

This article was downloaded by:[JHU John Hopkins University]
[JHU John Hopkins University]

On: 12 June 2007

Access Details: [subscription number 738314495]

Publisher: Informa Healthcare

Informa Ltd Registered in England and Wales Registered Number: 1072954

Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Microcirculation

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713723262>

A Computer-Based Method for Determination of the Cell-Free Layer Width in Microcirculation

Sangho Kim ^a; Robert L. Kong ^a; Aleksander S. Popel ^b; Marcos Intaglietta ^a; Paul C. Johnson ^a

^a Department of Bioengineering, University of California, San Diego, La Jolla, California, USA

^b Department of Biomedical Engineering, Johns Hopkins University, Baltimore, Maryland, USA

To cite this Article: Kim, Sangho, Kong, Robert L., Popel, Aleksander S., Intaglietta, Marcos and Johnson, Paul C., 'A Computer-Based Method for Determination of the Cell-Free Layer Width in Microcirculation', *Microcirculation*, 13:3, 199 - 207

To link to this article: DOI: 10.1080/10739680600556878

URL: <http://dx.doi.org/10.1080/10739680600556878>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article maybe used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

© Taylor and Francis 2007

A Computer-Based Method for Determination of the Cell-Free Layer Width in Microcirculation

SANGHO KIM,* ROBERT L. KONG,* ALEKSANDER S. POPEL,† MARCOS INTAGLIETTA,* AND PAUL C. JOHNSON*

*Department of Bioengineering, University of California, San Diego, La Jolla, California, USA; and †Department of Biomedical Engineering, Johns Hopkins University, Baltimore, Maryland, USA

ABSTRACT

Objectives: The cell-free layer between the erythrocyte column and the vessel wall is an important determinant of hydrodynamic resistance in microcirculatory vessels. The authors report a method for continuous measurement of the width of this layer.

Methods: The light intensity of a linear array of pixels perpendicular to the vessel axis is continuously determined from a video image of a microcirculatory vessel. A threshold level based on Otsu's method is used to establish the interface between the cell-free layer and the erythrocyte column. To test the method, video images at 750–4500 frames/s were obtained from venules and arterioles in rat spino-trapezius muscle at normal and reduced arterial pressures before and after induction of erythrocyte aggregation with Dextran 500. The current measurements were compared to manual measurements of the same images.

Results: Values obtained by the manual and the new methods were in agreement within the 95% confidence limit by the Bland-Altman analysis and within 90–95% range by the correlation coefficient (R^2). The more frequent measurements reveal substantial, rapid variations in cell-free layer width and changes in mean values with alteration of arterial pressure and red cell aggregability.

Conclusions: A new, computer-based technique has been developed that provides measurements of rapid, time-dependent variations in the width of the cell-free layer in the microcirculation. *Microcirculation* (2006) **13**, 199–207. doi:10.1080/10739680600556878

KEY WORDS: axial migration, hemodynamics, in vivo blood rheology, line intensity scanning method

Viscosity is one of the variables in the Hagen-Poiseuille relationship describing laminar flow in a circular tube. Application of this relationship to blood flow in microcirculatory vessels is often used for an approximation of the factors that determine microcirculatory flow and pressure gradients. One of the limitations of this application is that blood viscosity is not constant but is a function of a number of factors, including hematocrit, tube diameter, degree of red blood cell aggregability, and red blood cell flexibil-

ity. It is known from studies in small glass tubes that effective viscosity at low shear rates is significantly affected by the width of the cell-free layer between the red blood cell column and the tube wall (18–20). With the exception of studies of the glycocalyx in capillaries (25,26), information on the separation between the red blood cell column and the vessel wall has been limited to visual estimates with an eyepiece micrometer in vivo or manual determinations from video images (15,23,24).

The cell-free layer is formed by axial accumulation of red blood cells. This effect contributes in part to the decrease of local hematocrit in microvessels (Fahraeus effect) and to the decrease of apparent viscosity (Fahraeus-Lindqvist effect) (14). In vitro studies (2,7,9,19,20) have shown that flow resistance in narrow tubes is reduced with formation of a cell-free layer as red blood cell aggregation occurs. In contrast, some in vivo studies (4,5,15,24) showed that flow resistance in microvessels increases as high molecular weight dextrans are infused and flow rates are

Supported by NIH grants HL 52684, HL 64395, and HL 62354. The authors thank Scott Dunning and Jong Hoon Park for their expert technical assistance. Marcos Intaglietta is chairman of the board of the La Jolla Bioengineering Institute. Paul C. Johnson is a senior scientist at the La Jolla Bioengineering Institute.

Address correspondence to Paul C. Johnson, PhD, Department of Bioengineering, University of California, San Diego, La Jolla, CA 92093-0412, USA. E-mail: pjohnson@bioeng.ucsd.edu

Received 23 July 2005; accepted 6 October 2005.

reduced. To better understand the influence of red blood cell aggregation on the flow dynamics of red blood cells in microcirculatory systems, quantitative information on the relationship between the cell-free layer and the flow patterns of red blood cells and their aggregates is required.

Although the formation of the cell-free layer in vivo has been of interest for many years, due to the complexity of the vascular network, it has not been feasible to predict its occurrence and importance. Current methods of determining the width of the cell-free layer mainly rely on manual measurements from video microscopy and/or digitized images (1,2,7,15,23,24). For example, Maeda and coworkers (15,23,24) used recordings of successive images obtained with a video camera at a normal frame rate of 30/s to measure the width of the cell-free layer. Measurements were taken at multiple points along the vessel per frame and averaged. However, to obtain data on the time-dependent changes in width of the cell-free layer, a rapid succession of measurements at a single site is needed. The method described here provides such information at a spatial resolution less than the dimensions of a single red blood cell. This method provides new information on the width of the cell-free layer.

METHODS

Animal Preparation

Animal handling and care were provided in accordance with the procedures outlined in the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996). The study was approved by the local Animal Subjects Committee. Wistar-Furth rats weighing 160–220 g were anesthetized with an intraperitoneal injection of 50 mg/kg pentobarbital sodium (Abbott). The rat spinotrapezius muscle was exteriorized and prepared for intravital microscopy. An intravital microscope (Ortholux II, Leitz) transilluminated with a 100-W mercury lamp (model 1149, Walker Instruments, Scottsdale, AZ) was used with an Olympus $\times 40$ water-immersion objective and a long working distance condenser (Instec, Boulder, CO), which have numerical apertures of 0.7 and 0.35, respectively. A blue filter (model 59820, Spectra-Physics, Stratford, CT) was used to enhance the contrast between the red blood cells and the background field. Focused images were projected onto a high-speed video camera (model FASTCAM ultima SE, Photron USA), capable of recording up to

4500 frames/second and storing up to 24,576 frames in the internal memory. Details of the animal preparation and experimental setup used in the present study have been described previously (12), and the reader is referred to those studies for more complete information.

Pressure, Hematocrit, and Aggregation Measurements

Arterial pressure was continuously recorded and stored using a physiological data acquisition system (MP 100 System, BIOPAC Systems, Goleta, CA). Blood samples of approximately 0.1 mL were withdrawn from the carotid artery catheter during an experiment for hematocrit and degree of red blood cell aggregation measurements, which were taken during control as well as after infusion of Dextran 500, as described previously (12). The degree of red blood cell aggregation was determined with a photometric rheoscope (Myrenne Aggregometer, Myrenne, Roentgen, Germany) and is presented in terms of the M index where M is a function of the degree of aggregation. Experiments were performed on rats both under nonaggregating normal conditions ($M = 0$) and after infusion of Dextran 500 to increase red blood cell aggregability to the range of healthy humans ($M = 12$ – 16).

Experimental Protocol

In a previous study of aggregate size in venules (3), it was possible in some cases to construct images of red blood cell aggregates following hemodilution by monitoring light intensity across the vessel at a specific location. Dimensions of aggregates were then determined manually. With that experience as background, we sought to develop a technique to continuously measure cell-free layer width automatically.

Long segments of skeletal muscle venules or arterioles with no branches in the field of view were selected for the study using the criteria of stable flow as well as clear focus and good contrast of the image. The microscope was focused on the equatorial plane of the microvessel and the high-speed video camera was used to record the flow pattern of red blood cells in the vessel for 2–5 s. The frame rate was adjusted to maximize image clarity for the specific flow and background conditions. In the present study, frame rates of 750/s–4500/s were used. In general, higher frame rates gave us more distinct edge definition of red blood cell flow but lower contrast with the background. Recordings were obtained from microvessels

at normal arterial pressure. Red blood cell aggregation was then induced by infusion of Dextran 500 (total 200 mg/kg body wt) and the microcirculatory observation was repeated at reduced arterial pressure and flow rate achieved by removing blood via the carotid artery into a heparinized syringe.

Determination of Vessel Wall Location

Digital video clips stored in the control unit of the high-speed camera were transferred to a hard drive in the microcomputer for analysis and storage. Using Adobe Premier 5.1, the video files were then converted to image files (BMP format) with 640×480 resolution. Figure 1A shows a videomicrograph of a typical vessel studied under transillumination. In the present study, the interface between the endothelial surface and the plasma appeared to transition from dark to light over two pixels. Based on the criterion for the determination of the edge of the endothelial surface suggested by previous investigators (10,22), the light pixel was considered as the inner surface of the vessel. The location of the endothelial surface

along an analysis line was measured repeatedly on 10 randomly selected images. As reported in a previous study (12), the error associated with this technique was estimated to be $\pm 0.5 \mu\text{m}$.

Determination of Erythrocyte Velocity

Mean centerline and edge velocities of the red blood cell column were determined using a video sampler (27) and cross correlator (11). Pseudoshear rate (γ) was calculated from the following equation:

$$\gamma = \frac{V}{D} \quad (1)$$

where V is mean centerline velocity of red blood cells and D is vessel diameter. The edge velocity of the red blood cell column in the vessel is necessary to calculate the spatial resolution of the cell-free layer measurement. When it was not feasible to measure the edge velocity by the cross-correlation technique, the movements of red blood cell column edge were manually tracked across 10 digital frames using commercially available image analysis software (SigmaScan

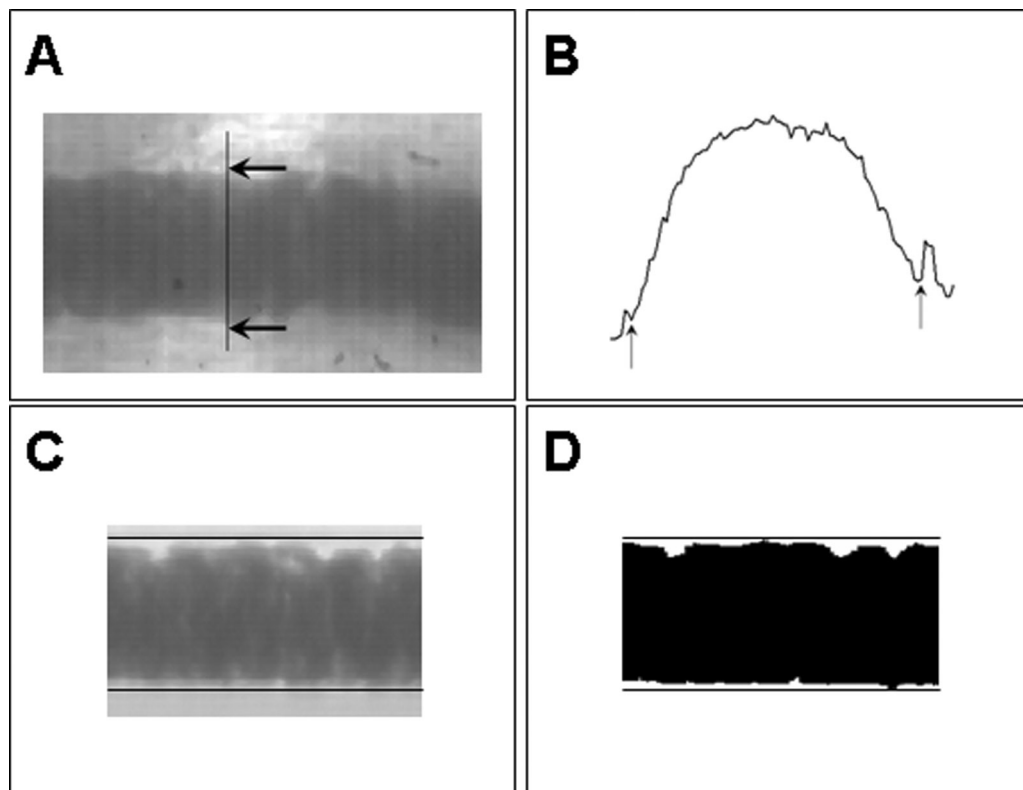


Figure 1. Computer-based method for cell-free layer determination. (A) Videomicrograph of red blood cell flow in a venule. (B) Intensity-level histogram for analysis line. (C) Grayscale intensity image. (D) Binary image. Arrows in (A) and (B) and solid lines in (C) and (D) represent location of vessel wall.

Pro, Aspire Software Intl, Leesburg, VA). The velocity of the column edge between each frame was then averaged. The edge velocity measurement was repeated at a total of 10 locations along the red blood cell column and averaged.

Statistics

All the data are reported as means \pm SD. All statistical tests were done using a commercially available software package (Prism 4.0 for Windows). For all tests, $p < .05$ was considered statistically significant.

IMAGE ANALYSIS

Establishment of Analysis Line

For an image such as shown in Figure 1A, the first frame of the control was examined for a specific area that had a clear vessel wall and red blood cell flow column with a high-contrast resolution. A line for intensity analysis was established, with the ends of the lines extending beyond the outer vessel wall and positioned perpendicular to the vessel wall. The recorded images were examined for movement of the vessel within the timeframe of the recording, as well as for any shift from the previous condition. If movement was observed, the line of analysis was shifted to compensate.

Light Intensity Data Analysis

Figure 1B shows an intensity-level histogram obtained along the line shown in Figure 1A. Usually more than 3000 successive frames were used for the analysis, depending on the frame rate used for recordings. Using a commercially available image processing software package (MATLAB, Mathworks, Natick, MA), each frame was converted from an RGB color image into a grayscale intensity image as shown in Figure 1C. This is accomplished by converting the RGB values to NTSC coordinates, which allows for the hue and saturation components of the picture to be reduced to zero. The image is then converted back into the RGB color space as an intensity image. Each digitized image was then filtered using a median filter, which removed "salt and pepper" noise (random pixels being set to black or white), and reduced impulsive noise (signals that deviate greatly from the surrounding signals). Maintaining the contrast between the background and cell column was critical to the determination of the diameter of red blood cell flow column. As expected, thinner areas in the mus-

cles were found to provide greater light transmittance and a better contrast between the red blood cell flow column and the background. The line intensity data was then stored into an image matrix, with each row representing the line intensity data for one frame.

The image-processing software package was used to convert the intensity image to a binary image. Otsu's method (16) was used to establish the gray-threshold level from the new image matrix. Otsu's method is an automatic thresholding method designed to choose a threshold level for the binarization of an intensity image. In short, the pixels are divided into 2 classes: the object pixels (black) and the background pixels (white). Those pixels whose intensity falls below the thresholding level are determined to be background pixels, and those that are above the thresholding level are determined to be object pixels. The variance between the two color classes is then calculated, based on the histogram of the image. The threshold level is shifted until the between-class variance is maximized. The edge of the cell column is assumed to be above the threshold determined by the Otsu's method. Once the image is binarized, the edge of the cell column is determined to be the first object pixel encountered in each row. It is necessary to convert the image into binary format to quantify the red blood cell flow column as well as to accurately determine the width of the cell-free layer. Figure 1D shows the binary image of the red blood cell flow column.

Analyses of Erythrocyte Flow Column and Cell-Free Layer

The binary images were used to determine the width of the cell-free layer near each wall and the width of the red blood cell column. Since black pixels represented the areas of light absorption by hemoglobin, the location of the outer edges of the cell column for each row was determined by the position of the outermost black pixels in the row. The position of the inner surface of the vessel wall together with that of the outer edge of the red blood cell column was used to determine the width of the cell-free layer near each wall.

Determining Validity of the Method

To validate our method for in vivo flow situations, the location of the edge of a red blood cell flow column in a venule was determined both manually and using the new method. Measurements were compared in every twentieth frame. Next, the width of the cell-free

layer at each side of wall was calculated and the results obtained by the two methods were statistically compared.

Considering the manual measurement as the reference, a Bland-Altman analysis (6) and a linear regression curve were used to determine the accuracy of the new method. The Bland-Altman analysis provides a more extensive analysis of the agreement between two different methods of measurement than the linear regression analysis. If the difference in resulting values obtained from the two techniques is not large enough to affect interpretation, then the new method is verified to be interchangeable with the established method.

RESULTS

Validation of the Method

Figure 2 shows the location of the edge of a red blood cell flow column in a venule ($39\text{-}\mu\text{m}$ i.d.) determined both manually and using the computer-based method. The measurements were taken in a dextran-treated rat ($M = 12.8$) at reduced arterial pressure (50 mmHg) using a frame rate of 750/s. The results obtained with our method appeared to be in good agreement with those determined manually, although the latter shows finer gradations; however, these gradations are smaller than the error ($0.5\ \mu\text{m}$) of the manual method (12).

Figure 3A shows the linear regression curve for the cell-free layer of one side of the vessel shown in Figure 2. The correlation coefficient was 0.904, indicating close agreement between the manual and line intensity scanning methods for that portion of the cell-free layer. Since the linear regression analysis may conceal some systematic differences between the two methods, the Bland-Altman analysis was also performed. Figure 3B shows the bias versus average chart for the Bland-Altman analysis of the same cell-free layer. The acceptable error (mean \pm 1.96 SD) was determined using the standard deviation (SD) of four repeated manual measurements. The bias of the two methods shown in the y axis indicates that the line intensity scanning method fell within the acceptable limit with a 95% confidence. No significant systematic difference between the two methods was detected. Therefore, the line intensity scanning method is interchangeable with the manual method for the determination of the cell-free layer. This process was repeated for the other side of the vessel, using the same analysis techniques. As shown in Figure 3C,

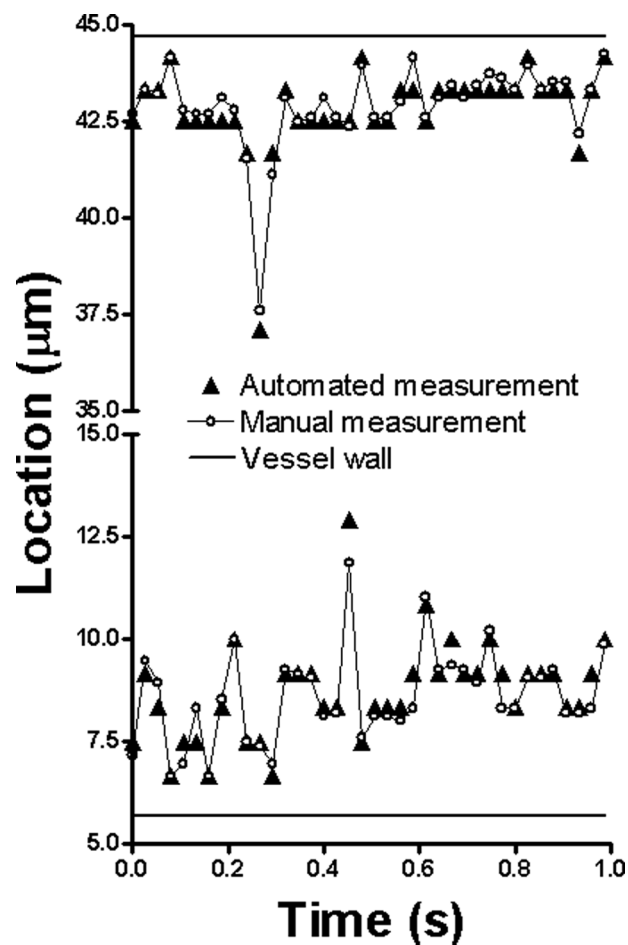


Figure 2. Location of the edge of the red blood cell column in a venule ($39\text{-}\mu\text{m}$ i.d.) measured manually and with the computer-based method. An overall close correspondence of the two measurements is evident. The manual method appears to show slightly finer gradations.

the correlation coefficient for the other cell-free layer was 0.943, also indicating high agreement between the two measurements. Figure 3D shows the Bland-Altman analysis for that portion of the cell-free layer. Thus, in these tests, no significant systematic difference between the two methods was found.

Cell-Free Layer Width

To test the system under conditions of an experiment, cell-free layer width measurements were performed in a venule ($35\text{-}\mu\text{m}$ i.d.) at control arterial pressure before dextran infusion (Figure 4A) and at reduced arterial pressure after dextran infusion (Figure 4B). Systemic hematocrit was 38% and the M value of aggregation was 0.0 in the control state and 14.6 after dextran infusion. The cell-free layer width on one

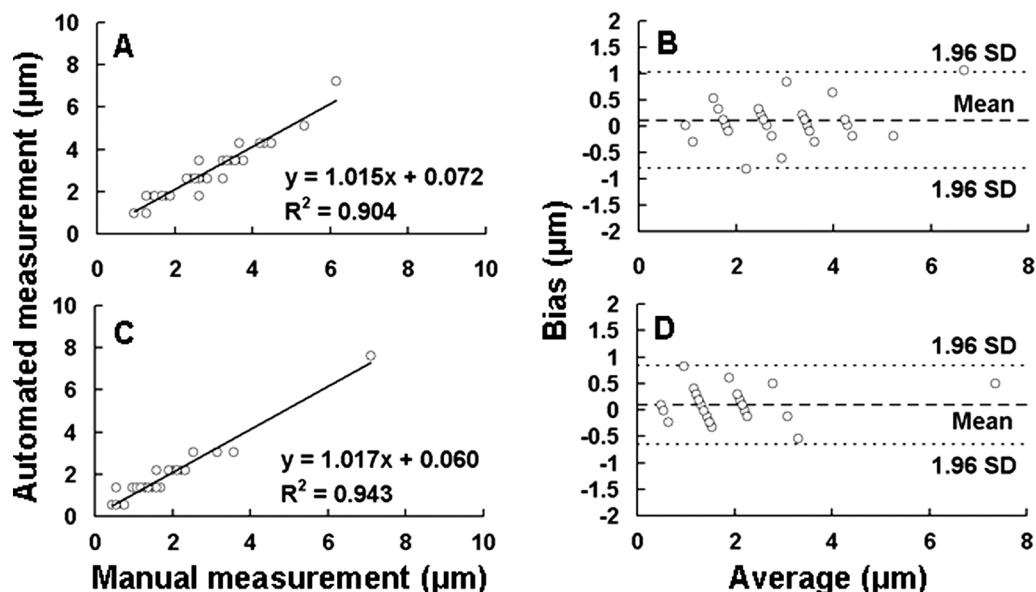


Figure 3. Statistical comparison of the manual and computer-based method for cell-free layer width measurement in a 39- μm venule. (A, C) Linear regression analyses for the two measurements of cell-free layer width on each side of the vessel. (B, D) Bland-Altman analyses for the same measurements. No significant systematic difference between the two methods was found by either analysis.

side of the vessel was compared under each condition. The length of the flow stream at the edge of red blood cell flow column is shown on the x axis and was calculated from the edge velocity. At an arterial pressure of 129 mmHg and pseudoshear rate of 100 s^{-1} before dextran infusion, the cell-free layer width was $3.1 \pm 0.6 \mu\text{m}$. Since the edge velocity of red blood cell column was approximately 1.9 mm/s and frame rate was 4500/s, the measurements in the axis direction were approximately 0.4 μm apart.

After dextran infusion and reduction of arterial pressure to 59 mmHg, pseudoshear rate was 46 s^{-1} and the edge velocity was 1.1 mm/s in the venule. A frame rate of 2250/s was used in this instance, providing repeated measurements in the axis direction approximately 0.5 μm apart. As shown in (B), the width of the cell-free layer increased to $5.3 \pm 1.4 \mu\text{m}$ and an increased amplitude of variations is evident in the axial as well as the radial direction, as would be expected with aggregate formation.

To test the applicability of our method to arterioles, measurements were performed in an arteriole (22- μm i.d.) without dextran ($M = 0$) at control arterial pressure (117 mmHg) and a pseudoshear rate of 450 s^{-1} , as shown in Figure 4C. Systemic hematocrit was 39%. The cell-free layer width in the arteriole was $1.0 \pm 0.5 \mu\text{m}$. Since the edge velocity of

red blood cell column was approximately 7.5 mm/s and frame rate was 4500/s, the measurements in the axis direction were approximately 1.7 μm apart.

DISCUSSION

The present study introduces a computer-based method of quantifying the cell-free layer in microcirculatory vessels. Rapid measurements of the width of the red blood cell column together with vessel diameter were obtained with a high-speed video camera and binary image processing. When the method was compared to manual measurement, no significant difference was apparent. The high time resolution of the computer-based method reveals a dynamic and variable structure of the cell-free layer in microcirculatory vessels. It would not be feasible to obtain such detailed information by manual methods.

Previous in vitro and in vivo studies (1,2,15,23,24) reported the formation of cell-free or cell-poor layers under various conditions. The methodology used in those studies for determining the width of the cell-free layer relied either on visual observation of the microcirculation and a calibrated eyepiece or manual analysis of video replay taken at several points along the blood vessel. Such determinations can in

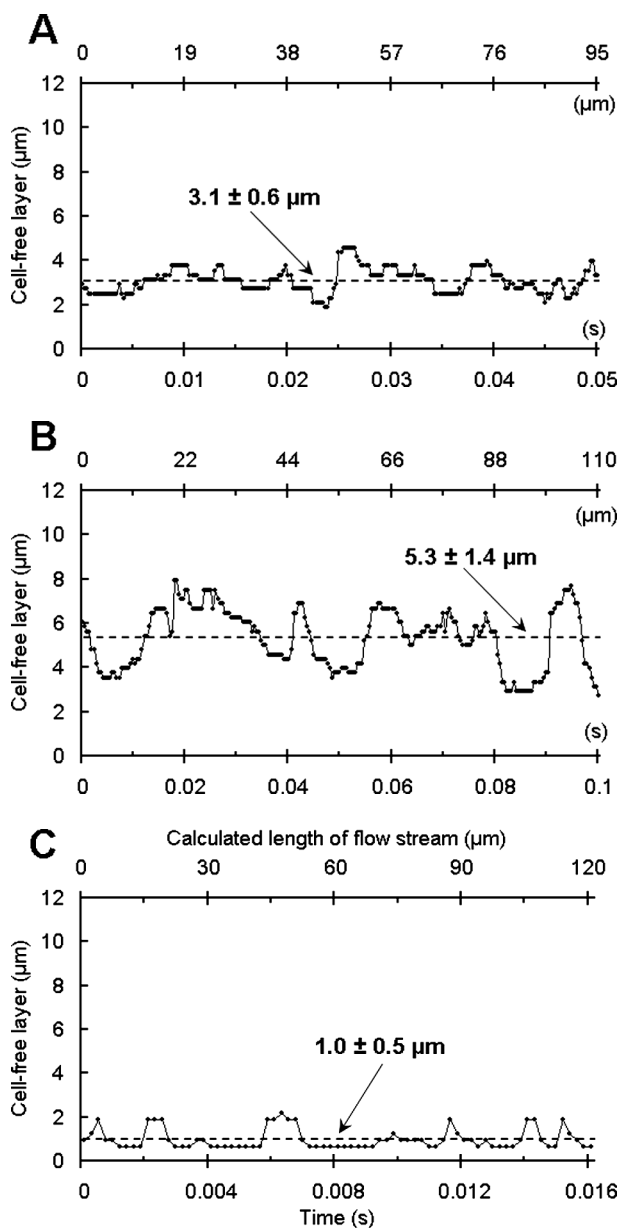


Figure 4. Cell-free layer width at one wall of microvessels. (A) 35- μm -i.d. venule at an arterial pressure of 120 mmHg before dextran infusion. (B) The same venule at an arterial pressure of 59 mmHg after dextran infusion. (C) 22- μm -i.d. arteriole at an arterial pressure of 117 mmHg without dextran treatment. Dotted lines represent mean values of cell-free layer width. The length of the flow stream shown on the x axis was calculated from the velocity at the interface between red blood cell column and cell-free layer.

principle provide an estimate of average and statistical variation, but are too infrequent to obtain detailed information on temporal or spatial variation of the cell-free layer.

In the present study, we assume that the glycocalyx layer at the endothelial surface is included in the cell-free layer since it is not observable with light microscopy (8,13). Its thickness would be approximately $0.5 \mu\text{m}$ in the microvessels used in this study based on studies by Damiano and coworkers (8,13).

For the determination of cell-free layer width, the position of the outermost red blood cell was used as the interface between the red blood cell column and the plasma layer. If individual cells are separated from the column, the intervening plasma space would be considered as part of the red blood cell column by our method in its current configuration. This is consistent with the definition of the cell-free layer, as opposed to a cell-poor layer. This method may not be useful in smaller vessels or in low hematocrit situations where there is not a distinct interface between a red blood cell column and a cell-free layer. Based on our experience, it would appear that this technique could be used successfully with other microcirculatory preparations and microscope systems in which image light intensity and contrast are sufficient for the video camera at the high frame rates used in the present study.

At the magnification used in the present study (i.e., field size of $200 \times 200 \mu\text{m}$), the limitation on precision of the cell-free layer measurement in the axial and radial directions imposed by the camera was the pixel size ($\sim 0.4 \mu\text{m}$). In Figure 2, it is evident that the resolution in the radial direction for this method is somewhat less than for the manual method, which is likely a result of thresholding process and the median filter. In addition, the manual method allows interpolation between pixels. To achieve a spatial resolution in the axial direction equal to pixel size in this study requires a ratio of frame rate to edge velocity in mm/s of 2500:1, while a spatial resolution equal to the minimal dimension of the red cell, $\sim 2 \mu\text{m}$ (12), requires a ratio of 500:1. The latter ratio is the minimum required to ensure that the measurement includes every red blood cell at the interface. If the edge velocity of the red blood cell column becomes higher than 9 mm/s , as would be expected in large arterioles at normal arterial pressures, a frame rate above 4500/s would be required to detect all red blood cells.

In a previous study with the same microscope and video system (12), we reported a mean error of $\pm 0.5 \mu\text{m}$ for the manual measurement of vessel diameter or red blood cell location. Those measurements were made with a frame rate to velocity ratio of 3600:1. Since our tests indicate that this method is

equivalent to manual measurement, we would expect that the error of our measurements would be similar at the same frame rate to velocity ratio. Errors in the axial direction would become proportionately greater as the ratio decreased and the separation between measurements exceeded $0.5 \mu\text{m}$.

As shown in Figure 4, this method can be applied to determine both mean cell-free layer width and its variations in arterioles and venules. While analysis of causal mechanisms will require separate study, it appears from Figure 4 that factors such as vessel diameter, flow rate, and degree of red cell aggregability may lead to differences in mean width and/or variability.

A key feature of the present technique is that it provides information on variations in cell-free layer width that apparently influence the effective viscosity of blood in microcirculatory vessels. As Popel and coworkers have pointed out previously (17,21), the presence of the “rough” interface between the red blood cell core and the cell-free layer should lead to additional viscous dissipation. They demonstrated that this dissipation contributed to an increase of the effective viscosity. Thus, it is important to obtain information on the variations of the cell-free layer as well as its mean value in order to better understand its possible influence in the microcirculatory system. The new method described in this report provides a means to obtain such information.

REFERENCES

- Alonso C, Pries AR, Kiesslich O, Lerche D, Gaehtgens P. (1995). Transient rheological behavior of blood in low-shear tube flow: velocity profile and effective viscosity. *Am J Physiol* 268 (*Heart Circ Physiol* 37):H25–H32.
- Alonso C, Pries AR, Gaehtgens P. (1993). Time-dependent rheological behavior of blood at low shear in narrow vertical tubes. *Am J Physiol* 265 (*Heart Circ Physiol* 34):H553–H561.
- Bishop JJ, Nance PR, Popel AS, Intaglietta M, Johnson PC. (2004). Relationship between erythrocyte aggregate size and flow rate in skeletal muscle venule. *Am J Physiol Heart Circ Physiol* 286:H113–H120.
- Bishop JJ, Nance PR, Popel AS, Intaglietta M, Johnson PC. (2001). Effect of erythrocyte aggregation on velocity profiles in venules. *Am J Physiol Heart Circ Physiol* 280:H222–H236.
- Bishop JJ, Popel AS, Intaglietta M, Johnson PC. (2001). Effects of erythrocyte aggregation and venous network geometry on red blood cell axial migration. *Am J Physiol Heart Circ Physiol* 281:H939–H950.
- Bland JM, Altman DG. (1986). Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1:307–310.
- Cokelet GR, Goldsmith HL. (1991). Decreased hydrodynamic resistance in the two-phase flow of blood through small vertical tubes at low flow rates. *Circ Res* 68:1–17.
- Damiano ER. (1998). The effect of the endothelial-cell glycocalyx on the motion of red blood cells through capillaries. *Microvasc Res* 55:77–91.
- Goldsmith HL. (1971). Red blood cell motions and wall interactions in tube flow. *Fed Proc* 30:1578–1588.
- Gretz JE, Duling BR. (1995). Measurement uncertainties associated with the use of bright-field and fluorescence microscopy in the microcirculation. *Microvasc Res* 49:134–140.
- Intaglietta M, Tompkins WR, Richardson DR. (1970). Velocity measurements in the microvasculature of the cat omentum by on-line method. *Microvasc Res* 2:462–473.
- Kim S, Popel AS, Intaglietta M, Johnson PC. (2005). Aggregate formation of erythrocytes in postcapillary venules. *Am J Physiol Heart Circ Physiol* 288:H584–H590.
- Long DS, Smith ML, Pries AR, Ley K, Damiano ER. (2004). Microviscometry reveals reduced blood viscosity and altered shear rate and shear stress profiles in microvessels after hemodilution. *Proc Natl Acad Sci USA*, 101:10060–10065.
- Maeda N. (1996) Erythrocyte rheology in microcirculation. *Jpn J Physiol* 46:1–14.
- Maeda N, Suzuki Y, Tanaka J, Tateishi N. (1996). Erythrocyte flow and elasticity of microvessels evaluated by marginal cell-free layer and flow resistance. *Am J Physiol* 271 (*Heart Circ Physiol* 40):H2454–H2461.
- Otsu N. (1979). A threshold selection method from gray-level histograms. *IEEE Trans Syst Man Cybern* 9:62–66.
- Popel AS, Johnson PC. (2005). Microcirculation and hemorrheology. *Annu Rev Fluid Mech* 37:43–69.
- Pries AR, Neuhaus D, Gaehtgens P. (1992). Blood viscosity in tube flow: dependence on diameter and hematocrit. *Am J Physiol* 263 (*Heart Circ Physiol* 32):H1770–H1778.
- Reinke W, Johnson PC, Gaehtgens P. (1986). Effect of shear rate variation on apparent viscosity of human blood in tubes of 29 to 94 μm diameter. *Circ Res* 59:124–132.
- Reinke W, Gaehtgens P, Johnson PC. (1987). Blood viscosity in small tubes: effect of shear rate, aggregation, and sedimentation. *Am J Physiol* 253 (*Heart Circ Physiol* 22):H540–H547.
- Sharan M, Popel AS. (2001). A two-phase model for flow of blood in narrow tubes with increased effective viscosity near the wall. *Biorheology* 38:415–428.
- Smith ML, Long DS, Damiano ER, Ley K. (2003). Near-wall μ -PIV reveals a hydrodynamically relevant

- endothelial surface layer in venules in vivo. *Biophys J* 85:637–645.
23. Soutani M, Suzuki Y, Tateishi N, Maeda N. (1995). Quantitative evaluation of flow dynamics of erythrocytes in microvessels: influence of erythrocyte aggregation. *Am J Physiol* 268 (*Heart Circ Physiol* 37):H1959–H1965.
 24. Tateishi N, Suzuki Y, Soutani M, Maeda N. (1994). Flow dynamics of erythrocytes in microvessels of isolated rabbit mesentery: cell-free layer and flow resistance. *J Biomech* 27:1119–1125.
 25. Vink H, Duling BR. (2000). Capillary endothelial surface layer selectively reduces plasma solute distribution volume. *Am J Physiol Heart Circ Physiol* 278:H285–H289.
 26. Vink H, Duling BR. (1996). Identification of distinct luminal domains for macromolecules, erythrocytes, and leukocytes within mammalian capillaries. *Circ Res* 79:581–589.
 27. Wayland H, Johnson PC. (1967). Erythrocyte velocity measurement in microvessels by a two slit photometric method. *J Appl Physiol* 22:333–337.