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(54) Title: DETERMINATION OF TISSUE OXYGENATION IN VIVO

(57) Abstract: A system and method for noninvasively determining the oxygenation of a tissue, for example, a muscle, in vivo uses optical methods to optically interrogate the tissue in both a visible wavelength range and a near infrared (NIR) wavelength range. The illuminating light is sculpted in intensity to approximately match the absorbance spectrum, for example, with the visible light having an intensity an order of magnitude greater than the NIR light. Training data is obtained from healthy patients in both the visible and NIR ranges simultaneously and used to calculate muscle oxygenation.



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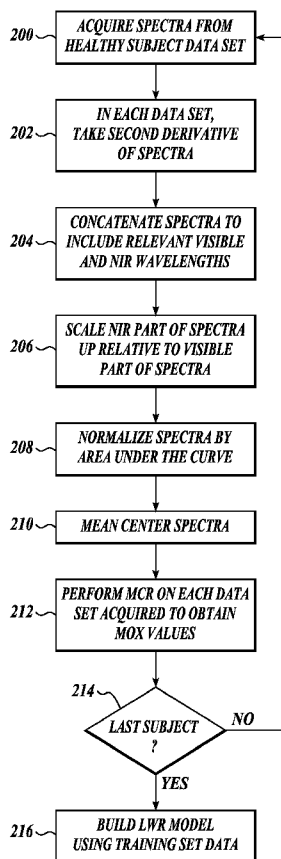


Fig. 4.



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DETERMINATION OF TISSUE OXYGENATION *IN VIVO*

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of Provisional Application No. 61/409,793,
5 filed November 3, 2010, the entire disclosure of which is hereby incorporated by
reference herein.

BACKGROUND

The benefits of non-invasive systems for detecting and/or monitoring for the
presence of a particular analyte are well known in the art, and in particular in the
10 biological arts. For example, in pulse oximetry a sensor is placed on a patient's body,
e.g., the patient's finger. The sensor emits a red light and an infrared (IR) light
sequentially through the patient, and detects the resulting light transmitted through the
patient. The changing absorbance of each of the two wavelengths as the heart beats is
measured and used to determine the oxygenation of the pulsing arterial blood alone. The
15 absorbance of the red and IR light is used to calculate the oxygenation of the blood.

Methods and systems for determining and/or monitoring *in vivo* the oxygenation
of tissue in a person or other mammal have a number of applications. For example:

- During surgical procedures when a patient is under general anesthesia, it is
valuable for clinicians to monitor the patient's perfusion and oxygenation. Low muscle
20 oxygenation will provide an early warning of perfusion and oxygenation problems. An
oxygenation monitor would be particularly useful during bypass surgery when pulsatile
flow to the body may be limited and pulse oximetry is not a viable option.

- Circulatory shock is a life-threatening medical condition that may result in
inadequate oxygen to the organs and tissues of the body. Shock includes, for example,
25 traumatic shock, cardiogenic shock, septic shock, neurogenic shock, anaphylactic shock,
and the like. In a particular example, a muscle oxygenation probe would be useful in to
improve triage and initial medical management, for example in battlefield situations,
multi-victim accident situations, and other scenarios involving multiple casualties.

- An oxygenation monitor or probe would also be beneficial for refining
30 oxygen titration, including monitoring of neonatal (and premature infant) patients.
Muscle oxygen measurement is more sensitive than other existing methods for titration of
supplemental oxygen. Better modulation of oxygen support in premature infants and
other patients will decrease risks of inadequate oxygen (which can result in brain or other

organ damage) as well as excessive oxygen therapy (which can result in long-term lung injury).

• A tissue oxygenation monitor would be beneficial in exercise physiology and sports medicine applications. In particular, muscle oxygenation is an indicator of aerobic capacity, muscle anaerobic metabolism, and performance.

• Measuring muscle oxygenation during transport of patients and during pre-hospital admission as part of early evaluation could lead to earlier treatment and better outcome.

• Often trauma can lead to blood vessel compression by the raised pressure within a body compartment, and ultimately to lack of oxygenation and tissue death. A tissue oxygenation probe would help identify decreasing oxygenation and increasing tissue death so treatment can be initiated early.

• An *in vivo* oxygenation probe may be used to determine viability of tissue to be transplanted and to monitor the progress of reperfusion in the transplanted organ.

• An *in vivo* oxygenation probe may be used to detect and/or monitor for peripheral vascular disease and/or during arterial and vascular surgery to aid in the identification of tissue perfusion status. The probe would be especially helpful in identification of tissue viability in extremities with vascular disease, for example, helping the surgeon to determine whether and where to perform amputations.

Muscle oxygenation or saturation can be used as an early or leading signal of circulatory shock. Shock is a life-threatening medical condition characterized by "inadequate substrate for aerobic cellular respiration." Silverman, Adam (Oct 2005). "Shock: A Common Pathway for Life-Threatening Pediatric Illnesses and Injuries," *Pediatric Emergency Medicine Practices* 2(10). In its early stages, shock may be described generally as inadequate oxygen levels in tissue. Several conditions can trigger the onset of shock, including, for example, sepsis, heart dysfunction, and hemorrhage. If left untreated, shock can lead to Multiple Organ Dysfunction Syndrome (MODS) and even death.

A clinically important aspect of circulatory shock is that it progresses by one or more positive feedback mechanisms, and therefore can rapidly escalate to permanent damage or death if left untreated. Early detection of shock is critical to optimize patient outcomes as well as to reduce the costs for medical treatment.

Current methods of recognizing shock are indirect, non-definitive, or slow. These methods include monitoring vital signs, which are non-specific, and sampling blood chemistry, which has associated metabolic and procedural delays. High lactate levels indicate a metabolic response to the presence of shock, but they lag decreases in muscle and systemic oxygenation that occur at the onset of shock. Invasive catheters are not used to detect shock, but may be used in cases of known severe shock. These catheters are expensive and must be inserted by highly trained personnel.

Continuous measurement of muscle oxygenation would also allow physicians to refine therapy as normalization of cellular oxygenation is achieved, giving patients what they need while preventing over-resuscitation. It is well established that unnecessary blood transfusions and excess fluid administration worsen outcome.

At this time, there are several commercial near infrared spectroscopy (NIRS) devices that are being used in various areas of health and physiology. Traditional NIRS devices measure 2 to 6 discrete wavelengths in the near infrared region. Exemplary NIRS devices are disclosed in U.S. Patent Application Publication No. 2011/0205535, to Soller et al., the disclosure of which is hereby incorporated by reference in its entirety.

There remains a need for improved methods, systems, and devices for detecting and monitoring the oxygenation of tissue. There is also a clinical need for improved methods, systems, and devices for monitoring patients to rapidly detect circulatory shock.

SUMMARY

This summary is provided to introduce a selection of concepts in a simplified form that are further described below in the Detailed Description. This summary is not intended to identify key features of the claimed subject matter, nor is it intended to be used as an aid in determining the scope of the claimed subject matter.

As discussed above, the detection of tissue oxygenation *in vivo* would have a number of uses. Methods and apparatus for detecting tissue oxygenation are disclosed herein.

A method for generating a set of training data is useful for determining tissue oxygenation *in vivo*, and includes providing a probe having (i) a first light source operable to emit light in an NIR spectrum and having a first radiant flux, (ii) a second light source operable to emit visible-spectrum light having a second radiant flux that is greater than the first radiant flux, and (iii) a light detector. For a plurality of healthy

° subjects, the probe is placed such that the detector detects absorbance spectra resulting from the emitted light. For each detected absorbance spectrum, [oxyMbHb] and [deoxyMbHb] (defined herein) are calculated, and these are used to calculate oxygenation of the tissue at the time the spectrum was detected. The calculation results are stored on a computer readable media. Data is collected and analyzed from a number of healthy subjects to generate a set of training data. In an embodiment of the method the second radiant flux is at least an order of magnitude greater than the first radiant flux. The first and second light sources produce a radiant-intensity-sculpted illumination that improves the ability to detect absorbance characteristics in the visible and NIR regions simultaneously.

In an embodiment, the first and second light sources emit from a distal face of the probe, and one or both of the light sources may comprise a plurality of lighting elements, for example light emitting diodes with or without phosphor coatings, that cooperatively produce the desired sculpted radiant flux illumination. The first and second light sources may emit light intermittently and synchronously.

In an embodiment of the method continuous first and second portions of the detected spectrum in the visible and NIR ranges are extracted and concatenated, and the new concatenated spectra are used in the analysis. The NIR portion of the new spectra may be scaled to have a magnitude (e.g., area or peak) similar to the visible portion. The visible portion may include the visible wavelength 580 nm, and the NIR portion may include the NIR wavelength 760 nm.

A method for determining tissue oxygenation *in vivo*, comprises generating training data as discussed above, and obtaining similar spectral data from a patient using a similar probe, and using the spectra data from the patient and the training data to calculate tissue oxygenation for the patient.

In an embodiment of the invention a locally weighted regression (LWR) model is constructed using the training data, and the LWR model receives the patient data to calculate the tissue oxygenation.

A method for generating a set of training data that is useful for determining tissue oxygenation *in vivo* includes providing a first probe having (i) a first light source operable to emit light in a near infrared spectrum having a first radiant flux, (ii) a second light source operable to emit light in a visible spectrum having a second radiant flux that is greater than the first radiant flux, and (iii) a light detector. For a plurality of healthy

subjects, placing the first probe such that the light detector detects absorbance spectra of light reflected from or transmitted through a tissue of the subjects resulting from light emitted by the first and second light sources. The detected absorbance spectra are used to calculate oxygenations of a portion of the tissue that is not solely pulsatile arterial blood.

5 Storing on computer readable media: (i) data calculated from the detected absorbance spectra, and (ii) the calculated tissue oxygenations; and repeating steps (b)-(d) for a plurality of subjects to generate a set of training data.

In one embodiment, tissue oxygenation may be defined as myoglobin saturation, where myoglobin saturation is calculated from [oxyMb] and [deoxyMb], and oxyMb is
10 oxymyoglobin and deoxyMb is deoxymyoglobin. The concentrations [oxyMb] and [deoxyMb] are calculated from each detected absorbance spectrum.

In another embodiment, tissue oxygenation may be defined as hemoglobin saturation, where hemoglobin saturation is calculated from [oxyHb] and [deoxyHb], and oxyHb is oxyhemoglobin and deoxyHb is deoxyhemoglobin. The concentrations [oxyHb]
15 and [deoxyHb] are calculated from each detected absorbance spectrum. Hemoglobin saturation includes contributions from pulsatile arterial blood, non-pulsatile arterial blood, and venous blood.

The same method for determining tissue oxygenation *in vivo* as described above, consisting of generating an *in vivo* training set, obtaining similar spectral data from a
20 patient using a similar probe, and using the spectral data from the patient and the training data to calculate tissue oxygenation from the patient may be used.

A probe for detecting *in vivo* tissue oxygenation in a subject includes (a) a first light source operable to emit light in a near infrared spectrum having a first radiant flux,
25 (b) a second light source operable to emit light in a visible spectrum having a second radiant flux that is greater than the first radiant flux, and (c) a light detector operable to detect absorbance spectra of light reflected from or transmitted through a tissue of the subject resulting from light emitted by the first and second light sources.

In an embodiment, the second radiant flux is at least an order of magnitude greater than the first radiant flux and comprises a plurality of lighting elements that cooperatively
30 produce the second radiant flux. The plurality of lighting elements are preferably disposed along a circular arc centered on the light detector, which in a particular embodiment extends for not more than 90°.

DESCRIPTION OF THE DRAWINGS

The foregoing aspects and many of the attendant advantages of this invention will become more readily appreciated as the same become better understood by reference to the following detailed description, when taken in conjunction with the accompanying drawings, wherein:

FIGURE 1 is a block diagram providing an overview of a system for measuring and/or monitoring the oxygenation of tissue *in vivo* in accordance with the present invention;

FIGURE 2 shows the absorbance spectrum of solutions of oxyhemoglobin and deoxyhemoglobin;

FIGURE 3 illustrates an embodiment of the probe shown in FIGURE 1;

FIGURE 4 is a flow chart illustrating a method for generating a useful set of training data in accordance with the present invention; and

FIGURE 5 is a flow chart illustrating a method for using the training data obtained by the method illustrated in FIGURE 4.

DETAILED DESCRIPTION

Non-invasive methods, apparatuses, and systems for detecting an analyte in a turbid medium such as a biological tissue are disclosed. There are many biological and medical applications wherein a non-invasive means for determining oxygenation in a tissue would provide benefits. For example, tissue oxygenation provides an early signal of potential problems in physiology and pathology. A system for monitoring muscle oxygenation could also be applied in clinical areas such as cardiac surgery, in sports medicine, and the like. In a particular embodiment, a device and method for monitoring muscle oxygenation (Mox) to provide early detection of shock in a patient is described. It will be readily appreciated by persons of skill in the art that the teachings of the present invention may be readily applied to other applications, including the exemplary applications discussed in the Background section above.

In U.S. Patent No. 5,931,779, to Arakaki et al., which is hereby incorporated by reference in its entirety, some of the present inventors disclosed a method and system for real-time *in vivo* measurement of myoglobin oxygen saturation. In U.S. Patent Application Publication No. 2007/0265513, to Schenkman et al., which is hereby incorporated by reference in its entirety, some of the present inventors disclosed a system

and method for determining a mitochondrial characteristic using calibration spectra and a reflectance spectrum. In U.S. Patent Application Publication No. 2008/0033263, to Marcinek et al., which is hereby incorporated by reference in its entirety, some of the present inventors disclosed a system and method for calculating a ratio of hemoglobin
5 and myoglobin based on peak positions between spectral peaks. In U.S. Patent Application Publication No. 2008/0200780, to Schenkman et al., which is hereby incorporated by reference in its entirety, some of the present inventors disclosed a system and method for generating an estimate of a cellular energetic parameter using a multivariate curve resolution algorithm.

10 In contrast to prior art near infrared spectroscopy systems, a system 100 in accordance with the present invention measures continuous wavelengths of light in both the visible spectrum and the near infrared (NIR) spectrum. The addition of at least a portion of the visible region of the spectrum allows enhanced sensitivity to muscle oxygenation and more rapid detection of circulatory shock. Visible light, as used herein,
15 is expressly defined to comprise electromagnetic radiation having a wavelength in the range of 400-700 nm. Near infrared light, as used herein, is expressly defined to comprise electromagnetic radiation with a wavelength in the range of 700-3,000 nm.

FIGURE 1 is a block diagram providing an overview of a system 100 for measuring and/or monitoring the oxygenation of tissue *in vivo* in accordance with the
20 present invention. The system 100 includes a probe 102 having a visible light source 104, an NIR light source 106, and a light detector 108. The visible light source 104 and NIR light source 106 illuminate a desired region of tissue adjacent the probe 102. For example, the visible and NIR light may transmit through a user's skin and fluids to illuminate the desired region of tissue.

25 The light detector 108 receives light reflected from or transmitted through the desired region of tissue, and transmits the detected light to a spectrometer, spectrophotometer, or the like 110. Various types of spectrometers 110 are contemplated, including those having a sensor and a prism, diffraction grating, or slit. Both the prism (or grating or slit) and the sensor may be stationary or swept. For example, an exemplary
30 system 100 includes a fiber-optic spectrophotometer 110 having a grating and a photodetector, such as a photodiode array, a charge-coupled device (CCD), or a complementary metal-oxide-semiconductor device such as an active pixel sensor (CMOS APS).

The detected spectra are transmitted to a processor, for example, a conventional computer system 112 for processing. The particular details of the computer system 112 are not critical to the present invention. In an exemplary system, the computer system 112 includes an input interface for coupling to an analog-to-digital
5 converter to receive spectral data. Well-known components may comprise the computer system 112, such as a keyboard, mouse, trackball, touchpad, printer, an interface, and other elements configured for controlling or monitoring components of system 100.

The computer system 112 may further control the visible and NIR light sources 104, 106. In a current embodiment, for example, the visible light source 104 and
10 the NIR light source 106 are controlled to synchronously emit light intermittently. The computer system 112 includes a processor configured to execute instructions for implementing an algorithm described elsewhere in this document. The instructions, algorithm, and data can be stored in memory or received from an input. The memory, in various examples, includes a volatile or non-volatile memory or storage device.
15 Typically, a display device 114 may be used to display the status of the system 100 and/or the results of the analysis of the spectra. For example, the display device 114 may include a visual or audible alert to notify a healthcare provider if the results of the analysis indicate the need for remedial action.

In an embodiment of the present invention adapted to monitor for shock, the
20 probe 102 is attachable to the patient, for example, over the thenar muscle group in the hand. The visible and NIR light sources 104, 106 illuminate muscle tissue, and the reflected light returning to the spectrometer 110 is recorded. The spectra are provided to the computer system 112 for analysis, as described below, to calculate muscle oxygenation (Mox). If the Mox indicates that the patient is experiencing circulatory
25 shock, the caregiver is alerted to take appropriate action.

Mox was found to be an early indicator of shock. Moreover, Mox can be used to differentiate between mild, moderate, and severe shock. Low Mox in critically ill patients can be used to identify the early stages of shock since blood flow is preferentially shunted away from skin and muscle as the body tries to preserve blood flow to the critical
30 organs of the body--the heart, brain, and liver.

Mox is a measure of cellular oxygenation in muscle tissue and is a weighted average of myoglobin and hemoglobin oxygen saturations. The weighting depends on the relative concentrations of total myoglobin and total hemoglobin in the light path.

In another embodiment, the computer system 112 can perform analyses to calculate tissue oxygenation defined as myoglobin saturation. Since myoglobin is contained within muscle cells, myoglobin saturation is a measure of intracellular oxygenation that will be useful in the detection of shock and the monitoring of critically
5 ill patients.

In another embodiment, tissue oxygenation can be defined as hemoglobin saturation. Hemoglobin saturation measurements would not depend on the patient having pulsatile blood flow; it is an aggregate saturation measurement of pulsatile arterial blood, non-pulsatile arterial blood, and venous blood. Hemoglobin saturation can be measured
10 in muscle tissue and in other tissues that do not contain myoglobin, such as the brain.

In an embodiment of the monitoring system 100, the probe 102 is configured to illuminate the tissue with a sculpted or shaped intensity (or radiant flux) illumination that improves the usefulness of the reflectance or transmission of the optical spectral information obtained. For example, in a current embodiment the visible light source 104
15 is configured to illuminate the desired tissue with light in a visible region (e.g., 545-580 nm) having a radiant flux that is an order of magnitude larger than the NIR illumination provided by the NIR light source 106. By matching the profile of the incident illumination to the expected absorbance of the tissue, the signal-to-noise ratio of the detected spectra can be optimized, resulting in higher quality optical spectra than can
20 be acquired with a traditional broadband light source. It is contemplated that the visible light source 104 will produce a significantly larger radiant flux than the NIR light source, although the difference may be less than, or greater than, an order of magnitude.

To understand the reason for the improved performance from the sculpted illumination refer to FIGURE 2, which shows the absorbance spectrum of solutions of
25 oxyhemoglobin and deoxyhemoglobin. Hemoglobin in the blood binds with oxygen and carries oxygen from the lungs to the body tissues where it releases the oxygen and carries the resultant carbon dioxide back to the lungs. Oxyhemoglobin is formed when hemoglobin binds with oxygen; deoxyhemoglobin is formed when no oxygen molecules are bound to hemoglobin. (Myoglobin is an oxygen-binding protein in muscle tissue and
30 may similarly be bound to oxygen molecules (oxymyoglobin) or not bound to oxygen molecules (deoxymyoglobin)).

It can be seen from FIGURE 2 that the absorbance spectrum for oxyhemoglobin differs from the absorbance spectrum for deoxyhemoglobin. The differences in the

absorbance characteristics may be used to optically evaluate the oxygenation of the hemoglobin. The ability to accurately evaluate the oxygenation will be improved by looking at features of the oxygenation curve in both the visible light region and the NIR region. However, the peaks of the absorbance or optical density for oxyhemoglobin in the visible region (and more particularly in the range of 545-580 nm) are about an order of magnitude larger than the peak of the absorbance of deoxyhemoglobin in the NIR region (e.g., approximately 760 nm). Therefore, if a conventional broadband light source is used, the amount of light collected in the visible region will be about ten times smaller than that collected in the NIR region. Moreover, FIGURE 2 illustrates a best-case scenario because the absorbance spectra were collected from a solution of hemoglobin that did not include a scatterer. When light scattering is significant, as in biologic tissue, the collected light from the visible region can be as much as three orders of magnitude smaller than in the NIR region. The absorbance spectrum for oxymyoglobin and deoxymyoglobin also differ in a qualitatively similar way. The differences in the absorbance spectra can be analyzed to calculate tissue oxygenation (e.g., Mox, myoglobin saturation, or hemoglobin saturation).

However, if a conventional broadband light source (across the visible and NIR regions) is used to acquire spectra from biologic tissue that includes hemoglobin, the detector may be unable to detect a measurable signal in the visible region (e.g., no visible range photons may be detected) or the detector may become saturated in the NIR region (e.g., more NIR photons arrive than the detector can count, so a maximum value is registered). In either case, the dynamic range of the detector may not be large enough to measure both the visible and the NIR region spectra simultaneously.

The probe light sources 104, 106 produce a sculpted illumination having an intensity or radiant flux profile that is generally matched to the expected absorbance spectrum. In other words, the radiant flux of the illumination in wavelength regions with high absorbances is higher than the radiant flux of the illumination in wavelength regions with lower absorbances. In a preferred embodiment, the visible light source 104 is configured to illuminate the target tissue with an radiant flux that is an order of magnitude, or more, greater than the corresponding radiant flux produced by the NIR light source 106.

A number of different methods may be used to approximately match the illuminating light with the absorbance characteristics of the target and are contemplated

by the present invention. For example, filters may be used with a broadband light source to produce the desired sculpted illumination. In a currently preferred embodiment, the visible light source 104 and the NIR light source 106 are implemented with light-emitting diodes (LED) disposed to emit light from a distal face of the probe 102. Although the disclosed probe uses LEDs that are disposed at the face of the probe 102, other configurations are contemplated. For example, in a fiber optic embodiment the light sources are disposed away from the probe 102 and light is transmitted to the probe face through fiber optic cables.

FIGURE 3 illustrates an embodiment of the probe 102 shown in FIGURE 1 having a housing 118 that is configured to be placed against the user or tissue to be examined. The housing 118 may be rigidly constructed or may be conformable to the user. The visible light source 104 comprises a plurality of LEDs 120 that emit light in visible wavelengths (e.g., 540-620 nm), and the NIR light source 106 is a single LED 122 that emits in the NIR wavelengths (e.g., 740-790 nm). In this embodiment the probe 102 includes 13 visible light LEDs 120. The probe 102 is configured to illuminate the target tissue such that the intensity of the visible light illumination is approximately an order of magnitude greater than the intensity of the NIR illumination.

A light detector 108, for example a conventional CMOS photodetector 124 is positioned in the housing 118 to detect light reflected from the target tissue. Preferably, the visible light LEDs 120 and the NIR LED 122 are all disposed along a circular arc centered on the detector 124 such that the LEDs 120, 122 are all the same distance from the detector 124. The distance from the LEDs 120, 122 to the detector 124 is selected to optimize light collection, depending on characteristics of the tissue and the spectral information being collected. In particular, the distance between the LEDs and the detector is selected to provide a desired depth of penetration into the tissue. This allows for homogeneous sampling of the tissue in a known tissue volume.

Although it is not critical to the present invention, it should be appreciated that the LEDs 120, 122 in this embodiment are disposed along a circular arc that comprises approximately a 90° arc segment rather than a complete circle centered on the detector 124. This configuration advantageously allows for large spacings between the LEDs and the detector (e.g., 15-20 mm), while keeping probe sizes reasonable. The known and constant spacings between LEDs and detector provide for a constant depth of light penetration into the tissue from the skin surface. The LEDs 120, 122 and/or the

detector 124 are preferably disposed in the housing 118 such that the detector 124 is shielded from stray light.

The probe 102 further includes a first connector 126 that engages the computer system 112 to control the visible and NIR LEDs 120, 122, and a second connector 128
5 that engages to transmit the light signal from the detector 124 to the spectrometer 110.

In another embodiment, the visible and NIR light sources 104, 106 are generated remotely from the face of the probe, and a first fiber optic system is used to transmit the light to the face of the probe, and a second fiber optic system returns the light from the detector 124 to the spectrometer 110.

10 The probe 102 is configured to collect reflectance spectral information non-invasively. The probe may be used to collect, for example, reflectance optical spectra in humans or other mammals for medical diagnostics, exercise physiology, and the like.

In studies by the present inventors, it was found that Mox is effective for the early
15 detection of shock. In a recent study, spectra were acquired from the thenar muscles of 20 healthy human subjects to build a locally weighted regression (LWR) model that is used to provide real-time measurement of Mox in other subjects. LWR is a nonparametric learning algorithm that modifies a conventional linear or nonlinear least squares regression model by introducing a weighting scheme to give greater effect to
20 "local" data points, and less weight to more distant data points.

The spectral data from the healthy subjects were obtained using the following basic protocol. Each of the subjects was monitored using a system functionally similar to the monitoring system 100 shown in FIGURE 1, with the probe 102 disposed over the subject's thenar muscle group. The subject breathed room air and then 100% oxygen, and
25 then underwent a 15-minute period of arm ischemia produced by inflation of a blood pressure cuff on the upper arm while spectral data were collected.

The spectra from each subject were analyzed using Multivariate Curve Resolution (MCR). MCR is a group of spectral techniques known in the art. In the present method, MCR is used to yield accurate measurements of Mox from the spectra obtained from the
30 subjects. However, MCR is not practical for real-time measurements because of its iterative nature. The MCR values for Mox and the spectra were used to train an LWR model and produce the model, as described more fully below.

The currently preferred protocol for obtaining the spectral data from the healthy subjects, and the preferred method for generating a useful set of training data will now be described in more detail, with reference to the flow chart of FIGURE 4. First, datasets of spectra from healthy subjects are obtained 200. Each of the healthy subjects undergoes a
5 period of 3 minutes of rest while breathing air followed by 3 minutes of rest while breathing 100% oxygen. Ischemia is then produced in the subject's arm for a 15-minute period by inflation of a blood pressure cuff around the subject's upper arm to 200 mmHg or 60 mmHg above systolic blood pressure, whichever is less. A 5-minute recovery period follows to ensure normal circulation has been established. Spectra are acquired
10 throughout the protocol using a system functionally similar to the system 100 described above.

Second derivatives with respect to wavelength are taken of each spectrum 202. Using the second derivative of the spectra makes the distinctions between oxygenated and deoxygenated spectra greater and removes some of the effects of scattering.

15 In the current embodiment, only those portions of the collected spectra that correspond to the interesting portions of the spectrum are used in the analysis. As discussed above, the interesting peaks of the absorbance spectrum for oxyhemoglobin, deoxyhemoglobin, oxymyoglobin, and deoxymyoglobin are generally in the visible region around 580 nm (e.g., 570-588 nm) and in the near infrared region around 760 nm
20 (e.g., 740-777 nm). The selected interesting portions of each of the collected spectra are extracted and concatenated to produce spectra for analysis that include only the most relevant portions of the visible and NIR spectra 204. Including only the relevant portions of the spectrum in the analysis filters out noise and makes the analysis more sensitive to Mox.

25 More generally, in biologic tissues or samples, many optically active species may be present. In order to improve multi-wavelength spectral analysis, aspects of the spectrum information can be amplified over background spectrum information (or noise) by selection of desired spectrum regions. When these selected spectrum regions are concatenated into a new spectrum array, the information can be analyzed by conventional
30 methods of analysis, for example, by MCR, and the result is improved measurement of analyte concentrations of interest.

In the present specific example, the analysis of concatenated portions of the spectrum data around known peaks for oxymyoglobin, deoxymyoglobin, oxyhemoglobin,

and deoxyhemoglobin enhances the analysis using a spectral methods approach, such as MCR. Measurement of reflected light in the visible region around 580 nm yields information about concentrations of oxymyoglobin and oxyhemoglobin because these chromophores absorb in this region. Absolute quantification of myoglobin and hemoglobin saturation is made possible by the addition of measurements of concentrations of deoxymyoglobin and deoxyhemoglobin. These chromophores absorb in the NIR region around 760 nm.

Keeping and analyzing only wavelength regions around where the oxygenated and deoxygenated forms of myoglobin and hemoglobin absorb has proven effective. MCR is an iterative algorithm that uses least squares fitting to determine the shape of each chromophore spectrum present in the sample. The inventors believe that MCR is given a better chance of converging on the correct absorbance characteristics of the chromophores by removing or ignoring the less-interesting portions of the spectra, including the noise and absorbance from other chromophores present in tissue. The selection and concatenation of non-contiguous wavelength regions before spectral analysis would benefit the analysis of other types of spectrum data. For example, in another application it is contemplated that spectrum regions around cytochrome oxidase or cytochrome c absorbances may be selected to improve measurement of cytochrome oxidase or cytochrome c concentrations or oxidations.

The NIR portion of each concatenated spectrum is scaled up relative to the visible portion of the spectrum 206. The magnitudes of the NIR absorbances are much smaller than those of the visible absorbances. Although the scaling step is not critical to the present invention, without scaling the normalization may bias MCR toward erroneously oxygenated Mox values.

The concatenated spectra are then scaled (normalized) 208, for example, such that the areas under each of the curves is equal to one. Normalization is needed as a preparatory step for MCR. Mean centering is then applied to all spectra 210.

Mox must be determined from each spectrum in the training set. In the currently preferred method, MCR is used to obtain Mox values for each spectrum in a data set 212. MCR is an iterative spectral analysis method that can determine the individual concentrations of absorbing species within complex spectra. In one embodiment, in each iterative step MCR simultaneously quantifies concentrations of two components in each spectrum, oxyMbHb and deoxyMbHb, where by definition:

$$[\text{oxyMbHb}] = [\text{oxy myoglobin}] + [\text{oxyhemoglobin}],$$

and

$$[\text{deoxyMbHb}] = [\text{deoxy myoglobin}] + [\text{deoxyhemoglobin}].$$

In every iterative step, MCR also uses the current estimates of component
 5 concentrations to determine the shape of the pure component spectra. When MCR has
 converged on the shape of the pure component spectra of oxyMbHb and deoxyMbHb and
 their relative concentrations in each spectrum in the data set, Mox is calculated from each
 spectrum in a subject's data set as:

$$10 \quad Mox = \{ [\text{oxyMbHb}] / ([\text{oxyMbHb}] + [\text{deoxyMbHb}]) \} * 100 \quad (1)$$

Myoglobin saturation (Mb sat) may also be calculated from MCR determination
 of [oxyMb] and [deoxyMb]:

$$15 \quad Mb \text{ sat} = \{ [\text{oxyMb}] / ([\text{oxyMb}] + [\text{deoxyMb}]) \} * 100$$

The concentrations of [oxyHb] and [deoxyHb] can also be determined by MCR,
 resulting in a calculation of hemoglobin saturation (Hb sat):

$$20 \quad Hb \text{ sat} = \{ [\text{oxyHb}] / ([\text{oxyHb}] + [\text{deoxyHb}]) \} * 100$$

The concentrations [oxyMb], [deoxyMb], [oxyHb], and [deoxyHb] may be
 determined simultaneously using MCR. Hemoglobin saturation may be determined using
 MCR in tissues where myoglobin is not present.

25 If additional healthy subjects are to be included 214, the data collection proceeds
 with the additional subjects. Spectra from a number of subjects and associated Mox,
 Mb sat, or Hb sat values for each spectrum, determined with MCR, comprise the training
 set. The set of training data calculated from the test subjects may then be used to build an
 LWR model 216.

30 The LWR model provides for real-time monitoring or testing of patients or other
 subjects. As illustrated in FIGURE 5, when spectra are collected from a patient 220, the

spectra are preprocessed in the same way the LWR training set spectra were processed, including taking the second derivative 222, concatenating the selected portions 224, scaling the NIR portion 226, normalization 228, and mean centering 230.

In prior work, the present inventors have used partial least squares (PLS) to effectively measure myoglobin saturation in skeletal and cardiac muscle. Unfortunately, the global PLS method trained with spectra collected *in vitro* does not allow real-time measurement of Mox. The currently preferred method therefore uses locally weighted regression (LWR) with PLS to calculate Mox from spectra in real time. In particular, for each new spectrum acquired from a patient, LWR builds a local PLS model from spectra in the *in vivo* training set 232 that are most similar to the new spectrum. The LWR method does not allow, for example, training set spectra with vastly different melanin and lipid characteristics from the new spectrum to contribute to its Mox measurement. This essentially creates an effective filter for spectrum characteristics that reflect melanin and lipid content and that are unrelated to Mox. Mox is calculated from each test set spectrum by application of the local PLS model 234.

When spectra in the LWR training set encompass a wide range of physical characteristics found in patients, the robustness of the Mox measurement is improved by assuring that appropriate training set spectra are available. Good accuracy in human subjects has been demonstrated using LWR with a relatively small training set.

Other types of LWR models can be built to measure tissue oxygenation. Mb sat may be measured from spectra in the training set and used to build an LWR model that will measure Mb sat from patient spectra. Similarly, Hb sat may be measured from training set spectra collected from tissue that either does or does not contain myoglobin. These Hb sat values may be used with the training set spectra to build an LWR model that will measure Hb sat from patient spectra.

Examples

Muscle oxygenation is an early indicator of shock, and it can differentiate mild, moderate, and severe shock from the healthy state. In a study, Mox was measured as soon as possible after emergency department admission for 15 minutes in each of 57 trauma patients. Shock severity in each patient was determined using a scoring system based on maximum arterial lactate or base deficit, maximum heart rate, and minimum systolic blood pressure recorded during the first hour after the start of spectral acquisition

Mox during mild, moderate, and severe shock were significantly different from healthy controls ($p < 0.01$).

New Zealand white rabbits were subjected to hemorrhagic shock by withdrawal of blood in three increments. The key result is that Mox can detect the onset of shock. After transitioning the rabbit from 100% O₂ to air, Mox significantly decreased from baseline values after the first blood withdrawal. At this same point, lactate was still normal. Mox progressively decreased during moderate and severe shock, and then increased with resuscitation. Mox closely followed trends in mean arterial pressure (MAP) that was measured via a catheter placed in the carotid artery. Lactate levels rose during shock and decreased with resuscitation, but lagged in time behind changes in MAP and Mox. Arterial blood saturation measured by pulse oximetry was not sensitive to shock. This study demonstrates the potential of Mox to detect shock early and to continuously monitor patient condition during resuscitation.

Spectra were acquired continuously in a rabbit subjected to hemorrhagic shock by withdrawal of about 40% of the total blood volume in three equal increments. A four-component Multivariate Curve Resolution (MCR) analysis was used to simultaneously measure concentrations of oxymyoglobin, deoxymyoglobin, oxyhemoglobin, and deoxyhemoglobin. Calculation of Mb saturation and Hb saturation from each spectrum followed. Prior to MCR, all spectra were preprocessed with the second derivative, concatenation that allowed retention of specific parts of the visible and NIR wavelength regions, visible/NIR scaling, normalization, and mean centering.

Hb saturation and Mb saturation were measured separately *in vivo*. Hb saturation showed larger fluctuations with blood withdrawal (first three dotted lines) and with blood re-infusion than did Mb saturation. This is expected since hemoglobin concentrations temporarily change a great deal with rapid blood withdrawals and infusions.

Arterial (pulsatile and non-pulsatile) and venous blood contribute to the Hb saturation measurement. Measurement of Hb saturation does not require pulsatile blood flow, as in pulse oximetry. Mb saturation is a measurement of the fraction of myoglobin that is bound to oxygen. Myoglobin is contained within muscle cells, and Mb saturation is an intracellular measure of oxygenation.

Although the specific exemplary embodiment described above is directed to a currently preferred embodiment wherein the oxygenation of muscle is calculated, it will be readily apparent to persons of skill in the art that the method may be extended

according to the teachings in the present application to calculate the oxygenation of other tissues where there is no myoglobin, such as in the brain. In such applications rather than using the detected absorbance spectrum of muscle, the spectrum of a mixture of arterial, capillary, and venous hemoglobin could be analyzed generally using one or all of a
5 suitably sculpted light sources, concatenated spectrum segments, spectral method to develop training data, and an LWR method to determine the oxygenation of the tissue that is not pulsatile arterial blood. Measurement of hemoglobin saturation using the present invention would not depend on the presence of a pulsatile signal, as pulse oximetry does.

10 While illustrative embodiments have been illustrated and described, it will be appreciated that various changes can be made therein without departing from the spirit and scope of the invention.

CLAIMS

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A method for generating a set of training data that is useful for determining tissue oxygenation *in vivo*, comprising:

- (a) providing a first probe having:
 - (i) a first light source operable to emit light in a near infrared spectrum having a first radiant flux,
 - (ii) a second light source operable to emit light in a visible spectrum having a second radiant flux that is greater than the first radiant flux, and
 - (iii) a light detector;
- (b) for a subject, placing the probe such that the light detector detects absorbance spectra of light reflected from or transmitted through a tissue of the subject, the detected absorbance spectra resulting from light emitted by the first and second light sources;
- (c) using the detected absorbance spectra to calculate sums of a concentration of oxymyoglobin and a concentration of oxyhemoglobin in the tissue [oxyMbHb];
- (d) using the detected absorbance spectra to calculate sums of a concentration of deoxymyoglobin and a concentration of deoxyhemoglobin in the tissue [deoxyMbHb];
- (e) calculating oxygenations of the tissue from [oxyMbHb] and [deoxyMbHb];
- (f) storing on a computer readable media:
 - (i) data calculated from the detected absorbance spectra, and
 - (ii) the calculated tissue oxygenations; and
- (g) repeating steps (b)-(f) for a plurality of subjects to generate a set of training data.

2. The method of Claim 1, wherein the second radiant flux is at least an order of magnitude greater than the first radiant flux.

3. The method of Claim 1, wherein the probe has a distal face, and further wherein the first and second light sources emit light from the distal face.

4. The method of Claim 1, wherein the second light source comprises a plurality of lighting elements that cooperatively produce the second radiant flux.

5. The method of Claim 4, wherein the plurality of lighting elements comprise light-emitting diodes.

6. The method of Claim 4, wherein the first and second light sources each emit light from positions on the distal face of the probe that are disposed along a circular arc.

7. The method of Claim 5, wherein the light-emitting diodes further comprise a phosphor coating that causes the light-emitting diodes to emit light having a peak intensity wavelength that is in the range bounded by a peak absorbance wavelength of myoglobin and a peak absorbance wavelength of hemoglobin.

8. The method of Claim 1, wherein the first light source emits near infrared light having a wavelength that is in a range bounded by a peak absorbance wavelength of myoglobin and a peak absorbance wavelength of hemoglobin.

9. The method of Claim 1, further comprising the step of extracting a first continuous portion of the detected absorbance spectrum and a second continuous portion of the detected absorbance spectrum, and wherein the step of using the detected absorbance spectrum to calculate [oxyMbHb] uses only the first continuous portion and the second continuous portion.

10. The method of Claim 9, wherein the first continuous portion includes the visible wavelength 580 nm, and the second continuous portion includes the near-infrared wavelength 760 nm.

11. The method of Claim 10, wherein the first continuous portion and the second continuous portion are concatenated prior to calculating [oxyMbHb] and [deoxyMbHb].

12. The method of Claim 11, wherein [oxyMbHb] and [deoxyMbHb] are calculated simultaneously.

13. The method of Claim 1, wherein the first light source and the second light sources emit light periodically and synchronously.

14. A method for determining tissue oxygenation *in vivo* comprising:

- (a) providing a first probe having:
 - (i) a first light source operable to emit light in a near infrared spectrum having a first radiant flux,
 - (ii) a second light source operable to emit light in a visible spectrum having a second radiant flux that is greater than the first radiant flux, and
 - (iii) a light detector;
- (b) for a subject, placing the first probe such that the light detector is operable to detect absorbance spectra of light reflected from or transmitted through a tissue of the subject, the detected absorbance spectra resulting from light emitted by the first and second light sources;
- (c) using the detected absorbance spectra to calculate sums of a concentration of oxymyoglobin and a concentration of oxyhemoglobin in the tissue [oxyMbHb];
- (d) using the detected absorbance spectra to calculate sums of a concentration of deoxymyoglobin and a concentration of deoxyhemoglobin in the tissue [deoxyMbHb];
- (e) calculating tissue oxygenations for the subject from [oxyMbHb] and [deoxyMbHb];
- (f) repeating steps (b)-(e) for a plurality of subjects to generate a training set of data;
- (g) providing a second probe operably similar to the first probe and having a similar first and second light source and a similar light detector, and placing the probe such that the similar light detector is operable to detect an absorbance spectrum of light reflected or transmitted through a tissue of a patient and emitted by the similar first and second light sources; and

(h) calculating a tissue oxygenation for the patient from the training set of data and the absorbance spectrum detected by the second probe.

15. The method of Claim 14, wherein the second radiant flux is at least an order of magnitude greater than the first radiant flux.

16. The method of Claim 14, wherein each of the subject tissue is muscle and the patient tissue is muscle.

17. The method of Claim 14, wherein the second light source comprises a plurality of lighting elements that cooperatively produce the second radiant flux.

18. The method of Claim 17, wherein the plurality of lighting elements comprise light-emitting diodes.

19. The method of Claim 14, wherein the training set of data is used to construct a locally weighted regression model, and the tissue oxygenation for the patient is calculated by applying the locally weighted regression model to data detected by the second probe.

20. The method of Claim 18, wherein the light-emitting diodes further comprise a phosphor coating that causes the light-emitting diodes to emit light having a peak intensity wavelength that is in the range bounded by a peak absorbance wavelength of myoglobin and a peak absorbance wavelength of hemoglobin.

21. The method of Claim 14, wherein the first light source emits near infrared light having a wavelength that is in a range bounded by a peak absorbance wavelength of myoglobin and a peak absorbance wavelength of hemoglobin.

22. The method of Claim 14, further comprising the step of extracting a first continuous portion of the absorbance spectrum detected by the second probe and a second continuous portion of the absorbance spectrum detected by the second probe, and wherein the step of calculating the tissue oxygenation for the patient from the training set of data and the absorbance spectrum detected by the second probe uses only the first and second continuous portions of the absorbance spectrum detected by the second probe.

23. The method of Claim 22, wherein the first continuous portion includes the visible wavelength 580 nm and the second continuous portion includes the near infrared wavelength 760 nm.

24. The method of Claim 23, wherein the first continuous portion and the second continuous portion are concatenated prior to calculating tissue oxygenation.

25. The method of Claim 14, wherein the second probe is operable to monitor a patient for detecting circulatory shock.

26. The method of Claim 14, wherein the first light source and the second light sources emit light periodically and synchronously.

27. A method for generating a set of training data that is useful for determining tissue oxygenation *in vivo*, comprising:

- (a) providing a first probe having:
 - (i) a first light source operable to emit light in a near infrared spectrum having a first radiant flux,
 - (ii) a second light source operable to emit light in a visible spectrum having a second radiant flux that is greater than the first radiant flux, and
 - (iii) a light detector;
- (b) for a subject, placing the first probe such that the light detector detects absorbance spectra of light reflected from or transmitted through a tissue of the subject resulting from light emitted by the first and second light sources;
- (c) using the detected absorbance spectra to calculate oxygenation of a portion of the tissue that is not solely pulsatile arterial blood;
- (d) storing on computer readable media:
 - (i) data calculated from the detected absorbance spectra, and
 - (ii) the calculated tissue oxygenations; and
- (e) repeating steps (b)-(d) for a plurality of subjects to generate a set of training data.

28. The method of Claim 27, wherein the second radiant flux is at least an order of magnitude greater than the first radiant flux.

29. The method of Claim 27, wherein the step of using the detected absorbance spectra to calculate an oxygenation of the portion of the tissue that is not solely pulsatile arterial blood comprises using the detected absorbance spectra to: (i) calculate concentrations of oxymyoglobin and concentrations of deoxymyoglobin in the tissue to determine myoglobin saturation; (ii) calculate concentrations of oxyhemoglobin and concentrations of deoxyhemoglobin in the tissue to determine hemoglobin saturation; or (iii) calculating a value representing the sum of the concentration of oxymyoglobin and the concentration of oxyhemoglobin [oxyMbHb] and calculating a value representing the sum of deoxymyoglobin and deoxyhemoglobin [deoxyMbHb], and using the calculated [oxyMbHb] and [deoxyMbHb] to determine oxygenation of the tissue.

30. The method of Claim 27, wherein the subject is a mammal.

31. A probe for detecting *in vivo* tissue oxygenation in a subject comprising:

(a) a first light source operable to emit light in a near infrared spectrum having a first radiant flux,

(b) a second light source operable to simultaneously emit light in a visible spectrum having a second radiant flux that is greater than the first radiant flux, and

(c) a light detector operable to detect absorbance spectra of light reflected from or transmitted through a tissue of the subject resulting from light emitted by the first and second light sources.

32. The probe of Claim 31, wherein the second radiant flux is at least an order of magnitude greater than the first radiant flux.

33. The probe of Claim 31, wherein the second light source comprises a plurality of lighting elements wherein the plurality of lighting elements cooperatively produce the second radiant flux.

34. The probe of Claim 33, wherein the first light source comprises a single lighting element, and the second light source comprises at least ten lighting elements, and wherein the second radiant flux is at least an order of magnitude greater than the first radiant flux.

35. The probe of Claim 34, wherein the single lighting element and the at least ten lighting elements are all disposed an equal distance from the light detector.

36. The probe of Claim 31, wherein the first light source comprises at least one NIR wavelength lighting element and the second light source comprises at least ten visible wavelength lighting elements, and further wherein the at least ten visible wavelength lighting elements are disposed along a circular arc centered on the light detector.

37. The probe of Claim 36, wherein the circular arc defined by the at least ten visible wavelength lighting elements extends not more than 90°.

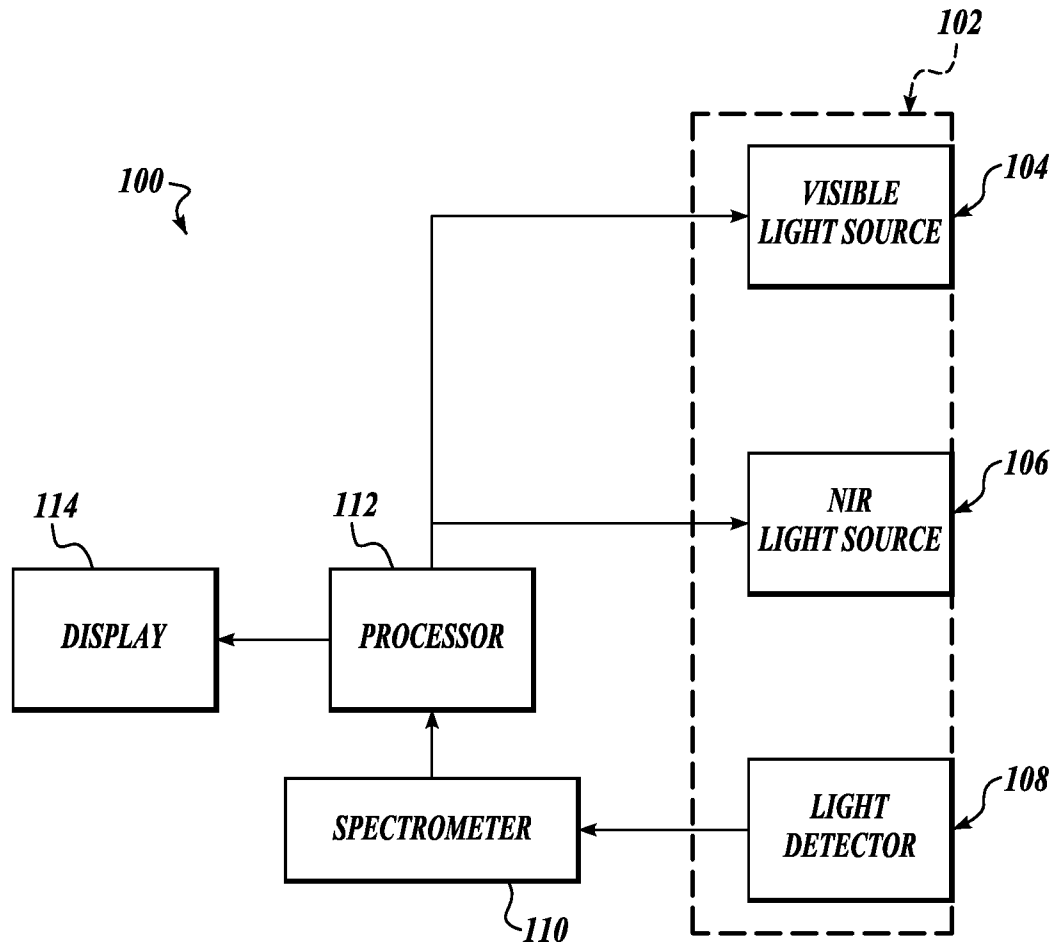


Fig. 1.

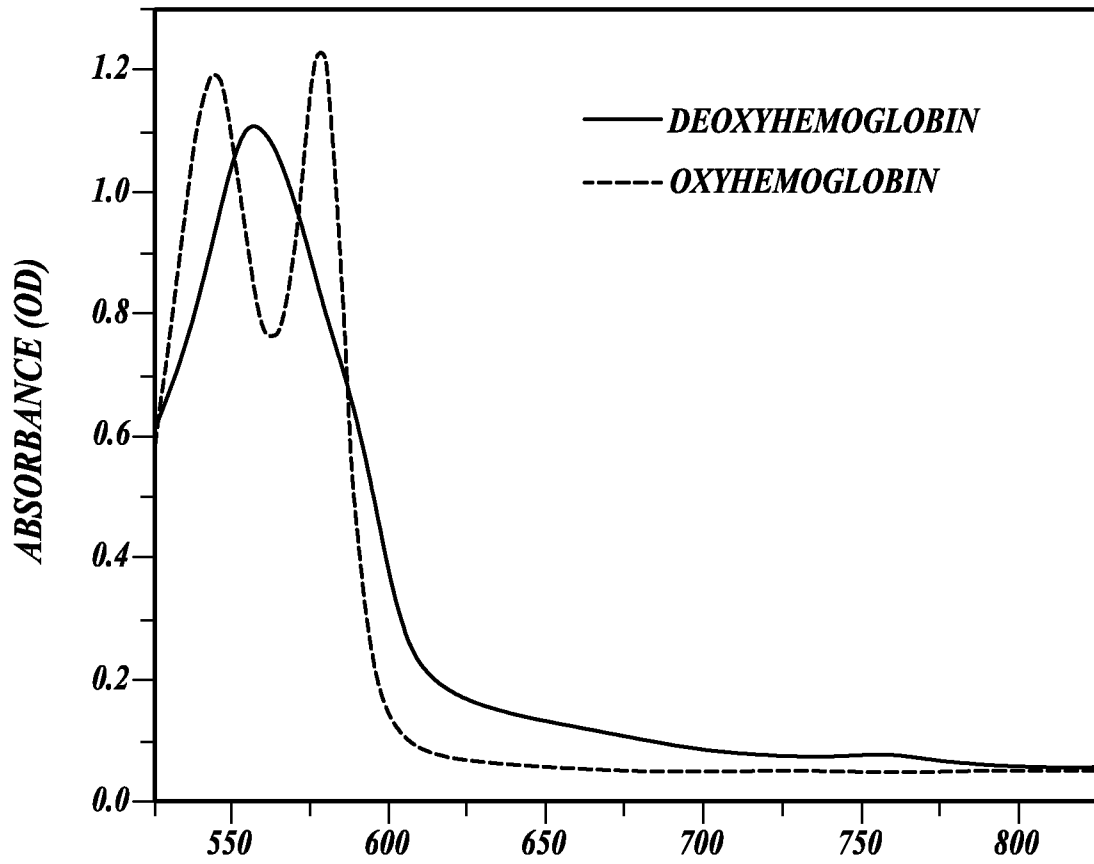


Fig.2.

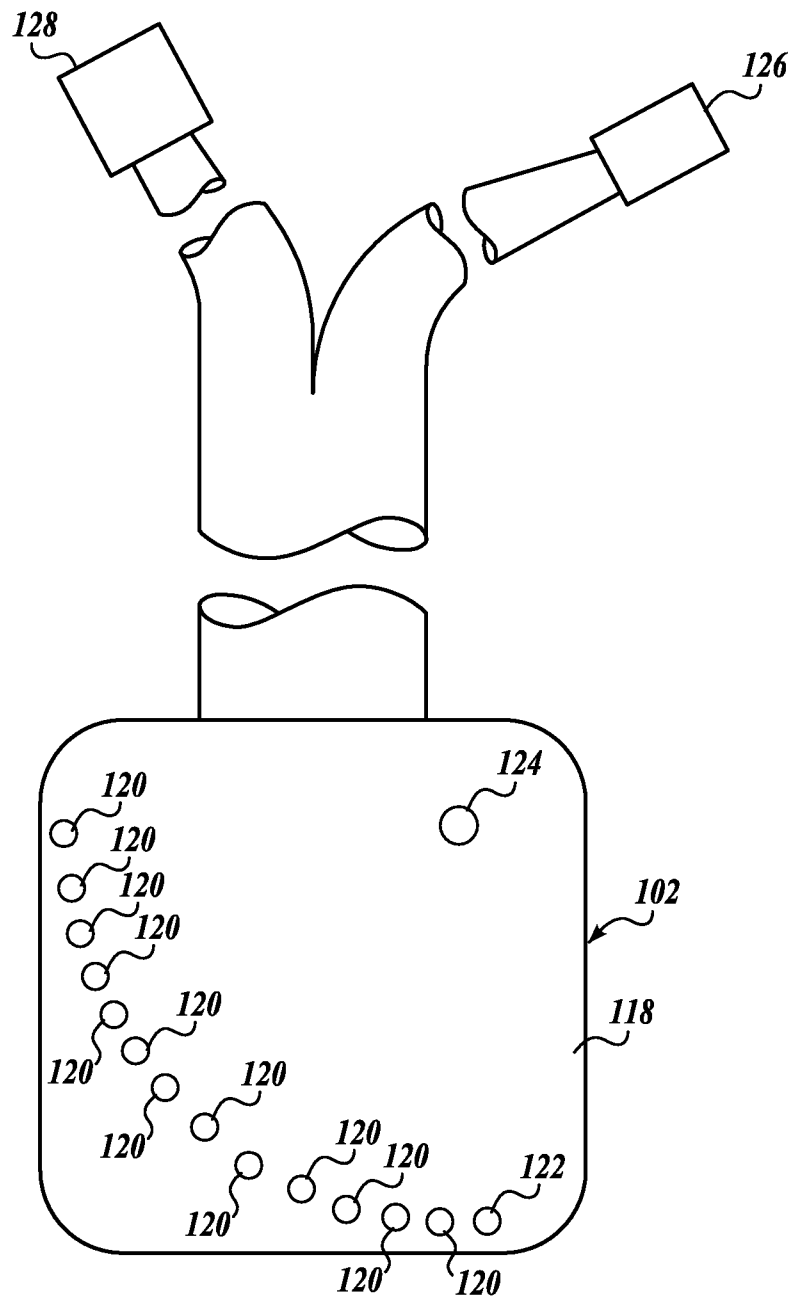


Fig. 3.

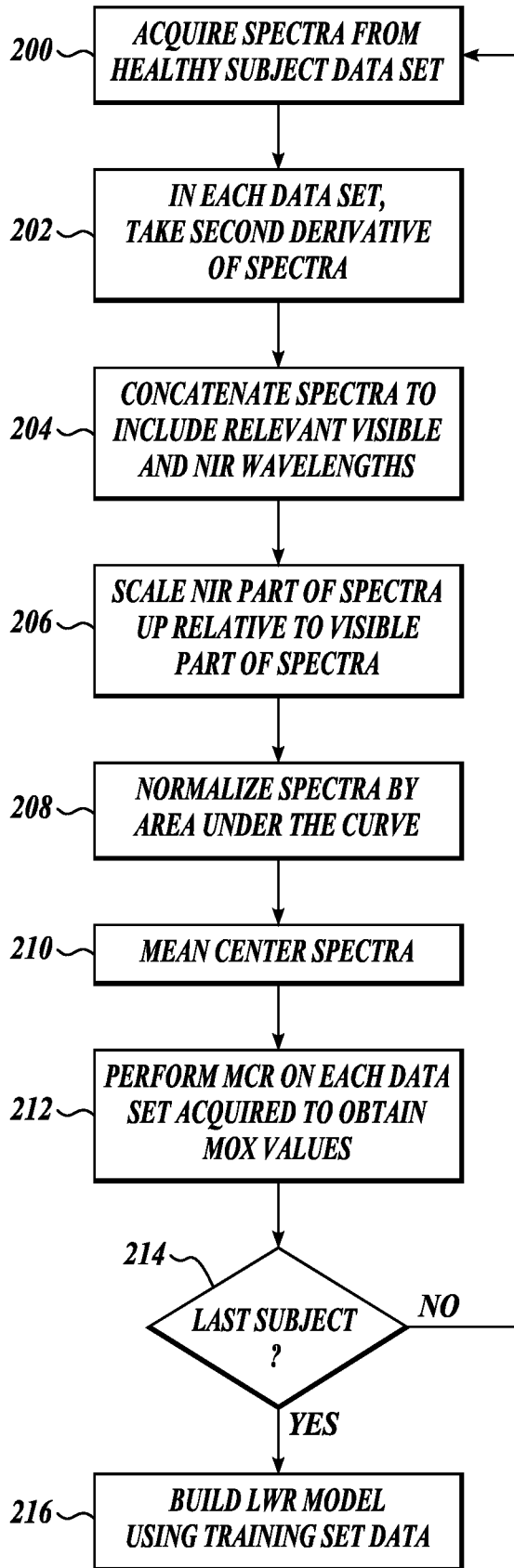


Fig. 4.

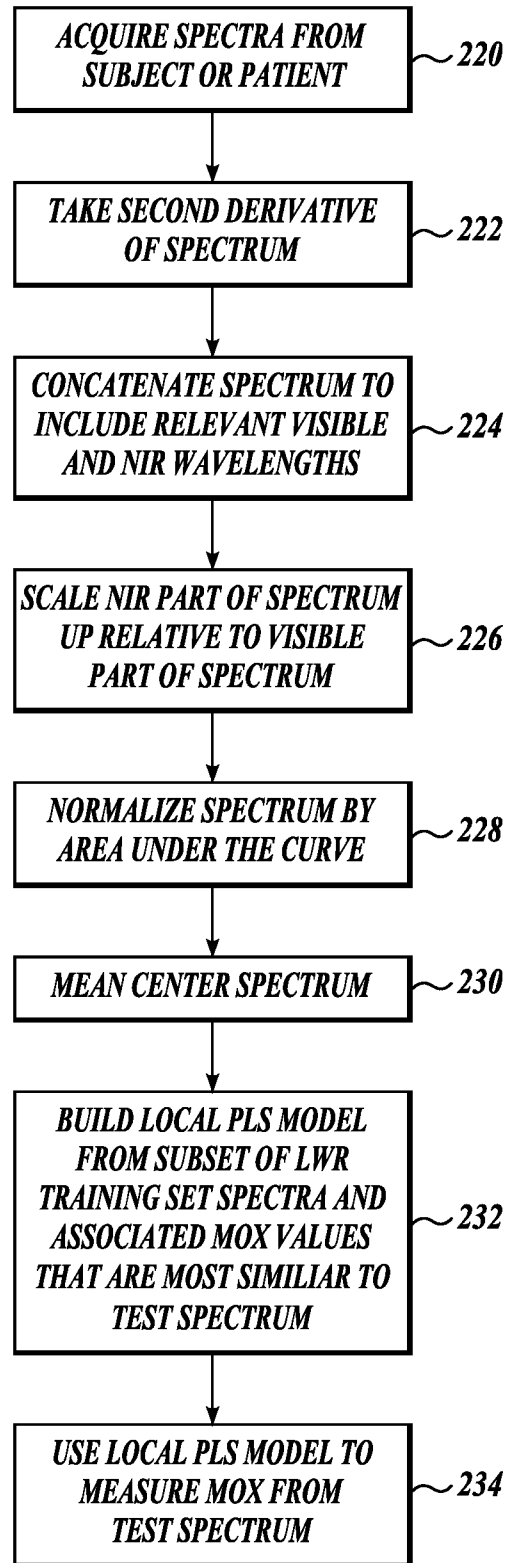


Fig. 5.