Effect of Image Quality, Color, and Format on the Measurement of Retinal Vascular Fractal Dimension

Alan Wainwright,[1] *Gerald Liew,*[1] *George Burlutsky,*[1] *Elena Rochtchina,*[1] *Yong Ping Zhang,*[2] *Wynne Hsu,*[3] *Janice MongLi Lee,*[4] *Tien Yin Wong,*[4,5] *Paul Mitchell,*[1] *and Jie Jin Wang*[1,4]

Purpose. Fractal dimension of retinal vasculature is a global summary measure of retinal vascular network pattern and geometry. This study was conducted to examine the effect of variations in image color, brightness, focus, contrast, and format on the measurement of retinal vascular fractal dimension.

Methods. A set of 30 retinal images from the Blue Mountains Eye Study was used for a series of experiments by varying brightness, focus (blur), contrast, and color (color versus monochrome). The original and the modified images were graded for fractal dimension (D_f) using dedicated retinal imaging software (IRIS-Fractal). A further set of 20 grayscale images was used to compare image format (.jpg versus .tif) with regard to the resultant D_f and processing time.

Results. The mean D_f of original images in this sample was 1.454. Compared with the original set of images, variations in brightness, focus, contrast, and color affected the measurements to a small to moderate degree (Pearson correlation coefficient, r, ranged from 0.47 to 0.97). Very dark or blurry images resulted in a substantially lower estimate of D_f. Monochrome images were also consistently associated with lower D_f compared with that obtained from color images. Using .jpg or .tif image formats did not affect the measurement or the time needed to process and measure D_f.

Conclusions. Variations in image brightness, focus, and contrast can significantly affect the measurement of retinal vascular fractals. Standardization of image parameters and consistent use of either monochrome or color images would reduce measurement noise and enhance the comparability of the results. (*Invest Ophthalmol Vis Sci.* 2010;51:5525–5529) DOI: 10.1167/iovs.09-4129

The human retinal vasculature is the only directly accessible microvasculature and conveys important information on the health of the circulation.[1,2] Many reports of studies have indicated that retinal vessel caliber is associated with an increased risk of systemic outcomes, such as cardiovascular disease, hypertension, diabetes, and obesity.[3–9] However, in most studies to date, limited measures have been examined, such as retinal vessel caliber in a small, defined region around the optic disc, which may not adequately reflect changes in the peripheral retinal vessels or alterations in vascular branching patterns. As the retinal vasculature exhibits fractal-like structural characteristics such as self similarity, fractal analysis may offer a more natural and complete description of retinal vessel structure and geometry.[10–12]

Methods of calculating the fractal dimension (D_f) of the retinal vasculature have generally relied on manual tracing of the retinal vessels to produce a skeletonized binary image from which the fractal dimension is calculated. Such methods are slow and prone to subjective error between graders. We have developed a new software program, International Retinal Imaging System—Fractal (IRIS-Fractal) that speeds this process by automating vessel segmentation. The automation of this step greatly increases the number of images that can be graded in a given time and results in minimal between-grader differences.[13] Our preliminary results indicate that change in D_f may be a sensitive indicator of retinal vascular damage from ocular and systemic disease processes such as diabetic retinopathy[14] (increased D_f) and elevated blood pressure (decreased D_f).[13] Thus, the D_f may be a marker of subtle changes in retinal vascular architecture, and its measurement from fundus photographs may be a rapid, noninvasive test for detection of early vascular disease. However, to maximize the clinical utility, we require an understanding of how variations in retinal image parameters influence the measurement of D_f.

Successful segmentation of a retinal image requires IRIS-Fractal to accurately distinguish vessel from nonvessel characteristics under different conditions of brightness, contrast, and image clarity. These image differences can arise from variations in photographic technique, pupil dilation, presence of cataract and other ocular media opacities that can cause blurring of retinal images and the contrast between retinal vessels and pigment density of the retinal pigment epithelium (RPE). Those who scan film-based images to obtain digital images before processing with IRIS-Fractal may find that the digitization process also imparts variations in regard to image resolution, color (either grayscale or color), and the image formats selected, which may be based on various methods and levels of image compression. In addition, the processing time for each

From the [1]Department of Ophthalmology, Centre for Vision Research, Westmead Millennium Institute, University of Sydney, Sydney, Australia; the [2]School of Electronic and Information Engineering, Ningbo University of Technology, Ningbo, China; the [3]School of Computing, National University of Singapore, Singapore; the [4]Centre for Eye Research Australia, University of Melbourne, Melbourne, Australia; and the [5]Singapore Eye Research Institute, Yong Loo Lin School of Medicine, National University of Singapore, Singapore.

Supported by Australian National Health and Medical Research Council (NHMRC) Project Grants 153948, 211069, and 302068; Singapore Bioimaging Consortium Grant SBIC RP C-011/2006; Biomedical Research Council Grant 501/1/25-5; Pfizer Australia Cardiovascular Lipid Grant 2007; and Diabetes Australia Research Trust (DART) Grant 2007. JJW is funded by a National Health and Medical Research Council Senior Research Fellowship (2005-2014). IRIS-Fractals was developed in Singapore funded by the national science agency, A*STAR, which owns the intellectual property and commercialization rights of this software. It is currently not commercially available but is freely available to researchers. WH, JM-LL, and TYW are named inventors of the patent for the software, but do not have a direct financial interest.

Submitted for publication June 12, 2009; revised December 15, 2009, and March 24, 2010; accepted May 18, 2010.

Disclosure: **A. Wainwright**, None; **G. Liew**, None; **G. Burlutsky**, None; **E. Rochtchina**, None; **Y.P. Zhang**, None; **W. Hsu**, P; **J.M-L. Lee**, P; **T.Y. Wong**, P; **P. Mitchell**, None; **J.J. Wang**, None

Corresponding author: Jie Jin Wang, Centre for Vision Research, University of Sydney, Westmead Hospital, Hawkesbury Road, Westmead, NSW, 2145, Australia; jiejin_wang@wmi.usyd.edu.au.

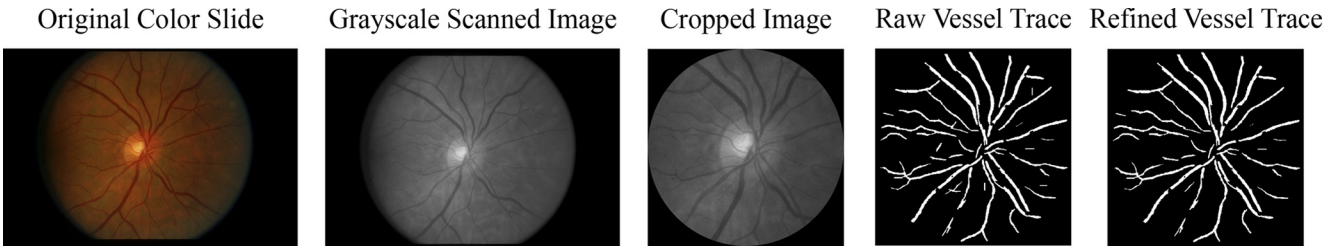

FIGURE 1. Pictorial view of the IRIS-Fractal grading process. The original color slide is scanned as a grayscale image. The grayscale image is then cropped and the raw vessel tracing is produced. The raw trace is graded by a trained grader and the D_f for the refined image is calculated.

image, which may depend on image file size, is important when grading large image sets for the purpose of screening.

In this study, we used retinal photographs from the Blue Mountains Eye Study, a large, population-based study of eye disease conducted in a defined area west of Sydney, Australia, to assess the effect of these possible limitations on measurement of D_f with IRIS-Fractal.

METHODS

We selected a subset of 30 color, positive film photographs of the retina of the right eye, processed as 35-mm slides from the Blue Mountains Eye Study baseline participants, conducted from 1992 to 1994. To examine the effect of variation in image brightness, contrast, and clarity on the segmentation process and thus the final refined fractal dimension measures, we selected the 30 slides to have good-quality (defined as having reasonable image brightness and clarity on visual inspection by the grader and to produce gradable vessel traces, according to protocol), disc-centered images from normotensive subjects who were free of known systemic disease. The 30 selected slides were digitized to produce .tif monochrome (grayscale) images (size = 9.6 Mb, 3888 × 2595 pixels) with a slide scanner (CanoScan FS2710; Canon Corp., Tokyo, Japan). IRIS-Fractal was used to process each image through to the image-cropping stage (Fig. 1).[13] The cropping of the image involved drawing a circular mask with a radius of 3.5 optic disc radii centered on the optic disc. This process is intended to provide a consistent area of measurement across individuals, while minimizing loss of focus and image artifact at the edge of the photographic field.[13] After they were cropped, the images were saved as a bitmap. Saving the cropped images allowed identical images to be used for each subsequent image set, formed by manipulation of brightness, blur, and contrast. It therefore ensured that an identical area was measured for each image under the various conditions studied. We then performed a series of experiments.

Experiment 1: Image Brightness Variation

The pixel density histogram of each cropped grayscale image was examined (Fig. 2). As there were no pure white pixels in any of the grayscale images and most of the numerous black pixels in the pixel density histogram were likely to have come from the black border that results from cropping, we examined the substantive middle section of the histogram and recorded the highest and lowest pixel values. From these data, it was determined that a maximum shift of 40-pixel brightness values could be made in both directions, for every image, without information loss. Thus, the position of the histogram, with respect to light and dark, was changed but without altering its shape. Eight brightness-variation image sets (−40, −30, −20, −10, +10, +20, +30, and +40) were created (Photoshop CS2; Adobe Systems, San Jose, CA). These image sets were graded with IRIS-Fractal and compared to the reference set.

Experiment 2: Image Focus Variation

The Lens Blur filter in Photoshop was used to produce three image sets that varied in image clarity (Fig. 3). The settings used were "hexagonal iris," with a blur radius of 10, 20, or 30. The images produced were graded and compared with the reference set.

Experiment 3: Image Contrast Variation

A maximum contrast–expansion grayscale image set was obtained, applying a 0.1% clip of the upper and lower pixels in each image (Fig. 4). This process was completed in Photoshop via the use of the Auto Levels function and standard settings. This setting was chosen over the Auto Contrast function, which ignores the top and bottom 0.5%, to

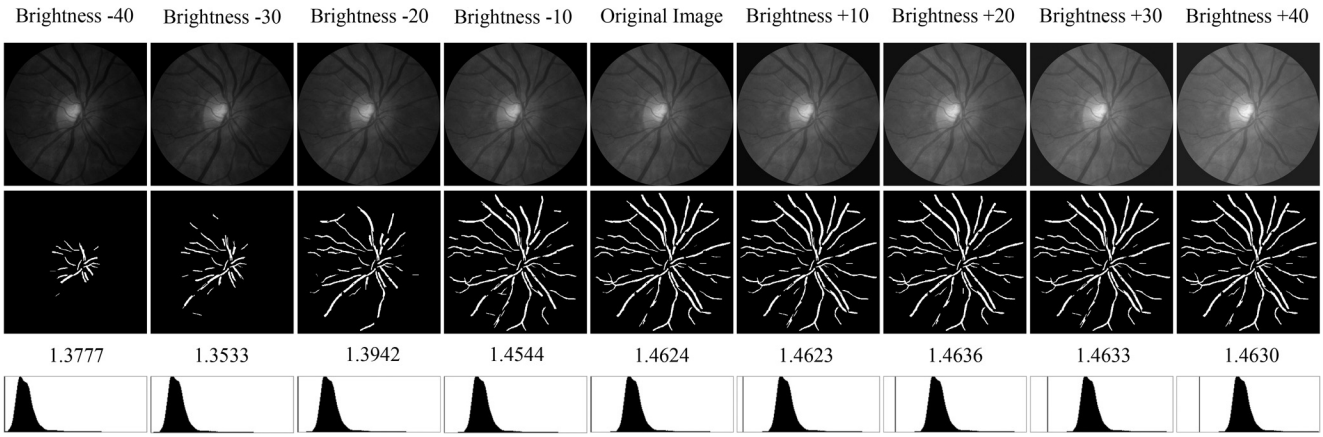

FIGURE 2. Brightness variations. This series shows the effect of brightness adjustments applied to a single original image. Stepwise, four 10-pixel adjustments were made in both the positive and negative directions (*top*). The resulting refined skeletonized line tracing is shown below each brightness-adjusted image (*middle*), along with its associated D_f measure. The pixel density histogram for each image is also shown (*bottom*). This process was repeated for each of the 30 images in the experimental dataset.

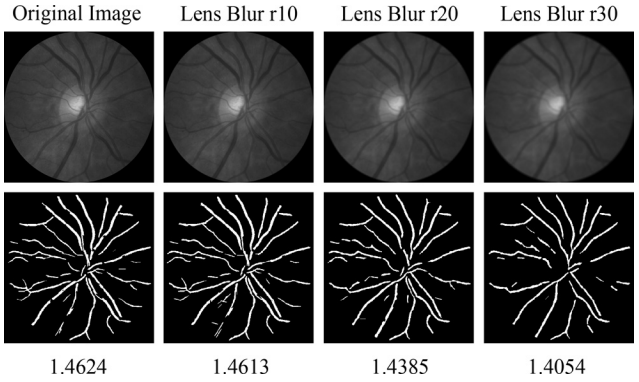

FIGURE 3. Focus (blur) variations. Three increasing levels of blur were applied to the original image. The resultant skeletonized line tracing and associated D_f are shown for each condition. Note loss of smaller vessel traces as blur increases. This sequence of image blurring was repeated for each of the 30 images in the experimental dataset.

retain as much of the information contained in the image as possible while still increasing the relative difference between gray values in the image.

Experiment 4: Image Color Variation

We determined whether there was a difference between the D_f obtained from color and grayscale images by scanning the original 30 color slides as color .tif images. Both this new color .tif image set and the original grayscale .tif image set were graded according to the standard IRIS-Fractal protocol, and the resulting means were compared. No image manipulation was conducted for this analysis.

Experiment 5: Image Format Variation

The effect of image format on D_f measurement was studied by comparing the fractal dimension from an image set scanned in .jpg with those scanned in .tif. An additional 20 color slides were chosen from the baseline BMES population, and each image was scanned, in grayscale, as both .jpg (average image size, 0.49 Mb) and .tif (9.61 Mb). This method gave an approximately 20:1 compression over the standard image and would be considered high (quality grade, 8) .jpg compression in Photoshop. The standard IRIS-Fractal grading process was then applied to each image set and the results compared.

Other Assessments

As the amount of time spent on grading an image is important, we also measured the mean time (in seconds) necessary for IRIS-Fractal to calculate the raw fractal dimension and produce the initial ungraded vessel trace. This comparison was conducted by using the 20-image grayscale .jpg and .tif image sets that were used for examining the effect of image format.

As a continued check of intragrader reliability, the original grayscale 30-image set was regraded by the same grader and the results compared with those obtained at the beginning of the study.

Statistical Analysis

The mean D_f obtained from each manipulated image set was compared with the mean D_f obtained from the unaltered, original cropped image set. The paired Student's *t*-test was used to compare the mean D_f of the reference image set and each of the brightness-, blur-, and contrast-adjusted image sets. The paired Student's *t*-test was also used for comparisons of grayscale versus color images, the .tif versus .jpg image sets, and for the analysis of processing time for the .tif and .jpg image sets. The Pearson correlation was also calculated for all comparisons, along with the Cohen d[15] as a measure of effect size (defined as the difference in means divided by the standard deviation of the original unaltered image set).

$$d = (\text{mean}_1 - \text{mean}_2)/\text{SD}_1 \quad (1)$$

For comparisons that were not between pre- and postalteration data sets (color versus grayscale, .tif versus .jpg, and processing time) the square root of the pooled variance was used instead of the standard deviation.

$$d = (\text{mean}_1 - \text{mean}_2)/\sqrt{[(\sigma_1^2 + \sigma_2^2)/2]} \quad (2)$$

$P < 0.05$ was considered significant. As statistical significance does not necessarily equal practical or clinical significance, effect sizes were used to describe the magnitude of the differences relative to the standard deviation of the measures from the original images. Effect size was graded as suggested by Cohen, with effects considered to be small at 0.2, moderate at 0.5, and large at 0.8 or greater.[15]

RESULTS

The mean D_f of the reference image set was at 1.4542, with a mean median pixel value of 99.9. Table 1 shows the results of experiments 1 to 3. Increasing brightness (experiment 1) was not found to significantly affect the measurement of D_f. A brightness increase of 40 resulted in a mean D_f of 1.4541, which was not significantly different from the mean D_f of the reference set (1.4542; $P = 0.60$, $d = 0.01$). However decreasing brightness by 4 units significantly lowered the measurement of D_f (mean D_f, 1.4113; $P = 0.0001$, $d = 3.14$; Table 1, Fig. 2).

Increasing the amount of blur in an image (defocusing, experiment 2) lowered the measured D_f significantly (mean D_f, 1.4118; $P < 0.0001$, $d = 3.11$; Table 1) In experiment 3, applying a maximum contrast operation to each image in the set increased the measured D_f. The increase was small (mean D_f, 1.4570; $P = 0.005$), as was the effect size ($d = 0.21$; Table 1).

There was a significant difference in the D_f gained from a color image set (experiment 4) compared with that gained from the same image set but scanned and processed in grayscale (1.4692 vs. 1.4562; $P < 0.0001$, $d = 0.95$; Table 2).

In experiment 5, image format (.tif versus .jpg) for grayscale images did not affect the measured D_f (Table 2).

Finally, image format also did not affect the processing time for each image, with production of the initial vessel trace

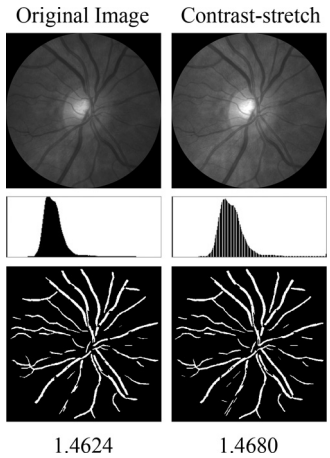

FIGURE 4. Contrast-stretch. A contrast-stretch operation involving a 0.1% clip of maximum and minimum pixel values. The pixel density histogram shows how the pixel values in the image are spread out to increase the difference between them and thus make small differences more obvious. D_f is given below the images. This adjustment was performed for each of the 30 images in the experimental dataset.

TABLE 1. Effects of Changes in Brightness, Focus, and Contrast on D_f

Image Manipulation	Mean D_f	*P*	*r*	Effect Size (Cohen *d*)*	Mean Median Pixel Value
Experiment 1: brightness adjustment					
Brightness −40	1.4113	0.0001	0.07	3.14	59.90
Brightness −30	1.4412	0.0046	0.47	0.95	69.90
Brightness −20	1.4479	0.0385	0.55	0.46	79.90
Brightness −10	1.4541	0.9081	0.95	0.01	89.90
Reference Set	1.4542	Reference	1.00		99.90
Brightness +10	1.4552	0.1160	0.97	0.07	109.90
Brightness +20	1.4544	0.2719	1.00	0.01	119.90
Brightness +30	1.4548	0.3300	0.97	0.04	129.90
Brightness +40	1.4541	0.5972	1.00	0.01	139.90
Experiment 2: image blurring					
Lens blur r10	1.4554	0.1185	0.96	0.08	99.37
Lens blur r20	1.4374	<0.0001	0.85	1.23	99.30
Lens blur r30	1.4118	<0.0001	0.77	3.11	99.23
Experiment 3: contrast expansion	1.4570	0.0054	0.93	0.21	137.30

n = 30 for each set. Brightness $\pm x$, the amount the reference image was lightened or darkened.
* d = (mean$_1$ − mean$_2$)/standard deviation. r = Pearson correlation coefficient.

taking a mean time of 90.15 seconds for the .tif image set versus 90.14 seconds for the .jpg image set (Table 3). Intragrader reliability was high (0.95), which agrees with the findings of Liew et al.[13]

DISCUSSION

Fractal analysis of retinal vessels may convey valuable information on microvascular structure that is absent from alternative measures such as retinal vessel caliber.[2,13,14] To fully use this potential, we examined how variations in retinal image quality may influence the measurement of D_f. The simulated degradation levels are comparable with the quality range of images in real life, particularly images taken from older persons who may have various pupil sizes and levels of cataract and other ocular media opacities. In five experiments, we attempted to recreate, via the use of software, the typical variations due either to differences in photographic technique (e.g., amount of flash used, the ability of the photographer to achieve correct focus, and the exposure) or to anatomic and physiological features that may not be related to the structure and complexity of the retinal vasculature (e.g., retinal pigment epithelial cell pigment density, pupil dilation in response to phenylephrine and/or tropicamide, and the presence of cataract or other ocular media opacities).

In this study, we found that variations in image brightness, clarity, and contrast all affected the ability of IRIS-Fractal to accurately segment a digitized image of the retinal vasculature and, thereby, the measurements of D_f produced by IRIS-Fractal.

TABLE 2. Results of Image Formats and Measure of Intragrader Reliability

Experiment 5 Image Format	Mean D_f	*P*	*r*	Effect Size (Cohen *d*)*
Grayscale .tif	1.4438	0.3353	0.97	0.05
Grayscale .jpg	1.4424			
Color .tif	1.4692	<0.0001	0.87	0.95
Grayscale .tif	1.4562			
Original crop	1.4542	0.0244	0.95	0.14
Full regrade	1.4562			

The image format comparisons were .tif versus .jpg and color versus grayscale (n = 20 for .jpg vs. tif, n = 30 for other).
* d = (mean$_1$ − mean$_2$)/$\sqrt{[(\sigma_1^2 + \sigma_2^2)/2]}$.

D_f assessment was thus also sensitive to these image variations. Effect sizes for most of these image variations (contrast expansion, small decrease in image brightness) were low to moderate (Cohen *d*, 0.21–0.46). Increased blur and decreased brightness both impaired the ability of IRIS-Fractal to accurately segment a retinal image, resulting in spuriously low D_f. However, these reductions in measured D_f were generally of small magnitude, except for the lowest range of pixel brightness values and greatest amounts of blur (Table 1). Conversely, an increase in image brightness was not associated with any significant change in the reported D_f measures, nor was a small amount of image blurring. Moderately improving contrast improved detection of vessels and measurement of D_f by a small but significant amount (D_f difference, 0.0028; P = 0.005, d = 0.21), considering that the images studied were all reasonable quality to begin with. The increase in measured D_f seen after contrast expansion is not due to the change in brightness of the images, as the median pixel value of the contrast expansion set, 137.3, is within the range of that produced by the brightness increases that we examined and which were found to have no effect. Similarly, the effect on D_f caused by the blurring of an image is not related to any change in brightness, as each image-blur set had a median pixel value similar that of to the others and also to the reference set.

We found that IRIS-Fractal produced different measures of D_f when analyzing color as opposed to color-scanned-as-grayscale images, with the color image generally giving higher D_f. This effect size was large (average D_f difference, 0.013; P < 0.0001, d = 0.95). From manual inspection, color images appeared to generate a more faithful tracing of the retinal vasculature and were thus less susceptible to false negatives (i.e., failure to detect and segment vessels), because the green channel of color images provides the greatest contrast and details of the images. This finding suggests that, preferen-

TABLE 3. Processing Time for Grayscale .tif and .jpg Images

Image Format	Image Size (MB)	Mean Processing Time (s)	*P*	*r*	Effect Size (Cohen *d*)*
.tif	9.61	90.15	0.8617	0.74	0.03
.jpg	0.49	90.14			

n = 20 for each set. r = Pearson correlation coefficient.
* d = (mean$_1$ − mean$_2$)/$\sqrt{[(\sigma_1^2 + \sigma_2^2)/2]}$.

tially, color slides should be scanned and graded in color rather than in grayscale when using IRIS-Fractal. Although grayscale-scanned-from-color images may provide useful information on associations between retinal vessel complexity and various physiological markers of interest (e.g., presence of retinopathy lesions), our results suggest that the D_f measurements gained from this process may not be directly comparable to those gained from color grading of the same images.

Brightness thresholds below the default used in this study appear to adversely affect the accuracy of IRIS-Fractal in tracing retinal vessels, and could thus result in spuriously lower measurements of D_f. As brightness variation across an image is common in retinal photographs, some form of image postprocessing to increase the uniformity of brightness across an image (e.g., shading correction, antivignetting, brightness adjustments, contrast expansion, or combinations of these and others) may help improve the accuracy of D_f measures. Further, images without extreme blur or defocus and with contrast similar to those in this study will provide consistent and reasonably accurate measurements of D_f.

IRIS-Fractal is not sensitive to the format of the image, allowing moderate compression of grayscale images (.jpg) to be used without unduly affecting the results. The size of the image file was also unrelated to the time taken to analyze retinal vascular structure. This finding supports the use of .jpg format in image storage, for measurement of retinal vascular fractal dimension.

Our study has several strengths including the use of actual epidemiologic study images and manipulation through a range of image degradations. Compared with the distribution of D_f in the whole Blue Mountains Eye Study (BMES) population (mean D_f, 1.441, SD 0.024; interquartile range, 1.428–1.457; Mitchell P, et al. *IOVS* 2008;49:ARVO E-Abstract 603; personal communication), the changes from most artificial image degradations were <1 SD and may be considered of less concern. However when the brightness was reduced by 40 units, the change in D_f (+0.043) was more than the interquartile range. Although this is a consequential difference, it is likely to occur only in a small number of images (being the maximum decrease in brightness that we examined). As our work focused on IRIS-Fractal and the segmentation algorithms that we used, it remains unclear how our results apply to other software with different algorithms or segmentation methods. Nonetheless, we believe that developers of other image-processing software should be aware of these aspects, particularly for subtle measurements. It also remains unclear how software-induced degradation of images is related to real-life image degradation due to photographic (e.g., angle of photography and defocus) and patient-related (e.g., cataract, pupil size and ethnicity) factors. We did not evaluate whether changes in measurements due to image degradation affect the clinical utility of this software, as the differences in measurements were of the same order of magnitude as the differences between normal control subjects and patients with different disease states (e.g., hypertension and diabetic retinopathy) reported in other studies.[13,14] This possible drawback may limit the feasibility of using the current software in comparative studies (when image quality is not similar) or in prospective studies in which D_f is used as a risk factor in the diagnosis or follow-up of patients with early subtle vasculopathy (when image quality is different at different time points). These limitations must be addressed before the current fractal software can be applied to clinical settings.

In conclusion, our results suggest that variation in brightness has only a small effect on the ability of IRIS-Fractal to measure D_f, provided the lowest ranges of brightness are avoided (i.e., keeping the median pixel value of the cropped images above 90). A small amount of image blurring is also well tolerated by IRIS-Fractal; however, an increase in image blur beyond this relatively small amount was associated with significantly lower D_f measures. Moderately increasing contrast has a small but positive impact on the ability of this program to accurately segment images and thereby measure D_f. A significant difference was seen in measured D_f between color and color-scanned-as-grayscale images, with color images generally providing higher measures of D_f. Our study has raised image quality as an issue that may also be applicable to other automated vessel-imaging programs using the same or different segmentation methods. These differences may have to be accounted for when measuring D_f of the retinal vasculature and examining associations with ocular and systemic disease. Further work to determine optimal parameters of image brightness, focus, and contrast for measurement of retinal fractals may allow the development of these measurements as novel tests for vascular disease in the eye and elsewhere in the body.

References

1. Wong TY, Mitchell P. Hypertensive retinopathy. *N Engl J Med.* 2004;351:2310–2317.
2. Liew G, Wang JJ, Mitchell P, Wong TY. Retinal vascular imaging: a new tool in microvascular disease research. *Circ Cardiovasc Imaging Sep.* 2008;1:156–161.
3. Wong TY, Mitchell P. The eye in hypertension. *Lancet.* 2007;369:425–435.
4. Sun C, Wang JJ, Mackey DA, Wong TY. Retinal vascular caliber: systemic, environmental, and genetic associations. *Surv Ophthalmol.* 2009;54:74–95.
5. Wang JJ, Liew G, Klein R, et al. Retinal vessel diameter and cardiovascular mortality: pooled data analysis from two older populations. *Eur Heart J.* 2007;28:1984–1992.
6. Wong TY, Klein R, Sharrett AR, et al. Retinal arteriolar narrowing and risk of coronary heart disease in men and women. The Atherosclerosis Risk in Communities Study. *JAMA.* 2002;287:1153–1159.
7. Wang JJ, Rochtchina E, Liew G, et al. The long-term relation among retinal arteriolar narrowing, blood pressure, and incident severe hypertension. *Am J Epidemiol.* 2008;168:80–88.
8. Ikram MK, de Jong FJ, Bos MJ, et al. Retinal vessel diameters and risk of stroke: the Rotterdam Study. *Neurology* 2006;66:1339–1343.
9. Wong TY, Islam FM, Klein R, et al. Retinal vascular caliber, cardiovascular risk factors, and inflammation: the multi-ethnic study of atherosclerosis (MESA). *Invest Ophthalmol Vis Sci.* 2006;47:2341–2350.
10. Stosic T, Stosic BD. Multifractal analysis of human retinal vessels. *IEEE Trans Med Imaging.* 2006;25:1101–1107.
11. Masters BR. Fractal analysis of the vascular tree in the human retina. *Annu Rev Biomed Eng.* 2004;6:427–452.
12. Avakian A, Kalina RE, Sage EH, et al. Fractal analysis of region-based vascular change in the normal and non-proliferative diabetic retina. *Curr Eye Res.* 2002;24:274–280.
13. Liew G, Wang JJ, Cheung N, et al. The retinal vasculature as a fractal: methodology, reliability, and relationship to blood pressure. *Ophthalmology.* 2008;115:1951–1956.
14. Cheung N, Donaghue KC, Liew G, et al. Quantitative assessment of early diabetic retinopathy using fractal analysis. *Diabetes Care.* 2009;32:106–110.
15. Cohen J. A power primer. *Psychol Bull.* 1992;112:155–159.