

PHOTONIC CRYSTAL FIBRE AS A LIGHT SOURCE FOR SHORT-WAVELENGTH TWO-PHOTON EXCITED FLUORESCENCE MICROSCOPY

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Two-photon excitation fluorescence (TPEF) microscopy revolutionized deep and live tissue imaging. It uses ultra-short pulsed laser sources that emit near-infrared radiation to excite VIS and UV fluorophores. It has the ability to image deep into optically thick specimens, while restricting photobleaching and phototoxicity of the area being imaged. Several important (endogenous) fluorophores, including NADH, tryptophan and the ratiometric Ca^{2+} indicators, are excited with wavelengths shorter than 360 nm. Unfortunately, efficient imaging of these endogenous fluorophores cannot be performed with TPEF at present, due to the lack of suitable laser sources delivering radiation at the wavelengths necessary for two-photon excitation. A reliable pulsed laser source with emission wavelengths between 500 nm and 700 nm would therefore considerably widen the application of TPEF microscopy.

We are investigating the potential of photonic crystal fibres (PCFs) as a laser source for visible wavelength TPEF microscopy. PCFs are micro-structured fibres, where incident light is guided by a large index difference, arising from periodically arranged air holes that extend longitudinally through the fibre. This configuration leads to a tight confinement of the light resulting in fibers that are single mode throughout the visible range [Birks *et al* 1997]. The high nonlinearity of the PCFs also leads to a supercontinuum generation (SCG) that can span the visible to the near-infrared spectral regions [Ranka *et al* 2000, Husakou and Hermann 2001]. We are presently studying the effects of fibre length, laser excitation wavelength and intensity on the spectrum, pulse-width and output power of the generated supercontinuum, more specifically in the spectral region between 500 nm and 600 nm.

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