Optogenetics and Multiphoton Excitation

June 2014

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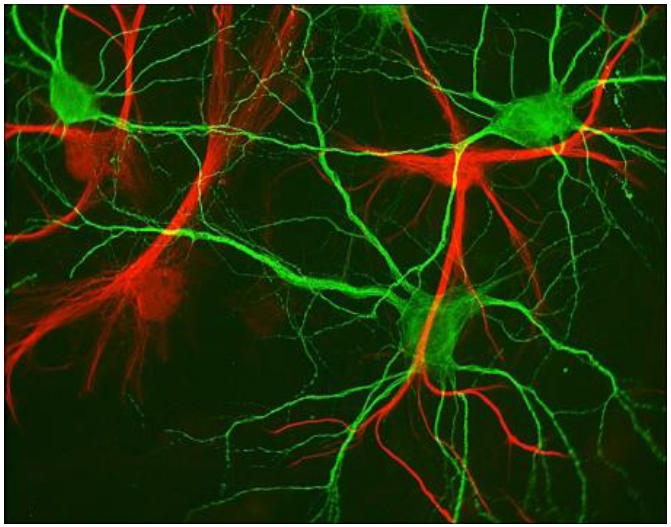
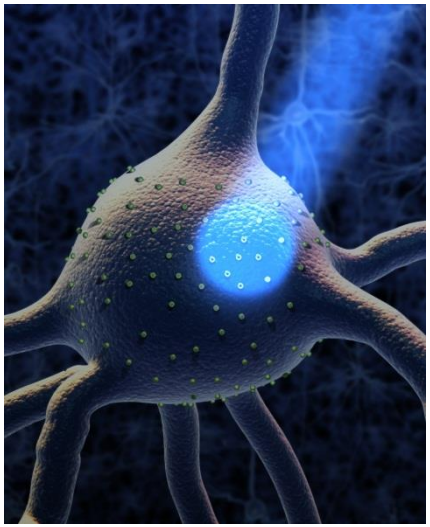
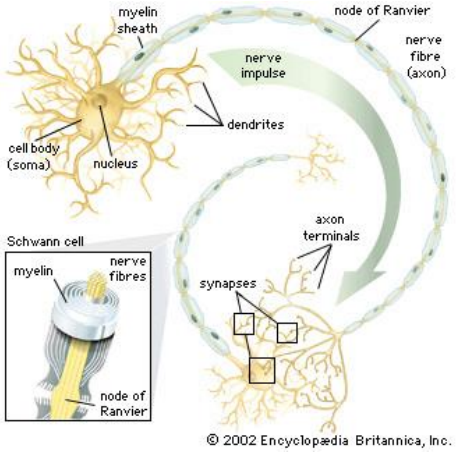
Optogenetics and Multiphoton Excitation (MPE)

- MPE is used in Optogenetics for the usual advantages related to non-linear excitation:
  - Deeper penetration (to ~1 mm)
  - Damage-free imaging
  - Precise targeting of individual neurons or selected group of neurons
- Studies in live animals are likely to be connected to behavioral patterns, but analyzed to the physiology of individual neurons or groups of neurons.
- This means that most MPE-based Optogenetics studies will also include MPE imaging of the brain and/or measurement of electrical signals visualized as fluorescence (see next slides)

The Genetically Encoded Neuroscience Revolution







MPE imaging
Imaging neurons
(Fluorescent proteins)

Optogenetics
Activating neurons
(Oopsins: Ch-R2)

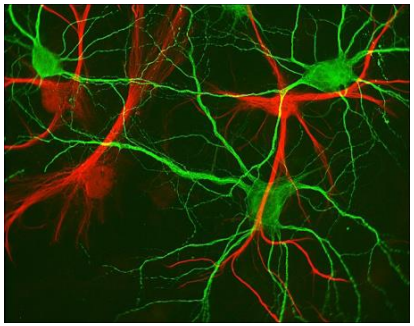
Ca signals detection
Monitoring neurons
(GECI: GCaMP)

A True Revolution in Neuroscience

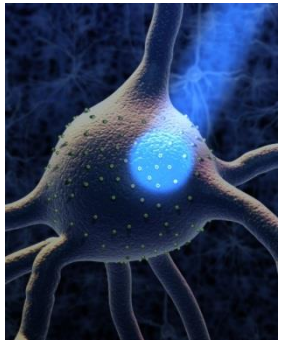
- Genetic expressions eliminate toxicity, unwanted effects and behaviors
- Live animal studies over long lifespans are now an acquired tool
- Perfect match with low damage and imaging depth of MPE

The Processes Involved

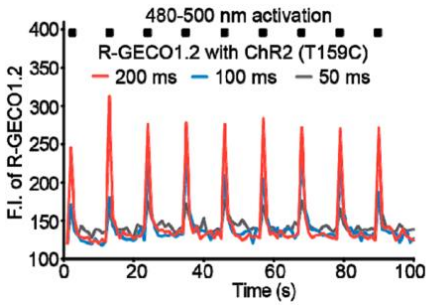
Imaging:
- Does not need to be repeated. Purpose is to find locations
- It can be excited at same wavelength of Optogenetic activation
- Relies on fluorescent protein



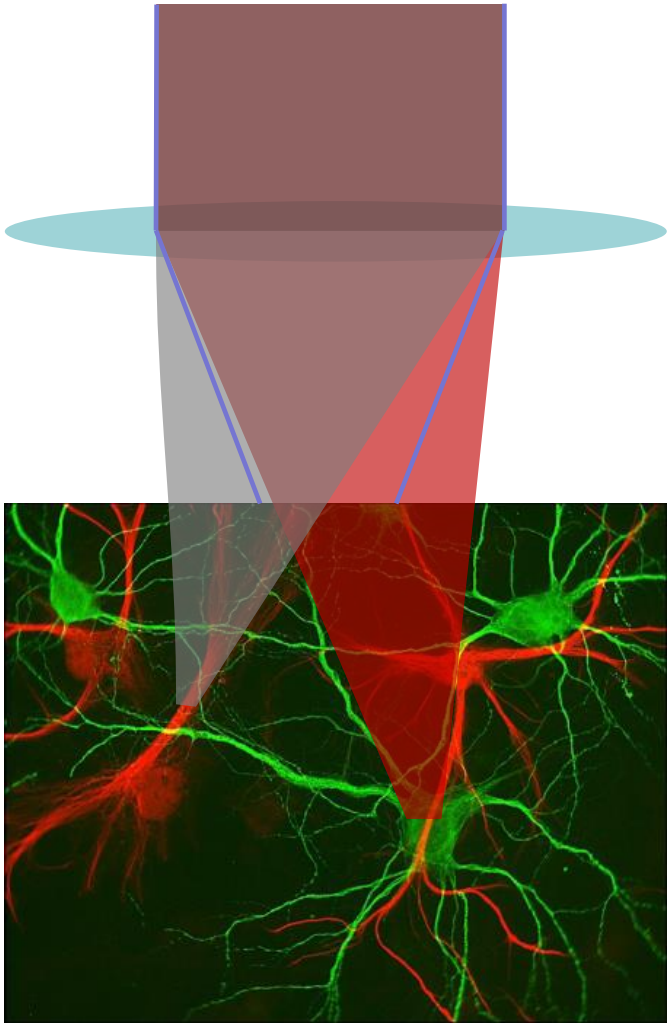
Optogenetics:
- 2P laser light is used to activate neurons. Activation does not result in fluorescence, unless also a fluorescent protein is expressed



Calcium signals:
- 2P laser light is used to excite a probe that fluoresces more in presence of Ca ions



Ideal Representation of an Optogenetics Microscope



Activate this neuron at 1050 nm (C1V1)

Detect signal in this neuron at 940 nm (GCaMP3)

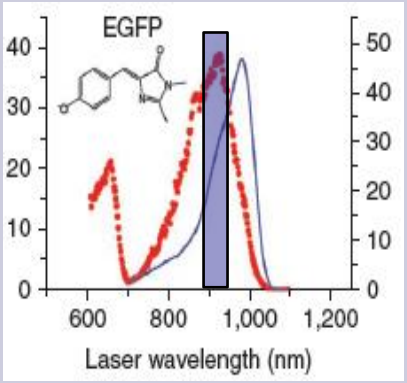
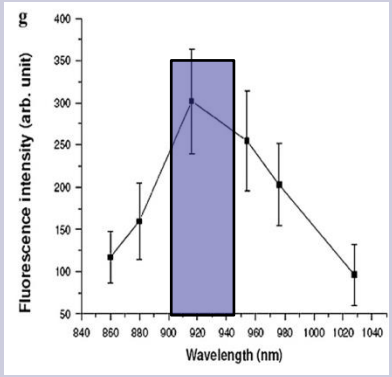
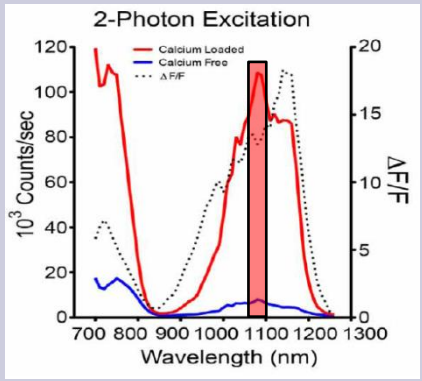
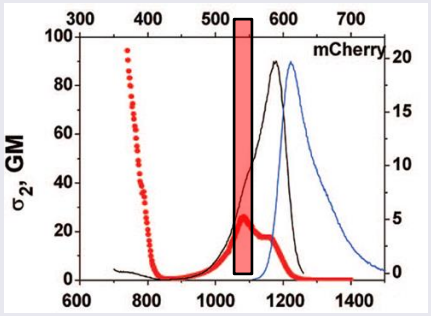
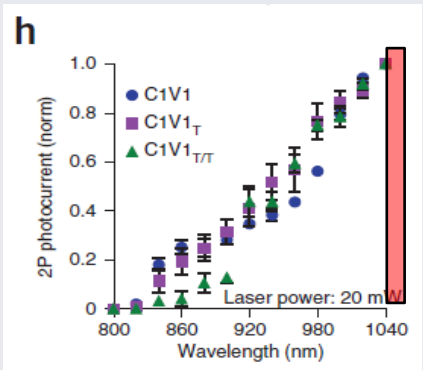
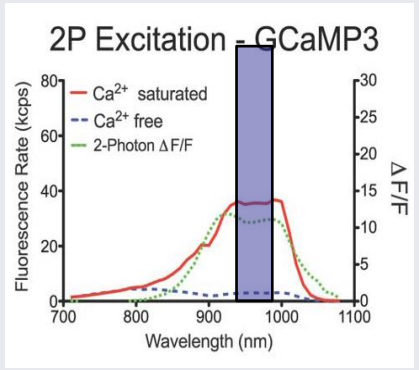
1) Image live specimen to find locations of neurons
2) Activate one or more neurons
3) Detect Ca signals in other (distant) neurons

General Considerations

- Not all 3 modalities need to be present
  - Stimulation can be visual or olfactory or tactile, not necessarily via illumination of a neuron. In this case is enough to image the sample and measure the Ca signals (2 wavelengths)
- Imaging probe can be co-expressed (i.e. in the same molecule) as Optogenetic probe, or it can be a separate probe
- Key thing is that Optogenetic and Ca signal excitation must be at wavelengths that minimize crosstalk

	Imaging	activation	Ca detection
Example 1	eGFP (~920 nm)	ChR2 (940 nm)	RCaMP (1,100 nm)
Example 2	mCherry (1,100 nm)	C1V1 (> 1,060 nm)	GCaMP (940 nm)

Two Examples

	Imaging	Activation	Ca Detection
Example 1	eGFP (~920 nm) 	ChR2 (940 nm) 	RCaMP (1,100 nm) 
Example 2	mCherry (1,100 nm) 	C1V1 (> 1,060 nm) 	GCaMP (940 nm) 

Colored bar is ~ 50 nm wide centered at peak of excitation spectrum

Chameleon/OPO MP/Fidelity – a World of Choices

Fidelity

Single wavelength
2 W at 1050 nm

2PE mFruits, SHG, C1V1, RCaMP, scanless imaging

Chameleon

680-1080 nm
Up to 3.5 W

MPE, SHG, Optogenetics, scanless imaging

Chameleon+Fidelity

680-1080 nm +
2 W at 1050 nm

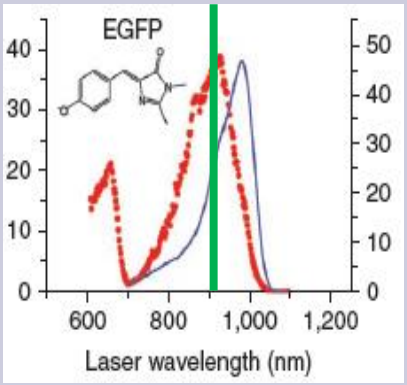
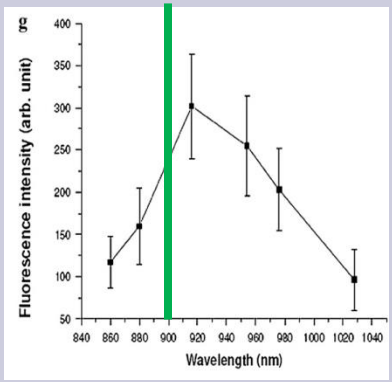
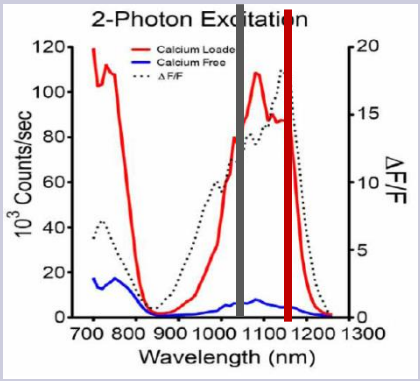
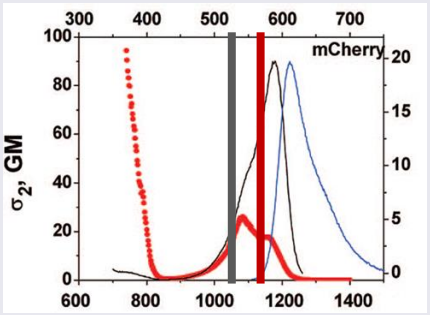
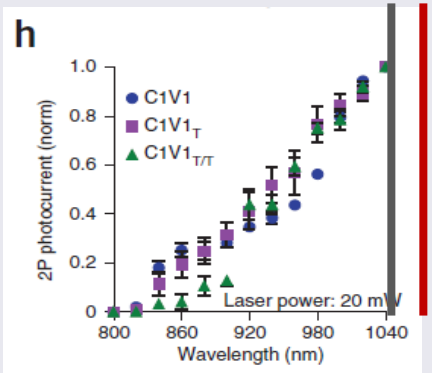
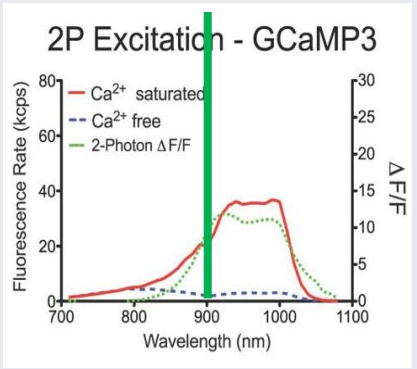
MPE, SHG,
Optogenetics, GECIs,
2-color imaging,
Scanless imaging

Chameleon+OPO MPX

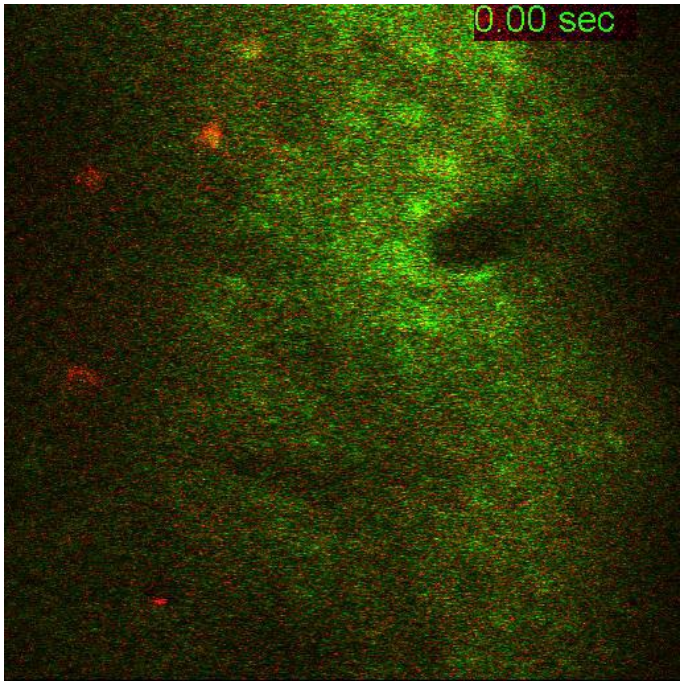
680-1080 nm +
1000-1600 nm
Independent tuning

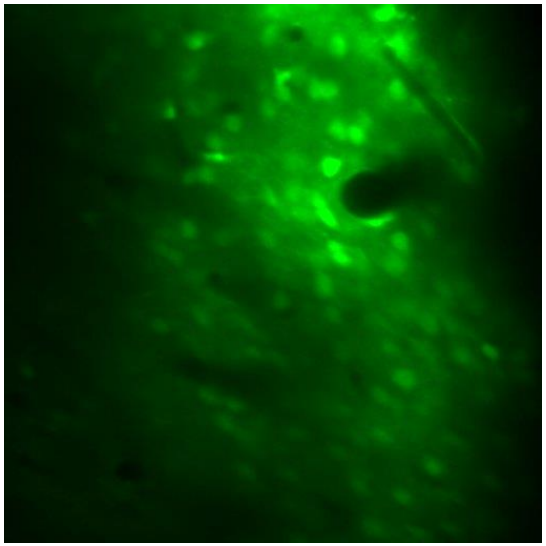
MPE, SHG, THG, fs CARS,
Optogenetics, GECIs,
3-color imaging,
Scanless imaging

Chameleon, Fidelity and Optogenetics

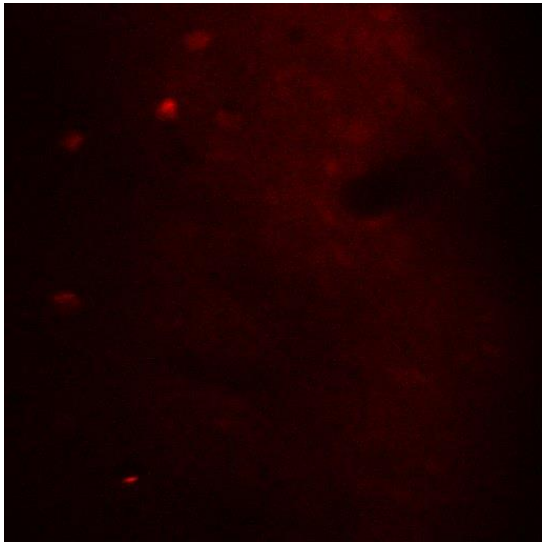
	Imaging	activation	Ca detection
Example 1 MPX (1150 nm) Fidelity (1055 nm) Ultra (900 nm)	eGFP (~920 nm) 	ChR2 (940 nm) 	RCaMP (1,100 nm) 
Example 2 MPX (1150 nm) Fidelity (1055 nm) Ultra (900 nm)	mCherry (1,100 nm) 	C1V1 (> 1,060 nm) 	GCaMP (940 nm) 

All Optical Physiology - Example





OGB-1

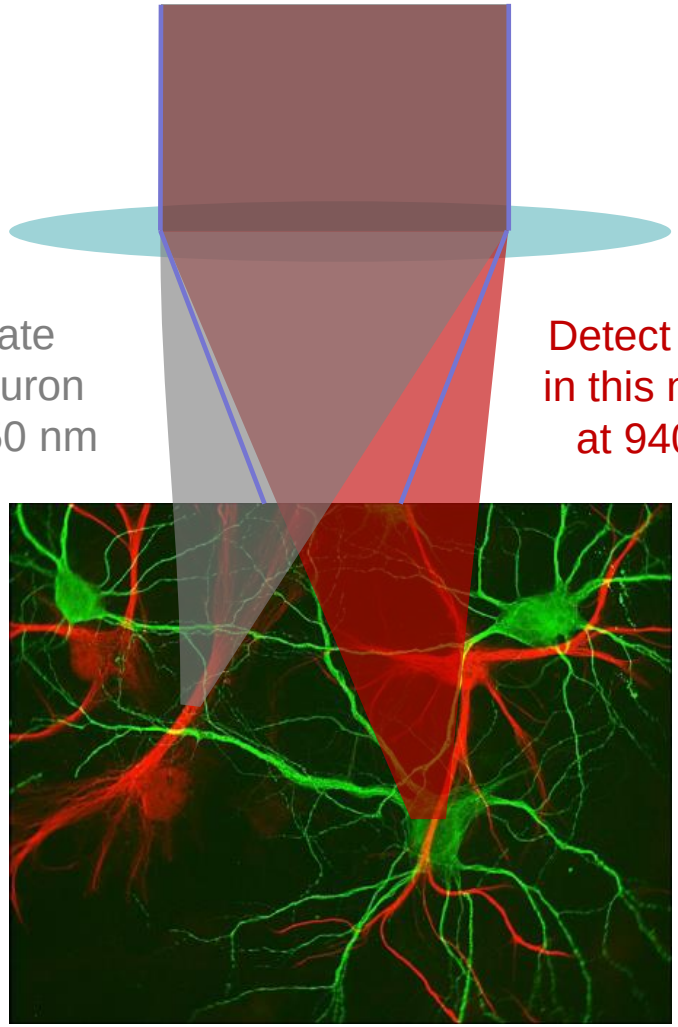


C1V1-mcherry

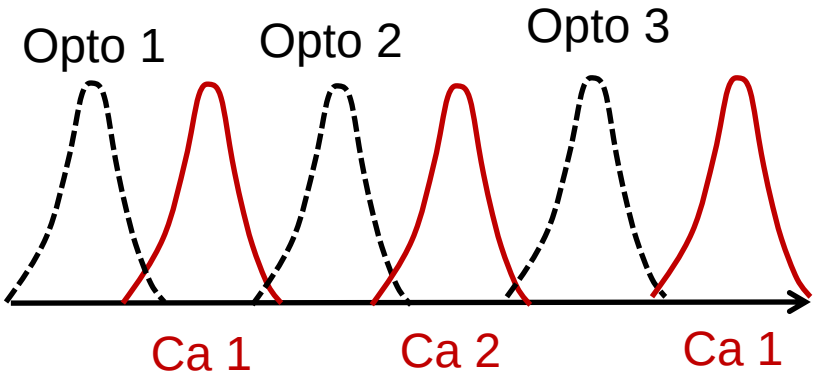
All optical physiology: 50 Hz frame rate 2P
Ca^{2+} imaging (OGB-1) and C1V1-mCherry Stimulation
Chameleon (800 nm) and Chameleon OPO (1100 nm)
Live mouse, Visual cortex layer II/III, 250 micron depth

Courtesy of Albrecht Stroh
Focus Program Translational Neurosciences (ftn) &
Institute for Microscopic Anatomy and Neurobiology
Johannes Gutenberg-University Mainz

The Optogenetics Microscope – the Grand Goal



1) Do it on 10,000 neurons
   - SLM approach (Oron, Emiliani, Vaziri, 3I)
   - 3D AOM approach (Saggau, femtonics
2) Do it with different opto-activation and monitoring tools

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