Critical considerations of pupil alignment to achieve open-loop control of MEMS deformable mirror in non-linear laser scanning fluorescence microscopy

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ABSTRACT

In this paper we present an alignment methodology for a non-linear laser scanning fluorescence microscopic imaging system integrated with a MEMS deformable mirror that is used to compensate microscope aberrations and improve sample image quality. The procedure uses an accurate open-loop control mechanism of the MEMS DM, a high resolution CMOS camera and a compact Shack-Hartmann wavefront sensor. The success of the indirect AO control method used by the microscope to compensate aberrations requires careful alignment of the optical system, specifically the DM conjugate planes in the scanning laser optical path. Considerations of this procedure are presented here, in addition to an assessment of the final accuracy of the alignment task is presented, by verifying the pupil conjugation and wavefront response. This method can also serve as a regular check-up of the system's performance and trouble-shoot for system misalignment.

Keywords: Adaptive optics, MEMS deformable mirror, open-loop control, Shack-Hartmann wavefront sensor

1. INTRODUCTION

Nonlinear fluorescence microscopic imaging, with its unique advantages of intrinsic three dimensional optical sectioning ability, deep tissue penetration, and reduced photo-toxicity, has been widely adapted to reveal biological processes at cellular level[1],[2][3]. However, like all other optical imaging techniques, nonlinear microscopy suffers from image quality degradation due to the optical aberrations in the system, from both the optical system and the specimen. Optical aberrations distort the wavefront of the excitation beam, and then affect the focal spot to be larger than diffraction limited. Since the fluorescence efficiency scales nonlinearly with the profile of the focusing excitation beam, aberrations further degrade the imaging quality more severely in this case.

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To correct for the aberrations and to improve the contrast of the imaged sample, adaptive optics (AO) has been adopted by many biomedical imaging platforms[6],[7],[8],[9],[10],[11]. AO was originally designed for ground-based observatory astronomy to correct for the aberrations from atmospheric turbulence while imaging distant stars and planets[4],[5]. The AO in our non-linear laser scanning fluorescence microscope uses a Boston Micromachines Corporation microelectromechanical system (MEMS) deformable mirror (DM) to compensate microscope aberrations that reduce image quality .

In typical AO systems, the DM is controlled in a closed-loop manner using direct feedback from a wavefront sensor that measures the error between the current DM shape and a compensatory shape, effectively driving the measured wavefront such that it becomes planar. In the nonlinear microscope, direct feedback of wavefront error does not exist, and the DM is controlled indirectly, using information contained in the microscope image, such as sharpness. Furthermore, in the microscope system presented here, the aberrations primarily consist of known Zernike modes, such as astigmatism and spherical aberrations, which can be compensated using a modal hill climbing control method, where each mode can be applied to the DM with variable amplitude and adjusted until the image metric in the indirect controller is maximized. The amplitude of this mode is then fixed and the next orthogonal mode of interest with variable is applied to the DM until the metric is maximized, and the process continues.

To implement this type of indirect AO control, known shapes need to be applied to the DM without the use of direct feedback on its current position, as is typically supplied by a wavefront sensor. Determination of the voltages for a desired shape for a continuous facesheet deformable mirror is made challenging due to mechanical coupling of adjacent DM actuators through the mirror surface, this is also known as the DM influence function. With the recent development of an accurate open-loop control mechanism[12],[13],[14] which predicts the control voltages and generates a prescribed surface shape on the MEMS deformable mirror, known aberrations in the system can be compensated using this modal and indirect control technique.

A central challenge in the implementation of this type of DM control method in the nonlinear microscope is the precise alignment of the active DM aperture with the pupil of the microscope objective. Any misalignment between the objective and the DM will lead to cross-talk between the modes compensated and reduce the overall AO performance of the microscope. This plane is also conjugate with two scanning mirrors in the optical train, compounding the problem. Open-loop compensation of known aberrations represented in Zernike modes requires precise alignment of the optical pupil. We show a careful alignment procedure with the aid of a Shack-Hartmann wavefront sensor and a CMOS camera, that can evaluate the alignment quality by manually imposing one Zernike mode on the DM and measuring with the Shack-Hartmann wavefront sensor at the conjugated pupil plane.

2. MATERIALS AND METHODS

2.1 Multiphoton scanning laser microscopic system

The imaging platform adopted in this study is based on an *in vivo* video rate laser scanning microscopic technique developed by Veilleux *et al*[14]. A schematic of the non-linear laser scanning fluorescence microscope integrated with a MEMS DM used in this study is shown in Figure 1. A solid state mode-locked Ti-Sapphire laser (Newport Corp., Irvine, CA) is used as excitation light source. A polarizing beam splitter cube (CVI Melles Griot, Albuquerque, NM) that reflects s-polarized light and transmits p-polarized light, when used together with a quarter wave plate (Edmund Optics, Barrington, NJ) enables normal incidence onto the MEMS DM. This MEMS DM with 140 electrostatic actuators (MultiDM®) from Boston Micromachines Corporation features 3.5μm stroke, 400μm pitch between actuators and a 4.4mm clear aperture. In order to utilize the maximum number of active actuators and to generate precise shapes of different Zernike orders, the center 10-by-10 actuators are chosen and hence define the entrance pupil diameter to be 4mm.

The raster scan is achieved through the use of a spinning polygonal mirror (Lincoln Laser, Phoenix, AZ) for horizontal (or fast) axis and a galvanometer-mounted mirror scanner (Cambridge Technology, Lexington, MA) for vertical (or slow) axis. The excitation beam is focused onto the sample with an 60X objective lens of 1.0 NA (Olympus America, Center Valley, PA) and the nonlinear signal is collected with a photomultiplier tube (PMT, Hamamatsu Photonics, Japan). The analog signal from the PMT is sent to a video acquisition board (Active Silicon, Chelmsford, MA) that is also synchronized with the scanning mirrors, to acquire images using a PowerMac G4 computer.
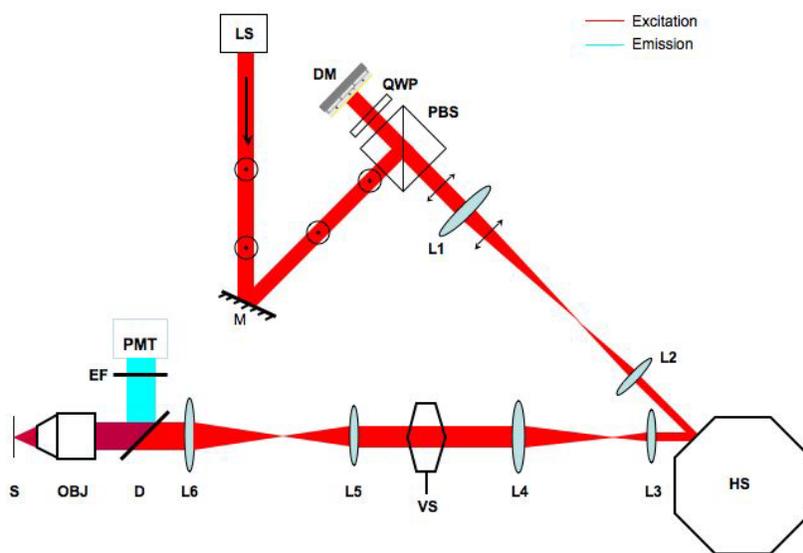

Figure 1. Optical layout of the nonlinear fluorescence microscopic imaging system integrated with MEMS DM. LS: laser source, M: mirror, PBS: polarizing beam splitter, QWP: quarter wave plate, DM: deformable mirror, L1 – 6: lenses, HS: horizontal scanner, VS: vertical scanner, D: Dichroic filter, OBJ: objective lens, S: sample, EF: emission filter, PMT: photomultiplier tube, ⊙: s-polarized wave before reaching the quarter wave plate, ↕: p-polarized wave after double-pass of the quarter wave plate.

2.2 MEMS DM Open-loop control

The open-loop control mechanism was previously described in publications by Stewart[12] and Diouf[13]. Arbitrary shapes can be made with this control approach, with errors typically <25 nm rms. The images presented in Figure 2 qualitatively show the capability of this open-loop control method.

In the work reported here, we use an active optical aperture diameter of 4mm, incorporating the center 100 actuators. Actuators outside of the optical aperture are used to assist in the open-loop control, by minimizing mirror forces at the aperture edge.

2.3 System alignment considerations

First, the collimation of the beam in between pairs of telescope lenses needs to be ensured. To eliminate divergence or convergence of the beam where it should be collimated, a Mode Master (Coherent, Santa Clara, CA) samples the beam at positions before L1, after L2, L4, and L6 to measure the M^2 value and the divergence angle of the laser. Proper adjustments of the distance between two telescope lenses should be made according to the results of the divergence angle measurement.
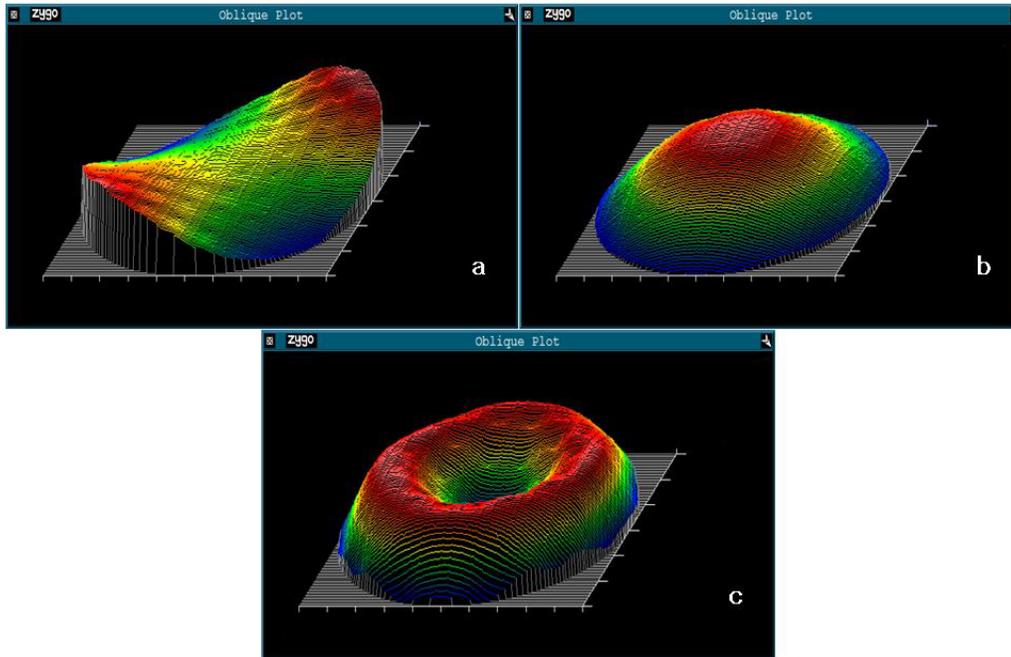
Figure 2: Different Zernike term shapes with 1 μm Peak-to-Valley difference in DM surface made using the open-loop control mechanism, as measured with a surface mapping interferometer: a) Astigmatism; b) Defocus; c) Spherical.

For the shapes generated on the DM by the open-loop control to be effectively translated to the sample, the surfaces of the DM (entrance pupil), the horizontal scanner, the vertical scanner and the back focal plane of the objective lens need to be optically conjugated by telescope lens pairs, in Figure 1, L1 and L2 conjugate the DM surface to the horizontal scanner surface. L3 and L4 conjugate the polygonal mirror to the galvanometer-mounted mirror, and also scale the scanning angle of the polygon to match with the one of the galvanometer scanner, resulting in a square scanning field. L5 and L6 conjugate the above three surfaces to the back focal plane of the objective lens, as well as magnifying the beam to fully fill the objective back aperture. The optically conjugated surfaces will form their respective images at the same axial position, where the high resolution USB 2.0 CMOS camera is used to image the overlap surfaces onto its sensor plane. The wavefront at the objective back aperture plane can also be examined using a Shack Hartmann type wavefront sensor.

3. RESULTS

3.1 Pupil conjugation

Figure 3 shows the image captured by the CMOS camera at the objective back focal plane. When the fine structures of the DM actuators and the silver mirror surfaces of the horizontal and vertical scanners all appear sharp, the three surfaces are optically conjugated.

Once the pupils are considered optically conjugated, a Shack-Hartmann wavefront sensor can be used to investigate the residual higher-order aberrations using Zernike decomposition[16][17]. For a flattened DM, one should adjust the system until minimal systematic aberrations present in the microscope imaging system observed by placing a Shack-Hartmann wavefront sensor where the beam should be collimated along the beam path and at the objective back focal plane[17]. With the same optical setup, the wavefront responses at the objective back focal plane are recorded according to various

Zernike shapes imposed on the DM using the open-loop controller. Figure 4 shows the correlation between the mirror shape and the Shack-Hartmann wavefront sensor readings.
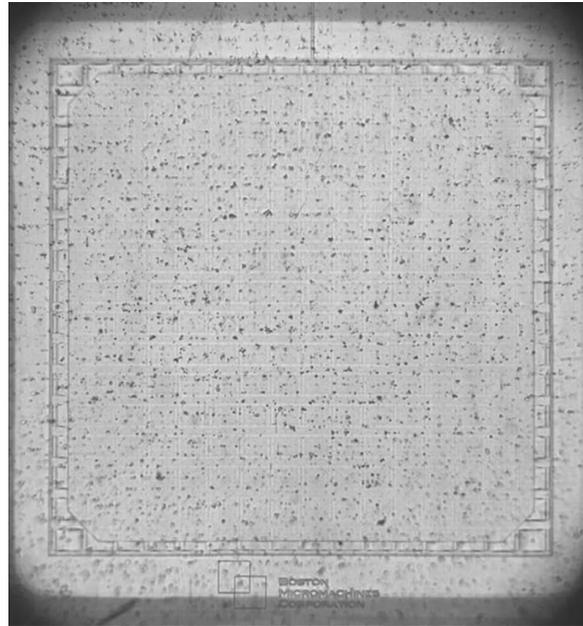

Figure 3: CMOS camera image of the DM actuators and structures on the surfaces of silver mirrors of the horizontal and vertical scanners.
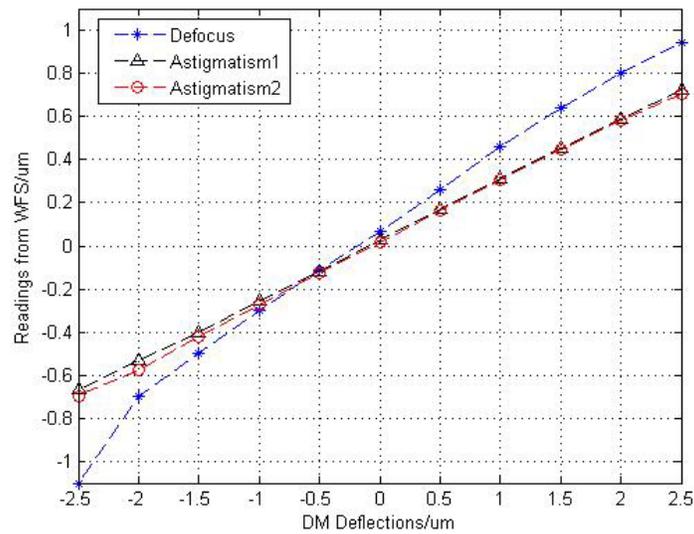

Figure 4: Linear response between the open-loop controlled DM shapes and the readings from the Shack-Hartmann wavefront sensor. Open-loop controlled DM deflection from Peak-to-Valley value of -2.5 μm to 2.5 μm, for defocus and two orthogonal astigmatism shapes.

4. CONCLUSIONS

In this paper, we have introduced an alignment method using various beam profiling combining a pupil finding equipment. The necessary assessment of the alignment quality using the same equipments is demonstrated. An accurately aligned optical imaging system is crucial for effective open-loop wavefront control. The procedure described in this paper can also serve as a regular check-up of the system's performance and trouble-shoot for system misalignment.

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