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(71) Applicant (for all designated States except US): UNIVER-
SIDADE DO MINHO [PT/PT]; Largo do Paço Braga,
P-4700-320 BRAGA (PT).

(72) Inventors; and

(75) Inventors/Applicants (for US only): GOMES COR-
REIA, José Higinio [PT/PT]; Rua dos Mouriscas, 6,
Gualtar, P-4710-494 BRAGA (PT). HENRIQUES MI-
NAS, Graça Maria [PT/PT]; Rua Dr. Elísio de Moura,
24 - 2º Esq., P-4710-422 BRAGA (PT). DE ALMEIDA
RIBEIRO, José Carlos [PT/PT]; Cavadas-Ossela,
P-3720-176 OLIVEIRA DE AZEMÉIS (PT).

(74) Agent: VIEIRA PEREIRA FERREIRA, Maria Silvina;
Clarke, Modet & Cº, Rua Castilho, 50 - 5º, P-1269-163
Lisbon (PT).

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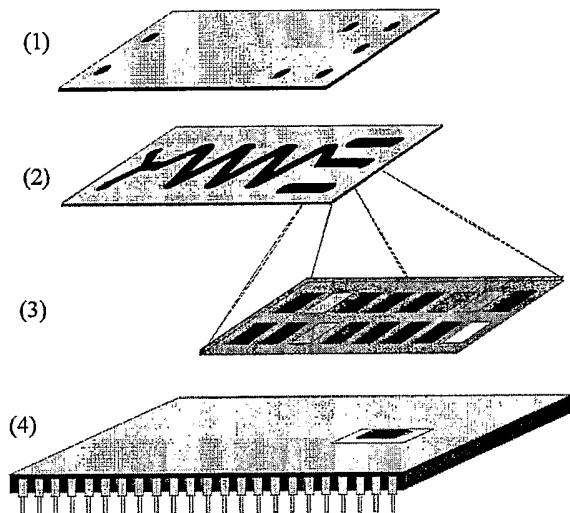
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(54) Title: MICROLABORATORY FOR BIOLOGICAL FLUIDS ANALYSIS USING WHITE LIGHT ILLUMINATION



(57) Abstract: The present invention relates a laborato-
rial microsystem for biological fluid analysis, especially
the concentration measurement of biomolecules in those
fluids, for application in clinical analyses. This device
combines in a single microsystem the microchannels, the
optical filters, the detectors and the readout electronics,
enabling the measurement of the concentration of
several biomolecules using white light source as
illumination, thus avoiding the use of a wavelength
dependent light source (such as a laser, for example). Its
operation is based on colorimetric detection by optical
absorption. A white light beam is guided through the
microchannels containing the samples to analyse. The
impinging light is filtered by a narrow passband optical
filter at the wavelength defined by the biomolecule
being analysed. The intensity of the selected spectral
component transmitted through the fluid, proportional
to the concentration of the biomolecule in analysis, is
measured using an underlying photo-detector, vertically
aligned with the optical filter.

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Description

MICROLABORATORY FOR BIOLOGICAL FLUIDS ANALYSIS USING WHITE LIGHT ILLUMINATION

Field of the invention

- [1] The invention relates a laboratorial microsystem for analysing biological fluids (such as urine, blood, saliva, cerebrospinal fluid, etc.), especially the measurement of the concentration of biomolecules in those fluids (such as uric acid, albumin, total protein, etc.).

Background of the invention

- [2] Automated equipments are commercially available and used in clinical laboratories performing several and simultaneously tests for each biological fluid. Nowadays, those equipments are extremely sophisticate, precisely and accurate. However, they use high reagent and sample volumes making the analysis systems expensive. In addition, to perform these analyses it takes several hours or even days.
- [3] For diagnostic reasons patients are often subjected to biochemical analysis of their biological body fluids. Usually the analyses are carried out in clinical laboratories. All this process needs long time and a reliable diagnosis cannot be performed within the consultation time (only after the receiving of the requested analysis results). Besides time delay, mistakes in the logistics, such as lost samples and mislabelling, may further delay diagnosis.
- [4] Outside the laboratory environment, reagent strips are commercially available for routine analyses of biological fluids (urine and blood). They can be used, read and interpreted directly by the patients and by the health care personal. Those strips are chemically impregnated with reagent and allow quantifying the concentration values of certain biomolecules in urine, using a visual comparison process codified by colours. The reaction times of the chemical biomolecules in the strips are standardised for each strip class. Actually, these reagent strips work as miniaturised laboratories, however, they are available for a limited set of biomolecules to be analysed (pH, total protein, glucose, bilirubin, nitrite, and haemoglobin) and the colour readout, even with controls, is merely qualitative.
- [5] There are several methods for measuring the concentration of biomolecules, such as: fluorescence, electrochemical and optical absorption. The fluorescence detection method has high detection sensitivity. However, the time of the fluorescent light emitted by the molecules is extremely short. Moreover, it is not easy to find a reagent that forms a strongly fluorescence complex. The electrochemical method has also high detection sensitivity, but its application is limited to only some compounds. The optical

absorption measurement method can be applied to a wide range of analyses and has the advantage that it is not necessary fluorescent compounds for the detection.

- [6] The patents US2003017079A1 - 'Absorbance detection system for lab-on-a-chip' and US6048498 - 'Microfluidic devices and systems' - use the optical absorption method for measuring the concentration of biomolecules, but they need a monochromatic light source and also the patent US2003017079A1 uses optical fibres to guide the light. The present invention does not need optical fibres to guide the light and does not need a specific monochromatic light source, once it only needs white light source and the required wavelength is selected by optical filters.
- [7] The patent WO0170400 - 'Multiblock micro-arrays or macro-arrays with lab-on-a-chip' - needs mixers to mix the reagents. The fabrication of those mixers, in that patent, is very complex due to their vertical multi-structures. The present invention does not need micromixers, once the mixing is performed by diffusion, which highly simplifies the device fabrication.
- [8] The patent US2003052281 - 'Apparatus to collect, classify, concentrate, and characterize gas-borne particles' - needs UV light source. The UV detectors are very difficult to fabricate in silicon. The present invention uses white light source, using optical filters for the filtering and detectors for the visible spectral range that are simple to fabricate in silicon.
- [9] The patents US6129896 - 'Biosensor chip and manufacturing method' - and US5755942 - 'Partitioned microelectronic device array' - need to have recourse to optical fibres to guide the light, which requires a monochromatic light source. The present invention does not need optical fibres to guide the light and does not need a specific monochromatic light source.
- [10] The patent US6100973 - 'Methods and apparatus for performing microanalytical techniques using photolithographically fabricated substrates having narrow band optical emission capability' - uses the fluorescence detection method, which limits its application to only some compounds. The present invention uses the optical absorption detection method, which can be applied to a wide range of compounds and consequently to a wide range of analyses and does not need fluorescent compounds for the detection.
- [11] None of these documents advances the object of the requested patent that now is intended to protect.

Summary of the invention

- [12] The objective of the invention is to quantify the concentration of biomolecules in human fluids, with instantaneous results and at any location, using a regular white light source for illumination, such as a commercially available fluorescent light, with low cost and without the use of complex and expensive analyses systems as the spec-

trophotometer.

[13] The present invention is a portable microlaboratorial equipment for clinical diagnosis. It combines in a single microsystem the microchannels, the optical filters, the detectors and the readout electronics. This device allows quantifying the concentration of biomolecules without external components. The equipment will allow performing clinical analyses in doctor's office during the consultation time, on-line (Point Of Care), in the clinical analyses laboratories and at patient's home, allowing the exact determination of the concentration of biomolecules in biological fluids.

[14] It measures the concentration value using white light source as illumination, with the help of the optical filters. This characteristic shows an important advantage, because it avoids the need of a specific monochromatic light source, like a laser, it does not need optical fibres for guiding and directing the light for polarising and, once optical absorption detection method is used, fluorescent biomolecules are not needed.

[15] The simplicity of its utilisation allows predicting that the own patients will be qualified to use the equipment and perform their own analyses.

[16] Its small dimensions, low-power consumption and portability, presents instantaneous results with the same viability an precision of the biological fluids analyses systems that are available nowadays in clinical laboratories, and using low quantities of reagents and samples.

Brief description of the drawings

[17] In attach there is a sheet with the drawings, without restrictive character, in which is described, schematically, the laboratorial microsystem for biological fluids analysis.

[18] Figure 1 presents the microlaboratory in its several parts, in which (1) presents the polystyrene die that contains the holes for the injection and the removing of the fluids, (2) presents the polystyrene die with the microchannels, (3) presents the optical filters group placed under the detection chamber (enlarged figure to point out the 16 optical filters), and (4) presents an integrated circuit with the silicon die that contains the photodetectors and readout electronics.

[19] Figure 2 presents the reader in which the microlaboratory (5) is inserted. The reader also includes a display (6), which allows visualising the quantitative result of the analysis.

Detailed description of the invention

[20] The Microlab measures the concentration value of the biomolecules in biological fluids with instantaneous results and at any location, combining in a single microsystem the microchannels, the optical filters, the detectors and the readout electronics, and is schematically described in the drawing of figure 1.

[21] The module for carrying the fluids is micromachined in polystyrene (using mi-

cromilling techniques for fabricating the microchannels, with SiO₂ passivation and annealing for eliminating the roughness and the residual stress) and is composed by two dies (1) and (2) each one with 1 mm thick, 25 mm long and 10 mm wide. The first die (1) has the holes for the injection and removing of the fluids (inlets and outlets) and the second (2) includes the microchannels.

- [22] The microlaboratory comprises basically three microchannels: one to obtain the baseline reference and to calibrate the light source, other allows the analysis of the mixed solution, it has two inlets and one outlet for allowing the automatic mixing between the fluid and the reagent, and the third microchannel is needed to calibrate the biomolecule concentration that will be measured (with a well-known concentration calibrator). The shape of the microchannels is rectangular due to the light reflexion, once the measurement method is by optical absorption.
- [23] The optical filters module (3) is placed under the module for carrying the fluids and is composed of a 0.5 mm thick die. It is on this die where the dielectric thin films will be deposited, with a multilayer structure, to form narrow pass-band optical filters. The thin films can be deposited by PVD (Physical Vapor Deposition), such as sputtering, electron beam, etc.
- [24] The optical filters select the wavelength, within the visible spectrum, suitable to the biomolecules in analysis. The use of the optical filters allows that the microlaboratory performs measurements using a regular white light source for illumination (with all wavelengths, such as a commercially available fluorescent light). The number of the optical filters depends on the number of the biomolecules to be analysed. It is necessary one filter for each biomolecule.
- [25] The detection system module (4) is placed under the other two and is fabricated by a standard CMOS microelectronics process. It includes an array of photodetectors to measure the intensity of the light beam transmitted through the mixture. This impinging light, with several spectral components, is filtered by the optical filters, to a narrow spectral band with only some spectral components. The photodetectors number depends on the optical filter number. The photodetectors array is placed under the optical filters array and vertically aligned with them. An analog to digital converter was integrated with the photodetectors (in the same fabrication process) to convert the analog signal into a digital signal.
- [26] After packaging the detection system, fabricated in silicon, it is placed on its top the die with the optical filters. The device is assembled with a reader containing a display connected to the integrated circuit that contains the detection system. The display is used to show the quantitative results. It avoids the connection to a computer, which gives portability to the microlaboratory. The microchannels module is placed on the reader in its right place, with the measuring area over the optical filters. This

module is disposable, avoiding the costs associated with the cleaning of the reagents. The remaining modules and the reader are used in several analyses.

[27] The number of biomolecules that can be determined with this equipment depends on the number of optical filters that are placed in the array. In a laboratorial example, it has been possible to determine the concentration of 16 different biomolecules in biological fluids, using 16 optical filters (3). The biomolecules analysed are indicated in table 1:

[28]

Biological biomolecules	Biological Fluid	Absorption spectra maximum peak (nm)	Filter position in the array
17 - Ketosteroids	U	478	1
Aldolase	S	484	2
Uric Acid	U, CSF	497	3
Cholesterol	S	500	4
Glucose	S	504	5
Glutamic oxalacetic	S, P, CSF	508	6
Urea nitrogen	U, S, P	514	7
Magnesium	S	518	8
Creatinine	U, S, P	523	9
Bile acids	S	528	10
Blood urea nitrogen	S, P	535	11
Salicylate	S	540	12
Hemoglobin	P	543	13
β -Glucuronidase	S, U	548	14
Bilirubin	S	558	15
Leucine aminopeptidase	U	567	16

[29] Table 1: Analysed biomolecules. S (serum), U (urine), B (blood), P (plasma) and CFS (cerebrospinal fluid). The 4th column indicates the filter that corresponds to the biomolecule in analysis.

Claims

- [1]
1. An equipment for biological fluids analysis by optical absorption, which uses in the measurements white light source, characterised in that it comprises a die that contains the holes for the injection and removing of the fluids (1), a die with the microchannels (2), a die with the optical filters (3), which allows the use of white light source as illumination, and a conventional integrated circuit (chip) (4), which includes photodetectors, placed under the filters, and the readout electronics.
 2. The equipment for biological fluids analysis by optical absorption according to claim 1, characterised in that it comprises three microchannels, one to obtain the baseline reference and to calibrate the light source, the second for the fluid to be analysed and the third to calibrate the biomolecule concentration that is being measured, with a well-known concentration calibrator.
 3. The equipment for biological fluids analysis by optical absorption according to claim 1, characterised in that in the die that comprises the holes for the injection and removing of the fluids (1), those holes are vertically aligned with the microchannels and placed at the beginning and ending of each microchannel.
 4. The equipment for biological fluids analysis by optical absorption, according to claim 1, characterised in that in the optical filter die (3) the optical filters are deposited by PVD (Physical Vapor Deposition) and this die (3) is placed in a such way that the optical filters are under the measurements area.
 5. The equipment for biological fluids analysis by optical absorption according to any of the preceding claims, characterised in that the die material is a transparent material, such as glass, quartz and polymeric materials, with a length equal or smaller than 25 mm, a wide equal or smaller than 10 mm and a thickness between 0.5mm and 1mm.
 6. The equipment for biological fluids analysis by optical absorption according to claims 1 and 2, characterised in that the micro-channels are micromachined in the die and exhibit a rectangular shape.

AMENDED CLAIMS

[received by the International Bureau on 07 December 2005 (07.12.2005) :
original claims 1-6 replaced by amended claims 1-10 [(3 pages)]]

Claims

- [1] An apparatus for optical absorption analysis of biological fluid samples characterized in that it uses any external white light illumination source and comprises in a multi-chip-module:
- (i) a microfluidic mixing system for automatic mixing two or more fluids, comprising a transparent die with the holes for injection and removal of the fluids (1) and a die with the microchannels, which are vertically aligned with the holes, being the holes placed in the beginning and in the ending of each microchannel, and 3 detection chambers (2), or multiples thereof, wherein one chamber contains the blank reagent, other chamber contains the reagent plus the sample to analyse and the other chamber contains a well-known concentration standard of the biomolecule to be analysed;
 - (ii) a die comprising arrays of highly selective optical filters (3), located vertically under the detection chambers of the microfluidic system, the optical filter arrays having fixed thicknesses;
 - (iii) a die with photodetectors and readout electronics integrated in the same fabrication process (4), placed under the filter arrays die;
- wherein the apparatus is free from any previous calibration, being self calibrated during the effected optical absorption measurements, which simultaneously analyse the light intensities transmitted through the 3 detection chambers.
- [2] The apparatus of claim 1, wherein the die comprising the microchannels (2) is a transparent material such as glass, quartz and polymeric materials, being a preferable embodiment the die made of a biocompatible photoresist, such as the octafuntional epoxidized novalac, providing a low sidewall roughness and deep rectangular vertical profile of the microchannels and also providing a disposable die.
- [3] The apparatus of claim 1, wherein the straight microchannels of the detection chambers have a length of 8 times and a width of 2 times of the length and width of one of the optical filters in the arrays and a deep thickness between 0.5 mm and 1 mm, providing high sensitivity optical absorption measurements.
- [4] The apparatus of claim 1, wherein the number of arrays is the same as the number of the detection chambers.
- [5] The apparatus of claim 1, wherein each array of highly selective optical filters includes from about 1 to about 32 optical filters and each one is sensitive to a single highly selective spectral band, allowing the simultaneous analysis of more than one biological fluid samples with the same apparatus.
- [6] The apparatus of claim 1, wherein all the optical filters have the same length and width from about 50 μm to about 1 mm and each optical filter is

- composed by a stack of several dielectric thin-film layers with only 2 different dielectric materials in the stack, such as TiO_2 and SiO_2 , and each optical filter stack having a fixed thickness.
- [7] The apparatus of claim 1, wherein the spectral band of each optical filter is due to the different thicknesses of one or two dielectric layers, the same layers for all optical filters arrays, obtained during fabrication and keeping the other dielectric layers with the same thicknesses in all optical filters arrays.
- [8] The apparatus of claim 1, wherein it is placed in a reader (5) with a display (6) for visualizing the results and with a keyboard for entering data, providing portability and the apparatus is free from any physical connection to a computer.
- [9] The apparatus of claim 8, wherein the reader, the die with the detection and readout electronics (4) and the die with the arrays of highly selective optical filters (3) are the same for all analysis and only the microfluidic mixing system die (1) and (2) is disposable for each analysis.
- [10] Use of the apparatus according to claims 1 to 9, characterized in that a non-calibrated external polychromatic light illumination source can be used, such as a lamp connected to the power supply, being the reliability of the measurements assured by the fact that said apparatus compensates the fluctuations associated to the use of polychromatic light sources, detecting simultaneously the optical absorption of the fluid samples within the 3 detection chambers.

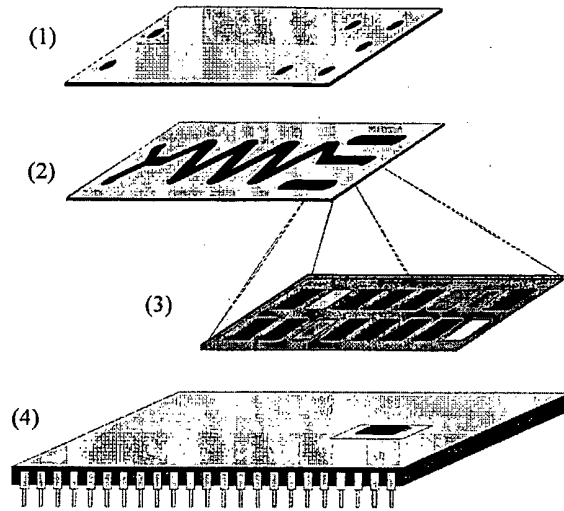


Figure 1

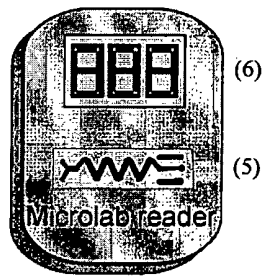


Figure 2

INTERNATIONAL SEARCH REPORT

Inter — Application No
PCT/IB2005/052200

A. CLASSIFICATION OF SUBJECT MATTER IPC 7 G01N21/05 G01N21/31 G01N33/487 B01L3/00		
According to International Patent Classification (IPC) or to both national classification and IPC		
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Minimum documentation searched (classification system followed by classification symbols) IPC 7 G01N B01L		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, WPI Data, BIOSIS, COMPENDEX, INSPEC		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2003/235924 A1 (ADAMS MARK L ET AL) 25 December 2003 (2003-12-25) paragraphs '0038! - '0059!, '0072!, '0074!, '0075!, '0084!; figures 1,2A,2B,3,4,5B -----	1-6
X	US 2004/115861 A1 (WONG WILLIAM S ET AL) 17 June 2004 (2004-06-17) paragraphs '0001!, '0002!, '0020! - '0022!, '0032!, '0041! - '0044!; figures 1,2 -----	1-6
<input type="checkbox"/> Further documents are listed in the continuation of box C.		
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A document defining the general state of the art which is not considered to be of particular relevance	*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	
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Date of the actual completion of the international search <p style="text-align: center;">26 September 2005</p>	Date of mailing of the international search report <p style="text-align: center;">07/10/2005</p>	
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer <p style="text-align: center;">Duijs, E</p>	

INTERNATIONAL SEARCH REPORT

International Application No
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2003235924 A1	25-12-2003	AU 2003303121 A1 EP 1561095 A2 WO 2004063681 A2	10-08-2004 10-08-2005 29-07-2004
US 2004115861 A1	17-06-2004	BR 0306028 A JP 2004198420 A	31-08-2004 15-07-2004

PCT REQUEST

Paper Copy for submission

VIII-4-1-2-1	Name (LAST, First)	HENRIQUES MINAS, Graça Maria
VIII-4-1-2-2	Residence: (city and either US State, if applicable, or country)	BRAGA, Portugal
VIII-4-1-2-3	Mailing address:	Rua Dr. Elísio de Moura, 24 - 2° Esq. 4710-422 BRAGA Portugal
VIII-4-1-2-4	Citizenship:	PT
VIII-4-1-2-5	Inventor's Signature: (if not contained in the request, or if declaration is corrected or added under Rule 26ter after the filing of the international application. The signature must be that of the inventor, not that of the agent)	<i>Henrique Henriques Minas</i>
VIII-4-1-2-6	Date (of signature which is not contained in the request, or of the declaration that is corrected or added under Rule 26ter after the filing of the international application)	14. July. 2005
VIII-4-1-3-1	Name (LAST, First)	DE ALMEIDA RIBEIRO, José Carlos
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VIII-4-1-3-3	Mailing address:	Cavadas-Ossela 3720-176 OLIVEIRA DE AZEMÉIS Portugal
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VIII-4-1-3-5	Inventor's Signature: (if not contained in the request, or if declaration is corrected or added under Rule 26ter after the filing of the international application. The signature must be that of the inventor, not that of the agent)	<i>José Carlos de Almeida Ribeiro</i>
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