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(54) MEASUREMENT OF A FLUORESCENT ANALYTE USING TISSUE EXCITATION

MESSUNG EINES FLUORESZIERENDEN ANALYTEN MITTELS GEWEBEERREGUNG

MESURE D'UN ANALYTE FLUORESCENT PAR EXCITATION TISSULAIRE

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Description

TECHNICAL FIELD

[0001] An apparatus and method for measurement of one or more fluorescent analyte concentration(s) in the blood of a patient by exciting the blood and the analyte at two wavelengths. More particularly, the apparatus and method measure the concentration of erythrocyte zinc protoporphyrin (referred to herein as "eZnPP" or "ZnPP") and erythrocyte protoporphyrin IX (referred to herein as "ePP" or "PP") in the red blood cells of a patient.

BACKGROUND

[0002] Iron deficiency remains the most common form of malnutrition worldwide, increasing the risk of disability and death among more than two billion people. Lack of iron causes anemia, decreases physical capabilities, impairs cognitive and behavioral development, compromises immune responsiveness and when severe, increases mortality during infancy and childhood. Iron supplements are needed for prevention of iron deficiency in those with increased iron requirements, especially infants, children and women of childbearing age, and for correction of iron deficiency anemia in all affected individuals.

[0003] However, in areas with endemic malaria, untargeted iron supplementation is no longer recommended as a means of providing additional iron because an increased risk of hospitalization and death was found in a trial of universal iron and folic acid supplementation for preschool children in Pemba, Tanzania. Using an elevated eZnPP/heme molar ratio (> 80 (μmol/mol heme) as the criterion for iron deficiency, iron-*deficient* children were found to benefit from supplementation. Their risk of severe illness and death decreased by 38%. In contrast, iron-*replete* children were harmed by supplementation. In fact, their risk of severe illness and death increased by 63% after iron supplementation. *See, e.g.,* Sazawal S. et al., Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised, placebo-controlled trial. Lancet 2006; 367: 133-143. In view of this risk, a World Health Organization (WHO) Consultation recommended that, in malaria-endemic areas, (i) iron supplements should be given to children only after screening for iron deficiency and (ii) the measurement of eZnPP was the preferred indicator for identifying iron-deficient children who could benefit from iron supplementation. *See,* WHO Conclusions and recommendations of the WHO Consultation on prevention and control of iron deficiency in infants and young children in malaria-endemic areas. Food Nutr Bull 2007; 28: S621-7.

[0004] In resource-limited settings, like those in regions with endemic malaria, the use of the existing front-face hematofluorometer technique for measurement of eZnPP is constrained by the requirement for a blood sample obtained by finger- or venipuncture, the necessity for a trained technician for operation, use of an electrical power supply, a need for frequent recalibration and expense. Other currently available means of assessing iron status also require blood samples and even more complex and costly laboratory facilities and processing. Because of the lack of means to determine iron status, the effective result of the WHO recommendation has been the cessation of programs of iron supplementation in almost all malarial areas.

[0005] Thus, there is a need for a new technique that overcomes the technical difficulties of existing invasive techniques for identifying those individuals in malarial areas who would benefit from iron supplementation to permit safe and effective prevention and correction of iron deficiency, while avoiding harm to those who are iron replete.

[0006] Globally, 30% to 70% of the populations in developing countries are iron deficient, with the highest prevalence among persons who have diets low in bio-available iron. In developed countries, despite increased amounts of dietary bio-available iron, iron nutrition nevertheless remains a problem in subpopulations with the highest iron requirements, especially among infants, children and women of childbearing age. Without iron supplementation, most women will become iron deficient during pregnancy. Thus, screening for iron deficiency is a crucial component of healthcare. Initially, iron deficiency may be asymptomatic or produce only nonspecific manifestations, such as weakness and easy fatigability. As iron deficiency becomes more severe, anemia develops and progressively restricts work capacity and tolerance of physical exertion. Early detection of iron deficiency permits prompt recognition and management of underlying causes. Most commonly, a diet with inadequate amounts of bio-available iron is responsible. In these individuals, iron deficiency may be corrected by nutritional approaches, such as consuming iron-rich food as well as food which helps the body absorb iron more effectively, such as food high in vitamin C, or by iron supplementation.

[0007] Thus, there is also a need for periodic iron monitoring in a safe, effective manner without the need for a blood sample. There is also a need to provide a technique and apparatus that can be used as a point-of-care screening device for iron deficiency in pediatric, obstetric and medical facilities, and in blood donation centers worldwide, and by individuals to monitor their own iron status in their homes or portably, without the need to be in a clinical setting. Because eZnPP is also elevated in lead poisoning, a noninvasive method would be useful for screening those people at risk from occupational or environmental exposure.

[0008] There is also a need to provide a technique and apparatus for measuring the concentration of an analyte in

the blood of a patient. For example, in certain settings - such as a hospital or clinical environment with access to sterile conditions and adequate equipment - it is acceptable and/or desirable to analyze iron levels in the blood. A technique and apparatus which provides greater accuracy and consistency when compared with existing techniques is needed.

[0009] Document WO 2011/063032 relates to detection of skin intrinsic fluorescence.

SUMMARY

[0010] An apparatus according to the invention is defined in claim 1. A method according to the invention is defined in claim 22.

[0011] In some embodiments, a tunable filter unit is provided which excites the blood and the analyte(s) at the first wavelength range and the second wavelength range. In some embodiments, the tunable filter unit includes a first optical filter and a second optical filter, the first and second optical filters are capable of independent variation of the angle of incidence of light provided by the light source. In some embodiments, the tunable filter unit includes two tunable bandpass filters, (such as Semrock Versachrome® filters). In some embodiments, the tunable filter unit includes a first optical filter and a second optical filter and a third optical element to correct for offset of the light passing through the first and second optical filters.

[0012] In some embodiments, the apparatus further includes one or more optical filters, wherein the emission spectra of the fluorescent analyte defines a wavelength range, and wherein the detector includes one or more light sensitive elements receiving light through the one or more optical filters transmitting light in the wavelength range of the emission spectra of the fluorescent analyte.

[0013] In some embodiments, the emission spectra of the fluorescent analyte define an emission maximum, wherein a first portion of the detector receives light through the optical filters transmitting light in the wavelength range of the emission spectra of the fluorescent analyte, and wherein a second portion of the detector receives light through optical filters transmitting light in a wavelength range outside the emission maximum of the fluorescent analyte.

[0014] In some embodiments, the light source is a lamp, one or more laser diodes, or one or more light emitting diodes.

[0015] In some embodiments, the apparatus further includes an optical fiber associated with the light source. In some embodiments, the apparatus further includes an optical fiber associated with the detector.

[0016] In some embodiments, the apparatus further includes a probe including an optical fiber associated with the light source and an optical fiber associated with the detector. In some embodiments, the probe includes a plurality of optical fibers associated with the light source surrounding an optical fiber associated with the detector.

[0017] In some embodiments, the interfiber spacing of the optical fiber associated with the light source and the optical fiber associated with the detector is selected such that the derived signal is insensitive to the blood volume fraction.

[0018] In some embodiments, the interfiber spacing of the optical fiber associated with the light source and the optical fiber associated with the detector is selected to achieve a maximum detection sensitivity at a selected depth of the tissue. In some embodiments, the selected depth of the tissue is selected as the depth having the highest expected concentration of the fluorescent analyte.

[0019] In some embodiments, the apparatus further includes a power source. In some embodiments, the power source is a rechargeable battery.

[0020] In some embodiments, the apparatus includes a housing adapted to receive the detector, the processor and the power source. In some embodiments, the housing is less than 6 inches in length.

[0021] In some embodiments, the apparatus includes an output component. In some embodiments, the apparatus includes a housing adapted to receive the detector, the processor and the output component. In some embodiments, the apparatus the output component is a display screen, a speaker or a vibrator.

[0022] In some embodiments, the output component provides an indication of the concentration of analyte in the tissue. In some embodiments, the output component provides an indication that the concentration of analyte exceeds a predetermined threshold.

[0023] In some embodiments, the apparatus includes memory storing at least one previous concentration of analyte, and wherein the output component is adapted to provide an indication that the concentration of analyte is increasing or decreasing from the previous concentration of analyte.

[0024] In some embodiments, the apparatus includes a communications component. In some embodiments, the communications component comprises an RF transmitter, a USB connector, an IR transmitter, a cellular phone or a WiFi transmitter.

[0025] A system for noninvasive measurement of a concentration of a fluorescent analyte in the blood of a patient is provided that includes the apparatus described hereinabove, and a monitor unit, wherein the communications component provides an output signal relating to the concentration of analyte to the monitor unit.

[0026] In some embodiments, the processor is adapted to provide the output signal to the monitor unit when the analyte concentration exceeds a predetermined threshold.

[0027] In some embodiments, the monitor unit comprises a user interface. In some embodiments, the user interface

provides an indication of the concentration of analyte in the tissue. In some embodiments, the user interface provides an indication that the concentration of analyte exceeds a predetermined threshold. In some embodiments, the monitor unit comprises memory storing at least one previous concentration of analyte, and wherein the user interface provides an indication that the concentration of analyte is increasing or decreasing from the previous concentration of analyte. In some embodiments, the user interface provides the user with the option of storing a health goal related to the concentration of analyte, and wherein the user interface provides an indication of a trend towards or away from the health goal.

[0028] In some embodiments, the user interface provides a treatment suggestion related to the concentration of analyte. In some embodiments, the treatment suggestion comprises ingestion of a quantity of nutrition. In some embodiments, the treatment suggestion comprises administration of a quantity of a pharmaceutical compound.

[0029] In some embodiments, the monitor unit is portable. In some embodiments, the monitor unit is a personal computer. In some embodiments, the monitor unit is a telephone.

[0030] An apparatus for noninvasive measurement of a concentration of erythrocyte zinc protoporphyrin (eZnPP) as the eZnPP/heme ratio in the blood of a patient is provided which includes a light source for providing excitation of the tissue at a first wavelength range and a second wavelength range, the first excitation wavelength range selected at the excitation peak of eZnPP and the second excitation wavelength range selected so that the absorbance of blood is similar to that of the first excitation wavelength range; one or more detectors for detecting a portion of the emission spectra at the first excitation wavelength range and the second excitation wavelength range; and a processor for determining the concentration of eZnPP based on the difference between the portion of the emission spectra excited at the first excitation wavelength range and the second excitation wavelength range.

[0031] In some embodiments, the apparatus is for measurement of a concentration of eZnPP in whole blood.

[0032] In some embodiments, a tunable filter unit is provided which excites the blood and eZnPP at the first wavelength range and the second wavelength range. In some embodiments, the tunable filter unit includes a first optical filter and a second optical filter, the first and second optical filters capable of independent variation of the angle of incidence of light provided by the light source. In some embodiments, the tunable filter unit includes two tunable bandpass filters, (such as Semrock Versachrome® filters). In some embodiments, the tunable filter unit includes a first optical filter and a second optical filter and a third optical element to correct for offset of the light passing through the first and second optical filters.

[0033] In some embodiments, the apparatus further includes one or more optical filters, wherein the emission spectra of eZnPP defines a wavelength range, and wherein the detector includes one or more light sensitive elements receiving light through the one or more optical filters transmitting light in the wavelength range of the emission spectra of eZnPP.

[0034] In some embodiments, the emission spectra of eZnPP define an emission maximum, wherein a first portion of the detector receives light through the optical filters transmitting light in the wavelength range of the emission spectra of eZnPP, and wherein a second portion of the detector receives light through optical filters transmitting light in a wavelength range outside the emission maximum of eZnPP.

[0035] In some embodiments, the light source is a lamp, one or more laser diodes, or one or more light emitting diodes.

[0036] In some embodiments, the apparatus further includes an optical fiber associated with the light source. In some embodiments, the apparatus further includes an optical fiber associated with the detector.

[0037] In some embodiments, the apparatus further includes a probe including an optical fiber associated with the light source and an optical fiber associated with the detector. In some embodiments, the probe includes a plurality of optical fibers associated with the light source surrounding an optical fiber associated with the detector.

[0038] In some embodiments, the interfiber spacing of the optical fiber associated with the light source and the optical fiber associated with the detector is selected such that the derived signal is insensitive to the blood volume fraction.

[0039] In some embodiments, the interfiber spacing of the optical fiber associated with the light source and the optical fiber associated with the detector is selected to achieve a maximum detection sensitivity at a selected depth of the tissue. In some embodiments, the selected depth of the tissue is selected as the depth having the highest expected concentration of eZnPP.

[0040] In some embodiments, the apparatus further includes a power source. In some embodiments, the power source is a rechargeable battery.

[0041] In some embodiments, the apparatus includes a housing adapted to receive the detector, the processor and the power source. In some embodiments, the housing is less than 6 inches in length.

[0042] In some embodiments, the apparatus includes an output component. In some embodiments, the apparatus includes a housing adapted to receive the detector, the processor and the output component. In some embodiments, the output component is a display screen, a speaker or a vibrator.

[0043] In some embodiments, the output component provides an indication of the concentration of eZnPP in the tissue. In some embodiments, the output component provides an indication that the concentration of eZnPP exceeds a predetermined threshold.

[0044] In some embodiments, the apparatus includes memory storing at least one previous concentration of eZnPP, and wherein the output component is adapted to provide an indication that the concentration of eZnPP is increasing or

decreasing from the previous concentration of analyte.

[0045] In some embodiments, the apparatus includes a communications component. In some embodiments, the communications component comprises an RF transmitter, a USB connector, an IR transmitter, a cellular phone or a WiFi transmitter.

[0046] A system for noninvasive measurement of a concentration of eZnPP in the blood of a patient is provided which includes the apparatus described hereinabove, and a monitor unit, wherein the communications component provides an output signal relating to the concentration of eZnPP to the monitor unit.

[0047] In some embodiments, the processor is adapted to provide the output signal to the monitor unit when the eZnPP concentration exceeds a predetermined threshold.

[0048] In some embodiments, the monitor unit comprises a user interface. In some embodiments, the user interface provides an indication of the concentration of eZnPP in the tissue. In some embodiments, the user interface provides an indication that the concentration of eZnPP exceeds a predetermined threshold. In some embodiments, the monitor unit comprises memory storing at least one previous concentration of eZnPP, and wherein the user interface provides an indication that the concentration of eZnPP is increasing or decreasing from the previous concentration of eZnPP. In some embodiments, the user interface provides the user with the option of storing a health goal related to the concentration of eZnPP, and wherein the user interface provides an indication of a trend towards or away from the health goal.

[0049] In some embodiments, the user interface provides a treatment suggestion related to the concentration of eZnPP. In some embodiments, the treatment suggestion comprises ingestion of a quantity of nutrition. In some embodiments, the treatment suggestion comprises administration of a quantity of a pharmaceutical compound.

[0050] In some embodiments, the monitor unit is portable. In some embodiments, the monitor unit is a personal computer. In some embodiments, the monitor unit is a telephone.

[0051] An apparatus for simultaneous measurement of a concentration of erythrocyte zinc protoporphyrin (eZnPP) and erythrocyte protoporphyrin IX (ePP) as the eZnPP/heme ratio and ePP/heme ratio in the blood of a patient is provided including a light source for providing excitation of the tissue at a first wavelength range and a second wavelength range, the first excitation wavelength range selected at the excitation peak of eZnPP and the second excitation wavelength range selected so that the absorbance of blood is similar to that of the first excitation wavelength range; one or more detectors for detecting a portion of the emission spectra at the first excitation wavelength range and the second excitation wavelength range; and a processor for determining the concentration of eZnPP and ePP based on the difference between the portion of the emission spectra excited at the first excitation wavelength range and the second excitation wavelength range.

[0052] An apparatus for noninvasive measurement of a concentration of erythrocyte zinc protoporphyrin (eZnPP) as the eZnPP/heme ratio in the blood of a patient is provided including a light source for providing excitation of the tissue at about 425 nm and about 407 nm; a detector for detecting a portion of the emission spectra excited at about 425 nm and about 407 nm; and a processor for determining the concentration of eZnPP based on the difference between the portion of the emission spectra excited at about 425 nm and about 407 nm.

[0053] An apparatus for measurement of a concentration of erythrocyte zinc protoporphyrin (eZnPP) as the eZnPP/heme ratio in the blood of a patient is provided including a light source for providing excitation of the tissue at about 425 nm and about 407 nm; a detector for detecting a portion of the emission spectra excited at about 425 nm and about 407 nm; and a processor for determining the concentration of eZnPP based on the difference between the portion of the emission spectra excited at about 425 nm and about 407 nm.

BRIEF DESCRIPTION OF FIGURES

[0054]

Figure 1 is a schematic view of an apparatus in accordance with an exemplary embodiment of the subject matter described herein.

Figure 1A is a simplified schematic view of an apparatus in accordance with an exemplary embodiment of the subject matter described herein.

Figure 1B is a simplified schematic view of an apparatus, indicating a first spacing between an excitation fiber and a detection fiber, in accordance with an exemplary embodiment of the subject matter described herein.

Figure 1C is a simplified schematic view of an apparatus, indicating a second spacing between an excitation fiber and a detection fiber, in accordance with an exemplary embodiment of the subject matter described herein.

Figure 2 is a view of an apparatus in a free-beam instrumental configuration in accordance with another exemplary

embodiment of the subject matter described herein, adapted for measurement of patient blood samples or tissue.

Figure 3 illustrates the excitation spectra of eZnPP bound to oxyhemoglobin.

Figure 4 illustrates the emission spectra of eZnPP bound to oxyhemoglobin.

Figure 5 is a perspective view of a fiber optic probe head in accordance with an exemplary embodiment of the subject matter described herein.

Figure 6 is a cross-sectional view of tissue receiving the stimulation from the light source of an exemplary apparatus as disclosed in the subject matter herein.

Figure 7 illustrates the tissue autofluorescence spectrum from oral mucosa obtained using an exemplary apparatus as disclosed in the subject matter herein.

Figure 8 illustrates the normalized blood absorbance and eZnPP excitation spectrum.

Figure 9 is a perspective view of an apparatus and tissue phantom in accordance with an exemplary embodiment of the subject matter described herein.

Figure 10 illustrates the excitation spectra for collagen with and without blood.

Figure 11 illustrates the emission spectra for the simple tissue phantom of the oral mucosa.

Figure 12 illustrates the emission spectra for the difference spectrum for alternating two-wavelength fluorescence excitation.

Figure 13 illustrates a comparison of the output of an apparatus in accordance with an exemplary embodiment shown in Figure 2 (on the vertical axis) with those provided by an Aviv hematofluorometer (on the horizontal axis) in measurements of the eZnPP/heme ratio on a series of patient blood samples.

Figure 14 is a side view of another apparatus in accordance with an exemplary embodiment of the subject matter described herein.

Figure 15 is a side view of a further apparatus in accordance with an exemplary embodiment of the subject matter described herein.

Figure 16 is a schematic view of an apparatus in accordance with an exemplary embodiment of the subject matter described herein.

Figure 17 is a view of an apparatus in a free-beam instrumental configuration in accordance with another exemplary embodiment of the subject matter described herein, adapted for measurement of patient blood samples or tissue.

Figure 18 is a view of a portion of an apparatus in accordance with another exemplary embodiment of the subject matter described herein.

Figure 19 is a schematic view of the apparatus of Figure 18 in accordance with an exemplary embodiment of the subject matter described herein.

Figure 20 illustrates the transmission of light through the apparatus of Figure 18 in accordance with an exemplary embodiment of the subject matter described herein.

Figure 21 is a schematic view of a portion of an apparatus in accordance with an exemplary embodiment of the subject matter described herein.

Figures 22-23 are schematic views of a portion of an apparatus in accordance with an exemplary embodiment of the subject matter described herein.

Figure 24 is a schematic view of a portion of an apparatus in accordance with an exemplary embodiment of the subject matter described herein.

Figures 25-30 illustrate the excitation and emissions spectra of tissue measured in accordance with an exemplary embodiment of the subject matter described herein.

Figures 31-36 illustrate a comparison of conventional techniques with the results obtained in accordance with an exemplary embodiment of the subject matter described herein.

Figure 37 illustrates an emission spectrum in accordance with an exemplary embodiment of the subject matter described herein.

Figure 38 illustrates an emission spectrum in accordance with an exemplary embodiment of the subject matter described herein.

Figure 39 illustrates a difference spectrum in accordance with an exemplary embodiment of the subject matter described herein.

Figures 40-41 illustrate a correlation between the eZnPP/heme ratio obtained by a hematofluorometer and by HPLC.

Figures 42-43 illustrate a correlation between the measured fluorescence intensity at 593 nm evaluated by HPLC and a method in accordance with an exemplary embodiment of the subject matter described herein.

Figures 44-45 illustrate a correlation between the measured fluorescence intensity of the difference spectrum at 593 nm evaluated by HPLC and a method in accordance with an exemplary embodiment of the subject matter described herein.

Figures 46-47 illustrate a correlation between the measured fluorescence intensity of the difference spectrum at 627 nm evaluated by a method in accordance with an exemplary embodiment of the subject matter described herein and the PP/heme ratio measured by HPLC.

Figure 48 illustrates the eZnPP/PP ratio calculated from the eZnPP and PP fluorescence intensities as evaluated by a method in accordance with an exemplary embodiment of the subject matter described herein.

DETAILED DESCRIPTION OF EMBODIMENTS

[0055] It is understood that the subject matter described herein is not limited to particular embodiments described, as such may, of course, vary. It is also understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present subject matter is limited only by the appended claims. Where a range of values is provided, it is understood that each intervening value between the upper and lower limit of that range, and any other stated or intervening value in that stated range, is encompassed within the disclosed subject matter.

[0056] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosed subject matter belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present disclosed subject matter, this disclosure may specifically mention certain exemplary methods and materials.

[0057] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present disclosed subject matter is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates, which may need to be independently confirmed.

[0058] As used herein and in the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise.

[0059] Nothing contained in the Abstract or the Summary should be understood as limiting the scope of the disclosure. The Abstract and the Summary are provided for bibliographic and convenience purposes and due to their formats and purposes should not be considered comprehensive.

[0060] As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the present

disclosed subject matter. Any recited method can be carried out in the order of events recited, or in any other order that is logically possible.

[0061] Reference to a singular item includes the possibility that there are plural of the same item present. When two or more items (for example, elements or processes) are referenced by an alternative "or," this indicates that either could be present separately or any combination of them could be present together, except where the presence of one necessarily excludes the other or others.

[0062] As summarized above and as described in further detail below, in accordance with the various embodiments of the present invention, there is provided an apparatus for measuring fluorescent analyte concentration in the blood and a method for using the apparatus. The apparatus measures the fluorescent analyte concentration noninvasively, *e.g.,* by exciting intact tissue in the patient, *e.g.,* the oral mucosa. In some embodiments, the apparatus measures the fluorescent analyte concentration by exciting blood samples or other tissue *ex vivo.*

[0063] The apparatus described herein measures the fluorescence of an analyte by excitation of tissue at two alternating wavelengths, or wavelength ranges. The two wavelengths are selected such that the analyte exhibits a greater difference in fluorescence at the two wavelengths (one of which may be at the excitation peak of the analyte) than that of background fluorophores, and at which blood exhibits substantially similar absorbance at the two wavelengths. The degree of similarity can be appropriately determined by those of ordinary skill in the art. To fulfill the requirement of sufficiently similar absorbance of light at the two excitation wavelengths in tissue, the excitation wavelengths can be determined experimentally in a way to minimize the effective penetration depth differences of light at both wavelengths or both wavelength ranges. This may be approximated on a representative sample, which does not contain the analyte, by setting the first excitation wavelength at the known wavelength for maximum excitation efficiency of the analyte and then scanning the excitation wavelength along the other side of the blood absorbance peak until the background fluorescence spectrum has the expected intensity and the most similar shape. An example of this is provided below.

[0064] Although the apparatus is described herein with respect to measurement of eZnPP using the absorbance characteristics of blood (*e.g.,* with hemoglobin as the predominant absorber), it is understood that the principles described herein are applicable to measurement of other analytes and reference materials having similar fluorescence and absorbance properties.

[0065] eZnPP is an indicator of iron supply to developing red blood cells. During hemoglobin synthesis, if iron deficiency makes iron unavailable to the developing red blood cell to form heme from protoporphyrin IX, then zinc is chelated instead to form eZnPP as one of the first biochemical responses to iron depletion.

[0066] The measurement of eZnPP by noninvasive tissue excitation requires a quantitative method to distinguish the fluorescence of eZnPP from that of other fluorophores in tissue, *i.e.,* from tissue autofluorescence. Since eZnPP is found inside erythrocytes only, it has been observed that the "dilution" of the blood by tissue that shows autofluorescence does not in first order destroy the quantitative nature of the derived signal. That is, measurement of eZnPP is insensitive to the concentration of blood within the tissue, *i.e.,* to the value of the blood volume fraction of the tissue, over a certain range. This range of insensitivity to the blood volume fraction can be modified by changing the probe head configuration, *i.e.,* by changing the spatial separation between excitation and detection fiber(s).

[0067] In an exemplary embodiment, the fluorometer noninvasively measures eZnPP fluorescence in red blood cells by examination of the microcirculation of the intact oral mucosa at the two alternating excitation wavelengths. The fluorometer can also be used to noninvasively examine other tissue, such as other mucosal surfaces, as well as the skin if permitted by the amount of skin pigmentation. The fluorometer illuminates the mucosa and transmits the induced fluorescence to a photodetector. Diode lasers may be used as the excitation light sources. The fluorometer can also be used to examine tissue samples or blood samples *ex vivo.*

[0068] An exemplary embodiment of the fluorometer 100 is shown schematically in Figure 1. Fluorometer 100 includes an excitation light source 102 for illuminating the tissue T of the patient, and a light detector 104, such as a spectrophotofluorometer, for analyzing the fluorescence. In some embodiments, two or more detectors are employed, in which part of these detectors receive light through optical filters transmitting light in the wavelength range of the emission wavelength range of the analyte and the other part of these detectors receive light through optical filters transmitting light in the wavelength range outside the emission maximum of the analyte. The fluorometer 100 measures the concentration of eZnPP found in erythrocytes E in the blood vessel V of the patient. A processor 106 determines the concentration of eZnPP based on the fluorescence detected by the detector 104.

[0069] The provision of light to the tissue T and transmission of fluorescence to the light detector 104 is performed by an optical probe head 108 in an exemplary embodiment. The excitation light source 102 provides radiation for tissue excitation at two wavelengths. In accordance with the invention, alternating wavelengths are provided by first and second light sources 110 and 112, such as lasers or LEDs which operate at two wavelengths, *e.g.*, 407 nm and 425 nm. A beam combiner 114 provides the light to the tissue T as a single source in an alternating fashion. The frequency of the alternation is chosen quickly enough to show intensity variations during the measurement, *e.g.*, due to movement of the patient, in both emission spectra in such a way that the variations are reduced or canceled in the difference of the spectra. It has been observed that there are high intensity variations if the patient moves during measurement. However, if the speed

of the alternation is sufficiently rapid, these variations are discernible in both emission spectra, and by subtracting them, the variations are canceled out. It has also been observed that measuring the emission spectra in parallel (*e.g.,* with a CCD detector such that all wavelengths are measured simultaneously) avoids the result in which intensity variations due to movement become wavelength-dependent intensity variations ("peaks") in the spectrum. A lens and/or filter 116 can be provided to focus and/or direct the light to the tissue T. Transmission of light from the probe 108 to the light detector 104 is accomplished by one or more optical fibers. A single optical fiber is used to illuminate the tissue and also to transport the fluorescence to the detector. A lens 120 and/or filter 118 can be provided to focus and/or remove noise from the light transmitted to the detector 104. After accounting for background tissue fluorescence, scatter, path length, geometric and other factors, the processor 106 correlates the intensity of the fluorescence to the eZnPP/heme ratio and provides the result in an output device, such as a display screen 130, speaker 132 or vibrating unit 134. An optional communications component 130 is provided in certain embodiments, as will be described in greater detail hereinbelow. An optional power supply 122, such as a battery, can be included in the fluorometer, particularly if it is a portable device. In some embodiments, the fluorometer 100 can be directly connected to the electrical power supply of the home or institution.

[0070] Apparatus 100 is illustrated in Figure 1A which indicates the optical fiber 111 associated with the light source 102, also referred to herein as the excitation fiber, and the optical fiber 109 associated with the light detector, such as spectrometer 104, also referred to herein as the detection fiber. Figure 1B illustrates a first spacing between excitation fiber 111 and a detection fiber 109, *i.e.,* "interfiber spacing." In Figure 1B, the interfiber spacing d = 0. Figure 1C illustrates a second interfiber spacing, *i.e.,* a spacing d of about 1200 μm. The interfiber spacing d in probe 108 is selected, *e.g.*, experimentally, to obtain minimal dependence on the blood volume fraction over a physiological relevant range. A photograph of an exemplary embodiment of a portion of the fluorometer is represented in Figure 2, adapted for measurement of patient blood samples, although such apparatus may be used for tissue measurements as well.

[0071] In measurements of a blood sample on a glass slide, eZnPP is one of the major fluorophores with a dominant, characteristic excitation peak at about 425 nm (Figure 3) and an emission peak at about 590 nm (Figure 4). Within the erythrocyte, eZnPP is bound to hemoglobin. Hemoglobin does not fluoresce but strongly absorbs light at 400 to 430 nm. This absorption by hemoglobin diminishes the eZnPP fluorescence. With a front-face hematofluorometer, the hemoglobin and eZnPP in a blood sample on a glass slide absorb almost all the excitation light within a thin surface layer that allows the emitted light to be collected with equal efficiency. The intensity of the emission at 590 nm is proportional to the eZnPP/heme molar ratio.

[0072] Figure 13 compares measurements of patient blood samples (diluted to 4%) taken by the fluorometer 100 (excitation at 425 nm; emission at 590 nm; measurements in a.u., arbitrary units) in the free-beam instrumental configuration shown in Figure 2 on the vertical axis, with those by an Aviv hematofluorometer, on the horizontal axis. Overall, the measurements are closely correlated; the remaining scatter may be explained by a greater specificity for eZnPP with the fluorometer 100 compared to the Aviv hematofluorometer.

[0073] In contrast to measurement of blood samples on a glass slide, in noninvasive measurement of erythrocytes within an examined tissue, *e.g.,* in the microcirculation of the oral mucosa, eZnPP is a minor fluorophore. Instead, connective tissue (collagen and elastin) is the principal source of autofluorescence from the stromal layers containing the microcirculation. In the thin overlying epithelium of non-keratinized oral mucosa (mucous membranes of the lip, buccal and sublingual mucosa), the dominant fluorophores are mitochondrial reduced nicotinamide adenine dinucleotide (NADH) and mitochondrial flavin adenine dinucleotide (FAD). The absorbance spectrum of NADH does not extend up to 425 nm and, accordingly, will not contribute to fluorescence at the 590 nm emission peak of erythrocyte eZnPP. As will be discussed below, the contribution of epithelial FAD to fluorescence at 590 nm can likely be minimized or eliminated by optimizing the configuration of the excitation and detection fibers in the probe head 108.

[0074] In Figure 5, an exemplary fiber-optic probe head 108 is illustrated. A central detection fiber 109 is surrounded by one or more (*e.g.*, six) excitation fibers 111. Figure 6 is a schematic diagram of non-keratinized oral mucosa. The excitation light (indicated by arrow L) must pass through a thin overlying epithelial layer EL with light-scattering elements to reach erythrocytes E in the microcirculation in the stromal layer S, also with light-scattering elements.

[0075] Using the fiber-optic probe head design disclosed in Figure 5, the tissue autofluorescence spectrum from the mucous membrane of the lower lip of a human patient is shown in Figure 7. Because the magnitude of the tissue autofluorescence is considerably greater than the fluorescence of erythrocyte eZnPP, some investigators have concluded that measurement in the oral mucosa is not feasible. *See, e.g.,* Chen X. "Feasibility test for noninvasive detection of zinc protoporphyrin in oral mucosa and retina." Biomedical Engineering 2007; M.S.: 1-71.

[0076] The fluorometer overcomes limitations in the prior art by providing an alternating two-wavelength fluorescence excitation method that is used to distinguish eZnPP fluorescence from tissue autofluorescence. As shown in Figure 8, the hemoglobin absorbance is the same at the excitation peak for erythrocyte eZnPP (425 nm) and at 407 nm. By alternating excitation at two wavelengths, *i.e.,* about 407 and about 425 nm, the fluorescence emission spectrum excited at 407 nm can be subtracted from that excited at 425 nm to obtain a difference measurement that is proportional to the eZnPP/heme molar ratio. In some embodiments that excitation occurs at two wavelength ranges, *i.e.,* about 405 to about

415 nm and about 420 nm to about 430 nm. In a tissue measurement, the intensities of the exciting light sources would be adjusted to give the same fluorescence emission intensities for autofluorescence. Such adjustment would depend on the light source being used by the apparatus, *e.g.*, whether a laser or some other light source is being used. Also, the emission intensities can be normalized (*e.g.*, scaled to the 407 nm emission spectrum). The resulting difference spectrum would be zero at the point of normalization. In the presence of eZnPP, the emission intensity on excitation at 425 nm would be greater than that at 407 nm. The difference of the two emission intensities would be virtually specific for eZnPP and would depend linearly on the concentration in the target volume.

[0077] The second excitation wavelength (around 407 nm) excites protoporphyrin IX ("ePP") fluorescence more efficiently than at 425 nm. Accordingly, information about zinc protoporphyrin (eZnPP and ePP) fluorescence can be gathered simultaneously from the difference spectrum.

EXAMPLE

[0078] A simple tissue phantom for oral mucosa (illustrated in Figure 9) consists of an overlying, diffusely scattering film to model the epithelial layer and - to model the stromal layer - a solution containing dissolved elastin, lipofundin as a light-scattering agent, and also a whole blood sample at a 1% dilution (eZnPP 60 μmol/mol heme, the upper limit of normal). Figure 10 shows the fluorescent properties of the tissue phantom, displaying the excitation spectra for collagen with and without blood. The vertical lines indicate excitation wavelengths of 407 and 425 nm.

[0079] The results are summarized in Figures 11 and 12. Each of the figures illustrates F_m (emitted fluorescence) at the indicated excitation wavelength. Simple measurement of the emission spectra at 590 nm, in accordance with conventional techniques, would be unable to detect an emission peak for eZnPP (Figure 11). By contrast, Figure 12 illustrates the use of alternating two-wavelength fluorescence excitation. Accordingly, the difference spectrum F_m(425nm) - F_m(407nm) clearly shows the characteristic emission peak at 590 nm for erythrocyte eZnPP in this tissue phantom whose concentration is at the upper limit of the normal range. In whole blood, the output is automatically quantitative for the ratio C(eZnPP)/C(Hemoglobin). Without being bound to a particular theory, it is understood that as long as the optical scattering inside the tissue doesn't vary significantly, the output will be quantitative as well. However, additional considerations include intra-/inter-patient variations and/or probe head geometry.

[0080] Fluorometer 200 is illustrated in Figure 14 and is generally identical to fluorometer 100 discussed above, with the substantial differences noted herein. In an exemplary embodiment, the fluorometer 200 is a portable unit that includes a power supply (not shown), such as a watch-type battery or a rechargeable battery. The fluorometer 200 may include a housing 226 in which the detector, the processor and the power supply are housed. In order to provide portability, the housing 226 may have an overall length of about 2 inches to 6 inches. Fluorometer 200 includes a probe 208 used to illuminate the tissue being examined, *e.g.*, the mucosa or a blood sample, and transmit the fluorescence to a light detector by depressing an activation switch 228.

[0081] The fluorometer 200 also includes one or more output components. In some embodiments, the output component is disposed in or on the housing. Exemplary output components include a display screen 230, a speaker 232, and/or a vibrating component (not shown). The display screen 230 may be an LCD display, an AMOLED display or the like. The output of the particular output components may be used to signal to the user that the analyte reading was successfully completed. For example, the display screen 230 may display an icon that is illuminated when successful analyte readings are obtained. The speaker 232 can provide an audible signal that the analyte reading was obtained successfully. The vibrating component similarly can provide a vibration signal to indicate successful analyte readings. Such tactile or audible outputs are particularly useful if the analyte reading is self-administered, or if the testing is performed in settings where bright sunlight or other conditions make it difficult to view the display screen.

[0082] The output component further provides an indication of the concentration of analyte in the tissue being examined. In some embodiments, the display provides a numerical indication of the analyte concentration 240. The speaker 232 may alternatively, or in addition, provide the numerical analyte concentration audibly.

[0083] For certain users of the fluorometer 200, the raw analyte information may not be meaningful. Accordingly, the fluorometer 200 may allow the user or a health care provider to enter threshold concentrations for a health range of analyte concentration. In some embodiments, the threshold concentrations may be entered at the time of manufacturing, *e.g.*, programmed in software or hard-coded. In use, the fluorometer 200 would determine whether the detected concentration of iron is below a preselected concentration of iron. Such concentration of iron may be selected based on the circumstances, *e.g.*, to determine whether an individual is iron-deficient or iron-replete. The display or other output device would provide an indication 242 to the user that the iron concentration was below this threshold. For example, the display would provide an indication "LOW" iron concentration or "IRON REPLETE" etc. The speaker audibly provides the same phrase. A vibrating component can be programmed to vibrate in a certain manner to indicate that a threshold has been exceeded, *e.g.,* two consecutive vibrations for low iron concentrations.

[0084] The fluorometer 200 may also be programmed to track trends in the analyte concentration over time. In some embodiments, the fluorometer 200 includes a memory that stores multiple analyte readings, which can be tagged with

a patient identification and a time stamp. When successive analyte readings are obtained for a particular patient, the fluorometer 200 can determine whether the analyte concentrations are increasing or decreasing as well as the rate of such increase or decrease. Trend indications 244 are provided by the display, *e.g.*, upward or downward trend arrows or alphanumeric indications such as "IRON CONCENTRATION INCREASING" or "IRON CONCENTRATION DECREASING." The speaker 230 and vibrating component can likewise provide such information to the user in a similar manner as described above for the analyte concentration.

[0085] Fluorometer 300 is generally identical to fluorometer 100 discussed above, with the substantial differences noted herein. In some embodiments, it is useful to provide a separate monitor unit 360 that allows the user to obtain information remotely from the patient. The fluorometer 300 includes a communications component for communicating with the monitor unit 360. The fluorometer 300 can include a wired connection to the monitor, *e.g.,* by use of a USB connection. As illustrated in Figure 15, the communications component may be wirelessly connected to the monitor and may include an RF transmitter, IR transmitter, Bluetooth transmitter or a WiFi transmitter for providing the detected eZnPP concentrations or other information about the patient or the fluorometer to a receiver on the monitor unit 360. Communications between the fluorometer and monitor may be achieved by providing a cellular transmitter (GSM, CDMA, etc.) or satellite transmitter on the fluorometer.

[0086] The communications component can provide signals to the monitor unit - signals that relate to the analyte concentration. Such communications may occur immediately or at a predetermined time after taking the analyte reading of the patient. In some embodiments, the fluorometer 300 may transmit the analyte reading when the analyte concentration is determined to exceed a threshold.

[0087] The monitor unit 360 includes a receiver that receives the signal from the fluorometer 300. In the case of a wireless transmission, the monitor can include an RF, IR, Bluetooth or WiFi receiver. For a wired connection, the receiver component 360 may include the electrical contacts for the wired connection. The monitor unit 360 also includes a processor, memory component, power supply and user interface. The display screen may be omitted from the fluorometer 300 in some embodiments.

[0088] A user interface is provided on the monitor unit 360, which can include a display unit 330, speaker 332, vibrating component, and input controls 336, *e.g*., switches, buttons, soft keys, keyboard, touch screen interface and the like. The user interface can provide an indication of the concentration of analyte in the tissue 340. The user interface provides an indication that the concentration of analyte exceeds a predetermined threshold 342, *e.g.,* by indicating that the iron concentrations are "LOW."

[0089] The memory provided on the monitor unit 360 stores successive analyte concentrations and can provide an indication 344 that the concentration of analyte is increasing or decreasing from the previous concentration of analyte, *e.g*., with trend arrows.

[0090] The monitor unit 360 can be programmed, *e.g*., through the user interface or by factory settings, to store health goals for the patient. Such health goals include overall fitness concentrations and may include a target analyte concentration, *e.g*., achieving a recommended iron concentration within a desired time frame. The monitor unit 360 can evaluate whether the patient is reaching the health goal. For example, the monitor may determine that the patient's iron concentrations are increasing. The user interface may then provide an indication to the patient that the trend of iron concentrations is towards the health goal and that the user has attained 50% of the patient's health goal with a bar graph-type display 346. In some embodiments, the display screen 330 may provide an icon that changes color *(e.g.,* from red to green) or increases in size as an indication that iron concentrations are improving. The speaker 332 can audibly provide the same information.

[0091] The user interface can provide a treatment suggestion to the patient after determining the analyte concentration and/or comparing the analyte concentration to the patient's health goals. For example, the user interface may provide a suggestion for consuming a nutritional supplement to address the analyte concentration, *e.g.*, consumption of iron-rich foods or supplementation, and the quantity of such supplementation 348. The user interface may suggest the patient take a pharmaceutical compound to address the particular analyte concentration.

[0092] The monitor unit 360 can be a fixed component in a clinical setting. In such case, the monitor unit can be a desktop or laptop personal computer and receive power by an AC household current. In some embodiments, the monitor unit 360 can be a portable unit. For example, the monitor can be a laptop computer, a cellular telephone, a tablet computer or the like. The monitor can be a portable dedicated handheld unit.

[0093] Fluorometer 400 is illustrated in Figures 16 and 17, and is identical to fluorometer 100 discussed above, with the differences noted herein. In an exemplary embodiment, the fluorometer 400 includes a "free beam" configuration (*e.g.*, a fluorometer without a fiber-optic probe). It is understood that fluorometer 400 can alternatively include a fiber-based configuration. In an exemplary embodiment, fluorometer 400 distinguishes iron deficient blood samples from iron replete blood samples. When testing is performed *in vitro,* the sample is tested with or without the presence of an agent to mimic light scattering in tissue. When testing is performed *in vivo,* the light source is applied to the intact patient tissue, *e.g.,* the oral mucosa, as discussed hereinabove. An embodiment of the instrumentation is shown schematically in Figures 17 and 18.

[0094] Fluorometer 400 includes an excitation light source 402 for illuminating the tissue T of the patient and a light detector 404 for analyzing the fluorescence. In some embodiments, the light source 402 is a 500W short-arc Xe-lamp (T-light. Karl Storz, Tuttlingen, Germany) white light source, and the light detector 404 is a cooled CCD spectrometer. The fluorometer 400 measures the concentration of eZnPP found in erythrocytes *in vivo* in the blood vessel of a patient, or *in vitro* in a blood sample T maintained in a cuvette 405. The *in vitro* measurements can be performed on diluted blood samples, with a concentration of 2% whole blood in phosphate buffered saline. The sample volume can be about 3000 μl, including 60 μl whole EDTA blood in a cuvette. As discussed above regarding fluorometer 100, a processor (not shown) determines the concentration of eZnPP based on the fluorescence detected by the detector 404.

[0095] The provision of light to the tissue T and transmission of fluorescence to the light detector 404 is performed by excitation light source 402, which provides radiation for tissue excitation. In an exemplary embodiment, alternating wavelengths are provided by a tunable optical filter 440. In some embodiments, the optical filter unit consists of the filter 440, as described in greater detail herein, with a tunable wavelength and a tunable bandwidth, a detection unit that can detect emission spectra from 520 to 1000 nm, and incorporates a free beam format that can be readily converted to a fiber-based configuration. In the testing configuration, light was optically filtered such that the transmitted light's central wavelength was tunable in the blue wavelength range, 395 nm to 431 nm, while preserving a spectral bandwidth h *(e.g.,* 5 nm full width/half maximum, "FWHM"). Light in the wavelength range 500 nm-750 nm was suppressed with OD > 10.

[0096] The fluorometer 400 implements the techniques described herein for measurement of a fluorescent analyte using alternating wavelengths (407 and 425 nm) for tissue excitation. Procedures were established for protoporphyrin measurements in whole blood samples using the reference HPLC method (Immundiagnostik AG) and the conventional front-face hematofluorometer 452 (Aviv; shown in Figure 17), and the eZnPP-fluorometer 400.

[0097] With continued reference to Figure 16, a collimating lens 415 and/or clean-up filter 417 can be provided to focus and/or direct the light to the sample T via a dichroic beam splitter 419 and lens 421. The blue light beam was focused onto the sample T, with a focus diameter of 2 mm. On the sample T, the total excitation light power was 6 mW (central wavelength 425nm, wavelength-dependent). The fluorescence light emitted from the sample T was transmitted backwards through the beam splitter 419 and filtered by a long-pass filter 418 (*e.g.,* OG515, Schott AG, Mainz, Germany) and lens 420, limiting the usable detection range to 520 nm-750 nm. Finally, the fluorescence light was coupled into a cross-section converting fiber 409, consisting of seven 200 um-diameter optical fibers, arranged in a circle. These fibers, linearly arranged at the other end of the fiber bundle, were coupled into a temperature regulated CCD spectrometer 404 (*e.g.,* detection range: 340 nm-1022 nm, S2000-TR, Ocean Optics, Inc., Dunedin, FL, USA), yielding an effective spectral resolution of 5 nm.

[0098] To optionally allow correction for wavelength- and time-dependent intensity variations, fluorescence standard measurements were performed. For example, the emission intensity of the short-arc lamp 402 is wavelength dependent, and the filtered light's intensity is also wavelength dependent. Also, the total power of the lamp 402 may change during usage. The fluorescence standard includes a 1 mm thick piece of commercially available solid polymethyl methacrylate containing Rhodamin B (1BF/RB, Starna GmbH, Pfungstadt, Germany), fixed at the wall of the cuvette 405.

[0099] After accounting for background tissue fluorescence, scatter, path length, geometric and other factors, the processor correlates the intensity of the fluorescence to the eZnPP/heme ratio and provides the result in an output device, such as a display screen, speaker or vibrating unit. An optional communications component is provided in certain embodiments, as will be described in greater detail hereinbelow. An optional power supply, such as a battery, can be included in the fluorometer, particularly if it is a portable device. In some embodiments, the fluorometer 400 can be directly connected to the electrical power supply of the home or institution.

TUNABLE FILTER

[0100] The instrumentation of tunable optical filter 440 is illustrated in Figures 16 and 18. The filter unit 440 allows the simultaneous selection of both the central filtered wavelength and the spectral bandwidth, for use in applications requiring detection of light or illumination by light at a small spectral bandwidth. The tunable optical filter unit 440 provides improved light transmission efficiency and makes possible spectral filtering of images and fiber bundles without a scanning device. Although the filter unit 440 is described herein in connection with fluorescence spectroscopy, the filter unit 440 can find application in fluorescence microscopy, fluorescent imaging, and in advanced microscopy applications such as fluorescence-lifetime imaging microscopy. For illumination, the filter 440 can be used with filtered incoherent light sources to achieve strong illumination intensities at small spectral bandwidths, such as illumination for fluorescence microscopy, fluorescence spectroscopy, and more generally, in applications in which a tunable laser is not practical, *e.g.,* due to cost.

[0101] As illustrated in Figure 18, filter unit 440 includes two tunable bandpass optical filters 444 and 446, *e.g.*, Semrock Versachrome® filters capable of independent rotation for selecting an angle of incidence with a beam of light passing through the filter unit 440. After acquiring the fluorescence emission spectra for the excitation wavelengths, shutter 442 was closed to prevent further illumination of the sample T. A dark spectrum can be recorded with the same settings. Step motor controller 448 is provided to allow independent rotation of the two step motors carrying the optical filters at

a high rotation speed with sub-degree precision.

[0102] The components, design and function of the filter unit 440 are shown schematically in Figures 19-24. In Figure 19, a beam of light passing through filter unit 440 is designated as beam portions 480, 482, and 484. Beam portion 480, a collimated beam of unfiltered light typically having a large spectral width, is transmitted through optical filter 444, *e.g.,* a Versachrome filter capable of rotation as indicated in the angular direction of arrow R1 (as well as in the opposite angular direction indicated by arrow R1). The filtered light beam 482, filtered by filter 444, has a fixed spectral bandwidth, and the central wavelength can be selected by the angle of filter 444 with respect to the incidence of beam 480 on filter 444. The light beam 482 is transmitted through filter 446, *e.g.,* a Versachrome filter capable of rotation as indicated in the angular direction of arrow R2 (as well as in the opposite angular direction indicated by arrow R2), with the resulting light beam 484. The results are illustrated in Figure 20, showing the proportion of effective transmission through the two filters 444 and 446. The solid lines represent transmission through the first filter 444 for two different angles defined with respect to the direction of the light beam. Typically, angle 2 is greater than angle 1. The dotted lines represent the transmission through the second filter 446 for two different angles. The total transmission characteristics of the filter unit 440 are the product of both transmission curves (filter 444 and filter 446), solid and dotted, shown as a shaded area under the overlap of the curves in Figure 20. In the exemplary embodiment, filter 444 and filter 446 provide about 60% transmission of light. If two similar filters are used for filters 444 and 446, the angles of the two filters have to be chosen independently because the angular dependency of the filter transmission is non-linear. Example: Angle 1: a(Filter 444)=20°, a(Filter 446)=0° resulting in a 5 nm spectral bandwidth; Angle 2: a(Filter 444)=40°, a(Filter 446)=35° also resulting in a 5 nm spectral bandwidth at a lower central wavelength.

[0103] As shown in Figure 21, after transmission of the light through filters 444 and 446, a beam splitter 419 can be added to the filter unit 440 for monitoring a part of the filtered light, *e.g.,* light beam 586. This part of the light can be detected, *e.g.,* by a spectrometer 404 or power meter to monitor the spectral bandwidth, the power or both.

[0104] As shown in Figures 22-25, during non-perpendicular transmission of a light beam through a filter, a parallel offset of the light beam occurs. If the filters are arranged as shown in Figure 22, the offset provided by filters 444 and 446 accumulates to a large net offset. As illustrated in Figure 22, the offset O1 of light beam 682 with respect to light beam 680, and the offset O2 of light beam 684 with respect to light bean 682 results in a total offset O3 with respect to light beam 680. (A dashed line is used to represent the hypothetical trajectory of light beam 680 in the absence of filters 444 and 446.) If the filters are rotated counter-wise, *i.e.,* in opposite directions, as shown in Figure 23, the offset O6 of light beam 684' with respect to light beam 680' (the cumulative offset of offset O4 and offset O5) is significantly reduced from offset O3 but not eliminated because the angles of both filters 444 and 446 are different. In some embodiments, as illustrated in Figure 24, a third optical element 449, *e.g.,* a planar piece of glass that provides 100% transmission and refractive index n > 1, can be inserted, in which case the offset O10 between light beam 680" and light beam 686" is eliminated (i.e., O10=0).

[0105] During *in vitro* testing, blood samples and tissue phantoms may contain a light-scattering agent to mimic the optical properties of tissue such as the oral mucosa. Latex microspheres having a diameter of about 0.5 μm were found to have little auto fluorescence and remained suspended during the time required for testing, and thus are a suitable, exemplary light-scattering agent.

[0106] Figures 25-30 illustrate the excitation and emission spectra of tissue. The z-axis represents arbitrary units (a.u.). A single excitation central wavelength, with a bandwidth of 5 nm full width at half maximum (FWHM), was used to obtain the associated emission spectra. To determine that 407 and 425 nm are the optimal alternating wavelengths for measurements of blood samples, excitation-emission matrices were obtained in blood samples (2% in saline) with eZnPP concentrations in the reference ("normal") range of 30 to 80 μmol/mol heme and iron deficient range (> 80 μmol/mol heme), as determined by conventional front-face Aviv hematofluorometer. (In the Figures, "ZnPP" refers to the erythrocyte zinc protoporphyrin concentration. In Figure 25, for example, "ZnPP=198" means erythrocyte zinc protoporphyrin concentration of 198 μmol ZnPP/mol heme.) Typical matrices are shown in Figures 25-30. The eZnPP peak 804 is illustrated in Figures 25-30 at excitation of 425 nm. For the determination of eZnPP levels in blood, 407 nm and 425 nm provide the optimal performance as the alternating wavelength pair.

[0107] As shown in Figures 31-36, the alternating (407 nm-425 nm) wavelength method significantly reduces or eliminates the background whole blood autofluorescence that produces an elevated baseline with conventional single wavelength (425 nm) studies. The y-axis represents arbitrary units (a.u.). The conventional single wavelength measurement (425 nm) is indicated by line 802 (upper line) and its associated eZnPP peak 804. (Figure 31 corresponds to the data illustrated in Figure 25 for an excitation wavelength of 425 nm and eZnPP = 75.) The alternating (407 nm-425 nm) wavelength method in accordance with the embodiments described herein is indicated by line 806 with an associated eZnPP peak 808.

EXAMPLE

[0108] A study was performed using 35 anonymous patient whole blood samples from the Institut für Laboratoriums-

medizin, Klinikum der Universität München, which were analyzed prospectively for erythrocyte zinc protoporphyrin ("eZnPP") concentration by the reference HPLC method (Immundiagnostik AG), by the Aviv hematofluorometer, and by the ZnPP-fluorometer 400 in a free beam configuration as described herein.

[0109] The reference ("normal") range of eZnPP concentrations was 30 to 80 μmol/mol heme. The iron deficient range of eZnPP concentrations was > 80 μmol/mol heme. The study included eZnPP-fluorometer measurements of the blood samples (i) without a light scattering agent, with a blood volume fraction of 0.02, (ii) with the smaller (0.5 μm) latex microspheres as a scattering agent to provide scattering coefficients over a physiologic range (reduced scattering coefficient about μ_s' = 1 to 4 mm^{-1}), in combination with (iii) a physiologic range of blood volume fractions (about 0.02 to 0.08). In aggregate, results were obtained for a series of studies of the 35 blood samples under 11 different combinations of light scattering and whole blood concentrations over the physiologic range for each of the 35 blood samples. In these samples, the prevalence of iron deficiency, as determined by the HPLC reference method, was 69%.

[0110] For all measurements, the light source was tuned to 425 nm and 407 nm (central wavelengths) with a spectral bandwidth of 5 nm FWHM. After acquiring the fluorescence emission spectra for the excitation wavelengths, a shutter was closed to prevent further illumination of the sample, and a dark spectrum was recorded with the same settings.

[0111] For the measurement of the Rhodamin B fluorescence standard, the CCD spectrometer's integration time was set to 40 ms, averaging internally over 16 spectra. Including the time required for the wavelength-tuning of the filter unit and the shutter, the measurement time was 4 s. For the measurements of blood samples, which showed much dimmer fluorescence, the integration time was set to 400 ms, averaging internally over 4 spectra, resulting in a total measurement time of 10 s. It was verified that during measurements the signal remained stable.

[0112] An exemplary spectral calibration and normalization process is described herein. From all raw, uncorrected spectra $F_{uncorrected}(\lambda)$, the corresponding dark spectrum $D(\lambda)$ was subtracted. The resulting spectrum was multiplied by the factor $C_{excitation}$ which depends on the excitation wavelength and is used to compensate for wavelength- and time-dependent excitation light intensity variations, as well as for optical adjustment variations. In addition, the resulting spectra were divided by the wavelength-dependent transmission of the detection filter $T_{filter}(\lambda)$, and multiplied by a wavelength-dependent factor including optical fiber transmission and spectrometer sensitivity $C_{spectrometer}(\lambda)$. These additional calibration factors allow comparing the corrected spectra $F_{corrected}(\lambda)$ to spectra that were measured using other devices, because influences of the spectral sensitivity of the detection optics and the spectrometer are compensated. The complete calibration procedure is shown in Equation (1):

$$F_{corrected}(\lambda) = [F_{uncorrected}(\lambda) - D(\lambda)] \frac{C_{spectrometer}(\lambda)}{T_{filter}(\lambda)} C_{excitation} \qquad (1)$$

[0113] To obtain $C_{excitation}$, at first the calibration procedures described above (dark subtraction, filter transmission, detection sensitivity calibration, except for the factor $C_{excitation}$) were applied also to the fluorescence standard measurement $F_{RhodaminB}(\lambda)$. Then, $C_{excitation}$ was calculated as described in Equation (2): The "real" value of the Rhodamin B fluorescence maximum from a reference measurement, $\max R_{RhodaminB}(\lambda)$ was divided by the maximum of the Rhodamin B fluorescence measured by the prototype measurement set-up $\max F_{RhodaminB}(\lambda)$. The reference measurement was recorded by a fluorescence spectrometer (Fluoromax-2, Jobin Yvon GmbH, Unterhaching, Germany), with excitation and detection monochromator adjusted to match excitation and detection bandwidth (5 nm FWHM) of the measurement set-up.

$$C_{excitation} = \frac{\max R_{Rhodamin\ B}(\lambda)}{\max F_{Rhodamin\ B}(\lambda)} \qquad (2)$$

[0114] For purposes of comparison with the novel two wavelength excitation method described herein, a method is described herein which requires one excitation wavelength band (*e.g.*, centered at 425 nm) and two emission wavelength bands (centered at 573 nm and 593 nm) (also referred to as "two wavelength emission method."). For *in vitro* testing, a sample measurement of patient blood (HPLC determined eZnPP/heme ratio = 333μmol(ZPP)/mol(heme) and PP/heme ratio = 605μmol(PP)/mol(heme)) is shown in Figure 37. Using the reference HPLC method, the eZnPP and PP concentrations C_{ZPP} and C_{PP} were determined as absolute concentrations in units (nmol/l). Separately, the hemoglobin concentration C_{Heme} was determined by standard laboratory tests in units (g/dl). From the HPLC determined eZnPP and hemoglobin measurements, C_{ZPP} and C_{Heme}, the eZnPP/heme ratio (and in the same way, from Cpp and C_{Heme} the PP/heme ratio) is calculated by Equation (3), using the hemoglobin subunit's molecular weight 64,458 g/mol.

$$\frac{c_{ZPP}\left[\frac{\mu mol}{mol}\right]}{c_{Heme}} = \frac{c_{ZPP}[nmol/l]}{c_{Heme}[g/dl]}\frac{64.458}{10^4} = \frac{c_{ZPP}[nmol/l]}{0.1551 \cdot c_{Heme}[g/dl]} \quad (3)$$

In Figure 37, a calibrated fluorescence emission spectrum F_{425} in the wavelength range 520 nm-750 nm is shown (solid line in Figure 37). The emission maximum of eZnPP is at 593 nm; the background fluorescence from blood plasma $F_{Background}$ was fitted as an exponential decay curve to the data in the spectral range (dashed line in Figure 37), without contribution from porphyrin emission.

[0115] With continued reference to Figure 37, the eZnPP fluorescence intensity I_{ZPP} was calculated according to Equation (4) below. The measured fluorescence intensities were taken from the calibrated emission spectrum by averaging over the wavelength range 590 nm-596 nm (I_{593}) and over 570 nm-576 nm (I_{573}). The background fluorescence intensity at 593 nm $I_{bkg,593}$ (double arrows, dashed line) cannot be measured directly, but can be calculated from the fluorescence intensity at 573 nm (I_{573}), *e.g.,* 0.8 times the fluorescence intensity at 573 nm. The difference of I_{593} and $I_{bkg,593}$ was used to quantify the eZnPP/heme ratio.

$$I_{ZPP} = I_{593} - I_{bkg,593} = I_{593} - 0.8 \cdot I_{573} \quad (4)$$

[0116] According to one aspect of the disclosed subject matter, a novel evaluation method is described herein for reducing the influence of background fluorescence on the detected intensity at 593 nm (also referred to as "two wavelength **excitation** method.") The method uses two excitation wavelength bands, *e.g.,* 407 and 425 nm. For quantification of the eZnPP/heme ratio, one emission wavelength band, centered at 593 nm, is used. In addition, the ePP/heme ratio is quantified by a second emission wavelength band centered at 627 nm.

[0117] The method is illustrated in Figure 38, in which two corrected emission spectra (F_{425} ; solid line in Figure 38 and F_{407}; dot-dashed line in Figure 38; left axis description) of the patient blood sample described above are shown; the central excitation wavelengths were 425 nm and 407 nm, respectively. For optimized overlap in the 520 nm-570 nm region, F_{407} was scaled by a factor 1.15. Additionally, the difference between these spectra is shown, which is referred to as "difference spectrum."

[0118] In Figure 38, the differences of the two spectra at 593 nm and at 627 nm are illustrated by dashed line (right axis description) and highlighted by arrows: As the excitation wavelength 407 nm approaches the PP excitation maximum at 397 nm and is far off the eZnPP excitation maximum at 424 nm, the emission spectrum F_{407} shows a pronounced PP fluorescence emission peak, which is found at 627 nm, compared to the lower eZnPP fluorescence peak at 593 nm. The difference in the range 520 nm-570 nm becomes nearly zero, which shows that the background fluorescence is eliminated by calculating the difference spectrum.

[0119] The difference spectrum is then used to evaluate both eZnPP and PP fluorescence. The eZnPP/heme ratio can be directly quantified by evaluating the fluorescence intensity at 593 nm (averaging over 590 nm-596 nm), as illustrated in Figure 39. Also, a eZnPP emission spectrum is drafted. At 627 nm, a linear combination of eZnPP and PP fluorescence intensities yields the signal at 627 nm: the eZnPP fluorescence (positive value in the difference spectrum) and the PP fluorescence (negative value in the difference spectrum). Accordingly, a measure for the PP/heme ratio I_{PP} can be calculated according to Equation (5): The PP fluorescence is the negative of the detected fluorescence at 627 nm I_{627} plus the eZnPP fluorescence intensity at 627 nm, which equals 1/3 of the eZnPP fluorescence intensity at 593 nm.

$$I_{PP} = -I_{627} + (1/3)I_{593} \quad (5)$$

The eZnPP/PP ratio was calculated by dividing the eZnPP/heme and the PP/heme ratios, I_{ZPP}/I_{PP}. As both ratios are given in arbitrary units, this calculated eZnpp/PP ratio is also given in arbitrary units.

[0120] MATLAB (R2010a, MathWorks®, Natick, MA, USA) was used for statistical data evaluation. For the statistical evaluation of the correlation of two methods, a linear regression was calculated using a least square fit (function polyfit) and the Pearson product-moment correlation coefficient (PCC) R-value was calculated (function corrcoef) as well as the p-value (*e.g.*, test against the hypothesis of no correlation, t statistics, correlation if $p < 0.05$).

[0121] Results of the testing described herein are illustrated in Figures 40-47. The eZnPP/heme ratio's correlation of the Aviv hematofluorometer and the reference standard HPLC is shown in Figures 40-41. The error bars indicate the precision of each method, being 9% (HPLC) and 15% (hematofluorometer). The linear regression is shown in Figure 40 (solid black line). The number of samples n=35, the correlation coefficient R = 0.967, with a p-value $p < 0.0001$. The relative residuals of the measurement and the linear regression are shown in Figure 41, being in the range -0.39 ... +0.46.

[0122] The precision of the eZnPP peak intensity evaluation of the fluorescence spectroscopic measurements was 10%, determined by repeated measurements of the same sample. The correlation of the eZnPP fluorescence intensity

at 593 nm evaluated by the two wavelengths emission method (y-axis) and the eZnPP/heme ratio (μmol eZnPP/mol heme) measured by HPLC measurements (x-axis) is shown in Figures 42-43. The error bars indicate the precision of each method for three exemplary measurements. The linear regression is shown in Figure 42 (solid black line). The number of samples n=35, the correlation coefficient R = 0.978, with a p-value p < 0.0001. The relative residuals of the measurement and the linear regression are shown in Figure 43, being in the range -0.48 ... +0.53.

[0123] The precision of the evaluated eZnPP peak intensity of the difference spectrum was 10%, determined by repeated measurements of the same sample. The correlation of the eZnPP fluorescence intensity at 593 nm evaluated by the two wavelengths excitation method (y-axis) and the eZnPP/heme ratio (μmol eZnPP/mol heme) measured by HPLC measurements (x-axis) is shown in Figures 44-45. The error bars indicate the precision of each method for three exemplary measurements. The linear regression is shown in Figure 44 (solid black line). The number of samples n=35, the correlation coefficient R = 0.976, with a p-value p < 0.0001. The relative residuals of the measurement and the linear regression are shown in Figure 45, being in the range -0.50 ... +0.57.

[0124] The precision of the PP peak intensity, calculated from the intensity of the difference spectrum at 627 nm and 593 nm using Equation (5), was 15%, determined by repeated measurements of the same sample. The correlation between the measured PP fluorescence intensity of the difference spectrum at 627 nm evaluated by the two wavelengths excitation method (y-axis) and the PP/heme ratio (μmol PP/mol heme) measured by HPLC measurements (x-axis) is shown in Figures 46-47. The error bars indicate the precision of each method for three exemplary measurements. The linear regression is shown in Figure 46 (solid black line). The number of samples n=35, the correlation coefficient R = 0.996, with a p-value p < 0.0001. The relative residuals of the measurement and the linear regression are shown in Figure 47, being in the range -0.37 ... +0.50.

[0125] The eZnPP/PP ratio, calculated from the eZnPP and PP fluorescence intensities for each patient blood sample (n = 35), is shown in Figure 48. The average eZnPP/PP ratio was 2.74 (arbitrary units), with a range 0.64 ... 7.91 (arbitrary units).

[0126] It is also understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting, since the scope of the present subject matter is limited only by the appended claims.

Claims

1. An apparatus (100) for noninvasive measurement of a concentration of a fluorescent analyte in the blood of a patient comprising:

   a light source (102) for alternately providing excitation of the analyte and the blood at a first wavelength range and a second wavelength range, the first and second excitation wavelength ranges selected such that the analyte exhibits a difference in emission intensities at the first and second excitation wavelength ranges and such that light absorbance of blood at the first and second wavelength ranges is similar;
   one or more detectors (104) for detecting a portion of the emission spectra of the fluorescent analyte at the first excitation wavelength range and the second excitation wavelength range; and
   a processor (106) adapted to determine a derived signal representative of the concentration of the analyte based on the difference between the portion of the emission spectra excited at the first excitation wavelength range and the second excitation wavelength range.

2. An apparatus of claim 1, wherein the first and second excitation wavelength ranges are selected such that the fluorescent analyte exhibits a difference in emission intensities at the first and second excitation wavelength ranges that is greater than that of background fluorophores.

3. An apparatus of claim 1, wherein the apparatus (100) is for measurement of a concentration of a fluorescent analyte in whole blood.

4. An apparatus of claim 1, further comprising a tunable filter unit (440) for providing excitation of the blood and the analyte at the first wavelength range and the second wavelength range.

5. An apparatus of claim 4, wherein the tunable filter unit (440) comprises a first optical filter and a second optical filter, the first and second optical filters capable of independent variation of the angle of incidence of light provided by the light source.

6. An apparatus of claim 4, wherein the tunable filter unit (440) comprises two tunable bandpass filters (444, 446).

7. An apparatus of claim 4, wherein the tunable filter unit (440) comprises a first optical filter (444) and a second optical filter (446) and a third filter to correct for offset of the light passing through the first and second optical filters.

8. An apparatus of claim 1, further comprising one or more optical filters (444, 446), wherein the emission spectra of the fluorescent analyte defines a wavelength range, and wherein the detector (104) includes one or more light sensitive elements for receiving light through the one or more optical filters transmitting light in the wavelength range of the emission spectra of the fluorescent analyte.

9. An apparatus of claim 8, wherein the emission spectra of the fluorescent analyte define an emission maximum, wherein a first portion of the detector (104) is configured to receive light through the optical filters transmitting light in the wavelength range of the emission spectra of the fluorescent analyte, and
wherein a second portion of the detector (104) is configured to receive light through optical filters transmitting light in a wavelength range outside the emission maximum of the fluorescent analyte.

10. An apparatus of claim 1, further comprising a probe (108) comprising an optical fiber (111) associated with the light source (102) and an optical fiber (109) associated with the detector (104).

11. An apparatus of claim 10, wherein the probe (108) comprises a plurality of optical fibers (111) associated with the light source (102) surrounding an optical fiber (109) associated with the detector (104).

12. An apparatus of claim 10, wherein the probe (108) comprises a plurality of optical fibers (109) associated with the detector (104) surrounding the optical fiber (111) associated with the light source (102).

13. An apparatus of claim 10, wherein interfiber spacing of the optical fiber associated with the light source and the optical fiber associated with the detector is selected such that the derived signal is insensitive to the blood volume fraction.

14. An apparatus of claim 10, wherein interfiber spacing (d) of the optical fiber (111) associated with the light source (102) and the optical fiber (109) associated with the detector (104) is selected to achieve a maximum detection sensitivity at a selected depth of the tissue.

15. An apparatus of claim 14, wherein the selected depth of the tissue (T) is selected as the depth having the highest expected concentration of the fluorescent analyte.

16. An apparatus according to claim 1, wherein the apparatus (100) is designed for noninvasive measurement of a concentration of erythrocyte zinc protoporphyrin (eZnPP) as the eZnPP/heme ratio in the blood of a patient wherein the first excitation wavelength range selected at the excitation peak of eZnPP and the second excitation wavelength range selected so that the absorbance of blood is similar to that of the first excitation wavelength range;
and wherein the processor (106) is adapted for determining the concentration of eZnPP based on the difference between the portion of the emission spectra excited at the first excitation wavelength range and the second excitation wavelength range.

17. An apparatus of claim 16, wherein the apparatus (100) is for measurement of a concentration of eZnPP in whole blood.

18. An apparatus of claim 16, further comprising one or more optical filters, wherein the emission spectra of the eZnPP defines a wavelength range, and wherein the detector includes one or more light sensitive elements receiving light through the one or more optical filters transmitting light in the wavelength range of the emission spectra of the eZnPP.

19. An apparatus of claim 18, wherein the emission spectra of the eZnPP define an emission maximum,
wherein a first portion of the detectors (104) is configured to receive light through the optical filters transmitting light in the wavelength range of the emission spectra of the eZnPP, and
wherein a second portion of the detectors (104) is configured to receive light through optical filters transmitting light in a wavelength range outside the emission maximum of the eZnPP.

20. An apparatus according to claim 1, wherein the apparatus (100) is designed for simultaneous measurement of a concentration of erythrocyte zinc protoporphyrin (eZnPP) and erythrocyte protoporphyrin IX (ePP) as the eZnPP/heme ratio and ePP/heme ratio in the blood of a patient, wherein
the first excitation wavelength range is selected at the excitation peak of eZnPP and the second excitation wavelength

range selected so that the absorbance of blood is similar to that of the first excitation wavelength range; and
wherein the processor (106) is adapted for determining the concentration of eZnPP and ePP based on the difference between the portion of the emission spectra excited at the first excitation wavelength range and the second excitation wavelength range.

21. An apparatus according to claim 1, wherein the apparatus (100) is designed for noninvasive measurement of a concentration of erythrocyte zinc protoporphyrin (eZnPP) as the eZnPP/heme ratio in the blood of a patient wherein the light source is adapted for providing excitation of the tissue (T) at about 425 nm and about 407 nm;
wherein the detector (104) is adapted for detecting a portion of the emission spectra excited at about 425 nm and about 407 nm; and
where the the processor (106) is adapted for determining the concentration of eZnPP based on the difference between the portion of the emission spectra excited at about 425 nm and about 407 nm.

22. A method for noninvasive measurement of a concentration of a fluorescent analyte in the blood of a patient comprising:

alternately exciting tissue of the patient at a first wavelength range and a second wavelength range, the first and second excitation wavelength ranges selected such that the fluorescent analyte exhibits a difference in emission intensities at the first and second excitation wavelength ranges that is greater than that of background fluorophores and light absorbance by blood at the first and second excitation wavelength ranges is similar;
detecting a portion of the emission spectra at the first excitation wavelength range and the second excitation wavelength range; and
determining the concentration of the fluorescent analyte based on the difference between the emission spectra excited at the first excitation wavelength range and the second excitation wavelength range.

Patentansprüche

1. Vorrichtung (100) zur nichtinvasiven Messung der Konzentration eines fluoreszierenden Analyten im Blut eines Patienten, umfassend:

eine Lichtquelle (102) zum abwechselnden Bereitstellen von Anregung des Analyten und des Bluts in einem ersten Wellenlängenbereich und einem zweiten Wellenlängenbereich, wobei der erste und der zweite Anregungswellenlängenbereich so ausgewählt sind, dass der Analyt einen Unterschied der Emissionsintensitäten in dem ersten und dem zweiten Anregungswellenlängenbereich zeigt, und so, dass die Lichtabsorbanz von Blut in dem ersten und dem zweiten Wellenlängenbereich ähnlich ist;
einen oder mehrere Detektoren (104) zum Erfassen eines Teils der Emissionsspektren des fluoreszierenden Analyten in dem ersten Anregungswellenlängenbereich und dem zweiten Anregungswellenlängenbereich; und
einen Prozessor (106), der dafür ausgelegt ist, ein abgeleitetes Signal, das für die Konzentration des Analyten repräsentativ ist, auf der Grundlage des Unterschieds zwischen den Teilen der Emissionsspektren, die in dem ersten Anregungswellenlängenbereich und dem zweiten Anregungswellenlängenbereich angeregt werden, zu bestimmen.

2. Vorrichtung gemäß Anspruch 1, wobei der erste und der zweite Anregungswellenlängenbereich so ausgewählt sind, dass der fluoreszierende Analyt einen Unterschied der Emissionsintensitäten für den ersten und dem zweiten Anregungswellenlängenbereich zeigt, der größer als jener der Hintergrund-Fluorophore ist.

3. Vorrichtung gemäß Anspruch 1, wobei die Vorrichtung (100) zur Messung der Konzentration eines fluoreszierenden Analyten in Vollblut ist.

4. Vorrichtung gemäß Anspruch 1, ferner umfassend eine einstellbare Filtereinheit (440) zum Bereitstellen von Anregung des Bluts und des Analyten in dem ersten Wellenlängenbereich und dem zweiten Wellenlängenbereich.

5. Vorrichtung gemäß Anspruch 4, wobei die einstellbare Filtereinheit (440) einen ersten optischen Filter und einen zweiten optischen Filter umfasst, wobei der erste und der zweite optische Filter zur unabhängigen Variation des Einfallwinkels von Licht, das von der Lichtquelle bereitgestellt wird, fähig sind.

6. Vorrichtung gemäß Anspruch 4, wobei die einstellbare Filtereinheit (440) zwei einstellbare Bandpassfilter (444, 446)

umfasst.

7. Vorrichtung gemäß Anspruch 4, wobei die einstellbare Filtereinheit (440) einen ersten optischen Filter (444) und einen zweiten optischen Filter (446) und einen dritten Filter zum Korrigieren der Versetzung des Lichts, das durch den ersten und den zweiten optischen Filter tritt, umfasst.

8. Vorrichtung gemäß Anspruch 1, ferner umfassend einen oder mehrere optische Filter (444, 446), wobei das Emissionsspektrum des fluoreszierenden Analyten einen Wellenlängenbereich definiert und wobei der Detektor (104) ein oder mehrere lichtempfindliche Elemente zum Empfangen von Licht durch den einen oder die mehreren optischen Filter, die Licht in dem Wellenlängenbereich des Emissionsspektrums des fluoreszierenden Analyten durchlassen, umfasst.

9. Vorrichtung gemäß Anspruch 8, wobei das Emissionsspektrums des fluoreszierenden Analyten ein Emissionsmaximum definiert,
wobei ein erster Teil des Detektors (104) dafür gestaltet ist, Licht durch die optischen Filter zu empfangen, die Licht in dem Wellenlängenbereich des Emissionsspektrums des fluoreszierenden Analyten durchlassen, und
wobei ein zweiter Teil des Detektors (104) dafür gestaltet ist, Licht durch optische Filter zu empfangen, die Licht in einem Wellenlängenbereich außerhalb des Emissionsmaximums des fluoreszierenden Analyten durchlassen.

10. Vorrichtung gemäß Anspruch 1, ferner umfassend eine Sonde (108), die eine optische Faser (111), die mit der Lichtquelle (102) verbunden ist, und eine optische Faser (109), die mit dem Detektor (104) verbunden ist, umfasst.

11. Vorrichtung gemäß Anspruch 10, wobei die Sonde (108) eine Vielzahl von optischen Fasern (111), die mit der Lichtquelle verbunden sind, die eine optische Faser (109) umgeben, die mit dem Detektor (104) verbunden ist, umfasst.

12. Vorrichtung gemäß Anspruch 10, wobei die Sonde (108) eine Vielzahl von optischen Fasern (109), die mit dem Detektor (104) verbunden sind, die die optische Faser (111) umgeben, die mit der Lichtquelle verbunden ist, umfasst.

13. Vorrichtung gemäß Anspruch 10, wobei der Zwischenfaserabstand der optischen Faser, die mit der Lichtquelle verbunden ist, und der optischen Faser, die mit dem Detektor verbunden ist, so ausgewählt ist, dass das abgeleitete Signal unempfindlich gegen den Anteil des Blutvolumens ist.

14. Vorrichtung gemäß Anspruch 10, wobei der Zwischenfaserabstand (d) der optischen Faser (111), die mit der Lichtquelle (102) verbunden ist, und der optischen Faser (109), die mit dem Detektor (104) verbunden ist, ausgewählt ist, um eine maximale Nachweisempfindlichkeit bei einer ausgewählten Tiefe des Gewebes zu erzielen.

15. Vorrichtung gemäß Anspruch 14, wobei die ausgewählte Tiefe des Gewebes (T) als die Tiefe ausgewählt ist, die die höchste erwartete Konzentration des fluoreszierenden Analyten aufweist.

16. Vorrichtung gemäß Anspruch 1, wobei die Vorrichtung (100) zur nichtinvasiven Messung der Konzentration von Erythrozyten-Zinkprotoporphyrin (eZnPP) als das eZnPP/Häm-Verhältnis im Blut eines Patienten ausgelegt ist, wobei der erste Anregungswellenlängenbereich an dem Anregungsmaximum von eZnPP ausgewählt ist und der zweite Anregungswellenlängenbereich so ausgewählt ist, dass die Absorbanz von Blut jener in dem ersten Anregungswellenlängenbereich ähnlich ist;
und wobei der Prozessor (106) dafür ausgelegt ist, die Konzentration von eZnPP auf der Grundlage des Unterschieds zwischen den Teilen der Emissionsspektren, die in dem ersten Anregungswellenlängenbereich und dem zweiten Anregungswellenlängenbereich angeregt werden, zu bestimmen.

17. Vorrichtung gemäß Anspruch 16, wobei die Vorrichtung (100) zur Messung der Konzentration von eZnPP in Vollblut ist.

18. Vorrichtung gemäß Anspruch 16, ferner umfassend einen oder mehrere optische Filter, wobei das Emissionsspektrum des eZnPP einen Wellenlängenbereich definiert und wobei der Detektor ein oder mehrere lichtempfindliche Elemente umfasst, die Licht durch den einen oder die mehreren optischen Filter, die Licht in dem Wellenlängenbereich des Emissionsspektrums des eZnPP durchlassen, empfangen.

19. Vorrichtung gemäß Anspruch 18, wobei das Emissionsspektrums des eZnPP ein Emissionsmaximum definiert,

wobei ein erster Teil des Detektors (104) dafür gestaltet ist, Licht durch die optischen Filter zu empfangen, die Licht in dem Wellenlängenbereich des Emissionsspektrums des eZnPP durchlassen, und
wobei ein zweiter Teil des Detektors (104) dafür gestaltet ist, Licht durch optische Filter zu empfangen, die Licht in einem Wellenlängenbereich außerhalb des Emissionsmaximums des eZnPP durchlassen.

20. Vorrichtung gemäß Anspruch 1, wobei die Vorrichtung (100) zur gleichzeitigen Messung der Konzentration von Erythrozyten-Zinkprotoporphyrin (eZnPP) und Erythrozyten-Protoporphyrin IX (ePP) als das eZnPP/Häm-Verhältnis und das ePP/Häm-Verhältnis im Blut eines Patienten ausgelegt ist, wobei
der erste Anregungswellenlängenbereich an dem Anregungsmaximum von eZnPP ausgewählt ist und der zweite Anregungswellenlängenbereich so ausgewählt ist, dass die Absorbanz von Blut jener in dem ersten Anregungswellenlängenbereich ähnlich ist;
und wobei der Prozessor (106) dafür ausgelegt ist, die Konzentration von eZnPP und ePP auf der Grundlage des Unterschieds zwischen den Teilen der Emissionsspektren, die in dem ersten Anregungswellenlängenbereich und dem zweiten Anregungswellenlängenbereich angeregt werden, zu bestimmen.

21. Vorrichtung gemäß Anspruch 1, wobei die Vorrichtung (100) zur nichtinvasiven Messung der Konzentration von Erythrozyten-Zinkprotoporphyrin (eZnPP) als das eZnPP/Häm-Verhältnis im Blut eines Patienten ausgelegt ist, wobei
die Lichtquelle zum Bereitstellen von Anregung des Gewebes (T) bei etwa 425 nm und etwa 407 nm ausgelegt ist;
wobei der Detektor (104) dafür ausgelegt ist, einen Teil der bei etwa 425 nm und bei etwa 407 nm angeregten Emissionsspektren zu erfassen; und
wobei der Prozessor (106) dafür ausgelegt ist, die Konzentration von eZnPP auf der Grundlage des Unterschieds zwischen den Teilen der bei etwa 425 nm und bei etwa 407 nm angeregten Emissionsspektren zu bestimmen.

22. Verfahren zur nichtinvasiven Messung der Konzentration eines fluoreszierenden Analyten im Blut eines Patienten, umfassend:

abwechselndes Anregen von Gewebe des Patienten in einem ersten Wellenlängenbereich und einem zweiten Wellenlängenbereich, wobei der erste und der zweite Anregungswellenlängenbereich so ausgewählt sind, dass der fluoreszierende Analyt einen Unterschied der Emissionsintensitäten in dem ersten und dem zweiten Anregungswellenlängenbereich zeigt, der größer als jener von Hintergrund-Fluorophoren ist, und die Lichtabsorbanz von Blut in dem ersten und dem zweiten Anregungswellenlängenbereich ähnlich ist;
Erfassen eines Teils der Emissionsspektren in dem ersten Anregungswellenlängenbereich und dem zweiten Anregungswellenlängenbereich; und
Bestimmen der Konzentration des fluoreszierenden Analyten auf der Grundlage des Unterschieds zwischen den Emissionsspektren, die in dem ersten Anregungswellenlängenbereich und dem zweiten Anregungswellenlängenbereich angeregt werden.

Revendications

1. Appareil (100) destiné à la mesure non invasive d'une concentration d'un analyte fluorescent dans le sang d'un patient, comprenant :

une source de lumière (102) destinée à fournir alternativement une excitation de l'analyte et du sang dans une première gamme de longueurs d'onde et une deuxième gamme de longueurs d'onde, les première et deuxième gammes de longueurs d'onde d'excitation étant sélectionnées de manière à ce que l'analyte présente une différence d'intensité d'émission dans les première et deuxième gammes de longueurs d'onde d'excitation et de manière à ce que les absorbances de lumière du sang dans les première et deuxième gammes de longueurs d'onde soient semblables ;
un ou plusieurs détecteurs (104) destinés à détecter une partie des spectres d'émission de l'analyte fluorescent dans la première gamme de longueurs d'onde d'excitation et la deuxième gamme de longueurs d'onde d'excitation ; et
un processeur (106) adapté pour déterminer un signal dérivé représentatif de la concentration de l'analyte sur la base de la différence entre la partie des spectres d'émission excités dans la première gamme de longueurs d'onde d'excitation et la deuxième gamme de longueurs d'onde d'excitation.

2. Appareil selon la revendication 1, dans lequel les première et deuxième gammes de longueurs d'onde d'excitation

sont sélectionnées de manière à ce que l'analyte fluorescent présente une différence d'intensité d'émission dans les première et deuxième gammes de longueurs d'onde d'excitation qui est supérieure à celle des fluorophores d'arrière-plan.

3. Appareil selon la revendication 1, dans lequel l'appareil (100) est destiné à la mesure d'une concentration d'un analyte fluorescent dans du sang entier.

4. Appareil selon la revendication 1, comprenant en outre une unité de filtrage accordable (440) destinée à produire une excitation du sang et de l'analyte dans la première gamme de longueurs d'onde et la deuxième gamme de longueurs d'onde.

5. Appareil selon la revendication 4, dans lequel l'unité de filtrage accordable (440) comprend un premier filtre optique et un deuxième filtre optique, les premier et deuxième filtres optiques étant susceptibles de varier indépendamment de l'angle d'incidence de la lumière fournie par la source de lumière.

6. Appareil selon la revendication 4, dans lequel l'unité de filtrage accordable (440) comprend deux filtres passe-bande accordables (444, 446).

7. Appareil selon la revendication 4, dans lequel l'unité de filtrage accordable (440) comprend un premier filtre optique (444) et un deuxième filtre optique (446) et un troisième filtre destiné à corriger le décalage de la lumière passant à travers les premier et deuxième filtres optiques.

8. Appareil selon la revendication 1, comprenant en outre un ou plusieurs filtres optiques (444, 446), dans lequel les spectres d'émission de l'analyte fluorescent définissent une gamme de longueurs d'onde, et dans lequel le détecteur (104) comprend un ou plusieurs éléments photosensibles destinés à recevoir de la lumière à travers le ou les filtres optiques transmettant la lumière dans la gamme de longueurs d'onde des spectres d'émission de l'analyte fluorescent.

9. Appareil selon la revendication 8, dans lequel les spectres d'émission de l'analyte fluorescent définissent un maximum d'émission,
dans lequel une première partie du détecteur (104) est configurée pour recevoir de la lumière à travers les filtres optiques transmettant la lumière dans la gamme de longueurs d'onde des spectres d'émission de l'analyte fluorescent, et
dans lequel une deuxième partie du détecteur (104) est configurée pour recevoir de la lumière à travers les filtres optiques transmettant la lumière dans une gamme de longueurs d'onde se situant à l'extérieur du maximum d'émission de l'analyte fluorescent.

10. Appareil selon la revendication 1, comprenant en outre une sonde (108) comprenant une fibre optique (111) associée à la source de lumière (102) et une fibre optique (109) associée au détecteur (104).

11. Appareil selon la revendication 10, dans lequel la sonde (108) comprend une pluralité de fibres optiques (111) associées à la source de lumière (102) entourant une fibre optique (109) associée au détecteur (104).

12. Appareil selon la revendication 10, dans lequel la sonde (108) comprend une pluralité de fibres optiques (109) associées au détecteur (104) entourant la fibre optique (111) associée à la source de lumière (102).

13. Appareil selon la revendication 10, dans lequel l'espacement interfibres entre la fibre optique associée à la source de lumière et la fibre optique associée au détecteur est sélectionné de manière à ce que le signal dérivé soit insensible à la fraction du volume sanguin.

14. Appareil selon la revendication 10, dans lequel l'espacement interfibres (d) entre la fibre optique (111) associée à la source de lumière (102) et la fibre optique (109) associée au détecteur (104) est sélectionné de manière à obtenir une sensibilité de détection maximale à une profondeur sélectionnée du tissu.

15. Appareil selon la revendication 14, dans lequel la profondeur sélectionnée du tissu (T) est sélectionnée comme étant la profondeur ayant la concentration attendue la plus élevée de l'analyte fluorescent.

16. Appareil selon la revendication 1, dans lequel l'appareil (100) est conçu pour la mesure non invasive d'une concen-

tration de protoporphyrine de zinc érythrocytaire (eZnPP) sous la forme du rapport eZnPP/hème dans le sang d'un patient, dans lequel la première gamme de longueurs d'onde d'excitation est sélectionnée au pic d'excitation de l'eZnPP et la deuxième gamme de longueurs d'onde d'excitation est sélectionnée de manière à ce que l'absorbance du sang soit semblable à celle de la première gamme de longueurs d'onde d'excitation ;
et dans lequel le processeur (106) est adapté pour déterminer la concentration d'eZnPP sur la base de la différence entre la partie des spectres d'émission excités dans la première gamme de longueurs d'onde d'excitation et la deuxième gamme de longueurs d'onde d'excitation.

17. Appareil selon la revendication 16, dans lequel l'appareil (100) est destiné à la mesure d'une concentration d'eZnPP dans du sang entier.

18. Appareil selon la revendication 16, comprenant en outre un ou plusieurs filtres optiques, dans lequel les spectres d'émission de l'eZnPP définissent une gamme de longueurs d'onde, et dans lequel le détecteur comprend un ou plusieurs éléments photosensibles recevant de la lumière à travers le ou les filtres optiques transmettant la lumière dans la gamme de longueurs d'onde des spectres d'émission de l'eZnPP.

19. Appareil selon la revendication 18, dans lequel les spectres d'émission de l'eZnPP définissent un maximum d'émission,
dans lequel une première partie des détecteurs (104) est configurée pour recevoir de la lumière à travers les filtres optiques transmettant la lumière dans la gamme de longueurs d'onde des spectres d'émission de l'eZnPP, et
dans lequel une deuxième partie des détecteurs (104) est configurée pour recevoir de la lumière à travers des filtres optiques transmettant la lumière dans une gamme de longueurs d'onde se situant à l'extérieur du maximum d'émission de l'eZnPP.

20. Appareil selon la revendication 1, dans lequel l'appareil (100) est conçu pour la mesure simultanée d'une concentration de protoporphyrine de zinc érythrocytaire (eZnPP) et de protoporphyrine érythrocytaire IX (ePP) sous la forme du rapport eZnPP/hème et du rapport ePP/hème dans le sang d'un patient, dans lequel
la première gamme de longueurs d'onde d'excitation est sélectionnée au pic d'excitation de l'eZnPP et la deuxième gamme de longueurs d'onde d'excitation est sélectionnée de manière à ce que l'absorbance du sang soit semblable à celle de la première gamme de longueurs d'onde d'excitation ; et
dans lequel le processeur (106) est adapté pour déterminer la concentration d'eZnPP et d'ePP sur la base de la différence entre la partie des spectres d'émission excités dans la première gamme de longueurs d'onde d'excitation et la deuxième gamme de longueurs d'onde d'excitation.

21. Appareil selon la revendication 1, dans lequel l'appareil (100) est conçu pour la mesure non invasive d'une concentration de protoporphyrine de zinc érythrocytaire (eZnPP) sous la forme du rapport eZnPP/hème dans le sang d'un patient, dans lequel la source de lumière est adaptée pour fournir une excitation du tissu (T) à environ 425 nm et à environ 407 nm ;
dans lequel le détecteur (104) est adapté pour détecter une partie des spectres d'émission excités à environ 425 nm et environ 407 nm ; et
dans lequel le processeur (106) est adapté pour déterminer la concentration d'eZnPP sur la base de la différence entre la partie des spectres d'émission excités à environ 425 nm et environ 407 nm.

22. Procédé pour la mesure non invasive d'une concentration d'un analyte fluorescent dans le sang d'un patient, consistant à :

exciter alternativement le tissu du patient dans une première gamme de longueurs d'onde et une deuxième gamme de longueurs d'onde, les première et deuxième gammes de longueurs d'onde d'excitation étant sélectionnées de manière à ce que l'analyte fluorescent présente une différence d'intensité d'émission dans les première et deuxième gammes de longueurs d'onde d'excitation qui est supérieure à celle des fluorophores d'arrière-plan et à ce que les absorbances de lumière par le sang soient semblables dans les première et deuxième gammes de longueurs d'onde d'excitation ;
détecter une partie des spectres d'émission dans la première gamme de longueurs d'onde d'excitation et la deuxième gamme de longueurs d'onde d'excitation ; et
déterminer la concentration de l'analyte fluorescent sur la base de la différence entre les spectres d'émission excités dans la première gamme de longueurs d'onde d'excitation et la deuxième gamme de longueurs d'onde d'excitation.

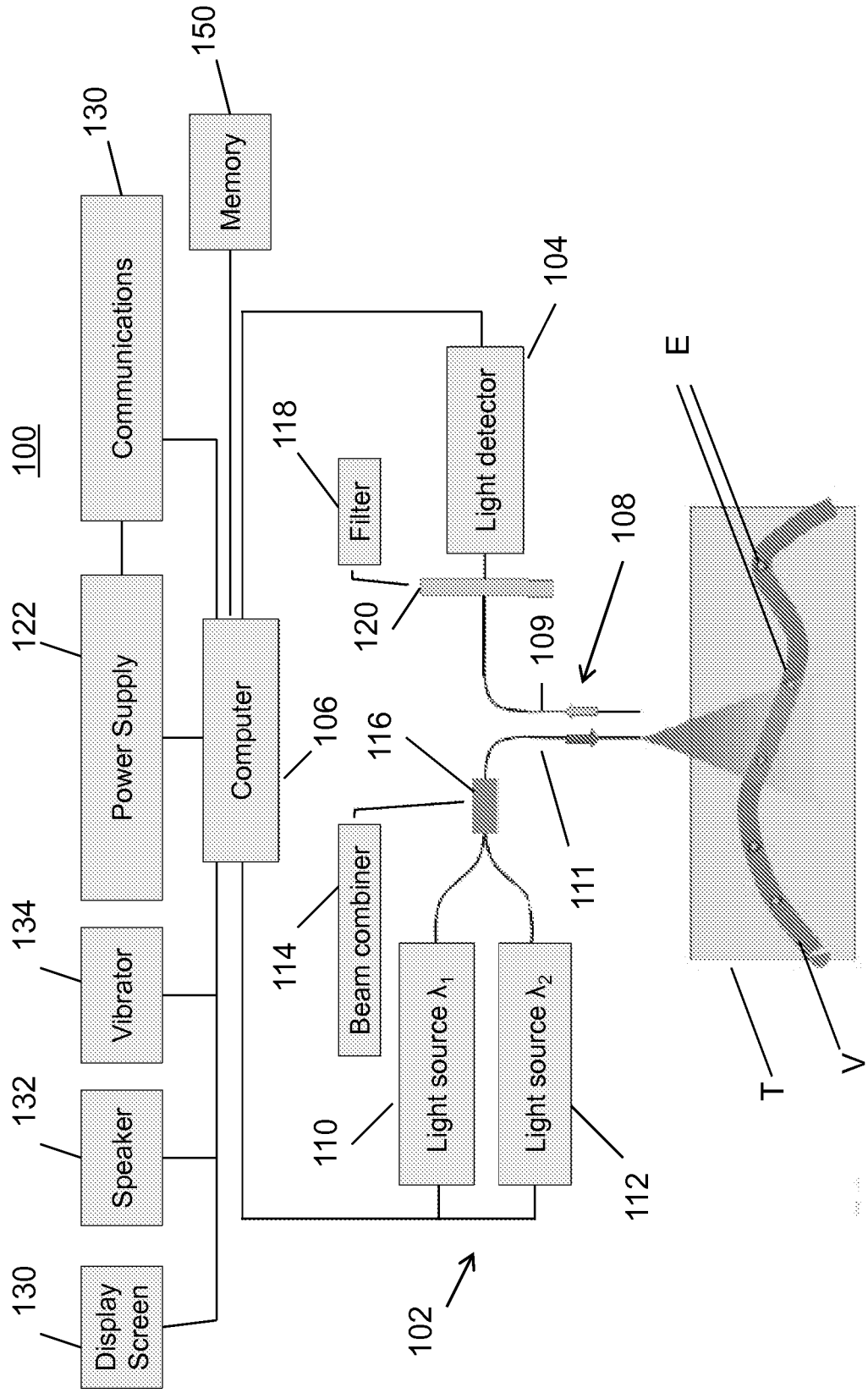

100
102
104
106
108
109
110
111
112
114
116
118
120
122
130
130
132
134
150
Communications
Power Supply
Display Screen
Speaker
Vibrator
Computer
Memory
Filter
Light detector
Beam combiner
Light source λ_1
Light source λ_2
E
T
V

FIGURE 1

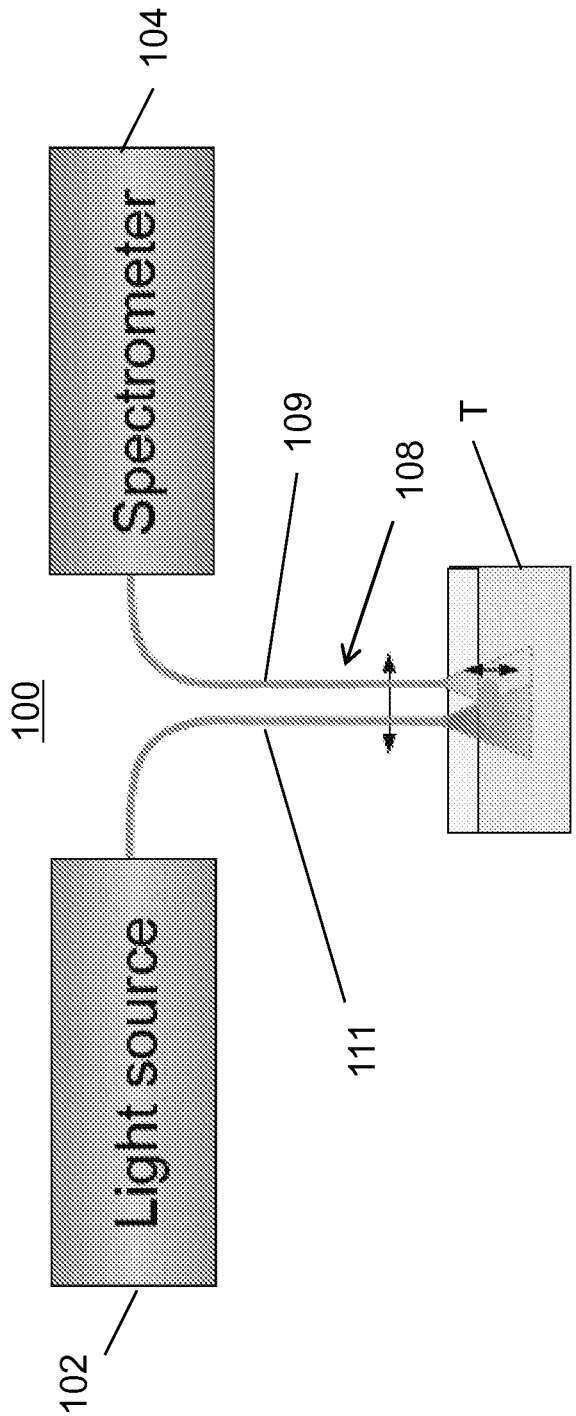
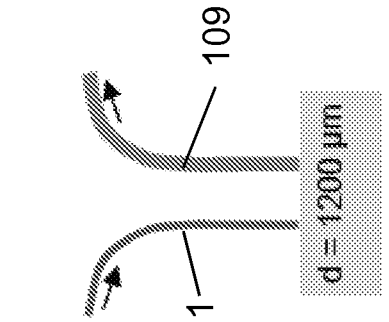


FIGURE 1A



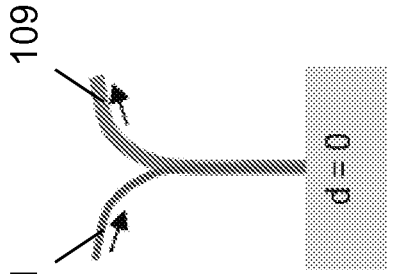


FIGURE 1B FIGURE 1C

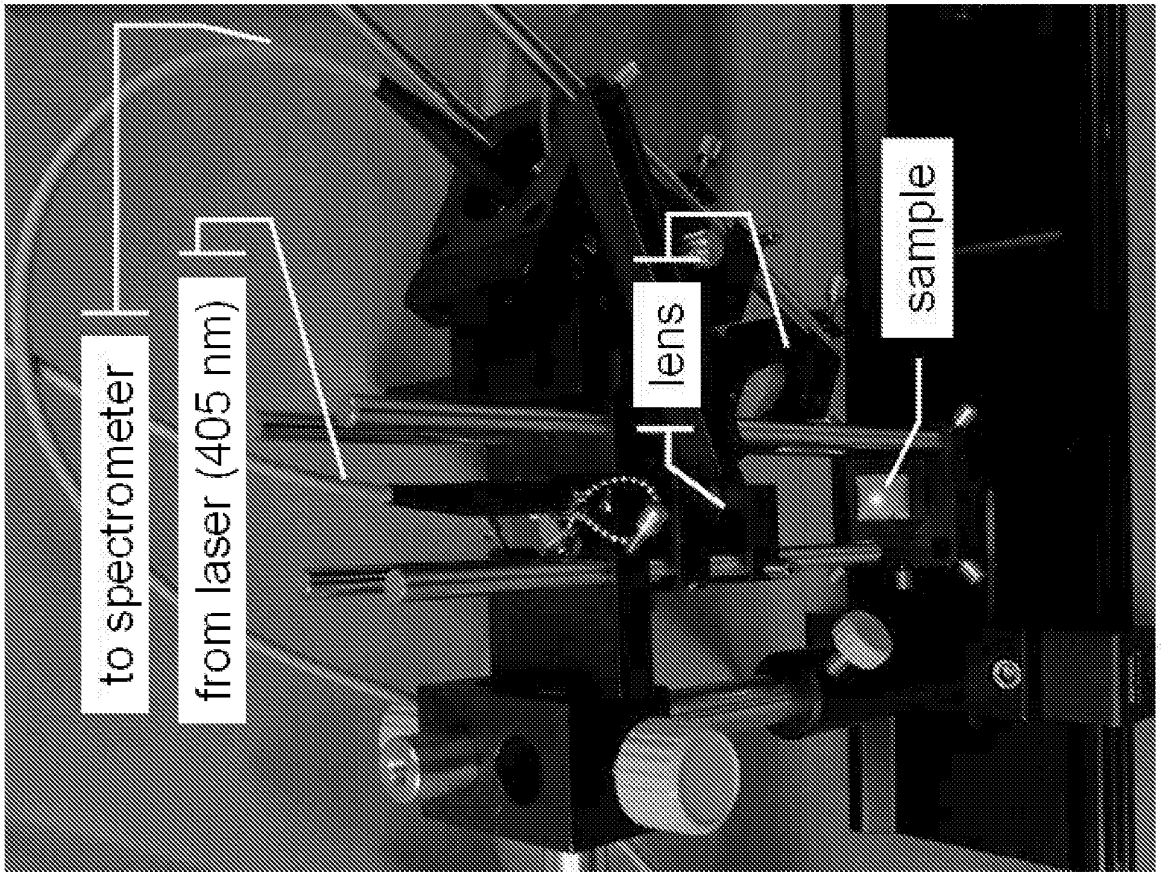


FIGURE 2

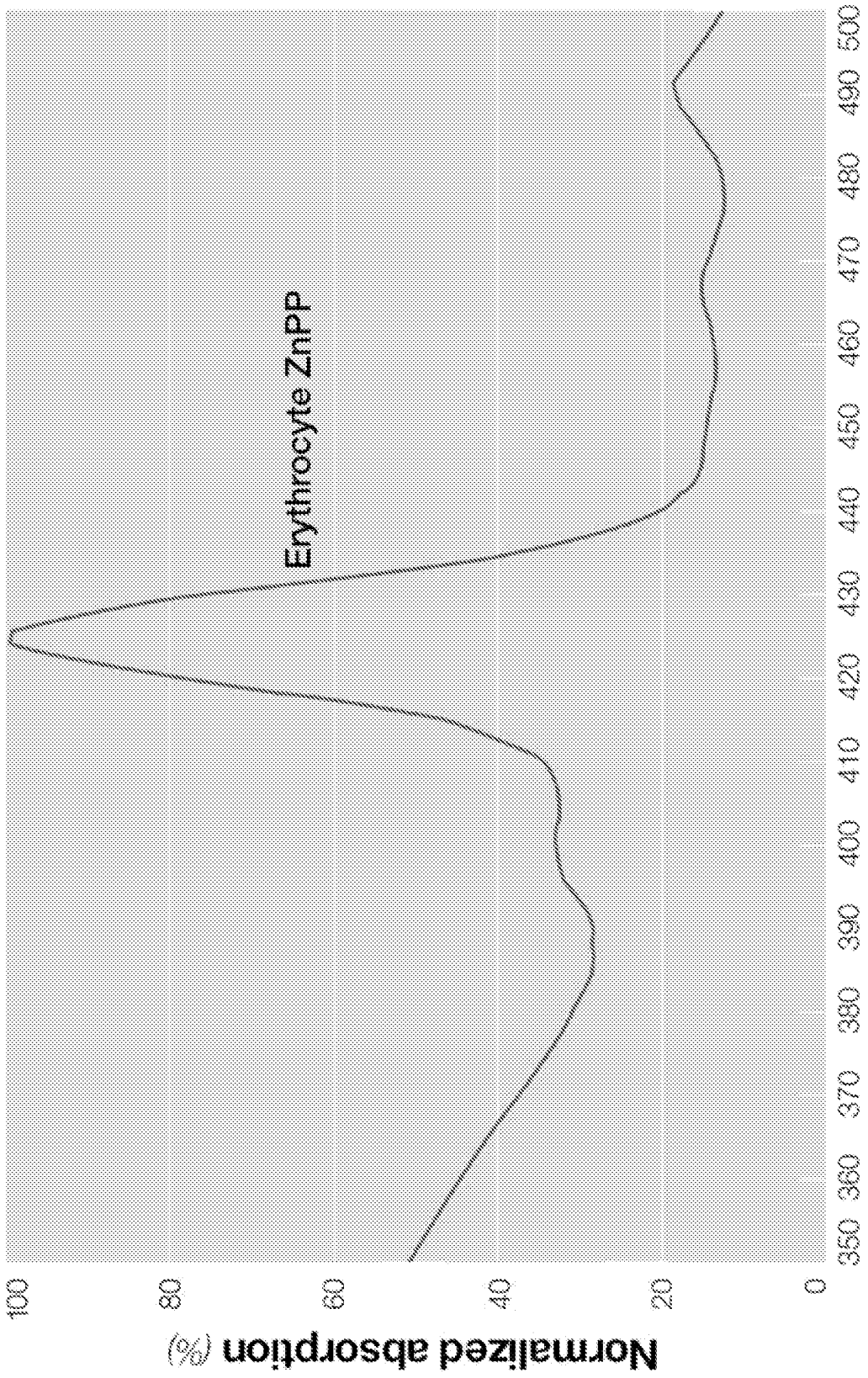

Erythrocyte ZnPP
Normalized absorption (%)
0
20
40
60
80
100
350
360
370
380
390
400
410
420
430
440
450
460
470
480
490
500
Excitation Wavelength (nm)
(Emission at 590 nm)

FIGURE 3

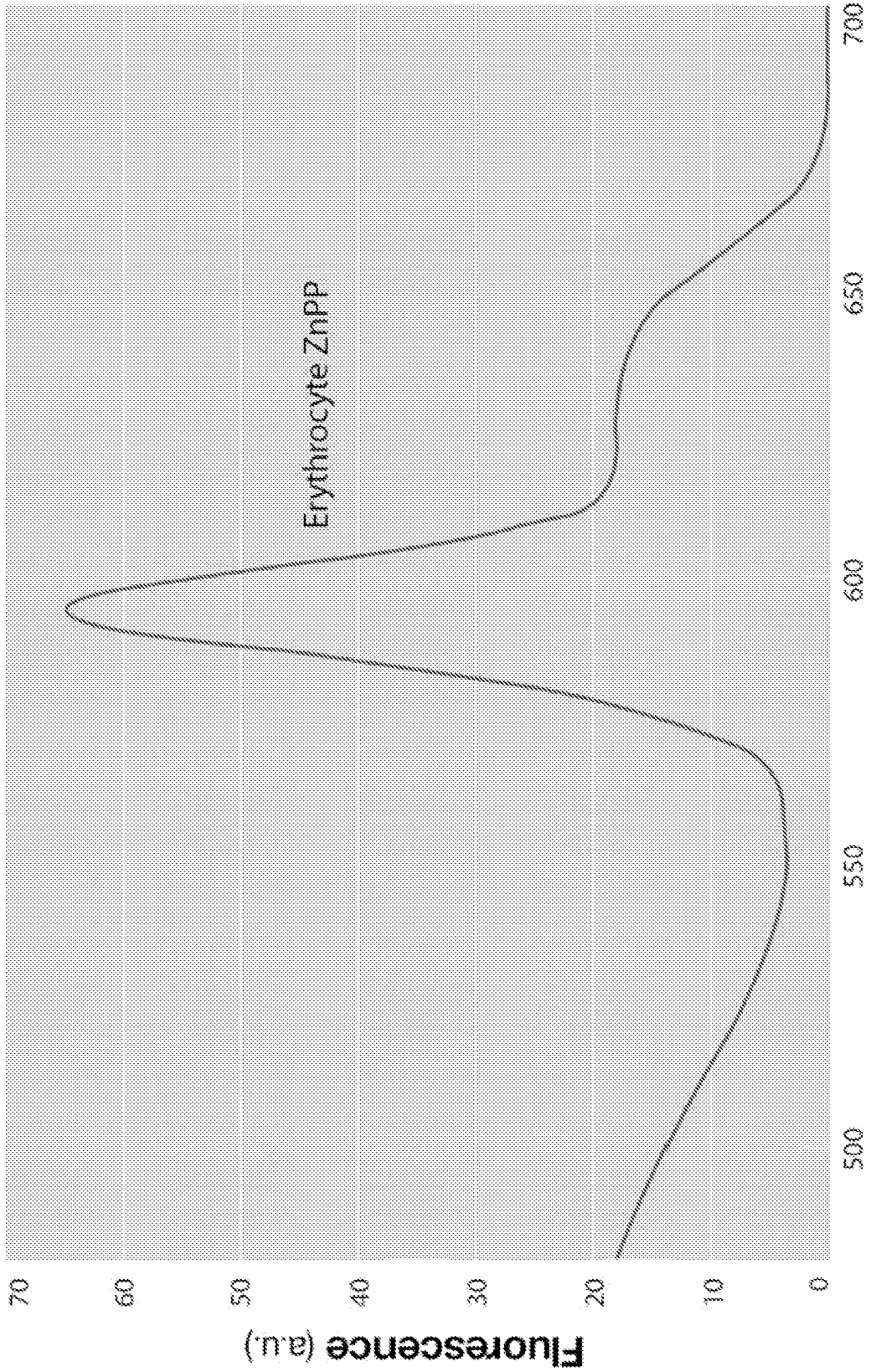

Erythrocyte ZnPP
Fluorescence (a.u.)
0
10
20
30
40
50
60
70
500
550
600
650
700
Emission Wavelength (nm)
(Excitation at 425 nm)

FIGURE 4

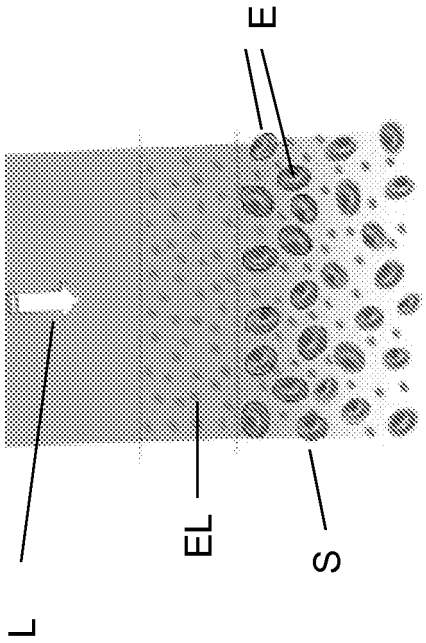


FIGURE 6

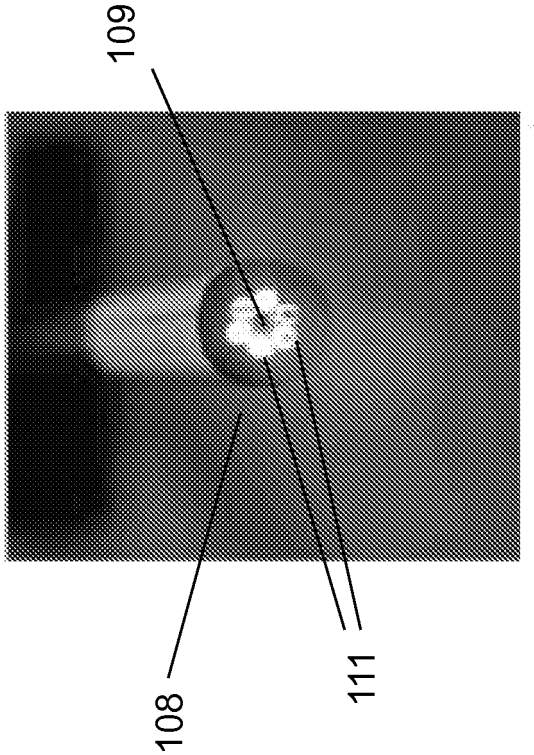


FIGURE 5

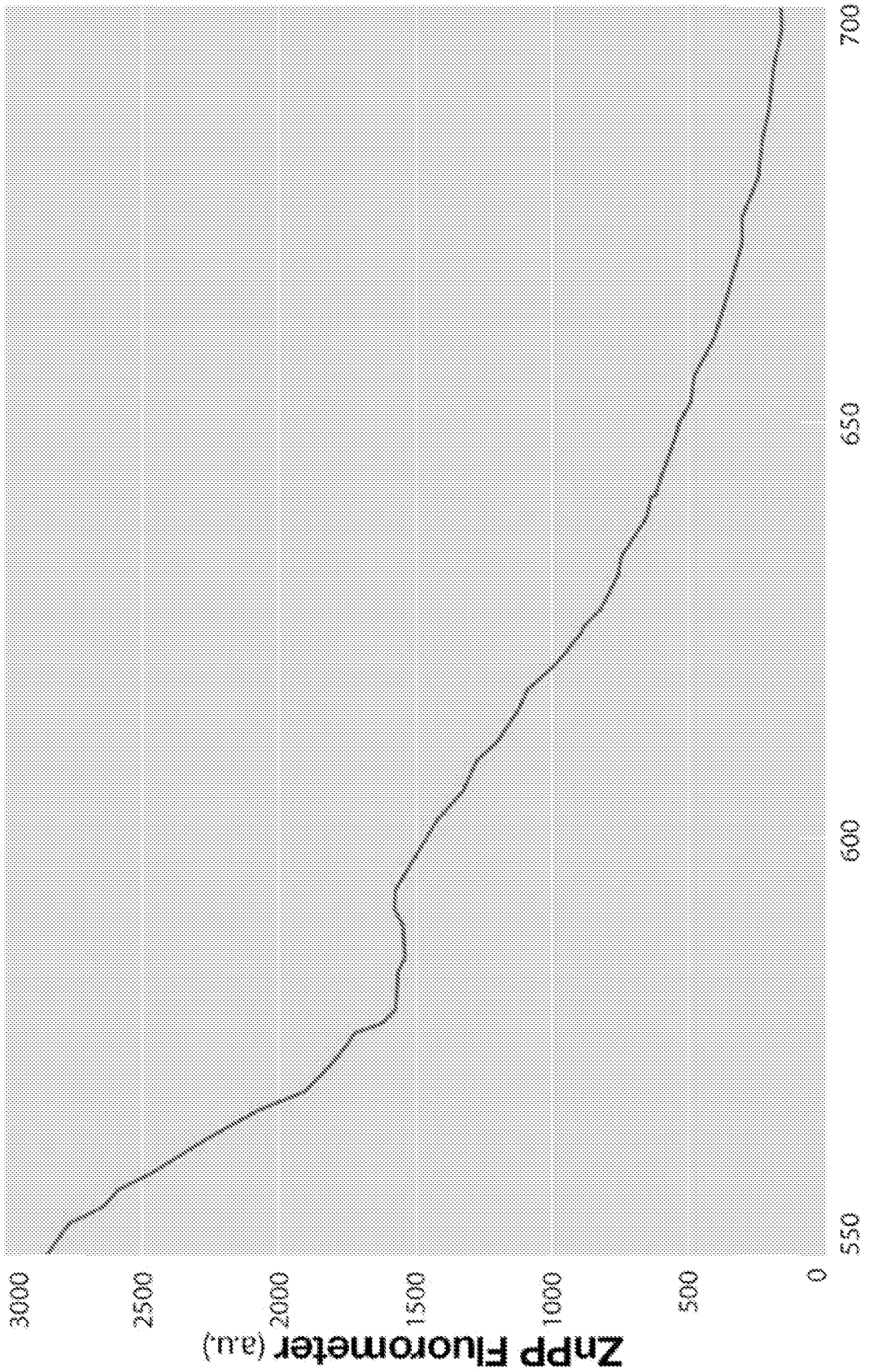

ZnPP Fluorometer (a.u.)
3000
2500
2000
1500
1000
500
0
550
600
650
700
Emission Wavelength (nm)
(Excitation at 425 nm)

FIGURE 7

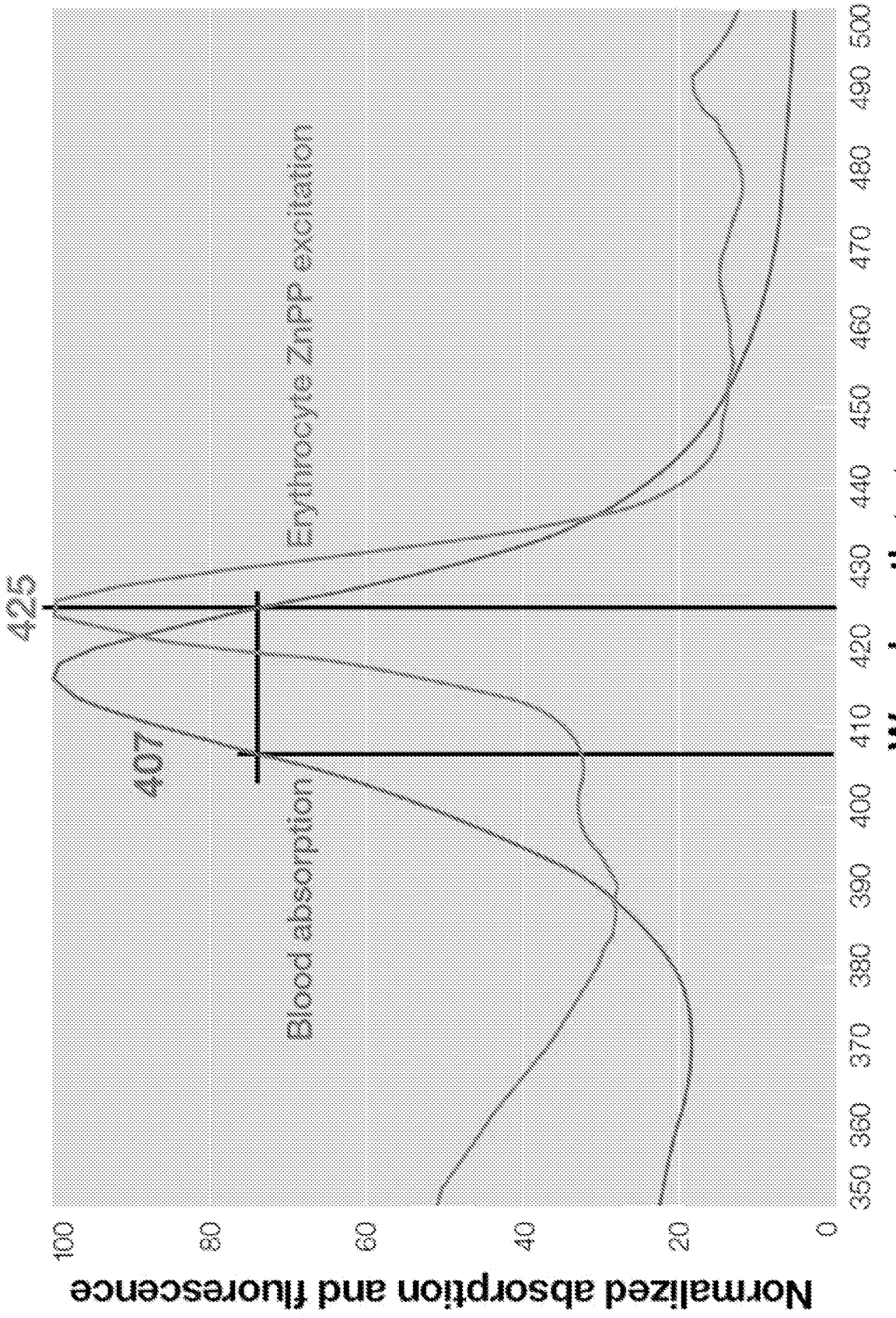

425
407
Erythrocyte ZnPP excitation
Blood absorption
Normalized absorption and fluorescence
Wavelength (nm)
0
20
40
60
80
100
350
360
370
380
390
400
410
420
430
440
450
460
470
480
490
500

FIGURE 8

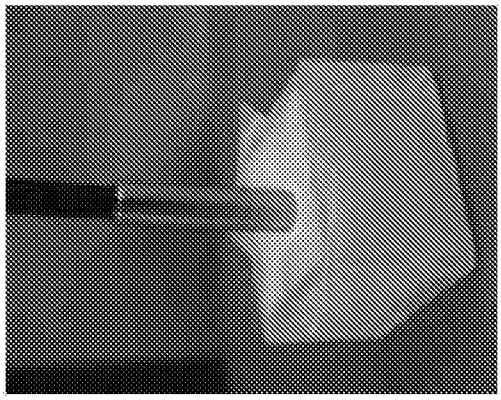


FIGURE 9

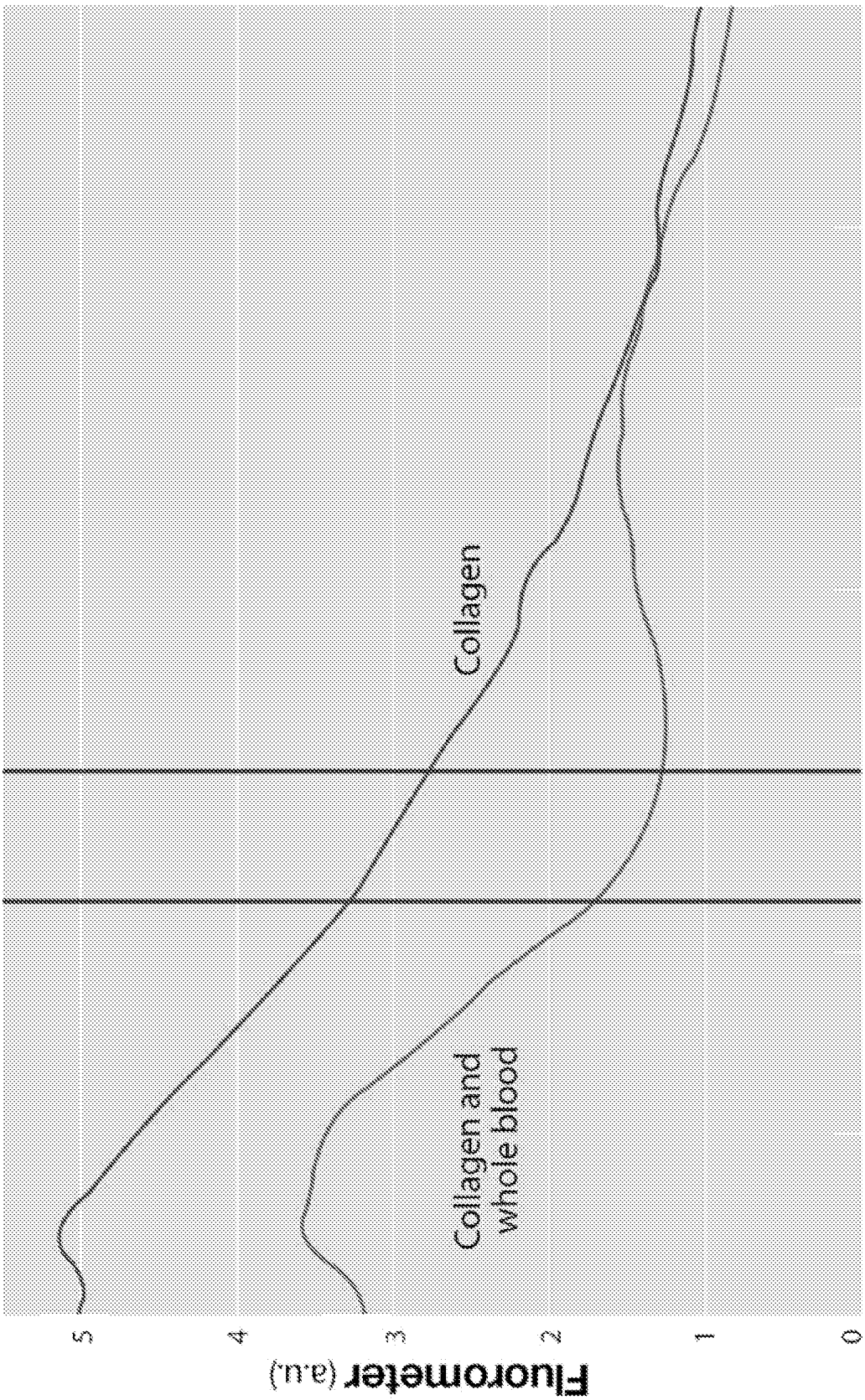

Fluorometer (a.u.)
0
1
2
3
4
5
350
375
407
425
450
475
500
Excitation Wavelength (nm)
(Emission at 590 nm)
Collagen
Collagen and whole blood

FIGURE 10

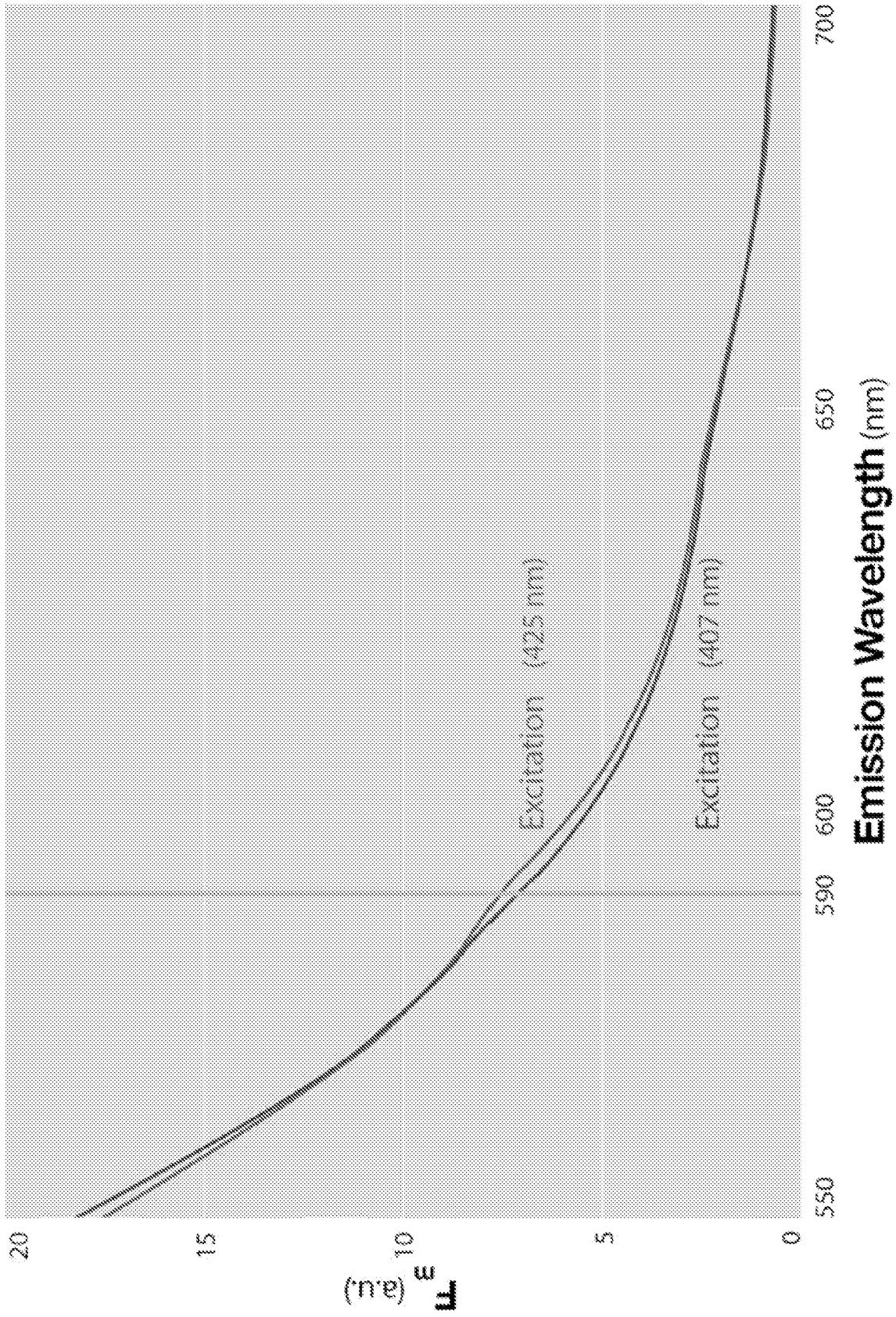

20
15
10
5
0
F_m (a.u.)
550
590
600
650
700
Emission Wavelength (nm)
Excitation (425 nm)
Excitation (407 nm)

FIGURE 11

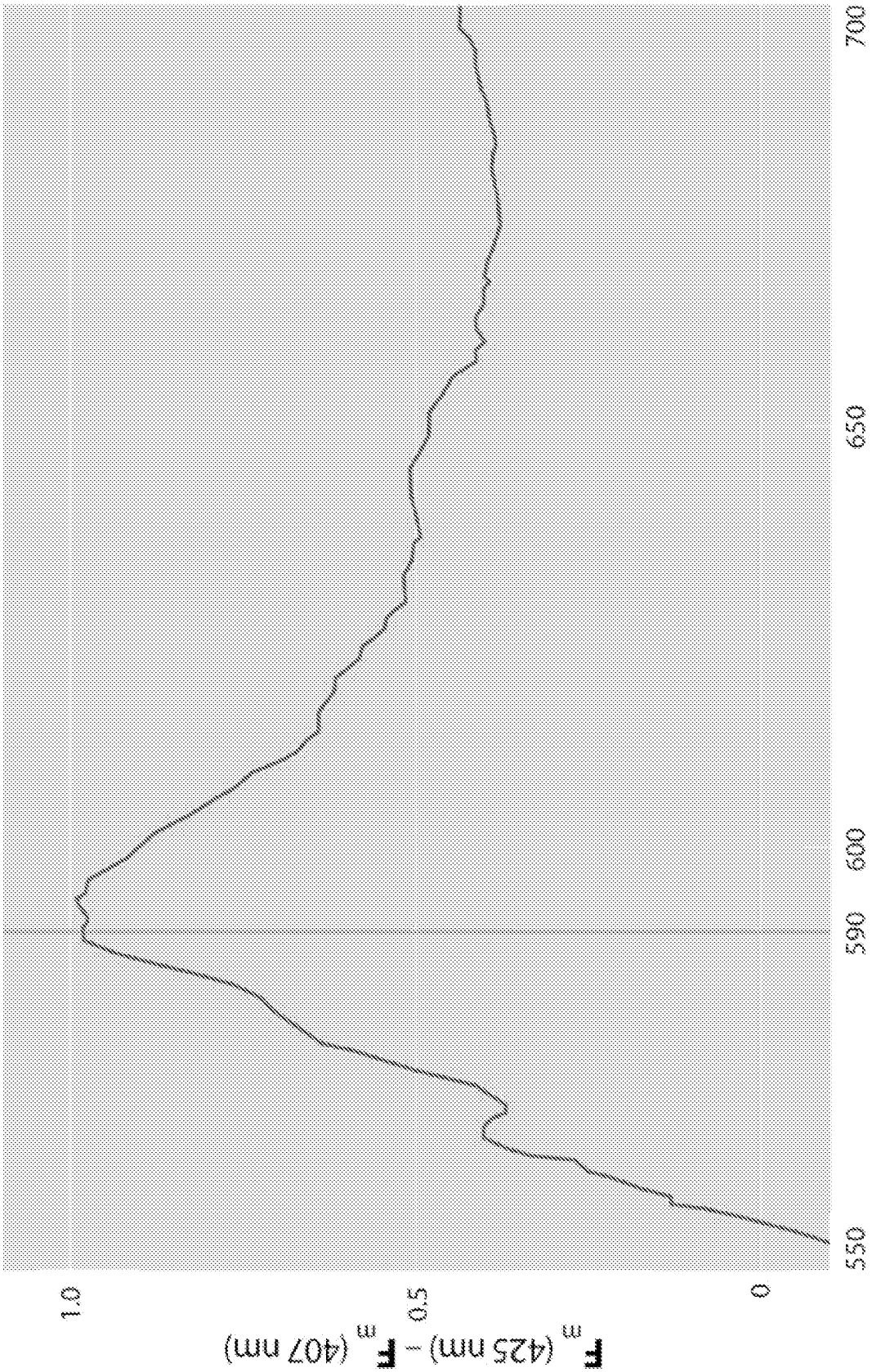

1.0
0.5
0
550
590
600
650
700
Emission Wavelength (nm)
r_m (425 nm) - r_m (407 nm)

FIGURE 12

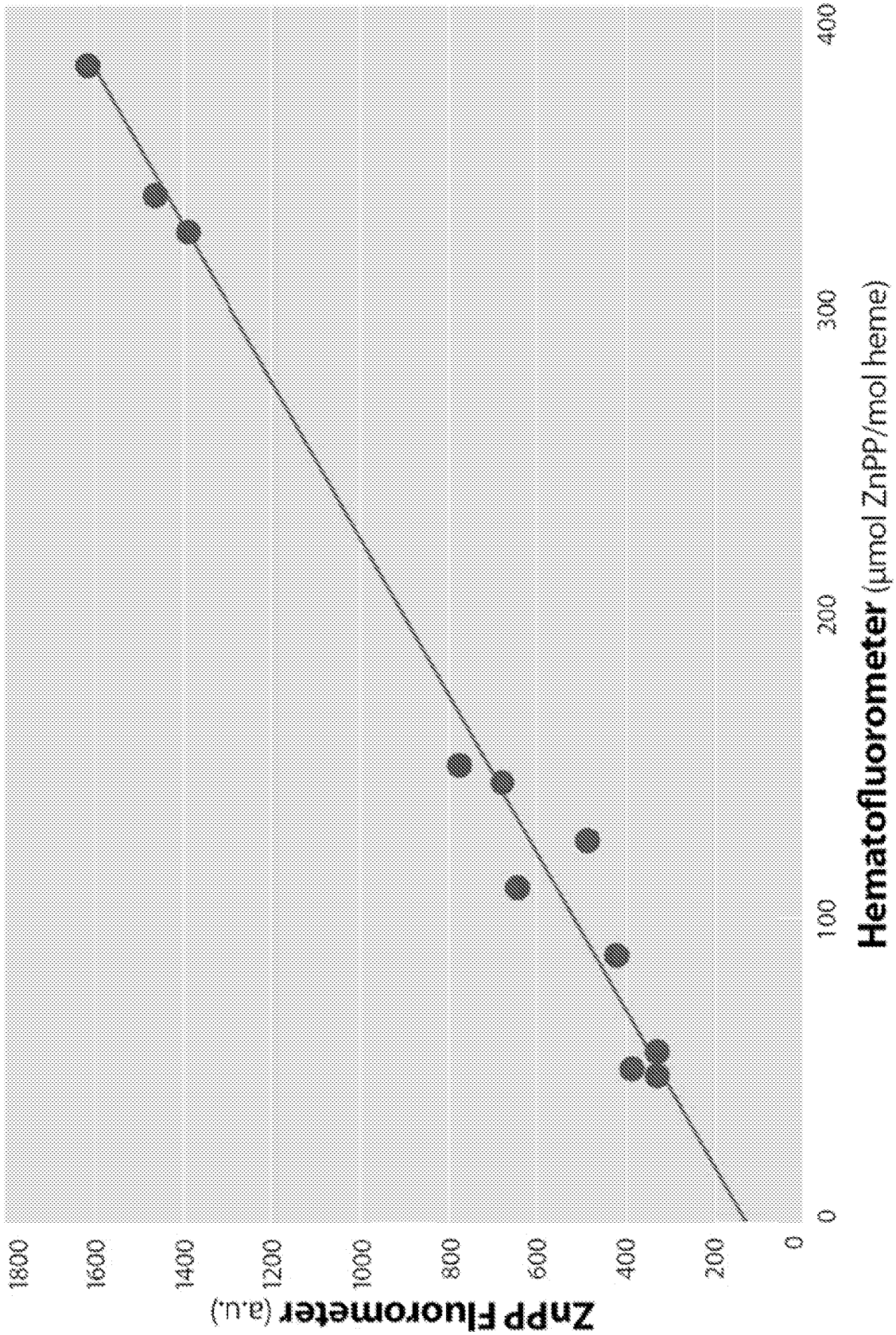

ZnPP Fluorometer (a.u.)
1800
1600
1400
1200
1000
800
600
400
200
0
0
100
200
300
400
Hematofluorometer (µmol ZnPP/mol heme)

FIGURE 13

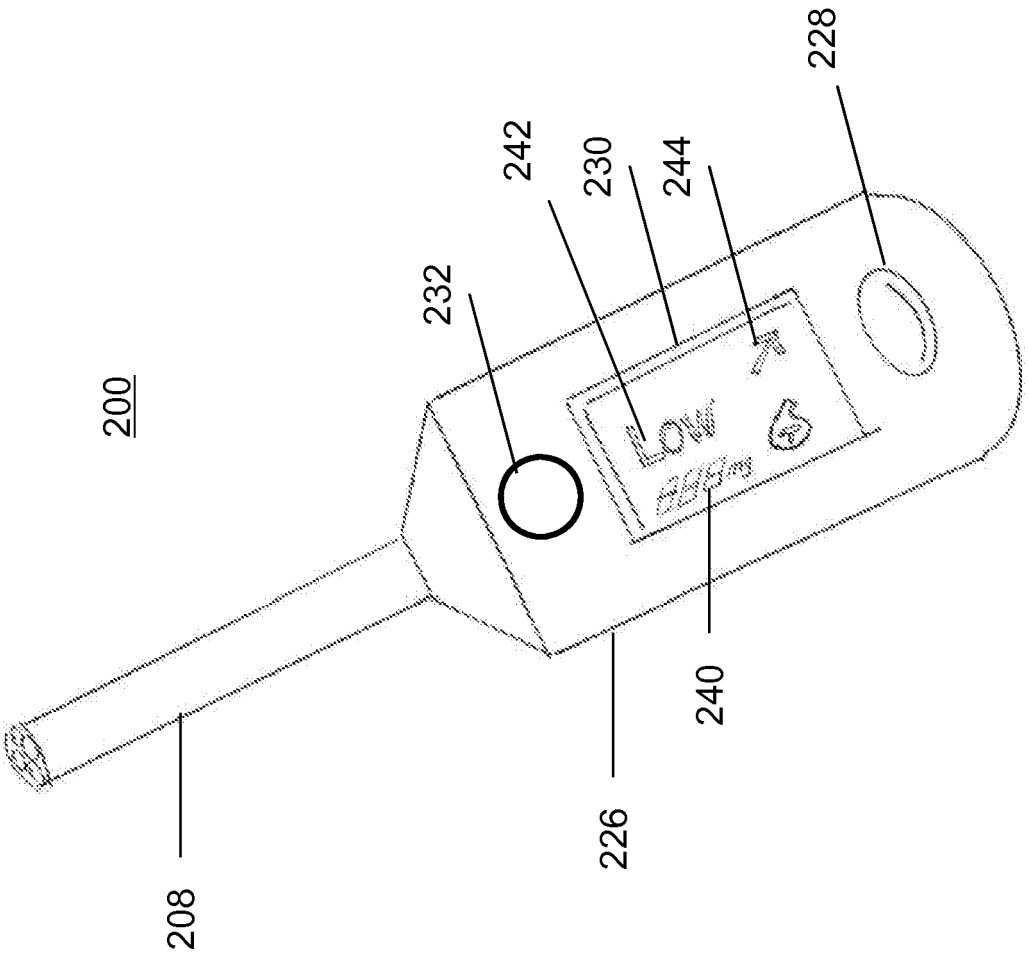


FIGURE 14

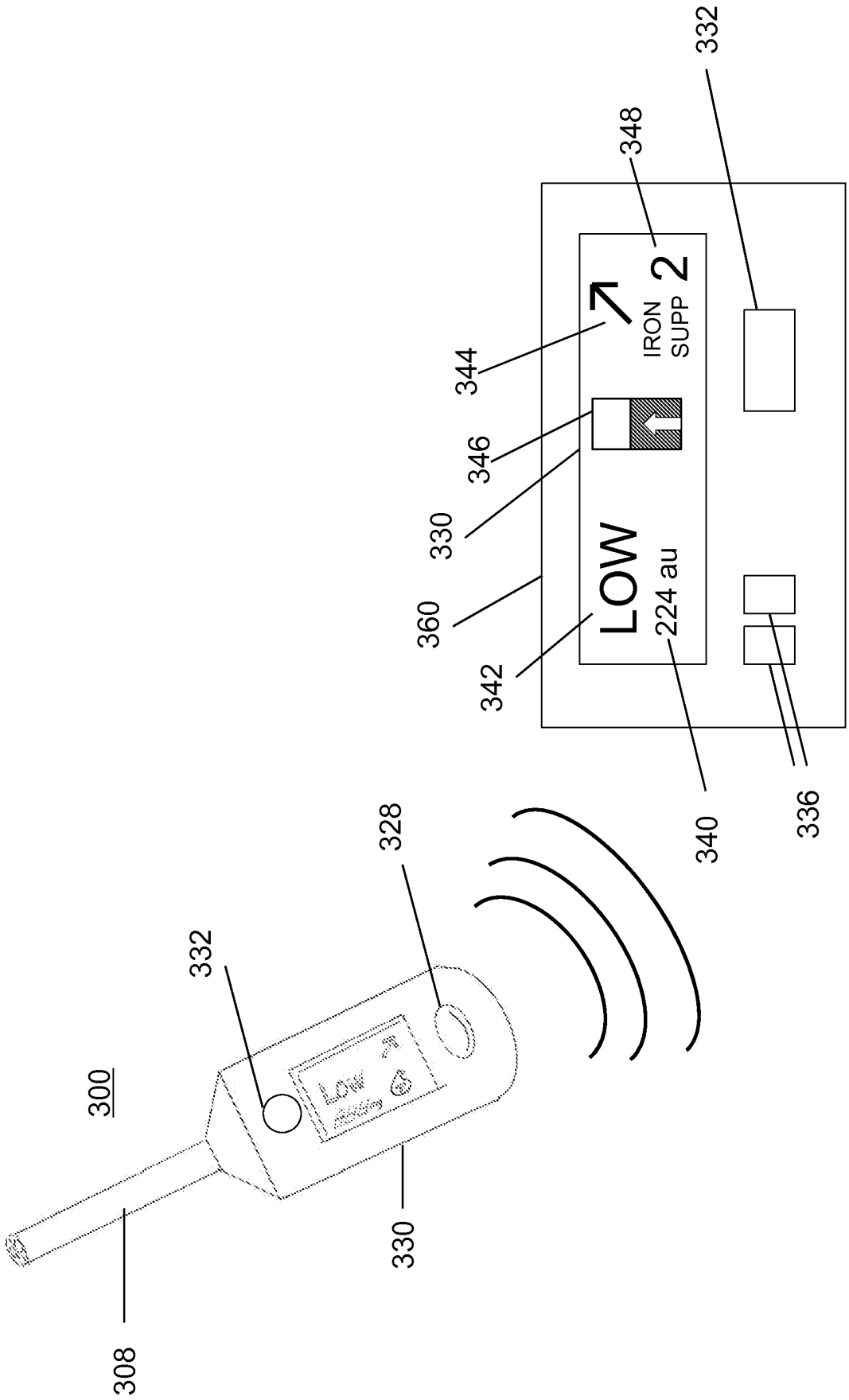

332
348
IRON SUPP
2
344
346
330
360
LOW
224 au
342
340
336
328
332
300
330
308

FIGURE 15

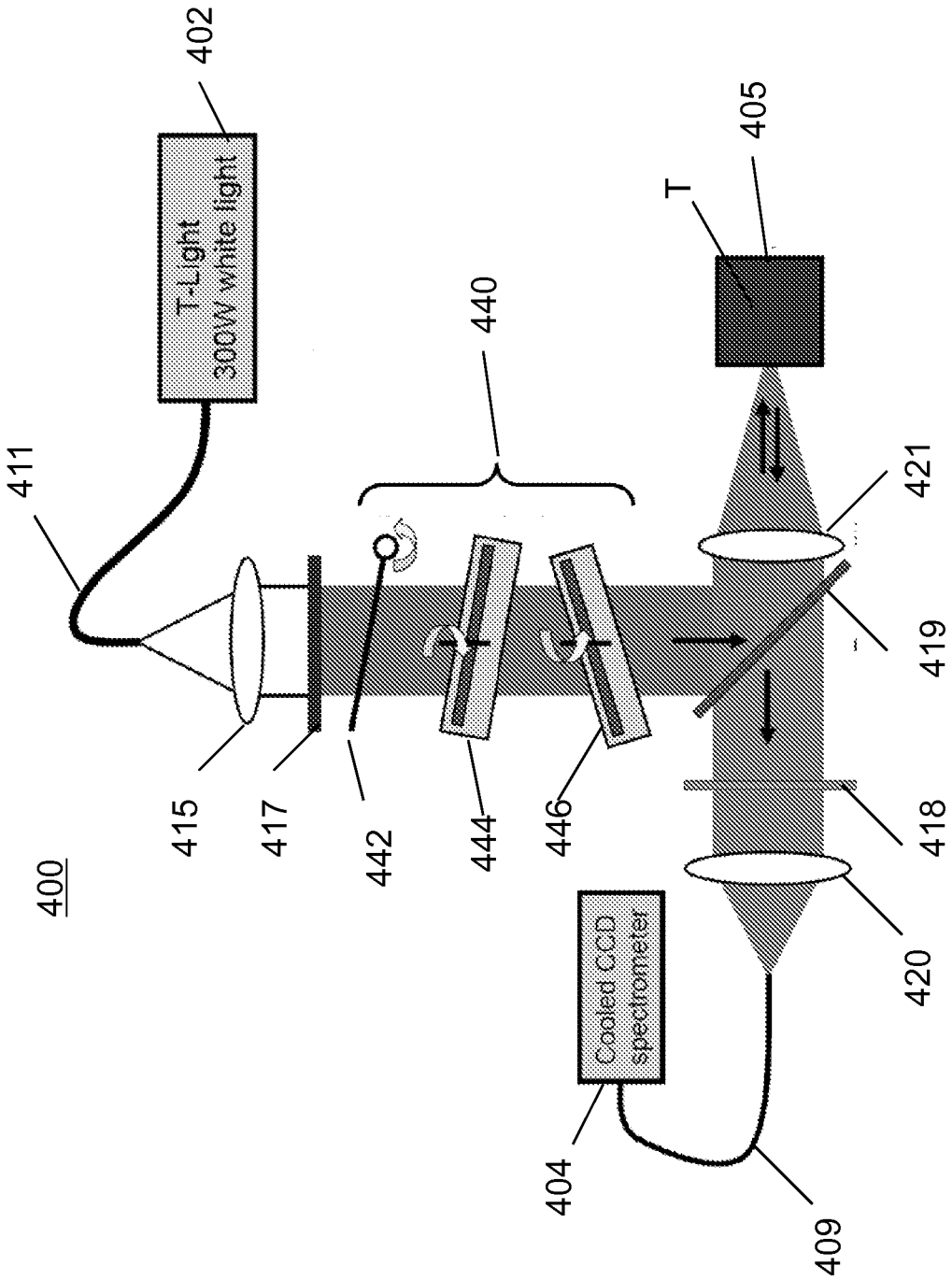

400
402
T-Light
300W white light
411
415
417
442
440
444
446
405
T
421
419
418
420
Cooled CCD
spectrometer
404
409

FIGURE 16

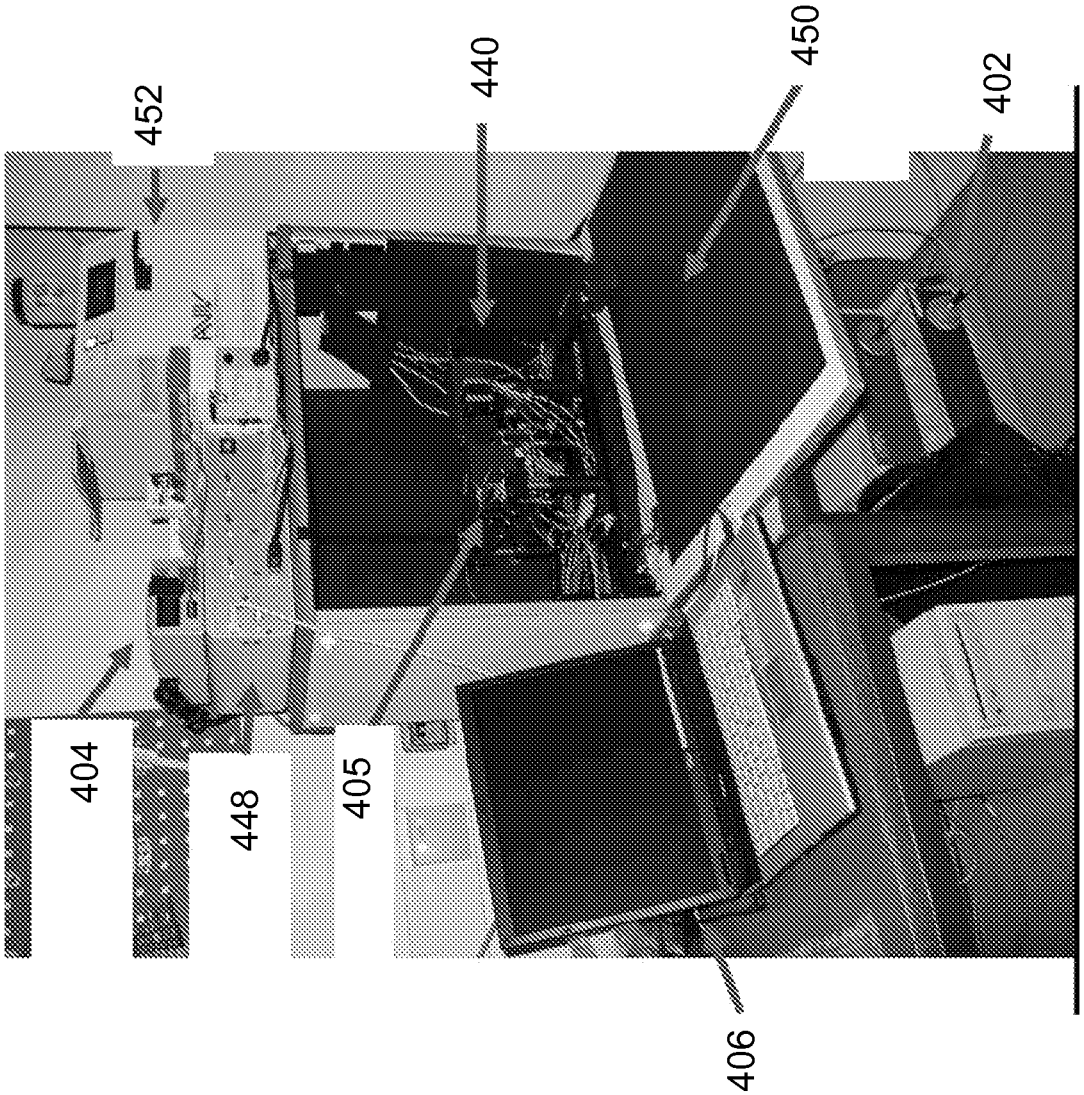


FIGURE 17

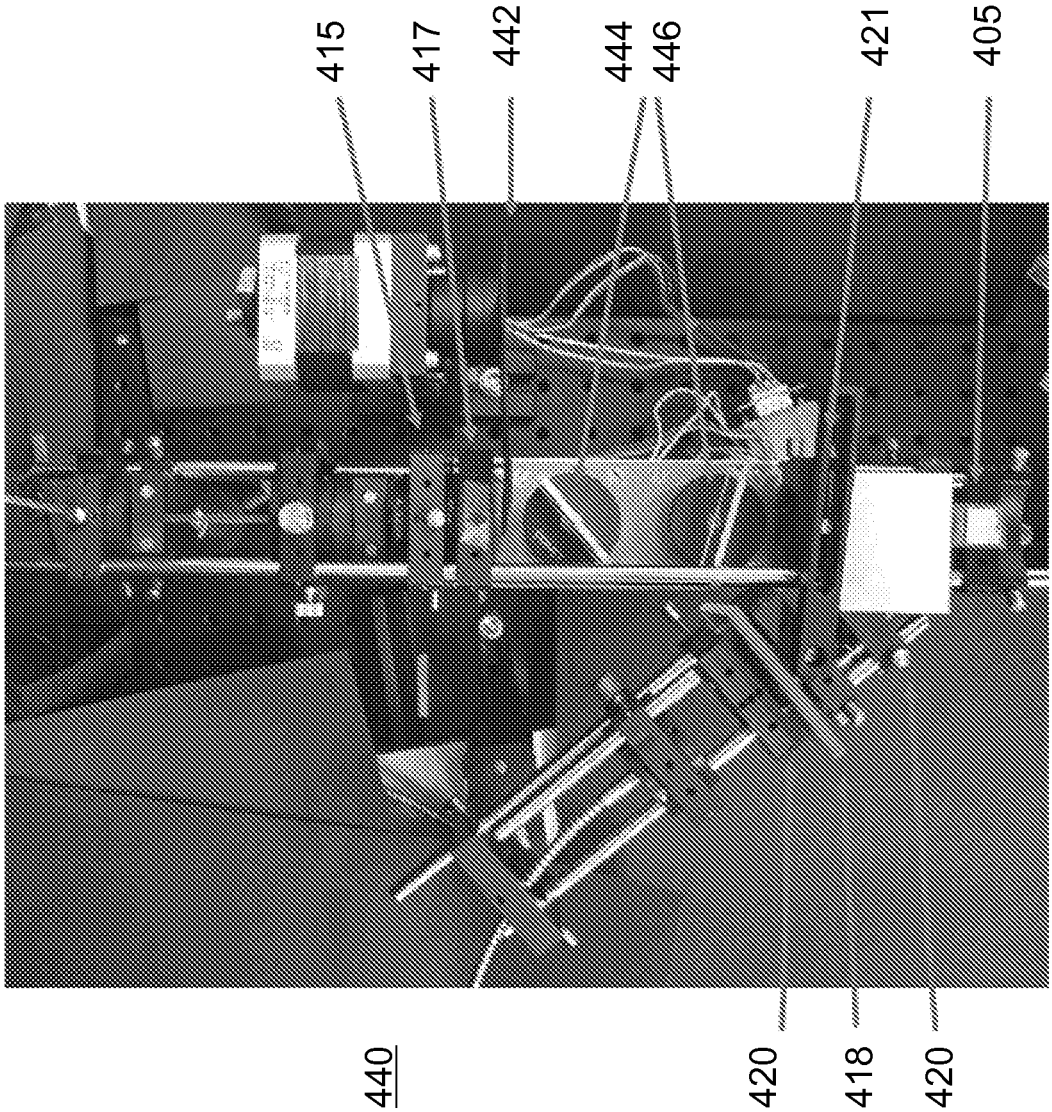


FIGURE 18

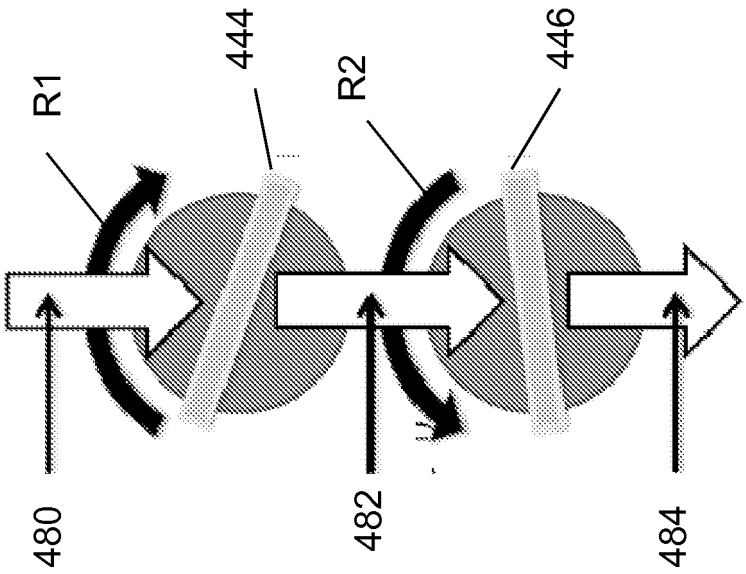


FIGURE 19

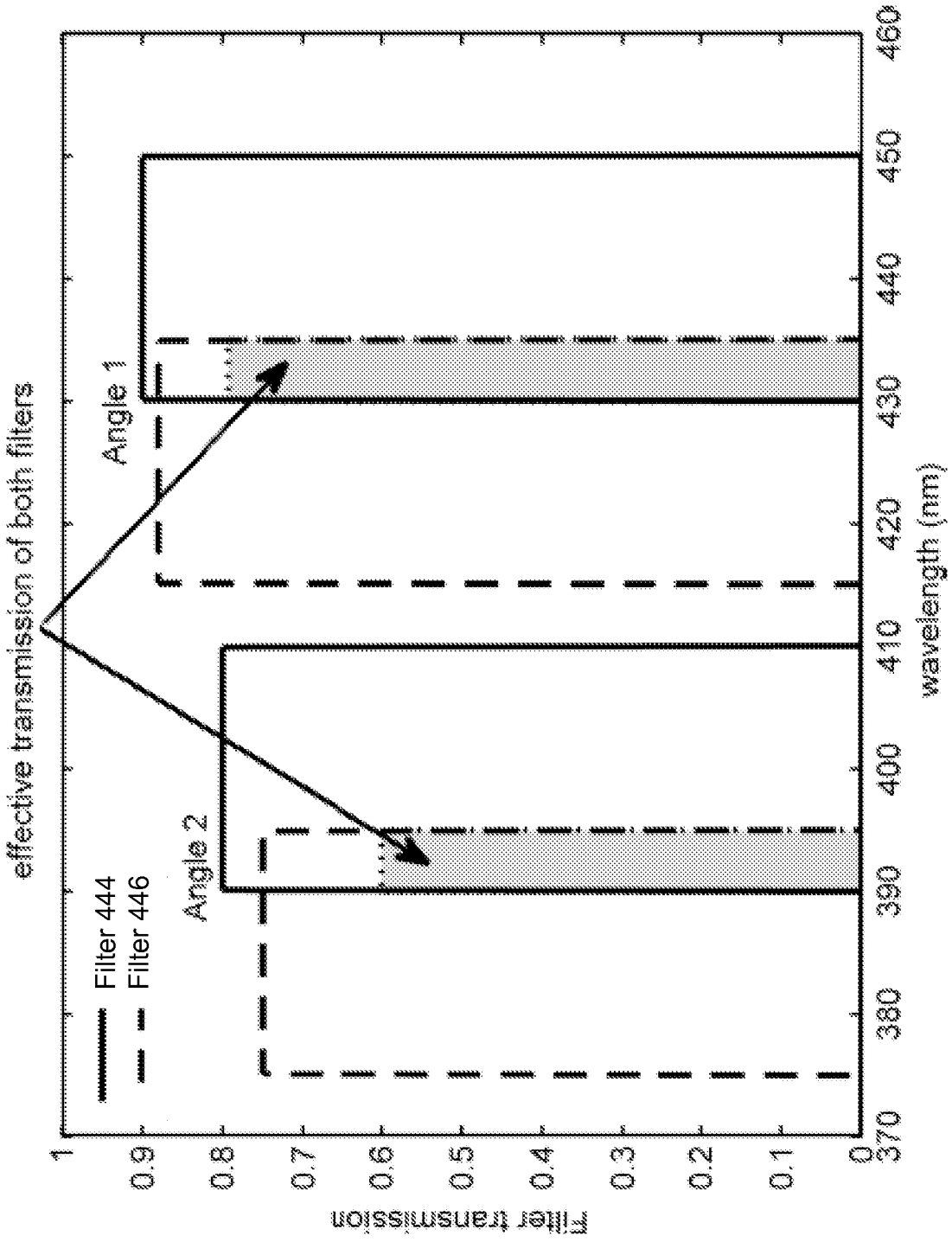

effective transmission of both filters
Filter 444
Filter 446
Angle 1
Angle 2
filter transmission
wavelength (nm)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
370
380
390
400
410
420
430
440
450
460

FIGURE 20

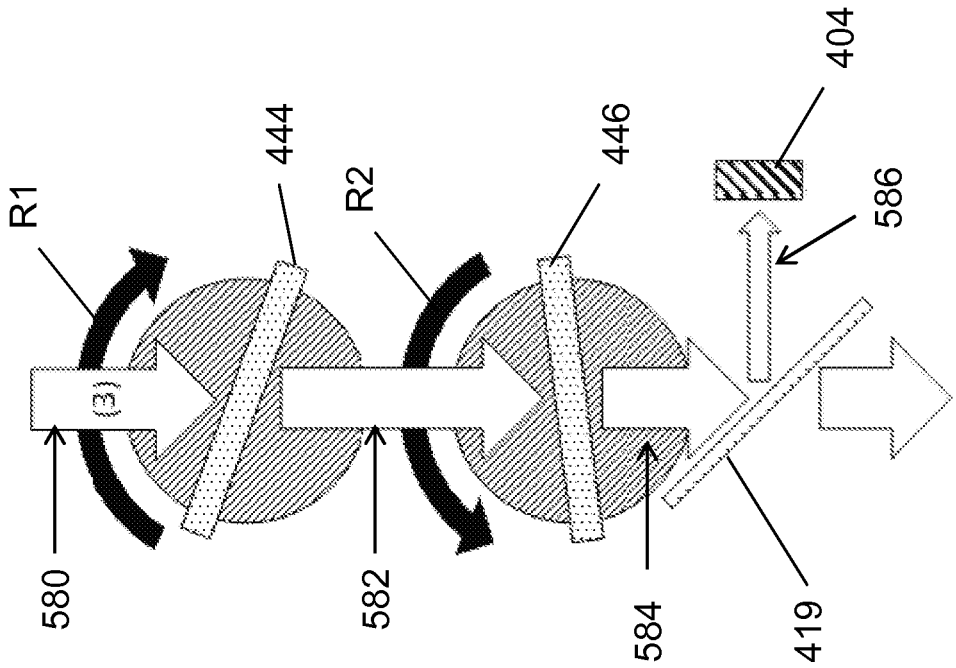


FIGURE 21

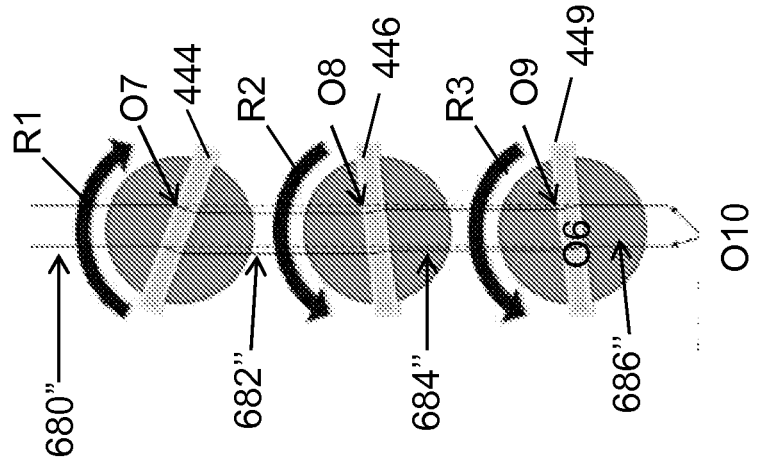


FIGURE 24

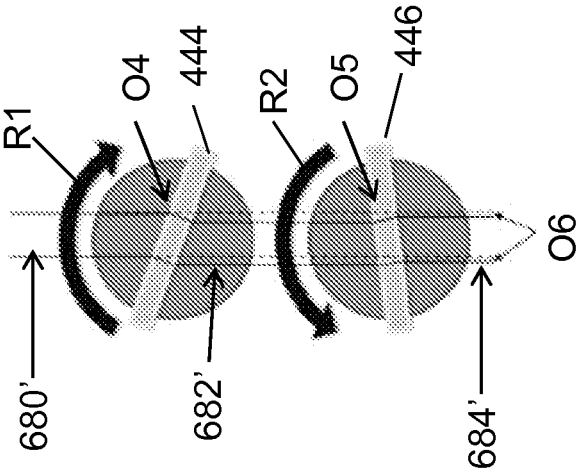


FIGURE 23

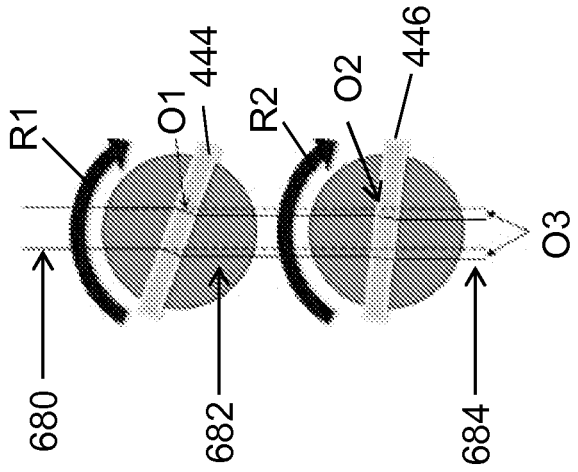


FIGURE 22

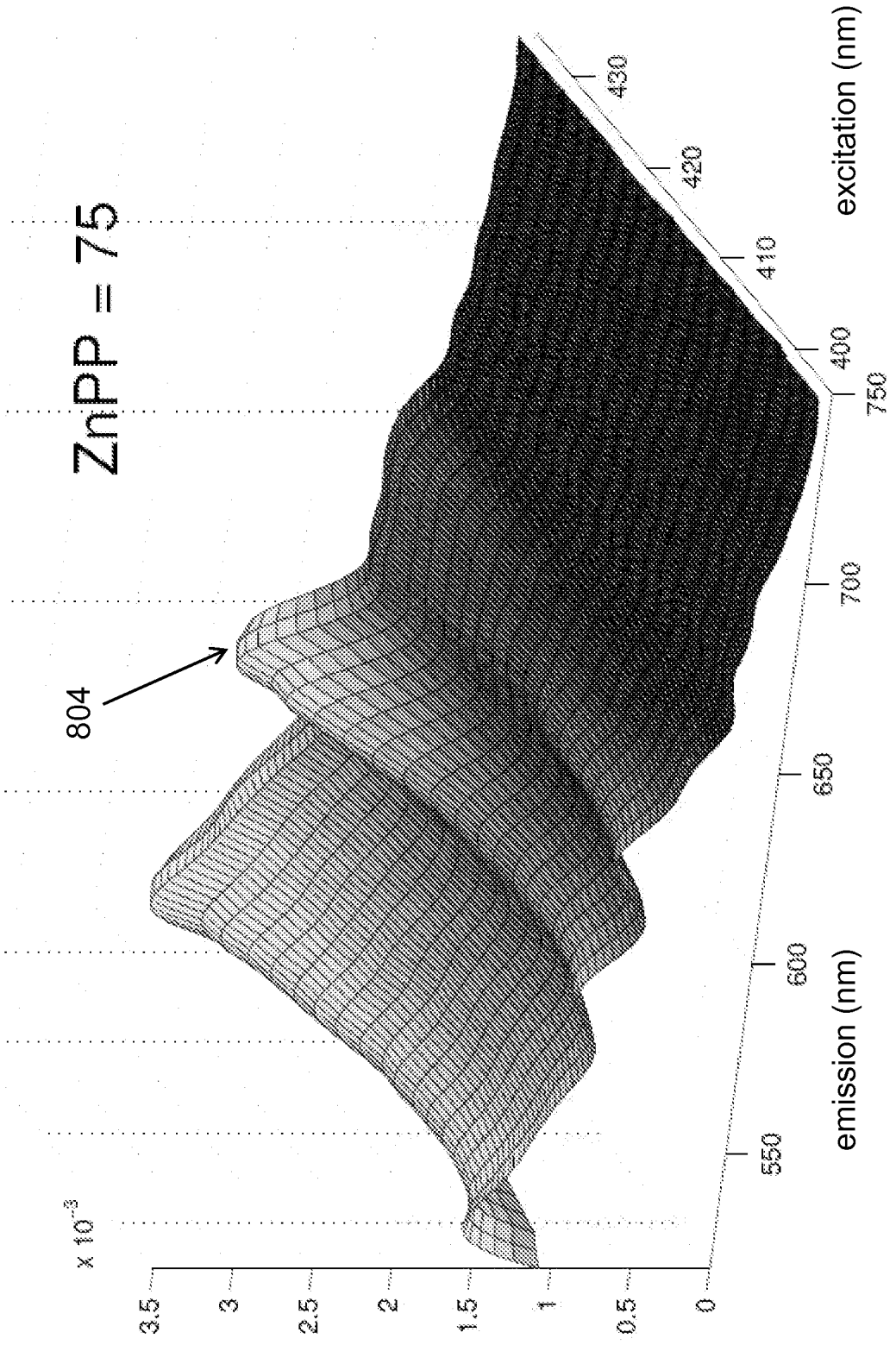

ZnPP = 75
804
x 10^-3
3.5
3
2.5
2
1.5
1
0.5
0
550
600
650
700
750
emission (nm)
400
410
420
430
excitation (nm)

FIGURE 25

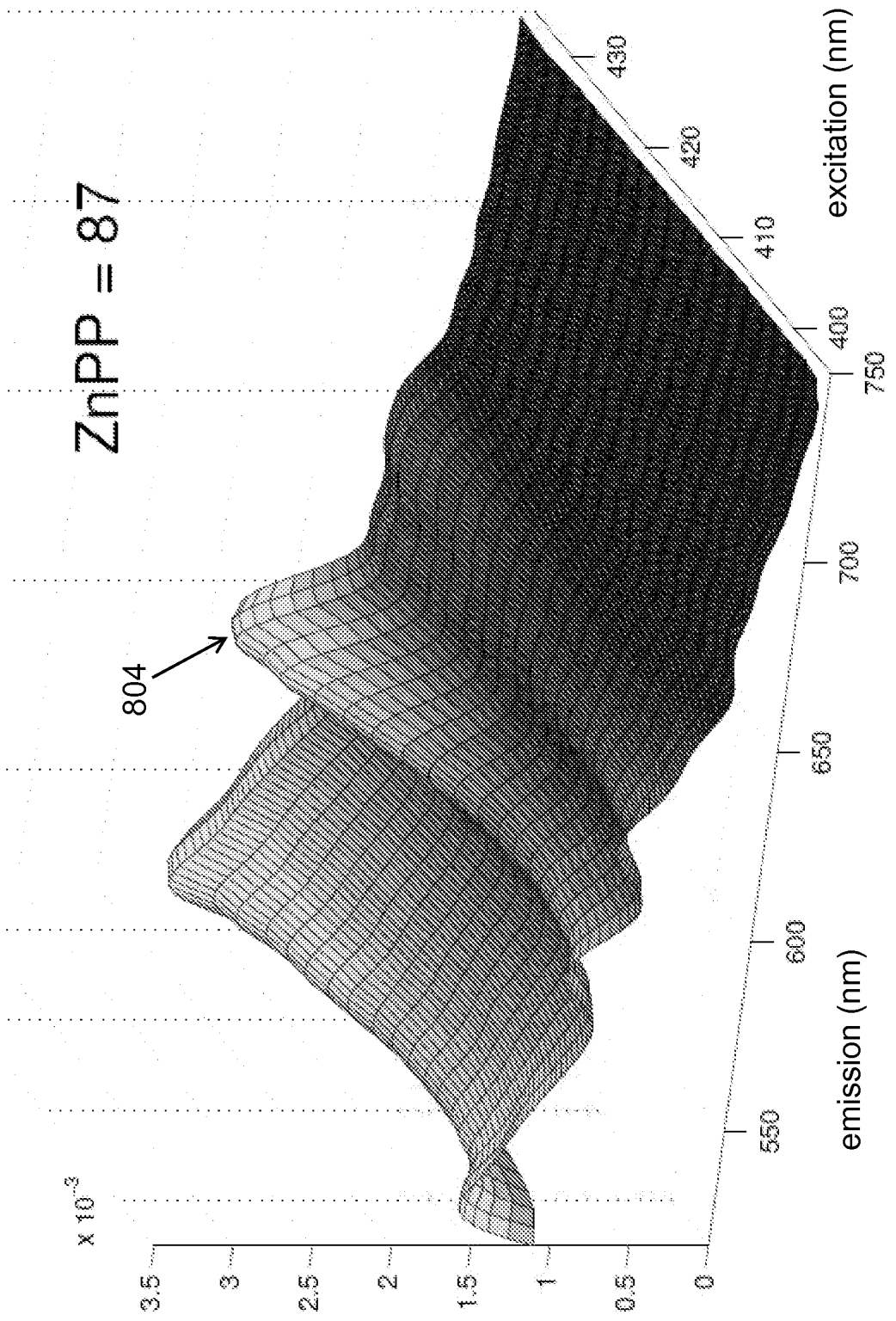

ZnPP = 87
804
x 10^-3
3.5
3
2.5
2
1.5
1
0.5
0
550
600
650
700
750
emission (nm)
400
410
420
430
excitation (nm)

FIGURE 26

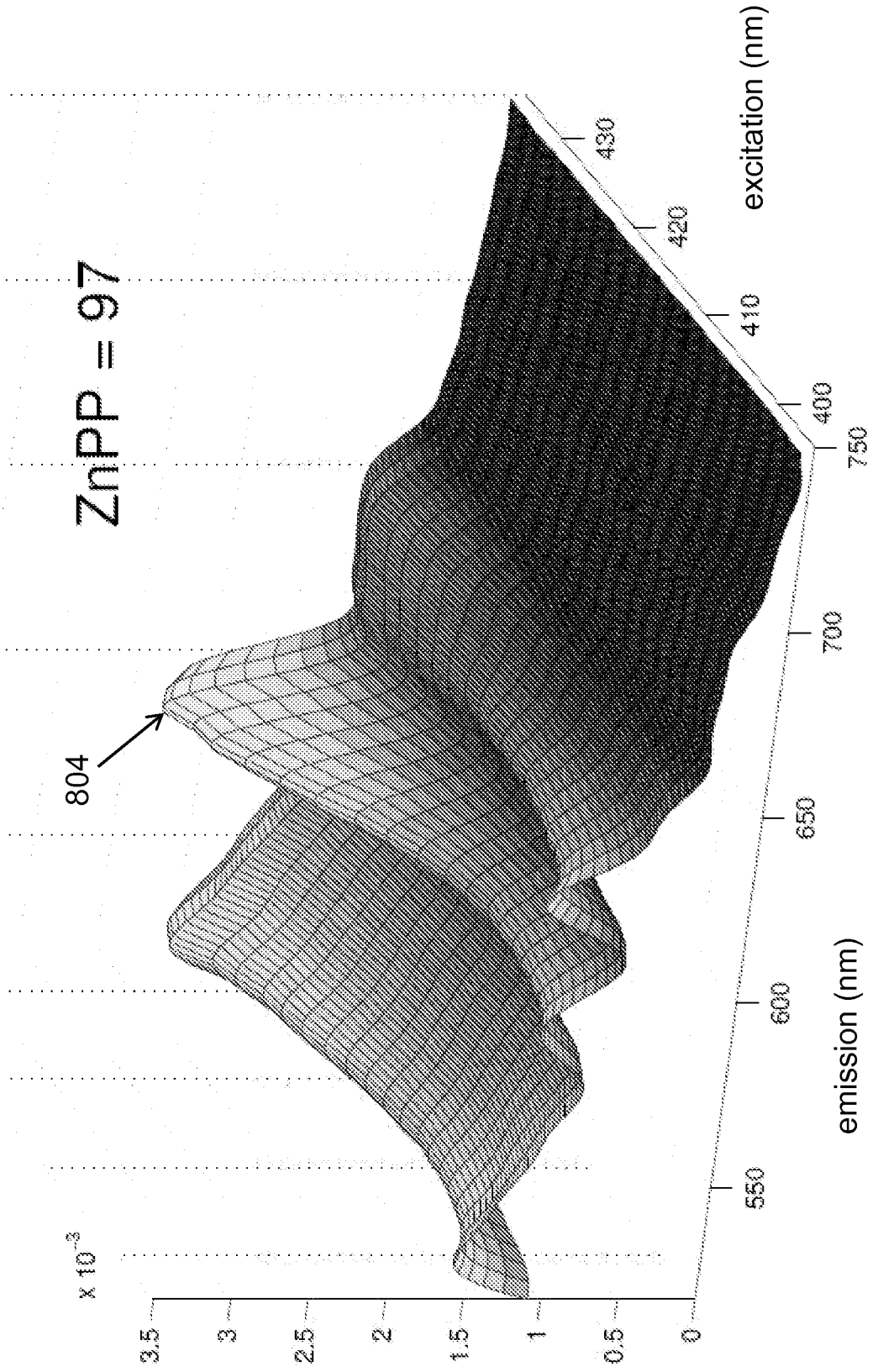

ZnPP = 97
804
x 10^-3
3.5
3
2.5
2
1.5
1
0.5
0
550
600
650
700
750
emission (nm)
400
410
420
430
excitation (nm)

FIGURE 27

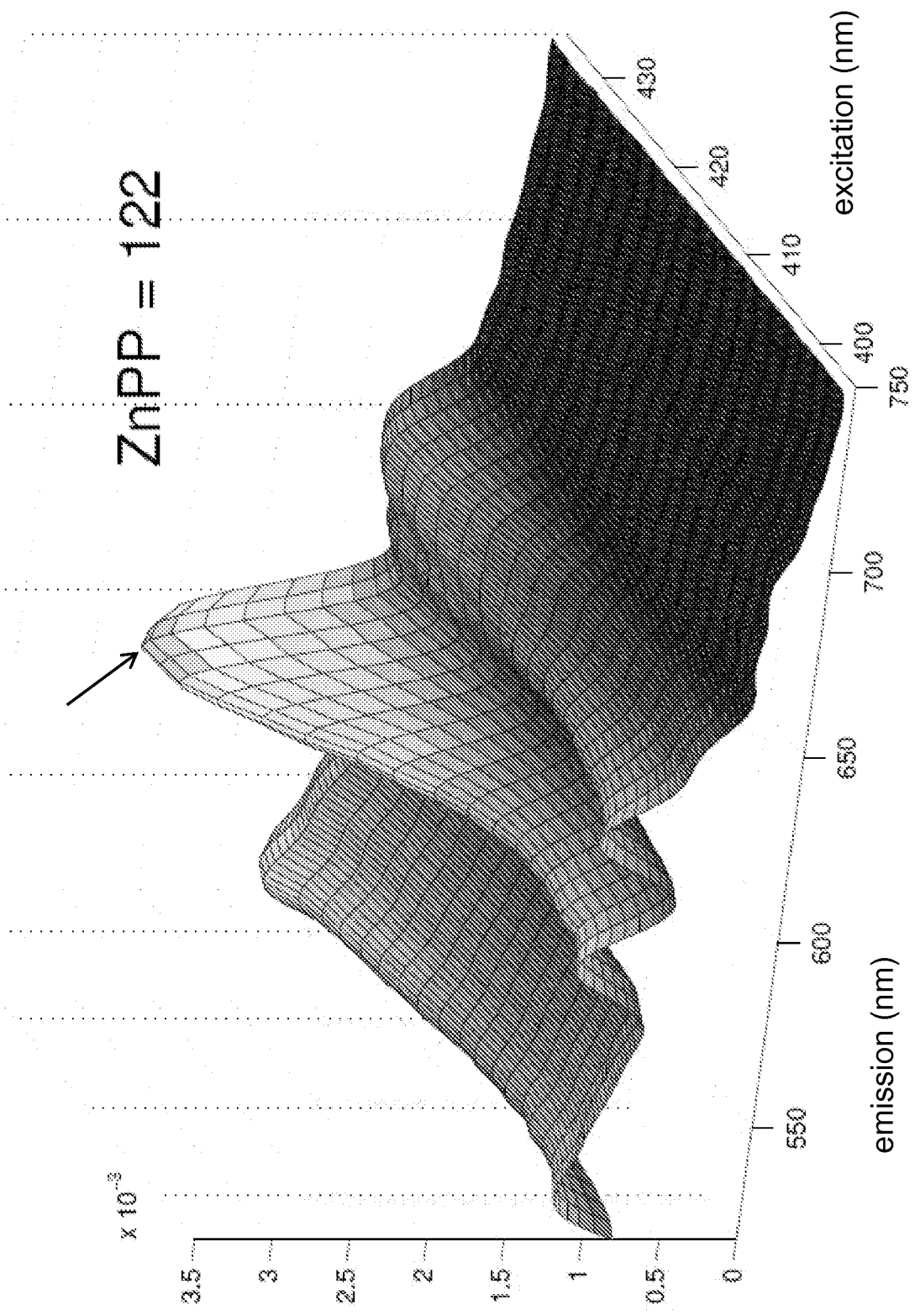

ZnPP = 122
x 10^-3
3.5
3
2.5
2
1.5
1
0.5
0
550
600
650
700
750
emission (nm)
400
410
420
430
excitation (nm)

FIGURE 28

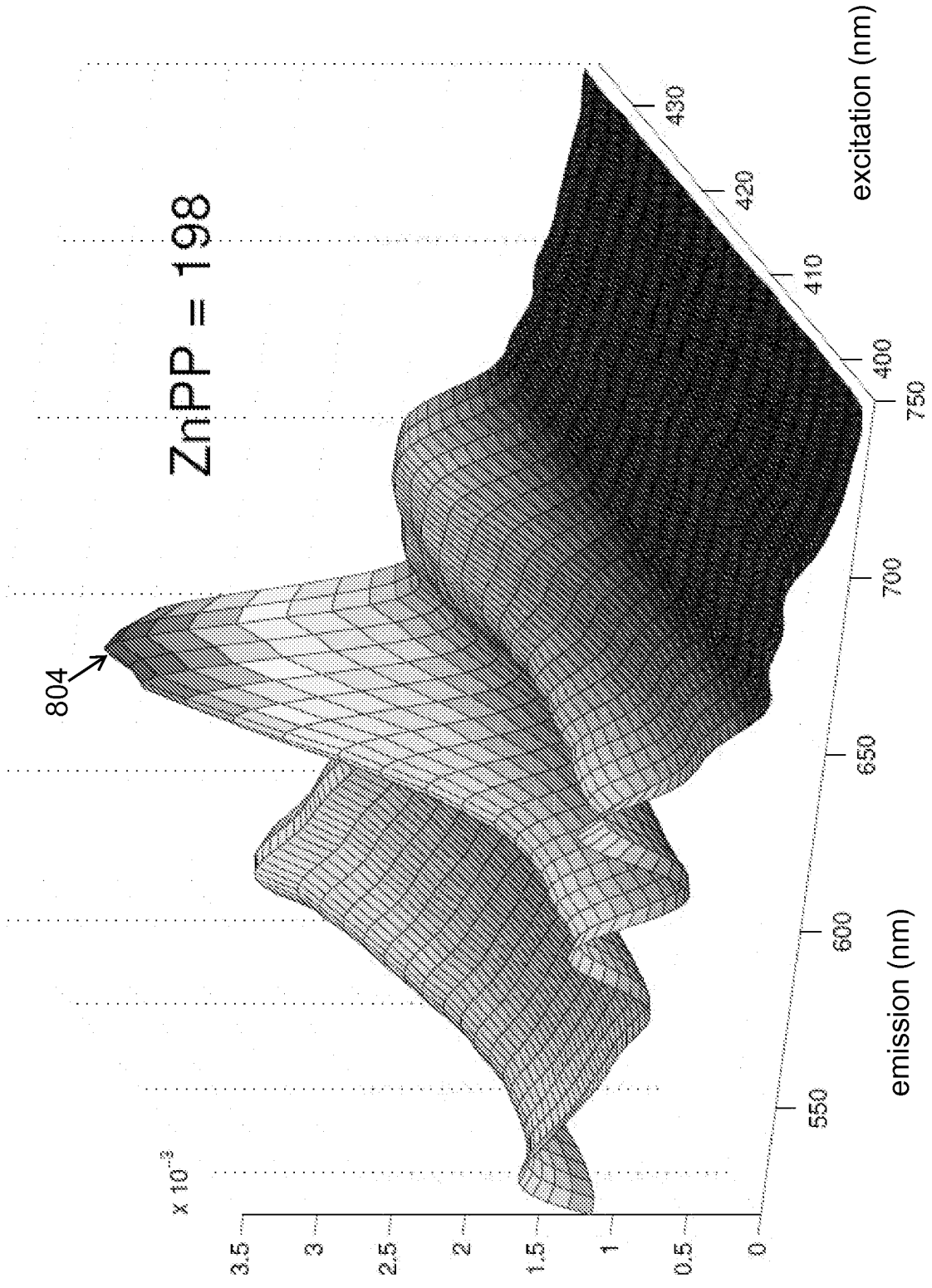

ZnPP = 198
804
x 10^-3
3.5
3
2.5
2
1.5
1
0.5
0
550
600
650
700
750
emission (nm)
400
410
420
430
excitation (nm)

FIGURE 29

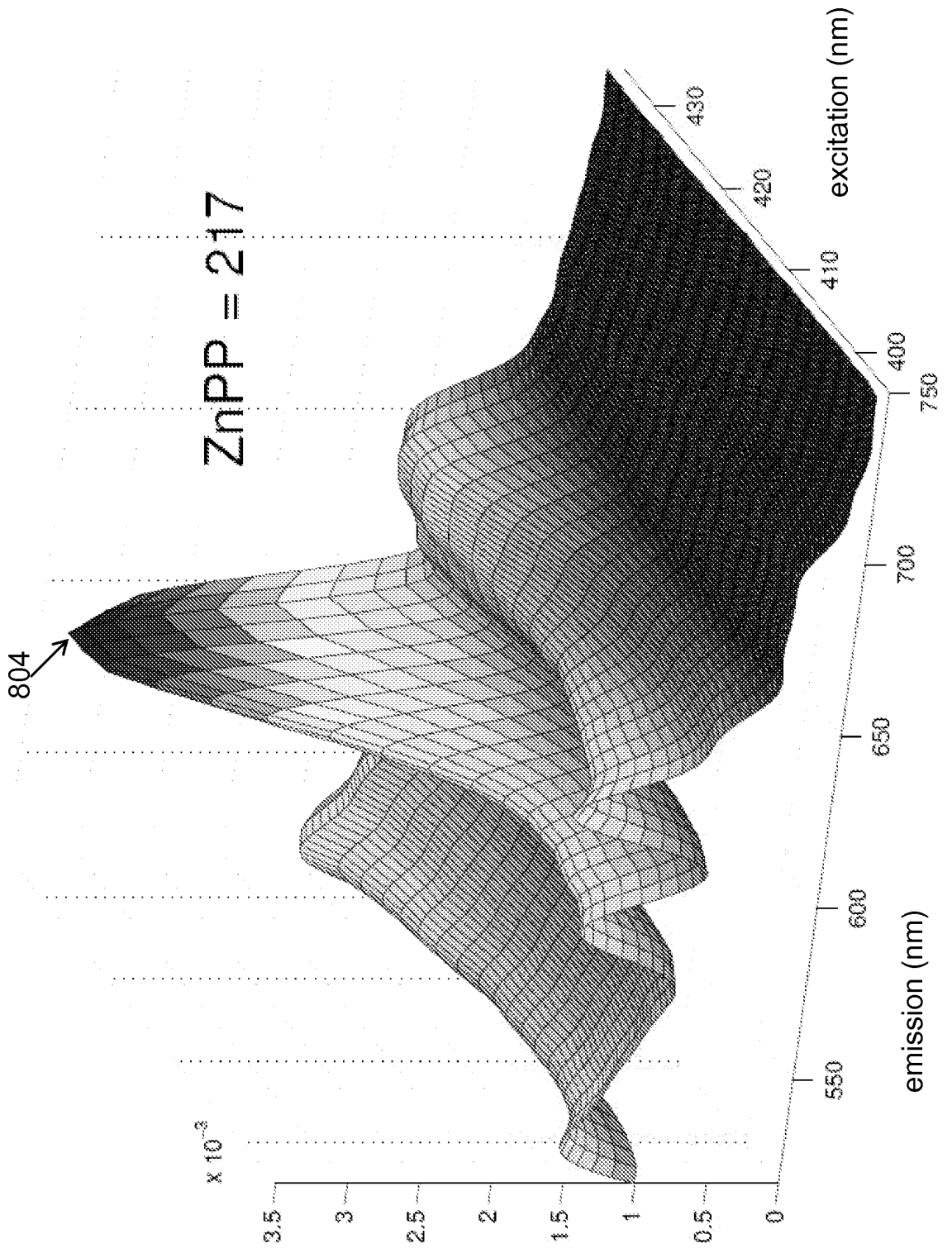

ZnPP = 217
804
x 10^-3
3.5
3
2.5
2
1.5
1
0.5
0
550
600
650
700
750
emission (nm)
400
410
420
430
excitation (nm)

FIGURE 30

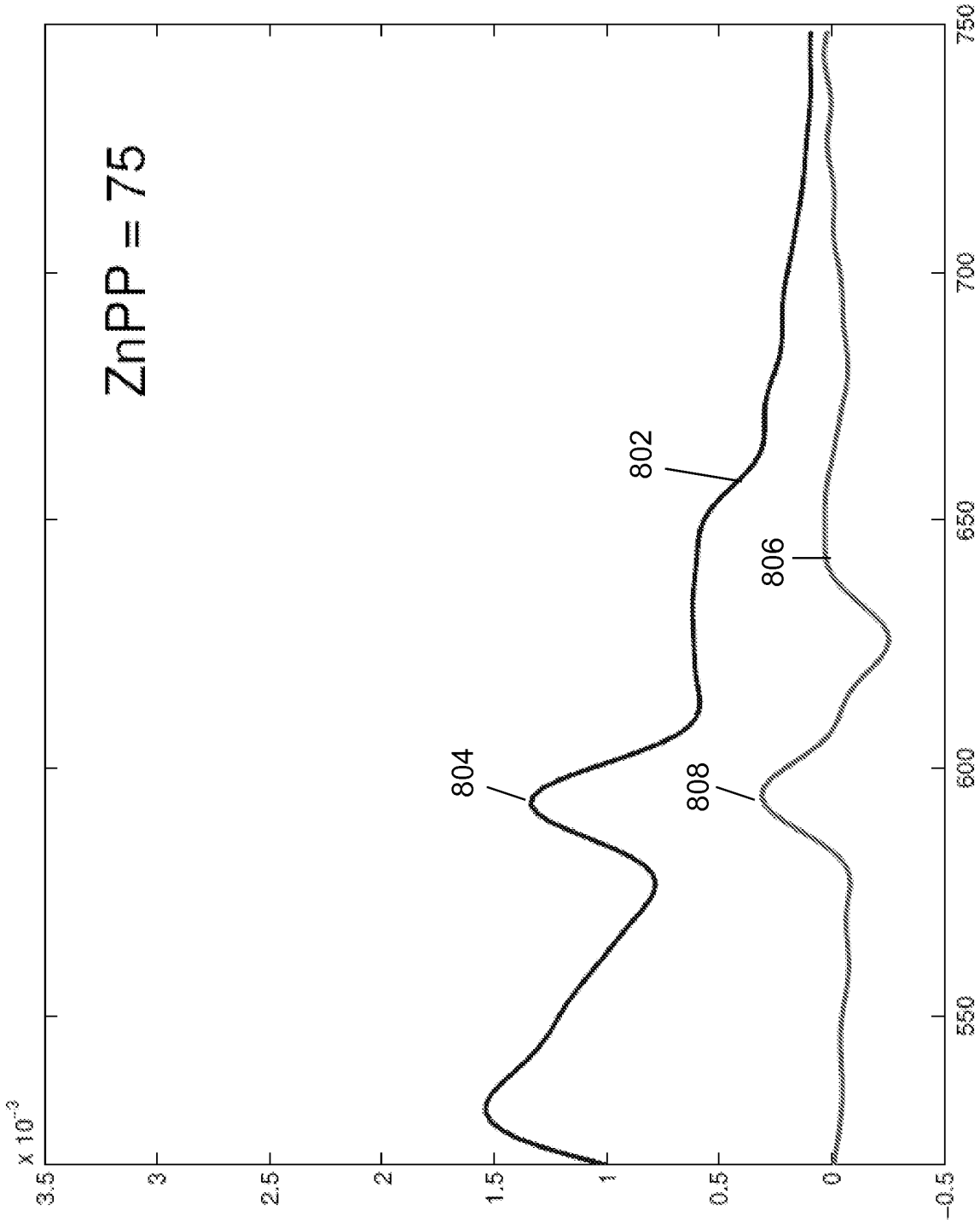

ZnPP = 75
x 10^-3
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
550
600
650
700
750
emission (nm)
802
804
806
808

FIGURE 31

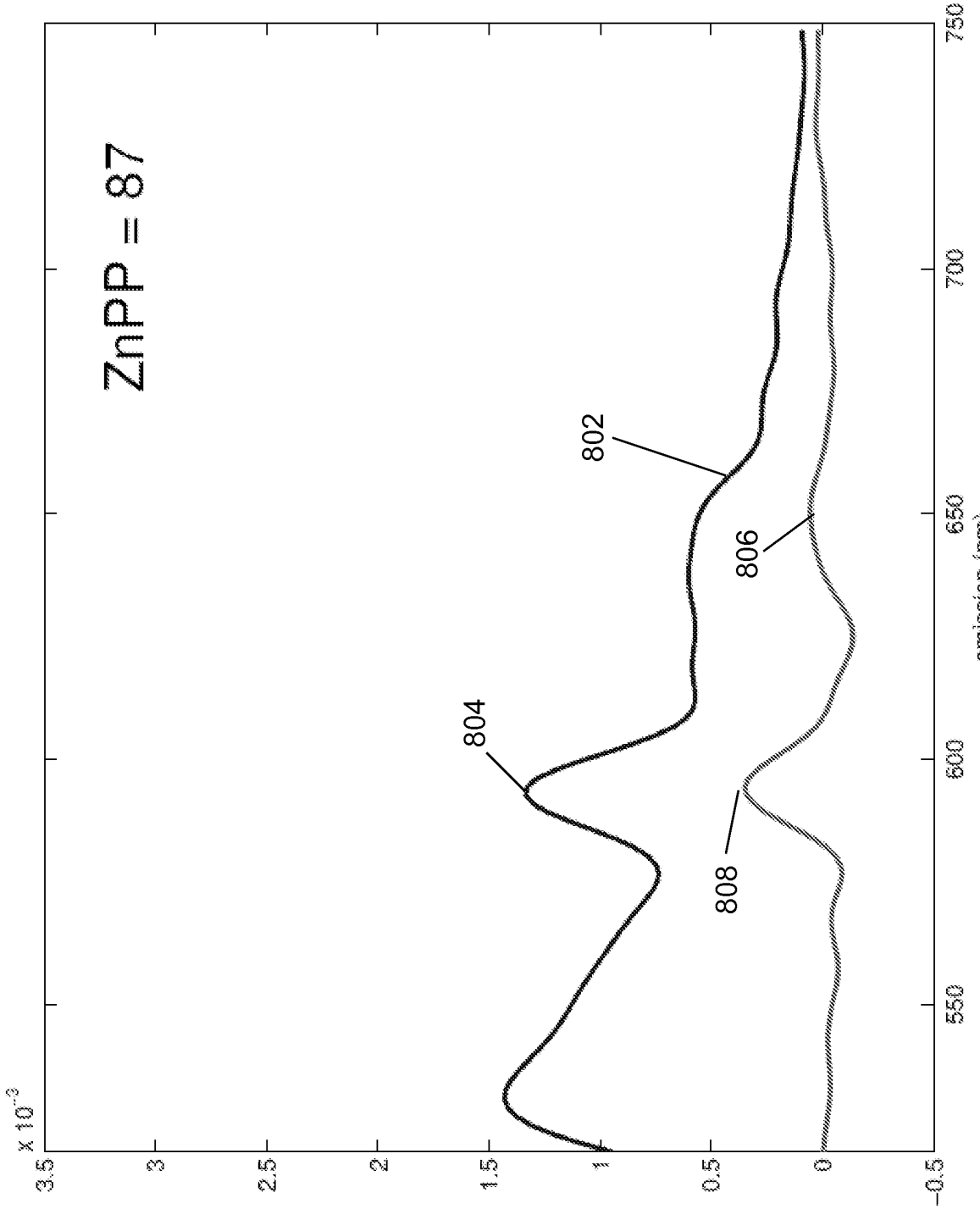

ZnPP = 87
x 10^-3
3.5
3
2.5
2
1.5
1
0.5
0
−0.5
550
600
650
700
750
emission (nm)
802
804
806
808

FIGURE 32

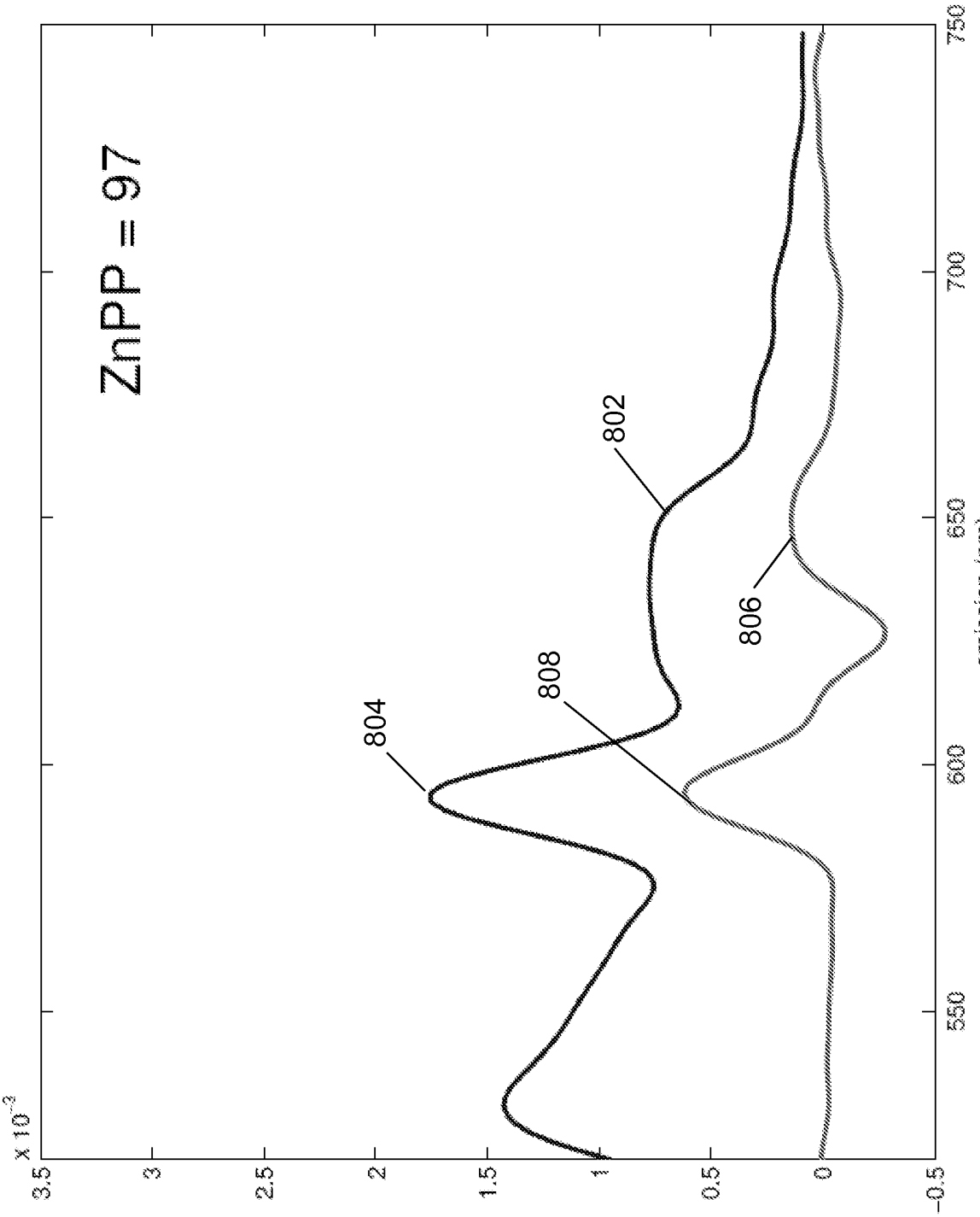

ZnPP = 97
x 10^-3
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
550
600
650
700
750
emission (nm)
802
804
806
808

FIGURE 33

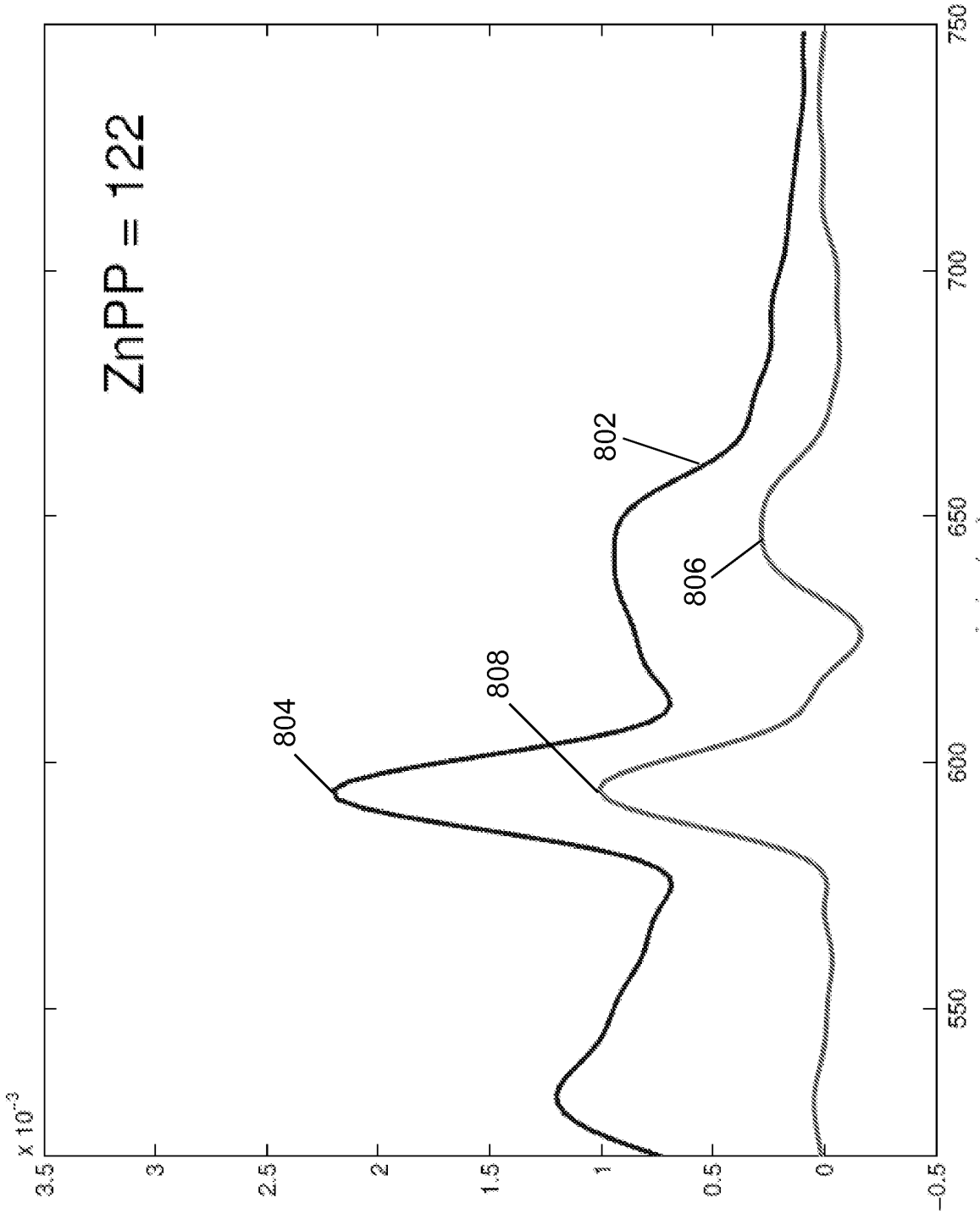

ZnPP = 122
x 10^-3
3.5
3
2.5
2
1.5
1
0.5
0
−0.5
550
600
650
700
750
emission (nm)
802
804
806
808

FIGURE 34

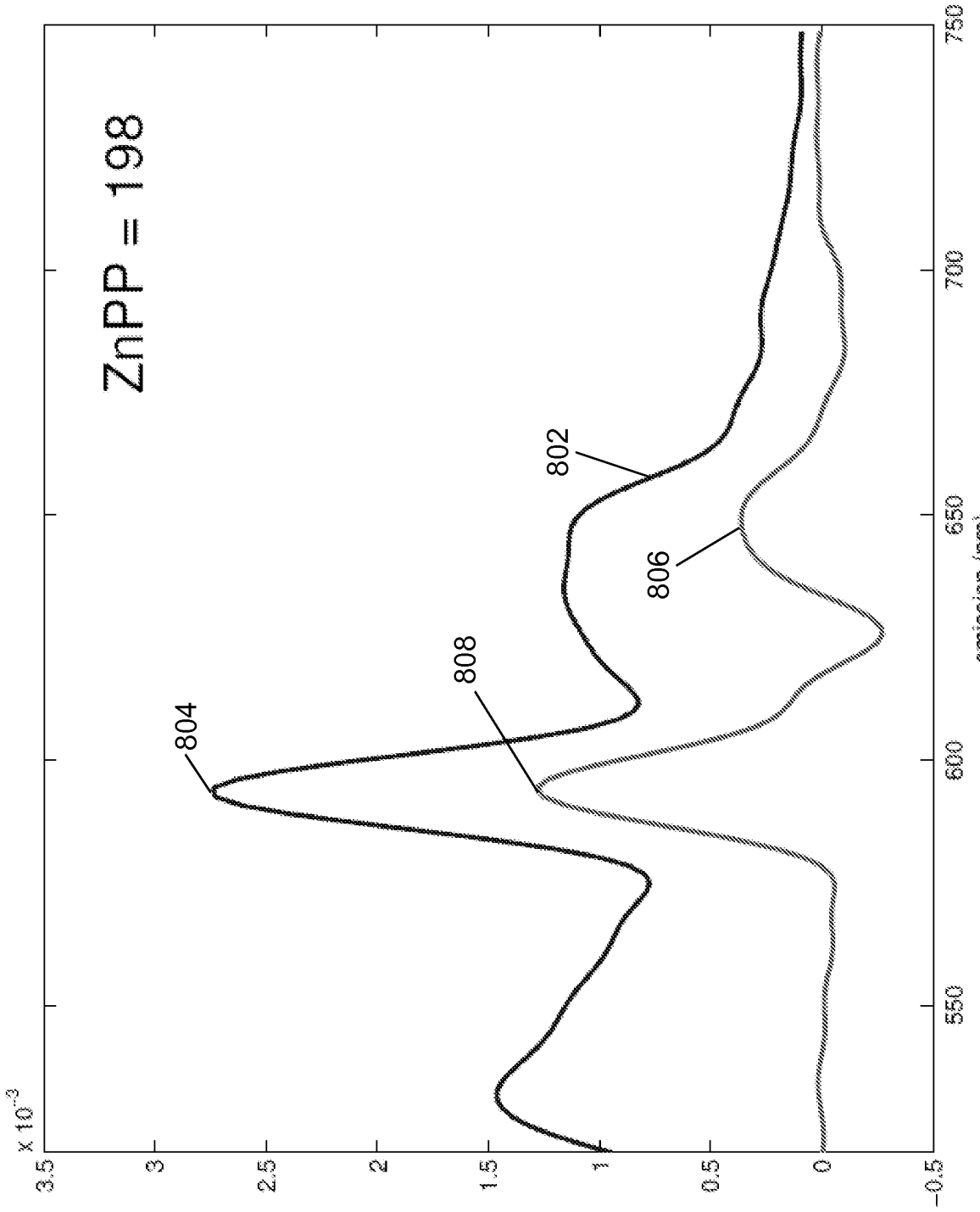

ZnPP = 198
804
808
802
806
x 10^-3
3.5
3
2.5
2
1.5
1
0.5
0
−0.5
550
600
650
700
750
emission (nm)

FIGURE 35

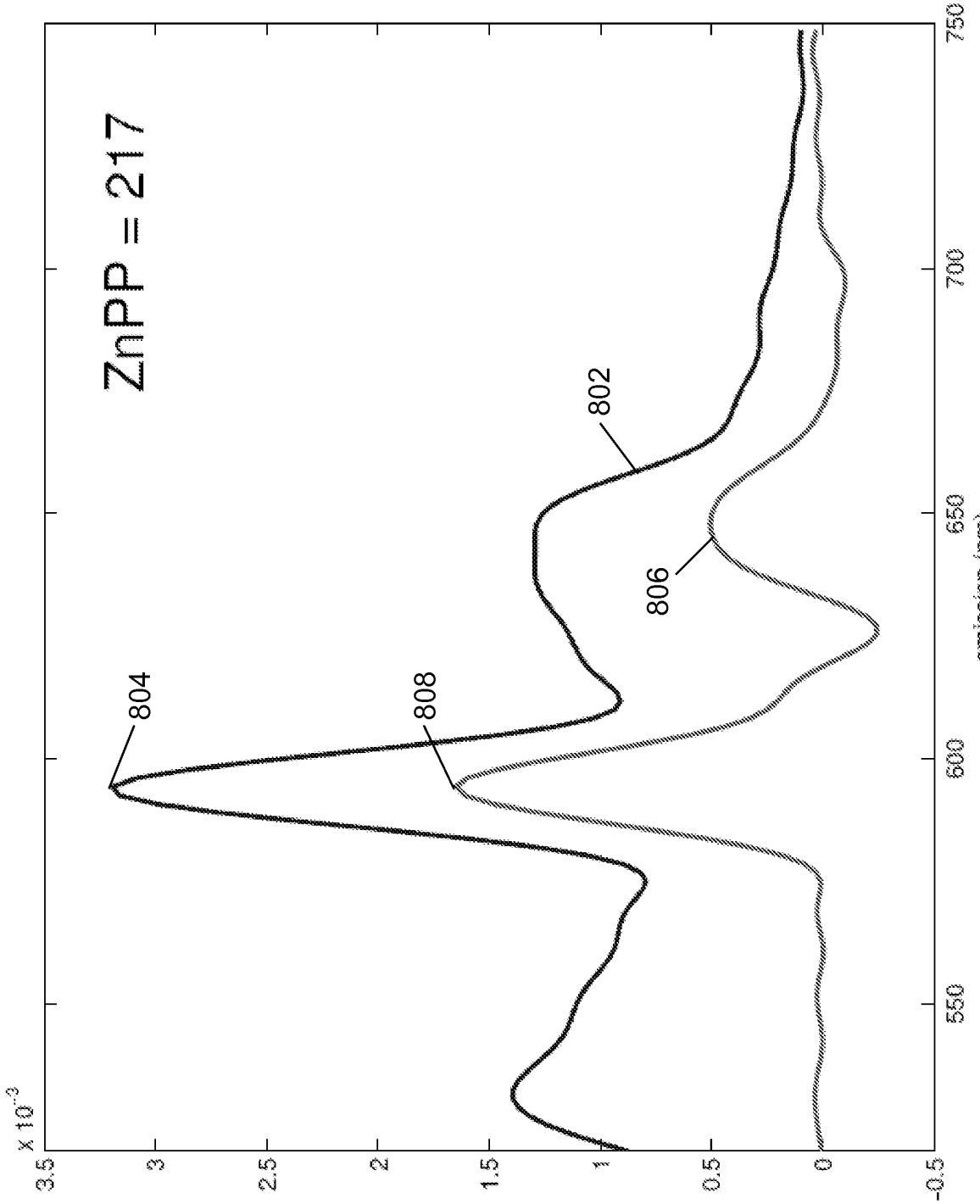

ZnPP = 217
804
808
802
806
x 10^-3
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
550
600
650
700
750
emission (nm)

FIGURE 36

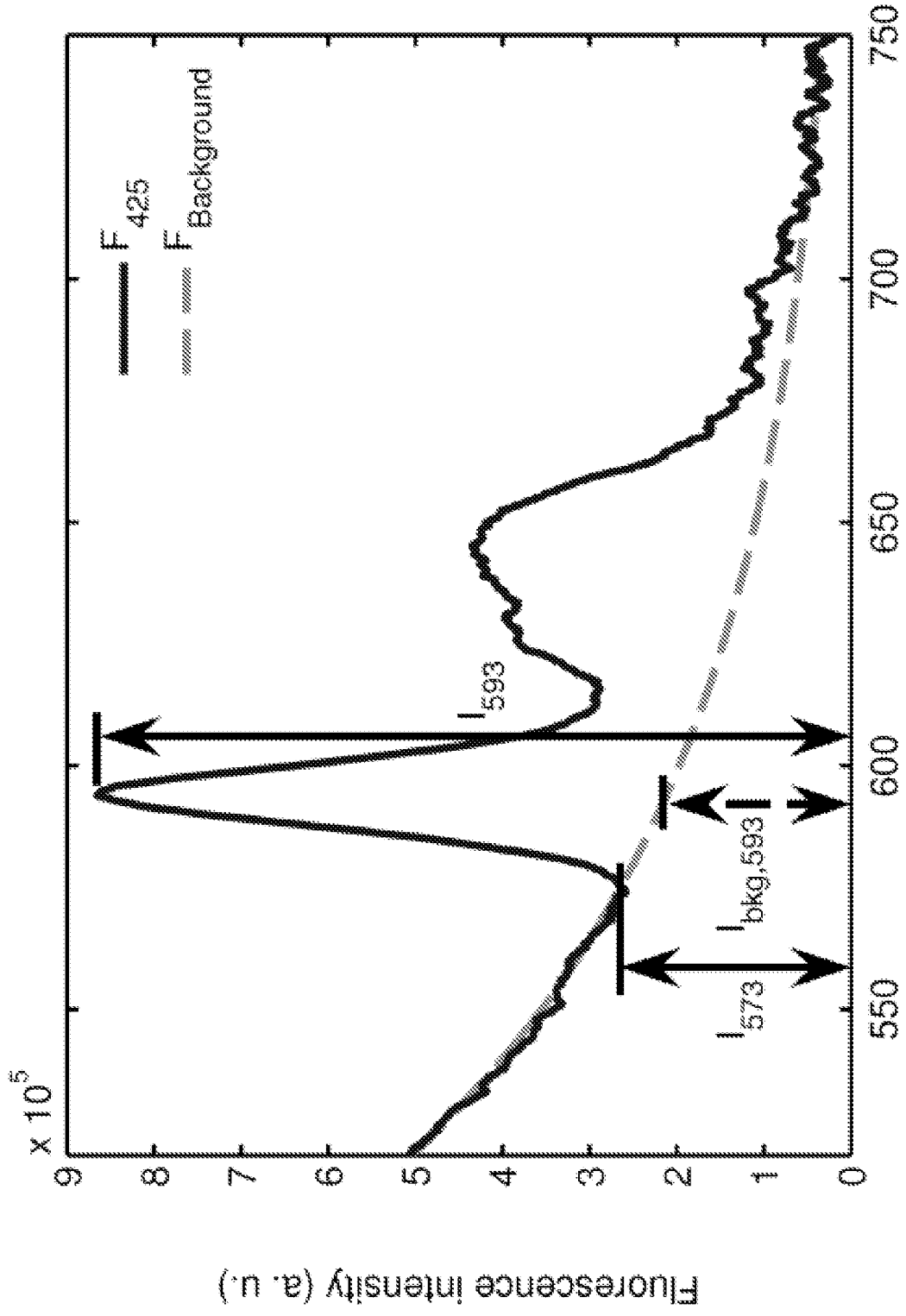

Fluorescence intensity (a. u.)
x 10^5
0
1
2
3
4
5
6
7
8
9
550
600
650
700
750
Wavelength (nm)
F425
FBackground
I593
I573
Ibkg,593

FIGURE 37

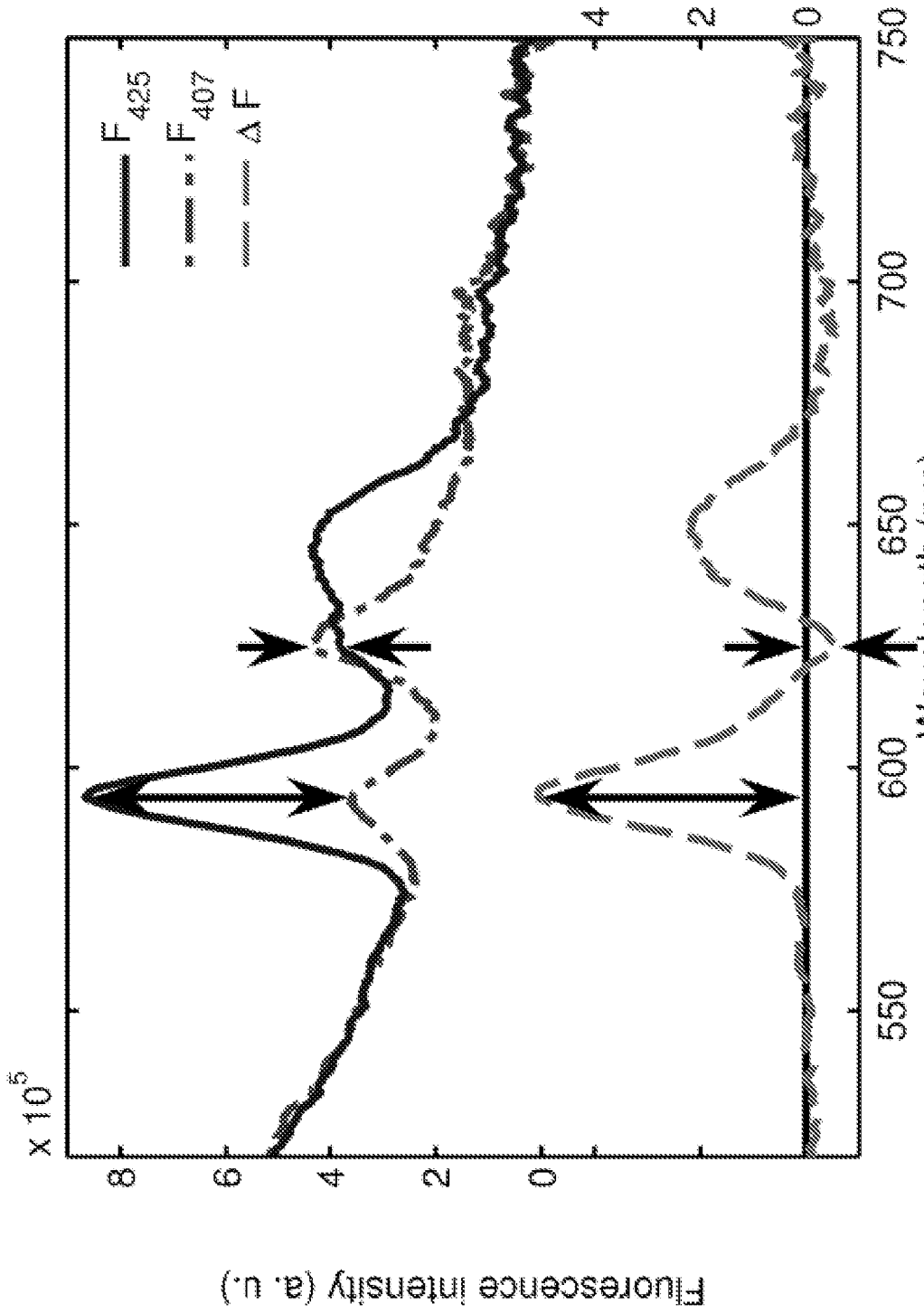

F425
F407
ΔF
x 10^5
8
6
4
2
0
4
2
0
550
600
650
700
750
Wavelength (nm)
Fluorescence intensity (a. u.)

FIGURE 38

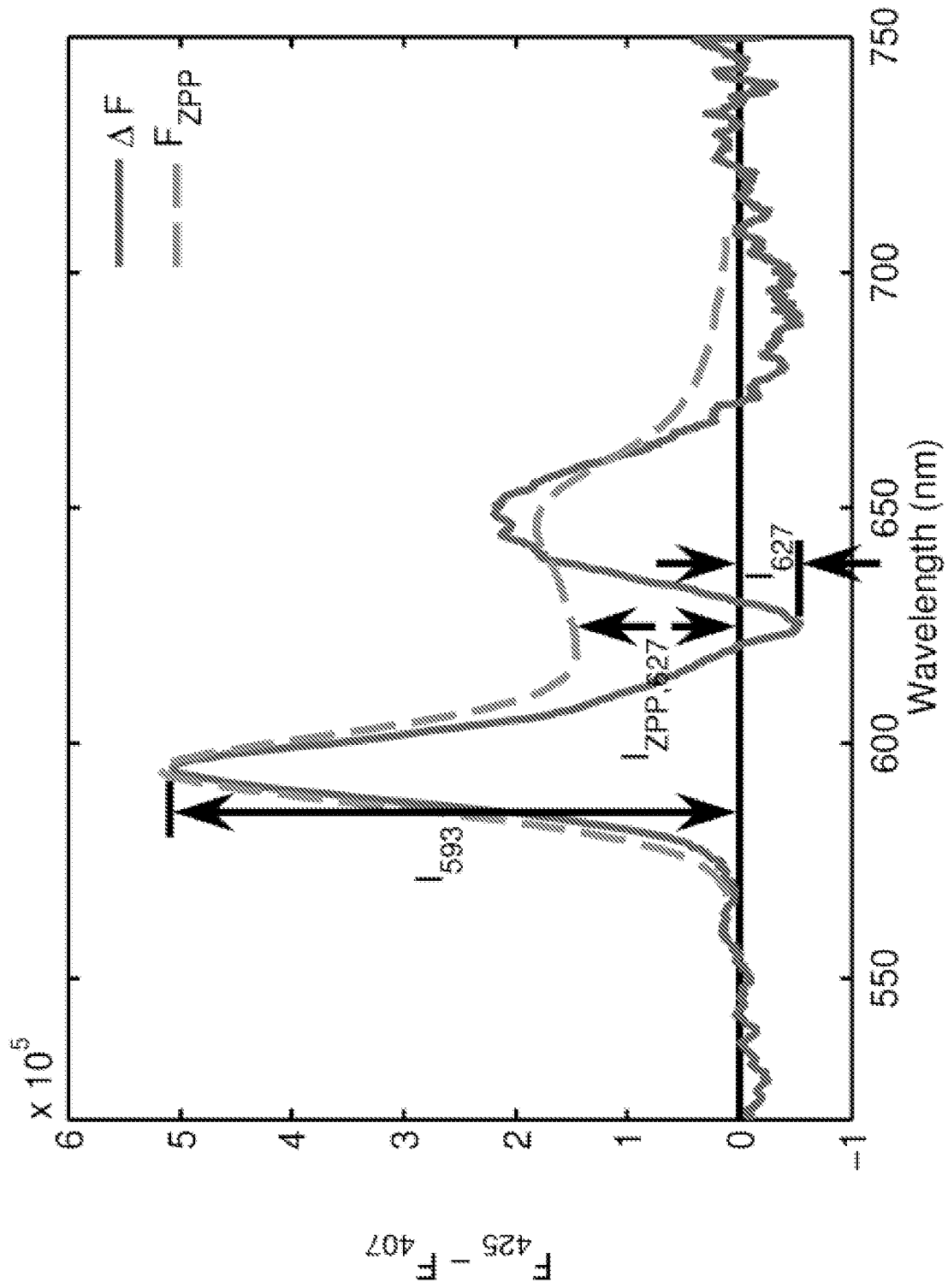

ΔF
F_ZPP
x 10^5
6
5
4
3
2
1
0
−1
550
600
650
700
750
Wavelength (nm)
I_593
I_ZPP,627
I_627
F425 − F407

FIGURE 39

FIGURE 40

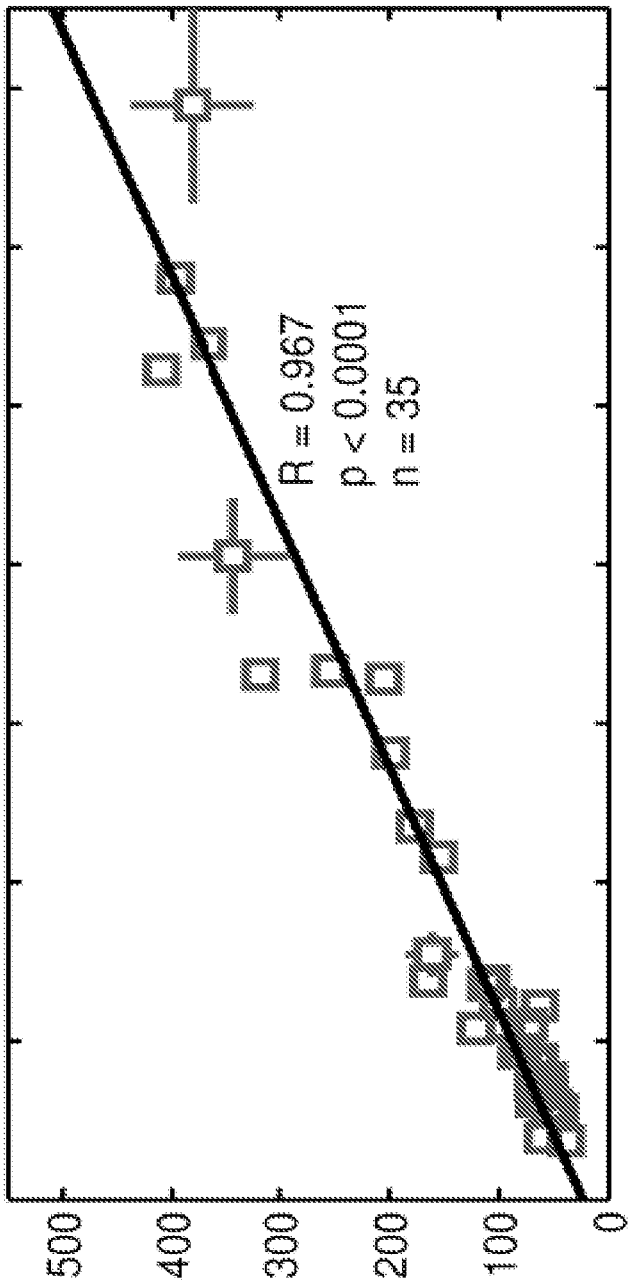


FIGURE 41

FIGURE 42

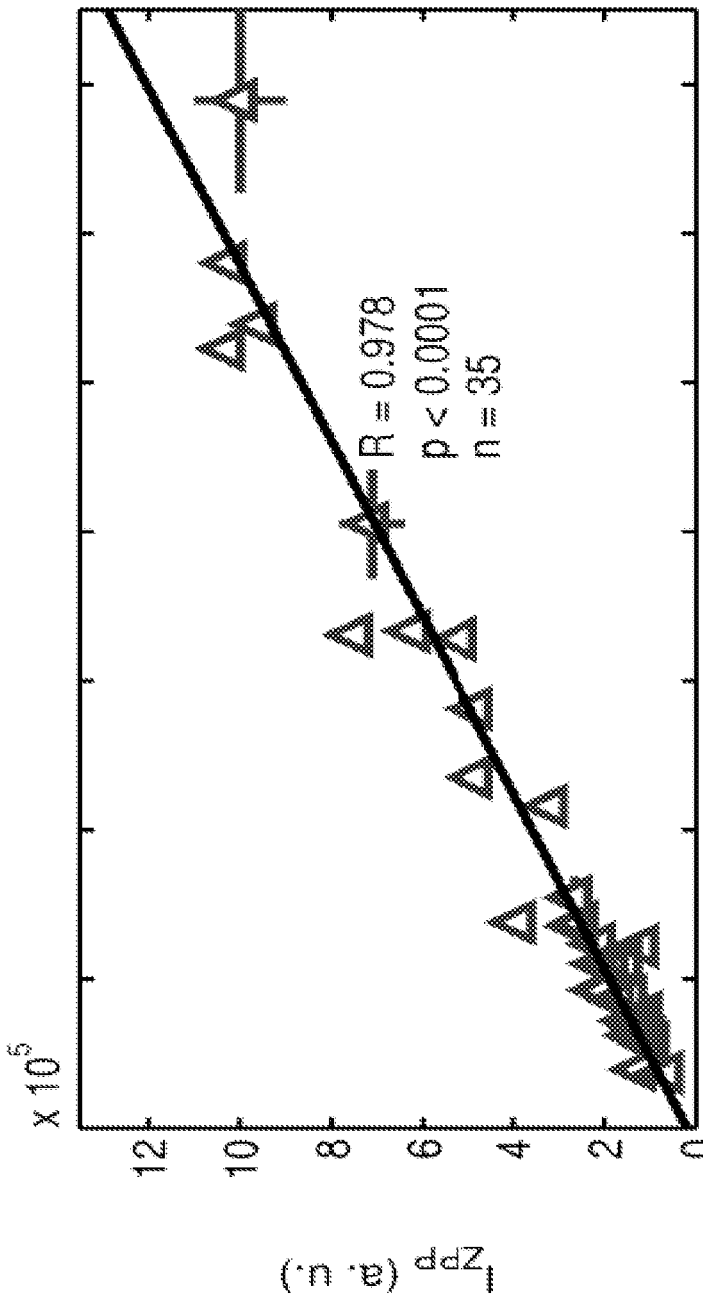


FIGURE 43

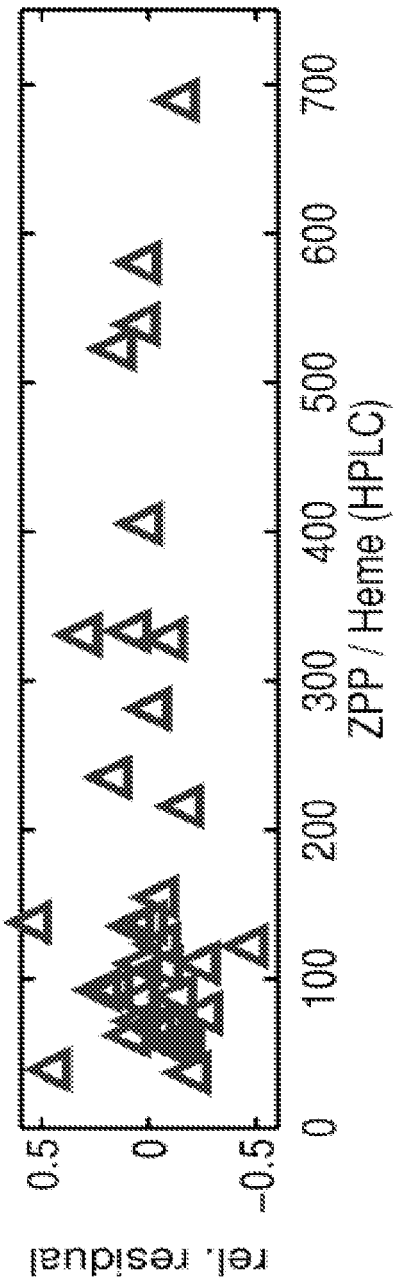


FIGURE 44

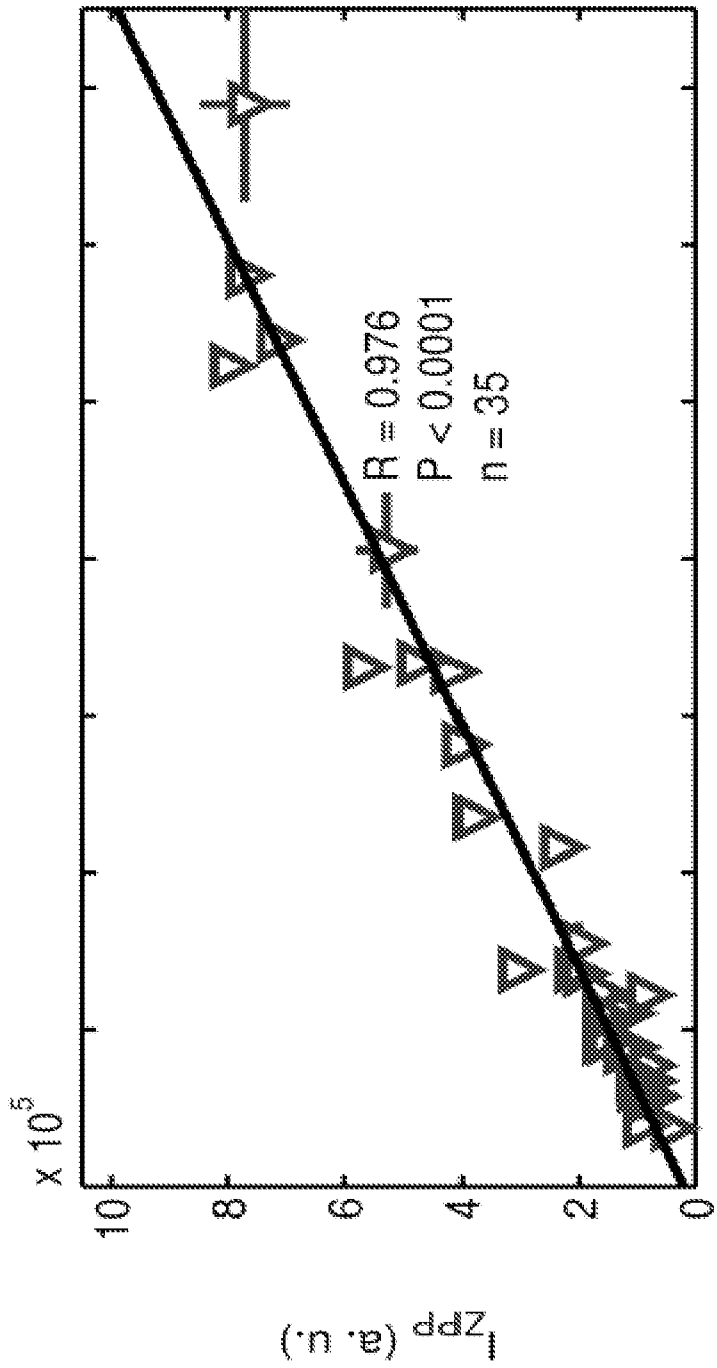
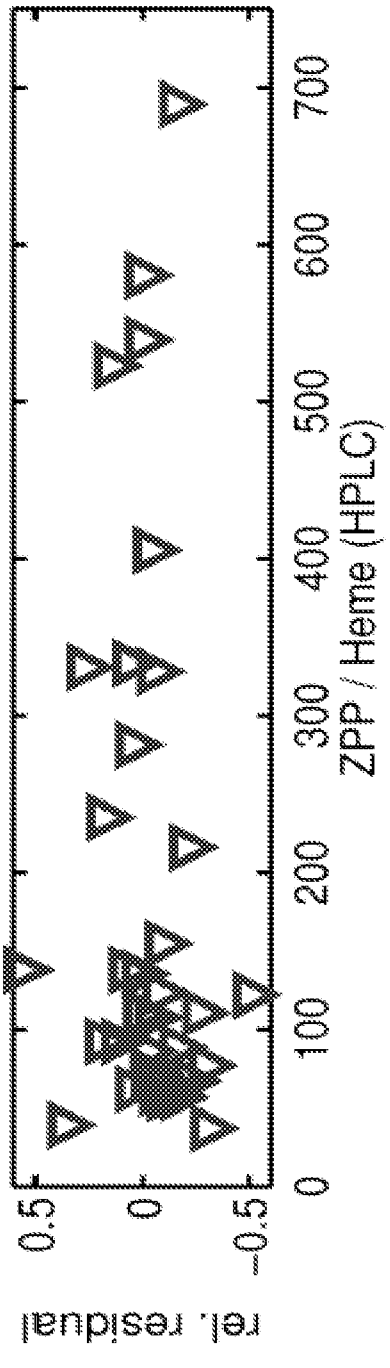


FIGURE 45



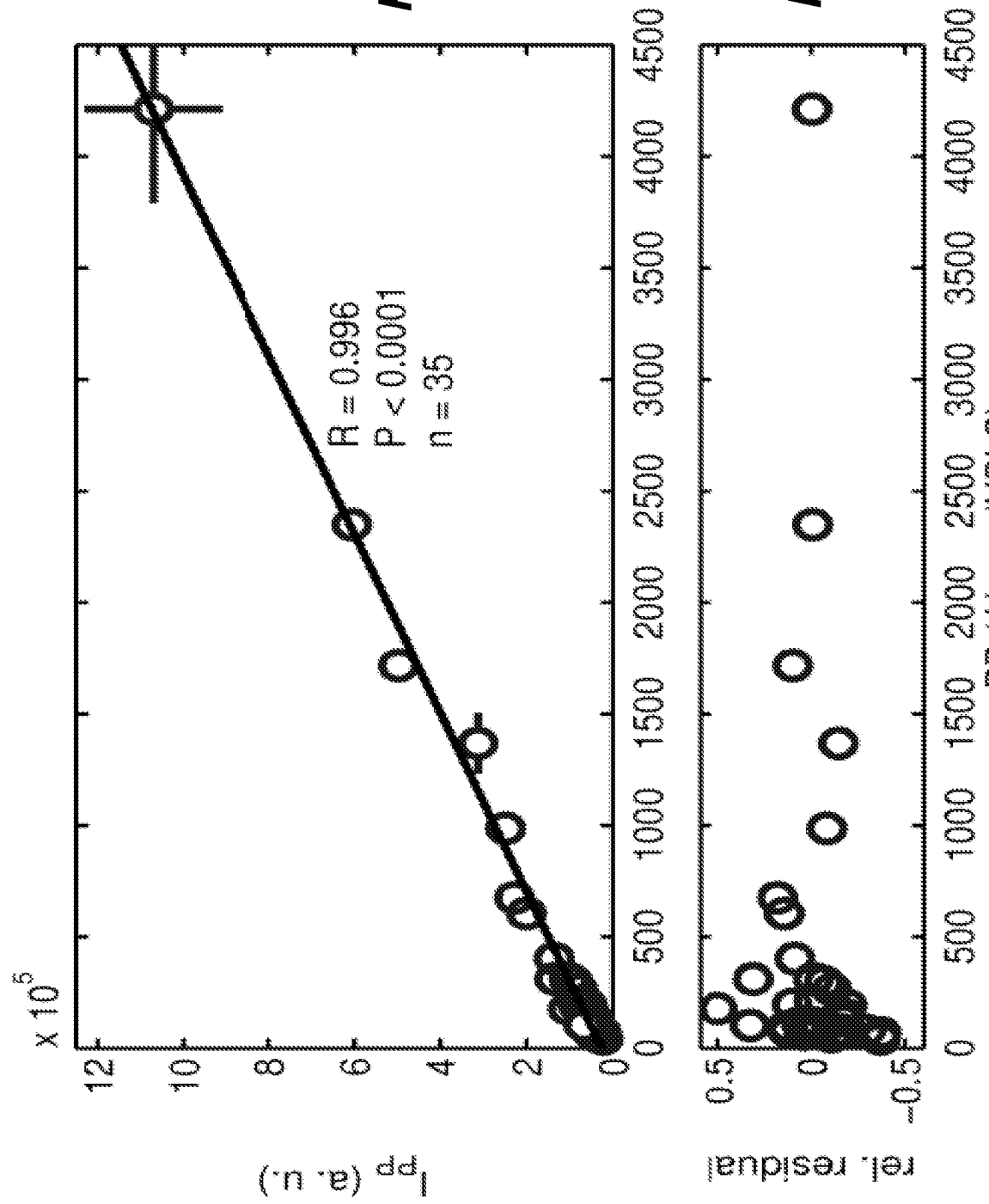


FIGURE 46

FIGURE 47

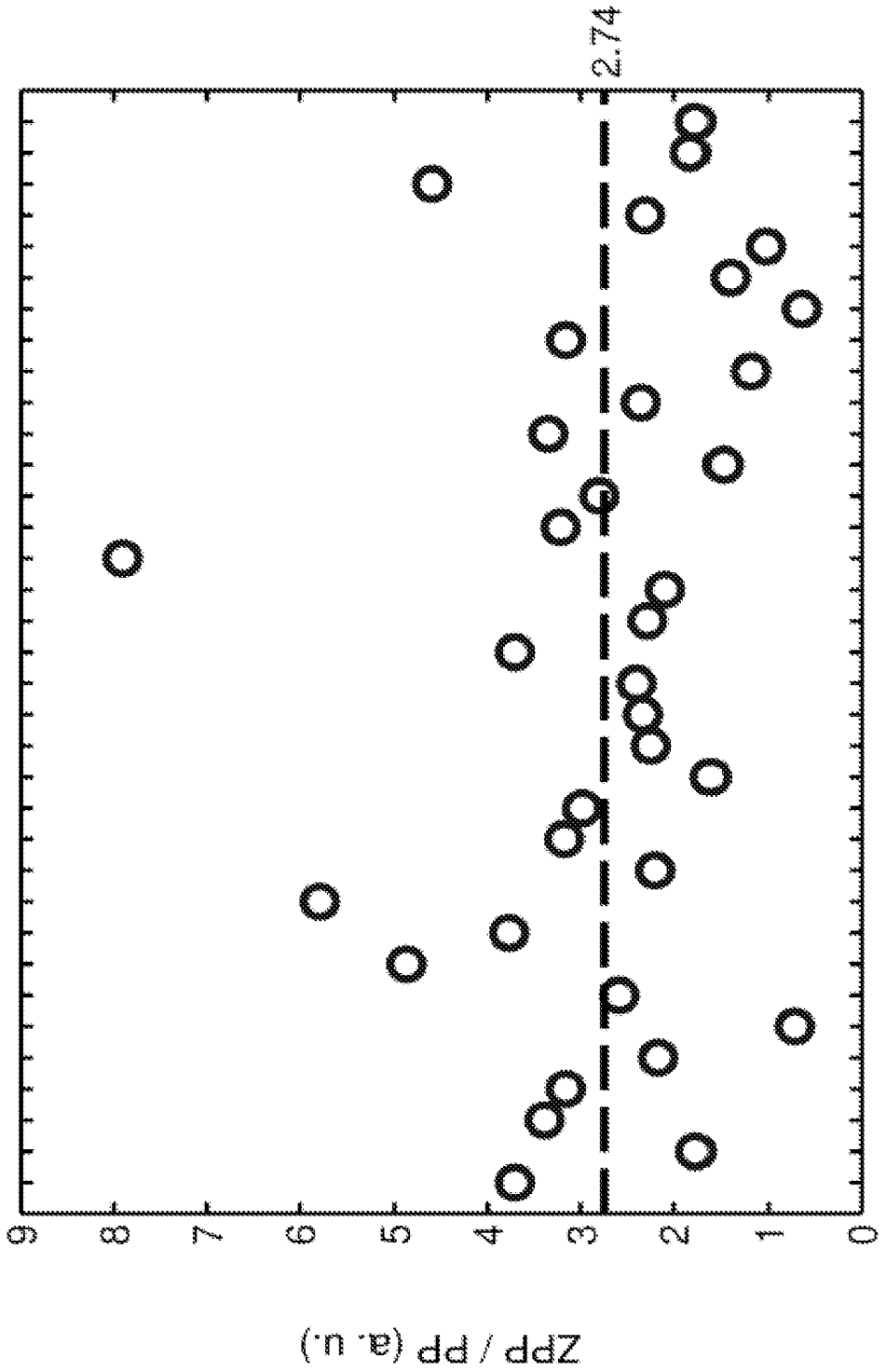


FIGURE 48

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- WO 2011063032 A **[0009]**

Non-patent literature cited in the description

- **SAZAWAL S. et al.** Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised, placebo-controlled trial. *Lancet,* 2006, vol. 367, 133-143 **[0003]**
- WHO Conclusions and recommendations of the WHO Consultation on prevention and control of iron deficiency in infants and young children in malaria-endemic areas. *Food Nutr Bull,* 2007, vol. 28, 621-7 **[0003]**
- **CHEN X.** Feasibility test for noninvasive detection of zinc protoporphyrin in oral mucosa and retina. *Biomedical Engineering,* 2007, 1-71 **[0075]**

专利名称(译)	使用组织激发测量荧光分析物		
公开(公告)号	EP2756303A4	公开(公告)日	2015-04-15
申请号	EP2012830997	申请日	2012-09-14
[标]申请(专利权)人(译)	纽约市哥伦比亚大学理事会		
申请(专利权)人(译)	哥伦比亚大学纽约市受托人		
当前申请(专利权)人(译)	哥伦比亚大学纽约市受托人		
[标]发明人	BRITTENHAM GARY M STEPP HERBERT HENNIG GEORG		
发明人	BRITTENHAM, GARY, M. STEPP, HERBERT HENNIG, GEORG		
IPC分类号	G01N33/53		
CPC分类号	A61B5/1455 A61B5/0071 A61B5/0075 A61B5/0088 A61B5/14546 A61B5/7275 A61B5/74 A61K49/0036 G02B26/001		
代理机构(译)	RUPP，CHRISTIAN		
优先权	61/535064 2011-09-15 US		
其他公开文献	EP2756303A1 EP2756303B1		
外部链接	Espacenet		

摘要(译)

一种用于通过在两个波长范围激发血液和分析物并且在（i）激发时的发射强度差异时测量荧光分析物的发射光谱来无创测量患者血液中的荧光分析物浓度的装置和方法荧光分析物的波长范围大于背景荧光团的波长范围，和（ii）当两个激发波长范围的血液吸收相似时。提供了一种用于测量患者血液中的荧光分析物浓度的装置和方法。