Extended depth-of-field microscopy through a multimode optical fibre

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Multimode optical fibres (MMF) can be used in point-scanning imaging devices when combined with wavefront control. The role of wavefront control is two-fold: to shape the light through the scrambling that occurs inside the fibre and to laterally shift the focus in the sample. The small diameter of MMF makes this approach of particular interest in neuroscience imaging applications. Indeed, the use of micro-endoscopes is required in order to achieve diffraction-limited spatial resolution beyond 2 mm from the surface of the brain while imparting minimal damage to tissue upon insertion[1,2].

In addition to providing high spatial resolution, micro-endoscopes should also have the ability to image large volume rapidly to capture fast dynamic events such as calcium transients. However, this capability is limited by scan speed. In order to mitigate against this limitation, we present a method for generating a focus with an extended depth-of-field at the distal end of a step-index MMF for multiphoton imaging[3]. The transmission matrix was first determined with the laser in continuous wave (CW) mode at 830 nm. The laser was then mode-locked to generate pulses at the same central wavelength, while still using the CW transmission matrix to form a focus. Chromatic dispersion from the limited spectral bandwidth of step-index MMF combined with the broader spectral content of the pulses compare to the CW light caused an axially elongated focus of constant lateral dimensions to form in the sample (axial FWHM: 24.4 ± 0.6 µm, lateral FWHM: 1.3 ± 0.1 µm, pulse duration: 308 ± 13 fs). This focus was then laterally scanned to acquire two-photon excited fluorescence images of beads and neurons. While the lateral dimensions were constant axially, the nonlinearity of the two-photon process increased the lateral variance of the focus.

We further show that a two-dimensional spatially variant deconvolution algorithm can be applied to extended depth-of-field images as they map the integrated axial intensity into a single plane[4]. Together, our results demonstrate the feasibility of spectral shaping through MMF for volumetric multiphoton imaging.

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