Multiphoton microscopy with frequency-doubled compact femtosecond erbium-doped fiber laser

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Abstract

Multiphoton microscopy (MPM) is a powerful imaging technique but the traditional design requires a Ti: sapphire laser which is bulky, expensive, not portable, and require precise alignment. Currently, there is a trend to replace the Ti:sapphire laser with a more compact, portable, and low cost femtosecond fiber laser as the light source. Although MPM systems using femtosecond fiber lasers at 1.55 µm or 1.0 µm wavelengths have the advantages of deep imaging depth and minimal autofluorescence background, they also have disadvantages such as higher water absorption, lower resolution, and requiring staining of the sample compared to systems using 800 nm wavelength. To fulfill the potential of MPM systems for *in vivo* imaging, we developed a compact MPM system based on a frequency-doubled femtosecond erbium-doped fiber laser source at 1.55 µm. By use of periodically poled MgO:LiNbO3, the frequency-doubled pulses at 790 nm with average power of 75 mW and pulse width of 200 fs are applied as the excitation source. The fiber laser is optimized for its pulsewidth, bandwidth, and dispersion in order to enhance the MPM excitation at the sample location. A gimbal-less two-axis MEMS scanner is utilized to perform XY scanning for MPM imaging. A miniature objective and multimode fiber are further used to build the compact MPM system. Two-photon excitation fluorescence (TPEF) and second harmonic generation (SHG) images are obtained from unstained biological samples, such as fish scale and mouse tail tendon. In conclusion, the MPM system with a compact, portable, low-cost, frequency-doubled fiber laser has a great potential to transform the current bench-top MPM system to a portable system for *in vivo* MPM imaging.