

ACTIVE ABERRATION CORRECTION IN CONFOCAL AND MULTIPHOTON MICROSCOPY

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A major advantage of confocal and multiphoton imaging is the ability to obtain optically sectioned images from deep within a biological sample. Unfortunately, as you image deeper into a sample, the image becomes highly aberrated and the resolution significantly reduced due to mismatches in the refractive index within the sample. To overcome this problem an active optical element can be introduced to shape the incoming wavefront compensating for any aberrations induced by the sample [1, 2, 3]. A similar technique is often used by ground based optical astronomers when correcting for atmospheric turbulence.

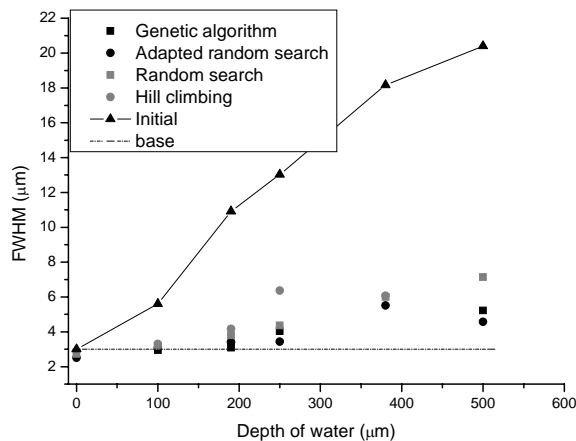


Figure 1: The FWHM measured before and after optimisation using several different optimisation algorithms. The samples consisted of increasing depths of water sandwiched between a coverslip and a flat mirror.

One method for determining the appropriate shape of the active element is to select a property of the image (e.g. brightness or contrast) and use an optimization algorithm to maximize its value. We present a successful implementation of this technique for both multiphoton and confocal microscopy, demonstrating an improvement in resolution of optical test samples and real tissue [1]. As a consequence of the increased signal intensity and increased resolution of the system a lower laser beam power is required to excite the equivalent fluorescence leading to a reduced risk of damage to the sample.

Figure 1 shows a decrease in the full width half maximum (FWHM) of the axial point spread function, relating to an improvement in axial resolution, when the shape of the active element is optimized using different optimization algorithms for a set of optical test samples. Results acquired with the various algorithms will be presented and compared in terms of time taken to complete the optimization and the resolution enhancement achieved.

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