LASER SPECKLE IMAGING: SPATIO-TEMPORAL IMAGE ENHANCEMENT

Ph.D. Thesis

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ABSTRACT

It is well known now that there exists a coupling between functional brain activity and regional blood flow response in the somatosensory cortex and other cortical areas. Various modalities, including functional magnetic resonance imaging and optical imaging (intrinsic signals as well as fluorescence), have been developed in the past to map functional brain activity. The complexity and fundamental physical constraints of the instruments preclude functional imaging in awake, behaving small animals. This thesis presents the method of Laser Speckle Imaging (LSI) of brain with high spatial and temporal resolution, and potential for imaging awake and behaving animals. The method has the potential to map brain activation with high sensitivity and spatiotemporal resolution without using any exogenous contrast agents.

In LSI, scattered laser light with different paths produces a random interference pattern known as speckle, fluctuations of which contain information about the motion of particles in the underlying medium. A post-processing step is needed to extract information out of the speckle images, two of which we introduce in details.

Our first method is based on Laser speckle contrast analysis (LASCA), which has been demonstrated as a full-field method for imaging the cerebral blood flow (CBF). However, conventional LASCA is limited to extremely low dynamic range because of the ambient background field, dark current and anomalies in the circuits of CCD camera, which makes it difficult to analyze the spatiotemporal variabilities in CBF. In this study, we propose an enhanced laser speckle contrast analysis (eLASCA) method to improve the dynamic range of LASCA based on monotonic point transformation (MPT). In addition, eLASCA greatly improves the CBF visualization, which is very helpful in demonstrating the details of CBF change.

Our second method involves the second order features (SOFs) of the image; they are derived from the cooccurrence matrix that in turn was calculated over the same spatial and
temporal window than for the contrast. The image quality metrics - equivalent number of looks, entropy and objective quality – showed superior performance of the SOFs comparing to the contrast analysis.
ACKNOWLEDGEMENTS

No big task can be undertaken, let alone fulfilled to completion, without the support of colleagues, friends and family. There are people and organizations that I would like to explicitly thank here.

I want to thank first my supervisor, Professor Anastasios Bezerianos for his optimism and administrative support.

I had many colleagues and friends in the laboratory for biosignal processing whom I want to thank for our enriching discussions. In particular Spiros Kostopoulos whose collaboration resulted in most of the source code written for the Chapter 5 and a joint publication.

The initial speckle data were handled by Nitish V.Thakor at the John Hopkins University, Baltimore, MD. One of his PhD students, Shanbao Tong, became professor at Shanghai Jiao Tong University, Shanghai, China. In turn, I collaborated with Peng Miao, a PhD student of Professor Tong. I want to thank Peng for our conversations over IM, and regular e-mail exchanges, which resulted in the Chapter 6 and a joint publication.

I want to thank Maria Stavrinou whom I met in our lab, for her constant support and encouragements.

This work was funded by the State Scholarship Foundation of Greece (IKY); and by the Research grant of the University of Patras, Karatheodoris (2004-B411): “Functional Laser Speckle Imaging (fLSI)”. It also received support, in form of conferences and travel awards, by NeuroMath, a European COST Action (BM0601) "Advanced Methods for the Estimation of Brain Activity and Connectivity".
### ACRONYMS

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>c.d.f.</td>
<td>Cumulative density function</td>
</tr>
<tr>
<td>DESP</td>
<td>Double exposure speckle photography</td>
</tr>
<tr>
<td>eLASCA</td>
<td>Enhanced laser speckle contrast analysis</td>
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<td>LASCA</td>
<td>Laser speckle contrast analysis</td>
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<td>LDF</td>
<td>Laser Doppler Flowmetry</td>
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<td>LDPI</td>
<td>Doppler perfusion imaging</td>
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<td>LDPM</td>
<td>Doppler perfusion monitoring</td>
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<td>LSFG</td>
<td>Laser speckle flowgraphy</td>
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<td>LSI</td>
<td>Laser speckle imaging</td>
</tr>
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<td>LSPI</td>
<td>Laser speckle perfusion imaging</td>
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<tr>
<td>LSTCA</td>
<td>Laser speckle temporal contrast analysis</td>
</tr>
<tr>
<td>MESI</td>
<td>Multi-exposure speckle imaging</td>
</tr>
<tr>
<td>p.d.f.</td>
<td>Probability density function</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SDCav</td>
<td>Spatial derived contrast with averaging</td>
</tr>
<tr>
<td>SESP</td>
<td>Single exposure speckle photography</td>
</tr>
<tr>
<td>sLASCA</td>
<td>Spatial laser speckle contrast analysis</td>
</tr>
<tr>
<td>SNR</td>
<td>Signal-to-Noise Ratio</td>
</tr>
<tr>
<td>SOF</td>
<td>Second-order features</td>
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<td>tLASCA</td>
<td>Temporal laser speckle contrast analysis</td>
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PART I

GENERAL PART
Chapter 1. INTRODUCTION

Objectives of the study

Laser Speckle Imaging (LSI) is an imaging technique which relies on a particular property of the laser. When a coherent light such as a laser illuminates a rough surface, it creates a pattern called speckle. The speckle phenomenon is present with any interfering wave front in fact, such as ultrasounds, and pollutes the signal. In LSI however this so-called “undesirable noise” reveals a “desirable” property: it carries information about the movement of the scatterers. In Cerebral Blood Flow (SBF) imaging, LSI reveals the velocities of the red blood cells within the capillaries of the illuminated tissue. This information does not come directly however, and requires several steps of signal and image processing, which in turn degrade the quality of the velocity map obtained.

The objective of this study is to improve the spatial resolution and/or, the temporal resolution of Laser Speckle Imaging. The research will look to either improve existing processing methods, either to develop new ones.

Original contributions

During the course of this PhD, and related to this thesis, Hugues Fontenelle has published the following papers:

- Hugues Fontenelle, Spiros Kostopoulos, Peng Miao, Shanbao Tong and Anastasios Bezerianos
  Second Order Features for Laser Speckle Imaging
• Peng Miao, Minheng Li, Hugues Fontenelle, Anastasios Bezerianos, Yihong Qiu and Shanbao Tong
  Imaging the Cerebral Blood Flow with Enhanced Laser Speckle Contrast Analysis (eLASCA) By Monotonic Point Transformation
  IEEE Transactions on Biomedical Engineering, 56(4):1127-33, 2009

  And the following poster:

• Hugues Fontenelle, Nitish V. Thakor, Anastasios Bezerianos
  A numerical model for Laser Speckle Imaging
  Neuroscience SfN 35th Annual Meeting, Washington, DC, November 12–16, 2005

  Outside Laser Speckle Imaging, but related to brain activity estimation, HF has contributed to:

• Katrina Wendel, Outi Väisänen, Jaakko Malmivuo, Nevzat G. Gencer, Bart Vanrumste, Piotr Durka, Ratko Magjarević, Selma Supek, Mihail Lucian Pascu, Hugues Fontenelle and Rolando Grave de Peralta Menendez
  EEG-MEG Source Imaging: Methods, Challenges, and Open Issues
  Computational Intelligence and Neuroscience, 2009

• Hugues Fontenelle and Anastasios Bezerianos
  Dynamic EEG source localization by Particle Swarm Optimization
  Frontiers in Human Neuroscience. 10th International Conference on Cognitive Neuroscience, Bodrum, Turkey, September 1–5, 2008

**Organization of the thesis**

The thesis is divided in two parts. Part I, immediately thereafter, introduce the background necessary to understand our contribution. Part II, starting on page 53, present the specific work done during the course of this PhD.

Chapter 2 is a brief reminder of diverse functional imaging modalities, with an emphasis on small animal research. The last modality introduced, Laser Doppler Imaging, is
particularly close to Laser Speckle Imaging, which is introduced in Chapter 3. The chapter discusses the physics of speckle; develops the maths behind the speckle contrast (which is the older and leading method in processing LSI) and connects it to velocity; compares scanning vs full-field technique; lists some applications but mainly the one this study is interested in: physiological studies in the rat’s brain. The last part of that chapter explains how to obtain speckles in silico, i.e. computer methods to simulate speckles. Following is a summary of existing methods for processing LSI in Chapter 4.

We present our contributions in Chapter 5 with High-Order Statistics of Speckle images and Chapter 6 with eLASCA. In Chapter 7 we discuss some issue of LSI that have arisen, then Chapter 8 concludes the present work. It is followed by a bibliography.
Chapter 2. **FUNCTIONAL IMAGING IN SMALL ANIMALS**

Anatomical imaging represents the sum of imaging techniques that allow inferring, non-invasively, the underlying anatomy of the body. Common modalities include: X-rays, Computed Tomography (CT), Magnetic Resonance Imaging (MRI), Ultra-Sounds (US)... As in anatomical imaging, the images provided do not convey any information as to the metabolic or electrical processes, or functions, of the organ being investigated. Functional imaging provides just that, and some techniques for imaging brain activity are summarized in this chapter.

It is to be noted that each technique is presented only briefly, just enough to put the next chapters into context. The references offered are usually reviews themselves.

**Correlates of Neural Activity**

It has been known for over 100 years that blood flow to the brain increases in a regionally specific manner according to mental activity. The father of modern psychology, William James, was aware of observations relating regional brain pulsation to mental activity. Paul Broca, known primarily for his observations of the effects of left frontal lesions on language and which now bears the eponym, Broca’s area, performed several experiments relating regional brain temperature to cognitive function. But it was not until the 1950’s when Seymour Kety and Louis Sokoloff developed the autoradiographic technique for quantitatively measuring regional blood flow that specific cognitive functions could be directly mapped in the living brain.

*Blood flow increases in areas where neuronal activity increases.*

Most cognitive neuroscience studies implicitly assume the verity of this relationship. The relationship between neural activity and the hemodynamic response is far more complicated than a simple direct relationship. We lack fundamental data regarding the nature of this relationship, and so there is no agreed upon physiological model.
Blood flow increases in both a spatially and temporally correlated fashion with synaptic activity. It is not clear, however, if blood flow is coupled to oxidative metabolism. This is surprising since the brain consumes 20% of the oxygen inhaled. Local oxygen consumption does not increase to the same extent as local glucose utilization, suggesting that neuronal activity may be an anaerobic process. One theory states that neuronal activation results in a transient increase in the extracellular pH, which stimulates glycolysis in the surrounding glial cells. This leads to the production of both pyruvate and lactate, with the lactate becoming available to diffuse back to the neuron, where it can be oxidatively metabolised. This model postulates that the glial cells serve as an energy reserve for the synapses, and oxidative metabolism occurs well after the electrical activity, replenishing the neuronal energy supplies. Optical imaging suggests that blood flow is, in fact, coupled to oxidative metabolism. Results suggest that a transient local decrease in oxygen concentration occurs in response to synaptic metabolic requirements (oxidative metabolism). The increase in blood flow occurs after a delay of 1–3 seconds, but overreacts, leading to the observation that the blood flow increase outpaces oxygen consumption. Unfortunately, there is little direct evidence for this reactive hyperemia, and it does not explain why glucose metabolism appears more tightly coupled to blood flow than oxygen consumption.

**functional Magnetic Resonance Imaging (fMRI)**

Increases in neuronal activity, specifically synaptic activity, result in a local increase in blood flow. Because oxygen consumption does not increase to the same extent, the increase in blood flow results in a lower concentration of deoxyhemoglobin. This relationship forms the basis of fMRI (see [14] for a review of Nuclear Magnetic Resonance spectroscopy and its imaging application, but put into the context of rodents).

When nerve cells are active they reduce their consumption of oxygen, switching to less energetically effective, but more rapid anaerobic glycolysis. The local response to this oxygen utilization is an increase in blood flow to regions of increased neural activity, occurring after a delay of approximately 1–5 seconds. This hemodynamic response rises to a peak over 4–5 seconds, before falling back to baseline (and typically undershooting slightly). This leads to local changes in the relative concentration of oxyhemoglobin and deoxyhemoglobin and changes in local cerebral blood volume in addition to this change in local cerebral blood flow.
Blood-oxygen-level dependent or BOLD is the MRI contrast of blood deoxyhemoglobin [62]. Neurons do not have internal reserves of energy in the form of glucose and oxygen, so their firing requires more energy to be delivered quickly. Through a process called the hemodynamic response, blood releases oxygen to them at a greater rate than to inactive neurons. Hemoglobin is diamagnetic when oxygenated but paramagnetic when deoxygenated. The magnetic resonance (MR) signal of blood is therefore slightly different depending on the level of oxygenation. Higher BOLD signal intensities arise from increases in the concentration of oxygenated hemoglobin since the blood magnetic susceptibility now more closely matches the tissue magnetic susceptibility. By collecting data in an MRI scanner with pulse parameters sensitive to changes in magnetic susceptibility one can assess changes in BOLD contrast. These changes can be either positive or negative depending upon the relative changes in both cerebral blood flow (CBF) and oxygen consumption. Increases in CBF that outstrip changes in oxygen consumption will lead to increased BOLD signal, conversely decreases in CBF that outstrip changes in oxygen consumption will cause decreased BOLD signal intensity. The signal difference is very small, but given many repetitions of a thought, action or experience, statistical methods can be used to determine the areas of the brain which reliably show more of this difference as a result, and therefore which areas of the brain are active during that thought, action or experience.

**Positron Emission Tomography (PET)**

Positron emission tomography (PET) is a nuclear medicine imaging technique which produces a three-dimensional image or picture of functional processes in the body (see [20] for a review of PET, put into the context for rodents). The system detects pairs of gamma rays emitted indirectly by a positron-emitting radionuclide (tracer), which is introduced into the body on a biologically active molecule. Images of tracer concentration in 3-dimensional space within the body are then reconstructed by computer analysis.

Most PET neuroimaging studies can be grouped into one of three categories: metabolic, blood-flow, or receptor.

Metabolic studies use $^{18}$FDG to measure regional glucose metabolism. $^{18}$FDG, like all $^{18}$F -compounds, has the advantage of a relatively long half-life (110 mins). This allows for the
synthesis to be performed in one location, the subject injection in another, and the scanning in
yet another. In fact, one can have a subject doing a particular task in a location remote from the
PET scanner and inject \(^{18}\text{FDG}\), which will be trapped in brain regions according to the local
metabolic rate. This has an obvious advantage in situations in which placing the subject in the
scanner would alter the conditions of the task. For example, \(^{18}\text{FDG}\) is used commonly in sleep-
studies. The main disadvantage is that the long half-life results in effectively no temporal
resolution. This offers a time-averaged snapshot of a particular brain state, and the state is
averaged over 20-60 mins.

Blood-flow studies use \(\text{H}_2\text{O}^{15}\) to measure changes in local brain blood flow. As noted
before, blood flow is an indirect measure of local synaptic activity. Because \(\text{H}_2\text{O}^{15}\) has a short
half-life (2 mins.), several administrations can be performed in one session. A typical \(\text{H}_2\text{O}^{15}\)
study would have 8-16 injections and scans for each subject. The experimental design would
manipulate what the subject does during each scan. Each scan lasts about 1 min., with 8-10 mins
between scans (5 half-lives). \(\text{H}_2\text{O}^{15}\) studies not only allow for multiple conditions to be studied,
but they also allow for the repetition of conditions, increasing statistical power. The main
disadvantage is that because of the short half-life, the \(\text{H}_2\text{O}^{15}\) must be produced reliably and in
close proximity to the scanner.

Receptor studies use radioligands-chemicals incorporating a positron emitting isotope
into a molecule whose pharmacokinetics are already known. Ideally, these ligands bind
specifically to one receptor type. Most of these studies are of the mapping type, which shows the
distribution of a particular receptor in the brain (e.g. D2 dopamine receptor). Here, the measured
radioactivity reflects both the local concentration of receptors (B\(_{\text{max}}\)) and the affinity of the
ligand for the receptor (measured by K\(_D\), the equilibrium dissociation constant). If the ligand acts
as a competitive antagonist, then the apparent affinity is also affected by the concentration of the
endogenous neurotransmitter. The analysis can be simplified by considering the ratio B\(_{\text{max}}/ K_D\)
termed the binding potential (BP). Ligands undergo both specific and nonspecific binding.
Typically, one is interested only in the specific binding, that is, to the receptor of interest. By
using a reference tissue, which is known to have a low receptor concentration, then the
nonspecific binding can be subtracted out, e.g. cerebellum has no D2 receptors. In this case, the
difference in distribution volume for the two tissues is directly proportional to the binding
potential. These molecules require a more involved synthesis than either water or $^{18}$FDG, and this is compounded by the problems of radiation exposure to chemists and a race against the clock as the isotope decays. The end product must meet several requirements: high specific activity (the amount of radioactivity/mole), high radiochemical purity, high chemical purity, and sterility. $^{18}$F-ligands are easier to synthesize because of the long half-life, but $^{11}$C-ligands (20 min. half-life) have a higher potential for biological relevance.

**Ultrasound Imaging**

Ultrasound imaging utilizes the interaction of sound waves with living tissue to produce an image of the tissue or, in Doppler-based modes, determine the velocity of a moving tissue, primarily blood (see [23] for a review). These dynamic, real-time images can be analyzed to obtain quantitative structural and functional information from the target organ. This versatile, noninvasive diagnostic tool is widely used and accepted in human and veterinary medicine. Until recently, its application as a research tool was limited primarily to larger, nonrodent species. Due to advances in ultrasound imaging technology, commercially available ultrasound systems now have the spatial and temporal resolution to obtain accurate images of rat and mouse hearts, kidneys, and other target tissues, including tumor masses. As a result, ultrasound imaging is being used more frequently as a research tool to image rats and mice, and particularly to evaluate cardiac structure and function. The developing technology of ultrasound biomicroscopy has even greater spatial resolution and has been used to evaluate developing mouse embryos and guide site-specific injections into mouse embryos. Additional ultrasound imaging technologies, including contrast-enhanced imaging and intravascular ultrasound transducers adapted for transesophageal use, have been utilized in rats and mice. This paper provides an overview of basic ultrasound principles, equipment, and research applications. The use of noninvasive ultrasound imaging in research represents both a significant refinement as a potential replacement for more invasive techniques and a significant advancement in research techniques to study rats and mice.
**Electroencephalography (EEG) and Magnetoencephalography (MEG)**

Magnetoecephalography (MEG) and electroencephalography (EEG) represent two noninvasive functional brain imaging methods, whose extracranial recordings measure extremely weak magnetic fields and electric potential differences, respectively. These recordings offer direct, real time, monitoring of spontaneous and evoked brain activity and allow for spatiotemporal localization of underlying neuronal generators. MEG and EEG share the following characteristics: 1) they have a millisecond temporal resolution; 2) magnetic fields and potential differences are nonlinear functions of source locations and linear functions of source strengths; 3) they are caused by the same neurophysiological events, i.e. currents from synchronously activated neuronal tissue often referred to as the primary source current density $J_p$ and both can be used equivalently for the localization of neuronal generators; 4) the measured external magnetic field and scalp potentials are generated by some completely unknown, current density $J$ inside the brain, which is a sum of the primary current of interest $J_p$ and $J_r$, which represents the extracellular, volume, or return currents that flow in accordance with Ohm’s law.

EEG and MEG do not measure correlates of neuronal activity, but rather the electrical activity itself (or the magnetic field resulting by this activity). However, in order to obtain a 3D image of mental activity, one must reconstruct from the scalp potentials. While tomographic techniques (e.g. CT, PET, MRI, etc.) are associated with well-posed mathematical problems, the non-invasive estimation of the brain activity is essentially an ill-posed problem due to the infinite number of solutions.

**Computed Tomography (CT)**

Computed tomography (CT) is a medical imaging method employing tomography. Digital geometry processing is used to generate a three-dimensional image of the inside of an object from a large series of two-dimensional X-ray images taken around a single axis of rotation.
SPECT

Single photon emission computed tomography (SPECT) is a nuclear medicine tomographic imaging technique using gamma rays. SPECT imaging is performed by using a gamma camera to acquire multiple 2-D images (also called projections), from multiple angles. A computer is then used to apply a tomographic reconstruction algorithm to the multiple projections, yielding a 3-D dataset. Like in PET, the injection of a contrast agent permits the imaging of specific biomolecules in the living brain. See Frankle et al. [34] for a review of neuroreceptor imaging using PET and SPECT.

Optical Imaging

Optical imaging is a collective term unifying all imaging techniques using the visible, ultraviolet, and infrared light, to determine the structure, texture, anatomic and chemical properties of the illuminated material.

Event-related optical signal (EROS)

The Event-Related Optical Signal (EROS) [40] is a technique is based on measures of the optical properties of cortical brain tissue, which change while the tissue is active. These changes are likely to be due to changes in light scattering, and are very rapid and localized, being related to phenomena occurring within or around the neuronal membrane. EROS, therefore yields images of cortical activity that combine spatial specificity (i.e. they can be related to patches of tissue less than a cubic centimeter in size) with temporal resolution (i.e. they depict the time course of the neural activity in the cortical areas under measurement). A limitation of this technique is its reduced penetration into the head (less than 3-5 cm). EROS appears to be a suitable technique for studying the time course of activity in selected cortical areas, and for providing a bridge between hemodynamic and electrophysiological imaging methods.

Near infrared spectroscopy (NIRS)

Near infrared spectroscopy (NIRS) is a spectroscopic method which uses the near infrared region of the electromagnetic spectrum (from about 800 nm to 2500 nm).
NIRS can be used for non-invasive assessment of brain function through the intact skull in human subjects by detecting changes in blood haemoglobin concentrations associated with neural activity, e.g. in branches of Cognitive psychology as a partial replacement for fMRI techniques. NIRS can be used on infants, where fMRI cannot (at least in the United States), and NIRS is much more portable than fMRI machines, even wireless instrumentation is available, which enables investigations in freely moving subjects. However, NIRS cannot fully replace fMRI because it can only be used to scan cortical tissue, where fMRI can be used to measure activation throughout the brain.

By employing several wavelengths and time resolved (frequency or time domain) and/or spatially resolved methods blood flow, volume and oxygenation can be quantified [92]. These measurements are a form of oximetry. Applications of oximetry by NIRS methods include the detection of illnesses which affect the blood circulation (e.g. peripheral vascular disease), the detection and assessment of breast tumors, and the optimization of training in sports medicine.

The use of NIRS in conjunction with a bolus injection of indocyanine green (ICG) has been used to measure cerebral blood flow [13] and cerebral metabolic rate of oxygen consumption [88] in neonatal models.

**Optical Coherence tomography (OCT)**

OCT is based on low coherence interferometry. In conventional interferometry with long coherence length (laser interferometry), interference of light occurs over a distance of meters. In OCT, this interference is shortened to a distance of micrometers, thanks to the use of broadband light sources (sources that can emit light over a broad range of frequencies). Light with broad bandwidths can be generated by using superluminescent diodes (superbright LEDs) or lasers with extremely short pulses (femtosecond lasers). White light is also a broadband source with lower powers.

OCT employs near-infrared light and is able to penetrate significantly deeper into the scattering medium, for example ~3× deeper than its nearest competitor, Confocal microscopy. See for example Maheswari et al. employing OCT for functionally imaging the brain surface [64].
Laser Doppler Flowmetry

Laser Doppler flowmetry (LDF) is a method whose most-known application is for the assessment of microvascular blood perfusion. “Doppler” refers to the frequency shift that arises in light that has been scattered by moving red blood cells. This fundamental principle constitutes the basics of LDF theory. In other words, by illuminating a tissue sample with single-frequency light and processing the frequency distribution of the backscattered light an estimate of the blood perfusion can be achieved.

The first experimental studies using LDF were performed in the 1970’s [80,84]. Various instrumentation setups have been used through the years and the original method has been developed and improved (see review [44]). In the early 1980’s the fibre-optic based laser Doppler perfusion monitoring (LDPM) technique was introduced. LDPM allows for temporal perfusion measurements in a small sampling volume. In order to enable spatial measurements, the laser Doppler perfusion imaging (LDPI) technique was developed. The surface of the tissue is scanned by a freely impinging laser beam, thus generating an image of the spatial variability of blood perfusion. An alternative method, utilizing a CMOS array, has been proposed by Serov et al, in order to speed up the imaging procedure [83]. Today both LDPM and LDPI instruments are commercially available.

Figure 1 Left: LDPM measurements on the heart muscle during surgery. Right: LDPI image of a hand. (Image reproduced from [35])
Currently, LDF does not give an absolute measure of blood perfusion. In the clinical setting this is a limiting factor and the reason why LDF instruments are not routinely used in health care. However, LDF has found its use in research. Among the applications are pharmacological trials, allergy patch testing, wound healing, physiological assessments and skin disease research. The skin is probably the most studied organ but also internal organs such as kidneys, liver, muscles, intestines, brain and heart have been investigated in a number of studies.

**Light source**

The light used in LDF must be monochromatic, i.e., consist of a single frequency. The frequency shifts caused by Doppler scattering will then result in a frequency broadening of the originally monochromatic light. It can be shown that the backscattered light forms an interference pattern on the photodetector and that the fluctuations in this pattern carry information about the Doppler shifts. In order for interference to occur, the coherence length of the laser must be much longer than the difference in path length for light waves that were emitted at the same moment. The coherence length is the interval between phase jumps in the laser light, see Figure 2. Wavelengths commonly used in LDF are 633 nm (red) and 780 nm (near-infrared). Output power is usually about 1 mW.

![Figure 2](image.png)

*Figure 2* The interval length between two phase jumps is the coherence length of the laser. (Note that this picture only illustrates the principle. The coherence length usually contains far more than 4-5 periods.) (Image reproduced from [35])
**Doppler shift**

When light is scattered by a moving object it will be frequency shifted depending on the movement of the object, the direction of the incoming light and the direction of the scattered light. Let $k_i$ describe the propagation vector (rad/m) of an incoming photon hitting a scattering particle with velocity $v$ (m/s) and $k_s$ the propagation vector of the photon after being scattered, Figure 3. The angular frequency shift, $\beta_D$ (rad/s), will then be:

$$\beta_D = -v \cdot q = -v \cdot (k_i - k_s) = -\frac{4\pi}{\lambda} |v| \sin \frac{\theta}{2} \cos \varphi \cos \alpha$$

Here $\lambda$ denotes the wavelength (m) of the photon in the surrounding medium, $\theta$ the scattering angle between $k_i$ and $k_s$, $\alpha$ the angle between $v$ and the plane of scattering and $\varphi$ the angle between the projection of $v$ in the plane of scattering and $(k_i - k_s)$. The difference between $k_i$ and $k_s$ is often denoted the scattering vector $q$.

![Figure 3](image)

A photon with propagation vector $k_i$ is scattered by a red blood cell with velocity $v$ and gets a new propagation vector $k_s$ after scattering. The velocity component in the plane of scattering is denoted $v_\parallel$ and the difference between $k_i$ and $k_s$, the scattering vector, is denoted $q$. The angle between $v$ and the plane of scattering is denoted $\alpha$, the angle between $k_i$ and $k_s$ is denoted $\theta$, the angle between $k_i$ and $v_\parallel$ is denoted $\psi$ and the angle between $q$ and $v_\parallel$ is denoted $\varphi$. (Image reproduced from [35])

**Advantages and disadvantages**

**Advantages:**

- LDF makes possible noninvasive recording and imaging of tissue perfusion with minimal impact on microcirculation.
- LDF devices are easy to use.
- Continuous recordings over unlimited periods of time can be made with LDPM.
• Two-dimensional perfusion maps can be visualized by LDPI.
• The theoretical basis of LDF is well established.

Disadvantages:

• No absolute calibration is possible, and results obtained from different organs cannot be directly compared because of variations in photon path lengths due to the different optical properties of the tissue.
• Results obtained by recording at a single site using LDPM may not be representative for the entire tissue. (*)
• LDF does not distinguish between nutritive (capillary) perfusion and global tissue perfusion.
• LDPI assumes steady-state conditions in perfusion during the image-capturing period, which may amount to 4 min or longer. (*)

In the next chapter, we will introduce a similar technique, Laser Speckle Imaging, which solved the disadvantages of LDF marked by an asterisk (*), that is, LSI is a full-field technique and instantaneous.
Chapter 3. LASER SPECKLE IMAGING (LSI)

Physics of speckles

When the laser was invented in the early 1960s (see Maiman's landmark paper on the first experimental LASER [66]), its first users noticed, when the laser light fell on a matt surface such as paper or unpolished metal or glass, a high-contrast grainy pattern. This effect was initially called “granularity” by Rigden and Gordon [79], but soon the name speckle became more popular.

When an image is formed of a rough surface which is illuminated by a coherent light (e.g. a laser beam), a speckle pattern is observed in the image plane; this is called a “subjective speckle pattern”, or simply “image speckle”. It is called "subjective" because the detailed structure of the speckle pattern depends on the viewing system parameters; for instance, if the size of the lens aperture changes, the size of the speckles change. If the position of the imaging system is altered, the pattern will gradually change and will eventually be unrelated to the original speckle pattern.

When laser light which has been scattered off a rough surface falls on another surface, it forms an “objective speckle pattern”. If a photographic plate or another 2-D optical sensor is located within the scattered light field without a lens, a speckle pattern is obtained whose characteristics depend on the geometry of the system and the wavelength of the laser. Objective speckles are usually obtained in the far-field (also called Fraunhofer region) and called “far field speckles”. Speckles can be observed also close to the scattering object, in the near field (also called Fresnel region, that is, the region where Fresnel diffraction happens). This kind of speckles are called “near field Speckles”.
Rigorously, a speckle pattern is a random intensity pattern produced by the mutual interference of a set of wavefronts. In optics and physics, a wavefront is the locus (a line, or, in a wave propagating in 3 dimensions, a surface) of points having the same phase. Thus the phenomenon can be observed with different media such as radio waves, or coherent light as in lasers.
Speckle is often regarded as a nuisance, or noise, and therefore speckle removal techniques or algorithms have been developed for many applications. Dainty however, studied speckle for its own sake [49]; and soon began to appear direct applications of the phenomenon. In the first chapter of book edited by Dainty, Goodman describes and analyses the statistical properties of laser speckle patterns [39]. Of this work, the most important contribution is the introduction of the speckle contrast.

The speckle contrast

Assuming ideal conditions for producing a speckle pattern—single-frequency laser light and a perfectly diffusing surface with a Gaussian distribution of surface height fluctuations—it can be shown that the standard deviation of the intensity variations in the speckle pattern is equal to the mean intensity. In practice, speckle patterns often have a standard deviation that is less than the mean intensity, and this is observed as a reduction in the contrast of the speckle pattern. In fact, it is usual to define the speckle contrast as the ratio of the standard deviation to the mean intensity.

Figure 6 The physical origin of speckle patterns: diffuse reflection of coherent light from a rough surface
No matter which picture is assumed, it is easily realized that the light field at a specific point \( P(x,y,z) \) in a speckle pattern must be the sum of a large number \( N \) of components representing the contribution from all points on the scattering surface, Figure 6. Under illumination by monochromatic and fully polarized light, the contribution to the field at \( P \) produced by any surface element, \( j \), is given by

\[
U_j(P) = |u_j| e^{i\phi_j} = |u_j| e^{ikr_j}
\]

Eq. 1

\( r_j \) being the (random varying) distance from the \( j \)-th scattering surface element to the point \( P \). The complex amplitude of the scattered field at point \( P \) can therefore be written as

\[
U(P) = \frac{1}{\sqrt{N}} \sum_{j=1}^{N} u_j(P) = \frac{1}{\sqrt{N}} \sum_{j=1}^{N} |u_j| e^{i\phi_j} = \frac{1}{\sqrt{N}} \sum_{j=1}^{N} |u_j| e^{ikr_j}
\]

Eq. 2

The summation in Eq. 2 can be considered as a random walk in the complex plane due to the random phases \( \phi_j = kr_j \). This erratic motion of the field components is represented in Figure 9 (on page 40) for a few of them.

Assuming that (1) the amplitude \( u_j \) and phase \( \phi_j \) of each field component are statistically independent of each other and are also independent of the amplitude and phase of all other field components and (2) the phases \( \phi_j \) are uniformly distributed on the interval \((-\pi, \pi)\), which means that the surface is rough in comparison to the wavelength, and with the additional hypothesis that the number of total scattering centers \( N \) is very large, thus ensuring validity of the central limit theorem, Goodman [39] has demonstrated that the real and imaginary parts of the resultant field are asymptotically Gaussian. The joint probability density function for them is thus given by

\[
P_{(r,i)}(U^{(r)},U^{(i)}) = \frac{1}{2\pi\sigma^2} \exp \left[ \frac{(U^{(r)})^2 + (U^{(i)})^2}{2\sigma^2} \right]
\]

Eq. 3

known as a circular Gaussian, where

\[
\sigma^2 = \lim_{N \to \infty} \sum_{j=1}^{N} \frac{|u_j|^2}{2}
\]

Eq. 4
From Eq. 3, and taking into account that the intensity $I$ and phase $\phi$ of the resultant field are related to the real and imaginary parts of the field according to

$$U^{(r)} = \sqrt{I} \cos \phi$$
$$U^{(i)} = \sqrt{I} \sin \phi$$  \hspace{1cm} \text{Eq. 5}$$

it follows that the probability density of the intensity $p(I)$ and of the phase $P(\phi)$ are given by

$$p(I) = \frac{1}{\langle I \rangle} e^{-\frac{I}{\langle I \rangle}} \text{ for } I \geq 0$$  \hspace{1cm} \text{Eq. 6}$$

And

$$p(\phi) = \frac{1}{2\pi} \text{ for } -\pi \leq \phi \leq \pi$$  \hspace{1cm} \text{Eq. 7}$$

respectively. In Eq. 6, $\langle I \rangle$ stands for the mean value of the intensity in the speckle diagram. Thus, according to the last two equations, the intensity distribution follows a negative exponential law, whereas the phase is uniformly distributed in the interval $(-\pi, \pi)$.

The moments of intensity distribution are defined as

$$\langle I^n \rangle = n! (2\sigma^2)^n = n! \langle I \rangle^n$$

and of special interest are the second-order moment and the variance:

$$\langle I^2 \rangle = 2\langle I \rangle^2 \text{ and } \sigma_I^2 = \langle I^2 \rangle - \langle I \rangle^2 = \langle I \rangle^2$$  \hspace{1cm} \text{Eq. 8}$$

This equation shows that the standard deviation of a polarized speckle pattern equals the mean value of the intensity. A usual measure of the degree of modulation of a speckle pattern is called the contrast, defined as

$$K = \frac{\sigma_I}{\langle I \rangle}$$  \hspace{1cm} \text{Eq. 9}$$
This definition, together with the result in Eq. 8 means that the contrast of a polarized speckle pattern is always unity, and the speckle pattern is said to be fully developed. In practice the pattern is not fully developed and

\[ K = \frac{\sigma_l}{\langle l \rangle} \leq 1 \quad \text{Eq. 10} \]

**Scanning technique**

Laser Doppler Flowmetry (LDF) has been reviewed by Humeau et al. [44]. LDF is a non invasive method enabling the monitoring of microvascular blood flow, which relies on the Doppler effect. When coherent light is directed toward a tissue, photons are scattered by moving objects and by static structures. If they encounter moving particles, the Doppler effect appears. The photon frequency is therefore modified. When the reemitted light is directed toward a photodetector, optical mixing of light frequency shifted and non frequency shifted gives rise to a stochastic photocurrent, whose spectrum can be analyzed to deduce the blood cells velocities. The above described system produces a measurement of tissue perfusion at a single point.

In order to produce an image, Laser Doppler perfusion imaging (LDI) scans a two-dimensional area by sweeping the laser beam over that area. It takes a long time to scan and the resolution is low, compared to the full field technique of laser speckle imaging [32]. However the two techniques are comparable in the sense that they both rely on the same physical principles.

**Full field techniques**

Laser speckle imaging (LSI) achieves full-field imaging, without scanning, of the area of interest. A laser beam illuminates the area and a photograph is taken, in its simplest early form, then called single –exposure speckle photography [31]. Using a very short exposure time would ‘freeze’ the speckle and result in a high-contrast speckle pattern, whereas a long exposure time would allow the speckles to average out, leading to a low contrast. Conversely, using an exposure time that is of the same order as the correlation time of the intensity fluctuations, would lead to high contrast in static parts of the image, but low contrast -blurred- in the moving parts of
the image. Then the velocity distribution in the field of view can be mapped as variations in speckle contrast.

**Time-varying speckles**

When an object moves, the speckle pattern it produces changes. For small movements of a solid object, the speckles move with the object, i.e. they remain correlated; for larger motions, they decorrelate and the speckle pattern changes completely.

Even when the object itself stays still, but a large number of individual scatterers move, such as particles in a fluid, decorrelation occurs.

If we assume that the frequency spectrum of the fluctuations is dependent on the velocity of the motion, then it should be possible to obtain information about the motion of the scatterers from a study of the temporal statistics of the speckle.

**Medical Applications**

Capillary blood flow in rats or pigs [17,29,58,59,91] as well as in human beings [3,36,87] has been monitored using speckle activity phenomena. Dynamic speckle has shown to be a useful and reliable tool to measure blood flow inside tissues in spite of the contributions of the activity of the surrounding layers and vessels. One of the accounts [60] showed the ability to measure the cerebral blood flow (CBF) through a (partially translucent) intact rat skull using temporal speckle contrast, or with the thinned preparation of the rat skull [29] in order to evaluate cerebral hemoglobin concentration (HbT) and CBF. The Doppler effect is the major contributor to activity values registered in capillary blood flow using dynamic speckle, with the other possible changes in the scatterers being concealed by the regular flux. Therefore, the LASCA method, using just one image, takes advantage of a phenomena that is considered best to be monitored by dynamic speckle [95], despite the complexity of human tissues. Blood flow measurements were recorded in applications such as retina microcirculation [52], and cadaveric human aortas requiring atherosclerotic plaque evaluation [87]. Other reports of specific usage can be seen in a new instrument proposed using endoscopic and laser imaging to achieve internal
tissues, [95] in an application on a knee evaluation using endoscopic laser speckle imaging eLSPI [37]. The usage in skin damages evaluation, [7,85] or skin thermal modification, [98] can be pointed out as additional examples. One report on the use of speckle imaging proposed the simultaneous usage of photodynamic therapy in tissues to acquire information for evaluating dynamically the changes in blood flow without interrupting the therapeutic approach [54].

Besides medical applications, there are a number of agricultural involving the determination of the mechanical properties and assessing the stage of maturation of fruits, seeds and vegetables. Some tests are performed on fruits, seeds, biscuits, ice cream, and biological films which can determine water content, moisture, monitor parasite mobility and the presence of fungi, etc.

Laser speckle techniques have been used in a variety of optical metrology techniques, including displacement, distortion and strain measurement, surface roughness assessment and velocity measurement. Laser speckle imaging is used for paint drying assessment, corrosion, salt efflorescence, foam stability, etc.

**Setup for laser speckle imaging in rodents**

A basic setup for laser speckle imaging is shown in Figure 7.

**Instrumentation.** A laser diode illuminates the target, i.e. a small area under the thinned skull of a rat in cerebral blood blow studies. The laser light may be collimated by an optic fiber. A charge-coupled device (CCD) camera images the reflected light, with the help of a magnifying lens or a microscope. A frame grabber controls the camera and sends the pictures to a computer terminal, where the image will be post-processed by custom code.
Animal preparation. A rat is anaesthetized and kept at stable temperature using a homoeothermic blanket. In cerebral blood flow studies, its skull is thinned using a dental drill (craniotomy), and then placed in a stereotactic frame. The thinned area is filled with glycerine to reduce the glaring reflection; or washed with saline or disinfected. Depending on application, the rat whiskers are stimulated, or is being electrically stimulated (stimulator in Figure 7), etc.

Physiological study. There varied studies, and the area being imaged depends on the study, of course.

Analysis software. The code is always custom-made, and implements either LASCA in its native form, either a new variation of it that the authors promote, along with a physiological study. Emphasis can be either on the method, either on the physiological study.

In Table 1 on page 35, four representative papers illustrate the small variations in the instrumentation, the animal preparation and the analysis.

Notes for the Table 1:

(1) Article is inconsistent (the text and the figure provide two different values)
(2) The focus was a new method, eLASCA. The physiological study is presented in another paper.

(3) Most probably the software used was Matlab.

(4) The method is referred herein simply as LSI. The authors emphasized that it is in the time domain.
<table>
<thead>
<tr>
<th>Paper</th>
<th>Dunn et al. [28]</th>
<th>Miao et al. [67]</th>
<th>Cheng et al. [16]</th>
<th>Li et al. [57]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Affiliation</strong></td>
<td>Harvard Medical School, Massachusetts, USA</td>
<td>Shanghai Jiao Tong University, Shanghai, China</td>
<td>Huazhong University of Science and Technology, Wuhan, China</td>
<td>Johns Hopkins University School of Medicine, Baltimore, MD, USA</td>
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<tr>
<td><strong>Imaging area</strong></td>
<td>right sensorimotor cortex (brain)</td>
<td>right barrel cortex (brain)</td>
<td>mesentery (abdomen)</td>
<td>5x5 mm area, 3.5 mm lateral to and 3 mm posterior to the bregma (brain)</td>
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<tr>
<td><strong>Study</strong></td>
<td>focal cerebral ischemia and cortical spreading depression</td>
<td>influences of Hypothermia on Capillary CBF (2)</td>
<td>dynamic responses of the rat mesenteric microcirculation to an incremental dose of phentolamine</td>
<td>vasomotor and blood flow responses to electrical stimulation in rat peripheral trigeminal system</td>
</tr>
<tr>
<td><strong>Instrumentation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Laser</strong></td>
<td>laser diode (Sharp LTO25MD; Thorlabs, Newton, NJ, U.S.A.)</td>
<td>laser diode (KL5650, Forward Co., Ltd., Shanghai, China)</td>
<td>He–Ne laser</td>
<td>He-Ne laser (JDSU, Milpitas, CA)</td>
</tr>
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<td>wavelenght λ.</td>
<td>780 nm</td>
<td>635 nm</td>
<td>632.8 nm</td>
<td>632 nm</td>
</tr>
<tr>
<td>power</td>
<td>30 or 10 mW (1)</td>
<td>20 mW</td>
<td>3 mW</td>
<td>n/a</td>
</tr>
<tr>
<td><strong>Lens</strong></td>
<td>collimating lens (C240-TM; Thorlabs)</td>
<td>trinocular stereo zoom boom microscope (XYH-05, Shanghai Optical Instrument Factory, Shanghai, China)</td>
<td>zoom stereo microscope (SZ6045TR, Olympus, Japan)</td>
<td>macro-lens (Nikon Inc., Melville, NY)</td>
</tr>
<tr>
<td><strong>Camera</strong></td>
<td>CCD camera (Cohu 4910; Scion, San Diego, CA, U.S.A.)</td>
<td>monochrome 12-bit CCD camera (Pixelfly QE, Cooke, USA)</td>
<td>CCD camera (PIXELFLY, PCO Computer Optics, Germany)</td>
<td>12-bit cooled . CCD camera (PCO, Kelheim, Germany)</td>
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<td>resolution</td>
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<td>1024 x 1392</td>
<td>480 x 640</td>
<td>SVGA</td>
</tr>
<tr>
<td>exposure time</td>
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<td>5 ms</td>
<td>20 ms</td>
<td>n/a (&quot;optimal&quot;)</td>
</tr>
<tr>
<td>frame rate</td>
<td>30 fps</td>
<td>23 fps</td>
<td>40 fps</td>
<td>10 fps</td>
</tr>
<tr>
<td><strong>Animal preparation</strong></td>
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<td>male Sprague-Dawley rats</td>
<td>Male Wistar rats</td>
<td>female Wistar rats</td>
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<td>250-350 g</td>
<td>120 - 160 g</td>
<td>200–250 g</td>
</tr>
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<td>Anaesthetic</td>
<td>urethane (1.2 g/kg, intraperitoneally)</td>
<td>pentobarbital (80mg/kg, intraperitoneally)</td>
<td>2% α-chloralose and 10% urethan (0.9 ml/100 g, intraperitoneally)</td>
<td>sodium pentobarbitone (3 ml/kg, intraperitoneally)</td>
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<td>Body T°</td>
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<td>variable (hypothermia study)</td>
<td>38°C</td>
<td>37°C</td>
</tr>
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<td>MacLab (AD Instruments, Mountain View, CA, U.S.A.)</td>
<td>Matlab (The MathWork, Massachusetts, USA)</td>
<td>easy-control software (PCO Computer Optics, Germany)</td>
<td>n/a (3)</td>
</tr>
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<td>Method</td>
<td>LASCA</td>
<td>eLASCA</td>
<td>LSI</td>
<td>tLSI (4)</td>
</tr>
</tbody>
</table>

Table 1  Comparison of setup for selected papers
The speckle contrast is a function of the exposure time, $T$, of the camera and is related to the autocovariance of the intensity temporal fluctuations in a single speckle, $C_t^{(2)}(\tau)$, by

$$\sigma_s^2(T) = \frac{1}{T} \int_0^T C_t^{(2)}(\tau) \, d\tau$$

$C_t^{(2)}(\tau)$ is defined as follows:

$$C_t^{(2)}(\tau) = \langle (I(t) - \langle I \rangle_t)(I(t + \tau) - \langle I \rangle_t) \rangle_t$$

where $I(t)$ is the intensity at time $t$, $\tau$ is the "lag", $\langle \rangle_t$ is the time average.

The normalized autocorrelation function of a field can often be approximated by a negative exponential function (for the case of a Lorentzian spectrum, for example, it is exactly negative exponential):

$$g_t^{(1)}(\tau) = e^{-\frac{\tau}{\tau_c}}$$

Where $\tau_c$ is the correlation time. $g_t^{(1)}(\tau)$ is defined as follows:

$$g_t^{(1)}(\tau) \equiv \frac{\langle E^+(t)E^+(t + \tau) \rangle_t}{\langle I \rangle_t}$$

Where $E(t) = E^+(t) + E^-(t)$ is the field at time $t$ and $I(t) = E^+(t)E^-(t)$ is the intensity.

The Siegert relationship is valid for the speckle fluctuations (strictly true only for Gaussian statistics):

$$g_t^{(2)}(\tau) = 1 + \left| g_t^{(1)}(\tau) \right|^2$$

Eq. 11

$g_t^{(2)}(\tau)$ is the normalized second-order autocorrelation function, i.e., the autocorrelation of the intensity, and is defined as follows:
From the definition of the various functions we have, assuming stationarity:

\[ g_t^{(2)}(\tau) = 1 + c_t^{(2)}(\tau) \]  

Eq. 13

Where \( c_t^{(2)}(\tau) \) is the normalized autocovariance,

\[ c_t^{(2)}(\tau) = C_t^{(2)}(\tau)/\langle I_T \rangle^2 \]

Eq. 14

Combining equations x to x we get:

\[ c_t^{(2)}(\tau) = \langle I_T \rangle^2 \left| g_t^{(1)}(\tau) \right|^2 \]  

Eq. 15

Assuming our negative exponential approximation for the normalized autocorrelation function, we combine equations x and x to get:

\[ C_t^{(2)}(\tau) = \langle I_T \rangle^2 e^{\frac{2T}{\tau_c}} \]  

Eq. 16

Substituting this expression in equation x we obtain the following expression for the spatial variance in the time-averaged speckle pattern:

\[ \sigma_s^2(T) = \left( \langle I_T \rangle^2 \frac{\tau_c}{2T} \right) \left( 1 - e^{-\frac{2T}{\tau_c}} \right) \]  

Eq. 17

Assuming ergodicity, we can replace the time average by the ensemble average to obtain:

\[ k = \frac{\sigma}{\langle I \rangle} = \frac{\tau_c}{\sqrt{2T}} \left( 1 - e^{-\frac{2T}{\tau_c}} \right) \]  

Eq. 18

Eq. 18 gives us an expression for the speckle contrast in the time-averaged speckle pattern as a function of the exposure time \( T \) and the correlation time \( \tau_c = 1/(ak_0\nu) \) where \( \nu \) is the mean velocity of scatterers, \( k_0 \) is the light wavenumber, and \( a \) is a factor that depends on the Lorentzian width and scattering properties of the tissue. As in laser Doppler measurements, it is
theoretically possible to relate the correlation times, $\tau_c$, to the absolute velocities of the red blood cells, but this is difficult to do in practice, inasmuch the number of moving particles that light interacted with and their orientations are unknown. However, relative spatial and temporal measurements of velocity can be obtained from the ratio of $2T/\tau_c$ that is proportional to the velocity and defined as measured velocity.

Eq. 18 can be rewritten this way:

$$K^2 = \frac{1}{(2ak_0T)v} \left(1 - e^{-(2ak_0T)v}\right)$$

Eq. 19

**Modeling of Speckle**

The speckle phenomenon is observed in any coherent imaging modality such as synthetic aperture radar, optical coherence tomography, ultrasound, or any number of nonimaging measurement schemes involving laser illumination. Quantitative interpretation of the data from such measurement schemes (whether imaging or nonimaging) often hinges on accurate knowledge of the statistical behavior of the speckle phenomenon. To complement experimental measurements, researchers often turn to computer simulation of the phenomenon of interest. One important situation is the temporal decorrelation of a speckle pattern. Such a behavior is of interest, for example, in the use of laser speckle dynamics to assess fluid flow or in quasi-elastic light scatter to determine molecular mass.

It is worth noting at this point that speckle is often treated as noise, and that one definition of speckle noise, as presented in most textbooks, is simply multiplicative noise where the random noise vector is drawn from a uniform distribution. For instance, given an image $I$, its speckled noisy version is $J = I + n \ast I$ where $n$ is uniformly distributed random noise with mean 0 and variance $v$. This is not the speckle noise that interests us, but the physical effect of coherent light scattering as introduced earlier.
In an early book on the laser speckle phenomenon edited by Dainty [1], Goodman [39] derives the first-order statistics of speckles based on the laser light beam behavior. That is, the intensity (irradiance) and the phase of the light both perform a random walk in the complex plane. The same random walk can in fact be used to generate speckle, and fully simulated laser speckle image as we will show in the next.

In another modeling study, Zakharov et al. [94], do a Monte-Carlo simulation of the multiple scattering of the laser light, using the photon packet approach of light propagation in turbid media, and handling the reflections and refractions according to the Fresnel formulas.

Recently, Duncan et al. [26], came up with a faster way to generate speckle, using the concept of copula, which basically requires only a Fourier transform. His group proceeds to analyze the statistic of the copula generated speckle pattern [27].

**Random Walk**

In this study, we aim at developing a numerical model (digital phantom) for speckle images. Such a phantom would in turn allow modeling various acquisition protocols and simulating the results.

Figure 8 is a schematic representation of the optical arrangement used to obtain speckles [71]. On the left, the object is called the scattering centers plane (x,y); in the middle, the optical system itself; on the right, the detector plane (X,Y).
Speckles are generated by a random walk [76]. The field $U$ at pixel $(X,Y)$ consists in a random phasor sum. The scattering centers around the one vis-à-vis the detector contributes to the sum (above, right). For each detector pixel, the sum runs over the scattering pixels surrounding the one in front (gray pixels). For static speckles, the diameter of the gray shaded area is constant; while for dynamic speckles it depends on the activity value.

![Random walk in the complex plane](image)

The field at pixel $(X,Y)$ is:

$$U(X,Y) = \sum_x A \frac{e^{ikR + i\Phi + ik\frac{|\vec{X} - \vec{x}|^2}{2R} - \frac{|\vec{x}|^2}{w^2}}}{R}$$  \hspace{1cm} \text{Eq. 20}

$$I = U \cdot U^*$$  \hspace{1cm} \text{Eq. 21}

In the equation the two firsts terms in the exponential are related to the generation of speckles itself, while the last two are related to the diffraction field geometry. $k$ is the wavenumber related to the wavelength of the laser beam, $l=632.8\text{nm}$ ($k=2\pi/l$); $R$ is the distance between the scattering plane and the detector plane, $R=0.2\text{m}$; $F$ is a random phase uniformly distributed in $[-\pi, \pi]$, $A$ is a random amplitude uniformly distributed in $[0,1]$, $w$ is the radius of the laser beam. $|\vec{X} - \vec{x}|^2$ is the square of the projected distance between the detector pixel and the scattering pixel (distance in red in the schema on Figure 10). The intensity $I = U \cdot U^*$ (where $U^*$ is the complex conjugate).
Static fully developed raw speckles are generated from the model, Figure 11. Their intensity histogram fits a decaying exponential perfectly \[39\], Figure 12. Goodness of Fit: \(r^2=1\). The Briers contrast is defined by \(K=s/<I>\) (where \(s\) is the standard deviation over a 5x5 window and \(I\) the average intensity). Its average value is close to unity \(<K>=0.98395\).
Monte Carlo

Zakharov et al. [94] are doing a Monte-Carlo simulation of the multiple scattering of the laser light and compares it to an experiment with a physical model.

The digital model uses the photon packet approach of light propagation in turbid media [74]. The Henyey-Greenstein phase function was used based on an average scattering angle \( \langle \cos \theta \rangle \) [74]. The degree of polarization was assumed to decay exponentially [97]. Depolarized photon packets back-reflected from the sample where registered within a numerical aperture 0:17. The field auto-correlation function (ACF) \( g_1(\tau) \) of the scattered light was determined as explained in [30].

The physical model (phantom) was realized with a homogeneous block of solid Teflon and a home-made heterogeneous sample. This medical phantom mimics a liquid inclusion in solid tissue. It is obtained by milling a cylindrical hole of diameter \( D = 3 \) mm in a block of solid Teflon. A layer of variable thickness 0.1 – 2.1 mm separates the cylindrical inclusion from the interface that is imaged. The void is filled with a dispersion of 710 nm polystyrene particles in
water. The particle concentration is adjusted to match the optical properties of the liquid dispersion to the solid such that no static scattering differences could be detected anymore.

Using those two models, Zakharov et al. studied the image blurring of an object buried in a turbid medium, and found that the resolution of the obtained images can be affected significantly by multiple scattering. They furthermore introduced a model that reflects the impact of the static scattering on the interpretation of LSI images. They suggest that a quantitative analysis in actual LSI experiments is possible though it, in our opinion, it may not be as simple to implement as they suggest, and require an additional processing step.

**Copula**

In [26], Duncan shows that a spatially band-limited speckle pattern can be synthesized easily by the following algorithm: Fill a circular region of diameter D of a square matrix of dimension LxL with complex numbers of unity amplitude and with phases uniformly distributed over $(0, 2\pi)$. Upon Fourier transforming the LxL array and multiplying point-by-point by the complex conjugate, one arrives at a synthetic speckle pattern with exponential probability distribution. The position of the circular region within the larger array is irrelevant, as the Fourier shift theorem attests, and the ratio of L to D sets the minimum size of the speckles. For example, if L/D=2, the Nyquist criterion is met and the smallest speckle is two pixels wide.

![Figure 13](image.png)

Figure 13  Synthetic speckle algorithm. Shaded region of the matrix is filled with complex numbers of unity amplitude and phases uniformly distributed over $(0, 2\pi)$. (Image reproduced from [26])
The speckle images generated, one of which is shown in Figure 14, obey the same statistics than those generated by a random walk. Their look differs slightly however, as the random walk images appear “grainy” while the copula-generated images appear “snake-like”.

Figure 14  Speckles generated by the copula (Image reproduced from [26])
Chapter 4. CURRENT METHODS FOR PROCESSING LSI

This chapter reviews the different full field techniques that appeared in the literature for processing laser speckle images. It is worth noting that, while we use the term laser speckle imaging (LSI) as a collective acronym for all full field methods, Cheng et a. [18] gave the same name for specifically for a contrast analysis exclusively in the time domain. All methods, except the Generalized Differences (below) and the second-order method (which is the object of Chapter 5), use the Briers contrast (the ratio of standard deviation of intensity over average intensity) but differ slightly in the domain over which this ratio is computed, and/or differ in their implementation. The following Table 2 summarize the methods; it is an extended version from the review of Draijer et al. [25].

Table 2  Methods of measuring tissue blood flow with laser speckle techniques

<table>
<thead>
<tr>
<th>Technique</th>
<th>Abbrev.</th>
<th>Domain</th>
<th>Year</th>
<th>Principle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Generalized Differences</td>
<td>GD</td>
<td>Spatial</td>
<td>2002</td>
<td>Difference between two consecutive frames</td>
</tr>
<tr>
<td>Laser speckle contrast analysis [9,11]</td>
<td>LASCA</td>
<td>Spatial</td>
<td>1995</td>
<td>Contrast is determined in 1 image over 5 x 5 or 7 x 7 pixels.</td>
</tr>
<tr>
<td>Laser speckle imaging [18]</td>
<td>LSI</td>
<td>Temporal</td>
<td>2003</td>
<td>Contrast is determined in 1 pixel over 25 or 49 images.</td>
</tr>
<tr>
<td>Double exposure speckle photography [41,46]</td>
<td>DESP</td>
<td>Spatial</td>
<td>1978</td>
<td>A sequence of two rapid speckle recordings is taken in 1 image. The resulting fringes contain information about the movement.</td>
</tr>
<tr>
<td>Laser speckle temporal contrast analysis [60]</td>
<td>LSTCA</td>
<td>Temporal</td>
<td>2006</td>
<td>Contrast is determined in one pixel over a sequence of images.</td>
</tr>
<tr>
<td>Laser speckle perfusion imaging [32,33]</td>
<td>LSPI</td>
<td>Spatial &amp; temporal</td>
<td>2002</td>
<td>Combination of LASCA and LSI.</td>
</tr>
<tr>
<td>Laser speckle flowgraphy [53]</td>
<td>LSFG</td>
<td>Spatial &amp; temporal</td>
<td>2002</td>
<td>The contrast is determined based on an area of 3 x 3 pixels, in 3 speckle images.</td>
</tr>
<tr>
<td>Spatial derived contrast with averaging [86]</td>
<td>SDCav</td>
<td>Spatial</td>
<td>2004</td>
<td>Contrast is determined based on averaging a sequence of LASCA-images.</td>
</tr>
<tr>
<td>Temporal laser speckle contrast analysis [55]</td>
<td>tLASCA</td>
<td>Spatial &amp; temporal</td>
<td>2007</td>
<td>Contrast is determined based on averaging a sequence of LSI-images.</td>
</tr>
</tbody>
</table>
Spatial laser speckle contrast analysis sLASCA Spatial 2007 Contrast is determined based on averaging a sequence of LASCA-images.

Multi-exposure speckle imaging MESI Spatial 2008 Contrast is determined in 1 image over 7 x 7 pixels. Exposure time is kept constant and T is controlled by laser pulse duration.

Enhanced laser speckle contrast analysis eLASCA 2009 Dynamic range of contrast is stretched (see Chapter 6).

Second-order features for laser speckle imaging SOF-LSI Frequency 2008 Other measures than contrast are used (see Chapter 5).

Early methods

Generalized Differences

A first and fast estimation of the activity distribution seems to be the subtraction of two consecutive frames. Pixels where the intensity does not change will appear dark, whereas those that suffered changes will appear proportionally bright. This representation depends strongly on the sampling time, and will show changes only if they occurred during the interframe lapse. Besides, as dynamic speckle is a statistical phenomenon, the resulting image will show a speckled appearance. To reduce that inconvenience, the averaging of several instances may be used. Stationarity must then be assumed, and acquisition and processing times increased.

More generally, instead of two consecutive frames, one can perform subtraction of an initial state to the following ones, leading to the Generalized Difference algorithm:

$$I'(i,j) = \sum_k \sum_l |I_k(i,j) - I_{k+1}(i,j)|$$

where $I_k(i, j)$ is the intensity at the point with coordinates $(i, j)$ in the $k$-th frame.

Double exposure speckle photography (DESP)

Archbold and Ennos invented double-exposure speckle photography in 1972 [4]. Two similar studies by Grousson and Mallick [41], then Iwata et al. [47] appeared later. The technique

---

1 There isn’t a specific reference to the “un-generalized” difference algorithm, but it is probably the earliest method tried.
is based on the principle that a photographic record of two identical and mutually displaced speckle structures gives rise to parallel straight fringes in the Fourier plane. The spacing and orientation of these fringes is related to the displacement and direction between both photographs.

The technique is only applicable for solid bodies or fluids with a stationary flow pattern [4], in fields like stress, strain and vibration analysis, as well as to non-destructive testing. Iwai and Shigeta [46] developed a digital version of double-exposure speckle photography. To obtain a velocity map, the whole image should be divided into small regions for analysis over which the velocity can be assumed to be spatially constant. The analysis of these fringe-patterns is complicated compared to analysis performed in speckle contrast techniques, which is a disadvantage.

**Single exposure speckle photography (SESP)**

The first real speckle contrast technique was single-speckle photography, invented by Fercher and Briers in 1981 [31]. The same authors would later go on to develop LASCA. As in DESP, conventional cameras were used, so making and developing a photograph and then analyzing the negative film was a laborious process. The paper investigates the flow velocity distribution of the blood vessels in the retina. The SESP is used to produce a map of such a flow in which velocity is coded as a variation in the variance of the speckle pattern. The basic math of the early paper of Goodman [38] is presented. The modern reader will be surprised to learn that paper fails to describe how a photograph can be filtered to obtain variance without the help of digital equipment.

**Laser speckle contrast analysis (LASCA)**

Briers and Webster [8-10] developed in 1995 a digital version of single speckle photography using a monochrome CCD and frame grabber linked to a computer. The digital photograph is processed by the computer and the local contrast is computed in a block of n x n pixels. This digital version was the first setup which uses Laser Speckle Contrast Analysis.
Figure 15 shows a schematic overview of a LASCA setup with an expanded laser beam, an imaging system comprising focusing optics, a variable diaphragm and a digital camera as essential features. Experiments showed [12,43] that in LASCA it was not possible to obtain the full contrast range from 0 to 1.0. Richards and Briers [12,78] suggested this was due to an offset in the pixel values termed pedestal and introduced by the CCD camera. Manually removing this offset resulted in an increase in contrast from 0.41 to 0.95 for a static speckle pattern. Besides improving the computer hardware and software, the development of the LASCA technique continued. An improved version was described by Richards and Briers who implemented a camera with a variable exposure time and ran trials with lasers in the green wavelength range instead of in the red [12,77,78]. Several researchers used a slightly different LASCA setup. For example, in one setup, the backscattered light was collected on the camera without making use of a lens but by making use of a singlemode fiber [100] or adjustable iris and camera in the diffraction plane [96]. Furthermore, a polarizer was positioned in between sample and camera to select the linearly polarized light [63] to increase the contrast of the grabbed speckle pattern [12,77,78,100].
Other methods

Laser speckle imaging (LSI)

LASCA lacks spatial resolution due to the fact that the contrast is analyzed for a group of pixels in one image. To overcome this disadvantage, Cheng et al. [18] developed Laser Speckle Imaging (LSI) into which the contrast is calculated based on one pixel in a time sequence, rather than based on multiple pixels in one image. LSI, therefore, is the temporal equivalent of LASCA. Note that no flow (i.e., no dynamic speckle pattern) and very high flow (i.e., complete blurred dynamic speckle pattern) both give a contrast equal to 0. This makes LSI unsuitable for sample with regions where no flow is present. Cheng et al. [18] showed that calculating the contrast with LSI gives as expected a five times higher spatial resolution compared to LASCA, at the expense of the temporal resolution of course.

Laser speckle temporal contrast analysis (LSTCA)

Nothdurft and Yao [70] showed that by adjusting the capture parameters (e.g., exposure time, incident power, and time interval between subsequent capture), LSI is able to reveal structures that are hidden under the surface. Surface and subsurface inhomogeneities depend differently on these capture parameters, so by tuning the capture parameters, the image contrast values of the surface and subsurface targets can be changed. When the contrast of the surface inhomogeneity is within the noise level of the background image, the surface effect is essentially removed from the image. They did not test LSI on tissue perfusion; that was done by Li et al. [60] who named the technique differently, Laser Speckle Temporal Contrast Analysis (LSTCA), but it is based on the same principle as LSI. They presented images of the cerebral blood flow of a rat through the intact skull by making use of temporal averaging of the speckle pattern. They used an exposure time of 5 ms, which is of the same order as that suggested by Yuan et al. [93] and an interval time of 25 ms, resulting in a real-time video frame rate of 33 Hz. They furthermore showed that LSTCA significantly improves the visualization of the blood vessels with respect to LASCA due to the fact that the speckle pattern on the detector is built up of a stationary and a dynamic part. They stated that the stationary part produced by the skull is mainly dependent on local properties of the skull and is therefore temporally homogeneous. So
the contrast value in the LSTCA process is not influenced by the stationary part, whereas in the LASCA process, the stationary part will influence the contrast value and lower the SNR.

**Laser speckle perfusion imaging (LSPI)**

In LSPI, the mean value of the speckle intensity, which is called speckle reduction by Forrester et al. [32,33], can be determined by spatial averaging (good temporal resolution), temporal averaging (good spatial resolution) or a combination of both (acceptable temporal and spatial resolutions). The nonfluctuating component of the measured intensity is quantified by the speckle reduction (i.e. mean value). To quantify the fluctuating component, the sum of the difference between the speckle reduction and the speckle intensity is taken and normalized with the speckle reduction, which is different from the formal definition of contrast, where the numerator is based on the standard deviation of the fluctuation instead of the mean absolute difference of the fluctuations.

**Laser speckle flowgraphy (LSFG)**

To determine the perfusion, the inverse relation of the normalized sum is taken. For obtaining high spatial resolution images, Forrester et al. [33] used a frame rate of just over 6 Hz, while with spatial averaging they obtained a semi-real-time imaging speed with a frequency of 15 Hz. The method of calculating the flow in LSFG, or mean blur rate (MBR) as Konishi et al. [53] termed it, is comparable to the combination of spatial and temporal averaging introduced by Forrester et al. [33]. In LSFG a 3 x 3 x 3 pixel matrix is taken and the MBR is defined as the mean intensity across these 26 pixels (the central point is not taken into account) divided by the mean difference of the central point and the 26 pixels. This is schematically shown in Fig. 3d. When using a CCD camera in LSFG, the interlace scanning mode of the camera requires compensation for the fact the odd lines are captured at different time to the even lines, so Konishi et al. [53] adjusted the definition of the MBR in LSFG to:

\[ MBR_{n,m,t} = \frac{2\langle I_{n,m,t}\rangle^2}{\langle I_{n,m,t}^2 \rangle - \langle I_{n,m,t} \rangle^2} \]

where the factor 2 in the numerator is related to the number of uncorrelated intensity data taken for the averaging (i.e., even and odd lines).
Spatial derived contrast with averaging (SDCav)

Tan et al. [86] modified the “classical” LASCA to SDCav by introducing averaging over multiple contrast maps, resulting in a decrease in root mean square (RMS) of the value of 1/τc with an increasing number of averages.

Spatial laser speckle contrast analysis (sLASCA)

A few years later in 2007, the SDCav technique of averaging over multiple contrast maps was presented by Le et al. [55] under the name sLASCA.

If \(K_{i,j,1}, K_{i,j,2}, \ldots, K_{i,j,n}\) denote the respective consecutive contrast values at pixel \((i,j)\) in frame 1, 2, ..., \(n\), the contrast \(K_{sLASCA}\) is given by

\[
K_{sLASCA(i,j)} = \frac{1}{n} \left( K_{i,j,1} + K_{i,j,2} + \cdots + K_{i,j,n} \right)
\]

Temporal laser speckle contrast analysis (tLASCA)

Le et al. [55] also introduced tLASCA in the same paper, a technique in which averaging in the spatial domain is performed on contrast maps obtained using LSI. They showed that tLASCA give better results and is faster than sLASCA and LSI.

\[
K_{tLASCA(i,j)} = \frac{1}{9} \sum_{r=1}^{i+1} \sum_{c=j-1}^{j+1} \delta_{i,j,t} \langle I_{i,j,t} \rangle
\]

Where \(\delta_{i,j,t}\) is the standard deviation of all pixels at \((i,j)\) and \(\langle I_{i,j,t} \rangle\) is the mean intensity of all pixels at \((i,j)\) in \(n\) frames along the temporal dimension, and \(K_{tLASCA}\) is calculated as an average over a \(3 \times 3\) spatial observation window.

Multi-exposure speckle imaging (MESI)

Parthasarathy et al. [72] presented a new multiexposure speckle imaging (MESI) instrument based on their robust speckle model that has potential to obtain quantitative baseline flow measures and overcomes their criticism of LASCA and LSI (e.g., lack of quantitative accuracy and the inability to predict flows in the presence of static scatterers such as an intact or thinned skull). To keep the noise contribution of the camera (e.g., readout noise, thermal noise)
constant while changing the integration time, they used a fixed exposure time for the camera and gated a laser diode during each exposure to effectively vary the speckle exposure duration $T$.

**Second-order features for laser speckle imaging (SOF-LSI)**

The second order features (SOFs), developed by us, differ radically from the other methods that they are not based on the ratio of variance on average intensity of the image. Instead, in a first step the cooccurrence matrix for each 3-by-3 pixels window is calculated. Then the Difference Moment, Difference Entropy, or Difference Variance is taken out of the cooccurrence matrix. SOF is explained in more details in Chapter 5.

**Enhanced laser speckle contrast analysis (eLASCA)**

eLASCA, also developed by us, enhances and improves the dynamic range of the basic Laser Speckle Contrast Analysis (LASCA). Its algorithm is based on monotonic point transformation (MPT). eLASCA is explained in more details in Chapter 6.
PART II

SPECIFIC PART
In this chapter, we introduce a new method that we have developed. The Second-Order Features (SOFs) method for Laser Speckle Imaging is a method based on the coocurrence matrix. We shall see that the new images have higher information content, are both smoother (in background) and sharper (along edges).

**Methods**

**Laser Speckle Images as a texture**

The texture of an image region has been associated with the structural arrangement of pixel’s intensities within the region [42]. The most customary approach utilizes the concept of the grey level co-occurrence matrix that provides statistics for the probability of joint relationship of grey level intensities between neighbor pixels. Using a domain of $3 \times 3 \times 23$, that is $3$ by $3$ pixels on $23$ successive frames, the Inverse Difference Moment, Difference Entropy, and Difference Variance were computed as described in Mahmoud-Ghoneim et al. [65].

Mathematically, a co-occurrence matrix $C$ is defined over an $n \times m$ image $I$, parameterized by an offset $(\Delta x, \Delta y)$,

$$C_{\Delta x, \Delta y}(i,j) = \sum_{p=1}^{n} \sum_{q=1}^{m} \{1, \text{if } I(p, q) = i \text{ and } I(p + \Delta x, q + \Delta y) = j \} \quad \text{Eq. 22}$$

Haralick [42] first presented how to create a gray-level co-occurrence matrix (GLCM) from an image $I$, by calculating how often a pixel with gray-level (grayscale intensity) value $i$ occurs horizontally adjacent to a pixel with the value $j$. Note that the intensity image is first scaled down to $n$ gray-levels. The following Figure 16 shows how the MATLAB function `graycomatrix` calculates several values in the GLCM of the 4-by-5 image $I$. Element (1,1) in the GLCM contains the value 1 because there is only one instance in the image where two,
horizontally adjacent pixels have the values 1 and 1. Element (1,2) in the GLC contains the value 2 because there are two instances in the image where two, horizontally adjacent pixels have the values 1 and 2. `graycomatrix` continues this processing to fill in all the values in the GLCM.

![Gray-Level Co-occurrence Matrix (GLCM)](image)

Figure 16  `graycomatrix` creates a gray-level co-occurrence matrix (GLCM) [Reproduced from MATLAB's help files]

Several GLCM can be computed for several offsets, such as

- \((\Delta x, \Delta y) = (1, 0)\)  horizontal
- \((\Delta x, \Delta y) = (0, 1)\)  vertical
- \((\Delta x, \Delta y) = (1, 1)\)  diagonal
- \((\Delta x, \Delta y) = (-1, 1)\)  other diagonal

Another way to look at those offsets is to use an orientation vector \((d, \theta)\), like in polar coordinates. Thus \(d = 1\) and \(\theta = \{0^\circ, 45^\circ, 90^\circ, 135^\circ\}\) for horizontal, diagonal, vertical, (other) diagonal offset, respectively.

In this work we extend the GLCM to the Z-axis in order to study gray-level co-occurencies on an image volume composed of multiple slices, so Eq. 22 becomes:

\[
C_{\Delta x, \Delta y, \Delta z}(i, j) = \sum_{p=1}^{n} \sum_{q=1}^{m} \sum_{r=1}^{l} \begin{cases} 1, & \text{if } I(p, q, r) = i \text{ and } I(p + \Delta x, q + \Delta y, r + \Delta z) = j \\ 0, & \text{otherwise} \end{cases} \tag{Eq. 23}
\]

The offsets in three dimensions are as follow:

\(\Delta (x, y, z) = \{(0,0,0); (0,1,0); (1,0,0); (-1,0,0); (0,0,1); (1,0,1); (0,1,1); (1,1,1); (-1,0,1); (0,-1,1); (1,-1,0); (1,1,-1)\}\)
The respective orientation vectors \((d, \theta, \Delta z)\) would be \(d = 1\) and

\[
\begin{align*}
\{ & \text{when } \Delta z = 0, \theta = \{0^\circ, 45^\circ, 90^\circ, 135^\circ\} \\
\{ & \text{when } \Delta z = 1, \theta = \{0^\circ, 45^\circ, 90^\circ, 135^\circ, 180^\circ, 225^\circ, 270^\circ, 315^\circ\}
\end{align*}
\]

The twelve possible GLCM’s for each of the twelve offsets are averaged.

Note that here, we downscale the 3 x 3 x 23 window to 8 gray-levels, that is the 3\textsuperscript{rd} quantization level \(2^3=8\). Each GLCM is thus a 8 x 8 matrix.

Then, some parameters such as the Inverse Difference Moment, Difference Entropy, and Difference Variance were computed, for each GLCM window:

\[
\begin{align*}
\text{Inverse Difference Moment} & = \sum_{i,j=1}^{N_g} \frac{1}{1 + (i - j)^2} P(i,j) \\
\text{Entropy} & = \sum_{i,j=1}^{N_g} P(i,j) \log P(i,j) \\
\text{Variance} & = \sum_{i,j=1}^{N_g} P(i,j)^2 - \left( \sum_{i,j=1}^{N_g} P(i,j) \right)^2
\end{align*}
\]

Where the number of gray-levels \(N_g = 8\); \(P(i,j)\) is the value of the GLCM at the \(i\text{th}\) row and \(j\text{th}\) column.

These are second-order statistics of the underlying image, representing texture properties. The rationale behind using texture properties for Laser Speckle Images is that the later are, by essence, texture images. In the next paragraph we go on to show that those transformed image do reveal more information than the raw speckles images, or even the LASCA processed images.

**Assessment criteria**

Three criteria were used to assess the quality of the second order features and the traditional contrast:

- The equivalent number of looks (ENL);
- The image Entropy (\(H_e\)).
• The difference of Peak Signal-to-Noise Ratio (ΔPSNR)

The average equivalent number of looks (ENL) measures smoothness in areas that should have a homogeneous appearance but are corrupted by speckle [2]. It is defined as

$$ENL_m = \frac{\mu_m}{\sigma_m}$$  \hspace{1cm} Eq. 27

where $\mu_m$ is the mean of the pixel values in the $m^{th}$ Region of Interest (ROI) and $\sigma_m$ is the standard deviation. We choose three ROIs and averaged the ENL’s.

The image Entropy describes the information content in an image [68]. If an image has $G$ gray-levels and the probability of gray-level $k$ is $P(k)$, then the entropy $H_e$ is defined as,

$$H_e = -\sum_{k=0}^{G-1} P(k) \log_2(P(k))$$ \hspace{1cm} Eq. 28

An objective quality criterion, called the difference of Peak Signal-to-Noise Ratio (ΔPSNR) by Le et al. [55], is an objective measure of sharpness. An image is sharp if it has many well-contrasted lines. In other words, thin and connected lines should be visible to the eyes, and intensity changes drastically from one side of a line to the other. Each image (I) has undergone a blurring ($I_b$) and a separate sharpening ($I_s$) operation. The image or is further discrete-cosine transformed (DCT), and high frequency coefficients are removed before reconstruction is made. The PSNRs of the respective images are calculated before and after DCT. The percent change in PSNR between the blurred and sharpened versions of the same image indicates the level of sharpness of that image. For two images $I_1$ and $I_2$, if $%\Delta$PSNR($I_1$) is greater than $%\Delta$PSNR($I_2$), then $I_1$ is sharper than $I_2$. This parameter can be used as the objective measure of speckle images.

**Experimental part**

**Animal preparation**

A male Sprague–Dawley rat (325 g) was used for the imaging. It was anaesthetized with pentobarbital (40mg/kg, IP) and mounted onto a stereotaxic frame (Benchmark, myNeurolab). A midline incision was made to expose the surface of the skull. The temporal muscle was freed and retracted with suture line. A window (6.5 x 6.5 mm) overlying the left barrel cortex (3.0 mm
lateral, 4.3 mm caudal from bregma) was thinned with a high speed dental drill. The thinned area was filled with glycerine to avoid the glaring in imaging.

**Imaging procedure**

A 20mW semiconductor laser diode (635 nm) was used to illuminate the imaging site. A monochrome 12-bit CCD camera (Pixelfly QE, PCO, Germany) with resolution of 1024x1392 pixels was positioned over the thinned skull and focused on the blood vessels overlying the cortical surface. A 2 by 2 hardware binning was performed by the camera, and the resulting image of size 512 by 696, was output to the PC for software processing. The acquisition began with 4 s of baseline activity, followed by 4 s of activity collected during stimulation and 10 s of activity collected post-stimulus offset for a total of 18 s. Camera exposure time was set at 5 ms and images were acquired at a rate of 23 frames per second, resulting in 414 images. In addition, a white light image was acquired for comparison.

**Data analysis**

Speckle is a random field intensity pattern produced by the mutual interference of partially coherent beams that are subject to instant temporal or spatial fluctuations. These patterns are seen when monochromatic coherent light is incident on a rough surface or a field of scattering particles. If the field of particles is non-static, photographing the pattern results in an image that is blurred over the exposure time of the recording device (see Figure 17).

---

*Figure 17*  Raw Laser Speckle Image  
*Figure 18*  White light image of the barrel cortex, and three selected Regions of Interest (ROI).
One measure is the local speckle contrast which is defined as the ratio of the standard deviation to the mean intensity in a small window of the image [48]. The depiction of the vascular structure can be improved by using several consecutive frames; thus the statistics of the standard deviation and mean intensity are computed in a small box of the dataset, leading to the temporal contrast $tK$, using the tLASCA method [55,69]. Here we use a box of size 3 by 3 pixels by 23 frames (one second in our dataset).

The texture of an image region has been associated with the structural arrangement of pixel’s intensities within the region [42]. The most customary approach utilizes the concept of the grey level co-occurrence matrix that provides statistics for the probability of joint relationship of grey level intensities between neighbour pixels. Using the same small box than for $tK$, the Inverse Difference Moment, Difference Entropy, and Difference Variance were computed [65] as described in this Chapter. Together we call them Second Order Features (SOF).

The criteria introduced previously, i.e. the Equivalent Number of Looks (ENL), the image Entropy ($H_e$) and the Peak Signal-to-Noise Ratio ($\Delta$PSNR), were used to assess the quality of the contrast and the second order features.

**Results**

The temporal-contrast ($tK$) and the three SOFs have been computed for each second of the same dataset. Three regions of interest (ROI, as in Figure 18), have been picked up and their average value plotted against time, Figure 19.

The four different features are assessed by different quality metrics as in Table 3.

<table>
<thead>
<tr>
<th>Statistic</th>
<th>ENL</th>
<th>Entropy</th>
<th>$\Delta$PSNR</th>
</tr>
</thead>
<tbody>
<tr>
<td>tLASCA</td>
<td>101.09</td>
<td>4.29</td>
<td>1.63</td>
</tr>
<tr>
<td>SOF1</td>
<td>149.44</td>
<td>5.61</td>
<td>2.23</td>
</tr>
<tr>
<td>SOF2</td>
<td>147.84</td>
<td>4.88</td>
<td>4.11</td>
</tr>
<tr>
<td>SOF3</td>
<td>56.46</td>
<td>6.58</td>
<td>9.28</td>
</tr>
</tbody>
</table>
Figure 19   Left, top to bottom: time-course plot of averaged features: Contrast (tK), Inverse Difference Moment (SOF1), Difference Entropy (SOF2), and Difference Variance (SOF3). The time course of the three Regions of Interest (marked by “•”, “+”, “*”) is plotted.
Discussion

We investigated Second Order Features, i.e. higher order statistics than the previously established temporal contrast. These SOFs, which are the Inverse Difference Moment, Difference Entropy, and Difference Variance, are found to contain more information than the widely used contrast, as assessed by the following quality metrics: the Effective Number of Looks (ENL), the Entropy, and the objective quality ($\Delta$PSNR). The ENL is higher for $SOF1$ and $SOF2$, the Entropy is higher for all three, as well as the Objective Quality, as shown in Table 3. Seen the other way, the Inverse Difference Moment ($SOF1$) performs better than tLASCA for all criteria. The Difference Entropy ($SOF2$) also performs better than tLASCA for all criteria. The Difference Variance ($SOF3$) performs better when assessed by the Entropy and the $\Delta$PSNR, but worse when assessed by ENL.

Besides, it has been demonstrated previously that there exists a mathematical relationship between the contrast and the cerebral blood flow. If our proposed high-order statistics are themselves proportional to the contrast, then they are also related to CBF. Empirically, it is the case, as shown in Figure 19, the Inverse Difference Moment ($SOF1$), Difference Entropy ($SOF2$), and Difference Variance ($SOF3$) which all follow closely the time-scale plot of contrast.

Therefore we would recommend using either the Inverse Difference Moment ($SOF1$), either the Difference Entropy ($SOF2$), as a new replacement measure for tLASCA.

Thanks to SOFs, images are now smooth in areas that should have a homogeneous appearance but were corrupted by speckle (that is, in background); have high information content (as explained by a higher Entropy measure); and are sharp in places were thin and connected lines should be visible to the eyes (that is along edges such as blood vessels).
Chapter 6. **NEW METHOD: ENHANCED LASCA**

According to the theory of laser speckle imaging, Goodman [39] gave the mathematical model of a fully developed speckle on an infinite rough surface where the contrast is always 1. So, the theoretical value of $K^2$ is within the range of $[0, 1]$. In practice however, the range of $K^2$, or called “dynamic range” hereafter, is extremely limited due to the ambient background field, the effect of dark current and other anomalies in the CCD camera [78, 82, 90]. For example, $K^2 < 0.01$ in Dunn’s work [28], $K^2 < 0.04$ in Cheng’s work [18] and $K^2 < 0.09$ in Yuan’s work [93]. In this study, the typical $K^2$ has all its values within $[0, 0.048]$ and 99% within $[0, 0.02]$.

There is no effective method to completely eliminate the influences of ambient background field, dark current, and other noise [78]. Besides, such a limited dynamic range not only influences the comparison of CBF changes, but also makes it difficult to analyze the transient spatiotemporal changes of blood flow in microvessels. Furthermore, small contrast values close to zero correspond to extremely large values of $\frac{1}{K^2}$ ($\propto v$), such that the velocity visualization would be problematic with a full-range color map.

In this chapter, we introduce a new method that we have developed. The enhanced LASCA (eLASCA), a method based on monotonic point transformation (MPT), aims at solving the aforementioned problems. We shall see that eLASCA improves the dynamic range of $K^2$ and keeps the variabilities of contrast values. Besides, the eLASCA method is fully adaptive and has a low computation load.

**Methods**

Suppose $L$ trials, $T$ frames each, of laser speckle images ($M \times N$ pixels) were acquired in a single experiment, $I_l(m, n, t)$ ($m = 1, ..., M; n = 1, ..., N; t = 1, ..., T; l = 1, ..., L$). The
corresponding contrast matrix \( K^2(m, n, l) \) can be obtained by traditional contrast equation, however taken in the time domain (as in LSI): \( K^2 = \frac{\sigma_i^2}{\langle l \rangle^2} \)

In order to improve the dynamic range, we reshape three dimensional \( K^2 \) into one-dimensional random variable \( f(i) (i = 1, ..., M \times N \times L) \) by

\[
f(m + (n - 1) \times M + (l - 1) \times M \times N) = K^2(m, n, l)
\]

Eq. 29

The profile of the probability density function (pdf) of \( f \), i.e. \( p(f) \), is very sharp in a small range close to zero as in Figure 20.

![Figure 20 probability density function of \( K^2 \)](image)

To improve the dynamic range, \( f \) is transformed into \( f_e \) satisfying \( p(f_e) = 1 \) by MTP theory [75]. The transform does not change the validity of \( f \) according to the monotonicity of the transform:

\[
\int_0^{f_e} p(f_e) df_e = \int_0^{f} p(f) df
\]

Eq. 30

where \( f_e \) is limited in any range (here it is [0,1]) and \( f \) is limited in [0,1] (actually in a small range).

Since \( p(f_e) \equiv 1 \), Eq. 30 can be solved easily
\[ f_e = \int_0^f p(f)df \]  
\text{Eq. 31}

According to Eq. 19, \( K^2 \) is approximately proportional to \( 1/v \), therefore, \( f \) can be represented as:

\[ f = \frac{b}{v} \]  
\text{Eq. 32}

where \( b \) is a constant.

Combining Eq. 31 and Eq. 32, we have:

\[ f_e = \int_0^{b/v} p(f)df \]  
\text{Eq. 33}

Since the contrast is always non-negative, the relation between \( v \) and \( f_e \) can be numerically deduced from Eq. 33 with the cumulative density function (c.d.f.) \( C(f) = \int_{-\infty}^f p(f)df \)

\[ f_e = C\left(\frac{b}{v}\right) - C(0) = C\left(\frac{b}{v}\right) \]  
\text{Eq. 34}

Clearly, higher velocity \( v \) results in lower \( f \), and then leads to lower \( f_e \) after MPT.

In practice, Eq. 34 can be approximately estimated with the percentage of contrast value less than or equal to \( f \), i.e. \( Num_f \), in \( K^2 \):

\[ f_e = \frac{Num_f}{M \times N \times L} \times 100\% \]  
\text{Eq. 35}

Therefore, the procedures of eLASCA can be summarized as follow.

1) Reshape 3 dimensional contrast \( K^2 \) to 1 dimensional vector \( f \) by Eq. 29.
2) Sort the vector \( f \) by ascending order and save it as \( g \).
3) Compute \( f_e \) based on \( g \) by Eq. 35.
4) Reshape the vector \( f_e \) back to 3 dimensional \( K^2 \) by Eq. 29.
Experimental part and Data processing

Animal Preparation

Figure 21 shows the experimental setup in our study. Seven male Sprague–Dawley rats (300 ± 50 g) were used to obtain the laser speckle images under experimental hypothermia protocols approved by the Committee for Animal Care and Use of Shanghai Jiao Tong University. The animals were anaesthetized with pentobarbital (80 mg/kg, intraperitoneal injection) and mounted in a stereotaxic frame (Benchmark Deluxe; MyNeurolab.com, St. Louis, MO). In the surgery, a midline incision was made to expose the skull. A window (9 × 6 mm²) overlying the right barrel cortex (see Figure 21, bottom left corner) was thinned with a high-speed dental drill (Stoelting, USA) equipped with 1.6-mm drill burr (Dentsply, Switzerland). The thinned area was filled with glycerine to reduce the glaring reflection. A feedback-controlled heating pad (SS20-2, Huaibei Zhenghua Bioinstrumentation Equipment Ltd., Anhui, China) was used to maintain the body temperature. A semiconductor laser diode (635 nm) (20-mW KL5650, Forward Company, Ltd., Shanghai, China) was used to illuminate the thinned window. A monochrome 12-bit CCD camera (Pixelfly QE, Cooke, USA) with resolution of 1024 × 1392 pixels was positioned over the thinned skull and focused on the cortical surface through a trinocular stereo zoom boom microscope (XYH-05, Shanghai Optical Instrument Factory, Shanghai, China). A 2 × 2 hardware camera binning was implemented such that 512 × 696 pixel laser speckle images were output to computer for CBF analysis. Exposure time of the camera was set to 5 ms [93] and the imaging rate was 23 ft/s throughout the experiments.
Data Recording

During the experiment, the temperature of the rat was monitored with a rectal probe and maintained by the feedback controlled heating pad and a radiator. Twenty minutes after the surgery preparation, the first trial’s laser speckle images \((l = 1, S = 200)\) were acquired as the baseline \((37 ^\circ C)\). Then, whole-body hypothermia was induced by 30 min of surface cooling with alcohol bathing. After maintaining the rectal temperature at \(32 \pm 0.5 ^\circ C\) for 20 min, another 200 frames of laser speckle images were captured as the hypothermia trial \((l = 2)\). The 30-min rewarming procedure started after 40 min of hypothermia until the rectal temperature reached \(37 \pm 0.5 ^\circ C\). Then, after another 20 min at \(37 ^\circ C\), the last trial \((l = 3)\) laser speckle images \((S = 200)\) were recorded.

Data Analysis

For each rat, three trials \((l = 1, 2, 3)\) of laser speckle images \((S = 200\) each trial) were obtained. After processing each trial by \((2)\), a \(512 \times 696 \times 3\) contrast matrix \(K^2\) \((m, n, l)\) was
obtained for baseline ($l = 1$), hypothermia ($l = 2$), and postrewarming ($l = 3$) trial, respectively. Then, $K^2$ of each rat was processed by the eLASCA method to improve the dynamic range.

**Results**

**Automatic Visualization of Contrast Data**

To visualize the contrast data, we need to convert $K^2$ into gray levels. Figure 22(a) shows the typical $K^2(m,n,l)$ in baseline trial ($l = 1$). Because the majority of contrast data in a baseline trial falls into an extremely small range $[0, 0.02]$ (see Figure 20), i.e., primary range hereafter, the image was too dark to show the details with a full-ranged color map from 0 to 1. Most literature linearly rescaled the primary range of the contrast data to gray levels for a better visualization, e.g., $K^2 \in [0, 0.02]$, as shown in Figure 22(b). Such a visualization processing not only needs manual intervention, but also loses the information of data outside the primary range. Figure 22(c) illustrated the automatic visualization by conventional contrast-limited adaptive histogram equalization (CLAHE) [51]. Compared with Figure 22(a)–(c), eLASCA presented more vascular details [see Figure 22(d)], particularly in the capillaries. Furthermore, the traditional method like CLAHE is not able to deal with the velocity visualization because an extremely limited low dynamic range [e.g., $[0, 0.048]$ in Figure 22(a)] results in an unlimited velocity range [e.g., $[20, \infty]$] while eLASCA visualizes the $1/K^2$ perfectly by normalizing the range of $1/K^2$ to $[0, 1]$. Figure 23(a)–(c) shows the changes of CBF under baseline, hypothermia, and postrewarming conditions.
Figure 22  Visualization of the contrast data from the baseline trial (37 °C). (a) Direct visualization of LASCA data in its full range of [0, 0.048]. (b) LASCA data in the range of [0, 0.02]. (c) LASCA data by conventional CLAHE. (d) Visualization of the corresponding eLASCA data after MPT. The dashed lines in (a) are highlighted for further comparing the variability in the data.

Figure 23  Visualization of the velocity of CBF, i.e., $1/K^2 \propto v$, under (a) baseline, (b) hypothermia, and (c) postrewarming trials. Such velocity visualization would be problematic with the LASCA data, i.e., $1/K^2$.

Influences of Hypothermia on Capillary CBF

Figure 24 shows the LASCA ($K^2$) for baseline [see Figure 24(a)], hypothermia [see Figure 24(b)], and postrewarming [see Figure 23(c)] trials in one experiment, respectively. As a comparison, the corresponding eLASCA ($K^2_e$) is also illustrated [see Figure 24(d)–(f)]. There were clear CBF differences between hypothermia and normothermia trials. In capillary area, eLASCA seemed to present more decrease of CBF under hypothermia. To quantitatively analyze
the CBF changes in capillary level, we segmented cortical arteries, veins [see Figure 24(g)], and capillaries [see Figure 24(h)] by Otsu multithreshold method [61] in The Insight Segmentation and Registration Toolkit (ITK) software [45] (bin = 256 and threshold number = 4). Considering the variation of CBF in baseline trial, we analyzed the relative contrast change, i.e., the ratio of contrast in hypothermia or postrewarming trial to that in the baseline trial. Figure 24(i) showed the relative changes of capillary CBF from seven rats in hypothermia and postrewarming trials by LASCA and eLASCA, respectively. Under hypothermia, eLASCA (relative contrast 189%) showed much greater decrease of capillary CBF than LASCA (relative contrast 137%). While in the postrewarming trial, capillaries demonstrated recovery by both eLASCA (151%) and LASCA (119%). However, the statistical analysis by paired t-test of CBF recovery indicated that eLASCA (p = 0.009) provided higher confidence level than LASCA (p = 0.013).

Figure 24  CBF change analysis by LASCA and eLASCA. The upper panels are corresponding to the contrast (K^2) of CBF from (a) baseline, (b) hypothermia, and (c) postrewarming trials, respectively, by the conventional LASCA. While the middle panels [(d), (e), and (f)] are their corresponding eLASCA analysis. In order to analyze
the capillary CBF change, we segmented the arteries, (g) veins, and (h) capillaries. (i) Statistical analysis of the CBF changes by LASCA and eLASCA corresponding to the capillaries in (h).

Figure 25  Comparison of the data variability by (a) LASCA, (b) histogram equalization, and (c) eLASCA at the dashed line in Figure 22(a). Compared with histogram equalization, eLASCA not only improves the contrast range, but also keeps the data variability (see highlighted data within the dash-dotted boxes).
Discussion

eLASCA not only provides an automatic visualization algorithm for the CBF change, but also keeps the CBF variability after the transform. Figure 25 plots the contrast values at the dashed line in Figure 22(a) by LASCA, histogram equalization, and eLASCA, respectively. Compared with the conventional LASCA, both histogram equalization and eLASCA greatly improve the dynamic range of the contrast data. However, histogram equalization loses the data variabilities when the fluctuations of CBF are within an extremely small range, as highlighted in the dashed windows in Figure 25. The histogram equalization is based on the image histogram (usually 64 or 128 bins are used to compute the image histogram), which leads to a loss of variation in the data when applied to a continuous random variable (e.g., K²) [see Figure 25(b)]. Keeping the data variability also helps to segment the vessels. For example, cortical capillary bleeding in surgery sometimes is not avoidable [see Figure 26(a)], which may result in artifacts in vessel segmentation [see Figure 26(b)]. Blood vessels in Fig. 7(c) are more easily discriminated after eLASCA transform than those in Figure 26(b) (see the circles in Figure 26). In our study, although eLASCA (p = 0.009) resulted in lower p-value than the tLASCA (p = 0.013), which is useful if a more rigorous level of significant difference is required, eLASCA also induced bigger error bars [as shown in Figure 25(i)]. In addition, eLASCA itself is very fast.
and has a low computational cost. Therefore, if the high spatial resolution is not required, eLASCA can be applied to sLASCA instead of tLASCA to produce real-time contrast images.

In conclusion, we proposed an MPT-based eLASCA method to improve the dynamic range of contrast, while maintaining the variability of data. eLASCA is fast and does not require supervision as a visualization tool. Compared with the conventional LASCA technique, eLASCA presents much better performances for analyzing the CBF variability.
Chapter 7. Discussion

The two new processing methods for LSI that we’ve introduced in this thesis are: Second-Order Features (SOFs), in Chapter 5, and enhanced LAser Contrast Analysis (eLASCA), in Chapter 6. The discussions regarding those methods were presented within their chapters for coherency. Below are discussed some additional issues on the use of Laser Speckle Imaging.

Exposure time

LASCA is fast and inexpensive, but there are technical details which should be taken into account for proper measurements. To adjust the “sensitivity” of the LASCA setup to a certain velocity, the integration time can be adjusted. As the integration time changes, the noise in the measurement also changes. Yuan et al. [93] identified a relation linking sensitivity, noise, and camera exposure time. They found that with an increasing exposure time up to 2 ms, the sensitivity to relative speckle changes increased. However, the noise in the speckle contrast also increases with increasing exposure time. The optimal contrast-to-noise ratio was found to be at 5 ms, so Yuan et al. suggested that ∼5 ms is an optimal exposure time for LASCA measurements in the brains of rodents.

Parthasarathy et al. [73] show that the velocities of fluid and exposure time of the camera are the most important factors that affect speckle contrast. In that study, microfluidic devices are used: these are tissue phantoms. They offer good flexibility to design channels of different sizes and shapes, and permit repeated measurements in a controlled environment.

Speckle size

To obtain good statistics, the speckle size should be carefully controlled. When speckle size and pixel size are of the same order, the error in calculated contrast is minimized [35, 41].
For image speckle (that is, when the speckle pattern is formed on through a lens, on a camera), the speckle size is dependent on the laser wavelength ($\lambda$), the f-number of the lens system (f#) and the magnification (M), as expressed by:

$$d = 2.44\lambda f_{\#} M$$

Therefore by controlling the f-number of the lens system (i.e., adjusting the iris of the lens system) the optimal speckle size can be chosen. However, this removes the facility to control the amount of light falling on the camera because Yuan et al. [93] showed that a fixed integration time of $\sim$5 ms gives the best contrast-to-noise ratio. One solution would be to use neutral density filters, in order to adjust the amount of light falling on the camera [35].

**Depth information**

With “classical” LASCA, all depth information about perfusion is lost, so Zimnyakov and Misnin [99] modified the setup by making use of a localized moving light source in combination with spatial filtering to reveal depth-resolved information about the micro circulation. When a dynamic layer below a static layer is imaged, the resulting speckle pattern will be composed of a stationary speckle pattern in the inner zone of the CCD camera and a dynamic speckle pattern in the outer zone. So by placing filtering diaphragms on the sample, depth information can be obtained. As a consequence of the stationary speckle pattern, the contrast will not drop to 0 for long integration times. To quantify that Zimnyakov and Misnin introduced the term residual contrast.

**Linearity / nonlinearity between measured and actual flow rates values**

Cheng et al. [16] in their Laser Speckle Imaging only assumed a linear relationship between the measured flow rate (that is $1/\tau_c$) and actual flow rate values, whereas Choi et al. [21,22] showed that there is a linear relationship between these parameters, the range over which this is valid depends on the integration time of the camera (e.g., 0 to 20 mm/s for $T = 1$ ms and 0 to 5 mm/s for $T = 10$ ms), as already was suggested by Yuan et al. [93].
Noise

Völker et al. [89] modified LSI by positioning a rotating diffuser, which can be controlled by a motor, to illuminate the sample with a random speckle pattern. In this way, they could suppress the noise in LSI. If the diffuser rotates slowly (e.g., one rotation per hour), temporal fluctuations will occur at time scale $\tau_0$. However, if the exposure time $T$ of the camera is chosen to be smaller than $\tau_0$, subsequent speckle images will be statistically independent and analyzing a large number of images results in the perfect averaging of the contrast without loss of spatial resolution. They showed that the noise level scales with $1/\sqrt{N}$, with $N$ being the number of independent speckle images.

Blood flow velocity profile

Bandyopadhyay et al. [6] and Zakharov et al. [94,94] pointed out recently that the commonly used LSI equation (i.e., Eq. 3) involves an approximation (i.e., $\tau c << T$ for Lorentzian velocity distribution) that could result in incorrect data analysis. Cheng and Duong [15] investigated the contribution of such approximation and its impact on LSI data analysis. They showed that the approximation is valid for calculating blood flow changes rather than absolute values for $\tau c << T$.

Qualitative method

LSI is qualitative rather than quantitative, that is, one can express the increase in CBF respect to some baseline, but expressing blood flow velocity is absolute term is difficult. This is because the auto correlation function of the system is unknown and one need to recourse to an approximation. However Parthasarathy et al. [73] propose to modulate the exposure time of the camera and thus collect several images, so to almost recreate the theoretical expression for speckle contrast for a lorentzian distribution of velocities, and derive the correlation time. So far this study has dealt with a microfluidic device, i.e. a phantom.
**Loss of spatial (temporal) resolution**

All image processing operations involving filtering induce a loss of spatial resolution (or temporal if the filtering is done in the time domain). Regarding LSI processing, only the Generalized Differences algorithm escapes the resolution decrease, but the images themselves are still speckled, and usually unusable in practice as many stationary images are required, defeating the dynamic purpose of LSI. See [81] for an exception in the imaging of seed germination process (beans) where the dynamic process is quite slow.

**Advantages and disadvantages**

**Advantages**

LSI is several orders of magnitude cheaper than most other functional imaging devices like fMRI, PET, CT, etc. In fact the image instrumentation devices (such as Laser, lenses, CCD camera) are usually part of a university physics lab, especially if it deals with optics. The small animal instrumentation devices (homeothermic blanket, stereotactic frame, etc…) are available in physiology lab using rats.

LSI devices are easy to use.

Continuous recordings over unlimited periods of time can be made with LSI, in order to measure blood flow changes over time, for example. Note that the images should not be compressed by traditional compressing methods as that would jeopardize the analysis of the speckles. The amount of data depends of camera resolution, frame rate, length of acquisition, number of trials and number of animals. For example, one of the datasets used in the SOF’s Chapter had the following characteristics: camera resolution 512x696, frame rate 23 Hz, length of acquisition 18s, giving 283 Mb of required space per trial/animal. This is hardly a problem with nowadays computer equipment.

Two-dimensional perfusion maps can be visualized by LSI, without scanning.

LSI does not assume steady-state conditions in perfusion. The exposure time of the camera is only 5 to 25 ms, capturing physiological phenomena which are slower.
Disadvantages

The vast majority of the literature claims that Laser Speckle Imaging is non-invasive. We note however that the technique still requires removing the scalp of the rodent, drilling through its skull and exposing its brain tissue, which can hardly be qualified of non-invasive. We agree however that LSI makes possible recording and imaging of tissue perfusion with minimal impact on microcirculation. In one particular study, Li et al. [59] image CBF through both the intact skull and without the skull. They note that LASCA does not resolve the blood vessels with the skull, but that LSTCA does.

Laser Speckle Imaging makes possible noninvasive recording and imaging of tissue perfusion with minimal impact on microcirculation. Note that while most work claims that LSI is “noninvasive”, in some cases, the technique still requires removing the scalp of the rodent, drilling through its skull and exposing its brain tissue.

The theoretical basis of LSI is not that well established. It is still unknown which velocity distribution (e.g., Voigt, Lorentzian, or Gaussian) should be used for the blood flow. The need to assume a specific velocity distribution to relate the speckle contrast to the tissue perfusion makes the technique less generally applicable. As a result, no absolute calibration is possible, i.e. the blood flow information is qualitative rather than quantitative. That is, one can estimate the increase (decrease) of blood flow velocity as a relative value, but not the absolute velocity.

LSI does not distinguish between nutritive (capillary) perfusion and global tissue perfusion.

LSI is an imaging technique that produces two-dimensional images only, and no information in depth. This disadvantage is intrinsic to the modality itself.
Chapter 8. CONCLUSIONS

LSI has been developed well over 25 years ago, and systematically marketed as “new” by the few who used it. Recently however there has been a surge in the number of publications related to laser speckle imaging. Few of those are actually neuro-physiological studies or, for that matter, strictly medical studies such as in retina analysis, skin burn assessment, etc. Instead the vast majority of the literature concentrated on two areas. Firstly, the early work by the group of Briers [7] contained a number of assumptions and shortcuts in the mathematical treatment of laser speckles. Many researchers then refined his model, corrected the mathematics, and there is a general consensus that the assumptions were in fact leading to usable results. The work of Briers at al. proved to be quite insightful. A second category of papers focused on improving the Spatio-temporal resolution of the speckle images. In other words, they described ways to improve the image processing and analysis pipeline, so that the images would provide better and more accurate information. This thesis fits in this second area.

Each of those papers, whose goal is to enhance LSI, implements a simple variation of the Briers contrast. While the original contrast is calculated in a 3-by-3 pixels window, in one single frame, the other papers improve upon the method by averaging the contrast over several frames, or by computing it in the time domain, or extending the window to a 3-by-3-pixels-by-10-frames (for example), or a combination of those methods. We’ve proposed two additional methods which differ radically from all the ones presented.

Second-Order Features is a method which uses higher-order statistics instead of the contrast, which results in higher quality images. Thanks to SOFs, images are smooth in areas that should have a homogeneous appearance but were corrupted by speckle (as in the image background); have high information content (as explained by a higher Entropy measure); and are sharp in places were thin and connected lines should be visible to the eyes (and intensity changes drastically from one side of a line to the other, a behavior observed along blood vessels).
Laser speckle contrast analysis (LASCA) has been demonstrated as a full-field method for imaging the cerebral blood flow (CBF). However, conventional LASCA is limited to extremely low dynamic range because of the ambient background field, dark current and anomalies in the circuits of CCD camera, which makes it difficult to analyze the spatiotemporal variabilities in CBF. For the second method, we proposed an enhanced laser speckle contrast analysis (eLASCA) method to improve the dynamic range of LASCA based on monotonic point transformation (MPT).

In an investigation on the influence of moderate hypothermia (32 § 0.5±C) on capillary CBF change, we used eLASCA. It presented much more significant (189%) decrease of CBF under hypothermia than LASCA (137%). Statistically, eLASCA resulted in a higher confidence degree (p = 0.009) of CBF change after the rewarming than LASCA (p = 0.013). In addition, eLASCA greatly improves the CBF visualization, which is very helpful in demonstrating the details of CBF change.

**Future work**

Regarding image processing there are now many tools available, from LASCA and its clones to SOFs and eLASCA. While in isolated cases we can demonstrate that one method is better over another method, it is possible that this is dependent upon the dataset. We have shown that eLASCA is better than tLASCA for CBF in hypothermia studies (tLASCA being better than LASCA was shown by [56]). We have shown that SOFs is better than tLASCA for CBF in whisker stimulation studies. What isn’t clear is whether eLASCA is better than SOFs, which we couldn’t test as we didn’t have the right to use the same dataset in both studies.

Le et al. [56] have shown that tLASCA is better than the “LSI” of Cheng et al. [19], which in turn performs better than LASCA. We cannot assume transitivity per se, therefore there is a need for a global study comparing all methods on the same dataset.

The wavelength of the laser light used in LSI is usually around 635 nm, which is red and in the visible spectrum. Modulating the wavelength in order to map tissue absorption and

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2 We wrote “LSI” between quotes because here it relates to the temporal version of the algorithm. Unfortunately Cheng and colleagues gave the same name for their algorithm than for the general method.
scattering properties has been done in illumination studies [24]. In the future, this method could be integrated and coregistered with laser speckle speckle imaging. Ultimately it could allow developing truly multidimensional methods for 3D tomography.

Speckle Interferometry [50] is a technique used in material science, allowing measurements of in-plane displacements and deformations. Particularly pertinent to blood flow is the direction of displacement. More research is needed to perhaps produce directional blood flow maps.


[99] D. A. Zimnyakov and A. B. Mishin (2001) "Blood microcirculation monitoring by use of spatial filtering of time-integrated speckle patterns: potentialities to improve the depth resolution" In: Optical diagnostics and sensing of biological fluids and glucose and cholesterol monitoring, SPIE proceedings series, San Jose, CA, vol. 4263, pp. 73-82