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Real-time monitoring of neurocritical patients with diffuse optical spectroscopies

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Cover Letter

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Dr. Jaydev Upponi
Science Editor
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Ref.: Submission of manuscript for publication in the JoVE

Dear Dr. Upponi:

We are enthusiastically submitting our manuscript, entitled "Real-time monitoring of neurocritical patients with diffuse optical spectroscopies" for consideration for publication in the Journal of Visualized Experiments (JoVE). In this work, we propose a complete potential solution for brain monitoring at the neuro intensive care unit (neuro-ICU). Our approach is based on an optical device that employs diffuse optical spectroscopies with custom made optical sensors and a friendly graphical user interface (GUI) for clinicians. By shining near-infrared light onto the scalp, the system is capable of monitoring cerebral oxygenation, blood flow and metabolism of neurocritical patients non-invasively, in real-time and at the bedside. These are important information that clinicians can assess to base their clinical treatment in the future.

We believe this work will be of great interest to the readership of *JoVE* as it provides a new perspective on the use of diffuse optics for monitoring neurocritical patients. Although diffuse optical spectroscopies have been previously used in clinical settings, to our best knowledge no studies have yet provided a complete environment to make the technique feasible in the neuro-ICU. In particular, the real-time friendly GUI we developed was inspired by current instruments already available to clinicians at the neuro-ICU, which facilitates the adoption of the optical system by hospital staff, such as neurointensivists and nurses - which can increase the use of diffuse optical systems at the clinic.

This manuscript has not been submitted to another journal. The results presented are novel. The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: RC Mesquita has one pending patent application and two other patents relevant to this work (United States patents 10,342,488 and 10,064,554). No author currently receives royalties or payments from these patents. Any clinical investigator and/or expert in diffuse optics would be able to review the current work.

Sincerely yours,

Dr. Rodrigo M. Forti

Prof. Rickson C. Mesquita

TITLE:

Real-Time Monitoring of Neurocritical Patients with Diffuse Optical Spectroscopies

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diffuse optical spectroscopy, diffuse correlation spectroscopy, cerebral blood flow, cerebrovascular disorders, neurocritical monitoring, stroke, intensive care unit

SUMMARY:

Presented here is a protocol for non-invasively monitoring cerebral hemodynamics of neurocritical patients in real-time and at the bedside using diffuse optics. Specifically, the proposed protocol uses a hybrid diffuse optical systems to detect and display real-time information on cerebral oxygenation, cerebral blood flow and cerebral metabolism.

ABSTRACT:

Neurophysiological monitoring is an important goal in the treatment of neurocritical patients, as it may prevent secondary damage and directly impact morbidity and mortality rates. However, there is currently a lack of suitable non-invasive, real-time technologies for continuous monitoring of cerebral physiology at the bedside. Diffuse optical techniques have been proposed as a potential tool for bedside measurements of cerebral blood flow and cerebral oxygenation in case of neurocritical patients. Diffuse optical spectroscopies have been previously explored to monitor patients in several clinical scenarios ranging from neonatal monitoring to

cerebrovascular interventions in adults. However, the feasibility of the technique to aid clinicians by providing real-time information at the bedside remains largely unaddressed. Here, we report the translation of a diffuse optical system for continuous real-time monitoring of cerebral blood flow, cerebral oxygenation, and cerebral oxygen metabolism during intensive care. The real-time feature of the instrument could enable treatment strategies based on patient-specific cerebral physiology rather than relying on surrogate metrics, such as arterial blood pressure. By providing real-time information on the cerebral circulation at different time scales with relatively cheap and portable instrumentation, this approach may be especially useful in low-budget hospitals, in remote areas and in monitoring in open fields (e.g., defense and sports).

INTRODUCTION:

Most of the complications that lead to poor outcomes for critically ill neurologic patients are related to secondary injuries caused by cerebral hemodynamic impairments. Therefore, monitoring cerebral physiology of these patients may directly impact morbidity and mortality rates[1–7]. Currently, however, there is no established clinical tool for the continuous real-time noninvasive monitoring of cerebral physiology in case of neurocritical patients at the bedside. Among the potential candidates, diffuse optical techniques have recently been proposed as a promising tool to fill in this gap[8–11]. By measuring the slow changes (i.e., on the order of tens to hundreds of ms) of the diffusively scattered near-infrared light (~650-900 nm) from the scalp, diffuse optical spectroscopy (DOS) can measure concentrations of the main chromophores in the brain, such as cerebral oxy- (HbO) and deoxy-hemoglobin (HbR)[12,13]. Additionally, it is possible to measure cerebral blood flow (CBF) with diffuse correlation spectroscopy (DCS)[10,14–17] by quantifying the rapid fluctuations in light intensity (i.e., from a few μs to a few ms). When combined, DOS and DCS can also provide an estimate of the cerebral metabolic rate of oxygen ($CMRO_2$)[18–20].

The combination of DOS and DCS has been explored to monitor patients in several pre-clinical and clinical scenarios. For example, diffuse optics has been shown to provide relevant clinical information for critically-ill neonates[21–24], including during cardiac surgeries to treat heart defects[23,25–28]. In addition, several authors have explored the use of diffuse optics to assess cerebral hemodynamics during different cerebrovascular interventions, such as carotid endarterectomy[29–31], thrombolytic treatments for stroke[32], head-of-bed manipulations[33–35], cardiopulmonary resuscitation[36], and others[37–39]. When continuous blood pressure monitoring is also available, diffuse optics can be used to monitor cerebral autoregulation, both in healthy and in critically ill subjects[11,40–42], as well to assess the critical closing pressure of the cerebral circulation[43]. Several authors have validated CBF measurements with DCS against different gold standard CBF measures[18], while $CMRO_2$ measured with diffuse optics has been shown to be a useful parameter for neurocritical monitoring[8,18,23,24,28,43–45]. In addition, previous studies have validated the optically-derived cerebral hemodynamic parameters for long-term monitoring of neurocritical patients[8–11], including for the prediction of hypoxic[46–48] and ischemic events[8].

The reliability of the diffuse optical techniques to provide valuable real-time information during longitudinal measurements as well as during clinical interventions remains largely unaddressed. The use of a standalone DOS system was previously compared to invasive brain tissue oxygen

tension monitors, and DOS was deemed to not have a sufficient sensibility to replace the invasive monitors. However, apart from using relatively small populations, the direct comparison of the invasive and non-invasive monitors may be misguided as each technique probe different volumes containing different parts of the cerebral vasculature. Even though these studies ultimately concluded that diffuse optics is not a replacement for the invasive monitors, in both studies DOS achieved a moderate-to-good accuracy, which may be sufficient for cases and/or places wherein invasive monitors are not available.

Relative to other approaches, the key advantage of diffuse optics is its ability to simultaneously measure blood flow and tissue blood oxygenation non-invasively (and continuously) at the bedside using portable instrumentation. Compared to Transcranial Doppler ultrasound (TCD), DCS has an additional advantage: it measures perfusion at the tissue level, whereas TCD measures cerebral blood flow velocity in large arteries at the base of the brain. This distinction may be particularly important when evaluating steno-occlusive diseases in which both proximal large artery flow and leptomeningeal collaterals contribute to perfusion. Optical techniques also have advantages when compared to other traditional imaging modalities, such as Positron-Emission Tomography (PET) and Magnetic Resonance Imaging (MRI). In addition to simultaneously providing direct measures of both CBF and HbO/HbR concentrations, which is not possible with MRI or PET alone, optical monitoring also provides significantly better temporal resolution, allowing, for example, the assessment of dynamic cerebral autoregulation[40–42] and the assessment dynamically evolving hemodynamical changes. Moreover, diffuse optical instrumentation is inexpensive and portable in comparison to PET and MRI, which is a critical advantage given the high burden of vascular disease in lower- and middle-income countries.

The protocol proposed here is an environment for real-time bedside neuromonitoring of patients at the intensive care unit (ICU). The protocol uses a hybrid optical device together with a clinical-friendly graphical user interface (GUI) and customized optical sensors to probe the patients (**Figure 1**). The hybrid system employed for showcasing this protocol combines two diffuse optical spectroscopies from independent modules: a commercial frequency-domain (FD-) DOS module and a homemade DCS module (**Figure 1A**). The FD-DOS module[49,50] consists of 4 photomultiplier tubes (PMTs) and 32 laser diodes emitting at four different wavelengths (690, 704, 750 and 850 nm). The DCS module consists of a long-coherence laser emitting at 785 nm, 16 single-photon counters as detectors and a correlator board. The sampling frequency for the FD-DOS module is 10 Hz, and the maximum sampling frequency for the DCS module is 3 Hz. To integrate the FD-DOS and DCS modules, a microcontroller was programmed inside our control software to automatically switch between each module. The microcontroller is responsible for turning the FD-DOS and DCS lasers on and off, as well as the FD-DOS detectors to allow interleaved measurements of each module. In total, the proposed system can collect one combined FD-DOS and DCS sample every 0.5 to 5s, depending on the signal-to-noise ratio (SNR) requirements (longer collection times leads to better SNR). To couple the light to the forehead, we developed a 3D-printed optical probe that can be customized for each patient (**Figure 1B**), with source-detector separations varying between 0.8 and 4.0 cm. The standard source-detector separations used in the examples presented here are 2.5 cm for DCS and 1.5, 2.0, 2.5 and 3.0 cm for FD-DOS.

The main feature of the protocol presented in this study is the development of a real-time interface that can both control the hardware with a friendly GUI and display the main cerebral physiology parameters in real-time under different temporal windows (**Figure 1C**). The real-time analysis pipeline developed within the proposed GUI is fast and takes less than 50 ms to compute the optical parameters (see the **Supplementary Material** for more details). The GUI was inspired by current clinical instruments already available at the neuro-ICU, and it was adapted through extensive feedback by clinical users during the translation of the system to the neuro-ICU. Consequently, the real-time GUI facilitated the adoption of the optical system by regular hospital staff, such as neurointensivists and nurses. The wide adoption of diffuse optics as a clinical research tool has the potential to enhance its ability to monitor physiologically meaningful data and can ultimately demonstrate that diffuse optics is a good option for non-invasively monitoring neurocritical patients in real-time.

PROTOCOL:

The protocol was approved by the local committee of the University of Campinas (protocol number 56602516.2.0000.5404). Written informed consent was obtained from the patient or a legal representative prior to the measurements. We monitored patients that were admitted to the Clinics Hospital at the University of Campinas with a diagnosis of either ischemic stroke or a subarachnoid hemorrhage affecting the anterior circulation. Patients with ischemic strokes affecting the posterior circulation, patients with decompressive craniectomies due to elevated intracranial pressure and patients with other neurodegenerative diseases (dementia, Parkinson's or any other disease that can be associated with cortical atrophy) were excluded from the study protocol.

1. Preparations before moving the system to the ICU

1.1. Connect all the fibers to the relevant lasers and detectors, and make sure they are properly attached to the optical probe (**Figure 1B**).

1.2. Check that optical probe is covered with a black cloth to avoid the lasers shining in the room.

1.3. Turn the system power switch to the 'ON' position. After powering the system, wait 30s and then turn the DCS laser key switch to the 'ON' position. The FD-DOS lasers are automatically turned on when the system is powered.

1.4. While the system is being prepared, obtain consent from either the participant or a legal representative. After obtaining consent, bring the cart to the patient's room.

NOTE: Since the hybrid system has a built-in battery that lasts up to 45 min, it does not need to be turned off during transport.

2. Calibration and gain settings of the DOS system

2.1. Upon arrival at the ICU, turn off the DCS laser by switching the key to the 'OFF' position.

2.2. Starting with the solid phantom marked '**Calibrate**', run the calibration process on the FD-DOS software (BOXY, ISS) by following the steps below.

2.2.1. On the '**File**' menu, load the appropriate settings file for the probe being used by clicking on the '**Load settings file**' option.

2.2.2. Place the probe on the curved side of the phantom, ensuring a good contact with the surface and then optimize the PMT bias voltage by clicking on the '**Optimize All Detectors**' button in the FD-DOS software.

2.2.3. Run the calibration for multiple source-detector separations by clicking on the option '**Calc. Waveform Calib. Values for Optical Props. and Multiple Distances**' from the '**Calibrate**' menu.

2.2.4. Open the '**User-defined Calculations**' option from the '**Text-Mon**' menu to check that the measured optical properties match the prespecified values (written in the solid phantom), and that the fitting R^2 is close to one.

2.3. Repeat the steps above (except step 2.2.3) to measure the optical properties of the phantom marked as '**Check**' to ensure the calibration was adequate. The measured optical properties should match, within 10%, the values specified in the phantoms.

CAUTION: Make sure to turn off PMTs (by clicking on the '**All Detectors OFF**' button) every time the probe is moved to avoid damaging PMTs due to direct illumination from ambient light.

2.4. If the calibration is not adequate, re-run the calibration process (steps 2.2 and 2.3). Ensuring a good calibration of the FD-DOS system is essential to the validity of the FD-DOS measurements.

3. Preparation of the participant at the bedside

3.1. Use sanitizing wipes to clean both the probe and patient forehead.

3.2. Place the double-sided tape on the probe (**Figure 1B**), ensuring the tape is not in direct contact with the optical fiber tips.

3.3. Place a laser safety googles on the subject.

3.4. Place the probes over the region-of-interest (ROI) and wrap the elastic straps around the subject's head. Although not strictly necessary for FD-DOS and DCS, it is advisable to cover the optical probe with a black cloth or black bandage to reduce noise due to ambient light.

NOTE: It is important to assure that the elastic strap is neither too tight, nor too loose. If the strap is too tight it may cause significant discomfort to the patient, and if the strap is too loose it may lead to poor data quality as the double-sided tape is not strong enough to keep the probes in place.

3.5. After the probe is properly secured to the patient forehead, turn on the DCS laser by switching the key to the 'ON' position.

CAUTION: The DCS system uses a Class 3B laser which is hazardous for eye exposure. It is very important to only turn on the lasers when the probe is properly attached to the patient's forehead.

4. Data quality assessment

4.1. Before starting to acquire data with the GUI, write the DCS source-detector separations in the '**Settings**' tab of the GUI.

NOTE: The DCS system does not require a calibration step, but the proper input of the source-detector separations is necessary for the real-time analysis (see **Supplementary Material** for details).

4.2. Start the acquisition software by pressing the '**Start**' button in the GUI and check the DOS signal in the FD-DOS software:

4.2.1. Click on the '**Optimize All Detectors**' button in the FD-DOS software to optimize the PMT bias voltage.

4.2.2. Check the optical properties and the R^2 of the DOS fitting in the '**User Defined Calculation**' option from the '**Text-Mon**' menu. The R^2 coefficient should be close to unity and, as a rule of thumb, the absorption coefficient of human patients should be within 0.05 and 0.2 cm^{-1}, while the scattering coefficient should be within 6 and 13 cm^{-1} [13].

4.3. Check the DCS signal in the '**Correlation curves**' tab of the GUI.

4.3.1. Turn on the DCS detectors by turning the switches to the 'ON' position.

4.3.2. Ensure that each DCS detector is measuring an adequate light intensity. As a rule of thumb more than 10 kHz is required.

4.3.3. If the measured intensity is higher than 800 kHz, use a neutral density filter to reduce the photon counts to avoid damaging the detectors. This is typically a problem for shorter (< 1 cm) source-detector separations.

NOTE: Apart from potentially damaging the DCS detectors, photon counts higher than 800 kHz

may also bring errors due to non-linear effects in the detector.

4.3.4. Check the autocorrelation curves to ensure a good skin coupling (see the **Representative Results** and **Figure 2)** and reposition the optical probe if necessary.

4.3.5. If the repositioning of the probe was necessary in the previous step, repeat Steps 4.2 and 4.3. These steps may need to be repeated multiple times.

NOTE: The DCS and the FD-DOS detectors must be turned off each time the probe is moved. To turn the DCS detectors off, manually move the switches to the 'OFF' position. The FD-DOS detector is turned off by clicking the 'All Detectors OFF' button in the FD-DOS software.

4.4. When a good contact between the probe and the skin is achieved, stop the data collection by clicking the '**Stop**' button in the GUI. Then, set the experiment and patient identifiers in the '**Folder**' textbox and write the ROI name in the '**File name**' textbox.

4.5. Start the data acquisition by pressing the '**Start**' button in the GUI.

4.6. Collect data in the first ROI for as long as required by the protocol. If necessary, move the probe to the other ROIs and repeat the measurement.

NOTE: The monitoring period may vary depending on the study goals.

5. Considerations for the experimenter during the measurement

5.1. After starting the measurement, write in the '**Experiment Info**' tab of the GUI the relevant patient information (e.g., type and location of the injury, drugs being administered, age, sex, etc.).

5.2. Ensure that any relevant event that occurred during the monitoring period is marked by clicking the '**Mark**' button on the GUI. After each mark, make sure to write the event description in the '**Experiment Info**' tab of the GUI.

6. Stop data collection

6.1. Stop the data collection by pressing the '**Stop**' button in the GUI.

6.2. Stop the FD-DOS software by pressing the stop data acquisition and recording button represented as two red squares in the FD-DOS software.

6.3. Turn off the DCS detectors by flipping the switches to the 'OFF' position and turn off the DCS laser by turning the key to the 'OFF' position.

6.4. Turn off the PMTs of the FD-DOS module by clicking the 'All Detectors OFF' button.

6.5. Remove the probe from the patient's head and remove the double-sided tape from the probe. Then, clean the probe with sanitizing wipes.

6.6. Repeat the measurement of the optical properties of each solid phantom as soon as possible to ensure the calibration remained adequate throughout the monitoring session (see step 4.2.2).

NOTE: Ideally, the calibration step should be done right after removing the optical probes from the patient head (step 6.6). However, due to timing issues, in the examples presented in the next section this was done in the storage facility.

6.7. Clean the system and its accessories with sanitizing wipes.

6.8. Wheel the cart back to the storage room.

REPRESENTATIVE RESULTS:

Ideally, the normalized autocorrelation curves obtained with the DCS module should be approximately 1.5 at the zero delay-time extrapolation (when using single-mode fibers[14]), and the curves should decay to 1 at longer delay times. The curve should be smooth, and it should have a faster decay for the longer source-detector separations. An example of a good autocorrelation is shown in **Figure 2A**. **Figure 2B** shows an example of a bad auto-correlation curve; in this example it is not possible to distinguish the curves for the different source-detector separations. **Figure 2C** shows another example of a bad auto-correlation curve, in which the tail of the curve did not match the model used. The issue in both curves (**Figure 2B,C**) are related either to a bad coupling of the probe onto the skin or to light leakage from the source directly into the shorter source-detector separations.

As an example of the importance of displaying the neurophysiology at different time windows to correctly interpret changes seen in real-time before relating changes to clinical behavior, **Figure 3** shows the time-series of a monitoring session from a sedated stroke patient, as seen on the GUI by the critical care personnel. During part of the monitoring session, the clinicians were suctioning the patient's bronchial and oral secretions (represented by the shaded area in **Figure 3**). The patient motion induced by the intervention clearly disturbs the optical signal, which leads to the unphysiological spikes in the optical parameters; therefore, it is hard to attribute any physiological meaning to these changes. Soon after the intervention, the hemodynamic parameters returned to approximately the same values before the intervention, as expected for a stable patient. This example illustrates the stability of the real-time system in the neuro-ICU, as well as the importance of analyzing a patient's hemodynamics at different temporal windows.

In order to illustrate the feasibility of the hybrid diffuse optical device to provide meaningful information in the neuro-ICU, we present the case of a 50-year-old woman with a history of diabetes, hypertension, and congestive heart failure, who was admitted with left sided hemiparesis and was found to have an ischemic stroke due to occlusion of the right MCA (NIH

stroke scale = 11). **Figure 4** shows the average optical-derived parameters and the CT scan at the thirteenth day after hospitalization, while the patient was intubated and sedated. During this monitoring session, CBF and $CMRO_2$ in the ipsilesional forehead were considerably lower than their contralesional parameters in the symmetric region. This result is consistent with a perfusion deficit and subsequent tissue necrosis caused by a large vessel ischemia. Notably, although CBF was lower in the ipsilesional hemisphere, a high OEF was found in both hemispheres. This may be consistent with the idea of misery perfusion, a state in which there is high oxygen consumption (high OEF) despite low (but non-zero) CBF as the tissue attempts to promote recovery[8,51,52]. Currently, misery perfusion is hard to diagnose at the neuro-ICU. Although a larger study with acute ischemic stroke patients is needed to assess the sensitivity of diffuse optical spectroscopies to detect misery perfusion, this example demonstrates the potential of the diffuse optical system to assess clinically important information in real-time.

Lastly, we present the longitudinal results obtained from a 62-year-old female who was admitted to the neuro-ICU due to a severe right middle cerebral artery (MCA) aneurysmal subarachnoid hemorrhage, with Grade V on the Hunt & Hess scale (i.e., predicting a poor outcome and a low likelihood of survival)[53] and Grade III on the Fisher Scale (i.e., low to high risk of vasospasm)[54]. This patient was monitored throughout the hospitalization, and all the cerebral hemodynamic parameters were consistent with the clinical evolution of the patient's condition. We refer the interested reader a recently published case-report that contains the complete description of this case[9]. To illustrate the feasibility of performing measurements on different days, **Figure 5** shows an offline analysis of data collected with the system at several sessions during hospitalization of the case described above and presented in details in ref.[9]. Here, the laterality index (LI) was computed for each physiological parameter as:

$$LI = \frac{X_{ipsi} - X_{contra}}{X_{ipsi} + X_{contra}},$$

where X represents the variable measured (i.e., CBF, OEF, CMRO2), and the subscript denotes the brain hemisphere. With the LI it is possible to directly compare the differences across each hemisphere over the entire hospitalization. The laterality index has been shown to be very useful for different clinical scenarios[52,55–57], and it can be readily assessed with the protocol presented here by sequentially measuring symmetrical regions in both hemispheres. The mean arterial pressure (MAP) was collected with an independent instrument available in the neuro-ICU, and it is also shown in **Figure 5** for reference.

Careful analysis of **Figure 5** reveals two significant periods of hemispheric impairment. The first period occurred between the first and the third days after hospitalization, in which all the neurophysiological parameters in the ipsilesional ROI increased more than in the symmetrical contralesional ROI. This increase in LI on the third day after hospitalization could be an indicative of a possible homeostatic attempt to restore the metabolic balance of the affected tissue. During the second period, starting after the third day of hospitalization, the LI continuously decreased, which was consistent with the worsening condition of the patient. In this case, the patient died after 9 days of hospitalization.

FIGURE AND TABLE LEGENDS:

Figure 1: The optical environment developed to monitor patients inside an intensive care unit. (**A**) The hybrid diffuse optical system combines a frequency-domain diffuse optical spectroscopy (DOS) module and a diffuse correlation spectroscopy (DCS) module. (**B**) The customizable probe proposed in this study has as a default 4 source-detector separations (0.7, 1.5, 2.5 and 3.0 cm) for DCS and 4 source-detector separations for DOS (1.5, 2.0, 2.5 and 3.0 cm). For simplicity, the examples presented here only used the 2.5 cm source-detector separation for DCS. (**C**) The real-time graphical user interface (GUI) controls the diffuse optical system, and displays the measured cerebral blood flow (CBF), the oxygen extraction fraction (OEF) and the cerebral metabolic rate of oxygen ($CMRO_2$) in real-time, both within a 5 min time window (left panels), and within a 2 h time window (right panels). On the bottom of the GUI, the researcher can press buttons to start and stop the data collection, to acquire a baseline period for comparison and to mark any relevant intervention(s).

Figure 2: Representative autocorrelation curves for the DCS module. (**A**) An example of a good autocorrelation, which was approximately 1.5 at the zero delay-time extrapolation and decayed to 1 at longer delay times. As expected, the autocorrelation curves decayed faster for the longer source-detector separations. (**B**) An example of a bad auto-correlation curve, where it is not possible to distinguish the curves for the different source-detector separations. (**C**) Another example of a bad auto-correlation curve, in which the tail of the curve did not match the model used. The issues in (B) and (C) are related to either bad coupling of the probe onto the skin or to light leakage from the source directly into the shorter source-detector separations. The researcher can look at the curves on the '**Correlation Curves**' Tab on the GUI.

Figure 3: Cerebral physiology of a monitoring session from a sedated stroke patient, as would be seen on the GUI by the critical care personnel. The GUI displays the cerebral blood flow (CBF, in red), the oxygen extraction fraction (OEF, in blue) and the cerebral metabolic rate of oxygen ($CMRO_2$, in green) in real-time for both (**A**) short (i.e., 5 min) and (**B**) long (i.e., 2 h) time windows as well as an (**C**) average value over the last 5 min. During part of this monitoring session, clinicians were suctioning the patient's bronchial and oral secretions (represented by the shaded area in **B**).

Figure 4: Neurophysiological information of a patient diagnosed with severe ischemic stroke in the right middle-cerebral artery on the thirteenth day after hospitalization. (A) Cerebral blood flow (CBF), oxygen extraction fraction (OEF), cerebral metabolic rate of oxygen ($CMRO_2$) and total hemoglobin concentration (HbT) measured with the diffuse optical system in the contralesional and the ipsilesional hemispheres. **(B)** Computed tomography (CT) scan from the single-day measurement of the patient. The red areas in the CT images represent the presumed optical sensitivity region and the purple ellipse shows the approximate injury location.

Figure 5: Temporal evolution of the laterality index for the optically derived physiological parameters in a 62-year old female patient following a high-grade aneurysmal subarachnoid hemorrhage (aSAH). The changes in the ipsilesional region of interest (ROI) compared to the

changes in the contralesional ROI are shown in the left axis for cerebral blood flow (CBF, red circles), oxygen extraction fraction (OEF, blue diamond) and cerebral metabolic rate of oxygen ($CMRO_2$, green triangles). Evolution of the mean arterial pressure (MAP, gray squares) were collected independently, and is shown in the right-axis for comparison. The error bars of each point represent the standard deviation of each parameter across the monitoring session. For some days, the standard deviation was too small to be shown.

DISCUSSION:

This paper presented a hybrid optical system that can provide real-time information about cerebral blood flow, cerebral oxygenation, and cerebral oxygen metabolism of neurocritical patients at the beside. The use of diffuse optical techniques had been previously addressed as a potential marker for non-invasive, bedside monitoring in clinical scenarios. A previous study focused on the clinical aspects and the feasibility of optical monitoring during hospitalization in the neuro-ICU through a case report [9]. The focus of this work is to detail relevant and innovative aspects related to real-time monitoring with diffuse optics. Specifically, this paper proposed a real-time GUI that provides clear and useful information for clinicians. The GUI allows for easy comparison of different time periods, which is important for interpreting clinically relevant data. The implementation of the GUI presented here can be easily translated for DCS system based on a software-correlator with the caveat that the real-time display frequency must be limited to ~20 Hz. Real-time averaging of the autocorrelation curves can be used to down sample faster acquisition rates. In the future, real-time information provided by the proposed protocol may be used to guide therapy, potentially improving the clinical outcome of neurocritical patients.

This work also proposes the use of a customizable optical probe that can address different settings and therefore suit different purposes and needs for clinicians. The proper selection of the source-detector separation is a critical step for maximizing the cerebral sensitivity of diffuse optics. In most cases, an optimal probe for DCS measurements in adults should have at least a short (< 1 cm) and a long (> 2.5 cm) source-detector separation. The long source-detector separation was shown to provide the best compromise between signal-to-noise ratio (SNR) and cerebral sensitivity[12,14,16], while the short separation is mostly sensitive to the extra-cerebral tissues and is useful to differentiate the extra-cerebral changes from cerebral changes[12,16]. For FD-DOS, a simple probe that provides a reasonable compromise between SNR and cerebral sensitivity in adults contains 4 source-detector separations (1.5, 2.0, 2.5 and 3.0 cm)[58]. The most critical step for a FD-DOS measurement is the calibration procedure that is necessary to compare the AC and phase changes from different fibers (Section 2 of the protocol). A poor calibration of a FD-DOS system can lead to large errors in the retrieved values of the optical properties of the tissue, which will affect the accuracy of both the cerebral oxygenation and cerebral blood flow values. Of importance, the protocol proposed in this study focus in an optical probe for FD-DOS that contains a single PMT and multiple light sources. The calibration procedure described here needs to be modified for experiments utilizing multiple detectors. For studies using multiple detectors, the bias voltage of the PMT should not be changed during the calibration procedure, and thus a careful selection of the optical properties of the calibration phantoms is necessary.

In addition to the cerebral oxygenation measurements, the DOS module also improves the

calculation of CBF, as the DCS model also depends on the optical properties of the tissue. This study employed a commercial FD-DOS system with a single modulation frequency to recover the optical properties and the cerebral oxygenation. However, there are other alternatives that could provide more accurate information, such as time domain DOS or multi-frequency FD-DOS systems[59–64]. These systems may reduce the experimental complexity as they require a single source-detector separation to recover the cerebral physiology, whereas the traditional FD-DOS employed here requires multiple source-detector separations and thus multiple fibers attached to the head. Additionally, since the main interest of this protocol was the long-term trends in the cerebral physiology, this study opted to conduct interleaved DOS and DCS measurements. In the future, to avoid cross-contamination and to increase the sampling frequency, it is possible to acquire simultaneous DOS and DCS measurements by including notch filters on the DOS and DCS detectors.

One limitation of the current protocol is the restriction of the probe placement to the forehead. As of now, it is difficult to acquire DCS measurements through hair. This is not an issue for insults covering a larger portion of the brain, as is mostly often the case in the neuro-ICU. However, measurements on the forehead may not be sensitive to small MCA or PCA strokes, for example. With further improvements of the optical probes, it may be possible to measure through the hair, and by combining the system with a neuro navigation device it would be possible to make measurements over a small local ROI. By gathering detailed spatial information onto the optical information, we expect a marked improvement in the sensitivity of diffuse optics to the hemodynamic impairments due to focal cerebrovascular disorders.

Finally, it is important to mention a few limitations of the diffuse optical techniques. First, diffuse optics is inherently sensitive to the extra-cerebral tissue, and better modeling of the data may be necessary to properly account for the difference in the extra-cerebral and cerebral physiologies[65–70]. Additionally, the DCS measurement of CBF is sensitive to the external pressure of the optical probe against the tissue. For example, by increasing the probe pressure we reduce the blood flow in the external tissues, which will also reduce the CBF measured by DCS[71–73]. Note, however, that although the CBF is reduced due to increasing probe pressure, the heart rate pulsatility of CBF is unchanged[72]. Interestingly, it is possible to use these changes in CBF due to the external probe pressure to separate the extra-cerebral and cerebral physiologies[73]. Last, the optical derived CBF has physical units (i.e., cm^2/s) rather than the more usual clinical units (i.e., ml/100g of tissue/min). Some authors have proposed the use of indocyanine-green (ICG) to recover absolute CBF from DOS and to calibrate the CBF index from DCS to absolute clinical units[74–78]. However, the accuracy of the calibration factor from ICG may not be directly translated to different situations due to abnormalities in the macro and microcirculation following brain trauma.

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DISCLOSURES:

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: RC Mesquita has one pending patent application and two other patents relevant to this work (United States patents 10,342,488 and 10,064,554). No author currently receives royalties or payments from these patents.

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Figure 1

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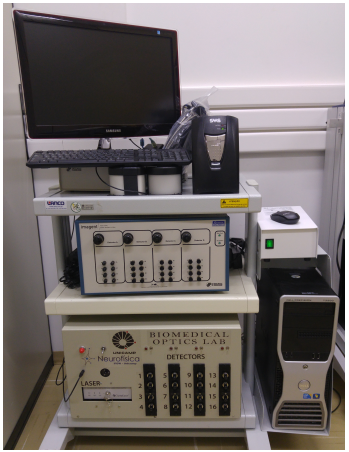
(A)



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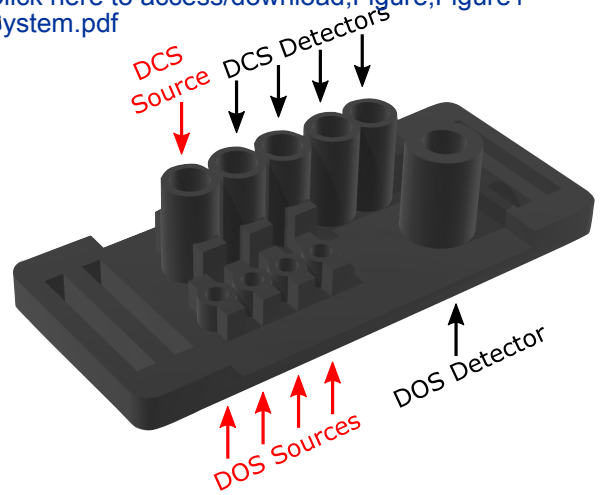

(C)

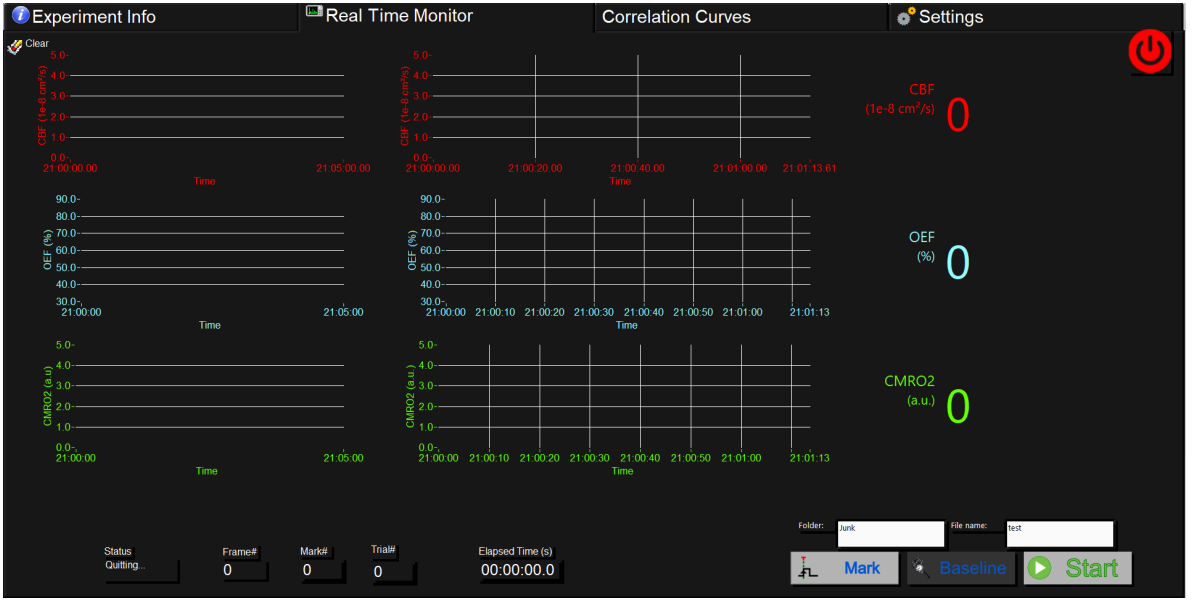

Figure 2

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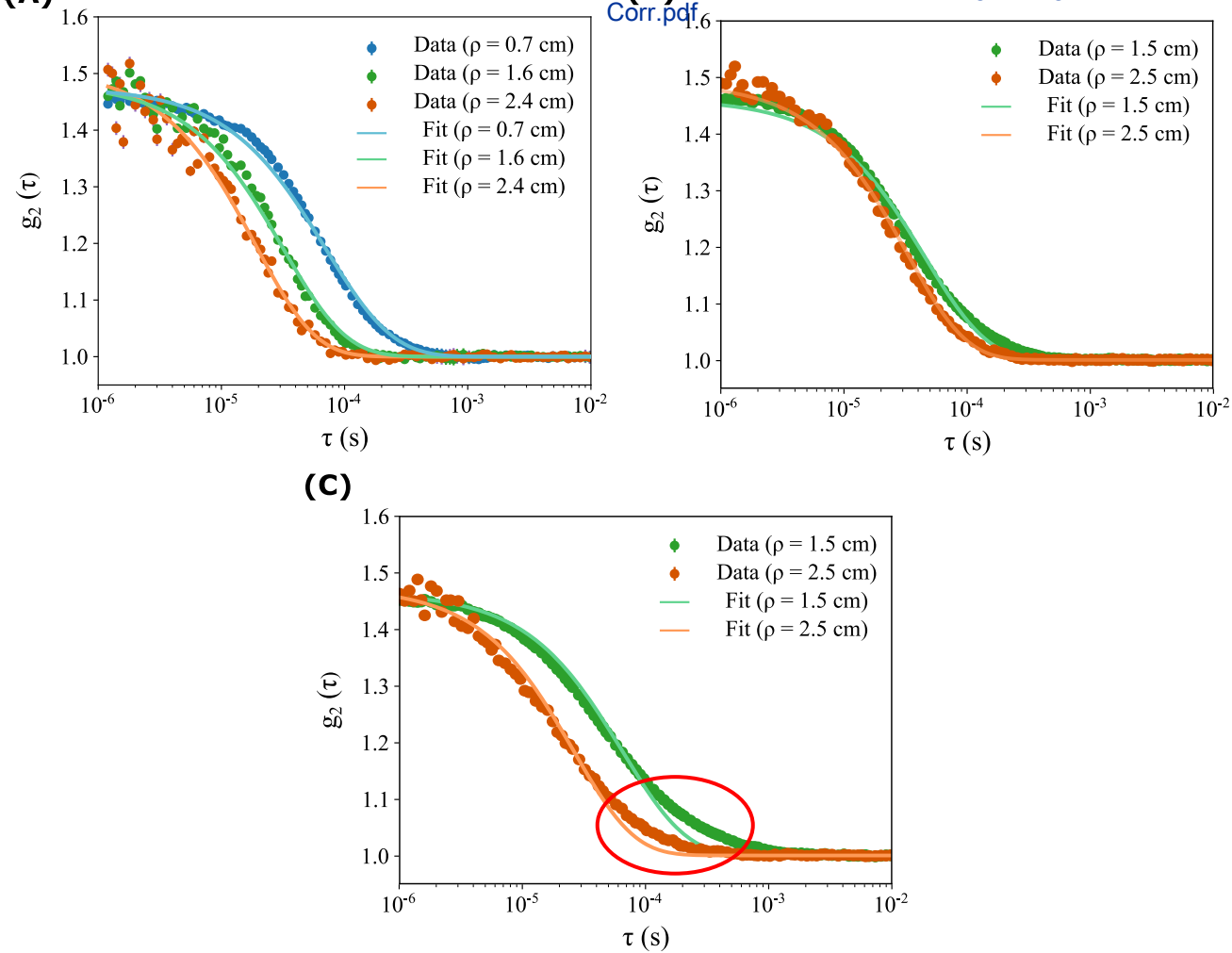

Figure 3

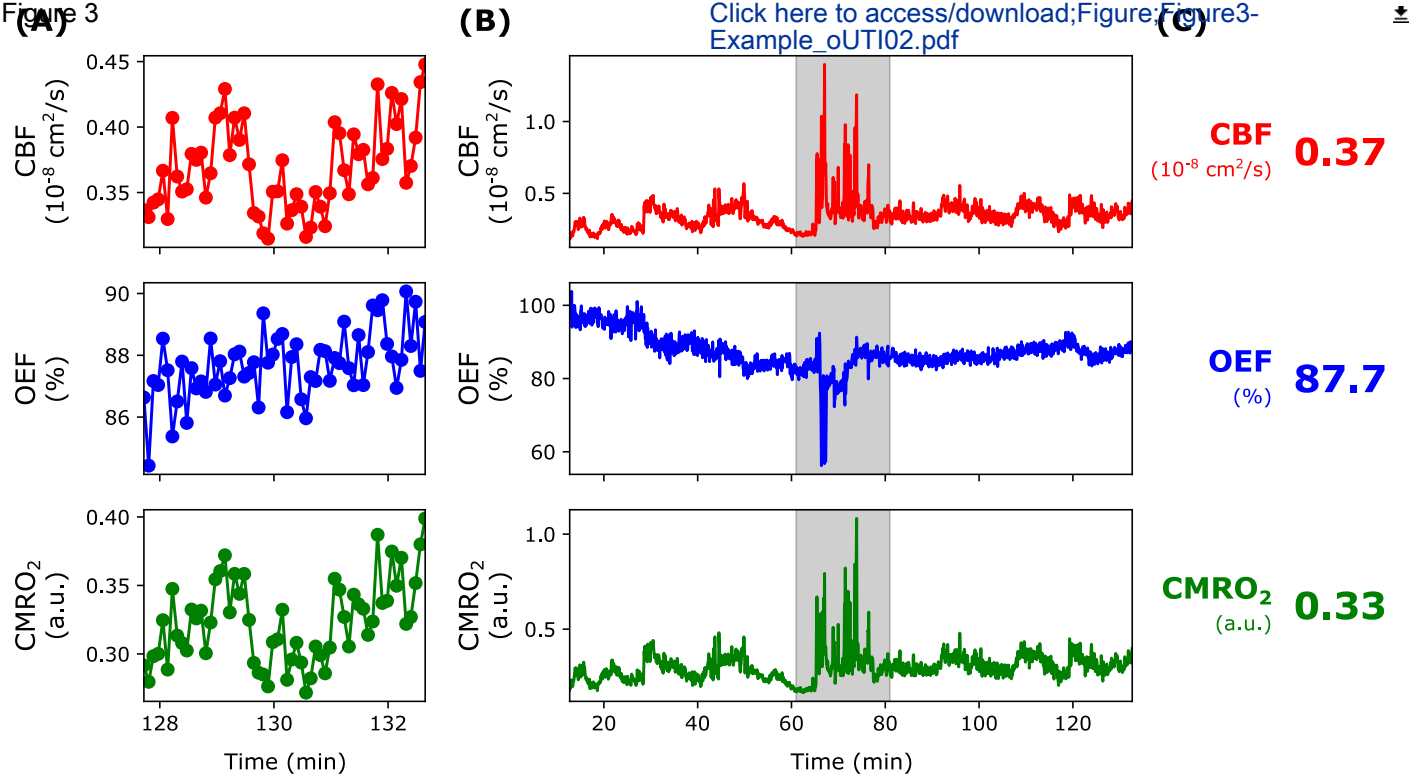

Figure 4

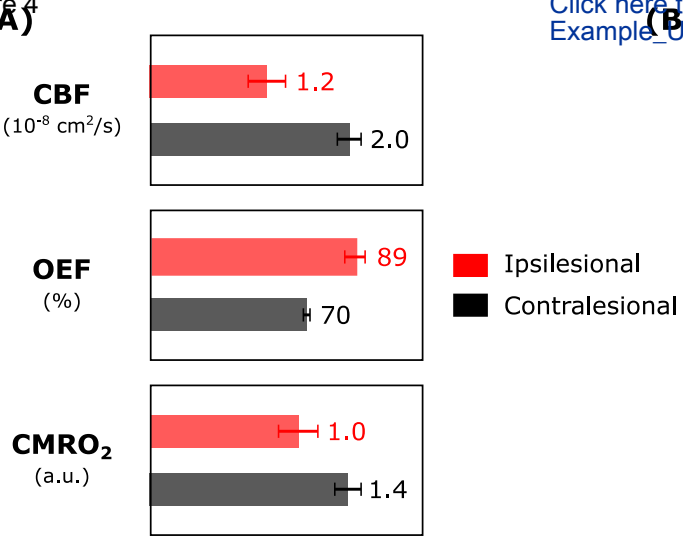

Figure 5

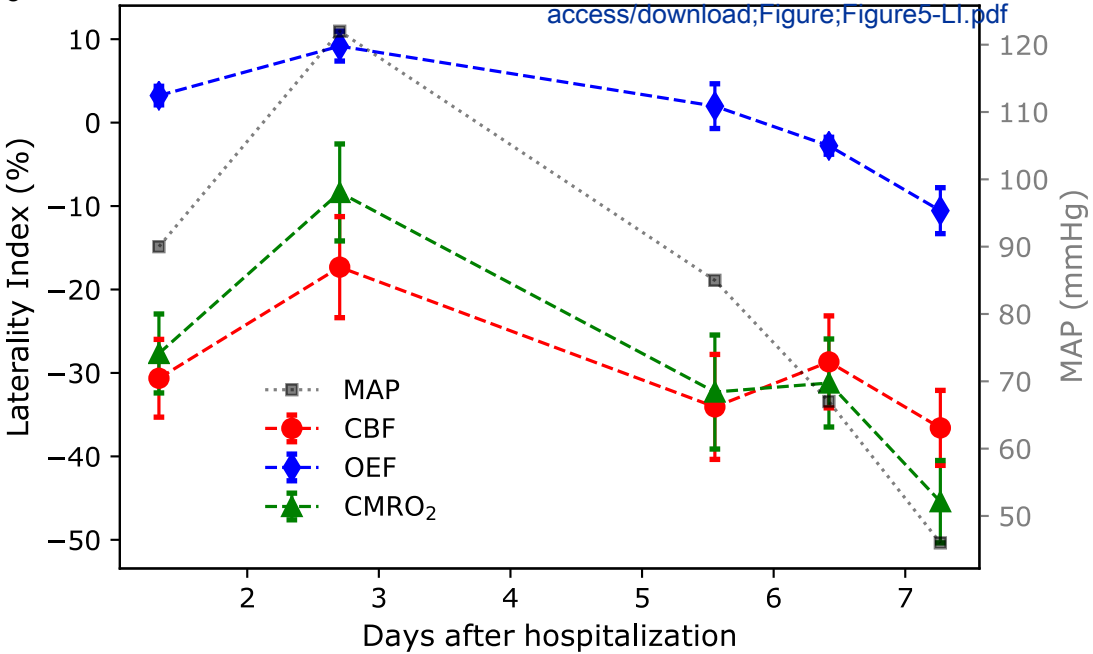

Table of Materials

Click here to access/download;Table of Materials;JoVE_Table_of_Materials (12).xlsx

Name of Material/Equipment	Company	Catalog Number
3D Printer	Sethi3D	S2
Arduino UNO	Arduino	UNO REV3
DCS Correlator	Correlator.com	Flex11-16ch
DCS Dectectors IO Boards	Excelitas Technology	SPCM-AQ4C-IO
DCS Detectors	Excelitas Technology	SPCM-AQ4C
DCS Laser	CrystaLaser	DL785-120-SO
DCS Power supply	Artesyn	UMP10T-S2A-S2A-S2A-S2A-IES-00-A
FD-DOS fibers	ISS	Imagent supplies
Flexible 3D printer material	Sethi3D	NinjaFlex
Imagent	ISS	Imagent
Laser safety googles	Thorlabs	LG9
Multi-mode fiber	Thorlabs	FT400EMT
Neutral density filter 1.0 OD	Edmund Optics	53-705
Single-mode optical fiber	Thorlabs	780HP
System battery	SMS	NET4

Comments/Description
3D-printer used to print the customizable probes
Microcontroller responsible to interleave the DCS and FD-DOS measurements
Component of the DCS module
Component of the DCS module
Component of the DCS module
Component of the DCS module
Component of the DCS module (power supply for the DCS detecto; 2, 5 and 30V)
The fibers used for FD-DOS detection and illumination are provived by ISS
Material used to print the flexible customizable probes
FD-DOS module
Multi-mode fiber used for DCS illumination
Neutral density filter for the short source detector separations
Single-mode optical fiber used for the DCS detectors
System battery used for transportation

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Re: Resubmission of Manuscript JoVE61608

Dear Editors of the Journal of Visualized Experiments,

We are grateful for the thoughtful consideration by the editors and the reviewers regarding our manuscript entitled "*Real-time monitoring of neurocritical patients with diffuse optical spectroscopies*". Please find attached a revised version of the manuscript, which we are resubmitting for consideration for publication in the *Journal of Visualized Experiments*. We also provide our complete set of responses to the reviewer comments.

We are pleased the reviewers found merit in the work. One reviewer, for example, described that "study has apparent merits for guide of healthcare in neuro-ICU rooms", and another described the manuscript as "generally well written". The reviewers had general concerns about the lack of details for some of the procedures described, as well as some questions about specific topics of the protocol proposed. We have carefully reviewed each comment, and we have provided responses and corresponding text changes.

In accordance on the editorial comments, we have also reorganized and rephrased a large portion of the manuscript. More specifically, the use of personal pronouns throughout the manuscript was drastically reduced to adhere to the JoVE policy and the protocol section was reorganized to only use statements in the imperative tense. Portions of the protocol were either moved to a Supplemental Material or to the discussion to reduce the usage of 'Notes', and the discussion was reorganized to adhere to the editorial guidelines.

We believe our responses should satisfy the reviewers and the editorial comments, and we note that the critique (and our responses) have improved the overall quality of the final manuscript. We thus hope the revised paper is suitable for publication in the Journal of Visualized Experiments.

If you have further questions, please do not hesitate to contact us.

Sincerely Yours,

Rodrigo M. Forti
Rickson C. Mesquita

RESPONSE TO REVIEWERS

Reviewer #1:

R1, Comment 1: *"87 to 89: I suggest to rephrase this sentence, as the application of optics in intensive care unit using commercially available devices has been tested, and it is currently deemed to have not a sufficient sensibility for clinical usage (e.g. Leal-Noval et al.; Davies et al.). If the authors are referring to the capacity to read the values real-time on the monitors, then this feature should be better highlighted to the readers."*

Response to 1: As mentioned by the reviewer, the use of a standalone DOS system was previously compared to invasive brain tissue oxygen tension monitors, and DOS was deemed to not have sufficient sensibility to replace the invasive monitors. However, apart from using relatively small populations, the direct comparison of the invasive and non-invasive monitors may be flawed as each technique probe different parts of the cerebral vasculature. Even though these studies ultimately concluded that diffuse optics is not a replacement for the invasive monitors, in both studies mentioned by the reviewer DOS achieved a moderate-to-good accuracy, which may be sufficient for cases (or places) wherein invasive monitors are not available. The variability in accuracy presented by both studies can potentially be explained by systematic errors, probably due to calibration issues, as well as to the extracerebral influence on the optical signal. Note that all these issues can be ameliorated with further developments of the technique.

> **TEXT CHANGES:** To better discuss this important topic in the manuscript, we have included a new paragraph in the Introduction (lines 88-97):
>
> *"The reliability of the diffuse optical techniques to provide valuable real-time information during longitudinal measurements as well as during clinical interventions remains largely unaddressed. The use of a standalone DOS system was previously compared to invasive brain tissue oxygen tension monitors, and DOS was deemed to not have a sufficient sensibility to replace the invasive monitors. However, apart from using relatively small populations, the direct comparison of the invasive and non-invasive monitors may be misguided as each technique probe different volumes containing different parts of the cerebral vasculature. Even though these studies ultimately concluded that diffuse optics is not a replacement for the invasive monitors, in both studies DOS achieved a moderate-to-good accuracy, which may be sufficient for cases and/or places wherein invasive monitors are not available."*

R1, Comment 2: *"97 to 99: please either put a reference or be more specific on the advantages of optics compared to the techniques mentioned."*

Response to 2: The advantages of optics compared to the imaging techniques is related to the ability of *simultaneously* measuring both cerebral blood flow and cerebral oxygenation with a faster sampling rate. This was already stated in the introduction, but we have slightly reworded the paragraph to clarify the advantages of optics (lines 105-113).

> **TEXT CHANGES:** Lines 105-112 were changed to better emphasize the advantages of diffuse optics when compared to the traditional imaging techniques:
>
> *"Optical techniques also have advantages when compared to other traditional imaging modalities, such as Positron-Emission Tomography (PET) and Magnetic Resonance Imaging (MRI). In*

addition to simultaneously providing direct measures of both CBF and HbO/HbR concentrations, which is not possible with MRI or PET alone, optical monitoring also provides significantly better temporal resolution, allowing, for example, the assessment of dynamic cerebral autoregulation 40–42 and the assessment dynamically evolving hemodynamical changes. Moreover, diffuse optical instrumentation is inexpensive and portable in comparison to PET and MRI, which is a critical advantage given the high burden of vascular disease in lower- and middle-income countries."

R1, Comment 3: *"110: The name of the company which made the FD device should be mentioned. Also, I suggest citing the name of the authors/groups who first described this module (i.e. Fantini, Franceschini, Gratton)"*
Response to 3: We are sorry that we omitted this information in the Introduction. In the revised manuscript we have now included the company name for the relevant components of each module, together with citations to the pioneer studies about multi-distance FD-DOS (Fantini et al. 1994, Fantini et al. 1995).

References:
Fantini, S., Franceschini, M.A., Fishkin, J.B., Barbieri, B., Gratton, E. Quantitative determination of the absorption spectra of chromophores in strongly scattering media: a light-emitting-diode based technique. *Applied Optics*. **33** (22), 5204 (1994).

Fantini, S., Franceschini, M.A., Maier, J.S., Walker, S.A., Barbieri, B.B., Gratton, E. Frequency-domain multichannel optical detector for noninvasive tissue spectroscopy and oximetry. *Optical Engineering*. 34 (1), 32 (1995).

TEXT CHANGES: Lines 120-124 now read:

"The FD-DOS module (Imagent, ISS) 49, 50 consists of 4 photomultiplier tubes (PMTs) and 32 laser diodes emitting at four different wavelengths (690, 704, 750 and 850 nm). The DCS module consists of a long-coherence laser emitting at 785 nm (DL785-120, CrystaLaser), 16 single-photon counters as detectors (SPCM-AQ4C, Excelitas) and a correlator board (Correlator.com)."

R1, Comment 4: *"150 to 152: there have to be taken, or are at least suggested, any precautions to reduce the ambient light in the ICU?"*
Response to 4: This is a valid and important question for diffuse optics applications. However, we do not expect ambient light to significantly affect the DCS and FD-DOS measurements. Ambient light is non-coherent and is not expected to affect the shape of the auto-correlation functions from DCS, except for a small decrease in SNR. For FD-DOS, we are only concerned about the detection of light modulated at 110 MHz, and thus the measured AC and phase changes should also not be affected by the room light. This is also an advantage of our hybrid system, as compared to standard commercial CW-NIRS systems that have been tested in clinical settings. Despite this fact, we agree that it is advisable to cover the optical probe with a black cloth or a black bandage to reduce measurement noise, and we have included a sentence in the protocol addressing this issue.

TEXT CHANGES: We have included the following sentence in lines 225-227:

"Although not strictly necessary for FD-DOS and DCS, it is advisable to cover the optical probe with a black cloth or black bandage to reduce noise due to ambient light.."

R1, Comment 5: *"193 to 196: was the elastic strap used to keep the probes in place (because the double-sided tape is not strong enough to keep the probes in place) or to reduce the ambient light?"*
Response to 5: The elastic strap was used because the double-sided tape is not strong enough to keep the probes in place. To avoid further confusions, we have explicitly included this point in the protocol.

> **TEXT CHANGES:** The 'NOTE' after Step 3.3 (lines 229-232) in the protocol was rephrased to clarify why the elastic strap is necessary.
>
> *"It is important to assure that the elastic strap is neither too tight, nor too loose. If the strap is too tight it may cause significant discomfort to the patient, and if the strap is too loose it may lead to poor data quality as the double-sided tape is not strong enough to keep the probes in place."*

R1, Comment 6: *"198 to 200: there is any advice if patients had a decompressive craniectomy and so more prone to increase the intracranial pressure if the elastic band is applied to them?"*
Response to 6: We share the reviewer's concern about using an elastic strap on patients with decompressive craniectomies, and we have excluded these patients from our pilot study for this reason. Based on the reviewer's and the editorial comments, we have included in the protocol the inclusion and exclusion criteria used in our study. Nonetheless, we think that in the future it may be possible to monitor this patient population by developing lighter probes and by using stronger double-sided tapes.

> **TEXT CHANGES:** We have included in the protocol the inclusion and exclusion criteria in the beginning of the Protocol section.
>
> *"The protocol was approved by the local committee of the University of Campinas (protocol number 56602516.2.0000.5404). Written informed consent was obtained from the patient or a legal representative prior to the measurements. We monitored patients that were admitted to the Clinics Hospital at the University of Campinas with a diagnosis of either ischemic stroke or a subarachnoid hemorrhage affecting the anterior circulation. Patients with ischemic strokes affecting the posterior circulation, patients with decompressive craniectomies due to elevated intracranial pressure and patients with other neurodegenerative diseases (dementia, Parkinson's or any other disease that can be associated with cortical atrophy) were excluded from the study protocol."*

R1, Comment 7: *"325 to 326: a film of water due to the wiping of the probes or movements of the probes while wheeling out the device from the intensive therapy unit may change the calibration and make the assessment of the calibration done before the recordings inaccurate. Can you please explain why the assessment of the status of the calibration should not be done immediately after removing the probes from the patients?"*
Response to 7: We agree with the reviewer that the assessment of the status of the calibration is best done immediately after removing the probe from the patient. In the case studies presented in this work, we assessed the calibration status at the storage facility to move the system out of the ICU as quickly as possible due to space restrictions in the ICU. To emphasize that it is best to assess the calibration status directly after removing the probe from the patient, we have reorganized steps 6.5-6.8 from the protocol.

> **TEXT CHANGES:** Steps 6.5-6.8 from the protocol now reads:

"6.5. Remove the probe from the patient's head and remove the double-sided tape from the probe. Then, clean the probe with sanitizing wipes.

6.6. Repeat the measurement of the optical properties of each solid phantom as soon as possible to ensure the calibration remained adequate throughout the monitoring session (see step 4.2.2).

NOTE: Ideally, the calibration step should be done right after removing the optical probes from the patient head (step 6.6). However, due to timing issues, in the examples presented in the next section this was done in the storage facility.

6.7. Clean the system and its accessories with sanitizing wipes.

6.8. Wheel the cart back to the storage room."

R1, Comment 8: *"427: there has been done any proper co-registration process which would make it possible to correlate the optical signal to the patients' CT scan? If not, I suggest rephrasing into "presumed optical sensitivity region"."*
Response to 8: Thanks for bringing this misleading sentence to our attention. In fact, we have not done any registration process, and the highlighted area is a *presumed* sensitivity region. Per the reviewer suggestion, we have included the word 'presumed' in the legend of Figure 4.

TEXT CHANGES: We have changed the legend of Figure 4 to include the word 'presumed':

"The red areas in the CT images represent the presumed optical sensitivity region and the purple ellipse approximately shows the injury location."

R1, Comment 9: *"461: I concur on stating the aims of the current paper in the discussion section. I would also suggest mentioning that the analysis of the accuracy of the values obtained using the tool described was one of the main objects of another paper from the same group, and they are not among the aims of the current paper. The description of the clinical cases only suggests that there is a relation between the values reported and the clinical status of the patient, and not that the values recorded are indeed the real values on patients."*
Response to 9: We thank the reviewer for the very good suggestion, and the Discussion section was reorganized to account for this and other comments.

TEXT CHANGES: To better discriminate between the current study and our previous publication, we have included in the Lines 463-468:

"A previous study focused on the clinical aspects and the feasibility of optical monitoring during hospitalization in the neuro-ICU through a case report [9]. The focus of this work is to detail relevant and innovative aspects related to real-time monitoring with diffuse optics. Specifically, this paper proposed a real-time GUI that provides clear and useful information for clinicians. The GUI allows for easy comparison of different time periods, which is important for interpreting clinically relevant data."

R1, Comment 10: *"512 to 514: are the authors suggesting that the ICG can be used with their system to retrieve absolute clinical units? If so, please clarify. Also, the analysis of the ICG kinetics may be impaired*

by abnormalities in the macro and microcirculation following a brain injury, so its validity still has to be tested in TBI patients. If the authors want to mention this technique, also the presumed difficulties on the usage of it in brain trauma should be mentioned."

Response to 10: Although we have not personally implemented the ICG calibration method with our system, we wanted to acknowledge that previous studies in the literature used a similar FD-DOS system to recover the absolute cerebral blood flow with the ICG bolus method (Kohl-Bareis et al., 2002), and that it would be possible to calibrate our CBF measurement to standard clinical units by obtaining the calibration factor either from the literature or from a direct ICG FD-DOS measurement. However, we agree with the reviewer that it is important to mention the presumed difficulties of the ICG calibration method for brain trauma, and we have included a sentence in the last paragraph of the discussion to address this missing information.

References:

Kohl-Bareis, M., Obrig, H., Steinbrink, J., Malak, J., Uludag, K., Villringer, A. Noninvasive monitoring of cerebral blood flow by a dye bolus method: Separation of brain from skin and skull signals. *Journal of Biomedical Optics*. 7 (3), 464 (2002).

> **TEXT CHANGES:** We have included the following at the end of the last paragraph of the Discussion (lines 526-531), following the reviewer's suggestion:
>
> *"Last, the optical derived CBF has physical units (i.e., cm2/s) rather than the more usual clinical units (i.e., ml/100g of tissue/min). Some authors have proposed the use of indocyanine-green (ICG) to recover absolute CBF from DOS and to calibrate the CBF index from DCS to absolute clinical units 74–78. However, the accuracy of the calibration factor from ICG may not be directly translated to different situations due to abnormalities in the macro and microcirculation following brain trauma."*

R1, Comment 11: *"50: cerebral physiology seems a too broad description, I suggest to be more specific and list the values measured real-time (as they are clearly stated in the introduction)."*

Response to 11: We have rephrased this sentence per the reviewer's suggestion.

> **TEXT CHANGES:** Lines 47-49 from the abstract now reads:
>
> *"Here, we report the translation of a diffuse optical system for continuous real-time monitoring of cerebral blood flow, cerebral oxygenation, and cerebral oxygen metabolism during intensive care."*

R1, Comment 12: *"80 to 82 and 99 to 102: I cannot see the purpose of the description of the capacity to assess the cerebral autoregulation using optics since this value has not been considered among the real-time parameters displayed by the device."*

Response to 12: It is true that we have not addressed the real-time calculation of cerebral autoregulation using optics in this work, mainly because we did not have access to a continuous monitor of blood pressure (BP). However, we included this information to highlight the full capacity of diffuse optics for clinical applications. In the future it is possible to include the real-time assessment of cerebral autoregulation in our proposed GUI by including a continuous BP monitor in the protocol. This can be accomplished either by

feeding the values from the clinical monitors directly to our system (e.g., via an ethernet cable), or by using a standalone BP monitor.

> **TEXT CHANGES:** To emphasize that cerebral autoregulation can only be assessed with a BP monitor, we have changed the sentence from lines 78-81:
>
> *"When continuous blood pressure monitoring is also available, diffuse optics can be additionally used to monitor cerebral autoregulation, both in healthy and in critically ill subjects 11, 40–42, as well to assess the critical closing pressure of the cerebral circulation 43."*

R1, Comment 13: *"441:the definition of "complete optical system" seems unprecise while I would refer to it as a hybrid system compounded by FD- DOS and DCS, as done in the introduction (phase 110)"*
Response to 13: We have followed the reviewer's suggestion and rephrased it as a 'hybrid optical system'.

> **TEXT CHANGES:** Following the Reviewer's suggestion we have rephrased the first sentence of the Discussion (lines 460-462):
>
> *"This paper presented a hybrid optical system that can provide real-time information about cerebral blood flow, cerebral oxygenation, and cerebral oxygen metabolism of neurocritical patients at the beside."*

R1, Comment 14: *"461: the description of "full […] system" seems unprecise, please rephrase."*
Response to 14: This sentence was removed from the reorganized Discussion.

> **TEXT CHANGES:** No text changes were made due to this comment.

Reviewer #2:

R2, Comment 1: *"How to minimize the ambient light influence on DOS and DCS measurements, or in other words, will the optical measurements be affected by the environmental noise?"*
Response to 1: This is a valid and important question for applications of diffuse optics. However, we do not expect ambient light to significantly affect the DCS and FD-DOS measurements. Ambient light is non-coherent and is not expected to affect the shape of the auto-correlation functions from DCS, except for a small decrease in SNR. For FD-DOS, we are only concerned about the detection of light modulated at 110 MHz, the measured AC and phase changes should also not be affected by the room light. Experimentally, we have not observed a significant effect of ambient light in the detected measures we use (in fact, it can affect the DC light intensity in FD-DOS but this is not used in our analysis). This is also an advantage of our hybrid system, as compared to standard commercial CW-NIRS systems that have been tested in clinical settings. Despite this fact, we agree that it is advisable to cover the optical probe with a black cloth or a black bandage to reduce measurement noise, and we have included a sentence in the protocol addressing this issue.

> **TEXT CHANGES:** We have included the following sentence in lines 225-227:
>
> *"Although not strictly necessary for FD-DOS and DCS, it is advisable to cover the optical probe with a black cloth or black bandage to reduce noise due to ambient light."*

R2, Comment 2: *"Is the data analysis approach (i.e., a semi-infinite model of the head to fit the DCS autocorrelation curves) fast enough to output the real-time blood flow curve?"*
Response to 2: Our real-time data analysis is fast enough to output the real-time blood flow, even when using the faster sampling rates achieved with software correlators; the real-time fitting process takes less than 50ms. In fact, in a recent study from our group, we have developed a simpler but similar GUI capable of displaying the real-time blood flow changes measured by a DCS system that uses a software correlator (Forti et al., *Journal of Stroke and Cerebrovascular Diseases*, 2019).

References:
Forti, R.M. et al. Transcranial Optical Monitoring of Cerebral Hemodynamics in Acute Stroke Patients during Mechanical Thrombectomy. *Journal of Stroke and Cerebrovascular Diseases*. 28 (6), 1483–1494 (2019)

> **TEXT CHANGES:** We have included a sentence in the last paragraph of the Introduction (lines 138-140) to highlight the speed of the real-time analysis algorithm:
>
> *"). The real-time analysis pipeline developed within the proposed GUI is fast and takes less than 50 ms to compute the optical parameters (see the Supplementary Material for more details)."*
>
> We have also included a sentence describing that it is straightforward to translate our real-time analysis to DCS systems based on software correlators in the discussion section (lines 469-472).
>
> *"The implementation of the GUI presented here can be easily translated for DCS system based on a software-correlator with the caveat that the real-time display frequency must be limited to ~20 Hz. Real-time averaging of the autocorrelation curves can be used to down sample faster acquisition rates."*

Reviewer #3:

R3, Comment 1: *"While the FD-NIRS instrument is a commercial instrument, for which a handbook would be supplied if ordered, the description of calibration and data quality check is not sufficient for actually using the instrument."*
Response to 1: We are sorry that our description of the calibration and data quality check for the FD-DOS module was not sufficient. We have changed most of the calibration steps for the FD-DOS module to better describe the necessary steps, and we have included a few sentences in the Discussion highlighting the necessity for the calibration. We would like to emphasize that a calibration step is not necessary for the DCS module, and that we have described the issues commonly encountered when using DCS in the first paragraph of representative results section.

> **TEXT CHANGES:** The description of the calibration and data quality for FD-DOS was completely rephrased in Steps 2.2-2.3 (please see revised manuscript for details). We have also included a discussion about the calibration steps of the FD-DOS in the discussion (lines 483-492):
>
> *"The most critical step for a FD-DOS measurement is the calibration procedure that is necessary to compare the AC and phase changes from different fibers (Section 2 of the protocol). A poor calibration of a FD-DOS system can lead to large errors in the retrieved values of the optical properties of the tissue, which will affect the accuracy of both the cerebral oxygenation and cerebral blood flow values. Of importance, the protocol proposed in this study focus in an optical probe for FD-DOS that contains a single PMT and multiple light sources. The calibration procedure described here needs to be modified for experiments utilizing multiple detectors. For studies using multiple detectors, the bias voltage of the PMT should not be changed during the calibration procedure, and thus a careful selection of the optical properties of the calibration phantoms is necessary."*

R3, Comment 2: ***"****The DCS system is not described in enough detail to actually build and operate the system. While other papers describe the layout of the instrument, this article should focus on actually building it or describing a path for obtaining an instrument, such as from ISS or Hemophotonics."*
Response to 2: Although building a DCS system was not the focus of this paper, we have increased the details of components required to construct a DCS module in the Table of Materials.

> **TEXT CHANGES:** We now indicate in the Table of Materials every component necessary to build a DCS module.

R3, Comment 3: *"The GUI developed is a nice addition for analysis and display of data. However, it is not clear if the GUI is compatible with other forms of DCS systems, such as software correlator based, and how one could obtain the GUI. Overall, while the paper describes how to in principle apply a FD-NIRS probe and DCS probe and what to expect from the data, the description is not detailed enough, and it is questionable whether the protocol is generalizable."*
Response to 3: Respectfully, we partially agree with the reviewer's concern that our protocol may be not generalizable. Although the protocol may need some minor changes for studies aiming specific goals different than ours, the general idea and steps of the protocol described in this work will remain the same. When using different systems, especially different DOS systems, the calibration steps of the protocol needs to be adjusted to the specificities of the system being used. However, the calibration steps are usually well described by the diffuse optics manufacturers. The best we can do is to make sure that the calibrated

versions of each system provide accurate values when compared to standard phantoms, as was proposed in a series of publications (Pifferi et al. 2005, Wabnitz et al. 2014a, Wabnitz et al. 2014b, Pifferi et al. 2015).

Regardless of small changes in the calibration of the protocol, the GUI that we introduce in this work is based on LabView and it is responsible for controlling the system as well as displaying the data. Thus, to use our GUI with different systems (such as DCS systems with software correlators or other DOS systems) some modifications to the back-end software will certainly be required. However, independently of the system used, the general idea of the front-panel does not need to be changed. In fact, we have previously developed a similar GUI for a hybrid system combining a software correlator DCS and a time-domain DOS system.

References:

Pifferi, A. *et al.* Performance assessment of photon migration instruments: The MEDPHOT protocol. *Applied Optics*. **44** (11), 2104–2114 (2005).

Wabnitz, H. *et al.* Performance assessment of time-domain optical brain imagers, part 1: basic instrumental performance protocol. *Journal of Biomedical Optics*. **19** (8), 086010 (2014a).

Wabnitz, H. *et al.* Performance assessment of time-domain optical brain imagers, part 2: nEUROPt protocol. *Journal of Biomedical Optics*. **19** (8), 086012 (2014b).

Pifferi, A. *et al.* Mechanically switchable solid inhomogeneous phantom for performance tests in diffuse imaging and spectroscopy. *Journal of Biomedical Optics*. **20** (12), 121304 (2015).

> **TEXT CHANGES:** We have included in the discussion a sentence describing that the real-time analysis from the GUI developed for our protocol can be translated to DCS systems based on software correlators (lines 469-472).
>
> *"The implementation of the GUI presented here can be easily translated for DCS system based on a software-correlator with the caveat that the real-time display frequency must be limited to ~20 Hz. Real-time averaging of the autocorrelation curves can be used to down sample faster acquisition rates."*

R3, Comment 4: *"Laser safety: The DCS laser is 3B. Please describe the laser safety procedures and laser safety goggles for subjects."*

Response to 4: We are sorry we have not included this information in the initial version of the manuscript. We have now included in the Protocol the placement of an optical safety googles (step 3.3), and instructed the user to turn off the lasers before placing the probe on the subjects. We have also highlighted that the DCS laser is 3B and hazardous for eye exposure.

> **TEXT CHANGES:** We have included the placement of an optical safety google on Step 3.3 of the protocol:
>
> "3.3. Place a laser safety googles on the subject."

The protocol now instructs the users to only turn the lasers on after it is attached to the patient's forehead (Step 3.5). We have also added a 'CAUTION' note after Step 3.5 to emphasize that the DCS laser is hazardous for eye exposure.

"3.5. After the probe is properly secured to the patient forehead, turn on the DCS laser by switching the key to the 'ON' position.

CAUTION: The DCS system uses a Class 3B laser which is hazardous for eye exposure. It is very important to only turn on the lasers when the probe is properly attached to the patient's forehead."

R3, Comment 5: *"Calibration of ISS: The detector optimization should be based on optical properties of the patient, not the phantom alone. It may be that the detectors are saturating on the patient in which case the calibration has to be repeated. This statement is based on assuming the authors use multiple detectors rather than multiple sources. Please clarify. Overall, the calibration step is a bit simplified. A discussion on the importance of the calibration would be good, otherwise the results will be faulty."*
Response to 5: We agree with the reviewer that when working with multiple detectors the detector optimization must be based on the optical properties of the patient. However, in the protocol proposed in this study, we employed a single detector and multiple sources, which facilitates the calibration procedure. We also agree with the reviewer that a discussion about the importance of the calibration was lacking, and we have included a brief discussion about the calibration procedure.

TEXT CHANGES: We have included in the manuscript a brief discussion about the calibration procedure (lines 483-492):

"The most critical step for a FD-DOS measurement is the calibration procedure that is necessary to compare the AC and phase changes from different fibers (Section 2 of the protocol). A poor calibration of a FD-DOS system can lead to large errors in the retrieved values of the optical properties of the tissue, which will affect the accuracy of both the cerebral oxygenation and cerebral blood flow values. Of importance, the protocol proposed in this study focus in an optical probe for FD-DOS that contains a single PMT and multiple light sources. The calibration procedure described here needs to be modified for experiments utilizing multiple detectors. For studies using multiple detectors, the bias voltage of the PMT should not be changed during the calibration procedure, and thus a careful selection of the optical properties of the calibration phantoms is necessary."

R3, Comment 6: *"The table of materials is lacking a lot of details. If one were to try to build a DCS system, many more components would be needed. Also missing: fibers for ISS and DCS; probe (if 3D printed, please provide cad file); switch between the two systems; GUI access"*
Response to 6: We have updated the Table of Materials with all the components necessary to build and use the proposed system.

TEXT CHANGES: Please see the revised version of the Table of Materials.

R3, Comment 7: *"It is not clear why real-time analysis is necessary. Also, is real-time analysis possible if a software correlator would be used with much higher sampling frequency? Clearly the analysis shouldn't have to be done offline, but real-time may not be necessary."*

Response to 7: We believe that the portability and the real-time capabilities of diffuse optics is one of the major advantages of the technique when compared to other clinically available techniques. In the future, with more validation studies, we envision that diffuse optics can be used to guide therapy in real-time, during the patient management in the ICU as well as during clinical interventions (e.g., carotid endarterectomy, endovascular treatment of stroke, etc). In fact, our real-time analysis can be used with a software correlator, albeit with a reduced sampling frequency; our algorithm has an upper limit on the display frequency of real-time values of approximately 20 Hz, whereas software correlators can go as fast as 100 Hz. We have also included a sentence indicating that the real-time analysis of our system is compatible with software correlators.

> **TEXT CHANGES:** We have included a sentence describing that the real-time analysis from our GUI can be easily translated to DCS systems based on software correlators and that the real-time information provided by diffuse optics has potential to guide therapy, potentially improving patient outcome (lines 469-473).
>
> *"The implementation of the GUI presented here can be easily translated for DCS system based on a software-correlator with the caveat that the real-time display frequency must be limited to ~20 Hz. Real-time averaging of the autocorrelation curves can be used to down sample faster acquisition rates. In the future, real-time information provided by the proposed protocol may be used to guide therapy, potentially improving the clinical outcome of neurocritical patients."*

R3, Comment 8: *"What are the sampling frequencies of the two systems?"*
Response to 8: The maximum sampling frequency of the DCS system is 3 Hz, while the sampling frequency of the FD-DOS is 10 Hz. The combined sampling frequency is variable and depends on the SNR requirements of the experiment: longer averaging time will increase the SNR of the measurement but will reduce the sampling frequency. In the examples presented in this work, the total acquisition time was approximately 5 s.

> **TEXT CHANGES:** We have described the sampling frequency of each module on lines 124-125:
>
> *"The sampling frequency for the FD-DOS module is 10 Hz, and the maximum sampling frequency for the DCS module is 3 Hz."*
>
> We have also included a sentence describing the overall sampling frequency of the proposed hybrid optical system (lines 129-131):
>
> *"In total, the proposed system can collect one combined FD-DOS and DCS sample every 0.5 to 5s, depending on the signal-to-noise ratio (SNR) requirements (longer collection times leads to better SNR)."*

R3, Comment 9: *"How are the two systems synchronized and which sampling rate is used for cmro2 calculation?"*

Response to 9: As described in the introduction, the acquisition process is controlled by a microcontroller that interleaves the measurements of each system. The synchronization of each module is achieved by time-tagging the start of the measurement cycle from each system. The sampling rate of the $CMRO_2$ calculation is the same as the total sampling frequency of the system and it can vary between 0.5 to 5s, depending on the SNR requirements.

TEXT CHANGES: No text changes were made due to this comment.

R3, Comment 10*: "Introduction: The authors say ms and ns for the 2 systems. That should be changed to seconds and milliseconds. Unless these were really just referring to sampling frequencies, in which case it is still misleading. Please clarify."*

Response 10: The references to ms and ns for the 2 modules refers to the timescale of the light intensity changes that each technique is trying to quantify. FD-DOS measures the AC and phase changes of the diffusely reflect light in the scale of tens to hundreds of ms (which is the same as the acquisition time). On the other hand, DCS measures the fast fluctuations in light intensity, from less than 1 μs up to tens of ms, by varying the delay-time in the autocorrelation computations. Note that the sampling time for DCS will be longer than the largest delay-time used in the auto-correlation computation. To better clarify this point, we have slightly rephrased lines 63-68 in the introduction.

TEXT CHANGES: To clarify the issue raised by the reviewer, we have modified lines 64-68, and it now reads as follow:

"By measuring the slow changes (i.e., on on the order of of tens to hundreds of ms) of the diffusively scattered near-infrared light (~ 650-900 nm) from the scalp, diffuse optical spectroscopy (DOS) can measure concentrations of the main chromophores in the brain, such as cerebral oxy- (HbO) and deoxy-hemoglobin (HbR) concentrations [12, 13]. Additionally, it is possible to measure cerebral blood flow (CBF) with diffuse correlation spectroscopy (DCS) [10, 14–17] by quantifying the rapid fluctuations in light intensity (i.e., on the order of from a few nμs to a few ms) one can also assess cerebral blood flow (CBF) with diffuse correlation spectroscopy (DCS) [10, 14–17]."

R3, Comment 11: *"How were the two systems combined such that the 785 nm laser didn't interfere with the ISS? If the detectors were switched on an off on the ISS, did that cause artifacts? Or if filters were used, which narrowband filter was used that was strong enough for 785 nm?"*

Response to 11: As described in the response to the reviewer's comment 9 and in the Introduction section, the two systems acquired data sequentially, therefore we sequentially turned the lasers from each system on and off, as well as the FD-DOS detectors. The switching of the FD-DOS from the ISS is not expected

to cause artifacts, and the ability to switch the detectors is provided by the company. To better clarify the measurement process, we have slightly clarified the last paragraph in the introduction.

TEXT CHANGES: Lines 127-134 were rephrased to better clarify the measurements process:

"The microcontroller is responsible for turning the FD-DOS and DCS lasers on and off, as well as the FD-DOS detectors to allow interleaved measurements of each module. In total, the proposed system can collect one combined FD-DOS and DCS sample every 0.5 to 5s, depending on the signal-to-noise ratio (SNR) requirements (longer collection times leads to better SNR). To couple the light to the forehead, we developed a 3D-printed optical probe that can be customized for each patient (Figure 1-B), with source-detector separations varying between 0.8 and 4.0 cm. The standard source-detector separations used in the examples presented here are 2.5cm for DCS and 1.5, 2.0, 2.5 and 3.0 cm for FD-DOS."

R3, Comment 12: "*Protocol: Why would you warm up the system before fibers are attached? How do you make sure the lasers aren't shining into the room? What do you mean the fibers are disconnected for safety reasons?"*

Response to 12: The warm-up steps in the protocol were written in the wrong order, and we are sorry about this. We only turn on the lasers after the fibers are attached, and we make sure the fiber tips are covered during all time. We always disconnect fibers to avoid them being damaged during storage, as the cart used to move the system does not have a front door to protect the optical system from accidental damage.

TEXT CHANGES: We have reordered the first section of the Protocol, and we have added a few sentences to highlight the points raised by the reviewer. Steps 1.1-1.3 are now written as:

"1.1. Connect all the fibers to the relevant lasers and detectors, and make sure they are properly attached to the optical probe (Figure 1-B).

1.2. Check that optical probe is covered with a black cloth to avoid the lasers shining in the room.

1.3. Turn the system power switch to the 'ON' position. After powering the system, wait 30s and then turn the DCS laser key switch to the 'ON' position. The FD-DOS lasers are automatically turned on when the system is powered."

R3, Comment 13: "*What source detector distances are used? What should they be based on subjects (kids vs adults)?"*

Response to 13: We are sorry for not clearly providing the source-detector separation information. In the examples presented in this study, we used a source-detector separation of 2.5 cm for DCS and 1.5, 2.0, 2.5

and 3 cm for FD-DOS. This work was only focused on adults and thus we have not described the required differences in the source-detector separations for measurements in infants (see Dehaes et al. *Biomed. Opt. Express*, 2011 for a discussion about this issue for FD-DOS). However, we included a brief discussion about the proper selection of the source-detector separations in the Discussion section.

References:

Dehaes, M., et al.. Assessment of the frequency-domain multi-distance method to evaluate the brain optical properties: Monte Carlo simulations from neonate to adult. *Biomedical Optics Express*, 2(3), 552. (2011)

TEXT CHANGES: To explicitly describe the source-detector separations used in this study, we have added a sentence in lines 133-134:

"The standard source-detector separations used in the examples presented here are 2.5cm for DCS and 1.5, 2.0, 2.5 and 3.0 cm for FD-DOS."

We have also included a Discussion about the choices of the source-detector separations in the Discussion section (lines 474-483):

"This work also proposes the use of a customizable optical probe that can address different settings and therefore suit different purposes and needs for clinicians. The proper selection of the source-detector separation is a critical step for maximizing the cerebral sensitivity of diffuse optics. In most cases, an optimal probe for DCS measurements in adults should have at least a short (< 1cm) and a long (> 2.5 cm) source-detector separation. The long source-detector separation was shown to provide the best compromise between signal-to-noise ratio (SNR) and cerebral sensitivity [12], 14, 16, while the short separation is mostly sensitive to the extra-cerebral tissues and is useful to differentiate the extra-cerebral changes from cerebral changes [12, 16]. For FD-DOS, a simple probe that provides a reasonable compromise between SNR and cerebral sensitivity in adults contains 4 source-detector separations (1.5, 2.0, 2.5 and 3.0 cm) [58]."

R3, Comment 14: "*Battery: Does the Imagent really have a battery that would allow operation? If not, make this clear."*

Response to 14: The Imagent module does not have a battery that allow operation, but we have included a small auxiliary battery to allow the whole system to be kept powered on for up to 45 minutes. This is enough time to keep the system powered on during transport. We have now included the battery model number in the Table of Materials.

TEXT CHANGES: We have included the auxiliary battery model number in the Table of Materials. Please see the revised version of the Table of Materials.

R3, Comment 15: "*Can the authors add a bit more information about what motion looks like in order to help someone less familiar with the signals?"*

Response to 15: This is a very good question raised by the reviewer. In general, a motion artifact will produce very fast spikes in the cerebral blood flow and cerebral oxygenation curves, sometimes coupled to a fast change in the baseline values. It is not a simple task to visually identify motion artifacts, and in our opinion, it is best to include motion sensors in the optical probes for measurements when a lot of movement is expected. Nonetheless, for monitoring neurocritical patients, which are usually sedated, we think that motion artifacts should not be a big issue, apart for periods during patient physical manipulations (which are best excluded from the analysis to avoid misinterpretation of the data). In the example presented in this study, although there may have been meaningful physiological oscillations during the manipulation of the patient, we could not precisely mark the timing of each head movement and we did not have a motion sensor in our optical probe. Thus, to avoid misinterpretation of the data, we opted classify this period as an artifact.

> **TEXT CHANGES:** Lines 355-357 were rephrased to avoid mislabeling the artifacts induced by the patient motion:
>
> *"The patient motion induced by the intervention clearly disturbs the optical signal, which leads to the unphysiological spikes in the optical parameters; therefore, it is hard to attribute any physiological meaning to these changes."*

R3, Comment 16: "*The authors are describing the placement of two probes, but the protocol only talks about one probe for calibration. This should be clarified / the protocol amended."*

Response to 16: We have not described the placement of two probes. Instead, we described the placement of the same one probe in two different regions of the brain for sequential measurements in different ROIs. To better clarify this point, we have rephrased the relevant protocol steps.

> **TEXT CHANGES:** We have reworded step 4.6:
>
> *"4.6. Collect data in the first ROI for as long as required by the protocol. If necessary, move the probe to the other ROIs and repeat the measurement.*
>
> *NOTE: The monitoring period may vary depending on the study goals."*

R3, Comment 17: *"Figure 1B: which holes are sources and which ones are detectors? What was spacing?"*

Response to 17: Thanks for bringing this issue to our attention. We have modified Figure 1 to better clarify the probe layout.

TEXT CHANGES: We have modified Figure 1 to describe which holes are sources and which ones are detectors. We have adjusted the Figure Legend accordingly:

*"**Figure 1** - The optical environment developed to monitor patients inside an intensive care unit. (A) The hybrid diffuse optical system combines a frequency-domain diffuse optical spectroscopy (DOS) module and a diffuse correlation spectroscopy (DCS) module. (B) The customizable probe proposed in this study has as a default 4 source-detector separations (0.7, 1.5, 2.5 and 3.0 cm) for DCS and 4 source-detector separations for DOS (1.5, 2.0, 2.5 and 3.0 cm). For simplicity, the examples presented here only used the 2.5cm source-detector separation for DCS. (C) The real-time graphical user interface (GUI) controls the diffuse optical system, and displays the measured cerebral blood flow (CBF), the oxygen extraction fraction (OEF) and the cerebral metabolic rate of oxygen (CMRO2) in real-tiime, both within a 5-minute time window (left panels), and within a 2-hour time window (right panels). On the bottom of the GUI, the researcher can press buttons to start and stop the data collection, to acquire a baseline period for comparison and to mark any relevant intervention(s)."*

Reviewer #4:

R4, Comment 1: "*I understand that there is little difference between this paper and the previous paper from the authors: Forti et al., Frontiers in Medicine (10.3389/fmed.2020.00147). Please state in the manuscript that this manuscript is based on the previous publication."*

Response to 1: Although there are similarities between this work and the paper previously published, the main goals of each paper are intrinsically different. Our previous work was focused on the clinical aspects of an interesting case-study of a patient monitored throughout hospitalization, which also demonstrated the feasibility of employing diffuse optical methods to longitudinally monitor patients in the neuro-ICU. The focus of this work is to introduce the methods developed for monitoring neurocritical patients in the ICU; we aimed to detail relevant and innovative aspects related to the real-time monitoring with diffuse optics and the control software we developed specifically for the neuro-ICU. As far as we are aware, this has not been published elsewhere. Nonetheless, we acknowledge there are overlaps, and we included a few sentences in the Discussion section stating that this work is a publication explaining the methodology behind the previously published case-study. Note that the present work tries to present a methodology that can be applied to different cases, as we trying to describe a more general protocol for neurocritical monitoring.

> **TEXT CHANGES:** We now clearly state that the main goal of this study and our previous publication are intrinsically different:
>
> *"A previous study focused on the clinical aspects and the feasibility of optical monitoring during hospitalization in the neuro-ICU through a case report [9]. The focus of this work is to detail relevant and innovative aspects related to real-time monitoring with diffuse optics. Specifically, this paper proposed a real-time GUI that provides clear and useful information for clinicians."*

R4, Comment 2: "*Line 65 and 85. Please limit the number of references to a few, most important. Linking 18! references to one sentence is not acceptable."*

Response to 2: We were genuinely trying to be as inclusive as possible in listing previous contributions from different research groups rather than our own. We have changed the references from that sentence and included only the relevant literature reviews.

R4, Comment 3: "*Why did not author conduct any regression, especially when they have values measured at short source-detector separation? Please explain."*

Response to 3: To properly remove the extracerebral influences from FD-DOS and DCS, a simple regression is not sufficient, and a multi-layer approach would be more appropriate. Here, we are interested in highlighting the real-time feature of the system developed and showing that the absolute values of optical parameters that we get with the simplest approach shows that the technique can be useful in real-time. The use of multi-layer methods is more complicated and requires further validation of the algorithms. This is

currently a topic of discussion in the diffuse optics community and will be the subject of future studies. The issue of using better modelling methods to remove the extracerebral influences was already highlighted in the last paragraph of the discussion.

TEXT CHANGES: No text changes were made due to this comment.

R4, Comment 4: "*Figure 3(b) Are the oscillation in the shaded area just movement artifacts? Please clarify?"*

Response to 4: As stated in the representative results section, during this period the clinicians were suctioning the patient's bronchial and oral secretions, which is expected to induce movement artifacts due to head movements. Although there may have been meaningful physiological oscillations during that time, we could not precisely mark the timing of each head movement and we did not have a motion sensor in our optical probe. Thus, to avoid misinterpretation of the data, we opted classify this period simply as an artifact. To clarify this issue, we have rephrased the description of this case in the revised manuscript.

TEXT CHANGES: Lines 355-357 were rephrased to avoid mislabeling the artifacts induced by the patient motion:

"The patient motion induced by the intervention clearly disturbs the optical signal, which leads to the unphysiological spikes in the optical parameters; therefore, it is hard to attribute any physiological meaning to these changes."

R4, Comment 5: "*Figure 3. Why CMRO2 have no units? Is it some relative change in CMRO2? If yes, please change the y-axis label."*

Response to 5: The unit of $CMRO_2$ is meaningless as we do not have access to the absolute blood flow values in clinical units. Thus, we opted to ignore the units of $CMRO_2$.

TEXT CHANGES: *No text changes were done due to this comment.*

R4, Comment 6: "*Reference 82 is dated. Please update it with more recent work: Khalid et al. Biomedical Optics Express, 10(9), 2019 (10.1364/BOE.10.004607)"*

Response to 6: We believe that it is important to highlight the pioneer studies, and thus we have just included the updated reference as an additional citation.

TEXT CHANGES: We have included the citation suggested by the reviewer in the last paragraph of the discussion.

Supplementary Material

The '*Real Time Monitor*' tab of the GUI displays the real-time CBF, oxygen extraction fraction (OEF) and cerebral metabolic rate of oxygen ($CMRO_2$) values at both a short-term (5-minute) and a long-term (2-hour) time window (Figure 1-C). Specifically, the software employs a Python script on the background to compute the cerebral physiology parameters from the diffuse optics models and the DOS/DCS data (Hunter, 2007; Newville et al., 2014; Oliphant, 2007). First, the software analyzes the FD-DOS data using a standard semi-infinite model of the head to recover the absorption and scattering coefficients (μ_a and μ_s', respectively) (T. Durduran et al., 2010; Shang et al., 2017). From the absorption coefficient, we can recover the oxy- and deoxy-hemoglobin concentration with:

$$\mu_a(\lambda) = \varepsilon_{HbO}(\lambda)HbO + \varepsilon_{HbR}(\lambda)HbR + 0.75\mu_{a_{H_2O}}(\lambda),$$

where we assumed a 75% water content in tissue. Then, from HbO and HbR we can compute the oxygen extraction fraction (OEF) as (Culver et al., 2003; Jain et al., 2014):

$$OEF \sim 1.33\left(1 - \frac{HbO}{HbO + HbR}\right).$$

For simplicity, we assumed that the oxygen saturation of the blood is 100% and assumed that the percentage of blood volume in the venous compartments is 75% (Jain et al., 2014).

To recover an index of CBF, we used the optical coefficients recovered from the FD-DOS data along with a semi-infinite model of the head to fit the DCS autocorrelation curves (T. Durduran et al., 2010; Turgut Durduran & Yodh, 2014; Yu, 2012). Since we are mostly interested in the cerebral physiological changes, the GUI only displays the largest source-detector separation, as it has the highest sensitivity to the cerebral tissues. Finally, we combine the CBF and oxygenation measurements to derive the cerebral metabolic rate of oxygen ($CMRO_2$) (Valabrègue et al., 2003). The $CMRO_2$ is computed using a steady state model and can be written as $CMRO_2 \propto OEF \times CBF$.

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