Clinical translation of laser speckle contrast imaging

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GENERAL INTRODUCTION AND OUTLINE OF THE THESIS

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GENERAL INTRODUCTION

Tissue- and organ perfusion have been of unremitting interest to surgeons, as both are strongly related to organ function and patient outcome. It is generally understood that adequate tissue perfusion prevents morbidity and mortality, as it is paramount for the natural healing process and a prerequisite for normal physiological function. This interest is widespread throughout medicine, ranging from reconstructive surgery, cancer surgery, organ transplantation and (burn-) wound management[1–4]. Understandably, surgeons have been looking for multiple ways in which to assess the state of perfusion in tissues and organs, such as by looking at bleeding of the resected edges, palpable pulsations of arteries, and tissue colour, as oxygenated blood is the ultimate read-out and the most important factor in adequate perfusion[5]. These subjective clinical indicators of tissue viability have proven to be effective, yet they heavily rely on the surgeons' experience, thus lacking quantitatively reproducible results and objectivity between surgeons[6]. During the last few decades, it has become possible to objectively measure arterial and venous perfusion with the use of traditional medical perfusion contrast enhanced imaging methods, such as computed tomography, ultrasound doppler, and magnetic resonance imaging. Although these imaging methods are now standard-of-care worldwide, they come with several limitations, such as large device size, the use of ionizing radiation, high costs, and the inability to be used during surgery in real time[7]. The vast increase in computing power, optical instrumentation, and detector technology have paved the way for a new class of imaging techniques which have the potential to solve these shortcomings: the so-called optical imaging modalities. These modalities share one commonality, as the information obtained is based on either light or special properties of photons in all cases. This has several advantages that facilitate easy implementation into standard-of-care, as these imaging techniques are generally available in a portable form, are cheaper to buy, and do not require strong magnetic fields or ionizing radiation[8].

This thesis is focussed on the clinical translation of a promising optical imaging method called Laser Speckle Contrast Imaging (LSCI). The scientific foundation for this technique was laid in 1981, when Fercher and Briers reported its first biomedical use, albeit far from clinically applicable due to the use of analogue camera systems[9]. The majority of the improvements that lead to the clinical applicability of LSCI took place in the past two decades. The user can now assess perfusion in the whole image (i.e. a full-field imaging technique) at real-time video framerates. LSCI has several advantages compared to other optical imaging techniques. Firstly, compared to the most common perfusion imaging technique, indocyanine green fluorescence imaging, LSCI has a major advantage, as there are no venous or arterial injections required. This makes LSCI theoretically suitable for many clinical applications without the use of a contrast agent[10]. It has to be noted that the traditional ultrasound doppler can measure perfusion deeper into the tissue. However, LSCI has the advantage of increased spatial resolution, whilst also

being a non-contact method. Lastly, even though laser doppler provides quantitative results, LSCI can approximate these results with the benefit of video frame rate imaging. This is clearly beneficial when compared to scanning times of up to several minutes for larger areas.

An LSCI setup is relatively simple, as only four components are required[11]. Firstly, a camera sensor is necessary to capture the images. Secondly, a laser light source is required to homogenously illuminate the tissue with coherent light. Thirdly, a diverging lens is used to produce a speckle pattern from the laser light, and fourthly, a computer is required to process the images. LSCI might sound complex, yet the principle is effectively explained in its name. In short: the *laser speckles* are projected onto the tissue and the loss *contrast* is *imaged* (i.e., calculated). These images are subsequently used to visualize perfusion. (In Dutch; de laser speckles worden op het weefsel geprojecteerd, waarna de vaagheid (*contrast*) wordt gemeten door de camera *(imaged)*)[12]. The laser light passing through the diverging lens (Figure 1A) projects laser speckles, which appear as random black and white dots (Figure 1B) caused by interference of the coherent light. The projected speckle pattern changes when there is movement. Due to the ability of coherent light to penetrate tissue, the movement can be detected either at the surface or subsurface. In the biomedical use case, the movement of interest is the subsurface movement of red blood cells. The contrast decreases when the speckle pattern changes (in other words; the picture gets more blurred when there is more movement of red blood cells). The contrast decrease can be quantified using a simple formula (Eq. 1), which can be related to blood flow.

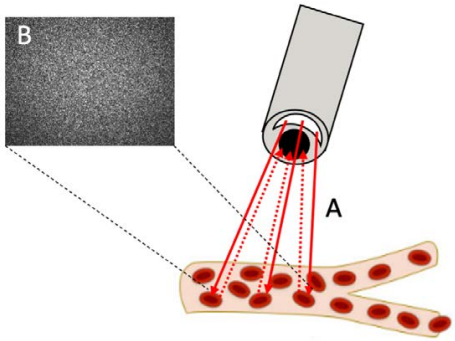

Figure 1 Figure 1 A schematic representation of laparoscopic laser speckle contrast imaging. The laser light (A) is used to project a speckle pattern on the tissue of interest (B). The speckle pattern changes based on movement, in this case movement of red blood cells. The change in speckle pattern is imaged using a camera and a simple formula is used to quantify the loss in contrast that corresponds to the amount of blood flow.

In more detail: The biomedical use of LSCI is based on the principle that backscattered light from tissue which is illuminated with coherent laser light (Figure 1A) forms a random interference pattern on the detector, the so-called speckle pattern (Figure 1B). The slightly different optical pathlengths cause waves to reach the observer during in- or out-phase, resulting in either bright or dark spots, respectively. The movement of particles inside the tissue causes fluctuations in this speckle pattern, resulting in the blurring of speckle images when

obtained with an exposure time equal to, or longer than, the speckle fluctuation time scale. This blurring can be related to blood flow, since the fluctuations are caused by the movement of red blood cells. Subsequently, the signal can be quantified in real-time using the simple mathematical formula (Eq. 1) for contrast K. Where σ is the standard deviation of the intensity I over the mean intensity $<I>$ calculated over a window in space or time.

$$K = \frac{\sigma}{< I >} \qquad (Eq. 1)$$

The contrast K can be calculated based on spatial or temporal changes. Spatial LSCI is calculated using a spatial window, typically of 7x7 or 5x5 pixels, in one frame[12]. Contrarily, temporal LSCI is calculated using the same pixel in multiple frames in time. This results in a high temporal resolution for spatial LSCI and vice versa[13]. Typically, a combination of both spatial- and temporal LSCI, the so-called spatiotemporal LSCI, is both tailored to, and subsequently used for, the intended use of the LSCI device. The calculated contrast K ranges between 1 for completely static and 0 for completely dynamic speckle regions. Generally, the inverse of *K* is calculated to relate contrast *K* to blood flow[12]. It remains indeterminate whether LSCI measures flow or velocity. However, the relation between the movement of red blood cells is clear, as the measured loss of contrast is directly related to the concentration and size of the scatterers[14].

To date, clinical acceptance of LSCI as a standard-of-care has not yet been reached. In this thesis, we show the successful clinical translation of LSCI by addressing methodological concerns with regards to robustness, reproducibility, and clinical applicability, showcased by introducing a laparoscopic laser speckle contrast imager for intraoperative perfusion measurements. This new form factor opens up unique opportunities for perfusion measurements during minimally invasive surgery.

OUTLINE OF THIS THESIS

The preclinical validation of LSCI as a non-invasive perfusion visualization tool will be performed on porcine kidneys and porcine small intestines. The cornerstones of an optical imaging protocol (i.e., standardized image acquisition, data processing, and image interpretation) are studied, and this knowledge is applied during the clinical translation of LSCI into a laparoscopic LSCI device called "PerfusiX-Imaging®" (previously named Lapvas-Imaging®). This device is used in clinical studies to evaluate whether it could help reduce anastomotic leakage. Anastomotic leakage is one of the most frequently occurring and feared complications in gastrointestinal surgery, and is defined as the leakage of enteric contents into the abdominal cavity, leading to high morbidity and even more significant mortality rates. Even though anastomotic leakage seems to be multifactorial, and the exact aetiology and sequence of events remain unknown, it is generally understood that adequate perfusion at the anastomosis site is a necessity for proper anastomotic healing. Thus, a reduction in anastomotic leakage could be achieved by

providing the surgeon with reliable and standardized perfusion images.
An in-depth explanation of the technical aspects of LSCI is described in **chapter 2**. This is followed by a review of the clinical applications of LSCI as they are currently described in literature. Even though LSCI is still in its infancy, applications in a wide variety of medical fields will be reviewed, such as in rheumatology, dermatology, neurology, ophthalmology, and gastro-intestinal surgery.

It is important to validate the ability of LSCI to measure small perfusion differences, as it remains unknown what a clinically relevant perfusion difference is, for instance with respect to anastomotic leakage. The ex vivo perfused porcine kidney model, commonly used to study novel transplantation and organ-perfusion preservation strategies, was deemed suitable[15]. The kidneys will be subjected to several models of hemodynamic changes. The LSCI perfusion data is compared with both the total renal blood flow and the side stream darkfield imaging[16,17]. The results of this preclinical bench study, which is the first to perform perfusion measurements on a human sized kidney using LSCI, is reported in **chapter 3**.

LSCI has the theoretical ability to detect the motion of a single red blood cell, which is the technological basis and underlying phenomenon for clinical use. However, this extreme sensitivity to motion is also its pitfall[13]. Unwanted motion is inevitable in a clinical setting, mainly in the form of breathing motions, pulsatile motions related to the heartbeat, and peristaltic movement of the colon. To correct for motion artefacts **Chapter 4** presents, validates, and discusses a new robust, multi-spectral, real-time LSCI motion correction and compensation model. The validation of the methodology is performed with a programmable 2D-motion platform on both a flow phantom and an *ex vivo* perfused porcine kidney, as is described in chapter 3. The improved LSCI model is the primary reason as to why the laparoscopic laser speckle contrast imager is able to produce clinically usable images, as it corrects for the peristaltic movement of the colon, the breathing motion of the patient, and the camera motion originating from the laparoscope operator.

LSCI is a fairly new, and relatively unknown, optical imaging method compared to other optical imaging modalities, such as fluorescence imaging. Fluorescence imaging uses contrast, either endogenous or exogenous, to optically enhance structures and tissues. The technology uses a light source to excite the fluorophore, which subsequently emits light in a different wavelength. This light is then detected by a camera sensor[18]. Fluorescence imaging can be used for perfusion imaging, biological structure identification, and tumour delineation. However, it often requires a (non-)targeted fluorescent tracer[19]. At present, fluorescence imaging is fairly common in clinical practice, especially in the form of indocyanine green fluorescence perfusion imaging in ophthalmology, and more recently in general surgery. LSCI and fluorescence imaging are both optical imaging methods that thus face similar challenges with respect to standardization, reproducibility, quantification, and establishment of clinical implementation. Chapter 5 and

chapter 6 are focused on the standardization of fluorescence imaging procedures, which is a necessity for successful clinical translation as already proven by computed tomography imaging, magnetic resonance imaging, and more recently by positron emission tomography imaging. **Chapter 5** provides a hands-on guideline for clinicians performing clinical optical imaging studies, and it ensures comparable data and outcomes by both minimizing and eliminating subjectivity and procedural irregularities. Furthermore, it provides better intra- and inter-observer output when using optical imaging. The guideline can be applied in both LSCI- and fluorescence imaging studies. **Chapter 6** provides a semi-quantitative model designed specifically for perfusion imaging using indocyanine green fluorescence imaging. The quantitative and reproducible modelling was directly applied in a prospective, observational, multi-centre, proof-of-principle study in ten patients undergoing a total thyroidectomy.

The findings from chapters 5 and 6 pertain to the laparoscopic laser speckle contrast imaging studies as described in chapters 7-10. The qualitative differentiation between well- and poorly perfused tissue is a valuable aspect of LSCI. In **chapter 7** we compare LSCI to blood lactate levels; the golden standard for ischemia (i.e., insufficient blood flow). As such, we can biologically validate ischemia with respect to LSCI values. Specifically, the laparoscopic laser speckle contrast imager is compared *in vivo* to the local capillary lactate levels in an ischemic bowel loop experiment on porcine small intestines. In **chapter 8** we demonstrate the use of laparoscopic LSCI during the construction of an intestinal anastomosis. We determine whether laparoscopic LSCI can distinguish between well- and poorly perfused tissue at the anastomosis site, in order to aid in the reduction of anastomotic leakage caused by insufficient blood flow. This hypothesis will be validated by purposefully creating differently perfused anastomoses in a porcine model.

Compared to *ex vivo* imaging, *in vivo* laparoscopic LSCI adds many complexities, such as specular reflections and movement of both the laparoscope and the tissue of interest. The first-in-human laparoscopic LSCI data can be found in **chapter 9**, describing perfusion measurements using two different wavelengths in ten patients undergoing a hemicolectomy. The laparoscopic laser speckle contrast imager will be validated using a classical ischemia-reperfusion experiment, which is induced by clamping the main feeding artery during surgery as part of the standard procedure. The setup is further validated on the nail fold of ten healthy volunteers by performing a tourniquet experiment. This model is used in multiple studies and allowed for comparison with currently available perfusion imaging techniques.

Chapter 10 is the culmination of all preceding chapters in this thesis. The study describes the largest LSCI clinical trial to date, with 67 patients undergoing an elective oncological colon resection. The prospective, observational multi-centre study is performed in order to study the interpretation of additional visual feedback, derived with the use of PerfusiX-Imaging, by surgeons, and the ability of the device to image colonic perfusion. Blinded to the surgeon,

perfusion images are made before intestinal transection at the intended location of the anastomosis. In order to assess the potential added clinical value, the surgeons are asked postoperatively whether the images are clear, and whether the location of the anastomosis would have been different based on the additional PerfusiX-Imaging derived perfusion images.

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