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Easy and versatile adaptive optics setup with deformable lens for high-resolution microscopy

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ABSTRACT

It has been widely proven in literature that most optical microscopy techniques can greatly benefit from the application of adaptive optics correction of phase aberrations through an adaptive optical element, such as a deformable mirror or a spatial light modulator. However, adaptive optics is not yet widely adopted in the life sciences community, mostly due to the lack of adaptive commercial microscopy systems, and the inherent technical difficulty in modifying an existing microscopy setup to integrate an adaptive element, both on the software and hardware sides.

We present a plug-and-play adaptive optics module for generic optical microscopes, based on a prototype refractive 18 actuators adaptive optical element[1], which can be inserted in any microscope between the objective and the microscope body. Correction is performed in a sensorless fashion, optimizing image quality metrics of the image presented to the user on screen. The results presented show how an end-user oriented commercial confocal laser scanning microscope (Leica SP5) can be upgraded with adaptive optics with minor hardware modifications, and no changes to the microscope control software.

Keywords: Adaptive optics, Optimization, Fluorescence microscopy

1. INTRODUCTION

Images acquired with high performance optical microscopy systems can be severely affected by the presence of phase aberrations introduced by the sample, or by defects and misalignments of the optical system itself [2]. Correction of phase aberrations has been proven possible through the use of active optical devices, such as deformable mirrors or spatial light modulators[3].

While the results presented in literature show how implementation of adaptive optics could provide significant advantages for the end users, the complexity of implementation of adaptive optics in an existing microscopy setup prevents the widespread adoption of the technique. In particular, the implementation of adaptive optics through a reflective optical element (i.e. a deformable mirror) requires significant modification to both the hardware and software of the microscopy system, which requires time and effort for a custom made setup, and is in general simply impossible in a commercial setup.

In this paper, we present a technique to easily implement adaptive optics on any optical microscopy system through the addition of a refractive adaptive element at the back of the microscope objective, and a standalone control software which performs sensorless wavefront optimization based on images acquired from the computer screen.

2. ADAPTIVE ELEMENT

The refractive adaptive element is a deformable lens[1] composed of two thin glass windows of 1 cm diameter, upon each of which is mounted a piezoelectric actuator ring. The space in between the windows is filled with a transparent liquid, mineral oil. The first window is used to generate defocus and astigmatism while the second one generates coma and secondary astigmatism. Both rings are divided into 8 sectors that can be actuated independently.

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The piezo rings are glued to the windows and act as a bimorph actuator, such that the application of a voltage generates a bending of the glass window. The actuators are controlled by a 16 channel high voltage driver. The 8 actuators on the top and bottom windows generate different effects because the top window is attached to the actuator by an elastomer foam that is free to move.

Due to the piezoelectric nature of the actuators, hysteresis is present when performing corrections through the deformable lens. Moreover, since the actuators movement is dampened by the compression of the fluid in the lens chamber, noticeable dynamic effects and creep can be observed in the actuator's influence on the wavefront.

To correct for both these effects, a model of the actuators dynamics is used to control the lens. The model consists in two parts: a generalized Prandtl-Ishlinskii model (GPI) with 7 play operators for the modeling of the Piezoelectric hysteresis, and a second order discrete time transfer function model for the modeling of the dynamics [4].
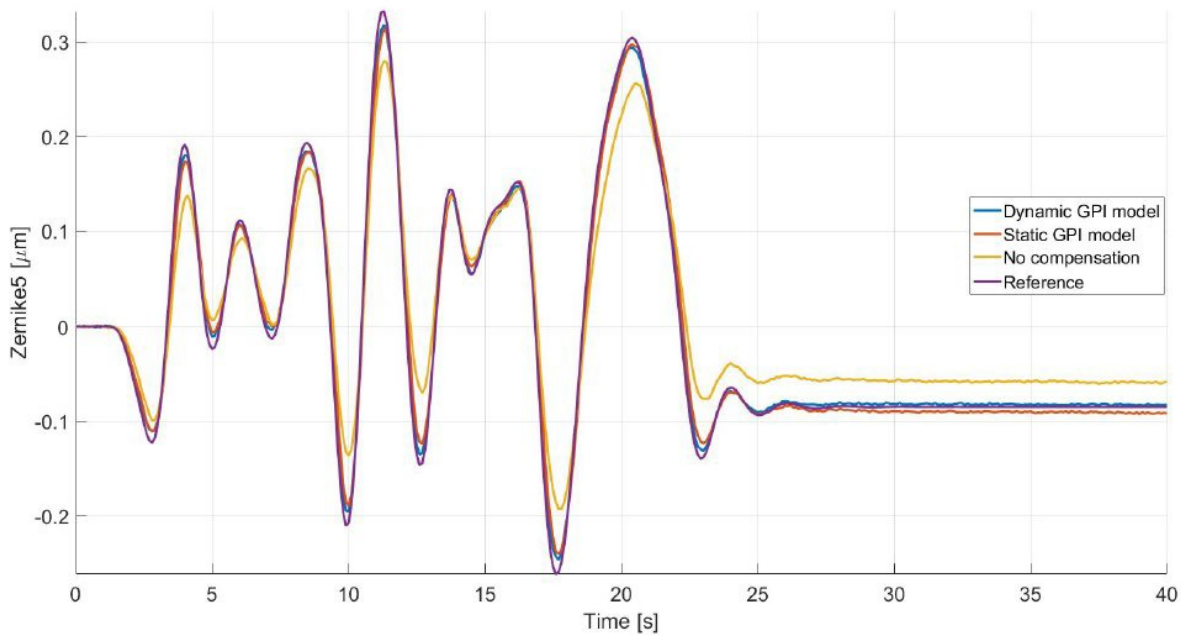

Figure 1. experimental measurement of the response of the adaptive element when generating a random variation of defocus aberration. Purple line is the input defocus, yellow line is the response without any correction, red line is the response when only correcting hysteresis, and blue line is the response when correcting for both hysteresis and dynamics. It can be observed how, without dynamics compensation, when fixing the value of the desired correction, the aberration drifts over time.

The lens was characterized on a simple adaptive optics benchmark setup. A collimated beam is shined through the lens, and the lens plane is conjugated with a Shack Hartmann wavefront sensor through a 4f optical system.

After poking each actuator to identify the influence functions of the actuators, a time dependent identification function is applied to each actuator, consisting in a series of steps movements of decreasing amplitude, to identify hysteresis parameters, followed by a frequency sweep, to identify the system dynamics. The response of the actuator is measured with the wavefront sensor, and the dataset is used to identify the parameters of the model.

The model is then inverted, and used to process the inputs to the lens during adaptive optics operation, in order to ensure a linear response of the actuators. A test measurement of the performance of the compensation is reported in Figure 1.

3. OPTICAL SETUP

In order to compensate for optical aberrations in microscopy systems, the deformable lens is simply screwed on the microscope objective holder of the microscope, and the objective is screwed on the opposite side of the lens.

Due to this modification, the position of the objective is shifted axially of a distance equal to the thickness of the mechanical enclosure of the adaptive lens, which is approximately 1cm. In most microscopes, focus control is achieved by shifting the objective axially, and the range of adjustment easily exceeds 1 cm, so this modification does not affect noticeably the performances of the microscope. The only hardware modification that may be necessary is a relocation of the sample holder, which, in the inverted setup used in the experimental results section, was easily achieved through the use of spacers on the sample holder mount.
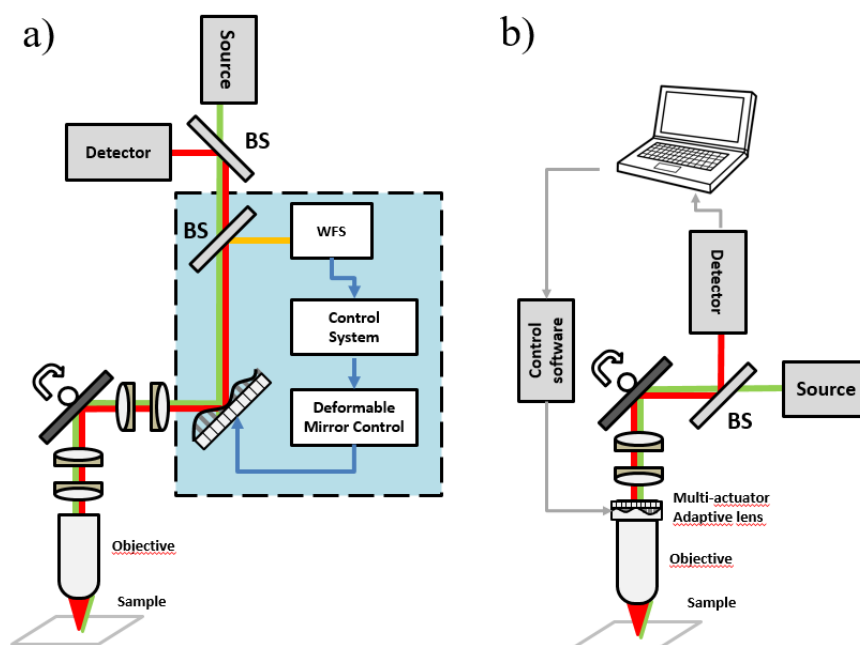

Figure 2. Scheme of adaptive optics implementation in a scanning microscopy system for **a)** a mirror based, wavefront sensing setup, and **b)** a lens based, sensorless setup. The blue square in panel **a** shows the components that should be added to an existing setup to implement adaptive optics. It is apparent how such modification is structural, and of difficult implementation in an existing system.

It is to be noticed that, since the back aperture plane of the objective is normally geometrically located within the objective itself, the adaptive optical element is not located exactly in the pupil plane of the system. This requires the diameter of the adaptive lens to be slightly wider than the aperture of the objective lens, to avoid vignetting artifacts in the image.

The 1cm diameter of the lens used in the experiments is appropriate for most high performance microscopy objectives, which have apertures in the order of 6-8 mm, but a different adaptive lens may need to be used for low-magnification, high aperture objectives, such as those used for high end in-vivo multiphoton imaging applications.

4. CORRECTION ALGORITHMS

Due to the low brightness of microscopy signals, especially in fluorescence microscopy applications, and to the absence of natural bright stars in microscopy samples, the use of closed loop adaptive optics techniques in microscopy is difficult

to implement. As a consequence, most reported implementations of adaptive optics in microscopy use wavefront sensorless correction techniques.

In the most common approach to sensorless aberration correction in imaging systems, an image metric is used to evaluate the performance of the correction applied, and an optimization algorithm is used to find the set of actuator voltages for the adaptive element which maximize (or minimize) the metric.

A variety of image metrics can be used to evaluate the performance of correction in microscopy, and the working principle of the microscope used should be carefully considered when selecting a metric.

In the case of both laser scanning confocal and laser scanning multiphoton microscopy, in the presence of an aberration, the total detected intensity decreases as the aberration increases, and can be therefore effectively used as a metric for sensorless aberration correction. This is due to the fact that the size of the point spread function depends on the amplitude of the aberration: in a confocal microscope a wider system point spread function will result in more light being rejected by the pinhole, while in a multiphoton microscope it will result in a lower irradiance at the focal point, which will reduce the two photon absorption due to its intrinsic non linearity.

The results presented in the following section were acquired on a laser scanning confocal microscope, and the metric employed was the total intensity of the image. However, the method reported could be easily implemented, with a different metric, in microscopy systems where the total image intensity is not dependent on the aberration (e.g. epifluorescence microscopy, lightsheet microscopy, structured illumination microscopy). Possible metrics to be used in such case are image sharpness, or image Fourier content.

The correction algorithm implemented was the Data-based Online Nonlinear Extremum-seeker (DONE) algorithm [5], previously successfully implemented in other imaging techniques in our group [6,7]. The DONE algorithm is an optimization algorithm based on the recursive creation of a nonlinear model of the metric function during the optimization procedure itself. Each time a new measurement is taken, the model of the metric function is updated with the new measurement, and the following measurement is performed in the maximum of the new model.

The main advantages in the use of the DONE algorithm are its robustness to measurement noise and small dynamic variations in the metric function, which could be introduced by a temporal variation in the sample itself (e.g. photobleaching, sample movement, focus drift) or by poor compensation of the actuator's hysteresis and dynamics.

5. RESULTS

Measurements were performed on a commercial laser scanning confocal microscope (SP5, Leica, Germany). Since the software controlling the microscope provides no options to transfer the images in real time to external software, we employed an internally developed software which grabs the screen output of the computer. We chose to apply our method to this microscope, as this represents the "worst case scenario" for implementation of adaptive optics in an existing optical system. The hardware of the microscope is extremely compact, precluding the possibility of adding a telescope system conjugating the aperture of the system to a deformable mirror, and the software is completely closed source, and does not allow access to the acquired images or the microscope settings to external scripts or softwares.

 Synchronization with the microscope timing was provided through a low cost digital I/O board (USB6501, National Instruments, USA), using the digital trigger ports of the microscope itself. Timings were set up so that, for each measurement, new voltages were applied to the lens actuators, and the acquisition of an image was triggered after waiting for a 50ms settling time for the actuators, and the screen image was grabbed 16 ms (the screen refresh time) after the end of the image acquisition.

Images were acquired at 1 frame per second, with a 63X, 1.4 N.A. microscopy objective. The metric used was the total fluorescence intensity of the images. Optimization was performed over 7 degrees of freedom, namely Zernike coefficients 5 to 11 according to Noll's notation, consisting on all coefficients up to Spherical aberration, escluding Piston, Tip, Tilt and Defocus. Optimization was run for 200 seconds.
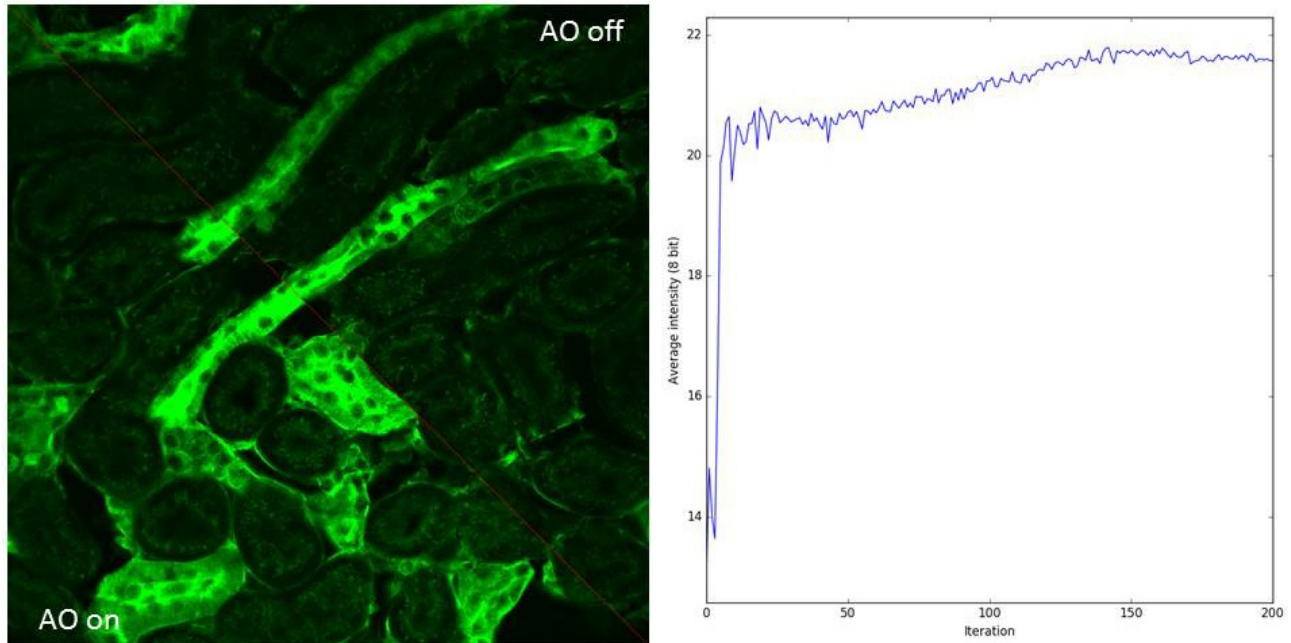
Figure 3. Representative results of the sensorless correction procedure. It can be observed how the metric improves consistently within a few iterations, and keeps increasing over time. At around 150 iterations, the metric reaches its maximum, and a slight decrease in observed due to photobleaching.

The sample used for the tests was a of a 16 μm thick prepared slide of mouse kidney stained with Alexa 488 (Fluocells prepared slide #3, Invitrogen, US), which is a sample introducing small, but detectable, phase aberrations. The optimization procedure could consistently increase the average image intensity by around 50%, producing most of the increase in the first 15-20 iterations.

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