Interpreting inverse correlation time: From blood flow to vascular network

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ABSTRACT

The inverse correlation time (ICT) is a key quantity in laser speckle contrast imaging (LSCI) measurements. Traditionally, ICT is regarded as a metric of blood flow, such as speed or perfusion. However, we highlight that ICT not only contains important information about blood flow, but also reflects the underlying structure of the vascular network. In the past, ICT has been found to be correlated with vessel diameter. Here, we further report that ICT exhibits a different sensitivity to blood flow depending on vessel orientation. Specifically, ICT is more sensitive to blood flow speed changes in vessels descending from or arising to the tissue surface, compared with those laying parallel to the surface. Those findings shift our understanding of ICT from purely blood flow to a combination of blood flow and vascular network structure. We also develop theories to facilitate the study of vascular network’s impact on ICT.

1. Introduction

Laser speckle contrast imaging (LSCI) has gained attention in recent years for the quantification of blood flow in biomedical imaging applications [1–3]. It is non-invasive, non-ionizing, and label-free. In general, the higher the blood flow speed, the more rapidly the speckle patterns will vary in time, leading to a lower speckle contrast when integrated over the camera exposure time [4,5]. Valuable information about blood flow perfusion in regions of interest can be extracted in real time from the continuous and wide-field 2-dimensional (2D) monitoring of speckle contrast [6,7]. Nevertheless, the speckle contrast only provides qualitative measurements of the underlying blood flow [8]. Numerous efforts have been put into advancing LSCI from qualitative to a quantitative imaging modality [9–17].

One promising path is to relate speckle contrast to the autocorrelation function of detected electric field, \( \gamma(t) \) and extract the inverse correlation time (ICT) as the index of blood flow. Within a limited set of conditions, ICT is proportional to the typical speed of blood flow within a certain range [18,19], and it has demonstrated great potential in quantifying cerebral blood flow and facilitating intraoperative flow monitoring [20–23].

In view of the promise of ICT in transforming LSCI to a quantitative imaging modality, the strategy to extract ICT from measured speckle dynamics is gaining the significant attention. Initially this was done with single-exposure LSCI [18,19], and more recently, multi-exposure speckle imaging (MESI) was developed to extract ICT more accurately from the confounding effects of static scattering, instrumentation noise and loss of correlation due to speckle averaging [24–26]. Recently, dynamic light scattering imaging (DLSI) was proposed to reduce the ICT estimating error owing to an inaccurate model of electric field autocorrelation function [27].

Though ICT has been mainly interpreted as a metric of blood flow, such as speed, or perfusion, there is increasing evidence that ICT is subject to the structure of the vascular network. Kazmi et al.’s experimental results showed that ICT might be quantifying neither volumetric flux nor flow speed but the product of the flow speed and vessel diameter [28]. Fredriksson et al. reported the vessel packaging effect in which the confinement of blood to vessels with an average diameter of 40 μm could lead to a 50% reduction in perfusion estimation by LSCI compared with homogeneous blood distribution inside the tissue [29]. Jafari et al. found that ICT could be shifted over 10 times under the homogeneous assumption compared with using the actual vascular geometry, highlighting the significant impact of vascular geometry on ICT [30].

The impact of vessel orientation on ICT has not been fully investigated yet. In this work, we conducted Monte Carlo simulations and found that ICT of vessels perpendicular to the tissue surface, i.e. descending from/arising to the surface, exhibits a higher sensitivity to blood flow changes than that of vessels laying on the surface. Such finding is confirmed by experimental validation in vivo combining MESI and 2-Photon (2P) imaging. Those results suggest that the structure of the underlying vascular network deserves more attention than it currently receives in the interpretation of ICT. We also develop a generalized theory to facilitate the study on the impact of vascular network structure. It is compatible with all extant \( \gamma(t) \) models and free of assumptions about groundtruth blood flow speeds.

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2. Theory and methods

2.1. A unified theoretical framework for ICT interpretation

According to the traditional dynamic light scattering (DLS) and diffusing wave spectroscopy (DWS) theories, in different scattering regimes (single vs. multiple) and flow conditions (ordered vs. unordered), different assumptions should be made about the form of the electric field autocorrelation function \( g_1(t) \) [31–35], as shown in Fig. 1a [27]. \( r_c \) is the correlation time and ICT is defined as \( 1/r_c \). The modulation number \( n \) is the main differentiating factor among those models.

In this paper, we first introduce a unified theoretical framework that is compatible with all extant \( g_1(t) \) models. After Monte Carlo simulation of photon migration inside the tissue, the electric field auto-correlation function \( g_1(t) \) can be calculated according to Eq. (1) (ref. [36])

\[
|g_1(t)| = \int_{-\infty}^{\infty} \Omega(\omega)e^{i\omega t} d\omega
\]

where \( \Omega(\omega) \) is the normalized power spectral density of the detected electric field, \( \omega \) is the frequency of the detected electric field, \( \Omega(\omega) \) is the Fourier transform of \( \Omega(\omega) \) and \( \Omega(\omega) \) is the power spectral density of the detected electric field. For clarity, the \( \Omega(\omega) \) is rewritten in terms of \( \Omega(\omega) \) as the Fourier transform of \( \Omega(\omega) \).

Considering the Wiener-Khintchine theorem, if \( \omega \) is the normalized power spectral density of the detected electric field, then \( g_1(t) \) would be the Fourier transform of \( \Omega(\omega) \) (Eq. (2)) (ref. [40]). Therefore, we arrive at Eq. (3) that \( \Omega(\omega) \) is a shifted version of \( \Omega(\omega) \).

\[
\Omega(\omega) = P(Y) = \int P(Y)e^{i\omega t} d\omega
\]

where \( \omega = \omega_0 + \omega_n \) and \( \omega_0 \) is the center frequency of the electric field.

Substituting \( P(Y) \) for \( \Omega(\omega) \) is advantageous in that \( P(Y) \) reveals the mechanism of spectrum broadening in LSCI since \( P(Y) \) is the accumulation of frequency shifts induced by dynamic scattering. \( P(Y) \) and specific forms of \( g_1(t) \) can be bridged by Eqs. (4) and (5). For \( g_1(t) = e^{-i\omega t}t_c \),

\[
\frac{1}{t_c} = \Gamma
\]

where \( \Gamma \) is the half width at half maximum of the Lorentzian \( P(Y) = \frac{1}{2 \pi} \left( 1 + \frac{\omega_n}{\omega_0} \right) \).

\[
\frac{1}{t_c} = \frac{1}{\sqrt{2\pi} \sigma}
\]

where \( \sigma \) is the standard deviation of the Gaussian \( P(Y) = \frac{1}{\sqrt{2\pi} \sigma} e^{-\omega^2/2\sigma^2} \).

See proof in Supplemental Material Section 2.

These two equations (Eqs. (4) and (5)) provide new interpretations of ICT (i.e., \( \frac{1}{t_c} \)) from the point of view of \( P(Y) \). For Lorentzian \( P(Y) \), ICT determines the half width at half maximum while in Gaussian \( P(Y) \), ICT is a scaled version of the standard deviation. Note that in both cases, ICT serves as a kind of measure of the broadening of the spectrum of \( Y \).

2.2. Characterizing the inherent properties of vascular network to produce ICT

In this section, we aim to decouple the effects of blood flow speeds and vascular structure on ICT and develop tools to study the vascular network individually. Further analyzing the structure of \( Y \) and separate the contributions to \( Y \) by the blood flow and others, we arrive at Eq. (6)

\[
Y = Y_f + Y_r = Y_f + k\tilde{v}
\]

where \( Y_f \) represents the contributions to \( Y \) by motions induced by blood flow while \( Y_r \) denotes the component in \( Y \) that is independent of the blood flow. \( Y_f \) can be expressed as the product of \( k \) and \( \tilde{v} \) where \( k = \sum_{i=1}^{N} |a_i| \cos q_i \) and \( q_i \) is the angle between \( \tilde{q} \) and \( \tilde{v} \). The \( \tilde{q} \) and \( \tilde{v} \) follow same definition as in Eq. (1).

Note that \( k \) represents the weighted average of absolute blood flow speeds and the weighting is determined by the photon’s scattering geometry, i.e., the scattering angle and the angle between the momentum transfer vector \( \tilde{q} \) and velocity vector \( \tilde{v} \). Substituting Eq. (7) in case of blood flow quantification. Either of the following two equations (Eqs. (7) and (8)) can be solved for any electric field auto-correlation functions taking the form of \( g_1(t) = e^{-i\omega t}/\sqrt{\gamma^2} \) (see Supplemental Material Section 3 for proof). This suggests that ICT in the various \( g_1(t) \) models shown in Fig. 1a can be unified as the reduced case of the standard deviation of \( Y \). Since \( P(Y) \) is essentially the same as the power spectral density of the electric field of detected light (Eq. (3)), ICT can be interpreted as the scaling factor of the variance of the detected optical spectrum as well. Since \( Var(Y) \) measures the variance of frequency shifts in the dynamics scattering process, we call it the variance of dynamic scattering.
of collected photons is fully randomized, i.e., $E(k)$ is 0. This is true for parenchyma regions where dynamic scattering is dominated by microvessels whose orientation is randomized. Our simulation results of $E(k)$ on parenchyma regions also support that as Section 3.1 will show.

$$Var(Y) = Var(Y_i) + Var(k)E(\tilde{v}^2)$$  \hfill (9)

With Eq. (9), we define the characteristic variance of dynamic scattering as $Var(k)$. The physical implications of $Var(k)$ are evident as the slope of the diagram shows in Fig. 1b if Eq. (9) is plotted. $Var(k)$ can be defined for each detection point (i.e., a pixel on the camera) in LSCI and it characterizes the ability of the sampled vascular network to decrease speckle contrast or increase ICT under the specified illumination/detection setup at the detection point. The larger the $Var(k)$, the stronger the ability of the vascular network to decrease speckle contrast or increase ICT under the same blood flow speed, at the detection point. In addition, $Var(k)$ is independent of the blood flow speed since it is defined with unit speeds. Hence, it reveals the inherent properties of the vascular network under the specified illumination/detection setup.

### 2.3. Monte Carlo simulation

The simulation algorithm is based on Ref. [30]. The uniform flat beam profile is used for simulation. The effects of ordered motion of the RBCs along the direction of vessels are investigated in this study. Though the diffusive motion of RBCs has been recently suggested as dominating the correlation decay in diffuse correlation spectroscopy (DCS) measurements [41], it is unclear whether the effects are due to the radially diffusive motion of RBCs within vessels or axially ordered motion of RBCs along the vessel but within a diffusive vascular network where vessels are curved and direction-randomized. Nor is it clear whether the observation holds in LSCI. Jafari et al. found that the axially ordered motion of RBCs along the vessel adequately describes the particle dynamics in LSCI given the strong agreement between experimental and simulated speckle contrast values [30]. Hence, the effects of radially diffusive motion of RBCs within vessels might be negligible in LSCI. The potential impact of radially diffusive motion of RBCs within vessels to findings of this study is also discussed in Section 4.1.

The simple vascular geometry for simulation is made of parallel and equally spaced vessels (similar to Ref. [41]). Vessels in the first geometry are parallel to y axis, and parallel to z-axis in the second geometry (Fig. 2a). Some of the major settings are as follows: geometry size: $3 \times 3 \times 3 \text{ mm}^3$, the radius of vessels: 0.01 mm, space between vessels: 0.1 mm, radius of the incident beam: 0.25 mm, radius of the detector: 0.01 mm, NA of the detector: 0.2. The detector is placed in the center of the field of view and right above the vessels. The optical properties of vessels and tissue are the same as in Ref. [41].

For the realistic vascular geometry (shown in Fig. 2b), the simulation flow for generating the photon trajectories through the 3D geometry is detailed by Jafari et al. [30]. Briefly, parallelized Dynamic Light Scattering Monte Carlo (DLS-MC) simulations were launched on the Stampede2 Skylake compute nodes on Texas Advanced Computing Center (TACC) using the Message Passing Interface (MPI) protocol to simulate $80 \times 10^6$ photon trajectories through the geometry.

The realistic vascular geometry was obtained through 2P imaging, followed by vectorization of the vascular structure [42]. The vectorized geometry was voxelized into a three-dimensional matrix of the size $277 \times 277 \times 303$ voxels in the X, Y, and Z directions, respectively. The voxel size is a cubic $2 \times 2 \times 2 \mu m^3$, yielding a total geometry size of $554 \times 554 \times 606 \mu m^3$. A circular collimated wide-field beam with a flat profile was set to illuminate 95% of the top surface of the geometry. A NA of 0.25 and detector size of $9.8 \times 9.8 \mu m^2$ were used in the simulation settings to reflect the typical configuration of LSCI experimental setup. For detected photons, both entry and exit locations as well as the photon trajectories through the volume and photon weights were recorded. The optical properties of capillaries, non-capillary vessels and extra-vascular tissue as used in Ref. [30] are adopted.

### 2.4. In vivo experimental validation

The Mesi imaging system detailed in Ref. [23,24] is used for microfluidics and in vivo speckle imaging experiments. The laser wavelength is 785 nm and the magnification of the system is 2x. 56 Mesi sequences with each sequence containing 15 speckle contrast images corresponding to 15 exposure times are acquired. The $K^2$ curves are calculated by averaging speckle contrast values over multiple Mesi sequences and then squaring it. ICT values are then extracted by fitting the $K^2$ curves based on the Mesi model [24].

The mouse cranial window preparation procedures were detailed by Kazmi et al. [28]. During imaging sessions, the mouse (C57BL/6, Charles River Laboratories Inc.) was anesthetized with medical grade O2 vaporized isofluran (3% induction, 1.5% maintenance).

For 2P imaging, images were acquired with a custom microscope and laser system [44,45]. The same anesthesia procedure as above-mentioned was used. In addition, 100 μl of 70 kDa dextran-conjugated
Texas Red diluted in saline at a 5% w/v ratio was added to the blood plasma through retro-orbital injection prior to imaging. The dye was then excited by an Yb fiber amplifier (λ = 1060 nm). 30 frames acquired with a resonant scanner were averaged at each depth to produce images.

Four mice in total were used in animal experiments. For each mouse, MESI imaging was performed first and then followed by 2P imaging. All animal procedures in this study were approved by The University of Texas at Austin Institutional Animal Care and Use Committee (IACUC).

3. Results

3.1. Monte Carlo simulation

The simulation results on the realistic vascular network under normal illumination are shown in Fig. 3. Fig. 3a–e shows the map of the number of detected dynamic photons (i.e., photons experiencing dynamic scattering at least once), Var(k), E(k), and Var(Y), respectively. To counter the effects of wavelength, the Var(Y), Var(k) and E(k) are normalized by the wavenumber, i.e. Var(Y/k₀), Var(k/k₀) and E(k/k₀), unless specified otherwise.

There are several interesting observations. First, note the bright blobs in Fig. 3b, d and blue/orange blobs in Fig. 3c whose positions are circled in Fig. 3a. The reason that those blobs appear in those places instead of elsewhere is suspected to be correlated with the underlying vascular structure and we find that there is always a major descending/ascending vessel in those spots (Fig. 2b and Supplemental Material Section 5).

Second, the major surface vessels extending in the x-y plane shown in Fig. 3a appear even darker than surrounding parenchyma regions in the Var(k) map (Fig. 3b). This suggests that Var(k) might be sensitive to the orientation of vessels, namely, small for x-y plane surface vessels while large for z-directional descending vessels under normal illumination. This hypothesis is verified by simulation results on the simple vascular geometries consisting of parallel and equally-spaced vessels (Fig. 2a). Var(k) of z-directional vessels is observed to increase by 7 times compared with that of y-directional vessels under normal illumination (Supplemental Material Section 6). In addition, it is observed that Var(k) is correlated with the proportion of dynamic photons among all detected photons. When the z-directional vessel is embedded deeper into the tissue and the distance between top surfaces of the z-directional vessel and the tissue is increased from 0 to 0.08 mm, the proportion of dynamic photons decreases from 61.2% to 19.3% as Var(k) drops from 0.202 to 0.063.

Third, among those blobs, some are blue while others appear orange in Fig. 3c. Further analysis on the flow vector assignment in simulation reveals that this is dependent on whether the flow vector is z-positive or z-negative (Supplemental Material Section 7). It is noticed that the sign of simulated E(k) is consistent with the expectation from the evidence of laser Doppler [46], i.e., when the flow direction is such that the angle between the flow and the incident beam is smaller than that between scattered beam and the flow, E(k) is negative and vice versa.

Finally, E(k) of parenchyma regions is 0 (Fig. 3c). Hence, Eq. (9) can be applied with good accuracy. With Var(Y) being 0 in simulation, we could calculate E(\(\gamma^2\)) by dividing Var(Y) with Var(k). As Fig. 3e shows, the E(\(\gamma^2\)) map reveals a clearer structure of surface vessels than Var(Y) (Fig. 3d). To evaluate accuracy of the absolute blood flow speed estimation given in Fig. 3e, the square of the maximum intensity projection (MIP) of blood flow speeds assigned in simulation is mapped in Fig. 3f. In terms of the resolved vessels, the shape of x-y plane surface vessels is well preserved in Fig. 3e and the intensity value also shows a good match with that in Fig. 3f. However, for z-directional descending vessels, the vessel boundary is expanded and there is no clear border, which highlights their distinct properties in LSCI from x-y plane surface vessels.

3.2. Experimental validation

The enhanced sensitivity of speckle contrast to blood flow speed in z-directional vessels compared with x-y plane vessels is also observed in vivo. As highlighted by white arrows in Fig. 4a and b, the orthogonal X–Y and X–Z cross-sections of 2P imaging data clearly reveal an inverted “L” shaped vessel. At the end of its surface strand, it develops into a descending strand into the tissue. Fig. 4c and d show the raw speckle image of the vascular structure and the histogram of pixel intensities. Fig. 4e and f show the speckle contrast and ICT squared images, respectively. The white boxes in Fig. 4f show ROI of the two strands of the inverted “L” shaped vessel. Notice that there is no other major vessel branch on this “L” shaped vessel and vessel diameters of the two strands are approximately the same (Fig. 4a and b). Thus, based on blood flow conservation [28], the flow speed should be approximately the same in these two strands. Nevertheless, we see a lower speckle contrast in areas corresponding to the descending strand as pointed out by the white arrow in Fig. 4e. It indicates an enhancement of the sensitivity of speckle contrast to blood flow speed in the descending strand compared with the surface strand. More specifically, the average ICT squared of the descending strand is \(\sim 78\%\) larger than that of its surface counterpart, as highlighted by white boxes in Fig. 4f. If the impact of Var(Y) is negligible, it implies that Var(k) of the descending...
Fig. 3. Simulation results on the realistic vascular network under normal illumination. a The map of the number of detected dynamic photons at each camera pixel. The dynamic photons refer to photons which experience at least one dynamic scattering event before exiting and being detected. b-f The map of \( \text{Var}(k) \), \( \text{Mean}(k) \), \( \text{Var}(Y) \), \( E(\tilde{v}^2) \), and \( \text{MIP}^2(v) \), respectively. \( \text{MIP}^2(v) \) represents the square of maximum intensity projection of flow speeds assigned in simulation. The position of bright spots in b, c and d is circled out correspondingly in a.

Fig. 4. One example of the \( z \)-directional descending vessel inducing a more significant decrease of speckle contrast and increase of ICT than its upstream \( x-y \) plane surface strand in vivo. a, b Orthogonal cross-sections of the vascular structure acquired from 2P imaging. a X–Y cross-section (depth: 27 \( \mu m \)); b X–Z cross-section along the yellow dashed line in a. The yellow line in b indicates the \( z \) position of a. White arrows in a and b indicate the direction of an upside-down “L” shaped vessel which has a surface strand extending horizontally on the surface followed by a descending strand deep into the tissue. Scale bar: 80 \( \mu m \). The spatial scale along the \( x \)- and \( z \)-axis is the same in b. c The raw speckle image of the vascular network at camera exposure time of 1 ms. d The histogram of pixel intensities of the raw speckle image in c. e The averaged speckle contrast image of the vascular network. Camera exposure time \( T = 1 \) ms. The white arrow indicates position of the descending vessel strand which shows a stronger decrease of speckle contrast than its connected \( x-y \) plane surface strand. f The map of ICT squared. The two white boxes highlight the descending strand and surface strand, respectively. The white arrow indicates the position of the descending strand exhibiting a larger ICT value than its connected \( x-y \) plane counterpart. The black arrow in b highlights another descending vessel branch from the main vessel. The black arrow in e and black boxes in f indicate the compound effects of vascular structure and blood flow in modulating speckle contrast and ICT values.
strand would be more than 75% larger than that of surface strand. Notably, similar border expansion is also observed here in the position of the descending strand as in simulation (Fig. 3e).

To further evaluate the statistical significance of the enhanced sensitivity, 9 pairs of z-directional vessels and x−y strand vessels from 3 mice are analyzed. Those vessel pairs are selected for analysis because their vascular structure has the same properties as in the example mentioned above, i.e., the upside-down L vessel shape and the approximately same diameter of the two strands. The location of four z and x−y strand pairs in a typical mouse cerebral window is shown in Fig. 5a and their vascular structure is acquired by 2P imaging (Fig. 5b). ROI of the z and x−y strand in each pair is selected in the similar way as shown by white boxes in Fig. 4d. The ICT squared of the z strand in each pair is plotted against that of the x−y strand (Fig. 5c). The linear fitting results in a slope of approximately 2. In addition, the difference between ICT squared of the z strand and that of the paired x−y strand is statistically significant (paired t-test, n = 9, p < 0.001). Those results provide direct experimental evidence for the enhanced sensitivity of ICT to blood flow changes in z-directional vessels compared with vessels extending in the x−y plane.

Finally, the compound effects of vascular structure and blood flow should be noticed. As highlighted by the black arrow in Fig. 4b, there is another descending vessel branch from the main vessel. The blood flow in the main vessel splits into two portions: one goes into the above-mentioned inverted “L” shaped vessel and the other goes into this second descending vessel. Therefore, the speckle contrast and ICT in the solid black rectangle in Fig. 4f result from not only an underlying descending vessel but also the larger blood flow in the main vessel. That is why they are not directly comparable with those of the descending strand in the white square in Fig. 4f. Interestingly, the enhanced sensitivity can be roughly examined if we compare the ICT squared in the solid-line black rectangle with that in the dashed black rectangle in Fig. 4f. Both areas cover the main vessel of the largest blood flow but the solid rectangle assumes larger ICT squared, which indicates the additional impact of the descending vessel in the solid square. Similarly, the joint effects of vascular structure and blood flow are observed in the region of vessel pair 2 in Fig. 5a where a second descending vessel is also present (Fig. 5b).

4. Discussion

4.1. Bridging ICT to physiologically meaningful blood flow variables

Given the volume integrated nature of LSCI and the complexity of the vascular network, it has been long hypothesized that if LSCI is measuring some physiologically meaningful blood flow variable, it measures the weighted average of that variable within the probed volume [24,41]. However, it is unclear how the weighting is determined. Our derivation in Sections 3.1 and 3.2 reveals that the weighting is determined by photons’ dynamic scattering process, according to the definition of (Eq. (6)). Note that is physiologically meaningful and represents the weighted average of blood flow speeds probed by detected photons. In the case that all dynamic scattering events sample the same blood flow speed, would be equal to regardless the weighting.

Combining the results in Sections 2.1 and 2.2, a general relationship between ICT and physiologically meaningful blood flow variables, i.e. , can be established. In Section 2.1, it has been shown that ICT squared is proportional to the variance of , which accommodates all current models in LSCI. In Section 2.2, Eq. (9) further points out that the variance of assumes a linear relationship with the expectation of squared. Hence, the linear relationship between ICT squared and the expectation of squared is reached by our theoretical derivation.

Contrary to the frequently adopted notion that ICT is proportional to blood flow speed, our theory concludes that it is the squares of the two that are linear to each other. The difference between the two notions is centered on whether is zero or not. Pragmatically, the existence of is indispensable to tackling the “biological zero” problem which refers to the non-zero residual signal even when no blood flow is present [39,47]. A non-zero might reduce the difference of ICT values between descending/ascending vessels and surface vessels.
As observed in simulation, the \( \text{Var}(k) \) of \( z \)-directional vessels is 7 times larger than that of \( y \)-directional vessels in the simple vascular geometry, which is expected to generate a ICT squared difference of 7 times according to Eq. (9) if \( \text{Var}(Y) \) is zero. However, ICT squared in \( \text{in vivo} \) is only 60% larger on average in descending/ascending vessels than vessels laying on the surface. A non-zero \( \text{Var}(Y) \) in \( \text{in vivo} \) could play a significant role in such discrepancy. In addition, the scatters’ movement could be more diverse in \( \text{in vivo} \) compared with our settings in simulation. For example, the radially diffusive motion of RBCs in vessels is not present in our simulation.

\( \text{Var}(k) \) plays a major role in bridging ICT and the physiologically meaningful blood flow variable, \( \bar{v} \), as Eq. (9) points out. It provides a theoretical basis for extracting the physiologically meaningful blood flow variable \( \bar{v} \) from ICT. Given the \( \text{Var}(k) \) already known of the vascular network, the estimation of absolute statistical blood flow speeds could be made by dividing the measured \( \text{Var}(Y) \) by \( \text{Var}(k) \) (Fig. 3e).

Finally, our findings showed partial support for Briers et al.’s method of converting measured ICT values to absolute blood flow speeds. Specifically, the conversion is performed by

\[
\overline{v} = \frac{\lambda}{2\pi\tau_s} \varepsilon I + \frac{\lambda}{2\pi\tau_s} \varepsilon J + \frac{\lambda}{2\pi\tau_s} \varepsilon K
\]

where \( v \) is named decorrelation velocity, \( \lambda \) is wavelength and \( \tau_s \) is correlation time [18,19]. Our theory shows that this is in fact acquired by assuming \( \text{Var}(Y) = 0 \) and approximating \( \text{Var}(k) \) with \( k_y^2 \) the square of wavenumber (Eq. (9)).

4.3. Physical mechanism and practical implications of the directionality susceptibility

The different sensitivity of ICT to the blood flow speed in \( x-y \) plane vessels and \( z \)-directional descending/ascending vessels is likely due to the different flow direction in those vessels. One might argue that in descending/ascending vessels, the ratio of blood volume in the overall volume sampled by detected photons might be larger than that in the surface vessels. And it might be the larger blood volume ratio in descending/ascending vessels that is causing the higher sensitivity of ICT to the blood flow. We exclude this theory by manipulating the flow direction in simulation. If the blood volume ratio theory is true, then \( \text{Var}(k) \) of \( z \)-directional vessels should remain larger than that of \( x-y \) plane vessels even if the direction of flow is changed since the blood volume ratio is invariant. However, it is observed that \( \text{Var}(k) \) of the \( z \)-directional vascular geometry is smaller than that of \( y \)-directional vascular geometry after switching the flow direction in \( z \)-directional vascular geometry to \( y \)-direction.

\[
\overline{v} = v_y i + v_x j + v_z k
\]

Finally, for \( x \)-directional vessels is highlighted. However, such phenomena would not appear if it is the amplitudes of \( x \) and \( y \) component that are swapped (Fig. 6). Instead, the position of \( y \)-component of the unit velocity vector of blood flow are swapped (Fig. 6a). Instead, the position of \( y \)-directional vessels is highlighted. Note that \( \text{Var}(Y) \) is assumed 0 in simulation.

4.2. Interpretation and practical implications of \( \text{Var}(k) \)

\( \text{Var}(k) \) can be interpreted at both the microscopic and macroscopic scales. Microscopically, by definition (Eqs. (6) and (8)), it provides an essential characterization for the variation of photons’ dynamic scattering process inside the probed medium in terms of the accumulated frequency shift. Macroscopically, as revealed by Eq. (9), it reflects the ability of the probed vascular network to induce a decrease of speckle contrast or increase of ICT for a given illumination and detection setup. The micro and macro-scale interpretations are connected due to the fact that photons’ dynamic scattering is mainly constrained within the vascular network.

\( \text{Var}(k) \) also illustrates that it is challenging, if not impossible, to do absolute blood flow speed measurements through a generalized calibration since \( \text{Var}(k) \) is unique for a given vascular network. When the probed vascular changes, \( \text{Var}(k) \) also changes.
The directionality susceptibility of LSCI has several practical implications. First, direct comparison of ICT values in $x$–$y$ plane vessels and in ascending/descending vessels should be avoided. If a descending/ascending vessel exhibits a larger ICT value, it does not necessarily imply a higher blood flow than its surface counterpart. Second, the special property of $z$-directional vessels, i.e., enhanced ability to induce the decrease of speckle contrast and increase of ICT under the same blood flow speed, might be useful in locating descending/ascending vessels. Note that those types of vessels are particularly prevalent in cerebral cortex and play an important role in the blood supply to deeper tissues.

4.4. The impact of vessel diameter

It is noticed that the largest $z$-directional vessel in either simulation or in vivo experimental validation is only 10–20 μm in radius. To examine the conclusion of this paper in case of vessels of larger diameters, we performed additional Monte Carlo simulation experiments in which an inverted L-shaped vessel is constructed to mimic the vascular geometry in Fig. 4. The radius of this vessel is varied from 10 to 100 μm across simulations (Fig. 7a–c). It is observed that the increased Var($k$) in $z$-directional vessels compared with that in $y$-directional vessels still holds for vessels up to 100 μm in radius (Fig. 7g–i). Considering the sampling volume of a single pixel in LSCI, the reason could be that the flow condition within that sampling volume is more uniform in the case of a large vessel. In contrast, when the vessel of interest is small and that the sampling volume involves multiple other vessels of diverse flow directions, Var($k$) could be less sensitive to the flow direction of the vessel of interest.

5. Conclusion

The interpretation of ICT is a key topic in quantitative LSCI. Though it has been mainly considered as a metric of blood flow, there is increasing evidence that it is susceptible to the structure of vascular network. We build a theoretical framework to facilitate the modeling of the vascular network’s impact on ICT and find that ICT is modulated by vessel orientation. In both simulation and in vivo experimental validation, ICT of descending/ascending vessels exhibits a higher sensitivity to blood flow changes than in surface-extending vessels. The different sensitivity is shown due to the flow direction instead of blood volume ratio by simulation. The single-scattering or few-scattering component of the detected light might play a major role in ICT’s susceptibility to vessel orientation. Those results suggest that the impact of vascular network structure warrants more attention and investigation in the interpretation of ICT.

CRediT authorship contribution statement

Qingwei Fang: Developed the theory, Designed the experiments, Did the Monte Carlo simulation experiments, Writing – original draft. Chakameh Z. Jafari: Did the Monte Carlo simulation experiments, Writing – original draft. Shaun Engelmann: Performed the in vivo mice imaging experiments, Writing – original draft. Alankrit Tomar:
Performed the in vivo mice imaging experiments, Writing – original draft. Andrew K. Dunn: Developed the theory, Designed the experiments, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.optcom.2023.129334. Supplementary data contains derivations and proofs of some of the expressions from this paper.

References


