Notes on two-photon microscopy

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Hardware for two-photon microscopy

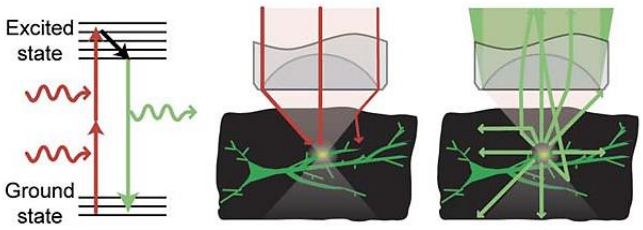
(Svoboda & Yasuda, 2006)

- In two-photon microscopy, a rapidly pulsed laser excites a fluorophore via two lower energy photons (which are typically red to infrared range). The fluorophore then emits a higher energy photon which is detected by the microscope.
- Two-photon microscopy is usually implemented using a laser scanning microscope. The laser is tightly focused on a small focal volume, then scanned over the sample's area to acquire information from each point within the sample. When the laser overlaps with fluorophores, photons are emitted from the given focal volume. All of these points are mapped to pixels and the full image is reconstructed computationally.
- Since the focal volumes used in two-photon microscopy are small, high excitation intensities are needed to generate enough emitted signal. This necessitates rapid trains of short pulses from the excitation laser. Mode-locked Ti:sapphire lasers have a frequency of about 100 MHz, a pulse duration of about 100 femtoseconds, and their wavelength can be tuned from about 700-1,000 nm. These properties are nearly ideal for most fluorophores. If the required wavelengths are over 1,000 nm, other types of lasers are available as well.
- Some examples of fluorophores used with two-photon microscopy include transgenic fluorescent proteins (i.e. XFPs), organic calcium indicator dyes, and other types of fluorescent dye molecules.

Advantages of two-photon microscopy

(Svoboda & Yasuda, 2006)

- The absorption rate of the fluorophore is given by the intensity of the laser squared. This nonlinear process allows excitation to occur only within the described small focal volumes. When using a high numerical aperture objective, the diameter of an individual focal volume can reach down to just 100 nm.
- Since two-photon microscopy uses long wavelengths, the light penetrates tissue more deeply than with most other techniques.
- As a result of the nonlinear excitation process which requires two photons in order to excite a fluorophore, single photons scattered by the tissue are too dilute to cause much off-target fluorescence.



- The only photons which do experience scattering are those which come from the localized focal volume. As such, they still provide useful signal if detected. In traditional fluorescence microscopy, scattered photons are typically lost or they contribute to background fluorescence.

- It is relatively easy to modify confocal fluorescence microscopes to create two-photon microscopes. Academic laboratories sometimes perform such modifications in-house.

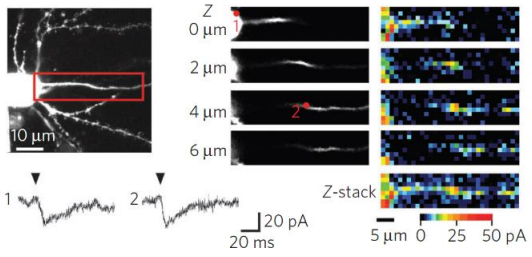
Selected applications of two-photon microscopy
(Ellis-Davies, 2011)

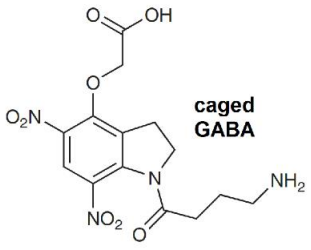
- Cortical neurons within transgenic mice expressing GFP and transgenic mice expressing YFP were imaged in vivo using two-photon microscopy by the Gan and Svoboda groups respectively. The fluorescent proteins were expressed only by a subset of neurons, allowing for higher contrast against the background. In this way, entire dendritic trees of pyramidal neurons were imaged at depths of up to 500-600 µm. The method established that, under the conditions used, most of the dendritic spines in the mouse neocortex remain fairly stable over a period of one month.

- Later studies using similar techniques but with deliberate ablations to various sensory systems showed greater destabilization of dendritic spines, indicating that sensory memories might be partly encoded in spine patterns.
- Two-photon microscopy also helped reveal that spines in the motor cortex show significantly greater stabilization during motor learning tasks than spines elsewhere in the cortex. This was demonstrated in both mice and songbirds.

Two-photon photolysis of caged neurotransmitters
(Matsuzaki, Hayama, Kasai, & Ellis-Davies, 2010)

- The Ellis-Davies group developed modified glutamate and GABA molecules that include moieties which block their biological function. Such caged neurotransmitters are initially inactive, but can undergo photolysis when exposed to the excitation lasers used in two-photon microscopy, releasing the active molecules.





- Two-photon photolysis was used on caged glutamate within neural tissue samples, causing localized release of glutamate and the induction of action potentials.
- Similarly, caged GABA was photolyzed by two-photon excitation. By combining this technique with patch-clamp recording, electrical activity was mapped on neural membranes, allowing GABA receptor distributions over the membranes to be estimated.

References

Ellis-Davies, G. C. R. (2011). Two-Photon Microscopy for Chemical Neuroscience. *ACS Chemical Neuroscience*, *2*(4), 185–197. http://doi.org/10.1021/cn100111a

Matsuzaki, M., Hayama, T., Kasai, H., & Ellis-Davies, G. C. R. (2010). Two-photon uncaging of γ-aminobutyric acid in intact brain tissue. *Nature Chemical Biology*, *6*, 255. Retrieved from http://dx.doi.org/10.1038/nchembio.321

Svoboda, K., & Yasuda, R. (2006). Principles of Two-Photon Excitation Microscopy and Its Applications to Neuroscience. *Neuron*, *50*(6), 823–839. http://doi.org/10.1016/j.neuron.2006.05.019