High-resolution imaging of the human retina with a Fourier deconvolution technique

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A high-resolution retinal imaging camera is described that uses a Shack–Hartmann wave-front sensor and a Fourier deconvolution imaging technique. The operation of the camera is discussed in detail and high-resolution retinal images of the human cone mosaic are shown for a retinal patch approximately 10 arc min in diameter from two different retinal locations. The center-to-center cone spacing is shown to be 2.5 μm for the retinal images recorded at 2° temporal from the central fovea and 4 μm for the retinal images recorded at 3° temporal from the central fovea. © 2002 Optical Society of America

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1. INTRODUCTION

Adaptive optics promises to increase the resolution of ophthalmoscopes by correcting the wave aberrations of the human eye. Deformable mirrors, such as the ones used by Liang et al.1 and Hofer et al.,2 have become the technology of choice for wave-front correction in adaptive optics. The great cost and size, however, have prohibited many vision scientists from taking advantage of this technology and have also hindered the development of commercial ophthalmic instruments based on adaptive optics.

The use of a Fourier deconvolution technique, based on multiple wave-front aberration data and simultaneously acquired degraded images to provide an ensemble maximum-likelihood estimate of the original object, is an alternative approach. The deconvolution method used here was first proposed by J. Primit et al.3 as a new technique for high-resolution imaging through atmospheric turbulence by ground-based telescopes. This method was applied to the eye by Iglesias and Artal,4 who used quasi-incoherent illumination to simultaneously record the ocular wave-front aberration and degraded retinal images. They used the same light source for both wave-front sensing and retinal imaging, with their retinal images subtending approximately 0.5° and collected at 4° eccentricity. Nine images and their associated wave fronts were recorded and used in the deconvolution process; no evidence of the retinal cone mosaic is present in their result.

We present a high-resolution retinal imaging camera, based on these ideas, that uses coherent illumination for wave-front sensing and incoherent illumination for recording the retinal images. The laser produces a spot size on the retina, where the diameter of the beam is limited only by the aberrations of the eye, and the incoherent light source produces a spot size approximately 0.07 mm in diameter. With incoherent illumination, an estimation of the original object can be obtained from the expression

\[
\text{object} = FT^{-1} \left[ \frac{\langle I_i P_i^* \rangle}{\langle P_i P_i^* \rangle} \right],
\]

where \( i = 1, \ldots, N \), \( FT^{-1} \) denotes inverse Fourier transform, the angle brackets denote an average, and \( I_i \) and \( P_i \) are the Fourier transforms of the degraded images and the point-spread function, respectively. \( P_i^* \) is the complex conjugate of the Fourier transform of the point-spread function. The point-spread function is computed from the measured wave-front aberration obtained with a Shack–Hartmann wave-front sensor. In addition to noise reduction by averaging, assuming random aberrations, the averaging process fills zero-valued regions in the denominator, \( \langle P_i P_i^* \rangle \), allowing the avoidance of division by zero. The calculation of the object is always possible, as the denominator is never equal to zero for frequencies less than the cutoff frequency if \( N \) is large enough.5 Primot et al.3 showed that there is a systematic bias in the estimation of the computed speckle transfer function, \( \langle P_i P_i^* \rangle \), which can be explained by the fact that a finite number of Zernike polynomials are used to reconstruct the aberrated wave-fronts. Consequently, the reconstructed wave fronts are slightly smoothed, and the aberration therefore is underestimated. Hence it is desirable to use as many Shack–Hartmann spots as possible to estimate the local slopes of the wave-front aberration and to reconstruct the wave front by using a large number of Zernike polynomials. Primot et al.3 used 88 Zernike polynomials and 100 recorded degraded images, with their associated wave-front aberrations, to resolve a complex object. Iglesias and Artal4 used nine retinal images and an unstated number of Zernike polynomials to reconstruct their wave fronts.

In the processing algorithm, each short-exposure image \( I_i \) is Fourier transformed and the associated optical transfer function \( P_i \) is computed from the measured and reconstructed wave front. By measuring the distorted wave-front phase, \( \Psi \), one can determine the optical transfer function \( P_i \) by the autocorrelation of \( \exp(i\Psi) \):

\[
P_i = \exp(i\Psi) * \exp(i\Psi).
\]

The average cross spectrum of the images, the optical transfer function, and the average squared modulus of

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the optical transfer function are then calculated over the $N$ records. Finally, the object is estimated by an inverse Fourier transform.

2. EXPERIMENTAL SETUP

The experimental high-resolution retinal imaging camera, shown in schematic form in Fig. 1, consists of an imaging arm and a wave-front-sensing arm. The illumination of the imaging arm consists of a shuttered 50-W halogen lamp, which is focused onto a small aperture. This arrangement semispatially filters the incoherent light beam. Lens $L_3$ collimates the light beam. A wavelength-selecting interference filter ($550 \pm 5$ nm) is placed in the path of the light beam between $L_3$ and the beam-splitting cube $BSC_1$.

The illumination for the wave-front-sensing arm consists of an 830-nm pigtailed laser diode that has been expanded, spatial filtered, and collimated. The collimator lens, $L_4$, is used to conjugate the 25-$\mu$m-diameter pinhole with the retina.

Lens $L_9$ is set one focal length from the artificial pupil and two focal lengths from lens $L_8$. Lens $L_9$ is set one focal length from the pupil of the eye. Hence the pupil of the eye is conjugated with the artificial pupil. Lenses $L_9$ and $L_8$ act as a focus corrector. Lens $L_8$ remains at a constant distance from the pupil of the eye. The subjects move their heads in combination with lens $L_8$ along the optical axis to achieve best focus.

The fixation target consists of concentric circles, crossed by perpendicular axes, printed on acetate sheet and placed in front of a ground-glass screen. The intersection of the circles with the axes determine different

![Fig. 1. Schematic diagram of the high-resolution retinal imaging camera.](image-url)
Table 1. Variation in the Value of the Zernike Coefficients during the Acquisition of the Data Set

<table>
<thead>
<tr>
<th>Zernike Coefficient</th>
<th>Meaning</th>
<th>Mean and Standard Deviation (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₃</td>
<td>Astigmatism with axis at ±45°</td>
<td>0.62 ± 0.07</td>
</tr>
<tr>
<td>C₄</td>
<td>Defocus</td>
<td>1.16 ± 0.09</td>
</tr>
<tr>
<td>C₅</td>
<td>Astigmatism with axis at 0°</td>
<td>-0.33 ± 0.07</td>
</tr>
<tr>
<td>C₆</td>
<td>Trifoil with axis at 30°</td>
<td>0.17 ± 0.03</td>
</tr>
<tr>
<td>C₇</td>
<td>Coma along x axis</td>
<td>-0.26 ± 0.09</td>
</tr>
<tr>
<td>C₈</td>
<td>Coma along y axis</td>
<td>0.12 ± 0.02</td>
</tr>
<tr>
<td>C₉</td>
<td>Trifoil with axis at 0°</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td>C₁₀</td>
<td>Tetrafoil with axis at 22.5°</td>
<td>-0.19 ± 0.07</td>
</tr>
<tr>
<td>C₁₁</td>
<td>Astigmatism, second order at ±45°</td>
<td>0.11 ± 0.03</td>
</tr>
<tr>
<td>C₁₂</td>
<td>Spherical aberration</td>
<td>0.17 ± 0.04</td>
</tr>
<tr>
<td>C₁₃</td>
<td>Astigmatism, second order at 0°</td>
<td>-0.16 ± 0.03</td>
</tr>
<tr>
<td>C₁₄</td>
<td>Tetrafoil with axis at 0°</td>
<td>0.15 ± 0.03</td>
</tr>
</tbody>
</table>

*Of the calculated value of the Zernike coefficients during acquisition of the 30 wave-front measurements.

Fig. 2. (a) Shack–Hartmann spot pattern for the first recorded wave-front aberration of subject 1’s left eye (320 × 240 pixels), (b) reconstructed wave front across a 6-mm pupil, where the contour spacing is 0.3 μm, and (c) the associated point-spread function (both 256 × 256 pixels).
retinal eccentricities (ranging from 0° to 3°) and locations (temporal, nasal, horizontal, and vertical) at which the observer fixates. Small circles printed on the target help the observer to maintain fixation. The target is back illuminated with a 1.5-V torch bulb placed in front of a green filter, with the intensity of the bulb adjusted so that the target is just visible.

The system uses an off-axis illumination method whereby the beam-splitting cube, $BSC_2$, is fractionally tilted so that light enters the eye’s pupil at a position displaced approximately 1 mm from the pupil center. This causes the corneal reflection to be displaced in the retinal plane. By adjusting to a minimum the aperture stops $A_2$ and $A_3$, which are conjugated with the retina and positioned between lenses $L_{10}$ and $L_{11}$ and $L_{13}$ and $L_{14}$, respectively, in both the imaging and the wave-front-sensing arms, the corneal reflection can be blocked at these locations.

A manually operated flip mirror is positioned after the beam-splitting cube $BSC_2$ so that the light beam can either be selectively directed to the wave-front-sensing arms (mirror raised) or to the imaging arm (mirror lowered).

In the wave-front-sensing arm the light beam is reduced in diameter by a 3:1 Keplerian telescope arrangement, lenses $L_{13}$ and $L_{14}$. The pupil of the eye and the surface of the Shack–Hartmann lenslet array are conjugate pairs.

The Shack–Hartmann wave-front sensor is constructed from a two-dimensional array of spherical lenslets with a CCD camera (SBIG ST-5C) placed at the focal point of the lenslet array. The lenslet array divides the tested wave front into a number of subapertures. The light passing through each subaperture is brought to a focus at the focal plane of the lenslet array. The Shack–Hartmann lenslet array has a center-to-center lenslet spacing of 203 $\mu$m, with each lenslet having a focal length of 5.6 mm.

The test of an ideal plane wave results in a regular array of focus spots. Each spot is located on the optical axis of the corresponding lenslet. This spot pattern of a plane wave is used as a reference pattern. If a deformed wave front is measured, the image spot at each subaperture shifts with respect to the corresponding point in the reference pattern by a factor proportional to the local tilt. The local slopes, or partial derivatives, of the tested wave front can therefore be detected by measurement of the shift of the focus spots. Once the local slopes are known, the wave front is reconstructed by using modal wave-front estimation\textsuperscript{6,7} with Zernike polynomials.\textsuperscript{8} The Shack–Hartmann pattern consisted of 88 spots for each measured wave front, and the wave front was reconstructed by using the first 35 Zernike polynomials, with tip and tilt removed. The imaging arm also consists of a 3:1 Keplerian telescope, $L_{10}$ and $L_{11}$, plus an imaging lens, $L_{12}$. The surface of the imaging scientific-grade CCD (SBIG ST-5C) is conjugated with the retina of the eye.

### 3. EXPERIMENTAL PROCEDURE

The subject’s left eye was dilated with 1% tropicamide, and the subject fixated on the 2° temporal point of the accommodation fixation target. The subject’s head was held in a stationary position by a head rest consisting of a chin rest and a forehead rest. The chin rest could be replaced with a bite bar if the subject found this a more comfortable arrangement.

A total of 30 wave fronts were recorded, for a 6-mm pupil, at an exposure time of 50 ms per recording, with a laser beam intensity of 4 $\mu$W/cm\textsuperscript{2} at the pupil plane.
Thirty retinal images were also recorded at an exposure time of 0.5 s per recording, at a wavelength of 550 nm, for an incoherent light intensity of 0.5 mW at the pupil. The series of wave-front measurements were recorded immediately before the series of retinal images. Ideally the wave fronts and the retinal images should be recorded simultaneously; indeed, for atmospheric turbulence this would be essential. For the eye, however, where the aberration varies less rapidly, it is more important that the recorded retinal images have a minimum amount of residual speckle noise. Table 1 shows the variation in the calculated values of the first 11 significant Zernike coefficients for the recorded data set of 30 measurements. The data, both wave-front measurements and images, were collected in less than 30 s. To test the validity of the method adopted in this work, many simulations and models were tested before the retinal camera was used on human subjects.

The centroid location of each spot in the Shack–Hartmann pattern was iteratively evaluated by placing a box around the spot that completely enclosed it with a minimum amount of space on each side. The box automatically moves, performing a subpixel averaging of the pixel intensities along the rows and columns of the box. The box centers itself on the center of the column and row of maximum intensity. The box is moved, and the process is repeated until the difference between the previous and the new maximum-intensity centroid position is less

![Fig. 4. Fourier deconvolved retinal images, from location 2° temporal. Image (a) was obtained after 10 realizations of the series of images and wave-front aberration point-spread functions. Image (b) was obtained after 20 realizations of the series of images and wave-front aberration point-spread functions. Image (c) was obtained from the full 30 realizations of the series of images and wave-front aberration point-spread functions. Each image is 144 × 144 pixels and represents a retinal patch approximately 10 arc min in diameter.](image-url)
than $10^{-4}$ pixels. Once the local slopes are known, the wave front is reconstructed by using modal wave-front estimation with Zernike polynomials.

Figure 2 shows the first recorded Shack–Hartmann spot pattern together with the reconstructed wave front and its associated point-spread function. Initially the image can be located, and removed, from the background by using a routine similar to the centroiding algorithm that is used to locate the center of the Shack–Hartmann spots. In this case, a box is chosen such that its dimensions completely enclose the image with a few (three or four is sufficient) blank pixel rows and columns on either side of the image. The algorithm assumes that the image is the brightest entity in the frame and centers itself on the area of maximum intensity. The box then moves in all directions until it completely encloses the entire image. The image is then removed from the background, and a feature is chosen in the image to use as a template for matching the other images in the sequential series. A small feature template from image 1 is used to search image 2 for the same feature. Once the feature has been located, image 2 is repositioned so that the features in both images are correlated. The first recorded retinal image for the subject’s left eye is shown in Fig. 3, before and after removal from the background. It is essential to remove the image from the surrounding frame of unexposed CCD surface area so that the deconvolution algorithm can operate only on the area of interest. The process was repeated with the subject fixated at 3° temporal, and 30 additional degraded retinal images and wave-front aberrations measurements were recorded.

4. RESULTS

Figure 4(a) shows the Fourier deconvolved retinal image obtained by applying Eq. (1) to a series of 10 degraded images and a series of 10 wave-front aberration measurements recorded at the 2° temporal location. There is no evidence of any retinal structure in this minimal number of averaged resolved images. Figure 4(b) shows the deconvolved retinal image from use of a series of 20 degraded images and wave fronts, and Fig. 4(c) shows the deconvolved image from use of the full 30 degraded images and wave fronts. Figure 4(b) and 4(c) both show bright spots that could be retinal cones.

Yellot showed that the power spectrum of the cone mosaic of excised human retinas has power concentrated in a ring about the origin. The radius of this ring corresponds to the cone sampling frequency, which is the reciprocal of the cone spacing. Miller et al. and Marcos et al. both demonstrated the existence of Yellot’s ring in the power spectrum—the square modulus of the Fourier transform—produced from their images of the cone mosaic in the living human eye. Both Miller and Marcos used power spectra obtained by averaging the square modulus of the Fourier transform from several images, thus:

$$\langle |I|^2 \rangle = \frac{1}{N} \sum_{k=1}^{N} |FT(\text{image}_k)|^2.\tag{3}$$

In this case, since the signal-to-noise ratio is low for a single power spectrum, the average power spectrum of Fourier deconvolution realizations 20 to 30 have been taken. The result is shown in Fig. 5(a). To enhance the higher frequencies, the power spectrum is expressed in a logarithmic scale. The horizontal cross section of the power spectrum (an intensity profile), Fig. 5(b), shows the radius of the ring to be $\approx 120$ cycles per degree, giving a cone spacing of $\approx 0.55$ arc min. This corresponds to a center-to-center cone spacing of $\approx 2.5$ μm, the conversion being based on the parameters of the Le Grand theoretical model eye.
The reason for using the parameters of a model eye is that the parameters of the subject’s eye, such as the total power and length, are not precisely known. The angular center-to-center spacing can be measured directly from the images or from the intensity cross section of the power spectrum. However, in order to convert the angular measurement into a unit of length inside the eye, one must know the radius of curvature of the various surfaces of the cornea and crystalline lens as well as the refractive index of the eye’s components.\textsuperscript{13}

This calculated value for the cone spacing is considerably less than the estimate of \( \sim 4 \, \mu m \) given by Curcio \textit{et al.}\textsuperscript{14} based on their measurements from excised human retinas, for this retinal location. It has been argued that owing to possible distortions in the preparation of the tissues, anatomical data could differ from the actual density in living eyes. If we take this into account, plus the large variation of spacing between individuals, the result obtained here seems reasonable.

Figure 6 shows the first recorded image for the 3° temporal location before and after removal from the background together with the first recorded wave-front aberration and associated point-spread function. Figure 7(a) shows the Fourier deconvolved retinal image obtained by applying Eq. (1) to a series of 10 degraded images and a series of 10 wave-front aberration measurements. There is, again, no evidence of any retinal structure for this minimal number of resolved images. Figure 7(b) shows the deconvolved retinal image obtained with an averaged series of 20 degraded images and wave fronts; Fig. 7(c) shows the deconvolved image obtained with the full 30 degraded images and wave fronts. Once more, Figs. 7(b) and 7(c) both show bright spots that could be retinal cones.

The power spectrum obtained from realizations 20 to 30 of the Fourier deconvolution process is shown in Fig. 8(a), along with a horizontal cross section taken through the center of the power spectrum shown in Fig. 8(b). The horizontal cross section of the power spectrum shows the radius of Yellot’s ring to be \( \sim 50 \) cycles/deg, giving a cone spacing of \( \sim 1.20 \) arc min. This corresponds to a center-to-center cone spacing of \( \sim 4 \, \mu m \), the conversion being

![Fig. 6. (a) First degraded retinal image recorded at the 3° temporal location; this image is 320 × 240 pixels. (b) Retinal patch removed from the background; this image is 144 × 144 pixels and represents a retinal patch approximately 10 arc min in diameter. (c) The first recorded wave-front aberration for this location and (d) the associated point-spread function (both 256 × 256 pixels). The pupil diameter is 6 mm.](image-url)
Evidence of the photoreceptor cone mosaic was found in four of the eight subjects tested. The average cone spacing for the four subjects at the 2° temporal location was $(0.82 \pm 0.27)$ arc min and $(1.20 \pm 0.25)$ arc min for the 3° temporal location. Our results seem perfectly consistent with those given by Miller et al.\(^{10}\) of $(1.18 \pm 0.12)$ arc min at 2.5° eccentricity and $(0.92 \pm 0.14)$ arc min at 1.25° eccentricity. No evidence of the cone mosaic was found for the central fovea location in any of the tested subjects, nor did the power spectrum reveal any evidence of a ringlike structure for this location.

5. CONCLUSION

We have shown that a Fourier deconvolution technique can be used successfully to resolve images of the photoreceptor cone mosaic in the living human eye. For this procedure to be successful, it is important to remove the degraded retinal patch from the surrounding unexposed area of the frame so that the calculated optical transfer function can operate only on the area of interest. The systematic bias in the estimation of the computed speckle transfer function that results from using a finite number of slope measurements and Zernike polynomials in the wave-front reconstruction means that a minimum number of 20 images and wave fronts have to be recorded for the cone mosaic to be resolved.

Although the Fourier deconvolution technique is a simpler, and far cheaper, method of imaging the retina than the use of an adaptive optics system based on a deformable mirror, it is a method that cannot be operated in real time. There is a delay between acquiring the degraded images and the deconvolution realizations of the initial
object. Multiple retinal images must be recorded, and those images must be correlated before the deconvolution algorithm is applied. A closed-loop adaptive optics system, such as that demonstrated by Hofer et al. or Le Gargasson et al. based on the use of a deformable mirror is therefore a more desirable option for producing real-time high-resolution retinal images, because the cone mosaic can be resolved in a single image. The cone classification technique pioneered by Roorda and Williams for determining the arrangements of the three types of cones in the eye, although difficult to perform under any conditions, would be almost impossible with the Fourier method. If cone classification is desired, then an adaptive optics system would seem to be the only viable option. However, although many improvements could be made to the experimental setup and data processing, the Fourier method is an alternative approach to high-resolution retinal imaging when cost is a limiting factor.

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REFERENCES