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Measurements of Blood Flow Velocity in the Microcirculation

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ABSTRACT

A method for the measurement of red blood cell velocity in the microcirculation is described. The technique is based on the on-line cross-correlation measurement of the delay between optical signatures derived from photodetectors aligned in direction of flow. A specialised photodiode holder which can be inserted directly in place of an ocular is described. The interdetector delay data is computed automatically from the cross-correlation function by a peak detector designed to provide a voltage proportional to the delay to maximum cross-correlation, regardless of intermediate lower peaks. Application of this system to measurements in the rabbit ear chamber, mesentery and skeletal muscle is described.

INTRODUCTION

The measurement of blood flow in the microcirculation has recently developed as a specialised technique incorporating distinct physical principles and methods which are not common to other types of flow measurement. The small size of the vessels excludes techniques which rely on volumetric determinations, and it has not been possible to use electromagnetic flow measuring systems which operate reliably in blood vessels smaller than 1 mm inside diameter.

Attention has therefore been directed to the optical properties of flowing blood as a means of making microvascular flow measurements. One of the first attempts on which many succeeding methods are based was that of Hugues (9) who devised a streak-image method to estimate velocity. At high magnification, blood acquires a granular appearance due to the presence of blood cells. The magnified image of flowing blood gives the appearance of streak lines parallel to the centerline of the blood vessel. When this image is projected onto photographic film, which is being moved in a direction at right angles to that of the blood vessel, the photographic image of the streaks will have an orientation determined both by the corpuscular velocity and the film velocity; thus, when the latter is known, the former can be calculated.

A modification of this method was used by Monro (15), who developed a rotating prism device from which visual estimates of red cell velocity can be made. In this modification, the speed of the prism (and thus the angle of the streak lines) is manually adjusted.

A simpler method can be utilised in capillaries where it is possible to compare the position of a red cell from one frame to another of either a cinefilm or a videotape recording, and thus the velocity of cells can be calculated in terms of their displacement as a function of time. This approach, used in conjunction with high-speed cinematographic methods, where individual cells could be discerned and their paths plotted, have been extensively utilised by Bloch (5) and have provided accurate measurements of flow in capillaries.

Brånemark & Jonsson (7) designed a system in which the velocity of the red blood cells was compared by eye with that of a moving illuminated spot. This technique, termed the "flying spot system", was utilised successfully in the measurement of flow velocity in capillaries.

Asano et al. (3) first proposed the use of the optical light transmission pattern of red blood cells, as they move across a photodetector, as a means of measuring their transit time, and Müller (16) implemented such a technique for capillaries. Wayland & Johnson (17), when studying the red blood cell flow in capillaries, small arterioles, and venules, utilised two

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photodetectors to measure the transit time of the projected image of red blood cells. In larger vessels, where individual red blood cells could not be discerned in the photometric signals, it was possible to measure the delay between the photometric signals from the upstream and downstream detectors by computing the time to maximum cross-correlation as a function of time delay.

This dual-slit technique was used on-line by Intaglietta et al. (10, 11, 12), with a specialised cross-correlation computer.

In this paper we describe the state-of-the-art of this methodology, as well as some of its applications in microcirculatory research.

Optical coupling

In the original schema of Wayland & Johnson (17) the preparation was transilluminated with a 100 Watt high-pressure mercury arc which was focused on the area under study by an objective lens utilised as a condenser. The image was then projected through the microscope optics onto a screen, so that the area under study could be viewed directly by reflection from the screen. The screen was penetrated by two slits (hence the name "dual-slit method"), and the back of the slit was optically coupled via a lucite rod to a photomultiplier. In this configuration, the vessel under study is first located by direct observation through the microscope ocular. Once it is identified, the intense light beam is turned on and the image is projected on the screen and focused sharply. The vessel is then aligned so that the two slits are located along the centerline of the vessel. This is accomplished by moving the preparation on the stage, fine adjustments being made by moving a projection prism mounted on the ocular. Angular adjustments are made by rotating the screen.

A somewhat different technique has been developed in our laboratories. The image of the vessel under study is projected on a ground glass surface, which is positioned along the extension of the optical axis of the microscope ocular (Fig. 1). This configuration allows the image of the vessel under study to be viewed direct (i.e., not by reflection), and therefore, utilises the available light somewhat more efficiently. The photodetectors used consist of two photodiodes (SP 50, thin film substrate: Hafo, Stockholm, Sweden) mounted with a 1.0 mm centre-to-centre separation. The diodes are placed directly on the proximal side of the ground glass, which avoids the need for complete darkening of

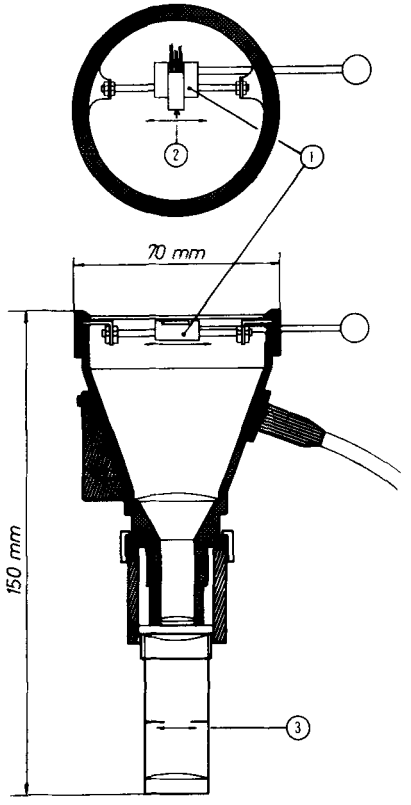

Fig. 1. Photodiodes assembly for velocity measurements. (*1*) Carriage for photodiodes mounted on micrometer thread which provides lateral adjustments. (*2*) Photodiodes. (*3*) Standard microscope ocular, which is integral with the unit and whose rotation provides for angular alignment.

the room during observations. Angular alignments are obtained by rotating the whole assembly within the ocular support. The preamplifier is mounted inside the ocular cone to lower electrical noise and obtain a better signal-to-noise ratio.

Photodetectors

Photomultipliers and/or different types of light-sensitive semiconductors are used to convert the optical data into electronic information. These devices modulate their output in proportion to the light that falls on then, so that changes in the received light are converted into electronic signals, referred to as "electronic signatures".

In terms of electrical performance, these devices are rated in terms of their sensitivity, frequency response, ratio of signal to self-generated noise, and dynamic range. The photo-multiplier tube has the

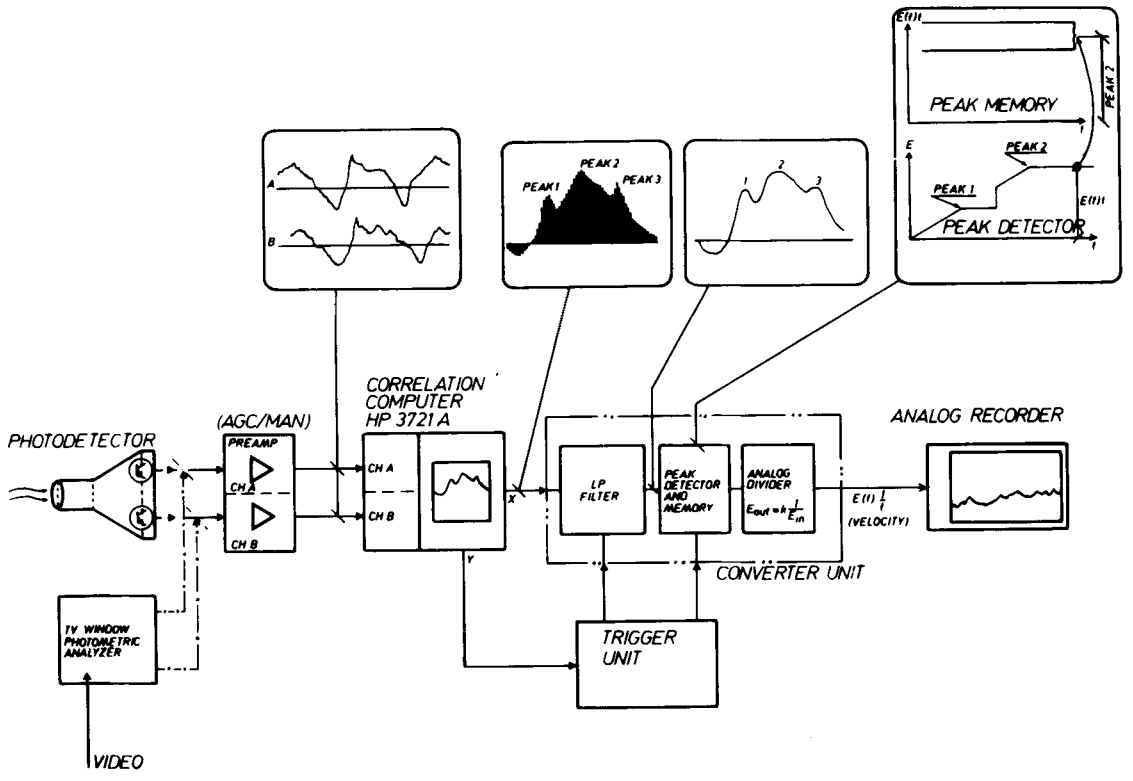

Fig. 2. Schematic representation of velocity measuring system. Either photodetectors or television windows can be connected directly to the automatic gain controlled (AGC) amplifiers. The correlator provides two outputs: (1) the correlogram proper, and (2) a pulse which marks the beginning of each correlogram. This later signal is utilised to synchronise the action of the peak detector to maximum cross-correlation. The peak detector operates by starting a ramp voltage at the beginning of each correlogram, which is stopped at the time at which the maximum cross-correlation product is reached. This value is held until the next correlogram, and applied to the denominator of a divider, whose numerator is set to a value equivalent to interdetector separation.

largest gain of any of the photo-detectors yet developed because the amplification is achieved directly within the device. It has the ability to amplify signals over a substantial input range without any changes in the electronic arrangement, i.e., it has a wide dynamic range. The superior electronic characteristics of the photomultiplier tube is offset by its need for high voltage excitation and its size, which makes it necessary to couple the image by perspex rods or fibre optics to the photomultiplier face plate. This causes loss of a substantial amount of light through absorption in the optical conduit, and reflection at the different interfaces, and takes up a considerable amount of space.

The sensitivity or gain of the photomultiplier tube can be further augmented by shielding it magnetically and cooling it to below ambient temperatures.

Semi-conductor devices can be used in most applications. They are small, comparatively inexpensive, and do not require high voltages to operate them. The most suitable photodetectors are phototransistors or photodiodes. In contrast to photomultipliers, they are limited in their dynamic range causing noise which is greater than that of the photomultiplier at low light levels, and they have a limited maximum output.

Television methods

The use of television in conjunction with quantitative microscopy was pioneered by Bloch (5) and Wiederhielm (18) who utilised the information in single television screen lines.

The performance of the cameras is primarily determined by their image tubes. The most common cameras utilise vidicon image tubes. These are adequate for generating images in situations where light

levels are comparatively high, and where the time resolution is not critical because of tube persistence. Higher frequency response and sensitivities can be obtained with plumbicon tube cameras, and with silicon diode matrix cameras. When the amount of light is too small even for these devices, the ultimate in sensitivity is obtained by using image intensifiers. This approach is needed in preparations such as tissue surfaces of organs which cannot be transilluminated, and therefore must be observed by reflected light.

The versatility of television methods has been substantially increased by the "window" techniques developed by Intaglietta & Tompkins (14). This method is based on the Video Photometric Analyzer, which is an instrument capable of isolating a temporal window from the composite video data and of routing the data contained within the window to signal-averaging circuitry, in such a way that the window acts as a photometer for the area which it overlies.

The position of the window is established by measuring the interval between the vertical and horizontal screen synchronisation pulses. When the time interval corresponding to the upper left hand corner of the window has elapsed, the system routes the video signal into an averaging circuit, until the upper right hand corner of the window is reached. The voltage thus obtained is sampled and held until the next raster line passes through the window. The process is repeated for all the screen lines within the window. When the window is completely scanned, the value of the averaging circuit is held in a second sample-and-hold circuit until completion of scanning of the window in the next television frame. Simultaneously, this voltage is presented at the output in analogue form and the window is delineated by white screen lines in the televised scene in such a fashion that its position can be observed.

The frequency response of this system is determined by the television framing rate, (i.e. 25 Hz in European television systems) provided that the television camera has a sufficiently fast response (i.e. low persistence). It is important to consider the relationship between magnification, resolution and velocity, because increases in linear magnification result in increased measured velocity.

The framing rate limitation of television systems can be partially overcome by decreasing the size of the scanned field, which increases the framing rate. Silicon diode matrix cameras make it possible to half

Fig. 3. Flow velocity in a 40 μm arteriole in the rabbit mesentery measured with photodiodes. At time zero the vessel is transected and the proximal flow velocity rises suddenly to value 6 times greater than the control. A haemostatic plug begins to form, eventually sealing the vessel, and the velocity falls to zero.

the image and therefore double the framing rate. The process can be applied to any integer of sub-division of the image, with the corresponding integer multiplication of the framing rate, up to the total number of lines in the television image.

Television methods make low light level viewing possible, and use of a video-tape recorder allows the data to be stored and analysed at leisure.

On-line velocity computation

The measurement of velocity is based on the computation of transit time between photometric windows. In the simplest case is that of a single red blood cell passing first one detector, and then the next. In these conditions the measurement of delay can be readily accomplished by means of an electronic timer, which measures the interval between events. In the more general case in which red blood cells pass in rapid succession with varying spacing, the delay between optical signatures can be measured by computing the cross-correlation between signals as a function of time delay (12).

The basic design of correlators is well established, but these instruments are not usually built to output on-line, and in analog format the delay to maximum cross-correlation. This feature must be added in terms of specialised circuitry, designed to function with the characteristic output of the given correlator. This output is usually in the form of a correlogram, which displays the cross-correlation value computed at each of a series of increasing delays within a given total range of delays. In the Hewlett-Packard cor-

relator 3721 A this correlogram is computed every 1/30 of a second over a total delay range from 100 sec to 0.1 millisec, each range being sub-divided into 100 incremental delays. The delay to maximum cross-correlation can be measured directly from the correlogram, either manually or electronically. Automatic means for measuring this delay are based on the operation of a circuit which compares the value of the cross-correlation at each delay increment with that of the previous one. Then if the previous one is greater than the new one, the older one is held, until either: (i) a new larger value appears in the correlogram, or, (ii) the correlogram is completed. In the first instance the comparison and subsequent hold is carried out again; the correlogram is completed. Finally the maximum value within the correlogram becomes available during each correlation computation. The delay corresponding to this maximum value is presented at the output of this system to the denominator of a divider circuit, whose numerator is proportional to the interdetector spacing and whose output is thus the required velocity (Fig. 2).

Applications

In microcirculatory research, continuous velocity measurements are of great importance. Any system with a frequency response permitting measurement of pulsations in the microcirculatory blood flow is of great advantage. Such a system makes it possible to obtain the characteristic wave-forms associated with pulsatile flow and to analyse their phase relation and phase shifts when studying the damping characteristics of the microcirculatory bed. In our laboratory we applied the techniques described to some of our problems and will examine some of the findings in greater detail.

Blood flow velocity during haemostasis

Bleeding of transected microvessels in the rabbit mesentery is stopped by haemostatic plug formation. Consistent differences in haemostatic plug formation time and stability exist between arterioles and venules (1, 2, 4).

When a blood vessel is transected, the blood flow velocity, because of the sudden loss of resistance, has an average increase to six times its control value. A plateau is then maintained while the haemostatic plug forms, and velocity drops rapidly to zero when an effective haemostatic plug seals the vessel (Fig. 3). In some arterioles (40–60 μm in diameter) blood

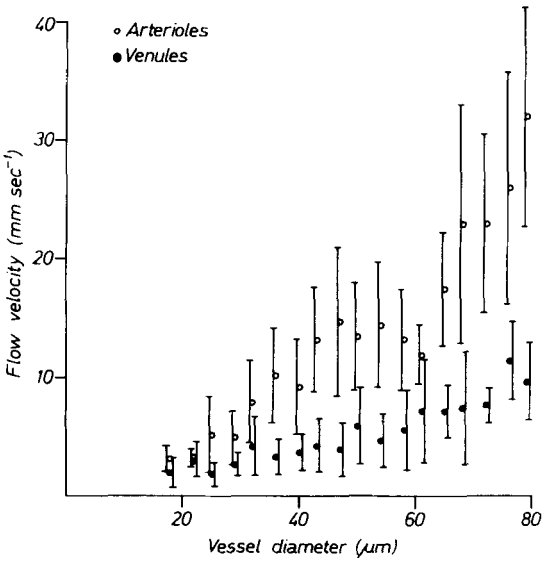

Fig. 4. Flow velocity in the arterioles, venules, small arteries and veins of the rabbit mesentery as a function of vessel diameter. Vertical lines indicate the standard deviation.

flow velocity increases to as much as 80 mm sec^{-1}. Although this is the highest value we have measured in the microcirculation, the upper limit of our on-line correlating system is three orders of magnitude higher. At the other extreme, when flow velocity is lower than 0.2–0.3 mm sec^{-1}, tissue tremor causes autocorrelation, thus making interpretation more difficult.

While measuring flow in the mesentery, peristaltic movements sometimes make it necessary to readjust rapidly the position of the two photodetectors, which is simplified by direct viewing on the ground glass.

Flow velocity related to vascular dimension in normal rabbit mesentery

As a preliminary study for the haemostatic experiments we measured the velocities in arterioles and venules of different sizes (Fig. 4). With increasing diameter there is an increase in flow velocity, especially in arterioles (2). Normal velocities in arterioles with diameters up to 30 μm range from 3.0 to 5.0 mm/sec. The comparatively high scatter in the data is a consequence of the fact that the local diameter is not the only determinant of the local velocity, since this is also affected by the upstream and downstream variations in calibre and hydraulic resistance. The comparatively high difference in velocity between

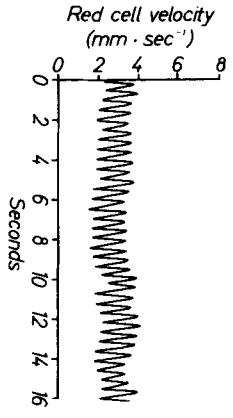

Fig. 5. Pulsatile flow velocity in a 25 μm arteriole of the rabbit ear chamber. The pulsatility is synchronised with the cardiac cycle. The respiratory cycle is also noticeable.

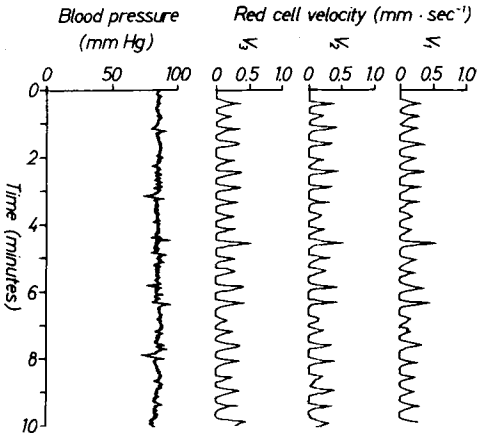

Fig. 6. Flow velocity in three contiguous capillaries in skeletal muscle. The measurements were obtained from a televised record.

arterial and venous vessels of the same diameter is a direct consequence of the comparatively greater number of venous vessels. This situation has also been noted in the ear chamber and skeletal muscle.

Pulsatile components in the microcirculation

The frequency responses of the photodiodes and the correlator make it possible to record pulsatile flow velocity in arterioles. The example shown (Fig. 5) is from a rabbit ear chamber in an awake animal. The pulsation related to heart beat and the slower waves (Hering-Breuer) caused by the animal's respiration can also be seen.

Capillary flow in skeletal muscle

In order to study blood flow velocity in single capillaries of skeletal muscle we have employed a television method utilised in conjunction with an image intensifier (ITT F 4715). The tenuissimus muscle in cat and rabbit (8) is a suitable skeletal muscle composed of mixed red and white fibres. The muscle is thin and can easily be transilluminated using a fibre optic light system. To enhance contrast in the preparation we use a narrow band filter (4200 Å). The narrow band wave of light is absorbed by the haemoglobin of the red cells, and increases the optical signatures given by cells passing in the capillary. Low light levels due to the action of the filter required the utilisation of an image intensifier to which a COHU-camera is optically coupled with a close-up objective (Micro-Nikkor). The information in the televised scenes can be stored in a videotape recorder (Sony) for analysis of multiple vessels in the same field. This is very advantageous, because, with repeated play-backs, many capillaries in the same field can be analysed simultaneously. As an example, three capillaries stemming from the same arteriole in one field of the tenuissimus muscle are shown in Fig. 6.

Concluding remarks

Red cell velocity determinations based on continuous cross-correlation of the optical signatures from contiguous photodetectors aligned in the direction of flow constitutes a method of measurement which has many applications in microcirculatory research. Depending on the nature of the problem being studied, different transducers (photodiodes, photomultipliers, and television) can be used and connected to the main system. Photodiodes and photomultipliers provide a frequency response fast enough to permit analysis of pulsatility in microvessels as small as capillaries. With ocular-mounted photodiodes it is possible to obtain mean velocity data from slowly moving tissue preparations affected by peristalsis or respiration. The television dual window photometric analysis makes possible tape recordings from which simultaneous measurements on many vessels can be made using the same time base.

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