

# Estimating the retinal thickness from Shack-Hartmann aberrometry data

Varis Karitans<sup>1</sup>, Liene Jansone<sup>2</sup>, Maris Ozolins<sup>1,2</sup>, Gunta Krūmina<sup>2</sup>

<sup>1</sup>Department of Ferroelectrics, Institute of Solid State Physics, University of Latvia, Riga, Kengaraga street 8, LV-1063

<sup>2</sup>Department of Optometry and Vision Science, Faculty of Physics and Mathematics, University of Latvia, Riga, Kengaraga street 8, LV-1063

E-mail: variskaritans@gmail.com

## Introduction.

Commonly, it is assumed in the Shack-Hartmann aberrometry that a retina is a single reflecting layer. However, there are several retinal layers each reflecting some amount of the light. Each of these reflections may form a spot pattern on the Shack-Hartmann wavefront sensor (SHWS). While these spot patterns would coincide with each other in the central part they would be displaced in the peripheral region due to difference in vergences of the outgoing beams (see the Figure to the right). The amount by which these spot patterns are displaced may contain information about the retinal thickness. First, this method was suggested by Liu et al. [1] during EMVPO-2012 held in Dublin. Liu et al. used a mathematical simulation in Matlab and successfully applied a global reconstruction algorithm to recover the retinal thickness from the Shack-Hartmann images. In this study we applied the classical centroiding algorithm to identify double spots and calculate the centroid of each component to recover the thickness of an artificial retina. The experiments were primarily done with an artificial eye although the double-spot pattern of a living eye was also captured. We aimed to evaluate applicability of this method in ophthalmology and to compare this method to the optical coherence tomography (OCT) which is widely used to determine the retinal thickness [2,3].

## Methods.

In the experiment a custom-built SHWS shown schematically in the Figure to the right was used. The artificial eye consisted of an anterior layer made of a tissue paper and a posterior plastic surface. The material of the anterior layer was chosen so that it is both transparent and diffusely reflecting whereas the material of the posterior surface was chosen so that adherence between the anterior layer and the posterior surface is minimized. The distance between the anterior layer and the posterior surface was varied using a motorized translation stage. A narrow collimated beam from an infrared laser diode was directed into the eye and illuminated the retina. The beam was reflected both by the anterior layer and the posterior surface and went out of the eye. The pupil plane and the lenslet array (Thorlabs) were conjugated using two relay lenses L1 and L2 ( $f = 160$  [mm]). The lenslet array had the focal length  $f = 18.6$  [mm]. In the focal plane of the lenslet array an astroimaging CCD camera OptiStar DS-335C-ICE was placed. The CCD has resolution 2048 [px] x 1536 [px] and the size of the pixel 3,45 microns. The CCD camera was controlled by software Maxim DL 5. The small inset window is the histogram of the Shack-Hartmann image which allows to control the brightness and contrast.

For each distance the double-spot pattern was captured and saved as a grayscale bitmap. Based on the threshold chosen the code separated the double-spots in two individual components. In MATLAB the classical centroiding algorithm [4] was used to calculate the centroids of both components of the double-spots. Next, from simple geometrical relationships the distance between the centroids was converted to the retinal thickness. Prior to applying the algorithm a Gaussian filter with radius  $r = 1$  [px] was superimposed on the Shack-Hartmann images to eliminate intensity variations within a single point.

The transversal resolution of the optical system was also determined. As in OCT, the transversal resolution is the full width of the intensity distribution. The transversal resolution of the system was calculated by placing the CCD in the focal plane of the eye (see the Figure below) and determining the full width of the intensity distribution of a single spot. The full width of the intensity distribution was determined using software ImageJ. The value was compared to that given by the equation (on the left side) used to calculate the transversal resolution in OCT.

$$R_L = \frac{4 \cdot \lambda}{\pi} \cdot \frac{f}{d}$$

As in OCT where the transversal resolution is independent of the axial resolution [5] these two types of resolution are also independent in this method. In OCT the axial resolution is determined by coherence length of the light source while in this method it is mostly determined by the size of the PSF on the CCD which in turn depends on the optical parameters of the lenslet array (focal length and diameter). It is also determined by the pixel size of the CCD.



## Results.

The Figure to the right shows both components of a double-spot marked in blue colour (top left image) and increase in distance between the components of the double-spots as the translation stage is moved. The increase in the distance is clearly visible. All images in the Figure are small cut-outs of a very peripheral region of the whole spot pattern. The second image from the left in the upper row shows the spot pattern for 0  $\mu\text{m}$  large movement distance of the translation stage while the first image from the right in the lower row was obtained for the movement distance 200  $\mu\text{m}$ . The distance increases in steps of 20  $\mu\text{m}$ . The spot pattern of a living eye is also shown. The red circles identify the possible double spots. These spots are orientated radially towards the centre indicating the both of these components could indeed come from separate layers and are not the result of, e.g., diffraction in the apertures of the lenslets or have not resulted from background.

A Bland-Altman plot showing the calculated distance vs the movement distance of the translation stage is shown in the Figure to the right. The error bars are the standard errors calculated for each distance. In Figure 3 it can be seen that the average measured distance increases linearly with the movement distance of the translation stage ( $R^2 = 0.91$ ). The minimal movement distance against which the calculated distance has been plotted is 100  $\mu\text{m}$  because for smaller movement distances the classical centroiding algorithm couldn't resolve both components of the double-spots. There is a very considerable bias thickness (about 200  $\mu\text{m}$ ). Most likely the bias thickness is due to the initial distance between the anterior layer and the posterior surface which is hard to control. The minimal distance which can be resolved is about 300  $\mu\text{m}$  which corresponds to the approximate retinal thickness in parafoveal region [6]. The last measurement point corresponds to the retinal thickness which can be observed if the angiographic macular edema is present (>400  $\mu\text{m}$ ) [7]. Increase in the retinal thickness may also be detected in the case of retinal detachment.

The transversal resolution of the system is shown in the Figure to the right. The image of a spot is shown in the upper left corner while the image on the right side plots intensity vs distance in micrometers. The full width of the intensity profile is about 75  $\mu\text{m}$  which is also the transversal resolution of the system. The equation given previously yields the corresponding value equal to 65  $\mu\text{m}$  comparable with the real value for the values = 850 nm,  $f = 21$  mm and  $d = 0.35$  mm.

## Discussion and conclusions.

Although the method described here may not be sufficiently accurate for precise estimate of the retinal thickness, i.e., the standard errors are quite large the results still prove the applicability of this method for qualitative analysis of changes in the retinal thickness. The results also confirm that Shack-Hartmann aberrometry may be vulnerable to errors when using the classical centroiding algorithm. Possible inaccuracies associated with the classical centroiding algorithm have already been discussed previously [1, 8-9]. Instead of a single centroid two centroids may be detected within the area of a single lenslet giving erroneous results of aberrometry. The minimal measurable distance between the retinal layers and the axial resolution depends on the optical parameters of the lenslet array which determine the size of the PSF of the spots formed on the CCD. The axial resolution of this method depends also on the resolution of the CCD. It must also be taken into account that the intensity of both double-spot components isn't the same, i.e., the intensity of the more myopic component is lower than that of the other component. When applying the Rayleigh criterion both peaks have approximately the same height. However, in this method both peaks have different height. In addition the retinal layers are a scattering media attenuating the light exponentially according to the Beer-Lambert law as it travels through these layers so that the peak of the spot could be displaced laterally from the centre. In the case of the current optical setup this method has low transversal resolution compared to optical coherence tomography [10] due to the small diameter of the infrared beam. It can be increased by increasing the beam diameter but in Shack-Hartmann aberrometry this gives rise to the corneal reflections which imposes difficulties in analyzing the images. The retinal layers are structures which reflect the light in the whole volume not only from the surfaces. That's why this method may not be suited for analyzing the thickness of an individual layer. A double-spot pattern from a real retina may consist of two elongated overlapping spots with different intensity unlike in this study where these spots were even apart from each other. From OCT images of a living retina it can be seen that all layers between the retinal nerve fiber layer (NFL) and the choriocapillaris have similar reflectance which is several times lower than that of both of these layers [5, 11]. In addition, the thickness of a single retinal layer is several tens of micrometers corresponding to the dioptric thickness equal to some hundredths of a diopter [12] while the total retinal thickness corresponds to the dioptric thickness equal to several tenths of a diopter. It suggests that this method could be more applicable for estimating the total retinal thickness rather than thickness of individual layers. Based on the results of this study which prove the applicability of this method for qualitative analysis of changes in the retinal thickness in future we plan to apply this method to evaluate the thickness of thin diffusively reflecting polymer films which would also eliminate the problems associated with the bias thickness.

## References.

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