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

Adaptive Optics for Microscopy

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Abstract

Contents

1 Introduction

actual techniques in the second part, methods

[2]

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.

[7]

Optical microscopes have long been essential tools in many scientific disciplines, particularly the biological and medical sciences. Conventional widefield microscopes—encompassing transmission, phase contrast and fluorescence imaging modes—are the workhorses of many laboratories. Over the last 25 years, researchers have also made significant developments in 3-D imaging using scanning laser microscopes. This progress started with the confocal microscope, which provides 3-D resolution by using a pinhole to exclude out-of-focus light. Rather than produce a whole image simultaneously, these microscopes scan a laser spot through the specimen, building the image point-by-point. This achievement was followed by several other laser-scanning methods, including the commonly used twophoton fluorescence microscope. Rather than using a pinhole to generate 3-D discrimination, this microscope relies on the nonlinear process of two-photon excitation to ensure that fluorescence is only generated in the focus, where the laser intensity is highest. Various advances in this field have led to improvements in resolution and contrast. Standard laboratory microscopes now regularly produce images revealing 3-D structure on the submicrometer scale. Several new methods of nanoscopy that combine optical and photophysical phenomena can even beat the diffraction limit to resolve details on the tens-of-nanometers scale.

These methods all rely on careful engineering to ensure that the optics operate at the diffraction limit, so that optimum resolution and efficiency are achieved. However, one part of the optical system—the specimen—lies outside the design specification. It is optically inhomogeneous and exhibits spatially varying refractive indices. Hence, the light focused into the specimen suffers from wavefront distortions— or phase aberrations—that degrade the resolution and imaging efficiency of the microscope. The aberrations vary from one specimen to another, so they cannot be corrected by a fixed optical design. Dynamic correction is necessary. This is where adaptive optics (AO) comes into play.

Adaptive optics was originally conceived for use in astronomical telescopes. These AO systems detect aberrations introduced by the atmosphere and use a deformable mirror to remove the aberrations before the light reaches the imaging detector. For imaging systems with small apertures, such as our eyes, the turbulence causes twinkling; for wider telescope apertures, it leads to severe image blurring that limits the resolution of the telescope.

The AO approach has been widely applied in astronomy, and it has also found application in ophthalmic imaging, laser-based fabrication, optical communications and, of course, microscopy. The adoption of AO for microscopes has brought new challenges that have required innovative solutions.

[2]

2 Aberration Measurement and Correction

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.

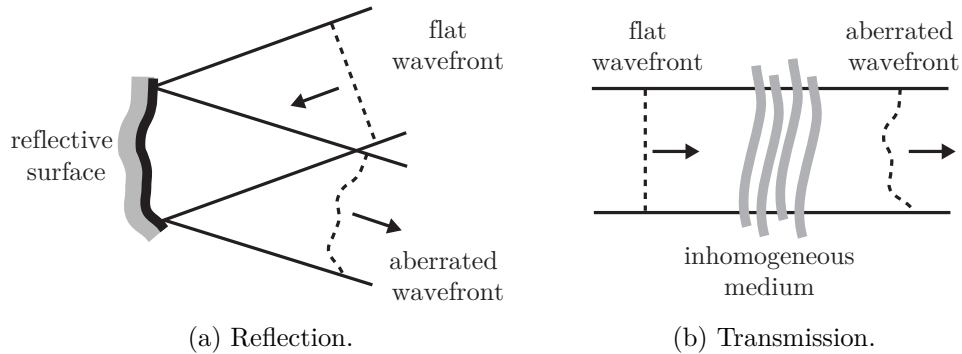(a) Reflection.   (b) Transmission.

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 [7].

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

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 different temperature (see Chapter 11 of Ref. 9).

2.1 Wavefront Sensing

2.1.1 Direct Wavefront Sensing

2.1.2 Indirect Wavefront Sensing

2.2 Aberration Correction

2.3 Control Strategies

3 Adaptive Optics Methods applied in Microscopy

AO has been demonstrated in a range of microscope modalities, including conventional widefield microscopes as well as laser scanning systems. The most common implementations have involved confocal and two-photon fluorescence microscopy, both of which are widely used methods in biomedical investigations. Due to aberrations, these microscopes suffer from a significant drop in signal and resolution as the focus is moved deeper into the specimen.

Various research groups have combined these microscopes with direct wavefront sensing and sensorless AO, normally using deformable mirrors for aberration compensation. Ji et al. developed another approach that uses an SLM to implement a pupil segmentation phasing method in a two-photon microscope. AO has also been applied to microscopes using more exotic contrast mechanisms based upon nonlinear optical processes, such as second- and third-harmonic generation or coherent anti-Stokes Raman scattering. Using these various methods, researchers

have demonstrated image improvement at depths of up to 100 ìm in mouse embryos and over 200 ìm in brain tissue.

Adaptive microscopy is also finding a role in the imaging of live specimens. It can help to reduce the time required for image acquisition by increasing signal generation and collection efficiency.

3.1 Widefield Microscopy

In conventional microscopes, widefield illumination is provided using either transmission optics or, in the case of re ection or uorescence modes, via the objective lens in an epi configuration. In either case, the image quality depends only on the optics of the detection path and is independent of the fidelity of the illumination path. Aberration correction is therefore only necessary in the detection path and a single pass adaptive optics system will suffice.

[1] [4]

get: `http://www.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&retmode=ref&cmd=prlinks&id=20096044`

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3.2 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.

3.2.1 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.

[10]

[3]

3.2.2 two-photon excitation

[11] [5] [8]

3.2.3 Harmonic Generation

[6] [9]

3.2.4 CARS

[12]

4 Conclusion

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