

# Optical monitoring of tissue viability parameters *in vivo*: from experimental animals to clinical applications

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**Abstract** Optical monitoring of tissue physiological and biochemical parameters in real-time is a new approach and a powerful tool for better clinical diagnosis and treatment. Most of the devices available for monitoring patients in critical conditions provide information on body respiratory and hemodynamic functions. Currently, monitoring of patients at the cellular and tissue level is very rare. Real-time monitoring of mitochondrial nicotinamide adenine dinucleotide (NADH) as an indicator of intra-cellular oxygen levels started 50 years ago. Mitochondrial dysfunction was recognized as a key element in the pathogenesis of various illnesses. We developed the “CritiView” — a revolutionary patient monitoring system providing real time data on mitochondrial function as well as microcirculatory blood flow, hemoglobin oxygenation as well as tissue reflectance.

We hypothesize that under the development of body O<sub>2</sub> insufficiency the well known blood flow redistribution mechanism will protect the most vital organs (brain and heart) by increasing blood flow while the less vital organs (gastrointestinal (GI) tract or urogenital system) will become hypoperfused and O<sub>2</sub> delivery will diminish. Therefore, the less vital organs will be the initial responders to O<sub>2</sub> imbalances and the last to recover after the end of resuscitation. The urethral wall represents a less-vital organ in the body and may be very sensitive to the development of emergency situations in patients. It is assumed that the beginning of deterioration processes (i.e., internal bleeding) as well as resuscitation end-points in critically ill patients will be detected.

In this paper, we review the theoretical, technological, experimental and preliminary clinical results accumulated using the “CritiView”. Preliminary clinical studies suggest that our monitoring approach is practical in collecting data

from the urethral wall in critical care medicine. Using CritiView in critical care medicine may shed new light on body O<sub>2</sub> balance and the development of body emergency metabolic state.

**Keywords** patients monitoring, tissue vitality, mitochondrial dysfunction, tissue blood flow, critical care medicine

## 1 Introduction

The need for a practical and simple medical device for *in vivo* monitoring of tissue physiology in real time had stimulated many scientists as well as various medical device companies. It is very clear that critical care medicine is the largest market that needs intensive monitoring in order to improve the quality of the treatment given to patients. Monitoring of organ and tissue physiology is very important and significant in patients hospitalized in various types of operating rooms (ORs) as well as in the various intensive care units (ICUs).

Patients undergoing severe operations or hospitalized in the various ICUs post-operatively are exposed to changes in systemic or peripheral hemodynamic [1]. In order to optimize the physiological condition of the patient, during the surgical procedure as well as during the early post operative period careful additional monitoring of the patient, at the tissue level, is required. Recently, Ospina-Tascón et al. [2] reviewed the literature regarding a very elementary question in patient monitoring. The intensivists that are treating the patients in the ORs and ICUs are looking for new monitoring devices that will provide real time data on the oxygen balance at the tissue level in patients [3].

Currently, most of the developed medical devices provide information on one or two parameters representing tissue physiology. A few companies are distributing laser Doppler flowmeters (tissue blood flow) [4,5], tissue

hemoglobin saturation levels ( $\text{HbO}_2$ ) [6], tissue oxygen level ( $\text{pO}_2$ ) [7], tissue  $\text{CO}_2$  and pH levels [8], and mitochondrial cytochrome oxidase redox state [9]. Nevertheless, none of the mentioned techniques is used in daily clinical practice in any medical field. Pulse oximeters are widely used in many clinical applications but the provided information represents the function of the respiratory and the cardiovascular system as reflected in the level of  $\text{HbO}_2$  saturation level.

The new device to be described in this paper, the CritiView, was developed in order to overcome all the disadvantages of the early developed devices for tissue and organ level monitoring. The CritiView was adapted to a continuous and real time single point measurement in critical care medicine including cardiovascular and other ORs and in the ICUs.

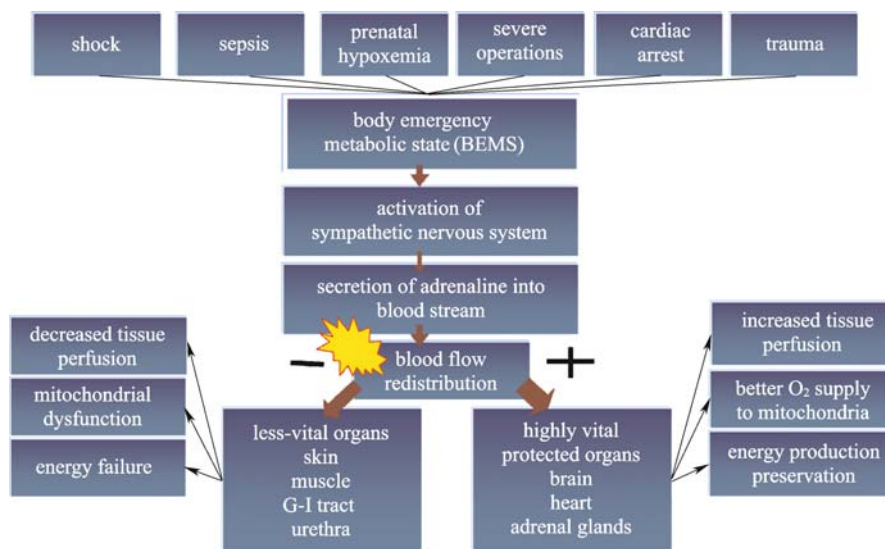
Pathophysiological events develop in many emergency clinical situations such as shock, hypoxemia, sepsis, and cardiac arrest, shown in Fig. 1, are mostly involved with the deterioration of body metabolic state, negative oxygen balance and energy failure. As a compensatory mechanism, the sympathetic pathways are activated, via the secretion of epinephrine and norepinephrine (NE), leading to blood flow redistribution in the body in such a manner that blood flow to the most vital organs (brain, heart and adrenal medulla) will increase whereas, blood flow to “less vital” organs (gastrointestinal tract, skin, kidney or urethral wall) will decrease. The brain, which is considered as the most vital organ, is highly affected by NE that modulates the response of the cerebral cortex to increase in brain metabolic demand.

Since catecholamines influence the cerebral blood flow (CBF) they also have an influence on the energetic state of the brain. As was observed, NE administration caused the

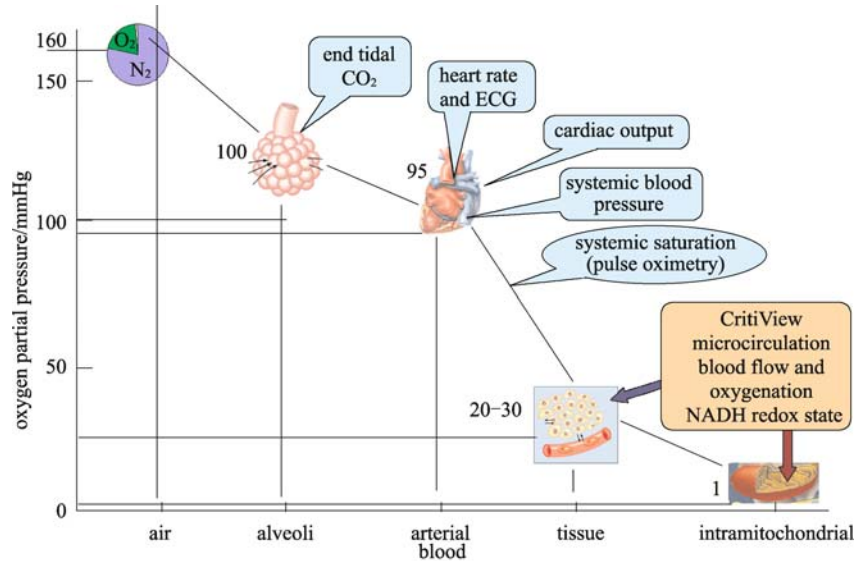
oxidation of nicotinamide adenine dinucleotide (NADH) [10] and a change in the rate of oxidative metabolism activity [11]. In 1962, Chance et al. [12] showed that NADH is the most sensitive component, in the respiratory chain, to tissue oxygen levels. Since then several studies used the fluorometric monitoring technique to evaluate renal energetic state [13–15]. It was demonstrated that NE increases microvasculature resistance and decreases vascular volume in a dose dependent manner in the liver [16,17].

Kraut et al. in 2004 [18] used the multiorgan monitoring approach (including vital and “less-vital” organs) in real time following NE administration in a rat model. By using NE injection in a rat, they were able to evaluate the hemodynamic and metabolic state of four different organs simultaneously. They estimated the differences in the responses of the brain, a vital organ and “less-vital” organs such as the kidney, liver and testis.

Mitochondrial function is a prerequisite condition for the continuous supply of energy in order to perform all cellular functions in the body. Most of the oxygen consumed in the body is utilized by the mitochondria [19], which produces 95% of the adenosine triphosphate (ATP) in normal tissues. As shown in Fig. 2, the levels of  $\text{pO}_2$  needed for normal mitochondrial function is around 1 mmHg. The exact level of  $\text{pO}_2$  that exists in the mitochondria is not known due to the lack of a direct monitoring technique for *in vivo* measurement. The  $\text{pO}_2$  levels at the other body locations, shown in Fig. 2, are well known and documented as shown by the average values. The determination of  $\text{pO}_2$  at the tissue levels is practical by using an appropriate oxygen electrode, representing the average levels of  $\text{O}_2$  in the intravascular, extracellular and intracellular compartments.



**Fig. 1** Various clinical pathological conditions that may lead to development of body emergency metabolic state (BEMS) in critically ill patients. These events may stimulate the mechanism of body blood flow redistribution that will provide more oxygen to the most vital organs in the body



**Fig. 2** Oxygen gradient in the body starting at the highest level in lung (100 mmHg) and the lowest levels in mitochondria (about 1 mmHg). The optical measurements of four parameters done by CitiView represent events occurring in the microcirculation level as well as in the intracellular space. (ECG: electrocardiogram)

The best approach to evaluate intracellular or intramitochondrial  $O_2$  levels is to measure the spectroscopic nature of the various components of the respiratory chain [20]. In this publication, Chance concluded that “For a system in equilibrium, NADH is at the extreme low potential end of the chain, and this may be the oxygen indicator of choice in mitochondrial and tissue as well.” The same conclusion was published by Lübbers stating “The most important intrinsic luminescence indicator is NADH, an enzyme of which the reaction is connected with tissue respiration and energy metabolism” [8]. The pioneering work by Chance and Williams [21] opened up an era of 50 years of intensive research dealing with mitochondrial function *in vivo*. The evaluation, *in vitro* as well as *in vivo* conditions, was done by monitoring NADH redox state using the auto-fluorescence approach [12]. Although the importance of normal mitochondrial function in various tissues is appreciated [9,22], the research effort done in this important field was not significant. During the last 30 years, our group had published about 150 articles in this important subject. We found that monitoring of mitochondrial NADH fluorescence alone is very practical but it is not enough to understand the metabolic state of the tissue. We therefore developed the multiparametric monitoring approach to evaluate the functional state of various tissues or organs in the body [23,24]. The other parameters that could be monitored provide information originating mainly from the vascular compartment. For this reason the correlation between those three parameters maybe significant under certain conditions such as ischemia (decrease in blood supply to the tissue). The decrease in

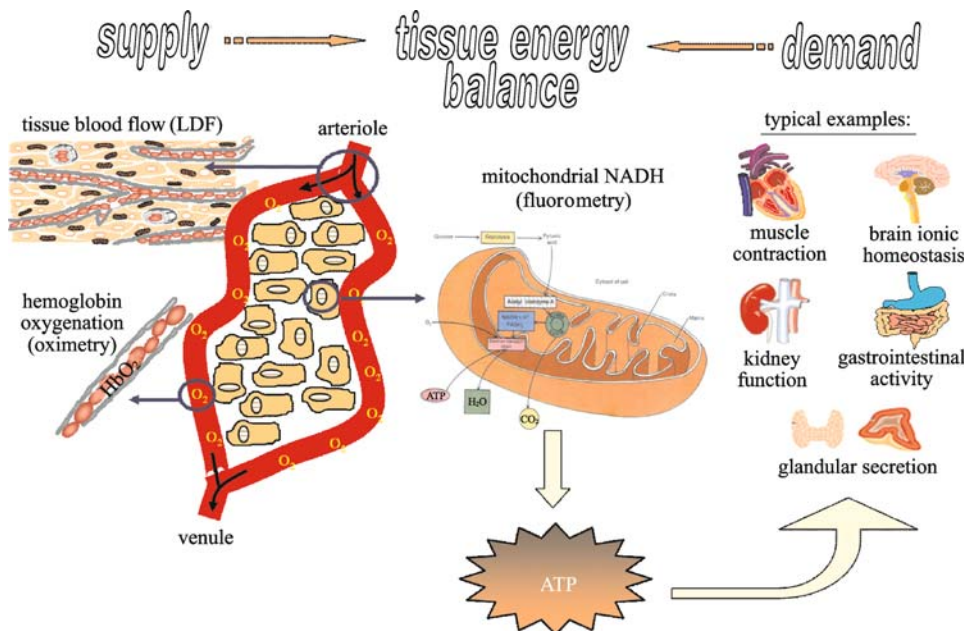
blood to the tissue (i.e., ischemia) will be accompanied by a decrease in  $HbO_2$  as was in blood volume (increase reflectance). The intramitochondrial NADH will show a significant increase as published earlier [25–27]. The contribution of  $HbO_2$  monitoring to the understanding of tissue energy metabolism is not clear since it was not monitored intensively with the other three parameters namely, blood flow, tissue reflectance and mitochondrial NADH [6,28,29].

Figure 3 presents in a schematic way the balance between energy or  $O_2$  supply and demand in a typical tissue. The supply of  $O_2$  is evaluated by monitoring of the microcirculatory blood flow using laser Doppler flowmetry as well as the level of hemoglobin oxygenation at the microvasculature. The evaluation of  $O_2$  demand was not done directly in the present study but  $O_2$  balance was evaluated by monitoring of mitochondrial function using the NADH fluorometry/reflectometry approach. All this three monitored parameters could be measured in all tissues or organs in the body and therefore could be used routinely in research as well as in clinical practice.

## 2 Material and methods

In our previous communication [30], we presented Tissue Spectroscopy (TiSpec-3) and CitiView [23,30,31], which were applied in critical care medicine.

The new device named CitiView is based on the principles of monitoring the four parameters as shown in Fig. 4 and described below.



**Fig. 3** Relationship between oxygen supply and demand. The supply of oxygen is measured by tissue blood flow and microcirculatory hemoglobin saturation. Tissue oxygen balance is evaluated by the integrity of mitochondrial function measured by the fluorescence of NADH *in vivo*

### 2.1 Mitochondrial function (A)

Mitochondrial function is evaluated by monitoring of NADH fluorescence. The measurement is done by excitation of the tissue by ultraviolet (UV) light (375 nm) and measuring the emitted fluorescence signal (420–480 nm). The principles of this method was established 50 years ago [12,21] and the main changes that were introduced since then were the type of light source used as well as the usage of a bundle of optical fibers to connect the tissue to the fluorometer. The main light source that was used was an Hg arc lamp having a strong emitted light at 366 nm absorbed by the reduced form, NADH and not by the oxidized form ( $NAD^+$ ). The introduction of 375 nm light emitting diode (LED) by Nichia as a new light source in the present instrument (CritiView) affects significantly the size and the price of this component in this medical device.

The use of optical fibers led to a more flexible connection between the monitored tissue and the fluorometer. This enabled us to monitor the unanesthetized brain [20,25] or at different locations in the same animal [18,32].

### 2.2 Tissue blood flow (B)

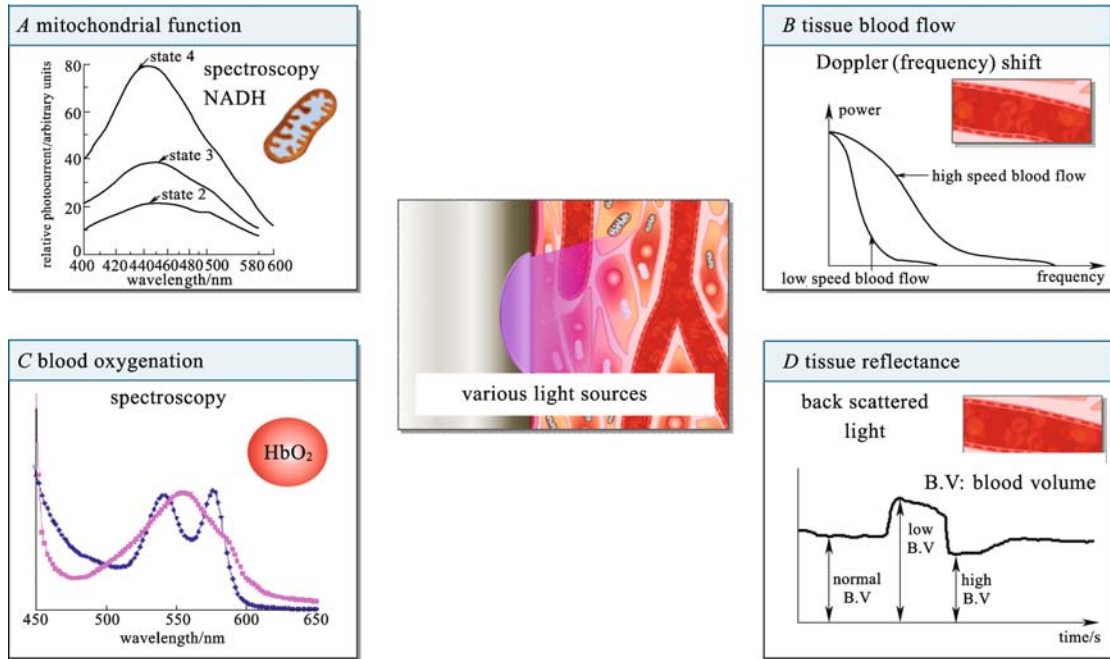
Monitoring of microcirculatory tissue blood flow using the laser Doppler flowmetry (LDF) approach started more than 30 years ago [33,34] and is widely used in

various animal models as well as in clinical applications [4]. The main advantage of the LDF is the real time data provided by the optical nature of the signal. Currently, the large number of publications (more than 5000) in experimental animals and patients indicate its applicability [35].

### 2.3 Microcirculatory blood oxygenation (C)

For the monitoring of blood oxygenation we utilized a two-wavelength reflectance technique to analyze the absorption of light in the blood, exploiting the difference in absorption spectra of oxyhemoglobin (hemoglobin saturated with oxygen) and deoxyhemoglobin. The principles of the technique were demonstrated years ago using a time-sharing system [6]. Generally, the absorption coefficient of oxyhemoglobin differs from that of deoxyhemoglobin, along most of the spectrum<sup>1)</sup>. However, in some certain isosbestic wavelength, the absorption curves cross each other and represent the same value. The change in reflectance in an isosbestic wavelength is affected only by blood volume, while a change in reflectance in a non-isosbestic wavelength is affected by the oxy-deoxy ratio as well. By subtracting the back-reflectance in these two wavelengths, one can consider the difference between the measurements as qualitative representation of blood oxygenation at the microcirculatory level. The previous time-sharing system

1) Prahl S. Optical absorption of hemoglobin. 1999, <http://omlc.bme.ogi.edu/spectra/hemoglobin/index.html>



**Fig. 4** Principles of tissue vitality monitoring by multiparametric monitoring system — CritiView. Three of these parameters (*B*, *C*, and *D*) are monitored from the intravascular compartment, while the NADH parameter (*A*) indicates the function of the intracellular organelle — mitochondrion

[6,35] used two Hg-lamp emission bands to determine the oxy-deoxy ratio *in vivo* by measuring the reflectance in 585 nm ( $R_{585}$ ) and 577 nm ( $R_{577}$ ). Calculating  $-(R_{577} - R_{585})$  yields the relative change in oxyhemoglobin state. In the present study, we used the same principle with contemporary 530 nm (nearly isosbestic) and 470 nm (non-isosbestic) super bright LEDs. The relative change in oxyhemoglobin state is therefore derived by  $-(R_{470} - R_{530})$ .

#### 2.4 Tissue reflectance (*D*)

The continuous changes in tissue absorption properties affect the intensity of tissue reflectance and fluorescence. It was found that reflectance could be used as a correction tool for the hemodynamic artifacts measured in the NADH fluorescence signal [20,25,32]. The main factor affecting the reflectance (*R*) signal changes by blood volume [6,23,25,36]. When ischemia is induced, the decrease in blood volume will lead to an increase in the *R* signal. During the recovery phase from ischemia the hyperemic response (increase blood volume) will be recorded as a decrease in the *R* signal.

#### 2.5 Device description

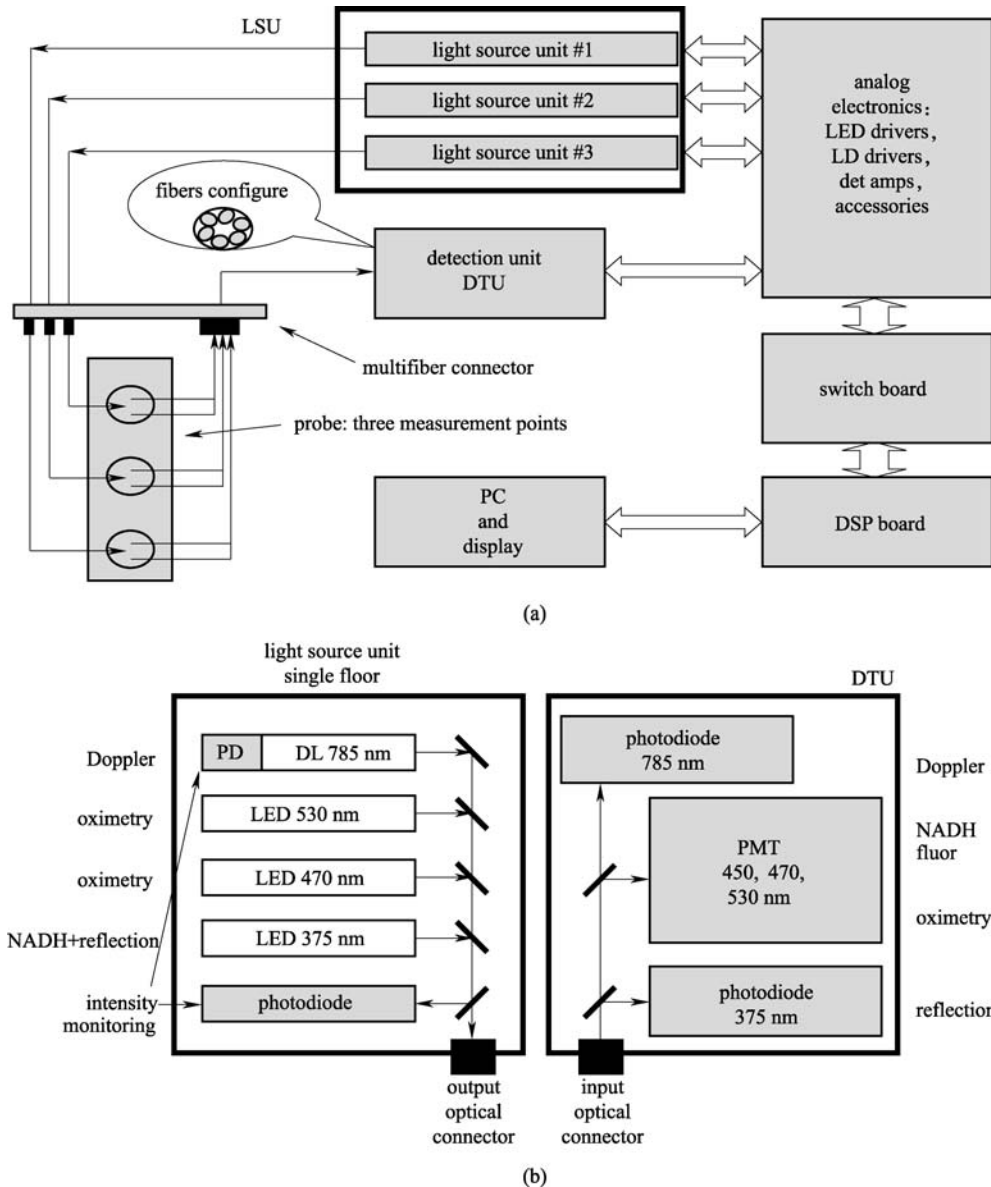
The general scheme of CritiView shown in Fig. 5 consists of a few units enabling the measurement of the four parameters from three different points or areas in the tissue.

#### 2.6 Light source unit

The light source unit consists of three identical floors. Each floor is responsible for supplying excitation light for a single excitation point and it comprises all excitation light sources required for CritiView measurements. In Fig. 5(b), one of the three light source floors and the detection unit are shown. It consists of near infrared (IR) laser diode at 785 nm for laser Doppler measurements, LEDs at 530 and 470 nm for tissue oxygenation measurements and UV LED at 375 nm for excitation of NADH fluorescence and reflection measurements. All discrete light sources are collimated (not shown in Fig. 5) and the different wavelengths are combined and coupled into a single excitation fiber by means of dichroic beam splitters. The light intensity of the LEDs is monitored by a discrete photodiode and the laser diode intensity is monitored by its internal photodiode.

#### 2.7 Detection unit

The detection unit consists of a photodiode for reflection measurements at 375 nm; photomultiplier (PMT) for NADH fluorescence at 450 nm and tissue oxygenation measurements by reflection measurements at two wavelengths of 470 and 530 nm; photodiode for laser Doppler measurements at 785 nm. The light incident into the detection unit distributed according to the different wavelengths to appropriate detectors by means of dichroic beam splitters.



**Fig. 5** (a) Schematic presentation of CriteView used in present study; (b) schematic description of one of floors of CriteView containing light source unit (LSU) and detection unit (DTU)

## 2.8 Electronics and digital signal processor (DSP)

The electronics unit includes all necessary drivers for LED and laser light sources and amplifiers for the detectors. The digital signal processor (DSP) P5 from Tern Inc. (Davis, California) is responsible for all the timing of the data acquisition. The system utilizes time sharing operation mode in order to acquire signals from various light sources for each measurement point of the probe. In order to enable a sufficient number of A/D and D/A inputs for all light sources and detectors additional multiplexers and demultiplexers are used. The data acquired by the system are processed by a dedicated software developed on Microsoft Visual Basic.

## 2.9 Fiber optic probes

In order to connect the monitored tissue to CriteView we developed various types of fiber optic probes. For animal studies we used a “needle” type probe [31] while for clinical application we developed a three point probe embedded in a three way Foley catheter shown in Fig. 6(b). This probe is built around the three measurement points concept (as shown in Fig. 5(a)). The main advantage of the usage of multiple measurement points for assessment of tissue vitality parameters is in spatial averaging of the measured parameters. Indeed microcirculatory tissue blood flow, tissue oxygenation, reflectance and NADH fluorescence may exhibit various levels as a function of the

measurement site. The averaging of these parameters over three measurement points enables us to retrieve a single value for each parameter that best represents the value of the parameter at the tissue under test. The third type of probe was applied to the skin (less vital organ) of neonates hospitalized in the ICU. The probe shown in Fig. 6(c) was built using the principles of the electroencephalogram (EEG) probe connected to the forehead skin of the neonates. Figures 6(b) and 6(c) show two probes developed for clinical applications.

### 3 Animal preparation and experimental protocols

All experimental protocols were approved by the institutional animal care committee under the instruction of the National Institute of Health.

Male Mongolian gerbils (50–80 g) were used. The animal was anesthetized by Equithesin (E-th is chloral hydrate 42.51 mg; magnesium sulfate 21.25 mg; alcohol 11.5%; propylene glycol 44.34%; pentobarbital 9.72 mg) intraperitoneal (IP) injection with 0.3 ml/100 g body weight. Body heat was measured by a rectal probe and was regulated to be at the range of 36°C–37°C using a heating blanket.

The gerbil was anesthetized and placed in a head holder. After a midline incision of the skin, a hole (5 mm in diameter) was drilled in the parietal bone of the right hemisphere. The dura mater remained intact and an appropriate light guide holder was placed in the hole. Two stainless steel screws in the parietal bone were used, with dental acrylic cement, to fixate the probes. The two common carotid arteries were isolated just before brain

surgery, and ligatures of 4-0 silk threads were placed around them.

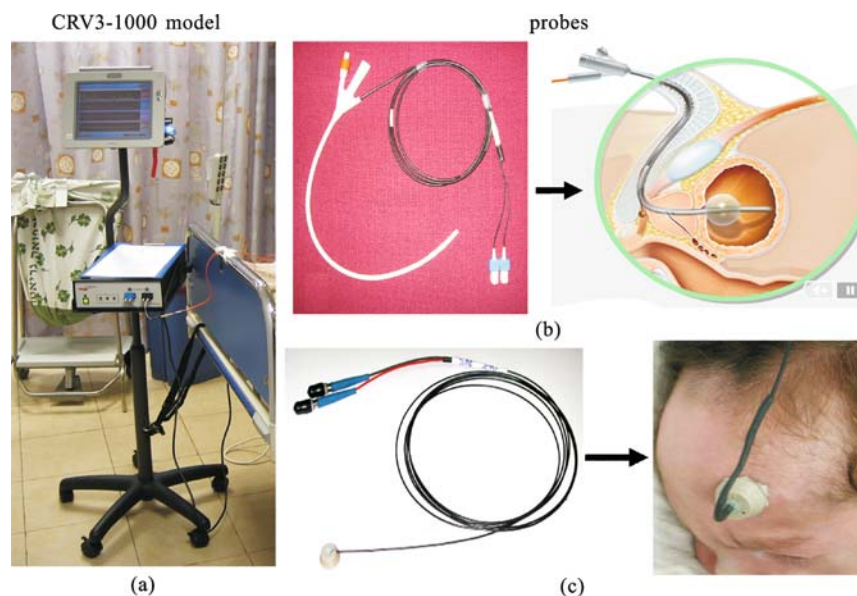
The gerbils were exposed to the following perturbations:

- Anoxia: The animals were exposed to oxygen deficient atmosphere by spontaneous breathing of 100% N<sub>2</sub>, for a short period (25 s).
- Ischemia: Reversible occlusion (~1 min) of one or two common carotid arteries (by constricting them with threads) led to brain ischemia in the gerbils.

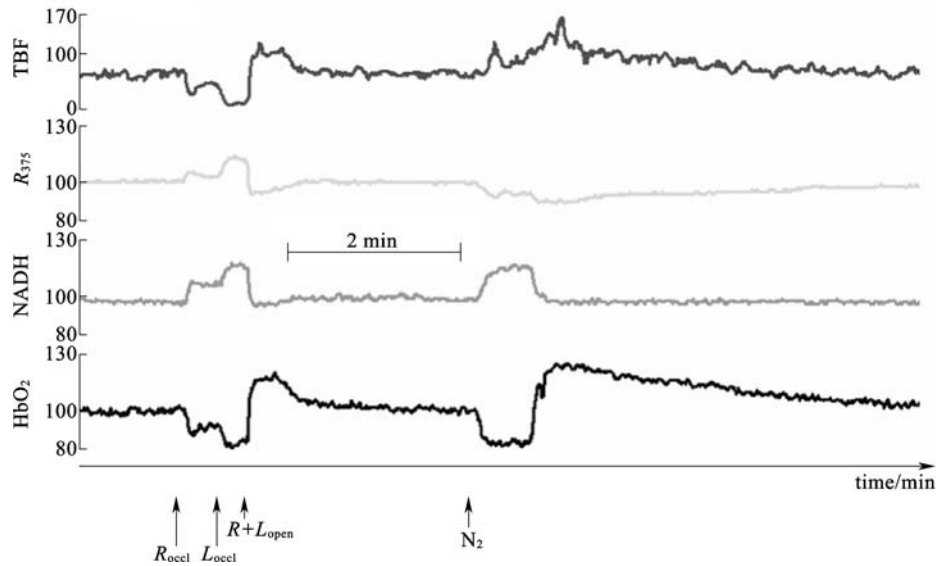
### 4 Results

The current version of CritiView was tested in gerbil's brain as well as in rat's brain and small intestine. In this communication we show the typical responses of the gerbil's brain to ischemia and anoxia. In Fig. 7, we present the four physiological monitored parameters namely tissue blood flow (TBF),  $R_{375}$ , NADH, and HbO<sub>2</sub>.

The raw signal of fluorescence is translated into the net NADH signal by subtracting the Ref from the Flu signal [37]. As seen in Fig. 7 (right side) under anoxia (100% N<sub>2</sub>) the initial response was the decrease in HbO<sub>2</sub> followed by an increase in NADH. The TBF showed a later increase which turned into a hyperemic response that was also recorded in the HbO<sub>2</sub> signal after recovery from anoxia. The responses to complete ischemia induced by bilateral occlusion of the carotid arteries are shown on the left side of Fig. 7. Due to the decrease in blood flow and volume to the brain (TBF signal), a large decrease in HbO<sub>2</sub> was correlated very well to the NADH. A small hyperemia was noticed in the TBF and HbO<sub>2</sub> traces. The time needed for recovery to base line value is much shorter in the NADH signal as compared to the TBF and the reflectance signal



**Fig. 6** (a) CritiView model used for monitoring of four different parameters from wall of urethra; (b) three way Foley catheter is inserted into bladder; (c) neonate probe is located on forehead skin

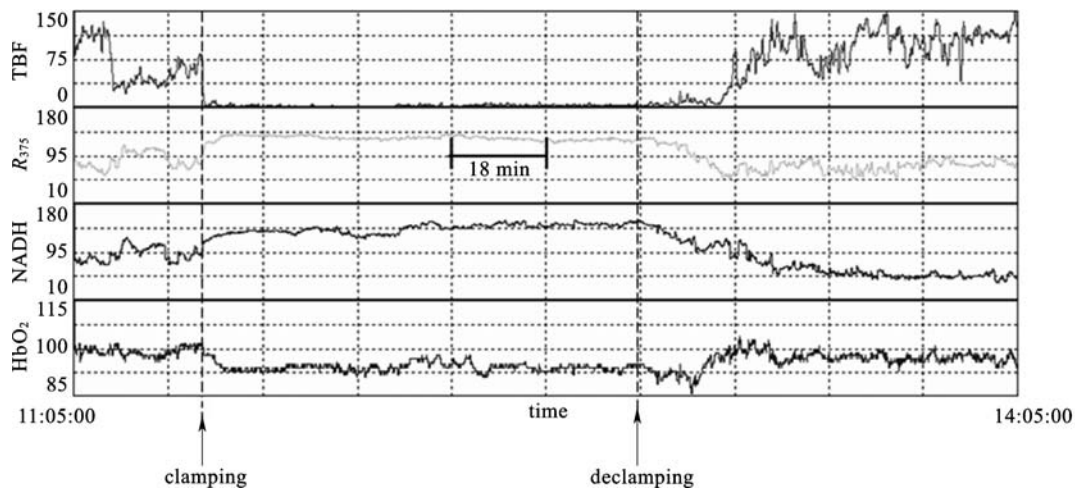


**Fig. 7** Responses of gerbil's brain complete ischemia ( $R_{occl}$  and  $L_{occl}$ ) and anoxia ( $N_2$ ) (TBF: tissue blood flow; NADH: corrected NADH fluorescence;  $R_{375}$ : reflected light at 375 nm wavelength;  $HbO_2$ : saturation level of hemoglobin)

( $R_{375}$ ). When ischemia was induced in two steps (Fig. 7 left side) namely occlusion of one carotid artery followed by the occlusion of the second artery (while the first remained occluded) the expected responses were recorded. The two levels of changes were recorded in the TBF, NADH, reflectance and  $HbO_2$  signals. A small recovery was noted shortly after the unilateral occlusion due to blood flow compensation through the anterior part of the circle of Willis of the gerbil's brain.

In order to assess the ability of CritiView to measure the vitality of the urethral wall in patients, a preliminary clinical study was performed. In patients we tested the viability of the urethral wall (a less vital tissue) by a three way Foley urinary catheter that contains the developed

optical probe. The catheter was introduced to patients who underwent open heart by-pass surgery or abdominal aorta aneurysm (AAA) operations. The monitoring started immediately after the insertion of the catheter to the patient and was stopped when the patient was discharged from the operating room. Figure 8 presents the data collected from the urethral wall during the occlusion period as well as after the reperfusion of the urethra in one of the patients monitored. During the preparation for the occlusion of the large arteries, a small transient decrease in microcirculatory TBF together with an increase in NADH levels. When the two arteries (Abdominal aorta and iliac artery) were occluded completely, the TBF decreased to near 0 levels while NADH reached its maximal levels. The



**Fig. 8** Effects of occlusion of iliac artery and abdominal aorta (clamping) on urethral wall parameters represent vitality at tissue level in AAA operation (time scale represents a period of 3 h)

HbO<sub>2</sub> decreased in parallel to the decrease in TBF. Under the no flow conditions developed, the reflectance signal increased due to the decrease in blood volume in the monitored area. Immediately after the reopening of the two blood vessels, all signals returned to the area of the baseline values. The results show that monitoring of the vitality of the urethral wall provides information in correlation to the surgical procedure performed. In patients who underwent heart bypass surgery the urethra vitality was decreased dramatically during the operation and recovery was noted in most patients after their discharge from the operating room.

## 5 Discussion

Real time optical monitoring of tissue vitality could be applied to many clinical situations in order to diagnose the metabolic state of the patient. The first issue is the addition of a new parameter to the three existing indicators of tissue vitality, namely, the oxygenation of hemoglobin. The second advantage is the technical improvement of the monitoring system and its smaller dimensions. Despite the fact that the presentation of the parameters are not in absolute values, it appears that the multiparametric nature of the system makes it very practical for real time evaluation as a function of time in the same tissue. The redox state of the NADH as measured *in vivo* is the most sensitive parameter of intracellular oxygen levels as compared to the other parameters monitored by CritiView. The changes in the NADH levels are also the best indicator of cellular energy balance in the tissue exposed to various pathophysiological conditions [37]. The other three parameters monitored by CritiView are very important for the understanding of tissue vitality but having them measured without the NADH decreased the level of data interpretation. For example, in Fig. 7, under anoxia the changes in CBF are not significant at the early stage while the HbO<sub>2</sub> and NADH showed a better correlation to the availability of oxygen. Under ischemia, all three parameters are very well correlated to the oxygen supply to the tissue. The decrease in dimensions of CritiView will enable to use it in the operating room and in the various ICUs. In critically ill patients the need for a better monitoring system is obvious and could be beneficial to the better outcome of the patients. These measurements may be performed on a so-called non-vital organ and will provide information that will serve as an early warning signal for the deterioration of the body.

The main progress, in our developmental effort to monitor tissue vitality, presented in the current report, is the significant increase in the better statistical based monitoring approach. Most of the devices developed in the past measured parameters from a single small spot in the tissue. Due to variation in the vascular as well as in O<sub>2</sub>

gradients across the tissue, the monitored parameter may not represent adequately the real behavior of the tissue. Only by increasing the number of monitored sites in the tissue the variations will decrease and the results will represent better the physiological and biochemical processes taking place in the tissue.

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