(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 November 2016 (24.11.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/184441 A1

(51) International Patent Classification:
A61N 5/06 (2006.01) *C12M 1/00* (2006.01)

(21) International Application Number:
PCT/CZ2015/000125

(22) International Filing Date:
23 October 2015 (23.10.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PV 2015-330 18 May 2015 (18.05.2015) CZ

(71) Applicant: **UNIVERZITA PALACKÉHO V OLOMOUCI** [CZ/CZ]; Křížkovského 511/8, 771 47 Olomouc (CZ).

(72) Inventors: **BAJGAR, Robert**; Nešverova 1, 779 00 Olomouc (CZ). **KOLÁŘOVÁ, Hana**; Hněvotín 505, 783 47 Hněvotín (CZ). **KOLÁŘ, Petr**; Hněvotín 519, 783 47 Hněvotín (CZ). **PÍŽOVA, Klára**; Ve Svahu 4, 73401 Karviná-Ráj (CZ). **HANÁKOVÁ, Adéla**; V hlinkách 2, 779 00 Olomouc (CZ).

(74) Agent: **SOUKUP, Petr**; Vídeňská 8, 772 00 Olomouc (CZ).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, 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, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

(54) **Title**: PLANAR IRRADIATION SOURCE ESPECIALLY FOR INDUCTION AND MONITORING OF PHOTODYNAMIC EFFECT IN VITRO

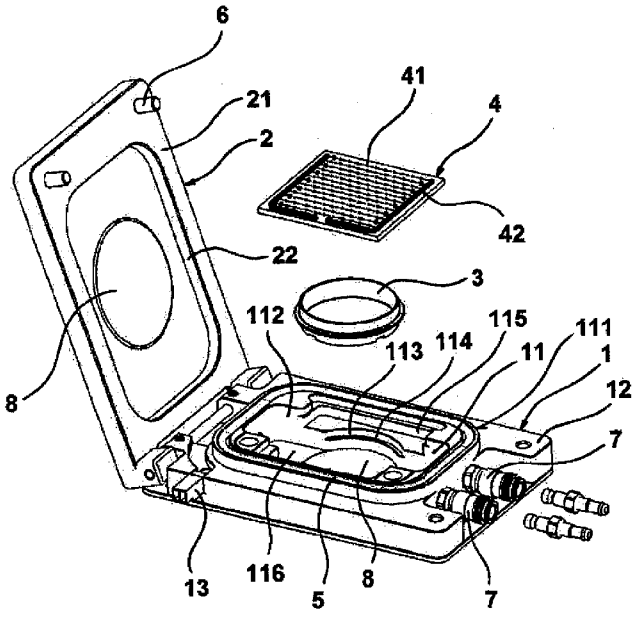

Fig. 2

(57) **Abstract**: The planar irradiation source especially for induction and monitoring of photodynamic effect in vitro in samples placed in a bed (3) which is formed with a sealable body (1) which is procured with a from above opened central chamber (11) modified not only for removable insertion of the bed (3) with analyzed sample but also for above it positioned removable positioning of a semitransparent thin walled irradiation plate (4) which is procured with diode chips (41) placed in the rows with mutual hexagonal arrangement whereas the diode chips (41) are in parallel connected with a bus (42), which is led along the perimeter of the irradiation plate (4) and is connected with an external power supply, and are placed in the irradiation plate (4) in the way that their light beams are directed straight down toward the bed (3).

WO 2016/184441 A1

Planar irradiation source especially for induction and monitoring of photodynamic effect in vitro

Art Domain

The invention concerns a layout of a planar source of irradiation with homogeneity of a light field designed especially for induction and monitoring of a photodynamic effect in vitro, mainly in standard analytic Petri dishes, with an option of continuous monitoring of changes in standardized analytical microplates for its transparency and possibility of carry out of changes in composition and pressure of surrounding atmosphere of analyzed sample.

Present Prior Art

The photodynamic effect represents combined effect of four components, namely a photosensitive compound (sensitizer), molecular oxygen, light and a substrate, most often of biological substance. Resulting product of this reaction is creation of different forms of reactive oxygen, an oxidised substrate and a regenerated sensitizer which was activated by the light. This effect finds its main use in a photodynamic therapy where the sensitizers are preferably trapped in pathologically changed cells. After subsequent irradiation of cells with visible radiation with wavelength identical with absorption maximum of sensitizer there comes to their destruction on which participate mainly singlet oxygen and other radicals. The lifetime of these products in cell environment is very short because they react very fast with surrounding biomolecules. In dependence on size of this way induced changes in their molecular structure, thus in extend of cellular damage, there may come to trigger a series of cellular processes which lead to destruction of the cell. The sensitizer is non-toxic without light activation and it is characteristic for its own fluorescence which is also used in diagnostics. Induced cellular damage is possible to observe by the help of various light activated probes through detection for example of their fluorescence by the help of a spectroflourimeter.

In a preclinical research phase are during the study of efficiency of this type of therapy used various tumour and non-tumour cell cultures are used and cultivated for example in Petri dishes or analytic microplates. Laser or diode light are most often used as a source of light energy for activation of the sensitizer. To form a wider irradiation area there is often placed an optic diffuser between a laser beam and an examined object. For light originating from LED diodes the irradiation field is formed with different arrangement of several LED diodes which form different level of homogeneity of light flow not only for in vivo applications as it is described for example in documents KR20090055891, EP2044974, US2009088824, CN101214403, US2007239233, US2007225778, WO02098508, WO2005035058, WO9321842, but also for applications in vitro as it is known from documents DE102008008875, CZ302829, CZ302084 or WO9321842. The arrangement of diodes in irradiators is either random or in rows with same or different mutual distance.

Suitable arrangement of diodes is connected with formation of a homogenous light field and is described in several files for example in WO2005035058, CZ302829, which solve problem of homogeneity during irradiation from above. The LED diodes are in this design arranged in a hexagonal shape in constant distance from each other and are bonded on a pad above a bed. In design according to the file CN203247266 (U) the homogeneity is reached by the movement of a plate with bonded LED elements and by the help of diffusion on supplementary diffusing glass. An analysis of the sample is commonly carried out by detection of a signal by the help of a detection device which is placed above or under the sample. A disadvantage of this design is that the analysis of final effect of photodynamic therapy in vitro, i.e. determination of total amount of oxidized compound, is possible to carry out after certain time from irradiation. Very often used and favourite detector is in fact a chemical marker which after penetration into cells and its oxidation changes to fluorescent product whose total yield can be easily determined by the help of commercially accessible fluorescent spectrophotometers or readers. Due to fast photophysical-chemical changes connected with formation of reactive forms of oxygen and autophotooxidation of the detection marker itself it is desirable to proceed the measurement continuously in time. Some sort of solution brings device according to CZ302084, where the irradiation is realized from side. This way of design is suitable for relatively small samples and it does not reach the same homogeneity as with direct irradiation from above.

The aim of the presented invention is to introduce for use a planar irradiation source which would be structurally quite simple, would provide light with relatively uniform light flow and at the same time would enable continuous monitoring of products formed during photodynamic effect by the help of commercially accessible spectrophotometers or readers and would have provided possibility of carry out of changes in composition and pressure of surrounding atmosphere of analyzed sample.

Essence of the invention

The given aim is achieved with an invention which is a planar irradiation source especially for induction and monitoring of photodynamic effect in vitro in samples placed in a bed which is formed with a sealable body which is procured with a from above opened central chamber modified not only for removable insertion of the bed with analyzed sample but also for above it positioned removable positioning of a semitransparent thin walled irradiation plate which is procured with diode chips placed in rows with mutual hexagonal arrangement whereas the diode chips are parallel connected with a bus which is led along the perimeter of the irradiation plate and is connected with an external power supply, and are placed in the irradiation plate in the way that their light beams are directed straight down toward the bed.

In an advantageous design is the central chamber is formed in the way that its upper frame overreaches above an upper face of the body and in its side external walls are formed arched grooves which are procured with collars for vertical positioning of the bed and above the arched grooves are formed basically rectangular grooves for placing of the irradiation plate.

It is likewise advantageous when the body is procured with a swing away lid on whose inner face is formed a central groove whose shape and size corresponds with the shape and size of upper frame of the central chamber of the body whereas on the upper frame is placed peripheral sealing which enables hermetical closure of the central chamber.

And finally it is advantageous when in the body are built in sealable passages which are sideway or frontally led into the central chamber and a side port which enables connection of conductors going from the bus of the irradiation plate with a power supply.

In an optimal design the central groove of the lid and also the bottom of the central chamber are procured with transparent windows which enable checking and monitoring of processes inside the central chamber.

The device according to the invention reaches new and higher efficiency in the fact that the structural design of the planar irradiation source enables continuous monitoring of products which are formed during photodynamic effect. Next advantage is that the irradiation plates which are bonded with diode chips are easily removable and it is possible to push in/out change them for other plates with other irradiation characteristic. Indispensable advantages of the device are its low purchase price, small build up size and minimal energetic demandingness of operation.

Description of the figures in enclosed drawings

Particular example of structural design of a planar irradiation source is clarified by the help of enclosed drawings where:

Fig. 1 is a general axonometric view of the irradiation source in closed condition,

Fig. 2 is a view of the irradiation source from the Fig. 1 in exploded design with uncovered lid,

Fig. 3 is a view of an alternative design of the irradiation source in exploded design with uncovered lid,

Fig. 4 is a detail view of a part of the irradiation plate with an arrangement scheme and electric connection of diode chips.

The drawings which illustrate presented invention and consequently described examples of particular designs do not in any case limit extend of the protection mentioned in definition yet only clarify essence of the invention.

Examples of invention design

A planar irradiation source is in its basic design formed with a plate body 1, preferably of rectangular shape which is procured with a from above opened central chamber 11, whose upper frame 111 overreaches an upper face 12 of the body 1. Side external walls 112 of the central chamber 11 are modified for placing of not only a bed 3, for example a Petri dish, with analyzed sample but also a semitransparent thin walled irradiation plate 4. In design according to Fig. 2 there are in side external walls 112 above each other formed arched grooves 113, which are procured with collars 114 for vertical positioning of the bed 3, and basically rectangular grooves 115 for placing of the irradiation plate 4. On upper surface of the rectangular grooves 114 are then placed non-illustrated conductive flexible connectors for possibility of electric connection of the irradiation plate 4 by the help of also non-illustrated conductors which are led out of the body 1 through its side port 13 to a supply source. The body 1 is procured with a swing away lid 2, on whose inner face 21 is formed a central groove 22, whose shape and size corresponds with the shape and size of the upper frame 111 of the central chamber 11 of the body 1. On the upper frame 111 is placed a peripheral sealing 5, which enables hermetical closure of the central chamber 11, for example by the help of screw connections 6, whereas in the body 1 are built in sealable passages 7, which are procured with sealable valves or rapid couplings and which are sideway or frontally led into the central chamber 11 and enable by the help of changes of pressure or kind of blown medium regulation of surrounding atmosphere of analyzed sample. The central groove 22 of the lid 2 and also the bottom 116 of the central chamber 11 are then procured with transparent windows 8 which enable checking and monitoring of processes inside the central chamber 11.

The semitransparent thin walled irradiation plate 4, which is basic function element of the planar irradiation source, is procured with diode chips 41 which are placed in rows with mutual hexagonal arrangement and are placed in the way that their light beams are directed straight down toward the bed 3. Particular rows with in series connected diode chips 41 are in parallel connected with a bus 42 which is led along the perimeter of the irradiation plate 4, as it is clear from Fig. 4. The bus 42 is then connected, by the help of non-illustrated conductors which are led out of the body 1, through its side port 13, to power supply.

The structure of entire planar irradiation source but especially arrangement of the diode chips 41 must be selected in the way to reach maximal homogeneity of the light field and for influence of partial shading to be relatively independent on positioning of the sample in the bed 3. Therefore is suitable the hexagonal arrangement of the diode chips 41. Surface density of the diode chips 41 then directly determines level of homogeneity and transparency of the source. The bigger it is the lower is the transparency and vice versa. During study of photodynamic processes is the issue of homogeneity is more important and from the point of view of theoretical calculations it is the most suitable when the transparency of the irradiation plate 4 does not uselessly exceed 50 %.

Efficiency and functionality of the planar irradiation source has been tested for contemporary, commercially produced highly illuminative diode chips 41 with maximal size 0.33 and 0.40 mm with irradiation performance of one diode chip 41 to 50 mW and with emission of radiation in the range of wavelength 415 and 660 nm. For arrangement of field with 195 chips was formed a homogeneous irradiated surface for detection area of the bottom of a Petri dish with diameter 35 mm. For achievement of higher homogeneity it would be optimal to increase this amount of chips in compliance with former paragraph. For the diode chips 41 with size 0.4 x 0.4 mm with neglect of narrow in series connection the amount of diode chips 41, which corresponds with surface with diameter 35 mm when kept 50% permeability, would be app. 6 013 (π x $17.5^2 / 0.4^2$).

The described structure of the planar irradiation source is not the only possible design of the invention but without influence on its essence can be solved own structure of the body 1 in another way and can be used different amount of the diode chips 41 according to their size and the size of analyzed area thus diameter of the bed 3. The sealable passages 7 can be led out from the body 1 sideway or frontally and hermetical sealing of the central chamber 11 can be realized another way than with the screw connection 6.

Industrial usability

The irradiation source according to the invention is possible to use for monitoring of photodynamic changes with in vitro methods in standard beds, especially for a photodynamic therapy which is used for destruction of tumour cells by the help of singlet oxygen and other radicals which are created in tumour tissue after its irradiation with light. By its size and transparency it enables study of photodynamic processes in common commercially accessible microplate analyzers.

PATENT CLAIMS

1. A planar irradiation source especially for induction and monitoring of photodynamic effect in vitro in samples placed in a bed (3) **wherein** it is formed with a sealable body (1) which is procured with a from above opened central chamber (11) modified not only for removable insertion of the bed (3) with analyzed sample but also for above it positioned removable positioning of a semitransparent thin walled irradiation plate (4) which is procured with diode chips (41) placed in the rows with mutual hexagonal arrangement whereas the diode chips (41) are in parallel connected with a bus (42), which is led along the perimeter of the irradiation plate (4) and is connected with an external power supply, and are placed in the irradiation plate (4) in the way that their light beams are directed straight down toward the bed (3).

2. The planar irradiation source according to the claim 1 **wherein** the central chamber (11) is formed in the way that its upper frame (111) overreaches above an upper face (12) of the body (1) and in its side external walls (112) are formed arched grooves (113), which are procured with collars (114) for vertical positioning of the bed (3), and above the arched grooves (113) are formed basically rectangular grooves (115) for placing of the irradiation plate (4).

3. The planar irradiation source according to the claim 1 or 2 **wherein** the body (1) is procured with a swing away lid (2), on whose inner face (21) is formed a central groove (22), whose shape and size corresponds with the shape and size of the upper frame (111) of the central chamber (11) of the body (1), whereas on the upper frame (111) is placed a peripheral sealing (5) which enables hermetical closure of the central chamber (11).

4. The planar irradiation source according to some of the claims 1 to 3 **wherein** in the body (1) are built in sealable passages (7), which are sideway or frontally led into the central chamber (11), and a side port (13) which enables connection of conductors going from the bus (42) of the irradiation plate (4) with the power supply.

5. The planar irradiation source according to some of the claims 1 to 4 **wherein** the central groove (22) of the lid (2) and also the bottom (116) of the central chamber (11) are procured with transparent windows (8) which enable checking and monitoring of processes inside the central chamber (11).

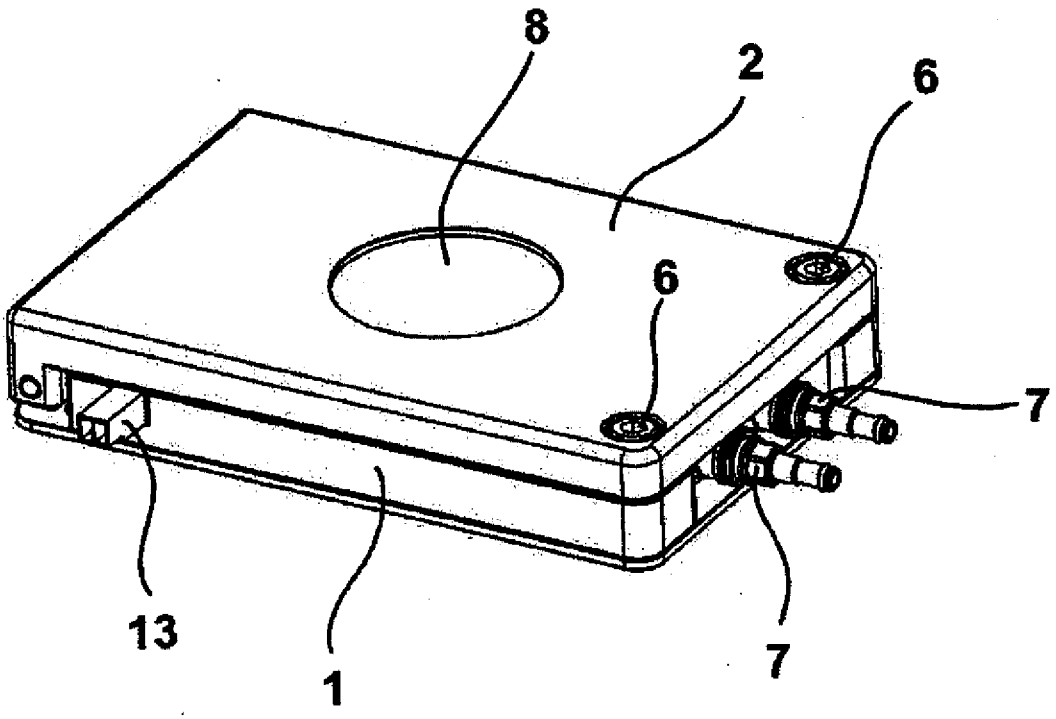

Fig. 1

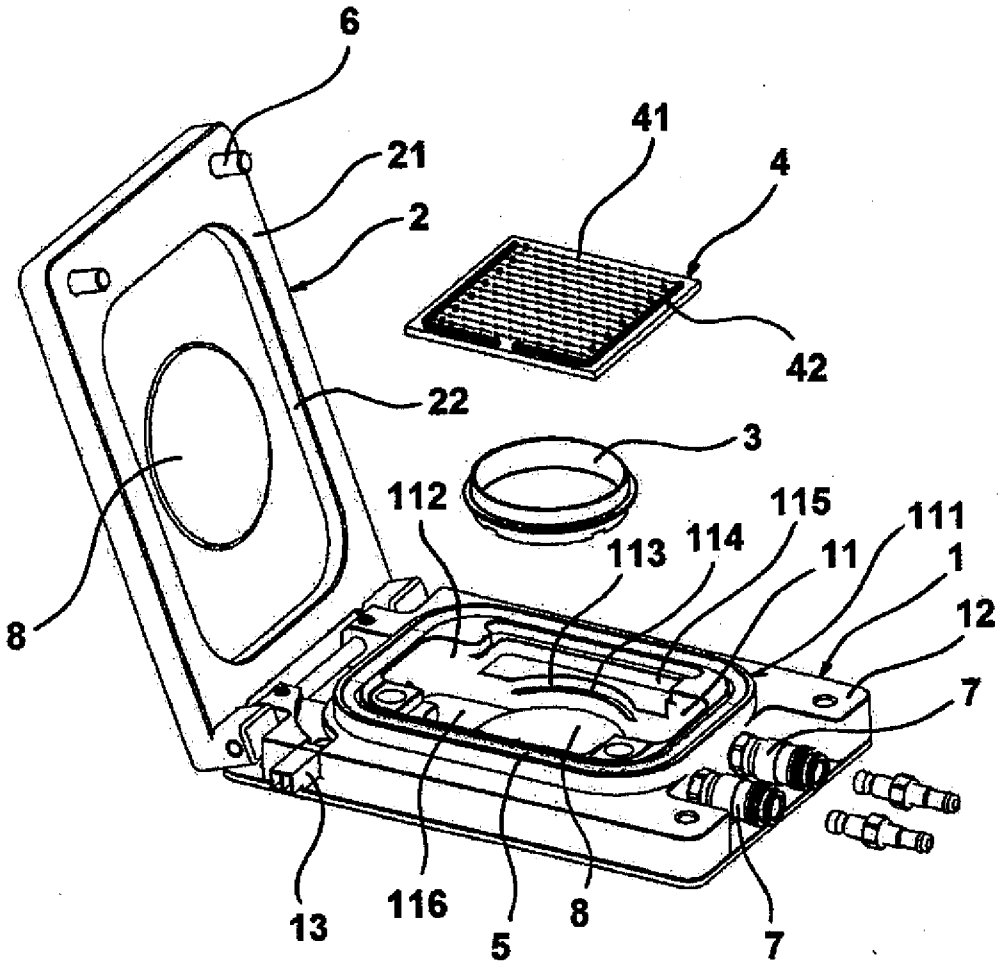

Fig. 2

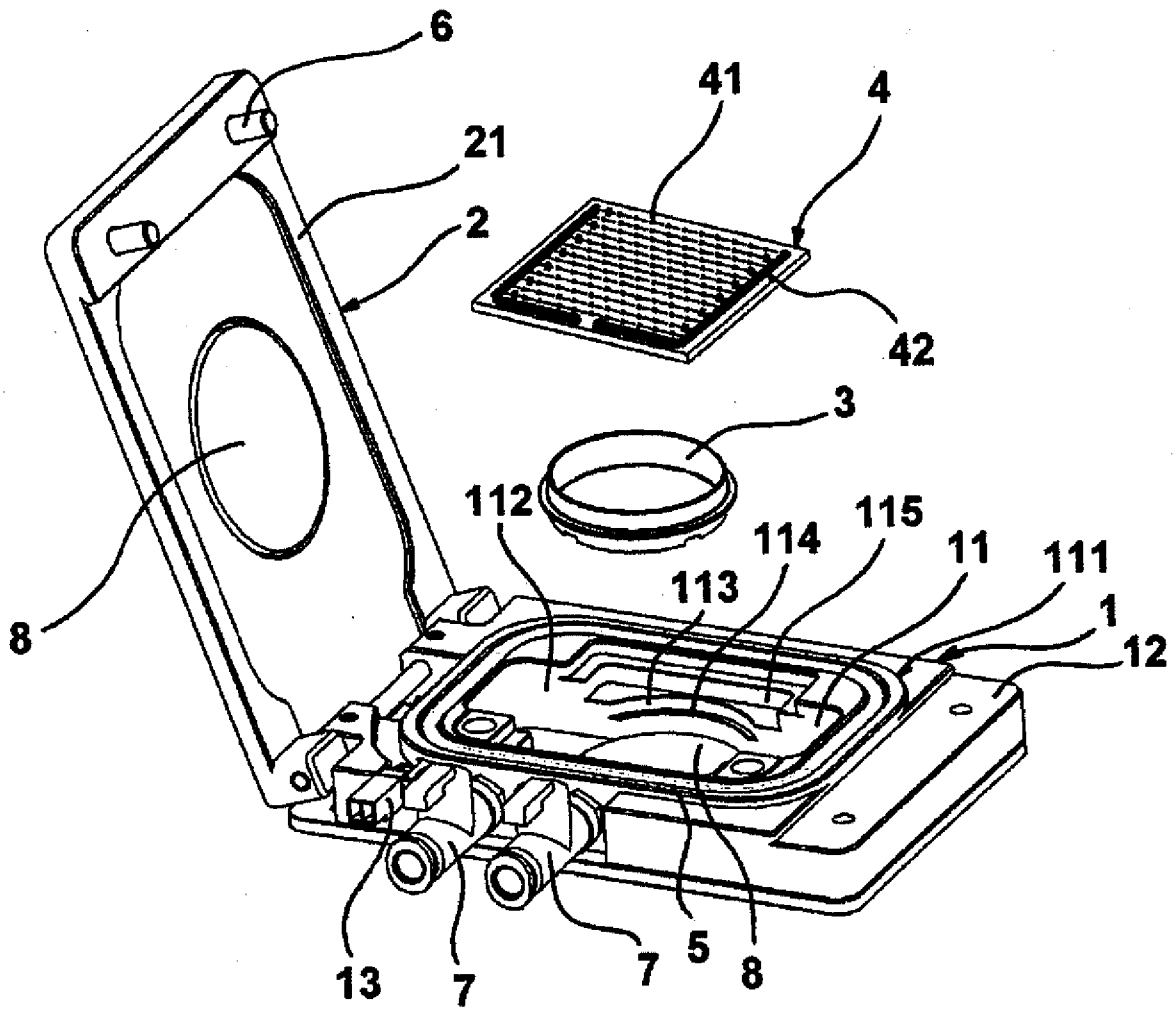

Fig. 3

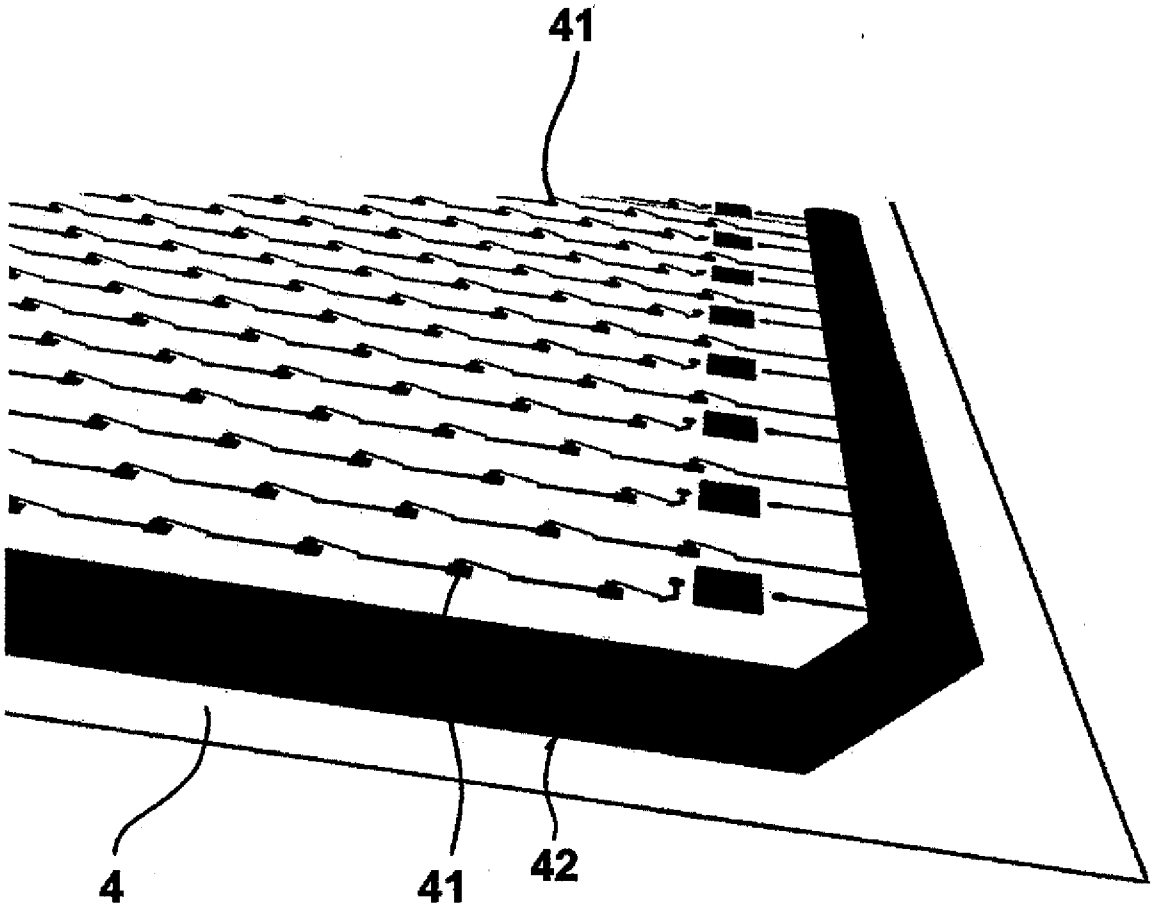

Fig. 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/CZ2015/000125

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61N5/06 C12M1/00
ADD.

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)
A61N C12M

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 practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CN 101 701 183 A (UNIV FUJIAN) 5 May 2010 (2010-05-05) claims 1-7; figures 1-8	1-5
Y	CN 203 174 082 U (XIAO YAGUO) 4 September 2013 (2013-09-04) claim 1; figures 1-4	1-5
Y	CZ 302 829 B6 (UNIVERZITA PALACKAHO V OLOMOUC [CZ]) 30 November 2011 (2011-11-30) cited in the application figures 1-4	1-5
Y	US 2003/048927 A1 (SATO TOSHIYUKI [JP] ET AL) 13 March 2003 (2003-03-13) figures 2,3	2

☐ 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 another 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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 March 2016	14/04/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Gentil, Tamara

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/CZ2015/000125

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
CN 101701183 A	05-05-2010	NONE	
CN 203174082 U	04-09-2013	CN 203174082 U	04-09-2013
		TW M437848 U	21-09-2012
CZ 302829 B6	30-11-2011	NONE	
US 2003048927 A1	13-03-2003	CN 1407331 A	02-04-2003
		KR 20030022661 A	17-03-2003
		TW 539853 B	01-07-2003
		US 2003048927 A1	13-03-2003