The role of optical spectroscopy in epilepsy surgery in children

SANJIV BHATIA, M.D.,1,2 JOHN RAGHEB, M.D.,1,2 MAHLON JOHNSON, M.D., PH.D.,3 SANGHOON OH, PH.D.,1,4 DAVID I. SANDBERG, M.D.,1,2 AND WEI-CHIANG LIN, PH.D.1,4

1Brain Institute, Miami Children’s Hospital, Miami; 2Department of Neurological Surgery, University of Miami Leonard M. Miller School of Medicine, Miami, Florida; 3Department of Pathology, Division of Neuropathology, University of Rochester Medical Center, Rochester, New York; and 4Department of Biomedical Engineering, Florida International University, Miami, Florida

Object. Surgery is an important therapeutic modality for pediatric patients with intractable epilepsy. However, existing imaging and diagnostic technologies such as MR imaging and electrocochleography (ECoG) do not always effectively delineate the true resection margin of an epileptic cortical lesion because of limitations in their sensitivity. Optical spectroscopic techniques such as fluorescence and diffuse reflectance spectroscopy provide a nondestructive means of gauging the physiological features of the brain in vivo, including hemodynamics and metabolism. In this study, the authors investigate the feasibility of using combined fluorescence and diffuse reflectance spectroscopy to assist epilepsy surgery in children.

Methods. In vivo static fluorescence and diffuse reflectance spectra were acquired from the brain in children undergoing epilepsy surgery. Spectral measurements were obtained using a portable spectroscopic system in conjunction with a fiber optic probe. The optical investigations were conducted at the normal and abnormal cortex as defined by intraoperative ECoG and preoperative imaging studies. Biopsy samples were taken from the investigated sites located within the zone of resection. The optical spectra were classified into multiple subsets in accordance with the ECoG and histological study results. The authors used statistical comparisons between 2 given data subsets to identify unique spectral features. Empirical discrimination algorithms were developed using the identified spectral features to determine if the objective of the study was achieved.

Results. Fifteen pediatric patients were enrolled in this pilot study. Elevated diffuse reflectance signals between 500 and 600 nm and/or between 650 and 850 nm were observed commonly in the investigated sites with abnormal ECoG and/or histological features in 10 patients. The appearance of a fluorescent peak at 400 nm was observed in both normal and abnormal cortex of 5 patients. These spectral alterations were attributed to changes in morphological and/or biochemical characteristics of the epileptic cortex. The sensitivities and specificities of the empirical discrimination algorithms, which were constructed using the identified spectral features, were all >90%.

Conclusions. The results of this study demonstrate the feasibility of using static fluorescence and diffuse reflectance spectroscopy to differentiate normal from abnormal cortex on the basis of intraoperative assessment of ECoG and histological features. It is therefore possible to use fluorescence and diffuse reflectance spectroscopy as an aid in epilepsy surgery. (DOI: 10.3171/FOC/2008/25/9/E24)

KEY WORDS • fluorescence spectroscopy • diffuse reflectance spectroscopy • pediatric epilepsy surgery

Surgery has become an acceptable form of management for intractable epilepsy in children. The management begins with a clear understanding of the semiology of habitual seizures. These symptoms help in understanding the possible site of onset of the seizure activity. The location of this site is then confirmed with surface and video EEG recordings, which may be performed on multiple occasions. Magnetic resonance imaging now is playing an increasingly prominent role in epilepsy surgery because it can be used to outline the structural abnormalities and demonstrate functional correlates.6,42 Both ictal SPECT and PET scans can outline the physiological abnormalities associated with the epileptic substrate6 and therefore assist in localizing the area of seizure onset and defining the target for more precise surgery. Often, these data must be supplemented by intraoperative or extraoperative electrocorticography32 and magnetoencephalography.29 Despite a number of attempts to localize seizure onset, results of resective surgery for pediatric intractable seizures are often less than optimal in certain situations. Difficulties
in precise localization, the episodic nature of the seizure discharge, and colocalization with eloquent cortex make the entire exercise challenging. Residual tumors, cortical dysplasia, and structurally normal epileptic tissue can account for recurrent seizures. The challenges are increased significantly in children with nonlesional epilepsy. Improvements in technology to facilitate better recognition of epileptic neural tissue can help to improve the results of resection. These measures have included coregistration of ictal SPECT scans with MR anatomical images, intraoperative frameless neuronavigation to recognize areas of dysplasia in the depths of sulci and assist in the placement and depth of electrodes. Our group has investigated the use of optical spectroscopy to recognize the unique pathophysiological features associated with cortical abnormalities in pediatric epilepsy. In the present study, we elaborate on the basics of optical spectroscopy as applied to neural tissue and summarize our preliminary experience with this technology in epileptic tissue in the pediatric population.

**Optical Spectroscopy**

The propagation of light in biological tissue is governed by the morphological, biochemical, and physiological characteristics of the tissue; therefore, light provides a convenient, noninvasive means of characterizing tissue diseases and injuries. There are 3 optical spectroscopy types that are used frequently in biomedicine to monitor light-tissue interaction and therefore to perform in vivo tissue characterization: diffuse reflectance spectroscopy, fluorescence spectroscopy, and Raman spectroscopy. Each of these spectroscopy types targets a particular type of light-tissue interaction and a subset of biological molecules (Fig. 1).

![Fig. 1. Schematic diagram summarizing the potential uses for optical spectroscopic techniques for characterizing the brain in vivo. The intrinsic biological and morphological characteristics of the brain, their corresponding unique interactions with light, and the detection mechanisms are shown.](image-url)

Optical spectroscopy, explored thus far, is to demarcate a brain tumor operatively. Several research groups have demonstrated the feasibility of using intrinsic fluorescence and optical characteristics to differentiate brain tumors from normal brain tissue in vivo over the past decade. In a recent clinical trial, the Vanderbilt group demonstrated high sensitivity with a combined optical spectroscopic method to detect the infiltrating brain tumor margins. The feasibility of using intraoperative optical spectroscopy to detect and demarcate epileptic lesions has been investigated on only very few occasions. In published studies to date, diffuse reflectance optical imaging has been used frequently to monitor the hemodynamics of an epileptic lesion in vivo in both animal models of epilepsy and humans. Although these results support the idea that the investigation of intrinsic physiological characteristics, especially hemodynamics, may be a viable alternative or addition to the current methods of epileptic lesion demarcation, the impact of such an approach on the outcome of epilepsy surgery has not yet been determined. Furthermore, the feasibility of using optical spectroscopy to delineate the margins of neocortical epileptic lesions in children has not yet been proven. We therefore performed a pilot clinical study to evaluate the feasibility of using static optical spectroscopic methods intraoperatively—specifically the combination of fluorescence and diffuse reflectance spectroscopy—to differentiate cortex with abnormal ECoG recordings and/or histological features from normal cortex during interictal periods in children.

**Methods**

We conducted our pilot in vivo patient study at Miami Children’s Hospital to investigate the utility of optical spectroscopy as an aid in pediatric epilepsy surgery. We were specifically interested in ascertaining whether cortex with abnormal electrical and/or histological features could be
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![Spectroscopic System](image)

**Fig. 2.** Intraoperative photograph of optical spectral acquisition. **Upper left inset:** Portable fiberoptic spectroscopic system. **Bottom right inset:** The configuration of the optical fibers in the optical probe. Red and white fibers are excitation fibers and the yellow fibers are collection fibers.

differentiated from normal cortex on the basis of static optical spectroscopy methods. This study was approved by the Western Institutional Review Board. All eligible patients were recruited by one of the neurosurgeons involved in this study, and informed, written consent was obtained from the parents or guardians of each participant.

We used a portable fiberoptic spectroscopic system for static fluorescence and diffuse reflectance spectral acquisition (Fig. 2). The system contained 2 light sources: a pulsed nitrogen laser (337 nm, 20 Hz, VSL-337; Spectra-Physics) for fluorescence spectroscopy, and a portable halogen lamp (LS-1; Ocean Optics) for diffuse reflectance spectroscopy. The selection of 337-nm excitation for fluorescence spectroscopy enabled probing for biological fluorophores, including nicotinamide adenine dinucleotide phosphate, flavin adenine dinucleotide, collagen and tryptophan, serotonin and dopamine. Broadband emission from the tungsten halogen light source enabled investigation of the hemodynamic, structural, and other compositional characteristics of the cortex.

A fiberoptic probe (RoMack Inc.) was used in conjunction with the spectroscopic system, for remote in vivo spectral acquisition. The probe was composed of seven 300-μm core fibers arranged in a conventional 6-surrounding-1 design (Fig. 2). Two fibers were used to conduct the fluorescence and diffuse reflectance excitation lights separately. The arrangement of the optical fibers produced an investigated volume < 1 mm³. The collection fibers of the optical probe were coupled directly to a spectrometer (USB2000-FL Spectrometer; Ocean Optics) with a spectral range of 350–900 nm and a spectral resolution of ~ 5 nm. To filter out the reflected nitrogen laser light in the collected fluorescence and diffuse reflectance signals, two 385-nm long-pass filters were placed at the entrance port of the spectrometer. The spectrometer was controlled by a laptop computer via a universal serial bus interface. The integration time for each spectral acquisition was set at 1 second to ensure a sufficient signal-to-noise ratio in the acquired spectra.

Optical spectral acquisition was performed in both normal and abnormal cortex, as defined on preoperative MR images and intraoperative ECoG studies. In each patient, the surgeon selected at least 3 unique sites from the normal and abnormal cortical area. At each investigated site, the baseline, fluorescence, and diffuse reflectance spectra were acquired sequentially, and this process was repeated at least 3 times. The time to acquire optical spectra from a single investigated site was < 12 seconds. The locations of the optical investigation sites and their spatial correlations with the ECoG electrode grids were documented with digital photography. Biopsy samples were obtained from the investigated sites located within the resection zones, and processed using conventional histological methods (such as H & E staining). The histological features of these samples were evaluated by a neuropathologist (M.I.).

Prior to their analysis, the spectra were processed to eliminate any spectral alterations induced by the spectroscopy system itself. Specifically, background subtraction was performed, which removed the corresponding baseline spectra from all acquired fluorescence and diffuse reflectance spectra. Next, instrument-induced spectral alterations were removed using a set of calibration factors. The system’s calibration factors were determined using a calibrated tungsten light source (LS-1-CAL, Ocean Optics Inc.). These spectral processing procedures allowed an accurate correlation between the optical spectral characteristics and the physiological/biochemical characteristics of the cortex.

The preprocessed spectral data were classified using either the corresponding histological or ECoG records. Specifically, spectral data were divided into 3 subsets: 1) normal cortex; 2) cortex with abnormal ECoG features but normal histological features; and 3) cortex with abnormal ECoG and histological features. Spectral comparisons were performed between the normal cortex and both types of abnormal cortex so that any spectral features unique to abnormal cortex, such as the spectral intensity at a given wavelength or the spectral profile, could be identified. In addition to the preprocessed fluorescence and diffuse reflectance spectra, normalized spectra were utilized in the spectral comparison and analysis procedures. The normalization methods incorporated here included normalization to the spectral intensity at an arbitrary wavelength, normalization to the mean spectrum of the subgroup, and normalization to an arbitrary point in the mean spectrum. The nonpaired, 2-tailed Student t-test was used to identify statistically significant differences between the spectra obtained in normal and abnormal cortex. All statistically significant spectral alterations, in turn, were used to construct discrimination algorithms to evaluate the effectiveness of optical spectroscopy in separating normal from epileptic cortex.

**Results**

Fifteen patients were evaluated using the methods we
have described in this pilot study. Patient demographic information and the gross histological diagnosis of their lesions are shown in Table 1. Note that the gross histological information presented in Table 1 was obtained from the general pathology report on the entire resected lesion.

The fluorescence and diffuse reflectance spectral data displayed in Fig. 3 are typical of our findings. In the fluorescence spectra acquired in both normal and abnormal cortex, a dominant peak was found at ~470 nm wavelength. In some fluorescence spectra obtained from the resected zone (ECoG abnormal cortex), a strong secondary peak was noticed at ~400 nm. In the diffuse reflectance spectra, 3 significant signal valleys were detected, these fell between 400 and 650 nm. Diffuse reflectance signals beyond 650 nm, in general, exhibited a steadily decreasing trend. We found that spectral intensity fluctuations in all investigated sites were more prominent than spectral profile alterations.

Comparisons of fluorescence and diffuse reflectance spectra, normalized to the arbitrary reference points in their

### TABLE 1

<table>
<thead>
<tr>
<th>Age (yrs), Sex</th>
<th>Gross Histopathological Characteristics</th>
<th>DR Elevation†</th>
<th>Peak at 400 nm‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>2, M</td>
<td>cortical tubers</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>14, M</td>
<td>heterotopic neurons, Chaslin gliosis</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>4, F</td>
<td>local cortical dyslamination</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>3, M</td>
<td>severe cortical dysplasia, Palmini Type Ib</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>4, M</td>
<td>microdysgenesis</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>10, M</td>
<td>left functional hemispherectomy</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>6, M</td>
<td>minimal cortical dysplasia</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>2, F</td>
<td>gliosis, areas of Palmini Type IA</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>14, F</td>
<td>mild multifocal Palmini Type IA cortical dysplasia</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>9, F</td>
<td>gliosis; no cortical dysplasia</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>2, M</td>
<td>multifocal cortical dysplasia</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>21, F</td>
<td>Chaslin gliosis</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
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<td>temporal lobe gliosis</td>
<td>no</td>
<td>no</td>
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<td>17, F</td>
<td>neuronal disorganization &amp; gliosis</td>
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<td>no</td>
</tr>
<tr>
<td>5, M</td>
<td>low-grade glioma</td>
<td>yes</td>
<td>no</td>
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</table>

* All resection sites showed abnormal ECoG patterns consistent with seizure discharge.
† Indicates diffuse reflectance (DR) intensity elevation at 500–600 nm and/or 650–850 nm.
‡ Indicates the presence of the secondary fluorescence peak at 400 nm.

Fig. 3. Representative fluorescence (A), and diffuse reflectance spectra (B) obtained in a single patient. The presented spectra are the mean spectra obtained in 3 repeated measurements. Inset: The spectral region and its associated primary biological molecular features.
corresponding subcategory mean spectra, identified many statistically significant spectral alterations between normal and abnormal cortex. We observed that the normalized diffuse reflectance intensities from normal cortex seemed to be higher than those from cortex with abnormal ECoG and/or histological features. Normalized fluorescence intensities from normal cortex were lower than those from cortex with abnormal histological features. However, this trend was reversed in the comparison between normal cortex and cortex with abnormal ECoG features. Using 1 fluorescence and 1 diffuse reflectance spectral feature, an empirically derived linear discrimination algorithm was constructed for each normal-abnormal cortex comparison (Fig. 4). The sensitivities and specificities of these empirical discrimination algorithms were all > 90%.

Discussion

Our results demonstrate that combined fluorescence and diffuse reflectance spectroscopy can be used to separate epileptic cortex from normal cortex with a high degree of sensitivity and specificity. The fluorescence and diffuse reflectance spectral features depict unique, static, in vivo characteristics of the epileptic cortex during the interictal period, which should not be confused with the dynamic diffuse reflectance features reported by other groups.3,8,14,17,28,37,40,41,44,49,52,53 These observations warrant further studies to ascertain the clinical utility of optical spectroscopy in pediatric epilepsy surgery.

In the diffuse reflectance spectra from the cortex, hemoglobin absorption produces the predominant spectral features that can be observed between 400 and 650 nm (val-leys at 400, 540, and 580 nm) due to its strong absorption of visible light. In fluorescence spectra from cortex, nicotinamide adenine dinucleotide phosphate is considered to be the primary contributor, because its peak emission location is very close to 460 nm. Although these spectral profile characteristics are observed consistently among all spectra acquired, the fluctuations in intensity are very prominent between investigated sites.

Cortex with definite pathological abnormalities often produces a stronger diffuse reflectance signal in both the visible and near-infrared wavelength regions than normal cortex does. In some cases, elevated diffuse reflectance signals also are observed from cortex with abnormal ECoG features, relative to normal cortex. It should be noted that this trend is reversed when the diffuse reflectance signal is normalized, as shown in Fig. 4. The increase in diffuse reflectance spectral intensity in a biological medium, especially between 650 and 900 nm, often indicates an increase in its scattering properties. This alteration in tissue-scattering properties may be attributed to the unique microscopic structural characteristics of epileptic brain lesions. It is known that several seizure-inducing brain lesions, including focal cortical dysplasia and cortical tubers, possess unique microscopic structural features. For example, the presence of focal cortical dysplasia and cortical tubers causes cortical laminar disorganization. Immature and dysmorphic neurons, giant cells, and balloon cells are hallmarks of cortical dysplasia. It has also been suspected that hyperactivity of the epileptic cortex could be caused by an increased number of mitochondria. These morphological alterations could in turn lead to an increase in the scattering properties of epileptic cortex. Further microscopic studies
are needed to elucidate the true correlation between the morphological and biochemical characteristics of epileptic cortex and its scattering properties.

A unique feature was observed in some fluorescence spectra acquired from epileptic cortex: a secondary peak of wavelength ~ 400 nm (Fig. 5). There are several potential biological contributors to this particular emission peak, namely collagen, tryptophan, serotonin, and dopamine. Although it is possible that this peak is caused by the presence of a large blood vessel (collagen) in the area under investigation, it was not observed consistently in all patients. It is also possible that this spectral feature reflects abnormal concentrations of neurotransmitters in the region. Further studies must be conducted to identify the true origin of this peak.

Although the results of the present study are promising, several obstacles must be overcome and improvements made before the true clinical utility of static fluorescence and diffuse reflectance spectroscopy in pediatric epilepsy surgery can be determined. A critical challenge to this in vivo study arises from the significant disparity in outcomes of ECoG and histological studies. Many biopsy samples obtained from cortex with abnormal ECoG readings were declared histologically normal by the study neuropathologist. There are several possible reasons for this discrepancy. First, the optical probe we used is limited to a depth of investigation ≤ 1 mm, and detection was performed solely on the cortical surface. It is possible that pathological abnormalities may actually be present in deeper layers of the cortex and hence not detected within the biopsy samples taken from the cortical surface. To address this concern, a new optical probe with a greater separation between source and detector has been designed and built and will be used in a future study. Another possible reason for the disparity is that the underlying mechanisms for the abnormal ECoG features may not be detectable using conventional histological methods. Third, because of the rapidly spreading nature of cortical electrical activities, the abnormal ECoG features detected may not originate at the site of investigation. These concerns undermine the use of either ECoG or histological examination alone as the true gold standard for data analysis. To address these concerns we are investigating a change in the design of the probe so that it can be integrated with the subdural grid electrodes, enabling a real-time simultaneous acquisition and analysis of ECoG and spectroscopic data.

Inconsistencies in spectral measurements with hand-held probes are particularly evident when spectral measurements are repeated at a single site. In a previous study, we found that when the probe contact pressure exceeds a certain threshold, the hemodynamics of the target tissue and therefore its fluorescence and diffuse reflectance spectra can be influenced drastically. The hallmark spectral alterations that result from excessive probe contact pressure are alterations in the spectral profile between 500 and 600 nm, caused by deoxygenation of hemoglobin and an increase in fluorescence emission. The possible presence of these artifacts in the acquired fluorescence and diffuse reflectance spectra may explain why absolute intensities of either signal type could not effectively differentiate normal from abnormal cortex. To obtain true fluorescence and diffuse reflectance spectra from brain in vivo, these types of motion and pressure effects must be minimized.

Establishing true baseline levels for the fluorescence and diffuse reflectance characteristics of normal cortex may be challenging in children, because their brains are continuously growing and developing. The size and weight of a child’s brain continues to increase significantly for several years after birth because of brain cell division and growth. The biochemical processes involved in this development, including cholesterol synthesis and the formation of new mitochondria, further influence both the morphological and biochemical characteristics of the brain. We therefore expect that “normal” measurements will vary with patient age. A separate study currently is underway to investigate the effects of patient age and brain lesion location on optical and fluorescence spectra features.

The spectroscopic methods we used in this pilot study only explore the static nature of the cortex. In other words, they provide only a snapshot of the physiological characteristics of the cortex during a 1-second integration time. The dynamic nature of brain metabolism and hemodynamics completely neglected in this statistical model. To address this potential shortcoming, we have designed and developed a new spectroscopic system that can be used to acquire broadband optical spectra (400–950 nm) from the brain at a rate of 33 Hz in a continuous manner. This new device should allow us to investigate both the static and dynamic nature of epileptic cortex, and to perform advanced analysis of tissue morphological characteristics, biochemistry, and composition. Current is this system is being tested in a pilot study, the results of which will be reported in a future publication.

Conclusions

In the present study, we investigated the feasibility of using fluorescence and diffuse reflectance spectroscopy to aid in pediatric epilepsy surgery. Static fluorescence and
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diffuse reflectance spectra were acquired in cortex with normal and abnormal ECoG and histological features. Spectral features separating normal from epileptic cortex were identified and used to produce tissue discrimination algorithms with high sensitivities and specificities. The spectral alterations observed may be attributable to the unique compositional and/or structural characteristics of the epileptic cortex.

Disclosure
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References


