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Interpretation and medical application of laser biospeckle

Oliver Bendix Thompson

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in Bioengineering, The University of Auckland, 2014
Abstract

Laser biospeckle is the dynamic laser speckle pattern produced by illuminating tissue with laser light. The dynamic nature of the speckle indicates the movement of particles in the tissue, particularly blood cells. Contrast analysis of laser biospeckle generates flow maps showing areas of higher or lower blood flow, for example vessels or background perfusion, and can detect changes in flow associated with inflammation, wounds or disease.

This thesis presents methods of analysing laser speckle contrast, particularly a method using multiple camera exposures to generate the same spectral information from laser speckle fluctuations as laser Doppler, allowing quantitative measurements of flow. This work also presents a correction for spatial averaging in speckle, and confirms the validity of that correction using simulation and experiment. Spatial effects on laser speckle imaging techniques in tissues are tested experimentally.

Multiple exposure speckle imaging was tested in vivo in a variety of situations, and clinically in measurements around diabetic foot ulcers. Measurements were made on three groups: patients with diabetic foot ulcers, patients with peripheral vascular disease, and normal control subjects. No difference in mean flow speed was found between the groups, but a significant difference in mean static contrast, indicating blood volume in tissue, was found.
Acknowledgements

Thanks to Diana Siew, for the inspiration and instruction to start a PhD; Evan Hirst for valuable discussions along the way and for taking speckle imaging into new areas; Mike Andrews for asking the hard questions; Wiendelt Steenbergen, Erwin Hondebrink, and Carla Kloese for their help in our collaborative experiments; John Baker, Ajith Dissanayake and others at Counties Manukau DHB and the Centre for Clinical Research and Effective Practice for their work on the clinical trials, and Poul Nielsen for his patience.
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**Chapter 4 contains work adapted from a paper previously published in Journal of Biomedical Optics**


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1 Introduction

1.1 Motivations and intentions
Laser biospeckle provides the basis of techniques that image and measure the movement of the constituents of biological tissues. Typical laser biospeckle implementations take a single image of the skin surface, illuminated with laser light, and calculate the motion in each area of the image as a function of the statistics of the laser speckle in that area. In medical applications, both clinical and research, laser biospeckle provides a method of imaging the blood flow near the surface of tissues. Blood flow in tissues can be generally described as perfusion, which is the process of the body delivering blood through capillaries to tissue. Near surface perfusion images can be used for a variety of tasks such as quantifying the progress of peripheral vascular disease in diabetes, monitoring the reperfusion of skin flaps in plastic surgery, observing the effects of stroke in the cerebral circulation\textsuperscript{1-3}, and establishing the viability of burned tissue. While imaging applications of laser biospeckle have a long history, there has been some doubt and confusion about quantification of laser biospeckle results and the reliability of the connection between speckle measurements and a quantitative description of blood flow.

Similar images and measurements of perfusion can be made using laser Doppler techniques\textsuperscript{4,5}. Laser Doppler measurements have a well-established theoretical base, providing a reliable connection to quantitative measurements of blood flow\textsuperscript{6-8}. They have, however, the disadvantage of requiring a continuous time series, or the related spectrum, at each measurement point. This reduces the applicability of Doppler methods to imaging tasks, as in order to generate an image the measurement point must be scanned over the imaging area and sufficient time allowed at each point to measure a power spectrum. Recent developments have allowed full-field laser Doppler measurements to be made using a very high speed (20 kfps) camera\textsuperscript{9}, but that technique remains excessively expensive for clinical use.

As laser Doppler and laser speckle rely on the same physical phenomenon, it should be possible to obtain equally reliable and quantitative measurements from speckle as from the established Doppler techniques. This thesis will show that this is the case – there is an analysis of multiple-exposure laser speckle that provides the same spectral and blood flow information as laser Doppler.
While laser biospeckle imaging is applicable to a large variety of biomedical imaging tasks, the applied medical part of this thesis will focus on one task in particular: biospeckle imaging of the dermal perfusion in the diabetic foot, with the hope of predicting the formation of diabetic foot ulcers. It is believed that the autoregulation of blood flow in the microvasculature is impaired in diabetes, and that this has implications for wound healing and ulcer formation. The capillary blood flow has been shown to be reduced in the feet of patients with peripheral neuropathy, a common complication of diabetes. Laser biospeckle images and measurements may provide a clinical indication of the severity of this impairment and thus indicate ulcer risk. The fact that laser biospeckle images are measured in real time and can cover a large area of tissue means that both the spatial and temporal variations in blood flow can be observed.

1.2 Background

1.2.1 Microcirculation

Laser speckle techniques are generally limited to interrogating only the outer layers of tissue, and thus generally only give information about the superficial microcirculation; the smaller vessels in the vasculature that supply blood to the tissue near the surface. The biospeckle applications considered in this thesis will concentrate on the dermal microvasculature.

1.2.2 Laser speckle

When an object is illuminated with coherent radiation, provided that the surface is rough with respect to the wavelength of the radiation, interference between many random paths of various lengths produces speckle. This phenomenon occurs in various fields where coherent radiation of one kind or another is used for imaging—in radar, in medical ultrasound and, particularly important to this work, in laser imaging. An image of almost any object illuminated by laser light appears speckled so long as the surface roughness is similar or larger than the wavelength of the laser light, regardless of the apparent texture of the object under white light. For example, Figure 1.1 shows an image of a section of a watch face under both white light and laser light, showing the speckled image produced on a relatively plain surface by laser illumination. Similarly, a laser diffusely reflected from a surface produces a speckled field in space which may be detected using a screen or a sensor such as a CCD camera chip. The phenomenon of speckle was discussed by early workers in laser science as an image defect, and the cause
of the effect was soon realised to be an interference effect between light travelling by randomly varying paths\textsuperscript{12}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image1.png}
\caption{A speckle image using laser illumination (left hand side), with a white light images of the same object (right hand side) for comparison.}
\end{figure}

In the simplest case of laser speckle, shown below in Figure 1.2, we look at the speckle pattern formed in space at some distance $L$ from an illuminated circular spot $D$ on an optically rough surface. The spatial speckle pattern can be captured using a sensor such as photographic film or a CCD camera chip. This is the far field speckle case, sometimes called the objective speckle case. At any particular point in view of the illuminated area, the amplitude of the light wave is given by a vector sum of contributions from a collection of many paths. These contributions are coherent but their path-lengths differ, so that they have a random phase offset from each other.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image2.png}
\caption{Far field speckle formation. The ensembles of path lengths illustrated in red and black will produce a different vector sum and different intensities at their respective observation points. A speckle pattern with speckle size $s$ is formed at a distance $L$ from a laser spot of diameter $D$.}
\end{figure}

Although the intensity measured at a single point is randomly distributed, with a negative exponential density function for the light intensity for ideal speckle\textsuperscript{6}, the spatial statistics of the pattern are determined by the geometry of the experiment. A larger laser spot
creates speckles with a smaller minimum size, as the angular movement required to make a phase change of $\pi$ between the most extreme path lengths is smaller. The minimum speckle size $s$ in the case illustrated is given by the equation\(^{13}\):

$$s \approx 1.2 \frac{\lambda L}{D}$$  \hspace{1cm} (1-1)

**Figure 1.3:** Imaging speckle formation. Adjacent points on the illuminated surface generate overlapping Airy disks on the sensor. The minimum speckle size $s$ at the sensor is then governed by the Rayleigh criterion.

In the case of *imaging* speckle, sometimes called *subjective* speckle, the speckle pattern is formed using a lens, on some imaging surface – the film or sensor in a camera or the retina of the eye. This situation is illustrated in Figure 1.3.

The minimum speckle size at the sensor is now determined by the lens aperture and is the minimum resolvable separation at the sensor given by the Rayleigh criterion: the separation at which the maximum of the Airy disk generated by one point on the illuminated surface overlies the first zero of the adjacent Airy disk. Assuming that the lens is focussed on the illuminated surface, this size is:

$$s \approx 1.2(1 + M)\lambda N$$  \hspace{1cm} (1-2)

where $M$ is the working magnification and $N$ the f-number of the lens\(^{13}\). It may be useful to relate the speckle size to the scale of the imaged surface. The apparent speckle size, referred back to the illuminated surface, is
\[ s \approx 1.2 \frac{(1 + M)\lambda N}{M} \] (1-3)

1.2.3 *Speckle as a random walk*

Goodman provides a good explanation of the cause and character of speckle, which also leads to his calculation of the statistical properties of speckle\(^{11,14}\). Speckle occurs in signals which are the sum of a multitude of independently phased complex components, having both an amplitude and a phase. Either the phases, or both the phases and amplitudes may vary randomly. When these components are added together, they comprise a “random walk”, and the amplitude of the resultant vector can be large, when constructive interference dominates, or small, when destructive interference dominates.

1.2.4 *Biospeckle and speckle contrast*

The discussion and illustrations of speckle above deal with static objects and single scattering – the light arrives at the measuring surface having been scattered once from a static object. In biomedical applications, the situation is generally more complex. The speckle field is generated by light that is both multiply scattered through tissue, and scattered from both moving and static scatterers.

Dynamic speckle patterns generated by illuminating living tissue can inform us about the movements within that tissue and are often referred to as *biospeckle*.

The multiple scattering of light in the generation of biospeckle is important – this scattering influences the way biospeckle analysis samples a particular tissue, and alters the fluctuation rates of the speckle patterns\(^{15}\). In multiple scattering situations, light is generally returned from tissue after multiple scattering events. The tissues accessible to optical imaging are generally strongly scattering\(^{16}\). In the visible and near infra-red spectrum, skin tissues are highly scattering with scattering coefficient in the dermis \(\mu_s = 187.5\) cm\(^{-1}\) for light with a wavelength \(\lambda = 633\) nm, and \(\mu_s = 175\) cm\(^{-1}\) for \(\lambda = 800\) nm, compared to the respective absorption coefficients \(\mu_a = 2.7\) cm\(^{-1}\) for \(\lambda = 633\) nm and \(\mu_a = 2.3\) cm\(^{-1}\) for \(\lambda = 800\) nm\(^{16}\). This scattering is highly forward biased with scattering anisotropy \(g = 0.8\) and 0.85 for \(\lambda = 633\) nm and 800 nm respectively.

The transport of light in multiply scattering tissue can be modelled as a process of diffusion\(^{17}\), or simulated using Monte Carlo techniques\(^{18}\).
The effects of multiple scattering determine the volume of tissue interrogated by a speckle measurement at any point. This effect is investigated empirically in this thesis in section 6.3. Interpretation of dynamic speckle measurements must take this multiple scattering into account. Unlike Doppler measurements in single scattering measurements such as Optical Coherence Tomography, in which the light returned from the detector has been backscattered directly from a moving object and hence the direction of the movement giving rise to the Doppler shift is known, the directions of particle movements giving rise to speckle dynamics in a multiple scattering situation are not known.

Figure 1.4 illustrates the formation of a biospeckle pattern in imaging speckle on human skin. Light returned to the camera has travelled via a multitude of multiply-scattered paths. Most paths intercept only relatively static scatterers, but some intercept moving blood cells in the capillaries, and so have a path length that varies with time. As some of the path lengths adding to the light amplitude are changing, the intensity at any point fluctuates.

![Laser scattering from skin and its capillaries generates a biospeckle pattern. The amplitude of the light scattered from the surface at point a and recorded at a' is generated by interference between an ensemble of paths, some varying, that pass through a volume of tissue.](image)

Figure 1.4: Laser scattering from skin and its capillaries generates a biospeckle pattern. The amplitude of the light scattered from the surface at point a and recorded at a' is generated by interference between an ensemble of paths, some varying, that pass through a volume of tissue.

The mean rate of intensity fluctuations at a point in the speckle pattern indicates the mean rate of movement of the moving scatterers. As the fluctuations are typically too fast to measure directly using an ordinary CCD camera, we measure the blurring effect of a finite camera exposure on the pattern. These relationships between movement, speckle fluctuations and blurring effect can be simply described as follows: faster movements of the scatterers generate faster fluctuations in the speckle pattern, and more blurring. The
degree of blurring is generally quantified using the parameter called speckle contrast, given the symbol $K$ and defined in equation (1-4) below as the ratio of the standard deviation of intensity to the mean intensity:  

$$K \equiv \frac{\sigma}{I}$$

(1-4)

In the simplest analysis, the speckle contrast is calculated over small regions of the image to generate a speckle contrast image. These regions are typically 5 pixel x 5 pixel or 7 pixel x 7 pixel squares of the original image, and may be overlapping or independent. Although they are not truly quantitative flow maps, speckle contrast images of tissue do show areas of relatively higher or lower blood flow, as illustrated in Figure 1.5 which shows both the raw speckle image, as recorded by the camera, and a speckle contrast image of a thumb with a small wound. Note the small central area of higher contrast, corresponding to a flap of skin, and the surrounding area of lower contrast due to increased blood flow in the vicinity of the wound.

![Figure 1.5: Raw image (Left side) and speckle contrast image (Right side) of a thumb, showing a small wound.](image-url)

Increasing the camera exposure $T$ used to capture biospeckle reduces the measured contrast, as shown in Figure 1.6. The curve is generally found to be sigmoidal, when plotted as $K$ against log($T$). The analysis of this curve and its parameters underlies much of the interpretation of biospeckle contrast, and will be a key topic of this thesis.

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* Speckle contrast takes the symbols $C$ and $K$ in the literature, depending on the author, and will be $K$ in this work to avoid confusion with autocorrelation functions using $C$.☆
Figure 1.6: Generalised speckle contrast vs. camera exposure curve. Speckle contrast measured at increased camera exposures reduces on living tissue.

The sigmoidal shape of $K$ plotted against log ($T$) is predicted by theory, as shown in section 4. The shape is generated by the integrating effect of the camera exposure, and the particular spectrum of the speckle fluctuations. For an ideal setup and fully developed speckle, $K$ approaches 1 at zero exposure as there is no integrating camera effect, and $K$ approaches 0 asymptotically as exposure increases. In the illustration above, $K$ does not approach 1 or 0 at its limits, showing the general non-ideal case, where system effects limit the maximum contrast and residual static contrast remains at long exposure. These two effects are explored in sections 5.1 (maximum $K < 1$) and 6.1 (minimum $K > 0$). Briefly, a maximum $K$ less than 1 is found to be generally due to spatial averaging of the speckle pattern by the finite sized camera pixels, and minimum $K > 0$ due to static scatterers, for example calloused skin, overlaying the dynamic scatterers such as red blood cells.

The typical approach to speckle analysis, used in current commercial systems and many research applications, has been to measure $K$ at a single camera exposure, and calculate a parameter related to blood flow from that measured $K$ value, as shown in several sections of the following chapter. The approach in this thesis has been to determine the entire shape of the curve as far as possible, so as to obtain both more information and more accurate information about the speeds of the dynamic scatterers.

1.3 An outline of the contents of this thesis

Chapter 2 covers the laser biospeckle literature, from the initial development of the technique in the 1970s.
Chapter 3 details the experimental setup and optimisation of laser speckle imaging systems, including the key components; laser illumination (section 3.1), and camera and imaging setup (section 3.2). This chapter also covers the optimisation of speckle contrast systems (section 3.3), including some simulations showing the effects of spatial averaging on speckle contrast patterns, and discussion of appropriate speckle size in speckle imaging systems.

Chapter 4 introduces a novel analysis of multiple exposure speckle contrast, which showed for the first time that the same information that laser Doppler systems can provide about the spectrum of intensity fluctuations in a speckle pattern can be obtained by multiple exposure laser speckle. This analysis provides a foundation for the connection between speckle contrast images and real physiological data and is confirmed in section 4.2 and 4.3 by simulations and experiments.

Chapter 5 covers some practical aspects of multiple exposure laser speckle imaging: a correction for spatial averaging, the effective frequency sensitivity of laser speckle measurements, choice of fitting functions for real speckle data, and synthetic generation of the long exposures required for multiple exposure speckle analysis. The correction for spatial averaging, introduced in section 5.1, justifies a correction that had been in use in the field but had never been experimentally established as accurate.

Chapter 6 covers laboratory work in laser speckle. Section 6.1 shows that accounting for the stationary contrast, the contrast patterns generated by static paths returning light to the camera, is both important and possible using multiple exposure laser speckle analysis. Chapter 6 also explores and elucidates the relationship between the speed of flows and the spectrum generated, and spatial effects caused by multiple scattering in speckle images.

Chapter 7 presents *in vivo* and medical trial results. Section 7.1 presents some lab-based *in vivo* measurements including an investigation of the speckle signal remaining in a limb under arterial occlusion, a comparison of multiple exposure laser speckle single exposure laser speckle and laser Doppler measurements of dermal inflammation changing with time, and an investigation of pulsatile effects in speckle measurements. Section 7.2 presents a trial of multiple exposure laser speckle to examine perfusion in the sole of the foot in diabetic foot ulcer patients.

Chapter 8 presents general conclusions to all of the work and shows examples of related continuing work beyond the scope of this thesis.
2 Laser biospeckle and laser Doppler: literature review

2.1 Laser biospeckle interpretation and applications

2.1.1 Early biospeckle measurements and theory

J.D. Briers applied biospeckle measurements to biological specimens in 1975, recording fluctuations in the intensity of speckle patterns generated by shining a laser on fruit and interpreting the different fluctuations recorded at different laser wavelengths as the result of scattering from different coloured moving chromophores in the fruit\(^\text{23}\). Briers developed the idea of obtaining information about the proportion of moving scatterers in a target of mixed moving and stationary scatterers using speckle contrast\(^\text{24}\) and established a connection between laser Doppler measurements – termed intensity fluctuation spectroscopy or light beating spectroscopy at the time – and speckle contrast by showing that in certain circumstances the spatial and temporal statistics of speckle are equivalent\(^\text{25}\). He expanded on this theme in 1996, publishing a reconciliation of the two methods, laser Doppler and speckle, based on consideration of a Michelson interferometer with one moving mirror\(^\text{26}\). This work is further explored in section 2.2.3 below.

The earliest application of laser speckle contrast to blood flow measurement was by Fercher and Briers in 1981\(^\text{27–29}\). Photographs were taken of a human retina under laser illumination, and optical high-pass filtering was used to convert these raw speckle images into contrast images. The optical high-pass filter has the effect of reproducing areas with fine detail, in this case speckle, at higher intensity than areas with little fine detail, in this case vessels where blood flow blurred the speckle, producing images of the blood flow in the retinal vessels as shown in Figure 2.1.
2.1.2 Digital realisation of biospeckle measurements.

As Briers and Fercher's original speckle work relied on traditional photography and optical filtering to generate a speckle contrast image, it was not a useful clinical tool. The development of camera and computer technology allowed Briers and others to develop a digital realisation of the technique in the 1990s\textsuperscript{30-32}. A CCD camera was connected via a framegrabber to a computer which provided speckle contrast analysis and a laser was used to illuminate various test targets\textsuperscript{30}. With only minor changes – for example using digital cameras instead of an analogue camera and framegrabber combination, and the continual increase in available computer power over the last two decades – the basic hardware used for speckle contrast imaging has not changed significantly since these first systems were built. A typical setup is shown in Figure 2.2. Subsequent work has focussed on improving the interpretation and developing applications of speckle contrast measurements. Commercial laser speckle imaging systems have been developed using essentially similar hardware to laboratory systems and applying a variety of speckle analyses\textsuperscript{33,34}. 

\begin{figure}
\centering
\includegraphics[width=\textwidth]{Figure2.1_High_pass_optically_filtered_speckle_photograph_of_the_human_retina_1981.png}
\caption{High pass optically filtered speckle photograph of the human retina (1981)\textsuperscript{27}}
\end{figure}
Figure 2.2: A typical laser speckle imaging setup. The object or tissue under test is illuminated with an expanded laser beam and imaged using a digital camera. Raw images are sent to a computer for analysis.

2.1.3 Developments of Briers’ biospeckle theory

Fercher and Briers’ early speckle contrast imaging work introduced an equation connecting the spatial variance of a speckle pattern to its temporal autocorrelation at a fixed point when the camera exposure is some finite value.

The temporal autocorrelation function used throughout this thesis is the correlation of the fluctuating intensity with itself, defined in the equation:

\[ C_t(\tau) = \lim_{T_{obs} \to \infty} \frac{1}{2T_{obs}} \int_{-T_{obs}}^{T_{obs}} I(t)I(t + \tau)dt \]

where \( T_{obs} \) is the observation time, \( \tau \) is the lag time and \( I(t) \) is the fluctuating speckle intensity.

At fixed camera exposure \( T \) the equation presented by Fercher and Briers\(^{27,28} \) shows that the spatial variance \( \sigma^2 \) is:

\[ \sigma^2 = \frac{1}{T} \int_0^T C_t(\tau)d\tau \]  

(2-2)

This equation is important in the later development of the field, as it leads to a more quantitative interpretation of speckle contrast as a blood flow measurement. It equates the spatial variance of a speckle pattern captured at a fixed camera exposure with the integral of its temporal autocorrelation function.

Equation (2-2) was published by Fercher and Briers in 1981, and made reference to a paper by Goodman from 1965\(^{35} \). This may have been a mistake; the equation as it stands
does not appear in that paper. It does appear in a different form in other work by Goodman\textsuperscript{12}

The equation as given by Fercher and Briers is missing a windowing term inside the integration. It ought to be:

\[
\sigma^2 = \frac{1}{T} \int_0^T [2(1 - \tau/T)]C_t(\tau)d\tau
\]  

(2-3)

according to Goodman\textsuperscript{11}. This error was subsequently pointed out by Duncan and Kirkpatrick et al.\textsuperscript{36,37}. The previously missing term is shown in square brackets. This term is a triangular window, generated by the autocorrelation function of the effective rectangular integration window which the camera exposure imposes on the speckle fluctuations\textsuperscript{11}. The triangular window has more effect in situations where the camera exposure time $T$ is similar to the width of the autocorrelation function $C_t(\tau)$, so neglecting it when using comparatively long camera exposures may be justifiable; however, in the this thesis the analysis will use the full equation (2-3) both for precision and because a wide exposure range is used.

Equation (2-2) without the windowing term and other results derived from it\textsuperscript{38–40} have been repeated in the literature over the years\textsuperscript{1,41}. Goodman's earlier work is impenetrable to those without deep statistical understanding, making the detection of this omission less likely.

In order to relate speckle contrast and perfusion, many researchers choose a function for $C_t(t)$, based on assumptions about the light scattering properties of the target and the velocity distribution of the scatterers.

For example, Briers\textsuperscript{32} and subsequently Choi et. al.\textsuperscript{38–40} chose to assume a Lorentzian flow velocity distribution. Given the assumption that the flow velocity distribution is Lorentzian, the autocorrelation is an exponential function, $C_t(t) = e^{-T/\tau_c}$. Using equation (2-2) the speckle contrast $K$ at camera exposure $T$ is a function of the correlation time $\tau_c$ of the intensity fluctuations:

\[
K = \sqrt{\frac{\tau_c}{2T}[1 - e^{-2T/\tau_c}]}
\]  

(2-4)

Taking an inverse of this function by generating a series expansion of for $\tau_c$ as a function of $K$ and $T$, and using $1/\tau_c$ as a relative perfusion measurement, allowed Choi to produce
good perfusion images, particularly of the rodent dorsal skinfold model.

The relative perfusion measurement produced using this analysis had good linearity with the actual flow rate in tube-in-phantom experiments, where whole blood was pumped using a syringe pump through tubes in an agar-based tissue phantom. It doesn’t, however, measure a true correlation time \( \tau_c \): my experiments showed that the \( \tau_c \) calculated using this method from contrast values measured on a tube with a fixed flow rate varies with \( T \), while the true \( \tau_c \) ought to remain constant. This analysis has also been used by other researchers in various applications including cerebral vessel imaging. The problems with this approach include the difficulty of dealing with populations of scatterers including groups with quite different velocity distributions, for example red blood cells flowing in both arterioles and capillaries, or cases where there are a proportion of static scatterers. There remains the complication that the scattering interactions with particles generally intercept only a small component of the particles’ velocities, as shown in section 6.2.

Parthasarathy and Dunn extended this approach to multiple camera exposures and proposed a model of biospeckle that accounted for the effects of static speckle patterns. Using equation (2-3), assuming a Lorentzian velocity distribution so that \( C(t) = e^{-T/\tau_c} \) and considering light scattered from both moving and static scatterers, they find the following expression for the speckle contrast:

\[
K(T, \tau_c) = \left\{ \beta \rho^2 \frac{e^{-2x} - 1 + 2x}{2x^2} + 4\beta \rho (1 - \rho) \frac{e^{-x} - 1 + x}{x^2} \right\}^{1/2} \tag{2-5}
\]

where \( x = T/\tau_c \), \( \rho = \frac{l_f}{l_f + l_s} \) is the proportion of the light that is scattered from moving scatterers, \( \beta \) is a normalisation factor to account for speckle averaging effects in the system, and \( \tau_c \) is the speckle decorrelation time. This expression generates a sigmoidal \( K \) vs \( \log(T) \) plot, similar to that plotted above in Figure 1.6. Measuring the contrast \( K \) at a range of exposures \( T \) and fitting the equation above allowed them to find \( \tau_c \). They found that this model produced consistent estimates of \( \tau_c \) for a phantom flow-tube with varying levels of static scattering. A similar approach was taken by Smausz et. al. Again using equation (2-3) and a Lorentzian velocity distribution then adding scaling parameters \( P_1 \) and \( P_2 \), they develop the expression:
\[ K(T, \tau_c) = P_1 \left( \frac{\tau_c^2}{2} T^2 \left[ \exp \left( -\frac{2T}{\tau_c} \right) - 1 + \frac{2T}{\tau_c} \right] + P_2 \right)^{1/2} \]  \hspace{1cm} (2-6)

which they fit to measured \( K \) vs. \( T \) curves to find \( \tau_c \).

Homogeneous, ordered flow in vessels and the inhomogeneous motions of capillary flow or Brownian motion require different assumed autocorrelation functions, as shown by Duncan and Kirkpatrick\textsuperscript{36,37,45}, though many researchers have assumed that analyses suitable for inhomogeneous velocity distributions are applicable to homogeneous flows. A collection of scatterers with a homogeneous velocity distribution give an exponential autocorrelation function. This happens, for example, in the dynamic speckle patterns generated by Brownian motion. Organised flow, corresponding to an inhomogeneous velocity distribution, generates a Gaussian autocorrelation function. The actual autocorrelation function on skin will likely fit neither of these limiting cases, having contributions from populations of scatterers with both inhomogeneous and homogeneous velocity distributions\textsuperscript{36}.

For speckle contrast less than 0.6 Gaussian and Lorentzian autocorrelation functions give the same values for \( \tau_c \) as a function of \( K \), provided that the autocorrelation functions use the same definition of decorrelation time. Ramirez-San-Juan\textsuperscript{46} used this characteristic to develop a approximate speckle imaging equation, called the speckle flow index (SFI) defined as follows:

\[ \frac{1}{\tau_c} = \frac{1}{TK^2} \]  \hspace{1cm} (2-7)

This equation is only usable when \( K < 0.6 \) and has no normalisation for cases where the speckle contrast does not approach 0 at long exposures and 1 at short exposures. A static scattering layer over the moving scatterers will cause the first condition, and various characteristics of the optical system design can result in the second due to spatial averaging of speckles.

Duncan and Kirkpatrick point out that the common assumption that \( \tau_c \) has a simple inverse relationship to a mean flow velocity is not necessarily justified, when \( \tau_c \) is simply calculated for some chosen autocorrelation function. They offer a solution\textsuperscript{36} that physically relates a decorrelation time \( \tau_c \) based on Goodman's phase screen model\textsuperscript{11} to the speed \( V \):
\[ \tau_c = \frac{w}{V} \]  
\[ (2-8) \]

where \( w \) is the characteristic width of the Airy function:

\[ w = \frac{\lambda z}{D} \]  
\[ (2-9) \]

It is important to consider the angle dependence of tissue and blood scattering when making a connection between \( \tau_c \) or other speckle parameters and velocity. Skin tissues and blood are strongly forward scattering, with anisotropy coefficients \( g \) approximately 0.8 for skin and > 0.99 for blood\(^47\). This implies that direct back-scatter, giving the maximum possible rate of path-length change, is unlikely for any optical path intercepting a moving scatterer. The scattering angle will generally be small and the rate of path-length change will be much less than the speed of the scatterer. The same caveat applies to the laser Doppler techniques, described below in section 2.2.

### 2.1.4 Empirical approaches to biospeckle analysis

More empirical approaches to relating laser speckle contrast and blood flow are possible. Forrester applied laser speckle imaging using an algorithm calibrated using flow tube models\(^48,49\). His analysis finds the absolute difference between the speckle pattern and a generated reference image, an image of the same scene in which the speckle is reduced using spatial or temporal filtering. The intensity \( I_{REF} \) of a pixel at position \((x,y)\) in the reference image is an average over a volume of speckle, given by the equation:

\[ I_{REF}(x,y) = \frac{1}{N_{MAX}} \sum_{N=1}^{N_{MAX}} \left[ \frac{1}{2i + 1} \sum_{x-i}^{x+i} \left( \frac{1}{2j + 1} \sum_{y-j}^{y+j} I_{SP,N}(x,y) \right) \right] \]  
\[ (2-10) \]

where \( I_{SP,N} \) is the intensity of the Nth pixel at position \((x,y)\) in a sequence of \( N_{MAX} \) captured speckle images and \( i \) and \( j \) give the size of speckle averaging region, chosen to be larger than the minimum speckle size. Intensity fluctuations \( I_{SD} \) at the pixels are calculated using a normalised sum of differences between the captured speckle intensities \( I_{SP,N} \) and the reference intensities \( I_{REF} \).
\[
I_{SD}(x, y) = \frac{1}{I_{REF}(x, y)} \sum_{N=1}^{N_{MAX}} \sum_{x-i}^{x+i} \sum_{y-j}^{y+j} \left( I_{SP,N}(x, y) - I_{REF}(x, y) \right) \]  \quad (2-11)
\]

\(I_{SD}\) is a similar measurement to speckle contrast, defined in equation (1-4) above, and like contrast falls with increased blood flow. Forrester converts the \(I_{SD}\) values to a blood flow index \(I_{BF}\) using the power series equation

\[
I_{BF}(x, y) = \sum_{c=0}^{C_{MAX}} A_c \left( \frac{1}{I_{SD}(x, y)} \right)^{B_c} \]  \quad (2-12)

in which the order and coefficients of the power series are determined empirically, using fluid flow in pipes and tissue phantoms, so that the blood flow index \(I_{BF}\) is as far as possible linearly related to blood flow. This analysis was compared directly with Laser Doppler perfusion Imaging (LDI) for determination of burn scar perfusion\(^50\). It was also used in an endoscopic speckle imaging system, described further below\(^51,52\). This analysis relies on a calibration using flow in relatively large (0.95 mm) tubes, which may not accurately model the real microcirculation in which 7 µm diameter erythrocytes travel through approximately 10 µm capillaries.

Another suggested in-vivo calibration method for speckle contrast measurements used the pulsatile flow in vessels. Duncan recorded time series of speckle contrast at spatially separated points in a vessel, and made an estimate of the speed of the pulse travelling along the vessel\(^45\). They suggest that this speed might be used to calibrate speckle contrast measurements. However, the velocity of the pulse pressure wave which generates the velocity surge in the vessels does not directly correspond to the mean flow velocity, introducing further problems of interpretation.

### 2.1.5 Time history of speckle patterns: an alternative approach.

Time history of speckle patterns (THSP) is an alternative use of biospeckle, useful for characterisation of biological samples\(^53,54\). The technique was initially developed by Oulamara in 1989\(^55\). Using a 2D CCD array and measuring the decorrelation between time-separated speckle patterns they characterised the biological activity of botanical specimens and could distinguish different species of fruit. The changing speckle patterns in this case are ascribed to continuous slow movements of the µm sized cell organelles, and faster movements of smaller particles in the cell vacuoles.

Other statistical analyses of THSP line-scan data produced by this method have also been
used to characterise biological activity, including the inertial moment of a co-occurrence matrix generated from THSP data\textsuperscript{53,56} and wavelet transform-based entropy calculations\textsuperscript{56–58}.

### 2.1.6 Applications of biospeckle measurements.

There have been many applications of biospeckle measurements to various tasks. The earliest experimental application by Briers, as mentioned above, was in imaging retinal blood flow\textsuperscript{28}. Subsequently, speckle techniques have been applied to measuring biological activity in botanical and agricultural subjects\textsuperscript{59} such as the detection of fungi in beans\textsuperscript{60}, measurement of the activity of other micro-organisms\textsuperscript{61,62}, assessment of seed viability\textsuperscript{63}, monitoring the leavening process in bread\textsuperscript{64}, and testing the ripeness of fruit\textsuperscript{65}. A speckle technique has even been applied to the proverbially dull task of watching paint dry\textsuperscript{66}, as well as other films\textsuperscript{67}.

The range of medical applications has been fairly broad following Fercher and Briers’ original speckle photograph imaging of retinal vessels, though none of these applications seem to have achieved widespread clinical use. Research on retinal measurements and images has continued, including the development of fundus cameras adapted to incorporate speckle imaging\textsuperscript{68,69}. There has been greater interest in applying speckle contrast imaging to cerebral blood flows – the high definition and frame rate achievable with speckle imaging mean that functional imaging of the blood flow in the surface vessels of the brain is possible in animal models\textsuperscript{1–3,70–76}. For example, speckle contrast imaging was used to image the development of an ischemic core following a photochemically induced cortical infarction\textsuperscript{2}. An infarction was induced in a rat's brain using a photo-sensitive dye and a small laser spot, and the subsequent changes in the local perfusion monitored using a laser speckle contrast system. These images were through a hole in the skull, with the dura mater removed, but imaging is also possible through the thinned skull of a rat, using a speckle analysis that accounts for static speckle patterns\textsuperscript{42}.

An endoscopic speckle imaging system was developed by Forrester in 2003\textsuperscript{51}, and used for measurements of perfusion in the tissues of the knee during arthroscopic surgery\textsuperscript{52}. Laser speckle imaging has also been regarded as a good candidate for determining the perfusion in burn scars, as it rapidly provides an image by a non-contact method that indicates the relative viability of damaged tissue\textsuperscript{50,77}. The immediacy of laser speckle imaging means that it can provide feedback on a procedure during treatment.
Huang et al. used a speckle imaging system during laser therapy of Port Wine Stain birthmarks, to indicate areas where the perfusion was not reduced by treatment.\textsuperscript{78}

Biospeckle measurements are sensitive to any moving particle. Kim et al. introduce a twist to the technique by altering the motion of fluids using super-paramagnetic iron oxide (SPIO) nanoparticles in an external AC or DC magnetic field.\textsuperscript{79} They suggest that this technique might aid in mapping the microvasculature, particularly the small vessels where the ordinary speeds of the blood cells are slow.

Using longer exposure times than those used to image blood flow, Kalchenko et al. have shown that speckle techniques can be used to image the flow in lymphatic vessels.\textsuperscript{80,81} They use a system with dual exposure times, long exposures (650 ms) imaging slowly flowing lymph and shorter exposure (33 ms) imaging blood flow.

Laser speckle perfusion imaging was validated in multi-modal instruments combining speckle imaging with Fluorescence Intravital Microscopy (FIM) and Spectrally Enhanced Microscopy (SEM) in work investigating cancer tumours by Kalchenko et al.\textsuperscript{82,83} Speckle contrast imaging was complementary to the other modalities in these studies, providing functional flow information while the other modalities mapped the tumour microenvironment (FIM) and vessel topology (SEM). This multi-modal approach suggests a possible role for speckle imaging in biomedical research in adding function information to existing imaging modalities which detect anatomical structure.

2.2 Laser Doppler and laser biospeckle

2.2.1 Laser Doppler flowmetry

Laser Doppler flowmetry and laser Doppler perfusion imaging are optical techniques for measuring blood flow.\textsuperscript{5,6} These methods rely on the same physical mechanism as laser speckle techniques but use a different measurement and analysis regime. Laser Doppler methods measure the intensity fluctuations in a speckle, sometimes called a coherence region in laser Doppler nomenclature, directly using a fast, sensitive optical detector such as a photomultiplier or photodiode. When coherent light is scattered and returned to the detector from the tissue, a proportion of the returned light which has been scattered from moving particles is Doppler shifted: its frequency is changed as a result of the changing path length between source and detector. The fluctuations detected are created by beating between Doppler-shifted light and non-shifted light which has scattered from static paths.

Riva et al. applied laser Doppler velocimetry to flow in capillary tubes and retinal
arteries in 1972\textsuperscript{84}. This measurement assumes light singly scattered from moving blood cells in small vessels. In 1975, M.D. Stern reported Doppler broadening in the spectral line of laser light which was multiply scattered from skin, and suggested that this effect could be used to measure the state of blood flow in the microcirculation\textsuperscript{85}. Subsequently, several instruments were developed\textsuperscript{86-91} by different groups, and commercial laser Doppler instruments are now available\textsuperscript{33,34}. Various laser Doppler arrangements allow differential measurements, excluding spurious results from changes in optical power\textsuperscript{86,92}, and use a variety of analyses to determine perfusion from the Doppler signal. Laser Doppler imaging techniques involving scanning the measurement point have been developed\textsuperscript{5,93}, as have full-field techniques using fast cameras (20 kfps) to collect the laser Doppler signal at every pixel\textsuperscript{9,94,95}.

2.2.2 Laser Doppler perfusion index.

Doppler perfusion measurements generally rely on a spectral analysis of the Doppler signal to estimate a perfusion index. Bonner and Nossal\textsuperscript{7,96}, Nilsson\textsuperscript{5} and other workers in laser Doppler flowmetry give the equation, for an arbitrary velocity distribution of moving scatterers,

\[
\int \omega^n P(\omega) d\omega \propto C_{RBC} \langle v^n \rangle
\]  

(2-13)

which scales with the concentration \( C \) of RBCs for \( n=0 \) and with the product of \( C \) and mean speed \( \langle v \rangle \) for \( n=1 \). This value (with \( n=1 \)) is the first moment of the spectrum, and provides a processing algorithm generally used as a perfusion index (PI) with arbitrary scaling. The proportionality between PI and the product of concentration and mean speed has been verified by both experiment\textsuperscript{7} and simulations\textsuperscript{97}.

2.2.3 Laser Doppler and time-varying speckle.

Briers showed clearly that laser speckle and laser Doppler measurements rely on the same physical phenomenon\textsuperscript{26}. As this fact is crucial to much of the work in this thesis, Briers' analysis is given below in some detail. His reconciliation of laser Doppler perfusion measurements and laser speckle contrast analysis starts with consideration of a coherently illuminated Michelson interferometer, as shown in Figure 2.3, in which one of the two paths includes a moving mirror \( M_2 \), with velocity \( u \). A detector receiving the combined light from both paths records a sinusoidally fluctuating signal, which is explainable using either Doppler shift or interferometric interpretations.
Figure 2.3: Michelson interferometer with one moving mirror. Coherent light from the laser source is split at the beam splitter to travel two paths, and a proportion of the light is recombined and sent to the detector. Interference between the paths results in a fluctuating signal at the detector.

A Doppler effect interpretation of the sinusoidal signal at the detector is that the frequency of the light which has travelled via the moving mirror $M2$ has changed from its original frequency $\nu$ to a Doppler-shifted frequency $\nu'$:

$$\nu' = \frac{c}{c-2u} \nu$$

(2-14)

The detector measures this light mixed with light which has travelled via the fixed mirror, unshifted in frequency. The detected signal fluctuates at the beat frequency – the difference $\delta \nu$ between the two frequencies $\nu$ and $\nu'$:

$$\delta \nu = \nu' - \nu = \nu \left( \frac{c}{c-2u} - 1 \right)$$

(2-15)

and as $c >> u$:

$$\sigma \nu = \nu \frac{2u}{c}$$

(2-16)

This gives the Doppler shift frequency recorded by the detector.
The interferometric interpretation is as follows: The two beams, travelling by their respective paths, interfere at the detector. The light intensity at the plane of the detector will be either uniform, if the two beams are completely parallel with flat wavefronts, or composed of interference fringes if they are not. Either way, the intensity measured at the small detector depends on the relative phase of the two beams at that point. If the moving mirror M2 moves a distance $\lambda/2$, where $\lambda$ is the wavelength of the light, the path difference between the two beams changes by $\lambda$, and the detector measures one full sinusoidal cycle of intensity. If $\delta \nu$ such cycles occur in 1 s, then the mirror M2 must have moved a distance $u$ in that second, where:

$$u = \sigma \nu \frac{\lambda}{2}$$  \hspace{1cm} (2-17)

As $u$ is the velocity of M2 and $\delta \nu$ is the frequency of the signal measured at the detector, we can use the relationship $c = \nu \lambda$ and rearrange to find:

$$\sigma \nu = \nu \frac{2u}{c}$$  \hspace{1cm} (2-18)

identical to the equation (2-16) above. We have the same result from two different models – one (Doppler) relying on changing the frequency of one of the waves, and detecting the resulting beat frequency, and the other (interferometric) assuming the same frequency for both waves and detecting correlation as one wave is moved past the other.

This result is extended from classical interferometry to laser speckle by considering an analogous experiment where the moving mirror is replaced by a moving diffusing surface as shown in Figure 2.4. Light scattered from this surface will produce a speckle pattern at the plane of the detector. This speckle pattern is still coherent with the light returned from the fixed mirror, so will interfere to generate another speckle pattern. The argument above still applies – there will still be a Doppler signal due to the changed frequency of the scattered light, or alternatively changing the relative path lengths by a distance $\lambda$ will still produce a single cycle fluctuation in the intensity measured at the detector. The interferometric explanation now becomes a speckle explanation – rather than interference fringes passing the detector, there is a changing speckle pattern.
Trivi restates this argument, and adds another permutation to the thought experiment: a cuvette containing a liquid with refractive index $n$ is placed in the variable arm of the interferometer as shown in Figure 2.5. In this case the effective path-length of the light returned though the cuvette depends on both the length and refractive index of the cuvette. Changing either of these parameters presents the same shift in path length to the detector as some movement of the mirror M2, and a continuous change in either $n$ or $d$ with time will produce a Doppler signal – or, a dynamic speckle pattern – indistinguishable from that produced by a moving mirror or diffuser. This situation is similar to many biospeckle configurations, where the both configuration and optical properties of the tissue under test may be variable and demonstrates a possible limitation of both laser Doppler and laser speckle perfusion measurement systems: we must generally be able to make the assumption that the signal we receive is generated by moving blood cells, not by other movements in the tissue under test.
Figure 2.5: Michelson interferometer with a cuvette interposed in one arm. Refractive index $n$ of the fluid contained in the cuvette is variable. The path length as seen by light travelling through the cuvette changes with $n$, producing a change in speckle pattern at the detector similar to that produced by moving the mirror M2.

2.3 Simulations of laser speckle

In order to test speckle analyses and develop a better understanding of the biospeckle phenomenon, it can be useful to produce computer simulated speckle data. Duncan and Kirkpatrick provided sets of algorithms for both generating simulated speckle fields and conveniently calculating Briers-style speckle contrast.

The central element of these simulations is the procedure for generating a speckle field by taking the 2D Fourier transform of a generating matrix. The simplest version of this procedure is illustrated in Figure 2.6. To generate a frame of simulated speckle, we start with a zero-filled square matrix $A$, containing a smaller area filled with complex numbers. These complex numbers all have unit magnitude, and their phase is randomly distributed on a uniform distribution. The speckle field $E$ is generated by taking the two dimensional Fourier transform of $A$. The speckle intensity $I$ is found by multiplying $E$ by its complex conjugate. The phase of the complex numbers in $A$ represents the random phase added to to each contributing light path during scattering from tissue. The ratio of the size $L$ of $A$ and the size $D$ of the filled area gives the speckle grain size: $s = L/D$. The complex number region is shown in this example as a square – other shapes may be used, with a circular region producing isotropic speckles and elliptical or oblong regions producing stretched speckles.
The algorithm above generates a single frame of simulated speckle data. In order to simulate dynamic biospeckle, it is necessary to generate a series of speckle frames which vary with time. Duncan and Kirkpatrick describe using a copula to generate speckle with defined inter-frame correlation: they generate a pair of uniformly distributed variables with defined, arbitrary correlation. These variables are inserted into the speckle frame algorithm above as the phases of the complex numbers in the generating matrix \( A \).

Variations on these simulations are used extensively in this thesis in sections 3.3, 4.2,
5.1.2, 5.1.3, and in associated papers\textsuperscript{20,22} as a useful tool for understanding and demonstrating various aspects of biospeckle measurements. Adaptations of the original code are described in the sections where they are applied.

2.4 A summary of the state of laser biospeckle research.

Though laser biospeckle provides an excellent imaging method, the simple analyses often used in speckle applications have not given quantitative measurements. More sophisticated analyses, particularly those using multiple exposures promise to increase the quantitative measurement power of biospeckle measurements\textsuperscript{20,21,42,44,46}. This will be important if laser biospeckle is to be applied to measurement of the effects of chronic diseases such as diabetes: measuring the change in perfusion over the course of a long treatment will require repeatable, quantitative measurements. There has been a degree of confusion over the connection of laser speckle and Doppler. This connection is becoming clearer; despite their apparent differences, laser speckle and Doppler are the same physical phenomenon, with different interpretations. Reconciling these interpretations enables the same quantitative information to be generated using laser speckle systems as laser Doppler systems.

The current medical applications, both research and clinical, of laser biospeckle show the promise of what is achievable with this technique. Developing a quantitative connection between blood flow and biospeckle measurements will make the method more suitable for research and clinical applications that require better reliability about what is being measured.
3 Speckle imaging hardware and software

The essential components of a laser speckle system are, as shown in Figure 2.2 above, an illuminating laser, a monochrome digital camera and a computer with appropriate software performing the speckle analysis and displaying the resulting images and data. The software must be designed to present useful results in an appropriate form for the end user – this form may be different for research and clinical purposes. Aspects of the various system components used in this work are discussed in the following sections.

3.1 Laser illumination

Initial implementations of speckle contrast imaging starting in the 1970s used Helium Neon (HeNe) lasers for illumination. Later implementations moved to diode lasers, which introduce issues as their coherence length is typically much shorter than that of gas lasers. As speckle contrast measurements rely on interference between light which has travelled through multiple paths, the lasers used for illumination must have coherence lengths longer than the maximum expected path length difference due to multiple scattering in the target being imaged. This requirement means that any diode laser selected must be single-mode, and must operate in single mode over the time course of the experiment. Any changes in coherence length in the laser illumination are detectable as a change in the maximum contrast achievable.

The lasers initially used in this work were single mode diode laser modules from World Star Tech\textsuperscript{103}, similar to those available from many other suppliers. As the lasers proved to be insufficiently stable, they were replaced with volume Bragg grating stabilised laser diode modules from Ondax\textsuperscript{104}.

The wavelengths chosen for this work were in the red region of the visible spectrum, 650 nm to 658 nm, which are not absorbed by dermal tissue and are strongly reflected from blood cells. Working in near infra-red, for example at 850 nm may provide some advantages as this wavelength gives a slightly lower scattering coefficient and hence penetrates deeper into the tissue\textsuperscript{16}.

3.1.1 Single mode temperature controlled diode lasers

Although the World Star Tech lasers are single mode, the maximum contrast achievable using them on a multiply-scattering target was found to fluctuate over time. This indicates
that the laser is not stably operating in a single longitudinal mode but changing between two or more modes, and occasionally producing reduced contrast while operating in two modes simultaneously. This effect is more apparent in multiply scattering targets than singly scattering surfaces, as the path differences in multiply scattering targets are longer and hence more likely to be affected by the reduced coherence length of a multi-mode laser. The modules were 50 mW at 658 nm, part number TECRL-50G-658. Both a fixed and variable power version of these modules was used.

The laser modules are thermoelectrically cooled, in order to stabilise their operation and increase the diode lifetime, and one of the modules used allowed the laser power to be varied continuously via a control voltage. The temperature set-point control for these modules was factory set and inaccessible, but an alteration involving drilling a hole in the module case and adding extra wires to allow measurement of the temperature setting voltage allowed the temperature to be user-adjustable. There is a linear conversion between this voltage $V_T$ and the diode temperature $T$ in °C, with the parameters supplied by the manufacturer: $T = -40.26 \, ^\circ\text{C} \cdot V^{-1} \times V + 80.74 \, ^\circ\text{C}$.

The module specification claims temperature stability of 0.01 °C. The temperature sensor in the control loop is at the back of the diode package, so the temperature at the chip may vary by more than this figure. Later iterations of the module design from this and other manufacturers have user adjustable temperature set-points, in recognition of the fact that the laser wavelength, and hence mode stability at a particular set-point, drifts over the medium term.

In order to determine the performance of these modules at various output powers and temperature set points, a series of phantom tests with a range of power and temperature settings were performed.

For the fixed power laser, speckle contrast and the laser's central wavelength were measured over a range of temperatures between 15 °C and 25 °C. The central wavelength was measured using an Ocean Optics spectrometer. Unfortunately the resolution of this instrument is not sufficient to separate the adjacent laser modes that are operating, so the central wavelength measurement is a mean wavelength generated from a spectral peak that probably covers two or more modes. Speckle contrast was measured on a multiply scattering target made of silicone and Al₂O₃ powder, with aperture and contrast calculation regions set at f/16 and 35 pixels respectively. The camera had both a
polarising and a bandpass filter installed.

The results from the fixed power laser are shown in Figure 3.1. There is a region of stable, high contrast between 20.0 °C and 20.7 °C, and another between 21.7 °C and 22.3 °C. Operating this laser in one of these two regions allows repeatable measurements with a high maximum contrast. The maximum contrast achieved in these trials was less than 1 due to spatial averaging, as discussed in section 3.3.1.

![Figure 3.1: Speckle contrast and laser wavelength produced using a fixed-power thermoelectrically cooled diode laser module at a range of temperatures.](image)

For the variable power laser (658 nm, 50 mW max power), the contrast was measured at a range of temperatures from 13 °C to 24 °C in steps of approximately 0.3 °C and a range of laser powers from 10 % to 100 % in steps of 10 %. Speckle contrast was measured on a multiply scattering PTFE phantom. These measurements were repeated, 18 days apart, and both sets of data are plotted as false-colour images in Figure 3.2. For this laser there is a stable single mode region of operation which extends from approximately 19 °C to 22 °C at 100% power. It would be useful in many experimental situations to use variable laser power to prevent saturation in the camera while changing the exposure – however, it is clear from these plots that the maximum power reduction maintaining single mode operation at a fixed temperature is from 100% to around 50%, at 20 °C.
The isocontrast regions in Figure 3.2 appear stretched on a consistent angle. As the temperature controller in this module appears to act on the back of the diode package, these might represent a constant chip temperature, as at a lower power there will be a lower temperature gradient through the chip and package assembly.

The second set of measurements were made 18 days after the first, as there was some long term variation in the maximum achievable contrast with no change in operating parameters while using these lasers. The re-measurement showed that the stable regions are similar over that time. The contrast variations observed may have been caused by operating the laser near the edge of a stable region.

3.1.2 Volume Bragg grating stabilised diode lasers

While good results can be achieved using the temperature controlled diode lasers described above, the requirement for long-term monitoring of the maximum achievable contrast and occasional adjustment of the operating power or temperature set-point means that they are not ideally suited to use in a clinical or medical research system, which should run reliably with minimal technical attention. For clinical trial use, the World Star Tech modules were replaced with volume Bragg grating stabilised diode laser modules available from Ondax\textsuperscript{104}. These modules have a fixed linewidth set by the characteristics of a volume Bragg grating, produced using a holographic technique and used to provide external feedback to the laser diode\textsuperscript{105}. The specified linewidth is 50 MHz at 658 nm, corresponding to a coherence length of 6 m.

These modules give reliable high contrast in a speckle system at the costs of increased
price over a standard laser diode module, and a darker region in the centre of the expanded beam caused by the external feedback, as shown below in the left hand panel of Figure 3.4.

### 3.1.3 Laser spreading optics

Speckle measurements require that the whole area to be imaged is illuminated by the laser beam. Though the inherent normalisation of the speckle contrast calculations allows some non-uniformity of the illumination, non-uniform illumination is undesirable at both short and long exposures approaching the dynamic range limits of the camera. Simply enlarging the laser beam using a lens is suitable in some situations\(^{40}\), but generally provides non-uniform illumination as the Gaussian or similar beam shape is reproduced at a larger size on the imaged field. This is a particular problem when using the volume Bragg stabilised laser diodes described above with a central dark region. Simple lens-based expanders are also susceptible to thrown shadows caused by dirt and dust. For these reasons, after some initial experiments, lens-based beam expanding setups were not used in this thesis.

Some researchers have used optical fibre illumination for speckle systems. Using a single-mode optical fibre to bring the laser light to the instrument head allows for the use of bulky laser modules without increasing the bulk of the instrument head\(^{1,49}\). These systems generate a Gaussian intensity profile over the working area, so the brighter central region of the illumination is used.

The method of beam spreading found most suitable for the speckle systems in this work was to use engineered diffusers, also called micro-optical arrays and produced by RPC photonics\(^{106}\). These diffusers consist of an array of micro-lenses, and spread a parallel beam into a variety of forms including top-hat profile circular and rectangular, with spreading angles from 0.25° to 120°.

![Figure 3.3 Laser expansion using an engineered diffuser, as used in speckle contrast systems.](image)

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[^40]: Number or reference
[^49]: Number or reference
[^106]: Number or reference
As engineered diffusers are refractive elements, they preserve coherence, unlike ground glass or similar scattering diffusers. In order to recruit as many of the micro-lenses as possible, giving uniform illumination, the approximately collimated beam from the laser diode modules was expanded using a pair of lenses to cover the 25 mm diameter of the engineered diffusers as shown in Figure 3.3. The illumination achievable using these diffusers is very uniform in the working region, as shown in Figure 3.4 which compares the top-hat profile of a 20° square engineered diffuser with a lens-expanded laser beam.

The engineered diffusers were found to have an interesting effect on the polarisation of the expanded light beam. When linearly polarised light is incident on an engineered diffuser a proportion of the transmitted light is converted to the orthogonal polarisation. This effect is caused by birefringence in the injection moulded material used to make the diffusers. This phenomenon was observed in the 20° square and circular pattern diffusers, and not in a 10° circular pattern diffuser. The proportion of transmitted light with polarisation orthogonal to the incident light varied with the angle between the diffuser pattern and the polarisation direction, from approximately 2% when the polarisation direction was oriented parallel to the edges of the square pattern in a 20° square diffuser, to 30% when the polarisation direction was oriented along a diagonal. This effect is important as cross polarisation was used in the speckle systems, as described below in section 3.2.2, to reduce specular reflections in the images and increase contrast. The engineered diffusers were always used with the polarisation direction aligned for minimum disruption to the polarisation.
Figure 3.4 Illumination examples - images and profiles of the beam from a Volume Bragg Grating (VBG) laser diode module expanded using a lens (left) and a 20° square engineered diffuser (right). The profiles plotted in the upper image are column means of the images between the dashed lines. Note the central dark area in the lens-expanded laser, due to the VBG feedback.

Given limited laser power and the requirement for a fixed aperture in speckle imaging, the illumination area chosen must always be a compromise between using a large enough area to produce a useful clinical image, and keeping the laser intensity sufficiently high that short exposures of under 1 ms collect sufficient light.

3.2 Imaging hardware

3.2.1 Cameras

Speckle images are generally recorded using monochrome industrial digital video cameras. The experimental work in this thesis uses two Sony cameras with Firewire connections to the computer, models XCD-SX910 and XCD-V60.

These cameras have adjustable electronic gain, with a usable range of 0-18 dB. The reported gain, as set in the software, was found to vary slightly from the real value – a heuristic correction developed by my colleague Evan Hirst and given below produces intensity values that are independent of gain for a given scene, provided that the camera is not saturated\(^{107}\).

\[
I = \frac{\text{counts} \times 0.49565}{\left(1.4207^{\text{gain/3}}\right)}
\]  \hspace{1cm} (3-1)

The constants in this equation were measured for the particular XCD-V60 camera generally used, and may be different for different cameras.
A selection of C-mount camera lenses were used for imaging speckle, with a 12 mm to 36 mm varifocal lens proving the most versatile.

3.2.2 Filters
Two filters were used in the laser speckle imaging path. A red coloured filter was used to reduce the effect of background light. Bandpass Fabry-Perot filters, matched to the laser line wavelength, are available and provide better background reduction but have the disadvantage of low working acceptance angles, so were found impractical for this work.

A polarising filter was also used, with two key effects: increasing measured contrast, and excluding specular reflections. Laser speckle from a single-scattering surface illuminated by coherent, linearly polarised light has a contrast of 1. Multiple scattering in tissue, and the resultant depolarisation, reduces speckle contrast by a factor of $1/\sqrt{2}$. Once multiple scattering depolarises the incident light, the two orthogonal polarisations may be described as two unrelated speckle patterns, which add at the camera on a sum of intensities basis rather than as a vector sum of fields. Selecting a single polarisation returns the achievable maximum speckle contrast to 1. The polarising filter excludes specular reflections as it is aligned to cross polarisation axes with the incident light, which is linearly polarised as it is produced in the laser diode module. All of the light collected at the camera sensor must then have been multiply scattered to change its polarisation, so has travelled through the tissue rather than reflecting from the surface.

3.2.3 Mechanical hardware and stability
Laser speckle contrast measurements are movement sensitive. As well as detecting the movement of blood cells in tissue, a speckle contrast-based measurement will detect any other movement between the camera, laser, and tissue under test. Speckle equipment therefore requires solid, stable stands that do not vibrate. The lab equipment used is shown in Figure 3.5. This setup was also used in the clinical trials described in Chapter 7.
3.3 Optimisation of speckle system parameters

There are a number of interrelated adjustable parameters in a speckle contrast imaging system. As these parameters have inter-related effects, they must all be optimised for a particular application. Figure 3.6 shows the interrelationship of these adjustable parameters, and their dependent effects. The adjustable parameters are shown in the green boxes on the left hand side of the diagram, and dependent effects in yellow boxes on the right. Dependencies are shown by arrows travelling from each parameter to the effects dependant on it. The following sections explain the effects of changing these parameters and their interrelationships.
Figure 3.6 Adjustable parameters and dependant effects in the design of a laser speckle system.

3.3.1 Lens aperture, speckle size and calculation region

A critical adjustable parameter in an imaging laser speckle system is the lens aperture, as it determines the speckle size (described in equation (1-2), and repeated below in equation (3-2)) where \( s \) is an approximation of the minimum speckle size for an imaging system with magnification \( M \) and f-number \( N \) working at wavelength \( \lambda \):

\[
s \approx 1.2(1 + M)\lambda N
\]  (3-2)

If the speckle size is significantly smaller than the pixel size, the speckle contrast falls as the square root of the number \( n \) of coherence regions inside the measurement area, as shown by Goodman:

\[
K = 1/\sqrt{n}
\]  (3-3)

It is therefore important that the speckle size is at least as large as the pixel size. Many of the earlier speckle imaging systems chose a lens f-number so that the speckle size is approximately equal to the camera chip pixel spacing \(^{38,41}\). Kirkpatrick et al. have shown through simulations that this effectively undersamples the speckle pattern, and that the minimum speckle size to avoid undersampling is twice the pixel spacing - a straightforward application of the Nyquist criterion \(^{108}\). Their simulations show that when the pixel size equals speckle size, the speckle contrast is significantly reduced. This contrast reduction is not caused by sampling points occurring too far apart, as might be implied by the use of the Nyquist theorem, but by spatial averaging of the light across
each individual pixel.

Repeating Kirkpatrick's simulations, with some variations, illustrates the effect of spatial averaging. A range of speckle images were developed from a speckle image with different effective speckle sizes relative to the pixel size. The initial image was generated, as described above in Section 2.3, with a minimum speckle size $s = 20$ pixel, and subsequent images with smaller effective speckle sizes were produced by low-pass filtering using box-car filters with the sizes 1 pixel to 128 pixel, followed by down-sampling, to generate a set of images with effective speckle sizes between 20 and 0.16 pixels. This procedure simulates the effect of increasing the lens aperture in a real speckle system and thus reducing the speckle size relative to the camera pixels. The size of all the simulated speckle images was 200 pixel by 200 pixel.

Figure 3.7 shows the results of this procedure. The speckle contrast rises continuously with increasing speckle size, approaching $K = 1$ as expected when speckle size is significantly greater than the pixel size. The annotated points in Figure 3.7 indicate the two speckle size criteria introduced above. At the commonly used speckle size of one pixel, the speckle contrast is approximately 0.7. At a speckle size of 2 pixel, corresponding to the Nyquist criterion, the speckle contrast is approximately 0.9. Despite meeting the Nyquist criterion for sufficient sampling, there is still a definite reduction in contrast at a speckle size of 2 pixels due to the spatial averaging effect of the finite pixel size.

![Figure 3.7](image)

*Figure 3.7 Change in speckle contrast $K$ with changing speckle/pixel size ratio. Contrast is reduced as speckle size is reduced due to the spatial averaging effect of pixels.*

The contrast values above were calculated by taking standard deviation and mean over the whole 200 pixel by 200 pixel speckle image. However, in speckle imaging the contrast is usually calculated over relatively small regions of the raw image. As the
speckle contrast is a statistical measurement, the calculation area needs sufficient pixels for the standard deviation and mean of the sample to be meaningful. The calculation region, generally a square, needs to be a multiple of the minimum speckle size and has often been set at 5 pixel x 5 pixel or 7 pixel x 7 pixel$^{32,38}$. In order to quantify the effect of changing region size, the speckle calculation region was varied in the range 2 pixel x 2 pixel to 200 pixel x 200 pixel and the contrast re-calculated. The effective speckle size was held at its original value of 20 pixel for these calculations. For each region size, the contrast was calculated for regions covering the whole simulated speckle image and the mean of the contrast found. The results are plotted in Figure 3.8.

The annotated points in Figure 3.8 indicate the effective region sizes generated by combinations of common speckle size and region size criteria.

![Figure 3.8](image)

*Figure 3.8 Change in speckle contrast with change in speckle calculation region with respect to speckle size.*

The two effects of speckle to pixel size and region size are considered independently here, but will clearly interact in practice – a restricted calculation region will produce a further reduction in measured speckle contrast if it is not sufficiently large.

The choice of lens aperture and its resultant speckle size, and the choice of calculation region, affect the maximum contrast achievable. Reducing the aperture to increase the speckle size clearly also reduces the light level transmitted to the chip, and thus constrains the usable gain and exposure camera settings. Due to these practical limitations in producing the perfect situation of very large speckles, workers in this field have allowed some spatial averaging as a compromise in system design, producing a maximum contrast less than 1. Many of these researchers use a system factor $\beta=1/K_{\text{max}}$, where $K_{\text{max}}$ is the
maximum achievable contrast for a particular system using a static target, to correct for spatial averaging effects\textsuperscript{42}. This correction is shown empirically to recover the true speckle contrast by simulation and experimental results, as discussed in Section 5.1 below.

3.3.2 Measuring speckle size
As the varifocal lens that was predominantly used in these experiments does not have f-number markings allowing the speckle size to be simply calculated, speckle size was measured from the image using a 1-D autocorrelation calculation incorporated into the speckle software for this purpose.

3.4 Software design
Several versions of a custom software programme \textit{IRL Speckle Viewer} were written in Borland Delphi for lab and clinical use. The Delphi programming environment was chosen for familiarity, and because it was already in use in the lab at the time. The key features of this software are the capability to control the camera and receive data from it, calculate speckle contrast, display both raw and processed images, and save raw and processed data. The software is written in an object oriented style. The general design of the software is illustrated in Figure 3.9.

![Diagram of software design](image)

\textit{Figure 3.9 Illustration of the software design for the \textquote{IRL Speckle Viewer} software.}

In ordinary operation, the software is controlled by a main working thread, written in the program logic module. Following initialisation, which starts the camera capturing images at a specified frame rate, this thread uses a waiting function supplied in the Sony camera library via the camera interface module to wait until there is an image available in the camera’s buffer. On receiving the message that a frame is available, the working thread uses subroutines in the ‘Program Logic’ section to copy the data into a working buffer,
process the data to generate a speckle contrast image, then display and save the raw and processed data as appropriate before waiting until another image is available.

The Sony cameras used support the DCAM/IIDC\textsuperscript{109} standard, a standard designed for operating industrial digital cameras over an IEEE 1394 (FireWire) connection. They were accessed from the software using a Sony supplied dynamically linked library (DLL) and header file, translated from C to Delphi. The camera control code was written in a separate module so that changing cameras in future implementations of the system will not require rewriting the whole software, simply replacing that module.

Figure 3.10 shows the lab version of the speckle viewer software. The left-hand (grayscale) image shows the raw speckle image, and the right (false colour) image shows a processed version of that image.

![Figure 3.10 Screen shot of 'IRL Speckle Viewer’ software. This is the working lab version, with full flexibility and consequent complicated GUI.](image)

The setup of experiments was controlled using text files which defined all of the working parameters of the camera and file storage options, which were loaded by the software to make sure that all settings remained the same in a particular set of experiments. An example experiment file is included in Appendix 2.
3.5 Clinical trial hardware and software considerations
For the clinical trials described in section 7.2, the speckle hardware and software was adapted to fit the particular clinical application: measuring the perfusion immediately around diabetic foot ulcers. This adaptation consisted of incorporating the hardware into a specialised trolley and simplifying the software user interface.

3.5.1 Hardware adaptations
The speckle camera and laser were incorporated into a trolley as shown in Figure 3.11. A padded footrest ensured that the patient’s foot was always placed at a consistent distance from the camera.

![Speckle hardware adapted for imaging around diabetic foot ulcers.](image)

3.5.2 Software adaptations
The user interface of the speckle software was adapted as shown in Figure 3.12. The workflow was simplified so that the clinician using the system did not have access to the camera controls, and so that the patient and site details were recorded consistently. The clinician’s workflow was as follows:

- Click the “New Patient” button. This triggers a pop-up dialog box in which the patient’s details are entered. Clicking Submit enters the patients details in the main window of the software.
For each site measurement, centre the site to be measured on the screen and click the “New Measurement” button, which triggers a dialog with a drop-down box of possible entries for the site.

Hitting “Submit” in this dialog starts the measurement. A multiple exposure speckle series is taken and saved in a date-stamped folder along with the patient and site details.

Figure 3.12 Speckle software with simplified user interface for clinical trials.

3.6 Speckle imaging hardware and software: conclusions
A flexible laser speckle system was designed, suitable for lab use and clinical trials in particular applications. Some aspects were found to be important in the design of the
system. In particular, laser specifications and decisions around the camera aperture, and hence speckle size, and speckle contrast calculation region, are critical to the performance of a laser speckle system. The effects of laser coherence length and spatial averaging influence the achievable laser speckle contrast and hence the interpretation of contrast reduction as a flow measurement.

The stability of the temperature-controlled single-mode WorldStar lasers first used was not found sufficient. While good results could be obtained intermittently the fact that the achievable maximum speckle contrast varied over time meant that a system using these lasers was not reliable and didn’t produce repeatable measurements. This was found to be due to the laser not operating reliably in a single-mode state. Adjusting the temperature set-point and laser power showed that there were regions of relatively stable operation available but we did not wish to rely on an individual laser module remaining in these stable regions, so moved to a different laser.

Volume Bragg grating stabilised laser modules from Ondax have proved stable in subsequent work, though they have the issue of a dark spot in the centre of the beam due to the feedback from the Bragg grating removing that light. This is a characteristic of the laser design, confirmed in discussion with the manufacturers.

Expansion of the laser beam using engineered diffusers has proved very satisfactory. These are not true diffusers, as they do not scatter light but rather refract it using a large number of micro-lenses, preserving coherence and producing even illumination over the field.

Optimisation of both speckle size and speckle contrast calculation region with respect to the camera pixel size is critical. Typical choices of these sizes trade off achieving maximum speckle contrast with resolution or signal-to-noise ratio. In other words, practical choices of speckle and calculation region size generally imply some spatial averaging. Section 5.1 below will show that a correction for this spatial averaging is possible.

For clinical applications a minimum speckle size of approximately 2 pixel is sufficient, generating a reduction in maximum contrast from 1 to 0.9. Given 2 pixel speckles, the typical 5 pixel x 5 pixel calculation region is barely sufficient, and in cases where the required system definition allows, larger calculation regions should be used.
4 Speckle analysis: from contrast to perfusion

Medical acceptance of laser speckle based imaging and development of research and clinical applications has been hindered by uncertainty about what physiological parameters are displayed in laser speckle contrast images. A solution to this uncertainty is to compare laser speckle methods to the physically similar laser Doppler technique as shown by Briers\cite{26}, and discussed in section 2.2 above. In that analysis it is clear that speckle contrast methods and laser Doppler rely on the same physical phenomenon. This chapter shows that exactly equivalent information can be drawn from laser speckle contrast measured at a range of exposures as from laser Doppler. This analysis is repeated from my publications \cite{19-21}.

Laser Doppler measures the spectrum of fluctuations within a general speckle in a speckle pattern, and then develops perfusion information from that spectrum. The fluctuations in intensity within a single speckle are interpreted as being the result of beating between Doppler-shifted light, which has encountered a moving blood cell in its multiply-scattered path through the tissue, and non Doppler-shifted light, which has encountered only static scatterers. Laser Doppler methods generally calculate a perfusion index, defined as the first moment of the power spectrum of these fluctuations\cite{6}. This perfusion index has been shown by both calculation and experiment to be proportional to both the concentration and mean speed of moving blood cells in tissue\cite{7}. The proportionality factor between the perfusion index, measured in Hz, and the blood flow in tissue, measured in an appropriate unit such as mL per second per gram of tissue, is not well defined in any of the optical perfusion measurement methods.

Laser speckle contrast records an entire full-field image of the dynamic speckle pattern produced in an image of tissue under laser illumination, generally using a digital video camera. Rather than measuring the fluctuating signal within each individual speckle grain as laser Doppler does, speckle contrast analyses use the blurring effect of a finite camera exposure to determine the dynamic change in the speckle pattern, by quantifying the degree of blurring at particular finite exposures using the speckle contrast parameter $K$.

How can laser speckle contrast, a quantification of the blurring effect of finite camera exposure, find the same spectral information as laser Doppler? This question can be answered by considering the way that speckle contrast $K$ falls with increasing camera
exposure $T$, illustrated in Figure 4.1 below:

Figure 4.1 Speckle contrast falls with increasing camera exposure, as higher frequencies are filtered out by the camera integration time.

For a dynamic speckle image at an infinitesimal exposure, or an exposure shorter than the period of the shortest intensity fluctuation, there is no blurring and the contrast of fully developed speckle is 1. Increasing the exposure to a particular finite value $T_f$ blurs those fluctuations that have periods shorter than $T_f$ while fluctuations longer than $T_f$ remain unchanged. The speckle contrast is reduced from 1 as the speckle pattern blurs, and the degree of reduction in contrast depends on both $T_f$ and the frequency spectrum of the intensity fluctuations. This dependency is developed more rigorously in the following section.

4.1 Calculating Doppler-like spectra from multiple-exposure speckle contrast.

Equation (2-3), introduced in chapter 2 and repeated as equation (4-1) below, relates the spatial variance $\sigma^2$ of a speckle pattern imaged using a camera with exposure $T$ to a time integral of the temporal autocorrelation function of the speckles $C_i(\tau)$. The autocorrelation function describes the temporal evolution of a typical speckle, and via the Wiener–Khinchin theorem also implies the power spectral density (PSD) of the speckle fluctuations. This PSD is the measurement made by laser Doppler systems, and used to generate the laser Doppler perfusion index described above in equation (2-13) It would, therefore, be useful to calculate the autocorrelation function $C_i(\tau)$, starting from speckle measurements. The analysis below shows how this can be done. Starting from Goodman’s equation$^{11}$,
\[ \sigma^2 = \frac{1}{T} \int_0^T [2(1 - \tau/T)] C_t(\tau) d\tau \]  

(4-1)

rearranging this equation to the form:

\[ \frac{\sigma^2 T^2}{2} = \int_0^T [T - \tau] C_t(\tau) d\tau \]  

(4-2)

then using integration by parts on the right hand side and differentiating with respect to T gives:

\[ \frac{d}{dT} \frac{\sigma^2 T^2}{2} = \int_0^T C_t(\tau) d\tau \]  

(4-3)

Differentiating a second time generates an equation for the temporal autocovariance function \( C_t(\tau) \) as a function of the spatial variance \( \sigma^2 \) and the camera exposure time \( T \):

\[ \frac{d^2}{dT^2} \frac{\sigma^2 T^2}{2} = C_t(T) \]  

(4-4)

In speckle analysis we measure the speckle contrast \( K \), which is the standard deviation normalised by the mean intensity over regions of speckle pattern:

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

(4-5)

Substituting \( KI \) for \( \sigma \), the autocovariance function can be expressed in terms of measured parameters \( K \) and \( T \).

\[ C_t(T) = \frac{d^2}{dT^2} K^2 T^2 \]  

(4-6)

This equation is scaled by the square of the mean intensity \( I^2 \), which is constant and arbitrary – it is convenient to drop this scaling factor in further calculations.

\[ C_t(T) \propto \frac{d^2}{dT^2} K^2 T^2 \]  

(4-7)

This equation allows us to find the autocorrelation function of speckle fluctuations.
without recording time series of the intensity at each point, instead calculating the autocorrelation curve from a measured $K$ vs. $T$ curve.

4.2 Test of the theory by simulations

4.2.1 Generating synthetic speckle data

The analysis shown above was tested using computer simulations based on those developed by Duncan$^{99,100}$, as described above in section 2.3. The MATLAB code for this simulation is reproduced below in appendix 1. A series of speckle images in which the speckle pattern evolved with time was generated. Duncan and Kirkpatrick describe using a copula to generate speckle frames with a defined inter-frame correlation$^{100}$ to produce this effect. In this case, I have used a simpler technique: adding a small, normally distributed random value to the phase of each of the vectors representing a scattering path in the generating matrix $A$ (Figure 2.6) and calculating a new speckle intensity matrix $I$. Repeating this process for the number of frames required produces a 3D volume of data as shown in Figure 4.2. A single speckle frame, as shown on the smaller square face of the speckle volume, shows typical speckle. I will refer to each element of one of these frames as a pixel, as they represent the pixels of the CCD camera in an experimental measurement. The longer sides of the speckle data volume show the evolution of the intensity of a typical line in the image with time.

![Figure 4.2 Synthetic dynamic speckle data shown as a 3D volume. Time is plotted along the long axis and spatial dimensions along the two short axes of the volume.](image)

For the following analysis a set of speckle data was generated with 1024 frames, each 100 pixel x 100 pixel, with a minimum speckle size of 2 pixel. Each frame is strongly correlated with the immediately previous frame, with a mean correlation $R$ between
adjacent frames of 0.96. The inter-frame correlation was set by adjusting the magnitude of the random phase addition described above and tested by calculation. The inter-frame time step was defined as 1 ms to make the results of the simulation comparable to skin measurements.

The synthetic data produced were analysed using two different methods: a laser Doppler style analysis, and a multi-exposure laser speckle contrast analysis. Both analyses provided the Power Spectral Density (PSD) of the light intensity at a typical pixel in the frame, so that direct comparison between the methods is possible.

### 4.2.2 Doppler style analysis of synthetic data

Doppler perfusion measurements are typically based on a power spectrum of photodetector current (See section 2.2.2). The Power Spectral Density (PSD) of intensity or detector current at any pixel can be found using a Fast Fourier Transform of the time series at that pixel. The PSD is the square of the magnitude of the FFT. The mean of these spectra over the whole spatial extent of the array gives a mean PSD using all of the synthetic data, reducing noise.

This PSD analysis is a fair approximation to a Doppler system that samples entirely within a single speckle. This simulation allows investigation of another common case, that of using a photodetector larger than the speckle size. By filtering each frame with a 4 pixel x 4 pixel averaging kernel, then calculating the PSD as above, we can see the effect of using a photodetector detector approximately twice the speckle size.

Both the single speckle PSD and filtered speckle PSD are plotted in Figure 4.3 below. It’s clear that the effect of using a larger photodetector than the speckle size is to reduce the overall AC level of the PSD, without changing the spectral shape.
Figure 4.3 Synthetic speckle Power Spectral Sensity (PSD) by laser Doppler-style analysis, both with and without an initial 4x4 pixel intensity average. The 4x4 pixel average before computing PSD reduces the AC level of the PSD at all frequencies but the spectral fall-off shape is unchanged.

4.2.3 Multi-exposure speckle contrast analysis of synthetic data

The basis of the multiexposure speckle contrast analysis is a $K(T)$ curve consisting of measurements of speckle contrast $K$ at a range of exposures $T$. Longer exposures in a camera integrate the speckle intensity over time, and this is equivalent to taking intensity sums of pixels over a number of adjacent frames. It is convenient to use exposures that increase exponentially in order to define the shape of the $K(T)$ curve over several orders of magnitude of $T$, and here the exposure length was increased by increasing the number of summed speckle frames by powers of two. A single simulated frame has fully developed speckle and $K=1$ and is therefore considered to have exposure $T=0$. It represents the unblurred speckle pattern, not affected by camera integration. $N$ summed frames are considered to have exposure $T=(N-1)$ ms as the inter-frame time step was defined as 1 ms. Any inaccuracies produced by quantization errors in this procedure will be most significant at short exposures, corresponding to high frequencies in the eventual PSD plot. Again, all of the data available was used for smoothing, by calculating the mean contrast $K$ for 1024 single frames ($T=0$), the mean $K$ of 512 sums of two frames ($T=1$ ms), and so on, with the entire frame used as the speckle calculation region.

The resulting $K(T)$ curve, plotted in Figure 4.4 resembles the sigmoidal plots with logarithmic x-axes we expect to see in skin or tissue measurements. The simple speckle-generating algorithm used will not accurately reflect speckle evolution in real tissue as it does not reflect the light scattering processes and speed distribution of erythrocytes in
tissue, so the PSD will have a different shape to those measured in tissue, but the comparison between a speckle and Doppler based measurement of this PSD remains valid.

![Graph](image)

Figure 4.4 Speckle contrast $K$ plotted against exposure time in computer simulation.

A curve was fitted to the calculated points using a spline function. From the smooth contrast versus exposure curve, the autocorrelation function was numerically computed using the double differential equation (2-3). The autocorrelation function produced from the speckle curve is plotted in in Figure 4.5, together with the temporal autocorrelation function from each pixel calculated using the MATLAB function `xcorr()` and then averaged over all pixels to reduce noise. The two curves are essentially the same. Since the temporal PSD at a pixel may be calculated via the Wiener–Khinchin relation from the temporal autocorrelation function, the PSD computed via speckle contrast must now be expected to equal that computed from an FFT of the photocurrent at a point.
Figure 4.5 Autocorrelation function produced by speckle analysis of simulated data, with autocorrelation estimated directly using the MATLAB function xcorr() for comparison.

This PSD calculated from the speckle-derived autocorrelation is shown in Figure 4.6, with the PSD previously calculated in the Doppler analysis for comparison. The power spectra calculated using the two methods are clearly consistent. The main difference is at high frequencies corresponding to short exposures in the contrast vs. exposure curve, where the simulation is most susceptible to quantisation errors.

Figure 4.6 Power spectral density, calculated by both Doppler and speckle contrast methods from simulated speckle data.
4.3 Experimental test of the theory

4.3.1 Experimental methods

In order to test the equivalence of the PSD measured using laser Doppler and multi-exposure laser speckle experimentally, a simple laser Doppler system was constructed and compared with a speckle system in a number of trials. Both systems were applied to two test objects: the skin of the volar (palm side) surface of the forefinger, and a fluid phantom. The volar forefinger was chosen as a test site purely for convenience, and has similar perfusion to most sites on the dermis. The fluid phantom consisted of homogenised low-fat milk in a 10 mm diameter transparent plastic tube, with the surface slightly roughened using fine sandpaper to reduce specular reflection.

![Diagram of experimental setup](image)

Figure 4.7 Layout of Doppler, objective, and subjective speckle experiments. Not to scale.

The simple laser Doppler system used, illustrated in Figure 4.7, consisted of a laser for illumination (WorldStarTech, 658 nm, 50 mW, single mode) and a photodiode sensor (Centronics AEPX65). The laser was focussed to a spot with effective diameter approximately 0.5 mm, and the photodiode placed at 250 mm from the target. The minimum objective speckle size at this range is about 0.4 mm, compared to the 0.85 mm diameter of the photodiode. Despite this mismatch, which reduced the recorded AC signal to some degree, there was a sufficient AC signal to record a Doppler spectrum. The spectrum of the photocurrent was recorded using a transimpedance amplifier giving 42 MV·A⁻¹ and an HP spectrum analyser (HP 3589A). Doppler spectra were recorded on both the skin and fluid phantom dynamic targets and a static, multiply scattering Teflon target as a control for amplifier, dark-current, and background light noise. This small background measurement was subtracted from the dynamic target spectra. The Doppler spectra are plotted in Figure 4.10 and Figure 4.13 as discrete points rather than continuous curves, as the values were recorded from the spectrum analyser using a cursor function.

Speckle contrast was measured using the same laser used for the Doppler measurements, incorporated into a normal speckle imaging setup. The laser beam was expanded using a
lens to cover the target, and images recorded using a monochrome digital industrial camera with band-pass and polarising filters. The camera aperture was set to f/16, giving a minimum speckle size at the sensor of 15 μm, larger than the Nyquist minimum size for this sensor of 9.3 μm. Speckle contrast calculation regions of 50 pixel x 50 pixel and a range of camera exposures from 0.1 ms to 100 ms on the milk target, and 0.05 ms to 400 ms on skin were used. The maximum contrast achievable using this setup was 0.91 on a multiply scattering static target.

As well as testing the standard subjective (imaged) speckle setup, which was used for both skin and the fluid phantom, the fluid phantom was also tested using an objective speckle setup in which the lens was removed from the camera leaving the bandpass and polarising filters directly in front of the sensor, and the laser concentrated to a spot as it was for the Doppler experiments. The objective speckle pattern was recorded directly at the chip, and had a minimum speckle size approximately 10 times the pixel size.

Both of these laser speckle configurations, and the laser Doppler setup, are illustrated in Figure 4.7.

4.3.2 Experimental comparison results – Brownian motion in fluid phantom
Laser speckle contrast results for the fluid phantom are shown in Figure 4.8. These are the subjective speckle results. Laser speckle contrast K reduces with increasing exposure, and the K against log of exposure plot shows the expected sigmoid shape. An interpolating polynomial function was fitted to this data set to provide a smooth curve to analyse.

![Image of graph showing measured contrast versus exposure curve for Brownian motion in a tube of milk, using subjective (imaged) speckle. Error bars show standard deviation.](image-url)

*Figure 4.8 Measured contrast versus exposure curve for Brownian motion in a tube of milk, using subjective (imaged) speckle. Error bars show standard deviation.*
A temporal autocorrelation curve based on the fitted polynomial was calculated numerically, using equation (4-7) above, and normalised to have a maximum value of 1. The autocorrelation curve is plotted in Figure 4.9. The irregularities between 1 ms and 3 ms are likely artefacts of the polynomial chosen and the numerical processing and might be removed by using a larger number of measured points.

![Autocorrelation function calculated from speckle curve for Brownian motion using subjective (imaged) speckle.](image)

Power Spectral Densities for the fluid phantom, computed from the autocorrelation functions found in both the objective and subjective speckle experiments, are plotted in Figure 4.10, together with the Doppler measurements described above for comparison. The spectra are arbitrarily scaled to overlay each other, as there is an effectively arbitrary gain factor in all of the systems. The Doppler and speckle generated spectra are equivalent.
Figure 4.10 Power spectral density measured by Doppler and by both objective (lensless) and subjective (imaged) multiexposure speckle contrast methods, for Brownian motion in milk.

4.3.3 Experimental comparison results – perfusion in skin

Speckle measurements were made on the volar skin of the forefinger, using the subjective (imaged) speckle setup as used for the fluid phantom. The $K(T)$ curve measured is plotted in Figure 4.11.

Figure 4.11 Measured contrast versus camera exposure for perfusion in skin, using subjective (imaged) speckle.

Again, fitting a polynomial then calculating the autocorrelation (Figure 4.12) and comparing the related Power Spectral Density to laser Doppler results (Figure 4.13) shows that the spectra measured using multiple-exposure laser speckle is equivalent to that measured using laser Doppler. The small difference between the PSD curves at high frequencies corresponds to the region of the speckle contrast curve extrapolated towards zero exposure, and it is possible that this extrapolation generated the error.
Figure 4.12 Autocorrelation function calculated from speckle curve for blood flow in the volar skin of the right forefinger using subjective speckle.

Figure 4.13 Power spectral density measured by Doppler methods and by multiexposure subjective speckle contrast, for blood flow in the skin of the right forefinger.

4.4 Implications of Doppler/speckle equivalence

It is clear from both the simulated and experimental results that the same spectral information can be obtained using either laser Doppler or multiple exposure laser speckle measurements. However, laser Doppler measurements are a single-point measurement, and require collecting a time series or the associated spectrum of the fluctuating speckle intensity at each measuring point. Imaging laser Doppler systems have been built, either scanning a laser point across the surface of the tissue to be measured, which requires several minutes per image or using a very fast (20 kfps), and consequently expensive, camera to record full field laser Doppler images. A multiple exposure laser speckle image, by contrast, can be recorded in a time not significantly longer than the longest exposure used.
As multiple exposure laser speckle finds the full Doppler-equivalent spectrum of the speckle fluctuations, it has the capacity to provide a more accurate measurement of blood flow as well as more information on the speed distribution of the scatterers. The typical use of equation (4-1) in speckle contrast analysis has been to choose an appropriate autocorrelation function $C_\tau(\tau)$ with some characteristic time parameter $\tau_c$, based on the assumption that a presumed velocity distribution for the red blood cells (RBCs) will generate an autocorrelation function directly linked to its velocities\textsuperscript{27,30,37,38}. Substituting the chosen autocorrelation function and then integrating equation (4-1) generates a function relating $\tau_c$ and contrast. This means that an estimate of $\tau_c$ and the related speed of RBCs can be made from a single contrast measurement. Single exposure speckle contrast analysis hence relies on a correct choice of the assumed velocity distribution for the RBCs. As I will show in section 6.1, single exposure LSPI also relies on there being no static paths returning light to the camera.

The approach of choosing an autocorrelation function and finding its parameters by fitting can be extended to incorporate multiple exposures. Parthasarathy et. al.\textsuperscript{42} use multiple exposure speckle imaging and a speckle model revised from Briers' to include static speckle patterns giving an improved linearity in relative flow measurements using flow tubes. Similarly, Smausz\textsuperscript{44} uses multiple exposures and a Lorentzian model for velocity distribution, and adds extra fitting parameters to the $K(\tau_c)$ function to obtain a better estimate of $\tau_c$ than those obtained by a single exposure measurement. These methods still rely on choosing the correct physical model for light scattering in tissue and moving blood, including an appropriate velocity distribution\textsuperscript{37}.

Using the approach of measuring a full contrast versus camera exposure curve and finding the autocorrelation function by calculation allows us to remove an assumption that is otherwise required.

Once the autocorrelation function and its related power spectrum are measured using multiple exposures and the analysis shown here, speckle measurements are in the same position regarding interpretation as Doppler measurements, and follow the same path to an estimate of blood flow. This interpretation requires a number of assumptions – these are, however, common to all of the analyses here. The assumptions are that: (1) there is a fixed matrix of tissue scatterers, with all moving scatterers being RBCs; (2) the velocity distribution of the RBCs is independent of their spatial position; (3) the individual blood cells move independently; and (4) there is a RBC concentration low enough that each
scattered photon encounters no more than one RBC\textsuperscript{5,96}. These assumptions will clearly not be met in all cases, particularly when imaging blood vessels directly, but in the case of dermal perfusion they should be reasonable. The difference between vessel and tissue imaging should also be considered when designing flow-tube in scattering phantom experiments to test perfusion measurements, as used by several groups\textsuperscript{39,40,49}. Though this approach may provide good calibration for measurement of flow in vessels, the extension of these calibrations to microvascular perfusion measurement is probably not reliable.

The ‘perfusion index’ defined in laser Doppler measurements by Bonner and Nossal\textsuperscript{96}, Nilsson\textsuperscript{5} and others, shown in section 2.2.2 above, is the first moment of the spectrum of the speckle fluctuations. As multi-exposure speckle contrast measures the same spectrum as laser Doppler, it can also measure this value.

Some problems remain with a direct interpretation of such a perfusion index, whether measured by multi-exposure speckle contrast or by Doppler. These problems relate to the assumptions above, particularly the assumption that only the movement in the tissue is blood flow. Some authors report a biological zero, a perfusion index remaining during occlusion of blood flow in Doppler measurements\textsuperscript{110}, and we have found the same phenomenon in speckle measurements (see section 7.1.1) suggesting that the tissue matrix should not, in fact, be considered totally static. The original explanation of this biological zero was that it represented the Brownian motion of blood and other particles which remains when blood flow is occluded. However, Mełginski \textit{et al} have showed that directed blood flow persists for some time following cessation of cardiac activity, falling over the course of 120 minutes after euthanasia in mice and rats\textsuperscript{111}.

This chapter shows that multiple-exposure speckle contrast measurements, using the analysis described, can recover the same spectral information as laser Doppler measurements. The work shown here confirms this both theoretically and experimentally. Speckle measurements can therefore measure dermal perfusion, with the same assumptions as required for laser Doppler perfusion measurement, but with the advantage over Doppler measurements of generating full images at video rates. This technique can therefore provide an improvement for any current application of laser Doppler imaging.

Multi-exposure speckle need not rely on choosing a particular intensity autocorrelation function to generate an estimate of blood flow but can instead calculate such a function from measurements, by analysis of the speckle contrast vs. exposure curve.
5 Practical biospeckle analysis

The previous chapter showed that multiple exposure laser speckle contrast measurements have the capacity to measure the spectrum of dynamic biospeckle fluctuations. This chapter will explore a number of practical considerations encountered in biospeckle analysis, in particular considering some of the corrections and simplifications which become necessary due to the compromises required when specifying laser speckle systems.

The first correction considered is related to the fact that the maximum speckle contrast achievable by most speckle systems will be less than the ideal value of 1. The work presented here answers the question “is a linear correction for $K_{\text{max}}$ less than 1 valid?”

This chapter also covers the effective frequency sensitivity of speckle contrast analysis, demonstrating the slightly counter-intuitive result that it is possible to find some information about speckle fluctuations using camera exposures significantly longer than the typical fluctuation time of the speckle intensity.

In experimental measurements, contrast is collected at a range of camera exposures. For the purposes of analysis, it is convenient to fit curves to these measurements. The selection of appropriate fitting functions and the interpretation of the fitting parameters of these functions is considered in section 5.3.

As multiple exposure laser speckle analysis requires a large dynamic range from the camera, there are advantages in generating the longer exposure frames synthetically, by taking sums of shorter frames using a variety of schemes. Two such schemes are considered here.

5.1 Correction for reduced maximum contrast: $K_{\text{max}}$ and $\beta$

An ideal speckle imaging system, with speckles several times larger than the pixel size and a linearly polarised, long coherence length illuminating laser gives a contrast $K = 1$ when imaging a static target. However, in practical systems, we do not always achieve the ideal setup described above. In particular, there is often a degree of spatial averaging, deliberately allowed for in the system design as part of the optimisation of parameters described above in section 3.3.
5.1.1  Introducing $\beta$

Many applications of laser speckle have recognised this reduction in maximum achievable speckle contrast and accounted for it using a linear system correction factor $\beta = 1/K_{\text{max}}$ to account for $K_{\text{max}}$ less than $1^{21,42}$. The measured speckle contrast is multiplied by this linear factor in order to estimate the true speckle contrast during data processing. It’s not clear in the literature to what extent this linear correction was supported by theory or experiment, rather than being a simple ‘fudge factor’ to correct $K_{\text{max}}$ back to 1. Indeed one of the early papers attributes a $K_{\text{max}}$ less than 1 to a baseline effect in the CCD camera used$^{112}$, and refers to other workers subtracting such a baseline in similar work. The $\beta$ correction has been used in the related field of Dynamic Light Scattering$^{113}$ and was adopted from that field for speckle contrast analysis$^{42,114}$.

Although the $\beta$ correction was widely used, it had not been tested and confirmed as valid in speckle contrast analysis. In order to test the correction, a number of simulations and experiments were carried out. These were reported in the paper in Biomedical Optics Express$^{22}$, and are shown in the sections below.

5.1.2  Test of $\beta$ correction for spatial averaging using simulated speckle.

The $\beta = 1/K_{\text{max}}$ correction was tested using simulated speckle data. The simulation was based on Duncan and Kirkpatrick’s method as described in sections 2.3 and 3.3.1. above in which the instantaneous speckle intensity is generated by a Fourier transform of a generating matrix.

For this simulation, the speckle generating algorithm was altered slightly to produce speckle patterns with both a dynamic and static speckle component. This simulates the real clinical case in which moving blood cells are often covered by relatively static tissue. Further implications of mixed static and dynamic speckle patterns, and measurement of the proportions of the respective patterns, are discussed in section 6.1.

In order to generate these patterns, a proportion of the generating matrix elements were held static. The static elements are distributed randomly, with a uniform spatial distribution throughout the $D \times D$ matrix in order that they will have approximately the same maximum separation, and the static and dynamic components of the speckle pattern will have the same minimum speckle size. The effects of temporal blurring are simulated as previously described, by taking sums of series of adjacent frames.

An original set of 256 images was generated with simulated exposures $T$ from 0 to 255.
arbitrary units. The images were generated with the static proportion of the speckle generating matrix at 0.4, representing the highest level of static scattering likely in a clinical situation. These images have a minimum speckle size $s$ of 20 pixels and contrast $K$ ranging between the ideal value of 1, for exposure of 0 units, and 0.41 for the longest exposure of 255 units as $K$ approaches the predicted minimum $K_0$ of 0.4.

Spatial averaging was applied to the original images by filtering and sub-sampling, as described in section 3.3.1. This simulates imaging using a range of lens apertures, generating speckle with a range of minimum speckle sizes $s$. The results of these simulations are plotted in Figure 5.1.

![Figure 5.1 Simulation: uncorrected speckle contrast vs. camera exposure at 4 speckle sizes.](image)

Each curve in Figure 5.1 represents the speckle contrast that will be measured at a range of camera exposures, given a particular speckle size $s$ relative to the camera pixels. For the $s = 20$ curve the speckles are significantly larger than the pixels, and are well oversampled, so there is no spatial averaging effect and the contrast starts at the ideal $K = 1$ at zero exposure, falling with increasing exposure and approaching the static contrast value $K = 0.4$ at long exposures. This simulates the ideal case in which no correction is necessary. The other curves, with smaller speckle size and some spatial averaging, have reduced speckle contrast $K$ at every exposure and simulate the results that require correction using the system correction factor $\beta$ in clinical measurements.

We can define a $K_{max}$ for each curve as the value of contrast that will be measured given no temporal blurring. In the case of these simulations, that is the contrast at $T = 0$. 
Normalising each curve by $K_{\text{max}}$ produces Figure 5.2. The curves now overlay each other – the normalisation by $\beta = 1/K_{\text{max}}$ successfully recovers the true speckle contrast, indicating that this linear correction is valid in this case.

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**Figure 5.2** Simulation: speckle contrast vs. camera exposure corrected using $\beta = 1/K_{\text{max}}$ factor.

5.1.3 Test of $\beta$ correction for background light addition using simulated speckle $K_{\text{max}}$ can also be reduced in some experimental or clinical situations due to addition of an incoherent background light source to the laser illumination. For example, despite using coloured or band-pass filters, sunlight incident on the skin in dermal imaging may be added to the speckle pattern detected. This situation was simulated by adding a constant background value to speckle images with speckle size $s = 20$. For background level $b$ and original image intensities $I_0$ the new image intensities are $I = (1-b)I_0 + b$. The background level $b$ was varied in steps from 0 to 0.4, reducing $K_{\text{max}}$ from 1 to 0.17. Figure 5.3 shows the uncorrected speckle contrast at a range of exposures for each background level, and Figure 5.4 shows the corrected curves. Again, a simple normalisation by $\beta = 1/K_{\text{max}}$ recovers the true speckle contrast.
5.1.4 Experimental test of $\beta$ correction

The linear $\beta=1/ K_{\text{max}}$ correction for reduced maximum achievable speckle contrast was also tested experimentally, using speckle contrast measured on both skin and a simple phantom at a range of camera exposures, with the maximum achievable contrast $K_{\text{max}}$ reduced by spatial averaging. Speckle images were recorded using the imaging system described above, consisting of the Sony CCD camera with polarizing filter and a 12 mm
to 36 mm varifocal lens, and the Ondax 658 nm, 30 mW laser diode module. The targets for these tests were the skin of the palm, and a fluid phantom consisting of a container of milk, providing a Brownian motion source, covered with a thin static scattering layer made from silicone and alumina powder.\textsuperscript{115}

Contrast vs. exposure curves at each degree of spatial averaging were produced. The degree of spatial averaging was progressively increased by opening the lens aperture until contrast at a particular exposure was significantly reduced, then repeating the contrast measurements over the exposure range. As the varifocal lens used has no marked f-stops and the speckle size is comparable to the pixel size, precluding a determination of $s$ by autocorrelation, the value of speckle size $s$ for these curves is not easily obtained. $K_{\text{max}}$ values used for correction were obtained by measuring the speckle contrast on a static multiply-scattering target, consisting of a block of the silicone/alumina mixture used above. All of the data recorded show some degree of spatial averaging, with $K_{\text{max}}$ ranging from 0.83 to 0.32.

The results of these experiments are plotted in Figure 5.5 – the uncorrected results in the upper two subplots, and corrected versions of these results in the lower two subplots. The linear $\beta$ correction is confirmed by these experimental results; normalising by $1/K_{\text{max}}$ makes the curves overlay satisfactorily.
Figure 5.5. Results of experimental testing of $\beta = 1/K_{\text{max}}$ correction for spatial averaging, showing that the linear system correction recovers true speckle contrast after significant spatial averaging.

5.1.5 Conclusion: $\beta = 1/K_{\text{max}}$ is empirically valid

It’s clear, from both the simulated results in section 5.1.3 and the experimental results here, that applying a linear system correction factor $\beta = 1/K_{\text{max}}$ is empirically valid. This will prove useful in practical clinical systems, where the compromises involved in optimising systems mean that a degree of spatial averaging is generally present.

5.2 Frequency sensitivity of K measurement

There is an interesting question posed by the practice of laser speckle contrast analysis. Laser Doppler cameras use very high frame rates (20 kFPS in the University of Twente’s TOPcam, for example) while speckle systems collect the same information using 10 ms range exposures. How does speckle contrast measured at camera exposures in the 10 ms range provide information about the temporal autocorrelation of the speckle pattern which may have time constants less than 1 ms?
Figure 5.6 shows both an autocorrelation curve and its associated speckle contrast curve plotted against log-scaled time. For the autocorrelation curve this time is the lag, as usually used, and for the contrast curve it is the camera exposure. Both are scaled by the autocorrelation time constant \( \tau_c \). The autocorrelation function used for this illustration is the exponential \( C_t = e^{-T/\tau_c} \) and the contrast curve is calculated from the autocorrelation function using the integral equation (5-1).

In laser speckle analysis, we usually measure the speckle contrast at integration times significantly longer than the autocorrelation time, as shown in the green block in Figure 5.6. For example, dermal blood flow generates speckle autocorrelation times in the sub-millisecond range, corresponding to kHz Laser Doppler signals, while laser speckle measurement times are generally an order of magnitude or more longer, in the range 10-25 ms. This looks, at first glance, like a classic case of undersampling – we infer information about shorter times (higher Doppler shift frequencies) than our measurement time.

The \( C_t \) and \( K \) curves plotted are connected by equation (5-1), repeated from sections above (equations (2-3) and (4-1)):

\[
\sigma^2 = \frac{1}{T} \int_0^T \left[ 2 \left( 1 - \frac{\tau}{T} \right) \right] C_t(\tau) d\tau
\]  

(5-1)

This can be transformed as shown in section 4.1 above to show that:
\[ C_t(T) \propto \frac{d^2}{dT^2} K^2 T^2 \]  \hfill (5-2)

where \( K \) is the speckle contrast measured at camera exposure \( T \).

A section in either the curve determines the shape of the other curve at the same range of \( T \), so we might expect that measurements at a range of \( T \) only define the autocorrelation function at that \( T \). However, the width of the peaks of the curves is also related: simply changing the time scale of one changes the time scale of the other, so measuring the width of the \( K \) curve might tell us the width of the \( C_t \) curve and hence \( \tau_c \). How well do we characterise the autocorrelation function using measurements well outside the autocorrelation time?

One phenomenon which helps make sense of this situation is that the contrast curve approaches zero at an exposure time orders of magnitude longer than the speckle autocorrelation time. In order for the contrast to approach zero, there need to be a large number of effective uncorrelated speckle patterns added due to the camera integration. In other words, if we consider the camera integrating for \( N \tau_c \) seconds to be similar to adding \( N \) uncorrelated speckle patterns, we expect the contrast to fall similarly to \( 1/\sqrt{N} \). This is a superficial argument, made more rigorous in the sections below.

### 5.2.1 Speckle contrast equation in different time ranges

Plotting parts of equation 5-1 is instructive. Figure 5.7 shows a representative exponential autocorrelation function \( C_t(\tau) \) plotted in blue, the windowing term \( [2(1-\tau/T)] \) plotted in green and the product \( [2(1-\tau/T)]C_t(\tau) \), which is the integrand in equation (5-1), plotted in red.
Figure 5.7 Parts of equation (5-1), plotted for several camera integration times $T$ which are multiples of the autocorrelation time $\tau_c$. $C_t$ is the speckle autocorrelation function, plotted in blue. The green line shows the windowing function in the integral, $[2(1-\tau/T)]$ and the red line shows their product $C_t[2(1-\tau/T)]$ which is the integrand in equation (5-1).

Part A of Figure 5.7 shows the case where the camera exposure $T$ is less than the autocorrelation time constant $\tau_c$. In this case, the shape of $C_t(\tau)$ contributes to the change in the integrand $C_t[2(1-\tau/T)]$ with changes in $T$, as the windowing function $[2(1-\tau/T)]$ sweeps through $C_t(\tau)$. However, when $T/\tau_c$ is significantly greater than 1 as shown in parts C and D the integrand varies little with changes in $T$, as the windowing function varies little across the range where $C_t(\tau)$ is significantly greater than zero. As the integral is near constant, we expect the variance $\sigma^2$ as described by equation (5-1) to fall as $1/T$, so the speckle contrast $K = \sigma/\bar{I}$ will fall at long exposures as $1/\sqrt{T}$. This corresponds to the speckle flow index (SFI) as described by Ramirez et al.\textsuperscript{46}

5.2.2 Contrast at long exposures: analytic results

We can determine the value of the contrast at long camera exposures analytically for
various autocorrelation functions. Exponential and Gaussian autocorrelations correspond, respectively, to two common assumptions about the speed distribution of moving scatterers: either that there is a homogeneous collection of scatterers with identical dynamic behaviour, as in Brownian motion; or that there is an inhomogeneous collection, as in organised flow within structured vessels where the dynamics of different scatterers are not identical\textsuperscript{36,37}. Exponential and Gaussian autocorrelations, with their associated speckle contrast curves, are plotted in Figure 5.8.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5_8.png}
\caption{Exponential and Gaussian autocorrelation functions and their associated speckle contrast curves. Blue and green lines show the exponential and Gaussian autocorrelation functions, and red and cyan lines show their associated speckle contrast vs. camera exposure curves calculated using equation (5-1).}
\end{figure}

For an exponential autocorrelation $C_t(\tau) = e^{-\tau/\tau_c}$, equation (5-1) becomes

$$\sigma^2 = \frac{1}{T} \int_0^T \left[ 2(1 - \tau/T) \right] e^{(-\tau/\tau_c)} \, d\tau$$

(5-3)

By integrating, we find the equation:
\[ \sigma^2 = \frac{2\tau_c}{T} \left[ \tau_c \left( e^{-T/\tau_c} - 1 \right) + \frac{1}{T} \right] \quad (5-4) \]

At \( T \gg \tau_c \) this equation simplifies to the form:

\[ \sigma^2 \approx \frac{2\tau_c}{T} \quad (5-5) \]

and the speckle contrast is:

\[ K \approx \sqrt{\frac{2\tau_c}{T}} \quad (5-6) \]

For a Gaussian autocorrelation \( C_t(\tau) = e^{-(\tau/\tau_c)^2} \) equation (5-1) becomes:

\[ \sigma^2 = \frac{1}{T} \int_0^T \left[ 2(1 - \tau/T) \right] e^{-(\tau/\tau_c)^2} \, d\tau \quad (5-7) \]

Integrating produces the equation:

\[ \sigma^2 = \frac{\tau_c}{T} \left[ \frac{\tau_c \left( e^{-T^2/(\tau_c^2)} - 1 \right)}{T} + \sqrt{\pi} \, \text{erf} \left( \frac{T}{\tau_c} \right) \right] \quad (5-8) \]

where \( \text{erf} \) is the error function, \( \text{erf}(x) = \frac{2}{\sqrt{\pi}} \int_0^x e^{-t^2} \, dt \).

At \( T \gg \tau_c \) this simplifies to:

\[ \sigma^2 \approx \frac{\sqrt{\pi} \tau_c}{T} \quad (5-9) \]

and the speckle contrast \( K \) becomes:

\[ K \approx \frac{\sqrt{\pi} \tau_c}{T} \quad (5-10) \]

So, at long exposures both the exponential and Gaussian autocorrelation speckle contrast curves have the same form, \( K \propto \frac{\tau_c}{\sqrt{T}} \). The form of the autocorrelation no longer influences the shape of the contrast curve and there is only a small difference, a factor of \( \sqrt{2/\sqrt{\pi}} = 1.06 \), between contrast generated using a Gaussian or an exponential
autocorrelation function. Figure 5.9 shows both the full contrast curve and the long exposure simplification, plotted for both exponential and Gaussian autocorrelations.

![Figure 5.9 Speckle contrast K vs. camera exposure T curve at longer exposures than plotted in Figure 5.8. Long exposure simplifications are also plotted, showing that they overlay the K curve at \( T \gg \tau_c \).](image)

### 5.2.3 Frequency Sensitivity Scaling

We can indicate the increase in apparent high frequency sensitivity of a speckle contrast system by estimating a scaling factor \( m_f \) for laser speckle contrast measurements, quantifying the ability of speckle contrast to measure the speckle autocorrelation time \( \tau_c \) at camera exposures \( T \approx m_f \tau_c \). This factor represents the increase in exposure time, or the consequent reduction in frame rate, required for a laser speckle contrast system compared to a laser Doppler system with equivalent frequency sensitivity.

We estimate \( m_f \) by finding the ratio of times for the respective curves to fall to \( 1/e \). For the Gaussian and exponential autocorrelation functions this is the characteristic autocorrelation time \( \tau_c \). Setting \( \tau_c = 1 \) and \( k = 1/e \) in Equations (5-4) and (5-8) provide the equivalent times for the contrast curves. For an exponential autocorrelation, \( m_f \approx 13.7 \) and for a Gaussian autocorrelation \( m_f \approx 12.5 \).
Figure 5.10 Illustration of the frequency sensitivity scaling effect. The scaling factor \( m_f \) is defined as the ratio of the 1/e times \( T_2 \) and \( T_1 \).

5.2.4 Conclusions on frequency sensitivity
The results above imply that we can measure the autocorrelation time \( \tau_c \) by using speckle contrast measurements at \( T \gg \tau_c \), but that the accuracy of this measurement depends on the correct assumption about the autocorrelation function. Measuring the autocorrelation time, if we know the autocorrelation function, gives us full information about the Doppler spectrum of the fluctuations in the speckles. It seems most likely that assuming that a frequency sensitivity scaling factor \( m_f > 10 \) will be valid for almost any autocorrelation function we are likely to encounter.

However, if we wish to determine the form of the autocorrelation function, we need to measure contrast at a full range of \( T \), particularly covering the region where \( T < \tau_c \).

5.3 Curve fitting to K/T data
When speckle contrast measurements are collected, it is usually necessary to fit a curve to the data, either as an intermediate stage in data analysis, or for parameterisation of the data to enable interpretation. These functions may be either physically justified equations relating speckle contrast to the underlying physical mechanism, as described by Parthasarathy et al\(^42\), or an empirically chosen function which fits the data, where the fitting parameters may be interpreted directly or transformed in further analysis.

Where sufficient points are available, spline and polynomial functions have been used in this thesis to interpolate between measured data. In in-vivo and clinical measurements, where the compromises necessary to generate multi-exposure speckle curves mean that there are generally fewer points in a measured speckle curve, it is useful to fit a simpler
function, and one in which some of the parameters may have some physical interpretation.

5.3.1 Developing an empirical fitting function

The empirical fitting function used in the majority of the in-vivo and clinical work in this thesis was developed as follows, producing sigmoidal curves in $K$ against log($T$) curves with adjustable limits $K_{\text{max}}$ and $K_{\text{th}}$, characteristic time parameter $\tau$ and shape parameter $\alpha$. The basic sigmoidal function used is:

$$K = \frac{1}{1 + \frac{T}{\tau}}$$

(5-11)

where $T$ is the camera exposure, and $\tau$ is the characteristic time parameter. This is not the same characteristic time as the $\tau_c$ defined for autocorrelation functions, though the two are linearly related for any particular autocorrelation function. Figure 5.11 shows the effect of changing $\tau$.

![Figure 5.11 Three curves for different values of the characteristic time parameter $\tau$ in a fitting function, equation (5-11).](image)
Adding a shape parameter $\alpha$ allows the slope of the curve at its inflexion point to change:

\[
K = \frac{1}{1 + \left(\frac{T}{\tau}\right)^\alpha}
\]  \hspace{1cm} (5-12)

Figure 5.12 Three curves illustrating the effect of changing the shape parameter $\alpha$ in a fitting function, using equation (5-12) with $\tau=1$ and $\alpha$ values 0.5, 1 and 2.

and adding adjustments to the asymptotic limits of the curve produces the fitting equation generally used in clinical applications in this work, where $K_0$ is the minimum contrast, discussed at length in section 6.1 and $\beta=1/K_{\text{max}}$ is the system correction factor discussed in section 5.1.

\[
K = \beta \left( \frac{1 - K_0}{1 + \left(\frac{T}{\tau}\right)^\alpha} + K_0 \right)
\]  \hspace{1cm} (5-13)
5.3.2 Relationship between fitting function and analytic speckle contrast curves

The fitting function (5-13) above was found to fit the speckle data adequately in measurements of Brownian motion in fluids, and measurements of dermal blood flow. We can also compare the fitting functions with the analytic speckle contrast functions produced for different autocorrelation functions in the previous section. Figure 5.14 shows two analytic $K$ functions, equations (5-4) and (5-8), for exponential and Gaussian autocorrelations and the respective best-fits for the fitting function (5-12). In these plots the characteristic time speckle autocorrelation $\tau_c = 1$ ms.

The fit is adequate for the speckle curve generated using an exponential autocorrelation function, with a shape parameter $\alpha \approx 0.8$, the same value generally found when this equation is fitted to real dermal measurements. The fit to a speckle curve based on Gaussian autocorrelation is poor – the simple sigmoid curve does not follow the sharper curve of the analytic speckle function around $T = 1$ ms.

Figure 5.13 Effect of changing limits $K_{\text{max}}$ and $K_0$ in fitting equation (5-13) with $\tau=1$ and $\alpha=1$. 
Figure 5.14 Comparison between speckle fitting function (5-12) and analytic speckle curves based on exponential and Gaussian autocorrelations. Autocorrelation characteristic time $\tau_c = 1$.

It is possible to use a more complicated fitting function, which accurately follows the analytic speckle contrast as shown in Figure 5.15. In this case, the fitting function is:

$$K = \frac{1}{\left(1 + \left(\frac{T}{\tau_c}\right)^\alpha\right)^b}$$  \hspace{1cm} (5-14)

Using this fitting function, we can fit the analytic curves perfectly, but using an exponential fitting parameter $\alpha$ raised to the power of another fitting parameter $b$ is numerically unstable, and the parameters do not lend themselves easily to physical interpretation.

Figure 5.15 Comparison between a 3 parameter speckle fitting function equation (5-14) and analytic speckle contrast functions based on two representative autocorrelation functions.
5.4 Synthetic long exposures in speckle measurements.

5.4.1 Why use synthetic long exposures?
As shown in Chapter 4, multiple exposure laser speckle allows measurement equivalent to the full laser Doppler spectrum. This produces a more accurate blood flow measurement, and gives the capability to measure several parameters and hence provide more information than single-exposure laser speckle. However, multiple exposures over several orders of magnitude generate some difficulties in system design due to dynamic range issues. A typical CCD camera, generally used for these systems, has 8 bit resolution, insufficient to cope with large differences in illumination level. It is not possible to change the camera aperture to adjust to the scene brightness, as would be done in most photography, since the camera aperture controls the speckle size and so must remain fixed. A number of solutions to this problem have been proposed and tested in the literature. In much of the work in this thesis, a camera with adjustable electronic gain, varying from 0 dB to 18 dB, was used and provided sufficient dynamic range for most experimental requirements. Other workers have adjusted the laser power. As adjusting the output power of a diode laser has generally detrimental effects on its stability, one approach uses an Acousto-Optic Modulator and diffraction effects to pulse the laser and regulate its intensity at the sample, while other workers have used filters to alter laser power.

Another possible solution to the problems of dynamic range in multiple exposure laser speckle is to keep the total illumination in each camera exposure constant, and find the contrast at long camera exposures synthetically by adding speckle patterns. Two such methods are presented in the following sections.

5.4.2 Double-flash laser speckle contrast
The first scheme is to use a constant, long camera exposure and generate changing contrast by flashing the laser illumination twice during the camera exposure. Using a frame exposed by two short flashes each with length $t_1$, separated by time $t_2$, we should be able to measure the autocorrelation function by a similar method to the varying exposure method, but without requiring dynamic range changes at the camera. The ‘flashing’ proposed here would use shutters or similar to chop the laser, preserving any correlation between the two speckle patterns. Figure 5.16 illustrates the concept. Considering the limiting cases, with very short $t_1$ and $t_2=0$, we expect the contrast measured to be $K_{max}$. With the same short $t_1$ and sufficiently long $t_2$ that the speckle pattern has completely
decorrelated between the two flashes, expected contrast is \( \frac{K_{\text{max}}}{\sqrt{2}} \) as for adding any two uncorrelated speckle patterns of equal intensity. We then expect that the correlation coefficient for lag \( t_2 \) will be a function of this fall in contrast.

**Equivalent short exposure measurement**

![Image of double-flash illumination schemes for laser speckle contrast using synthetic long exposures.]

**Equivalent long exposure measurement**

The speckle contrast measured from a sum of two partially correlated speckle patterns is calculable, as shown by Goodman\(^{11,118}\). Goodman’s calculations give the relationship between speckle pattern correlation coefficients and the speckle contrast of the summed speckle shown in Figure 5.17.

The speckle contrast is near linear with correlation coefficient. It would be simple to find a suitable function for speckle pattern correlation as a function of contrast, \( \rho(K) \) by fitting a polynomial or similar, so it is possible to use this scheme to measure and calculate speckle correlation as a function of inter-flash time, \( \rho(t_2) \). With limited assumptions, this function can be interpreted as the autocorrelation function of the speckle intensity, providing the same spectral information as found above in section 4.
Figure 5.17 The speckle contrast the sum of two partially correlated, equal intensity speckle patterns.

The technical implications of this result are that it is possible to build a system using a flashed laser to measure dynamic biospeckle, and find a speckle autocorrelation function without changing the total exposure, allowing fixed laser power and camera gain and thus avoiding some of the issues of dynamic range encountered in multiple-exposure schemes with a range of exposures.

This would require a flashable single mode laser source, synchronised with the camera exposure – though the laser power would need to be relatively high in order to use small $t_1$. This scheme does allow the use of ordinary cameras, not capable of high frame rates or short exposures, while still collecting information about the high frequency parts of the speckle fluctuations. No further experimental work has been carried out on this scheme, though it may prove useful in future applications of this technology.

5.4.3 Summed exposure speckle contrast
The second synthetic exposure scheme leaves the laser on continuously and collects a series of short speckle frames with a small inter-frame gap. This requires a camera capable of collecting such a frame series. This approach was used by my colleague Evan Hirst in his work based on my analysis, adapting multiple exposure laser speckle
perfusion imaging to retinal imaging applications using a modified fundus camera\textsuperscript{69,119}. The various exposures required are synthesised by calculating sums of the frames captured during data processing. For example, by capturing 10 frames each of 10 ms exposure, we can find the contrast at 10 ms exposure from each single frame, at 20 ms exposure by taking sums of two frames before calculating the contrast, and so on up to an exposure of 100 ms by taking the sum of all the frames. This is very similar to the method for simulating long exposures used in the MATLAB simulations described in section 2.3.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image.png}
\caption{Illustration of summed exposure scheme for synthetic long exposures.}
\end{figure}

Given availability of a suitable camera, this method makes optimal use of the time available to collect multiple-exposure speckle images, as the total capture time is the same as the longest exposure required.

5.5 Conclusions to this chapter.

Experimental and simulation results confirm that applying a $\beta=1/K_{\text{max}}$ linear system correction factor is valid. The validity of the correction has significant practical implications. The optimisation of inter-dependant system parameters, described in section 3.3, means that almost all practical system setups will introduce a degree of spatial averaging, due to the compromises made between illumination level, spatial resolution and speckle size. As the speckle size is proportional to the f-number of the lens, it follows that the mean intensity at the sensor is inversely proportional to the square of the speckle size. Reducing the aperture in order to reduce spatial averaging can significantly reduce the light available at the sensor due to this inverse square relationship. Given that the camera exposure will generally be set at a predefined value or range of values in order to capture dynamic biospeckle, the linear $\beta$ correction allows a trade-off between light levels and spatial averaging in the selection of an optimum working aperture.

A reliable $\beta$ correction also allows repeatable inter-experiment and inter-system
comparison of contrast-based perfusion measurements, which is important in many clinical and research applications. For example, an upcoming collaborative study into wound healing following excision of melanomas and suspect nevi, in which we will measure blood flow around wounds, will require measurements over a long period of time as we follow up individual patients, and inter-group comparisons between a intervention and control groups.

The frequency sensitivity results above answer a significant question, which has interested some of the participants in the speckle perfusion community for some time. It was easy to draw the erroneous conclusion that laser speckle, operating at a camera exposure time around 10 ms, could provide no information about dynamic fluctuations with much shorter periods. The results above show that such high-frequency Doppler information is found in speckle contrast images. In fact, the high frequency sensitivity of a speckle contrast system with a specific camera exposure is approximately 10 times what a naïve estimation would suggest, provided the approximate form of the speckle autocorrelation function is known. However, if we wish to determine the form of the speckle autocorrelation function using speckle contrast curves, we need to measure contrast at exposures in the range of the autocorrelation time.

The simple fitting function equation (5-13) accounts for changing flow speed, spatial averaging and static contrast, but will not be appropriate for all possible autocorrelation curves. However, in cases where the function is an acceptable fit to the data and provided that the shape value $\alpha$ is fixed, as is possible in most clinical applications encountered so far where $\alpha=0.8$ is a reasonable assumption, the inverse of the characteristic time $1/\tau_{fit}$ may be an acceptable perfusion index.

Methods for measuring long exposure speckle contrast by adding frames avoid the problems of large dynamic range created by multiple exposure laser speckle, while maintaining its accuracy and information advantages. Multiple frame long exposures also make optimal use of the available exposure time.
6 Laboratory exploration of biospeckle effects

Laboratory exploration of the effects of biospeckle, using phantoms that mimic tissues while allowing control over the geometry and optical properties, has been helpful in elucidating some of the phenomena seen in biospeckle measurements and has provided some of the foundation for interpretation of biospeckle results in clinical settings.

The effect of stationary scattering layers, which add a static speckle pattern to the dynamic biospeckle patterns, is important in several clinical applications. Section 6.1 below shows that neglecting to account for this effect can lead to inaccurate measurement of perfusion.

The connection between the speed of moving scatterers and the spectrum of fluctuations in the biospeckle is key to the interpretation of laser speckle contrast. A simple single-speed experiment exploring this connection is described in section 6.2.

The remainder of this chapter deals with spatial effects in laser speckle imaging, applying tissue simulating phantoms to investigate the depth sensitivity of laser speckle systems as compared to laser Doppler systems, and the volume of tissue contributing to laser speckle measurements.

6.1 Stationary and dynamic contrast and $K_0$.

The contrast of a totally dynamic speckle pattern, in which all the light paths include a moving scatterer, falls to zero at long exposures. However, in the case where a proportion of the light paths contributing to the pattern are static, the contrast does not fall to zero but to a minimum value $K_0$. Results presented in this section show that the value of $K_0$ is proportional to the thickness of a static layer over dynamic scatterers. This is relevant to many medical applications: for example, thickened callouses in dermal measurements on the soles of the feet show high values of $K_0$, and images of retinas show that the visibility of retinal vessels in speckle contrast images is partly due to reduced thickness of the static tissue layers over the vessels.$^{119}$

6.1.1 Measured static contrast results

The effect of static scattering in speckle contrast measurements is clearly shown in phantom experiments. As part of an inter-system comparison carried out at Twente University,$^{117}$ speckle contrast measurements were made on a static/dynamic fluid
phantom. The phantom was constructed as shown in Figure 6.1, by adding layers of Scotch tape to a transparent sheet, covering a Petri dish filled with 10% Intralipid. Brownian motion in the Intralipid contributes the dynamic motion, and the layers of tape add static paths.

Measurements were made using both the multiple exposure speckle system described in this thesis (called multiple exposure laser speckle perfusion imaging here, M-LSPI), a commercial single exposure speckle system from Perimed¹²⁰ (Perimed speckle imager, PSI) and an experimental full-field laser Doppler system developed at the University of Twente⁹ (Twente Optical Perfusion camera, TOPCam).

![Figure 6.1 Layout of the static layer phantom: numbers indicate the number of layers of Scotch tape over a 10% Intralipid fluid phantom.](image)

Normalised perfusion values calculated using the three systems are plotted in Figure 6.2. The perfusion index used for the multiple-exposure system in this experiment was $1/\tau_{fit}$ where $\tau_{fit}$ is the characteristic time value in equation (5-13) above, with $\alpha$ fixed at 0.8. Perfusion indices generated by all three systems were normalised by dividing by the 2-layer measurement and are plotted in Figure 6.2. These values are averages calculated using rectangular regions of interest centred over the tape patches.
Figure 6.2 Scaled perfusion values plotted against number of layers of Scotch tape for three perfusion systems.

Perfusion measured using multiple-exposure method remains constant, while the perfusion measured using laser Doppler and single-exposure laser speckle falls with increasing tape thickness. There is a shape common to the PSI and M-LSPI curves; these two curves differ only in their trend, indicating that this shape is generated by differences in the phantom rather than indicating inaccuracy in the systems. Figure 6.3 shows a perfusion Index $\text{PI} = 1/\tau_{\text{fit}}$ and $K_0$ images generated using the multiple-exposure system. The homogeneity of PI across the image is clear, as is in increase in $K_0$ with increasing tape thickness. $K_0$ is plotted against the number of tape layers in Figure 8 and can be seen to increase nearly linearly with tape thickness.

The TOPCam and PSI results show a fall in perfusion with increasing thickness of the scattering layer, despite the Brownian motion of the moving scatterers being the same. This is because they do not account for the static scattering layer in their analysis. Accounting for static scattering paths allows the multiple-exposure laser speckle perfusion imaging method to remove the effect of the static scatterers, so that the perfusion index measured by this method reflects the true motion of the dynamic scatterers.
6.1.2 Recovery of dynamic $K(T)$ curve after $K_0$ correction

We have assumed, as have other workers in this field\textsuperscript{42}, that a static contrast value $K_0$ can be linearly combined with the dynamic contrast curve, a function of the camera exposure, as shown in the equation below:

$$ K(T) = (1 - K_0)K_{\text{dynamic}}(T) + K_0 \quad (6-1) $$

If this assumption is correct then the dynamic contrast curve should be recoverable by a simple linear correction. The contrast curves $K(T)$ for the experiment above are plotted in Figure 6.5 and the extracted $K_{\text{dynamic}}$ curves are plotted in Figure 6.5. The $K_{\text{dynamic}}$ curves overlay satisfactorily, indicating that the simple model of adding static and dynamic contrast linearly is sufficient in this case. The maximum $K_0$ found in this experiment was approximately 0.15, and we expect that higher $K_0$ might be found in some clinical cases such as viewing dermal perfusion through a very thick callus on the foot. To simulate
these higher $K_0$ values the experiment was repeated using thicker scattering layers consisting of 10, 20 and 40 layers of Scotch tape. The results of this repeated experiment are shown in Figure 6.7 and Figure 6.8. In this case the maximum $K_0$ is approximately 0.5, and the dynamic curves still overlay satisfactorily.

Figure 6.5 Total speckle contrast curves for a range of thicknesses of Scotch tape over a fluid phantom. Thicknesses of 0 to 11 layers provide static contrast $K_0$ of approximately 0 to 0.15

Figure 6.6 Dynamic speckle contrast curves for a range of thicknesses of Scotch tape from 0 to 10 layers over a fluid phantom.
6.1.3 Some conclusions on $K_0$

Assuming a linear addition of static and dynamic contrast in $K$ vs $T$ analyses will recover the dynamic contrast curve of the moving scatterers, for static contrast $K_0$ in the range 0 to 0.5. This is an important consideration when there is significant static scattering, as not accounting for $K_0$ will lead to an underestimate of flow.

6.2 Relationship between speed of flow and spectrum

In order to confirm the relationship between the speed of the scatterers and the detected
speckle contrast curves, calculated Doppler spectrum and perfusion index, a simplified experiment investigating the spectrum generated by scatterers moving with a single speed was conducted. This removes any effect on the spectrum of the speed distribution we expect in a real tissue measurement or a fluid phantom. The single-speed phantom constructed consists of sheets of sand-blasted plastic, providing multiple scattering. Two pieces of the plastic are fixed to the bench and the third pushed between them using an improvised hydraulic pusher built using syringes and a syringe pump.

Figure 6.9 Phantom for experiments on scatterers with a single translating speed.

The phantom was measured at two speeds, 0.32 mm·s⁻¹ and 0.64 mm·s⁻¹, and with the phantom stationary. The speckle size was approximately 4 pixels, checked by autocorrelation of static images, and contrast of the static images was 0.93. Speckle exposures in a multiple-exposure experiment were 0.1 ms to 10 ms, with the camera aperture sized for sufficient illumination and high contrast. Speckle contrast curves recorded for both speeds and with the phantom stationary. These results are plotted in Figure 6.10 below. The fitted curves are based on the empirical equation:

\[ K = \beta \left( \frac{1 - K_0}{1 + \left( \frac{T}{\tau} \right)^\alpha} \right)^b + K_0 \]  

(6-2)

The parameters α and b are shape parameters. Fitting these shape parameters, particularly where one exponential is raised to the power of another makes any simple physical interpretation of the fitting parameters difficult but gives a better fit. As the further analysis of this data is a full calculation of the Doppler spectrum, disregarding the actual
value of these parameters, that is not a problem in this case.

![Figure 6.10](image)

Figure 6.10 Speckle contrast curves for single speed experiments, showing measured points and a fitted curve for two speeds of the translating phantom, and for the phantom while stationary.

Calculating the power spectral density of the speckle fluctuations, as described in section 4.1, produces the results in Figure 6.11 below.

![Figure 6.11](image)

Figure 6.11 Power spectral density of speckle fluctuations for single speed speckle contrast experiments, for two speeds of the translating phantom.

The highest expected Doppler shift on the returning light, hence the highest expected beat frequency speckle fluctuations, will occur when the light is backscattered from a moving
scatterer. The Doppler shift in this case is given by equation (6-3).

\[ \Delta f = \frac{2v}{\lambda_o} \]  

\( \text{(6-3)} \)

At the laser wavelength of 658 nm, the maximum Doppler shifts are 973 Hz for 0.32 mm/s and 1945 Hz for 0.64 mm/s. These values should be the maximum frequency threshold for the power spectra. The plots in Figure 6.11 show that the spectra approach zero below their respective cutoff frequencies. This is due to an effect that has been recognised since the earliest experiments in laser Doppler perfusion measurement: most of the Doppler shifts are lower than the maximum possible due to the angular diversity of the scattering producing them.\(^6\)\(^,\)\(^8\) The same effect that we observe here in plastic will also occur in tissue as tissue is highly biased to forward scattering, with anisotropy \(g\) in the range 0.7 to 1 for most human tissues.\(^4\)\(^,\)\(^7\) Most scattering interactions between light and a moving scatterer in tissue will only intercept a small component of the scatterer’s velocity.

\section{6.3 Spatial effects on laser speckle measurement}

\subsection{6.3.1 Point illumination experiments and depth effects}

The experiment described in this section, and previously published, was motivated by an apparent discrepancy between laser speckle and laser Doppler perfusion measurements. General opinion in the field was that laser Doppler detected deeper flows than laser speckle. In head-to-head comparison between the systems, there are differences in the images produced. Specifically, laser Doppler imaging appears to detect deeper flows than laser speckle using similar wavelengths – some Doppler images show deeper and larger vessels than speckle contrast images of the same subject. This difference may be due to differences in the illumination and measurement geometry between the systems.

Many laser Doppler systems use point illumination, where a small area is illuminated by a focussed or tightly collimated laser. To investigate the effects of this setup, speckle contrast measurements were made at a range of camera exposures using similar point
illumination. The phantom used for these experiments is shown in Figure 6.13, and consists of a bath of milk providing a Brownian motion target, covered with a transparent cover on which a 0.7 mm layer of a scattering silicone phantom was laid. The thin silicone layers used to construct the phantoms used in these experiments were produced following the procedures described by Saager.\textsuperscript{115} They consist of silicone (Sylgard 184 kit) with an addition of 0.1 kg·L\textsuperscript{-1} Al\textsubscript{2}O\textsubscript{3} powder as a scattering agent, spread in shallow moulds using a scraper before setting. The scattering coefficient $\mu_s$ of these phantoms is approximately 17 mm\textsuperscript{-1}, similar to that of skin.\textsuperscript{123} This phantom simulates detection of sub-dermal flows, as occurs in Doppler imaging where vessels can be seen.

![Figure 6.13 Phantom for point illumination experiments.](image)

The phantom was illuminated with a focussed laser spot, and images recorded at a range of exposures from 1.6 ms to 102.4 ms. The images produced by this method have a very large dynamic range due to the geometrical falloff in illumination from the centre of the laser spot, such that only a small proportion of the image will be within the working dynamic range of the camera. To overcome this problem, the laser illumination was varied by means of a polarising filter used at a range of angles from the laser polarisation. Valid regions of the resulting images were combined during processing in order to produce a composite speckle contrast image. The series of images with differing illumination at each single exposure were processed in MATLAB as follows:

1. Calculate a speckle contrast image for each raw image, producing a stack of contrast images
2. Exclude areas of the images outside the working dynamic range of the camera (raw mean < 20 counts, or including any camera saturation)
3. Assemble a composite image of valid contrast data from the contrast image stack by taking means of the valid K values at each point.
A typical composite speckle contrast image and an illustration of this process is shown in Figure 6.14.

Figure 6.14 Composite contrast image processing. The final image shown here was composed from 12 original images, only 3 of which are shown here. The ring delineated with black circles indicates an averaging area, used to calculate the mean contrast K at radius r.

The radial change in speckle contrast was calculated by taking the mean contrast of points falling within a 2-pixel thick ring at varying radius. These contrast against radius results are plotted as solid curves in Figure 6.15. The speckle contrast at a single exposure falls with increasing radius, and at a particular radius the contrast falls with increasing exposure. Changing the illumination to an expanded beam using a beam expander and an engineered diffuser, while holding the imaging setup constant, gives the contrast as measured by a conventional speckle contrast system. The mean values of the conventional-system speckle contrast at the four exposures in question are shown using dotted lines in matching colours.
Figure 6.15 Radial plots of speckle contrast, from static over fluid phantom with point illumination (curves), with mean expanded beam contrast at the same exposures for comparison (straight lines).

Figure 6.16 Radial plots of speckle contrast, on static phantom with point illumination.

Figure 6.16 shows the results of repeating the point illumination measurements and processing on a static, multiply scattering silicone target as a control experiment. The contrast doesn’t vary systematically with radius from the centre of the spot, indicating that no spurious results are being generated due to the spatial non-uniformity of the illumination – the speckle calculation region is sufficiently small that the illumination is adequately uniform over each calculation area.
Taking values at a set of particular radii to generate $K$ vs $T$ plots, and fitting curves as described in section 5.3 above using the fitting equation below shows that the value of the shape factor $\alpha$ and characteristic time $\tau$ do not change systematically with changing radius. These curves are shown in Error! Reference source not found.. The factor that changes most obviously is the static contrast $K_0$ which falls with increasing radius.

\[ K = \beta \left( \frac{1 - K_0}{1 + \left( T/\tau \right)^{\alpha}} + K_0 \right) \]  

(6-4)

![Graph showing speckle contrast curves measured at three radii from point illumination in a fluid phantom covered by a static scattering layer.](image)

**Figure 6.17** Speckle contrast curves measured at three radii from point illumination in a fluid phantom covered by a static scattering layer.

**Table 6-1** Fitting parameters for $K$ vs. $T$ curves in Figure 6.17.

<table>
<thead>
<tr>
<th></th>
<th>$\tau$</th>
<th>$\alpha$</th>
<th>$K_0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r = 2$ mm</td>
<td>0.67</td>
<td>0.77</td>
<td>0.33</td>
</tr>
<tr>
<td>$r = 4$ mm</td>
<td>0.96</td>
<td>0.68</td>
<td>0.13</td>
</tr>
<tr>
<td>$r = 6.25$ mm</td>
<td>0.79 ms</td>
<td>0.78</td>
<td>0.09</td>
</tr>
</tbody>
</table>

The results plotted in Figure 6.15 show that the contrast reduction observed on the static over fluid phantom increases with increased radius from the illuminated spot. A possible
interpretation of this effect is that measurements near the spot are biased towards the static surface layer, and measurements at a distance are biased towards the deeper fluid layer where Brownian motion makes the light paths dynamic. The expanded beam results, plotted as dashed lines, show that the depth probed by this method, corresponding to conventional laser speckle contrast imaging, is somewhere between the two extremes possible with a point illumination system.

This effect is expected for a fibre-optic probe based Doppler system, where the mean tissue depth probed increases with increased separation between the illuminating and receiving fibres.

The only significant difference in the fitted curves in Error! Reference source not found. is the value of $K_0$. The proportion of static to dynamic scattering changes, but the dynamic scattering generates intensity fluctuations with the same spectrum.

The depth sensitivity of a point-illumination based system will depend on the area contributing to the measurement – in other words, on the photodetector used and any imaging optics in front of it, whereas the depth sensitivity of an expanded beam system will depend only on the optical properties of the tissue under test. It is possible that this difference explains the apparent greater depth probed by some Doppler systems than some speckle systems at the same wavelength.

### 6.3.2 Contrast blurring effect of a vessel.

Determining the volume of tissue contributing to speckle contrast measurements is important for clinical applications, particularly in the dermis. Measurements made using fibre Doppler probes have reportedly large point-to-point spatial variations in the recorded perfusion. By comparison, experience with laser speckle measurements shows comparatively little spatial variation in perfusion images of undamaged tissue. Reducing the working field of view in the following experiments, previously published, allowed investigation of the spatial variation in speckle contrast measurements down to the scale of capillaries.

At the nailfold, by applying a drop of oil to the skin to reduce scattering at the skin surface, the ends of capillary loops are clearly visible in conventional microscopy using modest magnifications. The Sony camera, with the addition of a 75 mm lens and 45 mm spacers, can resolve the capillary structure in white light, as shown in Figure 6.18. The capillary loops are approximately 200 μm apart and the individual vessels are
approximately 20 μm diameter. However, at the same magnification they are not visible in the speckle contrast image, Figure 6.19, processed using 5 pixel x 5 pixel blocks to compute contrast. Since the object resolution is 7.4 μm per pixel with this lens and spacer setup, the contrast image could marginally resolve the capillary loops with 37 μm per contrast region, or at least delineate a zone of changed contrast.

Figure 6.18 Nailfold capillaries in white light, using the speckle imaging camera. Inset is magnified.

Figure 6.19 Speckle contrast image, using 5 pixel x 5 pixel contrast regions, of the same nailfold area as Figure 6.18.
Provided that the target is stationary, the same spatial resolution as that of the white-light image can be obtained by a temporal analysis of a pixel sequence$^{69,101}$. Reprocessing using a temporal contrast analysis over 11 frames of video produced an image with improved spatial definition of 7.4 μm per contrast region, shown in Figure 6.20, but the capillaries are still not visible. This suggests that resolution is being limited by a blurring effect produced because the speckle at a point is due to the summation of light paths which have been scattered in passage through large tissue volumes.

6.3.3 Point spread function in a skin simulating phantom

Spatial resolution in speckle imaging will always be limited by a point spread function, determined by the optical scattering properties of the tissue under test. A set of tissue phantom experiments were undertaken to investigate the region of a speckle contrast image affected by a small volume of moving scatterers in an otherwise static matrix, in order to find an effective spreading function for the contrast blurring effect from a vessel in skin.

The phantoms, simulating the blurring effects of a vessel in tissue, were cylinders 10 mm deep with a 1 mm diameter well. Filling the central well of the tissue phantom cylinders with milk creates a localised source of speckle blurring, as the particles in the milk show Brownian motion. This motion influences a volume of tissue by means of multiple scattering.
The permanent tissue phantoms used in these experiments, adapted from work by Lualdi\textsuperscript{123} and Forester\textsuperscript{49,125} were made from transparent silicone and used Al\textsubscript{2}O\textsubscript{3} powder as a scattering agent and methylene blue dye as an absorbing agent. Chloroform was used to dissolve the methylene blue and is soluble in uncured silicone. The permanency of these silicone phantoms, compared to agar based phantoms that can spoil over a few days, allows them to be reused or remeasured indefinitely.

The scattering coefficient $\mu_s$ is approximately proportional to the Al\textsubscript{2}O\textsubscript{3} concentration, with a concentration of 0.1 kg·L\textsuperscript{-1} giving $\mu_s = 17$ mm\textsuperscript{-1} at 660 nm wavelength, similar to skin\textsuperscript{47}. The absorption coefficient $\mu_a$ is approximately proportional to the methylene blue concentration, with 1\% by volume of a 1 g·L\textsuperscript{-1} mixture giving $\mu_a = 0.2$ mm\textsuperscript{-1}\textsuperscript{125}. 15 mg of methylene blue powder was mixed with 15 mL of chloroform to make the initial dye, which was added to the phantom material by drops, one 50 $\mu$L drop in 5 mL of phantom giving a dilution of approximately 1\%.

A range of scattering and absorbing configurations, shown in the table below, were made in order to both approximate the values for skin and find the sensitivity of the spreading function to $\mu_s$ and $\mu_a$.
Table 6-2 Concentrations of scattering and absorbing agents, and predicted scattering and absorption coefficients, for silicone phantoms.

<table>
<thead>
<tr>
<th>Varying scattering series</th>
<th>Al₂O₃ (g/L)</th>
<th>Methylene Blue Dye (% by vol.)</th>
<th>Predicted $\mu_s$ mm⁻¹</th>
<th>Predicted $\mu_a$ mm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
<td>0</td>
<td>3.4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0</td>
<td>6.8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0</td>
<td>10.2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>0</td>
<td>13.6</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Varying attenuation series</th>
<th>Al₂O₃ (g/L)</th>
<th>Methylene Blue Dye (% by vol.)</th>
<th>Predicted $\mu_s$ mm⁻¹</th>
<th>Predicted $\mu_a$ mm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
<td>1</td>
<td>17</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2</td>
<td>17</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Observing contrast images of the top surface of the phantom illuminated by uniform laser light, as shown in Figure 6.22, the lowest contrast (maximum motion) is seen on the liquid cylinder, with a region of reducing contrast on the phantom surrounding this. The ring of high contrast immediately outside the liquid is an artefact produced by an elevated rim of silicone about the cast well, caused by a meniscus in the liquid silicone. It serves to mark the liquid boundary.

![Figure 6.22 An example contrast image of a phantom containing a well of milk.](image)

As the images are circularly symmetrical mean contrast values from concentric rings of pixels were used to obtain spatial smoothing. The rings are 10 pixels wide on a 10 pixel radial spacing, with the central point chosen by eye for each image. This results in significantly smoother contrast data than those obtained taking a profile along a diameter.
Figure 6.23  Speckle contrast $K$ vs radius, showing the improvement in spatial smoothing using rings instead of profiles. 10 pixel concentric rings compared to a 20 pixel wide profile with 10 pixel moving average in a radial direction.

The effect of a moving scatterer, or a volume of fluid containing moving scatterers, is to reduce the local contrast. We define the speckle blurring effect, or contrast reduction $\Delta K = K_{\text{max}} - K_{\text{local}}$ where $K_{\text{max}}$ is the undisturbed contrast measured on the static phantom a large distance from the moving scatterers. Figure 6.24 shows both $K$ and $\Delta K$ for the data in Figure 6.23 above.

Figure 6.24  $K$ and blurring effect $\Delta K$ changing with radius $r$ from a well of milk in a tissue phantom.
Support for the validity of the speckle results from the phantoms was indirectly checked by comparing their optical properties to those of skin. For this the fall-off in diffuse reflectance in both phantoms and skin was measured from a focused laser spot, again using concentric ring means as described above. This technique was verified by Forrester as producing reasonable values of scatter and absorption.

Figure 6.25 and Figure 6.26 show the results for changing the scattering coefficient in 4 phantoms with approximately zero attenuation as shown in the first four rows of the table above, and the same measurements made on finger and palm skin for comparison. The phantoms fail to match the hand and finger tissue measurements in diffuse reflectance measurements. Speckle images of the phantoms under expanded, uniform laser illumination were then measured with the wells filled with milk. Despite their significantly different reflectance fall-off characteristics, there is no systematic difference in the falloff curves for ΔK.

![Figure 6.25 Light intensity falloff in tissue phantoms from point source laser illumination, with a range of scattering coefficients.](image-url)
Figure 6.26 $\Delta K$ fall off in tissue phantoms with a range of scattering coefficients from a small region of moving scatterers.

Figure 6.27 Intensity falloff in tissue phantoms with a range of attenuation coefficients from point source laser illumination.
Fixing the value of the scattering coefficient $\mu_s$ at 17 mm$^{-1}$, approximately the value expected for skin of 19 mm$^{-1}$\textsuperscript{47}, and varying the attenuation coefficient $\mu_a$ between approximately 0 and 0.4 mm$^{-1}$ by the addition of various amounts of dye, we find that the diffuse reflectivity for the phantom with 1 drop of dye, and $\mu_a = 2$ mm$^{-1}$ matches the form of skin well at higher radii, as shown in figure 8a. The $\Delta K$ results are similar for all the attenuating phantoms: blurring effect $\Delta K$ falls exponentially with radius from a small region of movement. Fitting a curve to the phantom most closely approximating skin, we get a distance for $\Delta K$ to reduce by a factor of $1/e$ of 0.7 mm. However, this characteristic length is not significantly sensitive to the attenuation coefficient.

6.4 Chapter conclusions.

The effects investigated here in laboratory measurements have significant implications for the clinical applications of laser speckle techniques.

The static scattering correction factor $K_0$ is important in any measurement where there are a significant number of static scatterers. Correctly estimating $K_0$ requires that sufficiently long camera exposures must be used where there are static scatterers.

The spatial effects experiments described in section 6.3 show that speckle perfusion
imaging systems interrogate a volume of tissue. There is a natural spatial averaging effect, as every point in the biospeckle pattern captured is informed by light paths that travel by multiple scattering through a volume of tissue. Using high magnification and white light illumination in the speckle imaging system, nailfold capillaries can be easily delineated; however we have shown there is no speckle contrast variation over a distances of the 200 μm separating the capillaries, though a speckle contrast variation should have been easily resolved were it present. The capillaries themselves, 20 μm diameter, should have been resolved at the lower limit of resolution, but were undetectable in the general perfusion signal.

The silicone phantoms which included scattering and absorbing material mimicked the point spread function of dermal tissue\textsuperscript{123,125}. When used under uniform defocused laser light, as in clinical use, where a capillary was modelled by Brownian motion of scatterers within a small cylindrical well, the speckle contrast blurring was observed to spread into the surrounding material approximately exponentially with a length to reduce by 1/e of 0.7 mm. This scale is not strongly affected by absorption levels in the first 2mm to 3mm.

Close to the point laser source, the radial gradient of intensity of surface scatter from skin and the preferred phantom can be seen in Figure 6.27 to be falling exponentially with a scale length of about 0.8 mm. The speckle point spread length and the close-to-source intensity attenuation approximately match. This is reasonable since significant speckle contrast changes require the interfering light (in this case the dynamically scattered light from the fluid well) to have an intensity approaching that of the local intensity.

The optical properties of skin tissue therefore preclude seeing structure with scales appreciably below 1 mm. This resolution should be strongly wavelength-specific. We suggest higher resolution may be achievable with a shorter wavelength laser.

The conclusion remains that speckle measurements at 660 nm wavelength do not show significant spatial variations on scales below 1 mm in intact skin, and cannot be expected to reveal structure below this. From our measurements it seems that the discrete nature of capillary loops (about 30 loops per mm\textsuperscript{2}) might account for the large spatial difference seen in fibre Doppler probes but not seen in diffuse-illuminated speckle experiments.

As speckle systems use expanded illumination, they will tend to be biased towards surface measurements in comparison to systems using focussed illumination, for example a scanned laser Doppler system, in which the depth of sampling will be determined to a
degree by the separation of illumination source and detection regions. This effect leads to 
an interesting possibility, already taken advantage of by some workers in the field in a 
limited way: the depth sensitivity of speckle imaging might be controllable by using 
structured illumination.
7 In vivo and medical results

The aim of this work is to produce clinically applicable measurements, based on sound physics, and providing measurements with a clear physiological interpretation. The in vivo and clinical tests presented in this chapter provide some foundation to understand what we measure when applying laser speckle techniques to tissue, explore some of the possible application areas and test the practicality of speckle-based clinical systems.

7.1 Preliminary in vivo measurements

7.1.1 Occlusions and biological zero

In laser speckle measurements there is always some reduction of speckle contrast even when the net blood flow ought to be zero. For example, on using a sphygmomanometer to produce arterial occlusion in the arm, the contrast measured at the fingers does not reach the contrast of a truly static target measured with the same equipment. The remaining contrast reduction bears some resemblance to a ‘biological zero’: a Doppler spectrum and related perfusion index remaining under arterial occlusion reported by some researchers in the laser Doppler literature\(^{110}\).

A time series of speckle contrast recorded under arterial occlusion, and showing this effect, is plotted in Figure 7.1. These measurements were made with an early iteration of the speckle setup, using the Worldstar thermally controlled laser module with relatively short coherence length, and produced a maximum contrast of 0.75 on a multiply-scattering static target. The maximum contrast reached for the skin under arterial occlusion was 0.63 – a small but definite reduction.

![Figure 7.1 Time series of speckle contrast measured on the finger under arterial occlusion. Blood flow was stopped at the upper arm at approximately 120 seconds, and restored at 250 seconds. Reactive hyperaemia causes the overshoot in blood flow and contrast reduction following 250 seconds.](image-url)
In order to investigate the spectrum remaining under occlusion, contrast curves with changing exposure were measured on the finger under arterial occlusion, using the later speckle setup with a long coherence length (6 m) Ondax laser as described in section 3.1.2. The camera aperture of f/16 and the speckle contrast calculation region of 25 pixel x 25 pixel were chosen to allow the contrast to approach 1 for a static target.

Contrast curves were measured on the volar surface of the forefinger (the surface nearest the palm), before, during, and after the brachial artery was occluded using a sphygmomanometer by taking the pressure to approximately 50 mmHg (6.7 kPa) over systolic pressure. Figure 5 shows raw contrast data with a fitted curve, using equation (5-12) repeated from above:

\[
K = \frac{1}{1 + \left(\frac{T}{\tau}\right)^\alpha}
\]

The autocorrelations plotted in Figure 7.3 were calculated numerically from points of the fitted function, using the double-differential equation (4-7) above, and the power spectral densities in Figure 7.4 and Figure 7.5 were obtained by taking discrete Fourier transforms of these autocorrelations, according to the Wiener-Khintchine theorem.

Perfusion indices calculated from each curve by finding the first moments of the power spectra are plotted against measurement time in Figure 7.6.

Figure 7.2 Contrast vs. exposure curves measured on the finger under arterial occlusion
Figure 7.3 Autocorrelations calculated from multiple-exposure speckle measurements on the finger under arterial occlusion.

Figure 7.4. Power spectra calculated from the autocorrelations.
Figure 7.5 The same power spectra as shown in Figure 7.4, restricting the axis to 0-1 kHz.

Figure 7.6. Perfusion indices showing the effect of arterial occlusion.

There is some apparent blood flow remaining in the tissue under arterial occlusion, when there is no net blood flow through the finger. This apparent blood flow may be microscopic movement of other constituents of the tissue, the biological zero as discussed above.

As well as a significant fall in perfusion index, from baseline 860 Hz to the occluded value 160 Hz, there was a significant rise in perfusion index to 1200 Hz following release of the occlusion which persisted for at least 600 seconds following the release of the sphygmomanometer.

7.1.2 Inflammation sensing

As part of three-system comparison testing at Twente University in 2011, experiments were carried out comparing perfusion images recorded over time of changing blood flow. The three systems, as described above in Section 6.1.1, were the M-LSPI system, a single exposure commercial laser speckle contrast system (Perimed) and an experimental wide-
field laser Doppler system. A capsaicin-based warming cream, Midalgan Extra Warm generates a large change in perfusion, due to local dilation of the capillaries. The volunteer test subject was a healthy 23 year old female. An area of approximately 10 mm by 30 mm was treated with the cream, and the perfusion imaged over 15 minutes using all three systems. The experiment was repeated twice. In order to measure with all three systems, they were arranged such that they could be easily swung on their respective stands to image the area under test, and repeatedly moved back into the measuring position in sequence. Figure 7.7 shows images from the three systems.

![Image of three systems comparing perfusion](image)

**Figure 7.7** Perfusion images of induced perfusion increase on forearm using three different perfusion imaging systems. The M-LSPI image shows Perfusion Index (1/τ), the TOPCam shows the first moment of the Doppler spectrum (mean Doppler frequency) and the PSI image shows Perimed’s proprietary Perfusion Index.

A small piece of paper was attached to the skin to provide a fiducial point and this paper is visible as the small square in the lower right of the images. The induced increase in the perfusion is not instantaneous; it occurs around 5 minutes after the cream is applied. This effect is visible in the M-LSPI images shown in Figure 7.8. The perfusion is relatively constant for 5 minutes, then changes suddenly.
Figure 7.8 Perfusion increase with Midalgan cream on the forearm, imaged with M-LSPI system. These images show Perfusion Index (1/τ).

The change in perfusion is plotted for all three systems in Figure 7.9 Perfusion time series for three instruments, the Twente Optical Perfusion Camera (TOPCam), the Perimed Speckle Imager (PSI) and the Multiple Exposure Laser Speckle system developed in this thesis (M-LSPI). Midalgan cream was applied at time 0, and perfusion increased with inflammation starting at approximately 3 minutes.

All three of the devices have measured the increase in perfusion similarly. The data in this plot were scaled, in order to start at the same point. It was found necessary to subtract a background level from the M-LSPI data before this scaling, determined by the apparent perfusion level measured on a static target. However, with this consideration in mind, the systems all show the same proportional change in perfusion between the baseline and increased flow state.
7.1.3 Pulsatile effects in speckle measurements

All live, perfused tissue has a large speckle contrast reduction compared to static targets or unperfused tissue. As well as the general contrast reduction, contrast images recorded at sufficiently high frame rates generally have a small pulsatile component superimposed. Near larger vessels, such as the radial artery, there is clearly movement of the skin at the pulse rate and this movement provides the pulsatile component. However, there is also a residual pulsatile component at a distance from major vessels, for example in the fingers, through the fingernail and in the toes. The interpretation of this observation is not immediately clear – the pulsatile component might be an indication that our light intercepts a truly pulsatile flow in the capillaries, or it might indicate a gross movement or a change in conformation of the imaged tissues. Similar pulsatile signals have been reported as being visible in laser Doppler records where they are generally regarded as motion artifacts. Whatever the physical origin of these signals, when they are clearly understood they will have clinical and research applications in investigating circulation. This section presents some measurements of the skin and of attached phantoms designed to elucidate the origin of the pulsatile measurements, and shows that a pulse shape can be extracted from the data which is different for different individuals, and which may be useful in clinical applications.

Speckle contrast was measured on various skin regions, using a small camera frame of 320 pixel x 60 pixel, corresponding to a skin area of approximately 10 mm x 2 mm, to
allow high processing rates. The frame rate was set at 30 fps, allowing adequate sampling over each pulse period to establish a pulse shape as well as the pulse rate. In order to establish whether the pulse measurements were related to pulsatile flow or movement of the skin, opaque tape and multiply scattering phantom materials were attached to the skin for control measurements.

Figure 7.10 shows a speckle contrast record made on the upper surface of the foot using 10 ms exposures, together with a control measurement made on a 2 mm thick piece of silicone phantom material attached to the skin using double sided Sellotape. The two records were made consecutively without changing the setup or moving the foot by simply removing the phantom material while recording, to ensure that precisely the same area is being imaged. These measurements were made using the Worldstar laser, which had gone into multi-mode operation and consequently had a short coherence length during the measurements, as they were taken before the purchase of the Ondax laser.

![Speckle contrast record on skin of foot and attached phantom material, 10 ms exposure 30 fps.](image)

**Figure 7.10 Pulsatile contrast signal recorded on the skin of the foot, with silicone phantom for attached to the skin for comparison.**

There is a clear pulse evident in the skin, and none in the phantom material. The power spectrum of the two traces, calculated by FFT and plotted in Figure 7.11 shows a clear peak at the pulse frequency in the skin record, at approximately 5 dB above the background level, but nothing above the background level at this frequency in the record of the phantom, indicating that in this case the phantom material was not moving at the pulse rate. There was either no gross movement of the foot or skin with pulse or this movement was insufficient to move the lightweight phantom.
Figure 7.11 Power spectra by FFT of contrast records on skin of foot and attached phantom from figure 9, showing a peak at the pulse rate in the skin – marked by the red dashed line – and no corresponding peak in the phantom.

Repeating this experiment on different areas of skin, we do not get consistent results. Measuring again on the upper surface of the foot, where there are large vessels relatively close to the surface, produced the data shown in Figure 7.12. In this case there is significant pulse in the phantom record. Tissue movement is contributing to the “perfusion” measurement through motion of the phantom, induced by the pulse in an arteriole. The background contrast in both of the curves in this record is reduced as the laser had gone into multi-mode operation, due to changing ambient temperature. It remains noteworthy that the bare skin result ($K = 0.35$) is significantly lower than that produced by the phantom motion alone ($K = 0.47$). It is noteworthy also that the bare skin record shows a more clearly defined profile.

Figure 7.12 Contrast records for skin and phantom, taken consecutively. In this case there is clearly a pulse in the phantom control, showing some movement of the skin with pulse.

It may be useful to measure an average pulse shape at a particular measurement site. For
example, the pulse shape at the toe may reveal the effects of peripheral vascular disease, through a reduced peak flow or reduced peak change in flow caused by sclerosis.

In order to extract an average pulse shape from a noisy record, we find the position of each pulse and construct a matrix of stacked pulses, allowing us to find a statistical average pulse. Positions of the pulses are extracted using a FIR filter in MATLAB. Given the variability of the pulse shape, a perfectly matched filter cannot be found, but an approximately matched filter can be chosen. The filter used is a section of a sawtooth wave with a period matching the pulse period found by spectral analysis of the contrast record. The sawtooth roughly approximates the pulse shape generally found in contrast data. Pulse start positions are identified as local maxima of the filtered signal. Figure 7.13 shows an example of this position calculation on a noisy record of the speckle contrast measured on the thumb nailbed.

![Figure 7.13 Pulse position calculation example. The marked vertical lines indicate the start of each pulse in the pulse record, for averaging purposes.](image)

Having found the pulse positions in a record, we can find a statistical average pulse, using means or medians of the contrast value at variable delays from the calculated pulse position. Five example mean pulse shapes are shown in Figure 7.14, with a minimum $K$ subtracted from each for co-plotting.
Figure 7.14 Mean pulse shapes for two subjects — subject 1 plotted in fine black lines was measured on the hand, finger and toe. Subject 2 plotted in thick blue lines was measured on the thumb.

Further investigation of pulse shape in a range of subjects has not been carried out within the scope of thesis. However, there may be some physiological significance and applicability to the measurements.

7.2 Diabetic foot ulcer trial

As an initial clinical test of M-LSPI in a real application perfusion around diabetic foot ulcers was imaged in a set of patients attending a podiatry clinic at Counties Manukau DHB (CMDHB). This research was conducted in collaboration with researchers from the Centre for Clinical Research and Effective Practice (CCREP). A speckle imaging system was supplied, and the measurements were made by CCREP staff. This trial was intended as a brief clinical test, collecting a small number of patients as an indication of the clinical usefulness of perfusion measurements in assessing diabetic foot ulcers. As it progressed, changes in CCREP staff, delays in patient recruitment, and other organisational problems meant there was some inconsistency in recording data. The trial also took much longer than initially proposed. However, the data recorded show some interesting results and there are useful conclusions to be drawn about the practical application of speckle techniques in clinical practice.

7.2.1 Trial outline – study design

The study design, as proposed, was a prospective cohort study, intending to examine dermal perfusion in the plantar aspect (sole) of the foot in non-diabetic and diabetic subjects with and without peripheral vascular disease (PVD), and to determine whether a baseline perfusion measurement in the soles of the feet could predict the healing rate of
foot ulcers in patients with diabetes mellitus. The study design called for 30 healthy control subjects, 30 patients attending a PVD clinic, and 30 patients with foot ulcer and diabetes.

Specific sites were chosen for measurements. One measurement was taken over the ball of the big toe (1st distal inter-phalangeal joint) and one over the ball of the little toe (5th distal inter-phalangeal joint). In diabetic foot ulcer patients, an extra measurement was taken covering the ulcer bed.

7.2.2 Diabetic foot ulcer trial methods

The speckle hardware and software were adapted for this trial as described above in section 3.5. Raw speckle images were collected in time- and date-stamped folders, as well as patient details which allowed the CCREP staff to correlate perfusion images with medical records. The raw images were post-processed in MATLAB, generating images in both perfusion index (PI, the first moment of the Doppler spectrum) and \( K_0 \). As described in sections above, PI indicates the mean speed of blood cells and \( K_0 \) indicates the proportion of static scattering paths in the returned light. \( K_0 \) will be high in callouses, or in any areas that have a reduced proportion of blood in the surface of the tissue.

The images covered relatively small areas of the foot, approximately 25 mm across. This small area was a compromise between accuracy of the measurements and field of view, given the light level constraints that affect this system.

7.2.3 Representative M-LSPI images

Figure 7.15 to Figure 7.17 below show representative M-LSPI images in both parameters, PI and \( K_0 \), plotted in false colours and overlayed on a digital photograph of the same patient’s foot. There are three diabetic foot ulcer patients represented here. Registration between the speckle images and the photograph was achieved using features identifiable on both the raw speckle images and the photograph.

The \( K_0 \) images show a low level in the centre of the ulcer, approximately zero indicating no static scattering layer there, and surrounding high values indicating a callous or exsanguinated area. The PI images shows an area of higher perfusion outside the ulcer.

The areas imaged using the speckle system were small, not covering the whole foot.
7.2.4 Inter-group comparisons

One aim of this study was to determine whether there was a difference in baseline perfusion or $K_0$ between the three different groups recruited: diabetic patients; patients with peripheral vascular disease; and normal controls. Collecting all of the valid data for each patient, and separating the results by cohorts, enabled this analysis. Baseline perfusion and $K_0$ was calculated as the mean of all of the valid perfusion and $K_0$ data for each subject. For ulcer patients the baseline perfusion values are the mean value of all the non-ulcer sites, and for non-ulcer patients all sites were included.

The data collected are presented here in several plots. Figure 7.18 shows the distributions
of the perfusion index for each of the three cohorts. Subject measurements were binned into bins 200 Hz wide for PI, and 0.05 wide for $K_0$. The mean perfusion index is the same in the three groups, approximately 890 Hz, while the mean $K_0$ varies significantly between groups. The control group mean $K_0$ is 0.25, with the peripheral vascular disease group having mean $K_0$ of 0.22 and the diabetic foot ulcer group having mean $K_0$ of 0.19. Statistical significance of these results were checked by single-factor analysis of variance (ANOVA) testing, which give $p$-values $p=0.79$ for the perfusion index data, and $p=0.004$ for the $K_0$ data.

![Baseline Perfusion Index Distributions](image1)

**Figure 7.18** The distribution of Perfusion Index is consistent between cohorts.

![$K_0$ Distributions](image2)

**Figure 7.19** Static contrast $K_0$ differs between cohorts.
7.2.5 Ulcer area change

The third goal of this study was to determine whether the baseline M-LSPI measurements could predict the healing rate of ulcers. Ulcer area for all the pictures was calculated by drawing a polygonal border around the ulcer by eye, as in the example in Figure 7.20. The scale of the pictures was calibrated by drawing a line on the photographed scale, and the ulcer area worked out from the recorded points in MATLAB.

![Example ulcer area measurement from digital photo.](image)

Repeated pictures were available for some patients, collected over several clinic visits. For these patients, the change in ulcer size was calculated in both mm/day and percentage/day. Negative rates of change indicate healing as the ulcer is reducing in size. Unfortunately, due to fewer patients returning to the clinic during the study time than were expected, there were only repeated photographs available for 5 patients which was insufficient to allow robust analysis of a possible correlation between ulcer rate of change and perfusion measurements.

7.2.6 Ulcer time to healing

Time to healing for the patients’ ulcers was collated and analysed by CMDHB biostatistician Alain Vandal\textsuperscript{126}. The metric used was remaining ulcer duration (RUD), measured in days.

Figure 7.21 and Figure 7.22 show times to healing plotted against PI and $K_0$, respectively.
There was no association found between perfusion index and time RUD. However, there was a significant association between $K_0$ and RUD, with larger values of $K_0$ indicating shorter time to healing after the measurement was made. It is possible that this measurement has simply detected the normal healing process: measurements made later in the progress of a healing ulcer may show larger static contrast $K_0$ as the epidermis recovers its thickness. It is also possible that the few measurements showing high $K_0$ are outliers.

Figure 7.21 Remaining ulcer duration (RUD) against Perfusion Index (PI). No correlation is found between these variables. Plot taken from Alain Vandal’s report\textsuperscript{126}.
Figure 7.22 Remaining ulcer duration (RUD) plotted against $K_0$. Statistical analysis shows a negative correlation between time to healing and $K_0$: high $K_0$ indicates a short time to healing. Plot taken from Alain Vandal’s report.\textsuperscript{126}
7.3 Conclusions to this chapter

7.3.1 Preliminary in vivo experiments
The preliminary in vivo experiments carried out covering biological zero, inflammation detection, and pulsatile effects provided a test ground for the speckle equipment. The experience gained in making these measurements influenced practical improvements in the hardware and software which carried through to the clinical trials. The data collected also indicate the possibilities of further applications of speckle measurements, particularly in detection of inflammation in tissue.

The measurements above show that some apparent blood motion remains under arterial occlusion. A convincing explanation of this value will be necessary to establish any interpretation of the perfusion index as an absolute measurement. Several possibilities exist: there may still be some net flow in the capillaries under arterial occlusion; the motion may be generated by microscopic movements of elements of the tissue other than blood cells; the effect may be generated by the effect of Brownian motion in the vessels; or there may be some gross bodily movement influencing the result. Depending on the final explanation, this value may have some research or clinical significance. Measuring the movement of tissue constituents other than red blood cells may provide insight into physiological processes not directly associated with blood flow.

Laser speckle measurements have been shown to be a reliable detector of inflammation, picking up the increase in blood flow due to application of capsaicin based cream. It will be useful in future work to find the lower limits of sensitivity for this method. A possible application of this capability is in detection of allergic reactions by developing a quantitative measurement of the dermal reaction in skin prick tests.

It is possible to detect pulse rate and shape using speckle contrast. The method of finding pulse times and averaging several pulses may prove to have clinical applications.

7.3.2 Clinical trial conclusions
One hypothesis tested in the diabetic foot ulcer trial was that there would be detectable differences in perfusion index between the normal group, the group with peripheral vascular disease, and the group with diabetic foot ulcers. This hypothesis was falsified – there is no detectable difference, as shown in the results in Figure 7.18. However, despite finding no difference between the baseline perfusion index measured in the three groups,
there was a significant difference between the mean static contrast $K_0$ for the three groups. $K_0$ is related to the ratio of static to dynamic scattering. In phantom measurements (section 6.1) $K_0$ has been shown to be approximately linearly related to the thickness of static layers overlaying dynamic scatterers. The differences in $K_0$ in these clinical measurements may reflect the thickness of callouses on the sole of the foot or other structural changes in the microvasculature leading to fewer light paths encountering moving red blood cells. Perfusion index does not offer any prediction of time to healing for the ulcer patients. However, again the value of $K_0$ showed a correlation with the medical data. $K_0$ has a negative correlation with tie to healing. This correlation is not strong, and may be simply indicating the normal healing process.

The unexpected result that perfusion index indicates little about the medical condition of the subjects in this trial, while static contrast $K_0$ does offer information, indicates that $K_0$ should not be neglected in further clinical applications of laser speckle measurements. Measuring $K_0$ requires the long exposures captured in multiple-exposure systems, or synthetic long exposures as described in section 5.4.

Collaborating in running the clinical trials, the first conducted with this system and the first conducted by our research group, provided a number of practical lessons applicable to future trials and applications of the system, particularly with respect to the setup of software for straightforward use by clinicians, facilitating consistent data collection by a number of workers, and the compromises made in choices of system parameters. A significant lesson learned was that the size of the imaged field, and definition of the image collected is important. In the measurements in this trial, the image area was restricted to 25 mm. This was done in order to collect frames at short exposures (1 ms) and large speckle size (approximately 5 pixel) given the restriction of limited laser power, to ensure that the speckle statistics could be correctly interpreted. The images collected, while informative of the perfusion in the area imaged, were difficult to interpret and to register with photographs. Compromising on the shortest exposure collected and the speckle size in order to collect images of larger areas would have resulted in more useful images. Given the availability of a correction for spatial averaging (section 5.1) and the fact that $K_0$ (determined from the longer exposures in the series) has significance in this application than the details of the higher frequencies in the speckle fluctuation spectrum (determined by shorter exposures), these compromises would not remove any useful information from the data collected. Future trials using this system will reflect this lesson.
8 Conclusions

The capacity of laser speckle imaging to generate good qualitative images of blood perfusion is well established and applications of these techniques have been developed by a number of groups. Quantitative analysis of speckle contrast, developing a reliable perfusion index from speckle contrast measurements, allows the extension of the technique from simply imaging where blood flow is present to showing the changes in blood flow in a linear and reliable fashion.

The work in this thesis shows a method to achieve such a quantitative analysis, by using multiple exposures in order to gather information about the spectrum of speckle fluctuations. Supporting work elucidates aspects of the design and specification of speckle imaging systems, as well as investigating many practical aspects of speckle imaging using simulations and phantom measurements to establish the physical basis underlying clinical measurements.

In vivo and clinical experimental work reported here shows some of the possibilities of speckle imaging applications. The diabetic foot ulcer measurements show an unexpected result – the significance of the $K_0$ measurement in these data may lead to further discoveries.

8.1 Multiple exposure speckle contrast theory and practice

8.1.1 Ambiguity of single exposure speckle contrast

Speckle contrast at a single exposure finds only limited information about the speckle fluctuations, and relies on assumptions which are not necessary in multiple exposure speckle analysis. Revisiting the speckle contrast versus camera exposure curve first introduced in chapter 1 illustrates one of the issues. Figure 8.1 illustrates a case in which the interpretation of a single exposure speckle contrast measurement would be ambiguous. The two curves differ in two parameters. The red curve represents tissue with a higher mean blood flow speed, so has a shorter characteristic time than the blue curve. However, the red curve also represents tissue with a higher static contrast $K_0$ than the blue curve as occurs where there is a layer of unperfused tissue, such as the thick epidermis in a calloused foot, overlaying the perfused tissue. At a camera exposure of 2 ms, the speckle contrast measured for both curves will be the same.
Measuring speckle contrast at a range of camera exposures is the only way to discriminate the effects of multiple parameters changing in speckle contrast curves.

![Graph](image)

Figure 8.1 Illustration of a speckle contrast curves generated by tissue with different blood flow mean speed and static contrast. The red curve is generated by tissue with higher mean speed and higher static contrast than the blue curve.

### 8.1.2 Multiple exposure speckle contrast curves find the full Doppler spectrum

Establishing that laser speckle methods can obtain the full spectral information of the speckle fluctuations using multiple exposures, and proving that this analysis is correct by simulation and experiment has laid the foundation for speckle methods to be used for quantitative measurement of perfusion.

This thesis and associated papers\textsuperscript{19–21} show that the same spectral information can be obtained from multiple exposure laser speckle measurements as can be measured using laser Doppler. The analysis recovering autocorrelation from speckle contrast curves was developed from speckle theory, specifically from the integral equation (4-1) given by Goodman\textsuperscript{11}. The analysis, once developed, was tested using both simulation and experimental tests and confirmed as valid.

In other words, given a curve consisting of speckle contrast $K$ measurements at a range of camera exposures $T$, it is possible to recover the autocorrelation function of the dynamic speckle intensity fluctuations which give rise to the contrast curve. This is equivalent to finding the spectrum of these fluctuations and, critically, allows the determination of their
mean frequency which is directly related to perfusion. This is important in medical applications of biospeckle analysis as the mean frequency of speckle fluctuations is established in laser Doppler theory as being linearly related to the product of blood cell concentration in tissue and their mean speed\(^6\). This analysis removes the need to assume an autocorrelation function for the speckle fluctuations.

The same caveats apply to laser speckle based perfusion measurements as to laser Doppler: the measurement made reflects the actual light path through the tissue. A particular scattered path from laser illumination to camera will include multiple scattering events, possibly intercepting a number of moving scatterers. The composition of the tissue and its light scattering characteristics will influence the final reading, making an absolute determination of flow difficult without calibration. Given a particular tissue makeup and distribution of particle speeds, a perfusion index inversely proportional to a characteristic time (hence proportional to a characteristic frequency) will be linearly related to the mean particle speed. However, the factor linking the two variables may not be easily determined. Multiple exposure laser speckle and laser Doppler both remain relative rather than absolute perfusion measurements.

### 8.1.3 A linear $\beta$ correction for $K_{\text{max}} < 1$ is valid.

Compromises in system setup mean that the maximum speckle contrast achievable, $K_{\text{max}}$, is often less than the ideal value of 1 for practical systems, due to spatial averaging of the speckles caused by the size of the camera pixels.

Experimental and simulation results confirm that a linear system correction factor $\beta = 1/K_{\text{max}}$ is valid. Confirming the validity of this correction factor enables a trade-off between light levels and spatial averaging, as it is possible to recover the original speckle contrast from a pattern degraded by spatial averaging. Reliable $\beta$ correction also allows comparison between systems and measurements with different $K_{\text{max}}$ values.

### 8.1.4 Speckle systems have higher frequency sensitivity than might be expected.

The frequency sensitivity result, shown in section 5.2, shows that speckle contrast measurements respond to speckle fluctuations with frequencies significantly higher than might be expected, given the camera exposure times used. If this was not the case, speckle measurements would be restricted to measuring slower flows than laser Doppler systems.

Defining a frequency sensitivity scaling factor $m_f$ as the ratio of the times for the
autocorrelation or the speckle contrast curve to fall by $1/e$ allows a quantitative estimate of this difference, and this factor is $\approx 13$ for the two autocorrelation functions (exponential and Gaussian) considered. It is a reasonable assumption that $m_f > 10$ will apply to any autocorrelation we are likely to encounter in biospeckle measurements.

Speckle based systems can then respond the same blood flows as a Doppler system with sampling rate at least 10 times higher.

### 8.1.5 Curve fitting to multiple exposure speckle data

The speckle contrast $K$ versus camera exposure $T$ curve measured in the multiple exposure speckle contrast technique, with its typically sigmoidal shape when $K$ is plotted against $\log(T)$, is the key to the technique. Finding the shape and position of this curve is sufficient to determine all the information available about the speckle fluctuations. In many cases the shape of the curve is relatively predictable, so that it is suitable for parameterisation. Section 5.3 suggests a possible equation (5-13) to fit to the curve in clinical measurements:

$$K = \beta \left( \frac{1 - K_0}{1 + \left( \frac{T}{\tau} \right)^\alpha} + K_0 \right)$$

When the shape factor $\alpha$ is fixed at 0.8, this curve has been found to fit the data collected in dermal perfusion experiments, and Brownian motion in phantom experiments. With a fixed shape factor $\alpha$ there is a simple practical interpretation of the other factors: $\beta$ is the system correction factor $1/K_{\text{max}}$, $K_0$ the static contrast, and $\tau$ is a characteristic time factor. The time factor $\tau$ is not the same as the autocorrelation $\tau_c$ but is linearly related, so $1/\tau$ can be used as a perfusion index.

This set of three parameters, one of which ($\beta$) can be measured in advance for a particular system and two of which ($\tau$ and $K_0$) found by fitting a curve to measured points, usefully simplify the analysis of multiple exposure speckle data for clinical applications.

### 8.1.6 Synthetic long exposure method for multi exposure laser speckle

Synthetic long exposures as introduced in section 5.4 are a practical method for finding the speckle contrast at long exposures, a key measurement in multiple exposure speckle contrast measurements particularly when considering the effect of static contrast $K_0$ as shown in section 6.1. Synthetic long exposures avoid the dynamic range problems created
by taking a large range of camera exposures while holding the camera aperture fixed in order to keep speckle size constant.

The double-flash technique allows a fixed laser power, fixed camera exposure and gain, and fixed aperture while generating the same measurements as a conventional multiple exposure series. While it has not been tested further experimentally, this method may prove useful in future speckle applications as it enables the use of ordinary low speed cameras while still collecting information on high frequency parts speckle fluctuations.

The summed exposure contrast method allows continuous laser illumination, and escapes the dynamic range issues of conventional multiple exposure series, by collecting a series of short exposure (10 ms) speckle frames with very short (< 1 ms) inter-frame time. This method has proved useful in my colleague Evan Hirst’s retinal imaging work using a modified fundus camera.

8.1.7 Static speckle contrast $K_0$

The value of static contrast $K_0$, the remaining contrast measured at sufficiently long camera exposure that all dynamic speckle is blurred, has proved to be an important measurement. Neglecting $K_0$ results in incorrect estimation of flow speed in situations where there is significant static scattering, as occurs in some experimental and clinical cases. $K_0$ also has some significance in the results of the diabetic foot ulcer clinical trial.

8.2 Applications of biospeckle – in vivo and clinical

The in vivo experiments reported in section 7.1 provided the background to the clinical application of multiple exposure speckle contrast measurements. Notable results from this work are that speckle is a sensitive detector of inflammation, that there is a biological zero value of apparent perfusion, and that pulse shape is detectable using speckle measurements.

The key clinical work in this thesis is the measurements in diabetic foot ulcer patients. The hypothesis that there would be a significantly different mean perfusion index between the three groups of normal subjects, patients with peripheral venous disease and diabetic foot ulcer patients was falsified. This is an interesting point, as differences in blood flow have been generally understood to play a key role in the pathology of diabetes. However, there is a significant difference in the mean static contrast $K_0$ between the groups. The further relevance of this discovery remains to be established and may reward further research.
Similarly, there was no prediction of healing time in ulcer patient using perfusion index, but some correlation between $K_0$ and healing time. The correlation was not strong and further investigation will be required to establish whether there is any information of clinical relevance, or whether this correlation merely reflects the normal healing process.

### 8.3 Contribution to the field and future work

The significant contributions to the field of laser speckle contrast perfusion measurements in this thesis are: the proposal of a multiple exposure speckle analysis; the demonstration that such an analysis recovers the full spectral information of the speckle fluctuations, equivalent to laser Doppler; the confirmation of the validity of a linear system correction $\beta$; and the establishment of the importance of static contrast $K_0$.

The discovery that the three cohorts in the clinical trial, normal, peripheral vascular disease, and diabetic foot ulcer, have the same mean perfusion index but differing mean static contrast $K_0$ shows the importance of considering $K_0$ in future speckle perfusion imaging research. This implies that multiple exposures must be used as it is impossible to separate the two parameters of perfusion index PI and static contrast $K_0$ using only a single exposure.

Work related to this research has continued at Callaghan Innovation. Evan Hirst’s work, relying on my analyses to measure and image retinal blood flow has produced good results, with a possible commercial instrument now proposed. Images from that work are shown in the final figure of this thesis as an illustration of the continuity of research, and to show that the multiple exposure speckle contrast method, with separation of static contrast and flow rates, has a bright future in medical applications.

![Figure 8.2 Perfusion index (left) and static contrast $K_0$ (right) images of a retina](image)

The perfusion index image shows the flow in vessels radiating from the optic disc, near the centre of the picture. These flow speeds, while not absolute, are quantifiable. The $K_0$ image shows that there is less static scattering material over the vessels and also indicates small regions of high static scattering, appearing as small white dots in the image.
Appendices

1. Simulation: speckle contrast and laser Doppler comparison

% This code generates a speckle cube by Kirkpatrick and Duncan's method, but
% using simple addition of a random phase to the randomly distributed initial
% scatterers rather than using a copula to generate specific inter-frame decorrelation.
% Then we calculate autocorrelation and power spectral density by both Doppler and
% speckle contrast / multiple exposure methods, for comparison.

% set up values etc. and generate a speckle cube
clear all;
M=100;%dimension of square speckle frames
fact=2;%size of speckles in pixels
Nframes=2^10+1;%number of frames. Less wasteful as a a ^2 number +1
stepfactor=0.06;
timestep=1e-3;%time step for calculating t and f axes
N = M^2;
t1=rand(N,1);
tn=t1;
specklecube=zeros(M,M,Nframes);
Kstore=zeros(Nframes,1);
for frame=1:Nframes
% first make speckle with this t
specklecube(:,:,frame)=speckle_gen_from_t(M,fact,tn);
% then change it
tn=tn+randn(N,1)*stepfactor;
% could do subsets with different factor to create variety of spectra
end

%% Calculate PSD
% Calculate PSD of speckle, Doppler style. Use all points to find an average PSD,
% for noise reduction.
row=1;
PSDpointmat=zeros(N,2*Nframes);
for x=1:M
    for y=1:M
        specklepoint=specklecube(x,y,:);
        PSDpointmat(row,:)=abs(fft([specklepoint(:)' mean(specklepoint).*ones(size(specklepoint(:)))'])).^2;
        row=row+1;
    end
end
PSDpoint=mean(PSDpointmat)./(2*Nframes);
% calculate f axis
Fstep=1./(2*Nframes*timestep);%factor of 2 because of padding
Faxis=(-Nframes:Nframes-1)*Fstep;
%% plot PSD 1
figure(1);semilogy(Faxis,fftshift(PSDpoint));
title('PSD, Doppler style');
xlabel('F (Hz)');ylabel('PSD (Hz^{-1})');

%% Calculate PSD - fibre
% Calculate PSD of speckle, Doppler style. Use all points to find an average PSD,
% for noise reduction. This version uses bigger speckle areas to find the PSD
% check whether this affects the spectrum shape or only the ac/dc ratio.

row=1;
meansize=4;%Size of fibre region in pixels
clear PSDpointmat;
for x=1:meansize:M-meansize
    for y=1:meansize:M-meansize
        specklepoint=mean(mean(specklecube(x:x+meansize-1,y:y+meansize-1,:),1),2);
        PSDpointmat(row,:)=abs(fft([specklepoint(:)' mean(specklepoint).*ones(size(specklepoint(:))))']).^2;
        row=row+1;
    end
end
PSDfibre=mean(PSDpointmat)./(2*Nframes);

%% plot image and fibre style PSD
figure(8);semilogy(Faxis,fftshift(PSDpoint),Faxis,fftshift(PSDfibre),'-');
xlabel('F (Hz)');ylabel('PSD (Hz^{-1})');
axis([-500 500 1e-7 1e2]);
legend('Mean single speckle PSD','Mean 4x4 pixel regions PSD','Location','South');

%% calculate autocorrelations
% Autocorrelation of speckle by xcorr. Use all points to find an average Ct,
% for noise reduction.
row=1;
% clear xcorrpointmat;
xcorrpointmat=zeros(N,2*Nframes-1);
for x=1:M
    for y=1:M
        specklepoint=specklecube(x,y,:);
        xcorrpointmat(row,:)=xcorr(specklepoint(:)-mean(specklepoint(:)),'coeff');
        row=row+1;
    end
end
xcorrpoint=mean(xcorrpointmat);

figure(2);plot(xcorrpoint);
title('Ct, xcorr');

%autocorrelation Ct is fft(PSD)
tempPSD=PSDpoint;
tempPSD(1)=0;%remove DC term

% plot Doppler Ct
Ctdoppler=fftshift(real(fft(tempPSD)));
figure(3);plot(Ctdoppler);
title('Ct, Doppler');

%% K/T plots
% K/T plot, as we measure them. Points at powers of 2 version
maxexp=2^floor(log2(Nframes));
maxindex=ceil(log2(Nframes));
Exp1=zeros(1,maxindex);
K1=zeros(1,maxindex);
for expindex=1:maxindex
  exposure=2^(expindex-1);
  Exp1(expindex)=exposure;
  % generate integrated frames and calculate their K
  count=1;
  tempK=zeros(maxexp/exposure,1);
  % clear tempK;
  for nn=1:exposure:maxexp;
    intframe=sum(specklecube(:,:,nn:nn+exposure-1),3);
    tempK(count)=std(intframe(:))/mean(intframe(:));
    count=count+1;
  end
  K1(expindex)=mean(tempK);
end
% display K/T plot
figure(4);semilogx(Exp1*timestep,K1,.-'-');
title('Contrast vs Exposure plot');
xlabel('Exposure (s)');ylabel('Contrast K');
axis([timestep Nframes*timestep 0 max(K1)]);

%% Autocorrelation from K/T data
% Put a spline through K/T points. Might be a better alternative to fit a
% smooth function.
Exp3=1:maxexp;
Kspline=exp(spline(Exp1,log(K1),Exp3));
% Calculate autocorrelation
Ct_KT=diff(diff((Kspline.*Exp3*timestep/2).^2))./timestep^2;
% normalise
Ct_KTn=Ct_KT./max(Ct_KT);
doubleCt_KT=[fliplr(Ct_KTn(2:end)) Ct_KTn];

%% do the plots
figure(5);semilogx(Exp1*timestep,K1,.-' ',Exp3*timestep,Kspline);
title('Speckle Contrast vs Exposure');
xlabel('Exposure (s)');ylabel('Contrast K');
axis([timestep Nframes*timestep 0 max([K1 1])]);
legend('Calculated points','Interpolating spline');

figure(6);semilogx(timestep*(1:Nframes:Nframes-1),xcorrpont,.-' ',timestep*(4-Nframes:Nframes-4),doubleCt_KT);
title('Ct by KT curve');
xlabel('Lag (s)');ylabel('Autocorrelation');
legend('Ct from MATLAB xcorr','Ct from contrast curve','Location','South');
axis([0 20 -0.4 1]);

%% calculate PSD from autocorrelation, W-K relation
Faxis2=(4-Nframes:Nframes-4)*Fstep;
PSD_KT=fftshift(abs(fft(doubleCt_KT)));
figure(7);semilogy(Faxis2,PSD_KT,Faxis,fftshift(PSDpoint).*2.*M);
axis([0 400 0 30]);
	title('PSD by KT curve and Doppler');
legend('Speckle','Doppler');
xlabel('F (Hz)');ylabel('PSD (Hz^{-1})');

2. Sample experiment file

This is the title speckleviewer will display:
title=Simple multi-exposure experiment

This is the camera format index. 0 gives 1280 by 960 for the Sony XCD-SX910.
Other values will apply to different cameras
ChosenFormat=0

The framerate can be set to 15, 7.5, 3.75 or 1.875 Hz, depending on the format
FrameRate=7.5

hsize and vsize don't do much in format 0 but give the (windowed) size in format 8.
hsize=320
vsize=60

blocksize is the speckle calculation region.
blocksize=30

nframes is the number of contrast frames buffered. Usually 1 in this implementation, but more available for other tricks in future.
nframes=1

nrecords is the number of records of mean contrast etc.
nrecords=16

These are camera settings that will be used when the experiment is first run, in dB and ms
gain=18
shutter=1.6
brightness=0

This value may be either true or false and tells us whether this is a multi-exposure experiment.
multiexp=true

We might use automatically adjustable gain
Autogain=false

If it is a multi-exposure experiment, we need to define the changing parameters.
We do this as comma-separated series. These are in dB and ms.
gainseries=18,18,16,11,5,0,0,0
expseries=0.8,1.6,3.2,6.4,12.8,25.6,51.2,102.4

We can repeat the series a number of times:
repeats=2

This option will automatically log processed data – the mean K, working parameters etc. and the message log, on running a multi-exposure series.
autosave=true

whereas this one automatically saves the raw image data when running a series, as well as the mean K, working parameters and message log etc. Takes a little longer but leaves all the info for later analysis.
autosaveraw=true
References


