Adaptive compensation of aberrations in ultrafast 3D microscopy using a deformable mirror

Adaptive compensation of aberrations in ultrafast 3D microscopy using a deformable mirror

L. Sherman⁴, O. Albert⁴, C. Schmidt⁴, G. Vdovin⁵, G. Mourou⁴, and T.B. Norris⁴

⁴Center for Ultrafast Optical Science, University of Michigan, 2200 Bonisteel Blvd, Ann Arbor, Michigan, 48109-2099, USA

⁵Department of Electronic Instrumentation, Technical University of Delft, P.O. Box 5031, 2600 GA Delft, The Netherlands

ABSTRACT

3D imaging using a multiphoton scanning confocal microscope is ultimately limited by aberrations of the system. We describe a system to adaptively compensate the aberrations with a deformable mirror. We have increased the transverse scanning range of the microscope by three with compensation of off-axis aberrations. We have also significantly increased the longitudinal scanning depth with compensation of spherical aberrations from the penetration into the sample. Our correction is based on a genetic algorithm that uses second harmonic or two-photon fluorescence signal excited by femtosecond pulses from the sample as the enhancement parameter. This allows us to globally optimize the wavefront without a wavefront measurement. To improve the speed of the optimization we use Zernike polynomials as the basis for correction. Corrections can be stored in a database for look-up with future samples.

Keywords: Scanning confocal microscopy, aberration correction

1. INTRODUCTION

Confocal microscopy with ultrashort pulses has been used extensively to image biological samples at high resolution⁴. Two- and three-dimensional imaging using the confocal method can be done either by scanning the sample or by scanning the beam. Beam scanning is often preferable since it leaves the sample undisturbed and allows for faster acquisition of the image. The range of diffraction-limited resolution in such systems is limited by the aberrations that arise from using the microscope optics off-axis, to achieve the surface scan, and on-axis aberrations such as spherical aberration when the beam is focused deep into a sample (since the objective is generally well corrected only for a specific focal plane). To increase the volume for near diffraction-limited resolution one should consider optical systems that can incorporate wavefront correction to reduce the aberrations. Two experiments are described here that use a computer-controlled deformable mirror in conjunction with a learning algorithm to adaptively compensate for the static aberrations and thereby increase the scan area.

1.1 The deformable mirror

The main element in our wavefront correction system is a deformable mirror (DM). The DM is a micromachined silicon membrane coated with silver, the deformation is controlled via the voltages on 37 electrostatic actuators⁵. These actuators form the surface of the DM into a smooth polynomial surface, much like a fun-house mirror, with the deflection of the surface relative to the voltage applied to the actuator. The 15 mm diameter mirror has a maximum displacement at the center of 3 microns. A telescope is used to image the DM onto the surface of the microscope objective that is generating the aberrations. Thus the wavefront change introduced by the deformation of the DM will correspond to a wave-front change on the objective without introducing amplitude modulation. The wavefront change due to the DM is essentially the conjugate of the wavefront aberration introduced by the objective; so a diffraction-limited spot can be obtained on the sample.
1.2 Machine learning

In most traditional adaptive optical systems, the optimal shape of the DM which will compensate wavefront aberrations is determined by first using a wavefront sensor to measure the aberrated wavefront. A wavefront error correction would then be calculated and fed to the DM to produce a flat (or spherical) wavefront. Because it is extremely difficult to measure the wavefront at the focus of a high-NA objective, we simplified the process by using nonlinear optics in conjunction with machine learning through a genetic algorithm. In multiphoton microscopy, the excitation of the sample by ultrashort pulses is nonlinear (typically quadratic or cubic); thus, if the total nonlinear signal generated at the focus is measured, the signal will be maximum if the spot is diffraction-limited. By using a genetic algorithm, the DM "learns" what the optimal correction is for any given position of the beam on the sample simply by maximizing the nonlinear signal. This learning process can be made even easier using Zernike polynomials, which orthogonally describe aberrations over a circular aperture. Therefore we can obtain diffraction limited focusing using adaptive optics without needing to know anything about the wavefront of the laser. Because the aberrations in the system are static, we find the optimal mirror shape for each beam position (pixel) on the sample, and store this shape (in the form of the voltages applied to each actuator) in a database. When imaging an unknown sample, one simply recalls the correction for each pixel from the database as the beam is scanned. The response time of the DM is approximately 1 μs, so video-rate scanning will not be hindered by the use of the database. Of course, if the objective is changed, a new database will be required.

![Diagram](https://www.spiedigitallibrary.org/conference-proceedings-of-spie)

Fig. 1. The experimental setup.

2. OFF-AXIS SCANNING

The first experiment determined the ease of correction in a surface scan mode. The experimental setup is shown in Figure 1. A Ti:sapphire laser providing 10-fs pulses at 800 nm with an 80-MHz repetition rate is used for the multiphoton excitation, as the use of the shortest possible pulses maximizes the nonlinear excitation for a fixed average power. We use a reflective optic, specifically a f:1 off-axis (60 deg) parabola, to focus the excitation beam to a 1 μm diameter spot. Use of reflective optics eliminates the need for dispersion compensation of the excitation pulses, and completely eliminates chromatic aberration. The parabola, however, is extremely sensitive to alignment and suffers from large astigmatism and coma when the incident beam angle is scanned, such a system therefore requires wavefront correction in order to be useful. A traditional confocal microscope using a well-corrected plan-apochromatic objective will have much less aberrations to correct, but the exceptional demonstration with the f:1 parabola shows the power of this correction scheme most dramatically. The beam is scanned across the sample by scanning the angle of incidence on the parabola. This is accomplished in our setup by moving lens f 3 in the transverse (x,y) plane. The magnitude of the displacement of the beam at focus dx' is given by the magnification of the telescope times the displacement of the lens dx, as shown in Figure 2.
2.1 Results of off-axis scanning

The corrected and uncorrected intensity distributions are shown in Figure 3 for several representative beam positions in the focal plane of the parabola. At the center (on axis), no correction from the DM is required, and the measured spot size is 1.0 \( \mu \text{m} \); this measurement is the convolution of the actual size of the spot with the 0.5 \( \mu \text{m} \) resolution of the microscope objective used for the measurement. The minimum spot size achievable with a top hat mode and f:1 parabola is 1.1 \( \mu \text{m} \), so the on-axis beam is diffraction limited within our experimental resolution. If the beam is scanned off-axis, strong aberrations become clearly visible (examples where the beam is scanned down or to the right are shown in Figure 3). After the optimal DM solution has been found, the corrected beam appears to recover the diffraction limit. The corrected spot is in the form of an Airy pattern, which appears since the laser beam slightly over fills the DM.

2.2 Comparison of Strehl ratio

A simple, single-parameter characterization of the focal spot is the Strehl ratio, i.e. the ratio of the measured peak power of the pulse at the focal spot to the theoretical peak power of the same pulse focused to the diffraction limit. For an on-axis spot, or a perfectly corrected off-axis spot, the Strehl ratio is almost 1. A variation of the Strehl ratio from 1 to 0.5 corresponds in our setup to an increase in the spot diameter from 1.1 to 1.6 \( \mu \text{m} \). Figure 4 shows a plot of the Strehl ratio during horizontal scanning for the corrected and uncorrected beam; if we take 0.5 to be a reasonable limit for the acceptable Strehl ratio, this Figure shows that an adaptive correction allows us to increase the scan range from 60 \( \mu \text{m} \) to 170 \( \mu \text{m} \), a factor of almost three. We also have measured the same factor of three improvement in scan range for vertical scanning, so that the total increase in scan area is a factor of nine. The (optimized) Strehl ratio for each pixel can also be stored in the database, which will allow the signal intensity from the sample to be normalized while reconstructing the image, thus minimizing important vignetting at the edges of the image.
Fig. 4. Strehl ratio measured for the horizontal and vertical scanning. The adaptive wavefront correction allows to extend the scanning area. The asymmetry of the plot is due to the use of an off-axis 60° deg parabola.

3. ON-Axis SCANNING

The second experiment looks at correcting for aberrations that occur when scanning into the depths of a sample. Microscope objectives are usually corrected for a specific amount of glass to accurately image a sample in a specific focal plane inside the sample. However, if you focus the beam deeper into the sample severe spherical aberration arises. This is due to a refractive index mismatch between the cover glass and the sample solution. The aberrations become worse the deeper into the sample, which severely limits the scanning range for confocal microscopy. We would like to correct for these aberrations at different depths in the sample, creating a database to extend the useful scanning range of the microscope.

3.1 On-axis scanning procedure

The system is very similar to the previous set-up shown however we do not use an off-axis parabola, but a conventional confocal microscope objective, with the sample attached to a mechanical scanner to vary the targeted depth into the sample. The sample consisted of a 70 μm cell of fluorescent dye. The ultrashort Ti:saphire laser was focussed into sample, starting from the inside surface of the coverslip, easily located by a scratch on the glass, to the end of the cell. The two-photon fluorescence signal was measured from the beginning of the cell, until the signal decreased due to aberrations. This formed a map of the normal two-photon fluorescence over the entire depth of the sample (corresponding to the solid-circle curve in fig. 5), without any aberration correction from the DM. The sample was then shifted 10 μm deep into the sample as measured from the scratch on the cover slip. The DM was then used to correct for aberrations by maximizing the two-photon fluorescence at that location. Keeping the corrected shape on the DM the sample was scanned through the length of the cell and the two-photon signal measured and mapped again. This procedure was repeated for 10 μm increments until the DM could no longer make significant correction to the output signal.

3.2 Results of on-axis correction

As shown in Figure 5, the uncorrected normalized two-photon fluorescence intensity is maximum at the top of the cell, and decreases sharply as the sample is penetrated as the aberrations due to the sample increases. For each of the maps taken with a correction applied to the DM the peak normalized two-photon fluorescence is at the location where the correction was made. In other words, if the DM corrected for aberrations 20 μm into the sample (the third map), the peak normalized fluorescence intensity occurs at 20 μm. The corrections for each focal plane can be made using a uniform dye solution, and stored in a database. To achieve the best resolution when imaging a particular plane in the sample, the appropriate correction would be recalled and applied to the DM.
Fluorescence in Flow Chamber

Figure 5. Normalized two-photon fluorescence signal over the length of the cell. Displacement is measured from the inside surface of the cover glass by the scanning knob.

4. SUMMARY

In summary, we have demonstrated how a deformable mirror can be used to correct the off-axis aberrations and initial investigation have shown promising correction for depth in beam-scanning confocal microscopy. The optimal correction can be found using a genetic algorithm, eliminating the need for a separate wavefront measurement. A nine times improvement in scan area is found when the objective is an f:1 off-axis parabola. Future work will include more investigation into correction of aberrations as the beam is focussed deep into the sample to deduce useable range of correction, and orders of wavefront correction possible.

REFERENCES