Detection of human brain cancer infiltration ex vivo and in vivo using quantitative optical coherence tomography

Carmen Kut, Kaisorn L. Chaichana, Jiefeng Xi, Shaan M. Raza, Xiaobu Ye, Elliot R. McVeigh, Fausto J. Rodriguez, Alfredo Quinones-Hinojosa, Xingde Li

More complete brain cancer resection can prolong survival and delay recurrence. However, it is challenging to distinguish cancer from noncancer tissues intraoperatively, especially at the transitional, infiltrative zones. This is especially critical in eloquent regions (for example, speech and motor areas). This study tested the feasibility of label-free, quantitative optical coherence tomography (OCT) for differentiating cancer from noncancer in human brain tissues. Fresh ex vivo human brain tissues were obtained from 32 patients with grade II to IV brain cancer and 5 patients with noncancer brain pathologies. On the basis of volumetric OCT imaging data, pathologically confirmed brain cancer tissues (both high- and low-grade) had significantly lower optical attenuation values at both cancer core and infiltrated zones when compared with noncancer white matter, and OCT achieved high sensitivity and specificity at an attenuation threshold of 5.5 mm$^{-1}$ for brain cancer patients. We also used this attenuation threshold to confirm the intraoperative feasibility of performing in vivo OCT-guided surgery using a murine model harboring human brain cancer. Our OCT system was capable of processing and displaying a color-coded optical property map in real time at a rate of 110 to 215 frames per second, or 1.2 to 2.4 s for an 8- to 16-mm$^3$ tissue volume, thus providing direct visual cues for cancer versus noncancer areas. Our study demonstrates the translational and practical potential of OCT in differentiating cancer from noncancer tissue. Its intraoperative use may facilitate safe and extensive resection of infiltrative brain cancers and consequently lead to improved outcomes when compared with current clinical standards.

INTRODUCTION

Patients with brain cancer have finite survival times with inevitable recurrence and subsequent death, and surgery is the first-line therapy. The median survival time for patients with high-grade brain cancer is about 14 months, but individual survival is heterogeneous (1, 2). There is a growing body of evidence showing that the extent of resection is the most important risk factor associated with delayed tumor recurrence and prolonged survival (1–4). However, surgery-inflicted neurological deficits are associated with poorer survival; therefore, it is imperative to achieve extensive resection of cancer tissue without compromising noncancer tissue (5). In particular, there is great utility in detecting cancer infiltration within white matter, especially because intraoperative deep white matter stimulation can be unreliable and current technologies are suboptimal in providing a real-time, efficient, and quantitative detection of cancer versus noncancer white matter.

Various technological advances have made major contributions in surgery, including intraoperative magnetic resonance imaging (MRI) and computed tomography (CT), ultrasound, Raman spectroscopy, and fluorescence-guided resections, but these technologies have pros and cons in providing quantitative, real-time, and three-dimensional (3D) continuous guidance in brain cancer detection (6–10). The performances of these different technologies in guiding surgical resection of brain cancer are detailed in table S1.

Optical coherence tomography (OCT) is a noninvasive, label-free, and cost-effective technique capable of imaging tissues in three dimensions in real time (table S1). OCT has been used previously to image various tissue pathologies in human and animal organs, including the retina, gastric tract, coronary artery, breast, and brain (11–13). In recent years, OCT has attracted increasing interest in its application for brain cancer detection and surgical guidance because it can provide high-resolution and continuous, quantitative feedback to the surgeons with imaging depth in millimeters (which is comparable with the resection depth for brain cancers near eloquent areas) (14). For example, OCT has been used ex vivo for 2D imaging of human brain cancer tissues (13, 15, 16), as well as en face imaging of 3D human brain cancer ex vivo (17). Despite these advances, there have been no 3D OCT imaging studies that provide quantitative diagnostic criteria in identifying brain cancer versus noncancer. Additionally, studies to date have not been able to provide direct visual cues for surgical guidance in real time (14). As a result, the utility of OCT in detecting and facilitating resection of brain cancer in humans remains unclear.

To bridge the gap between research and clinical use, we systematically investigated the potential of OCT for real-time and label-free imaging. After devising optical attenuation parameters and establishing a diagnostic threshold for pathologically confirmed cases, we applied these in a double-blinded study to identify the detection sensitivity and specificity of OCT. We then moved into rodent models of human brain cancer and applied the same optical attenuation diagnostic threshold during in vivo brain surgery. Our study provides a real-time method to construct a color-coded map that offers direct visual cues to distinguish cancer versus noncancer at high resolution.
and with a reasonably large field of view (FOV), thus demonstrating the translational potential and practicality of OCT during brain cancer surgery.

RESULTS

OCT imaging of human brain cancer versus noncancer (white matter) ex vivo

Fresh human brain tissues surgically removed from 37 patients (table S2) were scanned over a given volume to generate depth-dependent OCT signal profiles and tissue attenuation values using a swept-source OCT (SS-OCT) system and miniature handheld imaging probe (Fig. 1 and fig. S1). For accurate assessment of the attenuation properties, we developed a method to remove the influence of the depth-dependent effects of the beam profiles (18), where the OCT signal from the brain tissue sample is normalized with the OCT signal from a silicon oxide phantom with known attenuation coefficients.

At the high-speed setting [220,000 A-scans/s for 1024-2048 A-scans per 2D frame, or 110 to 215 2D frames per second (fps)], our OCT system took 1.2 to 2.4 s to scan, process, and display OCT imaging and tissue attenuation results for an 8- to 16-mm³ tissue block (movie S1). Although the default OCT FOV is set to 8 to 16 mm², we can extend the FOV using a robotic positioning device (fig. S2). On the basis of OCT results and corresponding histology of the imaged tissues, an optimal attenuation threshold was established for the identification of high-grade (grade IV) and low-grade (grade II) human brain cancer with high detection sensitivity and specificity (Fig. 1D). Finally, to facilitate the potential intraoperative use of this technology, OCT images were displayed in intuitive 3D and color-coded optical property maps to reflect tissue attenuation properties (Fig. 1E and movie S2).

Optical attenuation differences between human brain cancer and noncancer

Fresh ex vivo human brain tissues in the training data set (nine high-grade, two low-grade, and five noncancer) were characterized to establish
optical attenuation differences between cancer and noncancer white matter (Fig. 2). There was little to moderate overlap in the distribution of optical attenuation values between noncancer and cancer (9% versus 33% overlap for high-grade infiltrated zone versus cancer core and 6% versus 37% overlap for low-grade infiltrated zone versus cancer core). To ensure the accuracy of the optical attenuation values, the diagnosis of all cancer versus noncancer tissues was first confirmed with histopathological analysis by a neuropathologist (F.J.R.).

Lower attenuation values for cancer tissues were found in both cancer core and the infiltrated zone when compared with surrounding noncancer white matter (Fig. 2, A and C). For high-grade, the average optical attenuation value of noncancer \(6.2 \text{ mm}^{-1}\) was significantly higher than that of infiltrated zone \(3.5 \text{ mm}^{-1}\) and cancer core \(3.9 \text{ mm}^{-1}\); for low-grade, the average optical attenuation value of noncancer \(6.2 \text{ mm}^{-1}\) was significantly higher than that of infiltrated zone \(2.7 \text{ mm}^{-1}\), but not significantly higher than that of cancer core \(4.0 \text{ mm}^{-1}\) (Table 1). However, there were no statistically significant attenuation differences for low-grade versus high-grade in both infiltrated zone \(2.7 \pm 1.0\) versus \(3.5 \pm 0.8\); \(P = 0.45\) and cancer core \(4.0 \pm 1.4\) versus \(3.9 \pm 1.6\); \(P = 0.94\) using Welch’s t test.

From the training data set, the optimal threshold attenuation value—defined as maximum sensitivity with at least 80% specificity—was found to be \(5.5 \text{ mm}^{-1}\). Using this specified threshold, we determined the ROCs in detecting both high- and low-grade brain cancers (Fig. 3A). As an example, attenuation results of a brain cancer sample were shown with corresponding histology (Fig. 3B). Notably, the attenuation map represents optical properties of a 3D tissue block along the entire imaging depth \(1.8 \text{ mm}\), whereas the corresponding histology images represent a 2D section of this tissue block at a specific depth. During histopathological validation, the pathologist (F.J.R.) reviewed multiple sections at different depths and concluded that the sections showed areas with high cancer density (red regions in attenuation map), areas

**Table 1. Attenuation data in the training data set for patients with high- and low-grade brain cancer.** Quantitative attenuation values are provided for 16 patients in the training set (9 high-grade, 2 low-grade, and 5 control). \(P\) values were calculated using two-sample, one-tailed Welch’s t test based on the hypothesis that noncancer white matter has higher attenuation. N/A, not available.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>(n)</th>
<th>Attenuation mean ± SD ((\text{mm}^{-1}))</th>
<th>(P) (versus noncancer tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control patients (seizure)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noncancer white matter</td>
<td>5</td>
<td>6.2 ± 0.8</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>High-grade cancer patients</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancer core</td>
<td>9</td>
<td>3.9 ± 1.6</td>
<td>0.002</td>
</tr>
<tr>
<td>Infiltrated zone</td>
<td>3</td>
<td>3.5 ± 0.8</td>
<td>0.004</td>
</tr>
<tr>
<td>Noncancer resection margin</td>
<td>4</td>
<td>7.1 ± 1.0</td>
<td>0.902</td>
</tr>
<tr>
<td><strong>Low-grade cancer patients</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancer core</td>
<td>2</td>
<td>4.0 ± 1.4</td>
<td>0.120</td>
</tr>
<tr>
<td>Infiltrated zone</td>
<td>2</td>
<td>2.7 ± 1.0</td>
<td>0.037</td>
</tr>
</tbody>
</table>

Fig. 2. Establishing the optical attenuation threshold for high- and low-grade brain cancers in patients. (A to C) Histogram distribution (A), diagnostic sensitivity/specificity (B), and the optimal attenuation threshold (C) for both cancer core and infiltrated zone in tissue blocks freshly resected from nine high-grade, two low-grade, and five control patients within the training data set. At an optical attenuation threshold of \(5.5 \text{ mm}^{-1}\), maximum sensitivity was achieved while maintaining at least 80% specificity for differentiating cancer versus noncancer tissues in cancer core and infiltrated zone.
with medium cancer density (yellow regions in attenuation map), and areas with low cancer density, that is, diffusely infiltrated area with mostly white matter and some neoplastic cell components (green regions in attenuation map).

Validation of the optical attenuation threshold in an independent, blinded study of human brain tissue

Brain tissues were collected from 16 additional patients with cancer (7 high-grade and 9 low-grade), as the validation data set, to determine the sensitivity and specificity of the threshold value in an independent, blinded study. OCT imaging was performed, and attenuation values were obtained by a researcher blinded to tissue type and grade. Histological diagnosis was obtained by a neuropathologist (F.J.R.) blinded to the OCT results. With a diagnostic optical attenuation threshold of $5.5 \text{ mm}^{-1}$, the specificity was 100% and the sensitivity was 92% for high-grade patients ($n = 7$); for low-grade patients ($n = 9$), the specificity was 80% and the sensitivity was 100% (table S3).

During surgery and before OCT imaging of the tissues from the 16 patients in the validation cohort, the surgeon gave an independent evaluation of the tissue based on gross appearance and all intraoperative surgical navigations, including surgical microscopes and intraoperative MRI. The surgeon’s impression of the tissue was then compared directly with the blinded histological diagnosis for 16 patients in the validation cohort. On the basis of these data, the surgeon’s detection specificity/sensitivity was 50/100% for high-grade brain cancer and 40/100% for low-grade brain cancer (table S3).

Additional optical attenuation analyses and microscopic information offered by OCT

We used a consolidated data set (training and validation) to evaluate the optical attenuation differences among different subgroups of low- and high-grade cancer (Table 2). We found that, regardless of treatment, brain cancers had significantly lower attenuations compared with noncancer white matter, and there were no significant differences between attenuation in newly diagnosed and recurrent brain cancers (Table 2). Moreover, the cancer core had comparable attenuation with the infiltrated zone ($P = 0.51$ for high-grade and $P = 0.80$ for low-grade, Welch’s $t$ test). Last, noncancer gray matter had significantly lower attenuation when compared with high- and low-grade cancer cores, but not with high- or low-grade infiltrated zones (table S4).

In addition to optical attenuation analyses, OCT imaging was able to identify microscopic structures, which can complement attenuation data in differentiating cancer from noncancer white matter (Fig. 4). Using OCT, we were able to identify necrosis and hypercellularity, which appeared as heterogeneous regions of hypointense signals (necrosis) surrounded by hyperintense signals (hypercellularity) in high-grade cancer, as well as hypercellularity and microcysts in low-grade brain cancer (19).

Although our study primarily focused on the attenuation results of high-grade (grade IV) and low-grade (grade II) brain cancer, because the differentiation of grade III is traditionally difficult and often controversial (20), we also studied the attenuation results for three patients with grade III brain cancer (fig. S3). There was moderate to significant overlap in the distribution of optical attenuation values between noncancer and grade III brain cancer tissues (24 and 77% overlap in cancer core and infiltrated zone, respectively). Histological validation for the infiltrated zone revealed diffuse cancer infiltration, consisting of primarily noncancer white matter with some neoplastic cell components. Overall, there was a greater overlap between cancer and noncancer tissues for grade III brain cancer (when compared to grade II), which could be attributed to the limited sample size for grade III (Fig. 1).
Table 2. Optical attenuation differences between treated and untreated brain tissues for patients in the combined training and validation data sets. Data are provided for 32 patients and are reported as averages ± SD. *P* values were determined using a two-sample, one-tailed Welch’s *t* test based on the hypothesis that noncancer white matter (WM) has higher attenuation than cancer and newly diagnosed and recurrent brain tissues have equal attenuation, respectively.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>n</th>
<th>Attenuation (mm⁻¹)</th>
<th><em>P</em> (versus noncancer WM)</th>
<th><em>P</em> (new versus recurrent)</th>
</tr>
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<tr>
<td><strong>Control patients (seizure)</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Noncancer white matter</td>
<td>5</td>
<td>6.2 ± 0.8</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>High-grade patients (newly diagnosed)</strong></td>
<td></td>
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<tr>
<td>Cancer core</td>
<td>9</td>
<td>3.6 ± 1.6</td>
<td>&lt;0.001</td>
<td>0.225</td>
</tr>
<tr>
<td>Infiltrated zone</td>
<td>3</td>
<td>3.7 ± 1.3</td>
<td>0.030</td>
<td>N/A</td>
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<tr>
<td>Noncancer resection margin</td>
<td>5</td>
<td>6.4 ± 1.0</td>
<td>0.368</td>
<td>0.835</td>
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<td><strong>High-grade patients (recurrent)</strong></td>
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<tr>
<td>Cancer core</td>
<td>6</td>
<td>4.6 ± 1.4</td>
<td>0.022</td>
<td>0.225</td>
</tr>
<tr>
<td>Infiltrated zone</td>
<td>1</td>
<td>3.7 ± 0.7</td>
<td>N/A</td>
<td>N/A</td>
</tr>
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<td>Noncancer resection margin</td>
<td>2</td>
<td>6.2 ± 1.0</td>
<td>0.500</td>
<td>0.835</td>
</tr>
<tr>
<td><strong>Low-grade patients (newly diagnosed)</strong></td>
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</tr>
<tr>
<td>Cancer core</td>
<td>10</td>
<td>3.8 ± 1.3</td>
<td>&lt;0.001</td>
<td>N/A</td>
</tr>
<tr>
<td>Infiltrated zone</td>
<td>4</td>
<td>3.6 ± 1.3</td>
<td>0.005</td>
<td>N/A</td>
</tr>
<tr>
<td>Noncancer resection margin</td>
<td>3</td>
<td>5.9 ± 1.1</td>
<td>0.353</td>
<td>N/A</td>
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<tr>
<td><strong>Low-grade patients (recurrent)</strong></td>
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</tr>
<tr>
<td>Cancer core</td>
<td>1</td>
<td>3.2 ± 0.5</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Infiltrated zone</td>
<td>1</td>
<td>3.6 ± 1.6</td>
<td>N/A</td>
<td>N/A</td>
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</table>

In vivo OCT detection of human brain cancer in a murine model

To test the ability of OCT to detect cancer from noncancer intraoperatively in vivo, five mice with two different high-grade human brain cancer xenografts were studied. Mice were implanted with U87 cell line or GBM272, a patient-derived cell line (fig. S4). OCT attenuation maps were acquired and displayed over the resection cavity during surgery, which can resolve cancer versus noncancer on the scale of 0.004 mm⁻¹ (movies S3 and S4). OCT attenuation maps aided the user in identifying regions of cancer versus noncancer (white matter) before and after surgery, even for mice that displayed more infiltrative brain cancer characteristics with the patient-derived GBM272 cell line (Fig. 5). After imaging, mice brains were resected and the corresponding histological slides were reviewed by a neuropathologist (F.J.R.) for validation of the OCT results. These histological slides were sectioned in the same orientation as OCT cross-sectional images [that is, perpendicular to the tissue surface or perpendicular to the OCT attenuation map and along the dotted lines in Fig. 5 (B and C)]. In the postsurgery and control images, residual amounts of cancer (about 5 to 10% of the imaged area) were visible in the histological images.

DISCUSSION

The goal of surgery in many cancers and specifically in brain cancer is to achieve maximal resection of cancer while avoiding noncancer tissues. There is substantial survival benefit and a strong need in achieving a clean cavity margin (3, 4, 21). Patients who undergo gross total resection for high- and low-grade brain cancers have a 200 and 160% increase in median survival, respectively, as compared to those who only undergo subtotal resection (4, 21–23). Patients who incur surgery-related deficits, however, have a 25% decreased median survival than those without deficits, regardless of extent of resection (5).

This increasing need to identify cancer tissues readily and intraoperatively has led to the development of different surgical adjuncts including surgical navigation, ultrasound, fluorescence imaging and intraoperative CT and MRI (6–10). These modalities provide substantial contributions to neurosurgery, and their performance has been summarized in table S1 in terms of resolution, FOV, and other parameters. For example, preoperative CT/MRI provides excellent global surgical guidance with a whole-brain FOV but is sensitive to brain shifts and position errors (24). Intraoperative CT/MRI, on the other hand, enables surgeons to intraoperatively assess the resection cavity for residual cancer and often reduces the need for a second resection, but it is expensive, is time-consuming, and does not provide continuous, real-time intraoperative guidance.

Alternatively, intraoperative ultrasound provides real-time imaging with good penetration depth and FOV, and can identify blood flow; however, it has limited spatial resolution and contrast for brain cancer detection (25). 5-Aminolevulinic acid (5-ALA) enables intraoperative, fluorescence-guided detection of brain cancer at a wide FOV (for fluorescence applications) and good resolution. Clinical studies have shown an increased extent of resection and improved overall survival using this surface imaging technique (22, 23). Nevertheless, 5-ALA has limited sensitivity in delineating all cancer areas, and 5-ALA uptake can vary based on blood-brain barrier permeability, edema, cellular/vascular proliferation, and cancer grade; active research is currently under way in further improving its detection sensitivity, especially in infiltrative areas (26, 27). In addition, other emergent technologies, such as stimulated Raman scattering microscopy, provide impressive subcellular resolution and label-free imaging capabilities (7); however, its imaging depth (~0.1 mm), FOV (~0.35 mm × 0.35 mm), and imaging speed (~1 fps) remain to be further improved for clinical, intraoperative use. Conversely, visible and near-infrared spectroscopy provides very precise estimation of optical parameters such as scattering, absorption coefficient, and anisotropy factors (12) but does not provide sufficiently high resolution for brain cancer applications.

OCT is a modality that complements existing tools, and the technology we have developed is more effective than other surgical adjuncts for the specific application to reliably, quantitatively, and volumetrically distinguish cancer from noncancer tissues in real time and with continuous, intraoperative image guidance. OCT relies on the reflections of near-infrared and low-coherence light, which allows for its deep penetration (compared to visible light), label-free contrast, high-resolution imaging, and the ability to provide real-time quantitative values for optical attenuation (28); additionally, it is easy to use and provides noncontact imaging, which will minimize infection risks for intraoperative use. As a
The optical attenuation is governed by two key factors: (i) an increased cell density and NC ratio, which increases optical attenuation for a tissue specimen. For most systemic organs, cancers have a higher optical attenuation owing to high myelin content, whereas gray matter has a lower attenuation owing to the absence of myelin (16, 19, 32–35). Moreover, when brain cancer infiltrates into white matter, the invading cancer cells are known to break down and decrease the expression of myelin in white matter (32–34); this lowers the attenuation for both cancer core and infiltrated zone when compared with noncancer white matter. Consequently, our results are consistent with this reported phenomenon in that noncancer white matter had significantly higher attenuation when compared with brain cancer tissues (regardless of grade).

On the other hand, when brain cancer infiltrates into gray matter (which lacks myelin), optical attenuation is mainly determined by increased cell density and NC ratio; thus, as shown here, both cancer core and infiltrated zone had high attenuation when compared with noncancer gray matter. In addition, the attenuation in the cancer core was comparable with that in infiltrated zone, presumably because the cancer core has higher cell density (which increases attenuation) but more complete breakdown of myelin (which decreases attenuation). All attenuation results (noncancer versus cancer, low-grade versus high-grade, and cancer core versus infiltrated zone) were largely consistent for both newly diagnosed and recurrent patients. As a result, we are able to detect brain cancer from noncancer tissues based on optical attenuation properties. Thus, OCT has translational potential in guiding surgical resections for primary brain cancer patients, especially with complementary techniques, such as cortical stimulation mapping, to identify normal, eloquent cortex.

This study established an OCT diagnostic threshold in detecting cancer versus noncancer with excellent sensitivity (92 to 100%) and specificity (80 to 100%). High-grade brain cancer had a higher OCT detection sensitivity but lower specificity when compared with low-grade, although the differences are likely due to the limited number of tissue samples in each subgroup. Moreover, OCT exhibited comparable sensitivity but significantly greater specificity than the standard of care (100% sensitivity and 40 to 50% specificity), which is interpreted as the surgeon’s perception of cancer based on gross appearance and all available intraoperative surgical navigational systems. In comparison, previous studies showed lower sensitivity (26 to 87%) and specificity (42 to 100%) for other imaging technologies, as detailed in table S1 (6–10).

In addition to attenuation and diagnoses, OCT was also able to distinguish histologic cancer features (that is, microcysts, hypercellularity, and necrosis), which could potentially assist the surgeon in detecting cancer grade because microcysts are generally found in lower-grade brain cancer, whereas necrosis and hypercellularity are generally found in higher-grade brain cancer and are not characteristics of normal brain. Furthermore, OCT was able to reliably detect cancer from surrounding noncancer in vivo during surgery in a rodent model of human brain cancer by applying the same attenuation threshold obtained from the ex vivo human study. Thus, in vivo OCT detection of brain cancer is reliable even in the presence of brain motions, uneven tissue surface, and other surgical complications (for example, bleeding).

OCT represents a promising solution in brain cancer localization and mapping. Nevertheless, it does not provide molecular information. The imaging volume is also suboptimal for imaging the entire resection cavity. For eventual clinical translation, the OCT imaging area (and volume) can be substantially increased by robotic positioning and co-registering with surgical microscopes (29, 30). OCT can also be integrated with wide-field imaging technologies (for example, MRI...
and fluorescence) for complementary image guidance, which will further facilitate safe extensive resections. Moreover, additional processing of OCT images can also yield functional information such as Doppler to detect the presence of major embedded blood vessels and therefore prevent bleeding or stroke-related complications during surgery. Intraoperative OCT imaging will benefit from developing more advanced OCT light sources for further improved imaging speeds (>400 fps) (36) and/or resolution (<3 μm) (37).

In summary, OCT could have tremendous translational potential in brain cancer resections. Besides brain cancer, this modality may also be useful for distinguishing cancer from noncancer tissue for other intraparenchymal brain cancers including metastatic brain cancer. This study sets the stage for future clinical trials and technological refinement that will ultimately lead to an enabling technology for detecting brain cancer rapidly and efficiently, increasing the extent of resection and thus improving patient survival.

MATERIALS AND METHODS

Study design

This study evaluates the feasibility of OCT in delineating cancer from noncancer tissue for human patients undergoing brain surgery (Fig. 1). Tissues were obtained using intraoperative MRI-guided surgical navigation for patients with low-grade (grade II glioma) and high-grade [grade IV glioma or glioblastoma (GBM)] brain cancers. OCT cross-sectional images were acquired over the tissue ex vivo at a uniform spatial interval of 0.326 mm × 0.008 mm × 1.8 mm within the tissue block, with a step size of 0.033 mm in the x direction. Each histological image (C and E) represented a cross-sectional view of the tissue block: the image corresponds to a single perpendicular slice through the attenuation map, along the dotted lines in (B) and (D), respectively. Residual cancer cells were marked with black arrows and correspond to yellow/red regions on the attenuation maps (at the level of the dotted line). Scale bars, 0.2 mm.

Fig. 5. In vivo brain cancer imaging in a mouse with patient-derived high-grade brain cancer (GBM272). (A and B) Brain tissues were imaged in vivo in mice (n = 5) undergoing brain cancer resection. After imaging, the mice were sacrificed and their brains were processed for histology. Here, we show the representative results of a mouse brain at the cancer site before surgery (A) and at the resection cavity after surgery (B). (C) Corresponding histology for the resection cavity after surgery was also shown. (D and E) With the same mouse, control images were imaged at a seemingly healthy area on the contralateral, left side of the brain (D), with its corresponding histology (E). The red circle indicates cancer, gray circle indicated resection cavity, and square was the OCT FOV. 2D optical property maps were displayed using an attenuation threshold of 5.5 mm⁻¹. C, cancer; W, noncancer white matter; M, noncancer meninges. Aliasing artifacts at the image boundaries, which were produced when dorsal structures from outside the OCT depth were folded back into the image, were cropped out of image. 3D volumetric reconstructions were overlaid with optical property maps on the top surface. Optical attenuation properties were averaged for each subvolume of 0.326 mm × 0.008 mm × 1.8 mm within the tissue block, with a step size of 0.033 mm in the x direction. Each histological image (C and E) represented a cross-sectional view of the tissue block: the image corresponds to a single perpendicular slice through the attenuation map, along the dotted lines in (B) and (D), respectively. Residual cancer cells were marked with black arrows and correspond to yellow/red regions on the attenuation maps (at the level of the dotted line). Scale bars, 0.2 mm.
brain cancer implantation and resection protocols are well established in murine models (38–40), only five mice were selected to provide 15 proof-of-concept data sets for OCT detection of brain cancer under an in vivo environment and correlation with histology. A total of three OCT data sets were collected from each mouse brain over the 8-mm³ OCT imaging volumes at the following locations: (i) at a selected cancer site before resection, (ii) at the same cancer site after surgery, and (iii) at a selected noncancer site (control) in the contralateral hemisphere of the brain. For each imaging data set, 52 attenuation data points were collected per OCT cross-sectional image, with 256 OCT cross-sectional images per 8-mm³ imaging volume.

**Human brain cancer and noncancer samples**

For all 37 patients, brain tissues were removed using standard neurosurgical techniques (including microsurgical dissection when necessary and the use of intraoperative surgical navigation). Tissues necessary for clinical diagnosis of the patient were obtained before any excess tissues were sent for research purposes. Noncancer tissue samples were obtained from control, noncancer patients who required tissue resection as part of a standard surgical approach for seizure (lobectomy). Tissue samples from brain cancer patients were obtained from the cancer core and infiltrated zone, as categorized by the neurosurgeon (A.Q.-H.) and later validated by a neuropathologist (F.J.R.). In addition, tissues were also obtained from the resection margins of brain cancer patients who showed normal pathology (<5% cancer cells based on visual estimate). These resection margins from brain cancer patients were obtained either as part of the planned trajectory to the cancer core or along the surgically defined margins at the infiltrated-normal brain interface.

**SS-OCT system**

A home-built SS-OCT system (Fig. S1) was used for imaging all samples, as described in Supplementary Materials and Methods.

**Imaging and histological correlation**

Each tissue was cut into flat homogeneous samples at about 50 mm³ per sample. The tissue surfaces were marked with a 3-mm line using a yellow margin marking dye (MasterTech) for histological registration and correlation. A cover glass was placed to prevent dehydration and to flatten the tissue surface. The tissues were then kept on ice until transfer to the imaging stage. For this, the imaging beam was aligned with the 3-mm yellow line on the tissue. This marked our first OCT scan line, and the first cross-sectional (B-scan) OCT image was obtained. Afterward, cross-sectional images were acquired at 0.5-mm intervals along the horizontal plane. The image data were stored digitally in a computer for postprocessing and attenuation analyses.

After imaging, tissue samples were placed in 10% neutral buffered formalin overnight. Afterward, tissue samples were transferred to saline and resectioned at the first OCT scan line (yellow line) for histological processing and correlation. On the basis of histology, all tissue samples were divided into three categories (cancer core, infiltrated zone, and noncancer). Each histological slide contained multiple 5-µm-thick hematoxylin and eosin–stained tissue samples sectioned at an interval of 0.5 mm per slide, with close correspondence to the OCT cross-sectional images' location and orientation (Fig. 1).

**Optical attenuation**

During quantitative analyses of depth-dependent OCT signals, each OCT image was divided into one to three sections depending on tissue features and characteristics, such that each section roughly delineated a homogeneous tissue region. The optical attenuation value was computed as described previously (18) and in Supplementary Materials and Methods. After quantitative analyses, attenuation coefficients for the biological tissues were grouped into several categories for statistical analyses. These included cancer core, infiltrated zone, and resection margin for cancer patient samples, and noncancer samples as control.

**Diagnostic threshold**

Data in the training data set (n = 16) were used to establish an optimal attenuation threshold that distinguishes cancer versus noncancer white matter. The distribution of attenuation coefficients of noncancer versus cancer in cancer cores and infiltrated zones was presented in binned histograms at 0.5 mm⁻¹ per bin for high- and low-grade cancers. To find the attenuation threshold, sensitivity and specificity were estimated, and ROCs for distinguishing cancer versus noncancer were calculated. The sensitivity/specificity values obtained at different attenuation values (0 to 10 mm⁻¹ evaluated at intervals of 0.1 mm⁻¹) were estimated for both high- and low-grade tissues. On the basis of the results, the optimal attenuation threshold was determined based on a threshold value that yielded maximum sensitivity with at least 80% specificity.

**Calculating sensitivity and specificity for an established attenuation threshold**

Data from 16 patients were used to find the sensitivity and specificity associated with the established attenuation threshold in an independent blinded study. For this part of the study, the user was blinded to tissue type and grade when imaging the tissue samples and calculating the attenuation coefficients. A total of 59 tissue samples (19 high-grade and 40 low-grade) were obtained. To determine the test outcome, a tissue sample (about 50 mm³) was considered cancer if >50% of the OCT attenuation data points were lower than the threshold value, and considered noncancer if >50% of the OCT attenuation data points were higher than the threshold value. Histological diagnosis was obtained by a neuropathologist (F.J.R.) who was blinded to the OCT results. The tissue was then categorized as either true-positive, true-negative, false-positive, or false-negative based on the test outcomes (OCT attenuation data) and the histological diagnosis.

**OCT versus surgeon in determining cancer from noncancer tissue**

Diagnostic sensitivity and specificity values for OCT were compared with surgeon identification of brain tissues. True-positive, true-negative, false-positive, or false-negative was derived by comparing surgeon’s impression (that is, test outcome) with the blinded histological evaluation (that is, true outcome). Here, the surgeon’s perception of cancer was based on all preoperative and intraoperative imaging information available (for example, surgical microscope and intraoperative MRI), in addition to the gross appearance of the tissues resected and the clinical history of the patient.

**In vivo OCT imaging of mice implanted with human GBM cell lines**

To test the ability of OCT to detect cancer from noncancer in vivo, five 8-week-old nonobese diabetic/severe combined immunodeficient male mice (Charles River Laboratories) were stereotactically inoculated with cancer cells as previously described (38) but with coordinates X: 3.5, Y:
To distinguish cancer from noncancer white matter from the training data set, we performed two-sample, one-tailed Student’s t test of equal variance (or Welch’s t test), with the hypotheses that attenuation values for noncancer white matter are higher than those for cancer core, infiltrated zone, and/or resection margin tissues. For intergroup analyses based on treatment (newly diagnosed versus recurrent), cancer density (cancer core versus infiltrated zone), and grade (high-grade versus low-grade) for the attenuation data from the combined training and validation data sets, we performed two-sample, two-tailed Welch’s t test with the hypothesis that both groups have equal attenuation values. To detect cancer from noncancer gray matter for the combined training and validation data sets, we performed two-sample, one-tailed Welch’s t test with the hypothesis that gray matter has lower attenuation compared to both cancer and noncancer white matter tissues. The α value was 0.05, and we assumed a normal Gaussian distribution.

**SUPPLEMENTARY MATERIALS**

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Methods

Fig. S1. SS-OCT system.

Fig. S2. Improved OCT FOV with robotic positioning.

Fig. S3. Optical attenuation data set for grade III glioma.

Fig. S4. Human cell lines U87 and GBM272 demonstrate the range of cancer behavior in mice.

Table S1. Comparison of different technologies in surgical guidance of brain cancer detection.

Table S2. Patient characteristics.

Table S3. Sensitivity/specificity analyses for brain cancer patients in the validation data set.

Table S4. Optical attenuation differences between cancer and noncancer gray matter for patients in the combined training and validation data sets.

Table S5. Comparison of different technologies in surgical guidance of brain cancer.

**REFERENCES AND NOTES**


RESEARCH ARTICLE


Acknowledgments: We thank our collaborators for their help and contribution to this project: D. Herzk a for data analyses; A. Olivi, W. Anderson, and F. Lenz for clinical support and collaboration; J. Mavadia for technological development/assistance with the OCT imaging system; O. Wijesekera and H. Guerrero-Cazares for the rodent models; J. Rincon-Torrozuela for MR acquisition and video production/assistance; S. Manrique and J. J. Torres for MRI acquisition; T. Gao for OCT phantom development; and the tissue handling team for tissue transfers from the operating room to the laboratory (L. Chen, A. Abutaleb, J. C. Martinez, S. Dangelmajer, B. Patel, K. Refaey, and T. Buitrago).

Funding: Grants R01NS070024 (A.Q.-H.), R01CA120480 (XXL), R01EB007636 (XXL), and F30CA183430 (CK), as well as the Coulter Foundation (XXL and A.Q.-H). Author contributions: CK contributed to the study design, tissue acquisition, execution of experiments, software development, data analysis, and manuscript writing. KLC contributed to the study design, tissue acquisition, and manuscript writing. JX developed the technology and software for the SS-OCT system. SMR contributed to the study design and tissue acquisition. XY contributed to the statistical aspects of the study design. ERM contributed to the study design, data analyses, and manuscript editing and proofreading. FJR performed histology and gold standard determination of all tissue samples. AQ-H contributed to the study design, human tissue acquisition, and manuscript editing and proofreading. XL contributed to the study design, technology development, and manuscript editing and proofreading. Competing interests: We have filed an international patent application (PCT/US15/22432) entitled “Quantitative tissue property mapping for real-time tumor detection and interventional guidance” (CK, KLC, JX, ERM, A.Q.-H, and XXL). Data and materials availability: Raw data not included in this manuscript can be made available upon reasonable request.)

Submitted 9 September 2014
Accepted 7 May 2015
Published 17 June 2015
10.1126/scitranslmed.3010611

Detection of human brain cancer infiltration ex vivo and in vivo using quantitative optical coherence tomography
Carmen Kut, Kaisorn L. Chaichana, Jie Feng Xi, Shaan M. Raza, Xiaobu Ye, Elliot R. McVeigh, Fausto J. Rodriguez, Alfredo Quiñones-Hinojosa and Xingde Li (June 17, 2015)
Science Translational Medicine 7 (292), 292ra100. [doi: 10.1126/scitranslmed.3010611]

Editor's Summary

Observing cancer with OCT

The label-free imaging technology optical coherence tomography (OCT) is used routinely in the clinic for detecting abnormalities in certain tissues, such as in the eye. Now, Kut et al. demonstrate that OCT can differentiate different grades of human brain cancer from noncancer in rodents and in patient tissue samples. Fresh human brain cancer and noncancer tissues that had been surgically removed were used to generate "optimal attenuation thresholds" for high- and low-grade cancers. These thresholds were then used by blinded pathologists to diagnose cancer in a separate set of tissues, showing 100% specificity and 80% sensitivity for high-grade brain cancer (and 80% specificity and 100% sensitivity for low-grade brain cancer). To demonstrate the potential of this technology during neurosurgery, mice had their human tumors removed with the guidance of OCT "maps" that displayed color-coded optical properties of the tissue. Thus, surgeons were able to remove only the cancerous areas, as confirmed by histology. Such real-time, intraoperative imaging to ensure total cancer resection will markedly improve patient survival.