Handheld Laser Speckle Imaging System for Neonatal Blood Flow Imaging

THESIS

submitted in partial satisfaction of the requirements
for the degree of

MASTER OF SCIENCE

in Biomedical Engineering

by

Ryan Thomas Farraro

Thesis Committee:
Professor Bernard Choi, Chair
Professor Anthony Durkin
Professor Elliot Botvinik

2015
DEDICATION

To my parents

"You'll never know if you can fly unless you take the risk of falling."
-Nightwing
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ABSTRACT OF THE THESIS

Handheld Laser Speckle Imaging System for Neonatal Blood Flow Imaging

By

Ryan Thomas Farraro

Master of Science in Biomedical Engineering

University of California, Irvine, 2015

Professor Bernard Choi Irvine, Chair

Background and Objectives:

Although abnormal blood flow is linked to clinical risk in neonates, clinicians do not integrate flow measurements into routine monitoring in the neonatal intensive care unit (NICU). We and other research groups have previously demonstrated the ability of Laser Speckle Imaging (LSI) to measure changes in blood flow. We postulate that LSI, in a clinic-friendly form factor, can provide important hemodynamic information in the NICU. Here, I describe initial efforts to develop a handheld LSI system and deploy it to UC Irvine Medical Center NICU.

Study Design/Methods:

I designed and fabricated a handheld LSI system. I assessed the system performance of handheld LSI versus a traditional mounted configuration. I collected multi-user data (n=7) to assess the variation in flow measurements attributed to user performance. I collected data from two in-vivo occlusion models. Finally, I acquired data from an on-going NICU based clinical study.

Results:
I demonstrate that the handheld nature of the device has little effect on the flow measurement
sensitivity. Flow phantoms were characterized using both bench top and handheld LSI
instruments. I show that measurements of flow from these phantoms are not user dependent. I
demonstrate that the handheld LSI system measures trends in flow that are similar to those
reported previously. Lastly, I observed a difference between the maximum range of blood flow
measured at the abdomen in necrotizing enterocolitis neonates and a healthy population
(P=.015). These results suggest that LSI may be capable of providing additional screening for
neonates with suspected gut pathology.
INTRODUCTION

Microvascular blood flow is known to be an important indicator of illness severity in neonates $^{1,2}$. For example, several neonatal diseases, including polycythemia, necrotizing enterocolitis, and sepsis, are all linked to changes in the microcirculation $^{3,4,5}$. However, a gap in the knowledge base is the lack of data on normal microcirculatory parameters such as blood flow.

Researchers have studied neonatal microvascular flow with a variety of tools such as laser Doppler Imaging $^{1,2,9}$ and plethysmography $^{6,7,8}$. With these techniques, important cardiovascular phenomena such as adaptation to extrauterine life $^8$, the thermoregulatory response $^9$, and the response to feeding $^7$ have all been discovered. Unfortunately, these imaging techniques are cumbersome and disrupt the flow of routine care in the NICU. These shortcomings limit the frequency at which data can be collected and leave a large gap in the understanding of neonatal hemodynamics.

**Laser Speckle Imaging (LSI)** is a simple, non-invasive imaging technique that provides quantitative and qualitative assessment of blood flow in real-time $^{10}$. While extensive clinical data support the feasibility of using LSI for microcirculatory measurements in adults $^{28,29,30,34}$, such data does not exist for term and preterm infants. Despite this promise, it is not currently used for routine hemodynamic monitoring in neonates. This lack of use is presumably due to an absence of standardized, point-of-care instrumentation, making it difficult to obtain normative data in a clinical setting. In a clinic-friendly form factor, LSI has the potential to provide important hemodynamic information in the NICU that cannot be obtained by the current technologies.

In order to bridge the gap between research promise and clinical reality, this thesis will describe the design and validation of a handheld, point-of-care LSI device. I will also present LSI
data and results obtained from an exploratory pilot study of neonates at the UC Irvine Medical Center Neonatal Intensive Care Unit (NICU). The three specific aims for this project are 1.) build a handheld LSI device 2.) validate design with in-vitro and in-vivo experiments, and 3.) Deploy the handheld device to a NICU.
CHAPTER 1: Background and Significance

1.1 The Importance of Obtaining a Normative Perfusion Parameter in Neonates

Aberrations in microvascular function in neonates are indicative of clinical risk \(^1,2\) (Fig. 1). While peripheral blood flow has been studied using occlusion plethysmography \(^6,7,8\) and laser Doppler imaging \(^1,2,9\), limitations to these techniques, including long acquisition times and contact measurements, have prevented any widespread use for monitoring blood flow in the NICU. Several neonatal diseases have well documented accounts of decreased blood flow, yet no cohort study has been undertaken to investigate monitoring blood flow as a tool to either help diagnose or monitor treatment effects. The following sections will discuss neonatal disease states that are known to have significant hemodynamic changes and would potentially benefit from the addition of blood flow monitoring to routine care practices.

![Fig. 1. Diagram of neonatal diseases and the regions of interest in which they induce vascular changes.](image)
1.1.1 Neonatal Sepsis

Sepsis is a global epidemic that kills approximately 4 million neonates annually. The disease occurs when bacteria enters the blood stream of a neonate, causing a systemic immune response. Doctors commonly treat before confirming neonatal sepsis due to a high mortality rate and a relatively long diagnostic process (~1 day). The disease is only confirmed through either a positive blood or spinal fluid culture. In addition to a long diagnostic period, the clinical signs for neonatal sepsis are very non-specific including body temperature changes, vomiting, and reduced movement. These factors lead to a low specificity of the initial prediction.

Treatment is given prior to the results of the cultures to improve chance of survival. Sepsis is treated through the use of broad-spectrum antibiotics due to the uncertainty of the exact type of bacterial strain affecting the neonate. The low specificity associated with the disease has led to an overuse of antibiotics that causes antibiotic resistance in some patients. Some antibiotics are also ototoxic which can lead to sensorineural hearing loss.

Neonatal sepsis is associated with major cardiovascular changes. A systemic immune response occurs in the neonate that leads to widespread vasoconstriction due to the bacteria circulating in the blood stream. This leads to hypoperfusion and can cause pulmonary hypertension in some cases. Along with the systemic perfusion changes, a decrease in vascular density in the sublingual region has been found to be predictive of sepsis.

Monitoring perfusion would allow for both detection of a systemic decrease in perfusion along with perfusion changes in the sublingual region. Detection of these changes has the potential to provide better screening parameters to increase specificity of initial diagnosis. Several studies have recently attempted to use orthogonal polarization spectral imaging to detect early changes in
the microvasculature associated with sepsis 17,18. While this technique has proven useful for detecting abnormal vasculature, the lack of quantitative measurements limits their impact due to subjective analysis.

1.1.2 Necrotizing Enterocolitis

Necrotizing Enterocolitis (NEC) is the most common gastrointestinal disease that affects neonates. NEC causes an ischemic event in the mesenteric artery, inducing necrosis of the intestines 19. The disease can lead to perforation of the bowels and eventually death if left untreated. Little is known about the pathology of the disease making it difficult to diagnose. Surgery is available once perforation of the gut has been confirmed through x-ray; however the disease is still associated with a 15-30% mortality rate once the bowels have perforated 20. 90% of the proven cases of NEC affect babies born prematurely, and the incidence rate is inversely proportional to weight. Some studies have reported the incidence to be as high as 13.5% in the lowest weight group of premature newborns (<1150g) 21.

The addition of the capacity to make localized quantitative blood flow measurements would help identify regions of compromised blood flow and potentially diagnose NEC before the condition worsened. It is expected that local areas of compromised blood flow exist due to the necrotic tissue that localizes in the gut during the progression of the disease. Identifying these regions would also enable improved screening and planning for surgery.

Blood flow monitoring of the intestines would be useful during surgery to assess viability of tissue. Bowel resectioning surgery can be employed to remove portions of the bowel that are necrotic once perforation of the gut is confirmed. Neonates who have too much of their bowels
removed are at risk of short bowel syndrome which can lead to malnutrition\textsuperscript{22}. Therefore, it is important to remove the minimum amount of tissue during surgery.

\subsection{Polycythemia}

Polycythemia is a relatively common disorder in neonates (2-4\%) in which the venous hematocrit levels rise above 65\% \textsuperscript{23}. Due to the increase in hematocrit, the viscosity of the blood also increases. This increase in viscosity can lead to a number of problems if left untreated including a systemic decrease in oxygen saturation and a decrease in perfusion. These cardiovascular effects have been linked to neurodevelopment problems later in life \textsuperscript{24}.

Polycythemia is known to be asymptomatic, making it difficult to make an initial diagnosis. The disease is treated by reducing the hematocrit percentage of the baby through use of a partial exchange transfusion (PET). While this technique is proven to reduce the hematocrit content, it is questionable whether prolonged outcome is advantageous to the neonate’s health. PET also has the potential to lead to further health risks \textsuperscript{25}.

Monitoring of perfusion in neonates afflicted by polycythemia may help provide feedback during treatment of the disease. Because low blood flow is the major risk factor associated with polycythemia, identifying areas of compromised blood flow would allow clinicians to better screen their patients for treatment. With the addition of blood flow monitoring, PET could be investigated by monitoring if any therapeutic effect of increased perfusion exists after treatment.
1.2 Significance

Due to the asymptomatic nature associated with many neonatal diseases, clinicians in the NICU oftentimes must diagnose and treat preterm infants based solely using subjective information. While multiple criteria are used to determine if neonates have diseases, oftentimes diagnoses of preterm infants are difficult to classify into a single disease state, resulting in both high false positive and false negative rates. For any neonatal disease state, earlier treatment is important, as diagnosis within the first 24 hours of incidence greatly increases the rate of survival. For neonates that are misdiagnosed, longer hospital stays lead to risk of infection: 20% of neonates, whom are hospitalized for 48 hours or more, develop at least one infection; of those 20%, the mortality rate is increased by 6% \(^26\). The cost of this unnecessary, extended stay is estimated to add $23 billion dollars to healthcare costs annually \(^27\). Therefore, there is a critical need for non-invasive, point-of-care diagnostic tools such as LSI to address the timely diagnosis of neonates.

LSI is a non-invasive imaging technique that can obtain maps of microvascular blood flow in real-time. LSI provides both a qualitative and quantitative assessment of blood flow in-vivo. LSI is an established technique, used in a multitude of adult human studies \(^28\,29\,30\,34\). No study has been undertaken to use LSI to measure blood flow in neonates.

Forty years ago, pulse oximetry was a new optical technology and now it is an inexpensive point-of-care device that is in nearly every NICU around the world. For pulse oximetry, the absorption spectra of blood was an untapped resource, for LSI, it is skin microvascular blood flow. LSI has the potential to provide a wealth of hemodynamic information that is not currently available with other technologies.
CHAPTER 2: Instrumentation Design

2.1 Laser Speckle Imaging

2.1.1 Intro to Laser Speckle Imaging

Laser Speckle Imaging (LSI) is a wide-field imaging technique that is used to monitor blood flow changes in both a laboratory and clinical setting. LSI has been used for point-of-procedure applications such as monitoring vasculature during open brain surgery\(^{28}\) and point-of-care applications such as intraoperative monitoring of port-wine stain therapy\(^{29}\). Despite advances in the technology, LSI remains widely unused in a clinical setting mainly due to lack of standardized, clinic-friendly instrumentation.

The two main advantages of LSI over the current gold standard for measuring blood flow, laser Doppler imaging, are that 1.) blood flow maps can be obtained in real time and 2.) the images can be taken using low-cost components. LSI obtains blood flow maps on the order of milliseconds, while laser Doppler imaging requires scanning of a laser beam on the tissue surface and takes several minutes to generate images. To reduce the costs of LSI, researchers have studied the use of consumer-grade platforms such as color cameras and webcams\(^{30}\)\(^{31}\).

2.1.2 Speckle Physics

LSI is based on a phenomenon of highly coherent light in which an interference pattern is created as a single wavelength of light scatters off of a rough surface. This granular interference pattern, called a speckle pattern, is created due to phase differences of the coherent light as the individual photons scatter off different surface heights of a rough surface. The
resultant pattern is a series of dark and light speckles that appears static or unchanging (Fig. 2a). When the surface or subsurface that the coherent light reflects off of is moving, whether through bulk movement or Brownian motion, a time-varying speckle pattern can be observed that causes a blurring effect (Fig. 2b). The degree of blurring is dependent on the imaging system used to capture the speckle pattern for a given exposure time.

2.1.3 History of the Speckle Contrast Equation

In 1975, Goodman took a statistical approach to analyzing the speckle pattern and studied the spatial variance of the pattern as it varied with time. The decorrelation of the pattern was found to be proportional to the speed of optical scatterers causing the decorrelation. Goodman derived the relationship between speckle contrast and the spatial variance using the normalized autocorrelation function to produce the speckle contrast equation,

\[ K = \left( \int_0^T 2 \left(1 - \frac{\tau}{T} \right) |g(\tau)| d\tau / T \right)^{1/2} \]
where K is the speckle contrast, τ is the speckle correlation time, T is the exposure time, and 
g(τ) is the autocorrelation function 32.

The speckle contrast equation provided an important milestone in developing the field of speckle contrast imaging for biomedical applications. Goodman’s work led to the first use of LSI to measure blood flow in 1981 when Ferchers and Briers derived their speckle contrast equation,

\[ K = \frac{\sigma}{< I >} \]

where K is the speckle contrast, σ is the standard deviation, and <I> is the mean intensity, to obtain blood flow maps of the human retina 10.

2.1.4 Development of the Speckle Flow Index

Relative changes in speckle contrast are derived primarily by the ratio of the exposure time of an imaging system to the speckle correlation time, \( \frac{T}{\tau} \). To derive their speckle contrast equation, Ferchers and Briers assumed that the \( 1 - \frac{1}{\tau} \) term in Goodman’s equation could be approximated to unity 10. This approximation created some discrepancy when attempting to characterize physiological blood flow, as it was only reliable for large values of \( \frac{T}{\tau} \) 42. This led researchers to develop a flow index based on \( \frac{T}{\tau} \), calculating τ directly using a Newtonian iterative method to integrate the normalized autocorrelation function. To simplify this, Cheng and Duong approximated that \( 1/K^2 \) was proportional to the flow index, \( \frac{T}{\tau} \) 43. Ramirez-San-Juan et. al. furthered this effort by deriving the speckle flow index (SFI) equation,

\[ SFI = \frac{1}{\tau} = \frac{1}{TK^2} \]
where SFI is the speckle flow index, which satisfied both a Lorentzian and Gaussian definition of the correlation time\textsuperscript{35}.

### 2.1.5 Factors that Affect Speckle Contrast

While speckle contrast is reduced by light reflected from moving optical scatterers, internal image system factors also can affect speckle contrast measurements. The statistics of a speckle pattern are unique to the imaging system that observes the pattern. Speckle size is one such parameter that is governed by the equation

\[
S = 2.44\lambda(1 + M)f/#
\]

where S is the speckle size, \(\lambda\) is the optical wavelength, M is the magnification and f/# is the f-stop\textsuperscript{34}. This equation is important because the speckles must be at least twice the size of the image sensor pixels to satisfy the spatial Nyquist criterion\textsuperscript{39}.

Shutter speed or exposure time also can change to tune the dynamic range of a given system. To maximize sensitivity for applications, one must analyze relative speckle contrast changes at multiple exposure times to determine the linear dynamic range.

External factors such as noise also affect the dynamic range of LSI. Noise has the potential to cause a detrimental effect to measuring relative speckle contrast changes. If the noise of an LSI system primarily consists of a DC signal, the noise floor of the system will be raised, reducing the dynamic range of the device. Detection sensitivity depends on the ratio of the noise floor to the signal. Theoretically if the noise floor is smaller than the magnitude of the signal, sensitivity to the signal should be retained.
2.2 Design Approach & Requirements

The Handheld LSI device is designed to obtain point-of-care blood flow maps in a clinical setting (Fig. 3). In order to bridge the gap between use in the laboratory and use in the clinic, the handheld LSI device should follow three key design principles: 1.) portable and lightweight, 2.) usable with minimal training, and 3.) uses low-cost components. I now will describe in detail the efforts made to address each principle.

![Fig. 3. (a) Tablet-based LSI system. (b) Representative example of system in use to collect LSI images of a patient’s hand.](image)

2.2.1 Design Principle #1: Portability

In order to follow the first principle, the handheld device will be designed with components that are combined to a single apparatus. The components will be lightweight (<5lbs) and will be designed with a GUI that can be used while holding the device. The device will be designed with either a touch or voice controlled GUI to accomplish this.
2.2.2 Design Principle #2: Usability

In order to follow the second principle, settings such as the gain, f/#, exposure time, focus, and magnification should be pre-set for the user to insure consistency across measurements. The handheld LSI system will be designed in such a way that the user settings will enable non-experts to effectively employ the instrument. The GUI will be designed such that processed images will be easily viewable and saved to insure convenient retrieval.

To further satisfy the second principle, the handheld LSI system should incorporate a visual feedback system to ensure that all images are acquired in focus. The feedback system shall be designed such that there is no interpretation needed by the user.

2.2.2 Design Principle #3: Affordability

To satisfy the third principle, the handheld LSI device should be designed with a low power (<5mw) laser diode in the visible spectrum, a small monochrome or color CCD/CMOS camera, and a commercial-grade processor. A tablet computer or other handheld computer with supported touch or voice controls should be used to facilitate image acquisition and display of results. The camera used should be small enough to be handheld, and should have an output and input that are supported by the computer. The camera should also have a spectral sensitivity that encompasses the wavelength used by the laser diode.
2.3 Handheld LSI System Description

Fig. 4. System diagram of the handheld LSI system. The laser diode is angled at a slight angle from the lens to assure that the illumination spot is always in focus.

The following section will discuss components chosen to embody the design principles in section 2.2. The system diagram shown above (Fig. 4) was the basis for the design of the handheld LSI device.
2.3.1 Tablet

A tablet computer was chosen for the design of the handheld LSI system due to its portability. The tablet chosen was a Surface Pro 2 (Microsoft, Redmond, WA, USA). The Surface Pro 2 included a 4th generation i5 processor (2.7Ghz) and 8gb of RAM to enable real time processing of LSI images. The device weighed 2 pounds and was designed to be handheld. The tablet had a USB 3.0 port allowing a large selection of cameras available to be coupled with the setup.

2.3.2 Camera

An 8-bit monochrome CCD camera from Point Grey (CMLN-13S2M-CS, Point Grey, Richmond Canada) was chosen for the device. The camera had a wide spectral sensitivity (400-700nm) that allowed it to be coupled with several illumination sources. The pixels in the imaging sensor were small (3.75μm). Smaller pixels were ideal as they assured that the speckle size could be at least twice the pixel size to satisfy the spatial Nyquist sampling criterion (see section 2.1.5) \(^{39}\). The small, compact design (25.5mm x 44 mm x 41 mm, 37g) allowed coupling with the handheld setup. The Point Grey camera drivers were directly controllable with MATLAB’s Image Acquisition toolbox. The Camera used a USB mini b-to-USB a connector facilitating integration with the Surface’s USB port.

2.3.3 Illumination Source

A 650nm, 5mw laser pointer (Five Star Inc., West Chester, PA, USA) was chosen as the illumination source of the system. This wavelength was chosen to mitigate effects of absorption
by blood, yet still be in the visible spectrum. The class IIIa laser pointer provided an inexpensive alternative to a scientific grade laser. The low power and visible light emission made it ideal for a NICU, as higher power laser sources presented potential hazards to infants. The laser pointer could only be toggled on or off with a switch, so it had to be converted into a binary laser pointer. A solution was implemented by using a zip tie around the button. This allowed the laser pointer to be turned continuously on or off by rotating the zip tie to compress the button. A piece of scotch tape was placed in front of the laser pointer to diffuse the beam over a larger surface area.

2.3.4 GUI Development

The touch-based GUI was developed using MATLAB (Fig. 5). Because the device was intended to be usable by non-experts, all camera settings (gain, exposure, gamma, frames per second, number of images) were set as constants. The controls of the GUI allowed for five imaging modes: Color, Brightfield, Raw, Speckle Contrast, and Speckle Flow Index.

The raw mode was the primary mode to acquire images. The exposure time for this mode was set to 5ms due to the results of sensitivity testing discussed below (sections 3.11 and 3.12). In order to better visualize the speckle pattern on the screen, the “imadjust” function was applied to the raw image in MATLAB to provide increased contrast. Due to the added processing step, a decreased frame rate was observed during the speckle contrast and speckle flow index modes.

Processed Images could be visualized using the ‘Process Image’ button. A speckle flow index map of the data, indicated by the root name, is displayed in the center of the GUI. This feature was added to provide instantaneous feedback for clinicians attempting to use the
device. Along with the SFI map, the average SFI value for each set was also saved to enable quantitative assessment of blood flow.

![MATLAB GUI](image.jpg)

**Fig. 5.** MATLAB GUI as seen during a live feed prior to acquisition. A raw speckle image is displayed in the center of the GUI.

### 2.3.5 Visual Feedback

A visual feedback system was implemented to insure that all images acquired by the handheld device were in focus. The feedback system was created by having a field of view that is larger than the illumination spot of the laser pointer. This feature was designed to enable a user to determine that the device is positioned the same distance from the target, for each set of images. This was done by attaching the camera orthogonal to the tablet while orienting the laser at a slight angle to the lens (see Fig. 4). With this alignment, the center of the laser spot moved laterally in the displayed 2-d image as the user moved the device closer to or farther from the target. The device was characterized to ensure that the region of interest is always in focus as long as the illumination spot was on the screen. The characterization was verified by imaging a
solid phantom in a mounted configuration at several distances away from the lens (12-24cm). Three images were taken at each distance to produce error bars. A sequence of 250 images was obtained during each acquisition set of the phantom, and the mean contrast was calculated by obtaining the average contrast in an ROI of each image. The results (Fig. 6) indicate that speckle contrast is independent of distance away from the phantom. This finding supports the requirement that the visual feedback system be effective at determining an in-focus image.

2.4 Processing Scheme

In order to facilitate automatic processing of images, a processing scheme to locate a reliable ROI had to be created. This problem arose due to the field of view being larger than the illumination spot. The automation was performed by calculating the center of mass, or image moment, of the SFI map and then choosing an ROI that was centered on that point. Before the
centroid could be calculated, the image first had to be converted to a binary image. The threshold for converting the image to a binary image was calculated using the “graythresh” command in MATLAB. This command identifies a threshold range by minimizing the intra-class variance between the pixels with the highest and the lowest SFI. By minimizing the intra-class variance, the calculated threshold has the highest probability of eliminating background pixels when producing a binary image. Once the image was converted into a binary image using a high-pass filter, the center of mass of the binary image was calculated. A 50x50 pixel ROI was then created that was centered at the coordinates of the center of mass. The ROI was chosen and the average speckle contrast was obtained for each image. The average speckle contrast for each acquisition set was finally obtained by averaging again over all images.

2.5 Design Conclusions

The handheld LSI device was engineered with components and considerations that make it viable for point-of-care use in a clinical setting. The device was built with low cost and lightweight components that were controllable with a GUI designed specifically for non-experts to use. A visual feedback system was successfully implemented that allows for repeatability of handheld LSI measurements.
CHAPTER 3: Handheld Device Validation

3.1 Effect of Exposure Time on Handheld Motion

Because relative speckle contrast changes are resultant from the ratio of exposure time and motion, it is expected that handheld motion artifacts have an effect on obtaining speckle contrast measurements. The first test in validating the handheld system was to investigate how motion artifacts affected the ability to characterize \textit{in-vitro} phantoms at different exposure times. The initial hypothesis was that longer exposure times were expected to degrade the ability to characterize phantoms due to an overall increase in non-sample related blurriness. The goal of this testing was to identify a range of exposure times that would be sensitive to changes in speckle contrast but not sensitive to handheld motion.

3.1.1 Effect of Handheld Motion: Solid Phantom

The first test was performed by imaging a solid PDMS and titanium dioxide phantom at multiple exposure times. The guiding theory was that because the constituents in a solid are relatively static, the major source of changes in contrast would be primarily due to handheld motion. We hypothesized that if we imaged the solid phantom at decreasing exposure times, there existed a point in which handheld motion artifacts would begin to decay. The phantom was imaged using the handheld LSI system in both a handheld and mounted configuration. A sequence of 250 images was obtained during each acquisition set of the phantom, and the mean contrast was calculated by obtaining the average contrast in an ROI of each image. Analysis of the data was performed by calculating the percent error of the handheld measurements with the mounted data as the gold standard. The results (in Fig. 7) show that the percent error drops
approximately 36% from the images taken at a 5ms to 1ms exposure time. This finding supports
the hypothesis that lower exposure times reduce the effect of handheld motion.

![Graph showing motion error vs exposure time](image)

**Fig. 7.** Percent error of handheld measured speckle contrast compared to contrast measured using a benchtop LSI configuration. The percent error can be seen to drop off as the exposure time is decreased. The error bars represent the standard deviation of the percent error for each pair of handheld to mounted speckle contrast values (n=3).

### 3.1.2 Effect of Handheld Motion: Liquid Phantom

While a static phantom produces a speckle pattern that is unchanging in time, a liquid phantom produces a time varying speckle pattern due to the movement of the scattering centers within the liquid matrix. A system with inherent motion better represents the conditions of a physiological system. The guiding hypothesis for testing a liquid phantom was that an imaging
subject with inherent motion would be less sensitive to handheld motion than a subject that is not changing with time.

Tissue-simulating liquid phantoms were created with varying absorption coefficients (0 to 0.03mm\(^{-1}\)). The phantoms consisted of 1% Intralipid mixed with varying amounts of India ink by weight (0.01875%-0.075%) to mimic that of physiological tissue \(^{33}\). A single user acquired images from each of the four phantoms, with the device in both a handheld and a mounted configuration at 6 different exposure times. To compare the handheld data to the mounted data, we used logarithmic regression, and fit a line to the mounted data. We then calculated the sum of square errors from the handheld data, and obtained the goodness-of-fit for each phantom measurement. The R\(^2\) coefficients (0.72-0.87) (Fig. 8), using the handheld data, indicate a good fit allowing us to conclude that, with a single, experienced user, handheld motion artifacts had a negligible effect on the sensitivity of the device while imaging a system with inherent motion.
3.1.3 Validation: Invariance to User

The second test in validating the handheld system was done to ensure that results obtained using the device remain minimally sensitive to the experience of the person using the device. Because the device is handheld, it had to be shown that any user, regardless of the degree of motion, would be able to obtain accurate measurements. The test was performed by
using the same four tissue simulating liquid phantoms described in section 3.1.1. The phantoms used in the exposure time experiment were again used for the multi-user experiment. Seven volunteers used the handheld device to image the same four liquid phantoms described above. The exposure time $T$ was set at 5ms and the aperture at $f/22$. Each user was instructed on how to use the device and collected 3 sets of images from each phantom. After each user acquired images from all four phantoms, the device was mounted in a fixed position and an identical number of images were acquired.

We used a two-way ANOVA test to assess if the average measured speckle contrast differed significantly ($p<0.05$) as a function of the concentration of India ink, on the configuration, and on any interaction between concentration and configuration. Based on the ANOVA test, we did not identify any significant difference ($p>0.05$) between the measured speckle contrast values using either a handheld or mounted configuration. We also did not identify any significant effect from the interactions ($p>0.05$) between the configuration used during acquisition and the concentration of India ink. Lastly, we identified a significant difference ($p<0.05$) between the measured speckle contrast values of each concentration of India ink. The data (Fig. 9) indicate that the device was able to characterize each phantom (0.0%-0.075% India ink by weight in a 1% Intralipid solution) in both a mounted and handheld configuration with no significant loss in sensitivity when multiple users were introduced.
3.1.4 The Use of Motion Sensors for Artifact Correction

The following section will discuss an attempt to use built-in motion sensors to correct for handheld motion associated with the device. The Surface Pro 2 tablet is built with an embedded accelerometer and gyroscope. The initial hypothesis was that motion sensors data would be inversely proportional to speckle contrast values.

The raw motion sensor data was accessed using code written in C# using the Microsoft.Devices.Sensors library and imported into the MATLAB acquisition code. On execution of the code, the x, y, and z component of both the acceleration and rotational velocity were saved to a text file. The code was then executed after each raw image was acquired using the “dos” command in MATLAB to synchronize acquisition of both parameters.

Fig. 9. Comparison of multi-user (n=7 users) handheld measurements of speckle contrast with those obtained using a mounted device at a single exposure time. The mean contrast value was taken for each user’s set (n=3 sets per user) and combined to create each bar. The measured speckle contrast values were similar with both configurations. The average speckle contrast for both configurations can be seen to decrease with decreasing % India ink. Error bars represent the standard deviation of the mean contrast value (n=21 for each bar)
To test the relevance of the motion sensor data to LSI, motion sensor data was collected during LSI acquisition of a solid phantom at different exposure times. 1250 LSI images were obtained of the phantom at each exposure time (1ms, 5ms, 10ms, 20ms, 30ms). The raw components of the motion vectors were converted into magnitude. For acceleration, the inertial force was calculated by subtracting the gravitational force from the magnitude of the sensor reading.

A linear regression was performed to assess if a correlation existed between speckle contrast and the magnitude of the motion sensor data. The results of the linear regression showed that there was no statistical significance at any exposure time for a correlation between speckle contrast and motion sensor data. The best fit lines were graphed with the raw data (Fig. 10) and can be shown to indicate no intrinsic trend between the two parameters.

The lack of correlation with the motion sensor data is most likely due to the sensitivity of the motion sensors used. The sensitivity limitations of the sensors can be seen in the large number of recorded null measurements, suggesting that the sensors missed several handheld events. The gyroscope showed the most promise in correcting motion artifacts in LSI measurements, as the linear regression lines all showed negative trends. In conclusion, a gyroscope has the most promise in detecting changes in speckle contrast associated with handheld motion, but a more sensitive motion sensor must be implemented to further investigate this claim.
Fig. 10. (top) Speckle contrast plotted versus rotational velocity. The best fit lines are shown for each exposure time, but no statistically significant trend was found. The best fit lines show a weak inverse relationship between speckle contrast and rotational velocity. (bottom) Speckle contrast plotted versus inertial acceleration. The best fit line is shown, but no statistically significant trend was found.
3.2 Proof of Concept in a Preclinical In-vivo Model

3.2.1 Brachial Artery Reactive Hyperemia Testing

To demonstrate the device’s ability to provide a quantitative assessment of in vivo blood flow dynamics, we performed a reactive hyperemia experiment on one human subject in which we used a sphygmomanometer to apply a pressure of 180mmHg for one min to temporarily occlude the brachial artery. The handheld LSI device was then used to collect raw speckle images from the palm of the subject. We then released the pressure and continued to collect speckle images to visualize the hyperemic response to the occlusion challenge. We then selected a region of interest to obtain the average SFI for each frame. Here we show the ability of handheld LSI to detect both physiological increases and decreases in flow that is comparable to previous hyperemia challenges in turbid media (Fig. 11).

![Graph](image)

**Fig. 11.** Plot of average SFI versus time measured on a human palm during a brachial artery occlusion and release. A pressure of 180mmHg was applied at the 20 second mark and was held until the 95 second mark. The pressure was then released, causing a hyperemic response.
3.2.2 Finger Occlusion: Identifying Ischemic Regions

An occlusion model was used to assess the qualitative performance of the device to detect regions of compromised blood flow. This model consisted of a fingertip occlusion induced by wrapping a rubber band around the knuckle of one finger in a volunteer. To facilitate comparison with previously published data \(^3^4\), we converted speckle contrast values to maps of Speckle Flow Index (SFI) using the simplified speckle imaging equation, \(SFI = (2TK^2)^{-1}\). \(^3^5\)

We illuminated the entire hand to obtain a spatial blood flow map of the subject’s hand. With handheld device operation, we observed a clear decrease in SFI in the occluded fingertip (Fig. 12).

![Speckle Flow Index (SFI) image of a hand during fingertip occlusion with a rubber band. We collected the raw speckle images using the LSI device in handheld mode. The finger with the rubber band has a clear decrease in SFI, corresponding to a decrease in blood flow that is distinct from the other regions of the hand.](image)

Fig. 12. Speckle Flow Index (SFI) image of a hand during fingertip occlusion with a rubber band. We collected the raw speckle images using the LSI device in handheld mode. The finger with the rubber band has a clear decrease in SFI, corresponding to a decrease in blood flow that is distinct from the other regions of the hand.
3.3 Validation to Implementation

Chapter 3 has discussed the validation tests performed to assure that handheld motion artifacts have a negligible effect on measuring blood flow changes. The initial hypothesis was that a gyroscope and accelerometer could be used to correct for motion artifacts; however experimental results revealed that more sensitive motion sensors are needed to verify this claim.

*In-vitro* experiments performed with solid phantoms have shown that while handheld motion artifacts affect the accuracy of speckle contrast measurements, this accuracy can be improved by decreasing the exposure time.

In liquid phantoms, the inherent motion of the scattering constituents reduces the effect of handheld motion artifacts on measured speckle contrast values. This observation allows both single and multiple users to characterize the speckle contrast of liquid phantoms with varying absorption coefficients with similar accuracy to that of a mounted system.

Lastly, the *in-vivo* experiments described have shown that the handheld LSI device is capable of providing both qualitative and quantitative assessment of changes in blood flow.

The next step in validating the handheld LSI device is implementing the device in a clinical setting. The following chapter will discuss the implementation of the handheld LSI device to obtain LSI measurements of neonates at the UC Irvine medical center NICU.
CHAPTER 4: Laser Speckle Imaging of Term and Preterm Neonates

4.1 Longitudinal Changes of Bloodflow in Term and Preterm Neonates

In this study, thirty-one neonates were recruited who were admitted to the UC Irvine medical center NICU (IRB Protocol #NCT01483703). In order to widen the number of patients admitted to the study, there was no limitation on age or health. The babies were imaged using the handheld LSI device at various time points throughout their stay in the NICU. Dr. Omid Fathi, the lead neonatologist, also assisted in acquiring LSI images. Two regions, the abdomen and the heel, were selected for imaging because they were known to have alterations in microvascular blood flow during disease states. The nature of the study was intended to be exploratory and thus a wide range of experiments were undertaken to understand the feasibility of using LSI in a clinical setting. The data was analyzed to assess the importance of this data in improving patient care.

4.2 Laser Speckle Imaging of Healthy Neonates

4.2.1 Hourly Blood Flow Changes of a Neonate in a Single Day: Case Report

In order to assure that major blood flow changes seen in the longitudinal data were not due to measurement artifacts, an experiment was performed to measure the blood flow changes that occur in a healthy neonate over a period of nine hours. The initial hypothesis was that the variations of the LSI measurements would be primarily due to changes in blood flow and not measurement artifacts. The handheld LSI device was used to obtain hourly SFI measurements of the abdomen on a single neonate. The heart rate and respiration rate of the infant were also monitored to assess if the SFI values were linked to other vital parameters. The results were analyzed by comparing SFI to both respiration rate and heart rate in the time domain. SFI
measurements were obtained from the abdomen every hour and averaged to obtain a single value for each time point. The results were plotted in a time series plot with a second axis for the two vital parameters.

Figure 13 depicts a double Y plot of the hourly abdominal SFI measurements with both heart rate and respiration rate. The abdominal SFI values can be seen to follow a similar trend to heart rate. The SFI values do not follow the same trend as respiration rate, suggesting that breathing artifacts have no significant effect on measurements. We did not assess the data using a linear regression, as blood flow is not directly dependent on heart rate. Vascular resistance of blood vessels can reduce blood flow even with an increased heart rate. In conclusion, because the abdominal SFI values followed a similar trend to that of heart rate and not respiration rate, the results from this experiment suggest that the SFI values are of cardiovascular relevance and not due to motion artifacts.

**Fig. 13.** Hourly abdominal SFI values versus heart rate and respiration rate in a single neonate. (top) SFI of the abdomen plotted with a second y-axis with heart rate. The fluctuations in SFI can be seen to follow a similar trend to that of heart rate suggesting that SFI values hold some cardiovascular relevance. (bottom) SFI of the abdomen plotted with a second y-axis with respiration rate. No trend with respiration rate and SFI is seen.
4.2.2 Blood Flow Measurements After Feeding: Case Series

Past research using duplex Doppler Sonography has indicated that there is an increase in blood flow to the neonatal mesenteric artery 30 minutes after feeding\textsuperscript{36}. To further verify that the LSI device can detect changes in blood flow, two neonates were recruited to study this claim. LSI images were taken of the abdomen and heel, before and up to forty minutes after feeding for each neonate. The first case seen is a healthy, premature infant. Two feedings events were recorded on two separate days for this case.

The SFI maps obtained from the handheld LSI device depict blood flow maps of the neonatal heel and abdomen. The SFI maps are on a color scale in which blue represents lower blood flow and red represents higher blood flow. Figure 14 depicts the SFI maps obtained before and after feeding. For both feeds, an increase in SFI can be seen in the abdomen and not the heel. Because SFI changes are primarily seen in the abdominal region, the LSI data suggests that the changes in SFI are due to blood flow increasing to the mesenteric artery during feedings. The time that the peak value occurs coincides with previous literature\textsuperscript{36}.
Fig. 14. SFI Maps of the abdomen and heel of a healthy premature infant before and after feeding for two separate trials. Trial 1 was taken at the 27th day of extrauterine life, while trial 2 was taken on the 31st day of extrauterine life. The SFI value in the abdomen was observed to increase at time points up to 30 minutes after feeding for both trials. The SFI values in the heel were observed to stay relatively the same for both trials as would be expected. Red = highest perfusion, dark blue = lowest perfusion. All SFI maps are on the same color scale.

The second case is a healthy full term neonate. One feeding event was recorded for this patient. LSI images were taken of the heel and abdomen every ten minutes before and after feeds.

Figure 15 depicts SFI maps of the heel and abdomen obtained from the patient during feeding. The results show that there was not a subsequent increase in SFI of the abdomen after feeds. These results may be due to the fact that the infant had a longer gestational period. Ultrasonic imaging has been used to assess mesenteric flow in neonates and can penetrate through tissue to evaluate arterial flow. LSI is more superficial than ultrasonic waves, and
thus babies with mature tissue may limit the depth penetration of LSI. Further study is required to assess the effect of gestational maturity on imaging depth.

![Figure 15](image)

**Fig. 15. SFI Maps of the abdomen and heel of a healthy term infant before and after feeding for a single trial.** The trial was taken at the 42nd day of extrauterine life. The SFI value in the abdomen and heel fluctuate and show no trend of increasing perfusion after feeding. Red = highest perfusion, dark blue = lowest perfusion. All SFI maps are on the same color scale.

4.3 Laser Speckle Imaging of Sick Neonates

4.3.1 Visualization of a Blood Clot using Laser Speckle Imaging: Case Report

In Figure 16, we present LSI results obtained from a patient who developed a clot in the right forearm that was confirmed with Doppler ultrasound. Tissue downstream of the clot became hypoxic due to the blockage of flow. The peripheral vasculature in the thumb was most affected and led to irreversible tissue damage. LSI measurements were taken of the affected hand to assess if tissue damage could be identified through LSI. The LSI image of the hand (Fig. 16), indicates a region of decreased SFI in the affected digit. This data suggests that LSI enables visualization of impaired flow resulting from clotting.
In Figure 17 we present LSI data obtained from a patient that was born premature and at risk of hypoxic tissue damage. The mother of the infant had history of methamphetamine abuse during pregnancy. The baby was born with birth asphyxia due to complications at birth. This led to both metabolic acidosis and general encephalopathy. The neonate underwent therapeutic hypothermia to reduce the risk of systemic hypoxic damage. During therapeutic hypothermia, the infant’s body temperature was reduced to a core temperature of 33.5°C for 72 hours and rewarmed back to 37°C to prevent hypoxic injury. LSI images were acquired of the abdominal region and the heel during the entirety of the rewarming process (3 hours).

**Fig. 16.** Blood-flow map of the right hand of a neonate (Male 53 days) in the UCI NICU that has compromised flow. The thumb (located inside of the red rectangle) of the subject had a clear reduction in blood flow, which was associated with a clot in the right forearm. Red = highest perfusion, dark blue = lowest perfusion. Note that an outline (in black) of the hand and fingers was superimposed for clarity.
In Figure 17, SFI data obtained from the abdomen and heel are plotted every 30 minutes during the warming of the patient. The measured SFI values indicate a steady increase in blood flow up to 30 minutes after the start of the rewarming process and a return to baseline (Fig. 17). An explanation for this observation is that the rewarming triggered a reactive hyperemic response causing the initial increase in blood flow. We also observed that the changes in abdominal blood flow were greater than blood flow changes measured at the heel. Peripheral vasculature resistance significantly increases during induced hypothermia. During the rewarming process, blood flow increases more quickly to the core than the periphery, as the peripheral vascular has a reduced blood supply. The decreased rate of blood supply to the peripheral vascular during rewarming seen in previous literature provides a possible explanation for why core blood flow is seen to increase at a faster rate than peripheral blood flow in this experiment.

![Rewarming of a neonate undergoing therapeutic hypothermia](image)

**Fig. 17.** SFI measurements of the heel and abdomen of a neonate undergoing therapeutic hypothermia. LSI measurements were obtained during rewarming of the neonate. The SFI values can be seen to increase up to 150 minutes into the rewarming process and fall back down to baseline. These results suggest evidence of a reactive hyperemia effect.
4.3.3 Alterations in Blood Flow of Neonates with Gut Pathology: Case Series

In order to demonstrate the potential of handheld LSI to provide information about neonates affected by gut pathology, four patients were recruited who were confirmed to have NEC or intestinal perforation. It is expected that major ischemic events occur that would predicate tissue damage, and thus LSI would visualize the evolution of blood flow abnormalities associated with this. The guiding hypothesis was that LSI could probe through the tissue of the abdomen and provide insight into the progression of the disease.

Figure 18 (below) presents a comparison of the maximum SFI changes in the abdomen associated with the NEC population (n=4) and the non-NEC population (n=13). The maximum change in SFI was calculated by taking the lowest measured abdominal SFI for each patient and subtracting it from the highest measured abdominal SFI. It was expected that the NEC population would have the largest changes in SFI in the abdomen due to major perfusion changes following treatment.

The results (in Fig. 18) show a box and whisker plot of the two groups. The median and the maximum value for the NEC group are observed to be higher than that of the non-NEC group. To facilitate comparison of the results, a Wilcoxin rank-sum test was used to determine if the difference between the two groups was statistically significant. The results (P=0.013) indicate that there is a significant difference between the two groups. The results suggest that analysis of SFI changes in the abdominal region of NEC patients may provide a method of monitoring treatment parameters.
Case 1: Stage II NEC

The infant was born at a weight of 0.86kg. The infant was admitted to the NICU on the 64th day of life after signs of illness. Clinical evaluation revealed signs of NEC which was later confirmed. SFI Measurements were obtained from the abdomen and heel of the patient from the 64th day of postnatal life to the 107th day of postnatal life.

SFI measurements were acquired from both the abdomen and heel once per day. Figure 19 (below) depicts a time series evaluation of SFI measurements in the abdominal region for a single neonate with stage II NEC. In order to verify the results were of physiological relevance, two regions on the abdomen were selected for LSI analysis. Imaging protocol involved first taking an image of the right lower quadrant and then repositioning to acquire images from the left lower quadrant. The results indicate a similar

![Box and Whisker Plot Comparing NEC to non-NEC population](image)

**Fig. 18.** Box and whisker plot comparing maximum SFI changes measured in the abdominal region for a NEC (n=4) and non-NEC(n=13) population. The thick vertical line represents the 25th to 75th percentile, while the thinner lines represent the smallest and largest values in the data set. The grey horizontal line represents the median of each population. The NEC population is seen to have significantly higher changes in SFI compared to the non-NEC population.
trend between the right lower and left lower quadrant of the abdomen. A large difference (45%) is observed on the last day of measurements; however this difference may be due to patient motion artifacts.

![Graph showing SFI measurements of a neonate with confirmed stage II NEC.](image.png)

**Fig. 19. SFI measurements of a neonate with confirmed stage II NEC.** The right lower (RLQ) and left lower (LLQ) quadrant of the abdomen were measured over a period of several months. Both quadrants can be seen to follow a similar trend after surgery. A large difference (45%) is observed on April 6th, which may be due to patient motion artifacts.

No surgery was performed for this patient, and no significant events occurred that were responsible for the peaks or troughs associated with the data. The neonate also had the lowest change in abdominal SFI for the NEC group (Fig. 18). Overall, the results prove inconclusive regarding any useful information obtained from abdominal SFI measurements of a neonate with stage II NEC.

**Case 2: Intestinal Perforation**

The infant was born prematurely at a weight of 0.66kg. Clinical evaluation revealed radiologic signs of intraperitoneal gas indicating a perforated bowel. Surgery was performed on
30th day of postnatal life to resection the bowels. LSI images were taken of the abdomen from the 28th to the 106th day of postnatal life. In order to verify the results were of physiological relevance, two regions on the abdomen were selected for LSI analysis. Imaging protocol involved first taking an image of the right lower quadrant and then repositioning to acquire images from the left lower quadrant. The images were processed and the SFI value for each set was analyzed over time.

Figure 20 (below) presents the time series SFI measurements before and after surgery to resection the bowels. The results of the measurements show a similar trend in the measured SFI from the right and left lower quadrant of the abdomen. The similar trend found from the two quadrants supports previous results obtained in section 4.2.1 that suggest changes in SFI measurements are due to blood flow changes. After surgery the SFI values can be seen to fluctuate until July 1st when both values increase. The increase seen in SFI of both quadrants directly coincides with the patient’s recovery from surgery. These results suggest that SFI values can be monitored over time to assess recovery after bowel surgery.
Case 3: Stage III NEC

The infant was born prematurely at a weight of 0.67kg. Clinical evaluation revealed radiologic signs of intraperitoneal gas indicating a perforated bowel. Surgery was performed to resection the bowels. A Colostomy was also performed to alleviate the gut from the burden of digestion. Because the gut was heavily resected during surgery, a double barrel colostomy was performed and thus two ends of the intestines were attached to the abdominal wall. This resulted in two exposed stomas at the abdominal wall. LSI images were taken of the exposed stomas, the right lower quadrant of the abdomen, and the heel.

Figure 21 presents SFI flow maps obtained from the abdomen, exposed stoma, and the heel. The SFI maps denote blood flow by a color map in which blue represents low blood flow.
and red represents high blood flow. The SFI maps indicate a low SFI value for the stoma and abdomen prior to surgery. This result is expected, as the gut vasculature is severely damaged once perforation occurs \(^4\). The LSI images of the heel indicate a large measured SFI value for the peripheral vasculature. Because the exposed bowel and the abdomen followed similar trends in measured SFI, while the heel was largely unaffected, it can be suggested that SFI changes found in the abdomen are potentially correlated to SFI changes found in the intestines.

**Fig. 21. SFI maps of a neonate affected by stage III NEC.** The abdomen and the exposed stoma can be seen to have similar SFI values prior to surgery (top row) while the extremity is seen to be significantly higher. After surgery (bottom row) the abdomen and the exposed stoma show an increase in SFI suggesting that the two areas are correlated. All SFI maps are displayed on the same color scale.
4.4 Conclusion of Clinical Study

In this Chapter, a handheld LSI device (Chapters 2 and 3) was deployed to the UC Irvine medical center to obtain LSI measurements of neonates. The study focused on acquiring LSI images of the abdomen and heel of both healthy and sick neonates.

Both longitudinal hourly LSI measurements and LSI measurements before and after feeding were obtained for healthy neonates. The results from the hourly measurements suggested that the handheld LSI device was capable of detecting changes in blood flow in the abdomen. The results of the feeding experiment suggested that blood flow changes to the mesenteric artery could be detected by LSI in preterm neonates. Future research should investigate this observation in term neonates.

Several sick neonates were recruited as unique cases. The three conditions that were presented were neonatal blood clots, therapeutic hypothermia, and NEC/gut pathology. For neonatal blood clots, the handheld LSI device was able to identify a region of compromised blood flow. The therapeutic hypothermia case revolved around the rewarming process of the patient. The LSI data from this case provided evidence of a possible reactive hyperemic response during rewarming. The NEC data showed a significant difference in the changes in speckle contrast between the NEC population and non-NEC population. Furthermore, several time course abdominal LSI measurements were presented and suggest that LSI can be used to monitor treatment parameters during recovery of Stage III NEC patients/gut pathology. Future studies should recruit a larger population of stage II and stage I NEC patients to determine if LSI provides insightful information early on in the progression of the disease.

Chapter 5 will further discuss the implications of the clinical study and provide insight into the future of using LSI to study neonatal hemodynamics.
CHAPTER 5: Discussion and Implications for the Future

5.1 Discussion of Laser Speckle Imaging in a Clinical Setting

The clinical work done with handheld LSI has provided a demonstration of the feasibility of measuring point-of-care blood flow. The cases presented in this study have shown that the current knowledge on neonatal hemodynamics is widely unknown; however, due to the exploratory nature of this study, the results remain theoretical. Because the values obtained by LSI are not absolute measurements of blood flow, future clinical work using LSI should incorporate a method of accounting for tissue optics. The dynamic nature of neonatal skin provides a unique obstacle for optical techniques such as LSI. Several studies have performed analysis of maturation of neonatal skin and have shown large changes in the reduced scattering coefficients of the tissue.

The feed experiment revealed that not all cases exhibited an increase in abdominal blood flow after feeding. It is possible that more mature skin would limit the depth at which LSI interrogates. Because of this, it is important to take tissue optics into consideration. The addition of spatial frequency domain imaging (SFDI) to future LSI studies would allow for the quantification of neonatal tissue optics. This information would potentially allow for the correction of LSI measurements based on absorption and reduced scattering coefficients. This information would also help clarify the depth at which LSI penetrates in neonatal tissue.

Future studies should implement more exclusive recruitment criteria to better control for independent variables. Gestational age and postnatal age are two factors that were widely uncontrolled in the current study. Choosing a smaller range for these parameters would limit the intra-class variance associated with maturation of tissue. Babies that are born with lower
gestational ages are also more at risk for neonatal diseases, making it easier to perform a cohort study.

The small baby unit located at the Children’s Hospital of Orange County (CHOC) would be an ideal location for developing the next LSI study of neonates. The unit only admits patients that have undergone less than a 27 week gestational period or are less than 1000 grams. This restriction would filter recruitment and allow for a more controlled experimental design. Dr. Mustafa Kabeer would be an excellent clinical consult during the creation of the study and has already expressed interest in the use of LSI in the NICU.

5.2 Discussion of Future Clinical Laser Speckle Imaging Devices

The handheld LSI device was successfully integrated into the NICU setting and was usable by a non-expert. This is a promising step forward in transitioning LSI to a part of routine care. The handheld form factor proved to be an effective method for point-of-care use. While the motion artifacts associated with the device were of initial concern, clinical results prove cardiovascular relevance.

Future systems can be further improved to cater to different areas of neonatal research. A current limitation of the device is the use of a low-power laser pointer as an illumination source. Lower power light is difficult to use for LSI, as diffusing of the beam causes a decrease in intensity. The addition of a higher power source would allow for wider blood flow maps to be obtained. The current illumination spot has a diameter of 5 cm, requiring the complete abdominal region to be imaged in two adjacent images in most neonates.

If a higher power source were used, full body blood flow maps could be obtained. There were several times over the course of the study in which peripheral vascular measurements were missed. Full body SFI maps would eliminate the need to image multiple sites on the neonate.
Full body SFI maps could then be used to assess relations between different regions. For example for NEC, it would be useful to identify regions of compromised blood flow in the gut in relation to the peripheral vasculature. When taking multiple images, there is always a risk of introducing motion artifacts; however with a single image, all changes remain relative.
Conclusion

The design and build of the handheld LSI device along with an initial study on neonatal hemodynamic monitoring has been presented. Validation of using the handheld device was confirmed through use of *in-vitro* and *in-vivo* experiments with multiple minimally trained users. The handheld device was successfully implemented into the NICU and usable by a non-expert. The results of the data suggest that LSI has the potential to provide useful hemodynamic information to clinicians that is not currently available through routine care. Modifications to the LSI device, such as acquiring full body SFI maps, would further increase the usefulness of LSI in the clinic. Future studies targeting neonates should design experiments with consideration to tissue optical properties to better interpret their results.
References


