IEEE Journal of Translational Engineering in Health and Medicine

Received 24 July 2023; revised 2 November 2023; accepted 27 November 2023.
Date of publication 1 December 2023; date of current version 26 December 2023.

Digital Object Identifier 10.1109/JTEHM.2023.3338564

A Wearable Fluorescence Imaging Device for Intraoperative Identification of Human Brain Tumors

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This work was supported in part by the National Institutes of Health (NIH) Small Business Technology Transfer (STTR) Program under Grant R41CA243600 and Grant R42CA243600 and in part by the Kentucky Innovation Matching Fund Award Program.

This work involved human subjects in its research. Approval of all ethical and experimental procedures and protocols was granted by the Institutional Review Board of the University of Kentucky under Protocol No. 45877.

ABSTRACT Malignant glioma (MG) is the most common type of primary malignant brain tumors. Surgical resection of MG remains the cornerstone of therapy and the extent of resection correlates with patient survival. A limiting factor for resection, however, is the difficulty in differentiating the tumor from normal tissue during surgery. Fluorescence imaging is an emerging technique for real-time intraoperative visualization of MGs and their boundaries. However, most clinical grade neurosurgical operative microscopes with fluorescence imaging ability are hampered by low adoption rates due to high cost, limited portability, limited operation flexibility, and lack of skilled professionals with technical knowledge. To overcome the limitations, we innovatively integrated miniaturized light sources, flippable filters, and a recording camera to the surgical eye loupes to generate a wearable fluorescence eye loupe (*FLoupe*) device for intraoperative imaging of fluorescent MGs. Two *FLoupe* prototypes were constructed for imaging of Fluorescein and 5-aminolevulinic acid (5-ALA), respectively. The wearable *FLoupe* devices were tested on tumor-simulating phantoms and patients with MGs. Comparable results were observed against the standard neurosurgical operative microscope (PENTERO® 900) with fluorescence kits. The affordable and wearable *FLoupe* devices enable visualization of both color and fluorescence images with the same quality as the large and expensive stationary operative microscopes. The wearable *FLoupe* device allows for a greater range of movement, less obstruction, and faster/easier operation. Thus, it reduces surgery time and is more easily adapted to the surgical environment than unwieldy neurosurgical operative microscopes.

INDEX TERMS Fluorescence guided surgery, malignant glioma, neurosurgical operative microscope, wearable fluorescence imaging device.

***Clinical and Translational Impact Statement*—**The affordable and wearable fluorescence imaging device developed in this study enables neurosurgeons to observe brain tumors with the same clarity and greater flexibility compared to bulky and costly operative microscopes.

I. INTRODUCTION

THERE are nearly 700,000 people in the U.S. living with a brain tumor [1]. Malignant gliomas (MGs) constitute 35-45% of primary brain tumors with an incidence of approximately 5 out of 100,000 [2], [3]. MGs are aggressive, highly invasive, and neurologically destructive [4]. Surgical resection remains the cornerstone of therapy to treat MGs and the extent of resection correlates with survival [5], [6], [7], [8], [9], [10], [11]. However, gross-total-resection (GTR) rates in conventional surgeries are only 30-55% due

to difficulty in recognizing diffuse infiltrative tumor cells at the margin of resection and tissue shift during surgery [12], [13], [14], [15], [16].

Conventional tumor resection techniques are largely relying on surgeon's observation of subtle changes associated with tissue distortion by invasive tumors. More recently, neurosurgeons utilize imaging techniques such as magnetic resonance imaging (MRI) [17], computed tomography (CT) [18], and angiogram to identify tumor preoperatively [19]. Some MRI machines specially designed for intraoperative imaging help neurosurgeons navigate delicate structures surrounding the tumor [20]. However, incorporating MRI techniques is expensive and time consuming and significantly interferes with surgical workflow. Moreover, specific types of tumors such as high-grade MGs have ill-defined tissue boundaries with tendrils extending from the main tissue volume, which are difficult to locate during resection. Prevalence of residual cancerous cells on the surgical margin leads to repeat surgeries and is a significant burden on patients and healthcare systems [5], [6], [7], [8], [9], [10], [11].

Fluorescence imaging has emerged as an advanced adjunctive technique, allowing for real-time cancer-specific detection without the concern for brain tissue shift and with limited disruption to surgical workflow [9], [10], [11], [12], [13], [14], [15], [16], [21]. Surgeries guided by fluorescence imaging achieve GTR rates of 75-100%, which are significantly higher than the conventional surgeries with GTR rates of 30-55% [12], [13], [22], [23]. Fluorescence light is generated when a fluorophore absorbs the excitation light at the wavelength of its absorption peak, which excites fluorophore electrons to a higher energy level. The fluorophore then releases the extra energy as light (photons) while its energy level returns to the ground state. Therefore, the emitted fluorescence light has a longer wavelength compared to the excitation radiation [24].

Various fluorescent agents are increasingly being tested to distinguish tumors from normal parenchyma, thus improving surgical resection while sparing healthy tissue. Fluorescein and 5-aminolevulinic acid (5-ALA) are the two most frequently used fluorescent tracers for guiding MG surgeries [21], [25]. Fluorescein accumulates primarily in the extracellular space of MGs for hours while minimizing its leakage into the normal brain tissue [16]. The efficient clearance of Fluorescein from the body ensures its safe use in medical applications [16]. Fluorescein dye has a peak excitation from 465 to 490 nm and emission band between 500 and 550 nm [16]. 5-ALA is a precursor molecule that leads to the synthesis of phototoxic protoporphyrin IX (PpIX) in the heme biosynthesis pathway. When 5-ALA is systemically delivered to the body, it is selectively accumulated within MG cells, which often exhibit higher metabolic activity compared to healthy cells. As a result, the concentration of PpIX increases in cancerous cells. PpIX absorbs blue light from 375 to 440 nm and emits a violet-red fluorescence from 620 to 710 nm [26], [27].

Although 5-ALA is more expensive than Fluorescein, it is the only fluorescent agent so far tested in randomized controlled trials in Europe and results in significant GTR improvement and especially progression-free survival in high-grade MGs [21]. More recently in 2017, 5-ALA was granted approval by the Federal Drug Administration (FDA) as an optical agent for adjunct visualization of malignant tissue in patients with suspected World Health Organization (WHO) III or IV gliomas [28], [29].

Optimization of imaging technologies to enhance fluorescence contrasts have been explored over decades [12]. Current standards for intraoperative visualization of MGs are neurosurgical operative microscopes. Industries have also developed FDA-cleared supplementary fluorescence modules that attach to the standard neurosurgical operative microscopes for fluorescence imaging during surgery [12], [15], [16], [21], [22]. For example, Carl Zeiss Meditec (Oberkochen, Germany) has marketed the PENTERO® 900 microscope with a YELLOW 560™ filter kit for Fluorescein visualization and a BLUE 400™ filter kit for 5-ALA visualization. The PENTERO®900 with filter kits encompasses Xenon arc lamps (white light) with special excitation filters in the wavelength ranges of 460-500 nm (YELLOW 560™) and 400-410 nm (BLUE 400™) for exciting Fluorescein and 5-ALA contrasts, respectively. On the other hand, integration of emission filters into detection paths customize the emissions in the wavelength ranges of 540-690 nm (YELLOW 560™) and 620-710 nm (BLUE 400™) for both ocular visualization and monitor display of Fluorescein and 5-ALA fluorescence images, respectively [30]. However, most clinical grade neurosurgical operative microscopes are hampered by low adoption rates due to high cost, limited portability, limited operation flexibility, and lack of skilled professionals with technical knowledge. Currently available neurosurgical fluorescence microscopes are expensive and weigh hundred pounds [16], [22]. Resource poor environments may not have access to neurosurgical florescence microscopes. Many neurosurgeons prefer and continue to resect tumors while wearing low-cost surgical eye loupes that allow for easy operation. However, most commercial surgical eye loupes cannot visualize fluorescence due to the lack of excitation and fluorescence filtering systems.

To overcome limitations of currently available imaging modalities, we innovatively integrated miniaturized light sources, flippable filters, and a recording camera to the eye loupes to generate a unique wearable fluorescence eye loupe device, namely "*FLoupe*" (U.S. Patent #11,813,118, approved on Nov. 14, 2023; Provisional Application #62/530,613, filed on Jul. 10, 2017), for real-time identification of MGs during surgery. Two *FLoupe* prototypes have been constructed for intraoperative imaging of Fluorescein and 5-ALA, respectively. We have experimentally verified the dual-imaging ability of *FLoupe* prototypes against the standard PENTERO® 900 with fluorescence kits

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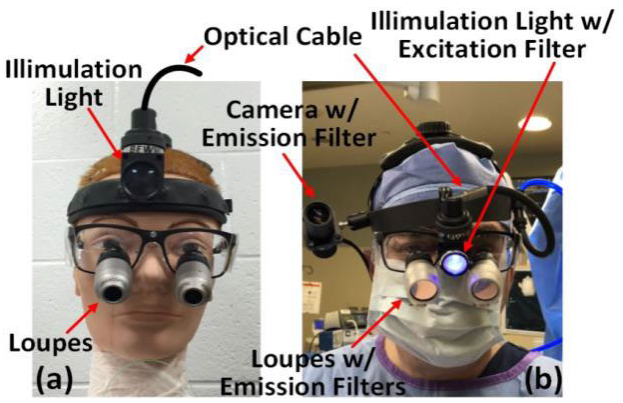

FIGURE 1. Development of *Floupe-1* prototype. (a) Conventional wearable eye loupes and fiber-optic headlight bracket for white light imaging. (b) *FLoupe-1* prototype for fluorescence imaging.

in the tumor-simulating fluorescence phantom and patients with MGs. Overall, our low-cost wearable *FLoupe* devices enable visualization of both color and fluorescence images with the same quality as the large and expensive stationary neurosurgical operative microscopes.

II. MATERIALS AND METHODS

Two FLoupe prototypes *(FLoupe-1 and FLoupe-2)* were developed to image Fluorescein and 5-ALA contrasts, respectively. The devices were tested in 11 patients with MGs (one with Fluorescein and 10 with 5-ALA) during surgery at the University of Kentucky (UK) Hospital. The study has been approved by the UK Institutional Review Board (IRB). Informed consents were obtained from all subjects. Patient demographics, tumor type, and WHO grade were listed in **Table** 1. The WHO grade classifies tumors based primarily on their malignancy, with particular emphasis on most aggressive regions of tumors. Low-grade tumors include WHO grade I (pilocytic astrocytoma) and WHO grade II (diffuse astrocytoma). High-grade tumors include WHO grade III (anaplastic astrocytoma) and grade IV (glioblastoma multiforme - GBM) [31]. Isocitrate dehydrogenase (IDH) wildtype GBM comprise over 90% of glioblastomas [32]. MG resection was performed following the standard protocols under the guidance of PENTERO® 900 with YELLOW 560™ for Fluorescein and PENTERO® 900 with BLUE 400™ for 5-ALA, respectively. The identified MGs were then imaged by the Floupe devices for comparisons.

A. FLOUPE-1 FOR IMAGING OF FLUORESCEIN CONTRASTS

We first developed a *FLoupe-1* prototype for imaging Fluorescein contrasts, which piggybacks on a commercial eye-loupe device (EyeZoom™ 5.0X, Orascoptic, **Fig. 1a**) and a headlight bracket (Halogen III, BFW). An excitation filter (MF475-35, Thorlabs) was installed in front of the fiber-optic headlight (coupled with a 300W Xenon arc lamp) to generate the narrow-band light (center wavelength = 475 nm; full width at half-maximum (FWHM) = 35) for Fluorescein excitation (**Fig. 1b**). A pair of emission filters (MF530-43, Thorlabs) was attached to the eye loupes for Fluorescein visualization (center wavelength = 530 nm; bandwidth = 43 nm). A video camera (FL3-FW-20S4C-C, FLIR) equipped with another filter (MF530-43, Thorlabs) was attached to the headlight bracket for fluorescence image recording.

We tested this *FLoupe-1* prototype on a tumor-simulating fluorescence phantom. The solid phantom was fabricated using a 3D printer (Gigabot® 3.0) and consisted of holes/spaces to test imaging sensitivity and spatial resolution. The bulk material used for making the solid phantom was acetal plastic, which acted as a diffusive tissue. To replicate various sizes of brain tumors, we created holes with diameters ranging from 2 to 10 mm. To delineate real tumorous tissues with different fluorescence contrasts, these holes were filling with the mixture liquid phantom solution consisting of varied Fluorescein concentrations (1 to 8 mg/kg), Intralipid solution to regulate the reduced scattering coefficient ($\mu_s' = 8cm^{-1}$), and India ink to adjust the absorption coefficient ($\mu_a = 0.05cm^{-1}$).

We also tested this *FLoupe-1* prototype in one patient with MGs. At the induction of anesthesia, patient received intravenous Fluorescein at a dosage of 5 mg/kg body weight [12], [22]. During MG resection, the PENTERO® 900 microscope equipped with a YELLOW 560™ filter were utilized as the standard for intraoperative identification of MGs to guide resection. The identified MGs (before resection) were then imaged by *Floupe-1* device for comparisons.

B. FLOUPE-2 FOR IMAGING OF 5-ALA CONTRASTS

More recently, the UK Hospital started routinely using the PENTERO® 900 with BLUE 400™ module to image 5-ALA contrasts in GMs for the guidance of tumor resection. To meet this need, we developed the *FLoupe-2* prototype for imaging 5-ALA contrasts. *FLoupe-2* was developed in collaboration with SurgiTel, a world leading manufactory and supplier for eye loupes, headlights, video cameras, and other medical devices. The standard SurgiTel products include white LED headlights driven by a rechargeable lithium portable battery pack (~8 hours) and surgical eye loupes with up to 10× magnification Eye Loupes (**Fig. 2a**)

To achieve our goal of imaging 5-ALA, the white LED in SurgiTel headlight was replaced with a narrow-band, high-intensity, blue LED (405 ± 5 nm, SST-10-UV, Luminus Devices) for 5-ALA excitation (**Fig. 2b**). We adjusted/calibrated the blue light intensity against the FDA-approved PENTERO® 900 with BLUE 400™ module to ensure the patient safety. Specifically, we measured the illumination power of the PENTERO® 900 with BLUE 400™ at neurosurgeon's working distance (~45 cm) using a power meter (Newport 843-R), and then limited the maximum illumination power of *FLoupe-2* to the same level. We then quantified the illumination powers and densities of *FLoupe-2* (with the restricted maximum power) at varied working distances from 10 to 100 cm. Based on the

TABLE 1. Patient demographics and tumor characteristics.

Contrast Agent	Age	Gender	Histology	Location
Fluorescein	76	Female	GBM, IDH Wildtype, WHO Grade IV	Left parietal lobe
5-ALA	47	Male	GBM, IDH Wildtype, WHO Grade IV	Left parietal
5-ALA	72	Male	GBM, IDH Wildtype, WHO Grade IV	Right temporal
5-ALA	60	Female	GBM, IDH Wildtype, WHO Grade IV	Right frontal
5-ALA	62	Male	GBM, IDH Wildtype, WHO Grade IV	Right posterior temporal with extension into the occipital and parietal
5-ALA	30	Male	Oligodendroglioma, WHO Grade III	Left mid anterior frontal lobe
5-ALA	66	Male	GBM, IDH Wildtype, WHO Grade IV	Right frontal lobe
5-ALA	35	Male	Minute residual GBM, IDH Wildtype, WHO Grade IV	Right frontal lobe
5-ALA	76	Male	GBM, IDH Wildtype, WHO Grade IV	Right temporal lobe
5-ALA	61	Female	GBM, IDH Wildtype, WHO Grade IV	Lateral temporal lobe
5-ALA	56	Male	Gliosarcoma, IDH Wildtype, WHO Grade IV	Right temporal/occipital/ parietal lobes

GBM: Glioblastoma Multiforme, IDH: Isocitrate Dehydrogenase, WHO: World Health Organization.

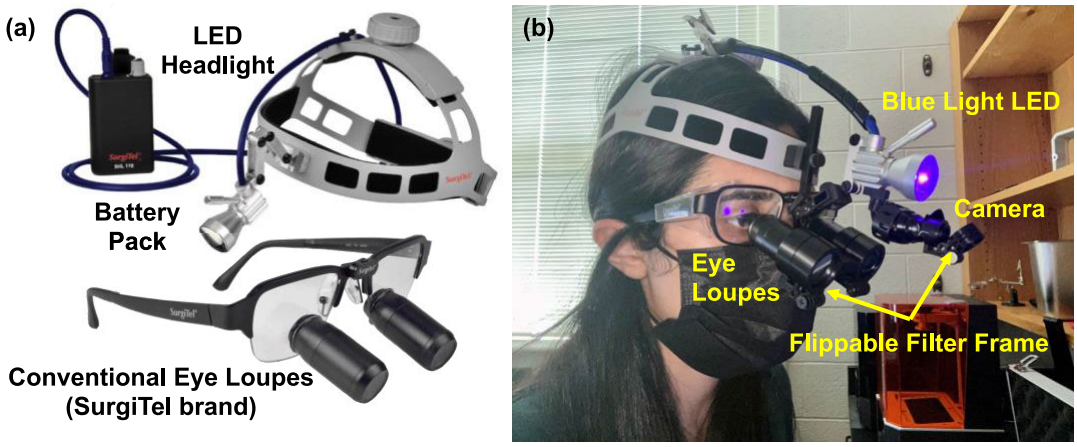

FIGURE 2. Development of *Floupe-2* prototype. (a) Commercial wearable eye loupes and headlight bracket (SurgiTel brand) for white light imaging. (b) *FLoupe-2* prototype for both white light and fluorescence imaging.

photobiological safety standards for LEDs (IEC 62471) [33], the *FLoupe-2* device operated at a working distance larger than 30 cm is safe for a patient illuminated by the blue LED (405 ± 5 nm). A video camera was attached to the headband for real-time recording of the surgical site. We also designed and fabricated a small 180° flippable filter frame by a 3D printer (SL1, Prusa) and integrated a long-pass optical filter (>550 nm, Diameter 12.5 mm, #15-213, Edmund Optics) in front of the video camera for fluorescence image recording. Another flippable frames were designed and fabricated to hold long-pass filters (>550 nm, Diameter 25 mm, #62-984, Edmund Optics) in front of eye loupes. These unique flippable filter frames attached to the video camera and eye loupes allow for easily changing from fluorescence to color vision/recording and vice versa. The video camera and eye loupes are aligned to the same field-of-view (FOV) at the surgical site. The integrated small video camera in *FLoupe-2* with a dedicated emission filter frame is used for continuous displaying on the Operating Room (OR) monitor and video recording of fluorescence images at the surgical site (**Fig. 3**). This video recording is critical for comparing *FLoupe-2* with the fluorescence surgical microscope (e.g., PENTERO® 900 with fluorescence kits) in real time.

We tested this *FLoupe-2* prototype in 10 patients with MGs and compared results against PENTERO® 900 with BLUE 400™ module. Patients were administered 5-ALA (Gliolan, Medac) at a dosage of 20 mg/kg body weight four hours prior to induction of anesthesia. The PENTERO® 900 microscope equipped with a BLUE 400™ filter were utilized as the standard for the guidance of MG resection.

III. RESULTS

Fig. 4a-4c show the results from tumor-simulating fluorescence phantom measurements using *FLoupe-1*. Fluorescence images of phantom "tumors" were clearly observed by the PENTERO® 900 with YELLOW 560™ (**Fig. 4a**) and our *FLoupe-1* (**Fig. 4b**). Holes with Fluorescein concentrations as low as 1 mg/kg, and diameters as small as 2 mm were detected by both imaging devices. These lower limits are generally sufficient to identify MGs for resection, given that patients receive 5mg/kg body weight of intravenous Fluorescein [12], [22]. As expected, when using our *FLoupe-1* device without

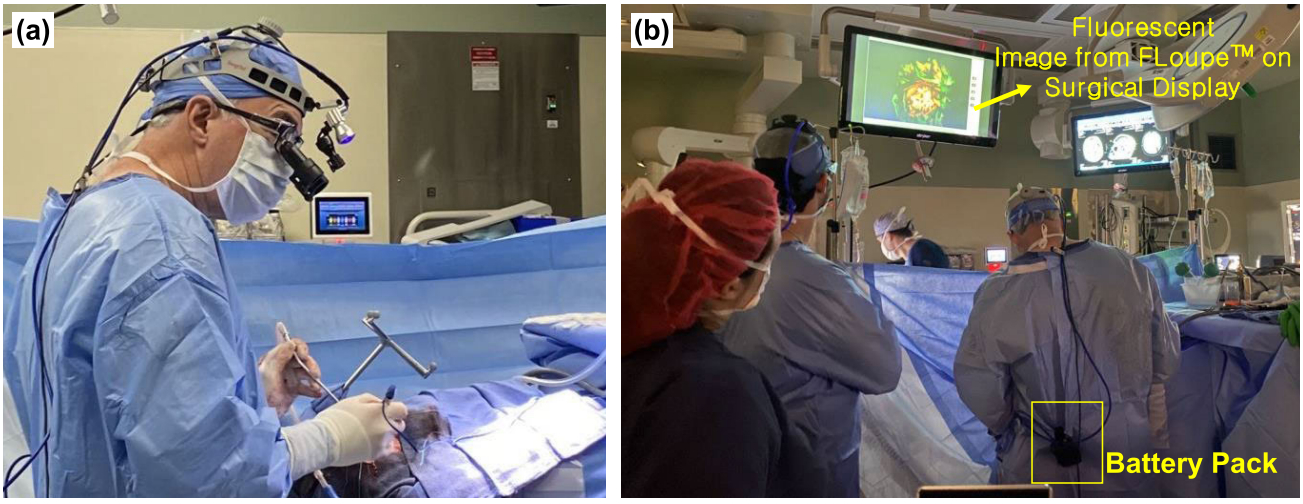

FIGURE 3. Use of *Floupe-2* for fluorescence imaging of MGs. (a) The neurosurgeon wears *FLoupe-2* during surgery. (b) Real-time display of MGs on the OR monitor allows the surgical team with immediate feedback and other trainees (e.g., residents) to observe and discuss the surgical operation.

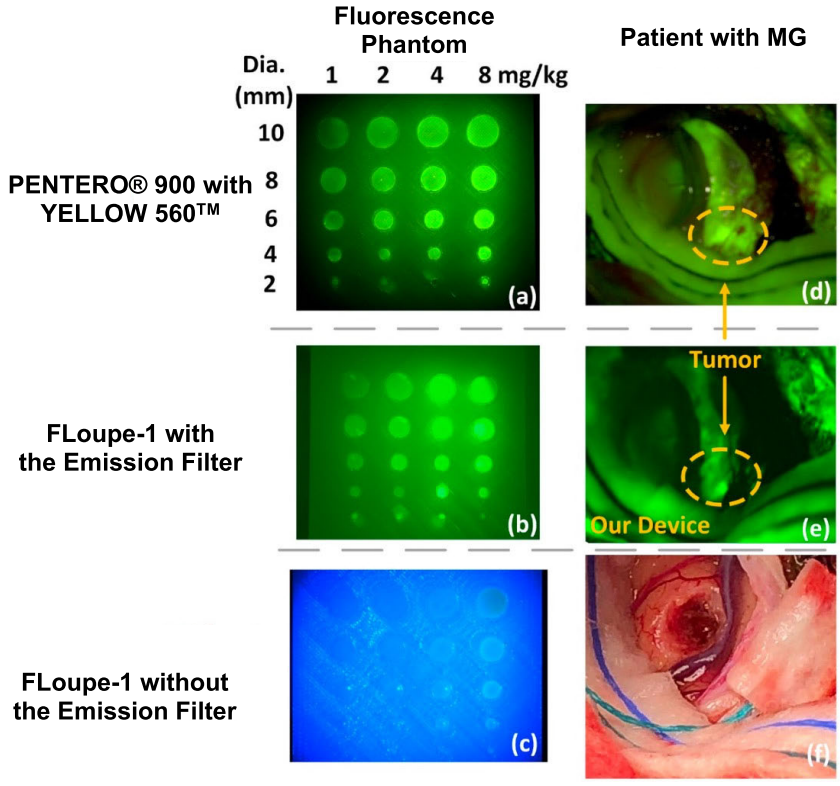

FIGURE 4. Comparison of imaging results in a fluorescence phantom (a-c) and a patient with MG (d-f). Images were obtained using PENTERO® 900 with YELLOW 560™ (a, d) and *FLoupe-1* with the emission filter (b, e) and without the emission filter (c, f), respectively. Note that the color image (f) was obtained using the white light illumination and *FLoupe-1* camera without the emission filter.

an emission filter, fluorescent contrasts were hidden by the high-intensity excitation light (**Fig. 4c**).

Fig. 4d-4f show fluorescence images taken from a patient with MG by the commercial PENTERO® 900 with YELLOW 560™ (**Fig. 4d**) and our *FLoupe-1*device (**Fig. 4e**). Although filter variances across the two devices lead to slight color differences between images, similar fluorescent signals/dots are observed on diffusive tumors.

Fig. 5 shows the color and fluorescence images from three patients (P1-P3) obtained by the PENTERO® 900 with

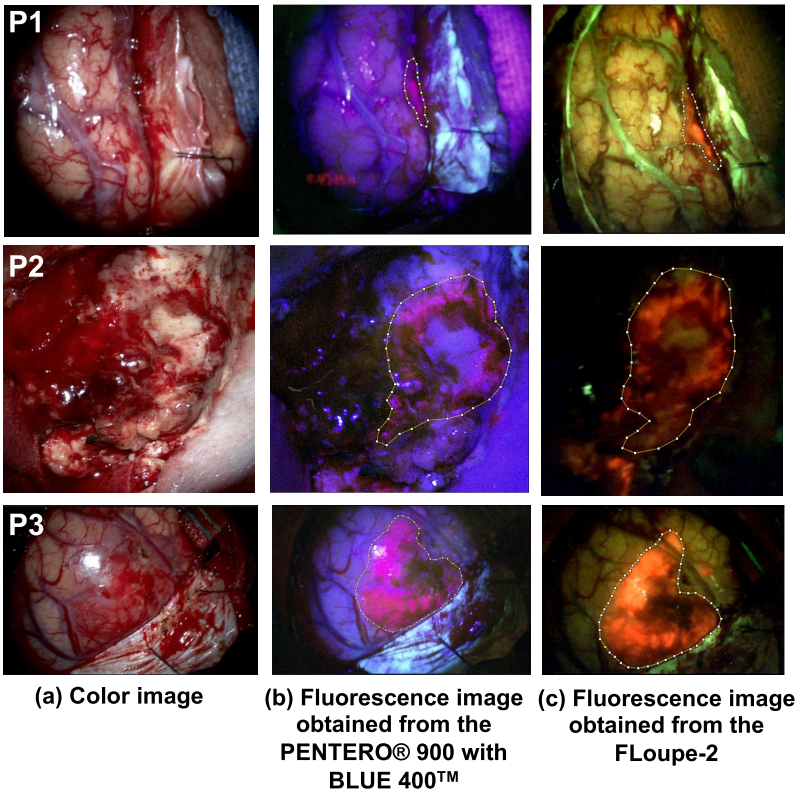

FIGURE 5. **Comparison of imaging results from three patients with MGs (P1-P3). (a) Color images obtained using the white light illumination and PENTERO® 900 camera without the emission filter. (b) Fluorescence images obtained from the PENTERO® 900 with BLUE 400™. (c) Fluorescence images obtained from the *FLoupe-2*. Note that the tumor contours were drawn manually based. on fluorescence visualization.**

BLUE 400™ and *FLoupe-2*, respectively. The two imaging modalities generated consistent results in visualization of fluorescent MGs (see the tumor contours drawn manually based on fluorescence visualization in **Fig. 5b** and **Fig. 5c**). The fluorescent tumorous tissues appeared more vivid with our *FLoupe-2* (**Fig. 5c**) as compared to the PENTERO® 900 with BLUE 400™ (**Fig. 5b**), which is likely due to the differences in excitation lights and emission filters used in the two modalities. The PENTERO® 900 with BLUE 400™ uses the Xenon arc lamp with an excitation filter to generate the blue light whereas *FLoupe-2* uses a narrow-band blue LED for 5-ALA fluorescence excitation (see **Fig. 2b**). Moreover, the color difference on the fluorescence images (**Fig. 5b** and **Fig. 5c**) is mainly due to the spectral difference of the emission filters used in the two imaging systems. Similarly, *FLoupe-2* was able to visualize fluorescent tumors in other patients with low or high grade of MG (see **Table 1**).

IV. DISCUSSION AND CONCLUSION

Fluorescence imaging of cancers during surgery allows real-time cancer-specific detection for guiding tumor removal, usually resulting in significant improvements in patient survival. However, most clinical-grade fluorescence imaging systems are hampered by high costs, limited portability, and lack of operation flexibility. Many surgeons prefer and continue to use wearable surgical eye loupes, which allow for convenient and fast operation, but are not capable of fluorescence visualization. We developed low-cost wearable *FLoupe* devices (U.S. Patent #11,813,118, approved on Nov. 14, 2023; Provisional Application #62/530,613, filed on Jul. 10, 2017), which are attached to wearable surgical eye loupes to help neurosurgeons easily and accurately identify fluorescent MGs for safe and maximal tumor removal. We have tested two *FLoupe* prototypes for real-time imaging of Fluorescein and 5-ALA contrasts respectively in a small group of patients with MGs. Comparable results were observed against the standard neurosurgical operative microscope (PENTERO® 900) with fluorescence kits.

Our *FLoupe* technology provides unique advancements over other competitors' fluorescence imaging technologies, specifically for the guidance of brain cancer resection. Compared to expensive, large, stationary, neurosurgical operative microscopes (e.g., PENTERO® 900) [16], [22], *FLoupe* devices are affordable and wearable and allow for a greater range of movement, less obstruction, and faster/easier operation. This affordable and wearable device significantly increases the ability of more surgeons to conduct fast and thorough operations, and thus improving patient safety and outcomes. Moreover, this low-cost allows for procurement of multiple devices, thus alleviating costs for maintenance and downtime for device repair/replacement.

Very recently, Design for Vision reported a fluorescence eye loupe prototype (*ReVeal*) for imaging of 5-ALA contrasts in MGs [34]. Pilot observation studies were performed in a small group of patients to test its usability for fluorescence imaging of high-grade MGs. As mentioned in their study limitations, several key studies are lacking before *ReVeal* is recommended for widespread clinical practice. Also, a comparison sensitivity/specificity histological study needs to be performed to fully assess the potential of *ReVeal*as a replacement for the neurosurgical microscopes. In comparison, *FLoupe* has the following unique features. First, *FLoupe* has designed and fabricated unique flippable filter frames that are attached to the eye loupes and video camera for easily switching from fluorescence to color vision and vice versa. Moreover, the flippable and modular design makes it easy to switch the LEDs and filters on the *FLoupe* for imaging different dyes. Second, *FLoupe* has designed and tested two prototypes (*FLoupe-1* and *FLoupe-2*) for real-time imaging of Fluorescein and 5-ALA, respectively. In other words, *FLoupe* has demonstrated the potential to incorporate into other surgical settings beyond brain tumor surgeries using different visible dyes. Third, *FLoupe* has integrated a small video camera with a dedicated emission filter frame for continuous video recording of fluorescence images at the surgical site and real-time displaying on the OR monitor. Real-time video recording is critical for comparison of *FLoupe* and PENTERO® 900 measurements, which supports a substantial equivalence determination between the two imaging systems. The comparison results can be used to apply for FDA clearance. Moreover, the real-time display on the OR monitor allows the surgical team with immediate feedback and other trainees (e.g., residents) to observe and discuss the surgical operation.

The development of *FLoupe* devices involved engineering design, experimental optimization, phantom tests, and clinical applications, which are essential steps to translate the innovative wearable fluorescence imaging technique (*FLoupe*devices) to the clinic. This Phase-1 study aims to demonstrate the feasibility of *FLoupe* prototypes and gather preliminary data and feedback for designing a Phase-2 study. Throughout a rigorous Phase-2 study using standardized tumor-simulating phantoms for performance characteristics and with enough patients for statistical analysis, low-cost and wearable *FLoupe* devices are expected to be calibrated and validated against the PENTERO® 900 system using established methods [35], [36], [37] for the equivalence to precisely image fluorescent MGs.

Based on neurosurgeons' feedback in this pilot study, we are currently further optimizing the headlights (LEDs), video camera, emission filters, electrical control system, and mechanical design of *FLoupe*devices in terms of weight, size, imaging quality, and ease to wear/operate. The optimized *FLoupe* devices will be calibrated and validated against the PENTERO® 900 system for equivalence to image fluorescent MGs in a large group of patients. We expect that fluorescence images taken at the surgical site and from biopsied samples by both devices will be equivalent and agree with the blinded histopathological analyses (the gold standard). Resulting data will be used for FDA clearance via the pre-market notification 510(k) submission with the PENTERO® 900 system as the primary predicate.

DISCLOSURES

Guoqiang Yu, Thomas Pittman, Chong Huang, and Nick MeGregor are inventors of the "Loupe-based intraoperative fluorescence imaging device for the guidance of tumor resection," U.S. Patent #11,813,118, approved on Nov. 14, 2023; Provisional Application #62/530,613, filed on Jul. 10, 2017. These inventors are currently collaborating with Bioptics Technology LLC to commercialize the *FLoupe* devices.

ACKNOWLEDGMENT

The authors would like to thank Jaimie L. Hixson for her invaluable assistance in patient recruitment, IRB documentation, and clinic trial management and also would like to thank SurgiTel for customizing eye loupes and LED headlights to meet their needs in the development of *FLoupe* devices. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH or Kentucky Innovation.

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