BENEFRI Neuroscience Workshop 2011

Calcium imaging and voltage - sensitive imaging in neurons and neuronal tissues

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Calcium imaging and voltage-sensitive imaging in neurons and neuronal tissues

Principles of imaging techniques

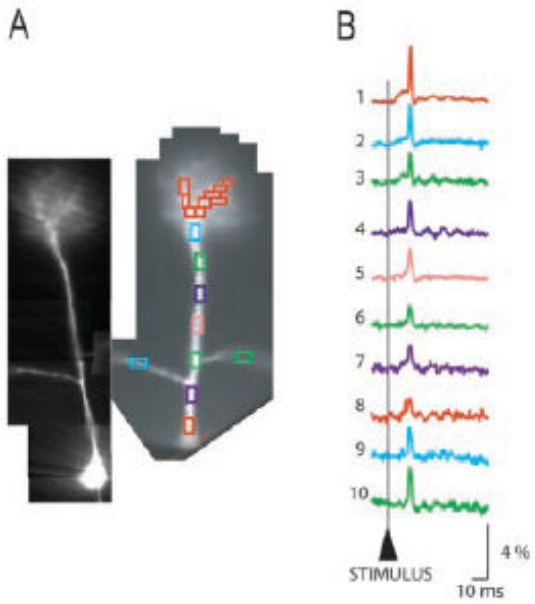
Detection of cellular and subcellular activity with CaSD and VSD imaging techniques

Detection of network activity with CaSD and VSD imaging techniques

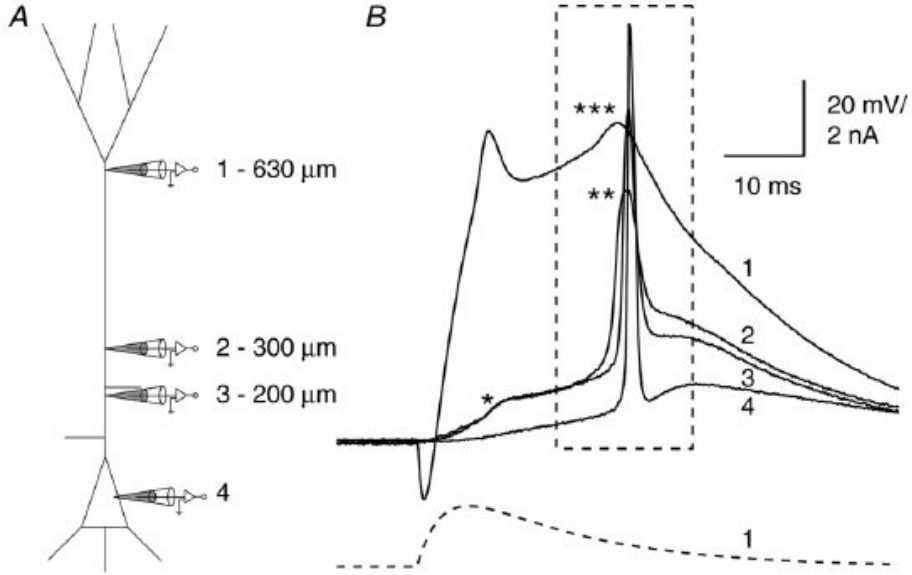
Why should one use
imaging techniques
on the cellular and subcellular level?

When are imaging techniques superior
to other physiological techniques like
electrophysiology?

Imaging: Easy access to tiny subcellular compartments : Dendrites



VSD imaging in the dendrite and soma of a mitral cell (olfactory bulb)

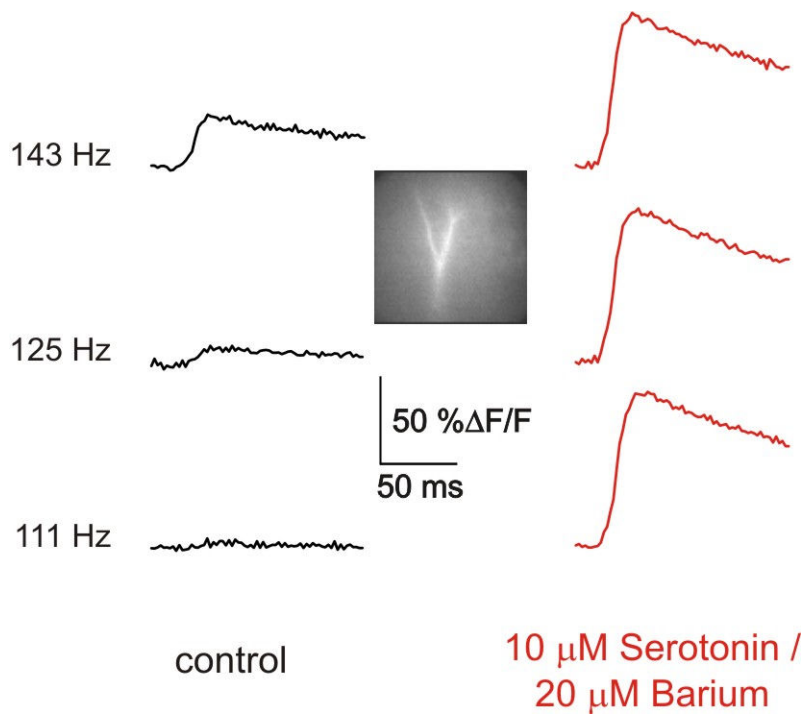


Quadruple patch-clamp recording on dendrite and soma of a cortical pyramidal cell

Imaging with CaSDs and VSDs gives you the information from every compartment of the cell at the same time (have the epifluorescence limitations in mind!). Electrophysiology is restricted in this respect.

Djurisic et al., J. Neurosci. 2004; Larkum et al., J. Physiol. 2002

Imaging: Long lasting access to tiny subcellular compartments : Dendrites



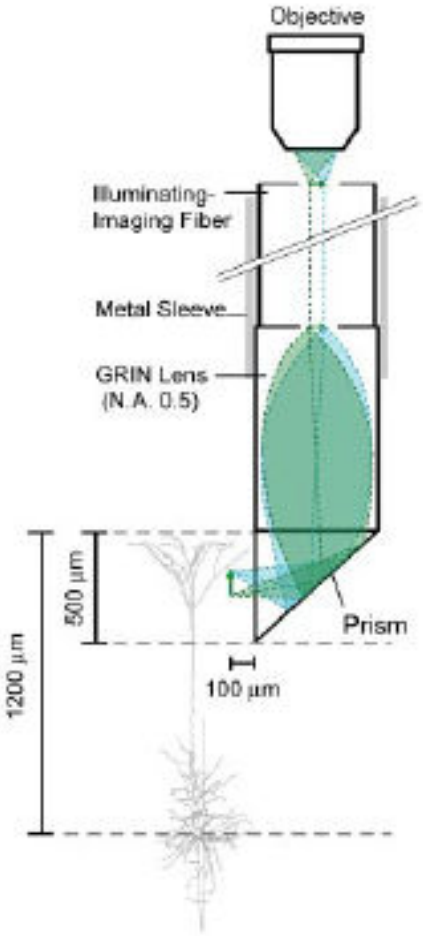
Calcium imaging in the distal dendrite (600 μm from the soma) in a cortical pyramidal cell. Epifluorescence imaging using OGB-1 applied to the cell interior via a somatic patch pipette. 40-50 min after obtaining the patch clamp recording, the dye is diffused into the dendrite and imaging can start.

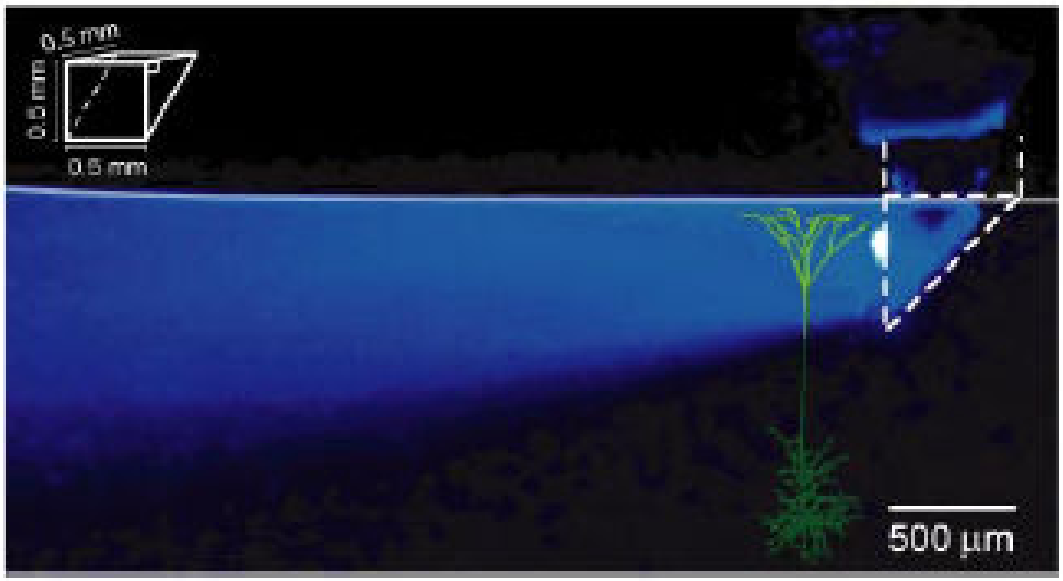
Changes in the intracellular calcium concentration at the distal dendritic calcium action potential initiation zone are modulated by pharmacological intervention.

Dendritic patch-clamp recordings are much more difficult to obtain and are often not stable enough for a long-lasting pharmacological study.

Neubauer & Berger, 2007

Imaging: Dendritic activity in behaving animals

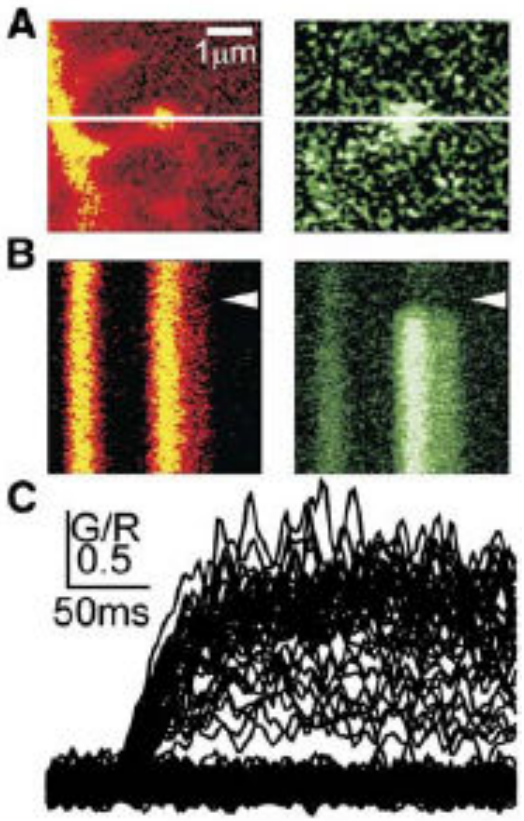




Fiberoptic-based epifluorecence microscope. Optical diagram of light rays for the single-fiber system. “Periscope” system was used for illumination, consisting of a 90° prism combined with a lens. Sketch (gray) of a pyramidal neuron drawn to scale shows the size of the periscope system relative to the apical dendrite. Picture of the 488-nm light passing through the periscope in a 2% agar block.

Murayama et al., J. Neurophysiol. 2007

Imaging: Access to tiny subcellular compartments : Spines



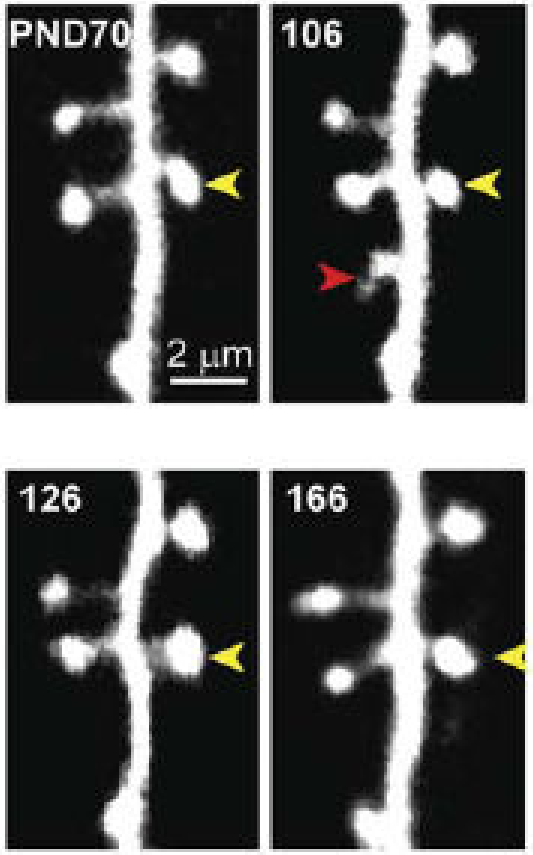
Two photon calcium imaging in single spines.

A: Dendritic shaft and spine are filled with a calcium-insensitive red fluorescent dye and a green fluorescent CaSD.
B: A linescan through shaft and spine shows a dramatic increase in intracellular calcium following stimulation of the corresponding synapse.
C: Traces of the changes in intracellular calcium in the spine show two populations of responses. On one hand a clear increase, but also clear failures reflecting the stochastic nature of glutamate release.

A study of single postsynaptic entities is in principal only possible with imaging techniques. The same is also true for presynaptic endings (exceptions are Calyx of Held and Mossy Fiber synapses).

Taken from: Svoboda & Yasuda, Neuron 2006

Imaging: Access to tiny subcellular compartments : Spines

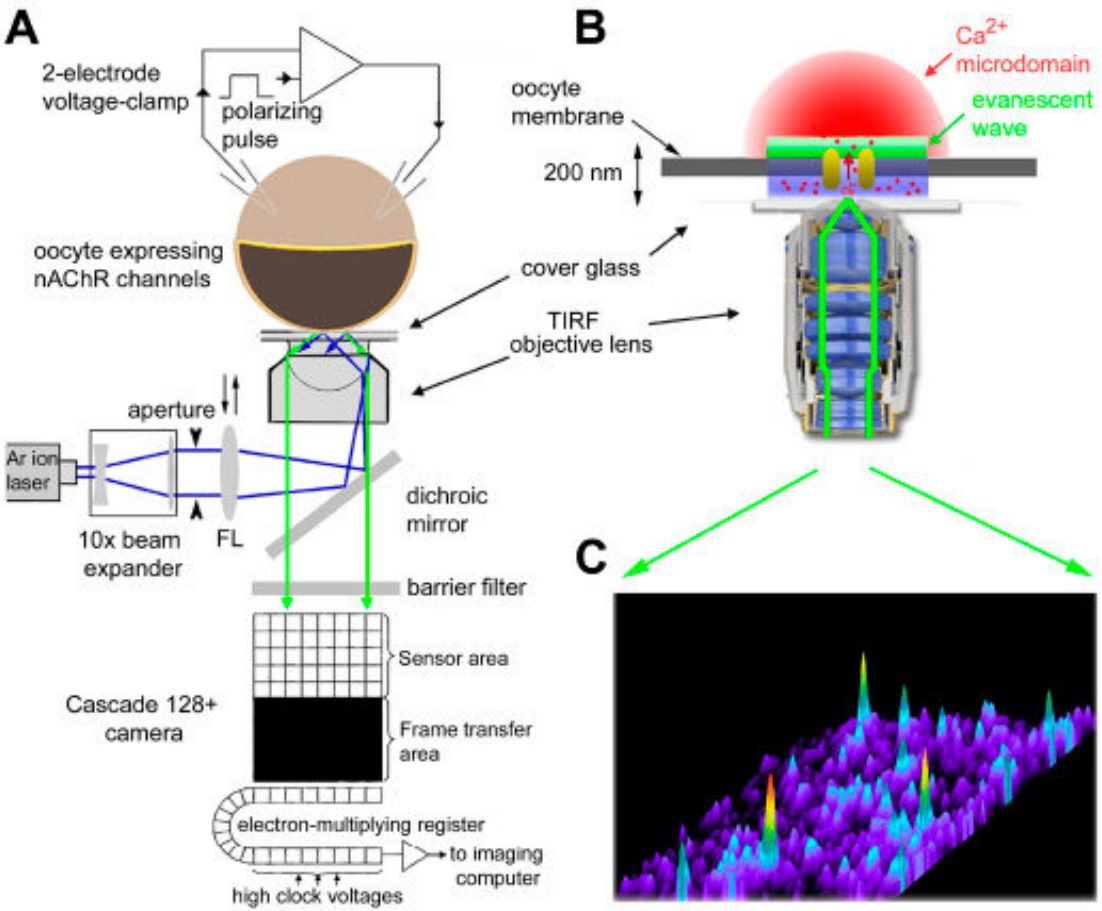


Two photon imaging of genetically encoded Green Fluorescent Protein (GFP) in dendrites and spines *in vivo*.

Imaging over months (between postnatal day 70 and 166) enables the study and quantification of spine protraction and retraction during development and as part of plasticity phenomena following learning paradigms.

Taken from: Svoboda & Yasuda, Neuron 2006

Imaging: Optical single-channel recordings



(A) Overview of the system using an inverted microscope. An oocyte expressing nicotinic acetylcholine receptor (nAChR) channels was loaded with a CaSD, and allowed to adhere to the bottom of the imaging chamber. Its membrane potential was controlled by a two-electrode voltage clamp. (B) Enlarged view illustrating the imaging of near-membrane fluorescent signals. (C) Single image frame illustrating calcium signals from simultaneous opening of nAChR channels in the oocyte membrane. Increasing calcium is denoted both by “warmer” colors and by height.

Taken from: Demuro & Parker, J. Gen. Physiol. 2005

Cellular Imaging: Genetically encoded CaSDs

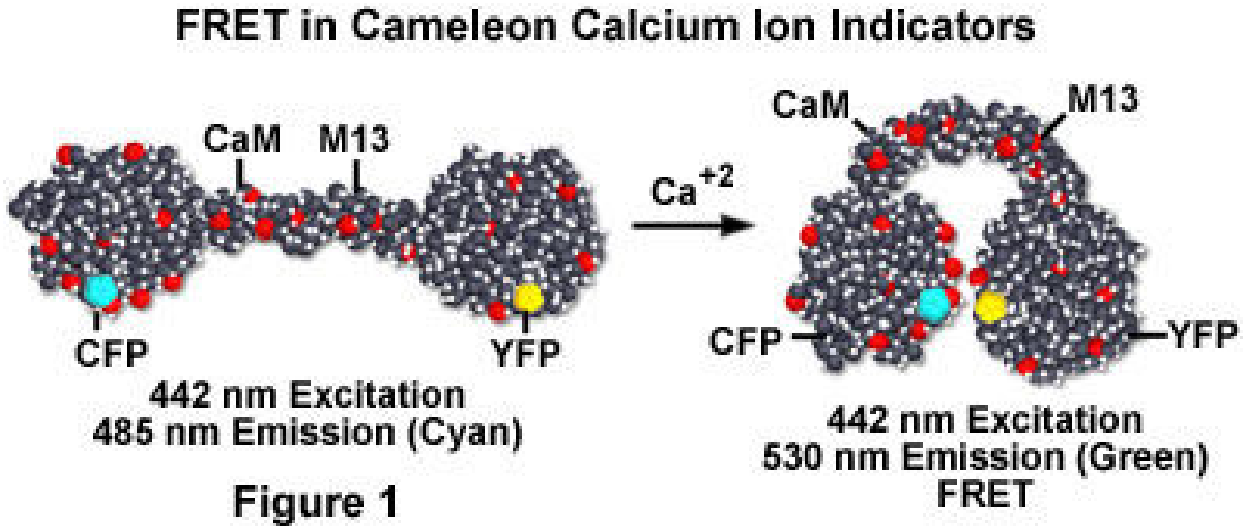
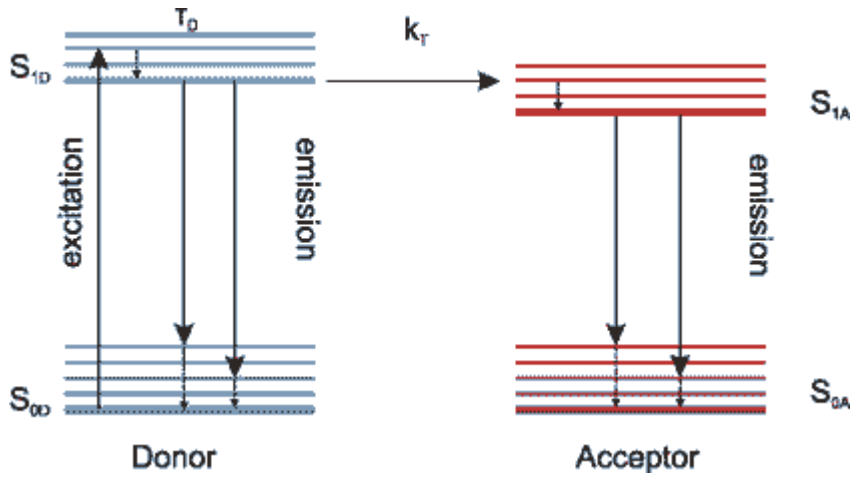


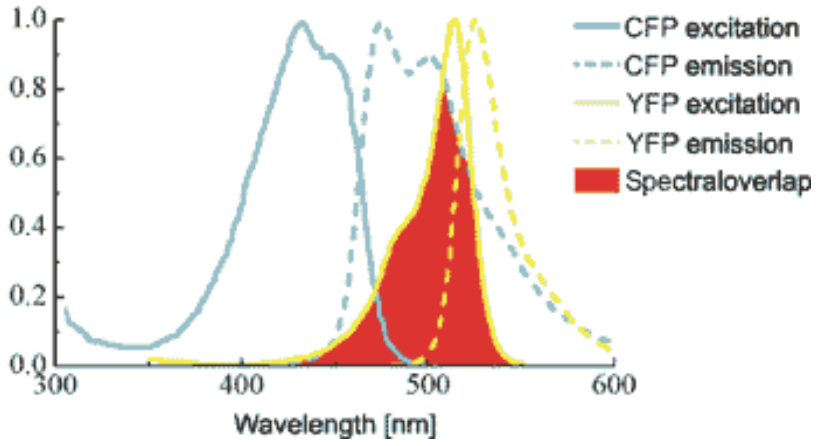
Figure 1

Genetically encoded calcium dyes like **Cameleon** are artificial proteins. The cameleon molecular structure is modeled as a fusion product between two fluorescent proteins like **CFP** and **YFP** (having differing excitation and emission characteristics), calmodulin (**CaM**), and the calmodulin-binding domain of myosin light chain kinase (**M13**). Calmodulin is capable of binding free calcium ions and the M13 chain can bind with calmodulin after it has bound the calcium ions. This leads to a conformational change bringing GFP aside YFP. Under these conditions, FRET can happen. The genes of these four proteins are joined linearly, and the fusion genes can be expressed in a variety of cells.

Source: Olympus Microscopy Source Center

Cellular Imaging: FRET
Förster (Fluorescence) Resonance Energy Transfer



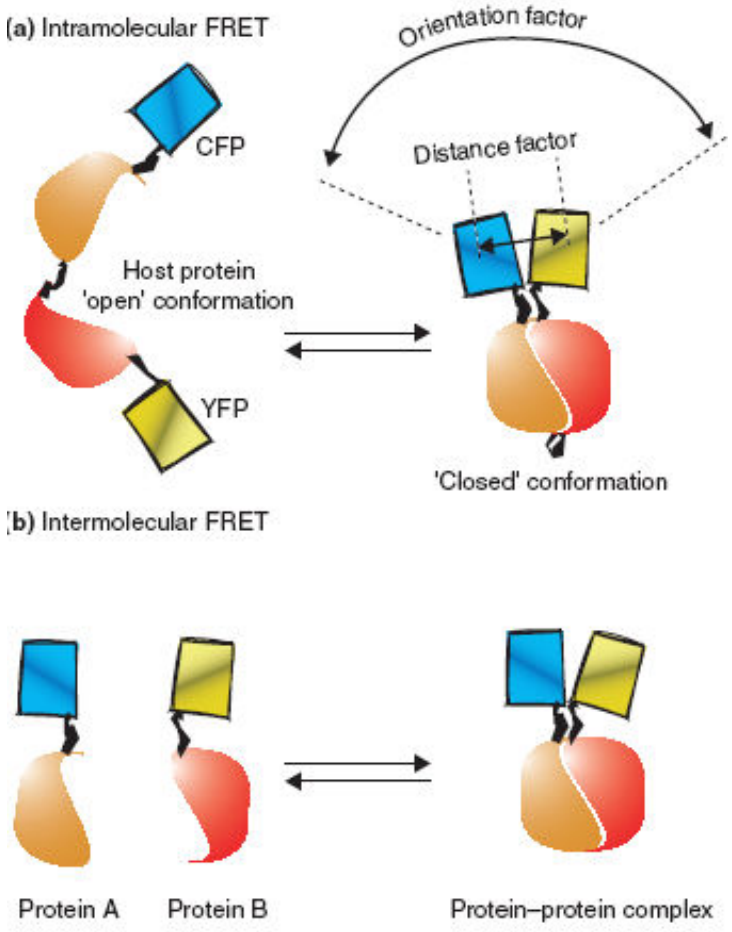


Occurrence of FRET
FRET is a phenomenon that occurs between a fluorescent dye (donor) and another dye (acceptor) when they are close (< 10nm) together. In that case the fluorescent energy is transferred without the emission of light form the donor to the acceptor.

Spectral requirements
Excitation and emission spectra of the FRET pair, CFP and YFP, illustrating the overlap of the CFP emission and the YFP excitation spectrum.
The emission specturm of the donor and the absorbtion spectrum of the acceptor are required to exhibit a significant overlap (approx. 30%) for FRET.

Taken from: http://www.bphys.uni-linz.ac.at/bioph/res/icg/fret.html

Cellular Imaging: FRET can also be used to study molecule – molecule interactions



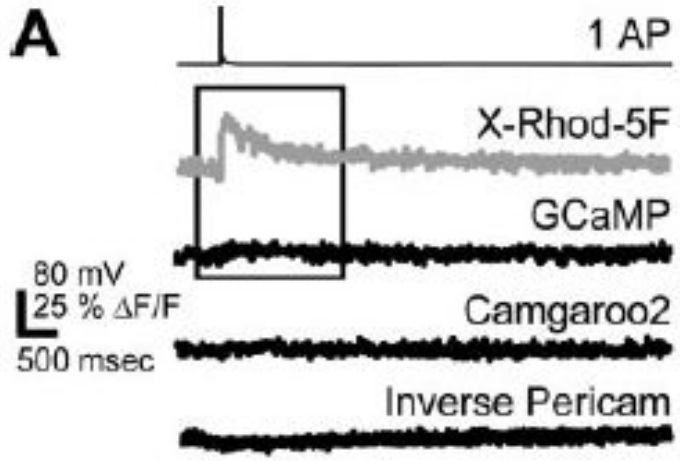
Intramolecular and intermolecular FRET.

(a) Intramolecular FRET can occur when both the donor and acceptor chromophores are on the same host molecule, which undergoes a transition, for example, between 'open' and 'closed' conformations.

(b) Intermolecular FRET can occur between one molecule (protein A) fused to the donor (CFP) and another molecule (protein B) fused to the acceptor (YFP). When the two proteins bind to each other, FRET occurs. When they dissociate, FRET diminishes.

Taken from: Truong & Ikura, Curr. Opin. Struct. Biol. 2001

Cellular Imaging:
Advantages and disadvantages of FRET



Taken from: Pologruto et al., J. Neurosci. 2004

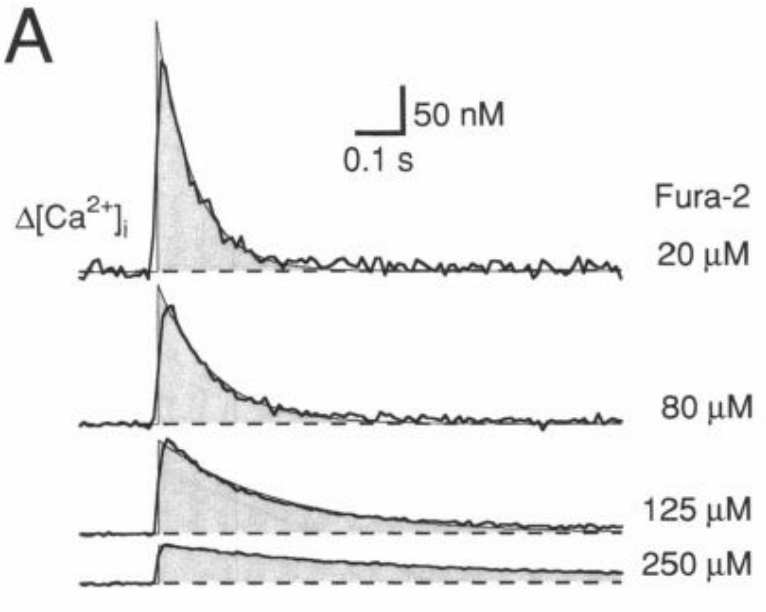
Advantages of FRET

Molecules which are able to generate FRET can be included in the genome of the cells of interest. The source of the calcium imaging signal is therefore defined. Different xFP pairs for FRET with different calcium affinities are available. The dye is already in the cell interior and it is not necessary to get it in.

Disadvantages of FRET

The kinetics of the response to changes in intracellular calcium are in principle the kinetics of the folding of the molecule which are slow in comparison to the electron shift in a classical CaSD. These dyes detect therefore primarily long-lasting spiking activity.

Cellular Imaging: FLIM
Fluorescence lifetime imaging microscopy



Dependence of dendritic $[Ca^{2+}]_i$ transients on Fura-2 concentration. $[Ca^{2+}]_i$ transients, evoked by single action potentials, imaged using 20, 80, 125, and 250 μM Fura-2, respectively. Exponential fits gave decay time constants of 78, 117, 265, and 703 ms, respectively.

Source: Helmchen et al., Biophys. J. 1996

Each fluorescent dye has its own lifetime in the excited state. FLIM can be utilized to visualize the factors that affect the fluorescence lifetime properties of dye molecules, i.e. the state of the environment around the molecule.

FLIM makes it possible to obtain information on the molecules while observing a living cell. The factors affecting the fluorescence lifetime include ion intensity, hydrophobic properties, oxygen concentration, molecular binding, and molecular interaction by energy transfer when two proteins approach each other. Lifetime is, however, independent of dye concentration, photobleaching, light scattering and excitation light intensity. Therefore, FLIM allows to perform accurate ion concentration measurement.

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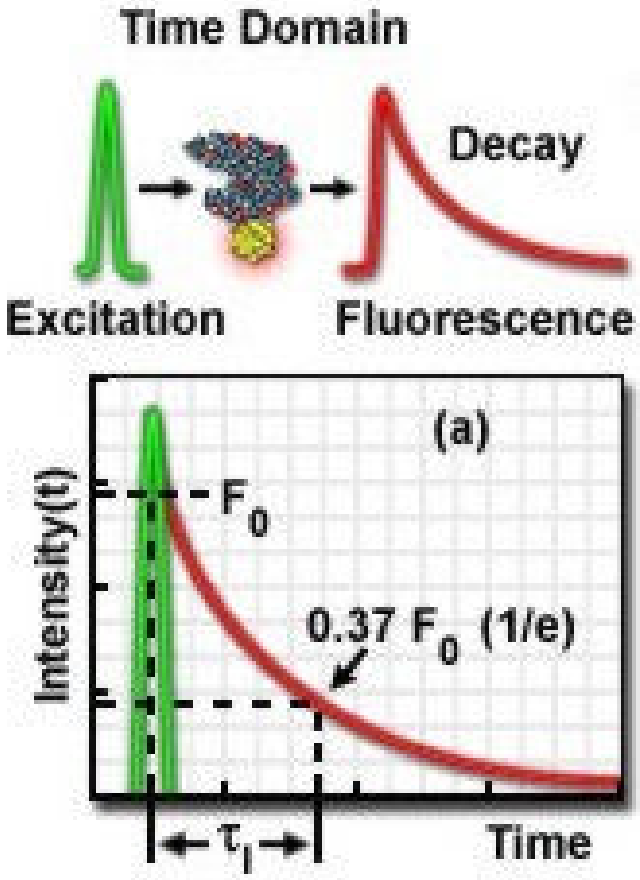
Cellular Imaging: FLIM



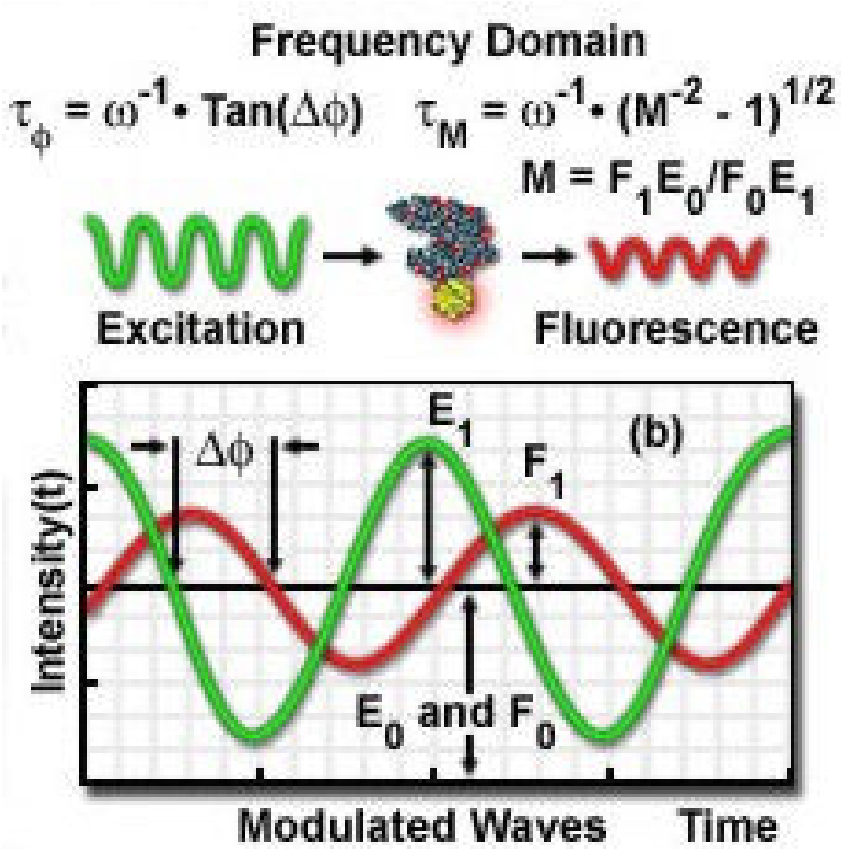
Two methods of FLIM: the time-domain method and the frequency-domain method.

Time-domain FLIM

When a calcium ion binds to a CaSD, both the fluorescence lifetime and the fluorescence intensity change. The conventional procedure for ion concentration measurement focuses on the change in intensity. However, also the ratio of dyes between bound and unbound calcium ion changes, and this subsequently leads to a change in the fluorescence lifetime of the measuring spot in the specimen. In addition to calcium ion probes, this technique is also applicable to the measurement of pH and other ions.

Source: Olympus Microscopy Source Center

Cellular Imaging: FLIM



Two methods of FLIM: the time-domain method and the frequency-domain method.

Frequency-domain FLIM

Fluorescence lifetime is calculated by measuring the phase shift of fluorescence ($\Delta\Phi$) and the reduction in its amplitude using a detector with a gain modulator when the laser used as the excitation light source is modulated (1 to 200 megaHertz). The measurement may be taken either by laser scanning (photomultiplier) or using a charge-coupled device (CCD).

Source: Olympus Microscopy Source Center

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Calcium imaging and voltage-sensitive imaging in neurons and neuronal tissues

Principles of imaging techniques

Detection of cellular and subcellular activity with CaSD and VSD imaging techniques

Detection of network activity with CaSD and VSD imaging techniques

Why should one use
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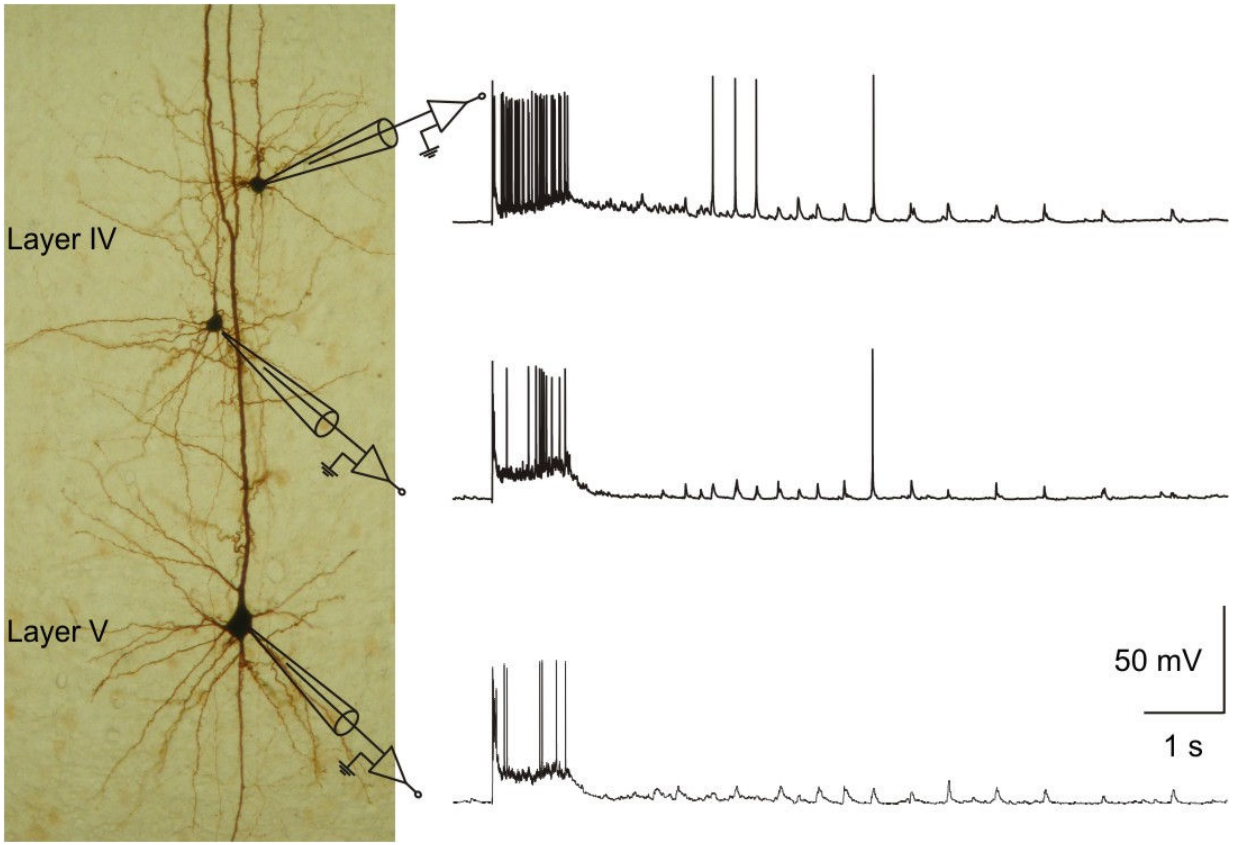
When are imaging techniques superior
to other physiological techniques?

Network activity studied in vitro : Possible techniques
1. Multi-cell patching



Advantages:

High temporal and spatial resolution

Recording of sub-threshold and action potential activity

Very good signal to noise ratio

Stimulation and recording from the same cell

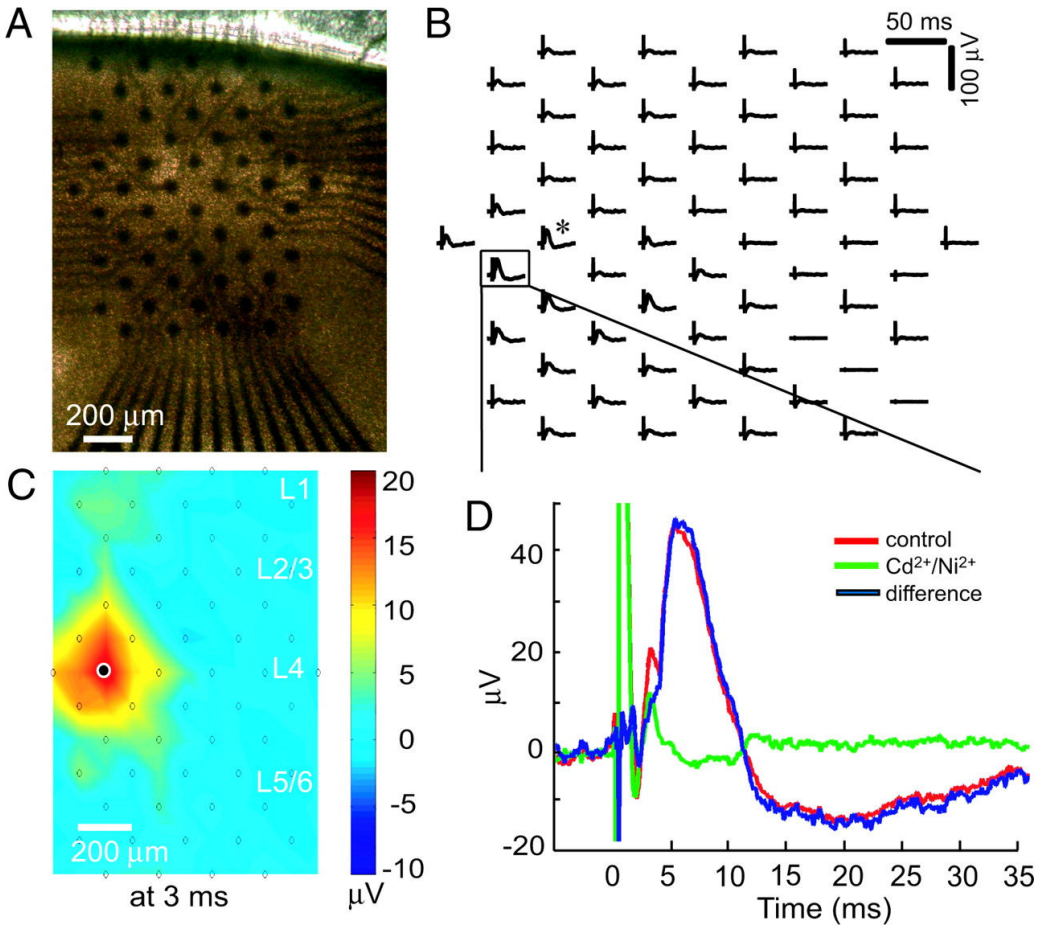
Disadvantages:

Limited number of recordings

Still the gold standard for imaging techniques !

Berger et al., 2006

Network activity studied in vitro : Possible techniques
2. Multi-electrode arrays (MEAs)



Advantages:

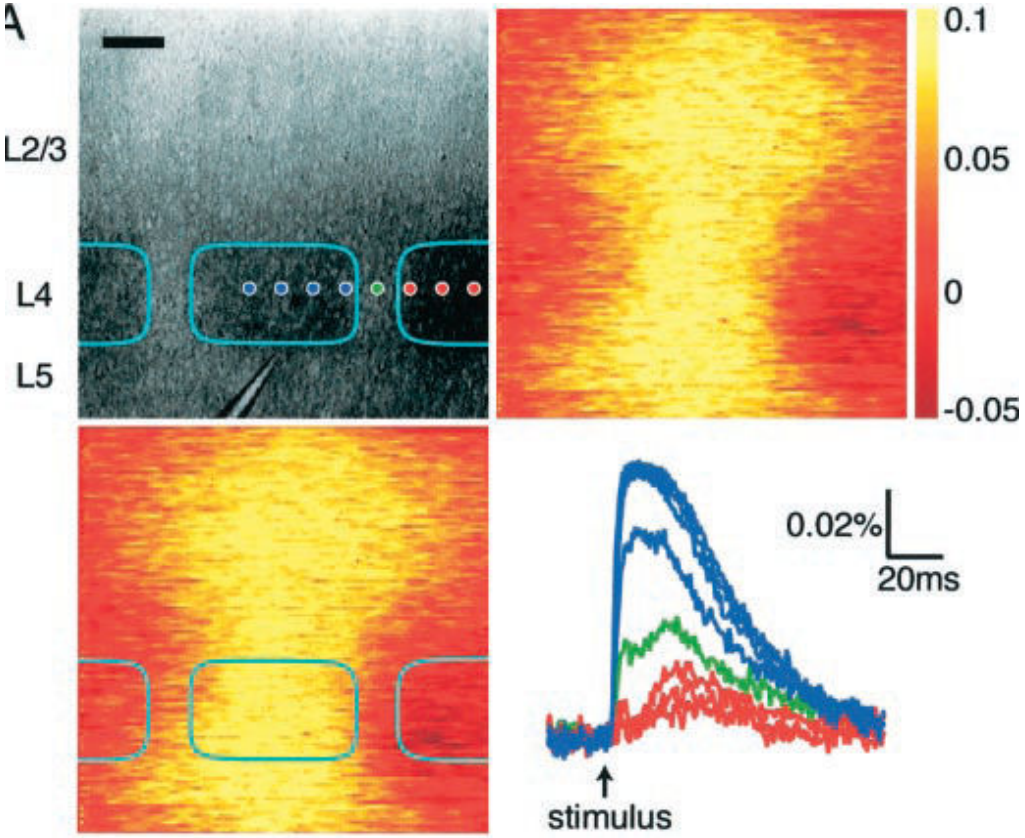
High temporal resolution
Recordings detect the activity in a large area of interest
Stimulation and recording from the same electrode possible

Disadvantages:

Limited spatial resolution (electrode distance ~200µm)
Underlying signal uncertain (synaptic activity versus action potentials)
Mediocre signal to noise ratio
Extraction of data can be complex

Wirth & Lüscher (2004); J Neurophysiol 91: 1635-1647

Network activity studied in vitro : Possible techniques
3. Optical imaging



Petersen & Sakmann (2001); J. Neurosci. 21: 8435-8446

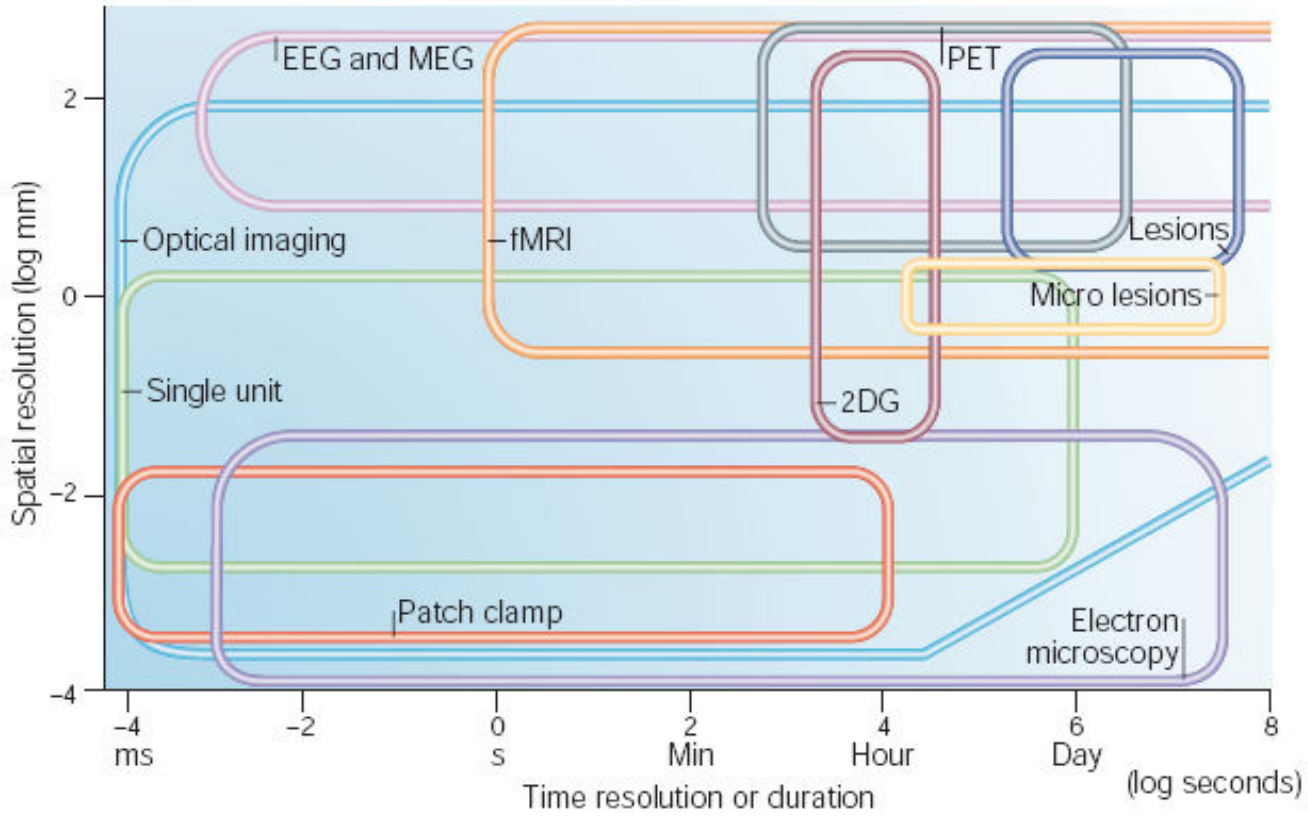
Advantages:

High temporal resolution (in dependence from the camera)
Spatial resolution sufficient (functional pixel size of ~10μm depending on camera sampling rate and optics)
Recordings detect the activity in a large area of interest
Stimulation and recording with different devices

Disadvantages:

Underlying signal uncertain (synaptic activity versus action potentials)
Signal can be indirect (e.g. imaging of the intracellular calcium)
Signal to noise ratio can be bad (e.g. imaging with VSDs)
Possible toxic dye effect (e.g. VSDs)
Dyes have to be applied, can be difficult (e.g. CaSDs)

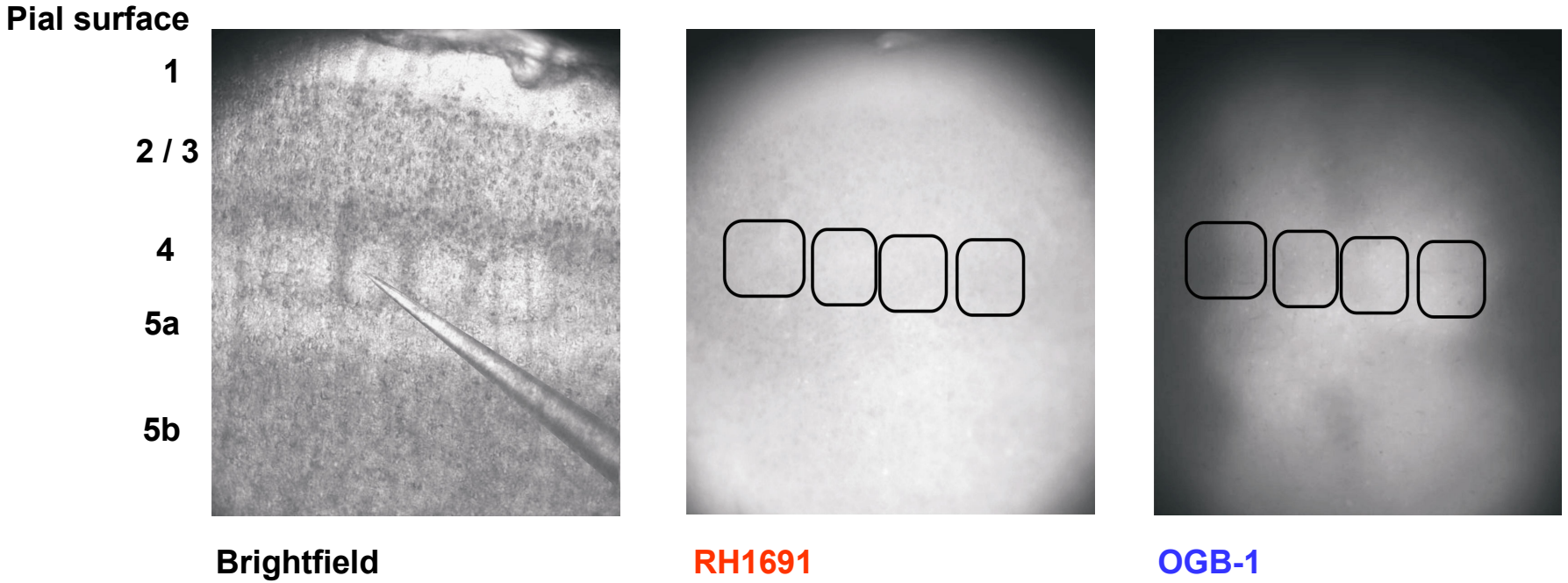
Network activity studied in vitro : Optical imaging has ideal properties with regard to spatial and temporal extent



Grinvald & Hildesheim, Nature Rev. Neurosci. 2004

Epifluorescence network imaging *in vitro*

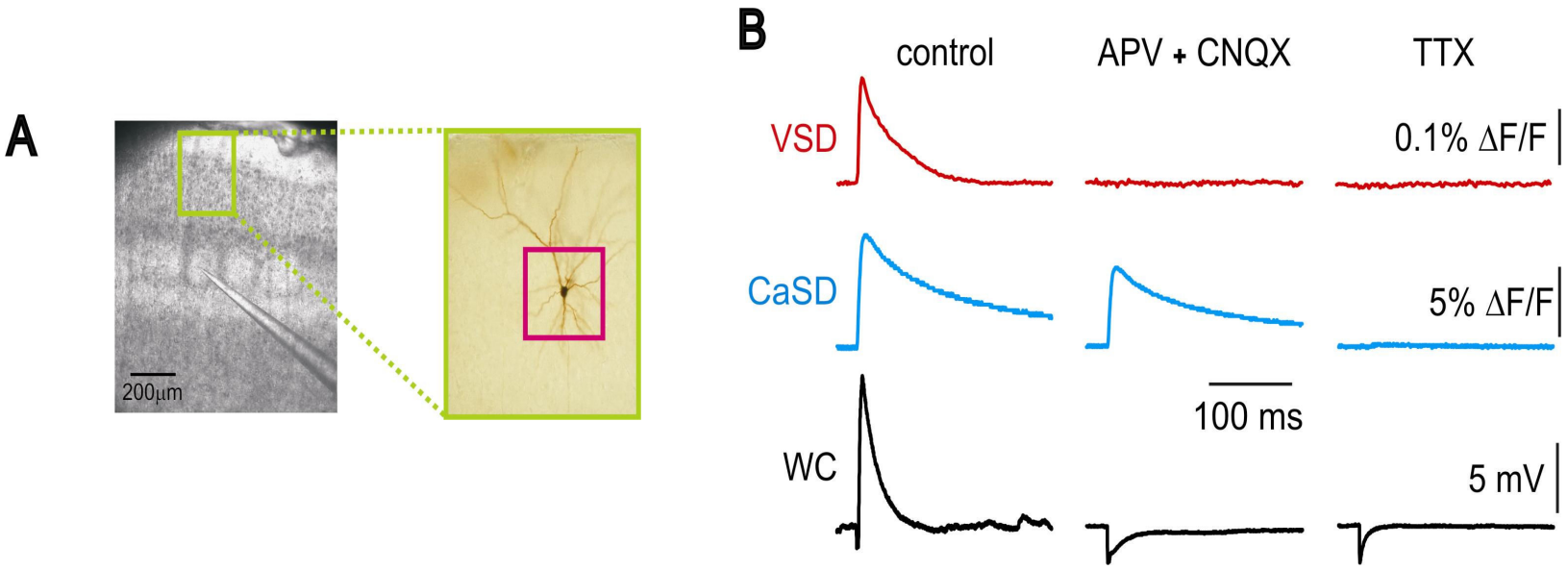
Epifluorescence imaging in the barrel cortex *in vitro*



Berger et al., 2007

Voltage-sensitive dye:
RH1691
Calcium-sensitive dye:
Oregon Green BAPTA - 1

The VSD response reflects postsynaptic activity, the CaSD response in contrast spiking activity



Imaging of membrane potential and intracellular calcium with RH1691 and OGB-1; combined with whole-cell patch-clamp recording and biocytin filling

Berger et al., 2007

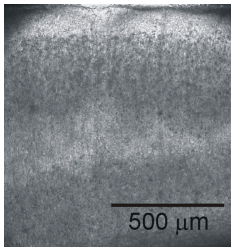
CaSDs and VSDs can be used to distinguish between input and output activity of a grey matter structure

1. Input activity reflected as postsynaptic events is detected by VSDs. These dyes do not “see” on the network level any spiking activity because the surface of axonal membranes is much less then the surface of dendritic membranes. Exception: White matter tracts.

2. Output activity reflected as action potentials in somata is detected by CaSDs. These dyes do not “see” subthreshold activity and do not “see” spikes in incoming fibers because their volume is too small in comparison to somata.

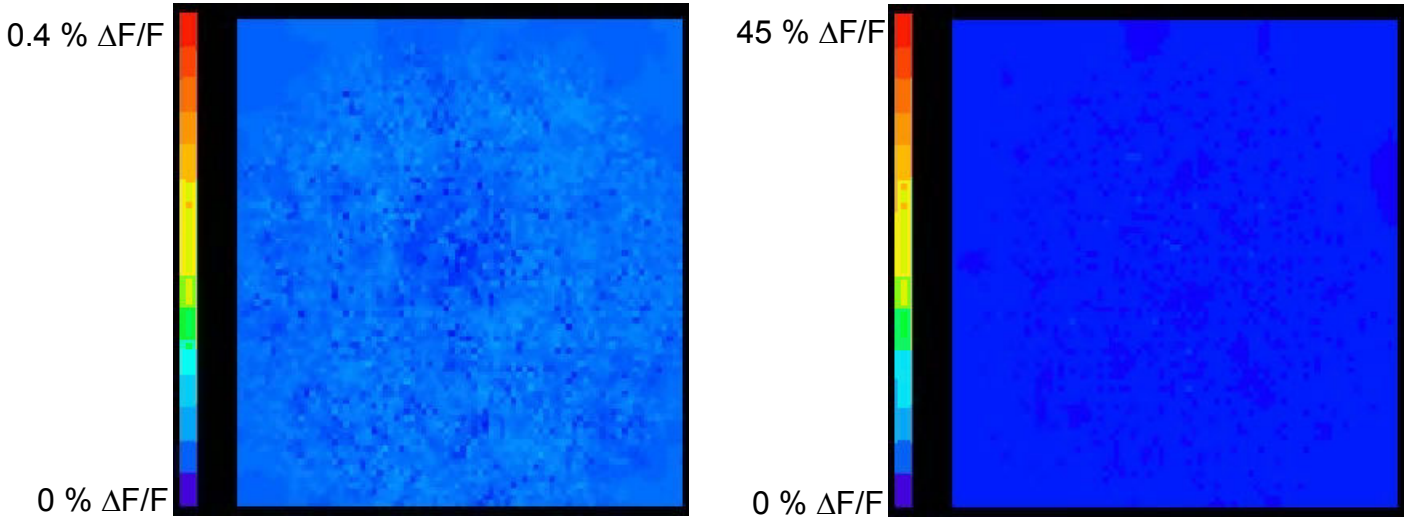
Be aware: This is only true for epifluorescence, not for 2-Photon !

Information flow within the barrel cortex



Membrane potential:
RH1691

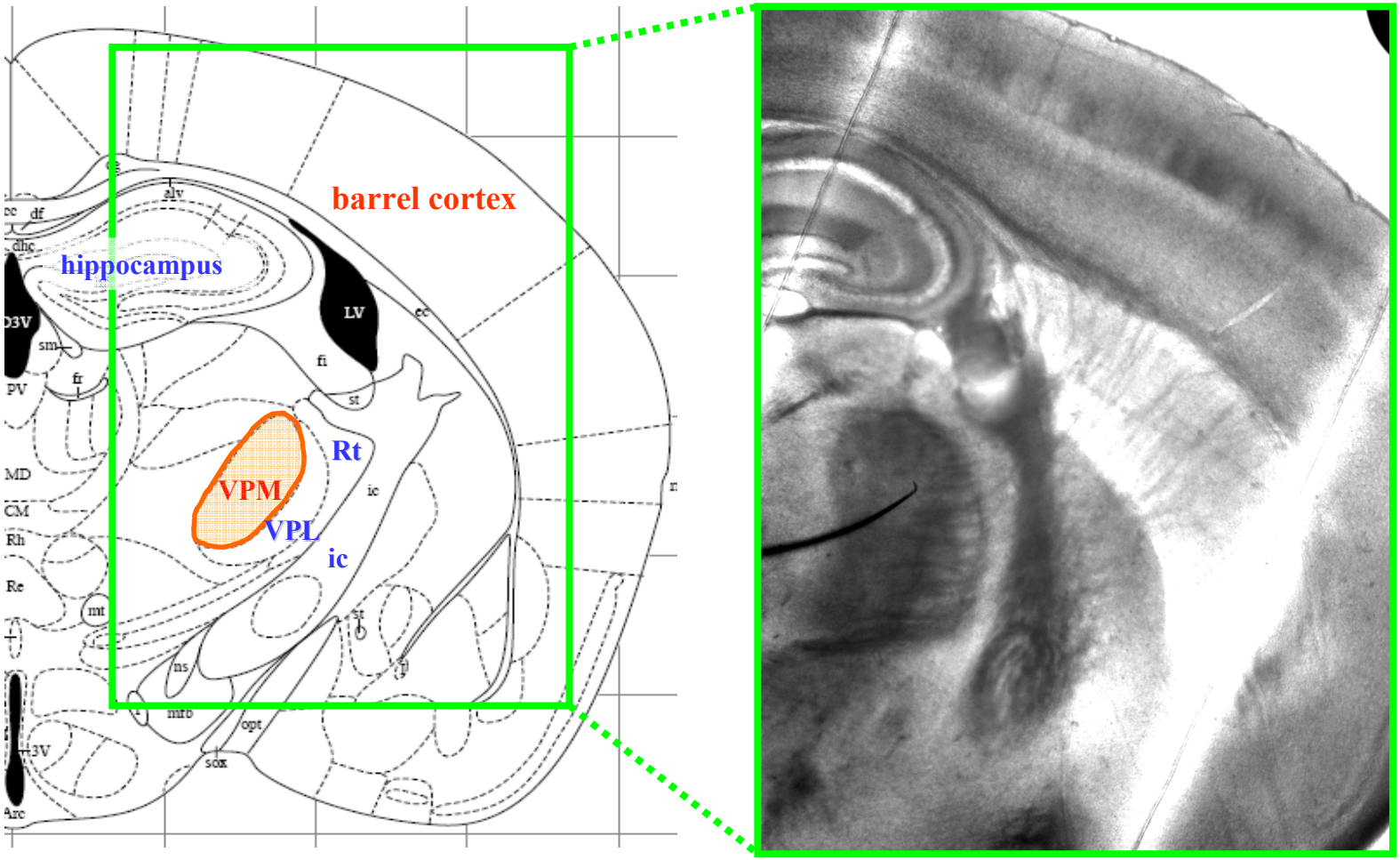
Intracellular calcium concentration:
OGB-1



Consecutive imaging of membrane potential and intracellular calcium with RH1691 and OGB-1
Total duration of the movies: 50 ms; Display slowed down 1:300; Sampling frequency 2 kHz

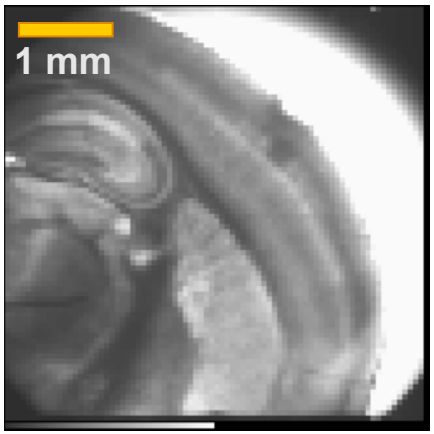
Berger et al., 2007

Information flow within the thalamocortical system: Thalamocortical Slices

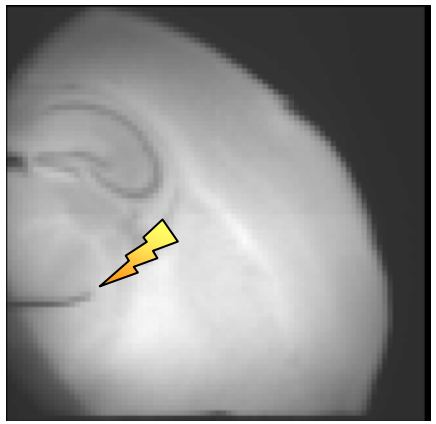


Taken from: Paxinos Mouse Atlas

Information flow from thalamus to barrel cortex: Subthreshold activity detected with VSDs

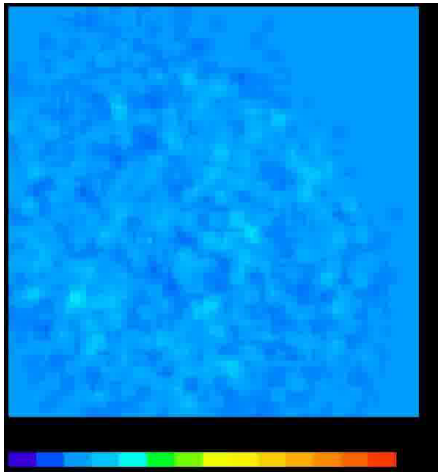


brightfield

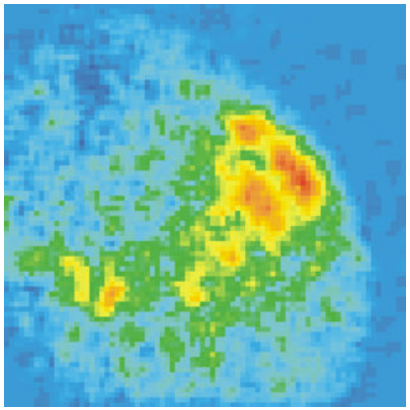


resting fluorescence
RH1691

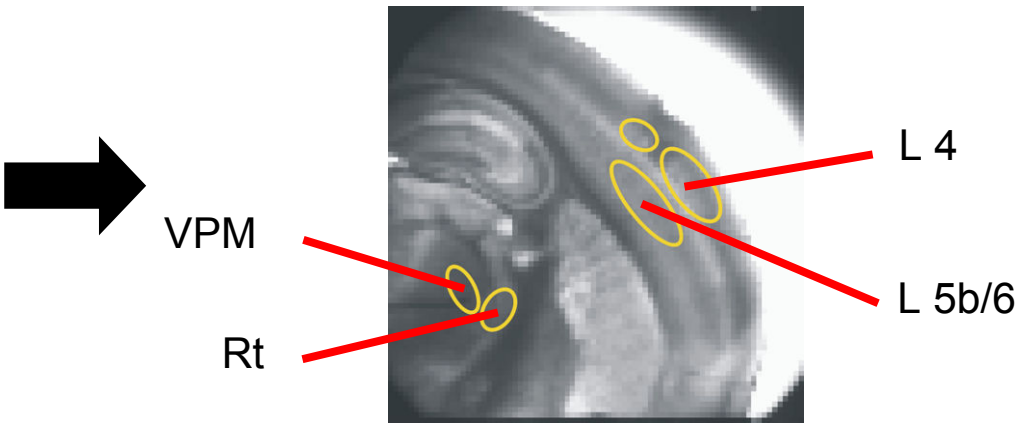
0% ΔF/F



0.1% ΔF/F



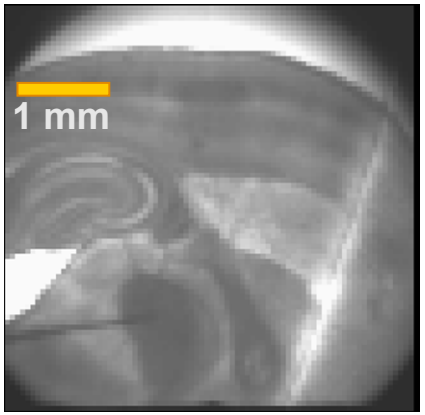
peak ΔF/F



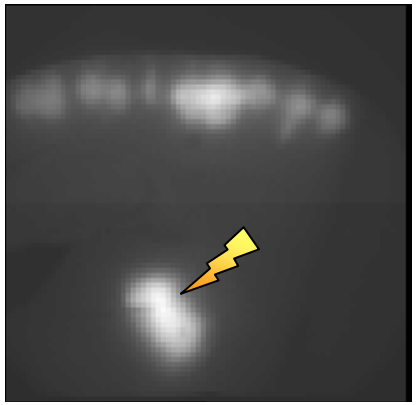
Imaging of membrane potential with RH1691; Activity upon a single 400 us stimulus in VPM
Total duration of the movie: 120 ms; Sampling frequency 1 kHz

Neubauer & Berger, 2008

Information flow from thalamus to barrel cortex: Suprathreshold activity detected with CaSDs

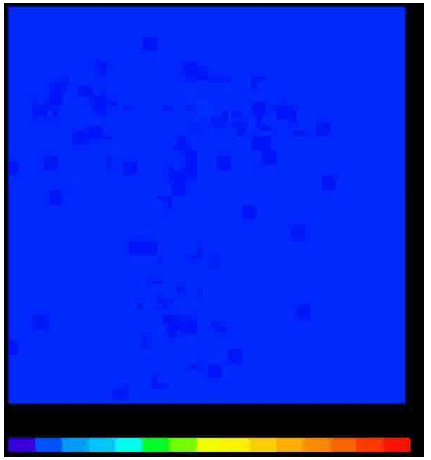


brightfield

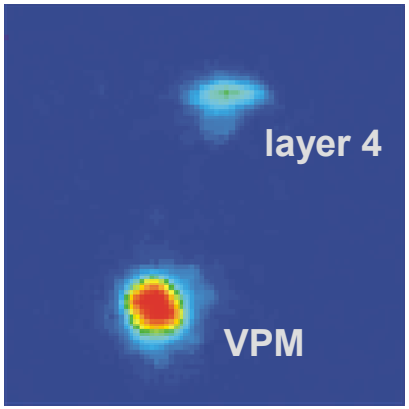


resting fluorescence
OGB1

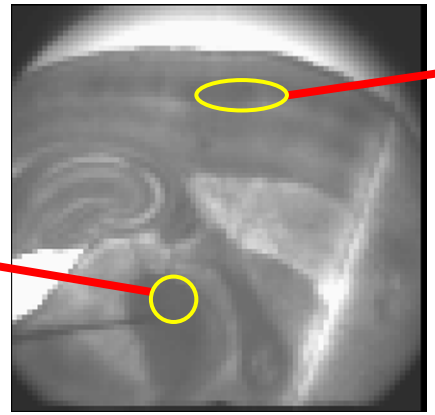
0% ΔF/F



3% ΔF/F



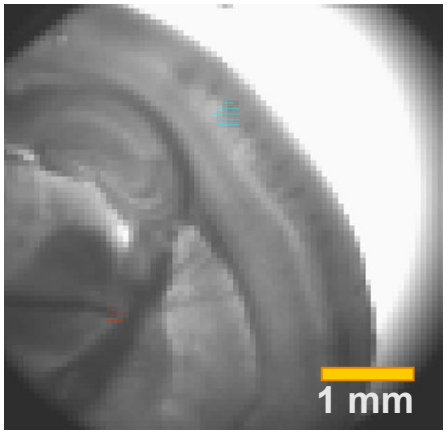
peak ΔF/F



Imaging of membrane potential with OGB1; Activity upon a single 400 μs stimulus in VPM
Total duration of the movie: 170 ms; Sampling frequency 1 kHz

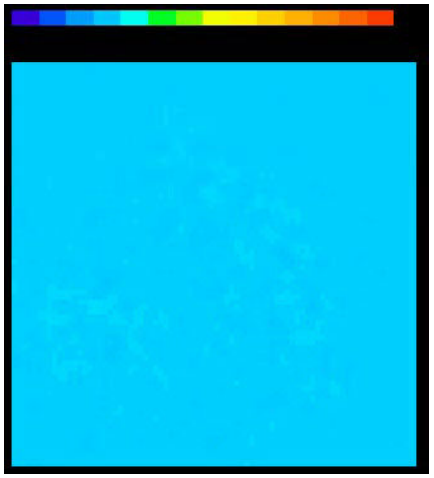
Neubauer & Berger, 2008

Initiation of cortical upstates following thalamic stimulation



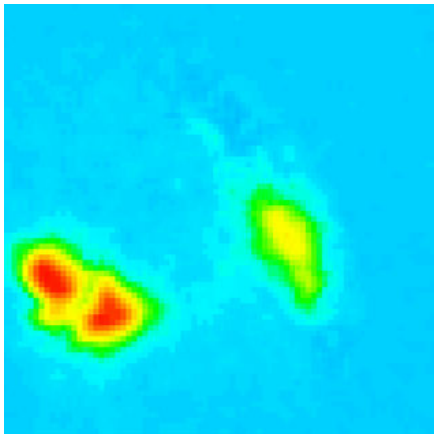
brightfield

0% ΔF/F

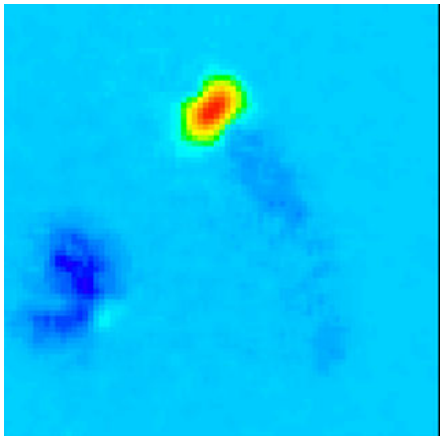


3% ΔF/F

OGB1 fluorescence changes



peak ΔF/F sensory input

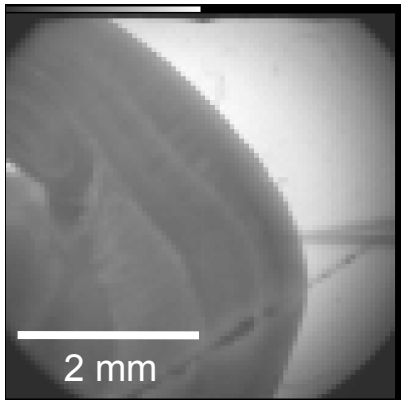


peak ΔF/F upstate

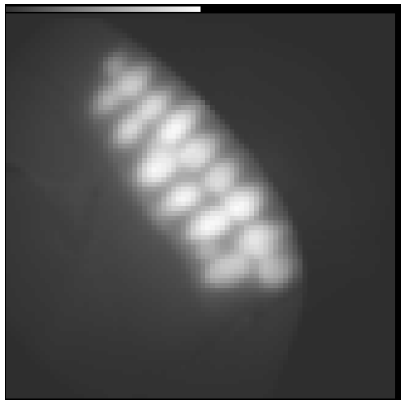
Imaging of intracellular calcium with Fluo-3; 500us stimulations in the thalamic VPM (4 pulses @ 40Hz)
Total duration of the movie: 2720 ms; Sampling frequency 1kHz

Neubauer & Berger, 2008

Initiation of cortical upstates using a modified ionic composition

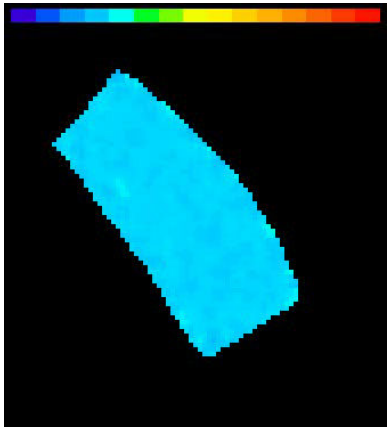


Brightfield



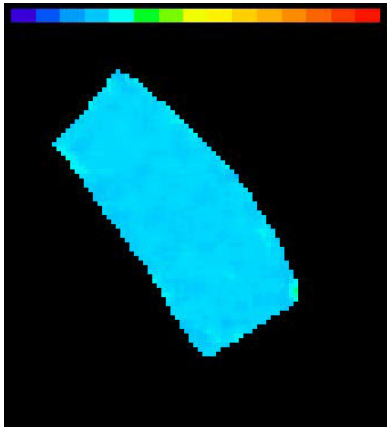
OGB-1 resting fluorescence

0% ΔF/F



ACSF

6.3% ΔF/F



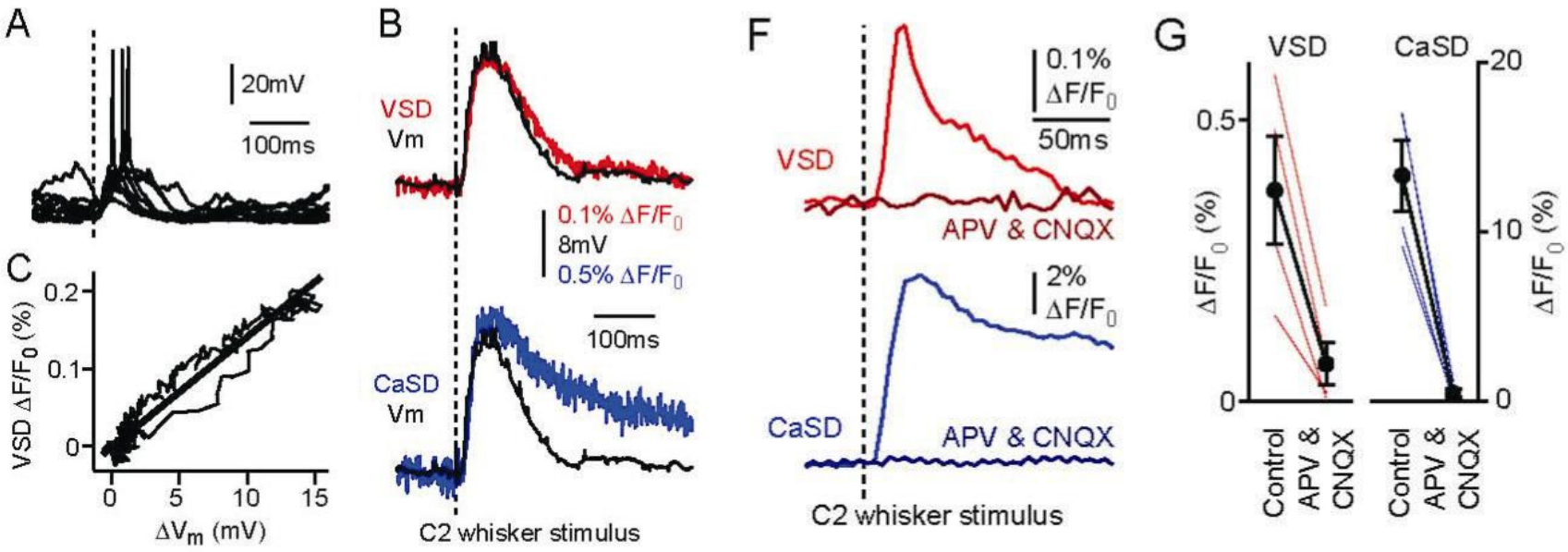
mACSF: 4.5 K, 1.5 Ca, 0.5 Mg

Imaging of intracellular calcium with OGB-1
Total duration of the movies: 30 s; Display fastened 1.25 : 1; Sampling frequency 125 Hz

Neubauer & Berger, 2008

Epifluorescence network imaging *in vivo*

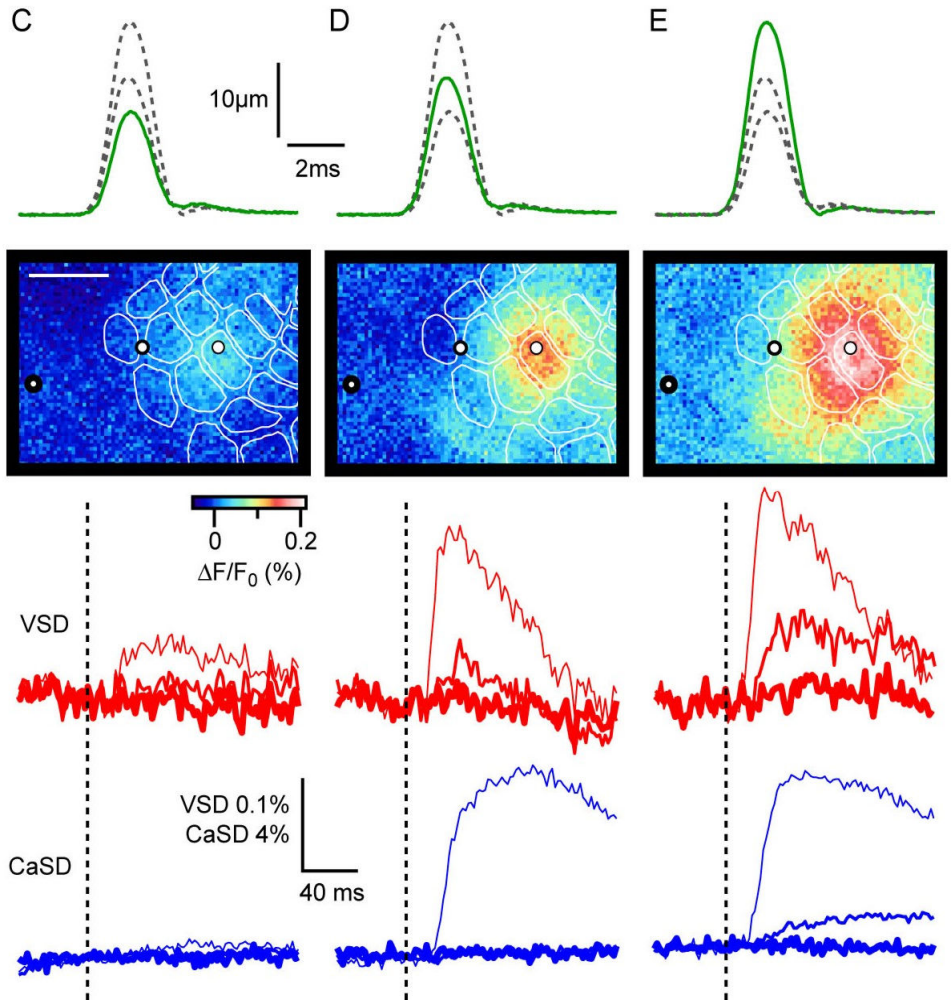
Combined VSD and CaSD imaging *in vivo*



Identified C2 barrel in vivo; whisker moved
Imaging of membrane potential and intracellular calcium with RH1691 and OGB-1;
combined with whole-cell patch-clamp recording

Courtesy of Aren Borgdorff,
Bruno Fauvet,
and Carl Petersen, EPFL

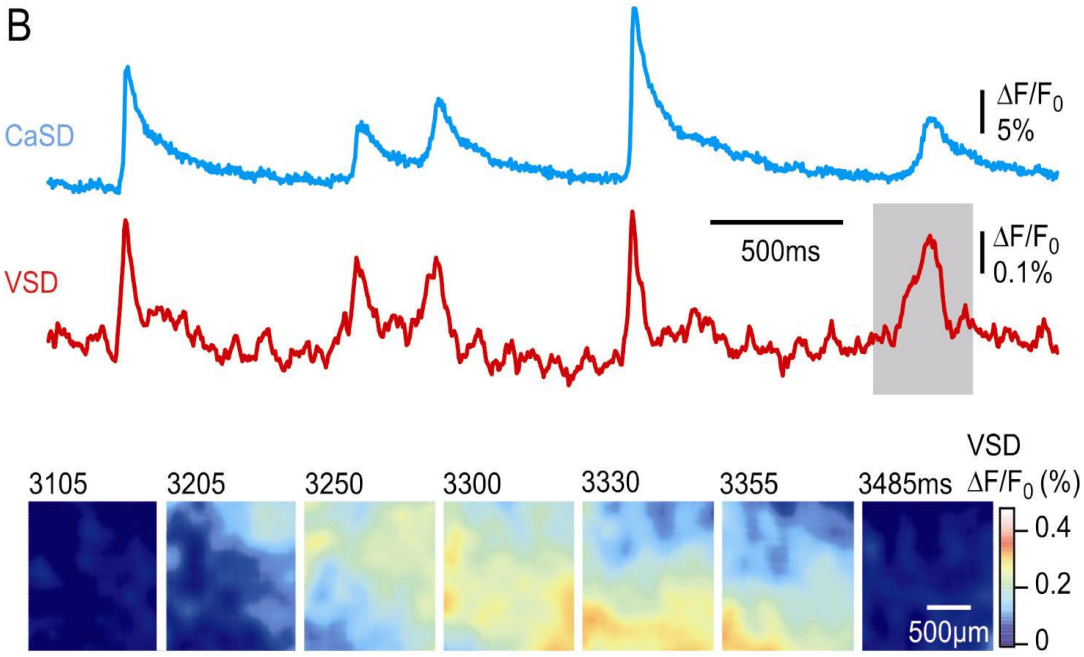
Spread of the VSD and CaSD signal *in vivo*



Identified C2 barrel in vivo; whisker moved at three intensities
Imaging of membrane potential and intracellular calcium with RH1691 and OGB-1;

Courtesy of Aren Borgdorff
and Carl Petersen, EPFL

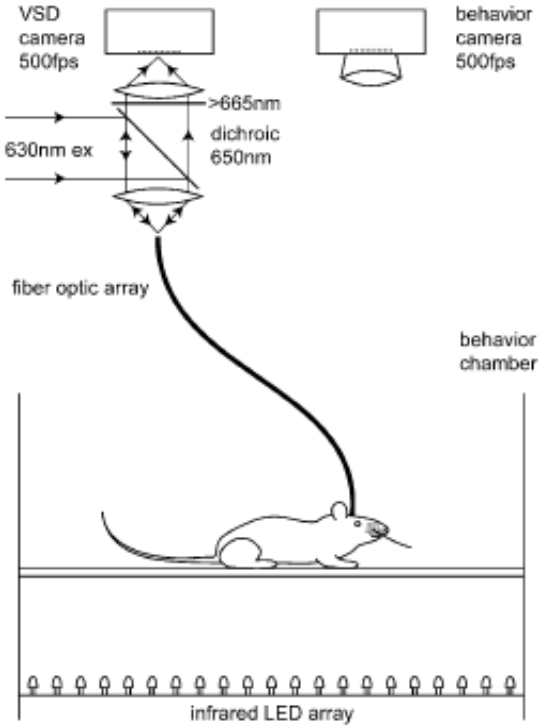
Simultaneous VSD and CaSD imaging of spontaneously ongoing activity *in vivo*

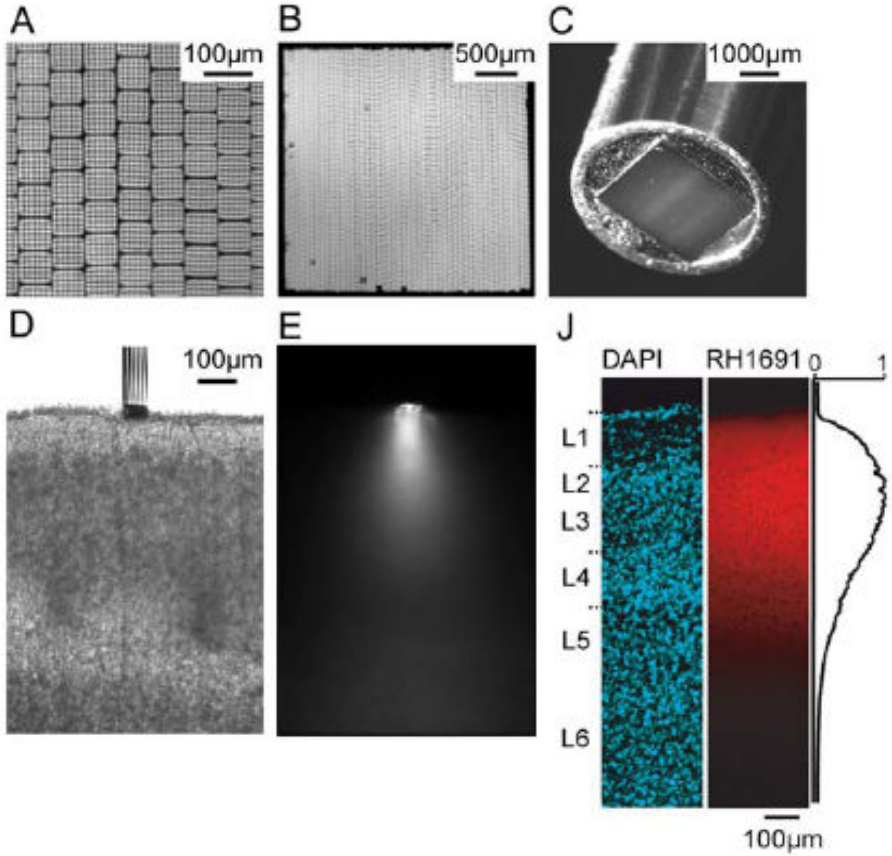


Identified C2 barrel in vivo
Imaging of membrane potential and intracellular calcium with RH1691 and OGB-1; simultaneous recording with two cameras

Courtesy of Isabelle Ferezou, Alan Carlton and Carl Petersen, EPFL

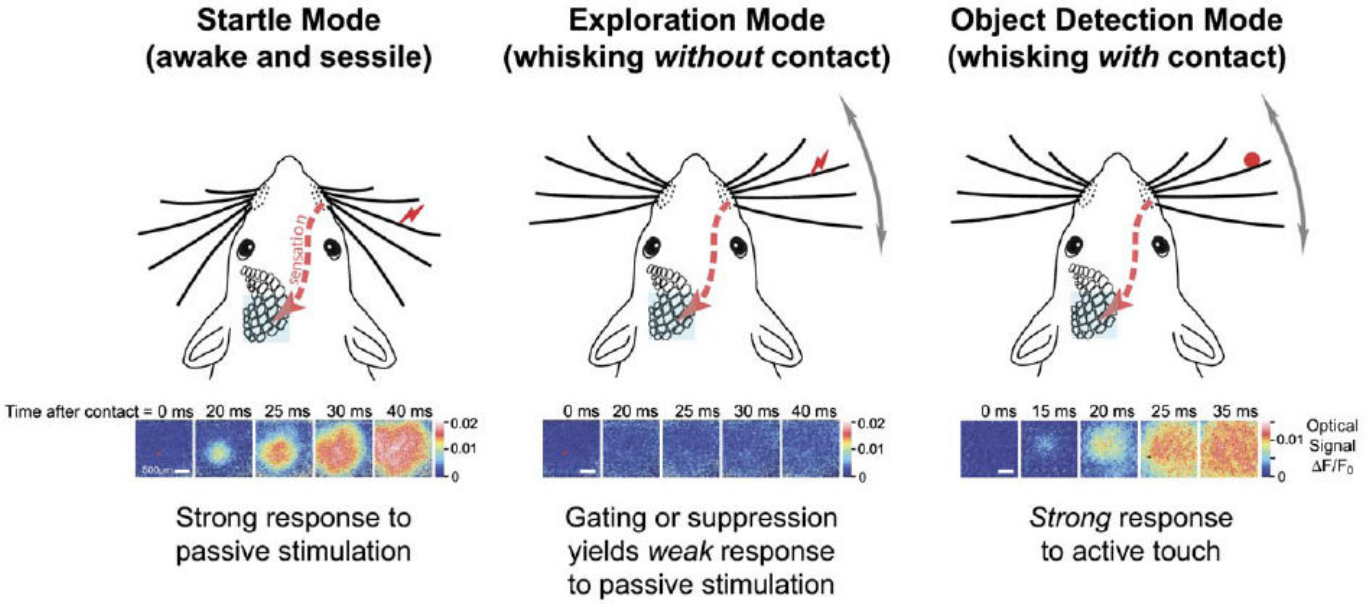
VSD imaging in behaving animals





Ferezou et al., Neuron 2006

VSD imaging in behaving animals

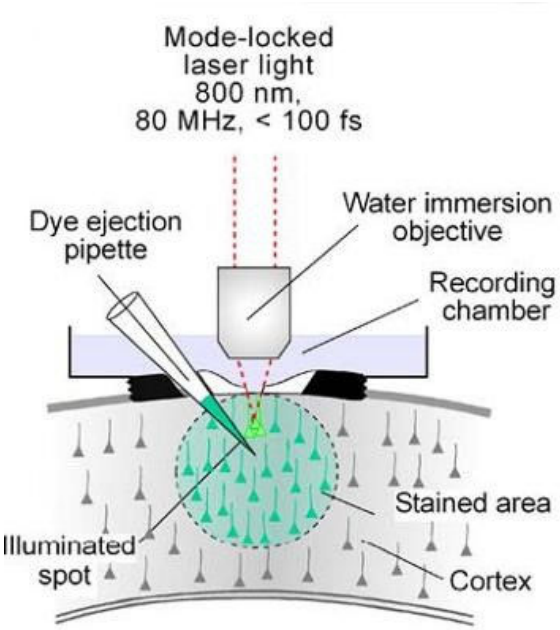


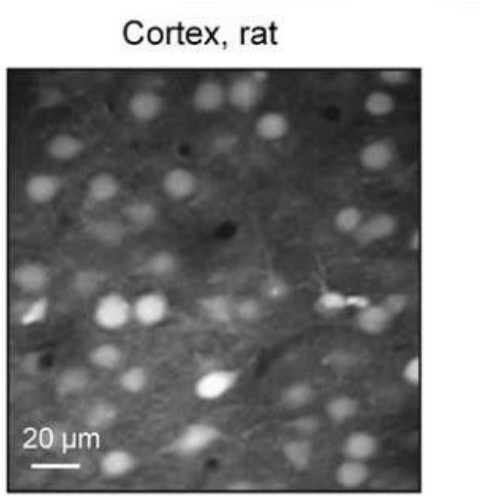
Summary of behavioral states and associated cortical responses in whisking mice. The upper panels are caricatures of the behavior of the mouse in three different awake states: an “immobility mode’’, an ‘‘exploration mode’’ that is accompanied by rhythmic sweeps of the vibrissae in air, and an ‘‘object-detection mode’’ that corresponds to palpation of an object with the vibrissae. A sequence of images, which reports the spatially averaged change in the membrane potential of neurons in superficial layers of vibrissa cortex, corresponds to a typical response for each of the three awake states. Note the large difference between active and passive stimulation in the two whisking states.

Ferezou et al., Neuron 2006

Two photon network imaging *in vivo*

Two photon imaging in vivo : Loading CaSDs



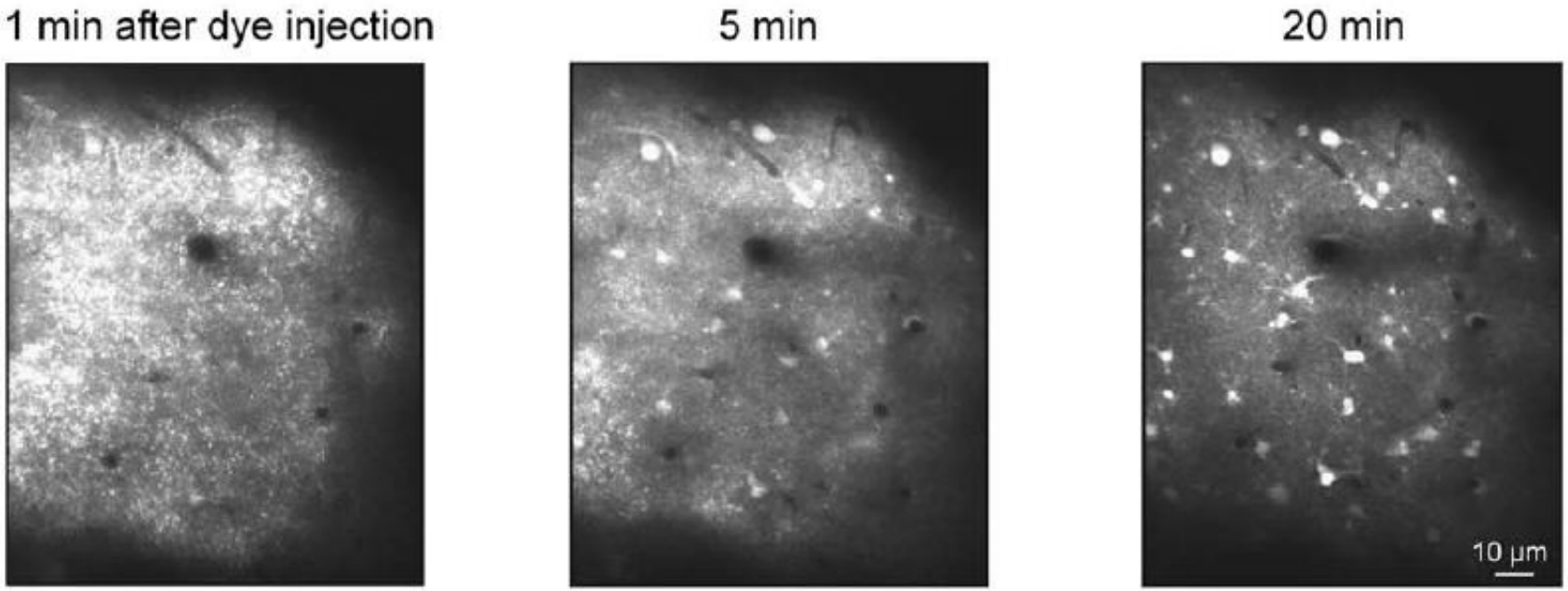


Multi-cell bolus loading enables in vivo staining of neural networks.

Schematic drawing of the experimental arrangement. In vivo image of OGB-1 stained cells in the cerebral cortex of a rat. The image was obtained using two-photon laser scanning microscopy.

Garaschuk et al., Pflügers Arch. 2006

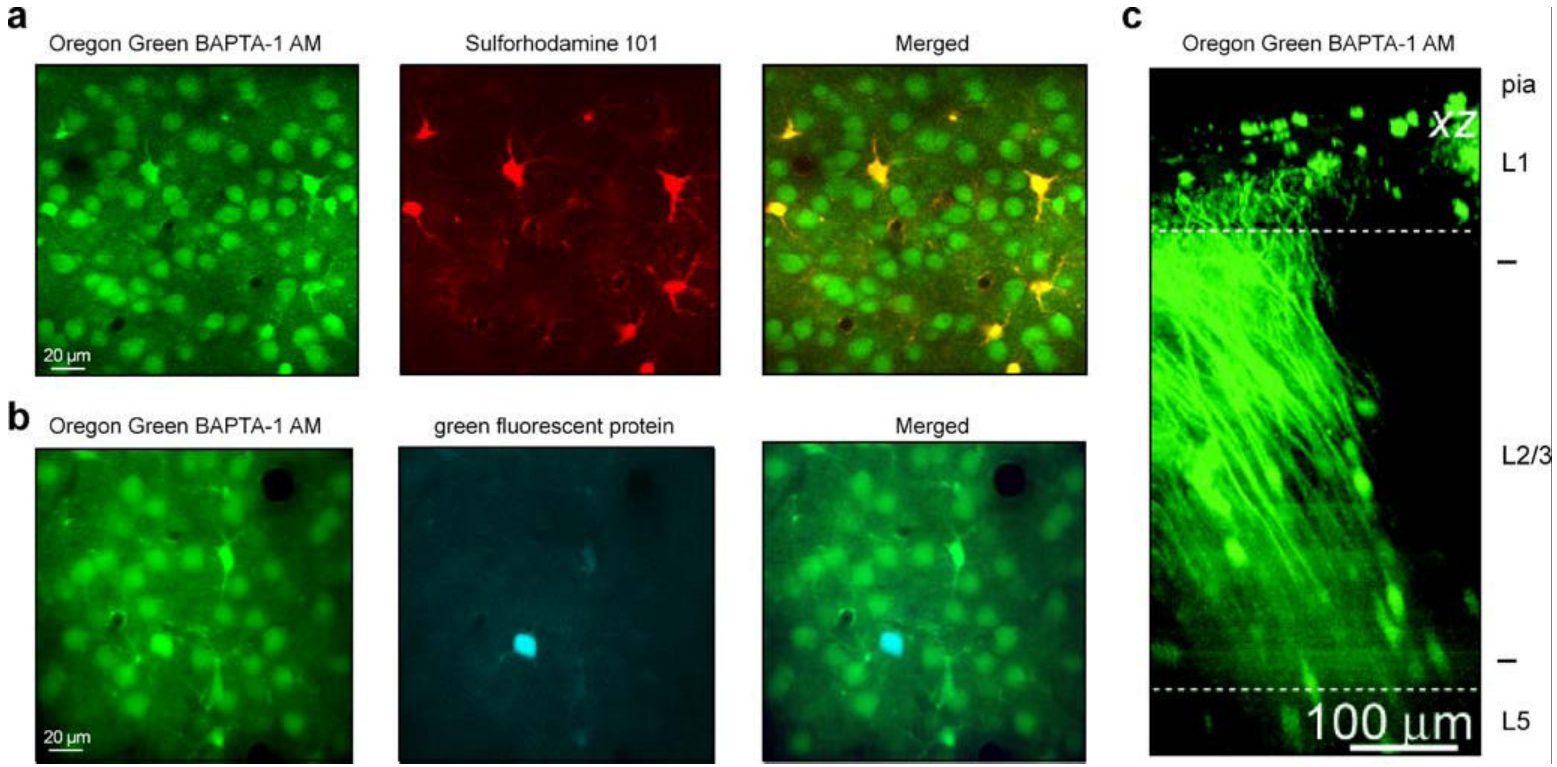
Two photon imaging in vivo : Uptake of the CaSDs



Removal of extracellular dye molecules and uptake into the cells. Images of layer 2/3 cells in the mouse visual cortex taken *in vivo* 1, 5, and 20 min after injection of the AM ester of the CaSD. Gradual removal of the dye from the extracellular space and an increasing brightness of individual cells.

Garaschuk et al., Pflügers Arch. 2006

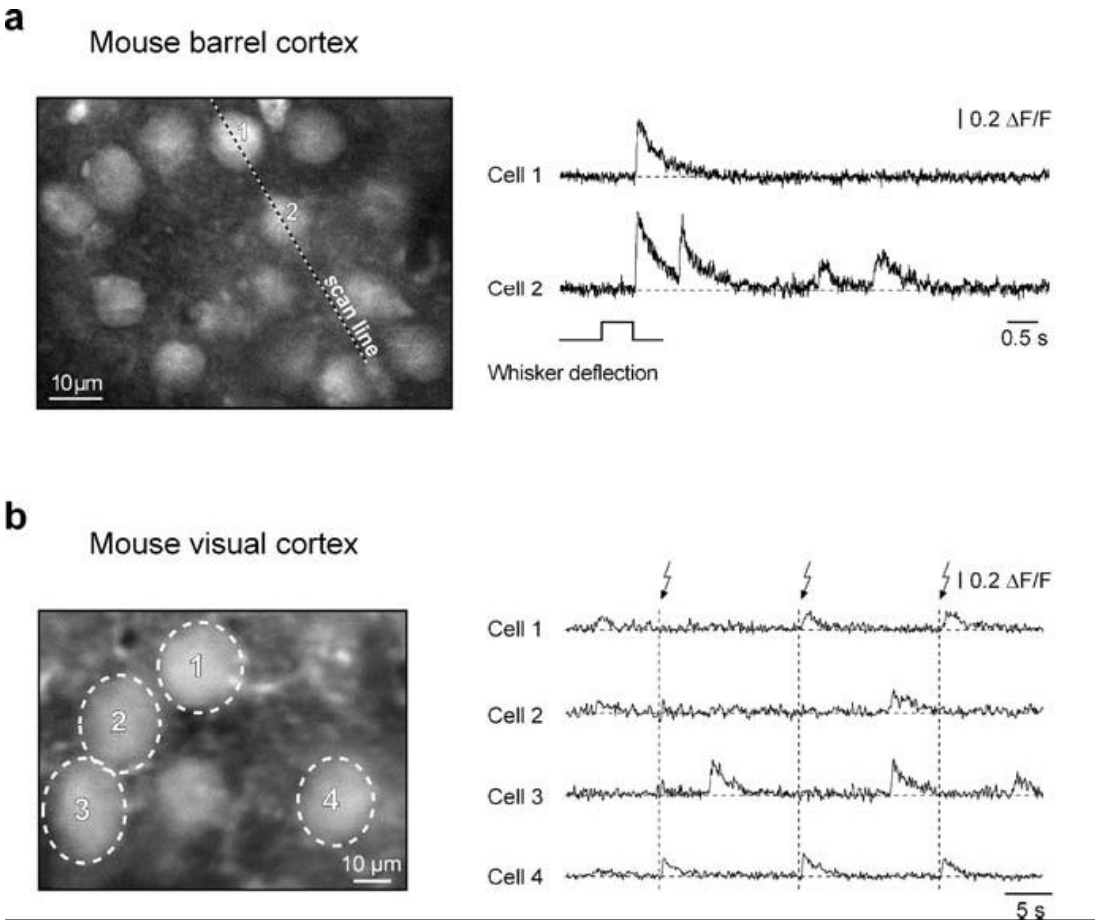
Two photon imaging in vivo : Identified cell types



(a) Microphotographs of layer 2/3 cells in the mouse visual cortex stained with a dye mixture of OGB-1 AM and a glial marker sulforhodamine 101. OGB-1 : green channel ; sulforhodamine 101: red. The merged image on the right shows neurons in green and glial cells in yellow. (b) OGB-1 staining of layer 2/3 cells (left) in a transgenic mouse expressing EGFP (middle) selectively in somatostatin-positive GABAergic interneurons. The merged image shows all cells in green and a somatostatin-positive interneuron in light blue. (c) A side projection of dendrites of layer 5 pyramidal neurons.

Garaschuk et al., Pflügers Arch. 2006

Two photon imaging in vivo : Sensory network responses

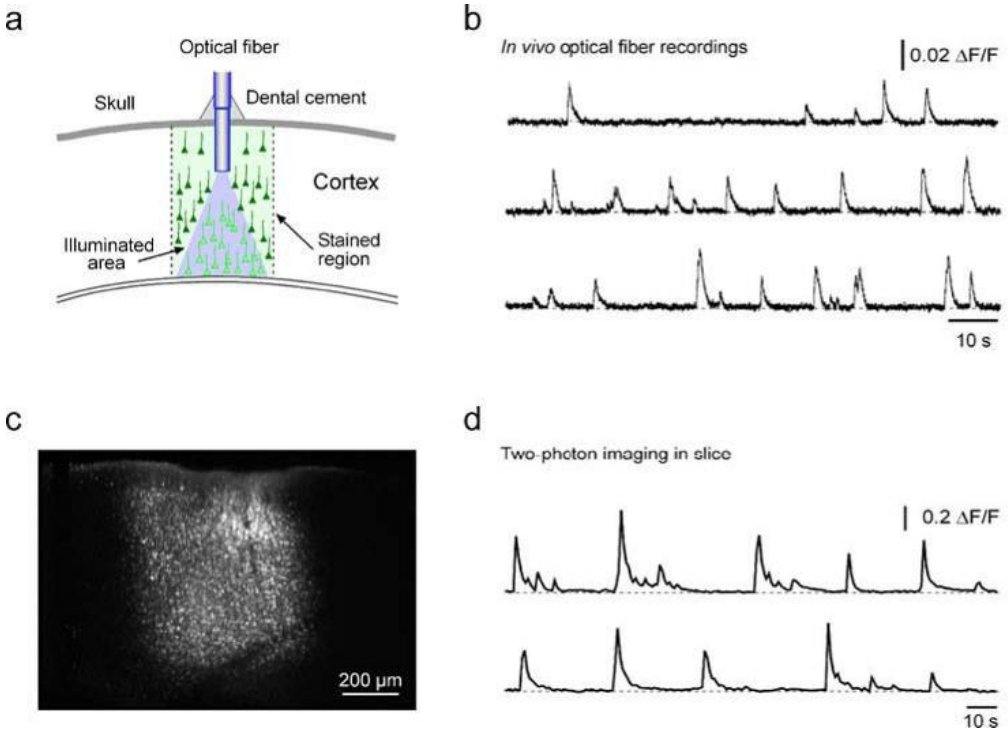


(a) Individual neurons in the mouse barrel cortex and the corresponding calcium transients evoked by the deflection of whiskers at the contralateral side of the snout. The transients were recorded using the line-scan mode (5 ms/line).

(b) Individual neurons in the mouse visual cortex and the corresponding calcium transients evoked by brief light flashes.

Garaschuk et al., Pflügers Arch. 2006

Two photon imaging in vivo : Fiber recordings

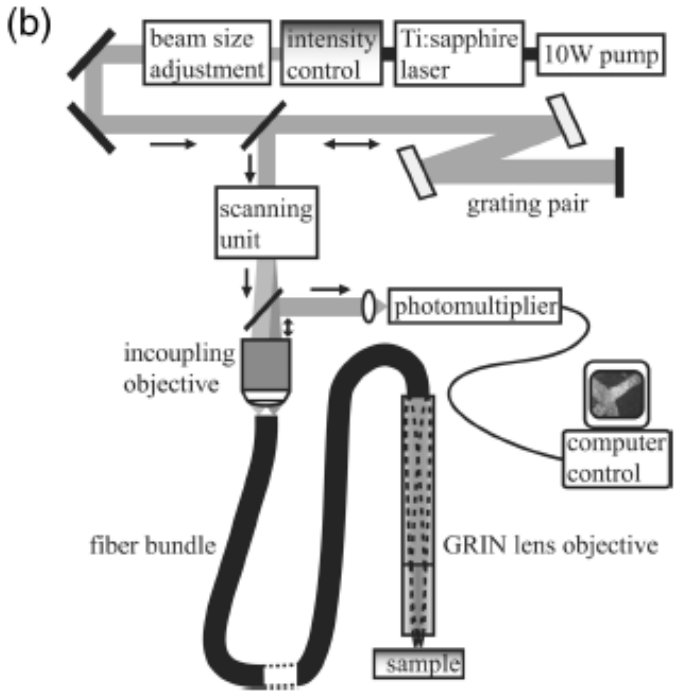


In vivo brain microendoscopy. (a) A schematic drawing illustrating an implanted optical fiber. (b) In vivo recordings of spontaneous cortical calcium waves in a non-anesthetized newborn mouse. (c, d) A two-photon image of the in vivo stained cortical area (c), where the calcium waves shown in d were recorded from. Slice preparation.

These fiber recordings detect only activity in networks active at the same time, not in single cells.

Garaschuk et al., Pflügers Arch. 2006

Two photon imaging in behaving animals



Scheme of the optical setup.
Laser pulses double pass a pair of diffraction gratings before they are coupled into the 1.4-m-long fiber bundle through a standard two-photon laser-scanning microscope. Fluorescent light is detected through the same fiber bundle and the incoupling objective by a photomultiplier tube.

Göbel et al., Optics Letters 2004