(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) **EP 2 082 223 B1**

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
20.02.2019 Bulletin 2019/08

(21) Application number: **07824529.7**

(22) Date of filing: **09.11.2007**

(51) Int Cl.:
G01N 21/21 *(2006.01)* ***G01N 33/487*** *(2006.01)*
A61B 5/00 *(2006.01)* ***A61B 5/1455*** *(2006.01)*
A61B 5/05 *(2006.01)* ***G01N 21/17*** *(2006.01)*

(86) International application number:
PCT/GB2007/004300

(87) International publication number:
WO 2008/056171 (15.05.2008 Gazette 2008/20)

(54) DEVICES AND METHODS FOR DETECTING HAEMATIN, HAEMOZOIN AND RELATED IRON CONTAINING COMPOUNDS

VORRICHTUNGEN UND VERFAHREN ZUM NACHWEIS VON HÄMATIN, HÄMOZOIN UND VERWANDTE EISEN-ENTHALTENDE VERBINDUNGEN

DISPOSITIFS ET PROCÉDÉS DE DÉTECTION DE LA -HÉMATINE ET DE L'HÉMOZOÏNE

(84) Designated Contracting States:
AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IS IT LI LT LU LV MC MT NL PL PT RO SE SI SK TR

(30) Priority: **10.11.2006 GB 0622450**

(43) Date of publication of application:
29.07.2009 Bulletin 2009/31

(60) Divisional application:
18214815.5

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Description

[0001] This invention relates to devices and methods for the detection of β-haematin and haemozoin, with particular, but by no means exclusive, reference to the detection of malaria.

[0002] Malaria remains a major health problem in many parts of the world. In regions where it is endemic, effective treatment and eradication is often compromised by lack of access to rapid, accurate and affordable diagnosis since, unfortunately, the best diagnostic tools currently available require a laboratory environment. Even in Europe the number of cases and fatalities increases year on year reflecting the increasing preference of Europeans to holiday in malarial prevalent areas coupled with a reluctance to take anti-malarial prophylaxes. Native Europeans have no immunity so that without early diagnosis infection can, and often does, have rapidly fatal consequences. This situation is moreover only likely to worsen as global warming is now predicted to facilitate the spread of malaria to areas previously free of the disease including southern Europe.

[0003] Examination by high-power microscopy, typically of 100 fields of Giemsa-stained thick blood smears, is still generally regarded as the so called "gold standard" for malarial diagnosis. Dominant for more than a century, this technique can in principle attain a sensitivity of 5 to 20 parasite infected cells per μl of blood. It is however time consuming and in reality subject to significant variability in its application, particularly in respect of the number of fields examined and the methodology employed to determine parasitaemia from parasite counts within the fields examined. Coupled with its dependence on the skill base of highly trained microscopists, the sensitivity discussed above is consequently rarely obtained outside specialist laboratories. Recognition of this and of the need for more rapid diagnosis has over the last decade or so driven the study and development of several alternative techniques. Fluorescent microscopy, laser desorption mass spectrometry and techniques involving PCR amplification to detect and identify nucleic acid sequences are currently acknowledged as the most sensitive and specific techniques available. However, in general this emerging generation of diagnostic procedures remains time consuming and again too costly and complex for dissemination beyond specialist laboratories. For field application rapid detection tests (RDTs), in the form of sensor sticks or wands, are now available from a variety of manufacturers. These employ immuno-chromatographic methods to detect malarial antigens such as the histidine-rich protein II (HRP-II) and/or parasite lactate dehydrogenase (pLDH) which are present in peripheral blood during infection. These tests generate results within 15 minutes and require only minimal operator training. However, they are relatively expensive, non-quantitative and have a limited usefulness in detecting low level parasitaemia (< 100 parasites/μl). There is still a pressing need for new diagnostic techniques affording rapid yet simpler to operate instrumentation. Furthermore, there is a need for such a technique which is quantitative. Furtherstill, there is a need for a device for detecting malaria which is suitable for field use or first point symptom presentation.

[0004] US 2006/025659 and JP 55 024614 describe devices which use the Faraday Effect to make measurements of biological analytes and materials such as glucose, haemoglobin, myoglobin, human serum and blood corpuscle. WO 03/029790 discloses an apparatus for measuring optical activity which uses the Faraday Effect to impart a modulation on the polarisation state of the interrogating beam. WO 02/16910 describes an absorption spectrometer for making measurements in biological media wherein the Faraday Effect is used to reduce the effects of scattering. Balasubramanian et al (Science, vol. 223, No. 4638, 1984, pp 828 - 830) discloses the use of photoacoustic spectroscophy to monitor the malaria parasites Plasmodium chabaudi and Plasmodium berghei, their pigment, and ferriprotoporphyrin IX. Nalbandian et al (American Journal of Clinical Pathology, vol. 103, No. 1, 1995, pp 57 - 64) discloses a test for malaria wherein a magnetic field is used to concentrate infected erythrocytes, followed by detection using a polarising microscope. The detection step, which is not performed in the presence of a magnetic field, exploits the birefringent properties of haemozoin.

[0005] The present invention, in at least some of its embodiments, addresses the above described problems and needs.

[0006] According to a first aspect of the invention there is provided a method of detecting the presence of an analyte in a sample, in which the analyte is haemozoin, β-haematin or an analogous iron containing compound, the method including the steps of:

providing the sample;
applying a magnetic field across at least a portion of the sample to orient the analyte along the direction of the applied magnetic field;
detecting a physical property of the sample by introducing polarised electromagnetic radiation into the sample and utilising a magneto-optical spectroscopic detection technique to detect a physical property of the analyte which varies in dependence with the orientation of the analyte with the square of the applied magnetic field; and
correlating the detected physical property with the presence of the analyte.

[0007] The term analogous iron containing compound' is understood to mean a compound having substantially identical spectroscopic, magnetic and crystallographic properties and characteristics to haemozoin and β-haematin.

[0008] Preferably the physical property is a property associated with the absorption of the electromagnetic radiation. The electromagnetic radiation may be in the range 400 to 700 nm. Preferably the electromagnetic radiation is greater

than 600 nm and most preferably is in the range 640 to 680 nm. It may be possible to utilise electromagnetic radiation of other wavelengths. For example, ultraviolet radiation or near infra-red radiation, especially in the 700-900nm region, might
be used.

[0009] The polarisation state of the electromagnetic radiation may be varied, and the presence of the analyte detected by detecting a dependence of the detected physical property on the polarisation state.

[0010] In preferred embodiments the transmission of the electromagnetic radiation through the sample or the generation of photo-acoustic energy is detected.

[0011] The detected physical property may be associated with a non-linear wave mixing process. In particular, radiation produced by second harmonic generation may be detected.

[0012] The detected physical property may be the generation of surface plasmons, said generation being augmented by the presence of the analyte and varying in dependence with the applied magnetic field.

[0013] Alternatively, the detected physical property may be the specific heat of the sample, the thermal conductivity of the sample, or a property associated with the transmission of ultrasound through the sample, such as the intensity of ultrasound transmission through the sample.

[0014] In some embodiments the magnitude and/or the direction of the applied magnetic field is varied, and the presence of the analyte is detected by detecting a dependence of the detected physical property on the variation of the applied magnetic field. For the avoidance of doubt, said variation of the applied magnetic field includes embodiments in which measurements are made with and without an applied magnetic field.

[0015] Phase sensitive detection of the physical property may be performed using modulation of the applied magnetic field and/or the electromagnetic radiation introduced into the sample.

[0016] According to a second aspect of the invention there is provided a device for detecting the presence of an analyte in a sample, in which the analyte is haemozoin, β-haematin or an analogous iron containing compound, the device including:

magnetic field applying means for applying a magnetic field across at least a portion of the sample to orient haemozoin along the direction of the applied magnetic field;
detection means adapted for detecting a physical property of the sample by introducing polarised electromagnetic radiation into the sample and utilising a magneto-optical spectroscopic detection technique to detect a physical property of the analyte which varies in dependence with the orientation of the analyte with the square of the applied magnetic field; and
correlation means for correlating the detected physical property with the presence of the analyte.

[0017] The magnetic field applying means may include a permanent magnet.

[0018] The magnetic field applying means may be controllable so as to vary the magnitude and/or the direction of the applied magnetic field. The magnetic field applying means may be an electromagnet.

[0019] The magnetic field applying means may include micro-magnetic elements. In general, the micro-magnetic elements are of dimensions comparable to the crystal length of the analyte, typically around 1-2 μm. The micro-magnetic elements may comprise pole pieces of a suitable material, such as soft nickel or NiFe. Typically the micro-magnetic elements are embedded in a substrate formed from an optically inert substrate which does not exhibit birefringence or dichroism.

[0020] The detection means may include means for providing electromagnetic radiation and sensing means for detecting a physical property associated with the absorption of the electromagnetic radiation by the analyte. The means for providing electromagnetic radiation provides polarised electromagnetic radiation. The wavelength of the electromagnetic radiation may be in the range 400 to 700 nm, preferably greater than 600 nm and most preferably in the range 640 to 680 nm.

[0021] The sensing means may be an acoustic detector.

[0022] The sensing means may be an electromagnetic radiation detector for detecting electromagnetic radiation transmitted through the sample.

[0023] The detection means may include means for varying the intensity of the electromagnetic radiation and means for detecting a dependence of the physical property on the variation of said intensity.

[0024] The presence of the analyte may be detected by varying the magnitude and/or the direction of the applied magnetic field.

[0025] Phase sensitive detection means may be employed.

[0026] The polarisation state of the polarised electromagnetic radiation may be controllably variable. In these embodiments the presence of the analyte may be detected by varying the polarisation state of the polarised electromagnetic radiation and observing a polarisation state dependence in the detected physical property. The means for providing electromagnetic radiation may provide pulsed, polarised electromagnetic radiation, and the sensing means may include

means for separating ballistic and quasi-ballistic photons from diffuse photons. A gating system may be used for this purpose. In this way the effects depolarisation occurring within the patient's tissue can be countered.

[0027] The means for providing polarised electromagnetic radiation may include at least one laser. The means for providing polarised electromagnetic radiation may include at least a pair of lasers, in which the first laser in the pair produces a first beam of electromagnetic radiation and the second laser in the pair produces a second beam of electromagnetic radiation, and wherein the first and second beams have differing, preferably orthogonal, polarisation states when introduced into the sample.

[0028] The first and second beams may be combined using an optical element so that the beams are co-linear when introduced into the sample.

[0029] The first and second beams of electromagnetic radiation may be intensity modulated 180° out of phase.

[0030] Preferably, each laser in the pair has an electronic driving arrangement which controls the production of the respective beams of electromagnetic radiation, and the device further includes control means for controlling the electronic driving arrangements so that the first and second beams of electromagnetic radiation are intensity modulated 180° out of phase.

[0031] The device may be configured so that the electromagnetic radiation propagates into the sample along an axis, and the magnetic field applying means applies a magnetic field which is aligned in parallel with said axis of propagation.

[0032] Alternatively, the device may be configured so that the electromagnetic radiation propagates into the sample along an axis, and the magnetic field applying means applies a magnetic field which is aligned orthogonal to said axis of propagation.

[0033] In preferred embodiments, the device is attachable to peripheral tissue of a patient, preferably an ear lobe or a finger, and means for detecting the presence of haemozoin comprising the magnetic field applying means and the detection means performs in vivo detection of the presence of haemozoin in the bloodstream of the patient.

[0034] A device of the invention may include a device for producing a beam of polarisation modulated electromagnetic radiation including a first laser producing a first beam of electromagnetic radiation, a second laser producing a second beam of electromagnetic radiation, a first polarising arrangement for polarising the first beam of electromagnetic radiation, a second polarising arrangement for polarising the second beam of electromagnetic radiation, in which the first and second polarising arrangements are configured so that the first and second beams are polarised with orthogonal polarisation states, an optical element for combining the polarised beams so that said beams are co-linear, and intensity modulation means for intensity modulating the intensities of the first and second beams.

[0035] This arrangement has been found to be with a highly advantageous way of producing an intensity modulated beam of orthogonal polarisation states. The device can be applied advantageously to the detection techniques discussed herein. However, the device may be usefully applied to other investigative techniques or experiments which require a source of electromagnetic radiation which alternates between orthogonal polarisation states.

[0036] Preferably, the lasers are semiconductor lasers.

[0037] Preferably, the first and second beams of electromagnetic radiation are intensity modulated 180° out of phase. Advantageously, the first and second lasers each have an electronic driving arrangement which controls the production of its respective beam of electromagnetic radiation, and the device further includes control means for controlling the electronic driving arrangements so that the first and second beams of electromagnetic radiation are intensity modulated 180° out of phase. The control means may include a digital clock producing a digital clock signal, and means for converting the digital clock signal into two out of phase digital clock signals such that each laser is alternately switched between two intensity levels, the switching of the first laser being 180° out of phase with the switching of the second laser.

[0038] The optical element for combining the beams may be a polarising beams splitter.

[0039] At lease one of the first and second beams may be directed by a polarising beam splitter to the optical element which combines the beams.

[0040] Prior to being combined by the optical element, each of the first and second beams may be polarised using separate Glan Taylor polarisers.

[0041] At least one of the polarising arrangements may include an adjustment stage for controllably adjusting the polarisation state of the first or second beam. The adjustment stage may be a rotary stage which controllably rotates at least one element of a polarising arrangement.

[0042] Whilst the invention has been described above, it extends to any inventive combination or sub-combination of the features set out above or in the following description or drawings or claims.

[0043] Embodiments of methods and devices in accordance with the invention will now be described with reference to the accompanying drawings, in which:-

Figure 1 shows (a) a first arrangement and (b) a second arrangement of a device of the invention;
Figure 2 shows observed signals of a function of applied magnetic fields;
Figure 3 shows differential absorptive signal against the concentration of β-haematin in blood;
Figure 4 shows the absorption spectrum of whole blood;

Figure 5 shows (a) the results of further experiments measuring differential transmittance against the concentration of β - haematin in blood and (b) the results of experiments measuring differential transmittance against the concentration of haemazoin in blood;

Figure 6 is a plan view of a device for producing a polarisation modulated laser beam;

Figure 7 shows the results of experiments measuring differential transmittance against the concentration of live parasitized red blood cells in suspension; and

Figure 8 shows device for making in vivo measurements of malaria infection which are attached to (a) an ear lobe and (b) a finger tip;

[0044] Although the present invention is applicable to the detection of haemozoin and β-haematin per se, an important aspect of the invention relates to the detection of the malarial parasite. The malarial parasite enters the red blood cells (erythrocytes) and converts the haemoglobin to haemozoin, which changes the magnetic state in the process. The erythrocytes subsequently burst, releasing haemozoin into the plasma, whilst the malarial parasite goes on to infect further erythrocytes. The present inventors have realised that it may be possible to detect the malarial parasite by detecting the presence of haemozoin in the blood.

[0045] In particularly preferred embodiments, the change in the magnetic state of the haemoglobin caused by the malarial infection is exploited by detecting suitable properties of haemozoin which are dependent on the application of a magnetic field.

[0046] In one preferred embodiment, photo-acoustic detection is used. Advantages associated with this approach are the possibility of making in vivo measurements, and the avoidance of problems of optical scattering associated with conventional optical measurements on turbid liquids such as whole blood. Figure 1 shows apparatus, shown generally at 10, for performing magneto-optical detection using photo-acoustic techniques. The apparatus 10 comprises a light source 12, producing a beam of optical radiation 14 which passes through a polariser 16, a variable LC retarder (0 or 180° retardance) 18, and a chopper 20, before impinging on a sample 22 held in a sample holder 24. The sample is in direct contact with an acoustic detector 26. The apparatus 10 further comprises an electromagnet 28, and a Gauss meter 30 can be utilised to measure the applied magnetic field strength. In the arrangement shown in Figure 1a, the electromagnet 28 is arranged orthogonal to the axis of the beam 14. In the arrangement shown in Figure 1b, the electromagnet 28 is arranged parallel to the axis of the beam 14, and a bore 32 is provided in the electromagnet 28 allowing the beam to travel to the sample 22. It will be apparent to the skilled reader that in the arrangement shown in Figure 1a the applied magnetic field is orthogonal to the axis of the beam 14, whereas in Figure 1b, the applied magnetic field is parallel to the axis of the beam 14. Typically the light source 12 is a laser of any suitable type, and in one set of experiments a semiconductor laser operating at 650 nm is utilised. Experiments were performed using laser light which is polarised in the plane of the paper of Figure 1 (henceforth termed p polarisation) and laser radiation polarised out of the plane of the paper (henceforth termed s polarisation). Experiments were performed on solutions of the haemozoin analogue β-haematin. With the arrangement shown in Figure 1a, i.e., with the magnetic field applied parallel to the plane of the sample 22, the recorded signal follows an M^2 relationship with the field. The signal decreases upon applied field for p polarisation and increases for s polarisation. When the arrangement shown in Figure 1b is employed, i.e., when the field is applied perpendicular to the plane of the sample 22, the signal is also M^2 in nature but increases with magnetic field irrespective of the polarisation state. Figure 2 shows a signal obtained using the arrangement shown in Figure 1a and laser radiation having p polarisation in an experiment in which the magnetic field was ramped from 0 to 14 kOe and then ramped down to 0 kOe. It can be seen that the signal decreases with increasing field strength.

[0047] Without wishing to be bound or otherwise limited by any particular theory, it is believed that the results discussed above can be explained as follows. Crystals of β-haematin are rod shaped. On excitation with lineally polarised radiation, the crystals of β-haematin absorb when the electric vector of the radiation is along the axis of the rod. In suspension in blood or other fluid the axes of the β-haematin crystals are randomly orientated and so the suspension expresses no preferred direction absorption on interrogation using linearly polarised radiation. However, on application of a magnetic field the crystals behave simply as weak bar magnets experiencing a torque that seeks to orient them along the applied field direction. This is opposed by the thermal energy of their environment that constantly acts to randomise the assembly. The experiments show that the crystals can be held in close alignment on application of fields around 14 kOe. In the arrangement shown in Figure 1a, differential absorptive signals which are characteristic of the presence of β-haematin can be obtained by switching the polarisation state of the optical radiation from parallel to the applied field direction to orthogonal to the applied field direction. Alternatively, the same measurement can be made by reversing the magnetic field along a direction orthogonal to the polarisation direction of the light wave. It will be apparent to the skilled reader that the arrangement shown in Figure 1b is not suitable for this differential absorption detection technique, since p and s polarised light produced the same results. However, it is possible to detect β-haematin using the Figure 1b arrangement by varying the magnitude of the magnetic field whilst interrogating the sample with linearly polarised radiation, and monitoring the dependence of the signal on the magnetic field. This approach can also be utilised with the Figure 1a arrangement.

[0048] Experiments have been performed interrogating samples of β-haematin in human blood. The Figure 1a arrangement was utilised and the difference between the signals obtained with p and s polarised light were investigated, i.e., a differential absorptive signal was obtained. Figure 3 shows a differential absorptive signal (designated as $\Delta PA/I_0$) against the β-haematin concentration in blood. As can be seen in Figure 3, it has been established that the presence of β-haematin in blood is readily detectable at levels at less than 10μgm/ml. This is broadly equivalent to a malarial infection or parasitemia level of 0.3%. However, it should be noted that at this concentration both the signal level and the signal noise ratio are such that previous experience allows confident expectation that the detectivity of the technique can be extended down to parasitemia levels of 0.05% or more. The limitation to calibration of parasitemia levels significantly below 0.3% is merely constrained by the difficulty of creating 0.1 ml samples containing β-haematin uniformly dispersed at concentrations less than 10μgm/ml.

[0049] Experiments were performed on β-haematin because it is a close analogue of haemozoin. For example, electron micrographs of β-haematin and haemozoin reveal a near identical rod-like crystal morphology. Furthermore, S.Pagola et al, Nature 404, 2000 and references therein state that" β-haematin is chemically, spectroscopically and crystallographically identical to haemozoin". It is thus envisaged that results obtained using β-haematin should be transposable to measurements on harvested malarial blood containing haemozoin.

[0050] Although the photo-acoustic experiments discussed above were performed at 650 mn, light of other wavelengths may be utilised. In particular, it may be possible to utilise light of shorter wavelengths, for example in the blue/green region of spectrum.

[0051] In another preferred embodiment, it has been found that it possible to detect β-haematin in blood using optical transmission detection. Figure 4 shows the absorption spectrum for whole blood. It can be seen that above 600 nm blood absorbs much less strongly. The present inventors have realised that β-haematin absorbs significantly above 600 nm, and in fact a peak is seen in the absorption spectrum of β-haematin at 659 nm. Furthermore, it appears that scattering from whole blood at wavelengths above 600 nm is at least a manageable concern. Thus, the detection of β-haematin can be accomplished using arrangements which are very similar to the arrangements shown in Figures 1a and 1b, with the exception that the photo-acoustic detector is replaced with an optical detector such as a silicon photo detector. Experiments were performed at 650 nm using a semiconductor laser light source to interrogate β-haematin in blood, and very similar results to those shown in Figure 3 were obtained using the differential absorptive signal approach to look at the differences between optical transmission using p and s polarised light.

[0052] β-haematin in blood was interrogated also with a very similar experimental set-up in which 660 nm light from a semiconductor laser is used in conjunction with a photodetector to measure transmission through blood samples. A 1T in plane magnetic field was utilised. The results are presented in Figure 5, which demonstrate that measurable differential signals are observed in connection with β-haematin concentrations down to the 1 μg/ml level or lower.

[0053] It was originally envisaged that late stage malarial infection (in which the erythrocytes have broken down, releasing free haemozoin into the plasma) can be readily detected using the techniques discussed above. Early stage infection, in which the haemozoin is still confined within the erythrocytes, was originally envisaged to be detectable, although it was not originally clear whether haemozoin which is still confined in the erythrocytes is fully mobile and free to rotate. It is possible that the presence of the erythrocytes will slow down the alignment of the haemazoin with the magnetic field, in which case a different response might be obtained that are obtained with free haemozoin in blood plasma. Alternating magnetic fields might be utilised in order to identify early stage infection, and to separate and independently evaluate the response of haemozoin in the plasma from that of haemozoin in the erythrocytes. For example, the response may be characteristically dependent on the frequency of the alternating magnetic field. These considerations are principally of relevance to in vivo detection. With ex vivo detection, for example using a device that analyses a pinprick of blood, it is possible to lyaise the red cells, for example by adding a small amount of detergent to the sample or for injecting the cells to a freeze thaw cycle.

[0054] Further experiments have confirmed that both later and early stage malarial injection can be detected.

[0055] In one set of experiments, fresh blood was doped with varying concentrates of haemazoin in the form of mature trophozoite stage parasitized red blood cells (PRBCs) grown in culture and lysed by freezing and thawing before diluting with the whole fresh blood. The samples were analysed using the experimental set-up employed to obtain the data shown in Figure 5a, ie, the fractional change in transmittance at 660 nm was measured. The results of the measurements on blood doped with haemazoin are shown in Figure 5b.

[0056] The linearity of the plots shown in Figures 5a and 5b is striking and continues unshown out to concentrations beyond at least 100μg/ml. Note also how the data point (◘), obtained with cells in which the infection is at the early ring stage of hemozoin formation when the crystals are believed to be much smaller, fits closely to the linear trend. The difference in the gradients of the two plots may be a consequence of at least two factors. Firstly, haemozoin crystals *in situ* exhibit a tendency to clump, which would reduce the dichroism when compared with an equivalent number of individual crystals, such as is the case with β-haematin. Secondly, cultured cells are supplied frozen to ensure all cells are at the same point in their infection cycle and to facilitate safe handling. Freezing however appears to only partially release the haemozoin so that cellular debris continues to adhere to the crystals, altering the dynamical forces acting

upon them and possibly constraining the rotation achieved in a given applied field. Adherence of diamagnetic cellular material to the crystals might for example result in a small torque opposing that seeking to orientate the crystals with the field. Alternatively, adhering cellular material might simply be providing a larger interaction cross-section for the thermal restoring mechanism. Plots obtained in the same way as Figure 5a for blood doped in this manner have exactly the same form but with reduced dichroism. Similarly, the rate of response of the dichroic signal to changes in the applied field is also observed to be reduced. It may be preferable in practice to use a detergent to completely release the haemozoin in order to restore the dichroic signal strength to that exhibited by an equivalent β-haematin concentration. The effectiveness of this procedure has been tested by extracting the haemozoin from two samples of parasitized red blood cells (PRBCs) used in producing Figure 5b. This is cleaned before reintroducing it at the same concentration into whole fresh blood. The fractional intensity change for these samples is plotted as (◊) in Figure 5a. It can be seen that the haemozoin samples treated is this way provide results which are in very close agreement with the β-haematin results.

[0057] Correlation between the results shown in Figures 5a and 5b and malarial parasitaemia is made initially by assuming whole blood contains $5x10^9$ RBCs/ml and that in mature parasitized cells conversion of 50% of the haemoglobin yields ≈ 0.6pg haemozoin per cell. On this basis, detecting 100 PRBCs/μl (0.002% parasitaemia), for example, requires the measurement of haemozoin concentrations of ≈ 0.06μg/ml. The lowest concentration actually measured to date is 0.1μg/ml but as shown by the linear separation of the lower data points in Figure 5, the resolution of the instrumentation is actually somewhat better than 0.05μg/ml. Ultimately, the sensitivity achievable will for the most part likely be determined by the degree to which the orthogonal polarisation states used to interrogate the induced dichroism are depolarised by scattering from cellular structures and other material during their passage through the blood sample prior to interacting with haemozoin crystals. Loss of polarisation after this point is unimportant since only the resulting intensity modulation is detected.

[0058] In a confidential clinical study, the technique of the present invention was used to analyse blood samples from thirteen patients. The blood samples were all lysed and thus haemazoin originally present in red blood cells was free at the time of the measurements. The apparatus used to obtain the data shown in Figure 5 was used to analyse the clinical blood samples. It is noted that the present invention provided a quantitative value which is related to the haemazoin load which is in turn related to the progression of the malarial parasitaemia. The clinical blood samples were also analysed using Rapid Diagnostic Tests (RDT) supplied by Orchid Biomedical Systems and Parascreen from Zephyr Biomedicals. The results are shown in Table 1, which indicates that the clinical study is 100% successful. It is considered particularly encouraging that sample #1 was correctly given a positive identification for malaria by the present invention, because in this case the infectious species was found to be Plasmodium Ovalis, which is known to be more difficult to detect.

Table 1. Results of clinical study. P = Positive diagnosis N= negative diagnosis

ID #	Patient Description	RDT diagnosis	Condition	Present Invention	ΔI/I
A	Nigerian child	P	Plasm. Falc.	P	8.2E-5
B	Nigerian child	P	Plasm. Falc.	P	4.7E-5
C	Nigerian child	P	Plasm. Falc.	P	7.8E-5
D	Nigerian child	P	Plasm. Falc.	P	9.5E-5
E	Nigerian child	P	Plasm. Falc.	P	5.8E-5
1	Netherlands Returned traveler	P	Plasm. Oval.	P	4.4E-5
2	N/A	P	Plasm. Falc.	P	5.9E-3
3	Tanzanian	N	Sickle cell anemia	N	-
4	Tanzanian	N	Beta-Thalassemia	N	-
5	Tanzanian	N	Genetic Disorder	N	-
6	N/A	N post dil.	-	N	-
7	N/A	inconclusive	-	N	-
8	Netherlands patient	-	N/A	N	-

[0059] In a further study, the fractional change in transmitted intensity was measured for suspensions of live parasitzed red blood cells grown in culture. The cell concentration in suspension was varied and for each suspension the haemazoin concentration of haemazoin was established by spectroscopy. In those experiments there was no free haemazoin external to the living cells. The instruments utilised to obtain the results shown in Figure 5 was used to analyse the

suspensions of live parasitical red blood cells, i.e., a photodetector was used to measure transmittance.

[0060] The results shown in Figure 7 demonstrate conclusively that at least some of the haemozoin within the living cell vacuoles is free to respond to orientation by an applied magnetic field.

[0061] A device for in vivo measurement of malarial infection in a patient can be constructed in which measurements of peripheral tissue are made. Preferred location for making in vivo measurements are the ear lobe, a finger and a toe, although other peripheral tissue such as webbing between fingers and toes might be contemplated. It is advantageous that the testing provided by such devices is non-invasive. A device of the type generally shown in Figure 1a, in which the magnetic field is aligned orthogonal to the incident beam and in the plane of the ear lobe, would be most preferred from the point of view of sensitivity. However, there may be merit in using the configuration shown generally in Figure 1b, in which the magnetic field is aligned parallel to the incident beam and orthogonal to the plane of the ear lobe. In this configuration it is relatively easy to position the magnetic field production means on either side of the ear lobe. The photo-acoustic or optical transmission techniques discussed above might be employed. For applications in which the magnetic field strength is not varied, it is preferable to use a permanent magnet. In applications in which the magnetic field is varied in some way, an electromagnet might be employed, although alternatively it may be possible to use a mechanical system which employs permanent magnets, such as a system of rotating magnets. In order to provide modulation of the polarisation state of the light, it may be convenient to use two lasers which are orthogonally polarised and intensity modulated 180° out of phase and which are directed in turn to the relevant site by way of a suitable optical element such as polarising beam splitter cube.

[0062] Further devices for making in vivo measurements will now be described. Figure 8 (a) shows a device 70 for making in vivo measurements of malarial infection in a patient which clips on to the patient's earlobe. The device 70 comprises a main body 72 which houses instrumentation of the sort described herein, and a wire 74 which transmits measurement data to a suitable recording/analysis device (not shown). In place of the wire 74, a telemetric system might be used instead for these purposes. Figure 8 (b) shows another embodiment of an in vivo device 76 which is adapted to fit onto a finger of a patient. Again, the device 76 comprises a main body 78 and wires 80, and identical comments to those made in respect of the device 70 apply to the device 76. The devices 70 and 76 may be attached to the respective body parts by any suitable means, although a clip arrangement is preferred.

[0063] It is believed that depolarisation caused by various mechanisms within the tissues of a patient can affect the working of embodiments of in vivo devices which use polarised radiation. One way in which this problem may be addressed is to utilise polarised pulsed optical fields to separate ballistic and quasi-ballistic photons from diffuse photons. Various gating techniques have been developed which can achieve this purpose (see, for example Opt. Comm. 241 (2004) 1-9, the entire contents of which are herein incorporated by reference). The delivery of the polarised light could be achieved by low birefringence spun fibres (see, for example, Optics Express 13 (10) (2005) 3841-3851, the entire contents of which are herein incorporated by reference) which will hold any polarisation state, even when the fibres are bent or twisted. Polarised laser techniques might be utilised, although alternatively special purpose LEDs as used in pulse oximetry, might be used.

[0064] Alternatively, as noted above, the configuration shown generally in Figure 1b might be utilised.

[0065] The present invention also provides an advantageous technique for achieving polarisation modulation at a predetermined wavelength which results in a single beam of electromagnetic radiation which is alternately and controllably altered between orthogonal polarisation states. An embodiment of this aspect of the present invention is shown in Figure 6, which depicts a first semiconductor laser 60 producing a first beam 60a of electromagnetic radiation and a second semiconductor laser 61 producing a second beam 61a of electromagnetic radiation. The first and second semiconductor lasers 60, 61 produce light of identical wavelength, although, at least in principle, differing wavelengths could be utilised. The first beam of electromagnetic radiation 60a passes through a Glan Taylor polariser 62 and then through a polarising beam splitter 63. The second beam 61a of electromagnetic radiation similarly passes through a Glan Taylor polariser 64 and is subsequently turned through 90° with a suitable polarising beam splitter 65 so as to be directed towards the polarising beam splitter 63, whereupon the first and second beams 60a, 61a of electromagnetic radiation are combined to produce a co-linear output beam 66. The linear polarisation states of the first 60a and second 61a beams are set at 90° to each other by means of the Glan Taylor polarisers 62, 64 and a micromanual rotary stage 67.

[0066] The extinction ratios of the Glan Taylor polarisers are 10^{-5} and the extinction ratios of the polarising beam splitters are 10^{-3}, leading to a very high polarisation purity for each beam of 10^{-8}. The orthogonality of the two beams is in principle only limited by the resolution of the rotary stage 67, which can be easily of the order of 15 arcsec, therefore giving an orthogonality of $90 \pm 4.17 \times 10^{-3}$°.

Each laser 60,61 has a respective laser driver 60b, 61b. Polarisation switching can be achieved by electronically modulating each laser 180° out of phase from the other using a control arrangement 68. This can be done using two out of phase digital clock signals such that the lasers are alternately switched between two adjustable intensity levels. Components such as a digital clock and digital invertors can be used to perform this function. In one specific embodiment, two circularised collimated (2mm diameter) laser modules emitting 50mW at 660nm (Blue Sky Research) are driven using separate laser drivers (Micro Laser Systems), each driver providing laser power stability of >0.1% over a two hour

period. Polarisation switching can be achieved by electronically modulating each laser 180° out of phase from the other at frequencies of up to 2MHz (the frequency limit of the driver). Phase shifting can be provided by an all-pass filter or a flip flop logic circuit such that the beam exiting the optical arrangement has a constant amplitude (stability >0.2%) whilst the polarisation is switched between orthogonal states. In principle, higher switching frequencies could be obtained with different driving electronics.

[0067] All of the optical components 62, 63, 64, 65, 67 shown in Figure 6 can be mounted on adjustable stages allowing fine adjustment of the co-linearity of the output beam 66.

[0068] The arrangement shown in Figure 6 can be advantageously used in conjunction with the detection techniques of the present invention. In particular, the general arrangement shown in Figure 1a can be used in conjunction with the device shown in Figure 6. In this instance, a stage containing the optical components 62, 63, 64, 65, 67 can be aligned so that one state of polarisation of the output beam 66 is parallel to the direction of the applied magnetic field. The beam 66 may be expanded to a desired extent (for example to around 4-5mm) prior to introduction to the sample by a suitable beam expander so that a large proportion of or indeed the entire sample volume is interrogated. In one embodiment, transmitted light through the sample is collected by a large area photodiode to ensure maximise light collection. A collective lens may be added.

[0069] The polarisation modulation technique described in relation to Figure 6 is advantageously incorporated into the detection techniques of the present invention. However, the skilled reader will appreciate that the polarisation modulation provided might be advantageously utilised in other experiments and detection techniques.

Claims

1. A method of detecting the presence of an analyte in a sample (22), in which the analyte is haemozoin, β-haematin or an analogous iron containing compound, the method including the steps of:

   providing the sample;
   applying a magnetic field across at least a portion of the sample to orient the analyte along the direction of the applied magnetic field;
   detecting a physical property of the sample by introducing polarised electromagnetic radiation into the sample and utilising a magneto-optical spectroscopic detection technique to detect a physical property of the analyte which varies in dependence with the orientation of the analyte with the square of the applied magnetic field; and correlating the detected physical property with the presence of the analyte.

2. A method according to claim 1 in which the physical property is a property associated with the absorption of electromagnetic radiation.

3. A method according to claim 2 in which the electromagnetic radiation is in the range 400 to 700 nm, preferably greater than 600 nm and most preferably in the range 640 to 680 nm.

4. A method according to any previous claim in which the polarisation state of the electromagnetic radiation is varied, and the presence of the analyte is detected by detecting a dependence of the detected physical property on the polarisation state.

5. A method according to claim 2, claim 3 or claim 4 when dependant on claim 1 in which the transmission of the electromagnetic radiation through the sample is detected.

6. A method according to any previous claim in which the magnitude and/or the direction of the applied magnetic field is varied, and the presence of the analyte is detected by detecting a dependence of the detected physical property on the variation of the applied magnetic field.

7. A device (10) for detecting the presence of an analyte in a sample (22), in which the analyte is haemozoin, β-haematin or an analogous iron containing compound, the device including:

   magnetic field applying means (28, 30) for applying a magnetic field across at least a portion of the sample to orient the analyte along the direction of the applied magnetic field;
   detection means (12, 16, 18, 20, 26) adapted for detecting a physical property of the sample by introducing polarised electromagnetic radiation into the sample and utilising a magneto-optical spectroscopic detection technique to detect a physical property of the analyte which varies in dependence with the orientation of the

analyte with the square of the applied magnetic field; and
correlation means for correlating the detected physical property with the presence of the analyte.

8. A device according to claim 7 in which the magnetic field applying means is controllable so as to vary the magnitude and/or the direction of the applied magnetic field.

9. A device according to claim 7 or claim 8 in which the detection means includes means for providing electromagnetic radiation (12) and sensing means (26) for detecting a physical property associated with the absorption of the electromagnetic radiation by the analyte, wherein the device is configured so that the electromagnetic radiation propagates into the sample along an axis, and the magnetic field applying means applies a magnetic field which is aligned in parallel with said axis of propagation.

10. A device according to claim 7 configured so that the electromagnetic radiation propagates into the sample along an axis, and the magnetic field applying means applies a magnetic field which is aligned in parallel with the axis of propagation.

11. A device according to claim 10 in which the detection means includes means for providing electromagnetic radiation and sensing means for detecting a physical property associated with the absorption of the electromagnetic radiation by the analyte, wherein the device is configured so that the electromagnetic radiation propagates into the sample along an axis, and the magnetic field applying means applies a magnetic field which is aligned orthogonal to said axis of propagation.

Patentansprüche

1. Verfahren zum Nachweis eines Analyten in einer Probe (22), wobei es sich bei dem Analyten um Hämozoin, Hämatin b oder eine analoge Eisen-enthaltende Verbindung handelt, und das Verfahren die folgenden Schritte umfasst:

Bereitstellen der Probe;
Anlegen eines Magnetfeldes über mindestens einen Teil der Probe, um den Analyten entlang der Richtung des angelegten Magnetfeldes zu orientieren;
Nachweis einer physikalischen Eigenschaft der Probe durch Einbringen polarisierter elektromagnetischer Strahlung in die Probe und Verwenden einer magnetoptischen, spektroskopischen Technik zum Nachweis einer physikalischen Eigenschaft des Analyten, die in Abhängigkeit von der Orientierung des Analyten zum Quadrat des angelegten Magnetfeldes variiert; und
Korrelieren der nachgewiesenen physikalischen Eigenschaft mit der Anwesenheit des Analyten.

2. Verfahren gemäß Anspruch 1, bei dem die physikalische Eigenschaft eine mit der Absorption der elektromagnetischen Strahlung assoziierte Eigenschaft ist.

3. Verfahren gemäß Anspruch 2, bei dem die elektromagnetische Strahlung im Bereich von 400 bis 700 nm, vorzugsweise größer als 600 nm und am stärksten bevorzugt im Bereich von 640 bis 680 nm liegt.

4. Verfahren gemäß einem der vorhergehenden Ansprüche, bei dem der Polarisationszustand der elektromagnetischen Strahlung variiert wird und die Anwesenheit des Analyten durch den Nachweis einer Abhängigkeit der nachgewiesenen physikalischen Eigenschaft vom Polarisationszustand nachgewiesen wird.

5. Verfahren gemäß Anspruch 2, 3 oder 4, bei dem in Abhängigkeit von Anspruch 1 die Übertragung der elektromagnetischen Strahlung durch die Probe nachgewiesen wird.

6. Verfahren gemäß einem der vorhergehenden Ansprüche, bei dem die Größe und/oder Richtung des angelegten Magnetfelds variiert wird und die Anwesenheit des Analyten durch den Nachweis einer Abhängigkeit der nachgewiesenen physikalischen Eigenschaft von der Variation des angelegten Magnetfelds nachgewiesen wird.

7. Verfahren zum Nachweis eines Analyten in einer Probe (22), wobei es sich bei dem Analyten um Hämozoin, Hämatin b oder eine analoge Eisen-enthaltende Verbindung handelt, und die Vorrichtung folgendes umfasst:

Mittel zum Anlegen eines Magnetfelds (28, 30) zum Anlegen eines Magnetfeldes über mindestens einen Teil

der Probe, um den Analyten entlang der Richtung des angelegten Magnetfeldes zu orientieren;
Nachweismittel (12, 16, 18, 20, 26), die zum Nachweis einer physikalischen Eigenschaft der Probe durch Einbringen polarisierter elektromagnetischer Strahlung in die Probe angepasst sind und Verwenden einer magnetoptischen, spektroskopischen Nachweistechnik zum Nachweis einer physikalischen Eigenschaft des Analyten, die in Abhängigkeit von der Orientierung des Analyten zum Quadrat des angelegten Magnetfeldes variiert; und
Korreliermittel zum Korrelieren der nachgewiesenen physikalischen Eigenschaft mit der Anwesenheit des Analyten.

8. Vorrichtung gemäß Anspruch 7, bei der die Mittel zum Anlegen des Magnetfelds so eingestellt werden können, dass die Größe und/oder Richtung des angelegten Magnetfelds variiert.

9. Vorrichtung gemäß Anspruch 7 oder 8, bei der die Nachweismittel Mittel zum Bereitstellen elektromagnetischer Strahlung (12) und Sensormittel (26) zum Nachweis einer physikalischen Eigenschaft umfassen, die mit der Absorption der elektromagnetischen Strahlung durch den Analyten assoziiert ist, wobei die Vorrichtung so konfiguriert ist, dass sich die elektromagnetische Strahlung entlang einer Achse in der Probe ausbreitet, und die Mittel zum Anlegen des Magnetfelds ein Magnetfeld anlegen, das parallel zur Ausbreitungsachse ausgerichtet ist.

10. Vorrichtung gemäß Anspruch 7, die so konfiguriert ist, dass sich die elektromagnetische Strahlung entlang einer Achse in der Probe ausbreitet, und die Mittel zum Anlegen eines Magnetfelds ein Magnetfeld anlegen, das parallel zur Ausbreitungsachse ausgerichtet ist.

11. Vorrichtung gemäß Anspruch 10, bei der die Nachweismittel Mittel zum Bereitstellen elektromagnetischer Strahlung und Sensormittel zum Nachweis einer physikalischen Eigenschaft umfassen, die mit der Absorption der elektromagnetischen Strahlung durch den Analyten assoziiert ist, wobei die Vorrichtung so konfiguriert ist, dass sich die elektromagnetische Strahlung entlang einer Achse in der Probe ausbreitet, und die Mittel zum Anlegen des Magnetfelds ein Magnetfeld anlegen, das orthogonal zur Ausbreitungsachse ausgerichtet ist.

Revendications

1. Procédé de détection de la présence d'une substance à analyser dans un échantillon (22), la substance à analyser étant l'hémozoïne, la β-hématine ou un composé analogue contenant du fer, le procédé comprenant les étapes de :

se procurer l'échantillon ;
appliquer un champ magnétique à travers au moins une partie de l'échantillon pour orienter la substance à analyser le long de la direction du champ magnétique appliqué ;
détecter une propriété physique de l'échantillon par introduction d'un rayonnement électromagnétique polarisé dans l'échantillon et utilisation d'une technique de détection spectroscopique magnéto-optique pour détecter une propriété physique de la substance à analyser qui varie en fonction de l'orientation de la substance à analyser avec le carré du champ magnétique appliqué ; et
mettre la propriété physique détectée en corrélation avec la présence de la substance à analyser.

2. Procédé selon la revendication 1, dans lequel la propriété physique est une propriété associée à l'absorption du rayonnement électromagnétique.

3. Procédé selon la revendication 2, dans lequel le rayonnement électromagnétique est compris dans la plage allant de 400 à 700 nm, de préférence est supérieur à 600 nm, et de la façon que l'on préfère le plus est compris dans la plage allant de 640 à 680 nm.

4. Procédé selon l'une quelconque des revendications précédentes, dans lequel l'état de polarisation du rayonnement électromagnétique est amené à varier, et la présence de la substance à analyser est détectée par détection d'une dépendance de la propriété physique détectée à l'état de polarisation.

5. Procédé selon la revendication 2, la revendication 3 ou la revendication 4 lorsque prises en dépendance de la revendication 1, dans lequel la transmission du rayonnement électromagnétique à travers l'échantillon est détectée.

6. Procédé selon l'une quelconque des revendications précédentes, dans lequel la grandeur et/ou la direction du

champ magnétique appliqué est amenée à varier, et la présence de la substance à analyser est détectée par détection d'une dépendance de la propriété physique détectée à la variation du champ magnétique appliqué.

7. Dispositif (10) pour détecter la présence d'une substance à analyser dans un échantillon (22), la substance à analyser étant l'hémozoïne, la β-hématine ou un composé analogue contenant du fer, le dispositif comprenant :

un moyen d'application de champ magnétique (28, 30) pour appliquer un champ magnétique à travers au moins une partie de l'échantillon afin d'orienter la substance à analyser le long de la direction du champ magnétique appliqué ;
un moyen de détection (12, 16, 18, 20, 26) apte à détecter une propriété physique de l'échantillon par introduction d'un rayonnement électromagnétique polarisé dans l'échantillon et utilisation d'une technique de détection spectroscopique magnéto-optique pour détecter une propriété physique de la substance à analyser qui varie en fonction de l'orientation de la substance à analyser avec le carré du champ magnétique appliqué ; et
un moyen de corrélation pour mettre la propriété physique détectée en corrélation avec la présence de la substance à analyser.

8. Dispositif selon la revendication 7, dans lequel le moyen d'application de champ magnétique est commandable de façon à faire varier la grandeur et/ou la direction du champ magnétique appliqué.

9. Dispositif selon la revendication 7 ou la revendication 8, dans lequel le moyen de détection comprend un moyen pour fournir un rayonnement électromagnétique (12) et un moyen de détection (26) pour détecter une propriété physique associée à l'absorption du rayonnement électromagnétique par la substance à analyser, le dispositif étant configuré de telle sorte que le rayonnement électromagnétique se propage dans l'échantillon le long d'un axe, et le moyen d'application de champ magnétique appliquant un champ magnétique qui est aligné en parallèle avec ledit axe de propagation.

10. Dispositif selon la revendication 7, configuré de telle sorte que le rayonnement électromagnétique se propage dans l'échantillon le long d'un axe, et le moyen d'application de champ magnétique applique un champ magnétique qui est aligné en parallèle avec l'axe de propagation.

11. Dispositif selon la revendication 10, dans lequel le moyen de détection comprend un moyen pour fournir un rayonnement électromagnétique et un moyen de détection pour détecter une propriété physique associée à l'absorption du rayonnement électromagnétique par la substance à analyser, le dispositif étant configuré de telle sorte que le rayonnement électromagnétique se propage dans l'échantillon le long d'un axe, et le moyen d'application de champ magnétique appliquant un champ magnétique qui est aligné orthogonalement audit axe de propagation.

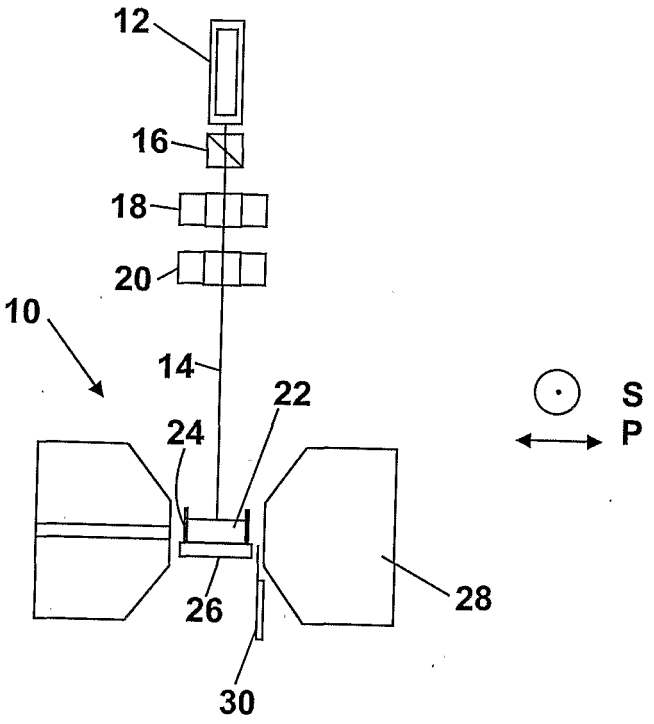

Fig. 1(a)

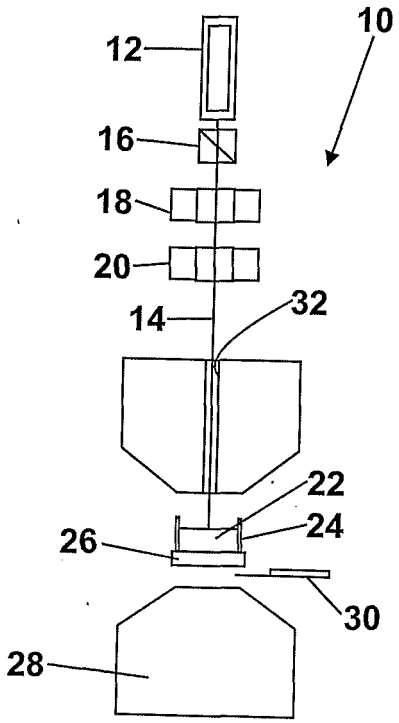

Fig. 1(b)

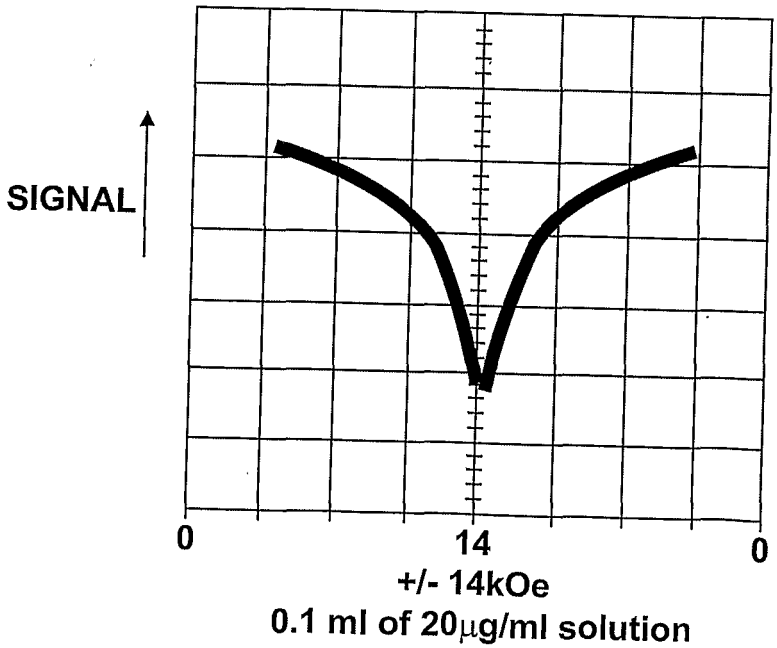

Fig. 2

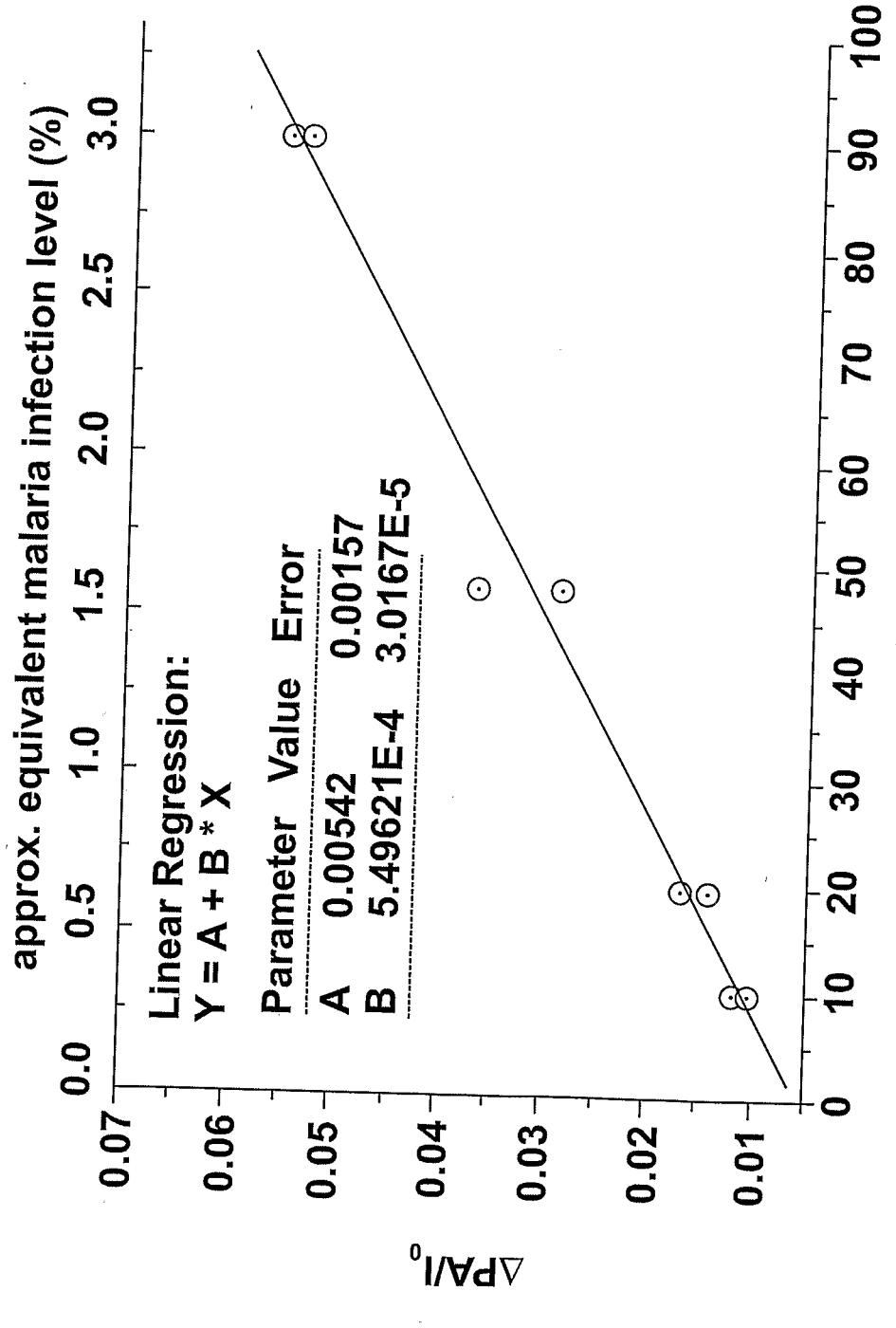
approx. equivalent malaria infection level (%)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Linear Regression:
Y = A + B * X
Parameter Value Error
A 0.00542 0.00157
B 5.49621E-4 3.0167E-5
ΔPA/I0
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
10
20
30
40
50
60
70
80
90
100
β-heamatin concentration in blood (µg/ml)

Fig. 3

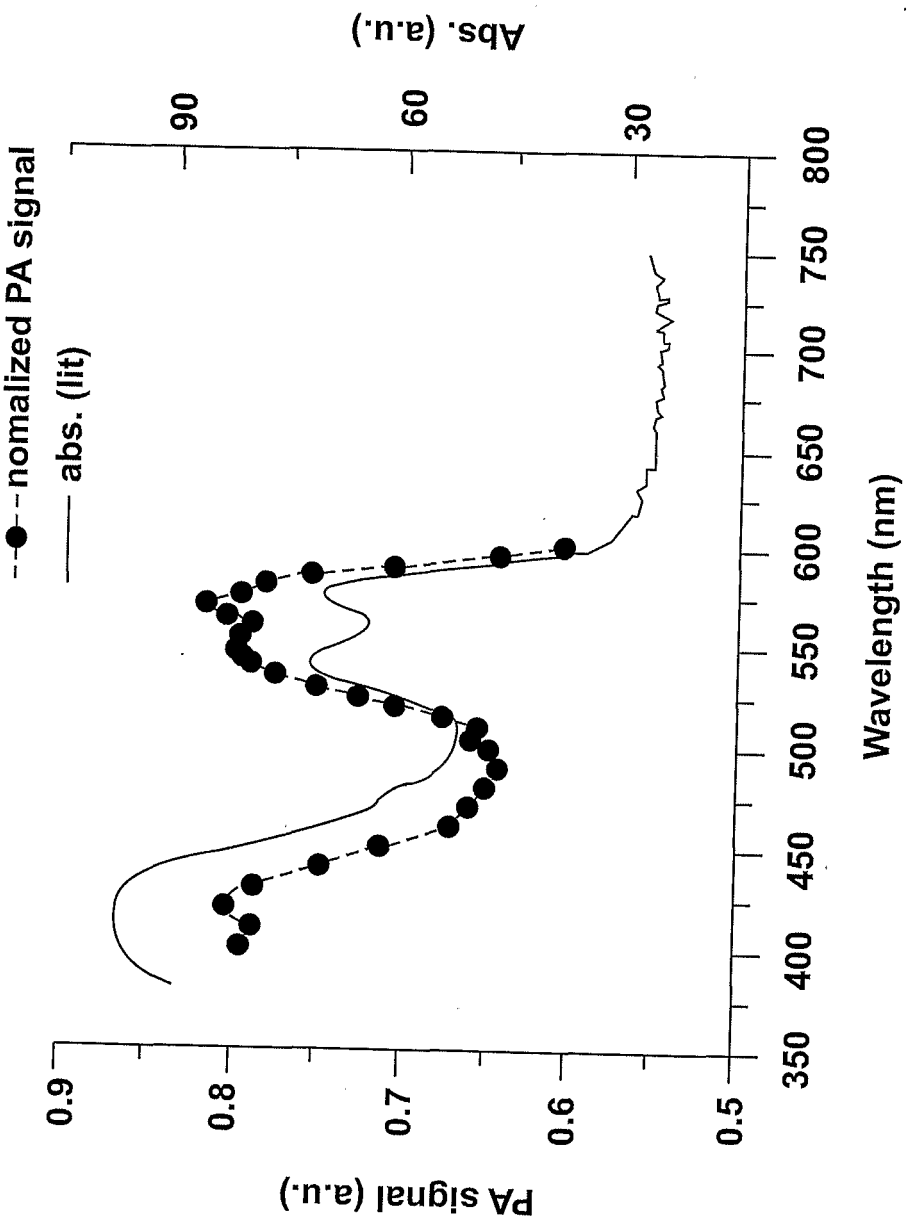
nomalized PA signal
abs. (lit)
PA signal (a.u.)
Abs. (a.u.)
Wavelength (nm)
0.9
0.8
0.7
0.6
0.5
90
60
30
350
400
450
500
550
600
650
700
750
800

Fig. 4

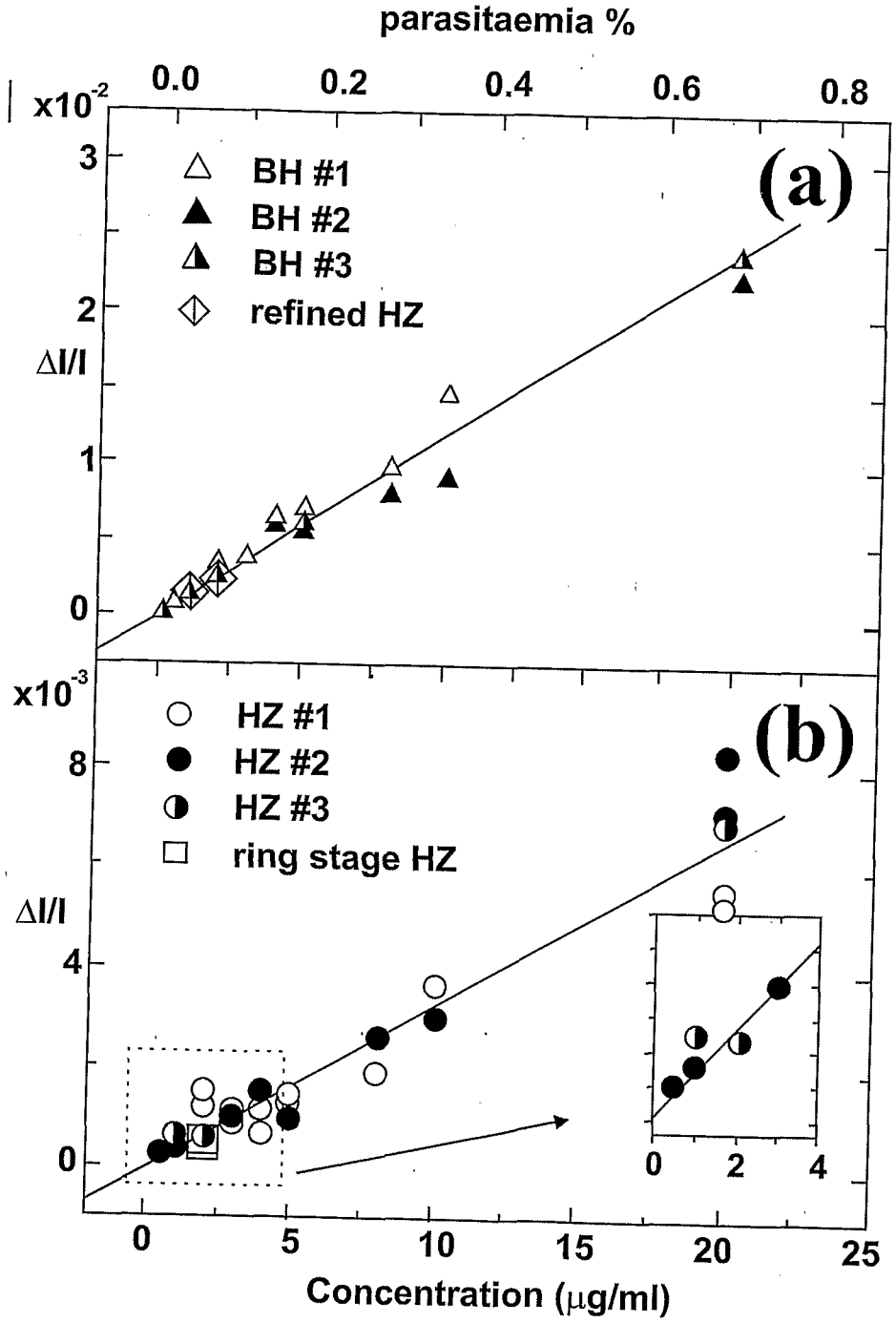
parasitaemia %
0.0
0.2
0.4
0.6
0.8
x10-2
3
2
1
0
ΔI/I
BH #1
BH #2
BH #3
refined HZ
(a)
x10-3
8
4
0
ΔI/I
HZ #1
HZ #2
HZ #3
ring stage HZ
(b)
0
2
4
0
5
10
15
20
25
Concentration (μg/ml)

Fig. 5

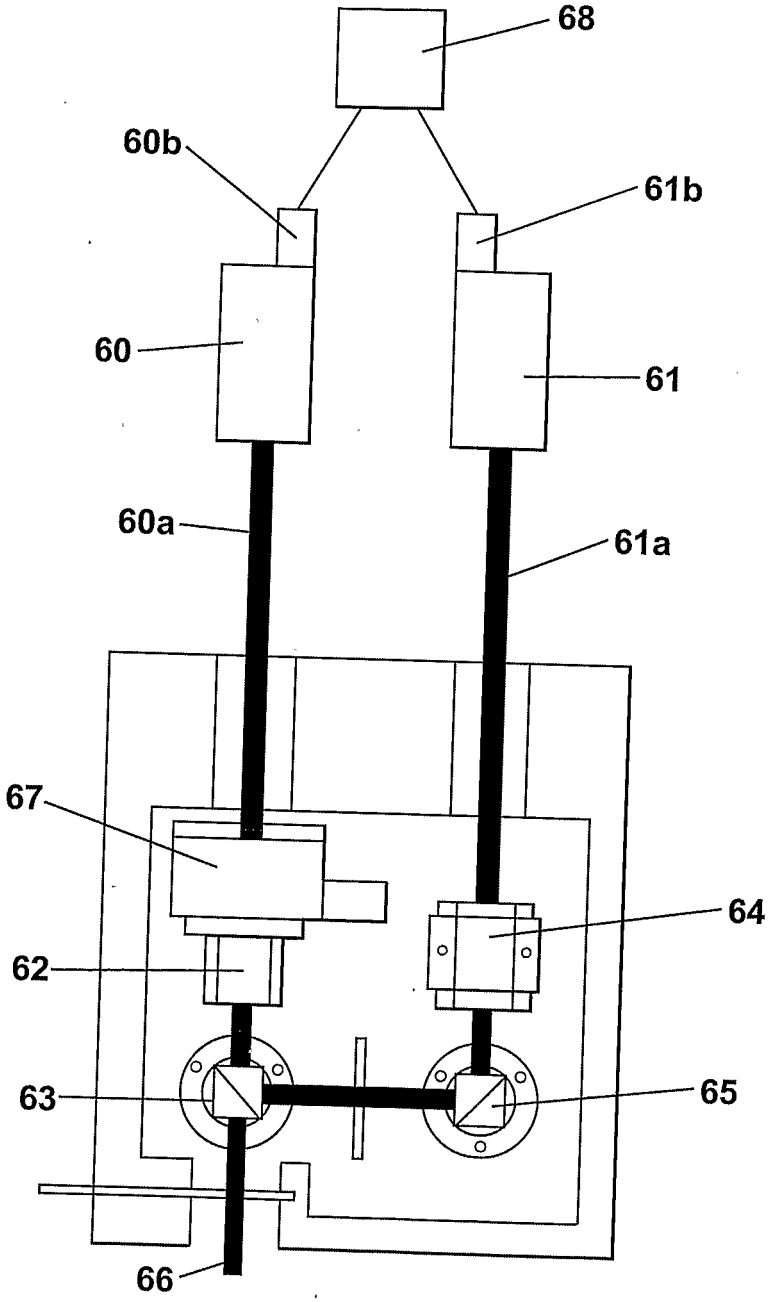

Fig. 6

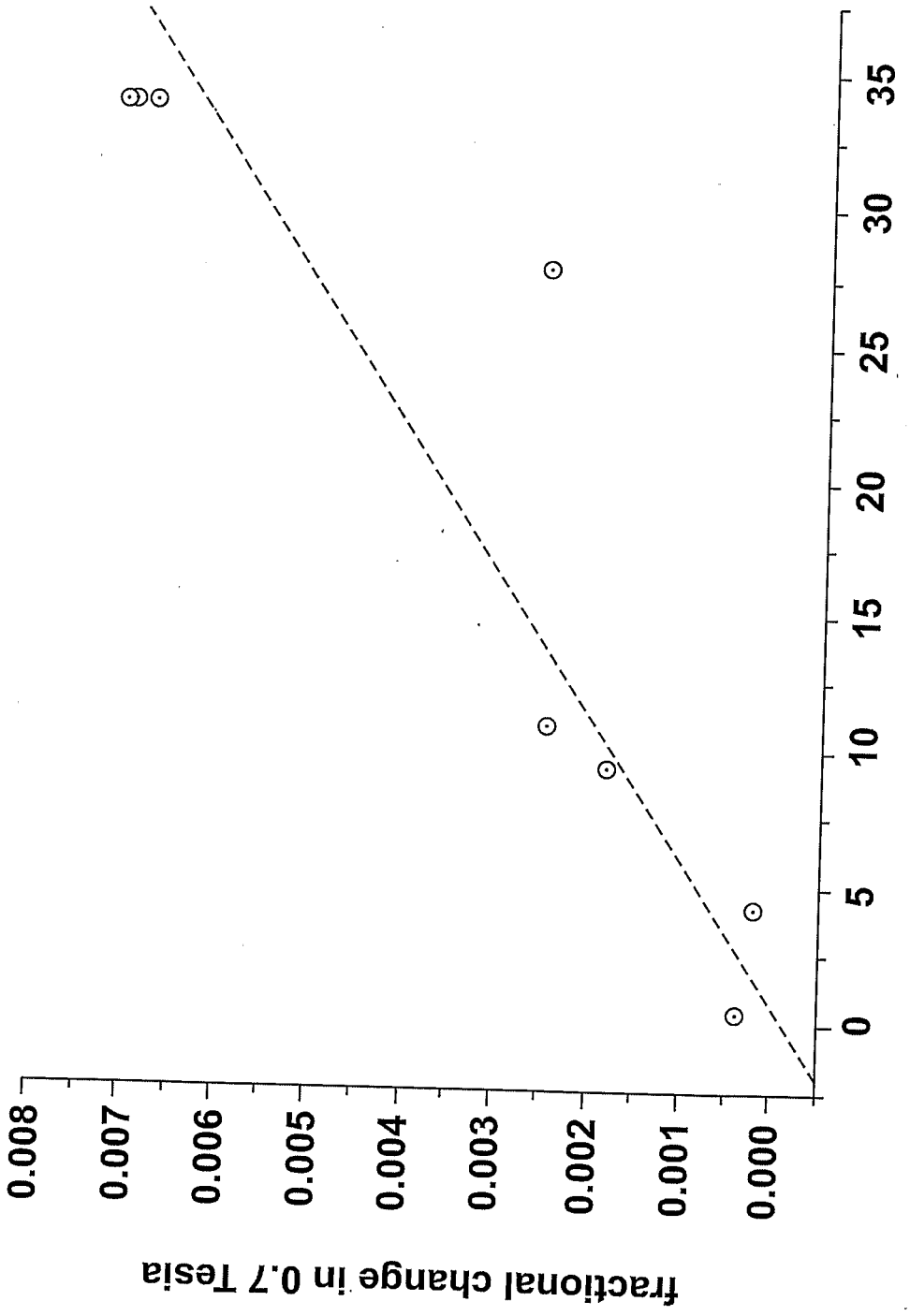
fractional change in 0.7 Tesla
0.000
0.001
0.002
0.003
0.004
0.005
0.006
0.007
0.008
0
5
10
15
20
25
30
35
HZ concentration (μg/ml)

Fig. 7

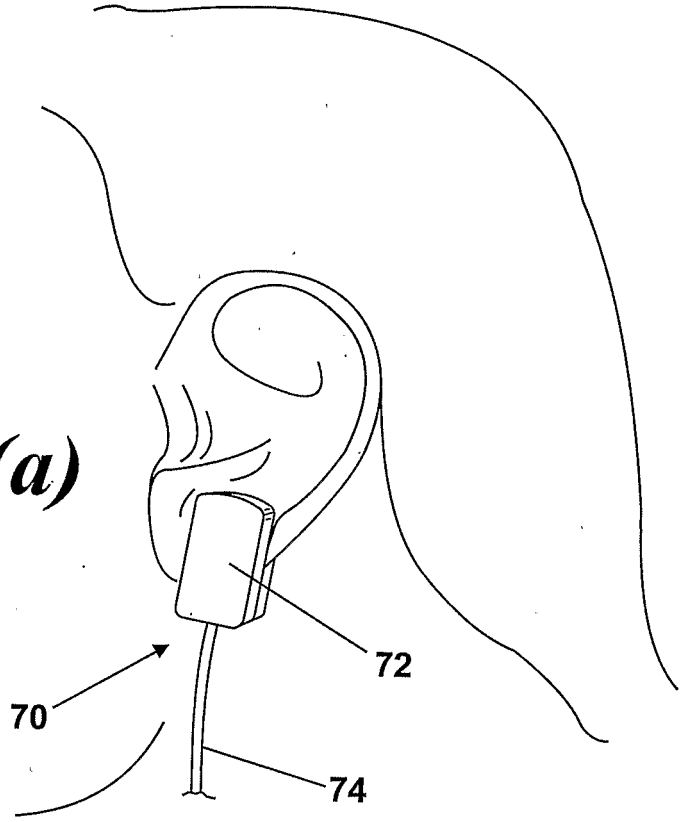

Fig. 8(a)

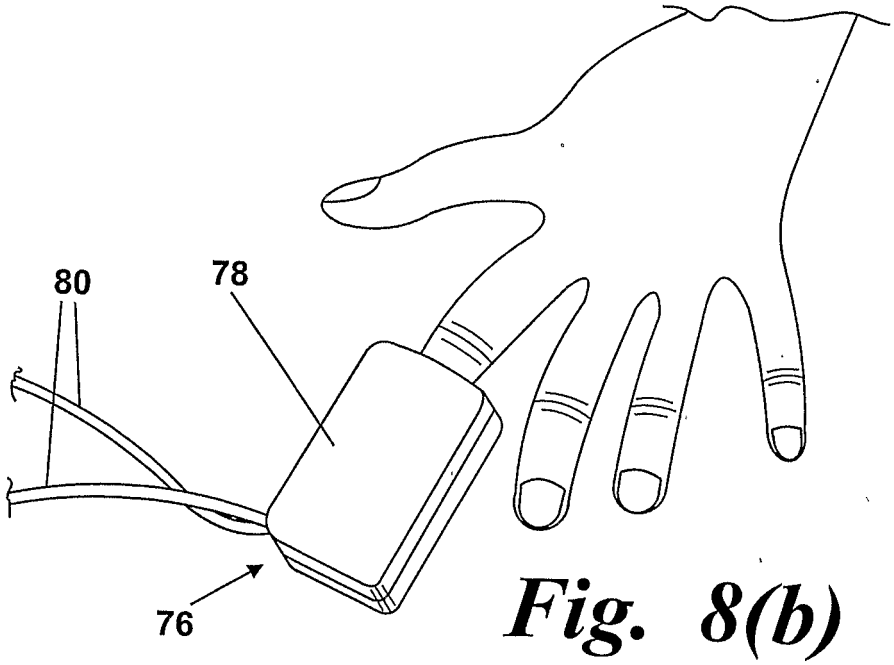

Fig. 8(b)

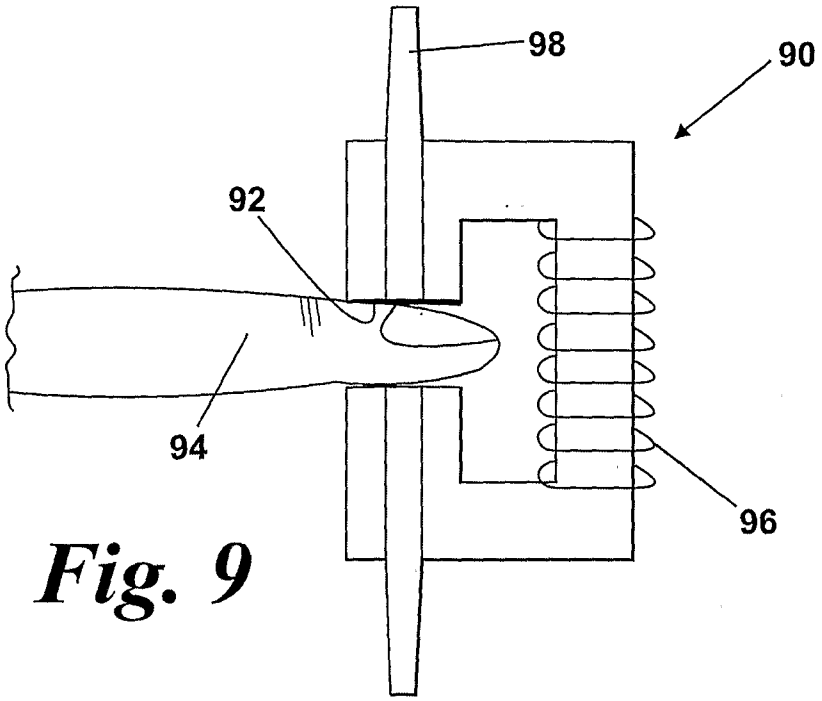

Fig. 9

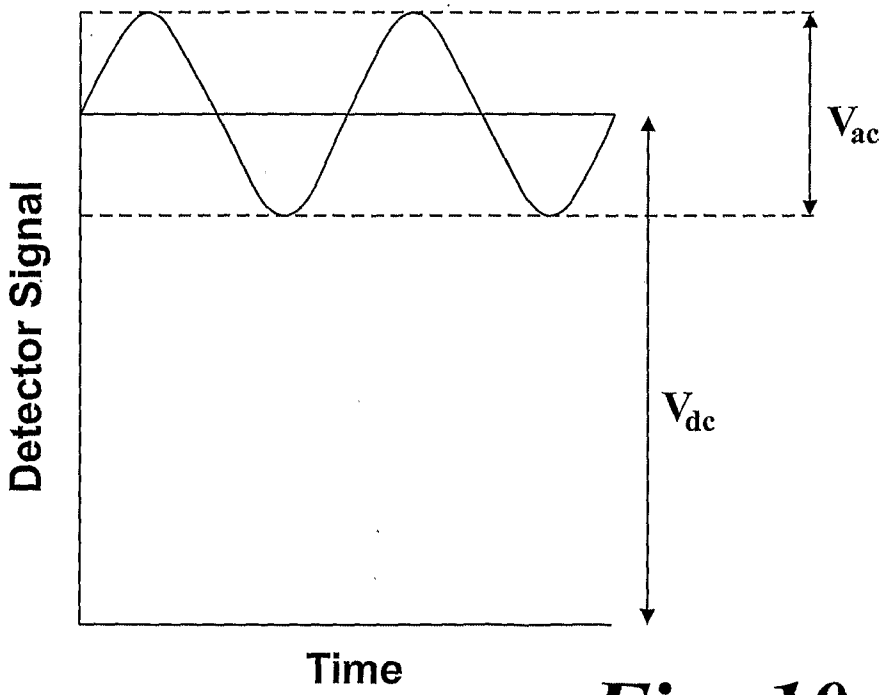

Fig. 10

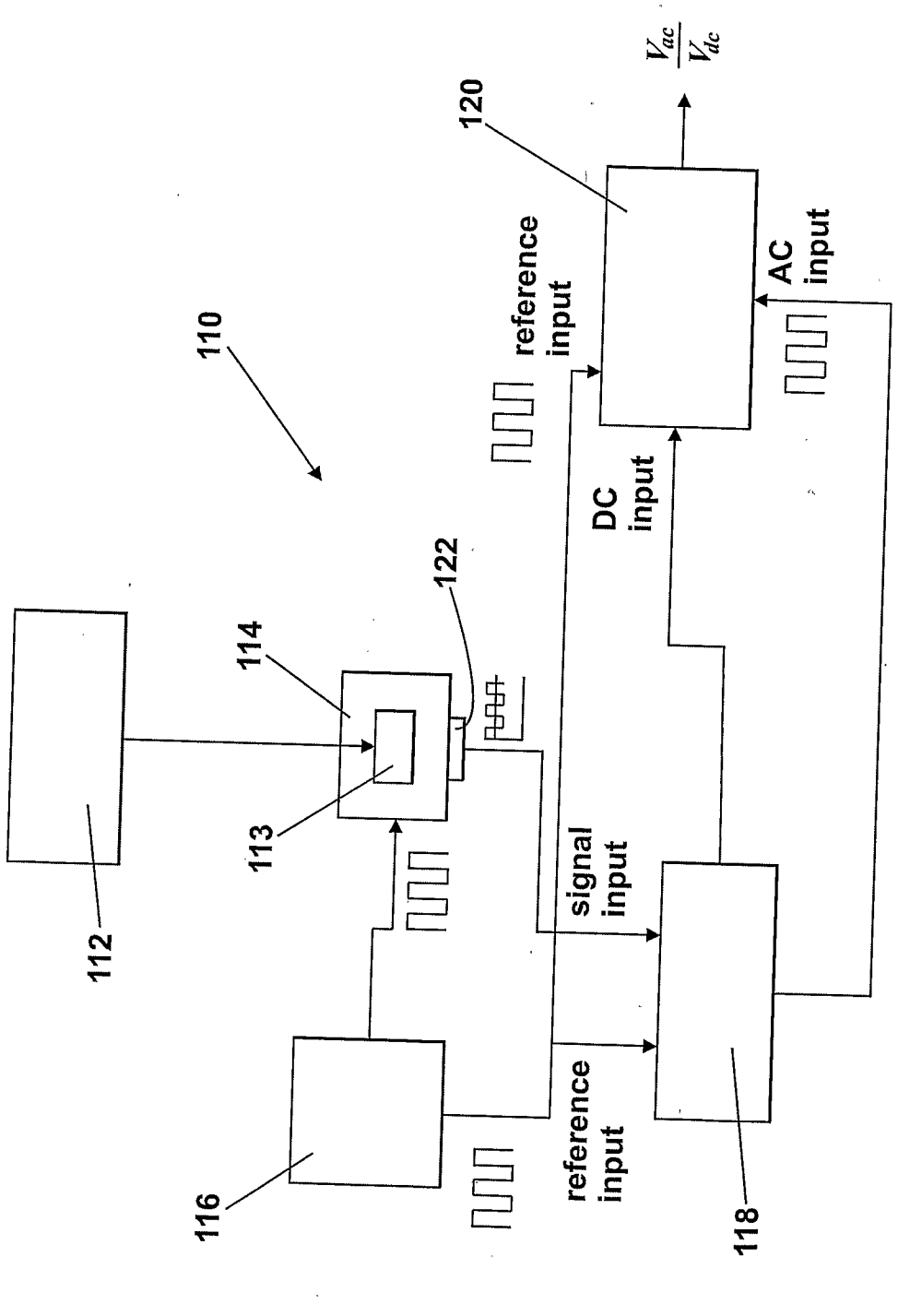
$\frac{V_{ac}}{V_{dc}}$
120
reference input
AC input
110
DC input
122
114
signal input
113
112
reference input
116
118

Fig. 11

REFERENCES CITED IN THE DESCRIPTION

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专利名称(译)	用于检测血红蛋白和血红蛋白的装置和方法		
公开(公告)号	EP2082223A2	公开(公告)日	2009-07-29
申请号	EP2007824529	申请日	2007-11-09
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IPC分类号	G01N33/487 A61B5/00 A61B5/1455 G01N21/21 A61B5/05 G01N21/17		
CPC分类号	G01N21/1717 A61B5/0059 A61B5/0095 A61B5/05 A61B5/6816 A61B5/6826 A61B5/6838 G01N21/21 G01N2021/1727 G01N2021/218 G01N2201/067 G01N2201/0697 G01N2333/445		
优先权	2006022450 2006-11-10 GB		
其他公开文献	EP2082223B1		
外部链接	Espacenet		

摘要(译)

在本申请中，通过检测依赖于施加磁场的haemozoin的合适性质来利用由疟疾感染引起的血红蛋白的磁性状态的变化。图。图1示出了通常在（10）处示出的用于使用光声技术执行磁光检测的装置。装置（10）包括光源（12），产生通过偏振器（16）的光辐射束（14），可变LC延迟器（0或180°延迟）（18）和（斩波器）20），在撞击保持在样品架（24）中的样品（22）之前。样品与声学检测器（26）直接接触。装置（10）还包括电磁铁（28），高斯计（30）可用于测量施加的磁场强度。与该方法相关的优点是进行体内测量的可能性，以及避免与诸如全血的混浊液体上的常规光学测量相关的光学散射问题。