Review Article

Role of Optical Spectroscopic Methods in Neuro-Oncological Sciences

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Abstract:

In the surgical treatment of malignant tumors, it is crucial to characterize the tumor as precisely as possible. The determination of the exact tumor location as well as the analysis of its properties is very important in order to obtain an accurate diagnosis as early as possible. In neurosurgical applications, the optical, non-invasive and in situ techniques allow for the label-free analysis of tissue, which is helpful in neuropathology. In the past decades, optical spectroscopic methods have been investigated drastically in the management of cancer. In the optical spectroscopic techniques, tissue interrogate with sources of light which are ranged from the ultraviolet to the infrared wavelength in the spectrum. The information accumulation of light can be in a reflection which is named reflectance spectroscopy; or interactions with tissue at different wavelengths which are called fluorescence and Raman spectroscopy. This review paper introduces the optical spectroscopic methods which are used to characterize brain tumors (neuro-oncology). Based on biochemical information obtained from these spectroscopic methods, it is possible to identify tumor from normal brain tissues, to indicate tumor margins, the borders towards normal brain tissue and infiltrating gliomas, to distinguish radiation damage of tissues, to detect particular central nervous system (CNS) structures to identify cell types using particular neurotransmitters, to detect cells or drugs which are optically labeled within therapeutic intermediations and to estimate the viability of tissue and the prediction of apoptosis beginning in vitro and in vivo. The label-free, optical biochemical spectroscopic methods can provide clinically relevant information and need to be further exploited to develop a safe and easy-to-use technology for in situ diagnosis of malignant tumors.

Keywords: spectroscopy; neuro-oncology; optics

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Introduction

Application of optical spectroscopic methods in diagnosis of cancer has been an attractive area of interest during the past decades. Optical spectroscopy is dealing with a group of methods that provide the structural or functional data from cells and tissues by optical exploration. All types of optical spectroscopic methods are dealing with light-tissue interplays. These interplays can be utilized for data extraction of the structure or chemistry of the investigated tissue. There are various famous optical spectroscopy methods such as diffuse reflectance, fluorescence, vibrational and Raman spectroscopy. In fluorescence spectroscopy, a tissue absorbs a wavelength of light and emits it at a longer wavelength. This technique can be used to obtain information about the endogenous fluorophores or an injected fluorophore. In reflectance spectroscopy, a tissue is illuminated by light which is scattered and/or directly reflected back. Due to the alternations of morphology and structure at the cellular and subcellular level, reflectance spectroscopy can easily differentiates gray and white

matter [1,2]. Another type of optical interrogation, Raman spectroscopy, relies upon the fact that a small fraction of light undergoes inelastic (Raman) scattering from the tissue. The scattered light have slight shifts in the wavelengths due to interactions between the incident light and biochemical groups such as amide bands, methyl groups, and ringed structures on subcellular structures such as nucleic acids and proteins. More information about the biochemistry of the underlying tissues may be collected from Raman spectra [3,4].

Approximately, 2.3% of all deaths related to cancer is due to the brain cancer [5]. In order to provide optimal and personalized therapy, it is crucial to precisely determine localization and boundaries of tumors as well as their exact properties and characteristics as early as possible to adjust therapy accordingly. The most prevalent neoplasms of the brain reported in adults are gliomas and brain metastases [6]. Gliomas demonstrate a diverse group of brain tumors with signed intra- and inter-tumor variation. They are the most common of primary brain tumors, accounting for over 60% of all cases [1,7]. In the case of for instance high-grade malignant gliomas, it is important to remove the overall tumors and to maintain the surrounding functional brain [8]. Malignant gliomas are a severe pathology due to their invading features and restricted intracranial region. MRI images before operation can give the localization and size information about the tumor. These images can be used for neuro-navigation during surgery but they cannot compensate for intra-operative tissue changes and alterations, eg, shifts [9]. Intra-operative MRI requires profound constructional changes of the operating theatre, and special, expensive equipment [10]; it is time-consuming (15-30 min) and is used to optimize the extent of resection [11]; for resection control and not during ongoing surgery. Information about localization of certain tumor types can also be obtained by fluorescence monitoring [12]. Nevertheless, there is no method available that allows the localization of every type of brain tumor during surgery. The exact normal and tumorous tissue delineation during surgery is an unsolved problem. Additionally, it is not possible to detect small micrometastases before the blood-brain barrier breakdown with the technology which is clinically available [13, 14]. To optimize the resection strategy and decide upon radical resection of aggressive and highly malignant glioma, a biopsy sample of suspicious tissue is removed and the morphology of tissue is appraised by a trained pathologist [15]. This method is time-consuming (approximately 30 min) and it is only applicable after removal of the tissue. On the other hand, neurosurgery renders it possible to visually access the tumour and therefore theoretically opens the possibility to perform in situ diagnosis without tissue removal through the application of non-invasive optical analysis of suspicious tissue. New advanced optical methods allow the label-free investigation of tissues and have the potential for histopathological diagnosis [16] which can address biochemical properties.

Therefore, the present paper focuses on the various optical spectroscopic method for the pathology assessment of brain cancer and novel methods for the in vivo discrimination of margins of the tumor. In this paper, we first discuss the advantages of optical techniques in clinical oncology, the optical spectroscopy principle and the common experimental setup. Furthermore, we describe several optical spectroscopy methods in diagnosis of cancer. Various famous optical spectroscopy methods including diffuse reflectance spectroscopy, fluorescence spectroscopy, vibrational spectroscopy and Raman spectroscopy. Our focus in this review is on optical spectroscopy in neuro-oncology [17-21]. So, due to relatively minor investigated in clinical applications, the alternative optical spectroscopy methods including light scattering spectroscopy [22], coherent backscattering spectroscopy [23], and low coherence spectroscopy [24], are not discussed. Some specific applications are discussed in the other section.

Advantages of optical techniques in clinical oncology

Nowadays, optical spectroscopic techniques can be served as acceptable methods in comparison to other imaging techniques including magnetic resonance imaging (MRI), x-ray computed tomography (CT) and ultrasonography in clinical oncology.

Optical techniques possess several advantages compared to above mentioned modalities which cause them to be acceptable in clinical management of cancer: the irradiation in optical range is not ionizing and does not cause a health problem even for a long time of exposure; the optical experiments have sensitivity to alternations of biochemistry and morphology that are related to carcinogenesis; technical improvements in the detectors and sources of light have made it possible to reach to real time measurements in several conditions; the fiber-optic probes development and miniaturization of detectors has made it practical to combine optical spectroscopy with systems of endoscopy to achieve to interior organs [25].

In general, the depth of penetration of light is small in comparison with x-ray or ultrasound. However, modulation of wavelengths of light can vary the sensing depth of optical experiments from a several micrometers to hundred centimeters [26]. The tunability of sensing depth can add extra flexibility and sensitivity for special clinical applications.

Principles of optical spectroscopy for cancer diagnosis

Several lighttissue interplays such as absorption, fluorescence and scattering are the basis of optical spectroscopy for tissue characterization. When a specie absorbs excitation light photons without radiating any photons, absorption occurred. In the whole optical spectrum, the major light absorber in tissues is hemoglobin [27]. Total concentration of hemoglobin shows the vascularization degree, which is important in testing angiogenesis within cancer progress. Depending on the degree of oxygenation, the absorption spectrum of hemoglobin can change. Therefore, hemoglobin oxygenation is the other significant factor as well as total hemoglobin concentration, which have effect on the absorption of tissue. The hemoglobin oxygenation changes could show the variations in the balance between the need and the reserve of the oxygen in tissues while the hemoglobin is the oxygen carrier in the blood. The absorption of lipid and water is in near infrared region of light. Several studies have represented that lipid and water amounts are considerably distinguished between normal and cancerous breast tissue [28].

Vibrational optical spectroscopy is relying on the specific wavelength of light absorption via vibrational levels of molecules. In case of biological samples and tissues, which consist of a variety of different biochemical compounds, vibrational spectra are the products of the complex overlap of multiple bands of the chemical bonds of all tissue constituents. Therefore, vibrational spectra comprise the entire information about cell or tissue biochemistry and are referred to as biochemical fingerprint. Vibrational spectroscopic techniques therefore have been gaining increasing attention for biomedical applications, investigation of disease mechanisms, and diagnosis over the past years [29, 30]. Fourier-transform infrared spectroscopy (FT-IR) is relying on the infrared radiation absorption by the sample and its use in biomedical science has been studied for many decades.

After interplaying with a molecule of the tissue, when one photon is bend from the direction of incident, elastic or inelastic scattering occurred. Elastic scattering or Raleigh scattering is a kind of scattering which happens without the alternation of frequency of the photon. Nuclei, mitochondria and collagen fibers [31] are the major tissue elastic scatterers. Due to the variations in the size of nuclei, the ratio of nucleustocytoplasm and the collagen fibers density in cancer cells, elastic scattering has been shown to be effective in detecting epithelial precancers [32, 33]. When the alternation in the frequency happens, inelastic scattering took place. Based on whether the reduction or addition of the frequency of scattered photons, inelastic scattering is named Stokes or antiStokes Raman scattering [34]. Raman scattering is the basis of Raman spectroscopy. Collagen, water, the nucleus and cytoplasm of the cell, fat and cholesterollike lipid deposits are Raman scatterers in tissues [35].

When a molecule return from an excited singlet state to the lower singlet state and emits light, fluorescence occurred. The wavelength of the radiation is often longer than the wavelength of excitation, because of the dissipation energy in the action. When a fluorescence light emitted from fluorophore molecules (molecules exhibiting fluorescence), it subjects to absorption and scattering. Flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide (NADH) are the most extensively studied fluorophore molecules which are metabolic coenzymes in the action of reductionoxidation for energy production for cellular functions. The fluorescence of these two fluorophores has been investigated to explore the metabolic rate change of tissues [36]. The range of wavelength in fluorescence spectroscopy is usually bigger than that in Raman spectroscopy. In diffuse reflectance spectroscopy, light intensity measurements are done at the excitation wavelengths, which are sensitive to the absorption and scattering features of the tissues.

Experimental Instrumentation

The main components of an optical spectroscopy system are listed below:

1. A source of light (such as laser);
2. Wavelengths selection elements (such as a band-pass filter) for the selection of both the illumination and emission radiations;
3. A fiber for illumination light delivery and emission light collection;
4. A detector for signal intensity measurements.

Xenon lamp is usually used to utilize a large range of wavelengths in diffuse reflectance spectroscopy and fluorescence spectroscopy. When high power excitation

light is needed, lasers can also be used. Monochromator can be used as dispersing element for detection that is usually connected to a single-channel detector such as a photomultiplier tube (PMT). Another dispersing element is a spectrograph, which can be connected to a multi-channel detector such as a charge coupled device (CCD). Usually, the excitation source in Raman spectroscopy is a high power laser, due to the weak Raman signals. A filter with transmission of laser light wavelength and suppression of other wavelength, is usually used. In the detection section, a long pass or notch filter is applied to remove the excitation wavelength.

Optical spectroscopy techniques in clinical cancer diagnosis

As it was mentioned, various optical spectroscopic methods can play important roles in many aspects of clinical brain cancer. In this section, some applications of these methods are reviewed separately.

Fluorescence and Diffuse Reflectance Spectroscopy

Diffuse reflectance and fluorescence spectroscopy have been investigated in the evaluation of tumor margin or characterization of the tissue during neurosurgical operation [37]. The application of diffuse reflectance spectroscopy in identification of tissue types for improving the intracerebral guidance within deep stimulation of brain has been investigated by Antonsson et al [38]. For various functional targets of the internal globus pallidus (GPi), subthalamic nucleus (STN) and zona incerta (Zi), diffuse reflectance spectroscopy experiments were performed in 10 patients. There are considerable discrimination between the white and gray matter intensities which is at least 14% ($P < 0.05$) for MRI and 20% ($P < 0.0001$) for spectral sorted information. The use of diffuse reflectance and fluorescence spectroscopy to discriminate pediatric neoplastic and epileptogenic brain from normal brain in an in vitro experiment was studied by Lin et al [39]. Diffuse reflectance spectroscopy was performed between wavelengths of 400 and 900 nm; fluorescence spectroscopy was performed at 337, 360, and 440 nm excitation wavelengths for every sample. The spectroscopic results are in good correlation with pathological results for classification of brain samples abnormalities. Statistically significant differences ($P < 0.01$) were obtaied for both raw and normalized diffuse reflectance and fluorescence spectroscopic data for three different matter of neoplastic and normal white matter of brain, neoplastic and normal gray matter of brain, and epileptogenic and normal gray matter of brain.

Diffuse reflectance spectra were used for assessment of the of optical nerve discrimination feasibilities by Stelzle et al to find the foundation in a feedback control system to improve nerve maintenance in maxillofacial and oral laser surgery [2,40]. In the range of 350–650 nm wavelength, diffuse reflectance spectroscopy were performed on nerve tissue, fat tissue, mucosa, skin, bone, muscle and cartilage of ex vivo pig heads. Tissue identification was made using principal components analysis (PCA) in addition to linear discriminant analysis (LDA) to discriminate nerve tissue from various kinds of soft and hard tissue in the facial section by means of diffuse reflectance spectroscopy. Nerve tissue was discriminated accurately from fat tissue, mucosa, skin, bone, muscle and cartilage with a 78% specificity of over and a sensitivity of more than 86%. These results demonstrated the overall feasibility of applying diffuse reflectance spectroscopy in remote discrimination of nerve.

Fourier-Transform Infrared Spectroscopy (FT-IR)

Compositional information about the biochemistry of nervous tissue, grey and white matter can be obtained from FT-IR spectra. Bands in the region of 1000–1350 cm–1 are dominated by the vibrations of phosphate groups and carbohydrates and mainly indicate the presence of phospholipids, DNA/RNA, and carbohydrates in the context of cells and tissue. Prominent bands of amide II and amide I bond vibrations of proteins are recognized at around 1550 and 1650 $cm^{-}1$, respectively. Bands related to C-H bond vibrations in lipids and proteins are found in the high-energy region from 2800–3000 cm^{-1} [41]. Used as an imaging technique, FT-IR can provide spatially resolved image of distribution of tissue components.

For investigation of brain tumors, FT-IR spectroscopy has been intensively used. Primary brain tumors as well as brain metastases of peripheral tumors can be localized and discerned from normal brain tissue with high specificity [42-44]. The type of primary tumor can be identified in case of brain metastases [45]. The main components that allow differentiation of normal and tumor tissues and tumor-grading are the tissue lipid content and alternations related to nucleic acids [46]. Furthermore, collagen content and distribution of collagen subtypes are changed in brain neoplasms which can be investigated by FT-IR spectroscopy [47].

It is possible to gain some diagnostic information

including the glioma grade, expression of hormones or tumor vascularization [48-50]. The pathologically and clinically increased hormone production could be extracted from the spectral information using chemometrical analysis as well as tumor identification [49]. Chemometrical analysis can sort spectral data according to similarities and differences [51]. They can be used to build colorful maps of the tumorous tissue [52].

Attenuated total reflection FT-IR (ATR FT-IR) is a type of infrared spectroscopy technique which can offer the advantage of measuring non-transparent samples such as bulk tissue. This technique requires tight contact between the sample of interest and the core of the ATR crystal or a fiber optic probe in an endoscopic setup [53]. Infrared radiation propagates in the crystal, generating an evanescent wave that penetrates a few micrometers of the sample. Spectral changes in the backscattered light are used to obtain information about the sample's biochemical properties. This is especially interesting for direct analysis of biopsy tissue [54]. Using fiber optics, it holds great potential for future clinical application and in situ diagnosis of malignant glioma. The use of optical ATR FT-IR spectroscopy for the analysis of native human brain tumor biopsies indicates the possibility of this method to find differences of extracellular matrix components among different tumor types.

Raman Spectroscopy

In comparison to FT-IR spectra, in the Raman spectra vibrational bands are better separated. So, the spectra contain a higher degree of information. It is worth to mention that the technique can be applied on non-dried tissue because the spectral contribution of water does not interfere with relevant bands of biological tissue as it does in FT-IR spectroscopy [55]. So, this property makes Raman spectroscopy suitable for in situ diagnosis.

Raman spectroscopy of tissue permitted the discrimination of healthy and tumor and necrotic tissues in rat brain tissue samples [56] and was used to study brain functions in living mice and rats [57]. Brain injury caused by traumatic insults related to caspase-3-activated apoptosis can also be detected by Raman spectroscopy [58]. Raman mapping can identify brain tumors in the living animal [59]. Ex vivo studies on human brain tumor samples have proven the capability of the method to distinguish normal and tumorous tissues of adults and children [60-62]. Raman micro-spectroscopy of primary brain tumors can provide diagnostic information on the malignancy grade and cell density [63].

Raman spectroscopy was carried out for in vivo mapping the surface of intercerebral tumors of rat brains and comparison with hematoxylin-and-eosin (H&E) stained coronal parts of the same area by Kirsch et al [59]. A multivariate chemometric method of k-means cluster analysis of the spectra was applied for colorful map construction of the rat brain surface. Surprisingly, the mapping by Raman spectroscopy could find a tumor below the surface that was not evident and cognizable in photomicrographs visually.

In another recent study, Raman spectroscopy was applied for the assessment of C6 glioblastomas implanted in rat brains by Beljebbar et al [64]. At first, Raman spectroscopy was applied for classification of tissue cut from the implanted brain tumors. The classification was based on a set of reference spectra acquired from purified DNA and lipids. The comparison was done between the Raman spectroscopy results and those from conventional histopathology as the classification standard. Principal component analysis (PCA) was applied as a chemometric method on the spectra acquired with 100% accuracy for classification. It was assessed that reducing the acquisition times from 100 s to 10 s had small impact on the signal averaging while obtaining a robust procedure. For demonstration of possibility of clinical utilization, by means of a handheld Raman microprobe, implanted tumors were investigated in vivo over 20 days. A distinct discrimination of spectra of normal tissue before tumor implantation from spectra acquired on days 4 and 20 after implantation was observed using hierarchical cluster analysis. The immunohistochemistry staining and the spectra were strongly correlated. Spectral signs were correlated with invasive and proliferative characteristics of the tumors. It is significant to find such spectral signs that can play an important role in diagnosis the biochemical change between cancerous and normal brain tissue [65].

The possibility of spectral signs determination relating to the lipids concentrations in the tissues was examined using Raman spectroscopy of lipid extracted from malignant tumors and normal tissues of brain. The concentration variations in the of phosphatidylcholine and cholesterol were significant between normal and cancerous cells [66]. Raman spectroscopic results of the lipid extracts were in good agreement by mass spectrometric data. Discrimination between neural crest-derived pediatric tumors were investigated using Raman spectroscopy [67, 68]. Raman spectroscopy of freshly resected tissue or biopsies obtained from 39 patients were acquired and analyzed by PCA statistical method. For discrimination between

nerve sheath tumors, neuroblastomas, ganglioneuromas, pheochromocytomas and normal adrenal glands, good sensitivity and specificity was attained [67].

For ex vivo Raman spectroscopy of fresh human tumor samples, grading of astrocytoma was proved using fiber optic probes [69]. There are many researches that focus on the application of Raman spectroscopy to perform optical biopsies for tumor recognition [63]. Technical advances in the development and miniaturizing of Raman fiber probes may allow short acquisition times of approximately 10 s in concert with high-quality spectra acquisition [64,70]. Raman endoscopy has already been performed in a clinical context (> 300 patients) for gastric cancer diagnosis and provided diagnostic information [71].

In order to increase the sensitivity and also reduce acquisition time, different techniques are used to enhance Raman signal intensity. A famous enhancing technique is surface-enhanced Raman scattering (SERS). This method exploits the electrochemical interaction of molecules adsorbed by nanostructures. Raman signal enhancements as much as approximately 1010 can be achieved by putting the sample onto a suitable surface. This technique is applicable for the analysis of chemical substances or single cells, but not for large tissue samples. Additionally, nanoparticles and compounds that exhibit strong SERS signals were employed as alternatives to fluorescent or colorimetric markers. In this context, spectroscopy is not used to reproduce tissue properties but to detect and reproduce the distribution of experimentally introduced compounds in a sample. This can be used for the detection and research of cancer and other diseases to visualize the distribution of known markers detected by classical immunohistochemistry [72]. Another enhancement technique is resonance Raman spectroscopy. If the energy of the beam of the exciting laser approaches the optical band gap of a tissue constituent, selected by appropriate tuning of the excitation wavelength, the amplification of the Raman signal takes place. The Raman signal intensity is increased around 1000-fold and the resulting Raman spectrum is dominated by the bands of the resonance-enhanced molecule. So, detection of specific compounds at very low molecular concentrations, such as NADH, flavins, collagens, carotenoid, elastin and the heme proteins can be done [73].

Some Specific Applications

Detection of Pediatric brain tumor

The utility of diffuse reflectance spectroscopy to discriminate intraoperatively between pediatric tumors and normal parenchyma of brain at the edge of resection cavities was evaluated using an in vivo human experiment. Diffuse reflectance spectra were obtained from normal and tumorous brain areas of 12 pediatric patients during their tumor resection procedures, using a spectroscopic system with a hand-held optical probe. Statistical methods were used and the results showed that diffuse reflectance spectral intensities between 600 and 800 nm are effective in terms of differentiating normal cortex from pediatric brain tumors. Furthermore, probe movements induce large variations in spectral intensities between 400 and 600 nm [74].

Infiltrating Tumor Margin (ITM) in Brain

Intraoperative identification of brain tumor margins using optical spectroscopy was studied in a pilot clinical trial of brain tumor of 26 patients. Diffuse reflectance and autofluorescence spectroscopy was used for identification of brain tumors and infiltrating tumor margins (ITM). Using autofluorescence and diffuse reflectance at 460 and 625 nm wavelengths, a two-steps empirical discrimination algorithm was constructed with 100% of sensitivity and 76% of specificity in discriminating of ITM and normal tissues of brain. The contamination of blood was the main difficulties that decrease the brain tumor demarcation accuracy using optical spectroscopy. Generally, this study indicates the feasibility of optical spectroscopy to conduct the resection of brain tumor intraoperatively with great sensitivity [2].

Many attempts at the use of optical systems in surgical resection of gliomas have relied upon the introduction of an exogenous fluorophore such as 5-aminolevulonic acid (5-ALA). Where the blood-brain barrier (BBB) breakdown has happened, 5-ALA is got by gliomas but not in normal brain [75, 76]. As a preliminary step of using optical spectroscopy, one type of portable, handheld system has been constructed and employed recently which can be used to obtain spectroscopic data quickly, and nearly real-time, in the operating room [37]. In one clinical trial for gliomas, spectral data of 24 patients with glioma and 11 patients with mesial temporal lobe epilepsy were acquired, in whom the pathologically-normal temporal cortex was used as control brain tissue. Results of the combination of diffuse reflectance and fluorescence spectroscopy obtained 80% sensitivity and 89% specificity in distinguishing solid tumor from normal tissues. Furthermore, infiltrating tumor margins were discriminated from normal tissues with 94%

sensitivity and 93% specificity [77]. These results suggest that it may be possible to develop an "optical biopsy" tool. A new optical spectroscopy probe compatible with a biopsy needle has been designed and has completed preclinical testing. A clinical trial is underway to examine the efficacy of the smaller probe as a surgical adjunct in stereotactic brain biopsy. It may be possible for these tools to prepare the surgeon with near real time feedback on the adjacency of tumor remnants. These techniques may develop the percentage of resected tumor, in the way that has been suggested in preliminary studies with 5-ALA described above. Additional advantages of these systems are that they are portable, fast, and easily put into the operative field. These inexpensive systems can reduce the need for more costly surgical adjuncts including intra-operative MR (iMR) and multiple frozen section tissue analyses [37].

Optical Spectroscopy of Radiation Necrosis

A common clinical problem in patients with malignant gliomas who have been under intensive adjuvant therapy is the development of new masses [78]. The lesions may demonstrate the recurrent of tumor, radiation necrosis, or a combination of tumor and radiation injury. Existing imaging methods including positron emission tomography (PET) and magnetic resonance spectroscopy (MRS) are often unable to discriminate recurrent tumor from radiation necrosis [79, 80]. Therefore, tissue biopsy stays the gold standard for clinical decision-making. Unfortunately, even needle biopsies of new areas of contrast enhancement are confounding due to difficulties related to sampling error [81]. Recently, a novel spectral feature was encountered in patients with radiation injury and radiation necrosis [82]. A shift of the predominant spectral peak, the fluorescence peak at 460 nm, 40 nm to the right has been identified as a hallmark of radiation injury to the central nervous system. A special peak has only been observed in patients with prior radiotherapy. Absence of this peak has a 94% positive predictive value in ruling out radiation injury of the cerebrum. These spectral characteristics may be exploited to improve the yield of stereotactic biopsies.

Optical Spectroscopy for Neuro-Navigation

Optical spectroscopy can also be applied for identification of specific brain structures, such as nuclei, which can be important in neuro-navigation. Diffuse reflectance and fluorescence spectra were obtained from cat brain in vivo to identify the optical and fluorescence characteristics of various anatomical components encountered on a trajectory from cortex through midbrain. A representative two-dimensional plot of a set of diffuse reflectance and fluorescence spectra was recorded from a single interrogation path. By comparison, it can be found that a depth-dependence in intensities and line-shape variations in fluorescence and diffuse reflectance spectra exist, which correlate with the anatomical structural encountered along the interrogation pathway [37].

In addition, spectroscopic techniques have been used in vivo to detect the excitatory amino acids glutamate and aspartate [83, 84]. Several neurotransmitters and precursors are endogenous fluorophores. Fluorescence can detect the characteristics of dopamine and other metabolites in brain and tumor, which may have utility in guiding surgical navigation, through metabolite detection as well as by assessing the effects of therapy, especially if certain metabolites, neurotransmitters, and endogenous fluorophores are produced in response to anti-tumoral agents [37].

Cell and Tissue Viability

Nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate [NAD(P)H] has long been considered as a dominant fluorophore of tissue, with an emission wavelength peak at 450-470 nm. Optical measurements of NAD(P)H have been used for decades to interrogate cellular physiology due to its primary role in oxidative phosphorylation and aerobic respiration [85]. In addition, NAD(P)H is involved in nucleotide donation for DNA repair and is consumed during active cell death during poly (ADP-ribose) polymerase (PARP) cleavage [86].

Optical Spectroscopic methods may be useful to rapidly assess the utility of promising agents or novel techniques in the treatment of neurological disorders. For example, an in vivo fluorescence spectroscopy probe system that can be implanted to monitor relative NAD(P)H levels during therapeutic interventions have been developed [37]. Continuous or intermittent monitoring could allow the identification, in near-real time (within minutes to hours, instead of days to weeks) of response to a therapeutic intervention. In animal models of tumors, for example, if there were no change in the NAD(P)H levels, it would be likely that this therapy was not producing cellular killing and would likely be ineffective.

Using the example of malignant brain tumors, a patient is usually treated with chemotherapy for 2-3 months prior to obtaining a MRI to assess the utility of treatment. If

the tumor has grown, the agent is abandoned and another chemotherapeutic regimen begun. Using a minimally-invasive optical spectroscopy device, information regarding the efficacy of chemotherapy as measured by NAD(P)H autofluorescence could be achieved within a few hours or days rather than awaiting the next MRI, several months later. Thus, optical spectroscopy could become an effective means of identifying useful chemotherapies tailored to individual responses [37].

Conclusions

Optical spectroscopic methods have been reviewed as a useful in vitro/in vivo method in the neuro-oncology science. Some specific applications including detection of glioma tumor margins, identification of cerebral radio-necrosis, and assessment of tissue viability, are also discussed. As a future prospective application, Optical spectroscopy has the ability to be a useful method for monitoring the therapeutic response in a variety of neurological conditions that rely upon cell killing (neoplasia) or tissue survival (stroke, neurodegenerative diseases). Furthermore, optical spectroscopy has the potential to track a new generation of fluorophores for surgical navigation, monitoring delivery of therapeutic agents, and pharmacokinetic studies. These developments may permit the rapid development of commercial optical spectroscopic tools useful for neuro-oncology in a variety of clinical applications. However, there is a need for continual improvement of optical spectroscopic instrumentation in order to accomplish at the necessary level for clinical applications.

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