Regional Variation in Capillary Hemodynamics in the Cat Retina

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PURPOSE. The behavior of the retinal microcirculation and its role in the progression of ocular disease is of considerable interest, yet few details are known about the flow of blood through the capillary networks of the retina. Although retinal vessels may be viewed through the pupil using standard optics, the optical limitations of the cornea and lens prevent the resolution of retinal features smaller than approximately 10 μm in size. Because red blood cells are smaller than this, fluorescent techniques such as angiography, specific cell labeling, and fluorescein-encapsulated liposomes have typically been used to observe the retinal microcirculation in vivo. Here the authors report a study of in vivo retinal capillary hemodynamics using white light GrAdient INdex of refraction (GRIN) lens endoscopy.

METHODS. GRIN lens endoscopy and robotic manipulation were used to directly observe and record the motion of erythrocytes within retinal capillary networks. Video images from the endoscope were analyzed to determine the regional variation of erythrocyte velocity and normalized optical density (an index of relative capillary hematocrit) in the superficial retinal capillaries of the cat.

RESULTS. A significant decrease in mean retinal capillary velocity coupled with a corresponding increase in red blood cell density was observed in peripheral regions of the retina when compared with regions of the retina near the optic disc. Stasis or intermittent flow was not observed in the unstained retina, nor were capillaries noted that contained only plasma.

CONCLUSIONS. Quantification of the bloodflow in retinal microcirculation was possible using GRIN lens endoscopy and showed significant regional heterogeneity in the cat retina. (Invest Ophthalmol Vis Sci. 1998;39:407-415)

The healthy retina regulates both flow1,2 and perfusion pressure3 over a wide range of intraocular pressure, and the time course of this vascular response points to autoregulation by local myogenic factors.2,3 However, in glaucoma it appears that autoregulatory mechanisms are compromised4-6 and predictable patterns of scotomas develop in response to elevated intraocular pressure.4,6 Abnormal or local variation in microvascular function, caused at least in part by altered autoregulation,7 may play a role in the progression of glaucomatous disease7 as demonstrated by capillary degradation8 and decreased arteriovenous transit times9 in glaucomatous eyes. Patients with diabetic retinopathy also exhibit abnormal microvasculatures and are characterized by regionally dependent retinal hemorrhages, protein and lipid exudates, and microaneurysms10,11 thought to result from ischemia caused by capillary closure.10,12

The vascular caliber and blood flow of larger retinal vessels has been studied using a variety of techniques,1,2,13,14 however, much less is known about the retinal microcirculation. Although the retina can be viewed with reasonable clarity through the pupil using standard optics, optical limitations of the cornea and lens prevent discerning retinal features smaller than approximately 10 μm in size.15 Red blood cells are smaller than this optical limitation, thus rendering individual, nonlabeled erythrocytes unobservable without use of microscope magnification. To overcome this limitation, several investigators viewed the retinal microcirculation using either a scleral window16 or intravitral microscopy,17-19 the latter of which entailed removing the cornea and lens to allow placement of a microscope objective next to the retina. Although they provide stunning clarity and dramatic pictures, these techniques are quite traumatic and the data collected by these methods may not represent the normal physiology.

More recently, various techniques have been implemented to quantify retinal microcirculatory hemodynamics including fluorescent dyes,20-23 laser Doppler flowmetry,24 and the blue-field entoptic phenomenon.25 Fluorescent dyes overcome the resolving limitations of the eye by labeling either blood cells or plasma with a fluorescent tag and then exciting the fluorescent dye in vivo. This in essence creates a source of fluorescent illumination on the surface of the retina which may be observed from outside of the eye, allowing the flow of blood through retinal capillary networks to be quantified.

In this study we used an alternate method for evaluating retinal capillary hemodynamics using GrAdient INdex of refraction (GRIN) lens endoscopy.26-28 The high-resolution, 20-gauge endoscope (Volpi, Zurich, Switzerland)29 was inserted through the sclera and positioned near the retina using a robotic micromanipulator of our own design.29 The velocity of red blood cells and normalized optical density (an index of relative capillary hematocrit) within superficial retinal capillary networks of six cats were determined at various locations.
within the superior and nasal regions of the retina. The results of this study show a decrease in retinal capillary velocity coupled with a corresponding increase in the density of red blood cells in peripheral regions of the retina as compared to regions of the retina near the optic disc.

**METHODS**

**GRadient INdex of Refraction (GRIN) Lens Endoscopy**

The GRIN lens endoscope used in this study was designed for ophthalmic use and modified slightly for our own purposes. Unlike fiberoptic bundles, the GRIN lens consists of a single rigid glass rod with an index of refraction that varies with radius. The lens is surrounded by fiberoptic strands used for illumination and encased in a 68-mm long, 20-g stainless steel tube. The magnification of the image formed by the lens increases exponentially with decreased focal length and gives a maximum in vivo resolution of approximately 1 μm per video scan line. When the tip of the lens is held sufficiently near the retina, the magnification of the lens is such that the microcirculation can be readily resolved. Light from the lens passed through a changeable filter and was focused onto a color, charge-coupled device (CCD) array (Sony, San Jose, CA) in the endoscope body. A video camera (Teli CS6100; Tokyo, Japan) converted the CCD information to an RGB video signal, which was recorded using a Betacam-SP video recorder (UVW-1400; Sony).

During use, the focus of the lens must be adjusted as the working distance changes. The original focus knob was housed in the body of the endoscope and required touching the endoscope whenever the image was out of focus. To observe individual erythrocytes, the tip of the lens must be placed very near the retina (<100 μm) and touching the endoscope while in this position could cause retinal damage. A hydraulic cylinder (model HO-22; Narishige, Greenvale, NY) was mounted onto the body of the endoscope, allowing the focus to be adjusted remotely. For each lens, a calibration was performed relating the focal depth of the lens as measured by the hydraulic cylinder to the scale of the image as measured using a reticle with 10-μm divisions. The mechanical advancement of the hydraulic cylinder was monitored by a computer and used to determine the scale of the recorded images based on the appropriate calibration curve for the lens being used.

**Robotic Manipulator**

The precision and stability with which the endoscope must be positioned to view in vivo capillary flow precluded the endoscope from being held by hand. Instead, the endoscope was mounted to a robotic manipulator of our own design. The device is parallel in structure and provided submicrometer placement of the GRIN lens at the retinal surface. The position of the lens within the eye was controlled directly by an operator using a hand-held trackball. Based on trackball movement, the manipulator either pivoted the endoscope about the point it penetrated the sclera or moved the endoscope into and out of the eye. An enable button on the trackball allowed the manipulator to respond to trackball motion, and the endoscope remained at a fixed position when this button was released.

**Animal Preparation**

In six experiments, 4- to 5-kg, mixed-breed cats were initially anesthetized with an intramuscular injection of ketamine hydrochloride (30 mg/kg) mixed with acepromazine (0.5 mg/kg). Both saphenous veins, the left femoral artery and the trachea, were cannulated. Subcutaneous atropine was given (0.04 mg/kg) to prevent buildup of fluids in the airways during ventilation. Thiopental sodium was delivered through one venous cannula for anesthesia during surgery and urethane through the other venous cannula for long-term anesthesia. Constant urethane delivery at a rate of 50 mg/kg-hour was maintained using an infusion pump after an initial loading dose (200 mg/kg in 0.5-ml increments every 20 minutes) was administered. Blood pressure was monitored using the arterial cannula and used in conjunction with the corneal reflex to ensure an adequate level of anesthesia. Body temperature was kept at 38°C using a rectal temperature monitor and warming blanket. Both eyes were used sequentially in a single experiment and after fixing the cat's head into a head holder, one or two drops of 0.03% flurbiprofen sodium (Ocuphen, Allergan Marhan, Ontario, Canada) were placed on the cornea of the first eye. The order in which the eyes were used alternated from experiment to experiment. Some tissue and a small amount of bone were removed from the temporal portion of the orbit to expose the eye after which the conjuctiva was attached to a fixed stainless steel eye ring. The animals were allowed to breathe spontaneously, however, PO₂, PCO₂, and pH were monitored periodically and a ventilator used if the values fell out of normal physiological range. Intraocular pressure was maintained and monitored at 20 mmHg by placing a 22-gauge needle into the anterior chamber and connecting it to an elevated saline reservoir and pressure transducer. The pupil was dilated with topical atropine (1%) and a plano-concave lens placed over the cornea. Guidelines set forth in the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research were strictly followed.

**Data Acquisition**

To allow passage of the endoscope into the eye, a trocar made from a thin-wall, 18-gauge needle was placed through the sclera at the equator after local administration of 0.5% proparacaine hydrochloride (Alcain, Alcon Laboratories, Fort Worth, TX). The retina and tip of the trocar were viewed through the pupil using an operating microscope (OPMI; Carl Zeiss, Thornwood, NY) as the endoscope, held by the robotic manipulator, was advanced through the trocar and into the eye. Once within the eye, the manipulator could position the endoscope over roughly two thirds of the desired sampling range. This necessitated varying the initial angle of approach of the endoscope within the eye between experiments, typically alternating between observing capillary networks near the optic disc and those further toward the periphery.

An advantage of using a robotic manipulator to hold the endoscope for data acquisition was that, after registering the robot to landmarks within the eye, the position of the endoscope tip could be determined in angular eye coordinates. A coordinate system was developed which defined Φ as the angular distance from the optic disc to the sampled region with the origin located at the center of the eye (Fig. 1). A second variable, Θ, described the angular distance around the equator of the eye from the superior vessels to the sample point.
Retinal coordinates were given in terms of angular distance from the optic disc, $\Phi$, and the angular distance around the equator from the superior vessels, $\Theta$. A single ($\Theta, \Phi$) coordinate corresponded to a unique location on the surface of the retina.

With the position of both the eye and robot fixed, a simple registration procedure was used to define transformation parameters required to convert robot coordinates into eye coordinates. This registration was performed by positioning the operating microscope such that the optic disc was centered within the pupil. Because a plano-concave lens was placed on the cornea to facilitate retinal viewing and the orientation of the plano-concave lens affected the accuracy of registration, care was taken to always position the lens normal to the microscope so as to minimize refractive effects. Data points were acquired with the tip of the endoscope placed at several locations along an imaginary line between the center of the pupil and the center of the optic disc. The linear best fit through the collected data points was used to determine the central axis of the eye in the robot coordinates. Once the central axis was determined, the tip of the endoscope was placed at the superior vein approximately three disc diameters from the optic disc, and another data point was recorded. This established the orientation of the eye near the central axis and provided enough information to fully register the eye with respect to the robot.

While acquiring data, the location of each data point in robot coordinates was saved to a data file. These locations, along with the entry point of the endoscope, were used to determine the center and radius of the eye using an algorithm that determined the best fit of the data to a sphere by minimizing the associated squared error terms. The center of the sphere approximated the center of the eye and served as the origin for the eye coordinate system. The vector starting from the center of the eye and pointing in the direction of the central axis vector defined the location of the optic disc ($\Phi = 0^\circ$) on the retina in robot coordinates.

We found that, although red blood cells could be resolved using standard xenon lamp illumination, contrast was enhanced by placing a high pass yellow filter (OG 515) in front of the CCD camera and allowing a single intravenous injection of fluorescein (0.025 mg/kg) to diffuse into the tissue for several minutes before acquiring data. Once some fluorescein had diffused out of the choroidal vessels, the background intensity of the images was brighter than both plasma and erythrocytes, making it easier to detect capillaries and their contents.

For each experiment, more than 30 retinal capillary networks located within the working range of the manipulator were randomly selected for data acquisition. At each of the selected points, 15 seconds of video footage was recorded on tape. The data acquisition system automatically placed a time stamp on the video tape and wrote a corresponding log file to disc with information including endoscope tip position, blood pressure, intraocular pressure, and image scale.

**Determining Red Blood Cell Velocity**

Data analysis was performed after the experiment in two steps. The first step was a conversion of data from video tape to reduced digital format, and the second step calculated retinal capillary red blood cell velocity and normalized optical density from the reduced data sets. The data was reduced from video to digital format using a real time PCI frame-grabber card (Imaging Technology, Bedford, MA) housed in a data acquisition computer (P100; Micron Computers, Boise, ID) and a custom data conversion program. With the video for a single 15-second data segment displayed on the computer monitor, an operator highlighted one of typically 10 visible capillary segments using the mouse (Fig. 2). After the desired capillary was highlighted, the video tape was rewound and started again from the beginning of the video sequence. The data acquisition program recorded the intensity along the capillary for each video frame and wrote the information to a data file when acquisition was complete. When the image intensity along the length of the capillary for sequential frames was examined, red blood cells within the capillary segment appeared as low intensity regions that moved along the capillary over time (Fig. 3).
FIGURE 3. Red blood cells within a capillary segment appeared as low intensity regions that moved with time as shown in the sequential frames above. Only the even frames are shown for compactness. By correlating the patterns of red blood cells within the capillary, the distance blood traveled between frames was determined and used to calculate the red blood cell velocity.

The acquired sequences formed a two-dimensional data map in which the abscissa represented the distance along the capillary and the ordinate represented the video frame number (time). The gray-scale intensity at each point in the map represented the intensity measured from the video at the corresponding frame number and distance along the capillary. A representative data map is shown in Figure 4, generated after removal of noise and illumination gradients from the original video footage. Note that each horizontal slice is equivalent to the traces shown in Figure 2 and that the sloping horizontal streaks are caused by red blood cells traversing the capillary.

The data maps were analyzed using a time-based correlation algorithm to determine the slope (time/distance) of distinct features in the data sets. The inverse of the slope (distance/time) represents red blood cell velocity within the given capillary. To determine the slope of features in the data sets, a correlation algorithm calculated the time shift of each time sequence along the length of the capillary, which best matched the profile of a reference time sequence. A time sequence is the intensity versus time data for a fixed location on the capillary. The correlation algorithm was repeated using all possible time sequences as reference profiles, while noting which reference profile resulted in the greatest strength of correlation.

The strength of correlation was determined by plotting the resulting time sequence shift versus distance along the capillary. The reference time sequence was given a position and time-shift value of zero, and a linear regression was performed (Fig. 5A). If the squared linear regression coefficient was not greater than 0.9, the time sequence corresponding to the data point furthest from the regression line was marked as invalid, and the regression was performed again. The marking of data points as invalid continued until a satisfactory regression coefficient was obtained (Fig. 5B). The removal of invalid data was a necessary step in the analysis to account for the operator placing part of the selected region off of the capillary and to account for regions along the capillary that were of particularly low contrast. The velocity was equal to the inverse slope of the linear regression, whereas the strength of correlation was evaluated as the percentage of sample points along the capillary that remained valid after forcing the squared linear regression coefficient to be greater than 0.9. Only data sets having at least 90% of the original data included in the regression were counted as valid data sets.

Determining Relative Red Blood Cell Density

Relative red blood cell density was determined from the normalized optical density of the data sets by comparing the

![Figure 4. The intensity information along the length of a capillary for sequential video frames was used to form a two-dimensional data map as shown above (noise and illumination gradients have been removed from the raw data). The abscissa represents distance along the capillary, and the ordinate represents video frame number (time). The sloping horizontal streaks were caused by red blood cells traversing the capillary, whereas the inverse slope of the streaks is red blood cell velocity (distance/time).](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933427/ on 12/03/2018)
average pixel intensity along a capillary segment during the sample interval with the minimum and maximum values which were measured. This required constructing an intensity data series with sufficient resolution to observe a single cell. Because a red cell may have moved further between video frames than the width of an individual cell, it was necessary to use the spatial resolution along the length of the capillary (approximately 1 μm) to create this series. The distance a cell travels between video frames was known from the calculated velocity and used to offset the intensity profile along the capillary for sequential frames. Valid points in the shifted profiles were averaged with overlapping regions from previous frames. Once the intensity versus distance profile had been constructed, the optical density algorithm described by Ellis was implemented using the following equation:

\[
\text{Optical density} = 1 - \frac{(I_{\text{ave}} - I_{\text{min}})}{(I_{\text{max}} - I_{\text{min}})}.
\]

The normalized average intensity was subtracted from unity to make samples with a greater percentage of red blood cells (darker average intensity) have a larger optical density value.

**RESULTS**

The data sets, which had a velocity linear regression coefficient of greater than 0.9 with more than 90% of the data set marked as valid, were used to determine variations in retinal capillary velocity and normalized optical density as a function of both Φ and Θ. In this series of experiments, the endoscope was placed through the temporal portion of the sclera at the equator thus limiting the data collection area to only the nasal and superior regions. Variations in retinal capillary velocity and optical blood density were tested for as a function of Θ within the sampled area but were not statistically significant. To test for variations as a function of distance from the optic disc (Φ), the sampled area was divided into two zones, each having a range of Φ = 30°. The number of valid data points within each region was \(N = 230\) for zone I (0° < Φ < 30°) and \(N = 391\) for zone II (30° < Φ < 60°).

The distribution of velocities for retinal capillary vessels as a function of Φ within the sampled region is shown in Figure 6. Erythrocyte velocities for zone I were found to range between 0.09 mm/second and 2.73 mm/second with a mean velocity of 0.70 mm/second and standard deviation of ± 0.39 mm/second. Zone II velocities ranged from 0.13 mm/second to 2.09 mm/second having a mean and standard deviation of 0.57 ± 0.28 mm/second. The mean (Z test) and variance (F test) of erythrocyte velocity in the peripheral capillaries (zone II) were found to be significantly lower (\(P < 0.005\)) than near the optic disc (zone I).

In contrast, the mean and variance of normalized optical density in the retinal capillaries were found to increase with increasing Φ and were significantly greater (\(P < 0.005\)) in the peripheral capillaries (zone II) than near the optic disc (zone I). The distribution of normalized optical density versus Φ is shown in Figure 7. The normalized optical density values for zone I ranged between 0.36 and 0.64 with a mean value and standard deviation of 0.50 ± 0.052. For zone II, the normalized optical density values ranged from 0.35 to 0.71 with a mean and standard deviation of 0.54 ± 0.061.

**DISCUSSION**

The velocity of erythrocytes within retinal capillaries near the optic disc (zone I) of the cat were found in this study to range between 0.09 mm/second and 2.73 mm/second with a mean...
The general heterogeneity of superficial retinal capillary velocity is apparent from the data in Figure 6 and has been noted by other investigators. From Figure 6, it is also apparent that there are trends in the regional velocity distribution which are noteworthy. In particular, whereas the minimum velocity values are approximately the same near the optic disc and in the periphery, the maximum velocity values decrease with increasing angular distance from the optic disc (Φ) resulting in decreased mean velocity and variance. This decrease in maximum capillary velocity from the disc to the periphery may in part be explained by a reduction in perfusion pressure across capillary networks. Glucksberg showed that the pressure of retinal arteries in cat decreases with vascular generation, whereas the pressure in the veins is both significantly higher than intraocular pressure and relatively constant. This means that perfusion pressure, the impetus for driving blood from the arterial to the venous circulation, decreases toward the periphery, possibly limiting the maximum capillary velocity. Although regional variation of capillary hemodynamics has not been previously reported, it is interesting to note that Laatikainen and Hill have both reported a decrease in velocity and standard deviation of 0.70 ± 0.39 mm/second. This compares well with the velocity of fluorescein-encapsulated liposomes in the macular capillaries of the cynomolgus monkey as measured by Khoobehi and Peyman who reported velocities ranging from 0.45 mm/second to 1.33 mm/second with a mean velocity of 0.76 mm/second. Nishiwaki et al. report a similar velocity for stained leukocytes within the perifoveal capillaries of 0.92 ± 0.32 mm/second as did Riva and Petrig who reported leukocyte velocities of 0.5 mm/second to 1.0 mm/second within human macular capillaries using the blue-field entoptic phenomenon.

The normalized optical density was used as a relative indicator of capillary erythrocyte concentration and was found to be significantly greater (P < 0.005) in the periphery than near the optic disc. Optical density for a capillary segment was calculated by comparing the average intensity measured along the capillary during the sample time with the minimum and maximum measured intensity values.
maximum arteriolar flow rates in the periphery when compared with regions near the optic disc.

The erythrocyte velocity within capillaries of any localized region of the retina was quite heterogeneous with distributions shown in Figure 6. This heterogeneity may be caused by several mechanisms, including active regulation of blood flow and vessel geometry. The retinal circulation has been shown to regulate retinal blood flow in a variety of studies including elevated intraocular pressure and hypoxemia. Evidence from intravital microscopy and oxygen tension measurements suggest that the retinal microcirculation, like circulation from other tissue, may exhibit flow variations in the form of vasomotion. We have not observed evidence of vasomotion in the feline retina, but this may be attributed to our short (15 second) data acquisition times.

The heterogeneity of capillary blood velocity may depend upon capillary geometry as well. Ben-nun has described at least two types of capillary morphologies in the cat retina consisting of simple (relatively straight with few curves) and complex (many curves) pathways that exhibit significantly differing flux rates. Several studies also suggest that circulating leukocytes move more slowly than erythrocytes, presumably because of their large size, reduced deformability, and potential to adhere to the vascular endothelium. Capillaries are small and cells move through the capillary network in single-file manner. A difference between leukocyte and erythrocyte velocities therefore leads to speculation that leukocytes travel through preferred channels and possibly contribute to the heterogeneity of capillary velocity as well.

It is interesting to note that, unlike Fillacier and Nishi-waki, who reported various degrees of transient leukocyte stasis, we concur with Ben-nun and report observing only a steady, unvarying flow in every capillary examined. There are two possible reasons for this. First, the GRIN endoscope did not reliably resolve leukocytes without fluorescent tagging and thus we might have missed some events. However, it is unlikely that a capillary could become plugged without affecting the erythrocyte motion, and in one experiment where we injected 15-µm fluorescent microspheres into a saphenous vein, the visible microspheres clearly stopped erythrocyte flow in nearby capillaries. The second possibility is that previous studies either introduced activated leukocytes from a donor animal, or insufficient spatial resolution to accurately measure instantaneous leukocyte velocity, or used staining procedures that might contribute to heightened leukocyte-endothelium interaction. It was only in several trials, in which we used acridine orange and followed the staining procedures described by Nishiwaki, where we observed stasis, sometimes lasting for several minutes. We attribute this stasis and its long duration to the phototoxic effects of acridine orange and the light required for endoscopic imaging. The scanning laser ophthalmoscope uses a lower light level and thus may not activate the dye in the same manner.

Retinal capillary hemodynamic quantification techniques, which rely on viewing the retina through the cornea and lens, do not have sufficient spatial resolution to ascertain instantaneous capillary velocity. In systems limited to 10 µm of spatial resolution at 30 frames/second, the minimum instantaneous velocity that can be accurately measured without the object mathematically appearing to “stick” is 0.3 mm/second. In capillaries with a blood velocity of 0.09 mm/second (the minimum value measured in this investigation) over three video frames would be required to see a leukocyte move one pixel. While the mean velocity and transit time of fluorescently labeled blood cells or plasma can be accurately measured through the pupil, the fact that a slow-moving leukocyte cannot be differentiated from a transiently sticking leukocyte must be considered. Previous studies reporting a transient plugging of leukocytes may have actually observed leukocytes with velocities below the measuring capabilities of their instrument.

The normal cat has three major artery vein pairs emanating from the optic nerve head, each responsible for supplying blood to roughly one third of the retina. The vessels are long, reaching from the optic disc to the periphery, and go through several generations of dichotomous and side-arm branching. Bifurcations of retinal arteries are rarely sized symmetrically, and the larger branch generally delivers blood to a more peripheral location than its smaller counterpart. Because erythrocytes have been shown to preferentially remain in the branch with the greatest flow, the retinal vessels have an efficient system for plasma skimming. As blood leaves the optic disc and travels toward the periphery, the erythrocytes preferentially stay in the larger vessels, whereas plasma is skimmed by the smaller dichotomous and side-arm branches. This tends to concentrate the red blood cells in the peripheral vessels and may, in part, explain the increased relative red blood cell density in the periphery as shown by Figure 7.

The combination of decreased blood velocity and increased erythrocyte concentration is apparent when viewing the microcirculation of the retina with the GRIN lens endoscope. In the periphery, most capillaries are perfused with long, slow-moving trains of red blood cells. Near the optic disc, however, the erythrocytes in general travel faster than in the periphery and are usually not clumped together. We suggest that this may help explain why retinopathy associated with sickle cell anemia, a disease characterized by abnormal erythrocytes, generally begins in the peripheral regions of the retina, in which capillary velocity is lower and the erythrocytes are more densely packed.

Although we have demonstrated a regional variation in retinal capillary velocity and erythrocyte density, a similar variation in retinal oxygen tension has not been reported. This is not surprising because most oxygen is carried by red blood cells, making the oxygen supplied to a region of the retina primarily dependent on the total number of erythrocytes passing through a capillary network per unit time (flux). Red blood cell flux (and hence the oxygen-carrying capacity of blood) will increase with both increased blood velocity and increased red blood cell density. Our techniques are currently not capable of measuring absolute red blood cell flux; however, the inverse relationship of capillary velocity to red blood cell density as a function of distance from the optic disc will tend to equilibrate oxygen delivery to the retina. While the anatomy of the eye possibly contributes to this inverse relationship, the retinal circulation regulates local flow in response to the oxygen content of blood and will contribute as well.

GRIN lens endoscopy provides an alternate high-resolution platform for performing in vivo retinal hemodynamic studies; however, there are several limitations of this technique that must be considered. The instrument itself is an endoscopic tool, which must be inserted through the sclera and held very near the retina to observe the retinal microcirculation. Although it is unlikely that using an endoscope affects the retinal circulation, the technique described in this study is invasive and currently appropriate only for use in animal models.
When used to observe capillary networks, the endoscope had a limited field of view (0.5 mm in diameter). This necessitated use of a manipulator not only to hold the endoscope steady during data acquisition but to aim the endoscope at different regions of the retina. The protocol used in this study called for inserting the endoscope through the temporal portion of the eye, leaving the area centrals inaccessible.

In addition to physical limitations, the device also has a maximum velocity limitation, measurable by using the data analysis algorithms described in this study. To accurately measure erythrocyte velocity within a capillary, the same erythrocyte must be visible within a given capillary segment for at least two frames, preferably more. At a video frame rate of 30 frames/second and a typical capillary segment length of 0.3 mm, the maximum velocity measurable was 4.5 mm/second. This value decreased for short capillaries and might prevent accurate measurement of short capillary segments containing high velocity erythrocytes.

Acknowledgments
The authors thank Raj Attariwala for experimental help, Dominick Beck and Pascal Rol for technical expertise with the endoscope, J. Edward Colgate and Kenneth Grace for technical assistance with the manipulator, and Frederick Bernstein and Michael Lin for data analysis.

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