An Electronically Tunable Ultrafast Laser Source applied to Fluorescence Imaging including Wide-Field Optically-Sectioned Fluorescence Lifetime Imaging using a Nipkow Disk Microscope

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Tunable visible ultrafast light sources are generally complex, expensive and usually require a significant degree of adjustment. We demonstrate a convenient, continuously electronically tunable (435-1150 nm) ultrafast source for fluorescence imaging applications that is derived from a visible supercontinuum generated by femtosecond pulses in a microstructured fibre [1]. It allows rapid tuning and multi-wavelength operation. We have applied it to confocal and wide-field fluorescence intensity and lifetime microscopy, as well as multiwell-plate imaging. We are currently applying it to hyperspectral imaging and are working towards a system for acquiring full fluorescence excitation-emission matrix data.

Fig. 1: (a) Experimental setup. Inset: confocal FLIM image of a GFP labelled cell. (b) and (c) Wide-field conventional and sectioned images and (d) and (e) FLIM maps of pollen grains obtained using Nipkow microscope. (a) reproduced from [2] © 2004 IOP.

Fluorescence lifetime imaging (FLIM) requires a rapidly modulated or pulsed light source, and provides more accurate lifetime images with optical sectioning. To realise this with the potential for fast acquisition using single photon excitation, we present a Nipkow disk FLIM microscope using this new tunable continuum source (TCS). Figures 1(b) to (e) illustrate this system applied to pollen grains. A particularly exciting future possibility is a low cost TCS based on compact, fibre amplifier technology. We will present first results towards such a system, which we believe could replace gas lasers for many imaging applications.

Abstract: We present a continuously electronically tunable ultrafast spatially coherent source based on spectral selection from a supercontinuum generated in a microstructured fibre. We demonstrate operation in fluorescence microscopy and both scanning and wide field fluorescence lifetime imaging including a Nipkow disk microscope.

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