MULTIPHOTON EXCITATION OF MULTIPLE FLUOROPHORES WITH A SINGLE WAVELENGTH

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The benefits of multiphoton microscopy in biological studies are now well known: Lower scattering in biological tissues, reduced auto-fluorescence, lower photo-bleaching and phototoxicity, etc. Infrared femtosecond lasers are typically used as excitation sources. Titanium-Sapphire lasers can be tuned from 700 to about 1000 nm but the operation is not always straightforward and leads to a significant decrease in output power at extreme wavelength.

We show that a new generation of fixed-wavelength Ytterbium doped lasers is an attractive alternative, and allows excitation of a wide range of fluorophores, from DAPI to DsRed. We used a simple, turn-key, Ytterbium diode-pumped femtosecond oscillator [1] with an emission wavelength of 1030 nm and an average power greater than 1.2 W as an excitation source for different intrinsically fluorescent proteins imaging (i.e. GFP, YFP or DsRed). We will report on different experiments showing the versatility of the technique:

- In-depth imaging of GFP-transfected in sliced brain of transgenic mice.
- Dynamic analysis on calcium orange dyes to study intracellular calcium concentration in primary culture neurons.
- Three photon excitation of DAPI marked cell nuclei.
- High efficiency excitation of DsRed transfected cells.

The versatility of the laser source used in these studies coupled with the easy to use feature represent a real powerful advance in multiphoton microscopy compared to classical Titanium-Sapphire lasers especially in high wavelength fluorescent dyes such as DsRed.

[1] model t-Pulse, Amplitude Systèmes, Talence, France