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

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Summary *Background:* Free-tissue transfer has become a standard procedure for reconstructive surgery in the head and neck area. Flap failures are relatively rare ($\leq 5\%$), and a high percentage can be salvaged if detected early. Indocyanine Green (ICG) angiography might be able to improve the detection of flap malperfusion at an early stage.

Methods: So far, 11 patients with free-flap reconstructions of the upper aerodigestive tract (UADT) have participated in this study. Each participant underwent three endoscopic ICG angiographies (24 h intra-operatively and 72 h postoperatively). The data obtained were evaluated online as well as offline on a personal computer (PC), and the results compared to the clinical outcome.

Results: There were no partial or complete flap losses. One flap was successfully salvaged following initial arterial kinking with impeded perfusion. The ICG fluorescence angiography was tolerated well in all patients. The free flaps showed a delayed yet equal ICG fluorescence as compared to the surrounding tissue. The timing and slope of fluorescence build-up were dependent on circulatory factors. The relative fluorescence maxima of flap versus surrounding were 33% in the initially failing flap and $\geq 64\%$ for all other examinations.

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Conclusions: It was possible to prove the feasibility of endoscopic ICG fluorescence angiography in patients undergoing free-flap transfer to the UADT. The method provides instant information about the perfusion state of the tissue and is easily performed without greater patient discomfort or risk of side effects. Due to the endoscopic approach, the method seems highly promising for this indication and merits further evaluation.

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For the reconstruction of larger defects, free-tissue transfers (free flaps) have become a routine procedure worldwide. For flap survival, the vessels of the free flaps must be joined to the recipient blood supply. If the anastomosis fails, then the flap is prone to die. Surgical salvage is possible when a failing flap is detected early. Therefore, close monitoring of flap perfusion is essential until the flap has an established blood supply.

Reliable assessment of the perfusion of free flaps still poses a challenge, as the complexities of flap microcirculation are difficult to assess. Routinely performed tests are clinical (texture, colour, capillary refill and temperature) or via Doppler ultrasound devices that pick up flow in larger blood vessels.¹ More advanced systems are discussed in a review by Whitaker et al. from 2005.² The authors state, however, that 'although many techniques to assess flap perfusion have been described, a standard, reliable, universally accepted method, other than bedside clinical observation ..., remains elusive.'

Fluorescence angiography using Indocyanine Green (ICG) has been described as a helpful indicator of free-flap perfusion in tissue transfers to the outer body.^{3–5} The dye is approved internationally for use in cardiac, (micro)circulatory and liver function diagnostics. The dye is administered intravenously, and the fluorescence detected via a modified video camera (IC View[®], Pulsion Medical Systems AG, Munich, Germany). The evaluation potentially relevant for long-term flap survival includes measurement of delay of fluorescence maximum values following administration and the ratio of maximum fluorescence intensity values of flap versus the surrounding.

For reconstructive surgery of the upper aerodigestive tract (UADT), flap-perfusion measurements need to be performed endoscopically. To our knowledge, however, endoscopically guided ICG-angiography has not been investigated so far. If successful, this method would be helpful for the assessment of free flaps in reconstructive head and neck surgery.

Materials and methods

Following approval by the Institutional Review Board, this ongoing, prospective, uncontrolled, unblinded and non-randomised diagnostic study was initiated in October 2007. The criterion for inclusion is a microvascular free-flap transfer to the UADT. The exclusion criteria correspond with those for the dye (previous allergic reaction to ICG, hypersensitivity to iodine, prematurely/newborn children and pregnancy).

After informed, written consent is obtained, fluorescence angiography is performed at three different times for each patient: intra-operatively (following vessel anastomosis and wound closure) and 24 h as well as 72 h

postoperatively. For each examination, ICG (ICG Pulsion[®], Pulsion Medical Systems AG, Munich, Germany) is administered intravenously in a single dose of 0.3 mg kg⁻¹ according to the manufacturer's instructions. At the same time, cardiovascular parameters (heart rate and blood pressure) are recorded. During and directly following administration, the free flap, a fluorescence standard and the surrounding tissue are monitored under red/near-infrared excitation (filtered Xenon Short Arc lamp, Karl Storz, Tuttlingen, Germany) with a filtered, rigid endoscope (0°, 4 mm, Karl Storz, Tuttlingen, Germany) coupled to an infrared-sensitive three-chip CCD camera (Karl Storz, Tuttlingen, Germany). Thereby, the detection filter blocks all the reflected excitation light, so that only genuine fluorescence is taken up by the camera. The acquired sequences are digitally stored (AIDA[®] Compact II System, Karl Storz, Tuttlingen, Germany) and subsequently evaluated on a personal computer (PC) using the IC CALC[®] Software Version 1.1 (Pulsion Medical Systems AG, Munich, Germany).

For each patient, the free-flap survival is followed closely on the ward and along regularly scheduled outpatient visits up to 4 weeks following surgery (Table 1).

Results

Patients and cases

To date, a total of 11 patients (four females and seven males; ages 48–74 years, mean age: 64.6 years) have been included in this study. All patients underwent reconstruction of an oral ($n = 8$) or oropharyngeal ($n = 3$) tissue defect following resection of a malignant tumour of the UADT. Nine patients received a free radial forearm flap (fRFF) and two patients received a free anterolateral thigh flap (fALT). All flap procedures were conducted by one surgeon (U. Harreus).

Clinically, all flaps healed well and showed a complete survival. In one patient, there was an initial kinking of the pedicle artery after anastomosis and closure of the neck, which led to a reduced blood supply, manifesting itself clinically in a pale appearance and a questionably missing capillary refill. Upon re-opening of the neck, a detangling and re-fixation of the pedicle was performed; this resulted in a stable perfusion of the flap from then on.

Clinical fluorescence examinations

All patients tolerated the additional monitoring procedures (approximately additional 5 min per examination) well, and no adverse events were encountered. However, in one case

Table 1 Standard protocol of postoperative clinical and Doppler aided free flap monitoring

Time after surgery	1–72 h post surgery	3–5 days post surgery	6–14 days post surgery	2–4 weeks post surgery
Frequency of clinical checks	hourly	every 2 h	once daily	at least once weekly
Frequency of Doppler examinations	hourly	every 2 h	none	none

where major parts of the base of the tongue were reconstructed using an rFFF, postoperative oedematous swellings made it impossible to adequately monitor the flap, so the second and third procedure had to be omitted. All other patients were examined 3 times as defined in the protocol.

In all 11 patients included in the study so far, the surrounding and transplanted tissue showed a positive ICG-fluorescence following administration of the dye. The examinations showed the following online results: after an initial phase of missing fluorescence (Figure 1a and 1b), ICG fluorescence first became visible in the tissue surrounding the free flap (Figure 1c). Shortly afterwards, the transplanted tissue picked up fluorescence as well, until both appeared to exhibit almost equal fluorescence (Figure 1d). The only exception to this was the first examination in the transplant procedure where the arterial kinking had occurred. Here, fluorescence intensities within the flap

kept staying well below the one observable in the surrounding, which supported the decision to re-open the neck. After revision surgery, the examination was instantly repeated, and the result was now comparable to all others.

Data evaluation

An objective, and in part statistical, evaluation of the recorded sequences was performed for 10 patients; the first case could not be used for further evaluation due to a high degree of movement artefacts.

It was found that the time span from ICG administration to the first noticeable fluorescence ranged from 6 to 22 s (mean: 13.5 s), but that it was equal for the transplanted flaps (even for the initially failing one) and for the surrounding tissues (Figure 2). There was a negative correlation between the time to an onset of fluorescence

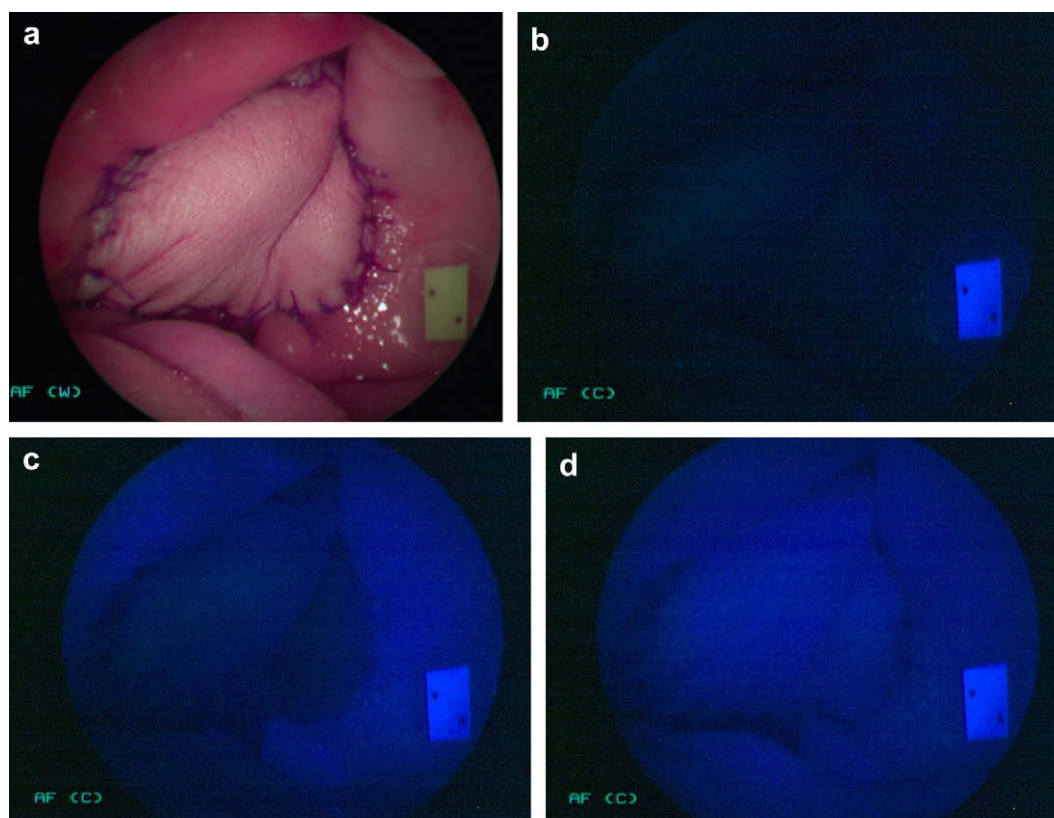


Figure 1 Exemplary ICG angiography of an rFFF transplanted to the right buccal area following resection of a squamous cell carcinoma. (a) Ordinary endoscopic finding. (b) Fluorescence image directly following injection of ICG; only the fluorescence standard is visible on the image. (c) Snapshot of fluorescence uptake in the flap (slow) and the surrounding tissue (fast) at approximately 15 s after ICG injection. (d) Fluorescence image at 30 s after dye application; fluorescence intensities within flap and surrounding tissue seem equal.

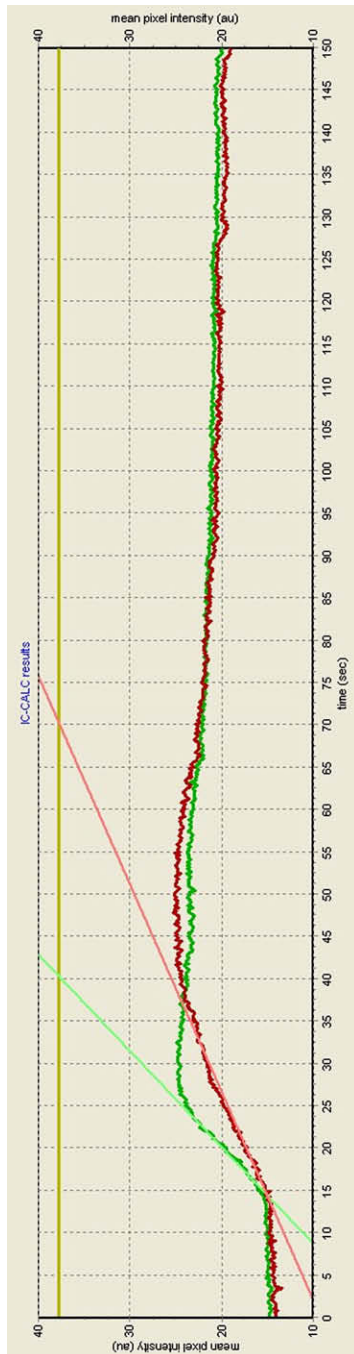


Figure 2 Exemplary evaluation of fluorescence intensities (in arbitrary units) over time (in seconds) for a transplanted flap (red line), the surrounding tissue (green line) and the fluorescence standard (yellow line).

and cardiovascular factors – a moderate correlation of 0.41 was determined for the rate–pressure product (RPP; Figure 3) and a weak correlation of 0.13 for the mean arterial pressure (MAP).

The slope of fluorescence build-up as well as maximum fluorescence intensities varied between transplanted flaps and surrounding tissues. Whereas there was a steep and short increase of ICG fluorescence in autochthonous tissue, this was usually prolonged in transplanted tissue (Figure 2). The relative slope values as well as the slope ratio between free flaps and surrounding tissues showed a high amount of inter- and intra-patient variability. However, there was an upwards trend for the relative slopes of fluorescence build-up of flap versus surrounding from the first two against the last examination (Figure 4). This difference, however, was not statistically significant ($p = 0.069$ in the Mann–Whitney U Test for paired samples). The relative slope values for the initially failing flap did not differ considerably from those of well-perfused flaps. Again, there was a negative correlation between the period of increasing fluorescence intensities and circulatory factors (correlation factor 0.40 for the RPP and 0.19 for the MAP). The ratios of maximum fluorescence intensities between flaps and surrounding tissues ranged between 64.00% and 95.65% (mean: 82.95%) for well-perfused flaps. Only in the case of the initially failing flap, this ratio read 33.33% only; following surgical intervention, this value immediately climbed to 92.86%.

Discussion

Free-flap surgery has advanced to become a routine procedure in aesthetic and functional treatment of tissue defects. For soft tissue reconstructions of the UADT, the rFRF is nowadays considered the 'workhorse',⁶ as it has a stable blood supply and can be perfectly moulded into complex anatomical structures. Depending on the volume and the type of tissue missing, a variety of other flaps are available.^{6–8}

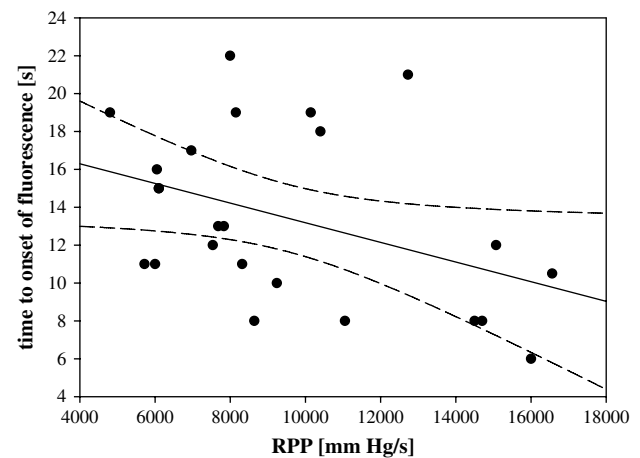


Figure 3 Scatter plot representing the time period from dye application to onset of fluorescence (in seconds) correlated with the rate–pressure product (RPP; in mmHg s^{-1}). The linear regression is depicted by the solid line; the dashed lines include the 95% confidence intervals.

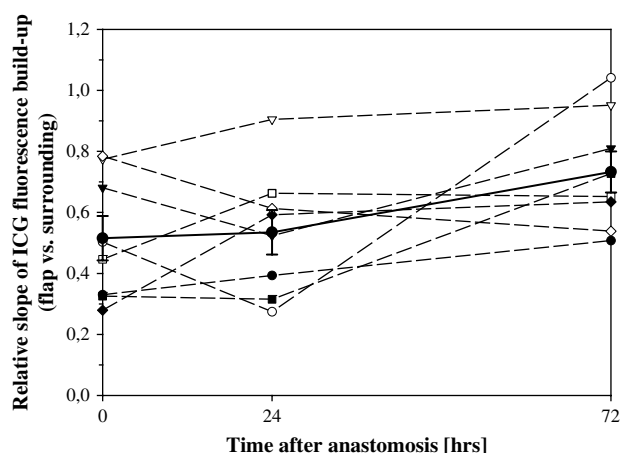


Figure 4 Multiple line and scatter plot showing the time dependency of the ratios (flap vs. surrounding) of ICG fluorescence build-up. The dashed lines represent single measurements; the solid line represents the mean, with the standard errors depicted as error bars.

For the most experienced centres worldwide, the survival of free flaps varies between 95.0% and 98.8%.^{9–12} Free-flap failures seem to be almost exclusively due to an impeded blood perfusion. The most common causes are pedicle thrombosis (53%; 3/4 venous and 1/4 arterial thrombosis) and haematoma/bleeding (30%).¹² More than 75% of all complications in the head and neck field develop during the first 5 days following surgery.¹² If a surgical re-exploration of the pedicle is performed within the first hours, then salvage of the flap is successful in 63–77% cases.^{9,12,13} It is therefore of utmost importance to detect any circulatory compromise as soon as possible and to keep a very low threshold for surgical re-exploration.

To date, clinical judgement of a flap's vitality by an experienced microsurgeon is still the most reliable monitoring method. It includes checking for changes in appearance, colour, texture, temperature and capillary refill.¹ The pinprick test is also frequently applied. Even though there are no standardised guidelines, it is generally understood that monitoring should be performed hourly for at least the first 72 h following anastomosis.¹⁴

Apart from clinical judgement, a number of methods for monitoring free-flap perfusion have been described. The one most commonly used is a handheld, acoustic Doppler probe that can provide reliable information about the blood flow within the pedicle through intact soft tissue.¹⁵ A slightly more advanced, related system is the colour flow Doppler (CFD), which measures blood-flow velocity and resistance to flow in the pedicle.¹⁶ There are several problems arising when relying on these methods: a detected flow can be originating from other vascular structures and may be mistaken for a patent pedicle; the correct location for measuring the Doppler signals might change due to postoperative swellings; there might be a misleading, persistent arterial Doppler signal in the event of an early venous congestion and the system does not pick up changes of the microvascular flow within the flap itself. Whereas the former problems might be overcome by implantable, continuously measuring Doppler probes,^{17,32} the last one still remains an issue.

Even though infrared thermography has been successfully applied elsewhere,¹⁸ this method seems unsuitable for the UADT as the tissue temperature in UADT is always approximating the body temperature independently of its perfusion.

Other methods for monitoring of free-flap perfusion include laser Doppler flowmetry,¹ microcatheter-based measurement of pO_2 ,¹⁹ 99 mTc scintigraphy,²⁰ near-infrared spectroscopy^{21–23} or angiography following application of contrast agents such as Fluorescein²⁴ or ICG.²⁵ All of these are aimed to measure microcirculatory changes. Especially a combination of laser-Doppler flowmetry and tissue spectrophotometry seems promising in predicting flap failure and to differentiate between the causes.²⁶ However, none of these methods has gained worldwide acceptance so far.²⁷

Encouraged by a study on 285 pedicled flaps where the authors showed that fluorescein angiography could accurately predict flap survival within a distance of 3–5 mm,²⁸ this method had first attracted much attention in the 1980s and early 1990s. Because of the severity of the potential side effects, the possibility for erroneous interpretations of marginal fluorescence and the dye's unfavourable pharmacokinetic properties, the method has not become a standard procedure.

In 1995, another fluorescent dye named ICG has first been successfully applied for free-flap monitoring in an experimental animal model.²⁵ The dye was already in use for other indications (e.g., visualisation of the choroidal vasculature or assessing liver function) and is still widely used in these fields with international approvals.^{29,30} It has far better pharmacokinetic properties than fluorescein and only a small potential for side effects such as an over-reaction to its iodide content as well as anaphylactoid or anaphylactic reactions (with an incidence of approximately 1:40 000). The ICG is excited in the red and emits in the near-infrared region, which is favourable over fluorescein because the depth of interrogation is increased and – as the dye is not visible under normal lighting conditions – the visual monitoring is in no way impeded. Another advantage is that ICG has a high binding affinity to plasma proteins and therefore remains strictly intravascular. The molecule is quickly degenerated in the liver with a plasmatic half-life of 3–4 min. Therefore, examinations can be repeated within 20 min. Last but not the least, the substance seems reasonably well priced at approximately 50 € per application for fluorescence angiographies.

Whereas the first study could demonstrate the feasibility of ICG angiography for quantifying flap microcirculation,²⁵ a second publication took things further by proving that areas of postoperative necroses within randomly elevated flaps could be accurately predicted by variations in ICG angiographies.³¹

These findings have paved the path for *in vivo* applications on humans, and the first report on clinical free-flap monitoring was published in 2002.⁵ In this prospective feasibility study on 20 patients, the authors found that intra-operative ICG fluorescence angiography allows for an 'evaluation of perfusion in all kinds of microsurgical flaps' and that there was a strong correlation between ICG fluorescence findings and clinical outcome. Another study by Mothes et al. on 11 free flaps concludes that intraoperative,

but not postoperative, measurements have a significantly better prognostic value concerning flap survival than clinical parameters.⁴ Krishnan et al. in their study on nine flaps, including three free flaps, remarked that the method in some cases might be too sensitive to adequately predict clinical outcome.³

Encouraged by these results, our group has tried to apply the method for assessing the viability of free flaps used for reconstructions of the UADT. At first, the IC-View®-System (Pulsion Medical Systems AG, Munich, Germany) was used, and the results were published in a brief report.³² However, the camera was not designed for endoluminal applications and did not allow an adequate imaging of the UADT. Therefore, a modified endoscopic setup that is not commercially available so far was used in this feasibility study. It could be shown in this intermediate analysis that the system works well and is patient- and user-friendly. The procedures were well tolerated by patients, and side effects were not encountered. This corresponds with the other published studies cited above.^{3–5} If the reconstruction was performed in the lower areas of the UADT and/or if a considerable postoperative swelling of the flap occurred, the system did not yet meet our expectations. This might be overcome by the construction of a 90° ICG endoscope, which seems like a minor technical challenge.

Clinically, there were no partial or complete flap losses. The ICG fluorescence angiographies that were performed at 0, 24 and 72 h following microvascular anastomosis showed slightly delayed, subjectively equal uptakes of the dye within the flaps compared to the surrounding. The only exception to this rule was the initially malperfused flap, which was correctly highlighted by a markedly and persistently reduced fluorescence intensity. In this case, the additional information provided by the ICG-angiography helped with our decision to re-open the neck. Therefore, it might be surmised that a reduced ICG-fluorescence within free flaps in relation to the surrounding could be a predictor of an impending flap failure. Obviously, however, we do not reach any statistical level of significance with this hypothesis yet.

For each patient (except the first), the data were subsequently evaluated on a PC. It would have been more desirable to perform these evaluations online, and we are currently working on software solutions to solve this problem.

Through data processing, it was shown that the dye uptake in the flap and the surrounding is inversely correlated to circulatory factors. The flap with the arterial kinking did not show a prominent delay of ICG uptake, which is somewhat surprising. The slope of fluorescence build-up was different in free flaps (slow) and surrounding tissues (fast), with considerable inter- and intra-patient variations. After surgery, the build-up of fluorescence within the flap increases considerably over time, when compared to the surroundings (Figure 4). This might be explained by a time dependent stabilization of the free-flap circulation. Nevertheless, fluorescence uptake values do not seem reliable indicators for free-flap malperfusions.

The opposite might be true for the maximum fluorescence intensities in flaps and surrounding tissue. Whereas all primarily successful flaps showed a relative fluorescence intensity of at least 64%, the initially failing one only

reached 33%. This stands in accordance with the results reported from the animal study by Giunta et al., who state that the surviving parts of the studied flaps 'had a mean perfusion index of 62% compared to reference skin,' whereas the 'distal parts of the flaps that necrotized showed an average perfusion index of only 19%.'³¹ Perhaps, it might be feasible to introduce a threshold around 40–50% as a minimum relative value predictive of free-flap survival, but this needs verification in larger patient numbers.

Unfortunately, obtaining reproducible fluorescence values from the recorded video sequences proved more difficult than originally thought. This had the following reasons:

- 1) Due to the complex anatomy of the UADT, the images obtained show surfaces in various distances and angles from the camera. This influences the measurable intensities, regardless of the actual concentration of the dye within the tissue.
- 2) The endoscopic illumination is strongest in the centre and shows a gradual decline towards the edges. Therefore, fluorescence intensities in the image centre are over-rated.
- 3) Due to slight movements, the flap, the surrounding tissue and the ICG standard are hard to track through the sequences, which is essential for a quantitative evaluation.

In order to keep errors as small as possible, the endoscope was angled perpendicular to the surface of interest (i.e., free flap and rim of surrounding tissue) and both patient and endoscope were kept as still as possible. The most reliable sequences were therefore obtained intra-operatively. We are currently working on software and hardware solutions to confront these problems.

In conclusion, we could prove the feasibility of endoscopic ICG fluorescence angiography in patients undergoing free-flap surgery of the UADT. The method provides instant information about the perfusion state of the transplanted tissue and is easily performed at reasonably low costs without patient discomfort or risk of side effects. Our results raise the question if reduced relative maximum fluorescence intensity within a free flap might be predictive of failure. Further studies with higher patient numbers are needed to verify this hypothesis.

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