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(54) **Rotor for a thermal cycler and thermal cycler**

(57) The present invention pertains to a rotor (1) for a thermal cycler providing an environment, the rotor comprising holding fixtures (2) each for holding a reaction container (3) capable of being filled with a reaction mixture providing a micro-environment, wherein at least two holding fixtures (2) are provided which have different distance from the axis of the rotor (1). The present invention also pertains to a thermal cycler comprising a receptacle for a rotor for placement of microenvironments in which a reaction mixture might be filled, the rotor comprising at least two holding fixtures (2) for a micro-environment, wherein the at least two holding fixtures (2) have different distance from the axis of the rotor.

The respective rotor (1) and the thermal cycler are advantageous for a thermal cycling process having high capacity.

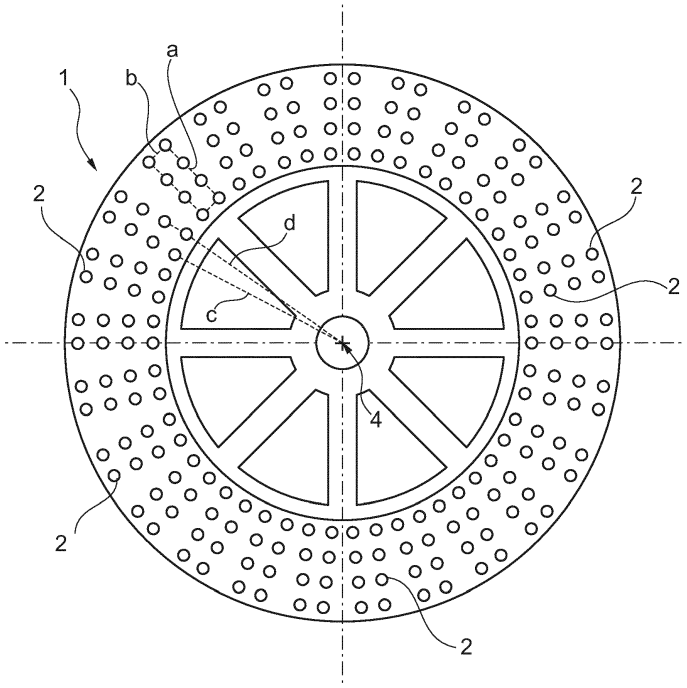

Fig. 1

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Description

[0001] The invention pertains to the field of laboratory equipment for chemical, biological or for biotechnological methods which require handling of multiple vessels. In particular the invention relates to a rotor for a thermal cycler.

[0002] Many laboratory methods require handling in such a way that the process or a particular step in the process is dependent on analysis and/or treatment of the respective reaction mixture..

[0003] One example for a process which requires specific handling of vessels and analysis of parameters is the polymerase chain reaction (PCR). PCR is a technique in molecular biology that allows polynucleotide sequences to be amplified. The basic principle of PCR is well known in the art and is described in many books, including, PCR: A Practical Approach M. J. McPherson, et al., IRL Press (1991), PCR Protocols: A Guide to Methods and Applications by Innis, et al., Academic Press (1990), and PCR Technology: Principals and Applications for DNA Amplification H. A. Erlich, Stockton Press (1989). PCR is also described in many U.S. patents, including U.S. 4,683,195; 4,683,202; 4,800,159; 4,965,188; 4,889,818; 5,075,216; 5,079,352; 5,104,792; 5,023,171; 5,091,310; and 5,066,584.

[0004] Each PCR cycle usually comprises three basic discrete temperature steps, a denaturation step, an annealing step and an extension step. Denaturation of DNA typically takes place at around 90 to 95°C, annealing a primer to the denatured DNA is typically performed at around 40 to 60°C, and the step of extending the annealed primers with a polymerase is typically performed at around 70 to 75°C. Therefore, during a PCR cycle the temperature of the reaction mixture must be varied repeatedly. The PCR technique has a wide variety of biological applications, including but not limited to for example, DNA sequence analysis, probe generation, cloning of nucleic acid sequences, site-directed mutagenesis, detection of genetic mutations, diagnoses of viral infections, molecular "fingerprinting" and the monitoring of contaminating microorganisms in biological fluids and other sources.

[0005] The PCR is usually carried out in a laboratory apparatus called thermal cycler or PCR cycler.

[0006] In addition to PCR, other in vitro amplification procedures, including ligase chain reaction as disclosed in U.S. Patent No. 4,988,617 to Landegren and Hood, are known and used in the prior art. More generally, several important methods known in biotechnology, such as nucleic acid hybridization and sequencing, are dependent upon changing the temperature of solutions containing sample molecules in a controlled fashion and also require monitoring.

[0007] Conventional techniques rely on use of individual wells or tubes cycled through different temperatures. For example, a number of thermal "cyclers" used for DNA amplification and sequencing are disclosed in the prior art in which a temperature controlled element or "block" holds a reaction mixture, and wherein the temperature of the block is varied over time. An advantage of these devices is that a relatively large number of samples can be processed simultaneously, e.g. 96 well plates are commonly employed.

[0008] Different designs of such thermal cyclers are known including devices for the thermal cycling of multiple samples. A common format of such devices uses a block of heat conductive material which has a plurality of channels or cavities for receiving vessels - such as reaction tubes or plates - in which the desired reactions are executed. Monitoring of temperature is relatively easy in such devices since a temperature sensor can be associated with the block.

[0009] However, such block devices suffer various drawbacks, e.g. in that they are relatively slow in cycling the reaction mixtures, they are relatively energy intensive to operate and detection of parameters of the reaction mixture in situ is difficult. In an effort to avoid several of these disadvantages, thermal cyclers have been developed in which a plurality of containers for holding reaction mixture(s) are supported on a rotor which is rotated in a controlled temperature environment (also known as "Rotor-Gene"). Temperature cycling is effected by heating and cooling of the environment. The rotor has apertures, receptacles or slots for the containers, vials or tubes located at a circumference of the rotor. Rotors are known, which have 60 or 72 receptacles for a corresponding number of vials or tubes. An example of such a device is disclosed, for example, in WO 98/49340 A1.

[0010] The rotor disclosed in WO 98/49340 A1 consists of a high temperature plastic and can be driven by a motor which is controlled by a computer. The rotor comprises a number of slots and glass capillary tubes positioned at the circumference of the rotor. The glass capillary tubes contain a reaction mixture, especially for carrying out the polymerase chain reaction. The rotor is rotated and the reaction mixtures are monitored by a real time fluorescence optical system and the resulting data transferred to a computer analysis.

[0011] The present invention seeks to solve the problem of providing an improved rotor and a thermal cycler.

[0012] The problem is solved by a rotor for a thermal cycler providing an environment, wherein the rotor comprises holding fixtures each for holding a reaction container providing a micro-environment capable of being filled with a reaction mixture, wherein at least two fixtures are provided which have different distance from the axis of the rotor.

[0013] The amount of reaction mixture during one run relates to the number of micro-environments which can be monitored in one run. In the prior art it was known to increase the number of micro-environments held by the rotor by providing an increased amount of holding fixtures at the periphery of the rotor thereby increasing the diameter of the rotor. The present invention provides a new approach by providing holding fixtures even in an inner

area of the rotor. With regard to the arrangement of the holding fixtures, the common thinking was overcome that the fixtures and the respective micro-environments need to be positioned at the peripheral circumference.

[0014] According to the invention a high speed thermal cycler can be realized with the high number of reaction containers which may be used in one run of the thermal cycleras the position of the holding fixtures is not limited to the peripheral circumference of the rotor. It allows an even higher number of reaction containers to be held or positioned in the rotor without increasing the diameter of the rotor. Thus, a high speed thermal cycler with a high capacity can be obtained, maintaining a small volume of the thermal cycler.

[0015] According to the invention a "thermal cycler" is a laboratory apparatus or device for carrying out thermal cycles with regard to a reaction process, especially a polymerase chain reaction. The thermal cycler is capable of raising and lowering the temperature of an environment in which micro-environments are provided in discrete, pre-determined steps.

[0016] It will be appreciated from the above that the term thermal cycler is particularly directed to a thermal cycler for nucleic acid amplification, wherein the reaction vessels are supported on a rotatable circular carousel or rotor rotatably mounted within a chamber. Particularly preferred thermal cyclers are the Rotor-Gene™ family of thermocyclers manufactured and distributed by Qiagen GMBH (www.qiagen.com), most preferably the Rotor-Gene Q. Other similar devices are disclosed in WO 92/20778 A1 and WO 98/49340 A1. However, it will be appreciated that other thermal cyclers are also to be intended.

[0017] The term "environment" according to the invention means any temperature-controlled environment established in a device, e.g. in a chamber of an apparatus. E.g. the temperature in said environment can be controlled for a process or a step within a process to be carried out at a specific temperature. The term "environment" preferably refers to a heatable reaction chamber, especially preferred a heatable reaction chamber of a thermal cycler. Temperature cycling is achieved in a respective thermal cycler by heating and cooling the environment, here the (insulated) chamber.

[0018] The term "micro-environment" according to the invention relates to a volume in which a reaction mixture can be filled. Usually, the volume of the micro-environment is in the range of 0.01 to 2.0 ml, preferably 0.1 to 0.6 ml. The term "reaction mixture" according to the invention means an amount of liquid, solution or suspension intended to be subjected to qualitative or quantitative determination of any of its properties, such as the presence or absence of a component, the concentration of a component, etc. The reaction mixture is subjected to cyclic heating and/or cooling, especially in accordance to carry out a polymerase chain reaction.

[0019] The term "holding fixture" according to the invention is intended to encompass any form of a bore, aperture, slot, receptacle or opening which is capable for holding a reaction container or otherwise providing the micro-environment in which the reaction mixture can be filled. The term "reaction container" is intended to encompass inter alia a vial, vessel, well, tube or any other container suitable for being filled with the reaction mixture.

[0020] The term "rotor" according to the invention is intended to encompass any disc, plate or device which may be rotated around an axis.

[0021] The term "circumference" according to the invention is intended to encompass the shape of a circle which surrounds the axisof the rotor. According to the invention the holding fixtures are preferably arranged such that the centers of the holding fixtures do not only lie on one circumference but on several different circumferences having a different distance to the axis of the rotor. Preferably holding fixtures are provided which are positioned at least partly one behind the other in the radial direction from the axis of the rotor.

[0022] Preferably, the holding fixtures are arranged in concentric circles around the axis of the rotor.

[0023] The holding fixtures can be arranged in concentric circles around the axis of the rotor.

[0024] Preferably, the holding fixtures of different circumferences are positioned on a straight line from the outside to the inside of the rotor. Such an arrangement is simple to handle and permits for an automated load and an automated removal to and from the micro-environments via a reaction mixture transfer device. With the reaction mixture transfer device a transfer of the reaction mixture and/or a reaction container comprising the reaction mixture is possible.

[0025] In a preferred embodiment the straight line of the holding fixtures is offset with regard to the axis of the rotor. The term "is offset" according to the invention is intended to encompass that the straight lines do not intersect the axis of the rotor. Such an embodiment provides a simple geometry and permits for an automated load and an automated removal because holding fixtures can be arranged such that at least two straight lines of holding fixtures adjacent to each other can be obtained wherein the at least two straight lines extend parallel to each other. The straight lines allow an automated transfer of the reaction mixtures from/to the micro-environments of the rotor. The micro-environments or reaction containers can be automatically filled by a sample transport device, which can aspirate reaction mixtures from a plate with a rectangular arrangement of wells (96-well plate) and transport it to the micro-environments in the rotor and inject the reaction mixtures therein. The transport device can have pipettes which are arranged in a rectangular shape in two columns and four lines. Alternatively, the transport device can be arranged for a transport of the reaction containers being arranged in a pad or plate in rectangular shape. The transport device may be arranged to move the reaction containers from the pad or plate into the holding fixtures of the rotor. The transport device can grip or pick two columns of four lines of reac-

tions containers arranged in a plate of twelve columns and eight lines. This enhances the efficacy of the reaction process because the time used for preparation is reduced.

[0026] Preferably, the holding fixtures are arranged in at least one group such that in the at least one group of holding fixtures a connection line between holding fixtures of a line around the center of the rotor spans an angle of 80° to 100° with a connection line of two holding fixtures of different distance from the center of the rotor. The specification of the angle as being 80° to 100° is intended to encompass all the values of angles in the mentioned interval, however, especially the angle of 90° with a small deviation is mostly preferred. The mentioned arrangement permits an simple automatic transfer from or to a well plate in which the reaction mixtures are held in an rectangular manner with regard to each other. The arrangement of the holding fixtures in the rotor is such that the rectangular arrangement of the reaction containers in a known 96-well plate can be transferred to the round cycler format of the rotor. As mentioned above it is also possible that the reaction mixture is not transferred together with the reaction container but aspirated by several pipettes arranged in rectangular shape, especially two columns with four lines, at once and then injected to reaction containers held by the holding fixtures of the rotor.

[0027] The reaction containers can be "bundled" such that several reaction containers can be transferred to and from the rotor at the same time.

[0028] In a preferred embodiment the number of holding fixtures is at least 100, more preferably 200. Preferably, the holding fixtures are arranged in four concentric circles around the axis of the rotor which permits the arrangement of at least 192 reaction containers in the rotor and eight fixtures for additional use.

[0029] Further, a thermal cycler is provided which comprises an optical system and a receptacle for a rotor e.g. as described above. In the thermal cycler preferably at least two optical systems are provided and arranged such that at least two micro-environments having a different distance from the axis of the rotor or being on a different circumference may be analyzed by the optical systems. Preferably, the number of optical systems corresponds to the number of circumferences of micro-environments around the axis of the rotor. Preferably, the optical systems are provided in proximity to each other, i.e. adjacent to each other, which simplifies the arrangement for the wiring of the optical systems. Thus, a thermal cycler is provided with a high sample or reaction mixture capacity which may not have bigger dimensions than known thermal cyclers.

[0030] The term "optical system" according to the present invention is intended to encompass an optical system which allows determination of at least one optical property of the reaction mixture in the micro-environment, i.e. the reaction container. The optical system can include an illumination source, such as an LED, and a corresponding optical detector, e.g. a photomultiplier for detecting reflected illumination or emitted fluorescence.

[0031] Preferably, the at least two optical systems comprise each two confocal systems which allows for two channels for each of the optical systems. When comprising two confocal systems per optical system it becomes possible to determine two channels (colours or wavelengths) for each of the lines of the micro-environments in the rotor at the same time. This determination enhances the efficacy of the process even more because the determination may include validation by two channels. Further, the occurrence of two different channels permits to use two different luminophores which can be determined with high accuracy.

[0032] Further, preferably the confocal systems comprise two excitation sources with different excitation wavelength which permits the use of two different luminophores and a simple detector system in which the focus or image point of one lens is the same as one focus of the next lens.

[0033] Other objects, features, advantages and aspects of the present application will become apparent to those skilled in the art from the following description and appended claims. It should be understood, however, that the following description, appended claims, and specific examples, while indicating preferred embodiments of the application, are given by way of illustration only. Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art reading the following.

[0034] Examples of the invention will now be described with reference to the accompanying drawings in which:

Figure 1 is a schematic diagram as a top view of a rotor according to the invention;
Figure 2 is a schematic diagram of the rotor according to Figure 1 in a thermal cycler indicating the position of optical systems of the thermal cycler; and
Figure 3 is a schematic diagram in an isometric view of the rotor according to Figure 1 in a thermal cycler and optical systems of the thermal cycler as well as a plate in which reaction containers are stored.

[0035] Figure 1 schematically depicts a rotor 1 for a thermal cycler, especially for real time PCR. A thermal cycler provides an environment which can be subjected to heating and cooling and the rotor can be inserted into a thermal cycler. The rotor 1 comprises holding fixtures 2. The holding fixtures 2 are arranged to hold a reaction container 3 (see Fig. 3) which provides a micro-environment in which a reaction mixture might be filled. The holding fixtures 2 are arranged in several circumferences around the axis/center 4 (indicated by an "+" in Fig. 1 and 2) of the rotor 1.

[0036] The holding fixtures 2 are arranged in circumferences around the center 4 of the rotor 1. The circumferences have the shape of concentric circles around the center 4 of the rotor 1. The number of concentric circles

around the center 4 of the rotor 1 is four. On each of the concentric circles fifty holding fixtures 2 are arranged. The holding fixtures 2 can be grouped in four groups containing 50 holding fixtures 2 each, each group containing holding fixtures 2 having the same distance with regard to the center 4 of the rotor 1.

[0037] The reaction containers 3 are held in the holding fixtures 2 substantially parallel to the rotation axis of the rotor 1 which is perpendicular to the paper plane in Fig. 1 and intersects the center 4 of the rotor 1. The reaction containers 3 extent through the holding fixtures 2 of the rotor 1. Thus, the reaction containers 3 extent through the rotor 1 parallel to the rotation axis of the rotor 1.

[0038] The holding fixtures 2 of different circumferences are arranged such that a straight line connects holding fixtures 2 (center of the holding fixtures 2) of different circumferences from the outside to the inside of the rotor 1. Two of the different circumferences are visualized by the dashed lines c and d which extend radially from the center 4 of rotor 1, wherein the line c denotes the circumference for the holding fixtures 2 next to the center 4 and the line d denotes the neighbouring circumference for the holding fixtures 2 which are arranged next to the holding fixtures with circumference c. The straight line connecting the holding fixture 2 of the outer circumference and the holding fixture 2 of the inner circumference is visualized by the dashed line denoted by "a". Each straight line is offset to the center 4 of the rotor 1 such that the straight lines do not intersect the center 4. The offset of the straight lines can be small.

[0039] Further, the holding fixtures 2 are arranged in at least one group such that in the at least one group of holding fixtures 2 a connection line (visualized by the dashed line denoted by "b") between holding fixtures 2 of a circumference spans an angle of substantially 90° with a connection line of two holding fixtures 2 of different distance from the center 4 of the rotor 1. In Figure 1 one of the afore-mentioned groups is indicated by a dotted line. The group comprises eight holding fixtures 2. The eight holding fixtures 2 are arranged in the shape of a rectangular with sides a and b. As can be seen from Fig. 1 the side a of the rectangulars is not parallel line which extends radially from the center 4 of the rotor 1. The rectangular shape of the eight holding fixtures 2 permits to transfer eight reaction containers 3 from/to a well by an automatic transfer device grabbing the eight reaction containers 3 at once.

[0040] In the embodiment shown in Figure 1 the number of holding fixtures 2 in the rotor 1 is two hundred.

[0041] As explained, the rotor 1 might be inserted into a thermal cycler. The thermal cycler includes a chamber establishing an environment and containing at least one reaction container as a micro-environment which contains the reaction mixture and is arranged in the rotor 1. The thermal cycler typically includes a controller, for example a processor, coupled to a heater and a cooler. The heater is typically a convection heater, or similar, arranged to heat air in the chamber. Heating/cooling the chamber will influence the temperature of the micro-environment and thus the temperature of the reaction mixture contained therein. In the chamber a temperature sensor is arranged which is connected to the controller. With the temperature sensor the temperature of the air inside the chamber can be sensed by the controller. Further, the controller is operatively connected to the heater and the cooler.

[0042] The rotor 1 is insertable into the thermal cycler and the thermal cycler provides a motor by which means the rotor 1 can be rotated around an axis which intersects the center 4 of the rotor 1 and which is perpendicular to the paper plane of Fig.1.

[0043] For investigation purposes each micro-environment can be placed by rotating the rotor 1 in a position that the micro-environment held in the respective holding fixture 2 can be irradiated by a light source, which is preferably a LED. For this purpose the reaction containers 3 are transparent with regard to the light which impinges the reaction container 3. Usually the light emitted from the light source passes a filter on the optical way. A light detector is provided for detecting light intensity as a function of wavelength. The detector can comprise a filter as well as a photomultiplier tube. The detector is operatively connected to the controller for receiving the measured fluorescence intensity as well as for setting a gain of the detector.

[0044] In Fig. 2 a schematic diagram of the rotor 1 according to Figure 1 in a thermal cycler is shown. The position of optical systems 6 of the thermal cycler is indicated. Three different positions of the optical systems 6 are indicated by reference signs 5', 5" and 5"'. For each of the lines (concentric circles) one optical system 6 is provided. The four optical systems 6 are shown for each of the depicted three positions 5', 5", 5"'. The arrangement of the four optical systems 6 of each of the three positions 5', 5", 5"' is such that four micro-environments /reaction containers 3 having different distance from the center 4 of the rotor 1 can be analyzed by the optical systems 6. For this purpose the motor that rotates the rotor 1 is controllable by the controller of the thermal cycler such that the reaction containers 3 can be moved / rotated such that each of the reaction containers 3 can be analyzed by the optical detectors.

[0045] Each optical system 6 comprises two confocal systems and the confocal systems comprise two excitation sources with different excitation wavelength. Further, the confocal systems comprise two detectors corresponding to the two different excitation wavelengths. Especially, the fluorescence of the reaction mixture in the reaction container 3 can be determined by the optical systems 6.

[0046] In Fig. 3 an isometric view of a part of the thermal cycler (the rotor 1 and the optical systems 6 of the thermal cycler) is shown. Further, 96-well plates 7 are shown which are capable of holding reaction containers 3. The arrangement of the reaction containers 3 in the 96-well plate 7 is such that the reaction containers 3 are held in

a rectangular shape in columns and lines. The geometry of the holding fixtures 2 in the rotor 1 permits to transfer the reaction containers 3 held by the 96-well plate 7 to the holding fixtures 2 in the rotor 1. The transfer can be carried out by a manipulator arranged to grip the eight reaction containers 3 shown in the 96-well plate 7 at once and put them in the holding fixtures 2 of the rotor 1.

Claims

1. A rotor (1) for a thermal cycler providing an environment, the rotor (1) comprising holding fixtures (2) each for holding a reaction container (3) capable of being filled with a reaction mixture providing a micro-environment, **characterized in that** at least two holding fixtures (2) are provided which have different distance from the axis (4) of the rotor (1).

2. The rotor (1) according to claim 1, **characterized in that** one of the at least two holding fixtures (2) is a member of a group of holding fixtures (2) having a first distance from the axis (4) of the rotor (1) and the other one of the at least two holding fixtures (2) is a member of a group of holding fixtures (2) having a second distance from the axis (4) of the rotor (1) wherein, the first distance is smaller than the second distance.

3. The rotor according to claim 1, **characterized in that** groups of holding fixtures (2) are provided in which the holding fixtures (2) of one group are arranged with respect to the mid point of the holding fixtures (2) along the shape of a rectangular with sides (a) and (b).

4. The rotor (1) according to any one of claims 1 to 3, **characterized in that** the holding fixtures (2) are arranged in circumferences around the axis (4) of the rotor (1).

5. The rotor (1) according to any one of claims 1 to 4, **characterized in that** the holding fixtures (2) of different circumferences extend in a straight line from the outside to the inside of the rotor (1).

6. The rotor (1) according to claim 5, **characterized in that** the straight lines are offset with regard to the center (4) of the rotor (1).

7. The rotor (1) according to any one of claims 1 to 6, **characterized in that** the holding fixtures (2) are arranged in at least one group such that in the at least one group of holding fixtures (2) a connection line (b) between holding fixtures (2) of a line around the center (4) of the rotor (1) spans an angle of 80° to 100° with a connection line (a) of two holding fixtures (2) of different distance from the center (4) of the rotor (1).

8. The rotor (1) according to any one of claim 1 to 7, **characterized in that** the number of holding fixtures (2) is at least 196.

9. A thermal cycler comprising a receptacle for a rotor (1) for placement of micro-environments in which a reaction mixture might be filled, the rotor comprising at least two holding fixtures (2) for a micro-environment, **characterized in that** the at least two holding fixtures (2) have different distance from the axis (4) of the rotor (1).

10. The thermal cycler according to claim 9, **characterized in that** at least two optical systems (6) are provided and arranged such that the at least two micro-environments having different distance from the center (4) of the rotor (1) can be analyzed by the optical systems (6).

11. The thermal cycler according to claim 10, **characterized in that** the at least two optical systems (6) comprise each two confocal systems.

12. The thermal cycler according to claim 11, **characterized in that** the confocal systems comprise two excitation sources with different excitation wavelength.

13. The thermal cycler according to claim 12, **characterized in that** the confocal systems comprise two detectors corresponding to the two different excitation wavelengths.

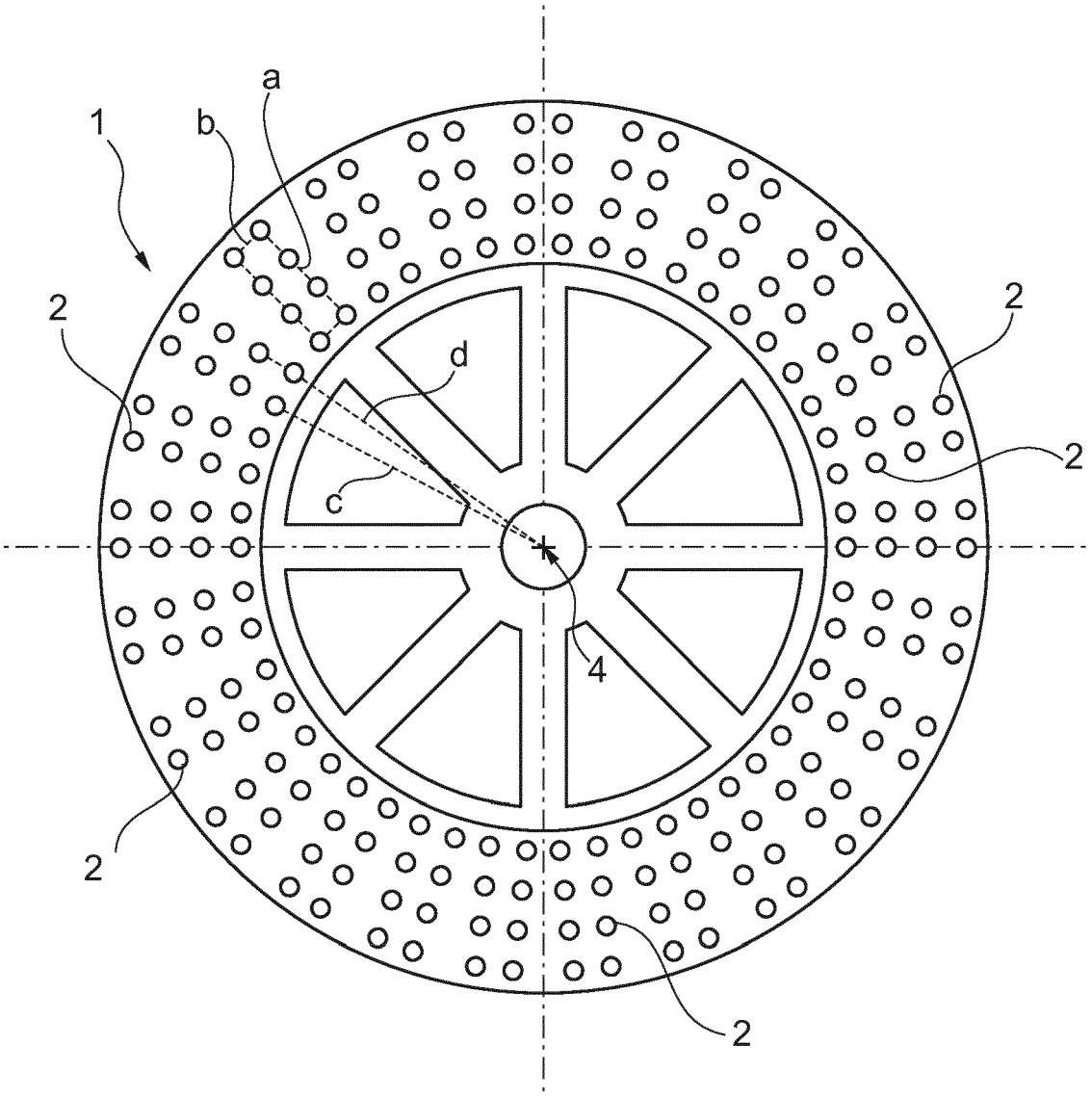

Fig. 1

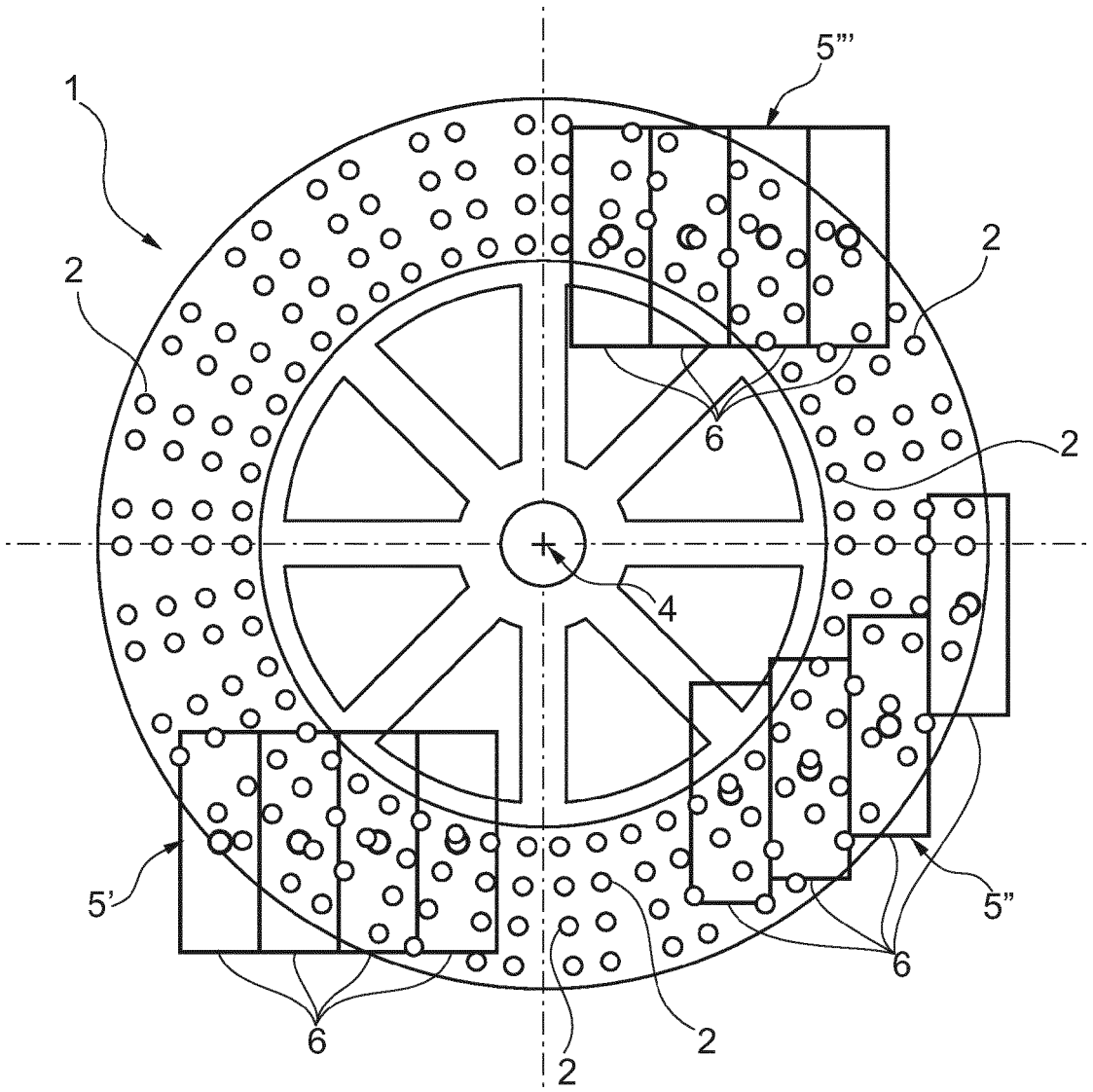

Fig. 2

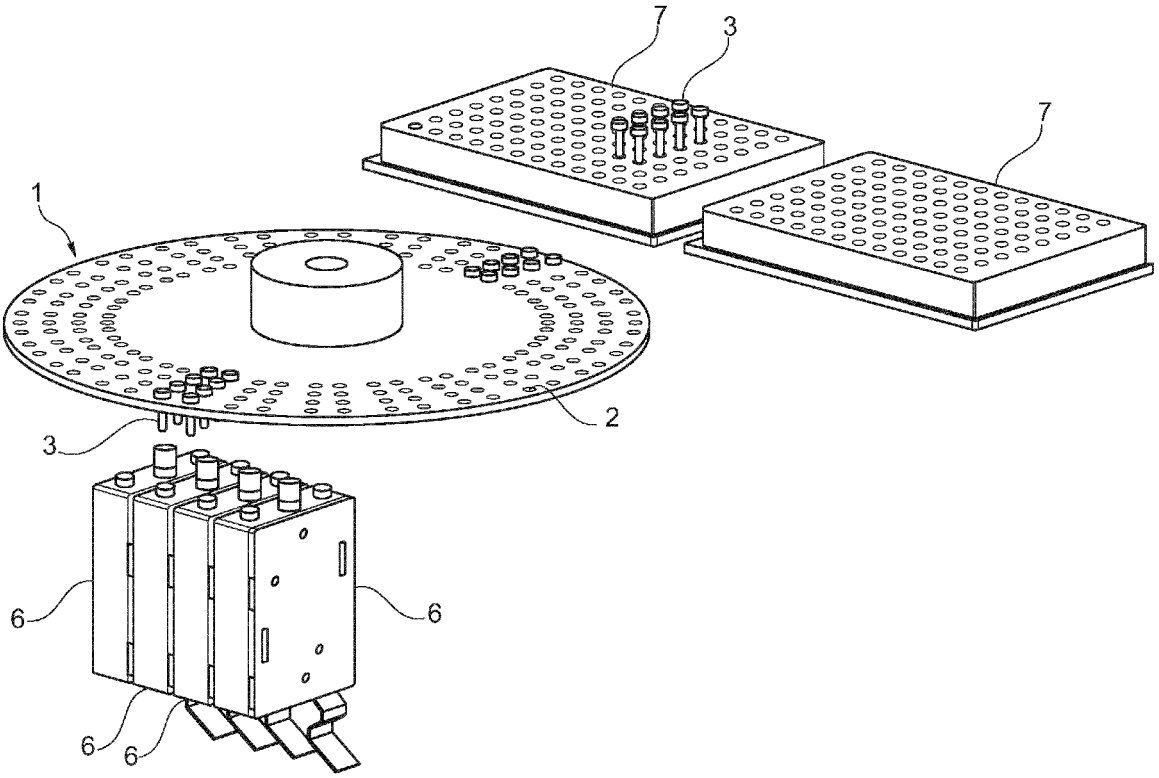

Fig. 3

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EUROPEAN SEARCH REPORT

Application Number
EP 12 17 6913

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
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X	US 2002/155591 A1 (FARINA EDWARD FRANCIS [US] ET AL) 24 October 2002 (2002-10-24) * abstract * * paragraphs [0022], [0053]; figure 1 * -----	1-8	TECHNICAL FIELDS SEARCHED (IPC) B01L B04B

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
The Hague	21 January 2013	Sinn, Cornelia

CATEGORY OF CITED DOCUMENTS

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EPO FORM 1503 03.82 (P04C01)

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 12 17 6913

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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

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