Aus der Klinik und Poliklinik für Hals-, Nasen- und Ohrenheilkunde
der Ludwig-Maximilians-Universität München

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Feasibility of endoscopic assessment of free flap perfusion in the head and neck region using Indocyanine Green fluorescence imaging

Dissertation
zum Erwerb des Doktorgrades der Humanmedizin
an der Medizinischen Fakultät der
Ludwig-Maximilians-Universität zu München

vorgelegt von
Sven Zhorzel
aus
Kaufbeuren
2017

Mit Genehmigung der Medizinischen Fakultät
der Universität München

Berichterstatter:	Prof. Dr. med. Christian Betz
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Dekan:	Herr Prof. Dr. med. dent. Reinhard Hickel
Tag der mündlichen Prüfung:	23.02.2017

Teile dieser Arbeit wurden publiziert in:

Endoscopic assessment of free flap perfusion in the upper aerodigestive tract using indocyanine green: a pilot study.

Betz CS, Zhorzel S, Schachenmayr H, Stepp H, Matthias C, Hopper C, Harréus U.

J Plast Reconstr Aesthet Surg. 2013 May;66(5):667-74.

Endoscopic measurements of free-flap perfusion in the head and neck region using red-excited Indocyanine Green: preliminary results.

Betz CS, Zhorzel S, Schachenmayr H, Stepp H, Havel M, Siedek V, Leunig A, Matthias C, Hopper C, Harreus U.

J Plast Reconstr Aesthet Surg. 2009 Dec;62(12):1602-8.

To

my parents

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1 Introduction

Cancer is the leading cause of death in developed countries and has long surpassed cardio-vascular diseases. Meanwhile it is also the second leading cause of death in developing countries. Also, incidence and mortality seem to be rising. Back in 2002, 10.9 million new cases of cancer and 6.7 million deaths related to cancer were reported[1]. In 2008 however, about 12.7 million cases and 7.6 million deaths related to cancer are estimated to have occurred worldwide[2, 3]. While incidence rates in developed countries are almost twice as high as in developing countries in men (77% higher) and women (57% higher), mortality is only slightly higher in men (18% higher) and women (1% higher)[2]. This reflects diagnosis in earlier stages of the disease as well as availability and use of treatment services.

Oral and pharyngeal cancer is the sixth most common cancer in the world. Worldwide, 275,000 new cases from oral cavity cancer and 130,300 new cases of oropharyngeal cancer occurred in 2008. Incidence of these forms of cancer varies widely between geographic regions, which is attributable to the risk factors prevalent in these regions[4].

In 2008, 91,900 new cases with oral and pharyngeal cancer could be found in Europe, 68,100 male and 23,800 female patients, making oral and pharyngeal cancer the seventh leading cancer in Europe. Overall mortality in 2008 was 41,700 (32,900 male and 8,800 female)[5].

For men, age standardized rates (ASR) of cancer incidence are generally higher in Central and Eastern Europe (19.7/100,000) than in Western (18.9/100,000) and Southern Europe (16.8/100,000). The lowest regional incidence rates were found in Northern Europe (12.0/100,000).

In women, the highest regional incidence rates are found in Western (5.9/100,000) and Northern Europe (5.3/100,000). Southern (4.5/100,000) und Central and Eastern Europe (3.6/100.000) report lower rates.

Alike for men and women, of all countries in the EU, Cyprus (4.3/100,000 men, 1.5/100,000 women) and Greece (6.8/100,000 men, 2.0/100,000

women) showed the lowest incidence of oral cancer, while the highest rate of new cases occurred in Hungary (46.1/100,000 men, 10.3/100,000 women)[5].

In Germany, 11,390 new cases (8,320 male and 3,070 female) were estimated in 2008 and 4,640 patients died of oral cancer (3,570 male and 1,070 female)[5].

Over 90% of malignancies in the upper aerodigestive tract are squamous cell carcinomas[4] (SSC) and have the same risk factors as the other head and neck carcinomas. Risk factors include consumption of alcohol and tobacco[6, 7], but only recently an association to Human Papilloma Virus (HPV) infections has been found[8]. The remaining malignancies form a heterogeneous group consisting mostly of salivary gland tumours, sarcomas of soft tissue and the jaw bones as well as melanomas. In rare cases, lymphoreticular malignancies, malignant odontogenic tumours and metastases from non-oral cancers can be found.

Like other malignancies, oral squamous cell carcinomas are classified according to the tumour-node-metastasis (TNM) staging system of UICC (Union for International Cancer Control). The TNM classification simply describes the cancers anatomical extend of the tumour at its primary site (T stage), the regional lymph nodes (N stage) and existence or absence of remote metastases (M stage)[9]. Derived from the TNM stages, cancer can be classified by certain UICC stages ranging from I to IV which are directly related to prognosis. A higher UICC stage means a worse overall prognosis and therefore usually leads to a more radical treatment.

The overall five-year survival rate for oral cancer is around 50%[8]. While UICC stage I oral cancer has a 5-year-survival rate of up to 80%, it is dramatically lower in patients who present themselves with advanced disease (UICC stage III/IV) and may drop to as low as 20%[4].

A basic principle of the treatment of oral cancer is to achieve permanent or longest possible locoregional control of the tumour. In the head and neck,

standard resection margins of 1-3cm usually desired in oncologic surgery are not always feasible. Because of the limited options for resection, loco-regional recurrence as well as metastasis to regional lymph nodes is relatively frequent in aggressive cancers and leads to poor survival rates[10]. Historically, ablative and extirpative surgery was the primary and only means for curative intention. Lately, chemotherapy and radiation therapy play an equally important role in the treatment concepts. For some cases, a targeted therapy might be a viable option. While the smaller defects corresponding with lower UICC stages can be often curatively treated with surgery or radiotherapy as a single modality, more advanced stages require a multimodal approach often making use of a combination of surgical treatment and postoperative radiotherapy and/or chemotherapy.

Depending on the localization of the tumour, radical surgical treatment can lead to a variety of impairments, the most common of which are disfigurement and severe impact on speech, swallowing and chewing. Serious disabilities lead to nutritional deficits - themselves associated with wound healing disorders - and possibly prolonged need of parenteral nutrition or feeding tubes. Disfigurement and inability to speak might lead to social deprivation and isolation leading to depression.

These dysfunctions can be due to primary resection of the involved and functionally unique tissues, when they have been infiltrated by the tumour, or be long-term complications like formation of fistulas or fibrosis and neural impairment often encountered after radiotherapy[11, 12]. Treatment of oral cancer and the collateral damages involved is therefore very complex and should be approached by a multi-profession team.

Two concepts are of major importance in regard to long-term outcome: The first and foremost aim must be the eradication of the disease often requiring extreme ablative or extirpative procedures. In the long run, late effects and control of the possible complications play an evenly important role and often have tremendous impact on quality of life which can be measured by scales like the Karnowsky Index, Functional Living Index[13] or ICF (International

Classification of Function)[14, 15]. The best possible reconstruction is desirable to regain a high level of function.

In reconstruction of the head and neck, restoration of integrity of the face, neck and alimentary tract takes highest priority to prevent fistula formation as well as life-threatening conditions like carotid artery blow-out. Next, restoration of functions like swallowing, mastication, facial expression and speech should be attempted. Last but not least restoring form in an aesthetically pleasing way should be considered, if the general condition of the patient allows[16].

Plastic and reconstructive surgery has come to use a plethora of different flaps, divided in pedicled and free microvascular flaps. While the first often offer better perfusion and shortened operative time in co-morbid patients and may often be better suited to previously irradiated patients, the later promise optimal results for recovery of function due to better tissue reconstruction[17, 18]. In 1957, Seidenberg et al. performed the first auto-transplanted heterotypical tissue transfer in a human patient, when they transplanted a jejunal segment for reconstruction of the cervical oesophagus using a small vessel anastomotic technique and started the development of many different free flaps frequently used nowadays[19].

Through improved techniques in surgery and anaesthesiology longer and more complex procedures evolved allowing for simultaneous ablative and reconstructive surgery. Both pedicled and free flaps have been used that way commonly and successfully for decades. For reconstruction of the head and neck the use of free flaps has been the means of choice for almost 20 years as they are more flexible than pedicled flaps[20]. It is clear, that the free radial forearm flap (fRFF) is considered the "workhorse" of reconstructive surgery in the head and neck region due to its stable blood supply, long pedicle length and the relative ease to achieve moulding to complex geometrical structures. It is used in 55-70% of all soft tissue transfers in the head and neck. Other commonly used fasciocutaneous free flaps are the rectus abdominis (10-15%) and anterolateral thigh flap (4-

8%). Each of this flaps has advantages and disadvantages and is used to meet the demands of the recipient site[21]. Still, there are many more types of highly specialized flaps available - like the free ileocolon flap used for speech rehabilitation[22].

Free flap transfer and microsurgery have become two of the fastest growing fields in head and neck surgery over the last two decades as more sophisticated methods of surgical tumour treatment evolved and the need to cover the defects and restore integrity and functionality arose. Nowadays most academic otolaryngology or head and neck surgical units employ at least one microsurgeon and this number seems to be raising still[23].

Success rates for free flap transfer are being reported from 95-99% percent for the most experienced centers with reexplorations needed in 6-14% of the cases[24, 25]. Salvage rates range from 33-81% and the vast majority of anastomosis related problems, up to 95.6%, occur within the first 72 postooperative hours[25]. Flap failures almost exclusively run back to vascular problems like impeded arterial inflow or venous outflow obstruction. In this time window, surgical reexploration goes along with high salvage rates, but must rely on the right clinical judgement beforehand to take the patient back to the operating theatre. To not risk flap survival, a very low threshold for reoperation is to be maintained, so that even the slightest hint of vascular compromise is taken seriously and eventually leads to revision. Logically, this can lead to unnecessary procedures that may endanger the patient and are costly as well, so they should be avoided if possible.

When so called buried flaps with no or minimal surface exposure are used, or the recipient site is usually inaccessible like in the hypopharynx, sufficient monitoring might be hard to achieve. One technique often applied in these cases is the use of a so called "monitor flap", a special externalized portion of the distal flap that is sewn in on skin level and facilitates clinical examination and is supposed to be indicative of the perfusion state of the buried portion of the flap[26-29].

A good monitoring method should be safe, mobile, reliable, easy to use and interpret and be able to immediately detect vascular compromise. Ideally, it should differentiate between venous and arterial problems. Although many different invasive and non-invasive monitoring techniques for free-flaps have been described, it is still to be proven whether any of these new methods brings any advantage over clinical judgment by an experienced surgeon[30]. In times of global economic crisis, a look at the costs should also be taken into consideration.

To date, clinical judgment is the most important source of information. Although most centers acknowledge, that the first 72 postoperative hours are crucial for flap survival, monitoring regimes vary greatly between these institutions from hourly checks for the first 72 hours in a controlled setting like an intensive care unit (ICU) or intermediate care unit (IMC) to daily checks and early discharge from hospital. Also, most centers use hand-held, transcutaneous Doppler-probes to assess arterial and venous flow in the pedicle vessels[23, 31]. Another way to assess perfusion is to do it optically. There are a different approaches to this, usually using fluorescent dyes like Fluoresceine or Indocyanine Green (ICG).

Fluorescence is a form of luminescence and describes the emission of light by a substance, which has previously absorbed energy either in form of photons (light) or electromagnetic radiation. An electron, orbiting a molecule, is excited to a higher quantum state by absorbing some kind of energy. When the electron falls back or relaxes to its former lower quantum state, the formerly absorbed energy is emitted. By definition, the emitted light has a longer wavelength and therefore lower energy than the absorbed radiation, which is called a Stokes shift.

Fluorescein angiography is often used in ophthalmology for visualization of retinal and choroidal circulation. It evolved in 1961 and is still in clinical use on a regular basis[32]. In the early 1970s, Flower and Hochheimer started to use Indocyanine Green (ICG) angiography in ophthalmology first[33], but since then has been refined by adding new technology like the

videoangiography and laser scanning ophthalmoscope[34]. Later its use has spread to other medical fields in need of visualization of circulation and also serves to evaluate liver perfusion and function[35].

In some surgical fields like plastic and reconstructive surgery[36, 37], coronary-bypass surgery und neurosurgery[38, 39], Indocyanine Green (ICG)-fluorescence angiography is already in frequent use for assessing perfusion of freshly anastomosed vessels or clipped aneurysms intraoperatively. Studies have shown that it corresponds well with bloodflow and is easy to use and interpret[40] and is able to predict necrosis in pedicled and free flaps[41, 42].

The presented method of endoscopic ICG-fluorescence-angiography might be beneficial in the head and neck area since it can easily adapt to the complex three-dimensional geometry and reach otherwise hidden flaps. This study was intended to prove the feasibility of the method and to our knowledge is the first to employ endoscopic equipment.

2 Patients, Material and Methods

2.1 Patient Recruitment

According to the study protocol, a total of 25 patients were to be included in order to prove the feasibility of the method. Beginning in December 2007 after the study design had been approved by the ethics committee of the LMU, recruitment of the patients started. All patients with a need for free flap transplantation into the oral cavity or oropharynx who were admitted to the Department of ENT were asked for their participation. The need for transplantation may have arisen from either primary resection of the cancer or secondary reconstruction after prior resection. All patients who agreed to participate in the study were included. There was no further prerequisite regarding gender, age, classification or location of cancer or type of flap. The participants have been informed of the purpose of the study and probable benefits for future patients as well as possible risks of the endoscopic examinations and the administration of ICG. They had to give their informed consent in written form after a consideration period of at least 24 hours to be included. Exclusion criteria primarily derived from the use of ICG and consisted of prior adverse anaphylactic or anaphylactoid reaction to ICG, severe liver dysfunction and known hypersensitivity to iodide. Newborn children and pregnant women were also to be excluded, since appropriate data on safety in these patients are lacking.

2.2 Medication and dosing of Indocyanine green

2.2.1 Physical and Physiological Properties of Indocyanine green

Indocyanine green (ICG) is a water-soluble, amphiphilic, tricarbocyanine dye (*see Figure 1*). It has been internationally licensed and used in clinical routine for decades for examinations of liver and cardiac function as well as the choroideal vasculature. Due to its pharmacokinetic and physical properties, ICG can also be used in fluorescence perfusion diagnostics.

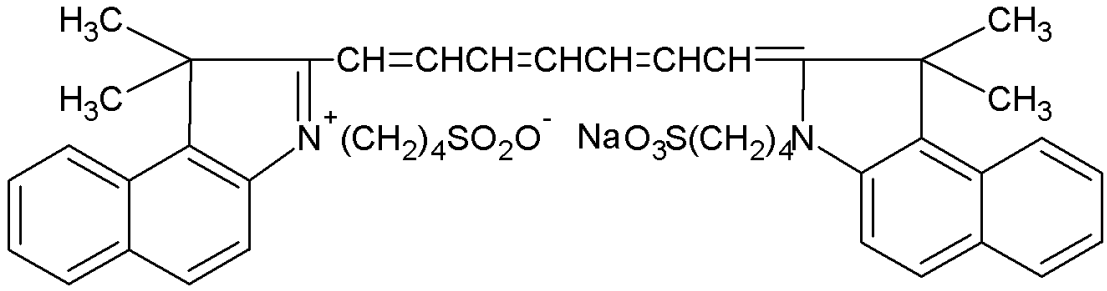

Figure 1: Indocyanine Green (ICG) - molecular structure

Within two seconds after intravenous injection ICG binds to plasma proteins, preferentially α_1-lipoproteins. Weak binding to Human Serum Albumin and phospholipids has also been reported. Therefore, the diffusion out of the healthy vessels and peripheral uptake are negligible. The molecules are then rapidly degraded in the liver and through their lipophilic properties eliminated via the biliary system, where they are not subject to enterohepatic recirculation. These pharmacokinetic properties lead to a plasmatic half-life of only 3-4 minutes and allow for the dye to be repeatedly injected after 20 minutes. Protein-bound ICG has a characteristic absorption maximum of 805nm or 810nm and an emission maximum at 820-830 nm.

With higher concentration over 80µg/ml, ICG tends to form aggregates. Through aggregation the fluorescence yield of the dye decreases, because ICG polymers have a weaker fluorescence yield than monomers. This process is called quenching[43].

The used, commercially available preparation of ICG used in this study, "ICG Pulsion" (Pulsion Medical Systems AG, Munich, Germany), contains a iodine component, that is needed to allow crystallization. Adverse reactions including anaphylactic and anaphylactoid reaction have been reported, but are very rare (approx. 1:42 000)[44].

2.2.2 Dosing of ICG

In this study, ICG was dosed based on the weight of the patients. The dose was 0.3mg/kg body weight in all the patients analogously to the

recommended dosage for other perfusion examinations using ICG. 25mg of ICG dry powder were diluted in 5ml of sterile H_2O (aqua), and then the appropriate volume of the ready-to-use ICG solution was injected intravenously.

2.2.3 Administration of the Drug

The dye was administered either via central venous lines through the subclavian or jugular vein or – if those were not available – via peripheral venous catheters. The appropriate dose was injected in one fast bolus and the i.v. system rinsed with at least 10 ml of normal saline solution to ensure the rapid rise of plasma ICG levels.

2.2.4 Provision of ICG

The required amount of ICG was provided for free by the manufacturer, Pulsion Medical Systems AG (Munich, Germany), for exclusive use in the study.

2.3 Technical equipment

All technical equipment needed was CE-marked and was provided for free for exclusive use in the study during the time of the examinations by the manufacturer Karl Storz GmbH (Tuttlingen, Germany). Since the primary aim of this study was to prove the value of an endoscopic ICG-Angiography for assessing the vitality of the flaps, there was a need for close cooperation with the manufacturer of the used optical equipment, which lead to further refinements of the optical equipment while the examinations and data acquisition was still ongoing. As soon as it was available, the next generation gear was used.

2.3.1 Endoscopes

In the course of the study a total of three different endoscopes in comparable configurations were used. After a short initial phase where a standard endoscope was used in combination with a snap-on ICG-filter, a provisional study endoscope with an integrated ICG-filter was provided by

KARL STORZ. The intensive feedback finally led to further refinements on the study endoscope and the development of a second generation ICG endoscope with improved imaging qualities due to the usage of better suited, coated lenses and filter systems.

- Provisional Karl Storz GmbH Hopkins II endoscope:
  10mm straight forward (0°) endoscope without built-in ICG-Filter and thus with a need for an external filter that is snapped between the camera and the endoscope (Karl Storz GmbH, Tuttlingen, Germany)
- Karl Storz GmbH ICG Hopkins Optik 0° endoscope (1st generation):
  10mm straight-facing (0°) endoscope, 20cm long, (Karl Storz GmbH, Tuttlingen, Germany) with build-in switchable ICG-Filter
- Karl Storz GmbH ICG Hopkins Optik 0° endoscope (2nd generation):
  5,8mm straight forward (0°) endoscope, 19cm long, (Karl Storz GmbH, Tuttlingen, Germany) with built-in switchable ICG-Filter and improved lenses for better light transmission the NIR wavelength and improved antireflection coating

2.3.2 Observation Filter

The observation filter was designed to block the excitation light and let only ambient light and ICG fluorescence pass and be registered by the camera. Therefore wavelengths below 800nm were blocked. It was either integrated in the endoscope as a part of a changeable filter block or a separate snap-on filter (Aufsteckfilter mit ALA-Filter, Karl Storz GmbH, Tuttlingen, Germany), which can be fitted to various endoscopes.

2.3.3 Light source

Red/near-infrared (NIR) excitation light at ~740 - 780 nm was emitted by a filtered Xenon Short Arc lamp (D-Light, Karl Storz GmbH, Tuttlingen, Germany) and transmitted to the endoscope via a standard fluid light guide (Fluid-Lichtkabel, 250cm, 5mm, Karl Storz GmbH, Tuttlingen, Germany).

2.3.4 Camera and controller

For imaging of the white light as well as the fluorescence examinations, two different NIR sensitive RGB three-chip CCD-endocamera-systems (Karl Storz GmbH, Tuttlingen, Germany) which could be set to IR/NIR mode were used (Tricam PDD Camera System & Image 1 Camera System, Karl Storz, Tuttlingen, Germany). The used camera controller was a routine off-the-shelf controller (Tricam PDD Control Unit, Karl Storz GmbH, Tuttlingen, Germany) that needed no further modification towards the specified use, because the IR/NIR signals received by the camera could be interpreted without problems.

A switch on the camera handle was programmed to change the settings of the light source and camera simultaneously from white light to fluorescence imaging and back again.

2.3.5 Video and audio recording system

All data were recorded and stored using an AIDA Compact NEO Standard system (Karl Storz GmbH, Tuttlingen, Germany). This allowed for simultaneous recording of a video and audio input; as well, it was possible to save high resolution still images. The resulting movies were stored in compressed mov-video format, single images used the TIFF-format. Both movies and still images had a resolution of 720 x 544 pixels. The AIDA system’s recording can be managed using the provided pedal switch or the standard computer input, mouse and keyboard.

2.3.6 ICG reference

To improve the reproducibility, a standardized platelet with ICG dye was placed as a reference next to the areas of interest in approximately the same distance from the endoscope.

The ICG reference was made of an ICG-dye stained piece of cloth of approximately 1,5 x 1 cm (provided by Pulsion AG), that had been laminated in plastic and could be sterilized and reused. While the ICG-standard proved

to be fairly resistant to photobleaching, the ideal concentration of the ICG dye the cloth was stained with had been experimentally determined during the preparation phase of the study. It was diluted and checked under NIR excitation until it the stained cloth was always the most intensely fluorescing item in a specified experimental setting without leading to total overexposure of the signal by reaching the sensitivity limit of the CCD-chip and thus leading to loss of information. To avoid involuntary accidental swallowing of the platelet by a conscious patient, a thread was attached to it, so it could easily and quickly be recovered if necessary.

2.3.7 Safety Acknowledgement

All equipment listed above was mounted in a single mobile video rack and was approved for safe usage in the operation room (OR) as well as the intensive care units (ICU). The light intensity emitted by the light source was set to levels that would not lead to thermal damages when used in the intended distance while providing optimal conditions for the fluorescence measurements at the same time.

2.4 Experimental Design

2.4.1 Usual Procedure After Surgery

After Surgery, the patients are brought from the OR to the ICU while they are still under general anaesthesia and kept fully sedated and ventilated for at least several hours to reduce the likelihood of damage to the anastomosed vessels through patient movement. Starting at admission to ICU, the flap and/or monitor flap are inspected hourly by trained personal. Additionally, the artery and vein of the pedicle are evaluated using a transcutaneous Doppler probe at the same time, even if there are no anomalous findings like paleness or swelling of the transplanted flap during inspection. These hourly examinations are continued for 72 hours, no matter whether the patient is still on ICU or has already been moved back to a general ENT ward. Afterwards the regime is loosened to some degree and the intervals for examinations are two hourly from then on for another 48

hours. So, a total of 120 hours is covered. After that time, the flap's vitality is clinically assessed at least once daily (*see Table 1*).

If only the smallest doubt relating to the perfusion situation arises, the patient is immediately taken back to the OR for reexploration and salvage operation, if necessary.

Table 1: Post-OP monitoring regime at LMU head and neck surgery department:

Time after surgery	**1-72 hrs.**	**3-5 ds**	**6-14 ds**	**2-4 wks**
clinical checks	Hourly	2 hourly	Once daily	weekly
Doppler examinations	Hourly	2 hourly	None	None

2.4.2 Study Design

The study was designed as a prospective clinical trial. Informed consent was acquired from the patients prior to inclusion. There was no randomization or blinding.

2.4.3 Patient data of interest

For all patients the following parameters were determined and written down in the study chart (also attached in the appendix):

- Age
- Weight
- Heart rate
- Blood pressure
- Localization and type of cancer
- Used flap for this procedure

If continuous invasive monitoring of blood pressure and heart rate was available – as it always was in the OR and on ICUs – the data at the moment of injection of ICG were those of importance. If there was only intermittent non-invasive monitoring as provided on general wards the data was taken directly before and after the examination and then the mean value was

recorded. As there was no intention to measure an effect of the examination or injection of the dye in the blood pressure, no further analysis of circulatory data was performed.

2.4.4 Time of examinations

According to study protocol, all patients had to undergo three planned ICG angiography examinations. These had to take place at the following points:

- 1st Examination: Intraoperatively (0h) following anastomosis
  The first examination was always performed in the OR directly after anastomosis of the vessels, while the patients were still under general anaesthesia and continuously monitored. For these first examinations, the endoscope was mounted on a stand to reduce artificial movements.
- 2nd Examination: 24h following surgery
  At this point the patients were still on ICU and either sedated or fully awake, depending on their overall physical condition. There was continuous measurement of vital parameters in all the patients at this time. For these examinations, the endoscope was handheld.
- 3rd Examination: 72h following surgery
  The last examination was performed either on ICU or general ward. The endoscope was again handheld.

2.4.5 Adaption of the schedule

Changes to this regime were only made, if circumstances demanded it:

- If the circulation seemed compromised in the OR following the original anastomosis, a second ICGA was performed after the probable cause of the problem was resolved.
- In case of an assumed vascular compromise in the postoperative phase, the ICGA was performed immediately before salvage surgery, if both hardware and trained personnel was available at that time.

If in any of these cases a revision of the anastomosis was performed, the additional ICGA again marked a new start and was followed by a 72 hour-postoperative monitoring period and 2 subsequent ICGAs.

2.4.6 Requirements for the examined region

The field of view during the examinations always included the following:

- Transplanted flap
- Surrounding, autochthonous tissue
- ICG reference

If the distance from the light source – in this case the tip of the endoscope – grows, the maximum excitation intensity and thus the returned fluorescence signal diminishes. Due to these optical and physical prerequisites, there were further demands on the placement of the investigating endoscope within the examined region (*see Figure 2*):

- Ideally, the flap, the ICG reference and the surrounding tissue had to have the same distance from the endoscope
- The endoscope had to be placed over the flap in a right angle

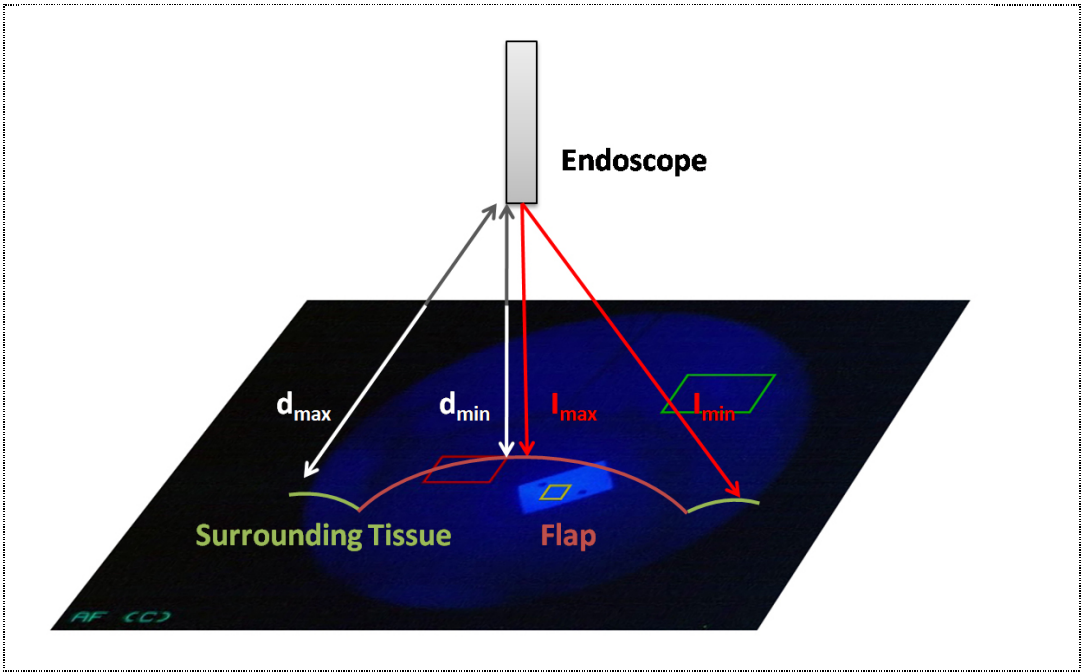

Figure 2: Optimal position of the endoscope: *perpendicular over the center of the flap. Maximum intensities of excitation light are reached at minimal distances from the endoscope in the center of the flap. In the peripheral field, the excitation intensity diminishes as the distance from the tip of the endoscope grows.*

2.4.7 Execution of the ICG angiography

First, the ICG solution was prepared for injection in the right concentration and dose. Second, the needed equipment consisting of the examination unit composed of endoscope, light source, camera with controller and video recording system was prepared. Also, additional instruments like spatula and sterile gauzes for revealing of the flap were put in place.

Finally, the equipment was placed next to the patient so that the examiner had clear view of the screen while handling the endoscope and spatula and the assistant had uninhibited access to the venous catheter through which the ICG was to be administered.

First, the field of surgery was cleaned of saliva and blood. Then, the region of interest (ROI) was shown in white light and the ICG reference was placed. After the flap region was clearly identified and pictures and a video sequence had been taken for reference, the view was switched to the fluorescence imaging mode and the light source was changed to red excitation light mode simultaneously. The video recording was then started and the ICG was administered intravenously, while the sequence was being recorded. The point of injection was marked by voice command on the video for later evaluation. On screen, the live uptake and maximum of fluorescence intensity within the flaps were viewed and compared to the surrounding tissue while being recorded. In the first examination (0h), the recording was longer than in the following because of better conditions, as a stand could be used and the patients were still under anaesthesia. In these longer examinations the beginning elimination of ICG could also be seen by falling fluorescence intensities, whereas the follow-ups were primarily intended to be as close to reality in later clinical use as possible and ended, when the maximum fluorescence was subjectively reached in flap and surrounding tissue.

2.5 Data acquisition

2.5.1 Video and audio recording

All examinations were recorded in the .mov-format by the AIDA system including the audio input via a headset.

2.5.2 Data storage

The acquired video data was first written on the internal hard drive of the AIDA system in real time. To ensure data protection, the data was copied to a CD or DVD and kept in a secure place, and the original data was wiped off the hard drive.

2.5.3 Data processing

For further interpretation of the acquired video sequences, Pulsion, the manufacturer of ICG, provided an appropriate evaluation software (IC Calc (Version 1.1, Pulsion Medical Systems, Munich, Germany)). It works by measuring the digitalized data from the video (RGB, values 0 – 255) over time as average values of user defined ROIs. The ICG reference was used as a calibration signal to normalize the acquired data according to changes in distance of endoscope and observed tissue.

Since IC Calc can only process .avi-format files, the AIDA system's .mov-files had to be converted to the IC Calc-conform .avi-format. To do this, Adobe Premiere Pro (Adobe Systems Inc., San José, CA, USA) was used to read the fluorescence videos, cut them according to the audio recordings and convert them to an uncompressed .avi-format.

Then the videos were imported into IC Calc. There again, regions of interest could be defined. In each sequence, three of these regions were determined:

- ICG reference
- Flap
- Surrounding tissue

In the process of evaluation, special care was taken with regards to the selection of these regions of interest, since only oval or rectangular and no free shaped regions could be defined due to restrictions of the used software. To guarantee that the examination was interpreted correctly, these regions were placed in approximately the same distance from the centre of the video and thus the light source and close to each other to increase comparability. IC Calc then plotted the curves of fluorescence intensity for each region of interest into the same figure. The program allowed for saving of the plots embedded in a HTML-file as well of the raw data in a text file for later use and import in a better suited plotting program for publication purposes.

For each sequence, both options for saving were used.

2.5.4 Important values for examination

The most important value that we aimed to derive from our measurements was the fluorescence index (FI), the quotient of gain in intensity of fluorescence in the flap versus the surrounding, autochtonous tissue. Another interesting value was the perfusion index (PI), the quotient of fluorescence slopes.

Through IC Calc, however, only the following basic values could be determined directly:

- Fluorescence intensity of ICG reference (I_{icg})
- Basal fluorescence intensity of the flap and surrounding tissue before injection of ICG (bI_{fl} or bI_{st})
- Time of onset of uptake (t_0)
- Maximum fluorescence intensity of the flap and surrounding tissue after injection (mI_{fl} or mI_{st})
- Time from injection to maximum fluorescence intensity in both flap and surrounding tissue (t_{fl} or t_{st})

All the fluorescence intensity data were provided as absolute values on a scale from 0, this means no light at all, to 100, which means maximum

intensity of fluorescence that can be registered by the camera and controller. IC Calc plotted the curves against time and the intensities had to be extracted by the examiner.

2.6 Data evaluation

2.6.1 Primary data

The data from IC Calc were then read and directly transferred to a table in Microsoft Excel (Microsoft Corp., Redmont, WA, USA). This table contained the following information on all patients:

- LMU study code for this patient
- Date of examination
- Time of examination after surgery
- Age (in years)
- Gender
- Localization and type of cancer
- Used flap
- All values listed under point 2.5.4 as shown by IC Calc
  (in s for time values and % for fluorescence intensities)
  Time of injection on the video (t_i in s)
- Heart rate (HR in bpm = s^{-1})
- Blood pressure (BP) systolic and diastolic (RR_{sys} and RR_{dia} in mmHg)
- Type of venous access (peripheral or central)

Time data was rounded to 0.5 seconds and fluorescence intensity was rounded to 0.5%.

2.6.2 Secondary Data

From the original data, some secondary data has been calculated and also entered into the same table. These were the calculations used:

- Circulation time from injection (t_i) until onset of uptake (TUO)

$$TUO = t0 - ti$$

- Time from onset of uptake to time of maximum FI in flap and surrounding tissue (TMF and TMS)

$$TMF = \Delta t_{fl} = t_{fl} - t0$$

$$TMS = \Delta t_{st} = t_{st} - t0$$

- Increase in fluorescence intensity in both flap and autochthonous surrounding tissue (ΔI_{fl} or ΔI_{st})

$$\Delta I_{fl} = mI_{fl} - bI_{fl}$$

$$\Delta I_{st} = mI_{st} - bI_{st}$$

- Fluorescence Index (FI) is the quotient of maximum increase of FI in flap and surrounding tissue (FI)

$$FI = \frac{\Delta I_{fl}}{\Delta I_{st}}$$

- Perfusion Index (PI)is the quotient of the mean slopes of uptake in the flap and the surrounding tissue (PI)

$$PI = FI \times \frac{TMS}{TMF} = \frac{\Delta I_{fl} \times \Delta t_{st}}{\Delta I_{st} \times \Delta t_{fl}}$$

- Mean arterial pressure (MAP in mmHg)

$$MAP = RR_{dia} + \frac{1}{3}(RR_{sys} - RR_{dia})$$

2.7 Randomization and blinding

The study was neither randomized nor blinded since its purpose was merely to prove the feasibility of the method.

2.8 Statistics

In the preparation phase of the study, there were no considerations on statistics as far as case numbers were concerned, since its main purpose was to show, whether endoscopic ICG angiography is a useful, feasible and easy to perform method of determining surface perfusion of transplanted free flaps in the UADT and predict the state of the anastomosis.

In the course of evaluating the study results, different statistical tests were applied. Because of the low case number, the evaluation was mainly of descriptive nature. The relatively small number of patients in the study led

to the use of the Mann-Whitney-U-Test to show the significance of the study results. For Correlations, Spearman's test was used.

All the secondary data was imported in SPSS (Version 17.0 for Windows, SPSS Inc., Chicago, IL USA) and then processed statistically. Graphics were created using either SPSS, Photoshop CS4 (Adobe Systems Inc., San José, CA, USA) or Powerpoint 2007 (Microsoft Corp., Redmont, WA, USA).

2.9 Interpretation survey

In order to assess the possible value of the real-time on-line interpretation of the examination in the OR, a video including 10 ICG-fluorescence sequences and the matching white light images was created using Adobe Premiere Pro CS 3 (Adobe Systems Inc., San José, CA, USA). The video included two sequences of patients who had to undergo revision surgery for arterial malperfusion und eight examinations of patent flaps with no abnormal findings for control.

A total of 21 persons from the Department of Otorhinolaryngology and Head and Neck surgery at the University Hospital Munich participated in our interpretation study. This included 5 nurses and 16 medical staff members with experience ranging from students to attending head and neck surgeons. All personnel was familiar with postoperative flap monitoring techniques and from the medical staff, 11 of whom have seen ICG-examinations performed in the OR during the course of the study.

All raters were shown the same 10 sequences with 8 patent and 2 insufficiently perfused flaps each. This added up to a maximum of 210 possible sequences to be assessed (168 patent, 42 insufficient), both for white light and ICGA sequences. The participants were asked to view the sequences only once and then give a statement on the actual perfusion situation. In writing, the participants then had to judge on the flap-perfusion on a 4-step-scale ranging from 1 (bad) to 4 (good) and need for operative revision first for the white light images and then second for the fluorescence videos.

2.10 Privacy policy

All patient data were evaluated anonymously. The patients' details were exchanged by alphanumeric codes (e.g. LMU XX) that had no relation to the names but indicated the sequence of surgeries and thus first examinations. In the table that included the results no names but only the codes were shown. All the consent forms had to show the full names and birthdays and were stored in a locked drawer, that only one person, Dr. med. Ch. Betz from the Department of ENT of the LMU University Hospital had access to. The other data, including tables, plots and the videos were completely anonymous and stored on either password or biometrically secured computer systems that only members of the research team could access.

3 Results

3.1 Patient collective

From December 6th 2007 to October 28th 2008, a total of 25 patients were included in the study according to the study protocol. Of those, five were female, 20 male. The patients were aged from 45 to 74 years (mean age 60.5 years*, Table 2*).

Table 2: Distribution of sex and average age in years

	Male	**Female**	**Total**
Number	20	5	25
Average Age (years)	58.53	68.54	60.52

All patients suffered from cancer in the upper aero-digestive tract (UADT) and had to undergo reconstructive flap surgery following cancer resection either primarily or secondarily. While 92% (23/25) of the malignant formations were pathologically identified as squamous cell carcinoma, only 8% (2/25) suffered from adenocarcinomas. The flaps used in the study were the free radial forearm flap (fRFF), free anterolateral thigh flap (fALT) and free fibula flap (fFF; *see Table 3*: **Localizations and flaps used**).

Table 3: Localizations and flaps used

		Localization of defect					
		Pharynx	**Tongue**	**Root of tongue**	**Floor of mouth**	**Cheek**	**Total**
Flap used	**fRFF**	8	3	5	2	2	20
	ALT	1	2	0	1	0	4
	FF	0	0	0	1	0	1
Total		9	5	5	4	2	25

All procedures were performed by one head and neck surgeon (Prof. Dr. med. U. Harréus) and there were no total or partial flap losses.

3.2 Anastomosis Techniques

All the arterial anastomoses were performed end-to-end in manual single suture technique with a monofilament 8-0 nylon suture and used either the A. thyreoidea, A. facialis or A. lingualis as donor vessels. For venous end-to-end anastomoses venous mechanical anastomotic coupler devices (vMACD; GEM Microvascular Anastomosis Coupler, Synovis Companies Alliance Inc., Birmingham, AL, USA) with sizes between 2.5-4.0mm were used. Receiving vessels were either the V. jugularis externa or V. facialis.

3.3 ICGA-Examinations

3.3.1 Course of the examination

The ICG angiography (ICGA) examinations could be performed on all patients at 0 hours in the OR. There were always two people needed to perform the angiography, since the examiner had to handle the endoscope and the attached camera and point it towards the region of interest as well as reveal the flap or improve view of it by using a spatula. An assistant had to inject the ICG solution.

The ICGA was very well tolerated by sedated and awake patients alike, and there was no harm inflicted during the examinations. In the course of the study no adverse effects such as anaphylactic reaction or shock occurred following the administration of ICG.

3.3.2 Duration of an examination

The time to perform a complete ICG angiography was about 15 minutes, consisting of approximately 5 minutes to ready and assemble the endoscopic equipment and prepare the ICG dye for injection, another 5 minutes of actual examination and finally up to 5 minutes for disassembly of the instruments and storage of the data.

3.3.3 Problems encountered during examinations

On one occasion, we encountered a technical difficulty with the foot pedal switch, which was used to start and stop recording of the video sequences and taking pictures. Apparently caused by a defective contact, it had to be repaired. During that time, the recording was managed by an assistant by using mouse and keyboard and the AIDA system's graphic user interface. This way, the issue could be resolved without problems.

A problem for recording and later off-line evaluation was excessive movement of an agitated or not fully oriented awake patient, when the level of sedation in the early postoperative phase on ICU was not deep enough anymore to still easily tolerate manipulation within the oral cavity. When sight of the ROIs was lost, it had to be found again in the ICG-enhanced view, since switching back to the white light mode could have missed the relevant phase of rising fluorescence. This was rather difficult in the early phases of each examination, because contrast of the basal fluorescence was very low and only rudimentary orientation was provided by the (always well visible) ICG reference. Rearranging the composition of ROIs was rather easy in the middle and late phases however, when contrast was higher and the good spacial resolution even revealed the stitching sutures for orientation. It was especially important to maintain the same distance from the flap, since this would have led to fluctuations in fluorescence intensities.

Placement of the ICG reference in the awake patients was not always ideal, because the examination was mostly performed in a sitting or half-sitting position of the patient. When the localization of the flap was on the roof of the oral cavity, the reference had to be placed on the tongue, since it could not be attached to the pharynx. Also very deep localizations like the root of the tongue were problematic and the reference had then to be placed on the back wall of the oro-/hypopharynx. In one case the platelet could not be placed due to postoperative swelling and the narrowness of the available space to perform the ICGA, as not even the entire flap could be revealed.

The flap could not always be completely revealed without risking damage to the tissue in very deep localizations like the root of the tongue. If that was the case, the flap was revealed as much as possible and then the ICGA was performed.

The regime of three planned examinations for each patient could be followed for all but one patient (LMU 05), who had a very deep glossopharyngeal cancer resection and reconstruction and showed a massive postoperative swelling despite the routine steroid treatment. To not risk damage to the flap and its vessels, the team decided to avoid further manipulation to the site of surgery to reveal the flap. Therefore, the 2nd and 3rd examinations were waived in this patient.

3.4 Cases

Of the 25 patients, five had to undergo revision surgery based on clinical findings – including reduced arterial and/or venous Doppler probe signal, pale aspect on inspection or absence of bleeding after pinprick testing. One of them had to be revised twice because of suspected impending flap failure.

The remaining 20 patients showed completely uneventful postoperative courses and normal healing and did specifically not require surgical re-evaluation of the perfusion situation.

3.4.1 Patients with immediate early revision following Anastomosis

Two patients had to be revised due to suspicious clinical findings in the OR following anastomosis directly after the neck had been closed.

The first patient, LMU 06, aged 66, has had a T3 primary cancer of the soft palate including the right tonsil. The procedure included resection of the cancerous lesion and neck dissection. After the radial forearm flap had been elevated, the flap was first sewn in place and then the pedicle artery was connected to the superior thyroid artery with single interrupted sutures and the vein was anastomosed to the external jugular vein using a venous mechanical anastomotic coupler device (vMACD, see 3.2). Intraoperatively, an excellent flow in the anastomosed vessels and good vitality of the flap

were verified. An additional monitor flap was sewn in the side of the neck and then the flap was sewn into the tonsil's loge. When the flap's vitality was checked one last time in the OR before transferring the patient to the ICU, the flap appeared pale and there was almost no bleeding after pinprick testing. Upon these findings it was decided to perform immediate revision surgery. While the preparations for a reopening of the neck took place, the ICGA was performed for a first time in this patient. In stark contrast to previous ICG examinations, there was subjectively barely any uptake of fluorescence in the flap, while fluorescence was present in the autochthonous tissue and showed the expected behaviour. The intra-operative assessment showed a lack of blood flow in the artery due to a kinking of the pedicle. After this problem had been resolved by shortening the pedicle and renewing the arterial anastomosis, the neck was closed again. Subsequently, the flap appeared rosy and showed appropriate bleeding upon pinprick testing and a second ICGA was performed (*see Figure 3*). In this examination, the flap also took up fluorescence as expected. After this intervention, the further postoperative course of LMU 06 was uneventful.

The later off-line ICGA analysis showed low FI of 0.33 and PI of 0.29 prior to revision which both rose after re-establishing perfusion to a FI of 0.93 and a PI of 0.68, respectively.

In a second case, on LMU 23, a 64 years old male patient, who also suffered from T3 oropharyngeal cancer including the right tonsil and the root of the tongue, a secondary reconstruction was performed one week after resection of the cancer, using a free radial forearm flap (fRFF). Again, the pedicle artery was anastomosed with the superior thyroid artery using button sutures, whereas a vMACD was used to connect the pedicle vein to the external jugular vein. At the end of surgery both, flap and monitor flap, appeared "unusually pale". The ICGA showed delayed and greatly reduced fluorescence of the flap compared to the surrounding tissue.

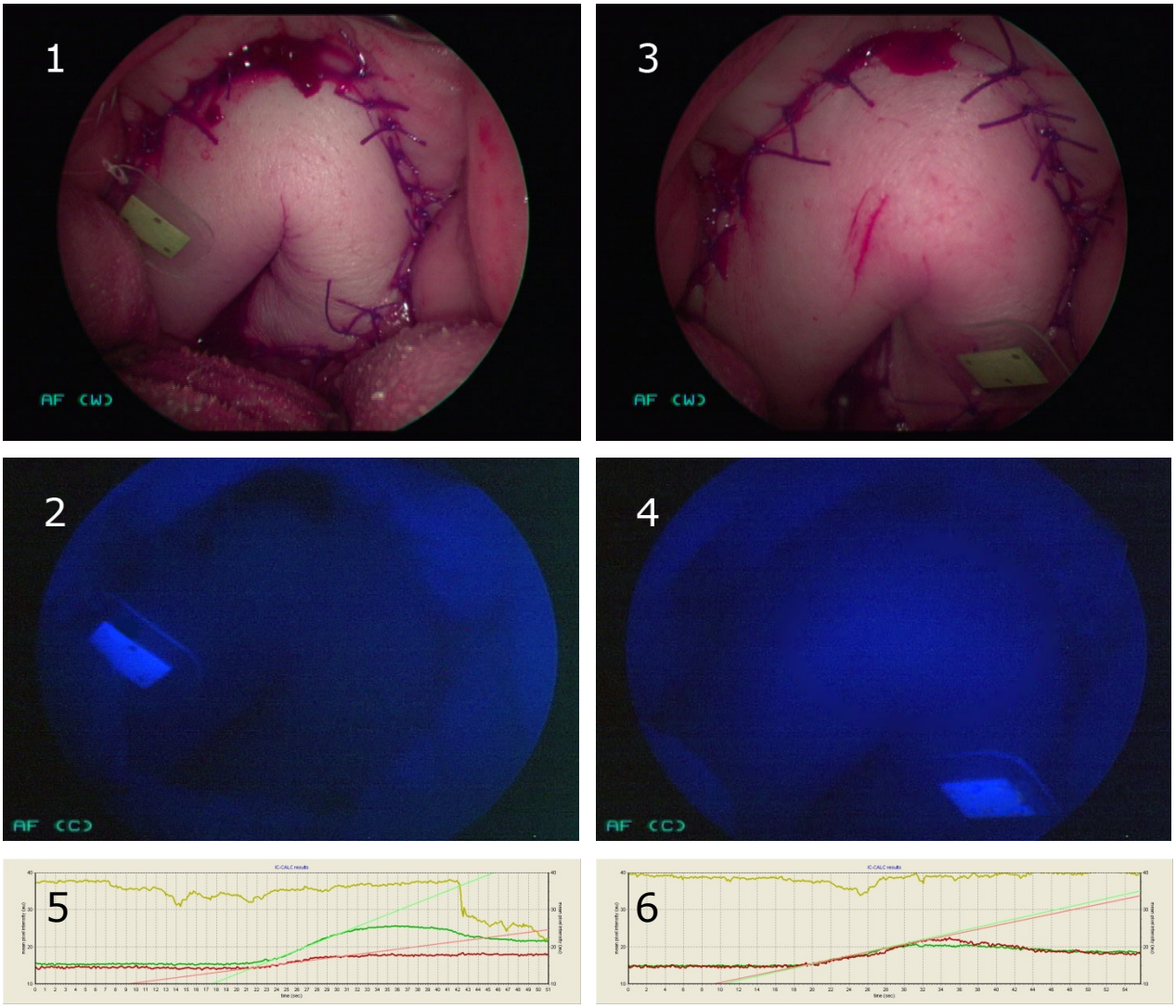

Figure 3: Images of Patient LMU 06 before (1 & 2) and after (3 & 4) revision in white light and ICG angiography mode.
The flap appears pale (1) and takes up less fluorescence than the surrounding tissue (2). In the pre-revision ICGA only a low increase in fluorescence intensity in the flap could be found corresponding with a low FI of 0.33 and PI of 0.29 (5). After re-exploration, the flap is of rosy colour and bleeds following pinprick testing (3) and shows stronger fluorescence then before (4). The postoperative ICGA shows better ICG uptake and fluorescence in the flap translating into a FI of 0.93 and a PI of 0.68 (6).

Due to the clinical findings it was decided to immediately revise the flap. Upon exploration, missing arterial blood flow was found. Although the definitive cause for this remained unclear, resection of the arterial anastomosis and renewed anastomosis to the more proximal stem of the superior thyroid artery succeeded in restoring flow in the pedicle artery. Intraoperative Doppler examination showed also good flow of the venous branch and so the neck was closed again. The flap now appeared well perfused and presented itself with healthy colour and active bleeding after

pinpricking. In the ICGA now better uptake of the dye could be seen (*see Figure 4*). In the postoperative phase, the patient had to undergo another revision due to a clinically pale aspect of the flap (*see 3.4.3 for details*).

When the ICGA sequences were further analysed, the FI before revision was 0.37 with a PI of 0.20. The ICGA analysis after renewing the anastomosis clearly improved to values of 0.73 for FI and 0.67 for PI.

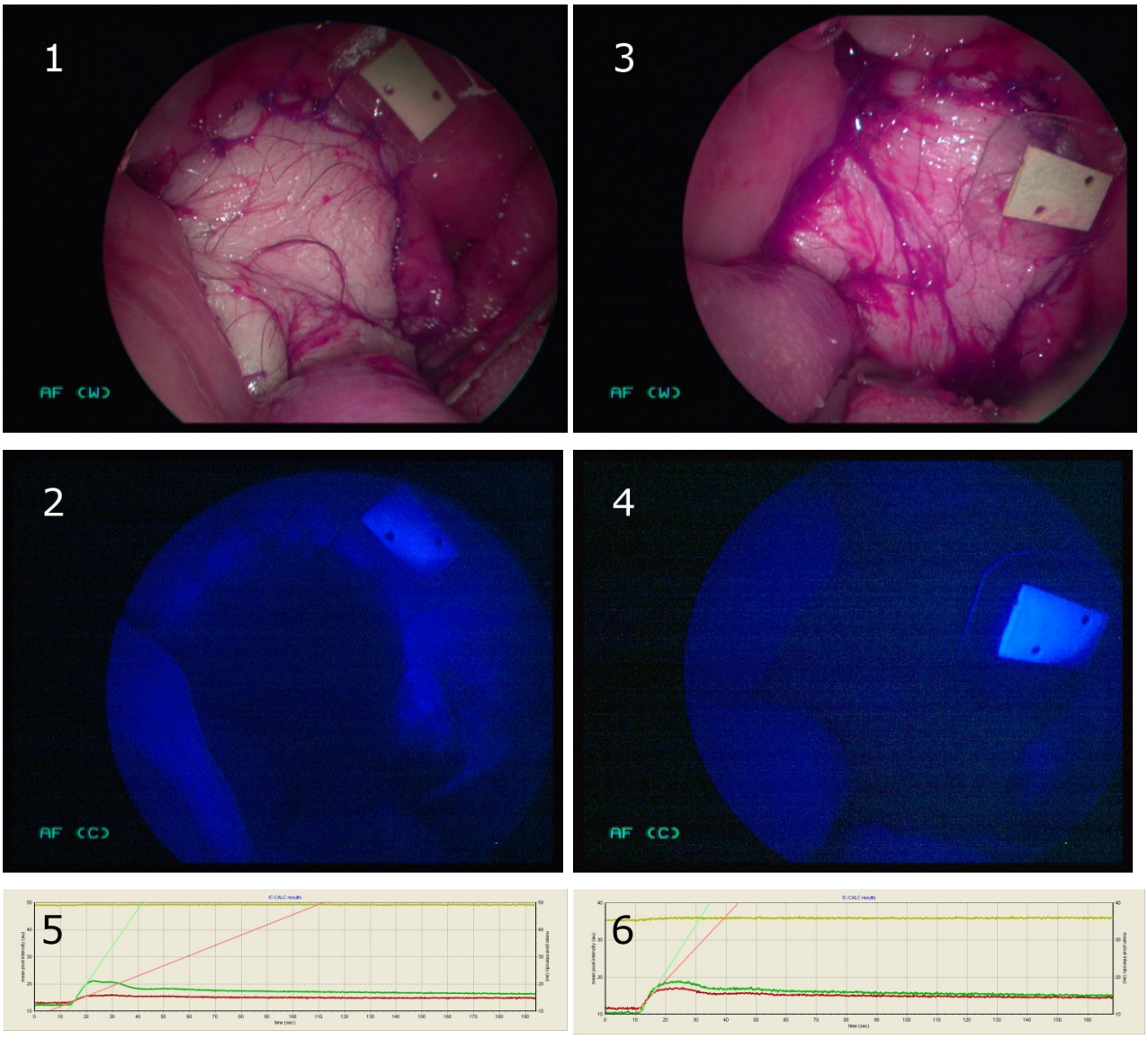

Figure 4: LMU 23 before and after revision:
again, pale aspect of the flap (1) combined with reduced ICG-uptake in the flap (2). The ICGA shows reduced uptake of ICG in the flap with a FI of 0.37 and PI of 0.20 before revision (5). After renewing the anastomosis, the flap appears more rosy (3) and the maximum ICG fluorescence increases (4). The ICGA confirms the clinically improved perfusion situation with values of 0.73 for FI and 0.67 for PI (6).

3.4.2 Revised Patients without pathologic intraoperative findings

Two patients, namely LMU 23 and LMU 25, underwent revision surgery, that showed no pathologic findings concerning perfusion intraoperatively.

LMU 23, who had also been previously revised when still in the OR from the original surgery, again showed a rather pale flap in clinical examination exactly 24 hours following anastomosis. Indication for the surgery was based on the clinical findings, because even the hint of vascular compromise should result in operative reassessment. While preparations for the re-exploration were underway, a preoperative ICGA was performed, which turned out with relatively fast and good uptake of fluorescence within the flap and thus stood in contrast to the clinical findings. Intraoperatively though, there were no pathologic findings, that could have accounted for the paleness of the flap. Because there was no true anastomotic revision, this ICGA was considered a regular 2nd examination 24 hours following anastomosis and there were neither an extended follow-up period nor additional ICG-angiographies afterwards. The flap healed in uneventful afterwards (*see Figure 5*).

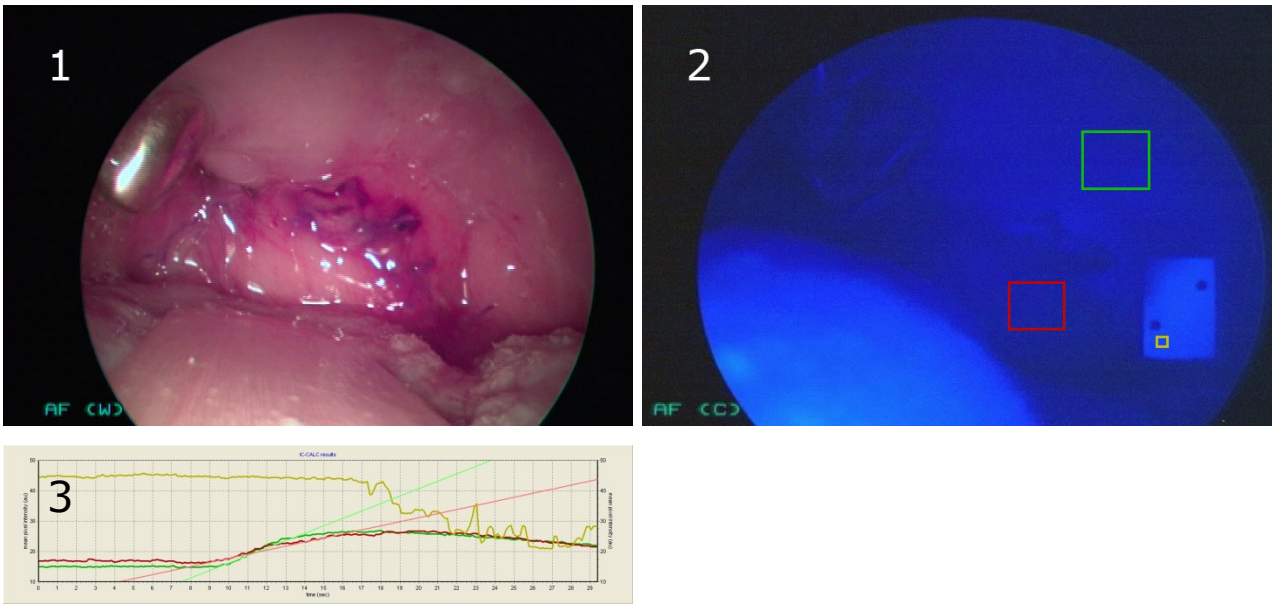

Figure 5: WL (1) and ICG imaging (2) of LMU 23 directly before revision:
The flap can hardly be completely revealed, the tongue being pushed away by a wooden spatula. It appeared rather pale on one of the hourly clinical checks, in the WL footage it seems quite well perfused, though. In the ICGA there is adequate fluorescence increase (Note: images taken from IC Calc hence the ROIs for evaluation). The pre-revision ICGA indicated good perfusion of the flap with a FI of 0.79 and a PI of 0.69 (3).

LMU 25, a 54 years old, 130kg obese patient with a T4 oropharyngeal cancer receiving a fRFF showed signs of vascular compromise 14 hours after anastomosis. The flap appeared pale, although adequate bleeding after pinpricking was present. A Doppler-probe examination was hardly possible due to the patients weight and extensive postoperative swelling of the neck combined with extended cutaneous emphysema surrounding and originating from the tracheostomy. The vessels could not be doubtlessly identified acoustically and no information on either arterial or venous flow could be obtained. Because of the possible compromise of the flap, immediate operative assessment of the anastomoses was indicated, although clinical signs were equivocal. The preoperative ICGA showed good and rapid uptake of ICG and good fluorescence intensities in the flap (*see Figure 6*). In the OR, there were no pathologic findings to the flap or pedicle and the neck was carefully closed again without intervention. Again, since there was no operative revision of the anastomosis, this was considered a regular 2nd examination and the schedule was adapted accordingly. The further postoperative course was uneventful.

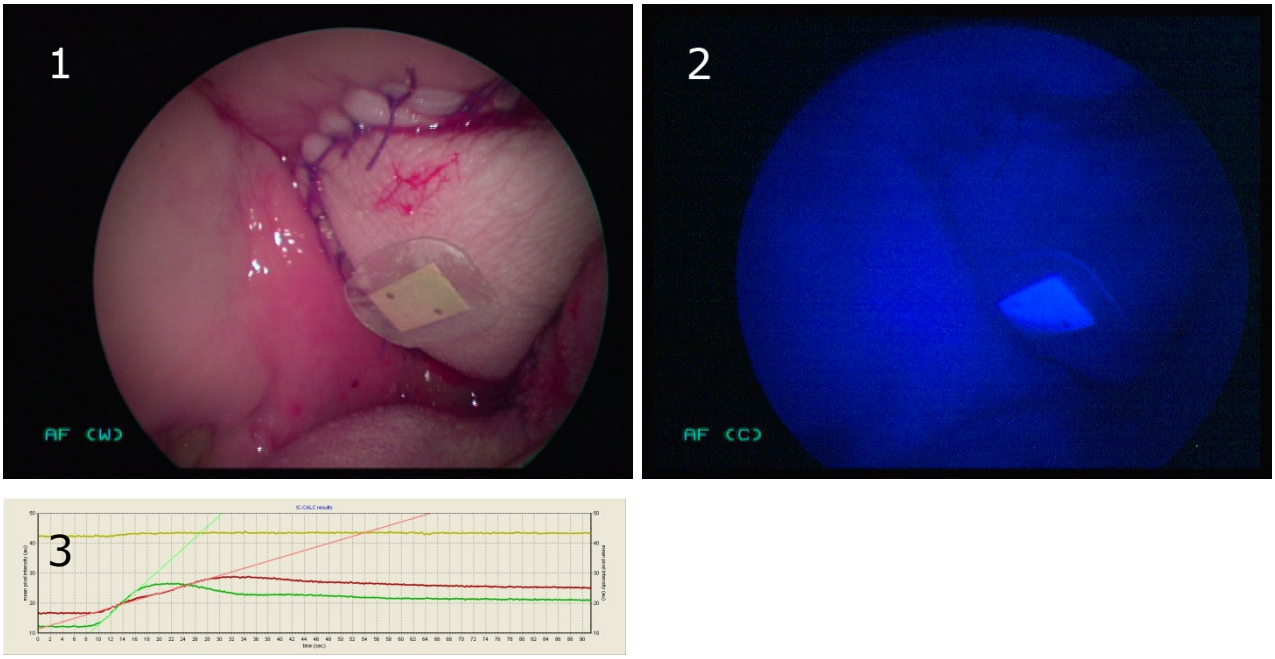

Figure 6: LMU 25 14 hours after anastomosis:
Pale aspect of the flap with arterial bleeding after pricking (1). Good and fast uptake and almost equal intensities of fluorescence in flap and surrounding tissue in the ICGA (2). Analysis later showed a good pre-revision perfusion with a FI of 0.93 and a PI of 0.50 (3).

3.4.3 Patients with revisions without preoperative ICGAs

Two patients showed clinical signs of vascular compromise, but could not be preoperatively examined by an ICGA. In this case postoperative ICGAs were performed and considered 1st examinations with 2 further ICGAs following, since there was an operative revision of the anastomosis.

The first patient, LMU 13, aged 45 years and male, had a T4 relapse of a carcinoma of the floor of the mouth with infiltration of the mandible. Due to the extent of the disease, partial resection of the mandible and reconstruction using a osteo-cutaneous fibula-flap was necessary. 11 hours after anastomosis, the neck and region around the floor of the mouth appeared swollen and the flap unusually pale. Since this was judged as an apparent sign of an acute vascular compromise, the situation called for emergency salvage surgery and it was decided there was no time to perform a preoperative ICGA. Intraoperative investigations showed a good flow within the pedicle vessels with accordingly assumed good perfusion of the bone transplant, but an impaired perfusion of the skin flap. After the skin flap had been released of some tension by undoing some of the sutures, it was immediately reperfused and sewn in again without tension. The postoperative clinical assessment showed a healthily coloured flap and the ICGA turned out with normal findings (*see Figure 7*). In the further postoperative course, a wound dehiscence resulted in the formation of a oro-cutaneous fistula that had to be operatively revised twice. Also, intraoperatively taken smears showed colonization with MRSA.

The second patient, LMU 19, a 60 years old male patient with T4 oropharyngeal carcinoma treated with a free radial forearm flap transplant, showed a livid and swollen monitor flap 22 hours after anastomosis. Due to suspicion of venous congestion, an emergency salvage surgery was scheduled. A preoperative ICGA could not be performed. Upon reopening the neck, complete kinking of the pedicle with venous congestion due to thrombosis was found. Resection and renewing of the venous anastomosis allowed for stable venous outflow and lead to rapid improvement of the clinical aspect. In the postoperatively performed ICGA, the findings were

unsuspicious of any problems (*see Figure 8*). The further postoperative course was uneventful.

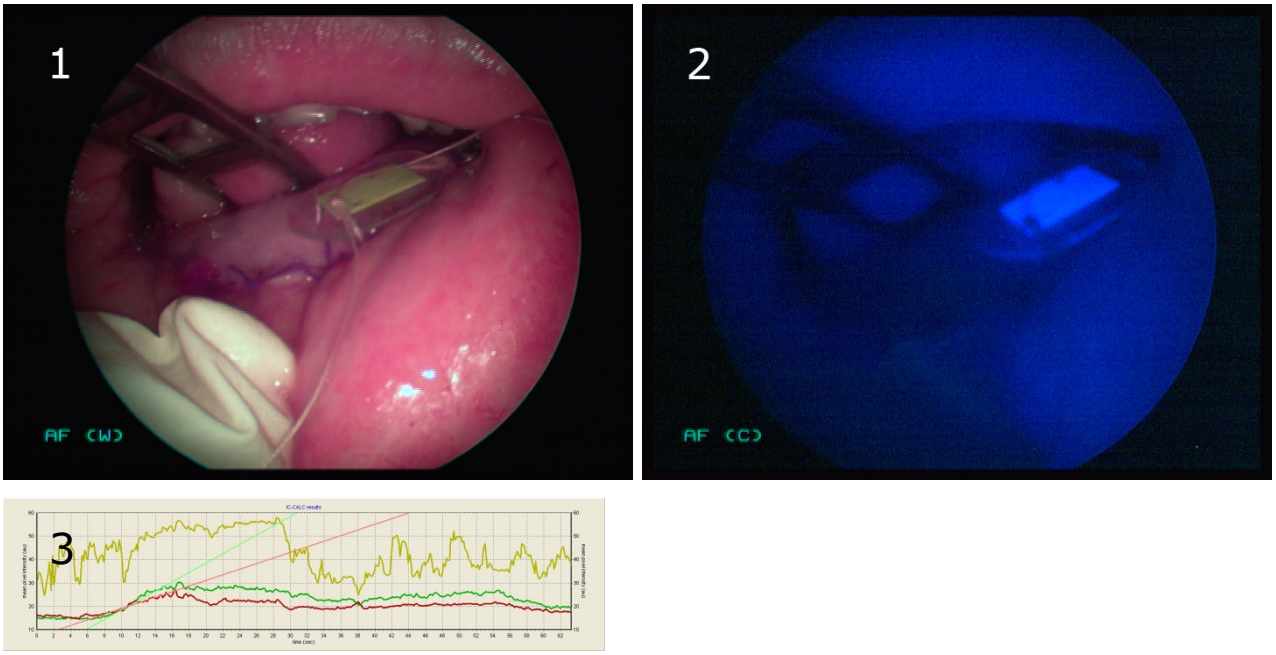

Figure 7: LMU 13 with reconstructed floor of the mouth:
Postoperative assessment showed clinically rosy flap (1), after tension on it had been released. The ICGA showed normal and arguably subjectively reduced fluorescence of the flap (2), which could not be confirmed, when analyzed later. The FI was 0.80 and the PI 0.72 (3).

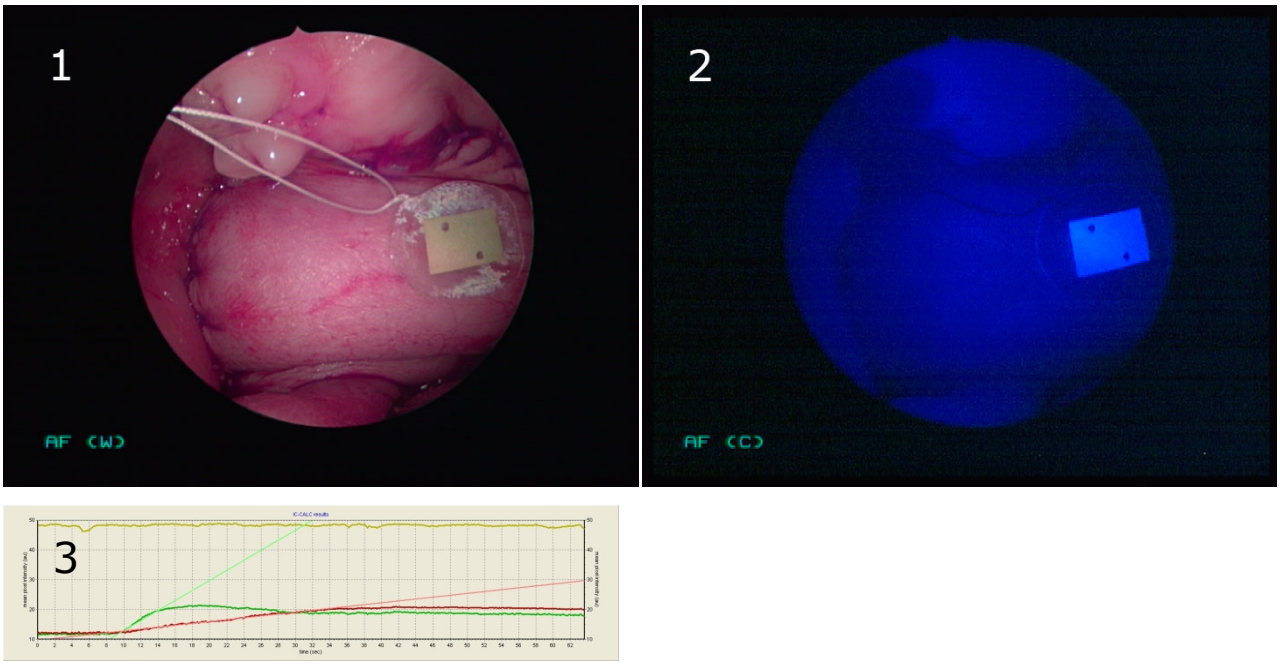

Figure 8: LMU 19, assessment after revision 22 hours following original anastomosis:
Very good perfusion situation of the flap clinically (1) and in the ICGA (2). The later evaluation showed good perfusion with an FI of 0.89 and PI of 0.39 (3).

3.5 Evaluation

3.5.1 On-screen interpretation of the videos

While the videos of the fluorescence angiography were recorded, the monitors were closely watched for apparent differences in flap perfusion. The examiners and the surgeon judged the situation based on their subjective impression. In the OR, two patients (LMU 06 and 23) subjectively showed lower fluorescence intensities in the flaps. When those were assessed intraoperatively during revision, loss of arterial blood flow was found. The arterial anastomoses had been renewed subsequently in both cases, and a normal blood flow and clinical findings returned afterwards.

When clinical assessment according to the standard post-op monitoring schedule for free-flap transplants lead to a surgical revision and no pathologic findings were obtained intraoperatively in two cases (LMU 23 and 25), also the additionally performed ICGA before revision surgery was subjectively normal with rapid and homogenous uptake in both flap and surrounding tissue.

In two cases (LMU 13 and 19), preoperative ICGAs could unfortunately not be performed, despite pathologic clinical findings (paleness of the flap in one, livid monitor flap in the other case), because the surgeries were considered to be highly urgent emergency salvage procedures and a delay through the ICGA was not desired. After the problems had been surgically resolved and the clinical findings were in the normal range again, the now obtained ICG footage also showed normal results.

In all other cases, a total of 58 examinations were performed, which all were subjectively unsuspicious. 19 patients had all 3 examinations (total of 57 ICGAs) while one patient only had a single ICGA before swelling impeded the subsequent examinations. There were also no extraordinary clinical findings at the times of those examinations.

Mere real time viewing of the ICG uptake showed, that in all patients the same sequence of fluorescence phases could be found. After an initial phase

of missing fluorescence following injection of the dye raising intensity was noticeable first in the surrounding tissue, then in the flap, before both areas reached their maximum levels of fluorescence intensity (*see Figure 9*).

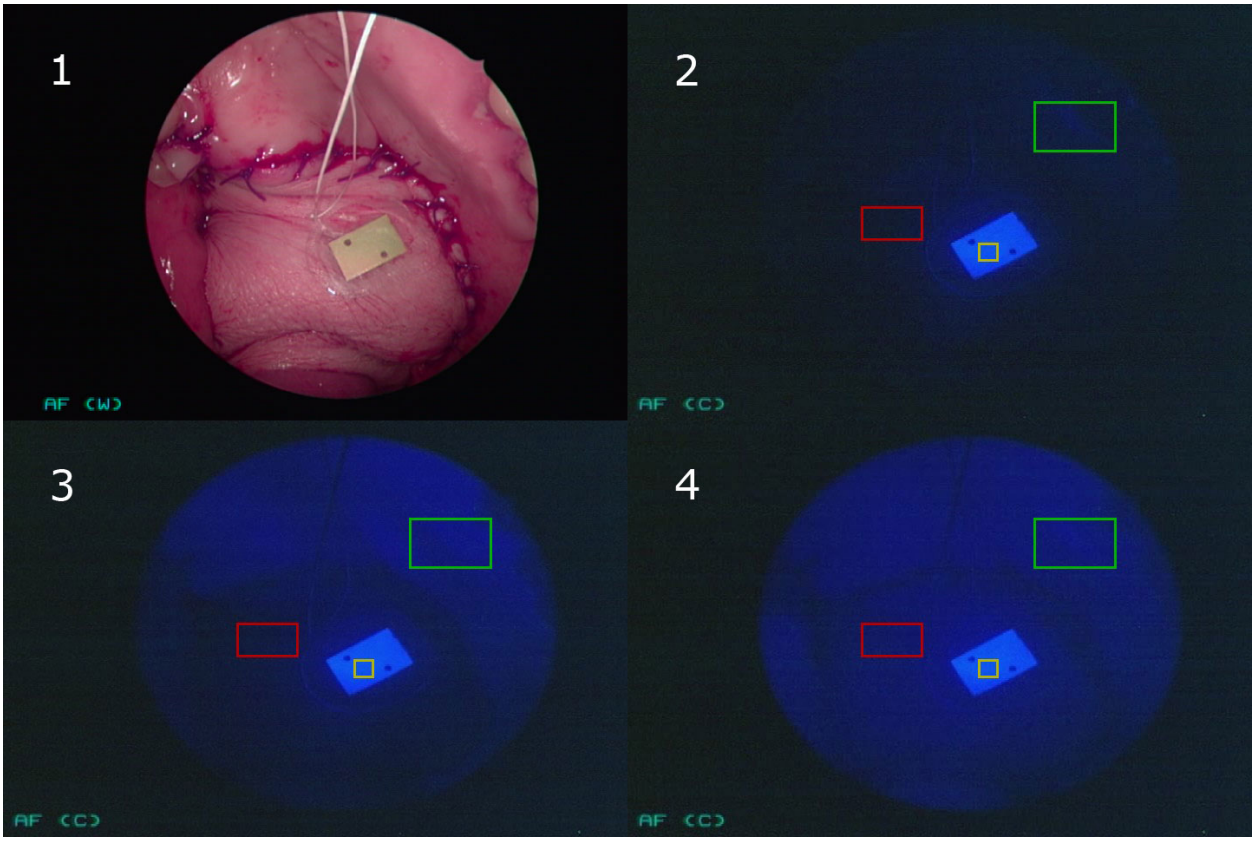

Figure 9: Typical sequence of raising ICG fluorescence (LMU19 0h):
Endoscopic image of the operational field in white light mode (1) showing flap and ICG reference with surrounding tissue. ICG fluorescence imaging of the same area before injection of ICG (2), during the arterial enrichment phase (3) and after maximum intensity is reached also in the flap (4).

3.5.2 After-Processing of Video Material

Each of the sequences taken during the ICGA was cut afterwards and transferred from .mov-format into the .avi-format. The videos were either used in their original form or cut so that the moment of injection, which had originally been marked on the recording acoustically, became the first frame of the re-cut clip.

Depending on the used PC hardware this procedure took about 5-10 minutes. There was no data loss during the transformation process.

3.5.3 Interpretation using IC Calc and extracting data

After the respective regions of interest had been identified and marked on the first frame, the .avi-files were fed into Pulsion's IC Calc program and processed to be put out as a two-dimensional graph showing the slopes of the fluorescence intensities of the ROIs in their respective colour, which had been previously assigned (*see Figure 10*).

Also, the slope is calculated by IC Calc based on a routine not further specified by Pulsion.

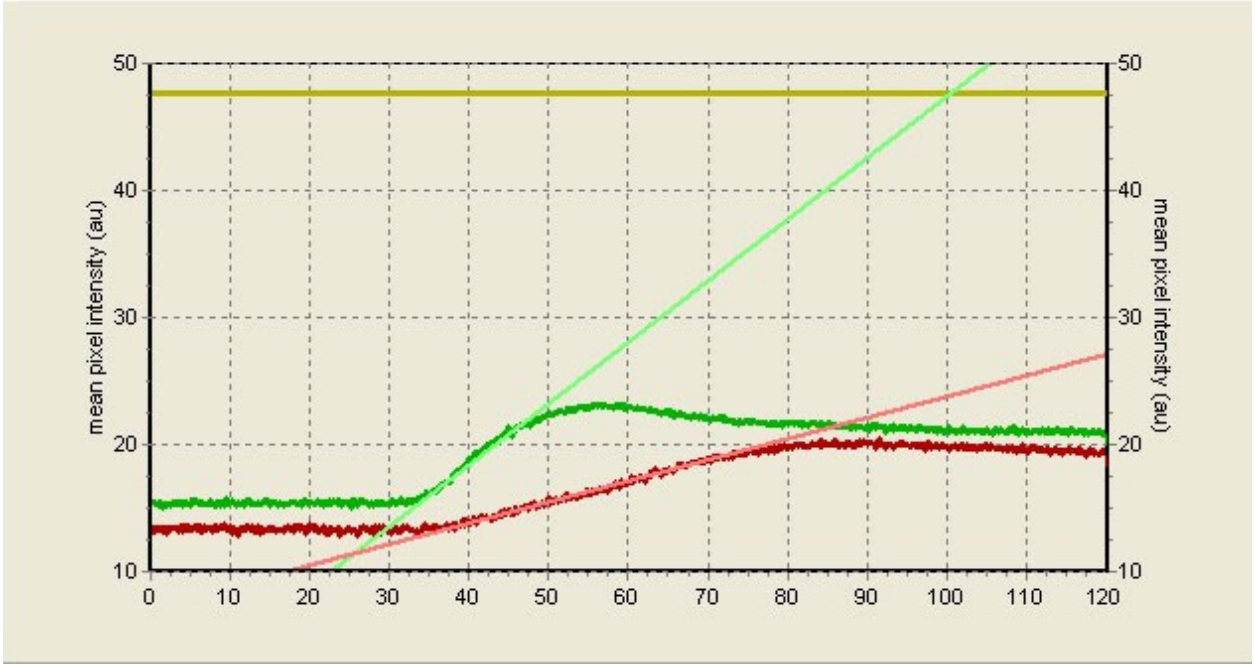

Figure 10: Typical IC Calc output file (LMU 19 0h):
Slopes of ICG reference (yellow), flap (red) and surrounding tissue (green) from injection (0s) until end of video recording (approximately 69s). Note the synchronous begin of raising fluorescence intensities at approximately 33s and that the surrounding tissue reaches maximum intensity earlier (approx. 56s) when compared to the flap (approx. 80s). Also included are the slopes which IC Calc determines.
The IC Calc plot depicts the aforementioned sequence of *more rapid onset of fluorescence in the autochtonous tissue (green) compared to the flap (red) graphically*, *which can be* regularly seen in each patient (also see *3.5.1* and *Figure 9)*

The acquired data - e.g. intensities at basal and maximum levels - was then transferred into a Microsoft Excel file. From the raw data, Excel calculated the derived data according to the formulas given in 2.6.2. Additionally, the Excel file was transformed into a SPSS sheet to allow more detailed analysis.

Sometimes the ICG reference's fluorescence intensity alternates, while the observed ROIs` intensities do not and vice versa (*see Figure 11*). In this particular case the fluctuation was caused by the thread attached to the reference, which involuntarily moved against the reference's predefined ROI. Other causes of ragged curves were movement of the endoscope against the flap and affected all ROIs (*see Figure 12*). Since the program could not eliminate these fluctuations, whatever the cause, automatically if they occurred, the examiner had to choose consistent values. Watching the sequence play along while the curves evolved, this was an easy task.

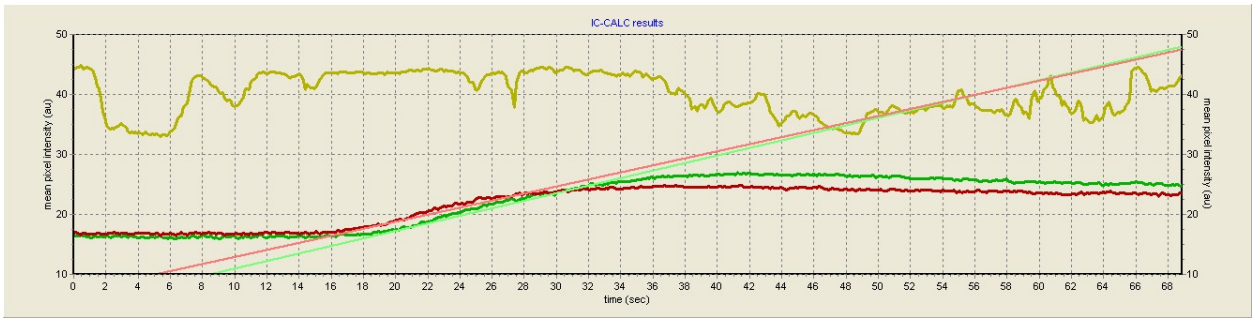

Figure 11: Fluctuating fluorescence intensity of the ICG reference with unaffected flap ROIs (LMU 22 0h).

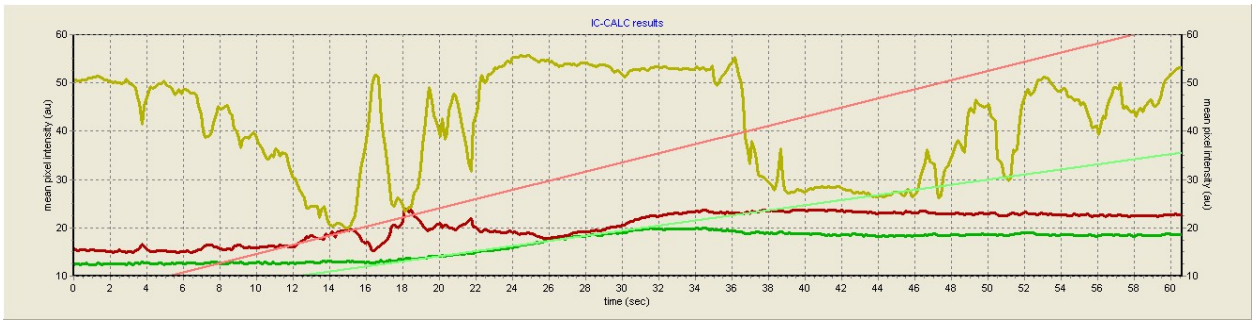

Figure 12: Extreme movement in an awake patient (LMU 20 72h) affecting both, the ICG reference as well as the flap ROIs.

In the first patient to be included into the study all three examinations were performed. Due to lack of experience with the method of analysis and its requirements - especially possibly still image - a later objective evaluation of the videos was not possible because of permanent movement of the endoscope and change of distance to the flap. In all the later patients great care was taken to improve conditions and minimize movement.

This fact reduces the count of patients statistically evaluated to 24, of whom one only got a first examination due to postoperative swelling that prevented an exposition of the flap (LMU 05).

3.6 Calculations

3.6.1 Number of evaluated Examinations

The examinations were primarily classified as first, second or third exam according to study protocol.

In case of revision the respective examinations were re-classified as "revision needed" if pathologic findings were identified intraoperatively or "no revision needed" if it turned out that there were no pathologic findings.

In that case, "no revision needed" replaced the examination due at that time (e.g. 1st exam – "no revision needed" – 3rd exam). If a revision turned out to be "needed", it was treated as a first exam with another two ICGAs to complete the cycle (e.g. 1st exam – "revision needed" – 2nd exam – 3rd exam).

Had a pre-revision examination not been possible, the following post-op check-up was classified as "only post-revision" and considered as a second with one last following ICGA (e.g. 1st exam – "only post-revision" – 3rd exam, *see Table 4*).

Table 4: Count of Examinations

Examinations	n=
1st Exam	25
2nd Exam	23
3rd Exam	24
Revision needed	2
No revision needed	2
Only post-revision	2
Total	78

3.6.2 Cardiac Parameters

Cardiac parameters were noted for the majority of the patients. Mean arterial pressure (MAP) and heart rate (HR) increased with growing timely distance from surgery in most patients (*see Table 5 and Table 6*).

Table 5: Statistics of mean arterial pressure (MAP) [mmHg].

Examinations	1st Exam (n=24)	2nd Exam (n=19)	3rd Exam (n=20)
Mean	79,04	88,30	94,55
95% conf. interval	74,1 - 84,0	81,8 - 94,8	89,3 - 99,8
Std. deviation	11,37	13,87	11,23
Minimum	58	69	70
Maximum	108	116	115
Range	50	47	45

Table 6: Statistics of heart rate (HR) [bpm].

Examinations	1st Exam (n=23)	2nd Exam (n=19)	3rd Exam (n=20)
Mean	57,17	82,84	85,45
95% conf. interval	52,8 - 61,6	76,8 - 88,9	80,4 - 90,5
Std. deviation	10,15	12,50	10,77
Minimum	43	57	64
Maximum	85	104	100
Range	42	47	36

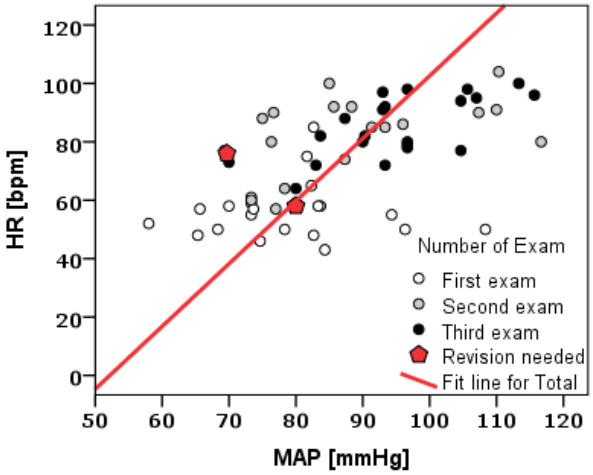

Figure 13: Plot of HR and MAP against each other:
Revised flaps do not behave differently than the uneventful flaps. Regression line added (red).

MAP and HR show a significant correlation of 0.568 (Spearman's Correlation; $p < 0.01$). Subgroups seem to form clouds around a regression line, with narcotized patients tending to have lower heart rate compared to the later measurements, which is most probably due to anaesthesia (*see Figure 13*).

In those patients who had to be revised, the HR (mean 67/min, range 58 - 76/min) and the MAP (mean 74,8 mmHg, range 69,7 - 80,0 mmHg) do not significantly differ from the measured values during regular first examinations (p=0.55 Mann-Whitney-U-Test).

3.6.3 Time intervals

Time from injection until onset of fluorescence (TUO; **t**ime **u**ntil **o**nset of fluorescence increase) ranged from 5 to 38 seconds and was identical for flap and surrounding tissue. After first signs of uptake of the dye, maximal fluorescence was reached earlier in the original tissue then in the transplanted (4 - 35s vs. 5 - 54s; *see Table 7*) in all patients.

The first value of interest was the time from injection until onset of fluorescence increase (TUO). The intervals were longest in the first examinations and became shorter with elapsing time from surgery. There is no obvious explanation, why TUO was shortest in the 2nd exam (*see Table 8*).

Table 7: Fluorescence build-up times:

Examinations	n=	Range	Mean	Std.
TUO	73	5 - 38	12,78	5,95
TMS	73	4 - 35	12,21	6,13
TMF	73	5 - 54	17,44	10,49

In flaps which needed revision in the OR directly after anastomosis the TUO did not differ significantly from regular first examinations (n=2, mean 15.50s, range 13 - 18s; p=1.0 Mann-Whitney-U-Test).

Table 8: Time intervals until onset of raising fluorescence intensities (TUO):

Examinations	1st Exam (n=23)	2nd Exam (n=21)	3rd Exam (n=23)
Mean	16.68	10.14	12.0
95% conf. interval	13.3 - 20.0	8.2 - 12.0	10.2 - 13.8
Std. deviation	7.51	4.27	4.17
Minimum	5	5	6
Maximum	38	21	20
Range	33	16	14

Also of interest were fluorescence build-up times (*see Table 7*). This means the times until the maximum fluorescence intensities were reached in the flap (TMF; **t**ime until **m**aximum fluorescence in the **f**lap) or the surrounding tissue respectively (TMS; **t**ime until **m**aximum fluorescence in the **s**urrounding tissue).

In the original surrounding tissue, the TMS (time until maximum fluorescence in the surrounding) is longer directly after anastomosis than after 24 or 72 hours (*see Table 9*). The same applies for the TMF (time until maximum fluorescence in the flap) (*see Table* **9** *10*). In all fluorescence examinations the maximum intensity was reached earlier in the surrounding tissue than in the transplanted flap. Shortly after surgery the mean time difference was 9.96 seconds and was reduced to 4.58 seconds after 24

hours and was negligibly low with a mere 0.73 seconds 72 hours postoperatively.

Table 9: Time intervals until maximum fluorescence in the surrounding (TMS):

Examinations	1st Exam (n=23)	2nd Exam (n=21)	3rd Exam (n=23)
Mean	16.39	8.90	11.57
95% conf. interval	13.0- 19.8	8.0 - 9.9	9.5 - 13.7
Std. deviation	7.96	2.01	4.87
Minimum	7	5	4
Maximum	35	15	27
Range	28	10	23

Table 10: Time intervals until maximum fluorescence in the flap (TMF):

Examinations	1st Exam (n=23)	2nd Exam (n=21)	3rd Exam (n=23)
Mean	26.35	13.48	12.30
95% conf. interval	20.7 - 23.0	10.6 - 16.4	10.5 - 14.1
Std. deviation	12.96	6.37	4.28
Minimum	8	5	5
Maximum	54	33	24
Range	46	28	19

3.6.4 Correlation between cardiac parameters and TUO, TMS and TMF

It was found that there is a low negative correlation between the cardiovascular factors (heart rate and blood pressure) and the time needed to register first signs of fluorescence and that it is stronger for heart rate than it is for blood pressure (HR -0.35; MAP -0.18; n=61). Also, circulatory factors were shown to have an even stronger influence on flap perfusion (HR -0.54; MAP -0.37; n=61) than on the original tissue (HR -0.37; MAP -0.22; n=61), since faster paced patients reached maximal flap fluorescence earlier than slower paced ones (*see Table 11*).

Table 11: Spearman's correlation between circulatory factors and fluorescence build-up:

Spearman's rho	TUO	TMS	TMF
HR Correlation coefficient	-0.348	-0.372	-0.537
n=	61	61	61
MAP Correlation coefficient	-0.178	-0.217	-0.372
n=	61	61	61

The revised flaps do not show different behaviour than the uneventful flaps (*see Figure 14*).

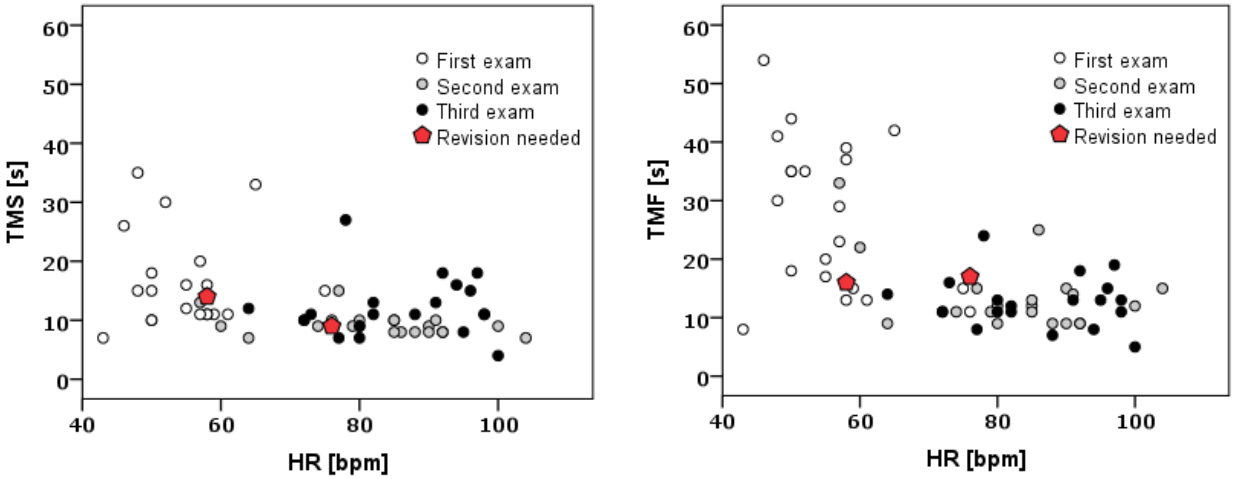

Figure 14: *Plots of HR vs. time until maximum fluorescence is reached in both surround tissue (TMS; left) and flap tissue (TMF; right) for all three examinations (color coded, see legend). The cases when revision was necessary are displayed with red pentagons and do not differ from the other examinations in regard to the distribution.*

3.6.5 Fluorescence Index (FI)

Fluorescence Index (FI), the ratio of maximum fluorescence intensity between flap and surrounding tissue, was calculated for all examinations except those of the first patient according to the formula provided in 2.6.2 (*see Table 12*).

Table 12: Fluorescence index (FI) with descriptives for all subgroups:

Examinations	n=	Mean	95% Conf. Int.	Std. Dev.	Range
1st Exam	23	0.819	0.77-0.86	0.105	0.60-0.96
2nd Exam	21	0.859	0.82-0.90	0.081	0.69-0.96
3rd Exam	23	0.883	0.84-0.92	0.096	0.68-1.00
Revision needed	2	0.351	0.12-0.57	0.025	0.33-0.37
No revision needed	2	0.860	0.00-1.73	0.097	0.79-0.93
Only post-op	2	0.847	0.25-1.45	0.067	0.80-0.89

In those examinations, which were not associated with a surgical re-evaluation or revision, a mean value of 0.85 was reached (n=71), with values ranging from 0.60 to 1.00. Most patients showed a continuously raising FI with growing time from initial surgery following the overall trend, although some showed falling FIs without obvious reason or postoperative complications. The mean FIs rose from 0.819 directly after anastomosis to 0.859 after 24 hours respectively to 0.883 after 72 hours (*see Figure 15 and Figure 16*).

In those cases, that had a truly compromised arterial flow (n=2), the FI was 0.33 or 0.37, respectively. After revision, these values returned to the normal range immediately, namely 0.73, respectively 0.93. Those cases, which underwent revision surgery lacking any true perfusion issues, the FIs were also in the normal range (0.79-0.93; mean 0.85; n=4) and mixed well with the regular measurements.

The revised flaps` FIs differed significantly from the other cases' first regular examination ($p<0.01$, Mann-Whitney-U-test).

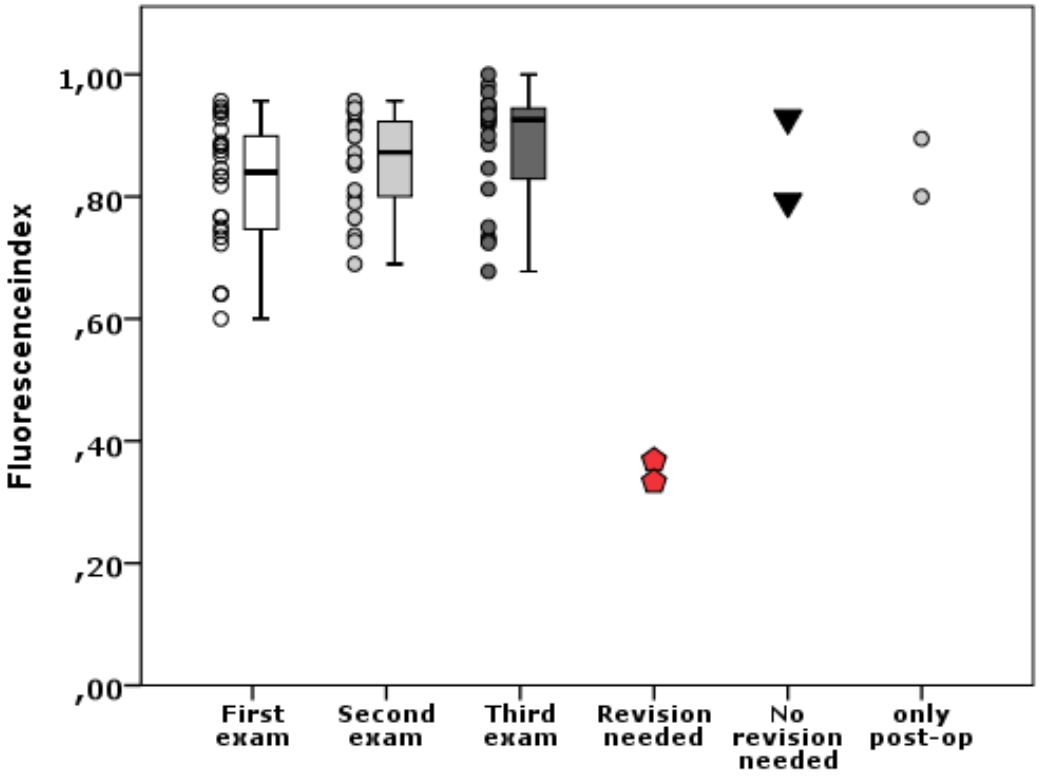

Figure 15: *FI shown in regular exams as combined scatter and box plots (n=23 for 1st and 3rd exam, n=21 for 2nd exam) and in revised flaps depicted as dots due to low numbers (n=2 for necessary revisions, n=2 each for non-arterial problems).*

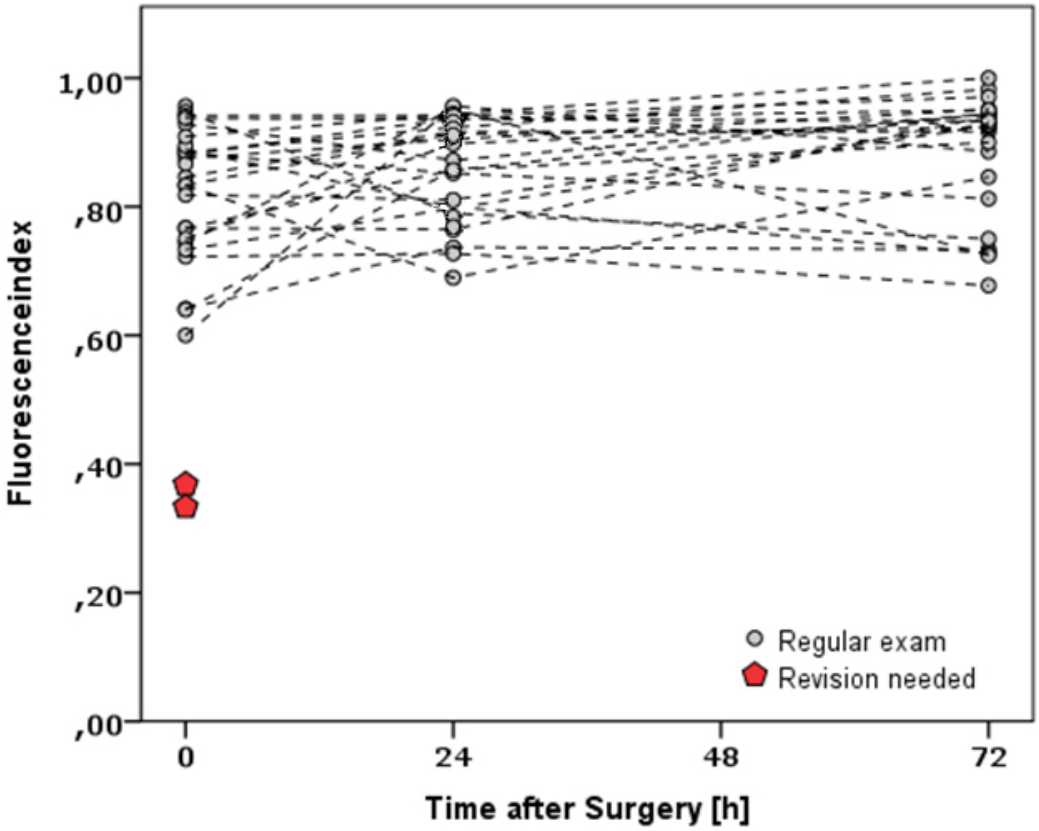

Figure 16: *Increasing ratio of fluorescence intensity with growing time from surgery within the observation period. Intrapatient development shown by dotted black line.*

If a cut-off value of 0.50 for the FI was drawn, only the flaps with a true, acute vascular compromise are found below this threshold whereas all other measurements lie above.

3.6.6 Perfusion Index (PI)

Perfusion index (PI; ratio between slopes of the curves of fluorescence gain in the flap and surrounding) was determined by calculating the mean slopes from the raw data and not using IC Calc's build-in feature since in some cases it could not be used due to movement artifacts in awake patients. The formula used was $PI = \frac{\Delta If \times \Delta ts}{\Delta Is \times \Delta tf}$. In this formula ΔI stands for gain in fluorescence intensity, Δt for time until maximum intensity, indices f and s for flap or surrounding, respectively.

PI ranged from 0.21 to 2.00 (mean 0.67; median 0.68). In patent flaps, PI was 0.21 - 2.00 and in the two insufficient flaps 0.20 or 0.29. Like the FI, PI improved with time from anastomosis (*see Table 13*).

Table 13: Perfusion index (PI) with descriptives for all subgroups:

Examinations	n=	Mean	95% Conf. Int.	Std. Dev.	Range
1st Exam	23	0.570	0.47-0.67	0.234	0.21-1.06
2nd Exam	21	0.635	0.55-0.73	0.192	0.27-0.91
3rd Exam	23	0.853	0.71-0.99	0.327	0.51-2.00
Revision needed	2	0.243	-0.37-0.86	0.068	0.20-0.29
No revision needed	2	0.598	-0.61-1.80	0.134	0.50-0.69
Only post-op	2	0.515	-2.09-3.12	0.290	0.31-0.72

The PI for flaps which needed revision were 0.20 and 0.29, which were low values, when compared to the other examinations, but were still within the normal range in contrast to the FI. 13 percent (3/23) of the PIs measured at 0 hours after anastomosis and 4.8 percent (1/21) at 24 hours postoperatively also showed a value below 0.30. All 72 hour PIs were above 0.30 (*see Figure 17 and Figure 18*).

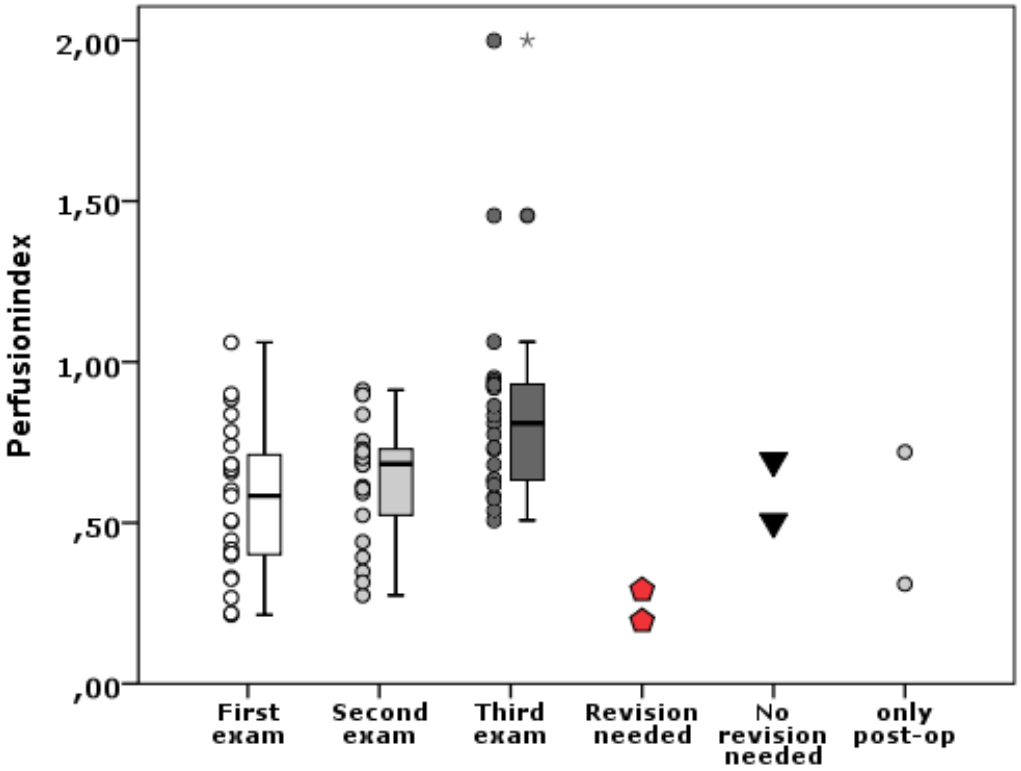

Figure 17: *Combined scatter plot and box plot of PI for all subgroups. Flaps with acute vascular compromise range below a threshold of 0.30 and are depicted with dots due to low case numbers (n=2).*

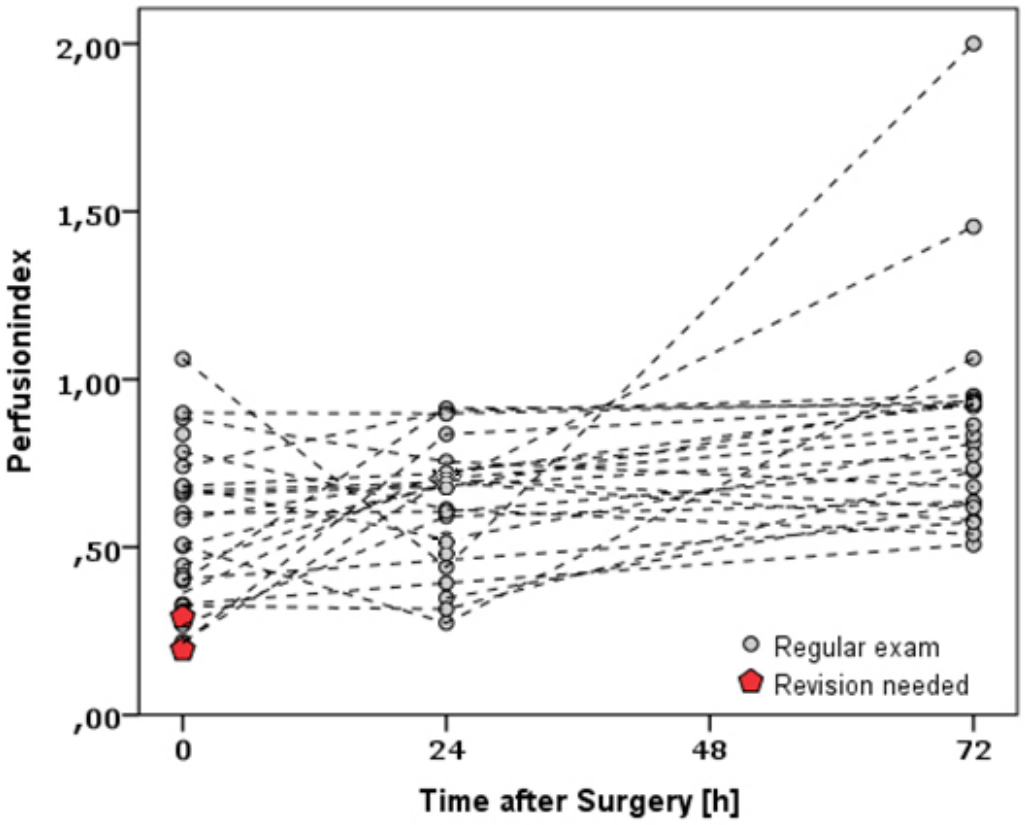

Figure 18: *Intrapatient development of PI over time.*

PI values for flaps suffering from vascular compromise differed significantly from the values taken from regular first examinations ($p<0.05$, Mann-Whitney-U-test).

3.6.7 Reproducibility of FI and PI

In order to check the reproducibility of each FI and PI, one fluorescence sequence of a well perfused flap was repeatedly analysed 14 times with different randomly assigned pairs of ROI for the flap and the surrounding tissue. Only a relatively minor scatter was found for the FIs with values ranging from 0.75 to 0.93 (mean 0.83, median 0.83; std. dev. 0.07). Even the lowest value of 0.75 stayed well above the assumed threshold for vascular compromise of 0.50.

At the same time, PI ranged from 0.34 to 0.86 (mean 0.52; median 0.44; std. dev. 0.44) and thus scattered a lot more (*see Figure 19*).

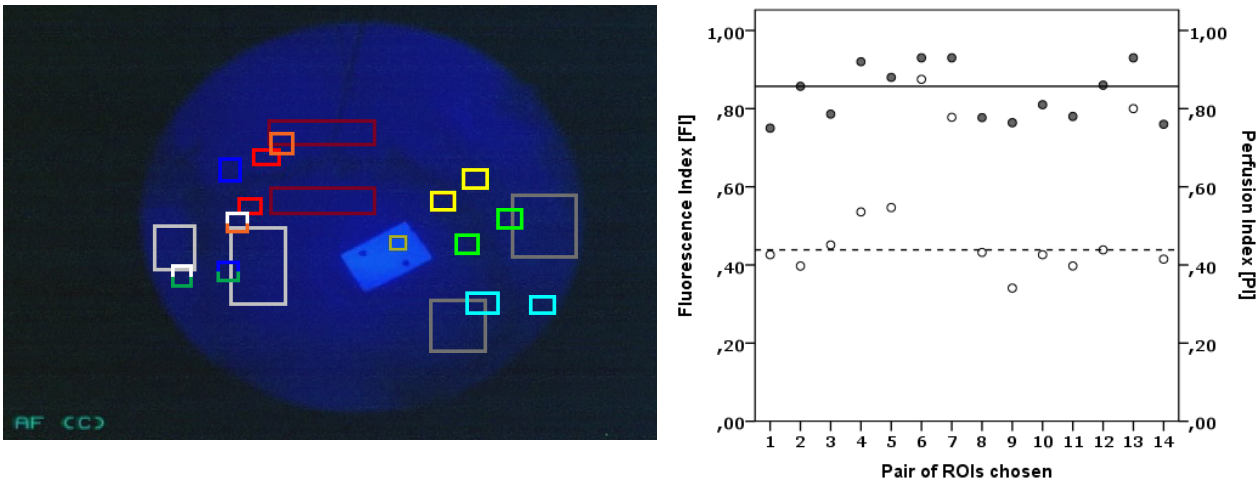

Figure 19: *Left: Endoscopic ICG-image showing some of the ROI pairs (left). Corresponding pairs are depicted the same color. ROIs were randomly selected and false colors in this CGI added during afterprocessing (no original data).*
Right: Values of the different measurements for FI (black) und PI (white) with median lines (massive line for FI, dotted line for PI). (n=14)

3.6.8 Correlation between FI and PI

A moderate significant correlation between FI and PI was found for all patients. Due to small numbers, a subgroup analysis was not possible (Spearman's rho; 0.437).

3.6.9 Summarized comparison of FI and PI

In summary, when FI and PI are compared, there is a definitive threshold of 0.50 for the FI below which no patent flap was found. In contrast, below a threshold of 0.30 for the PI, there were still 5.9 percent (4/67) of patent flaps which were also below this threshold. As well, the Mann-Whitney-U test suggests a better separation of values for insufficient and uneventful flaps when using the FI ($p < 0.01$) as in comparison to the PI ($p < 0.05$).

3.7 Possible value of real-time on-line interpretation

3.7.1 Interpretation of perfusion situation

As described in Materials & Methods, 21 raters were shown a total of 210 sequences and then asked for their judgement. From the 168 patent flaps shown, 167 (99.4%) were evaluated regarding the clinical perfusion status by the participants (in one form the according box was not checked by the participant). All 168 (100%) ICG sequences were judged. 127 (76,1%) were considered to have sufficient ("adequate" and "good") perfusion in the white light images as opposed to 148 (88,1%) in the ICG angiography. Also, 6 (3,6%) of the patent flaps were judged to suffer from insufficient ("bad" and "poor") perfusion from clinical aspect whereas there were none in this category in ICG view.

All 42 sequences of the malperfused flaps were evaluated by the participants for both white light (100%) and ICGA (100%). From the 42 insufficiently perfused flaps, that had to undergo revision surgery, only 28 (66,7%) were assumed to have insufficient ("poor" or "bad") perfusion from the clinical impression alone, while the ICG angiography lead to a correct identification in 38 cases (90,5%). In neither white light nor ICG imaging were any compromised flaps considered to have "good" blood supply (*see Table 14 and Figure 20*).

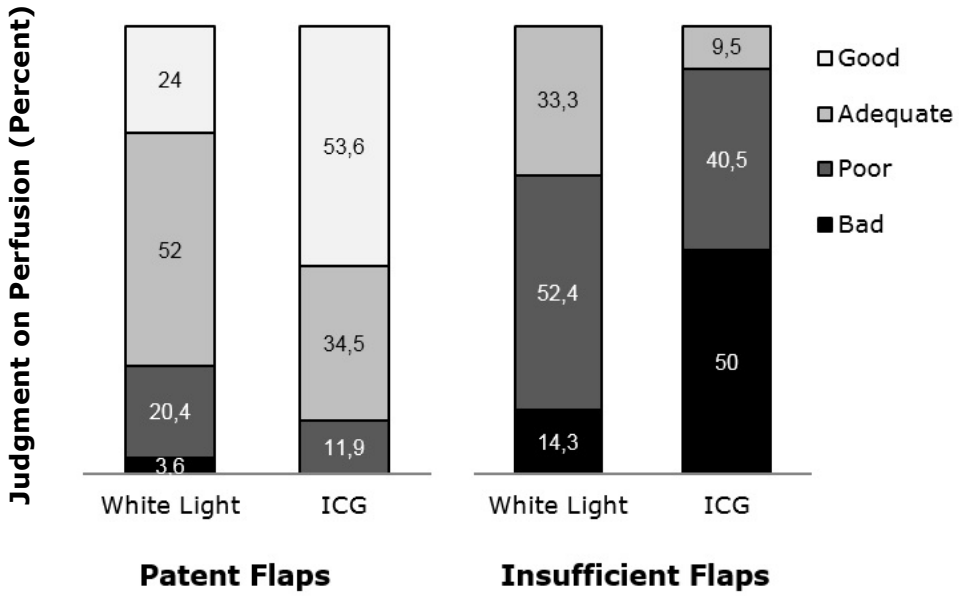

Figure 20: *Judgment on flap perfusion situation in four steps from 1 (bad) to 4 (good) for patent and insufficient flaps, based on both, white light and ICG imaging.*

Regarding the judgment of perfusion situation, the mere white light interpretation has a sensitivity of 76.0% and a specificity of 66.7%, when grouping "good" and "adequate" as sufficient perfusion against "poor" and "bad" as insufficient perfusion. ICG angiography has a sensitivity of 88.1% and a specificity of 90.5%.

Table 14: *Judgment on flap perfusion situation in four steps from 1 (bad) to 4 (good) for patent and insufficient flaps, based on both, white light (WL) and ICG imaging.*

	Patent Flap WL	**Patent Flap ICG**	**Insuff. Flap WL**	**Insuff Flap ICG**
Bad	6 (3.6%)	0 (0%)	6 (14.3%)	21 (50.0%)
Poor	34 (20.4%)	20 (11.9%)	22 (52.4%)	17 (40.5%)
Adequate	87 (52.0%)	58 (34.5%)	14 (33.3%)	4 (9.5%)
Good	40 (24.0%)	90 (53.6%)	0 (0%)	0 (0%)
Total	167 (100%)	168 (100%)	42 (100%)	42 (100%)

When applying a scoring system reaching from 1 for "bad" up to 4 for "good" there are differences in the mean scores between sufficiently and

insufficiently perfused flaps depending on whether they were seen under white light or in ICG-fluorescence (*see Table 15*).

Table 15: *Applying the scoring system to the judgments leads to the mean perfusion scores (1 point for "bad", 2 points for "poor" and so on). The point values per category (e.g. "good") were multiplied by how often they were given (n) for patent and insufficient flaps both under white light and ICG conditions.*

	Patent Flap WL	**Patent Flap ICG**	**Insuff. Flap WL**	**Insuff Flap ICG**
Bad x 1	n6 x 1 = 6	n0 x 1 = 0	n6 x 1 = 6	n21 x 1 = 21
Poor x 2	n34 x 2 = 68	n20 x 2 = 40	n22 x 2 = 44	N 17 x 2 = 34
Adequate x 3	n87 x 3 = 261	n58 x 3 = 174	n14 x 3 = 42	n4 x 3 =12
Good x 4	n40 x 4 = 160	n90 x 4 = 360	n0 x 4 = 0	n0 x 4 = 0
Total Score	495	574	92	67
Total n	167	168	42	42
Formula	495 / 167	574 / 168	92 / 42	67 / 42
Mean Score	2.96	3.42	2.20	1.60

In white light, the average score for patent flaps was 2.96 and for insufficient flaps it was 2.20. Accordingly, using ICG angiography, patent flaps had a higher score of 3.42 and insufficient flaps had a lower score of 1.60.

3.7.2 Interpretation of need of revision

Of the 210 sequences shown, 21 raters gave their opinion on need for intervention for 167 of the WL sequences (79.5%) and 199 of the ICG sequences (94.8%).

In WL, 47.1% (16/34) could be correctly identified and 12.0% (16/133) were wrongly indicated for revision. In contrast, ICG helped identify 74.4% (32/43) of insufficient flaps and only 4.5% (7/149) of patent flaps were assumed to be compromised.

4 Discussion

4.1 Usage of free flap transfer

Free flap transfer has become a standard procedure for aesthetic and functional treatment of soft tissue defects. A wide range of different flaps is available depending on size, localization and tissue type of the defect[17, 18, 20, 21, 45-49].

Disa[50] and Lutz[51] both suggest a simplifying approach to donor site selection, that shall lead to lower complication rates and reliable surgical results as the surgeons familiarize themselves more with the used flaps. In Disa's 13 years spanning study (1986-1999) at Memorial Sloan-Kettering Center (New York, NY, USA), 728 free flaps were performed in 698 patients. The center managed to perform 92% of all head and neck reconstructions using only four donor sites: the radial forearm, fibula, rectus abdominis and jejunum. In the oral cavity, the radial forearm flap was used in 75% of the cases and also in the midface or hypopharyngeal regions it was used on a regular basis (31-38%)[50]. While most centers still seem to favor the radial forearm flap for most oral reconstructions, Lutz reports good outcomes when using the anterolateral thigh flap as a primary means of reconstruction, but does not differentiate between regions in the presented study. During a 4 year period (1999-2003) 2587 flaps were used at Chang Gung Memorial Hospital (Taipei, Taiwan), 1315 of which were fALT flaps (50.8%), 425 forearm flaps (16.4%) and 407 osteoseptocutaneus Fibula flaps (15.7%). Using only these three flaps, 83% of all head and neck reconstructions could be performed[51].

During our study, the majority of our patients (20/25) were treated with a radial forearm flap, known to be the workhorse of free flap transfer in the oral cavity. In other patients, the anterolateral thigh flap (4/25) or fibula flap (1/25) were used.

4.2 Free flap survival and salvage

Survival rates for free flap transfers are reported to be as high as 95,0% - 98,8% by the most experienced centres worldwide and several studies with large numbers were published in the recent years[24, 25, 49, 52, 53]. Back in 1998, Hidalgo published results from a retrospective 10 years spanning study which included 716 consecutive free flaps over 10 years in 698 patients, 69% of which were located in the head and neck region. The overall flap survival rate was reported to be 98%[53]. In 2007, a study including a total of 1142 free tissue transfers was published by Chen. Again, most of the transfers were used in the head and neck region and the overall complete flap survival rate was 96.4 percent [25]. In Bui`s large study, also published in 2007, 1193 free flaps were examined - the largest subregion once more in the head and neck with 74 percent. The overall flap survival was reported even higher with 98.8%[24].

While the above studies report their survival rates for all regions and do not individually list rates for the head and neck regions, specific reports have looked into this region to see if there are differences or specific problems. Dassonville reviewed 213 free tissue transfers between 2000 and 2004. With 93.4% the overall success rate was reported a little lower than in the studies covering all body regions. Recipient site complications occurred in 20.9% of all patients, the most common of which were infection in 21 cases, associated with a strong correlation to salivary fistula formation. The second most common complication was neck hematoma requiring drainage in 16 cases[54]. Comparable results were presented by Haughey, who reported a success rate of 95 percent. In his series of 241 flaps in 236 patients, 10 total and 3 partial flap losses were reported[49]. Nakatsuka published a very impressive study including 2372 free flaps in 2301 patients during a period of 23 years, beginning in 1977. With 4.2% total and another 2.5% partial flap necrosis, the overall flap survival rate was 93.3%[55].

Another study focusing on free flaps in the head and neck by Suh examined 400 free tissue transfers in 288 patients by a single senior surgeon between

1995 and 2002. Of these, only 0.8% of total flap failures were reported, presenting one of the lowest failure rates in the world[56].

In our study, all flaps healed in well and no flap was lost, not even partial necrosis was observed. Given the rather small case number presented in this feasibility study we lie within the expected reach and with increasing numbers flap failures would most probably be observed. Also, in our study all procedures were performed by only one experienced surgeon.

When a flap is at risk of failing, immediate salvage surgery has to be performed. Flap re-exploration is a common procedure in free tissue transfer with rates between 4.8 to 16.0 percent within the first 7 days following surgery[24, 25, 49, 57, 58]. Brown et al. reports a comparably high surgical revision rate of 16%, but his data encompasses patients from 1992 to 1998 and is thus one of the oldest studies in this field[57]. When taking into account even larger and more recent studies, the overall re-exploration rate seems to be considerably lower. However, free flaps in the head and neck region seem to be slightly more prone to complications with need of exploration than other regions, according to Bui, and accounted for the highest revision surgery rate (6.6% compared to 6.0% total)[24]. Wu presented a study that ran over 20 years and included 1918 patients with a total of 2019 transplanted free flaps exclusively in the head and neck region and showed an even higher revision rate of 9.9%[59].

The rates of successful salvage surgery vary greatly between studies and range from 32% to 73%[53, 57, 59-62]. In up to 15% of cases, however, no pathologic findings concerning the flap perfusion are found during revision surgery according to Bui et al.[24]. Because the patient is then exposed to the risks of surgery and the associated anaesthesia unnecessarily, the rate of negative explorations should be minimized.

A total of 6 revisions in five patients were performed in course of our study. Two of them, however, were performed directly during the initial surgery before the patient left the OR. So with three out of 25 patients to undergo revision surgery (12%) – one of them twice – within the first postoperative

week our rate seems to be comparable to current literature[59]. On the other hand, all revised flaps eventually healed in without any partial or total flap loss resulting in a salvage rate of 100 percent. There were 33% percent (2/6) of negative re-explorations, which is rather high in comparison to current literature.

Failures of free transplants are almost exclusively caused by an impeded blood supply. Generally in free flap transfer, venous complications occur way more often (54-93%) than arterial thrombosis (7-23%)[53, 57, 61, 62]. All other possible reasons like mechanical stress, hematoma, infection or fistula formation only play a secondary role and usually present themselves later and can themselves induce thrombosis, as has been shown by Hyodo[60].

In the head and neck, venous and arterial causes for reexploration attribute for approximately the same percentage (38% venous, 35% arterial), followed by bleeding (14%) and hematoma (13%) [59].

When arterial thrombosis is present, flap survival rates are generally worse than in flaps which suffer from venous thrombosis. This is mainly because salvage rates vary between both groups. While total salvage of flaps with venous congestion can be achieved in 54-69%, the outcome is much more unfavorable when arterial malperfusion is present and only 15-51% rescued [55, 58, 60]. Again, Wu presents precise data for the head and neck region and shows that for venous congestion a salvage rate of 74% can be achieved while only 50.7% of arterially compromised flaps could be successfully salvaged[59].

Both intraoperative revisions in our study showed arterial problems and lead to the arterial anastomoses being revised. Of the four patients to undergo true revision surgery, only two (50%) showed pathologic findings - hematoma in one case and venous pedicle thrombosis in the other. Our data may imply a rather high rate of arterial problems (2/4 or 50%) compared to venous impairment (1/4 or 25%), in contrast to literature. A possible explanation might be our inclusion of intraoperative findings from the initial procedure, before the patient even left the OR, which no other study did. If

only the postoperative period is to be looked at in accordance to literature, there were no arterial complications during our study. A reason for low venous congestion rates might be found in our routine use of venous mechanical anastomotic coupler devices (vMACDs), which were used in each patient. Various studies showed significantly lower venous thrombosis rates when using vMACDs in comparison to using conventional hand sewn anastomoses. In reconstructive breast surgery, the usage of vMACDs decreased the venous thrombosis rate from 3-10 percent to as low as 0.6 percent [63]. Similar results have been presented in the head and neck region [64, 65].

Time of presentation of complications is a critical factor which largely influences the postoperative monitoring regime. The vast majority of all free flap complications presents itself within the first 24 hours postoperatively. Up to 82.3 percent can be identified within the first day, another 8.8 percent during the next and finally 4.4 percent on the third day. After the third postoperative day, complications are much less likely to occur [25]. Since up to 95.5 percent of all flap failures develop during the first 72 postoperative hours, this period is the most important and frequent monitoring is crucial[31, 61].

In our study, all patients who showed signs of impending flap failure presented these within the first 24 postoperative hours. Immediate revision during the initial surgery was performed in two patients (LMU 6 and LMU 23) and both showed arterial problems that could be solved by renewal of the arterial anastomosis. In the later revision surgeries hematoma and venous congestion were found in two patients (LMU 13 and LMU 19), but in two other patients no definitive intraoperative pathology could be determined and the perfusion of the anastomoses was normal (LMU 23 and LMU 25).

In 1996 Kroll reported, that salvage after the third postoperative day is unlikely as all of the revised flaps were unsalvageable irrespective of the cause of thrombosis[61]. In analogy, Yu showed a significant difference in successful salvage rates between flaps revised within the first 72

postoperative hours (61%) and after (13%)[66]. In the following years, other studies showed success in flap salvage even later, although the probability is much lower.

When salvage surgery is performed, the outcome depends on how much time lies between detection of impending flap failure and begin of the salvage surgery – it was shown that salvaged flaps were revised significantly earlier than failed flaps[24].

After revision surgery, all six flaps healed in well and the further hospital stay of the respective patients was uneventful, irrespective of cause of failure or timing of operative revision. All patients in our study were revised within the first 24 postoperative hours immediately after signs of impending flap failure were present. No signs of impairment were observed after this early postoperative period.

4.3 Postoperative monitoring techniques

To date, after over 50 years of free flap transfer, various methods of postoperative monitoring have been described and tested. Gold standard today is still clinical judgment of an experienced microsurgeon as it is still the most reliable method of monitoring. This includes checking for changes in appearance, color, texture, temperature and capillary refill. Pinprick bleeding can provide further information and help determining, whether an arterial or a venous problem is more probable. The use of hand-held acoustic Doppler probes is often considered an adjunct of conventional monitoring. These checks should be performed regularly and frequently within the first 72 hours following surgery, although there are no guidelines and not all centers use this regime routinely.

In 2005, Jallali published results from a survey among plastic surgery units in the UK that showed that only 28 percent of all units perform regular clinical monitoring for more than 72 hours postoperatively. The majority cover between 48 and 72 hours and only 5% of all units refrain from routine clinical assessment beyond the first postoperative day. Frequency of checks

also varied between units, but the most frequent regimen was hourly for the first 24 hours, 1-2 hourly for the following 24 hours and every 4 hours after the first 48 hours[31].

The efficacy of clinical monitoring (including the use of hand-held Doppler probes) was shown by Disa in 1999, when vascular compromise in non-buried flaps was usually identified within the first 48 hours postoperatively and lead to flap salvage in 77 percent and overall success rate of 98.2 percent. These non-buried flaps were monitored by clinical observation, Doppler examinations and pinprick testing. In contrast, buried flaps showed significantly worse outcome with overall success of only 93.5 percent due to very late (generally > 7 days) and therefore unsalvageable complications. These complications may develop much earlier, but are very hard to be detected. The author assumes the reasons in limited monitoring possibilities in these flaps, which could only be assessed by Doppler examinations[67].

As mentioned before, our unit performs these clinical and Doppler probe checks hourly for the first 72 hours, then every 2 hours on day 4 and 5 and then once daily (clinical checks only) within the first 2 weeks postoperatively. To overcome restriction in monitoring of buried flaps, we routinely externalized a part of the flap as a so coalled “monitor” to allow for direct and easy inspection - as was also recommended by Disa in his aforementioned publication[67]. Other studies also showed how using an externalized distal skin paddle can help in identifying impending vascular compromise earlier in buried flaps and raise the salvage rate to 67%[28]. The method has been put forward by Byun[68] and Iwasawa[27] in the 1990s for free forearm flap transfer. However, the described method went along with increased risk of fistula formation due to an additional suture and a number of false positive readings due to microvascular compromise of the distal island flap. Nonetheless, it provides an important additional tool for the evaluation of buried flaps, which would otherwise not be routinely examinable without the use of endoscopes [29].

To supplement clinical judgment, a number of methods have been put forward in the recent decades. The most commonly used is the hand-held acoustic Doppler probe that can reliably identify arterial and venous flow signals through intact soft tissue. It is based on the Doppler principle and can use the frequency shift of moving objects or fluids like blood and make the flow audible. The abundance of vessels in the head and neck region can complicate the identification of the pedicle vessels. Marked postoperative swelling, hematoma or emphysema may drastically reduce the value of the examination, as a regular flow might be present but undetectable due to physical limitations or displacement. The initial investment in the reusable hand-held Doppler probe is only several hundred dollars per device and they can be obtained from multiple manufacturers.

Apparent restrictions of the method lead to the emergence of several additional monitoring techniques [(29)].

An improved and slightly more advanced method is the use of Colour Doppler Imaging (CDI) with its widespread use throughout many medical fields from angiology, cardiology to different surgical specialties. Apart from flow, it can also determine the relative direction of the flow in relation to the probe. To achieve best results, an experienced examiner is required[(69)]. A further variation is the so-called Power Doppler Imaging (PDI) mode, which is even more sensitive to low flow velocities and can even show tissue vascularity, but yields no information about the direction of blood flow. Use of PDI for free flap monitoring in the head and neck has been demonstrated and is considered to be a welcome adjunct to CDI[(70)]. Recently, contrast agent based assessments like Contrast Harmonic Ultrasound Imaging (CHI) or Contrast Enhanced Ultrasound Imaging (CEUS) have been introduced as further refinements of previous systems and seem to detect even minimal flows and are considered to provide valuable additional information on flap perfusion to a depth of 3cm maximum[(71, 72)].

These methods all suffer from the same problems: neighbouring vascular structures could be mistaken for the pedicle vessels – a problem which can

be worsened due to postoperative swelling and bleeding and a possible displacement of the pedicle. Technical improvements like CHI or CEUS, however, might be able to identify the anastomosis by using sonographic contrast agents (e.g. Sonovue™) and thus overcome this problem[71]. Another problem that could be encountered with the above mentioned systems is based on the fact that in the early stage of venous congestion, the arterial flow may still be normal while microcirculation is already impaired. There is a huge array of colour duplex systems available by many different manufacturers (e.g. Siemens, GE, Toshiba) and - depending on the demanded specifications - they cost between $30,000 and $225,000[73]. Nonetheless, most units will employ at least one capable colour duplex ultrasound and most probably no new devices have to be purchased for mere use in flap monitoring.

Laser-Doppler flowmetry has been introduced in the 1980s and allows for measurement of blood flow velocity using the Doppler principle in conjunction with light (not sound)[74]. Tissue is illuminated with a coherent laser light of 820nm wavelength and a probe then collects the backscattered light and its frequency shift, which can be used to calculate the velocity of blood flow. It was shown, that Laser-Doppler flowmetry can detect vascular problems prior to clinical assessment and seem to be able to safely identify vascular compromise. The probe itself is rather flat like a spatula and use in the head and neck region has been proved feasible for non-buried flaps[75]. Although the probe is capable of delivering continuous online measurements, it is still only applied at specific intervals and not a continuous monitoring by definition. Continuous monitoring can be achieved though, by sewing the probe temporarily to the flap and due to marked differences in flow alteration, venous and arterial compromise can often be differentiated. While a marked and rapid or almost instant decrease in flow velocity is characteristic for arterial compromise, venous occlusion shows a continuous and significantly slower decrease [76, 77]. Prices begin at $5460 for the monitoring box and probes range upwards of $1015, but can be reused in least ten cases or for six months before they need to be replaced[73].

All of the presented devices are for use on the flap surface only and usually do not allow for continuous monitoring in a regular clinical setting. In 1988, Swartz presented his results from an experimental and clinical study in which he used an implantable ultrasound Doppler monitoring device with a 20MHz wavelength. Initially, the probe was attached to the pedicle artery distally of the anastomosis and allowed for precise and immediate detection of reduced arterial flow due to occlusion. There were a few cases (2/63) that underwent unnecessary revision due to dislocation of the probe and one malfunction (1/63) was reported, which could be resolved prior to re-exploration. The device failed to detect venous occlusion rapidly, although specific changes could be identified in retrospective[78]. The method has been further refined by attaching the probe to the vein instead of the artery and thus lead to a markedly improved detection of flap failure (16/16) and a high salvage rate of 75% (12/16) in one study. As well, there were no more negative re-explorations based on the misinterpretation of Doppler signals. Another advantage is the possible continuous use for monitoring with a very quick response time to changes[79]. The method has since then been subject of many studies which proved its worth in postoperative monitoring[80-82].

In 2009, Rozen et al. published their results of a study, where clinical monitoring was compared to continuous implantable Doppler probe monitoring alone in regard to overall flap survival and salvage rates. However, no significant differences between those two methods could be found, placing the method next to clinical judgment of an experienced surgeon, the actual gold standard[83]. The disadvantages on the other hand are also obvious: the probe itself can be detached from the pedicle and thus fail to measure correctly and finally lead to an unnecessary surgical revision. Since it is attached to the vessels, it is basically a pedicle monitoring. Changes to mircovascular flap perfusion might be present and lead to partial necrosis while blood flow in the vessels is normal and might thus be overlooked. Of-the-shelf systems (e.g. by Cook Medical, Bloomington, IN, USA) are available. The method itself is invasive and therefore might expose the patient to unnecessary risks, but no major complications with the used

devices have been reported yet, so that it can be considered a safe monitoring method. The costs amount to $3100 for the monitoring box and $412 for each disposable single-use probe[73].

Since 2010 a novel device - the GEM Flow Coupler was introduced (Synovis Micro Companies Alliance, Inc., Birmingham, Alabama, USA). It combines a regular venous mechanical coupling device with an implantable Doppler probe and makes monitoring the venous anastomosis easier. Clinical studies have shown similar results when compared to the conventional Cook-Swartz Doppler[84, 85]. A flow coupler monitor costs 2790 €, each single flow coupler unit 795 €, whereas a regular single coupler (without the implantable Doppler probe) costs only 210 €. Although this might seem expensive at first, the higher price for the combined device might even reduce costs of the entire procedure by saving OR time, since the correct placement of the Doppler probe at the anastomosis as a separate and usually difficult as well as time-consuming step is omitted[84].

In contrast to these pedicle-focused methods stand those, that are meant to register changes in microcirculation of the entire flap and not only within the larger vessels.

Measuring tissue temperature, infrared thermography has been successfully applied and may be a useful, noninvasive method after free flap transfer to the outer body surface[86, 87]. Thereby, the surface temperature has been shown to correlate with the tissue microcirculation. In order to work properly, a temperature difference to a reference tissue is needed. The method thereby may even be able to differentiate between arterial and venous compromise [88]. It probably loses its relevance in the UADT though, where the temperature of the flap always approximates the body temperature independently of its perfusion, although there are no studies on that subject[82]. The controversy about the method's value is still ongoing, since it reportedly failed to detect flap compromise in some reported cases[89].

Another method is perfusion weighted MRI , that can identify changes in tissue perfusion even in deeper layers of the flap or completely buried flaps. It encompasses the entire engrafted tissue and not only its surface, and allows precise prediction of flap viability. Obviously, an MRI tomograph is required, the patient has to be mobile or be moved – which can be cumbersome and even dangerous in some instances - and is time and cost intensive as it takes up to 30 minutes and an average MRI tomograph costs around one million dollars and subsequently the single evaluation can cost thousands of dollars[90].

Metabolic monitoring can be achieved by microdialysis when biochemistry of the flap is examined. A catheter is positioned during surgery and later a physiologic fluid is irrigated that equilibrates with the interstitial fluid around it. This fluid is then analysed for marked changes like falling glucose concentration or rising lactate to pyruvate ratio that can be indicative of arterial malperfusion. Venous occlusion is identified by increasing glycerol levels. In a laboratory setting, the method was shown to be working and has already been adapted for use in patients, especially with intraoral reconstruction[91]. Unfortunately, the analysis of the fluids takes up to 30 minutes of time, though, and for maximum flap survival, time is essential[82]. The costs consist of a high initial investment of almost $52,000 for the monitoring and analysing unit alone. Per each flap, additional costs for catheters, reagents and consumables sum up to around $570.

Oxygen-saturation of the flap tissue can be measured by tissue spectrophotometry or spectroscopy using the absorption of white light by oxygen-saturated haemoglobin and its “colour”. It determines the average oxygen saturation and haemoglobin concentration in a given examined volume of tissue. The method is non-invasive and the probe can be used covered in a sterile cover sheath, if necessary. Rising haemoglobin levels in the flap are indicative of venous occlusion, and falling levels of oxygen-saturation can mean arterial compromise[92]. The method lends itself to combination with simultaneous laser flowmetry and then allows for a precise

assessment of flap perfusion[75, 93, 94]. According monitoring devices are commercially available, e.g. the O2C system (Oxygen-to-see, LEA Medizintechnik GmbH, Gießen, Germany). A monitoring box usually costs around $16,500 and an additional $150 are needed for each disposable sensor.

Another method for flap monitoring are fluorescence angiographies following application of contrast agents such as Fluorescein or ICG.

None of these advanced and objective methods has reached wide-spread acceptance so far, however. While frequent clinical checks and simple Doppler examinations are performed by approximately 80% of all surgeons, less than 10% use advanced monitoring methods like the implantable Doppler probe[23].

Previous studies showed that fluorescence angiographies correlate well with tissue perfusion and are able to precisely predict necrosis in free and pedicled flaps. In 1977 the first experiences with Fluorescein-based tissue angiography in arterialized flaps were published and it was shown that it was able to accurately predict necrosis within a distance of 3-5 mm[95]. Encouraged by this study on 285 pedicled flaps, the method attracted lots of attention in the 1980s and early 90s[96], but was subsequently abandoned for flap monitoring because of the severity of possible side effects[97] as well as unfavourable pharmacokinetic properties of the dye and the possibility of erroneous interpretation of marginal fluorescence. Today, there are still plenty of other indications for Fluorescein use in other medical fields like ophthalmology [32, 33].

Indocyanine Green (ICG), another fluorescent dye, has been in clinical use in different fields and for various indications since the 1960s[35] (e.g. assessing cardiac or hepatic function as well as visualization of choroidal vasculature) until now and is internationally approved. ICGs pharmacokinetic properties are more favourable for the purpose of flap monitoring than Fluorescein's as it has a high affinity to plasma proteins and thus stays strictly intravascular. ICG is quickly degraded in the liver with a half-life of 3-4 minutes and is

then eliminated through the biliary system and not subject to enterohepatic recirculation. This makes it ideal for repeated administration, as it is almost completely eliminated from the blood after 20 minutes[43]. So far, there have been no reports of severe adverse effects, although anaphylactoid or anaphylactic reactions are possible (with an incidence of approximately 1:40000[44, 98]) as well as reaction to the iodide in the dye. The use of ICG is considered to be very safe and only few contraindications exist[99].

In 1995, Indocyanine Green was first successfully applied in an experimental rat model with axial flaps and showed high resolution images of superficial tissue perfusion[41]. After the potential for flap monitoring was seen, further experimental studies were performed and using computer-aided calculations predicting flap necrosis was possible for the first time[42]. Clinical trials confirmed the ability of the method to predict flap necrosis and even superficial epidermiolysis on the flap surface[100].

An example of a no longer commercially available device for surface ICG fluorescence angiography is the IC-View® System, which offered an integrated solution with near-infrared excitation light and a filtered video recording system (Pulsion Medical Systems, Munich, Germany). The costs depend on the needed hardware, but usually an infrared sensitive camera with appropriate optical filters and a near-infrared light source should suffice and cost no more than a few thousand dollars in a non-endoscopic setting. If use with an endoscopic unit is desired (for example for observing reconstructed areas within the upper aerodigestive tract), according to Karl Storz GmbH (Tuttlingen, Germany), a complete initial setup consisting of light source, NIR optimized camera head and controller, endoscopes with build in filters and video recording system would cost around 30,000€. Of course this equipment can be used for conventional endoscopic examinations or surgery as well, and would thus alleviate the initial investement. Also, existing gear like HD camera heads which already are NIR capable can be further used. At present (December 2015) there are several ICG-capable cameras available from Karl Storz (Tuttlingen, Germany), e.g. the TRICAM PDD-3-Chip Camera (20221037), the Image1 H3-Z FI 3 Chip-ICG-FULL-HD-

Camera (22220085-3) and the Image1 H3-Z FI SPIES 3-Chip-FULL-HD-Camera (TH 102). They are used in combination with a D-Light P SCB light source (20133701-1) and a TRICAM control unit or the IMAGE 1 HUB HD SCB camera controller (22201011-102), respectively. There are two endoscopes with build-in filters available – the HOPKINS 0° (26003 ACA) and HOPKINS 30° (26003 BCA). Both are 10mm in diameter and the former offers a straight forward view, while the latter provides a 30° angle.

A single dose of ICG runs at approximately 50€.

4.4 ICG Imaging for assessing patency of anastomoses

Holm et al published two papers, that showed how a modified operational microscope with ICG capability could lead to better results regarding overall salvage and failure rates. Shortly after anastomosis the vessels were examined by ICG angiography through the microscope that provided the surgeon with a real time assessment of patency of the arterial and venous branch of the flap. Integration of the needed filters into the optical path of surgical microscopes is meanwhile possible and commercially available, and has been shown to be useful and time-saving, as directly after anastomosis a test of patency is possible without removing the microscope[101, 102].

When pathologic findings (11/50 cases) lead to immediate intraoperative revision (n=3), flap survival was 100%[101].

As an additional instrument for determining need of re-exploration and probable cause of flap failure, Holm described the intrinsic transit time (ITT) of the flap – the time needed for the ICG fluorescence to travel from the arterial to the venous anastomosis – as a predictive factor towards postoperative complications. Patients with an uneventful postoperative course showed significantly lower ITTs (median: 31sec) compared to patients with flap loss or early revision (median >120sec)[103]. Holm suggested the method could be valuable in the re-exploration situation to assess patency of the pedicle and the anastomoses and possibly avoid unnecessary manipulation of an intact vessel. The intraoperative findings

could be compared to the video material obtained in the initial surgery, especially, when the revising surgeon was not present at the first procedure [40, 104]. Studies have proven the value of the method and its very high sensitivity (100%) and specificity (86%) when applied to the anastomosed pedicle vessels [104].

The method is not a monitoring technique, but a valuable tool to evaluate patency of the anastomoses intraoperatively before closing the neck or in a revision situation. It can therefore not be compared to the presented ICGA which does not assess pedicle patency, but surface perfusion of the flap.

4.5 Study Summary

In the presented study, the use of the dye and endoscopic devices was easy and non-problematic. Preparation time including dilution of ICG in aqua and assembly and setup of the endoscopic equipment took about 5 minutes. The period of actual measurement was about 2 minutes in most cases, including WL imaging prior to and after the proper ICG angiography. In patients that were still under general anaesthesia in the OR and when a stand for the endoscope was used, the recorded sequence was up to 5 minutes long. Sedated and fully awake patients alike tolerated the examinations very well and there were no adverse reactions or injuries. After that, it took about 5 minutes to save and record the data and disassemble the equipment for transportation and storage. Overall, a single measurement could be performed in less than 15 minutes.

Since this study was only intended to prove the feasibility of an endoscopic ICG-perfusion measurement, only 25 patients were to be included. To our knowledge, this is the first study using endoscopic ICG angiography in the head and neck region. In the course of the study, 78 ICG angiographies - including six additional measurements after revision in five cases - were performed. The majority of all patients could be examined according to the study protocol without any problems (24/25). Unfortunately, in one case, LMU 05, the flap could not be completely seen endoscopically in the follow-

up period due to massive postoperative swelling without risking to inflict damage to the transplant. Since this shows an obvious limitation of the method, the patient was not excluded from the study. Probably, the use of an angled or flexible endoscope instead of the forward looking one would have been beneficial in this case, but was not available in the study setting. As mentioned before, there are two 10mm endoscopes available at Karl Storz - the HOPKINS 0° (26003 ACA) and HOPKINS 30° endoscope (26003 BCA).

It is already also possible to use conventional endoscopes with a snap-in ICG-Filter, though. The drawback of this method is, that a prior identification of the flap in white light would have to be done without the filter since it blocks most of the ambient light. Through insertion of the filter, displacement is probable, that would thus lead to an erroneous depiction and examination of the flap. Karl Storz GmbH has meanwhile introduced new endoscopes with integrated changeable filters, which allow for instantaneous switching between white light and fluorescence imaging and are even easier to use.

A problem we encountered in the first patient is that with the existing IC Calc software, a reliable measurement is not possible, if there is a lot of movement of the endoscope relative to the ROIs. This issue could be overcome by implementing a tracking software, which is able to follow defined ROIs. Outside of the study our department performed experimental testing that enabled simultaneous white light and ICG fluorescence imaging using a single standard 3-chip camera head. Reflected blue light was filtered from the camera and later digitally reconstructed to recreate a "pseudo white light" image with only little impact on colour perception. Fluorescence could be displayed in a second window at the same time and changed to false colours, if desired. Implementing a motion tracking system proved helpful and allowed for more precise calculations[105].

For further advancement of the presented ICGA method, new evaluation software has to be designed, since IC Calc in its current form is no longer

supported by the manufacturer Pulsion Medical Systems and development of a new analysis program is not in planning according to representatives of the company.

The method of endoscopic ICG angiography is simple and easy to learn, since the used Karl Storz system is only a modified version of the normal endoscopic equipment and the additional functions are seamlessly integrated in the existing gear. OR personnel could be quickly trained to safely and correctly use the ICG angiography equipment, so that it could be made available even at nights in emergency situations.

In total, six surgical revisions were performed. In two of those, the patients were only examined AFTER re-exploration, as time was pressing and none of the study examiners were present at the time. These two cases are therefore not helpful for an early evaluation of the value of the method presented. Nevertheless, those two flaps then postoperatively showed "normal" ICGA behaviour after revision and healed in well. In a further two cases (LMU 6 and LMU 23), ICGA was performed prior to surgical revision due to a clinical indication, and the malperfused arterial anastomoses were successfully revised. In these cases, the on-line angiography showed that ICG uptake was significantly delayed and maximum fluorescence intensity remarkably lower than it was in prior measurements of patent flaps. In the last two cases that underwent revision surgery due to a retrospectively false clinical indication (LMU 23 (second revision) and LMU 25), ICGA was also performed before revision surgery and had shown subjectively "good" perfusion. Especially in LMU 25, an extremely obese patient with 130 kg and additional cutaneous emphysema, where clinical signs of malperfusion were equivocal and conventional monitoring with a hand-held Doppler probe could not be performed, the ICGA could have made the additional surgical procedure unnecessary retrospectively. In conclusion, a total of two unnecessary operations could have been avoided if the results from the ICGA measurements would have had an influence on the indication of a surgical revision (outside of this study protocol).

The off-line evaluation of the data objectified the subjective impressions: the fluorescence index (FI; relative maximum of fluorescence intensities) was 0.33 and 0.37 for the flaps with impaired perfusion and 0.84 (0.73 – 0.93) for the revised flaps without pathologic findings and intervention. All other flaps showed an FI of 0.83 (0.60 – 0.96) directly after anastomosis and 0.88 (0.68 – 1.00) 72 hours postoperatively. Since it can be assumed that ICG angiography corresponds with perfusion, as it correlates well with cardiovascular factors also in our study, the growing fluorescence index over time is most probably a sign of improving blood supply of the flap that comes from either consolidation of the anastomosis and improved pedicle patency, or the improvement of microperfusion over time. These results backup previous animal and clinical studies which presented a FI of 0.10 (0.06-0.42) for complicated postoperative courses in patients and 0.87 (0.07-2.26) for regular uneventful courses[42, 106]. Since the study design was slightly different and we experienced no partial or complete flap losses, these values might not be fully comparable as there was no further information on how the regions of interest were defined by the interrogator, but add weight to our results. In contrast to these other studies, there was no overlap between the groups "revision needed" and any other. In order to define a threshold, we would like to suggest an FI of 0.50 as a value below which a revision should be strongly recommended.

At the same time, the Perfusion index (relative gain of fluorescence over time) is also dependent on time after surgery and rises in a similar manner. But other than the fluorescence index, the perfusion index showed massive inter- and intrapatient variability. The PI was shown to scatter extremely depending on selection of the ROIs and is less reproducible within the same patient. When revision was necessary, the PI was 0.20 or 0.29; when the postoperative course was uneventful, it ranged from 0.21 to 1.06 following anastomosis. In our study, it overlaps in the area from 0.21 – 0.29 and does not as sharply discriminate between insufficient and patent transplants as in other studies[42, 100, 106, 107]. A threshold of 0.25 was suggested to predict necrosis – a value that does not stand in accordance with our experiences,

since we found only one insufficient (50%, 1/2) and two of the patent (9%, 2/22) flaps in the range from 0.20 – 0.25. We therefore favour the fluorescence index (FI) as the better marker, as there is a stronger difference (0.35 vs 0.86) and no overlapping, and it can be as easily determined.

To compare different monitoring devices, Ihbler just recently presented two new tool for evaluation: the revision success rate (RSR = salvaged flaps / revised flaps) and the flap failure reduction rate (FFRR = (lost flaps without monitor / n) – (lost flaps with monitor / n). He then used both tools to test the efficiency of the implantable Doppler probe to find an RSR between 50% and 92.3% in the five large studies on the subject. The FFRR ranged from 5.5% to 11.6%[108]. When we apply these new tools to our admittedly small study collective, our results compare favourably with a RSR of 100% and a FFRR of 8.0%.

A survey in our department, including ICGA videos and white light images of the flaps, showed the possible use of the method in clinical routine. When judgment is merely based on visible clinical aspect without other factors (e.g. pinprick bleeding), only 66.7% of poorly perfused flaps were indicated for re-exploration correctly, as opposed to 90.5% in ICG angiography. This is an improvement of 35.7 percent that is merely attributed to a change of method and could greatly improve overall flap results. Also, based on clinical aspect, 24.0% of the well perfused flaps were wrongly considered to have poor or bad perfusion, while ICG only lead to 11.9%. When assuming that flaps with a possibly impaired perfusion had to be immediately revised, ICG screening prior to surgery could possibly reduce the rate of unnecessary procedures by up to 50%. It is notable, that patent flaps were judged remarkably better (3.42 vs. 2.96) and compromised ones remarkably worse (1.60 vs. 2.20) in ICGA than in WL. This leads to a better separation of the groups, as under WL the subgroups lay less than one step from each other whereas in ICGA it is almost two steps. Of course, 21 raters are a very low

number and this may not be generalized, but still a trend may be recognized here, especially since the qualification of the participants varied greatly.

By using the ICG angiography in an endoscopic setting, even hidden and deep flaps otherwise inaccessible for conventional monitoring could be safely evaluated in a time and money efficient way. Of course, buried flaps could not be examined since – by definition – there is no surface to be monitored.

The method has shown itself to be a potentially valuable additional evaluation tool if ambiguous signs of malperfusion are present. Possibly the highest potential can be seen in performing preoperative ICG angiography before revision surgery, when clinical and advanced monitoring are equivocal. If ICG angiography shows good perfusion (e.g. FI>50%), exploration could possibly be postponed and the rate of negative explorations with its possible harm for the patient could be reduced. Obviously, this statement needs verification in a much larger clinical trial.

5 Summary

The presented study is the first to examine the possible value of an endoscopic fluorescence perfusion measurement after free flap transfer in the upper aero-digestive tract using the fluorescent dye Indocyanine Green (ICG).

25 patients who had free flap transfer were included in the study after obtaining informed consent. Each patient was to be examined three times: first shortly after anastomosis in the OR (0h), second after 24 hours and third after a total of 72 hours.

The examinations themselves took approximately 2 minutes each. Total time including assembly and later disassembly of the endoscopic gear and dissolving and application of the dye was about 15 minutes. It could be performed by one single examiner. The costs of one dose of ICG run about 50 Euros.

In total, six surgical revisions were performed. In two of those cases, clinical signs of impaired perfusion were present directly after anastomosis and closure of the neck access but still in the OR, and the ICG angiographies (ICGA) showed unusually low uptake of fluorescence in the transplanted flap. Both had a revision immediately in the OR and both cases had arterial problems. After these were resolved, the repeated ICG examination then showed greatly improved uptake of the dye in comparison to the earlier ICGA. Two further cases showed clinical signs of flap failure during the later postoperative stay and were indicated for urgent revision surgery. The immediately preoperatively taken ICG angiographies did not show any impairment of flap perfusion. In both cases there were no intraoperative pathologic findings that lead to any revision of the anastomoses. Due to an urgent indication for revision in the last two revision cases, no preoperative ICGA could be performed. One suffered from hematoma and the other from venous congestion, both of which had to be revised. The postoperative ICGAs then showed normal findings. All the other patients showed uncomplicated courses.

Video sequences of all examinations were recorded and stored for later offline evaluation using a dedicated software. There, regions of interest (ROI) had to be determined for the flap, the surrounding tissue and a prefabricated fluorescence reference platelet. Processing of the data lead to introduction of two derived parameters of flap perfusion. First, the Fluorescence Index (FI) which directly compared absolute maximum fluorescence intensities of the flap to the surrounding tissue. Second the Perfusion Index (PI), which compares the gain of fluorescence over time.

The FI appeared to be more reliable in detecting impeding flap failure and had better reproducibility than the PI. Also, FI is easier to determine, since it is influenced by less variables.

Later off-line evaluation of the data suggests the use of the fluorescence index instead of the perfusion index. A recommended cut-off-value would be 0.50, below which revision surgery should be strongly recommended. Possibly, values above 0.50 might justify a wait-and-see attitude with increased frequency of repeated ICGAs as long as no unequivocal clinical signs of malperfusion are present.

In order to evaluate a possible value of on-line interpretation during the ICGA, a survey among 21 medical staff members with experience in postoperative free flap monitoring from nurses to attending head and neck surgeons was performed. White light and ICGA sequences of the same ten flaps were shown to them and they were asked for their judgement regarding patency of the flap and need of revision. There, vascularly compromised flaps were much more accurately identified in the ICG views than under white light conditions.

In this limited number of patients, ICGA had a high predictive value in detecting flap malperfusion and was easily and quickly performed. The intraoperative, real-time perfusion angiography provides the surgeon with additional information that could lead to important therapeutic consequences, once ICGA is accepted as an adjunct monitoring method.

In two cases, we demonstrated correct identification of malperfusion of flaps via ICGA. In two other cases where surgical re-exploration was performed but no vascular compromise evident, ICGA showed patency of the flap preoperatively, while clinical judgment was ambiguous at best. Possibly, operative re-exploration could have been avoided if the results from the ICGA would have been considered for clinical decision making.

So far, ICG angiography is just another adjuvant method to assess flap perfusion, but our experience shows a great reliability. With a wider range of endoscopes including flexible endoscopes and an advanced software that can track the ROIs relative to a reference's position and produce on-line values for FI of the measurement, this method could be further refined.

Even if ICGA should not be established as a routine postoperative monitoring method, it could be used as an additional source of information, when clinical signs or other conventional monitoring methods are equivocal. Reduced surface perfusion should endorse the decision for operative revision while a normal perfusion situation could also allow for a wait and see approach.

Further studies with increased patient numbers are needed, though, to prove the definitive value of endoscopic Indocyanine Green Angiography beyond anecdotal value.

6 Zusammenfassung

Die vorgestellte Studie untersucht zum ersten Mal den möglichen Stellenwert einer endoskopischen Fluoreszenz-Perfusions-Messung nach Transplantation eines freien Lappens im oberen Aerodigestivtrakt unter Verwendung des Fluoreszenzfarbstoffs Indocyanin-Grün (ICG).

25 Patienten, die eine freie Lappentransplantation bekamen, wurden in die Studie eingeschlossen. Zuvor wurden sie aufgeklärt und erklärten ihre Einverständnis zur Teilnahme. Jeder Patient sollte insgesamt dreimal untersucht werden: zunächst nach der Anastomosierung im OP (0h), dann 24 Stunden und schließlich 72 Stunden postoperativ.

Die Untersuchungen selbst dauerten jeweils etwa 2 Minuten. Die Gesamtzeit inklusive Aufbau und späterem Abbau der Endoskopieausrüstung sowie Herstellung der Injektionslösung betrug ca. 15 Minuten. Ein einzelner Untersucher konnte die Untersuchung alleine durchführen. Eine Einzeldosis ICG kostet etwa 50 Euro.

Insgesamt erfolgten sechs chirurgische Revisionen. Bei zwei dieser Fälle zeigten sich bereits unmittelbar nach Anastomosierung der Gefäße und nach Hautverschluss des zervikalen Zugangs noch im OP klinische Zeichen einer Minderdurchblutung und die ICG-Angiographien (ICGA) zeigten ungewöhnlich geringe Fluoreszenzzunahmen in den transplantierten Lappen. Beide Patienten wurden unmittelbar im OP revidiert und beide litten unter arteriellen Beeinträchtigungen. Nachdem diese revidiert worden waren, zeigten die wiederholten ICG Untersuchungen dann eine stark erhöhte Aufnahme des Farbstoffes im Vergleich zur Voruntersuchung.

Bei zwei anderen Fällen mit klinischen Zeichen des Lappenversagens im weiteren postoperativen Verlauf wurde die Indikation zur dringlichen Revision gestellt. Die unmittelbar präoperativ erfolgten ICG Angiographien zeigten keine Einschränkungen der Lappendurchblutung. In beiden Fällen zeigten sich intraoperativ keinerlei pathologischen Befunde, die eine Revision der Anastomose erfordert hätten.

Wegen dringlicher Indikation zur Revision konnten bei zwei weiteren Fällen keine präoperativen ICGAs durchgeführt werden. Ein Patient litt unter einem Hämatom, der andere an einer venösen Thrombose, welche operativ revidiert werden mussten. Die postoperativen ICGAs ergaben dann unauffällige Untersuchungsergebnisse. Alle anderen Patienten hatten unkomplizierte Verläufe.

Die Videosequenzen aller Untersuchungen wurden aufgezeichnet und für spätere Offline-Evaluation mittels spezieller Software aufbewahrt. Dazu wurden jeweils für den Lappen, das Umgebungsgewebe und ein vorgefertigtes Fluoreszenzstandard-Plättchen Regions of Interest (ROI) definiert. Die Weiterverarbeitung der Daten führte zu zwei abgeleiteten Parametern für die Lappendurchblutung: Erstens den Fluoreszenzindex (FI), welcher direkt die maximalen Intensitäten der Fluoreszenz im Lappen und Umgebungsgewebe miteinander vergleicht, und zweitens den Perfusionsindex (PI), der die Fluoreszenzzunahme der Gewebe abhängig von der Zeit nach der Injektion vergleicht.

Der FI stellte sich als zuverlässiger heraus, wenn es darum ging, ein Versagen des Lappens zu erkennen und war besser reproduzierbar als der PI. Darüber hinaus ist der FI leichter zu bestimmen, da er von weniger Variablen beeinflusst wird.

Die nachträgliche Offline-Untersuchung der Daten legt die Verwendung des Fluoreszenzindex anstelle des Perfusionsindex nahe. Eine Schwelle von 0,5 scheint empfehlenswert, unterhalb welcher ein Revisionseingriff dringend angezeigt scheint. Möglichweise rechtfertigen Werte oberhalb von 0,5 eine abwartende Haltung mit vermehrten wiederholten ICGAs im Verlauf, solange keine eindeutigen klinischen Zeichen von Minderdurchblutung vorliegen.

Um den möglichen Stellenwert einer sofortigen Live-Interpretation während der ICGA zu untersuchen, wurde eine Umfrage unter 21 medizinischen Mitarbeitern mit Erfahrung im postoperativen Monitoring von freien Lappen von Pflegekräften bis hin zu Oberärzten durchgeführt. Dazu wurden ihnen Weißlicht- und ICGA-Videosequenzen von denselben zehn Lappen gezeigt

und um eine Einschätzung bezüglich der Vitalität des Lappens und der Revisions-Notwendigkeit gebeten. Dabei wurden die Lappen mit Gefäßproblemen unter ICG-Sicht deutlich besser identifiziert als unter Weißlicht-Bedingungen.

Bei dieser limitierten Zahl an Patienten hatte die ICGA einen hohen prädiktiven Wert, wenn es darum ging eine Minderdurchblutung des Lappens zu erkennen, und ist einfach und schnell durchzuführen. Die intraoperative Echtzeit-Perfusions-Angiographie stellt dem Chirurgen zusätzliche Informationen zur Verfügung und könnte zu entscheidenden therapeutischen Konsequenzen führen, sobald die ICGA als ergänzende Monitoringmethode anerkannt ist.

Wir konnten in zwei Fällen zeigen, dass die Minderdurchblutung von freien Lappen mittels ICGA erkannt werden kann. In zwei weiteren Fällen, bei denen chirurgisch reexploriert wurde, ohne dass sich intraoperative eine Gefäßproblematik zeigte, zeigte die ICGA die Vitalität des Lappens, während die klinische Untersuchung diesbezüglich nicht eindeutig war. Möglicherweise wäre eine operative Reexploration zu vermeiden gewesen, wenn die Ergebnisse der ICGA in die klinische Entscheidungsfindung miteingeflossen wären.

Bisher ist die ICG Angiographie lediglich eine weitere ergänzende Methode um die Lappendurchblutung zu beurteilen, sie zeigt aber in unserer Erfahrung eine große Zuverlässigkeit. Die Methode könnte mit einer größeren Auswahl an Optiken einschließlich flexibler Endoskope und einer weiterentwickelten Software, die die ROIs selbstständig in Abhängigkeit vom ICG Standard verfolgen und Echtzeitwerte für den Fluoreszenz Index liefern kann, weiterentwickelt werden.

Selbst wenn die ICGA nicht als ein postoperatives Routinemonitoring etabliert wird, könnte sie als zusätzliches Kriterium dienen, wenn die klinische Einschätzung und andere übliche Monitoringmethoden sich widersprechen. Eine reduzierte Oberflächendurchblutung sollte die

Entscheidung zu revidieren forcieren, während eine normale Durchblutung auch ein abwartendes Vorgehen rechtfertigen könnte.

Weitere Studien mit größeren Patientenzahlen werden jedoch nötig sein, um den endgültigen Stellenwert der endoskopischen Indocyanin Grün Angiographie über einen rein anekdotischen Wert hinaus zu zeigen.

7 Reference List

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA: a cancer journal for clinicians 2005;55(2):74-108.

2. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA: a cancer journal for clinicians 2011;61(2):69-90.

3. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010;127(12):2893-917.

4. Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. Oral oncology 2009;45(4-5):309-16.

5. Ferlay J, Parkin DM, Steliarova-Foucher E. Estimates of cancer incidence and mortality in Europe in 2008. Eur J Cancer 2010;46(4):765-81.

6. Lewin F, Norell SE, Johansson H, et al. Smoking tobacco, oral snuff, and alcohol in the etiology of squamous cell carcinoma of the head and neck: a population-based case-referent study in Sweden. Cancer 1998;82(7):1367-75.

7. Wynder EL, Bross IJ, Feldman RM. A study of the etiological factors in cancer of the mouth. Cancer 1957;10(6):1300-23.

8. Ragin CC, Modugno F, Gollin SM. The epidemiology and risk factors of head and neck cancer: a focus on human papillomavirus. J Dent Res 2007;86(2):104-14.

9. Patel SG, Shah JP. TNM staging of cancers of the head and neck: striving for uniformity among diversity. CA: a cancer journal for clinicians 2005;55(4):242-58; quiz 261-2, 264.

10. Blair EA, Callender DL. Head and neck cancer. The problem. Clinics in plastic surgery 1994;21(1):1-7.

11. Kolokythas A. Long-Term Surgical Complications in the Oral Cancer Patient: a Comprehensive Review. Part I. J Oral Maxilloc Res 2010;1(3).

12. Kolokythas A. Long-Term Surgical Complications in the Oral Cancer Patient: a Comprehensive Review. Part II. J Oral Maxilloc Res 2010;1(3).

13. Schliephake H, Ruffert K, Schneller T. Prospective study of the quality of life of cancer patients after intraoral tumor surgery. Journal of oral and maxillofacial surgery : official journal of the American Association of Oral and Maxillofacial Surgeons 1996;54(6):664-9; discussion 669-70.

14. Tschiesner U, Cieza A, Rogers SN, et al. Developing core sets for patients with head and neck cancer based on the International Classification

of Functioning, Disability and Health (ICF). European archives of oto-rhino-laryngology : official journal of the European Federation of Oto-Rhino-Laryngological Societies (EUFOS) : affiliated with the German Society for Oto-Rhino-Laryngology - Head and Neck Surgery 2007;264(10):1215-22.

15. Tschiesner U, Rogers S, Dietz A, Yueh B, Cieza A. Development of ICF core sets for head and neck cancer. Head & neck 2010;32(2):210-20.

16. Chim H, Salgado CJ, Seselgyte R, Wei FC, Mardini S. Principles of head and neck reconstruction: an algorithm to guide flap selection. Seminars in plastic surgery 2010;24(2):148-54.

17. Vartanian JG, Carvalho AL, Carvalho SM, Mizobe L, Magrin J, Kowalski LP. Pectoralis major and other myofascial/myocutaneous flaps in head and neck cancer reconstruction: experience with 437 cases at a single institution. Head & neck 2004;26(12):1018-23.

18. Schneider DS, Wu V, Wax MK. Indications for pedicled pectoralis major flap in a free tissue transfer practice. Head & neck 2012;34(8):1106-10.

19. Seidenberg B, Rosenak SS, Hurwitt ES, Som ML. Immediate reconstruction of the cervical esophagus by a revascularized isolated jejunal segment. Annals of surgery 1959;149(2):162-71.

20. Smith RB, Sniezek JC, Weed DT, Wax MK. Utilization of free tissue transfer in head and neck surgery. Otolaryngology--head and neck surgery : official journal of American Academy of Otolaryngology-Head and Neck Surgery 2007;137(2):182-91.

21. Smith RB, Sniezek JC, Weed DT, Wax MK. Utilization of free tissue transfer in head and neck surgery. OtolaryngolHead Neck Surg 2007;137(2):182-191.

22. Chen HC, Mardini S, Yang CW. Voice reconstruction using the free ileocolon flap versus the pneumatic artificial larynx: a comparison of patients' preference and experience following laryngectomy. Journal of plastic, reconstructive & aesthetic surgery : JPRAS 2006;59(12):1269-75.

23. Spiegel JH, Polat JK. Microvascular flap reconstruction by otolaryngologists: prevalence, postoperative care, and monitoring techniques. The Laryngoscope 2007;117(3):485-490.

24. Bui DT, Cordeiro PG, Hu QY, Disa JJ, Pusic A, Mehrara BJ. Free flap reexploration: indications, treatment, and outcomes in 1193 free flaps. PlastReconstrSurg 2007;119(7):2092-2100.

25. Chen KT, Mardini S, Chuang DC, et al. Timing of presentation of the first signs of vascular compromise dictates the salvage outcome of free flap transfers. PlastReconstrSurg 2007;120(1):187-195.

26. Kim SC, Kim EK. Externalized monitoring of totally buried free flap in hypopharyngeal reconstruction. The Journal of craniofacial surgery 2013;24(2):575-8.

27. Iwasawa M, Furuta S, Hayasi M, Ohtsuka Y, Kushima H. Use of a monitor muscle flap in buried free forearm flap transfer. Annals of plastic surgery 1996;37(4):364-6.

28. Tan NC, Shih HS, Chen CC, Chen YC, Lin PY, Kuo YR. Distal skin paddle as a monitor for buried anterolateral thigh flap in pharyngoesophageal reconstruction. Oral oncology 2012;48(3):249-52.

29. Ferguson RE, Jr., Yu P. Techniques of monitoring buried fasciocutaneous free flaps. Plastic and reconstructive surgery 2009;123(2):525-32.

30. Abdel-Galil K, Mitchell D. Postoperative monitoring of microsurgical free-tissue transfers for head and neck reconstruction: a systematic review of current techniques--part II. Invasive techniques. BrJOral MaxillofacSurg 2009;47(6):438-442.

31. Jallali N, Ridha H, Butler PE. Postoperative monitoring of free flaps in UK plastic surgery units. Microsurgery 2005;25(6):469-472.

32. Garcia CR, Rivero ME, Bartsch DU, et al. Oral fluorescein angiography with the confocal scanning laser ophthalmoscope. Ophthalmology 1999;106(6):1114-8.

33. Flower RW, Hochheimer BF. Clinical infrared absorption angiography of the choroid. American journal of ophthalmology 1972;73(3):458-9.

34. Owens SL. Indocyanine green angiography. The British journal of ophthalmology 1996;80(3):263-6.

35. Caesar J, Shaldon S, Chiandussi L, Guevara L, Sherlock S. The use of indocyanine green in the measurement of hepatic blood flow and as a test of hepatic function. Clinical science 1961;21:43-57.

36. Still J, Law E, Dawson J, Bracci S, Island T, Holtz J. Evaluation of the circulation of reconstructive flaps using laser-induced fluorescence of indocyanine green. AnnPlastSurg 1999;42(3):266-274.

37. Liu DZ, Mathes DW, Zenn MR, Neligan PC. The application of indocyanine green fluorescence angiography in plastic surgery. Journal of reconstructive microsurgery 2011;27(6):355-64.

38. Raabe A, Nakaji P, Beck J, et al. Prospective evaluation of surgical microscope-integrated intraoperative near-infrared indocyanine green videoangiography during aneurysm surgery. JNeurosurg 2005;103(6):982-989.

39. Moon HS, Joo SP, Seo BR, Jang JW, Kim JH, Kim TS. Value of indocyanine green videoangiography in deciding the completeness of cerebrovascular surgery. Journal of Korean Neurosurgical Society 2013;53(6):349-55.

40. Raabe A, Beck J, Gerlach R, Zimmermann M, Seifert V. Near-infrared indocyanine green video angiography: a new method for intraoperative assessment of vascular flow. Neurosurgery 2003;52(1):132-139.

41. Eren S, Rubben A, Krein R, Larkin G, Hettich R. Assessment of microcirculation of an axial skin flap using indocyanine green fluorescence angiography. PlastReconstrSurg 1995;96(7):1636-1649.

42. Giunta RE, Holzbach T, Taskov C, et al. Prediction of flap necrosis with laser induced indocyanine green fluorescence in a rat model. BrJPlastSurg 2005;58(5):695-701.

43. Desmettre T, Devoisselle JM, Mordon S. Fluorescence properties and metabolic features of indocyanine green (ICG) as related to angiography. SurvOphthalmol 2000;45(1):15-27.

44. Speich R, Saesseli B, Hoffmann U, Neftel KA, Reichen J. Anaphylactoid reactions after indocyanine-green administration. AnnInternMed 1988;109(4):345-346.

45. Urken ML. Advances in head and neck reconstruction. The Laryngoscope 2003;113(9):1473-6.

46. Hurvitz KA, Kobayashi M, Evans GR. Current options in head and neck reconstruction. Plastic and reconstructive surgery 2006;118(5):122e-133e.

47. Wong CH, Wei FC. Microsurgical free flap in head and neck reconstruction. Head & neck 2010;32(9):1236-45.

48. Thankappan K. Microvascular free tissue transfer after prior radiotherapy in head and neck reconstruction - a review. Surgical oncology 2010;19(4):227-34.

49. Haughey BH, Wilson E, Kluwe L, et al. Free flap reconstruction of the head and neck: analysis of 241 cases. OtolaryngolHead Neck Surg 2001;125(1):10-17.

50. Disa JJ, Pusic AL, Hidalgo DH, Cordeiro PG. Simplifying microvascular head and neck reconstruction: a rational approach to donor site selection. Annals of plastic surgery 2001;47(4):385-9.

51. Lutz BS, Wei FC. Microsurgical workhorse flaps in head and neck reconstruction. Clinics in plastic surgery 2005;32(3):421-30, vii.

52. Kubo T, Yano K, Hosokawa K. Management of flaps with compromised venous outflow in head and neck microsurgical reconstruction. Microsurgery 2002;22(8):391-395.

53. Hidalgo DA, Disa JJ, Cordeiro PG, Hu QY. A review of 716 consecutive free flaps for oncologic surgical defects: refinement in donor-site selection and technique. Plastic and reconstructive surgery 1998;102(3):722-32; discussion 733-4.

54. Dassonville O, Poissonnet G, Chamorey E, et al. Head and neck reconstruction with free flaps: a report on 213 cases. EurArchOtorhinolaryngol 2007.

55. Nakatsuka T, Harii K, Asato H, et al. Analytic review of 2372 free flap transfers for head and neck reconstruction following cancer resection. Journal of reconstructive microsurgery 2003;19(6):363-8; discussion 369.

56. Suh JD, Sercarz JA, Abemayor E, et al. Analysis of outcome and complications in 400 cases of microvascular head and neck reconstruction. Archives of otolaryngology--head & neck surgery 2004;130(8):962-6.

57. Brown JS, Devine JC, Magennis P, Sillifant P, Rogers SN, Vaughan ED. Factors that influence the outcome of salvage in free tissue transfer. The British journal of oral & maxillofacial surgery 2003;41(1):16-20.

58. Chubb D, Rozen WM, Whitaker IS, Acosta R, Grinsell D, Ashton MW. The efficacy of clinical assessment in the postoperative monitoring of free flaps: a review of 1140 consecutive cases. Plastic and reconstructive surgery 2010;125(4):1157-66.

59. Wu CC, Lin PY, Chew KY, Kuo YR. Free tissue transfers in head and neck reconstruction: complications, outcomes and strategies for management of flap failure: analysis of 2019 flaps in single institute. Microsurgery 2014;34(5):339-44.

60. Hyodo I, Nakayama B, Kato H, et al. Analysis of salvage operation in head and neck microsurgical reconstruction. The Laryngoscope 2007;117(2):357-60.

61. Kroll SS, Schusterman MA, Reece GP, et al. Timing of pedicle thrombosis and flap loss after free-tissue transfer. Plastic and reconstructive surgery 1996;98(7):1230-3.

62. Miyasaka M, Ichikawa K, Nishimura M, et al. Salvage operations of free tissue transfer following internal jugular venous thrombosis: a review of 4 cases. Microsurgery 2005;25(3):191-5.

63. Jandali S, Wu LC, Vega SJ, Kovach SJ, Serletti JM. 1000 consecutive venous anastomoses using the microvascular anastomotic coupler in breast reconstruction. Plastic and reconstructive surgery 2010;125(3):792-8.

64. Barker EV, Enepekides DJ. The utility of microvascular anastomotic devices in head and neck reconstruction. Current opinion in otolaryngology & head and neck surgery 2008;16(4):331-4.

65. Frederick JW, Sweeny L, Carroll WR, Rosenthal EL. Microvascular anastomotic coupler assessment in head and neck reconstruction. Otolaryngology--head and neck surgery : official journal of American Academy of Otolaryngology-Head and Neck Surgery 2013;149(1):67-70.

66. Yu P, Chang DW, Miller MJ, Reece G, Robb GL. Analysis of 49 cases of flap compromise in 1310 free flaps for head and neck reconstruction. Head & neck 2009;31(1):45-51.

67. Disa JJ, Cordeiro PG, Hidalgo DA. Efficacy of conventional monitoring techniques in free tissue transfer: an 11-year experience in 750 consecutive cases. PlastReconstrSurg 1999;104(1):97-101.

68. Byun JA, Baik BS, Park JS. An effective pharyngoesophageal reconstruction with free radial forearm flaps. Eur J Plast Surg 1992;15:173-179.

69. Khalid AN, Quraishi SA, Zang WA, Chadwick JL, Stack BC, Jr. Color doppler ultrasonography is a reliable predictor of free tissue transfer outcomes in head and neck reconstruction. OtolaryngolHead Neck Surg 2006;134(4):635-638.

70. Loh N, Ch'en IY, Olcott E, et al. Power Doppler imaging in preoperative planning and postoperative monitoring of muscle flaps. Journal of clinical ultrasound : JCU 1997;25(9):465-71.

71. Lamby P, Prantl L, Gais S, et al. Evaluation of the vascular integrity of free flaps based on microcirculation imaging techniques. ClinHemorheolMicrocirc 2008;39(1-4):253-263.

72. Prantl L, Pfister K, Kubale R, et al. Value of high resolution ultrasound and contrast enhanced US pulse inversion imaging for the evaluation of the vascular integrity of free-flap grafts. Clinical hemorheology and microcirculation 2007;36(3):203-16.

73. Smit JM, Zeebregts CJ, Acosta R, Werker PM. Advancements in free flap monitoring in the last decade: a critical review. Plastic and reconstructive surgery 2010;125(1):177-85.

74. Svensson H, Svedman P, Holmberg J, Wieslander JB. Detecting changes of arterial and venous blood flow in flaps. Annals of plastic surgery 1985;15(1):35-40.

75. Holzle F, Loeffelbein DJ, Nolte D, Wolff KD. Free flap monitoring using simultaneous non-invasive laser Doppler flowmetry and tissue spectrophotometry. JCraniomaxillofacSurg 2006;34(1):25-33.

76. Choi CM, Bennett RG. Laser Dopplers to determine cutaneous blood flow. Dermatologic surgery : official publication for American Society for Dermatologic Surgery [et al] 2003;29(3):272-80.

77. Yuen JC, Feng Z. Distinguishing laser Doppler flowmetric responses between arterial and venous obstructions in flaps. JReconstrMicrosurg 2000;16(8):629-635.

78. Swartz WM, Jones NF, Cherup L, Klein A. Direct monitoring of microvascular anastomoses with the 20-MHz ultrasonic Doppler probe: an experimental and clinical study. Plastic and reconstructive surgery 1988;81(2):149-61.

79. Swartz WM, Izquierdo R, Miller MJ. Implantable venous Doppler microvascular monitoring: laboratory investigation and clinical results. Plastic and reconstructive surgery 1994;93(1):152-63.

80. Guillemaud JP, Seikaly H, Cote D, Allen H, Harris JR. The implantable Cook-Swartz Doppler probe for postoperative monitoring in head and neck free flap reconstruction. Archives of otolaryngology--head & neck surgery 2008;134(7):729-34.

81. Paydar KZ, Hansen SL, Chang DS, Hoffman WY, Leon P. Implantable venous Doppler monitoring in head and neck free flap reconstruction increases the salvage rate. Plastic and reconstructive surgery 2010;125(4):1129-34.

82. Wax MK. The role of the implantable doppler probe in free flap surgery. The Laryngoscope 2013.

83. Rozen WM, Chubb D, Whitaker IS, Acosta R. The efficacy of postoperative monitoring: A single surgeon comparison of clinical monitoring and the implantable Doppler probe in 547 consecutive free flaps. Microsurgery 2009.

84. Zhang T, Dyalram-Silverberg D, Bui T, Caccamese JF, Jr., Lubek JE. Analysis of an implantable venous anastomotic flow coupler: experience in head and neck free flap reconstruction. International journal of oral and maxillofacial surgery 2012;41(6):751-5.

85. 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. Journal of plastic, reconstructive & aesthetic surgery : JPRAS 2014;67(7):960-6.

86. de Weerd L, Mercer JB, Setsa LB. Intraoperative dynamic infrared thermography and free-flap surgery. AnnPlastSurg 2006;57(3):279-284.

87. Tenorio X, Mahajan AL, Wettstein R, Harder Y, Pawlovski M, Pittet B. Early detection of flap failure using a new thermographic device. JSurgRes 2009;151(1):15-21.

88. Kraemer R, Lorenzen J, Knobloch K, et al. Free flap microcirculatory monitoring correlates to free flap temperature assessment. Journal of plastic, reconstructive & aesthetic surgery : JPRAS 2011;64(10):1353-8.

89. Busic V, Das-Gupta R. Temperature monitoring in free flap surgery. BrJPlastSurg 2004;57(6):588.

90. Jung EM, Prantl L, Schreyer AG, et al. New perfusion imaging of tissue transplants with Contrast Harmonic Ultrasound Imaging (CHI) and Magnetic Resonance Imaging (MRI) in comparison with laser-induced Indocyanine Green (ICG) fluorescence angiography. ClinHemorheolMicrocirc 2009;43(1):19-33.

91. Jyranki J, Suominen S, Vuola J, Back L. Microdialysis in clinical practice: monitoring intraoral free flaps. Annals of plastic surgery 2006;56(4):387-93.

92. Payette JR, Kohlenberg E, Leonardi L, et al. Assessment of skin flaps using optically based methods for measuring blood flow and oxygenation. PlastReconstrSurg 2005;115(2):539-546.

93. Holzle F, Swaid S, Nolte D, Wolff KD. Nutritive perfusion at donor site after microvascular fibula transfer. Microsurgery 2003;23(4):306-12.

94. Rothenberger J, Amr A, Schaller HE, Rahmanian-Schwarz A. Evaluation of a non-invasive monitoring method for free flap breast reconstruction using laser doppler flowmetrie and tissue spectrophotometry. Microsurgery 2013;33(5):350-7.

95. McCraw JB, Myers B, Shanklin KD. The value of fluorescein in predicting the viability of arterialized flaps. PlastReconstrSurg 1977;60(5):710-719.

96. Weisman RA, Pransky SM, Silverman DG, et al. Clinical assessment of flap perfusion by fiberoptic fluorometry. AnnOtolRhinolLaryngol 1985;94(3):226-231.

97. Ha SO, Kim DY, Sohn CH, Lim KS. Anaphylaxis caused by intravenous fluorescein: clinical characteristics and review of literature. Internal and emergency medicine 2013.

98. Garski TR, Staller BJ, Hepner G, Banka VS, Finney RA, Jr. Adverse reactions after administration of indocyanine green. JAMA : the journal of the American Medical Association 1978;240(7):635.

99. Obana A, Miki T, Hayashi K, et al. Survey of complications of indocyanine green angiography in Japan. American journal of ophthalmology 1994;118(6):749-53.

100. Holm C, Mayr M, Hofter E, Becker A, Pfeiffer UJ, Muhlbauer W. Intraoperative evaluation of skin-flap viability using laser-induced fluorescence of indocyanine green. BrJPlastSurg 2002;55(8):635-644.

101. Holm C, Mayr M, Hofter E, Dornseifer U, Ninkovic M. Assessment of the patency of microvascular anastomoses using microscope-integrated near-infrared angiography: a preliminary study. Microsurgery 2009;29(7):509-514.

102. Holzbach T, Artunian N, Spanholtz TA, Volkmer E, Engelhardt TO, Giunta RE. [Microscope-integrated intraoperative indocyanine green angiography in plastic surgery]. Handchirurgie, Mikrochirurgie, plastische Chirurgie : Organ der Deutschsprachigen Arbeitsgemeinschaft fur Handchirurgie : Organ der Deutschsprachigen Arbeitsgemeinschaft fur Mikrochirurgie der Peripheren Nerven und Gefasse 2012;44(2):84-8.

103. Holm C, Dornseifer U, Sturtz G, Basso G, Schuster T, Ninkovic M. The intrinsic transit time of free microvascular flaps: Clinical and prognostic implications. Microsurgery 2009.

104. Holm C, Dornseifer U, Sturtz G, Ninkovic M. Sensitivity and specificity of ICG angiography in free flap reexploration. JReconstrMicrosurg 2010;26(5):311-316.

105. Stepp H. Endoscopic ICG perfusion imaging for flap transplants: technical development. Head & neck oncology 2010;2(Suppl 1):O14.

106. Holzbach T, Taskov C, Henke J, et al. [Evaluation of perfusion in skin flaps by laser-induced indocyanine green fluorescence]. HandchirMikrochirPlastChir 2005;37(6):396-402.

107. Holm C, Tegeler J, Mayr M, Becker A, Pfeiffer UJ, Muhlbauer W. Monitoring free flaps using laser-induced fluorescence of indocyanine green: a preliminary experience. Microsurgery 2002;22(7):278-287.

108. Iblher N, Eisenhardt SU, Penna V, Stark GB, Bannasch H. A new evaluation tool for monitoring devices and its application to evaluate the implantable Doppler probe. Journal of reconstructive microsurgery 2010;26(4):265-70.

8 Appendix:

8.1 forms used during the study

Klinikum der Universität München
Klinik für Hals-, Nasen- und Ohrenheilkunde – Großhadern
Direktor: Prof. Dr. med. Alexander Berghaus

LMU Ludwig Maximilians Universität München

PROSPEKTIVE, DIAGNOSTISCHE MACHBARKEITSSTUDIE ZUR ENDOSKOPISCHEN GEWEBEPERFUSIONSMESSUNG FREIER GEWEBETRANSPLANTATE IN DER KOPF-HALS-REGION UNTER VERWENDUNG VON ROT ANGEREGTEM INDOCYANIN-GRÜN

DOKUMENTATIONSBOGEN – SEITE 1

PATIENT:	LMU-
GEWICHT:	kg
DATUM:	. . 2 0 0
ZEIT NACH OP: (HAUTNAHT)	Stunden
ICG-DOSIS:	mg
BLUTDRUCK:	/ mmHg
PULS:	Herzschläge / Minute
ICG ÜBER:	ZVK ☐ PVK ☐
TUMORLOKALISATION: (ANATOMISCHE EINHEIT UND UNTEREINHEIT)	
TRANSPLANTAT-TYP:	Radialis-Lappen ☐
	Anterolateraler Oberschenkel-Lappen ☐
	Fibula-Lappen ☐
	Fibula-Lappen ☐

Vorlage KUMOC – fe 05/03-19

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PROSPEKTIVE, DIAGNOSTISCHE MACHBARKEITSSTUDIE ZUR ENDOSKOPISCHEN GEWEBEPERFUSIONSMESSUNG FREIER GEWEBETRANSPLANTATE IN DER KOPF-HALS-REGION UNTER VERWENDUNG VON ROT ANGEREGTEM INDOCYANIN-GRÜN

DOKUMENTATIONSBOGEN – SEITE 2

BILDER:

Image		Lokalisation:	
		Bemerkung: (Weißlicht/Fluo., Blickwinkel, Fluo-Verhältnis,…)	
Image		Lokalisation:	
		Bemerkung: (Weißlicht/Fluo., Blickwinkel, Fluo-Verhältnis,…)	
Image		Lokalisation:	
		Bemerkung: (Weißlicht/Fluo., Blickwinkel, Fluo-Verhältnis,…)	
Image		Lokalisation:	
		Bemerkung: (Weißlicht/Fluo., Blickwinkel, Fluo-Verhältnis,…)	
Image		Lokalisation:	
		Bemerkung: (Weißlicht/Fluo., Blickwinkel, Fluo-Verhältnis,…)	

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DOKUMENTATIONSBOGEN – SEITE 3

VIDEOS:

Video		Lokalisation:	
		Bemerkung: (Anflutung, Weißlicht/Fluo., Blickwinkel, Fluo-Verhältnis, einsehbare Prozent der Oberfläche…)	
Video		Lokalisation:	
		Bemerkung: (Anflutung, Weißlicht/Fluo., Blickwinkel, Fluo-Verhältnis, einsehbare Prozent der Oberfläche…)	
Video		Lokalisation:	
		Bemerkung: (Anflutung, Weißlicht/Fluo., Blickwinkel, Fluo-Verhältnis, einsehbare Prozent der Oberfläche…)	

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DOKUMENTATIONSBOGEN – SEITE 4

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Klinik für Hals-, Nasen- und Ohrenheilkunde – Großhadern
Direktor: Prof. Dr. med. Alexander Berghaus

LMU Ludwig-Maximilians-Universität München

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UMFRAGE UNTER HNO-ÄRZTEN ZUR INTERPRETATION DER VIDEOSEQUENZEN

In dem Video werden insgesamt 10 Sequenzen von maximal 40 Sekunden Länge gezeigt. Jede dieser Sequenzen zeigt eine im ersten Teil endoskopische Weißlichtstandbilder vom OP-Situs, im zweiten Teil Videos der ICG-Fluoreszenz-Angiographie, die nach intravenöser Injektion von ICG in Echtzeit die Perfusion widergibt.

Bitte geben Sie für jede der Sequenzen 1 – 10 zuerst beim Weißlichtbild und anschließend beim zweiten Durchlauf beim ICG-Video an, wie Sie die Durchblutungssituation subjektiv einschätzen und ob Sie meinen, die Perfusion sollte operativ überprüft werden (Revision nötig)!

Bei der Auswertung der Videos liegt der Fokus auf dem Verhältnis zwischen Fluoreszenz im Lappen im Vergleich zum Stammgewebe. Zeigt der Lappen **am Ende der Sequenz** die gleiche Intensität und ist also genauso „hell" wie das Stammgewebe, wird die Durchblutung als gut gewertet. Vor Beginn der Videosequenz im Fluoreszenz-Modus wird für fünf Sekunden ein Weißlichtbild vom Situs angezeigt, um eine leichtere Orientierung zu ermöglichen.

Qualifikation:
Assistenzarzt ☐
Facharzt ☐
Oberarzt ☐

Abteilung:
HNO ☐
Plastische Chirurgie ☐
Andere: _____

Sequenz	**Perfusion**	*Gut*	*Eher gut*	*Eher schlecht*	*Schlecht*	**Revison nötig**	*Ja*	*Nein*	**Perfusion**	*Gut*	*Eher gut*	*Eher schlecht*	*Schlecht*	**Revison nötig**	*Ja*	*Nein*
Sequenz 01	**Teil 1: Weißlicht**								**Teil 2: Fluoreszenzvideos**							
Sequenz 02																
Sequenz 03																
Sequenz 04																
Sequenz 05																
Sequenz 06																
Sequenz 07																
Sequenz 08																
Sequenz 09																
Sequenz 10																

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8.2 Abbreviations

ASR	age standardized rates
BP	Blood pressure
CDI	Colour Doppler imaging
CEUS	Contrast enhanced ultrasound imaging
CHI	Contrast harmonic ultrasound imaging
ENT	Ear, nose and throat
EU	European Union
FI	Fluorescence index
fALT	free anterolateral tigh flap
fFF	free fibula flap
FFRR	Flap failure reduction rate
fRFF	free radial forearm flap
HPV	Human Papilloma Virus
HR	Heart rate
ICG	Indocyanine Green
ICGA	ICG (Indocyanine Green) angiography
ICU	Intensive care unit
IMC	Intermediate care unit
ITT	Intrinsic transit time
LMU	Ludwig-Maximilian-Universität, München

MAP	mean arterial pressure
MRI	Magnet resonance imaging
OR	Operation room
PDI	Power Doppler imaging
PI	Perfusion index
ROI	Region of interest
RSR	Revision success rate
SSC	Squamous Cell Carcinoma
TMF	Time until maximum fluorescence in the flap
TMS	Time until maximum fluorescence in the surrounding
TNM	Tumor-Nodes-Metastasis
TUO	Time until onset of fluorescence
UICC	Union for International Cancer Control
WL	white light

9 Acknowledgments

Mein Dank gilt in erster Linie Herrn Professor Dr.med. Ulrich Harréus für die Überlassung dieser interessanten und abwechslungsreichen Arbeit und die freundliche Unterstützung zu jeder Zeit.

Ganz besonderer Dank gebührt Herrn Prof. Dr.med. Christian Betz, der nicht nur meine Begeisterung für die Wissenschaft im Allgemeinen und dieses Thema im Speziellen weckte, sondern mir auch ein ausgezeichneter Betreuer und Freund war. Nach dem Weggang von Prof. Harréus übernahm er die Doktorvaterschaft, wofür ich ihm ewig dankbar sein werde. Ihm verdanke ich, dass diese Arbeit in der vorgelegten Form angefertigt werden konnte. Stets war er für alle Fragen erreichbar und dank seiner fundierten Kritik wurde die Arbeit kontinuierlich besser.

Auch danken möchte ich allen Mitarbeiterinnen und Mitarbeitern des Klinikums Großhadern, die mir zu allen möglichen und unmöglichen Zeiten bei meinen Messungen halfen. Besonders hervorzuheben sind darunter Fr. PD Dr. Pamela Zengel und Dr. Miriam Havel.

Im Laser-Forschungs-Labor konnte ich mich stets auf den Rat von Dr. Herbert Stepp und Hilmar Schachenmayr verlassen, die mich in wissenschaftlichen und statistischen Angelegenheiten berieten.

Meine neuen Arbeitskollegen in Agatharied steckten mich mit ihrer Arbeitswut und Liebe zur Wissenschaft erneut an, um mir den Schub zum Endspurt zu geben. Danke an Martin und Thomas von Ahnen.

Danke auch an alle meine Freunde, die mich auf dem langen Weg zur Promotion begleiten und regelmäßig fragten, wann ich nun endlich nicht mehr nur Arzt, sondern auch endlich Doktor sei.

Diese Arbeit wäre mir nicht möglich gewesen ohne meine Eltern. Ihre andauernde Unterstützung auf meinem gesamten bisherigen Lebensweg gab und gibt mir täglich neue Kraft. Ihnen gilt von ganzem Herzen mein Dank.

Eidesstattliche Versicherung:

Ich, Sven Matthias Zhorzel, erkläre hiermit an Eides statt, dass ich die vorliegende Dissertation mit dem Thema:

Feasibility of endoscopic assessment of free flap perfusion in the head and neck region using Indocyanine Green fluorescence imaging

selbständig verfasst, mich außer der angegebenen keiner weiteren Hilfsmittel bedient und alle Erkenntnisse, die aus dem Schrifttum ganz oder annähernd übernommen sind, als solche kenntlich gemacht und nach ihrer Herkunft unter Bezeichnung der Fundstelle einzeln nachgewiesen habe.

Ich erkläre des Weiteren, dass die hier vorgelegte Dissertation nicht in gleicher oder in ähnlicher Form bei einer anderen Stelle zur Erlangung eines akademischen Grades eingereicht wurde.

Ort, Datum, Unterschrift Doktorand