FULL ARTICLE

Imaging blood flow inside highly scattering media using ultrasound modulated optical tomography

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Received 19 January 2017, revised 30 March 2017, accepted 18 April 2017

Keywords: medical and biological imaging, imaging through turbid media, acousto-optics, laser Doppler velocimetry

We report the use of ultrasound modulated optical tomography (UOT) with heterodyne parallel detection to locally sense and image blood flow deep inside a highly scattering medium. We demonstrate that the UOT signal is sensitive to the speed of the blood flow in the ultrasound focus and present an analytical model that relates UOT signals to the optical properties (i.e. scattering coefficient, anisotropy, absorption, and flow speed) of the blood and the background medium. We found an excellent agreement between the experimental data and the analytical model. By varying the integration time of the camera in our setup, we were able

to spatially resolve blood flow in a scattering medium with a lateral resolution of 1.5 mm.

1. Introduction

The ability to measure blood flow in a noninvasive manner with high sensitivity and accuracy is of fundamental importance for studying a wide range of biological processes including local metabolism, neurovascular communication, pharmacodynamics, and stroke recovery. The commonly used imaging modalities, such as MRI, CT and ultrasound (US) enable imaging of blood flow [1–3]. However, each of these methods has its drawbacks: MRI is expensive and has a low throughput; CT relies on ionizing radiations; and ultrasound has a poor sensitivity and contrast unless a contrast agent is used [4]. Optical methods, on the other hand, can potentially be cheap, safe, have high throughput and provide ultimate sensitivity since a displacement in the order of the wavelength of the light can potentially be de-

tected. Currently, optical techniques such as laser speckle contrast analysis (LASCA), laser Doppler and optical coherence tomography (OCT) are important tools in the clinic [5–7]. However, these techniques are limited to the detection of superficial blood flow as the resolution deteriorates with depth due to multiple scattering. Because of the high clinical relevance of blood flow measurements [8,9] intense research is being conducted to develop techniques with improved resolution, penetration depth and quantification ability [6,10–12]. Among these techniques, photoacoustics (PA) is being intensely investigated for blood flow measurements due to its advantageous contrast mechanism based on absorption [12]. In PA the blood flow is measured by measuring the Doppler shift in ultrasound wave (i.e. optically generated). Similar to US flowmetry, however, the relatively long wavelength of the ultrasound

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makes the technique insensitive to small displacements.

Ultrasound modulated optical tomography (UOT) was originally developed to image absorbing objects inside turbid media [13,14], and it has recently found exciting new applications such as focusing light into turbid media [15], measuring optical fluence variations [16,17], and quantification of scattering inhomogeneities [18]. These applications of UOT are mainly motivated by the invention of a heterodyne parallel speckle detection (HPSD) method using a CCD camera [19,20]. HPSD is superior in terms of sensitivity compared to methods based on a single detector and allows for direct quantitative measurements of ultrasonically modulated light [21]. Due to the superior sensitivity of HPSD, the US burst that is used to modulate the light can be as short as a few cycles that improves the axial resolution [22], further pulse inversion approach is applied to improve the axial resolution [23]. However in our work we have only used short US bursts for axial resolution, which can be further improved by combining it with pulse inversion.

Recently, the use of ultrasonically modulated light has been suggested for sensing the flow of scattering particles. Chandran et al. [24] demonstrated that the temporal fluctuations in the reflected light intensity can be linked to the flow speed of the moving particles in the US focus. Tsalach et al. [25] proposed the use of ultrasonic modulation of light for depth selective flow measurements. They showed using simulation and experiment using single fast detector that the spectral width of the photocurrent fluctuation of ultrasonically modulated light is related to the movement of scattering microspheres in the ultrasound focus. However, the description by Tsalach et al. of the dependence of UOT signal on the flow of the optically scattering particles in the US focus and dynamics of the background medium is only qualitative. For truly quantitative estimation of flow using UOT, a robust detection method that allows direct measurement of ultrasonically modulated light in absolute terms and an analytical model that describes the dependence of the UOT signal on optical properties of the flowing particles and of the background medium is required.

Here we present the use of UOT with HPSD for imaging depth-resolved blood flow inside highly scattering media. We experimentally show the dependence of the HPSD signal on the speed of blood flowing through a tube embedded in a scattering medium. Further we present an analytical model that relates the HPSD signal to the optical properties (i.e. scattering coefficient, anisotropy, absorption, and flow speed) of moving particles and the background medium. Finally, we demonstrate a sim-

ple flow imaging method that relies on comparing UOT images taken with a short and a long integration time of the CCD. In comparison with work of Chandran et al. and Tsalach et al. [24,25] on sensing flow of scattering particle using UOT using a single, fast photodetectors, we employ a parallel detection scheme with a CCD camera. Our method relates to these studies as LASCA relates to laser Doppler flowmetry [26].

2. Material and methods

2.1 Phantom

We performed experiments on a slab of scattering tissue phantom of dimensions $3 \times 3 \times 1.5 \text{ cm}^3$ that contained a polyethylene tube mimicking a blood vessel. The acoustic impedance of polyethylene is 1.73 MRayls [27] which is close to the acoustic impedance of water 1.5 MRayls, leading to 92 % acoustic transmission when placed in water. The tube had an inner and outer diameter of 0.96 and 1.28 mm respectively. For experiment 1 and 2, the configuration of the sample used is illustrated in Figure 1b, where the tube is placed 5 mm under the surface that is illuminated. For experiment 3 the configuration of the sample used is illustrated in Figure 1c, where the depth of the tube under the surface at the first and second pass is 5 and 10 mm respectively. The tissue phantom with a reduced scattering coefficient (μ'_s) of approximately 0.7 mm^{-1} was prepared by mixing 7 % (w/v) of 20 % Intralipid (Sigma Aldrich) and 3 % (w/v) of Agar (Sigma Aldrich) in 90 ml of water. Blood was obtained from an anonymous healthy volunteer organized by the TNW-ECTM-donor service at the University of Twente. The total hemoglobin concentration of the blood samples was 15 g/dl and the oxygen saturation was 96 %. Oxygen saturation and total hemoglobin of the blood samples were measured using a blood oximeter (Avoximeter 1000E, ITC point of care) before and after the experiment for comparison and were found to remain unchanged over the duration of the measurement. Blood was pumped through the nylon tube in a closed system using two syringe pumps (Figure 1b and 1c), one pushing and the other withdrawing at the same rate, this configuration also prevented the blood from being exposed to air during the experiment.

2.2 Heterodyne parallel speckle detection method for ultrasound modulated optical tomography

In UOT, the medium is illuminated with coherent light of frequency ω_o , and a region of interest

Figure 1 (a) schematic of the ultrasound modulated tomography setup based on heterodyne parallel speckle detection (HPSD). AOM: acousto-optic modulator, BS: beam splitter, CCD: charge coupled device camera. Two syringe pumps are used for enforcing a controlled blood flow, (b) schematic of the sample used in experiment 1 and 2, (c) schematic of the sample used in experiment 3.

(ROI) is insonified with focused ultrasound (US) of frequency ω_{US} (see Figure 1a). The ROI can be placed anywhere inside the medium thanks to the low scattering of ultrasound. A fraction of the diffused light interacts with the ultrasound and gets modulated at $\pm\omega_{US}$. In effect, the insonified region becomes a source of ultrasonically modulated light of frequency $\omega_o \pm \omega_{US}$. The detection of the ultrasonically modulated light at the medium boundary was done using heterodyne parallel speckle detection (HPSD) [28]. Our setup, shown in Figure 1a, consists of a CCD camera, an US transducer and a Mach-Zehnder interferometer. The light in the reference arm reaches the CCD camera at an angle, so that we can perform off-axis holography. The reference arm angle is adjusted by rotating the beam splitter (BS) in front of the CCD camera (see Figure 1). The BS is fixed on a manual rotation stage and the angle of reference arm is chosen such that the hologram of the aperture (i.e. heterodyne term) is off axis enough so that it is isolated from the on axis background. The alignment is done by looking at the real time hologram of the aperture. Additionally, acousto-optic modulators (AOM) are used to shift the frequency of the light in the reference arm to $\omega_o + \omega_{US}$, in order to match the frequency of the modulated light emanating from the sample.

The CCD camera records the image of the intensity $I(\vec{r}) = \int_0^T E(\vec{r}, t) E^*(\vec{r}, t) dt$, where T is the integration time, $E(\vec{r}, t)$ is the total electric field, and $E^*(\vec{r}, t)$ is its complex conjugate. The electric field is a sum of the field of the reference beam, the diffuse

light modulated at frequency $\omega_o + \omega_{US}$, the non-modulated diffuse light, and light modulated at $\omega_o - \omega_{US}$ or higher ordered frequencies. Of these contributions, only the first two interfere with each other while the others only contribute to a background signal $I_{bg}(\vec{r})$. Therefore, we can express the recorded intensity at the CCD as

$$I(\vec{r}) = I_{bg}(\vec{r}) + \int_0^T \left[E_{US}^*(\vec{r}, t) E_{LO} e^{i\vec{k}_{LO} \cdot \vec{r}} + E_{US}(\vec{r}, t) E_{LO}^* e^{-i\vec{k}_{LO} \cdot \vec{r}} \right] dt \quad (1)$$

where E_{LO} is the electric field amplitude of the light from the reference arm, $\vec{k}_{LO}$ is the wave vector of the reference beam, $\vec{r}$ is the position vector and E_{US} is the electric field amplitude of the ultrasonically modulated light. The time dependence of the field amplitude E_{US} is due to the temporal variations in the field that are caused by moving particles.

The camera image (Figure 2a) contains fringes that correspond to the heterodyne term $E_{LO}^* E_{US}(\vec{r}, t)$. One can isolate the heterodyne term from the background light intensity $I_{bg}(\vec{r})$ in k-space after spatially Fourier transforming the CCD image [21], a method known as off-axis holography. In k-space I_{bg} is centered at the origin, and its spread is defined by the speckle size relative to the size of the camera pixels. The position of the heterodyne term is defined by the frequency of the fringes (Figure 2a). In turn, this fringe frequency depends on the angle of the wave vector ($\vec{k}_{LO}$) of the reference beam relative to the normal of the image sensor. The angle of the refer-

Figure 2 illustration of the principle of the HPSD method for sensing movement of scattering particles, the white box marks the position of heterodyne term in k-space. a) CCD camera image with integration time less than the decorrelation time of the medium, b) Fourier transform of a, c) CCD camera image with integration time longer than decorrelation time of medium, d) Fourier transform of c.

ence beam is chosen such that the heterodyne term is separated from the I_{bg} term, and still falls within the k-space image completely (Figure 2b). As a result, HPSD enables measurement of $E_{LO}^* E_{US}(\vec{r}, t)$ in isolation from the diffuse background light emanating from sample, written as

$$H(r) = E_{LO}^* \int_0^T E_{US}(\vec{r}, t) dt \quad (2)$$

where T is the integration time of the CCD camera. In case the medium is dynamic, the integration time of the camera suppress the interference fringes (see Figure 2c), hence reducing heterodyne term.

Further, we define the modulation depth as,

$$M \equiv \frac{\langle |H|^2 \rangle}{\langle |E_{LO}|^2 \rangle \langle |E_L|^2 \rangle T^2} \quad (3)$$

where $\langle \rangle$ denotes averaging over CCD pixels, $T|E_L|^2$ is the CCD signal corresponding to the total amount of diffuse light emanating from the sample and it is measured by switching off the reference beam. $T|E_{LO}|^2$ is the CCD signal corresponding to the light in the reference beam and it is measured while the sample arm is switched off. Using Eq.

2, the expression for the modulation depth M (i.e. Eq. 3) can be written as,

$$M = \frac{1}{\langle |E_L|^2 \rangle} \left\langle \int_0^T \int_0^T E_{US}(\vec{r}, t) E_{US}^*(\vec{r}, \tau) dt d\tau \right\rangle \quad (4)$$

$$= \frac{2\langle |E_{US}|^2 \rangle}{\langle |E_L|^2 \rangle T} \int_0^T (1 - \tau/T) C_E(\tau) d\tau$$

where, $C_E(\tau) \equiv \langle E_{US}(\vec{r}, t) E_{US}^*(\vec{r}, \tau) \rangle / \langle |E_{US}(\vec{r}, t)|^2 \rangle$ is the field autocorrelation function and τ is the time with respect to the start of acquisition of the detector. We arrived at Eq. 4 by assuming that E_{US} is ergodic and C_E is an even function of τ (see Ref. [29] for a similar derivation involving the intensity correlation function). Eq. 4 shows that the modulation depth M in HPSD is directly linked with the field autocorrelation function C_E of the ultrasonically modulated light. In areas with more flow, C_E will decrease as a function of time more rapidly. Therefore, we expect the modulation depth to decrease when the ultrasound focus is placed in areas with a higher blood flow.

2.3 Experimental setup

Details of the setup and the experiment are as follows. The sample is illuminated by a 760 nm CW laser (Coherent MBR EL Ti:sapphire, pumped by a Coherent Verdi 6), beam diameter 3 mm and ultrasonically modulated light is detected with a CCD camera (GRAS-14S5M-C, 1384 × 1036) through an aperture of diameter 3 mm. The CW laser beam is chopped into pulses of 1 μs at repetition rate of 25 kHz using AOM1. Laser power incident on the sample was measured to be 18 mW, which leads to the optical irradiance of 0.25 W/cm². The laser pulses are synchronized with ultrasound bursts of 1 μs to achieve a spatial resolution of 1.5 mm along the US propagation axis. The input laser beam is split into reference and sample arms using a beam splitter (BS). The reference arm is shifted in frequency by 5 MHz using AOM up (+80 MHz) and AOM down (−75 MHz) to match the frequency of the US transducer. The US transducer (Panametrics V309, diameter of 12.7 mm and focal length of 20.3 mm) was driven by a 5 MHz signal amplified using a power amplifier (ENI 350 L). Short bursts (0.6 μs) of five US cycles were used for local modulation of light resulting in a pressure of approximately 1 MPa in the US focal zone. The delays between the US burst and the laser pulse were set using a function generator, which allowed the ultrasound to propagate (z-axis) a

configurable distance before interrogating the region of interest with light.

2.4 Analytical model for blood flow measurements using ultrasound modulated optical tomography

To describe our blood flow measurements quantitatively, we apply the following physical model. We consider an external light source S that is 100 % correlated. The correlated light propagates from the source at $\vec{r}_o$ to the US focus at $\vec{r}$ with the propagator $G(\vec{r}, \vec{r}_o, \tau)$, where the light gets tagged with a tagging efficiency of $\eta(r)$. The ultrasonically tagged light propagates further to a point $\vec{r}_d$ at the boundary of the medium with the propagator $G(\vec{r}_d, \vec{r}, \tau)$ where it gets detected. The amount of ultrasonically tagged correlated light at point $\vec{r}_d$ is given as,

$$C_\eta(\vec{r}_d, \tau) = \iint G(\vec{r}_d, \vec{r}, \tau) \eta(\vec{r}) G(\vec{r}, \vec{r}_o, \tau) S(\vec{r}_o) dr_o dr \quad (5)$$

We describe the propagation of correlated light with the following three dimensional inhomogeneous diffusion equation [30],

$$D \nabla^2 G(\vec{r}, \vec{r}_o, \tau) - (\mu_d(\tau) + \mu_a) G(\vec{r}, \vec{r}_o, \tau) = -\delta(\vec{r} - \vec{r}_o) \quad (6)$$

where μ_d is the decorrelation coefficient, which is a function of time difference τ . $\vec{r}$ is the three-dimensional position vector, D is the diffusion coefficient in meter and μ_a is the absorption coefficient. During the propagation, light may encounter moving particles that decorrelate the light to some extent. Since the decorrelated light is not detected in our heterodyne setup (as described above), we consider it lost. Therefore, we can model the decorrelation as having the same effect on light as absorption. The decorrelation coefficient μ_d is defined as the number of scattering events per propagated meter (μ_s) times the fraction of light that gets decorrelated at each scattering event. In line with [30] we define, $\mu_d = \mu_s (1 - e^{-2\langle \Delta r^2 \rangle k_o^2 (1-g)})$, where g is the anisotropy factor and $\langle \Delta r^2 \rangle$ is the mean squared displacement of the moving particles, and $k_o \equiv 2\pi n / \lambda$ where n is the refractive index and λ is the wavelength of the light. In our experiments, we have blood flowing through a nylon tube, we treat it as a directed flow such that $\Delta r = v\tau$, with v the flow speed of the blood, and τ the time with respect to the start of the acquisition time of the detector.

To model the experiment in an analytically tractable way, we model the scattering medium as a cylinder of radius r_o , with a tube of radius r_T with blood flowing through it placed at $r=0$ (see Figure 3). As-

Figure 3 illustration of the model geometry. r_T is the tube radius, r_o is the radius of the object and the location of the source, r_{US} and ΔZ are the radius and length of the US focus.

suming radial symmetry, the diffusion equation in cylindrical coordinates reads

$$\begin{aligned} \frac{\partial^2 G(r, r_o, \tau)}{\partial r^2} + \frac{\partial G(r, r_o, \tau)}{r \partial r} - \frac{\mu_d(\tau) + \mu_a}{D} G(r, r_o, \tau) \\ = -\frac{\delta(r - r_o)}{D} \end{aligned} \quad (7)$$

where r is the radial coordinate. Further we define,

$\alpha = \sqrt{\frac{(\mu_{d,in} + \mu_{a,in})}{D_{in}}}$ and $\beta = \sqrt{\frac{\mu_{d,out} + \mu_{a,out}}{D_{out}}}$, where $D_{in} = (3(\mu'_{s,in} + \mu_{d,in} + \mu_{a,in}))^{-1}$ is the diffusion coefficient inside the tube with the blood flow and $D_{out} = (3(\mu'_{s,out} + \mu_{d,out} + \mu_{a,out}))^{-1}$ is the diffusion coefficient of the background medium in which the tube is placed. The solution to Eq. 7 is,

$$G(r, r_o, \tau) = \begin{cases} b K_o(\beta r) - \frac{r_o I_o(\beta r) K_o(\beta r_o)}{D_{out}} & r_T < r < r_o \\ a I_o(\alpha r) & 0 \leq r < r_T \end{cases} \quad (8)$$

Here, I_o and K_o are the Bessel functions of the first and second kind respectively, coefficients a and b are time dependent because of their dependence on μ_d and will be solved later. In Eq. 8 we chose $r_o > r_T$ and omitted the terms that diverge as $r \rightarrow \infty$ or $r \rightarrow 0$.

Next we solve for coefficients a and b ; considering the continuity of intensity $G(r, r_o, \tau)$ and the flux $(-D \frac{\partial G(r, r_o, \tau)}{\partial r})$ at the tube surface ($r = r_T$),

$$\begin{aligned} a &= -\frac{r_o K_o(r_o \beta)}{\alpha D_{in} r_T I_1(r_T \alpha) K_o(r_T \beta) + \beta D_{out} r_T I_0(r_T \alpha) K_1(r_T \beta)} \\ b &= \frac{r_o K_o(r_o \beta) (\alpha D_{in} I_1(r_T \alpha) I_0(r_T \beta) - \beta D_{out} I_0(r_T \alpha) I_1(r_T \beta))}{D_{out} (\alpha D_{in} I_1(r_T \alpha) K_o(r_T \beta) + \beta D_{out} I_0(r_T \alpha) K_1(r_T \beta))} \end{aligned} \quad (9)$$

Here, α , and D_{in} are both time dependent through their dependence on μ_d . We can now use Eq. 5 to calculate the fraction of correlated light that

propagates from the source, interacts with the US focus and reaches the detector. We modeled the ultrasound focus as a cylinder segment of radius r_{us} and height ΔZ concentric with the tube in which blood flows (see Figure 3), where part of the US focus lies outside the tube containing blood flow. At the ultrasound focus, the correlated light gets tagged, and locally acts as a source of frequency shifted correlated light. This tagged light propagates outwards from the ultrasound focus, and results in a net outgoing flux of frequency shifted correlated light. As a further simplification, we assume that the detector is placed at the same distance from the tube as the source ($r_d = r_o$), such that there is a symmetry in the propagation of inward flux from the source towards the ultrasound focus, and outward flux towards the detector. Using Eq. 5, the ultrasonically tagged correlated light that arrives at the detector is given as,

$$C_\eta(r, \tau) = 2\pi\Delta Z\eta \int_0^{r_{us}} G^2(r, r_o, \tau) r dr \quad (10)$$

Considering the definition of the modulation depth given in Eq. 4, the expression for the modulation depth based on the model is given as,

$$M = \frac{1}{T} \int_0^T (1 - \frac{\tau}{T}) \int_0^{r_{us}} G^2(r, r_o, \tau) r dr d\tau \quad (11)$$

We evaluated the Eq. 11 numerically to estimate the modulation depth by using the following parameters $r_o = 6$ mm, $r_{us} = 0.5$ mm, $\Delta Z = 1.5$ mm, $r_T = 0.48$ mm, $\lambda = 760$ nm, $\mu'_{s,out} = 0.7$ mm⁻¹, $\mu'_{s,in} = a \left(\frac{\lambda}{500(nm)} \right)^b$ where, λ is the wavelength in nm, $a = 220$ mm⁻¹ and $b = 0.66$ for the whole blood [31], $n = 1.33$, and $g = 0.99$ matching with the experimental situation and we fitted the proportionality constant to account for unknown tagging efficiency η and the total untagged light at the detector.

The model assumes a block flow of scattering particles in the tube whereas in reality the flow profile of blood is known to be parabolic. This flow profile reduces the effective radius of the tube in which the blood flows. We found that an effective tube radius of $r_{T-eff} = 0.7r_T$ matches well with the experimental data.

3. Results

To demonstrate the concept of depth-resolved measurements of blood flow, we performed three experi-

ments. In the first experiment, we investigated the relation between the ultrasound modulation depth and the acquisition time of the CCD camera for a fixed flow speed. We kept the flow speed of the blood fixed at 0.2 mm/s through the tube and varied the integration time of the CCD camera. Multiple line scans were made along the propagation direction of the US for integration times varying from 1.75 ms to 4 ms with a step of 0.25 ms. Here, 1.75 ms corresponds to the shortest integration time that still gave enough signal to be interpreted meaningfully.

Figure 4a shows the line scans along the z-axis, each line scan is acquired with a different integration time of the CCD camera. In Figure 4a, we compare two situations, one where the US focus overlaps the region with blood flow (at $x = 5$ mm), and one where the US focus is placed in the static background (at $x = 4$ mm). The modulation depth profile in the static background case is maximum when the US burst is centered at $z = 21.75$ mm. This is because that the probability of light to propagate from illumination aperture to the US burst and get modulated and propagate further to the detection aperture is maximum when the US burst is in the plane of illumination and detection aperture which is at $z = 21.75$ mm. The line scans at $x = 5$ mm in Figure 4a, correspond to the case where the US burst propagate through the tube with constant blood flow placed. Therefore, in these line scans we observe a local minima at the location of the tube (i.e $z = 22$ mm). This decrease in modulation depth at the location of tube is the result of the decorrelation of ultrasonically modulated light by the blood flow. It is apparent from Figure 4a that the modulation depth at these minima decreases with an increase in integration time. Figure 4b, shows the measured modulation depth and modulation depth predicted by the model as a function of the integration time of the CCD camera when the US focus was overlapping with the tube. The error bars on the experimental data are stand deviation of five measurements. The five measurement are performed after each other for a fixed integration time of the CCD, then the integration time of the CCD is changed and the next measurements are performed. Both the model and the experimental data show that the modulation depth decreases with increasing integration time of the CCD camera. The comparison between the model and the experiment data for an effective radius of the tube with blood is considered 0.7 times the geometric radius of the tube yielded R-Squared value of 0.92.

In the second experiment, we investigated the relation between the modulation depth and the local flow speed of the blood in the turbid medium. We kept the integration time of the CCD camera fixed

Figure 4 (a) UOT line scans through the tube ($x=5$ mm) and before the tube ($x=4$ mm) along the z -axis with varying integration time, (b) ultrasound modulation depth versus integration time of the CCD camera, for a flow speed of 0.2 mm/s of blood through the tube. The tube is placed in the US focus ($z=22$ mm, with $z=0$ being the surface of the transducer) and 5 mm (along x -axis) below the surface of the phantom. r_T is the actual radius of the tube, whereas $r_{T\text{-eff}}$ is the effective radius used in the model.

at 2.0 ms and changed the flow speed of the blood from 0 mm/s to 2.75 mm/s with increments of 0.23 mm/s per measurement. The delay time between US burst and light pulse for these measurements was such that the US focus overlapped with the tube. Figure 5 shows the measured modulation depth and modulation depth predicted by the model as a function of the flow speed of the blood. Without taking into account the data point corresponding to flow speed 0 mm/s the comparison between the ex-

Figure 5 Measured modulation depth as a function of flow speed of blood in the tube, for a fixed integration time of 2 ms. The tube is placed in the US focus ($Z=22$ mm) and 5 mm (along x -axis) below the surface of the phantom.

perimental data and the model yielded R-squared value of 0.87. In case of flow speed 0 mm/s the modulation depth predicted by model is factor of two higher than the experimental value. This discrepancy at a flow speed of 0 mm/s may be due to the fact that optical properties of the blood change as blood stops flowing [32], this aspect will be discussed further in the discussion session.

In the third experiment, we explored the feasibility of the technique to make depth-resolved maps of blood perfusion in turbid media. We scanned a region of 4×11 mm² in the scattering medium containing a nylon tube passed twice through the medium (see Figure 1c). The depth of the tube under the surface at the first and second pass was 5 and 10 mm respectively. The blood was pumped through the tube at flow speed of 0.2 mm/s. The medium was scanned with the US focus in the xz -plane, the scan along the x -axis was achieved mechanically while the z -axis was scanned by delaying laser pulses relative to US bursts. Two scans were performed consecutively, for the first scan the integration time of the camera was 1.5 ms (scan A) and for the second scan the integration time was 10 ms (scan B). Then the ratio (M_A/M_B) of the modulation depths in the two scans is calculated. Under ideal circumstances, in regions with blood flow the ratio will exceed one. Everywhere else the ratio will be unity, since the variation in modulation depth due to absorption is independent of the integration time of the CCD camera.

Figure 6a and b, show the two scans (A and B) in the xz -plane with an integration time of 1.5 ms and

Figure 6 UOT scans of turbid media having two nylon tubes embedded at $x=3$ and 8 mm relative to scan area with blood flow. a) scan A: modulation depth, integration time 1.5 ms, b) scan B: modulation depth, integration time 10 ms c) perfusion image: ratio of modulation depths (scan A and B,) d) comparison of line profiles along x -axis at $z=22$ mm for two scans A (dotted black) and B (dashed red) and ratio image (solid blue). The tubes with blood are located at $x=3$ mm and $x=8$ mm.

10 ms respectively. In both scans the regions close to the illumination and detection apertures show a high modulation depth which decreases towards the center of the sample. This is because, when the US focus is close to illumination/detection aperture it is more probable that the detected light has interacted with the US focus. In scan B the drop in modulation depth at the location of tubes is larger than in scan A, and it is caused by a combination of decorrelation and absorption of light by blood in the tubes. From scan A or scan B alone, it cannot be determined whether the drop in signal is caused by absorption or by blood flow, not is it possible to resolve the two tubes spatially.

Figure 6c shows the ratio of modulation depths of scans A and B. As expected, the ratio peaks at the centers of the two tubes, clearly demonstrating the ability to form depth-resolved images of the blood flow. Figure 6d shows the comparison of the line profiles of the conventional UOT scans A, B and the ratio image along the x -axis for $z=22$ mm. The two peaks in the line profile (blue solid line, d) of the ratio image correspond to the two tubes with blood flow. Based on the full width half maximum of these two peaks, indicated by vertical dotted lines in Figure 6d, we estimated the lateral along the z -axis is defined by the length of the US burst which is

equal to 1.5 mm in our experiment, given the US burst length of $1 \mu\text{s}$ and speed of sound 1500 m/s in water. The ratio image provides a depth-resolved semi-quantitative map of blood flow inside the turbid medium. Semi-quantitative here means that the signals are directly related to the flow speed of blood, given that the medium is acoustically homogeneous such that the shape and size of US focus remains unaltered while scanning a region of interest. The flow profile in c is slightly elongated along the optical axis (x -axis). This distortion may be caused by the highly forward scattering property of the phantom and blood in combination with a transmission mode experimental geometry.

4. Discussion and conclusion

We presented the use of ultrasound modulated optical tomography based on heterodyne parallel speckle detection to make semi-quantitative depth-resolved images of blood flow inside the scattering media. We showed theoretically that the modulation depth is directly linked to the field autocorrelation function of the ultrasonically modulated light field E_{US} and modeled the propagation of the field autocorrelation function of ultrasonically modulated light to better under-

stand the relation between the physical properties of moving particles and the measured modulation depth. Experimentally, we measured the modulation depth as a function of acquisition time of the CCD camera for a fixed flow speed of blood in the US focus and compared the results with the prediction of the model. We observed a good agreement between the model and the experimental data using effective radius of 0.7 times the actual tube radius to account for flow profile.

The observed decrease in modulation depth with increasing acquisition time is analogous to the LASCA signals, where the speckle contrast decreases with the integration time of the CCD camera [33]. Our findings suggest that our technique is similar to LASCA with the added benefits of depth selectivity. Further we observed that both the model and the experimental show that the modulation depth decreases with increasing speed of blood flow for a given integration time of the CCD camera. However, at a flow speed of 0 mm/s, the modulation depth predicted by the model is two times higher than experimentally measured modulation depth. One possible reason for this discrepancy might be the fact that the model assumes the same optical properties of blood at all flow speeds, while it has been demonstrated that the scattering of blood decreases as flow speed approaches zero due to Rouleaux formation [32]. The decrease in scattering in turn reduces the tagging efficiency of the US thereby decreasing the measured modulation depth, since it is dependent on the scattering coefficient of the medium [34]. Although our observation is consistent with the phenomenon, the hypothesis requires further investigation and quantification, either by quantifying the changes in optical properties at lower flow speed of blood and taking that into account in the model or by simulating the scattering fluid that resembles blood and its optical properties are not dependent on the flow speed. Our future work will focus on this aspect and also on quantifying the performance of UOT as a technique to measure blood flow in comparison with ultrasound Doppler and photoacoustic flowmetry in terms minimum/maximum measurable flow speeds and sensitivity.

The minimum flow speed that can be detected by this technique is only limited by the dynamics of the background medium. By increasing the integration time of the CCD, the system may be made sensitive to even the slowest movements inside the sample, with the caveat that red blood cells aggregate at such low flow speeds. Considering our results of experiment 2 we were able to accurately measure the minimum flow speed of 0.3 mm/s, which is 20 times more sensitive than the ultrasound power Doppler using a transducer with a center frequency of 6 MHz

[35]. The minimum detectable flow speed using power Doppler is dependent on the frequency of ultrasound transducer used which puts physical limit on minimum detectable flow speed and imaging depth [35]. In photoacoustic flowmetry the contrast is superior to ultrasound however the detection principle is similar in nature to that of ultrasound, therefore the minimum detectable flow speed limit is expected to be identical.

The maximum measurable flow speed is limited by the minimum integration time required to measure a signal on the CCD. By employing a different detection technique with tandem pulses designed for acousto-optic imaging [36], the effective integration time may be reduced to as low as 20 ns, which is 4 orders of magnitude shorter than the integration time used in our experiment number 2. In experiment number 2 we have seen that at the integration time of 2 ms the flow speed of 2.75 mm/s results in maximum measurable decrease in modulation depth, as a result the system becomes insensitive to flow speed higher than 2.75 mm/s. However, when using integration time of 20 ns the limit of maximum measurable decrease in modulation depth would occur at 27.5 m/s, making maximum measurable flow speed 4 orders of magnitude higher than in our current set up.

Our findings suggest that UOT with HPSD has a potential for depth-resolved blood flow imaging in highly diffusive media. Our model shows that the decorrelation rate of the background medium surrounding the blood vessel affects the quantitative results. Therefore, we expect that the ability of UOT for quantitative imaging can be further improved by combining it with LASCA to obtain the global decorrelation coefficient μ_d of the background medium, and using this measured coefficient in our model to compensate for the background dynamics. In diffusive media where the light trajectories are scrambled enough to form a near isotropic light irradiance, the situation similar to presented in experiment 3, the spatial resolution of the technique is approximated to be 1.5 mm. We expect the techniques ability to quantitatively measure flow speed of blood in two blood vessels would be compromised if the two blood vessels are spatially unresolvable. One other perceivable limitation may occur when a small blood vessel is situated close to large blood vessel with a high-speed blood flow in it. In this case, the larger blood vessel will cause a shadow since most of the light in its surrounding would have been decorrelated through its interaction with it and will affect the visibility of the small blood vessel. The quantification of such effects is outside the scope of this proof of principle paper and will be investigated in our future work.

In conclusion, we presented a method for depth resolved imaging of blood in highly scattering media based on UOT using HPSD method. We demonstrated through analytical model and experiment that UOT signal is dependent on the flow speed of blood in the US focus for a given integration time of the detector and vice versa. We demonstrated through a simple experiment that the ratio of the two UOT scans performed using different integration time of the CCD camera can be used to do depth resolved imaging of blood vessels inside the scattering media.

We acknowledge Steffen Resink, Berkan Basel and Robert Molenaar for their input and valuable discussions. This research is supported by the Technology Foundation in the Netherlands STW, under Demonstrator grant, project 14546 and the European Research Council under the European Union's Horizon 2020 Program / ERC Grant Agreement no 678919, and by the MIRA Institute of the University of Twente.

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