Smart microscope: an adaptive optics learning system for aberration correction in multiphoton confocal microscopy

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Off-axis aberrations in a beam-scanning multiphoton confocal microscope are corrected with a deformable mirror. The optimal mirror shape for each pixel is determined by a genetic learning algorithm, in which the second-harmonic or two-photon fluorescence signal from a reference sample is maximized. The speed of the convergence is improved by use of a Zernike polynomial basis for the deformable mirror shape. This adaptive optical correction scheme is implemented in an all-reflective system by use of extremely short (10-fs) optical pulses, and it is shown that the scanning area of an f:1 off-axis parabola can be increased by nine times with this technique. © 2000 Optical Society of America

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Confocal microscopes based on two- or three-photon fluorescence are now widely used for three-dimensional imaging of biological samples. Optical sectioning is obtained by use of a high-numerical-aperture objective to focus ultrashort laser pulses into the sample, as multiphoton excitation of fluorescent dyes occurs only in the high-intensity region ($\approx 1\ \mu m^3$ in volume) at focus.[1] Three-dimensional imaging with second- and third-harmonic generation as the nonlinear process has also been demonstrated.[2] The ability to achieve diffraction-limited resolution in such systems is ultimately determined by the ability to overcome aberrations, including chromatic aberrations, off-axis aberrations (if the excitation beam is not on the axis of the objective), and on-axis aberrations (especially spherical aberrations) when the beam is focused deep into a sample. Often the sample is scanned instead of the beam, to eliminate the off-axis aberrations, but beam-scanning systems are often preferable because they permit faster image acquisition rates and do not disturb the sample. Thus the scanned sample volume in which near-diffraction-limited resolution can be obtained is limited by aberrations, and it is desirable to consider optical systems that can incorporate wave-front correction to reduce them. The system that we describe here uses a computer-controlled deformable mirror in conjunction with a learning algorithm to compensate for the static off-axis aberrations and thereby to increase the scan area.

The experimental setup is shown in Fig. 1. A Ti:sapphire laser providing 10-fs pulses at 800 nm with an 80-MHz repetition rate[3] 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, an f:1 off-axis (60°) 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 wave-front correction if it is to be useful. A traditional confocal microscope that uses a well-corrected (typically plan-apochromatic) focusing objective will have greatly reduced aberrations compared with the parabola, but the principle of adaptive correction is most dramatically demonstrated with the f:1 parabola.

Aberration correction is provided by a deformable mirror (DM), which is a micromachined silicon nitride membrane coated with silver; the deformation is controlled by means of the voltages on 37 electrostatic actuators.[4] The maximum displacement at the center of the mirror is 3 μm. The Ti:sapphire beam is expanded with a telescope (f_1, f_2) to match the 15-mm diameter of the DM. A second telescope (f_3, f_4) is then used to match the beam size to the f:1 parabola and to image the DM onto the parabola. Thus the wave-front change introduced by the deformation of the DM will correspond to a wave-front change on the parabola without introducing amplitude modulation. The wave-front change that is due to the DM is essentially the conjugate of the wave-front aberration introduced by the parabola; thus a diffraction-limited spot can be obtained on the sample at the focus of the parabola. One scans the beam across the sample by scanning the angle of incidence on the parabola, simply by moving lens f_3 in the transverse (x, y) plane.

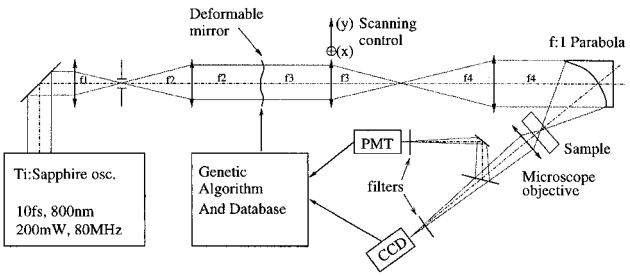

Fig. 1. Experimental setup: PMT, photomultiplier-tube; osc., oscillator.

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In most traditional adaptive optical systems, one determines the optimal shape of the DM that will compensate for wave-front aberrations by first using a wave-front sensor to measure the aberrated wave front, thus yielding a wave-front error correction, which can then be fed to the DM to produce a flat (or spherical) wave front.[5] Because it is extremely difficult to measure the wave front at the focus of a high-numerical-aperture objective, we desire a scheme that will determine the optimal correction by the DM without the necessity to perform a wave-front measurement. The scheme that we implement here uses nonlinear optics in conjunction with machine learning through an evolutionary algorithm. In multiphoton microscopy, the excitation of the sample by the 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. In an evolutionary algorithm (explained below), the DM learns what the optimal correction is for any given position of the beam on the sample simply by maximizing the nonlinear signal. Thus we can obtain diffraction-limited focusing by using adaptive optics without needing to know anything about the wave front of the laser on the parabola. 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.

We now provide some further details on the implementation and demonstration of the adaptive optics learning scheme. In most biological applications of multiphoton confocal microscopy, two- (or three-) photon excitation of fluorophores in the sample is used; to emulate this nonlinear process but to avoid the complications that might be introduced by permanent photobleaching of dyes, we implement the nonlinear correction scheme by using second-harmonic (2ω) generation in a thin β-barium borate crystal as the sample. The setup shown in Fig. 1 allows us simultaneously to measure 2ω power (using a photomultiplier tube) and to image the intensity distribution of the fundamental (using a CCD camera).

The 2ω power is used as the feedback signal for the computer-controlled DM by means of the evolutionary algorithm. Evolutionary algorithms are used as a form of machine learning to permit the optimization of nonlinear systems with a large number of variables.[6,7] The specific form of evolutionary algorithm utilized in our experiments is a genetic algorithm (GA), so named because of its analogy to the mechanism of evolution in nature. The basic idea is to create a population of individuals represented by chromosomes. In our case, each individual corresponds to a trial shape of the DM; each individual has 37 chromosomes, which correspond to the voltages on the 37 actuators of the DM. In the GA, the individuals are then tested for their fitness in the environment; in our case, the fitness parameter is the 2ω power generated at the focus of the parabola. Clearly, mirror shapes that yield smaller spots generate more 2ω and thus must be closer to the optimal shape.

The GA proceeds by testing the entire population of individuals on the system and selecting only a percentage of the most fit to become parents for the next generation. In our case, we start with 100 random mirror shapes and select only the 10% that generate the largest 2ω; we then form 100 new individuals (children) by randomly crossing the chromosomes of the 10 parents. Some random chromosome changes (mutations) are introduced in each generation to avoid convergence to a local rather than a global maximum. This new generation is then tested on the system, the ten individuals with the largest fitness parameter are selected, and so on. The evolution process stops when the population includes the optimum solution to the problem (i.e., the maximum attainable 2ω). The level of genetic mutation observed in the parents of each generation provides good information on the stability of the population and can be used to stop the evolution process. By going through this procedure, the GA gradually finds the globally optimum mirror shape that will yield the tightest focus for each beam position on the sample.

Using the 37 actuator voltages as the chromosomes for the trial solutions, we find that it takes a maximum of 15 generations for the algorithm to converge to a solution; this requires 3 min, limited by the 1-μs response time of the DM. The average fitness parameter (2ω power) for each generation is plotted in Fig. 2, which shows the gradual convergence to an optimal solution.

To speed up the convergence, one can reduce the size of the chromosome basis. The optimum basis for characterizing aberrations over a circular aperture is the Zernike polynomial basis.[8] Thus we can choose the chromosomes for each individual in the GA to correspond to the amplitudes of the Zernike polynomials instead of to the actuator amplitudes. The voltage applied to each actuator is determined by the sum of the Zernike polynomial amplitudes at the position of the actuator. According to the maximum spatial

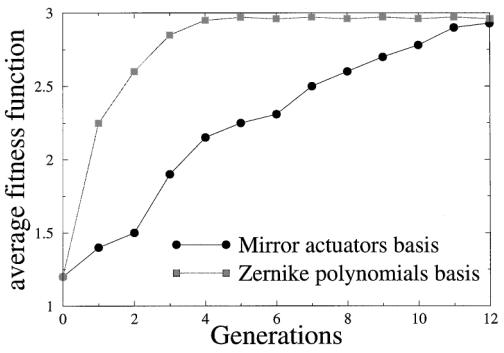

Fig. 2. Genetic algorithm improvement of the average fitness function with generations for two different populations. The first population is composed of 100 individuals with deformable mirror actuator chromosomes. The second is composed of the same number of individuals with Zernike polynomial amplitude chromosomes.

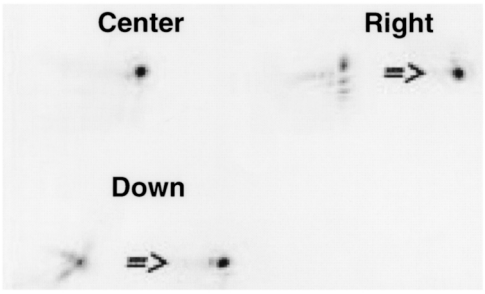

Fig. 3. Inverted images of the focus spot on axis, for a right scanning of 70 μm and a down scanning of 50 μm. The off-axis spots are displayed uncorrected and corrected. The uncorrected spots have been brightened to improved visibility in the figure. All the corrected spots and the centered one correspond to Airy patterns.

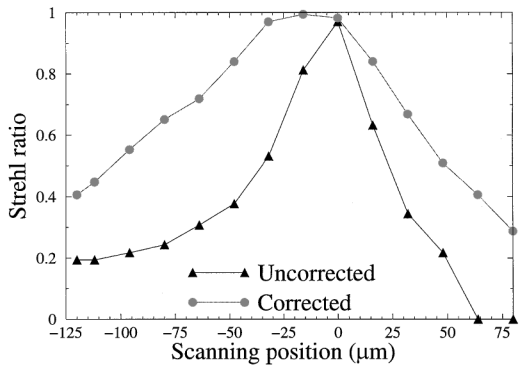

Fig. 4. Strehl ratio measured for the horizontal scanning. The adaptive wave-front correction allows the scanning area to be extended. The asymmetry of the plot is due to the use of an off-axis 60° parabola.

frequency that can be produced by the DM, we have limited the basis to the first four orders of the Zernike polynomials (except the zero order that corresponds to the piston); consequently the chromosomes correspond to a true 13-dimensional orthogonal basis instead of a 37-dimensional nonorthogonal basis. Using the Zernike basis permits the convergence to be achieved in five generations in less than 1 min. It should be noted that, because of the nonlinear response of the mirror to the actuator voltages, the amplitude of the DM deformation does not correspond exactly to the Zernike polynomial amplitudes (especially for the higher-order modes).[9] Nevertheless, because the correspondence is good for the lowest-order modes, which are the largest, a significant improvement in convergence is obtained, as shown in Fig. 2.

The corrected and uncorrected intensity distributions are shown in Fig. 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 $\approx$1.0 μm; this measurement is the convolution of the actual size of the spot with the 0.5-μm resolution of the microscope objective used for the measurement. The minimum spot size achievable with a top-hat mode and an f:1 parabola is 1.1 μm, 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 in which the beam is scanned down or to the right are shown in Fig. 3). After the GA has determined the optimal DM solution, the corrected beam appears to recover the diffraction limit.

A measure of the correction range for the DM can be taken by use of 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 μm. Figure 4 shows a plot of the Strehl ratio during horizontal scanning for the corrected and uncorrected beams; 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 to 170 μm, almost a factor of 3. We also have measured the same factor-of-3 improvement in scan range for vertical scanning, so the total increase in scan area is a factor of 9. The (optimized) Strehl ratio for each pixel can be stored to normalize the signal when the image is reconstructed, thus minimizing vignetting effects at the edges of the image.

In summary, we have demonstrated how a DM can be used to correct the off-axis aberrations in beam-scanning confocal microscopy. The optimal correction can be found by use of a genetic algorithm, eliminating the need for a separate wave-front measurement. A nine-times improvement in scan area is found when the objective is an f:1 off-axis parabola. Future research will include the correction of aberrations as the beam is focused deep into the sample. Typically spherical aberration limits the depth to which near-diffraction-limited imaging can be obtained in confocal microscopy; we expect that a significant improvement in the scan volume will result from this approach.

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