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A Newcomer's Guide to Functional Near Infrared Spectroscopy Experiments

Rand K. Almajidy[1,3], Kunal Mankodiya[2], Mohammadreza Abtahi[2] and Ulrich G. Hofmann[1,3]

1. Neuroelectronic Systems, Clinic for Neurosurgery, University Medical Center Freiburg, Germany
2. Wearable Biosensing Lab, Department of Electrical, Computer and Biomedical Engineering, University of Rhode Island, RI, USA.
3. Medical Faculty, University of Freiburg, Germany

Abstract:

This review presents a practical primer to functional near-infrared spectroscopy (fNIRS), with respect to technology, experimentation, and analysis software. Its purpose is to jump-start interested practitioners considering to open a non-invasive, versatile, but nevertheless challenging window into the brain by optical methods. We briefly recapitulate relevant anatomical and optical foundations and give a short historical overview. We describe competing types of illumination (trans-illumination, reflectance, and differential reflectance) and data collection methods (continuous wave, time domain and frequency domain). Basic components (light sources, detection, and recording components) of fNIRS systems are presented. Advantages and limitations of fNIRS techniques are offered, followed by very practical recommendations in its use. A huge variety of experimental and clinical studies with fNIRS are sampled, shedding light on a variety of brain-related ailments. Finally, we describe and discuss a number of freely available analysis and presentation packages suited for data analysis. In conclusion, we recommend fNIRS due to its ever-growing body of clinical applications, state-of-the-art neuroimaging and manageable hardware requirements. It can be safely concluded that it adds a new arrow to the quiver of neuro-medical examinations, due to both its versatility and limited costs.

Introduction:

The brain is undoubtedly one of the most complex structures known to humankind, as evidenced by its sheer numbers of neurons (ca. 10^{11}), supported in the cortex by about four times as many glial cells[1], and building some 10^{14} synaptic connections[2]. As such, grasping the inner

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workings and functions of the human brain is among the most - if not *the* most - profound and far-reaching challenges of our time. The quest for this understanding promises new treatments for brain disorders, fundamental discoveries about the brain's functions, and impactful applications spanning from neuro-medicine and live brain monitoring to new communication devices. Unfortunately, progress beyond a basic understanding is slow, and there are many open questions, not least because of a lack of unobtrusive, high resolution and fast measurement systems for natural environments. This lack leads to over-simplifications of the brain's workings. For example, it is a very common misconception to view the brain as simply a collection of neurons, thus ignoring both the exquisite role of glial cells and the essential role of blood supply in the brain. It is estimated that almost every neuron has its own nourishing capillary, altogether constituting a 400-mile supply infrastructure[3].

As neurons do not maintain any substantial provisions of oxygen or glucose, an increase in neural activity due to computational workload has to be followed by an increase in supply by vessels[4], most likely triggered by chemical signaling from neurons themselves[5]. However, the true relationship between local neural activity and resulting adaptations in cerebral hemodynamics, called neurovascular coupling (NVC) is not fully understood. Most investigations into the NVC employ expensive, bulky, stationary functional magnetic resonance imaging (fMRI) devices with limited resolution in time. Despite the superior spatial resolution offered by fMRI, the high cost and limited mobility represent a challenge for many researchers.

Thankfully, this situation might improve with the current emergence of functional near infrared spectroscopy (fNIRS) systems providing a portable and less costly imaging modality for cerebral hemodynamics[6]. Similar to the BOLD [Blood-Oxygen-Level Dependent] signal-the hallmark of fMRI, fNIRS data relies on NVC. However, fNIRS spatial resolution is limited when compared to the fMRI signals.

As the acronym suggests, fNIRS uses near-infrared light of longer than visible wavelengths from 750nm - 1200nm and benefits from the particular optical properties of tissue for this low energy radiation[6].

fNIRS Principles and Theory:

The different brain imaging techniques measure the changes in the tissues' physical or chemical properties, for example, during brain activity. These changes are then translated, in accordance with prior knowledge of the tissues' properties as well as the measuring techniques principles, into data that reflects the changes in brain activity.

fMRI BOLD signals employ changes in the blood's magnetic susceptibility during neural activity to measure the changes in brain activity [4]. fNIRS on the other hand, monitors the changes in the tissues optical properties, mainly blood's absorbance, during neural activity to give a measure of that activity. fNIRS employs light within the NIR range for that purpose. Hence its beneficial, for a fNIRS researcher, to understand the light propagation principles and tissues' optical properties that governs fNIRS.

Light propagation, in general, depends on the light's wavelength and the medium's optical properties which govern incident light's reflection, scattering, and absorption. Absorption is ruled by the medium's chemical constitution[6], whereas scattering, and thus deviation from a straight trajectory is influenced by many parameters such as the light's wavelength and the medium's particulate consistency[7]. Reflection, on the other hand, is related to the incident angle between light and tissue and its optical density[6].

Absorbed light is dissipated as heat in the absorber medium whereas its molecular makeup determines the specific wavelength at which maximal absorption occurs [12]. The most important chromophores, chemical groups absorbing light at specific wavelengths [9], in healthy perfused tissue are oxygenated hemoglobin HbO2, deoxygenated hemoglobin Hb, their sum - total hemoglobin HbT [13], and Cytochrome c oxidase[10], [11]. Their concentrations change with time and oxygen concentration[14].

Near infrared light displays advantageous propagation characteristics through biological tissue, originating in the limited absorbance by water or relevant chromophores in tissue ("Optical Window"). Light above 1200 nm is predominantly absorbed by the tissue's water content[6]. Absorption is quantified by the molar extinction coefficient *a* as a function of wavelength and shows to what extent the chromophore absorbs light at that wavelength. It results in a unique absorption spectrum for each chromophore[14]. The prominent Cytochrome c oxidase (Caa3 in Fig.1) is not used as an indicator for tissue oxygenation, as it is a mitochondrial enzyme representing intracellular oxygenation whose concentration relies on factors other than changes in oxygen[15]. Therefore, Hb and HbO_2 concentrations are of primary interest in tissue monitoring. The computations translating NIR photons collected from the body surface to information about tissue activation depend upon the optical properties of these illuminated tissues. In the following paragraphs we will introduce some of these optical properties, how to deduce the diffusion paths of NIR photons through tissue, and how to estimate the concentration changes of Hb and HbO_2 using these properties.

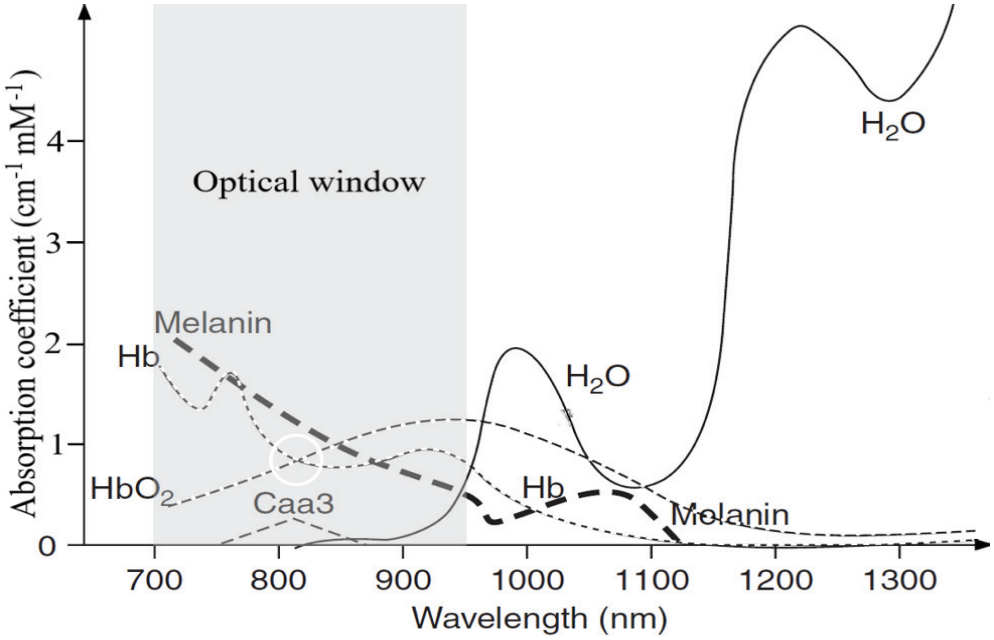

Figure 1: Absorption spectra of Hb, HbO_2, H_2O and other chromophores in NIR range (redrawn after Murkin and Arango009 [10]). The isosbestic point of the HB/HbO_2 absorption spectrum is in the optical window circled in white.

Beer-Lambert Law

As both absorption and scattering contribute to light attenuation, both parameters should be considered in NIRS. The Beer-Lambert law relates light attenuation by absorption to chromophore concentration:

$$A = -log_{10}\left(\frac{I}{I_o}\right) = a * c * d$$

The attenuation *A* of incident light is given by the logarithmic ratio of the intensity of the received light (*I*) to the intensity of the source light (I_0). This equals to a product from the molar extinction coefficient *a,* the molar chromophore concentration *c* and the distance between light source and detector *d*.

The second strong factor of light attenuation is scattering, where a photon's trajectory is changed by an interaction with matter without substantial energy loss. Scattering is the dominant mechanism of light propagation in biological tissue and Mie-scattering (where the scatterer's dimension is not too different from the incident wavelength) is weakly wavelength dependent as well. The human head is composed of many different layers with unique densities and thicknesses, resulting in many different scattering paths for NIR light. Thus, skin, bone and cerebral matter have to be treated carefully in simulations[13].

A photon crossing through a medium containing a uniform distribution of identical scatterers may be scattered away from its straight path with a probability $P_s(z)$ over a distance *z.* This probability is characterized by its scattering coefficient μ_s, the inverse distance a photon may cover without being deflected in 1/cm. The inverse of μ_s can be interpreted as the scattering free mean path length *mfps,* or the average distance a photon travels before a scattering event occurs.

To correct for a tissue's anisotropic scattering properties μ_s has to be corrected to the *reduced scattering coefficient* μ_s' which takes the *anisotropy factor g* into account [9], [16]:

$$\mu_s' = \mu_s(1 - g)$$

Typical reduced scattering coefficients for the brain (grey and white matter) are 11.8 1/cm and 11.1 1/cm at the 760 nm and 830 nm wavelengths commonly used in fNIRS.

Since NIR light photons suffer more from scattering than absorption in body tissues, NIR photon diffusion through the body can be described (and simulated) as a random walk with a step size of 1/(cm)[17].

Modified Beer-Lambert Law

As photons do not travel the distance from source to detector on a straight line, but instead follow a random path and thus travel greater distances than *d*, the actual distance is introduced as differential path length (DP). To modify the Beer-Lambert law, the differential path length factor (DPF) is introduced [18] as follows:

$$A = a * c * d * DPF + G$$

In the modified Beer-Lambert law, the attenuation is not linearly related to the extinction coefficient because of the unknown term G which includes the effects of the shape of the optodes and also the scattering factor. Therefore, it is not possible to calculate the exact concentration of the chromophores using the modified Beer-Lambert law. By assuming G to be constant for all the chromophores, it is possible to eliminate G from the equations and calculate the changes in the chromophores concentration[19]. Those can be found by assuming that *d* and DPF are constant for the time of the experiment.

Figure 1 depicts the NIR sweet spot of low water absorbance between 700nm and 950nm, with Hb and HbO_2 spectra crossing at an isosbestic point around 805nm. Taking advantage of this and solving the Beer-Lambert law for two (or more) measurement wavelengths (and below) on either side of the isosbestic point, helps to eliminate unknowns from the equations to actually find

changes in Hb and HbO_2 concentrations[14], [19], [20]. As we detect small changes in attenuation for both wavelengths

$$\Delta A_{\lambda_1} = a_{Hb}^{\lambda_1}.L.[Hb] + a_{HbO_2}^{\lambda_1}.L.[HbO_2]$$

$$\Delta A_{\lambda_2} = a_{Hb}^{\lambda_2}.L.[Hb] + a_{HbO_2}^{\lambda_2}.L.[HbO_2]$$

with *L* the total mean path length d*DPF, *a* the respective extinction coefficients and the respective concentrations [HbX], we can solve for the latter[21] :

$$[HbO_2] = \frac{a_{HbO_2}^{\lambda_2}.\Delta A_{\lambda_1} - a_{Hb}^{\lambda_1}.\Delta A_{\lambda_2}}{L.\left(a_{HbO_2}^{\lambda_1}a_{Hb}^{\lambda_2} - a_{HbO_2}^{\lambda_2}a_{Hb}^{\lambda_1}\right)}$$

$$[Hb] = \frac{a_{HbO_2}^{\lambda_1}.\Delta A_{\lambda_2} - a_{HbO_2}^{\lambda_2}.\Delta A_{\lambda_1}}{L.\left(a_{HbO_2}^{\lambda_1}a_{Hb}^{\lambda_2} - a_{HbO_2}^{\lambda_2}a_{Hb}^{\lambda_1}\right)}$$

Additional wavelengths may be used to measure the concentration of other chromophores such as Cytochrome c oxidase and water or to improve the accuracy of Hb and HbO2 concentration measurement[15], [19]. It is feasible to determine NIR wavelengths that can minimize the error, in calculations carried out by this equation, introduced by the assumptions above[20].

Chronology of fNIRS Evolution:

Glenn Millikan's attempt to measure oxygen concentration in well-perfused muscle (with an Oximeter) in the 1940s [22] is considered the origin of optical sensing methods [23], [24]. Frans Jöbsis presented one of his first efforts to measure blood oxygenation levels and its variations in a cat's brain by using trans-illumination spectroscopy in 1977[6]. He explained the relative transparency of brain tissues to NIR light and demonstrated the feasibility of monitoring changes in the brain's Hb oxygenation using NIRS[6]. These experiments and his subsequent research[25] make him the founder of in vivo NIRS.

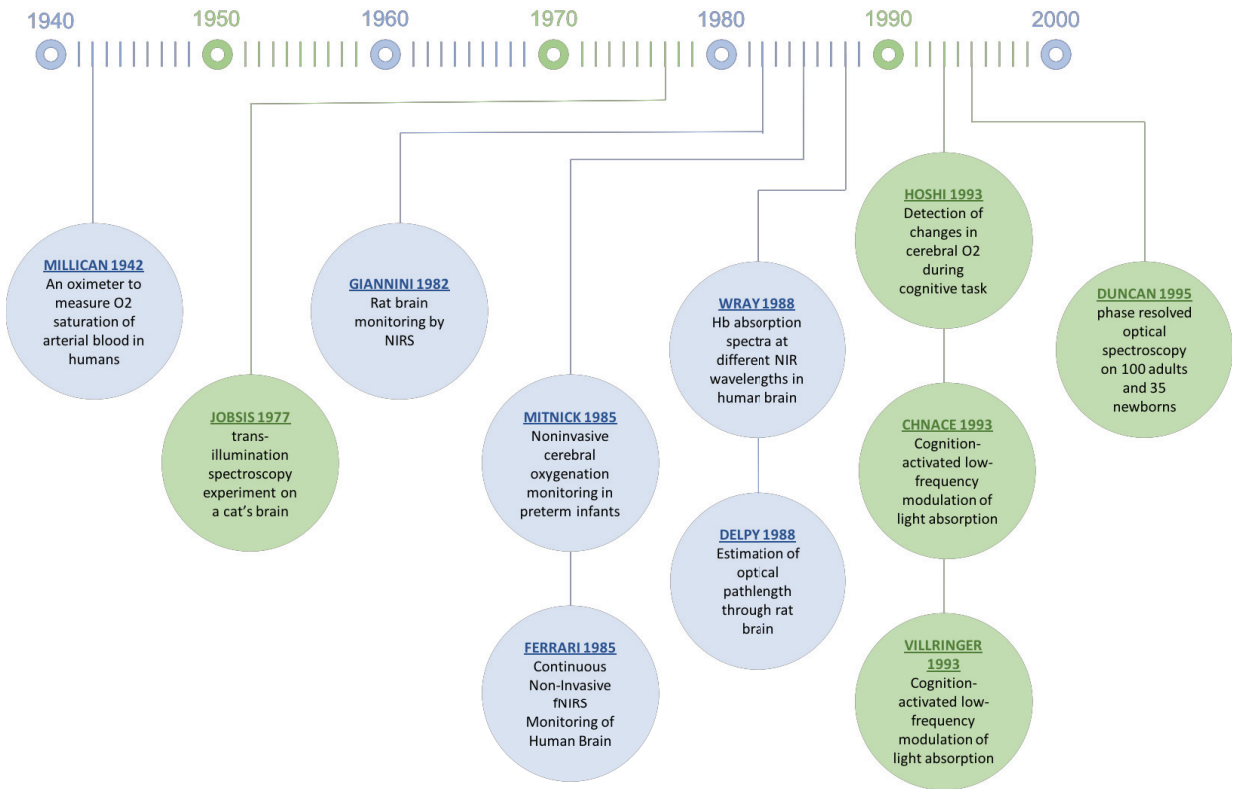

Figure 2: A chronology showing some of the early experiments and developments contributing to the evolution of fNIRS.

In the 1980s, Marco Ferrari started to measure brain oxygenation and its changes in animals. His results further confirmed that NIR light can efficiently detect blood oxygenation changes[26], [27]. In 1985 Ferrari, carried out experiments to monitor blood oxygenation changes in human adults using custom-made NIRS instruments[28]. These experiments, and the experiments by Brazy and his colleagues (including Jöbsis) to monitor preterm infants cerebral oxygenation[29], represent the first successful applications of NIRS in human patients. In 1986, Ferrari and colleagues presented more cerebrovascular measurements from neonates[30] and cerebrovascular patients. The data showed the effect of carotid artery compression on regional cerebral blood volume and oxygenation[31]. The same years witness the first quantitative data of HbO2, Hb and HbT changes collected from sick infants' cerebral blood by David Delpy and his colleagues (including M Cope). They employed a custom-made, four wavelength trans-

illumination NIRS system to monitor oxygenation level changes[32]. Their findings paved the way for NIRS' use as a bedside cerebral oxygenation monitor. Their experiments in 1988 provided the hemoglobin absorption spectra at different NIR wavelengths, facilitating the quantification of NIRS data collected from the brain[11] and estimation of the optical path length of NIR light through the rat brain[18]. They also presented a description of their system[33] which served as the base design for the first commercial NIRS system produced by Hamamatsu Photonics K.K. (Hamamatsu City, Japan) in 1989.

Delpy and his co-workers' effort to accurately calculate the optical path length for NIR photons with time-of-flight measurements[18] was extended by Duncan and colleagues, who collected precise differential path length factor (DPF) values, the absolute path lengths divided by the distances between sources and detectors, by phase resolved spectroscopy from 100 adults' and 35 newborn infants' heads. Their findings indicated differences between infants and adults, between males and females, and dependence on wavelength[34]. It was not until 1993 that the first human fNIRS systems measurement were published. These experiments utilized single-channel fNIRS systems and include the work of Hoshi[35], Chance[36], Villringer [37], Kato [38] and Okada[39]. Hoshi's data showed an increase in HbO2 and a decrease in Hb in the relevant area during brain activation by a cognitive task (solving an arithmetic problem). This change is associated with an increase in cerebral blood flow and was more prominent in younger subjects than in adults[35]. Chance and colleagues interpreted the variation in blood oxygen concentration in a relatively limited illuminated tissue (a banana shape) as a measure of brain activation during a similar cognitive task (problem solving). They concluded NIRS can monitor localized brain activity[36].

Villringer et. al. used a NIRS setup to assess hemodynamic changes in the brain during cognitive tasks and visual stimulation. Their results demonstrated that NIRS indeed can record changes in brain activity and not only hemodynamic changes in the skin [37]. Kato and colleagues investigated HbO_2 and Hb changes during visual stimulation[38]. Okada and colleagues (including

Hoshi and Tamura) documented differences due to handedness and gender[39]. They also published the first clinical use of fNIRS with schizophrenic patients in 1997[40].

Gratton and colleagues tested the feasibility of employing NIRS for optical scanning. They provided the first evidence of the indirect relationship between changes in the brain's optical properties and neuronal activity. They showed the feasibility of measurements in reflectance mode: near infrared light from a strong light source interacts with chromophores on its long, random path through tissue and a sensitive photodetector will detect some backscattered photons[41]. These efforts supported the emergence of the first multi-channel fNIRS system (for details see the review by Ferrari and Quaresima [42]).

Currently, fNIRS is a very useful neuroimaging technique because of its lower cost and greater portability than fMRI or PET. It has found its place in clinical and research settings monitoring cerebral functionality related to vision [43], hearing [44], speech[45], motor tasks [46], learning[47], and emotional stimuli[48].

NIRS signals are highly correlated with regional cerebral blood flow and thus cost-effectively augment PET or fMRI's BOLD measurements[49], [50]. They can therefore shed light on the coupling of hemodynamic responses with neuronal activity[51], [52] as revealed by electroencephalography (EEG)[53].

NIRS performance is influenced by handedness and gender[39] and in particular by aging[54] due to changes in the optical properties of scalp tissues[55] and decreased brain activation[56].

The increasing popularity of fNIRS is owed to its portability, its moderate spatial and temporal resolution, ease of use, and its ability to scan moving human subjects. All these advantages have encouraged researchers to utilize it individually or along with other modalities such as fMRI[12], [57]–[59] or EEG[60]–[65].

Different Types of NIRS:

The different applications of NIRS systems require a basic understanding of their principles. There are three main types of NIRS systems: I) Continuous Wave (CW), II) Time Domain and III)

Frequency Domain spectrometers (see figure 3). Each type has its strengths and weaknesses thus the researcher must design their experiments in accordance with the systems' characteristics.

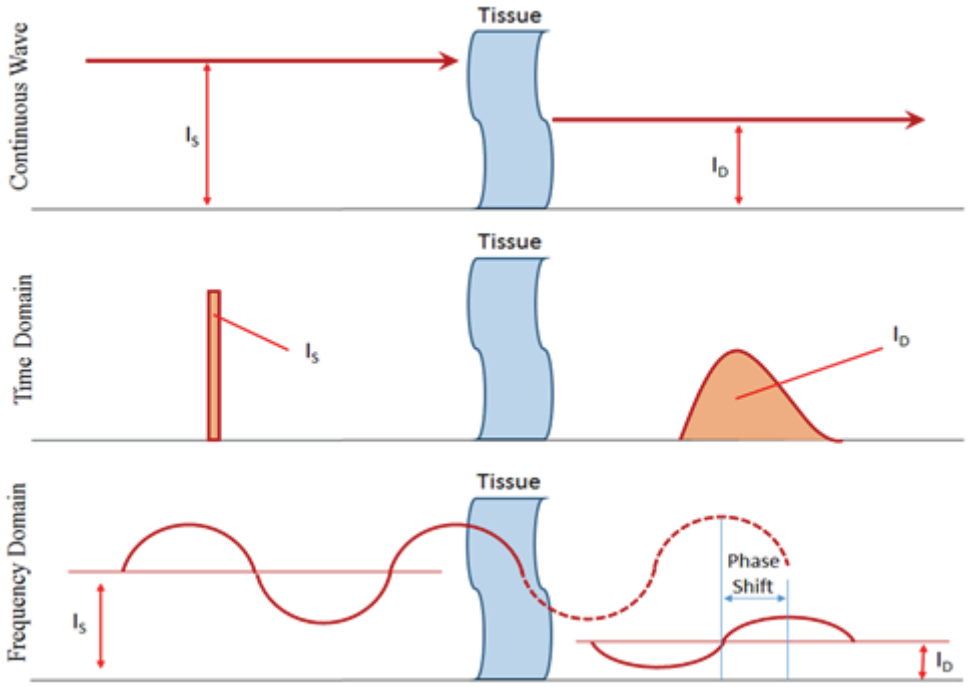

Figure 3: Principles of three different NIRS types.

- *Continuous Wave NIRS*: CW-NIRS is the oldest and the most common commercially used NIRS system (reviewed by[66]). This type of device uses multiple wavelength sources and measures the attenuation of light (see figure 3) by a photodiode or a photodetector[67]. Compared to the other types, CW-NIRS has advantages in simplicity, size, weight, and cost. However, it is very difficult to separate attenuation from absorption and scattering, and some systems have a small light penetration depth due to short source-detector distance (SDD)[67].
- *Time Domain NIRS*: Also known as time-resolved or time-of-flight NIRS systems, a solid-state laser is usually used to provide very short but powerful pulses. Light attenuation is measured by very sensitive special cameras or even single photon counters sorting them

based on their arrival time[67] (reviewed by[68]). This type has the advantages of higher accuracy and spatial resolution[69], [70] but is limited by the system's bulkiness and higher cost[71].

- *Frequency Domain NIRS*: Also known as frequency-resolved or intensity modulated NIRS systems, an LED, laser diode[69], [72], [73] , or white light source usually provides input light. These systems measure the attenuation, phase shift and modulation depth of the light with respect to the systems' incident light[71], [74]. They are exploiting the linear relation between the optical path length and a phase shift existing for frequencies < 200 MHz[74], [75]. A gain-modulated area detector or a photon counting device is used to take measurements[67], [76].

NIRS Illumination Modes (Figure 4)**:**

1) *Trans-illumination*: This mode is applicable for newborn infants[33]. Due to changes in the optical properties of scalp tissues as a function of aging, this mode is not used with adults.
2) *Reflectance*: This mode is utilized in most current NIRS devices[71], [77]. In reflectance mode, the penetration depth of NIRS is estimated at around 1/3 of the SDD[9].
3) *Differential reflectance*: More than one NIR detector (or source) is utilized to measure the difference between extra and intra cranial light paths[77].

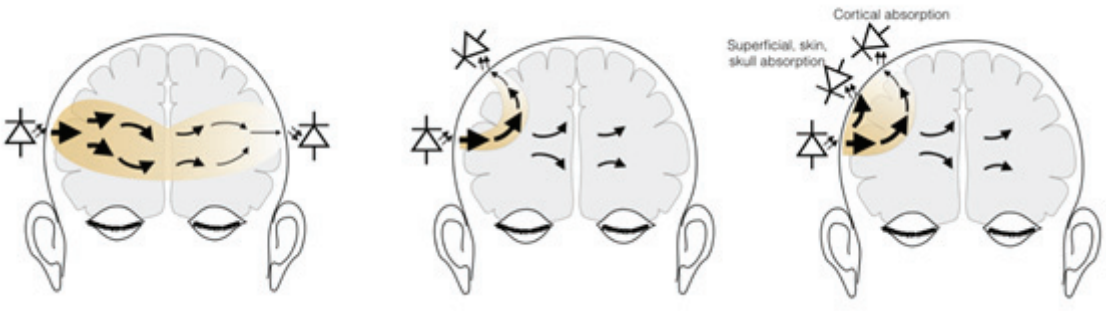

Figure 4: Different NIRS operation modes: Trans-illuminance, reflectance and differential reflectance (redrawn after [77], [78]).

NIRS Systems:

The major factors which control the efficiency of NIRS are 1) the type of NIR source and detector 2) the efficiency of NIR transmission into/collection from the tissue 3) the accuracy of tissue optical property coefficients and models used to calculate HbO_2 and Hb[79].

Previously, NIRS penetration's depth was limited to 3 mm of the skull[80]. However, current instrumentation allows the light to reach up to 1-2 cm in depth[9] depending on several factors including NIR light radiant energy[9], optical properties of the head beneath the NIRS optodes (NIR source/detector), the SDD[81] and the detector area[82].

Although increasing SDD is believed to increase the penetration depth[83], data quality deteriorates with increasing SDD above specific limits[84].

Hence an SDD between 2-4 cm[80], [82], [84], [85] is usually employed for NIRS systems. In implementations with longer SDDs, small detectors are associated with unstable DPF, and so detectors must be chosen in accordance with SDDs[82]. There are currently efforts underway to improve fidelity in both light sources and sensors to achieve timing precision permitting zero source-detector distances (0SD) and thus improving localization of hemodynamic response[68].

NIRS systems contain the following main components:

· *NIR Light Source:* In most cases it is either a light emitting diode (LED)[86]–[90] or a laser diode[35], [49], [91]–[93] with 670 and 890 nm[89], 730, 805, and 850 nm[94] or 760 and 850 nm (widely used combination by developers and commercial systems)[66], [86] wavelength. LEDs are often preferred due to safety precautions[95]. Picosecond lasers are used in experimental 0SD systems[68].

· *NIR Detector:* Photodiodes[86], [91], [96]–[98], avalanche photodiode[88], [89], [93], [99] or photomultiplier tubes (PMT) [33], [55], [66], [100] are usually utilized as NIRS detectors. They exhibit a low wavelength selectivity and thus caution must be taken to block or avoid ambient light.

· *Control and Data Collection Electronics:* NIRS sources and detectors need to be controlled by sophisticated electronic circuits. Data collected by the NIRS detectors are either amplified and saved on the same hardware, or transmitted tethered[86] or wirelessly[87], [88], [97], [101] to another electronic circuit or a computer where further amplification, noise reduction and signal analysis is performed.

· *NIR Light Transfer Module:* The NIR light is shone directly to the scalp from the NIR source[86], [98] or conveyed by optic fibers[102]. The reflected NIR is received from the head either directly by the NIR detector[98], [101] or guided via optic fibers to the NIR detector[86], [89], [103], [104].

Advantages:

Portable, Low Power and Low Cost: A 16 channel (=16 dual sources and 2 detectors) NIRS setup can be powered with a single 3.6V–1000mAh battery[101]. fNIRS devices can be designed portable and hence employed for freely moving subjects. It can be utilized at the bedside[105], in an emergency situation, or in an ambulance [90], [106]–[108]. A basic system may cost around $10.000[66], with lower operational costs as compared to MRI[9].

Non-invasive and Safe: LEDs and even laser diodes can stay very well below the critical heat deposition of 0.2 W/cm^2 (at 630 nm) -0.4 W/cm^2 (at 850 nm)[9] known to cause pain or even heat damage to the skin, [106], [109], [110].

Easy Preparation and Setup: No special skin adhesive is required to attach optodes to the scalp. Optodes, in most systems, are reusable and last for long periods and many measurements[111], [112]. Optodes' cleaning after employment is generally easy and sometimes depends on the manufacturers.

Motion Artifacts: Motion artifacts are less pronounced for minimized fNIRS systems where NIRS optodes and the controlling circuits are in close proximity and both attached to the body[108] as compared to fNIRS systems employing optical fibers. These may shift position during vigorous motion affecting optical coupling[113].

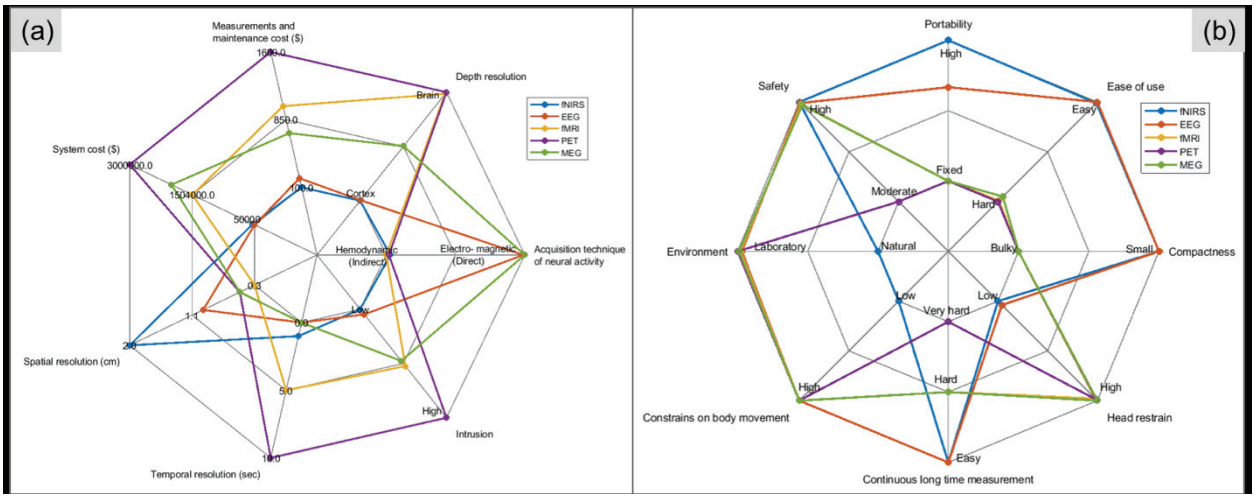

Figure 5: Multiparametric comparison of fNIRS (blue), EEG (red), fMRI (yellow), PET(purple) and MEG (green). (a) depth resolution, relation to neural activity, intrusivity, temporal resolution, spatial resolution, estimated system costs and running costs. (b) handling, size, head fixture, ease of long term studies, subjects' mobility, environment, safety and portability.

SNR and Temporal and Spatial Resolution. fNIRS sampling rate may exceed 25 Hz/channel versus 1Hz for fMRI [114]. In other words, fMRI provides the brain images at the rate of 1 frame/second while fNIRS could give 25 images per second. Therefore, the temporal resolution of fNIRS is considerably higher than fMRI, but slightly lower than EEG as illustrated in figure 5.a. However, the spatial resolution of fMRI is far greater than fNIRS. The spatial resolution of fNIRS is also slightly lower as well [115] compared to EEG. This is depicted in figure 5.a as well. Cui et al. (2011) performed a detailed comparison of fNIRS and fMRI signals in temporal and spatial domains. They have reported a weaker signal to noise ratio (SNR) in fNIRS compared to fMRI. Although the SNR is reported weaker, the signals are highly correlated. In the spatial domain, they reported that the banana-shaped path of the photons is strongly correlated to BOLD signal[116].

Application in Special Population: fNIRS is feasible for patients with implanted devices. For example, no interference in the fNIRS optodes have been detected in patients with pacemakers

[117]. fNIRS is more convenient for young and claustrophobic patients as compared to fMRI [9], [118]. Patient acceptance is better in fNIRS as a narrow “tube” of fMRI is avoided[108].

Response compared to fMRI: Minati et al. (2011) recorded NIRS and fMRI simultaneously in event-related visual stimulation. They measured inter-subject coefficients of variation (CVs) for the response peak amplitude and reported considerably larger CVs for NIRS compared to fMRI. The inter-subject CVs for the response latency and intra-subject CVs for response amplitude are reported comparable in their study[119].

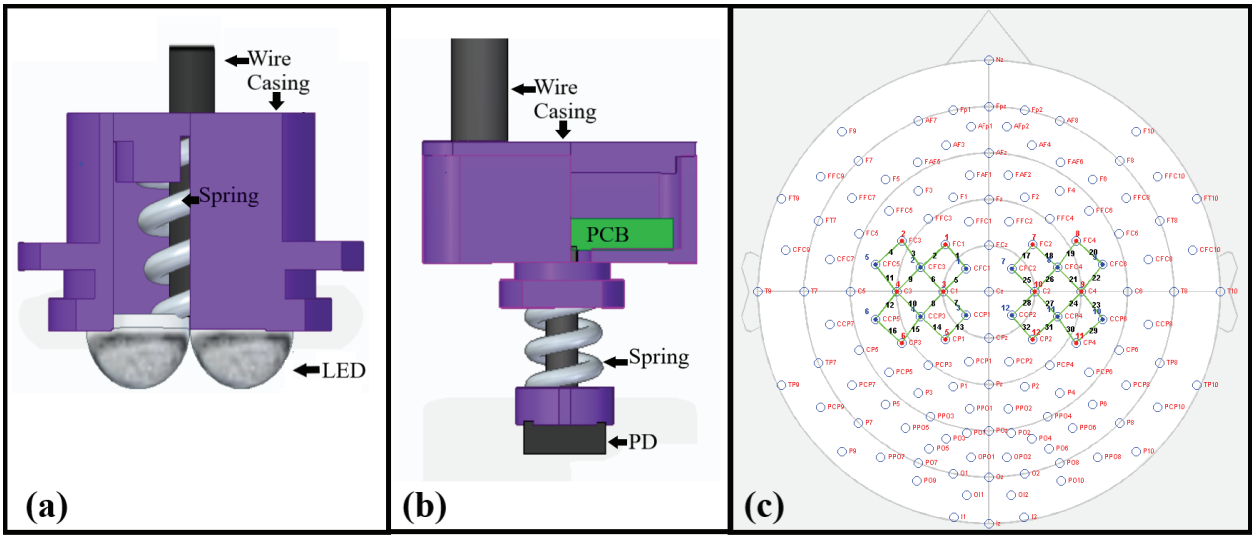

Figure 6: Example of the author’s spring loaded fNIRS a) source, b) detector, c) exemplary montage of optodes and comparable EEG channels map.

Limitations:

Susceptible to Ambient Light: As the light transfer module always contains a minimal air gap of some sort between transducer and skin or glass fiber and skin, it is challenging to avoid ambient light influencing measurements [95], [103]. Also, the placement of optodes on the head to send and receive the light in proper angle is important.

Shallow Penetration: fNIRS can’t reach the deeper areas in the brain and has a shallow penetration depth of around 1-3cm in the cortex[111]. As fNIRS in reflectance mode depends on

photons scattered towards the sensor, the number of photons decays exponentially with SDD. This cannot be easily compensated for by a higher flux as damage to the skin has to be avoided at all costs. Consequently, hemodynamic responses from deeper brain structures may not be measured with a simple fNIRS device[114].

Low Temporal Resolution (compared to EEG): Although fNIRS measurement is more rapid than fMRI, its temporal resolution is lower than that of EEG[121], [122] which displays between 1 msec[105] and 100 msec[123] time constants. Consequently utilizing it in applications such as brain-computer interface (BCI) requires longer task periods[108], [112], [124]. However, this may be inadequate to monitor the desired delay in activity between brain areas[125].

Low Spatial Resolution (compared to fMRI): fNIRS' spatial resolution is quite limited with about 1 cm[58], [114] as compared to fMRI's millimeter voxel sizes[105], [111]. Even with the picturesque simplification of a "banana-shaped" light path between source and sensor, it is hard to talk about a "spatial resolution", given that absorption acts in an integrative manner along each individual photons path.

Noise, Artifacts, and Interferences: As fNIRS offers sometimes noisy channels with a small bandwidth and in line with the Shannon-Hartley theorem, the reported information transfer rate is low with about ~ 4 bits/min[111], [126], [127]. As fNIRS is an optical method, the presence of hair -especially dark hair- in the region of interest (ROI) may block light and reduce signal strength both entering and exiting the skull[9]. A longer preparation period may be necessary to ensure minimal hair presence below optodes[107]. Hence, pre-experimental preparation time and signal strength depend on the ROI. Although the signals are not affected by muscle artifacts from body motion, signal quality might still be negatively affected by head movement[90], [113]. This may cause fluctuations in the efficiency of light transfer. Noise due to various physiological oscillations at around ~0.1 Hz Mayer waves are reported, which are caused by slow changes in the blood pressure[108]. Respiratory oscillations (0.2~0.5 Hz)[127] and heartbeat artifacts (1~1.5Hz)[108]

were found as well. Extracranial activity is reflected in NIRS data[80], [128]–[131]. This contribution may have, in some cases, a strong impact on data accuracy.

Participant Discomfort in Long-term Use: An easy way to minimize ambient light artifacts, optodes placements, and optimize coupling in the light transfer module is to press the working ends of glass fibers or LEDs into the skin. However, this becomes uncomfortable after a while, causing stress or headaches which can affect experimental trials [90], [103], [132], [133], [241].

Recommendations:

Spring-Loaded Optodes: One of the challenges with the fNIRS optodes and cap is to handle the hair. As shown in (Fig. 6 a and b)[9], [95], [120], spring-loaded mechanism have been developed and implemented to accomplish: 1) parting away the hair from the light path and 2) sustaining the secure pressure between the optode and skull [90], [107].

Optode Shielding and Special Caps: Studies have shown that ambient light has a significant influence on the performance of fNIRS [242]. It is generally a good practice to shield optodes from ambient light by dark caps. For example, 3D printing and laser cutting technologies are used to design optode capsules that are made of dark colored materials encapsulating optodes to reduce the influence of the ambient light [241]. In addition to the dark shields for the optodes, fNIRS cap is also covered with a black overcap to further reduce the ambient light. Employing special caps[132], [133], secondary caps to hold the optodes can offer a secure optode-skin coupling and minimize ambient light[90] and motion artifacts[113]

Safety: The long-term use of fNIRS may elevate the temperature at the contact point of light source and scalp skin [243]. In general, a commercial fNIRS system has to pass through a set of safety or regulatory standards. However, when employing a laser light source, care must be taken to prevent eye or skin injury [95]. Study participants should be communicated regarding safety limits of the fNIRS device in use. Laboratory designed fNIRS systems must meet the requirements

of IEC 80601-2-71:2015 that is designed to regulate the basic safety and essential performance of fNIRS equipment.

Signal Quality: To reduce extracerebral or, superficial influences optodes with different SDD are employed. Data collected from the short SDD will indicate superficial activity (Figure 7) which can be then by means of proper modeling be isolated from deeper brain activity[90]. It is always important to implement appropriate approach for filtering, noise reduction [9], [111], [113] and channel rejection for channels with weak / extremely noisy signals.

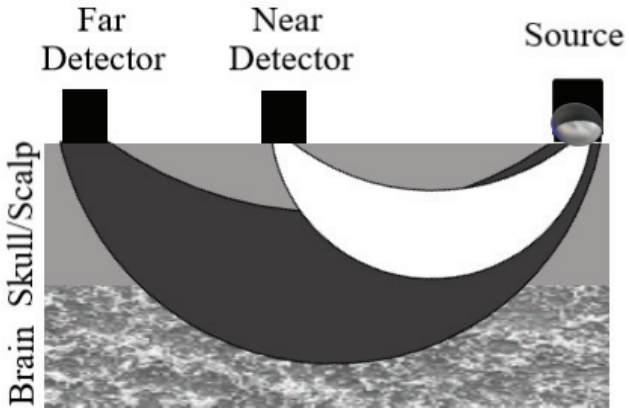

Figure 7: NIR light path between source and detector[131].

fNIRS Applications:

As fNIRS is meanwhile a rather mature technology and still open for new developments and creative implementations, it has led to an ever-increasing field of applications. Naturally, they are all based on the hemodynamic response of brain under a wealth of conditions and research paradigms. These experiments are at least augmenting, if not in some cases even replacing, the use of more expensive, stationary imaging modalities. It is thus used in functional connectivity and cognitive neuroscience experiments, as well as in neurological diagnostics or rehabilitation and neuroimaging and, not the least, as communication modality.

The following lists a deliberately not fully conclusive set of applications from literature.

Table 1: Selected studies using fNIRS technology for various applications.

Application	Neuroimaging modality	Citation (year)
Language studies	fNIRS	Lei et al. (2018)[134],Watanabe et al. (2016)[135], Takahashi et al. (2015)[136], Rossi et al. (2012, a review)[122] , Quaresima et.al (2012, a review)[137], Minagawa-Kawai et al. (2011)[138].
	fNIRS-EEG	Wallois et al. (2012, a review)[63].
Brain functional connectivity	fNIRS	Bu et al. (2018)[139], Vergotte et al. (2018)[140],Racz et al. (2017)[141], Gallagher et al. (2016)[142], Wang et al. (2016)[143], Medvedev et al. (2011)[144]. **Resting-state**: Wang et al.(2017)[145], Zhang et al. (2011)[146], Sasai et al. (2011)[147],
	fMRI–fNIRS	Sasai et al. (2012)[148], Duan et al. (2012)[149].
Psychiatry	fNIRS	Ohi et al. (2017)[150], Lin et al. (2017)[151], Okada et al. (2016)[152], Ehlis et al. (2014, a review)[153], Matsuzawa et al. (2012)[154]. **Schizophrenia**: Luo et al. (2018)[155], Kumar et al. (2017, a review)[156], Noda (2017)[157], Koike et al. (2013, a review)[158], Chou et al. (2017)[159], (2014)[160]. **Depression** : Nishi-zawa et al. (2019)[161], Kondo et al. (2018)[162], Fu et al.(2018)[163], Hirano et al. (2017)[164], Kawano et al. (2016)[165], Zhang et al. (2015, a review)[166].
	fNIRS-EEG	**Epilepsy:** Sannagowdara et al. (2018)[167], Bourel-Ponchel et al. (2017)[168], Manoochehri et al (2017)[169], Modir et al. (2017)[170], Peng et al. (2016, a review)[171].
Rehabilitation	fNIRS	**Neuro**: Miharaa and Miyai (2016, a review)[172], Balconi (2016, a review)[173], van Dokkum et al. (2015)[174] **Motor**: Bae et al. (2017)[175], Chang et al. (2014)[176], Rea et al. (2014)[177], Lin et al. (2009, a review)[115],
	fNIRS-EEG	Yamamoto et al. (2018)[178].
Anesthetic depth	fNIRS	Liang et al. (2016)[179], Sørensen (2016)[180], Hernandez-Meza et al (2015, a review)[181], Leon-Dominguez et al. (2014)[182].
Aging studies	fNIRS	**Cognitive**: Agbangla et al. (2017, a review)[183], Li et al. (2018, a review)[184], Uemura et al. (2016)[185]. **Sensory**: Lin et al. (2017)[186]. **Discourse comprehension**: Martin et al. (2018)[187]. **Anxiety:** Adorni et al. (2018)[188]. **Microvascular dysfunction**: Rosenberry et al. (2018)[189].
Cognitive neuroscience	fNIRS	Takeda et al. (2017)[190], Causse et al. (2017)[191], Ozawa and Hiraki (2017)[192], Keshmiri et al. (2017)[193], Fishburn et al. (2014)[194], Byun et al. (2014)[195], Cutini et al. (2012)[102].

	fNIRS-EEG	Liu et al. (2017)[196], Omurtag et al. (2017)[60].
BOLD signal	fMRI–fNIRS	Emir et al. (2008)[197], Schroeter et al. (2006)[198], Steinbrink et al. (2006, a review)[12].
Brain-computer interface (BCI)	fNIRS	Naseer et al.(2015, a review)[108], Qureshi et al. (2017)[199], Shin et al. (2016)[200], Chaudhary et al. (2015, a review)[105], Shin and Jeong(2014)[127] .
	fNIRS-EEG	Shin et al. (2018)[201], Hong et al.(2018, a review)[202], Min et al. (2010, a review)[112], Tomita et al. (2014)[203].
Brain motor activity	fNIRS	Herold et al.(2017, a review)[204], Abdalmalak et al. (2017)[205], Nishiyori et al. (2016)[206], Iso et al. (2016)[207], Drenckhahn et al. (2015)[125].
Driving research	fNIRS	Liu et al. (2016, a review)[208], Sturman et al. (2018)[209].

Analysis Software:

No matter which fNIRS hardware is used to perform the desired experiments, be it custom built or commercial, post-processing and data analysis take a huge toll on the workload of any researcher. But instead re-inventing the wheel another time it is very much worth the effort and gain proficiency and even improve on existing software tools.

The factors a researcher needs to consider when choosing fNIRS software are a) compatibility with the OS and fNIRS data format. b) the range, speed, and accuracy of the fNIRS data processing it offers. c) the software price. d) the software language, which can expedite or hinder understanding the software depending on the researcher's experience e) the software's extendibility and customizability. f) software-specific features or advantages. Table 2 summarize some currently available fNIRS software.

Table 2: A list of existing fNIRS Software

Package name	Available from	Features	Price	Software and OS compatibility	Data format	Advantages
EasyTopo 2.0[210]	https://sites.google.com/site/fenghuatian/software/easytopo	Diffuse optical topography algorithms. It visualizes the data which underwent angular interpolation, hence provides a more realistic representation of the data[211].	Free	MATLAB toolbox	MATLAB file .mat	Good computational efficiency
Functional Connectivity Analysis Tool for near-infrared spectroscopy data (FC-NIRS)[212]	https://www.nitrc.org/projects/fcnirs/	Functional connectivity calculation, visualization and network analysis. Signal quality control and batch processing are feasible. The package includes HOMER[213], thereby it allows data preprocessing. It also includes an fMRI based network analysis toolbox named GRaph thEoreTical Network Analysis (GRETNA)[214].	Free	MATLAB based package	fNIRS data with .csv or .nirs (HOMER2[213])format	Fast processing with an improved accuracy is to be expected
Fieldtrip[215]	http://www.fieldtriptoolbox.org/download/	The software offers to create optode layouts, data preprocessing and artifact correction.	Free, Open source	MATLAB toolbox	Artinis[216] NIRS data .oxy3, .oxy4, and XML files	The structure of the package allows the users to optimize or extend its functionality according to their requirements[215]. It can process EEG and MEG.
fNIRS Optodes' Location Decider (fOLD)[217]	https://github.com/nirx/fOLD-public	It facilitates positioning the NIRS optodes efficiently in accordance with the brain region of interest (ROI).	Free	MATLAB package or Windows standalone executable	.nii NIfTI[218], and .img (ANALYZE 7.5[219])	Default parameters are available for head tissue segmentation. Thus may be employed without loading subject-specific data

Functional optical signal analysis (FOSA)[220]	http://www.ucl.ac.uk/medphys/research/borl/nirs/current-projects/fosa	The user can perform fNIRS- optical topography data processing, and statistical analysis by employing SPM	Free, Open source	MATLAB based package		
Hemodynamic Evoked Response (HOMER2)[213]	http://homer-fnirs.org/, https://www.nitrc.org/projects/homer2	One of the most widely utilized NIRS analysis toolboxes. Tools to analyze the brain's hemodynamic changes, the optodes settings (wavelengths, geometry, etc.), and brain activation imaging (Figure 8 (a)). The latter two are realized by the AtlasViewer[221] a software that utilizes forward modeling.	Free, Open source	MATLAB package or Windows standalone executable	Many of the current commercial fNIRS systems data formats can be saved in a format to be imported	Batch processing and grand averaging capabilities. The former facilitates faster and more convenient processing of the data while the latter allows better understanding of the experiments' outcome.
Imperial College near infrared spectroscopy neuroimaging analysis (ICNNA)[222]	http://hamlyn.doc.ic.ac.uk/icnna/	The software allows limited data processing and visualization but offers graph theory-based connectivity analysis, statistical analysis and manifold-based topological analysis.	Free for academic use	toolbox developed under MATLAB	only supports[223] HITACHI ETG-4000[224]	Great analytical flexibility with focus on the experiment
Monte Carlo eXtreme(MCX)[225]	http://mcx.space/	The software exploits the high computational speed provided by modern Graphics Processing Units (GPU) for a parallelized computation of NIR photons propagation simulations in 3D turbid media.	Free	written in CUDA programming language and nightly builds for Windows, MacOS, Linux		It claims to reach up to 400 times the computational speed of a single-threaded CPU[226].
NIRS analysis package(NAP)[229]	https://sites.google.com/site/tomerfekete2/	Offers systematic noise and motion artifact reduction by various preprocessing methods. Statistical analysis of data by employing SPM and data visualization	Free, Open source	MATLAB	MATLAB format .mat or HITACHI excel format	

NIRFASTSlicer[227]	http://www.dartmouth.edu/~nir/nirfast/	multi-modal optical tomography software[228]. it allows optical computation and modeling of light propagation (in different wavelengths and luminescence), 3D image segmentation, 3D data visualization, and image reconstruction.	Free, open source	MATLAB package or Windows standalone executable	capable of importing data from different imaging systems	Users can integrate a customized version of 3DSlicer
NIRSlab	https://www.nitrc.org/projects/fnirs_downstate/ or https://nirx.net/nirslab-1/	The package offers analysis and processing of NIRS. The user can create an experiment-specific optode map (Fig. 6 c), data preprocessing (Fig. 8 c and d), artifact removal, employing SPM to extract data dynamic features[230], batch processing, grand averaging, connectivity analysis and brain activation visualization in 2D (Fig.8 b), 3D and in video format with the task displayed together with the localized brain activity.	Free [231] or included withNIRx[232] systems	Package of .p compiled MATLAB files or Windows standalone executable	data collected by NIRx-fNIRS systems)	Offers batch processing, and reducing the size of data files.
NIRS-Statistical Parameter Mapping(NIRS-SPM)	from the Bio Imaging Signal Processing lab (http://bispl.weebly.com/nirs-spm.html#/) and NITRC (https://www.nitrc.org/projects/nirs_spm/)	statistically analyzing the data. It allows the removal of global trends and computation of high-resolution maps of Hb, HbO2 changes in different brain areas of fNIRS data[233] as well as cerebral metabolic rate of oxygen (CMRO2) estimation from NIRS and fMRI[234].	Free, open source	software based on Matlab and statistical parameter mapping (SPM[235]) compatible with any computer with MATLAB	The software can process fNIRS data from at least 9 different commercial fNIRS systems [http://bispl.weebly.com/nirs-spm.html#/]	The software is based on a general linear model (GLM), and Sun's tube formula / Lipschitz-Killing curvature (LKC) based expected Euler characteristics [233], [236].

Nirstorm[237]	https://github.com/Nirstorm/nirstorm	A Brainstorm[175] plugin for fNIRS data analysis. Data Tools to filter, reduce signal noise and perform some statistical analysis on single data sets or in group form.	Free, open source	Developed mainly with MATLAB (and Java) Windows, MacOS, Linux	.nirs data in a HOMer-format.	Brainstorm can analyze EEG, EMG, and ECoG data.
Platform for optical topography analysis tools (POTATO)[238].	https://www.hitachihighteh.com/global/products/ind_solutions/ict/human/brain/ot/analysis/platform.html	Users can preprocess, analyze, and visualize fNIRS data by employing this software in either **Normal** mode, where the analysis steps and settings are fixed, or **Research** mode.	Free for academic use	MATLAB based package		The experienced researchers can utilize different data processing recipes according to their data and preferred settings
Toast++[239]	http://www.toastplusplus.org	NIR visualization in diffuse optical tomography (DOT). For image reconstruction and NIR light propagation simulations, various methods are employed such as finite element, sparse linear algebra, and others for solving the forward and inverse problems[239].	Open source software	C++software suite or precompiled binary for Linux, Windows, and Mac OS.		Interfaces for PYTHON and MATLAB are included in which exploits these packages' wide range of functions and allows researchers with different programming knowledge to benefit from this software.

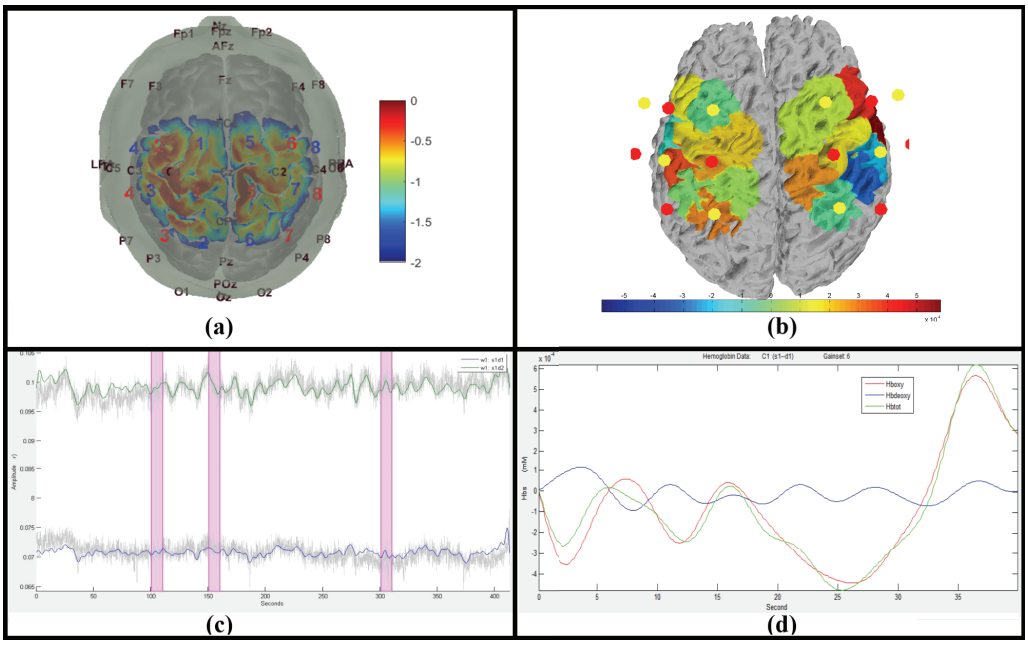

Figure 8: NIRS data visualization and analysis by employing different software a) 2D visualization of brain activation by employing HOMER2, b) 2D visualization of brain activation, c) NIRS data filtering and d) blood oxygenation change calculation utilizing NIRSlab.

Conclusion:

Although near-infrared spectroscopy is established and has widespread applications in non-destructive testing of agricultural, pharmaceutical or textile products[240], its more exciting applications deal with its ability to provide a versatile window into the processes of the human brain. fNIRS technology has come a long way from single optode systems capable of limited spectroscopy to multi-channel miniaturized wireless systems, easily deployed in natural settings and no longer limited by bulky lab systems. The relative simplicity of setting up and utilizing these systems to gain insightful data from elegant experiments is further supported by a range of high-powered software suites. They form a crucial link between experiment and understanding and build the backbone of many current publications. fNIRS technology and method is thus widely gaining ground in the health sciences and proved capable in more than intra-ICU monitoring applications. Due to its versatility and its increased coverage of the human cortex, fNIRS has

found its way into basic neuroscience shedding light on the most important activation patterns and connectivities in the cortex – on the very processes of being human. Most recently, a strong interest in combining fNIRS with other modalities such as EEG and fMRI with objectives ranging from validation to data fusion to brain-computer interfaces has grown, allowing fNIRS to widen our non-invasive and minimally obtrusive windows to the human brain.

The future prospects of fNIRS are promising, not least because the number of commercially available fNIRS systems has grown rapidly in the last decade. Nevertheless, further improvements in fidelity, sensors and analysis methods will expand its applications to true bedside and emergency health care as well.

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