



Article

Optical Neuroimaging to Discern Transitions in Anesthetic Depth from Systemic Hemodynamic Instability

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Abstract

Background: Reliable intraoperative monitoring of anesthesia depth is critical for patient safety, yet standard monitors can be confounded by systemic physiological changes. Non-invasive optical modalities that measure cerebral hemodynamics offer a promising approach, but their ability to differentiate brain neurovascular activity assessment versus changes due to systemic events requires validation. This study aims to explore the robustness of diffuse correlation (DCS) and near-infrared spectroscopy (NIRS) measures in differentiating changes in cerebral signals due to changes in anesthetic depth versus those resulting from alterations in systemic hemodynamics. **Methods:** A neonatal piglet model ($N = 18$) was used to assess anesthesia states and injury models creating systemic hemodynamic changes which may lead to instability in readings of hemodynamic responses and likely cause failure to detect the true anesthesia state. We continuously measured relative cerebral blood flow (rBFI), oxyhemoglobin (HbO), deoxyhemoglobin (HbR), and total hemoglobin (HbTotal) concentrations during three distinct conditions: (1) transition from deep to light anesthesia state in healthy piglets, (2) controlled hemorrhage, and (3) hypoxia induced under maintained deep anesthesia. Linear mixed-effects models were used to compare hemodynamic signatures across conditions. **Results:** Transition to light anesthesia was characterized by significant increases in rBFI ($p_{\text{adj}} < 0.01$), HbO ($p_{\text{adj}} < 0.01$), and HbTotal ($p_{\text{adj}} < 0.05$). Interaction analyses confirmed this distinct cerebral hemodynamic pattern was statistically different from the patterns of hemorrhagic shock (decreased rBFI, HbO, HbTotal) and hypoxic shock (decreased rBFI, HbO; increased HbR, HbTotal), with significant differences in trends ($p_{\text{adj}} < 0.05$). Optical biomarkers distinguished the transition to lighter anesthesia from early physiological changes during systemic insults, suggesting discriminative capability beyond severe shock states. **Conclusions:** These findings demonstrate that a DCS-NIRS system provides quantitative biomarkers capable of distinguishing changes in anesthetic depth from specific, controlled systemic insults causing subtle to severe hemodynamic instability, supporting its potential as a robust tool for improving intraoperative monitoring and patient safety.

Keywords: cerebral blood flow; cerebral hemodynamics; depth of anesthesia; diffuse correlation spectroscopy; near-infrared spectroscopy



Academic Editor: Patrick W. Stroman

Received: 31 March 2026

Revised: 29 May 2026

Accepted: 18 June 2026

Published: 3 August 2026

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Key Contribution: This study demonstrates that a multimodal DCS-NIRS system provides distinct cerebral hemodynamic signatures capable of differentiating transitions in anesthetic depth from systemic insults, including hemorrhage and hypoxia, even at early stages of physiological compromise.

1. Introduction

Monitoring the depth of anesthesia during surgery is critical for patient safety and for the success of the procedure. Unintended emergence from anesthesia is a significant clinical challenge, occurring when patients inadvertently emerge from maintenance anesthesia depth to a lighter state without a noticeable change in standard clinical monitors, potentially leaving the anesthesiologist unaware [1]. Such events can result in intraoperative awareness, physiological stress responses, hemodynamic instability, and increased risk of postoperative complications, including posttraumatic stress disorder [2,3]. Current estimates indicate that intraoperative awareness occurs in approximately 0.1–0.2% of general anesthesia cases, with higher incidences documented in high-risk surgeries, such as cardiac or trauma procedures [4].

Standard anesthesia care aims to mitigate such risks through careful patient management and monitoring, which typically includes measurement of vital signs such as heart rate and rhythm (via electrocardiogram, ECG), blood pressure, peripheral oxygen saturation (SpO₂), and body temperature. In addition, end-tidal gas concentrations are measured and titrated using Minimum Alveolar Concentration (MAC) defined as the concentration of gas required to prevent movement in 50% of subjects in response to surgical stimulus. MAC serves as a population-based guide for inhaled agents [5]. While essential for patient safety and detecting adverse events like hypoxia or hemodynamic collapse, and guiding supportive interventions, these systemic physiological variables often serve as indirect and non-specific indicators of the anesthetic effect on the brain [6,7]. Systemic variables like blood pressure or heart rate can be influenced by factors other than anesthesia depth, such as surgical stimuli or medications, and MAC does not account for individual patient variability [6,8,9]. Critically, from these measures alone, there is no objective ‘gold standard’ for determining anesthesia depth other than the clinical judgment of the anesthesiologist [10]. Moreover, in patients experiencing intraoperative complications such as hypoxia or hemorrhage, the resulting systemic physiological changes can confound these monitoring techniques, underscoring the need for monitoring modalities that can provide a direct assessment of brain state, even during periods of peripheral hemodynamic change [7,11].

To address this need for more direct assessment of brain activity at different anesthetic states, current standard monitoring predominantly relies on electroencephalography (EEG) based parameters, namely the Bispectral Index (BIS) and entropy monitors. While these EEG-derived technologies have significantly improved anesthesia management, they possess important limitations. These include delayed response to rapid anesthesia state transitions [12,13], susceptibility to electrical interference and signal artifacts in the operating room environment [11], variable performance across different anesthetic agents such as propofol versus ketamine [14,15], and inadequate sensitivity in certain patient populations, including pediatric or elderly patients [16]. These limitations underscore the necessity for complementary monitoring approaches that can provide direct cerebral physiological information during anesthesia, such as the use of optical imaging tools to measure cerebral hemodynamics.

Diffuse Correlation Spectroscopy (DCS) and Near-Infrared Spectroscopy (NIRS) are non-invasive optical modalities that offer promising alternatives or complements to EEG-based anesthesia monitoring methods [17–19]. DCS measures microvascular cerebral blood flow by analyzing the temporal fluctuations of scattered near-infrared light caused by moving red blood cells, providing a Blood Flow Index (BFI) and its relative change (rBFI), which reflect continuous cerebral tissue perfusion [20,21]. NIRS quantifies cerebral oxygenation and blood volume by measuring changes in oxyhemoglobin (HbO, an indicator of oxygen delivery), deoxyhemoglobin (HbR, reflecting tissue oxygen extraction), and total hemoglobin (HbTotal, a surrogate marker for cerebral blood volume) [22,23].

The simultaneous measures from DCS and NIRS offer continuous bedside monitoring of cerebral blood flow, volume, and oxygenation changes, thereby providing comprehensive physiological information beyond that available from EEG or other standard of care measures, e.g., vital signs. Furthermore, because they monitor distinct physiological mechanisms via neurovascular coupling, these optical modalities have the potential to provide complementary information to EEG-derived indices during anesthesia state transitions [24–26]. Additionally, DCS and NIRS directly measure cerebrovascular responses to anesthetic drugs, potentially reflecting drug-specific dose-dependent effects on cerebral perfusion and metabolism [18,27]. Optical monitoring is non-invasive and does not interfere with the surgical field or operating room workflow, facilitating continuous hemodynamic monitoring throughout the procedure [20,28].

However, before these optical modalities can be deployed clinically for anesthesia depth assessment, a fundamental validation requirement must be addressed: demonstrating that hemodynamic changes measured during emergence reflect brain state transitions rather than nonspecific systemic responses. Intraoperative complications such as hypoxia or hemorrhage can also produce hemodynamic changes that could confound anesthesia depth monitoring [18,29]. Without such validation, optical imaging modalities that quantify hemodynamic changes poses the risk of misclassifying adverse intraoperative events as signs of anesthetic emergence, limiting their clinical utility and patient safety.

Previous optical studies using NIRS have primarily examined anesthesia stages under stable hemodynamic conditions, with no record of confounding insults like brain injury or blood loss [10,18,24,25,30,31]. These studies report that cerebral hemodynamics can detect transitions in anesthesia depth, with significant changes reported in HbO, HbR or HbTotal observed during induction and emergence. More recently, the use of DCS for monitoring cerebral blood flow (CBF) during anesthesia transitions also confirmed this effect; in healthy patients, CBF were reported to decrease significantly during propofol induction and increase during recovery [32]. Our study builds on these findings by evaluating the discriminative capability of DCS-NIRS biomarkers by comparing hemodynamic responses during the transition to light anesthesia against systemic insults like hypoxia and hemorrhage.

To address the clinical need for reliable detection of changes in anesthetic depth, this study was designed to investigate the capability of DCS and NIRS derived biomarkers to detect the transition to a light anesthesia state. We wanted to evaluate if the hemodynamic signatures of this transition could be distinguished from those elicited by other intraoperative complications. Accordingly, this study employed a neonatal piglet model to compare cerebral hemodynamic responses across three distinct physiological conditions: (1) transition from deep to a light anesthesia state, (2) hypoxia, and (3) hemorrhagic shock. We hypothesized that the transition from deep to light anesthesia would produce a distinct hemodynamic response, characterized by increased cerebral blood flow and oxygenation, which is statistically distinguishable from the responses induced by the systemic insults. Overall, this work aimed to assess whether the DCS and NIRS derived optical

biomarkers could distinguish anesthesia state transitions from the tested systemic insults, a fundamental step towards their eventual clinical translation as anesthesia depth monitors.

2. Methods

2.1. Study Protocol

2.1.1. Animal Model

All animal procedures were conducted in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals, and the protocol was approved by the Institutional Animal Care and Use Committee (IACUC) at Drexel University (Protocol #20889). Newborn Yorkshire piglets (3–5 days old) provide an adequate model for anesthesia monitoring research due to their unique physiological characteristics. Their size allows for direct physiological monitoring and blood sampling while maintaining stable experimental conditions [33,34]. The piglets' head dimensions and skull thickness are ideal for DCS and NIRS sensor placement, enabling optimal penetration of near-infrared light for interrogation of cerebral tissues at varying depths [35]. Most importantly, piglets share neuroanatomical and cerebrovascular regulatory similarities with humans, including comparable cerebral autoregulation mechanisms under anesthesia [36,37]. Eighteen newborn Yorkshire piglets (3–5 days old; 2–3 kg, Meck Swine, LCC, Willow Street, PA, USA) were included in this study. Nine piglets were assigned to the depth of anesthesia study. From these animals, we analyzed data from sixteen distinct deep-to-light anesthetic transitions. Each transition encompasses the full physiological period from a stable deep anesthetic state to the subsequent light state. The 16 deep-to-light anesthesia transitions were obtained from 9 piglets, with individual animals contributing between 1 and 3 transitions each (Table A2). Some animals underwent repeated transition cycles within the same experiment, whereas others contributed a single transition before being allocated to different, unrelated experimental protocols. For animals contributing multiple transition episodes, each episode was temporally separated by the administration of a maintenance anesthetic bolus and the subsequent full duration of the stable deep anesthesia phase, ensuring physiological parameters were comparable across repeated transitions. Another set of nine animals were allocated to the systemic insult cohorts, which are detailed in the subsequent sections.

2.1.2. Depth of Anesthesia Study

This study focused on distinguishing between two distinct anesthetic states achieved during the anesthesia period: (1) deep anesthesia state, defined in this study as the period immediately after the intravenous anesthetic agent is administered that corresponds to the peak-effect of the drug based on pharmacokinetics, and (2) light anesthesia state, defined in this study to be the period of time shortly preceding the administration of the next dose of the intravenous anesthetic agent was required (Figure 1). During the deep anesthesia state, the animal is immobile and nonresponsive to pain (e.g., tail pinch), whereas during the light anesthesia state, the animal may be regaining responsiveness or becoming mobile or showing other signs indicating the waning effects of the previous ketamine dose and signaling the need for the next bolus. In this study, the deep and light anesthesia states were defined operationally using ketamine dosing interval and behavioral responsiveness criteria. Accordingly, these terms are used throughout the manuscript to denote protocol-defined anesthetic states within this animal model. Figure 1 illustrates the protocol with deep and light anesthesia regions annotated. Ketamine was selected as the primary intravenous anesthetic agent for this study protocol to minimize confounding hemodynamic variables. Unlike other common agents such as propofol or fentanyl, which may induce significant hypotension and bradycardia, ketamine maintains a favorable hemodynamic profile (preservation of heart rate and blood pressure) [38–41]. This stability

allows for the isolation of cerebral hemodynamic changes related to cortical state transitions rather than secondary responses to systemic circulatory depression.

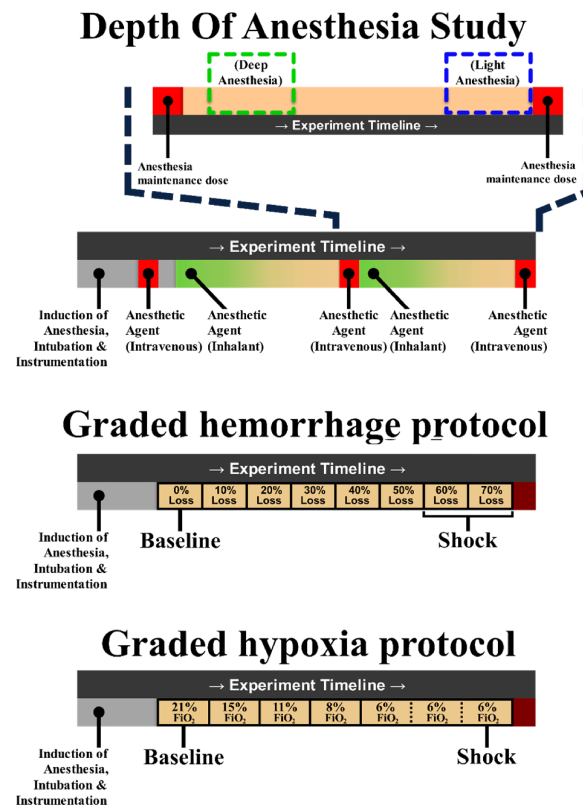


Figure 1. Experimental protocol timelines. **Top:** Transitions in anesthetic depth from deep (green) to light (blue) states. **Middle:** Graded hemorrhage (0–70% blood loss) under maintained deep anesthesia. **Bottom:** Graded hypoxia (FiO₂ 21% to 6%) under maintained deep anesthesia.

2.1.3. Systemic Insult Protocols

To evaluate the specificity of the optical biomarkers for detecting changes in anesthetic depth against different intraoperative events, data from two additional cohorts of neonatal piglets were included for comparative analysis. These animals were subjected to either controlled hypoxia or a graded hemorrhage protocol while maintained under stable anesthesia. The experimental procedures for these insults are explained below and have been previously described in detail in previous work by our group [36,42,43].

- *Graded hemorrhage protocol* (N = 4) was designed to model intraoperative blood loss. In these animals, a state of hypovolemic shock was induced by withdrawing 10% aliquots of the total estimated blood volume in graded manner (Figure 1). The protocol was continued until a state of shock was confirmed, defined as a mean arterial pressure (MAP) below 30 mmHg [36]. Cerebral hemodynamics were monitored continuously using DCS and NIRS throughout the baseline and hemorrhage phases of the experiment.
- *Graded hypoxia protocol* (N = 5) was designed to model the effects of systemic oxygen deprivation. Following a stable baseline period under normoxemic conditions, a global hypoxic challenge was initiated by reducing FiO₂ gradually to 6% (Figure 1). This level of FiO₂ was maintained until a clear physiological response, characterized by significant hypotension and bradycardia, was observed [36]. DCS and NIRS measurements were acquired continuously before and during hypoxia.

The hemorrhage and hypoxia cohorts were maintained under the same anesthetic protocol as the depth-of-anesthesia cohort. Thus, the primary difference between these groups was the induced physiological insult, rather than a change in anesthetic regimen.

2.1.4. Physiological Monitoring

Throughout the experimental period, the animals were maintained at normoxemic oxygen levels and did not experience any surgical or other painful interventions. Body temperature was maintained at euthermia (39 °C) using a servo-controlled temperature-regulated circulating water blanket (Gaymar Medi-Therm III, Gaymar Industries, Orchard Park, NY, USA), and an overhead radiant warmer (Ohio Neo-Natal Care Center, Ohio Inc., Madison, WI, USA). Oxygen saturation and heart rate were continuously monitored using a pulse oximeter placed on a limb, and ECG sensors connected to the PowerLab system and LabChart (Ver. 8.1.30) data acquisition software (AD Instruments, Colorado Springs, CO, USA). Blood pressure (BP) was collected every 15 min via an electronic sphygmomanometer using neonatal cuff (Contec, Hamburg, Germany) placed on a limb, from which Mean Arterial Pressure (MAP) was derived. Arterial or venous blood gases, glucose, and lactate levels were measured approximately every 60 min using a blood gas analyzer (i-STAT-1; Abbott, Princeton, NJ, USA) and a glucometer (Accu-Check, Roche, Indianapolis, IN, USA) to ensure physiological stability. Cerebral blood flow via DCS and cerebral oxygenated, deoxygenated, and total hemoglobin concentrations via NIRS systems were acquired as described below.

2.1.5. Euthanasia

Upon completion of the experimental protocol, animals remained under general anesthesia maintenance with continuous ECG monitoring while euthanasia was performed using Euthasol (pentobarbital sodium and phenytoin sodium; Virbac AH, Inc., Fort Worth, TX, USA) administered intravenously at a dose of 0.4 mL/kg, in accordance with American Veterinary Medical Association guidelines [44]. Following confirmation of asystole by ECG, the cadaver underwent decapitation and postmortem tissue harvest.

2.2. Optical Neuroimaging Instrumentation

2.2.1. Diffuse Correlation Spectroscopy (DCS)

The DCS system enables measurement of microvascular blood flow through analysis of the temporal fluctuations in scattered light intensity passing through tissue. DCS tracks the movement of red blood cells beneath the tissue surface, enabling the continuous monitoring of cerebral blood flow (CBF) without requiring contrast agents or exposure to ionizing radiation [20,21,45,46]. DCS is built on the fundamentals of photon correlation spectroscopy, which measures the intensity of coherent light within a speckle pattern. The speckle pattern comprises spots of both constructive and destructive interference. A small point in the speckle pattern is observed for fluctuating light intensity over time [47]. These temporal properties of such fluctuations are influenced by the scatterers' motion within the medium. The Temporal Correlation Function (TCF or $g_2(\tau)$) is the normalized temporal intensity autocorrelation function. In the context of a single-photon counting detector, this function is computed from the arrival times of photons and captures the temporal properties of the intensity fluctuations. For the assessment of blood flow, this general principle is adapted for multiple-scattering media like biological tissue. While related techniques use this principle to measure particle size in controlled solutions, in DCS the decay rate of the TCF is primarily influenced by the motion of scatterers within tissue, predominantly red blood cells. In such medium, photons from the long-coherence laser source undergo multiple scattering events before reaching the detector. Each scattering event from a moving cell introduces a phase shift, adding a consequent delay time at the detector. These cumulative

phase shifts define the decay rate of the TCF, which is therefore used to derive an index of microvascular blood flow. Figure 2 illustrates typical TCF curves corresponding to varying rates of blood flow [48].

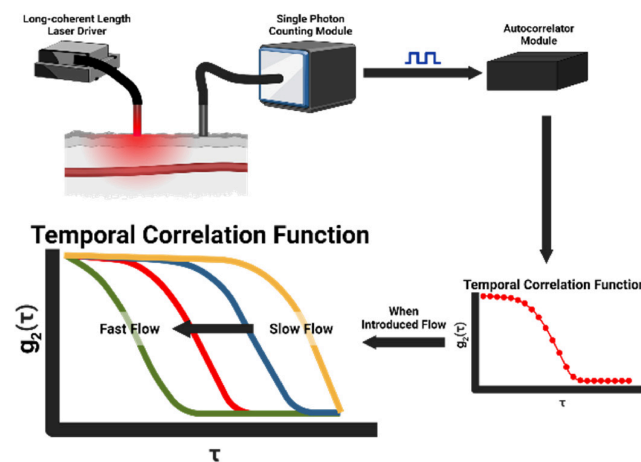


Figure 2. Diffuse correlation spectroscopy setup consists of a long-coherence laser source, a single photon counter, and an autocorrelator module.

The DCS system comprises three main components. A long-coherence length (>10 m) near-infrared laser illuminates the tissue region of interest. A single photon counter module collects light intensities at a given source-detector separation, followed by an autocorrelator module that computes TCF for each measurement. Figure 2 illustrates a typical DCS setup. The DCS system used in this study operates at a sampling rate of 5 Hz and employs a long-coherent length laser (~10 m) (DL785-100-S, CrystaLaser, Reno, NV, USA), a four-channel single photon counting module (SPCM-AQ4C, Excelitas, Pittsburgh, PA, USA) and a multi- τ correlator (Correlator.com, Bridgewater, NJ, USA). Step-index optical fibers (Thorlabs, Newton, NJ, USA) were used to guide light to and from the tissue. A multimode fiber (62.5 μm core diameter) delivered light from the laser source, while a single-mode fiber (5 μm core diameter) was used for detection. Additionally, a linear polarizer was placed before the detector fiber. These fibers were housed in a flat probe with 2.25 cm source-detector separation and placed over the temporal to parietal brain regions on the piglet head. The combination of a single-mode detection fiber and a polarizer ensure collection from a single coherence area (speckle), which maximizes the measured signal coherence, β .

2.2.2. Near-Infrared Spectroscopy (NIRS)

NIRS is a non-invasive optical imaging modality used to quantify changes in tissue chromophore concentrations, primarily oxyhemoglobin (HbO) and deoxyhemoglobin (HbR), by measuring the absorption of near-infrared light at multiple wavelengths [22,49]. The NIRS system used in this study employed continuous-wave light sources operating at two wavelengths, 730 nm and 850 nm [23]. These wavelengths were selected for optimal sensitivity to HbO and HbR concentration changes within the near-infrared optical window [50,51]. Backscattered photons through the tissue were collected by silicon (Si) photodiodes. A flexible flat NIRS probe, containing the source and detector components, was placed on the contralateral preorbital to temporal region of the piglet's head, opposite the DCS probe. The source-detector separation for the NIRS probe was 2.5 cm. The minor difference from the 2.25 cm DCS source-detector separation was a result of the custom fabrication of two independent probes. For the purpose of this study, these separations were considered comparable as they provide a similar depth of penetration of approximately 1–1.5 cm [52,53]. Data acquisition was managed by a custom-designed and programmed

field-programmable gate array (FPGA) based system, driving parallel digital lock-in amplifiers to process the detected light intensities for each wavelength. The NIRS data was acquired continuously at a sampling rate of 2 Hz simultaneously with the DCS system.

2.3. Biomarker Calculation

2.3.1. Cerebral Blood Flow Index Calculation with a Partial Curve Fitting Method

Blood Flow Index (BFI), a quantitative measure proportional to microvascular blood flow, is computed from DCS measurements. The primary output of DCS system is the TCF ($g_2(\tau)$), which quantifies the temporal fluctuations of scattered light intensity due to the movement of red blood cells. This function was first converted to the normalized electric field temporal autocorrelation function $g_1(\tau)$, using the Siegert relation [21,47,48]:

$$g_2(\tau) = 1 + \beta |g_1(\tau)|^2 \quad (1)$$

where τ is the autocorrelation delay time, and β is an empirical coherence factor. The value of β depends on the detection optics, primarily the number of collected light speckles (or coherence areas) relative to the detector size. It is typically determined numerically from the intercept ($\tau \rightarrow 0$) of the measured TCF (or $g_2(\tau)$). The decay characteristics of $g_1(\tau)$ reflect the motion of scatterers, predominantly red blood cells, within the tissue. This relationship is mathematically described by the correlation diffusion equation, typically solved assuming the measured tissue is a semi-infinite homogeneous medium, given by the Green's function [21,47,48]:

$$G_1(\tau) = \frac{3\mu'_s}{4\pi} \left(\frac{e^{-K(\tau)r_1}}{r_1} - \frac{e^{-K(\tau)r_2}}{r_2} \right) \quad (2)$$

and $g_1(\tau) = G_1(\tau)/G_1(0)$ where $K(\tau) = \sqrt{3\mu_a\mu'_s + 6\mu_s'^2k_0^2\alpha D_B\tau}$, $r_1 = \sqrt{\rho^2 + 1/\mu_s'^2}$, $r_2 = \sqrt{\rho^2 + (2z_b + 1/\mu_s')^2}$, and $z_b = \frac{2}{\mu'_s} \left(\frac{1+R_{\text{eff}}}{1-R_{\text{eff}}} \right)$. Here, $k_0 = 2\pi n_0/\lambda$ is the optical wavenumber in tissue, where λ is the wavelength of the light source in vacuum (or air) and n_0 is the refractive index of the measured tissue. Therefore, the solution models $g_1(\tau)$ as a function of autocorrelation delay times (τ), wavelength of the light source (λ), the source-detector separation (ρ), the effective reflection coefficient ($R_{\text{eff}} = -1.44n_0^{-2} + 0.71n_0^{-1} + 0.67 + 0.064n_0$) calculated using refraction indices of the measured tissue (n_0) and air (~ 1), tissue optical properties (absorption coefficient μ_a , reduced scattering coefficient μ'_s), and effective Brownian diffusion coefficient (D_B), which is proportional to the mean speed of the moving scatterers (primarily red blood cells). D_B is extracted by fitting this theoretical model to the experimentally measured autocorrelation data ($g_1(\tau)$) using MATLAB's *fminsearch* function (MathWorks Inc., Natick, MA, USA, R2024a), which implements the Nelder-Mead simplex algorithm for nonlinear optimization. αD_B is used as an index proportional to tissue blood flow also known as BFI, where α defines the fraction of light scattered by moving versus static components (taken 0.1 for brain tissue) [54].

However, fitting the standard correlation diffusion model to the entire measured TCF curve can reduce accuracy, particularly when aiming to measure deeper tissue (e.g., cerebral cortex) blood flow. Photons contributing to the later delay times (τ) of the TCF curve have typically undergone fewer scattering events or have traveled shorter pathlengths, preferentially sampling more superficial tissue layers like the scalp and skull [21,55–57]. These superficial layers often have different blood flow characteristics compared to the brain. To mitigate this well-known superficial contamination and enhance sensitivity to cerebral blood flow changes, our study implemented a partial curve fitting method. In

our method, instead of using the full decay TCF curve, the correlation diffusion model was fitted only to the early portion of the measured TCF data, specifically from $\tau = 0$ to the exponential decay time, τ_{exp} . This τ_{exp} limit was dynamically calculated for each acquired TCF curve, defined as the delay time τ at which the TCF curve decays from its maximum value $(1 + \beta)$ to $(1 + \beta/e)$ where e is Euler's number (the base of the natural logarithm) [47]. Unlike partial fitting methods that rely on a fixed cutoff, our proposed approach adapts the fitting range to the decay rate of each individual curve. This allows for a more robust representation of both faster and slower blood flow rates, ensuring that the fitting window consistently captures an appropriate range of photon scattering events for each measurement. By limiting the fit to this $0 - \tau_{\text{exp}}$ range, we aimed to increase contributions from photons that have travelled deeper into the tissue and experienced more scattering events, thus reflecting cerebral hemodynamics more accurately.

These custom fitting calculations were performed using a non-linear least squares algorithm implemented via the *fminsearch* function in MATLAB (Version R2024a, MathWorks Inc., Natick, MA, USA), minimizing the difference between the measured TCF (within the $0 - \tau_{\text{exp}}$ range) and the analytical model derived from the correlation diffusion equation combined with the Siegert relation (Equation (1)). During the in vivo fitting, the tissue optical properties were assumed constant, with an absorption coefficient (μ_a) of 0.15 cm^{-1} and a reduced scattering coefficient (μ_s') of 9.1 cm^{-1} , based on literature values for neonatal piglet brain tissue [50].

To provide quantitative validation of this fitting method, we evaluated both fitting approaches in a controlled flow phantom experiment. DCS measurements were acquired from a tubing phantom positioned 10 mm from the probe within a scattering medium, while intralipid flow was controlled at 0, 50, 100, and 150 mL/h using the same 2.25 cm source-detector separation employed in the in vivo measurements. For each flow condition, repeated DCS frames were analyzed using both the standard full-curve fit and the proposed partial fitting approach over the $0 - \tau_{\text{exp}}$ range. Figure 3 and Table A3 shows that partial fitting yielded a substantially more linear relationship between the extracted effective Brownian diffusion coefficient (D_B) and imposed flow than the standard full-curve fit, with higher *adjusted* R^2 (0.988 vs. 0.873) and lower RMSE (2.63×10^{-9} vs. 6.66×10^{-9}). These results support the use of the early decay portion of the correlation curve as a better estimator of flow changes, consistent with reduced influence from later-delay components that are more susceptible to superficial contamination.

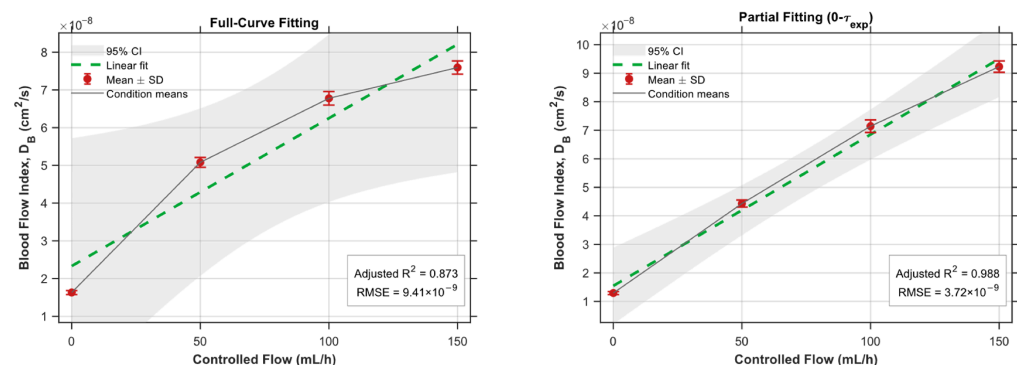


Figure 3. Phantom validation of DCS fitting methods under controlled flow conditions. Mean effective Brownian diffusion coefficient (D_B) values are shown for imposed flow rates of 0, 50, 100, and 150 mL/h for standard full-curve fitting (**left**) and partial fitting over the $0 - \tau_{\text{exp}}$ range (**right**). Dashed green lines indicate linear regression fits and shaded regions indicate the 95% confidence interval. Partial fitting demonstrated improved linearity with imposed flow (*Adjusted* $R^2 = 0.988$) and lower regression error ($RMSE = 2.63 \times 10^{-9}$) compared with full-curve fitting (*Adjusted* $R^2 = 0.873$, $RMSE = 6.66 \times 10^{-9}$).

To facilitate comparisons across experimental protocols and to minimize baseline variability, a relative Blood Flow Index (rBFI) was calculated for each episode. For anesthesia transitions, rBFI was obtained by dividing the BFI value within each anesthetic episode (deep and light) by the average BFI obtained from the immediately preceding deep anesthesia period. For hypoxia and hemorrhage protocols, rBFI was defined by dividing the BFI value in each episode (pre- and post-insult under deep anesthesia) to the average BFI obtained during a pre-insult baseline period. This approach enabled consistent cross-condition comparisons of blood flow changes across all datasets.

2.3.2. Cerebral Oxygenation and Blood Volume Calculations as Biomarkers for Cerebral Hemodynamic Changes

Changes in cerebral HbO and HbR concentrations were calculated from the NIRS light intensity measurements. Raw intensity data acquired at 730 nm and 850 nm were first pre-processed to isolate the slow neural responses of interest. A low-pass filter with a cutoff frequency of 0.09 Hz was applied to remove high-frequency physiological noise, including aliased cardiac and respiratory signals, followed by a wavelet-based motion artifact correction algorithm [58]. The filtered intensity data were then converted to changes in optical density (ΔOD) relative to a baseline period (selected from deep anesthesia or pre-insult state). These ΔOD values were used within the modified Beer-Lambert Law [49,59]:

$$\begin{bmatrix} \Delta OD_{\lambda_1} \\ \Delta OD_{\lambda_2} \end{bmatrix} = \begin{bmatrix} \epsilon_{\lambda_1}^{\text{HbO}} & \epsilon_{\lambda_1}^{\text{HbR}} \\ \epsilon_{\lambda_2}^{\text{HbO}} & \epsilon_{\lambda_2}^{\text{HbR}} \end{bmatrix} \cdot \begin{bmatrix} \Delta C_{\text{HbO}} \\ \Delta C_{\text{HbR}} \end{bmatrix} \cdot d \cdot \text{DPF} \quad (3)$$

where ϵ^{HbO} and ϵ^{HbR} are the known wavelength-dependent molar extinction coefficients of HbO and HbR [60], and $d \cdot \text{DPF}$ represents the effective optical pathlength through the tissue, incorporating the source-detector separation ($d = 2.5$ cm) and an assumed differential pathlength factor (DPF), calculated using the age and wavelength-dependent general equation [61] which adjusts the DPF based on subject age and measurement wavelength. For neonatal subjects, the calculated DPF values were 5.30 at 730 nm and 4.22 at 850 nm. By applying Equation (3) for both wavelengths (730 nm and 850 nm), a system of two linear equations was solved at each time point to determine the relative concentration changes, ΔC_{HbO} and ΔC_{HbR} (μM), used for HbO and HbR biomarkers used in this paper respectively. The change in total hemoglobin concentration (HbTotal), reflecting cerebral blood volume changes, was then calculated ($\text{HbTotal} = \text{HbO} + \text{HbR}$). This process yielded continuous time-series data for HbO, HbR, and HbTotal relative to a protocol-specific baseline (deep anesthesia for anesthesia transition episodes; pre-insult baseline for hypoxia/hemorrhage cohorts) for each experimental episode.

2.3.3. Statistical Methods

For each experimental protocol, biomarker values (rBFI, HbO, HbR, HbTotal) were averaged within protocol-specific analysis windows to yield one representative value per condition for each episode. For anesthesia transitions, stable four-minute segments were selected to represent the deep anesthesia state (immediately after bolus, with confirmed immobility/unresponsiveness) and the light anesthesia state (the four minutes preceding the next ketamine bolus). For hypoxia and hemorrhage, animals were maintained under a stable deep anesthesia throughout the experiment to isolate the effect of the systemic insult. The baseline windows were selected from the stable period prior to the insult.

The insult windows were defined by the most physiologically relevant segments after the confirmation of shock, while deep anesthesia was maintained. Specifically, for hemorrhage, values were averaged across the final 60–70% blood withdrawal phase, while for hypoxia, values were averaged using the last third of the sustained 6% FiO_2 epoch

(shown in Figure 1). To test the specificity of the optical biomarkers against more subtle physiological changes, a secondary early insult analysis window was defined. This window captured the initial onset of tachycardia, defined as either a 20% increase in heart rate from baseline or a heart rate exceeding 210 BPM. This specific threshold was selected to represent the earliest clinical sign of physiological distress typically detectable by standard monitors, allowing for a more challenging comparison between the early stages of anesthetic emergence and the early phases of systemic instability.

All statistical analyses of these biomarkers, including BFI, rBFI, HbO, HbR, and HbTotal changes were performed using *R* (Version 4.5.0; R Core Team, Vienna, Austria) and relevant packages, including *lme4* for model fitting, *lmerTest* for significance testing and *emmeans* for calculating estimated marginal means [62–64].

Normality of these averaged biomarker values was assessed using the Shapiro–Wilk test. Biomarkers deviating significantly from normality ($p < 0.05$) were Log-transformed to better meet model assumptions. Based on residual normality checks of preliminary models, Z-score transformation (standardizing to zero mean and standard deviation of 1) was applied to HbO, HbR, and HbTotal. Log transformation was applied to BFI and rBFI, as this yielded better residual normality compared to Z-scores.

To evaluate the effect of physiological state on cerebral hemodynamics and for comparison against systemic insults, two sets of linear mixed-effects (LME) models were created.

First, to evaluate the effect of anesthesia depth (Condition: Deep vs. light anesthesia) while accounting for the repeated measures design (multiple episodes nested within subjects) and inherent inter-subject variability, linear mixed-effects (LME) models were employed [65,66]. The anesthesia cohort included 16 transition episodes obtained from 9 piglets, with individual animals contributing between 1 and 3 transitions (Table A2). For each transformed biomarker, two models were fitted using maximum likelihood (ML) estimation. A null Model, which included only a random intercept for each subject to account for baseline differences between animals:

$$DV \sim 1 + (1|Animal_i) \quad (4)$$

and a condition model with the added fixed effect of anesthesia state (Condition: Deep vs. Light) to the null model:

$$DV \sim 1 + Condition + (1|Animal_i) \quad (5)$$

where DV is the dependent variable of interest, '1' is the intercept representing the overall mean, 'Condition' is a fixed effect of anesthesia state, and 'Animal_i' is the observation which the animal was in and evaluated as a random effect. Due to the limited number of repeated observations per animal (1–3), models incorporating random slopes were not utilized in order to avoid overfitting and model convergence issues (e.g., singular fits); thus, the random intercept model was retained as the most parsimonious approach. The significance of the anesthesia condition effect was determined by comparing the fit of the Condition Model against the Null Model using a likelihood ratio test (LRT).

Secondly, to determine whether biomarker responses during anesthesia transitions were distinct from those during hypoxia or hemorrhage, an interaction model was fitted. This model included fixed effects for Group (e.g., Anesthesia vs. Hypoxia), Condition (e.g., Deep/Light vs. Baseline/Insult), and their interaction term interaction, with a random intercept for each animal:

$$DV \sim 1 + Group * Condition + (1|Animal_i) \quad (6)$$

where $Group * Condition$ expands to $Group + Condition + Group : Condition$. The significance of the interaction term was tested to determine if the effect of Condition on the biomarker depended on the Group.

For all models, the LRT statistic (χ^2) and associated p -value were used to assess significance. To account for multiple comparisons across the eight biomarkers tested, the Benjamini–Hochberg procedure was applied to the LRT p -values to control the False Discovery Rate (FDR). To quantify the magnitude of the difference between the deep and light anesthesia states for each biomarker, effect sizes were calculated as the bias-corrected Hedges' g , as it provides a more accurate estimate than Cohen's d for smaller samples [67].

The key assumptions of the linear mixed-effects model, including the linearity of relationships and the normality and homogeneity of variance of the model's residuals, were assessed via visual inspection of diagnostic plots (e.g., Q-Q plots, residuals vs. fitted values). Additionally, potential influential observations were evaluated using Cook's distance. All observations showed Cook's distance values <1 (maximum $D = 0.52$), confirming that the significant effects observed were not driven by specific influential outliers. Statistical significance for all tests was set at an alpha level of $p < 0.05$ after FDR correction.

3. Results

The effects of transitioning from deep to light anesthesia and of systemic insults (hypoxia, hemorrhage) on cerebral hemodynamics were evaluated using the LME models as defined in statistical methods. This section first presents the hemodynamic signatures of transitions in anesthetic depth by quantifying the changes between deep and light anesthetic states. For comparison, the hemodynamic responses from hypoxia and hemorrhage groups are then summarized. Finally, an interaction analysis ($Group : Condition$) was performed to test whether the effects of the transition to light anesthesia are statistically distinguishable from those of the systemic insults.

Prior to analyzing cerebral hemodynamics, systemic physiological variables were monitored to confirm stability. In the depth of anesthesia study cohort, parameters remained stable during deep anesthesia, while heart rate increased during the transition to light anesthesia, consistent with decreasing anesthetic depth. In the insult cohorts, hypoxia produced marked desaturation ($SpO_2 < 70\%$) and metabolic acidosis, while hemorrhage produced profound hypotension ($MAP < 30$ mmHg) with compensatory tachycardia, confirming successful induction of systemic insults (Table A1).

3.1. Cerebral Hemodynamic Signatures During the Transition to Light Anesthesia

The effects of transitioning from deep to light anesthesia states on cerebral hemodynamics and systemic physiology were evaluated using linear mixed-effects models as described previously. Figure 4 shows representative time-series data from a single transition episode, with annotated analysis windows of stable Deep Anesthesia state and the subsequent rise in cerebral perfusion and oxygenation during the Light Anesthesia state. Table 1 summarizes the descriptive statistics and statistical results for all biomarkers (BFI, rBFI, HbO, HbR, and HbTotal). BFI, rBFI, HbO, and HbTotal significantly increased during the transition to light anesthesia. In contrast, changes in HbR did not show statistically significant differences between deep and light anesthesia states.

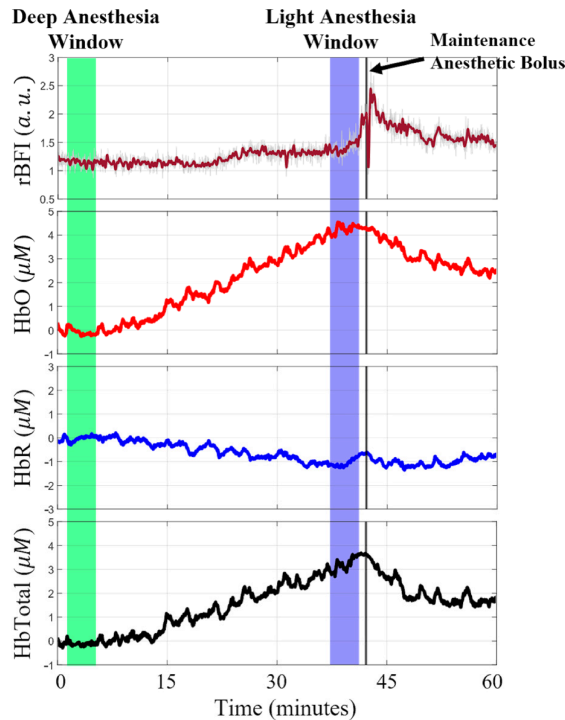


Figure 4. Representative time-series data from a single deep-to-light anesthesia transition episode. The plots show continuous measurements of relative Blood Flow Index (rBFI), displayed with low-pass filtering (cutoff frequency of 0.09 Hz) with the original unfiltered data plotted in gray in the background for visual clarity, oxyhemoglobin (HbO), deoxyhemoglobin (HbR), and total hemoglobin (HbTotal). The shaded regions indicate the 4 min analysis windows used for the Deep Anesthesia state (green) and the Light Anesthesia state (blue), immediately preceding the administration of a maintenance anesthetic bolus (vertical line).

Table 1. Descriptive statistics (Mean ± Standard Deviation [SD] on original scale) and Linear Mixed-Effects (LME) model results for the effect of anesthesia condition (Deep vs. Light) on cerebral hemodynamic and physiological biomarkers.

Biomarker	n	Deep Anesthesia Mean ± SD	Light Anesthesia Mean ± SD	χ^2	df	Adjusted p-Value	Hedges' g
BFI (DCS)	16	$1.068 \times 10^{-8} \pm 1.172 \times 10^{-8} \text{ cm}^2/\text{s}$	$1.875 \times 10^{-8} \pm 2.6695 \times 10^{-8} \text{ cm}^2/\text{s}$	9.23	1	<0.01	1.22
HbO (NIRS)	16	$-0.0146 \pm 0.0216 \text{ }\mu\text{M}$	$2.499 \pm 3.379 \text{ }\mu\text{M}$	8.28	1	<0.01	1.06
HbR (NIRS)	16	$0.00111 \pm 0.0248 \text{ }\mu\text{M}$	$0.712 \pm 3.330 \text{ }\mu\text{M}$	0.77	1	>0.05	0.30
HbTotal (NIRS)	16	$-0.0135 \pm 0.0247 \text{ }\mu\text{M}$	$3.211 \pm 4.838 \text{ }\mu\text{M}$	6.80	1	<0.05	0.95
rBFI (DCS)	16	$1.000 \pm 0 \text{ (baseline)}$	1.596 ± 0.978	10.54	1	<0.01	1.22

Statistics shown as Mean ± SD on original scale and include the likelihood ratio test (χ^2 , df, adjusted p-value) and effect size (Hedges' g).

BFI and rBFI, derived from DCS measurements, showed significant increase during the transition from the deep to light anesthesia state (BFI: $\chi^2(1) = 9.23$, p.adj < 0.01; rBFI: $\chi^2(1) = 10.54$, p.adj < 0.01). As shown in Figure 5a, this transition was associated with a large effect size (Hedges' g = 1.22), indicating a substantial and consistent rise in relative cerebral perfusion when emerging to light anesthesia.

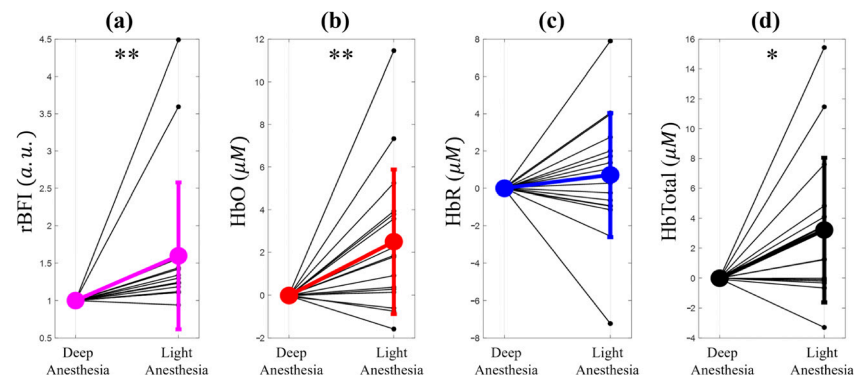


Figure 5. Effect of anesthesia depth on relative cerebral hemodynamic and physiological biomarkers across sixteen transition episodes. Changes from Deep to Light anesthesia periods are compared for (a) relative Blood Flow Index (rBFI) acquired via DCS, (b) change in oxyhemoglobin (HbO), (c) deoxyhemoglobin (HbR), and (d) total hemoglobin (HbTotal) acquired via NIRS, relative to their corresponding values during the preceding deep anesthesia period. Thick colored lines represent the group mean, with error bars indicating standard deviation. Thin black lines connect values from the same episode (* $p < 0.05$, ** $p < 0.01$).

Changes in HbO were significantly higher during light anesthesia compared to the deep anesthesia state ($\chi^2(1) = 8.28$, $p.\text{adj} < 0.05$). This represented a large effect size (Hedges' $g = 1.06$), suggesting increased cerebral oxyhemoglobin concentration accompanying the transition to light anesthesia (Figure 5b). Similarly, HbTotal, reflecting cerebral blood volume, showed a significant increase during light anesthesia ($\chi^2(1) = 6.80$, $p.\text{adj} < 0.05$), representing a large effect size (Hedges' $g = 0.95$) (Figure 5d). In contrast, changes in HbR did not show a significant difference between the deep and light anesthesia states ($\chi^2(1) = 0.77$, $p.\text{adj} > 0.05$).

3.2. Cerebral Hemodynamic Changes Due to Systemic Insults

To provide a comparison to the transitions in anesthetic depth, the hemodynamic responses to systemic insults were analyzed. In line with our previous findings, both hypoxic and hemorrhagic insults produced significant characteristic changes in cerebral hemodynamics compared to baseline (i.e., normoxia case in hypoxic injury and no blood loss case in hemorrhage) [36,42,43].

During the controlled hemorrhage study, a significant reduction in cerebral perfusion and volume was observed. Comparing the pre-insult baseline to the hemorrhagic shock condition revealed significant decreases in rBFI ($\chi^2(1) = 6.58$, $p.\text{adj} < 0.05$), HbO ($\chi^2(1) = 10.90$, $p.\text{adj} < 0.01$), and HbTotal ($\chi^2(1) = 12.10$, $p.\text{adj} < 0.05$).

The graded hypoxia protocol also induced significant changes in cerebral hemodynamics. This was characterized by a decrease in cerebral perfusion and oxygenation, met with an increase in deoxygenated hemoglobin and blood volume. The LME models comparing the normoxic baseline to the hypoxic shock condition, revealed significant decrease in rBFI ($\chi^2(1) = 32.95$, $p.\text{adj} < 0.01$) and HbO ($\chi^2(1) = 7.46$, $p.\text{adj} < 0.01$), alongside significant increases in HbR ($\chi^2(1) = 12.66$, $p.\text{adj} < 0.01$) and HbTotal ($\chi^2(1) = 9.54$, $p.\text{adj} < 0.01$). Table 2 shows the descriptive statistics and LME model results for both insult protocols.

3.3. Comparison of Cerebral Hemodynamic Signatures Between Anesthesia Transitions and Systemic Insults

To determine whether the hemodynamic trend during the transition from deep to light anesthesia was distinguishable from those due to systemic insults, LME models including a *Group:Condition* interaction were fit for each biomarker. The model then compared the anesthesia cohort to the hypoxia and hemorrhage cohorts. Significant interaction

effects across most biomarkers (specifically rBFI, HbO, and HbTotal for the hemorrhage comparison; and rBFI, HbO, HbR, and HbTotal for the hypoxia comparison) indicated that changes observed during the transition differed from those elicited by systemic insults. The results of this key finding are presented in Table 3.

Table 2. Descriptive statistics (mean $\pm$ SD and Linear Mixed-Effects (LME) model results for the effect of systemic insults (hemorrhage and hypoxia) on cerebral hemodynamic biomarkers.

Protocol	Biomarker	n	Baseline Mean $\pm$ SD	Shock Mean $\pm$ SD	χ^2	df	Adjusted p-Value	Hedges' g
Hemorrhage	rBFI (DCS)	4	1.000 $\pm$ 0 (baseline)	0.371 $\pm$ 0.304	6.58	1	<0.05	−1.96
	HbO (NIRS)	4	0.618 $\pm$ 1.016 μ M	−4.829 $\pm$ 2.403 μ M	10.90	1	<0.01	−2.97
	HbR (NIRS)	4	0.038 $\pm$ 0.457 μ M	0.568 $\pm$ 1.262 μ M	0.79	1	>0.05	0.56
	HbTotal (NIRS)	4	0.656 $\pm$ 0.762 μ M	−4.261 $\pm$ 2.091 μ M	12.10	1	<0.05	−3.89
Hypoxia	rBFI (DCS)	5	1.000 $\pm$ 0 (baseline)	0.304 $\pm$ 0.108	32.95	1	<0.01	−9.21
	HbO (NIRS)	5	−0.057 $\pm$ 0.683 μ M	−10.259 $\pm$ 7.633 μ M	7.46	1	<0.01	−1.90
	HbR (NIRS)	5	0.708 $\pm$ 0.562 μ M	25.378 $\pm$ 12.224 μ M	12.66	1	<0.01	2.97
	HbTotal (NIRS)	5	0.651 $\pm$ 1.132 μ M	15.119 $\pm$ 9.137 μ M	9.54	1	<0.01	2.51

Statistics shown as Mean $\pm$ SD on original scale and include the likelihood ratio test (χ^2 , df, adjusted p-value) and effect size (Hedges' g).

Table 3. Linear Mixed-Effects (LME) model results for the Group:Condition interaction between experimental group (anesthesia vs. insult) and condition (deep vs. light anesthesia; baseline vs. shock; baseline vs. early insult).

Protocol	Biomarker	Factor	χ^2	df	Adjusted p-Value
Hemorrhage (Shock)	rBFI (DCS)	Group:Condition	6.08	1	<0.05
	HbO (NIRS)	Group:Condition	16.98	1	<0.01
	HbR (NIRS)	Group:Condition	0.012	1	>0.05
	HbTotal (NIRS)	Group:Condition	10.18	1	<0.01
Hypoxia (Shock)	rBFI (DCS)	Group:Condition	8.39	1	<0.01
	HbO (NIRS)	Group:Condition	23.70	1	<0.01
	HbR (NIRS)	Group:Condition	37.32	1	<0.01
	HbTotal (NIRS)	Group:Condition	12.78	1	<0.01
Hemorrhage (Early Insult)	rBFI (DCS)	Group:Condition	3.56	1	0.059
	HbO (NIRS)	Group:Condition	11.83	1	<0.01
	HbR (NIRS)	Group:Condition	0.06	1	>0.05
	HbTotal (NIRS)	Group:Condition	5.67	1	<0.05
Hypoxia (Early Insult)	rBFI (DCS)	Group:Condition	1.18	1	>0.05
	HbO (NIRS)	Group:Condition	28.55	1	<0.01
	HbR (NIRS)	Group:Condition	27.95	1	<0.01
	HbTotal (NIRS)	Group:Condition	4.25	1	<0.05

Statistics shown include the likelihood ratio test (χ^2 , df) and adjusted p-value for the Group:Condition interaction term from the LME models.

When comparing the anesthetic state transition and hemorrhage cohorts, the Group:Condition interaction was significant for rBFI ($\chi^2(1) = 6.08$, $p_{\text{adj}} < 0.05$), HbO ($\chi^2(1) = 16.98$, $p_{\text{adj}} < 0.01$), and HbTotal ($\chi^2(1) = 10.18$, $p_{\text{adj}} < 0.01$), but not for HbR ($p_{\text{adj}} > 0.05$). During the transition from deep to light anesthesia, rBFI, HbO, and HbTotal

increased, whereas during hemorrhagic shock these biomarkers decreased relative to the baseline (no blood loss) condition, demonstrating different trajectories between anesthetic state transition and blood loss induced shock. These trends are depicted in Figure 6a–d.

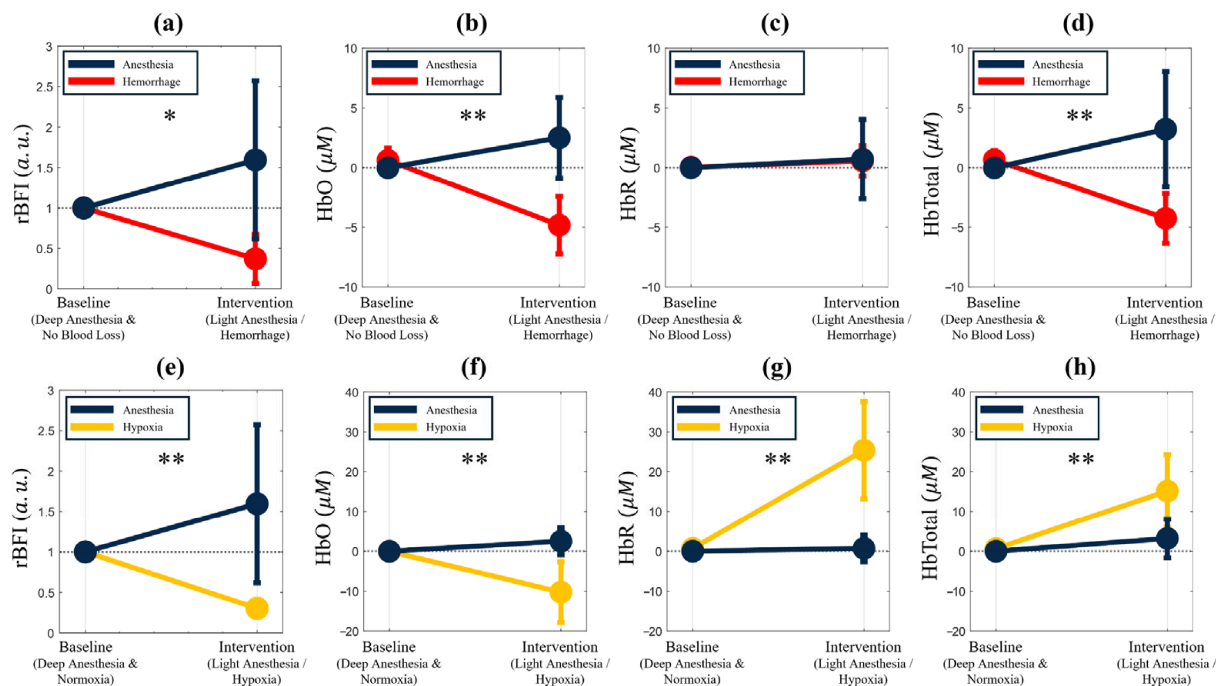


Figure 6. Contrasting cerebral hemodynamic changes during the transition to light anesthesia, hemorrhage, and hypoxia. The **top** row (a–d) compares biomarker trajectories during the transition to light anesthesia (n = 16, navy blue) versus hemorrhagic shock (n = 4, red). The **bottom** row (e–h) compares the same biomarkers between the two anesthesia states (n = 16, navy blue) versus hypoxic shock (n = 5, yellow). Asterisks indicate a significant Group:Condition interaction (* p.adj < 0.05, ** p.adj < 0.01).

A similar analysis comparing the anesthetic depth transition and hypoxia groups yielded significant *Group:Condition* interactions for all four biomarkers, including rBFI ($\chi^2(1) = 8.39$, p.adj < 0.01), HbO ($\chi^2(1) = 23.70$, p.adj < 0.01), HbR ($\chi^2(1) = 37.32$, p.adj < 0.01), and HbTotal ($\chi^2(1) = 12.78$, p.adj < 0.01). In contrast to the early emergence pattern, hypoxic shock was characterized by decreases in rBFI and HbO accompanied by increases in HbR and HbTotal from normoxia to hypoxia, again indicating a distinct hemodynamic signature compared to transitions in anesthetic depth, as shown in Figure 6e–h.

While distinguishing transitions in anesthetic depth from profound shock is critical, clinically relevant monitoring requires differentiating anesthetic depth transitions from early signs of physiological instability. To address this, we compared the hemodynamic signatures of anesthetic depth transition against an early insult phase of hemorrhage and hypoxia, defined by the initial onset of tachycardia.

Interaction analyses confirmed that the cerebral hemodynamic response to transitions in anesthetic depth remained statistically distinct from early systemic insults particularly in NIRS-derived biomarkers (Table 3). In the hemorrhage comparison, the Group:Condition interaction was statistically significant for HbO ($\chi^2(1) = 11.83$, p.adj < 0.01) and HbTotal ($\chi^2(1) = 5.67$, p.adj < 0.05). The rBFI biomarker showed a trend toward significance ($\chi^2(1) = 3.56$, p.adj = 0.059), while HbR did not show a significant interaction (p.adj > 0.05). In the hypoxia comparison, the interaction was significant for HbO ($\chi^2(1) = 28.55$, p.adj < 0.01), HbR ($\chi^2(1) = 27.95$, p.adj < 0.01), and HbTotal ($\chi^2(1) = 4.25$, p.adj < 0.05), whereas rBFI did not show a statistically significant interaction (p.adj > 0.05).

These results, visualized in Figure 7, demonstrate that even at the earliest signs of systemic distress (tachycardia), NIRS-derived biomarkers provided a distinct signature of hyperemia that contrasted with the early physiological responses to blood loss and hypoxia, while the DCS-derived flow index was less distinct during these early subtle changes.

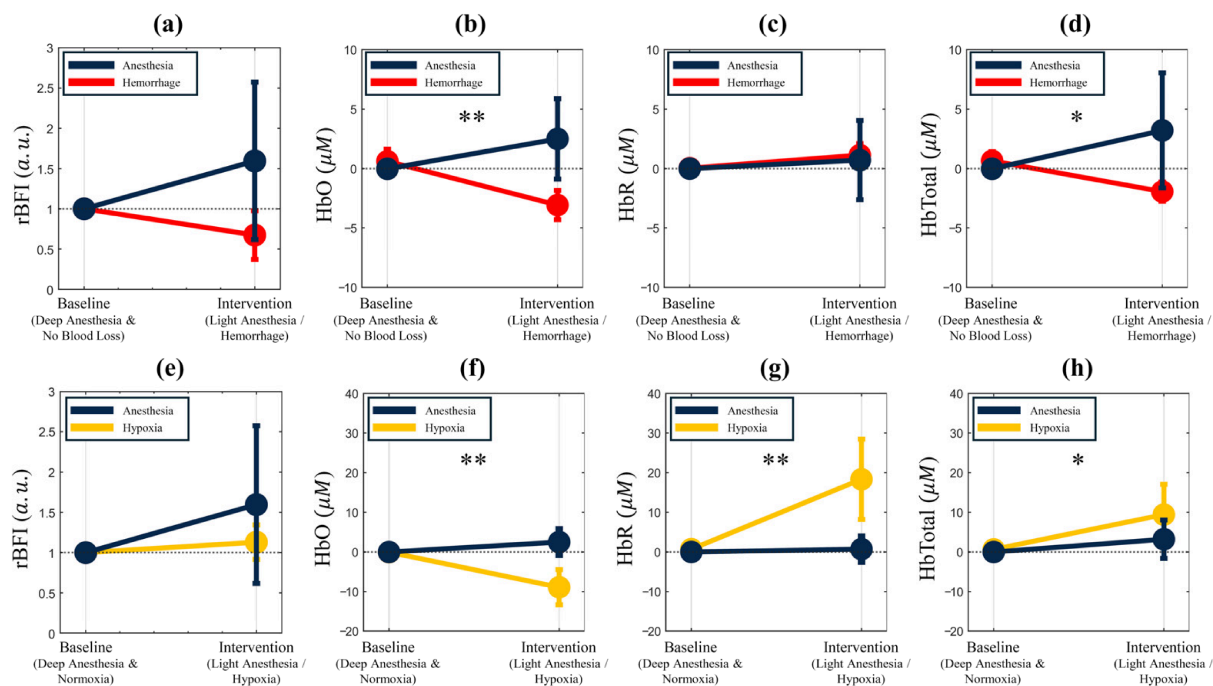


Figure 7. Contrasting cerebral hemodynamic changes during transitions in anesthetic depth versus early systemic instability. The **top** row (a–d) compares biomarker trajectories during anesthetic emergence (n = 16, navy blue) versus the early phase of hemorrhage (onset of tachycardia; n = 4, red). The **bottom** row (e–h) compares the same biomarkers between transition to light anesthesia (n = 16, navy blue) versus the early phase of hypoxia (onset of tachycardia; n = 5, yellow). Asterisks indicate a significant Group:Condition interaction (* p.adj < 0.05, ** p.adj < 0.01).

4. Discussion

This study investigated the efficacy of using DCS and NIRS systems in detecting cerebral hemodynamic changes during transitions in anesthesia depth. We also explored their robustness in their assessment of brain activity even in the presence of systemic physiological insults or hemodynamic instabilities. Our results demonstrated that DCS and NIRS derived biomarkers were significantly associated with changes during transitions from deep to light anesthesia. Specifically, the transition to a lighter anesthetic state was associated with significant increases in cerebral blood flow (BFI, rBFI), oxygenation (HbO), and total blood volume (HbTotal). In contrast, changes in deoxyhemoglobin (HbR) concentrations did not reveal statistically significant differences between the two anesthetic states. Our findings revealed that these hemodynamic patterns during transitions in anesthetic depth were statistically distinct and directionally opposite to those observed during the systemic insults induced by hemorrhage and hypoxia protocols. These results provide preliminary proof-of-concept that simultaneous DCS and NIRS measurements can detect physiological changes in response to anesthesia stage transitions and distinguish these patterns from potentially confounding systemic insults. However, we note that this specificity is currently demonstrated within the boundaries of these tested experimental conditions, and further work is needed to generalize this discriminative capability to broader intraoperative settings.

4.1. Changes in Optical Biomarkers in Deep and Light Anesthesia

The observed increase in rBFI during the transition to light anesthesia aligns with the expected physiological response to reduced anesthetic suppression. As the anesthetic effect diminishes, neuronal activity and cerebral metabolic rate increase, leading to a corresponding rise in cerebral blood flow via neurovascular coupling [27]. The large effect size observed for rBFI (Hedges' $g = 1.22$) indicates the sensitivity of DCS in detecting these perfusion changes associated with transition from deep to light anesthesia. This finding is consistent with previous studies demonstrating dose-dependent effects of various anesthetics on cerebral hemodynamics [18].

The significant increase in HbO towards light anesthesia suggests that the rise in cerebral blood flow increases oxygen delivery, likely to meet the increased regional oxygen demand. This results in a net increase in the concentration of oxyhemoglobin within the probed tissue. The large effect size for HbO (Hedges' $g = 1.06$) further supports its potential as a sensitive marker for monitoring anesthesia depth transitions. Furthermore, the significant increase in HbTotal, reflecting cerebral blood volume, indicates that the observed rise in cerebral perfusion during light anesthesia was accompanied by an increase in vascular volume within the probed region. Physiologically, HbTotal provides distinct information about changes in blood volume, which complements the information on oxygenation (HbO) and blood flow (rBFI). However, it is important to note the mathematical relationship ($\text{HbTotal} = \text{HbO} + \text{HbR}$); given the large significant increase observed in HbO and the lack of significant change in HbR in this study, the significant increase in HbTotal was expected and primarily driven by the HbO component. Regardless, observing concurrent significant increases in rBFI (blood flow), HbTotal (blood volume), and HbO (oxygenation) provides a comprehensive picture of the hemodynamic response transitioning to lighter anesthesia, consistent with increased perfusion and oxygen delivery meeting the metabolic demand during this transition, also described as functional hyperemia [68].

In contrast, HbR did not show a significant change between the deep and light states in this neonatal piglet model study. This could imply that the increase in oxygen extraction accompanying the increased metabolic activity during light anesthesia was minimal or effectively counterbalanced by the substantial increase in oxygenated blood supply (indicated by increased rBFI and HbO). While some studies have reported significant HbR changes during anesthetic transitions [24,25,69], it is important to note that those investigations typically involved adult human patients. Hence, one should be cautious when interpreting the current HbR findings, as they originate from a neonatal piglet model, which may exhibit different physiological responses compared to adult humans. This potential difference underscores the need for future studies involving human participants across various age groups for cross-validation.

4.2. Comparison of Cerebral Hemodynamic Responses Between Transitions in Anesthetic Depth and Systemic Insults

To validate the clinical utility of optical biomarkers for anesthesia monitoring, it is essential not only to quantify changes during anesthetic state transitions but also to demonstrate that these signatures are robustly distinct from those elicited by systemic insults. Similarly, one should not conclude that emergence is occurring while the patient is in a deep anesthesia state. However hemodynamic instability during hypoxia and hemorrhage conditions could potentially confound anesthesia state detection, especially when relying on changes in vital signs to assess transitions in depth of anesthesia. In this study, while both insult protocols induced significant hemodynamic changes, their specific patterns and directionality differed markedly from those observed during the transition to light anesthesia. Furthermore, evaluation of Cook's distance confirmed that these results

were robust to potential outliers (all values <1.0), ensuring that the observed hemodynamic patterns were not driven by isolated influential data points.

Under controlled hemorrhage, we observed significant reductions in CBF (rBFI; Hedges' $g = -1.96$), oxygenated (HbO; $g = -2.97$), and total hemoglobin (HbTotal; $g = -3.89$) concentrations. This is physiologically consistent with the reduction in circulating blood volume, which leads to decreased cerebral blood flow and oxygen delivery [70]. Similarly, during hypoxia decreases in rBFI ($g = -9.21$) and HbO ($g = -1.90$), accompanied by significant increases in HbR ($g = 2.97$) and HbTotal ($g = 2.51$) were observed. This pattern reflects the reduced arterial oxygen content and compensatory cerebral vasodilation, driving both increased blood volume and increased oxygen extraction, quantified by increasing deoxyhemoglobin concentration [37].

Statistical interaction analyses confirmed that the hemodynamic patterns observed during the transition from deep to light anesthesia (increased rBFI, HbO, HbTotal) were directionally and quantitatively distinct from those observed during systemic insult states (reduced rBFI, HbO, HbTotal in hemorrhage; reduced rBFI and HbO but increased HbR and HbTotal in hypoxia). The *Group:Condition* interaction was significant for all major biomarkers when comparing anesthesia to hypoxia (rBFI, HbO, HbR, HbTotal; $p_{\text{adj}} < 0.01$) and for flow, volume and oxygenation when comparing anesthesia to hemorrhage (rBFI, HbTotal; $p_{\text{adj}} < 0.01$ and HbO; $p_{\text{adj}} < 0.05$). This indicates that the optical cerebral hemodynamic biomarker changes during the transition to light anesthesia are not attributable to global systemic perturbation, but rather to neural responses.

While differentiating emergence from profound shock establishes the fundamental discriminative capability of these biomarkers, the more challenging clinical imperative is to distinguish anesthetic transitions from the earliest detectable signs of systemic disturbances, and well before shock develops. To address this more challenging scenario, we also analyzed the hemodynamic signatures at the onset of tachycardia, which represents an early objective sign of physiological instability typically detectable by standard monitors. This analysis revealed that even at these early stages of systemic insult, optical biomarkers (HbO, HbR, HbTotal) differentiated anesthetic emergence from hemorrhage and hypoxia groups (Table 3). Specifically, during early hemorrhagic instability, HbO ($p_{\text{adj}} < 0.01$) and HbTotal ($p_{\text{adj}} < 0.05$) showed significant *Group:Condition* interactions. During early hypoxia, all NIRS biomarkers remained highly significant (HbO, HbR, $p_{\text{adj}} < 0.01$; HbTotal, $p_{\text{adj}} < 0.05$).

These findings demonstrate the potential of simultaneous DCS and NIRS monitoring to not only detect the transition in anesthesia depth but also to distinguish these changes from those caused by systemic insults. The ability to differentiate early emergence from the early hemodynamic changes, where DCS-derived flow indices may be less distinct (Figure 7), highlights the value of multimodal optical monitoring. Such capability could prevent the misinterpretation of early instability as lightness of anesthesia, thereby avoiding false positives and enabling timely intervention before progression to irreversible shock [18,36].

4.3. Optical Biomarkers as Complementary Measures in Anesthesia Monitoring

While standard monitoring during anesthesia includes fundamental vital signs like heart rate, specific monitors aimed at assessing anesthesia depth, such as EEG-based Bispectral Index (BIS) or entropy monitors primarily assess the brain's electrical activity. These advanced EEG monitors have significantly improved anesthesia management; however, the literature notes potential limitations in certain contexts, including processing delays or variable performance across patient populations [11,12,14]. As demonstrated in our results, systemic insults like hemorrhage and hypoxia elicited profound hemodynamic changes that could be misinterpreted without direct cerebral monitoring. Our findings extend this dis-

tion beyond profound shock states to the earlier signs of systemic instability, a capability that standard vital sign monitoring may lack until significant decompensation has occurred. Therefore, we hypothesize that continuous optical monitoring of cerebral hemodynamics could serve as a valuable complementary tool alongside established electrophysiological and vital sign monitors.

In summary, the consistent hemodynamic changes observed in this study support that rBFI, HbO, and HbTotal biomarkers are significantly associated with anesthetic depth transitions. Unlike standard vital signs, these metrics statistically differentiated the hyperemic response of the transition from deep to light anesthesia from the ischemic or hypoxic patterns of shock (Figure 6) as well as from the more subtle hemodynamic shifts seen in early systemic instability (Figure 7). This discriminative capability posits rBFI, HbO, and HbTotal as robust optical indices for detecting transitions in anesthesia depth, even in the presence of confounding physiological perturbations ranging from early distress to profound shock.

Beyond complementary monitoring, the ability to distinguish anesthetic emergence from systemic instability offers clinical value. While changes in standard vital signs such as heart rate can occur during both light anesthesia and early systemic instability, our data suggested that optical biomarkers displayed distinct patterns for each condition (Figure 7). These distinct patterns could assist clinicians in correctly identifying the cause of hemodynamic changes, ensuring that systemic instability is not misinterpreted as anesthetic emergence. Such insight might support more accurate clinical decision-making, allowing for appropriate maintenance of anesthesia depth even in the presence of confounding physiological factors.

4.4. Study Limitations and Future Directions

Several limitations should be considered when interpreting the findings of the present study. Firstly, this study was conducted using a neonatal piglet model. While piglets share important cerebrovascular similarities with humans [33,34,37], direct translation of these specific findings to human subjects requires further validation. Secondly, the anesthesia protocol involved ketamine and nitrous oxide; the observed hemodynamic responses may differ with other commonly used anesthetic agents like propofol or volatile anesthetics [27]. Additionally, anesthetic depth was defined operationally via drug administration timing and behavioral signs rather than using a continuous neurophysiologic reference (e.g., EEG). The same anesthetic protocol was used across the anesthesia, hemorrhage, and hypoxia cohorts, reducing the likelihood that the observed Group:Condition differences were driven by between group differences in anesthetic regimen rather than by the physiological states under study. Moreover, real-world intraoperative complications involve dynamic, compounding factors that present more complex hemodynamic profiles than the isolated insults induced here. Therefore, the demonstrated specificity of these optical biomarkers serves as a proof-of-concept within these experimental boundaries, requiring future evaluation during mixed clinical instabilities. Thirdly, an a priori power analysis, based on preliminary data from our group comparing DCS and NIRS biomarkers (rBFI, HbO, HbR, and HbTotal) during shock versus no-shock conditions [36], estimated that $N = 5$ animals were required to detect a large effect size ($g = 1.4$) with 80% power ($\alpha = 0.05$) using a one-tailed paired t-test. While the overall sample size used in the present study was consistent with detection of large effects, the cohorts remained asymmetric, with more anesthesia-transition episodes than animals in the hemorrhage and hypoxia groups. Accordingly, although several biomarkers demonstrated robust differences across conditions, the study was primarily powered to detect large effects, and the findings should not be considered for a precise estimation yet. As such, more subtle or borderline findings should be interpreted

cautiously. Larger and more balanced cohorts will be important in future work to confirm these findings, better estimate effect magnitudes, and assess smaller or more subtle physiological differences. Finally, both DCS and NIRS techniques rely on modeling assumptions (e.g., semi-infinite homogeneous medium and assumed tissue optical properties for DCS, fixed DPF for NIRS) which may introduce potential inaccuracies. To mitigate superficial contamination in DCS, we employed a partial curve fitting method. Theoretically, photons penetrating deeper tissues undergo more dynamic scattering, causing a faster signal decay that dominates this early correlation window [21,55–57].

Future research should focus on validating these observations noted in optical biomarkers in human subjects across different age groups and anesthetic agents. Direct comparisons of the temporal dynamics of DCS and NIRS signals with current standards, such as EEG-based indices and continuous vital signs, during changes in anesthesia depth would allow for an investigation of their relative sensitivity and specificity in detection. Moreover, a key area for future device design and methodology is the creation of real-time, multimodal algorithms that can utilize DCS and NIRS data to classify brain states. Such algorithms could provide anesthesiologists with an intuitive brain state index that explicitly warns of impending emergence and distinguishes it from hemodynamic instability, facilitating rapid and robust clinical decision-making. However, real-time implementation will require robust signal quality control and rapid processing of multimodal data before such systems can be translated clinically. Finally, exploring the dose–response relationship between anesthetic agents and optical biomarkers could provide further insights for developing personalized dosing strategies.

5. Conclusions

This study suggests that DCS and NIRS monitoring can detect significant physiological changes during emergence from deep anesthesia in a neonatal piglet model. Specifically, we observed significant increases in cerebral blood flow (via BFI and rBFI), oxyhemoglobin concentration (via HbO), and total hemoglobin concentration (via HbTotal) during the transition to light anesthesia. Our results showed that these hemodynamic signatures of emergence were statistically distinct and often directionally opposite from the patterns observed during systemic insults, ranging from early subtler instability to hemorrhagic and hypoxic shock. These findings suggest the potential utility of non-invasive optical DCS and NIRS systems in assessing anesthesia depth by providing direct physiological insights into cerebral hemodynamics.

Further research, particularly validating these findings in human subjects across diverse anesthetic agents, is needed to fully evaluate the clinical utility of this approach for improving patient safety by minimizing the risk of both unintended intraoperative awareness and misinterpretation of early hemodynamic instability during anesthesia care.

Author Contributions: Conceptualization, M.D.P., K.I. and S.N.M.; methodology, M.D.P., K.I. and S.N.M.; software, M.D.P. and P.A.S.; validation, M.D.P., P.A.S., M.I. and S.N.M.; formal analysis, M.D.P.; investigation, K.I., P.A.S. and S.N.M.; resources, K.I. and S.N.M.; data curation, M.D.P. and P.A.S.; writing—original draft preparation, M.D.P.; writing—review and editing, M.D.P., K.I., P.A.S., M.I., M.G. and S.N.M.; visualization, M.D.P.; supervision, K.I. and S.N.M.; project administration, K.I.; funding acquisition, K.I. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the PA Department of Health, 2021 Commonwealth Universal Research Enhancement Program (CURE) and in part by the Office of the Assistant Secretary of Defense for Health Affairs through the Combat Readiness—Medical Research Program, under award number W81XWH-20-1-0899.

Institutional Review Board Statement: The animal study protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Drexel University (protocol code 20889).

Data Availability Statement: The data presented in this study are available upon reasonable request from the corresponding author.

Acknowledgments: The authors would like to acknowledge the contributions of Danielle Shoshany, Randolph Sinahon, and Juan Du; John Grothusen, Leonid Zubkov, and Sinan Ferit Tuzer for their valuable assistance with data collection and DCS device development.

Conflicts of Interest: K.I. owns stock options in InfraScan, Inc., the manufacturer of the NIRS system used in this study. The remaining authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Abbreviations

The following abbreviations are used in this manuscript:

BFI	Blood Flow Index
BIS	Bispectral Index
BP	Blood Pressure
BPM	Beats Per Minute
CBF	Cerebral Blood Flow
DCS	Diffuse Correlation Spectroscopy
DPF	Differential Pathlength Factor
ECG	Electrocardiogram
EEG	Electroencephalography
FDR	False Discovery Rate
FiO ₂	Fraction of Inspired Oxygen
FPGA	Field-Programmable Gate Array
HbO	Oxyhemoglobin
HbR	Deoxyhemoglobin
HbTotal	Total Hemoglobin
IACUC	Institutional Animal Care and Use Committee
LME	Linear Mixed-Effects
LRT	Likelihood Ratio Test
MAC	Minimum Alveolar Concentration
MAP	Mean Arterial Pressure
ML	Maximum Likelihood
NIRS	Near-Infrared Spectroscopy
OD	Optical Density
pCO ₂	Partial Pressure of Carbon Dioxide
rBFI	Relative Blood Flow Index
RMSE	Root Mean Square Error
SD	Standard Deviation
SpO ₂	Peripheral Oxygen Saturation
TCF	Temporal Correlation Function

Appendix A

Table A1. Summary of systemic physiological variables (Mean $\pm$ SD) across experimental groups. For the Anesthesia group, values represent stable deep anesthesia conditions, with Heart Rate reported for both Deep and Light anesthesia states. For Hypoxia and Hemorrhage groups, values compare Baseline versus intervention conditions to confirm physiological insult.

Parameter	Anesthesia Group	Hypoxia Group		Hemorrhage Group	
		Baseline	Hypoxia	Baseline	Hemorrhage
Heart Rate (BPM)	158.69 $\pm$ 30.92 (Deep) 176.56 $\pm$ 31.32 (Light)	184.64 $\pm$ 31.40	190.77 $\pm$ 46.94	152.13 $\pm$ 28.03	273.92 $\pm$ 44.74
Mean Arterial Pressure (mmHg)	65.88 $\pm$ 7.48	75.33 $\pm$ 13.09	49.60 $\pm$ 14.91	57.49 $\pm$ 5.90	28.09 $\pm$ 9.58
SpO ₂ (%)	97.17 $\pm$ 1.94	93.85 $\pm$ 2.94	<70	94.90 $\pm$ 3.84	93.42 $\pm$ 5.98
pH	7.39 $\pm$ 0.13	7.36 $\pm$ 0.16	<7.00	7.33 $\pm$ 0.08	7.32 $\pm$ 0.02
Lactate (mmol/L)	1.92 $\pm$ 1.08	1.35 $\pm$ 0.81	18.31 $\pm$ 1.47	1.11 $\pm$ 0.85	8.04 $\pm$ 4.30
pCO ₂ (mmHg)	43.3 $\pm$ 18.61	39.60 $\pm$ 2.35	38.87 $\pm$ 19.69	49.52 $\pm$ 20.67	34.93 $\pm$ 18.43

Table A2. Distribution of deep-to-light anesthesia transition episodes across animals in the anesthesia cohort.

Animal ID	A1	A2	A3	A4	A5	A6	A7	A8	A9
Number of transitions	2	2	1	3	2	1	1	1	3

Table A3. Descriptive statistics for phantom validation.

Method	Flow (mL/h)	N (Frames)	D_B Mean $\pm$ SD (cm ² /s)
Full-curve	0	25	$1.63 \times 10^{-8} \pm 5.26 \times 10^{-10}$
	50	23	$5.08 \times 10^{-8} \pm 1.30 \times 10^{-9}$
	100	23	$6.78 \times 10^{-8} \pm 1.80 \times 10^{-9}$
	150	21	$7.59 \times 10^{-8} \pm 1.74 \times 10^{-9}$
Partial (0 – τ_{exp})	0	25	$1.29 \times 10^{-8} \pm 5.13 \times 10^{-10}$
	50	23	$4.43 \times 10^{-8} \pm 1.24 \times 10^{-9}$
	100	23	$7.14 \times 10^{-8} \pm 2.21 \times 10^{-9}$
	150	21	$9.23 \times 10^{-8} \pm 1.99 \times 10^{-9}$

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