DIRECT MEASUREMENT OF THE OPTICAL FOCUS IN LASER SCANNING MICROSCOPY

Alison McDonald\textsuperscript{1}, William B. Amos\textsuperscript{2} and Gail McConnell\textsuperscript{1}

1. Centre for Biophotonics, SIPBS, University of Strathclyde, 161 Cathedral Street, Glasgow, G4 0RE
2. MRC Laboratory of Molecular Biology, Hills Road, CB2 2QH
E-mail: alison.mcdonald@strath.ac.uk

KEY WORDS: Multi-photon microscopy, resolution, ablation, SEM

The resolving power of a laser scanning microscope is the most important feature of the optical system and influences the ability to distinguish between fine details of a particular specimen. This is critical in life and materials science research, where sub-micron scale objects are frequently visualised using methods such as confocal and multi-photon laser scanning microscopy: the higher the spatial resolution, the more information on the structure (and hence function) of the specimen is available. The current gold-standard technique for measuring the resolution of a laser scanning microscope involves imaging fluorescently labelled beads or pre-fabricated etched gratings on a slide and then analysing the resultant image. However, this method is subjective, because at high magnification, an optical image of these objects may appear blurry even when they are resolved to the maximum ability of the objective lens, and the specimens are susceptible to damage by the high peak intensities of lasers used in multi-photon microscopy.

We have developed a technique to directly measure the size of the optical focus of a laser scanning microscope. This provides a measurement of the spatial resolution which is governed by the spot size. Our two-step approach firstly employs laser nanomachining of aluminium coated coverslips, whereby a raster scan of the laser beam through the objective lens ablates the aluminium film in the lateral (xy) direction. This leaves a trace of the beam path of the route usually taken to acquire an optical image, with the width of the scanned line related to the lateral resolution of the microscope. The second step is to use scanning electron microscopy (SEM), which, in our case, has a spatial resolution of 20nm, to re-image the ablated beam path. Using image analysis software, a direct measurement of the linewidth in the SEM image can then be made, with an accuracy of 20nm. An SEM image of laser ablation using a 20x/0.75 N.A. air objective lens is shown in Figure 1a-b. The beam radius measured in this way was within 10% of the theoretical value, as shown in Figure 1c.

Figure 1- SEM images of an ablated raster scan at 760nm with 160fs pulses at an average power of 28mW using a 20x/0.75 N.A. objective lens. a) 500x, b) 9000x magnification and c) experimental and theoretical data for varying wavelengths.