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Multiphoton Imaging of Mouse Intestine

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APPLICATION NOTE
Multiphoton Imaging of Mouse Intestine
AN_2022 Stuart Thomson, Angela Flock



Introduction

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Experimental Configuration

The sample to be imaged was a section of mouse intestine tissue stained with Alexa Fluor® 568. The RNS1000 was equipped with a motorised XYZ stage and a 40x NA = 0.75 objective. For spectral imaging, the RNS1000 was equipped with a back-illuminated CCD camera and for lifetime imaging a photon counting Hybrid Photodetector and time-correlated single photon counting (TCSPC) electronics. 2PEF and SHG both require a very high excitation intensity, which is achieved using a mode-locked femtosecond pulsed laser. The RNS1000 has external laser coupling ports that enable the optical coupling of femtosecond lasers into the microscope. The optical setup for the femtosecond excitation source is shown in Figure 2. The laser was a Chroma 1540 HP femtosecond fiber laser with an output wavelength of 1040 nm and an 80 MHz repetition rate (Chroma Ltd, UK). For lifetime imaging the output of the laser was pulse picked to the desired pulse frequency using a sub-femto-pulse picker (APE GmbH, Germany). A small fraction of the pulse picker output is picked-off into an Edinburgh Instruments OT100 optical trigger module to trigger the TCSPC electronics. For spectral measurements, the pulse picker is bypassed, and the 80 MHz laser output is coupled directly into the RNS1000.

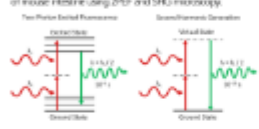


Figure 1. Two-Photon Excited Fluorescence vs Second Harmonic Generation.

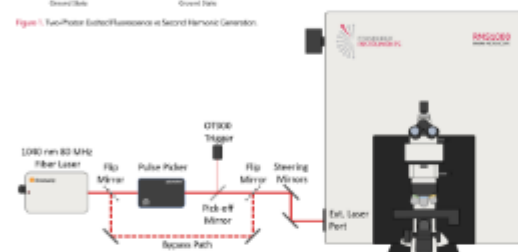


Figure 2. Optical setup for 2PEF and SHG microscopy with the Edinburgh Instruments RNS1000.

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