COMPARATIVE STUDY OF INFRARED FLUORESCENCE GENERATION EFFICIENCY IN MULTIPHOTON MICROSCOPY

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Using an infrared femtosecond laser as an excitation source in a multiphoton microscope offers many advantages, such as lower scattering in biological tissues, optimal wavelength of new intrinsically fluorescent proteins such as DsRed, or minimized auto-fluorescence [1]. Advances in laser technology have made available a new generation of simple, turn-key femtosecond lasers with an emission wavelength of 1030 nm [2].

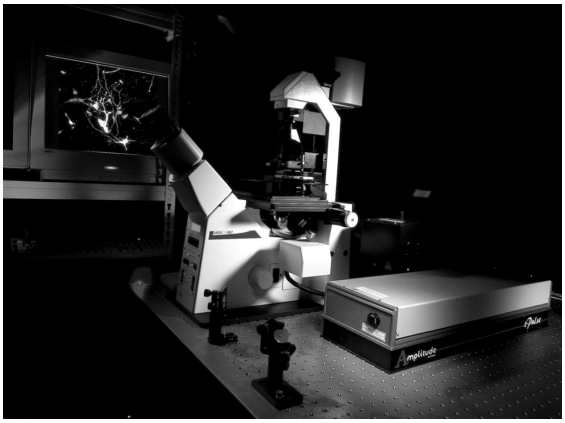

Fig. 1: Experimental set-up

GFP and DsRed are two spectrally separated intrinsically fluorescent proteins and are among the most commonly used fluorescent reporters. Their respective one photon excitation absorption spectra peak around 485 nm and 575 nm. The use of DsRed for multiphoton imaging has been limited due to the poor absorption at the wavelengths that can be efficiently generated by Titanium:Sapphire lasers, i.e. below 970 nm.

We have realized a quantitative comparison of the fluorescence efficiency vs. wavelength up to 1030 nm, for GFP and DsRed expressed in biological samples, using as excitation sources a Titanium Sapphire laser (model Mira from Coherent) and a Ytterbium femtosecond laser (model t-Pulse from Amplitude Systemes).

Results show that a 1030 nm excitation wavelength improves the fluorescence efficiency on DsRed by a factor of more than 10, and is comparable as a 970 nm excitation wavelength for GFP.

[1] N. Billinton, A.W. Knight, " A Review of Techniques for Distinguishing Green Fluorescent Protein from Endogenous Aufluorescence", *Analytical Biochemistry*, **291**, 175-197, (2001).
[2] N. Deguil, E. Mottay, F. Salin, P. Legros, D. Choquet, "Novel diode-pumped tunable infrared laser for multiphoton microscopy", Microscopy Research and Technique, 63, 23-26, (2004).