SCIENTIFIC REPORT

The effect of image alignment on capillary blood flow measurement of the neuroretinal rim using the Heidelberg retina flowmeter

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Aim: To examine the influence of image alignment on the repeatability of blood flow measurements of the optic nerve.
Methods: 10 normal subjects were examined. Heidelberg retina tomograph imaging was performed to establish best location and focus for the temporal neuroretinal rim. Two high quality Heidelberg retina flowmeter (HRF) images were acquired for three methods of alignment: central, nasal, and temporal. A 10×10 pixel measurement window was selected and exactly reproduced on all images. The interquartile pixel values were used to calculate capillary flow. ANOVA, intraclass correlation coefficients (ICC) and the coefficient of repeatability (CoR) were used for analysis.
Results: There was no difference between methods ($p=0.47$) or between visits ($p=0.51$). The ICCs were 0.83 for the central, 0.34 for the nasal, and 0.42 for the temporal alignment. The CoR was 31.5 for central (mean effect 235.1), 234.6 for nasal, and 256.7 for temporal alignment.
Conclusion: Central alignment was the most repeatable method for the measurement of neuroretinal rim capillary blood flow using the HRF.

The Heidelberg retina flowmeter (HRF, Heidelberg Engineering GmbH, Germany) is a scanning laser Doppler device used to estimate the ocular capillary blood flow.[1–6] Acquisition parameters influence the variability of flow values.[6–11] A recent study suggested placing the temporal rim margin tangent to the lateral image border for more reproducible measurement of the neuroretinal rim.[11] The purpose of this study was to examine the influence of different image alignments on the repeatability of blood flow measurements of the neuroretinal rim using the HRF.

METHODS

Institutional ethics approval was granted. Imaging was performed on one randomly selected eye of 10 normal subjects (age range 23–44 years, mean 29.9 (SD 7.6), four females, corrected VA⩾6/9).

Heidelberg retina tomograph (HRT) imaging was performed and the profile map used to determine the dioptric difference between the peripapillary retinal surface and the temporal rim. The best dioptric focus for the temporal rim was used for HRF imaging[12] using three different methods of alignment:

- central: the optic nerve head was positioned at the centre of the image;
- nasal: the nasal rim margin was placed tangent to the nasal side of the image;
- temporal: the temporal rim margin was placed tangent to the temporal side of the image.

The sensitivity was optimised for the temporal neuroretinal rim while avoiding oversaturation. One investigator performed all the measurements.

Images were included if they had an average DC value of ⩾175 at the retina, and no saccades greater than 30 ms.

A 10×10 pixel measurement window was placed on the temporal neuroretinal rim of a central alignment image, avoiding major vessels.[2] The image was then printed onto a transparency and placed on the computer monitor. The window was exactly reproduced for all images (fig 1). One hundred individual pixel values were extracted for each window. The upper and lower 25th percentile of flow values were excluded to reduce the outliers that may have been caused by, for example, heart beat or level of image saturation. The mean flow values of the remaining interquartile range were analysed using ANOVA to compare between alignments and across the two visits ($p<0.05$). The intraclass correlation coefficients (ICCs) and the coefficients of repeatability (CoR) were calculated to assess concordance and repeatability.[13]

RESULTS

There was no significant difference between alignments or visits for the mean ocular capillary blood flow (table 1). The mean and median of the original 100 pixel values within the 10×10 pixel window were compared to the mean of the interquartile range. There was a significant difference in the means of all alignments and visits ($p<0.001$). There was no significant difference for the median for central alignment (visit 1; $p = 0.13$; visit 2; $p = 0.53$).

The mean of the differences (MoD), CoRs, and ICCs for the mean of the interquartile range of flow values between visits are listed in table 2. Central alignment had the smallest MoD (4.3) and CoR (31.5) and temporal alignment had the largest MoD (81.6) and CoR (256.7).

These results were supported by the ICCs which were ''almost perfect'' at 0.83 for central, ''fair'' at 0.34 for nasal, and ''moderate'' at 0.42 for temporal alignment.[14]

DISCUSSION

We were interested in finding the most repeatable method for the measurement of ocular capillary blood flow of the neuroretinal rim. In particular we wanted to investigate the assertion that decentration of the optic nerve, such that the temporal aspect of the optic nerve head was positioned tangential to the edge of the image, improved the repeatability of the HRF measurements.[11] We found that the central alignment technique, with careful consideration of focal plane, image quality, and detector sensitivity, gave the most repeatable results when compared to nasal and temporal

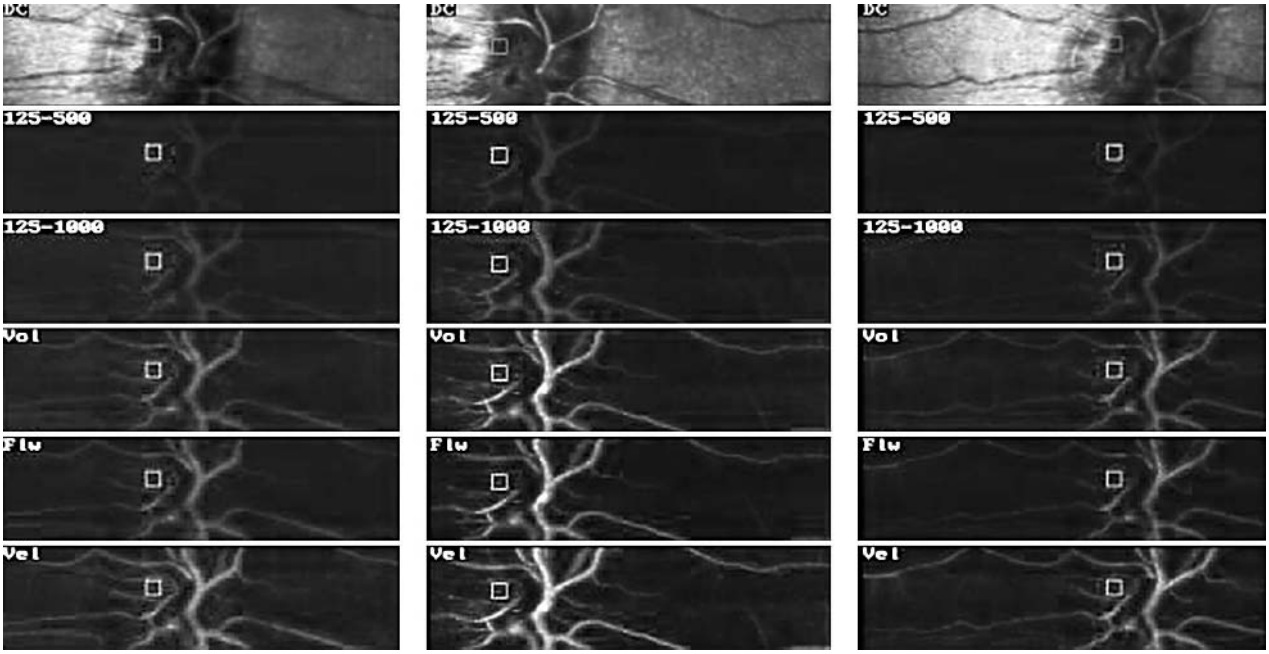

Figure 1 Heidelberg retina flowmeter measurements were performed using three different methods of alignment: central, temporal, and nasal. The 10×10 pixel window was exactly aligned for three different methods.

Table 1 The mean and standard deviation (SD) of the middle 50% of ocular capillary blood flow values at two visits

Visit	Alignment	Range of means (group mean)	Range of SDs (group SD)
1	Central	91.6–458.9 (230.8)	82.7–272.1 (125.9)
	Nasal	62.0–422.3 (251.5)	89.8–255.4 (91.8)
	Temporal	23.9–248.6 (154.9)	48.4–212.5 (84.7)
2	Central	97.4–469.8 (235.1)	84.9–276.1 (132.9)
	Nasal	73.8–419.9 (228.3)	78.6–165.8 (118.7)
	Temporal	41.1–559.3 (236.5)	71.0–184.0 (156.8)

alignment techniques. The difference in results was predictable given the methodologies used. Jonescu-Cuypers *et al*[11] measured the entire rim using a focus setting that was optimal for the retina, rather than the rim tissue, but their temporal alignment technique measured the rim with increased photodetector sensitivity compared to their central alignment technique.

In the majority of previous ocular flow studies using the HRF, the image was focused on the peripapillary retina when analysing the neuroretinal rim. Implicit within such a methodology is that the area of interest—that is, the neuroretinal rim, will be outside the optimal focal plane for the flowmetry measurements, a critical aspect of the technique.[2 11 15] A recent study by Hafez *et al*[16] confirmed that the location of the focal plane has an impact on the measured flow values and that the HRT images were useful in finding the optimal focal plane for the flowmetry of the neuroretinal rim. Also image saturation is a limiting factor for the operator that helps to acquire more repeatable images. The large difference between visits for the temporal alignment technique was consistent with the inherently large variability most likely caused by difficulties in judging image saturation. It has previously been suggested that two factors that decrease the variability of the neuroretinal rim flow values are photodetector sensitivity adjustment and the inclusion of single pixel values in the flow analysis.[11 17] We suggest image focus and image saturation as additional factors.

Different attempts have been made to increase the sampling size of the flow values to reduce the variability of the measurements.[6 9 18] The pointwise analysis of the entire

Table 2 Mean of differences (MoD), coefficient of repeatability (CoR), and intraclass correlation coefficient (ICC) of ocular capillary blood flow values for each method of alignment

Alignment	MoD (SD)	CoR (mean effect)	ICC of flow values
Central	4.3 (16.1)	31.5 (235.1)	0.83
Nasal	−23.2 (119.7)	234.6 (228.3)	0.34
Temporal	81.6 (131.0)	256.7 (236.5)	0.42

image has been considered to provide additional power for the analysis of flow values and increase the long term reproducibility.[15 16] However, it may not be appropriate for smaller areas of interest. Additionally, when the retinal plane is in focus and the rim area remains unfocused and dark, the proportion of outlier flow values from the rim that are excluded from the analysis would be high. Hosking *et al*[8] introduced a strategy in which a 10×10 pixel frame was repositioned within a 15×15 window and 36 mean flow values were extracted and ranked to find the highest and lowest local values of blood flow. The intrinsic effect of saturation and pulse on the maximum and minimum flow values is not excluded in this method.

We used the interquartile range for flow values within the measurement window to reduce the effect of variation as a result of underexposure and oversaturation of the image, and the cardiac cycle.[19 20] The HRF software generates the mean of data points within a measurement window. However, we found that in many cases the lower quartile of values consisted mainly of zeroes. Therefore the data were not Gaussian in distribution. The mean of such a distribution would result in a biased description of central tendency. In these cases the median is a better central descriptor. However, the median does not take into account the spread of data around the central measure. The interquartile range is a better descriptor of the spread and the central tendency of the data. The skewness of data was reduced by removing the zeroes, very low and very high values, as a result of undersaturation and oversaturation, and/or heart beat. Therefore, the distribution of the flow values was more Gaussian and the mean of the interquartile range and the median of the whole 100 data points were not significantly different. We therefore recommend that if a single average value for a measurement window is required then the median average of all pixels would be appropriate, but that if an estimate of the variance within the data is required the interquartile range and its mean would be more appropriate.

The ''automatic full field perfusion image analyser'' (AFFPIA) is a novel tool designed for the analysis of the retinal microvasculature flow.[21 22] It provides an overall analysis of the rim and analysis of the average flow within a measurement window after correcting for invalid DC values, large vessels, and saccades. However, we were unable to use AFFPIA for this study as it does not provide the flow values for each pixel within a measurement window, and does not permit the accurate placement of a measurement window on subsequent images of the same optic nerve head.

Our protocol for assessment of the capillary blood flow in a small area of the neuroretinal rim incorporates three essential aspects including previous imaging with the HRT to establish precise focus of the rim and peripapillary retina; central alignment of the optic nerve head during image acquisition; and point by point analysis of the interquartile range of flow values within the measurement window.

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