Intraoperative Near-Infrared Optical Imaging Can Localize Gadolinium-Enhancing Gliomas During Surgery

**BACKGROUND:** Although real-time localization of gliomas has improved with intraoperative image guidance systems, these tools are limited by brain shift, surgical cavity deformation, and expense.

**OBJECTIVE:** To propose a novel method to perform near-infrared (NIR) imaging during glioma resections based on preclinical and clinical investigations, in order to localize tumors and to potentially identify residual disease.

**METHODS:** Fifteen patients were identified and administered a Food and Drug Administration-approved, NIR contrast agent (Second Window indocyanine green [ICG], 5 mg/kg) before surgical resection. An NIR camera was utilized to localize the tumor before resection and to visualize surgical margins following resection. Neuropathology and magnetic resonance imaging data were used to assess the accuracy and precision of NIR fluorescence in identifying tumor tissue.

**RESULTS:** NIR visualization of 15 gliomas (10 glioblastoma multiforme, 1 anaplastic astrocytoma, 2 low-grade astrocytoma, 1 juvenile pilocytic astrocytoma, and 1 ganglioglioma) was performed 22.7 hours (mean) after intravenous injection of ICG. During surgery, 12 of 15 tumors were visualized with the NIR camera. The mean signal-to-background ratio was 9.5 ± 0.8 and fluorescence was noted through the dura to a maximum parenchymal depth of 13 mm. The best predictor of positive fluorescence was enhancement on T1-weighted imaging; this correlated with signal-to-background ratio (P = .03). Nonenhancing tumors did not demonstrate NIR fluorescence. Using pathology as the gold standard, the technique demonstrated a sensitivity of 98% and specificity of 45% to identify tumor in gadolinium-enhancing specimens (n = 71).

**CONCLUSION:** With the use of Second Window ICG, gadolinium-enhancing tumors can be localized through brain parenchyma intraoperatively. Its utility for margin detection is promising but limited by lower specificity.

**KEY WORDS:** Brain tumor, Fluorescence, Indocyanine green, Near-infrared

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**Each year, over 15,000 patients undergo resection of brain tumors in the United States.**¹ During surgery, identifying margins of brain tumors, particularly glioblastomas (GBMs) and highly invasive neoplasms, remains a technical challenge. Thus, for both benign and malignant brain tumors, the most common cause of relapse is local recurrence at the resection margins. At the time of the operation, surgeons typically use visual inspection and tactile discrimination to differentiate tumor margins from surrounding normal brain parenchyma. In addition, imaging adjuncts such as navigation and intraoperative ultrasound can provide value. However, this method has many limitations, which accounts for the high rate of local failure.

Intraoperative adjunctive technologies, such as imaging-based navigational systems, have been...
Based on preclinical animal and clinical data, we have been studying alternative approaches to using NIR dyes to develop contrast between tumor tissue and surrounding normal brain parenchyma. NIR dyes have several advantages. They have markedly improved depth of penetration, do not autofluoresce in the brain, and afford an opportunity for high-resolution data acquisition. Several groups have experimented with an NIR dye, indocyanine green (ICG), administered intravenously minutes before resection. However, this approach has failed to provide value as a means of localizing tumor margins. Although ICG provides vascular enhancement at the surface of the tumor, the dye rapidly clears and leaves significant background noise.

Our group has been studying an alternative approach. We have been studying a technique that we call “Second Window ICG” as a means to delineate tumor margins during surgery. In this technique, we administer intravenous ICG. We call it Second Window ICG to differentiate it from the standard ICG videoangiography bolus of 25 mg just before visualization. ICG for Second Window ICG is solubilized in a higher concentration using sodium chloride and administered 24 hours before imaging. ICG has a peak excitation of 780 to 790 nm and emission spectra of 805 to 820 nm in animal and human tissues. These make ICG an ideal fluorescent contrast agent within this spectral window, because other tissue components (water, hemoglobin-oxygen, deoxyhemoglobin, melanin) do not interfere with the excitation source.

Over 24 hours, the dye accumulates in the tumor tissue because of the enhanced permeability and retention (EPR) effect. This effect explains delivery in that small molecules may pass through a disrupted tumor blood-brain barrier and be retained because of a relative lack of drainage (by lymphatics, for example). Prior work has demonstrated that accumulation within may occur because of the relatively hypoxic microenvironment. As a molecule, ICG is amphiphilic; it has 2 ringed groups (1 with positive charge) and 2 negatively charged sulfonate groups. At high concentrations and at extreme temperatures (>60°C) when dissolved in vitro, chains may form based on noncovalent interactions between individual molecules. These aggregations (termed J-aggregates) can have different fluorescent properties. At the dose injected at the time of administration, ICG does not form J-aggregates; this has been verified in our laboratory. In circulation, the fluorescent compound is unlikely to form these aggregations given the relatively large plasma volume compared with the volume injected. Rather, we hypothesize that the amphiphilic properties of ICG may contribute in part to retention once delivered within the tumor microenvironment. Current tissue-processing methods limit our ability to support whether or not this mechanism contributes to retention within tumors. Based on preclinical animal and clinical data, we administered Second Window ICG for patients with intracranial gliomas based on this concept of EPR effect.

In this study, we hypothesized that Second Window ICG may be a reasonable approach to localize gliomas and to identify margins intraoperatively. The goal of this first-in-human pilot study with high-dose Second Window ICG was to determine whether gliomas would fluoresce during surgery. We found strong tumor-to-background fluorescence ratios, which appear to correlate with degree of gadolinium enhancement on preoperative magnetic resonance imaging (MRI) scans. Furthermore, we found that this approach could identify tumor margins with little autofluorescence or background noise.

METHODS

Study Design

This prospective cohort study was approved by the University of Pennsylvania Institutional Review Board, and all patients gave informed consent. The trial is registered under clinicaltrials.gov with identifier NCT02288095, and recruitment started in October 2014. Any patient undergoing craniotomy for brain tumor was considered eligible for this study. Adult patients (age >18 years) with brain tumors were eligible for the study. Pregnant woman and iodide allergy were the main exclusion criteria. All patients underwent preoperative MRI of the brain with intravenous gadolinium. The presence or absence of gadolinium enhancement and the tumor size on MRI were noted. Patients were consented and informed that the scope of surgery would not substantially change based on NIR findings, and biopsies would be taken only if deemed safe by the senior surgeon (JYKL).

NIR Imaging Contrast Agent

Patients were injected intravenously with a 5 mg/kg dose of ICG (Akorn Pharmaceuticals, Decatur, Illinois; C43H47N2O6S2.Na). Patients were injected 24 hours before surgery based on preclinical studies from our laboratory. There were no immediate complications from Second Window ICG administration at this high dose.

NIR Imaging System

All cases were imaged using the Visionsense Iridium camera system (Visionsense, Philadelphia, Pennsylvania; Figure 1). This system is FDA-
approved for perfusion imaging in plastic and reconstructive surgery (to assess for flap viability for example). The sensor is a silicon image sensor with an open field of view of 19 cm × 14 cm at a 40-cm nominal imaging distance. The emission filter band in visible light range is from 400 to 700 nm, whereas the emission filter band for NIR is much narrower from 825 to 850 nm (with recording at 720p). A heat map was used as an overlay on the visible light image that also provided fluorescence intensity. Image processing is done in real time and presented at 1080p video resolution.

FIGURE 1. Visionsense Iridium open visualization system (Visionsense). The extendable arm houses the camera and lens that can be suspended over the surgical field after being enclosed in a sterile camera drape. There are 2 light sources: a laser tuned to 805 nm (shown in bottom of cart) and a halogen for visible light (not shown). The camera system simultaneously acquires visible light images and an NIR image of the same scene. Since the 2 image types are acquired simultaneously and correspond to the same scene, an integrated image is created by showing the NIR image as a pseudo-color overlay on the visible image. The surgeon visualizes the scene on the monitor that is built into the camera system. NIR, near-infrared.

This camera system is not integrated into existing microscopes. It is a separate platform with its own xenon light source for white light (400-700 nm) and laser light source for NIR excitation (805 nm). It comes as a separate tower, and the integrated arm is used to position the camera system over the field in a sterile manner. The screen provides separate white light view (color spectrum) and separate NIR view (black/white). These 2 images can be superimposed in real time, and the NIR view can be pseudo-color-coded with more intense signal represented as yellow/red and weaker signal represented as purple/blue. Alternatively, the NIR view can be pseudo-color-coded as green and superimposed in real time over the white light view.

Study Procedure

Patients underwent anesthesia and a craniotomy using anatomic landmarks as well as neuronavigational imaging. Preoperative MRI was used for navigation in all subjects. All subjects underwent 3T MRI (Siemens Magnetom, Munich, Germany) at the University of Pennsylvania and were administered intravenous Multihance gadobenate dimeglumine 529 mg/mL (Bracco, Milan, Italy). A repetition time value of 1760 ms and echo time value of 3.1 ms were used with a flip angle of 15°. All patients were uniformly administered 0.5 mg/kg intravenous mannitol and 10 mg intravenous decadron before opening of the dura. Upon dural exposure, the operating room lights were dimmed, and the NIR imaging system was steriley draped and positioned above the operative field. The presence or absence of NIR signal was documented post hoc and recorded in binary yes/no format. The dura was then opened, and the camera was brought into the field, and the ability to localize NIR signal corresponding to the tumor before cortical incision was also recorded in binary yes/no format. Surgery then proceeded in the standard manner without the use of NIR-imaging adjuncts. If the NIR signal was not identified before corticectomy, an additional attempt was made at the time of tumor identification.

When the attending neurosurgeon was satisfied that a complete resection had been achieved, NIR imaging was used to determine if there were areas of residual disease. The surgeon’s impression of the NIR signal of the tumor itself and the margin biopsy samples were recorded in binary format as well as photo documented for post hoc analysis. Areas were biopsied at the discretion of the senior surgeon (JYKL).

While still in the operating room, all specimens were coded by the attending surgeon (JYKL) as consistent with tumor (white; yes/no) with bright light and with NIR fluorescence (NIR; yes/no). The histopathological diagnosis obtained several days later served as the gold standard and was catalogued by a single neuropathologist at our institution. The presence of tumor and gliosis and inflammation was noted and incorporated into our analysis.

Patients were admitted to the intensive care unit following surgery. There were no adverse outcomes. A postoperative MRI took place on the first postoperative day. Patients were seen at approximately 2 and 4 weeks from surgery.

Immunohistochemistry and Fluorescence Microscopy

Frozen tumor sections were prepared in standard fashion. Samples were examined using an Olympus IX51 fluorescent microscope (Olympus Corporation, Tokyo, Japan) equipped with an ICG-specific filter set (Chroma 49030; Chroma Technology Corporation, Bellows Falls, Vermont). Histopathological analyses were carried out cataloging tissue samples that showed fluorescence following intended resection. Permanent sections were chemically fixed and embedded in paraffin blocks at...
TABLE 1. Description of Patients—Demographics and Injection Parameters

<table>
<thead>
<tr>
<th>ID</th>
<th>Age</th>
<th>Sex</th>
<th>Final Pathology</th>
<th>Location</th>
<th>ICG Injection Dose, mg</th>
<th>Patient Weight, kg</th>
<th>Time From ICG Injection to Camera Visualization, h</th>
<th>Prior Surgery or Radiation</th>
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<td>81</td>
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<td>Glioblastoma</td>
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<td>354</td>
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<td>67</td>
<td>M</td>
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<td>Left medial temporal</td>
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<td>78.9</td>
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<td>Surgery</td>
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*ICG, indocyanine green; WHO, World Health Organization.

This patient had prior open biopsy at an outside institution that resulted in a change in imaging characteristics.
<table>
<thead>
<tr>
<th>ID</th>
<th>Pathology</th>
<th>Maximum Diameter on Preoperative MRI (mm)</th>
<th>Gadolinium Enhancement on MRI</th>
<th>Signal Visible With NIR Camera</th>
<th>Ratio of T1 Signal Intensity of Tumor Compared to Contralateral</th>
<th>Signal to Background Ratio (SBR)</th>
<th>Postoperative MRI—Gross Total Resection</th>
<th>Any True Negative Margin</th>
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<td>9.2</td>
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**TABLE 2. Near-Infrared Signal of Gross Tumor Specimen and Postoperative Results**

*MRI, magnetic resonance imaging; NIR, near-infrared; WHO, World Health Organization.

b This patient had prior open biopsy at an outside institution that resulted in a change in imaging characteristics.
a 5-μm thickness. Vertical sectioning was performed with hematoxylin and eosin staining. Each tissue sample was assessed for the presence of tumor-specific cells (matched to the bulk sample) and evidence of inflammation.

**Data Analysis**

The data from 15 patients were analyzed using Stata v.10 (StataCorp LP, College Station, Texas). To quantitate the amount of fluorescence from the tissue, we used region of interest (ROI) analyses within the Visionsense software (VSPlayer v1.8.05.01; Visionsense). Each reading was done in quadruplicate. In addition, a background reading was taken in quadruplicate from adjacent normal surrounding dura or brain tissue. Together, the signal-to-background ratio (SBR) was generated from dividing the mean tumor fluorescence as a proportion of the normal brain parenchyma fluorescence. MRI image analysis was performed using GE PACS software (GE Healthcare, Little Chalfont, United Kingdom). ROI analysis was performed on approximate area of 1-cm-diameter circles on axial T1 postcontrast studies. ROI analysis was performed in triplicate. Two-by-two contingency tables were constructed using STATA 10 (StataCorp), and sensitivity/specificity/receiver operating characteristic (ROC) analysis was computed. By calculating the area under the curve generated by plotting true-positive (y) vs false-positive (x) rates, we were able to determine the test discrimination, which reflects the ability of the test to properly classify those without and those with the disease.

**RESULTS**

**Clinical Design**

Between November 2014 and December 2015, 15 patients (n = 7 male) between the ages of 20 and 81 (mean 54) with a diagnosis of a solitary brain tumor and presumed glioma based on imaging or prior surgery/biopsy were evaluated in a neurosurgical clinic (Table 1: clinical parameters). T1- and T2-weighed MRI scan demonstrated a solitary brain mass (Table 2: 9-72 mm, mean 38 mm). All patients were deemed surgical candidates, and they consented to a craniotomy and the principles of maximal safe tumor resection.

**Tumor Fluorescence Is Independent of Tumor Histology, Tumor Size, and Dye Kinetics**

Once the tumor was exposed by the operating surgeon but before surgical debulking, every attempt was made to image the gross tumor specimen in situ with the NIR camera. The overall finding of this study is that 12 of the 15 gliomas demonstrated NIR positivity (Table 2). Three of the patients with gliomas did not demonstrate significant NIR fluorescence, despite opening the dura and despite obtaining direct visualization of the tumor (Table 2, subjects 5, 11, and 20). The 12 patients with tumors that did demonstrate NIR fluorescence had a mean SBR of 9.5 ± 0.8 (range, 5.5-14.8) vs a mean SBR 1.5 ± 0.15 for the 3 patients that did not demonstrate obvious fluorescence (Figure 2 and Table 2).

To understand the lack of fluorescence in these 3 patients, we examined several variables: tumor size, tumor histology, time from dye injection to NIR imaging, and MRI characteristics. First, we hypothesized that larger tumors may accumulate more dye, and hence we measured tumor size on preoperative MRI. The mean largest dimension of the 12 NIR-positive tumors was 41 mm, and the mean largest dimension of the non-NIR-positive tumors was 24.5 mm, but this was not a statistically significant difference (Wilcoxon rank sum \( P = .1939 \); Table 2).

Next, to determine if the tumor SBR was related to pathology, we correlated the final pathological diagnosis to the degree of fluorescence. On final pathology, all patients had gliomas ranging from World Health Organization (WHO) grade I to IV. Eight patients had newly diagnosed GBMs and 2 had recurrent GBMs. One patient had an anaplastic astrocytoma (WHO grade III), and 2 patients had low-grade gliomas (WHO grade II). Two patients had WHO grade I intraventricular gliomas, including 1 patient with a ganglioglioma of the third ventricle and 1 patient with adult-onset, juvenile pilocytic astrocytoma of the left lateral ventricle wall (Table 1). We did find that WHO grade II or III gliomas did not demonstrate tumor fluorescence, whereas WHO grade IV and I gliomas did demonstrate fluorescence. However, increasing and/or decreasing WHO grade of glioma was not the significant factor predicting fluorescence.

We also hypothesized that the timing of injection could lead to the absence of NIR visualization. On average, the 12 patients with tumors demonstrating NIR signal were injected 22.8 hours before the initial visualization, whereas the 3 patients who did not demonstrate NIR signal were injected 22.2 hours before the initial visualization. The difference in time was not statistically significant (Wilcoxon rank sum, \( P = .59 \)).

We also hypothesized that the volume of the tumor would correlate with the SBR, but upon linear regression, the \( R^2 \) was .0034. The correlation with maximum diameter was similarly poor at .027.
In Situ NIR Imaging Is Correlated With Gadolinium Enhancement by MRI

Finally, we postulated that preoperative gadolinium enhancement may predict NIR fluorescence. Indeed, every tumor that enhanced preoperatively on MRI with gadolinium was also visible with NIR fluorescence. In contrast, the 3 tumors that did not enhance were not visible with NIR fluorescence (Table 2). There was a direct correlation between gadolinium enhancement and NIR fluorescence. Preoperative T1-weighted, postcontrast MRI axial scans were used to obtain ROI signal intensity for all 15 tumors including the nonenhancing tumors. The MRI T1 signal ratio was mean 1.9 ± 0.16 (range, 1.1-3.4). Control areas of the brain were chosen as appropriate, usually in contralateral mirror image locations or contralateral uninvolved white matter, yielding MRI T1 signal ratio of mean 1.1 ± 0.06 (range, 1.0-1.2). The ratio of T1 signal intensity was plotted against the NIR SBR (Figure 3). Linear regression demonstrated that for every 1 full-point increase in MRI signal intensity compared with normal brain, the SBR on NIR increased by 4.7 ± 1.3 times (P = .03, adjusted $R^2 = .44$).

NIR Imaging Can Identify Gliomas Through the Intact Dura

NIR light possesses a longer wavelength than visible light, and thus tissue penetration is greater than visible light. Since ICG fluoresces in the NIR range, we hypothesized that Second Window ICG accumulation within tumors deep to the dura and deep to brain parenchyma may be visible with NIR imaging from the surface—both above the dura and above the cerebral cortex. During surgery, the operating surgeons performed a craniotomy and exposed the dura. Before opening the dura, the camera was placed above the closed dura to see if there was NIR signal at the approximate location of the presumed tumor (as determined by correlation with the neuronavigational BrainLab system [Brainlab, Munich, Germany]). In the 12 patients with contrast-enhancing tumors and with positive NIR signal, the mean distance from the dura to the nearest enhancing tumor edge was 13.5 ± 4.0 mm. Eight of the 12 patients demonstrated visible NIR signal above the dura. In the 4 patients (subjects 106, 112, 10, 17) where NIR signal could not confidently be identified above the dura, the enhancing tumor edge was located >20 mm from the dura based on MRI (Table 3). Hence, the deepest visualized tumor was 13 mm from cortical surface. No tumor located deeper than 13 mm was visualized at the dura surface with NIR technology (Figures 4-6). In cases where NIR signal could be identified above the dura (n = 8 patients), the SBR above the dura was 6.0 ± 0.85 (61% of final SBR; Table 3).

Next, the dura was cut open and reflected away from the cortical surface. The NIR camera was then positioned above the cortex. NIR imaging identified fluorescence in 12 of the 15 (78%) patients. The mean SBR in these cases increased to 7.5 ± 0.93 (77% of final SBR), ultimately improving to a mean 9.5 ± 0.8 (range, 5.5-14.8, representing 100% of final SBR) upon final exposure of the tumor (Figure 2, Table 3). Thus, the fluorescent signal was visible before opening the dura (61%), became stronger at the cortical surface after dural opening (77%), and demonstrated a strong (100%) SBR upon finally exposing the tumor via corticectomy.

Total NIR imaging time did not exceed 12 minutes in any patient.

NIR Fluorescence Is Sensitive But Not Specific for Residual Tumor at Surgical Margins

In addition to gross examination of the tumor mass, we studied the surgical margins during and at the completion of the surgical resection. After the senior surgeon deemed the surgery to be complete, the fluorescent camera was used to detect potential areas of residual disease. The NIR imaging device was used to scan the wound bed, and areas of residual fluorescence were biopsied as deemed safe based on adjacent anatomy. Diagnostic test characteristics were calculated concentrating only on the 12 patients with contrast-enhancing gliomas. Seventy-one specimens (12 bulk tumor specimens plus an additional 59 margin biopsies) were analyzed.

Of the 71 specimens in all enhancing tumors, 51 (71.8%) demonstrated glioma (tumor) tissue based on final histopathology and 61 (85.9%) were positive for NIR signal (see 2 × 2 contingency tables in Table 4). Using tumor on final pathology as the gold standard, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) based on the surgeon’s impression (visible light) alone, values were (respectively) 84.3%, 80%, 91.5%, 66.7%. In contrast, the sensitivity, specificity, PPV, and NPV of NIR intraoperative imaging for identifying tumor was (respectively) 98%, 45%, 82%, and 90% (Table 4A and 4B). Of the 11 samples with false-positive NIR signal, 2 specimens demonstrated no specific pathological change.
in normal brain, 3 specimens demonstrated necrosis, 4 specimens demonstrated reactive gliosis and atypical cells, 1 specimen demonstrated normal choroid plexus, and 1 specimen consisted of scar and fibrous tissue from prior surgery.

As a subset analysis, we calculated the test characteristics of NIR imaging for newly diagnosed GBM separately. Eight patients were diagnosed with new GBM, and a total of 34 specimen biopsies were performed. Twenty-seven (79%) were positive for tumor based on pathological examination and 30 (88%) specimens were positive for NIR signal. Using the presence of tumor on final pathology as a gold standard, the sensitivity, specificity, PPV, and NPV of NIR intraoperative imaging for identifying tumor were (respectively) 96.3%, 42.9%, 86.7%, and 75% (Table 4C and 4D). Of the 4 specimens with false-positive NIR signal, the pathology consisted of 1 specimen containing choroid plexus, 1 specimen demonstrating reactive gliosis, and 2 specimens demonstrating normal brain without specific pathological change. Of note, in contrast with experience in 5-ALA, we did find normal choroid plexus to retain ICG but did not see similar retention of dye in normal ependymal lining.

In order to determine the utility of NIR imaging to achieve a gross total resection, we performed a retrospective analysis of the data. Of note, we intentionally specified in our study protocol that the extent of surgery would not change based on the NIR results, and as such we do not believe we can adequately address the question of extent of resection with this current study. However, we did look at the postoperative MRI for a gross total resection as seen in Table 2. We correlated this with the presence of true negative margin biopsy specimens. Of the 12 enhancing gliomas, 4 patients had true-negative biopsy specimens and all 4 had gross total resection seen on MRI. In contrast, of the 12 enhancing gliomas, 8 patients did not have true-negative margin biopsy specimens and only 3 of these had gross total resection on postoperative MRI. This is suggestive of the benefit of a true-negative NIR signal after resection.

**DISCUSSION**

Better visualization (surgical microscope), intraoperative MRI and imaging-based navigational systems (with or without technologies to account for brain shift) have improved the ability of neurosurgeons to identify tumor and to distinguish it from normal tissues. Fluorescent contrast agents take visualization to another level with the potential for real-time imaging and tumor/cell-specific identification of disease. Although 5-ALA is not FDA approved for use in tumor imaging, surgical resection of glioma using 5-ALA has been favorably demonstrated through multiple studies, as best summarized by Zhao et al. The goal of this preliminary study was to determine if Second Window ICG delivery and NIR imaging could identify brain tumors during a craniotomy. In this pilot study, 15
ICG is rapidly cleared and leads us to the subject of this study. In a prior publication by Jiang et al., a syngeneic murine flank tumor model was used to test NIR imaging of ICG at various doses ranging from 0 to 10 mg/kg. Importantly, NIR imaging was performed serially from minutes to 72 hours after administration. The vascular visualization was contrasted with tumor visualization, and over time the videoangiography visualization dissipated, whereas the tumor visualization improved, peaked at 24 hours, and then dissipated as well. The optimal imaging of tumor rather than vasculature for “Second Window ICG” was seen at doses ranging from 5 to 10 mg/kg and tumor signal was best appreciated at 24 hours. Having developed this dose and timing schema in rodents, we now extend this work to intracranial tumors in this publication. The gross observation that 12 of the 15 patients’ brain tumor specimen retained measurable NIR signal parallels the experience previously published in lung cancer, and leads us to the tentative hypothesis that gadolinium-enhancing gliomas with inherently leaky vasculature can be visualized with a NIR camera by taking advantage of the EPR-mediated delivery of Second Window ICG. Given that ICG is delivered via passive diffusion, we posit that it remains extracellular in keeping with prior studies. We have tentatively termed this technique “DEPiCT”, which stands for “Delayed Enhanced Permeability and retention of near Infrared Contrast dye for Tumors.” The results from this cohort of patients demonstrate that Second Window ICG can be delivered systematically to provide NIR fluorescence in brain tumors at high signal strength (approximately 9 times higher than background). When delivered at the timing and dose specified, contrast-enhancing brain tumors demonstrated retention of Second Window ICG based on real-time fluorescence.

The second major finding in this study was tumor localization by real-time, NIR imaging provides similar information as preoperative MRI scanning data. Current neuronavigation techniques allow neurosurgeons to make judgments between preoperatively acquired MRI scans and areas felt to represent tumor in the operating room. However, even if the coregistration of the surface anatomy of the skin is precise, the brain can shift. Osmolar therapy/diuresis, opening of cerebral cisterns, and corticectomy/resection/tumor cavity deformation all create major challenges for intraoperative navigational accuracy. In addition to brain shift, increased setup times, and increased operative duration, its indirect methods of visualization detract from the known benefits of these technologies. Because of this situation, many centers have used an intraoperative MRI scanner within the...
Because of its operational room environment, usually of lower magnetic field strength in order to improve safety and maneuverability. Although intraoperative MRI can take account for brain shift (during the singular time of image acquisition) the timing, dose, and type of intraoperative contrast agent used may affect which areas of the tumor (or brain) are ultimately resected. Intraoperative MRI remains an expensive technology in terms of cost and time. These shortcomings continue to fuel interest in systemically delivered fluorescent compounds that produce a high SBR within tumors in real time.

The third major discovery in this series was the value of NIR imaging (λexcitation > 780 nm). At present, the FDA in the United States and the European Medicines Association have approved only a handful of systemic fluorescent contrast agents for use in humans. ICG remains the only NIR fluorescent contrast agent that has been approved for systemic injection in humans. The primary value of NIR imaging over other optical techniques is the ability to obtain superior depth of penetration and less autofluorescence and background noise from the surrounding cranium and brain parenchyma. Because of its longer wavelength, we were able to visualize Second Window ICG accumulation in enhancing gliomas even through the dura and through presumably uninvolved cortical parenchyma up to a depth of 13 mm. This technique allows us to localize intracranial glioma without opening the dura in real time (Figures 4–6).

A fourth major finding from this study was our ability to detect margins. We hypothesized that NIR intraoperative imaging in real time may be more sensitive than white light alone in identifying brain tumors. Indeed, we found that, in the identification of gliomas, there appeared to be a trend toward improved sensitivity of NIR at the expense of specificity compared with white light alone. Similarly, the NPV was improved at the expense of PPV with the use of NIR fluorescence. In our data set of 71 samples of contrast-enhancing gliomas, the surgeon’s sensitivity/specificity/PPV/NPV was 84.3%/80%/91.5%/66.7% compared with visualization mediated by NIR fluorescence (sensitivity/specificity/PPV/NPV of 98%/45%/82%/90%). The test accuracy as determined by the area under the curve appeared to be better for visible light alone vs NIR, 0.82 vs 0.72. This is most likely because of the low specificity of NIR signal. By comparison, 5-ALA has a summary ROC curve at 94% based on meta-analysis; pooled analysis in patients with GBM who underwent surgery with 5-ALA yielded a sensitivity of 87% and specificity of 89%. At this point, the use of Second Window ICG in the detection of tumor margins for oncologic control remains an area requiring further work. Hence, its use for detection of tumor margins for oncologic control remains an area requiring further work.

Although the test characteristics of Second Window ICG administered 24 hours to visualization demonstrate low specificity, 5-ALA, the only fluorescent drug in widespread use for glioma surgery, also demonstrates limitations as a diagnostic test. Valdes et al studied the test characteristics of 5-ALA fluorescence imaging and calculated a sensitivity of 47% and specificity 100% in a heterogeneous group of 14 patients. In a follow-up study focusing on 11 patients with newly diagnosed GBM, Roberts et al calculated the test characteristics of 5-ALA. Of the 124 specimens biopsied, 70% were positive for tumor, and thus 30% were not positive for tumor. The sensitivity of 5-ALA without the spectrophotometer but using the surgical microscope was 75%, with a specificity of 71%, PPV of 95%, and a NPV of 26%. Similarly, work by Stummer et al calculated a PPV of 82.8% and a NPV of 40% in a group of 29 patients with GBM (318 biopsy specimens). A recent meta-analysis published by Zhao et al has pooled data together and has concluded that 5-ALA has an overall sensitivity 87%, specificity 89%. Given that 5-ALA does not appear to be >90% sensitive or specific, we publish this article in an effort to explore ways that Second Window ICG, a nonspecific NIR dye, may improve glioma surgery.

The last major finding that warrants discussion is the correlation of NIR imaging and the preoperative MRI scanning. ICG is not receptor specific. Within the circulation, ICG binds to plasma albumin; we suspect that at high doses it remains trapped within the extracellular matrix of tumor tissue. As such, it is most likely not receptor bound or receptor specific. We are not surprised by the relatively low specificity with this technique, because ICG is not receptor specific. We posit that Second Window ICG as an intraoperative tumor imaging adjunct may be useful for initial localization of gliomas because it correlates with contrast enhancement on MRI, thus allowing the surgeon to “see” the contrast-enhancing portion of the tumor early, oftentimes before dural opening. Indeed, as seen in Figure 3, the degree of contrast enhancement appears to be directly correlated with the NIR signal.
signal, implying that the early amount of accumulation of gadolinium within a tumor correlates with the late (approximately 24 hours later) accumulation of Second Window ICG within the tumor. Indeed, gadolinium enhancement has been used as a proxy for disease in high-grade gliomas, with a diagnostic sensitivity (T1 contrast enhancement alone) of 72.5%, specificity of 65%, PPV of 86.1%, and NPV of 44.1%. The ROC characteristics are increased further with the interpretation of multiple sequences including diffusion and cerebral blood volume.

Although we now understand that tumor cells in gliomas may exist beyond the enhancing margins and into surrounding fluid-attenuated inversion recovery/T2 hyperintense regions, the primary aim continues to be to achieve as complete a resection of the enhancing portions of these tumors, as possible, while maintaining function.

Limitations
This pilot study demonstrated 1 major limitation to NIR imaging for brain tumors. NIR visualization of Second Window ICG does not appear to be specific for glioma. It is possible that areas of tumor cells...
adjacent edema or inflammatory change passively accumulate ICG by diffusion or by increased vascularity. Instead, we hypothesize that NIR visualization of Second Window ICG in delayed (24-h fashion) appears to best correlate with gadolinium enhancement. For high-grade gliomas, this may provide value for surgical resection, because the general goal is maximal resection of the contrast-enhancing portion of the tumor. In contrast, for grade II gliomas that generally do not enhance with gadolinium, Second Window ICG as administered via DEPICT technique may not provide any significant value.

We acknowledge that there are many caveats to this study. First, this is a pilot study; therefore, the study size is small (n = 15). In addition, SBR is an arbitrary number and may not be a true measure of tissue fluorescence. In addition, the SBR may vary with time after administration. Thus, correlating metabolism to fluorescence may not accurately represent the biology of the nodule. The test characteristics obtained in this study are largely determined by the number of biopsies taken at the time of surgery, and thus accurate test characteristic calculation is limited. Because of the limited number of biopsy samples, we combined the margin specimens with the bulk tumor specimens to maximize the power of this analysis, but of course this has the drawback of limiting the ability to draw conclusions regarding the utility of margin detection.

Another important caveat of this Second Window ICG technique of visualization is the difficulty in interpretation of the image on the screen. The VisionSense Iridium system (Vision-sense) provides a 2-dimensional view of a 3-dimensional scene. This handicap is also coupled to the concern that NIR wavelength, and, thus, the NIR signal may actually be slightly deeper or even behind normal brain parenchyma or even dura. Hence, the screen may show a bright object that is actually a few millimeters behind normal parenchyma. Careful dissection and understanding of the physical properties of the NIR signal and the 2-dimensional screen must be made by the trained observer before embarking on further surgical resection. We plan to investigate issues of parallax in 3 dimensions in future work.

### CONCLUSION

In conclusion, ICG is one of several NIR fluorescent agents, but it is the only NIR fluorescent contrast agent approved for use in humans by the FDA. It is low in cost, minimally toxic, and provides for real-time visualization. This is the first work to demonstrate practical sensitivity using Second Window ICG as an optical contrast agent during the neurosurgical resection of human brain tumors. The method of drug delivery used in this technique relies on passive diffusion. We demonstrate that gadolinium-enhancing tumors show measurable NIR contrast in the operating room with real-time visualization. The degree of gadolinium enhancement on the preoperative MRI appears to correlate with the NIR signal intensity within the tumor. The sensitivity of this technique appears to be better than bright light alone but at the expense of specificity. Second Window ICG may provide a practical and sensitive means of identifying glioma before dural opening, and before corticectomy for accurate localization. Its use for margin detection and establishing the extent of resection remains under study.
Disclosure
Dr Lee owns stock options in VisionSense. Dr Singhal holds patent rights over technologies presented in this article. Supported in part by the National Institutes of Health R01 CA193556 (SS), and the Institute for Translational Medicine and Therapeutics of the Perelman School of Medicine at the University of Pennsylvania (JYKL). In addition, research reported in this publication was supported by the National Center for Advancing Translational Sciences of the National Institutes of Health under Award Number UL1TR000003 (JYKL). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. The other authors have no personal, financial, or institutional interest in any of the drugs, materials, or devices described in this article.

REFERENCES
The authors present a timely pilot study on "Second Window ICG," repurposing of ICG for intraoperative real-time detection of gliomas. Although many questions about this agent’s efficacy still remain, the authors have demonstrated sensitive high uptake and retention of the fluorophore correlated with gadolinium-contrast MRI of tumor in a small cohort of 15 glioma patients. This approach is innovative repurposing of an already approved agent for human use, and there is a low barrier of entry because of an excellent safety record, available detection apparatus, long-imaging window, and experience in standard clinical use for vascular procedures. However, further work is necessary to study "Second Window ICG," to evaluate its efficacy and limitations compared with other fluorescent dyes such as fluorescein and 5-ALA. If the validation studies are completed successfully, these nonspecific dyes may be useful adjuncts in optical surgical techniques.

The authors provide a proof of concept that ICG can be used to visualize gliomas intraoperatively. Twenty-four hours ("second window") after ICG administration the fluorophore, accumulated in contrast-enhancing gliomas, is detected with a separate special camera system, brought in above the surgical field. Of special note is that using wavelength in the near-infrared spectrum, depth penetration of up to 13 mm below the surface is achieved. Further refinement and appropriate clinical evaluation for safety and efficacy are needed to determine whether this innovative technique is merely colorful or also helpful.

However, the described method illustrates that the spectrum of light has more potential (ie, multispectral analysis and penetration to various depths) but is presently not fully utilized to visualize the brain and its pathologies. Light provides more information than our eyes can detect. In order to use the full spectrum, without constantly changing cameras, digital image processing has to be improved and integrated directly into our surgical microscopes.

Investigating this avenue may bridge the gap between surface-visualization, as in fluorescence-guided resection (eg, 5-ALA), and depth visualization (intraoperative MRI, CT, US) as the current standards for intraoperative imaging. Articles like the current one open this avenue.

The authors are to be commended and encouraged to continue their investigations. As intriguing and beautiful as optical imaging techniques are, the real test remains: to prove that they provide practical information for surgical decision making toward our ultimate goal, improving our patients’ treatment.

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Even in the context of infiltrative, high-grade gliomas, maximal safe resection carries a clear survival benefit for patients, and, in this regard, intraoperative surgical adjuncts to achieving “gross total resection” as measured by postoperative MRI have potential clinical utility. The authors describe an interesting and novel variation on the fluorescent dye ICG, named Second Window ICG, which enables visualization of contrast-enhancing tumor through a near-infrared range (NIR) camera. This preliminary study is carefully performed, with pathology correlates and a tissue bank, but is not yet fully utilized to visualize the brain and its pathologies. The delays in US access to 5-ALA and the cost-prohibitive nature of intraoperative MRI capability for many institutions create a real opportunity for intraoperative tumor visualization techniques such as this in the brain tumor space. Integration of their technique with an operating microscope or endo/exoscope and additional data regarding the interpretation of what “positive” Second Window ICG signal represents—especially since NIR signal can be seen through “normal” tissue—are interesting points for future investigation.

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