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SUMMARY, FUTURE PERSPECTIVES AND CONCLUSION

SUMMARY AND FUTURE PERSPECTIVES

Humankind is moving forward in an astonishing pace in every scientific specialty. Interestingly enough, the current standard of care for perfusion assessment has remained unchanged since the beginning of medicine. The desire to 'see' intraoperative perfusion has never been more urgent with the growing understanding that adequate perfusion is an essential aspect of organ function or for instance wound healing and therefore plays a leading role in preventing mortality and morbidity throughout a multitude of disease states[1]. Current perfusion assessment is performed by the surgeon's naked eye and generally based on subjective clinical indicators of tissue viability (e.g., tissue colour, bleeding of the resected edges or pulsatile motion of tissue)[2]. The sum of these, sometimes ill-defined visual clues, is the surgeon's specific fingerspitzengefühl that takes a long time to develop and is lost at the end of his or her career, notwithstanding its reliability, accuracy and reproducibility. The high degree of inherent subjectivity of the surgeon's assessment of perfusion fuels the need for an objective imaging method capable of real-time imaging of perfusion in organs and tissues[3].

Perfusion imaging is of interest in many medical disciplines as the state of perfusion is indicative of the vitality of the tissue of interest. Disturbance of the normal perfusion state is an indication of imminent illness, immediate or threating loss of (organ) function or even the initiation of chronic disease. For example, perfusion imaging could be used to monitor the state of inflamed joints in patients with rheumatoid arthritis, foot ulcers in patients with diabetes and the state of the nailfold capillaries in patients with Raynaud's syndrome[4–6]. In the surgical setting, perfusion imaging is used intraoperatively to monitor the consequence of the inevitable vascular damage and the consequent failure of tissue healing, of great importance during and after surgical procedures.

It is important to realise that assessment of perfusion by means of imaging does not have any therapeutic effect by itself, yet, it can be regarded as a so-called 'red-flag' diagnostic tool. It is the surgeons' job to i) perform a standardized imaging, ii) to correctly interpret the images, and iii) act upon it in a sensible way, always weighing the consequences of either acting or reticent observation. Only when all of the three pillars previously mentioned are in place, a well-weighted and imaging-data based change in clinical outcome may be anticipated. Therefore, perfusion assessment itself functions as an additional source of important real-time information that the surgeon can tap into during clinical decision making at the moment it counts the most. Providing surgeons with sophisticated tools that allow them to 'see' perfusion in an objective manner is the essence of the added clinical value of perfusion imaging. Acting on the additional visual feedback could result in for instance the relocation of a colonic anastomosis to well perfused colon tissue thereby preventing a possible anastomotic leakage[7,8]. Or simply, that a transplanted kidney is repositioned in the recipient to remedy a twisted or obstructed artery or arterial anastomosis[9]. Today, few of these (potential) use cases have been established as standard-of-care for

intraoperative perfusion imaging. In this thesis we describe the development and validation of a new multi-organ perfusion imaging device based on laser speckle contrast imaging (LSCI).

The aim of this thesis is to understand, validate and improve LSCI for clinical perfusion imaging (part I), to establish a scientifically sound imaging protocol (part II) and to study the in-human implementation of laparoscopic LSCI in colon surgery to determine the optimal location for an anastomosis (part III).

Chapter 1 provides a general introduction of LSCI and perfusion imaging as well as the outline of this thesis. The first part of this thesis is focused on understanding and validating LSCI for perfusion imaging providing the basic concepts of the modality and its clinical potential.

Part I: Perfusion assessment using LSCI

Chapter 2 is a narrative review of the clinical applications of LSCI. The manuscript provides an outline of the mechanism of LSCI by explaining the basic mathematical principles. We review recent refinements and improvements of standard LSCI to provide quantitative results and improve motion artefact robustness, such as multi-exposure speckle imaging and movement artefact correction methods. Multi-exposure speckle imaging yields quantitative flow measurements as this model shows linearity with relative changes in flow speed over a broad range of velocities[10–12]. To the contrary, single-exposure becomes less accurate for larger variations. Movement artefacts are known as the foremost problem in LSCI as the biomedical application is based on the detection of the motion of red blood cells with a diameter of 0.0075mm[13]. This extremely high sensitivity to motion is also the potential pitfall. Movement of a patient, typically in the range of multiple centimetres (or 10^5 times larger), outweighs the LSCI signal of the motion of the red blood cells. This results in perfusion data heavily influenced by the motion of the patient instead of motion of the red blood cells. Several efforts were made to solve this problem, for example by using stickers to track the patient movement in combination with some form of image registration[14]. Another common method is to deduct the movement of a non-biological surface from the measure speckle contrast of the tissue of interest[15]. Although effective to some extent these methods far from real-time. Therefore, the need for a robust, real-time movement correction still exists. Our new solution to solving these problems is proposed in chapter 4, consisting of a real-time motion compensation and correction based on dual-wavelength optical flow algorithms. Quantification and movement artefact robustness are the main challenges for the clinical acceptance of LSCI. In this review we show that LSCI has not yet been established as a standard-of-care in any field of medicine. The low clinical acceptance might be caused by a relative unfamiliarity of the technique in hospitals in combination with the lack of commercially available camera systems, apart from quantification and movement artefact robustness. The clinical potential of LSCI as a future part of the standard-of-care is shown in data from studies in rheumatology, burn care, dermatology,

neurology and gastrointestinal surgery. With an improved and robust LSCI device, the technology could possibly expand to many more medical fields, obviously supported by sufficiently powered clinical diagnostic accuracy studies, standardized imaging and inter-observer agreement protocols on interpretation of the data.

Chapter 3 is focused on the validation of LSCI for sub-surface perfusion measurements on an isolated whole-blood machine-perfused porcine kidney model. The monitoring of the sub-surface perfusion seems clinically relevant in an explanted kidney since the functional tissues, such as glomeruli, are situated in the renal cortex[16–19]. A set of classical hemodynamic experiments was performed to validate LSCI. The perfusion model, designed by the transplantation surgery department of the University Medical Center Groningen, allows for high levels of control of hemodynamic parameters including temperature, blood pressure, renal resistance, renal function and most importantly total renal blood flow[20]. This model allowed us to manipulate the total renal blood flow whilst monitoring the cortical blood flow using LSCI. This study was the first to measure perfusion by means of LSCI in a human-sized kidney and gave us a better understanding of LSCI by applying various hemodynamic conditions during the experiments. We concluded that the correlation between the total renal blood flow and LSCI was influenced by the biological relocalization of flow during a state of hypoperfusion. In other words, when the kidney was presented with insufficient blood inflow, the cortical perfusion was prioritized over the medulla. This seems logical as the kidney relocates the blood flow to remain its vital function[21]. To confirm that LSCI was indeed measuring blood flow, the data was compared to sidestream darkfield imaging. sidestream darkfield imaging is a microscopy method that can optically track the movement of individual red blood cells resulting in quantitative blood flow measurements[22,23]. The good correlation between sidestream darkfield imaging and LSCI confirmed that LSCI measures sub-surface perfusion. This study showed that LSCI could detect small changes in the renal cortical microcirculation with high spatial and temporal resolution. Further exploration and implementation of LSCI during transplant surgery could help with the early establishment of an appropriate treatment plan directly after reperfusion of the organ in case of decreased perfusion, further increasing the functional outcome of renal grafts.

Chapter 4 presents a potential solution to the impact of motion artefacts in the form of a real-time multi-spectral movement artefact correction and compensation model. This model is the basis for the studies described in part III where measurements are affected by breathing artefacts and peristaltic movement of the colon. The effectiveness of the model is studied by subduing a flow phantom and the isolated perfused porcine kidney (from chapter 3) to computer-controlled movement artefacts. The new LSCI model uses optical flow and sudden changes in LSCI values to correct and compensate for movement artefacts. The images are corrected using image registration and compensated by means of a weighted average. It resulted in a large signal to noise ratio improvement and reduced overestimation of perfusion. This allowed us to differentiate between ischemic and non-ischemic tissue on a moving kidney whereas this was not possible using the standard LSCI model. The advantages over conventional movement artefact correction

methods are that this model is real-time and does not require any form of sticker or fiducial marker to be placed on the tissue of interest[14,24–27]. The implementation of this model into clinical practice could improve perfusion imaging in moving patients such as in burn wound patients who shiver often related to pain or in surgical settings where it is hard to place sterile fiducial markers such as during laparoscopic gastrointestinal surgery, described in chapters 7 through 10.

The studies presented in part I showed us that the potential of LSCI is the intrinsic ability to directly visualize even the slightest changes in perfusion. This is a valuable characteristic in the clinical setting since the gold standard, the surgeon's eye, requires a considerable amount of time before perfusion differences become apparent via tissue discoloration. In general, the predictive power of LSCI could be relevant in situations concerning a disturbance of the normal perfusion state. The studies presented in the first part of this thesis give rise to the subsequent question; what is the clinical value of the additional visual feedback which will be studied in part III.

Part II: Standardization and reproducibility in fluorescence imaging

The second part of this thesis is focused on the standardization and reproducibility of fluorescence imaging procedures. Fluorescence imaging and LSCI are different techniques, yet, both are optical imaging methods meaning that light (i.e., non-iodizing radiation) is the primary source of information used for imaging[28]. Consequently, all optical imaging methods, to some extent, are subject to the same physical principles. The principles described in the following two chapters should thus be integrated in fluorescence imaging and can be extrapolated to LSCI perfusion measurements.

Chapter 5 is a guideline for clinicians performing clinical studies with fluorescence imaging, focused on the standardization of the imaging procedures. Fluorescence imaging is an emerging optical imaging technique that has shown many benefits for clinical care[29–31]. The number of clinicians having access to fluorescence imaging systems is rapidly growing, whilst the knowledge required to choose the appropriate imaging approach and its subsequent use and interpretation for the clinical problem is often missing. The design of a scientifically substantiated protocol requires a basic understanding of the underlying physics of fluorescence imaging. In this chapter, the main constituents of a uniform fluorescence imaging protocol, that matches the clinical need, are discussed to ensure consistent study designs and reliable data collection for implementation in future standard of care in clinical trials. Clinical acceptance of fluorescence imaging requires standardized and reproducible clinical data based on a scientifically substantiated imaging approach that relies on the cornerstones of science; reproducibility and standardization. The manuscript goes over the constituents of a protocol in detail, such as clinical indication, the applied fluorescence imaging camera system, target moiety, signalling compound, standardized image acquisition, data processing and image interpretation. Also, the benchmarking of camera systems is described since results are greatly affected by camera characteristics such as detection sensitivity, depth sensitivity, field illumination homogeneity, exposure time, resolution and dynamic

range. Moreover, imaging procedures must be standardized regarding tracer administration (i.e., dose, volume, infusion rate), working distance, incident angle and ambient light. The results of the standardized procedure should be expressed by using the contrast-to-noise ratio and images should be presented using perceptually uniform scientific derived colour maps (e.g., Viridis)[32]. These specifically designed colour maps will maximize the interobserver agreement of imaging data interpretation. These factors, especially regarding the standardized imaging procedure, should also be considered when performing a LSCI clinical trial.

In **Chapter 6**, we describe a clinical study with the most commonly used perfusion imaging technique to date: indocyanine green fluorescence perfusion imaging. Indocyanine green fluorescence perfusion imaging has been used for many years to assess organ function and vascularization[33]. The aim of this study was to develop and implement a reproducible model for indocyanine green fluorescence perfusion imaging that was based on standardization and quantification in endocrine thyroid surgery. This is a necessity since the interpretation of indocyanine green fluorescence perfusion imaging results are subjective (i.e., qualitative observations instead of quantitative) and error-prone due to the lack of a standardized protocol[34]. The surgeons' visual interpretation was studied yielding interesting results. Three out of four surgeons misjudged the subjective visual interpretation of indocyanine green fluorescence perfusion imaging in one of the patients with hypoparathyroidism. Moreover, in five out of seven patients without hypoparathyroidism, a surgeon would perform unnecessary auto-transplantation based on the subjective qualitative assessment. This yields the surgeons' accuracy for assessing the viability of a parathyroid gland of 60%, stressing the need for quantification and standardization in imaging procedures. Our proposed standardization protocol minimizes the unwanted variation in the data, increasing the quality of the data. The quantification model is based on the hypothesis that a perfusion-related complication, either arterial or venous, will reveal itself in the presence of compromised inflow or outflow. Hence, the inflow-to-outflow curves are rated on five quantification endpoints. The model was implemented in parathyroid glands within a thyroidectomy procedure to study the clinical feasibility. During the clinical multi-centre study, the model showed promising results for hypoparathyroidism prevention based on the proposed quantification endpoints[35].

Part III: Laparoscopic laser speckle contrast imaging

The third part of this thesis is focused on the clinical feasibility and implementation of laparoscopic LSCI. Laparoscopic LSCI could have substantial clinical impact as the use of minimally invasive surgery is increasing worldwide. The development of laparoscopic LSCI was instigated by the desire to decrease the incidence of a severe complication in colon surgery: anastomotic leakage[36]. If a patient develops anastomotic leakage, the enteric content contaminates the peritoneal cavity causing significant morbidity and sometimes even mortality. Anastomotic leakage is multifactorial, including factors outside the surgeon's power (e.g., lifestyle, smoking, cardiovascular disease and body mass index among others)[37–39] and factors that can be controlled by the surgeons

(e.g., adherence to surgical standards and anastomotic technique)[40]. However, the general consensus is that adequate tissue perfusion is one of the most important prerequisites of optimal anastomotic healing[41]. The idea of intraoperative perfusion assessment was conceptualized in the LCSI PerfusiX-Imaging® project in 2014 (at that time named the Lapvas-Imaging project). PerfusiX-Imaging® is designed as a laparoscopic LSCI device that enables surgeons to see real-time the perfusion of tissues. In case of the colon, the output of the device could assure that the anastomosis is placed in well-perfused tissue and consequently, in term could possibly reduce anastomotic leakage rates. The findings from the previously described chapters have been the basis for the development of the device and are validated in the following chapters.

As mentioned above, ischemia (i.e., a shortage of oxygenated blood) at the site of the bowel anastomosis is thought to increase the chance of anastomotic leakage[3,42,43]. Hence, if anastomotic leakage is to be prevented, the device should be able to identify ischemic tissue correctly. **Chapter 7** validates PerfusiX-Imaging® using local capillary lactate levels. Lactate levels increase due to the lack of oxygen, and is one of the most used metrics in the world of medicine and sports (in Dutch; verzuring)[44,45]. For this experiment, an ischemic bowel loop model was used. Ischemic areas were created on the small intestine. The local capillary lactate was measured after a set amount of time. LSCI perfusion images were made outside the abdominal cavity directly after the creation of the loop, and in sync with capillary lactate measurements. As a result, the emergence of ischemia could be fittingly predicted immediately after perfusion induction, whereas decreased intestinal viability could only be seen with the human eye after 30 minutes. Additionally, a significant relation was found between local capillary lactate and LSCI values. In conclusion, PerfusiX-Imaging® achieves real-time intraoperative visualization of intestinal microperfusion deficits, potentially predicting postoperative ischemic complications than solely with the surgeon's eye.

Chapter 8 describes the demonstration of PerfusiX-Imaging® for visualization of local intestinal perfusion during the construction of a small bowel anastomosis. Three segments were isolated from the small intestine of a pig. The perfusion was compromised by coagulating several mesenteric feeding arteries. Bowel ends were used to create three differently perfused anastomoses of the afferent and efferent loop; a well/well perfused, a well/poorly perfused and a poorly/poorly perfused anastomosis. The laparoscopic PerfusiX-Imaging® camera allowed for continuous assessment of local intestinal perfusion. The perfusion deficits at the anastomoses were directly visualized and available to the surgeon for assessment of perfusion status. The study demonstrated that laparoscopic LSCI can distinguish between well- and poorly perfused tissue around the anastomosis.

The initial human clinical validation is described in **chapter 9** where we aimed to answer the fundamental question: is laparoscopic LSCI capable of measuring colonic blood flow? This was studied by means of the vascular occlusion test, a classical hemodynamic experimentwhere the feeding artery was clamped for a pre-defined period depriving the tissue of blood, followed by

reperfusion[46,47]. This phase of reperfusion is characterized by a short period in which the blood flow overshoots the baseline flow. After a short period, the blood flow resettles at the baseline level. To validate the LCSI device, this experiment was performed on the colon and compared to the ischemia reperfusion on the nail fold of healthy volunteers. In this prospective observational multi-centre study, a total of fourteen patients were included along with ten healthy volunteers for the nail fold evaluation. We show in this first in-human study show that the LCSI setup is capable of measuring blood flow intra-abdominally. However, improvements with regards to the image quality were required to become clinically usefull as the quality of the images is not yet sufficient.

The final chapter of this thesis, **chapter 10**, describes a clinical feasibility study, called the SCOUT-I study, where we investigated the potential clinical value of PerfusiX-Imaging® perfusion measurements. The perfusion images were made of the tissue surrounding the anastomosis. After surgery, the images were shown to the surgeon with the question whether they, in retrospect, would have changed the location of the anastomosis. A total of sixty-seven patients were included in this trial and all imaging procedures were successfully executed. The images showed eminent perfusion differences signifying the clinical relevance of perfusion imaging as the watershed area (i.e., the area where well- and poor perfused tissue meet) lies very close to the anastomosis. The data indicate that surgeons would relocate the anastomosis in a large number of patients based on the LCSI findings. The average added surgical time for assessment of the two imaging moments for evaluating the perfusion status was limited to 2.5 minutes. This is shorter compared to indocyanine green fluorescence perfusion imaging, since there is no fluorescent dye injection or inflow time required for LSCI[48]. The lack of intravenous injection of a dye when using LCSI also implies that surgeons can instantaneously, continuously and repetitively measure perfusion in real-time[49]. The low inter-operator variability observed shows promise for this technology as this indicates that, even though this is a qualitative measurement, the surgeons' collective judgement is quite objective. Lastly, the average relocation distance of 1.3 centimetre as observed is indicative of the delicacy of the anastomosis placement. Due to the prospective observational nature of this study, the data can only lead to speculative conclusions regarding anastomotic leakage. Hence, a large intervention study should be performed to establish the impact of LCSI application on anastomotic leakage.

Future perspectives

In this thesis, we have studied and validated LSCI perfusion imaging in pre-clinical and phase I observational studies. Additional phase II and III intervention studies are required to determine the actual benefit for the patient and possible implementation in standard-of-care. Relevant clinical parameters should be taken as endpoints to determine the added clinical value of LSCI as a perfusion imaging tool, such as reduction in anastomotic leakage rates, decreased hospitalization time, decreased mobidities and mortalities and lower re-operation rates. The study protocols should be set up according to the standardization principles described in part II of this thesis. This is especially relevant for larger multi-centre studies as the data collection often

takes place in multiple centres with different surgeons and investigators, whereas regulatory bodies will require intra- and inter-observer agreement with a clearly defined standard operating procedure for using the LCSI system.

The pre-clinical work described in chapters 2 and 3 suggests clinical relevance of LSCI also in transplantation surgery. As for renal graft function analysis, LSCI can measure the sub-surface perfusion which is representative of the kidney function since most functional structures like the glomeruli are situated in the cortex[16–19]. It is foreseeable that future LCSI imaging will be performed directly after anastomoses of the arteries to detect possible perfusion deficits9. Another potential fit could be kidney graft quality monitoring before transplantation to reduce graft rejection rates. This would require a transmogrification of a traditional kidney perfusion system into a transportable LSCI-kidney transplant system.

The clinical feasibility study with PerfusiX-Imaging® in colon surgery, named SCOUT-I study, was set up to be used to design a SCOUT-II pivotal study to confirm clinical efficacy, safety and risks. Similar studies regarding perfusion imaging and anastomotic leakage rates have reported quite mixed results[50]. However, a recent meta-analyses implies a strong downward trend towards a preventive impact on anastomotic leakage post-operatively when using perfusion imaging assessment, yet, not statistically significant[41,51]. This might be caused by underpowered studies due to the multi-factorial origin of anastomotic leakage or the lack of uniform executed imaging protocols – as outlined earlier.

This thesis includes several studies describing LCSI perfusion measurements of the skin, kidney, small intestine and colon. Naturally, this gives rise to the question whether this technology can be used on other organs. From the physics of LSCI follows that, in theory, perfusion imaging could be performed on more types of tissue, depending on the optical properties and accessibility. From this follows that the range of clinical applications of LSCI could be expanded as long as the clinical need can be solved by qualitative perfusion maps. For example, imaging of the gastric conduit during oesophageal resections could help with the assurance that the anastomosis is created with well-perfused tissue similar to the SCOUT-I as applied for colon anastomoses. Other possible clinical applications could be for free flap monitoring reconstructive surgery, burn wound monitoring, thoracoscopic segmentectomy, wound ulcer monitoring in peripheral arterial disease patients, monitoring of skin disease in dermatology, general wound monitoring, determination of amputation levels in peripheral vascular disease, endocrine surgery, vascular neurosurgery and possibly more.

Currently, LSCI is a fairly novel and unknown technique to clinicians that is clinically explored because of its potential for perfusion imaging. Phase II studies, such as those described above, would help with the general acceptance as long as the endpoints are clinically relevant and the scientific evidence is provided through sound and well-powered studies. LSCI's clinical acceptance could also benefit from comparative studies with the more generally accepted

imaging technique indocyanine green fluorescence perfusion imaging since this can, in most cases, be complemented or replaced with devices such as PerfusiX-Imaging®[48,49]. LSCI comes with several paramount advantages that all revolve around indocyanine green fluorescence perfusion imaging requiring a fluorescent dye to be systemically intravenously injected. Apart from possible health risks from the injection of a fluorescent dye, LSCI measures movement of red blood cells compared to the detection of the presence of fluorescence particles[48,49]. Indocyanine green fluorescence imaging can give the false impression that there is perfusion in a situation where blood flow is actually stagnant. In this situation, LSCI will not indicate perfusion since there is no movement of red blood cells, the carrier of oxygen. In essence, LSCI allows the surgeon to manipulate tissue and monitor the perfusion in real-time. The half-life of indocyanine green is relatively short resulting in an equally short imaging window compared to continuous imaging with LSCI. Also, even though the fluorescent dye is removed from the circulation by the liver relatively fast, remaining traces of the dye influences repeated measurements[52]. The prospect of repeated, continuous and instantaneous dye-free and real-time perfusion measurements could motivate surgeons to start using LSCI[48,49].

The currently available LSCI models allow for qualitative 2D-perfusion maps, however, in some situations, the quantification of the signal might become important(e.g., cut-off values to assist in clinical decision-making)[53]. Currently, general acceptance and applicability of a LSCI quantification method has yet to be achieved. The most promising and used model is the multi-exposure speckle imaging model first reported in 2008[10]. In this model, the velocity distribution is estimated resulting in linearity over a broader range of flow velocities. Only few groups have successfully applied this model, which could be attributed to the complexity of achieving real-time multi exposure speckle imaging [11,12,47,54,55]. Recently, an effort was made to solve this by combining the multi exposure speckle images with machine learning[56–59]. This combination effectively solves the non-real-time imaging issue, which is essential for clinical adaptation. The machine learning was used to combine shorter exposure time images (e.g., 1ms) into longer exposure time images (e.g., 2, 5, 10 and 20ms) in real-time to obtain the multiple exposures required for multiple-exposure speckle imaging. The quantification of the speckle signal should receive more attention even though it might not seem detrimental at the time since this could hinder the clinical acceptance of LSCI. Such as the current quantification issues in indocyanine green fluorescence perfusion imaging.

Imaging modalities typically show superiority in only one aspect of imaging (e.g., perfusion imaging, tumour delineation, tissue structure detection) combined with inferiority in other aspects. This signifies that clinician should investigate the use of multimodality systems for a complete image guided surgery approach. The combination of such technologies into one imaging device or imaging approach could give surgeons more guidance both intra- and post-operatively. It is probable that a combination of imaging techniques is developed for each clinical need, but is also strongly dependent on the commercialization and reimbursement opportunities of such systems. The optical imaging field is broader than the modalities mentioned in this thesis (i.e.,

LSCI and fluorescence imaging). For example, another promising modality is optoacoustic imaging for the detection of tissue chromophores based on the photoacoustic effect, earlier shown to be easily combined with fluorescence imaging60. Diffuse reflectance spectroscopy is a contact-method worth mentioning as this can be used to detect positive margins without having to deal with scattering properties of tissue. The intra-operative and post-operative use has been demonstrated to be effective in many different cancer types[61–64]. In case of oncological resections, the image guided surgery approach can entail the combination of a fluorescent tracer and fluorescence imaging system to delineate the tumour intraoperative. The fluorescence imaging would preferably be used in combination with spatial frequency domain imaging to help account for the scattering and optical properties of the tissue[65]. Once the tumour has been taken out, the surgeon could validate a negative margin using diffuse reflectance spectroscopy ex vivo on the specimen. Afterwards, the surgeon could close the wound bed and perform perfusion imaging using LSCI to ensure swift recovery of the patient.

An imaging supported approach of an oncological resection also could give rise to a new, specialized class of surgeons; the optical imaging interventional surgeon. These surgeons are experts in gathering and interpreting imaging data, knowing how to collect, interpret and analyse the real-time imaging data during surgical procedures. Surgeons with this kind of expertise already do exist, often unwittingly of their special expertise, mostly in university hospitals where they are the specialist in their department. However, with the vast increase of commercially available optical imaging systems, this technology now ends up with more inexperienced surgeons who are sometimes unaware of basic optical imaging principles and pittfalls as described in chapter 5, and thus objective, accurate, reproducible and reliable interpretation of imaging data. This could lead to the organic beginning of this new class of trained surgeons by instigating the awareness that operating and understanding optical imaging modalities is subject to training and thus a learning curve. This is nothing new as this is comparable to the classical imaging methods that now have their own class of doctor, i.e., the radiologist and nuclear physician.

One of the major advantages of optical imaging compared to classical medical imaging (e.g., computed tomography, magnetic resonance imaging, positron emission tomography, roentgen imaging) is that the components required are typically low cost and readily available. For example, a LSCI setup typically requires a low-powered laser, diverging optics, a camera and a processor to generate the perfusion images. Fluorescence imaging is relatively easy with the requirement of having a (LED or laser) light source, optical filters and a camera sensor. These low-cost components could help with solving global health challenges by bringing cutting edge technologies to low-income countries, such as ophthalmology for the detection of ocular malaria or malarial retinopathy. This has not been unnoticed as efforts have been made to create low cost systems, for example fluorescence imaging systems[66,67]. Yet, for the case of fluorescence imaging, the fluorescent tracers should also be made affordable for it to be used in low-income countries68. As for LSCI, as there are no tracers required, a low-cost setup could be used ideally created in an open-source platform. Efforts have been made and a system was produced using

a webcam for as low as 90$[69]. Others have made portable LSCI systems in combination with smartphones[70,71] all efforts lowering the bar for a world-wide adaptation of fluorescence and LCSI imaging in clinical practice.

The use cases described in this thesis are all related to surgery, yet, the use of LSCI in outpatient clinics and at the general practitioner could also help decrease the burden on the healthcare system. Early diagnosis and treatment of certain perfusion related diseases might prevent repeated hospital visits. For example, suppose the general practitioner or wound consultant monitors patients with peripheral arterial disease or diabetic ulcers, aggravation of ulcer forming and amputation can possibly be prevented. Moreover, the monitoring of chronic wounds and infections is a potential application for LCSI, as it could prevent damage both financially and environmentally. The financial costs are reduced since further care is prevented. The environmental impact of healthcare subsequently also will decrease. For example, in magnetic resonance imaging, the energy consumed by the imaging device itself only constitutes 38% of the total energy of one imaging procedure whilst most energy is consumed by transportation to the hospital plus the energy to manufacture the required disposable, consumable and reusable products[72]. This study implies that prevention is more effective compared to looking for greener alternatives (i.e., greener disposables still have a larger environmental impact that consuming no disposables). It also signifies that medical innovation should be designed to positively affect the healthcare system and more importantly our greatest challenge to date; the environment. Contrarily, a recent study was published on the contribution of healthcare sectors in driving environmental damage that in turn puts human health at risk[73]. Interestingly, that particular paper shows that health care practitioners put great effort in mitigating negative health effects of environmental damage. However, the global supply chains that feed into the enhanced activity of the healthcare sectors itself initiate adverse feedback cycles by increasing the environmental impact, thus effectively counteracting the mission of healthcare. Even though there are a multitude of papers that determine the environmental impact of healthcare, they all seem to agree its negative effect[74–77]. In the authors opinion, medical device companies should map their environmental impact and minimize carbon footprints and waste production. For example, by maintaining an environmental focus and limiting their environmental impact by eliminating single use disposables. Additionally, care givers should also become aware of their environmental impact.

CONCLUSION

This thesis describes the clinical translation of laparoscopic LSCI. The basic and more fundamental pre-clinical studies provide evidence that LSCI can measure the slightest of perfusion differences in real-time in both experimental and real-life studies. When this technology is used in a standardized and reproducible imaging procedure, it can yield reliable perfusion measurements supporting day-to-day clinical decision-making. The clinical value in minimally invasive surgery has yet to be determined using intervention studies, however, the observational studies show great potential. In short: 'LCSI is on the Move!'

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