

Optical coherence tomography as a promising imaging tool for brain investigations

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Abstract

This paper will review the newest results and directions for the usage of optical coherence tomography as an imaging tool for brain studies, focusing mostly on a rodent model. Together with state of the art in the field, based on some of the most recent work, this paper will include a brief look on some results obtained by our group. Brain injuries and stroke data obtained by optical coherence tomography analyzing will be presented as a possibility of detection and evaluation for affected tissue, using this imaging system.

Keywords: optical coherence tomography, traumatic brain injuries, ischemic stroke, rodent model.

Introduction

Optical coherence tomography (OCT) is an optical method for imaging on micron resolution range [1–3]. The method could be considered similar with B-mode ultrasound detection design but using light instead of ultrasounds [1]. Nevertheless, because of the high-speed value of light, simple measuring reflected intensity is not possible. Instead, in such an experiment, the suitable signal is obtained by using interference properties of light. There are several types of experimental set-ups systems, which are used now days for OCT investigations [4]. Historically, first experiments were carrying up using a time domain OCT (TD-OCT) design. This assumes usage of a Michelson interferometer with a moving mirror and a low coherence source of light. The analyzed sample is placed on one arm of the interferometer and, as long as the difference between the optical path for reference and sample arm is inside the coherence length of the light source, interference can occurs. Controlling the position of reference mirror allows the system to image different slices of the sample. Consequently, by analyzing the obtained interferogram (interference fringes) is possible to picture the investigated tissue with a resolution (axial) given by [1, 4]:

$$l_c = \frac{2 \ln(2)}{\pi} \frac{\lambda_0^2}{\Delta\lambda}$$

where λ_0 and $\Delta\lambda$ are the central wavelength and the bandwidth respectively; for holding inside this formula,

one should assumes that an incoming beam travels through air and has a Gaussian shape. Considering values of 50–60 nm for bandwidth and a central wavelength of about 1 μm one can observe that resolution stay in the range of microns as already stated. Such systems have a lateral resolution that is controlled by the numerical aperture of the lenses used to focus the optical radiation on the sample [4] and thus there is no direct connection between lateral and axial resolution.

Beside time domain technique, Fourier domain (FD-OCT) methods were also developed. Those methods use Fourier transformation of the spectrum in order to depict dependence between backscattering signal and sample depth [4–6]. This includes usually spectral domain (SD) and swept source (SS) OCT [4, 6, 7]. For these two types of investigations, a different set-up is used. In the case of SD-OCT, instead of a moving mirror in the reference arm a Fourier transformation on the received interferogram is performed. The obtained signal is capture using either a single detector and a dispersive element or a CCD type detector and will depict sample depth dependence. SS-OCT is a method which uses instead of the classical low coherence source a swept laser [8]. The advantage of these two methods over the TD OCT is the lack of moving parts, which allows a much faster scan of the target sample, while both axial and lateral resolution shows no significant differences from TD-OCT depending (almost) on the same parameters [6]. All OCT types of set-ups are able to perform investigations on living tissue with several main advantages: a high-resolution compares with

the classical methods (ultrasound, CT, MRI), contactless and noninvasive imaging due to usage of infrared radiation (low power; in the range of 800 nm or 1300 nm). Those advantages could focus OCT investigations towards desired optical biopsies, especially for cases where physical biopsies are not easy to be performed or because the procedure reveals dangers for the patient.

Taking into considerations these arguments, brain can be considered a good candidate for OCT investigations, especially due to the impossibility of physical biopsies and because it is situated very close to the skin surface. Brain is affected by an array of diseases and we are just starting to understand some underlying mechanisms behind the physiological aspects that they have on our life. While this is true for all of them, no other brain diseases affect more our quality of life as stroke, especially in elderly and traumatic brain injuries on younger patients. As such, we have focused our OCT research towards this two.

Stroke represents an acute brain injury due to sudden drop (at least 20%) in blood flow in one cortical area (ischemic stroke) or by cerebral blood vessel disruption causing hemorrhage (hemorrhagic stroke) [9, 10]. Stroke is the third most frequent cause of death in developed countries [11, 12] but also a major cause of disability, especially in elder populations.

On the other hand, traumatic brain injuries (TBI) represent a subgroup of head injuries that usually involved younger populations [13]. Nevertheless, during the last decades, as population aged, this classic pattern of TBI has shifted regarding incidence and localization, raising socio-economic costs [14].

While stroke has a linear evolution, TBI has a more unpredictable evolution, often evoking cerebral damage even after a long period of time although both have similar blood vessel and cellular damage [14].

In the last five decades, a large variety of experimental ischemic and TBI models were developed, trying to replicate effects of injuries on neural tissue with the purpose of developing new diagnostic and treatment methods, including electrophysiological and imaging methods [15].

Under these considerations, our group is oriented towards testing if OCT can be used as a detection imaging method for brain injuries, and to establish an experimental frame that will allow investigators to objectively evaluate occurring changes within the affected tissue (on a rodent model) after either TBI or stroke occurs.

☞ OCT applications

Use of OCT was first developed for imaging investigations in the field of ophthalmology and, early studies showed promising results in depiction of eye structure [2, 16]. In the present, researchers are extensively looking to expand the use of OCT in a variety of pathologies varying from detection and management of glaucoma [17, 18] up to monitoring and understanding the evolution of retinal degeneration using rodent models looking also at correlations between histological findings and OCT imaging [5, 19].

Optical coherence tomography was also use in dermatology, for monitoring healing of different types

of wound up to tumor diagnosis and sun burning effects [4, 6, 20].

More recently, with the developed of technology, OCT became a very efficient tool towards the desired task of optical biopsy [21]. Developing laser sources, as well as adaptive optic systems, special designed catheters and improvements in Doppler modules has allowed development towards other medical fields as gastroenterology [22] and cardiovascular investigations [22–24]. In gastric tract studies, OCT investigations have evolve from depiction and analyses of gastrointestinal layer structure [24–26] up to investigations of different grades of dysplasia [27] or towards putting into evidence intestinal metaplasia at squamo-columnar junction by almost endoscopic accuracy [28].

Because of their importance in determining the risk of cardiovascular events, characterization of atherosclerotic plaques is starting to have a greater and more profound impact in guiding arterial intervention. By using OCT criteria, some authors have showed that atherosclerotic plaques can be discriminated *in vitro* with a high degree of sensitivity and specificity [29], while others could not find the same degree of discrimination in using OCT for plaque characterization [30].

In brain investigations, OCT was used for the first in neurosurgery, to image and detect brain tumors by measuring the different optical backscattering of tumoral and normal cortex tissue [29]. It has been showed that using OCT during stereotactic surgery could depict valid data on micron scale, providing specific information about spatial relations between adjacent vessels real-time 3D visualization [4, 30, 31]. *In vivo* imaging of myelin sheath on living rodents using a special deep optical coherence microscopy method has allows quantitative comparison of normal and myelination deficit opening the possibility for myelin chronic imaging in demyelinating diseases and for minimally invasive medical diagnosis [32]. Some experimental studies have evidenced the possibility of measuring the density of individual myelinated fibers by using an OCT system [33], while other studies have use this imaging technique to evaluate cortical plasticity *in vivo* in adult mice [34].

In the last years, OCT research was orientated towards functional investigations using rat models [35–37]. These included evaluating the response of microvascularisation to establish neuronal activity [37] through metabolic and hemodynamic responses to brain activation [36] and also evaluating effects of stroke on structural and hemodynamic changes of the brain in a rodent model [38].

Additionally imaging retinal nerve fiber layer using OCT proved to be a good detection tool in degenerative disorders, including multiple sclerosis [39]. Along or complementary to optical intrinsic signal imaging (OISI), diffuse and two-photon microscopy the trend is to establish a core of optical, high-resolution noninvasive methods for optical biopsy.

Our research is following on this tendency, aiming to picture and characterize brain tissue following stroke or TBI by using optical coherence tomography, in order to outline a proper non-invasive description.

While the majority of experimental studies on stroke and/or TBI have been performed on young animals, the

incidence in humans of both lesions is higher in aged populations. From this reason, we have considered that models using both aged and young animals are more appropriate [40] and will closely replicate effects of injuries on neuronal tissue in aged humans [41–43].

A number of experimental stroke models using both rats and mice exist that are suitable with clinical observations, including global or focal ischemia, with or without craniotomy [44, 45]. Generally, most of the researchers use a focal stroke model, performed also by members of our group, using permanent or transient transcranial middle cerebral artery occlusion, with both internal carotid arteries transient simultaneous occlusion [46, 47]. While just a few stroke models exist, TBI are induced using a large number of experimental models, one of the issues being the heterogeneous conditions that it evokes within the brain, causing both focal and diffused injuries. Cellular death following TBI does not occur only as a direct mechanical event but it can also be initiated due to subsequent inflammation, excitotoxicity and oxidative stress [14].

Most TBI models involve either a focal or diffuse injury. While focal models, on which we are focused, can provide precise information on acute evolution of tissue scarring, diffuse injuries focuses more on long-term effects and secondary injuries cascades [48].

Imaging has completely change the management of brain lesions patients, both ischemic stroke and TBI being no exceptions.

With the development of new diagnostic imaging techniques, such as structural imaging with CT and MRI, the way we look at patients has radically change. Functional neuroimaging techniques like PET and perfusion/diffusion MR, which allows imaging of brain physiology, allowed researchers to better understand the dynamic and evolving process thus implementing better therapy [49].

All experiments performed by our group were conducted in accordance with statement regarding both care and use of animals and were approved by the Ethics Research Committee of the University of Medicine and Pharmacy of Craiova, Romania. Experiments were carried out on young and old rats, kept under normal laboratory conditions in the Animal Facility Building of the University of Medicine and Pharmacy of Craiova.

We induce cerebral infarction by transcranial reversible interruption of blood flow (90 minutes) in the right middle cerebral artery using a tungsten hook, as previously described [46]. TBI experimental models were performed through a short craniotomy (0.5 mm/2 mm) on the right part of the skull, parallel with sagittal suture, 1 mm lateral of it, and 1 mm anterior of lambda, with a 2.5 mm deep scalpel, similar with the common models used by others [46]. Throughout both types of surgery, anesthesia was induced and maintained by an injectable anesthetic cocktail (Ketamine/Xylazine).

From each animal, the whole brain was collected and placed in a 4% fresh depolymerized paraformaldehyde for five days and then subjected to OCT investigation.

For imaging, an OCT system from THORLABS (OCT1300SS) was used. The set-up is a SS-OCT having as a source a swept laser with central wavelength of 1325 nm and an average power of 12 mW. The device

allows 2D and 3D scans (with an A-scan rate of 16 kHz). Resolution (axial and lateral) is 12 μm and 15 μm respectively. The power on sample is 5 mW. Also, a Doppler module is available. The system is capable of an acquisition rate of 50 frames per second. An extensive description of the system was given elsewhere [23].

As already stated [38, 50] changes in the refractive index of damage tissue are occurring generating modifications in the scattered received signal by the OCT, which is representing a fair indicator of the status of the injure degree. By using different mathematical models [38, 50, 51], it is possible to establish parameters which may provide information about the extent and degree of the lesion.

Following this tendency, our measurements are also indicating a clear distinction between different regions inside the rodent brain, after both stroke and TBI but on different extent (Figures 1 and 2).

As both stroke and traumatic injury cause difference in brain tissue reflectance detected by optical coherence tomography, it is very likely that the molecular and cellular changes that generate these abnormalities are different in the two models. While in TBI the time difference between lesion and imaging did not allow for massive scarring formation, cell migration and network remodeling, in our stroke injury model one can clearly see difference in OCT image parameters, like penetration depth, due probably to cell death and cell migration. Furthermore, water density can also play an important role in tissue reflectance. While in the acute phase of a lesion tissue edema is possible, in a more chronic stage of scarring, water retention in the affected tissue is less likely to appear.

➤ Conclusions and future perspectives

Considering all the above results one may say that OCT represents a promising, fast developing method, which due to its characteristics like resolution in micrometer range, noninvasive, high acquisition speed, fills the gap between already classical imaging investigations (MRI and ultrasounds) and new subcellular resolution methods like two-photons microscopy. Obtained results in brain studies are promising and generate potential for qualitative and quantitative characterization; in brain injury models, depicting both structural changes and functional description of the affected tissue.

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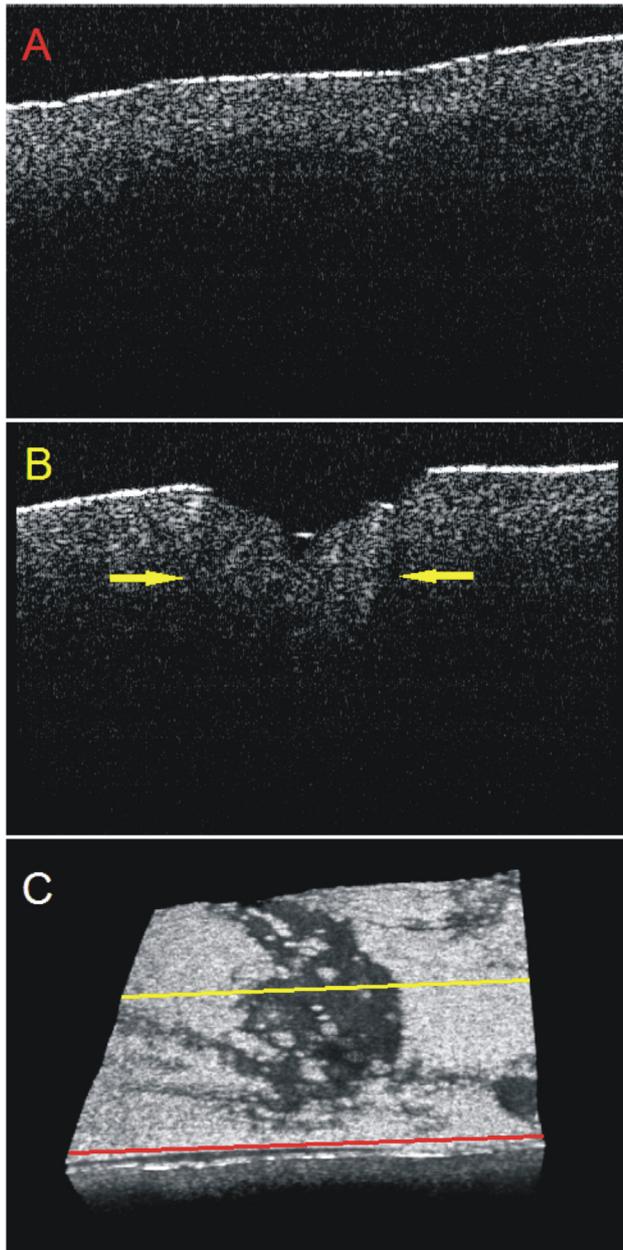


Figure 1 – Optical coherence tomography images of a rat brain, 12 hours after stab injury. Normal brain tissue (A) and affected one by traumatic lesion (B, between arrows). The 3D reconstruction shows in more detail the distinction between normal (red line) and injured tissue (yellow line) (C).

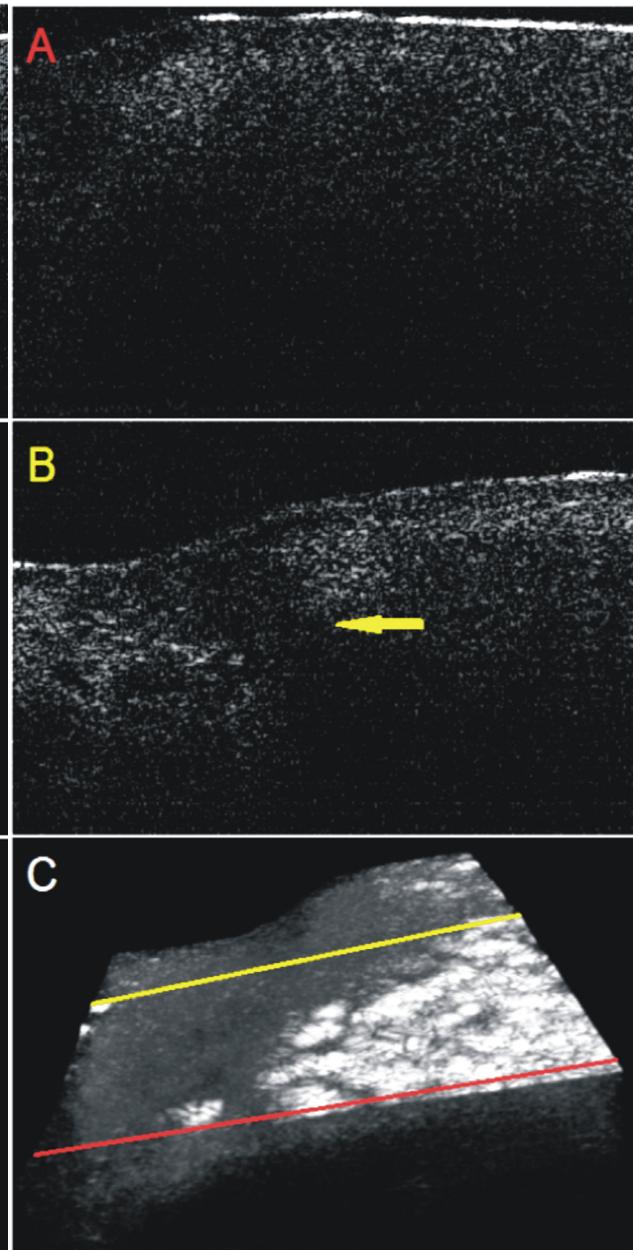


Figure 2 – Optical coherence tomography images of a rat brain, 28 days after middle cerebral artery occlusion induced stroke. Normal brain tissue (A) and necrotic brain tissue (B, starting from arrow, left side). The 3D reconstruction reveals details of both normal (red line) and scarred (yellow line) cortex (C).

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