LASER SCANNING MICROSCOPY WITH DYNAMICALLY CONTROLLED ILLUMINATION INTENSITY AND POLARIZATION PROPERTIES

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Keywords: Confocal microscopy, multi-photon microscopy, polarisation, fluorescence, programmable optics, holography, point spread function engineering, adaptive optics

Laser scanning confocal microscopy is a widely used technique for the electronic visualization of optically sectioned microscopic structures. The information available from such a system is limited by the intensity and polarisation properties of the point spread function (PSF) at the sample plane, which are in turn dependent on the optical system and the sample being imaged. Aberrations due to incorrect optics or the sample itself may significantly degrade the PSF there by reducing the resolution and the signal available for image formation. Also the information obtained from a laser scanning confocal system working in the epi-fluorescence mode will also depend on the polarization properties of the PSF, as molecular absorption and emission cross-sections are polarisation dependant. These polarisation properties can be particularly important for techniques such as STED and molecular alignment studies when high numerical aperture lenses are used that can result in complex polarisation structure within the PSF.

We describe a beam scanning optical microscope system that uses a programmable holographic optical element to control the optical field in the illumination pupil of the microscope objective [1]. The system uses a computer controlled, fast, ferroelectric liquid crystal spatial light modulator as the programmable optical element to display arbitrary computer generated holograms. In conjunction with simple polarisation optics these produce arbitrary optical wave fields that can be projected into the pupil plane of the scanning microscope by a galvo-mirror pair arranged to minimises the movement of the field across the pupil as the beam is scanned.

We demonstrate how the programmable optical element allows the control of aberrations in the illumination path and present a novel method for sensing those aberrations based on helical doughnut beams[2]. The control over polarisation afforded by the system also allows us to arbitrary polarisation distributions in the illumination pupil such as radially polarisation, which when focused in a high NA objective results in a dominant axially polarisation component in the focus. We investigate the use of this microscope to probe the orientation and reorientation of fluorescent markers on the molecular level.

[1] Neil, M. A. A., Wilson, T., and Juskaitis, R., "A wavefront generator for complex pupil function synthesis and point spread function engineering," J Microsc-Oxford 197, 219-223, (2000).

[2] B.R. Boruah and M.A.A. Neil, “Susceptibility to and correction of azimuthal aberrations in singular beams”, Optics Express, 14, 10377-10385, (2006)