Improved instrumentation for blood flow velocity measurements in the microcirculation of small animals

Jayme Alves de Mesquita, Jr. and Eliete Bouskela
Biomedical Instrumentation Laboratory, Institute of Biology, State University of Rio de Janeiro, 20550-013 Rio de Janeiro, Brazil; Faculty of Engineering, State University of Rio de Janeiro, 20550-013 Rio de Janeiro, Brazil; and Microcirculation Research Laboratory, Institute of Biology, State University of Rio de Janeiro, 20550-013 Rio de Janeiro, Brazil

Eliane Wajnberg
Coordination of Applied Physics, Brazilian Centre of Physics Research, 20550-013 Rio de Janeiro, Brazil

Pedro Lopes de Melo[a)]
Biomedical Instrumentation Laboratory, Institute of Biology, State University of Rio de Janeiro, 20550-013 Rio de Janeiro, Brazil; Faculty of Engineering, State University of Rio de Janeiro, 20550-013 Rio de Janeiro, Brazil; and Microcirculation Research Laboratory, Institute of Biology, State University of Rio de Janeiro, 20550-013 Rio de Janeiro, Brazil

(Received 5 January 2007; accepted 22 January 2007; published online 28 February 2007)

Microcirculation is the generic name of vessels with internal diameter less than 100 μm of the circulatory system, whose main functions are tissue nutrition and oxygen supply. In microcirculatory studies, it is important to know the amount of oxyhemoglobin present in the blood and how fast it is moving. The present work describes improvements introduced in a classical hardware-based instrument that has usually been used to monitor blood flow velocity in the microcirculation of small animals. It consists of a virtual instrument that can be easily incorporated into existing hardware-based systems, contributing to reduce operator related biases and allowing digital processing and storage. The design and calibration of the modified instrument are described as well as *in vitro* and *in vivo* results obtained with electrical models and small animals, respectively. Results obtained in *in vivo* studies showed that this new system is able to detect a small reduction in blood flow velocity comparing arteries and arterioles ($p<0.002$) and a further reduction in capillaries ($p<0.0001$). A significant increase in velocity comparing capillaries and venules ($p<0.001$) and venules and veins ($p<0.001$) was also observed. These results are in close agreement with biophysical principles. Moreover, the improvements introduced in the device allowed us to clearly observe changes in blood flow introduced by a pharmacological intervention, suggesting that the system has enough temporal resolution to track these microcirculatory events. These results were also in close conformity to physiology, confirming the high scientific potential of the modified system and indicating that this instrument can also be useful for pharmacological evaluations.
© *2007 American Institute of Physics.* [DOI: 10.1063/1.2668504]

I. INTRODUCTION

Microcirculation is the generic name of the finest level of the circulatory system. Adequate function of the microcirculation is a prerequisite for tissue nutrition and oxygen supply.[1,2] In studies of microcirculatory oxygen transport, it is important to know the amount of oxyhemoglobin present in blood and how fast it is moving. Blood flow velocity measurements in the microcirculation find applications in several physiological studies, as cerebral microcirculation,[3] and in numerous diseases, e.g., diabetes mellitus,[4] chronic ulcers, hypertension, or sepsis,[2,5] which are associated with structural changes of microvessels and/or functional defects, as well as evaluation of pharmacological treatments.

Despite the pivotal role of microcirculatory changes in numerous diseases, practical techniques for the measurement of blood flow velocity in the microcirculation remain limited. There is extensive literature data on this subject.[6–13] Therefore only a brief summary of the most popular technique used in physiological research, including its principles, merits, and also its drawbacks, is presented here.

A. Dual slit technique for the measurement of blood flow velocity in the microcirculation

The dual slit method proposed by Wayland and Johnson[11] and modified by Tompkins *et al.*[14] is the most widely used method for measuring flow velocity in the microcirculation. In this method, two photometric windows (slits) are positioned on the microscopic image perpendicular to the vessel axes in order to capture a time dependent signal for each window caused by the passage of red blood cells (RBCs) through the microvessels (Fig. 1).

This method is based on the observation that at the capillary level RBCs pass in a single file, with nonuniform

[a)]Author to whom correspondence should be addressed; FAX: 5521-2587-7760; electronic mail: plopes@uerj.br

 © 2007 American Institute of Physics

Downloaded 01 Mar 2007 to 152.84.252.224. Redistribution subject to AIP license or copyright, see http://rsi.aip.org/rsi/copyright.jsp

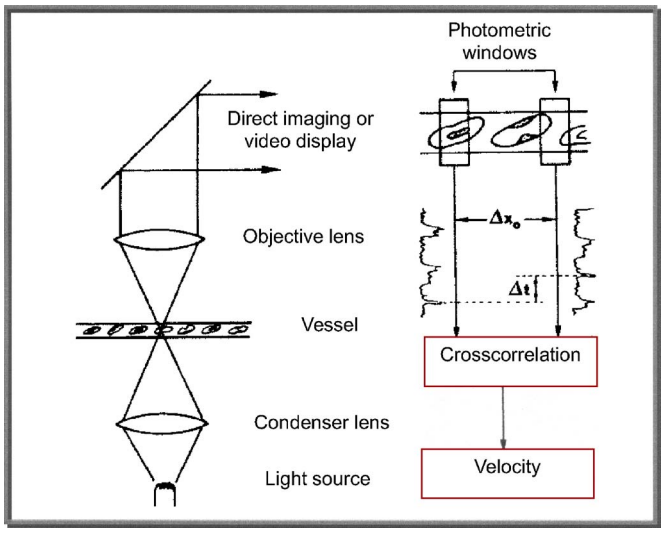

FIG. 1. Schematic representation of the temporal correlation method for red blood velocity measurements. The temporal light intensities are collected from two photometric windows (adapted from Ref. 7).

plasma spacing in between. Since these patterns remain stable over short distances, they can be used to determine transit time over a certain distance (Fig. 1). The downstream signal is delayed with respect to the upstream signal due to the separation between the detectors (d). Red blood cell velocity (V_{RBC}) may be calculated by the ratio of the slit separation and the time delay between the two signals (t_d, $V_{RBC} = d/t_d$).

The availability of a commercial instrument, which implements this technique, has been particularly valuable, allowing many individuals to measure RBC velocities in a variety of tissues (IPM, San Diego, California). This instrument is based on an online cross correlation[8,15] and can be used to measure high erythrocyte velocities.

1. Present technical limitations

Unfortunately, some limitations in this classical device have been recognized regarding practicability and dynamic characteristics. (1) The analog online system originally described by Intaglietta and Tompkins in 1971 (Ref. 8) works in real time, but the detection of the cross correlogram peak is not automatic. The system gives information about the velocity by means of manual tuning of instrument parameters (e.g., systems clock frequency), being susceptible to operator related biases.[6,16] (2) The manual search of the measured velocity results in a single velocity value, which may cause a nonreproducible measurement.[16] (3) The system does not follow the time changes of velocity and, therefore, it gives only average results.[16] (4) The results cannot be saved in digital format, which may hinder their reproduction and access after the experiment.

2. Goals

In order to overcome the limitations described above, the purpose of this work was twofold: (1) describe the modifications conducted in a commercially available unit and (2) evaluate the performance of the modified instrument, reporting *in vitro* and *in vivo* results obtained with electrical models and small animals, respectively.

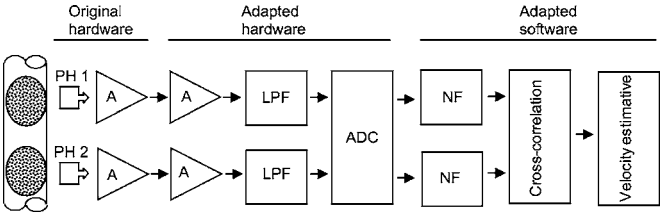

FIG. 2. Block diagram of the modified instrument. PH, photosensor; A, amplifier; LPF, low-pass filter; ADC, analog-to-digital converter; and NF, notch filter.

First a detailed description of hardware and software improvements is presented, together with details of the velocimeter calibration procedure and results of bench studies evaluating static errors. Then, *in vivo* results from the application of the modified device in several microcirculatory vessels are described and, finally, the performance of the modified instrument in pharmacological interventions is shown.

II. INSTRUMENT MODIFICATIONS

A commercially available unit from Instrumentation for Physiology and Medicine (IPM, San Diego, California) was selected for modification. The system is based on the dual slit technology[11] described in the Introduction section. A simplified block diagram of the implemented modifications is shown in Fig. 2.

The original instrument includes a photosensor head in which the signals associated with the passage of red blood cells flowing through the microvessels are sensed by two photodiodes and amplified by low-noise devices (AD OP-27). Using these analog signals, we developed a virtual cross-correlation analyzer for the velocimeter whose details are presented in the next sections.

A. Hardware

The modifications introduced in the instrument included an additional stage with variable gain and offset adjust, where the voltages from the original sensor head were amplified in order to adapt the photometric signals to the analog-to-digital conversion. Then, these signals were filtered using an analog 10 kHz low-pass filter (Butterworth, fifth order). This bandwidth was dimensioned in order to allow an adequate dynamic response to measure time variable velocities, minimizing the mean square error.[17] Processed signals were adapted to the virtual velocimeter, which was implemented on a platform constituted by a mother board Pentium IV 2.4 GHz with 256 Mbyte RAM and 38 Gbyte HD. Besides standard peripherics (17 in. monitor, keyboard, and mouse) the system includes a board (PCI 6024, National Instruments, Austin, TX) for signal acquisition.

Downloaded 01 Mar 2007 to 152.84.252.224. Redistribution subject to AIP license or copyright, see http://rsi.aip.org/rsi/copyright.jsp

B. Cross-correlation design and software implementation

The velocity measurement, which was determined using digital cross-correlation techniques, is based on the estimation of the time delay between the upstream and downstream signals. In this implementation, based on the works of Stott[18] and Beck,[19] the first problem was the definition of the sampling frequency and the number of acquired samples. Low sampling frequencies and/or number of samples do not allow accurate determination of blood flow velocity. On the other hand, high sampling frequency would increase the analysis time. These variables were calculated taking into consideration the following parameters:

- Minimal blood flow velocity (V_{min}=0.2 mm/s).
- Maximal blood flow velocity (V_{max}=50 mm/s).
- Sensor spacing (L=10 μm).
- Worst case errors in the delay detection (ε=2%).[19]
- Interval between measurements (T_m=1 s).

Minimal and maximum velocities used (V_{min}, V_{max}) were obtained from microcirculatory studies[20] and from information obtained with the technical staff of the Microcirculation Laboratory, respectively. Using V_{min}, V_{max}, and L values, minimum (τ_{min}) and maximum (τ_{max}) delays were determined applying the relationship $v=\Delta x/\Delta t$, corresponding to τ_{min}=200 μs and τ_{max}=50 ms. The minimal number of samples in the correlation (n_{min}) was determined from ε ($\varepsilon=1/2n_{min}$), corresponding to n_{min}=25 samples. The range (R_g) of the instrument, the ratio of the maximum and minimum velocities we want to measure, was determined (R_g=250), which allowed us to calculate the maximum number of samples (n_{max}), which is related with R_g and n_{min} through the relationship $R_g=n_{max}/n_{min}$, resulting in n_{max}=6250 points. The temporal resolution (δt) of the cross correlation was determined from the relationship $V_{max}=L/n_{min}\delta t$, corresponding to a δt=8 μs, resulting in a sampling frequency of 125 kHz.

The cross correlation was implemented using the LABVIEW™ 5.1 graphical "G" programing environment (National Instruments, Austin, TX). Since the microscopic optical signals were obtained from an inverted microscope (Zeiss, Model Wetzlar, Germany), whose illumination is derived from an ac lamp, the first signal processing is composed by notch filters (eight order centered in 60, 120, 180, and 240 Hz) in order to minimize spurious signals. Filtered signals were correlated in the time domain using standard LABVIEW™ cross-correlation algorithm, and the delay was determined from the detection of the cross-correlation peak. It is important to point out that the time delay values were accepted only if the corresponding correlation coefficient was larger than 0.7, as suggested by Hudetz *et al.*[3] The length of each sampling period was 500 ms, allowing the collection of 62 500 data points. With the objective of reducing the typical biological variability, existing in such systems, it is offered to the operator the possibility of using direct evaluations or to average the velocity values by 2, 4, or 8 sampling periods.

In order to make clinical applications easier for nontechnical personal, a dedicated user-friendly front panel was developed. This interface is shown in Fig. 3. In the front panel of the virtual instrument (VI) correlation coefficient, real-time RBC velocity, and a control to select the number of sampling periods used to average velocity are displayed. Indication of the beginning and the end of the evaluation, a real-time display of blood flow velocity versus time in a window of 10 s, together with the mean and standard deviation of the red blood cell velocity in this interval, and ASCII file format save option are some of the additional facilities provided to the user. A similar graphical interface, showing the two input optical signals, is also included in the software, allowing the user to adjust the offset and gain of the two associated signals to the range of the data acquisition board input (±10 V) before the beginning of the experiment. It allows optimizing the use of data acquisition board dynamic range.

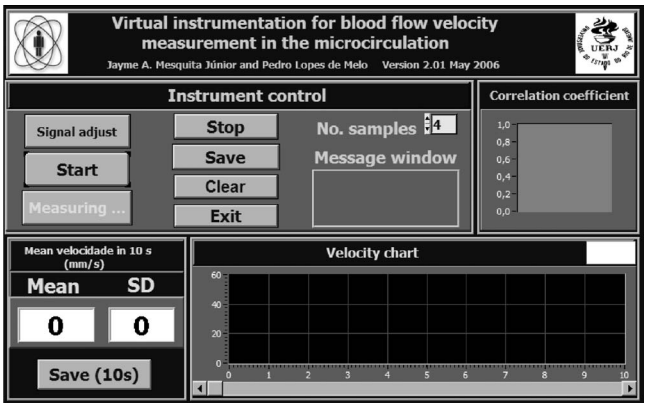

FIG. 3. Graphical user interface of the instrument providing real-time display of blood flow velocity vs time and calculating instantaneous velocity, as well as the correlation coefficient. The VI allows easy user configuration for a variety of experimental needs.

Red blood cell velocity includes a mean value and variations around it, associated with vasomotion cycles. These cycles are due to spontaneous rhythmic variations of vessel diameter, and their periods vary from 2 s to about 1 min.[7] Accurate data should reveal the vasomotion effect. Thus, maximal measurement interval of 1 s is adequate. The time length of the records being correlated (500 ms), summed with the computation time used to deal with uncorrelated data for each cycle of acquisition (380 ms), results in a total system measurement interval of 880 ms. This interval does not introduce a significant loss of information, since it is adequate to identify the vasomotion effect.

C. Cross-correlation errors

The analog signal conditioning, the analog-to-digital (A/D) conversion, the quantization processes, and the digital filtering involved can be considered as error sources in the cross correlation. The accuracy of the cross correlation was evaluated measuring electrical signals with well-defined time delays simulating relevant practical conditions. It consisted of a crystal-controlled 5 Hz square wave generator filtered by a first-order low-pass filter. Four analog delay lines with delays of 250 μs (τ_{min}), 15 ms, 30 ms, and 50 ms (τ_{max}) were studied. The delay lines were calibrated using a digital oscilloscope (Tektronics, TDS220). Table I shows that the

Downloaded 01 Mar 2007 to 152.84.252.224. Redistribution subject to AIP license or copyright, see http://rsi.aip.org/rsi/copyright.jsp

TABLE I. Errors in the cross-correlation subsystem.

Delays	Cross correlation (s)	Error (%)
250 μs	248 μs	0.8
15.00 ms	15.01 ms	0.1
30.00 ms	30.04 ms	0.1
49.00 ms	48.78 ms	0.5

percentage errors in the time delay range of the cross correlator found in the identification of the electrical delay lines were less than 1%, which were considered appropriate.

D. Velocimeter calibration procedure

The system was calibrated by a comparative analysis between experimental and predicted values obtained in a well-known mechanical model simulating RBC velocities from 1 to 50 mm/s. It consisted of a perfusion pump (Harvard Apparatus, 55-2222), with a 10 ml syringe adapted to a small animal plastic catheter (internal diameter of 500 μm). The blood was simulated using a graphite powder suspension in silicone oil. Theoretical values were calculated considering an incompressible fluid and using the classical continuity relationships.[21] Theoretical (V_{ref}) and experimental velocity (V_p) values are presented in Fig. 4.

The correlation between velocity values obtained in the reference system and in the modified instrument was very close to 1 (R=0.994). Errors described by the least squares method (V_p=−1.957+V_{ref}) were corrected by software.

III. PERFORMANCE TESTS

A. *In vivo* applications

Male golden hamsters (*Mesocricetus auratus*), seven to ten week old, weighing approximately 100 g, were used in *in vivo* measurements. Experiments were performed according to protocols approved by the Ethical Committee of the State University of Rio de Janeiro. Anesthesia was induced by an injection of sodium pentobarbital (Pentobarbital Sodique, Sanofi, Paris, France, 60 mg/ml) and maintained with α-chloralose [1,2-*O*-(2,2,2-trichloethylidene)-D-glucofuranose] (Merck, Darmstadt, Germany, 100 mg/kg). Throughout surgical procedures and during subsequent experiment, the temperature of the animals was kept at 37.5 °C

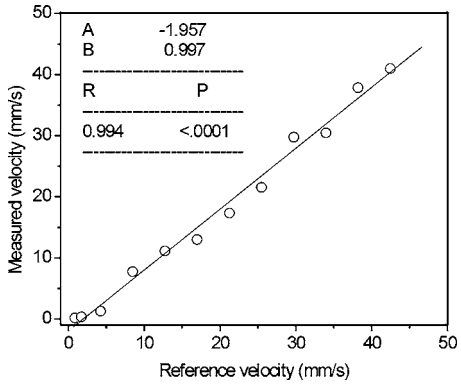

FIG. 4. Calibration curve of the instrument.

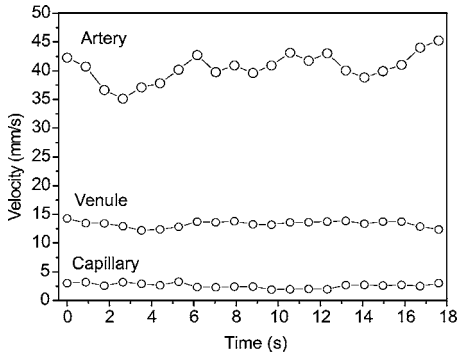

FIG. 5. Representative examples of time series of blood flow velocities in arteries, venules, and capillaries.

with a heating pad controlled by a rectal thermistor. A tracheal tube was inserted to facilitate spontaneous breathing. The hamsters were placed on a flexiglass stage similar to that described by Duling[22] and modified by Svensjo.[23] The tissue was transilluminated and the microcirculatory vessel image was aligned in the focal plane of the microscope (Zeiss, Model Wetzlar, Germany) using a 10× magnification objective (0.22).

Representative examples of typical results obtained measuring arteries, venules and capillaries are presented in Fig. 5. Variations in red blood cell velocity with time are consistent with those observed by Fleming *et al.*[6] and that reported by Renkin[24] and Slaaf *et al.*[7] In agreement with these researchers, we observed a pattern of temporal flow variability coherent with the physiological process of vasomotion. This process introduces continuous changes in microcirculatory vessel diameter, and hence flow, even under normal conditions.[7,25] These changes are part of the complex matching between oxygen supply and nutrients by blood and the demand of tissues.[25] Another explanation for these variations of flow velocity is the dependency of flow velocity on the cardiac cycle.[25,26] Red blood cell velocity in arteries, venules, and capillaries fluctuated within the ranges of 35.1–42.7, 12.2–14.3, and 1.9–3.3 mm/s, respectively. An interesting point in Fig. 5 is the reduction of variations of flow velocity comparing values obtained in a venule and a capillary with that obtained in an artery. This reduction reflects the condition that the pulsatile flow due to cardiac cycle is increasingly attenuated in more distal vessels.[25,26] These results give additional support to the use of the modified instrument as a versatile tool for microcirculatory studies.

Figure 6(a) presents results comparing mean blood flow velocities measured in ten samples of each type of microvessel studied (i.e., arteries, arterioles, capillaries, venules, and veins). The results were presented as mean±standard deviation. Comparisons between results were performed using t-test, and a value of $p<0.05$ was considered statistically significant. The total cross-sectional area of the cardiovascular system, the summation of the cross-sectional area of each vessel in parallel, increases as the aorta branches to arteries, arterioles, and capillaries [Figs. 6(b) and 6(c)]. The total flow is the same throughout the system, but the velocity in a segment is dependent on the total cross-sectional area of many parallel branches. Correspondingly, average blood flow ve-

Downloaded 01 Mar 2007 to 152.84.252.224. Redistribution subject to AIP license or copyright, see http://rsi.aip.org/rsi/copyright.jsp

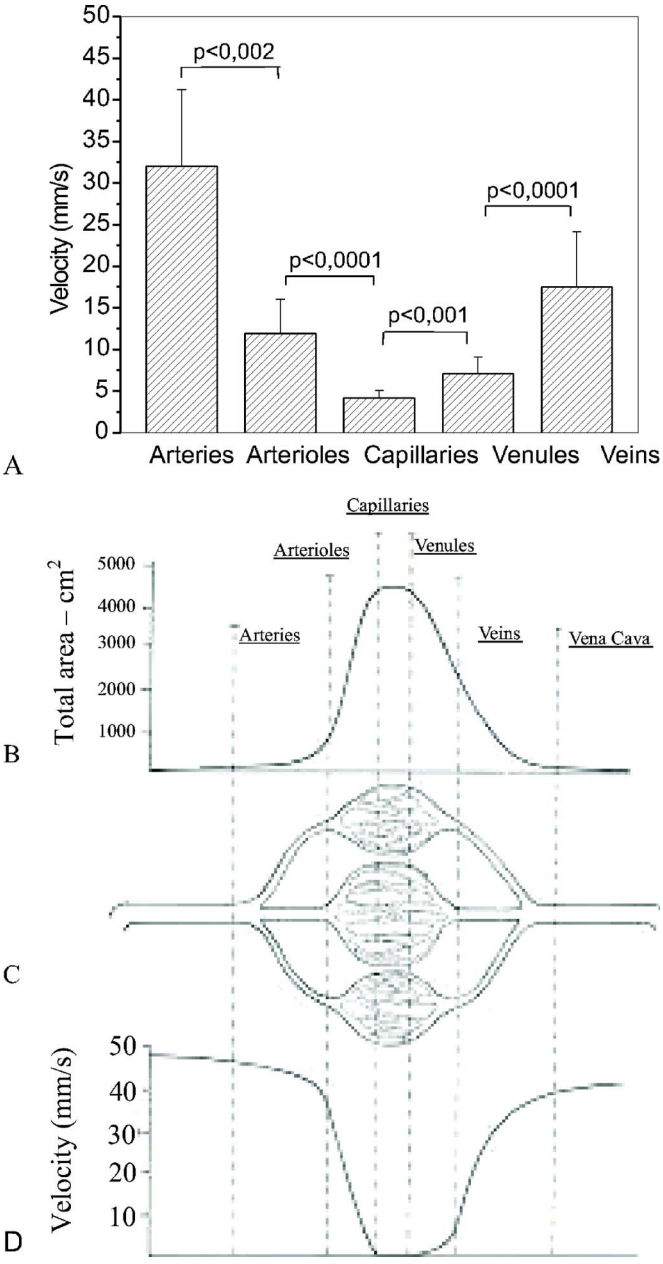

FIG. 6. Mean red blood cell velocities measured in arteries, arterioles, capillaries, venules, and veins (A). Estimated cross-sectional area (B), branching of the cardiovascular system (C), and blood flow velocity (D) for the cardiovascular tree. (B)–(D) adapted from Ref. 27.

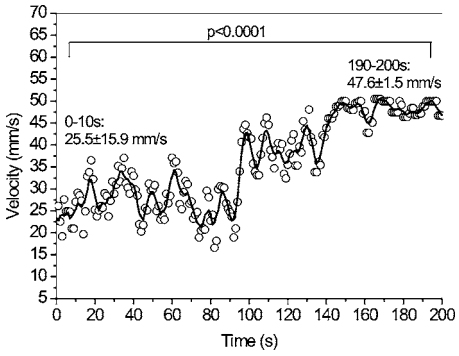

FIG. 7. Temporal change in blood flow velocity in response to local administration of phenylephrine. Circles indicate individual measurements, while the continuous line indicates mean of four samples.

locity decreases in small vessels in comparison with large arteries or veins [Fig. 6(d)].[27]

In close agreement with these biophysical principles, blood flow velocity was significantly reduced comparing arteries and arterioles ($p<0.002$) and further reduced in capillaries ($p<0.0001$) due to the increase in total cross-sectional area. The significant increase in velocity observed comparing capillaries and venules ($p<0.001$) and venules and veins ($p<0.0001$) was also consistent with these principles. In fact, the modifications observed in Fig. 6(a) are very consistent with theoretical predictions of Feigl, illustrated in Fig. 6(d).[27]

The results described in Fig. 6(a) are also consistent with the study developed by Slechta and Fulton[28] in hamsters, in which blood flow velocities in arterioles and in veins were measured producing results from 0.9 to 12.9 and from 0.4 to 6.6 mm/s, respectively. Similar values of velocities in arterioles and venules were also reported by Sato.[12] Measured values of velocity in arterioles described in Fig. 6(a) are also coherent with a study conducted by Intaglietta *et al.*,[29] which reported mean blood flow velocity measurements in arterioles around 10 mm/s. These results are also in close conformity with that obtained by Anand *et al.*[13] using the video shuttering technique and image analysis, in which mean velocities in arterioles were reported to be around 11.1 mm/s. Blood flow velocity values in capillaries described in Fig. 6(a) are consistent with the work of Schmidt-Schoenbein and Zweifach.[30] In the cited work, it was reported that in normal conditions, blood flow velocity in capillaries of rabbits ranges from 0.5 to 5 mm/s. These velocities are also similar to those recently observed by Jeong *et al.*,[31] using a high-speed video camera (between 1.25 and 2.95 mm/s), and those obtained by Hudetz *et al.*[3] measuring velocities in cerebral capillaries in mouse, who pointed out that 65% of the measurements were between 0.5 and 1.8 mm/s. Thus, the results presented in Fig. 6(a) are in agreement with physiological basis and previously reported results, confirming the adequate system performance.

Anand *et al.*[13] suggested that the capacity to measure velocities until 20 mm/s could be sufficient for several microvascular measurements. In line with these authors, Fig. 6(a) shows that, in hamsters, velocities in this range could be measured in arterioles, capillaries, and venules. However, to measure higher velocities, as observed in arteries and veins, a greater capacity is needed.

B. Pharmacological intervention

The blood flow velocity in one arteriole was analyzed before and after phenylephrine administration (Fig. 7). This pharmacological agent is a well-known vasoconstrictor, causing a decrease in vessel diameter in a few minutes. The blood flow velocity during the initial period, in the absence of pharmacological effect (phase 1 in Fig. 7), was characterized by smaller values. Continuous changes in observed blood flow velocity are consistent with the process of vasomotion[7] and the fact that flow velocity in arterioles varies during the cardiac cycle.[25,26] The reduction in arteriole

Downloaded 01 Mar 2007 to 152.84.252.224. Redistribution subject to AIP license or copyright, see http://rsi.aip.org/rsi/copyright.jsp

diameter, associated with the pharmacological effect, is clearly described by the increase in blood flow velocity (phase 2 in Fig. 7). More precisely, the pharmacological effect caused by phenylephrine was due to arteriolar smooth muscle contraction, resulting in a progressive narrowing of arteriolar internal diameter and, consequently, in larger values of blood flow velocity. Figure 7 also shows that, after the application of phenylephrine, blood flow velocities remained at increased values (phase 3). Sometimes, these abnormal velocities reached values larger than 50 mm/s, the maximal blood flow velocity specified in the instrument. This, indeed, is not a problem since the pharmacological effect is clearly described in the range of blood flow velocity measured by the instrument. The cited effect is clearly described in Fig. 7 by the highly significant increase in blood flow velocity observed considering a 200 s interval ($\Delta t_1 = 0-10$ s, $V_{RBC1} = 25.5 \pm 15.9$ mm/s; $\Delta t_2 = 190-200$ s, $V_{RBC2} = 47.6 \pm 1.5$ mm/s; t-test, $p < 0.0001$). Moreover, the improvements introduced in the device allowed us to observe continuous variations in blood flow in the microcirculation introduced by a vasoconstrictor agent. These results show that the system temporal resolution is adequate to track blood flow velocity changes in the microcirculation induced by phenylephrine, indicating that this instrument can be useful in pharmacological evaluations as well.

Manually operated procedures used in the classical instrument are time consuming and may not be sufficiently accurate. The usefulness of these procedures is further restricted due to the high number of measurements necessary to obtain statistical significant data. During the elaboration of these *in vivo* application examples, it was confirmed that, in comparison with the classical hardware-based instrument, the modified system demands much less operator evaluation or intervention in use. It requires only focusing, monitoring of vessel alignment on the photometric windows (slits), and adjustment of the offset and gain of the two associated signals. Therefore, there is a reduced possibility of operator error or bias with the modified system.

In conclusion, we have described significant improvements in a classical hardware-based device for the measurement of blood flow velocity in microvessels. Hardware- and software-based modifications were introduced to allow the automation of the measurement system. The new measurement procedure is organized as an open-architecture virtual instrument that samples optical signals from the microvessel under investigation and gives instantaneous velocity. The low cost modifications described in this article could easily be incorporated into existing classical hardware-based systems, which, otherwise, remain unchanged. The improved instrument can easily be modified further, in case of changes in the measurement task. It permits continuous visualization of blood flow velocity, and the possibility of saving the results in digital format, being notably more flexible than the hardware-based version currently in use. Moreover, automation of the measurement system contributes to reduce operator related errors. Examples of measurements in several microcirculatory vessels showed data in close agreement with previously published results and biophysical fundamentals, confirming the good features achieved in engineering tests. Based on these promising results, it can be anticipated that the proposed system can contribute to the production of more detailed descriptions on microcirculatory blood flow velocity.

ACKNOWLEDGMENTS

The authors wish to thank Fátima Cirino, Fabiana Gomes, and Cristiane M. S. Conde, for their assistance in the *in vivo* studies. This work was supported by FAPERJ and CNPq.

[1] B. I. Levy, G. Ambrosio, A. R. Pries, and H. A. Struijker-Boudier, Circulation **104**, 735 (2001).
[2] I. Tritto and G. Ambrosio, Cardiovasc. Res. **42**, 600 (1999).
[3] A. G. Hudetz, C. G. M. Weigle, F. J. Fenoy, and R. J. Roman, Microvasc. Res. **43**, 334 (1992).
[4] J. E. Tooke, Cardiovasc. Res. **32**, 764 (1996).
[5] H. A. Lehr, F. Bittinger, and C. J. Kirkpatrick, J. Pathol. **190**, 373 (2000).
[6] B. P. Fleming, B. Klitzman, and W. O. Johnson, Am. J. Physiol. **249**, H899 (1985).
[7] D. Slaaf, G. J. Tangelder, and R. Reneman, in *The Physics of Heart and Circulation*, edited by J. Strackee and N. Westerhof (Institute of Physics, Bristol, 1993), Chap. 19, pp. 383–416.
[8] M. Intaglietta and W. R. Tompkins, IEEE Trans. Biomed. Eng. **18**, 376 (1971).
[9] P. I. Brånemark and I. Johnson, Biorheology **1**, 143 (1963).
[10] P. A. G. Munro, in *Advances in Optical and Electron Microscopy*, edited by R. Barer and V. E. Cosslett (Academic, New York, 1966), pp. 1–40.
[11] H. Wayland and P. C. Johnson, J. Appl. Physiol. **22**, 333 (1967).
[12] M. Sato and N. Ohshima, Biorheology **25**, 279 (1988).
[13] A. P. Anand, A. J. Shruti, and N. P. Roland, Ann. Biomed. Eng. **27**, 313 (1999).
[14] W. R. Tompkins, R. Monti, and M. Intaglietta, Rev. Sci. Instrum. **45**, 647 (1974).
[15] W. R. Tompkins and M. Intaglietta, Rev. Sci. Instrum. **42**, 616 (1971).
[16] F. Sapuppo, D. Longo, M. Bucolo, M. Intaglietta, P. Arena, and L. Fortuna, in Proceedings of the 26th Annual International Conference of the IEEE EMBS, 2004, pp. 2219–2222.
[17] J. S. Bendat and A. G. Piersol, *Random Data, Analysis and Measurement Procedures*, 2nd ed. (Wiley, New York, 1980).
[18] A. L. Stott, Ph.D. thesis, University of Manchester, (1987).
[19] M. S. Beck and A. Plaskowski, *Cross Correlation Flowmeters: Their Design and Application* (Hilger, Bristol, 1987).
[20] R. A. Hoffman, P. F. Robinson, and H. Magalhães, *The Golden Hamster: Its Biology and Use in Medical Research* (The Iowa State University Press, 1968).
[21] D. Halliday, R. Resnick, and J. Walker, *Fundamentals of Physics Extended*, 5th ed. (Wiley, New York, 1997).
[22] R. Duling, Microvasc. Res. **5**, 423 (1973).
[23] E. Svensjö, Prostaglandins Med. **1**, 397 (1978).
[24] E. M. Renkin, in *Handbook of Physiology: The Cardiovascular System*, edited by E. M. Renkin and C. C. Michel (American Physiological Society, Bethesda, MD, 1984). Vol. IV, pp. 627–687.
[25] S. A. Popel and P. C. Johnson, Annu. Rev. Fluid Mech. **37**, 43 (2005).
[26] S. S. Singh and M. Singh, Med. Biol. Eng. Comput. **40**, 269 (2002).
[27] E. O. Feigl, in *Physiology and Biophysics*, 20th ed., edited by T. C. Ruch and H. D. Patton (W. B. Sounders, Philadelphia, 1974), pp. 10–22.
[28] R. F. Slechta and G. P. Fulton, in *The Golden Hamster: Its Biology and Use in Medical Research* edited by Roger A. Hoffman, Paul F. Robinson, and Hulda Magalhães (The Iowa State University Press, 1968).
[29] M. Intaglietta, W. R. Tompkins, and D. R. Richardson, Microvasc. Res. **2**, 462 (1979).
[30] G. W. Schmid-Schoenbein and B. W. Zweifach, Microvasc. Res. **10**, 153 (1975).
[31] J. H. Jeong, Y. Sugii, M. Minamiyama, and K. Okamoto, Microvasc. Res. **71** 212 (2006).

Downloaded 01 Mar 2007 to 152.84.252.224. Redistribution subject to AIP license or copyright, see http://rsi.aip.org/rsi/copyright.jsp