Title Page

Title

Validation of concentric rings method as a topographical measure of retinal non-perfusion in ultra wide-field fluorescein angiography

Short Title

Concentric rings method in wide-field image analysis

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Structured Abstract

Purpose:

To validate the use of concentric rings as a method to measure topographical area of retinal non-perfusion in ultra wide-field angiography with the ischemic index method, which is the most frequently used method to measure non-perfusion in ultra wide-field angiography.

Design:

Validation study and reliability analysis

Methods:

Setting: Single centre study performed at National Institute for Health Research Moorfields Biomedical Research Centre, London, United Kingdom.

Study Population: 28 ultra wide-field angiogram images of eyes with central retinal vein occlusion.

Observation Procedure: The concentric rings method consist of six macula centered concentric rings divided into 12 segments each. Each image was graded by five graders using both the concentric rings and the ischemic index methods.

Main Outcome Measures: Agreement between the two methods was calculated using the intraclass correlation coefficient. Intertest agreement, intergrader agreement, test-retest reliability and the time taken to grade using these two methods were compared.

Results:

The intertest agreement between concentric rings method and ischemic index method was 0.965. The intergrader agreement was 0.910 for concentric rings method and 0.898 with the ischemic index method. The test-retest reliability was 0.975 for the rings and 0.979 for the ischemic index. Average grading time per image was 187s and 297s for concentric rings method and ischemic index method respectively, p=<0.001.

Conclusion:

The concentric rings method has an ‘almost perfect’ intergrader agreement and intertest agreement with the ischemic index method with a shorter grading time.

(234 words)

Text

Introduction

Fundus fluorescein angiography (FA) remains the gold standard imaging tool for the measurement of retinal perfusion status in retinal vascular diseases. Previous seminal research papers such as the Early Treatment Diabetic Retinopathy Study[1] and the Central Vein Occlusion Study[2] classified retinal perfusion status based on the montage of overlapping 7-field FA images. However, 7-field imaging only allows visualisation of approximately a third of the retinal surface area. The acquisition of these 7-field images are also technically challenging and require well-trained photographers, well-dilated pupils and good patient co-operation to undergo multiple flash photography. Furthermore, a single 7-field montage image is formed of images acquired at different FA phases. These limitations have been overcome by the introduction of ultra wide-field imaging. The ultra wide-field imaging has a shorter image capture time and allows visualisation of significantly larger areas of the retina using less number of images compared to the conventional 7-field imaging. For example, the ultra wide-field image obtained by Optos Plc, Dumfermline, Scotland, United Kingdom covers approximately a 200 degree field of view in a single image compared to the 30 to 50 degree conventional fundus cameras and is now regularly used in clinical practice[3–9].

The conventional measurement of non-perfusion in 7-field FA is in total number of disc areas[2]. The SCORE study group applied a grid consisting of four concentric circles with nine subfields over the macula to better quantify the percentage involvement of capillary loss, blood, edema and fluorescein leakage in each subfield and the results were converted to disc areas[10]. With the advent of ultra wide-field imaging, an ischemic index method has been developed to quantity non-perfusion by taking into account the much larger retinal area visualized[3]. In brief, the ischemic index method is measured as a perfused ratio or percentage of the total area of perfused retina to the total area assessed by manually delineating the boundaries of the retina that are in focus and also the perfused retina[3–7]. Other investigators have quantified the total area of non-perfusion by manually or software assisted delineation of the non-perfused boundaries without taking into account the total area visualized and reported the area of non-perfusion in pixels or as total disc areas[11,12]. Marking the boundaries of non-perfusion is a labor intensive exercise and may be subject to variability. Although there has been a significant increase in the use of the ischemic index method, the intergrader agreement of the ischemic index method has not been formally reported. However, Wessel et al. have reported a Lin Correlation coefficient of >0.80 for measurement of areas of ischemia in ultra wide-field FA by manually delineating the boundaries of non-perfused retina and this represents an almost perfect agreement[11].

The drawback of the ischemic index method is that this measurement only provides a quantification of the total area of non-perfusion and does not give a topographical representation of the location of ischemia.

We believe the introduction of concentric rings similar to that used in the SCORE study will better quantify the area of peripheral retinal non-perfusion in ultra wide-

field FA images and enable us to qualify the location of ischemia. The concept of superimposing grids and templates to assess FA images is not a novel method. Templates have also been used to assess macular non-perfusion[10], peripheral changes in age related macular degeneration[8,13] and retinal non-perfusion using the Manchester Grid[14].

The objective of our study is to assess the feasibility of measuring non-perfusion in ultra wide-field FA, using a template of concentric rings centered on the fovea. We assessed (1) the agreement between this newly proposed method with the ischemic index method, (2) the intergrader agreement, (3) the test-retest reliability and (4) the time taken to grade non-perfusion using both methods.

Methods

This validation study was performed in the National Institute for Health Research Moorfields Biomedical Research Centre and the Institute of Ophthalmology, University College London, United Kingdom. Approval for retrospective anonymized image analysis from the institutional review board of Moorfields Eye Hospital was obtained and the study was conducted in accordance to the tenets of the Declaration of Helsinki.

Image acquisition

The ultra wide-field FA images of consecutive patients with a diagnosis of central retinal vein occlusion that were imaged with Optos 200TX (Optos Plc., Dumfermine, Scotland) ultra wide-field system were reviewed. Only images that were acquired according to local Optos FA protocol and were not obscured by media opacities were included. The images also had to be of sufficient clarity, defined as the ability to clearly discern perfused from non-perfused capillaries. Patients with previous panretinal photocoagulation treatment were excluded. Ultra wide-field FA images were obtained using a standard protocol after intravenous bolus infusion of 5ml of 20% fluorescein sodium. The protocol consisted of acquiring images in transit phase (up to 45 seconds), arteriovenous phase (1-2 mins), and late frames at 3-4 minutes and 7-8 minutes. A single investigator (L.N.) identified the best FA image in the arteriovenous phase from the FA series of each patient. A correction factor was applied for the flattening of the 3-dimensional image to a 2-dimensional image using a non-commercial research tool under development by Optos.

The Concentric rings method

Development and description of the template of concentric rings

The concentric rings method consist of seven fovea centered concentric circles (Figure 1). The innermost circle is centered on the fovea and is one disc diameter. This area is not used in the grading method as the foveal avascular zone is excluded from grading. The second circle, represents the macular ring with a radius of 2.5 disc diameters centered on the fovea. Each of the next five subsequent rings are placed at increments of 2.5 disc diameters in radius from the foveal center, therefore, the radii of these inner, inner middle, outer middle, outer and far outer peripheral rings are 5 disc diameters, 7.5 disc diameters, 10 disc diameters, 12.5 disc diameters and 15 disc diameters from the center. This results in the diameter of each ring

increasing by 5 disc diameters with the central macular ring also being 5 disc diameters in diameter. Each of these rings is further subdivided into 12 equal segments with each segment subtending 30 degrees at the center.

We applied a mathematical formula with the assumption that the mean axial length is 24mm to calculate the size of the concentric rings. We excluded 2mm from this to account for the cornea and part of the anterior chamber. In our model eye, the radius is 11mm (22mm in diameter), and therefore the full circumference would be 69.1mm (π=3.142). Optos is able to image up to 200 degrees of the retina and we used this to calculate the average diameter of retina obtained in a single central image. This was calculated to be 38.4mm. Using the disc diameter as 1.8mm, this would mean, the diameter of the central image will be 21.3 disc diameters. A diameter of 21.3 disc diameters will result in the need for a macular + three further rings with some involvement of the fourth ring. Taking into account future applications such as steering protocols, we made the assumption of gaze to be 45 degrees in each direction. This would mean, the total diameter potentially imaged is 290 degrees (200+45+45). This would then result in a total diameter of 55.7mm which equates to 30.9 disc diameters and thus, six rings (macular + five rings) are required on average.

The rings were then superimposed on each of the selected ultra wide-field FA images using Photoshop CS4 (Adobe Inc. San Jose, CA). As optic disc size varies between individuals, the innermost circle is first placed at the optic disc to individualise the disc area and the concentric rings are then sized proportionately. Once completed, the rings were then repositioned to be fovea centered (Figure 2). The concentric rings template can be downloaded from AJO.com as supplemental file 1 and available to use with appropriate citation.

Grading

The images were graded using ImageJ, an open source software by the National Institute of Health (NIH) that can be downloaded from http://imagej.nih.gov/ij/index.html. Using the software, each grader can adjust the brightness, contrast, and magnification to assist in grading. The definition of 'non-perfusion' was adopted from the SCORE study and is characterised by absence of retinal arterioles and/or capillaries and is detected by characteristics such as pruned appearance of adjacent arterioles and a darker appearance of the choroid[10]. The corresponding color fundus image was provided so that blocked fluorescence from retinal haemorrhages is not misjudged as ischemia. 'Perfused' retina was defined by its ground-glass appearance and 'ungradeable' areas were defined as areas where no clear definition of presence or absence of perfusion could be identified and this includes retinal vasculature not in focus and exclusion of 'blurred' areas in the periphery.

The 12 segments in each of the 6 rings totalling 72 segments were graded as one of three outcomes, 'perfused', 'non-perfused' or 'ungradeable' if more than 50% of the segment consisted of any one of the three. If these outcomes are in equal proportion in a segment, the segment is classified as ungradeable. The grading results for each image were recorded using a table (Table 1).

This also allowed a qualitative result of the distribution of ischemia by inputting a subscript of the number of segments judged to be non-perfused in each segment.

The first ring is the macula, M, and subsequent rings numbered 1-5. An example of a recording is $M_2 1_1 2_0 3_4 4_7 5_5$.

This grading can also be converted to a ratio of perfused to total area assessed to resemble the ischemic index by using the table provided (Table 2) which shows the total area in disc areas represented by each segment in each ring.

Although the perfused ratio allows the comparison with ischemic index, the examples given (Figure 3 and Figure 4) shows why the concentric rings method provides more useful information than the ischemic index method. Both Figure 3 and Figure 4 have similar perfused ratios but the location of ischemia is dissimilar. With the concentric rings, the additional notation, $M_0 1_0 2_1 3_3 4_0 5_0$ and $M_2 1_5 2_3 3_0 4_0 5_0$ allows one to picture the distribution of retinal non-perfusion.

The time taken from opening an image file to completion of table 1 was defined as the time required to grade the images and this was recorded.

The ischemic index method

This method has been described previously[3]. It reports ischemia as a ratio of the total area perfused to the total area assessed or gradeable (Figure 5). Images were graded using ImageJ. The total area gradeable was defined as total area whereby the grader was able to grade the presence or absence of perfusion which includes retinal vasculature in focus and exclusion of 'blurred' areas in the periphery. The definition for perfusion and non-perfusion are similar to that used in the concentric rings method. The ischemic index method is reported in a ratio and no units are used. To quantify areas of non-perfusion, in this study, the total number of pixels for the area of non-perfusion is divided by the number of pixels in the 'disc circle' in the concentric rings method to obtain a unit of disc areas.

The area is calculated using the freehand measure image tool in ImageJ. The size in pixels of each area can then be read off using the measure function. The results are then recorded, in pixels, for the total area assessed, perfused and non-perfused areas. Again, the corresponding color fundus image is provided so that blocked fluorescence from retinal haemorrhages is not misjudged as ischemia. Each grader was not required to sum up the total of perfused, non-perfused and gradeable area and was only required to record the size of each island measured. This is because the time taken to calculate the total areas will extend the grading time when compared to the concentric rings method and could be done by entering the measurements into a programme such as Microsoft Excel (Microsoft Corp.) to obtain the results. The grading time was defined as the time taken from opening the image file to completion of the grading process and this was recorded.

Inter-grader agreement

In a single session, five investigators, each with a minimum of four years of ophthalmology experience, graded 28 separate ultra wide-field FA images each, resulting in a total of 140 graded images for the concentric rings method and 140 for the ischemic index method. A Hewlett Packard LE2002xi LCD 20inch monitor with 1600 x 900 pixel resolution was used by each investigator. 14 images were graded with the concentric rings method first, followed by the ischemic index method and the

remaining 14 images were graded in the reverse order. This was done to reduce any learning bias that can affect the time taken for each method in grading the images.

Test-retest reliability

To obtain the test-retest reliability, six weeks after the initial grading session, a second session was completed using the same images. From a possible 140 image-grader combinations, 50 image-grader combinations were selected and re-graded using both methods. All 28 images were re-graded at least once.

Data analysis

The primary outcome was the perfused ratio of perfused to total area assessed - area perfused / (area perfused + non-perfused) - henceforth referred to as the 'perfused ratio' using both techniques. This is represented as an index whereby 0 is no perfused retina and 1 is completely perfused retina. The total area of non-perfused retina was also recorded. These areas were analysed in disc areas. Calculation of the area was performed using Microsoft Excel 2007. Time taken for each image is also recorded and collected for analysis. These parameters - perfused ratio, perfused area, and non-perfused area - were used to study the intertest agreement, intergrader agreement and test-retest reliability.

Statistical analysis

Statistical analyses were performed using MedCalc version 15.4 (MedCalc Software, Belgium). The intergrader agreement was assessed using the intraclass correlation coefficient.The inter-test agreement was analysed using the intraclass correlation coefficient and a Bland-Altman plot. For the test-retest reliability study, we utilised the intraclass correlation coefficient, a Bland-Altman plot and the coefficient of variation for duplicate measurements. The difference between the times taken and means between the two tests were analyzed using the Wilcoxon Matched paired test if normal distribution was rejected using the Kolmogorov-Smirnov test. The significance level was set at 0.05.

An absolute agreement intraclass correlation coefficient with a significance level of 0.05 was set. Single measure agreement levels were used to report the agreement levels. Agreement levels were interpreted according to the guidelines proposed by Landis and Koch[15]. Almost perfect agreement is described for values between 0.81 and 1.00, substantial agreement for values between 0.61 and 0.80, moderate for values between 0.41 and 0.60, fair for values between 0.21 and 0.40, and slight for values under 0.20. The sample size was calculated using the formula by Walter et al[16].

With a type 1 error, α, of 0.05, type 2 error, β, of 0.20, P_0 of 0.8 and P_1 of 0.9, and five graders, the sample size required was 26.1, therefore, 28 images were used in our study. For the test-retest reliability, the calculated sample size was 45.2 and 50 was the sample size decided upon in our study.

Results

Twenty eight images were selected for this validation exercise. Using the concentric rings method, Table 3 shows the mean number of segments with non-perfused, perfused and ungradeable in each ring. The mean area of gradeable retina in the concentric rings method was 447.67 disc areas (range, 117.76 - 843.54). Using the ischemic index method, the mean total area of gradeable retina was 475.38 disc areas (range, 117.79 - 731.02).

The mean perfused ratio for all 28 images from five graders, therefore a total of 140 results for each method, was 0.6588 for the concentric rings method and 0.6700 for the ischemic index method*, p=0.07*. The range of perfused ratios was 0 to 1 for the concentric rings method and 0.0016 to 0.9836 for the ischemic index method. The mean area of non-perfusion was 163.86 disc areas for the concentric rings method and 169.14 disc areas for the ischemic index method, *p=0.658*. The range of non-perfused areas was 0 to 630.26 disc areas and 5.01 disc areas to 636.59 disc areas for the concentric rings method and ischemic index method respectively.

The intertest agreement for 280 results (140 for each method) between the concentric rings method and ischemic index method was 0.965, 95%CI [0.951, 0.975] for the perfused ratio and the intertest agreement for area of non-perfusion was 0.963, 95% CI [0.948, 0.973]. The intertest agreement is also represented in a Bland-Altman plot in Figure 6.

The concentric rings method intergrader agreement for the perfused ratio was 0.910, 95% CI [0.839, 0.954] and 0.924, 95% CI [0.869, 0.960] for area of non-perfusion. The intergrader agreement for the ischemic index method was 0.898, 95% CI [0.809, 0.949] for the perfused ratio and 0.897, 95% CI [0.821, 0.947] for non-perfused areas. The intergrader agreements for number of perfused and non-perfused segments in each ring for the concentric rings were calculated and the results tabulated in Table 4.

Test-retest reliability six weeks apart using intraclass correlation coefficient was 0.975, 95% CI [0.956, 0.986] for the concentric rings method perfused ratio and 0.967, 95% CI [0.942, 0.981] for the area of non-perfusion. coefficient of variation for the test-retest perfused ratio was 9.18% and 17.02% for the area of non-perfusion using the concentric rings method. The mean concentric rings method perfused ratio for session 1 of the test-retest reliability study (n=50) was 0.5711 (range 0.0000 to 0.9394). For the ischemic index method, the test-retest intraclass correlation coefficient was 0.979, 95% CI [0.961, 0.989] for the perfused ratio and 0.973, 95% CI [0.947, 0.985] for area of non-perfusion. The test-retest coefficient of variation is 8.45% for the perfused ratio 15.50% for the area of non-perfusion (Table 5). The test-retest reliability for both methods are also represented in Bland-Altman plots in Figure 7 and Figure 8. The mean time taken for each image was 187± 73s with the concentric rings and 297±219s with the ischemic index, *p*=<0.01.

Discussion

In our study, we topographically quantified peripheral retinal non-perfusion using a template of concentric rings. We have shown that this method has almost perfect agreement with the ischemic index method but requires a shorter grading time. This method also has good intergrader agreement and test-retest reliability.

Ultra wide-field FA is not without its limitations. Firstly, we need to correct for the image projection error from a three dimension spherical object, in our case, the retina, to a two dimension flat image, the fluorescein angiogram[17,18]. Although this technical challenge applies to both grading methods, this correction factor has to be made available within the ultra wide-field imaging software to apply these grading methods in clinical practice.

The current standard of assessing peripheral non-perfusion is the ischemic index. However, its repeatability and intergrader agreement have not been reported. Our study also shows that the intergrader agreement and test-retest reliability of the ischemic index method is also near perfect. However, individually identifying and marking the boundaries of perfused and non-perfused retina does consume a significant amount of time and is not practical in clinical practice or in a reading center for large multicenter studies.

The ischemic index does have another limitation in that it does not provide a qualitative assessment on the location or distribution of ischemia. Despite giving a valuable index of ischemia, information of the pattern of non-perfusion is lacking. It is important to understand the patterns of non-perfusion in retinal vascular diseases[19–21]. The concentric rings method incorporates this qualitative information whilst still providing comparable accuracy and intergrader agreement to the ischemic index method.

The introduction of rings which anatomically identifies the location of ischemia is a promising concept. In our study, the intergrader agreement was almost perfect (substantial for perfused segments in Ring 2 and 3) for the macula, ring 1, 2, and 3, however, the agreement is only moderate to fair for the final ring, ring 5. This is likely due to the image quality in the peripheral retina affecting the subjective interpretation of perfused/non-perfused or ungradeable. This is further substantiated with 10.5 segments in Ring 5 and 7.9 segments in Ring 4 deemed to be ungradeable on average. This does not come as a surprise, as in our model eye, we anticipated that a majority of images will reach only part of the fourth ring. The introduction of steering protocols and a montage of ultra wide-field FA images may also help in improving the visibility of the peripheral retina as postulated in the model eye with the need for Ring 4 and 5. The improved clarity of the peripheral retina expected with steering is likely to improve the agreement in the periphery.

We have included a wide range of ischemia ranging from a perfused ratio of 0.0016 to 0.9836 so as to not limit our findings to only a certain level of ischemia. The intertest agreement between the concentric rings and the ischemic index was almost perfect at 0.965. This suggests that the concentric rings provide a comparable result in measuring the ratio of ischemia as the ischemic index. The intertest agreement for area of non-perfusion was equally good with a score of 0.963.

The concentric ring's intergrader agreement for the perfused ratio is similar to the ischemic index with an 'almost perfect' agreement, intraclass correlation coefficient of 0.910. As for measuring areas of non-perfusion, the concentric rings was again comparable to the ischemic index with an almost perfect agreement of 0.924. Therefore, the intertest and intergrader agreement have shown that the concentric rings is comparable to the ischemic index.

The ischemic index test-retest reliability has not been reported previously. We have shown that the test-retest reliability of the concentric rings method, coefficient of variation of 9.18% is slightly poorer compared to the ischemic index, with a coefficient of variation of 8.45%. This is because the concentric rings are graded as perfused, non-perfused or ungradeable based on more than 50% evidence of either outcome which is at the investigators discretion. Therefore, a single segment difference will lead to larger difference in the retest results compared with the ischemic index which is manually segmented. Despite this, the difference in coefficient of variation between the two methods is only 0.73% for the perfused ratio. The test-retest repeatability is an important factor especially for studies investigating change and progression of non-perfusion and more recently, reperfusion of non-perfused retina[22].

In regards to grading duration, the concentric rings method required significantly less time to complete. An average of 110s less time is needed compared to the ischemic index method. The time taken for the rings also appears to be fairly consistent with a smaller standard deviation of 73s compared with that of the ischemic index method with a standard deviation of 219s. This is due to the fact that the concentric rings method involves grading a total of 72 (12 segments X 6 rings) segments requiring an average of 2.6s per segment. A largely ischemic retina or a near completely perfused retina, the time taken does not differ significantly. This is unlike the ischemic index method which is likely to vary depending on the type of ischemia mentioned. With the ischemic index method, manually delineating perfused and non-perfused retina requires a significant amount of time especially for eyes with multiple islands of ischemia.

The concentric rings method is applicable to other wide-field imaging systems; however, the field imaged by other modalities will require the use of different numbers of rings. The concentric rings can still be used on imaging systems with smaller fields of view as the outer rings are just not used without any effect on quantifying area. The Heidelberg system (Heidelberg, Germany) using a Staurenghi 150 degree contact lens for example can image up to 150 degrees and will therefore require the macular + two further rings.

In conclusion, we have shown that the use of a superimposed template of rings such as the proposed concentric rings method gives a comparable result for measuring retinal non-perfusion in ultra wide-field FA to the current standard method, the ischemic index method. Furthermore, the time taken to grade is significantly shorter by 37% and it gives a qualitative description of the location of ischemia without compromising on the accuracy when compared with the ischemic index method.

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Figure Captions

FIGURE 1: The proposed concentric rings template which is designed to aid the measure of topographical areas of retinal non-perfusion in ultra wide-field fluorescein angiography. The concentric rings consists of a central circle and six rings. The first ring, the macular ring, is 5 disc diameters in diameter (2.5 disc diameters in radius). Each subsequent ring is in 5 disc diameter increments (2.5 disc diameter increments in radii). Each ring has 12 segments subtending 30 degrees to each other with the fovea.

FIGURE 2: An example of the concentric rings method used in an ultra wide-field fluorescein angiogram image. The concentric rings was superimposed onto an ultra wide-field fluorescein angiogram image. The rings were initially centered at the disc and sized such that the central disc circle is the same size as the optic disc. The rings were then repositioned to be fovea centered and can be graded.

FIGURE 3. An ultra wide-field fluorescein angiogram of a patient with predominantly peripheral retinal non-perfusion. In this image, the area of non-perfusion is mainly outside the macula with a perfused ratio of 0.8584 using the concentric rings and recorded as $M_0 1_0 2_1 3_3 4_0 5_0$.

FIGURE 4. An ultra wide-field fluorescein angiogram of a patient with predominantly central retinal non-perfusion. In this image the area of non-perfusion is mainly in the peripapillary area and macula. The perfused ratio of this image was 0.8280 and recorded as $M_2 1_5 2_3 3_0 4_0 5_0$.

FIGURE 5: An example of the ischemic index method. The top image is an ultra wide-field fluorescein angiogram of a patient with central retinal vein occlusion. The bottom image represents the boundaries delineated when determining the ischemic index. The solid line marks the boundaries of the total area gradeable. The areas of non-perfusion is in blue. The ischemic index is calculated using the sum total of perfused retina in pixels, divided by the sum total of gradeable retina in pixels and expressed in ratios or as a percentage.

FIGURE 6. The agreement between the concentric rings method and the ischemic index method for the perfused ratio which is the area of perfused retina divided by the total area gradeable is displayed in a Bland-Altman plot. The limits of agreement are -0.1608 and 0.1383.

FIGURE 7. The agreement between repeated measurements of the perfused ratio which is the area of perfused retina divided by the total area gradeable using the concentric rings method is displayed in a Bland-Altman plot. This plot presents the test-retest reliability of the concentric rings method. The limits of agreement are -0.1637 and 0.1246.

FIGURE 8: The agreement between repeated measurements of the perfused ratio which is the area of perfused retina divided by the total area gradeable using the ischemic index method is displayed in a Bland-Altman plot. This plot presents the test-retest reliability of the ischemic index method. The limits of agreement are -0.1538 and 0.1058.

Table of Contents Statement

AJO-15-680R1, "Validation of concentric rings method as a topographical measure of retinal non-perfusion in ultra wide-field fluorescein angiography"

This article describes the proposal of the concentric rings as a method to measure topographical areas of retinal non-perfusion in ultra wide-field angiography. The authors compared the concentric rings method with the current standard method, the ischemic index and found that the concentric rings method had an almost perfect agreement with the ischemic index, an almost perfect intrergrader agreement and takes significantly less time to complete compared to the ischemic index.

Table1: Table used by each grader in assessing retinal perfusion in ultra wide-field fluorescein angiogram images using the concentric rings method.

	Non-Perfused	**Perfused**	**Ungradeable**
Mac			
Ring 1			
Ring 2			
Ring 3			
Ring 4			
Ring 5			
Total			

Table 2: Conversion to disc areas for each segment in the six rings of the concentric rings method.

	Area per segment (in disc areas)
Macula (Ring 0)	2
Ring 1	6.25
Ring 2	10.42
Ring 3	14.58
Ring 4	18.75
Ring 5	22.92

Table 3: The mean number of perfused, non-perfused and ungradeable segments in each ring using the concentric rings method when assessing ultra wide-field fluorescein angiogram images of central retinal vein occlusion.

	Non-Perfused	**Perfused**	**Ungradeable**
Macula	3.1	8.7	0.1
Ring 1	3.5	8.4	0.1
Ring 2	3.6	7.5	0.8
Ring 3	3.2	5.1	3.7
Ring 4	1.9	2.2	7.9
Ring 5	0.6	0.9	10.5

Table 4. The intergrader agreement for number of perfused and non-perfused segments in each ring of the concentric rings method when assessing ultra wide-field fluorescein angiograpm images of central retinal vein occlusion.

	Non-Perfused (ICC[a] and 95% CI)	Perfused (ICC[a] and 95% CI)
Mac	0.903 (0.840, 0.949)	0.879 (0.804, 0.934)
Ring 1	0.926 (0.875, 0.961)	0.891 (0.821, 0.941)
Ring 2	0.868 (0.787, 0.928)	0.791 (0.670, 0.884)
Ring 3	0.894 (0.827, 0.943)	0.767 (0.632, 0.871)
Ring 4	0.726 (0.591, 0.842)	0.576 (0.412, 0.738)
Ring 5	0.572 (0.408, 0.735)	0.316 (0.156, 0.519)

[a]Intraclass Correlation Coefficient

Table 5. Test-retest agreement in assessing ultra wide-field fluorescein angiogram images using the concentric rings method and the ischemic index method.

	Rings[a] (ICC[b])	Index[c] (ICC[b])	Rings[a] (CoV[d], %)	Index[c] (CoV[d], %)
Perfused ratio	0.975	0.979	9.18	8.45
Area of non-perfusion	0.967	0.973	17.02	15.50

[a]Concentric Rings method

[b]Intraclass Correlation Coefficient

[c]Ischemic Index method

[d]Coefficient of Variation

Figure 6

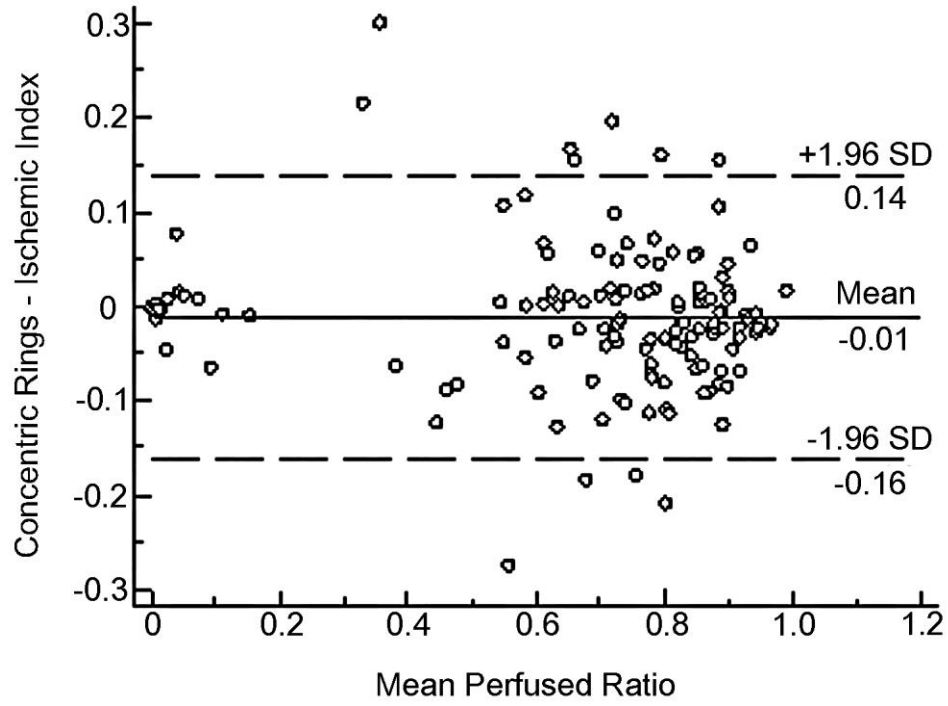


Figure 7

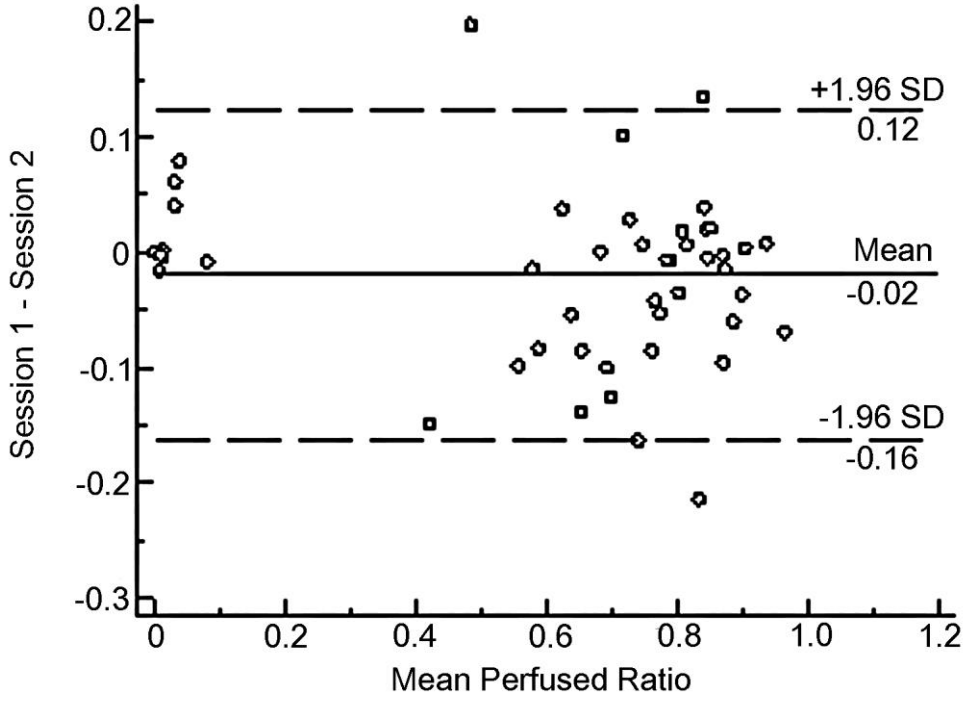
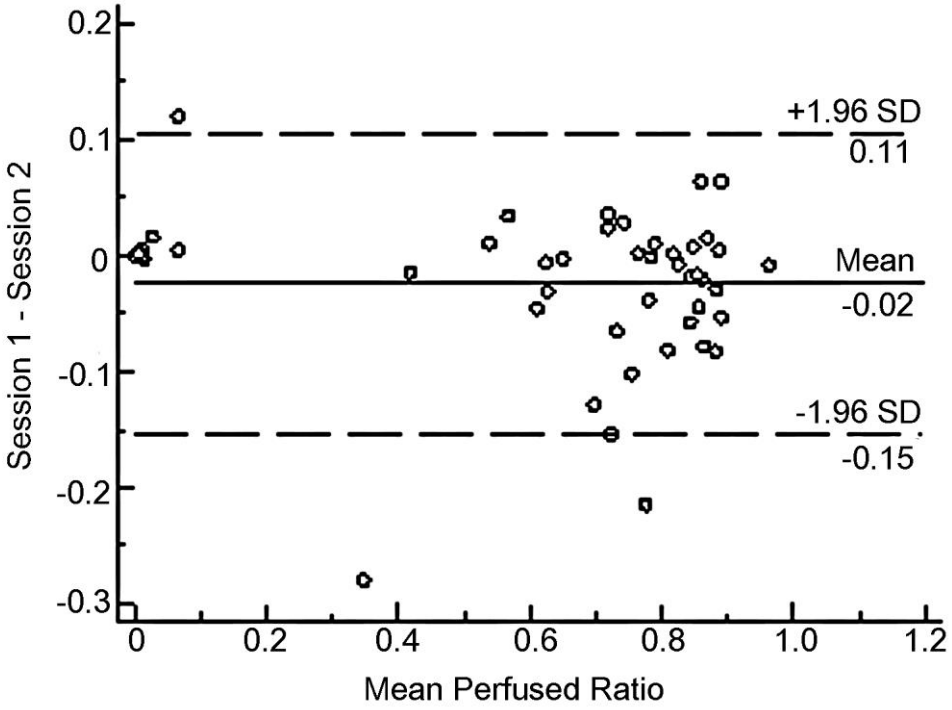


Figure 8


0.2
0.1
0
-0.1
-0.2
-0.3
Session 1 - Session 2
+1.96 SD
0.11
Mean
-0.02
-1.96 SD
-0.15
0
0.2
0.4
0.6
0.8
1.0
1.2
Mean Perfused Ratio