AWARD NUMBER: W81XWH-20-1-0899

TITLE: Portable Diffuse Optical Sensors for Point-of-Care Monitoring in Prolonged Field Care

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REPORT DATE: OCTOBER 2023

TYPE OF REPORT: Annual Technical Report

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release; Distribution Unlimited

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

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1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
OCTOBER 2023	Annual	1 Oct 2022 – 30 Sep 2023

4. TITLE AND SUBTITLE

Portable Diffuse Optical Sensors for Point-of-Care Monitoring in Prolonged Field Care

5a. CONTRACT NUMBER
W81XWH-20-1-0899

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The main purpose is to develop the prototype of a portable diffuse correlation spectroscopy (DCS) and near infrared spectroscopy (NIRS) based optical system with multiple capabilities to monitor tissue blood flow, blood volume and oxygenation. The proposed system includes multi-distance multi-wavelength NIRS-based sensors and DCS-based optical fibers. The proof of concept and system test studies include design and study of phantom and animal models, tests in hemorrhage assessment during pre-shock, shock, after fluid resuscitation, and during hypoxia and edema development. Final proof of concept study will be conducted using a real-life clinical scenario with adult pig model (uncontrolled hemorrhagic shock). A fully operational DCS optical system with all the components including the sensor array, data acquisition box and software was developed in Year 1. The novel head phantoms and dynamic microvasculature model (mimicking adult human head with extracerebral layers) were also designed and implemented to model varying cerebral blood volume and cerebral blood flow in Year 2. In Years 2 and 3, animal models and test plans were developed and conducted for detection of graded hemorrhage, hemorrhagic and hypoxic shock. The results validate that the prototype system is capable of measuring microvascular blood flow rate, volume and oxygenation changes in response to various type of brain injuries.

15. SUBJECT TERMS

Prolonged field care, point-of-care imaging, diffuse correlation spectroscopy, DCS, near infrared spectroscopy, NIRS, traumatic brain injury, hemorrhagic shock.

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRDC
U	U	U	UU	34	**19b. TELEPHONE NUMBER** *(include area code)*

TABLE OF CONTENTS

1. **INTRODUCTION:**

The ability to combine local microcirculatory blood flow measures (which is the critical physiological biomarker for various injuries, in particular, hemorrhagic shock) and local tissue oxygen saturation via hemoglobin oxygenation and deoxygenation lends itself to an advanced approach and accelerated medical device development, specifically for a point-of-care monitoring in prolonged field care. The purpose is to develop the prototype of a portable diffuse correlation spectroscopy (DCS) and near infrared spectroscopy (NIRS) based optical system with multiple capabilities to monitor tissue blood flow, blood volume and oxygenation. The proof of concept and system test studies include design and study of dynamic phantom models mimicking brain tissue and animal models for the tests in hemorrhage assessment during pre-shock, shock, after fluid resuscitation as well as during hypoxia and edema development. Final proof of concept study will be conducted using a real-life clinical scenario with adult pig model for the uncontrolled hemorrhagic shock.

2. **KEYWORDS:** *P*

Prolonged field care, diffuse correlation spectroscopy, DCS, near infrared spectroscopy, brain injury, hemorrhagic shock, hypoxic shock, tissue oxygenation, blood flow.

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?

Major Goals & SOW Tasks	**Timeline (Month)**	**Progress**
Aim 1 - Develop and test the prototype of the integrated diffuse correlation spectroscopy (DCS) - near infrared spectroscopy (NIRS) DCS-NIRS system		
Major Task 1. Develop & Test Prototype	1-12 Month	Completed
Subtask 1.1: Develop the sensors and control box unit of the DCS system.	1-3 Month	100% (Complete)
Subtask 1.2: Re-design and develop an oximetry/edema board (NIRS system) by implementing new digital lock-in amplifiers.	2-6 Month	100% (Complete)

Subtask 1.3: Integrate DCS hardware components into NIRS system. Subtask 1.4: Perform initial prototype testing with phantoms, including but not limited to: *a. initial system tests on linearity, drift, depth of penetration, and noise analysis* *b. device performance evaluation in terms of repeatability and accuracy under hypoxia, ischemia, varying blood flow and edema development conditions.*	3-12 Month 6-12 Month	100% (Complete) 100% (Complete) *a. 100% (Complete)* *b. 100% (Complete)*
Aim 2 - Perform animal tests for feasibility and validation		
Major Task 2. Test prototype in piglet model	1-15 Month	Completed
Subtask 2.1 Prepare and submit the research protocol with animals for the review and approvals by IACUC and ACURO. Subtask 2.2 Test prototype in piglet model of graded hemorrhage: a. *Measure physiological data, including but not limited to heart rate (HR), blood pressure (BP), pulse oximetry; and DCS-NIRS somatic and cerebral signals for the tissue oximetry, blood flow and blood volume.* b. *Analyze physiological data and DCS-NIRS signal synchrony in response to change in the phase of hemorrhagic shock.* Subtask 2.3 Test the prototype for changes in cerebral and somatic signals following cerebral edema development in a piglet model of hypoxia-induced cerebral edema:	1-6 Month 9-15 Month 9-15 Month	100% (Complete) 100% (Complete) a. *100% (Complete)* b. *100% (Complete)* 100% (Complete)

a. *Measure intracranial pressure (ICP), and physiological data including but not limited to HR, BP, pulse oximetry, and DCS-NIRS signals for the tissue oximetry, blood flow, blood volume and water content.* b. *Analyze to determine the relationship between changes in DCS-NIRS signals of cerebral and somatic tissue oxygen saturation, cerebral edema, cerebral blood flow and changes in intracranial pressure (ICP) and pulse oximetry.* c. *Analyze physiological data and DCS-NIRS signal synchrony in response to edema development.*		a. *100% (Complete)* b. *100% (Complete)* c. *100% (Complete)*
Subtask 2.4: Modify the prototype based on the piglet model tests.	12-15 Month	100% (Complete)
Aim 3 - Perform validation tests with adult pig models mimicking real-life clinical scenario		
Major Task 3. Test prototype in adult pig model of controlled and uncontrolled hemorrhagic shock	12-24 Month	In Progress
Subtask 3.1: Test the prototype in an adult pig model of controlled hemorrhagic shock.	12-16 Month	75% (In progress)
Subtask 3.2: Test the prototype in an adult pig model of uncontrolled hemorrhage by liver laceration.	17-20 Month	0% (Not Yet Initiated)
Subtask 3.3: System fine-tuning and final validation analysis.	21-24 Month	0% (Not Yet Initiated)
Aim 4 - Provide technical progress reports for the findings and system prototypes and disseminate research findings.		
Major Task 4. Reporting	3-24 Month	In Progress

Subtask 4.1 Technical progress reports (Quarterly).	3-48 Month ('NCE')	75% (In Progress)
Subtask 4.2 Annual technical report.	12th Month & 24th Month and 36th Month	100% (Complete)
Subtask 4.3 Prepare and disseminate findings by attending a DoD-sponsored meeting.	16-48 Month ('NCE')	100% (Complete)
Subtask 4.4 Final technical report	48th Month ('NCE')	0% (Not Yet Initiated)

What was accomplished under these goals?

Before discussing all the major findings, key outcomes and significant results with pertinent data and graphs in detail, a summary for major tasks, objectives and a brief discussion are provided below for Year 3.

Summary of the major activities during Year 3, objectives, and brief discussion:

SOW Major Task #2

I. The first major activity was to complete the tests with piglet models for the validation of simultaneous DCS and NIRS measures in response to different injury mechanisms, including controlled blood loss (hemorrhage), hemorrhagic shock, hypoxia-induced brain injury, i.e., edema, and hypoxic shock.

Objective: Primary objective for this activity was to demonstrate the ability of system, algorithms, and associated index/biomarkers in measuring changes of cerebral blood flow and oximetry in response to different injury types and sham controls.

Discussion of stated goal: The main goal for this major activity was met during this annual reporting period. **The tests with n=30 animals were completed.** We demonstrated the proposed system's ability to detect changes in cerebral flow rate and local oxygenation. Regardless of brain injury types/mechanisms, cerebral blood flow (CBF) measures via DCS sensors can be used as reliable biomarkers in detecting and managing brain injuries. During both brain injury tests, **graded hemorrhage for hemorrhagic shock and hypoxia-induced brain injury such as, cerebral edema and hypoxic shock elicited the significant changes in CBF**,

suggesting that such brain injuries can be detected by early CBF acceleration followed by severe deceleration of CBF. **Tissue oximetry measures via oxygenated hemoglobin (HbO) and deoxygenated hemoglobin (HbR) changes passed the test** by providing the expected changes **in terms of hemodynamic response to hemorrhagic condition**, i.e., decrease in HbO and HbT with graded blood loss and slight change in HbR; and decrease in HbO and increase in HbR in response to **hypoxic condition**. All these major findings, key outcomes and significant results are explained below including pertinent data and graphs. Analysis of the physiological data (vital signs) including heart rate (HR), Oxygen saturation (SpO2), mean arterial pressure (MAP) using measures of systolic blood pressure (SPB) and diastolic blood pressure (DPB) is provided in Appendix A.

II. Another major activity was to conduct control studies with no injuries and compare the data acquired via proposed DCS-NIRS system report between injury and sham controls.

Objective: Objective for this activity was to develop statistical model and conduct detailed analysis to compare all the biomarkers derived from proposed DCS-NIRS measures within and between injury versus control (sham) data.

Discussion of stated goal: The main goal for this major activity was met and a linear mixed effects regression (LMER) was developed and used to quantitatively verify reliability of the measures when compared with control group. That is, ***the data from sham control reveal no significant changes in response to 'no-brain injuries,' whereas there are significant differences between hemorrhagic shock vs. control and hypoxia-induced injury/hypoxic shock condition vs. control***.

SOW Major Task #3

III. Following controlled brain injury and sham control study with piglet models, another major activity was to kick off and conduct large animal studies using pig model of graded hemorrhage and uncontrolled hemorrhagic shock to mimic real time clinical scenario.

Objective: Primary objective for this activity was to conduct system performance tests with adult pig models of hemorrhage and in real-life clinical scenario via uncontrolled hemorrhagic shock test.

Discussion of stated goal: The main goal for this major activity was partially met as this study is ongoing. The proposed system (DCS-NIRS) was delivered to the clinical site (Hebrew University) to conduct adult/pig model study for the tests mimicking real-life clinical scenario. The Drexel team traveled to the location and set up the system and trained all the necessary clinical personnel**. The tests with n=10 animals were completed.**

All these major findings, key outcomes and significant results are explained below including pertinent data and graphs in detail.

Major Activity I & II (SOW Major Task # 2). Test prototype in piglet model:

This major task included evaluation of the DCS-NIRS system performances using piglet models of brain injury mechanisms, i.e., hemorrhage and hypoxia-induced as well as no-injuries (sham controls). We recruited n=30 animals, completed all the tests and calculated the following biomarkers from the proposed DCS-NIRS measurements: blood flow index – **BFI** values calculated by nonlinearly fitting the analytical and measured autocorrelation functions [1-5]) and oxygenated

hemoglobin – **HbO**, deoxygenated hemoglobin – **HbR**, total hemoglobin or blood volume – **HbT** calculated by using modified Beer-Lambert law [6]).

Study Designs and Measurement Protocols:

Graded hemorrhage and hemorrhagic shock measurement protocol*:* The following test protocol was followed for graded hemorrhage: Blood was withdrawn manually 7 times over a two-hour period (Figure 1). A total of ~66% of the animal's total blood volume, estimated to be 90 mL/kg [7], was removed. All the sensor measurements were collected at baseline and throughout each hemorrhagic epoch.

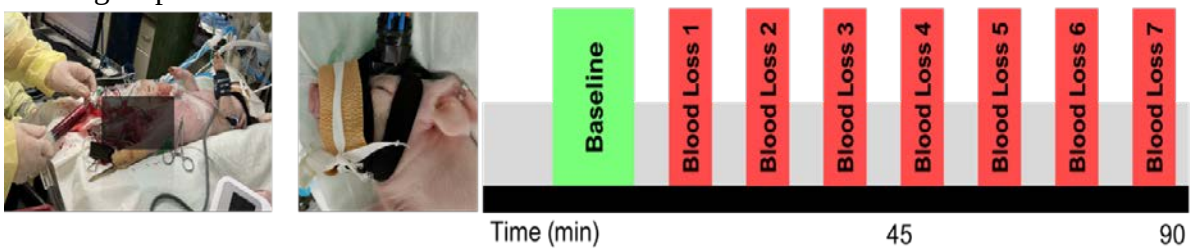


Figure 1. Experimental protocol for graded hemorrhage: Hemorrhagic shock was induced through controlled hemorrhaging via syringe from a femoral artery cannula.

Hypoxia-induced brain injury and hypoxic shock measurement protocol: Anesthetized and ventilated piglets were subjected to approximately 3 hours of hypoxic fraction of inspired oxygen (FiO_2), i.e., fraction of inspired oxygen (FiO_2) was lower than ~15 (Figure 2). We compared cerebral blood flow (BFI), and oximetry measures from body and head including oxygenation (HbO) and deoxygenation (HbR)

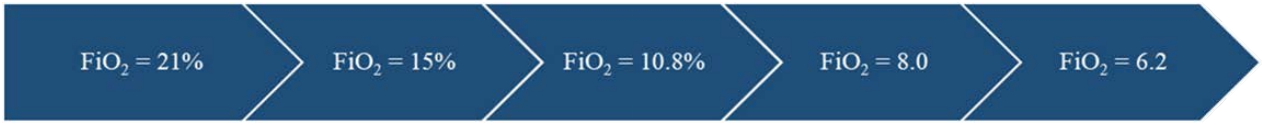
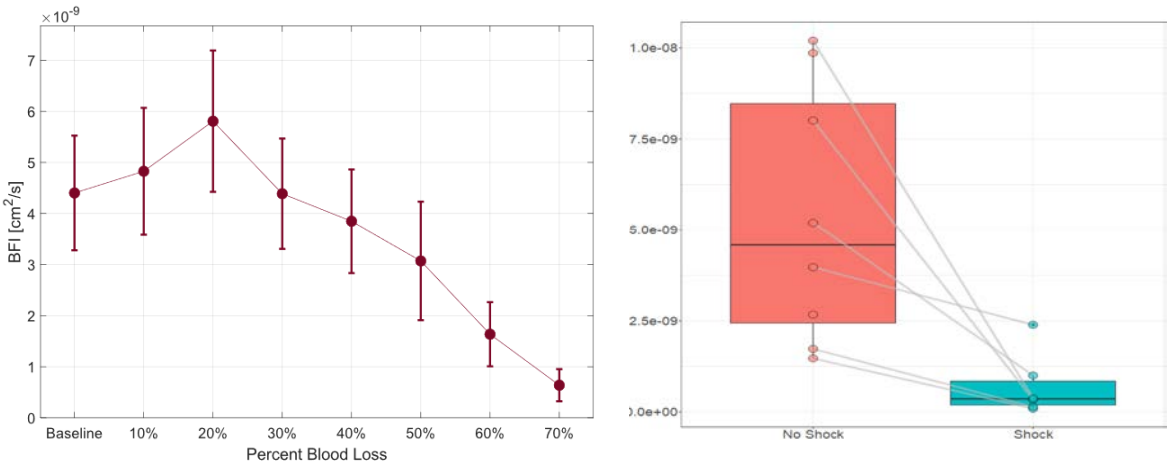


Figure 2. Hypoxic-induced ischemic injury and cerebral edema study protocol. Anesthetized and ventilated piglets were subjected to ***hypoxic*** fraction of inspired oxygen (FiO_2), i.e., fraction of inspired oxygen (FiO_2) was lower than ~15%.

Results and Key Findings:

Graded hemorrhage and hemorrhagic shock detection results: The simultaneous DCS and NIRS results are reported here for different types of injury mechanisms and hemorrhagic shock. The figures 3.A and 3.B reveal biomarker of ***BFI*** for all graded blood loss and detection of hemorrhagic shock, respectively. Figure 3.A shows each episode of blood loss and corresponding averaged blood flow measures with standard error of mean (n=9 animals). As for the hemorrhagic shock detection, the statistical analysis revealed significant difference between no shock versus shock ($p < 0.05$). Figure 3.B shows the shock condition (6^{th} blood drawn) versus no shock conditions (1^{st} and 2^{nd} blood drawn in which animal was not in the hemorrhagic shock).

The key finding of DCS measures is that early blood flow acceleration consistent with initial compensatory homeostatic response though cerebral vasodilation (up to second blood loss in the Figure 3.A), followed by severe deceleration of flow, indicative of decompensated circulatory shock.



A. **B.**

Figure 3. A. ***Progression of hemorrhagic*** **shock** is monitored by the blood flow measures (blood flow index; BFI) averaged across n=9 animals; **B.** ***Detection of hemorrhagic shock*** via BFI is significant; no-shock vs. shock ($p<0.05$).

Figures 4.A and 4.B reveal biomarkers of the oximetry measures, i.e., HbO, HbR, and HbT via NIRS.

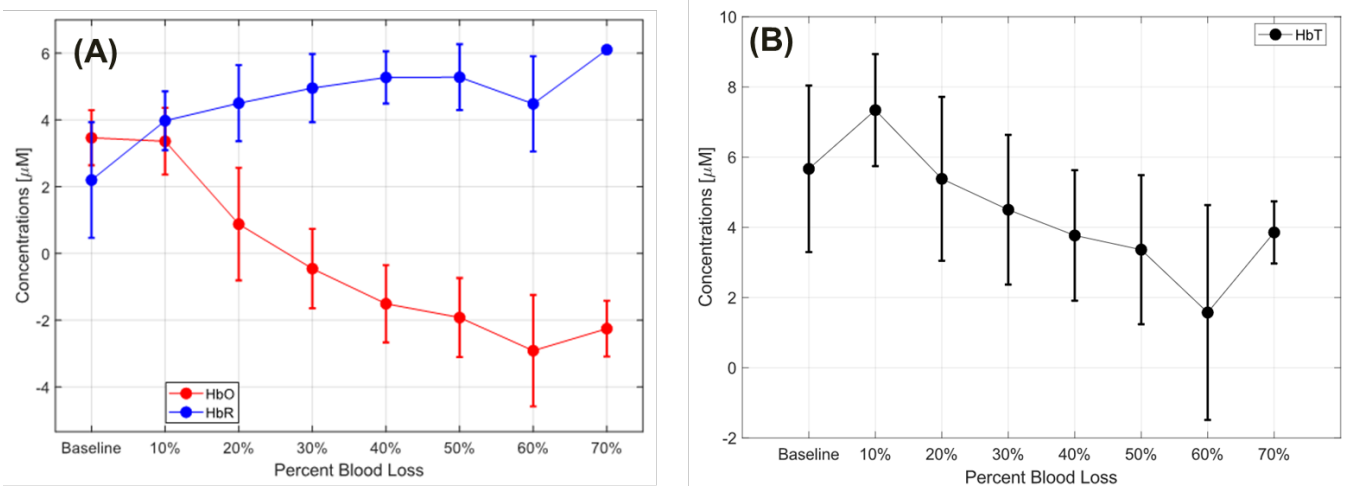


Figure 4. A. ***Progression of hemorrhagic*** **shock** is monitored by the Cerebral oxygenated hemoglobin (**HbO**), de-oxygenated hemoglobin (**HbR**) changes; **B.** Total blood volume, **HbT**, changes.

Hypoxia-induced brain injury and hypoxic shock results: We performed detailed data analysis for the averaged responses of n=11 animals to investigate the relationship between changes of hypoxic fraction of inspired oxygen (FiO2) and the measured cerebral blood flow (CBF). Figure 5 shows each critical change in oxygenation and corresponding *mean* of cerebral blood flow measures for each hypoxic fraction of inspired oxygen. The results show the averaged compensatory response up to FiO2 = ~11% followed by significant decrease of CBF right after FiO2< ~8%. This consistency

is significant as our proposed prototype system provides similar measures and trend for each brain injury type, suggesting that the system can be reliably used in response to different injury mechanisms.

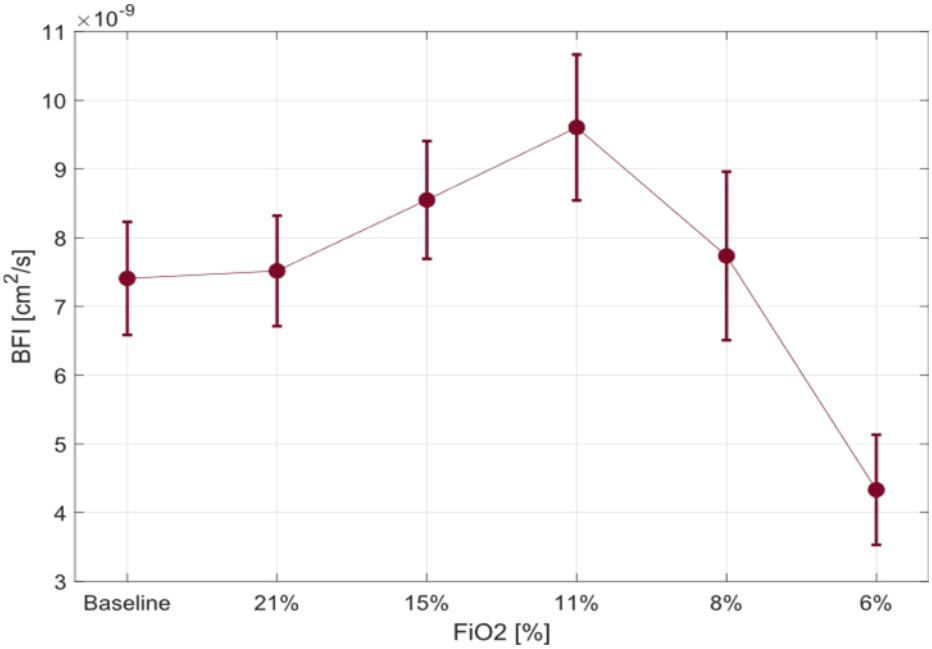


Figure 5. Averaged CBF measures across animals (n=11) in response to changes in hypoxic fraction of inspired oxygen with standard error of mean (mean ± SEM).

As for the tissue oximetry measures via NIRS, we applied the same analysis to investigate the relationship between changes of hypoxic fraction of inspired oxygen (FiO2) and the measured hemodynamic responses, i.e., cerebral blood oxygenation (HbO) and de-oxygenation (HbR). Figure 6 shows the averaged response for each animal *(n=5 animals for head measurements; n=6 for body measurements; totaling 11 animals)*.

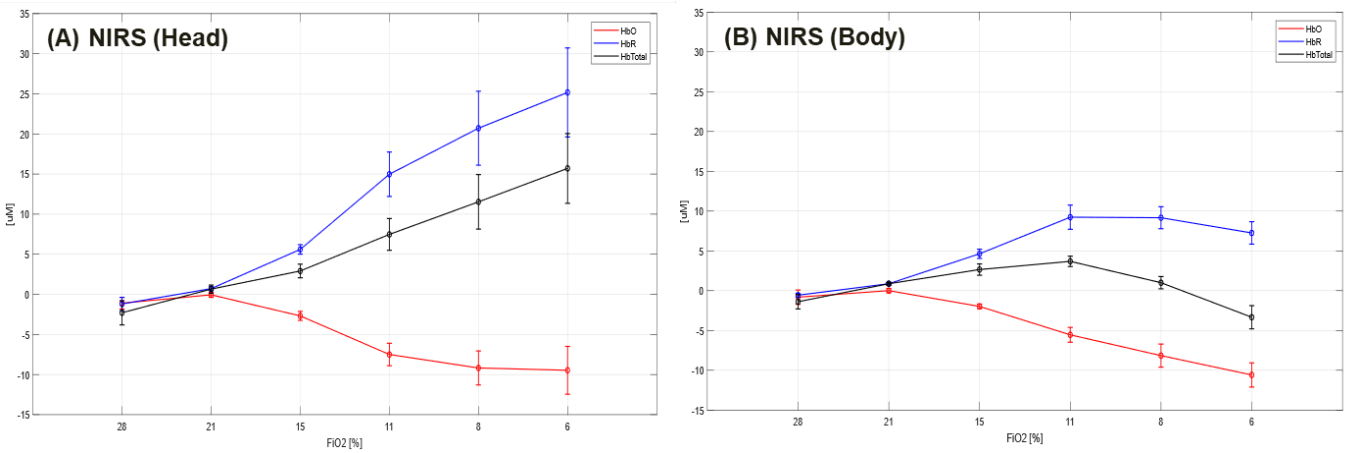


Figure 6. A. Tissue oxygenation measurements from head - Averaged cerebral blood oxygenation (HbO, ‘red’) and de-oxygenation (HbR, ‘blue’ and HbTotal ‘black’) in response to changes in hypoxic fraction of inspired oxygen; (n=5; mean ± SEM); B. Tissue oxygenation measurements from body; (n=6; mean ± SEM).

The observations and critical results for Major Task #2 are:

i. Note early cerebral blood flow (CBF) acceleration consistent with initial compensatory homeostatic response though cerebral vasodilation, followed by severe deceleration of CBF, indicative of decompensated circulatory shock (Compensatory response up to 20% blood loss, followed by significant decrease of CBF which is indication of decompensated shock in Figure 3).

ii. The statistical analysis revealed significant difference between no shock versus hemorrhagic shock ($p < 0.05$).

iii. Oxygenated hemoglobin (HbO) and blood volume (HbTotal) decreased with each blood withdrawn as expected, yet deoxygenated hemoglobin (HbR) slightly changed (Figure 4).

iv. Regardless of brain injury types/mechanisms, CBF measures via DCS sensors can be used as reliable biomarkers in detecting and managing brain injuries. During both brain injury tests, graded hemorrhage for hemorrhagic shock and hypoxia-induced cerebral edema elicited the significant changes in CBF, suggesting that such injuries can be detected by early CBF acceleration followed by severe deceleration of CBF (Figure 5).

v. Hemodynamic responses via HbO and HbR biomarkers measured by the proposed system from head and body verified the hypothesis that HbO decreases while HbR increases when the animal is in hypoxic condition (Figure 6).

Brain Injury versus Control Group Comparison: Statistical Analysis and Results

A group of animals served as sham-controls with no blood loss or hypoxia for the duration of the study to compare DCS-NIRS measurements. [Cerebral blood flow (n=6 via DCS sensor); Cerebral oxygenated hemoglobin and deoxygenated hemoglobin (n=5 via NIRS sensors)]

Statistical Model: A linear mixed effects regression (LMER) modeling has been used to accommodate the complex nesting structure. LMER model represented by (DV ~ 1 + Group + Group: Session + (1|Animal), was used to evaluate the

- main effects of Group (Hemorrhage injury group vs. Control group Hypoxia-induced injury vs. Control group), and
- interaction effects of Block (graded hemorrhage blood loss percentage 0-70% with 10% increments, graded hypoxia FiO2 settings 26-6% inhaled Oxygen, control group measurement blocks divided into 6 or 8 equal blocks depending on the injury type (Table 1)), for the four biomarkers:
    - BFI: Blood flow index,
    - HbO: oxygenated hemoglobin; HbR: de-oxygenated hemoglobin, HbTotal: Total blood volume, i.e., HbO+HbR

Table 1. Blocks used for the statistical analysis as defined based on the experimental procedure.

Control	Block 1	Block 2	Block 3	Block 4	Block 5	Block 6	Block 7	Block 8
Hemorrhage	Baseline	10%	20%	30%	40%	50%	60%	70%

Control	Block 1	Block 2	Block 3	Block 4	Block 5	Block 6
Hypoxia	26% FiO2	21% FiO2	15% FiO2	11% FiO2	8% FiO2	6% FiO2

Significance of fixed effect terms was evaluated using likelihood ratio tests, where the full effects model was compared against a model without the effect in question. Maximum likelihood estimation was used to conduct likelihood ratio tests, while restricted maximum likelihood was used to evaluate post hoc comparisons. If the interaction term was significant, then planned comparisons (30 per biomarker for Hypoxia vs Control, 56 for biomarker for Hemorrhage vs Control comparison models) were performed between the blocks within each group (e.g., Hemorrhage: Block 1/Baseline vs Block 4/40 % Blood loss). Homogeneity of variance, and normality of residuals and random effects were conducted using visual inspection. If model predictions showed heteroscedasticity or non-normal distribution, then log10 transformations were performed on the response variables. For all statistical analyses, the level of significance was set at $\alpha = 0.05$. Adjustments using false discovery rate (FDR) were made on p values to account for Type I error inflation per dependent variable. All statistical analyses were conducted in R (R Core Team, 2023) using *lme4, lmerTest, and emmeans* functions.

Hemorrhage vs. Control Group: Key Findings

The graded hemorrhage tests are split into two parts: i) *Pre-Shock* including the first 3 blocks, i.e., blocks corresponding to Baseline, 10%, 20% and 30% blood loss. ii) *Hemorrhagic Shock* including the last four blocks, 5-8, corresponding to 40%, 50%, 60% and 70% blood loss. Figure 7 shows each biomarker for the graded blood loss and sham controls (no blood withdrawn).

Hemorrhagic Shock vs. Control: The effect of Group was **significant** for BFI as expected ($\chi^2(4) = 8.52$, $p = 0.0035$), HbO ($\chi^2(4) = 9.08$, $p = 0.0026$), and HbTotal ($\chi^2(4) = 4.38$, $p = 0.036$), but not significant for HbR ($\chi^2(4) = 0.992$, $p = 0.319$). Hence, these results may posit that BFI, HbO and HbTotal would be reliable biomarkers for detection of hemorrhagic shock.

On the other hand, when we compare no hemorrhagic shock, i.e., Pre-Shock including baseline, versus control group, the effect of Group difference was **not significant**, BFI ($\chi^2(4) = 0.09$, $p = 0.922$)); HbO ($\chi^2(4) = 0.024$, $p = 0.875$), HbR ($\chi^2(4) = 0.051$, $p = 0.0.819$), HbTotal ($\chi^2(4) = 0.007$, $p = 0.931$). This is also in line as the pre-shock, which also includes baseline, should not have drastic changes compared to control group (see Figure 7).

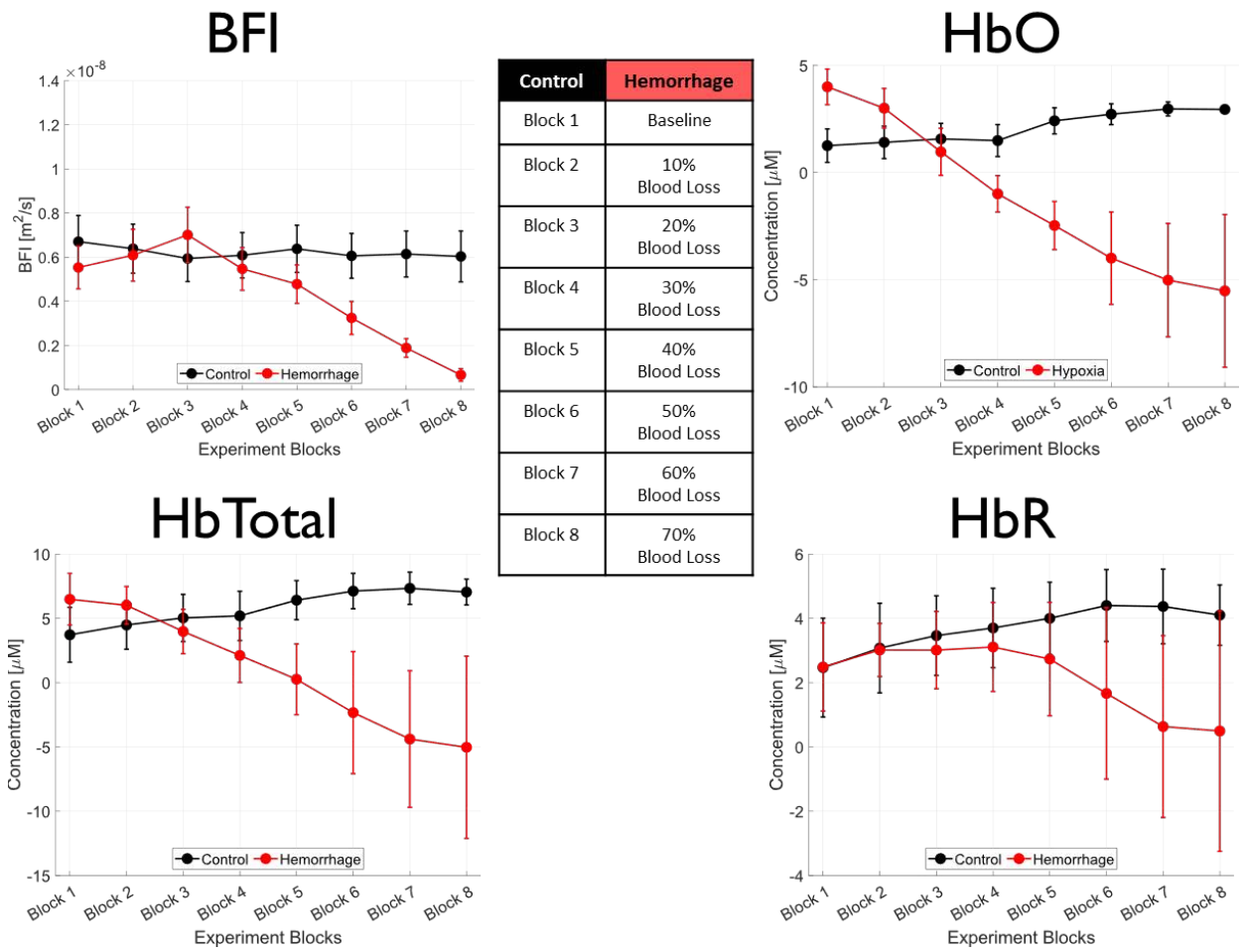


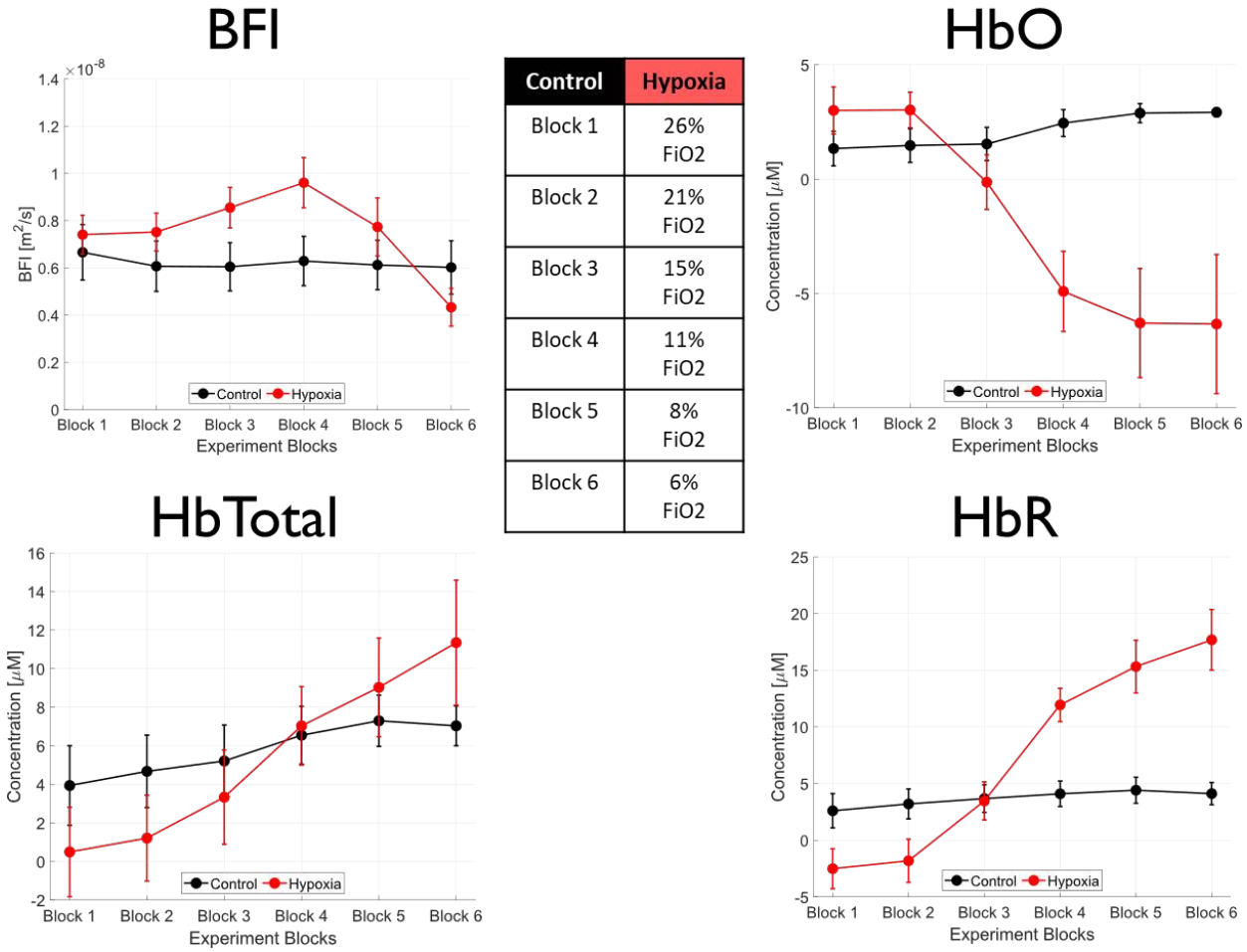
Figure 7. Hemorrhage versus sham controls data for each biomarker, BFI, HBTotal, HbO and HbR. Black lines show changes in control group and red lines show changes in response to blood loss with standard error of mean (mean ± SEM).

<u>Hypoxia Induced Brain Injury vs. Control Group: Key Findings</u>

Like the graded hemorrhage tests, hypoxia data are also split into two parts: i) *Normoxia* including the first 2 blocks, i.e., blocks corresponding to Baseline and 21% hypoxic fraction of inspired oxygen (FiO2) ii) *hypoxia* including the last blocks, corresponding to 15%, 11%, 8% and 6% FiO_2. Figure 8 shows each biomarker for the hypoxic conditions and sham controls (no changes in FiO_2).

Hypoxia vs. Control: The effect between group, i.e., hypoxic vs. control was **<u>significant</u>** only for HbO ($\chi^2(4) = 8.24$, $p = 0.002$) and HbR ($\chi^2(4) = 12.70$, $p < 0.0003$). but not significant for BFI and HbTotal ($p > 0.05$). Moreover, the interaction between group and Block was significant for all the biomarkers including BFI ($\chi^2(10) = 65.93$, $p < 0.001$), HbO ($\chi^2(10) = 30.27$, $p < 0.001$), HbR ($\chi^2(10) = 40.92$, $p < 0.001$) and HbTotal ($\chi^2(10) = 23.26$, $p < 0.001$). These results reveal different trends in the NIRS measures compared to hemorrhage study, thus this difference between two injury types could be used to determine the type of injury in the field.

On the other hand, when we compare normoxia, i.e., 26% and 21% FiO2, versus control group, the effect of Group difference was **not significant** for BFI ($\chi^2(4) = 0.87$, $p = 0.348$)); HbO ($\chi^2(4) = 2.13$, $p = 0.143$), , HbTotal ($\chi^2(4) = 1.54$, $p = 0.214$), yet it is significant for HbR ($\chi^2(4) = 4.71$, $p = 0.029$). This finding is also in agreement that the changes during normoxia should have similar changes compared to control group (see Figure 8).



Control	Hypoxia
Block 1	26% FiO2
Block 2	21% FiO2
Block 3	15% FiO2
Block 4	11% FiO2
Block 5	8% FiO2
Block 6	6% FiO2

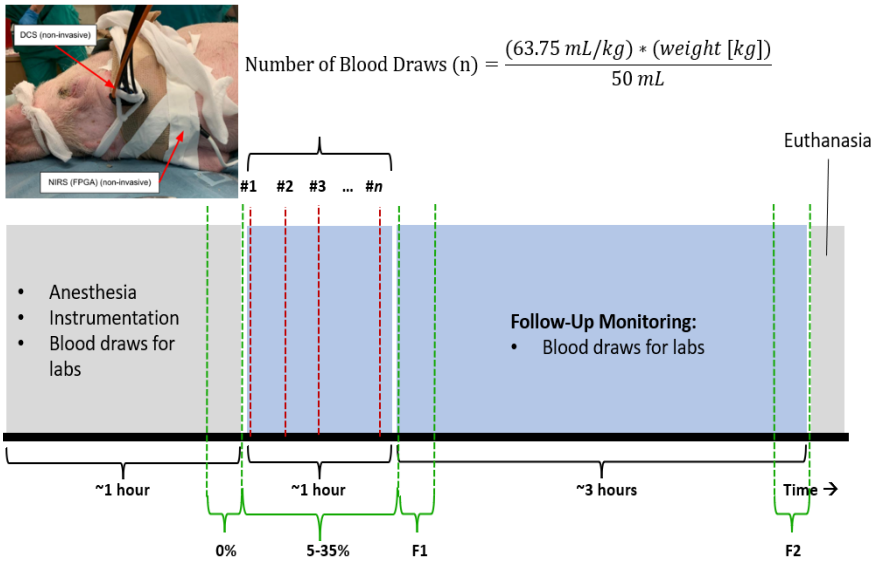
Figure 8. Hypoxia induced brain injury versus sham controls data for each biomarker, BFI, HBTotal, HbO and HbR. Black lines show changes in control group and red lines show changes in response to changes in hypoxic fraction of inspired oxygen (FiO2) with standard error of mean

Major Activity III (SOW Major Task # 3). Test prototype in adult pig model of controlled and uncontrolled hemorrhagic shock:

This major task included adult/pig animal data collection and data analysis. The team kicked off real-life clinical scenario tests and was able to complete the first cohort of adult pigs. The initial goal is to verify whether the test results from these new adult models are in agreement with the findings from piglet models of hemorrhage and hemorrhagic shock. The results from **n=10 domestic swine** weighing (>50 kg) revealed the cerebral blood flow decreases during each episode of blood loss.

Study Design and Measurement Protocol (Adult pig model):

Animals were anesthetized and mechanically ventilated. Then they were cannulated through the left femoral artery for arterial line blood pressure monitoring and hemorrhagic shock induction (Figure 9). Drugs and IV fluids were given through an auricular IV-line. Furthermore, a Swan-Ganz catheter was introduced to the pulmonary artery for hemodynamic monitoring. Hemorrhage was induced by removing **35% of the animal's calculated blood volume.** Blood was withdrawn manually from the femoral artery using a syringe in 50 ml.



Timepoint	Description
0% Blood Loss	2 minutes before the first blood draw.
5% Blood Loss	All blood draws between 0-7.5% total blood volume loss.
10% Blood Loss	All blood draws between 7.5-12.5% total blood volume loss.
15% Blood Loss	All blood draws between 12.5-17.5% total blood volume loss.
20% Blood Loss	All blood draws between 17.5-22.5% total blood volume loss.
25% Blood Loss	All blood draws between 22.5-27.5% total blood volume loss.
30% Blood Loss	All blood draws between 27.5-32.5% total blood volume loss.
35% Blood Loss	All blood draws between 32.5-37.5% total blood volume loss.
F1	From start of follow-up monitoring to 10 minutes after.
F2	10 minutes before euthanasia to euthanasia.

Figure 9. Experimental protocol for adult pig model of controlled hemorrhagic shock.

Results and Key Findings: In these tests, we use two, well established, swine models: one for the controlled hemorrhagic shock and the second for uncontrolled hemorrhage by liver laceration. The Institute for Research in Military Medicine (IRMM) at the Hebrew University-Hadassah Medical School has profound experience with both swine models and we already deliver and trained them to use the proposed DCS-NIRS prototype in these tests. In the first phase, they recruited 10 domestic swine weighing (>50 kg) for the validation of controlled hemorrhage study previously done with piglet models. We first performed the data analysis to investigate the relationship between progression of hemorrhagic shock and the measured cerebral blood flow (CBF). Figure 10 shows each episode of blood loss and corresponding averaged blood flow measures with standard error of mean (n=10 animals) including two follow-up monitoring periods, i.e., Follow-up 1(F1): 10 minutes after last blood draw and Follow-up 1(F2): 10 minutes before euthanasia). Figure 11 shows an individual animal result.

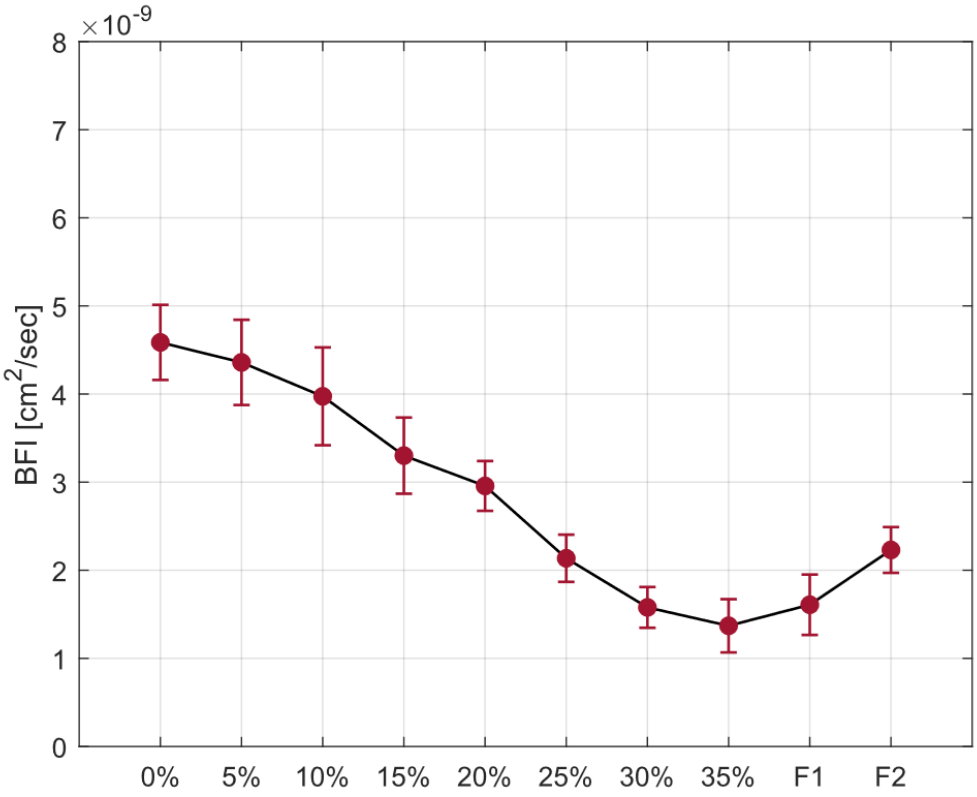


Figure 10. Adult pig model (***averaged response of n=10 pigs***): Progression of hemorrhagic shock by blood flow index (BFI). Note blood flow decreases with the graded blood loss and increases after the blood loss has stopped, i.e., during follow-up (F1).

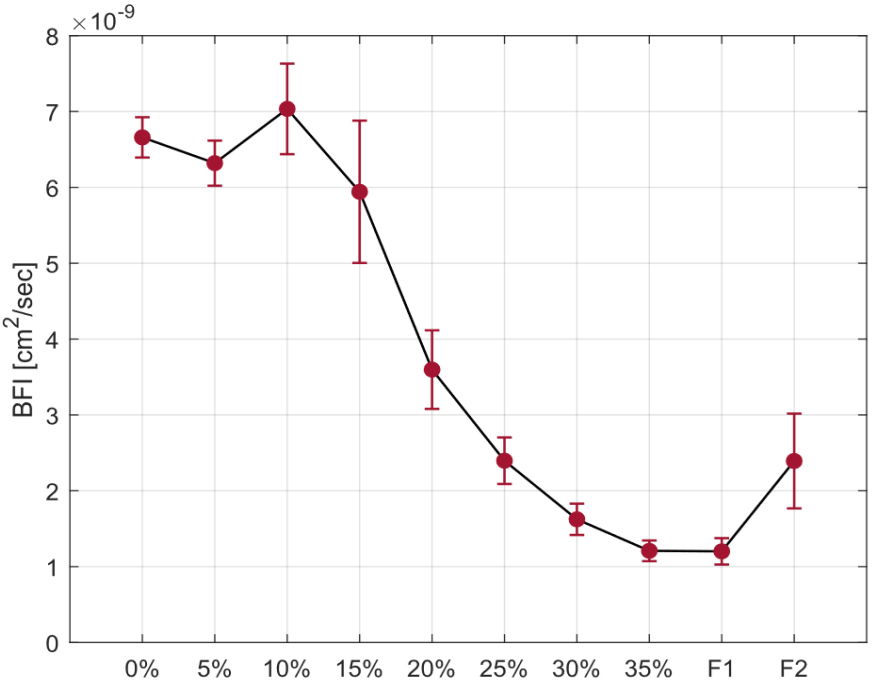


Figure 11. An ***individual pig*** result - Progression of hemorrhagic shock by relative blood flow index (BFI). Note the compensatory response, then deceleration of cerebral blood flow.

As for the tissue oximetry measure via the proposed system, Figures 12.A and 12.B reveal biomarkers of the oximetry measures, i.e., cerebral oxygenated hemoglobin (**HbO**), de-oxygenated hemoglobin (**HbR**) changes; total blood volume, **HbT**, changes measured from adult pigs.

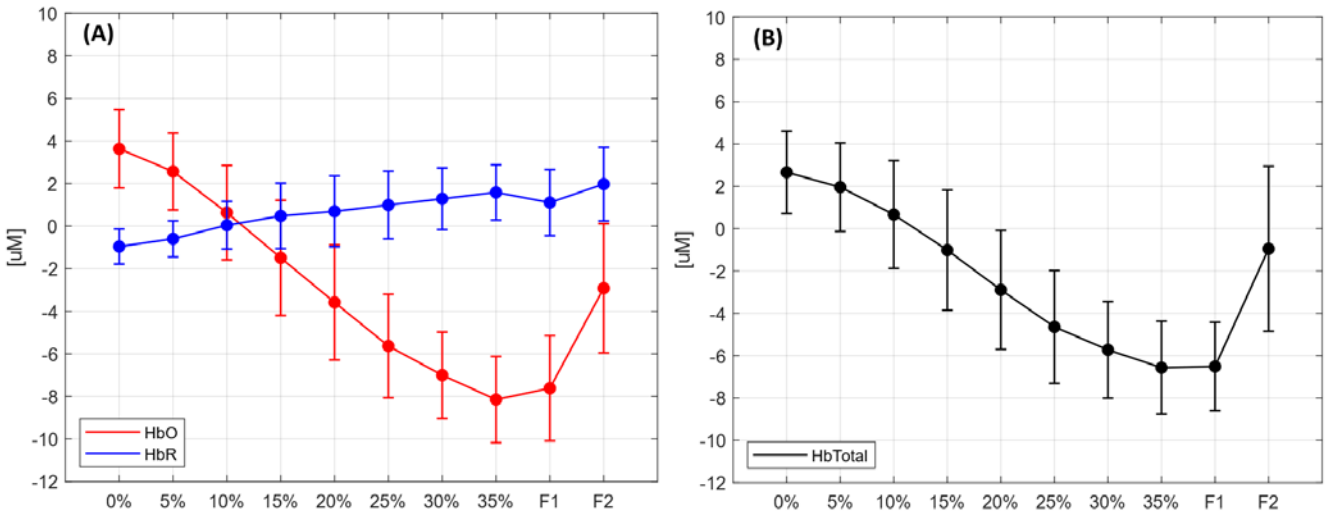


Figure 12. A. Progression of blood loss is monitored by the cerebral oxygenated hemoglobin (**HbO**), de-oxygenated hemoglobin (**HbR**) changes; **B.** Total blood volume, **HbT**, changes.

The observations and critical results

i. The average cerebral blood flow (CBF) of n=10 animals revealed a significant decrease following graded hemorrhage (Figure 10) which is in line with the results from piglet tests.

ii. Like the piglet tests, averaged response reveals a deceleration of flow. Individual animal data showed the compensatory response (Figure 11), yet initial CBF acceleration was not observed for all the animals (compensatory response was observed in the piglet tests prior to severe deceleration of flow). Further analysis will be performed once all the pig data is collected.

iii. Oxygenated hemoglobin (HbO) and total blood volume (HbT) decreased with each blood withdrawn as expected, and deoxygenated hemoglobin (HbR) slightly changed (Figure 12).

Describe the Regulatory Protocol and Activity Status as applicable**.**

Animal Use Regulatory Protocols

PROTOCOL(S): 2

			Enter information regarding number of subjects					
ACURO Protocol Number	**Protocol PI Name**	**Organization (Site)**	**# Target**	**# Screened**	**# Recruited**	**# Enrolled**	**# Completed**	**Other**
RC190294.e001	Dr. Shadi Malaeb	Drexel University	30	30	30	30	30	
RC190294.e002	Dr. Dean Nachman	Hebrew University	20	10	10	10	10	
		This annual reporting period	20	20	20	20	20	
		Cumulative	50					

Protocol (1 of 2 total):

Protocol [ACURO Assigned Number]: RC190294.e001

Title: Portable Diffuse Optical Sensors for Point-of-Care Monitoring in Prolonged Field Care

Target required for statistical significance: 30

Target approved for statistical significance: 30

Submitted to and Approved by:

Protocol Development: This protocol was developed to conduct proof of concept study and system tests in piglets. The experimental procedures and interventions, i.e., graded hemorrhage, fluid resuscitation, and induction of hypoxia ischemia are described in detail and reviewed.

Submission and Approvals: The protocol was first submitted to the Drexel University Institutional Animal Care & Use Committee and approved by the IACUC (*IACUC Protocol Number: 20889*) The protocol was reviewed and approved by the US Army Medical Research and Development Command (USAMRDC) Animal Care and Use Review Office (ACURO Protocol Number: RC190294.e001) on 10/15/2020.

Amendments: None

Status:

Progress Status: **Completed.**

Number of animals recruited/original planned target:	30 / 30
Number of animals enrolled/original planned target:	30 / 30
Number of animals completed/original planned target:	30 / 30

Protocol (2 of 2 total):

Protocol [ACURO Assigned Number]: RC190294.e002

Title: Portable Diffuse Optical Sensors for Point-of-Care Monitoring in Prolonged Field Care

Adult Pig Models for Real-life Clinical Scenario

Target required for statistical significance: 20

Target approved for statistical significance: 20

Submitted to and Approved by:

Protocol Development: This protocol has been developed to conduct validation tests with adult pig models mimicking a real-life clinical scenario. The experimental procedures including uncontrolled hemorrhage by liver laceration are described in detail and reviewed.

Submission and Approvals: The protocol was first submitted to the Hebrew University Institutional Animal Care & Use Committee and approved by the IACUC (*IACUC Protocol Number: MD-21-16440-3).* The protocol was reviewed and approved by the US Army Medical Research and

Development Command (USAMRDC) Animal Care and Use Review Office (ACURO Protocol Number: RC190294.e002) on 11/24/2021.

Amendments: None

Status:

Progress Status: In progress.

Number of animals recruited/original planned target:	10 / 20
Number of animals enrolled/original planned target:	10 / 20
Number of animals completed/original planned target:	10 / 20

Administrative, Technical or Logistical issues: None.

What opportunities for training and professional development has the project provided?

3 PhD level graduate students have participated in animal tests which help their training and degrees to conduct preclinical animal studies.

How were the results disseminated to communities of interest?

The team has disseminated these new development and test results by attending and presenting at the *2023 Military Health System Research Symposium (MHSRS)*. We have been also preparing manuscript for a journal in this year to report these models, implementation of the algorithms and test results which are highly significant (see the publication, presentation and journal list below).

What do you plan to do during the next reporting period to accomplish the goals?

We will accomplish following goals for the next annual reporting period:

Year 4 Quarter 1:

- Continue tests the prototype in an adult pig model of controlled hemorrhagic shock and uncontrolled hemorrhage by liver laceration.

Year 4 Quarter 2:

- Analyze the measurements from the adult swine tests.

Year 4 Quarters 3 & 4:

- Analyze and report validation analysis using physiological data and DCS and NIRS signal synchrony in response to change in the phase of hemorrhagic shock during this clinical model.
- Submit final report.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Using same modality, i.e., optics, with implementation of two different techniques, namely near infrared spectroscopy (NIRS) and diffuse correlation spectroscopy (DCS) techniques, multiple biomarkers were extracted and tested via piglet and adult swine animal models. The critical accomplishment and key contribution here is that the new prototype DCS-NIRS system developed with the support of this award revealed distinct changes in local cerebral blood flow (CBF) and tissue oxygenation/hemodynamics (HbO, HbR, HbT) that followed each hemorrhagic shock and hypoxic shock. In summary, our results strongly support that this prototype can probe the hemodynamic status of local cerebral cortical tissue, and gain insight into the underlying changes of cerebral tissue perfusion at the microvascular level.

What was the impact on other disciplines?

Specific to this period and previous period in this project, the head models we custom developed for this project can easily be used to test other clinical modalities or systems, such as efficacy and reliability of new local tissue oxygenation sensors which have been recently key sensory device in detecting some of COVID-19 symptoms. Decision making or regulatory agencies, such as the U.S. Food and Drug Administration can use these test models for independent evaluations. These models are modular, dynamic and can be easily tailored to mimic different tissue oxygenation, cerebral blood flow or water content changes for different injury types.

Use of the prototype system we are developing revealed the measures for cerebral tissue perfusion at the microvascular level which can also be extended to other clinical uses or field applications, such as depth of anesthesia and sedation monitoring for ambulatory anesthesia.

What was the impact on technology transfer?

Nothing to report for this period.

What was the impact on society beyond science and technology?

As previously mentioned, decision making or regulatory agencies, such as the U.S. Food and Drug Administration (FDA) can use these phantom/head models for independent evaluations.

This project helps three PhD level graduate students to improve their knowledge and training on pre-clinical studies by participating in animal tests.

5. CHANGES/PROBLEMS:

Nothing to report.

Changes in approach and reasons for change

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

We experienced following delays throughout Year 3:

During the first and 2nd quarter of Year 3, we experienced delays in recruiting more animals and our orders were cancelled because there were no mother sows scheduled to give birth in the time frame requested.

Changes that had a significant impact on expenditures

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/ or select agents

Significant changes in use or care of human subjects
Not applicable

Significant changes in use or care of vertebrate animals
Nothing to report.

Significant changes in use of biohazards and/or select agents
Nothing to report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Conference Papers

1. Izzetoglu, K., Malaeb, S., & Izzetoglu, M. (2023). Noninvasive Monitoring of Changes in Cerebral Blood Flow and Volume During Prolonged Field Care for Hemorrhagic Shock and Hypoxia-Induced Injuries with Portable Diffuse Optical Sensors. In *Military Health System Research Symposium (MHSRS)*. Kissimmee, FL, USA.
2. Sinahon, R., Polat, M.D., Izzetoglu, M., Izzetoglu, K., Malaeb, S. (2023). Noninvasive Monitoring of Hemorrhagic Shock in Piglets using Diffuse Correlation Spectroscopy. In *Pediatric Academic Societies Meeting (PAS)*. Washington, DC, USA.

Acknowledgement of federal support (yes)
Conference Presentation:

1. *"Noninvasive Monitoring of Changes in Cerebral Blood Flow and Volume During Prolonged Field Care for Hemorrhagic Shock and Hypoxia-Induced Injuries with Portable Diffuse Optical Sensors***"** at the *2023 Military Health System Research Symposium (MHSRS)*.

Journal publications.

1. Gomero, L.M., Izzetoglu, M., Malaeb, S., & Izzetoglu, K. (Under Preparation). Blood Flow Estimation from DCS Measurements Using a Frequency Domain Approach: Experimental animal modelling for brain injury. *IEEE Transactions on Instrumentation and Measurement.*
   Acknowledgement of federal support (yes)

2. Izzetoglu, K., Malaeb, S., & Izzetoglu, M. (Under Preparation). Noninvasive Monitoring of Changes in Cerebral Blood Flow and Volume During Prolonged Field Care for Hemorrhagic Shock and Hypoxia-Induced Injuries with Portable Diffuse Optical Sensors. *MHSRS Supplement to Military Medicine*.

   Acknowledgement of federal support (yes)

Books or other non-periodical, one-time publications.

c
Nothing to report.

Other publications, conference papers and presentations.

Apr 23-24, 2022. Poster at the 48th Northeast Bioengineering Conference, New York City, NY. Title: ‘Monitoring Cerebral Blood Flow with Diffuse Correlation Spectroscopy During Hemorrhagic Shock.

- **Website(s) or other Internet site(s)**

  Nothing to report.

- **Technologies or techniques**

  - A complete fully functional diffuse correlation spectroscopy (DCS) with a multi-distance optical probe/sensor and data acquisition software.
  - A novel algorithm is implemented to determine cerebral blood flow indices from experimental data recordings.
  - Animal models of different brain injuries are developed and studied.

- **Inventions, patent applications, and/or licenses**

  Nothing to report.

- **Other Products**

Custom developed dynamic phantom models, techniques and set ups to mimic the changes in blood flow and blood volume; and custom developed animal models to study different brain injuries.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Kurtulus Izzetoglu
Organization:	Drexel University
Project Role:	PI
Researcher Identifier (ORCID ID):	0000-0001-5304-7361
Nearest person month worked:	3.0
Contribution to Project:	Dr. Izzetoglu has been leading overall conduct of the project and ensure the successful completion of the SOW tasks, the efforts include but not limited to, tests and modification of the device, animal tests, data analysis and modelling in Year 3, and led internal technical team meetings, and presented at the 2023 MHSRS.

Name:	Shadi Malaeb
Organization:	Drexel University
Project Role:	Co-Investigator
Researcher Identifier (ORCID ID):	0000-0001-6523-5984
Nearest person month worked:	0.6
Contribution to Project:	Dr. Malaeb as a clinician has led the animal tests. This Year, he recruited 10 more animals (n=30 total) and developed the study for piglet model of graded hemorrhage and hypoxia-induced injuries.

Name:	John R. Grothusen
Organization:	Drexel University
Project Role:	Animal study technician
Researcher Identifier (ORCID ID):	0000-0001-8483-6339
Nearest person month worked:	0.5
Contribution to Project:	Mr. Grothusen supported the piglet studies and tests.

Name:	Sinan F. Tuzer
Organization:	Drexel University
Project Role:	Animal study coordinator
Researcher Identifier (ORCID ID):	0000-0001-8483-6339
Nearest person month worked:	1.0
Contribution to Project:	This year, Dr. Tuzer supported animal recruitment and screening of 10 piglets (supervised by Dr. Malaeb). He also provided support with the animal tests.

Name:	Juan Du
Organization:	Drexel University
Project Role:	Lab technician & manager
Researcher Identifier (ORCID ID):	0000-0002-7978-0665
Nearest person month worked:	9.0
Contribution to Project:	This year, Ms. Juan Du guided all the animal studies at the clinical setting and worked on animal tests and system performance tests. She has been coordinating the lab and clinical animal facility for the device setups and measurements.

Name:	Deniz Polat
Organization:	Drexel University
Project Role:	Graduate student
Researcher Identifier (ORCID ID):	0000-0002-0980-1416
Nearest person month worked:	4.5
Contribution to Project:	Mr. Polat actively participated in supporting clinical studies and worked on all the animal studies and data collection from 10 piglets this year.

Name:	Pratusha Reddy
Organization:	Drexel University
Project Role:	Graduate student (PhD)
Researcher Identifier (ORCID ID):	0000-0002-6589-9075
Nearest person month worked:	4.5
Contribution to Project:	Ms. Prat has been assisting with the tests and working on the development of signal processing algorithms for cerebral blood flow (DCS) and blood volume (NIRS) measures. She developed linear mixed effect regression models for the data and statistical analyses included in this annual report.

Name:	Meltem Izzetoglu
Organization:	Villanova University
Project Role:	Subaward PI
Researcher Identifier (ORCID ID):	0000-0002-1768-3384
Nearest person month worked:	*1*.0
Contribution to Project:	Dr. Meltem Izzetoglu has been working on algorithm development for the blood flow and cerebral volume indices using measures of the optical sensors developed in this project. She supported the data analysis with particular focus on modeling and statistical data analysis of NIRS data during this year. She has been leading the efforts on preparing the first manuscript for dissemination of the results in a journal.
Name:	Luis Gomero
Organization:	Villanova University
Project Role:	Graduate student (MSc) (Subaward)
Researcher Identifier (ORCID ID):	0000-0001-6374-3096
Nearest person month worked:	6.0
Contribution to Project:	Mr. Gomero, who is supervised by Subaward PI (Dr. Meltem), has worked on blood flow and oximetry data analysis and supported the animal studies. He has been preparing the first manuscript for dissemination of the results in a journal.
Name:	Dean Nachman
Organization:	Hebrew University-Hadassah Medical School
Project Role:	Subaward PI
Researcher Identifier (ORCID ID):	
Nearest person month worked:	0.5
Contribution to Project:	Dr. Nachman, as a clinician, has led the adult/pig tests. This Year, he recruited 10 pigs and developed the study for adult swine model of graded controlled and uncontrolled hemorrhage.

Name	***Project Role***	***Organization***	***Calendar Months (Year 1)***	***Contribution to Project***
Kurtulus Izzetoglu	PI	Drexel University	*3.0*	Dr. Izzetoglu has been leading overall conduct of the project and ensure the successful completion of the SOW tasks, the efforts include but not limited to, tests and modification of the device, animal tests, data analysis and modelling in Year 3, and led internal technical team meetings, and presented at 2023 MHSRS.

Shadi Malaeb	Co-Investigator	Drexel University	0.6	Dr. Malaeb as a clinician has led the animal tests. This Year, he recruited 10 more animals (n=30 total) and developed the study for piglet model of graded hemorrhage and hypoxia-induced injuries.
John R. Grothusen	Animal Study Technician	Drexel University	0.5	Mr. Grothusen supported the piglet studies and tests.
Sinan F. Tuzer	Animal Study Coordinator	Drexel University	1.0	This year, Dr. Tuzer supported animal recruitment and screening of 10 piglets (supervised by Dr. Malaeb). He also provided support with the animal tests.
Juan Du	Lab Technician & Manager	Drexel University	9.0	This year, Ms. Juan Du guided all the animal studies at the clinical setting and worked on animal tests and system performance tests. She has been coordinating the lab and clinical animal facility for the device setups and measurements.
Deniz Polat	Graduate Student (PhD)	Drexel University	4.5	Mr. Polat actively participated in supporting clinical studies and worked on all the animal studies and data collection from 10 piglets this year
Pratusha Reddy	Graduate Student (PhD)	Drexel University	4.5	Ms. Prat has been assisting with the tests and working on the development of signal processing algorithms for cerebral blood flow (DCS) and blood volume (NIRS) measures. She developed linear mixed effect regression models for the data and statistical analyses included in this annual report.
Meltem Izzetoglu	Subaward PI	Villanova University	1.0	Dr. Meltem Izzetoglu has been working on algorithm development for the blood flow and cerebral volume indices using measures of the optical sensors developed in this project. She supported the data analysis with particular focus on modeling and statistical data analysis of NIRS data during this year. She has been leading the efforts on preparing the first manuscript for dissemination of the

				results in a journal.
Luis Gomero	Graduate student	Villanova University	6.0	Mr. Gomero, who is supervised by Subaward PI (Dr. Meltem), has worked on blood flow and oximetry data analysis and supported the animal studies. He has been preparing the first manuscript for dissemination of the results in a journal.
Dean Nachman	Subaward PI	Hebrew University-Hadassah Medical School	0.5	Dr. Nachman, as a clinician, has led the adult/pig tests. This year, he recruited 10 pigs and developed the study for adult swine model of graded controlled and uncontrolled hemorrhage.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

Organization Name: Hebrew University-Hadassah Medical School
Location of Organization: (if foreign location list country) Jerusalem, Israel
Partner's contribution to the project (identify one or more)

- Collaboration/Subcontractor: Hebrew University is a subawardee and develops adult pig animal models and experimental protocols and conduct these studies. Colleagues at the Hebrew University developed the research protocol during this Year 1 and secured the approvals in Year 2 and conducting the animal studies in Year 3.

Organization Name: Villanova University
Location of Organization: (if foreign location list country) Villanova, PA
Partner's contribution to the project (identify one or more)

- Collaboration/Subcontractor: Villanova University is a subawardee and collaborating with the lead institute on analyzing animal tests reported during this year 3. They have also been supporting algorithm development for data analysis, particularly blood flow indices using measures of the optical sensors developed in this project.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

Updated quad chart is submitted.

9. APPENDICES:

Please see APPENDIX A

APPENDIX A: Analysis of the physiological data (vital signs)

In Year 3, we performed analysis on the physiological measures including heart rate (HR), Oxygen saturation (SpO2), mean arterial pressure (MAP) using measures of systolic blood pressure (SPB) and diastolic blood pressure (DPB). Figure A.1 shows all biomarkers and measurements.

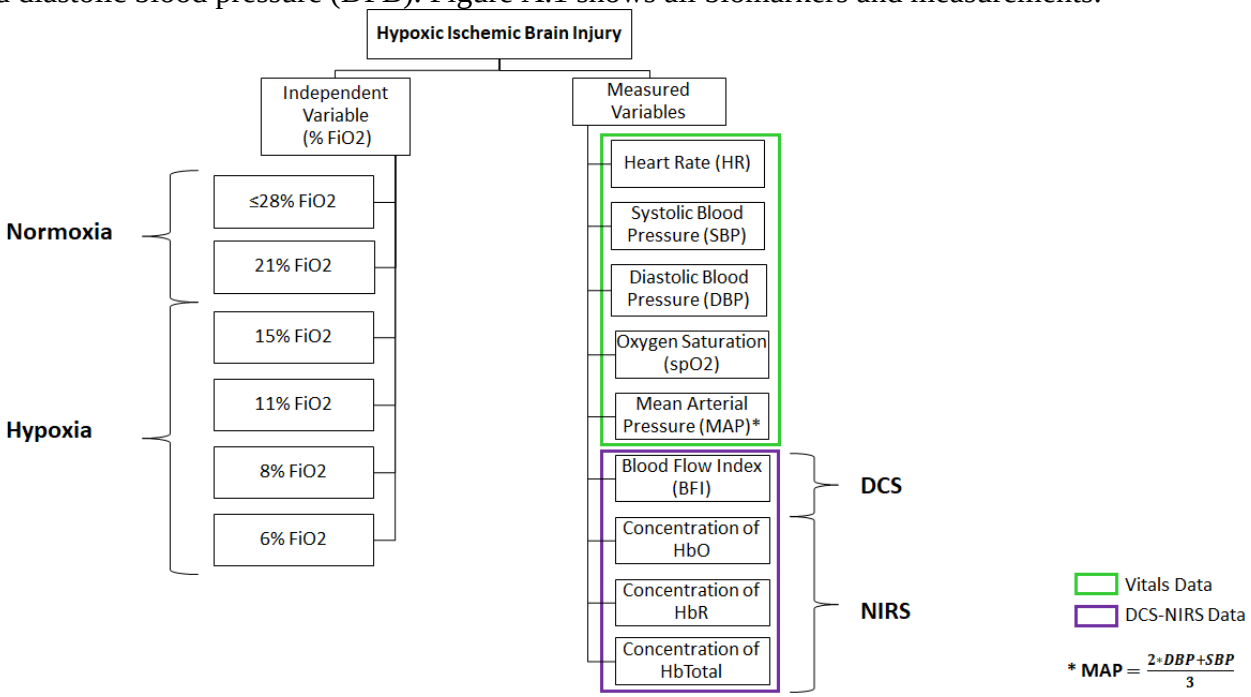


Figure A.1. All the physiological data (vital signs including HR, SB, DB, MAP), and DCS-NIRS signals for the tissue oximetry, blood flow and blood volume.

As shown in Table A.1 and figure A.2, heart rate reveals expected changes in response to hypoxic shock condition and was found to be significant between normoxic and hypoxic conditions ($p<0.05$).

Table A.1. Heart rate (HR) changes in response to hypoxic conditions

Normoxia v. Hypoxia	% FiO2 Interval	n*	mean (bpm)	median (bpm)	std (bpm)	SEM (bpm)
Normoxia	≤28% to ≥21%	11	**170**	159	33.2	10.0
Hypoxia	≤15% to ≥6%	11	**191**	192	27.0	8.14

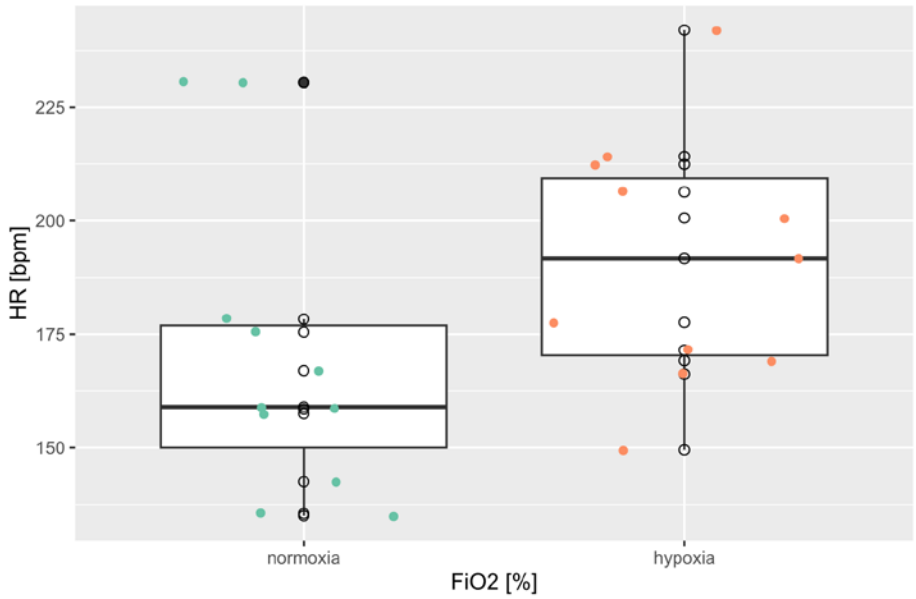


Figure A.2. Heart rate measures in response to hypoxic conditions.

Systolic and diastolic blood pressure changes were not significant between normoxic and hypoxic conditions ($p>0.05$) (Figure A.3)

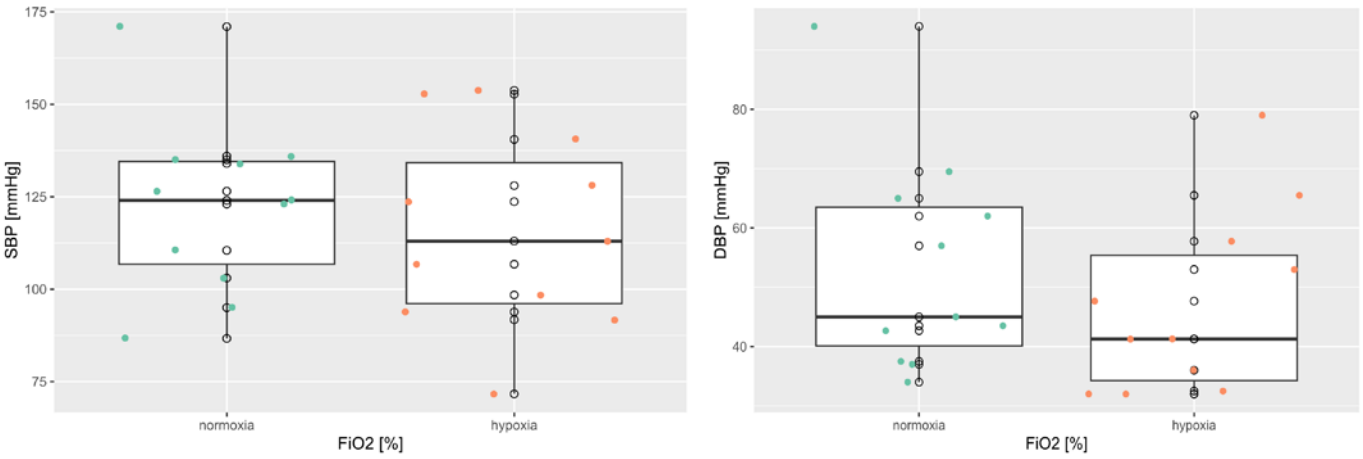


Figure A.3. (Left) Systolic, (Right) diastolic blood pressure changes

There was a significant difference in peripheral blood oxygen saturation (as measured by pulse oximetry) between normoxic and hypoxic conditions ($p< 0.05$; Figure A.4).

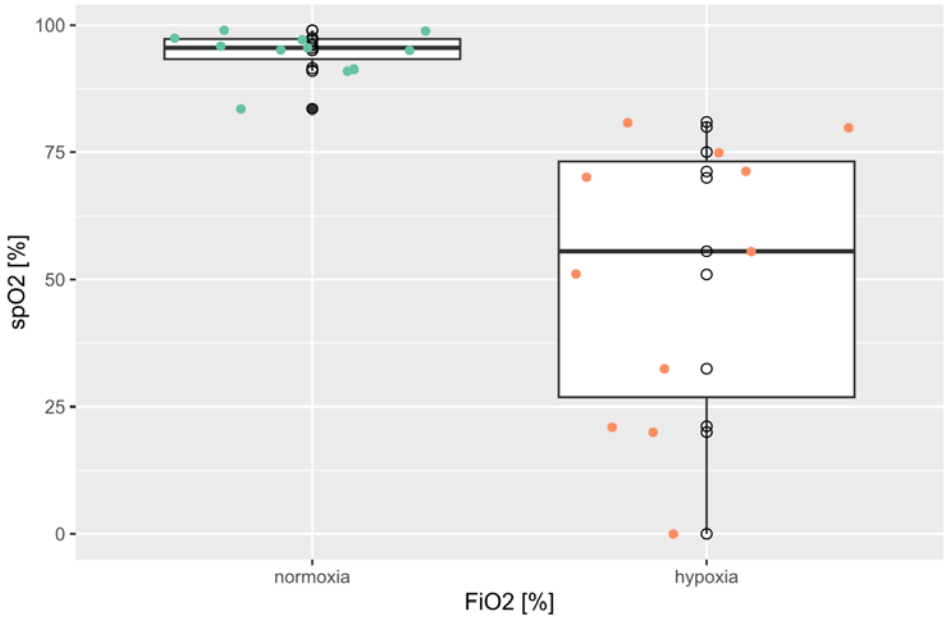


Figure A.4. Peripheral blood oxygen saturation (SpO2) measures

Similar to the results in systolic and diastolic blood pressure changes, mean arterial pressure (MAP) was not significant between normoxic and hypoxic conditions ($p>0.05$).

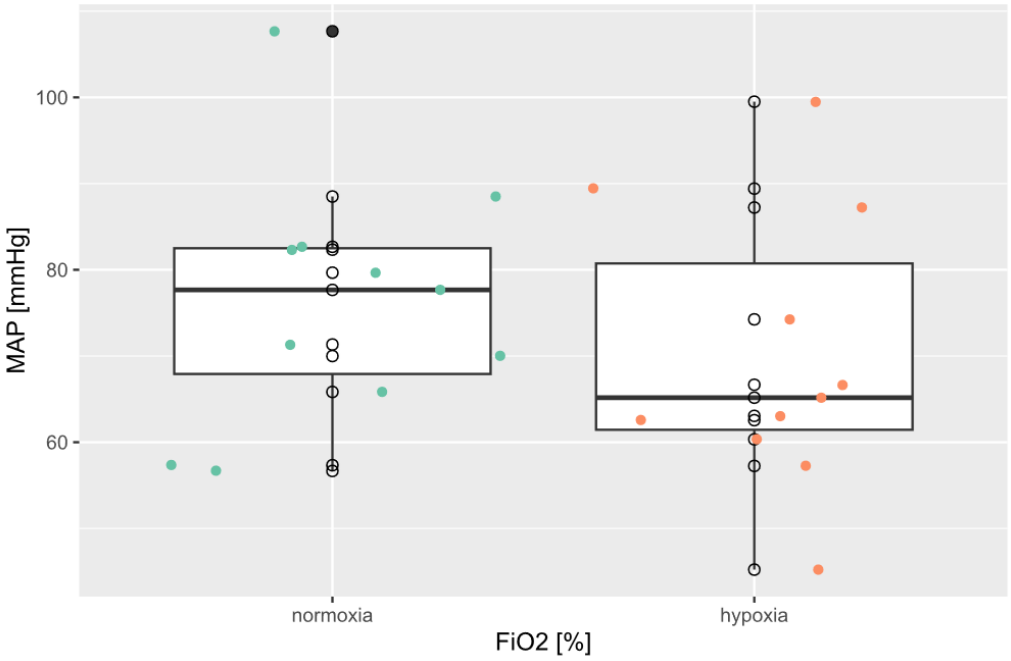


Figure A.5. Mean arterial pressure (MAP measures

References Cited:

1. Buckley, E. M., Parthasarathy, A. B., Grant, P. E., Yodh, A. G., & Franceschini, M. A. (2014). Diffuse correlation spectroscopy for measurement of cerebral blood flow: future prospects. *Neurophotonics*, *1*(1), 011009.
2. Verdecchia, K., Diop, M., Lee, A., Morrison, L. B., Lee, T. Y., & Lawrence, K. S. (2016). Assessment of a multi-layered diffuse correlation spectroscopy method for monitoring cerebral blood flow in adults. *Biomedical optics express*, *7*(9), 3659-3674.
3. Gagnon, L., Desjardins, M., Jehanne-Lacasse, J., Bherer, L., & Lesage, F. (2008). Investigation of diffuse correlation spectroscopy in multi-layered media including the human head. *Optics express*, *16*(20), 15514-15530.
4. Durduran, T., & Yodh, A. G. (2014). Diffuse correlation spectroscopy for non-invasive, micro-vascular cerebral blood flow measurement. *Neuroimage*, *85*, 51-63.
5. Tamborini, D., Farzam, P., Zimmermann, B. B., Wu, K. C., Boas, D. A., & Franceschini, M. A. (2017). Development and characterization of a multidistance and multiwavelength diffuse correlation spectroscopy system. *Neurophotonics*, *5*(1), 011015.
6. Izzetoglu, M., Pourrezaei, K., Du, J., & Shewokis, P. A. (2021). Evaluation of Cerebral Tissue Oximeters Using Multilayered Dynamic Head Models. *IEEE Transactions on Instrumentation and Measurement*, 70, 1-12.
7. Linderkamp, O., Betke, K., Güntner, M. *et al.* Blood Volume in Newborn Piglets: Effects of Time of Natural Cord Rupture, Intra-Uterine Growth Retardation, Asphyxia, and Prostaglandin-Induced Prematurity. *Pediatr Res* 15, 53–57 (1981). https://doi.org/10.1203/00006450-198101000-00013