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Quantitative Cerebral Blood flow measurement with Multi Exposure Speckle Imaging

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Quantitative Cerebral Blood flow measurement with Multi Exposure Speckle Imaging

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Dedicated to my parents.

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Quantitative Cerebral Blood flow measurement with Multi Exposure Speckle Imaging

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Cerebral blood flow (CBF) measures are central to the investigation of ischemic strokes, spreading depressions, functional and neuronal activation. Laser Speckle Contrast Imaging (LSCI) is an optical imaging technique that has been used to obtain CBF measures *in vivo* at high spatial and temporal resolutions, by quantifying the localized spatial blurring of backscattered coherent light induced by blood flow. Despite being widely used for biomedical applications, LSCI's critical limitations such as its tendency to underestimate large flow changes and its inability to accurately estimate CBF through a thinned skull have not been overcome.

This dissertation presents a new Multi Exposure Speckle Imaging (MESI) technique that combines a new instrument and mathematical model to overcome these limitations. Additionally, in a pilot clinical study, an adapted neurosurgical microscope was used to obtain intra-operative LSCI images of CBF in humans. The MESI instrument accurately estimates experimental constants by imaging backscattered speckles over a wide range of the camera's exposure durations. The MESI mathematical model helps account for light that has scattered from both static and moving particles.

In controlled flow experiments using tissue simulating phantoms, the MESI technique was found to estimate large changes in flow accurately and the estimates of flow changes were found to be unaffected by the presence of static particles in these phantoms. In an *in vivo* experiment in which the middle cerebral artery in mice was occluded to induce $\sim 100\%$ reduction in CBF, not only was the reduction in CBF accurately estimated by the MESI technique but these estimates of CBF changes were found to be unaffected by the presence of a thinned skull. The validity of statistical models used to derive the MESI mathematical model was confirmed using *in vivo* dynamic light scattering (DLS) measurements of CBF in mice. The MESI technique's potential to estimate absolute values of CBF *in vivo* was demonstrated by comparing CBF estimates obtained using the MESI technique to DLS measurements.

The MESI technique's ability to measure CBF changes quantitatively through a thinned skull makes it particularly useful in chronic and long term studies leading to the development of better, more accurate stroke models.

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Chapter 1

Introduction

Blood flow is one of the key indicators of tissue health. Interruption of nutrient and oxygen supply is commonly associated with tissue and cell death. Since blood is the primary mechanism through which the body distributes nutrients, monitoring blood flow helps asses tissue viability. Blood flow is also a physiological parameter that can be easily monitored using a variety of techniques. Magnetic Resonance Imaging (MRI) [2,3], Doppler Ultrasound (D-US) [4, 5], Laser Doppler Imaging (LDI) [6, 7], Computed Tomography (CT) angiography [8, 9], Fluorescence angiography [10, 11], Doppler Optical Coherence Tomography (D-OCT) [12] are some of the techniques that have been used to measure and monitor blood flow.

Monitoring blood flow is especially crucial in the brain because reduction in blood flow to the brain can cause neuronal cell death leading to possibly permanent damage to motor, speech and/or sensory skills. For this reason monitoring cerebral blood flow (CBF) is important for research and clinical uses. Accurate measurements of cerebral blood flow changes during ischemic strokes [13,14] in animals help to develop different stroke models and methods to alleviate the effects of stroke [15]. In these models, tissue viability is typically classified based on the extent of reduction in blood flow [13, 16]. Neuronal depolarization events like spreading depressions are associated with an increase in cerebral blood flow [17] and measuring this increase quantitatively is the first step in indirectly estimating the extent of depolarizations. These Cortical Spreading Depressions (CSDs) have been associated with the migraine aura [18]. Clinically during neuro-surgical techniques such as tumor recession and aneurysm removals, monitoring cerebral blood flow is important to estimate and reduce collateral tissue damage in the brain. Also, monitoring cerebral blood flow during motor and sensory activation can be used to functionally localize and map sensory, motor and speech centers in the brain during surgery.

Ideally, an instrument that measures cerebral blood flow should have the following characteristics: high spatial resolution to resolve individual vessels and capillaries & high temporal resolution to measure transient blood flow changes in situations such as functional activation and spreading depressions. In addition the technique has to be quantitative to accurately measure changes in blood flow and characterize regions in the cortex based on this change. Finally, the technique has to be at the least, moderately invasive to enable long term studies. In addition to these factors, cost and ease of instrumentation are desirable.

Techniques like Magnetic Resonance Imaging (MRI) and Computed Tomography (CT) while being non invasive, are expensive to setup and have poor spatial resolution especially for animal experiments. Doppler Ultrasound (D-US) in spite of being affordable and non invasive is not suited for cerebral blood flow measurements since it cannot make measurements through the skull and requires the probe to be in contact with the sample. Optical techniques typically have good spatial resolution. Fluorescence measurements like Indo-Cyanine Green Angiography (ICG-A) are not quantitative and need exogenous contrast agents. Doppler based flow measurement techniques like Laser Doppler Imaging (LDI) and Doppler OCT (D-OCT) both can produce images at high resolution and are quantitative. However, their spatial resolution is achieved through scanning and hence at the expense of temporal resolution. Also, Doppler flow measurements are more applicable for large blood vessels, and these instruments cant image through the skull.

Laser Speckle Contrast Imaging (LSCI) has been widely used to image flow *in vivo* in a wide variety of applications [13, 19–27], primarily due to advantages like high spatial and temporal resolution, low cost and ease of instrumentation. Despite its popularity, LSCI instruments and the accompanying mathematical models have largely been unchanged since it was first introduced by Fercher and Briers [28] in 1981. Consequently, most of LSCI's limitations such as its inability to produce quantitative measures of blood flow and its inability to image through the skull are yet to be overcome. The first limitation has restricted LSCI's use in assessing models of severe ischemia and monitoring large hyperemia during CSDs. Typically the second limitation has been overcome by performing a craniotomy. While this is acceptable for short time measurements, retaining at least a part of the skull (in a thinned skull preparation) is preferable for long term studies [15]. LSCI cannot produce consistent flow measures in the presence of thinned skull. The purpose of this research is to improve the traditional LSCI instrument and speckle models and remove or diminish its disadvantages while retaining most of its advantages.

In Chapter 2, the basic principles behind blood flow measurement with Laser Speckle Contrast Imaging are explained. Chapter 3 introduces the new Multi Exposure Speckle Imaging (MESI) technique. The new technique involves using a MESI instrument in conjunction with a new mathematical model. The instrument helps obtain quantitative measures of blood flow, while the new physical model helps account for the influence of the thinned skull. The MESI technique is used to image flow in a micro-fluidic flow phantom and the blood flow changes associated with an ischemic stroke *in vivo* in a mouse in Chapter 4. In Chapter 5 the speckle autocorrelation function is measured *in vivo* to establish speckle physics on a firmer base. Finally, in Chapter 6 the use of LSCI to image blood flow during neuro-surgery is presented as a novel clinical application.

Chapter 2

Laser Speckle imaging of Cerebral Blood Flow

2.1 Introduction

Optical imaging techniques based on the phenomenon of light scattering combine high resolution imaging with the advantage of using intrinsic contrast agents. The study of fluctuations in the intensity of coherent light scattered from dynamic systems is Dynamic Light Scattering (DLS). DLS has been used extensively to estimate particle sizes, molecule concentrations and study chemical kinetics [29]. It also forms the basis for light scattering based flow measurement techniques including laser Doppler imaging and laser speckle imaging. When coherent light interacts with moving particles, the photons undergo a momentum change or a Doppler shift, which causes temporal fluctuations in the backscattered intensity. The speed of moving particles is inversely proportional to time scale of these temporal intensity fluctuations.

Figure 2.1 illustrates the basic concepts and principles involved with obtaining blood flow measurements using laser speckle imaging techniques. Briefly, in Laser Speckle Contrast Imaging (LSCI), coherent light that has backscattered from dynamic particles (red blood cells) is imaged onto a camera. The captured images are converted into a qualitative pseudo-blood flow measure called 'speckle contrast' (K) which is an estimate of the variance of instantaneous intensity. Mathematical models based on DLS principles are then used to relate speckle contrast to a more quantitative measure of blood flow called correlation time (τ_c). In fact, τ_c is the time domain analog of frequency shifts measured by laser Doppler imagers. Relating τ_c to the speed of the red blood cells (and hence blood flow) is very difficult due to the probabilistic nature of light tissue interaction. However, $1/\tau_c$ has been shown to be a reproducible measure of CBF (ml/100g/min) [24, 25]. Hence the ultimate goal of any DLS based blood flow measurement system is to obtain τ_c .



Figure 2.1: Blood flow measurement using laser speckle - an outline

2.2 Laser Speckle Contrast Imaging

Laser Speckle Contrast Imaging (LSCI) is a popular optical technique to image blood flow. It was introduced by Fercher and Briers [28] in 1981, and has since been used to image blood flow in the brain [13, 19–23], skin [30–32] and retina [33]. Unlike traditional flow measurement techniques like scanning Laser Doppler Imaging; being a full field imaging technique, LSCI's spatial resolution is not at the expense of scanning time. Hence by using a fast camera, temporal resolutions of up to 100 frames per second can be obtained. Images can then be averaged to produce better statistical estimates of blood flow. High spatial and temporal resolution, low cost and ease of instrumentation are some of the reasons why LSCI has been used to dynamically image cerebral blood flow (CBF) changes in stroke models, in the rat [13] and mouse brain [24, 25], functional activation studies [20, 26, 27] and in clinical studies [34]

Laser speckle is a random interference pattern produced by the coherent addition of scattered laser light with slightly different path lengths. When the scattered photons encounter moving particles in the sample such as red blood cells, they undergo a change in momentum causing phase shifts in the interfering photons. These changes in phase lead to temporal fluctuations in the speckle pattern. When imaged with a camera, these temporal fluctuations manifest themselves as localized blurring of the image as shown in Figure 2.2. Since the cerebral cortex can be considered an ergodic system |13, 28, 29|, the spatial and temporal fluctuations are equivalent and hence either can be used to detect the speed of the scatterers [28,35]. In laser Doppler, the temporal fluctuations are analyzed by estimating the distribution of frequency shifts from a distribution of photons detected at a point, to measure blood flow [6]. In LSCI, the localized spatial blurring is estimated by calculating a quantity called speckle contrast (K) over a small window (usually 7×7 pixels) of the image. Figure 2.2 illustrates the estimation of speckle contrast from raw speckle images.

$$K = \frac{\sigma_s}{\langle I \rangle},\tag{2.1}$$

where σ_s is the standard deviation and $\langle I \rangle$ is the ensemble average of intensities of the pixels in the window.



Figure 2.2: Estimating speckle contrast. Regions with low flow have greater temporal fluctuations and hence a higher speckle contrast value

The ergodic nature of the system also allows the speckle contrast to be calculated temporally, in which case, the 'window' used to compute the contrast is in time rather than space [36]. To distinguish between the two techniques, the former is referred to as speckle contrast, while the later is typically called temporal contrast. In either case, the speckle contrast is the square root of the normalized variance of intensities within the window. As shown in Figure 2.2, for slower speeds, the pixels decorrelate (blur) less and hence K is large and vice versa. Speckle contrast can vary from a minimum value of 0 to a maximum value of 1. However, instrumentation factors typically restrict the maximum measurable speckle contrast to an arbitrary value $\beta <$ 1 [1,27,37,38]. Section 2.3 describes the origins and the role β plays in LSCI in greater detail. Later in Sections 3.2 and 3.3, the importance of accurately measuring β is discussed.

2.2.1 Speckle Instrumentation

One of LSCI's strengths is its simple and cost efficient instrumentation setup. The traditional LSCI instrument consists of a camera, a lens and a laser. Fast 8 bit cameras are ideal for LSCI. As mentioned earlier, the high frame rate of these cameras enable increased averaging of speckle contrast images to obtain better statistical estimates of blood flow. Since speckle is an interference based technique, it requires illumination from a coherent laser source. While any single mode laser can be used, single mode diode lasers are preferred for their portability and ease of handling. Such diode lasers are also available at a wide variety of wavelengths and powers. The choice of wavelength depends on the application for which LSCI is used. Table 2.1 suggests some appropriate wavelengths suitable for applications in which LSCI is most commonly used.

Fig. 2.3 shows a schematic of the LSCI instrumentation setup. Slightly diverging light from a laser diode is incident on the sample. Diode lasers pro-

Application	Suggested	Comments	
	wavelengths		
CBF measure-	Red $(> 630 \text{nm})$	Away from Hemoglobin absorption	
ments (animals)	to Near IR	wavelengths	
Clinical CBF	Visible (> $630 <$	Avoid Hemoglobin absorption	
measurements	700nm)	wavelengths, remain in visible	
(humans)		region for ease of navigation	
Skin	Near IR	Increased penetration depth	
Retina	Red $(> 630 \text{nm})$	Away from Hemoglobin absorption	
	to Near IR	wavelengths	

Table 2.1: Some suggested wavelength choices for LSCI

vide the flexibility to vary the laser divergence and produce a diffuse beam using collimation optics. A diffuse illumination beam is an easy method to obtain uniform illumination over the sample. The back scattered light is collected through a lens and recorded on a camera. While using the LSCI setup, care should be taken to avoid saturating the camera. Saturation will artificially reduce the contrast and reduce LSCI's sensitivity to speed. Care should also be taken to ensure that the laser diode is driven at its operating current and hence is emitting coherent light. Ideally, the histogram of intensities of the raw speckle image should resemble a Gaussian function centered over the midpoint of the camera's dynamic range. This ensures that the dynamic range of the camera is fully utilized.

Raw speckle images are collected and stored in a computer, where they can be converted to (pseudo flow) speckle contrast images using Equation 2.1. Using mathematical models described in Section 2.3, the speckle contrast val-



Figure 2.3: Schematic of Laser Speckle Contrast Imaging instrument

ues can be converted to τ_c values. While typically most computations are not real time, recent advances in computer and programming technologies have enabled real time computation and display of speckle contrast and correlation time images at high resolutions [39, 40].

2.3 Speckle models

Speckle contrast (K) provides qualitative information about blood flow. However, a more quantitative measure of flow is preferred, especially if such information is to be used for stroke models. The influence of speed of scattering particles on speckle contrast can be described using the theory of dynamic light scattering (DLS) [29]. LSCI differs from DLS in its use of a camera to image speckle intensity fluctuations. Cameras are not fast enough to sample the instantaneous intensity fluctuations of the speckles. Hence they temporally integrate the intensity at the pixels over the exposure duration. The speckle contrast is hence expressed in terms of the correlation time of speckles and the exposure duration of the camera [1,28]. The correlation time of speckles is the characteristic decay time of the speckle electric field autocorrelation function.

2.3.1 Basic Principles

In typical Dynamic light scattering measurements, the scattered light is collected using a point detector such as a photo multiplier tube or a photo diode. The fluctuations in the intensity of the light due to photons interacting with moving particles is a measure of blood flow. These fluctuations are estimated by computing the autocorrelation function of the intensity, $g_2(\tau)$ as expressed in Equation 2.2 [1,29].

$$g_2(\tau) = \frac{\langle I(t)I(t+\tau)\rangle}{\langle I\rangle^2}$$
(2.2)

The dynamics of the system can only be expressed in terms of the electric field autocorrelation function $g_1(\tau)$ (Equation 2.3). The correlation time τ_c , is the characteristic decay time of the electric field correlation function $g_1(\tau)$, and is a measure of the dynamics of the system; which in the case of LSCI is blood flow.

$$g_1(\tau) = \frac{|\langle E(t)E^*(t+\tau)\rangle|}{\langle E(t)E^*(t)\rangle}$$
(2.3)

Since sensors are square law detectors, they can only measure the intensity and subsequently only $g_2(\tau)$. The intensity autocorrelation function, $g_2(\tau)$ is related to the electric field autocorrelation function, $g_1(\tau)$ through the Siegert Relation (Equation 2.4).

$$g_2(\tau) = 1 + |g_1(\tau)|^2 \tag{2.4}$$

In DLS measurements, an appropriate form is assumed for $g_1(\tau)$ depending on dynamics of the system. The corresponding $g_2(\tau)$ is computed using Equation 2.4 and experimentally obtained intensity auto correlation function is fit to this form to estimate τ_c [1,29]. The limiting factor in the accuracy of flow measurements using DLS, LSCI or laser Doppler, is hence the choice of the form of electric field autocorrelation function $g_1(\tau)$.

Table 2.2 lists some commonly used expressions for $g_1(\tau)$ and the corresponding dynamics they represent. Clearly, the choice of the appropriate expression depends not only on dynamics of the sample but also the nature of light scattering in the sample. DLS and LSCI measurements are typically used in the single scattering regime i.e. the photons are assumed to have scattered off only one moving particle. For blood flow measurements, the most common expression of $g_1(\tau)$ is a negative exponential, corresponding to a Lorentzian distribution of velocities in the sample volume.

$$g_1(\tau) = \exp(-\tau/\tau_c) \tag{2.5}$$

This expression was first proposed [41] and subsequently verified [1, 42] by Jakeman, Pike and others. Equation 2.5 has been used in multiple LSCI studies beginning with the first study by Fercher and Briers [28] to more

Scattering regime	Sample Dynamics	$g_1(x)$ where $x = \tau / \tau_c$
Single scattering	Diffusive dynamics	$\exp(-x)$
Single scattering	Ballistic dynamics	$\exp(-x^2)$
Diffuse scattering	Ballistic dynamics	$\exp(-\sqrt{x})$

Table 2.2: Sample dynamics and their corresponding electric field autocorrelation functions [1]

recent and popular applications [13, 20]. Chapter 5 examines some of these expressions and provides justifications for the appropriate choice.

The relationship between correlation time (τ_c) and velocity of scatterers (v) is difficult to obtain, due to the probabilistic nature of light scattering. Nevertheless, calibrated measurements of velocity can be made if τ_c can be accurately estimated. Researchers have shown that $1/\tau_c$ (in arbitrary units) correlate well with CBF (ml/100g/min) [24,25]. Under certain condutions, the correlation time of the speckles can be assumed to be inversely proportional to the speed of the scatterers [6],

$$\tau_c \propto \frac{1}{v} \tag{2.6}$$

Strictly speaking this assumption is appropriate only 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 [6]. This is consistent with the assumption that LSCI and DLS measurements occur in the single scattering regime (Table 2.2 and Equation 2.5). The ability of LSCI instruments to obtain quantitative flow measurements is limited by the accuracy of the instrument and that of the model in estimating τ_c .

2.3.2 The Briers Expression

Fercher and Briers [28] developed the first speckle model in 1981, using basic DLS principles. As described earlier (Section 2.3.1), the fundamental difference between DLS measurements and LSCI is that the latter uses cameras to record speckle intensity fluctuations. Since cameras cannot sample these fluctuations as fast as a point detector, the instantaneous intensity is integrated over the exposure duration of the camera (T). The speckle contrast is hence expressed as a function of the camera exposure duration T and the intensity autocorrelation function $g_2(\tau)$ as [1, 28, 29, 43]:

$$K^{2} = \int_{0}^{T} g_{2}(\tau) d\tau$$
 (2.7)

Using Equation 2.5 with the Siegert relation (Equation 2.4) and substituting the resulting expression for $g_2(\tau)$ in Equation 2.7, Fercher and Briers derived the first speckle expression;

$$K(T,\tau_c) = \left(\frac{1 - e^{-2x}}{2x}\right)^{1/2},$$
(2.8)

where $x = T/\tau_c$, T is the exposure duration of the camera and τ_c is the correlation time.

Equation 2.8 has since been extensively used for many LSCI applications [13, 15, 17, 19–25]. Recently, Bandyopadhyay [1] pointed out a significant mathematical error in Equation 2.8 and derived an updated expression (Equation 2.13). The use of this approximate model (Equation 2.8) is one of the reasons for the lack of quantitative accuracy of τ_c measurements using LSCI [1]. While the Briers model has mathematical inconsistencies, it is still important to recognize that this is the first expression that related speckle contrast to correlation time. Further, the differences between the two models are insignificant if the exposure durations used are sufficiently long [37, 43].

2.3.3 The Bandyopadhyay Expression

Bandyopadhyay et. al. [1] recognized two significant deficiencies with the Briers expression (Equation 2.8). One, the Briers expression ignores speckle averaging effects; and two, it did not account for triangular weighting of the speckle autocorrelation function while relating $g_2(\tau)$ and K.

The speckle averaging effect arises from a mismatch between the size of the image of single speckle and that of the the sensor. When the average size of the image of a singe speckle is smaller than that of a camera pixel,more than one speckle is imaged onto a single pixel and, the intensity recorded by the pixel becomes a spatial average of intensities from these speckles. This leads to a condition where the speckle pattern is not fully evolved and the maximum observable speckle contrast is reduced by a factor β . Yuan et. al. [27] showed that the size of the image of a single speckle (d) can be expressed in terms of the magnification of the imaging system (M), the wavelength of light used (λ), and the f-number of the imaging lens (f/#),

$$d = 2.44\lambda f / \# M, \tag{2.9}$$

In addition to the mismatch between the pixel size of the camera and speckle size (d), the polarization of the light source, imaging geometry and coherence

fluctuations of the laser can reduce the speckle decorrelation further, affecting the value of β [44]. In their first speckle expression, Fercher and Briers assumed that $\beta = 1$.

To make this assumption valid, researchers have suggested matching the size of the image of a single speckle to the size of a pixel, by limiting the aperture of the detection optics [13,27]. However, this prevents high magnification imaging [17] and is difficult to implement accurately, leading to inconsistencies in τ_c estimates. Hence it is important to account for the speckle averaging effect in the model and estimate it *in situ* [1]. Bandyopadhyay et. al. [1] utilized an unnormalized version of the Siegert equation [44] (Equation 2.10) to account for speckle averaging effects.

$$g_2(\tau) = 1 + \beta |g_1(\tau)|^2$$
(2.10)

The second inconsistency in the Briers equation is the absence of triangular weighting of the autocorrelation function. Bandyopadhyay et. al. [1] accounted for this by expressing the second moment of the speckle intensities as,

$$v_2(T) = K^2 = \int_0^T \int_0^T \left(g_2(t' - t'') - 1\right) dt' dt'' / T^2.$$
 (2.11)

Then, using Equation 2.10, Bandyopadhyay et. al. obtained a new speckle visibility expression:

$$K^{2}(T) = 2\beta \int_{0}^{T} \frac{1}{T} \left(1 - \frac{t}{T}\right) \left[g_{1}(t)\right]^{2} dt \qquad (2.12)$$

The terms which weight the electric field autocorrelation function, $(1 - \frac{t}{T})$, were missing in the original Briers equation. When $T \to \infty$, these weighting
terms reduce to 1, confirming that at sufficiently high exposure durations, the Briers and Bandyopadhyay expressions would converge. By substituting for $g_1(t)$ from Equation 2.5 in Equation 2.12, Bandyopadhyay et. al. derived their new speckle expression:

$$K(T,\tau_c) = \left(\beta \frac{e^{-2x} - 1 + 2x}{2x^2}\right)^{1/2},$$
(2.13)

where β is an instrumentation factor that arises due to speckle averaging, $x = T/\tau_c$, T is the exposure duration of the camera and τ_c is the correlation time.

The Bandyopadhyay expression (Equation 2.13) is a physically and mathematically robust expression to relate the experimentally obtained speckle contrast values to τ_c the correlation time. However, its accuracy at quantitatively estimating τ_c is limited by the accuracy of estimating β . In typical DLS measurements, β is estimated *in situ* by extrapolating the experimentally computed $g_2(\tau)$ to 0, and using the intercept to measure β ($g_2(0) = 1 + \beta$) [1]. The traditional LSCI instrument (Figure 2.3) is incapable of this measurement because the average intensity must be maintained while reducing the exposure duration of the camera to a value close to 0. Hence, the inability of the LSCI instrument to measure experimental noise has prevented LSCI instruments from obtaining quantitative measures of τ_c even with the Bandyopadhyay expression. Furthermore, both expressions (Equations. 2.8 and 2.13) assume that the backscattered light is ergodic and has interacted with only moving scatterers [44]. This assumption is violated when imaging blood vessels in a static background like skin, or through a layer of static tissue, like imaging cerebral blood vessels through a thinned skull. This limitation can only be fixed by updating the mathematical models to account for the effect of light scattered from static tissue elements. Hence there is a need to improve existing instrumentation and theory to move towards quantitative imaging. In Sections 3.2 and 3.3, we introduce a new speckle model and a new Multi Exposure Speckle Imaging instrument that can overcome these limitations and obtain consistent τ_c measures in the presence of static scatterers.

Chapter 3

The Multi Exposure Speckle Imaging Technique

The Multi Exposure Speckle Imaging (MESI) technique is an optical technique used to measure blood flow. It is a full field imaging technique based on the principles of dynamic light scattering, and works by acquiring backscattered laser speckle images at multiple camera exposure durations. Experimental data obtained from these images are then fit to a mathematical model to produce estimates of blood flow. The primary advantages of the MESI technique are:

- 1. The ability to quantitatively estimate large changes in blood flow, and
- 2. The ability to estimate blood flow changes consistently in the presence of static scatterers

The Multi Exposure Speckle Imaging (MESI) technique comprises of a new instrument to obtain speckle images at multiple exposure durations and a new mathematical model. This chapter provides a description of the Multi Exposure Speckle Imaging instrument (Section 3.3) and derives the new mathematical model (Section 3.2).

3.1 Motivation

The primary motivation behind the development of the Multi Exposure Speckle Imaging (MESI) technique is a need to image cerebral blood flow (CBF) quantitatively at high spatial and temporal resolutions. Optical imaging techniques based on the phenomenon of light scattering combine high resolution imaging with the advantage of using intrinsic contrast agents. Laser Speckle Contrast Imaging (LSCI) is one such technique that has been widely used to image cerebral blood flow for a variety of applications [13, 15, 17, 20, 24, 26]. LSCI's spatial resolution enables imaging the cortical micro vasculature in small animals like mice [45], while its high temporal resolution enables imaging dynamic flow changes due to an ischemic stroke [13], spreading depressions [17], ischemic depolarizations [46] and functional activation [26].

As described in the Chapter 2, traditional LSCI instruments provide good measures of relative flow, but fall short of providing quantitative baseline values. Additionally, they have been shown to underestimate large changes of flow [38], and do not produce reliable flow measurements in the presence of static scatterers [38] such as thinned skull [45]. The MESI technique is aimed at removing these limitations.

The ability to obtain baseline measures of blood flow is important for comparing CBF estimates from multiple animals and from multiple studies. In applications like ischemic strokes, the ability to quantitatively measure large CBF decreases is crucial in developing better disease models and treatment strategies. For chronic and long term studies, it is desirable to image CBF through an intact (but thinned) skull [45], and hence there is a need to obtain CBF measures that are unaffected by the presence of a thinned skull.

3.1.1 Cortical Spreading Depression and Ischemic stroke

Cortical Spreading Depression (CSD), a wave of negative potential that propagates across the cortex at a rate of a few mm/min, was first identified in rabbits by Leao [47] in 1944. They have been studied extensively, and their role in the pathophysiology of stroke, migraine and sub arachnoid hemorrhage is well documented [18, 48]. A CSD is triggered by an increase in extracellular K^+ , and can be induced in a normal brain by localized injury such as a pinprick. After being induced, the CSD propagates across the cortex and is associated with large transient changes in DC potential. A short duration after being depolarized, the cerebral neurons begin to re-polarize. The increase in the metabolic demand of these re-polarizing cells cause the CSD to be accompanied by a large transient increase in blood flow (hyperemia). This increase in blood flow is referred to as the hemodynamic response of the CSD. The hemodynamic response enables us to monitor the progression of the CSD via optical methods. In a normally perfused brain, a CSD is considered harmless [49], since the brain is almost always able to meet the increased demand for blood and oxygen. However a brain with significantly reduced blood flow, such as during hypoxia or ischemia, cannot meet the increased metabolic demand placed by the repolarizing neurons. As a result, in a diseased brain, the CSD can lead to a worsening of the hypoxic conditions leading to permanent damage of the cortex [50–52]. Other clinical studies have also emphasized the importance of CSDs in the human brain [53–55]. Since the hemodynamic response is an indirect method of monitoring the CSD, it is crucial that this response be accurately measured. Hemodynamic studies of CSDs in normal brains of rats and mice report a transient hyperemia (increase in blood flow) of up to 300% of baseline blood flow levels [56, 57], while some other studies report that a brief hypoperfusion preceeds this hyperemia [17, 58]. Laser Speckle Contrast Imaging could underestimate this large change. Measuring these large changes in blood flow due to CSDs is one of the motivations for the development of the MESI technique.

In focal cerebral ischemia, blood flow is reduced in a localized region of the brain leading to tissue damage. A stroke of this kind can be modeled as having three regions; a core, a penumbra and healthy tissue [16]. These regions are identified based on the extent of reduction in blood flow compared to preischemic baselines values. The ischemic core, which can be identified as those regions in the cortex where the blood flow is less than 20% of baseline values, is characterized by unsalvagable dead tissue [13,16]. The penumbra region, where the reduction in blood flow is not as severe as in the core, is characterized by electrically silent neurons which retain the ability to maintain ion homeostasis. Since the reduction in blood flow is not severe in the penumbra, these neurons are considered salvagable with the right treatment. The first step towards developing stroke models is the proper identification of the core and penumbra regions. Since these regions are identified based on the extent of decrease in blood flow, it is crucial to measure these changes accurately. The need to measure the blood flow changes quantitatively and resolve these changes spatially is another motivation for the development of the MESI technique.

The reduction in blood flow in the penumbra, if untreated, can lead to secondary effects like peri-infract depolarizations (PIDs) and inflammations leading to cell death [59]. For these reasons, the penumbra has been the target of many stroke treatment strategies [15], and restoration of blood flow is a key indicator of return to normalcy of tissue. The aforementioned peri-infract depolarizations (PIDs) are one of the major mechanisms for the progression of stroke from the core into the penumbra. The PIDs in a diseased brain resemble CSDs in a normal brain and like CSDs, can be triggered by increased extracellular K⁺. A detailed mechanism of the effect of PIDs on stroke progression was given by Hossmann |59|. Studies |46| have shown that the hemodynamic response of different ischemic territories to PIDs varied, suggesting that PIDs are an extension of CSDs, when baseline oxygenation is reduced. Models of ischemic stroke and CSDs are hence possibly interlinked through baseline oxygenation. Sonn et. al. [50] showed that baseline blood oxygenation levels prior to a CSD can significantly impact the ensuing hemodynamic response. The inability to quantify baseline blood flow has prevented the development of a complete physiological model of the progression of CSDs and consequently of PIDs. Specifically, the effect of baseline blood flow on CSD is yet to be determined. Further it is unclear if a linear relationship exists between brain

oxygenation prior to CSDs and the amplitude of the hemodynamic response to CSDs. As described in Section 2.3.3, the primary reason for the inability of LSCI to obtain quantitative blood flow measurements, is its inability to estimate instrumentation constants in its models, specifically β in Equation 2.13. The motivation for the development of a new instrument (Section 3.3) is the need to fix this limitation, so quantitative baseline measures can be obtained.

In order to develop appropriate treatment strategies to 'reclaim' the penumbra, an accurate model of stroke progression due to PIDs has to be evaluated. Again, lack of baseline flow measurement techniques have hindered the investigation of the relationship between baseline blood oxygenation levels and the hemodynamic response of PIDs. Further, in order to evaluate any treatment strategy like hyperoxia [15, 60], one would inevitably need a large sample of test subjects. Quantitative comparisons across many subjects cannot be performed without analyzing baseline flow levels. This need for quantitative measurements has also been indicated in other studies. Shin et. al. [15] remarked that the type and amount of anesthesia used during surgery influences the resting CBF. Shin et. al. [15] also commented on the need for quantitative blood flow measurements in comparing CBFs across animals belonging to different age groups. Conclusions from studies related to CBF changes would be more complete and comparable when quantitative CBF measurements are used.

From these arguments we can summarize that the motivation behind the development of MESI technique is two fold. One, to design and develop a technique to accurately measure large changes in blood flow *in vivo*, and two, to develop a technique that can obtain baseline measures of blood flow *in vivo*. In Section 3.3 we describe the design of the MESI instrument, discuss its functioning and capabilities. The MESI technique involves using the MESI instrument in conjunction with a new speckle model described in Section 3.2.

3.1.2 Imaging through static tissue

One of the limitations of using LSCI to image blood flow is its inability to accurately predict blood flow in the presence of static tissue elements. In applications like imaging blood flow in the skin or retina, the presence of static tissue has prevented accurate and quantitative measurement of blood flow. For imaging CBF, the skull is the primary static tissue which affects measurements. For this reason, imaging CBF in vivo in animals usually involves a full craniotomy procedure. Ayata et. al. [24] pointed out many benefits of avoiding a full craniotomy and using a thinned skull preparation instead. Using a thinned skull preparation for imaging eliminates motion artifacts due to cortical swelling and prevents herniation during hyperemic responses. Having the skull intact reduces brain temperature fluctuations leading to a more stable baseline CBF. In addition, leaving the skill intact reduces surgical trauma. This has multiple advantages. Reduced trauma implies that it is easier to keep the animal alive for a longer time and places lesser stress on the subject leading to more stable blood flow conditions. Most importantly, reduced trauma implies that a lower amount of anesthesia is required, further stabilizing baseline CBF.

This inability to estimate blood flow in the presence of static scatterers is one that is common to both speckle and Doppler flow measurement systems. Removing this limitations is one of the primary motivations behind the development of the MESI technique. The new mathematical model 3.2 developed as part of the MESI technique is designed to account for the presence of static scatterers such as the thin skull, and still produce consistent and accurate measurements of changes in blood flow.

3.2 A Robust Speckle model

Sections 2.3.2 and 2.3.3 described the development of two mathematical models that have been used to express speckle contrast K, as a function of correlation time τ_c and exposure duration T. The primary limitation of both the Briers [28] (Equation 2.8) and Bandyopadhyay [1] (Equation 2.13) models is that they do not account for the light scattered from static tissue elements like thin skull. This is primarily because these models rely on the Siegert relation (Equation 2.10) which assumes that the speckles follow Gaussian statistics in time. In the presence of static scatterers, the fluctuations of the scattered field remain Gaussian but the field acquires an extra static contribution causing the recorded intensity to deviate from Gaussian statistics. Hence Equation 2.10 cannot be applied [44, 61] to situations where the scattered speckle intensity arises from a mixture of static and dynamic particles. This can be corrected by modeling the scattered field [44, 61] as

$$E_h(t) = E(t) + E_s e^{-i\omega_0 t},$$
 (3.1)

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 [44, 61]

$$g_{2}^{h}(\tau) = 1 + \frac{\beta}{\left(I_{f} + I_{s}\right)^{2}} \left[I_{f}^{2} |g_{1}(\tau)|^{2} + 2I_{f}I_{s} |g_{1}(\tau)|\right]$$

= 1 + A\beta |g_{1}(\tau)|^{2} + B\beta |g_{1}(\tau)|, (3.2)

where $A = \frac{I_f^2}{(I_f + I_s)^2}$ and $B = \frac{2I_f I_s}{(I_f + I_s)^2}$, $I_s = E_s E_s^*$ represents contribution from the static scattered light, $I_f = \langle EE^* \rangle$ represents contribution from the dynamically scattered light and β is a normalization factor that accounts for speckle averaging effects.

This updated Siegert relation (also called the heterodyne Siegert relation) can be used to derive the relation between speckle variance and correlation time as with the other models [1,62]. Following the approach of Bandyopadhyay et. al. [1] the second moment of intensity can be written using the modified Siegert relation as

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

$$(3.3)$$

The reduced second moment of intensity or the variance is hence

$$v_2(T) \equiv \int_0^T \int_0^T \left[A\beta \left(g_1(t' - t'') \right)^2 + B\beta g_1(t' - t'') \right] dt' dt'' / T^2.$$
(3.4)

Since $g_1(t)$ is an even function, the double integral simplifies to [62]

$$v_2(T) = A\beta \int_0^T 2\left(1 - \frac{t}{T}\right) [g_1(t)]^2 \frac{dt}{T} + B\beta \int_0^T 2\left(1 - \frac{t}{T}\right) [g_1(t)] \frac{dt}{T}.$$
 (3.5)

Equation 3.5 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 [28], which gives $g_1(t) = e^{-t/\tau_c}$ and recognizing that the square root of the variance is the speckle contrast [1], Equation 3.5 can be simplified to:

$$K(T,\tau_c) = \left\{\beta\rho^2 \frac{e^{-2x} - 1 + 2x}{2x^2} + 4\beta\rho(1-\rho)\frac{e^{-x} - 1 + x}{x^2}\right\}^{1/2}, \qquad (3.6)$$

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, $I_s = 0$, $\rho \to 1$ and Equation 3.6 simplifies to Equation 2.13. However Equation 3.6 is incomplete since in the limit that only static scatterers are present ($I_f = 0$ and $\rho \to 0$), 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 3.6 refers to the temporal (temporally sampled) speckle contrast, as described in section 2.2. The initial definition of K (Equation 2.1) 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 [28]. In the presence of static scatterers this assumption is no longer valid [61]. However it is still preferable to use spatial (ensemble sampled) speckle contrast since it helps retain the high temporal resolution of LSCI. To use Equation 3.6 with spatial (ensemble sampled) speckle contrast, a constant term is added to the speckle visibility expression (Equation 3.5). This constant is referred to as *nonergodic variance* (v_{ne}) .

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 3.6. However the spatial (or ensemble) speckle contrast would be a nonzero constant due to spatial averaging of the random interference pattern produced. This nonzero constant (v_{ne}) would primarily be determined by the sample, illumination and imaging geometries. Since the speckle contrast is normalized by the average intensity, v_{ne} would be independent of intensity. These factors are clearly independent of the exposure duration of the camera, and hence the assumption that v_{ne} is a constant is valid. These assumptions are further investigated and shown to be acceptable experimentally in Section 3.4.2. The addition of v_{ne} should enable the use of spatial (or ensemble) speckle contrast in the presence of static scatterers. The addition of the nonergodic variance is a significant improvement over existing models.

An additional factor that has been neglected in previous models (Equations 2.8 and 2.13) 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 more significant source of noise, and it is primarily determined by the average intensity 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. This can be made independent of exposure by holding the camera exposure duration constant. Section 3.3 presents an instrument that holds camera exposure duration constant, yet obtains speckle images at multiple exposure durations by pulsing the laser and hence maintaining the same average 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 3.6 can be rewritten as:

$$K(T,\tau_c) = \left\{\beta\rho^2 \frac{e^{-2x} - 1 + 2x}{2x^2} + 4\beta\rho(1-\rho) \frac{e^{-x} - 1 + x}{x^2} + v_{ne} + v_{noise}\right\}^{1/2},$$
(3.7)

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 3.7 is a rigorous and practical robust 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. For these reasons, these factors can be combined to a single static spatial variance v_s , where $v_s = v_{ne} + v_{noise}$.

3.3 The Multi Exposure Speckle Imaging Instrument

The objective for developing a new speckle imaging instrument is the need to acquire speckle images that will help obtain quantitative values of correlation times (τ_c), by fitting data extracted from these images to Equation 3.7. The MESI instrument should hence have the capability to obtain speckle images at multiple camera exposure durations. Additionally, as discussed in section 3.2, to ensure that the noise variance, v_s is constant over exposure duration, a constant average intensity should be maintained over a wide range of exposures.

The easiest method to obtain speckle images at multiple exposure durations would be to vary the camera integration time either using hardware or software triggers. However, by doing so the noise variance, v_{noise} would be dependent on the camera exposure duration. More importantly the average intensity would not be constant over different camera exposure durations and hence shot noise variance would change with camera exposure duration. At sufficiently long exposure durations, the average intensity and hence the shot noise overwhelm the speckle signal. The alternative to this method is to hold the camera exposure constant and pulse the laser instead [27]. By doing so, the camera noise variance can be held constant, and by appropriately modulating the intensity of the laser, the average intensity of the image and hence shot noise variance can be held constant. Directly pulsing the laser however suffers from bandwidth limitations, i.e. very short pulses cannot be effectively generated. More importantly at large exposure durations, when the intensity of the laser has to be very low (Figure 3.2), there is a possibility of the laser falling below its lasing threshold. Under such conditions no speckle signal would be generated. Therefore directly pulsing the laser poses limitations on the smallest and largest exposure durations that can be generated.

The limitations posed by direct pulsing of the laser can be overcome by using an acousto-optic Modulator (AOM). An AOM is a device that can be used to modulate and deflect light. It is a passive device that has two transparent apertures through which light can enter and exit. It is activated by using a radio frequency (RF) wave, which is delivered to the device through electrical connections. The RF wave is converted into a pressure wave in the device, which in turn acts as a diffraction gating, effectively deflecting the light. By modulating the amplitude of the RF wave, the intensity of the first diffraction order can be varied. In the MESI instrument (Figure 3.1), this concept was used to pulse the laser through the AOM.

The Multi Exposure Speckle Imaging (MESI) instrument is shown in Figure 3.1a. Speckle images at different camera exposure durations were acquired by triggering a camera (Basler 602f, Basler Vision Technologies, Germany) and simultaneously pulsing a laser diode ($\lambda = 660$ nm, 95mW, Micro laser Systems Inc., Garden Grove, CA, USA) using an acousto-optic modu-



Figure 3.1: Multi-Exposure Speckle Imaging instrument (a) Schematic (b) Speckle Contrast image at 0.1 ms exposure duration (c) Speckle Contrast image at 5 ms exposure duration (d) Speckle Contrast image at 40 ms exposure duration (scale bar = 50 μ m)

lator. The acousto-optic modulator was driven using an amplified radio frequency (RF) wave that was generated using a high frequency function generator, and then amplified using an RF power amplifier. The electrical power output was optimized to match the maximum electrical power that can be delivered to the AOM. To control the amplitude of the diffraction grating in the AOM, the amplitude of the input RF wave to the AOM was varied by amplitude modulating the RF wave at the function generator. The modulating voltage was generated by the computer and output to the function generator. This modulating voltage was a low frequency square wave, whose amplitude determines the strength of the diffraction order, and width determines the duration of the pulse. The first diffraction order was directed towards the sample, and the backscattered light was collected by a microscope objective (4X or 10X) and imaged onto the camera through a 150mm tube lens. Software was written to control the timing of the AOM pulsing and synchronize it with image acquisition. Using the MESI instrument, field of views of upto $\sim 800 \times 500 \mu \text{m}$ were obtained. Laser speckle images from, 15 exposure durations ranging from $50 \mu \text{s}$ to 80ms were used to compile 1 MESI frame.



Figure 3.2: MESI instrument control signals. Solid lines represent control signals for modulating the AOM, while the dashed lines are the control signals for camera exposure. The two signals are synchronized in time.

Figure 3.2 shows the camera exposure control waveforms and the AOM

control voltages that were generated by the computer, for one MESI set (15 exposures). The solid red lines represent the control voltages used to drive the AOM. The width of each pulse is equal to the desired exposure duration, while its amplitude is varied such that the area under pulses (and hence the pulse energy) are equalized. Hence, the lowest exposure duration is associated with maximum modulation, i.e. the amplitude of the RF wave is not attenuated. These control signals are synchronized in time with the control signals for the camera exposure, illustrated in Figure 3.2 using dashed lines. Since the exposure time of the camera is held fixed, each camera exposure is 80ms long, which is the longest exposure duration that is measured.

From Figure 3.2 a lot of dead time can be observed during the lower exposure durations. For example, in the 50μ s exposure, the camera is acquiring and collecting useful data for only 50μ s of its 80ms exposure. While this long camera exposure is required to hold v_{noise} constant, higher temporal resolutions can be obtained by minimizing this dead time. The trade off in this case would be that v_{noise} would no longer be independent of exposure. However, this can be acceptable because v_{noise} is typically dominated by shot noise. Figure 3.3 illustrates an alternative triggering mechanism called 'strobe triggering'. Here, both the camera exposure and the modulation signals have the same pulse width, and are synchronized in time. Hence, the camera is exposed only when it is collecting light, and dead time is minimized. This triggering technique is almost two times faster and is useful for *in vivo* applications. Using the strobe triggering mode a temporal resolution of ~ 1.5s per MESI frame was obtained.



Figure 3.3: MESI instrument control signals - Strobe triggering mode. Solid lines represent control signals for modulating the AOM, while the dashed lines are the control signals for camera exposure. The two signals are synchronized in time. Data acquisition using strobe triggering is more than two times faster.

Aside from synchronizing all signals in time, estimating the actual values of modulation control signals through a calibration procedure is important for the proper functioning of the MESI instrument. It is this calibration procedure that ensures that shot noise variance is constant across all exposure durations. Since reflectivity of the surface varies with the sample being imaged, this calibration procedure has to be performed before every new MESI experiment. In order to properly calibrate the values of the modulation control signals, the following steps were followed:

- 1. Reference measurement using a reflectance standard: A standard 20% reflectance standard was imaged using the MESI optical setup (Figure 3.1), with the sensor of a power meter at the image plane. The power was measured as a function of increasing modulation voltage and stored as a reference calibration file. This step needs was done only once to generate the reference calibration file.
- 2. Initial guess of modulation control values: An initial guess of the modulation control values was obtained by equalizing the energy in each pulse. The energy in each pulse would be the maximum possible modulation voltage multiplied by the minimum exposure duration. Guesses for the modulation control voltage for the remaining exposure durations were obtained by dividing this energy value by the required exposure duration.
- 3. Adjusting modulation control values *in situ*: During each experiment, a set of 15 raw speckle frames were obtained using the initial guesses for modulation control voltages. The average intensity in these images were calculated. The average intensity at the lowest exposure duration was used as a reference and the recorded intensities at the other exposure durations were compared to this reference. The modulation control voltages for these exposures were then changed based on the results of the comparison. This process was repeated recursively, till the intensities at all exposure durations were found to vary by less than 2%.

3.4 Preliminary model validation

In order to validate the new speckle model (Equation 3.7), controlled flow measurements were made on micro-fluidic flow phantoms. These controlled flow measurements helped verify the assumptions on non ergodicity that were made in deriving Equation 3.7. Also, since the speed of the scatterers can be precisely controlled, large flow changes can be simulated and flow estimates obtained using the MESI technique can be compared to true values.

3.4.1 Microfluidic phantoms



Figure 3.4: Cross-section of microfluidic flow phantom (not to scale) (a) Without Static scattering layer, (b) With Static scattering layer. Samples were imaged from the top

A microfluidic device as a flow phantom has the advantage of being a realistic, cost effective and flexible device that has a large shelf life and enables robust operation. The micro-fluidic devices used in these experiments were fabricated by the Zhang lab in the Department of Biomedical Engineering at The University of Texas at Austin. These flow phantoms, consisted of a single rectangular channel (300 μ m wide x 150 μ m deep) through which fluid can be pumped in a static background. They were fabricated in poly dimethyl siloxane (PDMS) using the rapid prototyping technique [63]. Briefly, a photo mask is made based on the design of the microfluidic device. This photo mask is used to etch the channels on a silicon wafer using UV light. The silicon wafer acts as a die using which microfluidic devices can be cast and fabricated in PDMS. Titanium dioxide (TiO₂) was added to the PDMS [64] (1.8 mg of TiO₂ per gram of PDMS) to give the sample a scattering background and mimic tissue optical properties. The prepared samples were bonded on a glass slide to seal the channels as shown in Figure 3.4. The sample was connected to a mechanical syringe pump (World Precision Instruments, Saratosa, FL, USA) through silicone tubes, and a suspension ($\mu_s = 250 \text{ cm}^{-1}$) of 1 μ m diameter polystyrene beads (Duke Scientific Corp., Palo Alto, CA, USA) was pumped through them.

To simulate a superficial layer of static scattering such as a thinned skull, a 200 μ m layer of PDMS with different concentrations of TiO₂ (0.9 mg and 1.8 mg of TiO₂ 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, (Figure 3.4b). The reduced scattering coefficients of the 200 μ m static scattering layer was estimated using an approximate collimated transmission measurement through a thin section of the sample. A collimated laser beam ($\lambda = 635$ nm) was directed through an optically thin section of the static scattering layer, of known thickness. The laser was adjusted to be normally incident on the sample, and the power of the laser in front (I_i) of and behind (I_t) the sample was measured. If the sample is assumed to not be absorbing, the loss in laser power due to transmission through the sample would be due to scattering alone. The reduced scattering coefficient μ'_s , can then be calculated using Equation 3.8,

$$I_{t} = I_{i}e^{-\mu'_{s}t}$$

$$ln\left(\frac{I_{t}}{I_{i}}\right) = -\mu'_{s}t$$

$$\mu'_{s} = -\frac{1}{t}ln\left(\frac{I_{t}}{I_{i}}\right)$$
(3.8)

Figures 3.5 and 3.6 show pictures of the 'clear' (without background scattering) and the realistic (with background scattering) phantoms respectively.



Figure 3.5: Picture of 'clear' microfluidic flow phantom showing flow channels

3.4.2 Validation Experiments

The experimental setup (Figure 3.1) was used in conjunction with Equation 3.7 to perform controlled experiments on the microfluidic samples.



Figure 3.6: Picture of microfluidic flow phantom with flow channels. TiO_2 has been added to mimic tissue optical properties

The microfluidic sample without the static scattering layer (Figure 3.4a) was used to test the basic functioning of the multi-exposure speckle imaging instrument (MESI) and the mathematical model (Equation 3.7). The suspension of micro spheres 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 raw speckle images were obtained at each of 15 different exposure durations, ranging from 50μ s to 80ms. 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. The speckle variance (K^2) in this region was then plotted as a function camera exposure duration for different speeds. These data points are shown in Figure 3.7. The dependence of speckle contrast on exposure duration can be clearly seen in Figure 3.7. The solid lines in Figure 3.7 are the fits of the



Figure 3.7: Multi-Exposure Speckle Contrast data fit to new speckle model. Speckle variance as a function of exposure duration for different speeds. Measurements were made on samples with no static scattering layer (Figure 3.4a)

experimental data to Equation 3.7.

In this fully dynamic case, the static spatial variance v_s is very small. It would be dominated by the experimental noise v_{noise} , since in this situation, the ergodicity assumption would be valid and $v_{ne} \approx 0$. β is one of the unknown quantities in Equation 3.7 describing speckle contrast. Theoretically, β is a constant that depends only on experimental conditions. An attempt to estimate β using a reflectance standard however would yield inaccurate results due to the presence of the static spatial variance v_s . 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} . Instead the value of β was estimated, by performing an initial fit of the multi-exposure data to Equation 2.13 with the addition of v_s , while having β , τ_c and v_s as the fitting parameters. The speckle contrast data was then fit to Equation 3.7 using the estimated value of β and these fits are shown in Figure 3.7. Figure 3.7 clearly shows that the model fits the experimental data well (mean sum squared error: 2.4×10^{-6}). The correlation time of speckles was estimated by having τ_c as a fitting parameter. Other fitting parameters were v_s , the static spatial variance and ρ , the fraction of dynamically scattered light. A priori knowledge of ρ is 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 argument and assumptions on nonergodicity, the experimental MESI curves were obtained from speckle contrast computed using spatial [13,28] and temporal analysis [36,65]. Multi-exposure speckle contrast measurements were obtained using the microfluidic devices with different levels of static scattering in the static scattering upper layer (Figure 3.4a: $\mu'_s = 0 \text{ cm}^{-1}$ and Figure 3.4b: $\mu'_s = 4 \text{ cm}^{-1}$ and $\mu'_s = 8 \text{ cm}^{-1}$). A suspension ($\mu_s = 250 \text{ cm}^{-1}$) 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 3.8.

Figure 3.8 shows that the MESI curves obtained using temporal contrast analysis (dotted lines) do not possess a significant constant variance, and that the experimentally measured variance approaches zero at long exposure durations. The small offset that is observed is likely due to v_{noise} which remains constant in the presence of static scattering. This constant does not vary with addition of static scatterers in the 200 μ m static layer, confirming that it is due to v_{noise} . However, the MESI curves obtained using spatial (ensemble sampled) contrast analysis (solid lines) show a clear offset at large exposure durations when static scatterers were present. This offset is seen to increase with an increase in the extent of static scattering in the 200 μ m static layer. When no static scatterers were present, the spatial (ensemble sampled) contrast curve does not posses this offset. 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. These measurements (Figure 3.7 and Figure 3.8) validate the MESI technique. They also demonstrate the use of the MESI instrument in conjunction with the speckle model, to estimate blood flow. The MESI technique can now be used to estimate blood flow *in vivo*.



Figure 3.8: 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 μ m static scattering layer. $\mu'_s = 0 \text{ cm}^{-1}$: No static scattering layer (Figure 3.4a), $\mu'_s = 4 \text{ cm}^{-1}$: 0.9 mg/g of TiO₂ in static scattering layer (Figure 3.4b), $\mu'_s = 8 \text{ cm}^{-1}$: 1.8 mg/g of TiO₂ in static scattering layer (Figure 3.4b). 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.

Chapter 4

Imaging Blood flow using the Multi Exposure Speckle Imaging Technique

The primary design goals of the Multi Exposure Speckle Imaging technique are:

- 1. Accurately estimate large changes in blood flow
- 2. Consistently estimate changes in blood flow in the presence of static tissue elements such as thinned skull
- 3. Provide a framework for baseline blood flow measures

This chapter presents results from experiments conducted using the MESI technique, to demonstrate its capabilities in estimating blood flow. First, in Section 4.1, results from controlled flow measurements in microfluidic phantoms are described. Controlled flow measurements also serve as a validation tool for the MESI technique. Then in Section 4.2, results from flow changes measured *in vivo* during an ischemic stroke are shown. The *in vivo* measurements serve as a practical demonstration of the MESI technique.

4.1 Flow measurements in tissue simulating phantoms

Microfluidic devices such as the ones described in Figure 3.4, are convenient and realistic phantoms that can be used to perform controlled flow experiments. They provide the flexibility to systematically vary flow rates, and compare them to experimental estimates. In section 3.4.2, micro-fluidic devices were used to perform flow measurements and validate the MESI technique. This section further demonstrates the capabilities of the MESI technique using these devices. As described in Section 3.4.2, for each experiment, at each of the 15 different exposure durations, 30 raw speckle images of flow in a micro-fluidic channel were obtained. These raw images were converted to speckle contrast images using Equation 2.1 and then averaged. The average speckle variance in a region within the channel was then used to compile sets of 15 measurements or 1 MESI set. These MESI sets were then fit to Equation 3.7 to estimate blood flow.

4.1.1 Estimating large flow changes

To verify that the MESI technique can estimate large changes in blood flow, controlled flow measurements were made using the MESI technique and the microfluidic sample without the static scattering layer (Figure 3.4a). The sample in Figure 3.4a was used to limit the number of objectives under test. The aim of this experiment is simply to compare the ability of MESI and LSCI techniques to estimate large changes in flow.

Similar to the validation experiments in Section 3.4.2, a suspension of

polystyrene microspheres ($\mu_s = 250 \text{cm}^{-1}$) was pumped through the flow channel in the microfluidic device (Figure 3.4a) at speeds varying from 0mm/sec to 10mm/sec, in 1mm/sec increments. The MESI technique was then used to obtain flow measurments, and time integrated speckle autocorrelation curves (or MESI curves), such as the ones shown in Figure 3.7, were generated. These curves were fit to Equation 3.7 using the procedure outlined in Section 3.4.2 to obtain estimates of correlation time, τ_c .

One of the criticisms of LSCI has been the lack of quantitative accuracy of correlation time measures. This lack of quantitative accuracy can be attributed to several factors including inaccurate estimates of β , 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. The MESI technique is aimed at reducing this experimental variability in measurements. Since images are obtained at different exposure durations the integrated auto correlation 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 of the speckle field autocorrelation time or correlation time τ_c , experimental noise and ρ , the fraction of light that is dynamically scattered. The MESI instrument also removes the dependence of v_{noise} on exposure duration. The MESI technique allows for determination of noise with a constant variance by decoupling the variances due to speckle decorrelation and the lumped variance due to noise and nonergodicity effects.

Since it is difficult to directly relate τ_c to speed, the accuracy of τ_c estimates obtained using the MESI technique can be indirectly evaluated using relative correlation time measures. Relative correlation time measures can be defined as

relative
$$\tau_c = \frac{\tau_{co}}{\tau_c},$$
 (4.1)

where τ_{co} is the correlation time at baseline speed and τ_c is the correlation time at a given speed. The τ_c estimates obtained with the MESI technique were compared to traditional single exposure estimates of τ_c at 1 ms and 5 ms exposures for their efficiency in predicting relative flows. These exposure durations were selected for their prevalence in many *in vivo* measurements. Ideally, relative correlation time measures would , be linear with relative speed in accordance with Equation 2.6. For this analysis, relative correlation times were obtained for a baseline flow of 2 mm/sec.

Figure 4.1 shows that MESI technique maintains linearity of relative correlation time measures over a long range. Single exposure estimates of relative correlation time measures are linear for small changes in flows, but the linearity breaks down for larger changes. The MESI technique addresses 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 such as imaging hemodynamic responses of CSDs, and in imaging spatial extent of severe ischemic strokes. These measurements show that the MESI technique is better suited for estimating large changes in flow.



Figure 4.1: Performance of different models to relative flow. 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. Measurements made using microfluidic phantom with no static scattering layer (Figure 3.4a).

4.1.2 Effect of Static Scattering

One of the significant improvements that the MESI technique provides is its ability to estimate correlation times consistently in the presence of static scatterers. To demonstrate this capability, flow measurements were done on three microfluidic phantoms; (1) the sample with no static scattering layer shown in Figure 3.4a, (2), the sample with the 200 μ m layer of static scattering shown in Figure 3.4b, with 0.9 mg of TiO₂ per gram of PDMS in static scattering layer ($\mu'_s = 4 \text{ cm}^{-1}$) and (3), the sample with the 200 μ m layer of static scattering shown in Figure 3.4b, with 1.8 mg of TiO₂ per gram of PDMS in static scattering layer ($\mu'_s = 8 \text{ cm}^{-1}$). The suspension of micro spheres was pumped through the sample using the syringe pump at different speeds from 0 mm/sec (Brownian motion) to 10 mm/sec in 2 mm/sec increments. Measurements on the sample with no static scattering layer (Figure 3.4a) served as reference (or 'true') estimates of correlation times.

Figure 4.2 shows the results of this analysis at two different speeds. The addition of the static scattering layer drastically changes the shape of the time integrated speckle autocorrelation function curves. It is also seen that Equation 3.7 fits to the experimental data very well for all cases. For a given speed, in the presence of the static scattering layer the decrease in variance at low exposure is due to the relative weighting of the two exponential decays in Equation 3.7 which is consistent with results obtained with DLS measurements [44]. The increase in variance at the larger exposure durations when the static scattering layer is added, is due to the addition of the nonergodic variance v_{ne} . Also, values of ρ are found to decrease with the addition of static scattering, implying a reduction in the fraction of total light that is dynamically scattered. Most importantly, for a given exposure duration and speed, the measured speckle contrast values are 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 Equation 3.7 in conjunction with the MESI instrument, the contributions of β , ρ and v_s to the variance

can be decoupled from variance due to speckle decorrelation, and hence more quantitative measures of τ_c can be obtained.



Figure 4.2: Multi-Exposure Speckle Contrast data from two samples fit to the new speckle model. Speckle variance as a function of exposure duration for two different speeds and two levels of static scattering. Solid lines 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 μ m static scattering layer. μ'_s values $\mu'_s = 0 \text{ cm}^{-1}$: No static scattering layer (Figure 3.4a), $\mu'_s = 8 \text{ cm}^{-1}$: 1.8 mg/g of TiO₂ in static scattering layer (Figure 3.4b).

To quantify the effects of the static scattering layer on the consistency of the τ_c estimates, the deviations in τ_c for a given speed, due to a change in the amount of static scatterers were estimated. For each speed, the variation in the estimated correlation times over the three scattering cases (Figure 3.4a: $\mu'_s = 0 \text{ cm}^{-1}$, Figure 3.4b: $\mu'_s = 4 \text{ cm}^{-1}$ and $\mu'_s = 8 \text{ cm}^{-1}$) was estimated
by calculating the standard deviation of their corresponding correlation time estimates. This deviation was normalized to the base (or 'true') correlation time estimates. The results for the MESI technique and the single exposure case are plotted in Fig. 4.3.

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

Fig. 4.3 shows that the single exposure estimates are not suited for speckle contrast measurements in the presence of static scatterers. The deviation in the correlation time estimates away from their expected (or 'true') value is high and this deviation increases with speed. The deviation in the correlation time estimates obtained with the MESI technique however, is less than 10% for all speeds. These results clearly show that the MESI technique can estimate correlation times consistently even in the presence of static scattering.

4.1.3 Discussion

The results in Figure 4.3 show that correlation time estimates obtained using LSCI are inconsistent in the presence of static scatterers. However, even if the baseline flow values are inconsistent, there is a possibility that relative flow changes (Equation 4.1) can be estimated consistently in the presence of static scatterers. In typical LSCI measurements through the thinned skull, researchers use only the relative correlation time measures to obtain a measure of changes in blood flows. Therefore, while it would be useful to obtain consistent baseline measures, it is more important that techniques estimate these relative flow changes accurately.



Figure 4.3: Percentage deviation in τ_c over changes in amount of static scattering for different speeds. τ_c estimates with the new speckle model have extremely low deviation

In section 4.1.2, correlation time estimates were obtained for different flow rates in a microfluidic channel, using devices with varying levels of scattering in the 200 μ m static scattering layer. For each static scattering case, the relative flow changes was calculated using Equation 4.1, using the measures at 2mm/sec as baseline. Relative correlation time estimates obtained using the MESI technique, and the single exposure (5ms) LSCI technique were compared. The results of this comparison are shown in Figure 4.4.

Figure 4.4 again illustrates 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 4.4a), while



Figure 4.4: Quantifying the effect of static scattering on relative τ_c measurements. Plot of relative correlation time (Equation 4.1) to relative speed. Baseline Speed - 2 mm/sec. The three curves on each graph represent different amounts of static scattering. μ'_s values refer to the reduced scattering coefficient in the 200 μ m static scattering layer. $\mu'_s = 0 \text{ cm}^{-1}$: No static scattering layer (Figure 3.4a), $\mu'_s = 4 \text{ cm}^{-1}$: 0.9 mg/g of TiO₂ in static scattering layer (Figure 3.4b), $\mu'_s = 8 \text{ cm}^{-1}$: 1.8 mg/g of TiO₂ in static scattering layer (Figure 3.4b). The MESI technique retains the linearity of relative τ_c estimates.

the flow estimates obtained using the MESI technique, maintain the linearity of relative correlation time measures even in the presence of static scatterers (Figure 4.4b). These results reinforce the observation that the MESI technique can predict consistent correlation times in the presence of static scatterers. Given the observation that the baseline correlation time estimates obtained using the MESI techniques do not deviate significantly from their expected ('true') value (Figure 4.3) and that the relative correlation times obtained using these 'true' correlation time values are linear (Figure 4.1), the results from Figure 4.4 are not entirely surprising. However, it is striking to notice that relative correlation time measures obtained using traditional LSCI measurements are not linear for even small changes in flow. This observation is precisely why the LSCI technique is completely unsuitable for flow measures in the presence of static scatterers, and it is this limitation that the MESI technique is designed to overcome.

Although the MESI technique yields robust estimates of τ_c in all cases that were tested, the technique will only work if the speckle signal from dynamically scattered photons is strong enough to be detected in the presence of the static background. Therefore, the technique will ultimately be limited by the signal to noise of the measurements. If the fraction of dynamically scattered photons is small compared to statically scattered photons, the dynamic speckle signal would be insignificant and estimates of τ_c would 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 MESI technique can be used to obtain consistent estimates of correlation times.

4.2 In vivo measurement of blood flow changes in mice cortex during ischemic stroke with Multi Exposure Speckle Imaging

The primary advantages of using the Multi Exposure Speckle Imaging(MESI) technique are its ability to image large flow changes accurately and its ability to image flow changes consistently through static tissue elements. Experimental flow measurements on microfluidic phantoms confirm these advantages (Section 4.1). In this section, these advantages are demonstrated by imaging the blood flow changes due to ischemic stroke. An $\sim 100\%$ reduction in blood flow was caused by photothrombosis of the middle cerebral artery (MCA) in a mouse and imaged using the MESI technique.

4.2.1 Experimental methods

The Multi Exposure Speckle Imaging (MESI) instrument shown in Figure 4.5a was used for the *in vivo* flow measurements. The only change in the experimental setup compared to Figure 3.1 is the addition of a photothrombosis inducing laser. This laser was used to excite a photo-sensitive dye (Rose Bengal) and cause thrombosis, which leads to ischemic stroke. As in previous flow measurements (Section 4.1), speckle images at different camera exposure durations were acquired by triggering a camera (Basler 602f, Basler Vision Technologies, Germany) and simultaneously gating a laser diode ($\lambda = 660$ nm, 95mW, Micro laser Systems Inc., Garden Grove, CA, USA) with an acoustooptic modulator, to equalize the energy of each laser pulse. The first diffraction order was directed towards the animal, and the backscattered light was collected by a microscope objective (10X) and imaged onto the camera. By appropriately controlling the acousto-optic modulator, the intensity of light in the first diffraction order and hence the average intensity recored by camera is maintained a constant over different exposure durations [38]. Laser speckle images were collected at 15 different exposure durations from 50μ s to 80ms, and the entire setup was controlled by custom software. Figure 4.5b shows some speckle contrast images of the mouse cortex at different camera exposure durations. While this is not the first in vivo demonstration of speckle images at multiple exposure durations [27], we note that these images span almost 3 orders of magnitude of exposure duration which is possible with an inexpensive camera using the MESI approach. The MESI data obtained using the instrument (Figure 4.5a) was fit to Equation 3.7 to find unknown constants β , ρ , v_s , and τ_c which is a measure of blood flow.



Figure 4.5: (a) Schematic of the Multi Exposure Speckle Imaging (MESI) instrument (b) Representative speckle contrast images of mouse cortex obtained at various camera exposure durations using the MESI instrument

The MESI technique was used to image cerebral blood flow changes that occur during ischemic stroke in mice. Mice (CD-1; male, 25 - 30 g, n = 5) were used for these experiments. All experimental procedures were approved by the Animal Care and Use Committee at the University of Texas at Austin. The animals were anesthetized by inhalation of 2-3% isoflurane in oxygen through a nose cone. Body temperature was maintained at 37°C using a feedback controlled heating plate (ATC100, World Percision Instruments, Sarasota, FL, USA) during the experiment. The animals were fixed in a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA) and an ~ 3 mm $\times 3$ mm portion of the skull was exposed by thinning it down using a dental burr (IdealTM Micro-Drill, Fine Science tools, Foster City, CA, USA). Further, part of this thinned skull was removed to create a partial craniotomy (shown in Figure 4.6). Care was taken to ensure that the boundary between the thin skull and the craniotomy was over a vessel and that the boundary was away from major branches. This ensured that one can expect the same blood flow changes across the boundary. The partial craniotomy was completed by building a well around the region using dental cement and filling it with mineral oil. The surgery was supplemented with subcutaneous injections of Atropine (0.4mg/kg) every hour to prevent respiratory difficulties and intraparetonial injections of dextrose-saline (2ml/kg/h of 5% w/v) for hydration.

To induce an ischemic stroke, the middle cerebral artery (MCA) was occluded using photothrombosis [66,67]. During animal preparation, the temporalis muscle in the same hemisphere of the craniotomy was carefully reseted



Figure 4.6: Speckle contrast image (5ms exposure duration) illustrating the partial craniotomy model. The regions within the closed loops (Regions 1, 3 and 5) are in the craniotomy. Regions outside the closed loops (Regions 2, 4 and 6) are in the thin skull region

from the temporal bone. The temporal bone was then thinned using the dental burr till it was transparent and the MCA was visible. A laser beam $(\lambda = 532 \text{nm}, \text{Spectra Physics}, \text{Santa Clara, CA, USA})$ was directed towards the MCA through an optical fiber. Typical laser power delivered to the animal during the experiment was $\sim 0.5 - 0.75$ W. During the experiment, a 1ml bolus intraparetonial injection of a photo sensitive thrombotic agent Rose Bengal (15mg/kg) was administered to the animal. The laser light acts on Rose Bengal to cause thrombosis in the MCA and cause occlusion. Figures 4.7a & b



Figure 4.7: Speckle Contrast images of a branch of the MCA, illustrating ischemic stroke induced using photo thrombosis (a) Before stroke (b) After stroke

show LSCI images (at 5ms exposure) before and after the stroke was induced. Occluding the MCA creates a severe stroke and reduced blood flow by almost 100% in the cortical regions downstream.

The experimental setup shown in Figure 4.5 was used to acquire multi exposure images before, during and after the stroke. Laser speckle images at 15 exposure durations ranging from 50μ s to 80ms, were used to compile one MESI frame. Typically, 3000 MESI frames were collected for each experiment. Each MESI frame takes ~ 1.5 seconds to acquire. The field of view of the cortex as measured by the MESI instrument is ~ $800 \times 500\mu$ m. Specific regions of interest as shown in Figure 4.9a were identified, and the average speckle contrast in these regions were computed for all MESI frames to produce the time integrated speckle contrast curves shown in Figure 4.9b. Each curve was then fit to Equation 3.7 to estimate blood flow (τ_c).





Figure 4.8: (a) Speckle contrast image (5ms exposure) illustrating regions of different flow (b) Time integrated speckle variance curves with decay rates corresponding to flow rates. The data points have been fit to Equation 3.7

Figure 4.8b illustrates the first step in obtaining blood flow estimates using the MESI technique. In this example, the MESI instrument (Figure 4.5a) was used to obtain raw speckle images at multiple exposure durations of a mouse brain whose cortex had been exposed by performing a full craniotomy. After converting these raw images to speckle contrast images, specific regions of interest were identified (Figure 4.8a), and the average speckle contrast in these regions were computed and plotted as a function of camera exposure duration (Figure 4.8b). These experimentally measured time integrated speckle variance, $K(T, \tau_c)^2$ curves were then fit to Equation 3.7 to obtain estimates for blood flow (through τ_c , the characteristic decay time of the speckle autocorrelation function [24, 38]). The curves correspond to different regions shown in Figure 4.8a. From these curves, it can be observed that the variance decays much faster in region 1 which is in the middle of a major vessel (a vein), when compared to region 4 which is in the parenchyma. A faster rate of decay corresponds to a lower τ_c value and hence higher flow.



Figure 4.9: (a) Illustration of Partial craniotomy model. The regions enclosed by the closed loops (Regions 1, 3 & 5) are located in the craniotomy. Regions outside of the closed loops (Regions 2, 4 & 6) are located in the thinned (but intact) skull. (b) Time integrated speckle variance curves illustrating the influence of static scattering due to the presence of the thinned skull. A decrease in the value of ρ indicates an increase in the amount of static scattering. Regions 2 and 4 show distinct offset at large exposure durations. This offset it due to increased v_s over the thinned skull

For stroke experiments, the partial craniotomy procedure was followed during animal preparation. A representative image of this model is shown in Figure 4.9a. Regions 1, 3, and 5 are in the craniotomy, while regions 2, 4 and 6 are under the thin skull. MESI images were obtained and the blood flow was estimated using the procedures described in Section 4.2.1. Figure 4.9b shows how the time integrated speckle variance curves are different for two regions across the thin skull boundary. The primary points of difference between the curves obtained from regions across the boundary are (a) an apparent change in the shape of the time integrated speckle variance curve over the thin skull due to variation in ρ (the fraction of light that is dynamically scattered [38]), and (b) an increase in the variance at the longer exposure durations due to an increase in v_s (the constant spatial variance that accounts for nonergodicity and experimental noise [38]). This difference is more apparent in the regions on the vessel (regions 1 and 2) than it is in regions in the parenchyma (regions 3 and 4). With LSCI at a single exposure, regions 1 and 2 measure vastly different speckle contrast values even though the actual blood flow is likely identical. Under baseline conditions, the ratio of the correlation time in region 1 to the correlation time in region 2 was found to be 0.6238 ± 0.0238 using the MESI technique, while this ratio was estimated to be 0.3771 ± 0.0215 using the LSCI technique. While the ideal value for these ratios should be 1, these estimates suggest that the MESI technique predicts τ_c values that are more consistent across the thin skull boundary. The ratio of the correlation time in region 3 to the correlation time in region 4 was found to be 0.883 ± 0.055 using the MESI technique, while this ratio was estimated to be 0.889 ± 0.019 using the LSCI technique. The MESI and LSCI estimates of these ratios are similar over the parenchyma regions because the thickness of the thinned skull

is non uniform and was found to be thinner, as evidenced by higher values of ρ in region 4 compared to region 2.

Each stroke experiment was performed after waiting for about 30 minutes after surgical preparation. The first 10 minutes of the data was used as baseline measures to compute the relative blood flow change. The thrombosis inducing laser was kept on during the entire course of the experiment. Rose Bengal was injected 10 minutes after start of the experiment and data collection was continued for about an hour. Data acquisition was not stopped while the dye was being injected. Immediately after the completion of data acquisition, the animal (n = 2) was sacrificed and 30 MESI frames (1 MESI frame consists of 15 exposure durations) were collected as a zero flow reference.

Since β is an experimental constant, its *in vivo* determination is important to obtain accurate flow measures [38]. In addition to β , ρ and v_s also have to be determined *in vivo*. However, we contend that changes in the physiology can change ρ and v_s , and hence these parameters were not held fixed during the fitting process. First, β was estimated under baseline conditions for the regions in the craniotomy (regions 1, 3 and 5 shown in figure 4.9a), by using equation 3.7 and holding $\rho = 1$. A statistical average of the estimated values of β were found for each region and this average value was used for the corresponding pair. For example, the value of β estimated from region 1, would be used for regions 1 and 2. The MESI curves from entire data set were then fit to Equation 3.7 using the estimated value of β , and holding it constant. Unknown parameters ρ , v_s and the flow measure τ_c were estimated from this



Figure 4.10: Relative blood flow change caused due to the ischemic stroke in the branch of the MCA (Region 1 in Figure 4.9a). (a) Time course of relative blood flow change in Region 1 as estimated using the MESI technique. The flow estimates in first 10 minutes were considered as baseline. The reduction in blood flow due to the stroke, is estimated to be $\sim 100\%$, which indicates that blood supply to the artery has been completely shut off. (b) MESI curves illustrating the change in the shape of the curve as blood flow decreases. The MESI curve obtained after the stroke is found to be similar in shape to that obtained after the animal was sacrificed. This is a qualitative validation of $\sim 100\%$ decrease in blood flow in the artery

fitting process.

Figure 4.10a shows the relative blood flow change as measured using the MESI technique in region 1, in the same animal as in Figure 4.9. Since τ_c can be assumed to be inversely related to blood flow [6], relative blood flow may be defined as the ratio of $\tau_{baseline}$ to $\tau_{measured}$. Here, $\tau_{baseline}$ is the statistical average of the correlation time estimates during the first 10 minutes. From time t = 10min to t = 30min, the blood flow is seen to fluctuate. These fluctuations are due to the increase and decrease of blood flow while the clot is being formed in the MCA. For the MCA to be completely occluded, the photo thrombosis process has to create enough thrombus to occlude the vessel and its downstream branches. Since the MCA is a major artery, partially formed thrombus can be washed down by blood pressure. The partially formed clots break down and produce blood flow fluctuations. These fluctuations were observed in all animals before the stroke was formed. Once the thrombosis process is complete, the blood flow settles to a stable value. Figure 4.10a shows that the relative blood flow drops to almost 0 after the clot is fully formed. The average percentage reduction in blood flow in the blood vessel, due to the ischemic stroke in all animals was estimated be 97.3 \pm 2.09% using the MESI technique and 87.67 \pm 7.04% using the LSCI technique.

In Figure 4.10b, we show three representative time integrated speckle variance curves estimated from Region 1 (Figure 4.9a) as a function of camera exposure duration, illustrating the progression of the stroke in one representative animal. The first two curves are the time integrated speckle variance curves before and after ischemic stroke. The drastic change in the shape of the curve reinforces the observation that the change in blood flow is drastic, as previously noted in Figure 4.7 and Figure 4.10a. The shape of the curve after the stroke has been induced is indicative of Brownian motion. This trend was observed in all animals, and is comparable to similar measurements in literature [1, 38]. An experimental measurement of the time integrated speckle variance curve after the animal has been sacrificed (comparing the blue and black curves in figure 4.10b) further confirm these observations. In Region 1

we estimated the average percentage reduction in blood flow due to death in all animals to be about 99% using the MESI technique and 92% using the LSCI technique. Since after death, the blood flow in the animals should be zero, we conclude that the MESI technique has greater accuracy in predicting large flow decreases. This observation is consistent with our previous measurements in phantoms [38].

While the post stroke and post mortem time integrated speckle variance curves are similar, the variances are different. The increase in measured speckle variance after the animal has been sacrificed is indicative of a further drop in blood flow. This drop is measured as a mild increase in τ_c . One of the reasons for the difference in speckle variance between the post stroke and the post mortem cases, is that in the post stroke case, the speckle contrast can still be affected by blood flow from deeper tissue regions (though not spatially resolved) which could possibly be unaffected by thrombosis. Additionally, the pulsation of the cortex in a live animal contributes to a reduction in variance. In the post mortem case, this pulsation is absent, and the blood flow is truly zero over the entire cortex. The only motion we detect is due to limited (thermal induced) Brownian motion that can be associated with the dead cells. These factors coupled with physiological noise contribute to the difference in variance between the post mortem and the post stroke cases. From these observations, we conclude that the magnitude of the blood flow reduction measured by the MESI technique is accurate.

Figure 4.11 compares the relative blood flow measures as estimated by



Figure 4.11: MESI technique can predict consistent blood flow changes across the thin skull - craniotomy boundary. (a) Relative blood flow changes estimated using the MESI technique in 3 pairs of regions across the boundary (Figure 4.9a). The change in blood flow is found to be similar for each pair of regions. (b) Relative blood flow changes estimated using the LSCI technique (at 5ms exposure) in 3 pairs of regions across the boundary (Figure 4.9a). The change in blood flow is not similar for each pair of regions. This difference is especially prominent over the vessel (regions 1 and 2)

(a) MESI technique and (b)LSCI technique at 5ms exposure duration. 5ms exposure duration was selected for comparison because it has been demonstrated to be sensitive to blood flow changes *in vivo* [27]. Considering the first pair of regions across the thin skull boundary (Regions 1 and 2 in Figure 4.9a), the relative blood flow measures as estimated by the MESI technique (Solid and dashed blue lines in Figure 4.11a) were found to be similar. This indicates that the relative blood flow measures obtained using the MESI technique are unaffected by the presence of the thin skull. The LSCI estimates (Figure 4.11b) however show two significant differences. One, the relative blood flow estimate

for Region 1 is not close to 0 after the stroke, but is rather close to 0.2 and two, the relative blood flow measures across the boundary (Solid and dashed blue lines in figure 4.11b) are different. The first observation is an *in vivo* reproduction of LSCI's underestimation of large flow changes previously observed in tissue phantom studies (Section 4.1) [38], and the second observation is the very limitation that the MESI technique is designed to overcome. These observations can also be made in relative blood flow measures from the other two pairs of regions, Regions 3 & 4 and Regions 5 & 6, both in the parenchyma. In these regions we see a similar trend, but the difference between the two techniques is not as drastic as it is in the blood vessel. Typically, each pixel in the image samples a large distribution of blood flows. The statistical models we use to describe speckle contrast assume that there is one value of blood flow (and hence one τ_c) in the sampling volume. This assumption is more valid over large blood vessels (or in a microfluidic phantom [38]), where there is a clear direction and rate, for flow. However in the parenchyma, the photons can sample a larger distribution of blood flow rates and we measure a statistical average of these different flow rates. It should be noted that this limitation is common to any dynamic light scattering based measurement. For these reasons, the MESI measurements are likely to be more accurate over the large blood vessel than the parenchyma. We still notice that the MESI estimates of relative flow are more consistent than the LSCI estimates, and that the MESI technique estimates a larger reduction in blood flow.

Figure 4.12 provides a full field perspective of the relative blood flow



Figure 4.12: Full field relative correlation time maps obtained using the (a) MESI technique (b) LSCI technique (5ms exposure). Three corresponding regions marked in the figures illustrate the superior performance of the MESI technique. The boundary (corresponding to the boundary between the thin skull and the craniotomy) indicated by the red arrow is clearly visible in (b), but not in (a). There is a clear change gradient in the region indicated by the star in (b), but this gradient is invisible in (a). The vessel circled is more visible in (a) compared to (b). Relative correlation time estimates obtained using the MESI technique are not affected by the presence of thinned skull. Hence, similar estimates of blood flow changes are obtained across the boundary between the thin skull and craniotomy regions, leading to the absence of any gradients in blood flow change in (a)

changes. These are full field maps of the relative correlation time, computed by taking the ratio of τ_c under baseline conditions to τ_c at a single time point after the stroke, as estimated using the MESI technique (Figure 4.12a) and the LSCI technique at 5ms exposure duration (Figure 4.12b). Both images are displayed on a scale of 0 to 1. The thin skull boundary is clearly visible in the LSCI estimate (Figure 4.12b), while the demarcation between the craniotomy and the thin skull is less obvious in the MESI estimates (Figure 4.12a). This difference is illustrated in the figures using (1) a red arrow and (2) a green star. Additionally, we see that some vessels are more visible in the MESI estimate. One example of this is illustrated by the blue oval. These images show that the MESI technique is better in estimating relative blood flow than LSCI and that these estimates are not affected by the presence of a thin skull.

4.2.3 Discussion

The change in the shape of the time integrated speckle variance curves due to the presence of static tissue elements is consistent with our previous measurements in flow phantoms (Section 4.1) [38]. While in the case of the tissue phantoms, the change in the shape was affected in equal parts due to the influence of ρ and v_s , in the *in vivo* measures, we find that the static speckle variance v_s is the more dominant factor. In the microfluidic device we used earlier, the flow channel was the only part of the device containing dynamic scatterers. We believe that in the microfluidic device, the influence of ρ was greater due to the opportunity for a photon to interact with static particles on the sides of the channel and below the channel. This is clearly not the case in vivo, because the only place where a photon can interact from a static particle is from the thin skull. This could explain a comparatively reduced role that ρ plays in the *in vivo* measurements. Nevertheless, there is no way of accurately determining the value of ρ or v_s without using Equation 3.7 and the MESI instrument. Hence, the MESI technique is better suited to obtain consistent and accurate measurements of blood flow changes in the presence of a thin

skull.

Recently, Duncan et. al. [37] pointed out that a Gaussian function $(g_1(\tau) = e^{-\tau^2/\tau_c^2})$ is a better statistical model to describe the dynamics of ordered flow in a vessel as opposed to the traditionally used negative exponential model [28] $(g_1(\tau) = e^{-\tau/\tau_c})$. The former corresponds to a Gaussian distribution of velocities, while the negative exponential model corresponds to a Lorentzian distribution of velocities in the sample volume. In order to test this hypothesis, we proceeded to derive a new MESI expression using the Gaussian function to describe speckle dynamics, and account for scattering from static tissue elements. We substituted $g_1(\tau) = e^{-\tau^2/\tau_c^2}$ in Equation 9 in our previous publication [38] and evaluated the integral to arrive at the new expression.

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

$$(4.2)$$

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, and v_s is the static spatial variance. Here again we combine the non ergodic variance v_{ne} and the noise variance v_{noise} into v_s .

We estimated the relative blood flow changes in Regions 1 and 2 (Figure 4.9a) using Equation 4.2 using the MESI technique. We compared these estimates to those we already obtained using Equation 3.7 and to the corresponding LSCI estimates at a few exposure durations other than 5ms. These results are plotted in Figure 4.13.



Figure 4.13: Comparison of the percentage reduction in blood flow obtained in Regions 1 and 2 (Figure 4.9a) using the MESI technique using two different speckle expressions (Lorentzian: Equation 3.7 and Gaussian: Equation 4.2) and multiple single exposure LSCI estimates. MESI estimates are found to be more consistent in estimating blood flow decreases across the boundary between the thin skull and the craniotomy. There is no significant difference between the two models in estimating blood flow decrease

From Figure 4.13 we observe that using the Gaussian statistical model and Equation 4.2 do not change our estimates of relative flow *changes* significantly. By incorporating the principles of heterodyne mixing into Equation 4.2 and by using the MESI technique, we are still able to obtain consistent flow measures across the boundary of the thin skull. Duncan et. al [37] also pointed out that the differences between the Lorentzian and the Gaussian models are more prominent at the lower exposure durations. By sampling a range of exposure durations, we are minimizing the difference between the two models. Also as we explained earlier, each speckle samples a wide range of flow rates. The differences between the two models are not significant enough to overcome the statistical variability in value of τ_c . In addition, physiological noise and variability are bigger sources of uncertainty in the fitting process than a small change affected by using a different model. Our observations are in agreement with Cheung et. al [68] and Durduran et. al. [69] who showed that the Lorentzian model is a better fit for *in vivo* blood flow measurements, using noninvasive diffuse correlation spectroscopy measurements, due to the complex fluid dynamics of blood flow in vessels.

In Figure 4.13, while comparing the LSCI estimates of relative blood flow decrease at multiple exposure durations, we observe that at 5ms the percentage reduction in blood flow is about 10% lower than those obtained with the MESI technique. We also notice that the choice of exposure time in LSCI can drastically change the estimated blood flow reduction. For example, at 1 ms (another popular choice for *in vivo* measurements), LSCI predicts a 70% drop in blood flow due to stroke, which is almost 30% lower than the MESI estimates. This is not surprising because the sensitivity to change in blood flow has previously been shown to depend on the choice of exposure duration [27]. This is another reason why the LSCI estimates did not completely pick up the drop in blood flow in a small vessel circled in Figures 4.12a & b. It is hence impossible to accurately measure with a single exposure duration, the change in blood flow of all vessels in a field of view that consists of vessels of different diameters (and hence different blood flows). Also, we note that the estimates of relative blood flow change are not consistent across the thin skull boundary for any of the single exposure measurements. From this we can conclude that for experiments where a relatively small change in blood flow is expected, the LSCI technique may be adequate, provided the sample is free from static tissue elements and the right choice of exposure duration is made before the experiment. For imaging large changes in blood flow or for imaging samples where dynamic and static scatterers are mixed, the MESI technique is likely to yield more accurate estimates of flow changes.

Chapter 5

In Vivo measurement of speckle autocorrelation function using Dynamic Light Scattering

5.1 Introduction

The fundamental basis for blood flow measurements based on light scattering is the field of Dynamic Light Scattering (DLS). The basic theory of DLS was described in Section 2.3.1. Briefly, in DLS measurements, the instantaneous intensity of backscattered coherent light is recorded using point detectors such as photomultiplier tubes. Blood flow measures are computed from this intensity by computing the autocorrelation function of intensities $(g_2(\tau))$, as described in Equation 2.2. Since the dynamics of the system is described via the electric field autocorrelation function $(g_1(\tau))$, the Siegert relation (Equation 2.10) is used to relate $g_1(\tau)$ and $g_2(\tau)$. The electric field autocorrelation function is assumed to follow a particular statistical model and the experimental data is fit to this assumed model (Table 2.2) to obtain τ_c , the characteristic decay time of the electric field autocorrelation function. As described in Sections 2.3.1, 2.3.2, 2.3.3 and 3.2, τ_c is a measure of blood flow and accurate estimation of τ_c is the primary goal of both Laser Speckle Contrast Imaging (LSCI) and Multi Exposure Speckle Imaging (MESI) techniques. Since, DLS techniques form the physical basis for both LSCI and MESI techniques, estimates of τ_c obtained using DLS methods can be considered a 'gold standard' for evaluating the quantitative accuracy of LSCI and MESI estimates of τ_c . The primary motivation for experiments in this chapter, is to compare the absolute values of τ_c estimates obtained with the MESI and LSCI techniques, to τ_c estimates obtained using single point DLS measurements.

Flow measurements using DLS techniques are similar to Doppler techniques. The temporal speckle intensity fluctuations can also be quantified using the power spectrum of recorded intensity $(S(\omega))$, which can be related to the speckle intensity autocorrelation function $(g_2(\tau))$ using Equation 5.1 [6,61,70].

$$S(\omega) = \frac{\langle I \rangle^2}{2\pi} \int_{-\infty}^{\infty} \cos(\omega\tau) \left[g_2(\tau) - 1 \right] d\tau, \qquad (5.1)$$

where $\langle I \rangle$ is the average intensity. Laser Doppler techniques measure the width, $\Delta \omega$, of the power spectrum $S(\omega)$. This width is analogous to the characteristic decay time of the speckle electric field autocorrelation function τ_c .

While τ_c estimates obtained using DLS techniques can be used as a point of reference, such estimates ultimately depend on the statistical model that is assumed for the electric field autocorrelation function $g_1(\tau)$ (Table 2.2). For blood flow measurements *in vivo*, this form has always been assumed to follow a negative exponential model, which corresponds to a Lorentzian distribution of velocities (Equation 2.5) [6,28,71] for both LSCI and laser Doppler measurements. Recently, Duncan et. al. provided some arguments for using a statistical model that corresponds to a Gaussian distribution of velocities [37]. This argument was examined in Section 4.2.3 for its validity in estimates of blood flow changes. Additionally, Cheung et. al. [68] and Durduran et. al. [69], used diffuse correlation spectroscopy measurements to show that the Lorentzian model was more accurate for blood flow measurements *in vivo*. Hence, there is some controversy on the assumption of an expression for $g_1(\tau)$, that would be appropriate for blood flow measurements with MESI or LSCI. In this chapter, *in vivo* measurements of the speckle electric field autocorrelation functions were made using DLS, to evaluate different statistical models of $g_1(\tau)$ and estimates of τ_c obtained using MESI and DLS techniques were compared.

5.2 Instrumentation

The objective for measuring the electric field correlation function of the backscattered speckles, is to compare τ_c estimates obtained using DLS principles to those estimated by LSCI and MESI techniques. Such a comparison of absolute values of τ_c would be appropriate only if these measurements were obtained from the same site in the cortex of the animal. Further, since instrumentation factors such as β can potentially affect the τ_c estimates, it would be most appropriate to perform both measurements using the same instrumentation setup. Figure 5.1 provides a schematic of the MESI instrument adapted to perform simultaneous DLS measurements using a single mode optical fiber



Figure 5.1: Multi Exposure Speckle Imaging instrument adapted to perform simultaneous Dynamic Light Scattering measurements. A small fraction of the backscattered light is imaged onto an optic fiber, the other end of which is connected to a photomultiplier tube. Instantaneous intensity is recorded on a computer after amplification using a current amplifier.

and a photo mutiplier tube (PMT).

The basic functioning of the instrument follows the description in Section 3.3. Briefly, speckle images at multiple exposure durations were obtained by simultaneously triggering a camera and pulsing a laser diode through an acousto-optic modulator. By appropriately varying the intensity of the first diffraction order (which is directed to the sample) using the AOM, the average camera intensity at the camera was held constant across all exposure durations. A small fraction ($\sim 4\%$) of the backscattered light was picked off and imaged onto a single mode optic fiber $(MFD = 10\mu \text{m})$. The diameter of the core of the optic fiber was matched to that of a pixel of the camera, ensuring that instrumentation factors such as β would remain similar for both techniques. The need to finely control the aperture of the collected light necessitated the use of an optic fiber. By, using a single mode fiber, it was ensured that the speckle intensity fluctuations were appropriately transmitted to the detector. Light coming out at the other end of the optic fiber was collimated and coupled onto the active surface of a photomultiplier tube (PMT) (Hamamatsu Corporation, Bridgewater, NJ, USA). The (current) output of the PMT was converted to voltage signals using a high bandwidth current amplifier (Stanford Research Systems, Sunnyvale, CA, USA). These voltage signals, corresponding to the intensity fluctuations of the backscattered speckles, were recorded on a computer for post processing.

The instrumentation setup shown in Figure 5.1 should ideally image the point from the center of the field of view of the camera onto the optic fiber. In practice, the point on the sample that was imaged onto the fiber was found to be a little off center. This shift occurs because the light imaged onto the fiber is shifted in its optical axis due to the use of a thick piece of glass to pick of the backscattered light. This shift, while reduced with thinner glass slides, cannot be completely eliminated, and hence the point from which DLS measurements were made was identified for each experiment by back illumination. Laser light was coupled into the fiber at the PMT (detector) end and was imaged as a focused spot on the sample using the camera. Since the sample plane, plane of the optical fiber's aperture and the plane of the camera sensor form conjugate imaging planes; the spot of back illuminated light that is imaged on the camera is the point on the sample that is mapped on the surface of the fiber. To image different areas in the cortex, the animal was moved using an X-Y translation stage, and back illumination was performed to identify which cortical region was imaged onto the fiber.

5.3 Experimental Methods

Cerebral blood flow was measured *in vivo* almost simultaneously with DLS and MESI techniques using the adapted MESI instrument shown in Figure 5.1. The animal surgical procedure was similar to those in experiments performed in Section 4.2. All animal procedures were approved by the Animal Care and Use Committee at the University of Texas at Austin. Briefly, mice (CD-1; male, 25 - 30g, n=3) were anasthetized by inhalation of 2 - 3% isoflurane in oxygen through a nose cone. After being fixed in a stereotaxic frame, a full craniotomy procedure was performed on the animals to expose $\sim 3 \text{mm} \times 3 \text{mm}$ of the cortex. The craniotomy was finished by building a well around the exposed area using dental cement, and filling it with mineral oil. The surgery was supplemented by subcutaneous injections of Atropine (0.4mg/kg) and intraparetonial injections of 5%w/v dextrose-saline (2ml/kg/hr). Like in Section 4.2, a blood flow change (ischemic stroke) was induced in these animals by occluding the middle cerebral artery (MCA) using photothrombosis [72]. The right temporalis muscle was carefully resected,

and the temporal bone was thinned until the MCA was visible. A laser beam of wavelength $\lambda = 532$ nm, (not shown in Figure 5.1) was directed towards the MCA using an optical fiber. During the experiment, 1ml of Rose Bengal (15mg/kg), a photothrombotic agent, was injected into the intraparetonial cavity of the mouse, before the thrombosis inducing laser was turned on to induce the stroke. More details about the animal procedure is available in Section 4.2.1.

The instrument shown in Figure 5.1 was used to obtain blood flow measurements almost simultaneously using MESI and DLS techniques. For each animal, DLS measurements were obtained from an artery (branch of the MCA), a vein and the parenchyma; except in one animal where no veins could be identified near downstream branches of the MCA. Before each DLS measurement, the observation spot was verified by back illuminating through the optical setup. For each point, 10^6 samples at a sampling rate of 10^5 samples/second were obtained using a data acquisition board connected to the computer. The PMT was operated at its maximum gain (to maximize its sensitivity and dynamic range) and the current amplifier was set to have a bandwidth of 50KHz. After DLS measurements were obtained at each point, 30 full field multi exposure frames were obtained at each of 15 different exposure durations using the camera. These 30 raw images were converted to speckle contrast images using Equation 2.1, and then averaged. The region of interest corresponding to the DLS measurement region was identified and MESI data was extracted from the averaged speckle contrast images. These data points were fit to Equation 3.7

to obtain estimates of τ_c . The acquisition and analysis of the MESI data is described in greater detail in Sections 4.2 and 4.1. After obtaining DLS and MESI measurements under baseline conditions, Rose Bengal was injected into the animal and an ischemic stroke was induced. The experimental procedures were repeated under post ischemic conditions.

5.4 Results

Figure 5.2 illustrates two DLS measurement sites from one animal. The DLS measurement site in Figure 5.2a is in the parenchyma, while the measurement site in Figure 5.2b is over a branch of the MCA. According to the theory of dynamic light scattering, the intensity and field autocorrelation functions obtained from the measurements over the artery should decay faster. The first step in estimating blood flow with DLS measurements is to compute the intensity and field autocorrelation functions of the backscattered speckles. The intensity of the backscattered speckle is measured as I(t). As described in Section 2.3.1, the intensity autocorrelation function can be defined as:

$$g_2(\tau) = \frac{\langle I(t)I(t+\tau)\rangle}{\langle I\rangle^2}$$
(5.2)

The experimentally recorded I(t) was used to compute the intensity autocorrelation function, $g_2(\tau)$ using Equation 5.2. Since, the dynamics of the system can only be described by the electric field autocorrelation function $g_1(\tau)$, the Siegert relation (Equation 5.3) was used to relate the electric field



Figure 5.2: LSCI (5ms exposure duration) images illustrating the DLS measurement regions in (a) parenchyma (b) an artery. The DLS measurement areas are illustrated by the red squares. It can be observed that the measurement sites are not centered in the image.

and intensity autocorrelation functions.

$$g_2(\tau) = 1 + \beta |g_1(\tau)|^2 \tag{5.3}$$

where, β is an instrumentation factor that accounts for speckle averaging effects. Hence, $|g_1(\tau)|^2$ was computed as:

$$|g_1(\tau)|^2 = \frac{g_2(\tau) - 1}{\beta}$$
(5.4)

The value of β was estimated by extrapolating $g_2(\tau) - 1$ to 0 and recognizing that $\beta = g_2(0) - 1$. The experimentally computed $|g_1(\tau)|^2$ for regions represented in Figures 5.2a and 5.2b., is shown in Figure 5.3. Although no model has been assumed for $g_1(\tau)$, the rate of decay of $|g_1(\tau)|^2$ is faster in the artery than in the parenchyma, which is along expected lines.



Figure 5.3: Experimentally computed electric field autocorrelation function represented as $|g_1(\tau)|^2$. In both (a) and (b), the experimentally computed $|g_1(\tau)|^2$ (blue curve) is superimposed over the raw data or the normalized intensity trace (red curve) (a) $|g_1(\tau)|^2$ and normalized intensity trace obtained from the parenchyma (Figure 5.2a) (b) $|g_1(\tau)|^2$ and normalized intensity trace obtained from the artery (Figure 5.2b). $|g_1(\tau)|^2$ decays faster in the artery.

5.4.1 Choosing the right statistical model for $g_1(\tau)$

To obtain blood flow measures using DLS measurements, the experimentally measured $|g_1(\tau)|^2$ has to be fit to an appropriate statistical model for the electric field autocorrelation function $g_1(\tau)$. Hence, to obtain accurate τ_c measures, an appropriate statistical model has to be selected. Some statistical models were previously listed in Table 2.2.

When light scatters off moving particles, the phases of the scattered photons change randomly and independently. This random change in phase causes intensity fluctuations in the backscattered speckles. The rate of change of phase is related to the speed of the moving particles and hence the time scale of intensity fluctuations. The electric field autocorrelation function $g_1(\tau)$ quantifies these intensity fluctuations. If the moving particles are assumed to be independent of each other and uniformly distributed, then the electric field autocorrelation function can be expressed as,

$$g_1(\tau) = \exp\left[-\frac{1}{6}q^2 \langle \Delta r^2(\tau) \rangle\right], \qquad (5.5)$$

where, q is the momentum transfer vector and $\langle \Delta r^2(\tau) \rangle$ is the mean square displacement of the photons. For a condition where the particles undergo Brownian motion (similar to the velocities being distributed according to a Lorentzian distribution), $\langle \Delta r^2(\tau) \rangle = 6D_B\tau$, where D_B is the Brownian diffusion coefficient [29, 61].

The most popular model for $g_1(\tau)$ is a negative exponential (Equation 5.6), which corresponds to a Lorentzian distribution of velocities in the sample volume. Equation 5.6 has been used for a wide variety of flow measurement applications using multiple techniques such as LSCI [13, 28, 71], laser Doppler [6] and diffuse correlation spectroscopy [68]. This statistical model is also the assumption made in all speckle mathematical models (Equations 2.8, 2.13 and 3.7). The Lorentzian model (Equation 5.6) is characterized by,

$$g_1(\tau) = \exp(-x) \tag{5.6}$$

where $x = \tau/\tau_c$, and τ_c is the characteristic decay time of the electric field autocorrelation function.

Recently, Duncan et. al. [37] argued that, since blood flow in large vessels are characterized by bulk flow; the proper statistical model for $g_1(\tau)$ should reflect a Gaussian distribution of velocities in the sample volume. This Gaussian model (Equation 5.7) is characterized by,

$$g_1(\tau) = \exp(-x^2) \tag{5.7}$$

where $x = \tau/\tau_c$, and τ_c is the characteristic decay time of the electric field autocorrelation function. Another commonly considered statistical model for $g_1(\tau)$ is given in Equation 5.8.

$$g_1(\tau) = \exp(-\sqrt{x}) \tag{5.8}$$

where $x = \tau/\tau_c$, and τ_c is the characteristic decay time of the electric field autocorrelation function. This Diffusive model (Equation 5.8) is typically used to describe intensity fluctuations due to diffsion of particles [1].

The applicability of the Lorentzian (Equation 5.6), Gaussian (Equation 5.7) and Diffusive (Equation 5.8) statistical models in describing intensity fluctuations in blood flow were investigated using the experimentally obtained $|g_1(\tau)|^2$ from an artery and the parenchyma.

Figure 5.4b shows the experimentally computed $|g_1(\tau)|^2$ values (Blue circles) fit to the three statistical models (Equations 5.6, 5.7 and 5.8). The experimental values were computed from intensity traces obtained from the arterial region shown in Figure 5.4a. This procedure was repeated for measurements obtained from the parenchyma (Figure 5.5). Figure 5.5b shows the experimentally computed $|g_1(\tau)|^2$ values (Blue circles) fit to the three statistical models (Equations 5.6, 5.7 and 5.8). Again, the rate of decay of the electric


Figure 5.4: Experimentally computed $|g_1(\tau)|^2$ from an artery fit to three statistical models. (a) Circle represents region from which DLS measurement were obtained. (b) Experimentally obtained $|g_1(\tau)|^2$ fit to Equations 5.6, 5.7 and 5.8, with residuals. The Lorentzian (Equation 5.6) and Diffusive (Equation 5.8) fit the data best.

field autocorrelation time is found to be faster over the artery (Figure 5.4b) than over the parenchyma (Figure 5.5b).

Two observations can be made from the fits and residuals in Figures 5.4 and 5.5. One, the experimentally obtained $|g_1(\tau)|^2$ is best represented by the Lorentzian model (Equation 5.6). The Diffusive model (Equation 5.8) is also found to be a good fit to the data. However, it is typically used in the diffuse (multiple scattering) regime, and hence is not an appropriate fit for DLS and MESI experiments. Two, the Gaussian statistical model (Equation 5.7) is not found to fit the experimental data. The fits with Equation 5.7 were



Figure 5.5: Experimentally computed $|g_1(\tau)|^2$ from the parenchyma fit to three statistical models. (a) Circle represents region from which DLS measurement were obtained. (b) Experimentally obtained $|g_1(\tau)|^2$ fit to Equations 5.6, 5.7 and 5.8, with residuals. The Lorentzian (Equation 5.6) and Diffusive (Equation 5.8) fit the data best.

characterized by poor R^2 values and large residuals. From these experiments, it can be concluded that the Lorentzian statistical model is most appropriate for *in vivo* blood flow measurements using DLS based techniques.

5.4.2 Influence of ischemic stroke on the electric field correlation function

As described in Section 5.3 a large blood flow change was induced by occluding the branches of the middle cerebral artery in a mouse. Figure 5.6 shows speckle contrast images (5ms exposure duration) obtained before and after the ischemic stroke. These images are similar to the strokes induced



Figure 5.6: Speckle contrast (5ms exposure duration) images illustrating the occlusion of an artery due to ischemic stroke. (a) Baseline (b) Post Stroke

in Section 4.10. The artery, from which DLS measurements were obtained previously (Figure 5.4), that is clearly visible in the speckle contrast image in Figure 5.6a, gets completely occluded by thrombosis (Figure 5.6b). This drastic change in blood flow should change the rate of decay of the electric field autocorrelation function.

Figure 5.7 shows the experimentally computed $|g_1(\tau)|^2$, from the region over the artery (Figure 5.4a), under baseline and post stroke conditions. The experimental measurements (circles and squares) are fit (solid lines) to the Lorentzian statistical model for $g_1(\tau)$ (Equation 5.6). Two observations can be made from Figure 5.7. One, the Lorentzian model fits the experimental data well, with high goodness of fit values and low residuals. This observation is



Figure 5.7: Comparing experimental $|g_1(\tau)|^2$ measurements obtained after stroke (squares) to baseline (circles) measurements. This data is fit to the Lorentzian statistical model (Equation 5.6). The model fits the data well as evidenced by low residual values.

consistent to good fits obtained in two previous cases (Figures 5.4b and 5.5b). Two, $|g_1(\tau)|^2$ obtained after the stroke is found to decay at a rate which is slower than baseline conditions. This is decrease in the rate of decay is representative of a decrease in blood flow. Slower decay of the field correlation function leads to larger τ_c values, which corresponds to lower values for blood flow.

5.4.3 Comparing τ_c measures estimated using the MESI technique with DLS estimates

One of the motivations for performing simultaneous DLS and MESI measurements of blood flow is to compare the absolute values of τ_c estimated by the two techniques. This comparison is important to establish if the MESI technique has potential to obtain quantitative baseline measures of blood flow.

The intensity traces obtained from 16 regions (3 animals, multiple regions of the cortex, i.e. arteries, veins and parenchyma), were processed to compute $|g_1(\tau)|^2$ using the techniques described in Section 5.3. These experimentally computed $|g_1(\tau)|^2$ were then fit to the Lorentzian statistical model for the electric field autocorrelation function (Equation 5.6), to produce estimates of correlation times τ_c . τ_c was also estimated in these regions using the simultaneously recorded MESI data, using the techniques outlined in Section 5.3 and Chapter 3. These correlation time values were compared to τ_c estimates obtained using single exposure (5ms) LSCI measurements. The results of this comparison are listed in Table 5.1. In general, while not being exactly equal, the τ_c estimates obtained using the MESI technique were found to be similar to and in the same range as the τ_c estimates obtained using DLS measurements. The τ_c estimates obtained using LSCI techniques were found to be at least 1 order of magnitude different from the DLS τ_c estimates.

#	Region	Condition	τ_c -MESI	τ_c -LSCI	τ_c -DLS	R^2
1	Artery	Baseline	0.561ms	0.035ms	0.7ms	0.9227
		Post Stroke	8.9ms	0.149ms	$3.7\mathrm{ms}$	0.9474
2	Vein	Baseline	1.1ms	0.035ms	1.2ms	0.9793
		Post Stroke	3.8ms	$0.096 \mathrm{ms}$	$2.3 \mathrm{ms}$	0.9815
3	Parenchyma	Baseline	6.6ms	0.126ms	2.3ms	0.8967
		Post Stroke	$15.6\mathrm{ms}$	0.183ms	$6.0 \mathrm{ms}$	0.8975
4	Artery	Baseline	0.087ms	0.009ms	0.197ms	0.827
		Post Stroke	$6.8 \mathrm{ms}$	0.089ms	$4.9 \mathrm{ms}$	0.9071
5	Vein	Baseline	0.269ms	0.012ms	0.6ms	0.9644
		Post Stroke	3.0ms	0.081ms	$2.7\mathrm{ms}$	0.9229
6	Parenchyma	Baseline	0.679ms	0.035ms	1.0ms	0.8027
		Post Stroke	$6.1 \mathrm{ms}$	0.115ms	$6.0\mathrm{ms}$	0.9519
7	Artery	Baseline	0.178ms	0.017ms	0.33ms	0.8761
		Post Stroke	7.9ms	0.408ms	$7.3 \mathrm{ms}$	0.9928
8	Parenchyma	Baseline	2.0ms	0.173ms	1.9ms	0.8542
		Post Stroke	6.8ms	$0.352 \mathrm{ms}$	9.0ms	0.9870

Table 5.1: Comparing absolute values of τ_c estimates from LSCI, MESI and DLS measurements

5.5 Discussion

The quantitative accuracy of DLS based flow measurement techniques such as laser speckle and laser Doppler, ultimately depends on an appropriate choice for the form of the electric field autocorrelation function. The model is the final and most important step in relating τ_c to a qualitative measure such as speckle contrast. Figure 5.4 and 5.5 suggest that the Gaussian model is not a good fit (Goodness of fit: ~ 22%) for *in vivo* measurements. One probable reason for this could be the absence of large blood vessels in the field of views tested. The Gaussian model is most applicable for bluk flow, where the velocities can be expected to be normally distributed. This situation cannot be satisfied in the parenchyma or in smaller arteries.

The Lorentzian model (Equation 5.6, Goodness of fit:~ 90%) and the Diffusive model (Equation 5.8, Goodness of fit:~ 90%) were both found to accurately represent the experimental data. The Lorentzian model has been widely used in many DLS based flow measurement techniques including Laser Speckle Contrast Imaging [13, 28, 38, 71] and Laser Doppler Imaging [6]. Its appropriateness, was also verified using Diffuse Correlation Spectroscopy by Cheung et. al. [68]. The results from Figure 5.4 and Figure 5.5 reinforce the observations made in the aforementioned studies and in Section 4.2.3. The results from Figure 5.4 and Figure 5.5 also suggest that the Diffusive model is equally effective. However, this model is most appropriate only for systems with diffusive backscattering geometries. Since LSCI and MESI are assumed to operate in the single scattering regime, it would be more appropriate to use Equation 5.6 as a statistical model for the electric field autocorrelation function, $g_1(\tau)$.

A comparison of absolute values of τ_c listed in Table 5.1 show that the MESI technique hold promise for quantitative baseline measures of blood flow. Since the same statistical model of $g_1(\tau)$ was used to obtain τ_c estimates using the LSCI, MESI and DLS technique; the same correlation time value can be expected from all three techniques. The results from Table 5.1 suggest that the τ_c estimates using the MESI technique and the DLS technique are similar but not equal. However, upon closer inspection, if only those measurements where

Region	Situation	τ_c - MESI	τ_c - DLS	R^2
Vein	Baseline	$1.1 \mathrm{ms}$	1.2ms	0.9793
Vein	Post Stroke	$3.8\mathrm{ms}$	$2.3 \mathrm{ms}$	0.9815
Vein	Post Stroke	$3.0\mathrm{ms}$	$2.7\mathrm{ms}$	0.9229
Artery	Baseline	$0.56\mathrm{ms}$	$0.7\mathrm{ms}$	0.9227
Artery	Post Stroke	$7.9\mathrm{ms}$	$7.3 \mathrm{ms}$	0.9928
Parenchyma	Post Stroke	$6.8\mathrm{ms}$	$9.0\mathrm{ms}$	0.9970

Table 5.2: Comparing τ_c estimates with high goodness of fit

the DLS estimates were obtained with high goodness of fit were considered, an interesting trend can be observed. Table 5.2 lists a subset of Table 5.1 which satisfy this condition.

The τ_c estimates in Table 5.2 clearly show that when both MESI and DLS techniques fit to their respective data points well, the correlation time estimates converge. Considering that DLS measurements can be considered as the standard of comparison for estimates of blood flow, these results suggest that the MESI technique can indeed produce quantitative baseline measures of blood flow.

The absolute values of τ estimated using the MESI technique and the DLS technique fall within the range of correlation time measures for microcirculation obtained by Bonner et.al. [6], using laser Doppler measurements. This provides additional evidence in favor of the potential for the MESI technique to obtain absolute measures of CBF.

This claim however needs to be validated by rigorous experimentation. The primary reason for the lack of consistency in the goodness of fits of the DLS measurements is limited signal to noise of the DLS measurements. As mentioned earlier, the use of a piece of glass shifts the optical axis of the light coupled into the PMT (Section 5.2). Since any such piece of glass would have two reflective surfaces, reflections from both surfaces are imaged onto the optical fiber. This presence of a phantom image could possibly restrict the signal to noise of the DLS measurements. This phantom image can possibly be removed by using another conjugate imaging plane, and spatially filtering out the phantom measurement point.

In conclusion, for *in vivo* blood flow measurements, a negative exponential model corresponding to a Lorentzian distribution of velocities, is the most appropriate statistical model to represent the speckle electric field autocorrelation function. Also, the absolute values of τ_c estimated using the MESI technique and the DLS technique were found to be similar. This suggests that the MESI technique has the potential to obtain quantitative baseline measures of τ_c and hence blood flow. Measurements from an improved DLS instrument are required to confirm this claim.

Chapter 6

Pilot Clinical Application: LSCI imaging of blood flow during neurosurgery

In the previous sections (Section 4.2) and in numerous other research publications [13,15,17,24,26,45,46,69], blood flow images obtained using Laser Speckle Contrast Imaging (LSCI) have been used to study a wide variety of physiological phenomena like ischemic strokes, spreading depressions and functional activation. Optical blood flow measures can also be used for a number of clinical applications, even though the lack of penetration depth of optical wavelengths restrict their use to surface vasculature. Similar to their applications in research studies, clinical measurements of blood flow can provide vital information about tissue perfusion and viability (for diagnosis) and vessel functionality (during surgery). In this Chapter, results from a pilot clinical study (n = 2), where Laser Speckle Contrast Imaging (LSCI) was used to image cerebral blood flow in humans during neurosurgeries, are presented.

6.1 Motivation

Being restricted to measurements on the surface or a few millimeters below, optical measures of blood flow have the potential to have a huge impact in the treatment of surface wounds and during surgical procedures. For example, monitoring blood flow in burn victims can help quickly estimate tissue viability. Currently, the standard method of assessing burn depths, and hence categorizing burns, is a combination of visual inspection and patient (or witness) questionnaires. This method is empirical and hence unreliable [73]. Accurate assessment of tissue viability is important to evaluate and direct treatment options, estimate survival rates and calculate volume of fluid that has been lost, and hence volume needed for resuscitation. Some studies have shown LSCI's potential for estimating burn depths [74, 75]. LSCI can also be used during surgical recession of burnt tissue, to estimate the blood flow of healthy tissue.

Another area where LSCI can be useful is the real time monitoring of blood flow during surgeries, especially brain surgeries. Monitoring Cerebral Blood Flow (CBF) during surgery is important during a variety of surgical procedures. In procedures like clipping of aneurysms or vessel bypass, CBF measurements can help assess if blood flow has returned to pre-surgical baseline levels. In other procedures like tumor resections, CBF measurements can help assess post surgical tissue viability. CBF measures can also be used to identify motor and sensory centers in the cortex in procedures that require functional localization. While the importance of imaging CBF during surgery is well known there are not many procedures that can produce real time CBF images with minimum interference to the surgery. For this reason, *in vivo* measurements of blood flow during surgeries have been underutilized as a surgical tool. Traditional flow measurement techniques cannot be used for intra-operative imaging because they are usually intrusive. Intra operative flow measurement techniques like Magnetic Resonance Angiography and Diffusion Weighted Imaging are difficult to use since they need special operating room conditions [76].

The most promising technique that is currently being used for imaging CBF is Indocyanine Green (ICG) based angiography [10,77]. ICG angiography involves injecting a dye, Indocyanine Green into the blood stream, exciting it with an infrared light source and imaging the fluorescence intensity with a camera. While commercial systems have managed to integrate ICG measurements into existing microscope systems, since the contrast mechanism for imaging blood flow is fluorescence intensity, these measurements can at best detect the presence or absence of blood flow in a vessel and hence are not quantitative. Additionally, the clearing time of ICG from the blood stream is less than 5 minutes. Therefore only a few measurements can be made, since repeated injections of large volumes of dye are not practical. Lack of quantitative measures, long acquisition times, and the need to repeatedly inject a dye to make measurements are the major limitations of ICG-based angiography. Hence, there is a clear need for a technique to image CBF during surgery, which (a) is minimally intrusive to the surgical procedure, (b) can produce quantitative measures of changes in blood flow, and (c) has real time capabilities.

Optical flow measurement techniques have the potential to satisfy these requirements. Laser Doppler techniques can provide quantitative flow information, but are traditionally restricted to single point measurements with probes which are cumbersome to handle. Recently, Raabe et. al. demonstrated CBF measurements intra operatively using a full field laser Doppler instrument [78] albeit without real time capabilities. LSCI measurements combine the advantages of being non contact, easy to use and posses the ability to produce quantitative measures of flow changes. Recently, Hecht et. al. demonstrated its use in measuring CBF during surgery with a commercial laser speckle instrument [34]. While this was the first intra operative use of LSCI, the use of a commercial instrument had its disadvantages. Primarily, the instrument while functional, was not integrated with the surgical procedure, and hence needed additional setup time. Further, the use of the instrument to measure blood flow was intrusive towards the actual surgery. Finally, the real time capabilities of the instrument were minimal. In this chapter, results are presented from a pilot clinical study, where LSCI images are obtained during neurosurgery using an existing neurosurgical microscope.

6.2 Instrumentation and Experimental methods

Laser Speckle Contrast Imaging (LSCI) automatically lends itself to address two of the three desired characteristics for measuring cerebral blood flow during surgeries. LSCI's ability measure flow changes quantitatively has been well documented [13, 26]. While LSCI has been shown to underestimate large changes of flow [38], such situations rarely arise during neurosurgeries. During surgeries, LSCI would be used obtain spatial maps of blood flow distributions and estimate small changes in blood flow due to functional activation, both of which fall within the capabilities of the technique. Additionally, high temporal resolutions that can be obtained with LSCI instruments, make them inherently real time. New processing techniques [39] have also enabled real time computation of blood flow measures.



Figure 6.1: Zeiss Pentero OPMI microscope adapted to perform LSCI (a)Camera is attached to an existing viewing port in the microscope and a diode laser is attached to an add-on laser adapter. Microscope's existing controls make it easy for the surgeon to position and focus it. (b)Schematic of the instrumentation. The laser adapter delivers light which is co-located with the microscope's light source.

Hence, the primary design consideration for an LSCI instrument that would be used as a neurosurgical tool, was to develop an instrument that would offer minimal interference to the surgical procedure. To achieve this, an existing Zeiss Pentero OPMI neurosurgical microscope (Carl Zeiss Meditec Inc., USA) was retrofitted with a camera and a laser, to obtain speckle images. Figure 6.1a shows a picture of the microscope with the camera and laser attached. An 8 bit camera (Basler 602f, Basler Vison Technologies, Germany) was connected through a camera adapter to one of the existing viewing ports of the microscope. This enabled the use of optics and mechanics present in the microscope to produce, and focus the image on the camera. As an added advantage, since existing microscope controls were used, the instrument was easily operated by the surgeons, who were already familiar with its functioning and use the microscope for their surgeries. To illuminate the brain, a laser diode ($\lambda = 660$ nm, P = 130mW, Thor Labs Inc, Newton, NJ) was connected to a microscope add-on laser adapter. The adapter delivered laser light through a pair of relay lenses to a mirror mounted on the inside of the microscope illumination and viewing aperture. The mirror has steering mechanisms in place to direct the laser beam over the field of view. This setup is illustrated in the schematic in Figure 6.1b.

The existing microscope had built in mechanical servos to move and position the microscope head over the field of view, and had the flexibility to position the microscope head at an appropriate angle for imaging. Figure 6.2 illustrates this flexibility in a picture of the microscope head positioned over the field of view of the cortex at an appropriate angle.

Cables for the camera data, camera exposure and the laser were run



Figure 6.2: Adapted neurosurgical microscope in use during imaging. The microscope has sufficient flexibility to angle the microscope head towards the field of view

through the microscope extension arm (and hence not interfering with the surgical procedure) to a computer and laser diode controller placed on a cart. The camera and laser were hence remotely controlled, away from the sterile surgical area, while a surgeon handled, operated and used the microscope in the sterile field. Additionally, the patient's ECG was recorded by the computer as a reference point for the measurements. The ECG waveform was obtained from existing monitoring systems present in the operating room. It was used to develop an ad-hoc filter, that would filter out the effect of pulsatile blood



Figure 6.3: The adapted microscope in use during an imaging session. The microscope has been covered in a sterile drape. The surgeon is seen viewing the images on a remote monitor after positioning the microscope over the field of view.

flow in the brain. Since additional equipment was not brought in to perform CBF imaging, the time taken for these measurements is reduced significantly and the surgical procedure is not interrupted. This is a significant advantage of this system over previous attempts [34]. Additionally, the temporal resolution of this instrument was found to be almost 10 times greater than previous efforts [34].

The primary focus of the pilot clinical study (n = 2) was to use the mod-

Patient	Surgery	Surgery Details	Imaging Details
#1,M,52yrs	Tumor	Right temporal lobe	Imaged before tumor
	Resection	(just under the surface)	recession
#2,M,33yrs	Tumor	Right hemisphere	Imaged after tumor
	Resection		recession

Table 6.1: List of experiments. Both imaging sessions were during tumor recession surgeries. Imaging of the cortex was done before the tumor was resected in the first patient, and after the tumor was resected in the second patient. The flexibility of the instrumentation can be seen in its ability to image the cortex in a variety of different cortical regions.

ified surgical microscope to image cerebral vasculature during neurosurgery, and demonstrate the feasibility of using LSCI in a minimally obtrusive way to obtain intra operative CBF measures. Hence, a blood flow change was not induced or imaged in these experiments, and the pilot study was used to obtain baseline images of CBF. All experiments were performed during tumor resection surgeries performed by Dr. Douglas J. Fox and Dr. Mark G. Burnett of the NeuroTexas Institute at the St. Davids Hospitals in Austin, TX. All experimental procedures were approved by the Institutional Review Boards of the University of Texas at Austin and St. Davids Hospitals.

Before each experiment, the microscope was setup, initialized and then draped using a standard surgical microscope drape as shown in Figure 6.3. Only the surgeon operated the microscope after it was draped. After the surgeon completed the craniotomy over the surgical area, images were obtained using the microscope for about 15 minutes. Table 6.1 lists the two experimental procedures, their specific case details and imaging details. All measurements were completed within 15 minutes. This comprised of ~ 5 minutes for setup and ~ 10 minutes for imaging. The setup time includes the time it took for the surgeon to position the microscope head over the surgical field of view, focus the camera and direct the laser using the builtin positioning system. For each patient, about 3000 laser speckle images were obtained at 5ms exposure duration per imaging session (which typically lasted for 1 minute). These sessions were repeated depending on the time available for measurements. During each imaging session, the surgical area was flushed with physiologically sterile warmed saline, to reduce specular reflections. Some experiments included a real time display of speckle contrast images, while most of the data processing was done after the experiment.

6.3 Results

Raw speckle contrast images obtained in the experiments were converted to speckle contrast images using Equation 2.1. 30 of these speckle contrast images were then averaged and the averaged speckle contrast image was then converted into a more quantitative measure of blood flow, correlation time τ_c using Equation 2.13. The Bandyopadhyay expression was used to compute τ_c , since it is more mathematically rigorous than the common Briers expression (Equation 2.8). Since the exact value of β was unknown, and because this value can change with magnification, β was assumed to be equal to 1 for computing the correlation time τ_c . Admittedly, this assumption is not entirely accurate. However, for the purposes of the pilot study, it is a good



Figure 6.4: Average of 30 LSCI images obtained using the adapted neurosurgical microscope from the cortex of a 52yr old male patient before tumor resection. Field of view of the image is $\sim 2 \text{cm} \times 1 \text{cm}(a)$ Speckle contrast image showing cerebral vasculature. Regions with higher flow are represented by lower speckle contrast values. (b) Correlation time map (visualized on a logarithmic scale). The dark spots on the images correspond to specular reflection from the cortex.

starting point to assume that the size of the speckles match the size of camera pixels.

The speckle contrast image obtained by imaging the cerebral cortex of the first patient is shown in Figure 6.4a. These images were obtained before the tumor was resected and are primarily surface vasculature. Despite flushing the surgical field of view with saline, some spots of specular reflection are visible. Specular reflection causes camera to saturate in a localized area in the raw image, and hence manifest themselves as dark spots in the speckle contrast image. Figure 6.4b shows the spatial correlation time map (displayed on a logarithmic scale) computed using Equation 2.13 as described earlier. Vessels of different sizes and flow rates can be clearly visualized in both the speckle contrast and correlation time maps.

Figure 6.5 shows the speckle contrast and correlation time maps obtained from the cerebral cortex of patient 2. While vasculature is still clearly visible, the quality of the image is not as good as in Figure 6.4a. The primary reason for this is that these images were obtained after the tumor was removed. Consequently, these images are not from surface vasculature, but rather from deeper cortical regions, exposed by the resection of brain tissue during surgery. Additionally, this specific patient had previous history of brain surgery, which coupled with the current surgery caused bruising in the brain.

Additionally, since the cortical regions imaged were deeper, imaging was performed at a steep angle, utilizing the flexibility of the microscope (Figure 6.2). However, this led to illumination inhomogeneities as visible in the top right hand corner of Figure 6.5a. These regions being on the surface of the cortex, were not within the depth of field of the instrument, and hence no speckle was recorded (leading to saturation in the speckle contrast image). Figure 6.5b shows the correlation time map computed using Equation 2.13. Vasculature and blood flow are spatially resolved in both the speckle contrast and correlation time spatial maps.



Figure 6.5: Average of 30 LSCI images obtained using the adapted neurosurgical microscope from the cortex of a 33yr old male patient after tumor resection. Field of view of the image is $\sim 2 \text{cm} \times 1 \text{cm}(a)$ Speckle contrast image showing cerebral vasculature. Regions with higher flow are represented by lower speckle contrast values. (b) Correlation time map (visualized on a logarithmic scale). The field of view that was imaged in this experiment was fairly deep in the cortex. The sides of the cortex is visible as in the top right corner of the image characterized an absence of vasculature.

6.4 Discussion

One of the challenges in clinical measurement of blood flow is the presence of artifacts, due to the pulsatile nature of blood flow in physiological systems. This effect is especially pronounced in humans, because of the presence of a more advanced circulatory system coupled with the presence of large blood vessels with significantly higher flow rates. Figures 6.6b shows the time course of measured correlation times (τ_c) from a region over a large vessel (Region 1 in Figure 6.6a) from cortical imaging of patient 1. This time course



Figure 6.6: (a) Speckle contrast image (5ms exposure duration) obtained from patient 1. (b) Average correlation time measure in spatial region 1, synchronized with ECG recorded from patient 1. The pulsatile nature blood flow is seen in the τ_c time course. The fluctuations are found to be synchronized with the heart beat.

is superimposed and synchronized in time with the ECG that was recorded from patient 1. From Figure 6.6b, the pulsatile nature of blood flow can be observed. It is also observed that these pulsations are synchronized in time with the heat beats. Similar observations were made from cortical blood flow measures obtained from patient 2. These measures are shown in Figure 6.7a and 6.7b.

These pulsatile blood flow measures can be tolerated for basic visual inspection of cerebral blood flow, and would not hinder applications such as imaging blood flow during aneurysm surgeries and vessel bypass procedures. Also, these artifacts would not affect large blood flow changes, since such a



Figure 6.7: (a) Speckle contrast image (5ms exposure duration) obtained from patient 2. (b) Average correlation time measure in spatial region 2, synchronized with ECG recorded from patient 2. The pulsatile nature blood flow is seen in the τ_c time course. The fluctuations are found to be synchronized with the heart beat. A similar trend to those viewed in patient 1 is observed here.

change would shift the baseline of correlation time measure by an order of magnitude. However, applications such as functional activation are typically associated with a $\sim 5 - 10\%$ change in blood flow. In such cases, these fluctutions in correlation time measures would limit the signal to noise of estimated blood flow changes. Hence, there is a need to eliminate these fluctuations.

Since these fluctuations in the blood flow are synchronized and collocated with the heart beat, an ad-hoc ECG de-trending filter was designed to filter out these fluctuations. This filter is similar to an adaptive filter technique used for MRI time series data designed by Deckers et. al. [79]. The filter was designed based on the time of a camera exposure relative to the ECG cycle. The camera exposure signals and the ECG waveform were recorded during the imaging session. These waveforms were used to determine the actual times at which each speckle image was obtained, and the actual times of each heart beat. A filter function, the correlation time value as a function of 'normalized time', was generated from these times. The 'normalized time' was defined as the time of image acquisition relative to the nearest heart beat. For example, if the camera was found to have exposed at a time t_{frame} seconds, and the nearest heart beat (of width t_{RR} seconds) was recorded at time t_{beat} seconds, then the normalized time for this frame would be:

$$t_{normalized} = \frac{t_{frame} - t_{beat}}{t_{RR}} \tag{6.1}$$

Figure 6.8 illustrates this filter function. The blue circles are the experimentally measured τ_c values as a function of $t_{normalized}$, while the red line is the filter function generated by averaging the experimental values using a moving average filter of width 100. Each value of the filter function represents the average ('artificial') rise or fall in correlation time at a specific time in the ECG cycle. Finally, the filter was applied to the data, by estimating the normalized time of each data point, subtracting the corresponding filter value and adding back the median correlation time value.

Figure 6.9 shows the time course over a 10 second duration (to illustrate filtering), of correlation time τ_c measured from cortical blood flow imaging of patient 1. Figure 6.9a is the time course from Region 1 (Figure 6.6a) - a large vessel, while Figure 6.9b is the time course from Region 2 (Figure 6.6a) - the



Figure 6.8: Ad-Hoc ECG de-trending filter designed from experimental values

parenchyma.

Two observations can be made from the raw (unfiltered) time course measurements. One, the correlation time measures in the parenchyma is approximately two orders of magnitude lower than those measured from the vessel, which is around expected lines. Two, fluctuations are present in measurements from both regions. The fluctuations in region 1 can be explained by arterial blood flow. However, pulsatile arterial supply to the brain causes the entire brain to 'throb' with every heart beat. This motion is especially pronounced when the skull has been removed, like it is during neurosurgery.



Figure 6.9: Time course of correlation time (τ_c from (a) Region 1 and (b) Region 2; obtained from patient 1 (Figure 6.6a). The red trace in both time courses represent the result of correlation time course filtered to remove the pulsatile effect of blood flow. The rise and fall of τ_c due to the heart beat is removed by filtering, while changes in the baseline τ_c values are retained.

This motion explains the fluctuations observed in the time course of correlation time from region 2. The red curve in both cases, represent the filtered time courses of correlation times. The ad-hoc filter is found to work well in removing the ECG trend from experimental data. Similar trends were observed in measurements made on patient 2. These measurements are shown in Figures 6.10a and b.

6.5 Future Directions

By incorporating the LSCI instrument into an existing surgical microscope, the instrument has been made minimally intrusive to the surgery as well as being easy for surgeons to use. Additionally, high temporal resolutions



Figure 6.10: Time course of correlation time (τ_c from (a) Region 1 and (b) Region 2; obtained from patient 2 (Figure 6.7a). The red trace in both time courses represent the result of correlation time course filtered to remove the pulsatile effect of blood flow. The rise and fall of τ_c due to the heart beat is removed by filtering, while changes in the baseline τ_c values are retained.

and real time capabilities make it a potential useful tool for neurosurgeries. Results from the pilot study have demonstrated the feasibility of using LSCI to obtain blood flow images during neurosurgeries. The next logical step is to use the instrument to measure flow changes in a clinical and surgical setting. An ability to measure blood flow changes due to functional activation would be useful as a guidance tool for surgeons. Functional activation measurements are routinely performed in neurosurgeries, with limited spatial resolution using electrodes, when surgeries need to be performed near the sensory or motor cortex areas. LSCI, with its high spatial and temporal resolution, can be used to monitor the associated blood flow changes due to functional activations, similar to how its use in animal studies [20, 26]. The filtering techniques need to be perfected before these measurements, since blood flow changes due to functional activation is usually $\sim 5-10\%$, and pulsatile blood flow can possibly be a large source of noise.

The biggest advantage of the design of the clinical LSCI instrument (Figure 6.1) is its modular nature. It would be easy to adapt this instrument to other microscopes and help extend the study. A multi-institution study would provide access to a larger number of patients and a wider variety of surgical procedures. However, the following instrumentation and processing improvements are needed before a wider study can be undertaken.

6.5.1 Processing improvements

Eliminating motion artifacts is the most important processing improvement that needs to be done. Figure 6.11 shows speckle contrast images obtained by averaging upto 2000 speckle contrast images from data obtained from patient1. The image displays a ghosting effect, leading to the vasculature not being properly spatially resolved. This is due to the field of view shifting spatially, due to a combination of motion of the microscope and motion of the cortex. To measure blood flow trends in a vessel accurately, this motion artifact needs to be eliminated or reduced using automatic segmentation procedures.

The ad-hoc filtering technique described in Section 6.4 has been implemented as a post processing technique, primarily because of the need to generate a unique filter function for each region of interest. It would be desirable for the filtered output be displayed in real time, so that the surgeon can



Figure 6.11: Speckle contrast images from patient 1. Increasing the number of frames for averaging reduces image sharpness. Motion between frames contributes to this blurring.

visualize blood flow changes during the surgery. Hence the filtering method needs to be refined and made more robust, so that a generic function can be used to measure blood flow changes in real time.

6.5.2 Instrumentation improvements

Speckle contrast images from Figures 6.4a and 6.5a, show a need to eliminate specular reflection. Flushing the surgical area with saline helps in reducing the effect of specular reflection, but is inadequate. Incorporating a polarizer in front of the camera can drastically cut down specular reflection. Polarized laser light that diffuses through the cortex, loses its polarization and emerges backscattered as randomly polarized. The polarization of the laser light that is specularly reflected does not change. By aligning axis of the polarizer to be cross polarized with respect to that of the laser, the camera is more sensitive to photons that have diffused through the tissue.

There is a need to move toward a fiber based illumination setup. Laser diodes typically have an elliptical beam profile. By coupling the laser light into a fiber, and using the output of the fiber in the laser adapter, a circularized beam can be obtained. A circular beam provides homogeneous illumination and can possibly prevent illumination artifacts such as the ones in Figure 6.5a. Researchers have shown that it is possible to perform speckle imaging through an optical fiber [80].

Finally, the instrument can be adapted to perform Multi Exposure Speckle Imaging (MESI). The MESI technique would improve the quantitative accuracy of blood flow changes. While it would be difficult to mount an AOM on the microscope head, the AOM and other associated MESI instrumentation can be placed on an isolated optical bench on a cart with light from the first diffraction order being coupled into an optical fiber. The other end of the optical fiber can be used with the add-on laser adapter in Figure 6.1.

Chapter 7

Conclusions and Future Work

This dissertation presents a new Multi Exposure Speckle Imaging (MESI) technique to quantitatively image cerebral blood flow *in vivo*. The MESI technique was designed to remove two significant limitations of the more traditional Laser Speckle Contrast Imaging (LSCI) technique. By using a new instrument, the MESI technique addresses LSCI's tendency to underestimate large changes of flow. By improving mathematical models used to describe speckle contrast and by using these models with the instrument, the MESI technique enables quantitative imaging of cerebral blood flow through a thinned skull.

In Chapter 4, controlled flow experiments on microfluidic tissue-simulating phantoms demonstrated that estimates of flow changes obtained using the MESI technique were more accurate than LSCI. The MESI technique was found to extend the range over which relative flow estimates were linear, addressing one of the limitations of LSCI. Additionally, the MESI technique was found to consistently estimate flow changes in the presence of static scatterers. These experimental results demonstrate the ability of the MESI technique to overcome LSCI's critical limitations.

The ability to quantitatively image large reductions in blood flow make

the MESI technique an ideal flow measurement technique for imaging and modeling ischemic strokes. In Section 4.2 the MESI technique was used to image a large reduction in blood flow caused due to an ischemic stroke. The MESI technique was found to be more accurate in imaging the stroke than LSCI. Estimates of reduction in blood flow obtained using the MESI technique were found to be quantitatively accurate. Additionally, these estimates of blood flow changes were found to be unaffected by the presence of the thinned skull. These experimental results demonstrated the ability of the MESI technique to obtain consistent and accurate estimates of blood flow changes *in vivo* through a thinned skull.

In Chapter 5, *in vivo* estimates of cerebral blood flow obtained using the MESI technique were compared to dynamic light scattering (DLS) measurements. The estimates of blood flow obtained using the MESI technique were found to be more accurate and quantitative than LSCI. These experiments demonstrated the MESI technique's potential for obtaining baseline blood flow measures. The ability of the MESI technique to image baseline blood flow is significant, because it enables comparing blood flow measures across animals and studies.

Finally, in Chapter 6 the potential for clinical applications of optical blood flow measurements was demonstrated. In a pilot clinical study, Laser Speckle Contrast Imaging (LSCI) was used to image cerebral blood flow during neurosurgery, by adapting an existing neurosurgical microscope. The pilot study demonstrated the simple instrumentation required for intra-operative imaging of blood flow. Adapting an existing microscope provided flexibility in operation and enabled surgeons to use the instrument with little or no previous experience in optical imaging.

7.1 Future work

Multi Exposure Speckle Imaging (MESI) is an exciting new technique for quantitative imaging of blood flow *in vivo* at good spatial and temporal resolutions. While the MESI technique in its current stage of development is fairly robust, a few instrumentation improvements can be made that would enable better characterization of blood flow *in vivo*.

While τ_c measures are reproducible estimates of blood flow, the exact relation between τ_c and velocity of scatterers v is unknown. It is possible that τ_c and v are related through the concentration and hence the scattering coefficient μ'_s of the moving particles. There is also a school of thought [6] that the correlation times depend only on the speed of the scatterers and not on their concentration. Resolution of this question will enable researchers to determine if τ_c is a measure of the speed of blood (in mm/sec) or the rate of blood flow (uL/min). This question can be addressed by performing flow measurements with different concentrations of polystyrene microspheres using the microfluidic sample (Figure 3.4a).

Advancing the development of the MESI instrument requires an understanding of the influence of camera bit depth on the accuracy of τ_c estimates. The bit depth of the camera sensor influences the camera dynamic range. A 16 bit camera would have higher dynamic range than an 8 bit camera. A higher dynamic range would enable the MESI instrument to better discriminate a fluctuating speckle signal from a static background. Additionally, higher bit depth cameras usually have better noise performances, while 8 bit cameras are relatively inexpensive. The trade off between dynamic range and cost can be evaluated by obtaining and comparing blood flow measurements simultaneously using the MESI technique, with an 8 bit and a 16 bit camera.

Almost all DLS based flow measurement techniques (like LSCI and MESI) assume that a photon has scattered of only one moving particle [6, 38]. While this assumption is true in most *in vivo* cases, a careful study of its limits has not been undertaken. The extreme case of a photon scattering off multiple moving scatterers requires an alternate approach - Diffusing Wave Spectroscopy [61]. Studying the validity of this assumption in an experiment would be difficult because it is practically impossible to keep track of each photon individually. Monte Carlo simulations have long been used to perform experiments that are difficult to set up in the laboratory [61]. In such a simulation, the path travelled by photons in tissue is statistically modeled in a computer. The behaviour of the photon in the tissue, i.e absorption, scattering, scattering angles, trajectory etc., are governed by probabilities dependent on the tissue optical properties. Monte Carlo simulations can be used to estimate the depth sensitivity of blood flow measurements with LSCI and MESI techniques. These simulations can also help numerically compute the shape of the electric field autocorrelation function, by tracking the change in

momentum of each photon for each scattering event.

With its ability to image blood flow changes quantitatively without interference from static tissue elements, the MESI technique can be used to image blood flow in a wide variety of applications, where previously LSCI could not be used. For example, the MESI technique can be used to evaluate the ability of stroke alleviating drugs and techniques. In such a study, the MESI technique's ability to estimate baseline blood flow measures would help better characterize baseline stroke conditions and hence the efficiency of the drug or technique in returning the animal back to baseline conditions. Additionally, baseline blood flow measures would enable comparison of results across animals and more importantly across species.

In this dissertation, the ability of the MESI technique to image blood flow changes due to an ischemic stroke was demonstrated. Another situation where the accurate estimation of large changes in blood flow would be crucial is in the imaging of Cortical Spreading Depressions (CSDs). As described in Chapter 3, LSCI estimates of flow changes during CSDs have been contradictory. It has been suggested that the hemodynamic response to a CSD depends on baseline blood flow conditions. The ability to estimate baseline blood flow measures make the MESI technique a more robust method to image the hemodynamic response of a CSD.

Producing consistent estimates of blood flow through a thinned skull make the MESI technique an attractive option in studies where a craniotomy cannot be performed to image the cortex. For example, the MESI technique
can be used to image long term blood flow changes in mice due to diseases such as brain tumors, alzhimer's disease and parkinson's disease. The MESI technique would be able to account for the change in thickness of the skull as the mouse ages.

Finally, one of the significant advantages of LSCI for imaging flow is its ability to provide flow maps at high spatial and temporal resolution. The MESI instrument adds to this by providing quantitative flow information. The intra-operative LSCI instrument can be used to provide neurosurgeons with blood flow measures that could be useful surgical information. One primary concern during brain surgery is damage to healthy brain tissue, which can cause severe loss of motor or sensory ability in the patient. Consistent blood flow is a good indicator of the health of tissue. During invasive and risky brain surgery, constant monitoring of blood flow would help significantly reduce the risk of accidental injury and also help assess the effectiveness of surgery. Thus the LSCI/MESI instrument can be used as a surgical aid. One example of where quantitative measures of blood flow would be clinically useful is during aneurysm surgeries.

An aneurysm is a collection of blood in a blood vessel (more commonly arteries) caused by disease or weakening of the vessel wall, causing a bulge in the vessel. The bulge in the blood vessel is prone to rupture, which can lead to death. Rupture of an aneurysm in the brain (cerebral aneurysm) is especially dangerous since it can lead to intra-cranial hemorrhage. One of the treatment methods for an aneurysm is to perform surgery to clip the aneurysm at its base



Figure 7.1: Schematic demonstrating aneurysm surgery

and isolate it from the blood vessel. Hence if the aneurysm ruptures, it does not cause the blood vessel to collapse. The clip also prevents the aneurysm from growing larger. This is schematically represented in Figure 7.1. During surgery, the primary concern for the surgeon is to clip the aneurysm without restricting blood flow in the artery. The intra-operative LSCI instrument can be used to monitor blood flow at regions in the blood vessel, upstream and downstream from the aneurysm, as schematically represented by Regions 1 to 3 in Figure 7.1. This information will enable the surgeon to judge if the clip is appropriately placed, and that the aneurysm is blocked while the vessel still has blood flow comparable to pre-surgical levels.

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Vita

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