THREE-PHOTON EXCITATION FLUORESCENCE MICROSCOPY OF LIVE CELLS USING A NOVEL BI-DIRECTIONAL PUMPED OPTICAL PARAMETRIC OSCILLATOR

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Use of wavelengths in the near infrared wavelength has previously been implemented as an excitation source for fluorescence and harmonic imaging [1]. When considering a three photon process, the typically the wavelength range used for is often somewhat restricted to 700 – 1280 nm. This is due to a combination of high losses incurred by objective lenses at longer wavelengths and the lack of cost effective excitation sources to overcome this issue. Coherent sources in the range of 1400 – 1600 nm are of particular interest within the life sciences as this corresponds to the three photon absorption peaks of many commonly used fluorescent dyes. Additionally, the use of longer wavelengths has been previously shown to improve the viability of biological samples [2].

We report three-photon laser scanning microscopy (3PLSM) using an optical parametric oscillator (OPO) bi-directionally pumped by a Yb:fibre laser, with a signal wavelength output tunable from $\lambda = 1400 - 1600$ nm. This novel laser geometry was used to boost the output power of the OPO in order to overcome the high optical loss in the infra-red spectral region observed in laser scanning microscopes and objective lenses that renders them otherwise difficult to use for imaging.

To test the 3PLSM system, autofluorescent 3PLSM imaging of live plant cells at $\lambda = 1500$ nm was performed, specifically *Spirogyra*. This was then compared to the performance with two-photon excitation (2PLSM) imaging using a femtosecond pulsed Ti:Sapphire laser at $\lambda = 780$ nm. Analysis of cell viability was based upon cytoplasmic organelle streaming and structural changes of cells and revealed that with continuous imaging at similar peak powers, 2PLSM caused gross cell damage after 5 minutes whereas 3PLSM showed little or no interference with cell function after prolonged exposure.