Managing the dispersion of objective lenses in two-photon laser scanning microscopy

By

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A thesis presented in partial fulfillment

of the requirements for the degree of

Master of Philosophy

Department of Physics

University of Strathclyde

November 2011

Declaration of authenticity and authors rights

This thesis is the result of the authors' original research. It has been composed by the author and has not been previously submitted for examination which has led to the award of a degree.

Signed:

Date: 29th November 2011

Acknowledgements

First and foremost I wish to thank my supervisors Dr Gail McConnell and Professor Erling Riis for their expert knowledge and supervision throughout the course of my studies. Both of which have provided me with the encouragement and support in completing my thesis and without them it would not have been possible.

I would like to thank Dr Brad Amos and Mr Esmond Reid for providing the Mesolens data and also Ewan and Bob at the Photonics Group workshop that supplied me with the bits and bobs required for the experimental part of my project. Without any of this, the project would not have been easy.

I am also grateful to my fellow students Alison, Greg, Rumelo and Debbie for their encouragement, help and particularly their friendship during our time together at University. Working amongst everyone has made this year an enjoyable one despite all my moaning, thanks for all the advice, help and the laughs we shared!

Last but by no means least, my deepest thanks goes to my Mum and Dad for their constant support, patience and unconditional love. Both of who have constantly encouraged me to further my studies even though they have had to suffer my mood swings for far too long. I can't make any promises but hopefully it will stop now! Thank you for being so patient.

Abstract

The dispersion in standard objective lenses used in multi-photon laser scanning microscopy and a novel mesoscopic imaging lens, the “Mesolens”, has been studied.

A numerical model to calculate the pulse stretching through lenses has been derived and applied. This involved calculating the pulse stretching through lenses (in the case of the standard lenses, for both on and off-axis propagation) and determining the effect this had on the probability of two-photon absorption during two-photon laser scanning microscopy.

The pulse stretching caused by the Mesolens has been shown to have an increased effect on the temporal spreading of ultrashort pulses compared to a standard 10x/1.2NA Cytometry lens and a 20x/0.75NA Nikon objective lens. The on-axis pulse durations for a pulse with an initial duration of 100 fs at a wavelength of 800 nm were calculated to increase by 21 fs, 25 fs and 127 fs for the Cytometry, Nikon and Mesolens respectively. Off-axis measurements were also obtained for the standard lenses, but these did not display any significant change in output pulse duration as the laser beam scans across the back aperture of an objective lens and therefore can be considered negligible.

It was mathematically illustrated that a simple grating pair was able to introduce the correct quantity of anomalous dispersion to compensate for the normal dispersion introduced during propagation of each lens by choosing the optimum line spacing and grating separation. In doing so, there is a reduced pulse at the sample plane, which maximizes the probability of two-photon absorption during two-photon laser scanning microscopy. Even for the highly dispersive Mesolens, it was calculated that the group velocity dispersion introduced by the Mesolens was 1.05×10^{-1} ps^2/m and could be compensated for in practice by using a grating pair with a line separation of 900 lines/mm separated by 25 mm.

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