Single-pass measurements of the wave-front aberrations of the human eye by use of retinal lipofuscin autofluorescence

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We describe a technique for making single-pass measurements of the wave-front aberration of the eye. The technique utilizes the natural fluorescence of the retina that is produced by lipofuscin to form an incoherent pointlike source for conventional Shack–Hartmann sensing. © 1999 Optical Society of America

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In measuring the wave-front aberrations of the eye objectively it is necessary to illuminate the eye and to use reflected and scattered light. The well-known problem[1,2] of information loss in the double-pass process in optics has been discussed by Artal *et al.*[3,4] The basic problem is that the odd aberrations can be incorrectly estimated, a problem that is minimized in the double-passage case by breaking or reducing of the symmetry between the input and the output, for example, by use of different numerical apertures for entering and exiting light.

The presence of speckle is also a problem: Because of retinal scattering, any instantaneous double-passage data form a speckle pattern. To overcome this problem, one time integrates the outgoing signal by means of long exposure times, thus averaging over eye trembling and effects related to the heartbeat. Recently Hofer *et al.*[5] suggested scanning over a small retinal patch to space integrate and thus reduce the speckle. These integrations are carried out over the double-pass wave front, so part of the information can still be lost.

The basic problem in many double-pass methods for determining wave-front aberration, such as those based on the Shack–Hartmann sensor, shearing interferometry, and the curvature sensor, is that reflection and scattering at the retina retain the coherence of the light. This means not only that speckle is present but, of equal importance, that the double-pass imaging is coherent unless some averaging process is employed; that is, the phase information accumulated in the first pass is retained in the second pass. If we could devise a means of decohering the light after the first pass, the phase information from the first pass would be lost and thus the measurement would be truly single pass and give an unambiguous measure of the wave-front aberration. This is the case in laser guide-star adaptive optics that uses the emission of light from the mesospheric atmospheric layer.[6]

To solve these problems we suggest using a naturally occurring fluorophore at the retina, lipofuscin, to generate a source of fluorescent light that is spatially and temporally incoherent with the first-pass light. Delori *et al.* have studied the autofluorescence of lipofuscin in detail.[7] Because we can now have a small incoherent source of light on the retina, conventional wave-front sensing (by any one of a variety of methods) will yield an unambiguous instantaneous measurement of the wave-front aberration over the wavelength bandpass of the detector system.

The fluorescence spectrum was measured for the left eye of subject LD, aged 26 years and with emmetropic eyes. The retina was illuminated by light of 543-nm wavelength, and the outgoing light was analyzed with a simple prism spectrometer. The pupil size was 3 mm (accommodation was not paralyzed, and the natural undilated pupil was used). Eighteen 15-s exposures were taken, with relaxation times longer than 3.5 min between successive exposures. The total measured power reaching the cornea was 6 μW, which, according to the British Standard BS EN 60825,[8] permits 500 s of continuous exposure without any risk of injury.

The intensity average of the 18 images is shown in Fig. 1(a). The isolated spot on the left-hand side of the image is produced by the green light reflected from the retina and not filtered out, whereas the line on the right-hand side corresponds to the dispersed fluorescent light. Figure 1(b) shows the relative intensity profile of the dispersed light, plotted against wavelength. The dashed curve in Fig. 1 is a 12th-order polynomial interpolation obtained with standard mathematical software (Matlab).

The results obtained are qualitatively similar to those reported by Delori *et al.*[7]; the principal difference is the low-wavelength cutoff of the diochroic and filter used in our experiment. A broadband fluorescent spectrum, from 580 to 740 nm, was detected, with a maximum intensity of 610–570 nm. The lower bound of 580 nm was forced by the edge filter used; the spectrum is probably broader, as reported by Delori *et al.*

Figure 2 shows the experimental setup that we used to measure the wave-front aberration of the eye. Laser 1 is a green He–Ne laser emitting at 543 nm. The beam is spatially filtered with a 15-μm pinhole and collimated by means of doublet L1. Beam splitter B1 is a dichroic filter that reflects light at 543 nm and transmits all longer frequencies in the visible and the near infrared. Entrance (P1) and exit (P3) pupils are conjugated with the eye pupil (P2) with the help of doublets L2 and L3. The lens of

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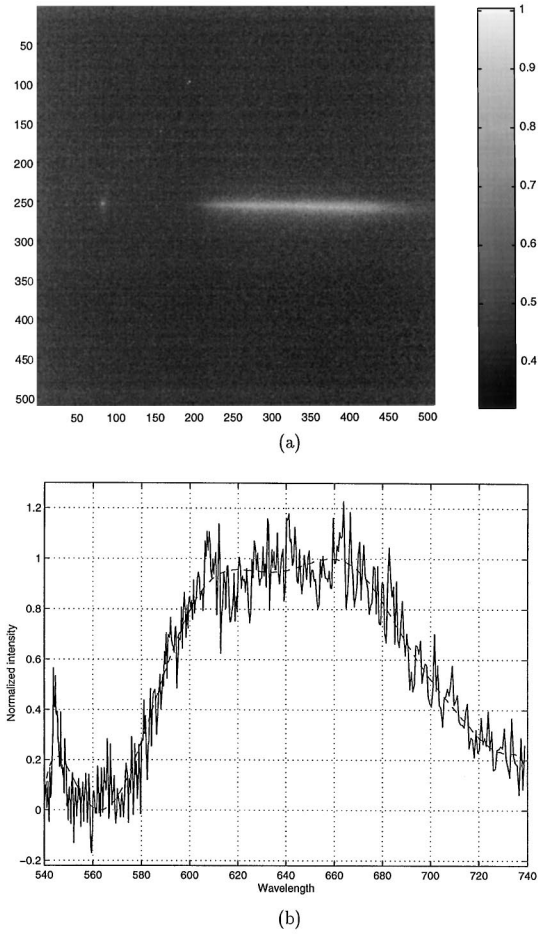

Fig. 1. (a) Retinal autofluorescence light spectrum as detected by the CCD array. The axes are labeled in pixels. (b) One-dimensional scanning of the retinal autofluorescence light spectrum.

the eye forms an image of the source at the retina, exciting lipofuscin. Reflected and fluorescent light propagates back through the system. Doublet L5 is used to form a Fourier image at the pupil plane (P4); spatial filtering at this plane filters out most of the reflections from the system and the cornea and also autofluorescence light from ocular tissues other than the retina. Doublet L6 recollimates the beam. Filter F1 is an edge filter that rejects virtually all the light below 550 nm that is not reflected by dichroic beam splitter B1. Note that all the doublets in the system are used with a low numerical aperture to minimize any additional aberration introduced by the optics. A Shack–Hartmann sensor is conjugated with pupils P2 and P3. A reference beam is introduced via a removable beam splitter (B2). A collimated beam from a laser diode (Laser 2, ≈630 nm) is used for this purpose; doublet L4 collimates this beam. The lenslets of the Shack–Hartmann sensor were 0.8 mm × 0.8 mm square (in the eye pupil plane).

The Shack–Hartmann sensor casts an array of spots upon the CCD array. By comparing the array of spots produced by the reference beam with that produced by the beam outgoing from the eye it is possible to reconstruct the wave front of the latter beam. Its operation principle was described by Tyson.[9]

It is important to note that the extended nature of the incoherent fluorescent spot of the retina, caused by aberrations on the forward pass, does not bias the measurement of the wave-front aberration on the second pass. The more extended the spot on the retina, the larger the random error in the determination of the centroid of each Shack–Hartmann subimage[10] and hence the larger the random error in the estimated wave front. Thus the extended nature of the spot lowers the signal-to-noise ratio of the measurement, but it does not bias the measurement in any way. Our measurement is therefore unambiguously a single-pass measurement.

To check the calibration, a Spindler & Hoyer Model 063127 40-mm focal-length doublet (reversed, to reveal spherical aberration) was used. Using a commercial Fizeau interferometer operating with a He–Ne laser at a wavelength of 633 nm, we measured the spherical aberration of the doublet for a pupil diameter of 7 mm. The same doublet with the same pupil was tested with the system shown in Fig. 2. The retinal fluorescence was simulated with Rhodamine 640 dye. The chromatic variation of spherical aberration in the red is expected to be negligible, and therefore the slight difference between using a He–Ne laser wavelength and that of Rhodamine 640 can be ignored. The Zernike spherical aberration measured with our setup and that measured with the Fizeau interferometer differ by less than 3%.

The wave-front aberration of the left eye of subject LD was measured. Four different exposure times were used: 0.25, 0.5, 1, and 1.5 s, at the same laser illumination level as for the spectrum measurements. For each exposure time 30 Shack–Hartmann records were taken. Accommodation was paralyzed, and the

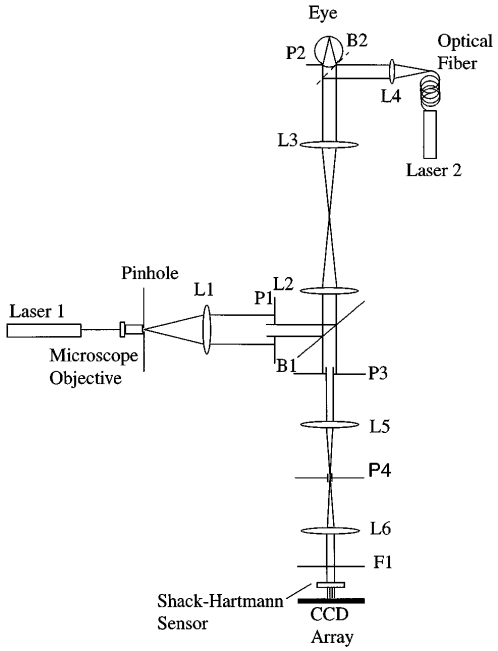

Fig. 2. Experimental setup used to measure the wave-front aberration of the human eye.

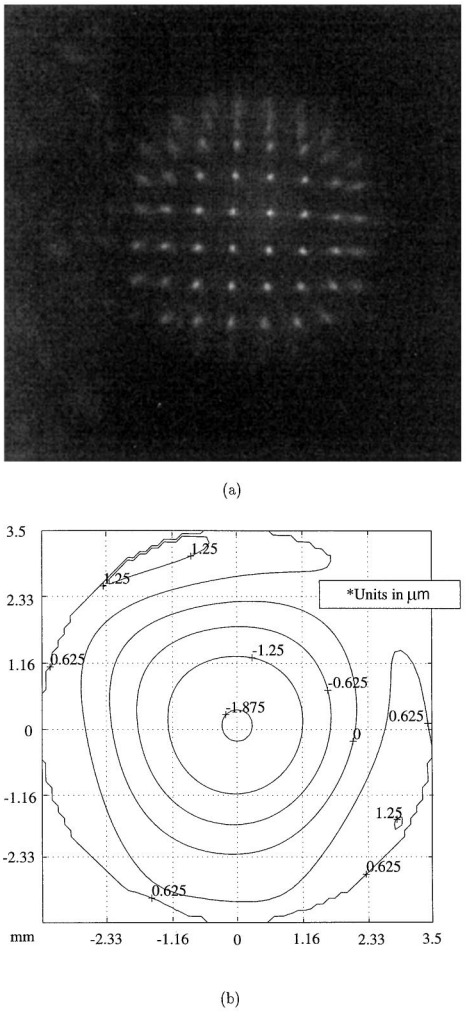

Fig. 3. (a) Array of Shack–Hartmann spots obtained from retinal autofluorescence for an exposure time of 0.25 s. (b) Wave-front reconstruction from Shack–Hartmann data. Subject LD.

pupil was dilated with solutions of Phenliephrine 2.5% and Tropicamide 1%. The position of the eye was held steady by a bite bar. The clean signal that is characteristic of noncoherent light was obtained for all exposure times. Figure 3(a) shows the array of spots obtained for an exposure time of 0.25 s.

We used a polynomial expansion with the set of orthonormal Zernike polynomials to reconstruct the wave front over a 7-mm-diameter circle in the pupil from the Shack–Hartmann data. Figure 3(b) shows the contour plot obtained from an average over 30 exposures of 0.25 s. The predominant aberrations are defocus and spherical aberration in this case. Similar results were obtained for the other reported exposure times.

The intensity of the fluorescent signal was measured for subject LD. For a 3-μW light input and a 7-mm-diameter pupil, the total output was approximately 0.0015 nW. Exposure times were typically 0.25 s.

Lipofuscin accumulates in the retina with age, and this fact will probably limit the use of this technique only to adults. The main advantage of using autofluorescence is the possibility of having a way to calibrate other techniques of measurement, because the uncertainty introduced by the double-pass process is eliminated.

An established way to measure the wave-front aberration of the eye is by use of an aberroscope.[11] Our proposed method, in which the incoming light loses its coherence at the retina because of autofluorescence, provides an alternative technique that is unambiguously a single-pass measurement. The original arguments that justify the validity of the measurement provided by the aberroscope would appear to be worth a thorough reexamination by the rigorous approach of coherence theory,[1] and a detailed comparison of our method with other methods that use double passage is currently being made.

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