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TITLE: **Development and Testing of New Noninvasive Monitoring Tools for Prolonged Field Care Goal-Directed Therapy**

PRINCIPAL INVESTIGATOR: Kevin Ward, MD

CONTRACTING ORGANIZATION: Regents of the University of Michigan
Ann Arbor, MI 48109

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6. AUTHOR(S)

Kevin Ward, MD
Mohamad H. Tiba, MD
Amanda Pennington, MS
Brandon Cummings, BS

E-Mail: keward@med.umich.edu

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

University of Michigan
Michigan Center for Integrative Research in Critical Care
2800 Plymouth Road, NCRC
Building 10, Room A107
Ann Arbor, Michigan 48109-2800

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Management of the polytrauma patient with or without TBI in the prolonged field care (PFC) setting especially when prolonged damage control resuscitation (pDCR) is required represents an extraordinary challenge. While there is a desire to develop new therapeutic agents to improve survival and mitigate tissue injury and organ failure, we have not yet developed tools which assist in helping providers maximize use of supportive treatments such blood transfusion, volume expansion, vasopressor use, etc. in a precision manner for goal directed therapy (GDT). The use of goal GDT has been demonstrated to be life saving for both surgical and medical populations with severe hemodynamic compromise but is difficult to implement with current invasive and noninvasive tools because of their lack of precision or form factor and expense. This proposal will scale testing of two novel noninvasive measures that could allow for real-time use of GDT in the PFC/pDCR setting. These include: 1) Resonance Raman Spectroscopy (RRS) to measure tissue hemoglobin oxygen saturation (StO2) of the buccal mucosa as a substitute for central or mixed venous hemoglobin oxygen saturation (ScvO2) and potentially lactate, and 2) Dynamic Respiratory Impedance Volume Evaluation (DRIVE) of the limb as a substitute for ultrasound of the inferior/superior vena cava and central venous pressure (CVP). RRS-StO2 uses a special wavelength of light to determine how much oxygen a tissue is receiving. DRIVE uses a small amount of electricity passed through tissue to measure blood volume moving in and out of the tissue during breathing. Hypothesis: The use of noninvasive RRS-StO2 and DRIVE will provide information of sufficient value in complex surgical patients regarding tissue oxygenation and intravascular volume to allow consideration of their use for GDT in PFC and pDCR.
Specific Aims/Objectives:
1) Test and compare RRS-StO2 with other measures and surrogates of tissue oxygenation including lactate and ScvO2 in polytrauma and complex operative and post-operative surgical patients.
2) Test and compare DRIVE to other measures and surrogates of intravascular volume monitoring including ultrasound of the IVC and SVC, CVP, and stroke volume variation (SVV) (when measured) in polytrauma and complex operative and post-operative surgical patients.
3) Compare time series measurement RRS-StO2 and DRIVE to patient outcomes including mortality and organ failure in order to support future clinical intervention trials.

This is a clinical research study examining two prototype noninvasive devices (RRS-StO2 and DRIVE) to compare their performance to a range of standard invasive and noninvasive monitoring that may not be suitable for PFC and pDCR. Trauma and surgical critical care patients undergoing invasive monitoring (CVP, ScvO2 stroke volume variation (SVV), etc.) and noninvasive or minimally invasive monitoring (IVC ultrasound, TEE, etc.) will have these measures compared to RRS-StO2 and DRIVE over time. Responses to treatment such as transfusion, volume loading, vasoactive medication administration, mechanical ventilation, etc. will be tracked and compared. Additional data such as lactate levels, injury severity scores, surgical interventions, organ failure scores, and finally outcome will be compared to understand how DRIVE and RRS-StO2 perform compared to other traditional measures. An attempt will be made to enroll 200-300 subjects.

15. SUBJECT TERMS
Tissue Oxygenation, Resonance Raman Spectroscopy, Bioimpedance, Intravascular Volume, Hemodynamics, Goal Directed Therapy

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INTRODUCTION:

Trauma frequently leads to a state of shock usually through significant hemorrhage. Hemorrhage continues to be the leading cause of death on the battlefield. The ability is lacking to both quickly determine the severity of hypoperfusion and tissue hypoxia after injury as well as during resuscitation as a means to guide therapy and optimally resuscitate victims early in their care and between echelons of care. Cellular dysfunction, organ damage, coagulopathy and death are known to occur proportional to the degree of shock. Early goal directed therapy (GDT) is a resuscitation strategy developed over the last decade based on the physiologic principle of reversing tissue dysoxia, and restoring basic oxygen transport metrics to a level that meets the body's oxygen demands in an early and individualized targeted fashion.

Management of the polytrauma patient with or without TBI in the prolonged field care (PFC) setting especially when prolonged damage control resuscitation (pDCR) is required represents an extraordinary challenge. While there is a desire to develop new therapeutic agents to improve survival and mitigate tissue injury and organ failure, we have not yet developed tools which assist in helping providers maximize use of supportive treatments such blood transfusion, volume expansion, vasopressor use, etc. in a precision manner for GDT. The use of goal GDT has been demonstrated to be life saving for both surgical and medical populations with severe hemodynamic compromise, but is difficult to implement with current invasive and noninvasive tools because of their lack of precision or form factor and expense. This proposal will scale testing of two novel noninvasive measures that could allow for real-time use of GDT in the PFC/pDCR setting.
These include:

1) Resonance Raman Spectroscopy (RRS) to measure tissue hemoglobin oxygen saturation (StO_2) of the buccal mucosa as a substitute for central or mixed venous hemoglobin oxygen saturation ($ScvO_2$) and potentially lactate
2) Dynamic Respiratory Impedance Volume Evaluation (DRIVE) of the limb as a substitute for ultrasound of the inferior/superior vena cava and central venous pressure (CVP).

   RRS-StO2 uses a special wavelength of light to determine how much oxygen a tissue is receiving. DRIVE uses a small amount of electricity passed through tissue to measure blood volume moving in and out of the tissue during breathing.

KEYWORDS:

Tissue Oxygenation, Resonance Raman Spectroscopy, Bioimpedance, Intravascular Volume, Hemodynamics, Shock, Resuscitation, Goal Directed Therapy

ACCOMPLISHMENTS:

- **What were the major goals of the project?**

- **Major Task 1:** Test and compare RRS-StO_2 with other measures and surrogates of tissue oxygenation including lactate and $ScvO_2$ in polytrauma and complex operative and post-operative surgical patients. Months 0-36
- **Major Task 2**: Test and compare DRIVE to other measures and surrogates of intravascular volume monitoring including ultrasound of the IVC and SVC, transthoracic echo, CVP, and SVV in polytrauma and complex operative and post-operative surgical patients. Months 0-36

- **Major Task 3:** Compare time series measurement RRS-StO_2 and DRIVE to patient outcomes including mortality and organ failure in order to support future clinical intervention trials. Months 6-36

- **What was accomplished under these goals?**

  Major Task 1: Test and compare RRS-StO_2 with other measures and surrogates of tissue oxygenation including lactate and $ScvO_2$ in polytrauma and complex operative and post-operative surgical patients. Months 0-36

  1) Specific objectives:
  a) IRB approval January 22, 2013
  b) HRPO approval November 15, 2017
  c) Patient recruitment: 27 patients

  Patients who were admitted to the University of Michigan cardiovascular ICU with a central line were consented and enrolled into the study. In cases where the patient was unable to consent, the legally authorized representative consented on their behalf. A signed copy of the informed consent document was provided. 2 females and 22 males were enrolled with an average age of 64(15).

  The RRS probe was covered in a sterile sleeve and placed on the buccal mucosa of the patient. Data were collected for 20 minutes. Both sides of the buccal mucosa were tested independently. Near the end of testing, 3cc of mixed venous blood was collected from the pulmonary artery port of the central line and the reading from the Raman spectrophotometer was recorded for comparison. Blood was tested for $ScvO_2$ and compared to tissue oxygen saturation (StO_2) as measured by RRS.

  2) Significant results:

  A) Data analysis
  Table 1 lists descriptive statistics for both StO_2 and $ScvO_2$.

	StO2	ScvO2
Mean	63.8	67.7
Std. Deviation	11.0	11.3
Lower 95% CI of mean	58.3	62.1
Upper 95% CI of mean	69.3	73.3
Minimum	42.0	48.0
Maximum	78.0	96.0

  There was significant correlation between $StStO_2$ and $ScvO_2$ (*$r=0.57$, $p<0.013$*) (Figure 1).

  A paired t-test showed no significant difference between the StO_2 and $ScvO_2$ with a mean(SD) difference of 4 (10.3)% (*95%CI = [-1.3, 9], $p = 0.13$*).

  Receiver Operator Characteristic (ROC) curves for predicting $ScvO_2$ at thresholds of $ScvO_2$ above and below 65, and 70% demonstrated the high predictive power of StO_2

with areas under the curve of 0.74 and 0.83 respectively with high sensitivity and specificity (Figure 2). These values are chosen since they represent cutoff values that would produce actionable therapeutic decision making.

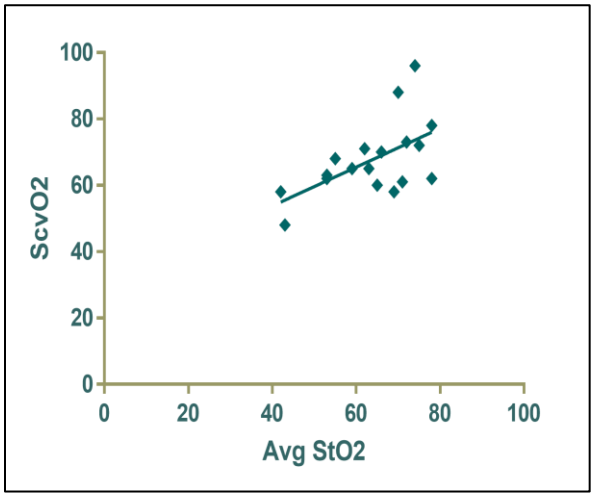


Figure 1: Correlation between StO_2 and $ScvO_2$

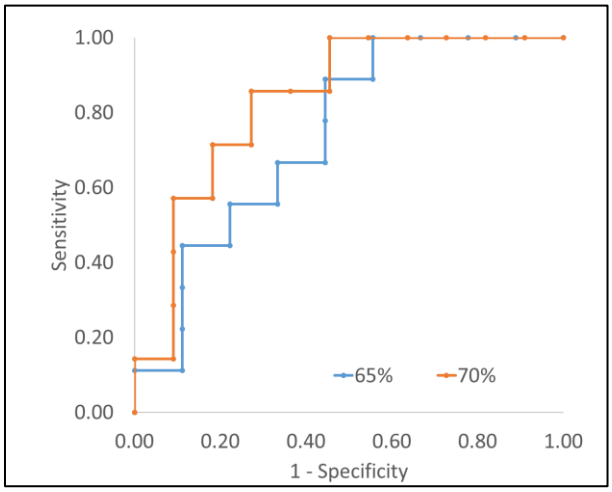


Figure 2: Receiver-Operator Curve for StO_2 at a threshold of 65% and 70% for $ScvO_2$

B) Probe enhancement

Probe and clip holder enhancement is currently underway to allow faster and more stable placement of the probe (Figures 3 and 4).

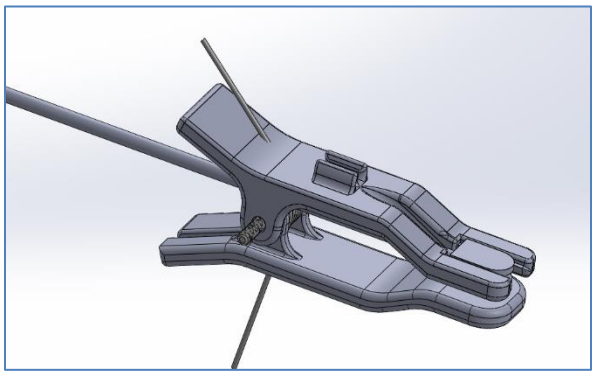


Figure 3: New probe holder configuration

Figure 4: RRS Probe

Major Task 2: *Test and compare DRIVE to other measures and surrogates of intravascular volume monitoring including ultrasound of the IVC and SVC, transthoracic echo, CVP, and SVV in polytrauma and complex operative and post-operative surgical patients. Months 0-36*

1) Specific objectives:

a) IRB approval January 22, 2013
b) HRPO approval November 15, 2017

c) Patient recruitment: 75 patients

Patients who were admitted to the University of Michigan trauma ICU, emergency department, or emergency critical care center were consented and enrolled into the study. In cases where the patient was unable to consent, the legally authorized representative consented on their behalf. A signed copy of the informed consent document was provided. 31 females and 44 males were enrolled with an average age of 49.9(18.7).

Subjects underwent an abdominal ultrasound to measure changes in IVC diameter during normal tidal breathing as well as during respiratory maneuvers such as deep breathing and sniff. Of these patients, a small subset was selected for bioimpedance monitoring with a new portable prototype device. These subjects were monitored using the prototype for 15-30 minutes, using a bipolar electrode array. The amplitude of the cardiac and respiratory components of the bioimpedance signal was quantified and compared to changes in IVC diameter as measured by ultrasound. The bioimpedance signal, comprised of distinct cardiac and respiratory components, was quantified by taking the ratio of the amplitude of these components.

A new prototype device that will no longer require the use of a laptop and cart is currently being developed (New Vital Signs, Inc) and tested alongside the Biopac system. The improvements in this device will allow greater mobility and reduce reliance on electricity.

2) Significant results

A) Data analysis

The objective of the Dynamic Respiratory Impedance Volume Estimation (DRIVE) technology is to noninvasively assess intravascular volume status using limb bioimpedance. When measured in a limb such as the arm, the bioimpedance signal represents a cumulative effect of the individual bodily tissues. Blood, however, has a unique and distinct effect on the signal due to its relatively conductive properties and its ability to flow. As the volume of blood in a localized area such as the limb varies with respiration and the cardiac cycle, these variations are reflected by corresponding changes in the impedance signal (Figure 1). We hypothesize that these respiratory and cardiac variations contain information which can be predictive of the a patient's intravascular volume status.

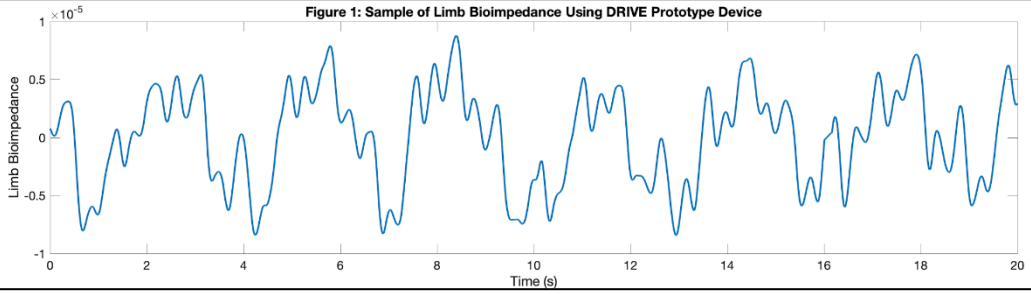


Figure 5: Sample bioimpedance waveform. Note the prevalent respiratory cycle (low frequency) and the higher-frequency cardiac component. This sample signal was obtained from a human subject using the prototype device.

During the first year of the award, we have made significant progress toward our goal of validating this technology. These achievements include both the development of a portable

prototype device capable of measuring bipolar bioimpedance and the characterization of several novel metrics based on the bioimpedance signal.

The portable prototype is capable of measuring limb bioimpedance effectively in our benchtop testing, and we have recently begun to collect human subjects' data using the device. Currently, ten patients in the ICU and ED have been tested using the device and their data is being analyzed. In all cases the signal quality has been excellent and minimal filtering is required to prepare them for processing. Note that the signal presented in Figure 5 was collected from a human subject using this prototype, with very clear cardiac and respiratory components.

We have also developed several novel, automated metrics to characterize the bioimpedance signal. Before the award, analysis of the waveform had been done through manual visualization and annotation of the waveform. This is clearly not scalable as the technology matures into a wearable device. These new automated measures, however, are better suited for real-time implementation and analysis. They include quantification of the respiratory component, which is in-line with our previous manual analysis technique, as well as assessing the relative contributions of the respiratory and cardiac components of the signal.

Both techniques involve filtering out the necessary frequencies and quantifying their relative amplitudes using the root-mean-square (RMS) envelope. In contrast to many analytic approaches in the frequency domain, this approach is agnostic of changes in respiratory and heart rate. Additionally, we have discovered that by indexing the magnitudes of the cardiac and respiratory envelopes we are able to significantly cut down on impulse artifacts which may otherwise disrupt the signal.

In addition to both these analytic techniques, we have begun to investigate techniques to automatically detect and mitigate excessive movement artifact, which will play an important role as the prototype is used for extended durations. While developing these techniques, we have focused on ensuring that they can easily be brought online and implemented in real-time as data is streaming. The current approach involves using a mean absolute deviation (MAD) threshold to recognize possible movement artifact, then excise it.

As described above, both the respiratory variation in the bioimpedance signal (termed *dz*) as well as the relative contribution of the cardiac and respiratory components (*dz* ratio) are compared to inferior vena cava (IVC) collapsibility index (dIVC) as a gold standard of volume status. The IVC is visualized longitudinally via B-mode ultrasound by a trained physician. Video clips of the IVC are recorded as the patient undergoes various breathing exercises such as deep breathing and forceful inhalation through the nose ("sniffing"). The minimum and maximum diameter of the IVC during these exercises is annotated by the physician, and the dIVC is calculated as the relative change in IVC diameter during the maneuvers. Equation 1 below displays the calculation of the collapsibility index.

Equation 1. $$dIVC = \frac{IVC\ Diameter_{max} - IVC\ Diameter_{min}}{IVC\ Diameter_{max}}$$

For the 75 subjects tested to date, analysis of dIVC to changes in impedance continues in response to respiratory maneuvers such as a sniff or deep breath continues. Using the new RMS methodology described above, we are now attempting a new analysis which will not require subjects in the future deployment of DRIVE to perform any respiratory maneuver. In this analysis we are comparing RMS changes produced by basal respiration to dIVC produced during deep breathing or sniff maneuvers. If this method is successful, a more continuous assessment of

volume status may be possible. We anticipate the potential to produce a simple binary assessment of volume status (fluid responsive vs. not fluid responsive or normo/hypervolemic vs hypovolemic).

For each of the ten patients on whom we collected bioimpedance data using the prototype, dIVC during the deep breathing and "sniff" maneuvers are compared to both the respiratory magnitude of the bioimpedance signal (Figure 6) as well as the cardiac contribution relative to the respiratory component (Figure 7). Even though we have only tested the prototype on 10 patients, the relationship between our bioimpedance metrics and the dIVC indices is approaching significance at a 5% level with p-values ranging from 0.06 to 0.14.

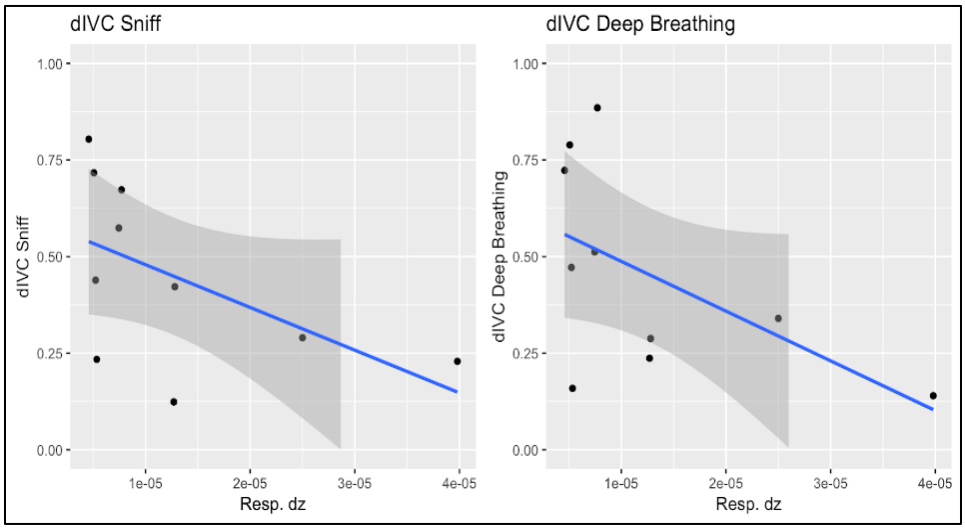


Figure 6: dIVC during maneuvers vs. respiratory bioimpedance magnitude.

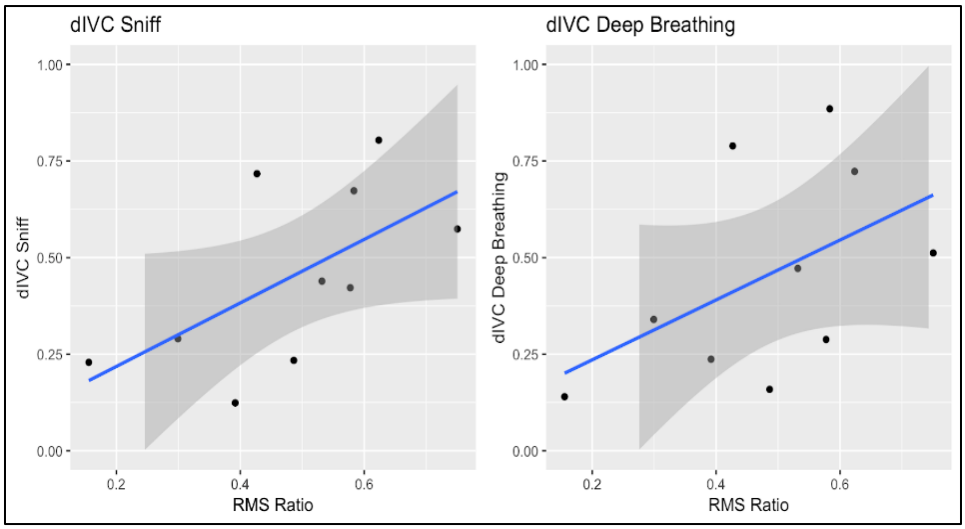


Figure 7: dIVC during maneuvers vs. cardiac contribution relative to the respiratory magnitude.

Other achievements
Development of a reduced size device prototype with a handheld tablet in collaboration with New Vital Signs (NVS). The unit is currently being tested alongside Biopac. This prototype has received positive feedback from patients for comfort and ease of application. This prototype will be used during patients testing in conjunction with Biopac system (Figure 8).

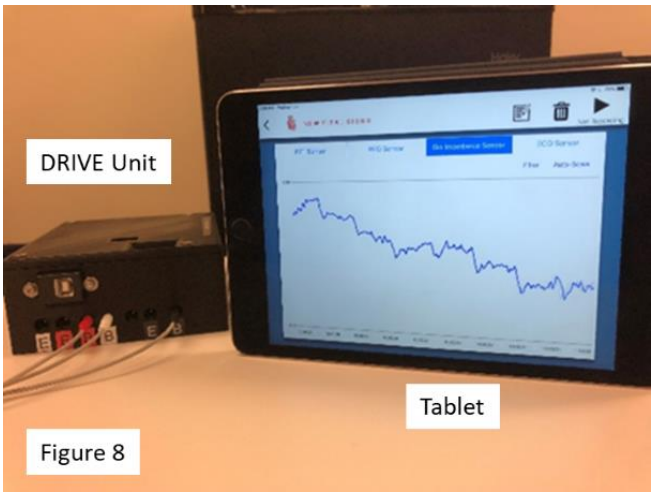


Figure 8

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

There are currently two undergraduate students participating on the RRS portion of this project as a part of their research coursework. The material collected will be presented in poster format for the Undergraduate Research Opportunity Program (UROP) research day on April 24, 2019. Poster will be included in the appendices

B. How were the results disseminated to communities of interest?

Two of our study clinical coordinators have presented data in a poster format at the Annual University of Michigan Barsan Research Forum.

- Abdelrahman Awad, MD; Amanda Pennington, MS; Brandon Cummings, BS; Christopher Fung, MD; Nik Theyyunni, MD; Michael Cover, MD; Kyle Gunnerson, MD; Mohamad Hakam Tiba, MD, MS; Kevin Ward, MD. Novel Non-Invasive Methodology for Dynamic Respiratory Impedance Volume Evaluate (DRIVE).

- Amanda Pennington, MS; Hakam Tiba, MD, MS; Brandon Cummings, BS; Varisha Essani; Claire Roberge; Kyle Gunnerson, MD; Kevin Ward, MD. Novel Monitoring of Tissue Microvasculature Oxygenation Using Resonance Raman Spectroscopy

Posters are provided in the appendices

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

The activities in all major task areas will be continued for the duration of the next reporting period. Human subjects' recruitment and testing will be continued with a target of 15 patients/ quarter. Data and project progress will continue to be divulged via presentations at scientific meetings both locally at the University of Michigan and nationally at the MHSRS during the next reporting period. Lastly, scientific manuscript writing will begin this reporting period with a target of 2-4 publications in major scientific and clinical journals covering all major tasks outlined in the report.

i. Continue patient recruitment
ii. Data analysis
iii. Data presentation (national and local)
iv. Manuscript writing
v. Technology and signal processing refinement

IMPACT:

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

Nothing to report at this time as we are still in testing phase. However, we are expecting a high level of impact by the end of the project on the understanding of volume status and tissue oxygenation and the ability to monitor and track these events noninvasively using bioimpedance and resonance Raman spectroscopy. The principle disciplines expected to be impacted by successful completetion include the clinical disciplines of emergency medicine, surgery, anesthesiology, critical care, nursing, and paramedical professionals.

b. What was the impact on other disciplines?

Nothing to report at this time, but we feel that in the future that the biomedical engineering and data science disciplines will also be impacted

c. What was the impact on technology transfer?

The RRS technology has been licensed to Pendar Technologies. RRS data and the performance of the device is being shared with Pendar to allow for continuous improvement and to develop regulatory approval and commercialization strategies.

The DRIVE technology has been licensed to New Vital Signs. DRIVE data and performance of the new prototype device is being shared with New Vital Signs to support continuous improvement of the technology and approaches to develop regulatory approval and commercialization strategies. New analytic approaches such as the RMS technique described above may be sources of new intellectual property in the future.

d. What was the impact on society beyond science and technology?

While there has been no direct societal impact of the project to date, the *long-term* impact of this research is expected to result in the development and deployment of technologies that noninvasively measure tissue oxygenation and volume status at earlier points of care that may:

1- Allow for rapid point of care diagnostic indicators of compensated shock states allowing for significantly earlier intervention in more far forward echelons of care.
2- Allow for improved therapeutic allocations by helping to drive therapy to objective measurable endpoints thus optimizing use of important resources such as resuscitation fluids including blood.
3- Allow for a greater uninterrupted continuum of care as casualties move from lower to higher levels of care including en-route care.
4- Allow for improved outcomes by preventing the early under or over-resuscitation of casualties.
5- Reducing iatrogenic and nosocomial complications associated with invasive monitoring.
6- Allow for improved resource allocation by providing indications for invasive monitoring.
7- Allow earlier termination of the use of invasive monitoring (when they are indicated) by transitioning invasive monitoring for noninvasive monitoring.
8- Allow for additional diagnostic and therapeutic end-points for casualties in intermediate care settings.
9- Allow for the eventual development of simpler closed-loop and decision assist algorithms and devices for early and late echelon of care settings including en-route care.

CHANGES/PROBLEMS:

a. Changes in approach and reasons for change

Nothing to report

b. Actual or anticipated problems or delays and actions or plans to resolve them
Nothing to report

c. Changes that had a significant impact on expenditures
Nothing to report

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

i. **Significant changes in use or care of human subjects:** None to report
ii. **Significant changes in use or care of vertebrate animals:** None to report
iii. **Significant changes in use of biohazards and/or select agents:** None to report

PRODUCTS:

a. Publications, conference papers, and presentations

i. **Journal publications.** Nothing to report
ii. **Books or other non-periodical, one-time publications.** Nothing to report
iii. **Other publications, conference papers, and presentations.**

An abstract has been submitted to the upcoming 2019 Military Health System Research Symposium (MHSRS) and is under consideration.

- Amanda J. Pennington, MS, Mohamad Hakam Tiba, MD, MS, Brandon C. Cummings, BS, Varisha Essani, Claire Roberge, Kyle Gunnerson, MD, Kevin R. Ward, MD. Novel Monitoring of Tissue Microvasculature Oxygenation Using Resonance Raman Spectroscopy.

Abstracts are included in the appendices

b. **Website(s) or other Internet site(s)**
Nothing to report

c. **Technologies or techniques**
The new DRIVE RMS and noise/movement detection analysis is new and will be reviewed in the near future for their potential/need for intellectual property protection. Discussion regarding these analytic techniques will take place with New Vital Signs in order to consider their incorporation into the DRIVE prototypes.

The new RRS clip will continue to be utilized and evaluated for ease of use. These were designed and 3-D printed by our team. Their final utilization and incorporation into the RRS system will be discussed with Pendar Technologies.

d. **Inventions, patent applications, and/or licenses**

i. RRS technology previously licensed (exclusively) to Pendar Technologies. Patent issued: Tissue Interrogation Spectroscopy: Ward KR, et al: United States Patent: 7,113,814. September 9, 2006

ii. DRIVE technology licensed (exclusively) to New Vital Signs Inc. U.S. Application No. 14/445,926. Evaluating Cardiovascular Health Using Intravascular Volume Filed July 29, 2014. Notice of claims allowance issued March 15, 2019.

e. **Other Products**
Created a one-page description of the methodology for patients and their families. Material is provided in the appendices.

f. Research material (e.g., Germplasm; cell lines, DNA probes, animal models);
Nothing to report

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

a. What individuals have worked on the project?

Name:	***Kevin Ward, MD***
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	*Oversight of data collection and analysis*
Funding Support:	

Name:	*Mohamad Hakam Tiba, MD, MS*
Project Role:	*Co-I*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*2*
Contribution to Project:	*Oversight of data collection and analysis*
Funding Support:	

Name:	***Kyle Gunnerson, MD***
Project Role:	*Co-I*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*medical consultation*
Funding Support:	

Name:	***Lena Napolitano, MD***

Project Role:	*Co-I*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*medical consultation*

Name:	***Pauline Park, MD***
Project Role:	*Co-I*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*medical consultation*

Name:	***Nik Theyyunni, MD***
Project Role:	*Co-I*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Perform ultrasounds*
Funding Support:	

Name:	***Christopher Fung, MD***
Project Role:	*Co-I*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Perform ultrasounds*
Funding Support:	

Name:	***Michael Cover, MD***
Project Role:	*Co-I*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	Perform ultrasounds
Funding Support:	

Name:	***Amanda Pennington, MS***
Project Role:	*Clinical Research Project Manager*

Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*6*
Contribution to Project:	*Subject screening and enrollment, regulatory and compliance management, data collection*
Funding Support:	

Name:	***Christopher Gillies, MS***
Project Role:	*Data Scientist*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*12*
Contribution to Project:	*data analysis*
Funding Support:	

Name:	***Brandon Cummings, BS***
Project Role:	*Research Staff*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	8
Contribution to Project:	Data analysis
Funding Support:	

Name:	***Anne Weitzel, BS***
Project Role:	*Research Staff*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	5
Contribution to Project:	*data analysis*
Funding Support:	

Name:	***Denise Poirier***
Project Role:	*Administrative Staff*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	*Scheduling of meetings*
Funding Support:	

Name:	***Abdelrahman Awad, MD***
Project Role:	*Clinical Coordinator*

Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	3
Contribution to Project:	*Screening and consenting patients, data collection*
Funding Support:	

Name:	***Justin Massey, BS***
Project Role:	*Research Staff*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Screening and consenting patients, data collection*
Funding Support:	

Name:	***Erin Bisco, BS***
Project Role:	*Research Staff*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Screening and consenting patients, data collection*
Funding Support:	

b. Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to report

c. What other organizations were involved as partners?
Pendar Technologies and New Vital Signs as manufacturers and commercial partners in developing the RRS and DRIVE technologies respectively.

d. Other.
Nothing to report

SPECIAL REPORTING REQUIREMENTS

a. COLLABORATIVE AWARDS:
None

b. **QUAD CHARTS:** Included with this report before the appendices

APPENDICES:

a. 2019 MHSRS Abstract

b. Clinical Coordinators Posters for Fourth Barsan Department of Emergency Medicine Research Forum
c. Student poster for University Research Opportunity Program Annual Symposium
d. Educational Material for
    i. One-page Protocol Description for DRIVE
    ii. One-page Protocol Description for RRS
e. Patent Materials
    i. DRIVE: Patent application
    ii. DRIVE: recent notice of patent allowance
    iii. RRS issued patent
f. PI Curriculum Vitae

Development and Testing of New Noninvasive Monitoring Tools for Prolonged Field Care Goal Directed Therapy

DM160225 Prolonged Field Care Research Award

PI: Kevin R. Ward, MD **Org:** University of Michigan **Award Amount:** $2,998,209

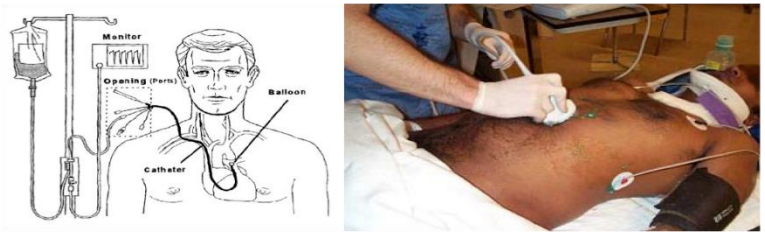
Study/Product Aim(s)

- Test and compare resonance Raman spectroscopy tissue oxygenation (RRS StO2) with other measures of tissue oxygen in polytrauma and complex surgical patients.
- Test and compare dynamic respiratory impedance volume evaluation(DRIVE) to other measures and surrogates of intravascular volume monitoring in polytrauma and complex surgical patients.
- Compare time series measurement RRS StO2 and DRIVE to patient outcomes including mortality and organ failure in order to support future trials supporting their use in PFC and pRDC.

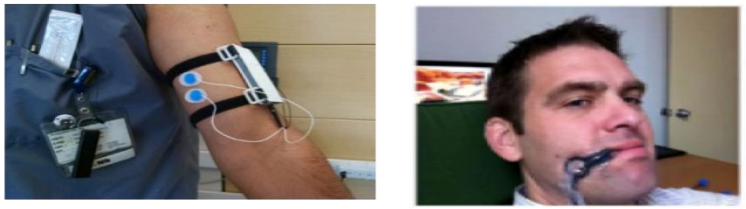
Approach

We will compare two newly developed noninvasive hemodynamic monitoring technologies to traditional hemodynamic monitoring in surgical patients for their suitability in goal directed therapy for potential future use in PFC and pRDC.

Comparing CVP, ScvO2 and IVC Ultrasound monitoring to



DRIVE and RRS StO2 monitoring for GDT in PFC



Clinical comparison of newly developed DRIVE and RRS StO2 buccal mucosa monitoring to traditional CVP, ScvO2, IVC ultrasound and other measures for potential in goal directed therapy in PFC

Timeline and Cost

Activities	CY18	CY19	CY20
Enroll 200-300Subjects			
Data comparisons to gold standard monitoring			
Monitoring comparisons to outcomes and interventions			
Estimated Budget ($K)	**$967,249**	**$1,003,281**	**$1,027,679**

Goals/Milestones

CY18Goal –

- ✓ Patient recruitment
- ✓ Comparison of new tools to standard measures

CY19 Goals –

- ✓ Additional patient recruitment
- ✓ Comparison of new tools to standard measures
- ✓ Begin comparisons of monitoring to outcomes and interventions

CY20 Goal – Production readiness

- ✓ Complete patient enrollment
- ✓ Complete data comparisons of monitors and comparisons to outcomes and interventions
- ✓ Work with industry partners on commercialization, trialing and scaling strategies

Comments/Challenges/Issues/Concerns. None.

Budget Expenditure to Date: $787,260

NOVEL MONITORING OF TISSUE MICROVASCULATURE OXYGENATION USING RESONANCE RAMAN SPECTROSCOPY

Amanda J. Pennington, MS[1,2], Mohamad Hakam Tiba, MD, MS[1,2], Brandon C. Cummings, BS[1,2], Varisha Essani[1,2], Claire Roberge[1,2], Kyle Gunnerson, MD[1,2], Kevin R. Ward, MD[1,2]

[1] Department of Emergency Medicine, University of Michigan. Ann Arbor Michigan.
[2] Michigan Center for Integrative Research in Critical Care (MCIRCC), University of Michigan, Ann Arbor, Michigan.

Introduction: The ability to monitor the critically ill patient noninvasively remains a challenge especially in settings outside the intensive care unit. Measurements of oxygenation at the level of a specific tissue might offer sensitive information to guide therapeutic modalities and decision making in the management of the critically ill. We examined the ability of Resonance Raman Spectroscopy (RRS) to monitor tissue hemoglobin oxygenation (StO_2) noninvasively in a post-surgery setting and compared its performance with conventional central venous hemoglobin oxygen saturation ($ScvO_2$). RRS is a novel optical technique capable of providing information on the vibrational and electronic properties of compounds, including oxy- and deoxyhemoglobin. RRS can be used to interrogate tissue hemoglobin levels (StO_2) by producing signals heavily dominated by venous blood. Thus, the resulting aggregate StO_2 is reflective of the post-extraction compartment of the tissue similar to $ScvO_2$.

Methods: Post-surgery patients who had a central venous catheter in place were consented and recruited. StO_2 measurements were obtained using RRS with a sensor placed on the buccal mucosa inside the mouth. Simultaneous blood samples were drawn from the indwelling central catheter. An algorithm that utilizes the spectral peaks was used to calculate the StO_2 which were compared to $ScvO_2$ measured by co-oximetry (gold standard).

Results: Eighteen patients with a mean(SD) age 64(15) years old were consented and recruited. Mean(SD) StO_2 and $ScvO_2$ were 64(11.1)% and 67(8.0)% respectively ($r=0.57$, $p<0.013$). A paired t-test showed no significant difference between the StO_2 and $ScvO_2$ with a mean(SD) difference of 4(10.3)% (*95%CI = [-1.3, 9], p = 0.13*). Receiver Operator Characteristic (ROC) curves for predicting $ScvO_2$ at thresholds of $ScvO_2$ above and below 65, and 70% demonstrated the high predictive power of StO_2 with areas under the curve of 0.74 and 0.83 respectively. Improvements to the clip are currently underway to allow the sensor to penetrate deeper into the tissue of the buccal mucosa.

Conclusions: RSS is showing promise as a non-invasive alternative to $ScvO_2$. StO_2 measurements taken using RSS are highly correlated with $ScvO_2$, which is an important measure of tissue oxygenation. Because of its non-invasive nature, RSS may serve as a faster, safer, and more cost-effective way to assess patient tissue oxygenation, aiding in the diagnosis and treatment of conditions such as sepsis, trauma, heart failure and other critical states.

Novel Monitoring of Tissue Microvasculature Oxygenation Using Resonance Raman Spectroscopy

Amanda Pennington, MS[1,2]; Hakam Tiba, MD, MS[1,2]; Brandon Cummings[1,2]; Varisha Essani[1,2]; Claire Roberge[1,2]; Kyle Gunnerson, MD[1,2]; Kevin Ward, MD[1,2]

[1] Department of Emergency Medicine, [2]Michigan Center for Integrative Research in Critical Care (MCIRCC)
University of Michigan, Ann Arbor, MI

CENTER FOR INTEGRATIVE RESEARCH IN CRITICAL CARE
UNIVERSITY OF MICHIGAN

DEPARTMENT OF EMERGENCY MEDICINE
UNIVERSITY OF MICHIGAN HEALTH SYSTEM

ABSTRACT

Introduction: The ability to monitor the critically ill patient noninvasively remains a challenge especially in settings outside the intensive care unit. Measurements of oxygenation at the level of a specific tissue might offer sensitive information to guide therapeutic modalities and decision making in the management of the critically ill. We examined the ability of Resonance Raman Spectroscopy (RRS) to monitor tissue hemoglobin oxygenation (StO_2) noninvasively in a post-surgery setting and compared its performance with conventional central venous hemoglobin oxygen saturation ($ScvO_2$). RRS is a novel optical technique capable of providing information on the vibrational and electronic properties of compounds, including oxy- and deoxyhemoglobin. RRS can be used to interrogate tissue hemoglobin levels (StO_2) by producing signals heavily dominated by venous blood. Thus, the resulting aggregate StO_2 is reflective of the post-extraction compartment of the tissue similar to $ScvO_2$.

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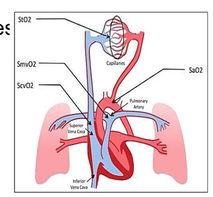
Conclusions: RSS is showing promise as a non-invasive alternative to $ScvO_2$. StO_2 measurements taken using RSS are highly correlated with $ScvO_2$, which is an important measure of tissue oxygenation. Because of its non-invasive nature, RSS may serve as a faster, safer, and more cost-effective way to assess patient tissue oxygenation, aiding in the diagnosis and treatment of conditions such as sepsis, trauma, heart failure and other critical states.

INTRODUCTION

Resonance Raman Spectroscopy (RRS) is promising as a non-invasive method to monitor tissue oxygenation status

- Provide early diagnosis of shock
- Reduce oxygen debt and allow better treatment resources
- Increase patient comfort
- Decrease common problems associated with central venous catheters such as infection and injury to the vein



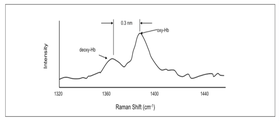
Approximately 70-80% of the blood in tissue resides in the venous system, making the hemoglobin oxygen saturation (StO_2) as measured by RRS a hopeful alternative to the invasively measured $ScvO_2$

INTRODUCTION

Changes in peripheral VO_2 resulting in lowering of StO_2 will parallel those of more central locations such as those used for $ScvO_2$ in the right atrium/superior vena cava or the $SmvO_2$ in the pulmonary artery.

Spectroscopic techniques produce hemoglobin signals that are heavily dominated by venous blood.

RRS provides information on the unique vibrational and electronic properties of compounds based on the well-defined and narrow Resonance Raman Spectral fingerprint of oxy and deoxy-hemoglobin and direct measurement of hemoglobin concentration in the illuminated volume.



Interfering chemicals such as melanin, tissue composition or myoglobin will not affect the signal.

RRS in the near ultraviolet spectrum matches the electronic energy state of heme, allowing measurement of both the oxygenated and deoxygenated heme states.

METHODS

- This study is approved for human subjects research by the University of Michigan IRB HUM00067675
- Critically ill patients with a central venous catheter in place were recruited from the cardiovascular ICU as well as the catheterization lab.

- Informed consent was obtained prior to any testing.
- The RRS sensor was placed on the buccal mucosa and StO_2 measurements were obtained and collected for 15 minutes.

- At the conclusion of testing, a blood sample was collected from the distal port of the central catheter and the RRS measurement at that time was recorded.
- An algorithm that utilizes the spectral peaks was used in GraphPad Prism 6 statistical software to calculate the StO_2 which were compared to $ScvO_2$.

RESULTS

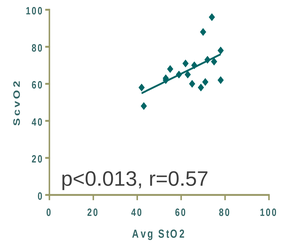
Eighteen patients with an average(SD) age of 64(15) y/o were tested

3 females, 15 males

All $StVO_2$ values are multiplied by 0.9

RESULTS

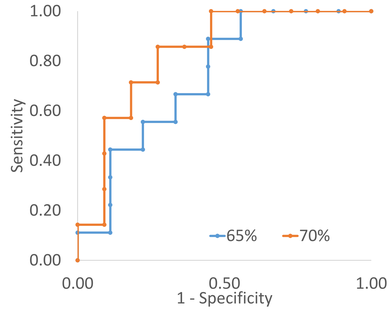
	StO2	ScvO2
Mean	63.8	67.7
Std. Deviation	11.0	11.3
Lower 95% CI of mean	58.3	62.1
Upper 95% CI of mean	69.3	73.3
Minimum	42.0	48.0
Maximum	78.0	96.0



The scatter plot demonstrates the significant correlation between StO_2 and $ScvO_2$.

A paired t-test showed no significant difference between StO_2 and $ScvO_2$. Average(SD) difference of 4(10)% (*95%CI: 0.3% - 1.2%, p=0.128)*

Receiver operating characteristic (ROC) curve for StO_2 at thresholds of $ScvO_2$ between 65% and 70% have demonstrated high predictive power of StO_2 with area under the curve between 0.74 and 0.83 (*p < 0.006*).



CONCLUSIONS

RRS is showing promise as a non-invasive alternative to $ScvO_2$.

StO_2 measurements taken using RRS are highly correlated with $ScvO_2$, which is an important measure of tissue oxygenation.

Because of its non-invasive nature, RRS may serve as a faster, safer, and more cost-effective way to assess patient tissue oxygenation, aiding in the diagnosis and treatment of conditions such as sepsis, trauma, heart failure and other critical states.

ACKNOWLEDGEMENT

This work has been supported by a grant award from the Department of Defense DM160294

Michigan Center for Integrative Research in Critical Care (MCIRCC)

MICHIGAN MEDICINE
UNIVERSITY OF MICHIGAN

MCIRCC

Non-Invasive Methodology for Dynamic Respiratory Impedance Volume Evaluation (DRIVE)

Abdelrahman Awad MD[1,2], Amanda Pennington MS[1,2], Brandon Cummings BS[1,2], Christopher Fung MD [1], Nik Theyyunni MD[1], Michael Cover MD [1] , Erin Bisco[1,2], Kyle Gunnerson MD[1,2], Hakam Tiba MD[1,2], Kevin Ward MD[1,2]

[1] Department of Emergency Medicine, [2] Michigan Center of Integrative Research in Critical Care (MCIRCC).

CENTER FOR INTEGRATIVE RESEARCH IN CRITICAL CARE, UNIVERSITY OF MICHIGAN

DEPARTMENT OF EMERGENCY MEDICINE, UNIVERSITY OF MICHIGAN HEALTH SYSTEM

INTRODUCTION

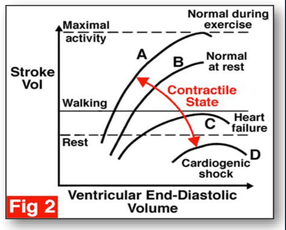
- Accurate assessments of circulating volume status is an integral part of the critically ill patient management.
- *Dynamic relationship between venous return, the function of the right ventricle, and its interaction with lung mechanics as key determinants of estimating intravascular volume status.*

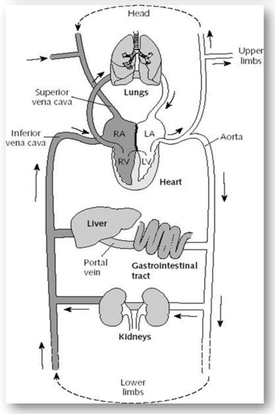
Systemic Venous System:
- 30x more compliant than arteries
- Conduit and Reservoir of the circulating blood

What changes Venous Return (VR)
- Changes in mean systemic pressure (Total blood volume)
- Changes in right atrial pressure or venous resistance

- ***Respiratory pump***
  - Positive pressure ventilation decreases VR
  - Tidal ventilation increases VR
- Therapeutic Interventions (fluids, vasopressors)

Current assessment of Intravascular Volume Status
- Traditional physical exam
  - Compensatory mechanisms
  - Flip of a coin
- Monitoring of CVP or PAP
  - Skilled physician
  - Severe complications
  - Only pressure is reported
- Stroke Volume Variation (SVV) and Pulse Pressure Variation (PPV)
- Passive leg lift and NICO



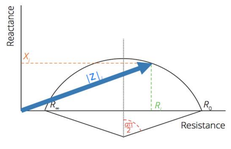


BIOIMPEDANCE

Opposition to an electrical current flow through tissues
- Passive bioelectricity. Tissues' response to external electrical excitation.
- Cumulative effect of individual impedances

Blood has a distinct effect on bioimpedance:
- Good conductor of electricity
- More blood present → lower bioimpedance

RESPIRATION affects bioimpedance indirectly
- Thoracic pressure gradient
- Changes in venous return



Normal respiration

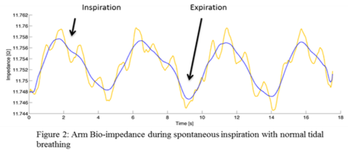


Figure 2: Arm Bio-impedance during spontaneous inspiration with normal tidal breathing

Forced inspiration

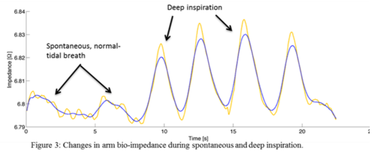


Figure 3: Changes in arm bio-impedance during spontaneous and deep inspiration.

AIM

- **Utilize bioimpedance measurements of the arm to assess dynamic changes in the peripheral venous volume in response to respiration.**
- **Compare it to changes in IVC diameter due to the same respiration.**

METHODS

Demographics:
- Total number 72 subjects have been tested.
- Mean age is 50 years, with standard deviation of 18.

Clinical Presentation:
- Polytrauma subjects.
- Complex operative and post-operative surgical subjects.
- Subjects undergoing hemodialysis.

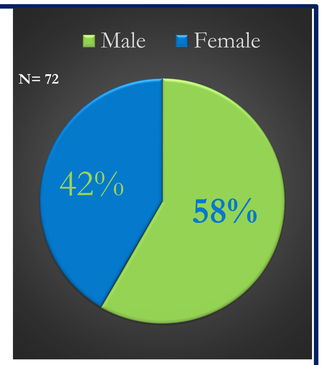
Ultrasound:
- Performed by three EM US- physicians.

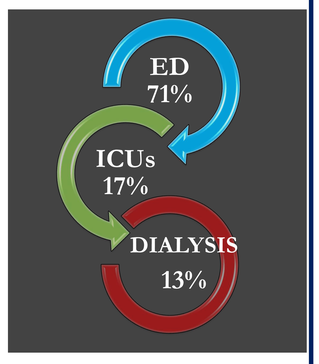
IVC abdominal ultrasound:
- Long axis B-mode,
- 2-3 cm from the cavoatrial junction.
- IVC minimum and maximum diameter measurements during normal breathing, during a deep breath and during a sniff.

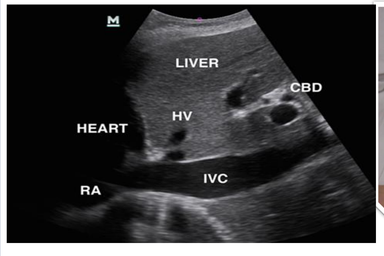
Carotid Artery ultrasound:
- Measured carotid artery flow time and heart rate to calculate corrected carotid artery flow time.

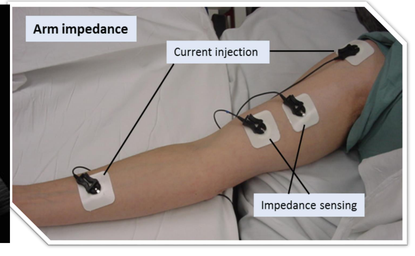
Echocardiograph:
- Measured left ventricle outflow tract velocity time integral.

Bioimpedance:
- Average of 30 minutes of running a (0.1-1) mA electrical current through a tetra-polar lead system.
- Data collected and saved using Biopac software.
- Data marked during the respiratory maneuvers to compare it to the ultrasound results.



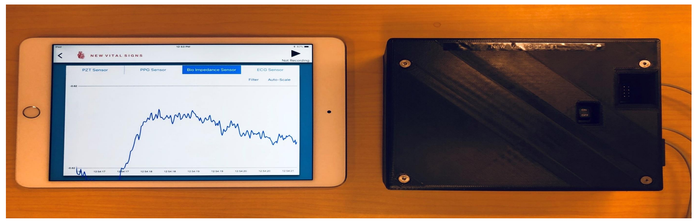




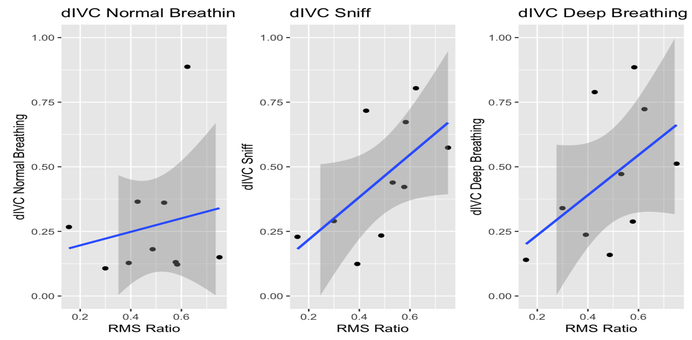


RESULTS

- The new prototype, developed by New Vital Signs, Inc., allows for a consistent, clean signal to be captured.



- Using the bioimpedance signal from the eight subjects tested and analyzed using the new device, the cardiac-respiratory amplitude ratio was able to track changes in IVC during maneuvers with moderate precision (R2 = 0.45).



	R-Squared	R	p
dIVC Normal Breathing	0.27	0.52	0.46
dIVC Sniff	0.54	0.73	0.11
dIVC Deep Breathing	0.55	0.74	0.10

- Further testing with the prototype is currently underway.

CONCLUSION

- Real-time dynamic changes in limb bioimpedance may be able to reflect a wide range of dynamic dIVC changes.
- This technique might be a suitable surrogate for monitoring intravascular volume status of critically ill patients.

ACKNOWLEDGEMENT

- This work has been supported by a grant award from the Department of Defense (DM160294)
- MCIRCC

Novel Monitoring of Tissue Microvasculature Oxygenation Using Resonance Raman Spectroscopy

Claire Roberge[1,2]; Varisha Essani[1,2]; Amanda Pennington, MS[1,2]; Brandon Cummings[1,2]; Kevin Ward, MD[1,2]; Mohamad H Tiba, MD, MS[1,2]

[1] Department of Emergency Medicine, [2]Michigan Center for Integrative Research in Critical Care (MCIRCC)
University of Michigan, Ann Arbor, MI

ABSTRACT

The Raman Resonance Spectroscopy (RRS) technology was developed in order to find an alternative and a potential surrogate to the measurements of mixed venous oxygen saturation (SmvO2) using a central line, which is an invasive way to monitor cardiac patients or other hospitalized patients. RRS is used through a light probe that is placed on the inner cheek of the patient. The StO2 values will be compared to a blood case sample. We utilized several statistical methods including Bland/Altman, t tests, and regression models that allow us to compare oxygen saturation in the blood versus the RRS tissue levels. Our data analysis already suggests that the RRS technology is very accurate and analogous to the SmvO2 measurements. The hope is that RRS can be incorporated into modern medicine in support of a central line as a noninvasive and safer alternative.

INTRODUCTION

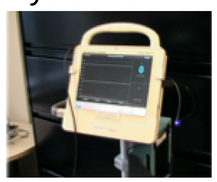
Measuring oxygen saturation of mixed venous blood (SmvO2) is an important factor in monitoring critically ill patients, offering information essential to the prognosis and treatment management of these patients. In typical clinical settings, monitoring venous oxygenation can be an invasive process and carries the risk of complications. The use of non-invasive technology can help reduce the risk and make these measurements more accessible. Resonance Raman Spectroscopy (RRS) is a novel optical technique capable of providing information on the vibrational and electronic properties of compounds, including oxy- and deoxyhemoglobin.

INTRODUCTION

Spectroscopic techniques produce hemoglobin signals that are heavily dominated by venous blood. In this study, RRS was used to monitor tissues oxygen saturation (StO2) noninvasively in a post-surgical setting and compared to the mixed venous oxygen saturation standard. We hypothesize that StO2 will reflect changes in ScvO2 and act as a surrogate non-invasive measure. Thus, the resulting aggregate StO2 should be reflective of the post extraction compartment of the tissue similar to SmvO2.

METHODS

Critically ill patients with a central venous catheter were recruited from the Cardiovascular Center. Informed consent was obtained from each patient before beginning any measurements. The RRS was placed in both the right and left buccal for five minutes each, approximately ten minutes in totality. StO2 measurements were collected over this time period. At the end, a blood sample was obtained from the most distal port of the central line and a final RRS measurement was obtained at this time, as well. The StO2 values were compared to the ScvO2 values using various statistical analysis methods.

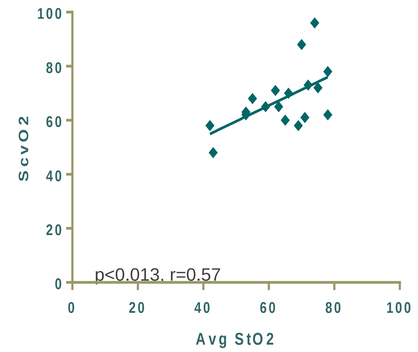


RESULTS

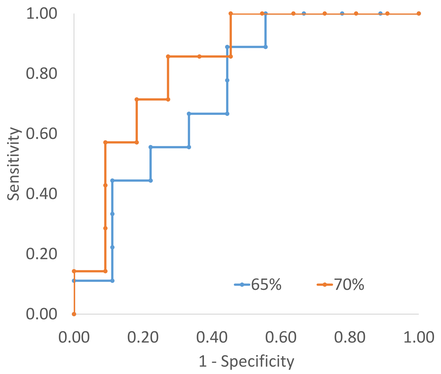
Twelve patients with an average(SD) age of 64(15) y/o were tested.
__ males and ___ females
All StO2 values are multiplied by 0.9.

RESULTS

	StO2	ScvO2
Mean	63.8	67.7
Std. Deviation	11.0	11.3
Lower 95% CI of mean	58.3	62.1
Upper 95% CI of mean	69.3	73.3
Minimum	42.0	48.0
Maximum	78.0	96.0



- The scatter plot demonstrates the significant correlation between StO_2 and $ScvO_2$.
- A paired t-test showed no significant difference between StO_2 and $ScvO_2$. Average(SD) difference of 4(10.)% (*95%CI: -1.2%, 9%*, *p=0.128)*
- Receiver operating characteristic (ROC) curve for StO_2 at thresholds of $ScvO_2$ between 65% and 70% have demonstrated high predictive power of StO_2 with area under the curve between 0.74 and 0.83 (*p < 0.006*).



CONCLUSIONS

RRS is showing promise as a non-invasive alternative to ScvO2. StO2 measurements taken using RRS are highly correlated with ScvO2, which is an important measure of tissue oxygenation. Because of its non-invasive nature, RRS may serve as a faster, safer, and more cost effective way to assess patient tissue oxygenation, aiding in the diagnosis and treatment of conditions and critical states of health.

Assessing Intravascular Volume Status using Limb Bioimpedance Changes (DRIVE)

Background: Many acute and chronic medical and surgical conditions require providers to make accurate assessments of a patient's circulating volume status and whether the patient will favorably respond to being given additional intravenous fluids or if providing these fluids will place them at risk for additional complications.

Recently, clinicians and physiologists have realized the significance of a patient's functional response to addition or removal of volume. As a result, the use of real-time ultrasound to view movement of the wall of the inferior vena cava (IVC) during respiration has been demonstrated to provide a powerful tool in estimating volume status and guiding intravenous fluid treatment. However, the use of ultrasound is expensive, requires an experienced user to make the measure, cannot be used continuously, and has limitations based on body habitus. Bio-impedance of an extremity is based in large part on the volume of blood within that extremity. Since blood is a good electrical conductor, impedance will decrease or increase if more or less blood volume exists in the extremity, respectively.

Since spontaneous inspiration pulls blood from the extremities thus increasing its impedance, the degree to which this will happen is dependent on how full the right heart central venous circulation is. It is thus possible to design methods to provocatively determine the degree of volume movement form the extremity as a mean to determine if patients are in need of fluid administration or if they have excess circulatory volume.

Our previous investigations show that these changes in limb impedance parallel those changes in the IVC that are noted on ultrasound during spontaneous breathing and deep breathing. As indicated earlier, this geometric change in the IVC produced by spontaneous respiration has been used as an indicator of volume responsiveness to guide fluid resuscitation and demonstrated performance accuracies of over 90% in predicting and managing fluid volume decision over the physical exam or invasive measures such as CVP monitoring.

Specific Aim: The aim is to evaluate the use of bioimpedance measurements of the upper or lower extremities as a non-invasive method to assess intravascular volume status by evaluating the dynamic change in peripheral venous volume in response to ventilation and by comparing those changes to the dynamic changes in the Inferior Vena Cava (IVC) diameter in response to the same ventilation.

We hypothesize that Dynamic changes in limb impedance during respirations will parallel those changes in IVC diameter during both spontaneous and positive pressure ventilation. Bioimpedance will have similar performance characteristics to IVC Ultrasound used in humans undergoing traditional monitoring. This level of performance will be high enough to allow substitution of non-invasive measures in future clinical trials.

For questions, comments, or concerns please see the contact information listed below. This study has been approved by IRBMED as HUM0067675. This study is funded by the Department of Defense (Grant #DM160294).

Contact Information

PI: Kevin Ward
Co-I: Kyle Gunnerson, Ross Kessler, Lena Napolitano, Pauline Park, Nik Theyyunni, Hakam Tiba
Research Staff: Amanda Pennington (734-936-5947), Justin Massey, Erin Bisco

Non-Invasive Monitoring of the Critically Ill and Injured Patient (Raman)

Background: The ability to use traditional vital signs such as blood pressure, heart rate, respiratory rate, temperature, and even pulse oximetry to determine the severity of illness or injury in patients or as a guide to therapy is acknowledged to pose severe limitations. Reasons for this include but are not limited to the ability of individuals to compensate using physiologic reserve, the type of injury or illness incurred and the effects of chronic physiology and polypharmacy such as hypertension and the use of cardiovascular medications. There is a great need to develop monitoring variables which are more reflective of the underlying physiology of injury and determined less by chronic disease or baseline states of health.

It is possible now to monitor aspects of physiology in a noninvasive manner that provide greater insight into the actual metabolism of the individual. While this type of metabolic monitoring is helpful, it is not always practical from either an economic or technical aspect. However, under proper protocols it would be helpful to understand what other more easily obtained monitoring information when analyzed by newer methods may provide a reasonable and informative substitute for these types of advanced monitors. In addition the relationship of these signals to inflammatory and coagulation changes which occur during the course of critical illness and injury will be helpful in understanding their potential value. The ability to use newer signals or combinations of existing signals analyzed by newer techniques to monitor patients would have great value. The ability to detect patient deterioration earlier would allow health care providers to intervene earlier and save lives. Use of this same approach could help guide treatments to more refined endpoints ensuring that patients are neither under or over resuscitated. These techniques would be particularly helpful for triage of mass casualties in helping to determine who is in most urgent need of treatment or who is simply too ill to benefit from treatment. Part of the major impetus for this research is to develop better monitoring approaches for wounded military personnel. However, such monitoring would also be useful for home health monitoring. Thus a wide spectrum of patients will find benefit from this research.

Specific Aim: The Pendar Medical Raman Spectroscopy device uses a special probe which can be placed on the buccal mucosa for measurement of oral mucosa oxygen saturation. The probe's diameter is less than the size of a nickel. Several pictures and a video are provided for the device. The probe might be left in the oral cavity for up to 4 hours at a time. Patients in whom probes will be left for this period of time will be those who are mechanically ventilated and sedated. All probes are disposable or can be chemically sterilized with Cidex between uses. The device is experimental and uses the technology of resonance Raman spectroscopy. This spectroscopy method relies on a laser producing light at a wavelength of 405 nm at power of less than 5 mW.

For questions, comments, or concerns please see the contact information listed below. This study has been approved by IRBMED as HUM0067675. This study is funded by the Department of Defense (Grant #DM160294).

Contact Information

PI: Kevin Ward
Co-I: Kyle Gunnerson, Ross Kessler, Lena Napolitano, Pauline Park, Nik Theyyunni, Hakam Tiba
Research Staff: Amanda Pennington (734-936-5947), Justin Massey, Erin Bisco

US 20150031966A1

(19) **United States**

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(10) **Pub. No.: US 2015/0031966 A1**

(43) **Pub. Date: Jan. 29, 2015**

Ward et al.

(54) **EVALUTATING CARDIOVASCULAR HEALTH USING INTRAVASCULAR VOLUME**

(71) Applicant: **THE REGENTS OF THE UNIVERSITY OF MICHIGAN**, Ann Arbor, MI (US)

(72) Inventors: **Kevin Ward**, Superior Twp., MI (US); **Mohamad Hakam Tiba**, Ann Arbor, MI (US); **James M. Blum**, Ann Arbor, MI (US)

(21) Appl. No.: **14/445,926**

(22) Filed: **Jul. 29, 2014**

Related U.S. Application Data

(60) Provisional application No. 61/859,615, filed on Jul. 29, 2013.

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A61B 5/026	(2006.01)
A61B 5/0295	(2006.01)
A61B 5/0205	(2006.01)

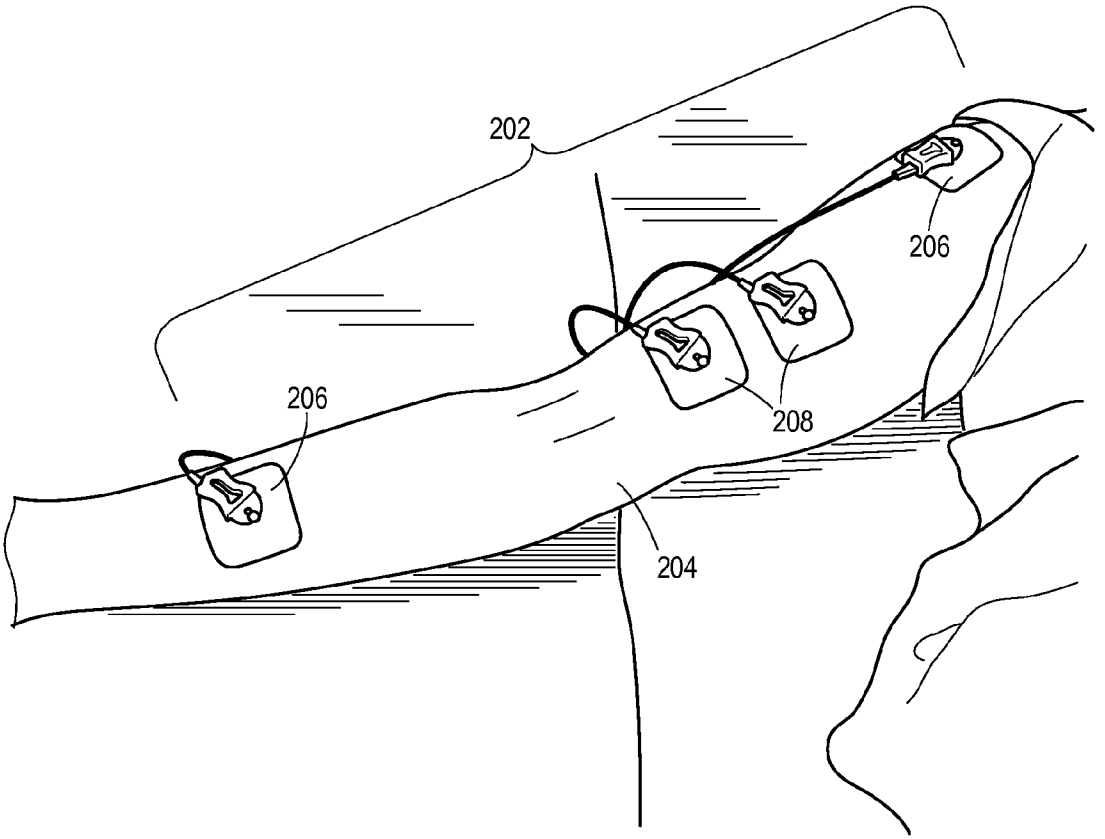
(52) **U.S. Cl.**

CPC ***A61B 5/4836*** (2013.01); ***A61B 5/0295*** (2013.01); ***A61B 5/0205*** (2013.01); ***A61B 5/0261*** (2013.01); ***A61B 5/7282*** (2013.01); ***A61B 8/06*** (2013.01); ***A61B 5/0075*** (2013.01); *A61B 5/02108* (2013.01)

USPC **600/301**; 600/506; 600/484

(57) **ABSTRACT**

Non-invasive monitoring of cardiovascular health is performed by monitoring changes in the volume of blood in the venous side of the vascular system. The blood volume changes are determined from measurements of bioimpedance of limbs or neck, in particular changes in bioimpedance in response to blood modulating events performed on the limbs or neck, where bioimpedance is measured and compared before and after such events.



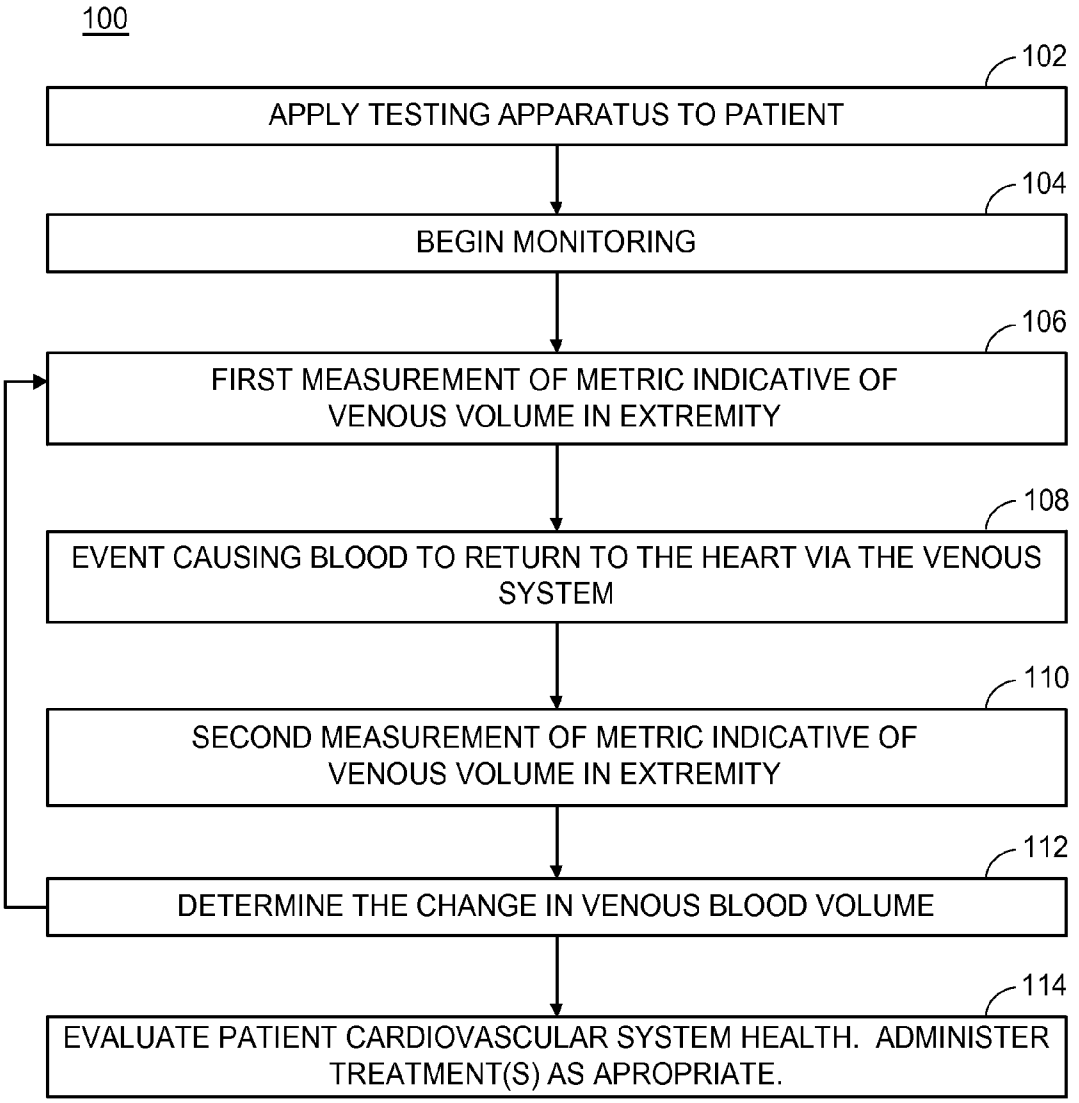

100
102
APPLY TESTING APPARATUS TO PATIENT
104
BEGIN MONITORING
106
FIRST MEASUREMENT OF METRIC INDICATIVE OF VENOUS VOLUME IN EXTREMITY
108
EVENT CAUSING BLOOD TO RETURN TO THE HEART VIA THE VENOUS SYSTEM
110
SECOND MEASUREMENT OF METRIC INDICATIVE OF VENOUS VOLUME IN EXTREMITY
112
DETERMINE THE CHANGE IN VENOUS BLOOD VOLUME
114
EVALUATE PATIENT CARDIOVASCULAR SYSTEM HEALTH. ADMINISTER TREATMENT(S) AS APROPRIATE.

FIG. 1

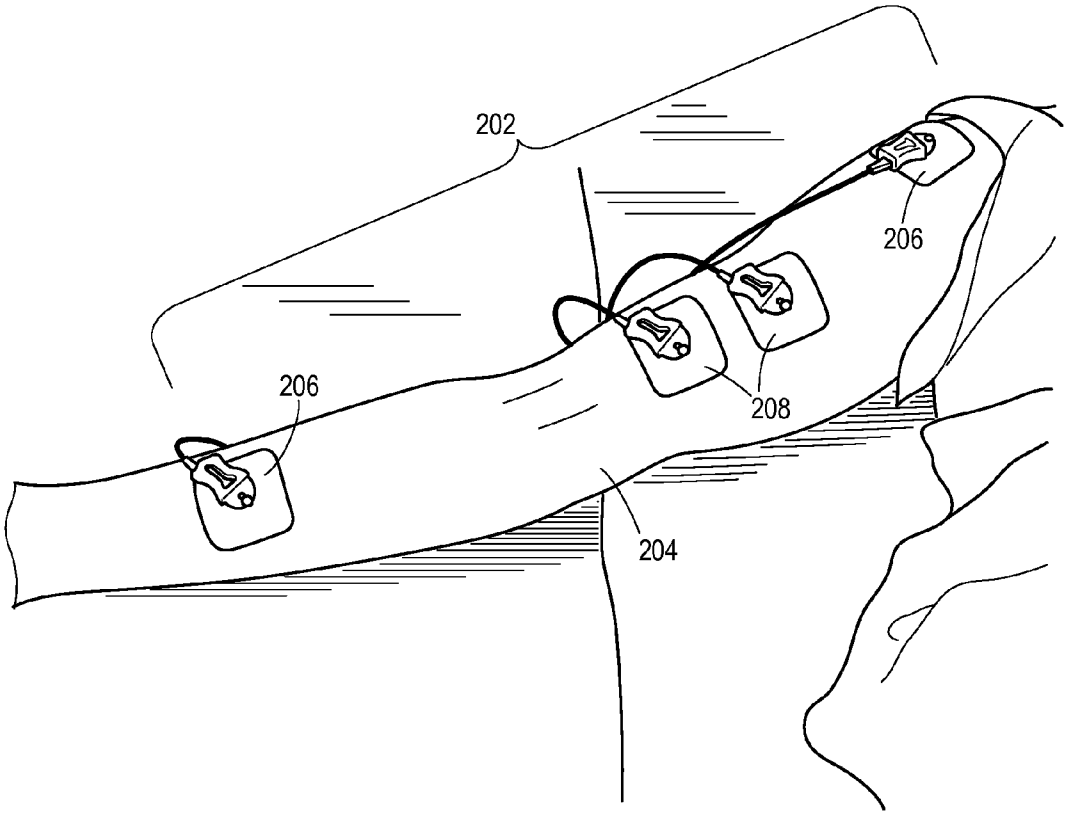


FIG. 2

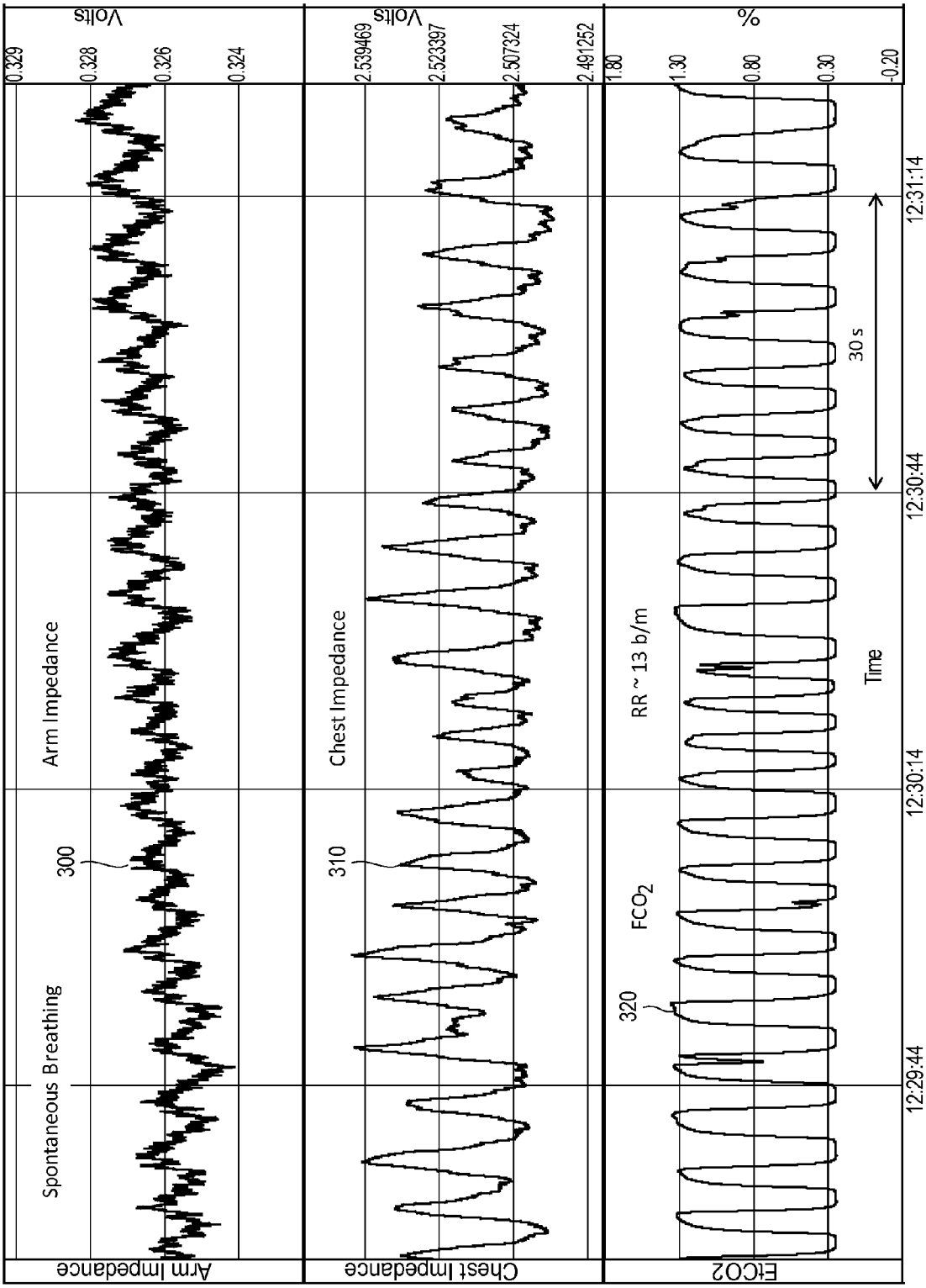

Arm Impedance
Volts
0.329
0.328
0.326
0.324
Spontaneous Breathing
300
Arm Impedance
Chest Impedance
Volts
2.539469
2.523397
2.507324
2.491252
310
Chest Impedance
EtCO2
%
1.80
1.30
0.80
0.30
-0.20
320
FCO2
RR ~ 13 b/m
30 s
Time
12:29:44
12:30:14
12:30:44
12:31:14

FIG. 3

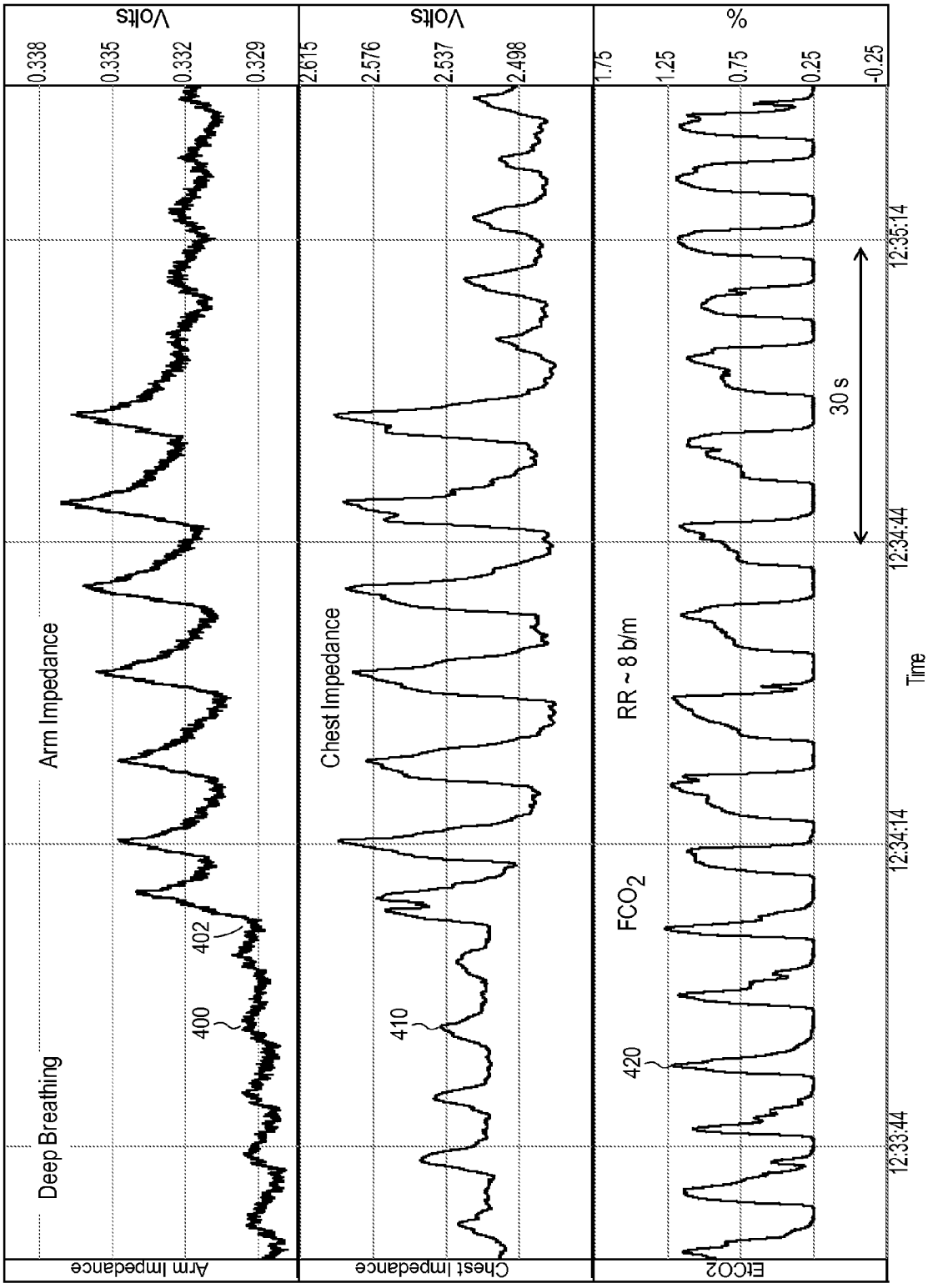

Arm Impedance
Volts
0.338
0.335
0.332
0.329
Deep Breathing
400
402
Chest Impedance
Volts
2.615
2.576
2.537
2.498
410
ETCO2
%
1.75
1.25
0.75
0.25
-0.25
RR ~ 8 b/m
FCO2
420
30 s
12:33:44
12:34:14
12:34:44
12:35:14
Time

FIG. 4

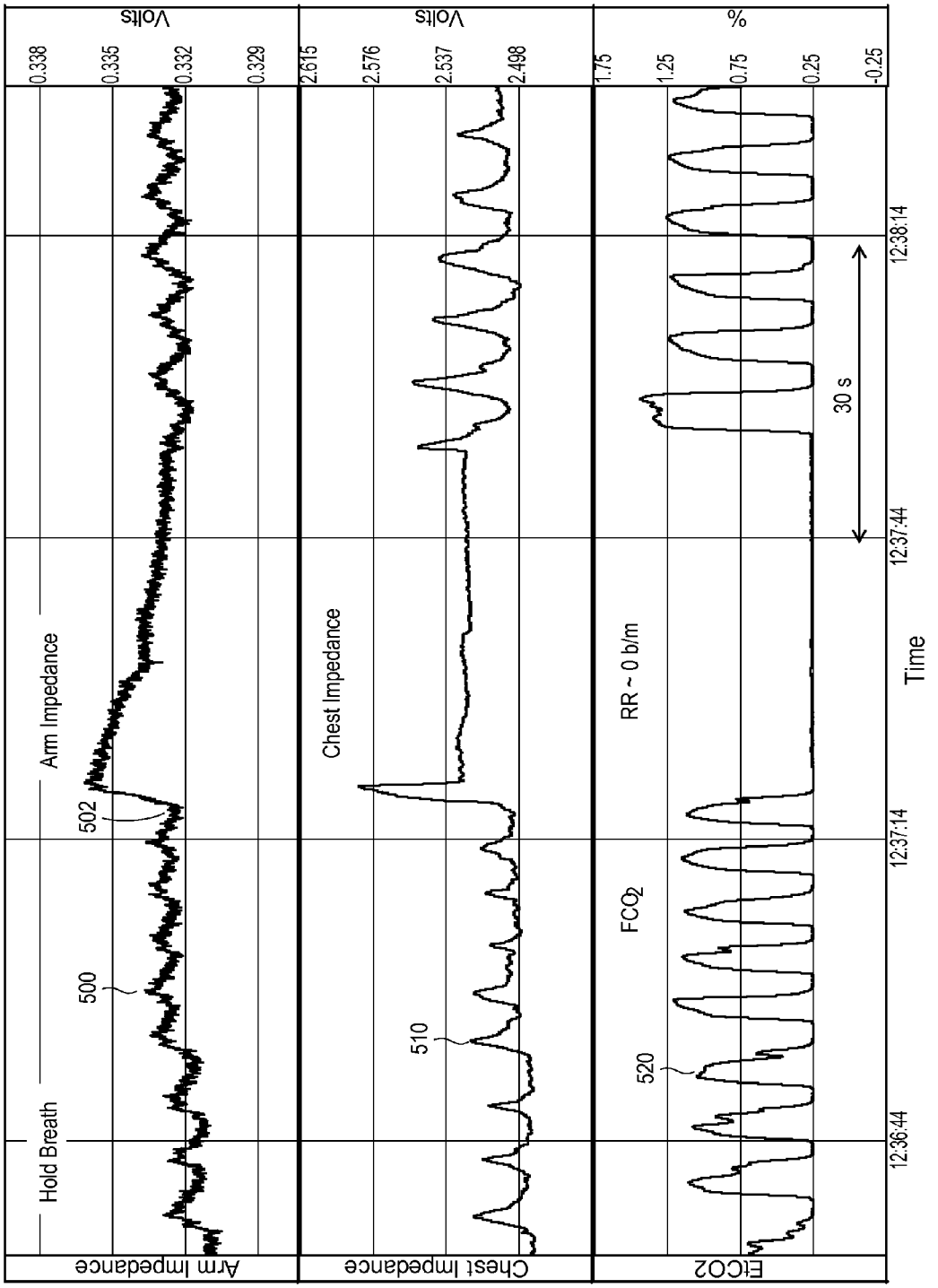

Arm Impedance
Volts
0.338
0.335
0.332
0.329
Hold Breath
Arm Impedance
500
502
Chest Impedance
Volts
2.615
2.576
2.537
2.498
Chest Impedance
510
EtCO2
%
1.75
1.25
0.75
0.25
-0.25
RR ~ 0 b/m
FCO2
520
30 s
12:36:44
12:37:14
12:37:44
12:38:14
Time

FIG. 5

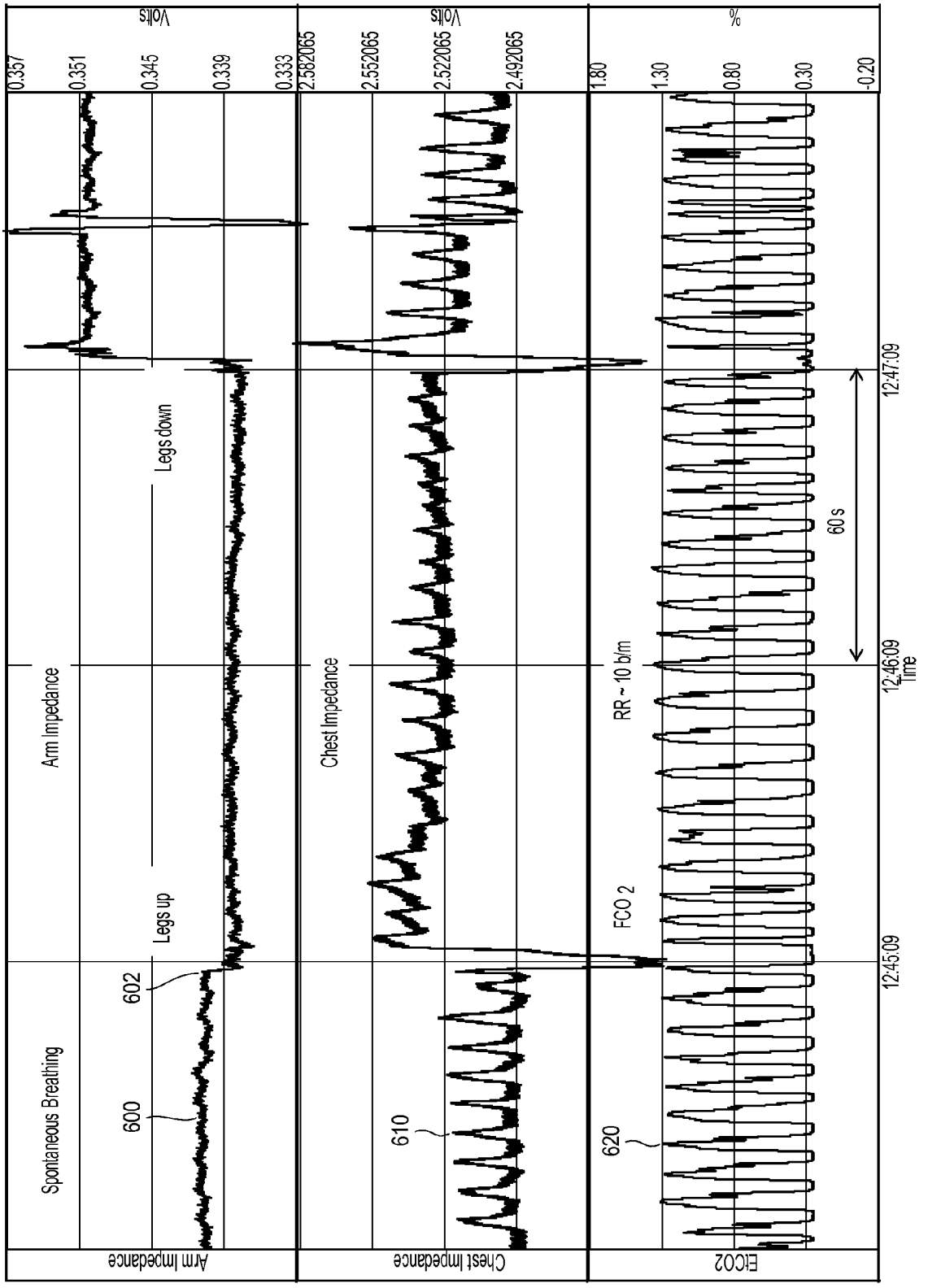

Arm Impedance
Volts
0.357
0.351
0.345
0.339
0.333
Spontaneous Breathing
Arm Impedance
Legs up
Legs down
600
602
Chest Impedance
Volts
2.582065
2.552065
2.522065
2.492065
Chest Impedance
610
EtCO2
%
1.80
1.30
0.80
0.30
-0.20
FCO 2
RR ~ 10 b/m
620
60 s
12:45:09
12:46:09
12:47:09
Time

FIG. 6

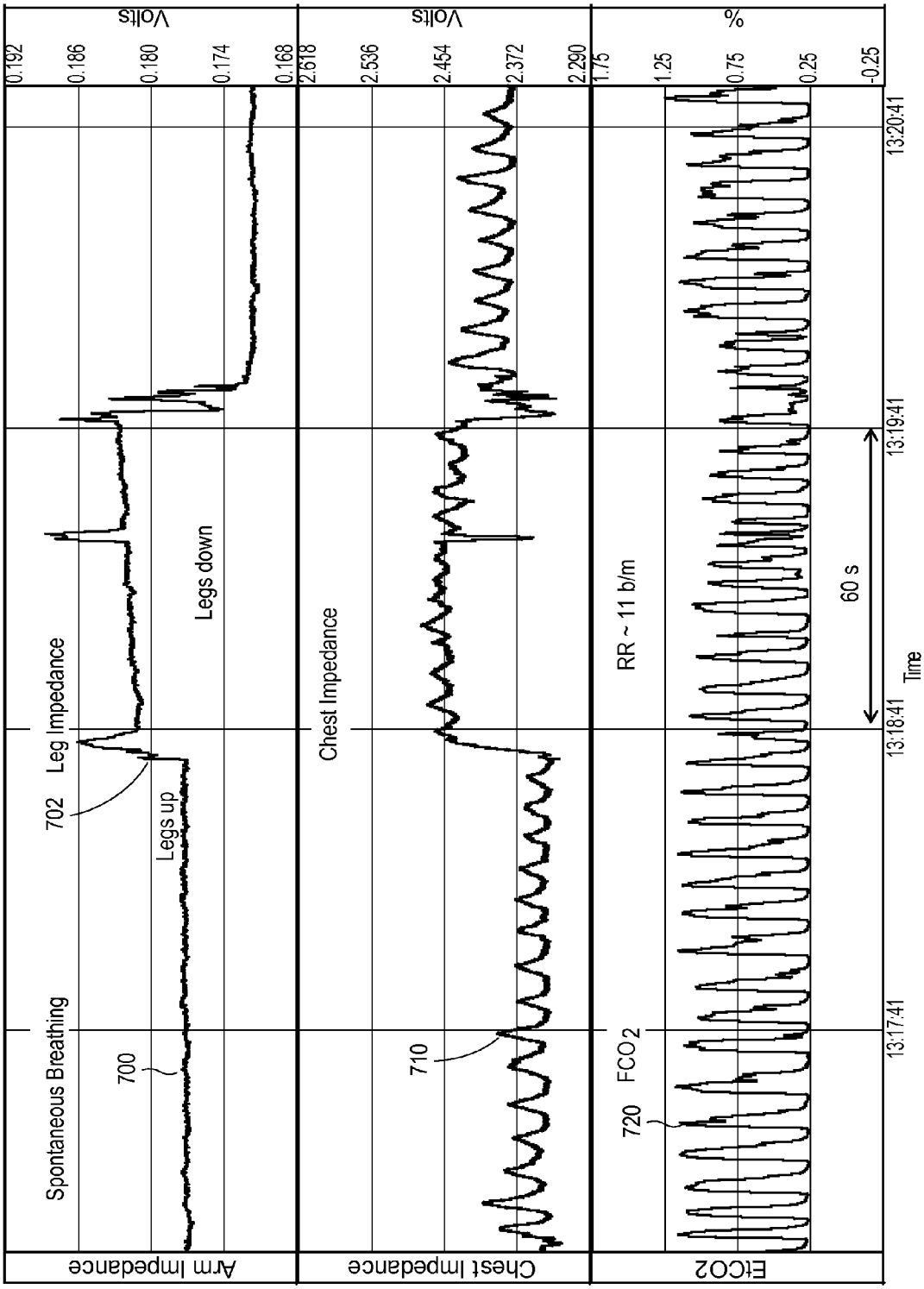

Arm Impedance
Volts
0.192
0.186
0.180
0.174
0.168
Spontaneous Breathing
700
Leg Impedance
702
Legs up
Legs down
Chest Impedance
Volts
2.618
2.536
2.454
2.372
2.290
710
EtCO2
%
1.75
1.25
0.75
0.25
-0.25
720
FCO2
RR ~ 11 b/m
60 s
13:17:41
13:18:41
13:19:41
13:20:41
Time

FIG. 7

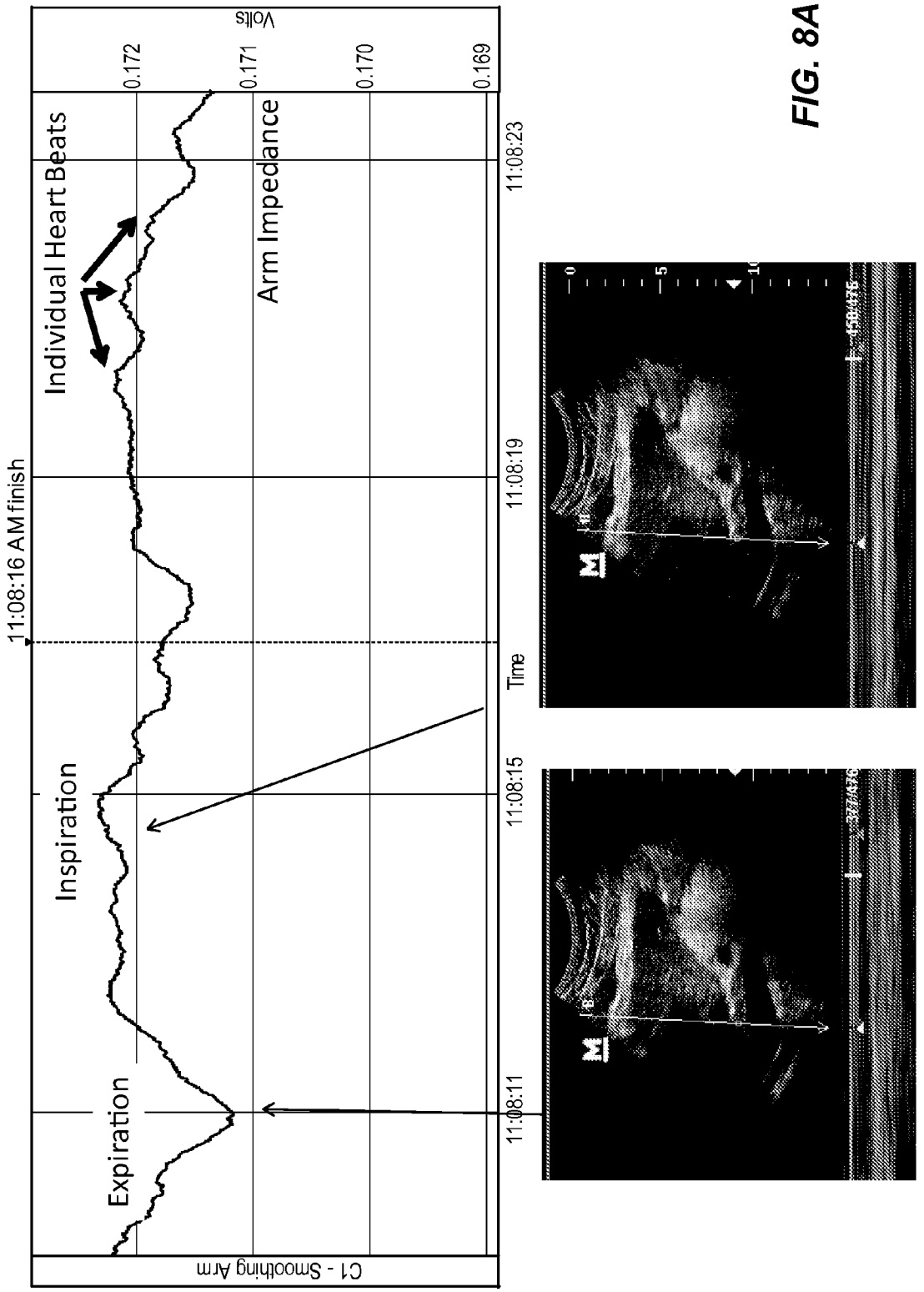

Volts
0.172
0.171
0.170
0.169
Individual Heart Beats
Arm Impedance
11:08:16 AM finish
Inspiration
Expiration
C1 - Smoothing Arm
11:08:11
11:08:15
Time
11:08:19
11:08:23

FIG. 8A

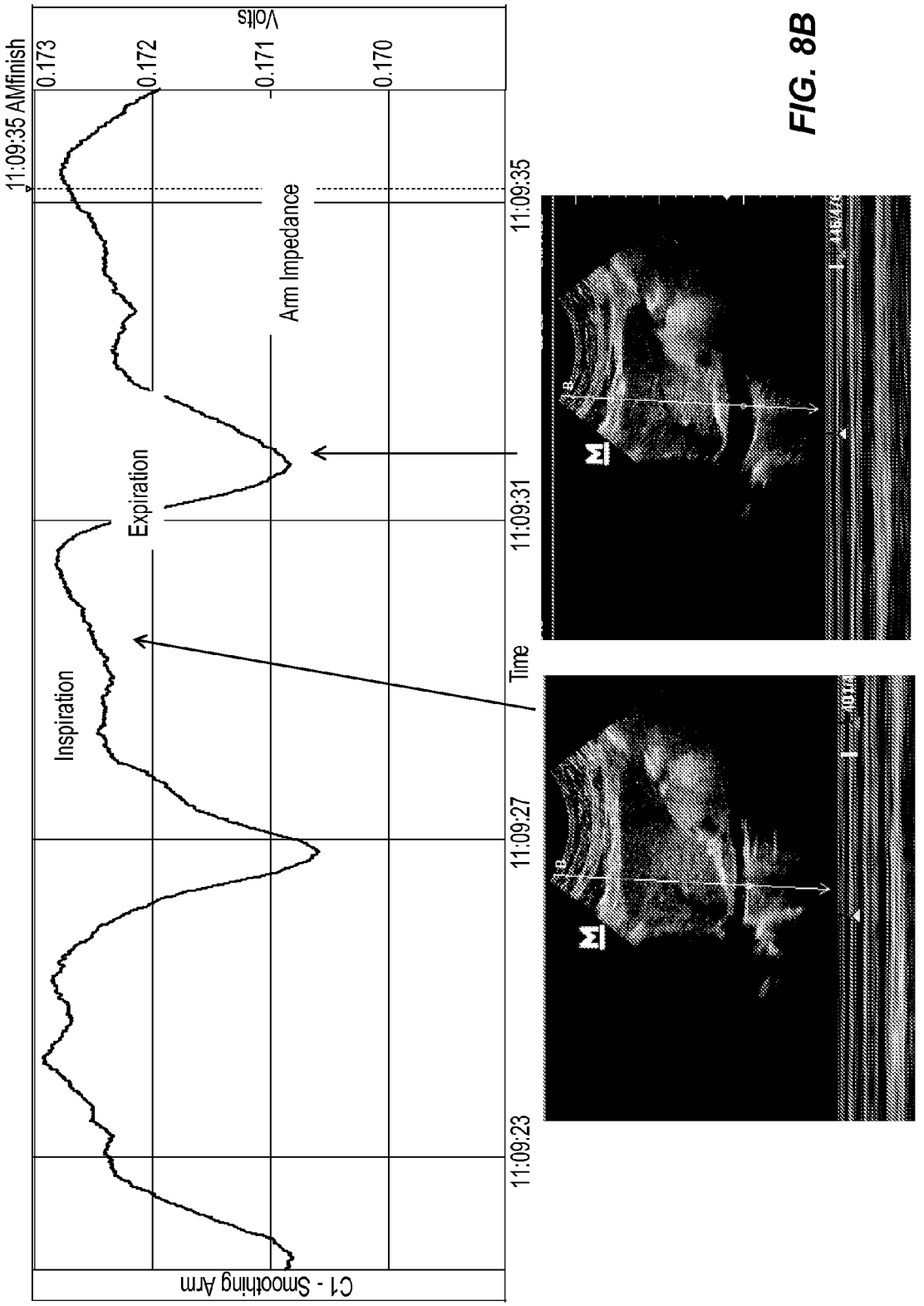

C1 - Smoothing Arm
Volts
0.173
0.172
0.171
0.170
11:09:35 AMfinish
Inspiration
Expiration
Arm Impedance
11:09:23
11:09:27
11:09:31
11:09:35
Time

FIG. 8B

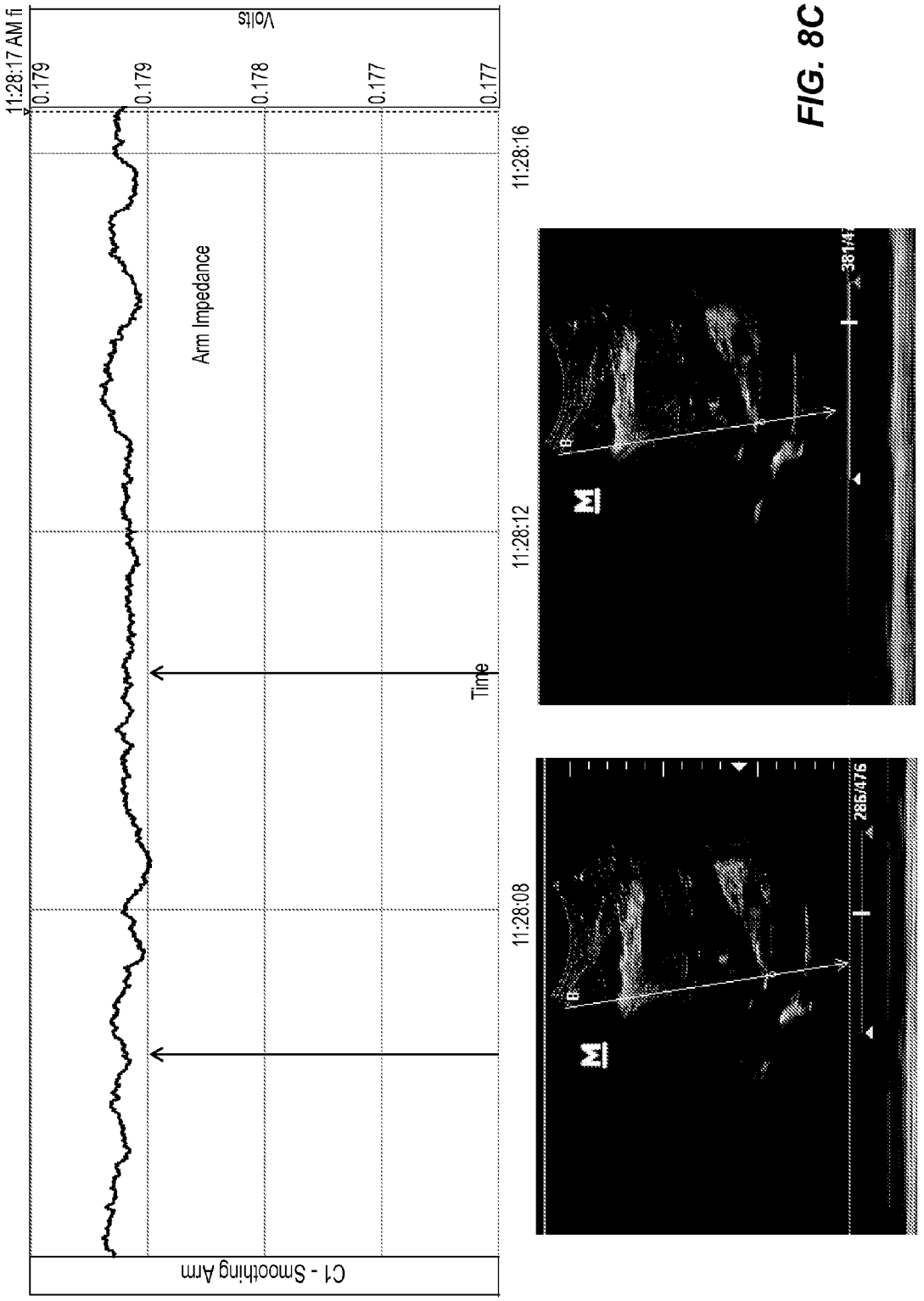

C1 - Smoothing Arm
11:28:17 AM fi
0.179
0.179
0.178
0.177
0.177
Volts
Arm Impedance
Time
11:28:08
11:28:12
11:28:16

FIG. 8C

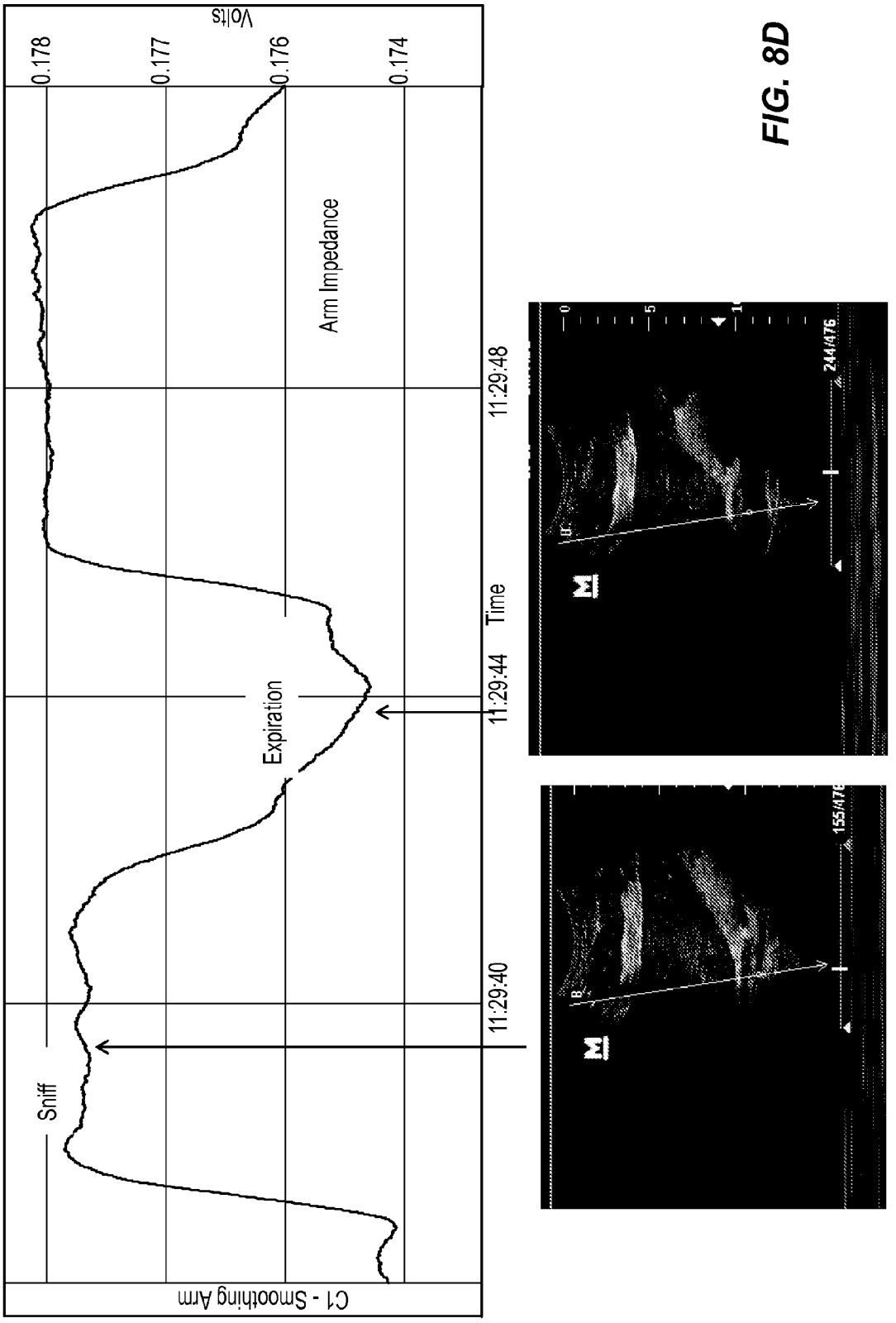

Volts
0.178
0.177
0.176
0.174
Arm Impedance
Sniff
Expiration
11:29:40
11:29:44
Time
11:29:48
C1 - Smoothing Arm

FIG. 8D

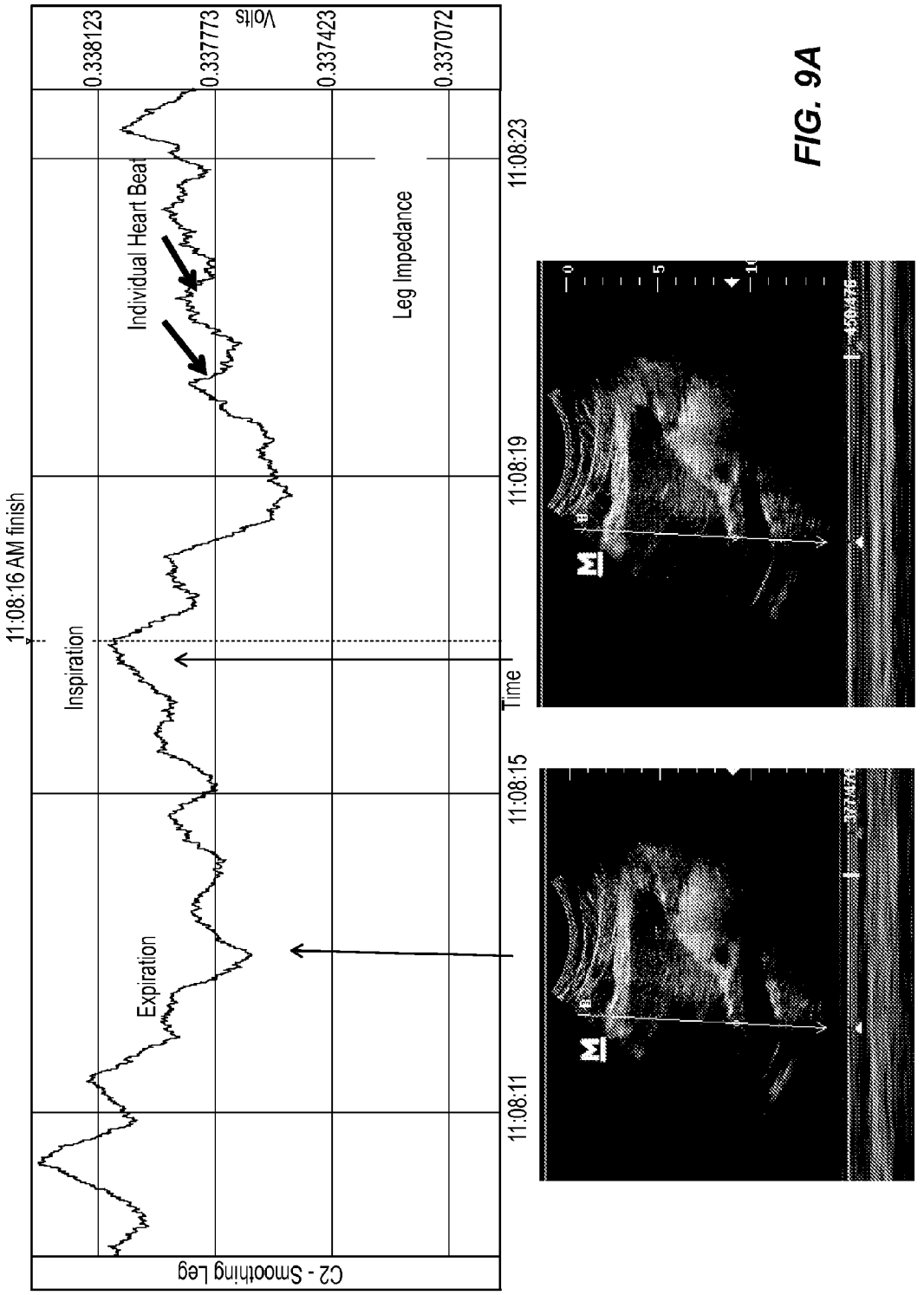

C2 - Smoothing Leg
Volts
0.338123
0.337773
0.337423
0.337072
11:08:16 AM finish
Inspiration
Expiration
Individual Heart Beat
Leg Impedance
Time
11:08:11
11:08:15
11:08:19
11:08:23

FIG. 9A

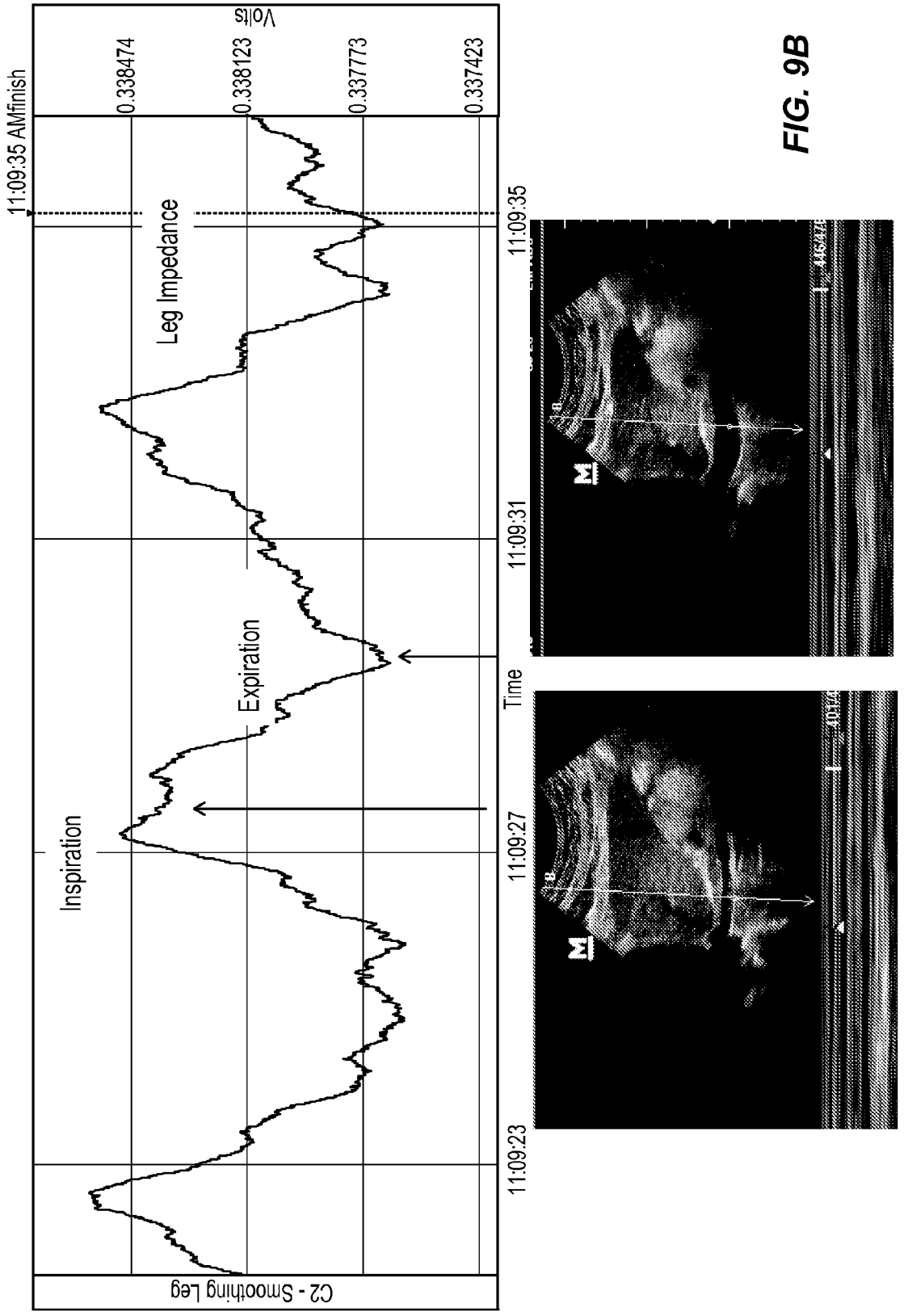

11:09:35 AMfinish
Volts
0.338474
0.338123
0.337773
0.337423
Leg Impedance
Inspiration
Expiration
C2 - Smoothing Leg
Time
11:09:23
11:09:27
11:09:31
11:09:35

FIG. 9B

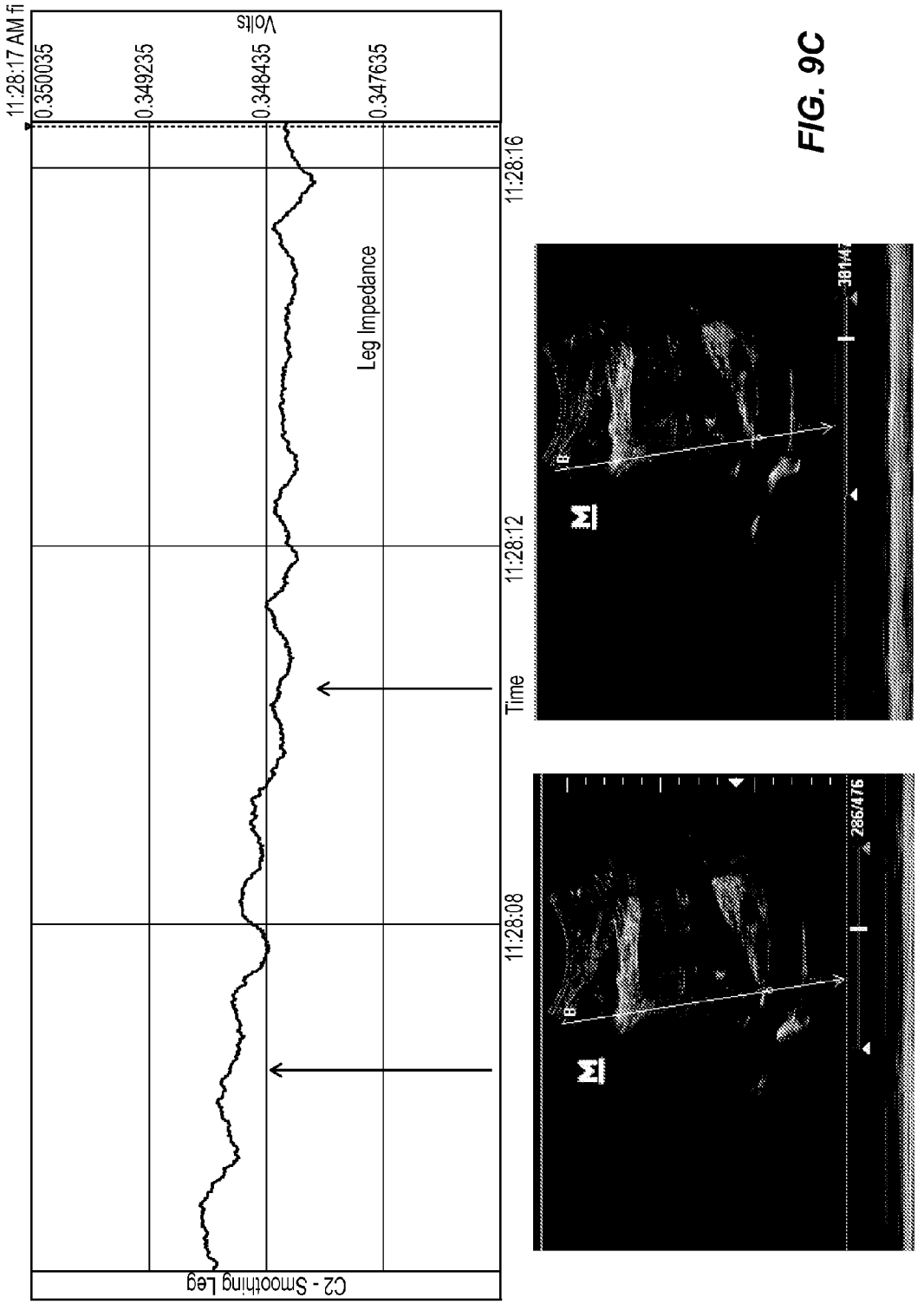

11:28:17 AM fi
0.350035
0.349235
0.348435
0.347635
Volts
Leg Impedance
11:28:16
11:28:12
11:28:08
Time
C2 - Smoothing Leg
M
B
286/476

FIG. 9C

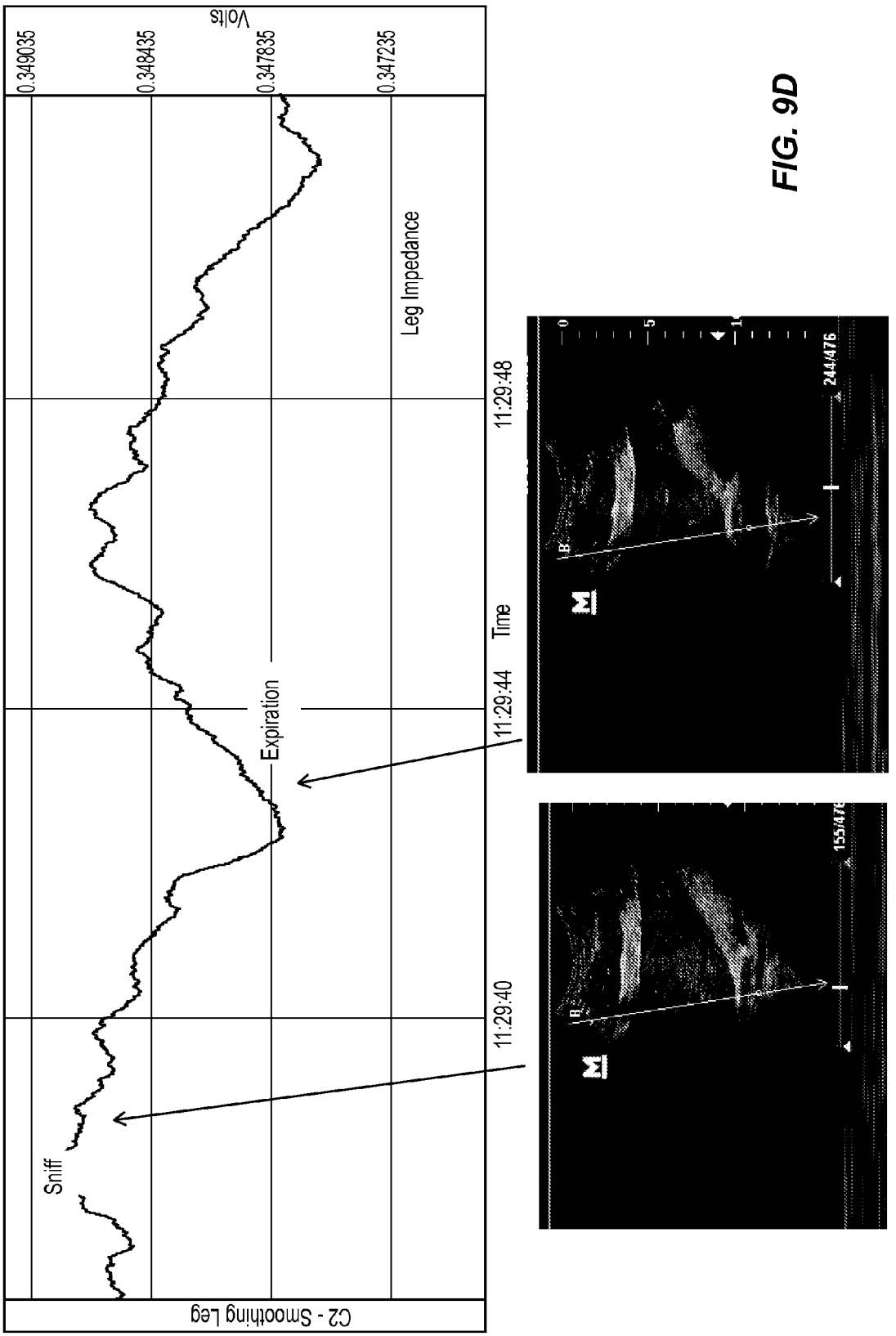

C2 - Smoothing Leg
Volts
0.349035
0.348435
0.347835
0.347235
Leg Impedance
Sniff
Expiration
11:29:40
11:29:44
Time
11:29:48

FIG. 9D

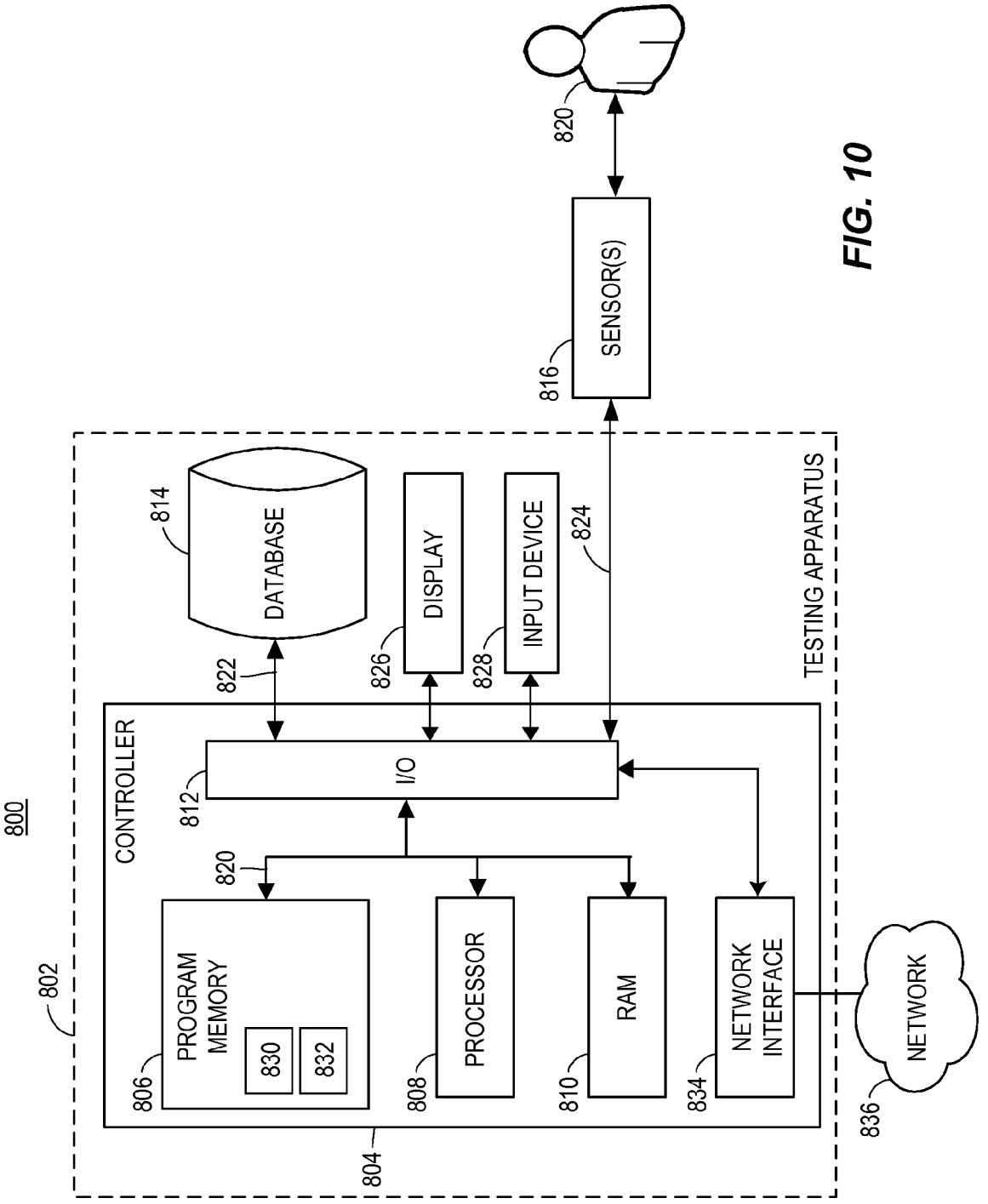

800
802
804
TESTING APPARATUS
CONTROLLER
I/O
812
820
PROGRAM MEMORY
806
830
832
PROCESSOR
808
RAM
810
NETWORK INTERFACE
834
NETWORK
836
DATABASE
814
DISPLAY
826
INPUT DEVICE
828
822
824
SENSOR(S)
816
820

FIG. 10

EVALUTATING CARDIOVASCULAR HEALTH USING INTRAVASCULAR VOLUME

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of U.S. Provisional Application No. 61/859,615, entitled "Evaluating Cardiovascular Health Using Intravascular Volume," filed on Jul. 29, 2013, which is hereby incorporated by reference herein in its entirety.

FIELD OF INVENTION

[0002] The present disclosure generally relates to a system and a method for evaluating a patient's cardiovascular health and, more particularly, to a non-invasive method for determining a patient's intravascular volume status by measuring the change in peripheral venous volume in response to an event causing blood to return to the heart via the venous system.

BACKGROUND

[0003] For patients suffering from a variety of injuries or disease states such as cardiac arrest, burns, trauma, heart failure, sepsis, dehydration from any cause, renal failure, or dialysis, it is important to monitor the relationship between the volume of circulating blood and the patient's ability to circulate that volume of blood. Further, in many medical conditions, it is important to know if patients will hemodynamically respond in a favorable manner to providing intravenous fluids and/or if they are volume overloaded. This is especially important in complex states such as sepsis and cardiogenic shock.

[0004] However, determination of a patient's intravascular volume status in a noninvasive manner has been problematic. Methods of monitoring cardiac output are commonly used to assess the condition of patient's suffering from a variety of conditions. However, many of the methods that are non-invasive fail to quantify the volume of blood circulating within the patient relative to the patient's ability to circulate that volume. These parameters are important because ideally the physician could adjust the volume of circulating blood (for example via intravenous fluids) in order to achieve optimum cardiovascular circulation or output. Recently, impedance cardiography has been used to measure changes in cardiac output (and thus stroke volume) in response to temporary central fluid provision by raising of the lower extremities. This approach, however, is expensive, generally does not provide sufficient measurement sensitivity or accuracy, and may not be an option to some patients. In particular, impedance cardiography may necessitate that a patient's lower extremities be raised by a health care provider and many further necessitate repetitive raising if used as an endpoint measure. In many instances, raising of the legs will not be possible due to lower extremity injury, pelvic fracture or in situations where the patient may have limb amputation. In addition, passive leg lifting, as a provocative volume challenge maneuver may be ill suited since limb volume will greatly vary between individuals and even potentially within an individual if it is used repetitively when impedance cardiography is used as the end-point of the maneuver. Further, impedance cardiography has not been used to guide a reduction of intravascular volume. Thus, a passive extremity lift when used in conjunction with impedance cardiography as a hemodynamic endpoint cannot be used as a continuous measure to guide therapy.

[0005] Another approach, using ultrasound of the inferior and superior vena cava, has been used to look at the changes in these large venous vessels in response to spontaneous and mechanical ventilation with great accuracy. The collapsibility of these large vessels during respiration is indicative of volume status including right atrial pressure and whether or not the patient will increase their cardiac output in response to intravenous fluid administration. However, despite its utility such monitoring is prohibitively cumbersome and expensive and requires an experienced ultrasound operator. Furthermore, ultrasonic measurement of the inferior and superior vena cava cannot be performed continuously for a relatively long period of time. Other technologies like pulse pressure variation and stroke volume variation have been used to examine arterial changes produced by volume induced changes in cardiac output caused by respiration. However, measuring the volume variation of the arterial system has been problematic for various reasons (e.g., various pharmaceuticals may alter arterial vascular stiffness and volume largely independent of total intravascular volume). Additionally, it is unknown whether such a technique will work in patients with very stiff arterial systems from calcific and chronic hypertension conditions. Also such techniques may also require that the tidal volume of the patient be carefully controlled.

SUMMARY

[0006] The present application describes techniques to non-invasively monitor cardiovascular system health by monitoring changes in the volume of blood in the venous system of the arms, legs, or neck of patients by using one or more methods of determining tissue volume and/or volume changes of an extremity such as an arm, leg, or neck of the patient. The volume or volume changes may be determined using impedance plethysmography, near infrared spectroscopy, photoplethysmography, galvanic skin response, laser Doppler flowmetry, or ultrasound; although in the illustrated examples techniques using impedance measurements and changes in impedance are detailed.

[0007] In an example, a method for evaluating the cardiovascular condition of a patient, the method includes: (a) recording a first impedance of a limb or extremity of the patient at a first time in response to receiving a first impedance reading from a plurality of sensors on a limb or extremity or neck; (b) after the occurrence of an event modulating blood return to the heart via the venous system of the patient, recording a second impedance of the limb or extremity or neck at a second time in response to receiving a second impedance reading from a plurality of sensors on a limb or extremity or neck, wherein the first impedance and the second impedance each correspond to a volume of blood flowing within the limb or extremity or neck; and (c) determining a change in venous blood volume between the first time and the second time by comparing the first impedance and the second impedance to determine a change in volume of blood.

[0008] In accordance with another example, a testing apparatus for evaluating the cardiovascular condition of a patient, the testing apparatus includes: one or more electrodes; one or more processors; a computer-readable memory storing non-transient instructions that when executed by the one or more processors cause the testing apparatus to: (a) use the one or

more electrodes to record a first impedance of a limb or extremity or neck of the patient at a first time in response to receiving a first impedance reading from a plurality of sensors on a limb or extremity or neck; (b) after the occurrence of an event modulating blood return to the heart via the venous system of the patient, use the one or more electrodes to record a second impedance of the limb or extremity or neck at a second time in response to receiving a second impedance reading from a plurality of sensors on a limb or extremity or neck, wherein the first impedance and the second impedance each correspond to a volume of blood flowing within the limb or extremity or neck; and (c) determine a change in venous blood volume between the first time and the second time by comparing the first impedance and the second impedance to determine a change in volume of blood.

[0009] In accordance with yet another example, a closed-loop cardiovascular condition evaluation system including: a testing apparatus; and a processor and a memory, the memory storing instructions that when executed by the processor, cause the processor to evaluate a cardiovascular condition of a subject in response to determining the change in the venous blood volume between the first time and the second time determined by comparing the first impedance and the second impedance, for different treatment cycles.

[0010] In accordance with yet another example, a method for evaluating the cardiovascular condition of a patient, the method includes: (a) determining a first volume of blood of a limb or extremity or neck of the patient at a first time; (b) after the occurrence of an event causing blood to return to the heart via the venous system of the patient, determining a second volume of blood of the limb or extremity or neck at a second time; (c) determining a change in venous blood volume between the first time and the second time by comparing the first volume of blood and the second volume of blood; and (d) determining one or more of: (1) how the patient will hemodynamically respond to one or more of an addition of cardiovascular fluid or removal of cardiovascular fluid, (2) how the patient will hemodynamically respond to one or more cardiovascular drugs which promote changes in cardiac output, changes in cardiovascular preload, and changes in cardiovascular afterload, or (3) determining how the patient will response to changes in mechanical or noninvasive ventilation.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] The figures described below depict various aspects of the system and methods disclosed herein. It should be understood that each figure depicts an embodiment of a particular aspect of the disclosed system and methods, and that each of the figures is intended to accord with a possible embodiment thereof. Further, wherever possible, the following description refers to the reference numerals included in the following figures, in which features depicted in multiple figures are designated with consistent reference numerals.

[0012] FIG. **1** depicts an example intravascular volume status monitoring process for implementing the intravascular volume status monitor in accordance with an example;

[0013] FIG. **2** depicts example placement of an impedance measuring device on an extremity of a patient (e.g., an arm), in accordance with an example application of the monitoring process of FIG. **1**;

[0014] FIGS. **3-7** depict example graphs of impedance measured from an arm of a patient, impedance measured from the chest wall of the patient, and end-tidal carbon dioxide (CO_2) measured from the nose of a patient as functions of time;

[0015] FIGS. **8A-8D** depict graphs of arm impedance measurements measured under different breathing conditions, specifically, normal breathing (FIG. **8A**), deep breathing (FIG. **8B**), holding breath (FIG. **8C**), and sniff breathing against a partially closed glottis (FIG. **8D**). Simultaneous changes in IVC diameter are also noted.

[0016] FIGS. **9A-9D** depict graphs of leg impedance measurements measured under different breathing conditions, specifically, normal breathing (FIG. **9A**), deep breathing (FIG. **9B**), holding breath (FIG. **9C**), and sniff breathing against a partially closed glottis (FIG. **9D**). Simultaneous changes in IVC diameter are also noted.

[0017] FIG. **10** depicts an example block diagram illustrating the various components used in implementing an exemplary embodiment of the intravascular volume monitoring method.

DETAILED DESCRIPTION

[0018] Although the following text sets forth a detailed description of numerous different embodiments, it should be understood that the legal scope of the invention is defined by the words of the claims set forth at the end of this patent. The detailed description is to be construed as exemplary only and does not describe every possible embodiment, as describing every possible embodiment would be impractical, if not impossible. One could implement numerous alternate embodiments, using either current technology or technology developed after the filing date of this patent, which would still fall within the scope of the claims.

[0019] It should also be understood that, unless a term is expressly defined in this patent using the sentence "As used herein, the term '_____' is hereby defined to mean . . . " or a similar sentence, there is no intent to limit the meaning of that term, either expressly or by implication, beyond its plain or ordinary meaning, and such term should not be interpreted to be limited in scope based on any statement made in any section of this patent (other than the language of the claims). To the extent that any term recited in the claims at the end of this patent is referred to in this patent in a manner consistent with a single meaning, that is done for sake of clarity only so as to not confuse the reader, and it is not intended that such claim term be limited, by implication or otherwise, to that single meaning. Finally, unless a claim element is defined by reciting the word "means" and a function without the recital of any structure, it is not intended that the scope of any claim element be interpreted based on the application of 35 U.S.C. §112, sixth paragraph.

[0020] FIG. **1** is a flow diagram depicting an example embodiment of an intravascular volume status monitoring process **100**. It may be advantageous to monitor the intravascular volume status of a patient for any of a number of injuries or diseases such as cardiac arrest, burns, trauma including combat trauma, heart failure, sepsis, dehydration from any cause, renal failure, dialysis, etc. Further, it may also be advantageous to conduct general status monitoring including respiratory rate and respiratory quality monitoring as discussed herein. Further still, a longer term use of the process **100** may be to monitor the status of a patient suffering from edema. Prior to commencing monitoring, the testing apparatus, such as the testing apparatus **802** illustrated in FIG. **10** discussed below, would be applied to the patient (block **102**).

As described herein, the testing apparatus **802** may be any of a number of devices and sensors used to gather the venous volume and other data used to evaluate the patient's intravascular volume status.

[0021] FIG. **2** is an illustration of how the testing apparatus **802** and the sensor(s) **816** operatively connected thereto may be used to measure impedance (and thus measure venous volume changes as discussed below). The testing apparatus **802** and the sensors **816** (not shown in FIG. **2**) may collectively be referred to as an impedance measuring device **202**. In some embodiments, the impedance measuring device is an impedance plethysmograph. The impedance measuring device **202** may be coupled to the patient via electrodes **206** and **208**. As shown in FIG. **2**, the electrodes **206** and **208** may be placed on the arm **204** of a patient. The impedance measuring device **202** may include two sets of electrodes. A first set of electrodes **206** are used to inject electrical current (e.g., 1 mA of alternating current) into the arm **204**. A second set of electrodes **208** is used to monitor the impedance of the arm **204**. Of course it will be appreciated that more than or less than four electrodes may be used to measure impedance. Further, different placement patterns for the electrodes **206** and **208** may be used (e.g., a circumferential pattern around the arm **204**). Additionally, instead of placing the electrodes **206** and **208** on the arm **204**, it will be understood that electrodes could be placed on the leg or neck of the patient to measure impedance as discussed herein. It will be appreciated that the electrodes **206** and **208** are placed peripherally (i.e., on the arm, neck, or legs) in order to take advantage of the venous volume modulation that will be produced through ventilation that will be reflected in relative blood volume changes in these peripheral sites. These sites are also insulated by distance so that they are not affected by motion of the chest. Traditional chest wall impedance used to measure respiratory rate use the impedance changes produced by small distance changes between impedance electrodes to make the respiratory rate measure. These changes in impedance at the chest are independent of cyclical blood volume changes. It will also be appreciated that the electrodes **206** and **208** do not have to be aligned along a vein. Volume changes are being made across a segment of tissue at the periphery which is dominated by movement of venous blood at that site. Accordingly, the testing apparatus **802** may be used to provide information about the relationship between ventilation, venous return, and heart function as described herein.

[0022] In another embodiment, the testing apparatus **802** may include any of a number of apparatuses useful for determining the volume of blood in one or more of the patient's extremities. For example, the testing apparatus may be a photoplethysmograph, a galvanic skin response monitor, a near infrared spectroscopy device, a laser Doppler device with our without speckle tracking, or an ultrasound device with or without speckle tracking. The present techniques, which focus on venous-side blood volume assessment, can be combined with other vascular techniques, including arterial-side measurement devices. As such, the testing apparatus **802** could be a device that also provides impedance cardiography, pulse pressure variation measurements, and stroke volume variation measurements, or the testing apparatus **802** could be a device coupled to one or more such devices (not shown), through a network, where such devices are coupled to a subject. Coordinating the present venous-side techniques with arterial-side measurements can provide additional information on vascular condition, such as preload, venous return, cardiac output, afterload, and vascular compliance.

[0023] As shown in FIG. **1**, with the testing apparatus **802** in place, the monitoring of the patient's intravascular volume status may commence (block **104**). Monitoring may be conducted at any of a number of physical locations (e.g., an intensive care unit, an operating room, a dialysis clinic, emergency department, ambulance, home health care, etc.). Once monitoring has begun, the testing apparatus **802** may take a first measurement of the extremity (block **106**), that measurement being of a physical metric indicative of venous volume in the extremity. For example, if the testing apparatus **802** is an impedance measuring device such as the one illustrated in FIG. **2**, the testing apparatus **802** may take a first impedance measurement of the extremity as the physical metric indicative of venous volume. The impedance of the extremity is based in large part on the blood volume within the extremity. Because blood is a good electrical conductor, impedance will decrease or increase if more or less blood volume, respectively, exists in the extremity. The first measurement may be a single data point, but the first measurement may also include multiple data points taken over a first period of time. Further, the first measurement may include a series of data points taken over a first period of time to establish a baseline measurement of the patient's intravascular volume cycle over the course of a plurality of respirations. As discussed herein, such respirations may be spontaneous and/or mechanically induced.

[0024] Subsequent to the first measurement of the extremity, an event causing blood to return to the heart via the venous system may occur (block **108**). These events causing blood to return to the heart via the venous system may also be referred to as "respiratory challenges" herein. For example, taking a deep breath produces a larger negative intrathoracic pressure thus pulling more blood from the extremity. Spontaneous respiration creates negative pressure in the thorax which in turn creates a pressure gradient between the chest and the limbs/abdomen. This negative pressure gradient sucks blood into the large veins of the abdomen and chest which then empty this volume into the heart. Because spontaneous inspiration pulls blood from the extremities, the degree to which this will happen will depend on how full the right heart is and how forcefully the patient inspires. The degree to which spontaneous or negative pressure ventilation enhances venous return as it relates to central venous volume has been shown to be reflected by ultrasound imaging in the diameter of the inferior and superior vena cava and their ability to collapse. Similar but converse changes will be noted for patients undergoing positive pressure mechanical ventilation. In particular, the increase in intrathoracic pressure may cause the blood flow returning from the limbs and abdomen to slow, which may be detected by monitoring the change in volume as discussed herein. Centrally, this has been demonstrated using ultrasound imaging to change the diameter of the superior and inferior vena cava. It will be appreciated that such changes may be caused by higher intrathoracic pressures which in turn may cause expansion or collapse of the inferior and superior vena cava. Such expansion or collapse may change the volume of the inferior and superior vena cava and this in return will be manifest by volume changes peripherally. Because of this, the process **100** may be used to optimize vascular volume and even mechanical ventilation parameters by understanding the effects of mechanical ventilation on the central cardiovascular system's venous component. Additionally,

changing the elevation of the extremities also may result in both local and remote blood volume changes that can be detected and are likely reflective of the patient's cardiovascular status as discussed herein. Raising the extremity will cause blood to flow into the central circulatory system, but the volume of blood flow will be dependent on the volume of blood already present in the central circulation system as well as the height of the extremity. Further, blood volume in the extremities may be affected by applying pressure to the patient's chest or abdomen. Accordingly, in an attempt to cause blood to return to the heart via the venous system, the patient may be instructed to take a deep breath, the patient may be instructed to inspire or expire against an impedance valve which produces a set negative inspiratory or positive expiratory pressure respectively, one or more of the patient's limbs may be raised or to a particular height, pressure (positive or negative) may be applied to the patient's chest or abdomen, and/or the parameters of positive pressure mechanical ventilation may be adjusted. The change in the volume of blood in the extremity being monitored may be used to determine the patient's intravascular health similar to changes in IVC diameter. In fact changes in IVC diameter as measured by ultrasound during respiration (spontaneous and mechanical ventilation) have been accurately correlated with right atrial or central venous pressure. FIGS. **8** and **9** demonstrate quantifiable changes in limb impedance and changes in vena cava diameter. In essence the limbs or neck would be treated as extensions of the vena cava, such that one can determine changes in blood volume in the limbs or neck as well as the volume and diameter changes in the vena cava, from changes in impedance in limbs or neck. These determinations can be explicit, e.g., with actual volumetric or length units, or inferred, e.g., maintaining units as impedance units. In this way, measure of changes in impedance, or other physical metric, may be used to determine changes in changes in blood volume on the heretofore unmeasured venous-side of the vascular system. Moreover, the changes in blood volume may be determined, explicitly from the impedance data or inferentially as represented by the impedance data. Moreover still, the changes in limb or neck volume, as measured by impedance or other method, also correlate with changes in right atrial pressure and central venous pressure.

[0025] During and/or after the occurrence of the event causing modulation of blood return to the heart via the venous system, the testing apparatus **802** may take a second measurement of the extremity (block **110**), for example, of the same physical metric indicative of venous volume in the extremity as first measured (block **106**). For example, if the testing apparatus **802** is an impedance measuring device such as the one illustrated in FIG. **2**, the testing apparatus **802** may take a second impedance measurement of the extremity. As with the first measurement, the second measurement may include a series of data points taken during a second period of time. Using the first measurement and the second measurement, the process **100** may then determine the change in venous volume in the extremity (e.g., by subtracting the mean value of the first measurement from the mean value second measurement) (block **112**). The block **112** may determine the change in venous volume from the values of the physical metric (e.g., impedance) taken at the first (block **106**) and the second (block **110**) points in time. When impedance (also termed bioimpedance) is used as the physical metric, then the changes in impedance will provide a relatively linear correlation to the changes in volume when compared to the changes in the diameter of the vena cava. In some examples, the actual change in blood volume is determined from the changes in impedance, i.e., calculated, while in other examples, the change is determined inferentially. Thus, in these ways, the present techniques are, in some implementations, able to determine changes in venous blood volume without needing to measure absolute values for the physical metric, e.g., without needing to measure absolute impedance. Instead changes in the physical metric, e.g., changes in impedance, can be measured.

[0026] In some examples, the blocks **106** and **110** may take the first and second measurements, respectively, over a period of time and determine a local peak value of the physical metric over those periods of time value, and provide those local peak values to the block **112**. In some examples, the blocks **106** and **110** may take the first and second measurements, respectively, over a period of time and determine a peak-to-peak value for the physical metric over those periods of time, and provide those peak-to-peak values to the block **112**. In any of these examples, the first and second measurements may be normalized to each individual's breathing baseline. In some examples, the normalization can occur from plotting this data versus changes in the diameter of an individual's inferior vena cava. In an example experimental implementation using control subjects and patients who were critically ill, an exponential model was fit to measured impedance data and was shown to have high predictive value of impedance changes on both a per individual basis (e.g., R^2=0.91±0.05 and R^2=0.96±0.03 for control subjects and patients, respectively) and a per subject group basis.

[0027] Referring again to FIG. **1**, the process of taking measurements of volume before and after a blood moving event and calculating the change in volume may be repeated one or more times. That is, the processes for blocks **106-112** may be repeated numerous times to monitor and assess the effects of different events on blood volume. Additionally various types of blood moving events may occur during monitoring. For example, a patient may be instructed to breathe normally for several cycles, instructed to take several deep breaths, and then have a limb be raised and/or lowered as discussed herein. By monitoring the patient's intravascular volume status before and after several occurrences of events causing blood to return to the heart via the venous system, a healthcare provider may assess whether the addition or subtraction of intravascular fluid would be advantageous to the patient's health as discussed below. The length of time the patient may be monitored may vary and may depend in part on (a) how ill or injured the patient is and (b) what treatments are being undertaken to optimize the patient's cardiovascular system and how the patient's cardiovascular system responds to this treatment discussed below. Accordingly, the patient may be monitored for minutes, hours, or days. Further, it may be advantageous to continuously monitor the extremity to detect changes in circulating volume over time in order to detect hydration states and continuing blood loss.

[0028] The event(s) modulating blood return may result from direct or indirect instruction from health care personnel, and as part of a treatment efficacy determination. Example events include spontaneous inspiration, positive pressure mechanical ventilation, raising a limb or extremity or neck of the patient, applying negative or positive pressure to the abdomen or chest, inspiration against a negative impedance valve, or discrete maneuvers performed with mechanical ventilation. Those discrete maneuvers performed with mechanical

ventilation may include, by way of example, adjusting positive pressure ventilation, negative pressure ventilation, maintaining inspiratory or expiratory pause, or a combination thereof.

[0029] Personnel may instruct patients to engage in events modulating blood return, at, before, or during a diagnosis test or administration of treatment. The change in venous blood volume is then determined based on changes in measured impedance before and after the events. The personnel may instruct the patient to engage in one event or in a protocol of events, e.g., performing different breathing exercises in a prescribed manner (such as, rapid breaths followed by deep expiration and deep inspiration breaths, different types of breaths taken at different limb positions, etc.). For example, an administering physician may instruct a patient to perform a blood-modulating event, from which changes in venous blood volume is determined between different time points, to determine how the patient will hemodynamically respond to treatment by a cardiovascular fluid, or how the patient will respond to the removal of a cardiovascular fluid. The physician thus uses the blood-modulating events to assist in determining patient's likely responsiveness a treatment. From here, the physician can assess which treatments will be most effective. The technique can be used to determine responsiveness to any number of conditions, including hemodynamic responsiveness to administration or removal of one or more cardiovascular drugs, such as drugs intended to promote cardiac output, changes in cardiovascular preload, changes in cardiovascular afterload, etc. In yet other examples, the blood modulating events may be used to determine how a patient will respond to changes in mechanical or noninvasive ventilation.

[0030] Mechanical ventilation may be from adjusting positive pressure ventilation, negative pressure ventilation, maintaining inspiratory or expiratory pause, or a combination thereof.

[0031] In some examples, instructions for performing blood modulating events may be supplied to the patient automatically by a system **800**, for example, using a display **826**, and under instruction by instructions executed by a processor **808** and stored in a program memory **806**, described further hereinbelow.

[0032] The instructions can be provided to a patient that is remote from a medical facility connected to the testing apparatus through a network, such as at the patient's home. Whether at a medical facility or remotely, the technique may be performed on a patient undergoing a general examination or undergoing a specific treatment, such as treatment for cardiac arrest, edema, burns, trauma, heart failure, sepsis, dehydration, renal failure, or dialysis.

[0033] FIGS. **3-7** illustrate graphs of the impedance measured in an extremity before, during, and after the occurrence of the event causing blood to return to the heart via the venous system (e.g., spontaneous respiration, moving one or more limbs, etc.). Each of FIGS. **3-7** includes three graphs. As discussed below, the top graph in each of FIGS. **3-7** is a graph of the impedance measured at an extremity of the patient (e.g., arm, leg, etc.). The middle graph in each of FIGS. **3-7** is a graph of the impedance measured at the chest wall of the patient. The electrodes were placed in a pattern similar to patterns used in known in-hospital respiratory monitoring. However, unlike the impedance measured at the patient's extremity, the changes in impedance in the chest level does not represent blood volume changes in the chest but rather changes in the distance between the electrodes in response to chest expansion. It will be appreciated by one of ordinary skill in the art that in each of FIGS. **3-7**, a comparison of the top graph of impedance measured at an extremity to the middle graph of impedance measured at the chest wall shows the greater sensitivity conferred by the methods discussed herein. In particular, the top graph may illustrate a more pronounced response to respiratory challenges relative to regular, spontaneous breaths. The bottom graph in each of FIGS. **3-7** is a graph of the end-tidal CO_2 monitoring from the nose. End-tidal CO_2 is a direct measurement of respiration, however the measuring apparatus used to generate FIGS. **3-7** introduced a slight delay in registering the measurements, which caused the bottom graph to be slightly out of phase with the top and middle graphs due to the side-stream sampling nature of the CO_2 measurement. However, because the amplitude and frequency of the top graph of impedance track with the peaks and troughs of the bottom graph of end-tidal CO_2, it will be appreciated by one of ordinary skill in the art that measuring impedance at an extremity can serve as an indirect measurement of respiratory rate and the degree of respiratory effort. The performance of impedance in this aspect is improved over end-tidal CO_2 monitoring.

[0034] FIG. **3** includes an illustration of a graph **300** of the impedance measured in a patient's arm while the patient engages in normal, spontaneous breaths. FIG. **3** also includes a graph **310** of chest impedance and a graph **320** of end-tidal CO_2. It will be appreciated that each of the graphs **300**, **310**, and **320** track one another and exhibit a substantially-regular amplitude and frequency as the patient breathes. FIG. **4** is an illustration of a graph **400** of the impedance measured in a patient's arm while the patient engages in deep, spontaneous breaths starting at point **402**. FIG. **4** also includes a graph **410** of chest impedance and a graph **420** of end-tidal CO_2. It will be appreciated that amplitude of waveform of the graphs **400** and **410** increase after point **402** while the patient is taking deep breaths, however the rate of breathing has not substantially increased. Additionally, it will be appreciated that the frequency of the graphs **400**, **410**, and **420** have not increased. Additionally, FIG. **5** is an illustration of a graph **500** of the impedance measured in a patient's arm while the patient takes and holds a deep breath at point **502**, with a characteristic spike and gradual tapering of impedance. FIG. **5** also includes a graph **510** of chest impedance and a graph **520** of end-tidal CO_2. As discussed herein, the relatively larger change in impedance may indicate a greater flow of blood toward the heart caused by greater negative intrathoracic pressure caused by a deep breath. The differences between FIG. **3** and FIGS. **4** and **5** will be appreciated by one of ordinary skill in the art. In particular, it will be noted that the change in impedance in FIG. **4** and FIG. **5** while the patient was taking deep breaths or taking and holding a deep breath, respectively, are larger relative to the change in impedance in FIG. **3**.

[0035] FIG. **6** is an illustration of a graph **600** of the impedance measured in a patient's arm while the patient's legs are raised and lowered while the patient is taking spontaneous breaths. At point **602**, the patient's legs are raised, causing the impedance measured in the arm to decrease slightly as more blood is present in the patient's arm. Then, at point **604**, the patient's legs are lowered, causing impedance in the arm to spike as less blood is present in the patient's arm. FIG. **6** also includes a graph **610** of chest impedance and a graph **620** of end-tidal CO_2. FIG. **7** is an illustration of a graph **700** of the impedance measured in a patient's leg as the patient's legs are

raised and lowered while the patient is taking spontaneous breaths. At point **702**, the patient's legs are raised, causing the impedance measured in the leg to increase as less blood is present in the leg. Then, at point **704**, the patient's legs are lowered, causing the impedance measured in the leg to decrease as more blood is present in the patient's leg. FIG. **7** also includes a graph **710** of chest impedance and a graph **720** of end-tidal CO_2.

[0036] Referring again to FIG. **1**, after calculating the change in venous volume at the extremity, the cardiovascular health of the patient can be evaluated and appropriate treatments or interventions may be planned and applied (block **114**). A healthcare provider may use the process **100** to assess the condition of the patient to determine whether it may be beneficial to increase the volume of circulating blood in order to achieve optimum cardiovascular circulation or output. If the patient is volume deficient, the volume of circulating blood may be increased, for example, by a blood transfusion, administering intravenous (IV) fluids, or other known ways of administering fluids. If the patient is hypervolemic, the volume of circulating blood may be decreased, for example, by diuresis or other known ways of decreasing fluids. In the past, a healthcare provider might hypothesize that a patient is volume depleted based merely on the patient's injury or illness (e.g., severe burns) and administer IV fluids without having the capability of determining whether such fluids may be beneficial beforehand. However, using the disclosed embodiments, if a healthcare provider initially believes that a patient has a condition that would be respond favorably to the addition of IV fluids, but the change in volume (e.g., as determine by measuring the change in impedance as discussed herein) is small in response to various respiratory challenges (e.g., deep breaths, manipulation of limbs, etc.), then the healthcare provider may determine that the patient will not respond to being given additional IV fluids. Conversely, if the change in volume is large, then the healthcare provider may determine that the patient will respond favorably to fluids. Similarly, if the healthcare provider hypothesizes that the patient's condition may be improved by removing fluids, the healthcare provider may use the example process **100** to determine that the change in volume is small, indicating that removing fluids may be beneficial. Additionally, the process **100** may be used to titrate positive pressure or negative pressure ventilation and the administration of either pharmaceuticals or mechanical maneuvers that increase blood flow dependent or independent of making the heart pump more efficiently.

[0037] Further, it is possible to use the technology as a respiratory monitor to determine not only the respiratory rate but also the degree of respiratory effort. Accordingly, the process **100** may be used to estimate central venous pressure (CVP) levels noninvasively similar to how Ultrasound of the superior or inferior vena cava have been used, as well as to detect changes in the cardiorespiratory system which may signal the deterioration or improvement of a patient's condition. Because there are no valves in the proximal large veins in the neck and limbs, the geometric changes in these vessels in response to the respiratory challenges discussed above may parallel those of the superior and inferior vena cava. Accordingly, the volume measuring techniques discussed above may be useful for indirectly measuring the volume of blood in the superior and inferior vena cava.

[0038] FIG. **10** is an example block diagram **800** illustrating the various components used in implementing an example embodiment of the intravascular volume monitoring process **100** discussed herein. A testing apparatus **802** may be coupled to a patient **820** via sensors **816** in accordance with executing the functions of the disclosed embodiments. The testing apparatus **802** may have a controller **804** operatively connected to the database **814** via a link **822** connected to an input/output (I/O) circuit **812**. It should be noted that, while not shown, additional databases may be linked to the controller **804** in a known manner. The controller **804** includes a program memory **806**, the processor **808** (may be called a microcontroller or a microprocessor), a random-access memory (RAM) **810**, and the input/output (I/O) circuit **812**, all of which are interconnected via an address/data bus **820**. It should be appreciated that although only one microprocessor **808** is shown, the controller **804** may include multiple microprocessors **808**. Similarly, the memory of the controller **804** may include multiple RAMs **810** and multiple program memories **806**. Although the I/O circuit **812** is shown as a single block, it should be appreciated that the I/O circuit **812** may include a number of different types of I/O circuits. The RAM(s) **810** and the program memories **806** may be implemented as semiconductor memories, magnetically readable memories, and/or optically readable memories, for example. A link **824** may operatively connect the controller **804** to a sensor **816** through the I/O circuit **812**. The sensor **816** may be operatively connected to the patient **820**. The sensor **816** may include the impedance measuring device **202** and electrodes **206** and **208** discussed in connection to FIG. **2**.

[0039] The program memory **806** and/or the RAM **810** may store various applications (i.e., machine readable instructions) for execution by the microprocessor **808**. For example, an operating system **830** may generally control the operation of the testing apparatus **802** and provide a user interface to the testing apparatus **802** to implement the process **100** described herein. The program memory **806** and/or the RAM **810** may also store a variety of subroutines **832** for accessing specific functions of the testing apparatus **802**. By way of example, and without limitation, the subroutines **832** may include, among other things: a subroutine for taking measurements with the sensor **816** and other subroutines, for example, implementing software keyboard functionality, interfacing with other hardware in the testing apparatus **802**, etc. For example, the process **100** of FIG. **1** (and instructions elsewhere described herein) may be stored on the program memory **806** for execution by the processor **808**. The program memory **806** and/or the RAM **810** may further store data related to the configuration and/or operation of the testing apparatus **802**, and/or related to the operation of one or more subroutines **252**. For example, the data may be data gathered by the sensor **816**, data determined and/or calculated by the processor **808**, etc. In addition to the controller **804**, the testing apparatus **802** may include other hardware resources. The testing apparatus **802** may also include various types of input/output hardware such as a visual display **826** and input device(s) **828** (e.g., keypad, keyboard, etc.). In an embodiment, the display **826** is touch-sensitive, and may cooperate with a software keyboard routine as one of the software routines **832** to accept user input. It may be advantageous for the testing apparatus to communicate with a broader medical treatment network (not shown) through any of a number of known networking devices and techniques (e.g., through a commuter network such as a hospital or clinic intranet, the Internet, etc.). For example, the testing apparatus may be connected to a medical records database, hospital management processing system, health care professional terminals

(e.g., doctor stations, nurse stations), patient monitoring systems, automated drug delivery systems such as smart pumps, smart infusion systems, automated drug delivery systems, etc. Accordingly, the disclosed embodiments may be used as part of an automated closed loop system or as part of a decision assist system. By way of example, a network interface **834** is coupled to the I/O interface **812** for connecting the testing apparatus **802** to a network **836**, through a wired or wireless connection.

[0040] In this way, the system **800** may be used to determine the cardiovascular condition of the patient and whether that condition has improved or deteriorated over the period of time, e.g., by measuring changes in venous blood volume over time, and in response to procedures performed by the patient and in response to different treatments provided to the patient. Ventilatory effort of the patient can be determined, e.g., how much effort does it take for a patient to reach a desired volume of inspiration or expiration as measured by impedance and as that correlates to venous blood volume. The cardiovascular condition of a patient can be monitored over time to determine if the condition has improved or deteriorated, e.g., by determining a baseline impedance pattern for a patient and then comparing subsequent impedance measures to that baseline do determine variations from the baseline.

[0041] The system **800** may be used to further determine, from the impedance measurements and determined changes in venous blood volume, a central venous pressure or right atrial venous pressure. The system **800** may be further used to determine respiratory rate and respiratory effort, from the change in venous blood volume data. The change in venous blood volume, the respiratory rate, and respiratory effort may be monitored over time to determine if the patient's condition has improved or deteriorated, for example, in response to different treatment cycles and different treatment conditions. In some examples, the system **800** may detect patterns in the changes in volume of blood over a period of time and detect outlines in changes in volume of blood over that time, as indicators of various conditions. In any of these cases, the memory **806** may store the appropriate instructions that are executed by the processor **808** to automatically affect such monitoring and determinations.

[0042] Throughout this specification, plural instances may implement components, operations, or structures described as a single instance. Although individual operations of one or more methods are illustrated and described as separate operations, one or more of the individual operations may be performed concurrently, and nothing requires that the operations be performed in the order illustrated. Structures and functionality presented as separate components in example configurations may be implemented as a combined structure or component. Similarly, structures and functionality presented as a single component may be implemented as separate components. These and other variations, modifications, additions, and improvements fall within the scope of the subject matter herein.

[0043] Additionally, certain embodiments are described herein as including logic or a number of routines, subroutines, applications, or instructions. These may constitute either software (e.g., code embodied on a non-transitory, machine-readable medium) or hardware. In hardware, the routines, etc., are tangible units capable of performing certain operations and may be configured or arranged in a certain manner. In example embodiments, one or more computer systems (e.g., a standalone, client or server computer system) or one or more hardware modules of a computer system (e.g., a processor or a group of processors) may be configured by software (e.g., an application or application portion) as a hardware module that operates to perform certain operations as described herein.

[0044] In various embodiments, a hardware module may be implemented mechanically or electronically. For example, a hardware module may comprise dedicated circuitry or logic that is permanently configured (e.g., as a special-purpose processor, such as a field programmable gate array (FPGA) or an application-specific integrated circuit (ASIC)) to perform certain operations. A hardware module may also comprise programmable logic or circuitry (e.g., as encompassed within a general-purpose processor or other programmable processor) that is temporarily configured by software to perform certain operations. It will be appreciated that the decision to implement a hardware module mechanically, in dedicated and permanently configured circuitry, or in temporarily configured circuitry (e.g., configured by software) may be driven by cost and time considerations.

[0045] Accordingly, the term "hardware module" should be understood to encompass a tangible entity, be that an entity that is physically constructed, permanently configured (e.g., hardwired), or temporarily configured (e.g., programmed) to operate in a certain manner or to perform certain operations described herein. Considering embodiments in which hardware modules are temporarily configured (e.g., programmed), each of the hardware modules need not be configured or instantiated at any one instance in time. For example, where the hardware modules comprise a general-purpose processor configured using software, the general-purpose processor may be configured as respective different hardware modules at different times. Software may accordingly configure a processor, for example, to constitute a particular hardware module at one instance of time and to constitute a different hardware module at a different instance of time.

[0046] Hardware modules can provide information to, and receive information from, other hardware modules. Accordingly, the described hardware modules may be regarded as being communicatively coupled. Where multiple of such hardware modules exist contemporaneously, communications may be achieved through signal transmission (e.g., over appropriate circuits and buses) that connect the hardware modules. In embodiments in which multiple hardware modules are configured or instantiated at different times, communications between such hardware modules may be achieved, for example, through the storage and retrieval of information in memory structures to which the multiple hardware modules have access. For example, one hardware module may perform an operation and store the output of that operation in a memory device to which it is communicatively coupled. A further hardware module may then, at a later time, access the memory device to retrieve and process the stored output. Hardware modules may also initiate communications with input or output devices, and can operate on a resource (e.g., a collection of information).

[0047] The various operations of example methods described herein may be performed, at least partially, by one or more processors that are temporarily configured (e.g., by software) or permanently configured to perform the relevant operations. Whether temporarily or permanently configured, such processors may constitute processor-implemented modules that operate to perform one or more operations or func-

tions. The modules referred to herein may, in some example embodiments, comprise processor-implemented modules.

[0048] Similarly, the methods or routines described herein may be at least partially processor-implemented. For example, at least some of the operations of a method may be performed by one or more processors or processor-implemented hardware modules. The performance of certain of the operations may be distributed among the one or more processors, not only residing within a single machine, but deployed across a number of machines. In some example embodiments, the processor or processors may be located in a single location (e.g., within a home environment, an office environment or as a server farm), while in other embodiments the processors may be distributed across a number of locations.

[0049] The performance of certain of the operations may be distributed among the one or more processors, not only residing within a single machine, but deployed across a number of machines. In some example embodiments, the one or more processors or processor-implemented modules may be located in a single geographic location (e.g., within a home environment, an office environment, or a server farm). In other example embodiments, the one or more processors or processor-implemented modules may be distributed across a number of geographic locations.

[0050] Unless specifically stated otherwise, discussions herein using words such as "processing," "computing," "calculating," "determining," "presenting," "displaying," or the like may refer to actions or processes of a machine (e.g., a computer) that manipulates or transforms data represented as physical (e.g., electronic, magnetic, or optical) quantities within one or more memories (e.g., volatile memory, non-volatile memory, or a combination thereof), registers, or other machine components that receive, store, transmit, or display information.

[0051] As used herein any reference to "one embodiment" or "an embodiment" means that a particular element, feature, structure, or characteristic described in connection with the embodiment is included in at least one embodiment. The appearances of the phrase "in one embodiment" in various places in the specification are not necessarily all referring to the same embodiment.

[0052] Some embodiments may be described using the expression "coupled" and "connected" along with their derivatives. For example, some embodiments may be described using the term "coupled" to indicate that two or more elements are in direct physical or electrical contact. The term "coupled," however, may also mean that two or more elements are not in direct contact with each other, but yet still co-operate or interact with each other. The embodiments are not limited in this context.

[0053] As used herein, the terms "comprises," "comprising," "includes," "including," "has," "having" or any other variation thereof, are intended to cover a non-exclusive inclusion. For example, a process, method, article, or apparatus that comprises a list of elements is not necessarily limited to only those elements but may include other elements not expressly listed or inherent to such process, method, article, or apparatus. Further, unless expressly stated to the contrary, "or" refers to an inclusive or and not to an exclusive or. For example, a condition A or B is satisfied by any one of the following: A is true (or present) and B is false (or not present), A is false (or not present) and B is true (or present), and both A and B are true (or present).

[0054] In addition, use of the "a" or "an" are employed to describe elements and components of the embodiments herein. This is done merely for convenience and to give a general sense of the description. This description, and the claims that follow, should be read to include one or at least one and the singular also includes the plural unless it is obvious that it is meant otherwise.

[0055] This detailed description is to be construed as examples and does not describe every possible embodiment, as describing every possible embodiment would be impractical, if not impossible. One could implement numerous alternate embodiments, using either current technology or technology developed after the filing date of this application.

What is claimed:

1. A method for evaluating cardiovascular condition of a patient, the method comprising:

(a) recording a first impedance of a limb or extremity or neck of the patient at a first time in response to receiving a first impedance reading from a plurality of sensors on a limb or extremity or neck;

(b) after the occurrence of an event modulating blood return to the heart via the venous system of the patient, recording a second impedance of the limb or extremity or neck at a second time in response to receiving a second impedance reading from a plurality of sensors on a limb or extremity or neck, wherein the first impedance and the second impedance each correspond to a volume of blood flowing within the limb or extremity or neck; and

(c) determining a change in venous blood volume between the first time and the second time by comparing the first impedance and the second impedance to determine a change in volume of blood.

2. The method of claim **1**, wherein (a)-(c) are performed repeatedly over a period of time, the method further comprising:

(d) determining whether the cardiovascular condition of the patient has improved or deteriorated over the period of time.

3. The method of claim **2**, wherein determining whether the cardiovascular condition of the patient has improved or deteriorated over the period of time includes monitoring the ventilatory effort of the patient and ventilatory dynamics of the patient.

4. The method of claim **2**, wherein determining whether the cardiovascular condition of the patient has improved or deteriorated over the period of time includes:

(1) using a first portion of the recorded impedances to determine a baseline impedance pattern for the patient, and

(2) comparing a second portion of the recorded impedances to the baseline impedance pattern to detect deviations from the baseline impedance pattern.

5. The method of claim **1**, wherein placing electrodes on one of a limb or extremity or neck to monitor the impedance of the limb or extremity or neck includes placing two electrodes to inject electrical current and two electrodes to measure impedance.

6. The method of claim **1**, wherein the event modulating blood return to the heart includes one or more of spontaneous inspiration, positive pressure mechanical ventilation, raising a limb or extremity or neck of the patient, applying negative or

positive pressure to the abdomen or chest, inspiration against a negative impedance valve, or discrete maneuvers performed with mechanical ventilation.

7. The method of claim **6**, wherein the discrete maneuvers performed with mechanical ventilation comprise adjusting positive pressure ventilation, negative pressure ventilation, maintaining inspiratory or expiratory pause, or a combination thereof.

8. The method of claim **1**, wherein (a) and (b) are performed outside a medical facility.

9. The method of claim **1**, wherein the patient is undergoing treatment for one or more of cardiac arrest, edema, burns, trauma, heart failure, sepsis, dehydration, renal failure, or dialysis.

10. The method of claim **1**, further comprising:

(d) determining one or more of:

(1) how the patient will hemodynamically respond to one or more of an addition of cardiovascular fluid or removal of cardiovascular fluid,

(2) how the patient will hemodynamically respond to one or more cardiovascular drugs which promote changes in cardiac output, changes in cardiovascular preload, and changes in cardiovascular afterload, or

(3) determining how the patient will respond hemodynamically to changes in mechanical or noninvasive ventilation.

11. The method of claim **1**, further comprising: determining changes in vena cava diameter, central venous pressure, or right atrial venous pressure from the change in venous blood volume.

12. The method of claim **1**, further comprising: determining respiratory rate and effort from the change in venous blood volume.

13. A testing apparatus for evaluating cardiovascular condition of a patient, the testing apparatus comprising:

one or more electrodes;

one or more processors;

a computer-readable memory storing non-transient instructions that when executed by the one or more processors cause the testing apparatus to:

(a) use the one or more electrodes to record a first impedance of a limb or extremity or neck of the patient at a first time in response to receiving a first impedance reading from a plurality of sensors on a limb or extremity or neck;

(b) after the occurrence of an event modulating blood return to the heart via the venous system of the patient, use the one or more electrodes to record a second impedance of the limb or extremity or neck at a second time in response to receiving a second impedance reading from a plurality of sensors on a limb or extremity or neck, wherein the first impedance and the second impedance each correspond to a volume of blood flowing within the limb or extremity or neck; and

(c) determine a change in venous blood volume between the first time and the second time by comparing the first impedance and the second impedance to determine a change in volume of blood.

14. The testing apparatus of claim **13**, wherein the non-transient instructions include instructions that when executed by the one or more processors cause the testing apparatus to determine whether the cardiovascular condition of the patient has improved or deteriorated over the period of time.

15. The testing apparatus of method of claim **14**, wherein the instructions to determine whether the cardiovascular condition of the patient has improved or deteriorated over the period of time includes instructions to monitor a respiratory rate of the patient and a respiratory effort of the patient.

16. The method of claim **15**, wherein the instructions to determine whether the cardiovascular condition of the patient has improved or deteriorated over the period of time includes instructions to:

(1) use a first portion of the recorded impedances to determine a baseline impedance pattern for the patient, and

(2) compare a second portion of the recorded impedances to the baseline impedance pattern to detect deviations from the baseline impedance pattern.

17. The testing apparatus of claim **13**, wherein the one or more electrodes include two electrodes to inject electrical current and two electrodes to measure impedance.

18. The testing apparatus of claim **13**, wherein the event modulating blood to return to the heart includes one or more of spontaneous inspiration; positive pressure mechanical ventilation, negative pressure ventilation, or raising a limb or extremity or neck of the patient.

19. The testing apparatus of claim **13**, further comprising a photoplethysmograph, a galvanic skin response monitor, a near infrared spectroscopy device, a laser Doppler device with or without speckle tracking, or an ultrasound device with or without speckle tracking.

20. The testing apparatus of claim **13**, further comprising impedance cardiography measurement, pulse pressure variation measurement, or stroke volume variation measurement.

21. A closed-loop cardiovascular condition evaluation system comprising:

the testing apparatus of claim **13**; and

a processor and a memory, the memory storing instructions that when executed by the processor, cause the processor to evaluate a cardiovascular condition of a subject in response to determining the change in the venous blood volume between the first time and the second time determined by comparing the first impedance and the second impedance, for different treatment cycles.

22. The closed-loop system of claim **21**, wherein the different treatment cycles comprising, determining the change in venous blood volume under a first treatment condition and under a second treatment condition.

23. The closed-loop system of claim **22**, wherein the first treatment condition is a pre-cardiovascular treatment condition and the second treatment condition is after a cardiovascular treatment has been applied.

24. The closed-loop system of claim **21**, wherein the memory stores instructions that when executed by the processor, cause the processor to adjust a cardiovascular treatment in response to the determination in the change of venous blood volume.

25. A method for evaluating the cardiovascular condition of a patient, the method comprising:

(a) determining a first volume of blood of a limb or extremity or neck of the patient at a first time;

(b) after the occurrence of an event causing blood to return to the heart via the venous system of the patient, determining a second volume of blood of the limb or extremity or neck at a second time;

(c) determining a change in venous blood volume between the first time and the second time by comparing the first volume of blood and the second volume of blood; and

(d) determining one or more of,

(1) how the patient will hemodynamically respond to one or more of an addition of cardiovascular fluid or removal of cardiovascular fluid,

(2) how the patient will hemodynamically respond to one or more cardiovascular drugs which promote changes in cardiac output, changes in cardiovascular preload, and changes in cardiovascular afterload,

(3) determining how the patient will response to changes in mechanical or noninvasive ventilation, or

(4) determining respiratory rate and magnitude of respiratory effort.

26. The method of claim **25**, wherein the first and second volume of blood in a limb is determined by measuring the diameter of a major vein in the limb or extremity or neck using ultrasonic imagery with our without speckle tracking, or laser Doppler Flowmetry with or without speckle tracking, or near infrared spectroscopy, or photoplethysmography, or galvanic skin response.

27. The method of claim **26**, wherein (a)-(c) are performed repeatedly over a period of time, the method further comprising:

(e) determining whether the cardiovascular condition of the patient has improved or deteriorated over the period of time.

28. The method of claim **27**, wherein determining whether the cardiovascular condition of the patient has improved or deteriorated over the period of time includes using a learning algorithm to

(1) detect a pattern in the changes of volume of blood over the period of time, and

(2) detect outliers in the changes of volume of blood over the period of time.

* * * * *

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NOTICE OF ALLOWANCE AND FEE(S) DUE

4743 7590 03/15/2019

MARSHALL, GERSTEIN & BORUN LLP
233 SOUTH WACKER DRIVE
6300 WILLIS TOWER
CHICAGO, IL 60606-6357

EXAMINER	
NATNITHITHADHA, NAVIN	
ART UNIT	PAPER NUMBER
3791	

DATE MAILED: 03/15/2019

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/445,926	07/29/2014	Kevin Ward	30275/47374A	8346

TITLE OF INVENTION: EVALUTATING CARDIOVASCULAR HEALTH USING INTRAVASCULAR VOLUME

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	$500	$0.00	$0.00	$500	06/17/2019

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Maintenance fees are due in utility patents issuing on applications filed on or after Dec. 12, 1980. It is patentee's responsibility to ensure timely payment of maintenance fees when due. More information is available at www.uspto.gov/PatentMaintenanceFees.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via EFS-Web.

By mail, send to: Mail Stop ISSUE FEE
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By fax, send to: (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

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Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

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I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via EFS-Web or by facsimile to (571) 273-2885, on the date below.

(Typed or printed name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/445,926	07/29/2014	Kevin Ward	30275/47374A	8346

TITLE OF INVENTION: EVALUTATING CARDIOVASCULAR HEALTH USING INTRAVASCULAR VOLUME

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	$500	$0.00	$0.00	$500	06/17/2019

EXAMINER	ART UNIT	CLASS-SUBCLASS
NATNITHITHADHA, NAVIN	3791	600-507000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-09 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,

(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 _____

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3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE

(B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. Fees submitted: ❑ Issue Fee ❑ Publication Fee (if required) ❑ Advance Order - # of Copies _____

4b. Method of Payment: *(Please first reapply any previously paid fee shown above)*

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5. **Change in Entity Status** (from status indicated above)

❑ Applicant certifying micro entity status. See 37 CFR 1.29

❑ Applicant asserting small entity status. See 37 CFR 1.27

❑ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
NATNITHITHADHA, NAVIN	
ART UNIT	PAPER NUMBER
3791	

DATE MAILED: 03/15/2019

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

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The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

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2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
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5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No. 14/445,926	Applicant(s) Ward et al.	
	Examiner NAVIN NATNITHITHADHA	Art Unit 3791	AIA (FITF) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☑ This communication is responsive to the Amendment, Remarks, and Information Disclosure Statement, filed 30 April 2018.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

3. ☑ The allowed claim(s) is/are 1-9,13-24 and 29-35 . As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information , please see **http://www.uspto.gov/patents/init_events/pph/index.jsp** or send an inquiry to **PPHfeedback@uspto.gov.**

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☐ All b) ☐ Some *c) ☐ None of the:

   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____ .

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file areply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____ .

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☑ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____.
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____.
4. ☑ Interview Summary (PTO-413), Paper No./Mail Date. _____.
5. ☑ Examiner's Amendment/Comment
6. ☑ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/NAVIN NATNITHITHADHA/
Primary Examiner, Art Unit 3791

EXAMINER'S AMENDMENT

1. An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with Paul B. Stephens on 01 March 2019.

The application has been amended as follows:

The following claims have been amended as follows:

- -

1. (Currently amended) A method for evaluating cardiovascular condition of a patient, the method comprising:

(a) recording a first impedance of a limb or extremity or neck of the patient at a first time in response to receiving a first impedance reading from a plurality of sensors on a limb or extremity or neck;

(b) after the occurrence of an event modulating blood return to the heart via the venous system of the patient, recording a second impedance of the limb or extremity or neck at a second time in response to receiving a second impedance reading from a plurality of sensors on a limb or extremity or neck, wherein the first impedance and the second impedance each correspond to a volume of blood flowing within the limb or extremity or neck;

(c) determining a change in venous blood volume between the first time and the second time by comparing the first impedance and the second impedance to determine a change in volume of blood; and

(d) determining, based on the change in venous blood volume between the first time and the second time, one or more of:

(1) how the patient will hemodynamically respond to one or more of an addition of cardiovascular fluid or removal of cardiovascular fluid,

(2) how the patient will hemodynamically respond to one or more cardiovascular drugs which promote changes in cardiac output, changes in cardiovascular preload, and changes in cardiovascular afterload, or

(3) how the patient will hemodynamically respond to changes in mechanical or noninvasive ventilation.

13. (Currently amended) A testing apparatus for evaluating cardiovascular condition of a patient, the testing apparatus comprising:

one or more electrodes;

one or more processors;

a computer-readable memory storing non-transient instructions that when executed by the one or more processors cause the testing apparatus to:

(a) use the one or more electrodes to record a first impedance of a limb or extremity or neck of the patient at a first time in response to receiving a first impedance reading from a plurality of sensors on a limb or extremity or neck;

(b) after the occurrence of an event modulating blood return to the heart via the venous system of the patient, use the one or more electrodes to record a second impedance of the limb or extremity or neck at a second time in response to receiving a second impedance reading from a plurality of sensors on a limb or extremity or neck, wherein the first impedance and the second impedance each correspond to a volume of blood flowing within the limb or extremity or neck;

(c) determine a change in venous blood volume between the first time and the second time by comparing the first impedance and the second impedance to determine a change in volume of blood; and

(d) determine, based on the change in venous blood volume between the first time and the second time, one or more of:

(1) how the patient will hemodynamically respond to one or more of an addition of cardiovascular fluid or removal of cardiovascular fluid,

(2) how the patient will hemodynamically respond to one or more cardiovascular drugs which promote changes in cardiac output, changes in cardiovascular preload, and changes in cardiovascular afterload, or

(3) how the patient will respond hemodynamically to changes in mechanical or noninvasive ventilation.

29. (Currently amended) A method for evaluating cardiovascular condition of a patient, the method comprising:

(a) recording a first impedance of a limb or extremity or neck of the patient at a first time in response to receiving a first impedance reading from a plurality of sensors on a limb or extremity or neck;

(b) after the occurrence of an event modulating blood return to the heart via the venous system of the patient, recording a second impedance of the limb or extremity or neck at a second time in response to receiving a second impedance reading from a plurality of sensors on a limb or extremity or neck, wherein the first impedance and the second impedance each correspond to a volume of blood flowing within the limb or extremity or neck;

(c) determining a change in venous blood volume between the first time and the second time by comparing the first impedance and the second impedance to determine a change in volume of blood; and

(d) determining, based on the change in venous blood volume between the first time and the second time, one or more of changes in vena cava diameter, central venous pressure, right atrial venous pressure from the change in venous blood volume, or respiratory rate and effort from the change in venous blood volume.

- -

REASONS FOR ALLOWANCE

2. The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

3. According to the Amendment, filed 30 April 2018, the status of the claims is as follows:

Claims 1, 2, 5, 13, and 18 are currently amended;

Claims 3, 4, 6-9, 14-17, and 19-24 are as originally filed;

Claims 29-35 are new; and

Claims 10-12 and 25-28 are cancelled.

4. In the prior Office Action, p. 12, mailed 22 January 2018, Claims 10-12 were indicated as allowable if rewritten in independent form including all of the limitations of the base claim. Applicant amended independent claims 1 and 13 to include the allowable subject matter of claim 10, and added new independent claim 29 to include the allowable subject matter of claim 11 and all of the limitations of original base claim 1. Thus, claims 1-9, 13-24, and 29-35 are allowable for the reasons stated in the prior Office Action, and the rejections of claims 1-9, 13-18, and 20-24 under 35 U.S.C. 102(a)(1) as being anticipated by Ward, U.S. Patent Application Publication No. 2009/0287102 A1 ("Ward"), and the rejection of claim 19 under 35 U.S.C. 103 as being unpatentable over Ward, as applied to claim 13 above, and further in view of Shelley et al, U.S. Patent No. 8,251,912 B2, are withdrawn.

5. In an Interview, on 01 March 2019, Examiner and Applicant's Representative, Paul B. Stephens, agreed on amending claims 1, 13, and 29 by the Examiner's Amendment above to overcome the rejection of claims 1, 13, and 29 under 35 U.S.C.

112(b) and to place the Application in condition for allowance. See Interview Summary, mailed with the present Office Action.

6. Claims 1-9, 13-24, and 29-35 are allowed.

7. Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

8. Any inquiry concerning this communication or earlier communications from the examiner should be directed to NAVIN NATNITHITHADHA whose telephone number is (571)272-4732. The examiner can normally be reached on Monday - Friday 6:00 am - 8:30 am & 10:30 am - 4:00 pm.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Patricia Mallari can be reached on 571-272-4729. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only.

For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

NAVIN NATNITHITHADHA
Primary Examiner
Art Unit 3791

/NAVIN NATNITHITHADHA/
Primary Examiner, Art Unit 3791
03/04/2019

Examiner-Initiated Interview Summary	Application No.	Applicant(s)	
	14/445,926	Ward et al.	
	Examiner	Art Unit	AIA (FITF) Status
	NAVIN NATNITHITHADHA	3791	Yes

All participants (applicant, applicant's representative, PTO personnel):

(1) NAVIN NATNITHITHADHA. (3) _____.

(2) Paul B. Stephens (Applicant's Representative). (4) _____.

Date of Interview: 01 March 2019.

Type: ☑ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☑ No.
If Yes, brief description: _____.

Issues Discussed ☐ 101 ☑ 112 ☐ 102 ☐ 103 ☐ Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: 1,13 and 29.

Identification of prior art discussed: none.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

See Continuation Sheet.

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

/NAVIN NATNITHITHADHA/ Primary Examiner, Art Unit 3791	

Continuation of Substance of Interview including description of the general nature of what was agreed to if an agreement was reached, or any other comments:
1) Examiner stated that claims 1, 13, and 29 are rejected under 35 U.S.C. 112(b) as being indefinite for not clearly defining how the last determining steps in each of these claims are determined. Examiner proposed an amendment to obviate this rejection.
2) Applicant's Representative agreed to the proposed amendment of claims 1, 13, and 29 in order to advance examination to allowance.
3) The proposed amendment will be included in an Examiner's Amendment.

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14/445,926	Ward et al.
	Examiner	Art Unit
	NAVIN NATNITHITHADHA	3791

CPC - Searched*		
Symbol	**Date**	**Examiner**
A61B5/4836 OR A61B5/0205 OR A61B5/0535 OR A61B5/4884 OR A61B5/7278 OR A61B5/0075 OR A61B5/0261 OR A61B5/0295 OR A61B5/0533 OR A61B5/0816 OR A61B5/1073 OR A61B5/1075 OR A61B5/14551 OR A61B5/6822 OR A61B5/6824 OR A61B5/7275 OR A61B8/06	01/11/2018	/NN/
A61B5/4836 OR A61B5/0205 OR A61B5/0535 OR A61B5/4884 OR A61B5/7278 OR A61B5/0075 OR A61B5/0261 OR A61B5/0295 OR A61B5/0533 OR A61B5/0816 OR A61B5/1073 OR A61B5/1075 OR A61B5/14551 OR A61B5/6822 OR A61B5/6824 OR A61B5/7275 OR A61B8/06	02/28/2019	/NN/

CPC Combination Sets - Searched*		
Symbol	**Date**	**Examiner**

US Classification - Searched*			
Class	**Subclass**	**Date**	**Examiner**
600	504-507	01/11/2018	/NN/
600	504-507	02/28/2019	/NN/

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	**Date**	**Examiner**
EAST (attached): USPAT, US-PGPUB, EPO, JPO, Derwent, Inventor, Assignee, IDS, Forward/Backward Citation, CPC, USPC searches	01/11/2018	/NN/
EAST (attached): USPAT, US-PGPUB, EPO, JPO, Derwent, Inventor, Assignee, Art of Record, IDS, Forward/Backward Citation, CPC, USPC, Interference searches	02/28/2019	/NN/

/N.N/ Primary Examiner, Art Unit 3791	

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14/445,926	Ward et al.
	Examiner	Art Unit
	NAVIN NATNITHITHADHA	3791

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner
	A61B5/4836 OR A61B5/0205 OR A61B5/0535 OR A61B5/4884 OR A61B5/7278 OR A61B5/0075 OR A61B5/0261 OR A61B5/0295 OR A61B5/0533 OR A61B5/0816 OR A61B5/1073 OR A61B5/1075 OR A61B5/14551 OR A61B5/6822 OR A61B5/6824 OR A61B5/7275 OR A61B8/06	03/01/2019	/NN/

/N.N/ Primary Examiner, Art Unit 3791	

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14/445,926	Ward et al.
	Examiner	**Art Unit**
	NAVIN NATNITHITHADHA	3791

CPC						
Symbol					**Type**	**Version**
A61B	/	5	/	4836	F	2013-01-01
A61B	/	5	/	0205	I	2013-01-01
A61B	/	5	/	0535	I	2013-01-01
A61B	/	5	/	4884	I	2013-01-01
A61B	/	5	/	7278	I	2013-01-01
A61B	/	5	/	0295	A	2013-01-01
A61B	/	5	/	0261	A	2013-01-01
A61B	/	8	/	06	A	2013-01-01
A61B	/	5	/	0075	A	2013-01-01
A61B	/	5	/	0533	A	2013-01-01
A61B	/	5	/	0816	A	2013-01-01
A61B	/	5	/	1073	A	2013-01-01
A61B	/	5	/	1075	A	2013-01-01
A61B	/	5	/	14551	A	2013-01-01
A61B	/	5	/	6822	A	2013-01-01
A61B	/	5	/	6824	A	2013-01-01
A61B	/	5	/	7275	A	2013-01-01

CPC Combination Sets								
Symbol					**Type**	**Set**	**Ranking**	**Version**
	/		/					

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	28	
/NAVIN NATNITHITHADHA/ Primary Examiner, Art Unit 3791	04 March 2019	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	1

U.S. Patent and Trademark Office

Part of Paper No.: 20190306

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14/445,926	Ward et al.
	Examiner	**Art Unit**
	NAVIN NATNITHITHADHA	3791

INTERNATIONAL CLASSIFICATION				
CLAIMED				
A61B	/	5	/	0205
A61B	/	5	/	0295
A61B	/	8	/	06

NON-CLAIMED				
	/		/	

US ORIGINAL CLASSIFICATION	
CLASS	**SUBCLASS**

CROSS REFERENCES(S)						
CLASS	**SUBCLASS (ONE SUBCLASS PER BLOCK)**					

NONE (Assistant Examiner) (Date)		**Total Claims Allowed:** 28	
/NAVIN NATNITHITHADHA/ Primary Examiner, Art Unit 3791 (Primary Examiner)	04 March 2019 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure 1

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14/445,926	Ward et al.
	Examiner	**Art Unit**
	NAVIN NATNITHITHADHA	3791

☑ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIMS

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	28	
/NAVIN NATNITHITHADHA/ Primary Examiner, Art Unit 3791	04 March 2019	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	1

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	11	(("20100222696") or ("20060122540") or ("20130172691") or ("20100036455") or ("20050080460") or ("20110046505") or ("20080097230") or ("20080190430") or ("20030167012") or ("20090287102") or ("20120016246")).PN.	US-PGPUB; USPAT; USOCR	OR	OFF	2019/03/01 13:55
L2	1	("20150031966").PN.	US-PGPUB; USPAT; USOCR	OR	OFF	2019/03/01 13:55
L3	235805	(REGENTS AJD3 UNIVERSITY AJD2 MICHIGAN).AS. OR ((Ward NEAR2 Kevin) OR (Tiba NEAR2 (Mohamad ADJ2 Hakam)) OR (Blum NEAR2 James)).INV.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2019/03/01 13:55
L4	5	(venous ADJ2 blood ADJ2 volume).clm. AND L3	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2019/03/01 13:55
L5	1	(((venous ADJ2 blood ADJ2 volume) WITH (chang$3 OR variation)) SAME (impedance OR bioimpedance)) AND L1	US-PGPUB; USPAT; USOCR	OR	ON	2019/03/01 13:55
L6	1	(((venous ADJ2 blood ADJ2 volume) WITH (chang$3 OR variation))) AND L1	US-PGPUB; USPAT; USOCR	OR	ON	2019/03/01 13:55
L7	9	(((venous OR blood OR pulse) ADJ3 volume) WITH (chang$3 OR variation)) AND L1	US-PGPUB; USPAT; USOCR	OR	ON	2019/03/01 13:55
L8	346	("20050197551" | "20060241506" | "20060258921" | "20080066753" | "20080076992" | "20080167564" | "20080190430" | "20080262326" | "20090043179" | "20090069647" | "20090076399" | "20110270094" | "4800495" | "4860759" | "5396893" | "5482036" | "5490505" | "5769082" | "5865756" | "6002952" | "6036642" | "6067462" | "6081742" | "6129675" | "6206830" | "6319205" | "6322515" | "6325761" | "6334065" | "6361501" | "6463311" | "6480729" | "6501975" | "6506161" | "6561984" | "6616613" | "6658277" | "6669632" | "6699194" | "6702752" | "6709402" | "6714804" | "6826419" | "6869402" | "6896661" | "6898452" | "7001337" | "7027850" |	US-PGPUB; USPAT; USOCR	OR	ON	2019/03/01 13:55

		"7209775" | "7471971" | "7489958" | "7499741" | "7515949" | "7976472").PN. OR ("2003/0167012" | "2005/0080460" | "2006/0122540" | "2008/0097230" | "2008/0190430" | "2009/0287102" | "2010/0036455" | "2010/0222696" | "2011/0046505" | "2012/0016246" | "2013/0172691" | "2015/0031966" | "8251912").URPN.				
L9	107	(((venous OR blood OR pulse) NEAR5 volume) WITH (chang$3 OR variation)) AND L8	US-PGPUB; USPAT; USOCR	OR	ON	2019/03/01 13:55
L10	39	(((venous OR blood OR pulse) NEAR5 volume) WITH (chang$3 OR variation)) AND (blood NEAR6 (return$4 OR reperfus$4)) AND L8	US-PGPUB; USPAT; USOCR	OR	ON	2019/03/01 13:55
L11	10	(((venous OR blood OR pulse) NEAR5 volume) WITH (chang$3 OR variation)) AND (blood NEAR6 (return$4 OR reperfus$4)) AND ((impedance OR bioimpedance) SAME (electrode OR bioelectrode OR sens$5 OR biosens$5 OR transducer OR biotransducer)) AND L8	US-PGPUB; USPAT; USOCR	OR	ON	2019/03/01 13:55
L12	10	((volume) WITH (chang$3 OR variation)) AND L11	US-PGPUB; USPAT; USOCR	OR	ON	2019/03/01 13:55
L13	10	(((venous OR blood OR pulse) NEAR5 volume) WITH (chang$3 OR variation)) AND (blood NEAR6 (return$4 OR reperfus$4)) AND L12	US-PGPUB; USPAT; USOCR	OR	ON	2019/03/01 13:55
L14	1	((((venous OR blood OR pulse) NEAR5 volume) WITH (chang$3 OR variation)) SAME (blood NEAR6 (return$4 OR reperfus$4))) AND ((impedance OR bioimpedance) SAME (electrode OR bioelectrode OR sens$5 OR biosens$5 OR transducer OR biotransducer)) AND L8	US-PGPUB; USPAT; USOCR	OR	ON	2019/03/01 13:55
L15	34675	(600/504-507).CCLS. OR (A61B5/4836 OR A61B5/0205 OR A61B5/0535 OR A61B5/4884 OR A61B5/7278 OR A61B5/0075 OR A61B5/0261 OR A61B5/0295 OR A61B5/0533 OR A61B5/0816 OR A61B5/1073 OR A61B5/1075 OR A61B5/14551 OR A61B5/6822 OR A61B5/6824 OR A61B5/7275 OR A61B8/06).CPC.	US-PGPUB; USPAT; USOCR	OR	OFF	2019/03/01 13:55
L16	7	((((venous OR blood OR pulse) NEAR5 volume) WITH (chang$3 OR variation) WITH (determin$6 OR estimat$4 OR calculat$4 OR monitor$4 OR compute OR computing OR deriv$6)) SAME ((blood OR bloodflow$4) NEAR6 (return$4 OR reperfus$4))) AND ((impedance OR bioimpedance) SAME (electrode OR bioelectrode OR sens$5 OR biosens$5 OR transducer OR biotransducer)) AND L15	US-PGPUB; USPAT; USOCR	OR	ON	2019/03/01 13:55
L17	37	((((venous OR blood OR pulse) NEAR5 volume) WITH (chang$3 OR variation)	US-PGPUB; USPAT;	OR	ON	2019/03/01 13:55

		WITH (determin$6 OR estimat$4 OR calculat$4 OR monitor$4 OR compute OR computing OR deriv$6)) SAME ((blood OR bloodflow$4) NEAR6 (return$4 OR reperfus$4))) AND L15	USOCR			
L18	11	(("20100222696") or ("20060122540") or ("20130172691") or ("20100036455") or ("20050080460") or ("20110046505") or ("20080097230") or ("20080190430") or ("20030167012") or ("20090287102") or ("20120016246")).PN.	US-PGPUB; USPAT; USOCR	OR	OFF	2019/03/01 13:55
L19	9	(((venous OR blood OR pulse) ADJ3 volume) WITH (chang$3 OR variation)) AND L18	US-PGPUB; USPAT; USOCR	OR	ON	2019/03/01 13:55
L20	34675	(600/504-507).CCLS. OR (A61B5/4836 OR A61B5/0205 OR A61B5/0535 OR A61B5/4884 OR A61B5/7278 OR A61B5/0075 OR A61B5/0261 OR A61B5/0295 OR A61B5/0533 OR A61B5/0816 OR A61B5/1073 OR A61B5/1075 OR A61B5/14551 OR A61B5/6822 OR A61B5/6824 OR A61B5/7275 OR A61B8/06).CPC.	US-PGPUB; USPAT; USOCR	OR	OFF	2019/03/01 13:55
L21	6	((((central OR artrial) ADJ2 venous ADJ2 pressure) OR (vena ADJ2 cava ADJ2 (diameter OR radius))) WITH (determin$6 OR deriv$6 OR estimat$4 OR calculat$4 OR compute OR computing) WITH ((blood OR pulse) ADJ2 volume)) AND L20	US-PGPUB; USPAT; USOCR	OR	ON	2019/03/01 13:55
L22	21	(((((central OR artrial) ADJ2 venous ADJ2 pressure) OR (vena ADJ2 cava ADJ2 (diameter OR radius))) WITH (determin$6 OR deriv$6 OR estimat$4 OR calculat$4 OR compute OR computing)) SAME ((blood OR pulse) ADJ2 volume)) AND L20	US-PGPUB; USPAT; USOCR	OR	ON	2019/03/01 13:55
L23	166640	(600/$).CCLS. OR (A61B5/4836 OR A61B5/0205 OR A61B5/0535 OR A61B5/4884 OR A61B5/7278 OR A61B5/0075 OR A61B5/0261 OR A61B5/0295 OR A61B5/0533 OR A61B5/0816 OR A61B5/1073 OR A61B5/1075 OR A61B5/14551 OR A61B5/6822 OR A61B5/6824 OR A61B5/7275 OR A61B8/06).CPC.	US-PGPUB; USPAT; USOCR	OR	OFF	2019/03/01 13:55
L24	16	(((((central OR artrial) ADJ2 venous ADJ2 pressure) OR "CVP" OR (vena ADJ2 cava ADJ2 (diameter OR radius))) WITH (determin$6 OR deriv$6 OR estimat$4 OR calculat$4 OR compute OR computing)) SAME ((blood OR pulse) ADJ2 volume)) AND (impedance OR bioimpedance) AND L23	US-PGPUB; USPAT; USOCR	OR	ON	2019/03/01 13:55
L25	16	(((((central OR artrial) ADJ2 venous ADJ2 pressure) OR "CVP" OR (vena ADJ2 cava ADJ2 (diameter OR radius))) WITH (determin$6 OR deriv$6 OR estimat$4 OR calculat$4 OR compute OR computing)) SAME ((blood OR pulse) ADJ2 volume)) AND L24	US-PGPUB; USPAT; USOCR	OR	ON	2019/03/01 13:55

L26	17	(((((central OR artrial) ADJ2 venous ADJ2 pressure) OR "CVP" OR (vena ADJ2 cava ADJ2 (diameter OR radius))) WITH (determin$6 OR deriv$6 OR estimat$4 OR calculat$4 OR compute OR computing)) SAME ((blood OR pulse) ADJ2 volume)) AND (impedance OR bioimpedance)	US-PGPUB; USPAT; USOCR	OR	ON	2019/03/01 13:55
L27	0	(((((central OR artrial) ADJ2 venous ADJ2 pressure) OR "CVP" OR (vena ADJ2 cava ADJ2 (diameter OR radius))) WITH (determin$6 OR deriv$6 OR estimat$4 OR calculat$4 OR compute OR computing)) SAME ((blood OR pulse) ADJ2 volume)) AND (impedance OR bioimpedance)	FPRS; EPO; JPO; DERWENT	OR	ON	2019/03/01 13:55
L28	3	(((((central OR artrial) ADJ2 venous ADJ2 pressure) OR "CVP" OR (vena ADJ2 cava ADJ2 (diameter OR radius)))) SAME ((blood OR pulse) ADJ2 volume)) AND (impedance OR bioimpedance)	FPRS; EPO; JPO; DERWENT	OR	ON	2019/03/01 13:55
L29	3	(((((central OR artrial) ADJ2 venous ADJ2 pressure) OR "CVP" OR (vena ADJ2 cava ADJ2 (diameter OR radius)))) SAME ((blood OR pulse) ADJ2 volume)) AND (impedance OR bioimpedance)	FPRS; EPO; JPO; DERWENT	OR	ON	2019/03/01 13:55
L30	76	(((((central OR artrial) ADJ2 venous ADJ2 pressure) OR "CVP" OR (vena ADJ2 cava ADJ2 (diameter OR radius)))) SAME ((blood OR pulse) ADJ2 volume)) AND (impedance OR bioimpedance)	US-PGPUB; USPAT; USOCR	OR	ON	2019/03/01 13:55
L31	60	L30 NOT L24	US-PGPUB; USPAT; USOCR	OR	ON	2019/03/01 13:55
L32	60	(((((central OR artrial) ADJ2 venous ADJ2 pressure) OR "CVP" OR (vena ADJ2 cava ADJ2 (diameter OR radius)))) SAME ((blood OR pulse) ADJ2 volume)) AND L31	US-PGPUB; USPAT; USOCR	OR	ON	2019/03/01 13:55
L33	166640	(600/$).CCLS. OR (A61B5/4836 OR A61B5/0205 OR A61B5/0535 OR A61B5/4884 OR A61B5/7278 OR A61B5/0075 OR A61B5/0261 OR A61B5/0295 OR A61B5/0533 OR A61B5/0816 OR A61B5/1073 OR A61B5/1075 OR A61B5/14551 OR A61B5/6822 OR A61B5/6824 OR A61B5/7275 OR A61B8/06).CPC.	US-PGPUB; USPAT; USOCR	OR	OFF	2019/03/01 13:55
L34	16	(((((central OR artrial) ADJ2 venous ADJ2 pressure) OR "CVP" OR (vena ADJ2 cava ADJ2 (diameter OR radius))) WITH (determin$6 OR deriv$6 OR estimat$4 OR calculat$4 OR compute OR computing)) SAME ((blood OR pulse) ADJ2 volume)) AND (impedance OR bioimpedance) AND L33	US-PGPUB; USPAT; USOCR	OR	ON	2019/03/01 13:55
L35	76	(((((central OR artrial) ADJ2 venous ADJ2 pressure) OR "CVP" OR (vena ADJ2 cava ADJ2 (diameter OR radius)))) SAME ((blood OR pulse) ADJ2 volume)) AND (impedance OR bioimpedance)	US-PGPUB; USPAT; USOCR	OR	ON	2019/03/01 13:55

L36	60	L35 NOT L34	US-PGPUB; USPAT; USOCR	OR	ON	2019/03/01 13:55
L37	60	(((((central OR artrial) ADJ2 venous ADJ2 pressure) OR "CVP" OR (vena ADJ2 cava ADJ2 (diameter OR radius)))) SAME ((blood OR pulse) ADJ2 volume)) AND L36	US-PGPUB; USPAT; USOCR	OR	ON	2019/03/01 13:55
L38	18	(US-20130172691-$ or US-20120016246-$ or US-20110046505-$ or US-20100222696-$ or US-20100036455-$ or US-20090287102-$ or US-20080190430-$ or US-20080097230-$ or US-20060122540-$ or US-20050080460-$ or US-20030167012-$ or US-20150031966-$ or US-20110077474-$ or US-20140249384-$ or US-20170239408-$).did. or (US-8251912-$ or US-8388545-$ or US-9675294-$).did.	US-PGPUB; USPAT	OR	ON	2019/03/01 13:55
L39	8	L38 AND (photoplethysmograph$5 OR "PPG" OR "GSR" OR (galvanic ADJ2 skin ADJ2 respons$5) OR ((IR OR spectroscop$3 OR doppler OR ultrasound OR ultrason$5 OR speckle) NEAR4 (sens$5 OR transducer OR monitor$4 OR probe OR meter$4)))	US-PGPUB; USPAT	OR	ON	2019/03/01 13:55
L40	8	L38 AND (((venous OR blood OR pulse) ADJ3 volume) WITH (chang$3 OR variation)) AND (photoplethysmograph$5 OR "PPG" OR "GSR" OR (galvanic ADJ2 skin ADJ2 respons$5) OR ((IR OR spectroscop$3 OR doppler OR ultrasound OR ultrason$5 OR speckle) NEAR4 (sens$5 OR transducer OR monitor$4 OR probe OR meter$4)))	US-PGPUB; USPAT	OR	ON	2019/03/01 13:55
L41	18	(US-20130172691-$ or US-20120016246-$ or US-20110046505-$ or US-20100222696-$ or US-20100036455-$ or US-20090287102-$ or US-20080190430-$ or US-20080097230-$ or US-20060122540-$ or US-20050080460-$ or US-20030167012-$ or US-20150031966-$ or US-20110077474-$ or US-20140249384-$ or US-20170239408-$).did. or (US-8251912-$ or US-8388545-$ or US-9675294-$).did.	US-PGPUB; USPAT	OR	ON	2019/03/01 13:55
L42	11	(("20100222696") or ("20060122540") or ("20130172691") or ("20100036455") or ("20050080460") or ("20110046505") or ("20080097230") or ("20080190430") or ("20030167012") or ("20090287102") or ("20120016246")).PN.	US-PGPUB; USPAT; USOCR	OR	OFF	2019/03/01 13:55
L43	7	L41 NOT L42	US-PGPUB; USPAT; USOCR	OR	OFF	2019/03/01 13:55

3/1/2019 2:03:48 PM
H:\EAST\Workspaces\FY2018\14445926.wsp

US007113814B2

(12) **United States Patent**
Ward et al.

(10) **Patent No.: US 7,113,814 B2**
(45) **Date of Patent: Sep. 26, 2006**

(54) **TISSUE INTERROGATION SPECTROSCOPY**

(75) Inventors: **Kevin R. Ward**, Glen Allen, VA (US); **R. Wayne Barbee**, Richmond, VA (US); **James Terner**, Richmond, VA (US); **Rao R. Ivatury**, Richmond, VA (US); **Fred Hawkridge**, Glen Allen, VA (US)

(73) Assignee: **Virginia Commonwealth University**, Richmond, VA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 277 days.

(21) Appl. No.: **10/332,613**

(22) PCT Filed: **Jul. 13, 2001**

(86) PCT No.: **PCT/US01/22187**

§ 371 (c)(1),
(2), (4) Date: **Jul. 29, 2003**

(87) PCT Pub. No.: **WO02/07585**

PCT Pub. Date: **Jan. 31, 2002**

(65) **Prior Publication Data**

US 2004/0039269 A1 Feb. 26, 2004

Related U.S. Application Data

(60) Provisional application No. 60/218,055, filed on Jul. 13, 2000.

(51) **Int. Cl.**
A61B 5/00 (2006.01)

(52) **U.S. Cl.** **600/310**; 600/476

(58) **Field of Classification Search** 600/310, 600/322, 323, 473, 476; 356/301, 303, 317–320; 250/341.1

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,769,081 A 6/1998 Alfano et al.
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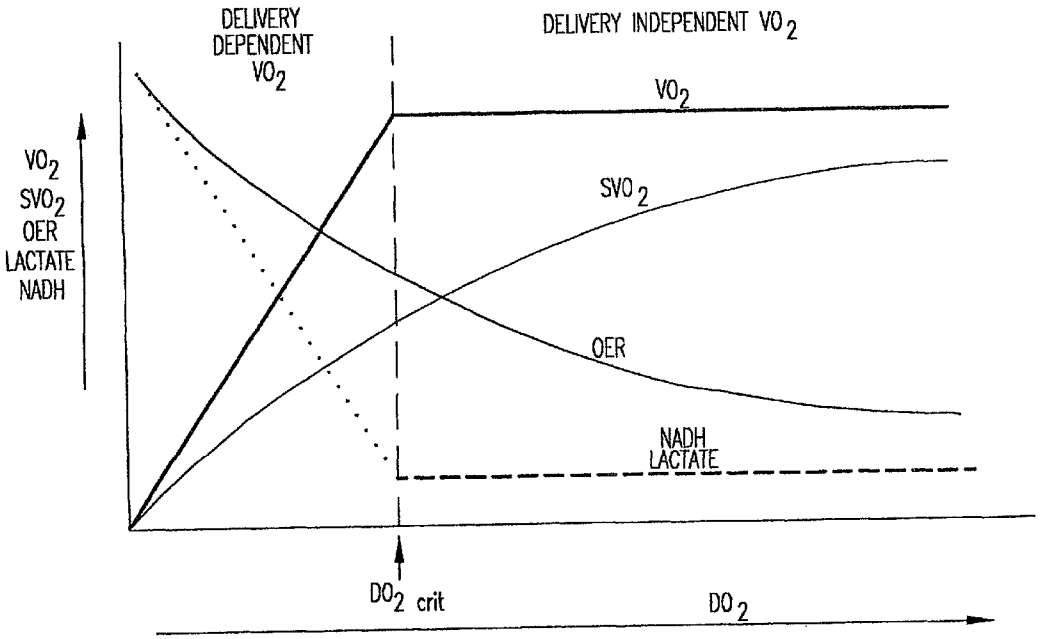
Primary Examiner—Eric F. Winakur
(74) *Attorney, Agent, or Firm*—Whitham, Curtis & Christofferson, PC

(57) **ABSTRACT**

In an emergency medicine patient, accurate measurement of change or lack thereof from non-shock, non-ischemic, non-inflammation, non-tissue injury, non-immune dysfunction conditions is important and is provided, as practical, real-time approaches for accurately characterizing a patient's condition, using Raman (**3**) and/or fluorescence (**30**) spectroscopy with a high degree of accuracy. Measurement times are on the order of seconds. High-accuracy measurement is achieved with Raman spectroscopy interrogation of tissue. Simultaneous interrogation by NADH fluorescence spectroscopy may he used. Measurements may be non-invasive to minimally invasive. Preclinical (ultra-early) states of shock can be detected (**5**), severity can be determined, effectiveness of various treatments can be determined.

70 Claims, 35 Drawing Sheets



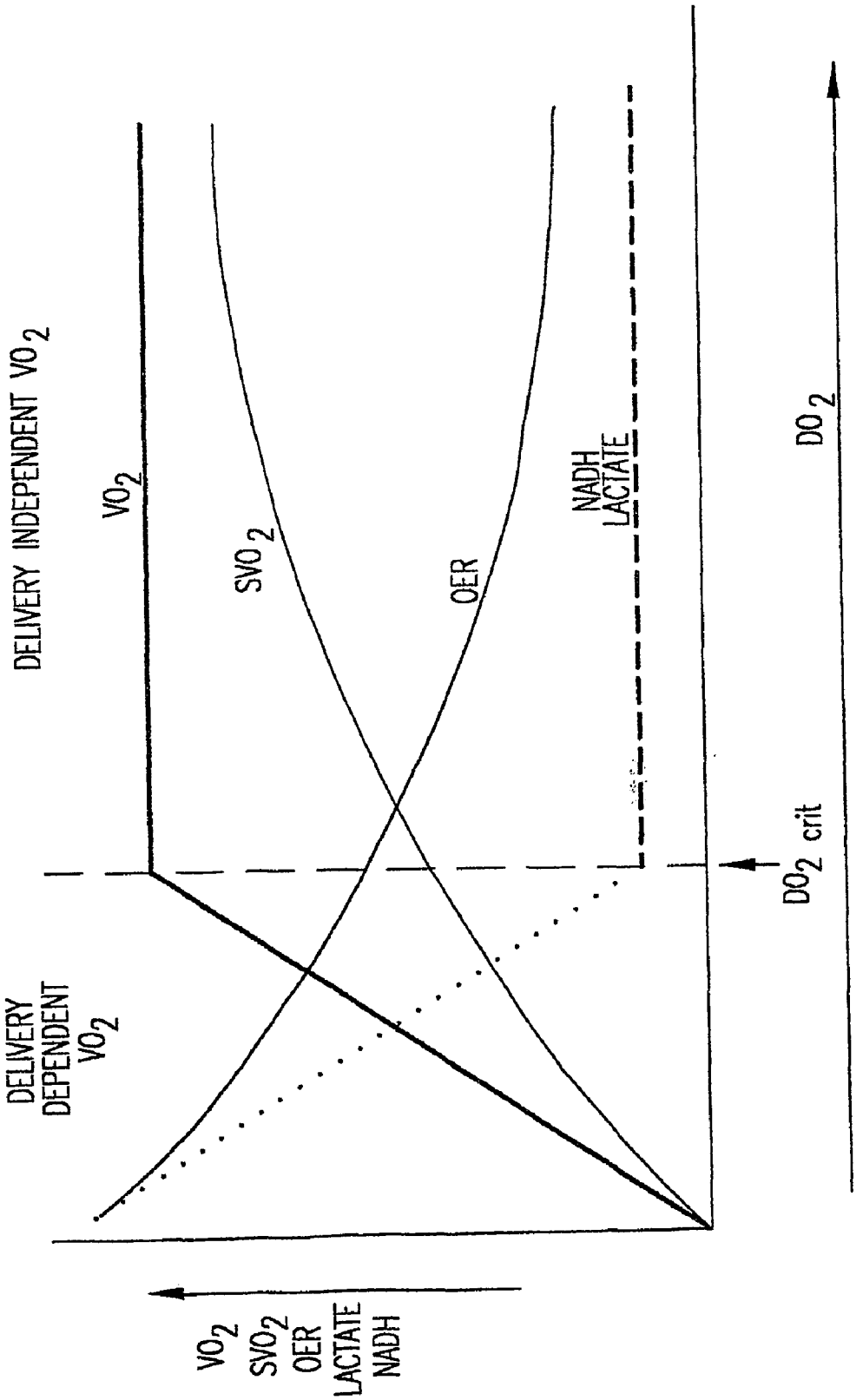

DELIVERY INDEPENDENT VO_2
VO_2
SVO_2
OER
NADH
LACTATE
DO_2
DO_2 crit
DELIVERY DEPENDENT VO_2
VO_2
SVO_2
OER
LACTATE
NADH

FIG. 1

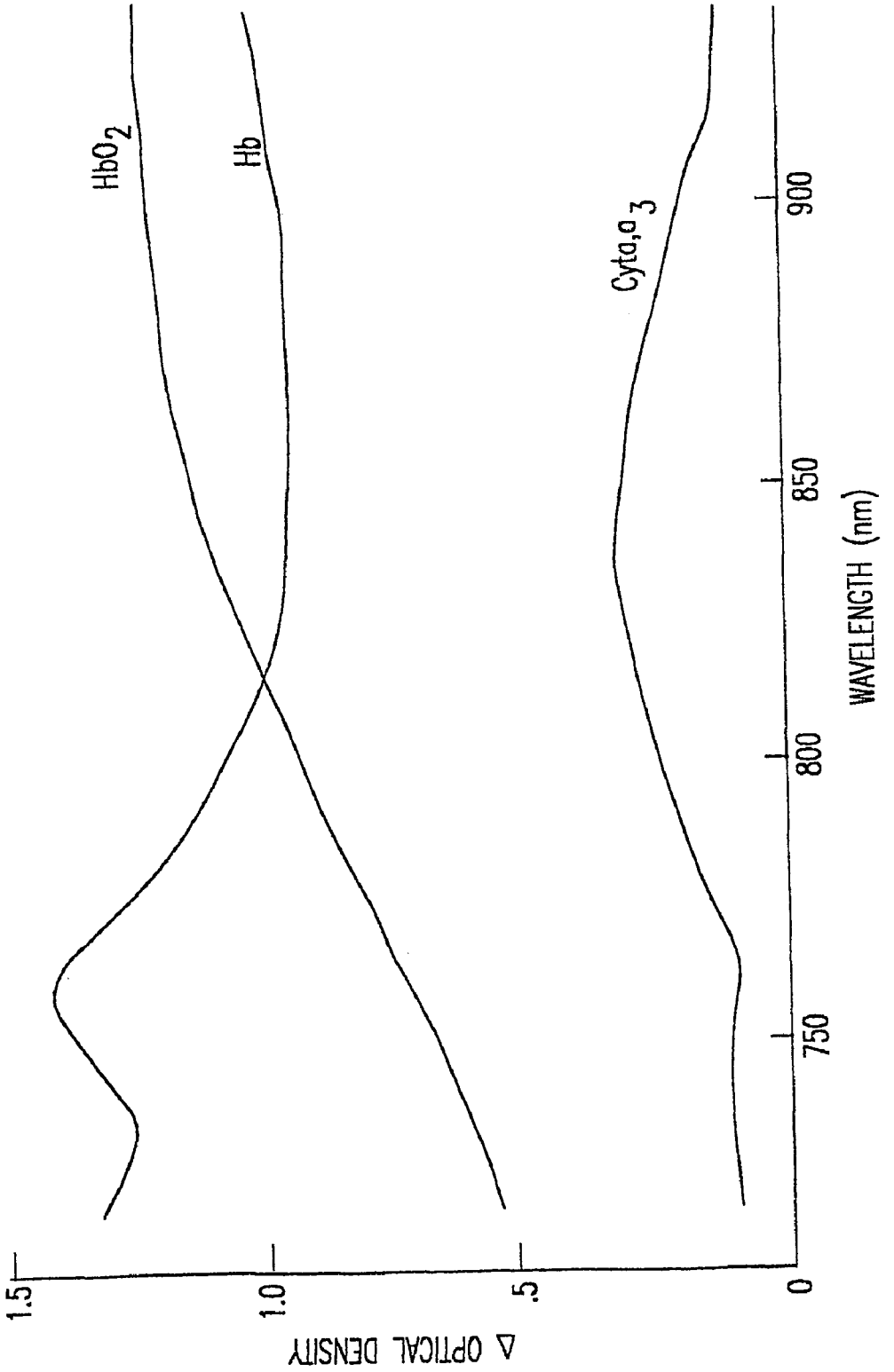

HbO_2
Hb
$Cyta,a_3$
1.5
1.0
.5
0
Δ OPTICAL DENSITY
750
800
850
900
WAVELENGTH (nm)

FIG.2

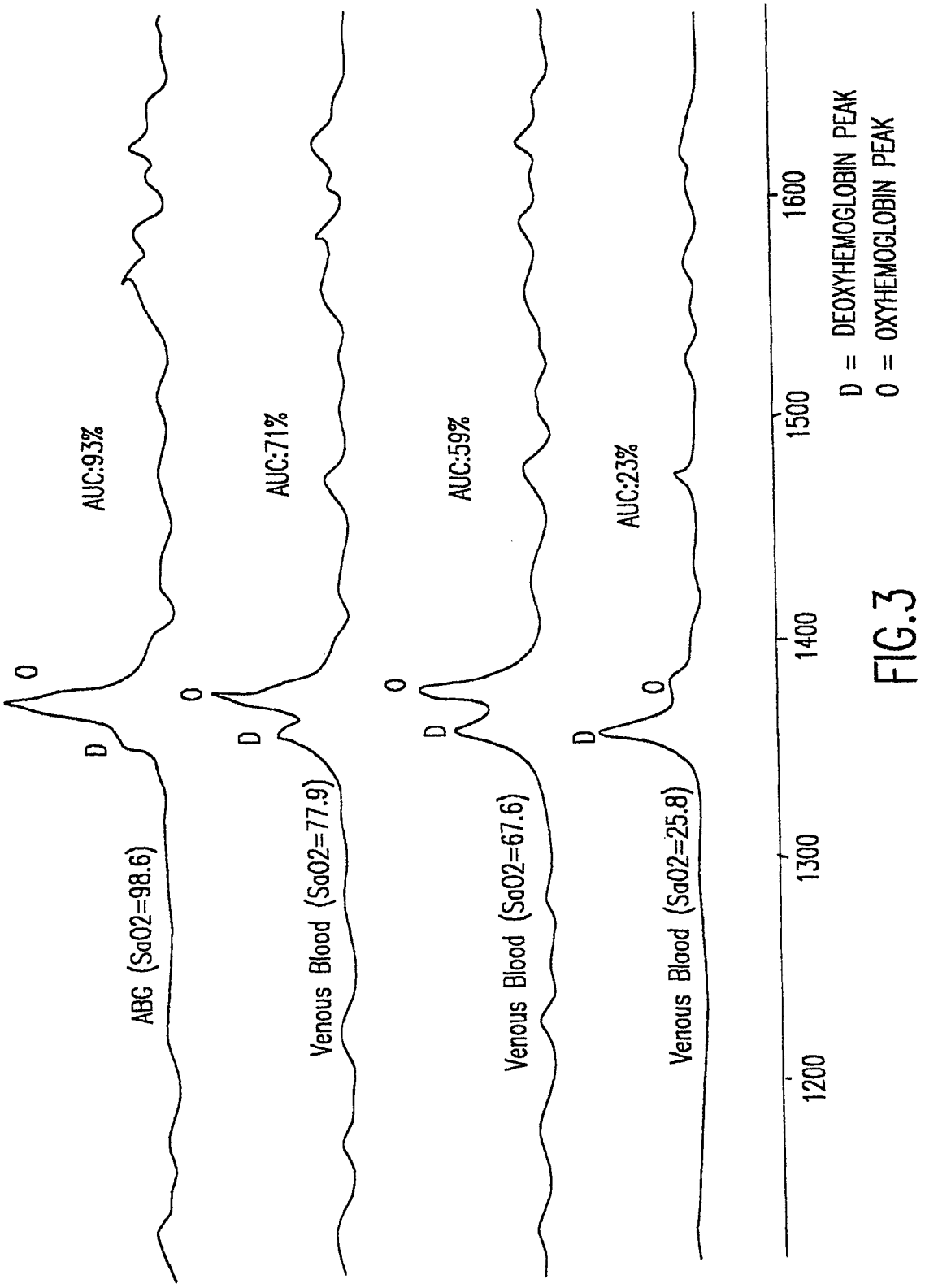

ABG (SaO2=98.6)
AUC:93%
Venous Blood (SaO2=77.9)
AUC:71%
Venous Blood (SaO2=67.6)
AUC:59%
Venous Blood (SaO2=25.8)
AUC:23%
D
O
1200
1300
1400
1500
1600
D = DEOXYHEMOGLOBIN PEAK
O = OXYHEMOGLOBIN PEAK

FIG.3

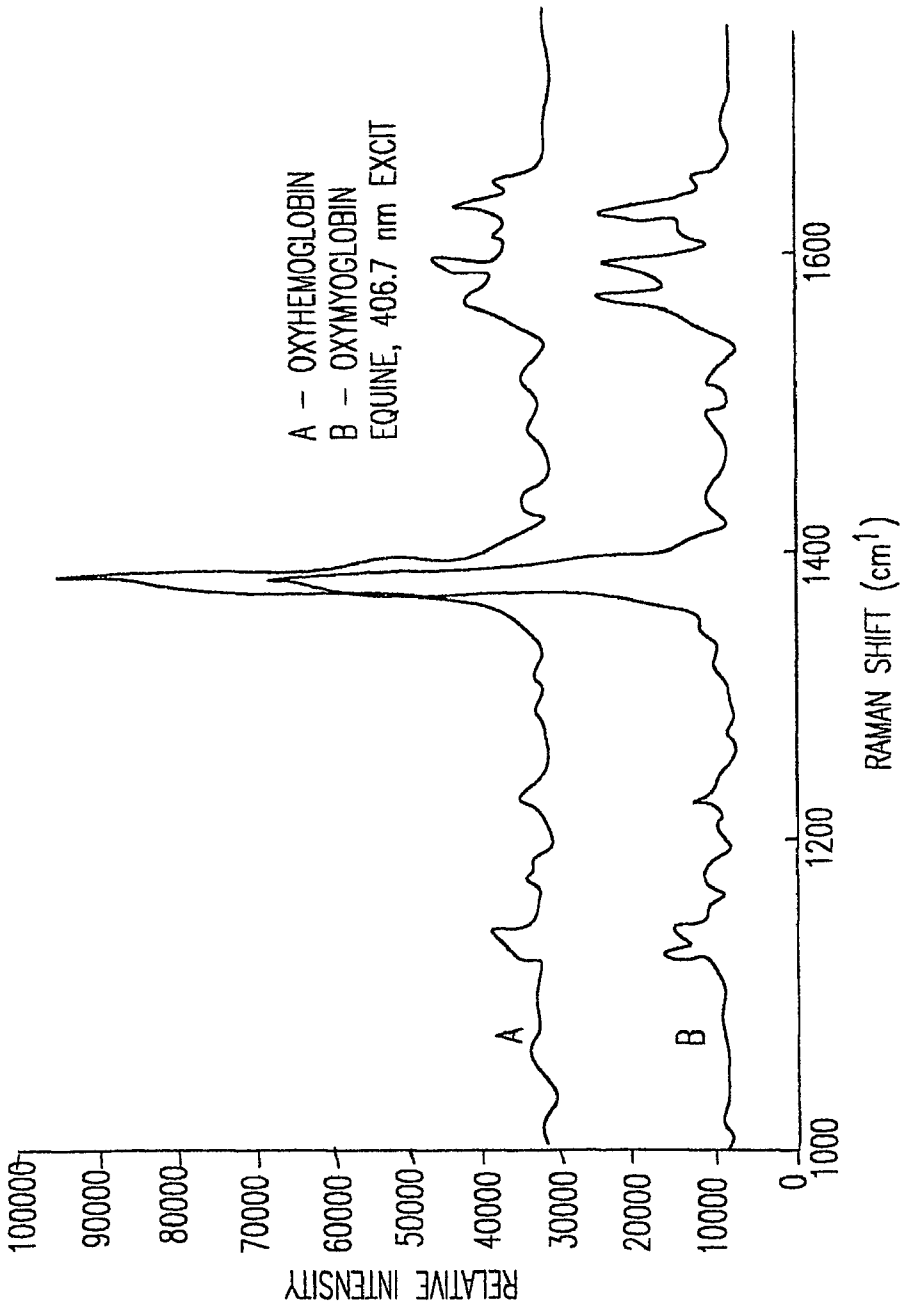

A – OXYHEMOGLOBIN
B – OXYMYOGLOBIN
EQUINE, 406.7 nm EXCIT
A
B
RELATIVE INTENSITY
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
1000
1200
1400
1600
RAMAN SHIFT (cm-1)

FIG.4

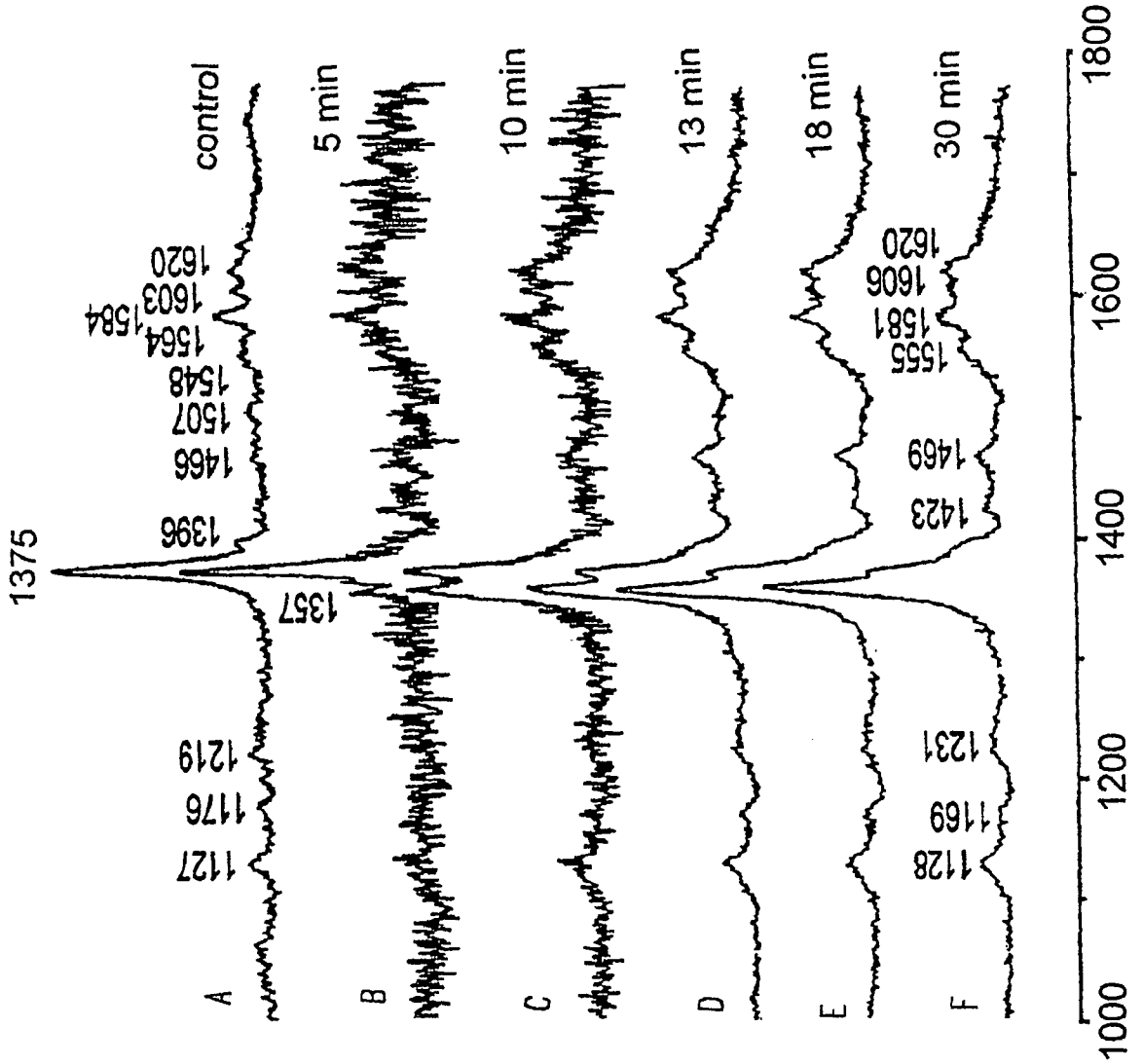

control
5 min
10 min
13 min
18 min
30 min
A
B
C
D
E
F
1375
1357
1396
1466
1507
1548
1564
1584
1603
1620
1219
1176
1127
1423
1469
1555
1581
1606
1620
1231
1169
1128
1000
1200
1400
1600
1800

FIG.5

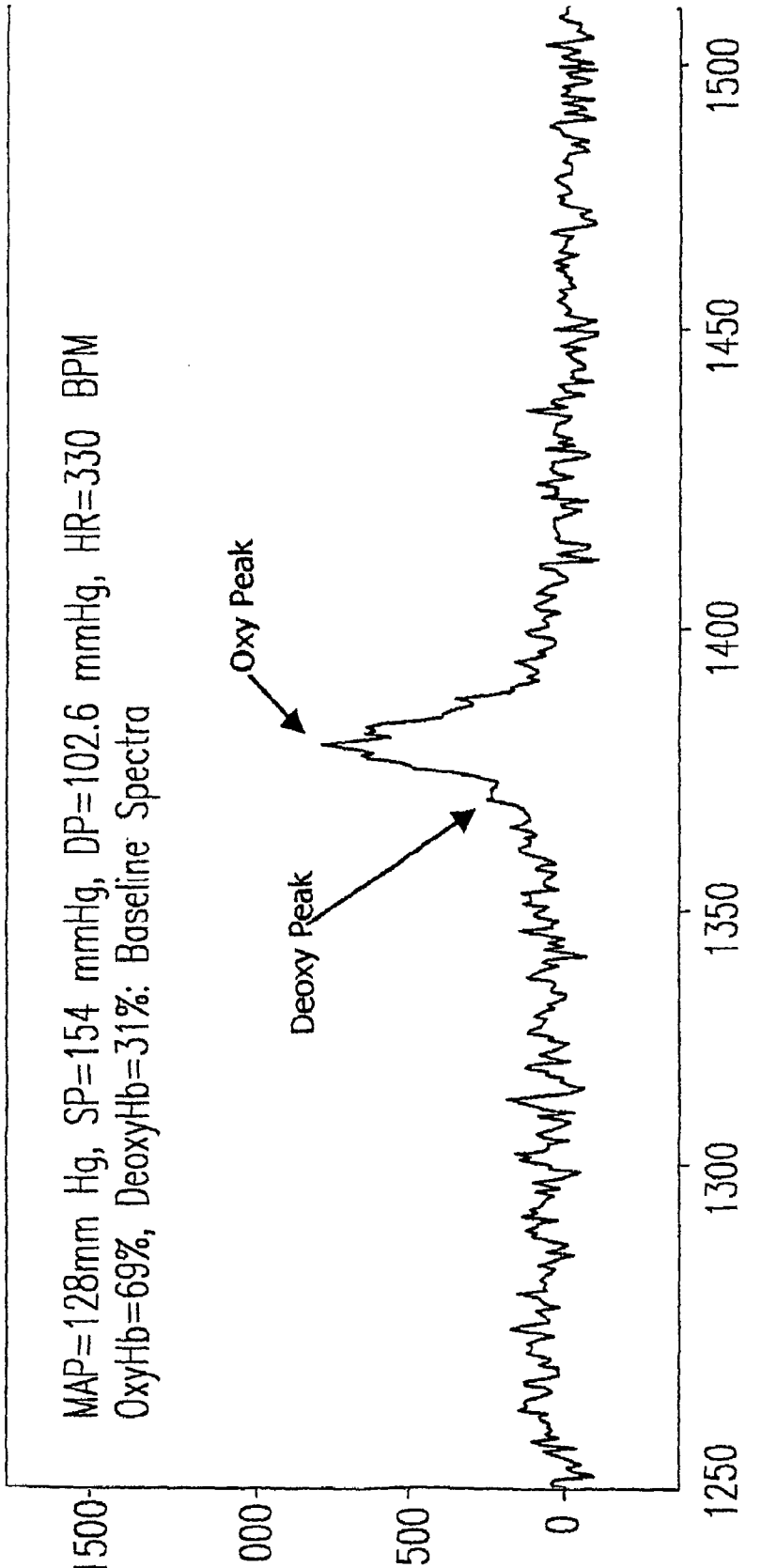

MAP=128mm Hg, SP=154 mmHg, DP=102.6 mmHg, HR=330 BPM
OxyHb=69%, DeoxyHb=31%: Baseline Spectra
Oxy Peak
Deoxy Peak
1500
1000
500
0
1250
1300
1350
1400
1450
1500

FIG.6

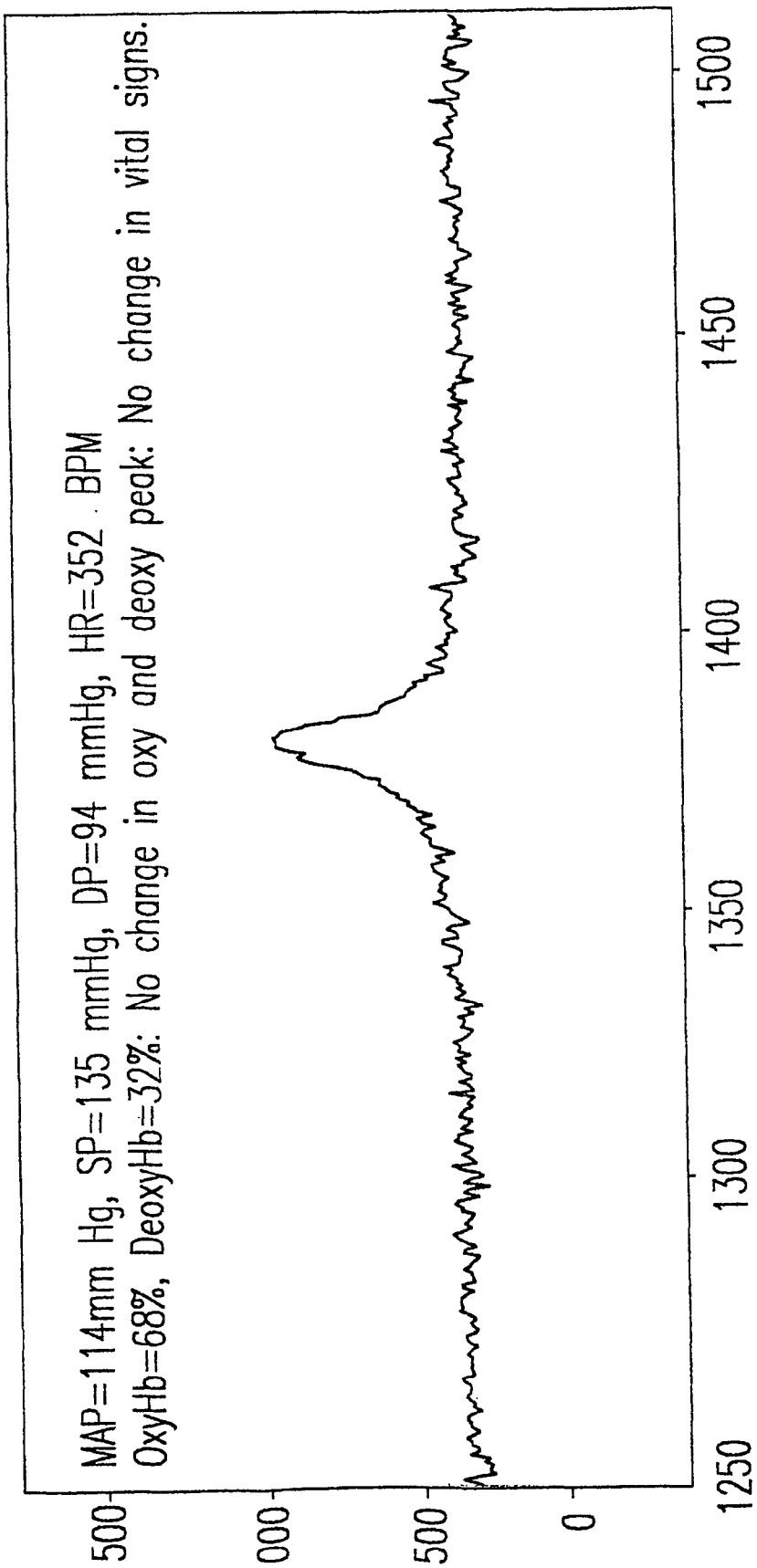

MAP=114mm Hg, SP=135 mmHg, DP=94 mmHg, HR=352 BPM
OxyHb=68%, DeoxyHb=32%: No change in oxy and deoxy peak: No change in vital signs.
1500
1000
500
0
1250
1300
1350
1400
1450
1500

FIG.7

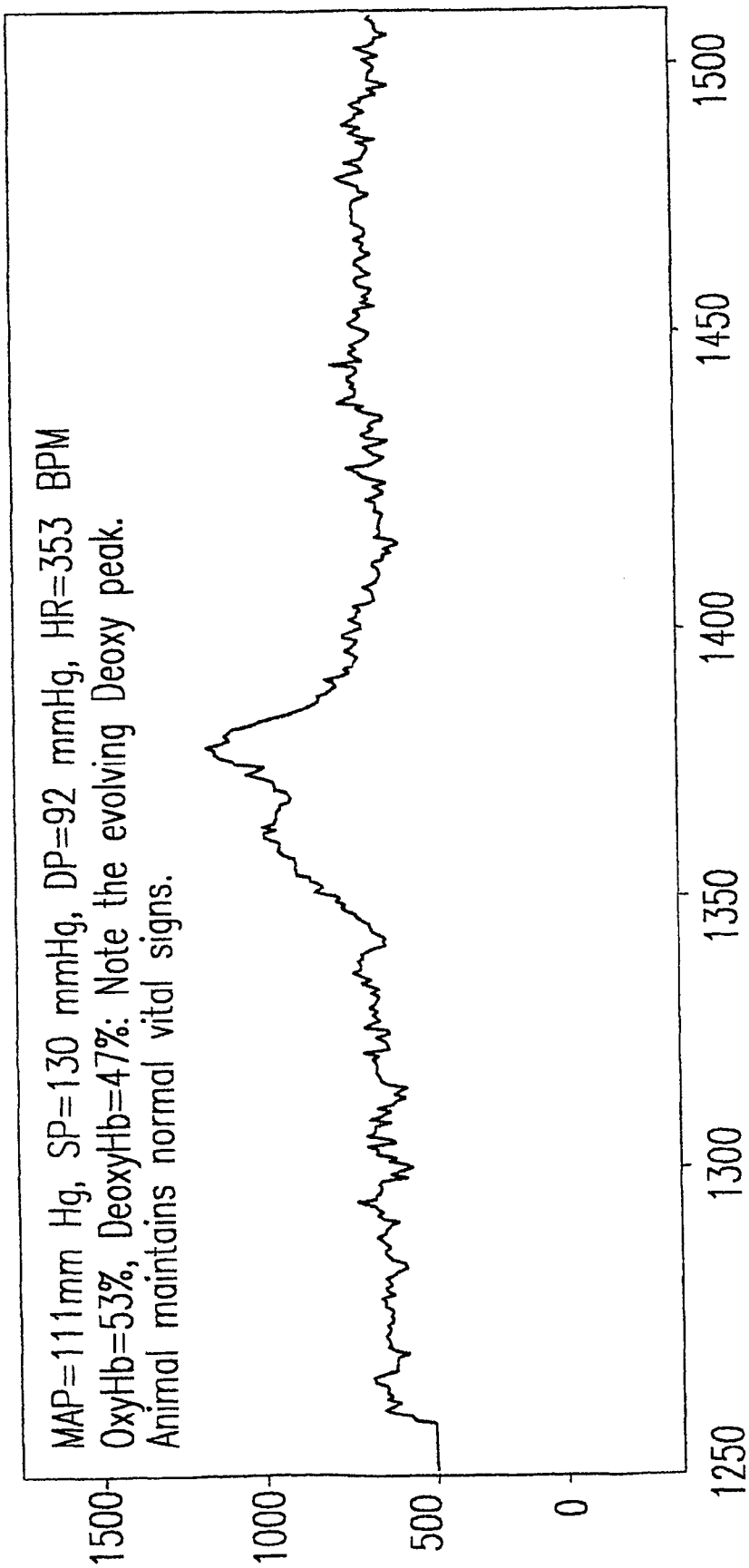

MAP=111mm Hg, SP=130 mmHg, DP=92 mmHg, HR=353 BPM
OxyHb=53%, DeoxyHb=47%: Note the evolving Deoxy peak.
Animal maintains normal vital signs.
1500
1000
500
0
1250
1300
1350
1400
1450
1500

FIG.8

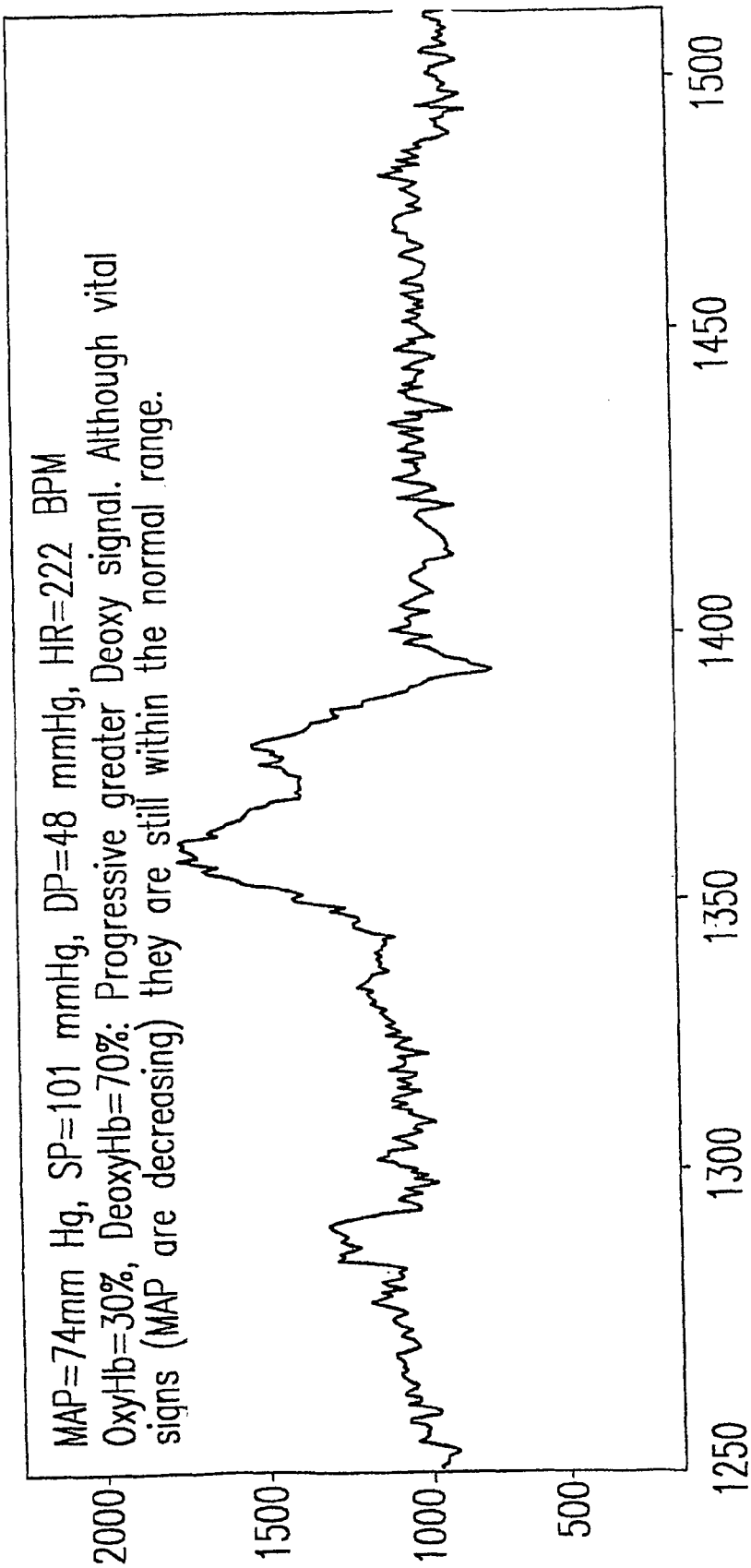

MAP=74mm Hg, SP=101 mmHg, DP=48 mmHg, HR=222 BPM
OxyHb=30%, DeoxyHb=70%: Progressive greater Deoxy signal. Although vital signs (MAP are decreasing) they are still within the normal range.
2000
1500
1000
500
1250
1300
1350
1400
1450
1500

FIG.9

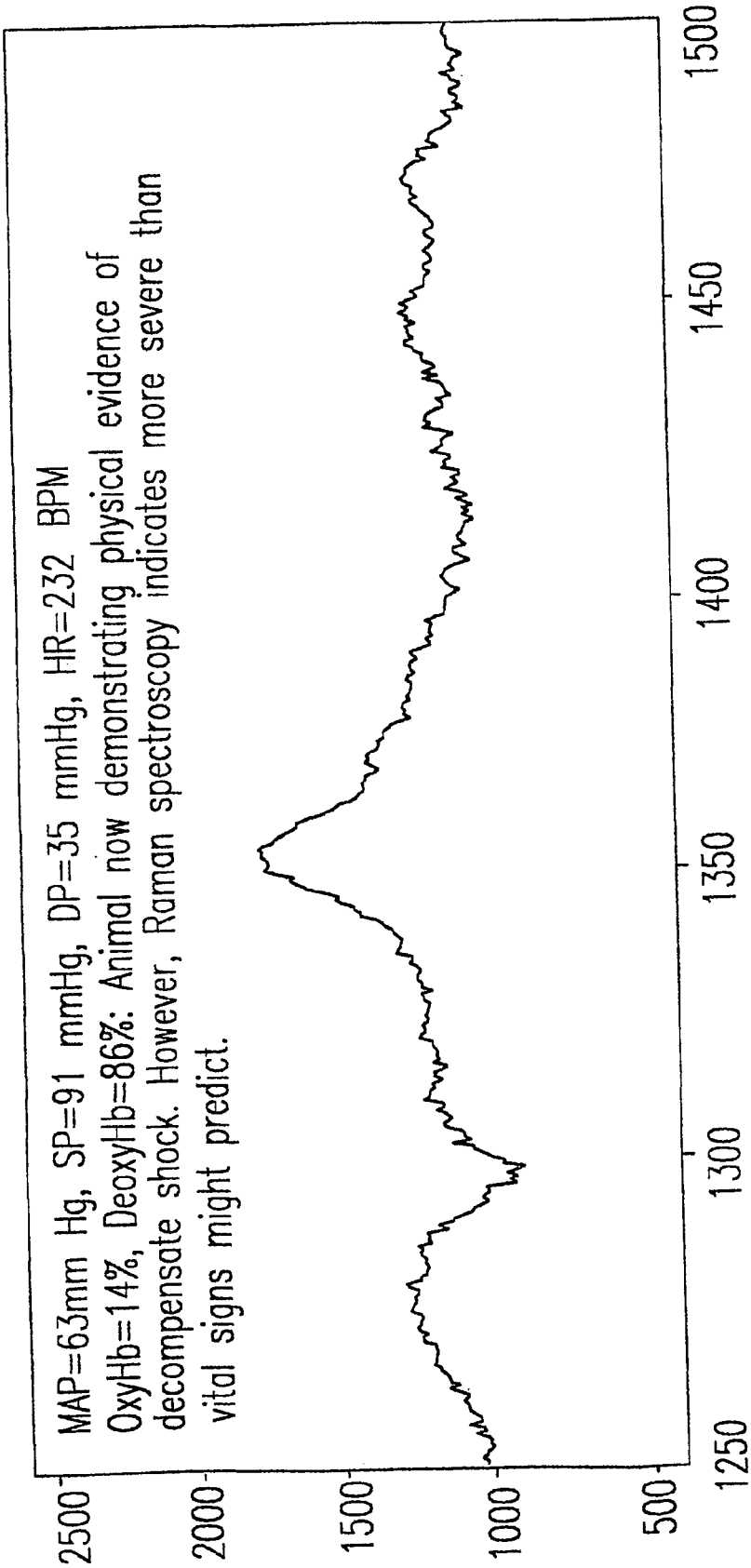

MAP=63mm Hg, SP=91 mmHg, DP=35 mmHg, HR=232 BPM
OxyHb=14%, DeoxyHb=86%: Animal now demonstrating physical evidence of decompensate shock. However, Raman spectroscopy indicates more severe than vital signs might predict.
2500
2000
1500
1000
500
1250
1300
1350
1400
1450
1500

FIG.10

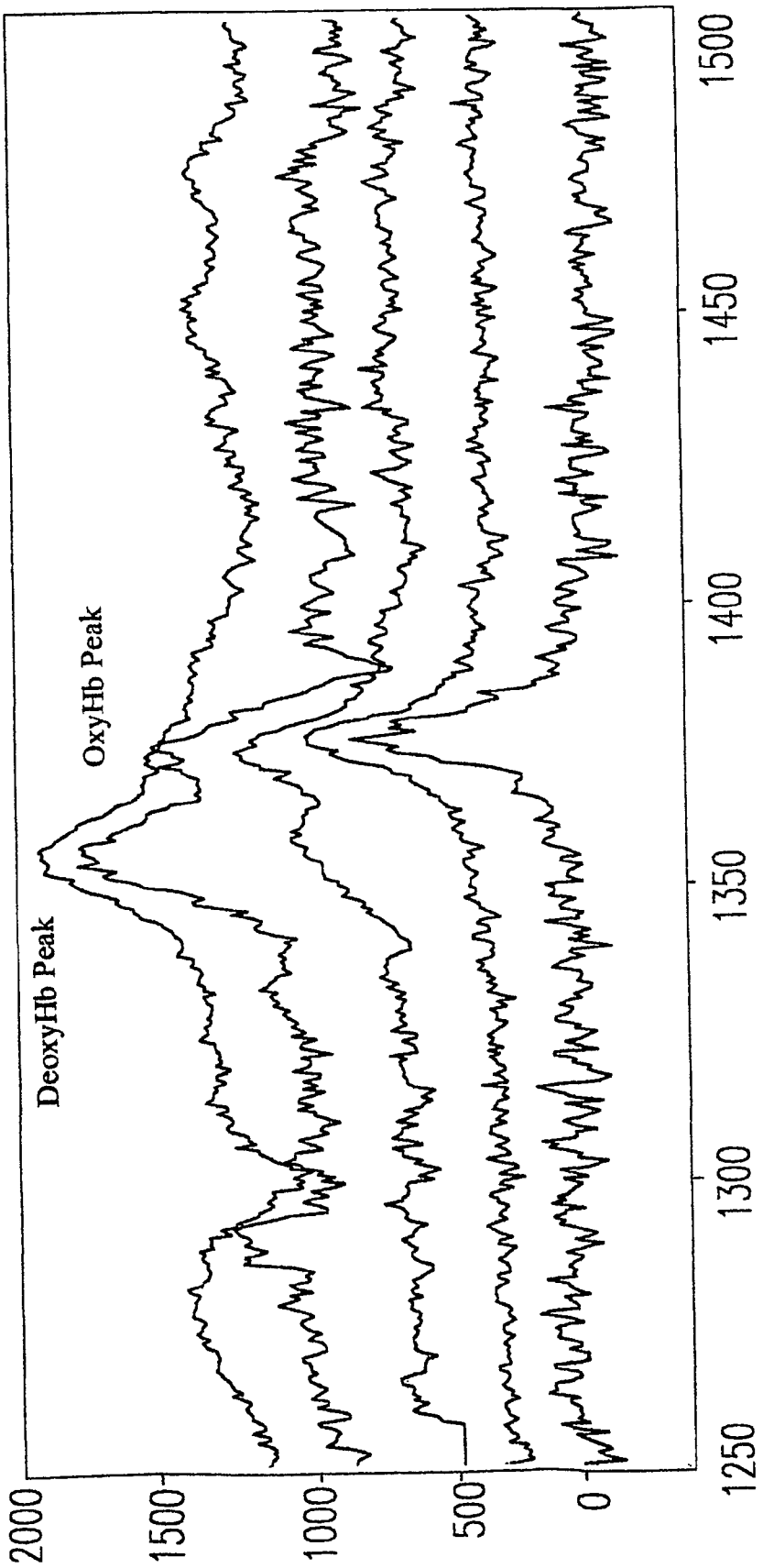

DeoxyHb Peak
OxyHb Peak
2000
1500
1000
500
0
1250
1300
1350
1400
1450
1500

FIG. 11

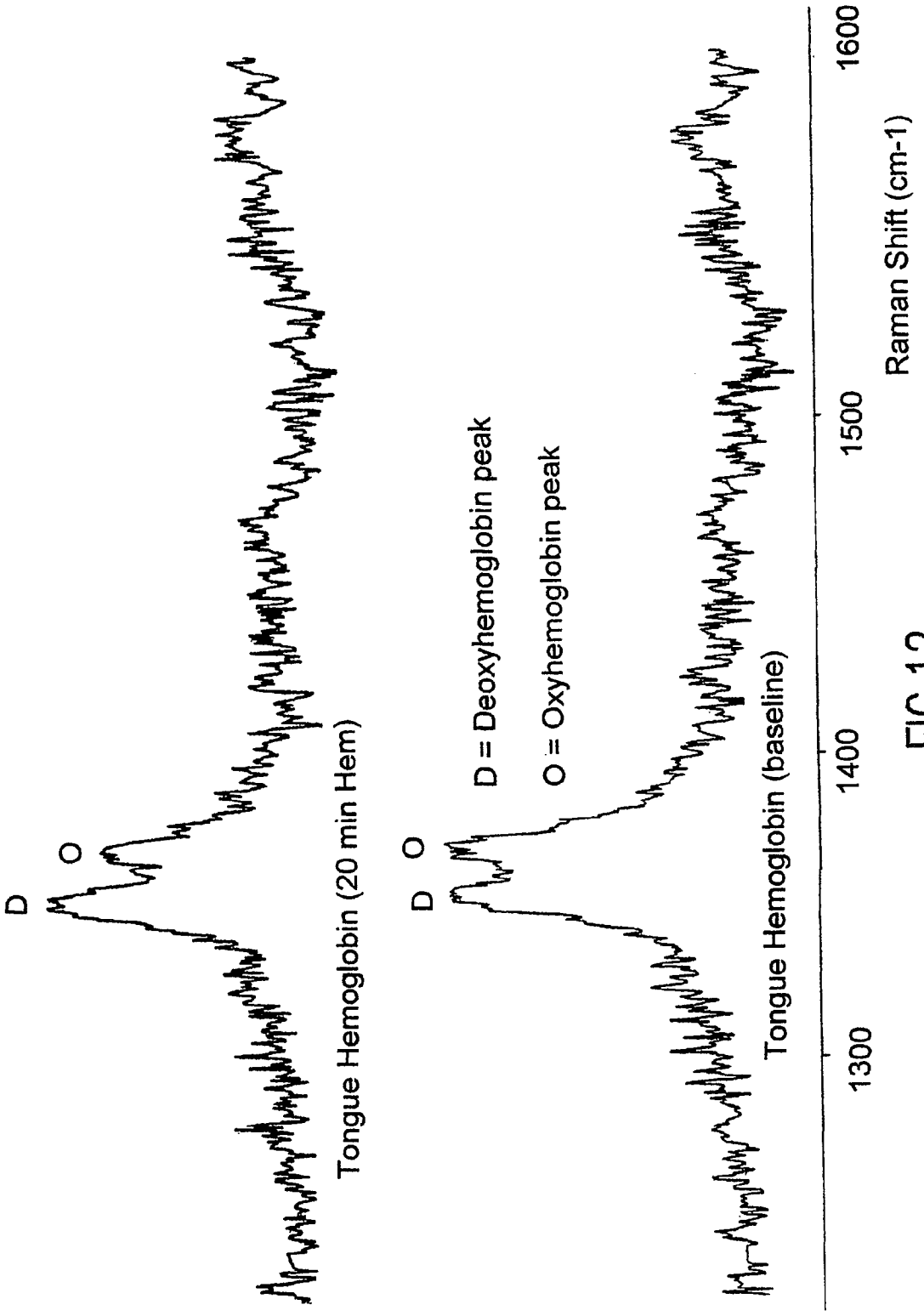

D
O
Tongue Hemoglobin (20 min Hem)
D
O
D = Deoxyhemoglobin peak
O = Oxyhemoglobin peak
Tongue Hemoglobin (baseline)
1300
1400
1500
1600
Raman Shift (cm-1)

FIG.12

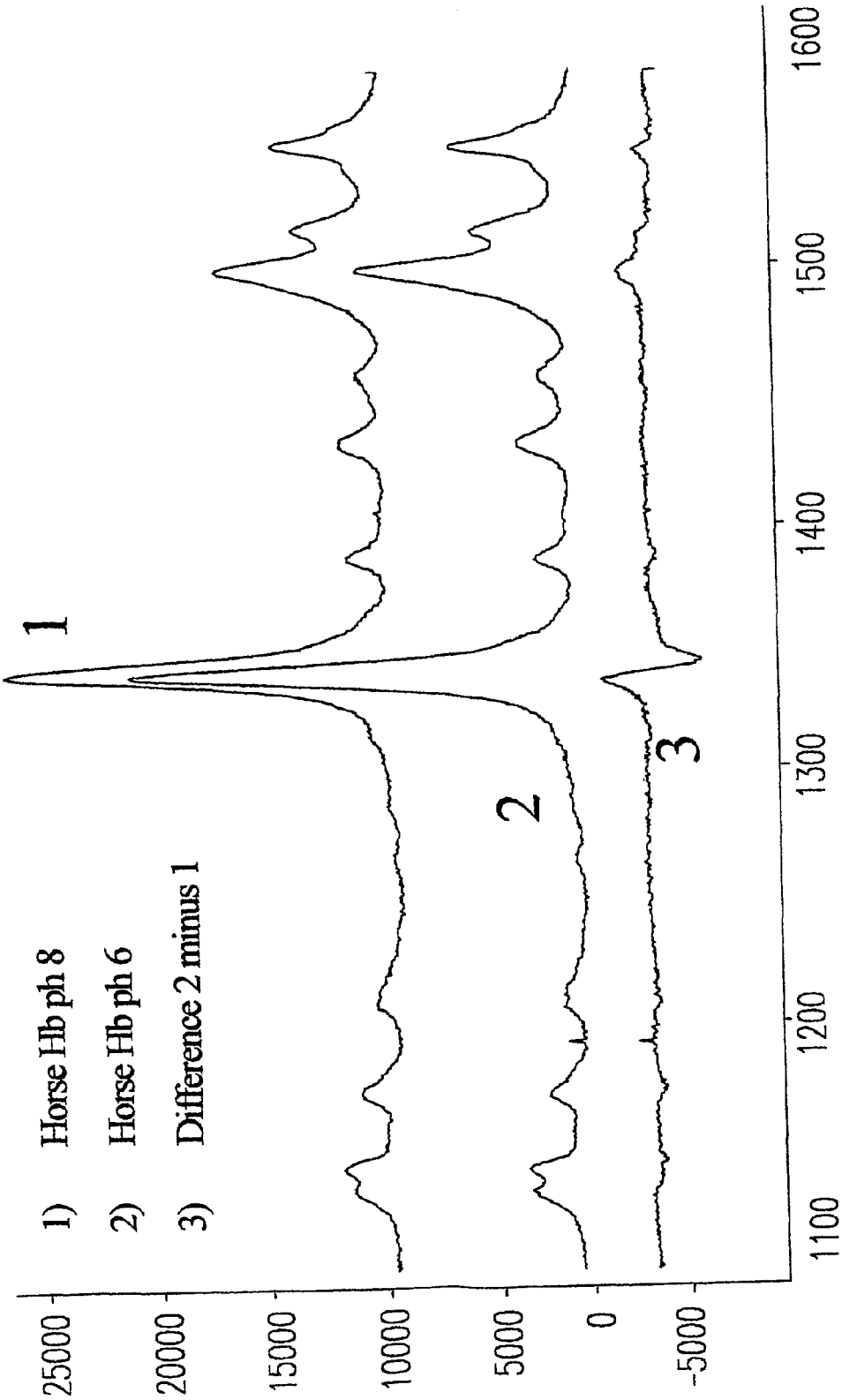

1) Horse Hb ph 8
2) Horse Hb ph 6
3) Difference 2 minus 1
1
2
3
25000
20000
15000
10000
5000
0
-5000
1100
1200
1300
1400
1500
1600

FIG.13

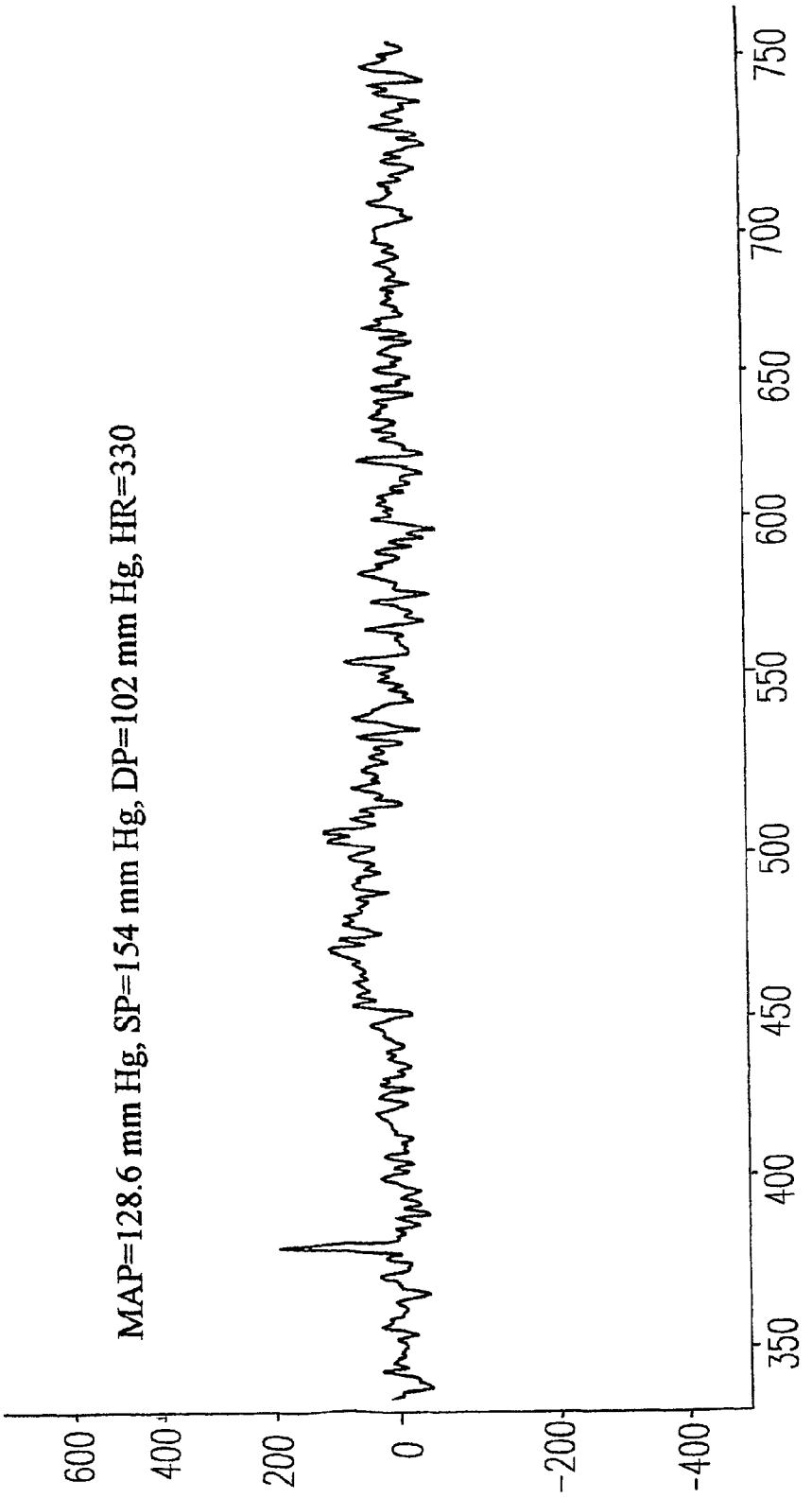

MAP=128.6 mm Hg, SP=154 mm Hg, DP=102 mm Hg, HR=330
600
400
200
0
-200
-400
350
400
450
500
550
600
650
700
750

FIG.14

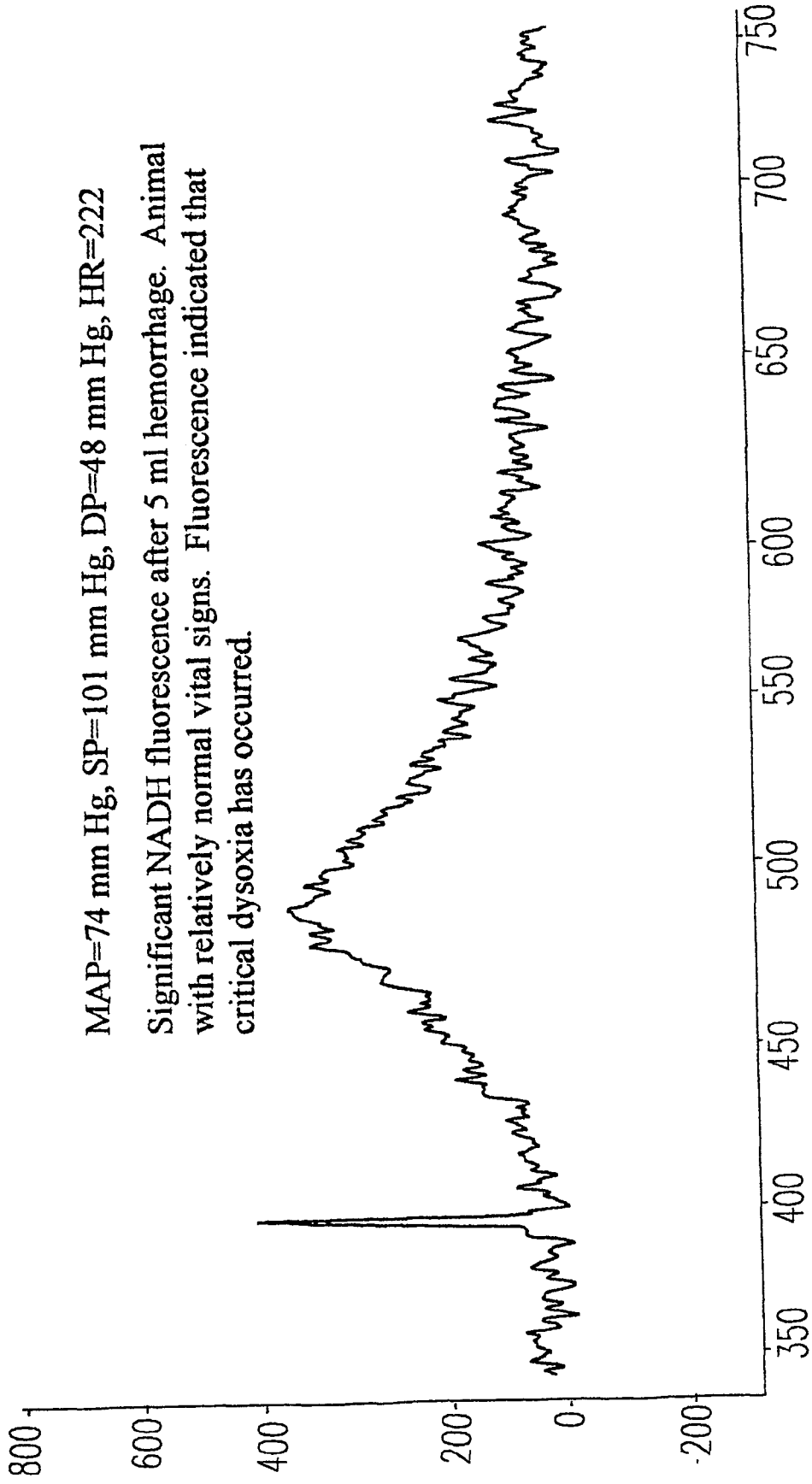

MAP=74 mm Hg, SP=101 mm Hg, DP=48 mm Hg, HR=222
Significant NADH fluorescence after 5 ml hemorrhage. Animal with relatively normal vital signs. Fluorescence indicated that critical dysoxia has occurred.
800
600
400
200
0
-200
350
400
450
500
550
600
650
700
750

FIG.15

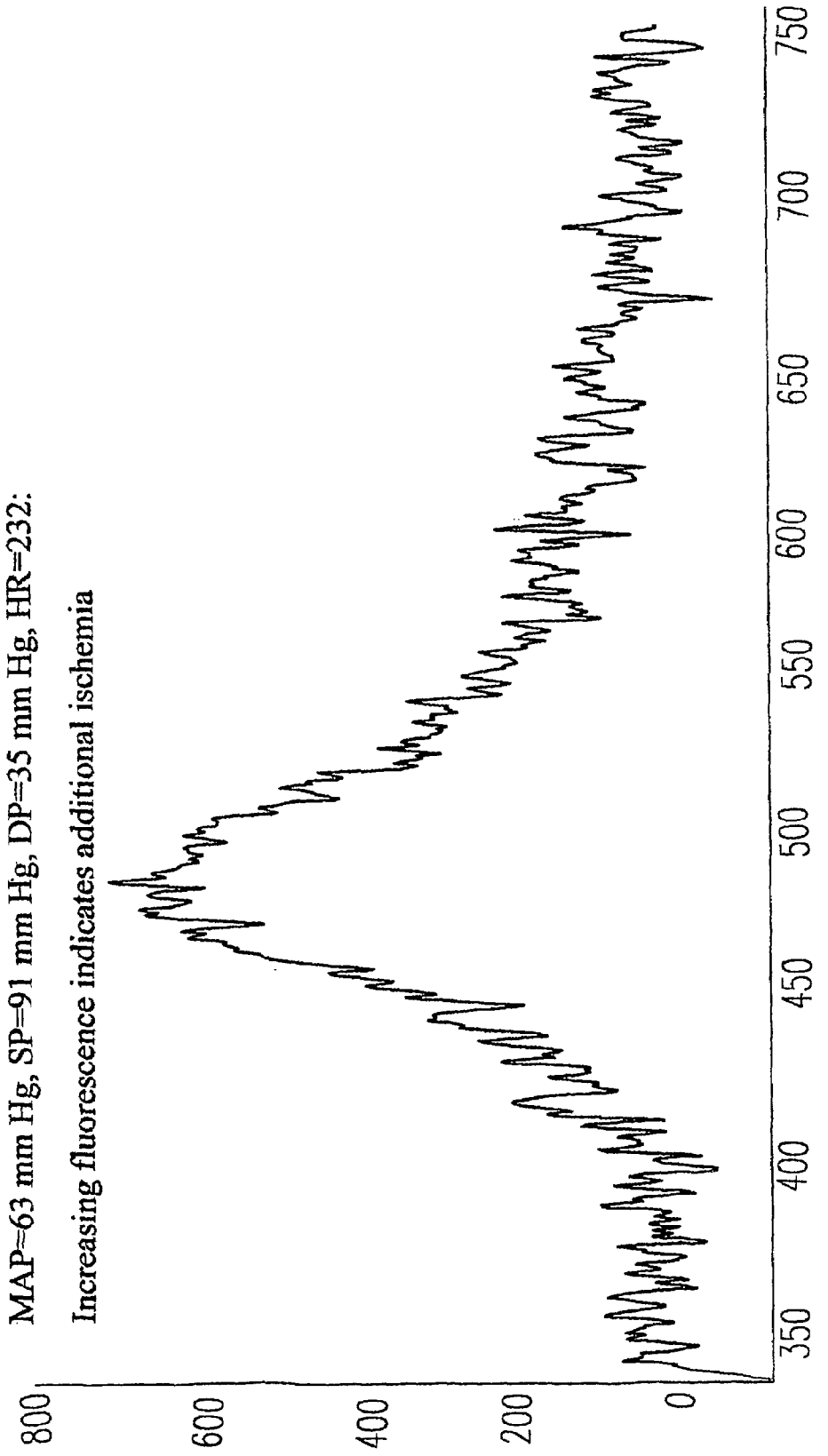

MAP=63 mm Hg, SP=91 mm Hg, DP=35 mm Hg, HR=232:
Increasing fluorescence indicates additional ischemia
800
600
400
200
0
350
400
450
500
550
600
650
700
750

FIG.16

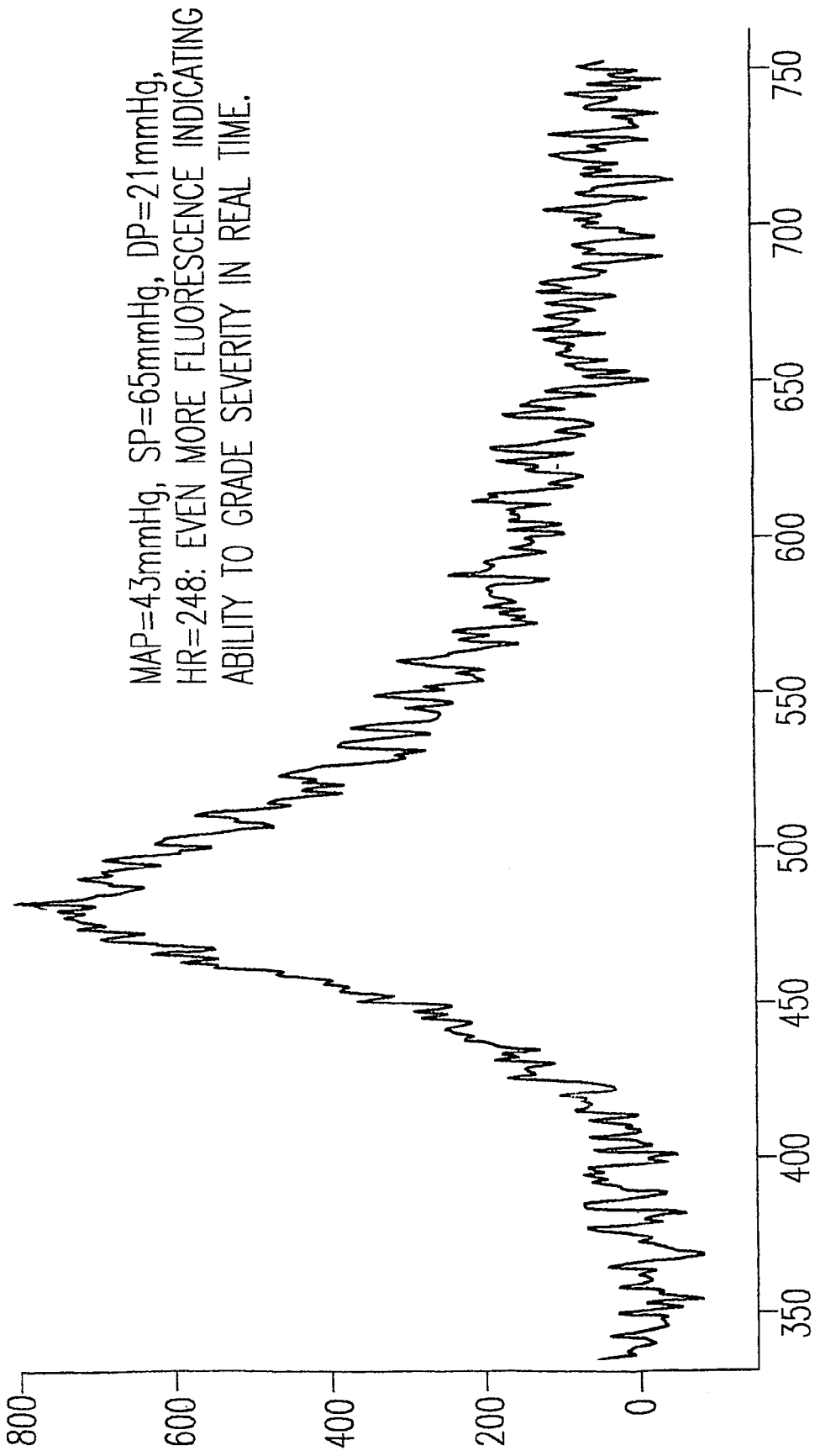

MAP=43mmHg, SP=65mmHg, DP=21mmHg,
HR=248: EVEN MORE FLUORESCENCE INDICATING
ABILITY TO GRADE SEVERITY IN REAL TIME.
800
600
400
200
0
350
400
450
500
550
600
650
700
750

FIG.17

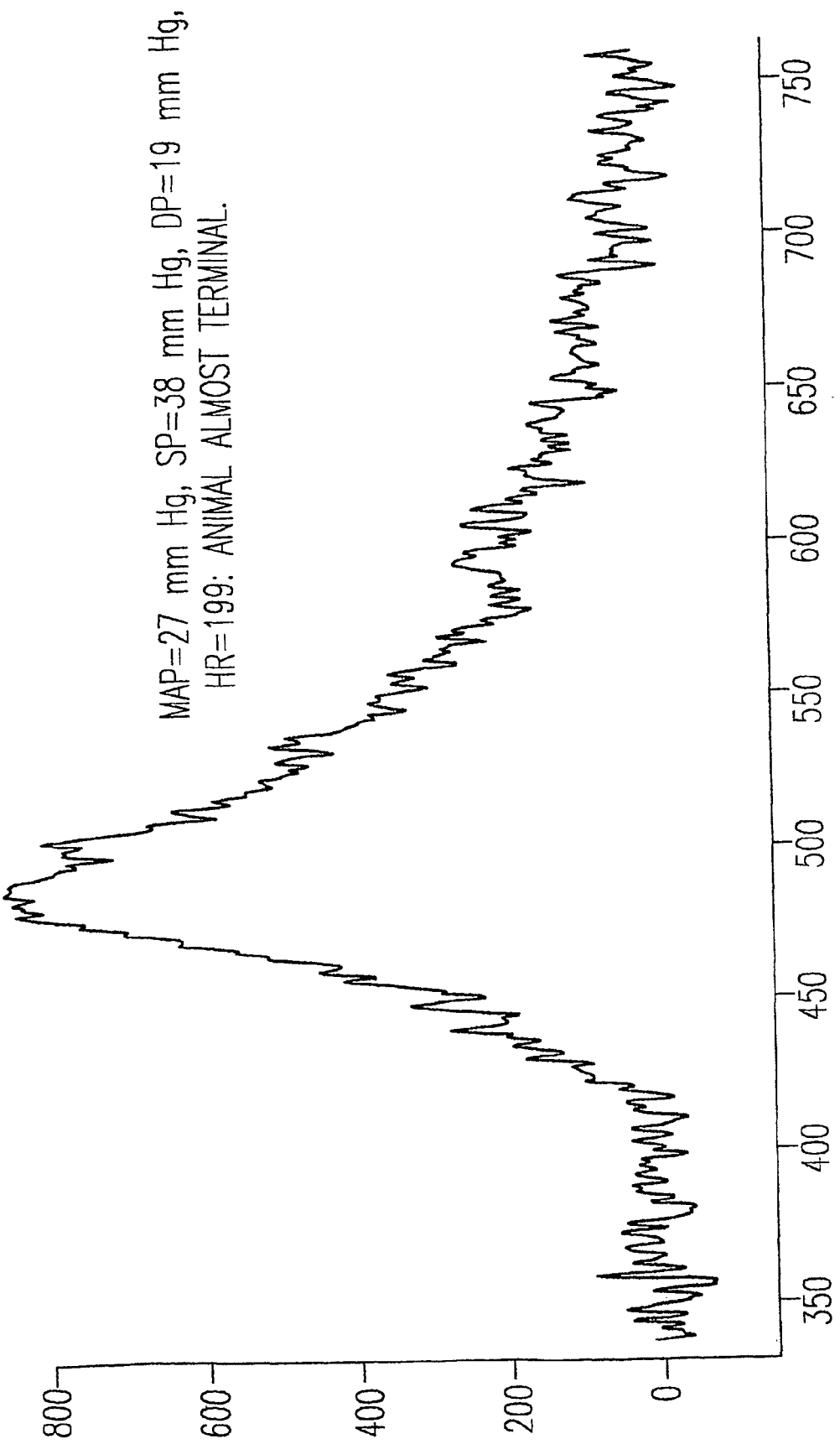

MAP=27 mm Hg, SP=38 mm Hg, DP=19 mm Hg,
HR=199: ANIMAL ALMOST TERMINAL.
800
600
400
200
0
350
400
450
500
550
600
650
700
750

FIG.18

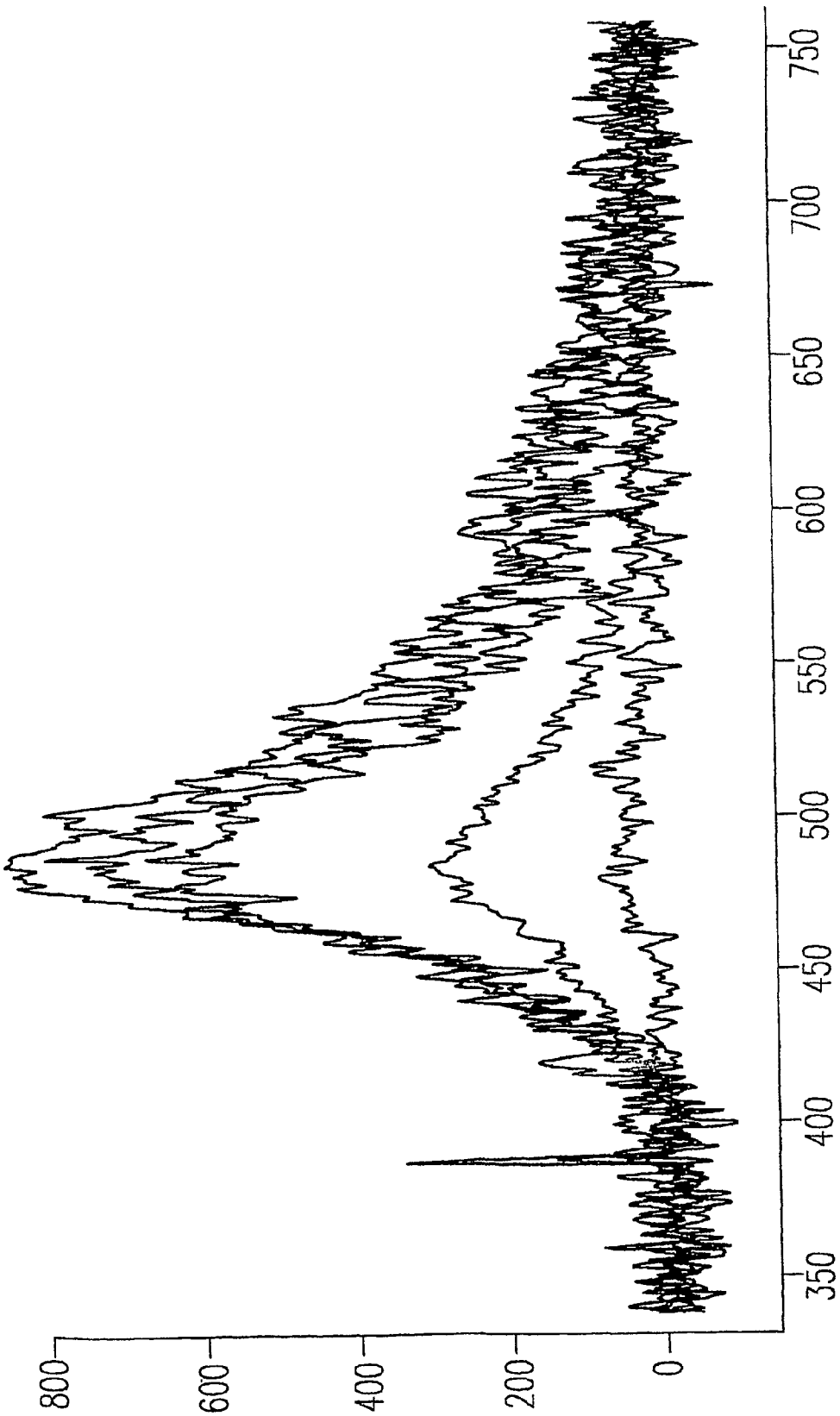


FIG.19

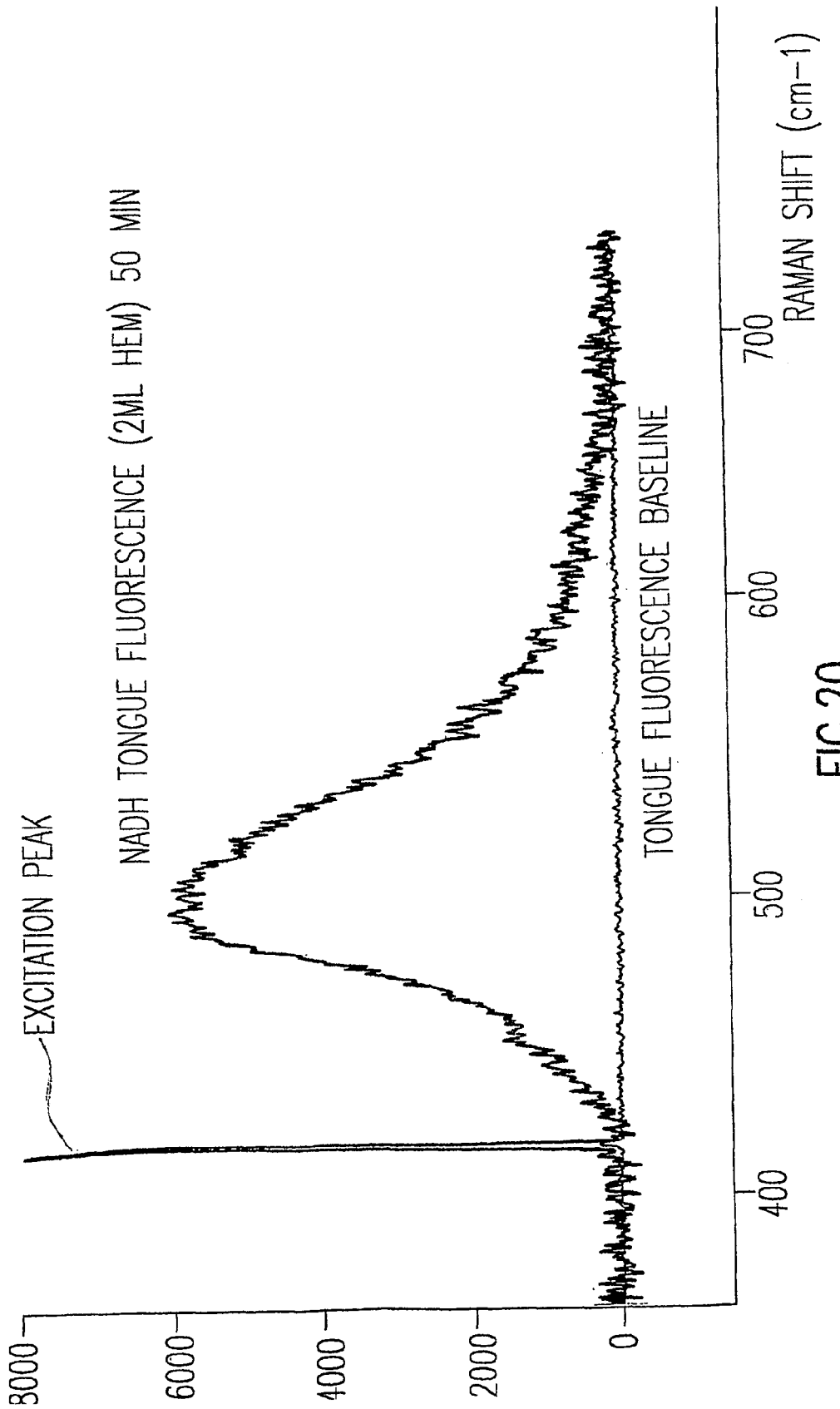

EXCITATION PEAK
NADH TONGUE FLUORESCENCE (2ML HEM) 50 MIN
TONGUE FLUORESCENCE BASELINE
8000
6000
4000
2000
0
400
500
600
700
RAMAN SHIFT (cm-1)

FIG.20

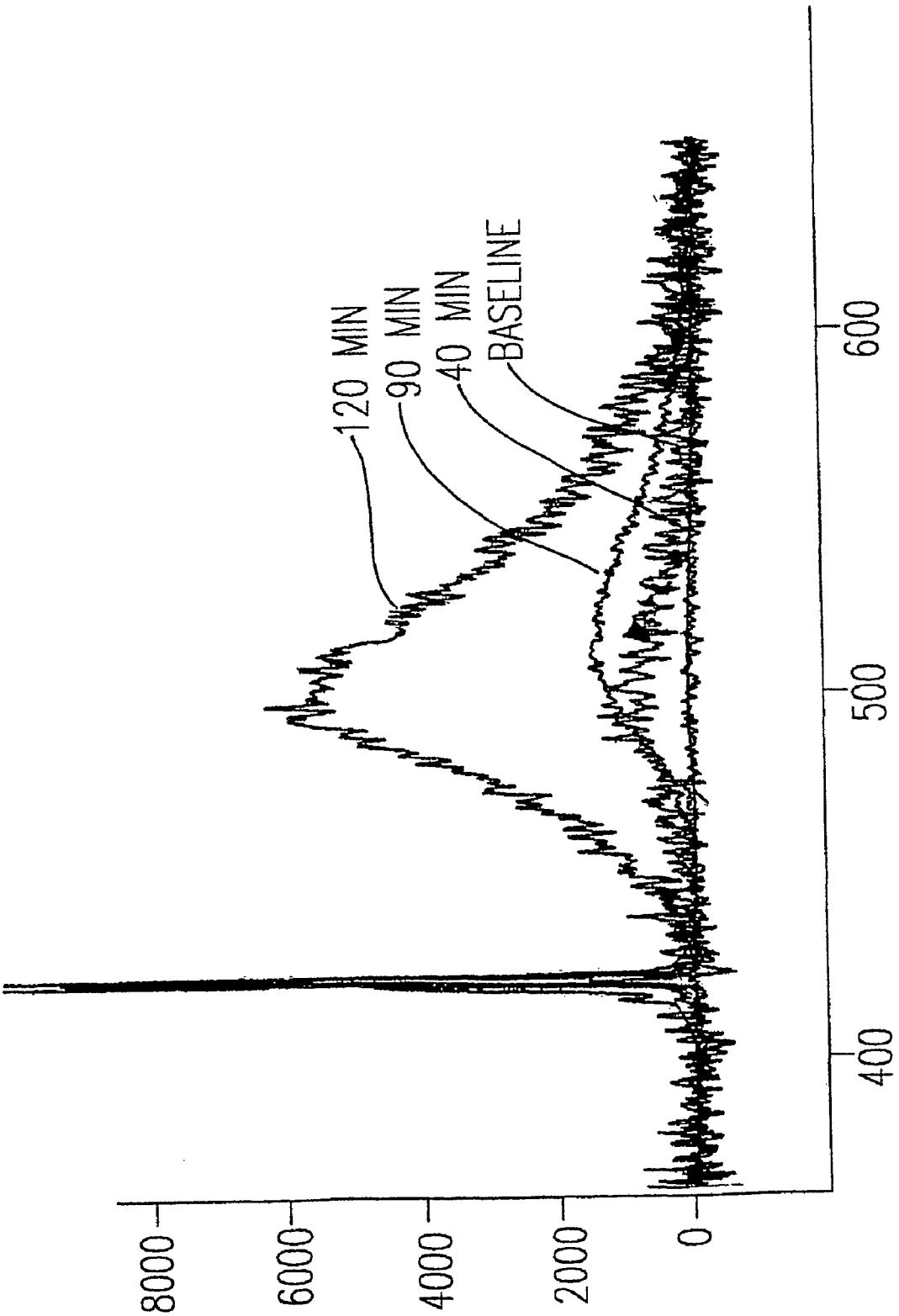

120 MIN
90 MIN
40 MIN
BASELINE
8000
6000
4000
2000
0
400
500
600

FIG.21

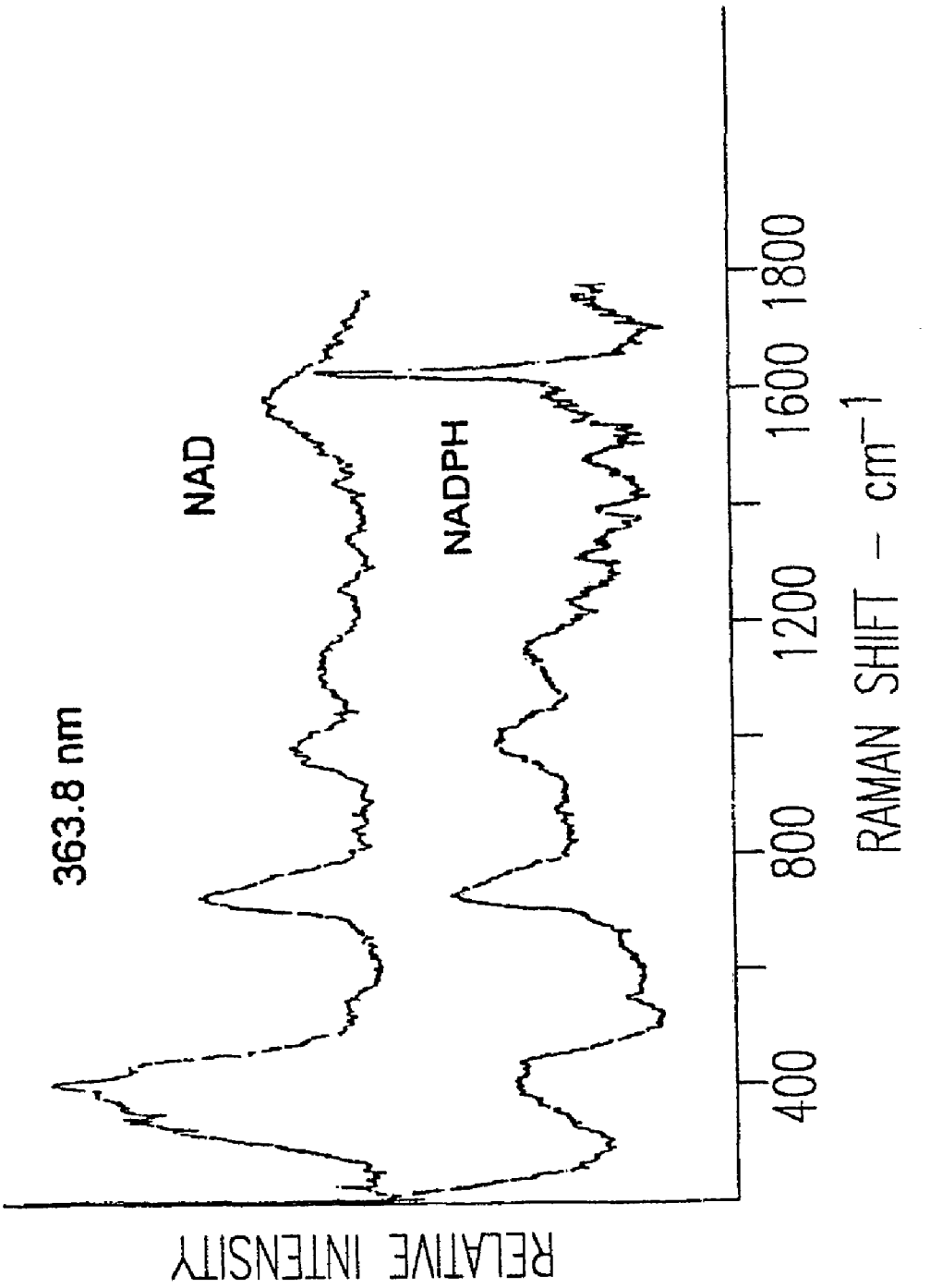

363.8 nm
NAD
NADPH
400
800
1200
1600
1800
RAMAN SHIFT – cm^{-1}
RELATIVE INTENSITY

FIG.22A

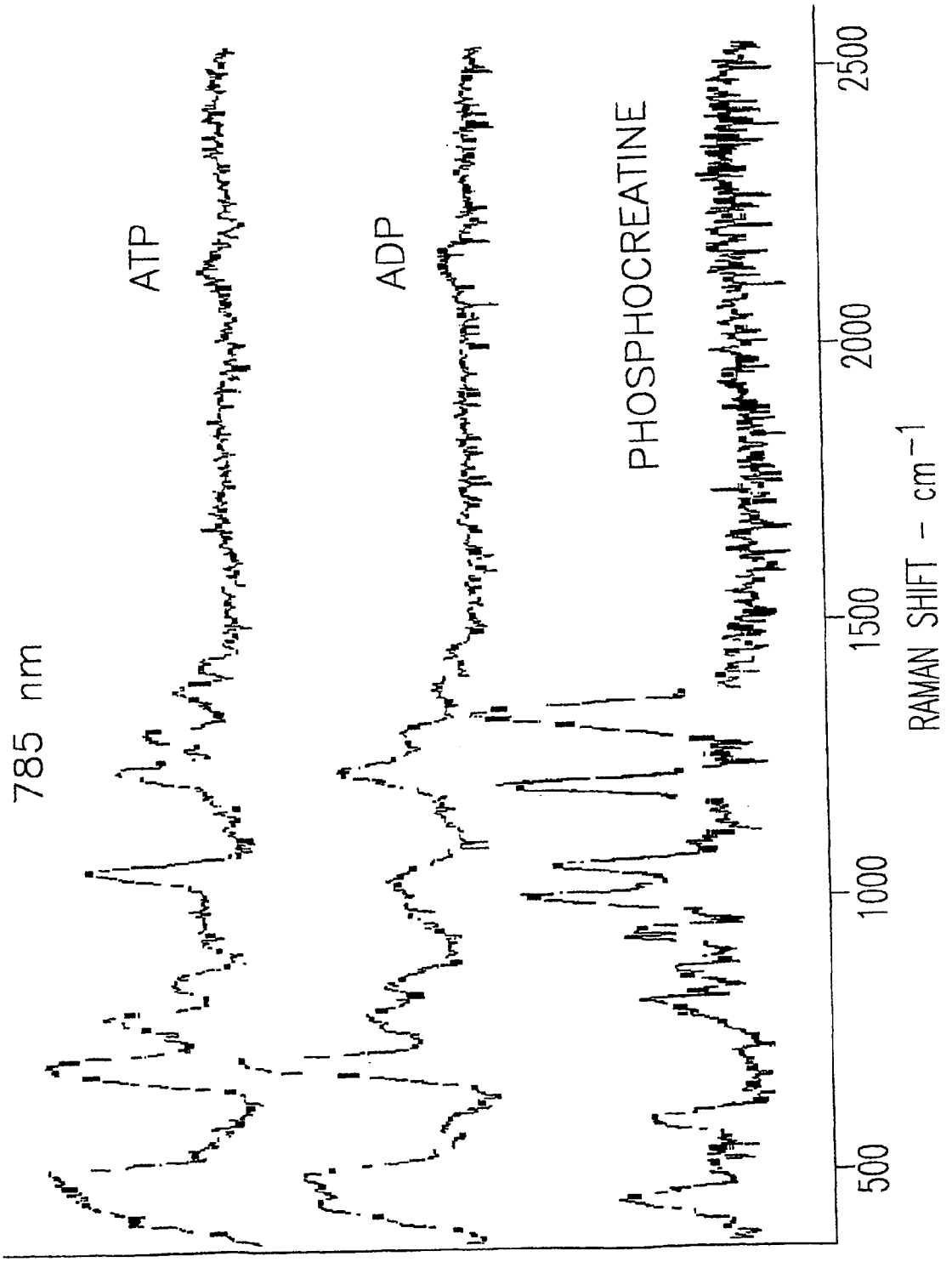

785 nm
ATP
ADP
PHOSPHOCREATINE
RELATIVE INTENSITY
500
1000
1500
2000
2500
RAMAN SHIFT - cm^{-1}

FIG.22B

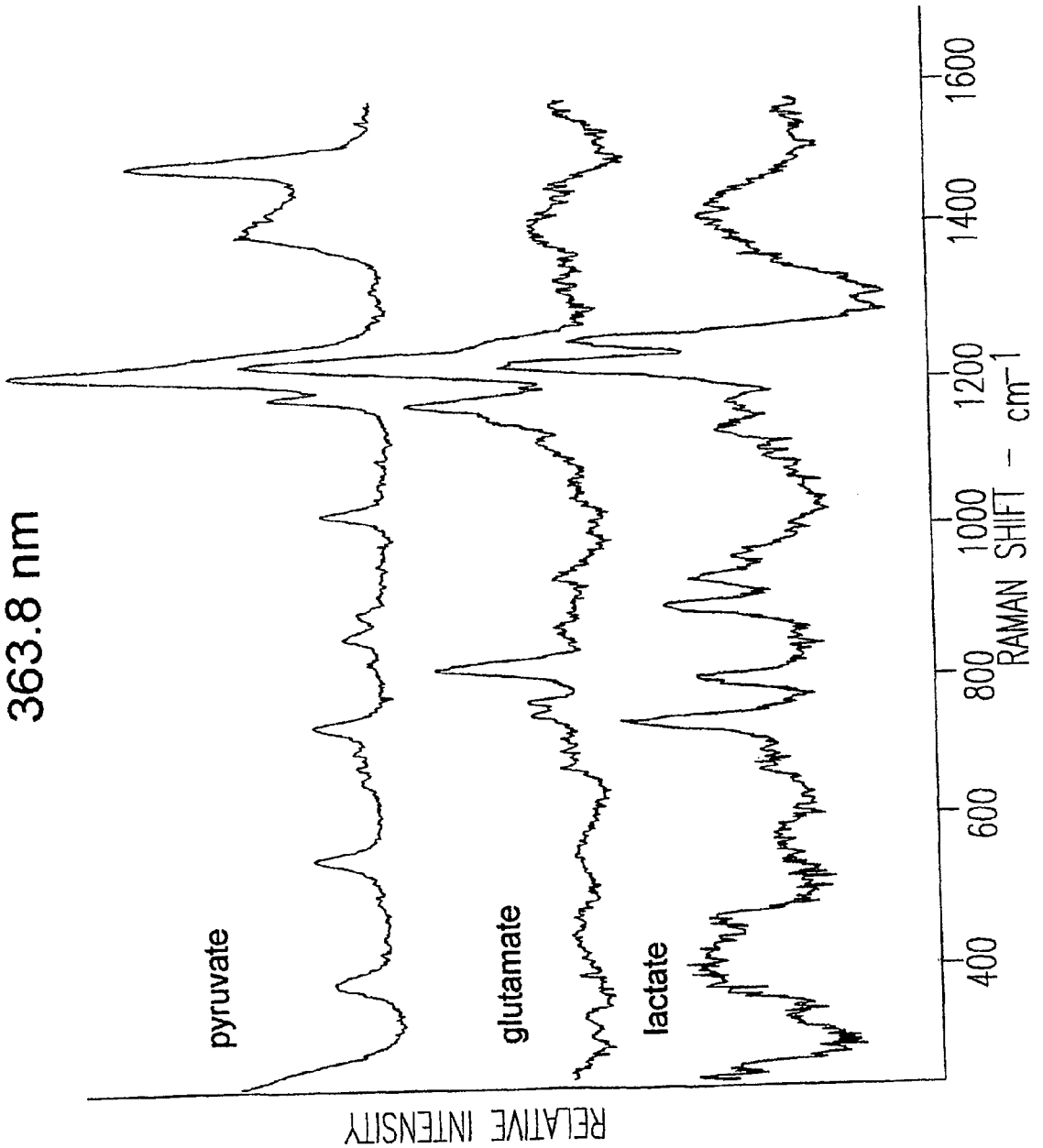

363.8 nm
pyruvate
glutamate
lactate
RELATIVE INTENSITY
400
600
800
1000
1200
1400
1600
RAMAN SHIFT - cm⁻¹

FIG.22C

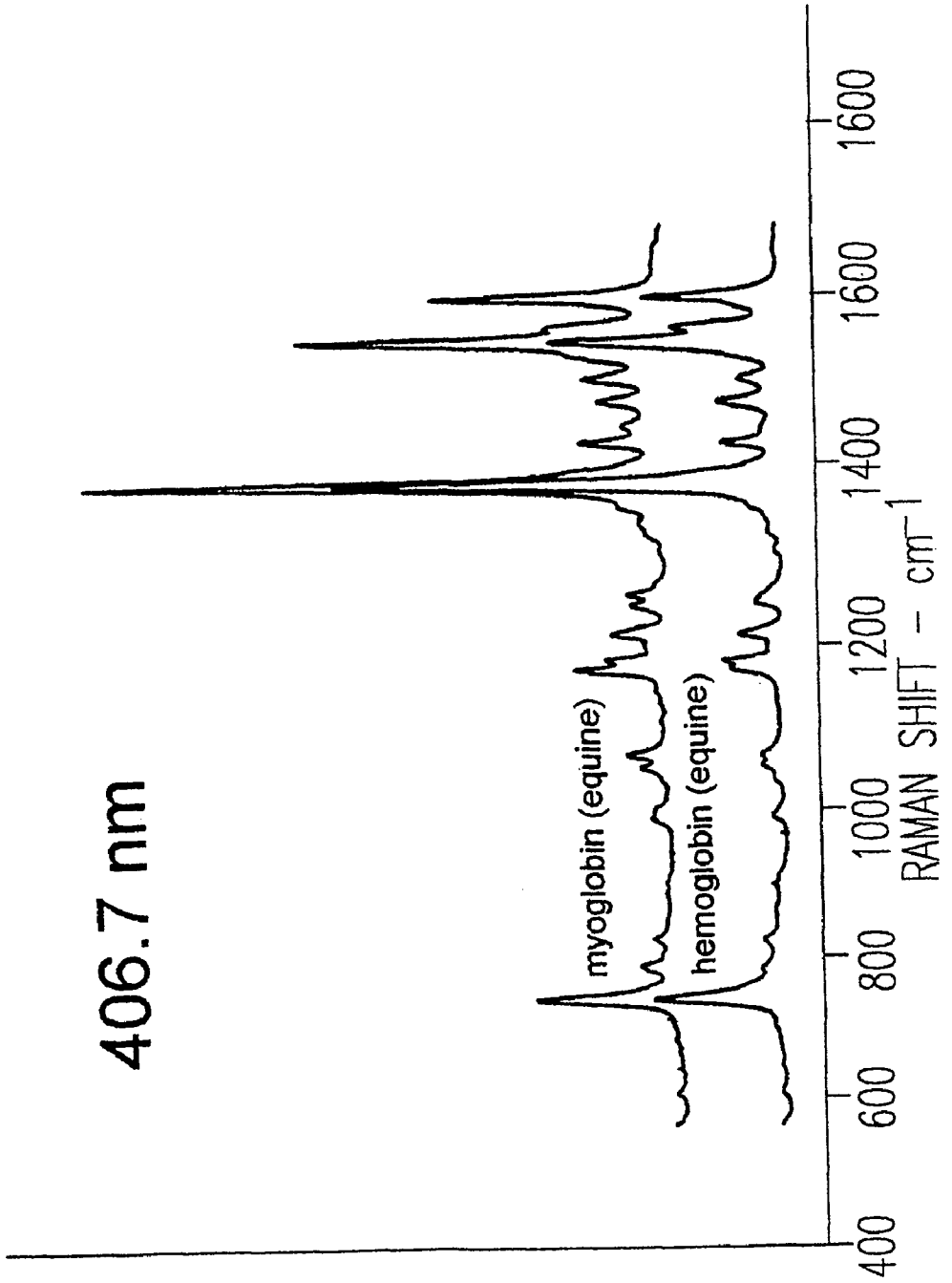

406.7 nm
myoglobin (equine)
hemoglobin (equine)
400
600
800
1000
1200
1400
1600
1600
RAMAN SHIFT – cm^{-1}

FIG.22D

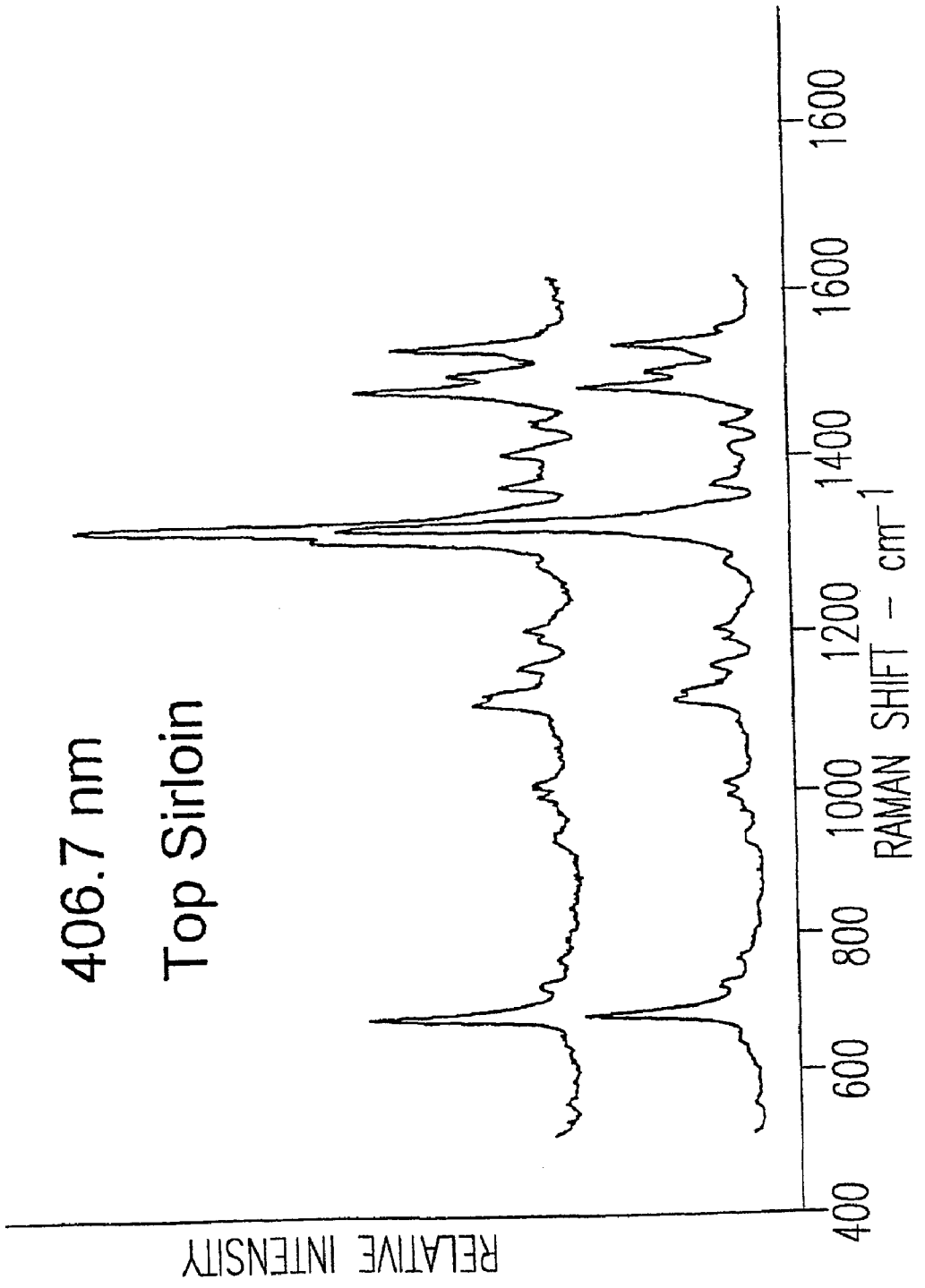

406.7 nm
Top Sirloin
RELATIVE INTENSITY
400
600
800
1000
1200
1400
1600
1600
RAMAN SHIFT - cm-1

FIG.22E

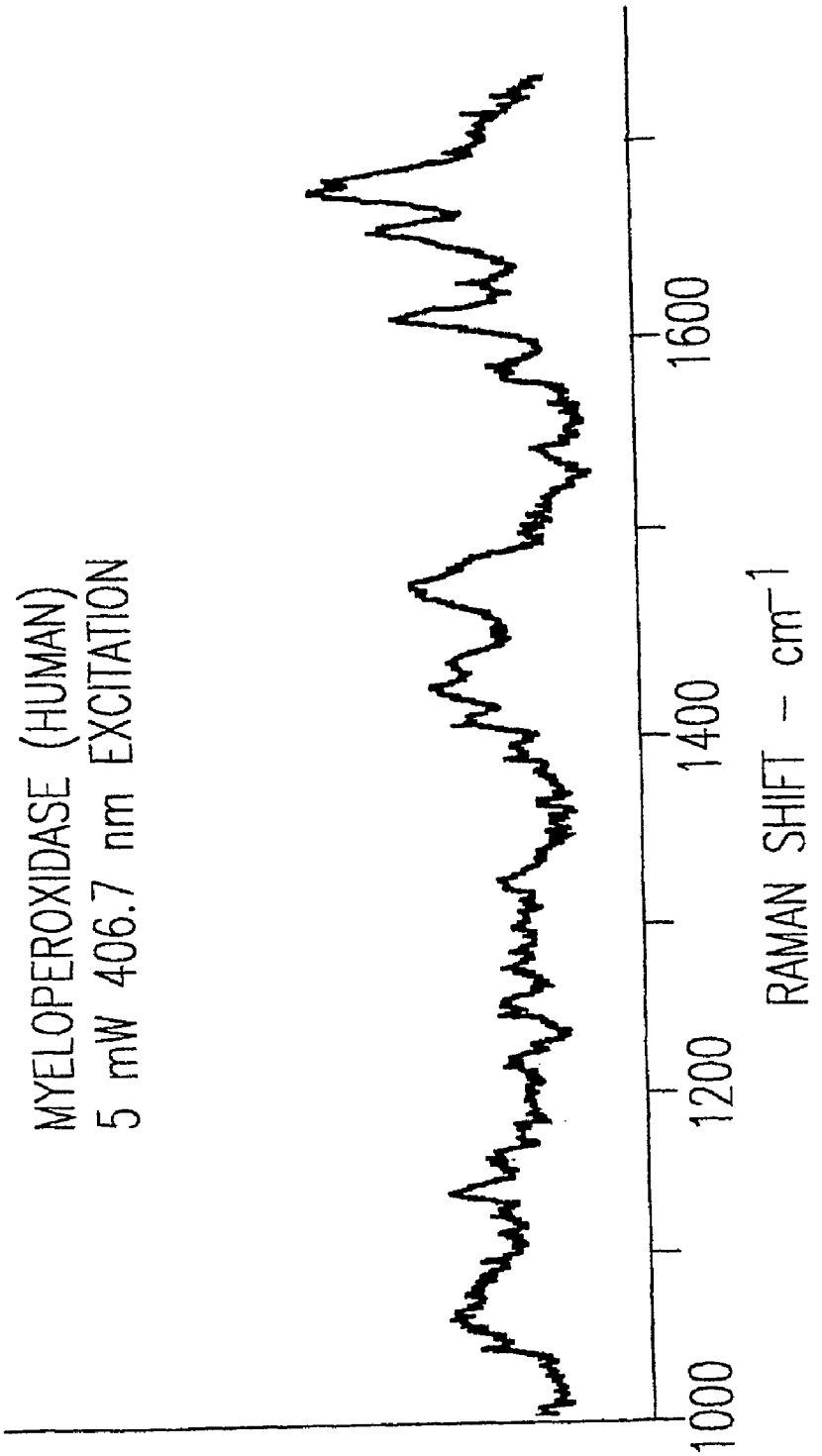

MYELOPEROXIDASE (HUMAN)
5 mW 406.7 nm EXCITATION
1000
1200
1400
1600
RAMAN SHIFT – cm^{-1}

FIG.23

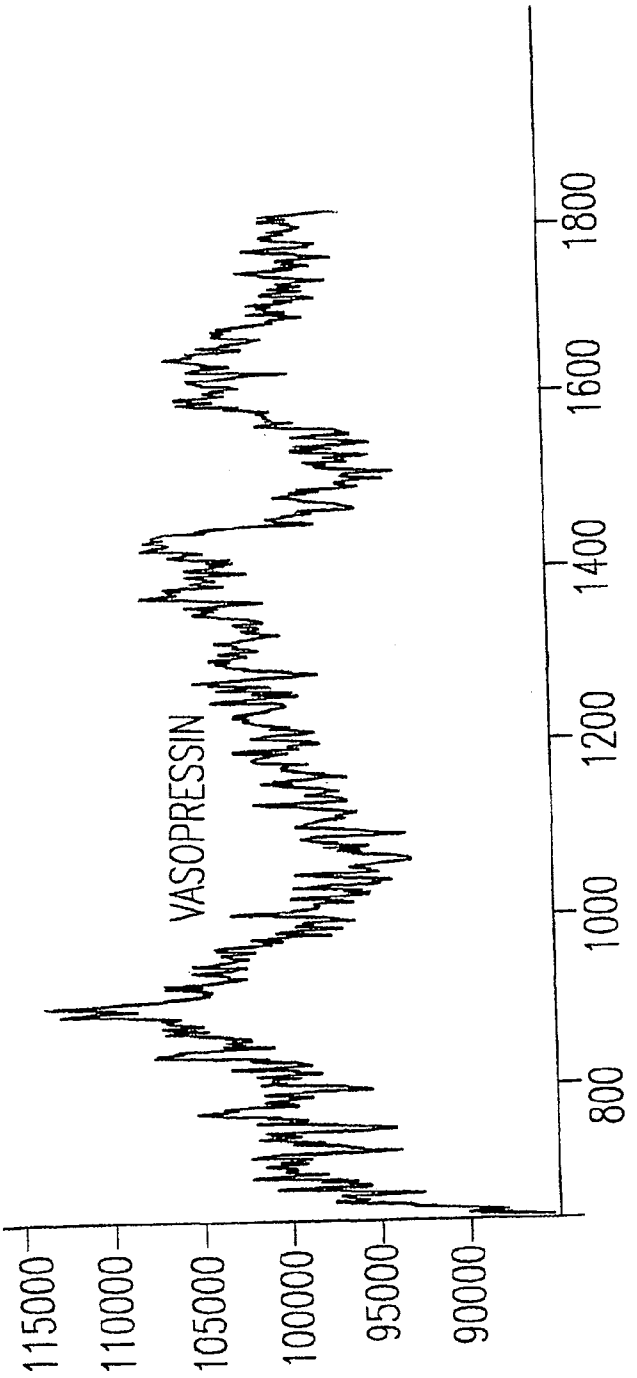

VASOPRESSIN
115000
110000
105000
100000
95000
90000
800
1000
1200
1400
1600
1800

FIG.24

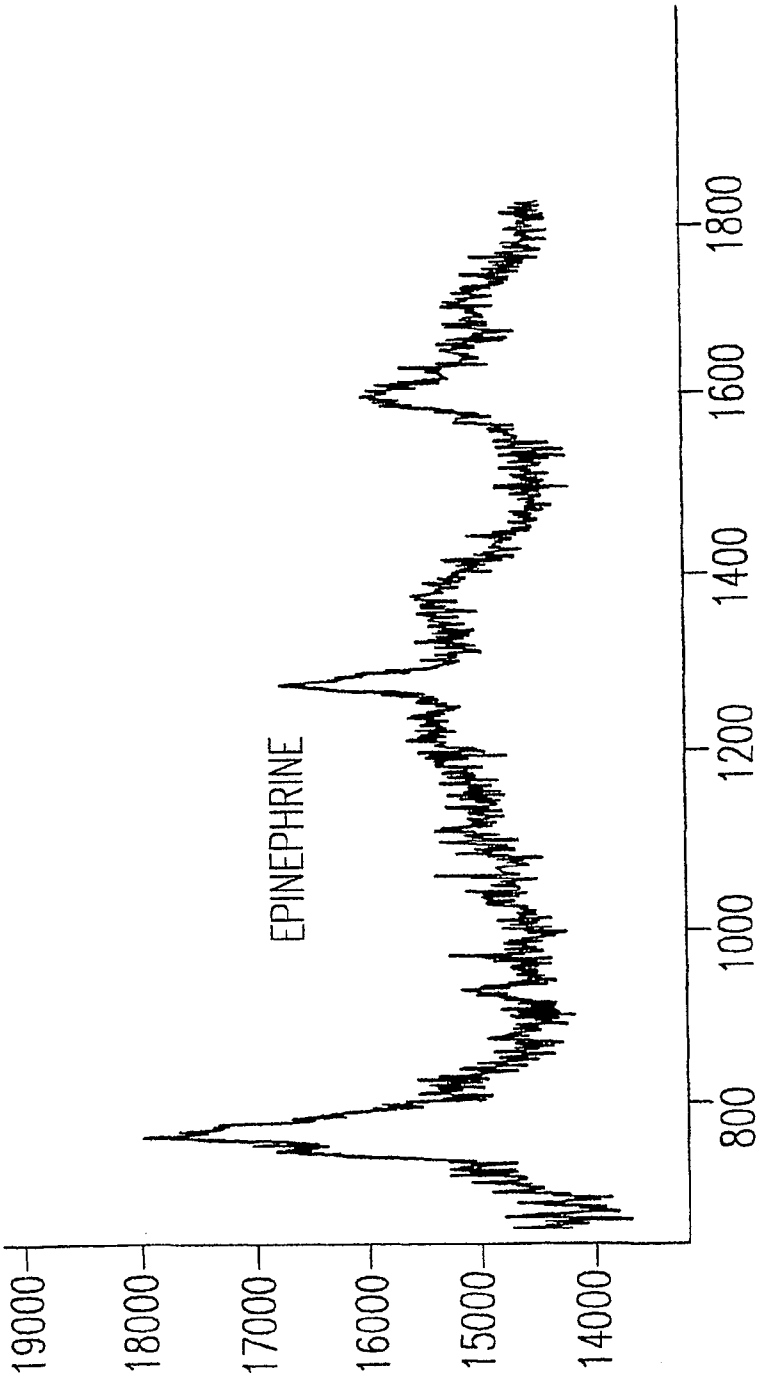

EPINEPHRINE
19000
18000
17000
16000
15000
14000
800
1000
1200
1400
1600
1800

FIG.25

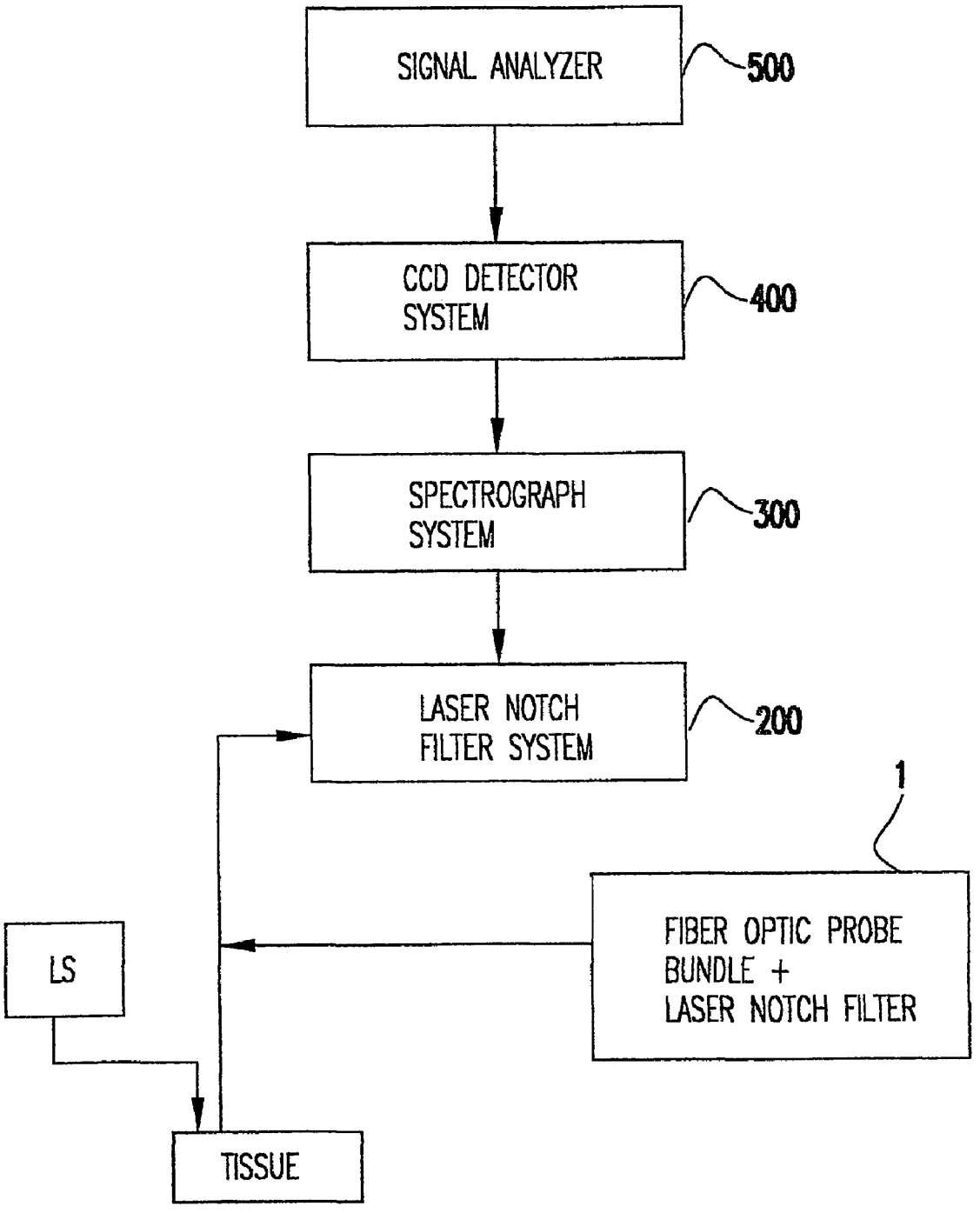

SIGNAL ANALYZER
500
CCD DETECTOR SYSTEM
400
SPECTROGRAPH SYSTEM
300
LASER NOTCH FILTER SYSTEM
200
1
FIBER OPTIC PROBE BUNDLE + LASER NOTCH FILTER
LS
TISSUE

FIG.26A

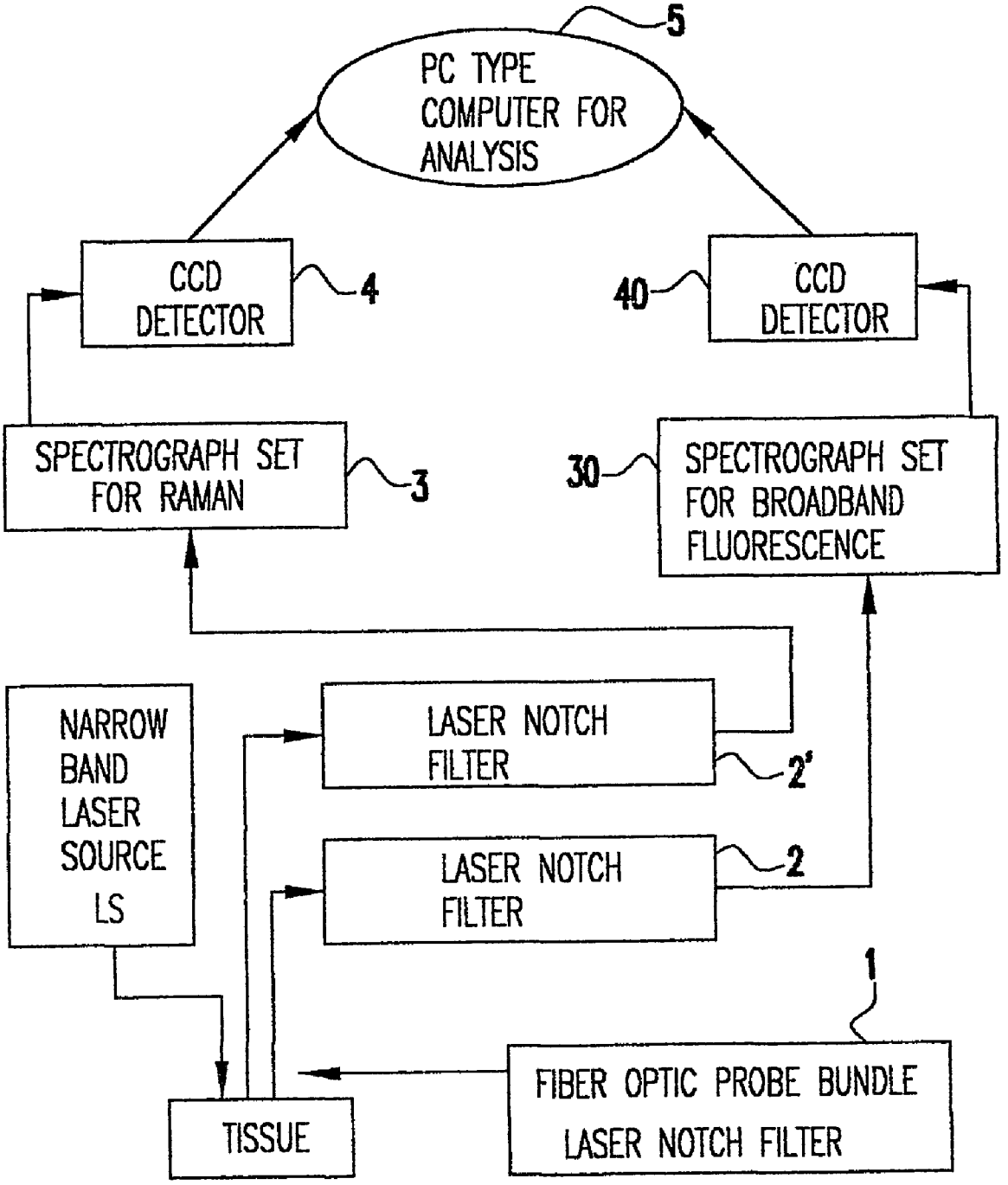

5
PC TYPE COMPUTER FOR ANALYSIS
CCD DETECTOR
4
40
CCD DETECTOR
SPECTROGRAPH SET FOR RAMAN
3
30
SPECTROGRAPH SET FOR BROADBAND FLUORESCENCE
NARROW BAND LASER SOURCE LS
LASER NOTCH FILTER
2'
LASER NOTCH FILTER
2
1
FIBER OPTIC PROBE BUNDLE LASER NOTCH FILTER
TISSUE

FIG.26B

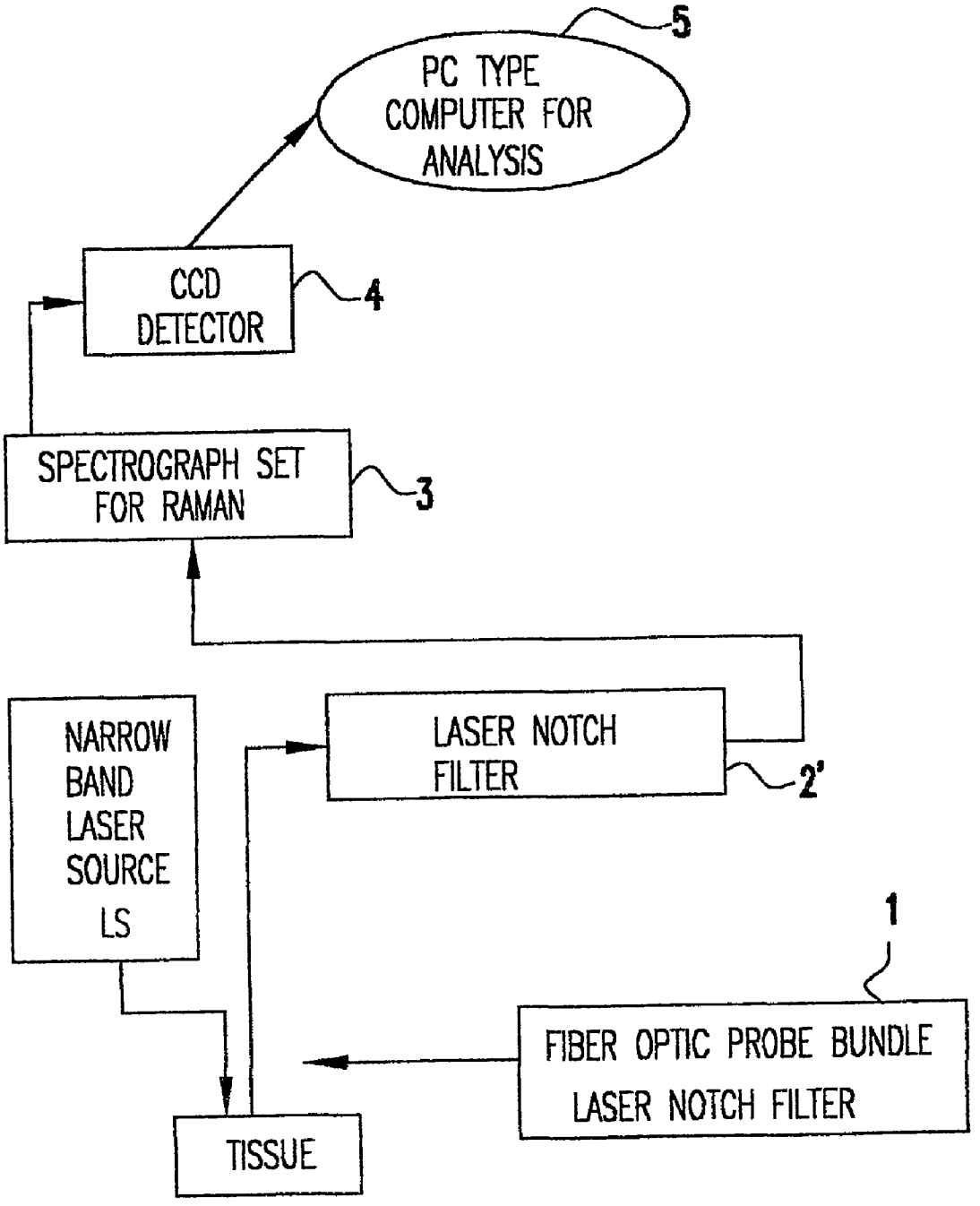

5
PC TYPE COMPUTER FOR ANALYSIS
CCD DETECTOR
4
SPECTROGRAPH SET FOR RAMAN
3
NARROW BAND LASER SOURCE LS
LASER NOTCH FILTER
2'
1
FIBER OPTIC PROBE BUNDLE
LASER NOTCH FILTER
TISSUE

FIG.26C

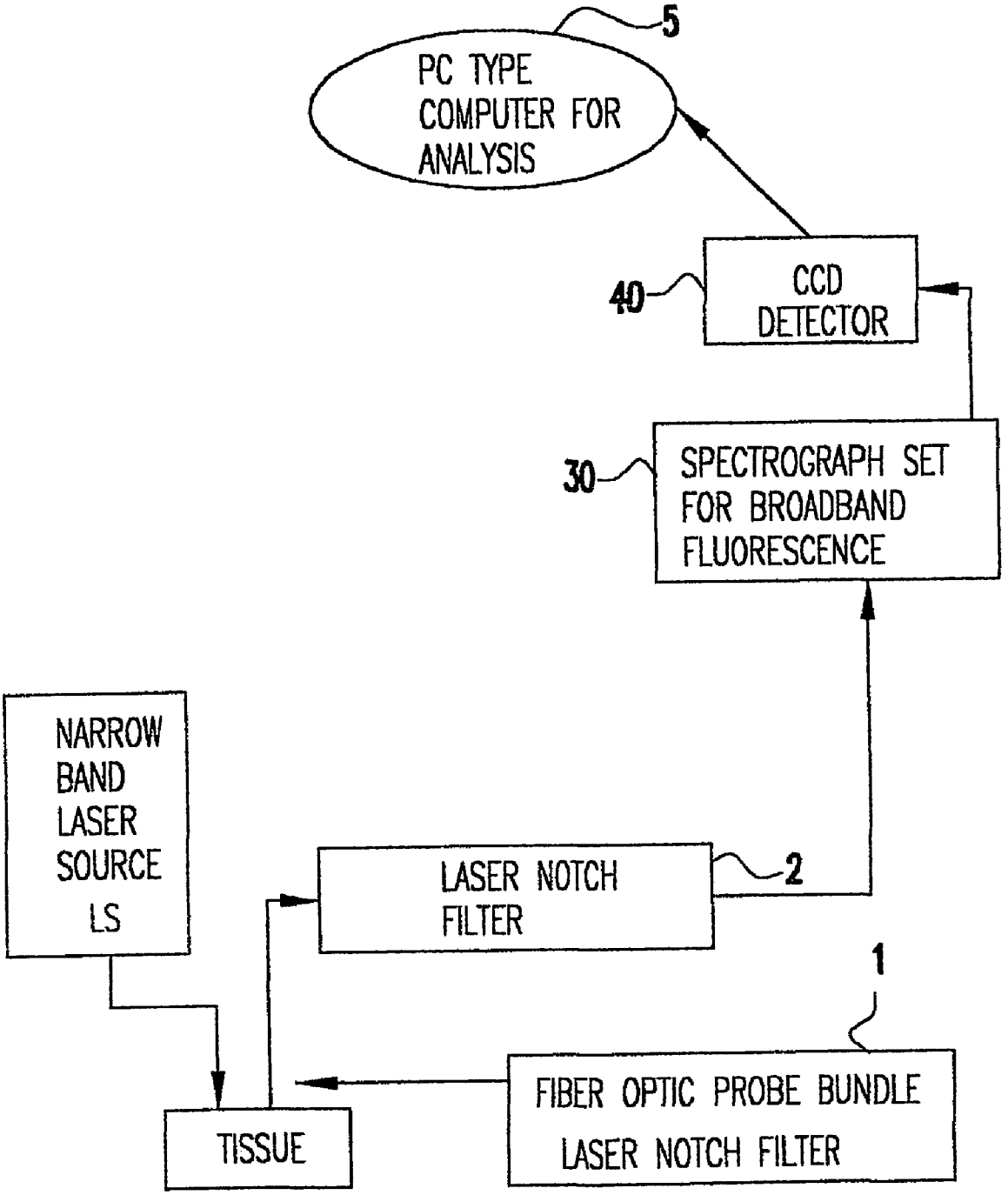

5
PC TYPE COMPUTER FOR ANALYSIS
40
CCD DETECTOR
30
SPECTROGRAPH SET FOR BROADBAND FLUORESCENCE
NARROW BAND LASER SOURCE LS
LASER NOTCH FILTER
2
1
FIBER OPTIC PROBE BUNDLE
LASER NOTCH FILTER
TISSUE

FIG.26D

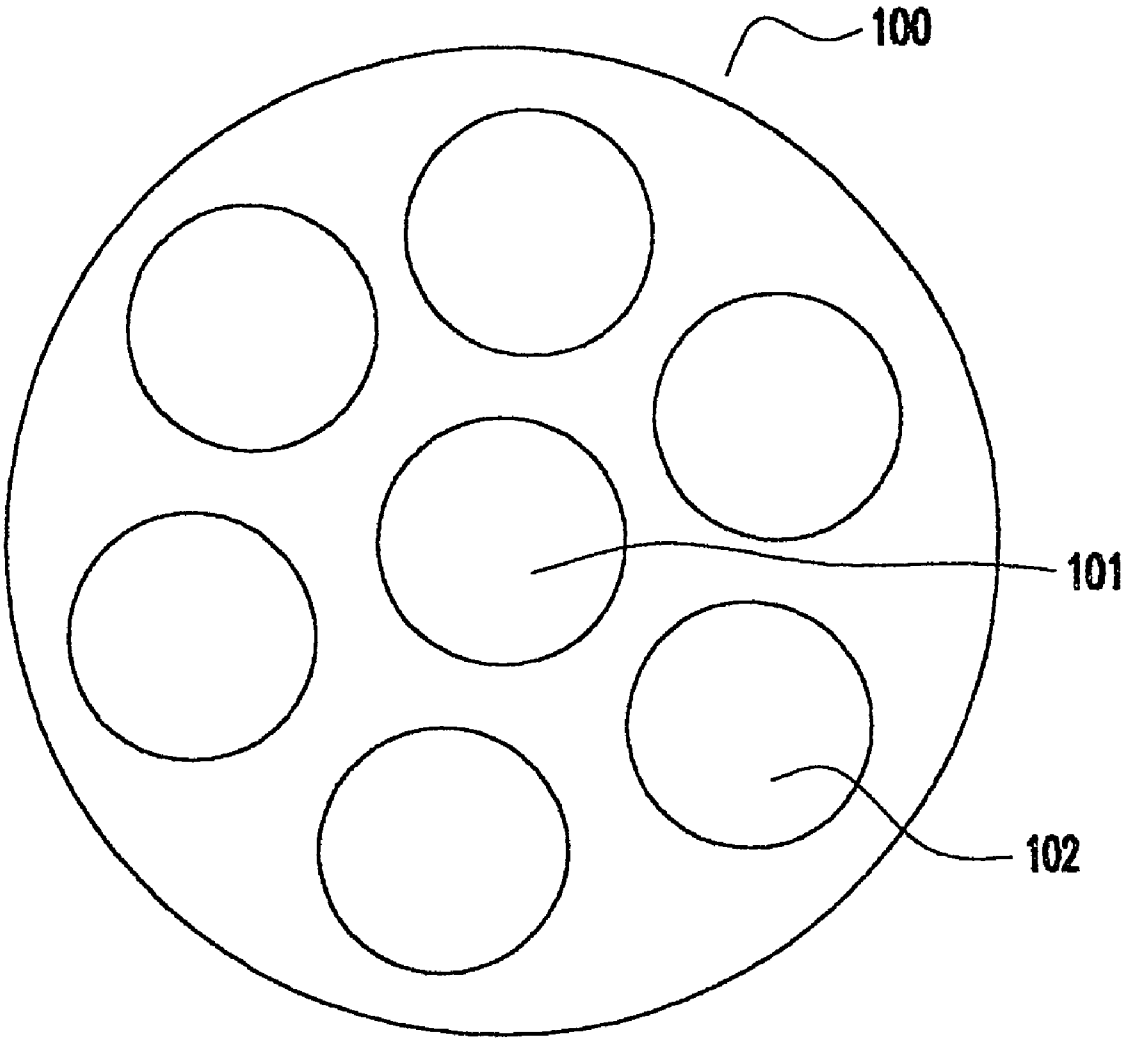


FIG.26E

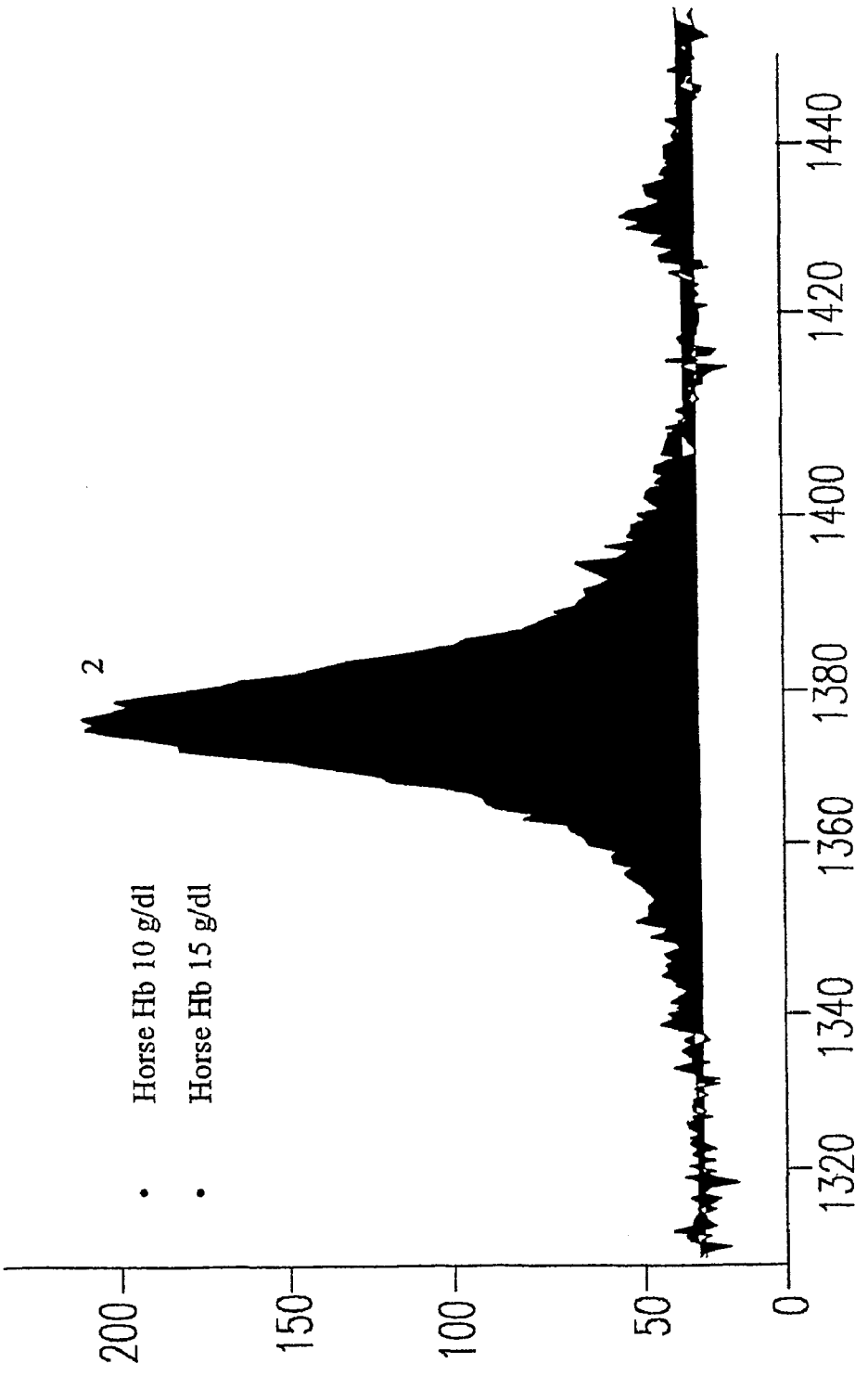

2
Horse Hb 10 g/dl
Horse Hb 15 g/dl
200
150
100
50
0
1320
1340
1360
1380
1400
1420
1440

FIG.27

TISSUE INTERROGATION SPECTROSCOPY

This application claims the benefit of Provisional Application No. 60/218,055, filed Jul. 13, 2000.

FIELD OF THE INVENTION

The invention generally relates to emergency medicine, and especially relates to shock states and critical illnesses and disease states.

BACKGROUND OF THE INVENTION

Shock is a complex entity, which traditionally has been defined as a state in which the metabolic demands of tissues are not matched by sufficient delivery of metabolic substrates, with the major substrate being oxygen. This mismatch commonly results from altered states of organ perfusion such as hemorrhage. Shock additionally involves complex inflammatory and immune mediated events which result from, and may further exacerbate, this initial metabolic mismatch. Many of these events play an important role in the development of subsequent multiorgan dysfunction, failure and death, with this latter mode responsible for over 60% of trauma deaths. Haljamae, H., "Cellular metabolic consequences of altered perfusion," in Gutierres, G., Vincent, J., eds., "Update in Intensive Care and Emergency Medicine: Tissue oxygen utilization (Springer Verlag, 1991), pp. 71–86. Despite the complexities of the inflammatory and immune components of trauma and hemorrhage, there is little debate on linking the severity of these events to the severity of initial perfusion deficits and tissue hypoxia. It is therefore essential to recognize and correct perfusion deficits at their earliest possible time. Although this seems intuitive, up to 80% of trauma patients on close monitoring continue to demonstrate evidence of tissue hypoxia secondary to perfusion deficits after what was considered to be complete resuscitation. Abou-Khalil, B., Scalea, T. M., Trooskin, S. Z., Henry, S. M., Hitchcock, R., "Hemodynamic responses to shock in young trauma patients; need for invasive monitoring," Crit. Care Med., 22:633–639 (1994).

Traditional clinical signs of tissue perfusion such as capillary refill, mental status, heart rate, pulse pressure and systemic blood pressure are very gross indicators of tissue perfusion and can only be considered to be of historic interest except at extreme values. Porter, J., Ivatury, R., "In search of optimal end points of resuscitation in trauma patients," J. Trauma, 44:908–914 (1998). Current markers of tissue perfusion include systemic lactate and base deficit measurements; transcutaneous and subcutaneous gas measurements, gastric and sublingual tonometry and spectroscopic techniques such as NIR absorption spectroscopy, fluorescence quenching, and orthogonal polarization spectral imaging. While these techniques have respective advantages, each is plagued by the relative singularity of its measure, lack of tissue specificity, inability to quantitate, or inability to easily apply or adapt for field use. Identification of any other useful markers is an important objective, and the search continues for further markers of shock states and the like. Effectively measuring and working with both known markers as well as markers being discovered would be highly beneficial to emergency medicine but is not provided in conventional technology. Information about biochemistry in shock states and disease states has not yet fully found its way and been used in practical applications. Rather, currently emergency medicine is left to rely on physical examination not much advanced by conventional, relatively limited spectroscopic measurement technology.

That is, much still turns on observation of simple vital signs. Yet, the diagnosis of shock and its severity can be difficult, and cannot be accomplished with certainty, from simple vital signs. A physical exam, including vital signs, is inadequate in detecting states of uncompensated shock. Ward, K. R., Ivatury, R. R., Barbee, R. W., "Endpoints of resuscitation for the victim of trauma," J. Intensive Care Med., 16:55–75 (2001). Dysoxia can be present despite normal vital signs. Ward et al., id.; Abou-Khalil, B., Scalea, T. M., Trooskin, S. Z., Henry, S. M., Hitchock, R., "Hemodynamic response to shock in young trauma patients: need for invasive monitoring," Crit Care Med. 22(4):633–9 (1994); Scalea, T. M., Maltz, S., Yelon, J., Trooskin, S. Z., Duncan, A. O, Scalafani, S. J., "Resuscitation of multiple trauma and head injury: role of crystalloid fluids and inotropes," Crit Care Med. 22(10):1610–5 (1994); Ivatury, R. R., Simon, R. J., Havriliak, D., Garcia, C., Greebarg, J., Stahl, W. M., "Gastric mucosal pH and oxygen delivery and oxygen consumption indices in the assessment of adequacy of resuscitation after trauma: a prospsective, randomized study," J. Trauma, 39(1):128–34; discussion 34–6 (1995).

In addition, resuscitation of victims of uncompensated shock back to "normal" vital signs is inadequate as a resuscitation endpoint. Unrecognized continued accumulation of additional oxygen debt is still possible and may contribute to later development of multisystem organ failure and death. Shoemaker, W. C., Appel, P. L., Kram, H. B., "Tissue oxygen debt as a determinant of lethal and nonlethal postoperative organ failure," Crit. Care Med., 16(11):1117–20 (1988).

Adding, to a physical exam, global measures of oxygen transport still does not ensure detection of early shock states or provide adequate information to act as sole end-points of resuscitation once shock is recognized and therapy instituted. For an outline of all of the current major technologies that have been used to detect the presence of shock and to guide its treatment, see Ward, Ivatury et al., supra. For various reasons, all have been problematic.

To better understand the difficulties in detecting shock states it is helpful to examine the biphasic relationship between oxygen delivery (DO_2) and consumption (VO_2) to understand the potential inadequacies of currently available monitoring systems. FIG. **1** demonstrates that VO_2 can remain constant over a wide range of DO_2. This is possible because cells have the ability to increase their extraction of oxygen (OER) in the face of decreased delivery. This is generally reflected by lower hemoglobin oxygen saturations in blood leaving the organ system (SvO_2), which may change before it is apparent in the physical exam. Scalea, T. M., Hartnett, R. W., Duncan, A. O., Atrweh, N. A., Phillips, T. F., Sclafani, S. J., et al., "Central venous oxygen saturation: a useful clinical tool in trauma patients," J. Trauma, 30(12):1539–43 (1990); McKinley, B. A., Marvin R. G., Cocanour, C. S., Moore, F. A., "Tissue hemoglobin O2 saturation during resuscitation of traumatic shock monitored using near infrared spectrometry," J. Trauma, 48(4):637–42 (2000). However, there is a point at which OER cannot keep pace with reductions in delivery. At this point VO_2 of the cell or organ falls (critical oxygen delivery: DO_2crit) and cells become dysoxic. This results in an increase in the oxidation-reduction (redox) value of the cell, effectively blocking the flow of electrons through the NADH-cytochrome a, a3 cascade in the mitochondria which prevents the formation of ATP. Cytochrome a,a3 (cytochrome oxidase) is the terminal electron acceptor in the mitochondrial electron transport

chain. Dysoxia can be recognized by the accumulation of a number of metabolic products such as lactate and intracellular reduced nicotinamide adenine dinucleotide (NADH). NADH offers one of the main sources of energy transfer from the TCA cycle to the respiratory chain in the mitochondria. NADH is situated on the high-energy site of the respiratory chain and during tissue dysoxia it accumulates because less NADH is oxidized to NAD+. The redox state of the mitochondria (NADH/NAD+) therefore reflects the mitochondrial energy state, which in turn is determined by the balance of oxygen availability in the cell and the metabolic rate of the cell. Siegemund, M., van Bommel, J., Ince, C., "Assessment of regional tissue oxygenation," Intensive Care Med., 25(10):1044–60 (1999). Conventional monitoring and measuring used in emergency medicine do not adequately take into account such biochemistry of shock states and the like. Knowing the biochemistry of shock states and the like but not being able to measure and monitor pertinent information thereto has been a frustrating, unresolved problem in emergency medicine.

Conventionally, a primary means of assessing tissue perfusion is through infrared (IR) or near-infrared (NIR) spectroscopy. Human skin and tissue are semi-transparent to wavelengths in this range. However, problems with IR technology arise because water strongly absorbs IR radiation. While NIR absorption spectroscopy does not suffer from water absorption as does classical IR, and NIR absorption spectroscopy is useful for the relative quantification of several specific chromophores such as hemoglobin, myoglobin, and cytochrome oxidase. Nakamoto, K., Czernuszewicz, W. S., "Infrared Spectroscopy," in: *Methods in Enzymology,* 226:259–289 (1993); Piantadisu, C., Parsons, W., Griebel, "Application of NIR Spectroscopy to problems of tissue oxygenation," in Gutierres, G., Vincent, J., eds., *Update in Intensive Care and Emergency Medicine: Tissue oxygen utilization* (Springer Verlag, 1991) pp. 41–44. Other recent work reports the ability of using NIR absorption shift of hemoglobin to measure pH. However, disadvantageously, NIR signals are so broad as to not be well-suited to quantification of overlapping species. Examples of NIR absorption spectroscopy signals being too broad to lend themselves to quantification of overlapping species include the spectra for oxy and deoxy hemoglobin and cytochrome oxidase (see FIG. **2**). Owen-Reece, H., Smith, M., Elwell, C. E., Goldstone, J. C., "Near infrared spectroscopy," Br J Anaesth, 82(3): 418–26 (1999). FIG. **2** is a graph of typical broad signals of oxy and deoxy hemoglobin and cytochrome oxidase obtained by NIR absorption spectroscopy. (In FIG. **2**, the HbO_2 and Hb signals also would include those from myoglobin.)

Conventional NADH-fluorescence techniques are more specific and quantitative than classical NIR absorption spectra but can only measure a single marker. The technique has relied on use of excitation wavelengths in the carcinogenic UV region and has not been reduced to clinical practice. Conventional noninvasive or minimally invasive measures of tissue perfusion include transcutaneous and tonometric (gastric or sublingual) monitoring of various gases such as oxygen and carbon dioxide. The major limitations of these devices are that they are limited to monitoring those specific gases and cannot provide additional information that, if provided, could be useful in diagnosis and stratification of patients. Methods such as tonometry can be cumbersome due to its invasive nature. These methods are also prone to deviations through changes either in minute ventilation or inspired oxygen concentration. Transcutaneous gas monitoring, gastric tonometry, and even sublingual tonometry are one-dimensional and are prone to non-flow related changes caused by hypo or hyper ventilation. Also, with the exception of sublingual tonometry, application of these methods in the field is problematic. Weil, M., Nakagawa, Y., Tang, W., et al., "Sublingual capnometry: A new noninvasive measurement for diagnosis and quantification of severity of circulatory shock," Crit. Care Med., 27:1225–1229 (1999).

Another concern associated with measurement of shock states, and balanced with other factors relating to measurement, is invasiveness. NIR absorption spectroscopy is being aggressively studied to use signals from these chromophores to noninvasively monitor oxygen transport at the tissue level. McKinley et al., supra. Perhaps the best-known use of this technology is in the monitoring of cerebral hemodynamics. The basis for this is that the majority of blood volume in an organ is venous and thus the tissue hemoglobin saturation should reflect the state of oxygen consumption of the tissue. Again, broad overlap of signals in addition to needing to know the pathlength of light presents challenges in quantification and differentiation of signals. For example it is difficult to distinguish hemoglobin and myoglobin making NIR use in hemorrhage problematic since myoglobin has a p50 of only 5 mmHg. Gayeski, T. E., Honig, C. R., "Direct measurement of intracellular O2 gradients; role of convection and myoglobin," Adv Exp Med Biol, 159:613–21 (1983). Because soft tissue and bone are translucent to NIR light, NIR can penetrate to significant depths, a feature with both advantages and disadvantages. Monitoring the redox state of cytochrome oxidase is also difficult unless baseline absorptions are known. There is also significant overlap between the cytochrome oxidase and hemoglobin signals. Despite this, NIR measurements of tissue saturation (StO_2) are being marketed.

Although some manufacturers of NIR absorption spectroscopy equipment claim to differentiate between the two species of oxygen hemoglobin and myoglobin, no work to this effect exists in the medical literature. In fact, evidence exists that a major portion of the NIR absorption spectroscopy signal reported from hemoglobin actually originates from myoglobin.

Another problem for NIR is that in terms of use on hollow organ systems such as the stomach, data from NIR absorption spectroscopy would likely include signals from non-stomach organs and thus not reflect data from the mucosal surface of the stomach.

Surface NADH fluorescence has been used to detect cellular dysoxia in a number of organ systems. Siegemund et al., supra. The traditional technique uses unique excitation light sources and detection filters to take advantage of the fact that NADH will fluoresce (emit light at 460 nm) when excited at a wavelength of 360 nm (near-UV). This technique has been used in video microscopy/fluorometry experiments. Van der Laan, L., Coremans, A., Ince, C., Bruining, H. A., "NADH videofluorimetry to monitor the energy state of skeletal muscle in vivo," J. Surg. Res., 74(2):155–60 (1998). However, such conventional methods do not necessarily provide optimum resolution.

Adverse effects of certain compounds (such as vasopressin and norepinephrine) on oxygen transport and the immune/inflammatory response are now beginning to be appreciated with manipulation of their actions being studied as therapeutic strategies. Kincaid, E. H., Miller, P. R., Meredith, J. W., Chang, M. C., "Enalaprilat improves gut perfusion in critically injured patients," Shock, 9(2):79–83 (1998); Catania, R. A., Chaudry, I. H., "Immunological consequences of trauma and shock," Ann. Acad. Med. Singapore, 28(1):120–32 (1999). However, satisfactory

measurement of such compounds in vivo without invasive probing has not yet been provided.

Thus, current technology includes pulmonary artery catheters, repetitive measures of lactate and base deficit, splanchnic tonometry, sublingual tonometry, NIR absorption spectroscopy, transcutaneous gas monitoring, phosphorescence quenching and fluorescence technology (indwelling blood gas/pH catheters). No such technology is without a substantial disadvantage. Civilian prehospital emergency medical services systems, emergency physicians, trauma surgeons, intensive care physicians, cardiologists, anethesiologists, and military medical personnel continue to be plagued by the insensitivity of the physical exam, lack of readily available physiologic and metabolic markers to judge the presence and severity of shock states, and lack of real-time relevant measurement approaches. In addition, it has been difficult to use singular measures to guide treatment or predict outcome. These problems are greatly magnified as the scale of the wounded population increases (such as on the battlefield and the various pre-definitive echelons of care provided to wounded soldiers or in a natural disaster). To the inventors' knowledge, currently no conventional techniques are available for real-time monitoring of a broad range of potentially valuable emergency medicine markers of shock, tissue ischemia, tissue injury, tissue inflammation, or tissue immune dysfunction.

SUMMARY OF THE INVENTION

The invention realizes methods, profiles, medical measurement devices, and other products for accurate measurement of change or lack thereof from non-shock, non-ischemic, non-inflammation, non-tissue injury, non-immune dysfunction conditions which are referred to herein as "baseline conditions". In attention to advantageous accuracy in such measurement, the invention provides practical, real-time approaches for accurately characterizing a patient's condition with respect to baseline conditions. With Raman and/or fluorescence spectroscopy according to the invention, change from baseline conditions is measured, characterized, monitored, identified and/or followed with a high degree of accuracy with measurement times on the order of seconds. Such high-accuracy measurement is achieved with Raman spectroscopy (such as resonance Raman spectroscopy) interrogation of tissue, optionally with simultaneous interrogation by fluorescence spectroscopy of compounds such as NADH. The tissue interrogation advantageously may be non-invasive to minimally-invasive to totally invasive. With methods and products according to the present invention, advantageously preclinical (ultra-early) states of shock, tissue ischemia, tissue injury, and tissue inflammation can be detected, severity can be determined, and the effectiveness of various treatments aimed at resolving the shock state can be determined, and other beneficial effects for patient care can be achieved.

In order to accomplish these and other objects of the invention, the present invention in a preferred embodiment provides a tissue analysis method, comprising interrogating a biological material (such as a biological tissue or a bodily fluid) with Raman spectroscopy and fluorescence spectroscopy to obtain spectroscopy results.

In another preferred embodiment, the invention provides a method of diagnosing shock, tissue ischemia, tissue inflammation, or tissue immune dysfunction, comprising: (A) for a target molecule population, taking a sample Raman spectroscopy, and/or fluorescence spectroscopy, profile for a patient; (B) comparing the sample spectroscopy profile with a pre-established Raman spectroscopy and/or fluorescence spectroscopy profile for the target molecule population under baseline conditions.

A further preferred embodiment provides a spectroscopy comparative profile, comprising: a pre-established Raman spectroscopy and fluorescence spectroscopy profile for a target molecule population under baseline conditions; and a sample Raman spectroscopy and fluorescence spectroscopy profile for the target molecule population.

The invention also provides for a preferred embodiment which is a method of diagnosing abnormalities in vivo and in situ, comprising: (A) for a target molecule population, taking a sample Raman spectroscopy and/or fluorescence spectroscopy profile for a patient; (B) comparing the sample Raman spectroscopy or fluorescence spectroscopy profile with a pre-established Raman spectroscopy or fluorescence spectroscopy profile for the target molecule population under baseline conditions; and (C) using differences identified in said comparing step to identify an abnormality.

In a particularly preferred embodiment of the inventive methods, simultaneous fluorescence spectroscopy probing of NADH and resonance Raman spectroscopy are performed.

Another preferred embodiment of the invention provides a medical measurement device comprising: a spectrometer with multiple wavelength settings for resonance Raman spectroscopy; and a biological probe electrically connected to the spectrometer.

Additionally, the invention in another preferred embodiment provides a spectroscopy comparative profile, comprising: a pair of Raman spectroscopy or fluorescence spectroscopy profiles for a target molecule population, wherein one profile was taken from a patient after a medical event concerning the patient.

A further preferred embodiment of the invention provides a computer system comprising: a database of stored baseline Raman spectroscopy and/or fluorescence spectroscopy profiles and a means to store patient Raman spectroscopy and/or fluorescence spectroscopy profiles.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing and other objects, aspects and advantages will be better understood from the following detailed description of the preferred embodiments of the invention with reference to the drawings, in which:

FIG. **1** is a traditional biphasic oxygen delivery and consumption curve.

FIG. **2** is a graph of oxy and deoxy hemoglobin and cytochrome oxidase obtained by NIR absorption spectroscopy, with Δoptical density plotted versus wavelength (nm).

FIG. **3** shows near-UV resonance Raman spectroscopy according to the invention for human blood at various oxygen saturation levels.

FIG. **4** shows resonance Raman spectroscopy according to the invention of both oxygen hemoglobin and myoglobin.

FIG. **5** shows near-UV resonance Raman spectra of isolated ischemic rat skeletal muscle over time.

FIG. **6** is a baseline Resonance Raman spectrum for rat muscle, with the signal obtained in one second.

FIGS. **7**, **8**, **9** and **10** each is a Resonance Raman spectrum for the same muscle as FIG. **6**, with respective bleeding of 1 ml, 2 ml, 5 ml and 7.5 ml.

FIG. **11** is an overlay of the Raman spectra of FIGS. **6**, **7**, **8**, **9** and **10**.

FIG. **12** are resonance Raman spectra of a rat tongue.

FIG. **13** are near UV resonance Raman spectra of hemoglobin.

FIG. **14** is a spectra of baseline NADH fluorescence of the same quadricep muscle from the same animal in which NADH fluoresces after being excited with light at 406.5 nm which is the same wavelength used to produce the previous resonance Raman spectroscopy of FIGS. **5**–**10**.

FIGS. **15**, **16**, **17** and **18** each is a spectra of NADH fluorescence for the same muscle as FIG. **14**, with respective bleeding of 5 ml, 7.5 mls, 9 mls and 12 mls.

FIG. **19** is an overlay of NADH fluorescence spectra (FIGS. **14**–**18**) from the quadriceps muscle.

FIG. **20** is an overlay of NADH fluorescence spectra of a rat tongue, for baseline and after 2 cc hemorrhage for 50 minutes.

FIG. **21** is an overlay of NADH fluorescence spectra of a rat liver during graded hemorrhage over time (baseline, 40 min, 90 min and 120 min).

FIG. **22**(*a*) are preliminary Raman spectra of β-nicotinamide adenine dinucleotide in the oxidized (NAD) and reduced (NADPH or NADH) forms. FIG. **22**(*b*) are preliminary Raman spectra of the high energy phosphates ATP and ADP. FIG. **22**(*c*) are preliminary Raman spectra of the glycolytic end-products pyruvate and lactate, along with the excitatory amino acid and neurotoxin glutamate. FIG. **22**(*d*) are preliminary Raman spectra of the oxygen transporters hemoglobin (Hb) and myoglobin (Mb), from an equine. FIG. **22**(*e*) includes preliminary Raman scans of uncooked beef, with the top scan taken in a darker area, and the bottom scan taken in a lighter area with more saturated myoglobin.

FIG. **23** is a near-UV resonance Raman spectrum of myeloperoxidase.

FIG. **24** is a UV resonance Raman spectrum of vasopressin.

FIG. **25** is a UV resonance Raman spectrum of norepinephrine.

FIGS. **26**(*a*), **26**(*b*), **26**(*c*) and **26**(*d*) are schematic views of devices according to the invention. FIG. **26**(*e*) is a top view of a fiber optic bundle shown schematically in FIGS. **26**(*b*), **26**(*c*) and **26**(*d*).

FIG. **27** depicts resonant Raman spectra for horse Hb dilutions.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS OF THE INVENTION

The present invention provides methods and products in which resonance Raman spectroscopy interrogates biological material (such as tissue or a bodily fluid) at near-UV excitation. The Raman spectroscopy may proceed with or without simultaneous fluorescence spectroscopy (such as NADH fluorescence spectroscopy). The interrogation advantageously may be in a non-invasive to minimally-invasive manner, but is not required to be so and if desired may be invasive. Data from interrogating tissue according to the invention may be used to detect preclinical (ultra-early) states of shock and other tissue injury and disease states, determine severity, and determine the effectiveness of various treatments aimed at resolving the shock or tissue disease/injury state of a patient.

In a preferred embodiment of the invention, a tissue analysis method comprises interrogating a biological tissue with Raman spectroscopy and fluorescence spectroscopy to obtain spectroscopy results. The Raman spectroscopy used in the present invention is that based on the Raman effect, which has been known for over 70 years and is caused by absorption of light leading to the transition of a molecule from the ground state to an excited state, followed by the emission of light with a different wavelength. Raman, C. V., Krishnan, K. S., "The colour of the sea," Nature (London), 121:619 (1928). The Raman effect has only recently, through the advancements and miniaturization of fiber optic, laser, and detector technology, become a practical technique for clinical use. Because each molecular species has its own characteristic molecular vibrations, a Raman spectrum provides a unique "fingerprint" useful for sample or marker identification. Hanlon, E. B., Manoharan, R., Koo, T. W., Shafer, K. E., Motz, J. T., Fitzmaurice, M., et al., "Prospects for in vivo Raman spectroscopy," Phys Med Biol, 45(2): R1–59 (2000); Diem, M., "Introduction to modern vibrational spectroscopy," New York: Wiley (1993). While any wavelength of light theoretically can be used as an excitation source to provide a Raman spectrum, visible excitation can produce strong broadband fluorescence, which undesirably can overwhelm Raman signals. Nevertheless, wavelengths can be chosen that produce resonance due to matching of the excitation wavelength and the electronic energy state of the scattering molecule. While Raman scattering is a rather low energy phenomenon requiring sensitive detectors, the signal is greatly enhanced when the molecule of interest is resonant (absorption maximum near the laser wavelength). This signal enhancement at a resonant frequency may be referred to as "resonance Raman spectroscopy" and allows for the selective detection of individual species of very low concentration within a complex mixture. Hanlon et al., supra.

If the excitation wavelength does not induce fluorescence within the wavelength region of interest, then remarkably high resolution Raman spectra can be obtained. If fluorescence does occur, this can be reduced or even eliminated in many instances by tuning of the excitation wavelength. Thus, while interfering fluorescence may occur with a particular excitation wavelength, it may not occur within the UV or NIR range where one could detect signals either above or below the fluorescing region, as the case may be. Hanlon, E. B., Manoharan, R., Koo, T. W., Shafer, K. E., Motz, J. T., Fitzmauric, M., Kramer, J. R., Itzkan, I, Dasari, R. R, and Feld, M. S., "Prospects for in vivo Raman spectroscopy," Phys. Med. Biol.; 45: R1–R59 (2000).

In the invention, the wavelengths for the Raman spectroscopy and/or fluorescence spectroscopy are wavelengths for which such spectroscopy equipment may be set, suitably for interrogating biological tissue in a living patient. Preferably resonance Raman spectroscopy according to the invention is performed at a deep ultraviolet wavelength, i.e., at 390 to 420 nm. Modifications of Raman spectroscopy that can be applied include Fourier Transform Raman Spectroscopy, Nonlinear Raman Spectroscopy, Raman difference spectroscopy, and Raman Optical Activity.

Examples of Raman spectra are FIGS. **3**, **4**, **5**, **6**, **7**, **8**, **9**, **10**, **11**, **12**, **13**, **22**(*a*)–(*e*), **23**, **24**, **25**. Examples of NADH fluorescence spectra are FIGS. **14**, **15**, **16**, **17**, **18**, **19**, **20**, **21**.

The inventive methods, products and profiles may include signal enhancement at a resonant frequency for a target molecule of the target molecule population. The inventive methods may include operating an electromagnetic radiation generator at a range of selectable wavelengths from about 270 nm to about 20,000 nm. Spectroscopy may be performed for multiple wavelengths. Preferably the Raman spectroscopy is resonance Raman spectroscopy at 390 to 420 nm wavelength. Because basic Raman scattering is a rather low intensity phenomenon requiring sensitive detectors, preferably Resonance Raman Spectroscopy (RRS) techniques are used, to enhance the signal when the mol-

ecule of interest is resonant (absorption maximum near the laser wavelength). The signal strength of Raman can be boosted by several orders of magnitude by providing areas of resonance. Also, use of resonant wavelengths will allow limiting laser power density to a minimum (well below the skin damage threshold of 4 watts/cm^2). Fluorescence can be avoided by choosing wavelengths not prone to this phenomenon, and through fluorescence quenching. Conversely, fluorescence may be advantageously used for quantification if a particular target is found to have identifiable Raman spectra in one light range such as the NIR but fluoresces at another light range such as the UV. Use of near UV wavelengths (violet, ~406 nm) will avoid the mutagenic potential of UV radiation, while insuring a strong Raman signal.

The use of Raman spectroscopy in the near-UV range within the clinical settings according to the invention has several advantages with respect to other optical techniques such as IR and NIR absorption spectroscopy. Use of resonance Raman spectroscopy in the near-UV range (406.7 nm) may overcome many problems associated with NIR absorbance spectroscopy and other markers of tissue perfusion. Raman spectroscopy in the NIR takes advantage of the remark transparency of tissue at these wavelengths, while at the same time providing high-resolution vibrational signals. Spiro, T. G., "Resonance Raman spectroscopy: A new structure probe for biological chromophores," Accts. Chem. Res., 7:339–344 (1974); Terner, J., El-Saye, M. A., "Time-resolved resonance Raman spectroscopy of photobiological and photochemical systems," Accts. Chem. Res., 18:331–338 (1985). Hemoglobin has strong absorption and resonance properties in the near-UV range. FIG. **3** depicts data from human blood samples in the laboratory demonstrating the sharp peaks of oxy and deoxy human hemoglobin samples. (The area under the curve (AUC) in FIG. **3** of the Raman spectra produce oxygen saturations comparable to that from a multiwavelength co-oximeter.) Comparison of area under the curves of the oxy-peak and blood gas saturations yielded a correlation coefficient of 0.997. These sharp peaks should be compared to the broad overlapping peaks of oxy and deoxy hemoglobin obtained by NIR absorption spectroscopy in FIG. **2**. Furthermore, the resonance Raman effect for hemoglobin is so specific that it can be differentiated from the resonance Raman effect of myoglobin (see FIG. **4**). FIG. **4** shows resonance Raman spectroscopy of both oxygen hemoglobin and myoglobin, demonstrating an ability to distinguish between the two.

Analyzing the spectroscopy data by computing the AUC has been mentioned. Alternately to computing area, peak height analysis may be performed. For example, tissue hemoglobin oxgyen saturation (StO_2) may be determined by either comparing area under the curve of the spectra for both oxyhemoglobin and deoxyhemoglobin and/or comparison of the peak heights between the two species. Each computation provides a percentage. The latter technique is likely to be preferable.

A characteristic of near-UV light is that it can only penetrate tissue to a depth of 1–2 mm and is noncarcinogenic. Although at first thought, depth of penetration might seem to be a disadvantage, actually there is not such a disadvantage. Pathlength becomes less important in this case in terms of quantification issue. Probe contact may be unnecessary, and fiber optics may be simplified. In terms of shock states, blood flow to the surface of any organ is compromised first. In terms of use on hollow organ systems such as the stomach, signals from near-UV resonance Raman spectroscopy would be only from the mucosal surface (an advantage over data from NIR absorption spectroscopy which would likely include signals from non-stomach organs and thus not reflect data from the mucosal surface of the stomach.)

While taking the Raman spectroscopy profile and the fluorescence spectroscopy measurement on the patient at the same time is a preferred embodiment, it will be appreciated that in other embodiments the invention does not require using both Raman and fluorescence spectroscopy.

The fluorescence spectroscopy (such as broadband fluorescence spectroscopy) of the present invention, is performed at between 390 and 800 nm. A most preferred example of fluorescence spectroscopy according to the invention is NADH surface-subsurface fluorescence spectroscopy.

As examples of the biological tissue according to the invention may be the brain, heart, lung, liver, blood, tongue or other oral mucosa, eye (such as the cornea or retina), the esophagus and stomach, peripheral skeletal muscle, skin, intestines, pancreas, kidney, bladder, urethra, skin, nailbed, cervix, uterus, oropharynx, nasopharynx, esophagus, blood etc. Probing on the tongue/oral mucosal, skin, or cornea/retina optionally may be totally noninvasive, without the requirement for probe contact with tissue. For probing the esophagus or stomach, simple fiber optics are constructed into a nasogastric tube for measurements at the level of the esophageal or stomach mucosa. Fiber optics also can be used in urinary catheters for monitoring of substances in the urine or for interrogation of the bladder mucosa. Skeletal muscle or dermis can be assessed with fiber optics of a size insertible through small needles, inserted into a muscle belly such as the deltoid or quadriceps.

The inventive methods may include monitoring a specific tissue bed (brain, heart, lung, liver, eye, blood, etc.) in the patient; placing a probe on or near any mucosal or epithelial covered surface of a body or an organ; detecting exhaled markers or mediators of organ injury (such as by placing a detector at the airway of the patient). Examples of exhaled markers or mediators are isoprostanes and/or myeloperoxidase.

Markers also may be present in a biological material according to the present wherein the markers are contained in urine, saliva, wound exudates, vitreous humor, aqueous humor, tissue exudate, gastric contents, fecal matter, or other biological materials.

The interrogating of biological material such as tissue according to the invention may be, but is not required to be, noninvasive. To maximize the number of markers and mediators that can be measured, a minimally invasive approach is preferred. Interrogating may be intermittently or continuously. A preferred example of minimally invasive probing is by minimally invasively probing the patient by a fiber optic probe or probe array inserted into a tissue bed. The tissue may be in vivo and in situ, but is not required to be. Alternately, the tissue may be removed from a patient before the tissue interrogation. As examples of interrogating tissue are mentioned inserting a probe or probe array into a muscle. Preferably interrogation is by a minimally invasive probe approach, with muscle and interstitium being interrogated directly. Such a minimally invasive approach is preferred for several reasons. UV light does not significantly penetrate epidermis. NIR light can penetrate several centimeters of tissue and can thus probe epidermis, dermis, and muscle. The inability to know the path length of light and to separate the signal of myoglobin from hemoglobin make interpretation of data for the noninvasive use of NIR absorption spectroscopy at any site other than the brain to be

conventionally difficult, and a drawback to be avoided. The large number of valuable markers, which can be detected in the UV and NIR range by Raman spectroscopy, more than outweigh any drawback to placing a small probe intramuscularly. Bench experiments have allowed measures to be made of such substances as hemoglobin within cells in the UV range. (See J. Terner, T. G. Spiro, M. Nagumo, M. F. Nicol, and M. A. El-Sayed, "Resonance Raman spectroscopy in the picosecond timescale: the CO hemoglobin photo-intermediate," J. Amer. Chem. Soc., 102: 3238–3239 (1980); J. Terner, J. D. Stong, T. G. Spiro, M. Nagumo, M. F. Nicol, and M. A. El-Sayed (1980), "Picosecond resonance Raman spectroscopic evidence for excited state spin conversion in carbonmonoxy-hemoglobin photolysis," Proc. Natl. Acad. Sci. USA, 78: 1313–1317; J. Terner, T. G. Spiro, D. F. Voss, C. Paddock and R. B. Miles, "Picosecond resonance Raman spectroscopy of oxyhemoglobin photolysis," J. Phys. Chem., 86: 859–861 (1982)). Such results indicate that cell penetration of the near-UV wavelength in the interstitium will not pose a major problem.

In the inventive methods and products, the obtained spectroscopy results preferably may be for at least one mediator or marker associated with a shock state and/or tissue injury; tissue ischemia, tissue inflammation and/or tissue immune dysfunction; for presence and/or proportions for the at least one shock state and/or tissue injury mediator or marker; for at least one mediator associated with a shock state and/or tissue injury or tissue ischemia, inflammation or immune dysfunction and/or for at least one marker of tissue perfusion or injury.

A marker and/or mediator according to the present invention may be within intracellular, interstitial or intravascular space or within exhaled air from a patient. The marker and/or mediator may be selected from the group consisting of lactate, pyruvate, ATP, PCr, AMP, ADP, Pi, NAD, NADH, albumin, endotoxin, exotoxin, microbes, cytokines-chemokines, procalcitonin, hormones, myeloperoxidase, elastase, xanthine oxidase, xanthine dehydrogenase, fatty acid binding proteins, catecholamines and vasoactive peptides. The marker or mediator may be a metabolic or pro or anti-inflammatory marker or mediator. Cardiac biomarkers, GI markers, cerebral markers, skin markers, lung markers, blood markers, and/or eye markers, etc. are mentioned as examples.

Examples of spectroscopy results according to the invention may be, e.g., data relating to diagnosing and/or following progression or resolution of shock states and/or tissue injury (such as inflammatory or immune dysfunction), and/or tissue ischemia; determining whether the tissue has insufficient oxygen delivery to meet metabolic demands of the tissue while simultaneously determining whether mitochondrial dysfunction or injury exists; monitoring for appearance of one or more tissue markers specific for a specific disease state; determining tissue viability; diagnosing tissue injury, tissue inflammation or tissue immune dysfunction; and/or continuously interrogating the patient for appearance of abnormal tissue markers specific for a suspected disease state. A preferred example of spectroscopy results are results relating to diagnosing shock.

As examples of spectroscopy results according to the present invention may be given data for tissue hemoglobin oxygen saturation including amount of oxyhemoglobin and deoxyhemoglobin by Raman spectroscopy; data for NADH presence and/or accumulation by fluorescence spectroscopy; data for oxygenated hemoglobin, deoxygenated hemoglobin and/or NADH; data for myoglobin oxygenation saturation; data for cytochrome oxidase redox status; data for pH of the tissue. A most preferred example of spectroscopy results is data for tissue hemoglobin oxygen saturation by Raman spectroscopy combined with data for NADH presence and/or accumulation by fluorescence spectroscopy.

The spectroscopy results according to the invention may be for absolute concentration (such as absolute concentration of hemoglobin in the tissue) or for relative concentration. Examples of relative concentrations are NAD/NADH; lactate/pyruvate; Pcr-ATP; ATP-ADP; Pcr-Pi; oxidized cytochrome oxidase to reduced cytochrome oxidase, and/or oxyhemoglobin with deoxyhemoglobin.

The spectroscopy results according to the invention advantageously are available on the order of seconds. Signal processing and computer algorithms may be used to process the spectroscopy data.

Another preferred embodiment of the invention provides a medical measurement device comprising: a spectrometer with multiple wavelength settings for resonance Raman spectroscopy; and a biological probe electrically connected to the spectrometer. Inventive medical measurement devices optionally may include a fluorescence spectrometer electrically connected to the biological probe; a laser source (such as a laser tunable to multiple wavelengths) and a charge coupled device. By using a laser tunable to multiple wavelengths, multiple target molecules may be detected. Such multiple target molecules may have useful detectable absorption, resonance Raman and fluorescence spectra at differing wavelengths. Exemplary devices according to the invention may be seen with regard to FIG. **26**(*a*)–(*e*).

With reference to FIG. **26**(*a*), a fixed frequency laser (preferably such as a laser LS including, but not limited to, wavelengths of approximately 290 to 420 nm) is piped **1** through one leg of a fiber optic bundle. An example of fiber optic bundle **100** is seen in further detail in FIG. **26**(*e*), shown in a configuration of one emitting fiber optic **101** (in the center) surrounded by eight collecting bundles **102**. A fiber optic bundle **100** is only one example, and the fiber optic bundle may be otherwise configured, such as containing one emitter and one or several sensor fibers, in a ratio of one emitter to one sensor up to one emitter to twelve sensors. The number of emitters could be increased and the spaces between emitters and detectors changed. The emitters and detectors might also be placed along the length of the probe as opposed to its end. The fiber optic bundle **100** may be positioned on or within a tissue sample. Re-emission from the tissue sample is collected in back-scattering configuration by the same fiber optic bundle. The end of the fiber optic bundle preferably is placeable directly onto the surface of a tissue such as the oral mucosal or heart. Alternatively, the fiber optic bundle is placeable directly into a tissue such as the brain or liver. The fiber optic arrangement does not require contact with the tissue especially when extraneous light (ambient light) is prevented from entering the fiber optic sensor.

A preferred example of an invasive fiber optic probe is one that is less than 0.2 mm, and which can be rapidly placed singularly or in an array in a muscle bed through a small gauge hypodermic needle. When such a needle is used to place a probe, after insertion the needle may then be removed and the probe secured in place such as by medical tape.

Again referring to FIG. **26**(*a*), the light collected by the fiber optic is notch filtered by a laser notch filter system **200** (comprising at least one and preferably two laser notch filters) and then distributed to a spectrograph system **300** (preferably such as a spectrograph system comprising a Raman spectrograph system and a fluorescence spec-

trograph system). The spectrograph system **300** has a respective CCD detector system **400** associated with it. The CCD detector system preferably comprises a CCD detector for each spectrograph system, i.e., when using a Raman spectrograph system with a fluorescence system, the Raman spectrograph system has an associated CCD detector and the fluorescence system has an associated CCD detector. The CCD detector system **400** provides signals to a signal analyzer **500** (such as a PC type computer). It will be appreciated that respective systems **200**, **300** and **400** each respectively may include one, two, three or more components, with some examples of such systems being given in FIGS. **26**(*b*), **26**(*c*) and **26**(*d*). Preferably, laser notch filter system **200** comprises at least one laser notch filter, spectograph **300** comprises at least one spectrometer and CCD detector system **400** comprises at least one CCD detector.

In a particularly preferred embodiment of the invention, an exemplary device is provided incorporating both Raman spectroscopy and fluorescence spectroscopy. The exemplary device according to FIG. **26**(*b*) is an example of such an inventive device combining Raman and fluorescence spectroscopy. With reference to FIG. **26**(*b*), the light collected by the fiber optic is notch filtered **2**, **2'***b* and distributed to spectrometers such a spectrograph system set for Raman **3** and a spectrograph set for broadband fluorescence **30**. An example of the Raman spectroscopy system **3** may include two spectrometers containing high groove density gratings, one set to collect Raman scattering between 300 and 3700 cm^{-1} from the laser line (collecting the Raman signal of water to use as an intensity standard) and one set to collect Raman scattering between 1200 and 1700 cm^{-1} (heme vibrations). An example of the fluorescence spectroscopy system **30** contains a low groove density grating and is set to collect broadband fluorescence emission within the region 200 to 800 nm. The Raman spectrograph system **3** has a respective CCD detector system **4** associated with it, and the fluorescence spectroscopy system **30** has a respective CCD detector system **40** associated with it. The CCD detector systems **4**, **40** provide signals to an analysis system, such as a PC type computer **5**.

With an equipment set-up according to FIG. **26**(*b*), levels of oxyhemoglobin, deoxyhemoglobin (and thus tissue hemoglobin saturation), and NADH accumulation, may be determined. Information necessary to determine blood pH within the tissue as well as the absolute concentration of hemoglobin within the tissue may be obtained. As for computing concentration, an example may be appreciated with reference to FIG. **27**, demonstrating that the resonant Raman spectroscopy technique can detect differences in the amount of hemoglobin present. Hemoglobin levels (in absolute terms) may be determined in tissue, by examining the intensity of the signals (y-axis in FIG. **27**). The more hemoglobin present in the tissue, the higher the resulting signal intensity. These intensities may be compared to known standards for the determination of hemoglobin amount.

Another example of an exemplary inventive device is one comprising an electromagnetic radiation generator (such as a laser) with a wide range of selectable wavelengths (such as deep ultraviolet, less than 270 nm to shortwave infrared all the way to 20,000 nm), filters, lenses, fiber optics, a charge coupled device (CCD), a spectrograph, and the software necessary to interpret the Raman shifts. The device can obtain resonance Raman spectra at a variety of wavelengths corresponding to the "fingerprint" or "signature" of molecules associated with tissue oxygen metabolism (such as hemoglobin (Hb), myoglobin (Mb), cytochrome oxidase (cyt a, a3), dissolved or free gases (i.e., O_2, CO_2, CO, NO, etc. in tissues, exhaled respiratory gas or intraluminal gastrointestinal gas), glucose, lactate, pyruvate, and bicarbonate. Various mediators associated with shock such as tumor necrosis factor (TNF) and other pro and anti-inflammatory cytokines, catecholamines (epinephrine, norepinephrine and dopamine), general and destructive proteins such as albumin and myeloperoxidase respectively, high energy phosphates (ATP, PCr, ADP, Pi), metabolic energy intermediates (NAD, NADH), excitatory amino acids (glutamate, aspartate), and vasocative peptides (vasopressin, angiotensin II, natriuretic peptides, etc.) can also be measured with such a technique.

Devices according to the invention may be used in a multiparametric system for non-invasive or minimally invasive monitoring of tissue perfusion and metabolism in critically ill or injured patients. Because the technique permits identification of an almost unlimited number of target compounds, ultra-early detection may be provided, as well as complete characterization and differentiation of various pathologic states. The invention provides also for determining when treatment is complete. The inventive methods and devices may be applied with regard to various shock states, and ischemia of various organ or organ systems such as the heart, brain, and gastrointestinal tract. Probes for the device may be placed within any tissue bed to monitor the state of a specific tissue. The probes and techniques also may be used to reflect the state of the organism as a whole. Probes may be constructed for intravascular placement as well as placement into other devices such as urinary catheters, gastrointestinal tubes and endoscopes, heart catheterization equipment, brain and other tissue monitoring devices.

Devices may used in the operating room to examine target molecules and the status of various organs such as the liver, GI tract, brain or heart or other tissues of interest. Implantable probes may be placed in transplanted tissues to allow for their interrogation at subsequent time points to monitor for rejection.

In another preferred embodiment, the invention provides a method of diagnosing shock, tissue ischemia, tissue inflammation, or tissue immune dysfunction, comprising: (A) for a target molecule population, taking a sample Raman spectroscopy, and/or fluorescence spectroscopy, profile for a patient; (B) comparing the sample spectroscopy profile with a pre-established Raman spectroscopy and/or fluorescence spectroscopy profile for the target molecule population under baseline conditions. A further preferred embodiment provides a spectroscopy comparative profile, comprising: a pre-established Raman spectroscopy and fluorescence spectroscopy profile for a target molecule population under baseline conditions; and a sample Raman spectroscopy and fluorescence spectroscopy profile for the target molecule population.

The profiles according to the invention may be of relative amounts, or of absolute amounts. The sample profile may be taken from a tissue or a space in a body, or taken from a tissue or a space out of the body. The respective profiles are not required to be from the same species. The comparative profiles in a preferred example include a pre-established fluorescence spectroscopy profile for NADH under baseline conditions and a sample fluorescence profile for NADH. In another preferred example, a spectroscopy comparative profile includes a pair of Raman spectroscopy profiles and a pair of fluorescence spectroscopy profiles (such as one Raman spectroscopy profile and one fluorescence spectroscopy profile taken from a patient after a medical event concerning the patient).

Preferred examples of target molecule populations are NAD/NADH; lactate/pyruvate; PCr-ATP; ATP-ADP; PCr-Pi; oxidized cytochrome oxidase to reduced cytochrome oxidase, and/or oxyhemoglobin with deoxyhemoglobin. However, it will be appreciated that further potential target molecule populations may be screened and selected as target molecule populations according to the invention.

Desired features of a marker(s) of tissue perfusion are its early change after injury; and, that its normalization would indicate that resuscitation is complete. This would help to ensure that shock is detected at its earliest possible time point and that resuscitation would not be prematurely stopped. In addition, the marker(s) would not be subject to misinterpretation from factors such as changes in minute ventilation, pain, etc.

Experimentation: Markers

Using lab bench versions with diode array detection, Raman spectroscopy was used in the UV and NIR in both reflectance and transmission mode to identify several compounds having utility as markers of hemorrhage severity and its sequelae. The first group are oxygen sensitive markers of ischemia and include hemoglobin (Hb), myoglobin (Mb) and cytochrome oxidase (Cyt aa_3). The second group is of exquisitely sensitive oxygen-related metabolic markers of shock including lactate, pyruvate, nicoteinamide adenine dinucleotide phosphate (NAD) and NAD reduced form (NADH). A third group includes the high-energy phosphates phosphocreatine (PCr), adenosine-5'-triphosphate (ATP) and adenosine-5'-diphosphate (ADP). The fourth group includes the vasoactive chemicals epinephrine and norepinephrine.

Raman spectra were obtained of post-hemorrhage markers in inflammation such as lipopolysaccharide (LPS) and cytokines such as tumor necrosis factor-alpha (TNF-α). Sample spectra of lactate, pyruvate NADH, NAD, PCr, and ATP are shown in FIGS. **27**(*a*), **27**(*b*) and **27**(*c*). Lactate and pyruvate can be easily discriminated by comparing the intensities measured at 1625 cm^{-1}. NAD/NADH can be discriminated by examining the peak around 1690 1625 cm^{-1}. PCr can be separated from other high-energy phosphates with the intensity at 1475 cm^{-1} while ATP and ADP can be separated with the peaks at 1100 and 1400 cm^{-1}.

Of particular interest in detecting the presence and severity of hemorrhagic shock are lactate/puruvate, NADH/NAD, PCr/ATP ratios and the redox status of cytochrome oxidase in skeletal muscle. In addition, hemoglobin concentration, oxygen saturation, and potentially myoglobin oxygen saturation may be obtained. The lactate/pyruvate ratio provides information on the coupling of glycolysis to oxidative phosphorylation, the NADH/NAD ratio provides information concerning the mitochondrial energy state, and the PCr/ATP ratio provides information concerning utilization of high-energy phosphate stores. Haljamae, H., "Cellular metabolic consequences of altered perfusion," in Gutierres, G., Vincent, J., eds., "Update in Intensive Care and Emergency Medicine: Tissue oxygen utilization (Springer Verlag, 1991), pp. 71–86. These indices are considered significantly more sensitive than the redox status of cytochrome oxidase or the local level of hemoglobin concentration, oxygen saturation or pH. Even so, monitoring of current NIR absorption spectroscopy derived parameters such as the redox status of cytochrome oxidase and hemoglobin concentration and saturation may be obtained with Raman spectroscopy and can be performed with greater confidence for potential quantification. One of the major advantages of the use of Raman spectroscopy over NIR absorption spectroscopy is its potential to differentiate the signal of hemoglobin from myoglobin.

Measuring lactate alone is known to be problematic because of the contribution of increased aerobic glycolysis on lactate production secondary to elevations in systemic catecholamine levels. This may occur in the absence of continuing tissue hypoxia. Luchette, F., Roboinson, B., Friend, L., McCarter, F., Frame, S. B., James, J. H., "Adrenergic antagonist reduce lactic acidosis in response to hemorrhagic shock," J. Trauma, 46:873–880 (1999). However, knowing the lactate/pyruvate ratio along with NADH/NAD and PCr/ATP ratios will provide the operator clear insight into whether true tissue hypoxia is occurring and its severity. In addition, because there is a definite lag in metabolism of lactate, restoration of adequate perfusion will likely result in return of the above ratios before normalization of lactate, thus informing the operator that instituted therapies are working or failing. Additional sensitivity could be added by external stimulation of a few muscle fibers to examine the rate of degradation and restoration of the above metabolic intermediates. Failure to normalize these values in a timely manner indicates a state of intractable shock.

The use of Raman in the near-UV and NIR has additional advantages of allowing caretakers to detect the progression of hermorrhagic shock to more complex forms of shock such as sepsis. Spectra have been obtained for inflammatory markers such as myeloperoxidase and cytokines such as TNF-α. In addition, spectra have been obtained on lipopolysaccharide, and d-lactate, which are markers indicative of intestinal barrier breakdown. Spectra on the catecholamines epinephrine and norepinephrine have been obtained. These vasocactive substances are now being recognized as sensitive markers of the level of hypoperfusion and stress caused in various shock states. These observations may be extended to vasocative peptides such as vasopressin and angiotensin, which have been measured in rat pheochromocytoma cells by Schulze et al. Schulze, H. G., Greek, L. S., Barbosa, C. J., Blades, M. W., Gorzalka, B. B., Turner, R. F. B., "Measurement of some small-molecule and peptide neurotransmitters in-vitro using a fiber-optic probe with pulsed ultraviolet resonance ultraviolet resonance Raman spectroscopy," J. Neurosci. Meth., 92:15–24 (1999).

Thus, markers mentioned herein have remarkable utility when examined in a manner of ratios. Also, absolute quantification can be obtained using embedded standards in probes placed in parallel with other emitting and sensing probes, from which can be determined an exact path length of light. The markers mentioned in this experiment were found to be detected by UV and NIR Raman spectroscopy in both the reflectance and transmission mode gives flexibility of design of methods and products according to the invention.

In Vivo Spectroscopic Experimentation

Techniques according to the invention have been successfully applied to several tissue sites in animals, demonstrating feasibility. Techniques according to the invention require no probe contact (although probe contact with tissue can take place if desired) with tissue and acquisition times are on the order of seconds.

FIG. **5** represents near UV resonance Raman signals taken from skeletal muscle subjected to isolated tourniquet ischemia. Signals were obtained in one minute (i.e., scans were acquired in one minute segments). The oxyhemoglobin signal (1375) decreases simultaneous to the increase in the deoxy hemoglobin signal (1357).

FIGS. **6**–**11** represent near UV-resonance Raman spectroscopy data of oxy and deoxy hemoglobin (with only gross signal processing) from the exposed quadriceps muscle from a rat during hemorrhage. Using area under the curve and

peak height comparison analysis, tissue saturations are demonstrated to decrease during hemorrhage. Of importance is that significant tissue desaturation occurs despite the maintenance of normal vital signs. These values for oxyhemoglobin are very similar to those reported with NIR absorption spectroscopy.

FIG. **7** is for 1 ml bleeding of the same muscle as FIG. **6**, which is the baseline spectrum. At 1 ml bleeding, no change in the oxy or deoxy peak was observed, and there was no change in vital signs. FIG. **8** is for 2 ml bleeding of the same muscle as FIGS. **6** and **7**. At 2 ml bleeding, an evolving deoxy peak is to be noted, while the animal maintained normal vital signs. At 5 ml bleeding (FIG. **9**) for the same muscle as FIGS. **6–8**, a progressively greater deoxy signal is observed, and, although vital signs (MAP decreasing) are still within the normal range. Where 7.5 ml of bleeding (FIG. **10**) has occurred for the same muscle as FIGS. **6–9**, the animal demonstrated physical evidence of decompensate shock; however, Raman spectroscopy indicates greater severity than the vital signs might predict. Results from FIGS. **6–10** are summarized in Table 1 below.

TABLE 1

	OxyHb	DeoxyHb
Baseline (FIG. 6)	69%	31%
1 ml bleed (FIG. 7)	68%	32%
2 ml bleed (FIG. 8)	53%	47%
5 ml bleed (FIG. 9)	30%	70%
7.5 ml bleed (FIG. 10)	14%	86%

A similar experiment to that of FIGS. **6–11** was performed using the tongue as the target organ, as seen with reference to FIG. **12**, which shows resonance Raman spectra demonstrating saturation changes during 3 cc hemorrhage. The signal was obtained in 5 seconds. Again, changes in tissue saturation occurred prior to changes in vital signs, demonstrating that the use of Raman spectroscopy according to the invention can be totally noninvasive.

FIG. **13** demonstrates that near-UV resonance Raman spectroscopy of hemoglobin may be used to monitor tissue pH in a manner similar to that of NIRS. The spectra of FIG. **13** are from pure oxyhemoglobin samples at different pH levels. Subtraction of the two scans provides clear evidence for a difference indicating that pH alone was responsible for the effect. FIG. **13** is for Horse Hb. Near UV resonance Raman spectra of hemoglobin are shown at pH of 8 (scan 1) and 6 (scan 2) with a subsequent subtraction scan (scan 3) demonstrating the likelihood of pH sensitive changes in the spectra.

Another important finding according to the present invention is that at this same near-UV wavelength, NADH demonstrates significant fluorescence. The present inventors have observed significant fluorescence from tissue excited in the near-UV range (406.7 nm) using a portable spectrometer. Exciting tissue in the near-UV range (406.7 nm) according to the invention provides better resolution than traditional filtering in which unique excitation light sources and detection filters are used for the conventional set-up relying on NADH fluorescing (emiting light at 460 nm) when excited at a wavelength of 360 nm (near-UV).

Based on the importance of NADH in cellular oxygen utilization (as set forth above), from this aspect of the invention may be determined the point of tissue dysoxia or critical DO_2 (ischemia) prior to being able to note increases in systemic lactate. Although NADH also exists in the cytoplasm, it does so in insignificant amounts compared to those produced within the mitochondria during states of dysoxia. In conjunction with the tissue saturation experiments above, NADH fluorescence from quadriceps (FIGS. **14–19**), tongue (FIG. **20**) and additionally liver (FIG. **21**) was obtained during graded hemorrhage.

FIG. **14** is a baseline, and FIG. **15** reports spectra after 5 mls bleeding for the same quadriceps muscle. Significant NADH fluorescence is observed after 5 ml hemorrhage. Although the animal has relatively normal vital signs, fluorescence indicated that critical dysoxia has occurred. (FIG. **15**.) At 7.5 mls of bleeding (FIG. **16**) for the same muscle, increasing fluorescence indicates additional ischemia. At 9 mls of bleeding (FIG. **17**), even more fluorescence is observed, indicating the ability to grade severity in real time. At 12 mls of bleeding (FIG. **18**), the animal is almost terminal.

In the experiment in which blood pressure was monitored, significant fluorescence occurred prior to and after changes in vital signs. With equipment according to the present invention, tissue oxygen saturation and NADH fluorescence may be simultaneously obtained. Again, the depth of tissue interrogated is the same as that for tissue oxygen saturation. Thus may be determined the point at which critical oxygen delivery (dysoxia) occurs. Significant warning prior to this time will occur as reflected in reductions in tissue oxygen saturation.

Continued NADH fluorescence after restoration of oxygen delivery indicates ongoing dysoxia despite the potential for normalization of tissue oxygen saturation. The normal heart shows little or no NADH surface fluorescence. The beginning of ischemia and maximum ischemia may e observed. Continued patch fluorescence may be observed after reperfusion. Thus, NADH fluorescence data has value in monitoring tissue even after perfusion has been restored.

Because of the kinetics of lactate production and transport, it is very likely dysoxia detected by NADH accumulation and fluorescence will occur significantly earlier than with detection of regional or systemic lactate or even CO_2. In short, the combined use of near-UV resonance Raman spectroscopy and near-UV NADH fluorescence serve as an exquisitely sensitive early warning system for pending defects in tissue perfusion and in ensuring the completeness of resuscitation.

Other heme proteins, which have importance in ischemia-reperfusion diseases can be detected using resonance Raman spectroscopy at the same near-UV wavelength (406.7 nm). These include myeloperoxidase, which is an injurious enzyme produced and released by neutrophils, and xanthine oxidase, which is converted from xanthine dehydrogenase after reperfusion of ischemia and is responsible for the production of free radicals. Hayward, R., Lefer, A. M., "Time course of endothelial-neutrophil interaction in splanchnic artery ischemia-reperfusion," Am J Physiol 275 (6 Pt 2): H2080–6 (1998); Tan, S., Yokoyama, Y., Dickens, E., Cash, T. G., Freeman, B. A., Parks, D. A., "Xanthine oxidase activity in the circulation of rats following hemorrhagic shock," Free Radic Biol Med, 15(4):407–14 (1993).

A resonance Raman spectroscopy spectrum taken for a human is shown for myeloperoxidase (FIG. **23**). The ability to detect myeloperoxidase would be helpful in evaluation of wounds and systemic reperfusion injury and sepsis.

Another group of potentially useful markers of preclinical shock secondary to hypovolemia are endogenously produced catecholamines such as epinephrine and norepinephrine and the vasoactive peptides such as angiotensin, vasopressin, endothelin, and adrenomedullin, all of which are known to be significantly elevated in the setting of hypov-

olemia and other shock states. Jakschik, B. A., Marshall, G. R., Kourik, J. L. Needleman, P., "profile of circulating vasoactive substances in hemorrhagic shock and their pharmacologic manipulation," J Clin Invest, 54(4):842–52 (1974); Lanza, V., Palazzadriano, M., Scardulla, C., Mercadante, S., Valdes, L., Bellanca, G., "Hemodynamics, prolactin and categchloamine levels during hemorrhagic shock in dogs pretreated with ap prolactin inhibitor (bromocriptine)," Pharmacol Res Commun., 19(4):307–18 (1987); Yilmazlar, A., Yilmazlar, T., Ozcan, B., Kutlay, O., "Vasopressin, renin, and adrenocorticotropic hormone levels during the resuscitation of hemorrhagic shock in dogs," J Emerg Med, 18(4):405–8 (2000); Kitajima, T., Tani, K., Yamaguchi, T., Kubota, Y., Okuhira, M., Mizuno, T., et al., "Role of endogenous endothelin in gastric mucosal injury induced by hemorrhagic shock in rats," Digestion, 56(2): 111–6 (1995); Fujioka, S., Ono, Y, Kangawa, K., Okada, K., "Plasma concentration of adrenomedullin is increased in hemorrhagic shock in dogs," Anesth Analg, 88(2):326–8 (1999); Lindner, K. H. Strohmenger, H. U., Ensinger, H., Hetzel, W. D., Ahnefeld, F. W., Georgieff, M., "Stress hormone response during and after cardiopulmonary resuscitation," Anesthesiology, 77(4):662–8 (1992); Lindner, K. H., Haak, T., Keller, A., Bothner, U., Lurie, K. G., "Release of endogenous vasopressors during and after cardiopulmonary resuscitation," Heart, 75(2):145–50 (1996).

Raman spectra of vasopressin in the UV spectrum at 350 nm is shown at FIG. **24**, and for norepinephrine in the same UV spectrum at FIG. **25**. Ability to detect, quantitate, and trend these markers can be used with regard to evaluating and treating numerous disease states such as shock, congestive heart failure, pain states, burns, etc. These and other similar mediators can be detected and quantitated using resonance Raman spectroscopy. Schulze, H. G., Greek, L. S., Barbosa, C. J., Blades, M. W., Gorzalka, B. B., Turner, R. F., "Measurement of some small-molecule and peptide neurotransmitters in-vitro using a fiber-optic probe with pulsed ultraviolet resonance Raman spectroscopy," J. Neurosci Methods, 92(1–2):15–24 (1999).

The ability to detect dysoxia and ensure its resolution at the earliest possible time has great value for the triage of ill or injured patients. Therapy and resources can be better allocated and victim's progress better monitored, reducing the incidence of under-resuscitation as well as provision of needless resuscitation. Based on the biphasic relationship of oxygen delivery and consumption of a tissue, the present invention measures hemoglobin saturation in conjunction with NADH as a reflection of the oxygen dependent bioenergetic state of the cell.

Based on experimental results herein, it is seen that near UV excitation can be exploited to simultaneously perform Raman resonance spectroscopy of oxy and deoxyhemoglobin, and surface-subsurface fluorescence of NADH. Also, UV and NIR RRS have been obtained from a number of compounds (including high-energy phosphates PCr, ATP and ADP, NAD, NADH, the glycolytic metabolites pyruvate and lactate, and the excitatory amino acid glutamate) in solid and aqueous states.

From the experimental data set forth above, it may be seen that with the use of combined UV, near UV and NIR Raman spectroscopy, identification and monitoring of a large number of useful target compounds may be accomplished, for detecting and monitoring the presence and severity of hemorrhagic shock and development or resolution of its sequelae such as sepsis. The present inventors have successfully shown that the various compounds discussed above are amenable to detection by UV, near UV and NIR Raman.

It has recently been demonstrated that NIR absorption spectroscopy can be used to determine tissue pH by examining shifts in the broad bands of hemoglobin. This is based on the known fact that histadine residues of hemoglobin are pH sensitive. NIR absorption spectroscopy is being examined to determine hematorcrit in a similar manner. Because pH sensitive shift is observed in all of the oxy and deoxy bands of the resonance Raman spectra and hemoglobin concentration differences in the heights of the bands, it may be concluded (by such techniques as partial least squares) that tissue pH and hematorcit/hemoglobin levels may be determined using resonance Raman spectroscopy.

Of recent importance has been the development of the concept of cytopathic hypoxia as an explanation for the oxygen transport abnormalities, which exist during sepsis. This theory suggests that such inflammatory compounds as tumor necrosis factor and endotoxin damage mitochondria. This damage prevents the mitochondria from utilizing oxygen. Data for studies using NIR absorption spectroscopy appear to support this theory. The combination of near-UV resonance Raman spectroscopy of hemoglobin tissue saturation and NADH fluorescence would help detect this if it existed. In such a setting, tissue saturation would be normal or elevated but NADH fluorescence would be significant. This entity of cytopathic hypoxia may be one factor which confounds the use of other monitoring modalities such as splanchnic tonometry and monitoring of mixed venous oxygen saturation.

Methods and devices according to the invention may be used in various manners, such as to exploit shifts in the spectra of molecules such as hemoglobin and myoglobin to calculate blood and tissue pH as well as detect and determine the actual hemoglobin and myoglobin oxygen saturation. Spectroscopy equipment may be coupled with probe(s) and sensor(s) to construct a device to interrogate the perfusion and metabolic status of individual tissues as well as the organism as a whole, advantageously in a noninvasive or minimally invasive manner. For instance, the invention provides a minimally invasive fiber optic probe or arrays of probes (each probe less than 0.2 mm) which are insertible into a muscle or other tissue bed with a small gauge needle. Resonance Raman spectroscopy in the deep ultraviolet wavelength (less than 270 nm) is used for interstitial fluid analysis (micron level penetration), while longer UV or near UV wavelengths are used for cellular analysis, due to slightly longer wavelengths that could penetrate to levels near 1 mm. Both the deep and near UV wavelengths also may be used with a probe placed on the oral cheek mucosal epithelium. NIR Raman spectroscopy may be used with non-invasive optical fibers placed on the skin or within tissue beds. Surface Raman spectroscopy is used to interrogate standards of detected substances for quantification.

When spectroscopy according to the present invention operates at multiple wavelengths, additional valuable metabolic and humoral targets may be selected for identification and tracking. The one-dimensionality problem of conventional emergency medical measurement technology is avoided. As has been mentioned above, for conventional IR or NIR technology, problems arise because water strongly absorbs IR radiation, and thus presents strong interference to the use of IR absorption spectroscopy in the clinical setting. The present invention is not burdened with such problems because water is a rather weak Raman scatterer. Raman spectroscopy can be used to provide the same vibrational information as the more common NIR absorption spectroscopy with no significant interference from water. In addition, the use of Raman allows one to take advantage of the

resonance Raman enhancement effect, plus polarization effects, neither of which have parallels in IR absorption spectroscopy. Thus, a principal advantage of the invention is that Raman spectroscopy does not suffer the same problems with water as normal IR spectroscopy, and, additionally, Raman spectroscopy is not limited to a single wavelength but can use a variety of wavelengths to interrogate molecules of interest at different tissue depths (skin, muscle, etc). Normal and transmission spectroscopy may be used to complement Raman spectroscopy for calibration, determination of tissue depth, and other enhancement of obtained information. Issues such as weakness of signal, potential tissue damage, and interference from fluorescence can be managed.

The ability to target multiple compounds in real-time provided by the present invention is a substantial advantage for detection and treatment of certain disease states and shock states. For example, the ability to monitor levels of catecholamines and vasoactive peptides may prove to be more sensitive of early shock states or states of intractable shock. Elevations of these compounds may indicate the severity of such states as acute sickle cell pain crises. Thus, resuscitation and treatment of such states using markers such as catecholaimnes or vasoactive peptides as endpoints may provide a relatively objective means to determine treatment efficacy.

Another example of methods according to the invention are uses in conjunction with maneuvers such as simple muscle contraction or use of a tourniquet. Monitoring rates of high-energy phosopahte degradation and regeneration or intermediary compound ratios such as lactate-pyruvate or NAD-NADH in disease states may provide relatively sensitive information concerning the state of the microvasculature.

Outpatient applications also are provided. Depending on the sensitivity of the device, the technology of the invention may be used in the outpatient setting for determination of various target compounds such as hemoglobin. The device may be used to diagnose certain precancerous or cancerous lesions (such as skin melanoma, etc.) in vivo. Point of care or continuous real-time tissue monitoring is provided with the inventive method and its components.

Because probes may be relatively small, patients may be continuously interrogated for the appearance of abnormal tissue markers specific for a suspected disease state. Examples of such markers are cardiac biomarkers such as troponin or myocardial fatty acid binding protein, GI markers of ischemia scuh as D-lactate and intestinal fatty acid-binding protein, and cerebral markers such as neuronal enolase.

Combining resonance Raman spectroscopy of tissue hemoglobin saturation and NADH fluorescence of the same region provides for detecting ultra-early perfusion deficits and determining adequacy of resuscitation. The present invention provides at least the following advantages: 1) little or no tissue contact (point and click technology); 2) rapid acquisition (such as acquisition times on the order of 1 second); 3) data (tissue saturation and point of critical oxygen delivery) that is not confounded by hypo or hypercarbia; 4) differentiation of sepsis (cytopathic hypoxia) from flow dependent dysoxia; 5) true data from mucosa (oral or other points in the GI or GU tract); 6) pH and hemaglobin (on a par with NIR absorption technology); and 7) other important markers of tissue injury such as myeloperoxidase, xanthine oxidase, vasocative substances, etc.

The present invention may be applied to known markers and also to markers as newly reported, because of the nature of resonance Raman spectroscopy. As a new marker (such as procalcitonin, etc.) is reported, the present invention provides for its study by resonance Raman spectroscopy.

The inventive methods and devices may be used for evaluation of any general shock state (trauma, cardiogenic, septic). Applications include hypoxic-hypoxia, hemorrhagic shock, cardiogenic shock, septic shock, and isolated organ ischemia (including wounds).

The inventive methods and devices may be used to evaluate the oxygen status of any organ during surgery (e.g., the heart during cardiopulmonary bypass surgery, the brain during neurosurgery, and various organs during transplant); to evaluate donor organs prior to transplant; to include in devices such as pacemakers to interrogate areas of myocardium at risk of injury; to evaluate a patient with congestive heart failure (such as at the hospital, office, home, etc.) to determine symptom etiology (such as fluid overload versus deterioration in heart function); to determine if a patient requires blood transfusion; to care for wounds.

The invention fills the current void of no universally accepted way of determining when a patient requires blood transfusion. Because each patient may have a different requirement based on past medical history and the current event, the invention is highly advantageous in allowing repetitive noninvasive measures of a sensitive tissue.

The invention benefits wound care (chronic and acute), by providing information about the oxygenation status of wounds. Care of chronic wounds is improved by the present invention providing the ability to determine oxygenation status of wounds. The use of near-UV resonance Raman spectroscopy determines tissue oxygen saturation at multiple points within the wound (within seconds) with tissue contact being unnecessary. In conjunction with the Raman spectroscopy, NADH fluorescence may be used to determine if the wound is becoming necrotic. The wound may be sampled for injurious substances interfering with wound-healing, such as myeloperoxidase.

The methods and products of the present invention have civilian and military uses. Using UV, near UV or NIR Raman spectroscopy according to the present invention provides for real-time monitoring of a broad range of valuable markers. An operator (such as a combat medic) may use a portable probe according to the invention, with the probe being pluggable into a hand held UV-NIR Raman spectrometer (such as a device the size of a hand held palm PC device). The Raman spectrometer before use on a patient may be programmed to perform V, near UV and NIR Raman spectroscopy for the markers of interest and to report them in a manner readily interpretable to indicate the presence and degree of shock. In the case of a combat medic using such a portable probe that plugs into a Raman spectrometer, the medic may then institute or order appropriate therapy while instrumenting and interrogating the next soldier using the same hand held spectrometer. In this manner, the medic can move back and forth between patients to determine the effect of the instituted therapy and to make triage decisions. When a marker or a combination of markers indicates intractable shock, an appropriate triage decision may be more readily reached than without the measurement information according to the present invention, and thus other more salvageable patients may be more likely to have access to resources and have increased chances of survival.

Data collected according to the present invention may be stored and/or transmitted. For example, data collected by a hand held device (such as a combat medic device) may be transmitted to a remote data bank. As the patient is transported, medics and physicians taking over care may use their

own devices (which may be hand held devices) to hook into the previously implanted fiber optic probe. Data measured during a new hook-up may be compared to data previously collected on the patient (such as data transmitted earlier from the field). New or additional probes optionally may be placed during surgery. The same devices, once placed, may be maintained in place and used to continuously interrogate tissue to monitor the efficacy of ongoing resuscitative efforts and to detect the development of post-hemorrhagic shock and surgical sequelae such as early sepsis. Multiple tissues beds may be interrogated, especially by using small, disposable probes.

While an operator has been referred to hereinabove, it will be appreciated that the inventive methods do not necessarily require a human operator, and that the invention may include partly and entirely automatic, such as computer-assisted, methods and devices. Such automatic and semi-automatic methods and devices may include those in which, upon measurement of ratios or amounts in a certain pre-determined adverse range, pre-formulated reactive therapies are applied. The invention provides for an optional computer system, such as a computer system comprising a database of stored baseline Raman spectroscopy and/or fluorescence spectroscopy profiles and a means to store patient Raman spectroscopy and/or fluorescence spectroscopy profiles. Such a computer system preferably includes a computing system for comparing patient profiles to baseline profiles.

While the invention has been described in terms of its preferred embodiments, those skilled in the art will recognize that the invention can be practiced with modification within the spirit and scope of the appended claims.

We claim:

1. A tissue analysis method, comprising:

interrogating a biological tissue with Raman spectroscopy and fluorescence spectroscopy to obtain spectroscopy results; analyzing the obtained spectroscopy results for at least one mediator or marker associated with a shock state.

2. The method of claim **1**, wherein the tissue interrogating is noninvasive.

3. The method of claim **1**, wherein the tissue is in vivo and in situ.

4. The method of claim **1**, wherein the tissue is removed from a patient before the tissue interrogation.

5. The method of claim **1**, including measuring NADH presence and/or accumulation by fluorescence spectroscopy.

6. The method of claim **1**, including measuring tissue hemoglobin oxygen saturation by Raman spectroscopy and measuring NADH presence and/or accumulation by fluorescence spectroscopy.

7. The method of claim **1**, including determining whether the tissue has insufficient oxygen delivery to meet metabolic demands of the tissue while simultaneously determining whether mitochondrial dysfunction or injury exists.

8. The method of claim **1**, including measuring myoglobin oxygenation saturation.

9. The method of claim **1**, including determining cytochrome oxidase redox status.

10. The method of claim **1**, including determining absolute concentration of hemoglobin in the tissue.

11. The method of claim **1**, including determining pH of the tissue.

12. The method of claim **1**, including intermittently or continuously interrogating the tissue of a patient.

13. The method of claim **1**, including determining tissue viability.

14. The method of claim **1**, including diagnosing shock.

15. A tissue analysis method, comprising:

interrogating a biological tissue with Raman spectroscopy and fluorescence spectroscopy to obtain spectroscopy results, including:

measuring tissue hemoglobin oxygen saturation including amount of oxyhemoglobin and deoxyhemoglobin by Raman spectroscopy; and/or

measuring tissue hemoglobin oxygen saturation by Raman spectroscopy and measuring NADH presence and/or accumulation by fluorescence spectroscopy; and/or

determining whether the tissue has insufficient oxygen delivery to meet metabolic demands of the tissue while simultaneously determining whether mitochondrial dysfunction or injury exists.

16. The tissue analysis method of claim **15**, wherein the obtained spectroscopy results are for at least one mediator or marker associated with a shock state and/or tissue injury.

17. The method of claim **16**, wherein the obtained spectroscopy results are for presence and/or proportions for the at least one shock state and/or tissue injury mediator or marker.

18. The method of claim **16** including determining the concentration of at least one mediator or marker.

19. The method of claim **18**, including determining absolute concentration.

20. The method of claim **18**, including determining relative concentration.

21. The method of claim **16** including determining the presence of at least one mediator or marker.

22. The method of claim **16**, wherein the obtained spectroscopy results are selected from the group consisting of at least one mediator associated with a shock state, tissue injury or tissue ischemia, inflammation or immune dysfunction, and at least one marker of tissue perfusion or injury.

23. The method of claim **22**, wherein the marker may be within intracellular, interstitial or intravascular space or within exhaled air from a patient.

24. The method of claim **22**, wherein the marker is selected from the group consisting of lactate, pyruvate, ATP, Pcr, AMP, ADP, Pi, NAD, NADH, albumin, endotoxin, exotoxin, microbes, cytokines-chemokines, procalcitonin, hormones, myeloperoxidase, elastase, xanthine oxidase, xanthine dehydrogenase, fatty acid binding proteins, catecholamines and vasoactive peptides.

25. The method of claim **22**, wherein the marker or mediator is a metabolic or pro or anti-inflammatory marker or mediator.

26. The method of claim **16**, including monitoring for appearance of one or more tissue markers specific for a specific disease state.

27. The method of claim **16**, including diagnosing tissue injury, tissue inflammation or tissue immune dysfunction.

28. The method of claim **15**, including diagnosing and/or following progression or resolution of shock states and/or tissue injury, and/or tissue ischemia.

29. The method of claim **28**, wherein the tissue injury includes inflammatory or immune dysfunction.

30. The method of claim **15**, including determining absolute concentration of hemoglobin in the tissue.

31. The method of claim **15**, including determining pH of the tissue.

32. The method of claim **15**, including continuously interrogating the patient for appearance of abnormal tissue markers specific for a suspected disease state.

33. The method of claim 32, wherein the markers are cardiac biomarkers, GI markers, cerebral markers, skin markers, lung markers, blood markers, and/or eye markers.

34. The tissue analysis method of claim 15, including measuring tissue hemoglobin oxygen saturation including amount of oxyhemoglobin and deoxyhemoglobin by Raman spectroscopy.

35. The tissue analysis method of claim 15, including measuring tissue hemoglobin oxygen saturation by Rama spectroscopy and measuring NADH presence and/or accumulation by fluorscence spectroscopy.

36. The tissue analysis method of claim 15, including determining whether the tissue has insufficient oxygen delivery to meet metabolic mends of the tissue while simultaneously determining whether mitochondrial dysfunction or injury exists.

37. A method of diagnosing shock, comprising:

(A) for a target molecule population, taking a sample Raman spectroscopy, and/or fluorescence spectroscopy, profile for a patient;

(B) comparing the sample spectroscopy profile with a pre-established Raman spectroscopy and/or fluorescence spectroscopy profile for the target molecule population under baseline conditions; and,

(C) diagnosing shock based on results of the comparing step.

38. The method of claim 37, wherein the method is non-invasive.

39. The method of claim 37, wherein the target molecule population comprises oxygenated hemoglobin, deoxygenated hemoglobin and/or NADH.

40. The method of claim 37, wherein the profiles are of relative amounts.

41. The method of claim 40, including operating an electromagnetic radiation generator at a range of selectable wavelengths from about 270 mm to about 30,000 nm.

42. The method of claim 37, wherein the profiles are of absolute amounts.

43. The method of claim 37, including taking the profiles by Raman spectroscopy.

44. The method of claim 43, including signal enhancement at a resonant frequency for a target molecule of the target molecule population.

45. The method of claim 37, including monitoring a specific tissue bed in the patient.

46. The method of claim 45, wherein the specific tissue bed is a brain, heart, lung, liver, eye, intestines, stomach, pancreas, kidney, bladder, urethra, skin, nailbed, cervix, uterus, oropharynx, nasopharynx, eosphagus or blood.

47. The method of claim 37, including calculating pH of blood and/or tissue.

48. The method of claim 37, including minimally invasively probing the patient by a fiber optic probe or probe array inserted into a tissue bed.

49. The method of claim 48, wherein the probe or probe array is inserted into a muscle.

50. The method of claim 37, including analysis of interstitial fluid.

51. The method of claim 32, including resonance Raman spectroscopy at 390 to 420 nm wavelength.

52. The method of claim 51, wherein the sample profile is taken from a tissue or a space in a body.

53. The method of claim 51, wherein the sample profile is taken from a tissue or a space out of the body.

54. The method of claim 37, including cellular analysis.

55. The method of claim 37, including placing a probe on or near any mucosal or epithelial covered surface of a body or an organ.

56. The method of claim 37, including simultaneously performing fluorescence spectroscopy probing of NADH while performing Raman spectroscopy.

57. The method of claim 37, wherein spectroscopy is performed for multiple wavelengths.

58. A method of diagnosing shock, tissue ischemia, tissue injury, tissue inflammation, or tissue immune dysfunction, comprising:

(A) for a target molecule population, taking a sample Raman spectroscopy, and/or fluorescence spectroscopy, profile for a patient;

(B) comparing the sample spectroscopy profile with a pre-established Raman spectroscopy and/or fluorescence spectroscopy profile for the target molecule population under baseline conditions, wherein the profiles are of relative amounts of NAD/NADH; lactate/pyruvate; Pcr-ATP; ATP-ADP; Pcr-Pi; oxidized cytochrome oxidase to reduced cytochrome oxidase, and/or oxyhemoglobin with deoxyhemoglobin.

59. A method of diagnosing shock, tissue ischemia, tissue injury, tissue inflammation, or tissue immune dysfunction, comprising:

(A) for a target molecule population, taking a sample Raman spectroscopy, and/or fluorescence spectroscopy, profile for a patient;

(B) comparing the sample spectroscopy profile with a pre-established Raman spectroscopy and/or fluorescence spectroscopy profile for the target molecule population under baseline conditions, including detecting exhaled markers or mediators of organ injury.

60. The method of claim 59, wherein exhaled markers or mediators of lung injury are detected.

61. The method of claim 59, wherein a detector is placed at the airway of the patient.

62. The method of claim 59, wherein the exhaled markers indicate organ injury.

63. The method of claim 59, wherein the exhaled markers or mediators are isoprostanes and/or myeloperoxidase.

64. A method of diagnosing abnormalities in vivo and in situ, comprising:

(A) for a target molecule population, taking a sample Raman spectroscopy and/or fluorescence spectroscopy profile for a patient;

(B) comparing the sample Raman spectroscopy or fluorescence spectroscopy profile with a pre-established Raman spectroscopy or fluorescence spectroscopy profile for the target molecule population under baseline conditions;

(C) using differences identified in said comparing step to identify an abnormality associated with a shock state.

65. The method of claim 64, including, while taking the Raman spectroscopy profile, also performing fluorescence spectroscopy measurement on the patient.

66. A computer system comprising:

a database of stored baseline Raman spectroscopy and/or fluorescence spectroscopy profiles and

a means to store patient Raman spectroscopy and/or fluorescence spectroscopy profiles;

including a computing system for comparing patient profiles to baseline profiles with regard to a shock state.

67. A biological material analysis method, comprising:

interrogating a biological material with Raman spectroscopy and

fluorescence spectroscopy to obtain spectroscopy results;

analyzing the obtained spectroscopy results for at least one mediator or marker associated with a shock state.

68. The biological material analysis method of claim **67**, wherein the biological material is bodily fluid.

69. The biological material analysis method of claim **67**, wherein the biological material is tissue.

70. The method of claim **67**, wherein the marker is contained in a biological material selected from the group consisting of urine, saliva, wound exudates, vitreous humor, aqueous humor, tissue exudate, gastric contents, and fecal matter.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,113,814 B2
APPLICATION NO. : 10/332613
DATED : September 26, 2006
INVENTOR(S) : Kevin R. Ward et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Specification

At Column 1, line 5, please insert the following:

--STATEMENT OF GOVERNMENT INTEREST

This invention was made with government support under contract number GM57042 awarded by the National Institutes of Health. The government has certain rights in the invention.--

Signed and Sealed this
Twenty-third Day of December, 2014

Michelle K. Lee

Michelle K. Lee
Deputy Director of the United States Patent and Trademark Office

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Ward, Kevin R

eRA COMMONS USER NAME (credential, e.g., agency login): KRWARD

POSITION TITLE: Professor Emergency Medicine and Biomedical Engineering, Executive Director: Michigan Center for Integrative Research in Critical Care

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Louisiana State University (Baton Rouge, LA)	B.S.	08/1985	Physiology-Zoology
Tulane University School of Medicine (New Orleans, LA)	M.D.	06/1989	Medicine
University of Pittsburgh (Pittsburgh, PA)	Residency	06/1992	Emergency Medicine Residency
The Ohio State University (Columbus, OH)	Fellowship	06/1994	Resuscitation Research Fellowship

A. Personal Statement

As a specialist in emergency medicine in treating the critically ill and injured, I have a great appreciation for the constant need to develop new collaborative approaches that produce the next best-in-class innovation for patients, their families and their health care providers. Emergency Medicine represents an ideal intersection clinical discipline to help develop and lead new clinical and research paradigms that impact the critically ill and injured. In this regard there is a tremendous unmet need to develop approaches in critical care team science that empower innovation allowing for the right care at the right time by the right individuals. I have lamented the lack of new technology that can be used to save lives with this approach. Integrative team science drawing from multiple medical, engineering, and information science disciplines became the new model for my approach, leading me to really understand what innovation was and how it should be executed. As Executive Director of the Michigan Center for Integrative Research in Critical Care (MCIRCC) and the architect and former Executive Director of a new Medical School-wide Innovation program called Fast Forward Medical Innovation, I have a solid track record in developing and leveraging multi and interdisciplinary teams of scientists to solve complex clinical problems in emergency, trauma, and critical care. I have successfully developed monitors for measuring tissue oxygenation, volume status, coagulation monitoring, redox monitoring, image and physiologic signal analysis, breath analysis and other physiologic parameters leading teams of engineers, basic scientists and clinicians bridging the translation gap. My expertise in the areas of innovation, emergency medicine, critical care, and interdisciplinary collaboration make me well suited to participate in efforts to develop new technologies for the care of critically ill and injured patients.

B. Positions and Honors

Positions and Employment

1994-1998: Senior Staff Physician and Physician Scientist Henry Ford Health System, Detroit, MI
1999-2003: Assistant Professor of Emergency Medicine and Director of Research VCU
2002-2010: Member VCU Office of Research Subjects Protection: Human Institutional Review Board:
2003-2008: Member U.S. Army Combat Casualty Care Program Task Area: Remote Triage
2004-2012: VCU Medical Site Director: Special Operations Combat Medic Training Program: U.S. Army Joint Special Operations Medical Training Center

2010-2012: Professor and Associate Chair: Department of Emergency Medicine: VCU
2004-2012: Director: VCU Reanimation Engineering Science Center (VCURES)
2010-12 Professor of Emergency Medicine and Physiology and Biochemistry
2012-Present: Professor, Department of Emergency Medicine: University of Michigan
2012-Present: Executive Director: Michigan Center for Integrative Research in Critical Care
2013-2018: Executive Director: Fast Forward Medical Innovation: University of Michigan Medical School
2013-2015: Oversight Committee: Coulter Translational Research Partnership
2017-Present Professor Biomedical Engineering: University of Michigan

Other Experience and Professional Memberships

1990-Present: Society of Critical Care Medicine;
1994-Present: Fellow and Founding Member America Academy of Emergency Medicine;
1994-present: Fellow American College of Emergency Physicians
2000- Present: Shock Society; Editorial Board: Resuscitation: Editorial Board: Shock. Manuscript reviewer for Annals of Emergency Medicine, Academic Emergency Medicine; American Journal of Emergency Medicine, Critical Care Medicine, Critical Care, Intensive Care Medicine, Executive Committee: Traumatic Hemostasis and Oxygenation Research (THOR) Network.

Selected Honors or Awards:

1992: Peter Safar Award for Excellence in Graduate Research: U of Pittsburgh
1992&94: Emergency Medicine Foundation Research Fellowship Award;
1996&97: Educator of the Year Award: Department Emergency Medicine, Henry Ford Hospital
1998: Henry Ford Health System New Clinical Investigator Award
2000: Society for Academic Emergency Medicine Young Investigator Award;
2003: Outstanding Achievement in Research VCU School of Medicine. .
2008: DoD Advanced Technologies Applications in Combat Casualty Care Award for Excellence
2010: VCU Innovator of the Year (Inventor of the Year) Award.
2012: Department of the Army Certificate for Patriotic Civilian Service
2013: Louisiana State University Alumni Hall of Distinction.
2017: Innovation and Commercialization Award: University of Michigan Medical School

C. Contribution to Science

1. Moving the Intensive Care Unit Far Forward: Death or survival from a sudden episode of critical illness and injury may be determined in minutes. Determining the severity of the critical state cannot be done with the physical exam and routine use of invasive monitoring has severe limitations. Being on the front lines of in the Emergency Department, I have led teams to develop noninvasive equivalents of technologies ranging from resonance Raman spectroscopy to impedance as a means to interrogate tissue and the cardiovascular system that is equivalent to invasive technologies used in the intensive care unit. These technologies are now being commercially transitioned and are entering trials for regulatory approval.
   a. **Ward KR**, Tiba MH, Draucker GT, Proffitt EK, Barbee RW, Gunnerson KJ, Reynolds PS, Spiess BD: A novel noninvasive impedance-based technique for central venous pressure measurement. Shock 2010;33:269-273. PMID 19487978
   b. Tiba MH, Draucker DT, Barbee RW, Terner J, Torres IF, Romfh P, Vakshoori D, **Ward KR**. Tissue oxygenation monitoring using resonance Raman spectroscopy during hemorrhage. J. Trauma and Acute Care Surg 2014;76:402-408. PMID 24378619
   c. Tiba MH, Belmont B, Heung M, Theyyunni N, Huang RD, Fung CM, Pennington AJ, Cummings BC, Draucker GT, Shih AJ, **Ward KR**. Dynamic limb impedance and inferior vena cava ultrasound in patients undergoing hemodialysis**.** ASAIO J. 2016;62:463–469. PMID: 26919184
   d. Tiba MH, McCraken B, Ansari S, Belle A, Cummings BC, Rajajee V, Patil PG, Alam HB, **Ward KR:** Novel noninvasive method of cerebrovascular blood volume assessment using brain bioimpedance. J Neurotrauma 2017: 15;34(22):3809-3096: PMID 28657491 .

2. Hemostasis, Coagulation, and Metabolic Monitoring: One of the greatest challenges in caring for the victim of trauma and shock is achieving hemostasis and controlling some of the overriding factors which dictate the function of these integrated systems. Failure to approach the system as integrated has stunted our ability to develop new innovations, which may be lifesaving. New technologies require an understanding of

a combination of materials science, biochemical function, and knowledge of the care process allowing for the development of new means to both monitor and treat. I have developed integrated teams which are developing new hemostatic materials, new insights into how the coagulation system functions, and new measures such as whole blood redox potential which may will provide critical insights in the metabolic drivers of coagulation and hemostasis.

a. White NJ, Wang Y, Fu X, Cardenas JC, Martin EJ, Brophy DF, Wade CE, Wang X, St John AE, Lim EB, Stern SA, **Ward KR**, López JA, Chung D. Post-translational modification of fibrinogen is associated with coagulopathy after traumatic injury Free Radic Biol Med. 2016 Apr 20;96:181-189 PMID: 27105953
b. Li Z, Li X, McCraken B, Shao Y, **Ward K**, Fu J: A Miniaturized Hemoretractometer for Blood Clot Retraction Testing. Small 2016 (Epub ahead of print). PMID 27248117.
c. Daniels RC, Jun H, Tiba MH, McCracken B, Herrera-Fierro P, Collinson M, **Ward KR**: Whole blood redox potential correlates with progressive accumulation of oxygen debt and acts as a marker of resuscitation in a swine hemorrhagic shock model. Shock 2018;49(3): 345-351. PMID 28658006
d. Li Y, **Ward KR**, Burns MA: Viscosity measurement using microfluidic droplet length. Anal Chem 2017 Apr 4;89(7):3996-400. PMID 28240541

3. Medical Innovation, Entrepreneurship, Team Science, and Mentoring: Sadly in the last 30 years, there has been very little innovation in Emergency and Critical Care Medicine resulting in new life-saving technologies. One of the reasons for this is a lack of inter and multidisciplinary collaboration especially outside the immediate scope of medicine. Creating such an approach requires a cultural shift and great patience since the language of disparate disciplines such as medicine, engineering and information science are significantly different. Innovation then becomes less about the ah-ha moment and increasingly more about a strategic and systematic approach to processes that allow for the rapid progression and iteration of the science that promotes a true solution. I have engaged in such approaches for the last 16 years at two large universities (Virginia Commonwealth University and now at the University of Michigan as the Executive Director of the Michigan Center for Integrative Research in Critical Care. At each of these institutions I developed critical care innovation programs In these programs I have had an opportunity to mentor over 60 students ranging from undergraduates and graduate students (MS and PhD) to post-doctoral, medical students, and residents. I have also mentored a great many junior faculty. A significant number of these mentoring relationships revolved around projects that intersected translational science, the development of intellectual property, and industry transition. The combination of the above experiences resulted in my appointment as the inaugural Executive Director of the University of Michigan Medical School's acclaimed Fast Forward Medical Innovation program. This program was developed to provide strategic innovation assets, which greatly expedite the movement of science into product development and commercialization. I am a serial innovator and entrepreneur in the field of critical care with over 60 issued and pending patents, 10 products licensed to industry, and 4 companies launched. My work has resulted in being awarded the Innovator of the Year at Virginia Commonwealth University, the University of Michigan Medical School and the Department of Defense for innovative work in hemostasis.

a. Servoss JM, Chang C, Fay J, **Ward K**: The early tech development course: Experiential commercialization education for the medical academician. Acad Med 2017;92:506-510. PMID 28351064.
b. Servoss JM, Chang C, Olson D, **Ward KR**, Mulholland MW, Cohen MC: The Surgery innovation & entrepreneurship development program (SIEDP): An experiential learning program for surgery faculty to ideate and implement innovations in healthcare. J Surg Educ. 2017; 75(4):935-941 PMID:28989009
c. Servoss J, Chang C, Fay J, Lota KS, Mashour GA, **Ward KR**: *fast*PACE Train-the-Trainer*:* A scalable new educational program to accelerate training in biomedical innovation, entrepreneurship, and commercialization. Journal of Clinical and Translational Science 2017 Oct;1(5):271-277. PMID:29707247

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/kevinr..ward.1/bibliograpahy/48065982/public/?sort=date&direction=ascending

D. Research Support
Ongoing Research Support

1R21HL139156-01 Fan(PI)12/15/17-11/30/19
Sponsor:NIH
Rapid breath analysis for acute respiratory distress syndrome diagnostics
Description: Project to create and test a 3-D microgas chromatography unit to diagnose and track ARDS in humans
Role: Co-Investigator

NCAI-17-7-APP-UMICH Fan(PI) 07/01/2017-06/30/2018
Sponsor: NIH/NCAI
Micro Gas Chromatography and Breathomics for Acute Point-of-Care Diagnostics of Acute Lung Injury
Description: The major goal of this award is to develop and refine a microgas chromatography device the diagnose and follow the trajectory of the acute respiratory distress syndrome.
Role: Co-Investigator

DM160299 Ward (PI) 01/30/18-12/30/21
Sponsor: DoD
Gastroesophageal Resuscitative Occlusion of the Aorta (GROA)
Description: This project will develop a minimally invasive device and method capable of occluding the descending aorta from the stomach for control of massive abdominal hemorrhage.
Role: Principal Investigator

DM160294 Ward (PI) 01/30/18-12/30/21
Sponsor DoD
Development and Testing of New Noninvasive Monitoring Tools for Prolonged Field Care Goal-Directed Therapy
Description: Project clinically test two novel noninvasive sensing technologies to test tissue oxygenation and circulatory volume in critically ill and injured patients.
Role: Principal Investigator

DM160225 Tiba/Ward (Co-PI) 07/01/17-06/30/20
Sponsor: DoD
Novel Noninvasive Methods of Intracranial Pressure and Cerebrovascular Autoregulation Assessment: Seeing the Brain Through the Eyes
Description: This project will develop several noninvasive means to evaluate cerebral autoregulation and ICP using bioimpedance and ultrasound technologies.
Role: Co-Principal Investigator:

W81XWH-16-R-BAA1 BA150235 Najarian (PI) 03/01/17-02/26/20
Sponsor: DoD
Title: A Multimodal Integrative Platform for Continuous Monitoring and Decision Support during in Cardiac Patients
Description: This project will develop an innovative, real-time clinical decision support (DSS) platform, including Big Data analytic methods, novel algorithms, and software tools to integrate and analyze disparate sources of continuous and non-continuous patient data
Role: Co-investigator

RFA-HL-16-019 Neumar/Pinsky (PIs) 01/02/17-06/30/20
Sponsor: NIH
Career Development Program in Emergency Care Research (K12)
Description: This K12 provides training to produce the next generation of translational Emergency-Critical Care scholars with an emphasis on integrating biomedical engineering into their research.
Role: Co-Investigator

Completed Relevant Research Support:

14-PAF03993 Ward (PI) 1/30/14-12/31/16
Sponsor: William Davidson Foundation
Title: Fast Forward Medical Innovation
Description: This grant provides important funding to supplement the University of Michigan's new Fast Forward Medical Innovation initiative allowing investment in development of early stage technologies to accelerate their commercialization as well as develop important entrepreneurial educational initiatives
Role: PI

15-PAF03360 Ward/Tiba (PI) 1/30/15-7/30/15
Sponsor: Baxter Healthcare Corporation
Title: Comparison of Respiratory Induced Limb Bioimpedance with Inferior Vena Cava Diameter Changes to Assess Intravascular Volume
Description: This grant will assess the ability of limb impedance as an accurate surrogate of functional intravascular volume in the management of dialysis and critical care patients.
Role: PI

W81XW H-1120089 Ward (PI) 01/10/11-01/09/13
Sponsor: Department of Defense: US Army Medical Research and Materiel Command
Title: Defining Platelet Function During Polytrauma.
Description: This project will characterize longitudinal platelet function in human victims of polytrauma
Role: PI

ONR N000140710526 Ward (PI) 01/29/07-01/10/2014
Sponsor: Department of Defense: Office of Naval Research
Title: Novel Acute Rescue Strategies using Non-pulmonary Oxygenation
Description: This project explores the creation of special compounds and delivery methods that provide tissue oxygenation via nonpulmonary routes.
Role: PI

NSF 0969062 Pidapart i (PI) 08/10-07/13
Sponsor: National Science Foundation
Title: Multiscale Study of the Respiratory Airway Mechanics for Cellular Inflammation
Description: This study utilizes several advanced computation techniques to model multiple levels of acute lung injury.
Role: Co-PI

H92239-09-003 Ward (PI) 09/09-09/12
Sponsor: Department of Defense: U.S. Army
Title: Preceptor Support Servces at VCU for Joint Special Operations Combat Medic/Special Forces Course
Description: This is a contract to provide clinical training to Special Operations Combat Medics prior to deployment
Role: PI