ADVANCES IN ULTRAFAST LASERS IMPROVE MULTIPHOTON MICROSCOPY

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The emergence of compact, fully automated, and widely wavelength-tunable femtosecond lasers has triggered explosive growth in their use in a broad array of multiphoton microscopy techniques. Over the past decade laser manufacturers have constantly improved the performance characteristics of these sources to meet the requirements of the biomedical user community for imaging with longer cell viability and deeper tissue penetration. We will summarize the latest advances in the development of user-friendly and flexible sources for nonlinear and multimodal imaging, and how they benefit the end user and progress application development. This will include a special focus on the new Spectra-Physics® InSight™ compact and fully automated one-box ultrafast laser [1]. This novel source delivers conveniently from one output beam sub-120 femtosecond pulses, which are continuously wavelength-tunable from 680 nm through 1300 nm. This 620 nm tuning range is about twice that of the Ti:sapphire lasers, which are presently the workhorses in multiphoton microscopy. Most importantly this new laser provides easy access to the long wavelength half of the relative transparency window of tissue in vivo beyond 1,000 nm, where scattering of excitation light is reduced and tissue penetration depth is increased [2]. Integrated, automated dispersion compensation allows for optimizing the pulse width within the specimen to further improve image quality and penetration depth. The laser also features an optional second femtosecond output beam at 1,040 nm, which can be utilized for dual-wavelength excitation and multimodal nonlinear imaging applications, including femtosecond Coherent Anti-Stokes Raman Scattering (CARS) microspectroscopy and microscopy [3].