Cr:f Multi-photon Imaging

A Ti:sapphire Alternative

The Mavericks Cr:forsterite laser from Del Mar Photonics is an all solid state laser system that is an affordable source of sub 60 femtosecond pulses in the ~1250 nm region. The small footprint, easy alignment and low cost is due in part to being pumped by a 6-10 W Ytterbium Fiber Laser, which is considerably cheaper than the pump lasers used for Ti: sapphire systems. The femtosecond Cr:forsterite laser is tunable over wavelengths from 1230 to 1270 nm, making it ideal for multi-photon imaging and biomedical applications. The extremely short time duration of a femtosecond pulse gives enormous peak powers and power densities. Laser output can be directly/fiber coupled into microscope systems for confocal florescence and multi-photon imaging.

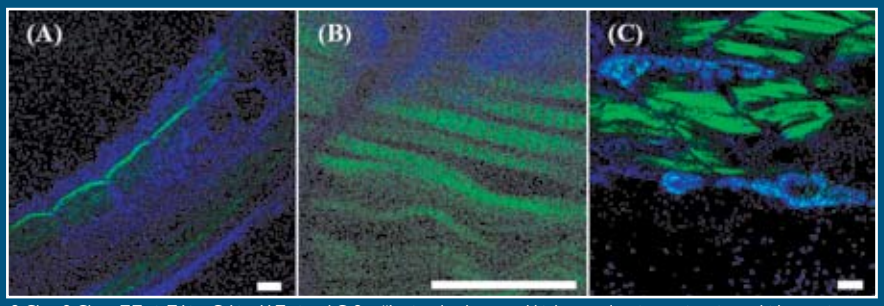
Laser scanning confocal florescence microscopy is a tool used to obtain high resolution three-dimensional images of biological samples by scanning the focal point of a diverging laser beam across a sample. Fluorescence information is passed through an aperture and then collected at each point by a detector. There are several disadvantages associated with florescence microscopy. Resolution is related to the size of the aperture, thus increasing the resolution shrinks the aperture and limits the number of photons incident on the detector leading to poor signal to noise ratios. The small number of detectable photons also means that high average power lasers are often required that can damage biological samples and make imaging of living tissue difficult. There are also photobleaching and toxicity problems related to the florescent dyes.

By taking advantage of nonlinear effects caused by the extremely high peak power (yet low average power) of ultrafast lasers many of these problems can be overcome with multi-photon imaging. In multi-photon imaging, second harmonic (SHG) and third harmonic (THG) light is generated by a tightly focused short-pulse laser beam. The harmonic generation is highly intensity dependent, thus it only occurs in a small area of the focal point. This allows the non-linear interaction region to act as the "aperture" and any SHG or THG photons that reach the detector are from the desired region at the focal point[1]. All materials have nonzero third-order susceptibilities, thus THG microscopy can be used to image biological samples without the need for fluorescence dyes.

Cr:forsterite laser sources offer several advantages over the Ti:sapphire lasers most often used in multiphoton imaging of living biological samples. The THG light from a Cr:forsterite laser is produced at 410 nm and is easily detectable by standard photodetectors. It has been shown that the longer wavelength (1230 nm) for Cr:forsterite lasers allows much higher power to be used without compromising cell viability. In a recent study, in vivo images of cell proliferation processes inside a zebra fish embryo were made using SHG and THG for a Cr:forsterite femtosecond laser[2]. No optical damage was observed in the cells when average powers of up to 100 mW were used, compared to a cell damage threshold of about 10 mW in similar studies conducted with Ti:sapphire lasers. Heating from water absorption was not a limiting factor and no damage from linear absorption nor reduced damage from multi-photon absorption was observed.

[1]R. Boyd: Nonlinear Optics (Academic, New York 1992)

[2]S. Chu, S. Chen, T. Tsai, T. Liu, C. Lin, H. Tsai, and C. Sun, "In vivo developmental biology study using noninvasive m ulti-harmonic generation microscopy," Opt. Express 11, 3093-3099 (2003)



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In vivo harmonic optical microscopy (HOM) sectioning inside a live zebrafish larva at the 20-somite stage. (a) An optical section of the larva showing the segments inside the vacuolated notochord and the distribution of somites alongside the notochord. (b) The enlarged view inside a somite showing individual muscle fiber and the sarcomeres on it through SHG, as well as the interface between somites through THG. (c) (281 kB) Depth resolved optical series showing that it is possible to visualize through a whole zebrafish larva with HOM. Scale bar: 20-µm.

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