Real-time Multiparametric Spectroscopy as a Practical Tool for Evaluation of Tissue Vitality *In Vivo*

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In medical practice the monitoring of organ and tissue vitality is a critical need in operating rooms as well as in intensive care units (ICUs). The concept of multiparametric monitoring of tissue vitality was described in details in our previous publication [1]. The device, called "Tissue Spectroscope" (TiSpec), contained a single light source (325 nm) used as an excitation light. The emitted-reflected light from the tissue was analyzed to provide real-time information on the following three parameters: microcirculatory tissue blood flow, mitochondrial NADH redox state and tissue reflectance. Those 3 parameters represent the main components of tissue O_2 balance under in vivo conditions. The 325 nm He:Cd laser used was a large bulky and expensive to operate as a critical component in a modern medical device unit. The development of an ultraviolet laser diode by Nichia, Japan, enabled us to replace the light source of the TiSpec with a 390 nm laser diode, stabilized by a system developed by Toptica Photonics AG. This change in light source permitted the construction of a second model of the TiSpec, having the following advantages: 1. Smaller in dimensions, 2. Safer in terms of UV radiation effects, 3. Better stabilized for long term monitoring. The new TiSpec was tested in various animal studies as well as in various clinical applications. In order to monitor the brain during neurosurgical procedures, two special fiber optic probes were developed and used. Preliminary studies have shown that the 390 nm based TiSpec could be used in monitoring of various organs.

Keywords: Laser Doppler Flowmetry, NADH redox state, Mitochondrial function, Multiparametric monitoring, Tissue vitality

1. INTRODUCTION

Monitoring of organ and tissue physiology in clinical practice include patients hospitalized in various types of operation rooms (ORs) as well as all types of intensive care units (ICUs). Fig 1 shows the two approaches towards tissue and body vitality monitoring of patients in clinical practice. In operation rooms and ICUs a number of vital signs are monitored routinely as shown in part A of the figure. The monitored parameters represent the integrity and function of the cardiovascular and respiratory systems. The second approach of real-time monitoring is related to the vitality of a specific organ (B) in a patient exposed to severe operation or hospitalized in the neurosurgical ICU where intracranial pressure is monitored. It has to be noted that the number of devices available for specific organ monitoring is very small.

The purpose of the present article is to describe the state of the art of the updated Tissue Spectroscope (TiSpec02) developed in our laboratory in order to provide a new medical device to be used in daily clinical practice.

In our previous publication [1] the principles of monitoring of tissue vitality were described in details using the Tissue Spectroscope. In this device we had used a single light source – the He-Cd laser (325 nm) as an excitation light. This TiSpec01 has provided three different parameters represent tissue vitality. The main disadvantage of the 325 nm-based TiSpec01 was the large dimensions of the device, which limits its clinical practicability.

The development of Ultraviolet (UV) laser diode by Nichia, Japan enabled us to redesign the TiSpec into a significant smaller device. The present study is aiming to describe the new TiSpec02 based on a laser diode emitting at 390 nm.

1.1 Monitoring of Tissue Vitality by the Tissue Spectroscope

In our previous publication (Mayevsky et al., 2002) the device called Tissue Spectroscope (TiSpec) was described. This TiSpec enables the in-vivo monitoring of three physiological parameters: microcirculatory tissue blood flow (TBF) by laser Doppler flowmetery (LDF), mitochondrial NADH redox state by measurement of NADH autofluorescence and tissue blood volume by measurement of total back reflectance. Those three parameters represent the main components of tissue O_2 balance under in-vivo conditions. All three parameters were measured (in clinical studies) optically by single use sterile fiber optic probe. The system incorporates single excitation light source for measurement of all three

Advanced Biomedical and Clinical Diagnostic Systems, Tuan Vo-Dinh, Warren S. Grundfest, David A. Benaron, Gerald E. Cohn, Editors, Proceedings of SPIE Vol. 4958 (2003)

parameters (Fig. 2). This light source for the TiSpec01 was built around a bulky He-Cd gas laser, which has several disadvantages as described below.

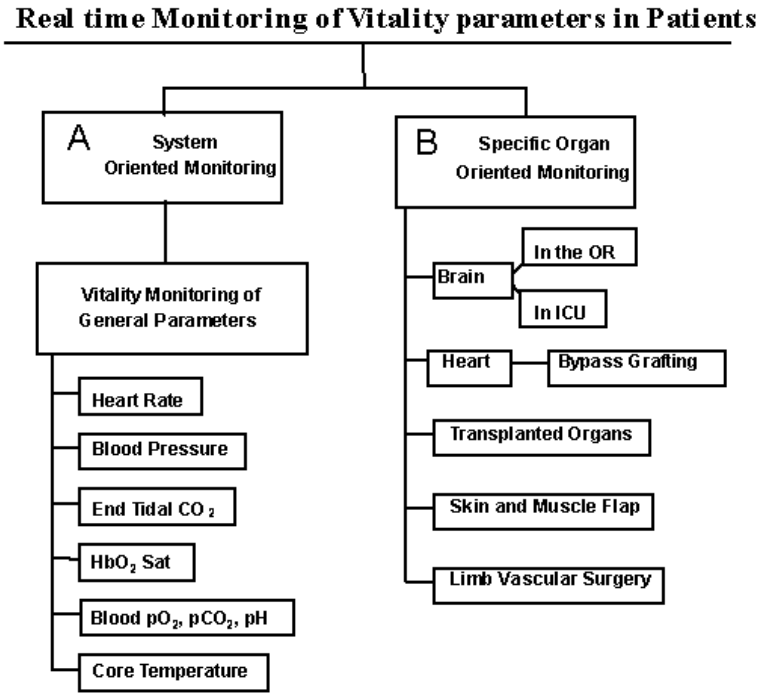

Fig. 1: General presentation of the main two approaches used in real time monitoring of patients in the operating room or in the various intensive care units.

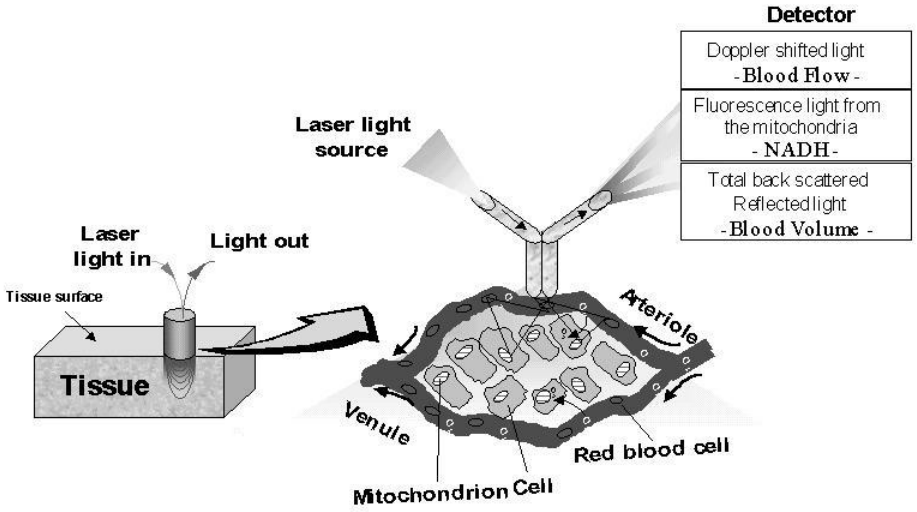

Fig. 2: The technical principles of the TiSpec. A single light source was used and the emitted light from the tissue is collected by optical fiber to the detection system.

The physical dimensions of the laser i.e. its length and the weight of the power supply emerges in relatively bulky final product. The limited floor space of the operating room can hardly incorporate such bulky TiSpec therefore smaller TiSpec device was highly desirable.

In order to perform LDF measurement single transverse mode He-Cd laser was utilized. This He-Cd laser has 11 longitudinal modes spaced over the 3GHz gain profile. This multi-mode nature along with non-negligible intensity noise (RIN ~ 0.5 to 1% RMS DC-20 KHz) deteriorates the LDF measurements. In order to enable the performance of reliable LDF measurements differential detection scheme was used [2]. This differential detection imposes the use of two sets of collection fibers and two detection channels. This increases the complexity of the TiSpec device and complicates the construction of the single-use fiber optic probe. The preferred design will incorporate single detection channel.

Fig. 3: The new TiSpec02

The UV light intensity needed for reliable LDF measurements is close to the safety limit as defined by various laser radiation safety standards. The maximum permitted exposure (MPE) is 1 mW/cm^2 for UVA laser radiation from 315 nm to 400 nm [3]. In order to reduce the mean irradiation intensity the TiSpec utilizes chopping excitation and synchronous detection scheme. It is generally known that the shorter UV wavelengths have more adverse influence on tissue then longer ones [4]. At longer wavelengths the responsively of the photodiode detector is higher therefore the excitation intensity for LDF measurements may be lower. Additionally too high intensity of irradiation inside the absorption spectrum of NADH can be potentially problematic for fluorescence measurements as the photo-bleaching effect may be significant. Therefore excitation at higher wavelength and lower intensity is favorable.

Recently released to the market UV-Violet laser diodes by Nichia (Nichia Chemical Industries Ltd., Anan, Japan) have emission wavelengths at UVA that coincide with the absorption spectrum of NADH. The light source based on laser diode may be much smaller in weight and dimensions then the He-Cd based system. Additionally the noise characteristic of laser diodes is much better then He-Cd laser therefore single channel detection system can be utilized. These considerations led us to design a new model of TiSpec device called now TiSpec02 shown in Figure 3. This TiSpec02 device is based on the new laser diode light source made by Nichia. Here we describe the new TiSpec02 device and show some preliminary measurements performed in experimental animals.

2. METHODS

2.1 Description of the TiSpec

2.1.1 Overview

The TiSpec02 device consists of several sub-units as described at Figure 4. The Light Source Unit (LSU) comprises the grating stabilized laser head DL100 from Toptica Photonics AG (Munich, Germany) with Nichia laser diode. All necessary accessories for laser diode beam shaping and intensity modulation included in the LSU.

The light produced by the LSU is coupled to the excitation optical fiber of the fiber optic probe. The excitation and emission fibers connected to the LSU and to the Detector Unit (DTU) respectively by SMA optical connectors. The excitation light guided to the tissue through multi-mode step-index silica fiber. The distal tip of the fiber optic probe is attached to the tissue by means of miscellaneous distal end attachments, as will be described later. A small fraction of the light that exits from the inside the bulk of the tissue to its surface is collected by the collection fibers and guided to the DTU. Several detectors are used to measure the light intensity correlated to the three different monitored parameters and convert the light intensity into electric signals. The Electronics Unit (EU) processes these electrical signals and the output of the EU is relatively slowly changing voltages that represent the three measured parameters and several auxiliary parameters. These signals feed into the Analog to Digital (A/D) converter. The A/D card enables data acquisition and device control through the Personal Computer (PC). The collected data stored and displayed on the screen by dedicated user-friendly software, which enables the user to control the entire operation of the TiSpec02 device. The Laser Stabilization Controller (LSC) provides the laser head, which is a part of the LSU, the correct laser diode current and temperature settings in order to operate in single longitudinal mode.

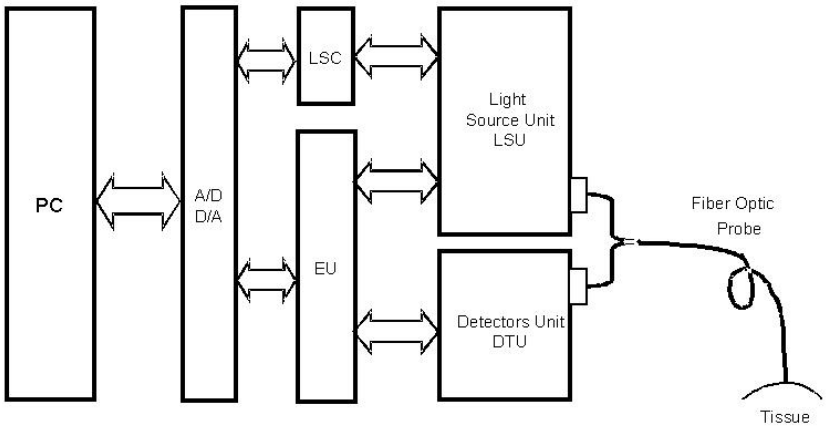

Fig. 4: TiSpec02 sub-units.
LSC – Light Stabilization Controller EU - Electronics Unit
A/D - Analog to Digital Converter PC - Personal Computer

2.1.2 LSU and LSC

The schematic diagram of the LSU and LSC is shown in Figure 5. The LDF measurements require high coherence length or narrow line-width and as low as possible amplitude noise. In laser diodes in general there is a strong coupling between the amplitude noise and the frequency noise. The free running Nichia UV violet laser diode emits several longitudinal modes that span over relatively broad line-width of about 400 MHz. Mutual competition of these modes expresses in relatively high intensity noise. This problematic line-width may be dramatically reduced by utilizing the external cavity [5]. The laser head comprises the external cavity stabilized laser diode (ECLD) DL100 from Toptica Photonics AG (Munich, Germany). The light from Nichia UV laser diode at the wavelength of about 390 nm is collimated by lens toward the grating. The grating serves as the front mirror of the ECLD resonator while rear facet of the laser diode comprising the back mirror. The grating is used to feed back part of the light, after spectral filtration, back to the laser diode. The first order is back reflecting to the laser while the zero order is coupled out. This kind of grating settings is called Littrow design [6]). The resulting output of DL100 with Nichia's UV-violet laser diode is single longitudinal mode with typical line-width of about 4 MHz. Such narrow line-width is sufficient for LDF measurements. Also when laser diode operates in such longitudinal single mode the amplitude noise is also reduced. As a result of various instabilities the system may drift after a while from the single longitudinal operation mode to some other adverse operation mode. The LSC system monitors the laser status and changes the laser current in order to ensure single longitudinal operation mode. The laser head comprises also the Peltier cooler and temperature stabilization controlling circuit (not shown on the scheme) that operated by the temperature controller, which is a part of the LSC. The laser head operates in Continuous Wave (CW) mode in order to obtain the lowest possible noise characteristic. The chopping modulation performed by means of Acousto-Optic Modulator (AOM) (AA sa Opto-Electronic Division, St Remy les Chevreuse, France). In order to get better chopping efficiency the laser ray passes through lambda/2 polarization plane rotator and anamirphic prism pair prior (not shown on the scheme) to modulation by AOM. In order to ensure separation between first and zero order of the AOM the minimum ray path length must be established. The total small footprint of the TiSpec02 is ensured by bending the laser ray twice by two banding mirrors M1 and M2. The chopping frequency and duty cycle is determined by the clock, which is a part of the EU. The output intensity of the excitation light controlled by the voltage of the clock pulse that supplied to the AOM. This voltage is set by the software, via the D/A, during the initialization procedure. The output intensity is monitored by the intensity sensing photodetector, which detects a small portion of the excitation intensity that passes through the second bending mirror. This photodetector enables the monitoring of momentary and total laser excitation intensity in order to ensure safe operation below the MPE. Additional safety feature incorporated into the LSU is the electro-mechanical shutter. The shutter precludes irradiation of the tissue unless explicit command received from the user through the software. In order

to enhance safety, the shutter automatically blocks the excitation light when one of the interlocks open. This feature enabled by the shutter controller, which is a part of the EU. The most important interlock monitors the connection of the excitation SMA connector of the fiber optic probe to the fiber coupler of the LSU precluding laser light come out of the LSU unless the fiber optic probe connected firmly to the TiSpec02.

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2.1.3 DTU and EU

The collecting optical fibers, at the DTU end of the fiber optic probe, are connected to the DTU by the SMA connector. The light from the fibers collimated by lens and passes through the dichroic beam-splitter which separates backscattered and Doppler shifted light at 390 nm from the NADH fluorescence light at 420-480 nm. Most of NADH fluorescence light passes through the beam splitter while the backscattered and Doppler shifted signals are reflected towards the photodiode detector, which converts it to a voltage. The output of the detector is fed into a synchronous detector allowing for the detection of the chopped light as initialized by the light source unit. The output of the synchronous detector is a combination of DC and AC voltages that are similar to those that would be acquired from a regular CW (non-chopped) excitation. The reflected light is a very slow changing parameter represented by DC voltage level at the amplifier output. The Doppler shifted light signal produces rapid voltage level changes in the audio frequencies range (from several Hz to a few kHz). Those changes appear as AC ripple on the relatively high DC level. Both signals appear at the output of the synchronous detector. The Doppler signals pass through an AC amplifier while the backscatter signals pass through a DC amplifier. After amplification, both signals are fed into the Doppler processor and its output consists of two signals. The first signal is correlated to Doppler shifted light that is related to microcirculatory Tissue Blood Flow (TBF). The seconds signal is correlated to the backscattered light intensity that is related to Tissue Blood Volume. When leaving the Doppler processor both signal types are slow changing, with a time constant of 3 sec. The AC and DC amplifiers and Doppler processor are part of the EU.

The light that passed through the dichroic beam splitter is focused on the active area of the photomultiplier (PMT) detector by a lens. Before reaching the PMT fluorescence detector, the light passes through a band pass filter that transfers only NADH fluorescence wavelengths (420-480 nm). The fluorescence detector signal, after amplification, is transferred to the A/D. The amplifier also works as a synchronous detector by accepting the timing signal from the clock. Fluorometer controller, who is a part of the EU, performs the set of PMT gain. The software starts the initialization of PMT gain setting by DIO of the A/D card. When leaving the EU all signal types are slow changing, with a time constant of 3 sec. The signals at the output of the EU are forward to the TiSpec02 software through the A/D.

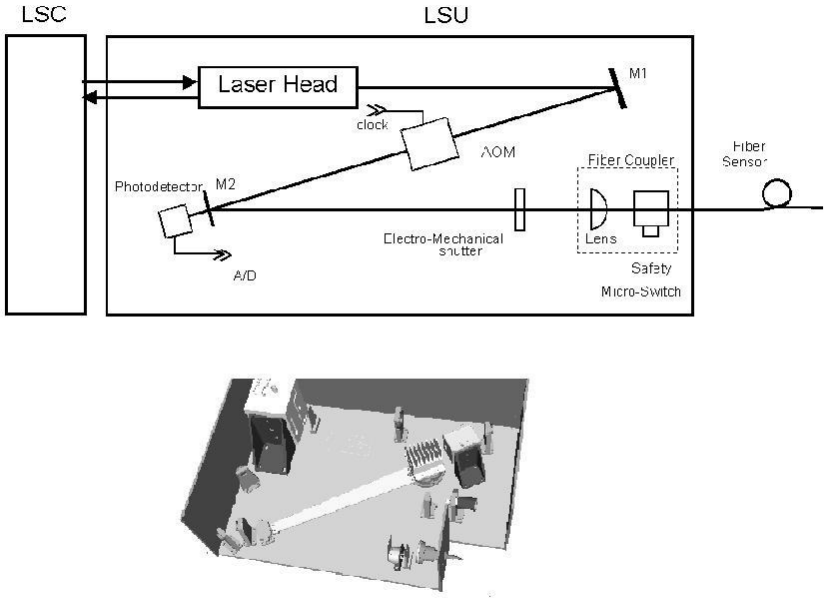

Fig. 5: Schematic diagram of the LSU and LSC

2.1.4 A/D Converter, PC and Software

The analog signals undergo A/D conversion, in order to be processed on the digital computer. TiSpec02 utilizes DAQPad-1200 from National Instruments (Austin, TX, USA). This standard multi-channel A/D converter enables also several Digital to Analog (D/A) outputs. The device also has several Digital Inputs-Outputs (DIO) that enable switching between ON/OFF states. This feature is useful for control of various TiSpec02 sub-units such as the detectors, amplifiers, electro-mechanical shutter, etc. Since all three TiSpec02 signals i.e. Doppler, NADH fluorescence and backscatter are slow changing signals, the sampling rate for these channels is as slow as 10 Hz.
The A/D connected to the parallel port of the Pentium II computer running Win2000 operating system.
The TiSpec02a device controlled by dedicated software – the TiSpec Operating Software (TiSpecOS). All of the TiSpec02 functions such as laser operation, measurement initialization and system calibration are controlled through software. The acquired data automatically displayed on the screen and stored on the hard disk for further data analysis. The TiSpecOS program is based on LabView development environment (National Instruments, Austin, TX, USA).

2.1.5 The probes

Fiberoptic probe is required in order to enable excitation light and optical signal transmission between the TiSpec and the monitored patient. Since the TiSpec is used mainly in OR and ICUs, the probe cable should be long enough to enable the device to be located relatively remotely from the patient, minimizing the disturbance to the routine medical practice.
The probe is based on a bundle of optical fibers, divided into two optical channels: The excitation channel and the collection channel. In the probe tip, all fibers are adjunct, while in the machine-end, the bundle is split into separate excitation channel and collection channel. The excitation channel is connected to the TiSpec light source by an optical SMA connector, and the collection channel is connected to the device's detection system (the DTU) by another SMA connector.
The probe is equipped with a special tip, carefully designed to obtain continuous contact with the monitored tissue. A reliable contact with the tissue is crucial, however no extensive force is allowed, since the physiological situation is very sensitive to applied pressure. Two probe types were developed: the TSP2000-2 and TSP-NB1.
The TSP2000-2 probe was designed to monitor exposed brain cortex, in various locations under the craniotomy during neurosurgical procedures. The tip is formed from a thin metal tube, which accommodates the bundled optical fibers. A 5mm OD cylindrical element is attached to the end of the metal tube, in order to enlarge the footprint of the probe and enables the probe to float upon the cortex (Fig. 6). Since the human brain is not static during the surgery, an adjustable floating arm was designed in order to track the restless brain tissue. The basis of the floating arm is firmly connected to a Yasargil arm, which is a common spatial positioning device in neurosurgery. Yet, the floating arm exploits the inherent flexibility of the probe cable, to produce spring-like movements of the tip, applying slight thus harmless pressure upon the brain cortex. This mechanism provides the required continuous contact of the probe and the tissue (Fig. 7).

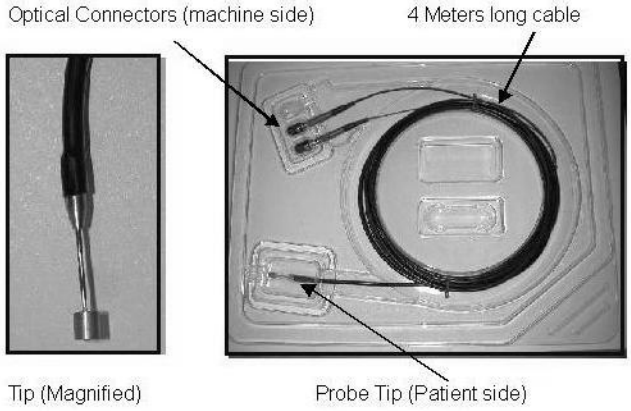

Fig. 6: The TSP2000-2 probe for brain cortex monitoring

The TSP-NB1 probe was designed to monitor neonates hospitalized in ICU. The probe was designed to be attached to the neonates' skin, since their skin is very thin and lack of epidermis. The extremely thin neonatal skin enables, on one hand, the penetration of the highly-absorbed UV light to labile tissues under the skin, but on the other hand ban the use of adhesives or medical adhesive tapes, due to aggressive peeling.
In order to attach the probe to the neonatal skin, yet not exposing the delicate skin to the dangerous effect of peeling an adhesive, a button shaped probe was designed. The probe features a low-profile cylindrical tip, in which the fiberoptic bundle is 90 degrees bent within. The tip has two round grooves on its face, concentric with the fiberoptic bundle. The outermost groove is to be overfilled with an EEG/ECG gel, then pressed upon the skin. The gel sticks the probe to the skin while excessive gel is forced out of the probe or inside into the innermost groove that serves as a moat. The moat keeps the optical fibers from being screened with gel.

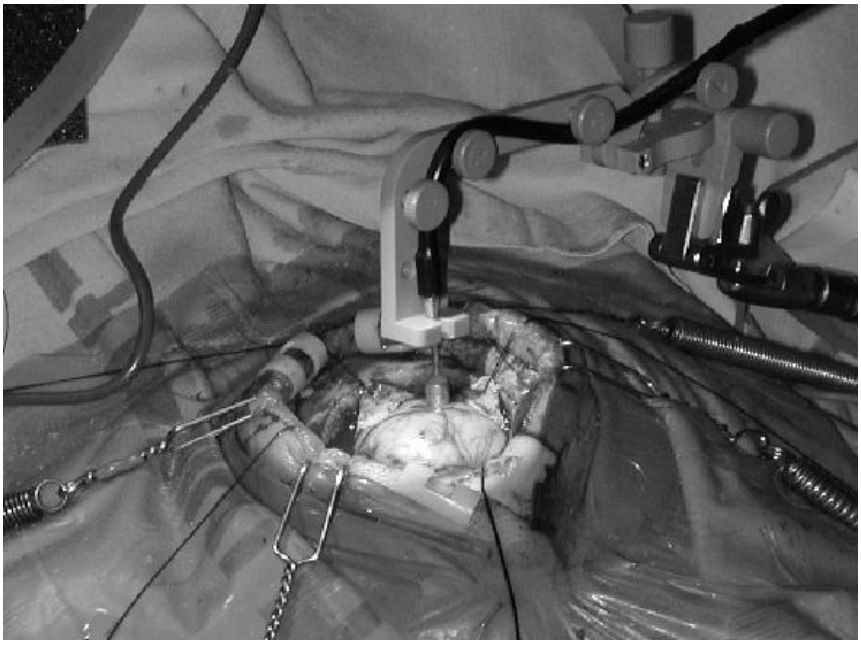

Fig. 7: The TSP2000-2 in a neurosurgical procedure. The probe is attached to a floating arm mounted on Yasargil positioning arm.

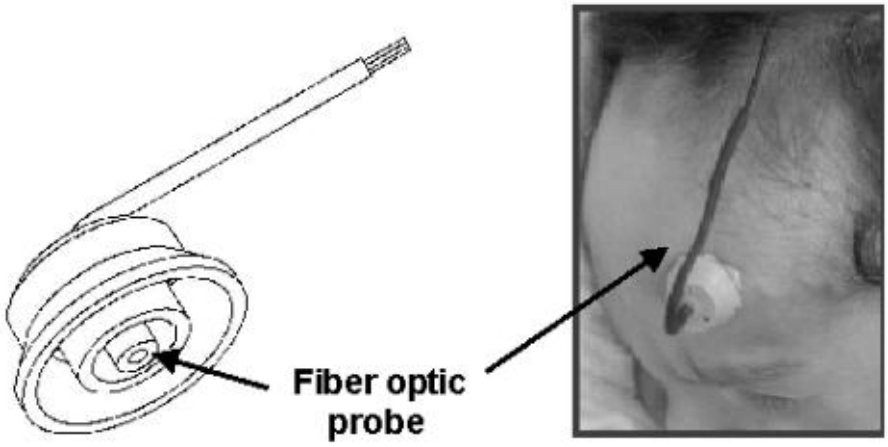

Fig. 8: The TSP-NB1 Neonates monitoring probe (Left) attached to a baby's forehead skin (right)

2.2 Surgical preparation for animal experiments

The animals (gerbils, 50-70 gr) were anesthetized by an IP injection (0.3 ml/100 gr) of Equithesin (each ml contains: pentobarbitoral 9.72 mg, chloral hydrate 42.1 mg, magnesium sulfate 21.25 mg, propylene glycol 44.34% w/v, alcohol 11.5% and water). We have been using this anesthetizing agent for approximately 25 years and it has never shown significant effects on mitochondrial activity. Furthermore, we have never been able to change the response of the rat brain to spreading depression by using an increased dose of Equithesin, suggesting that this is a safe drug. The skull was exposed and an appropriate hole was drilled in the parietal bone. The light guide holder was located on the brain so that the undue pressure on the brain is avoided. The cannula and screws were fixed to the skull by dental acrylic cement in 2-4 different locations. If needed, additional injections of anesthetic were given to the gerbils every 30 minutes during the operation (0.1 ml Equithesin/100 gr body weight).

3. RESULTS

In order to test the performance of the new TiSpec, in which the excitation light is 390 nm, animal studies in rats and gerbils were performed. In gerbils we have tested the effect of anoxia and ischemia on brain energy metabolism. In rats we have tested the effect of anoxia, ischemia or adrenaline injection on the three monitored parameters from the small intestine, skeletal muscle or the internal side of the skin covering the head (result not shown).

Figure 9 shows typical response of the brain to ischemia induced in a gerbil by occlusion of the two carotid arteries. As seen, the cerebral blood flow (CBF) was decreased initially to a very low level followed by a small spontaneous recovery during the occlusion period. The decrease in blood flow led to decrease in O_2 supply to the brain as seen in the large increase in the mitochondrial NADH. The net change in NADH is calculated by the correction of the fluorescence for the hemodynamic artifacts measured as seen in the reflectance trace. During the ischemia, the reflectance was elevated due to a decrease in blood volume followed by slow recovery to the baseline towards the end of the occlusion. The recovery processes recorded after the reopening of the two carotid arteries were very fast as seen by the increase in CBF having also a hyperemic phase. The NADH was reoxidized showing also a small undershoot immediately after the reperfusion, probably due to the large amount of blood releasing more O_2 to the tissue. The complete recovery of all traces to its baseline levels were recorded 2 minutes after the reperfusion.

In Figure 10 the responses to terminal anoxia, induced by breathing 100% N_2, is shown. The initial CBF change was small in parallel to the increase in NADH, followed by a fast decrease to the minimal level of flow. About 2 minutes after breathing N_2 a large increase in the reflectance trace was noted at the stage that the blood flow was minimal and the NADH was stable at its maximal level. This phenomenon of spontaneous reflectance increase (SRI) was noted many years ago in rat studies and is probably due to a massive increase in extracellular level of potassium leading to a large vasoconstrictive effect [7,8]. Due to this SRI event a large decrease in NADH was recorded (in the absent of O_2) and it is due to the correction procedure that can not take care on the large change in tissue absorption properties and not by blood volume change alone. This decrease in NADH was recovered back to thee maximal level recorded previous to this artifact.

In Figure 11 the two episodes shown were recorded from the same gerbil brain. Here, we have compared the responses of the brain to ischemia to those induced by anoxia. In the left side, a typical response to carotid arteries occlusion in the gerbil is shown. The large increase in the reflectance is clear but the fluorescence change is larger during the ischemic episode as seen clearly in the CBF trace. During anoxia, shown on the right side, the NADH reached its maximal level while the CBF showed a clear decrease. Upon recovery a clear hyperemic response is shown in the CBF trace although the NADH was stable in its baseline level.

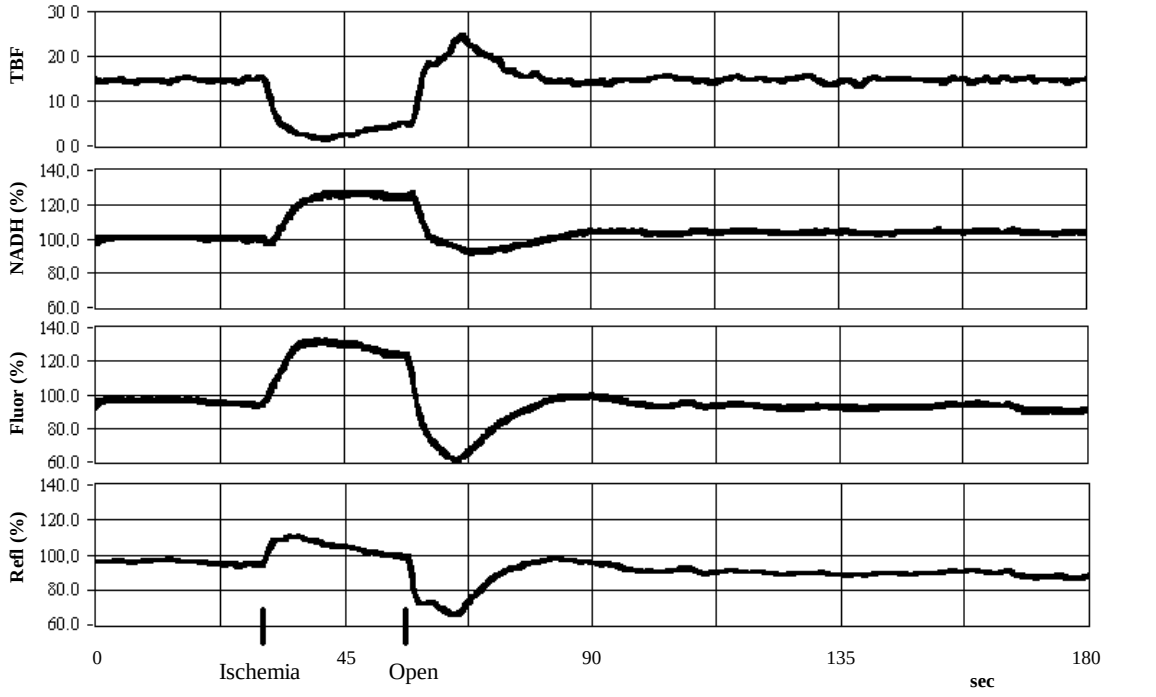

Fig. 9: Effects of ischemia of brain energy metabolism in the gerbil. TBF – Tissue Blood Flow, Fluor – Fluorescence, Refl - Reflectance

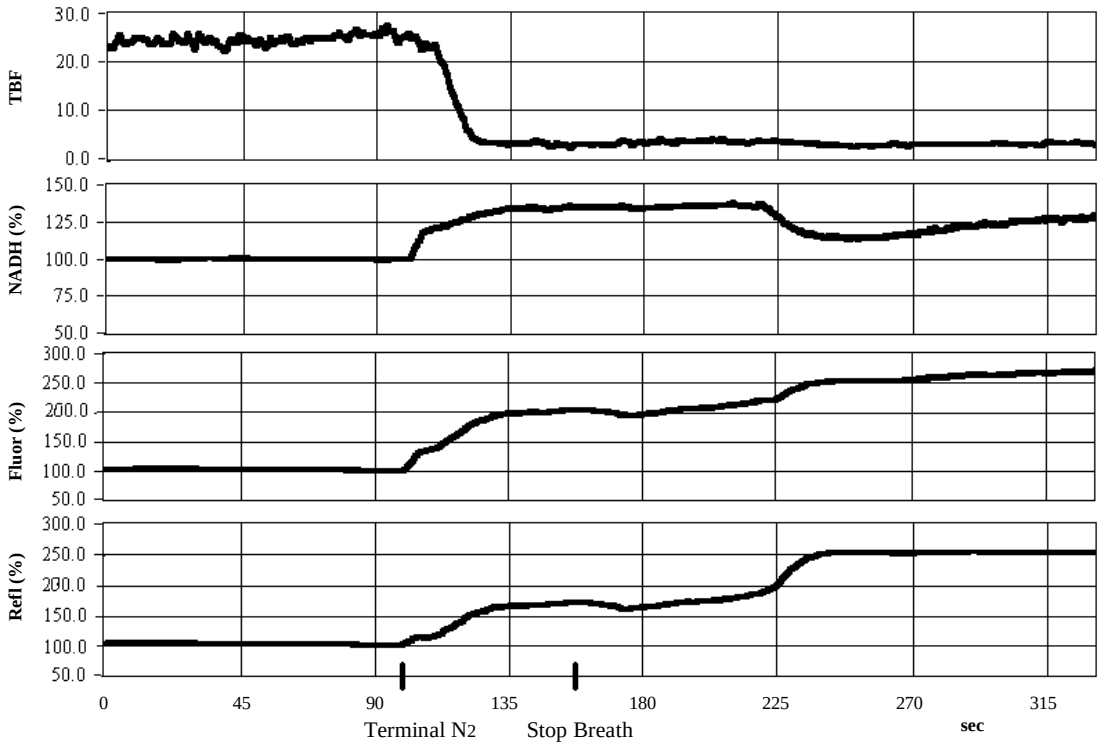

Fig. 10: Responses of the gerbil brain to terminal anoxia induced by exposing the animal to 100% N_2. Abbreviations are as in Fig. 8.

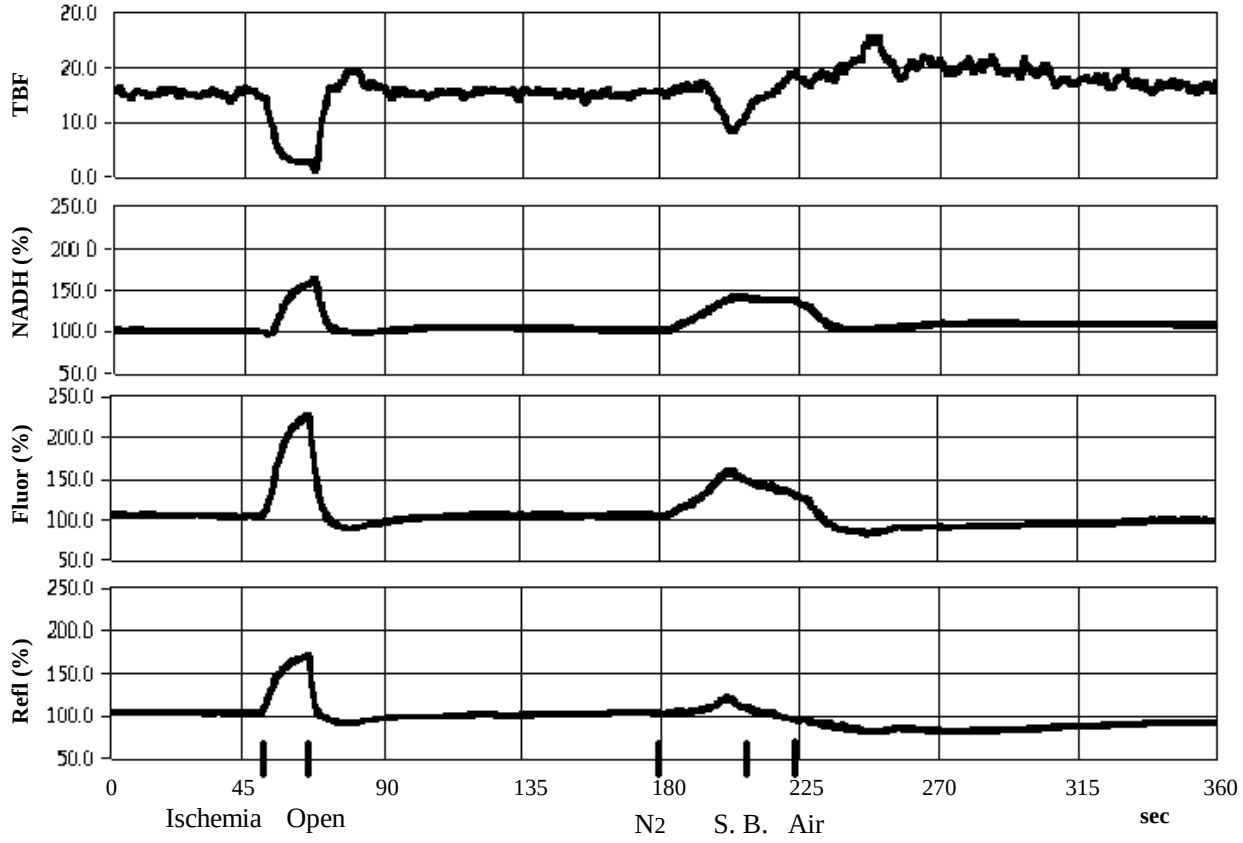

Fig. 11: Responses of the gerbil brain to ischemia (left side) and anoxia (right side). Abbreviations are as in Fig. 8

4. DISCUSSION

The TiSpec described in the present article has small dimensions that could be used in operating rooms and ICUs which are limited in free space for new medical devices. The search for reliable medical device that could be used in real-time is an ongoing process and became more critical due to the high mortality rate recorded in the various ICUs around the world. In addition to the monitoring approaches shown in figure 12, a new principle of patient's monitoring was developed in the last 10 years. This new approach is seeking to identify an early warning signal that will be monitored in real-time and will be detected significantly earlier before the existing monitoring devices.

Under conditions where the BEMS is developed, the body is responding by the activation of homeostasis mechanisms dominated by the sympathetic nervous system [9]. The immediate response to such situation is blood flow redistribution between more vital organs and less vital organs. The vital organ will receive more blood and the better oxygenation will ensure to continuous supply of energy. The perfusion to the non-vital organs will decrease and as a result O_2 supply to the mitochondria will diminish significantly. Ischemia will be detected in the non-vital organs and energy failure will occur. It is assumed that these changes in the non-vital organs could be used as an early warning signal to the development of BEMS.

The assumption is that the standard monitored systemic parameters such as systemic blood pressure, heart rate or even the pulse oximeter will respond to the BEMS in a late stage, therefore monitoring of non-vital organ is the solution for such situation. At present time we were able to test the TiSpec02 in neurosurgical operating room. The dimensions of the TiSpec02 were acceptable in the OR and routine activity of the medical team was not disturbed.

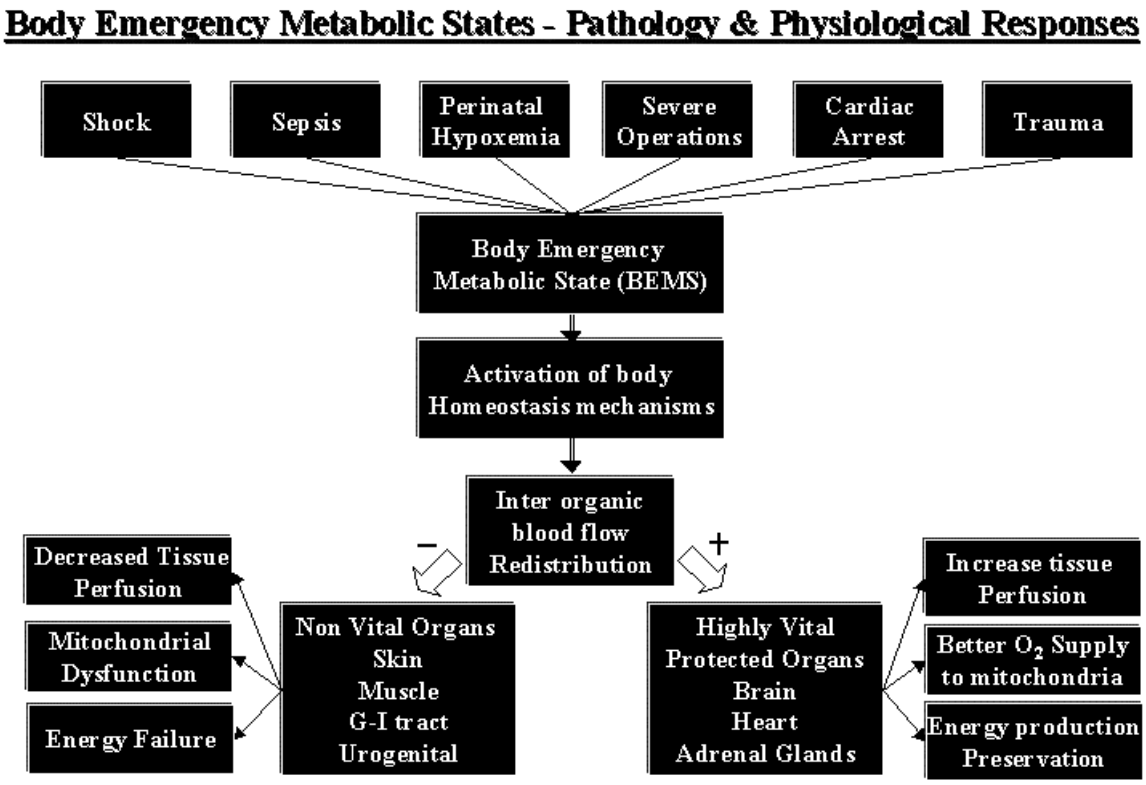

Fig. 12: Various clinical pathological conditions (e.g. shock, sepsis) that lead to the development of Body Emergency Metabolic State – BEMS in critically ill patients (see text for detailed explanation).

ACKNOWLEDGEMENT

Authors express their thanks to Dr. W. G. Kaenders, Dr. A. Deninger and Dr. T. Mueller-Wirts from Toptica Photonics AG, Munich, Germany for the help and support in the development of the laser stabilization controller (LSC) of the TiSpec02.

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