Adaptive optics can be used to correct the aberrations that frequently affect the performance of high-resolution microscopes, particularly when imaging thick specimens. As the light propagates to and from the focus it passes through regions of the specimen with differing refractive indices, inducing aberrations into the optical wavefronts. These aberrations distort the focal spot causing a reduction in resolution and, often more importantly, reduced signal level and contrast. The techniques of adaptive optics have been used to measure and correct the aberrations, restoring image quality in a number of microscopes [1]. Conventional adaptive optics systems use a wavefront sensor to measure aberrations, which are in turn corrected using an adaptive element, such as a deformable mirror. Some wavefront sensor based systems have been successfully implemented in microscopy. However, in microscopes, as with many other imaging systems, direct wave front sensing is not always straightforward and wave front sensor-less schemes are often employed. In these systems, the adaptive element is reconfigured in order to maximise a metric related to image quality.

Most existing implementations of microscope adaptive optics have used single aberration correction settings for each image. This method is appropriate if aberrations are approximately constant across a field of view, which is often the case if the imaged field is small. However, the light contributing to one point in the image follows a different path through the specimen than that for another point. So for many specimens, when fields of view are above a few micrometres, aberrations can vary significantly across the image [2]. Consequently, different aberration correction would be needed in different parts of an image for diffraction-limited operation to be maintained. With a single compromise correction setting, some areas of the image would benefit from more complete correction than other areas. It is also possible that in some image regions the aberrations are actually made worse.

We consider the challenges that these spatial variations present for the measurement and adaptive correction of aberrations. We investigate how the measurement of field dependent aberrations can be performed using both direct wavefront sensing and indirect, sensorless schemes. The measurements give rise to three-dimensional aberration maps that show the spatial variation of each aberration mode. This information can be used to reconfigure the deformable mirror as different regions of the image are acquired. We further discuss different approaches to aberration correction that can be adapted to deal with the spatially varying aberrations.