Acoustic resolution photoacoustic Doppler flowmetry: practical considerations for obtaining accurate measurements of blood flow

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ABSTRACT

An assessment has been made of various experimental factors affecting the accuracy of flow velocities measured using a pulsed time correlation photoacoustic Doppler technique. In this method, Doppler time shifts are quantified via cross-correlation of pairs of photoacoustic waveforms generated in moving absorbers using pairs of laser light pulses, and the photoacoustic waves are detected using an ultrasound transducer. The acoustic resolution mode is employed by using the transducer focal width, rather than the large illuminated volume, to define the lateral spatial resolution. This enables penetration depths of several millimetres or centimetres, unlike methods using the optical resolution mode, which limits the maximum penetration depth to approximately 1 mm. In the acoustic resolution mode, it is difficult to detect time shifts in highly concentrated suspensions of flowing absorbers, such as red blood cell suspensions and whole blood, and this challenge supposedly arises because of the lack of spatial heterogeneity. However, by assessing the effect of different absorption coefficients and tube diameters, we offer an alternative explanation relating to light attenuation and parabolic flow. We also demonstrate a new signal processing method that surmounts the previous problem of measurement under-reading. This method is a form of signal range gating and enables mapping of the flow velocity profile across the tube as well as measurement of the average flow velocity. We show that, using our signal processing scheme, it is possible to measure the flow of whole blood using a relatively low frequency detector. This important finding paves the way for application of the technique to measurements of blood flow several centimetres deep in living tissue.

Keywords: Doppler, photoacoustic, pulsed, acoustic resolution, cross-correlation, flow, velocity, blood

1. INTRODUCTION

Since the basic principles of photoacoustic Doppler flowmetry were first outlined over a decade ago [1], several methods have been developed to measure the speed of blood flow using photoacoustics [2,3,4]. Essentially they all involve detecting the movement of red blood cells (RBCs) from the changes (such as time, phase or frequency shifts) in the photoacoustic waves they emit. However, *in vivo* velocity measurements have so far only been achieved by focussing the laser light (the optical resolution mode); this achieves a penetration depth of less than approximately 1 mm, which strictly limits the pre-clinical and clinical utility. In contrast, measurements in the acoustic resolution mode have the potential to penetrate several millimetres or centimetres into tissue, but *in vivo* flow measurements have not yet been achieved.

It is widely considered that the challenge in making acoustic resolution velocity measurements in blood relates to its optical heterogeneity and the ability to resolve this using a finite detector bandwidth. This is not a problem in the optical resolution mode, since the excitation spot is only a few micrometres in diameter and is therefore comparable to that of a single RBC allowing the spatial heterogeneity in the optical absorption of blood to be resolved directly. However, in the acoustic resolution mode, where localization is achieved using the ultrasound transducer focus rather than the laser excitation beam, it is supposed that the spatial resolution is limited by the minimum detectable acoustic wavelength. Spatial separations of the order of 7.5 µm (a typical RBC diameter) correspond to ultrasound frequencies of 200 MHz (sound speed 1500 m/s), and therefore blood may appear spatially homogeneous using ultrasound detectors with bandwidths of only a few tens of MHz. This may compromise the ability of time, phase or frequency correlation flowmetry methods to make blood velocity measurements.

In this paper, we present evidence to refute the assumption that there is insufficient spatial heterogeneity on the scale of a few tens of MHz for photoacoustic time correlation flowmetry to be successfully operated in the acoustic resolution mode. Section 2 outlines the underlying principle of the time correlation technique and the experimental methods used to evaluate it in blood flow phantoms. Section 3 briefly describes the signal processing used to calculate the average flow velocity, and also a new method, similar to "range-gating" applied in Doppler ultrasound, used for estimating velocities at different points across a vessel diameter. The velocity measurements for different absorber colours and concentrations flowing in various tube diameters are presented in section 4; a novel explanation is provided for the measurement under-reading, and the new signal processing method is used to improve the measurement accuracy of flowing blood.

2. EXPERIMENTAL METHODS

Velocity measurements were made in fluids using a Doppler flowmetry approach, which is described in detail in reference [5]. The method is based on tracking a moving cluster of absorbers, such as red blood cells, by delivering a pair of laser pulses and measuring the time shift in the respective photoacoustic signals. Velocity range and resolution are scalable with excitation pulse separation allowing it be optimised for a wide range of physiologically realistic flow velocities, and it lends itself to both the acoustic and optical resolution modes of photoacoustic sensing.

The results in this paper were acquired using the setup described in reference [6]. Pairs of photoacoustic signals were generated by exciting flowing absorbers with pairs of laser light pulses (Nano L 200-15, Litron, wavelength 532 nm) separated by a time $T = 0.5$ ms, and the photoacoustic signals were detected using a focussed PZT ultrasound detector with a centre frequency of 30 MHz and a -6 dB bandwidth of 24 MHz. The diameter of the illuminated region was significantly larger than the diameter of the detector focal beam in order to be representative of the acoustic resolution mode of photoacoustic detection.

Two types of absorbers were investigated: polystyrene microspheres in different sizes and colours, and red blood cells. The black-dyed polystyrene microspheres (24293-5, Polysciences) had a mean particle diameter of 6 μm and were supplied in a suspension of de-ionized water at a concentration of approximately 5.3% relative to a physiologically normal blood haematocrit (Ht ≈ 0.45). The 3 μm polystyrene microspheres were obtained in dark red (42922-5ML-F, Sigma-Aldrich) and dark blue (68553-5ML-F, Sigma-Aldrich), both in suspensions of deionized water with a concentration of approximately 7.4% relative to Ht. The red blood cells were obtained from the UCLH Blood Transfusion Unit and diluted with phosphate buffered saline (P4417-100TAB, Sigma-Aldrich) to give concentrations ranging from 2% to 46% relative to Ht. Measurements were also made using fresh, whole human blood, which provided a red blood cell concentration of 100% Ht. Fluid flow was generated using a syringe pump (B. Braun Space®) with a 60 ml syringe (BD Plastipak™) and a polymer tube (Paradigm Optics). The majority of the results are for the absorbers flowing in a tube made from THV polymer and with an inner diameter (I.D.) of 390 μm. In addition, results are presented for PMMA polymer tubes with inner diameters of 250 μm and 600 μm, and a THV polymer tube with an I.D. of 800 μm.

3. SIGNAL ACQUISITION AND PROCESSING

Up to 25 waveforms were captured in real time using the FastFrame™ Segmented Memory feature of the oscilloscope. This enabled the waveforms to be concatenated in a single record and downloaded to a PC. The unbiased cross-correlation function was evaluated for each photoacoustic waveform pair, and the mean cross-correlation amplitudes at each time point were used to compute a mean cross-correlation function $C(t)$. The peak of C was isolated and the maximum amplitude of a ten-point interpolant fit was used to determine the measured time shift t_s and hence the calculated velocity value. The final measurement V' was the mean of up to twenty velocity values and the resolution $\pm\Delta V'/2$ was calculated from the standard deviation of these values.

Measurements $V'\pm\Delta V'/2$ were made for speeds in the range 0 to 88 mm/s. The syringe pump could be programmed to deliver rates in steps of 0.01 ml/hr, and the pre-selected rate and the inner diameter of the tube were used to calculate the corresponding average flow velocity V in mm/s. Uncertainties ΔV were based on the tolerance in the diameter of the tubing. These "known" velocity values and uncertainties $V\pm\Delta V/2$ were compared with the measured $V'\pm\Delta V'/2$ acquired via cross-correlation of the photoacoustic waveform pairs.

Both the measured *V'* and the known *V* are values of average flow velocity. In addition, a new signal processing method was developed in order to measure the profile of velocity values across the tube. This method entails cross-correlation not of the entire photoacoustic waveforms, but rather of small segments of the photoacoustic waveforms corresponding to specific parts of the tube.

4.   RESULTS AND DISCUSSION

Sub-section 4.1 presents velocity measurements made using various concentrations of red blood cells, and the difference between the measurements with the black spheres and a comparable concentration of the RBCs is discussed. Sub-section 4.2 compares velocity measurements made for the red microspheres flowing in tubes of various diameters. An explanation for the results in sub-sections 4.1 and 4.2 is provided in sub-section 4.3, which describes the challenge relating to laminar flow and light attenuation. This challenge is addressed by analysing waveform segments corresponding to different parts of the parabolic flow profile, and the measurements corresponding to the average flow velocity are shown for whole blood.

4.1 Absorber heterogeneity

For a 2% concentration of RBCs, the measured velocities under-read the known velocities *V*, which are defined using the pre-set average flow velocity. The under-reading becomes worse with increasing RBC concentration as shown in Figure 1. An increase in RBC concentration produces an increase in the homogeneity of the suspension, which would suggest that the loss of velocity accuracy is associated with the loss of heterogeneity. However, Figure 2 shows measurements made with a 5.9% concentration of RBCs (average diameter 7.5 µm) and with a similar concentration of black polystyrene spheres (average diameter 6 µm). It is clear that the accuracy is much improved in the case of the latter, even though, based on the comparable particle sizes and concentrations, the heterogeneity can be assumed to be near-identical for the two suspensions.

The major difference between two suspensions in Figure 2 is the absorption coefficient. At a wavelength of 532 nm, the RBC suspension has an absorption coefficient of $\mu_a \approx 1.7$ mm^{-1} and the suspension of black spheres has an absorption coefficient of $\mu_a \approx 0.6$ mm^{-1}. Since the lower absorption coefficient enables better velocity accuracy irrespective of absorber concentration, it seems that the under-reading is not due to the due to the degree of spatial heterogeneity but to the degree of absorption, which is proportional both the absorber concentration and the absorption coefficient.
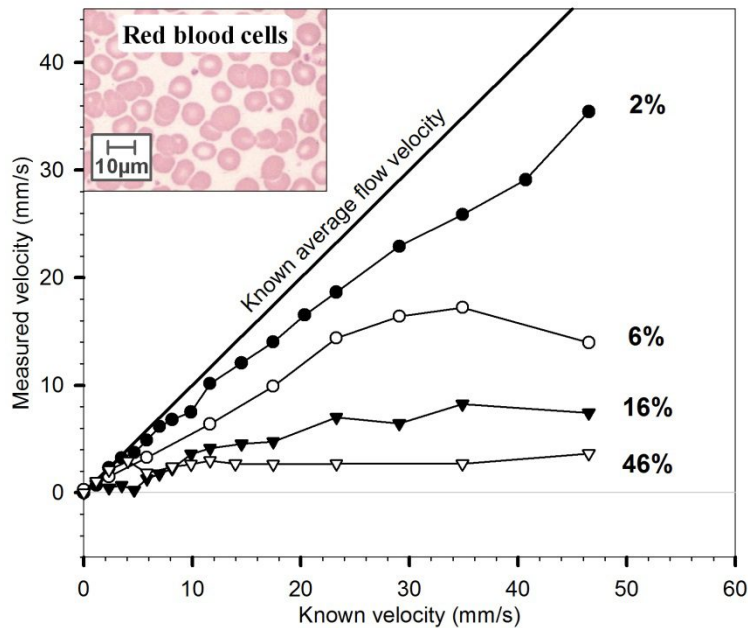
Figure 1. The effect of red blood cell concentration, expressed relative to the haematocrit of whole blood, on the accuracy of velocity measurements made for the cells flowing in a tube of diameter 390 μm. The measurements were acquired with a laser pulse separation of $T = 0.5$ ms and a 30 MHz focused transducer. Error bars have been omitted for clarity.
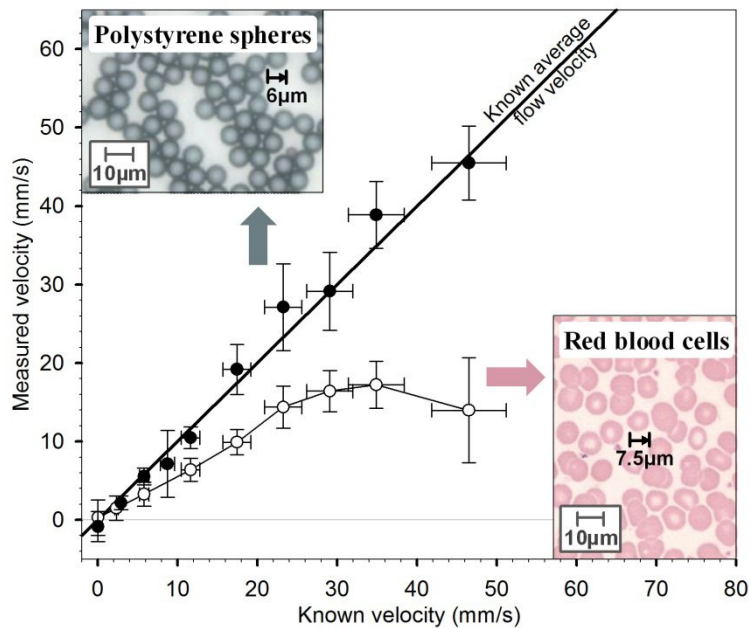

Figure 2. Velocity measurements for two different absorbers: red blood cells (unfilled data points) and 6 μm black polystyrene spheres (solid data points). Both absorbers were flowing in suspensions of the same concentration (5.6 ± 0.3 % relative to the haematocrit of whole blood) in a tube of diameter 390 μm. The measurements were acquired with a laser pulse separation of $T = 0.5$ ms and a 30 MHz focused transducer.

4.2 Tube diameter

Figure 3 shows that the measurement accuracy varies with tube diameter, with greater measurement under-reading observed in larger tubes. In each case the measurements were made using the same concentration (approximately 7% relative to Ht) of the 3 μm red polystyrene spheres.
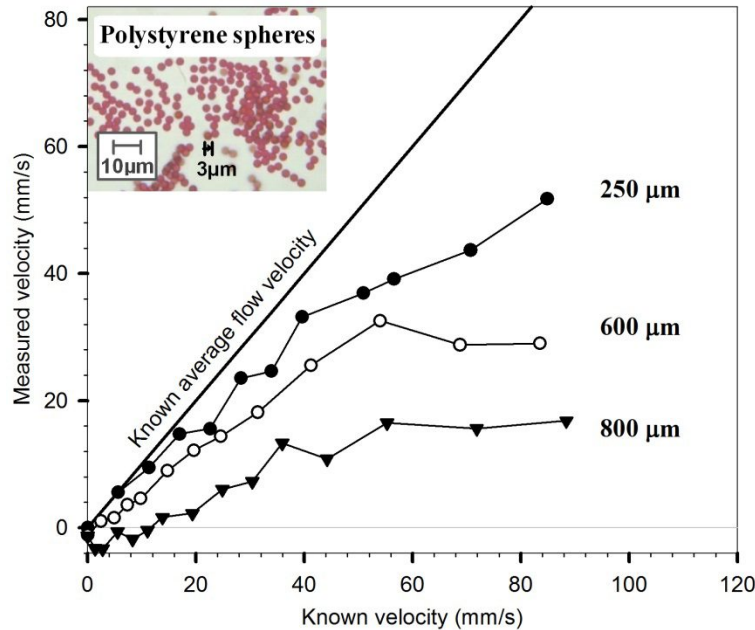

Figure 3. Velocity measurements for 3 μm red polystyrene spheres flowing in tubes of three different diameters: 250 μm (solid circle data points), 600 μm (unfilled circle data points) and 800 μm (solid triangle data points). In each case, the spheres were flowing in suspensions of the same concentration (approximately 7% relative to the haematocrit of whole blood). The measurements were acquired with a laser pulse separation of $T = 0.5$ ms and a 25 MHz focused transducer. Error bars have been omitted for clarity.

4.3 Laminar flow and light attenuation

It was previously thought that measurement under-reading was a consequence of the limited detector bandwidth and its ability to resolve the spatial heterogeneity of absorbers. However, the under-reading presented in sub-sections 4.1 and 4.2, which becomes worse with increasing absorption and increasing tube diameter, can rather be explained by the limited penetration of light into the tube, and the flow profile of the absorbing suspensions. The flow in the tubes is laminar, and therefore in the presence of strong light attenuation the photoacoustic signal is generated predominantly from the slower-moving absorbers in the parabolic flow profile. This effect is illustrated in Figure 4.

As the absorption increases, the photoacoustic signal generation becomes increasingly confined to the absorbers close to the edge of the tube. This explains why the measurement under-reading becomes worse with increasing concentration and absorption coefficient, as shown in sub-section 4.1. In addition, as the tube diameter is increased, the light penetration depth is smaller relative to the tube radius, and thus there is greater measurement under-reading even if the absorption remains constant. This explains the results in sub-section 4.2.
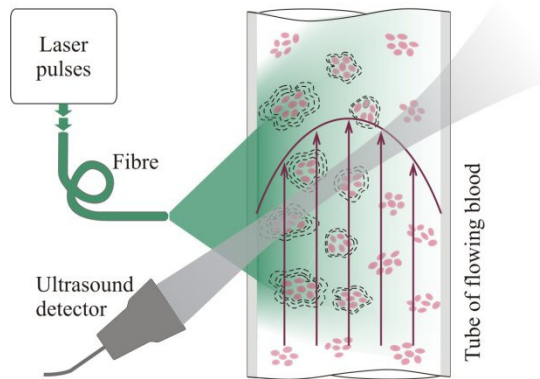
Figure 4. Illustration of the effect of laminar flow and light attenuation, resulting in generation of the largest amplitude photoacoustic signals in slow-moving absorbers at the near edge of the tube.

The under-reading caused by laminar flow and light attenuation can be addressed by analysing small segments of the photoacoustic waveforms corresponding to specific parts of the tube. By advancing the segments in time, it is possible to map out the distribution of flow across the vessel. Also, by choosing the appropriate waveform segment it is possible to recover the known average flow velocity, as illustrated in Figure 5, which is data for whole blood flowing in the 390 μm diameter tube.

(a) (b)

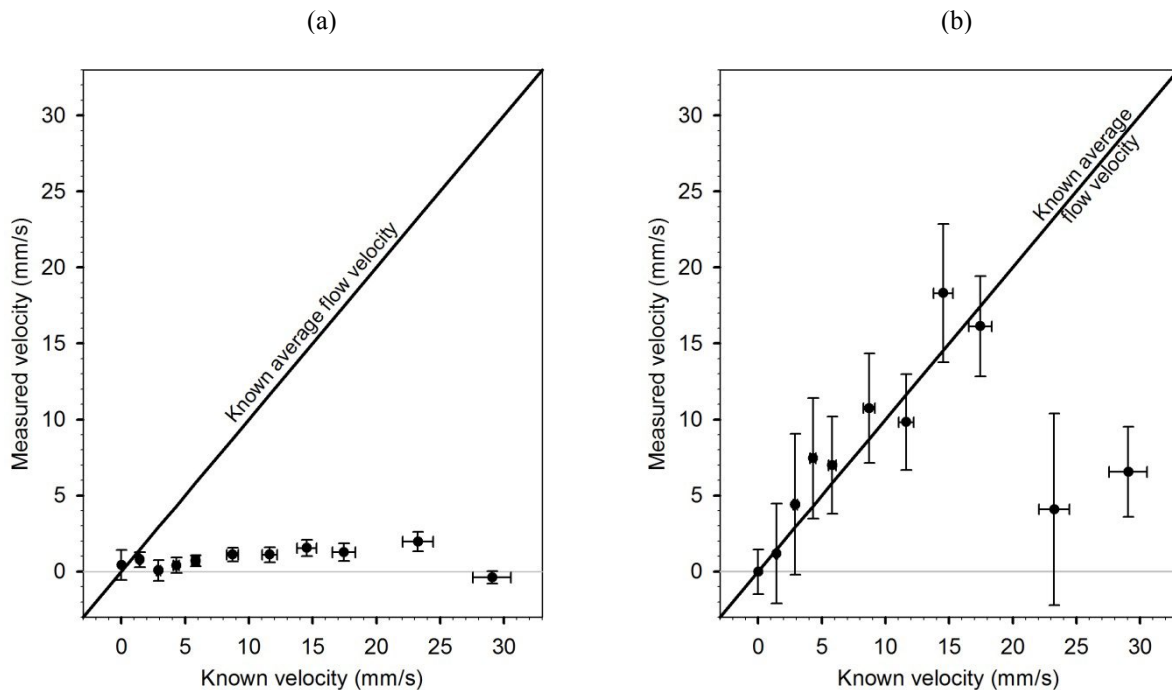
Figure 5. Velocities measured for fresh, whole blood flowing in a tube of diameter 390 μm. The data were acquired with a laser pulse separation of $T = 0.5$ ms and a 30 MHz focused transducer. In (a) the velocities were calculated by cross-correlating entire photoacoustic waveforms, whereas the velocities in (b) correspond to a single waveform segment.

5. CONCLUSIONS

This work suggests that, contrary to common expectation, it is possible to measure blood flow using photoacoustic time-correlation flowmetry in the acoustic resolution mode. Velocity measurements made for absorbers flowing in an optically transparent tube initially underestimated the known average flow velocity values, and the measurement under-reading became worse with increasing absorption coefficient and with increasing tube diameter. This is most likely to be due to light attenuation principally by slow-moving absorbers near the edge of the laminar profile. However, a new method involving analysis of small segments of the photoacoustic signals can be used to measure velocities at different points across the tube diameter, and also to recover the average flow velocity. Preliminary results have been obtained using fresh, whole blood flowing in a tube of diameter 390 μm, for which average flow velocities up to 20 mm/s were accurately measured.

The studies add further weight to the evidence that spatial heterogeneity is not such a stringent limitation to acoustic resolution flowmetry as is commonly supposed. All the photoacoustic signals were acquired using a detector with a centre frequency of 30 MHz and a -6 dB bandwidth of 24 MHz, which enabled the flow velocity of whole blood to be accurately measured. This suggests there is detectable heterogeneity in whole blood even at frequencies of a few tens of MHz, even though the spatial separations of RBCs typically correspond to ultrasound frequencies of about 200 MHz. In fact, studies of RBC aggregation have shown that, whilst changes in PA signals due to the spatial organisation of the cells are most significant for frequencies greater than 100 MHz [7], there are also detectable changes at frequencies less than 20 MHz [8]. In addition, encouragement may be taken from the success of pulsed wave Doppler ultrasound [9] and cross-correlation pulse-echo ultrasound measurements of blood flow [10], which also utilise detectors of only a few MHz.

Further work will apply the photoacoustic Doppler flowmetry technique using multiple wavelengths of light to verify the explanation relating to light absorption and laminar flow. Determination of the optimum wavelength will elucidate the feasibility of using the acoustic resolution mode to measure the flow of whole blood *in vivo*.

ACKNOWLEDGEMENTS

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