Non-invasive intraoperative optical coherence tomography of the resection cavity during surgery of intrinsic brain tumors

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ABSTRACT

Optical coherence tomography (OCT) is a non-invasive imaging technique with a micrometer resolution. It allows non-contact / non-invasive analysis of central nervous system tissues with a penetration depth of 1-3,5 mm reaching a spatial resolution of approximately 4-15 µm. We have adapted spectral-domain OCT (SD-OCT) and time-domain OCT (TD-OCT) for intraoperative detection of residual tumor during brain tumor surgery. Human brain tumor tissue and areas of the resection cavity were analyzed during the resection of gliomas using this new technology. The site of analysis was registered using a neuronavigation system and biopsies were taken and submitted to routine histology. We have used post image acquisition processing to compensate for movements of the brain and to realign A-scan images for calculation of a light attenuation factor.
OCT imaging of normal cortex and white matter showed a typical light attenuation profile. Tumor tissue depending on the cellularity of the specimen showed a loss of the normal light attenuation profile resulting in altered light attenuation coefficients compared to normal brain. Based on this parameter and the microstructure of the tumor tissue, which was entirely absent in normal tissue, OCT analysis allowed the discrimination of normal brain tissue, invaded brain, solid tumor tissue, and necrosis. Following macroscopically complete resections OCT analysis of the resection cavity displayed the typical microstructure and light attenuation profile of tumor tissue in some specimens, which in routine histology contained microscopic residual tumor tissue.
We have demonstrated that this technology may be applied to the intraoperative detection of residual tumor during resection of human gliomas.

Keywords: glioma, spectral domain optical coherence tomography, spectral radar, Invasion, intraoperative imaging, neurosurgery

1. INTRODUCTION

Because glial brain tumors are highly invasive, these tumors lack a true boarder to the normal brain (12). From the highly cellular tumor a gradient of invasive tumor cells can be found extending several centimeters into the adjacent brain. The path of individual invasive tumor cells follows preformed anatomical structures, predominantly the myelinated fiber tracts, but also structures such as the basement membrane of blood vessels, the pial lining of the brain surface and the walls of the ventricular system (8, 9, 11, 10). Because of this diffuse growth pattern and a low inherent contrast of highly cellular tumor, invaded brain, and adjacent normal brain the detection of residual tumor during neurosurgical resections remains an important challenge. Specifically, because the extent of resection of highly cellular tumor correlates with the survival of the patient (12, 13, 14). Technologies in biomedical optics may offer novel imaging modalities for intraoperative tissue analysis and detection of residual tumor, which may be used for guidance of the neurosurgical resection.

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Few studies have been undertaken to image central nervous system anatomy or pathology by optical coherence tomography (3, 15, 16). Recently, ultra high resolution optical coherence tomography (UHR OCT) has demonstrated to resolve brain tissue morphology from a single cell level to a whole animal brain ex vivo and in vivo (1). First investigation in human formalin fixed specimens of cortex, meningeoma and ganglioglioma have shown that UHR OCT carries the potential of discrimination between normal brain and brain tumor in biopsy specimens (2). However, UHR OCT works only in close contact to the tissue and is very difficult to implement in the operation room. We have recently shown that formalin fixation of brain and brain tumor specimen resulted in a significant loss of intra-tissue microstructure and definition of the OCT signal arguing for the need of studies in native tissue and in situ studies (4). Optical analysis of highly scattering tissue such as brain is limited by the fact that biologically save intensities of light only allows few millimeters of penetration into the tissue. For both time-domain and spectral-domain optical coherence tomography we have demonstrated in native experimental brain tumors and clinical samples of human brain and brain tumor specimens that interpretable signal in B-scan images can only be obtained from the tissue surface up to a depth of 1.5 – 2.0 millimeter, depending on the tissue characteristics (4). However, in a pilot study we have recently shown that time-domain OCT can differentiate normal brain, tumor infiltration of the brain parenchyma, highly cellular tumor, and areas of necrosis in clinical specimen of malignant gliomas in situ (5). We have further demonstrated that SD-OCT allows discrimination of both the microstructure and the light attenuation profile of normal brain and different areas of tumor at a higher resolution and definition than TD-OCT (4). A feasibility study using OCT analysis of clinical biopsy specimens from solid tumor and the wall of the resection cavity ex vivo has demonstrated that OCT may identify microscopic tumor in what appeared macroscopically normal cortex and white matter (6). These findings suggest that optical tissue analysis of the advancing edge of the resection cavity may provide the surgeon with information on tissue microstructure and light attenuation profile, which despite a limited penetration of less than two millimeters provides visualization of residual tumor and may help to guide neurosurgical resections of intrinsic brain tumors.

In this study we demonstrate the use of time-domain optical coherence tomography and post image acquisition processing in the analysis of the resection edge during resection of brain tumors. The spectral-domain implementation of OCT has improved the diagnostic value of this technology in the detection of residual tumor within the wall of the resection cavity. We present the first intraoperative application of a neuronavigation guided SD-OCT integrated prototype of an operating microscope.

2. MATERIAL AND METHODS

2. 1 Specimens and Histology

Specimens of human brain tumors were obtained at surgery under protocol # 05-004 granted by the ethics committee of the University of Lübeck. Tumor tissue was removed using standard microsurgical techniques and the resection site of individual tissue blocks was documented by marker acquisition using a VectorVision^2 neuronavigation system (BrainLab, Heimstetten, Germany), which allowed correlation of MRI signal characteristics and OCT B-scan images obtained in situ or ex vivo. For ex vivo OCT imaging the tissue was immediately placed on ice and the resection plain was imaged. Following analysis the tissue was fixed in formalin 4.5% and paraffin embedded for standard histological processing and H&E staining.

2. 2 Optical coherence tomography

In this study two optical coherence tomography systems were used. A clinical study of intraoperative analysis of intrinsic brain tumors and the resection cavity was done in six patients using a time-domain optical coherence tomograph. Spectral-domain tomography was used to analyze brain tumor tissue and tissue specimens obtained from the wall of the resection cavity ex vivo. The spectral domain optical coherence tomography was subsequently integrated into a neurosurgical operating microscope, which has been used for a pilot study ex vivo and in vivo.

2. 3 Time domain optical coherence tomography
We have used a Sirius 713 Tomograph (4optics AG, Lübeck, Germany) developed at the Medical Laser Center, Lübeck, Germany, which uses a superluminescence diode (SLD) emitting light at a near infrared wavelength with a central wavelength of 1310 nm and a coherence length of 15 µm. The light is launched into an optical mono mode fiber, which guides the radiation to a modified OCT adapter containing a lens system with a working distance of 10 cm and an integrated pilot laser. This allows measurements of brain tissue and brain tumor tissue in a no-touch technique producing 2D B-scan like images of 4 mm width and 1.5-2.0 mm depth depending on the tissue characteristics. The image acquisition rate was 50 A-Scans per second. The configuration used in this study allows an axial and lateral optical resolution of about 15 µm.

2. 4 In vivo imaging
For intraoperative time domain OCT imaging, the OCT applicator containing lenses, rotating mirror and pilot laser, was mounted to a flexible arm attached to the operating table. For measurements of in situ tumor tissue or the walls of the resection cavity the probe was placed above the craniotomy at a working distance of 10 centimeters and the target tissue volume was brought into the focal plain by adjusting the probes distance. A 4 mm scan line was measured at 0.5 mm / second. The scanned area was documented by marker acquisition using the neuronavigation as above and histological specimens were obtained.

2. 5 Spectral-domain optical coherence tomography
A spectral domain optical coherence tomography prototype developed at the Institute for Biomedical Optics, University of Luebeck, now being manufactured by the Thorlabs, Inc. (Newton NJ, USA), was used for the analysis of human brain tumor tissue ex vivo. This system used one superluminescence diode at a central wavelenth of 910 nm and achieved an axial and lateral resolution of 4 µm in air. This system allowed measurements of brain tissue and brain tumor tissue in a no-touch technique at a working distance of 20 mm. SD-OCT in the configuration used here produced a 2D B-scan like image of up to 8 mm width and up to 1,2 mm depth depending on the tissue characteristics. The data were acquired at a rate of 333 kHz resulting in approximately 330 A scans / second, which were downloaded to a host computer with a resolution of 16 bits per pixel. Data acquisition software was written in Labview and post processing of the raw data was done using OCTEval (4optics AG).

In addition we combined a second SD-OCT system with a neurosurgical Möller-Wedel microscope (Möller-Wedel, Wedel, Germany). The central wavelength of the th SD-OCT sysem is 840 nm, the axial resolution is about 11 µm, the measurement speed is 1,25MHz (1220 Ascans/sec) and the depth of the easurement window is 3,5 mm. The SD-OCT uses the same optic as the microscope. Therefore the working discance (231 mm) of visible microscope image and the OCT measurement are the same. The lateral OCT resolution is about 27 µm.

3. RESULTS

3. 1 Intraoperative time-domain optical coherence tomography
We have used a modified Sirius 713 Tomograph during the resection of brain tumors in six patients with glial tumors. In all patients prior to resection of tumors the cortical surface of macroscopically normal brain was imaged. TD-OCT showed the arachnoid fibers and the subarachnoid CSF filled space of varying depth. The cortical tissue below showed no intra-tissue microstructure, but a typical light attenuation profile similar to our previous findings (4, 5). Using the neuronavigation, areas of tumor at the brain surface were identified and TD-OCT analysis of gross tumor through the intact arachnoid was done in three patients. These areas demonstrated some poorly defined microstructure on B-scan images, but a distinct loss of the light attenuation profile observed for normal cortex, which results in light attenuation factors of approximately 20 cm^{-1} (illustrated in figure 1). OCT imaging was done during different stages of the resection and the sites of analysis were registered using the neuronavigation. Histologically confirmed tumor within the wall of the resection cavity, similar to the tumor surface, showed a variable tumor microstructure and the loss of the light attenuation profile typical for normal brain. Following macroscopically complete resection the edge of the resection cavity was analyzed in several areas. Histologically tumor free or lightly invaded margins showed a similar light attenuation profile to normal brain (fig.1).

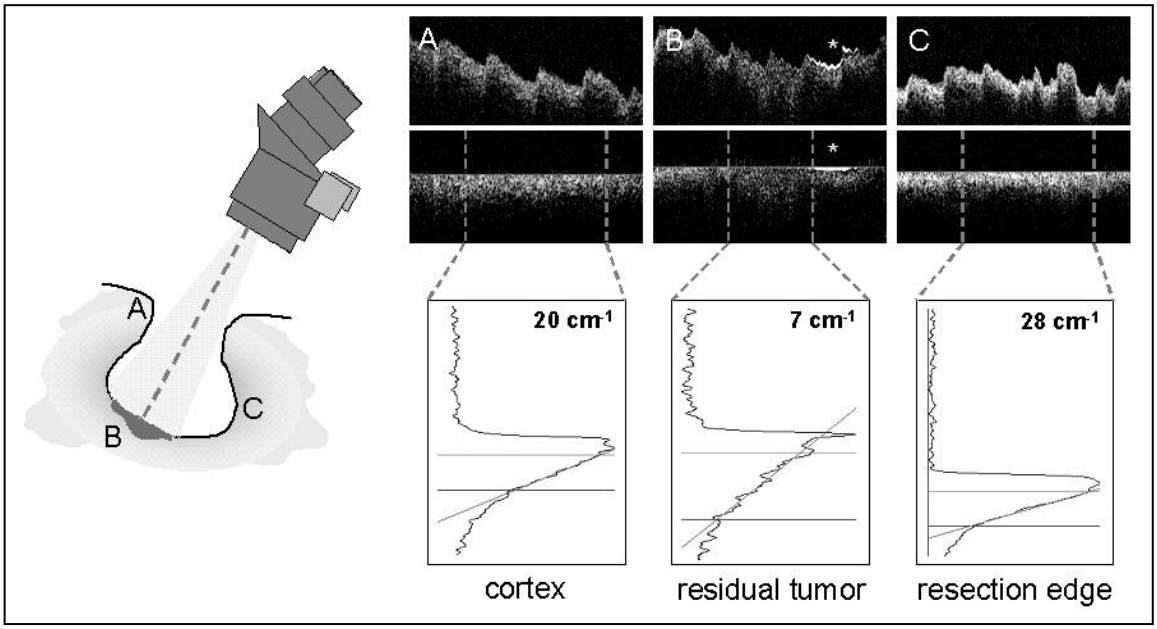

Figure 1: Intraoperative TD-OCT imaging during resection of a glioblastoma. TD-OCT imaging parallel to the optical path of the operating microscope was performed at different stages during resection of a glioblastoma. Macroscopically normal cortex was imaged after corticotomy prior to entering gross tumor tissue (A) and gross tumor was imaged prior to completion of the resection (B). Following a macroscopically complete resection the wall of the resection cavity was imaged (C). The corresponding light attenuation factors calculated from realigned B-scan images are shown in the lower panel. Note, high signal intensity at the tissue surface (*) is a consequence of the laser entering a fluid collection at the tissue surface at a 90° angle.

The open brain follows the arterial and respiratory cycle, which results in a distortion of the B-scan image. Post image acquisition processing and realignment of A-scans by automated surface recognition was used to compensate for movements of the target tissue during analysis. The realigned images were used for averaging of A-scans for specific regions of interest. This analysis demonstrated that normal brain and gross tumor showed distinct light attenuation factors. Tumor tissue of glial tumors consistently showed a light attenuation factor significantly lower than normal brain (tumor < 10 cm^{-1} , normal brain 20cm^{-1} or higher). The relationship of light attenuation and histoarchitecture requires more detailed studies, but may in the future provide some basis for an automation of optical tissue analysis.

3. 2 Spectral-domain OCT imaging of brain tumor tissue

Earlier data obtained by our group have demonstrated a higher diagnostic value of the new SD-OCT over the TD-OCT device in experimental gliomas (4). We therefore favor this technology for further clinical developments. An ex vivo analysis of normal human cortex and tumor specimens of different grades of glial tumors showed that SD-OCT displayed the profound microstructure of malignant gliomas, but also detects low grade gliomas, which histologically show mild hyper cellularity and little vascularization. Both low and high grade tumors could be delineated from normal brain (fig.2). In a specimen of a brain metastasis SD-OCT showed the transition between highly cellular tumor and adjacent normal brain. Histology confirmed the highly cellular and well delineated tumor. Based on these findings we have analyzed by SD-OCT specimens taken from gross tumor and from the edge of the resection cavity after what appeared to be a complete resection of an astrocytoma WHO grade III. Gross tumor was readily identified by its microstructure and light attenuation profile. Two specimens from the resection edge were suggestive of normal brain because of the lack of microstructure and a normal light attenuation profile. Histology in these two specimens revealed normal brain or brain infiltrated by a low density of single invasive tumor cells. One specimen of the *macroscopically tumor free* resection edge by SD-OCT showed a pronounced microstructure similar to gross tumor and no light attenuation profile typically observed in normal brain. Histologically this specimen contained highcellular tumor and pathological vascularization (fig.3).

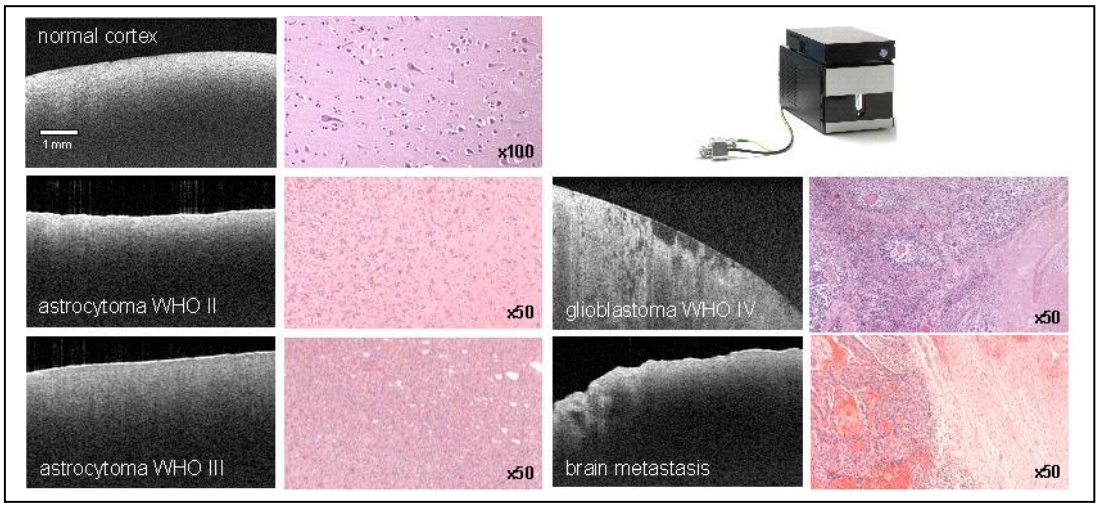

Figure 2: Ex vivo SD-OCT imaging of the resection plains of clinical brain tumor specimens and corresponding

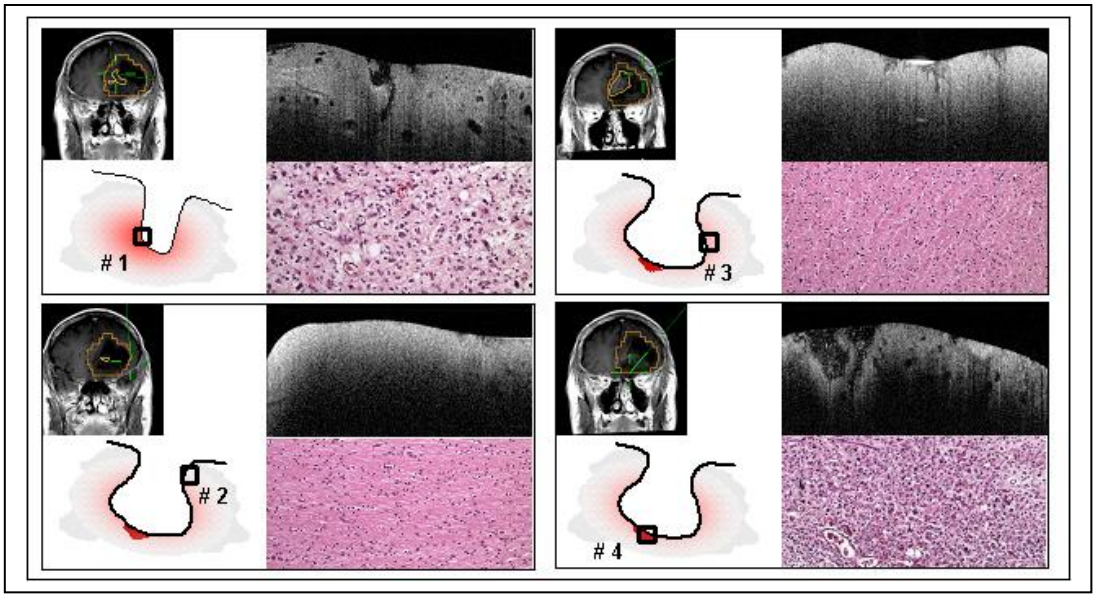

Figure 3: SD-OCT analysis of the resection cavity. During resection of a microcystic astrocytoma WHO grade III tissue biopsies were taken at different stages of the tumor resection and the biopsy sites were registered using a neuronavigation system. Solid macroscopic tumor by SD-OCT showed a microcystic structure (#1). After a macroscopically complete tumor removal OCT imaging of the walls of the resection cavity showed the typical light attenuation profile of normal brain for specimens #2 and #3. A single specimen showed a microcystic tissue structure and loss of the light attenuation profile of normal brain (#4). This specimen histologically contained highly cellular tumor.

3. 3 Neuronavigation guided SD-OCT integrated operating microscope

The probe arm and the reference arm of the spectral-domain optical tomography were integrated into a housing, which could be fitted to the side view of a Möller-Wedel HI R-20-1000 operating microscope (fig.4a). Ex vivo imaging of human brain tumor specimens demonstrated a similar image quality, although the resolution was decreased due to a reduced numerical aperture compared to the standard SD-OCT set up (data not shown). The HI R-20-1000 operating microscope in its commercial version may be integrated into a neuronavigation systems for display of the trajectory and focal plain of the microscope on three dimensional data sets of preoperative MRI. In this study the microscope was used

with a VectorVision[2] neuronavigation system (BrainLab), which allowed the registration of the SD-OCT integrated microscope and display of the SD-OCT analysis site on preoperative MRI (fig.4).

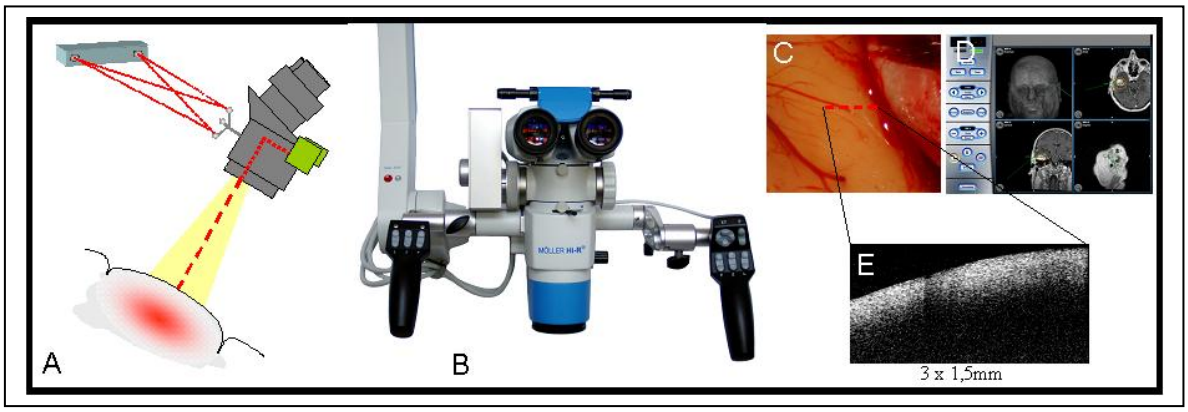

Figure 4: Neuronavigation registered SD-OCT integrated operating microscope (A). A neurosurgical operating microscope was equipped with a spectra domain OCT unite integrated into the optical path and fitted with a pilot laser (B). The Möller-Wedel Microscope can be integrated into a commercially available neuronavigation system, which tracks the field of view and trajectory of the microscope and the position of the pilot laser relative to preoperative MRI scans (D). This allows the correlation of in situ optical tissue analysis with the MRI findings (C). Post image processing and realignment of the A-scans facilitates easier interpretation of the OCT images (E).

4. DISCUSSION

Intraoperative neurosonography and MRI have proven their value in detection of residual tumor during neurosurgical operations. However, these imaging modalities by now have clearly demonstrated some principal limitations. Because of their complexity or time requirements both neurosonography and MRI require a pause in the operative workflow and may realistically only be performed a limited number of times during an operation. Furthermore, as a consequence of surgical microtrauma normal brain tissue seems to undergo time-dependent alterations that within a few minutes result in macroscopic changes of normal brain. This tissue contusion may have similar appearance to gross tumor under the view of the operating microscope and may result in imaging phenomena that may be mimicking tumor on neurosonography and also MRI (18). Conceptually, the problems caused by normal brain contusion during neurosurgical resection of tumors may be solved by early analysis of the resection edge before contusion results in non-specific imaging changes. Because optical coherence tomography can be performed as a non-invasive and no-contact technique allowing a focal distance of several centimeters it can be integrated into neurosurgical operating microscopes. This allows a continuous analysis of the resection plain providing an optical tomography adding a third dimension to the microscopic view. Such a setup integrates the optical tissue analysis into the workflow of the operation without the need to discontinue the resection or requirements for additional instruments. Because such an analysis would take palace at the time the resection plain is created secondary tissue changes as a consequence of tissue contusion, which like in intraoperative neurosonography and MRI, influence the OCT signal (Giese unpublished data), would be of no relevance.
However, intraoperative optical tissue analysis has to take into account that not only the tissue reaction to manipulation, but also the surgical resection techniques may influence the OCT signal and complicate the B-scan interpretation. Generally, optically opaque or highly scattering tissues will cause shadowing effects also known from ultrasound imaging. Generally these effects are readily identified in the OCT images (6, 7, 17). Several changes of the tissue surface associated with surgical procedures will alter the OCT signal. We have recently demonstrated that accumulation of blood or hemostatic materials applied to the tissue surface result in a loss of the OCT signal below, which may result in non-interpretable B-scan images. Irrigation fluid on the tissue surface resulted in opening of tissue gaps and clefts, which depending on the surgical resection technique have varying appearance and may be mistaken for elements of the tissue microstructure (6).

These findings illustrate that optical technologies offer the potential of a high resolution imaging modality of the structure and optical properties of the resection plain. This technology can be fully integrated into the existing

microsurgical equipment of a neurosurgical operating room and into the neurosurgical workflow. However, not only will have surgeons to get familiar with the interpretation of B-scan OCT images, but also will have to take into account the potential influence of resection techniques and time-dependent tissue changes on the OCT signal characteristics.

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