



# Intraoperative spectral imaging versus ICG fluorescence for gastric conduit viability in esophagectomy

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## Abstract

**Background** Anastomotic leakage remains a critical complication following esophagectomy, occurring in 8–20% of patients. While indocyanine green fluorescence imaging (ICG-FI) is commonly used to assess perfusion, it requires dye injection and does not directly measure tissue metabolism. This study evaluates a real-time, contrast-free spectral imaging (SI) system for quantifying gastric conduit oxygenation (StO<sub>2</sub>) and compares its performance to ICG-FI.

**Methods** Intraoperative SI was successfully performed in 33 patients undergoing laparoscopic Ivor Lewis esophagectomy using a video-rate snapshot spectral camera, acquiring 16 spectral bands in the 460–600 nm range. A deep learning model estimated StO<sub>2</sub> of the gastric conduit and quantitative perfusion metrics were derived from ICG-FI time-intensity curves. Local capillary lactate levels were measured as a physiological reference for tissue hypoxia. Statistical analyses compared StO<sub>2</sub> and ICG-FI metrics.

**Results** SI successfully differentiated well-perfused from ischemic tissue (median StO<sub>2</sub>: 69.16% vs. 31.58%,  $p < 0.001$ ). The estimated StO<sub>2</sub> showed a strong negative correlation with local capillary lactate ( $r = -0.72$ ,  $p < 0.001$ ). While quantitative ICG-FI metrics ( $T_{1/2}$  and  $AUC_{50\%}$ ) revealed significantly reduced perfusion at the anastomosis site compared to well-perfused tissue ( $p < 0.001$ ), they showed only a moderate correlation with StO<sub>2</sub> ( $r = 0.50$ ,  $p < 0.001$ ). Notably, in a patient who developed anastomotic leakage, both modalities detected ischemia intraoperatively, but they show different transition borders, with SI indicating a more extensive hypoxic area than ICG-FI.

**Conclusion** Snapshot SI provides a real-time, objective assessment of tissue oxygenation without the need for contrast agents. Its strong correlation with lactate suggests it may offer potential complementary value to dye-based perfusion imaging, potentially improving anastomotic site selection, although outcome-based validation studies in larger cohorts are needed.

**Keywords** Spectral imaging · Fluorescence imaging · Tissue ischemia · Graft oxygenation · Esophagectomy · Deep learning

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Esophageal cancer is the eleventh most common and seventh most lethal malignancy worldwide [1]. Despite advances in multimodal therapy, surgical resection remains the cornerstone of curative treatment. Following esophagectomy, the stomach is most commonly used to restore continuity of the upper gastrointestinal tract. Anastomotic leakage (AL) remains the most feared complication, occurring in 8–20% of cases and associated with substantial morbidity and mortality [2]. Adequate vascularization and oxygenation of the gastric conduit are critical determinants of anastomotic healing. However, intraoperative selection of the anastomotic site is still largely based on subjective assessment of tissue color, bleeding, and vessel pulsation. A quantitative, non-invasive method for intraoperative assessment of tissue ischemia could substantially improve surgical decision-making.

Indocyanine green fluorescence imaging (ICG-FI) is widely used for intraoperative perfusion assessment, yet important limitations remain. The technique requires intravenous dye administration, a wash-out time, and primarily reflects blood flow rather than actual tissue oxygenation. Alternative approaches, including laser speckle contrast imaging, gastric tonometry, Doppler-based techniques, and spatial frequency domain imaging, have been investigated [3–6], but limited integration into minimally invasive workflows and insufficient reproducibility have hindered their use in everyday practice.

Spectral imaging (SI) has recently emerged as a promising modality for assessing gastric conduit perfusion and oxygenation [7–9]. Comparative studies indicate that SI provides complementary information on tissue status beyond ICG-FI [9, 10]. However, conventional spectral imaging systems rely on scanning mechanisms that are bulky and require several seconds per acquisition, limiting their applicability in real-time laparoscopic surgery. Snapshot spectral cameras overcome the speed limitations of scanning-based systems by capturing all spectral bands simultaneously in a single exposure, enabling video-rate tissue oxygenation ( $\text{StO}_2$ ) monitoring without disrupting the surgical workflow.

Recent advances in deep learning have further improved estimation of  $\text{StO}_2$  from spectral data, replacing traditional linear unmixing approaches with robust neural network-based methods [11, 12]. Training on physics-based simulations of light–tissue interaction enables accurate oxygenation mapping without the need for invasive reference measurements. To address the domain gap between simulated and clinical data, we developed a domain-invariant deep learning framework based on previous work [12].

In this study, we integrated a snapshot spectral camera into a laparoscopic system to enable real-time intraoperative  $\text{StO}_2$  monitoring during esophagectomy in 33 patients. Previous work has either employed slow scanning-based

systems [7, 8, 13], estimated an ischemia index rather than quantitative  $\text{StO}_2$  directly [11], or demonstrated real-time  $\text{StO}_2$  imaging only in a single-animal preclinical model [14]. To our knowledge, the present study is therefore the first to apply a video-rate spectral imaging system for real-time intraoperative  $\text{StO}_2$  estimation in a clinical human cohort undergoing minimally invasive esophagectomy. The  $\text{StO}_2$  represents the ratio of oxygenated hemoglobin to total functional hemoglobin, within the microvascular bed of the sampled tissue. It is conceptually analogous to pulse oximetry, but reflects microvascular oxygenation rather than systemic arterial oxygenation.

We evaluated the clinical utility of this technology by comparing spectral oxygenation measurements with quantitative perfusion metrics derived from ICG-FI time-intensity curves and with local capillary lactate levels as a physiological reference for tissue hypoxia. Figure 1 illustrates the complementary data content and processing pipelines of SI and ICG-FI. The primary endpoint of this study was the feasibility of real-time intraoperative quantification of gastric conduit oxygenation using deep learning-enhanced snapshot SI. Secondary endpoints included comparison with quantitative perfusion metrics derived from ICG-FI and validation against local capillary lactate levels. Accordingly, the study aims were:

1. **Primary endpoint:** To evaluate the feasibility of a deep learning-enhanced snapshot SI system for real-time, intraoperative quantification of gastric conduit oxygenation.
2. **Secondary endpoint:** To compare the diagnostic value of SI versus quantitative perfusion metrics derived from ICG-FI.
3. **Secondary endpoint:** To validate spectral-based  $\text{StO}_2$  estimates against local capillary lactate levels as a physiological reference for tissue hypoxia.

## Methods

### Study population, design and ethics

This single-institution, single-arm prospective observational study included 38 patients scheduled for elective laparoscopic esophagectomy for cancer between February 2024 and November 2025 at Ghent University Hospital. The sample size of 38 patients was determined by logistical feasibility for this initial observational study, rather than formal power calculations, as the primary aim was to evaluate the technical feasibility. Five of the 38 enrolled patients were excluded due to technical acquisition issues (e.g., incorrect

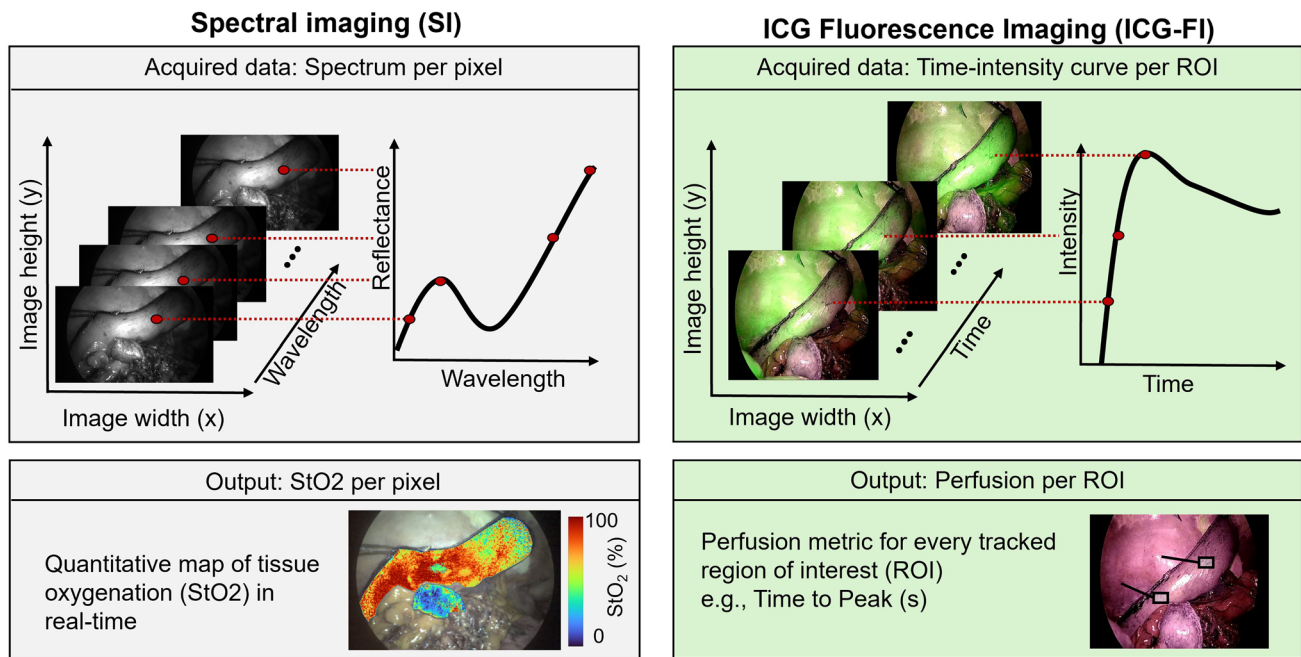


**Fig. 1** Comparison of spectral imaging (SI) and indocyanine green fluorescence imaging (ICG-FI). SI acquires pixel-wise spectra, which can be processed using deep learning models to generate tissue oxy-

genation maps. In contrast, ICG-FI captures time–intensity curves from selected regions of interest (ROIs), allowing for post hoc calculation of perfusion metrics

light cable connection, lens condensation or corrupted data files), resulting in a final cohort of 33 patients with complete data. This same subset of 33 patients was used consistently across all comparative analyses, ensuring a matched dataset between SI and ICG-FI. Patient demographics, clinical status and tumor characteristics are summarized in Table 1. In this cohort, five patients had postoperative AL (15%). The study was approved by the ethical committee of Ghent University Hospital (B670201836427), and written informed consent was obtained from all patients. The trial is registered at ClinicalTrials.gov (NCT03587532).

Inclusion criteria were: (a) age between 18 and 75 years, with ability and willingness to provide informed consent, (b) planned minimally invasive Ivor Lewis esophagectomy, and (c) intrathoracic circular stapled esophago-gastric anastomosis.

Exclusion criteria were: (a) pregnancy or lactation, (b) participation in other investigational studies, (c) use of anti-VEGF agents (e.g., Avastin) within 30 days prior to surgery, (d) intraoperative findings preventing study procedures, (e) non-standard anastomotic technique, and (f) excessive bleeding (> 500 mL) prior to anastomosis.

### Operative workflow and ROI selection

Standard minimally invasive Ivor Lewis esophagectomy was performed by experienced upper-GI surgeons, with intrathoracic circular stapled anastomosis. During the

thoracic phase, the gastric tube was mobilized into the chest. Prior to constructing the anastomosis and performing any imaging, three regions of interest (ROIs) were demarcated with sutures (Fig. 2): the surgeon-chosen anastomotic site (ROI-A), a well-perfused proximal region 2–3 cm from the pylorus (ROI-P), and an ischemic control region (ROI-I) excised from the proximal stomach. As this was an observational study, an a priori decision was established that SI and ICG-FI findings would not influence the surgeon's choice of anastomotic site, which was based on standard clinical criteria. Intra-operative decision-making and anastomosis site selection thus remained entirely dependent on the surgeon's clinical judgment. Selection of the ROIs is illustrated in Fig. 2.

Video-rate SI was performed with all ROIs in the field of view, immediately followed by ICG-FI after dye injection. ROI locations were matched across modalities. Local capillary lactate (LCL) was measured at all ROIs via serosal puncture using a handheld lactate analyzer (EDGE Analyzer, Apex Biotechnology, Taiwan, margin of error of 0.35 mmol/L) to further assess tissue hypoxia.

### Spectral imaging

#### Imaging hardware and data acquisition

Spectral recordings of the gastric graft were obtained using the XIMEA SNAPSHOT VIS camera (Imec, Belgium),

**Table 1** Baseline demographics, clinical status, and tumor characteristics of the esophagectomy cohort

| Characteristic                                        | Overall Cohort (n = 33) |
|-------------------------------------------------------|-------------------------|
| <i>Baseline demographics</i>                          |                         |
| Age (years), median (IQR)                             | 69.97 (61.67–74.44)     |
| Sex, n (%)                                            |                         |
| Male                                                  | 23 (69.70%)             |
| Female                                                | 10 (30.30%)             |
| BMI, median (IQR)                                     | 24.50 (22.50–27.63)     |
| Smoker, n (%)                                         | 9 (27.27%)              |
| <i>Clinical status</i>                                |                         |
| Physical status (ASA score), n (%)                    |                         |
| I–II (Healthy/mild systemic disease)                  | 5 (15.15%)              |
| III–IV (Severe systemic disease)                      | 28 (84.85%)             |
| Patients with $\geq 1$ Comorbidity, n (%)             | 16 (48.49%)             |
| <i>Tumor characteristics</i>                          |                         |
| Primary tumor location, n (%)                         |                         |
| Esophagus (middle third)                              | 5 (15.15%)              |
| Esophagus (lower third)                               | 13 (39.40%)             |
| Gastric cardia                                        | 15 (45.45%)             |
| Histology, n (%)                                      |                         |
| Adenocarcinoma                                        | 23 (69.70%)             |
| Squamous cell carcinoma                               | 10 (30.30%)             |
| Clinical stage (cTNM), n (%)                          |                         |
| Early (T1–T2)                                         | 8 (24.24%)              |
| Advanced (T3–T4)                                      | 23 (69.70%)             |
| Tx                                                    | 2 (6.06%)               |
| <i>Clinical outcomes</i>                              |                         |
| Anastomotic leakage (Clavien–Dindo at 90 days), n (%) |                         |
| IIIb                                                  | 4 (12.12%)              |
| IVa                                                   | 1 (3.03%)               |

capturing 16 spectral bands in the 460–600 nm range ( $460 \pm 9, 469 \pm 10, 475 \pm 16, 484 \pm 10, 496 \pm 16, 504 \pm 19, 514 \pm 15, 526 \pm 19, 532 \pm 17, 546 \pm 15, 550 \pm 15, 564$

$\pm 16, 570 \pm 13, 581 \pm 18, 589 \pm 17, 596 \pm 16$  nm (peak  $\pm$  full width at half maximum). This spectral resolution and range has shown to be sufficient for reliable StO<sub>2</sub> estimation [11, 15–17]. The sensor resolution is 2048×1088 pixels (5.5  $\mu$ m pixel pitch). The camera was mounted to a standard laparoscope via a C-mount zoom adapter (18–35 mm, RVA Synergies, UK) and coupled to a Xenon light source (CLV-S190, Olympus, Japan). The field of view was adjusted to include all three ROIs.

### Preprocessing and reflectance estimation

Radiometric calibration was applied [18]. Raw images underwent dark subtraction, followed by demosaicking using an algorithm developed by Muszyński and Luong [19]. Pixel spectra were corrected using the manufacturer-supplied matrix and normalized by the min-max spectrum of the light source. Further area-under-the-curve normalization compensated for variations in imaging distance and angle, preserving spectral shape critical for oxygenation estimation.

To create images familiar to the surgeon, synthetic RGB (sRGB) images were reconstructed by mapping bands at 600, 540, and 450 nm to R, G, and B channels, respectively, followed by histogram equalization and gamma correction to enhance visual clarity.

### Neural network-based oxygenation estimation

The deep learning framework for StO<sub>2</sub> estimation has been described in detail previously [12]. Briefly, a pixel-wise fully connected neural network (DA-FCN) was trained on  $6.4 \cdot 10^5$  Monte Carlo-simulated reflectance spectra with ground-truth oxygenation [12]. Domain-adversarial training addressed the gap between simulated and real in vivo data. The network generates real-time StO<sub>2</sub> maps at 16.8 ms per frame on an Nvidia GeForce RTX 4060 Laptop GPU.

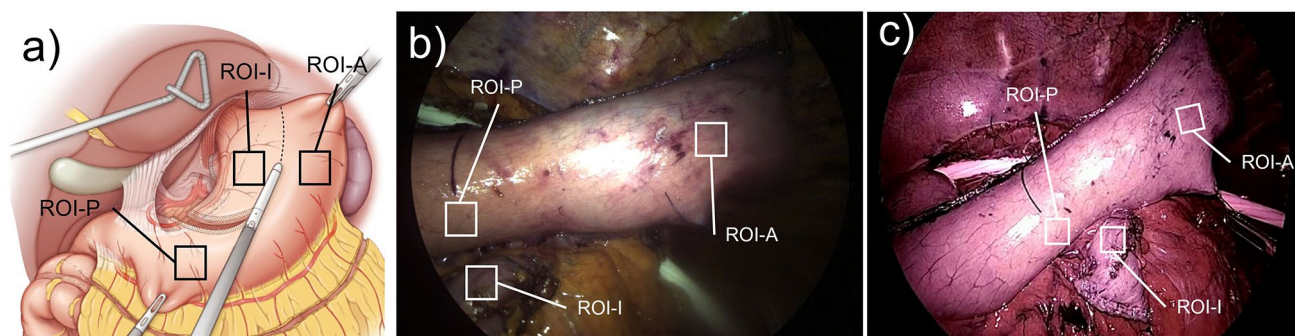


**Fig. 2** Region of interest (ROI) demarcation using sutures: ROI-A (anastomosis), ROI-P (well-perfused), and ROI-I (ischemic control). Shown on schematic of the gastric tube (a), reconstructed RGB image (b), and fluorescence imaging frame (c)

**Table 2** Definitions of perfusion metrics

| Metric                   | Definition                                    | Unit |
|--------------------------|-----------------------------------------------|------|
| <i>Ingress</i>           |                                               |      |
| $I_{\max}$               | Maximum intensity                             | AU   |
| Onset                    | Time when intensity exceeds 5% of maximum     | s    |
| Rise time                | Time from 10% to 90% of maximum               | s    |
| Inflow slope             | Rate of intensity increase (10%–90%)          | AU/s |
| TTP                      | Time from onset to maximum                    | s    |
| $T_{1/2}$                | Time from onset to 50% of maximum             | s    |
| $AUC_{50\%}$             | Area under curve from onset to 50% of maximum | AU   |
| $AUC_{100\%}$            | Area under curve from onset to maximum        | AU   |
| <i>Egress</i>            |                                               |      |
| $T_{80\%}$               | Time to drop from maximum to 80%              | s    |
| Outflow slope            | Rate of intensity decrease (max → 80%)        | AU/s |
| $I_{\text{end}}$         | Relative intensity at end of recording        | AU   |
| <i>Perfusion indices</i> |                                               |      |
| TR                       | $T_{1/2}/TTP$                                 | /    |
| Inflow/Outflow ratio     | Inflow slope/Outflow slope                    | /    |

## ICG fluorescence imaging

### Acquisition and preprocessing

A 0.5 mg/kg bolus of ICG (ICG-Pulsion, Pulsion Medical Systems, Germany) was administered via central venous catheter. Fluorescence imaging (Visera Elite II, Olympus, Japan) was recorded at 15 fps from immediately before injection for three minutes. ROIs were manually annotated based on sutures and tracked using PerfusionWorks software (PerfusionTech, Denmark), obtaining the time-intensity curves. Baseline subtraction used the first five seconds to correct for background fluorescence. The ischemic ROIs were excluded from analysis, as no dye perfused these resected areas.

### Perfusion metrics

The obtained time–intensity curves were low-pass filtered (4th-order Butterworth) and normalized by maximum intensity to reduce noise and mitigate inter-subject variability. This preserves relative perfusion dynamics and facilitates comparison between ROIs and across patients. Absolute metrics were computed, as summarized in Table 2, grouped into: ingress (wash-in), egress (wash-out), and global perfusion indices. Metric selection was guided by comprehensive reviews [20–22] and informed by prior studies on esophagectomy [23–25] and bowel or colonic perfusion [9, 10, 26, 27] with particular focus on anastomotic healing.

### Mapping perfusion and oxygenation along the graft

Points were manually annotated along the graft centerline on the first ICG-FI frame (avoiding specular reflections) and

tracked along the video recording using CoTracker [28]. For each  $5 \times 5$  pixel ROI around these points, mean green-channel intensity generated time–intensity curves, and were processed to obtain perfusion metrics as in Sect. "ICG fluorescence imaging".

Spectral images were registered to the first ICG-FI frame using their sRGB reconstructions. Registration was performed in two stages: (1) a perspective transformation based on LoFTR features [29], followed by (2) a non-rigid B-spline transformation using SuperGlue-based correspondences [30]. After registration, the tracked ICG-FI points were mapped onto the spectral image for pointwise comparison of StO<sub>2</sub> and perfusion metrics (Fig. 3).

### Clinical validation against capillary lactate measurements

The StO<sub>2</sub> predictions were compared with the measured LCL. This was normalized to each patient's preoperative systemic lactate to account for baseline variability. The relationship was modeled exponentially [12]:

$$LCL = e^{A \cdot \text{StO}_2 + B} \quad (1)$$

where  $A$  and  $B$  are fitted parameters. For ICG-FI perfusion metrics, linear regression was used [31, 32]:

$$LCL = C + D \cdot \text{Perfusion metric} \quad (2)$$

where fitting parameters  $C$  and  $D$  were estimated via ordinary least squares, and the quality of the fit was quantified using the mean absolute error (MAE) and the correlation coefficient ( $r$ ).

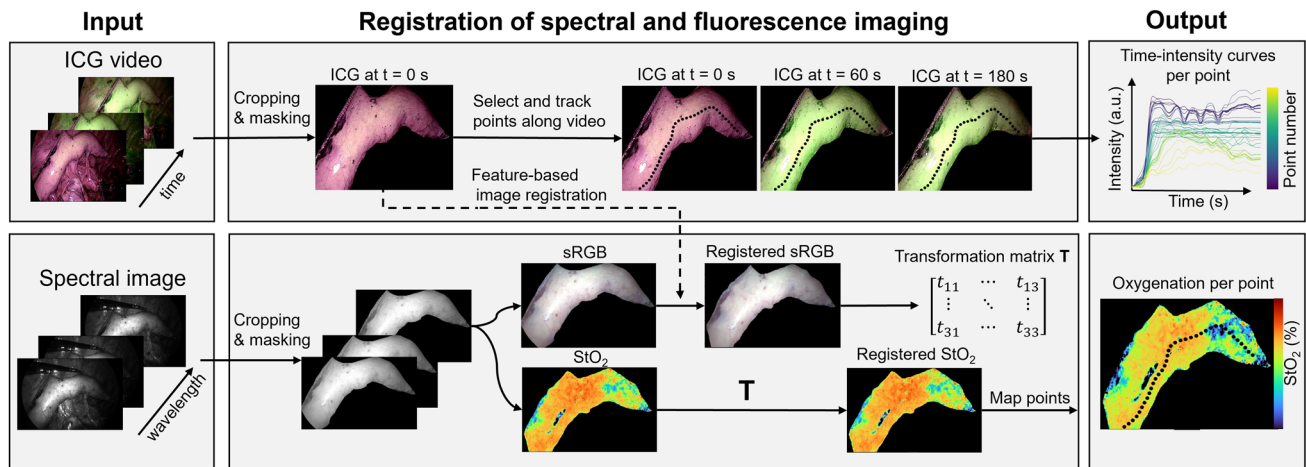


**Fig. 3** Workflow for registering tissue oxygenation maps to fluorescence imaging frames and extracting paired pointwise metrics along the graft

## Results

The primary endpoint of this study was to investigate the ability of spectral imaging–derived  $\text{StO}_2$  to discriminate between perfused and ischemic tissue. Secondary endpoints included comparison with quantitative ICG-FI perfusion metrics and local capillary lactate levels.

In each patient, three ROIs were defined intraoperatively, before any imaging was performed: a well-perfused control region located 2–3 cm proximal to the pylorus (ROI-P), the selected anastomosis site (ROI-A), and an ischemic region consisting of excised gastric tissue (ROI-I). LCL levels were measured for each ROI as a physiological reference for tissue hypoxia. Spectral imaging results were obtained for all three ROIs, whereas ICG-FI metrics were available only for ROI-P and ROI-A, as excised tissue does not receive fluorescent dye.

Due to the limited sample size of this study, it lacks statistical power to evaluate the predictive value of SI and ICG-FI

for AL. As an exploratory analysis, the predicted  $\text{StO}_2$  values and ICG-FI perfusion metrics at the anastomosis site are compared between the patient cohort with and without AL. In addition, an anecdotal case study is presented comparing the two modalities in a patient who developed leakage versus one who did not, illustrating their potential clinical utility for guiding anastomosis placement.

### Spectral imaging reveals impaired oxygenation at ischemic tissue sites

This analysis addresses the primary endpoint. Figure 4 presents boxplots of the neural network–estimated  $\text{StO}_2$  across the different ROIs. For comparison, values derived from conventional linear unmixing are also shown [33].

The  $\text{StO}_2$  values are expressed as Median [IQR], and were 69.16 [66.26 – 72.46]% for ROI-P, 68.73 [62.58 – 72.03]% for ROI-A, and 31.58 [24.13 – 36.11]% for ROI-I. The  $\text{StO}_2$  was significantly lower in ROI-I compared with ROI-P and ROI-A, using a Mann–Whitney U-test ( $p < 0.001$ ). No significant difference was observed between ROI-P and ROI-A ( $p = 0.46$ ).

A logistic regression classifier trained to distinguish ROI-P from ROI-I achieved an accuracy of  $0.92 \pm 0.18$  and an area under the receiver operating characteristic curve (AUC) of  $0.97 \pm 0.17$  using leave-one-patient-out cross-validation. The optimal discrimination threshold was  $52.90 \pm 15.54\%$ . Values are expressed as mean  $\pm$  standard deviation.

Compared with linear unmixing, neural network–derived estimates for perfused and anastomotic tissue were lower and demonstrated a reduced interquartile range.

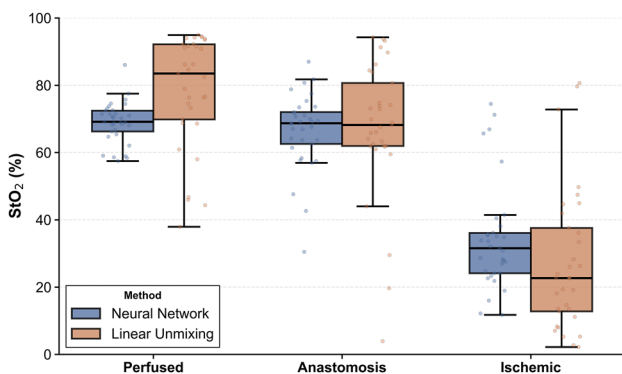


**Fig. 4** Boxplot of tissue oxygenation ( $\text{StO}_2$ ) values across perfused (ROI-P), anastomosis (ROI-A), and ischemic (ROI-I) tissue regions ( $n = 33$  patients)

**Table 3** Comparison of ingress, egress and perfusion metrics values between anastomosis (ROI-A) and perfused (ROI-P) regions. Median, first quartile (Q1), and third quartile (Q3) values are reported, along with their scaling factors ( $\cdot 10^n$ ) and corresponding p-values

| Metric                   | Anastomosis |      |      |     | Perfused |      |      |     | Scale<br>( $\cdot 10^n$ ) | $p$ -value                     |
|--------------------------|-------------|------|------|-----|----------|------|------|-----|---------------------------|--------------------------------|
|                          | Median      | Q1   | Q3   | $n$ | Median   | Q1   | Q3   | $n$ |                           |                                |
| <b>Ingress</b>           |             |      |      |     |          |      |      |     |                           |                                |
| T <sub>1/2</sub>         | 4.47        | 3.93 | 5.07 | 33  | 3.27     | 3.13 | 3.67 | 33  | —                         | <b>4.43</b> · 10 <sup>−9</sup> |
| AUC <sub>50%</sub>       | 1.12        | 0.98 | 1.32 | 33  | 0.80     | 0.78 | 0.90 | 33  | —                         | <b>5.80</b> · 10 <sup>−9</sup> |
| Inflow slope             | 0.81        | 0.61 | 1.05 | 33  | 1.38     | 1.12 | 1.43 | 33  | −1                        | <b>1.41</b> · 10 <sup>−6</sup> |
| Rise time                | 0.99        | 0.76 | 1.31 | 33  | 0.58     | 0.56 | 0.71 | 33  | 1                         | <b>1.41</b> · 10 <sup>−6</sup> |
| TTP                      | 1.75        | 1.28 | 2.37 | 33  | 1.25     | 0.89 | 1.79 | 33  | 1                         | <b>2.58</b> · 10 <sup>−3</sup> |
| AUC <sub>100%</sub>      | 1.20        | 0.84 | 1.69 | 33  | 0.81     | 0.55 | 1.43 | 33  | 1                         | <b>1.33</b> · 10 <sup>−2</sup> |
| Onset                    | 2.19        | 1.92 | 2.63 | 33  | 1.91     | 1.63 | 2.19 | 33  | 1                         | <b>1.51</b> · 10 <sup>−2</sup> |
| I <sub>max</sub>         | 1.04        | 0.86 | 1.15 | 33  | 0.89     | 0.74 | 1.13 | 33  | 2                         | 1.33 · 10 <sup>−1</sup>        |
| <b>Egress</b>            |             |      |      |     |          |      |      |     |                           |                                |
| T <sub>80%</sub>         | 6.25        | 5.40 | 7.75 | 27  | 6.18     | 5.03 | 8.37 | 26  | 1                         | 6.44 · 10 <sup>−1</sup>        |
| I <sub>end</sub>         | 5.67        | 4.16 | 7.64 | 33  | 6.59     | 3.96 | 7.95 | 33  | −1                        | 8.03 · 10 <sup>−1</sup>        |
| Outflow slope            | 0.80        | 0.60 | 1.25 | 27  | 0.80     | 0.50 | 1.38 | 26  | −2                        | 9.57 · 10 <sup>−1</sup>        |
| <b>Perfusion Indices</b> |             |      |      |     |          |      |      |     |                           |                                |
| Inflow/Outflow ratio     | 1.14        | 0.80 | 1.73 | 27  | 1.44     | 0.84 | 2.68 | 26  | 1                         | 3.15 · 10 <sup>−1</sup>        |
| TR                       | 2.94        | 2.30 | 3.37 | 33  | 3.06     | 1.92 | 3.65 | 33  | −1                        | 8.27 · 10 <sup>−1</sup>        |

Bold p-values indicate statistical significance

### Fluorescence imaging highlights lower perfusion at anastomotic sites

This analysis addresses the secondary endpoint comparing quantitative ICG-FI perfusion metrics between ROIs. For egress-phase parameters, six patients (in addition to the five excluded earlier) were excluded because of incomplete dye wash-out acquisition, resulting in  $n = 27$  and  $n = 26$  for ROI-A and ROI-P, respectively.

Table 3 summarizes median, first quartile (Q1), and third quartile (Q3) values for all metrics, ordered by statistical significance. Definitions are provided in Sect. "Perfusion metrics". Metrics are categorized as ingress (arterial inflow), egress (venous washout), and combined perfusion indices.

Among ingress-related parameters, all metrics except  $I_{\max}$  differed significantly between ROI-P and ROI-A at  $\alpha = 0.05$ . The most significant parameters were  $T_{1/2}$  and  $AUC_{50\%}$  ( $p < 0.001$ ). The median  $T_{1/2}$  was 27% lower in ROI-P (3.27 s vs 4.47 s), and the median  $AUC_{50\%}$  was 29% lower in ROI-P (0.80 vs 1.12 AU), indicating faster and more efficient dye inflow dynamics in well-perfused tissue. Additionally, significant differences were observed for the inflow slope, rise time and time-to-peak ( $p < 0.001$ ). Furthermore,  $AUC_{100\%}$  ( $p = 0.013$ ) and onset time ( $p = 0.015$ ) showed significant differences. In contrast, none of the egress-related parameters reached statistical significance, including  $T_{80\%}$ ,  $I_{\text{end}}$ , and outflow slope. Similarly, combined perfusion indices such as inflow/outflow ratio and TR did not differ significantly between groups ( $p > 0.05$ ). The interquartile ranges

were generally larger in ROI-A compared with ROI-P, indicating greater variability at the chosen anastomotic site.

### Correlation of tissue oxygenation, perfusion, and capillary lactate

This analysis addresses the secondary endpoint comparing SI-derived oxygenation and quantitative ICG-FI perfusion metrics with the measured LCL levels.

The normalized LCL levels were 1.88 [1.50–2.41] mmol/L in ROI-P and 1.88 [1.50–2.51] mmol/L in ROI-A, with no significant difference between these regions ( $p = 0.74$ ). In contrast, ROI-I showed significantly higher lactate levels compared with ROI-P and ROI-A ( $p < 0.001$ ), with a median of 7.44 [5.22–9.00] mmol/L. Values are expressed as Median [IQR] and taken from the patient cohort of 33 patients.

Figure 5 illustrates the relationships between  $StO_2$ ,  $AUC_{50\%}$ , and LCL. Prior to model fitting, outliers were excluded using a Z-score threshold of 3. An exponential model captured the inverse relationship between  $StO_2$  and LCL, yielding a strong negative correlation ( $r = -0.72$ ,  $p < 0.001$ ,  $n = 32$ ) with a median absolute error (MAE) of 0.90 mmol/L:

$$\text{Normalized local capillary lactate} = e^{-0.023 \cdot StO_2 + 2.48} \quad (3)$$

This indicates that LCL increases exponentially as  $StO_2$  decreases, consistent with physiological expectations of anaerobic metabolism under hypoxic conditions [34]. Linear

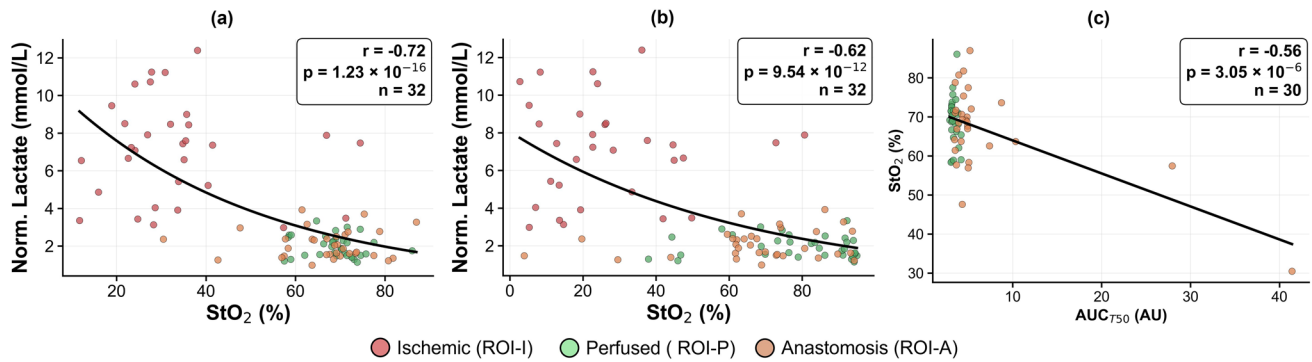


**Fig. 5** Relationships between tissue oxygenation ( $\text{StO}_2$ ), perfusion metric ( $\text{AUC}_{50\%}$ ), and normalized local capillary lactate (LCL). Exponential fits are shown for  $\text{StO}_2$  estimated using the neural network (a) and linear unmixing (b) approaches versus LCL, while a

linear fit is shown for  $\text{StO}_2$  versus  $\text{AUC}_{50\%}$  (c). Pearson correlation coefficients ( $r$ ), corresponding  $p$ -values, and the number of patients ( $n$ ) are indicated

unmixing-derived  $\text{StO}_2$  showed a weaker correlation with LCL ( $r = -0.62$ ,  $p < 0.001$ ,  $n = 32$ ) and a higher MAE of 0.99 mmol/L.

A significant linear relationship was also observed between  $\text{StO}_2$  and  $\text{AUC}_{50\%}$  ( $r = -0.56$ ,  $p < 0.001$ ,  $n = 30$ ), suggesting that higher perfusion (indicated by lower  $\text{AUC}_{50\%}$ ) is associated with increased tissue oxygenation. The corresponding linear fit had an MAE of 3.55%:

$$\text{StO}_2 = -0.85 \cdot \text{AUC}_{50\%} + 72.42 \quad (4)$$

No significant correlation was observed between LCL and  $\text{AUC}_{50\%}$  ( $p = 0.80$ ).

### Oxygenation and perfusion in patients with and without anastomotic leakage

Table 4 summarizes the  $\text{StO}_2$  and five most significant ICG-FI perfusion metrics at the anastomosis site for patients

who developed AL versus those who did not. In addition,  $p$ -values comparing these values among the two groups were obtained from Mann-Whitney U-tests. Due to the limited sample size, these results are purely exploratory.

The five patients with AL had a mean  $\text{StO}_2$  of  $63.20 \pm 26.90\%$ , which was lower than the mean  $\text{StO}_2$  of  $75.7 \pm 15.00\%$  observed in patients without AL, although this difference did not reach statistical significance ( $p = 0.51$ ). However, significant differences were observed for all the given ICG-FI perfusion metrics ( $p < 0.05$ ). Patients with AL exhibited a longer rise time, a slower inflow slope, a longer  $T_{1/2}$ , a higher  $\text{AUC}_{50\%}$ , and a higher relative intensity at the end of recording  $I_{\text{end}}$ , compared with patients without leakage.

Leakage patients 1 and 3 had notably low  $\text{StO}_2$  values of 48.02% and 23.22%, respectively, which were below the optimal discrimination threshold of 52.90% identified in the primary analysis. Remarkably, these two patients also exhibited the most impaired perfusion metrics among the leakage

**Table 4** Comparison of  $\text{StO}_2$  and ICG-FI perfusion metrics at the anastomosis site between patients with and without anastomotic leakage. Values are expressed as mean  $\pm$  standard deviation

|                      | Spectral Imaging     | ICG-FI               |                      |                      |                    |                    |
|----------------------|----------------------|----------------------|----------------------|----------------------|--------------------|--------------------|
|                      | $\text{StO}_2$ (%)   | Rise time            | Inflow slope         | $T_{1/2}$            | $\text{AUC}_{50}$  | $I_{\text{end}}$   |
| Leakage              |                      |                      |                      |                      |                    |                    |
| 1                    | 48.02                | 75.87                | 0.011                | 27.93                | 9.44               | 1.00               |
| 2                    | 75.94                | 13.07                | 0.061                | 4.73                 | 1.26               | 0.62               |
| 3                    | 23.22                | 58.27                | 0.014                | 41.40                | 11.33              | 0.95               |
| 4                    | 83.67                | 10.20                | 0.078                | 5.00                 | 1.25               | 0.57               |
| 5                    | 85.19                | 16.33                | 0.049                | 4.87                 | 1.28               | 0.79               |
| Overall ( $n = 5$ )  | 63.20<br>$\pm 26.90$ | 34.75<br>$\pm 30.23$ | 0.043<br>$\pm 0.029$ | 16.79<br>$\pm 17.00$ | 4.91<br>$\pm 5.04$ | 0.79<br>$\pm 0.19$ |
| No leakage           |                      |                      |                      |                      |                    |                    |
| Overall ( $n = 28$ ) | 75.7<br>$\pm 15.00$  | 13.43<br>$\pm 15.26$ | 0.089<br>$\pm 0.034$ | 4.80<br>$\pm 1.58$   | 1.19<br>$\pm 0.37$ | 0.55<br>$\pm 0.22$ |
| p-value              | 0.51                 | 0.011                | 0.011                | 0.042                | 0.022              | 0.026              |

group, with rise times of 75.87 s and 58.27 s, inflow slopes of 0.011 AU/s and 0.014 AU/s,  $T_{1/2}$  values of 27.93 s and 41.40 s,  $AUC_{50\%}$  values of 9.44 AU and 11.33 AU, and  $I_{end}$  values of 1.00 AU and 0.95 AU, respectively. Leakage patients 2, 4, and 5 had  $StO_2$  values above this threshold (75.94%, 83.67%, and 85.19%, respectively), suggesting that  $StO_2$  alone may not be sufficient to predict AL risk in all cases.

### Case study: tissue ischemia monitoring to guide anastomosis placement

Figure 6 compares SI and ICG-FI in two patients: one without postoperative AL and one who developed AL. According to Kumagai et al. [35], a suitable anastomosis site should exhibit a clear fluorescent signal within 60 s after dye injection. Accordingly, the first and 60 s post-injection frames of the ICG-FI video are shown. The black dots indicate the selected points along the stomach graft, allowing a direct comparison between perfusion metrics and  $StO_2$ . The black line denotes the anastomosis site chosen by the surgeon, and the dotted line represents the ischemic threshold of 52.90%  $StO_2$  determined by the classifier in Sect. "Spectral imaging reveals impaired oxygenation at ischemic tissue sites". As such, the dotted line corresponds to the point along the graft where  $StO_2$  falls below the threshold for distinguishing perfused from ischemic tissue.

Figure 7 illustrates the gradient of  $StO_2$  and perfusion metrics along the points. The perfusion metrics  $AUC_{50\%}$ ,

rise time, inflow slope, and  $I_{max}$  were chosen for their robust display of the perfusion gradient along the graft.

For the patient without AL, ICG-FI displays high signal intensity 60 s post-dye injection at the anastomosis site, matching the high  $StO_2$  levels. Proximal to the ischemic threshold, the anastomosis line resides at a well-oxygenated margin ( $StO_2 = 68.30\%$ ). Key perfusion metrics reinforce this viability:  $AUC_{50\%} = 2.95$  AU, rise time = 19.20 s, inflow slope =  $0.042\text{ s}^{-1}$ , and  $I_{max} = 96.31$ . Toward the graft tip,  $StO_2$  and perfusion gradually decrease.

The patient with AL shows a high intensity 60 s post-dye injection at the anastomosis site, although adjacent to a low-intensity boundary. In contrast, SI shows a low  $StO_2$  region surrounding the anastomosis, with the ischemic threshold occurring more proximally. Quantitatively, this patient's anastomosis site showed an  $StO_2$  of 54.44%,  $AUC_{50\%}$  of 9.59 AU, rise time of 71.80 s, inflow slope of  $0.011\text{ s}^{-1}$ , and  $I_{max}$  of 78.21. This suggests that the anastomotic site was positioned too distally within the oxygenation and perfusion gradient, potentially contributing to the developed AL.

### Discussion

This study demonstrates the feasibility of SI-based intra-operative  $StO_2$  to assess gastric conduit ischemia during esophagectomy, providing a direct quantitative comparison

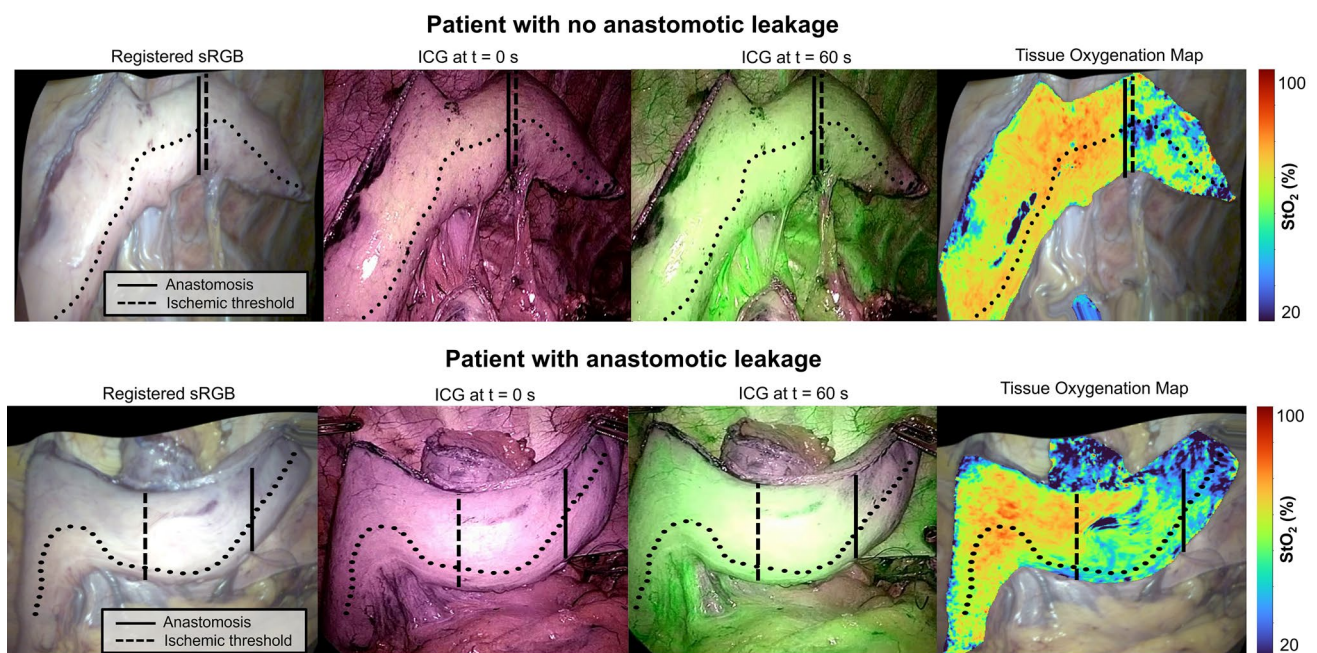


**Fig. 6** Comparison of spectral and fluorescence imaging in two patients: one without anastomotic leakage (AL) in the top row and one with AL in the bottom row. From left to right: registered sRGB image, first ICG-FI frame, frame at 60 s post-injection, and registered

SI-derived tissue oxygenation map. Black dots indicate tracked points along the graft, while the black line shows the selected anastomosis site and the black dotted line marks the ischemic threshold

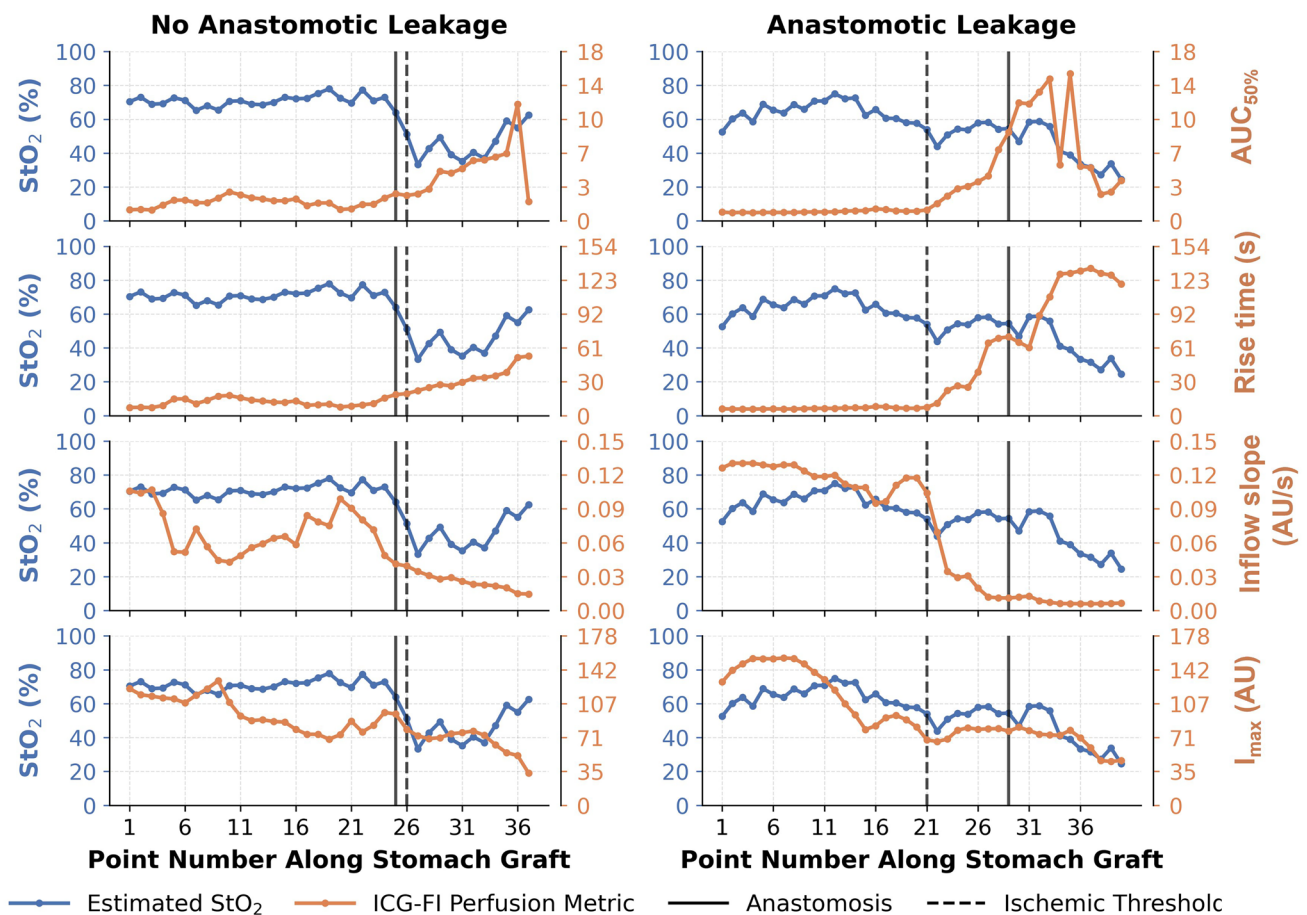


**Fig. 7** Comparison of  $\text{StO}_2$  with selected ICG-FI perfusion metrics along annotated points on the stomach graft for patients with and without anastomotic leakage. Lower perfusion is indicated by higher

$\text{AUC}_{50\%}$  and Rise time, and lower Inflow slope and  $I_{\max}$ . Vertical black lines indicate the anastomosis site and vertical dashed lines indicate the ischemic threshold

with ICG-FI. The primary challenge in preparing the gastric conduit and preventing AL is the identification of the demarcation between perfused and ischemic tissue by the operating surgeon. Our findings indicate that both SI and ICG-FI successfully identify tissue ischemia, and capture the ischemic gradient toward the distal tip of the graft, with SI providing a real-time assessment of actual tissue hypoxia. The compact snapshot spectral camera allowed for seamless OR integration by attachment to a standard laparoscope. It provided continuous, video-rate  $\text{StO}_2$  mapping without the need for large scanning equipment, contrast injections, or added operative time.

The median  $\text{StO}_2$  in well-perfused gastric tissue was approximately 69.16% and significantly different from ischemic tissue, aligning with other spectral imaging studies, reporting values around 70% [7, 8, 36, 37]. However, reported values vary in the context of conduit formation. Safi et al. observed an average  $\text{StO}_2$  decrease of 16% after creation of the gastric conduit, reflecting the physiological

impact of vascular division, which should be considered when comparing absolute  $\text{StO}_2$  values across studies and clinical settings [36]. Moreover, we found no significant difference in  $\text{StO}_2$  or LCL between the selected anastomosis site and the well-perfused control region, suggesting the surgeons generally selected suitable anastomosis sites in this cohort.

Based on receiver operating characteristic analysis, an  $\text{StO}_2$  threshold of 52.90% was identified to discriminate perfused from ischemic gastric tissue. This range is consistent with previously proposed thresholds of approximately 60% in colorectal surgery [27] and around 50% in plastic and vascular surgery [8]. While promising, such thresholds remain system- and cohort-dependent and are derived from the most perfused and ischemic regions. In clinical practice, the relevant threshold for anastomosis selection would likely be higher. Moreover, the leave-one-patient out calculation showed a relatively high standard deviation of 15.54% for this threshold, indicating that the optimal threshold may vary

significantly between patients. This underscores the need for larger studies to establish robust, generalizable thresholds for clinical decision-making.

StO<sub>2</sub> demonstrated a statistically significant correlation with LCL, supporting its physiological validity as a marker of tissue hypoxia. In contrast, ICG-derived perfusion metrics showed only moderate or non-significant correlations. This likely reflects the fact that ICG-FI metrics are calculated solely from clinically well-perfused tissue, as no dye enters the ischemic control tissue. Detecting significant associations between fluorescence metrics and LCL likely requires sampling more physiological states. However, previous pre-clinical studies also reported stronger correlations between StO<sub>2</sub> and LCL compared to ICG-FI parameters, although most were conducted in small animal models [6, 10, 31].

Among quantitative ICG-FI parameters, ingress-related metrics ( $T_1$ , AUC<sub>50%</sub>, rise time, and inflow slope) demonstrated the strongest discriminatory performance, whereas intensity-based parameters such as  $I_{\max}$  appeared less robust, consistent with prior reports [20, 22]. Egress-related metrics did not reach statistical significance, likely due to the limited three-minute acquisition window. Longer recordings are recommended in future studies to enable reliable assessment of wash-out dynamics, which may provide additional information regarding venous congestion. Because this study did not include a sufficiently large cohort with clinically confirmed leakage, no definitive ICG-FI thresholds were established. Nevertheless, previously reported reference values provide useful context. Suggested cut-offs include TTP > 30 s,  $T_1 > 10$  s, and abnormal inflow slope values, although these vary widely depending on imaging platform and analysis methodology [21, 24, 26, 27]. In esophagectomy specifically, delayed fluorescence intensity beyond 60 s has been associated with impaired perfusion [35]. These observations underscore the ongoing need for standardization of quantitative fluorescence metrics.

In the exploratory comparison between patients with and without AL, quantitative ICG-FI ingress metrics at the anastomosis site were significantly different between groups, whereas StO<sub>2</sub> alone did not reach significance. This likely reflects the multifactorial nature of AL. While reduced perfusion and tissue hypoxia frequently co-occur, as illustrated by leakage patients 1 and 3, other contributing factors including technical, mechanical, and patient-related variables may lead to AL, as seen in leakage patients 2, 4, and 5. Together, these observations suggest that perfusion and oxygenation metrics provide complementary rather than redundant information. However, given the small leakage cohort ( $n = 5$ ), these findings are exploratory and should not be interpreted as evidence of superiority of either modality for outcome prediction.

Both imaging modalities demonstrated a clear decline in oxygenation or perfusion toward the distal conduit. In an anecdotal case study of a patient who developed AL, ICG-FI demonstrated high fluorescence intensity at the chosen anastomosis site, whereas SI revealed that this region was already hypoxic. This finding suggests that although StO<sub>2</sub> mapping and ICG-FI are complementary, they visualize different physiological phenomena. Similar observations have been reported by Hennig et al. and Studier-Fischer et al., who described a more peripheral ischemic boundary with ICG-FI compared with SI in esophageal and small bowel surgery [9, 13]. This discrepancy likely stems from the different light penetration depths of the two modalities. ICG-FI captures fluorescence signals up to 6 mm deep, reflecting deeper vascular inflow and potential dye pooling in the tissue bed. In contrast, SI evaluates the superficial serosal and subserosal microcirculation at a depth of up to 2 mm. Additionally, diffusion of ICG dye into partially ischemic regions may further alter perfusion boundaries, as discussed by Diana et al. [38]. As such, both modalities could offer complementary insights, with StO<sub>2</sub> potentially providing a more direct assessment of the tissue's metabolic viability, while ICG-FI reflects macro-perfusion dynamics.

An important consideration when interpreting these metrics is the differentiation between arterial inflow-related hypoxia and venous congestion. SI measures StO<sub>2</sub>, which can drop in both scenarios, either due to a lack of freshly oxygenated arterial blood or the pooling of deoxygenated blood in congested veins, as demonstrated by Fischer et al. [39]. ICG-FI, particularly its wash-out dynamics, is theoretically better suited to specifically evaluate venous congestion. Our limited three-minute acquisition window restricted the evaluation of egress metrics. Future studies with extended monitoring could clarify the modalities' combined ability to differentiate these states.

Due to the limited sample size of this study, it lacked statistical power to formally evaluate the predictive value of SI and ICG-FI for AL. Crucially, the lack of outcome-based validation limits the definitive clinical applicability of these findings. The evidence supporting the potential of SI is primarily derived from an exploratory analysis and an anecdotal case presentation. Therefore, conclusions regarding the superiority of one modality over the other cannot be drawn. Rather, they should be viewed as providing complementary insights. Future large-scale, multicenter trials are essential to correlate StO<sub>2</sub> and ICG-FI metrics with clinical outcomes, such as the Clavien–Dindo classification, and to establish statistically robust predictive models.

## Conclusion

This study demonstrates the clinical feasibility of utilizing intraoperative spectral imaging (SI) and indocyanine green fluorescence imaging (ICG-FI) to assess gastric conduit viability during minimally invasive esophagectomy. While both modalities successfully map the progressive ischemic gradient toward the distal tip, they yield distinct physiological insights. SI provides continuous, contrast-free mapping of superficial tissue oxygenation (StO<sub>2</sub>) showing a strong correlation with local capillary lactate, suggesting it may be sensitive to early metabolic hypoxia. In contrast, ICG-FI captures deeper vascular macro-perfusion dynamics, with ingress-related metrics serving as robust indicators of blood inflow. Crucially, our findings indicate that both modalities are complementary.

Future large-scale trials with outcome-based validation are essential to translate the obtained metrics into clinically validated thresholds, ultimately aiming to optimize anastomosis site selection and reduce postoperative anastomotic leakage.

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## Declarations

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