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Real-time image guidance for brain tumor surgery through stimulated Raman scattering microscopy

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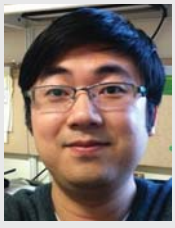

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Brain tumor surgery is one of the key factors in prolonging survival in patients with low- and high-grade gliomas. However, resections of these infiltrative lesions have historically been limited by the inability to accurately detect tumor margins. New methods in microscopy and dye injection have enabled more complete resections, but continue to lack biochemical specificity or high-resolution image acquisition. Stimulated Raman scattering microscopy represents an improvement over past techniques in the ability to differentiate intraparenchymal tissues on the basis of biochemical attributes, and is available for use in real-time, a feature that facilitates its translation to the surgical setting.

The treatment of malignant gliomas remains a formidable challenge. Among all cancers, these tumors carry one of the most dismal prognoses, with a 2-year survival of about 2% for patients >65 and 30% for those <45 years [1]. Recent advances in chemotherapy, radiation and surgery have resulted in modest survival improvements, with current mean survival estimated at 14.6 months [2].

Historically, glioma surgery has been controversial with some studies failing to show a survival benefit after maximal resection [3]. More recently, however, meticulous volumetric analyses have shown stepwise improvements in survival with increased tumor removal or extent of resection, leading many to advocate maximal cytoreduction [4]. Unfortunately, optimal surgical results are rarely and inconsistently achieved due to the difficulty of delineating tumor from normal brain in the operating room. In addition, tumor-infiltrated tissue is often left behind in an effort to minimize damage to normal brain [5]. Consequently, technologies that enable surgeons to carry out maximal, safe resection are needed.

While distinguishing tumor from normal brain on a macroscopic level is challenging; clear differences between tumor- and non-infiltrated brain exist microscopically. Consequently, multiple techniques have been proposed to assess the extent of resection during surgery. Confocal fluorescence microscopy can image the histomorphology of brain tumors in a surgical setting [6,7] but is reliant upon the specific uptake of dyes that are unevenly distributed within tumors [8]. Optical coherence tomography can assess architecture of tissue planes exposed during brain tumor resection, but currently lacks the specificity and resolution required for studying glioma infiltration and proliferation [9]. Third-harmonic generation microscopy offers improved resolution, but does not allow for delineation of structures based on biochemical differences [10]. We recently demonstrated the use of stimulated Raman scattering (SRS) microscopy, a label-free vibrational imaging technique for brain tumor delineation [11].

Raman scattering measures vibrational spectra by noninvasive laser spectroscopy [12], allowing rich, chemical-specific contrast based on intrinsic properties of the bonds within a sample. Each chemical bond has a specific stiffness (e.g., C=C is stiffer than C–C) and associated

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mass (e.g., C–C is heavier than C–H) and, consequently, a unique vibrational frequency (measured in wavenumbers per cm). The vibrational spectrum of a molecule is determined by total vibrational motions of each chemical bond, and the spectrum of a sample is determined by its molecular components [13]. This allows differentiation of tissues containing different concentrations of macromolecular components, such as lipids, proteins and DNA, eliminating the need for dye to visualize tissue architecture. The ability to collect vibrational spectra based on reflected signal eliminates the need for removing or processing tissue prior to imaging [14], making it well suited for *in vivo*, *in situ* use.

The Raman spectrum of normal rodent brain reveals two strong peaks at 2845 and 2930 cm^{-1}, corresponding to CH2 and CH3 vibrations, respectively. Due to their biochemical properties, lipid-rich regions, such as white matter tracts, will have high 2845 cm^{-1} and low 2930 cm^{-1} signal intensities. In contrast, densely cellular regions tend to have a lower lipid content and higher protein content, resulting in low 2845 cm^{-1} and high 2930 cm^{-1} signal intensities. Through a linear combination of the 2845 and 2930 cm^{-1} signals, images of tissue architecture can be created that correlate closely with H&E microscopy. We created a blue/green virtual color scheme in which high 2930 signal appears blue and high 2845 signal appears green. Consequently, densely cellular areas appear blue, while hypocellular, lipid-rich structures appear green.

Though highly specific, spontaneous Raman scattering has been limited by its weak signal and slow imaging speeds. In contrast, SRS [15] employs a second beam tuned to the Stokes frequency of a target vibration, which enhances the signal-to-noise ratio by up to 10,000-times compared with spontaneous Raman. Consequently, imaging at speeds up to video rate has been demonstrated with SRS [14].

SRS microscopy is well suited to detect tumor infiltration within brain tissue. Tumor-infiltrated regions are characterized by hypercellularity that have a blue appearance, in contrast to noninfiltrated tissues that typically appear greenish with well-spaced cells. The accuracy of SRS to delineate tumor, even in modestly infiltrated tissues, parallels that of H&E microscopy, providing high-resolution images that preserve characteristic histological features. Importantly, the success of SRS in delineating tumor margins in glioblastoma animal models translates well into the imaging of fresh, unprocessed human specimens. SRS imaging of surgical specimens removed during glioblastoma resection reveals the pathologic hallmarks of glioblastoma: hypercellularity, nuclear and cellular pleomorphism, pseudopalisading necrosis and microvascular proliferation. Preliminary work in our laboratory also suggests that the features of low-grade gliomas are also well-demonstrated by SRS microscopy. The ability of SRS microscopy to detect histopathological features characteristic of gliomas suggests that it will ultimately deliver actionable data that can be used for guiding surgical resection.

The true promise of SRS microscopy is evident in animal models where tumor margins are invisible. SRS microscopy can detect the presence of tumor in regions of brain, which appear entirely normal under standard bright-field conditions in living mice with implanted human glioblastoma xenografts. Importantly, the interface between densely cellular tumor and normal cellular brain is well-demonstrated with SRS microscopy [11]. Moreover, brain pulsations during the respiratory and cardiac cycles do not appreciably degrade image quality.

The ability to acquire SRS images in reflectance mode, advances in fiber-optic lasers and previous work to minimize the size of SRS imaging components [16] suggest the feasibility of building a handheld SRS device that can image human tissues during surgery. We anticipate that handheld SRS microscopy could improve the precision of cancer surgery, both inside and outside [17] the CNS, by providing real-time assessment of the histoarchitecture of tissues encountered during surgery. The use of SRS microscopy would be based on an iterative cycle of operating and imaging during resection. After the removal of all grossly abnormal tissue, the surgeon applies the handheld probe to the surface of the resection cavity. If microscopic imaging predicts the presence of dense tumor, then deeper dissection is continued until the majority of dense tumor has been removed. If microscopic imaging suggests the region is free of dense tumor, then resection is considered complete. This iterative scheme has been demonstrated to be feasible [7] for use with an intraoperative handheld confocal microscopy system in the context of a clinical trial in brain tumor patients and is an area of active investigation in our laboratories at this time.

Numerous questions must be answered concerning performance of SRS microscopy in a surgical setting. We previously demonstrated that high-quality SRS images can be acquired under simulated surgical conditions in human glioblastoma xenograft models. However, to judge its true performance and durability, SRS must be tested in an actual human surgical field to assess the performance of SRS imaging in surgically manipulated tissues. In addition, before human studies can be carried out, safety studies must be completed, despite preliminary safety studies that show no damage to SRS-imaged tissues. Logistic questions remain about how a relatively large region, possibly with the surface area of a 5-cm sphere, can be efficiently imaged with a device that will likely have a small (500 μm) field of view.

While treatment of gliomas and other brain tumors is most effective by attacking from several fronts, a consistent factor in prolonging survival is cytoreduction of the tumor. The ability to see the boundaries of an intraparenchymal tumor with microscopic resolution, in distinct contrast from surrounding normal brain and in real-time, would represent a major advancement in brain tumor surgery.

Surgeons need to see what they are removing and the ability to define the boundary between normal functional brain tissue and resectable tumor-infiltrated tissue. Functional mapping and imaging techniques must be established to meet these goals. SRS microscopy creates the possibility that brain tumor surgery may be carried with microscopic accuracy in the future.

Financial & competing interests disclosure

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