Adaptive Optics Microscopy:

Easy and automatic aberrations correction in microscopy

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Introduction

Adaptive optics revolutionized astronomical telescopes producing unprecedented image quality that allowed astronomers to detect objects in space at a higher clarity and at distances not previously possible. In the case of telescopes, adaptive optics is implemented with the use of deformable mirrors to compensate atmospheric turbulence distortions. Unfortunately, traditionally used deformable mirrors are not able to be inserted inside microscopes without significant modifications. Modern day advancements of deformable lenses and high-speed automatic optimization algorithms enable the same revolutionary methods to be applied in microscopy in a streamlined plug and play workflow.

Adaptive optics microscopy

Optical systems are usually conceived with fixed optical components. Astronomers demonstrated that deformable mirrors can be successfully used to compensate for optical aberrations generated by atmospheric turbulences. Since the sky aberrations changes rapidly, a wavefront sensor is necessary to real time measure them and to compute the deformable mirror shape that compensate for those distortions. Many deformable mirrors are currently installed in large scale telescopes. A layout of adaptive optics for astronomy is shown in Fig. 1 left. Unfortunately, deformable mirrors and wavefront sensors are very difficult to be installed on microscopes without completely re-design the instrument.

The use of deformable lenses allows an easy and plug and play integration inside any optical instruments. For example, in the case of a microscope it is possible to install the lens on the back pupil aperture of the objective as shown in Fig. 1 right.

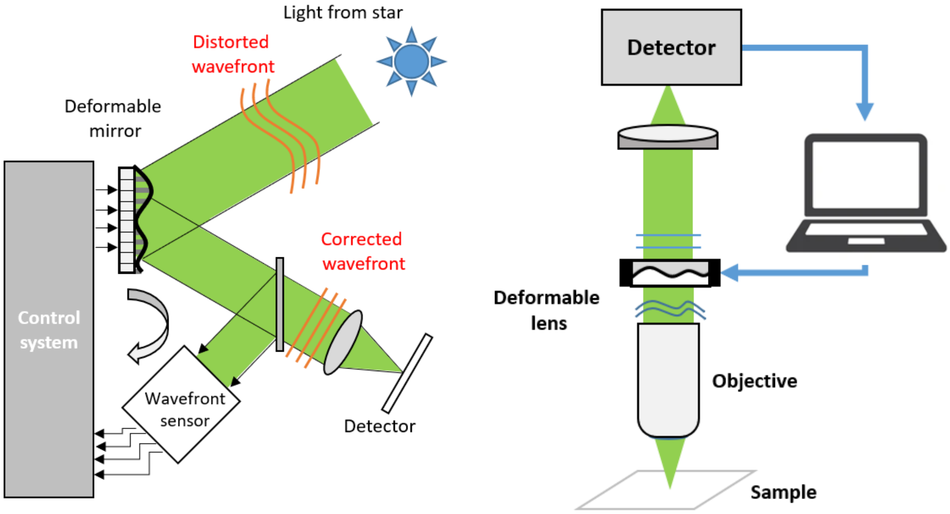


Fig. 1, left: adaptive optics system for astronomy with a deformable mirror and a wavefront sensor. Right: adaptive optics system for microscopy based on deformable lenses and an image optimization algorithm.

The concept is presented in Fig. 2. In an ideal system, a point source on the sample emits perfect spherical wavefronts that are collected by the objective and imaged on the microscope camera (Fig. 2a). The light rays passing through inhomogeneous media, are deviated from the ideal spherical wavefront and the image on the sensor loses resolution and contrast (Fig. 2b). Sources of aberrations can be many: inhomogeneities of biological samples, layers and films with refractive index mismatch, alignment errors etc. Using a deformable lens, it is possible to compensate for the optical aberrations and restore a perfect flat wavefront after the microscope objective leading to a diffraction limited image. Dynamic Optics Multi Actuators deformable lens can achieve this goal changing its own shape (Fig. 2c).

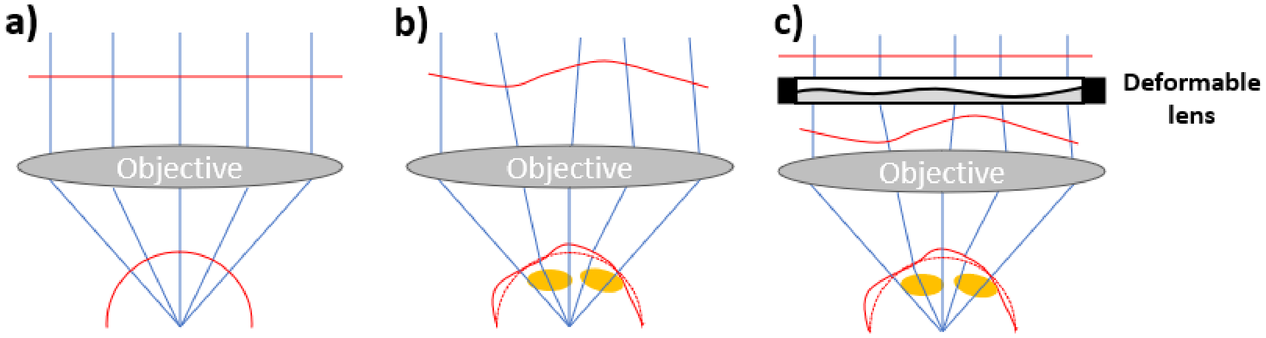


Fig. 2: a) ideal wavefront propagation from a point source in the object. b) In real samples the spherical wavefront emitted by each point source is distorted by the sample inhomogeneities. c) Deformable lenses can correct for the wavefront aberrations and compensate them.

Fig. 3: left) cross section of the deforamable lens. Center) image of the deformable lens (10mm clear aperture). Right) Deformable lens mounted on a microscope objective.

The lens is composed of a container sealed with two thin glass membranes and filled with a transparent liquid. Both lens faces are bonded to a piezoelectric ring actuator divided in many independent segments that can bend the membrane upward or downward (Fig.3). This lens is the equivalent of a free form programmable lens. Its surface deformation can generate up to the 4th order aberrations and correct for up to 30 Zernike modes. The lens has a fast response time of about 2ms allowing for a fast aberration correction.

Main deformable lens specs:

- High transmission >92%
- AR coated surfaces
- Up to 30 modes correction
- Fast response time
- No pixels or segments inside the clear aperture
- Low dispersion

The implementation of the lens in the microscope is easy and straight forward. The lens can directly placed on the back aperture of the microscope objective (see Fig. 3 and Fig. 4) or in any image plane of the microscope pupil.

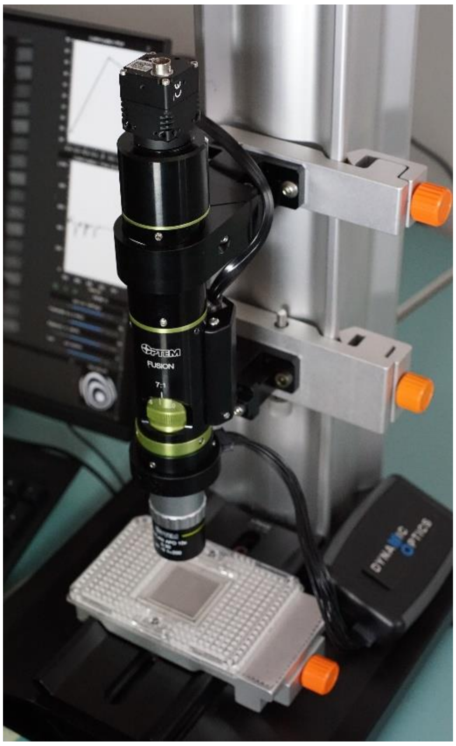


Fig. 4: Image of a brightfield microscope objective with deformable lens.

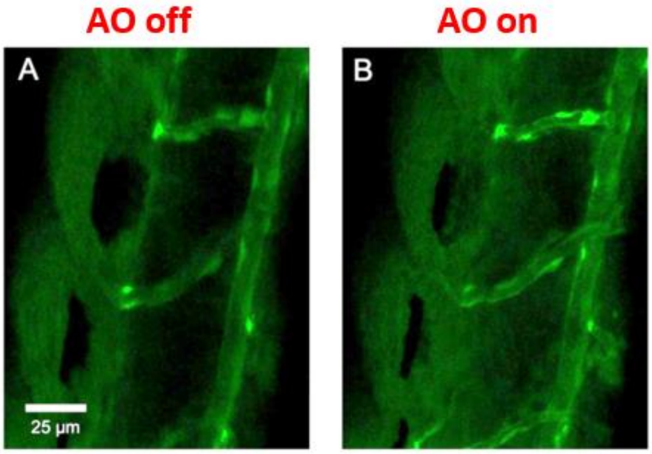
To find the optimal lens shape it is possible to use an automatic optimization algorithm. The sharpness of the acquired images can be measured with many functions such as for example the image variance. The algorithm tests the system with some bias aberrations while measuring the sharpness function for each of them. For example, the lens applies three different amount of defocus and then is set to the one with the best sharpness value. The same procedure is iteratively repeated for the other aberrations until the merit function does not increase.

APPLICATIONS

Deep Tissue Penetration

What is the problem, how does the tunable lens solve this and example.

Problem:
-Inhomogeneites and scattering from biological material cause localized aberrations of the optical wavefront, resulting in degraded image quality. Furthermore each sample is spatially unique and could potentially also be time varying. Adaptive optics have



- Fig. 6: confocal microscope Leica SP5. Confocal microscope P. Pozzi, et al, "Plug-and-play adaptive optics for commercial laser scanning fluorescence microscopes based on an adaptive lens,"Opt. Lett. 45, 3585-3588 (2020)

Biological Imaging with Varying Coverslip Thickness
Problem:
Imaging of biological samples at higher and higher NA, varying thicknesses of coverslip material which degrades image quality.

Multilayer Flat Panel Display Defect Inspection
Problem:
FPD construction process involves many deposited layers that required defect inspection during production. Seeing into buried layers to inspect for defects degrades the image quality.
Solution:
Adaptive optics allows for spherical aberration correction when viewing through transparent multilayer materials, enables accurate defect inspection not only at the first surface.

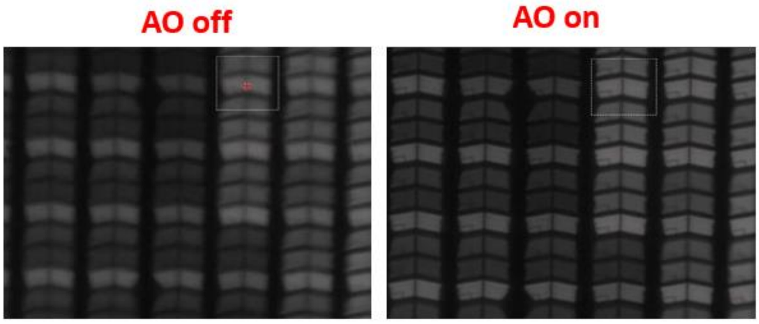


Fig. 5: image of the pixels of an iphone without (left) and with (right) adaptive optics correction with 10mm clear aperture Dynamic Optics deformable lens.

Cryochamber Imaging
Problem:

For some material research and electronic testing, imaging must be done in a cryo-chamber. For practical reasons the imaging system needs to reside on the outside of the chamger. Imaging through the porthole causes a reduction in image quality due to the thickness of the window, stress-induced refractive index changes and turbulence from the temperature delta between the cryochamber and the air.

Solution:

Adaptive Optics is able to correct for both of these aberrations and improve image quality.

Microfluidics / Lab-on-Chip
Problem:

Scattering and inhomogeneities caused by transparent medium flows within a channel.

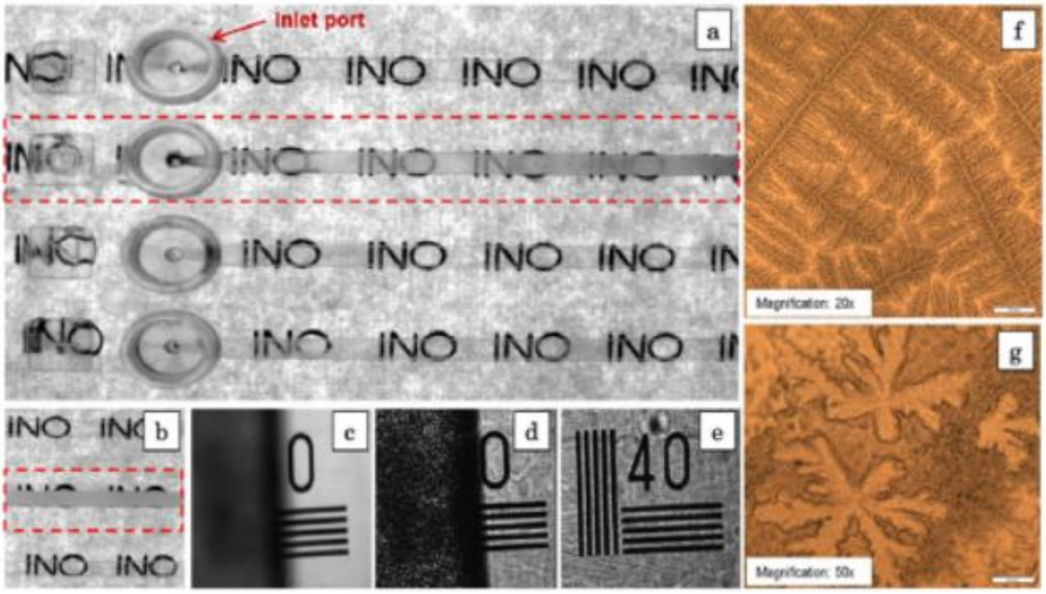
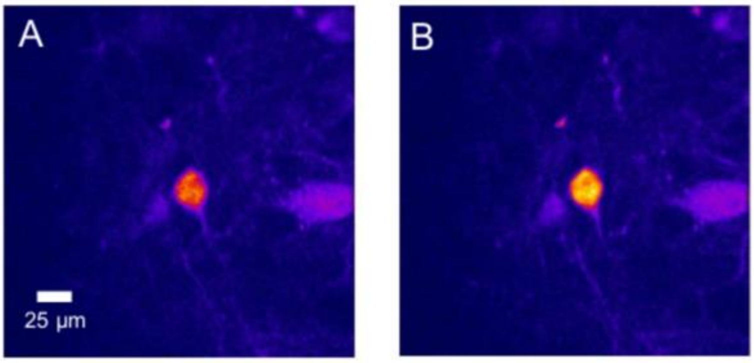
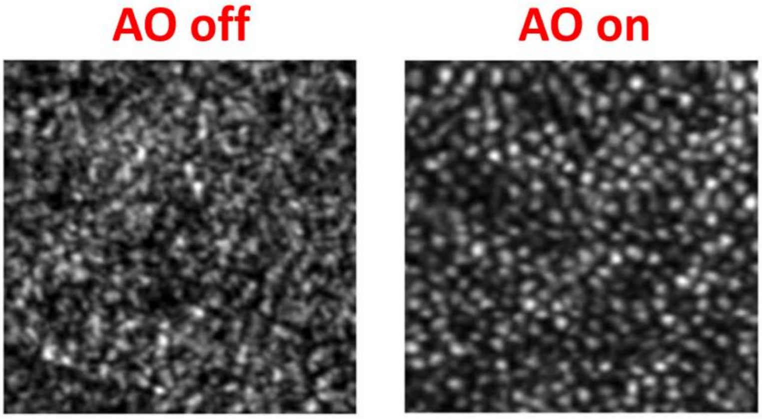


Fig. 1 Imaging through scattering microfluidics. (a) White-light image of a microfluidic chip with four channels. A salt deposit is settled only in the second channel (red dashed box), with opacity increasing from left to right. (b) White-light view of a different portion of the chip, with the maximum layer thickness. (c-d) Only the left part of a test target is placed behind a scattering channel imaged respectively by (c) white-light microscopy and (d) coherent laser microscopy at λ = 632,8μm. (e) Coherent laser microscopy of the target in absence of the scattering layer. (f-g) White-light images of the salt deposit inside the chip obtained with (f) 20x and (g) 50x magnification.

Interesting results have been obtained in many types of microscopes such as for example: fluorescence bright field, light sheet, optical coherence tomography, 2 photon and confocal. As an example, we show in this paper the correction of aberrations present observing the pixels of a mobile phone screen. The image has been acquired mounting the deformable lens on a microscope equipped with 0.28NA, 10x objective. The optimization was carried out in the selected square area highlighted with the dotted line. An image of the microscope is show in Fig. 5. The result shows that the image is much sharper than in the beginning. This system has already been implemented and tested on many types of complex microscopes such as Confocal (Fig. 6), Multiphoton (Fig. 7) and Optical Coherence Tomography (Fig. 8), see the references for further details.



- Fig. 7: Two photon Microscope. In vivo imaging of mouce neurons. P. Pozzi, et al, "Plug-and-play adaptive optics for commercial laser scanning fluorescence microscopes based on an adaptive lens,"Opt. Lett. 45, 3585-3588 (2020)



- Fig. 8: Optical Coherence Tomography. Yifan Jian, et al, Lens-based wavefront sensorless adaptive optics swept source OCT, Scientific Reports 6, Article number: 27620

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