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Abstract. Fluorescence imaging is a well-established optical modality that has been used to localize and track fluorophores *in vivo* and has demonstrated great potential for surgical guidance. Despite the variety of fluorophores currently being researched, many existing intraoperative fluorescence imaging systems are specifically designed for a limited number of applications. We present a modular wide-field fluorescence overlay tissue imaging system for intraoperative surgical guidance that is comprised of commercially available standardized components. Its modular layout allows for the accommodation of a broad range of fluorophores, fields of view (FOV), and spatial resolutions while maintaining an integrated portable design for intraoperative use. Measurements are automatic and feature a real-time projection overlay technique that intuitively displays fluorescence maps directly onto a 15 × 15 cm² FOV from a working distance of 35 cm. At a 20-ms exposure time, 10 μM samples of indocyanine green could be measured with high signal-to-noise ratio and was later tested in an *in vivo* mouse model before finally being demonstrated for intraoperative autofluorescence imaging of human soft tissue sarcoma margins. The system's modular design and ability to enable naked-eye visualization of wide-field fluorescence allow for the flexibility to adapt to numerous clinical applications and can potentially extend the adoption of fluorescence imaging for intraoperative use. © 2018 Society of Photo-Optical Instrumentation Engineers (SPIE) [DOI: 10.1117/1.JMI.5.2.021220]

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1 Introduction

Fluorescence imaging is a well-established optical modality that spatially measures the spectral emissions arising from fluorescent chemical molecules when excited by a light source. Fluorophores can be naturally occurring or synthesized and can span a wide range of excitation and emission wavelengths. Over the past two decades, there have been many major advances regarding the utilization of fluorescence imaging for noninvasive diagnosis and surgical guidance. Exogenous ultraviolet (UV) to near-infrared (NIR) contrast agents, such as 5-aminolevulinic acid-induced protoporphyrin IX (PpIX), and FDA-approved indocyanine green (ICG) have demonstrated a great potential for visualizing angiographic applications, improving the gross resection of malignant gliomas, and maximizing the survival benefits associated with surgical excisions, in which tissue conservation is a high priority.^{1–6} In particular, PpIX has absorption peaks ranging from the UV to visible wavelengths at 405, 510, 545, 584, and 635 nm while emitting in the NIR at 635 and 705 nm.⁷ Conjugation of visible to NIR dyes, such as Cyanine5.5, DyLight 680, and IRDye 800CW, with tumor-specific peptides and antibodies can even allow for the development of specialized probes capable of improving the detection of cancers *in vivo* and has been explored in various

animal models so far.^{2,8–12} In addition, UV to visible autofluorescence has been demonstrated to differentiate between normal and neoplastic tissue based on the changes of endogenous fluorophores and environment related to disease progression.^{13,14} This has been researched as a possible diagnostic tool in various organ systems, including the brain,^{15–18} bronchus,¹⁹ colon,²⁰ cervix,²¹ bladder,²² esophagus,²³ skin,²⁴ breast,²⁵ and arterial wall.²⁶ Recently, probe-based NIR autofluorescence spectroscopy has been shown to have potential for differentiating soft tissue sarcomas (STS) from the surrounding normal muscle and fat within a surgical bed without the use of any exogenous contrast agents.¹⁴ This in itself can allow for the detection of residual tumors during surgical resection and help decrease the need for additional therapies, such as postoperative radiation and/or re-excision. With an extensive variety of applications and fluorophores each with their own unique optical properties that span a wide range of wavelengths, currently, there does not exist a single fluorescence imaging system that can be conveniently configured to measure a broad range of fluorophores over a wide field of view (FOV) while being optimized for use in an operating room setting.

Fluorescence imaging at its core consists of three primary components: a light source for fluorophore excitation, an imaging sensor for collecting light emission, and a set of optical filters for selective filtering of the emitted light. Despite the

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relative simplicity in hardware, there are many design criteria that must be accounted for to translate a laboratory research system into an intraoperative environment. These include considerations of working distance, FOV, sensitivity, physical size, ability to work in a sterile environment, and software for control and data processing. Additionally, emphasis must also be directed toward ergonomics and the ability to relay accurate spatial fluorescence information back to the surgeon in a manner that is both intuitive, real-time, and nonobtrusive to normal surgical workflow. It is estimated that 30% to 40% of errors in surgical imaging are due to interpretive errors related to lack of image context, time-consuming image analysis, and the need to mentally coregister images displayed on a distant monitor to the actual surgical bed.^{27,28} As a whole, these challenges lessen the effectiveness of image-guided surgery, and it is crucial that many of these design constraints be addressed for intraoperative fluorescence imaging to gain widespread use in the operating room.

Currently, there are numerous FDA 510(k)-approved clinical fluorescence imaging systems available commercially with varying feature sets related to illumination wavelengths, emission collection wavelengths, sensitivity, cost, ergonomics, optics, and image output.^{29,30} Lower cost small profile systems, such as the Hamamatsu PDE Neo, are capable of hand-held operation but can only provide qualitative images at relatively small FOVs ($\sim 10 \times 6.7 \text{ cm}^2$), low pixel dimensions (640×480), short working distances ($\sim 25 \text{ cm}$), and low bit-depth (8 bit).³⁰ More widely utilized devices, such as the higher-end Novadaq SPY Elite articulated arm system, are capable of measurements at similar FOVs but with higher pixel dimensions (1024×768) and long working distances ($\sim 34 \text{ cm}$).³⁰ Newer research grade devices that are not FDA-approved, such as the Curadel Lab-Flare articulated arm system, are much more fully featured and can operate at large FOVs ($\sim 25.3 \times 25.3 \text{ cm}^2$), high pixel dimension (1024×1024), long working distances ($\sim 45 \text{ cm}$) and are capable of simultaneous multifluorophore imaging

over a range of distinct wavelengths from 470 to 760 nm.²⁹ Some devices, such as the Quest Spectrum feature methods, for background light corrected measurements while many others do not.³⁰ Despite variations in terms of cost and features, many are qualitative, dependent on remote monitors for displaying fluorescence maps, and are hardware confined to a small number of excitation and emission windows. As intraoperative needs are continually changing along with the discovery of newer fluorophores and applications, these strict hardware limitations can hinder the clinical adoption of fluorescence imaging as a whole.

Here, we present a modular fluorescence overlay tissue imaging system (OTIS) aimed at wide-field intraoperative surgical guidance. Unlike most commercial systems that rely on remote monitors, the device presented here works in real time to project fluorescence maps directly on to the surgical field in a manner similar to vein finders, an intraoperative system from the Akers group,^{31,32} and a benchtop system described by Gan et al.³³ In addition to providing a surgeon with intuitive feedback, the system is built entirely from standardized commercial components and features a modular layout that can allow the system's FOV, spatial resolution, working distance, and spectral range to be easily customized depending on application. Measurements are automatic and features background light correction that allows for use in low ambient lighting environments. The system was validated in preliminary *ex vivo* studies using ICG and tested in an *in vivo* mouse model before being demonstrated for intraoperative autofluorescence imaging in human patients undergoing STS resections.

2 Methods

2.1 Fluorescence Overlay Tissue Imaging System

A diagram illustrating the basic design and layout of the imaging head along with a photograph of the OTIS setup can be seen in Fig. 1.

Fig. 1 (a) Diagram illustrating the basic design and layout of the OTIS imaging head and (b) image of the entire fluorescence imaging system, including the transport cart and articulated arm.

Fig. 2 Filter spectral artifacts when collecting at nonnormal angles of incidence during wide-field imaging: (a) example of a traditional thin-film interference filter and (b) example of a hybrid longpass filter under the same imaging conditions.

For studies presented in this paper, the device's excitation source was fitted with a 785-nm diode laser (Innovative Photonic Solutions I0785MM0500MS, Monmouth Junction, New Jersey) fiber coupled via a SMA905 connector to a 785-nm notch filter (LL01-785, Semrock Inc., Rochester, New York) and a double concave lens for beam divergence. Image collection was accomplished using a 12-bit NIR complementary metal–oxide–semiconductor (CMOS) camera (Baslar AG acA1300-60gmNIR, Ahrensburg, Germany) equipped with a c-mounted manual iris lens (Ricoh FL-CC1214A-2m, Tokyo, Japan) and a c-mounted machine vision 830-nm long-pass filter (Midwest Optical Systems Inc., LP830, Palatine, Illinois). Unlike traditional coated interference filters, this particular hybrid longpass filter design also includes an absorptive filter glass at the leading edge of the filter passband that helps to minimize short-shifting wavelength aberrations caused by non-normal angles of incidence³⁴ when imaging large FOVs as seen in Fig. 2.

Color images were collected using a webcam (Logitech HD Webcam C930e, Lausanne, Switzerland), and fluorescence projection overlay was accomplished using a 300-lumen digital light projector (AAXA Technologies P5, Irvine, California). The system as a whole is controlled with a 64-bit laptop (Intel Core i7-6700HQ, Santa Clara, California), running a custom MATLAB® (MathWorks, Natick, Massachusetts) graphical user interface suite that utilizes the graphics processing unit (GPU) (NVIDIA Geforce GTX 960M, Santa Clara, California) for simultaneous capture, data processing, and projection overlay in real time. For intraoperative applications, the imaging head is attached to a ball mount that allows it to be positioned at any angle required during surgery. The mount is connected to a double articulated arm supported by a mobile cart that transports the entire system. The imaging head can be shielded using a 3-D printed enclosure and wrapped in a universal c-arm sterile drape without overheating. Additionally, a machined slot on the arm allows for the insertion of a disposable sterile handle similar to a surgical microscope. In its most compact transport configuration, the imaging system occupies a space of about $160 \times 64 \times 55 \text{ cm}^3$.

With the configuration described already, the OTIS is capable of measuring a $15 \times 15 \text{ cm}^2$ FOV at a pixel dimension of 1024×1024 and a working distance of 35 cm. The optical irradiance at this distance is $\sim 0.7 \text{ mW/cm}^2$. As all the optical components utilize standardized SMA905 and C-mount threading, each major element can be modified and replaced to best suit different application needs.

2.2 Flat-Field Reference Calibration

Prior to imaging, the system must be calibrated to acquire measurements and to correct for any system response due to spatial variations in source illumination and other optical aberrations. This is accomplished by acquiring a single reference measurement from a custom designed $20 \times 20 \times 5 \text{ cm}^3$ polydimethylsiloxane phantom consisting of pheophorbide-a (Sigma-Aldrich, St. Louis, Missouri) as a visible to NIR fluorophore, titanium dioxide as a scattering agent, and India ink as an absorber.³⁵ Optical property homogeneity and long-term stability of the phantom were validated using a probe-based spectrometer described in previous studies.^{14,36} For each subsequent measurement, flat-field correction is accomplished through the following equation:

$$C(x, y) = \frac{\langle R \rangle}{R(x, y)} * I(x, y), \quad (1)$$

where C is the corrected image, R is the reference measurement, and I is the raw sample image. The correction factor, $\langle R \rangle / R(x, y)$, is a ratio of the reference's total intensity average to each pixel's raw intensity.

2.3 Imaging Workflow

Depending on the application, the signal-to-noise ratio (SNR) performance can be increased through background subtraction of ambient light and by averaging multiple frames of images at the cost of longer total acquisition times. Fluorescence thresholding is automated using a method described by Otsu³⁷ based on a user-defined threshold value and can be simultaneously

projected back on the sample's surface in bright green at the same rate that images are being acquired. Green was chosen for the overlay image because it provides a stark visual contrast against tissue and does not contribute to the NIR fluorescence measurements. Single snapshot fluorescence measurements can also be recorded along with color and grayscale images by utilizing the projector's white light as illumination.

2.4 Indocyanine Green Sensitivity Test

To characterize the sensitivity of the fluorescence imaging system, ICG was diluted with water at concentrations of 10, 7, 5, 3, 1, 0.75, 0.5, 0.25, 0.10, 0.05, and 0 μ M. At a volume of 250 μ l per well, these values are equivalent to 7.75, 5.42, 3.87, 2.32, 0.77, 0.58, 0.39, 0.19, 0.08, 0.04, and 0 μ g/ml, respectively. Each sample was spaced about 2 cm apart from each other to prevent signal contamination from the surrounding wells and imaged at a relatively short exposure time of 20 ms to prevent the CMOS detector from oversaturating.

2.5 Mouse Indocyanine Green Tail Vein Injection

A total of four adult (8- to 12-week old) female CD1 wild-type mice (Charles River Laboratories, Wilmington, Massachusetts) with average weights of 30 g were used in an ICG tail vein

injection study aimed at validating the imaging system's performance *in vivo*. Housing and care for the animals were performed in accordance with the regulations described in the NIH Guide for the Care and Use of Laboratory Animals, and all experiments were performed under approval from the Vanderbilt University Medical Center's Institutional Animal Care and Use Committee (IACUC #M/15/111).

During this study, each mouse was anesthetized through a nose-cone delivery of 2% isoflurane mixed with 100% oxygen using a precision vaporizer (E-Z Anesthesia, Palmer, Pennsylvania). Each anesthetized mouse was given a tail vein injection of 0.1 ml ICG at 10 μ M concentration, which is approximately equivalent to a 0.026-mg/kg dosage and is well below the 2.0-mg/kg threshold for FDA-approved human clinical applications.³⁸ Ten minutes after imaging of tail vein injections, each mouse was then euthanized using a primary overdose of isoflurane and subsequent cervical dislocation. Postmortem images of the liver were then acquired after exposing the abdominal cavity to confirm each successful tail vein injection.

2.6 Intraoperative Soft Tissue Sarcomas Tumor Bed Autofluorescence Imaging

To demonstrate the system's intraoperative performance, 15 subjects undergoing STS excision were recruited under approval

Fig. 3 Resolving power of the fluorescence imaging system as tested by a United States Air Force 1951 resolution test chart with two different iris lens. Top images are of the targets, while bottom images are the line plots through each element of a particular target group. Limits of resolving power were determined by the largest group and element pair, in which the spacing pattern is no longer discernable within the line plot. Resolving power under (a) a 12-mm focal length lens and (b) a 50-mm focal length lens.

of the Vanderbilt University Institutional Review Board (IRB #120813). Subject eligibility was determined during preoperative evaluation by a surgical oncologist, and informed written consent was obtained from each subject prior to measurements. Along with images of the exposed tumor mass, measurements were also acquired from within each patient's surgical cavity.

3 Results

3.1 Spatial Resolution

The OTIS's maximum resolvable power was tested using a 1951 United States Air Force resolution test chart. This particular test target consists of reference bar patterns with well-defined thickness and spacings. By identifying the largest group and element pair of nondistinguishable bars, the maximum resolving power of a given optical system can be determined. The results of this test can be seen in Fig. 3, in which two c-mounted iris lenses (12- and 50-mm focuses) equipped with 830-nm longpass filters were tested under white-light illumination. Line plots of each pattern's grayscale intensities are also included to better validate the resolving power limits. The 12-mm focus lens was found to have a maximum spatial resolution of $250\ \mu\text{m}$ (group 1, element 1) and a maximum FOV of $15 \times 15\ \text{cm}^2$ while the 50-mm focus lens was shown to have a maximum spatial resolution of $78.75\ \mu\text{m}$ (group 2, element 5) and a maximum FOV of 4.3×4.3 .

3.2 Reference Calibration

Flat-field correction was validated using two small pheophorbide-a phantoms with slightly different optical properties

confirmed with a probe-based spectrometer.^{14,36} The samples were imaged in the center of the $15 \times 15\ \text{cm}^2$ FOV and then positioned on the far edge for another measurement. The results of the flat-field correction method can be seen in Fig. 4. Before calibration, the samples measured at the FOV's far edge exhibited a 38% decrease in fluorescence intensity than when measured at the FOV's center. After calibration using Eq. (1), the samples measured at the FOV's far edge exhibited a 2% difference than when measured at the FOV's center. An intensity cross section of the imaging surface before and after calibration can also be seen in Fig. 5.

Fig. 5 Line plot cross section of a measured surface before and after reference calibration.

Fig. 4 Validation of flat-field correction: (a) raw fluorescence images of two miniature phantoms measured at the center and far edge of the $15 \times 15\ \text{cm}^2$ FOV prior to calibration, (b) raw image of the reference calibration phantom (top) and the correction image derived from it (bottom), (c) fluorescence images of the two miniature phantoms after calibration, and (d) bar plots of averaged sample intensities with standard deviations.

Fig. 6 Results of ICG sensitivity tests using a 785-nm excitation and 830-nm longpass filter configuration at a 20-ms exposure. (a) Combined grayscale and NIR fluorescence intensity image of ICG samples, their concentrations (top), and averaged intensity values (bottom). (b) Intensity line plots across each well with ICG concentrations labeled at the top. (c) Plot of average ICG fluorescence intensity at each measured concentration of ICG. (d) Plot of SNR at each measured concentration of ICG.

3.3 Indocyanine Green Sensitivity

The results of the ICG sensitivity test can be seen in Fig. 6 where the ICG fluorescence signal is observed to decrease with lower concentrations following a second-degree polynomial ($R^2 = 0.99$). In terms of an SNR as defined as the ratio of sample mean intensity to the standard deviation of the background noise, a clinically relevant ICG concentration of 10 μM demonstrated an SNR of 531 as seen in Fig. 6(d). At the lowest concentration of 0.05 μM, the signal could still be measured with an SNR of 5.6 at an exposure time of 20 ms.

3.4 Projection Overlay

To demonstrate the ability of OTIS for simultaneous acquisition and projection overlay, 30 gelatin capsules filled with pheophorbide-a were scattered in random orientations across a $15 \times 15 \text{ cm}^2$ area and imaged by the system along with an *ex vivo* STS tumor margin. After thresholding, the binary image was then cast back onto the FOV using the digital light projector as seen in Fig. 7. In both cases, fluorescence measurements and data processing were accomplished simultaneously and projected onto the sample in real time. At a 30-ms exposure time, the acquisition and projection frame rate is ~ 33 frames per seconds (fps). Additional tests were also able to demonstrate a projection accuracy within 0 to 1 mm and a maximum projection resolution of $\sim 2 \text{ mm}$ when imaging at the 35-cm-distance focal plane.

Fig. 7 Example of real-time projection overlay at 33 fps using a random arrangement of pheophorbide-a capsules within a $15 \times 15 \text{ cm}^2$ area (top) and an *ex vivo* STS tumor margin (bottom). (a) Color images before projection overlay and (b) projection overlay in effect.

Fig. 8 Mouse ICG tail vein injection study as measured by the fluorescence imaging system at a 30-ms exposure. Images at the top correspond to a representative ICG tail vein injection measurement, while images at the bottom correspond to postmortem abdominal imaging of ICG accumulation in the liver within the same mouse after 10 min. (a) Color images of mouse tail and abdominal cavity and (b) NIR fluorescence images of ICG injected mouse tail (SNR: 22.9) and abdominal cavity with ICG accumulation within the liver (SNR: 90.7).

3.5 Mouse Indocyanine Green Tail Vein Injection

An ICG tail injection experiment was carried out to demonstrate the performance of OTIS in an *in vivo* animal model. In these experiments, each mouse was given a tail vein injection of 0.1 ml ICG at 10 μ M concentration prior to imaging at a 30-ms exposure time. Ten minutes after tail vein imaging, the mouse was then euthanized, and postmortem images of the liver were acquired after opening the abdominal cavity to confirm the success of each injection by imaging the final accumulation of ICG in the liver. Representative results of this study can

be seen in Fig. 8 with similar outcomes in all mice. With the background set as an adjacent nonfluorescing portion of the mouse, the SNR of the ICG tail vein injection measurement was ~ 22.9 while the SNR of the postmortem liver measurement was ~ 90.7 .

3.6 In Vivo Soft Tissue Sarcoma Imaging

The fluorescence imaging system was finally demonstrated in an intraoperative environment through measurements of

Fig. 9 Intraoperative autofluorescence imaging of an excised STS mass with the tumor exposed. (a) Color image of the STS mass and (b) NIR autofluorescence image of the same STS mass.

Fig. 10 Intraoperative NIR autofluorescence imaging of an STS tumor bed. (a) White-light image and (b) composite image of NIR autofluorescence map and white-light image, (c) color image of tumor bed, (d) real-time projection overlay of the NIR autofluorescence map directly into the tumor bed.

human STS and tumor bed autofluorescence acquired during STS excision surgeries. No exogenous contrast agents were utilized. Following excision of the STS tumor mass from the body, images were collected from the tumor bed *in vivo* before further measurements were acquired from the resected mass with the tumor exposed. Figure 9(a) shows a color image of an intraoperatively excised leiomyosarcoma mass with the underlying tumor exposed from its surrounding skeletal muscle margin, and Fig. 9(b) shows the NIR autofluorescence image of the same tumor mass. Here, a noticeably strong signal can be observed from the skeletal muscle margin while the exposed tumor itself exhibits a significantly decreased signal. In addition to the STS mass, an example of tumor bed autofluorescence imaging can be seen in Fig. 10. Figure 10(a) shows a white-light image of the right thigh tumor bed while its NIR autofluorescence map can be seen in Fig. 10(b). Again, skeletal muscle is observed to have high signal compared to everything else in the tumor bed, including fat, bone, skin, and the blood vessel sheath running through the center of the thigh. Figure 10(c) shows a color image of the tumor bed and Fig. 10(d) shows the real-time projection overlay technique in effect.

4 Discussion

In this paper, we have developed an intraoperative ready wide-field fluorescence imaging system, referred to as OTIS, which is comprised of readily available commercial components and features a modular layout that can be adapted to various applications in both laboratory research and clinical use. Furthermore, the system features an alternative visualization strategy for image-guided surgery by projecting fluorescence maps directly onto the imaging surface and can thus be used

to intuitively track the location of both exogenous and endogenous fluorophores in real time without the use of external monitors and the associated need for mental coregistration. The 12-bit CMOS detector with analog gain does not require active temperature control, has a manageable spectral response from 400 to 850 nm, and can be fitted with any standardized c-mount lens and filter setup. This, along with the flexibility to accept any fiber-coupled light source, allows the OTIS to accommodate a wide range of spectral, FOV, and spatial resolution requirements without the need for dedicated instrumentation. Through the use of a hybrid longpass filter design, the OTIS is capable of imaging a $15 \times 15 \text{ cm}^2$ FOV at a $250\text{-}\mu\text{m}$ resolution from a working distance of 35 cm away as shown in Fig. 3(a) without severe spectral short-shifting. This is comparable to many of the higher-end light-emitting diode array-based systems³⁰ that require further working distances. Unlike these systems, however, the OTIS can easily be modified to a $4.3 \times 4.3 \text{ cm}^2$ FOV at a $78.75\text{-}\mu\text{m}$ resolution as shown in Fig. 3(b), which may be useful for precision-sensitive operations such as glioma resection. As shown in Fig. 1, most of the optical components are compact, portable, and can be contained within a single unit mountable on to an articulated arm for flexible and mobile use by both clinicians and researchers.

As shown in Fig. 7, the system features a visualization strategy for fluorescence-guided surgery that allows for hands-free continuous image collection and overlay similar to previously described research systems.^{31–33} Additional research-based approaches for improving image visualization have been based on heads-up displays, either in the form of microscope oculars,³⁹ semitransparent mirrors,^{40,41} or wearable goggles.⁴² By leveraging the computer's GPU for parallel processing of basic arithmetic functions, the OTIS is able to perform real-time NIR fluorescence imaging, thresholding, and projection of images simultaneous with acquisition. This serves a dual purpose of intuitively displaying fluorescence maps to the surgeon with minimal disruption to workflow and amplifying the naked-eye detection of low-level fluorescence signal that may not be apparent due to intensity scaling.

While the system is capable of many different configurations, for validation purposes, an ICG-targeted setup was used for comparisons to other currently existing intraoperative fluorescence imaging on the market. As shown in Fig. 4, the OTIS is capable of spatially corrected measurements with the use of calibration phantoms. Additionally, the *ex vivo* sensitivity tests shown in Fig. 6 demonstrate the system's capability to measure ICG with a nonlinear trend consistent with previous literature⁴³ down to $0.05 \text{ }\mu\text{M}$ concentration with an SNR of 5.6 at an exposure time of 20 ms. In the same figure, variations in intensity across each ICG well due to the meniscus's effect on ICG self-shielding and reabsorption can also be observed. Further imaging capabilities were subsequently demonstrated in the *in vivo* results as shown in Fig. 8, in which the system was able to track mice tail vein injections of ICG at a dosage level well below the 2.0-mg/kg threshold for FDA-approved human applications.³⁸ While the system's relatively low optical irradiance in the current configuration limits its dynamic range and prevents it from measuring pM concentrations with the 1-ms exposure times of other devices, the fluorescence imaging system presented here is regardless more than capable of measuring clinically relevant concentrations of ICG in real time at $\sim 33 \text{ fps}$, which is comparable to many other commercial fluorescence imaging systems.³⁰

The OTIS was intraoperatively evaluated with contrast agent-free measurements of excised human STS tumor and *in vivo* tumor bed NIR autofluorescence as shown in Figs. 9 and 10. These results, along with the tumor margin as shown in Fig. 7, are consistent with previous probe-based studies, in which normal skeletal muscle was found to have a stronger NIR autofluorescence signal compared with normal fat and STS.¹⁴ This is most apparent in the tumor mass images as shown in Fig. 9 from which the exposed tumor incision exhibits a significantly decreased signal compared with the surrounding skeletal muscle margin. The tumor bed image in Fig. 10 also exhibits a strong NIR autofluorescent signal from the surrounding skeletal muscles along with some weak reflections from bone. Through the use of a real-time projection overlay system, these regions of high NIR autofluorescence could then be highlighted using a visible color to demonstrate the system's potential for real-time and automated feedback for intraoperative applications, such as tumor margin assessment during STS excision.

As a preliminary research device, the current OTIS implementation is not without its limitations, many of which are directly related to the additional challenges associated with wide-field imaging. The biggest issue is associated with measurement and projection of samples with varying surface topography. In its current form, OTIS field coregistration is accomplished by having the fluorescence imaging and projection maps aligned along a single plane of focus 35 cm away from the imaging head. Within that plane, the flat-calibration method helps to correct for nonhomogenous lighting conditions and detector response. Additionally at that plane, the projection coregistration is accurate from 0 to 1 mm and its maximum resolvable resolution is 2 mm, sufficient for tumor margin assessment applications where biopsies are often 5 mm wide or larger. While adequate for relatively flat imaging surfaces and samples as shown in Fig. 7, most intraoperative surfaces cannot reliably be assumed to be flat. An example of a topographic effect can be seen in Fig. 9(b) where the apex of the tumor mass's skeletal muscle margin exhibits a relatively high autofluorescence signal compared with the lower regions near the edges. While a method for planar image correction was developed, an additional method for surface topography and correction must also be implemented to ensure accurate measurements and projection mapping. In particular, a benchtop fiducial-based image registration algorithm that corrects for distance, imaging angle, and surface curvature has been demonstrated previously by Gan et al.³³ Because the OTIS is equipped with a digital light projector, a potential intraoperative solution may exist in fiducial-free fringe-phase profilometry for the generation of topographic maps for height intensity calibrations^{44,45} as mediated by the solid calibration phantom. In addition, the solid calibration phantom could also be utilized as a secondary reference to an National Institute of Standards and Technology-certified white-light standard to achieve quantitative measurements in future studies.

Another limitation of the OTIS configuration used in this study is the low surface irradiance of the 785-nm illumination light source at the imaging surface. At a distance of 35 cm away and an illumination diameter of over 15 cm, the recorded irradiance at the sample's surface is only 0.7 mW/cm² despite the laser diode itself outputting over 500 mW of power. While the theoretical interrogation depth of a 785-nm light source should be greater than that of the visible or UV light commonly used in

fluorescence imaging due to the low absorption and scattering of NIR light in tissue, a low irradiance may still confine OTIS measurements to a superficial portion of the sample. This factor in combination with the challenges posed by varying surface topography within a surgical FOV as described earlier might account for the increase in SNR when imaging ICG-perfused mice liver as compared with the tail as shown in Fig. 8. While the 0.026-mg/kg dosage of ICG used in the mice experiments is relatively low compared to the 2.0-mg/kg FDA-approved dosage for humans, the rate of elimination and depths is quite different among species. In particular, a mouse's lateral tail vein has a diameter of ~300 μ m and is about six times smaller than the diameter of a human median cubital vein commonly used for vascular access and is much more superficial, allowing for high SNR detection at low concentrations of ICG.⁴⁶⁻⁴⁸ This may also explain why certain regions of skeletal muscle within the tumor bed in Fig. 10 have little to no autofluorescence values as they may be surrounded by a thin layer of fascia or connective tissue. However, because the OTIS was designed to be modular, the laser diode currently being used could potentially be replaced with much more powerful light source for future studies.

The fluorescence imaging system (OTIS) presented here is currently a research prototype designed to demonstrate the concept of a modular intraoperative imaging platform and surgical navigation method. With the many advances in the development of exogenous fluorophores and the utilization of fluorescence imaging as a noninvasive surgical guidance tool, the adaptability of the OTIS allows it to be easily customized for numerous applications. In addition, its ability to simultaneously detect and display fluorescence to the surgeon in a direct and intuitive manner presents an enhancement for the adoption of image-guided surgery.

5 Conclusion

In this paper, we have developed and validated a multipurpose wide-field fluorescence imaging system for intraoperative surgical guidance that is comprised of commercially available standardized components. Its modular layout allows it to accommodate for a broad range of optical properties, FOV, and spatial resolution needs while maintaining a portable design for intraoperative use. Measurements are automatic and feature a real-time projection overlay technique that intuitively displays fluorescence maps directly onto the interrogation surface without the use of remote monitors.

Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

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Biographies for the authors are not available.