Micron 42 (2011) 318–323

Contents lists available at ScienceDirect

Micron

journal homepage: www.elsevier.com/locate/micron

A demonstration of the effectiveness of a single aberration correction per optical slice in beam scanned optically sectioning microscopes

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ARTICLE INFO

Article history:
Received 1 March 2010
Received in revised form 17 May 2010
Accepted 19 May 2010

Keywords:
Confocal microscopy
Biological imaging
Multiphoton microscopy
Adaptive optics
Aberration correction

ABSTRACT

In this paper we report the use of adaptive optics to correct for sample induced aberrations in optical microscopy, crucially comparing individual pixel-by-pixel correction against a single correction for an entire optical section. Sample induced optical aberrations in slices of rat brain tissue were corrected with a deformable membrane mirror. Using axial resolution measurements, we demonstrate that a single aberration correction per optical slice achieves around 80% of the maximum possible improvement compared to individual pixel-by-pixel correction in both confocal and multiphoton microscopy. A single aberration correction per depth, compared to pixel-by-pixel aberration correction, significantly decreases scan times and therefore reduces photobleaching and phototoxic effects enabling more rapid microscopy with active aberration correction. The results confirm that the use of a "look-up" table, based upon sample type and depth, may be the most practical way of implementing adaptive optic aberration correction in beam scanning optical sectioning microscopy.

1. Introduction

Confocal and two photon excitation microscopy are now invaluable and routine tools for biologists and life scientists (Diaspro, 2002). Both techniques are able to produce optically sectioned images with significantly improved axial and marginally improved lateral resolution compared to more conventional widefield fluorescence microscopy. However, when imaging deep inside biological samples the image resolution is degraded due to specimen induced aberrations (Hell et al., 1993). Several static aberration correction methods have been proposed (Booth and Wilson, 2000; Sheppard and Gu, 1992; Sieracki et al., 1995), however, such static corrections only apply at fixed depths within the sample. These static corrections have an added disadvantage in that they are not sample specific.

A more flexible approach to aberration correction is based on the application of astronomical adaptive optics technology (Tyson, 1998), utilizing an active deformable optical element to counteract the sample aberrations (Girkin et al., 2009). The significant challenge is to determine the most appropriate wavefront correction to be applied by the adaptive element to compensate for the aberrations present. Demonstrations have been made by several groups using a variety of search techniques to determine the appropriate wavefront shape required to improve image resolution and intensity (Albert et al., 2000; Booth et al., 2002; Marsh et al., 2003; Wright et al., 2005; Booth, 2006; Rueckel et al., 2006; Wright et al., 2007; Poland et al., 2008), and hence the overall image quality, in a beam scanning microscope.

In a laser scanning confocal system (the practical system most commonly used by life scientists) the ultimate correction using adaptive optics would be obtained by using a different correction for each pixel of the image (i.e. each point on the raster scan). However, for a 512×512 pixel image, with an update rate of 1 kHz on the active element, each optical section would require 262 s, assuming instantaneous determination of the wavefront correction required. Although this point-by-point optimization would undoubtedly offer the best image quality the imaging time would significantly increase the risk of tissue damage and photobleaching as well as ruling out the observation of any fast events.

We report on the removal of sample aberrations using a single wavefront correction per optical section, an approach which greatly reduces the total scan time compared to a point-by-point technique. This method is further enhanced through the use of a "look-up" table for a generic sample type, at a particular depth.

2. Materials and methods

The experimental layout shown in Fig. 1 was based around a laser scanning confocal microscope, operating in reflection mode.

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0968-4328/$ – see front matter.
doi:10.1016/j.micron.2010.05.018

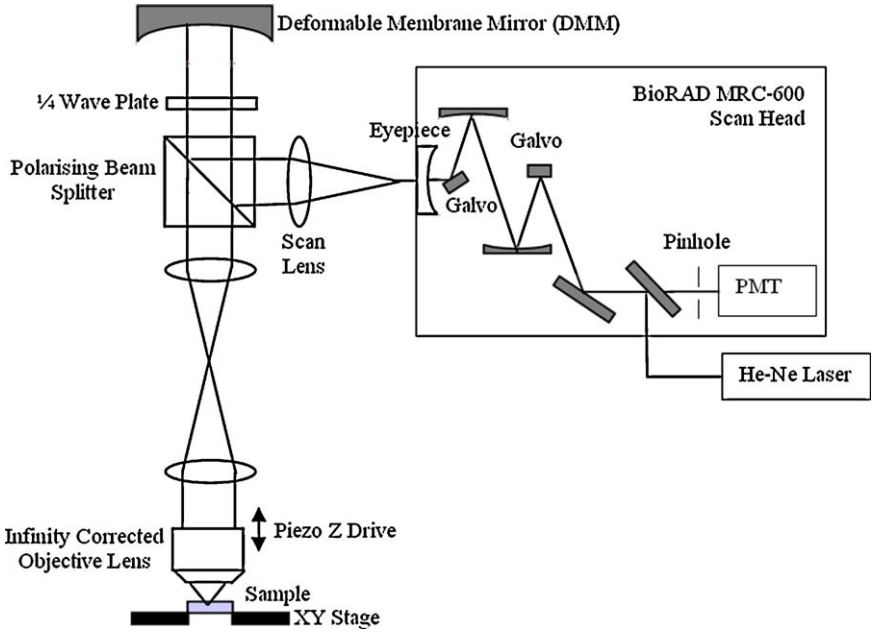

Fig. 1. Confocal reflection microscope setup including the DMM.

A Helium-Neon laser (633 nm, 10 mW maximum output power) was directed into a BioRad MRC 600 scan head, with an infinity corrected Nikon air-objective (coverslip compensating), numerical aperture of 0.5 and 20× magnification, being used to focus the laser beam onto the sample. The detection photomultiplier tube (PMT) and confocal aperture were housed in the scan head.

The adaptive optic element was a deformable membrane mirror (DMM) made by OKO tech/Flexible Optical which has a maximum update rate of 1 kHz and a maximum stroke of 8 microns. The DMM consisted of a silicon nitride membrane mounted above 37 electrostatic actuators with a maximum of 175 V being applied to each actuator to pull the membrane. The DMM was placed in the beam path after the scan head, with beam expansion optics relaying the stationary image point onto the mirror and back onto the back aperture of the objective, creating a double pass adaptive optic system. In order to enable the DMM to apply both positive and negative aberration correction, a bias was applied to the DMM pulling the mirror to a mid point in its travel. This had the effect of focusing the beam, which was counteracted by a defocusing bias introduced by the lens immediately after the DMM.

The objective was mounted on a piezoelectric translational device (PZT) (E662 LVPZT Physik Instumente, Germany) to control the position of the beam focus along the optical axis (Zucker and Price, 2001). This enabled easy and precise depth scanning. The DMM shape required to overcome sample induced aberrations was determined through the use of a genetic optimization search algorithm in which the DMM shape was rapidly changed to optimize a particular image quality (in general intensity was used in this paper). The tissue samples were placed on a plain mirror (see Fig. 2a) and the intensity of the reflection from a point on the mirror used as the optimization parameter, with the light passing twice through the tissue sample. Samples were prepared with different tissue thickness in order to control the level of sample aberrations present. This approach reduces the optical power density of laser light the tissue itself was exposed to (thus reducing the probability of photodamage) and would be the ideal approach to use for building a "look-up" table of aberration corrections.

2.1. Sample preparations

Experiments were performed in accordance with the U.K. Animals (Scientific Procedures) Act, 1986. Two male Wistar rats (~300 g) were deeply anesthetized with pentobarbital (Sagital, 150 mg/kg i.p.) prior to intracardiac perfusion with first saline (10 s)

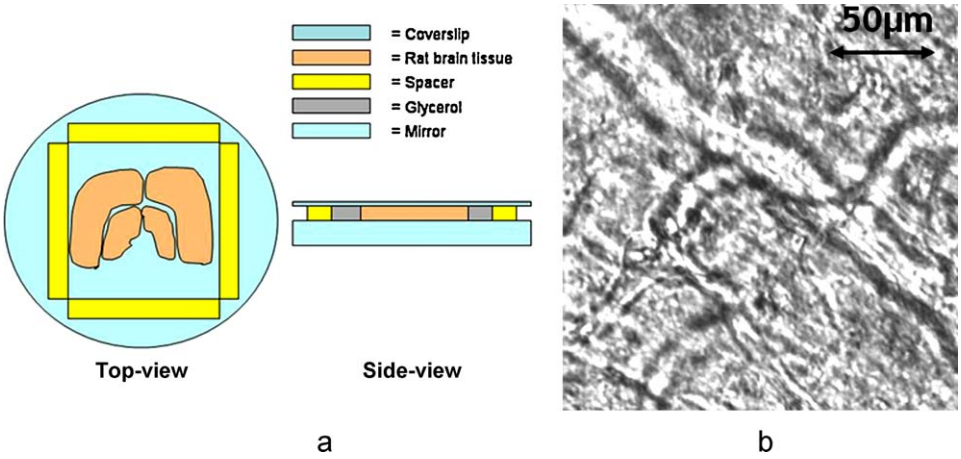

Fig. 2. (a) Layout of rat brain tissue samples and (b) a transmission image of a 20 μm thick brain slice.