Laser Speckle Contrast Imaging in Reconstructive Surgery

Johan Zötterman

LINKÖPING
UNIVERSITY

Linköping University Medical Dissertation

No. 1735

Laser Speckle Contrast Imaging in Reconstructive Surgery

Johan Zötterman

Department of Biomedical and Clinical Sciences

Linköping University

Supervisors

Simon Farnebo, MD, PhD
Adj. Professor
Department of Biomedical and Clinical Sciences
Linköping University

Erik Tesselaar, PhD
Assoc. Prof.
Department of Biomedical and Clinical Sciences
Linköping University

Jacob Wikner, PhD
Assoc. Prof.
Department of Electrical Engineering
Linköping University

Opponent

Henry Svensson, MD, PhD
Professor
Department of Clinical Sciences, Malmö
Faculty of Medicine
Lund University

Committee board

Karl-Eric Magnusson, MD, PhD
Professor emeritus
Department of Biomedical and Clinical Sciences
Linköping University

Malin Malmström, MD, PhD
Professor and Senior Consultant
Department of Clinical Sciences Lund,
Ophthalmology, Lund University, Sweden

Ingemar Fredriksson, PhD
Assoc. Prof., Adjunct Senior Lecturer
Department of Biomedical Engineering
Linköping University.

©Johan Zötterman

Printed in Sweden by LiU-tryck, Linköping, Sweden 2020.

Front page: "The Italian method" Typ 525.97.820, Houghton Library, Harvard University

Illustrations by Johan Zötterman unless otherwise stated.

ISBN: 978-91-7929-872-2

ISSN 03450082

To my family

Table of contents

Abstract	7
List of abbreviations	9
List of original papers	11
Introduction	12
Reconstructive surgery	13
Historical review	13
Modern reconstructive surgery	15
The reconstructive ladder	16
Microsurgery	17
Microsurgical free flaps	17
Breast reconstruction with free flaps	18
Deep inferior epigastric artery perforator flap	19
The dermal circulation in reconstructive surgery	21
Perforators and angiosomes	22
Assessing microcirculation and tissue monitoring in reconstructive surgery	24
Intraoperative perfusion mapping in reconstructive surgery	25
Postoperative monitoring in reconstructive surgery	27
Using laser to assess microcirculation in the skin	29
Laser Doppler flowmetry	30
Laser speckle contrast imaging	32
Aim of thesis	37
Methods	39
Subjects and environmental conditions	39
Methyl nicotinate	39
Animal model	40
Equipment	40
Laser speckle contrast imaging	40
Laser Doppler flowmetry	41
Flow probes	41
Laboratory shaker	41
Data processing and statistics	41
Review of papers and results	43
Paper I: Methodological concerns with laser speckle contrast imaging in clinical evaluation of microcirculation	43
Summary	43

Methods.....	43
Results.....	44
Conclusion	46
Paper II: Monitoring of partial and full venous outflow obstruction in a porcine flap model using laser speckle contrast imaging.....	47
Summary	47
Methods.....	47
Results.....	48
Conclusion	49
Paper III: The use of laser speckle contrast imaging to predict flap necrosis: an experimental study in a porcine flap model	50
Summary	50
Methods.....	50
Results.....	51
Conclusion	52
Paper IV: Intraoperative laser speckle contrast imaging in DIEP breast reconstruction: a prospective case series study.....	53
Summary	53
Method.....	53
Results.....	54
Conclusion	55
Discussion.....	57
General comments	57
Methodological factors that affect the measurements	58
Physiological factors that affect the measurements	59
The animal model.....	60
Laser speckle contrast imaging in reconstructive surgery.....	60
Is laser speckle contrast imaging observer independent?.....	62
The future of tissue monitoring with laser speckle contrast imaging	63
Conclusion	64
References.....	67
Populärvetenskaplig sammanfattning	75

Abstract

Objectives

Reconstructive surgery aims to restore function or normal appearance by reconstructing defective organs after trauma or disease. In patients undergoing reconstructive surgery, previous trauma, surgery or radiotherapy can result in compromised blood supply. This will affect the viability of the tissue and increases the risk for postoperative complications, such as ischemia and infection. It is therefore important to assess the tissue viability, both before, during and after the surgery. This can be done using different techniques that monitor the perfusion of the skin covering the affected area. In this thesis, LSCI have been evaluated for tissue monitoring in reconstructive surgery. The technique allows for a fast and non-invasive assessment of superficial tissue perfusion over a wide field. Based on previous work on the technology, we have seen clear advantages with LSCI compared to other methods, for example laser Doppler flowmetry (LDF). We have evaluated laser speckle contrast imaging (LSCI) as a tool for tissue monitoring in reconstructive surgery in four studies.

Methods

In study I we used a bench top model and healthy subjects to address methodological concerns subjected to the LSCI technology. We investigated the effect of motion distance and angle on the assessed perfusion value

In study II we used a porcine model to compare LSCI and LDF as tools to detect partial and full venous outflow obstruction. We used both methods to assess a flap based on the cranial gluteal artery perforator with partial and complete occlusion of the vein and artery.

In study III we used the same porcine model as in study II to investigate the possibility to use LSCI intraoperatively to identify flap areas with compromised circulation and thereby predict areas with a high risk of postoperative necrosis.

In study IV we used LSCI for intraoperative evaluation of tissue viability during deep inferior epigastric perforator (DIEP) free flap surgery and to investigate the perfusion distribution according to the Hartrampf zones, as measured with LSCI, in relation to the selected perforator in the deep inferior epigastric perforator free flap.

Results

In study I we saw that tissue perfusion as measured with LSCI increases with increasing tissue motion, independent of frame rate, number of images, and tissue perfusion. Measured perfusion will decrease when images are acquired at an angle larger than 45° but distances between 15 and 40 cm do not affect the measured perfusion.

In study II we observed significant decreases in perfusion during both partial and complete venous occlusion with both LSCI and LDF. However, higher variability seen with LDF, measured as % coefficient of variation.

In study III a decrease in perfusion during the first 30 min after raising the flap and a perfusion value below 25 PU after 30 min was a predictor for tissue morbidity 72h after surgery.

In study IV the highest perfusion values were found in zone I and higher perfusion in zone II compared to zone III, directly after the flap was raised. No remaining significant difference between zone I, II and III could be seen after anastomosis of the vessels. All flaps with a minimum perfusion <30 PU, measured after the flap was shaped and inserted, later suffered from partial flap necrosis.

Conclusion

LSCI is a technology that has the potential to contribute to tissue monitoring in reconstructive surgery. It has many advantages over other techniques, such as the fast acquisition time, the spatial resolution and the fact that it is completely non-invasive. However, the current system is still too bulky to be easily introduced into a clinical setting and the technology is also subject to certain drawbacks which limit its usability. It is sensitive to motion artefacts; only superficial tissue is assessed and cannot offer absolute perfusion data. If these disadvantages could be addressed, LSCI could contribute to a more accurate survey of tissue perfusion and thus better outcome in reconstructive surgery.

List of abbreviations

ANOVA	Analysis of variance	<i>A statistical method used to analyze the differences among group means in a sample.</i>
AU	Arbitrary units	<i>Units used in perfusion measurements.</i>
CGAP	Cranial gluteal artery perforator	<i>A blood vessel used in the porcine flap model.</i>
CTA	Computed tomography angiography	<i>A radiological method to visualize the blood circulation.</i>
ECG	Electrocardiogram	<i>Measurement of the electrical activity of the heart.</i>
ECMO	Extra-corporeal membrane oxygenation	<i>External oxygenation of blood.</i>
FA	Fluorescence angiography	<i>A method to visualize perfusion.</i>
ICG	Indocyanine green	<i>An intravenous dye.</i>
LASCA	Laser speckle contrast analysis	<i>A method to visualize perfusion.</i>
Laser	Light amplification by stimulated emission of radiation	<i>A method to create monochromatic coherent light.</i>
LDF	Laser doppler flowmetry	<i>A method to measure perfusion</i>
LDI	Laser Doppler imager	<i>A method to visualize perfusion</i>
LDPI	Laser Doppler perfusion imager	<i>A method to visualize perfusion. Synonym for LDI</i>
LDPM	Laser Doppler perfusion monitoring	<i>A method to measure perfusion. Synonym for LDF</i>

LSCI	Laser speckle contrast imaging	<i>A method to visualize perfusion. Synonym for LASCA</i>
MN	Methyl nicotinate	<i>A vasoactive substance.</i>
MRA	Magnetic resonance angiography	<i>A radiological method to visualize the blood circulation.</i>
PU	Perfusion units	<i>Units used in perfusion measurements.</i>
ROI	Region of interest	<i>An area marked in the LSCI software from which an average perfusion is given</i>
SGAP	Superior gluteal artery perforator	<i>A blood vessel used in flap surgery.</i>
SIEA	Superficial inferior epigastric artery	<i>A blood vessel used in flap surgery.</i>
TRAM	Transverse rectus abdominis myocutaneous	<i>A type of flap.</i>

List of original papers

I

Zötterman J, Mirdell R, Horsten S, Farnebo S, Tesselaar E. Methodological concerns with laser speckle contrast imaging in clinical evaluation of microcirculation.
PLoS One. 2017;12(3).

II

Zötterman J, Bergkvist M, Iredahl F, Tesselaar E, Farnebo S. Monitoring of partial and full venous outflow obstruction in a porcine flap model using laser speckle contrast imaging.
J Plast Reconstr Aesthetic Surg. 2016;69(7)

III

Zötterman J, Tesselaar E, Farnebo S. The use of laser speckle contrast imaging to predict flap necrosis: An experimental study in a porcine flap model.
J Plast Reconstr Aesthetic Surg. 2019;72(5):771-777

IV

Zötterman J, Opsomer D, Farnebo S, Blondeel P, Monstrey S, Tesselaar E. Intraoperative Laser Speckle Contrast Imaging in DIEP Breast Reconstruction.
Plastic and Reconstructive Surgery. Global Open, 2020;8(1)

Introduction

The work that is compiled into this thesis is about tissue monitoring in reconstructive surgery, focusing on the use of a technology called laser speckle contrast imaging or LSCI.

Reconstructive surgery aims to restore function or normal appearance by reconstructing defective organs after trauma or disease. For healing to be possible after surgery, it is crucial that the operated tissue is supplied with a sufficient amount of oxygenated blood. In patients undergoing reconstructive surgery however, previous trauma, surgery or radiotherapy sometimes has resulted in compromised blood supply. This will affect the viability of the tissue and increases the risk for postoperative complications, such as partial or complete necrosis and infection. It is therefore important to assess the tissue viability, both before, during and after the surgery. This can be done using different techniques that monitor the perfusion of the skin covering the affected area. Perfusion is a measure of the amount of blood that flows through a given volume of tissue, during a given time frame, and is often used as an approximation of tissue viability.

The principles of the LSCI technique is based on the fact that coherent laser light is scattered by moving particles in the illuminated tissue and will form a speckle pattern which contains information about the concentration and speed of the moving particles. When skin tissue is assessed it is the moving red blood cells that will affect the signal so that the LSCI can assess the tissue perfusion. It will however not provide an absolute perfusion value, instead it will present an arbitrary value of the perfusion that is believed to be a function of the concentration of the red blood cells and their velocity. This is in most situations sufficient, as the surgeon typically is more interested in the change of perfusion over time than in absolute values.

LSCI was chosen to be evaluated for tissue monitoring in reconstructive surgery, as the technique allows for a fast and non-invasive assessment of superficial tissue perfusion over a wide field, with the possibility to find a threshold value with LSCI below which the risk of complications will increase.

In the following sections, a brief summary of the history and current status of reconstructive surgery will be presented. The LSCI technique will also be describe in more detail and compared to other techniques for tissue monitoring. Finally, a review of the four papers included in this thesis is presented, and the method used in them and how this technique can be used in the practice of reconstructive plastic surgery is discussed.

Reconstructive surgery

Reconstructive surgery is surgery with the aim to restore function or normal appearance by reconstructing defective organs.

Historical review

The word surgery is derived from the Greek words cheiros, hand, and ergon, work, but the oldest recorded texts on surgery are found along the Nile. The so called “Edwin Smith Papyrus” is an ancient Egyptian document, probably from the early dynastic period around 1500 BC, but maybe older, was found in a tomb in Thebes. It describes a variety of different surgical procedures such as suturing of wounds and treatment of skull and nasal fractures (fig. 1).¹

It is possible that Egyptian medicine was influenced by contacts with the Sumerian civilization. A historical record of medical practice in this region was found in Susa, Iran, in 1901. The so-called Code of Hammurabi, containing 282 laws, was carved into a black stone monument, or stela, almost 4000 years ago. Hammurabi was a king that reigned in Babylon between 1795 and 1750 BC, and one of his laws concerns surgical malpractice: “If a physician makes a large incision with an operating knife, and kill him, or open a tumor with an operating knife, and cut out the eye, his hands shall be cut off”. This gives an indication that at a majority of the surgical procedures performed must have been successful, otherwise a shortage of physicians soon would have arisen in ancient Babylon.^{2,3}

Figure 1. Part of the Edwin Smith papyrus, the complete papyrus is 4.7 m long. It is a surgery manual from the early dynastic period that describes 48 cases of injuries, fractures, wounds, dislocations and tumors and how they are treated. (Picture from Wikipedia, public domain)

Many of these surgical concepts were adopted by Greek physicians via their contacts with Asia Minor and Egypt. In the 5th century BC medicine was taught in Cos by Hippocrates. In writing associated with him, surgical treatments of fractures, hemorrhoids and ulcers are described. The ancient Roman medicine was strongly influenced by Greece, and many Greek physicians were employed in Rome. The most famous of the Roman physicians was probably Galen, living in the second century AD. He was the imperial physician to Marcus Aurelius and described methods for operations of varicose veins, repair of cleft lips and even suture of intestines. Much of medical knowledge of the ancient world was forgotten after the fall of the Roman empire, but partly survived in southern Italy, the Byzantine Empire and in the Islamic world.⁴

Around the 6th century BC the Indian physician Sushruta wrote the *Sushruta Samhita*. In this Ayurvedic text he describes, among many other surgical methods, a pedicled cheek flap for nose reconstruction, ear reconstruction, repair of a lacerated lip and skin grafting.⁵ Knowledge of these surgical methods probably traveled to Europe on different routes in the late medieval, and renaissance period, maybe through interaction with the Islamic cultural sphere. The first record of a nasal reconstruction in Europe is from the 15th century and is referred to the Sicilian surgeon Branca de Branca. He used a method similar to Sushruta's cheek flap, and his son, Antonius Branca, later modified the technique using the skin of the forearm instead of the cheek.⁶

In the mid-15th century a German surgeon, Heinrich von Pflazpaint (1400–1464), describes a method for nasal reconstruction using skin from the upper arm, a method that came to be known as the “Italian method”. In 1597 Gaspare Tagliacozzi (1545–1599), Professor of Anatomy at University of Bologna, publish a work entitled *De Curtorum Chirurgia per Insitionem Libri Duo*, where he describes both the Italian method and a technique

Figure 2. Surgical instruments described by Tagliacozzi. (Picture from Wikipedia, public domain)

Figure 3. The "Italian method" for nose reconstruction from "De curtorum chirurgia per insitionem" (1597) by Tagliacozzi. Source: Typ 525.97.820, Houghton Library, Harvard University

resembling Sushruta's for reconstruction of the nose (fig. 2 and 3).⁷

After this "Golden Age" of reconstructive surgery during the renaissance, much of the knowledge unfortunately declined, maybe because of regulations by the Roman Catholic Church. It is not until the end of the 18th century that the knowledge is rediscovered in Britain and English surgeons start to use the "Italian method" for nose reconstruction.

Modern reconstructive surgery

The modern era of reconstructive surgery began during the nineteenth century, when the possibility of prolonged anesthesia made more complex surgical procedures possible. The term plastic surgery first appeared about two hundred years ago in a publication called *Rhinoplastik oder die Kunst den Verlust der Nase organisch zu ersetzen* by Karl Ferdinand von Gräfe (1787–1840). The method that he described was in many ways the same as was described by Sushruta 2500 years earlier.⁸

During world war one, knowledge of hygiene and trauma care had advanced and health care logistics improved. This meant that soldiers, who in earlier conflicts would have died on the battlefield, now survived the initial critical time frame. The large number of war casualties, especially those with facial trauma, required new methods for reconstruction and surgeons on both sides of the conflict contributed with novel methods and operation techniques. A notable name is Sir Harold Gillies (1882–1960), a New Zealand born ear, nose and throat surgeon, who was active during both world wars. He contributed with many new approaches on facial reconstruction and flap techniques.⁹

During the 20th century, reconstructive surgery evolved further, with larger leaps during major military conflicts such as world war two and the conflicts in Korea and Indochina. One of the major steps was the introduction of various optical techniques to magnify the operating field. This allowed for more advanced reconstructions where tissue could be moved longer distances to cover defects and for reimplantation of traumatically amputated limbs.¹⁰

The reconstructive ladder

Reconstructive and plastic surgery today includes a large variety of procedures and techniques, but the main aim is still the same, to restore function while maintaining the best possible esthetics. As a guideline in the choice of reconstructive method for closing a wound or other defect, the concept of the reconstructive ladder can be used. The basic principle is that the more complex wound, the higher up the surgeon must climb (fig. 4). There is no absolute definition of the steps in the ladder, but the first steps are usually considered secondary healing, where a wound is left open and coverage is obtained by re-epithelialization of skin from the wound edges. Primary closure of a defect can be done with different kinds of suture techniques. Skin grafts are used for larger defects but rely on revascularization from the

wound bed and is therefore not possible in areas with compromised circulation. Split thickness skin grafts consist of epidermis and the superficial part of dermis and can cover large areas since the donor site is left to secondary heal. Full thickness skin grafts consist of the entire dermis and offers better skin quality in the transplanted area, but the donor site needs to be closed with a primary closure. Random pattern flaps rely on random cutaneous microcirculation for their blood supply, there is therefore a limit to how long a flap can be relative to its width, and they are also sensitive to tension and torque. If the flap is based on a known vessel, a longer, so called pedicled flap can safely be harvested. It is also possible to include the underlying deep fascia and/or a muscle in the flap to cover larger defects. If no options for local flaps exist to cover a large defect where skin grafts cannot be used, tissue has to be moved from other body locations using microvascular techniques. The transferred tissue is called a microsurgical free flap and is considered the uppermost step of the reconstructive ladder.¹¹

Figure 4. The reconstructive ladder is often used to illustrate the increasing complexity of different reconstructive methods.

Microsurgery

Microsurgery is the application of magnification techniques to basic surgical principles and the functional restoration of body structures by means of the direct union of parts or transfer of tissue using microsurgical techniques.¹² This means that the surgeon uses some form of optical magnification, such as a microscope or loupes, and fine surgical instruments, so-called microsurgical instruments, to be able to manipulate and dissect very small tissue structures.

The Swedish otorhinolaryngology professor Carl-Olof Nylén (1892-1978) introduced the operating microscope for ear surgery in the early 1920s. He used a monocular microscope for a few cases of chronic otitis and pseudo-fistula formation in 1921, and in 1923, his colleague Gunnar Holmgren (1875-1954) reported the first microsurgical fenestration for otosclerosis, using a binocular operating microscope.^{13,14} The possibility to use a microscope in the operating room opened up for microsurgical procedures within many surgical disciplines, but it took until the 1950s before the optical technique had evolved sufficiently for the method to be more widely spread. In the 1960s it found its way into the hands of reconstructive surgeons, initially mainly for replantation of limbs after traumatic amputations, the first successful replantation of a forearm was performed by Malt and McKhann in 1962.¹⁵

Microsurgical free flaps

A free flap is an autologous tissue transfer from one site of the body to another, in order to reconstruct an existing defect. The tissue is completely detached from its blood supply and the circulation in the tissue re-established by anastomosis of arteries and veins.

In reconstructive surgery today, microsurgery is mainly used for so called free flaps, in situations where more traditional reconstructive methods are insufficient. Microsurgical free flaps is a technique that allows a surgeon to move tissue from healthy donor sites to areas with tissue deficit after cancer surgery or trauma.

A free flap can be defined as an autologous tissue transfer from one site of the body to another, in order to reconstruct an existing defect. The tissue is completely detached from its blood supply and the circulation in the tissue re-established by anastomosis of artery(s) and vein(s) at the site of reconstruction. This distinguishes them from pedicled flaps, which always have their original vascular supply preserved.

The microsurgical free flap technique is based on the fact that the surgeon can identify vessels and sometimes nerves that supplies the tissue to be moved. This requires a good knowledge of vascular anatomy, both in the donor site and in the area that shall be reconstructed. The vessels are often very delicate, with diameters of a few millimeters, which requires special sutures and suturing techniques. The requirements for an area of the body to be used as a free flap is that the included tissue structures are supplied by a common artery and vein of sufficient length and that the donor site has an accessible artery and vein for the anastomosis.

One of the first described microsurgical flap procedures was done in 1971, when McLean and Buncke transferred an omental flap to a skull defect. In 1973, Daniel and Taylor used a groin flap, based on the superficial circumflex iliac artery, to cover a defect in the lower extremity. Soon after, microsurgeons started to identify new donor sites and define more complex flaps, consisting of different types of tissue such as muscle, skin, bone or combinations of these.^{16,17}

Many of the free flap techniques that were developed in the 1970s are still in use, such as the latissimus dorsi musculocutaneous flap, the fibular osseous flap and the radial forearm flap, and more have been added to the reconstructive tool box to be used by surgeons to restore functionality and cosmetics after trauma and cancer surgery.

Breast reconstruction with free flaps

Reconstruction of the female breast after radical mastectomy has been one of the central areas of plastic and reconstructive surgery for the last century. Initially, traditional reconstructive methods were used, such as tube pedicled flaps, and later pedicled muscle flaps.¹⁸

In the 1950s abdominally based flaps were increasingly popularized, much because of a better understanding of the vascular anatomy of the abdominal tissue. This led at the end of the 1970s to the development of a method that used one of the rectus abdominis muscle together with an abdominal skin island. This flap was initially described as a pedicled flap based on the superior epigastric artery, but later as a free flap based on the inferior epigastric artery.^{19,20}

These methods offered an improved cosmetic result, and during the 1980s this was the method of choice for many free flap breast reconstructions. A down side of the method was however the rather high frequency of donor site morbidity, such as abdominal wall weakness and bulging, and the need to include the rectus abdominis muscle in the flap was eventually questioned.¹⁸

Deep inferior epigastric artery perforator flap

In 1983 Taylor described the use of a microvascular free flap based on a perforator of the deep inferior epigastric artery without the rectus muscle included. This was an important step, since this spared the important abdominal muscles and resulted in fewer postoperative complications. In the beginning of the 1990s the deep inferior epigastric artery perforator or DIEP free flap began to be used for breast reconstruction more frequently, but a similar technique was described by Holmström already in 1979.^{21,22}

The flap is composed of skin and subcutaneous fat from the lower part of the abdomen and is supplied by blood from a thin (around 2mm in diameter) vessel perforating the underlying abdominal wall.²³ The flap is raised by carefully dissecting the tissue along the abdominal wall. When the perforators are identified they can be followed through the rectus muscle fascia, through the muscle tissue, down to one of the deep inferior epigastric arteries which reside deep to the inguinal ligament, where they are cut. Depending on if it is bilateral or unilateral breast reconstruction and how much volume that is needed, the surgeon excises and discards redundant parts of the flap. This is normally done according to known “safe zones” in the microcirculation of the flap (see section “Perforators and Angiosomes”). The flap tissue is shaped to a new breast and the vessels that supply the flap are anastomosed to vessels on the thoracic wall. The internal mammary (thoracic) vessels, that runs along the inside of the thoracic, wall are often chosen as recipient vessels (fig. 5).²⁴

Figure 5. The DIEP free flap. Skin and subcutaneous tissue is harvested from the abdomen together with the supplying vessels. The tissue is shaped into a new breast and inserted in its new location. The vessels are microsurgically anastomosed to recipient vessels on the thoracic wall.

The DIEP flap has in a relatively short time been popularized and is now one of the main choices for free flap breast reconstruction. The method has many advantages compared to other methods, such as reconstruction with expander/implant or other flap-based techniques. Reconstruction with autologous tissue has been shown to have a lower rate of complications, the volume and texture of the abdominal tissue often matches the

breast, the scar is easy to hide and the patient does not have to be turned on the operating table.²⁵

Several other types of free flaps for breast reconstruction are based on the same principle as the DIEP flap, i.e. they consist only of skin and fat and therefore cause relatively little donor site morbidity. Examples of such flaps are superficial inferior epigastric artery (SIEA) flaps and superior gluteal artery perforator (SGAP) flaps.^{26–28}

The dermal circulation in reconstructive surgery

“The microcirculation, in addition to capillaries, include certain small arteries and veins, immediately adjacent to capillaries, that form pre- and postcapillary units. Their inclusion is based on wall structure.”²⁹

Many techniques for evaluation of tissue viability in conjunction with reconstructive surgery have in common that they make an estimate of the local microcirculation, on the basis of an assessment of the circulation in the superficial capillary bed of the skin. This information can be used as an approximation of the viability of the flap. The skin is the external barrier of body, protecting the inner organs from the surrounding environment. It is composed of two primary layers, epidermis and dermis. The superficial epidermis is largely waterproof and composed mainly of keratinocytes, Merkel cells, melanocytes and Langerhans cells, arranged in layers on top of a basement membrane. The thickness varies between 0.05 mm and 1 mm, depending on the location on the body. The underlying dermis also varies greatly in thickness depending on location, usually between 1 and 10 mm. In the dermis, fibroblasts and various forms of connective tissue forms a supporting scaffold, containing organs such as sensory receptors, sweat glands, sebaceous glands and hair follicles.³⁰

The dermis contains the cutaneous microcirculation, which is organized in two horizontal plexuses; one deeper in the junction between the dermis and the underlying subdermal fat, and one superficial under the basement membrane approximately 1–1.5 mm below the surface of the skin. These plexuses are composed of capillaries, arterioles and venules with a diameter between 10 and 35 μm and are connected via ascending arterioles and descending venules. The superficial layer of the dermis is organized into folds, so-called papillae, and forms the substrate for the basement membrane of the epidermis. This part of the dermis is called the papillary dermis and, in each papilla, papillary loops that provide the avascular epidermis with oxygen and nutrition, arise from the superficial vascular plexus (fig. 6).^{29,31}

Figure 6. Dermal (D) microcirculation is organized in two separate plexuses. One deeper (DP) in the junction to the subcutis (SC) and one more superficial (SP). Papillary loops (PL) provide blood supply to the avascular epidermis (ED).

In addition to its protective function, the skin also plays a major role in the thermoregulation of the body. Excess heat is transported from muscles and other deeper structures to the skin via the circulating blood. This can lead to a dramatic increase of the total blood flow through the skin in certain situations. The normal flow rate is about 250 ml/min but can increase to 8 L/min to meet the body's need for cooling during heavy exertion or when the temperature of the environment increases.³²

The large fluctuations in the perfusion of the skin are made possible because the vessel walls in many of the skin's blood vessels contain smooth muscle cells and pericytes. Both cell types can contract and relax in response to various forms of stimuli such as nerve signals or local chemical mediators, such as nitric oxide and possibly also local O₂ concentration. An increase in diameter of vessel lumen will lead to a decrease of the lumen vascular resistance and thereby an increased blood flow. This effect is mainly mediated through arteriovenous shunting of blood in the deep plexus. The possibility of local regulation of the microvascular flow also results in a large spatial heterogeneity in blood flow in the skin. This becomes especially clear when point-by-point measurements of perfusion in the skin are made, where values can differ considerably within distances of a few millimeter.^{31,33}

Perforators and angiosomes

The blood vessels that supply the skin and the underlying tissue normally emerge from the underlying fascia in specific locations. These vessels are called perforators and can be found in approximately the same locations in different individuals. A tissue area provided by one of these perforators is sometimes called an angiosome. The human skin is made up of about 80 such angiosomes. Knowledge of the anatomy of the perforators is often used in reconstructive surgery and many types of flaps are based on one or more perforators. There are also indications that the angiosome principle can be used in the planning of different types of reconstructions, even if the extent of its usefulness sometimes has been questioned. The basic idea is that the safe clinical vascular territory of a cutaneous perforator, used for example for a flap, extends beyond the anatomical territory of that perforator to include the anatomical territory of the adjacent cutaneous perforator, but if further territories are used, the risk of necrosis increases.^{34–36}

An example of this is the above-mentioned transverse rectus abdominis musculocutaneous (TRAM) flap. In 1983 plastic surgeons Michael Schefflan and Melvyn Dinner published a paper where they showed, based on anatomical studies, that there is a communication between the perforators that supply the skin over the flap. Based on this another surgeon, Carl Hartrampf, suggested that the skin of the TRAM flap could be horizontally divided into four zones based on their relation to the perforator. The one right above the perforator, zone I, is considered the safest with the lowest risk of

postoperative complications such as necrosis. Zone II is on the contralateral side closest to zone I, zone III on the ipsilateral side and zone IV, the least safe zone, is lateral of zone II (fig. 7).^{19,37}

It has been discussed whether zones II and III should change places, but the basic idea of subdividing the flap follows the theory of the angiosomes. The same idea has later been applied on the more modern deep inferior epigastric perforator (DIEP) free flap.³⁸

The knowledge of vascular zones is used when plastic surgeons are planning a reconstruction with TRAM and DIEP flaps. As zone IV is considered least safe, this part of the flap is often discarded when all tissue is not needed, when for example a new breast is constructed.

Figure 7. DIEP flap with the Hartrampf zones marked.

Assessing microcirculation and tissue monitoring in reconstructive surgery

In many cases where reconstructive surgery is used, the conditions for good healing are impaired, even before surgery. The area that needs to be reconstructed has often been subjected to trauma or radiation, and the tissue may be contaminated with microorganisms, which increases the risk of infections. It is therefore crucial to regularly assess the viability of the tissue, both before, during and after surgery. This need also increases with increasing complexity of the surgery.

Surgeons have always used their senses to evaluate the results of their operations. Most probably, the previously mentioned Italian renaissance surgeon Gaspare Tagliacozzi followed the healing progress in his patients by looking at the color of the tissue, feeling the temperature of the skin and smelling the odor from the dressings. By gently pressing on the skin and then releasing and observing how quickly the vessels in the skin fill up, one can make an estimate of the so-called capillary refill. This has also been shown to be correlated with the viability of the underlying tissue.³⁹

These ways to assess the tissue are both fast and easily accessible and are therefore still widely used by surgeons in the whole world. One problem is however that these methods are highly subjective and dependent on experience. There is a risk that different examiners will make different judgments, which of course may have an impact on the outcome of the surgery, especially if a flap with impaired perfusion is neglected due to a poor clinical decision. Therefore, it has been desirable to find an alternative to the traditional methods of tissue monitoring.

The very rapid technological development that has taken place throughout the past and the present century has offered a large number of observer-independent alternatives for tissue evaluation. For example, in microvascular free flap surgery, many surgeons today use computer tomography angiography (CTA) or magnetic resonance angiography (MRA) to localize and evaluate the feeding artery before surgery. These techniques are excellent for identifying perforators and evaluating their quality in the DIEP flap surgery with high accuracy.^{40,41} This allows the surgeon to have a clear picture of the flap's vascular supply even before surgery and has been shown to reduce the risk of some post-operative complications.⁴²

Intraoperative perfusion mapping in reconstructive surgery

In addition to the preoperative mapping of the circulation, situations often arise in reconstructive surgery where it is important for the surgeon to intraoperatively evaluate the viability of the tissue. Techniques for intraoperative assessment have the potential to identify areas at high risk of tissue morbidity after all types of reconstructive surgery. As reconstructive surgery increasingly uses small perforators to supply large tissue areas, there is room for new methods that can identify these risk areas. Situations involving tension, twisting and torque of the flap and the pedicle, and tissue areas exceeding the feeding vessels blood supply capacity, are of special interest. This may be the case, for example, in trauma surgery when there is suspicion that tissue areas are in such poor condition that they need to be excised, or in flap surgery where excess parts of the flap may need to be discarded, as they are found redundant for esthetic reason or, more importantly, will not be viable at the end of the surgical procedure. In the latter case, poorly perfused parts of the flap are often chosen for excision. Overall, techniques that have allowed for fast mapping of the perfusion over larger tissue areas with relatively high resolution have obviously attracted larger interest. There are a few techniques for intraoperatively perfusion mapping available today.

In microvascular free flap surgery and breast reconstruction surgery, one of the most widely used methods is fluorescence angiography (FA) (fig. 8). In FA the patient is given an intravenous injection of indocyanine green (ICG), a water-soluble dye that emits fluorescence when illuminated with near infrared light. By monitoring the assessed tissue area with an infrared camera, the ICG can be visualized when it reaches the perforators. This way, the smallest vessels can be tracked as the dye spreads through the tissue, and the microcirculation that supplies the tissue can be visually mapped during the procedure. This gives a dynamic picture of the perfusion pattern of the assessed tissue and can for example help the surgeon in the decision to excise poorly vascularized tissue in the periphery of a free flap. The technique

Figure 8. Fluorescence angiography image of a DIEP flap. The black ring in the upper part of the image is the umbilicus. The ICG dye can be seen as white lines as it reaches the subdermal vessels. In the more diffuse white area in the left half of the picture, the ICG dye has reached the dermal microcirculation.

has been shown to reduce the incidence of postoperative complications after for example DIEP surgery.⁴³

Laser Doppler imager (LDPI) is a full field technique for perfusion assessment that is further described below. It has been used for intraoperative evaluation in reconstructive surgery, mainly in clinical studies. For example, Schmidt et al used LDPI to assess replanted fingers. Venous occlusion that required reoperation could be detected with the method. Zdolsek et al used LDPI for perfusion monitoring of radial forearm flaps and Tindholdt et al for evaluation of the perfusion zone of DIEP flaps.⁴⁴⁻⁴⁶

Laser speckle contrast imaging (LSCI) is the main focus of this thesis. It is a laser-based imaging technique, which, in addition to our studies, has been evaluated by Nguyen et al for assessment of perfusion in eyelid flaps on patients undergoing eyelid surgery. They investigated the effect of flap length and the use of diathermy and could show a successive decrease in perfusion from the proximal to the distal end of the flaps and when diathermy was used. They also used LSCI in a porcine model and to assess stretched eyelid flaps and showed a similar decrease in perfusion. Sheikh et al used LSCI to follow revascularization of full thickness skin grafts on patients receiving tarsoconjunctival eyelid flaps and the effect of adrenaline on porcine eyelid flaps.⁴⁷⁻⁵⁰ This knowledge of the perfusion pattern in various forms of flaps has not only a theoretical value. The information can be used to evaluate the tissue intraoperatively and decrease the risk of for example partial flap necrosis. In a study by To et al LSCI was used to assess flap perfusion on patients undergoing free flap breast surgery. The study was in many ways similar to our study on DIEP flaps and in analogy with our own results, they could see a significantly lower perfusion in Hartrampf zone IV compared to remaining zone on the flaps, but no other significant differences.⁵¹ Rauh et al used LSCI to assess 27 free flaps intra-operatively. They could see significantly lower perfusion in flaps that later developed postoperative complications.⁵²

In thermal imaging an infrared camera is used to detect the thermal radiation from the tissue. It can present a full field color coded image showing warmer and cooler areas of, for example, skin. In the above-mentioned study on tarsoconjunctival eyelid flaps by Sheikh et al thermal imaging was done in addition to LSCI.⁵⁰ Hardwicke et al used the FLIR ONE (FLIR Systems, Inc., Wilsonville, Ore.) smartphone-compatible thermal imaging camera to detect and map perforators in flap surgery LSCI. Pereira et al compared the same system to computed tomographic angiography (CTA) for identifying perforators in anterolateral thigh flaps and concluded that thermographic images have a high concordance with the radiological method.^{53,54}

Other methods that have been used for intraoperative tissue evaluation are oxygen saturation measurements and microdialysis. These methods only offer point measurements and are not further described here.^{55,56}

Postoperative monitoring in reconstructive surgery

In all reconstructive surgery, postoperative assessment of the affected tissue is essential. This is even more valid in microvascular procedures, where the survival of the tissue is dependent on a maintained flow through the anastomosed artery and vein. One of the most feared postoperative complications in microvascular surgery is a compromised flap circulation, which is often caused by venous thrombosis. This usually occurs within the first day(s) after the surgery, why monitoring during these first days postoperatively is of utmost importance.⁵⁷ The sooner an occlusion is identified, the better chances the surgeon has to salvage the flap.⁵⁸ It is therefore crucial that the flap is assessed regularly during this initial time frame. As with other reconstructive surgery, conventional methods such as assessment of temperature, color and capillary refill are often used, but many surgeons also use different monitoring techniques as a complement.

There are basically two different principles in microvascular free flap monitoring today. The first option is to use different techniques to assess exposed superficial flap tissue, normally the skin. This has the advantage of easy accessibility, but the downside is the large spatial heterogeneity in the dermal perfusion that may give different values depending on where the measurements are done.³³ Laser Doppler flowmetry (LDF) is a method for assessment of superficial tissue by applying a small, 1cm in diameter, probe to the exposed skin. The LDF system continuously produces an arbitrary perfusion value that is presented as a number and a curve on a bedside monitor. This gives a postoperative monitoring that can be easily assessed by the health care staff. The ease of use and relatively simple data analysis has led to a wide spread of this technique among many microsurgical centers. This technique is further described later. Other systems for superficial assessment are near-infrared spectroscopy and thermography.^{59–61}

The other principle is to measure blood flow directly on the anastomosed vessels. Since this value represents the total blood flow in the flap, it gives a very good estimation of the viability of the flap. This can be achieved by using a simple handheld ultrasound Doppler pen, or by visualizing the vessels using more advanced color

Figure 9. An implantable Doppler probe (Cook-Swartz) for postoperative monitoring of free flaps. A silicon cuff (A) is wrapped around an artery (B) and fastened with a surgical clip (C). An ultrasonic probe (D) is held in a small pocket in the sleeve and measures the blood flow. It is connected to a monitor via wires (E) protruding through the wound. When the monitoring is completed, the probe is disconnected from the pocket by twitching the wire.

duplex ultrasonography. These methods require repeated assessments and will therefore not allow for continuous measurements. It is also possible to use implantable Doppler systems. There are two commercial systems offering this possibility. The Cook-Swartz implantable Doppler probe (Cook Medical, Bloomington, IN) uses an ultrasonic Doppler probe attached to a silicon sleeve around the vessel to assess the blood flow continuously. A wire protruding through the wound leads the signal to a bedside monitor (fig. 9). The second system is the Flow-Coupler (Synovis Life Technologies Inc, St. Paul, MN). It combines a vascular coupler that are used to anastomose the vein in microsurgical procedures, with an ultrasonic flow probe. Implantable systems offer continuous evaluation of the blood flow, but require a second procedure to remove the implanted probe.^{61–65}

Using laser to assess microcirculation in the skin

A laser is a device that generates an intense beam of coherent monochromatic light (or other electromagnetic radiation) by stimulated emission of photons from excited atoms or molecules.

Laser is an acronym for Light Amplification by Stimulated Emission of Radiation. A laser consists of a gain medium, a source to provide it with energy and a mechanism to provide optical feedback. The gain medium has the ability to amplify light of a certain frequency that passes through it by stimulated emission. Energy, typically in the form of light of different wavelengths, is fed into the medium in a process called pumping. Atoms in the medium are first excited by the energy source and then stimulated to emit photons of an extremely narrow wavelength, depending on the composition of the medium. The light emitted is coherent, which means that the light waves are in phase in time and space. Most lasers use an optical cavity to enhance the light, usually a pair of mirrors on both ends of the gain medium, of which one is partially transparent. The light will be amplified each time it passes through the gain medium, but some of the light will escape through the semi-transparent mirror and form the laser beam.⁶⁶

The first theoretical ground for the laser technique was in a proposal by Albert Einstein in 1916. He suggested that atoms irradiated by photons of a specific wavelength, would be excited and emit photons of an identical wavelength.⁶⁷ The first laser was however not developed until 1960, when Theodore Maiman presented a working laser with ruby as gain medium and a flash lamp as energy source (fig. 10).⁶⁸ Once the genie was out of the bottle, other research groups quickly introduced a series of new laser techniques, based on different gain mediums, for example helium-neon (He-Ne), carbon dioxide (CO₂) or neodymium-doped yttrium aluminum garnet (Nd-YAG) lasers. Depending on the choice of medium, laser light of different wavelengths can

Figure 10. A schematic description of a ruby laser: A energy source, in this case a xenon lamp (A) is pumping photons into the gain medium, a ruby rod (B). In the gain medium coherent light is amplified by stimulated emission. A mirror (C) and a semireflective mirror (D) forms an optical cavity in which optical feedback is gained. Some light will escape through the semi-transparent mirror and form the laser beam (E).

be obtained, from long-wave infrared light, through the entire visible spectrum, to short-wave ultraviolet light.

The introduction of the semiconductor diode laser working in room temperature, in the beginning of the seventies, made it possible to build very compact lasers. This improvement of the technique widened the possible applications of the technology considerably. During the relatively short period of time that has passed since the introduction of the technology, lasers have become an integrated part of our everyday life and can be found everywhere in our society: in lighting, consumer electronics, information technology, science and not least medicine. The first successful use of lasers for treating a medical condition was already in November 1961 when Charles J. Campbell used a ruby laser to treat a patient for a detached retina. Further medical applications soon followed in a large variety of fields, such as dermatology, otorhinolaryngology and thorax surgery.^{69,70}

Laser Doppler flowmetry

The coherent light wave produced by a laser can be used for flow measurements in fluids.⁷¹ To achieve this, a phenomenon described in 1842 by the Austrian physicist Christian Doppler is used. Doppler noticed that a change in frequency or wavelength of a wave could be seen in relation to an observer who is moving relative to the wave source. This is called the Doppler effect or the Doppler shift. This phenomenon can be observed in many everyday situations where a source of sound is moving in relatively high speed towards, passing close to, and then moving away from an observer (i.e. for example an ambulance or a train whistle). The phenomenon can also be observed when light is reflected on a moving object. The wavelength of the reflected light will be a fraction shorter when the object is moving towards the observer and a fraction longer when it is moving away from the observer.⁷²

In Laser Doppler Flowmetry (LDF), velocity is derived from the Doppler frequency-shift

Figure 11. When the Doppler effect is used to measure speed, it is difficult to assess the absolute change of the wavelength of the light or sound that is reflected from the moving object. Instead a phenomenon called beating is used: when two waves with a slightly different wavelength (red and green) are added, the interference will form a beat (blue) that is easier to detect. The smaller the difference between the original wavelengths is, the longer is the wavelength of the beat frequency.

in the backscattered light, caused by the movement of the particles in a fluid. When skin is illuminated by coherent laser light of a frequency that penetrates the surface, some of it will be scattered by moving red blood cells in the capillary bed and some by static tissue. The light reflected by moving blood cells will have a slightly altered wavelength when it blends with the unchanged light reflected by static tissue. This will cause an interference that can be picked up as an optical beating by a sensitive photodetector (fig. 11).⁷³

The penetrance of the light will differ depending on the wavelength of the laser, the concentration of red blood cells in the measured volume, and the distance between the optical fibers. This makes it difficult to define the measurement depth and volume of the technique. Usually, when a laser within the infrared or near infrared spectrum is used, the maximum penetration depth is about 1 mm and the volume assessed in a single measurement with LDF is about 1 mm³.⁷⁴

LDF is used in many clinical situations for tissue assessment, for example after reconstructive surgery. An optical fiber leads the laser light to a probe attached to the tissue surface and another optical fiber picks up the scattered light. In free flap surgery, the probe is attached to the skin over the flap. Information about the speed and concentration of red blood cells can be derived from the signal and the information is interpreted by a computer as perfusion, which is a measure of the amount of blood that passes a certain amount of tissue over a certain period of time. As the microvascular anatomy in the measured volume is unknown, it is not possible to give an exact value of the perfusion in milliliter per second per cm³ with the method, instead it is presented as an arbitrary value. Changes in perfusion can be followed in real time on a bedside monitor.

To overcome the problem with the small sampling area in LDF and the large spatial heterogeneity in the microcirculation of the skin, a technique called laser Doppler perfusion imaging (LDPI) can be used. In LDPI, a laser beam scans the skin and the scattered light is detected by a photodetector in the same way as in the LDF technique. This means that a larger skin area, and not only a single point can be assessed without the need to move the probe. The technique has been used for tissue monitoring in conjunction with reconstructive surgery in a few studies, but is not in clinical use to any extent, maybe because of the slow acquisition time. There are attempts to overcome this disadvantage and the technique is used in some centers for burn wound assessment.^{75,76}

Laser speckle contrast imaging

It is a long-known phenomenon in optics that a speckle pattern can be seen if a surface is illuminated with a fairly coherent light source. The phenomenon occurs because small irregularities in a surface cause the distance between the surface and the image plane (the eye or a camera) to vary. If the distance difference between two irregularities corresponds to a multiple of the wavelength of light, light waves reflected on the two irregularities will amplify each other. If the distance instead corresponds to half a wavelength, they will cancel each other out. This will create a pattern of lighter and darker areas on the surface, called a speckle pattern. The phenomenon can be seen in many everyday situations, for example when the light from an optical computer mouse is illuminating your desk (fig. 12).⁷⁷

Figure 12. Constructive interference (A) is seen when a crest of a wave (green) meets a crest of another wave (red) of the same frequency at the same point. The amplitude of the resulting wave (blue) is the sum of the individual amplitudes. In destructive interference (B) the crest of one wave (green) instead meets the trough of another wave (red) and the resulting wave (blue) has a low amplitude. In a speckle pattern (C) small differences in the length of the path the light must travel will cause a mix of constructive and destructive interference that creates the typical pattern of bright and dark spots.

If the light is scattered from a moving object, the speckle pattern will decorrelate and change over time. The same is true if the light is scattered from a large number of moving particles. This is called a “time-varying” speckle and can be seen in real time as a fluctuation in the speckle pattern. If these fluctuations are captured by a camera with a finite exposure time, there will be a blurring on the exposure caused by the movements of the speckles. The higher the velocity, the faster are the fluctuations and the more blurring occurs in a given integration time. If a digital camera connected to a computer

is used, the blurring can be quantified by calculating local speckle contrast. The contrast K is calculated using the formula:

$$K = \frac{\sigma}{\langle I \rangle}$$

where σ is the standard deviation of the intensity I and $\langle I \rangle$ is the mean intensity, calculated over a window in space or time. Spatial contrast uses an area of multiple pixels in one frame. The number of pixels over which the speckle contrast is computed will affect the result: too few, and the statistics will be questionable, too many and spatial resolution will be lost. Usually a square of 7×7 or 5×5 pixels is used. Temporal LSCI uses the same pixel in multiple frames to calculate the contrast in a time window (fig. 13).⁷⁸

Figure 13. In time-varying speckle the exposure time of the camera will cause a blurring of the speckles just like a moving object will be blurred in an ordinary photo (A). The blurring can be quantified by calculating the contrast, or difference between dark and bright areas in the image. In the example above, the intensity of each pixel of the image is given a number between 1 and 10. The contrast K is calculated from the standard deviation of for example 7×7 pixels divided with the mean intensity of the same pixels. Low or no motion of the light scattering particles will give a static speckle pattern with high contrast.(B) Faster moving particles will cause a blurring of the speckle pattern with low standard deviation in the pixels and thereby low contrast.(C)

LSCI was first used in biomedical applications in the beginning of the eighties, initially to assess retinal blood flow. It was then a complicated photographic process, requiring two exposures to achieve a picture that could be analyzed, which limited the clinical and experimental use of the technology. When digital imaging and analyzing methods became more widespread in the beginning of the nineties the method became more useful.

Depending on the camera and laser, a relatively large area can be assessed with high resolution. Early systems used a He–Ne laser operating at a wavelength of 633 nm. In more modern, commercially available systems, a near infrared laser operating at a wavelength of 785 nm is used. Longer wavelengths penetrate deeper, but the measurement depth is depending on a number of other factors, such as vascular anatomy and concentration of red blood cells in the upper dermis. It is therefore hard to determine an absolute measurement depth, although attempts have been made. Traditionally the measurement depth has been approximated to 300 μm . Davis et al used three-dimensional Monte Carlo simulations of photon propagation combined with high resolution fluorescence microscopy of the vascular anatomy to show that 95% of the detected LSCI signal comes from the top 700 μm of tissue.⁷⁹

Figure 14. LSCI image of human face. The red and yellow areas are areas with high perfusion, the blue are areas with lower perfusion and the green are intermediate. The color codes are not fixed but can normally be set in the software of the LSCI equipment to best visualize the perfusion in the assessed area. (Reprinted with permission from PeriMed)

Since its introduction, LSCI has been evaluated and used as a tool for perfusion assessment in a number of studies but is still not widely used in everyday clinical situations. As mentioned above, the initial studies were on retinal blood flow and this use has continued, both in clinical and research applications. Retinal perfusion is involved in many diagnoses, such as glaucoma, retinopathy, and macular degeneration and can therefore be assessed and evaluated using LSCI. The technique has also been used in dermatological studies, for example to follow the progress of laser treatment of port wine stains. In trauma care, LSCI has been used in burn wound assessment to discriminate between deeper and more superficial burns. This can provide information about the expected healing time and also help the surgeon to determine if the injury needs revision and coverage with a split thickness skin transplant (fig. 14–16).^{78,80}

Figure 15. The LSCI system used in all papers. The camera house (A) contains an infrared laser that creates the speckle pattern, a CCD camera that capture the speckle pattern, a visible red laser that projects a positioning pattern on the assessed tissue and a color camera used for documentation. The speckle pattern is analyzed by a computer (B) and the image is shown on a monitor (C).

Figure 16. A DIEP flap the replaces the right breast after mastectomy for breast cancer. The left image (A) is taken three days postoperative and the right (B) four days postoperative. Note the bluer color of the whole flap in the left image representing a lower perfusion. This particular flap suffered from healing problems and eventually had to be removed. The grey shape in the central of the flap is the LDF probe.

Aim of thesis

The overall aim of this thesis was to evaluate laser speckle contrast imaging (LSCI) as a tool for objective non-invasive tissue monitoring in reconstructive surgery. Specifically, the aims of the papers were:

1. To address methodological concerns subjected to the LSCI technology. We specifically addressed situations that may produce false high, or low perfusion values. These include motion of examined tissue, distance from object to camera, and curvature of the tissue as well as how high and low perfusion states affect the results. We also addressed how altered settings (frame rate and number of frames per image) affected the perfusion values.
2. To evaluate and compare LSCI and LDF as tools to detect partial and full venous outflow obstruction, a condition that often results in postoperative complications such as partial or complete flap failure in reconstructive surgery.
3. To evaluate LSCI as a method for peri-operative assessment in reconstructive surgery, based on the assumption that there is a perfusion threshold below which tissue viability is threatened.
4. To investigate the perfusion distribution, as measured with LSCI, in relation to the supplying perforator in the deep inferior epigastric perforator free flap.

In order to achieve these aims four studies were done: one methodological, two experimental animal studies, and one experimental clinical study. The methodological study was however published after the first animal study, but is, for logical reasons, referred to as study I in this thesis.

Methods

Subjects and environmental conditions

For study I, ten healthy, non-smoking subjects were included after informed consent had been obtained. The mean age was 32.4 (range 23–43) years. None of the subjects used regular medication, except for oral contraceptives.

The subjects were seated in an upright position during the measurements. All measurements were performed at a room temperature of 21 ± 1 °C.

In study IV twenty-three patients who underwent a DIEP procedure at Linköping University Hospital (Sweden) or Gent University Hospital (Belgium) were included after informed consent had been obtained. The age of the patients ranged between 38 and 55 years and the mean (SD) body mass index was 24.4 (3.6) kg/m². None of the patients were current smokers or diabetics. One patient had a history of Hodgkin lymphoma and arterial hypertension and one suffered from arterial hypertension alone.

Methyl nicotinate

Methyl nicotinate (MN) is a nicotinic acid and it is the methyl ester of nicotinate, commonly known as the B vitamin niacin. When applied on the skin, it induces a temporary increase in the skin perfusion through a vasodilatory response in the microcirculation. The vasodilatory actions in the skin of topically applied MN is believed to primarily be mediated through the prostaglandin pathway. The increasing vasodilatory effect of MN will plateau and reach its maximum within approximately 5 to 10 minutes.^{81,82} (fig. 17)

Figure 17. The maximum effect of MN is reached after 5–10 minutes (illustration by Sherif Elawa)

Animal model

In study II and III mixed breed pigs (Swedish Landrace pigs) were used as subjects. They had a mean age of 4 months and a mean weight of 45 kg. They were operated on and stabled at the KMC (Katastrofmedicinskt centrum) facility in Linköping. They were pre-anaesthetized with Dexdomitor (0.1 mg/kg), Zoletil (5 mg/kg) and atropine 0.05 mg/kg. Anaesthesia was withheld with pentobarbital sodium (8 mg/kg/h) and fentanyl (0.5 mg/kg/h).

The flap model is based on the cranial gluteal artery perforator, and is similar to a model described by Russell et al 2006 (fig. 18).⁸³

Figure 18. The CGAP flap. The pedicle with its vessels and branches can be seen in the lower end of the flap.

Equipment

Laser speckle contrast imaging

In all studies a laser speckle contrast imager was used (PeriCam PSI System, Perimed AB, Järfälla, Sweden). It uses a divergent near infrared (NIR) laser beam at a wavelength of 785 nm to create the speckle pattern and a visible red laser (650 nm) for positioning. A monochrome CMOS camera captures the speckle image for the analysis and a separate color camera is used for documentation (fig. 19).

The instrument was calibrated in accordance with instructions from the manufacturer.

Figure 19. PeriCam PSI from Perimed (reprinted with permission from PeriMed).

Laser Doppler flowmetry

In study II, in addition to LSCI, perfusion was also monitored continuously using two laser Doppler flow probes (Probe 45781-1, Perimed AB, Järfälla, Sweden) connected to a laser Doppler monitor (PeriFlux, Perimed AB, Järfälla, Sweden, fig. 20). It uses a 780 nm NIR laser for the measurements, and the probes have a fibre separation of 0.25 mm. The instrument was calibrated in accordance with instructions from the manufacturer.

Figure 20. PeriFlux 5000 from Perimed (reprinted with permission from PeriMed).

Flow probes

In study II transonic perivascular flow probes (Transonic Precision Perivascular Flow Probe MA-2PSB Transonic Systems Inc. 34 Dutch Mill Road, Ithaca, NY 14850 USA) were used to monitor the blood flow in the arteries and veins. The first probe was placed at the base of the artery and the second at the base of the vein. Both were fixated with sutures to the surrounding tissue. The probes were connected to a Transonic TS420 Perivascular Flow Metre Module (Transonic Precision Perivascular Flow Probe MA-2PSB Transonic Systems Inc. 34 Dutch Mill Road, Ithaca, NY 14850 USA). The system allows for real time absolute blood flow measurements presented as milliliter per second.

Laboratory shaker

In study I, a laboratory shaker (RK-30, Premiere, Shanghai, China) was used to generate a controlled, translational motion with a circular path. The shaker could be set to different number of revolutions and the speeds were determined by calculating the circumference of the circle described by the shaker and by measuring the time it took the shaker to complete ten turns.

Data processing and statistics

LSCI images were processed using the system analysis software (PSIWin, Perimed, Järfälla, Sweden). The software allows for regions of interest (ROI) to be selected in the LSCI images and mean perfusion for each region is calculated and presented as mean PU $\pm$ SD.

In study I, one-way analysis of variance (ANOVA) was used to analyze the effect of measurement distance and angle. For the analysis of the effect of different speeds of

motion using different absolute perfusion levels, two-way ANOVA was used. The relation between the speed of the tissue motion and the measured perfusion was analyzed using linear regression analysis.

In study II, Shapiro-Wilk normality tests were performed to confirm that the data were in consistency with a Gaussian distribution. Two-way ANOVA for repeated measures with Sidak's multiple comparisons test were performed to test whether changes from baseline were significant, for the respective measurement techniques. Intersubject variability was assessed by percent coefficient variability.

In study III, two-way ANOVA for repeated measures with Tukey's multiple comparisons test was performed to test whether changes from baseline and differences between $t = 0$ and $t = 30$ min were significant.

In study IV, two-way ANOVA and Sidak's multiple comparisons test were performed to test whether differences in perfusion between ROI were significant.

All statistical calculations were done using GraphPad Prism (GraphPad Software, San Diego California USA, 'www.graphpad.com'). For all analyses, probabilities of <0.05 were accepted as significant.

Review of papers and results

Paper I: Methodological concerns with laser speckle contrast imaging in clinical evaluation of microcirculation

Summary

The aim of paper I was to address some methodological concerns with LSCI that may result in diagnostic errors in a clinical setting. Previous studies indicate that the LSCI technique is sensitive to motion of the tissue that is being assessed. Another possible issue when LSCI is used clinically to measure larger areas is the possible effect the measuring distance and the curvature of the tissue has on the measured perfusion. We therefore wanted to investigate to what extent tissue motion affect the measured perfusion using different combinations of frame rate and number of frames/image and to investigate how the angle and distance between the measured surface and the LSCI camera affect the measured perfusion.

Methods

The effect of motion on the measured perfusion was investigated by using a laboratory shaker. The shaker could be set to different speeds, generating a controlled, translational motion, with a circular path between 25 and 205 mm/s. In the first part of the study, a small dish with a cloth soaked in a colloidal suspension of polystyrene microspheres was mounted on the shaker. Measurements of the cloth were done at different speeds, different frame rates and different number of frames per image. The change of frame rate and number of frames per image were done to investigate if the settings of the LSCI equipment affected the measured perfusion.

In the second part of the study, the effect of motion and settings on the measured perfusion was investigated on eight healthy, non-smoking subjects in a controlled environment. The forearm of the subject rested on the laboratory shaker to simulate motion artefacts.

To investigate how an altered tissue perfusion affects the appearance of motion artefacts after a motion provocation, three circular areas with a diameter of 4 to 6 cm were marked on the forearm. First, one reference measurement was made on the skin for every speed setting. Then, methyl nicotinate was applied in three different concentrations, 2.5 mM, 10 mM and 40 mM, to the marked areas to induce a prolonged vasodilatation to simulate

the higher perfusion often seen in clinical situations. Measurements were done after the perfusion increase had reached a plateau at approximately 15 minutes.

In the third part of the study the influence of angle and distance between the skin and the camera on measured perfusion was evaluated on the volar side of the forearm of five subjects. Methyl nicotinate was used to achieve prolonged vasodilatation. The LSCI camera was held at different angles and distances in relation to the assessed skin. Measurements were done in steps of 15° from 0° to 60° and in steps of 5 cm from 15 to 40 cm.

Results

The relation between the speed of the tissue motion and the measured perfusion value in calibration fluid was linear ($1.70 \pm 0.02 \text{ PUmm}^{-1}\text{s}$, linear regression analysis), varying between $66 \pm 2.3 \text{ PU}$ for zero speed and $430 \pm 3.8 \text{ PU}$ for 205 mm/s. The relation was independent of the frame rate and number of averaged image frames. The variation in perfusion between the different system settings varied between 0.7% and 3.5% (fig. 21).

There were small but significant differences in perfusion between sites treated with 2.5 mM and 10 mM of methyl nicotinate and between sites treated with 10 and 40 mM methyl nicotinate for all speeds ($p < 0.001$). There were small but significant differences in the increase in perfusion in relation to motion for skin sites with different baseline perfusion levels ($p = 0.03$). Unlike the measurements in calibration fluid, the relation between the speed of the motion and the measured

Figure 21. Relation between motion and measured perfusion in calibration fluid using different system settings (frame rate and number of frames).

perfusion value in healthy subjects was not linear, probably because the forearm was unable to follow the motion of the shaker at high speeds (fig. 22).

Figure 22. A-C. The relation between speed (mm/s) and mean measured Perfusion (PU) in the skin of the forearm after application of different concentrations of Methyl Nicotinate (MN), depending on the frame rate and the number of frames over which the perfusion is averaged ($n = 8$).

The measured perfusion decreased with an increasing camera angle. At an angle of 45° perfusion decreased 9% ($p = 0.03$), while at 60° perfusion decreased 16% ($p = 0.01$). There was a decrease in perfusion at increasing angles, however this effect was only significant when comparing 0° to 45° ($p = 0.03$) and 0° to 60° ($p = 0.01$) but not 0° to 15° and 0° to 30° . No significant variation in perfusion was observed between different distances (%CV: 0.9%, $p = 0.77$) (fig. 23).

Figure 23. Influence of distance and angle on the measured perfusion in vivo on the dorsal side of the forearm.

Conclusion

Tissue perfusion as measured with LSCI increases with increasing tissue motion, independent of frame rate, number of images, and tissue perfusion. Consequently, to reduce the risk for random motion artefacts during clinical use of LSCI, image acquisition time should be kept as low as possible while maintaining adequate image quality. During periodic tissue motion, images should be acquired continuously, because in the resulting images sequence, the images with the lowest perfusion values can be used to represent a value that is as close as possible to the true tissue perfusion. The measured perfusion will decrease when images are acquired at an angle larger than 45° , and this should be considered in certain clinical settings. Different distances between the camera and the assessed tissue within the focus range of the camera (15 to 40 cm) do not affect the measured perfusion.

Paper II: Monitoring of partial and full venous outflow obstruction in a porcine flap model using laser speckle contrast imaging

Summary

Laser Doppler flowmetry (LDF) is often used for postoperative monitoring of blood flow in microvascular flaps. The technique measures the microvascular perfusion in the skin which is proportional to the concentration and velocity of red blood cells. A drawback of the technique is that it only measures perfusion in a small volume of tissue (single point measurement). As the skin perfusion has a large spatial heterogeneity it may be preferable to measure over a larger skin area.

In this study we used LSCI and LDF to assess a porcine pedicled flap model based on the cranial gluteal artery perforator (CGAP). We simulated the postoperative situation of a free tissue transfer where the vessels have been anastomosed and there is a threatening thrombosis occluding either the artery or vein. The model allowed for a controlled venous and arterial occlusion and an exact assessment of the blood flow in the supplying vessels.

The aim of this study was to compare LSCI with LDF as tools to detect partial and full venous outflow obstruction, as well as complete arterial obstruction.

Methods

Five pigs were used in the study. Flaps were raised on both buttocks, one side at a time. The pedicle, containing the cranial gluteal artery perforator and vein, was isolated and perivascular flow probes were placed around both vessels. An inflatable vascular occluder was placed around the vein, distal to the probe. The amount of occlusion could be controlled using a 2 cm³ saline filled syringe. LDF probes connected to a laser Doppler monitor were placed central on the

Figure 24. LSCI images from one representative flap showing typical perfusion images during the six different phases of the of the protocol (left); (A) baseline, (B) 50% venous occlusion, (C) 100% venous occlusion, (D) recovery, (E) 100% arterial occlusion and (F) recovery.

flaps and a the LSCI camera was placed approximately 20-25 cm above the surface of the flap.

The protocol started with baseline measurements. Flap arterial flow, flap venous outflow and skin temperature were measured every 5 min during the whole protocol. Skin perfusion was continuously measured using LDF and LSCI. LSCI images were acquired every 5 min during baseline and every minute during occlusion. After 30 min of baseline measurements, the flap outflow vein was partially (~50%) occluded and after 30 min of partial occlusion, the flap vein was clamped with a removable microvascular clamp to achieve complete occlusion. The clamp was removed after 30 min and a 40-min venous recovery phase was initiated. The protocol ended with 30 min arterial occlusion with the vascular clamp applied to the flap artery followed by 30 min of arterial recovery (fig. 24).

Results

Both LDF and the LSCI detected significant changes in flap perfusion. During partial venous occlusion, mean perfusion over all regions of interest (ROI) decreased from baseline, although this was only significant for the LSCI measurement: LSCI: 63.5 ± 12.9 PU ($p=0.01$) vs. 76.8 ± 9.9 PU at baseline, LDF 31.3 ± 15.7 vs 36.6 ± 17.3 PU at baseline ($p=0.64$). After complete venous occlusion, a further decrease in perfusion was observed: LSCI 54.6 ± 14.2 PU ($p < 0.001$) and LDF 16.7 ± 12.8 PU ($p < 0.001$). After release of the venous cuff, LSCI detected a return of the perfusion to a level slightly, but not significantly, below the baseline level 70.1 ± 11.5 PU ($p=0.39$), while the LDF signal returned to a level not significant from the baseline 36.1 ± 17.9 PU ($p > 0.99$). Perfusion during complete arterial occlusion decreased significantly as measured with both methods. LSCI: 48.3 ± 7.7 (PU, $p<0.001$) and LDF: 8.5 ± 4.0 PU ($p<0.001$). However, when looking at perfusion values as measured with LSCI in individual ROIs, no significant change could be seen in the

Figure 25. Perfusion in absolute AU (mean, SEM) as measured by LSCI in the well-vascularized zone ROI 1, and the poorly vascularized zone ROI 3 for the six different phases of the protocol: (A) baseline, (B) 50% venous occlusion, (C) 100% venous occlusion, (D) recovery, (E) 100% arterial occlusion and (F) recovery. **($p < 0.005$) and *($p < 0.05$) indicate that there is a significant difference in perfusion compared with baseline, ns = no significant difference in perfusion compared with baseline.

distal ROI (3) (fig. 25). During partial and complete venous occlusion, LSCI showed a 20% and 26% intersubject variability (CV%), respectively, compared to 50% and 77% for LDF.

Conclusion

The main finding of this study was that LSCI could detect regional changes in skin perfusion in flaps after partial and complete venous obstruction. We observed significant decreases in perfusion during both partial and complete venous occlusion with LSCI, while changes in perfusion observed with LDF during venous occlusion were less consistent. This difference in sensitivity between LSCI and LDF is most likely explained by the higher variability seen with LDF (measured as % coefficient of variation), which is related to the heterogeneity in the perfusion in the skin, which considerably affects the LDF results. Placement of the probe will thus greatly affect all LDF measurements, whereas LSCI offers the possibility to assess skin perfusion over larger areas.

We observed a more pronounced reduction in skin perfusion during complete arterial occlusion than during both partial and complete venous occlusion, but the perfusion was not reduced to zero, neither with venous nor arterial occlusion. This was expected since the biological zero does not equal zero PU, but part of the measured remaining perfusion during occlusive phases may be attributed to a venous backflow effect. Local variations in perfusion could be observed in the flap during all phases of the protocol, which also indicates a remaining blood flow, even in the occlusive phases.

We were unable to see any significant post occlusive reactive hyperemia after the arterial occlusion, even if there was a more pronounced rise in perfusion in well-vascularized compared to poorly vascularized areas of the flap. Post occlusive reactive hyperemia is normally seen after arterial occlusion and we speculate that absence might be caused by the fact that we applied a venous occlusion before the arterial occlusion, which could have affected the vascular reactivity.

Paper III: The use of laser speckle contrast imaging to predict flap necrosis: an experimental study in a porcine flap model

Summary

In this experimental study, we evaluated laser speckle contrast imaging (LSCI) as an alternative method for perioperative assessment in reconstructive surgery. The aim of this study was to investigate whether LSCI can be used intra-operatively to identify flap areas with compromised circulation and thereby predict areas with a high risk of postoperative necrosis. Our hypothesis was that a decrease in perfusion, as measured with LSCI, could be seen in areas with compromised circulation during the first 30 min after the flap was raised. We also hypothesized that threshold values of the perfusion could be identified, which may potentially be used intraoperatively to assist in planning the flap and for safe removal of tissue parts that will have a poor viability postoperatively.

Methods

We used a porcine pedicled flap model based on the cranial gluteal artery perforator (CGAP). The model was similar to the one used in paper II, with the difference that the flap was considerably elongated distal to the pedicle to produce flap regions with a compromised circulation in the distal end. A 10×20 cm fasciocutaneous island flap was raised from the right buttock of each pig. The flap was dissected along the surface of the muscle including the skin, subcutaneous tissue, and muscle fascia. The pedicle containing the perforator artery along with comitant veins was isolated. The flap was then reinserted in its original place. Clinical evaluation and measurements with LSCI (PeriCam PSI System, Perimed AB, Järfälla, Sweden) was done before the flap was raised (baseline), directly after the operation ($t = 0$) and 30 minutes after ($t = 30$). After the initial measurements, the animal was returned to its box and awakened from anesthesia. After 72h, the animal was anesthetized, and after the final measurements were made, the animal was euthanized.

Digital photographs of the flaps were taken at the respective time points, and demarcation lines were overlaid on each image to indicate where the surgeons believed that viability was questionable. In each flap, the distance from the pedicle base to the demarcation line was measured using ImageJ software.

Figure 26. Example of changes in visual appearance (A–C) and perfusion (D–F) in a CGAP flap, directly (A, D) and 30 min (B, E) after raising the flap. The dashed black line represents the proximal border of the area with compromised circulation as predicted by clinical assessment. The dashed white line (C) represents the proximal border of the manifested ischemic area at $t = 72$ h. The colored dots indicate the regions of interest (ROI) wherein perfusion was measured. ROIs are numbered as 1–10, starting from the left to right (proximal to distal). (F) shows the change in perfusion from $t = 0$ to $t = 30$ min in different ROIs (green = viable, yellow intermediate, and red ischemic) in the same flap as that shown in A–E.

Results

After the flaps were raised ($t = 0$ min), a darker area interpreted as an area with compromised circulation could be seen on all flaps at a mean (SD) distance of 10.7 (1.9) cm from the proximal border of the flaps, and beyond. At $t = 30$ min, the distance to the area with stasis was 10.1 (0.8) cm. After 72h, a demarcation line was seen at a distance of 15.8 (0.4) cm from the proximal border of the flap. The area distal to this line had no capillary refill or arterial bleeding when punctured with a biopsy punch (fig. 26).

Ten regions of interest (ROI) were chosen along the central axis of the flaps. The mean perfusion of the flaps at baseline, $t = 0$, and $t = 30$ min for 10 different ROIs is shown in Figure 2. At baseline, the highest perfusion in the flap was 86 ± 17 PU (ROI 2) and the lowest perfusion was 68 ± 15 PU (ROI 9). The perfusion decreased slightly from the proximal to the distal regions of the flap (at most a 20% decrease in ROI 9 compared to that in ROI 1, $p < 0.001$). At $t = 0$, a general decrease in perfusion was observed in all ROIs compared to that at baseline (ranging from 75.6 ± 17.8 PU in ROI 1 to 30.8 ± 5.4 PU in ROI 10). The decrease in perfusion from the proximal to the distal side of the flap became more pronounced with time, with ROI 10 having a 59% and 70% decrease in perfusion compared to ROI 1 at $t = 0$ and $t = 30$ min, respectively ($p < 0.001$).

Changes in perfusion between baseline and either $t = 0$ or $t = 30$ min were significant in all ROIs ($p < 0.001$). Between $t = 0$ and $t = 30$ min, a significant decrease in perfusion could only be seen in the distal parts of the flap (ROI 8–10, $p = 0.03$). In the proximal and medial ROIs, perfusion either increased or was stable during the first 30 min after raising the flap. At $t = 30$ min, no ROI distal to the demarcation line at $t = 72$ h had a perfusion above 25 PU. At $t = 72$ h, perfusion in the area proximal to the demarcation line (ROI 1–8) had recovered to values that did not show significant difference with baseline values. In ROI 9, perfusion increased to a value that did not show significant difference with $t = 0$ values, and in ROI 10, the perfusion remained on a value that did not show significant difference with $t = 30$ min values (fig. 27).

Figure 27. The mean (SD) perfusion in ten different regions of interest (ROI) of the flaps (proximal to distal) at three different time points; baseline (before surgery), directly after raising the flap ($t = 0$ min), and 30 min after raising the flap ($t = 30$ min). Changes in perfusion between baseline and either $t = 0$ or $t = 30$ min were significant in all ROIs ($p < 0.001$). Between $t = 0$ and $t = 30$ min, a significant decrease in perfusion could only be seen in the distal ROI (ROI 8–10, $p = 0.03$).

Conclusion

The main finding of this study is that a decrease in perfusion during the first 30 min after raising the flap could be used as a predictor for tissue morbidity 72h after surgery in a porcine flap model. Flap areas with a perfusion below 25 PU were at risk for necrosis, however two out of six flaps included areas with PU below 25 which later recovered. If this data had been applied in a clinical setting, a lower threshold value would have led to less unnecessary excisions, but instead later ischemic areas would have been missed.

Based on visual appearance and capillary refill during the first 30 min after the flap was raised, observers typically overestimated the areas that would present clinical signs of ischemia three days after the surgery.

Paper IV: Intraoperative laser speckle contrast imaging in DIEP breast reconstruction: a prospective case series study

Summary

In this study we used laser speckle contrast imaging (LSCI) for intraoperative evaluation of tissue viability during deep inferior epigastric perforator (DIEP) free flap surgery. We measured perfusion for different perfusion zones of the DIEP flap according to the previous description of the transverse rectus abdominis flap done by Schefflan, Dinner and Hartrampf. The aim of this study was to investigate the perfusion distribution, as measured with LSCI, in relation to the selected perforator during DIEP surgery and to evaluate whether LSCI can assist in predicting postoperative complications.

Method

The study included twenty-three patients who underwent a unilateral DIEP flap procedure for breast reconstruction in two different centers, University Hospital Gent, Belgium and University Hospital Linköping, Sweden. LSCI was used to measure perfusion in four zones of the flaps according to Hartrampf (fig. 28). Measurements were done before surgery (baseline), after the flap was raised and after anastomosis of the vessels. Measurements were also done after shaping and inserting the flap, but it was not possible to identify the different zones at this point, and data from these measurements were therefore not included in the part of the study comparing the different zones. The perfusion pattern in relation to the selected perforator and the accuracy of LSCI in predicting complications, as observed within two weeks postoperatively, were analyzed.

Figure 28. A LSCI image of a DIEP flap with the Hartrampf zones separated by the vertical white lines and indicated by the numbers in circles (I-IV). Perfusion is higher in yellow and red areas and lower in blue and green. The perforator is situated in the upper half of zone I.

Results

At baseline, the perfusion in zone I was significantly higher than the perfusion in zone III (61 ± 16 and 51 ± 13 PU, $P = 0.006$), and in zone IV (53 ± 12 , $P = 0.02$). Zone II was significantly higher than zone III (62 ± 15 and 51 ± 13 PU, $P < 0.004$). After raising the flap, the perfusion in zone I was significantly higher than that in zone III (65 ± 10 and 53 ± 10 PU, $P = 0.002$) and zone IV (45 ± 11 , $P < 0.001$), but not zone II (58 ± 12 PU, $P = 0.21$). There was no longer a significant difference between zones II and III ($P = 0.45$). After anastomosis, perfusion was significantly lower in zone IV compared with all other zones ($P < 0.02$) and there were no significant differences in perfusion between zones I, II, and III (fig. 29).

Figure 29. Perfusion, specified in PU, in different Hartkamp zones of the DIEP flaps. The perfusion was significantly lower in zones III and IV compared with zone I at baseline ($P = 0.006$, respectively, $P = 0.02$) and when the flaps were raised ($P = 0.002$, respectively, $P < 0.001$). When the vessels were anastomosed, the perfusion in zone IV was significantly lower than that in zones I–III ($P < 0.02$).

There were postoperative complications in five flaps. All the flaps with a minimum perfusion < 30 PU directly after surgery had postoperative complications and required revision. In 3 flaps, partial necrosis occurred either in the medial or lateral parts, and perfusion in those parts was < 30 PU directly after surgery (18, 22, and 26 PU, respectively). In one patient, necrosis occurred in the adjacent mastectomy skin that showed low perfusion after surgery (16 PU). In another patient, stiffness of the lower

Figure 30. All cases of postoperative complications were found in the group with minimum perfusion values at 30 PU or below. *The flap that went necrotic after 2 weeks due to infection.

pole was observed but without need for revision. The affected area had a perfusion of 30 PU directly after surgery. In another patient, the flap had adequate perfusion (>50 PU) and was without complications for 2 weeks. However, in the same patient a full necrosis eventually developed, but this was attributed to an infection in the flap (fig 30). Receiver operator characteristic analysis of the perfusion data revealed a cutoff of 30 PU to predict postoperative complications with 100% accuracy.

Conclusion

In this study the highest perfusion was found in zone I and higher perfusion in zone II compared to zone III, directly after the flap was raised. However, there was no remaining significant difference between zone I, II and III after anastomosis of the vessels, contrary to what we had expected. This may be related to spatial differences of the blood flow due to localization of the perforator. In previous studies, medial perforators have been found to more often give branches that cross the midline, whereas lateral perforators seldom branch across the midline. In our study we did not discriminate between medial and lateral perforators.

All flaps with a minimum perfusion <30 PU, measured after the flap was shaped and inserted, later suffered from partial flap necrosis. However, the necrotic areas could not be correlated to the different perfusion zones or to the actual assessed area. A level of 30 PU can therefore, based on this study, not be used as a threshold value that can guide the surgeon in the resection of redundant flap tissue. Instead it should, before further studies are done, be used as an indicator of later healing problems.

Discussion

General comments

An ideal technique for tissue monitoring should be easy to use, non-invasive, reliable, and affordable. In addition, a high sensitivity and specificity is necessary for such a technique to identify the relatively few complications that are associated with modern reconstructive surgery. LSCI can; non-invasively, with high resolution and relatively low cost, assess superficial dermal perfusion over a relatively large area in real time. LSCI however only assesses the superficial dermal microcirculation, and it has not been known if a decrease in measured perfusion is associated with an increase in tissue morbidity, and at what perfusion level such a risk occurs. There are currently a relatively large number of methods for assessing tissue viability, and we wanted to explore if the LSCI technique has the potential to identify tissues at risk, especially in cases of impaired vascular supply, impaired venous drainage or in areas of a flap that include a tissue mass that exceeds the capacity of the supplying pedicles vascular tree. One of the clinically most used methods for postoperative microcirculatory monitoring today is LDF. This technique with high temporal resolution, enables continuous measurements in a single measurement point through a small probe that is attached to the skin at the time of surgery. LSCI enables measurements of a larger spatial area and is mostly used for instantaneous measurements. Other than this, LDF and LSCI are in many ways related techniques. Both use lasers of similar wavelength and effect, which penetrate the skin within the same range and can thereby roughly assess the microcirculation in the same vascular compartment. They also share some limitations; there is no defined biological zero, they are both relatively sensitive to motion artefacts only superficial tissue is assessed and none of the techniques can so far offer absolute perfusion data. In addition, unlike LDF, which presents the user with a single numerical value, LSCI requires additional analysis of the regions of interest in the image in order to obtain a measurement value. This may lead to more inter-observer variation.

In the following sections some methodological and physiological factors that may affect the measurements and limit the usefulness of LSCI in reconstructive surgery will be discussed. The animal model used in the first two studies will also shortly be discussed. Finally, the advantages of perfusion assessment with LSCI in reconstructive surgery and the possibilities of the technique, based on conclusions drawn from the studies presented in this thesis and work by other research groups will be addressed.

Methodological factors that affect the measurements

In study I methodological factors that may affect the LSCI measurements were addressed. These included distance from object to camera, curvature of the tissue, how high and low perfusion states affect the results and motion of examined tissue. Of these factors, only motion of the examined tissue had any significant effect on the measured value. Doppler techniques are also highly sensitive to motion artefacts, but the problem with LDF is not as pronounced as the probe usually is attached to the assessed tissue. As was shown in study I, even small motions will affect the measurements and the tissue perfusion as measured with LSCI will increase more or less linear to the increasing tissue motion. Different solutions to this problem have been suggested. Mahé et al proposed the use of an opaque patch taped to the skin within the field of vision. The motion artefacts measured over the patch is subtracted from artefact over the region of interest. Another approach taken by Richards et al is to record the patient's electrocardiogram (ECG) during the assessment. Correction for pulsatile artifacts can then be made using an algorithm after the acquisition. Our own suggestion to address the problem is to keep image acquisition time as low as possible while maintaining adequate image quality. From our study one can also conclude that the lowest measured perfusion value is what best represents true perfusion. Images should therefore be acquired continuously, so that the images with the lowest perfusion values can be extracted from the resulting images sequence. All these approaches require data processing after the measurement and are thus more difficult to use for real time assessment. The problem of not being able to interpret LSCI measurements in real time without considering possible motion artifacts is still waiting for its solution.⁸⁴⁻⁸⁸

In study II, even at complete arterial occlusion, the perfusion, measured with both LDF and LSCI, never reached zero PU. Both these techniques have the problem of the biological zero in common. Under certain conditions, when for example the circulation in an extremity is completely strangulated by pressure cuff, one would expect the perfusion value to drop to zero. However, both LDF and LSCI still give an output signal, higher than the electronic zero output caused by photoelectronic white noises of the instrument. Mahé et al showed in a LSCI study from 2011 that perfusion dropped to 9.1 PU during complete arterial occlusion of the forearm in healthy subjects. This could be considered an approximation of the biological zero and it is thought to be caused by random movement, for example Brownian motions, of blood cells in the tissue. This does not cause any concerns as long as the assessed tissue is well perfused with high perfusion values, but it may be harder to interpret the measurement when the perfusion values decrease in areas with questionable circulation.^{84,89,90}

Physiological factors that affect the measurements

Perfusion measurement methods that use laser speckle contrast analysis and laser Doppler technique both analyze laser light reflected from red blood cells to estimate the concentration and velocity of the cells in the assessed tissue. The measurement depth is therefore depending on how deep the photons penetrate and how many photons that are reflected and is captured by the light detector. This in turn depends on several factors, such as the wavelength of the laser, concentration and distribution of red blood cells in the assessed volume and in the case of LDF, the fiber separation in the probe. Biological factors such as thickness of the skin and pigmentation may also affect the measurement depth, but this has not been investigated to any extent. The blood flow that mainly contributes to the measured perfusion value in both LDF and LSCI occurs within the upper 0.5 mm of skin.^{79,91} This means, that the methods only assess the perfusion in the superficial dermal plexus in the papillary dermis. The deeper plexus in the junction between dermis and the subdermal tissue do not contribute to any extent to the measured perfusion value. The consequence of this is, that both techniques may fail to assess the arteriovenous shunts located in the deeper dermis that accounts for a large part of the dermal circulatory regulation. These are involved in for example thermoregulation, which is an important function of the skin. The dermal blood flow can, if needed, increase from a baseline of 250 ml / min to 8 L / min to meet an increased need for cooling, for example in the case of hard work or high ambient temperatures.^{31,32}

There are situations where systemic factors may affect the dermal microcirculation. Several studies that indicate a correlation between the dermal microcirculation and conditions that affect the more central hemodynamics, for example diabetes, hypothermia or cardiovascular shock.⁹²⁻⁹⁴ An experimental study by Wester et al from 2011 has shown a correlation between severe sepsis and decreased perfusion as measured with laser doppler perfusion monitor of auricular and lingual skin in a porcine model. This study was followed up by a clinical study on patients with acute cardiogenic shock treated with extra-corporeal membrane oxygenation (ECMO). Here, laser Doppler measurements of the skin showed no significant difference between healthy controls, survivor and non-survivors but examination with skin vital microscopy of the latter group, revealed major skin microvascular pathology, such as pericapillary bleedings, pericapillary dark haloes and capillary micro-thrombi.^{95,96} The possible inability of LSCI to detect such conditions can of course be seen as a disadvantage of the method. However, the significance of this possible discrepancy between actual perfusion and measured perfusion will probably not be as great in the clinical context of reconstructive surgery as this thesis highlights. The temperature in the operating room is strictly regulated and patients with conditions that affect the body temperature and microcirculation in the skin are normally not operated on.

The animal model

In study II and III, a porcine flap model based on the cranial gluteal artery perforator was used. A similar model was developed by Russell et al as a model to study skin flap physiology in response to varying levels of occluded venous outflow in free flaps.⁸³ It may be questionable to use a pedicled flap as a free flap model. Unlike a pedicled flap, a free flap will be exposed to ischemia before it is inserted in its new location and the anastomosed vessels are to some extent always traumatized with an increased risk of thrombosis. However, in our case, we were not specifically studying pathophysiology behind ischemia and vascular thrombosis in free flaps, but rather the possibility to predict any kind of impaired viability measured as a decrease in perfusion in the skin of the flap. Therefore, a free flap model would not have contributed with any additional information, instead it would have made the study set up more complicated with an increased risk of bias. An advantage of the choice of species is the similarity of skin features between human and pig. The thickness of both epidermis and dermis is comparable in the two species and the dermal microcirculation in the porcine dermis is similar to vessels in human skin.^{97,98} Another advantage of the porcine model is the possibility to raise large flaps based on a single vessel suited for adequate measurements.

Laser speckle contrast imaging in reconstructive surgery

Laser Doppler flowmetry has been used for more than three decades for assessment of free flaps and other reconstructive procedures, so the knowledge of the pros and cons of the technique is profound.⁹⁹ There is a number of studies that consolidate the usefulness of LDF in a clinical setting, in reconstructive surgery as well as in other surgical and medical fields. In microvascular free flap surgery, the technique is in many ways considered the golden standard with both high positive and high negative predictive value.⁴⁰ Yuen et al presented in 2000 a retrospective study on 232 microvascular transfers to head and neck. Thirteen flaps developed vascular complications, and all of these were detected with LDF without false positives or negatives.¹⁰⁰ In our study on partial and full venous occlusion in a porcine flap model, and in a study from 2010 by Gimbel et al on compromised venous outflow in a rabbit skin flap model, the decreasing perfusion as measured with LDF correlates very well with the grade of venous obstruction in the pedicle of the flap.

It seems hard to question the usefulness of Doppler techniques in reconstructive surgery and that the dermal microcirculation assessed with LDF seems to correlate to the tissue viability. The similarities between LSCI and LDF, a highly established method for perfusion assessment and tissue monitoring, makes it motivated to evaluate the utility of the LSCI technology in reconstructive surgery. However, techniques using laser speckle contrast analysis are not investigated to the same extent as laser Doppler techniques.

To the authors' knowledge, there are no studies directly comparing LSCI with LDF for assessing tissue viability in reconstructive surgery, except from our study on partial and total venous occlusion (study II). The aim of this study was to evaluate if LSCI was able to detect a simulated postoperative thrombosis and we could show that LSCI was able to detect both venous and arterial occlusion, as well as or better than LDF with less intersubject variability. Venous occlusion is one of the most common reasons for postoperative complications after microvascular free flap surgery, however, the conclusion was that LSCI, in its current configurations, cannot replace LDF for postoperative monitoring of free flaps. The technique is not suited for continuous monitoring, it cannot assess non-exposed tissue, it is motion sensitive and the commercially available systems offered today are relatively bulky. Instead, the technique could preferably be used for intra-operative assessment and planning during reconstructive surgery.

Today, one of the more established methods for intra-operative evaluation of tissue perfusion is fluorescence angiography (FA). This technique is sometimes called non-invasive, but it requires intravenous injection of indocyanine green (ICG). There are some disadvantages with ICG FA compared to LSCI. New dye must be injected for every assessment and there are case reports of severe allergic reactions to indocyanine green, in a few cases fatal. Another drawback of this technique is the price. The cost of one of the more widespread systems for ICG angiography, the SPY Elite, is about \$275,000 for an operating unit, with an additional cost of \$275 for the ICG dye and drapes for every case.¹⁰¹ This can be compared to the cost for the LSCI system PeriCam PSI, which is about \$56,000 according to the manufacturer Perimed AB, with no additional cost per case.

One of the aims of study III and IV was to, if possible, identify a threshold value for perfusion measured with LSCI which could be predictive for postoperative complications. As with ICG FA, it is not possible to achieve absolute perfusion values with LSCI. The measured perfusion is instead presented in arbitrary units and it is not linear relative to absolute flow. This could make it harder to compare different individuals and different body locations. Instead, the technique might be more fitted to assess temporal changes in predetermined areas. Still, a rather low intersubjective variability was found, at least in the porcine model. In study III all flap areas with perfusion below 20 were necrotic after 72 hours and we also found all cases of postoperative complications in study IV in the group with minimum perfusion values at 30 PU or below. Although it is not known if the areas of low perfusion were related to necrotic areas in the most recent study, it is interesting that the perfusion values in both studies are within approximately the same range. It is also interesting that the perfusion in the distal part of the flaps in study I was between 20 and 30 PU both in partial and complete venous occlusion with no significant change from baseline. Based on this reasoning, it does not seem farfetched to assume that perfusion values below 30 PU are

an indication that the area is at risk of developing postoperative complications. However, it is not certain that it is possible to set a certain threshold that applies in all situations. A larger study, including different types of flaps with a more durable marking to help identify the areas at risk until postoperative complications occur, may make it possible to narrow the threshold value further. Until then, perfusion values below 30 PU, as measured with LSCI, can be used together with traditional methods as color of the skin or slow capillary blink as an indicator of possible future complications.

In Study IV the perfusion distribution in DIEP flaps in relation to the perfusion zone described by Schefflan, Dinner and Hartrampf was investigated.^{19,37} This is interesting because knowledge of the perfusion pattern in flaps aids surgeons with surgical planning and in deciding which parts of the flaps are viable. As described in the section about perforators and angiosomes, the optimal perfusion in DIEP flaps is found in the skin above the perforator. It has been debated whether the adjacent contralateral or ipsilateral zone is best perfused thereafter.³⁸ We could see no significant difference between these zones in our study. However, it has been shown that medial perforators more often give branches that cross the midline, whereas lateral perforators seldom branch across the midline.¹⁰² There were unfortunately no discrepancy between lateral and medial perforators in the study, which may explain the lack of significant difference in the measured perfusion between the zones.

Is laser speckle contrast imaging observer independent?

In study II, the usefulness of LSCI in postoperative monitoring of microvascular free flaps was questioned. In this setting there is a need for a more continuous assessment to be able to early detect threatening vascular occlusions and the added value of a high spatial resolution is not as high as in intraoperative evaluation of tissue viability. Another issue that may make LSCI considered a less optional alternative for post-operative monitoring is the interobserver variability. As the monitoring normally takes place over several days after the surgery, the task is often delegated to several individuals. This also requires that the technique is easily interpreted to be used by other professional categories than experienced surgeons. In a LSCI image, the perfusion is presented as a color-coded map with lower perfusion shown in blue scales and higher perfusion red scales, with green and yellow in between. The mean perfusion presented in arbitrary units can be extracted by marking regions of interest in the picture. Therefore, in a clinical setting, the observer will mainly rely on the interpretation of the color map or will have to select regions of interest to extract mean perfusion values. This, of course, involves a risk that different observers make different interpretations of the image. It seems, at least in burn wound assessment, that perfusion measurements with LSCI are observer independent.¹⁰³ However, intermittent assessment with LSCI would only provide snapshots of the

perfusion and the benefits of the high resolution would not outweigh the risks of delayed detection discovery of a possible vascular occlusion.

The future of tissue monitoring with laser speckle contrast imaging

Laser speckle contrast imaging is a relatively straightforward technique which has a number of advantages over other techniques, including its fast acquisition time, its spatial resolution and the fact that it is completely non-invasive. The systems available today are however subject to certain drawbacks which limit its usability. Some of these drawbacks are unfortunately fundamental limitations in the technology which are hard to overcome, such as the limited depth of measurement and its inability to present absolute perfusion values, whereas other limitations could easily be overcome by relatively simple improvements.

The current systems are still too bulky to be easily introduced into clinical use in a surgical setting. The discrete components of a LSCI system are, however, relatively small. If the problem with motion artefacts could be addressed, for example with the above-mentioned methods, a handheld LSCI system could easily be assembled. It might also be beneficial to include an optical projector in the system. This would make it possible to project the perfusion pattern directly on the assessed tissue to give instant feedback and clearly visualize the perfusion in different areas of the assessed tissue. With these modifications, LSCI could contribute to a more accurate pre- and perioperative assessment of the tissue perfusion, and thus may better help improve outcome in reconstructive surgery (fig. 31).

Figure 31. The Tagliacozzi flap described in the historical review can still be used for reconstructions in the face, when free flap surgery for some reason is not possible. The flap can for example cover a defect on the forehead as in the illustration above. The pedicle of the flap is usually divided after three weeks. LSCI can be used to assess the flap before the pedicle is divided to assure a sufficient perfusion of the tissue.

In postoperative monitoring, on the other hand, systems for full field perfusion assessment offers unnecessarily high resolution and will not give a continuous assessment. Many microvascular surgeons instead use LDF for postoperative assessment, that offers continuous monitoring that can be easily assessed by the health care staff.^{100,104} However, the drawback with all single point measurement systems (including LDF) is the spatial

heterogeneity in the dermal perfusion.³³ As we saw in study II, LDF shows a higher variability in the assessment of dermal perfusion compared to LSCI. This means that the perfusion value might differ considerably depending on where the probe is placed. A solution to this is to measure the blood flow directly on the anastomosed vessels. Since this value represents the total blood flow in the flap, it gives a very good estimation of the viability of the flap. The two commercial systems taking advantage of this fact, the Cook-Swartz implantable Doppler probe and the Flow-Coupler, both are depending on a wire protruding through the wound that leads the signal to a bedside monitor. The wire is removed from its attachment by twitching it gently. This procedure can, of course, be perceived as risky as the surgeon may accidentally dislocate the vessel or damage the anastomose. It would therefore be appealing to use a wireless probe to avoid manipulating the vessel. Several research groups, including our own, are working on such solutions.¹⁰⁵⁻¹⁰⁷ The probe would use a small battery or some kind of inductive energy source and transfer data to an external monitor. The probe would not be removed after the postoperative monitoring period. This would give the surgeon a prolonged access to the blood flow over the anastomosed vessels without the risk in manipulating them and potentially damage the anastomose.

Conclusion

Laser speckle contrast imaging (LSCI) is a laser based, non-invasive technique for perfusion measurement. In study I presented in this thesis, some limitations to the technique was addressed. The most prominent of these is motion artefacts. There are different methods partially overcome this, including shorter sampling times and careful selection of appropriate images for analysis. In study II, LSCI was used to assess a pedicled flap with the aim to detect a decrease in both venous and arterial blood flow. It was concluded that the technique has the potential of early detection of postoperative vascular occlusions with the same accuracy as LDF, but with lower variability. Despite this, the technique has limitations in postoperative monitoring that makes it more suited for peri-operative tissue assessment. In study III LSCI was used to assess pedicled flaps in a porcine model and in study IV the technique was used to assess DIEP flap during the operation. In study III and IV, it was shown that LSCI can be used to assess tissue perfusion in flap surgery intra-operatively and thereby predict areas at risk for compromised viability post-operatively. In both these studies, there were indications that there exists a threshold value for perfusion as measured with LSCI, below which the risk of postoperative complications increases. Study IV also showed a significantly higher perfusion in the skin area above the perforator, but no difference between the adjacent zones.

The conclusion that can be drawn from this thesis is, that laser speckle contrast imaging is a technology that has the potential to contribute to tissue monitoring in reconstructive surgery. It has many advantages over other techniques, such as the fast acquisition time, the spatial resolution and the fact that it is completely non-invasive. However, the

current system is still too bulky to be easily introduced into a clinical setting and the technology is also subject to certain drawbacks which limit its usability. If some of these disadvantages could be addressed, LSCI could contribute to a more accurate assessment of tissue perfusion and help the surgeon to identify tissue at risk during surgery.

Acknowledgements

Simon Farnebo

The most positive, energetic and helpful supervisor one could wish for, even if it sometimes is hard to keep up with your pace. Hopefully this is just the beginning of a long and fruitful collaboration.

Erik Tesselaar

One of the smartest and friendliest people I know. A Dutch who sometimes corrects my Swedish and more often my statistics. Ad Astra!

Jacob Wikner

Although it did not manifest itself in this thesis, I have very much appreciated our collaboration over the last years and hope that it will continue for many more. I truly believe that we eventually will be able to work out how to make that wireless implantable blood flow meter.

The Department of Hand and Plastic Surgery and Burns (HPK)

A fantastic workplace that combines two of the most fascinating surgical specialties into one creative and competent team. It has very seldom felt hard to go to work with such colleagues.

Jennie

For all the love and support. What unexpected paths life can take after a brief meeting in an elevator in Dallas.

Einar, Hedvig and Vidar

My amazing children, who in a couple of years will beat me in everything that I think that I'm good at (just as it should be). I know that on and off I have been a little grumpy in recent months. Hopefully it gets better soon.

References

1. Barr J. Vascular medicine and surgery in ancient Egypt. *J Vasc Surg.* 2014;60(1):260-263. doi:10.1016/j.jvs.2014.04.056
2. Halwani T, Takrouiri M. Medical laws and ethics of Babylon as read in Hammurabi's code (History). *Internet J Law, Healthc Ethics.* 2006;4(2).
https://print.ispub.com/api/0/ispub-article/10352.
3. Adamson PB. *Surgery in ancient mesopotamia.* Vol 35.; 1991.
4. Ellis H, Abdalla S. *A History of Surgery.* Third edit. CRC Press; 2019.
5. Bath K, Aggarwal S, Sharma V. Sushruta: Father of plastic surgery in Benares. *J Med Biogr.* 2019;27(1):2-3. doi:10.1177/0967772016643463
6. Champaneria MC, Workman AD, Gupta SC. Sushruta: Father of plastic surgery. *Ann Plast Surg.* 2014;73(1):2-7. doi:10.1097/SAP.0b013e31827ae9f5
7. Mazzola IC, Mazzola RF. History of reconstructive rhinoplasty. *Facial Plast Surg.* 2014;30(3):227-236. doi:10.1055/s-0034-1376868
8. von Gräfe CF. *Rhinoplastik Oder Die Kunst Den Verlust Der Nase Organisch Zu Ersetzen.* Nabu Press
9. Thomas R, Fries A, Hodgkinson D. Plastic Surgery Pioneers of the Central Powers in the Great War. *Craniofac Trauma Reconstr.* 2019;12(01):001-007.
doi:10.1055/s-0038-1660443
10. Macnamara AF, Metcalfe NH. Sir Archibald Hector McIndoe (1900-1960) and the Guinea Pig Club: The development of reconstructive surgery and rehabilitation in the Second World War (1939-1945). *J Med Biogr.* 2014;22(4):224-228.
doi:10.1177/0967772013480691
11. Boyce DE, Shokrollahi K. Reconstructive surgery The “reconstructive ladder.” *BMJ.* 2006;332:710-712. doi:10.1136/bmj.332.7543.710
12. Gropper PT, Kester DA, McGraw RW. Introduction to microsurgery. *Clin Obstet Gynecol.* 1980;23(4):1145-1150. doi:10.1097/00003081-198012000-00020
13. Dohlman GF. Carl Olof Nylén and the Birth of the Otomicroscope and Microsurgery. *Arch Otolaryngol.* 1969;90(6):813-817. doi:10.1001/archotol.1969.00770030815025
14. Mudry A. The history of the microscope for use in ear surgery. *Am J Otol.* 2000;21(6):877-886.
15. Malt RA, Mckhann CF. Replantation of Severed Arms. *JAMA J Am Med Assoc.* 1964;189(10):716-722. doi:10.1001/jama.1964.03070100010002
16. McLean DH, Buncke HJ. Autotransplant of omentum to a large scalp defect, with microsurgical revascularization. *Plast Reconstr Surg.* 1972;49(3):268-274.
doi:10.1097/00006534-197203000-00005

17. Daniel RK, Taylor GI. Distant transfer of an island flap by microvascular anastomoses: A clinical technique. *Plast Reconstr Surg*. 1973;52(2):111-117. doi:10.1097/00006534-197308000-00001
18. Champaneria MC, Wong WW, Hill ME, Gupta SC. The evolution of breast reconstruction: A historical perspective. *World J Surg*. 2012;36(4):730-742. doi:10.1007/s00268-012-1450-2
19. Schefflan M, Dinner MI. The transverse abdominal island flap: part I. Indications, contraindications, results, and complications. *Ann Plast Surg*. 1983;10(1):24-35. <http://www.ncbi.nlm.nih.gov/pubmed/6219607>. Accessed November 21, 2018.
20. Robbins TH. Rectus abdominis myocutaneous flap for breast reconstruction. *Aust N Z J Surg*. 1979;49(5):527-530. doi:10.1111/j.1445-2197.1979.tb05869.x
21. Holmström H. The free abdominoplasty flap and its use in breast reconstruction: An experimental study and clinical case report. *Scand J Plast Reconstr Surg Hand Surg*. 1979;13(3):423-427. doi:10.3109/02844317909013092
22. Allen RJ, Treece P. Deep inferior epigastric perforator flap for breast reconstruction. *Ann Plast Surg*. 1994;32(1):32-38. doi:10.1097/00000637-199401000-00007
23. Boucher F, Brosset S, Shipkov H, et al. An anatomic study of deep inferior epigastric artery diameters at the origin from external iliac and at the lateral border of rectus abdominis muscle by computed tomographic angiography from autologous breast reconstruction patients. *Ann Chir Plast Esthet*. April 2019. doi:10.1016/j.anplas.2019.03.003
24. Lhuire M, Hivelin M, Dramé M, et al. Determining the best recipient vessel site for autologous microsurgical breast reconstruction with DIEP flaps: An anatomical study. *J Plast Reconstr Aesthetic Surg*. 2017;70(6):781-791. doi:10.1016/j.bjps.2017.01.008
25. Canizares O, Mayo J, Soto E, Allen RJ, Sadeghi A. Optimizing Efficiency in Deep Inferior Epigastric Perforator Flap Breast Reconstruction. *Ann Plast Surg*. 2015;75(2):186-192. doi:10.1097/sap.0000000000000559
26. Stern HS, Nahai F. The versatile superficial inferior epigastric artery free flap. *Br J Plast Surg*. 1992;45(4):270-274. doi:10.1016/0007-1226(92)90050-8
27. Fujino T, Harashina T, Aoyagi F. Reconstruction for aplasia of the breast and pectoral region by microvascular transfer of a free flap from the buttock. *Plast Reconstr Surg*. 1975;56(2):178-181. doi:10.1097/00006534-197508000-00010
28. Healy C, Allen RJ. The evolution of perforator flap breast reconstruction: twenty years after the first DIEP flap. *J Reconstr Microsurg*. 2014;30(2):121-125. doi:10.1055/s-0033-1357272
29. Reid L, Meyrick B. Microcirculation: definition and organization at tissue level. *Ann N Y Acad Sci*. 1982;384(1 Mechanisms of):3-20. doi:10.1111/j.1749-6632.1982.tb21357.x
30. Rorsman H, Björnberg A, Vahlquist A. Hudens struktur och funktion. In: *Dermatologi Venerologi*. Studentlitteratur; 2007:13-32.

31. Braverman IM. The Cutaneous Microcirculation. *J Investig Dermatology Symp Proc.* 2000;5(1):3-9. doi:10.1046/j.1087-0024.2000.00010.x
32. Charkoudian N. Skin blood flow in adult human thermoregulation: How it works, when it does not, and why. *Mayo Clin Proc.* 2003;78(5):603-612. doi:10.4065/78.5.603
33. Wardell K, Braverman IM, Silverman DG, Nilsson GE. Spatial heterogeneity in normal skin perfusion recorded with laser Doppler imaging and flowmetry. *Microvasc Res.* 1994;48(1):26-38. doi:10.1006/mvre.1994.1036
34. Taylor GI, Palmer JH. The vascular territories (angiosomes) of the body: experimental study and clinical applications. *Br J Plast Surg.* 1987;40(2):113-141. doi:10.1016/0007-1226(87)90185-8
35. Taylor GI, Corlett RJ, Dhar SC, Ashton MW. The anatomical (angiosome) and clinical territories of cutaneous perforating arteries: development of the concept and designing safe flaps. *Plast Reconstr Surg.* 2011;127(4):1447-1459. doi:10.1097/PRS.0b013e318208d21b
36. Cannon F V, Taylor AO GI, Gascoigne AC, Corlett R, Ashton MW. Understanding the vascular anatomy of zone IV in DIEA perforator flaps. *Australas J Plast Surg.* 2018;1(1):71-80. doi:10.34239/ajops.v1i1.38
37. Hartrampf CR, Schefflan M, Black PW. Breast reconstruction with a transverse abdominal island flap. *Plast Reconstr Surg.* 1982;69(2):216-224. doi:10.1097/00006534-198202000-00006
38. Holm C, Mayr M, Höfter E, Ninkovic M. Perfusion zones of the DIEP flap revisited: A clinical study. *Plast Reconstr Surg.* 2006;117(1):37-43. doi:10.1097/01.prs.0000185867.84172.c0
39. Pickard A, Karlen W, Ansermino JM. Medical intelligence article: capillary refill time: is it still a useful clinical sign? *Anesth Analg.* 2011;113(1):120-123. doi:10.1213/ANE.0b013e31821569f9
40. Smit JM, Zeebregts CJ, Acosta R, Werker PM. Advancements in free flap monitoring in the last decade: a critical review. *Plast Reconstr Surg.* 2010;125(1):177-185. doi:10.1097/PRS.0b013e3181c49580
41. Cina A, Barone-Adesi L, Rinaldi P, et al. Planning deep inferior epigastric perforator flaps for breast reconstruction: A comparison between multidetector computed tomography and magnetic resonance angiography. *Eur Radiol.* 2013;23(8):2333-2343. doi:10.1007/s00330-013-2834-x
42. Ghattaura A, Henton J, Jallali N, et al. One hundred cases of abdominal-based free flaps in breast reconstruction. The impact of preoperative computed tomographic angiography. *J Plast Reconstr Aesthet Surg.* 2010;63(10):1597-1601. doi:10.1016/j.bjps.2009.10.015
43. Varela R, Casado-Sanchez C, Zarbakhsh S, Diez J, Hernandez-Godoy J, Landin L. Outcomes of DIEP flap and fluorescent angiography. *Plast Reconstr Surg.* September 2019;1. doi:10.1097/prs.0000000000006393

44. Schmid M, Seyed Jafari SM, Haug L, et al. Laser doppler imaging as additional monitoring after digital replanting: A prospective study. *Microsurgery*. 2018;38(6):627-633. doi:10.1002/micr.30318
45. Zdolsek JM, Droog EJ, Thorfinn J, Lidman D. Laser Doppler perfusion imaging of the radial forearm flap: a clinical study. *Scand J Plast Reconstr Surg hand Surg*. 2006;40(2):101-105. doi:10.1080/02844310500415319
46. Tindholdt TT, Saidian S, Pripp AH, Tønseth KA. Monitoring microcirculatory changes in the deep inferior epigastric artery perforator flap with laser doppler perfusion imaging. *Ann Plast Surg*. 2011;67(2):139-142. doi:10.1097/SAP.0b013e3181f3e39b
47. Nguyen CD, Sheikh R, Dahlstrand U, Lindstedt S, Malmjö M. Investigation of blood perfusion by laser speckle contrast imaging in stretched and rotated skin flaps in a porcine model. *J Plast Reconstr Aesthet Surg*. 2018;71(4):611-613. doi:10.1016/j.bjps.2017.08.030
48. Nguyen CD, Hult J, Sheikh R, et al. Blood Perfusion in Human Eyelid Skin Flaps Examined by Laser Speckle Contrast Imaging-Importance of Flap Length and the Use of Diathermy. *Ophthal Plast Reconstr Surg*. 2018;34(4):361-365. doi:10.1097/IOP.0000000000001010
49. Sheikh R, Dahlstrand U, Memarzadeh K, Blohmé J, Reistad N, Malmjö M. Optimal Epinephrine Concentration and Time Delay to Minimize Perfusion in Eyelid Surgery: Measured by Laser-Based Methods and a Novel Form of Extended-Wavelength Diffuse Reflectance Spectroscopy. *Ophthal Plast Reconstr Surg*. 2018;34(2):123-129. doi:10.1097/IOP.0000000000000883
50. Sheikh R, Memarzadeh K, Torbrand C, Blohmé J, Lindstedt S, Malmjö M. Blood Perfusion in a Full-Thickness Eyelid Flap, Investigated by Laser Doppler Velocimetry, Laser Speckle Contrast Imaging, and Thermography. *Eplasty*. 2018;18:e9. <http://www.ncbi.nlm.nih.gov/pubmed/29520316>. Accessed February 16, 2020.
51. To C, Rees-Lee JE, Gush RJ, et al. Intraoperative Tissue Perfusion Measurement by Laser Speckle Imaging: A Potential Aid for Reducing Postoperative Complications in Free Flap Breast Reconstruction. *Plast Reconstr Surg*. 2019;143(2):287e-292e. doi:10.1097/PRS.0000000000005223
52. Rauh A, Henn D, Nagel SS, Bigdeli AK, Kneser U, Hirche C. Continuous Video-Rate Laser Speckle Imaging for Intra- and Postoperative Cutaneous Perfusion Imaging of Free Flaps. *J Reconstr Microsurg*. 2019;35(7):489-498. doi:10.1055/s-0039-1681076
53. Hardwicke JT, Osmani O, Skillman JM. Detection of Perforators Using Smartphone Thermal Imaging. *Plast Reconstr Surg*. 2016;137(1):39-41. doi:10.1097/prs.0000000000001849
54. Pereira N, Valenzuela D, Mangelsdorff G, Kufeke M, Roa R. Detection of perforators for free flap planning using smartphone thermal imaging: A concordance study with computed tomographic angiography in 120 perforators. *Plast Reconstr Surg*. 2018;141(3):787-792. doi:10.1097/PRS.0000000000004126
55. Hölzle F, Loeffelbein DJ, Nolte D, Wolff KD. Free flap monitoring using simultaneous non-invasive laser Doppler flowmetry and tissue spectrophotometry. *J Cranio-Maxillofacial Surg*. 2006;34(1):25-33. doi:10.1016/j.jcms.2005.07.010

56. Edsander-Nord Å, Røjdmark J, Wickman M. Metabolism in pedicled and free TRAM flaps: A comparison using the microdialysis technique. *Plast Reconstr Surg*. 2002;109(2):664-673. doi:10.1097/00006534-200202000-00037
57. Carruthers KH, Tiwari P, Yoshida S, Kocak E. Inpatient Flap Monitoring after Deep Inferior Epigastric Artery Perforator Flap Breast Reconstruction: How Long Is Long Enough? *J Reconstr Microsurg*. 2019;35(9):682-687. doi:10.1055/s-0039-1693454
58. Yoon AP, Jones NF. Critical time for neovascularization/angiogenesis to allow free flap survival after delayed postoperative anastomotic compromise without surgical intervention: A review of the literature. *Microsurgery*. 2016;36(7):604-612. doi:10.1002/micr.30082
59. Newton E, Butskiy O, Shadgan B, Prisman E, Anderson DW. Outcomes of free flap reconstructions with near-infrared spectroscopy (NIRS) monitoring: A systematic review. *Microsurgery*. 2019. doi:10.1002/micr.30526
60. Cruz-Segura A, Cruz-Domínguez MP, Jara LJ, et al. Early Detection of Vascular Obstruction in Microvascular Flaps Using a Thermographic Camera. *J Reconstr Microsurg*. 2019;35(7):541-548. doi:10.1055/s-0039-1688749
61. Kääriäinen M, Halme E, Laranne J. Modern postoperative monitoring of free flaps. *Curr Opin Otolaryngol Head Neck Surg*. 2018;26(4):248-253. doi:10.1097/MOO.0000000000000467
62. Um GT, Chang J, Louie O, et al. Implantable Cook-Swartz Doppler probe versus Synovis Flow Coupler for the post-operative monitoring of free flap breast reconstruction. *J Plast Reconstr Aesthet Surg*. 2014;67(7):960-966. doi:10.1016/j.bjps.2014.03.034
63. Wax MK. The role of the implantable Doppler probe in free flap surgery. *Laryngoscope*. 2014;124 Suppl 1(S1):S1-12. doi:10.1002/lary.24569
64. Han ZF, Guo LL, Liu LB, et al. A comparison of the Cook-Swartz Doppler with conventional clinical methods for free flap monitoring: A systematic review and a meta-analysis. *Int J Surg*. 2016;32:109-115. doi:10.1016/j.ijsu.2016.06.034
65. Fujiwara RJT, Dibble JM, Larson S V., Pierce ML, Mehra S. Outcomes and reliability of the flow coupler in postoperative monitoring of head and neck free flaps. *Laryngoscope*. 2018;128(4):812-817. doi:10.1002/lary.26944
66. Schawlow AL. Principles of Lasers. *J Clin Laser Med Surg*. 1995;13(3):127-130. doi:10.1089/clm.1995.13.127
67. 1. Einstein A. Strahlungs-emission und -absorption nach der Quantentheorie. *Verhandlungen der Dtsch Phys Gesellschaft*. 2016 18:318-323.
68. Maiman TH. Stimulated Optical Radiation in Ruby. *Nature*. 1960;187(4736):493-494.
69. Hecht J. Short history of laser development. *SPIE Rev*. 2010;1(1). doi:10.1364/ao.49.000f99
70. Choy D. History of Lasers in Medicine. *Thorac Cardiovasc Surg*. 1988;36(S 2):114-117. doi:10.1055/s-2007-1022985

71. Yeh Y, Cummins HZ. Localized fluid flow measurements with an HeNe laser spectrometer. *Appl Phys Lett*. 1964;4(10):176–178. doi:10.1063/1.1753925
72. Ballot B. Akustische Versuche auf der Niederländischen Eisenbahn, nebst gelegentlichen Bemerkungen zur Theorie des Hrn. Prof. Doppler. *Ann der Phys und Chemie*. 1845;142(11):321–351. doi:10.1002/andp.18451421102
73. Ware BR. Optical Techniques in Biological Research. In: Rousseau D, ed. *Optical Techniques in Biological Research*. ; 2002:32–33.
74. Fredriksson I, Fors C, Johansson J. *Laser Doppler Flowmetry-a Theoretical Framework*; 2007. www.imt.liu.se/bit/ldf/ldfmmain.html. Accessed August 29, 2019.
75. He D, Nguyen HC, Hayes–Gill BR, et al. Laser doppler blood flow imaging using a CMOS imaging sensor with on-chip signal processing. *Sensors (Switzerland)*. 2013;13(9):12632–12647. doi:10.3390/s130912632
76. Draijer M, Hondebrink E, van Leeuwen T, Steenbergen W. Twente Optical Perfusion Camera: system overview and performance for video rate laser Doppler perfusion imaging. *Opt Express*. 2009;17(5):3211–3225.
77. Goodman JW. Statistical Properties of Laser Speckle Patterns. In: Dainty JC, ed. *Topics in Applied Physics: Laser Speckle and Related Phenomena*. ; 1975:9–10. <http://www.ncbi.nlm.nih.gov/pubmed/18213052>. Accessed February 16, 2020.
78. Heeman W, Steenbergen W, van Dam G, Boerma EC. Clinical applications of laser speckle contrast imaging: a review. *J Biomed Opt*. 2019;24(8):1–11. doi:10.1117/1.JBO.24.8.080901
79. Davis MA, Kazmi SMS, Dunn AK. Imaging depth and multiple scattering in laser speckle contrast imaging. *J Biomed Opt*. 2014;19(8):086001. doi:10.1117/1.JBO.19.8.086001
80. Mirdell R, Iredahl F, Sjöberg F, Farnebo S, Tesselaar E. Microvascular blood flow in scalds in children and its relation to duration of wound healing: A study using laser speckle contrast imaging. *Burns*. 2016;42(3):648–654. doi:10.1016/j.burns.2015.12.005
81. Elawa S, Mirdell R, Farnebo S, Tesselaar E. Skin blood flow response to topically applied methyl nicotinate: Possible mechanisms. *Ski Res Technol*. 2019. doi:10.1111/srt.12807
82. Elawa S, Mirdell R, Tesselaar E, Farnebo S. The microvascular response in the skin to topical application of methyl nicotinate: Effect of concentration and variation between skin sites. *Microvasc Res*. 2019;124:54–60. doi:10.1016/j.mvr.2019.03.002
83. Russell JA, Conforti ML, Connor NP, Hartig GK. Cutaneous tissue flap viability following partial venous obstruction. *Plast Reconstr Surg*. 2006;117(7):2259–2266; discussion 2267–8. doi:10.1097/01.prs.0000225472.57337.2e
84. Mahe G, Rousseau P, Durand S, Bricq S, Leftheriotis G, Abraham P. Laser speckle contrast imaging accurately measures blood flow over moving skin surfaces. *Microvasc Res*. 2011;81(2):183–188. doi:10.1016/j.mvr.2010.11.013

85. Zötterman J, Mirdell R, Horsten S, Farnebo S, Tesselaar E. Methodological concerns with laser speckle contrast imaging in clinical evaluation of microcirculation. *PLoS One*. 2017;12(3). doi:10.1371/journal.pone.0174703
86. Richards LM, Kazmi SS, Olin KE, Waldron JS, Fox DJ, Dunn AK. Intraoperative multi-exposure speckle imaging of cerebral blood flow. *J Cereb Blood Flow Metab*. 2017;37(9):3097-3109. doi:10.1177/0271678X16686987
87. Lertsakdadet B, Yang BY, Dunn CE, et al. Correcting for motion artifact in handheld laser speckle images. *J Biomed Opt*. 2018;23(3):1-7. doi:10.1117/1.JBO.23.3.036006
88. Lertsakdadet B, Dunn C, Bahani A, Crouzet C, Choi B. Handheld motion stabilized laser speckle imaging. *Biomed Opt Express*. 2019;10(10):5149-5158. doi:10.1364/BOE.10.005149
89. Humeau-Heurtier A, Abraham P, Mahe G. Linguistic Analysis of Laser Speckle Contrast Images Recorded at Rest and During Biological Zero: Comparison With Laser Doppler Flowmetry Data. *IEEE Trans Med Imaging*. 2013;32(12):2311-2321. doi:10.1109/TMI.2013.2281620
90. Zhong J, Seifalian AM, Salerud GE, Nilsson GE. A mathematical analysis on the biological zero problem in laser Doppler flowmetry. *IEEE Trans Biomed Eng*. 1998;45(3):354-364. doi:10.1109/10.661160
91. Fredriksson I, Larsson M, Strömberg T. Measurement depth and volume in laser Doppler flowmetry. *Microvasc Res*. 2009;78(1):4-13. doi:10.1016/j.mvr.2009.02.008
92. Fuchs D, Dupon PP, Schaap LA, Draijer R. The association between diabetes and dermal microvascular dysfunction non-invasively assessed by laser Doppler with local thermal hyperemia: A systematic review with meta-analysis. *Cardiovasc Diabetol*. 2017;16(1). doi:10.1186/s12933-016-0487-1
93. Ince C. Hemodynamic coherence and the rationale for monitoring the microcirculation. *Crit Care*. 2015;19 Suppl 3:S8. doi:10.1186/cc14726
94. Allen J, Howell K. Microvascular imaging: Techniques and opportunities for clinical physiological measurements. *Physiol Meas*. 2014;35(7). doi:10.1088/0967-3334/35/7/R91
95. Wester T, Awan ZA, Kvernebo TS, Salerud G, Kvernebo K. Skin microvascular morphology and hemodynamics during treatment with veno-arterial extra-corporeal membrane oxygenation. *Clin Hemorheol Microcirc*. 2014;56(2):119-131. doi:10.3233/CH-131670
96. Wester T, Häggblad E, Awan ZA, et al. Assessments of skin and tongue microcirculation reveals major changes in porcine sepsis. *Clin Physiol Funct Imaging*. 2011;31(2):151-158. doi:10.1111/j.1475-097X.2010.00994.x
97. Meyer W, Schwarz R, Neurand K. The skin of domestic mammals as a model for the human skin, with special reference to the domestic pig. *Curr Probl Dermatol*. 1978;7:39-52. doi:10.1159/000401274

98. Sullivan TP, Eaglstein WH, Davis SC, Mertz P. The pig as a model for human wound healing. *Wound Repair Regen.* 2001;9(2):66-76. doi:10.1046/j.1524-475X.2001.00066.x
99. Svensson H, Pettersson H, Svedman P. Laser doppler flowmetry and laser photometry for monitoring free flaps. *Scand J Plast Reconstr Surg Hand Surg.* 1985;19(3):245-249. doi:10.3109/02844318509074510
100. Yuen JC, Feng Z. Monitoring free flaps using the laser Doppler flowmeter: Five-year experience. *Plast Reconstr Surg.* 2000;105(1):55-61. doi:10.1097/00006534-200001000-00009
101. McGregor A, Pavri SN, Tsay C, Kim S, Narayan D. Use of Indocyanine Green for Sentinel Lymph Node Biopsy. *Plast Reconstr Surg - Glob Open.* 2017;5(11):e1566. doi:10.1097/GOX.0000000000001566
102. Wong C, Saint-Cyr M, Mojallal A, et al. Perforasomes of the DIEP flap: vascular anatomy of the lateral versus medial row perforators and clinical implications. *Plast Reconstr Surg.* 2010;125(3):772-782. doi:10.1097/PRS.0b013e3181cb63e0
103. Mirdell R, Farnebo S, Sjöberg F, Tesselaar E. Interobserver reliability of laser speckle contrast imaging in the assessment of burns. *Burns.* 2019;45(6):1325-1335. doi:10.1016/j.burns.2019.01.011
104. Heller L, Levin LS, Klitzman B. Laser Doppler flowmeter monitoring of freetissue transfers: Blood flow in normal and complicated cases. *Plast Reconstr Surg.* 2001;107(7):1739-1745. doi:10.1097/00006534-200106000-00015
105. Cannata JM, Chilipka T, Yang HC, et al. Development of a flexible implantable sensor for postoperative monitoring of blood flow. *J Ultrasound Med.* 2012;31(11):1795-1802. doi:10.7863/jum.2012.31.11.1795
106. Oda H, Beker L, Kaizawa Y, et al. A Novel Technology for Free Flap Monitoring: Pilot Study of a Wireless, Biodegradable Sensor. *J Reconstr Microsurg.* November 2019. doi:10.1055/s-0039-1700539
107. Kifle Y, Wikner JJ, Zötterman J, Ryden L, Farnebo S. NFC Powered Implantable Temperature Sensor. In: *Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society, EMBS.* Vol 2019. Institute of Electrical and Electronics Engineers Inc.; 2019:4359-4362. doi:10.1109/EMBC.2019.8856607

Populärvetenskaplig sammanfattning

Denna avhandling handlar om vävnadsövervakning vid rekonstruktiv kirurgi, med fokus på användning av en teknik som kallas laser speckle contrast imaging (LSCI).

Rekonstruktiv kirurgi syftar till att återställa funktion och utseende efter skada eller andra ingrepp såsom cancerkirurgi. För att läkning ska vara möjlig efter operationen är det avgörande att den opererade vävnaden förses med en tillräcklig mängd syresatt blod. Hos patienter som genomgår rekonstruktiv kirurgi har trauma, tidigare kirurgi eller strålbehandling ibland resulterat i försämrad blodtillförsel. Detta kommer att påverka vävnaden negativt och öka risken för komplikationer efter operationen. Det är därför viktigt att bedöma vävnaden, både före, under och efter operationen. Detta kan göras med olika tekniker som övervakar genomblödningen i huden som täcker det drabbade området. Vi har utvärderat om LSCI kan användas för vävnadsövervakning vid rekonstruktiv kirurgi, eftersom tekniken möjliggör en snabb bedömning av genomblödningen utan att påverka vävnaden.

Principerna för LSCI-tekniken bygger att vävnaden belyses med laserljus som reflekteras av röda blodkroppar i de ytliga blodkärlen. Det bildas då ett mönster av fläckar (speckle) som innehåller information om koncentrationen och hastigheten hos de rörliga partiklarna. Utifrån en algoritm kan en dator beräkna den relativa genomblödningen i den undersökta vävnaden. Detta värde används för att uppskatta om det finns en ökad risk för komplikationer efter kirurgin.

Vi har i den första delstudien undersökt hur känslig tekniken är för rörelse hos vävnaden som undersöks och hur vinkeln och avståndet till kameran påverkar det uppmätta värdet. Vi kunde i den studien konstatera att det uppmätta värdet ökade med ökande rörelsehastighet hos vävnaden men att avstånd och vinkel endast påverkade värdet i liten grad.

I den andra studien använde vi en djurmodell för att jämföra LSCI och flödesmätning med hjälp av laser Doppler (LDF) som verktyg för att upptäcka hotande proppbildning i blodkärl som försörjer en lambå, dvs vävnad som har flyttats för att täcka en defekt efter till exempel skada eller cancerkirurgi. Vi kunde se att både LSCI och LDF kunde upptäcka förändring i flödet i kärlen men att det var större spridning i värdena uppmätta med LDF.

I studie tre använde vi samma djurmodell som i den andra studien för att undersöka möjligheten att använda LSCI under operationer för att upptäcka områden med försämrad cirkulation och därmed förutsäga områden med en hög risk för komplikationer efter kirurgin. Vi såg att genomblödningen i huden i lambån mätt med LSCI kort efter operationen minskade påtagligt i de områden som tre dagar senare visade tecken på att

inte överleva. Vi kunde också se att risken för denna komplikation var mer påtaglig om det uppmätta värdet var under 25.

I den fjärde studien använde vi LSCI för utvärdering av genomblödningen av vävnaden under en typ av rekonstruktiv kirurgi där man använder sig av hud och fett från buken för att skapa ett nytt bröst. Vi kunde i denna studie visa att genomblödningen var som högst i huden över kärlet som försörjer vävnaden i lambån och att de lambåer som hade områden med uppmätta värden under 30 löpte hög risk att drabbas av postoperativa komplikationer.

LSCI är en teknik som har många fördelar jämfört med andra tekniker för vävnadsövervakning, såsom den korta undersökningstiden, den höga upplösningen och att den inte påverkar den undersökta vävnaden. Emellertid är det nuvarande systemet fortfarande för skrymmande för att enkelt kunna användas i den kliniska vardagen. Tekniken har även nackdelen att den är rörelsekänslig och endast kan bedöma yttlig vävnad. Med en del modifieringar kan dock LSCI bli en teknik som har hög potential att bidra till vävnadsövervakning vid rekonstruktiv kirurgi.

Papers

The papers associated with this thesis have been removed for copyright reasons. For more details about these see:

<http://urn.kb.se/resolve?urn=urn:nbn:se:liu:diva-164327>

FACULTY OF MEDICINE AND HEALTH SCIENCES

Linköping University medical dissertations, No. 1735

Department of Biomedical and Clinical Sciences

Linköping University
SE-581 83 Linköping, Sweden

www.liu.se