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Corresponding Author:	Nora Bruns, M.D. Universitatsklinikum Essen Essen, North Rhine-Westphalia GERMANY
Corresponding Author's Institution:	Universitatsklinikum Essen
Corresponding Author E-Mail:	Nora.Bruns@uk-essen.de
Order of Authors:	Nora Bruns, M.D.
	Julia Moosmann
	Frank Münch
	Christian Dohna-Schwake
	Joachim Woelfle
	Robert Cesnjevar
	Sven Dittrich
	Ursula Felderhoff-Müser
	Hanna Müller
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TITLE:

How to Administer Near-Infrared Spectroscopy in Critically Ill Neonates, Infants, and Children

AUTHORS AND AFFILIATIONS:

Nora Bruns[1], Julia Moosmann[2], Frank Münch[3], Christian Dohna-Schwake[1], Joachim Woelfle[4], Robert Cesnjevar[3], Sven Dittrich[2], Ursula Felderhoff-Müser[1], Hanna Müller[4]

[1]Department of Pediatrics I - Neonatology, Pediatric Intensive Care, Pediatric Neurology, University Hospital Essen, University of Duisburg-Essen, Germany
[2]Department of Pediatric Cardiology, University Hospital Erlangen, University of Erlangen-Nürnberg, Germany
[3]Department of Pediatric Cardiac Surgery, University Hospital Erlangen, University of Erlangen-Nürnberg, Germany
[4]Division of Neonatology and Pediatric Intensive Care, Department of Pediatrics, University Hospital Erlangen, University of Erlangen-Nürnberg, Germany

Email addresses of co-authors:

Julia Moosmann	(julia.moosmann@uk-erlangen.de)
Frank Münch	(frank.muench@uk-erlangen.de)
Christian Dohna-Schwake	(christian.dohna-schwake@uk-essen.de)
Joachim Wölfle	(joachim.woelfle@uk-erlangen.de)
Robert Cesnjevar	(robert.cesnjevar@uk-erlangen.de)
Sven Dittrich	(Sven.Dittrich@uk-erlangen.de)
Ursula Felderhoff-Müser	(ursula.felderhoff@uk-essen.de)
Hanna Müller	(hanna.mueller@uk-erlangen.de)

Corresponding author:

Nora Bruns	(nora.bruns@uk-essen.de)

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NIRS, near-infrared spectroscopy, neonatal, pediatric, soma sensor, cerebral, somatic, renal, intestinal, surgery, intensive care, ECMO, cardiopulmonary bypass

SUMMARY:

This protocol is designed to assist clinicians to measure regional tissue oxygenation at different body sites in infants and children. It can be used in situations where tissue oxygenation is potentially compromised, particularly during cardiopulmonary bypass, when using non-pulsatile cardiac-assist devices, and in critically ill neonates, infants and children.

ABSTRACT:

Near infrared spectroscopy (NIRS) calculates regional tissue oxygenation (rSO_2) using the different absorption spectra of oxygenated and deoxygenated hemoglobin molecules. A probe placed on the skin emits light that is absorbed, scattered, and reflected by the underlying tissue. Detectors in the probe sense the amount of reflected light: this reflects the organ-specific ratio of oxygen supply and consumption - independent of pulsatile flow. Modern devices enable the simultaneous monitoring at different body sites. A rise or dip in

the rSO_2 curve visualizes changes in oxygen supply or demand before vital signs indicate them. The evolution of rSO_2 values in relation to the starting point is more important for interpretation than are absolute values.

A routine clinical application of NIRS is the surveillance of somatic and cerebral oxygenation during and after cardiac surgery. It is also administered in preterm infants at risk for necrotizing enterocolitis (NEC), newborns with hypoxic ischemic encephalopathy and a potential risk of impaired tissue oxygenation. In the future, NIRS could be used in multimodal neuromonitoring, or applied to monitor patients with other conditions (e.g., after resuscitation or traumatic brain injury).

INTRODUCTION:

Near-infrared spectroscopy (NIRS) noninvasively measures the regional tissue oxygen saturation (rSO_2) in brain, muscle, kidneys, liver or intestines[1-8,9]. It is applied in intensive care and cardiac surgery to monitor "real-time" oxygen consumption and somatic tissue saturation[10].

A probe on the skin emits near-infrared light (700 - 1000 nm)[11] that penetrates tissue and bone up to a depth of approximately 1-3 cm, thereby being scattered, absorbed and reflected[12]. Detectors in the probe sense the amount of reflected light – representing the relative amount of deoxygenated haemoglobin – and calculate a numerical value that indicates the regional oxygenation saturation in percent (%)[2]. Unlike pulse oximetry (which reflects systemic oxygen supply and requires pulsatile flow), NIRS reflects venous oxygen saturation and does not require pulsatile flow, thus making it suitable for low-flow situations such as cardiopulmonary bypass[7].

The rSO_2 reflects the balance between oxygen supply and consumption in the tissue – changes in either become visible even before alterations become otherwise clinically evident. Changes relative to the baseline are more important than the absolute measured values themselves[10,13-16]. Measuring rSO_2 helps clinicians monitor patients during heart surgery and cardiopulmonary bypass, in the intensive care unit; it can also assist in guiding oxygen therapy in preterm infants and monitor kidney, splanchnic, and systemic perfusion[12,17-21].

NIRS is a safe, feasible[22], and easy way to monitor tissue oxygenation continuously. Combined with other cerebral biomarkers and neuromonitoring techniques (e.g., continuous or amplitude-integrated EEG), NIRS will likely play a role in future (multimodal) monitoring in neonates and children[23,24]. In this article, we show clinicians how to set up NIRS monitoring for different organ systems, explain how rSO_2 values evolve corresponding to changes in physiology, and present typical results from different clinical settings.

PROTOCOL:

NIRS is conducted as part of the hospital's clinical routine. It is recommended in pediatric cardiac surgery interventions within the scope of quality assurance from the Competence Network for Congenital Heart Defects (http://www.kompetenznetz-ahf.de), the Pediatric Cardio Anesthetic Working Group and the German Society for Cardiovascular Engineering[25].

The protocol follows the guidelines of the institution's human research ethics committee. We obtained written informed consent regarding filming and publication of the material from both parents of each infant appearing in the video. The protocol we present corresponds to the clinical practice in the hospital and applies to infants and children of any age. If there are special concerns for a specific age group, we indicate this in a note in the protocol.

1. Preparation

1.1. Plug in and turn on the NIRS device. Enter the patient's data according to the device's setup.

1.2. Select the proper probe according to the patient's weight and intended site of use. The weight range is given on the probe's packaging and depends on the manufacturer (see **Table 1** for an overview of the weight ranges in common manufacturers).

1.3. Make sure the patient's skin is clean and dry for optimum adhesion. Dry the skin with a swab if necessary. Be very careful or omit cleaning if the skin is vulnerable.

2. Place the probe

2.1. After identifying the correct probe position, carefully bend the center of the probe toward the side of the white cover until it starts to come off. Gently peel off the cover without touching the probe's sticky surface.

2.2. Place the sensor on the skin from the center of the probe to the sides. Make sure the edges of the probe are firmly connected to the skin. If the probe disconnects, wrong NIRS values will be obtained. Disconnection in a bright environment causes false high values; disconnection in a dark environment causes false low values.

NOTE: To avoid skin lesions, do not place the probe on very immature or vulnerable skin. If the probe must be placed on vulnerable skin, use a layer of cellophane between the skin and the probe, or leave the cover on. When fixing the probe, avoid putting pressure on it (e.g., via an infant flow cap or headband) as this can impair skin perfusion and cause an erroneous measurement.

3. Select the probe position

3.1. Cerebral: Place the NIRS probe in the supra-orbital region on the forehead below the hairline to obtain values from the frontal cortex. Do not place the probe above hair, the frontal sinus, the temporal muscle, nevi, the superior sagittal sinus, intracranial hemorrhages or other anomalies, as that can alter the measurement and the values obtained will not represent regional tissue oxygenation. Placement of two probes, one on each forehead allows selective analysis of both hemispheres if the clinical setting requires this. Neighboring probes emit and measure signals alternately to avoid interference.

NOTE: The rSO_2 value only reflects the oxygenation status of the tissue underneath the

probe – for a large organ like the brain, obtained values do not reflect the entire organ‘s oxygenation status.

3.2. Somatic: Select a position above the region of interest. Avoid fat deposits, hair, and bones. Do not place the probe above nevi, hematoma, and injured skin. Always remember that the depth of the NIRS signal is approximately 2.5 cm - if the organ of interest is farther away from the probe, it cannot be analyzed. For renal or hepatic NIRS, use ultrasound to ensure correct placement.

3.2.1. Kidneys: Locate the kidney via dorsal sagittal sonogram before placing the probe. Make sure that the skin-to-organ-distance does not exceed the maximum depth of the probe.

NOTE: The use of ultrasound may interfere with the minimal-handling principle (e.g., in very preterm infants).

3.2.2. Intestines: Place the probe in the region of interest (e.g., below the umbilicus or in the right or left lower quadrant).

NOTE: Free air or liquid in the abdomen can make measuring the desired organ’s tissue oxygenation impossible.

3.2.3. Liver: Place the probe exactly above the liver. If possible, confirm its position by ultrasound. To avoid measuring the wrong organ, make sure the liver tissue underneath the probe is at least as deep as the emitted light penetrates (1-3 cm, according to the probe selected).

3.2.4. Foot: Place the probe on the plantar portion of the foot. Measuring NIRS in the most distant part of the body gives information about peripheral perfusion during hypothermia, in patients with shock or in any situation where pulse oximetry does not work.

3.2.5. Muscle: Place the probe over the muscle of interest.

4. Set the baseline

4.1. 1-2 minutes after placing the probe, set the baseline by pushing the corresponding button on the device. Baseline reflects the starting point of the measurement. The evolution of tissue perfusion in each monitored area can be observed and interpreted individually by relying on the change from the baseline value.

5. Check for problems with the device or complications

5.1 If the device indicates bad recording quality or values are implausible, confirm that all the aforementioned steps have been taken correctly. If necessary, replace the probe and preamplifer, and check all electrical plug contacts.

5.2 Check for external light sources that might affect the sensor and contact. Cover the

probe light-tight if disturbing light sources cannot be eliminated.

5.3 After ruling out technical problems, check the patient for clinical complications.

REPRESENTATIVE RESULTS:

The measured rSO_2 value results from the ratio between oxygen supply and consumption (**Figure 1A**); differing metabolic characteristics lead to slightly different normal values depending on age and organ (**Table 2**). Note that - except for the brain – scientifically evaluated reference values exist only for preterm infants and newborns[26-31] and most of the protocol steps rely on manufacturers' recommendations, personal experience, and expert opinion (**Table 3**). This is due to the fact the values depend on the device and sensors used and reveal high inter-individual variability[30,32]. Critically low values and critical changes relative to the baseline originate from experience and expert opinion.

If the oxygen supply and demand are balanced at physiological values, tissue oxygenation is within normal range. Changes in either oxygen supply or consumption cause the rSO_2 value to fall or rise (**Figure 1B,1C**). A typical curve revealing normal cerebral and renal NIRS values is displayed in **Figure 2** from the beginning until 14:25.

In the following, we provide examples to show how changes in underlying physiologic conditions affect rSO_2. During heart surgery, doctors manipulate circulation in a controlled manner - therefore the effects on rSO_2 are easy to observe. For example, clamping the descending aorta causes cerebral perfusion and the corresponding rSO_2 to rise; perfusion of the lower body results in an rSO_2 decrease (**Figure 2**). Another – non-surgical - cause of increased cerebral blood flow and elevated cerebral rSO_2 is hyperdynamic shock in conjunction with high cardiac output (**Figure 3**).

In cold shock, a dropping renal rSO_2 together with stable cerebral rSO_2 can be the first sign; a decrease in both renal and cerebral rSO_2 can occur later in the course[23]. Combined cerebral and renal NIRS can help identify early stages of shock in which cerebral perfusion is maintained at a normal level, but somatic perfusion is already impaired[23].

When using two cerebral NIRS probes, values from the right and left sides should be similar - dissonance between the right and left channel NIRS can be caused by the NIRS sensor's incomplete adhesion (**Figure 4**, red star) or indicate a complication: During some heart surgeries, the brain is perfused selectively via one carotid artery, making use of intracerebral collaterals (the circle of Willis) to supply the opposite side. Throughout this procedure, dissonance between the two cerebral NIRS channels can help diagnose a dysfunctional circle of Willis (**Figure 5**).

Another example of a complication discovered by NIRS is a dislocated vena cava superior cannula during cardiopulmonary bypass leading to venous stasis and lowered cerebral oxygen supply (**Figure 6**). The use of NIRS can help to identify impaired cerebral perfusion that would otherwise remain undetected and result in severe brain damage.

Besides heart surgery and cardiac intensive care, rSO_2 measurements can also facilitate "standard" pediatric intensive care – complications and changes in therapy can be

accompanied by changes in cerebral rSO_2 (**Figure 7**).

FIGURE AND TABLE LEGENDS:

Figure 1: Balancing the ratio between oxygen supply and demand. (**A**) Under physiologic conditions, oxygen supply and consumption are in equilibrium, and regional tissue oxygenation is within normal range. (**B**) A falling cerebral rSO_2 results from either increased oxygen consumption or decreased oxygen supply. Reasons for low or falling cerebral NIRS values are illustrated in the figure. For example, fever increases cerebral oxygen consumption by 10-13% per 1 °C increase in body temperature. Cerebral spasms can increase oxygen consumption by up to 150-250%. (**C**) An increase in cerebral rSO_2 results from reduced oxygen consumption or increased oxygen supply. Reasons for high or rising cerebral NIRS values are provided in the figure. A cerebral rSO_2 above 80%, caused by high cerebral blood flow after the loss of cerebral vascular autoregulation, is also called "luxury perfusion".

Figure 2: Evolution of cerebral and renal rSO_2 during clamp out of the descending aorta. Initially, cerebral (blue) rSO_2 is lower than renal rSO_2 (yellow) as in physiological conditions. During clamp-out of the descending aorta, cerebral blood flow increases while the lower half of the body is undersupplied. Thus, cerebral rSO_2 rises and renal rSO_2 drops. The red area indicates that renal rSO_2 values are critically low because they fell down by more than 25% below the baseline. After surgical reconstruction of the aorta and establishing normal circulation, both rSO_2 curves normalize.

Figure 3: Hyperdynamic shock. After arriving at the intensive care unit after cardiac surgery and changing respirator tubes, we experienced severe problems with mechanical ventilation (reaching only low tidal volumes at high ventilation pressures due to a defective filter). The patient developed hyperdynamic shock and respiratory acidosis with increased central venous saturation of 90% and increasing cerebral rSO_2 up to 92%. After changing the filter, fluid resuscitation, and vasopressor treatment, the patient stabilized quickly and cerebral rSO_2 normalized.

Figure 4: Evolution of NIRS values during hypothermia and deep hypothermic cardiac arrest. This figure illustrates how cerebral and renal NIRS values change under hypothermia, adjustment of cardiopulmonary bypass flow and in deep hypothermic cardiac arrest (switch surgery in a patient presenting transposed grand arteries and ventricular septal defect). The patient's baselines rSO_2 values are 59% (left, yellow) and 64% (right, blue) for the brain and 32% (green) for the left kidney. The blood supply to the lower half of the body depends on the ductus arteriosus. Intraoperatively-induced hypothermia reduces oxygen consumption, which leads to rising NIRS values, especially in the kidney. With increasing NIRS values we reduced the flow rate of cardiopulmonary bypass. Due to falling NIRS values caused by an altered metabolic situation (e.g., due to more superficial anesthesia), the flow was adjusted again. During deep hypothermic cardiac arrest, renal and cerebral rSO_2 fell to critically low values and rose again immediately after reestablishing physiological circulation. The red star with arrows shows two dips in the right cerebral NIRS curve due to incomplete probe adhesion. After gently remolding the sensor onto the skin, the values again run parallel to the left side's.

Figure 5: Dysfunctional circle of Willis during aortic arch surgery. As soon as the brain is selectively perfused via the right carotid artery (red arrow), the rSO_2 measured on the left side (dark blue) decreases because the intracerebral collaterals via the circle of Willis are insufficient. After placing an additional cannula in the left carotid artery, sufficient perfusion of both hemispheres and thus normal NIRS values are achieved.

Figure 6: Detection of upper vena cava obstruction caused by a dislocated cardiopulmonary bypass cannula. Shortly after the start of cardiopulmonary bypass (for closure of an atrial septal defect), cerebral NIRS values dropped. Troubleshooting showed that the venous cardiopulmonary bypass cannula had become dislocated, leading to occlusion of the superior vena cava and obstructed cerebral venous drainage. This caused a cerebral undersupply of oxygen, which was only detected through the low rSO_2 value. After repositioning the superior vena cava cannula, venous flow was restored and NIRS values normalized. No. 6: start cardiopulmonary bypass; No. 36 aorta clamped; No. 11 end of ischemia.

Figure 7: Changes in cerebral rSO_2 in a pediatric patient. After near drowning, this patient was put on extracorporeal membrane oxygenation. Due to side differences in arterial blood gas analyses, we put a second cerebral NIRS sensor in place. The end of muscle relaxation (**A**), change of extracorporeal membrane oxygenation system (**B**), blood pressure fluctuations (**A, C**), and the effect of a hemothorax (**C**) are reflected by changes in the NIRS curves.

Figure 8: Placing the NIRS probe over hair. (**A**) This patient has a lot of hair on the forehead. (**B**) The NIRS probe was still put in place. (**C**) The device indicates that the signal intensity is suboptimal. (**D**) The NIRS curve develops as expected during cardiac surgery (reconstruction surgery in Ebstein's anomaly). Please note that the absolute values cannot be interpreted, even if they seem normal.

Table 1: NIRS probes by manufacturer and weight range

Table 2: Typical rSO_2 values by organs and age group

Table 3: Levels of evidence of the protocol steps

DISCUSSION:

This article illustrates how cerebral and somatic NIRS is set up in infants and children. Cerebral NIRS is used for monitoring purposes during procedures such as patent duct closure, surfactant administration, heart surgery and cardiopulmonary bypass; it is also used to monitor critically ill patients in intensive care, to predict necrotizing enterocolitis (NEC) in preterm infants, and to predict outcome after hypoxic ischemic encephalopathy[2,5,6,33-40]. Further, NIRS can assist in guiding oxygen therapy in preterm infants[17-19]. Somatic NIRS helps to monitor kidney, splanchnic, and systemic perfusion[12,20,21] and may also be valuable to detect complications during or after liver transplantation[8,41,42]. The simultaneous use of multiple probes (multisite NIRS) facilitates the detection of systemic hypoperfusion[23,43].

For the NIRS measurement to function accurately, selecting the appropriate probe and

position is crucial. Vulnerable skin may require the use of non-adhesive probes (for example by leaving the cover or attaching a layer of cellophane to the sticky side). However, the entire probe must be in firm contact with the skin; otherwise, the sensors will not function properly (**Figure 4** and **Figure 8**). A bright environment causes false high and dark environment false low values if the probe is not firmly attached to the skin. In case of poor recording quality (indicated by the device) or implausible values, troubleshooting starts by checking whether the above-mentioned essential steps have been carried out. If the problem persists, the probe and preamplifier should be replaced and all electrical plug contacts checked. External light sources acting on the sensor can also trigger incorrect values; covering the probes with a light-impermeable cover will remedy this. If abnormal NIRS values persist, the patient must be examined to rule out complications. The following parameters should be assessed and optimized: arterial blood pressure, systemic oxygenation, pH, hemoglobin, cerebral oxygen return (when the patient is on cardiopulmonary bypass)[44].

For modifying the standards use, the sky is the limit: It is possible to place a NIRS probe on any site of interest provided the skin is intact. Deriving values simultaneously from several sites enables a great variety of setups according to each specific clinical or scientific question. For example, NIRS and multisite NIRS can be used outside of critical care and even during exercise[12].

Despite its ease of application and use, measuring rSO_2 has some limitations that must be considered when interpreting values and curves. The values measured depend on the device and sensors used[32]. Absolute values should therefore be interpreted with caution - reference values cannot be transferred easily between devices and setups[32]. rSO_2 values for organs other than the brain vary highly between individuals[30]. But even within one recording, values can fluctuate by up to 6% if a probe becomes detached and is then reattached[45]. Additionally, NIRS values depend on the individual's metabolic state, which is altered by interventions such as therapeutic hypothermia and medication[24].

Changes in tissue boundary conditions – for example the entry of blood or air due to surgery – also yield incorrect NIRS values[46]. In preterm infants' first days of life, the transition from meconium to regular stool alters the fecal absorption spectra and can affect the measured intestinal rSO_2 values[47]. Placing a NIRS probe over tissue other than the intended location produces inaccuracies in absolute values, but may still be helpful for monitoring trends[7].

Despite its limitations, NIRS is a good means of noninvasively and continuously monitoring the oxygenation of a specific region in real time. Alternative methods for assessing global tissue perfusion are invasive and discontinuous: arterial blood draws, serum lactate concentration, central venous saturation (SvO_2) or oxygen saturation of the jugular bulb. These can be particularly problematic in preterm infants, who frequently develop iatrogenic anemia due to repeated blood draws and whose cerebral rSO_2 is impaired during arterial blood drawing[48]. In cases of low cardiac output, during extracorporeal membrane oxygenation or when non-pulsatile cardiac assist devices are in use, NIRS still functions – in contrast to pulse oximetry – as it does not require pulsatile flow and can even selectively monitor areas at risk for hypoxia[7,49]. RSO_2 changes in these regions can serve as early signs of reduced cardiac output[7]. By these features, NIRS provides essential clinical information

that currently cannot be obtained from other measures of tissue saturation.

The scope of applying rSO_2 monitoring in neonatal and pediatric intensive care is likely to expand in the future. One potential application is monitoring cerebral hemodynamics after traumatic brain injury, which is already being investigated in adults[50-55]. In preterm infants, goal-directed O_2 supplementation may lead to better neurodevelopmental outcomes by reducing cerebral hypoxemia[17-19]. The combination of cerebral NIRS with other cerebral biomarkers may also be promising. For example, combining amplitude-integrated EEG and NIRS can help to determine prognosis in moderate hypoxic ischemic encephalopathy[56]. Possible further applications for this combination include compromised hemodynamics or seizures[23].

In summary, NIRS is a promising technology with the potential for even broader application. Correctly applied and interpreted, rSO_2 measurements help to detect complications or deteriorated clinical conditions at an early stage and guide therapy in various clinical settings. This protocol provides clinicians with the tools to set up and interpret rSO_2 measurements at different body sites, and to interpret those results.

DISCLOSURES:

The authors have nothing to disclose.

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Figure 1

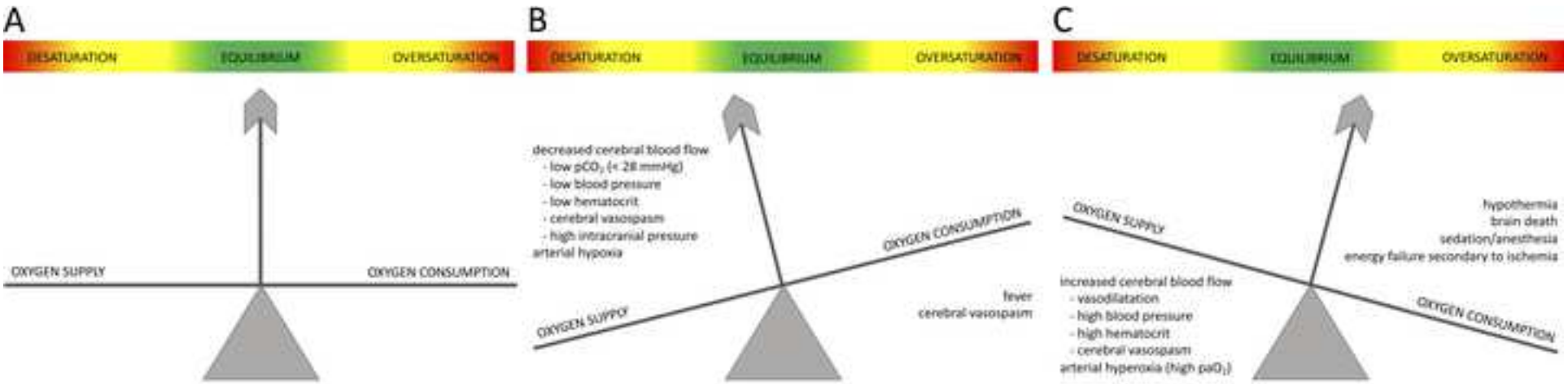

Figure 2

Click here to access/download;Figure;Revision Figure 2.tif

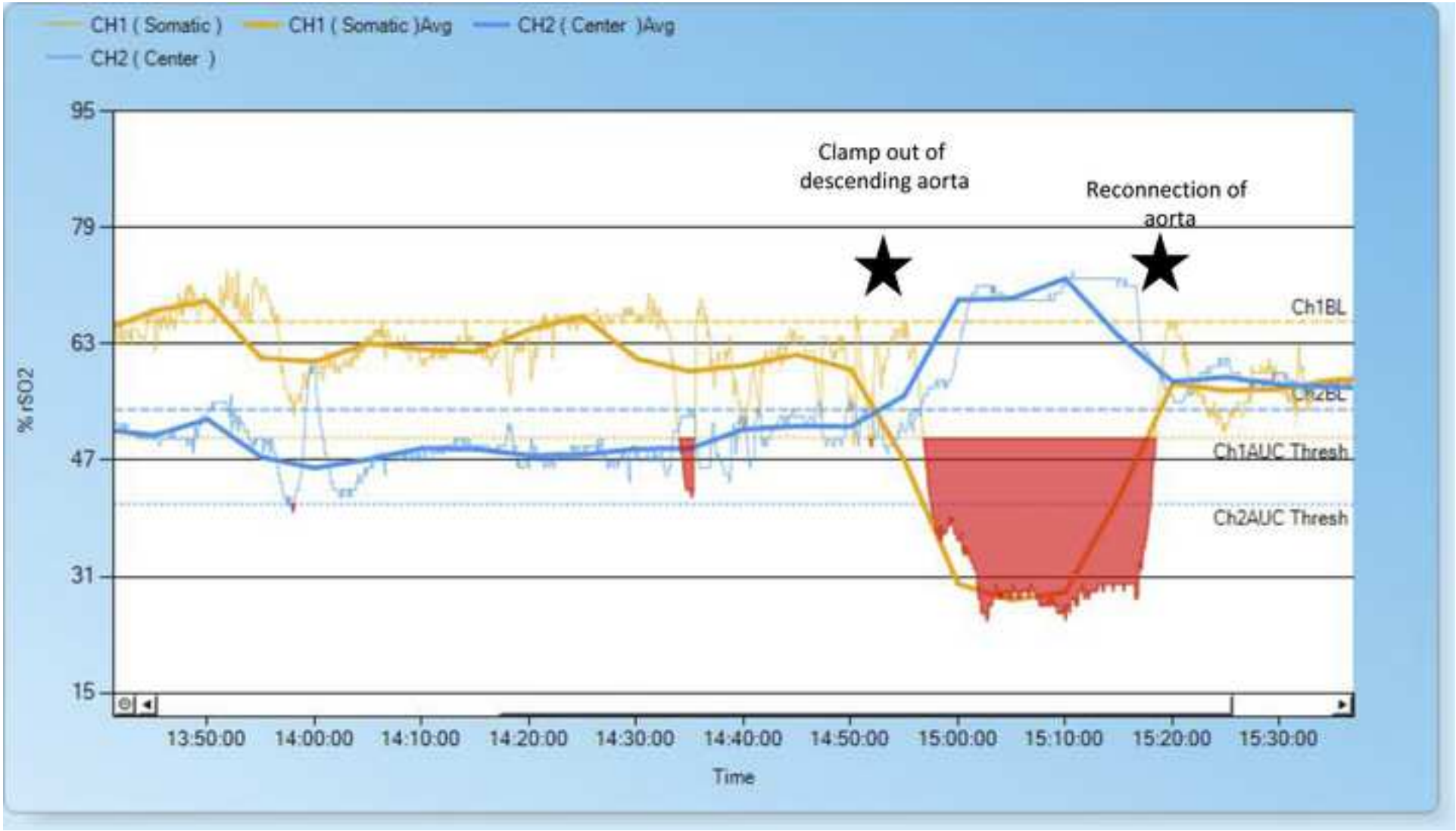

Figure 3

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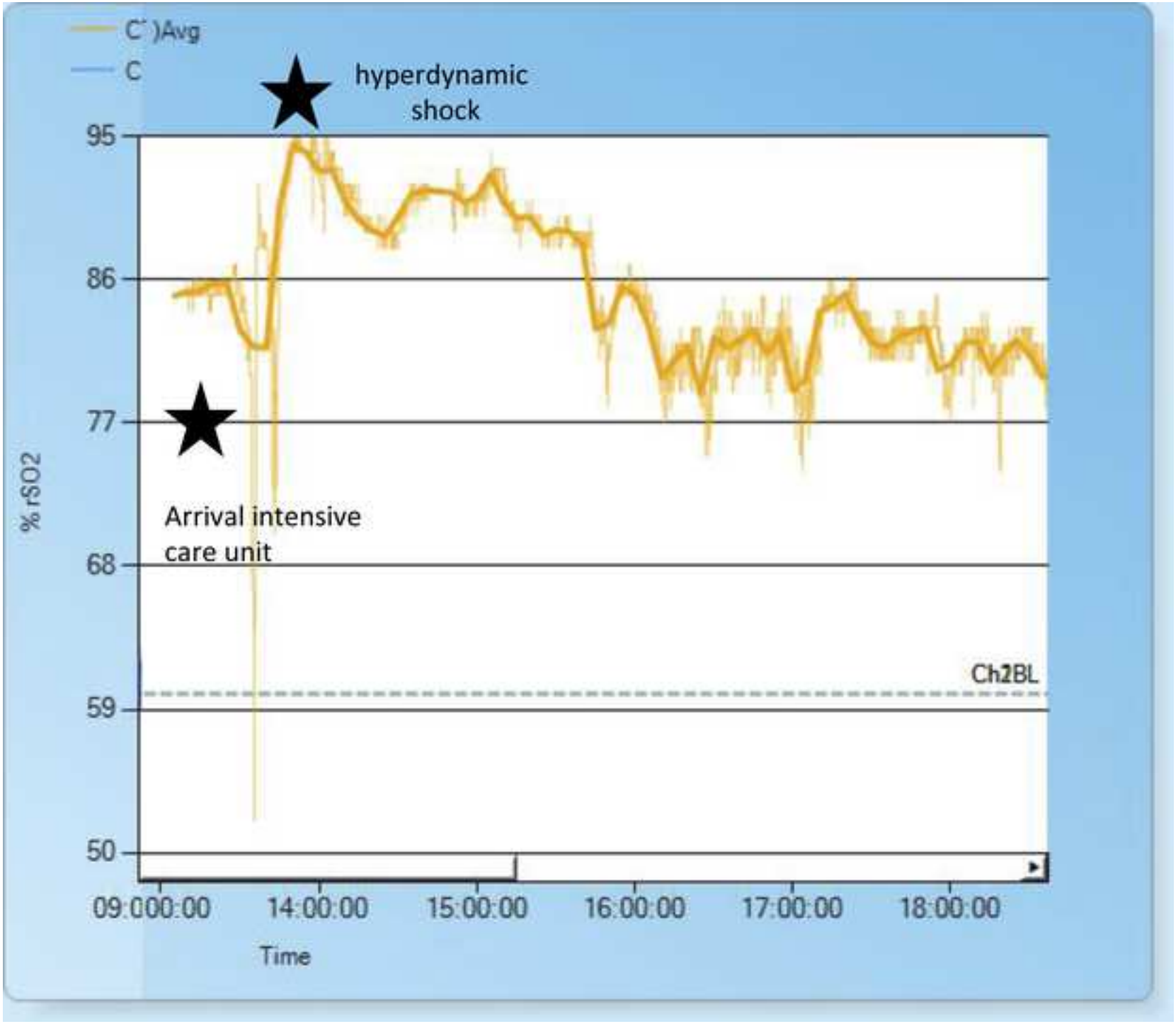

Figure 4

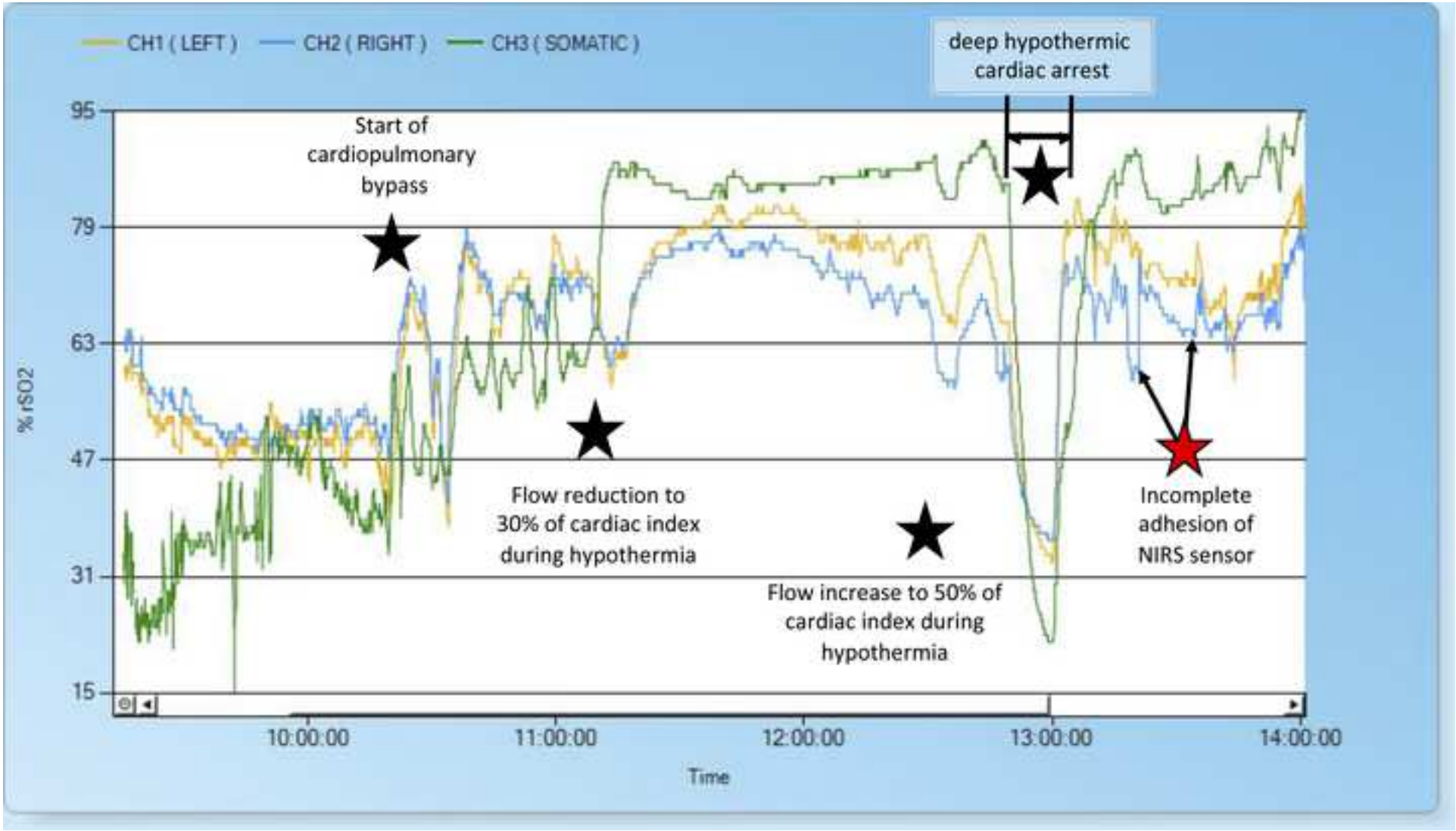

Figure 5

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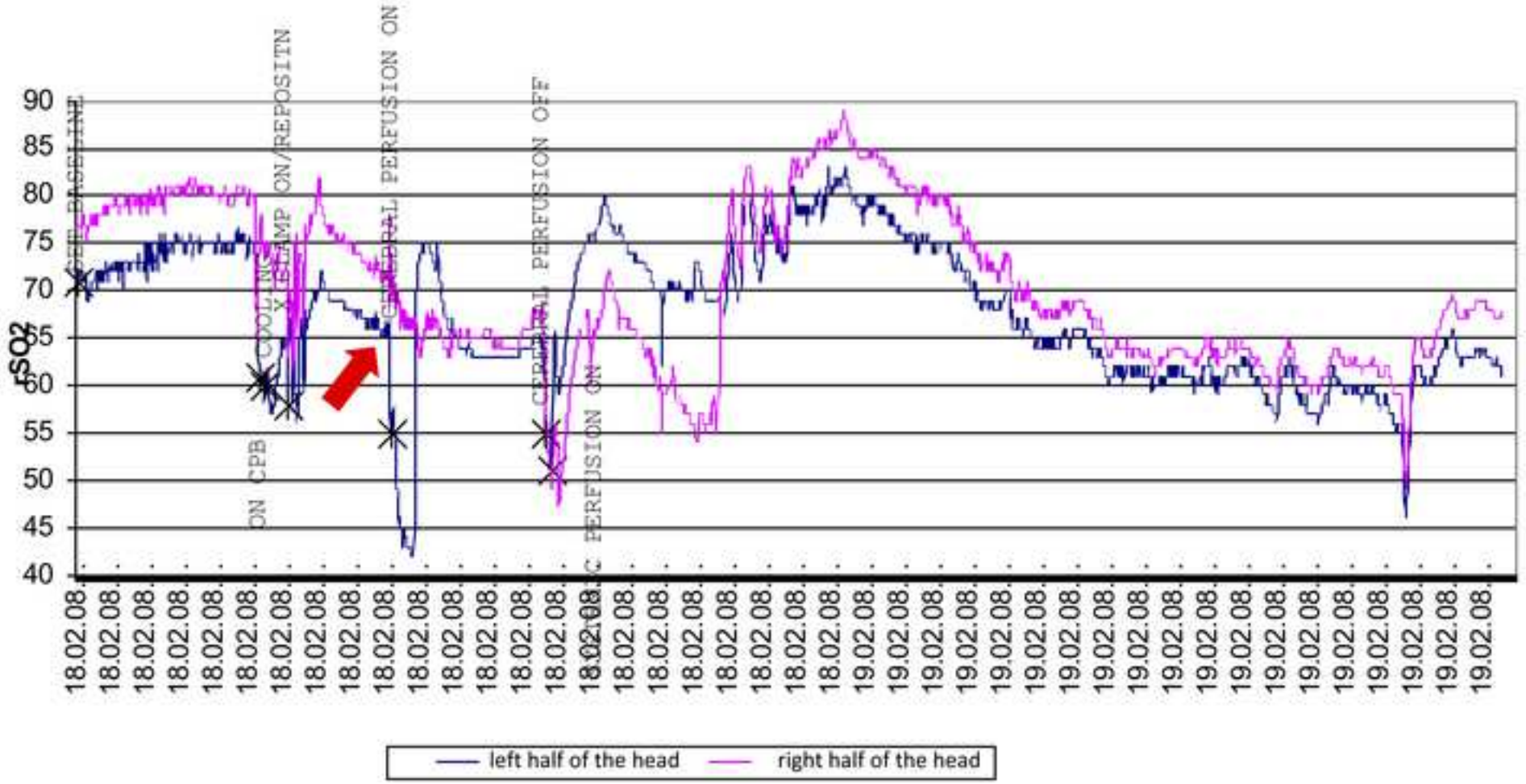

Figure 6

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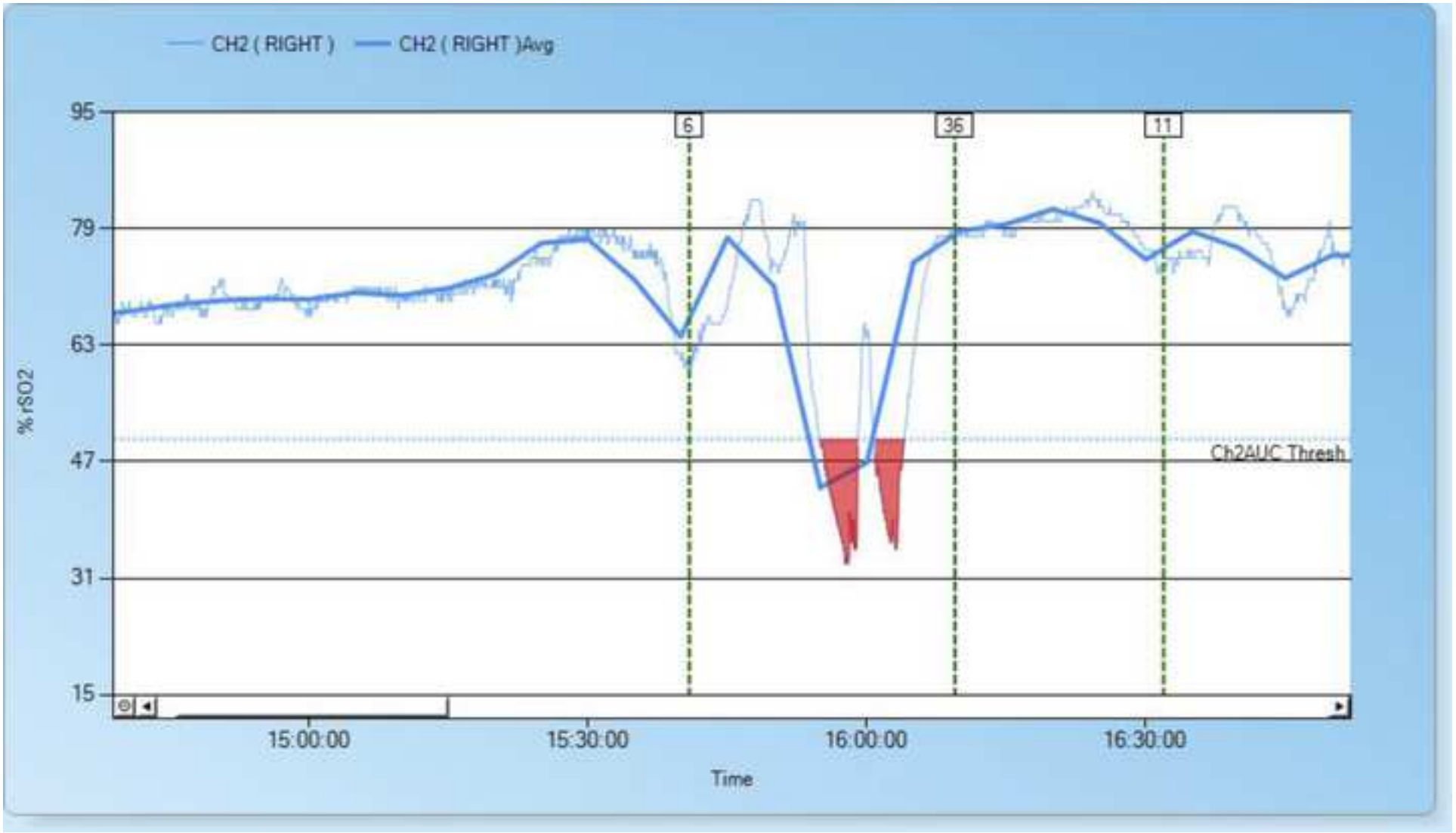

Figure 7

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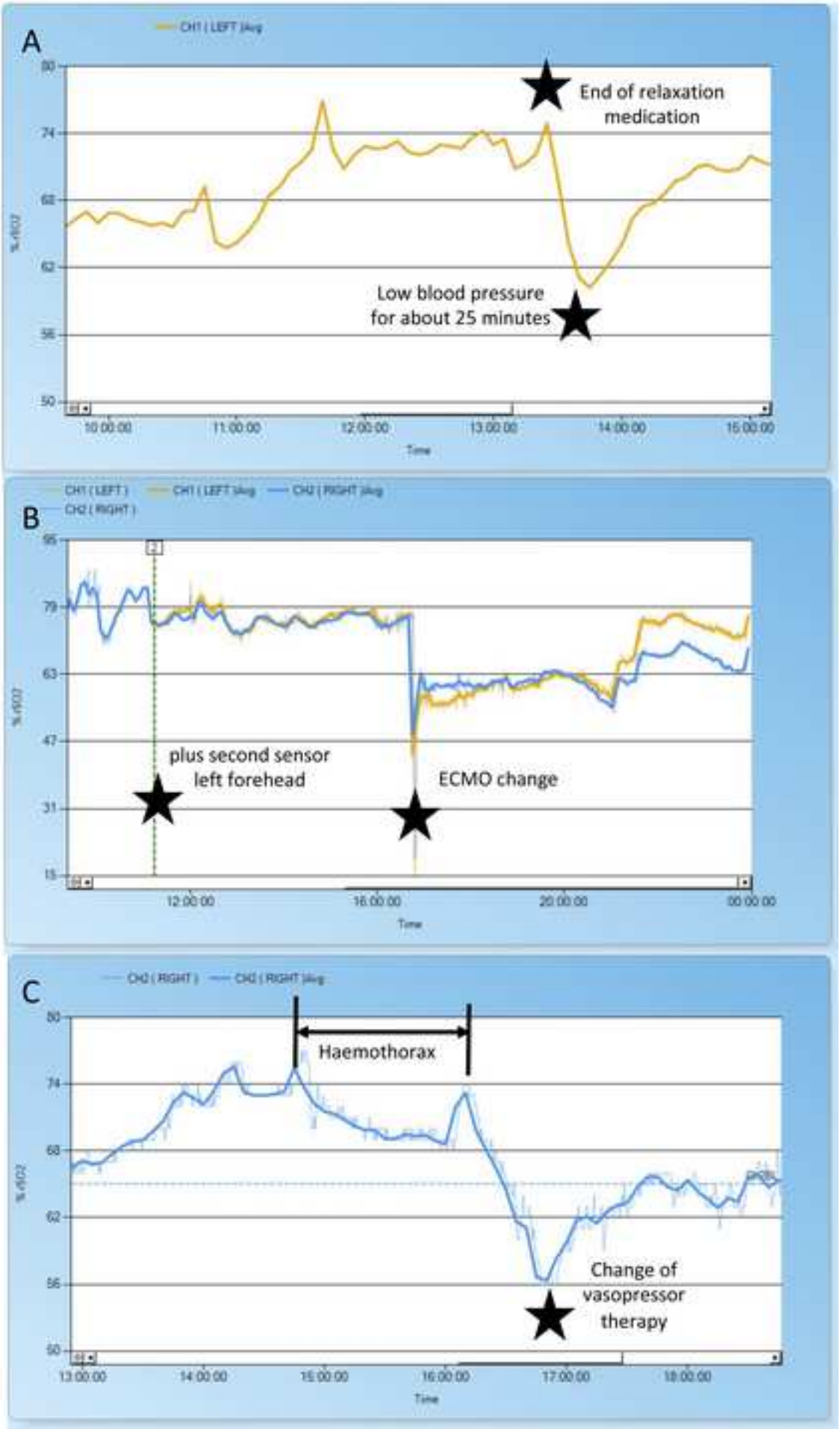

Figure 8

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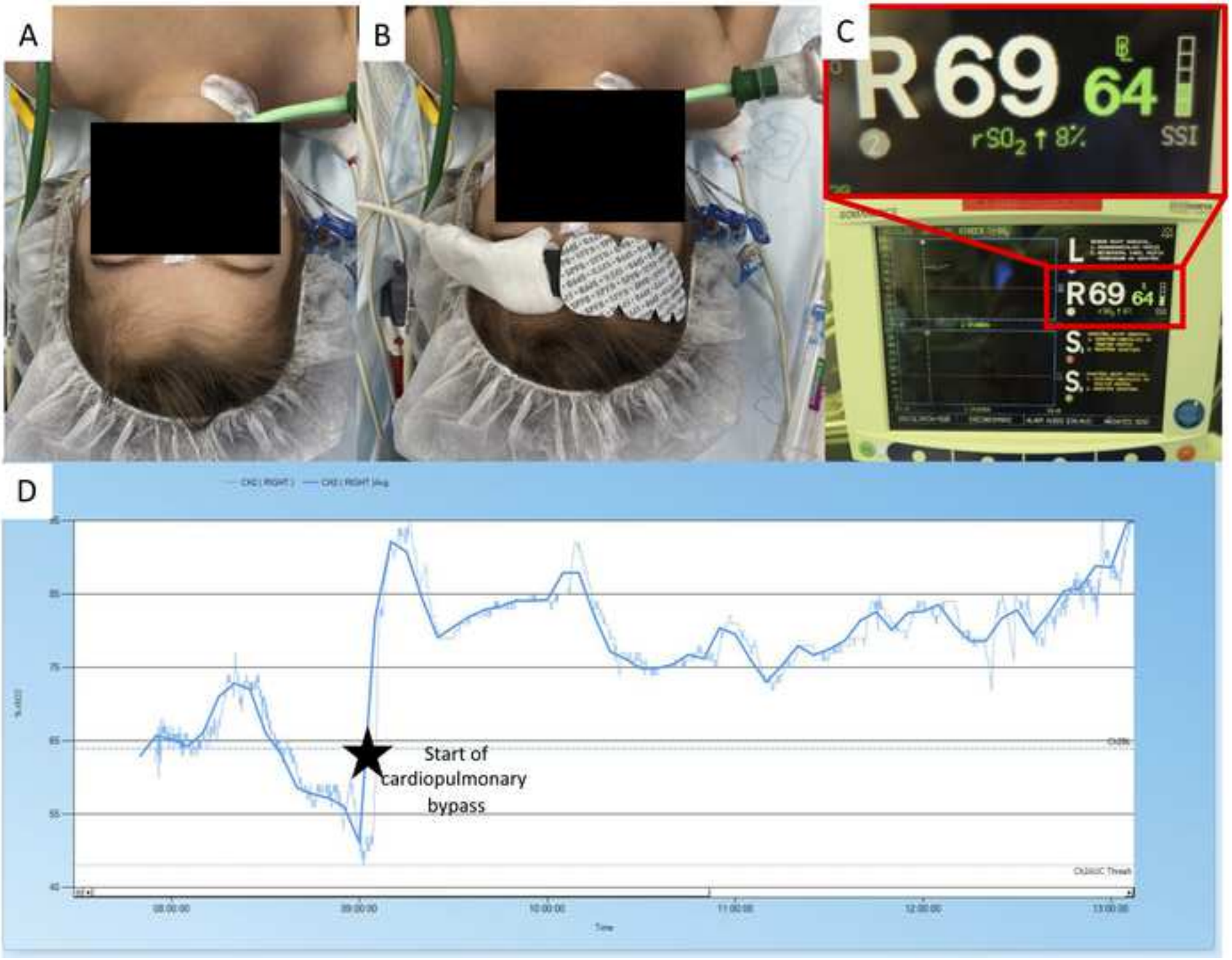

Table 1

		Age group		
Manufacturer	**Device**	**Neonates**	**Infants/Children**	**Adult**
Casmed	Fore-Sight Elite	< 8 kg	≥ 3 kg	≥ 40 kg
Masimo	Root with O3 Oximetry	< 40 kg	< 40 kg	≥ 40 kg
Medtronic	INVOS 5100C	< 5kg	5-40 kg	> 40 kg
Medtronic	INVOS 7100C	-	-	> 40 kg
Nonin	SenSmart Model X-100	< 40 kg	< 40 kg	> 40 kg

Table 2

Organ	Age group	Approximate values under physiologic conditions [%]	Critically low values [%] [E]	Critically high values [%] [E]
Brain	Preterm infants	60 – 90[26,27,30]	< 45	> 90
	Newborns	60 – 90[26,29,E]	< 45	> 80
	Infants/Children	60 – 80[26,E]	< 45	> 80
Kidneys	Preterm infants	70 – 90[28,30]	< 40	Not defined
	Newborns	80 – 95[26,29]	< 40	
	Infants/Children	Not defined, tend to be 5-15 % higher than cerebral values[26,31,E]	< 40	
Intestines	Preterm infants	18 – 80 [26,30]	Not defined	Not defined
	Newborns	55 – 80[26,29]		
	Infants/Children	Not defined, tend to be 5-15 % higher than cerebral values[26,E]		
Liver		Not defined	Not defined	Not defined
Muscle		Not defined	Not defined	Not defined

Experience/expert opinion

Absolute values depend on the device and the sensors used, on the metabolic state, and show high interindividual vari
with caution – if in doubt, the change relative to the baseline is more meaningful.

Critical relative change to baseline [%] [E]
> 25
> 25
> 25
> 25
> 25
> 25

Not defined

Not defined
Not defined

iability. They should be interpreted

Step
Cleaning the skin before placing the NIRS probe
Use of NIRS in neonates, infants and children of different ages
Use of two NIRS sensors on the forehead
Use of ultrasound to ensure correct placement of NIRS probes
Placing NIRS probe in different positions (brain, liver, intestine, kidney, foot, muscles)
Interpreting NIRS values with respect to reference values

*According to the Oxford Center of Evidence Based Medicine Evidence Levels: 1 – Systematic trials/randomized controlled trials with narrow confidence interval; 2 – Systematic reviews of cohor quality randomized controlled trials; 3 – Systematic review of case-control studies/individual case-c quality cohort and case-control studies; 5 – Expert opinion.

Level of evidence*
5
5-Jan
5
5
(1-)2-5
5-Feb

reviews of randomized controlled
t studies/individual cohort study or low
ontrol studies; 4 – Case series and poor

Name of Material/ Equipment	Company	Catalog Number
cotton swab		
INVOS (Adult Regional Saturation Sensor)	Covidien/Medtronic	SAFB-SM
INVOS (Pediatric Regional Saturation Sensor)	Covidien/Medtronic	SPFB
INVOS (preamplifier with Cable)	Covidien/Medtronic	5100C- PA (Ch 1&2)
INVOS (preamplifier with Cable)	Covidien/Medtronic	5100C- PB (Ch 3&4)
INVOS (Reusable Sensor Cable)	Covidien/Medtronic	RSC-1 - RSC-4
INVOS 5100C Monitor (Cerebral/Somatic Oximeter)	Covidien/Medtronic	5100C
INVOS Analytics Tool	Covidien/Medtronic	Version 1.2
OxyAlert NIRSensor (Cerebral/somatic -Neonatal)	Covidien/Medtronic	CNN/SNN
USB Flash Drive	Covidien/Medtronic	5100C-USB

Comments/Description

for skin cleaning

The adult regional saturation sensor Model SAFB_SM has been designed for cerebral-somatic monitoring of site-specific regional oxygen saturation (rSO_2) in adult patients > 40 kg.

The pediatric regional saturation sensor Model SPFB has been designed for cerebral-somatic monitoring of site-specific regional oxygen saturation (rSO_2) in pediatric patients < 40 kg.

Amplifier connects NIRS sensors (Canal 1&2) to monitor 5100C.

Amplifier connects NIRS sensors (Canal 3&4) to monitor 5100C.
The Reusable Sensor Cables are intended for multiple use. For use with SomaSensor SAFB-SM and SPFB.

Monitor for displaying and recording NIRS data.
Evaluation and display of "Real Time" and Case History data.

OxyAlert NIRSensors disposable sensor has a small adhesive pad with a gentle hydrocolloid adhesive for use with peadiatric, infant an neonatal patientes. Suitable for patients <5kg.
Collects and transfers Date to INVOS Analytics Tool

Dear Editors, dear Reviewers,

We deeply appreciate your critical input and the opportunity to improve our manuscript entitled " How to apply and use near-infrared spectroscopy in critically ill neonates, infants, and children." Changes to the original manuscript are highlighted in yellow.
All comments are addressed in the point-to-point fashion below:

Editorial Comments:

• Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.
We've thoroughly proofread and spellchecked the manuscript.

• Avoid use of the pronoun "your" throughout.
We've eliminated the pronoun "your" throughout the manuscript.

• Protocol Detail:
1) Please include an ethics statement before your numbered protocol steps indicating that the protocol follows the guidelines of your institutions human research ethics committee.
We've included the statement as requested.

2) 2.2: Is there a specific skin site? 3) Should section 3 be before section 2?
The probe can be placed in many skin sites, as described in section 3. When first writing the protocol, we also considered interchanging sections 2 and 3 or merging them. However, we decided to keep the original order, because the technique of applying the probe is basic information and is independent of where the probe is placed. The idea was to explain how to apply it to the skin in general and then focus on the different skin sites in section 3.

• Discussion: JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form (3-6 paragraphs): 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.
We've ensured that all required paragraphs are now included, and have added subheadings to the discussion.

• If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-

print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."

We've removed figure 5 from the manuscript, as it was taken from a CC-BY 4.0 license publication. The publisher agreed, but the authors did not give their final approval.

Comments from Peer-Reviewers:

Reviewer #1:

Manuscript Summary:

The aim of this manuscript was to provide a protocol to support clinicians to correctly apply NIRS at different body sites in critically ill infants and children. This is an interesting topic. There are, however, some adjustments needed before the manuscript can be considered for publication. My major concern is the lack of evidence that is provided by the authors to support the protocol. I think it is very important to provide an adequate level of scientific evidence when a protocol is proposed for publication to support clinicians. Furthermore, the authors should consider to correct the English language by a native speaker. My major and minor comments are listed below.

Major Concerns:

COMMENT 1

In my opinion, the introduction is quite long and lacks some focus. The authors should focus on what is NIRS, how can it be used, and why is it necessary to provide a protocol. At this moment, the structure is lacking while a lot of information is provided, but the main massage is not clearly presented.

We've shortened the introduction considerably, focusing on the points mentioned above.

COMMENT 2

In the introduction the authors state that this manuscript provides a protocol for the use of NIRS in infants and children. However, throughout the manuscript it is unclear whether the suggested guidelines concern infants or children (of what age?), or both. Next, in the discussion the authors state again that this is a protocol for neonates and pediatrics wards. In my opinion the authors should provide information separately for the different ages, for example neonates, young children and older children. Furthermore, I think it is important to include information on which evidence and which level of evidence the suggested steps in the protocol are based. I would suggest to write a protocol per step including the scientific evidence for each step. For example, the authors state that cleaning the skin before the placement of the NIRS probe is important for a proper measurement, but perhaps in cases of a vulnerable skin, cleaning the skin might result in damage to the skin.

The protocol applies to patients of any age - We've added a corresponding statement at the beginning of the protocol and added limitations regarding special age groups as a note in the protocol steps.
Evidence of the protocol: We illustrate our in-house protocol for NIRS, which represents the current state-of-the-art in perioperative and intensive care monitoring. This is included in the statement at the beginning of the protocol. The different steps about how the probe is applied to the skin correspond to the manufacturer's instructions. We've added some additional information in the notes that derives from our experience and was also suggested by the other reviewers.
The remaining steps in the protocol mainly explain how to identify the external body site to monitor the tissue of interest. The cited references explain how body sites were selected for conducting the studies. We gained this information by reviewing the literature and combined it with our own experience. As far as we understand, the protocol section is not supposed to contain references. For that reason we've added a table to the representative results section that presents evidence levels for each step (table 3).
On the concerns with skin preparation: We deliberately did not write "clean the skin", but "make sure the skin is clean and dry" to contribute to the fact that if the skin does not look dirty, it need not be additionally cleaned. To make this point very clear, we've added a sentence to step 1.3. and extended the note for step 2.

COMMENT 3

The protocol paragraph 1 preparation: 1.2 reads Select the proper probe according to Your patient's weight. I would suggest to add which probe should be used in which range of body weights, for example via an overview presented in a table.

Information on weight is given on the package and differs between manufacturers. We've added that information to the protocol step and added a table containing the probes of common manufacturers (Table 1).

COMMENT 4

The message of the representative results is not clear to me. It would in my opinion be better to provide an overview of the assumed range of normal values for the cerebral, renal, intestinal and hepatic measurements and possibly also for the muscles and other described tissues in paragraph 2 and 3 of the protocol, keeping in mind that normal values are not yet available. This way it becomes more clear what a certain measurements means and what a decrease or increase of the rSO2 of the specific tissue means. Next, I would suggest to add a new paragraph in which the different examples will be described.

There are no existing reference values for rSO2 for children. However, we've added a table summarizing published values and values derived from experience/expert opinion, giving information about the source for each value (Table 2). We've also revised Figure 2 (now Figures 1A-C) to enhance visualization of the changes in rSO_2.

Minor Concerns:

COMMENT 5

In paragraph 3.1 the authors mention the option to place to probes on the forehead, one at each side. It is however not yet scientifically proven that this is of added value. And in small children it is not clear yet of the probes might influence one another if applied close to each other. I would suggest to add a note addressing the lack of evidence of the use of two sensors on the forehead should be added.

The probes emit and measure signals alternately to avoid interference from neighboring probes. We've added this information, along with a comment that the two probes are only required in selected clinical settings.

COMMENT 6

In the introduction the abbreviation cNIRS is not fully written out before using the abbreviation. Do the authors mean cerebral NIRS measurements? Maybe consider to use the cerebral regional oxygen saturation (rcSO2) instead of using cNIRS.

We've edited the document by writing out the word cerebral and changing NIRS to rSO_2 where it seemed appropriate.

COMMENT 7

There are situations in which peeling off the cover of the probe may damage the skin, for example in preterm infant or in situations that children are having a very vulnerable skin. There is no mention of other methods to apply the probe without peeling off the cover to prevent the sticky surface harming the skin.

We've added information about not peeling the cover off or using cling film when applying the probe on vulnerable and immature skin to the note in the corresponding section of the protocol and discussion.

COMMENT 8

Paragraph 3.2 Somatic reads: For renal or hepatic NIRS, use ultrasound to ensure the correct placement. I think that it might not always be possible to perform an ultrasound (or a sagittal sonogram). Although an ultrasound and a sonogram can be performed to verify the correct placement of the probe to measure the renal or hepatic rSO2, the non-invasive character of the NIRS measurement, in my opinion, is lost, particularly in the very small infants, while this non-invasive character is one of the advantages of NIRS. When the authors decide to mention the ultrasound and sonogram to ensure correct placement, I would suggest to present this as an extra option instead of that is should be used I would also suggest to add the scientific evidence and to add a note that this may be quite invasive.

We've adjusted the sentence about the use of ultrasound so that it is a recommendation and added a note that it may interfere with minimal handling e.g. in very preterm infants. However, with some experience, identifying the kidneys or liver by ultrasound takes little time and ensures that the measured values really do derive from the intended region.

COMMENT 9

They recommend applying NIRS over the right kidney, as it is located slightly further caudal than the left kidney. What is the evidence to place the probe on the right side to measure the renal oxygen saturation? Is it possible that one would measure the liver oxygen saturation instead of the renal oxygen saturation by placing the probe at the right side? As the liver is also located on the right.

We use ultrasound to confirm the positioning of the probe. The right kidney is easier to locate via ultrasound. If ultrasound isn't applied, it may make sense to use the left kidney as well. We've eliminated that sentence from the protocol.

COMMENT 10

In the "representative results" they state that the cerebral rSO2 is lower than the somatic tissue in physiological conditions. Do they mean in older children? In neonates the cerebral rSO2 is often higher than intestinal tissue, but the renal rSO2 can be higher than the cerebral rSO2. As the intestinal, renal en liver rSO2 values show different rSO2 patterns. I would suggest to separately mention the different tissues rather than using the term somatic throughout the representative results.

We've adjusted the terms as suggested to avoid confusion.

Reviewer #2:

Abstract: This makes NIRS an ideal tool for real-time monitoring of patients during cardiopulmonary bypass (CPB) and other situations with potential compromise of tissue oxygenation. It seems sensible to move this to later in the abstract to precede A routine clinical …

Where did the adult come from?

We've rearranged the abstract as suggested to be more logical. The adult was mentioned to emphasize that NIRS is used in all age groups including adults. However, we've deleted it to make our content more harmonious.

Introduction: Normal values for cerebral NIRS values have been reported in Preterm infants. Pediatric Research volume 79, pages55-64(2016), for instance

We've added a table to provide an overview of existing normal values for each age group and region (Table 1).

Protocol: Why is Your with a capital letter?

As requested by the editorial team, we've removed "you" and "yours" throughout the manuscript.

You can place a sensor on very preterm skin, just do not remove de adhesive barrier and use a bandage for support

We've added that information as a note.

Discussion: The method of positioning was described earlier, it seems odd to repeat is in the discussion. Also the bad recording, perhaps add that to the protocol? What to do when you do not trust the results.
JoVE requires the discussion section to contain critical steps and trouble shooting. We've shortened and edited this part. As suggested, we've added that as a section in the protocol (Section 5).

Figures: The relation between CBF and desaturations is unclear. Lower CBF will lead to a desaturation, in this picture it can be interpreted that a higher CBF will lead to desaturation.
We've revised figure 2 (now figures 1A-C) to make it easier to understand.

Reviewer #3:
Major Concerns:
1. Please condense the introduction section. It's too long.
We've shortened the introduction considerably.

2. When placing the probe, it is important to ensure that the probe is firmly connected to the skin. If the probe disconnects later, you will get wrong NIRS values.
We've emphasized this in section 2.2 and in the discussion.

Minor Concerns:
1. Please give the full name of cNIRS
We've edited that term throughout the manuscript.

We hope that the changes we made address all concerns.

With kind regards

Dr. Nora Bruns (corresponding author)
Department of Pediatrics I
University Hospital Essen
University of Duisburg-Essen
Hufelandstr. 55
45147 Essen, Germany
Nora.bruns@uk-essen.de