

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

By

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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 $1.05 \times 10^{-1} \text{ ps}^2/\text{m}$ 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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