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Evaluating multi-exposure speckle imaging estimates of absolute autocorrelation times

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Multi-exposure speckle imaging (MESI) is a camera-based flow-imaging technique for quantitative blood-flow monitoring by mapping the speckle-contrast dependence on camera exposure duration. The ability of laser speckle contrast imaging to measure the temporal dynamics of backscattered and interfering coherent fields, in terms of the accuracy of autocorrelation measurements, is a major unresolved issue in quantitative speckle flowmetry. MESI fits for a number of parameters including an estimate of the electric field autocorrelation decay time from the imaged speckles. We compare the MESI-determined correlation times in vitro and in vivo with accepted true values from direct temporal measurements acquired with a photoncounting photon-multiplier tube and an autocorrelator board. The correlation times estimated by MESI in vivo remain on average within $14 \pm 11\%$ of those obtained from direct temporal autocorrelation measurements, demonstrating that MESI yields highly comparable statistics of the time-varying fields that can be useful for applications seeking not only quantitative blood flow dynamics but also absolute perfusion. © 2015 Optical Society of America

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Multi-exposure speckle imaging (MESI) is a wide-field imaging technique that can be used to quantitatively visualize blood flow dynamics relatively rapidly [1]; however, the accuracy of its measurements has never been verified against temporal autocorrelation measurements of exposed blood flows, particularly in an absolute fashion [2,3]. We seek to compare the MESI estimates of the characteristic autocorrelation decay time of the electric field with those obtained directly from temporal intensity recordings through *in vitro* microfluidic and *in vivo* neurovascular imaging experiments.

The physics of speckle imaging is rooted in dynamic light scattering [4,5], as moving scatterers cause fluctuations in the backscattered light intensity resulting in a dynamic speckle pattern. This phenomenon has been leveraged for applications in soft-matter physics ranging from particle sizing and diffusion to mechanical elastography and flowmetry. Typically, the rate of the intensity fluctuations is characterized by computing the intensity correlation function, $g_2(\tau)$, which can be related to the electric field correlation function, $g_1(\tau) = \langle E(t)E^*$ $(t + \tau) \rangle / \langle E^2(t) \rangle$, through statistical approximations, such as the Siegert relationship [4,6]. The characteristic decay time of $g_1(\tau)$ is commonly denoted as the field autocorrelation time, τ_c , and is inversely related to the encountered motion (e.g., diffusion, flows) [7], although the exact physical relationship is complex and poorly understood.

The experimental setup for both direct temporal autocorrelation and multi-exposure speckle imaging (MESI) is shown in Fig. 1. A laser diode ($\lambda = 660$ nm, 90 mW, Micro Laser Systems, Garden Grove, California) is used to illuminate the sample obliquely at approximately 30–40 deg incidence. The collection optics consisted of a CMOS camera (A602f, 656 × 492 pixels, Basler Vision Technologies, GmbH, Germany) for imaging the remitted light with an effective 5× (0.25 NA)



Fig. 1. Combined MESI and autocorrelation instrument highlighting speckle contrast exposure dependence and intensity recording. Camera sensor (9.9 μ m × 9.9 μ m pixel size) and single-mode fiber (4.5- μ m mode field diameter) are both conjugate to sample plane. Specimen area of ~4 μ m² imaged by a single pixel and fiber. L1 (f = 100 mm, d = 25.4 mm), L2 (f = 20 mm, d = 12.7 mm), and L3 (f = 11 mm, d = 7.2 mm aspheric).

objective and imaging lens configuration. For direct temporal recordings, a 70R/30T beam splitter directs some of the collected light into a single-mode fiber for transmission to a photomultiplier tube (PMT, 5-mm cathode diameter, 38% quantum efficiency at $\lambda = 660$ nm, H7422P-40, Hamamatsu Photonics, Japan) connected to a photon correlator board (DPC-230, Becker & Hickl, GmbH, Germany).

The single-mode fiber collection region also lies in a conjugate plane to the specimen and matches the sample plane imaging area of approximately one pixel. This fiber collection region was located in the camera field of view by back-illumination with a laser diode at the same wavelength through the fiber and optical system. Direct temporal intensity measurements were collected over a span of 10 s from individual regions selected by translating the sample. A modified Siegert relation-ship [Eq. (1)] is used for approximating the field autocorrelation function due to the effects of heterodyne mixing of statically, E_s , and dynamically, E_f , scattered fields, $E(t) = (E_f(t) + E_s) \exp(-i\omega t)$ [1,8] within the perfused tissue samples under consideration. Specifically, the heterodyne Siegert relationship relates the normalized intensity autocorrelation to that of the field for such specimens by

$$g_{2}^{b}(\tau) = 1 + \beta \rho^{2} |g_{1}(\tau)|^{2} + 2\beta \rho (1-\rho) |g_{1}(\tau)| + \beta (1-\rho)^{2}, \quad (1)$$

where $\rho = \frac{I_f}{I_f + I_r}$, $I_s = E_s E_s^*$ and $I_f = E_f E_f^*$ are the intensity contributions from the statically and dynamically scattered light, respectively. β is a normalization factor dependent on speckle sampling, polarization, and coherence effects. The traditional form of g_1 is based on assumptions of unordered motion of scatterers and single dynamic scattering (e.g., Lorentzian profile) or alternatively bulk flow and multiple scattering (e.g., Gaussian profile), both of which approximate to $g_1(\tau) = \exp(-\tau/\tau_c)$ when scattering is random and uncorrelated [9]. Incorporating this form into the modified Siegert relationship, we arrive at the intensity autocorrelation function for the direct measurements [Eq. (2)]:

$$g_{2}^{b}(\tau) = 1 + \beta \rho^{2} \exp(-2\tau/\tau_{c}) + \cdots$$

$$2\beta \rho (1 - \rho) \exp(-\tau/\tau_{c}) + \beta (1 - \rho)^{2}.$$
 (2)

The autocorrelation function of the normalized intensity measurements are fit to Eq. (2), (Fig. 2) for extracting an estimate of the field autocorrelation time, τ_c .

The MESI implementation utilizes the same collection objective, camera, and laser illumination, which is intensity controlled through an acousto-optic modulator (AOM, 23080-2-LTD, NEOS Technologies). Fifteen camera exposures, T, ranging from 50 µs to 80 ms [1,10–12], were recorded for every MESI computation in order to sample the range of speckle visibility for the given specimen dynamics [13]. Acquired frames were initially converted to images (Fig. 1) of spatially computed speckle contrast, $K(T) = \sigma_s(T)/\langle I \rangle$, defined as the standard deviation over the mean of pixel intensities in a 7 × 7 pixel window. Fields of view (FOVs) that covered areas sampled in direct temporal autocorrelation were imaged to facilitate comparison.

The speckle variance (i.e., K^2) is related to a weighted integral of $g_2(\tau)$ over the camera exposure duration, T, that has been described in detail previously [4,5]. By assuming a specific form of $g_1(\tau)$, this integral can be evaluated analytically



Fig. 2. (A) Temporal autocorrelation measurements fit with intensity autocorrelation function [Eq. (2)]. (B) Speckle contrast image of flow in microfluidic channel. (C) MESI fit [Eq. (3)] of speckle visibility versus exposure duration. (D) Regression of inverse correlation times (ICT) from direct measurements and MESI.

yielding an expression relating the measured speckle contrast, K, to the camera exposure time, T, and speckle decorrelation time, τ_c [Eq. (3)]. Specifically, by taking the second central moment of the normalized intensity autocorrelation function [Eq. (2)] and equating the variance to the observed speckle contrast [1,8], we arrive at the following speckle visibility expression:

$$K(T,\tau_c) = \begin{cases} \beta \rho^2 \frac{e^{-2x} - 1 + 2x}{2x^2} + 4\beta \rho (1-\rho) \frac{e^{-x} - 1 + x}{x^2} + \cdots \\ +\beta (1-\rho)^2 + v_{\text{noise}} \end{cases}^{1/2},$$
(3)

where $x = \frac{T}{\tau_c}$ and ν_{noise} is a lumped term accounting for measurement noise and any residual nonergodic variances [1]. The MESI estimates of the field autocorrelation times were obtained by fitting K(T) to Eq. (3), as shown for a flowing region in the microfluidic channel [Fig. 2(C)] [1,14,15].

To assess the measurement accuracy of MESI against direct temporal autocorrelation, the autocorrelation times from each method were extracted and analytically compared. As noted, the same regions of interest as those used in direct temporal autocorrelation measurements were selected for MESI analysis. Fitting was performed for all parameters in Eqs. (2) and (3) to facilitate cross-modality comparison with the maximum level of variability. This process ensured that both techniques best fit the critical decaying portion of the data, without susceptibility to the effects of *a priori* fixing of parameters, such as β , due the disparate temporal sampling of each technique.

In vitro samples consisted of straight 115- μ m-square microfluidic channels made from a PDMS substrate mixed with TiO₂ to mimic the optical properties of tissue. Figure 2 presents flow measurements from a microfluidic channel obtained by direct intensity autocorrelation [Eq. (2), Fig. 2(A)] and MESI [Eq. (3), Fig. 2(C)]. A range of flows from 1 μ L/min to 4 μ L/min were used in the microfluidic with a dilution of 1- μ m polystyrene beads in water to mimic the reduced scattering properties of whole blood ($\mu'_s \sim 2/mm$), shown flowing in the speckle contrast image in Fig. 2(B).

The four flow rates measured as inverse correlation times (ICT) from the direct and MESI measurements are juxtaposed in Fig. 2(D). The inverse correlation times have been posed to be proportional to the average speed and scatterer density product for multiple scattering [16,17]. If scatterer density is conserved, such as in a single flow channel, the inverse correlation times would translate directly to an estimate of particle speed. In support of this theory, the ratios of the speckle dynamics appear to closely mirror the ratio of flow rates through the channel and suggest potential for calibration. Particularly, there is a strong linear relationship between the measures from the two methods, given the $1.8 \pm 1.2\%$ deviation, demonstrating that correlation time approximations measured by MESI closely align with the values returned by temporal autocorrelation on a one-to-one basis [Fig. 2(D)]. However, further examination was needed for in vivo samples consisting of live mice.

For in vivo imaging, mice were implanted with glass cranial windows to allow collection and imaging from the microcirculation (i.e., resolved and unresolved microvessels) with high spatial fidelity [11]. Measurements from a prominent single vessel and parenchymal region [Fig. 3(A)] of the tissue are shown using the MESI [Fig. 3(B), Eq. (3)] technique and temporal autocorrelation [Fig. 3(C), Eq. (2)], respectively. Again, these expressions retain the same underlying DLS statistical assumptions, namely the modified Siegert relation [Eq. (1)] and particle speed distributions. Similar to the microfluidic results, the *in vivo* measurements appear to display a strong correspondence between the two methods, despite the disparity in temporal range due to light collection limitations precluding camera exposures shorter than 50 µs. The accuracy of the DLS assumptions that affect the formulation of g_1 may account for some of the residual variations, particularly with reduced fitting accuracy at early temporal delays in parenchymal regions [Fig. 3(C)]. These variations, however, appear less evident in MESI, likely due to selected temporal range of exposures [Fig. 3(B)].

Additionally, from seven mice, n = 70 cortical regions were selected for absolute correlation-time comparison (Fig. 4). 35 regions sampled largely a single surface vessel and another 35 sampled depth-distributed, multi-vascular parenchymal regions



Fig. 3. (A) Speckle contrast image of mouse cortex with selected vascular (circle) and parenchymal (square) regions. (B) Multi-exposure speckle visibility measurements and modeling [Eq. (3)]. (C) Intensity autocorrelation function approximation [Eq. (2)] of direct temporal autocorrelation measurements.



Fig. 4. Temporal autocorrelation versus MESI-computed inverse correlation times regressed over 35 single-vessel and 35 parenchymal regions across seven animals. Inset highlights range indicated by dashed box.

[18]. On average, the correlation time estimates by MESI deviated by 14% from those predicted by direct temporal autocorrelation measurements. This difference quantifies the accuracy of the MESI technique in estimating the absolute temporal autocorrelation dynamics. The higher discrepancy observed *in vivo* rather than in controlled flow microfluidics may stem from the larger range of flows encountered in the animals along with drifts in the physiology between the sequential acquisitions. The correlation time magnitudes from the seven animals sample a flow distribution as expansive as that seen in several MESI studies in multiple animal species [10,12,13,19]. Additionally, MESI-computed correlation times determined from spatially and temporally computed speckle contrast were found to be statistically insignificant (p > 0.8).

Further analysis into the appropriateness of the statistical assumptions (i.e., uncorrelated and random scattering, particle dynamics, single or multiple dynamic scattering) and their impact on the formulations [Eqs. (2) and (3)] for modeling the sample dynamics would benefit study. On average, parenchymal fits ($R^2 = 0.945$ autocorrelation, 0.98 MESI) were slightly worse than vascular ($R^2 = 0.985$ autocorrelation, 0.995 MESI). Increased MESI exposure sampling may be needed to fairly assess the DLS model accuracy for the specimen-specific and regionally varying sample dynamics. Neurovascular imaging was performed in this study since it is a common biomedical application of speckle contrast imaging, as exhibited by the selected range of camera exposures. The observed cross-modality correspondence suggests a strong potential for absolute autocorrelation measurements with the MESI technique. Applications quantifying diffusion dynamics, particle sizing, and mechanical properties will likely require a different range of exposure durations to accurately capture the dynamic range of speckle visibility associated with the sample motion of interest.

Reliance on single exposure speckle contrast imaging for estimating correlation times would likely lead to significant discrepancies with the directly measured intensity autocorrelation, as sensitivities are restricted to a range around the camera exposure duration [13,20]. The selected range and sample of exposures for MESI appears suitable for the flows observed *in vivo*, as exhibited by the correspondence observed at the



Fig. 5. Temporal autocorrelation versus MESI and single exposure inverse-correlation times across seven animals. Simplified model used for single-exposure speckle visibility expression, equivalent to Eq. (3) when $\rho = 1$ and $\nu = 0 \cdot \beta$ value for each single-exposure measurement was set by speckle variance observed at shortest utilized exposure (0.05 ms), which is therefore excluded from analysis.

extrema of the flow range (Fig. 4) and observed previously for relative flow changes [11]. Given that the majority of the correlation times (τ_c , Fig. 4) lie between 0.5 and 1 ms, it follows that the single-exposure speckle imaging performs best when the exposure durations are also in this range (Fig. 5). However, within this optimal exposure range, there remains a 40% discrepancy in the correlation time estimates, which is nearly threefold higher than that observed with MESI. At shorter and longer single exposures, greater deviations are observed, approaching 90% at the longest exposure. As there is significant overlap in the theoretical motion sensitivity between exposures (i.e., $\pm 50\%$ exposure) [13,20], the observed discrepancy (Fig. 5) will likely be similar for another set of absolute single exposures within the presented range.

In summary, the prevailing form of MESI closely approximates the measurements returned by direct temporal autocorrelation measurements. Speckle imaging has advantages of detection simplicity over the direct intensity recordings, which coupled with camera imaging enable rapid multi-point DLS quantification. Although $1/\tau_c$ is known to be a spatially integrated measure of the underlying motion [7,18], the spatial specificity to flows in various tissue types and regions remains to be examined. However, the technical confidence with multiple-exposure speckle imaging, exhibited in estimating the absolute inverse correlation times, will facilitate this calibration to absolute perfusion levels.

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