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Abstract. We highlight a significant problem that needs to be considered and addressed when performing functional near-infrared spectroscopy (fNIRS) studies, namely the possibility of inadvertently measuring fNIRS hemodynamic responses that are not due to neurovascular coupling. These can be misinterpreted as brain activity, i.e., "false positives" (errors caused by wrongly assigning a detected hemodynamic response to functional brain activity), or mask brain activity, i.e., "false negatives" (errors caused by wrongly assigning a not observed hemodynamic response in the presence of functional brain activity). Here, we summarize the possible physiological origins of these issues and suggest ways to avoid and remove them. [DOI: 10.1117/1.NPh.3.3.031405]

Keywords functional near-infrared spectroscopy; hemodynamic response.

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1 Changes in Functional Near-Infrared Spectroscopy Signals Represent Neuronal Activity—But Not Always

Functional near-infrared spectroscopy (fNIRS) is an increasingly used method in neuroscience research. It is a hemodynamic-based functional brain imaging technique, like functional magnetic resonance imaging (fMRI), relying on the detection of neurovascular coupling to infer changes in neuronal activity. Neurovascular coupling refers to the fact that changes in local neural activity are correlated with changes in local cerebral blood flow (CBF). The magnitude and spatial location of CBF changes are tightly linked to changes in neuronal activity.[1,2] Neuronal excitation causes changes in oxidative metabolism (neurometabolic coupling) which is linked to neurovascular coupling; blood vessel dilation causes an increase in CBF and cerebral blood volume that overcompensates the metabolic demand. This oversupply of oxygenated blood (functional hyperemia), the cause of which is still a matter of debate,[3] causes the fNIRS measurement of oxygenated hemoglobin ($[O_2Hb]$) to increase, the deoxygenated hemoglobin ([HHb]) to decrease, and the total hemoglobin ($[tHb] = [O_2Hb] + [HHb]$) to increase. Although this physiological phenomenon is well known and expected, often it is not completely presented in publications (authors tend to focus only on the $[O_2Hb]$ response). In addition, there is a possibility that this pattern of $[O_2Hb]$, [HHb], and [tHb] changes may not uniquely be caused by neurovascular coupling.

The aims of this opinion article are (i) to define and highlight the issue of false positives and negatives in fNIRS, (ii) to describe some of the challenges in recognizing these, and finally (iii) to suggest a way forward in resolving this issue. While recently a substantial number of research and review articles (including ours) have discussed this issue; here, we will attempt to offer a concise and summarized view of the topic that will also represent our opinion. (In this opinion article, we explicitly assume that fNIRS instrumentation can accurately monitor and resolve the concentration changes in $[O_2Hb]$ and [HHb]. The technical specifications and configuration of the fNIRS instrumentation (e.g., type and number of light sources and of photodetectors) can dictate the performance and accuracy of the device in monitoring $[O_2Hb]$ and [HHb]. One aspect, in particular, is the number and choice of wavelengths used for resolving $[O_2Hb]$ and [HHb] as it has been reported that certain wavelength combinations can result in "crosstalk" between the chromophores. By crosstalk we mean to describe the phenomena that a genuine change in one chromophore concentration is also inducing a spurious measured concentration change in another; for a more detailed discussion of this issue see section "Selection of optimum wavelengths" in the recent review article by Scholkmann et al.[4] and the recent study by Arifler et al.[5] It is considered good practice for authors to report the wavelengths of their fNIRS instrument used in their study).

2 What Are False Positives and False Negatives in Functional Near-Infrared Spectroscopy?

We use the terms "false positives" or "false negatives" to state that during a functional experiment a change in the fNIRS signals ($[O_2Hb]$, [HHb], and [tHb]) over a measurement channel that overlays a certain area of the cortex might not be due to the effect of neurovascular coupling, but could also be due to changes in (i) intracerebral hemodynamics caused by task-related systemic activity and/or (ii) extracerebral hemodynamics. These task-related systemic changes may mimic the

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presence of a neuronally induced hemodynamic response (false positives), or can attenuate (mask) the neuronally induced hemodynamic response (false negatives).

The presence of false positives and false negatives is not a problem exclusively for fNIRS, but is an issue for all hemodynamic-based functional brain imaging techniques including fMRI. However, the sensitivity of fNIRS to hemodynamic and oxygenation changes in the extracerebral (superficial) compartment is a particular confounding factor of this technique.

fNIRS is increasingly being used to investigate complicated cognitive functions, some of which can produce significant changes in systemic variables, which lead to non-neuronal driven changes in hemodynamics/oxygenation happening in the cerebral and extracerebral compartment mainly associated with changes in heart rate (HR), blood pressure, breathing rate, CO_2 concentration in the blood, and autonomic nervous system (ANS) activity.

The presence of these systemic effects, and in an extension their consequences for fNIRS measurements, largely depends on the functional protocol (e.g., passive versus active tasks) and the individual subject studied (e.g., two people can react very differently to the same stressor).

3 What Factors Cause False Positives and False Negatives in Functional Near-Infrared Spectroscopy?

As described recently in detail,[4] each fNIRS signal comprises different components that can be classified according to their (i) source (cerebral versus extracerebral), (ii) stimulus/task relation (evoked versus nonevoked), and (iii) physiological cause (neuronal versus systemic). The monitoring of the hemodynamic response due to neurovascular coupling that fNIRS aims to detect is only one of these components (i.e., the component neuronal/task-evoked/cerebral), while all the other components are the physiological noise that act as confounders in fNIRS studies (see Fig. 1).

Task-evoked changes in respiration and thus partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$), a reliable and accurate estimate of the partial pressure of carbon dioxide in the arterial blood ($PaCO_2$), was demonstrated in multimodal fNIRS studies (i.e., fNIRS studies including the measurements of systemic physiological changes using additional monitoring devices) investigating different tasks, e.g., speaking (maximal effect: $\Delta PaCO_2 \approx -9$ mm Hg),[6] mental arithmetic ($\Delta PaCO_2 \approx 0.5$ to 3 mm Hg),[6,7] and inner speech (i.e., speaking without vocalization, $\Delta PaCO_2 \approx -0.5$ mm Hg).[8]

Task-evoked change in mean arterial blood pressure (ΔMAP) has been demonstrated for a number of different experimental fNIRS protocols, e.g., arm-raising ($\Delta MAP \approx 6$ mm Hg),[9] visual stimulation ($\Delta MAP \approx 2$ mm Hg),[10] anagram solving ($\Delta MAP \approx 3$ to 5 mm Hg,[11,12] 6 to 7 mm Hg),[13] or hypercapnia ($\Delta MAP \approx 4$ mm Hg),[14] hypocapnia ($\Delta MAP \approx 4.5$ mm Hg;[14] $\Delta MAP \approx -10$ mm Hg),[15] hypoxemia ($\Delta MAP \approx 2$ mm Hg),[15] and hyperoxia ($\Delta MAP \approx 2$ mm Hg).[15]

Since MAP is a function of cardiac output (CO), systemic vascular resistance (SVR), and central venous pressure (CVP) ($MAP = CO \times SVR + CVP$), and since CO is the product of HR and stroke volume (SV) ($CO = HR \times SV$), task-evoked changes in HR and SVR will also have an effect on MAP.

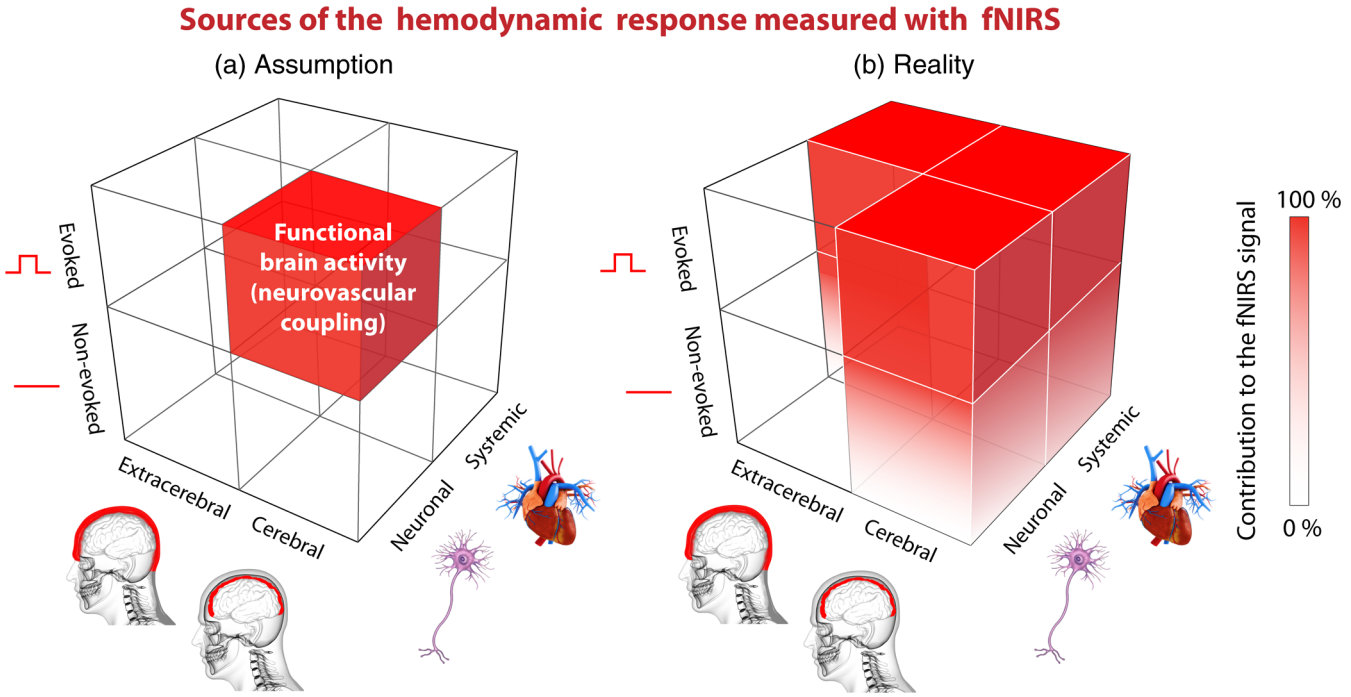

Fig. 1 Visualization of the six components of the fNIRS signals ([O_2Hb], [HHb], [tHb]). (a) An erroneous assumption is that the fNIRS signal represents only changes associated with functional brain activity due to neurovascular coupling. (b) In reality, the fNIRS signals comprise six components so that five components are potentially confounders in every fNIRS study. The contribution of the components to the fNIRS signal is visualized by color-coding (red: 100%, white: 0%). The nonevoked/cerebral/neuronal and nonevoked/cerebral/systemic components can contribute to the fNIRS signal to a significant degree; however, the evoked changes can be generally stronger in an experimental paradigm assessing functional, task-related, brain-activity.

A task-evoked change in the activity of the ANS is another factor potentially able to influence fNIRS signals since the ANS has a direct influence on CO, SVR, and in particular on blood flow (BF) in the extracerebral layer. All three of these physiological variables (i.e., respiration leading to CO_2 changes, blood pressure, and ANS activity) affect the BF both in the cerebral compartment and in the extracerebral compartment (i.e., the scalp BF, ScBF). Changes in either the CBF or ScBF may have an impact on the recorded fNIRS signals.

The coupling strength between changes in blood pressure, CO_2, or ANS activity with BF changes depends at least on two factors: (i) the type of compartment (cerebral versus extracerebral) and (ii) the speed of change (slow versus fast, i.e., minutes range versus seconds range). Table 1 shows the coupling strength values based on the present physiological knowledge. It is interesting to notice that the coupling is generally quite strong in all cases except between slow changes in blood pressure and CBF, where the coupling is limited by dynamic cerebral autoregulation. Note, however, that the efficiency of cerebral autoregulation has also been controversially discussed in the recent literature, with doubt cast on the shape and boundaries of the CBF-transfer function as traditionally described.[16]

Extracerebral confounders are an issue because the diffusely reflected light in fNIRS is attenuated by a significant degree in the extracerebral tissue layer of the head and not only in the cerebral compartment.[18–21] The penetration depth (D) of light emitted in the head tissue layers is substantially less than half the source–detector separation (SDS) as predicted by the diffusion approximation[22] (i.e., $D < 0.5$ SDS). Changes in hemodynamics and oxygenation in the extracerebral layer will have a strong impact on the fNIRS signals. This issue has been known in principle for many years (e.g., Refs. 23–27) but the real significance becoming increasingly apparent with a growing number of studies showing that fNIRS is affected by these extracerebral changes and also that the extracerebral hemodynamic changes are spatially heterogeneous.[28–31]

4 How Can We Avoid and/or Remove False Positives and Negatives in Functional Near-Infrared Spectroscopy Studies?

False positives and negatives can be avoided, minimized, and/or dealt with by adopting one or more of the following approaches (listed in no particular order of significance): (i) careful experimental design (e.g., avoiding tasks with strong systemic activation, avoiding additional stress and discomfort for the subject), (ii) depth-resolved fNIRS techniques [e.g., multidistance (MD) measurements], (iii) appropriate statistical and/or signal processing methods [e.g., independent-component analysis (ICA), principal component analysis (PCA), adaptive filtering, analysis with a general linear model (GLM) with systemic regressors], (iv) multimodal monitoring (e.g., measurement of local ScBF or measurements of signals from the systemic physiology).

(i) *Designing the fNIRS protocol:* As in any neuroscience study, careful consideration should be taken in selecting the trial design, defining the experimental tasks, designing the contrasts, and choosing the repetition and duration times. The fundamental aspect to consider when designing an fNIRS protocol is that brain activation must be assessed relative to a control condition or baseline. The functional protocol needs to have the best possible baseline condition to subtract out "spurious" hemodynamic/oxygenation responses from the experimental task. fNIRS protocols must thus create a high contrast between either the experimental condition and the baseline condition or between two experimental conditions.

(ii) *fNIRS instrumentation:* Concerning fNIRS hardware optimization, there are different possibilities to reduce the influence of physiological confounders in the recorded signals. Time-domain fNIRS devices offer the possibility to distinguish between measured changes in the cerebral or extracerebral layer automatically due to the measured temporal photon point-spread function. New algorithms and methods to separate the contribution from the extracerebral and cerebral compartment were published recently, e.g., Refs. 32 and 33. For continuous-wave fNIRS (cw-fNIRS) and frequency-domain fNIRS systems, MD measurements enable increased sensitivity to the cerebral compartment by calculating the slopes of the decrease in light intensity as a function of SDSs. The MD approach can be implemented via two main techniques "spatially resolved spectroscopy" and the "self-calibrating method" (for a more detailed description see the recently published review.[4]) Another possibility is to record with extra short channels. The signal from these short channels can then be used to regress out influences from the extracerebral layer.[34] The optimal SDS for such short-channel regression was determined recently to be 8.4 mm for adults and 2.15 mm for a term-age infant.[35] The number and location of short channels used should be guided by anatomical/physiological constraints, as discussed recently by Zhang et al.[28] Specific signal processing and data analysis methods to reduce physiological confounding include approaches that use information from the short channels or that are based on the different dynamics of [O_2Hb]

Table 1 Overview (according to our own opinion based on published literature and own experience) of the coupling strength between BF and systemic variables, i.e., blood pressure, $PaCO_2$, and ANS activity. "*" indicates that at present the coupling strength is not known or controversially discussed. For a critical review of the coupling factors, see Refs. 16 and 17. Coupling between hemodynamic changes in the cerebral layer concern the CBF, whereas changes in the extracerebral compartment are linked to scalp BF.

	Coupling strength between MAP and BF		Coupling strength between $PaCO_2$ and BF		
Tissue compartment	Slow change	Fast change	Slow change	Fast change	Coupling strength between ANS and BF
Extracerebral (→ScBF)	High	High	*	*	High
Cerebral (→CBF)	Low	High	High	High	Low

and [HHb]. For a general overview of these methods, see the section "Signal analysis methods to extract the functional brain activity" in Ref. 4 as well as the review of Tak and Ye.[36]

Another aspect to consider concerns the optode attachment. It is generally assumed that a tight attachment of the optodes to the scalp can eliminate or minimize the extracerebral contamination by squeezing out the scalp blood. While this is certainly true and may help to reduce the influence of the hemodynamic changes in the extracerebral layer, it must also be noted that the high probe pressure on the head can result in discomfort and local pain which activates the ANS leading to an increased strength of the systemic confounders.

The probe pressure can also be varied and, in combination with an MD measurement, an approach recently developed by Baker et al.[37] can be used to remove the hemodynamic influences from the extracerebral layer from the fNIRS signals.

(iii) *Analysis tools and approaches*: The analysis of all these signals within a single framework is challenging. One possibility is to employ a GLM where the physiological signals are additional regressors, as demonstrated already in a few studies.[29,30,38] In addition, the simultaneous use of [O_2Hb] and [HHb] in the same model may enhance the correct detection of the hemodynamic response and improve the statistics. Another useful approach is to use ICA in combination with MD measurements as recently shown.[39–42] Combining the ICA approach with systemic measurements is promising.[43] In a recent study, Kirilina et al.[30] proposed a denoising algorithm that uses regressors derived from the time dependency between the fNIRS and systemic signals. And the usefulness to use PCA in order to separate the global and local components in fNIRS signals was shown recently.[44]

Since the strength of task-evoked systemic physiological changes depends significantly on the specific psychophysiological state of the subject, single-level data analysis might also be important to avoid false positives and negatives. A combination of single-level and group-level analyses may be the optimal approach.

(iv) *Multimodal monitoring*: The fNIRS experimental environment allows easy interfacing with additional instruments that can monitor systemic variables during the functional experiment. These can be devices enabling the measurement of MAP, respiration, $P_{ET}CO_2$, HR, skin conductance, and ScBF. These measurements may then be used to identify individuals that exhibit large task-related systemic changes that can interfere with the brain functional response and thus exclude them from further analysis. These measurements can also be employed to validate the systemic similarities between the baseline and experimental task conditions. Importantly, these measurements can be used in statistical and signal processing methods as regressors and independent components to account for the non-neuronal-related functional response.

Although we have focused above on fNIRS monitoring during functional paradigms, there are additional issues to be considered when there is no functional contrast, i.e., in a study assessing the resting-state brain activity. Both neuronal and non-neuronal hemodynamic generating factors are present during the resting-state and should be considered when designing the experimental protocol [e.g., possible environmental noise, change in resting-state activity due to increased drowsiness during the experiment, increased subject stress due to the fNIRS optode(s) attachment which can cause unpleasant sensations and thus induce a change in ANS activity]. When conducting resting-state fNIRS studies one should be aware of the fact that more and more studies report the influence of systemic hemodynamic changes on resting-state fNIRS and fMRI signals. For example, recently the findings of Tong et al.[45] suggested, based on an fMRI study, that the resting-state "may reflect, to some extent, vascular anatomy associated with systemic fluctuations, rather than neuronal connectivity." Also, Zhu et al.[46] recently concluded from their resting-state (rs) fMRI findings that they "raise a critical question of whether a large portion of rs-fMRI signals can be attributed to the vascular effects produced from upstream changes in cerebral hemodynamics." Since fNIRS signals are additionally affected by changes in ScBF, careful interpretation of resting-state fNIRS signals is warranted. For resting-state fNIRS studies, it thus makes sense to name the measured connectivity accordingly, i.e., "vascular connectivity"[47] or "sum of intracerebral and extracerebral hemodynamic functional connectivity."[48]

5 Way Forward

There are many factors that should be considered in order to improve future fNIRS studies. What needs to be recognized immediately is the imperativeness of considering the time dynamics of both [O_2Hb] and [HHb] signals in fNIRS data analysis, and their interrelationship before formulating any neuroscientific conclusions. Reporting both [O_2Hb] and [HHb] changes, instead of only one of them, allows better physiological interpretation of the functional experimental results (e.g., when both [O_2Hb] and [HHb] show a strong increase and/or when both [O_2Hb] and [HHb] show a strong decrease during the task periods then the signals are probably confounded by systemic changes). However observing the typical pattern of the hemodynamic response during functional activation (i.e., increase in [O_2Hb] and decrease in [HHb]) is not sufficient for concluding that no systemic confounding effect took place. Synchronous measurements of systemic physiology, currently easily achievable with highly portable and relatively inexpensive instrumentation, can then also provide an accessible way to regress out systemic interference and identify individuals with strong task-related systemic changes.

Both [O_2Hb] and [HHb] can be influenced by systemic physiological changes (occurring in the cerebral and/or the extracerebral compartment). However, the degree of the influence often varies and also depends on the origin and type of the systemic change. Kirilina et al.[29] demonstrated that [O_2Hb] signals are more strongly affected by global processes in both extracerebral and intracerebral compartments, and local scalp BF regulation, while [HHb] signals are less contaminated by extracerebral processes. The physiological reason for this may be the fact that arterioles are enervated to a larger degree by the fibers from the sympathetic nervous system than the venules;[49,50] during activity of the ANS, the [O_2Hb] signals seem to be more affected than the [HHb] signals—a conclusion also supported by recent findings of Haeussinger et al.[51] In addition, it is well known that cardiac oscillations are more

prominent in the [O_2Hb] compared to the [HHb]. Although there is evidence of the [HHb] signal to be less contaminated by systemic changes, this does not imply that the [HHb] signal is not confounded by systemic changes.

In the present state of fNIRS research, most studies are using cw-fNIRS systems that implement only measurements at a fixed 3 cm SDS. In this case, it is recommended that ScBF effects be corrected by either (i) adding additional short channels and using them to regress out the superficial hemodynamics (by using one of the short-channel regression methods developed so far, e.g., Refs. 39, 41, and 52–56), (ii) by calculating the mean signal over all channels and using this channel as the superficial regressor (e.g., Refs. 57 and 58), or (iii) by combining both of these approaches.[59]

More and more fNIRS instrumentation companies currently provide alternative and/or add-on solutions to allow the implementation of MD probe geometries with subsequent short-channel regression frameworks. In the near future, such instrumentation advances will allow fNIRS measurements that better represent the neuronal, cerebral, and evoked responses.

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