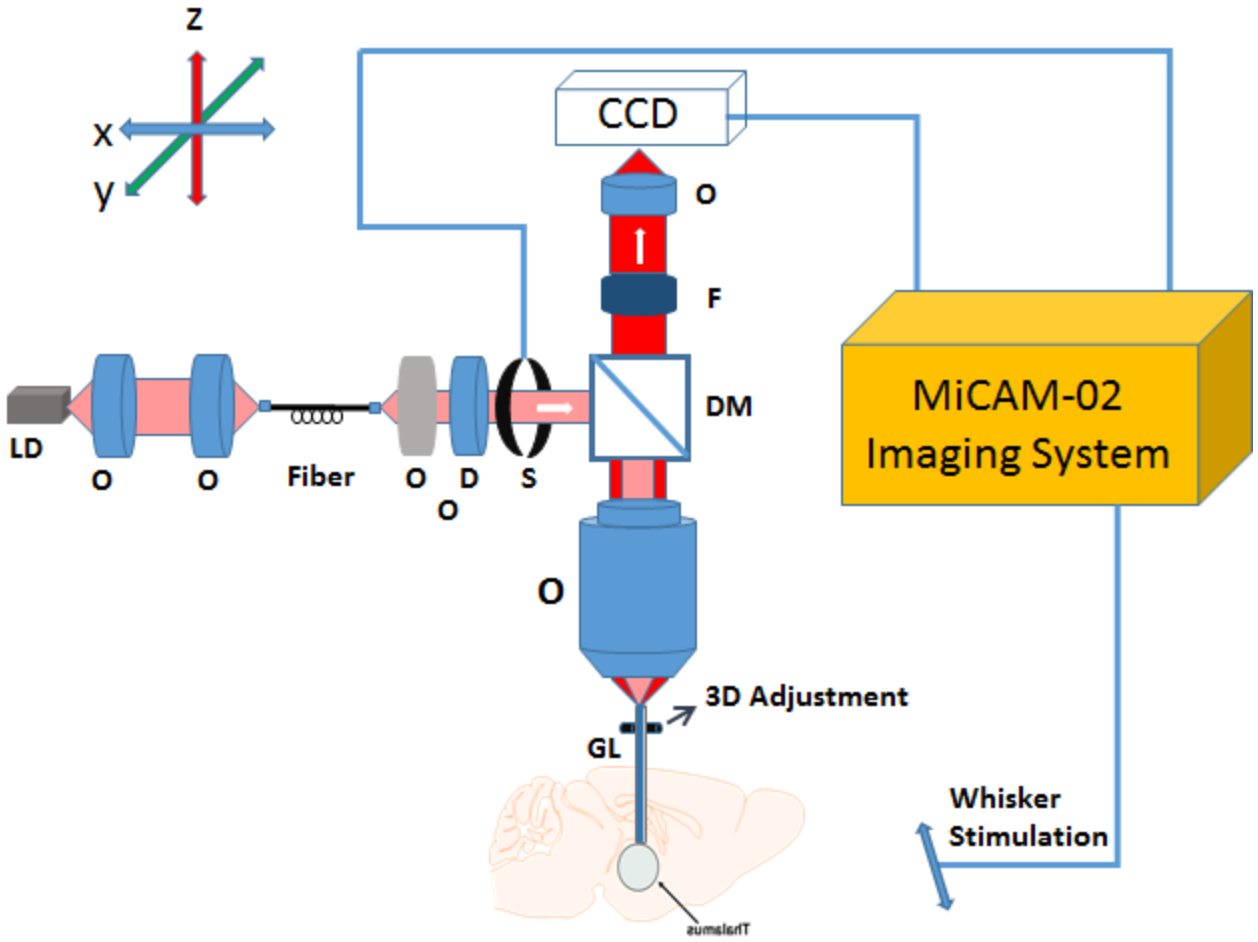


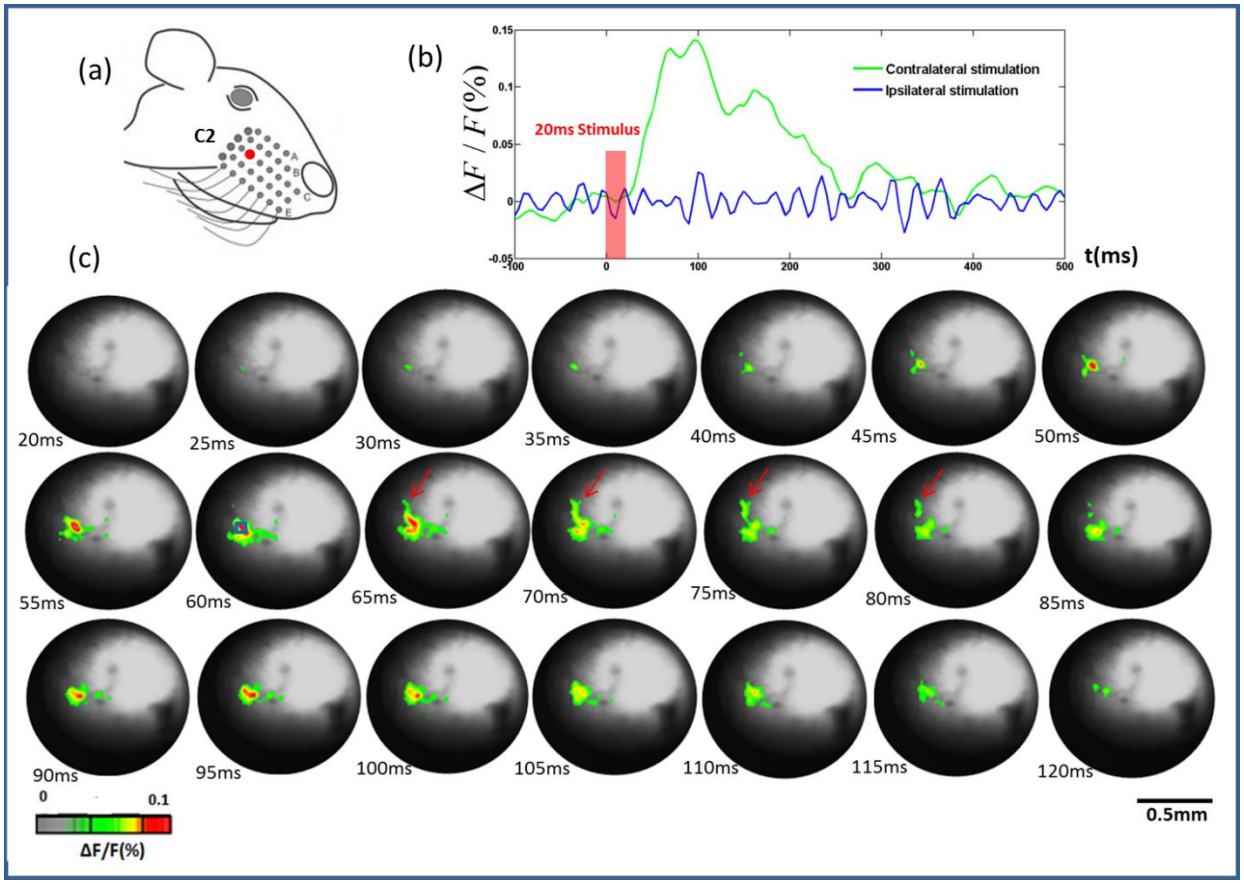
Voltage-sensitive *versus* Ca-sensitive Dye Imaging



Voltage-sensitive **Ca-sensitive**

GRIN optics: imaging of the deep brain structures





(A) C2 whisker for stimulation (labeled in red); (B) Change in fluorescence (ΔF/F(%), ordinate) in response to C2 whisker stimulation. Fluorescence signal was recorded in the small blue square marked on the cortical surface in Figure 3C, 60 ms; (C) Voltage-sensitive dye optical images showing single-whisker (C2) stimulation fluorescence changes in thalamus.

Thank you very much for your attention