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Resolving directional ambiguity in dynamic light scattering-based transverse motion velocimetry in optical coherence tomography

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Abstract

Dynamic Light Scattering-based Optical Coherence Tomography approaches have been successfully implemented to measure total transverse (xy) flow speed, but are unable to resolve directionality. We propose a method to extract directional velocity in the transverse plane by introducing a variable scan bias to our system. Our velocity estimation, which yields the directional velocity component along the scan axis, is also independent of any point spread function calibration. By combining our approach with Doppler velocimetry, we show three-component velocimetry that is appropriately dependent on latitudinal and longitudinal angle.

Many clinically relevant measurements, including retinal blood flow velocity and cilia-driven fluid flow velocity, require the quantification of transverse motion. Standard Doppler can measure only axial velocity (optical or z -axis). Since dynamic light scattering (DLS) methods of analyzing a time-varying scattering signal can determine various physical properties of the underlying particles, there is increasing interest in using DLS to access transverse velocity information [1]. Indeed, DLS-based Optical Coherence Tomography (OCT) methods [2-7], have been recently implemented to measure axial velocity and transverse flow speed (*en face* or xy -plane). All these DLS-OCT methods rely on the fundamental principle that a time-varying signal, generated from the convolution of moving scatterers with the imaging point spread function (PSF), can be autocorrelated to recapitulate the PSF with the width modulated by flow speed. As a simple example, if the PSF has the general form of $\exp[-2(x^2+y^2)/w_{xy}^2]$, where w_{xy} is the symmetric transverse beam waist, then the autocorrelation function becomes $\exp[-(v_x^2+v_y^2)\tau^2/w_{xy}^2]$, where v_y and v_x are the x and y components of flow velocity, and τ is the correlation lag variable. By numerically fitting DLS data to a specific form of autocorrelation function, which may be a simple

Gaussian, total transverse speed can be extracted [5, 7]. Because the autocorrelation function depends on the sum ($v_y^2 + v_x^2$) and not independently on v_x and v_y , however, DLS cannot measure directional velocity unless a mechanism for symmetry breaking is introduced.

Previous attempts to break symmetry and resolve directional ambiguity have included the implementation of dual focal volume cross-correlation in OCT [8]. In fluorescence correlation spectroscopy (FCS), the analogous incoherent technique in fluorescence microscopy, linear and circular scanning schemes previously were used as symmetry breaking mechanisms [9, 10]. Here, we use directional scanning as a method of directional symmetry breaking to isolate a single transverse velocity component with a conventional spectral domain OCT system. By doing so, we demonstrate that we can quantify transverse vectorial velocities using DLS-OCT, and in combination with Doppler, obtain three-component velocimetry. We note that our method, which effectively modulates the velocity along a single axis to measure that velocity, is one example of a more general method of estimating a physical parameter by measuring the deviation from a known perturbation.

We define our stationary, complex-valued OCT signal at some spatial location $\mathbf{r}=(x,y,z)$ at time t as $S(\mathbf{r},t)$, with complex conjugate $S^*(\mathbf{r},t)$. Our autocorrelation function $G(\mathbf{r},\tau)$ is defined as $G(\mathbf{r},\tau)=\langle\delta S(\mathbf{r},0)\delta S^*(\mathbf{r},\tau)\rangle/\langle|\delta S(\mathbf{r},0)|^2\rangle$, where δS is the deviation of the signal from its mean, $\delta S=S-\langle S\rangle$. More generally, given a three-component velocity field $\mathbf{v}=(v_x,v_y,v_z)$, a diffusivity D , an imaging wavenumber $k=2\pi/\lambda$, a $1/e^2$ coherence length w_z , and a lateral beam waist w_{xy} , our autocorrelation function takes the form [5]:

$$G(\mathbf{r},\tau)=e^{2ikv_z\tau}e^{-4k^2D|\tau|}e^{-v_z^2\tau^2/w_z^2}e^{-(v_x^2+v_y^2)\tau^2/w_{xy}^2} \quad (1)$$

The right hand side consists of four exponential terms. The first term is the complex Doppler term [11]. The second term is a simple negative exponential decay that depends on D and k and arises from diffusion along fringes. The final two exponential terms are Gaussian decay terms, dependent on the velocity flow vector $\mathbf{v}$ as well as w_{xy} and w_z , and arises from translational motion across the illumination profile. We note that Eq. 1 is the simplification of a more complicated expression that contains an additional decay term resulting from molecules randomly diffusing through the illumination profile. This decay term, however, causes decorrelation on a much slower scale than the other terms and can be neglected, as previously described [5]. Additionally, because the autocorrelation function decays with the quantity $(v_x^2+v_y^2)$, multiple independent measurements are required to separately estimate v_x and v_y .

Symmetry can be broken and directional ambiguity can be resolved by explicitly acknowledging three frames of reference and imparting a symmetry breaking modulation in one of those frames, as shown in Fig. 1a. Specifically, the three frames are flow, scanning, and measurement. The flow velocity of the sample, the quantity we wish to estimate, is labeled as $\mathbf{v}^{\text{flow}}=(v_x^{\text{flow}},v_y^{\text{flow}},v_z^{\text{flow}})$. We experimentally impart a scan velocity $\mathbf{v}^{\text{scan}}=(v_x^{\text{scan}},v_y^{\text{scan}},0)$. Finally, the apparent velocity in the measurement frame of reference $\mathbf{v}^{\text{meas}}$ is given as the difference between the scan and flow velocities, $\mathbf{v}^{\text{meas}}=\mathbf{v}^{\text{flow}}-\mathbf{v}^{\text{scan}}$.

If we restrict ourselves to scanning along a single axis e.g. $\mathbf{v}^{scan}=(v_x^{scan},0,0)$, and consider only the magnitude of our autocorrelation function, then we can express our measured decay rate γ^{meas} as $|G(\mathbf{r}, \tau)|=\exp[-(\gamma^{meas} \tau)^2]$, such that:

$$\gamma^{meas}=\left\{\left(v_x^{flow}-v_x^{scan}\right)^2/w_{xy}^2+\left(v_y^{flow}\right)^2/w_{xy}^2+\left(v_z^{flow}\right)^2/w_z^2+4k^2/\tau}\right\}^{1/2} \quad (2)$$

γ^{meas} is the sum of three translational terms and one diffusive term. Three of those terms depend only on the flow frame of reference, but the term arising from translation in the x -direction contains components from both the scanning and flow frames. Because the scanning frame of reference can be independently modulated, symmetry breaking is made possible.

If we modulate the scan bias v_x^{scan} at a variety of different speeds and estimate the resulting decay rates γ^{meas} , then all the terms in Eq. 2 besides v_x^{scan} remain constant. We trace out a curve similar to Fig. 1b (solid), which we refer to as the decay rate-scan bias curve. We can then perform a non-linear fitting to three independent parameters, A , B , and C , which are all invariant with scan rate:

$$\gamma^{meas}=A\left\{\left(B-v_x^{scan}\right)^2+C}\right\}^{1/2} \quad (3)$$

where A is a function of w_{xy} , B is equal to v_x^{flow} , and C is a function of v_z^{flow} , v_y^{flow} , D , k , w_{xy} , and w_z . Importantly, C lumps together diffusion and motion orthogonal to the scan axis. To give some physical intuition to this equation, we note that if we are measuring flow that is effectively non-diffusive and only in the direction of the scan bias, Eq. 2 simplifies to $\gamma^{meas}=|v_x^{flow}-v_x^{scan}|/w_{xy}$ (Fig. 1b dashed). The interpretation of the decay rate-scan bias curve is that as the scan rate is changed, the decorrelation rate is also modulated (Fig. 1c). The scan rate is varied until it best matches the flow rate, at which point a single particle is “tracked” by the scan and the decorrelation rate approaches zero. Orthogonal motion and diffusion may prevent the curve from reaching zero in a non-idealized circumstance, but Eq. 2 will still reach a minimum when $v_x^{flow}=v_x^{scan}$. Thus, by varying scan speed in a single direction, a single velocity component can be estimated by solving for the best fit parameter B , the value which also minimizes the decay rate-scan bias curve. We note additionally that the actual value of our $A=1/w_{xy}$ parameter does not affect our best fit for B , and thus our method calculates v_x^{flow} independent of any beam waist calibration. Moreover, particle diffusion modulates only the C parameter, and thus velocity estimates are in principle independent of diffusive motion as well.

According to Eq. 3, solving for our best fit parameter B gives us the projection of the velocity vector along our scan axis, up until now taken to be along the x -axis. By extension, if we scan in an orthogonal direction in a separate v_y^{scan} curve, we can also estimate v_y^{flow} . In other words, two sets of linearly independent scans will give us two linearly independent components of flow. In principle, a third axis of scanning could be implemented in the z -

direction, but practically, scanning axially is more difficult, and our DLS signal already measures v_z^{flow} via the Doppler term.

As an initial demonstration, we tested our methodology in a cylindrical flow phantom that consisted of a 500 μm diameter capillary tube that was placed horizontally and aligned with our direction of scanning (Fig. 2a, $\theta=0^\circ$). We used a spectral domain OCT (Thorlabs Telesto) running at 91 kHz A-scan rate (interscan time $\tau_0 \sim 11.0 \mu\text{s}$) and employed pressure-driven flow with an expected midline velocity of 32 mm/s. Our scattering solution consisted of 100 nm diameter polystyrene beads in a physiological solution. The solution additionally contained 0.1% Triton X-100 in order to prevent aggregation of the beads.

We scanned 250 μm lengthwise over the middle of our capillary tube, initially with the tube horizontal and 8 scan biases ranging from $\pm(6-44)$ mm/s, leading to B-scans consisting of 250-2000 A-scans. After computing $|G(\tau)|$ from our B-scan data (Fig. 1c inset), we estimated γ^{meas} . This decay parameter can be estimated in multiple ways, including with non-linear fitting algorithms to find the width of a Gaussian peak. Here, we directly computed the decay parameter by taking the natural log of our signal and averaging the expression

$$\ln [|G(n\tau_0)| / |G(\tau_0)|] = -(\gamma^{meas})^2 (n^2 - 1) \tau_0^2$$

over $2 \leq n \leq 8$, where $\tau = n\tau_0$. This expression accounts for the fact that our autocorrelation function $|G(\tau)|$ may have an experimental prefactor, denoted as β , arising from delta-correlated noise, i.e. $|G(\tau)| = \beta \exp[-(\gamma^{meas}\tau)^2]$ [5]. β is eliminated in the above expression by normalizing the data by the first non-zero time lag.

Plotting γ^{meas} across the cross-section of the capillary tube for a single scan bias yields a parabolic curve, consistent with a parabolic Poiseuille flow profile. Moreover, plotting γ^{meas} for a series of different scan biases shows a family of parabolic curves consistent with Poiseuille flow with an imparted velocity offset (Fig. 2b). As in Figs. 1b and 2c, we next plotted γ^{meas} , averaged over 5 repeats, at a single capillary depth as a function of scan biases. We then fit the experimental relationship between scan velocity and γ^{meas} to Eq. 3 to solve for our best fit parameter B . As discussed above, the B parameter is our estimate of directional flow velocity along the direction of scanning. That parameter B also corresponds to the minimum of the decay rate-scan bias curve highlights an important physical insight of our approach. Our method seeks to minimize decorrelation by best matching the scan bias with the flow velocity. Finally, we plotted our estimate of v_x^{flow} as a function of depth in the tube (Fig. 2d, *circle line*), along with the predicted velocities based on Poiseuille flow (*solid line*), and a Doppler signal obtained by tilting the tube (*dashed line*). Our measurements of directional transverse velocimetry are in excellent agreement with predicted values.

Once we had confirmed that we could estimate the directional velocity of a fluid known to be aligned in the direction of our scan bias, we also wanted to show that we could estimate flow of an arbitrary orientation. Based on Eq. 2, we should be able to scan in two orthogonal directions in two separate curves in order to recover both x and y velocities simultaneously.

To demonstrate our prediction, we rotated a flow phantom in the xy -plane at various longitudinal (azimuthal) angles ϕ to modulate v_x^{flow} versus v_y^{flow} . We simultaneously measured these two flow rates using our scanning DLS-OCT method with orthogonal scan axes i.e. $\mathbf{v}^{scan1}=(v_x^{scan},0,0)$ and $\mathbf{v}^{scan2}=(0,v_y^{scan},0)$. In order to minimize spatial variations of $\mathbf{v}^{flow}$, which is characterized by a parabolic profile as one scans cross-sectionally across a cylindrical tube, we employed a 0.2×5 mm rectangular capillary tube with a flat surface. This rectangular tube, with its large aspect ratio, approximates pressure driven flow between two infinite plates and is more representative of ciliary-driven flow than a cylindrical capillary tube is. Due to our lower predicted peak flow speed of 2.3 mm/s, we decreased our scan bias speeds to better match our range of velocity measurements, with a scan velocity range of $\pm(1.3-13)$ mm/s. Fig. 3a shows that our x -velocity component $|v_x^{flow}/v|$ (dark circles) falls as a $\cos\phi$ dependence (dark line), while $|v_y^{flow}/v|$ (light circles), plotted as absolute value for ease of viewing, increases with a $\sin\phi$ dependence (light line), as we would expect in changing our azimuthal angle. Additionally, the vector sum of the two components yields an invariant total peak speed $|v|$ regardless of tilt angle (Fig. 3b), with a value of 1.9 ± 0.1 mm/s (SD), which is close to our predicted value.

Having shown we could measure two-component flow in the lateral plane, we next confirmed that our measurement would be independent of the latitudinal angle θ . We modulated θ while measuring v_x^{flow} with our method and v_z^{flow} using Doppler. Tilting the capillary tube in the vertical direction θ caused us to scan over multiple flow velocities in the cross section of the capillary tube, violating our underlying assumption of stationarity; to address this issue, we took shorter scan segments (50 -150 μm) at the higher tilt angles θ and averaged over 10 repeats. Fig. 3c shows that, similar to our results in the xy -plane, measuring x and z velocities simultaneously yields a $\cos\theta$ and a $\sin\theta$ dependence of the two components. The overall speed in the transverse plane is similarly independent of polar angle (Fig. 3d), with a value of 2.4 ± 0.3 mm/s (SD). Overall, our ability to measure three orthogonal vector components independent of flow angle indicates that our method is capable of three-component velocimetry.

In comparison to previous decorrelation-based protocols, our method allows for directional determination of transverse velocity where only measurement of magnitude was previously possible. Our method has the advantage that velocity estimation is not dependent on either beam width calibration or decorrelation due to diffusion. Our method comes with the tradeoff of requiring multiple scans at different velocity biases, thereby increasing acquisition time. Thus, at the moment our method is most suitable for measuring steady-state flows with slowly varying spatial profiles, such as ciliary- driven surface flow.

One source of uncertainty in our measurements, and more generally in DLS, arises from the fact that short temporal acquisitions capture the dynamics of only a few independent ensembles of moving scatterers. As a consequence, measurements require aggregate statistics in order to reduce velocity estimation error. Indeed, the FCS literature [12] may serve as a starting point for understanding how factors such as the number of repeated measures and the number of particles per focal volume influence measurement signal-to-noise ratio (SNR).

Another source of uncertainty in our measurements is the appropriateness of the autocorrelation model. The autocorrelation model presented in this Letter makes two important assumptions that may be violated: (1) the underlying particle dynamics are constant over the profile of a single PSF, and (2) the signal is stationary over all autocorrelation lags, both temporally and spatially. Regarding the first assumption, recent work has demonstrated that the autocorrelation function $G(\tau)$ can be modified to take into account a spatially varying flow profile over the axial dimension of the focal volume [13], and would provide an appropriate modification to our autocorrelation function. With regards to the second assumption, as with Doppler OCT, stationarity is violated by scanning over finite extents of a flow velocity profile. Violation is minimized, however, if flow profile gradients are small relative to the dimensions of the scan distances.

The dynamic range of our system is likely determined by two major factors. Our maximum measurable speed is expected to be a function of our maximum scan speed. Our minimum measurable speed is expected to be determined by the density of sampling scan biases as well as by measurement noise arising from diffusion, scan non-linearity, and scan jitter at low speeds. Future studies will further characterize SNR and dynamic range properties of our scan protocol.

In sum, our novel OCT velocimetry method demonstrates that DLS-OCT can be used to quantify directional flow velocity measurements in the transverse (xy) plane. Our method is free from the kinds of directional ambiguity that are inherent to previous DLS methods. We implement directional symmetry breaking through the introduction of a scan bias. Our approach is analogous to other measurement methods such as pump probe in which specific physical parameters are modulated in order to measure those parameters. Overall, we show measurement of three orthogonal velocity components in scattering media using DLS-OCT.

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Fig. 1.

Symmetry breaking and directional ambiguity resolution using a scan bias. (a) Three frames of reference: flow, scanning, and measurement, with angles defined. Definition of latitudinal angle θ and longitudinal (azimuthal) angle ϕ (b) Theoretical decay rate-scan bias curve, based on Eq. 1, showing the case where v_x^{flow} is the only component of fluid flow (*dashed*) versus the case of non-zero orthogonal motion and diffusion (*solid*). (c) Theoretical autocorrelation curves of two scan biases, $v_x^{scan} = 0$ (*dark*) and $v_x^{scan} = -v_x^{flow}$ (*light*), the widths of which represent two points along curve 1b. (*inset*) Sample autocorrelation curve from data.

Fig. 2.

Estimation of directional transverse flow. (a) Orientation of capillary tube (b) Decay rate γ^{meas} across capillary tube at three different scan biases: -44 mm/s (*dark*), 22 mm/s (*medium*), 44 mm/s (*light*). (c) Representative decay rate-scan bias curve (*circles*) with fit to Eq. 3 (*line*), where the minimum corresponds to $v_{\text{x}}^{\text{scan}} = v_{\text{x}}^{\text{flow}}$. (d) Plot of estimate of $v_{\text{x}}^{\text{flow}}$ as a function of depth of capillary tube (*circle-line*), theoretical prediction (*solid line*), and Doppler measure (*dashed line*).

Fig. 3.

(a) Measurement of $|v_x^{flow}/v|$ (dark circles) and $|v_y^{flow}/v|$ (light circles) both with scanning DLS-OCT as a function of azimuthal angle ϕ . (b) Total speed $|v|$ as a function of azimuthal angle. (c) Measurement of $|v_x^{flow}/v|$ with scanning DLS-OCT (dark circles) and $|v_z^{flow}/v|$ using Doppler (light circles) as a function of latitudinal angle θ . (d) Total speed $|v|$ as function of latitudinal angle.