Impact of simulated light scatter on the quantitative, noninvasive assessment of retinal arteriolar hemodynamics

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Abstract. We determine the impact of artificial light scatter on quantitative, noninvasive assessment of retinal arteriolar hemodynamics. One eye from each of 10 healthy young subjects between the ages of 18 and 30 (23.6±3.4) is randomly selected. To simulate light scatter, cells comprising a plastic collar and two plano lenses are filled with solutions of differing concentration of polystyrene microspheres (Polysciences Inc., USA). We prepare 0.002, 0.004, 0.006, and 0.008% microsphere concentrations as well as distilled water only. The Canon laser blood flowmeter (CLBF) is used to noninvasively assess retinal arteriolar blood flow. After a preliminary screening to confirm subject eligibility, seven arteriolar blood flow measurements are taken by randomly placing the cells between the instrument objective lens and the subjects' cornea. To achieve a baseline, subjects are first imaged with no cell in place. Both low- and high-intensity CLBF laser settings are assessed. Our light scatter model results in an artifactual increase of retinal arteriolar diameter ($p<0.0001$) and thereby increased retinal blood flow ($p<0.0001$). The 0.006 and 0.008% microsphere concentrations produce significantly higher diameter and flow values than baseline. Centerline blood velocity, however, is not affected by light scatter. Retinal arteriolar diameter values are significantly less with the high-intensity laser than with the low-intensity laser ($p=0.0007$). Densitometry assessment of vessel diameter is increasingly impacted as the magnitude of artificial light scatter increases; this effect can be partially negated by increasing laser intensity. A cataract is an inevitable consequence of aging and, therefore, care must be exercised in the interpretation of studies of retinal vessel diameter that use similar densitometry techniques. [DOI: 10.1117/1.2750292]

Keywords: retinal blood flow; Canon laser blood flowmeter; light scatter; densitometry; vessel diameter.

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1 Introduction

The potential of noninvasive retinal imaging techniques to investigate the physiological mechanisms regulating blood hemodynamics in the human retina have long been recognized. However, techniques are now available that enable the noninvasive quantification of volumetric retinal blood flow in absolute units by simultaneously measuring vessel diameter (in micrometers) and centerline blood velocity (in millimeters per second) to derive flow in real units[1–3] (microliters per minute). Blood flow assessment using the Canon laser blood flowmeter (CLBF) is repeatable and reproducible with low variability.[4–6]

Disturbance of retinal blood flow is a feature of many ocular diseases,[7,8] including diabetic retinopathy,[6–10] age-related maculopathy,[11] and a subset of patients with glaucoma.[4,12] In the future, the assessment of retinal blood flow may play a diagnostic role in the early detection of some of these ocular diseases.[10] Retinal hemodynamic assessment techniques may also be used to monitor aspects of disease progression and to evaluate the retinal vascular response to surgical and drug treatments.[4]

Lens opacity, or cataract, frequently occurs concomitantly with other eye diseases, including diabetic retinopathy, age-related maculopathy, and glaucoma. The impact of lens opacity on the assessment of retinal blood flow is unknown, despite the fact that the technique has been used widely in elderly subjects. The clarity of fundus visualization using any fundus camera-based imaging device is limited in the presence of lens opacity due to intraocular light scatter that results in reduced image contrast. Recent studies from our group have quantified the influence of artificial light scatter on vari-

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ous retinal imaging instruments.[13–15] This study was designed to determine the impact of artificial light scatter on quantitative measurements of retinal arteriolar hemodynamics.

2 Methods

One eye from each of 10 healthy young subjects between the ages of 18 and 30 [mean age 23.6 years, standard deviation (SD) 3.4] was randomly selected. All volunteers were informed about the details of the study and gave their consent to participate. The research followed the tenets of the Declaration of Helsinki and was approved by the Research Ethics Board of the University Health Network and also by the Office of Research Ethics of the University of Waterloo. All subjects were drug free with no systemic disease, no ocular abnormalities, and no history of any ocular surgery. Subjects had no lens opacity, exhibited intraocular pressures less than 21 mm Hg, a logMAR (logarithm of the minimum angle of resolution) visual acuity of 0.0, or better, and a refractive error $<\pm 6.00$ DS and $<\pm 2.50$ DC.

2.1 *Artificial Light Scatter Model*

The details of the artificial light scatter model have been published elsewhere.[13–15] In brief, cells comprising a plastic collar and two plano lenses were filled with solutions of differing concentrations of polystyrene microspheres[14] (Polybead© Polysciences Inc., USA). The diameter of the microspheres was chosen to be similar to the mean diameter of aggregated lens proteins (500 nm) that are thought to produce intraocular light scatter in the normal aging lens. Microsphere concentrations of 0.002, 0.004, 0.006, and 0.008% were made up from a 0.16% stock solution and a distilled water only cell was also utilized to act as a further control, i.e., anticipated to not be different from baseline. The microsphere concentrations were determined empirically prior to commencing the study by imaging subjects using the CLBF and determining the point at which the instrument could no longer reliably stabilize a given measurement site. Solutions were made up and the cells were emptied, cleaned, and refilled every week. Cells were also checked regularly with a spectrophotometer to ensure consistency of the optical transmission and absorption characteristics throughout the course of the study.

2.2 *Quantitative Assessment of Retinal Arteriolar Blood Flow*

Quantitative assessments of retinal arteriolar blood flow were acquired using the CLBF, model 100. The CLBF utilizes bidirectional laser Doppler velocimetry (LDV) to quantify the centerline blood velocity in the large retinal vessels, densitometry to measure vessel diameter, and an image stabilization system to minimize the impact of eye movement.[4,16] A red diode laser (675 nm, 80×50 μm oval) is used to acquire bidirectional LDV measurements. The frequency shift of the light scattered by the moving blood cells at the illuminated site is simultaneously focused by two distinct photodetectors separated from each other by a fixed, known angle.[17] The maximum frequency shift detected by each photodetector is subtracted to enable the absolute quantification of centerline blood velocity, irrespective of the angle between the moving particle and reflected beam.[18,19] The resulting Doppler signal is analyzed using a previously described algorithm.[18] The frequency shift is determined as the frequency at which there is an abrupt reduction in the amplitude of the fluctuations in the Doppler shift power spectrum. This determination does not depend on any presumed shape of the average power spectral density curve. Velocity measurements are acquired automatically every 0.02 s throughout the 2-s measurement window, resulting in a velocity-time trace that depicts the systolic-diastolic variation of blood velocity.

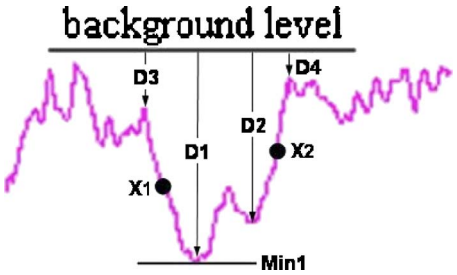

Fig. 1 Densitometry signal of a cross-sectional image acquired from a retinal arteriole: Min_1, minimum point in the central portion of the image designated as the nominal vessel center; D_1, signal level of Min_1 from the background level; D_2, signal level of the second minimum point in the neighborhood of Min_1; D_3 and D_4, signal levels of the maximum points on either side designated as the vessel edges; X_1 and X_2, the half-height points whose signal levels are $(D_1+D_3)/2$ and $(D_2+D_4)/2$, respectively. The width between X_1 and X_2 is taken to represent the uncorrected vessel diameter.

Retinal vessel diameter is determined by projecting a rectangular (1500×150 μm) green (543 nm) HeNe diode laser perpendicular to the vessel segment measurement site. Densitometry analysis of the cross-sectional vessel image on the CLBF array sensor is used to calculate vessel diameter (Fig. 1). The minimum point (Min_1) in the central portion of the image is designated as the nominal vessel center with D_1 being its signal level from the background level. When mirror reflection occurs from the vessel wall, the second minimum point is observed in the neighborhood of Min_1 with signal level D_2. The maximum points on either side are detected as the vessel edges with D_3 and D_4 signal levels. The half-height points (X_1 and X_2) are points on the image whose signal levels are $(D_1+D_3)/2$ and $(D_2+D_4)/2$, respectively. The width between X_1 and X_2 is taken to represent the uncorrected vessel diameter and is converted into micron units after correction for axial and refractive magnification effects.[16,20] Diameter measurements are acquired every 4 ms during the first and last 60 ms of the 2-s velocity acquisition window.

An integral "eye tracking" mechanism stabilizes the laser system on the selected measurement site. Light from the red laser is projected into the center of the green rectangle.[17] The green laser is manually adjusted perpendicular to the vessel segment. The resulting cross-sectional image of the vessel is focused on the CLBF array sensor. The array sensor detects any lateral motion of the vessel and, via a negative feedback loop, controls the galvanometer steering system to stabilize the green tracking laser on the selected measurement site. This system also enables identification and postacquisition rejection of velocity measurements impacted by significant saccades.

Two sequential measurements (path 1 and path 2) both of blood velocity and of vessel diameter are taken to ensure con-

sistency of each parameter and are then averaged to give one reading. The time average of the centerline blood velocity V_{mean} is used along with the diameter D to calculate retinal blood flow F assuming that the vessel has a circular cross section and that the flow characteristics obey Poiseuille's law. Retinal blood flow is derived using the formula: $F = \frac{1}{2}[\pi(D^2/4)](V_{mean} \times 60)$, where flow is in microliters per minute, diameter is in micrometers, and V_{mean} is in millimeters per second. Magnification effects associated with refractive and axial components of ametropia are corrected to provide absolute measurements of diameter (in micrometers), velocity (in millimeters per second), and flow (in microliters per minute).

2.3 *Procedures*

Each subject underwent screening that included the assessment of general and ocular health, best corrected logMAR visual acuity, anterior segment examination, assessment of crystalline lens status (using the lens opacity classification system, LOCS, III) and fundus examination. The randomly selected study eye was dilated using Mydriacyl 1% (Alcon Canada Inc., Mississauga, Canada). Retinal arteriolar hemodynamics were then measured in a superior temporal arteriole. The measurement site was selected in a relatively straight vessel segment, distant from any bifurcations and within 1 disk diameter of the optic nerve head. Initial measurements were acquired with no cell in place as a baseline to optimize and set the instrument focus and positioning. Retinal hemodynamic measurements were then acquired at the same measurement site (using the fixation target memory feature of the CLBF) while placing cells of various microsphere concentrations in front of the subjects' eye in random order. The randomization was implemented to negate the impact of any potential drift of CLBF values or systematic change of hemodynamic parameters. The cells were mounted to the objective of the CLBF using a custom-made adaptor that incorporated a 20-deg tilt to minimize surface reflections. Seven separate measurements were acquired with the CLBF for each light scatter condition, including the no-cell situation. Background fundus illumination and room lighting were kept constant. Subjects were encouraged to blink before each measurement and, if necessary, artificial tears were used to avoid image degradation due to corneal tear film breakup. Laser intensity was maintained on low for all measurements. Axial length was measured using A-scan ultrasound (I^3 Innovative Imaging Inc, Sacramento, California) to correct for magnification effects due to ametropia. These values were entered into the CLBF database prior to calculation of hemodynamic parameters.

The CLBF enables the operator to use either low (standard) or high laser intensity. To investigate the impact of laser intensity on hemodynamic measurements, we also acquired retinal arteriolar hemodynamics both at low- and at high-intensity laser settings on four of the original 10 subjects using the no-cell condition (baseline) and the 0.006 and 0.008% microsphere concentration cells.

2.4 *Statistical Analysis*

Repeated measures analysis of variance (reANOVA) was used to determine the relationship, if any, between microsphere concentration and each of the hemodynamic parameters, i.e., retinal arteriolar diameter, centerline blood velocity and blood flow. For those situations in which a significant effect was detected by the reANOVA, a least-squares difference (LSD) *post hoc* comparison test was used to determine the magnitude of artificial light scatter concentration, relative to the no cell condition, that significantly impacted hemodynamic measurements. The high-laser-intensity results were compared to the standard laser intensity results for the baseline (i.e., no cell) and 0.006 and 0.008% microsphere concentration cell conditions for the same four subjects using repeated measures ANOVA and tukey HSD *post hoc* analysis.

Table 1 Group mean (± SD) retinal arteriolar diameter, centerline blood velocity, and flow as a function of artificial light scatter condition.

	No Cell ($N=10$)	Water ($N=10$)	0.002% ($N=10$)	0.004% ($N=10$)	0.006% ($N=10$)	0.008% ($N=7$)
Diameter (μm)	105.88 (13.47)	105.66 (13.20)	107.67 (13.74)	111.79 (11.14)	120.29 (14.25)	133.18 (14.16)
Velocity (mm/s)	31.61 (8.70)	31.49 (8.53)	32.34 (9.93)	32.02 (8.74)	33.95 (8.79)	33.92 (7.30)
Flow (μl/min)	8.73 (3.72)	8.58 (3.58)	9.21 (4.23)	9.73 (3.92)	11.78 (4.45)	14.54 (4.75)

3 Results

Group mean (± SD) retinal arteriolar diameter, centerline blood velocity and blood flow as a function of artificial light scatter condition are summarized in Table 1. The change of each of the retinal hemodynamic parameters relative to the baseline is shown in Fig. 2.

The group mean retinal arteriolar diameter and blood flow significantly increased (reANOVA, $p < 0.0001$) with increase in microsphere concentration; however, centerline blood velocity was unaffected by artificial light scatter (reANOVA, $p = 0.4740$). The LSD *post hoc* test revealed that the 0.006 and 0.008% microsphere concentrations produced significantly higher diameter and flow values than the baseline condition (i.e., the no-cell condition) (Fig. 2). Importantly, the water-only cell condition produced almost identical values as the baseline for each of the three hemodynamic parameters (Table 1). The laser tracking system of the CLBF had difficulty stabilizing the laser on the measurement site for the highest microsphere concentration; for this reason, it was impossible to acquire any measurements on three subjects using the 0.008% cell.

Table 2 and Fig. 3 depict the hemodynamic assessment of 4 of the original 10 subjects using both high and low laser intensities. Diameter values attained using the high-intensity laser were closer to the baseline; only the 0.008% concentration cell resulted in significantly greater diameter values than the baseline ($p = 0.0008$). Diameter values were significantly less with the high-intensity laser setting than the equivalent measurement with the low-intensity laser ($p = 0.0007$). Diameter values measured with the 0.006% concentration cell were also significantly smaller when the high-intensity laser was utilized ($p = 0.0306$). Centerline blood velocity was unaf-

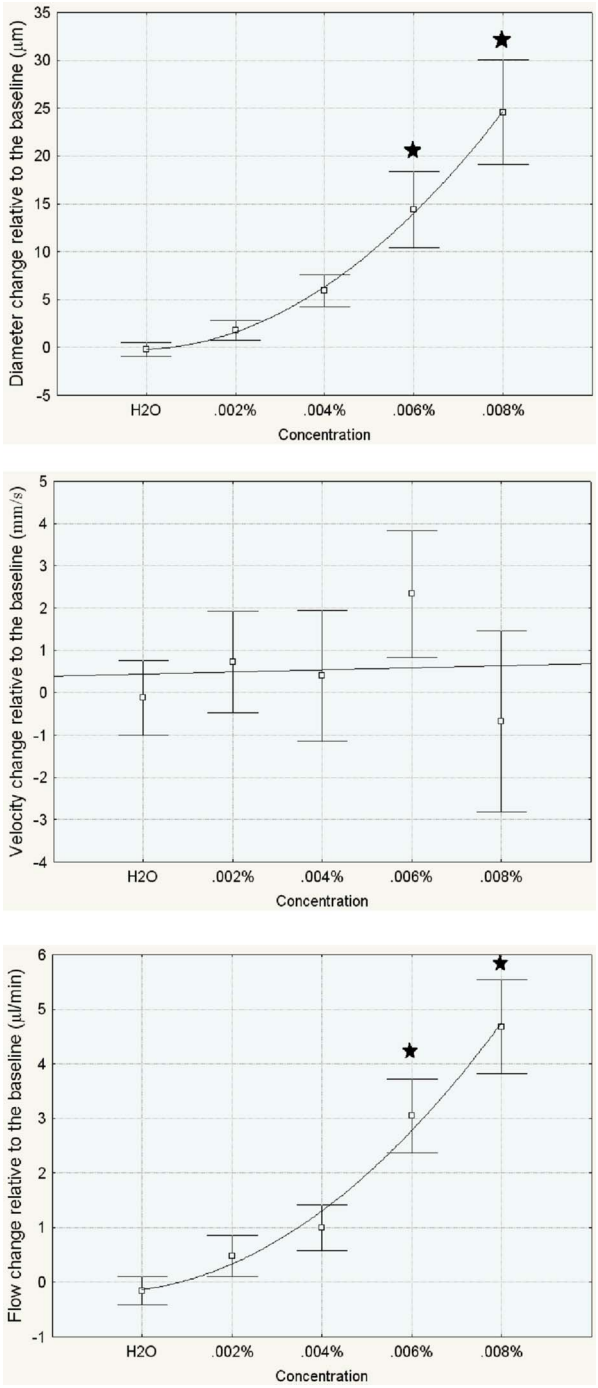

Fig. 2 Change in retinal arteriolar diameter (upper), centerline blood velocity (middle), and blood flow (lower) relative to baseline for all cell conditions. The error bars represent ±1 standard error of the mean (* denotes significant difference from baseline, i.e., the no-cell condition).

fected by laser intensity ($p=0.2817$). Similarly, blood flow values attained using high-intensity laser were closer to the baseline with only the highest light scatter condition being significantly greater than the baseline ($p=0.0313$) and were significantly less than the equivalent measurement with the low-intensity laser ($p=0.0221$).

Table 2 Group mean (± SD) retinal arteriolar diameter, centerline blood velocity, and flow as a function of artificial light scatter condition and laser intesity.

	Low (Standard) Laser Intensity			High Laser Intensity		
	No Cell ($N=4$)	0.006% ($N=4$)	0.008% ($N=4$)	No Cell ($N=4$)	0.006% ($N=4$)	0.008% ($N=4$)
Diameter (μm)	117.76 (9.84)	126.68 (13.02)	141.10 (6.79)	117.68 (10.62)	121.12 (12.74)	129.15 (9.70)
Velocity (mm/s)	39.52 (7.59)	37.10 (7.28)	38.72 (10.15)	34.37 (9.76)	37.11 (10.68)	36.47 (9.67)
Flow (μl/min)	13.14 (3.97)	14.51 (5.52)	18.30 (5.33)	11.41 (4.13)	13.24 (5.50)	14.73 (5.36)

4 Discussion

Our light scatter model resulted in an artifactual increase of retinal arteriolar diameter ($p<0.0001$), which in turn led to increased retinal blood flow values ($p<0.0001$). The increase in blood flow was secondary to the induced change in arteriolar diameter since flow is directly proportional to the second power of diameter, according to the formula used by the CLBF. Centerline blood velocity, however, was not affected by light scatter.

The densitometry technique that is used by the CLBF to measure vessel diameter is increasingly impacted as the magnitude of artificial light scatter increases (Fig. 2). We hypothesize that as the light scatter increases, the densitometry image of the vessel loses contrast and the slopes of the densitometry profile broaden (Fig. 4). This results in a greater separation of the half-height points causing an artifactual increase in the diameter of the vessel. Ultimately, light scatter will result in breakdown of the CLBF laser tracking system but artifactually increased vessel diameter values can be acquired prior to this point. On the other hand, it is clear that the bidirectional LDV technique used to measure centerline blood velocity is robust to the optical effects of artificial light scatter.

It is apparent that the impact of light scatter on the densitometric estimation of retinal vessel diameter can, in part, be negated by increasing the intensity of the HeNe laser (Fig. 3). Future instrumentation could incorporate a negative feedback loop that automatically increases HeNe laser intensity in situations where the slope of the densitometry profile becomes shallow. In addition, when assessing retinal blood flow in patients with concomitant cataract, we recommend using the high-intensity laser setting of the CLBF. CLBF parameters that do not rely on diameter estimation but still provide useful hemodynamic information, such as centerline blood velocity and maximum to minimum velocity ratio, should be given greater attention when assessing retinal blood flow in patients with concomitant cataract.

The data preclude the possibility that a real change in diameter occurred. To explain, if the apparent increase in arteriolar diameter was real then this would be reflected by an increase in blood velocity (since vascular resistance would decrease). The magnitude of apparent increase in arteriolar diameter, if real, would be predicted to result in an increase of

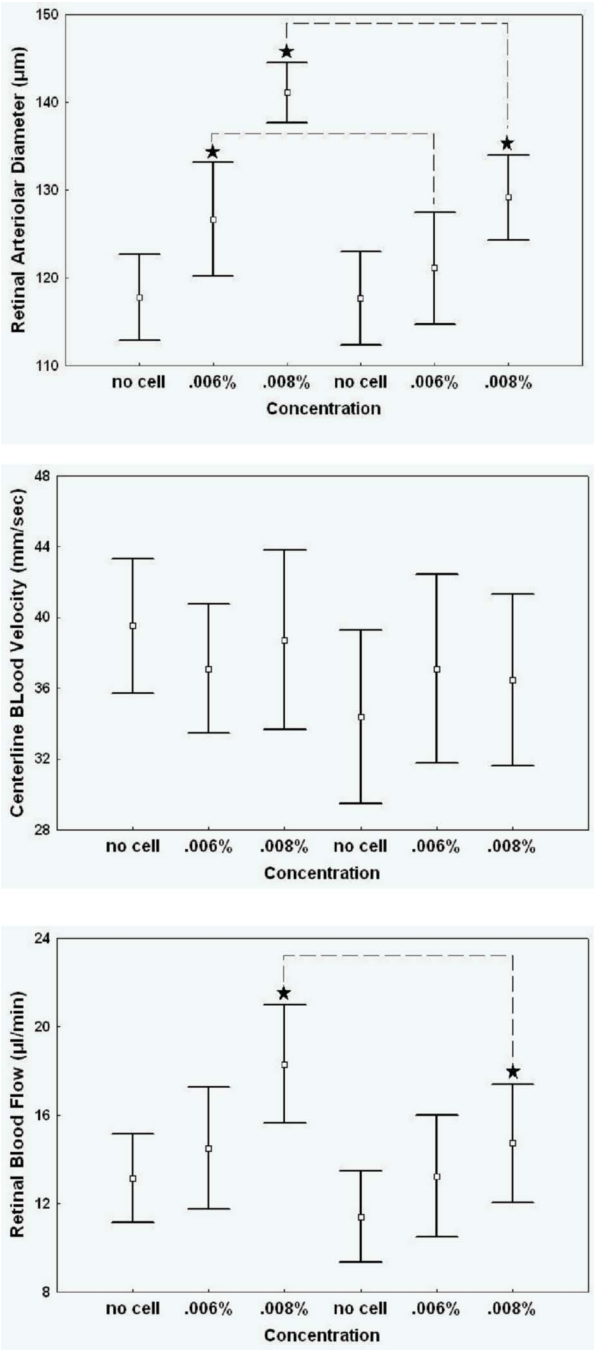

Fig. 3 Retinal arteriolar diameter (upper), centerline blood velocity (middle), and blood flow (lower) attained at the low (left) and high (right) intensity laser settings for the no-cell, 0.006 and 0.008% microsphere concentrations. The error bars represent ±1 standard error of the mean (* denotes significant difference from the baseline). The dashed line denotes a significant difference between identical cell conditions for the low and high laser settings.

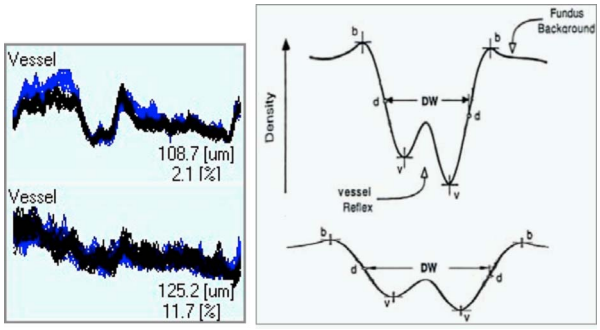

Fig. 4 Densitometry profiles of a vessel measured by the CLBF (left) and schematic representation (right): Upper, baseline (no-cell condition); lower, 0.006% microsphere concentration. DW is the half-height width.

blood velocity. An increase in retinal arteriolar diameter of 3.2±1.4% following hypercapnic provocation resulted[21] in an increase of blood velocity of 26.4±7.0%. In effect, the finding of an increase in arteriolar diameter in the absence of an increase in blood velocity is contradictory and can be explained only as an artifact of the light scatter cells on the densitometric estimation of vessel diameter. Furthermore, the magnitude of apparent increase in diameter for the 0.008% light scatter cell is outside the physiological range of diameter change produced by extreme hypercapnic and hyperoxic provocations.[21,22]

Are the results of this study relevant to only the limited number of CLBF users worldwide and to those individuals with a specific interest in retinal hemodynamic assessment? A number of techniques designed to assess retinal vessel diameter, including the retinal vessel analyzer[23] and the assessment of retinal photographs used in the Atherosclerosis Risk in Communities Study,[24] have been introduced. In particular, recent results from the Atherosclerosis Risk in Communities Study found that retinal vascular abnormalities are predictive of cardiovascular disease.[25] However, both of these techniques[23,24] use algorithms to assess retinal vessel diameter that are very similar to that used in the CLBF and therefore may be susceptible to an artifact resulting from light scatter. Cataract is an inevitable consequence of aging and, therefore, care must be exercised in the interpretation of studies of retinal vessel diameter that utilize cohorts with age as a covariable.

The results derived from any model, however, must be interpreted with caution since the model may not accurately simulate the impact of true cataract. From a clinical perspective, we previously demonstrated the impact of each artificial light scatter condition on fundus visualization achieved with a digital fundus camera that utilizes a polychromatic light source.[14,15] Direct comparison of our artificial light scatter model with a subjective, slit-lamp-based cataract grading scale is difficult; however, the magnitude of light scattering medium used in this study is far less than that used to assess confocal optics based instrumentation.[15] Unpublished data from our lab demonstrates that the artificial light scatter model impacts visual acuity and letter contrast sensitivity in a very similar manner to that of nuclear sclerotic cataract. Future work will investigate the impact of light scatter induced by cataract on retinal blood flow assessment.

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