Reliable Measurements from Fundus Photographs in the Presence of Focusing Errors

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When measuring the width of the blood column and the superimposed central light reflex of retinal vessels from fundus photographs, we find that the measurements are affected by focusing error, unless certain steps are taken to reduce its effect. Thus, we recommend using a scanning microdensitometer to extract the density profile of the blood column from the negative, and convert the density profile to an intensity profile by means of the characteristic curve of the film. In this paper we model the imaging process on a computer, and find that the half value width of the intensity profile is relatively insensitive to focus error. However, the maximum intensity of the central light reflex is still sensitive to focus error. Both these results are in agreement with experimental studies. Invest Ophthalmol Vis Sci 30:674-677, 1989

We find that the error of focusing the fundus camera is important for measurements of the size of retinal structures in spite of telecentric operation of the camera. Fortunately, since the camera is telecentric, it is possible to reduce the effect of focusing error by performing the measurements according to a method previously described. The method consists of scanning the photographic negative by means of a microdensitometer, and converting the density readings to light intensity by means of the characteristic curve of the film. The last step is of crucial importance, as will be discussed below.

The current paper is a theoretical study in which we model the effect of defocusing on a computer, and show that the “half value width” of a structure is relatively unaffected by defocusing. This theoretical result corresponds well with experimental studies of defocusing, thereby increasing confidence in both the theoretical modelling and the experimental method.

The retinal structure of special interest to us is the blood column of retinal vessels, with its superimposed central light reflex. A reliable measurement of the diameter of the blood column is important for the accurate determination of retinal blood flow. Further, changes in the dimensions of the reflex may be related to hemodynamic or structural changes due to disease or other stresses on the human body.

In our first experimental work we studied the effect of defocusing by visual inspection of extreme enlargements of fundus photographs. By this means we found that we can detect changes in sharpness with a defocusing of only 1/32 or 2/32 of a turn of the focusing knob of the original Zeiss 30° camera.

Such tight tolerance on focusing is not normally achieved in practical use of the camera. In more recent experimental work, we found that the necessary tolerance can be increased to at least ±1/8 by measuring the half value widths by a microdensitometric technique. The focusing tolerance thereby comes within reach in careful practical use of the camera.

Another parameter of interest is the intensity of the reflex. We find both experimentally and theoretically that this parameter is affected by focusing error to a much larger degree than the half value widths.

We wish by this paper to draw attention to the problem of focusing error, and point to a way of reducing its effects.

Materials and Methods

Our theoretical approach to this question is based on the way an object is imaged by an optical system. When a lens makes an image of an object, the image can never be absolutely sharp. Every point in the object is imaged as a somewhat blurred “spot” of light which all add up to form the whole image. This adding up of blurred spots is known as convolution and can be efficiently simulated by a computer. We are thus able to study image formation theoretically, by computer modelling.

The size and shape of the spot is determined by diffraction due to the finite wavelength of light, aber-
rations of the optics of the camera and the eye, and the amount of defocusing. Previous investigations indicate that the aberrations of the fundus camera are very small, and we assume aberration-free optics of the eye for this study. The spot is therefore only determined by diffraction and defocusing. Since the retinal vessels are linear structures, we simplify our problem further by using a blurred "line" instead of a blurred "spot." This blurred line is called the line spread function.

According to what has been said above, we investigate this problem in the following way:

1) We first make a mathematical function to simulate the real intensity distribution across the vessel, as shown in Figure 1.

2) We compute the intensity distribution of the line spread function for various amounts of defocusing by means of diffraction theory, as shown in the left hand column of Figure 2.

3) Then we compute the convolution of the function in Figure 1 with the line spread functions in Figure 2. We thereby get the right hand column of Figure 2, which shows the theoretical prediction of the intensity distribution on the film for the given amount of defocus.

4) Finally, from these theoretical curves we find the half value widths of the blood column (W₀), the central light reflex (Wᵣ), and the intensity of the light reflex (Iᵣ), and display the variation of these parameters with defocusing, as shown in Figure 3.

At this point we draw attention to the fact that the modelling is performed using the intensity distribution of the light. What is available from the exposed negative is the distribution of density. The half value width of intensity is quite different from the half value width of density. We must therefore convert the density to intensity before drawing conclusions from experimental results.

As mentioned above, the object, or the real intensity distribution across the vessel, is shown in Figure 1. The real intensity distribution cannot be measured directly in the living eye because various ocular tissues overlay the retina. We therefore simulate the real distribution by a mathematical function which contains the characteristic features of the real distribution.

In this model we have set the half value width of the blood column to 100 µm, the half value width of the reflex to 25 µm, and the intensity of the reflex to 50% of the surrounding retina. Due to the magnification of the fundus camera, which has been found...
earlier to be about 2.43, the theoretical image of the film of the blood column is 243 μm.

The method of computing the defocused line spread function of a perfect optical system is given by Stamnes.

The line spread function depends upon the wavelength of the light, the F/No or relative aperture of the fundus camera and the defocus distance expressed in mm. The F/No of the fundus camera is 20.7, as found from the formula:

\[
F/No = \frac{m f}{a}
\]

Here \( f = 17.055 \) mm is the focal length of Gullstrand's schematic eye, \( m = 2.43 \) is the magnification of the camera, and \( a = 2 \) mm is the diameter of the entrance pupil of the fundus camera.

The wavelength is chosen to be 0.5 μm, as representing light in the visible region. The amount of defocusing is found by measuring the movement of the film plane with one turn of the focusing knob. This movement was 29 mm.

Since we assume that the camera is aberration-free, the line spread functions for positive and negative defocusing will be equal. The line spread functions have been computed for the perfect focus and for 1, 2 and \( \frac{3}{32} \) of a turn of the focusing knob.

### Results

The line spread functions are shown in the left hand column in Figure 2. We see that the line spread functions become wider as the amount of defocus increases; the line becomes less sharp. The various peaks at \( \frac{1}{32} \) and \( \frac{1}{32} \) arise because of diffraction. They are of minor importance compared to the width of the line spread function.

In the right hand column in Figure 2 are shown the theoretical curves representing the image on the film of the object intensity curve in Figure 1. These curves are obtained by convolving the object intensity in Figure 1 with the line spread function.

Figure 3 shows the half width of the blood column and the central reflex and the intensity of the reflex as a function of defocus. These values have been obtained by measurement of the curves in the right-hand column in Figure 2. These theoretical curves show the same type of variation with defocus as found from microdensitometric scans of real fundus photographs. The half value widths of both the blood column and the reflex are relatively insensitive to defocus, whereas the intensity of the reflex is noticeably reduced upon defocus.

### Discussion

We have made a simplified theoretical investigation of the effect of focusing error of the fundus camera on the measurement of half value widths of the blood column and its central light reflex and the intensity of the reflex of retinal vessels. We find that the half value widths of the blood column and the reflex are largely insensitive to focusing error of the fundus camera. It can be seen from Figure 2 that the slope of the intensity curve at the edge of the blood column and the reflex changes with focusing. This means that a width measurement which is not at the 50% intensity level will be more sensitive to focus error. It is very important here that the camera is telecentric, so that the magnification (m) is independent of defocus.

Our findings show that one must be careful when taking measurements from fundus photographs. Such photographs have been used for taking measurements by: (1) directly measuring a positive enlargement by a ruler; (2) by projecting the negative on a screen for measurements; and (3) by studying the negative in a microscope.
By these means we have no control over the intensity level at which the measurements are taken. First, we do not check the intensity level at which the measurement is taken. Second, the reflectivity of the enlargement or the transmittivity of the negative are determined by the characteristic curves of the photographic materials and need to be converted to image intensity before measurements are taken.

Using a high-contrast film makes it easier to decide where to take the measurement because the edge is sharper, thereby reducing random errors of the measurements. But we have no assurance that the edge is at the 50% intensity level. Consequently, we must expect a measurement taken in this way to be affected by focusing error.

The current study shows that by using microdensitometry directly on the negative and converting the densities to intensities by the film characteristic curve, we obtain an objective way of measuring photographs. Various densitometric techniques have been used for measurement of diameters of retinal vessels.4,5,9-15 Densitometry has been criticized, however, for not having solved the problem of objectively locating the boundary between the vessel edge and the retinal background.10,11 Another difficulty is the determination of the intensity level of the surrounding retina. In our experimental studies,1,2 we chose the maxima of the background intensity level on either side of the vessel within one vessel diameter from its center. Averaged values were used as starting points of the measurements, and satisfactory reproducibility was obtained. We found a standard deviation of less than 4%, which included variation of normal vascular pulsation.1 This increased to about 5% upon defocusing, as inferred from experiments.2

The results show that the theoretical curves behave in the same way as the curves obtained by microdensitometric scans of real fundus photographs2 inside a focusing range of ±3/4 of a turn of the focusing knob.2 This correspondence indicates that the experimental measurements are reliable. The insensitivity of the half value widths shows that these are useful measures of the size of the corresponding structures on the retina. The sensitivity of the central intensity, however, means that special care must be taken in the use of these values.

Key words: retinal vessels, fundus photography, caliber measurements, densitometry, focusing

References