

# RECENT DEVELOPMENTS IN WIDELY TUNABLE AND HIGH PEAK POWER ULTRAFAST LASER SOURCES AND THEIR ADOPTION IN BIOLOGICAL IMAGING

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Multiphoton excited fluorescence (MPEF) microscopy plays an important role in many areas of bio-imaging and beyond. The technique has seen rapid growth and adoption in thousands of research laboratories worldwide, powering both commercial and homemade multiphoton microscopes [1, 2]. MPEF microscopy offers unique advantages for in-vivo imaging. Thanks to the nonlinear dependence of the fluorescence signal with the intensity of the excitation light, it ensures three-dimensional resolution down to a femtoliter volume, reduces photo-damage in the out-of-focus regions and allows for extended penetration depth in scattering live tissue.

Next-generation ultrafast lasers are fueling the next wave of innovation in the multiphoton imaging community. Specifically, novel platforms are providing straightforward and automated access to long excitation wavelengths (>1000nm) to take advantage of red shifted fluorescent markers and achieve deep in vivo penetration [3]. Leveraging these laser platforms, MPEF microscopes can now perform sustained imaging of fragile live samples, such as mouse cortex, or entire organisms, such as zebrafish or *C. elegans*, over many hours.

On the other hand, simple and cost effective fixed-wavelength ultrafast lasers based on rare earth doping elements (Erbium, Ytterbium) can enable specific applications that do not require spectral agility and broad wavelength tuning. These lasers are suitable for multiphoton excitation of specific markers such as YFP, mCherry and others. Long cavity oscillators and amplified systems, with higher pulse energy and peak power, offer optimal performance for power hungry applications such as three-photon excited fluorescence microscopy to image with high resolution ever deeper in mice brain. In optogenetics studies, these lasers may also be used for photo-stimulation of red-shifted opsins (C1V1, ReaChR) using holographic patterning techniques.

In this paper, we will discuss various types of ultrafast laser technologies and describe their respective strengths and attributes in the context of bio-imaging diverse and specific requirements.

We will present application examples with two-photon and three-photon excitation of commonly used fluorophores (eGFP, eYFP, mCherry, etc.) and the targeting of structural proteins such as collagen with SHG and cell membrane interface with THG. We will also discuss the use of flexible dual beam laser sources for resonant Raman imaging techniques (CARS, SRS). Finally we will specifically review the expansion of the neuroscientist optogenetics toolbox and discuss its impact on ultrafast laser technology roadmaps.

[1] W. Denk et al (1990). Two-photon laser scanning fluorescence microscopy. *Science*, Vol. 248, pp. 73-76

[2] A. Krueger (2010). Multiphoton microscopy: Turnkey femtosecond lasers fuel growth of multiphoton imaging. *Laser Focus World*, Vol. 46, pp. 39-43.

[3]. D. Kobat et al (2009). Deep tissue multiphoton microscopy using longer wavelength excitation. *Opt Express*, Vol. 17, pp. 13354-13364