

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
17 April 2008 (17.04.2008)

PCT

(10) International Publication Number  
**WO 2008/044822 A1**

(51) International Patent Classification:  
A61B 6/00 (2006.01)

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(21) International Application Number:  
PCT/KR2007/003355

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(22) International Filing Date: 11 July 2007 (11.07.2007)

(25) Filing Language: Korean

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(26) Publication Language: English

(30) Priority Data:  
10-2006-0099033 11 October 2006 (11.10.2006) KR

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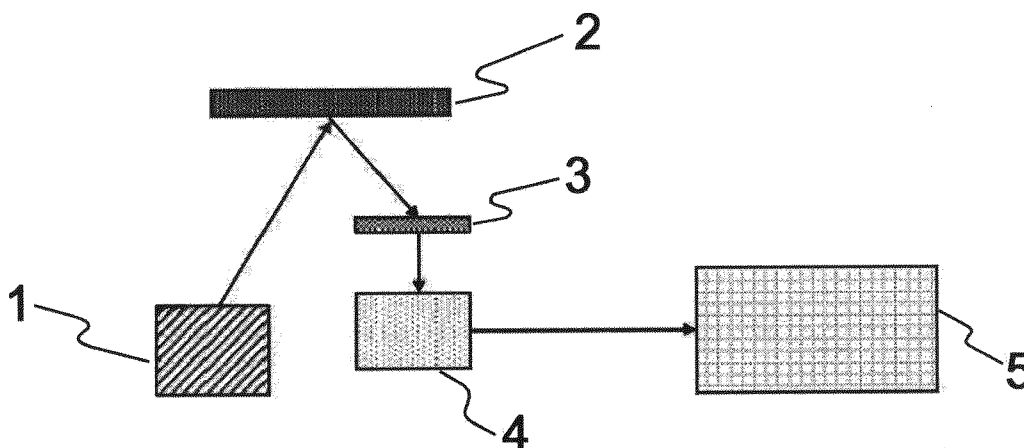
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(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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(54) Title: SYSTEM FOR ANALYZING TISSUE PERFUSION USING CONCENTRATION OF INDOCYANINE GREEN IN BLOOD



(57) Abstract: The present invention relates, in general, to a system for analyzing tissue perfusion using the concentration of indocyanine green and a method of measuring the perfusion rate using the system and, more particularly, to a system for measuring tissue perfusion by injecting indocyanine green into a living body, detecting variation in the concentration of indocyanine green with the passage of time, and analyzing the detected variation, and a method of measuring the perfusion rate using the system. The present invention provides a method of measuring perfusion in a living body, which enables accurate measurement for respective regions in a wide range from a perfusion rate decreased to less than 10% of normal perfusion to a perfusion rate increased to greater than normal perfusion using the above-described mechanism of ICG in a living body, which cannot be conducted using the conventional technology.

WO 2008/044822 A1



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**Published:**

— *with international search report*

**TITLE OF INVENTION**

**SYSTEM FOR ANALYZING TISSUE PERFUSION USING  
CONCENTRATION OF INDOCYANINE GREEN IN BLOOD**

**TECHNICAL FIELD**

5           The present invention relates to a system for  
analyzing tissue perfusion and a method of measuring the  
perfusion rate using the system. More particularly, the  
present invention relates to a system for measuring tissue  
perfusion by injecting indocyanine green into a living  
10 body, detecting variation in the concentration of  
indocyanine green with the passage of time, and analyzing  
the detected variation, and a method of measuring the  
perfusion rate using the system.

**BACKGROUND**

15           The conventional tissue perfusion measuring method  
'laser Doppler imaging' is a method of measuring the degree  
of scattering of laser light depending on the speed of  
blood flow in a skin surface, but has a shortcoming in that  
it is unsuitable for a method of measuring variation in the  
20 state in which blood flow is low because sensitivity is low  
when blood flow rate decreases to less than 20% of the  
normal of the blood flow rate.

Another conventional blood vessel imaging method 'X-

ray blood vessel imaging (X-ray angiography)' shows X-ray images using a blood vessel contrast agent. However, it is a structural imaging technique that shows the configuration of the internal diameters of blood vessels, rather than the actual flow of blood (Helisch, A., Wagner, S., Khan, N., Drinane, M., Wolfram, S., Heil, M., Ziegelhoeffer, T., Brandt, U., Pearlman, J. D., Swartz, H. M. & Schaper, W. (2006) *Arterioscler. Thromb. Vasc. Biol.*, 26: 520-526). Accordingly, it is currently impossible to clinically measure the precise rate of tissue perfusion using this method.

The safety of a conventional blood vessel imaging method using indocyanine green (ICG angiography) has already been verified (Sekimoto, M., Fukui, M. & Fujita, K. (1997) *Anaesthesia* 52: 1166-1172), and the method has been clinically used for the detection of the formation of blood vessels in grafted skin (Holm, C., Mayr, M., Hofter, E., Becker, A., Pfeiffer, U. J. & Muhlbauer, W. (2002) *Br. J. Plast. Surg.* 55: 635-644) and the measurement of newly created blood vessels in a diabetic patient's eyeballs (Costa, R. A., Calucci, D., Teixeira, L. F., Cardillo, J. A. & Bonomo, P. P. (2003) *Am. J. Ophthalmol.* 135: 857-866). ICG receives near infrared rays, having wavelengths ranging from 750~790 nm, and radiates near infrared rays, having longer wavelengths ranging from 800 to 850 nm, and the

radiated near infrared rays can be measured using a CCD camera or a spectrometer. Near infrared rays have high penetration power, and thus they can penetrate several centimeters deep into tissue and don't scatter much, with the result that they are the object of extensive research towards human body imaging technology (Morgan, N. Y., English, S., Chen, W., Chernomordik, V., Russo, A., Smith, P. D. & Gandjbakhche, A. (2005) *Acad. Radiol.* 12: 313-323). This method is also used as a technique for structural blood vessel imaging. This method is used for the test of the permeability of newly created blood vessels in a diabetic patient's eyeballs, not for the measurement of tissue perfusion.

The above method is used for an 'ICG elimination test' as well as the above purpose. When ICG is injected into a vein, ICG is attached to protein in a blood vessel, such as albumin, and is rapidly spread into the body via blood vessels. When it is transferred to the liver, it is separated from protein and discharged in the form of bile and excreted from the body finally while the protein is degraded. As a result, the concentration of ICG is rapidly reduced in the blood vessels, so that the intensity of ICG fluorescence signals is reduced to half of the intensity of initial ICG fluorescence signals 4 to 6 minutes later, and is then diminished and deviates from an accurate

measurement range. One use of the 'ICG dynamics', in which ICG is rapidly eliminated by the liver, is a liver function test (Sinyoung Kim, et al., (2003) *Kor. J. Lab. Med.*, 23: 88-91), but the ICG dynamics has been known to have  
5 shortcomings when applied to *in vivo* imaging (Sekimoto, M., Fukui, M. & Fujita, K. (1997) *Anaesthesia* 52: 1166-1172). Accordingly, the present inventors have completed the present invention through the ascertainment of the fact that accurate measurement can be conducted in a wide range  
10 from a perfusion rate decreased to less than 10% of that of normal perfusion to a perfusion rate increased to greater than that of normal perfusion using the ICG dynamics in the living body.

#### **DISCLOSURE**

##### 15 **TECHNICAL PROBLEM**

An object of the present invention is to provide a method of measuring perfusion in a living body, which enables to provide absolute value of perfusion for respective regions in a wide range from a perfusion rate  
20 decreased to less than 10% of normal perfusion to a perfusion rate increased to greater than normal perfusion using the above-described ICG dynamics in a living body, which cannot be conducted in the prior art.

**TECHNICAL SOLUTION**

In order to accomplish the above object, the present invention provides a tissue perfusion analysis apparatus, a method of measuring tissue perfusion and a tissue perfusion measurement apparatus, which calculate a perfusion rate by injecting indocyanine green into a living body and measuring variation in the concentration of the indocyanine green.

**DESCRIPTION OF DRAWINGS**

10 Fig. 1 is a photo showing the fact that there is no correlation between laser Doppler images and the rates of tissue necrosis.

Fig. 2 is a schematic diagram showing the overall construction for detecting ICG fluorescence signals:

15 Description of reference numerals of principal elements in the accompanying drawings:

1: light source,                      2: living body stand,  
3: 800~850nm filter,              4: ICG near infrared ray  
fluorescence detector, and

20 5: ICG image analysis apparatus.

Fig. 3 is a graph (upper side) showing the experimental results of the dynamics of the intensity of ICG fluorescence, directly acquired with the passage of time from the blood in a living body, and a photo (lower

side) showing the results of analysis of indocyanine fluorescence.

Fig. 4 is a simulation graph showing the mechanism of ICG fluorescence based on the perfusion rates of ischemic tissue.

Fig. 5 is a simulation graph showing the relationship between  $T_{\max}$  and perfusion rates.

Fig. 6 is an imaging view of a blood vessel acquired after the injection of ICG into the tail vein of a nude mouse.

Fig. 7 is an image outputted by mapping perfusion rates as colors, using an ischemia pattern analysis method in a mouse from which a thigh artery has been removed.

Fig. 8 is a series of images showing showing the probability of tissue necrosis (center) based on a perfusion map of the ischemia leg indicating ischemia rates measured immediately after an operation (left side), and a photograph showing the actual rates of tissue necrosis seven days after an operation (right side).

Fig. 9 is a graph showing the experimental results of the dynamics of the intensity of ICG fluorescence in blood collected from a living body; and

Fig. 10 is a diagram illustrating an example of the principle of mapping between perfusion rates and colors.

**BEST MODE**

The present invention will be described in detail below.

The present invention provides an analysis apparatus for measuring the rate of tissue perfusion by injecting indocyanine green (ICG) into a living body and measuring variation in concentration, and then presenting information about the probability of the necrosis of tissue.

The analysis apparatus includes:

1) an input means for receiving signals from a photodetector, 2) a numerical conversion means for processing the input signals into the intensities of fluorescence with the passage of time in a region of interest, 3) a perfusion rate calculation means for calculating the rates of perfusion for respective regions of a tissue using the numerical values, and 4) an output means for outputting the results of the calculation.

In the analysis apparatus, the photodetector is a device for detecting fluorescence in a typical infrared ray, visible ray or ultraviolet ray region. Although not limited to following devices, a photo-detection device, such as a photoresistor, a photovoltaic cell, a photodiode, a photomultiplier tube (PMT), a phototube, a phototransistor, a charge-coupled device (CCD), a pyroelectric detector, a Golay cell, a thermocouple, a

thermistor, a complementary metal oxide semiconductor (CMOS) detector or a cryogenic detector, may be used as the photodetector.

It is preferable to use RSC 232, a parallel port, IEEE 1394 or USB as the input means, although the invention is not limited thereto.

The numerical conversion means is operably connected to the input means, and consists of a microprocessor, and operation software embedded therein. The intensity of fluorescence may be numerically converted using a graph, with the intensity of fluorescence being plotted on the y axis and the time of measurement being plotted on the x axis, as shown in Figs. 3 and 4, but may be numerically converted using some other methods.

The calculation means includes a microprocessor, and operation software embedded therein and configured to drive an algorithm including a calculation equation for calculating perfusion using the numerical value of the intensity of fluorescence and the time of measurement, obtained from the numerical conversion means. The microprocessor may be a microprocessor included in the numerical conversion means, or may be a separate microprocessor.

The calculation uses the fact that the intensity of fluorescence in a normal tissue decreases exponentially

with the passage of time of measurement, and the fact that, in the case where ischemia occurs in a tissue that is the target of analysis, the ICG fluorescence particles of normal tissue enter at the perfusion rate of an ischemic tissue and the ICG fluorescence particles of the ischemic tissue exit at the same perfusion rate.

In the present invention, the rate of tissue perfusion can be calculated using the time at which the intensity of fluorescence in a target tissue for analysis is maximized. This uses the fact that, in the case where ischemia occurs in the target tissue for analysis, the time at which the intensity of ICG fluorescence in the ischemic tissue is highest is the point at which the time-differential value is 0. In the present invention, the time is defined as  $T_{\max}$ .

In a specific embodiment of the present invention,  $T_{\max}$  is obtained using the following method. First, a graph (See Fig. 3), in which images of the ICG fluorescence of a living body, obtained with the passage of time after the injection of ICG using the above-described method, were converted into numerical values with the highest brightness set to 1, was constructed. Since this graph shows a curve that decreases exponentially according with time, calculation can be conducted using the following equation:

$$FI_{nor} = A \times e^{-\frac{t}{\tau}}.$$

In the above equation,  $FI_{nor}$  (Fluorescence Intensity normal) means the intensity of ICG fluorescence of normal tissue, 'A' means the intensity of fluorescence obtained from ICG images during the first minute (in the present invention, A is calculated as 1),  $\tau$  is defined as  $t_{1/2}/\ln 2$  through calculation, and  $t_{1/2}$  means the time when the intensity of fluorescence of ICG is half the highest value.

The extent of variation in the intensity of ICG fluorescence in an ischemic tissue, in which the rate of tissue perfusion is lower than that of a normal tissue, can be calculated using the following equation based on the assumption that "the ICG fluorescence particles of the normal tissue ( $FI_{nor}$ ) enter at the rate of perfusion of the ischemic tissue (P) and the ICG fluorescence particles of the ischemic tissue ( $FI_{isc}$ ) exit at the same perfusion rate (P)":

$$FI_{isc} = \frac{PA}{P-1/\tau} (e^{-\frac{t}{\tau}} - e^{-Pt}).$$

The variation of ICG dynamics, in case when the perfusion rate of the ischemic tissue is lowered, was simulated using the above equation (See Fig. 4). Through the simulation, the result, in which  $T_{max}$ , which is the time at which the intensity of fluorescence was maximized, increased as the perfusion rate of a tissue decreased, was

obtained (See Fig. 5).

The above relationship between  $T_{\max}$  and the rates of blood perfusion is expressed by the following Equation:

$$- \ln \tau - \frac{T_{\max}}{\tau} = \ln P - PT_{\max} \quad (1).$$

5           The rate of tissue perfusion (P) is calculated based on  $T_{\max}$  using Equation 1.

However, the measurement of the perfusion rate of a tissue based on  $T_{\max}$  is only an embodiment of the present invention, and it is apparent to those skilled in the art that the scope of protection of the present invention is not limited by the above embodiment. Accordingly, analysis apparatuses for injecting indocyanine green, measuring the concentration of indocyanine green in tissue with the passage of time, and measuring the perfusion rate of the tissue using the mechanism of the dynamics of fluorescence of indocyanine green in tissue fall within the scope of the rights of the present invention, regardless of the algorithm or calculation equation used therein. Meanwhile, in the present invention, the calculated perfusion rates are outputted as a perfusion map wherein the calculated perfusion rates mapped to specific colors. Although it is preferable to use a color gradient for colors corresponding to the rates of perfusion, the present invention is not limited thereto, and a programmer may arbitrarily select a

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color representation method. The perfusion map as a means for visualizing the rate of tissue perfusion, is outputted through a screen, a printer or the like, and makes an operator of the perfusion rate analysis apparatus of the present invention perceive the rate of tissue perfusion visually and intuitively.

Through the embodiment, a relationship between the perfusion rates, measured using the above-described method, and the probability of future necrosis of a tissue due to ischemia was obtained. In a specific embodiment, the case where the rate of tissue perfusion is about 50 %/min is indicated by a yellow color in the perfusion map (See the left side of Fig. 8), and, in the case of this yellow color, the probability of necrosis of a tissue was statistically calculated to be in the range of 0 ~ 10%, and it may be colored blue in a tissue necrosis probability map (See the center of Fig. 8). Furthermore, the present invention provides a tissue perfusion measurement apparatus, comprising a stand configured to allow light to pass therethrough, a light source adjustable to be located to radiate light onto an ICG-injected living body which is disposed on the stand, a filter adjustable to be located to filter only near infrared ray wavelengths of 800 to 850 nm from fluorescence signals emitted from the living body under the action of the light source, a detector configured

to detect fluorescence light passed through the filter, and an analysis apparatus operably connected to the detector and configured to image light detected by the detector and measure the rates of tissue perfusion through the analysis  
5 of the ICG dynamics with the passage of time.

With regard to the light source, a Light Emitting Diode (LED) having a wavelength of 770 +/- 20 nm, a light source in which a filter having a wavelength of 770 +/- 20 nm is attached to a white light source, and a laser light  
10 source having a wavelength of 770 +/- 20 nm may be all used as the light source radiating light for detecting ICG fluorescence.

The above-described filter passes only light having a wavelength ranging from 800 to 850 nm therethrough. Using  
15 this filter, fluorescence generated in a living body itself is not detected by the detector.

The detector detects near infrared rays having wavelengths ranging from 800 to 850 nm, which are ICG fluorescence signals. An infrared detection camera or a  
20 spectrometer may be used as the detector. The detector continuously detects images immediately from the injection of ICG, and transmits signals, capable of exhibiting an ICG dynamics with the passage of time, to the analysis apparatus.

25 In the present invention, when a Region Of Interest

(ROI) of a living body, in which the perfusion rate is desired to be measured, is designated, the intensities of ICG fluorescence with the passage of time are automatically converted into numerical values from continuous ICG image data. Using this, the intensities of ICG fluorescence, measured as in Fig. 3, are processed into temporal and spatial information.  $T_{\max}$  is obtained based on the data, and then the rates of tissue perfusion are analyzed using the simulation data of Fig. 4, the correlation between  $T_{\max}$  of Fig. 5 and the rates of tissue perfusion, and the Equation 1. Furthermore, using the results of the analysis, a tissue perfusion map (See Fig. 7 and the left side of Fig. 8) and a tissue necrosis rate prediction map (See the center of Fig. 8) are provided. This prediction exhibited a high prediction level in the embodiment of the present invention (See the right side of Fig. 8).

In order to measure an ICG dynamics in a living body using the above measuring apparatus, ICG must be injected through an intravenous injection and variation in the concentration of ICG in tissue with the passage of time must be detected and analyzed. The variation in the concentration of ICG in tissue may be measured through the acquisition of a blood sample, through the direct acquisition of a near infrared ray image of the tissue, or using a spectroscopic technique.

Furthermore, the present invention provides a method of measuring the rate of tissue perfusion, comprising:

- 1) detecting sequentially concentration of ICG until the concentration of ICG is sufficiently decreased by injecting the ICG into a living body, radiating light onto the living body using a light source, and measuring the intensities of fluorescence, generated in the living body with the passage of time after the injection, using a photodetector;
- 2) processing the sequential concentration of ICG in the living body detected in the step 2 into numerical data by converting the intensities of ICG fluorescence into numerical values for respective regions and respective times; and
- 3) calculating the rates of perfusion from the numerical data.

In an embodiment, it is preferred that the method of measuring the rates of tissue perfusion further comprises the step of outputting the calculated perfusion rates as a perfusion map. It is preferred that the perfusion map is constructed by painting particular pixels with respective colors corresponding to the rates of perfusion according to a color gradient map.

According to another embodiment, the present invention provides a method of measuring the rates of

tissue perfusion, comprising:

1) detecting sequentially concentration of ICG until the concentration of ICG is sufficiently decreased by injecting the ICG into a living body, radiating light onto the living body using a light source, and measuring intensities of fluorescence, generated in the living body with the passage of time, using a photodetector and obtaining an ICG blood vessel image diagram using the intensities of fluorescence;

2) acquiring  $T_{\max}$  by analyzing dynamics of the intensities of ICG fluorescence per respective pixel in the ICG blood vessel image diagram; and

3) calculating the rates of perfusion rate based on the  $T_{\max}$ .

In this case, it is preferred that the method of measuring the rates of tissue perfusion further comprises the step of outputting the calculated perfusion rates as a perfusion map. It is preferred that the perfusion map is constructed by painting particular pixels with respective colors corresponding to the rates of perfusion according to a color gradient map, wherein the rates of perfusion is calculated through the analyzed data.

Furthermore, the present invention provides a method of predicting the rate of tissue necrosis, comprising representing a tissue necrosis probability map, indicating

the probability of necrosis of a tissue, based on the rates of tissue perfusion acquired using the above-described measurement method, as a result (See the right side of Fig. 8).

5           In the measurement method, with regard to the light source, an LED having a wavelength of 770 +/- 20 nm, a light source in which a filter having a wavelength of 770 +/- 20 nm is attached to a white light source, and a laser light source, having a wavelength of 770 +/- 20 nm, may all  
10 be used as a light source radiating light for detecting ICG fluorescence. Any device using the above-described photo-detection device may be used as the photodetector. Although the photodetector is not limited, the photodetector must detect near infrared rays having wavelengths ranging from  
15 800 to 850 nm, which are ICG fluorescence signals, and an infrared detection camera or a spectrometer may be used as the photodetector. The photodetector sequentially detects images immediately after an injection of ICG, and transmits signals, capable of exhibiting an ICG dynamics with the  
20 passage of time, to the analysis apparatus.

          Meanwhile, with regard to fluorescence emitted from a living body, it is preferred that fluorescence generated from a living body itself is not detected using a filter capable of passing only light having wavelengths ranging  
25 from 800 to 850nm therethrough.

It is preferred that the data processing step and the perfusion rate analyzing step are performed using a microprocessor and software embedded in the microprocessor, or stored in external storage, such as a hard disk drive, an optical drive or a flash memory. This software is not limited to a specific algorithm, as described above, and may be implemented through one of various platforms, such as Windows series operating systems, for example, Microsoft Windows XP, 2000, Me, 98 and 95, Linux, OS/2, and Unix.

Although the correlation coefficient at the perfusion rate analysis step is not limited thereto, it is preferred that the correlation coefficient is the time at which the intensity of ICG fluorescence of a target tissue to be analyzed (ischemic tissue) is maximized.

Although the acquisition of  $T_{\max}$  at the step of calculating perfusion rates from the dynamics of ICG fluorescence intensity of the target tissue (ischemic tissue) does not limit the method of analyzing the perfusion rates, it is preferable to perform calculation using the principle in which the time  $T_{\max}$ , at which the differential value of the intensity of fluorescence is 0, is proportional to the perfusion rate in the target tissue.

The step 3 may be embodied by the following steps: acquiring perfusion rates of region of interest (ROI) according to (a) average data of pixels of particular

regions, (b) respective pixels, or (c) respective ROIs representing perfusion rates; and representing the perfusion rates using a perfusion map by illustrating the perfusion rates for the ROI with corresponding colors designated according to the perfusion rates. The above process may be performed by calculations using software having an appropriate algorithm and loaded on a microprocessor and a display through an output device of a computer. Fig. 7 shows an example of the perfusion map, which may be acquired by sequentially obtaining fluorescence images with the passage of time after injecting ICG, measuring the perfusion rates through the analysis of the ICG fluorescence dynamics of respective pixels, and painting the pixels with colors corresponding to the measured perfusion rates. An example of matching between the perfusion rates and colors is illustrated in Fig. 10.

Although the output device is not limited thereto, it is preferred that the output device is a monitor, a printer or a plotter. It is possible to store data in various graphic formats using an external storage device.

The present inventors carried out a surgical operation of removing an artery and vein from the thigh of a leg of a nude mouse, acquired ICG images using the above-described method, and obtained the  $T_{\max}$  value. Since the

rate of tissue necrosis was greatly affected by the rate of tissue perfusion, whether the 'precise perfusion rates' were measured was evaluated through the observation of rates of tissue necrosis. As a result, as shown in Fig. 8, the method of the present invention showed the significant relationship between measured perfusion values and the rates of tissue necrosis. This indicates that measurement can be performed in the case where the perfusion rate is decreased to 0.04~50 %/min (the perfusion rate of a normal leg: 300 %/min), unlike the measurement using Doppler imaging (refer to Fig. 1), that is, a comparative example, in which a subtle difference in the considerably decreased 'perfusion rate' could not be measured.

#### **MODE FOR INVENTION**

The present invention will be described in detail below in conjunction with embodiments.

However, the following embodiments are only to illustrate the present invention, but do not limit the content of the present invention.

#### **Comparative example 1: Prediction for tissue necrosis through Doppler imaging**

In Fig. 1, showing the present inventors' experimental data, the three left views thereof show data

obtained through laser Doppler imaging one to four days after a surgical operation of removing an artery and vein from the thigh of one leg of a mouse so as to construct a blood perfusion reduction model, and the right view thereof shows the rate of necrosis of the leg tissue a week thereafter. With regard to the rates of tissue necrosis, the case A shows no necrosis, the case B shows necrosis reaching the center of the sole of the foot, and the case C shows necrosis reaching the ankle, and the rate of necrosis is proportional to the rate of tissue perfusion (Helisch, A. et al., (2006) *Arterioscler. Thromb. Vasc. Biol.* 26: 520-526, the case A shows 160% of normal perfusion, the case B shows 20% of normal perfusion, and the case C shows 5% of normal perfusion). Respective pieces of Doppler imaging data corresponding to the cases did not exhibit any difference in tissue perfusion between the cases.

**Example 1: Establishment of method of measuring perfusion using ICG**

The present inventors derived an equation through the following steps so as to establish a method of measuring perfusion based on the ICG concentration dynamics in blood.

Fig. 9 is a graph that is obtained by injecting ICG (1.5 mg/kg, Sigma, USA) into a vein, collecting blood over time, measuring the intensities of ICG fluorescence, and

converting the intensities into the concentrations of ICG in blood. The closed squares of the graph indicate the intensities of ICG fluorescence, while the open squares thereof indicate the concentrations of ICG in blood. Fig. 3 shows variation in ICG fluorescence over a period of time from 1 minute to 12 minutes after the injection of ICG. That is, in Fig. 3, an image of the ICG fluorescence of a living body acquired with the passage of time is converted into numerical values, with the highest brightness set to 1, and is represented in the form of a graph. When variation in ICG in normal tissue is formulated using a graph acquired from a normal tissue, as shown in Fig. 3, the graph decreases exponentially with respect to time, and thus the following formulation can be realized:

$$FI_{nor} = A \times e^{-\frac{t}{\tau}},$$

Wherein the  $FI_{nor}$  (Fluorescence Intensity normal) is the intensity of ICG fluorescence in the normal tissue,  $A$  is the intensity of fluorescence obtained from an ICG image for a first minute (in the present invention,  $A$  is calculated as 1), and  $\tau$  is defined as  $t_{1/2}/\ln 2$  through calculation. Furthermore,  $t_{1/2}$  is the time at which the intensity of ICG fluorescence is half of the highest value.

A simulation was made under the expectation that, when tissue perfusion decreases, the speed of dispersion of

ICG decreases, with the result that variation in the intensity of ICG fluorescence differs from that of normal tissue. In this case, the 'perfusion rate' is 'the rate at which blood in a region of interest is exchanged for blood in the other regions for one minute with respect to the total amount of blood.' The unit of the perfusion rate is %/min, and the 'perfusion rate' of a normal leg was calculated as 300 %/min. The rate of variation in the intensity of ICG fluorescence with the passage of time in an ischemic tissue, the perfusion rate of which is lower than in a normal tissue, is expressed by the following equation on the assumption that "ICG fluorescence particles  $FI_{nor}$  in the normal tissue enter at the perfusion rate  $P$  of the ischemic tissue, and ICG fluorescence particles  $FI_{isc}$  in the ischemic tissue exit at the same perfusion rate  $P$ ."

The following equation is given for  $FI_{isc}$  ( $FI_{ischemia}$ ) as follows:

$$FI_{isc} = \frac{PA}{P - 1/\tau} (e^{-\frac{t}{\tau}} - e^{-Pt}).$$

In this case, the simulation graph of Fig. 4 is obtained by substituting a perfusion rate of 20~300 %/min for  $P$ . In this graph, as the perfusion rate decreases, the maximum value of the intensity of ICG fluorescence decreases, and the time at which the intensity of ICG fluorescence is maximized is delayed.

An actual ICG fluorescence intensity graph differs from a simulated graph. The decrease in the intensity of ICG fluorescence in a normal tissue varies with the state of a living body, with the result that absolute comparison is possible only when the fluorescence in an ischemic tissue is corrected. As a result, a 'correlation coefficient', which is a standard criterion for obtaining 'perfusion rates' from respective pieces of experimental data, is required, and, in the present invention,  $T_{\max}$  is selected as the correlation coefficient.  $T_{\max}$  is the time at which the differential value of the ICG fluorescence intensity dynamics of an ischemic tissue is 0.

Since  $T_{\max}$ , which is the time at which the intensity of ICG fluorescence in an ischemic tissue is highest, is the point at which the time-differential value is 0, the following equation can be given:

$$\frac{dFI_{isc}(T_{\max})}{dt} = 0, \text{ and}$$

$$\frac{dFI_{isc}(T_{\max})}{dt} = \frac{PA}{P-t/\tau} \left[ -\frac{1}{\tau} e^{-\frac{T_{\max}}{\tau}} + P e^{-PT_{\max}} \right] = 0.$$

These can be summarized as follows:

$$-\ln \tau - \frac{T_{\max}}{\tau} = \ln P - PT_{\max} \quad (1).$$

A graph for  $T_{\max}$  and the 'perfusion rate' is shown in Fig. 5.

**Example 2: Measurement of perfusion using indocyanine green and the construction of perfusion map and tissue necrosis probability map based on correlation coefficient**

The present inventors constructed a blood perfusion reduction model to obtain ICG images in an actual ischemic tissue. A surgical operation of removing an artery and vein from one leg of each of 50 nude mice (Charles River Japan, Inc.) was carried out, ICG images were acquired using the measurement apparatus of Fig. 2 four hours after the surgical operation, and the perfusion rates of tissues were obtained using the above-described method.

The obtained perfusion rates of tissues were arranged in a perfusion map (the left side of Fig. 8). In Fig. 8, a perfusion rate of 300 %/min corresponds to 9 in the perfusion map, 0 %/min corresponds to 0.

Since the rate of tissue necrosis is considerably affected by the rate of tissue perfusion, whether a 'perfusion rate' has been precisely measured can be evaluated through the observation of the rate of tissue necrosis. The rates of tissue necrosis of 50 laboratory mice were observed seven days after operations (the right side of Fig. 8), and the relationship between perfusion rates and the rates of tissue necrosis, which were obtained four hours after the operations, was statistically analyzed using the statistical data. As a result of the analysis, it

was ascertained that the probability of tissue necrosis corresponding to a perfusion rate of 50 %/min fell within a range of 0 ~ 10%, and the probability of tissue necrosis corresponding to a perfusion rate ranging from 15 to 5 20 %/min fell within a range of 75 to 80%. The present inventors assigned a red color to the case where the probability of tissue necrosis was 1 and a blue color to the case where the probability of tissue necrosis was 0, and constructs a necrosis probability map for the tissue 10 using a color gradient between the cases (the center of Fig. 8). The result, in which the constructed tissue necrosis probability map corresponded to the actual rates of tissue necrosis, was acquired.

The result exhibited the fact that the probability of 15 necrosis of tissue increased as the perfusion rate measured using the method decreased, and thus verified the precision of 'perfusion rates' acquired using the method. This means that it was impossible to measure a subtle difference in considerably decreased 'perfusion rate' using the 20 conventional technology, as shown in Fig. 1, but it is possible to perform measurement using the method of the present invention in the case where the perfusion rate is decreased to 0.04~50 %/min (the perfusion rate of a normal leg: 300 %/min). This coincides with the rate of tissue 25 necrosis, which means that the method of the present

invention has an ability to predict the rate of tissue necrosis.

#### **INDUSTRIAL APPLICABILITY**

According to the method of the present invention, it  
5 is possible to measure perfusion rates precisely in a wide  
range from a perfusion rate decreased to less than 10% to a  
perfusion rate increased to the normal perfusion rate, and  
it is possible to predict the rate of tissue necrosis based  
on the perfusion rate. In addition, since it is possible to  
10 measure variation in blood perfusion, the present invention  
can be used for the diagnosis of blood perfusion rate and  
the measurement of variation in perfusion rate after an  
operation in a region in which the necrosis of a tissue  
does not appear in actual clinical situations. Furthermore,  
15 the present invention can be used for a blood vessel  
endothelial cell function test by comparing an ICG  
fluorescence dynamics when the ICG is injected in a normal  
blood vessel with an ICG fluorescence dynamics varied by  
drugs inducing activation of blood vessel endothelial cells  
20 or external pressure.

**CLAIMS**

1. An apparatus for measuring a rate of tissue perfusion using time-series analysis of fluorescence images of indocyanine green (ICG).

5           2. The apparatus as set forth in claim 1, comprising:

          1) an input means for receiving signals from a photodetector, 2) a numerical conversion means for processing the input signals into intensities of fluorescence over time in a region of interest, 3) a  
10 perfusion rate calculation means for calculating rates of perfusion for respective regions of a tissue using the numerical values, and 4) an output means for outputting results of the calculation.

          3. The apparatus as set forth in claim 2, wherein  
15 each of the perfusion rates is calculated using dynamics of the intensity of fluorescence of the tissue measured at a specific time.

          4. The apparatus as set forth in claim 3, wherein the perfusion rates are obtained using the following equation:

20           
$$FI_{isc} = \frac{PA}{P-1/\tau} (e^{-\frac{t}{\tau}} - e^{-Pt}),$$

          wherein the  $FI_{isc}$  (Fluorescence Intensity ischemia) is

an intensity of ICG fluorescence of a measurement target tissue, the P is a rate of perfusion, the A is an intensity of fluorescence obtained from a first ICG image, the  $\tau$  is  $t_{1/2}/\ln 2$ , and the  $t_{1/2}$  is a time at which the intensity of ICG fluorescence is half of a highest value.

5. The apparatus as set forth in claim 3, wherein the perfusion rates are calculated using the following equation:

$$- \ln \tau - \frac{T_{\max}}{\tau} = \ln P - PT_{\max} ,$$

10 wherein the P is perfusion rate, and the  $\tau$  is  $t_{1/2}/\ln 2$ .

6. An apparatus for measuring tissue perfusion, comprising a stand configured to allow light to pass therethrough, a light source adjustable to be located to radiate light onto an ICG-injected living body which is disposed on the stand, a filter adjustable to be located to filter only near infrared ray wavelengths of 800 to 850 nm from fluorescence signals emitted from the living body under the action of the light source, a detector configured to detect fluorescence light passed through the filter, and an analysis apparatus operably connected to the detector and configured to image light detected by the detector and

measure the rates of tissue perfusion through the analysis of the ICG dynamics with the passage of time.

7. The apparatus as set forth in claim 6, wherein the light source is a Light Emitting Diode (LED) having a wavelength of 770 +/- 20 nm, a light source in which a filter having a wavelength of 770 +/- 20 nm is attached to a white light source, or a laser light source having a wavelength of 770 +/- 20 nm.

8. The apparatus as set forth in claim 6, wherein the detector is an infrared ray detection camera or a spectrometer.

9. A method of measuring a rate of tissue perfusion, comprising:

1) detecting sequentially concentration of ICG until the concentration of ICG is sufficiently decreased by injecting the ICG into a living body, radiating light onto the living body using a light source, and measuring the intensities of fluorescence, generated in the living body with the passage of time after the injection, using a photodetector;

2) processing the sequential concentration of ICG in the living body detected in the step 2 into numerical data

by converting the intensities of ICG fluorescence into numerical values for respective regions and respective times; and

3) calculating the rates of perfusion from the numerical data.

10. The method as set forth in claim 9, further comprising outputting the calculated perfusion rates as a perfusion map.

11. The method as set forth in claim 10, wherein the perfusion map is constructed by painting particular pixels with respective colors corresponding to the rates of perfusion according to a color gradient map.

12. A method of measuring a rate of tissue perfusion, comprising:

1) detecting sequentially concentration of ICG until the concentration of ICG is sufficiently decreased by injecting the ICG into a living body, radiating light onto the living body using a light source, and measuring intensities of fluorescence, generated in the living body with the passage of time, using a photodetector and obtaining an ICG blood vessel image diagram using the

intensities of fluorescence;

2) acquiring  $T_{\max}$  by analyzing dynamics of the intensities of ICG fluorescence per respective pixel in the ICG blood vessel image diagram; and

3) calculating the rates of perfusion rate based on the  $T_{\max}$ .

5           13. The method as set forth in claim 12, further comprising outputting the calculated perfusion rates as a perfusion map.

10           14. The method as set forth in claim 13, wherein the perfusion map is constructed by painting particular pixels with respective colors corresponding to the rates of perfusion according to a color gradient map.

15           15. A method of predicting a rate of tissue necrosis, comprising representing a tissue necrosis probability map, indicating the probability of necrosis of a tissue, based on the rates of tissue perfusion acquired using the the method set forth in claims 9 or 12.

16. The method as set forth in claims 9 or 12, wherein the perfusion rates are calculated using a time at which the intensity of ICG fluorescence is highest,

obtained using a fluorescence mechanism pattern in a measurement target tissue.

17. The method as set forth in claim 16, wherein the perfusion rates are calculated using the following equation:  
5

$$- \ln \tau - \frac{T_{\max}}{\tau} = \ln P - PT_{\max} ,$$

wherein the P is a perfusion rate, and the  $\tau$  is  $t_{1/2}/\ln 2$ .

18. The method as set forth in claims 10 or 13,  
10 wherein the perfusion map is constructed using a method of acquiring perfusion rates of region of interest (ROI) according to (a) average data of pixels of particular regions, (b) respective pixels, or (c) respective ROIs representing perfusion rates; and representing the  
15 perfusion rates using a perfusion map by illustrating the perfusion rates for the ROI with corresponding colors designated according to the perfusion rates.

DRAWINGS

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FIG. 1

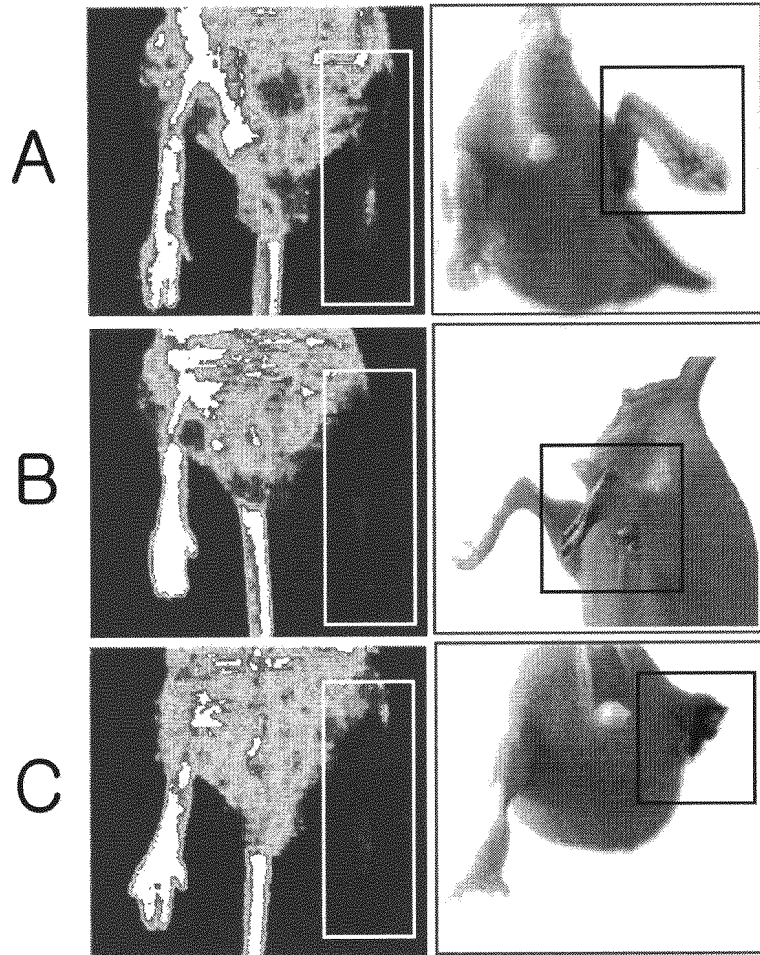
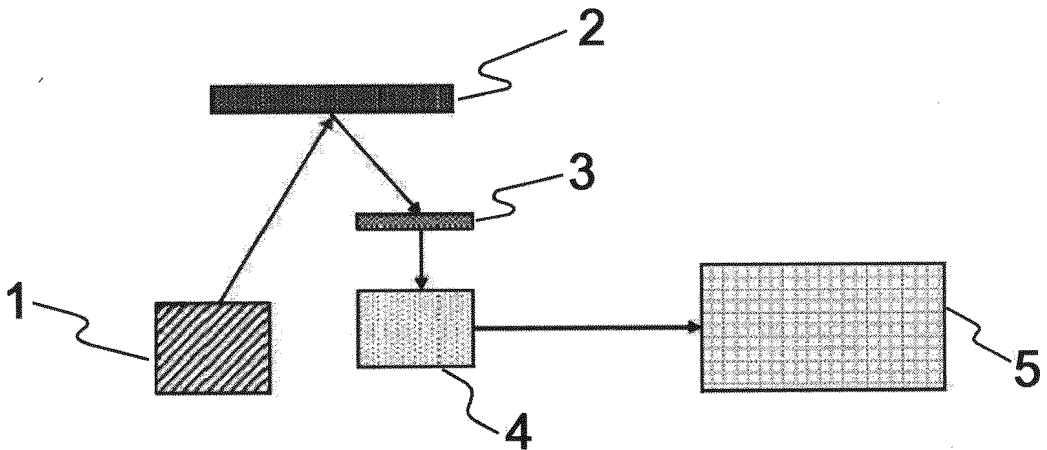


FIG. 2



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FIG. 3

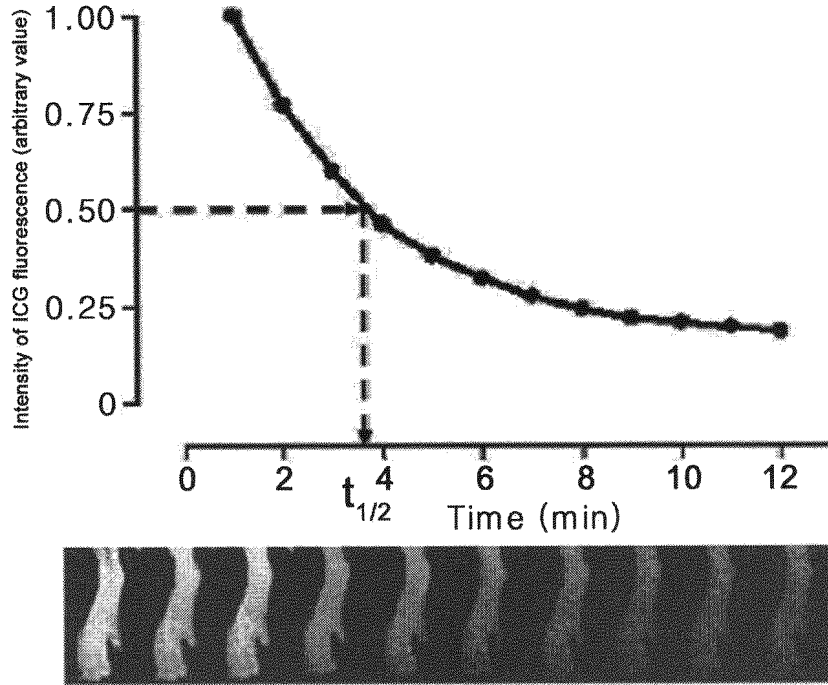
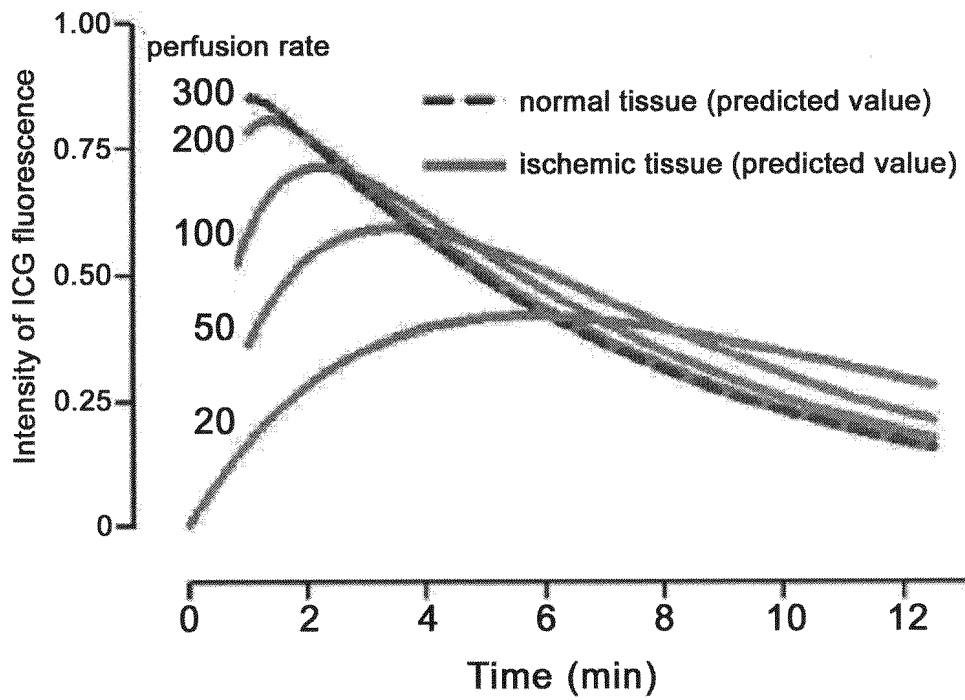


FIG. 4



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FIG. 5

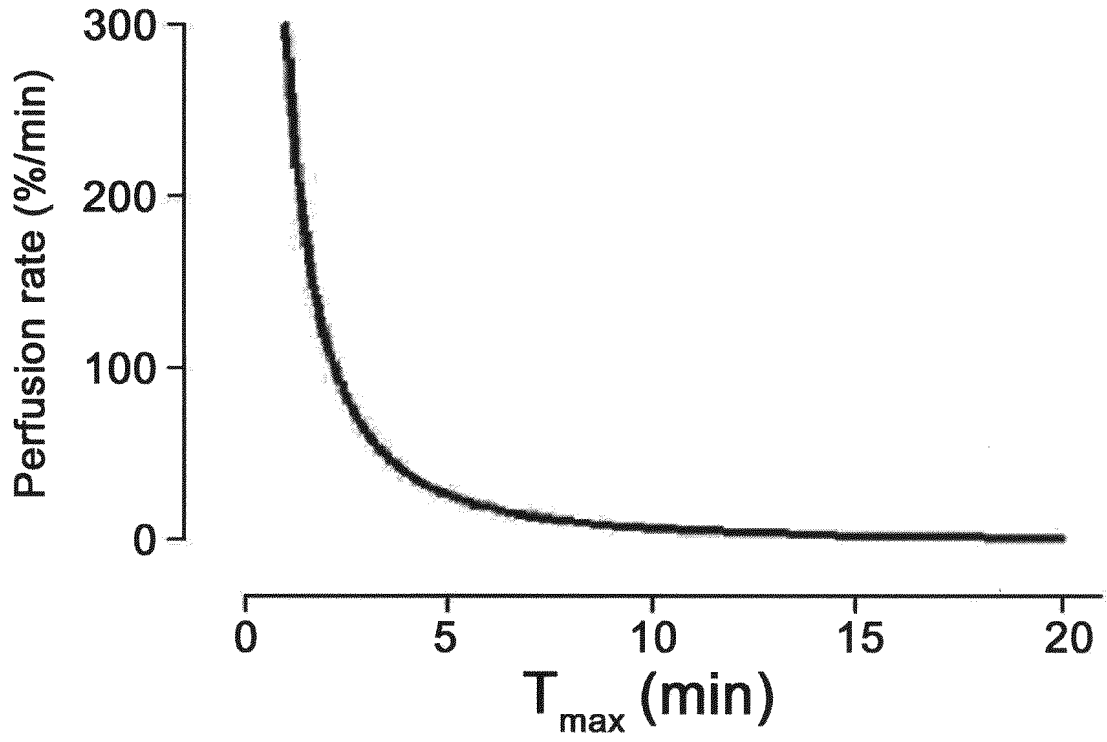
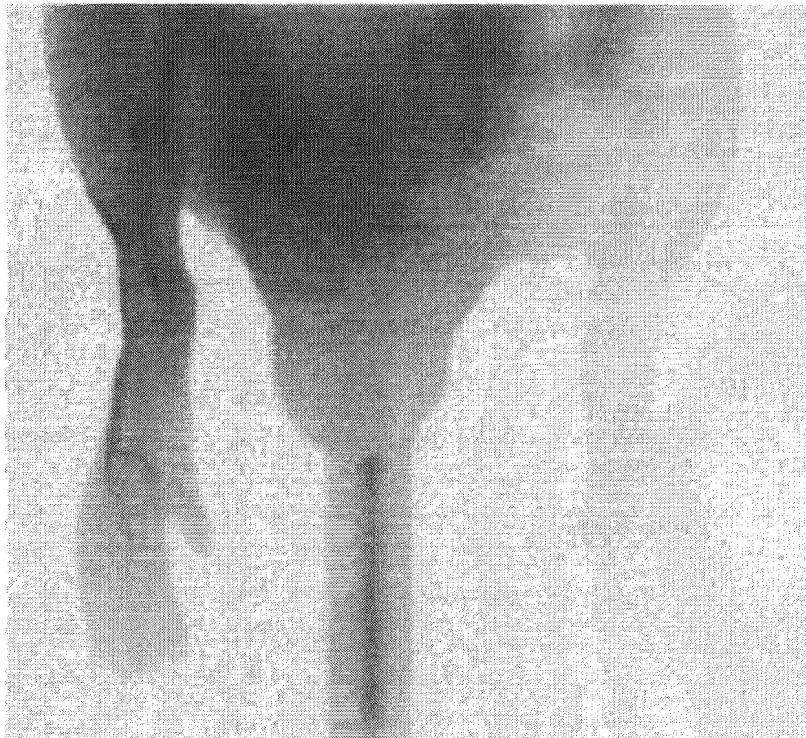


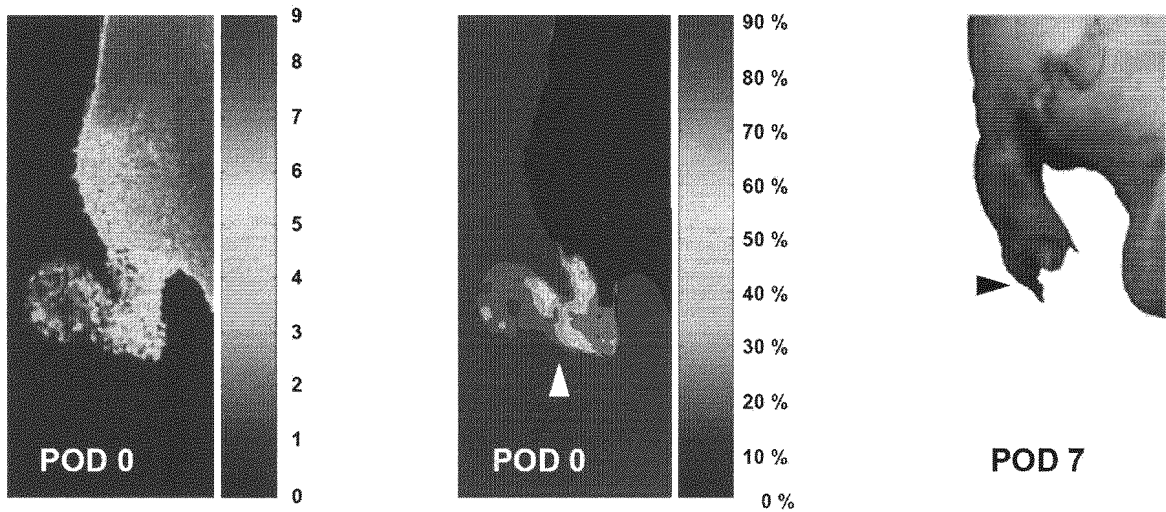
FIG. 6



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FIG. 7



FIG. 8



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FIG. 9

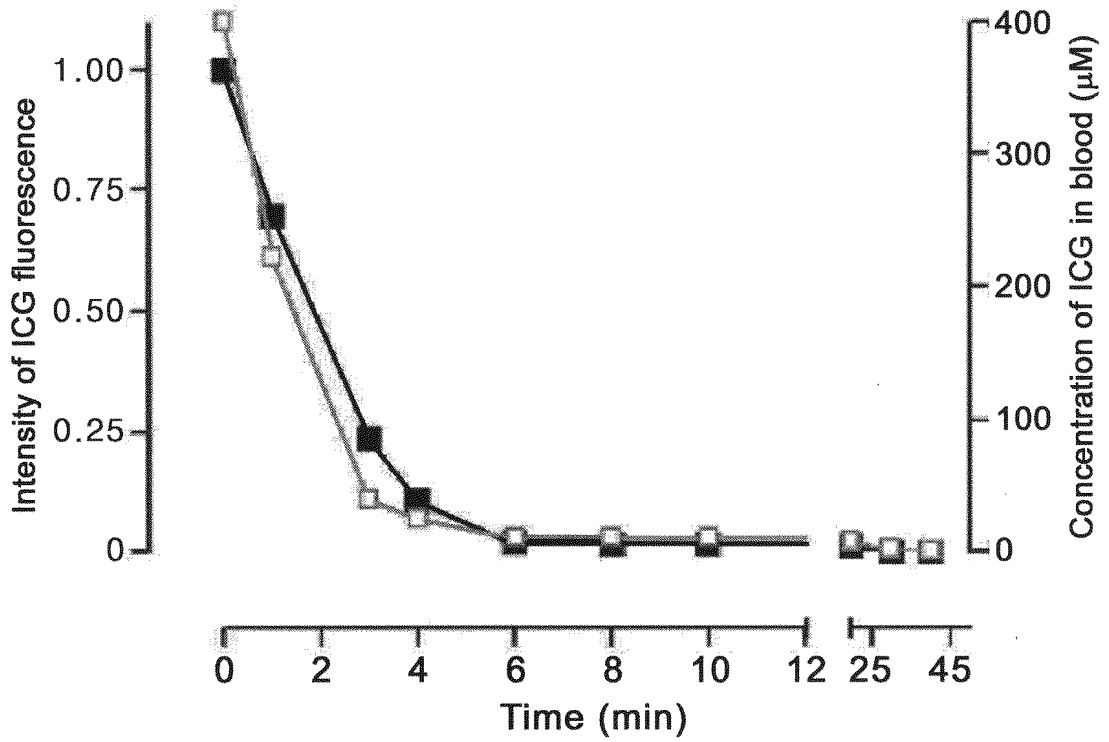
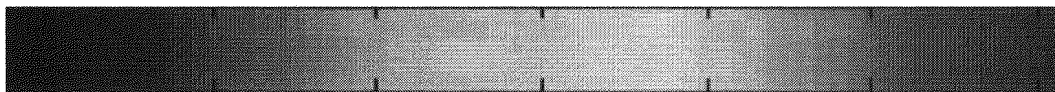


FIG. 10

**Perfusion Rate (%/min)**

**<3      7      20      45      130      300      700>**



**A. CLASSIFICATION OF SUBJECT MATTER***A61B 6/00(2006.01)i*

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 8 : A61B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean Utility models and applications for Utility models since 1975

Japanese Utility models and applications for Utility models since 1975

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKIPASS (KIPO internal) "perfusion", "tissue"

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	WO 01/22870 A1 (DOCHERTY, J. et al.) 05 April 2001 See claims and figure 1	1 2-18
A	WO 00/00080 A1 (EGOZI, N) 06 January 2000 See abstract and figure 2	1-18
A	US6542769 B2 (SCHWAMM, L. H.; SORENSEN A. G.) 01 April 2003 See abstract and figure 4	1-18

 Further documents are listed in the continuation of Box C. See patent family annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&amp;" document member of the same patent family

Date of the actual completion of the international search

23 OCTOBER 2007 (23.10.2007)

Date of mailing of the international search report

**24 OCTOBER 2007 (24.10.2007)**

Name and mailing address of the ISA/KR

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Facsimile No. 82-42-472-7140

Authorized officer

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Telephone No. 82-42-481-8458



**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International application No.

**PCT/KR2007/003355**

Patent document cited in search report	Publication date	Patent family member(s)	Publication date		
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US6542769 B2	01.04.2003	NONE			

专利名称(译)	使用血液中吲哚菁绿浓度分析组织灌注的系统		
公开(公告)号	<a href="#">EP2073706A4</a>	公开(公告)日	2010-07-28
申请号	EP2007768689	申请日	2007-07-11
[标]申请(专利权)人(译)	韩国科学技术院		
申请(专利权)人(译)	韩国高等学院科技		
当前申请(专利权)人(译)	韩国高等学院科技		
[标]发明人	CHOI CHULHEE KANG YUJUNG CHOI MYUNGHWAN		
发明人	CHOI, CHULHEE KANG, YUJUNG CHOI, MYUNGHWAN		
IPC分类号	A61B6/00 A61B5/00		
CPC分类号	A61B5/0261 A61B5/0059 A61B5/0275		
优先权	1020060099033 2006-10-11 KR		
其他公开文献	EP2073706A1 EP2073706B1		
外部链接	<a href="#">Espacenet</a>		

#### 摘要(译)

本发明一般涉及使用吲哚菁绿浓度分析组织灌注的系统和使用该系统测量灌注速率的方法，更具体地说，涉及通过将吲哚菁绿注入吲哚菁绿来测量组织灌注的系统。生物体，随着时间的推移检测吲哚菁绿浓度的变化，并分析检测到的变化，以及使用该系统测量灌注速率的方法。本发明提供了一种测量活体内灌注的方法，该方法能够在从灌注速率降低到小于正常灌注的10%到灌注速率增加到大于正常灌注的宽范围内对各个区域进行精确测量。上述ICG在生物体内的机制，不能用传统技术进行。