Fiber-coupled, sub-ps Ti-sapphire Laser for Multi-Excitation Wavelength, Head-Mounted Two-Photon Excitation Fluorescence Microscopy of the Brain

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Abstract: A fiber-coupled, sub-ps Ti-sapphire laser operating in three wavelength regimes at around 810 nm, 920 nm and 1000 nm is introduced for *in vivo* nonlinear microscopy of the brain.

1. Introduction

Fiber- or fiber-coupled ultrafast lasers are widely used in nonlinear microscopy, such as two-photon excitation fluorescence (2PEF), second-harmonic generation (SHG) or Coherent anti-Stokes Raman Scattering (CARS) microscopy [1,2]. In life science, there are several special requirements for these laser systems depending on experimental conditions: typically one needs several fluorophores to distinguish different tissue components during imaging, they require different wavelengths for optimum excitation (resulting in a minimum heat load of the biological tissue during laser exposure), emission spectra of the fluorophores used for labelling should not overlap, pulse duration and the focal spot after the objective must be minimized for maximum nonlinear effect (maximum fluorescence, SHG or CARS signal). In a few experiments, one needs *in vivo* imaging of the brain in freely moving animals, where a small size, light-weight, head-mounted scanning microscope [3] is used for imaging, in which ultrafast laser pulses are delivered by an optical fiber to the scanning head with focusing optics, while the emitted fluorescence is collected by the same (double-clad) optical fiber and delivered back to the (APD or PMT) photodetector(s). The near-infrared pulsed laser beam used for excitation and the backward propagating fluorescence signal are separated by a dichroic mirror between the laser and the fiber coupler, where properly designed laser blocking (LBF) and bandpass filters (BPF) protect the detectors from the back-scattered laser light.

Fiber-delivery of ultrafast laser pulses is difficult due to two main physical effects arising in optical fibers: nonlinearity (self-phase modulation due-to Kerr-nonlinearity) and dispersion (different propagation speeds in an optical waveguide at different wavelengths). Low thermal load of biological samples is a main requirement for *in vivo* imaging, which calls for high peak intensity (short and/or low repetition rate) laser pulses for two-photon excitation. Unfortunately, the shorter the laser pulse the higher the transform limited spectral bandwidth is, which results in a higher and higher sensitivity for dispersion of the fiber. In a recent experiment [2], we minimized the problem of fiber-dispersion by increasing the pulse duration of our Ti-sapphire lasers from ~150 fs to the ~0.6 ps and reducing the repetition rate of the laser from ~80 MHz to ~20 MHz in order to obtain nearly the same peak intensity of the pulses after a ~2 m long hollow core photonic bandgap (HC-PBG) fiber (HC-800-2, NKT Photonics). Depending on their central wavelength, the bandwidth of commercially available hollow core PBG fibers (HC-800-2, HC-920, HC-1030) were limited at around ~100 nm during our studies. Recent development in hollow core anti-resonant fibers resulted in designs with attenuation and slowly varying, moderate waveguide dispersion over the 820-990 nm spectral range (Perfos ARF-41-240), which would allow fiber delivery of sub-ps, ~20 MHz, ~10 nJ laser pulses (with average power of ~200 mW) with tolerable nonlinear and dispersive effects, while keeping a nearly Gaussian beam profile [4].

For a head mounted scanning microscope system (where we do not have space for optics and detectors mentioned above), however, we still have to collect the emitted fluorescent light in the visible, so the only option is to use double-clad, large mode area (LMA) solid core fibers for delivery ultrafast laser pulses between the laser source and the scanning head. In such a fiber, both peak intensity and spectral bandwidth of the optical pulses are limited by nonlinearity and fiber dispersion, respectively, the latter one is dominated by material dispersion of fused silica.