Versatility in Fluorescence Guided Surgery with the GLOW Camera System

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Background

Fluorescence Guided Surgery (FGS) is a novel and promising technique, whereby a contrast agent is used to demarcate targeted tissue and guide surgeons intraoperatively[1]. This is particularly useful in cancer operations where surgeons want to spare normal tissue[2] (neurosurgery, breast conserving surgery, etc.) or prevent damage to commonly injured structures[3] (e.g., ureters). FGS is becoming increasingly popular as it fulfils numerous pre-requisites for clinical translation[4], being safe (no ionizing radiation), user-friendly, cost-effective, and seamlessly blends into clinical workflow. So why has it not yet diffused into routine clinical practice? At present, there is a lack of specific targeted fluorophores, as the only European Medicines Agency approved drugs are passive in their mechanism of action and thus only applicable to a limited range of operations[5] (e.g. angiography, neurosurgery, urological procedures).

Given the vast potential of fluorescence guidance, the development of active fluorophores is rapidly evolving. Probes are being designed to target specific proteins in cancer, with 11 probes already undergoing clinical trials[6] (and hundreds more in pre-clinical trials). However, currently available fluorescence guided surgery systems (SpyPhi, Firefly, etc.) have not been developed with these new probes in mind, and furthermore are limited in the field-of-view which they can provide[7]. In view of addressing the technical challenges, our team has developed and tested a fluorescence imaging device with modular design, capable of accommodating a wide range of working distances and fluorescence probes.

2. Methodology & Results

The developed imaging device (Figure 1), uses interchangeable lenses and a beam-splitter to separate light into a colour and monochrome camera of high sensitivity with interchangeable fluorescence filters. Illumination is provided with the use of a Xenon light source with electronically controlled interchangeable filters. This system can be customized to facilitate imaging with different VIS-NIR fluorophores – available currently or in the future – by adjusting the corresponding excitation/emission filters (Figure 1a). The system is capable of being operated by a single user (Figure 1b) through its intuitive interface design (Figure1c).

The device performance was studied for the clinically approved and well-studied fluorescent agent Indocyanine Green11. Optimal excitation (bandpass, 500-750 nm) and emission (bandpass, 775-875 nm) were identified following ICG fluorescence spectroscopic measurements both in-vitro and ex-vivo Figure 2. Prior to clinical testing, the device was fully characterised with regards to its sensitivity to ICG fluorescence signals, working distance/ field of view coverage, and tested against a commercial analogue.

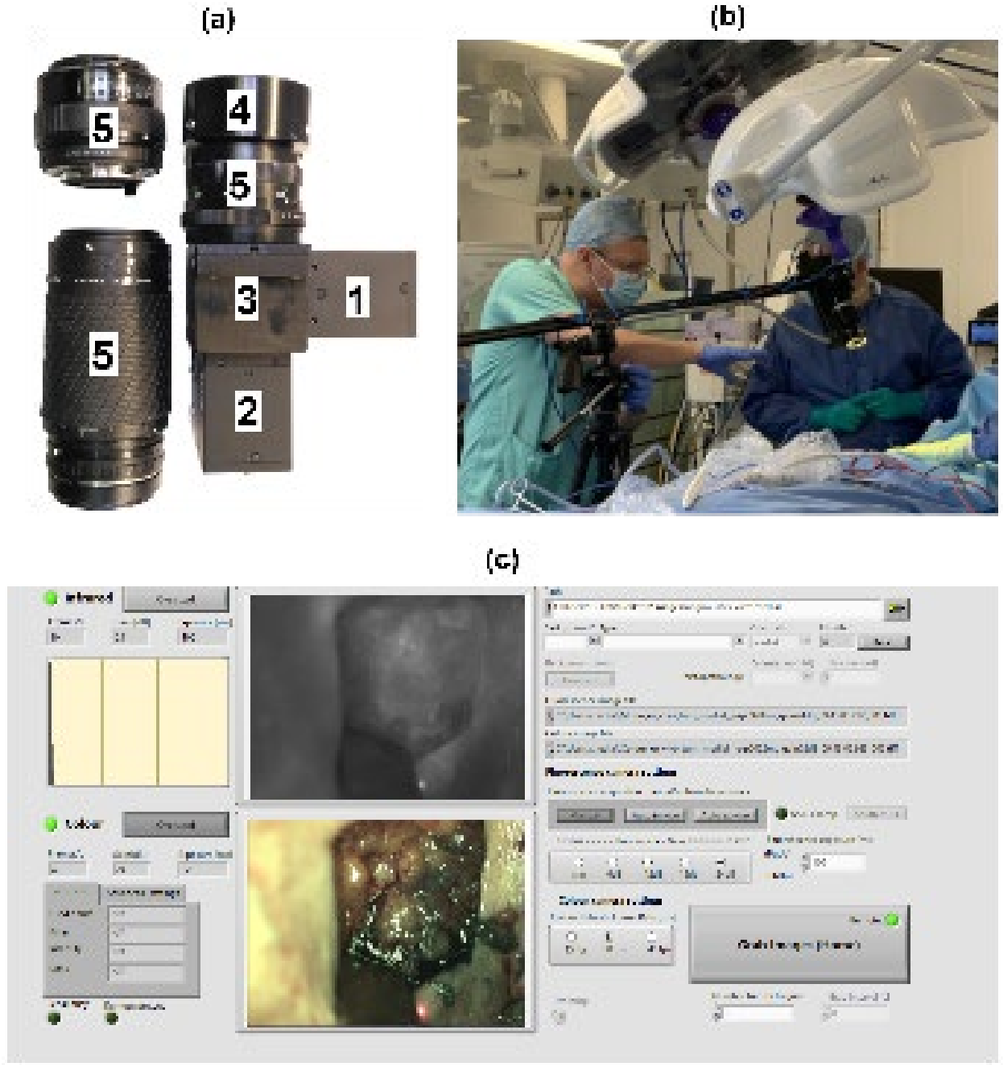

Figure 1. a) Modular GLOW setup for intraoperative tumour in-situ fluorescence imaging upon intravenous injection in BCS: 1) colour camera, 2) high sensitivity mono camera with built in interchangeable fluorescence filter 3) beam-splitter 4) illumination light ring connected to Xe lamp with interchangeable filters 5) interchangeable lenses, b) GLOW setup in operating theatre; c) clinician-oriented software interface.

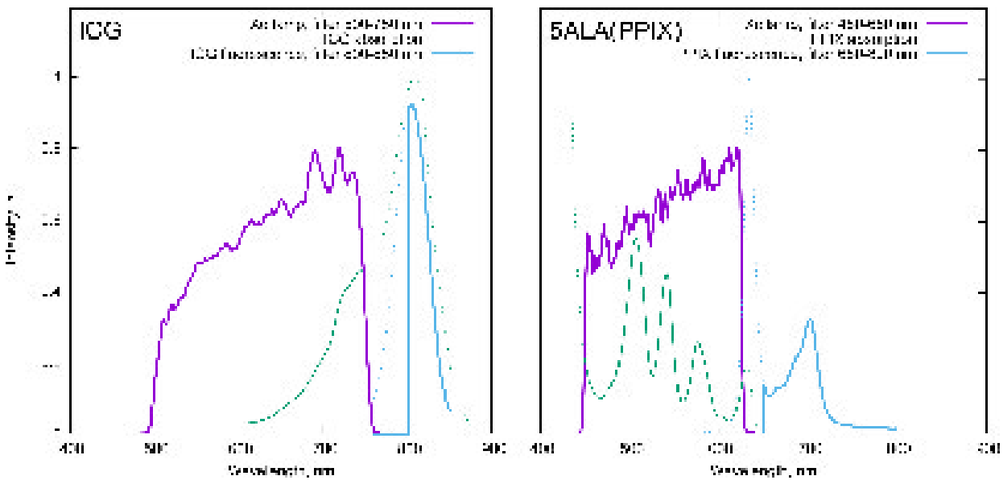

Figure 2 : Demonstration of GLOW setup adaptation for imaging with different fluorophores. ICG: spectrum of Xe lamp filtered in range 500-750 nm for simultaneous colour imaging/ICG fluorescence excitation with overlapping part of ICG absorption spectrum8 and ICG fluorescence spectrum in breast tissues. 5ALA (PPIX): spectrum of Xe lamp filtered in range 450-650 nm for simultaneous colour imaging/PPIX fluorescence excitation with overlapping part of PPIX absorption spectrum9 and PPIX fluorescence10, filtered in range 750-800 nm.

Experiment 1: Prototype development and performance characterisation

For the fluorescence sensitivity characterisation, ICG aqueous solutions at a concentration range of 1 to 100 10-9 M have been imaged to extract the signal to noise ratio:

SNR=(mean(fluorescence signal)-mean(background signal))/(standard deviation of background), at variable working distance close to the shortest acceptable distance between the patient and the camera (~10 cm) (Figure 3).

Working distance coverage and corresponding resolution have been measured with the help of a USAF 1951 (MIL-S-150A Standard). For every combination of aperture size, focal length and object distance of the lens, the corresponding optimal working distance, field of view and resolution have been calculated. The clinically relevant results are displayed in Table 1.

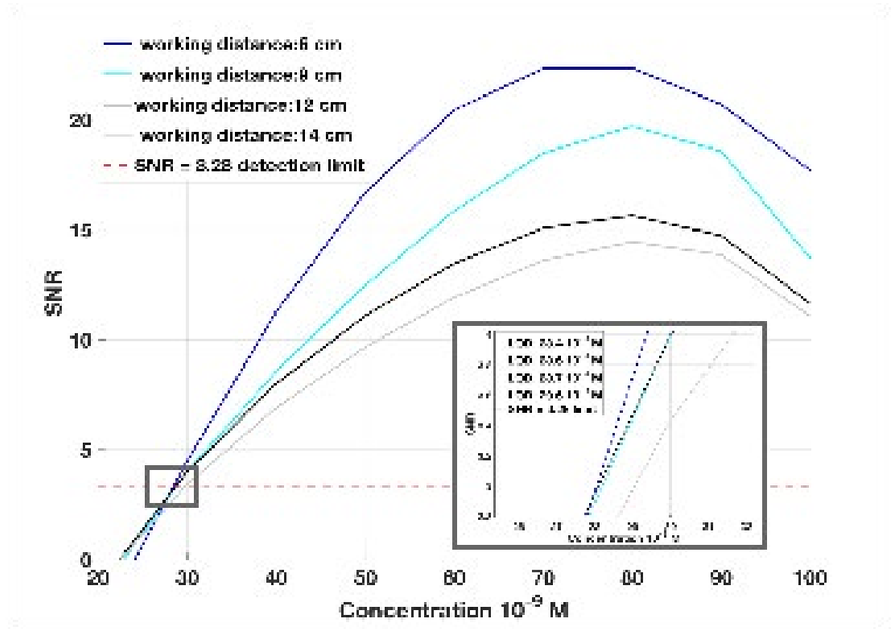

Figure 3: a) Sensitivity to ICG fluorescence characterized at different working distances. Limit of detection (LOD) is identified as the intersection point between curve and horizontal threshold of SNR=3.28 in line with Rayleigh's criterion. The magnification (grey rectangle) shows the LOD points of each curve.

Clinically relevant working distance range [cm] ±0.12	*Field of view (horizontal x vertical) [cm²] (±0.00, ±0.04)*	*Resolution [µm] (±2)*
10 cm~minimum allowable	3.1 x 2.2	40
30 cm~typical in surgery	**6.1 x 4.4**	**50**
56 cm~maximum in surgery	8.4 x 6.3	80

Table 1: Key-performance characteristics of the fluorescence imaging device. The standard error is also given in each column's top. The resolution reported here is the minimum detectable distance between adjacent features (measured in mm) on the USAF target for which the image contrast remains above 26.4 %, as defined by the Rayleigh criterion.

The lowest ICG concentration that can be detected at a working distance of 14cm was 29.6 10-9 M or ~0.023 mg L-1 (the system's limit of detection) (Figure 3). The superiority of our system's performance (SNR=28) in detecting the expected in-vivo ICG signals (3.2 10-6 M or 2.5 mg L-1) is statistically significant ($p<0.001$) compared to that of the SpyPhi commercial system12 (SNR=12).

Also, as demonstrated in Table 1, the system is capable of high acuity at a wide range of working distances (10-56 cm), capable of distinguishing adjacent features as close as 50 μm at the clinically relevant working distance of ~30 cm.

Experiment 2: In-vivo feasibility study

The prototype was trialled in-vivo in 10 BCS patients (REC 18/LO/2018) to investigate feasibility as well as improving the hardware and software design.

12.5 mg ICG was administered intraoperatively once skin flaps were raised (thus exposing the tumour), and images were taken of the tumour in-situ (Figure 4), ex-vivo, and of the cavity post excision. After exclusion of five patients in which the specimen had healthy margins (tumour was >0.4cm deep), the ex-vivo images of five specimens were analysed to extract image texture metrics and detect the tumour boundaries in the image. The result of this preliminary analysis was compared to gold-standard histopathology to estimate the accuracy of the method in identifying cancer. The texture metrics: 'slope' and 'intercept', which are indicators of the images' spatial frequency content, have been found to be potentially useful in differentiating between cancer and healthy tissue[13].

Moreover, in this trial, the surgeons' feedback was used for iterative improvement of the software and user-interface, to develop a reliable and user-friendly platform that allows for single user operation. The developed interface is intuitive and supports advanced image processing including:

- optional full manual control

- automatic dark frame acquisition

- original flexible camera autoexposure algorithms, optimised for both in vivo and ex vivo imaging

possibility of image sequence recording with variable time lapse

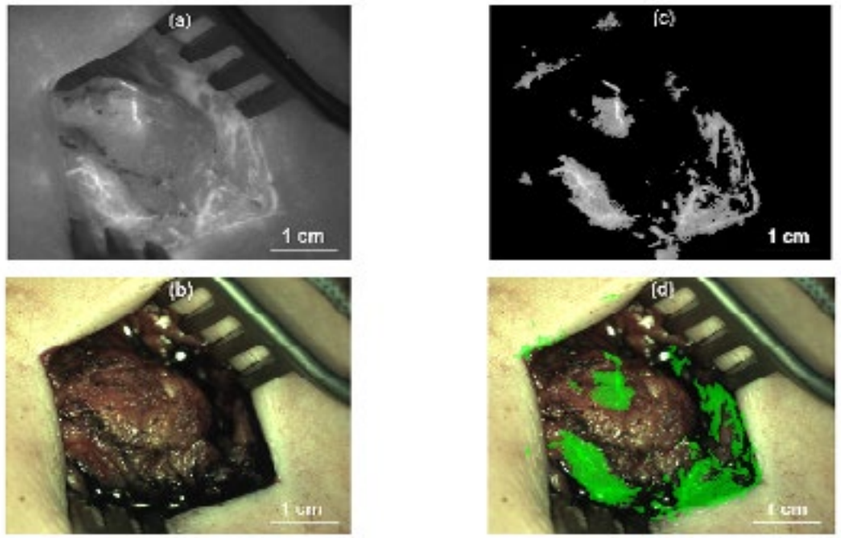

Figure 4: a) Raw fluorescence and b) colour image of the tumour in-situ, acquired with the device c) example of fluorescence image processing, where the pixel values below a global threshold are supressed to zero and d) the remaining fluorescence pixel values after the suppression are overlaid as a green pseudo-colour map on top of the colour image.

Discussion

FGS is a strong candidate in tackling the clinical need of real-time macroscopic tumour visualisation, empowering surgeons to make intraoperative decisions guiding patient treatment. However, commercially available systems which are restricted by filtration spectra and fixed lenses limit evaluations in new clinical interventions. In these trials, we have demonstrated that our system has higher sensitivity to fluorescence, improved resolution when compared to commercially available systems, and is ready for in-vivo applications due to its intuitive design.

Being able to detect the expected ICG concentration of 3.2 10-6 M opens up the potential of using a smaller dose of the fluorophore to the patients. This has both direct patient safety and cost implications, which in the case of cytotoxic and expensive immunotherapy agents (i.e. cetuximab-800/ panitumab-800), as the side effect profile is substantial and dose-dependent, one could improve tolerability through a reduced dose[14].

Device	Working distance [cm]	FOV (h x v) [cm]	Resolution [μm]	Sensitivity to contrast agent [nM]	Adaptable to fluorophores	Gain setting/ room light correction	Full Manual Control
GLOW system	10-56	3.1 x 2.2 – 8.4 x 6.3	40-80	29.6 (ICG)	YES	YES/YES	YES
LAB-Flare (Curadel)	30-45	0.9 x 0.8 - 25.3 x 25.3	50-500	NA	Only 85-735 & >791 [nm]	YES/NO	Partial
Spy Elite (Novadaq)	~30	10 x 14	NA	~5 (IRDye 800CW)	Only 825-850 [nm]	NO/NO	NA

Table 2: Comparison of the GLOW system's technical specifications against those of the commercially available devices7. FOV – field of view

As required for the majority of surgical applications[4], our system can cover a wide range of working distances with sub-mm resolution. At the clinically relevant WD range, we achieve superior resolution compared to commercial competitors (Table 2)[7]. These encouraging results push the boundaries of using FGS as a macroscopic visualisation tool.

Although the system is sensitive to in-vivo fluorescence, high tumour diagnostic accuracy - a key pre-requisite for clinical adaptation- was not achieved when ICG was used as contrast agent. This result is consistent with current literature15 where accuracy in the context of ICG is low as this fluorophore does not actively target tumour cells. However, the image texture metrics 'slope' and 'intercept' of the images' spatial frequency content analysis could be potentially useful in detecting tumour location. Therefore, in future studies we will aim to: a) investigate the diagnostic accuracy of the system in an additional 40 patients using ICG and b) modify the camera for its use with the fluorophore protoporphyrin IX in another 40-patient trial (Figure 2) (19/LO/0927).

Conclusion

FGS is a strong candidate in tackling the clinical need of real-time intraoperative macroscopic tumour visualisation as it is patient-safe, user-friendly, cost-effective and provides seamless integration with the clinical workflow. Since FGS is still in its infancy, it is limited in its clinical applications by the performance limitations of existing camera systems and the availability of targeted fluorophores. To address these issues, we have developed an imaging platform for fluorescence guided surgery with higher fluorescence sensitivity, better resolution and flexibility in choice of diagnostic fluorophores (as compared to commercially available systems). Iterative testing and design optimization through laboratory, in-vivo, and ex-vivo studies, enabled the development of a functional user- and problem-oriented prototype. Through facilitating interchangeable illumination/ acquisition filtration and adjustable lenses, a wide range of contrast agents and working distances/ scales can be accommodated, thus enabling the application of this system well beyond breast cancer surgery.

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Consent/ethical approvals

Ethical approval has been obtained for our previous1 and current2 work ('GLOW') which covers the intraoperative testing of fluorescence and fluorescence combined with multispectral devices, respectively:

[1]Study short title: Real time tissue validation using NIR fluorescence imaging: Surgery.

IRAS ID: 222629, REC reference: 18/LO/1362, Date study commenced: 08/10/2018, Date study ended: 25/10/2019

[2]Study short title: GLOW -Guiding Light Optimising Wide local excisions

IRAS ID: 260633, REC reference: 19/LO/0927, Date study commenced: 09/03/2020, Planned study end:07/2022

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