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The assessment of potential vessel segmentation pitfalls in the analysis of blood flow velocities by the Retinal Function Imager

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Abstract

Purpose—The purpose of our study was to investigate the potential pitfalls linked to different vessel segmentation methods when using the built-in software of the Retinal Function Imager (RFI) for the analysis of retinal blood flow velocities (BFVs).

Methods—Ten eyes of nine healthy subjects were enrolled in the study. Retinal blood flow measurements were obtained with the RFI device with a 20° field of view imaging. The same grader segmented the retinal vasculature using the RFI software in two sessions with segments ranging from 50 to 100 pixels (“short segments”) or 100–200 pixels long (“long segments”). The blood flow velocities for the arteriolar and venular system were calculated and the percentage of excluded vessel segments with high coefficients of variation (>45%) was recorded and compared by paired t-test. Spearman correlation was used to analyze the link between the two measurements by the two vessel segmentation methods.

Results—The number of analyzed vessel segments did not differ significantly in the two groups (28.6±2.6 short and 26.7±4.6 long segments, respectively), while the percent of acceptable segments was significantly higher in the long segment group (65.2±11.4% vs 85.2±5.87%, $p=0.001$). All subjects in the short segment group had more than 15% of vessel segments rejected, while in the long segment group only three subjects had a rejection rate of 15% (16.7%, 18.7% and 28%). Both arteriolar and venular velocities were lower in the short segment group, although it reached significance only in the case of the arteriolar velocities (3.93±0.55 vs 4.45±0.76 mm/s, $p=0.036$ and 2.95±0.56 vs. 3.17±0.84 mm/s, $p=0.201$ for arterioles and venules, respectively). Only the venular velocities showed significant correlation ($p=0.003$, $R^2=0.67$) between the two groups.

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Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

Conclusions—Our results suggest that BFV measurements by the RFI may be affected by the segment length and therefore care should be taken when choosing the vessel segment lengths used during the analysis of RFI data. Long segments of 100-200 pixels (400-800 μm) seem to provide more robust measurements which can be explained by the analysis methodology of the RFI device.

Keywords

retinal blood flow; Retinal Function Imager; reproducibility; image analysis

Introduction

Retinal and choroidal circulation has been evaluated invasively and noninvasively by several techniques, including video fluorescein angiography [1], ultrasound flowmetry [2], the blue-field simulation technique [3] and scanning laser Doppler flowmetry [4, 5, 6].

The Retinal Function Imager (RFI) is a new optical imaging device that performs direct, noninvasive qualitative and quantitative imaging of the retinal blood flow velocities (BFVs) in the secondary and tertiary branches of the main retinal arteries and veins using a stroboscopic fundus camera. [7] The blood flow velocity is calculated by measuring the movement of red blood cells within the retina taken with red-free (green) illumination in a short interval less than 140 ms. Besides the BFV measurements the RFI can provide the capillary perfusion map of the macula without the need of any contrast agent. The usefulness of RFI measurements has been shown in various pathologies involving the retinal circulation such as diabetic retinopathy (DR) [8, 9, 10], age-related macular degeneration (AMD) [11, 12], central serous chorioretinopathy [13], idiopathic juxtafoveal teleangiectasia [14] and even in severe hyperlipidemia [15].

Although the RFI is widely used for the measurement of BFV, we have found that there is some controversy in the literature about the methodology used to obtain these measurements regarding the field of view, the vessel segment lengths (VSLs) and other technical details of the analysis. Therefore, the purpose of this study was to review the relevant literature that has used different approaches for the BFV measurements using the RFI, and then investigate the potential pitfalls as a result of assuming two different vessel segmentation methods when using the built-in software of the Retinal Function Imager (RFI) for the BFV analysis.

Methods

Ten eyes of nine healthy subjects were enrolled in the study. All patients underwent RFI scanning at the Bascom Palmer Eye Institute, Miami, USA. The protocol was approved by the Institutional Review Board at the University of Miami Miller School of Medicine. All subjects gave written informed consent before the examinations. We enrolled 4 males and 5 females with ages ranging from 21 to 49 years (mean 37.9 ± 9.7 years). Ophthalmic exclusion criteria were ocular media opacity, any previous intraocular surgery except uneventful cataract extraction at least 6 months prior to enrollment and myopia of more than 6 diopters.

The RFI system (RFI-3005, Optical Imaging, Rehovot, Israel) is based on a standard fundus camera extended by a customized stroboscopic flash lamp system and a digital camera. [7]

The blood flow velocity was measured by quantifying the movement of hemoglobin-containing erythrocytes as hemoglobin is a natural, high-contrast chromophore that marks the flow of blood and thus facilitates the calculation of the blood flow velocities. [16] A green (“red-free”) interference filter is used for the illumination with transmission centered at 548 nm at a bandwidth of 17nm. The fundus camera employs a 60-Hz, $1,024 \times 1,024$ pixel digital imaging system, delivering 8 consecutive flashes with an interval of typically 17.5 milliseconds to generate 8 consecutive fundus images. Patient heart beats are being monitored by a probe attached to the fingertips or the earlobe of the subjects in order to synchronize image acquisition at a given period of the cardiac cycle and thus neutralize the effects of pulsation of arterial blood flow velocity.

We obtained three or more good-quality sessions for each eye by the same experienced photographer with at least five good images per session that were selected for statistical analysis. Images were evaluated for optical quality, exposure and focus. The digital images were stored and analyzed using the built-in software of the RFI device. The fundus vessels were traced using the RFI's custom-built vessel detection algorithm. Secondary and tertiary vessels were traced, avoiding the intersections of vessels in order to avoid optical interference between arterial and venous flow in these regions. Care was taken to draw the segments only until the bifurcation or branching of the secondary vessels and thus avoid measurement bias in vessels with different diameters. (Figure 1.) Preferentially all vessels in the image were used to mark the vasculature, i.e. no secondary or tertiary vessels were left out of the analysis and care was taken to highlight an equal number of secondary and tertiary vessels. Blood flow velocity measurements were calculated by using a path-constrained cross-correlation technique giving results for blood flow velocity in arteries and veins along with a value for total velocity. [7]

The same grader segmented the retinal vasculature using the RFI software in two independent sessions with segments of 50-100 pixels or 100-200 pixels long (“short segment” and “long segment technique, respectively). In both segmentation procedures the same set of image sessions were linked in order to maintain identical imaging background of the analyses.

The variability of the segment velocity measurements between different sessions was also calculated and used as a reliability measure. If a coefficient of variance (SD/mean) exceeded 45% the vessel was excluded from the analysis. [17] This was mostly due to poor focusing, poor illumination or artifacts near the edges of the images. An image was considered to be of “poor” quality if the number of rejected vessels exceeded 15%, while “bad” quality was assumed when it exceeded 33%. Images with less than 15% rejection rate were considered “good”. [9, 18]

The blood flow velocities for the arteriolar and venular system, the percentage of excluded vessel segments and the difference in the coefficients of variability (including all rejected and non-rejected segments) were calculated and compared by paired t-test. Spearman correlation was used to compare arterial and venous flow measurements by the two methods. The level of significance was set at $p < 0.05$.

Results

The number of analyzed vessel segments did not differ significantly in the two groups (28.6 ± 2.6 short segments vs. 26.7 ± 4.6 long segments), while the percent of acceptable segments was significantly higher in the long segment group ($65.2 \pm 11.4\%$ vs $85.2 \pm 5.87\%$, $p=0.001$). All subjects in the short segment group had more than 15% of vessel segments rejected (5 “poor” and 5 “bad” images), while in the long segment group only three subjects had a rejection rate of 15% (all falling into the “poor” category). (See Table 1.)

Both arteriolar and venular velocities were lower in the short segment group, although this difference reached significance only in the case of the arteriolar velocities (3.93 ± 0.55 vs 4.45 ± 0.76 mm/s, $p=0.036$ and 2.95 ± 0.56 vs. 3.17 ± 0.84 mm/s, $p=0.201$ for arterioles and venules, respectively). (see Table 2.) There was a significant correlation between the two measurement groups only in the case of the venular velocities ($p=0.003$, $r=0.83$) and not the arteriolar measurements ($p=0.12$, $r=0.53$).

The mean of coefficients of variability (including all rejected and non-rejected vessel segments) was significantly lower in the long segment group (0.40 ± 0.21 vs. $0.29 \pm 0.18\%$, $p<0.001$). The scatterplot of the mean velocities in all segmented vessel segments versus their standard deviation (SD) showed that the long segment methodology provided not only less rejections but also more robust measurements in the low velocity ranges below 3 mm/s. (Figure 2.) In the short group 100 measurements (34.96%) were rejected from a total of 286 segments while in the long segment group it was 40 measurements (14.98%) out of a total of 267 segments.

Discussion

The RFI device has been introduced less than a decade ago with noninvasive imaging capabilities that are very attractive for the clinical evaluation of retinal blood flow velocity in the arterioles and venules of the retina. The device also offers the possibility to image the capillary perfusion map with the foveal avascular zone along with the option to measure vessel oxygenation and even metabolic mapping of the retinal tissue. [7, 19] Although the last two modalities are currently not supported in the commercially available devices, the RFI has received significant attention to describe various vascular pathologies of the retina [14, 20-24] and to compare or correlate its output with other imaging devices, like fluorescein angiography [19, 25] or optical coherence tomography.[8]

Images of the retina provided by the RFI are very similar to the ones obtained with the standard fundus imaging as they are based on a standard Topcon (TRC-50DX) digital fundus camera (Topcon Inc., Nagoya, Japan) This means that there are three field of view (FOV) settings available for imaging at 20, 35 and 50 degrees, offering different levels of magnification. According to the user manual of the RFI device, the 20 degree high-resolution setting is suggested for the measurement of BFV with an approximately 4.3 micron/pixel resolution. [26] Also, the user manual recommends the segmentation of approximately 100-200 pixel long vessels segments which thus corresponds to 400-800 microns in actual length by 20 degree imaging. It should be noted that the vessel diameters

cannot be measured with by the built-in software of the RFI and therefore flow measurements are not obtainable with the device.

We reviewed the available literature in the field of BFV measurements using the RFI and found that despite the above, clinical studies performed so far were employing a number of different settings for BFV analysis. Some studies are reporting the use of either 20 or 35 degree FOV images, while some papers are not giving a direct description of the FOV setting used, it can only be speculated from the figures provided by the authors. Similarly, we found controversy reporting the methodology used to calculate the retinal blood flow velocities as some authors are describing the use of segments of less than 100 pixels while in other reports various lengths are being used.

In order to review the methodological approaches used by various groups we performed a web-based search in PubMed and Google Scholar to identify all studies published so far that are based on the RFI and BFV measurements. We noted the following details of the methodologies used in these studies:

- The fact of reporting the field of view (yes/no). In case it was not reported in the methodology of the paper the figures of the paper were used to describe the FOV;
- The fact of reporting the vessel segment lengths employed in the study (yes/no); if no specific statement is available in the methodology of the paper the figures of the paper were used to describe the length, being defined as short, long or mixed. The latter is used when there are obviously very short and long segments mixed on the image(s) provided in the publication;
- Whether the vessel segment markings respect the vessel crossings where the measurements may be imprecise. We note that this fact was only marked in Chablani et al. 2013 [27]) but in the cases of all other reports it is an observational description based on the figure(s) published;
- Whether only a few vessels are marked around the fovea or an attempt is given to mark most secondary and tertiary vessels in the foveal region. This may be of importance in providing a balanced measurement profile along all secondary and tertiary retinal vessels. This is not reported elsewhere but it is our assumption based on our experiences.

The results of the literature overview are summarized in Table 3. Of the available 14 clinical studies using the RFI for blood velocity measurements only four stated clearly in the methodology what FOV was used in the study, in the rest it needed to be determined by ourselves. Four studies were using 35 degrees images and only two of these noted the vessel segment length used for the analysis. It is of interest that Chablani et al [27] refers to VSL of <100 microns, while Burgansky-Eliash et al. [10] are referring to an optimal length of 100-150 microns which correspond to 25 and 38 pixels, respectively. As VSL was not discussed in the methodology of most papers it needed to be determined by the published images which was not available in two papers, it tended to be long segments in four, mixed in five and short in two cases. (See Table 3.) In four papers we were not able to extract whether the crossing were avoided, while in two papers the images contained the analysis of

vessel crossings; the rest of the papers avoided it. (See Table 3.) Finally, in four cases only a low number of vessels were marked according to the imagery without any explanation; this information was not available in two cases. (See Table 3.)

Based on the misunderstanding in the currently available literature, our current study aimed to investigate whether there are any differences between the analysis methods using short and long segment lengths for the analysis of retinal blood flow velocity with a 20 degree FOV.

Our findings show that there may be substantial differences in the RFI velocity measurements obtained by various segment lengths. Long segments of 100-200 pixels (400-800 microns) are providing measurements with significantly lower coefficients of variance and thus more reliable results for velocity analysis. There were significantly less rejections in the long segment group, with 70% of eyes falling in the range of good quality with less than 15% rejections while no eyes reached this criterion in the short segment group. Also, the long segment methodology showed to be more robust overall and giving more reliable measurements in the slower velocity ranges below 3 mm/s. One potential explanation for these observations could be the fact that motion contrast information is not uniform along the vessels, i.e. a reliably readable signal may be present only in a subsegment of a vessel. A short measurement path along a vessel may exclude this portion with high motion contrast and thus yield to a large variability of velocity readings.

Also, the velocities obtained by the short segment method were lower, reaching significance in the arteriolar measurements. In contrast, the arteriolar measurements obtained by the short and long segment methods did not show significant correlation while the venular measurements showed a significant, high correlation. These trends are difficult to explain by our currently available information in the field but are pointing towards the significant differences in measurements between the quantification methodologies.

Despite the promising results above, our study has a few limitations. First, it might have been advisable to perform imaging with both 20° and 35° FOV in order to compare the velocity readings by the two modalities. However, it would have been out of the scope of our study which was primarily aimed at refining our analytical approach of the 20° imaging. Second, we believe the number of eyes involved in the study was sufficient for our purposes but undoubtedly a larger cohort could have yielded a more refined view of the differences. Finally, our results might be biased by any confounders associated to the measurements such as background noise of vessel segment velocity readings or other factors that are currently unknown to us.

In conclusion, the RFI is a promising tool for the understanding of retinal microcirculation but it would be advisable to use a universal methodological approach to the image analysis. Future developments may enable a more robust, fully automated vessel tracing of retinal vessels with more reliable velocity readings and thus potentiating the clinical use of functional retinal imaging of blood flow in health and disease.

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Figure 1.

Assessment of retinal blood flow velocities by the retinal function imager using long and short vessel segments (A and B, respectively). Red segments denote arteries, purple segments denote veins. Note that there are no segmentation lines drawn where vessels are overlapping and that bifurcations and branching are also respected for vessel segmentation.

Figure 2.

Scatterplot of the standard deviation (SD) versus the mean velocities in all segmented vessels obtained in the short (A) and long (B) segment groups. The dashed grey line represents the 45% rejection threshold in the coefficient of reproducibility. All points located above this line are velocity readings that exceed this value and are thus rejected from analysis. In the short group 100 measurements (34.96%) were rejected from a total of 286 segments while in the long segment group it was 40 measurements (14.98%) out of a total of 267 segments.

Table 1

Clinical characteristics of the eyes and subjects involved in the study and their individual measurements obtained with the “short” and “long” segment approaches. Vart and Vvein: arteriolar and venular velocities, respectively; N: number of segments; N<45% number of segments with a coefficient of variation <45%; %: the percentage of unrejected segments, the color code can be seen at the bottom of the table; TotN: total number of segments (arteriolar and venular).

Subject	OD/OS	Short segment										Long segment									
		V art (mm/s)	N	N<45%	%	V vein (mm/s)	N	N<45%	%	TotN	%	V art (mm/s)	N	N<45%	%	V vein (mm/s)	N	N<45%	%	TotN	%
1	OD	3.17	18	13	72.2	2.24	13	9	69.2	31	71.0	3.25	14	13	92.9	2.43	12	10	83.3	26	88.5
2	OD	3.78	12	6	50.0	2.71	14	5	35.7	26	42.3	4.80	11	10	90.9	3.49	21	16	76.2	32	81.3
3	OD	3.49	16	11	68.8	2.82	15	5	33.3	31	51.6	4.25	14	11	78.6	2.60	16	15	93.8	30	86.7
4	OS	4.18	12	7	58.3	3.84	16	11	68.8	28	64.3	4.86	10	7	70.0	4.45	11	11	100.0	21	85.7
5	OS	4.39	12	5	41.7	2.48	17	15	88.2	29	69.0	4.51	8	8	100.0	2.47	12	11	91.7	20	95.0
6	OS	3.62	16	9	56.3	3.75	17	12	70.6	33	63.6	4.73	10	7	70.0	3.68	12	12	100.0	22	86.4
7	OS	3.61	15	12	80.0	2.33	12	9	75.0	27	77.8	3.14	15	12	80.0	2.36	14	13	92.9	29	86.2
8	OD	4.12	16	11	68.8	3.35	12	9	75.0	28	71.4	5.68	11	9	81.8	4.58	14	9	64.3	25	72.0
9	OD	3.84	10	6	60.0	3.04	14	13	92.9	24	79.2	4.47	16	12	75.0	2.94	14	13	92.9	30	83.3
	OS	5.14	10	3	30.0	2.95	19	15	78.9	29	62.1	4.76	19	16	84.2	2.65	13	12	92.3	32	87.5

% Color codes: 85-100 (good quality) 66-85 (poor quality) <66% (bad quality)

Table 2

Descriptive characteristics of the “short” and “long” segment methods groups.

	Short segments	Long segments	p
Arterial velocity (mm/s)	3.93 (0.55)	4.45 (0.76)	0.036
Arterial segments (n)	13.7 (2.8)	12.8 (3.4)	NS
Acceptable arterial segments (%)	58.6 (15.0)	82.3 (9.9)	0.004
Venous velocity (mm/s)	1.95 (0.56)	3.17 (0.84)	NS
Venous segments (n)	14.9 (2.3)	13.9 (2.9)	NS
Acceptable venous segments (%)	68.8 (19.7)	88.7 (11.1)	0.014
Total segments (n)	28.6 (2.63)	26.7 (4.55)	NS
Acceptable vessel segments in total (%)	65.2 (11.4)	85.2 (5.87)	0.001
Coefficients of variability (all segments in group) (%)	0.40 (0.21)	0.29 (0.18)	0.000

Values are means (standard deviation). NS: not significant.

Table 3

Overview of the image analysis methodologies reported in clinical studies employing the RFI device.

Authors [ref.]	FOV reported (Y/N)	FOV (20°/35°/NA)	VSL reported (Y/N)	S/L/Mixed segments	A/V crossings marked (Y/N)	Most vessels marked (Y/N)	Pathology studied
<i>Arbel et al. [23]</i>	N	20°	N	Long (mixed)	N	Y	Slow coronary flow (n=28)
<i>Barak et al. [11]</i>	N	20°	N	Long (mixed)	N/A	N	wet AMD after Avastin (n=8)
<i>Beutelspacher et al. [13]</i>	N	35°	N	Mixed	N	Y	Central Serous Chorioretinopathy (n=12)
<i>Birger et al. [15]</i>	N	20°	N	Mixed	N	N	severe hyperlipidemia (n=1)
<i>Burgonsky-Eliash et al. [9]</i>	Y	20°	N	Mixed	N	Y	NPDR (n=58 NPDR / 51 Healthy)
<i>Burgonsky-Eliash et al. [12]</i>	N	20°	N	Long (mixed)	N	N	wet AMD (n=63 wet AMD / n=53 Healthy)
<i>Burgonsky-Eliash et al. [10]</i>	N	20°	Y	Short	N	Y	Physiological parameters in healthy (n=67)
<i>Chablani et al. [27]</i>	Y	35°	Y	Short	Y	N	Reproducibility (n=18)
<i>Gutfreund et al. [18]</i>	N	20°	N	Long (mixed)	N	Y	Metabolic syndrome (n=20 MetS / 21 Healthy)
<i>Izhaky et al. [19]</i>	N	20°	N	Mixed	N/A	Y	review with an image
<i>Landa and Rosen [14]</i>	Y	35°	N	N/A	N/A	N/A	Idiopathic Juxtafoveal Teleangiectasia (n=10)
<i>Landa Jangi et al. [24]</i>	Y	20	N	Long (mixed)	N	Y	Healthy subjects (n=27 subj/54 eyes)
<i>Landa et al. [20]</i>	N	N/A	N/A	N/A	N/A	N/A	DME (n=39)
<i>Landa et al. [8]</i>	N	35°	N	Mixed	Y	Y	Various (n=17 of 14 subjects)

Please note that in cases where the Field of View (FOV) was not reported, it was described by the figure(s) published in the given study. Y: yes, N: no; N/A: not available, VSL: vessel segment length, S: short segments (<100 pixels or <400µm), L: long segments (100-200 pixels or 400-800µm), "Long (mixed)": mostly long segments with some short segments visible in the illustration shown in the reference. A/V crossing: arterio-venous crossing, AMD: age-related macular degeneration, NPDR: non-proliferative diabetic retinopathy, DME: diabetic macular edema.