

## **A SINGLE LASER SOURCE FOR THE OPTIMAL EXCITATION OF EGFP AND MRFP IN MULTIPHOTON TIME LAPSE LASER SCANNING MICROSCOPY**

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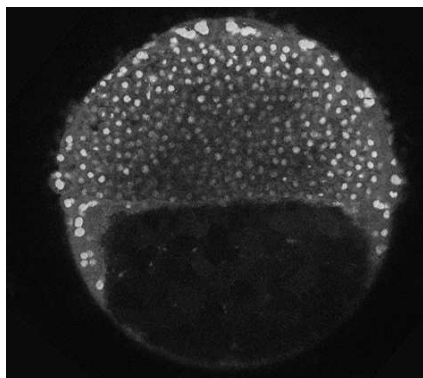
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Using infrared excitation wavelengths in multiphoton microscopy enables in-depth in vivo imaging and limits the phototoxicity processes when performing time lapse laser scanning microscopy over several hours. Infrared femtosecond lasers are typically used as excitation sources. Titanium-Sapphire lasers can be tuned from 700 to about 1100 nm but the operation is rather slow and leads to a significant decrease in output power at extreme wavelengths. In addition, a single wavelength cannot optimally excite two different fluorescent proteins such as eGFP and mRFP.

We used a novel infrared femtosecond excitation source that allowed us to adequately excite eGFP and mRFP expressed at rather low levels in stable transgenic zebrafish lines. The excitation laser consists of a compact Ytterbium diode-pumped femtosecond laser (Amplitude Systemes model t-Pulse). The laser beam at 1030nm is split to send part of it through a zero dispersion photonics crystal fiber providing a femtosecond infrared output from approximately 950 nm to 1150 nm with most of the average power between 980 nm and 1000 nm. Combination of the spare 1030 nm original beam and fiber output allows us to get an optimal spectrum for the simultaneous excitation of GFP and mRFP in live zebrafish embryos.



Transgenic fish embryos expressing ubiquitously mRFP-F (our own results unpublished) and H2A.F/Z:GFP (Pauls et al. Genes Dev. Evol. 2001) were imaged (see figure 1) from the one cell stage with a Leica upright microscope, 20x/0.95NA water deeping lens objective from Olympus, Leica SP5 LSM and infrared laser excitation as described below. The image displayed here is taken from the time lapse image data set. It corresponds to 4 hours of development at 28°C and is taken at 80 microns from the surface of the embryo.