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Experimental setup for the ultrasonic fractionation of flowing whole blood in a capillary

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Abstract: If a long ultrasound driving pulse is chosen such, that its frequency is greater than the resonance frequency of one cell type and less than the resonance frequency of another cell type, the cells are separated in different nodes and antinodes of the standing sound field. The purpose of this study was to build an experimental setup for the ultrasonic fractionation of whole blood in flow conditions. Blood fractionation is the separation of whole blood into its separate components, commonly used for analysis and transfusions.

The experimental setup comprised a container with single-element ultrasound transducers customisable in elevation and azimuth, focussed on a capillary through which blood was flowing. The acoustic focus coincided with the optical focus of a microscopic system to which a high-speed camera was attached. Ultrasound transducers were used with centre frequencies between 1 MHz and 20 MHz and with pressure amplitudes less than 1 MPa. The duty cycle was greater than 10% in all experiments. The field of view was $0.2 \times 0.2\,\mathrm{mm}^2$. The optical resolution and camera speed allowed for visibility of red and white blood cells during flow conditions.

Preliminary results show that red and white blood cells could be forced to move in opposite directions. The acoustic frequencies and pressures used were representative for those used in clinical diagnosis.

We have shown that ultrasonic whole blood fractionation is feasible using ultrasound. Sonic fractionation speed might be an indicator for diseases such as malaria.

Keywords: Whole blood fractionation, sonic cell separation, radiation forces, particle manipulation, blood sonication.

1 Introduction

Blood fractionation is the separation of whole blood into its separate components, commonly used for analysis and transfusions [1], commonly done using centrifuging or microfluidics [2]. Red blood cells have been found to move and line up in nodes or antinodes of standing ultrasound fields [3]. This phenomenon has been attributed to the compressibility of pockets inside cell membranes [4] subjected to radiation forces [5]. The acoustic response of a blood cell in an ultrasound field has been modelled [6]. Aggregation speeds were measured previously using high-frequency ultrasonics *in-vitro* [7], *in-vivo* [8, 9], and *in-silico* [10]. Ultrasonic blood cell manipulation has also been proven feasible using surface-acoustic waves [11], at frequencies too high to be applicable in clinical situations.

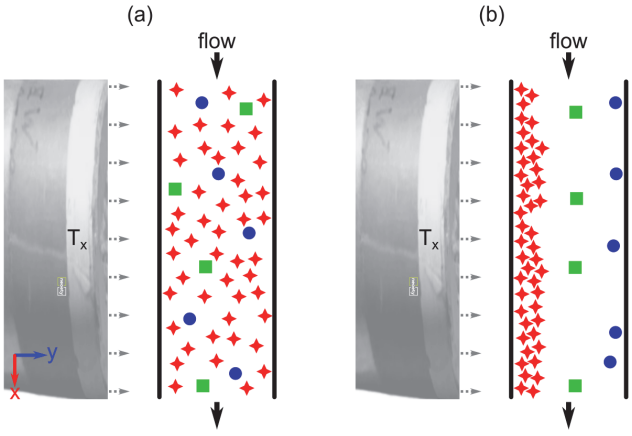

Fig. 1: Schematic top-view representation of ultrasonic cell separation in a capillary before (a) and after (b) sonication with a single-element transducer T_x.

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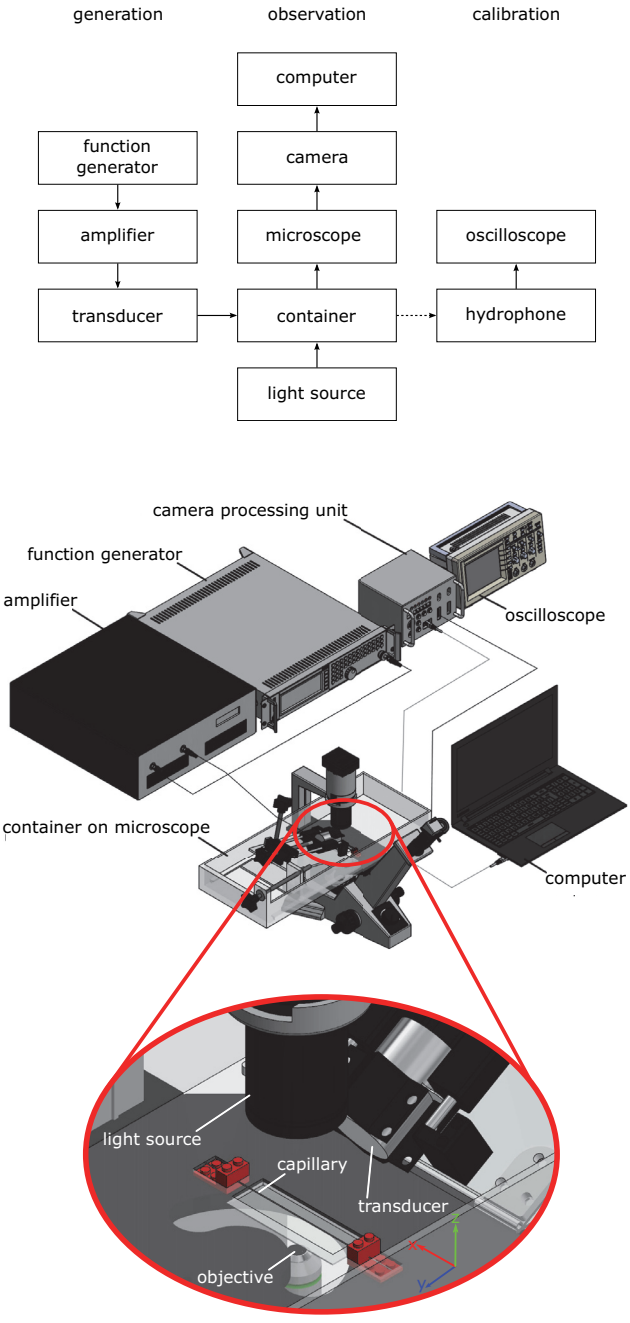

Fig. 2: Schematic overview of the experimental setup, a line drawing, and a close-up of the capillary.

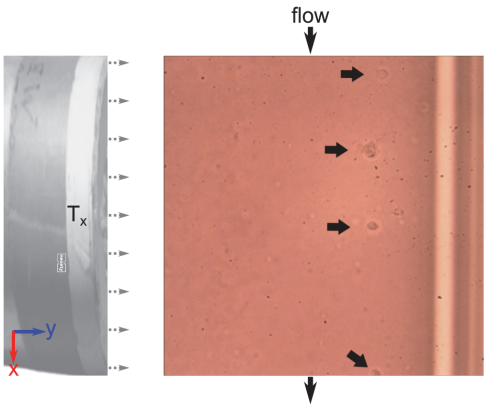

Fig. 3: White blood cells (arrows) lined up at positions distal to the ultrasound transducer T_x. Red blood cells have accumulated proximal to the transducer. The field of view corresponds to an area of $211\times211\,\mu m^2$ in the plane z=0.

Based on this model, cells with a different geometry and membrane viscoelasticity respond differently to the same incident pulse. It has been demonstrated in culturing wells, that if an ultrasound driving pulse is chosen such, that its frequency is greater than the resonance frequency of one cell type and less than the resonance frequency of another cell type, the cells are separated in different nodes and antinodes of the sound field [12]. The concept of ultrasonic cell separation in flow conditions is shown in a schematic in Figure 1, where the interaction of a multicycle plane wave with the flow capillary geometry creates a standing sound field, as demonstrated in a separate study on shell-encapsulated microbubbles [5].

In this study, we investigated the feasibility of an ultrasonic means to fractionate whole blood by building an experimental setup combining acoustics, microscopy, and microfluidics. Apart from common clinical applications, acoustic fractionation might be used for the detection of parasitic diseases, such as malaria.

2 Materials and methods

The experimental setup used is shown in Figure 2.

An AFG 3021B arbitrary function generator (Tektronix Incorporated, Beaverton, Oregon, USA) was used to generate a pulse sequence. An A-150 55dB linear power amplifier (ENI Technology, Inc., Rochester, NY, USA) amplified the signal. The power amplifier was connected to a ultrasound transducer of choice. Custom-manufactured single-element ultrasound transducers (Neoety AS, Kløfta, Norway) with centre frequencies between 1 MHz and 20 MHz were used throughout this study. The transducer was placed in a bracket that was positioned at a variable distance to the optical region of interest. The bracket was attached to a plate that was manually tilted to set the angle of incidence with respect to the y-axis that lies in the horizontal plane. This plate was positioned in a perspex container with internal dimensions $580\times235\times65\,mm^3$. The container was filled with degassed water which served as propagation medium for the ultrasound generated. The container was placed on the stage of a CKX31 inverted microscope (Olympus Corporation, Shinjuku, Tokyo, Japan) with a C-PlanN 20× objective lens (Olympus) with a numerical aper-

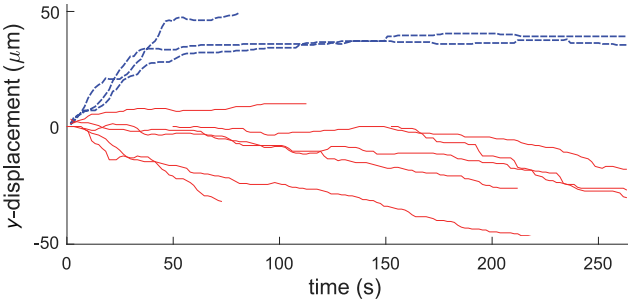

Fig. 4: Movement perpendicular to the flow direction as a function of time in a single experiment for sonicated white blood cells (- - -) and red blood cells (—).

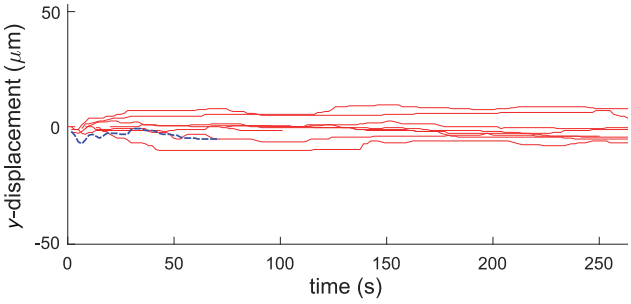

Fig. 5: Movement perpendicular to the flow direction as a function of time in a single experiment for an unsonicated white blood cell (- - -) and red blood cells (—).

ture of 0.4 and a working distance of 1.2 mm. The microscope had a built-in 6V, 30W halogen lamp light source.

A CUPROPHAN® RC55 cellulose capillary (Membrana GmbH, Wuppertal, Germany) with an inner diameter of 200 μm and a wall thickness of 8 μm was positioned along the x-axis of the system in the coinciding optical and acoustical focus in the $z = 0$ plane. Capillary action ensured the filling of the capillary. An HGL-0200 bullet hydrophone (Onda Corporation, Sunnyvale, CA, USA) was used for field calibration. The hydrophone was connected to an AH-2010 pre-amplifier (Onda) that was connected to a DPO 2014 digital phosphor oscilloscope (Tektronix).

The charge couple device of a FASTCAM MC1 colour high-speed camera (Photron (Europe) Limited, West Wycombe, Bucks, United Kingdom) was mounted to the microscope eyepiece through a custom three-dimensionally printed C-mount adaptor and connected to its processing unit. The high-speed camera recorded with frame rates up to 10 000 frames per second. The camera was controlled by a laptop computer. Image sequences were stored in the tagged image file format. Video recordings were continuous throughout experiments and lasted up to three minutes after the commencing of sonication. The recorded data were stored offline. All image processing was done in MATLAB® (The Mathworks, Inc., Natick, MA, USA), using code published separately [13].

All experiments with blood followed the same procedure. For each experiment, 60 ml of blood was drawn from a blood unit stored at 4°C using a syringe. The sample was transferred to vacutainers and agitated five times to mix the anticoagulant with the blood. Blood was drawn from the vacutainer using a pipette and mixed with Tyrode's buffer in a tube. Preparation of Tyrode's buffer has been described elsewhere [14]. The tube was agitated four times. A capillary was placed in the tube and left to fill. Once filled, the capillary was positioned in the container, after which sonication experiments commenced. Flow was induced by pressing one capillary end.

Sonication was done at the centre frequency of the transducer of choice, using a duty cycle greater than 10% in all experiments. Acoustic amplitudes were varied in the range $\langle 0, 1 \rangle$ MPa.

3 Preliminary results

Figure 3 shows an example of cell separation after three minutes of sonication with a driving pulse of centre frequency 5.8 MHz. The red blood cells were observed to have shifted to positions proximal to the transducer, whilst the white blood cells had lined up at positions distal to the transducer. The optical resolution and camera speed allowed for visibility of red and white blood cells during flow conditions.

A different example of the measurement of the movement of blood cells measured from camera footage is shown in Figure 4. The driving frequency used was 5.8 MHz and the peak-negative acoustic pressure 0.85 MPa. Again, white blood cells were observed to move in distal direction, whilst red blood cells were observed to move in proximal direction.

The results during sonication are in stark contrast with measurements of movement without sonication, shown in Figure 5. In these controls, hardly any movement was observed in the direction perpendicular to the flow. Consequently, we may attribute the fractionation observed in Figure 4 to the ultrasound applied.

The driving frequencies in the experiments presented in this study were substantially lower than those of prior studies with surface-acoustic waves. The main advantage of lower frequencies is in the applicability in clinical situations, where higher penetration depths are required. Furthermore, a lower driving frequency would require a lower acoustic driving pressure to create a similar mechanical effect.

Throughout the experiments, the points of accumulation did not change with acoustic amplitude, confirming they were nodes and antinodes of the ultrasound field. Although the aver-

age speed at which the white blood cells move to the nodes or antinodes appears to correlate to the acoustic amplitude (data not shown here), the standard deviations of these first measurements overlap.

It is noted that the driving frequency in these examples is substantially less than anticipated resonance. Yet, the differences in geometry between the two cell types may account for the differences in acoustic response causing their movement.

In this study, the flow rate was not controlled. As the transducer surface was parallel with respect to the flow direction, we presumed no influence of the flow rate on the blood cell translation. This was not confirmed experimentally.

4 Conclusions

Preliminary results show that red and white blood cells could be forced to move into opposite directions. The acoustic frequencies and pressures used were representative for those used in clinical diagnosis.

We have shown that ultrasonic whole blood fractionation is feasible using ultrasound. Sonic fractionation speed might be an indicator for diseases such as malaria.

Author statement

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