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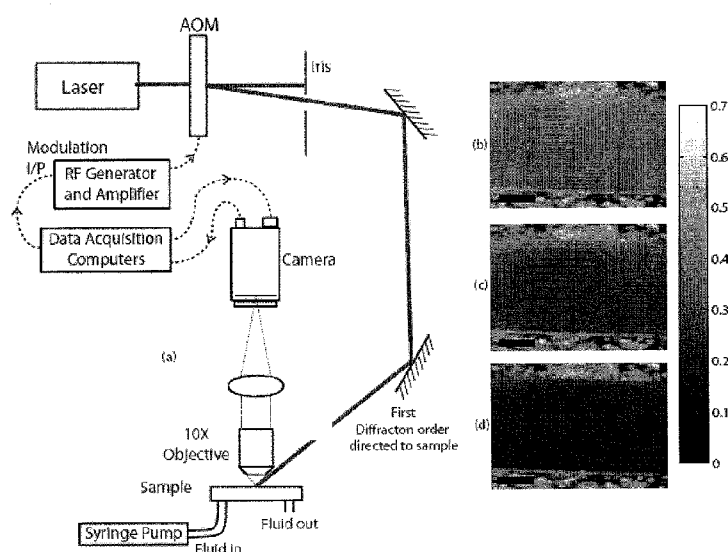
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(54) Title: QUANTITATIVE IMAGING WITH MULTI-EXPOSURE SPECKLE IMAGING (MESI)

Figures 1A-1D



(57) Abstract: Methods and systems relating to multi-exposure laser speckle contrast imaging are provided. One such system comprises a laser light source, a light modulator, and a detector for the measurement of reflected light comprising at least one camera, at least one magnification objective, and at least one microprocessor or data acquisition unit.

QUANTITATIVE IMAGING WITH MULTI-EXPOSURE SPECKLE IMAGING (MESI)

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Patent. App. Ser. No. 61/153,004 filed February
5 17, 2009, which is incorporated herein by reference.

STATEMENT OF GOVERNMENT INTEREST

The present invention was made with government support from the National Science
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BACKGROUND

Laser Speckle Contrast Imaging (LSCI) is a popular optical technique to image blood
flow. It was introduced by Fercher and Briers in 1981, and has since been used to image blood
flow in the brain, skin and retina. Since LSCI is a full field imaging technique, its spatial
resolution is not at the expense of scanning time unlike more traditional flow measurement
15 techniques like scanning Laser Doppler Imaging (LDI). For these reasons LSCI has been used to
quantify the cerebral blood flow (CBF) changes in stroke models and for functional activation
studies.

The advantages of LSCI have created considerable interest in its application to the study
of blood perfusion in tissues such as the retina and the cerebral cortices. In particular, functional
20 activation and spreading depolarizations in the cerebral cortices have been explored using LSCI.
The high spatial and temporal resolution capabilities of LSCI are incredibly useful for the study
of surface perfusion in the cerebral cortices because perfusion varies between small regions of
space and over short intervals of time.

One criticism of LSCI is that it can produce good measures of relative flow but cannot
25 measure baseline flows. This has prevented comparisons of LSCI measurements to be carried out
across animals or species and across different studies. Lack of baseline measures also make
calibration difficult. This limitation has been attributed to the use of an approximate model for
measurements. Another limitation of LSCI, especially for imaging cerebral blood flow, has been
the inability of traditional speckle models to predict accurate flows in the presence of light
30 scattered from static tissue elements. Traditionally this problem has been avoided in imaging
cerebral blood flow by performing a full craniotomy (removal of skull). Such a procedure is
traumatic and can disturb normal physiological conditions. Imaging through an intact yet thinned
skull can drastically improve experimental conditions by being less traumatic, reducing the

impact of surgery on normal physiological conditions and enabling chronic and long term studies. One of the advantages of imaging CBF in mice is that LSCI can be performed through an intact skull. However variations in skull thickness lead to significant variability in speckle contrast values.

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SUMMARY

The present disclosure generally relates to imaging blood flow, and more specifically, to quantitative imaging with multi-exposure speckle imaging (MESI).

In certain embodiments, the present disclosure provides a MESI system comprising: a laser light source for the illumination of a sample; a light modulator; and a detector for the measurement of reflected light comprising at least one camera, at least one magnification objective, and at least one microprocessor or data acquisition computer.

In some embodiments, the present disclosure also provides methods for quantitative blood flow imaging that comprise: providing a MESI system comprising a laser light source for the illumination of a sample; a light modulator; and a detector for the measurement of reflected light comprising at least one camera, at least one magnification objective, and at least one microprocessor or data acquisition computer; illuminating a sample and detecting a speckle pattern using the MESI system; and computing a quantitative blood flow image. In some embodiments, a quantitative blood flow image may be computed using a speckle model of the present disclosure.

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DRAWINGS

Some specific example embodiments of the disclosure may be understood by referring, in part, to the following description and the accompanying drawings.

Figure 1A shows a schematic of a multi-exposure speckle imaging (MESI) system, according to one embodiment.

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Figure 1B is a speckle contrast image at 0.1 ms exposure duration obtained by a MESI system of the present disclosure.

Figure 1C is a speckle contrast image at 5 ms exposure duration obtained by a MESI system of the present disclosure.

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Figure 1D is a speckle contrast image at 40 ms exposure duration (scale bar = 50 μm) obtained by a MESI system of the present disclosure.

Figure 2A depicts a cross-section of a microfluidic flow phantom (not to scale) without a static scattering layer. The sample was imaged from the top.

Figure 2B depicts a cross-section of microfluidic flow phantom (not to scale) with a static scattering layer. The sample was imaged from the top.

Figure 3 is a graph depicting the Multi-Exposure Speckle Contrast data fit to the speckle model of the present disclosure. Speckle variance as a function of exposure duration is shown for different speeds. Measurements were made on a sample with no static scattering layer (Figure 2A).

5 Figure 4 is a graph depicting the Multi-Exposure Speckle Contrast data analyzed by spatial (ensemble) sampling (solid lines) and temporal (time) sampling (dotted lines). Measurements were made at 2 mm/sec. The three curves for each analysis technique represent different amounts of static scattering. μ'_s values refer to the reduced scattering coefficient in the 200 μm static scattering layer. $\mu'_s = 0 \text{ cm}^{-1}$: No static scattering layer (Figure 2A), $\mu'_s = 4$
10 cm^{-1} : 0.9 mg/g of TiO_2 in static scattering layer (Figure 2B), $\mu'_s = 8 \text{ cm}^{-1}$: 1.8 mg/g of TiO_2 in static scattering layer (Figure 2B).

Figure 5 is a graph depicting the Multi-Exposure Speckle Contrast data from two samples fit to the speckle model of the present disclosure. Speckle variance as a function of exposure duration is shown for two different speeds and two levels of static scattering. Solid lines
15 represent measurements made on sample without static scattering layer. Dotted lines represent measurements made on sample with static scattering layer. μ'_s values refer to the reduced scattering coefficient in the 200 μm static scattering layer. $\mu'_s = 0 \text{ cm}^{-1}$: No static scattering layer (Figure 2A), $\mu'_s = 8 \text{ cm}^{-1}$: 1.8 mg/g of TiO_2 in static scattering layer (Figure 2B).

Figure 6 is a graph depicting the percentage deviation in τ_c over changes in amount of
20 static scattering for different speeds (estimated using Equation 4). Data from all three static scattering cases $\mu'_s = 0 \text{ cm}^{-1}$: No static scattering layer (Figure 2A), $\mu'_s = 4 \text{ cm}^{-1}$: 0.9 mg/g of TiO_2 in static scattering layer (Figure 2B), $\mu'_s = 8 \text{ cm}^{-1}$: 1.8 mg/g of TiO_2 in static scattering layer (Figure 2B) was used in this analysis.

Figure 7 is a graph depicting the performance of different models to relative flow.
25 Baseline speed: 2 mm/sec. Plot of relative τ_c to relative speed. Plot should ideally be a straight line (dashed line). Multi-Exposure estimates extend linear range of relative τ_c estimates. Error bars indicate standard error in relative correlation time estimates. Measurements were made using a microfluidic phantom with no static scattering layer (Figure 2A).

Figure 8A is a graph that quantifies the effect of static scattering on relative τ_c
30 measurements. Plot of relative correlation time (Equation 12) to relative speed. Baseline Speed - 2 mm/sec. The three curves represent different amounts of static scattering. μ'_s values refer to the reduced scattering coefficient in the 200 μm static scattering layer. $\mu'_s = 0 \text{ cm}^{-1}$: No static scattering layer (Figure 2A), $\mu'_s = 4 \text{ cm}^{-1}$: 0.9 mg/g of TiO_2 in static scattering layer (Figure 2B), $\mu'_s = 8 \text{ cm}^{-1}$: 1.8 mg/g of TiO_2 in static scattering layer (Figure 2B).

Figure 8B is a graph that quantifies the effect of static scattering on relative τ_c measurements. Plot of relative correlation time (Equation 12) to relative speed. Baseline Speed - 2 mm/sec. The three curves represent different amounts of static scattering. Error bars indicate standard error in estimates of relative correlation times. μ'_s values refer to the reduced scattering coefficient in the 200 μm static scattering layer. $\mu'_s = 0 \text{ cm}^{-1}$: No static scattering layer (Figure 2A), $\mu'_s = 4 \text{ cm}^{-1}$: 0.9 mg/g of TiO_2 in static scattering layer (Figure 2B), $\mu'_s = 8 \text{ cm}^{-1}$: 1.8 mg/g of TiO_2 in static scattering layer (Figure 2B).

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While the present disclosure is susceptible to various modifications and alternative forms, specific example embodiments have been shown in the figures and are described in more detail below. It should be understood, however, that the description of specific example embodiments is not intended to limit the invention to the particular forms disclosed, but on the contrary, this disclosure is to cover all modifications and equivalents as illustrated, in part, by the appended claims.

DESCRIPTION

The present disclosure generally relates to imaging blood flow, and more specifically, to quantitative imaging with multi-exposure speckle imaging (MESI).

LSCI is a minimally invasive full field optical technique used to generate blood flow maps with high spatial and temporal resolution. The lack of quantitative accuracy and the inability to predict flows in the presence of static scatterers, such as an intact or thinned skull, have been the primary limitation of LSCI. Accordingly, in one embodiment, the present disclosure provides a Multi-Exposure Speckle Imaging (MESI) system that has the ability to obtain quantitative baseline flow measures. Similarly, in another embodiment, the present disclosure also provides a speckle model that can discriminate flows in the presence of static scatters. In some embodiments, the speckle model of the present disclosure, along with a MESI system of the present disclosure, in the presence of static scatterers, can predict correlation times of flow consistently to within 10% of the value without static scatterers compared to an average deviation of more than 100% from the value without static scatterers using traditional LSCI. The details of a MESI system and speckle model of the present disclosure will be discussed in more detail below.

In general, speckle arises from the random interference of coherent light. When collecting laser speckle contrast images, coherent light is used to illuminate a sample and a photodetector is then used to receive light that has scattered from varying positions within the sample. The light will have traveled a distribution of distances, resulting in constructive and destructive interference that varies with the arrangement of the scattering particles with respect to the photodetector. When this scattered light is imaged onto a camera, it produces a randomly varying intensity pattern known as speckle. If scattering particles are moving, this will cause fluctuations in the interference, which will appear as intensity variations at the photodetector. The temporal and spatial statistics of this speckle pattern provide information about the motion of the scattering particles. The motion can be quantified by measuring and analyzing temporal variations and/or spatial variations.

Using the latter approach, 2-D maps of blood flow can be obtained with very high spatial and temporal resolution by imaging the speckle pattern onto a camera and quantifying the spatial blurring of the speckle pattern that results from blood flow. In areas of increased blood flow, the intensity fluctuations of the speckle pattern are more rapid, and when integrated over the camera exposure time (typically 1 to 10 ms), the speckle pattern becomes blurred in these areas. By acquiring a raw image of the speckle pattern and quantifying the blurring of the speckles in the raw speckle image by measuring the spatial contrast of the intensity variations, spatial maps of relative blood flow can be obtained. To quantify the blurring of the speckles, the speckle contrast (K) is calculated over a window (usually 7×7 pixels) of the image as,

$$K = \frac{\sigma_s}{\langle I \rangle}, \quad \text{Equation 1}$$

where σ_s is the standard deviation and $\langle I \rangle$ is the mean of the pixels of the window. For slower speeds, the pixels decorrelate less and hence K is large and vice versa.

Although speckle contrast values are indicative of the level of motion in a sample, they are not directly proportional to speed or flow. To obtain quantitative blood flow measurements from speckle contrast values, two steps are typically performed. The first step is to accurately relate the speckle contrast values, which are obtained from a time-integrated measure of the speckle intensity fluctuations using Equation 1 above, to a speckle correlation time (τ_c). The second step is to relate the speckle correlation time to the underlying flow or speed.

The relationship between speckle contrast values, K , and speckle correlation time, τ_c , is rooted in the field of dynamic light scattering (DLS). The correlation time of speckles is the characteristic decay time of the speckle decorrelation function. The speckle correlation function is a function that describes the dynamics of the system using backscattered coherent light. Under

conditions of single scattering, small scattering angles and strong tissue scattering, the correlation time can be shown to be inversely proportional to the mean translational velocity of the scatterers. Strictly speaking this assumption that $\tau_c \propto 1/v$ (where v is the mean velocity) is most appropriate for capillaries where a photon is more likely to scatter off only one moving particle and succeeding phase shifts of photons are totally independent of earlier ones. Hence great care should be observed when using this expression. The measurements in the present disclosure are made in channels that mimic smaller blood vessels and hence this relation between the correlation time and velocity can be used.

The uncertainty over the relation between correlation time and velocity is a fundamental limitation for all DLS based flow measurement techniques. Nevertheless, quantitative flow measurements can be performed through accurate estimation of the correlation times. The correlation times can be related to velocities through external calibration. The speckle contrast can be expressed in terms of the correlation time of speckles and the exposure duration of the camera. The MESI system of the present disclosure obtains speckle images at different exposure durations and uses this multi-exposure data to quantify τ_c . Previous efforts to obtain speckle images at multiple exposure durations have been limited to a few durations or to line scan cameras.

In one embodiment, the present disclosure provides a MESI system that is able to obtain images over a wide range of exposure durations (50 μ s to 80 ms). Accordingly, a MESI system of the present disclosure is able to obtain better estimates of correlation times of speckles.

A. Speckle Model

Speckle contrast has been related to the exposure duration of a camera and correlation time of the speckles using the theory of correlation functions and time integrated speckle. The theory of correlation functions has been widely used in dynamic light scattering (DLS) and LSCI is a direct extension of it. The temporal fluctuations of speckles can be quantified using the electric field autocorrelation function $g_1(\tau)$. Typically $g_1(\tau)$ is difficult to measure and the intensity autocorrelation function $g_2(\tau)$ is recorded. The field and intensity autocorrelation functions are related through the Siegert relation,

$$g_2(\tau) = 1 + \beta |g_1(\tau)|^2, \quad \text{Equation 2}$$

where β is a normalization factor which accounts for speckle averaging due to mismatch of speckle size and detector size, polarization and coherence effects. In prior art, it was assumed that $\beta = 1$ and Equation 2 was used, along with the fact that the recorded intensity is integrated over the exposure duration, to derive the first speckle model,

$$K(T, \tau_c) = \left(\frac{1 - e^{-2x}}{2x} \right)^{1/2}, \quad \text{Equation 3}$$

where $x = T/\tau_c$, T is the exposure duration of the camera and τ_c is the correlation time. Equation 3 has been widely used to determine relative blood flow changes for LSCI measurements.

Recently, it has been shown that Equation 3 did not account for speckle averaging effects. Arguing that β should not be ignored and also using triangular weighting of the autocorrelation function, a more rigorous model relating speckle contrast to τ_c was developed,

$$K(T, \tau_c) = \left(\beta \frac{e^{-2x} - 1 + 2x}{2x^2} \right)^{1/2}. \quad \text{Equation 4}$$

One disadvantage of these prior models is that they breakdown in the presence of statically scattered light. This is primarily because these models rely on the Siegert relation (Equation 2) which assumes that the speckles follow Gaussian statistics in time. However, in the presence of static scatterers, the fluctuations of the scattered field remain Gaussian but the intensity acquires an extra static contribution causing the recorded intensity to deviate from Gaussian statistics, and hence the Siegert relation (Equation 2) cannot be applied. This can be corrected by modeling the scattered field as

$$E_h(t) = E(t) + E_s e^{i\omega_0 t}, \quad \text{Equation 5}$$

where $E(t)$ is the Gaussian fluctuation, E_s is the static field amplitude and ω_0 is the source frequency. The Siegert relation can now be modified as,

$$\begin{aligned} g_2^h(\tau) &= 1 + \frac{\beta}{(I_f + I_s)^2} \left[I_f^2 |g_1(\tau)|^2 + 2I_f I_s |g_1(\tau)| \right] \\ &= 1 + A\beta |g_1(\tau)|^2 + B\beta |g_1(\tau)|, \end{aligned} \quad \text{Equation 6}$$

20

$$\text{where } A = \frac{I_f^2}{(I_f + I_s)^2} \text{ and } B = \frac{2I_f I_s}{(I_f + I_s)^2}, \quad I_s = E_s E_s^*$$

represent contribution from the static scattered light, and $I_f = \langle EE^* \rangle$ represent contribution from the dynamically scattered light.

This updated Siegert relation can be used to derive the relation between speckle variance and correlation time as with the other models. Following the approach of Bandyopadhyay et. al. the second moment of intensity can be written using the modified Siegert relation as

25

$$\begin{aligned}
\langle I^2 \rangle_T &\equiv \left\langle \int_0^T \int_0^T I_i(t') I_i(t'') dt' dt'' / T^2 \right\rangle_i \\
&= \langle I \rangle^2 \int_0^T \int_0^T \left[1 + A\beta \left(g_1(t' - t'') \right)^2 + B\beta g_1(t' - t'') \right] dt' dt'' / T^2.
\end{aligned}$$

Equation 7

The reduced second moment of intensity or the variance is hence

$$v_2(T) \equiv \int_0^T \int_0^T \left[A\beta \left(g_1(t' - t'') \right)^2 + B\beta g_1(t' - t'') \right] dt' dt'' / T^2.$$

Equation 8

Since $g_1(t)$ is an even function, the double integral simplifies to

$$v_2(T) = A\beta \int_0^T 2\left(1 - \frac{t}{T}\right) [g_1(t)]^2 \frac{dt}{T} + B\beta \int_0^T 2\left(1 - \frac{t}{T}\right) [g_1(t)] \frac{dt}{T}$$

Equation 9

Equation 9 represents a new speckle visibility expression that accounts for the varying proportions of light scattered from static and dynamic scatterers. Assuming that the velocities of the scatterers have a Lorentzian distribution, which gives $g_1(t) = e^{-t/\tau_c}$, and recognizing that the square root of the variance is the speckle contrast, Equation 9 can be simplified to:

$$K(T, \tau_c) = \left\{ \beta \rho^2 \frac{e^{-2x} - 1 + 2x}{2x^2} + 4\beta \rho(1 - \rho) \frac{e^{-x} - 1 + x}{x^2} \right\}^{1/2},$$

Equation 10

where $x = \frac{T}{\tau_c}$, $\rho = \frac{I_f}{(I_f + I_s)}$ is the fraction of total light that is dynamically scattered, β is a normalization factor to account for speckle averaging effects, T is the camera exposure duration and τ_c is the correlation time of the speckles.

When there are no static scatterers present, $\rho \rightarrow 1$ and Equation 10 simplifies to Equation 4. However Equation 10 is incomplete since in the limit that only static scatterers are present ($\rho \rightarrow 0$), it does not reduce to a constant speckle contrast value as one would expect for spatial speckle contrast. This can be explained by recognizing that K in Equation 10 refers to the temporal (temporally sampled) speckle contrast. The initial definition of K (Equation 1) was based on spatial sampling of speckles. Traditionally, in LSCI, speckle contrast has been estimated through spatial sampling by assuming ergodicity to replace temporal sampling of speckles with an ensemble sampling. In the presence of static scatterers this assumption is no longer valid. It is preferred to use spatial (ensemble sampled) speckle contrast because it helps retain the temporal resolution of LSCI. In order for the current theory to be used with spatial (ensemble sampled) speckle contrast, a constant term is added to the speckle visibility expression

(Equation 9). This constant is referred to as *nonergodic variance* (v_{ne}). It is assumed that this is constant in time.

The speckle pattern obtained from a completely static sample does not fluctuate. Hence the variance of the speckle signal over time is zero as predicted by Equation 10. However the spatial (or ensemble) speckle contrast is a nonzero constant due to spatial averaging of the random interference pattern produced. This nonzero constant (v_{ne}) is primarily determined by the sample, illumination and imaging geometries. Since the speckle contrast is normalized to the integrated intensity, v_{ne} does not depend on the integrated intensity. These factors are clearly independent of the exposure duration of the camera, and hence the assumption is valid. The addition of v_{ne} allows the continued use of spatial (or ensemble) speckle contrast in the presence of static scatterers. This addition of the nonergodic variance is a significant improvement over existing models.

An additional factor that has been previously neglected is experimental noise which can have a significant impact on measured speckle contrast. Experimental noise can be broadly categorized into shot noise and camera noise. Shot noise is the largest contributor of noise, and it is primarily determined by the signal level at the pixels. This can be held independent of exposure duration, by equalizing the intensity of the image across different exposure durations. Camera noise includes readout noise, QTH noise, Johnson noise, *etc.* It can also be made independent of exposure by holding the camera exposure duration constant. The present disclosure provides a MESI system that holds camera exposure duration constant, yet obtains multi-exposure speckle images by pulsing the laser, while maintaining the same intensity over all exposure durations. Hence the experimental noise will add an additional constant spatial variance, v_{noise} .

In the light of these arguments, Equation 10 can be rewritten as:

$$K(T, \tau_c) = \left\{ \beta \rho^2 \frac{e^{-2x} - 1 + 2x}{2x^2} + 4\beta \rho(1 - \rho) \frac{e^{-x} - 1 + x}{x^2} + v_{ne} + v_{noise} \right\}^{1/2}, \text{ Equation 11}$$

where $x = \frac{T}{\tau_c}$, $\rho = \frac{I_f}{(I_f + I_s)}$ is the fraction of total light that is dynamically scattered, β is a normalization factor to account for speckle averaging effects, T is the camera exposure duration, τ_c is the correlation time of the speckles, v_{noise} is the constant variance due to experimental noise and v_{ne} is the constant variance due to nonergodic light.

Equation 11 is a rigorous and practical speckle model that accounts for the presence of static scattered light, experimental noise and nonergodic variance due to the ensemble averaging.

While v_{ne} and v_{noise} make the model more complete, they do not add any new information about the dynamics of the system, all of which is held in τ_c . Hence v_{ne} and v_{noise} can be viewed as experimental variables/artifacts. In the present disclosure, v_{ne} and v_{noise} may be combined as a single static spatial variance v_s , where $v_s = v_{ne} + v_{noise}$.

5 Accordingly, the speckle model of the present disclosure (Equation 11) accounts for the presence of light scattered from static particles. The model of the present disclosure applies the theory of time integrated speckle to static scattered light. The model of the present disclosure also takes into account the assumption that ergodicity breaks down in the presence of static scatterers and thus proposes a solution to account for nonergodic light. Furthermore, the speckle
10 model of the present disclosure provides a model that accounts for experimental noise. The influence of noise and nonergodic light have been neglected in most previous studies.

The methods of the present disclosure may be implemented in software to run on one or more computers, where each computer includes one or more processors, a memory, and may include further data storage, one or more input devices, one or more output devices, and one or
15 more networking devices. The software includes executable instructions stored on a tangible medium.

It should be noted that the speckle model of the present disclosure generally works when the speckle signal from dynamically scattered photons is strong enough to be detected in the presence of the static background signal. If the fraction of dynamically scattered photons is too
20 small compared to statically scattered photons, the dynamic speckle signal would be insignificant and estimates of τ_c breakdown. For practical applications, a simple single exposure LSCI image or visual inspection can qualitatively verify if there is sufficient speckle visibility due to dynamically scattered photons and subsequently the model of the present disclosure can be used to obtain consistent estimates of correlation times.

25 B. Multi-Exposure Speckle Imaging System

In addition to the speckle model presented above, the present disclosure also provides a MESI system. In some embodiments, a MESI system of the present disclosure is able to acquire images that will obtain correlation time information. Additionally, in some embodiments, a MESI system of the present disclosure is able to vary the exposure duration, maintain a constant
30 intensity over a wide range of exposures and ensure that the noise variance is constant.

In one embodiment, a MESI system of the present disclosure generally comprises a laser light source; a light modulator; and a detector for the measurement of reflected light comprising at least one camera, at least one magnification objective, and at least one microprocessor or data

acquisition unit. Examples of suitable light modulators may include, but are not limited to, an acousto-optic modulator, an electro-optic modulator, or a spatial light modulator.

A MESI system of the present disclosure may also comprise additional electronic and mechanical components such as a gated laser diode, a digitizer, a motion controller, a stepper motor, a trigger, a delay switch, and/or a display monitor. One of ordinary skill in the art, with the benefit of this disclosure, will recognize additional electronic and mechanical components that may be suitable for use in the methods of the present invention. Furthermore, a MESI system of the present disclosure may also be used in conjunction with custom-made software. An example of an embodiment of a MESI system is depicted in Figure 1.

The need for high-resolution blood flow imaging spans many applications, tissue types, and diseases. Accordingly, the MESI systems of the present disclosure may be used in a variety of applications, including, but not limited to, blood imaging applications in tissues such as the retina, skin, and brain. In another embodiment, the MESI systems of the present disclosure may be used during surgery.

EXAMPLE 1

The examples provided herein utilize a tissue phantom to show that the speckle model of the present disclosure, used in conjunction with a MESI system of the present disclosure, can predict correlation times consistently in the presence of static speckles.

In order to test the model experimentally, flow measurements were performed on microfluidic flow phantoms. To do this, the exposure duration of speckle measurements had to be changed, while ensuring that certain conditions were satisfied. To obtain speckle images at multiple exposure durations, the actual camera exposure duration was fixed and a laser diode was gated during each exposure to effectively vary the speckle exposure duration T as in Yuan *et. al.* This approach ensures that the camera noise variance and the average image intensity is constant. Directly pulsing the laser limited the range of exposure durations that can be achieved. The lasing threshold of the laser diode dictated the minimum intensity and hence the maximum exposure duration that could be recorded. Consequently, the minimum exposure duration was limited by the dynamic range of the instruments. To overcome this limitation, the laser was pulsed through an acousto-optic modulator (AOM). By modulating the amplitude of the radio-frequency wave fed to the AOM, the intensity of the first diffraction order could be varied, enabling control over both the integrated intensity and the effective exposure duration.

Figure 1 provides a schematic representation of the MESI system used in this example. A diode laser beam (Hitachi HL6535MG; $\lambda=658\text{nm}$, 80mW Thorlabs, Newton, NJ, USA) was directed to an acousto-optic modulator (AOM) (IntraAction Corp., BellWood, IL, USA). The

AOM was driven by signals generated from an RF AOM Modulator driver (IntraAction Corp., BellWood, IL, USA) and the first diffraction order was directed towards the sample. The sample was imaged using a 10X ∞ corrected objective (Thorlabs, Newton, NJ, USA) and a 150 mm tube lens (Thorlabs, Newton, NJ, USA). Images were acquired using a camera (Basler 602f; Basler Vision Technologies, Germany). Software was written to control the timing of the AOM pulsing and synchronize it with image acquisition.

A microfluidic device was used as a flow phantom in this example. A microfluidic device as a flow phantom has the advantage of being realistic and cost effective, providing flexibility in design, large shelf life and robust operation. A microfluidic device without a static scattering layer (Figure 2A) and with a static scattering layer (Figure 2B) were prepared. The channels were rectangular in cross section (300 μm wide x 150 μm deep). The microfluidic device was fabricated in poly dimethyl siloxane (PDMS) using the rapid prototyping technique disclosed in J. Anderson, D. Chiu, R. Jackman, O. Cherniavskaya, J. McDonald, H. Wu, S. Whitesides, and G. Whitesides, "Fabrication of Topologically Complex Three-Dimensional Microfluidic Systems in PDMS by Rapid Prototyping," Science 261, 895 (1993). Titanium dioxide (TiO_2) was added to the PDMS (1.8 mg of TiO_2 per gram of PDMS) to give the sample a scattering background to mimic tissue optical properties. The prepared samples were bonded on a glass slide to seal the channels as shown in Figures 2A and 2B. The sample was connected to a mechanical syringe pump (World Precision Instruments, Sarasota, FL, USA) through silicone tubes, and a suspension ($\mu'_s = 250 \text{ cm}^{-1}$) of 1 μm diameter polystyrene beads (Duke Scientific Corp., Palo Alto, CA, USA) ("microspheres") was pumped through the channels.

For the static scattering experiments, a 200 μm layer of PDMS with different concentrations of TiO_2 (0.9 mg and 1.8 mg of TiO_2 per gram of PDMS corresponding to ($\mu'_s = 4 \text{ cm}^{-1}$) and ($\mu'_s = 8 \text{ cm}^{-1}$ respectively) was sandwiched between the channels and the glass slide, to simulate a superficial layer of static scattering such as a thinned skull (Figure 2B). The reduced scattering coefficients of the 200 μm static scattering layer were estimated using an approximate collimated transmission measurement through a thin section of the sample. Figures 2A and 2B show a schematic of the cross-section of the devices.

The experimental setup (Figure 1) was used in conjunction with the exposure modulation technique to perform controlled experiments on the microfluidic samples. The microfluidic sample without the static scattering layer (Figure 2A) was used to test the accuracy of the MESI system and the speckle model. As detailed earlier, the suspension of microspheres was pumped through the sample using the syringe pump at different speeds from 0 mm/sec (Brownian motion) to 10 mm/sec in 1 mm/sec increments. 30 speckle contrast images were calculated and

averaged for each exposure from the raw speckle images. The average speckle contrast in a region within the channel was calculated.

In this fully dynamic case, the static spatial variance v_s is very small. v_s would be dominated by the experimental noise v_{noise} as the ergodicity assumption would be valid and $v_{ne} \approx 0$. β is one of the unknown quantities in Equation 11 describing speckle contrast. Theoretically, β is a constant that depends only on experimental conditions. An attempt to estimate β using a reflectance standard would yield inaccurate results due to the presence of the static spatial variance v_s . Here the ergodicity assumption would breakdown, and v_{ne} would be significant. It would not be possible to separate the contributions of speckle contrast from β , v_{ne} and v_{noise} .
 10 Instead, the value of β was estimated, by performing an initial fit of the multi-exposure data to Equation 4 with the addition of v_s , while having β , τ_c and v_s as the fitting variables. The speckle contrast data was then fit to Equation 11 using the estimated value of β and the results are shown in Figure 3. Holding β constant ensures that the fitting procedure is physically appropriate and makes the nonlinear optimization process less constrained and computationally
 15 less intensive. Figure 3 clearly shows that the model fits the experimental data very well (mean sum squared error: 2.4×10^{-6}). The correlation time of speckles was estimated by having τ_c as a fitting parameter. The standard error of correlation time estimates was found using bootstrap resampling. Correlation times varied from 3.361 ± 0.17 ms for Brownian motion to 38.4 ± 1.44 μ s for 10 mm/sec. The average percentage error in estimates of correlation times was 3.37%,
 20 with a minimum of 1.99% for 3 mm/sec and a maximum of 5.2% for Brownian motion. Other fitting parameters were v_s , the static spatial variance and ρ , the fraction of dynamically scattered light. A *priori* knowledge of ρ was not required to obtain τ_c estimates. Hence this technique can be applied to cases where the thickness of the skull is unknown and/or variable.

In order to verify the arguments on nonergodicity, the speckle contrast obtained using
 25 spatial analysis and temporal analysis was compared. Spatial speckle contrast was estimated by using Equation 1 and the procedure detailed earlier, while temporal speckle contrast was estimated by calculating the ratio of the standard deviation to mean of pixel intensities over different frames at the same exposure duration. Multi-exposure speckle contrast measurements were performed on the microfluidic devices with different levels of static scattering in the static
 30 scattering upper layer (Figure 2A: $\mu'_s = 0$ cm^{-1} and Figure 2B: $\mu'_s = 4$ cm^{-1} and $\mu'_s = 8$ cm^{-1}). A suspension ($\mu_s = 250$ cm^{-1}) of 1 μ m diameter polystyrene beads was pumped through the channels at 2 mm/sec. The experimentally obtained temporal contrast (temporal sampling) and spatial contrast (ensemble sampling) curves for each static scattering case is shown in Figure 4.

From Figure 4, it can be seen that the temporal contrast curves (dotted lines) do not possess a significant constant variance since the variance approaches zero at long exposure durations. The small offset that was observed was likely due to v_{noise} which remains constant even in the presence of static scattering and does not change as the amount of static scattering increases. However, the spatial (ensemble sampled) contrast curves (solid lines) show a clear offset at large exposure durations when static scatterers were present. This offset increases with an increase in static scattering. Again, when no static scatterers were present, the spatial (ensemble sampled) contrast curve does not possess this offset. The speckle variance curves show that the nonergodic variance v_{ne} is absent in all three temporally sampled curves and in the completely dynamic spatially (ensemble) sampled curve. v_{ne} is significant in the cases with a static scattered layer, when the data is analyzed by spatial (ensemble) sampling. This provides evidence in favor of the argument that the increase in variance at large exposure durations is due to v_{ne} , the nonergodic variance. For the same static scattering level, the variance obtained by temporal sampling is greater than the variance obtained by spatial sampling. This could be due to different β . The objective was not to compare temporal speckle contrast with spatial speckle contrast, but to utilize the two curves to provide evidence in favor of the model.

One of the significant improvements that the speckle model of the present disclosure provides is its ability to estimate correlation times consistently in the presence of static scatterers. The flow measurements as detailed earlier were repeated, at speeds 0 mm/sec to 10 mm/sec in 2 mm/sec increments. Measurements on the sample with no static scattering layer (Figure 2A) served as base (or 'true') estimates of correlation times. Figure 5 shows the results of this analysis at two different speeds. The addition of the static scattering layer drastically changed the shape of the curve. For a given speed, the decrease in variance at the low exposures was due to the relative weighting of the two exponential decays in Equation 11 which was consistent with results obtained with DLS measurements. The increase in variance at the larger exposure durations was due to the addition of the nonergodic variance v_{ne} . The speckle model of the present disclosure fit well to the data points. Also, the ρ values decreased with the addition of static scattering, implying a reduction in the fraction of total light that was dynamically scattered. It is important to note that for a given exposure duration and speed, the measured speckle contrast values were different in the presence of static scattered light when compared to the speckle contrast values obtained in the absence of static scattered light. Hence accurate τ_c estimates cannot be obtained with measurements from a single exposure duration without an accurate model and a *priori* knowledge of the constants ρ , β and v_s . These constants are typically

difficult to estimate. By using the multi-exposure data and the speckle model of the present disclosure, this problem was overcome and τ_c was reproduced consistently.

To quantify the effects of the static scattering layer on the consistency of the τ_c estimates, the deviations in τ_c were estimated for each speed as the amount of static scatterer was varied. For each speed, the variation in the estimated correlation times over the three scattering cases (Figure 2A: $\mu'_s = 0 \text{ cm}^{-1}$ and Figure 2B: $\mu'_s = 4 \text{ cm}^{-1}$ and $\mu'_s = 8 \text{ cm}^{-1}$) was estimated by

$$\% \text{ Deviation in } \tau_c = \frac{\text{Standard deviation in } \tau_c}{\tau_c \text{ in the absence of static scatters}} \times 100$$

calculating the standard deviation of the correlation time estimates. This deviation was normalized to the base (or 'true') correlation time estimates. Single exposure estimates of correlation time was obtained using Equation 3. Equation 3 was used in estimating the correlation time because of its widespread use in most speckle imaging techniques to estimate relative flow changes, and was hence most appropriate for this comparison. The correlation time was estimated from a lookup table. A lookup table which relates speckle contrast values to correlation times was generated using Equation 3 for the given exposure time. The correlation time was then estimated through interpolation from the lookup table for the appropriate speckle contrast value. For an appropriate comparison, β was prefixed to Equation 3, and same value of β was used for both the single exposure and MESI estimates. The results for the speckle model of the present disclosure and the single exposure case are plotted in Figure 6.

Figure 6 shows that the single exposure estimates are not suited for speckle contrast measurements in the presence of static scatterers. The error in the correlation time estimates is high and increases drastically with speed. The speckle model of the present disclosure performed very well, with deviation in correlation times being less than 10% for all speeds. τ_c estimates with the speckle model of the present disclosure have extremely low deviation. This shows that the speckle model of the present disclosure can estimate correlation times consistently even in the presence of static scattering.

The lack of quantitative accuracy of correlation time measures using LSCI can be attributed to several factors including inaccurate estimates of β and neglect of noise contributions and nonergodicity effects. The absence of the noise term in traditional speckle measurements can also lead to incorrect speckle contrast values for a given correlation time and exposure duration. A MESI system of the present disclosure reduces this experimental variability in measurements. Since images are obtained at different exposure durations, the integrated autocorrelation function curve can be experimentally measured, and a speckle model can be fit to it to obtain unknown

parameters, which include the characteristic decay time or correlation time τ_c , experimental noise and in the speckle model of the present disclosure, ρ , the fraction of dynamically scattered light. A MESI system of the present disclosure also removes the dependence of v_{noise} on exposure duration. The speckle model of the present disclosure and the τ_c estimation procedure allows for
 5 determination of noise with a constant variance. Without these improvements it would be very difficult to separate the variance due to speckle decorrelation and the lumped variance due to noise and nonergodicity effects.

EXAMPLE 2

Another experiment was conducted to test whether the τ_c estimates obtained using a
 10 MESI system of the present disclosure were more accurate than traditional single exposure LSCI measures by comparing the respective estimates of the relative correlation time measures. Correlation time estimates from traditional single exposure measures were obtained using the procedure detailed earlier. Relative correlation time measures were defined as

$$\text{relative } \tau_c = \frac{\tau_{co}}{\tau_c}, \quad (12)$$

15 where τ_{co} is the correlation time at baseline speed and τ_c is the correlation time at a given speed. Correlation time estimates were obtained from the fits performed in Figure 3, on multi-exposure speckle contrast data obtained with measurements made on the fully dynamic sample (Figure 2A). The τ_c estimates obtained with the MESI instrument were compared with traditional single exposure estimates of τ_c at 1 ms and 5 ms exposures for their efficiency in predicting relative
 20 flows. Ideally, relative correlation measures would be linear with relative speed. Relative correlation times were obtained for a baseline flow of 2 mm/sec.

Figure 7 shows that the speckle model of the present disclosure used in conjunction with a MESI system of the present disclosure maintains linearity of relative correlation measures over a long range. Single exposure estimates of relative correlation measures are linear for small
 25 changes in flows, but the linearity breaks down for larger changes. A MESI system and the speckle model of the present disclosure address this underestimation of large changes in flow by traditional LSCI measurements. This comparison is significant, because relative correlation time measurements are widely used in many dynamic blood flow measurements. Traditional single exposure LSCI measures underestimate relative flows for large changes in flow. This example
 30 shows that a MESI system of the present disclosure and the speckle model of the present disclosure can provide more accurate measures of relative flow.

Figure 7 also shows that even in a case where there is no obvious static scatterer like a thinned skull, there appears to be some contributions due to static scatterers, in this case possibly

from the bottom of the channel in Figure 2A. While the fraction of static scatterers is not too significant, it appears to affect the linearity of the curve, and a MESI system of the present disclosure with the speckle model of the present disclosure can eliminate this error.

EXAMPLE 3

5 As shown earlier, the presence of static scatterers significantly alters the shape of the integrated autocorrelation function curve in Figure 5, for different speeds. Also, it was previously shown that the speckle model of the present disclosure fits well to the experimentally determined speckle variance curve (Figure 5) and that the speckle model provides consistent estimates of τ_c even in the presence of static scatterers (Figure 6). This example tested whether
10 the correlation time estimates obtained with a MESI system of the present disclosure and the speckle model maintained linearity for relative flow measurements (as in Figure 7) in the presence of static scatterers.

Relative correlation time measures were obtained as detailed earlier (Equation 12) using 2 mm/sec as the baseline measure. The speckle model of the present disclosure and traditional
15 single exposure measurements (5 ms) were evaluated, and the results are shown in Figure 8. Figure 8 shows again why traditional single exposure methods are not suited for flow measurements when static scatterers are present. The linearity of relative correlation time measurements with single exposure measurements breaks down in the presence of static scatterers (Figure 8A) while the speckle model of the present disclosure maintains the linearity
20 of relative correlation time measures even in the presence of static scatterers (Figure 8B). This again reinforces the fact that a MESI system and the speckle model of the present disclosure can predict consistent correlation times in the presence of static scatterers.

Therefore, the present invention is well adapted to attain the ends and advantages mentioned as well as those that are inherent therein. The particular embodiments disclosed
25 above are illustrative only, as the present invention may be modified and practiced in different but equivalent manners apparent to those skilled in the art having the benefit of the teachings herein. Furthermore, no limitations are intended to the details of construction or design herein shown, other than as described in the claims below. It is therefore evident that the particular illustrative embodiments disclosed above may be altered or modified and all such variations are
30 considered within the scope and spirit of the present invention. While compositions and methods are described in terms of "comprising," "containing," or "including" various components or steps, the compositions and methods can also "consist essentially of" or "consist of" the various components and steps. All numbers and ranges disclosed above may vary by some amount. Whenever a numerical range with a lower limit and an upper limit is disclosed, any number and

any included range falling within the range is specifically disclosed. In particular, every range of values (of the form, “from about a to about b,” or, equivalently, “from approximately a to b,” or, equivalently, “from approximately a-b”) disclosed herein is to be understood to set forth every number and range encompassed within the broader range of values. Also, the terms in the claims have their plain, ordinary meaning unless otherwise explicitly and clearly defined by the patentee. Moreover, the indefinite articles “a” or “an”, as used in the claims, are defined herein to mean one or more than one of the element that it introduces. If there is any conflict in the usages of a word or term in this specification and one or more patent or other documents that may be incorporated herein by reference, the definitions that are consistent with this specification should be adopted.

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CLAIMS

What is claimed is:

1. A multi-exposure laser speckle contrast imaging system for quantitative blood flow imaging comprising:

5 a laser light source;
a light modulator; and
a detector for the measurement of reflected light comprising at least one camera, at least one magnification objective, and at least one microprocessor or data acquisition unit.

2. The system of claim 1 wherein the system is automated, semi-automated, or both.

10 3. The system of claim 1 wherein the detector comprises a plurality of cameras.

4. The system of claim 1 wherein the detector detects reflected light.

5. The system of claim 1 wherein the laser light is pulsed to create multiple exposures.

6. The system of claim 1 wherein the light modulator varies the intensity of the laser light source.

15 7. The system of claim 1 wherein the light modulator is an acousto-optic modulator, an electro-optic modulator, or a spatial light modulator.

8. A method for quantitative blood flow imaging comprising:

providing a system according to claim 1;

illuminating a sample and detecting a speckle pattern using the system according to claim

20 1; and

computing the quantitative blood flow images.

9. The method of claim 8 wherein quantitative blood flow imaging is conducted in the presence of a static scatter.

10. The method of claim 8 wherein quantitative blood flow imaging is conducted in the
25 presence of a static scatter, and wherein computing the quantitative blood flow images uses the following equation:

$$K(T, \tau_c) = \left\{ \beta \rho^2 \frac{e^{-2x} - 1 + 2x}{2x^2} + 4\beta \rho(1 - \rho) \frac{e^{-x} - 1 + x}{x^2} + v_{ne} + v_{noise} \right\}^{1/2},$$

where $x = \frac{T}{\tau_c}$, $\rho = \frac{I_f}{(I_f + I_s)}$ is the fraction of total light that is dynamically scattered, β is a normalization factor to account for speckle averaging effects, T is the camera exposure duration, τ_c is the correlation time of the speckles, v_{noise} is the constant variance due to experimental noise and v_{ne} is the constant variance due to nonergodic light.

- 5 11. The method of claim 10 wherein the static scatter is bone.
12. A method of measuring blood velocity in a tissue comprising:
 illuminating a tissue surface with coherent light from a laser light source;
 receiving reflected and scattered coherent light from the tissue on a photodetector;
 obtaining a speckle pattern from the reflected and scattered coherent light;
 10 computing a quantitative blood flow image using the speckle pattern and the following equation:

$$K(T, \tau_c) = \left\{ \beta \rho^2 \frac{e^{-2x} - 1 + 2x}{2x^2} + 4\beta \rho(1 - \rho) \frac{e^{-x} - 1 + x}{x^2} + v_{ne} + v_{noise} \right\}^{1/2},$$

where $x = \frac{T}{\tau_c}$, $\rho = \frac{I_f}{(I_f + I_s)}$ is the fraction of total light that is dynamically scattered, β is a normalization factor to account for speckle averaging effects, T is the camera exposure duration,
 15 τ_c is the correlation time of the speckles, v_{noise} is the constant variance due to experimental noise and v_{ne} is the constant variance due to nonergodic light.

13. The method of claim 12 further comprising evaluating the quantitative blood flow image and thereby determining blood velocity and perfusion in the tissue.

Figures 1A-1D

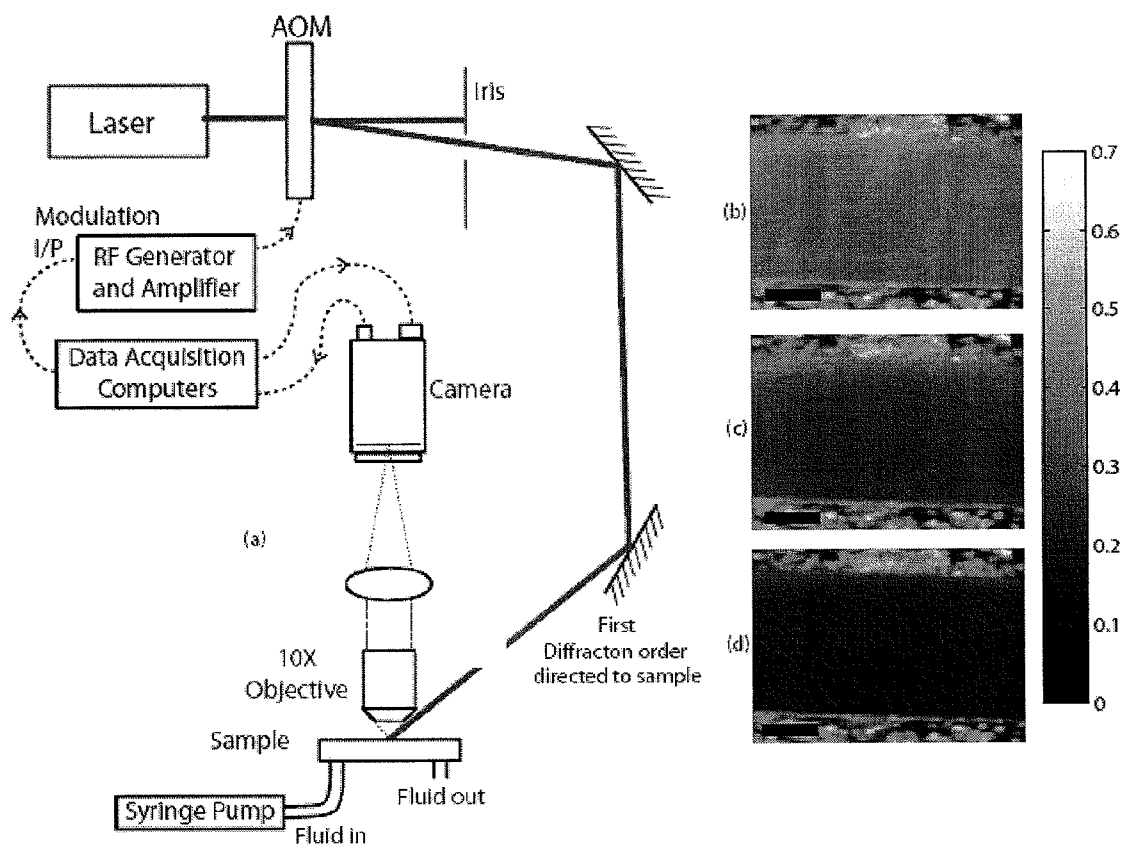


Figure 2A

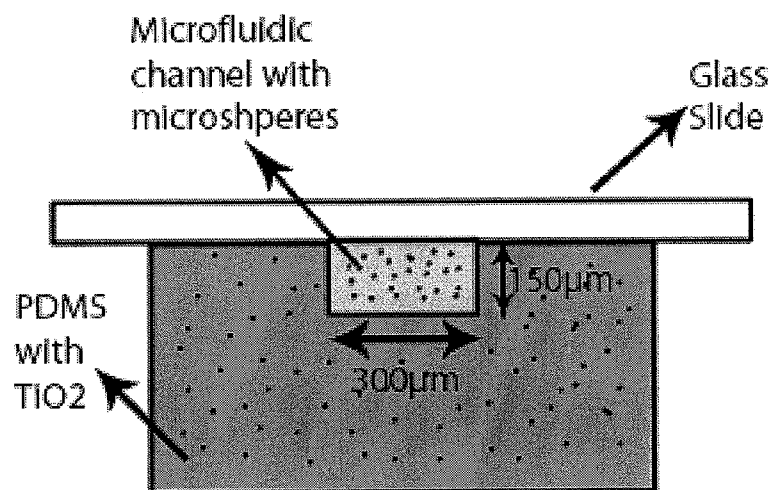
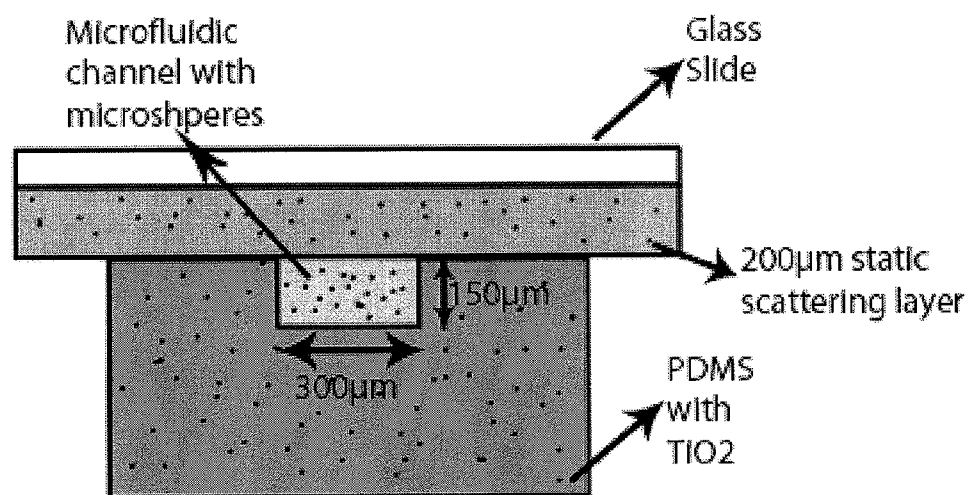
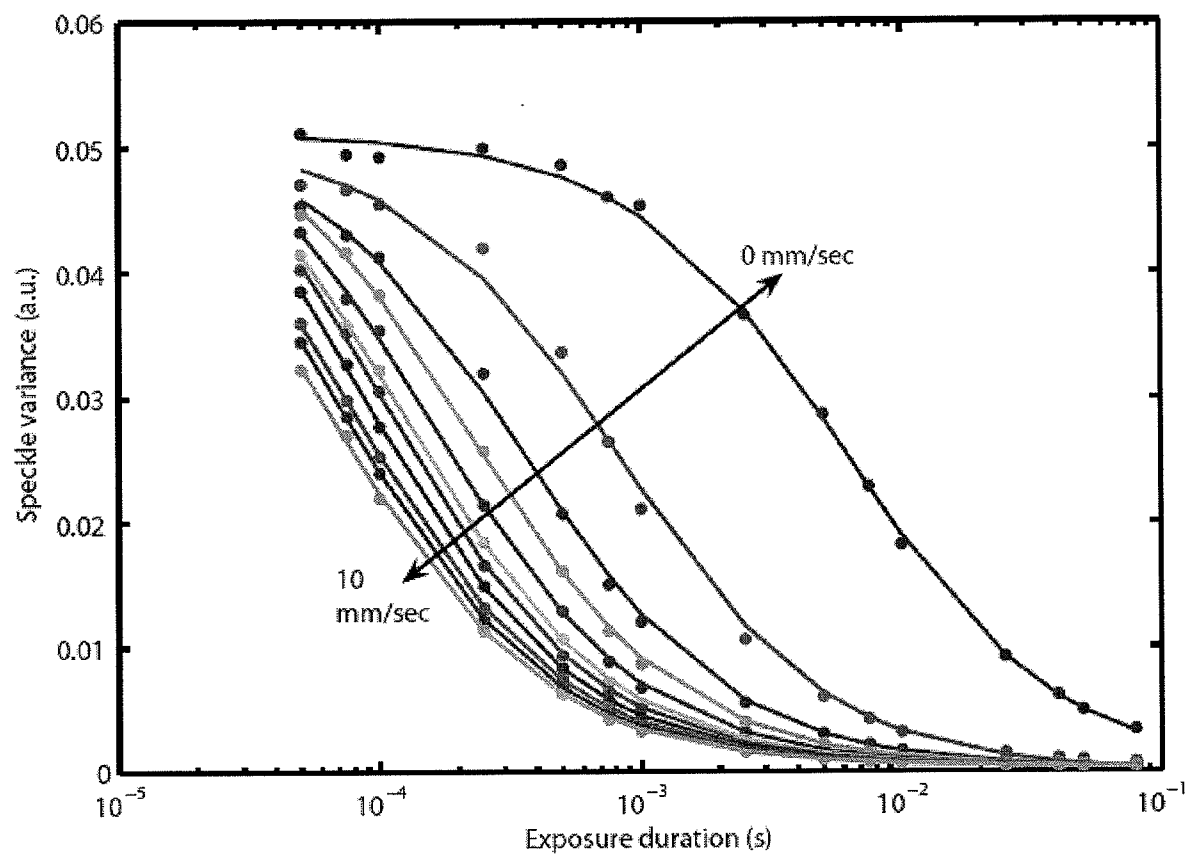


Figure 2B



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Figure 3



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Figure 4

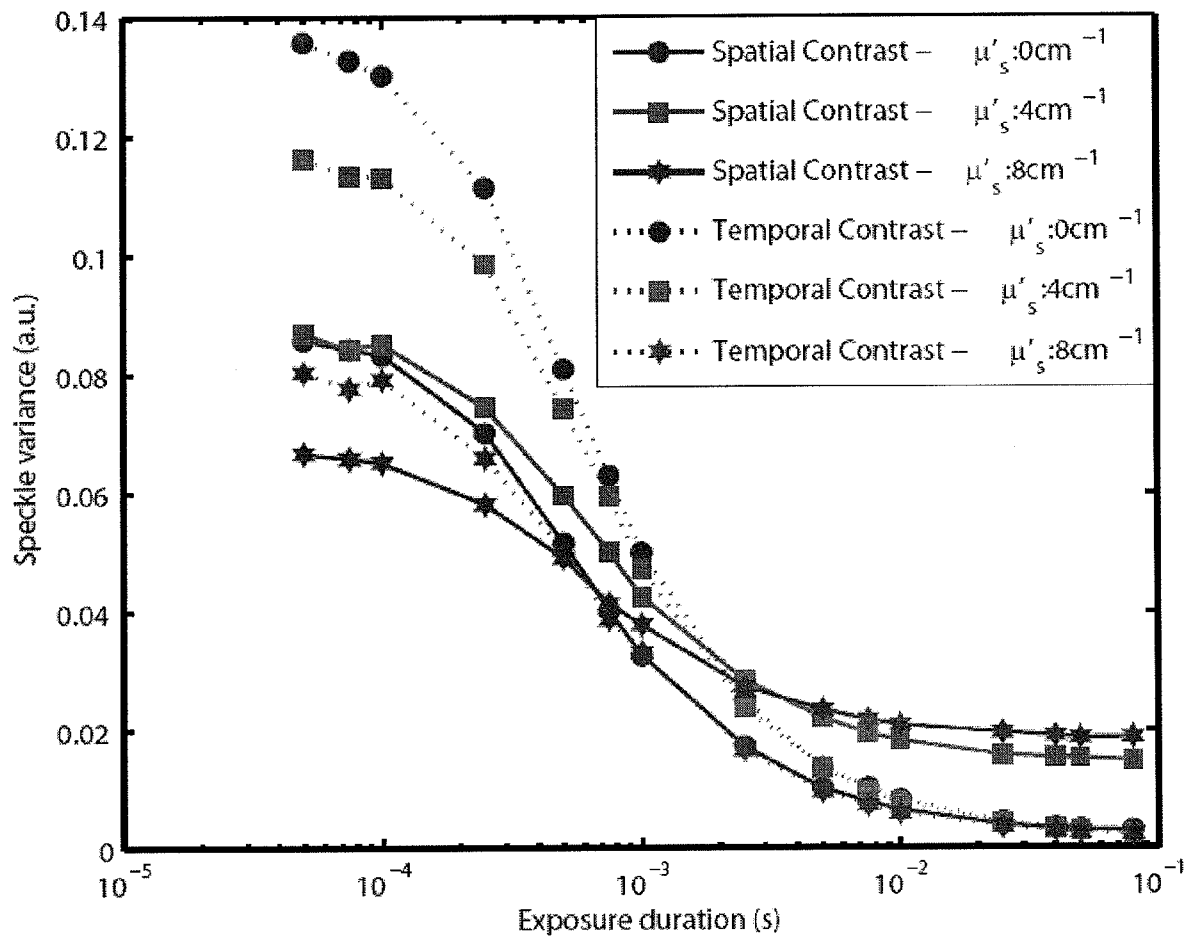


Figure 5

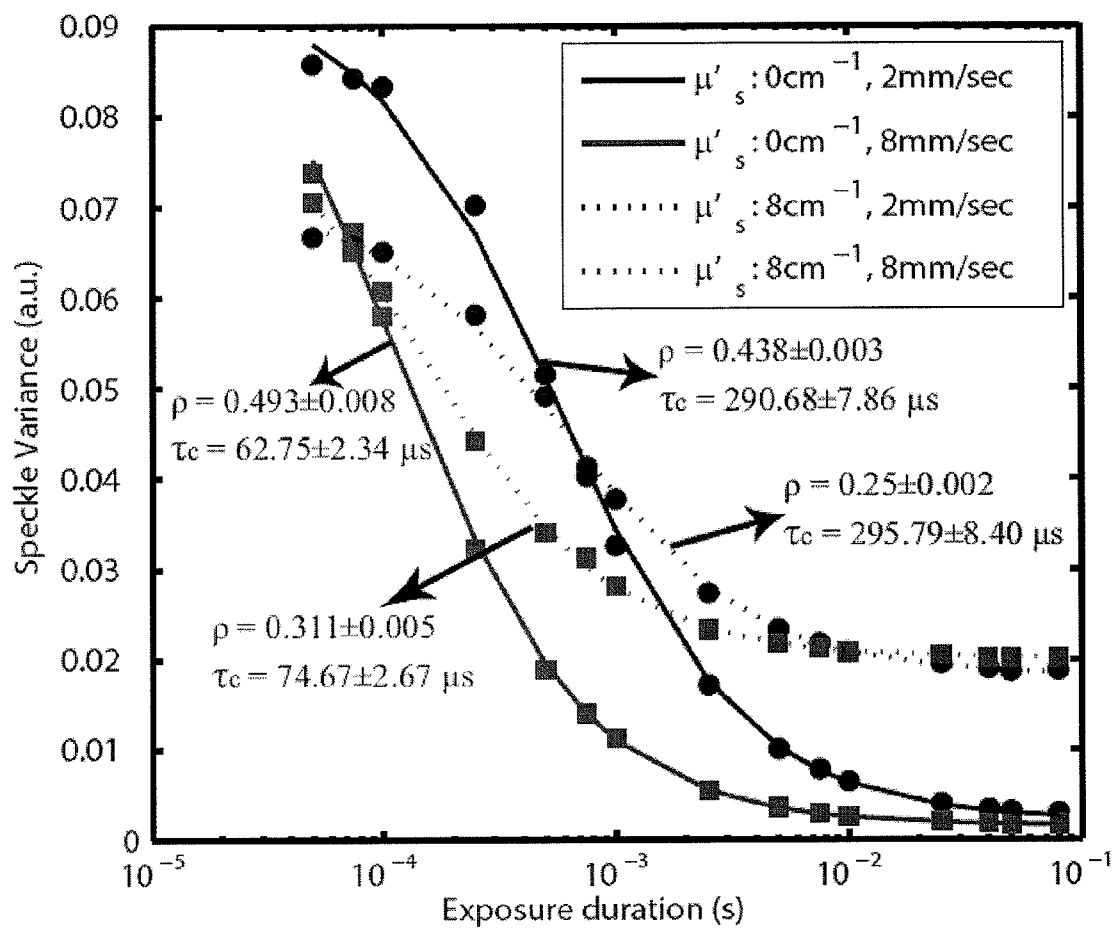
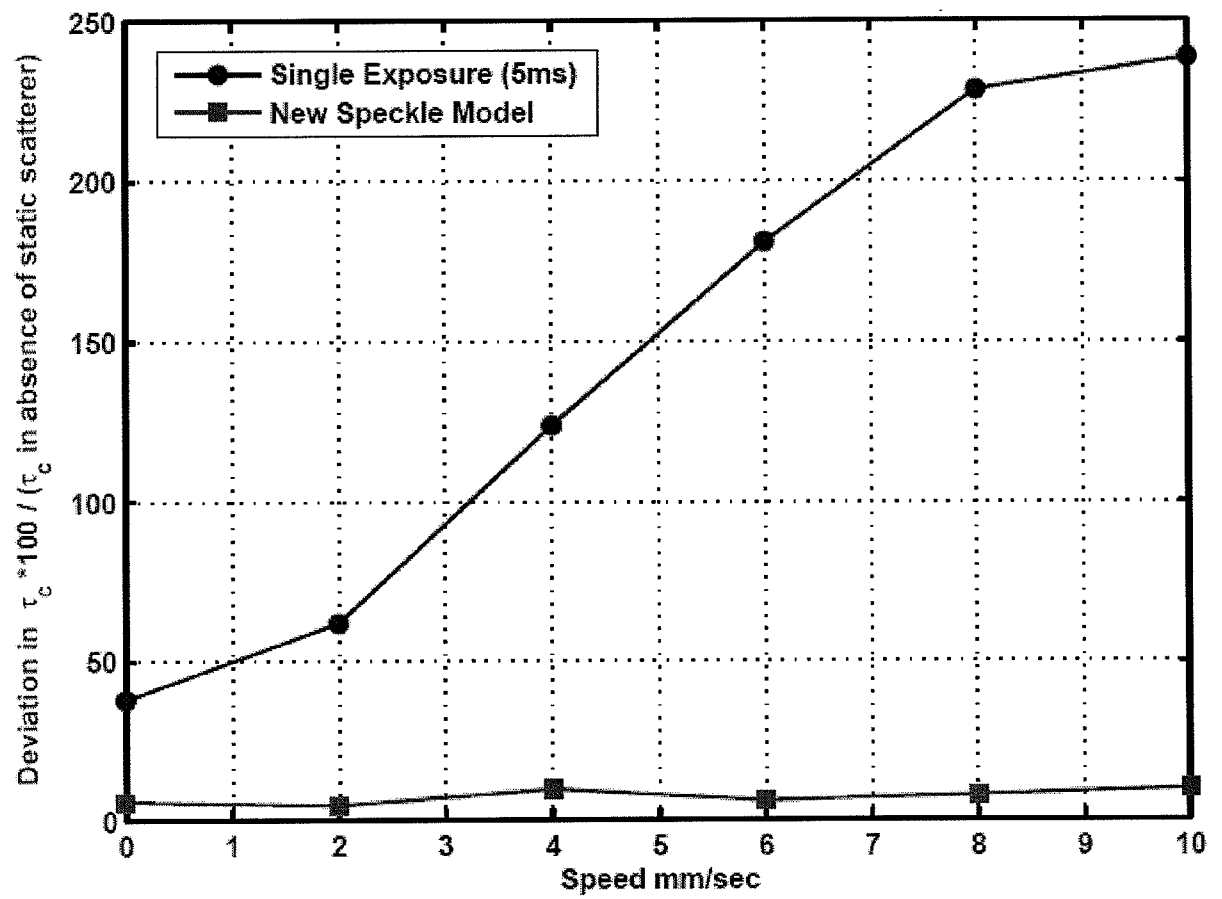
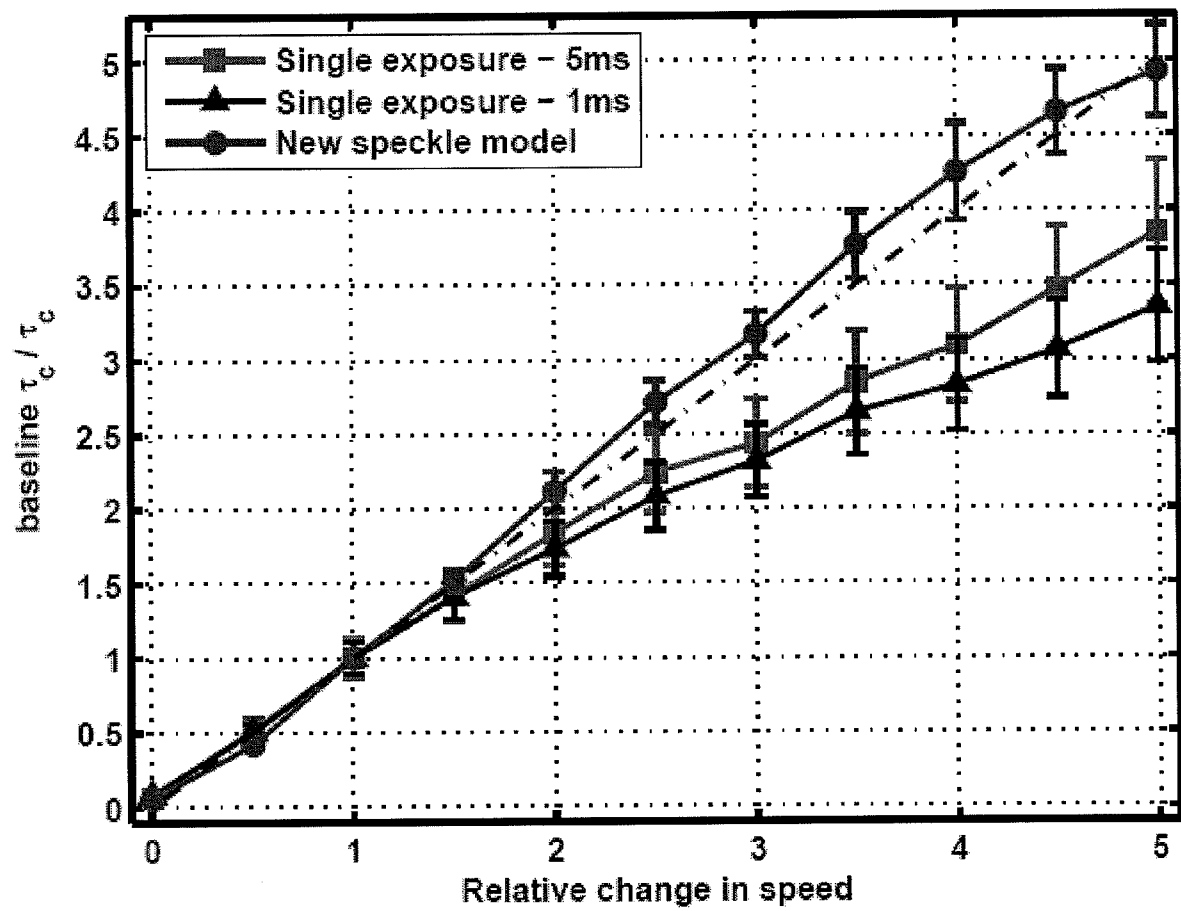


Figure 6



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Figure 7



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Figure 8A

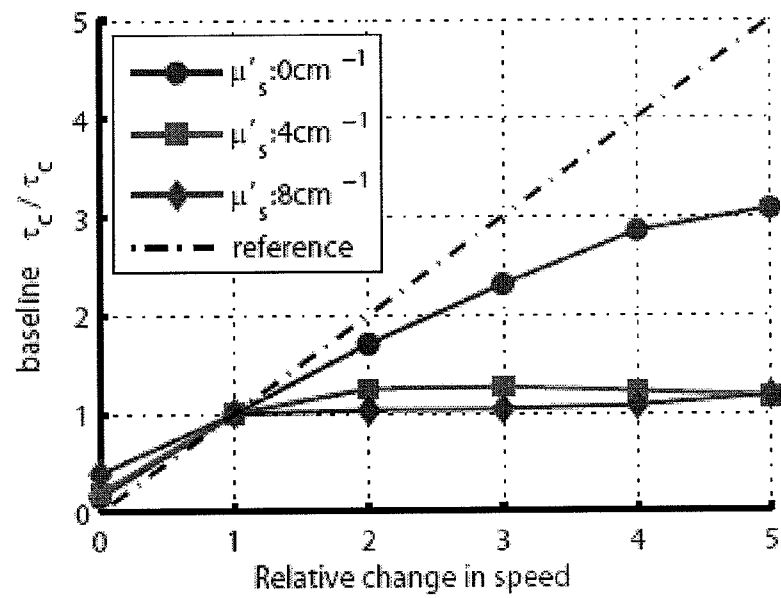


Figure 8B

