

INTENSITY CORRELATION ANALYSIS OF A WHITE-LIGHT SUPERCONTINUUM SOURCE FOR MULTIPLE WAVELENGTH LASER SCANNING CONFOCAL MICROSCOPY

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The most striking manifestation of the high nonlinearity in an anomalously dispersive photonic crystal fibre (PCF) is the generation of a white-light supercontinuum. By spectrally filtering a supercontinuum source based on a PCF pumped by a fs-pulsed Ti:Sapphire laser, confocal laser scanning microscopy (CLSM) of cellular samples labelled with a single fluorophore has recently been demonstrated to provide a novel yet simple wavelength-flexible alternative to the existing approach [1]. Source stability is particularly important where quantitative measurements are to be made from the resultant CLSM images, however previous reports of such supercontinuum sources have described up to 50% amplitude noise for certain input pulse parameters [2]. To ascertain the effect of the described noise fluctuations on the resultant CLSM image on a pixel-pixel basis, intensity correlations of both the Ti:Sapphire platform laser and the white-light supercontinuum were performed. These intensity correlations were then compared with the source most typically applied for CLSM, the Kr/Ar laser. By sampling data corresponding to the pixel dwell time for 128^2 , 256^2 , 512^2 and 1024^2 pixel CLSM images, intensity correlations across a two-pixel sample ($n=1000$) of up to 98% and 94% were measured for the Ti:Sapphire and white-light supercontinuum source respectively. The intensity correlation of the Kr/Ar source revealed a correlation of 95% and thus compared similarly to the white-light supercontinuum source. A full report of the experimental system used to measure the intensity correlations of the various sources and details of the computational method for calculating the correlation factors will be presented. To substantiate the source reliability and further demonstrate the flexibility of the system, the white-light supercontinuum source was used to perform sequential CLSM of multiple-labelled guinea pig detrusor, as shown in Figure 1.

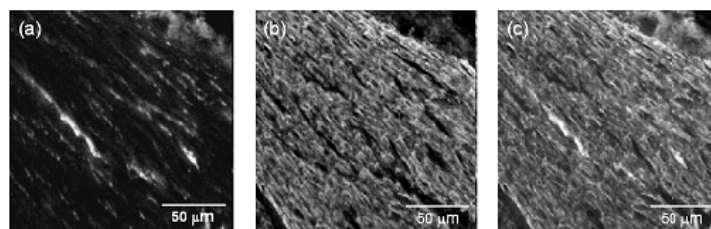


Figure 1: CLSM images of guinea pig detrusor labeled with (a) anti-PGP 9.5 and Alexa 488 and (b) anti-smooth muscle myosin and Alexa 594, obtained using the filtered visible supercontinuum at a depth of 13 μm . Fig.1(c) is a merge of (a) and (b).

References

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