Prospects for *in vivo* blood velocimetry using acoustic resolution photoacoustic Doppler

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ABSTRACT

Acoustic resolution photoacoustic Doppler flowmetry (AR-PAF) is a technique that has the potential to overcome the spatial resolution and depth penetration limitations of current blood flow measuring methods. Previous work has shown the potential of the technique using blood-mimicking phantoms, but it has proved difficult to make accurate measurements in blood, and thus *in vivo* application has not yet been possible. One explanation for this difficulty is that whole blood is insufficiently heterogeneous. Through experimental measurements in red blood cell suspensions of different concentrations, as well as in whole blood, we provide new insight and evidence that refutes this assertion. We show that the velocity measurement accuracy is influenced by bandlimiting not only due to the detector frequency response, but also due to spatial averaging of absorbers within the detector field-of-view. In addition, there is a detrimental effect of limited light penetration, but this can be mitigated by selecting less attenuated wavelengths of light, and also by employing range-gating signal processing. By careful choice of these parameters as well as the detector centre frequency, bandwidth and field-of-view, it is possible to make AR-PAF measurements in whole blood using transducers with bandwidths in the tens of MHz range. These findings have profound implications for the prospects of making deep tissue measurements of blood flow relevant to the study of microcirculatory abnormalities associated with cancer, diabetes, atherosclerosis and other conditions.

Keywords: Doppler, photoacoustic, flow, blood, heterogeneity, acoustic resolution, cross-correlation, bandlimiting

1. INTRODUCTION

Photoacoustic flowmetry (PAF) is an emerging technique that has excited interest on account of its significant advantages compared to existing methods for measuring blood velocity. The general principle of photoacoustic flowmetry involves illuminating red blood cells with modulated laser light in order to generate ultrasound (photoacoustic) waves via the photoacoustic effect. The velocity of moving red blood cells can then be calculated from measureable changes such as time, phase or frequency shifts in the photoacoustic waves they emit. Unlike Doppler ultrasound, photoacoustic methods are well suited to measuring the low flow velocities in microvessels: this is a result of the high optical absorption difference between blood and the surrounding tissue, which enables superior blood vessel contrast.

Various photoacoustic flow and velocity measuring methods have been developed and successfully validated using blood flow phantoms [1]–[6]. These methods can be categorised into two modes: acoustic resolution and optical resolution. In acoustic resolution photoacoustic flowmetry (AR-PAF) the tissue is diffusely illuminated; photoacoustic signals are localised in the optical diffusion regime using an acoustic focus, thus enabling several millimetres of penetration. By contrast, optical resolution photoacoustic flowmetry (OR-PAF) relies on focussed illumination in order to localise the signal. Thus the penetration depth is limited to the optical ballistic regime of less than approximately 1 mm below the tissue surface.

OR-PAF has been successfully implemented to measure blood velocity *in vivo* [7]–[12]. However, it has proved extremely difficult to accurately measure the velocity of whole blood at physiologically realistic concentrations of RBCs even in well-controlled phantoms using AR-PAF. One possible explanation for this challenge relates to the heterogeneity of the red blood cells. If the spatial scale of the RBC distribution is small relative to the minimum detectable wavelength,

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then it will not be possible to spatially resolve the granularity of the RBC distribution. In this case the medium is perceived to be a homogeneous continuum and is photoacoustically indistinguishable from static blood thus precluding the detection of flow.

Consideration of the spatial scale of RBCs and their distribution would suggest that accurate tracking of RBC motion would only be possible using detectors sensitive to frequencies of the order of hundreds of MHz. However, in this paper, we present evidence to refute this assertion, and also identify light penetration as an additional factor affecting the accuracy of AR-PAF measurements. We show that there is sufficient heterogeneity in whole blood (at physiologically realistic haematocrits) on a spatial scale equivalent to frequencies of a few tens of MHz; however, examination of the frequency content of various different RBC suspensions illustrates that signal bandlimiting is not only due to the detector frequency response: we identify a second bandlimiting effect due to spatial averaging of the RBCs within the detector field-of-view.

The principle of the time correlation AR-PAF technique employed is described in Section 2 and the signal processing in Section 3. Section 4 shows velocity measurements for whole blood and for a red blood cell suspension diluted to 25% of the whole blood haematocrit. The light penetration is varied using different illumination wavelengths and the depth is also probed using a range-gating method analogous to that in Doppler ultrasound.

2. EXPERIMENTAL METHODS

Velocity measurements were made in fluids using a time-correlation 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 using the cross-correlation function. Velocity range and resolution are scalable with excitation pulse separation allowing it be optimised for a wide range of physiologically realistic flow velocities.

The results in this paper were acquired using the setup illustrated in Figure 1. Pairs of photoacoustic signals were generated by exciting flowing red blood cells with pairs of laser light pulses generated by two OPO laser systems (Innolas Spitlight 600 and Newport Spectra-Physics Quanta-Ray / GWU GmbH VisIR) 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.

Experiments were carried out with various blood samples at different concentrations expressed as a percentage of the normal case. For the velocity measurements in Figure 2, the blood was sourced from a healthy human volunteer and then diluted to 25% of the original haematocrit (Ht = 0.56) using phosphate buffered saline (PBS) to give Ht = 0.14. In order to produce the range of haematocrits shown in Figure 3 the human blood samples were first washed with PBS and then diluted to concentrations ranging from 3% to 80%, and the haematocrits verified in each case (Sysmex XE-5000™ Automated Hematology System).

3. SIGNAL ACQUISITION AND PROCESSING

To obtain a single velocity estimate, the tube was irradiated by a series of 25 laser pulse pairs. The pulses within each pair were separated by $T = 0.5$ ms, which was optimal for the range of velocities investigated. Measurements $V' \pm \Delta V'/2$ were made for average flow speeds $|V|$ in the range 0 to 40 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 (390±10 μm) were used to calculate $|V|$ in mm/s. Uncertainties ΔV were based on the tolerance in the diameter of the tubing. These "known" values and uncertainties correspond to the average flow velocity $V \pm \Delta V/2$ and were compared with the measured $V' \pm \Delta V'/2$ acquired via cross-correlation of the photoacoustic waveform pairs, as described in references [5], [13]. The average flow velocity was calculated from the mean of all 25 cross-correlation functions computed using the entire 5000-point (1.25 μs) time series of each signal pair; alternatively, the signals were range-gated such that they were evaluated in 250-point (62.5 ns) time windows, as described in reference [13].

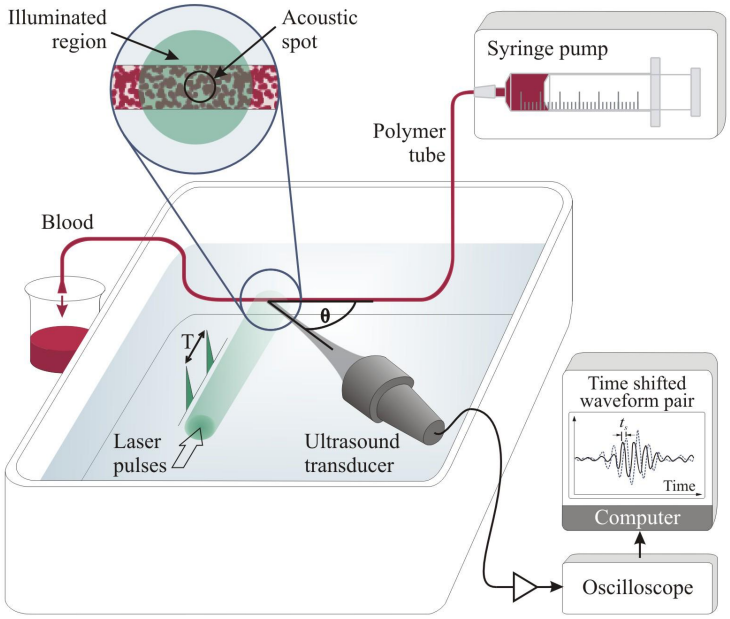

Figure 1. Experimental setup for pulsed photoacoustic Doppler blood flow measurements. Laser pulses separated by a time T are used to generate pairs of photoacoustic waveforms which are detected by an ultrasound receiver positioned at an angle θ to the flow axis. This angle was measured to the nearest degree using a turntable with angular markings at 1˚ intervals, and verified by horizontally translating the tube and comparing the measured distances with those calculated from cross-correlation of photoacoustic signals acquired before and after translation. The inset shows that whilst a large area (at least 5 mm diameter) of the red blood cells (RBCs) is illuminated, photoacoustic signals are collected from a smaller region defined by the transducer focal spot in order to be representative of the acoustic resolution mode of photoacoustic detection.

4. RESULTS: BLOOD VELOCITY MEASUREMENTS

For a haematocrit Ht = 0.14 (25% of the concentration of RBCs in whole blood), the measured velocities under-read the known velocities V. This is illustrated in Figure 2(a). For whole blood (Ht = 0.56) the under-reading is so severe that the measurements appear to be consistently zero across the velocity range (0-40 mm/s).

The difference in measurement accuracy for the two RBC concentrations could be explained by the difference in absorber heterogeneity, but also by the difference in light penetration: in the whole blood (100%) case the strong light absorption results in photoacoustic signals predominantly generated by the slower-moving absorbers at the edge of the tube. This bias towards the low velocities is less pronounced for the 25% concentration since the lower absorption enables deeper light penetration.

The effect of limited light penetration can be alleviated using one or both of two approaches. The first entails selecting an illumination wavelength at which the blood absorption coefficient is relatively low. Figure 2(b) shows that selection of a longer wavelength enables deeper light penetration and therefore improves the accuracy of both the 25% and 100% suspensions. In the second approach, the photoacoustic signals are time-windowed in order to select segments corresponding to specific range gate locations along the transducer axis within the vessel. Selection of time windows corresponding to regions deeper within the tube allows sampling of the faster moving RBCs closer to the tube centre. By avoiding the slow moving RBCs near the tube wall, this range-gating method results in measurements that are more representative of the average flow velocity, as illustrated in Figure 2(c).

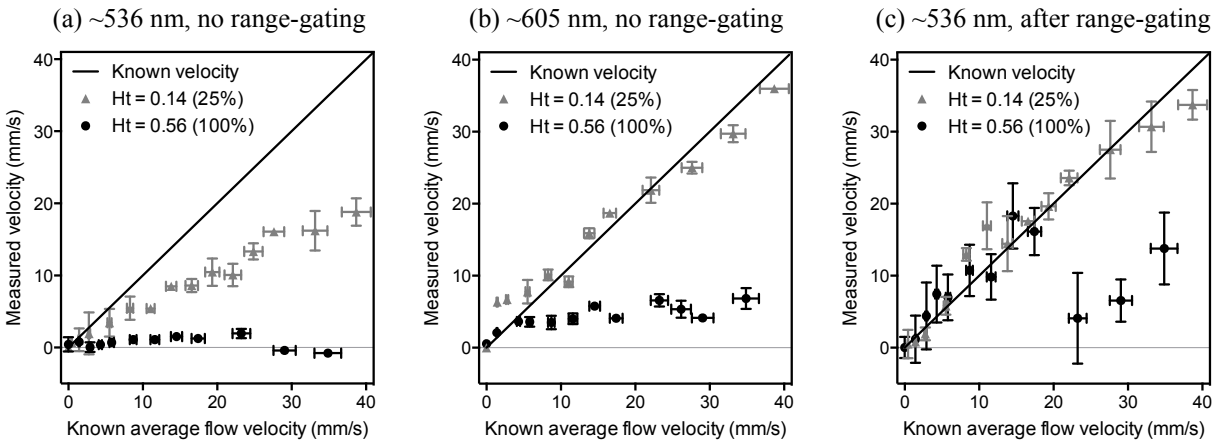

Figure 2. Velocities measured for fresh, whole blood at its original (100%) concentration (haematocrit Ht = 0.56) and also at a 25% concentration (haematocrit Ht = 0.14). In (a) the 25% and 100% suspensions were illuminated with wavelengths of 540 nm and 532 nm respectively, and the length of the signal range gate was set such that it was greater than the tube diameter. The same signal processing was applied in (b) but the suspensions were illuminated with wavelengths of 590 nm and 610 nm. In (c) the illumination wavelengths were the same as in (a) but time-windowing was applied to the photoacoustic signals in order to remove the bias towards low velocities due to greater light absorption at the edge of the tube. In each case the suspensions were flowing in a tube of diameter 390±10 μm. The data were acquired with a laser pulse separation of T = 0.5 ms and a 30 MHz focused transducer.

The results in Figure 2 illustrate that the blood motion can be tracked using a detector with a 30 MHz centre frequency. This suggests that there is heterogeneity in whole blood (Ht = 0.56), as well as in the diluted RBC suspension (Ht = 0.14), on a spatial scale equivalent to frequencies of a few tens of MHz. This is somewhat surprising if blood is composed of a random distribution of red blood cells (diameter ~ 7.5 μm): this would suggest that detector frequencies of hundreds of MHz would be required in order to resolve the spatial distribution. However, RBCs typically form aggregates and clusters leading to heterogeneity on a larger scale, which may explain how it is possible to track blood motion using a detector with a centre frequency of 30 MHz.

The question remains as to whether it is possible to improve the accuracy and resolution of the results in Figure 2 by choosing different detector characteristics. It might be expected that a detector with a higher centre frequency and larger bandwidth may be beneficial, since this will mitigate the effects of bandlimiting due to the detector frequency response. However, examination of the frequency content of photoacoustic signals acquired using the 30 MHz detector illustrates that there is an additional bandlimiting effect that is concentration dependent. Figure 3(a) shows the frequency content for the 100% (whole blood) case, and also for a haematocrit of 0.01 (3% relative to whole blood). For the 3% case, the majority of the frequency content is at 12 MHz, which is well below the detector centre frequency of 30 MHz. For the 100% case, this downshifting is even more pronounced, with the dominant frequency components around 5 MHz. These frequency spectra can be characterised using a weighted mean value, and Figure 3(b) shows that the mean frequency decreases with increasing absorber concentration (RBC haematocrit), reaching a plateau of about 20 MHz. This frequency downshifting is due to spatial averaging of the photoacoustic signals generated by absorbers within the detector field-of-view. Spatial averaging results in smoothing of the RBC distribution so that high RBC concentrations are perceived by the detector to be homogenous. Thus the frequency content is no longer defined by the microscopic scale of the RBCs but the relatively large size of the tube, and therefore low frequencies tend to dominate.

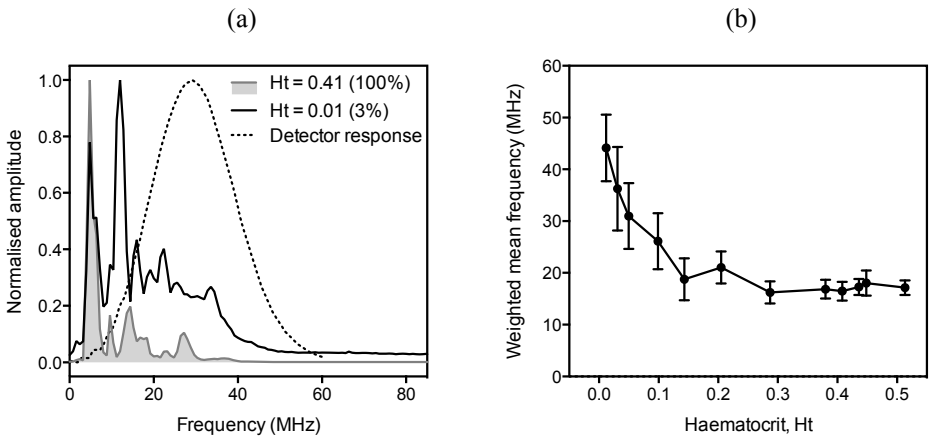

Figure 3. Frequency downshifting due to spatial averaging arising from the detector field-of-view (a) Comparison of normalised photoacoustic signal frequency content for a high red blood cell (RBC) concentration (100% of a physiologically normal haematocrit of Ht = 0.41) and a low RBC concentration (Ht = 0.01, which corresponds to about 3% of the physiologically normal value). The normalised frequency spectra are the means of fast Fourier transforms (FFTs) calculated for over 5000 PA signals, and the dotted line shows the normalised frequency response of the detector. (b) Weighted mean frequencies (WMFs) calculated from the fast Fourier transforms (FFTs) of photoacoustic (PA) signals acquired for different red blood cell (RBC) haematocrits. The WMF was calculated by summing the product of the amplitudes and the frequencies of the FFT and normalising by the sum of the amplitudes. The data points show the mean WMF of over 1500 FFTs calculated for a set of the same number of PA signals acquired for each of the relevant RBC concentrations, and the error bars represent the standard deviation. The blood samples were taken from human volunteers, centrifuged and washed with PBS three times before diluting with PBS to give the range of haematocrits shown. In both (a) and (b) the PA signals were generated using 532 nm laser pulses and acquired using the 30 MHz focussed transducer positioned at an angle of $\theta = 45°$ relative to a 390 μm tube containing the blood suspensions.

5. DISCUSSION AND CONCLUSIONS

This paper has described the principle of acoustic resolution photoacoustic flowmetry (AR-PAF) and demonstrated that it can be used to accurately measure the velocity of whole blood (haematocrit Ht = 0.56). The results in Figure 2 show that these measurements were possible using a detector with a centre frequency of 30 MHz. Range-gating the photoacoustic signals enabled calculation of velocities in specific regions of the flow vessel, and improved measurement accuracy. Greater light penetration was achieved by selecting an appropriate wavelength for the illuminating light, and thereby mitigated the bias towards the slower velocities due to limited light penetration thus improving accuracy.

Bandlimiting reduces the perceived heterogeneity and thereby compromises the ability to track RBC motion. Two bandlimiting effects have been identified: bandlimiting due to the detector frequency response, and also due to spatial averaging over the detector field-of-view as evidenced by the results in Figure 3. For accurate, high-resolution velocity measurements it is desirable to minimise both bandlimiting effects. So whilst an increase in the detector centre frequency and bandwidth would mitigate bandlimiting, this must be done in conjunction with a reduction in detector spot size otherwise the frequency downshifting due to spatial averaging will remain the limiting factor. These compensatory measures also have implications for the depth, velocity range, accuracy and resolution of the measurements. Increasing the detector frequency beyond a few tens of MHz will compromise the penetration depth of the measurements due to frequency-dependent acoustic attenuation. Reducing the detector spot size to diameters smaller than about 100 micrometres would compromise the maximum measurable velocity at which all the absorbers move out of the detection focal region during the time T between successive laser pulses. Reducing T would preserve the upper velocity limit, but would reduce the measured time shift t_s and thereby compromise the velocity accuracy and minimum measurable value (the velocity resolution).

For successful implementation of AR-PAF *in vivo* it therefore seems that a careful choice of experimental parameters is necessary. First, range-gating and/or a suitable wavelength of light must be used in order to ensure that inadequate light penetration does not introduce a bias towards slower moving absorbers. Second, the detector characteristics must be selected in order to minimise the effects of bandlimiting: this involves choosing a high centre frequency and wide bandwidth to reduce the bandlimiting effect due to the detector frequency response, but also reducing the detector spot size so as to minimise bandlimiting due to spatial averaging. In achieving adequate light penetration, there is a tradeoff with the photoacoustic signal-to-noise ratio, suggesting that it would be challenging to reach depths of more than a few millimetres *in vivo*. Provided that the above challenges can be suitably addressed, the accurate measurement of blood flow velocity using AR-PAF would therefore surpass the penetration depth limit of OR-PAF whilst improving on the contrast and spatial resolution of deeper flow imaging modalities such as Doppler ultrasound. This would be useful in many clinical conditions: for example, measurement of blood flow velocity in conjunction with blood oxygen saturation could provide a measure of the oxygen consumption rate in tumours; also determination of blood velocity would assist in the diagnosis of microcirculatory abnormalities such as those associated with diabetes and cardiovascular disease.

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REFERENCES

[1] S. L. Chen, T. Ling, S. W. Huang, H. Won Baac, and L. J. Guo, "Photoacoustic correlation spectroscopy and its application to low-speed flow measurement," Opt. Lett., 35 (8), 1200–1202 (2010).
[2] A. Sheinfeld, S. Gilead, and A. Eyal, "Photoacoustic Doppler measurement of flow using tone burst excitation," Opt. Express, 18(5), 4212–4221 (2010).
[3] A. Sheinfeld, S. Gilead, and A. Eyal, "Simultaneous spatial and spectral mapping of flow using photoacoustic Doppler measurement," J. Biomed. Opt., 15(6), 0660101-0660108 (2010).
[4] J. J. Yao and L. H. V Wang, "Transverse flow imaging based on photoacoustic Doppler bandwidth broadening," J. Biomed. Opt., 15(2), 0213041-0213045 (2010).
[5] J. Brunker and P. Beard, "Pulsed photoacoustic Doppler flowmetry using time-domain cross-correlation: accuracy, resolution and scalability," J. Acoust. Soc. Am., 132(3), 1780–1791 (2012).
[6] P. J. van den Berg, K. Daoudi, and W. Steenbergen, "Review of photoacoustic flow imaging: its current state and its promises," Photoacoustics, 3(3), 89–99 (2015).
[7] J. Yao, R. C. Gilson, K. I. Maslov, and L. L. V Wang, "Calibration-free structured-illumination photoacoustic flowgraphy of transverse flow in scattering media," J. Biomed. Opt., 19(4), 0460071-0460076 (2014).
[8] R. Zhang, L. Wang, J. Yao, C.-H. Yeh, and L. V Wang, "In vivo optically encoded photoacoustic flowgraphy," Opt. Lett., 39(13), 3814–3817 (2014).
[9] Y. Zhou, J. Liang, K. Maslov, and L. Wang, "Calibration-free in vivo transverse blood flowmetry based on cross correlation of slow time profiles from photoacoustic microscopy," Opt. Lett., 38(19), 3882–3885 (2013).
[10] L. Wang, K. Maslov, and L. V Wang, "Single-cell label-free photoacoustic flowoxigraphy in vivo.," Proc. Natl. Acad. Sci. U.S.A., 110(15), 5759–5764 (2013).
[11] J. Liang, Y. Zhou, A. W. Winkler, L. Wang, K. I. Maslov, C. Li, and L. V Wang, "Random-access optical-resolution photoacoustic microscopy using a digital micromirror device," Opt. Lett., 38(15), 2683–2686 (2013).
[12] S.-L. Chen, Z. Xie, P. L. Carson, X. Wang, and L. J. Guo, "In vivo flow speed measurement of capillaries by photoacoustic correlation spectroscopy," Opt. Lett., 36(20), 4017–4019 (2011).
[13] J. Brunker and P. Beard, "Acoustic resolution photoacoustic Doppler velocimetry in blood-mimicking fluids," Sci. Rep., 6, 209021-2090214 (2016).
[14] P. J. van den Berg, K. Daoudi, and W. Steenbergen, "Pulsed photoacoustic flow imaging with a handheld system.," J. Biomed. Opt., 21(2), 0260041-0260046 (2016).