REVIEW ARTICLE

In vivo Near Infrared Spectroscopy: a novel approach for simultaneously estimating molecules and hemodynamic parameters in the human and rat brain: a review

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Abstract

There have been great advances in optical brain imaging over the last 50 years and the technique has grown into a richly diverse field. *In vivo* recording and imaging using light provides extraordinary sensitivity to functional changes through intrinsic contrast, blood, and can even exploit the growing availability of exogenous optical contrast agents. Light can be used to analyze microscopic structures and function *in vivo* in the exposed animal brain, while also allowing noninvasive imaging of hemodynamics and metabolism in a clinical setting. This review is an overview of approaches that have been applied *in vivo* optical brain recording, in both animals and humans. The basic principles of each technique are described, emphasizing the techniques used in our laboratory.

Techniques include imaging of exposed cortex, *in vivo* functional spectroscopy of the living brain using optic fibers, and the broad range of noninvasive topography and tomography approaches to near-infrared imaging of the human brain. The basic principles of each technique are described, followed by examples of current applications to cutting-edge neuroscience research. In summary, it is shown that optical brain recording continues to grow and evolve, embracing new technologies and advancing to address ever more complex and important neuroscientific questions.

KEY WORDS: Spectroscopy, optical imaging; two-photon microscopy; near-infrared spectroscopy; diffuse optical tomography; neuroimaging; neurovascular coupling

1. Introduction

The interaction of light in tissue to recognize disease has been widely researched since the mid-19th century when Joseph von Fraunhofer developed diffraction grating. A large number of scientists have brought optical spectroscopy forward and enabled it to become a precise and quantitative scientific technology.

In 1963, when Franz F. Jöbsis published a new optical method in an original article(1), near-infrared spectroscopy (NIRS) was seen as the technique which could deliver a solution to a clinical need. In 1977, this author demonstrated the possibility of detecting changes in adult cortical oxygenation during hyperventilation(2). NIRS has become an established research and clinical tool for measuring changes in cerebral oxygenation, in particular, changes in oxygenated (HbO_2) and deoxygenated (HbR) hemoglobin concentration.

The technology has gained interest in the medical field in numerous biomedical applications for its advantages over existing conventional techniques. Optical spectroscopy at infrared and visible wavelengths avoids the use of ionizing radiation, is non-destructive, utilizes relatively inexpensive equipment, and can be performed near real-time without pharmaceutical means to enhance contrast, i.e., contrast agents.

Different optical recording techniques, both in visible and near infrared, have been used in animal experimentation. One of the most widely used techniques has been the exposed-cortex imaging; its use in animal studies has been widespread. Although it has been widely used by many groups, optical imaging in experimental animals has been only one step towards the study of imaging in clinical diagnosis and an excellent tool to learn much more about the basic mechanisms of brain function both in physiology and in pathology. These results can be useful to help the development of new drugs and treatments. These studies can also contribute to the interpretation and better comprehension of results from other imaging modalities such as functional magnetic resonance imaging (fMRI) or positron emission tomography (PET). Some of these applications of animal imaging have included studies of Alzheimer's disease(3), stroke(4), epilepsy(5) and published by our group, the mechanisms of neurovascular coupling(6).

The obvious advantage of optical imaging over other modalities is its reduced cost and infrastructure requirements (such as shielded rooms, synchrotrons etc...).

This review describes a selection of optical approaches to detect functional brain activity. The basic principles of each technique are described, highlighting the techniques used in our laboratory, 1) Invasive optical brain techniques, including: a) optical techniques for exposed cortex imaging, b) recording functional activity using optic fibers, 2) noninvasive clinical optical imaging of the living brain.

2. Invasive optical brain techniques.

2.1. Optical techniques for exposed-cortex imaging.

The exposed-cortex imaging in animals rather than humans provides significantly more flexibility, since preparations can be much better controlled and all types of experiments can be systematically compared. Extrinsic dyes and cross-validation techniques such as voltammetry, amperometry or electrophysiology can even be used simultaneously(6).

This technique therefore also offers significant technical advantages for small animals, allowing higher resolution imaging and improved sensitivity. The cortex can be

surgically exposed to obtain high resolution imaging, allowing direct optical imaging of the brain's surface with only minimal disturbance to brain activity. Exposed cortex is highly accessible, and most commonly performed in experimental animals, although it has also been achieved on the intra operatory human brain(7)(8).

In the neuroscience literature, the exposed-cortex is the simplest optical imaging techniques. The most useful are, HbO_2 and HbR dynamics(6); imaging extrinsic voltage sensitive dyes(9); speckle-flow imaging(4), capable of imaging the blood flow dynamics in the superficial cortex.

Exposed-cortex imaging has been applied to an extensive range of research areas. These can be summarized as: 1. functional imaging to improve understanding of the basic mechanisms of the hemodynamic and neuronal response to stimulus, 2. functional imaging to investigate the sensory and cognitive processing functions of the brain, and 3. a study of the effects of diseases and treatments on normal brain behavior.

2.1.1. Technical procedure.

Surgical preparation for exposed-cortex recordings, the experimental subject, commonly a rat, mouse, cat or primate is anesthetized while the scalp is retracted and the skull is carefully removed from over the brain area of interest. In some cases, the skull may be carefully thinned to obtain a good vision of an area of the brain's surface, avoiding contact with the brain cortex. Many imaginative tricks have been developed to ensure that correct brain function is not affected. Most often, these techniques are performed with anesthetized animals. However, in many other cases a cranial window can be implanted in the animal to perform chronic optical studies.

While the techniques described above make it possible to obtain information from the cortex (2D-imaging), there is the possibility of recording images with tomographic information (3D) using Optical Coherence Tomography (OCT)(10) of functional brain activation, Laminar Optical Tomography(LOT)(11), Fluorescence Lifetime Imaging and Microscopy (FLIM)(12) and Two-Photon Tomography(13)(14).

Brain *in vivo* imaging for research using Two-Photon Tomography has also found applications in areas of functional mechanisms, functional processing and pathology/treatment research(13)(14). These applications exploit a variety of methods to introduce fluorescent contrast into the brain, including intravenous injection of dextran-conjugated dyes to show blood vessels, topical application, or pressure injection of dyes into the cortex, transgenic mutation of cells to express fluorescent proteins and systemic delivery of dyes.

2.2. Recording functional activity using optic fibers.

We will now review fiber optic probe scattering spectroscopy of turbid tissues using visible and infrared light. A spectroscopic system incorporates a light source, an optical analyzer with a detector, and a light transport conduit, which, in many cases, is made of optical fibers, figure 1. The excitation or illumination light source is usually a laser or a white light source, such as a xenon or incandescent lamp. The coupling optics adapts the f-number of the light source to the numerical aperture of the fiber and guarantees optimal irradiance into the fiber.

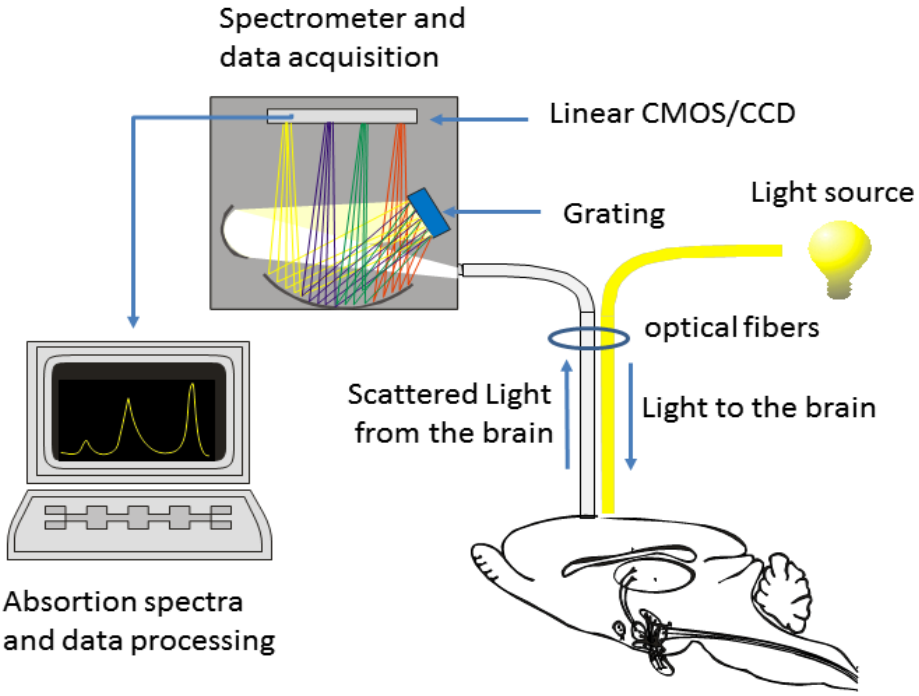

Figure 1. Fiber optic-based spectroscopy system, with separate illumination (excitation source, LED or incandescent light bulb). Optical elements couple the excitation light into the flexible probe, a probe collects the emitted light, coupling optics adapt the numerical aperture of the probe to the miniature spectrometer and an optical detector (CCD, or CMOS linear element) is read out and digitized.

Single fiber solutions are used and well-aligned coupling optics to achieve the smallest probe diameters. Single-fiber solutions are the most commonly used because of the small diameter 50-100µm and the fact that single fiber-based probes require a minimal amount of components for the probe and can be used to create the smallest illumination spots as well as having excellent light collection efficiency. The simplest way to setup is Y-shaped assemblies with two fibers of the same diameter side-by-side in the common end, which then diverges into two separate legs. The fibers in the assembly may be UV-VIS, VIS-NIR or one of each in a mixed bifurcated assembly(15). See figure 2 for more details.

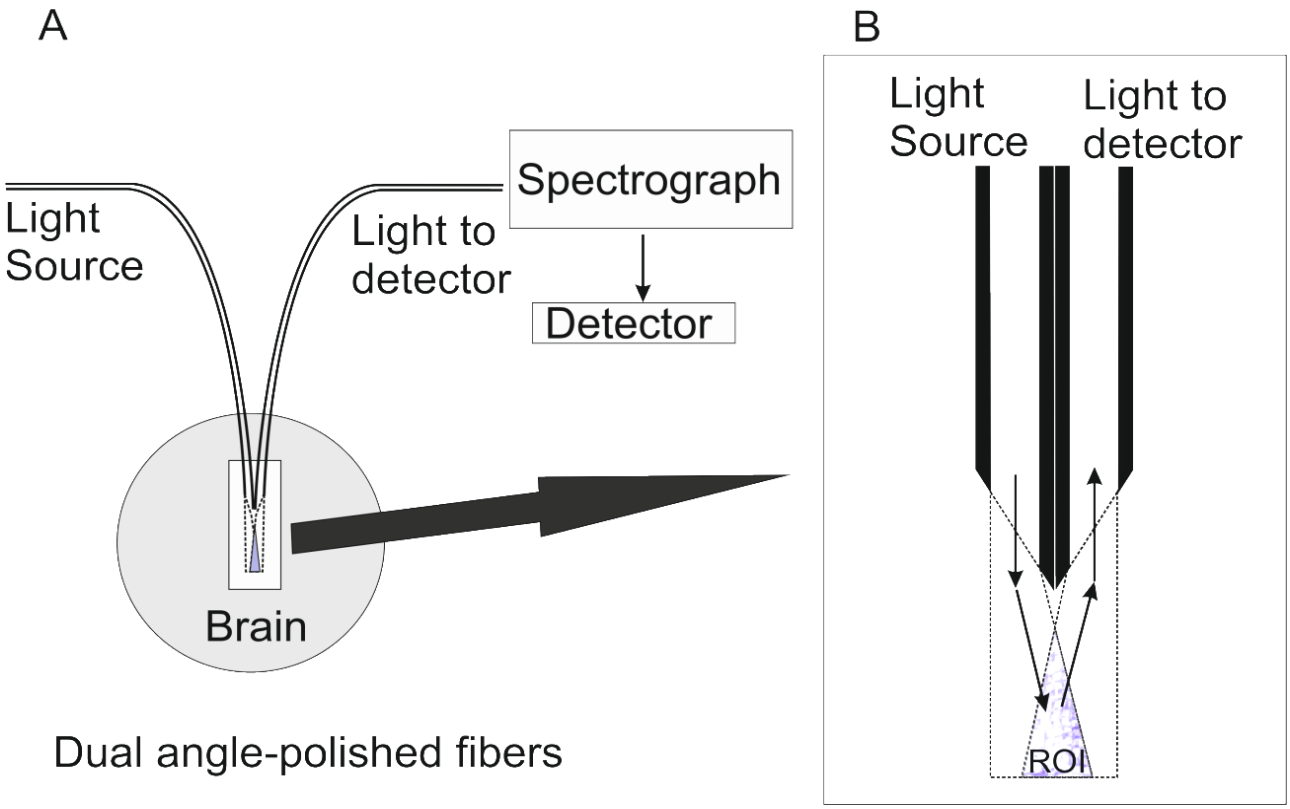

Figure 2. A. Y-shaped assembly with two fibers of the same diameter side-by-side in the common end implanted in brain, which then diverge into two separate legs. B. Zoom of distalendtips of fiber optic in dual angle-polished fibers configuration. The arrangement of the fibers and their angle of polishing try to prevent damage to the cerebral parenchymaas much as possible.

Another of the additional advantages of simultaneously using optical fibers is the possibility of using other neurotechniques (voltammetry(6), amperometry(16), electro-physiology(17), microdialysis(18), etc.), that complement the obtained information. The optical registration techniques do not generate interferences or electronic noise that could alter the results.

As an example of what was said above, using microdialysis and fNIRS, we found that intracerebral infusions of amphetamine increase the extracellular concentration of glutamate, dopamine, aspartate, GABA, and taurine. This study(18) also shows that an alpha-noradrenergic receptor antagonist is able to attenuate the effects of amphetamine on the release of glutamate, dopamine, GABA and taurine, which further suggests a vasoconstrictor effect of amphetamine as a result of which hypoxia could develop.

2.2.1. In vivo spectroscopy: for simultaneously estimating nitric oxide and hemodynamic parameters

Nitric oxide (NO) is a well-known signaling molecule involved in a wide range of biological processes. Under physiological conditions, NO reacts with HbO_2 to form methemoglobin (MetHb) at a very high rate. Microdialysis studies have used hemoglobin solutions as a trapping method to quantify NO *in vivo*. The methodology described here uses the microcapillary network (capillary bed) with endogenous

HbO_2 instead of a microdialysis probe with exogenous HbO_2 for monitoring MetHb as an indirect index of NO levels by *in vivo* spectroscopy using optical fibers.

This method has been validated using in vivo voltammetry and selective NO microelectrodes. We have used in vivo local infusion of NO into the tissue surrounding the probe (optodes) in both methods, NOS inhibitors to decrease the NO production and local infusion of NMDA agonists to increase NO production in the cerebral cortex. Thus, the association between in vivo voltammetry and in vivo spectroscopy as we have described for our group could be very advantageous, because by using both methodologies it is possible to measure the NO directly in the extracellular fluid (voltammetry) and its deactivation by its principal in vivo scavenger (spectroscopy). Moreover, the latter technique makes simultaneous measurements of hemodynamic parameters such as oxygenation rate and blood volume (cerebral blood flow) possible, see figure 3.

NO is extremely unstable *in vivo* and its half-life has been estimated as a few seconds(18). In accordance with its role as a paracrine mediator, NO can travel to reach target cells in neighboring areas of the NO-generating cell. During the paracrine migration, in particular at high concentrations, this reactive molecule can interact with molecular oxygen to form higher nitrogen oxides (e.g. NO_2 andN_2O_3), which can either react with other biomolecules such as thiols and amines or be hydrolyzed to nitrite (NO_2^-) and nitrate (NO_3^-)(16). However, the most important reactions are with ferrous hemoproteins and especially with hemoglobin (Hb)(19), (figure 3A), such as those which yield nitrosylhemoglobin or methemoglobin. Nitrosylhemoglobin formation has a very low rate(16), whereas the interaction with HbO_2 is characterized by a very high rate even under saturating oxygen concentrations, and it has been estimated to be at least 26 times faster than the auto-oxidation of NO in aqueous solution. Thus, MetHb levels are proportional to the NO concentration and they can be used as an indirect index of NO (20)(21)(22), as we can observe when this technique is compared with another technique, see figure 3, C and D.

Many results indicate that this spectroscopy technique is able to record large increases in MetHb levels and to detect reductions of its basal levels(16)(17)(23)(24)(22). In addition, data show that similar changes and kinetics can be observed with both techniques. Thus, intravascular MetHb can be used as an indirect index of NO levels. It is proposed that *in vivo* spectroscopy may be a useful tool to gain insight into the roles of NO in hemodynamic parameters and in other physiological processes such as the regulation of the mitochondrial respiratory chain(18)(16).

Finally, this technique offers the possibility of monitoring the neuronal activity, bearing in mind that it is widely accepted that changes or alterations in regional cerebral blood flow and cerebral oxygen consumption rate can be used as an index of neuronal activity. Other groups have made interesting contributions using our method(23)(24).

3. Non-invasive optical imaging of the human brain

Functional near infrared spectroscopy (fNIRS) commonly used the topography approach in brain research covering different fields such as physiology (25), psychiatry (26), alterations in disease (27), and its application in the brain computer interface (BCI) (28),in neuroimaging studies has recently been developed. However, the method has some

disadvantages as the relative positions of the measurement channels to brain anatomy vary between subjects or fNIRS measurements in cortical areas are always affected by the hemodynamic changes in the scalp layer affecting the interpretation of results in cortical activity(29)(30).

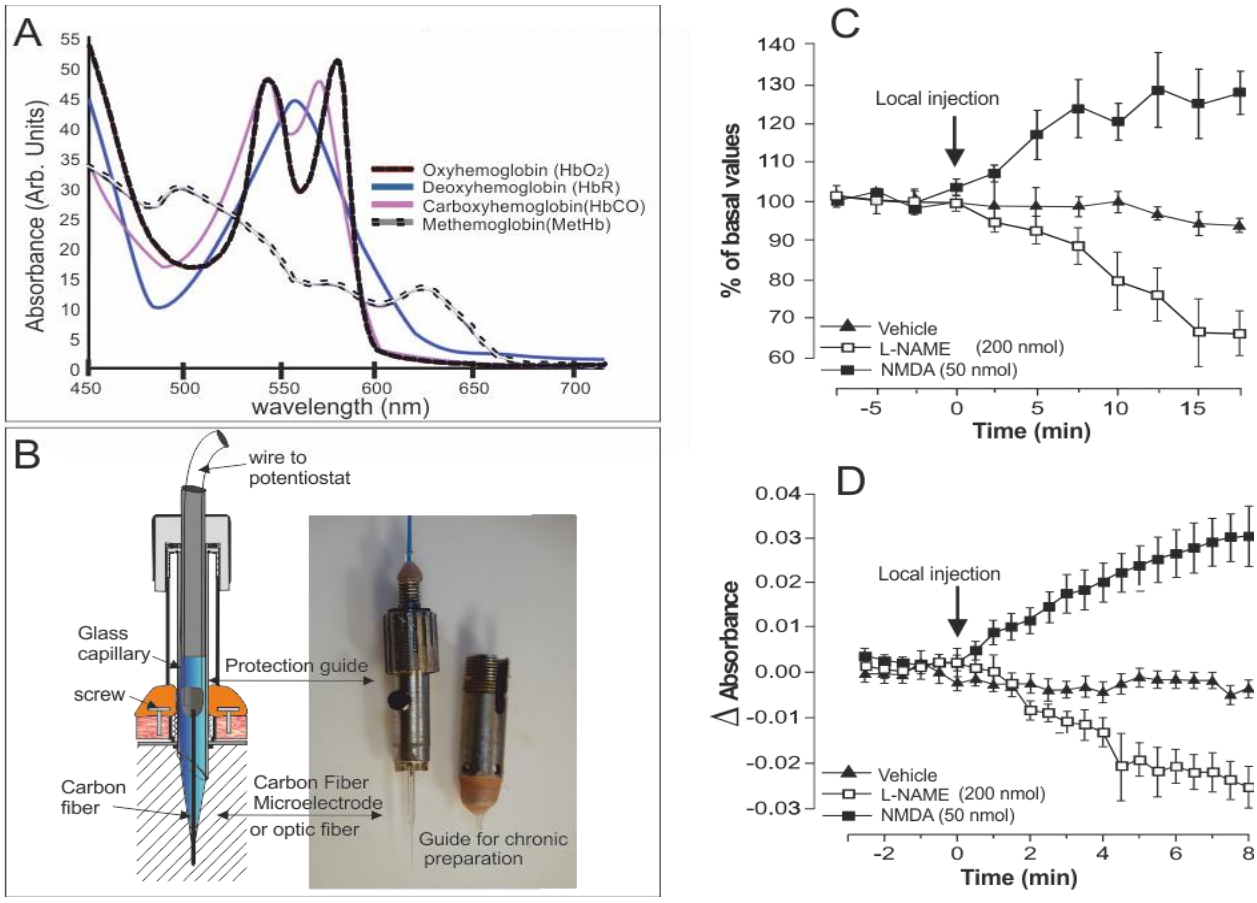

Figure 3. A, Absorbance in arbitrary units of oxyhemoglobin (HbO2), deoxyhemoglobin (HbR), carboxyhemoglobin (HbCO) and methemoglobin (MethHb) as a function of wavelength. B, Mechanical device for selective microelectrode brain implantation for nitric oxide (NO) and fiber optics. Schematic drawing and photography. C and D, Kinetic effects of the manipulation of NO synthesis on MetHb band and free NO levels in rat cerebral cortex determined by *in vivo* spectroscopy (D) and *in vivo* voltammetry (C), respectively. Absorbance values at 635 nm (A) were averaged every 30 s (scan rate: 40 spectra min^{-1}. Voltammetric peaks of NO (oxidation peak at approximately 650 mV) were recorded every 2 min and percentages of modification from basal values were calculated. Data represent mean ±SEM (n = 5-8 rats per group) and arrows indicate the infusion of drugs close to the microsensor (see publication 17 for more detail) As illustrated in C and D, NO and MetHb levels were decreased by the potent NO-synthase inhibitor L-NAME, white squares, but were not modified by vehicle solution (PBS), black triangles. In addition, NO and MetHb levels were markedly enhanced by the administration of NMDA, black squares. Time scales for spectroscopic and voltammetric studies differ due to the differences in scan rate between both techniques.

3.1. Advantages of optical techniques

Functional brain imaging has provided substantial information about how dynamic neural processes are distributed in space and time. A large number of brain studies based on task or resting state imaging studies of neural networks through healthy subjects have been reported(31)(32)(33). Some imaging modalities to study brain functioning use fMRI which require costly infrastructure, while optical imaging instruments are less expensive. Moreover, technique limitations in fMRI devices such as a fixed scanner, contraindication with metal implants, scanner noise and stress associated with fear are avoided or reduced in optical imaging devices.

fMRI measures changes in the *blood oxygen level dependence* (BOLD) signal associated with hemodynamic changes after the neural activity to visualize functional changes in the brain. Although the BOLD signal has been associated to a decrease of HbR(34), an increase of BOLD signal could be associated to an increase of HbO_2(35) or a combination of both. Initially, the HbR decreases and then the HbO_2 increases due to the vasodilatation that washes the local HbR. The above controversy disappears with the use of optical imaging techniques, which measure each hemoglobin state separately (HbR & HbO_2), using at least two wavelengths to measure each hemoglobin state. Moreover, the optical imaging techniques provide more comprehensive information of hemodynamic and metabolism than the BOLD signal, due to the complicated connection of the BOLD signal to the neurovascular coupling.

Optical imaging techniques can measure changes in HbO_2, HbR and HbT at a much higher sampling rate than fMRI, and this could be a fundamental tool for the study of the neurovascular coupling in humans, especially when the neurovascular coupling is either unknown or altered. The principal advantage of fMRI measurements is that they can cover the whole brain, while optical measurements only reach the cerebral cortex because its penetration depth is around of 3-4 centimeters could anatomically reach the gyral level(36).

Finally, unlike other imaging modalities such as the PET(37) or x-ray computed tomography(38), functional brain measurements using optical imaging do not need a contrast agent, whose doses are limited in infants and could induce anaphylactic reactions in certain populations.

All these aforementioned circumstances have potentiated the use and developments of optical imaging techniques in recent years for research, diagnosis and prognostic studies.

3.2. Instrumentation

A wide variety of NIRS instruments has been created for different types of measurements, with the most common being the following: continuous wave (CW), time domain (TD) and frequency domain (FD).

- In CW measurements, the light is emitted at a constant intensity by sources into the tissue, and the same device detects the transmitted light intensities. CW uses frequency-encoded intensities to acquire data and can simultaneously measure wavelength(39) or light sources(40).
- TD uses ultrashort laser pulses to irradiate the tissue, and the light intensity detected is recorded over time to show a temporal point spread function (TPSF) with a resolution of picoseconds (41)(42).

- In FD measurements, the light source is modulated at radio frequencies (100-1000 MHz)(43), and measures the phase delay of the light detected from the tissue(44). The parameters of FD measurements are phase shift, the intensity of light (DC component) and the amplitude of the intensity oscillations (AC component) at given wavelengths and for different distances between the light source and detector. The FD instrumentation is more complex and expensive than CW systems, thus a combination of CW measurements and small frequency-domain measurements have been proposed to provide good spatial resolution and quantitative accuracy(45).

3.3. Tomography approach

The most significant improvements in optical imaging came when image reconstruction techniques were proposed in the 1990s using diffusive photons(46)(47). Diffuse optical tomography (DOT) is an fNIRS approach that transforms the detected light from different measuring distances on the surface of the head into depth information providing three-dimensional images of cerebral activations(48). DOT uses the multi-distance approach with the purpose of increasing spatial resolution and positional accuracy of optical brain imaging(49). Unlike the topography approach which directly maps the changes in optical properties from the midway between a source and detector into a 2-D image(50).

3.3.1. Image reconstruction

The **forward model** is used by DOT to model light migration processes to create functional images. The forward model relates the activity inside the head tissue with the measured light intensity changes, using the radiative transfer equation (RTE) or diffusion approximation (DA).The mathematical forward model must be implemented in computational models. There are three types of computational modeling approach, which are the following: analytical modeling, stochastic modeling and deterministic modeling.

- Analytical modeling uses Green´s function for the solution of partial differential equations such as the RTE or DA in a homogeneous semi-infinite medium(51) and simple geometries. It has been used to validate stochastic and deterministic models.

- Stochastic modeling whose distribution of optical properties is calculated by Monte Carlo simulations which model the light propagation inside a 3-D realistic head obtained from MRI scans, where heterogeneous structures are incorporated to the simulation(52). This method allows heterogeneity and a flexible shape of the medium.

- Deterministic modeling is based on the finite element method to solve the DA. FEM is capable of dealing with heterogeneity in arbitrary geometries. This is the most commonly used system in diffuse optical imaging.

The relationship between the measurement of light intensity and optical property is non-linear. However, the relationship is assumed to be linear for DOT images, and is known as the **inverse problem.** Two approaches are used to solve the inverse problem: linearization and nonlinear iterative approaches.

The linearization approach does not correctly predict changes in the optical properties, showing as results qualitative

image reconstructed of measured changes in the brain(53). This approach is good enough for neuroimaging studies, but not for clinical studies because, for it, it´s necessary to have quantitative images of hemoglobin states, whereas the nonlinear iterative approach applies an iterative optimization method to minimize the differences between the calculated and measured data of the distribution of optical properties. In addition, a Jacobian matrix or sensitivity is found as a product of both approaches, which relates the number of measurements on the surface and changes in the optical properties, and must be computed. Methods have been proposed such as the perturbation method(54) or the gradient-based method(55) to compute the Jacobian matrix

Finally, the inverse problem is ill conditioned suggesting that the reconstructed images are sensitive to noise during the measurements. Some research groups have attempted to solve this using regularization methods(56)(57) such as the use of decomposition singular values(58).

In addition, anatomical information of a subject can be a problem during the image reconstruction. The DOT technique cannot provide anatomical information making it difficult to solve the forward model, unlike fMRI or x-ray CT which provide anatomical information. In neuroimaging studies using DOT technology, the optical model is constrained to the tissue geometry through segmented MRI scans. In order to solve this problem, some research groups have proposed other methods to perform DOT studies without MRI scans such as MNI-guided DOT(59) or DOT images reconstructed on a generic head model(60).

The same problem occurs when NIRS technology is applied on other tissues such as prostate or breast. X-ray CT(61) or ultrasound(62) or MRI scans can be used to improve the image reconstruction.

3.4. Applications in functional brain imaging

Optical measurements can play a role in determining underlying brain physiology, especially when investigating the relationship between neural activity and hemodynamic changes known as neurovascular coupling from animals(63) to humans(64). fNIRS has been used in a wide variety of applications in neuroimaging studies to measure functional changes associated to a stimuli or paradigm. Some examples are listed below:

- Cognitive stimuli based on go/no-go paradigms(65). Different letters are presented on a screen for a few seconds followed by an inter-stimulus period of 1-2 seconds. On the one hand, the participants are instructed to press a button with their right index finger each time a letter appears on the screen (during the go). On the other hand, the participants are instructed to push the button for all letters except X (during the no-go). Although, the go/no-go paradigm is one of the most used in cognitive studies, some authors, as is the case of our laboratory, have used other cognitive tasks such as mental arithmetic tasks(66), see figure 4.

- Somatosensory and motor stimuli based on finger tapping(67), tactile stimulation(68) or finger flexion/extension(69) tasks are some of the most used examples in neuroimaging studies. Due to the fact that cerebral activation amplitudes are higher than the amplitudes given by other paradigms e.g. cognitive, the cerebral activations are reproducible and spatial localizations of the motor

activity are known, the motor paradigms are especially used in the new method applications such as image reconstruction algorithms(56), filtering procedures(70) or corroboration of simulated models(57).

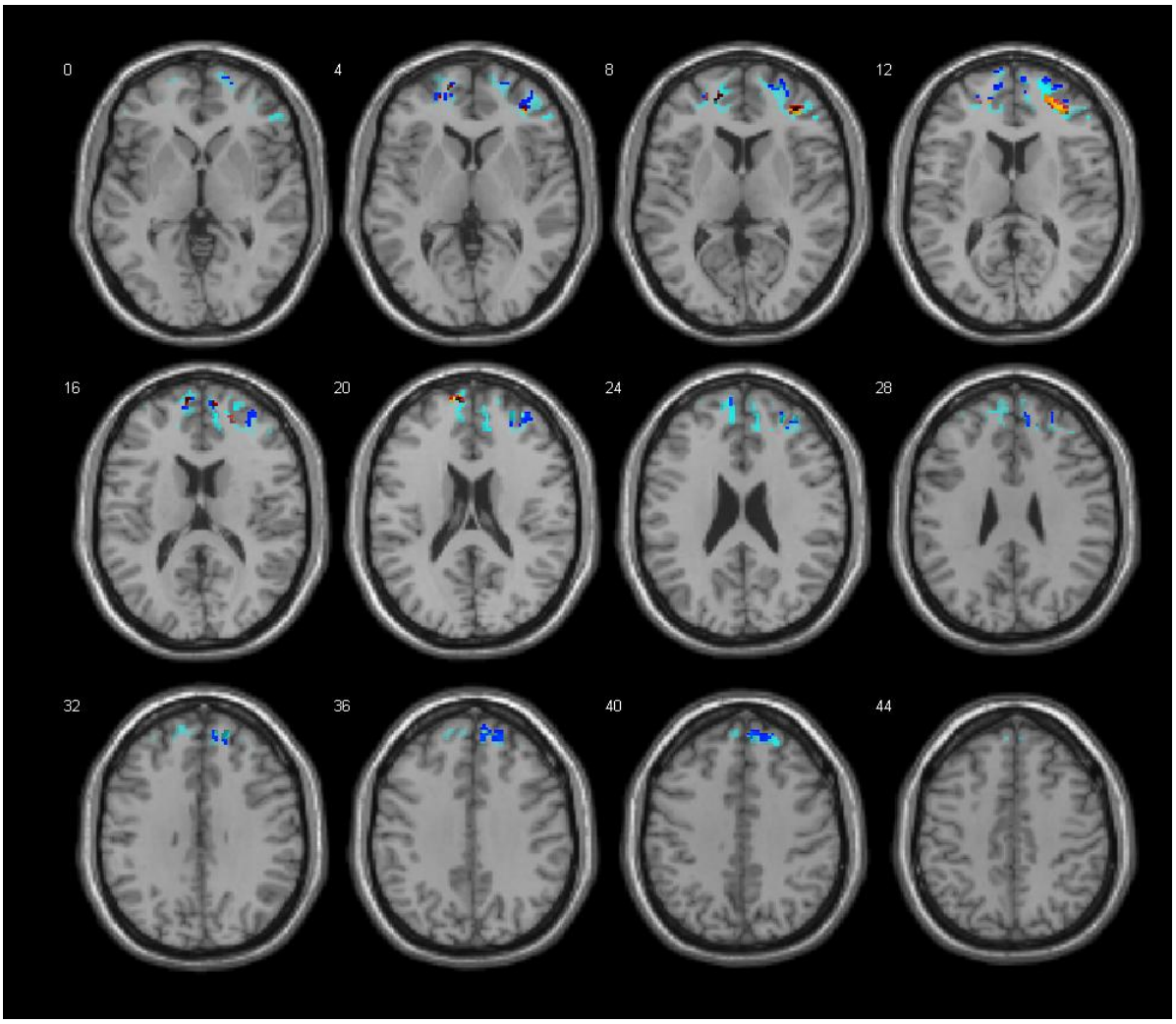

Figure 4. Spatial conjunction of HbO (yellow), HbR (red), HbT (blue) and BOLD (cyan) signals during a mental arithmetic task based on easy count<difficult count, performance by a subject in 6 sessions on subsequent days. Easy count refers to counting backwards from a 3-digit number for 1s. e.g. "136 for 1s". Difficult count refers to counting backwards from a 3-digit number for 7s, e.g. "136 for 7s", for 30 seconds of the task period. Each condition was repeated twelve times in both DOT and fMRI devices with a random order of the instructions. All resulting t-images of contrast selected were fitted a normalized anatomical space. Threshold p-value < 0.05, FDR corrected.

- Visual stimuli based on random dot stereo pairs where the stimuli are presented as a pair of images, one to each eye, then when viewed binocularly a strongly fused perception of depth is produced(71). A visual paradigm is used because binocular vision allows the fusion of

each image presented from our retinas using the difference between them to estimate relative depths. A wide variety of studies to measure the relationship of HbO_2/HbR with perception have been performance(72).

- Resting-state based on the study of the functional architecture of the brain(73). Monitoring HbO_2 and HbR using fNIRS during a rest state of the participants can exhibit patterns of functional connectivity. Given that fNIRS uses a sampling rate higher than classical fMRI, optical measurements allow the study of low frequency components (aim of resting-state studies), thereby avoiding the mix with high frequency components(74).

3.4.1. Applications in infants and neonates

Portable and noninvasive measurements are characteristic of NIRS devices, which allow blood flow and oxygenation monitoring in infants and neonates especially in brain injuries. It is essential to control hemodynamic changes during the development of neurological disorders in these patients. Various studies have reported the suitability of fNIRS to measure changes in saturation, blood volume and relative cerebral metabolic rate of oxygen(75), even in hemorrhage in a premature baby(76).

3.4.2. Clinic applications

NIRS has been used in populations for which other imaging modalities are impractical, such as the elderly and infants, because fNIRS devices are flexible, minimally invasive and can be portable. Despite its limited depth penetration and difficulty to apply on darker skins or hairs, fNIRS devices can still be used for neurologic and psychiatric disorders studies such as the following:

Neurologic disorders such as Parkinson's(77), Alzheimer's(78), epilepsy(79), ischemia(80) or aging(81), have been evaluated using fNIRS devices. Moreover, psychiatric disorders such as schizophrenia(82) or anxiety disorders(83), have also been monitored by fNIRS devices.

In spite of the wide use of fNIRS devices for brain imaging, an essential application is, without doubt, for breast cancer imaging, one the most common cancers in women. Currently, the most usual screening is x-ray mammography combined with physical examination. Tumors are normally associated with an increase in vascularization. In these cases, NIRS can play an important role because it can measure blood volume and oxygenation to determine the presence of a tumor. A variety of studies have shown the capability and feasibility of fNIRS to identify the increased vascularization associated with a tumor(84)(85), despite the fact that the poor spatial resolution is still insufficient for a diagnosis.

3.4.3. Other applications

fNIRS not only offers potential applications in diagnosis and evaluation of diseases, but can also be used to monitor the saturation of oxygen in the blood during the neurorehabilitation in stroke patients, allowing the evaluation of the recovery degree. Some research reports the use of fNIRS devices to monitor functional changes during neurorehabilitation processes in cognitive disabilities(86), motor disabilities(87) or aphasia(88). A new application of fNIRS is being developed based on the application of transcranial magnetic stimulation (TMS)

whose electrical changes produced inside the brain lead to hemodynamic changes which can be monitored by fNIRS. Electroencephalography (EEG) has been used to monitoring the cerebral changes generated during the TMS (89). The EEG allows the measurement of changes in the bioelectric activity while the TMS is applied, with a good temporal resolution but does not provide hemodynamic information. Some authors have stimulated previous to or between intervals of radio frequency pulses inside fMRI(90) to measure cerebral functional changes generated by TMS. It is not possible to monitor functional changes during the stimulation period because of the electromagnetic incompatibility with fMRI. In these cases, NIRS offers the possibility of functional change monitoring while the TMS is applied without interferences that can affect the results, an example of this, recorded in our laboratory can be seen in figure 5.

Although, there are some discrepancies about the hemodynamic changes measured by fNIRS during the TMS application, which depending on the cerebral area stimulated, TMS coil angulation, intensity, and frequency of the stimulation, the results are variable. In spite of these discrepancies, simultaneous fNIRS and TMS are potential tools for the study of the physiology that underlies the stimulation, neurovascular coupling and as clinic tools.

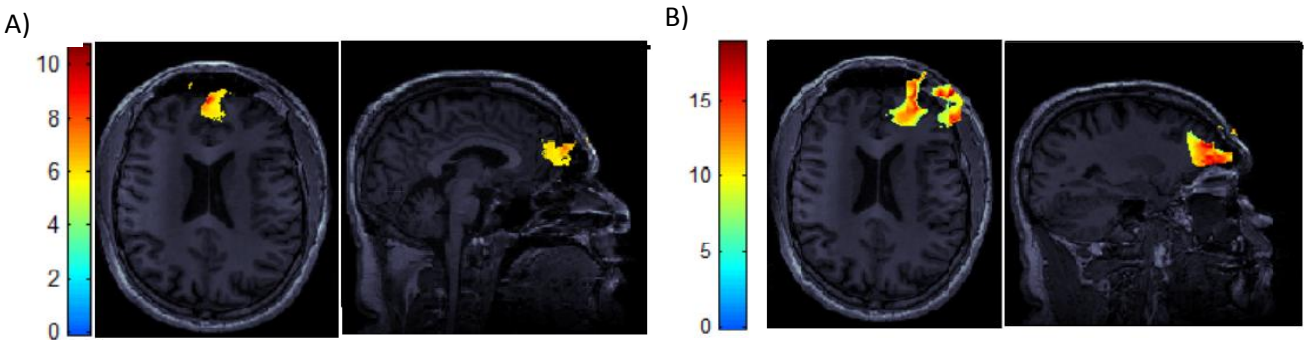

Figure 5. T-maps of brain activation during rTMS at high-frequency (>10Hz) vs resting period, simultaneously measured by DOT on A) the middle of the prefrontal cortex and B) the right lateral prefrontal cortex. All results were mapped onto the subject´s anatomical scans. Threshold $p < 0.001$; $p < 0.05$ corrected FDR, at the voxel level for HbO signals. Color bars show HbO changes during rTMS.

In summary, for clinical applications, noninvasive optical imaging can provide complimentary information to other modalities such as fMRI and provide a low-cost alternative in some cases. This is in addition to serving populations often unable to receive MRI or PET scans such as young infants or the critically ill. Clinical optical brain imaging is generally noninvasive and uses NIR light to obtain improved penetration through the scalp, skull, and brain. To conclude, optical imaging's key advantage is the ability to measure a range of functional contrasts, it can readily be exploited in functional brain imaging via a wide range of approaches from animal studies of the intricate cellular mechanisms of normal and diseased brain to *in vivo* noninvasive clinical brain imaging.

In addition, optical recordings or brain imaging is finding widespread applications as a research tool for both clinical and animal studies of basic brain function and

disease. At present, so little is known about the way that the normal brain functions, in part due to the difficulties of measuring such a complex organ without disturbing or damaging the brain's *in vivo* functioning. Optical imaging allows the living brain to be closely observed, as well as investigation into many functional interactions and changes over many length scales.

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