

MAKING LIGHT WORK IN MICROSCOPY

Tony Wilson

Dept. of Engineering Science, University of Oxford, Parks Road, Oxford, OX1 3PJ, UK.

tony.wilson@eng.ox.ac.uk

<http://acara.eng.ox.ac.uk/som/>

There are many factors that influence the final design of a confocal microscope. Among these are the choice of light source and the method of achieving optical sectioning or confocal operation. The choice of illumination has traditionally been restricted to available laser lines, however the recent introduction of white light supercontinuum (WLS) sources has provided much greater freedom and, in combination with spectral detection, greater versatility in confocal fluorescence microscopy. We will demonstrate the use of a WLS source in a reflection mode confocal microscope together with spectral detection to provide spectrally resolved images in three-dimensions. This approach has enabled the imaging of specimen features that are not apparent in either laser illuminated confocal or conventional microscopy.

Microscopic imaging of thick biological specimens is often detrimentally affected by specimen-induced aberrations that cause a loss in signal strength together with reduced resolution. Aberrations can be corrected using adaptive optics, where a deformable mirror introduces equal but opposite aberrations into the optical path. We will discuss the design of a wave front sensorless adaptive system for conventional fluorescence microscopy and optical sectioning microscopy using a structured illumination approach.

Whichever light source and method of obtaining confocality is chosen it is still necessary to focus the microscope. This operation is the bottleneck to high-speed image acquisition since it usually requires a mechanical system to physically move the specimen so that the region of interest is brought into the focal region of the objective lens. In scanning microscopes it is also necessary to introduce a scanning mechanism so that the focal spot is scanned across the focal plane of the specimen. It is straightforward to introduce this scan optically using, for example, galvanometer mirrors since the objective lenses are usually designed according to the sine condition. It is clearly desirable to develop an optical method of focussing which would leave the specimen stationary. We will describe such a system where the refocusing may be achieved remotely from the specimen at high speed without introducing spherical aberration that is common to other optical refocusing systems. In this way can position the focal spot at an arbitrary point in the focal region of a high-aperture microscope objective at high speed.