Optical Imaging in Biology and Medicine
Master in Photonics & Europhotonics Master Program

Adaptive Optics for Microscopy

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November 6, 2013

Abstract

Contents

1 Introduction

The performance of these microscopes is often compromised by aberrations that lead to a reduction image resolution and contrast.

These aberrations may arise from imperfections in the optical system or may be introduced by the physical properties of the specimen.

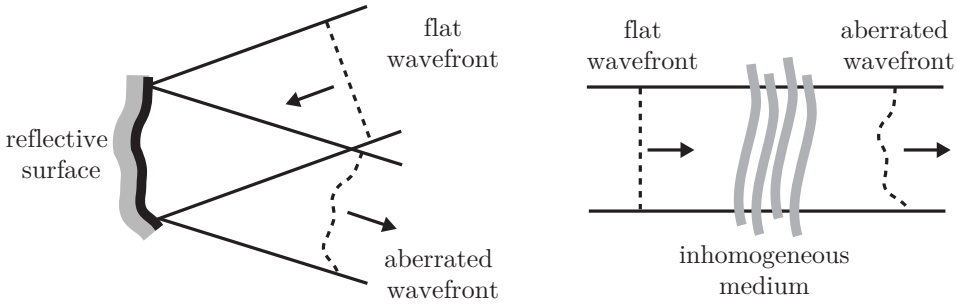
The problems caused by aberrations can be overcome using adaptive optics, whereby aberrations are corrected using a dynamic element, such as a deformable mirror.

This technology was originally conceived for the compensation of the aberrating effects of the atmosphere and was first developed for military and astronomical telescopes.

Adaptive optics systems have also been introduced for other applications such as laser beam shaping, optical communications, data storage, ophthalmology and microscopy.
Hellooooooooo

[5]

For the purpose of understanding the operation of an adaptive optical system, it is best to think of aberrations in terms of distortions of an optical wavefront.



(a) Reflection. (b) Transmission.

Figure 1: Wavefront aberrations due to (a) reflection from a non planar surface and (b) caused by propagation through a non-uniform refractive index distribution. Image after [5].

Representing aberrations in this way can simplify the design, control and characterisation of adaptive optics. The choice of modes for a particular application is often influenced by some aspect of the system, such as the deformation modes of a deformable mirror or the statistics of the induced aberrations. Otherwise, the modal representation may be chosen through mathematical convenience. For example, Zernike polynomials are often used for systems with circular apertures as they form a complete, orthogonal set of functions defined over a unit circle

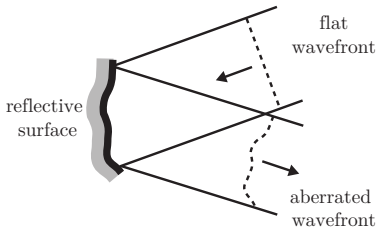


Figure 2: This is my first image.

As with all optical systems, microscopes can also suffer from aberrations due to imperfections in the optical components. In practice, no system can be totally free from aberrations and so systems are designed to maintain aberrations below a particular tolerance for a given set of imaging conditions, such as wavelength, magnification and field of view. Significant aberrations can be introduced if a microscope is used outside its design specifications, for example at the incorrect wavelength or at a difierent temperature (see Chapter 11 of Ref. 9).

2 Adaptive Optics Methods applied in Microscopy

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2.1 Point Scanning Microscopes

Scanning optical microscopes are widely used for high resolution imaging, mainly because certain implementations provide three-dimensional resolution with optical sectioning and are thus particularly useful for imaging the volume structures of biological specimensz. In these microscopes, illumination is provided by a laser that is focused by an objective lens into the specimen. The light emitted from the specimen is collected, usually through the same objective lens, and its intensity is measured by a single photodetector. The focal spot is scanned through the specimen in a raster pattern and the image is acquired in a point-by-point fashion. The resulting data are stored and rendered as images in a computer.

Several other point scanning microscope modalities have been introduced, including two-photon excitation uorescence (TPEF) microscopy, second harmonic generation (SHG) and third harmonic generation (THG) microscopy, and coherent anti-Stokes Raman (CARS) microscopy.

2.1.1 Confocal Microscopes

The most common example of this type is the confocal microscope, which can be operated in reflection or fluorescence mode. Three-dimensional resolution is achieved by the placement of a pinhole in front of the photodetector. In a reflection mode confocal microscope, the illumination is scattered by objects not only in the focal region, but throughout the focusing cone. In fluorescence mode, emission is generated in the focus but also in out-of- focus regions. The pinhole ensures that mainly light from the focal region falls upon the detector and light from out-of-focus planes is obscured. It is critical in the confocal microscope that both the illumination and detection paths are diffraction limited. This ensures that i) the illuminating focal spot is as small as possible, and ii) that the focus is perfectly imaged on to the detector pinhole. Therefore, in an adaptive confocal microscope, aberration correction must be included in both paths. This dual pass adaptive system can usually be implemented using a single deformable mirror, if the path length aberrations are the same for both the illumination and the emission light. This is the case if there is no significant dispersion in the specimen or chromatic aberration in the optics.

A pinhole is not required to obtain three-dimensional resolution, so most TPEF microscopes use large area detectors to maximise signal collection. Although they rely upon other physical processes, non-linear imaging modalities such as SHG, THG and CARS exhibit similar resolution properties. When using large area detectors, the fidelity of imaging in the detection path is unimportant so the effects of any aberrations in this path are negated. It follows that single pass adaptive optics is appropriate for these microscopes as aberration correction need only be implemented in the illumination path.

Adaptive optics systems have been successfully combined with several point- scanning microscope systems including confocal,13 TPEF,6, 14, 15 harmonic generation,16, 17 CARS.18 Example images of aberration correction in an adaptive THG microscope are shown in Fig. 10.

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[2]

2.1.2 two-photon excitation

[9] [3] [6]

2.1.3 Harmonic Generation

[4] [7]

2.1.4 CARS

[10]

3 Results and Discussion

4 Conclusion

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