Laser Innovations in Non-Linear Microscopy: the Techniques and Benefits

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Both users and suppliers of non-linear microcopy setups recognize the rapid growth of non-linear imaging techniques – from advanced neuroscience to embryology, from H&E histology augmentation/replacement to clinical endoscopy. This variety of applications, together with the expanding number of probes, beam manipulation, delivery and excitation schemes is a strong driving force for the continuous advancements in parameters and functionality of the commercial femtosecond laser sources used for these applications.

Especially in the last few years, multiple innovative approaches were adapted by commercial femtosecond laser sources. In one segment, lasers for multiphoton microscopy can cover more than octave in wavelength range, produce energy/pulse from tens of nJ to tens of microjoules with variable repetition rates, and include complex functions like pulse pre-chirping and fast output modulation. The flexibility and available parameters of these sources highly benefited the advanced neuroscience imaging setups using 2-photon or 3-photon excitation schemes for structural and functional imaging of in-vivo biological tissues. On other end portable and dedicated femtosecond laser sources emerged that will benefit multiphoton microscopy in clinical endoscopy or the surgical room environments. These compact and cost efficient sources are designed specifically for nonlinear microscopy applications and provide reliable, easy to integrate operation. The variety of these lasers sources has been driven by the success of multiphoton imaging applications and – in turn – is enabling further progress and popularization of these non-linear imaging techniques.

In this presentation we will review these novel developments in femtosecond lasers and will look into how they help to shape the multiphoton microscopy. In particular, rapidly growing 3-photon excitation microscopy [1], 2-photon holographic imaging [2] and optogenetic stimulation techniques enabling all-optical investigation of neural activities [3] as well as increasing level of optical integration within experiment which helps to streamline the laser usage in the laboratory and (pre-)clinical environment.

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