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(54) **THERMAL CYCLER.**

(57) The invention refers to a system for thermal cycling, comprising at least one vessel holder for taking up a reaction vessel providing a contact surface for the reaction vessel, wherein the vessel holder is surrounded by liquid channels for cooling and at least one electrical heater is attached to the vessel holder; and a multilayer liquid port for attachment of at least one vessel holder for providing the cooling liquid to the at least one reaction vessel holder.; and at least one control unit for connecting with the at least one electrical heater.

THERMAL CYCLER

Field of the Invention

[0001] The invention relates to a system for thermal cycling enabling PCR (polymerase chain reaction).

Background of the invention

[0002] Automated analyser systems for use in clinical diagnostics and life sciences are produced by a number of companies. For example, the Stratec Biomedical AG, Birkenfeld, Germany, produces a number of devices for specimen handling and detection for use in automated analyser systems and other laboratory instrumentation.

[0003] Polymerase chain reaction (PCR) thermal cycling is a method for amplification of specific DNA target sequences. The reaction solutions comprise a reaction buffer, enzymes, primer and nucleotides. This mixture has to undergo repeated changes of temperature to enable amplification of the desired DNA sequence. The repeated change of temperature is referred to as thermal cycling A quantitative analysis during PCR cycling may be achieved by measurement of fluorescence emission.

[0004] PCR thermal cyclers are widely used as laboratory standalone devices to amplify segments of DNA via the polymerase chain reaction. They usually have one thermal block with holes where single tubes, stripes of tubes, or micro plates holding reaction mixtures can be inserted.

[0005] Some of these commonly used heater blocks allow for performing thermal gradients over several tubes at a time. They however cannot treat each tube individually and are not suitable to perform different assays with individual timings and temperature profiles simultaneously.

[0006] Thermal cycling (heating/cooling) inside the devices is usually performed by thermos-electrical devices (Peltier elements) and with electrical (resistive) heaters. Only a few other cyclers use different approaches like hot or cool air, previous types of cyclers used several oil or water baths with different temperatures.

[0007] Various steps of pre-treatment (e.g. extraction of DNA) and the addition of reaction mixes are typically necessary prior to applying a sample onto a thermal cycler. These steps are often performed manually in a lab environment. Loading and unloading of reaction vessels is usually done manually.

[0008] The number and types of fluorescent dyes that can be used and measured in know thermal cycler is limited and refer to the commonly used fluorophores to label DNA probes. It is a disadvantage of known systems that all necessary pre-treatment steps like extraction and purification steps needs to be done externally and manually prior to loading the reaction mix onto a thermal cycler.

[0009] A further disadvantage of systems known from the state of the art is that the reaction vessels have to be applied or removed manually. Individual assay processing in different reaction vessels cannot be achieved on known thermal cycler. That prevents a desired variability of assay processing on automated analyser systems.

[0010] Standard thermal cycler work usually with quasi standard vials or reaction vessels which still introduce a wide range of mechanical, thermal, and optical performance properties and variations. User are restricted to use only limited numbers of fluorescent dyes that can be used on a standard thermal cycler. Thus, changing to different dyes which might be intended for use is often not possible.

[0011] An automatic or robotic handling of standard vial geometries (particularly with individual tubes) is difficult or not feasible within automated analyser systems known from the state of the art.

[0012] The ramping speeds related to changes of temperature is limited to the properties of standard vials thus restricting standard heating and cooling technology. As a consequence, the overall processing speed and throughput in a system is limited as well. Scalability like up scaling is only possible by using multiple stand-alone devices.

[0013] Commonly used heating and cooling designs generate waste heat close to the core functional area of a thermal cycler making the implementation of heat sinks, fans, air channels etc. necessary resulting in space restrictions at various areas of the thermal cycler.

[0014] Document US 2004/0209348 A1 discloses a PCR apparatus with a specimen chamber, a heating conduit which communicates with the specimen chamber, a cooling conduit which communicates with the specimen chamber, and a pumping device for pumping a gaseous or liquid medium through the heating conduit and/or the cooling conduit to the specimen chamber. The PCR apparatus further has a heating device, disposed separately from the cooling conduit, which communicates with the heating conduit and heats the medium located in the heating conduit c. The PCR apparatus has further a mixing device, which communicates with the heating conduit and with the cooling conduit, such that a ratio between a volume of the medium flowing per unit of time through the heating conduit and a volume of the medium flowing per unit of time through the cooling conduit to the specimen chamber is adjustable.

[0015] In document WO 2016074910 A1 a thermocycler and to a method for operating a thermocycler are disclosed. The method for operating the disclosed thermocycler comprises the steps of setting a first temperature in a first temperature region of the sample chamber by means of a first means to denature the nucleic acid strands, setting a second temperature in a second temperature region of the sample chamber by means of a second means to hybridize a primer onto the nucleic acids, adding a sample containing nucleic acid into the first temperature region of the sample chamber of the thermocycler, denaturing the nucleic acids, moving a movable section of the first outer wall in such a way that the section is moved toward the opposite second outer wall and the sample is moved from the first to the second temperature region, hybridizing the primer-nucleic acid molecules onto the nucleic acid strands, moving the movable section of the first outer wall in such a way that the section is moved away from the opposite second outer wall and the sample is moved from the second to the first temperature region, elongating the nucleic acid strands.

[0016] Document WO 1995018676 describes a method and apparatus for thermal cycling of nucleic acid assays including a blended fluid stream produced from a plurality of constant velocity, constant volume, constant temperature fluid streams wherein to provide a variable temperature, constant velocity, constant volume fluid stream which is introduced into a sample chamber for heating and cooling samples contained therein. By diverting and altering the ratio of the constant temperature fluid streams relative to one another, the blended fluid stream is rapidly variable in temperature, providing for almost instantaneous temperature change within the environment defined by the sample chamber.

[0017] In document US 9,656,265 B2 a random access, high-throughput system and a method for preparing a biological sample for polymerase chain reaction (PCR) testing are disclosed. The disclosed system includes a nucleic acid isolation/purification apparatus and a PCR apparatus. The nucleic acid isolation/purification apparatus magnetically captures nucleic acid (NA) solids from the biological sample and then suspends the NA in elution buffer solution. The PCR testing apparatus provides multiple cycles of denaturing, annealing, and elongating thermal cycles. The disclosed PCR testing apparatus includes a multi-vessel thermal cycler array that has a plurality of single-vessel thermal cyclers that is each individually-thermally-controllable so that adjacent single-vessel thermal cyclers can be heated or cooled to different temperatures corresponding to the different thermal cycles of the respective PCR testing process.

[0018] Further thermal cyclers are described in US 2013143272 A1 and US 2015020532 A1.

Object of the Invention

[0019] It is an object of the present invention to provide a system that can be part of analyser systems that allows for a highly scalable real-time thermal cycling.

Summary of the Invention

[0020] The present disclosure provides a device for a system for thermal cycling, comprising at least one vessel holder for taking up a reaction vessel providing a contact surface for the reaction vessel, wherein the vessel holder is surrounded by liquid channels for cooling and at least one electrical heater is attached to the vessel holder; and a multilayer liquid port for attachment of at least one vessel holder for providing the cooling liquid to the at least one reaction vessel holder.; and at least one control unit for connecting with the at least one electrical heater.

[0021] The system may encompass in a further aspect that said at least one vessel holder has an optical path access area at its lower side.

[0022] It is further envisaged that the optical path access area of the at least one restriction vessel holder may have a ventilation channel for its cleaning.

[0023] The at least one vessel holder may have in a further embodiment an inlet and an outlet for the liquid for cooling at the upper side of the at least one vessel holder.

[0024] It is further intended that the at least one vessel holder may have an internal fluidic path conveying the liquid for cooling on one side of said vessel holder from its upper to its lower side and on its opposite side from its lower to its upper side.

[0025] The at least one vessel holder may have in a further aspect an acceptance for a maximum of two of the at least one electrical heater at opposite sides.

[0026] The at least one control unit can be a printed circuit board (PCB), wherein the PCB can be connected with at least one and a maximum of eight of the at least one vessel holder and at least one electrical heating, wherein four of the at least one vessel holder and attached at least one electrical heating form a unit.

[0027] It is envisaged that the multilayer port may have at least one control valve for each of the at least one vessel holder, wherein the at least one control valve can be a 3-port/2-way valve. The at least one control valve may be connected to two membrane valves.

[0028] In a further aspect, the membrane valves can be 2-por/2-way valves.

[0029] The at least one control valve may be connected to pressurized air for switching over the membranes between the ports.

[0030] It is envisaged that the at least one vessel holder may have stands for attachment to the PCB.

[0031] The e at least one electrical heating may have electrical connections for attachment to electrical connections on the PCB for transmission of electricity and controlling the at least one electrical heater.

[0032] The four vessel holder, one multilayer port and one PCB may form a unit.

[0033] In a further aspect, the system may additionally comprise an optical detection device. The optical detection device may comprise for each of the at least vessel holder one optical fiber and a dichronic mirror for excitation and emission path.

[0034] In a further embodiment, the optical detection device may comprise one optical fiber for excitation and one optical finer for the emission pathway.

Summary of the Figures

[0035] The invention will be described on the basis of figures. It will be understood that the embodiments and aspects of the invention described are only examples and do not limit the protective scope of the claims in any way. The invention is defined by the claims and their equivalents. It will be understood that features of one aspect or embodiment of the invention can be combined with a feature of a different aspect or aspects and/or embodiments of the invention. It shows:

Figure 1	Single reaction vessel holder with a reaction vessel, liquid channels and stands at the bottom
Figure 2	Vessel holder with attached electrical heaters
Figure 3	Optical path access area of the vessel holder
Figure 4	Internal fluidic path of a reaction vessel holder
Figure 5	Arrangement of vessel holder
Figure 6	Multilayer liquid port
Figure 8	Thermal profile of a thermal cycle for PCR amplification

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Detailed Description of the Invention and the Figures

[0036] The PCR thermal cycler device according to the present description provides a design that best supports integration in automated analyzer systems. It is designed to be highly scalable in terms of numbers of assay independent compartments (reaction vessel holders) and to process each PCR reaction vessel individually and assay dependent in terms of timing, temperature profile and optical measurement.

[0037] In parallel, a specific reaction vessel is designed to be used with this module to improve thermal, optical, and mechanical performance and support robotic handling. The specific reaction vessel design is part of this description. However, some vessels shown in the context of breadboard and prototype development are off the shelf PCR reaction vessels.

[0038] The present disclosure relates to the use of a combination of fluidic based cooling and electrical heating to individually temper single reaction vessel holders and the vessels contained therein in a very dense spatial packaging. Document US 9,656,265 B2 does not refer or disclose to the technical solution provided by the present disclosure regarding the combination of fluidic based cooling and electrical heating.

[0039] The single reaction vessel holder is designed for low mass and high thermal conductivity to best support fast thermal ramping. It incorporates liquid channels for cooling and provides contact surfaces for electrical heating and conducts to the reaction vessel.

[0040] The fluidic approach for cooling allows for high thermal ramping speeds after the denaturation process in a PCR cycle. It also allows for separating the location of where energy for cooling ramping is generated from where it needs to be applied. This provides chances for reduction or elimination of spatial constraints in the vessel holder's arrangement.

[0041] An indirect valve based fluidic control is introduced as a means to expose the PCR reaction vessel holder and vessel to cold fluid for thermal ramp-down. Electrical heating in combination with a thermal sensor allows for heating ramp-up and permits to control the temperature during the thermally constant periods of the PCR cycling processes.

[0042] The disclosure further relates to specific designs for the electronics architecture, for power supply, temperature control, fluidic paths, as well as for the optical measurement.

[0043] All mechanical, fluidics, electronics and optical concepts are designed in a way to best support scalability to make the module fit in various instruments with various throughput. This variability and scalability is another core topic of the invention.

[0044] The reaction vessel as the final part of the overall concept is designed to carry an estimated volume of up to 50µl for the reaction mix. Its design needs to best support thermal contact for heating and cooling, optical contact for fluorescence measurement with optical paths for excitation and emission, protection against evaporation and support for robotic handling

[0045] Advantages of the invention can be summarized for manufacturing, for automation and for the end user as follows:

[0046] Figure 1 shows a single reaction vessel holder 1 with a reaction vessel 1, liquid channels 10 and stands 15 at the bottom. The vessel holder 1provides a contact surface 2 for the reaction vessel 5, the cooling liquid, the electrical heaters and thermal sensor(s) and paths for optical measurement and access and stability for automated pick and place of the vessel.

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The vessel holder 1 shown in figure 1 is designed for low mass and high thermal conductivity resulting in fast thermal ramping.

[0047] Figure 2 shows the arrangement of figure 1 in a 3D view, wherein an electrical heater 20 is attached to the vessel holder 2.

[0048] Figure 3 shows in a bottom view the optical path access area 3 of the vessel holder 1 with indications for thermal sensor placement 4 and a ventilation channel 6 on the left. The ventilation channel 6 serves as a part to keep the top surface of optical fibers that end below reaction vessel 1 clean. Pressurized air is used to blow away dust or particles that might collect on top of the fibers over time. Stands 15 are also indicated.

[0049] Figure 4 shows on the left a side view of a vessel holder 1 with reaction vessel 5 as shown in figure 2 with electrical heater 20 and on the right a sectional view rotated by 90 degree to illustrate a key functional element of the reaction vessel holder 1 which is its internal fluidic path. The arrows indicate the flow direction and meandering paths of the liquid. The fluid enters and exits the vessel holder 1 at its top. The fluid stream meanders from the top to the bottom of the holder, then changes sides (left to right) and meanders back up again. The meandering path allows for a reliable exchange of cooling liquid with pressurized air and vice versa. The opposite side of the liquid inlet and outlet ports of the vessel holder is closed.

[0050] Scalability of the concept concerning the vessel holders 1 with electrical heater 20 can e.g. be achieved by a linear, circular or elliptical array arrangement of vessel holders 1 (Figure 5). A linear arrangement of four vessel holders 1 with reaction vessel 5 and stands 15 is shown in figure 5. This illustrated package of four holders is the smallest unit that is envisaged in one embodiment of the invention. It can be regarded as minimum array for all of the following liquid, optical and electrical scalability considerations and descriptions

[0051] The liquid connectivity of the four vessel holders is established by a multilayer liquid port 30 as shown in figure 6A, which has four sealing contact surfaces 35 for vessel holder with inlet and outlet openings. Figure 6B shows an embodiment with membrane chambers 40 (circles) on one sealing contact surface. The most left sealing contact surface is hidden by a vessel holder that is attached to it. Beyond the interfaces to the vessel holders (figure 6A) it contains the liquid interfaces to the control valves.

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[0052] One control valve 45 is intended per vessel holder. Figure 6B shows a more detailed depiction of the membrane valves and membrane chambers 40 inside a liquid port. In total, the liquid port carries four control valves 45 that are visible at the left side of the port. The control valves are 3-port / 2-way valves that use pressurized air as a medium to switch over little membrane valves inside the port. The membrane valves are 2-port / 2-way valves. Each control valve 45 handles two membrane valves. One of the two membrane valves per vessel holder opens/closes the liquid stream, the other one opens/closes the pressurized air flow through the associated holder. The two membrane valves are alternatingly switched by their corresponding control valve so that either liquid or pressurized air is routed through the holder. The functionality of the membrane valves of the membrane chambers 40 in figure 6B are described in more detail below in connection with figure 9. Water 50 enters the multilayer liquid port 30 as indicated on the top left and air 51 enters the multilayer liquid port 30 as indicated at the bottom left. An opening for water and air 51/52 is indicated as well as an opening for a reflow 52.

[0053] A cooling liquid and pressurized air (at ambient temperature) will be necessary as a supply medium for the system. One 3 port / 2 way valve for each vessel holder switches between pressurized air and cooling liquid running through the holder. The default condition is pressurized air.

[0054] Figure 7 shows exemplary a thermal profile of one thermal cycle for a PCR amplification. After an initial heating-up (1-2), a denaturation cycle (2-3) follows and for a temperature drop from e.g. 95°C to 40-60°C (3-4), the valve switches to cooling liquid. When afterwards a stable temperature level is required for primer annealing (4-5) the temperature is increased (5-6) for elongation of sequences (6-7) and a ramp for another cycle initiated with denaturation may begin (7-8) or the valve switches back to the default state and the pressurized air pushes liquid residue out of the vessel holder. The electrical heaters are switched on and controlled with the input of the thermal sensor(s).

[0055] A collector bottle takes up all drained cooling liquid coming from the vessel holders and cools it down to the required temperature before it is transferred back to the also actively cooled supply reservoir. The pressurized air is ventilated to ambient via a channel in the collector bottle.

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[0056] The fluidics schematic shows the scalability of the system as each subassembly of vessel holder and switch valve can be added to an existing number of holders (named as 'stackable' in the schematics). As already introduced, a package of four vessel holders with their liquid and electronics supply is the smallest unit for upscaling in this description. Details for that follow below.

[0057] Further functionalities like temperature sensing, level sensing, and pressure regulation, further parts like pumps and compressors, liquid and air reservoirs, ventilation and restriction channels are necessary and documented in the schematics to give an overview of the whole fluidic concept.

[0058] Figure 8 shows in detail the functionality of a membrane valve. In figure 8A, the control valve 45 ventilates the membrane chamber to ambient 53. As a result, the incoming liquid / air pressure from the supply line 47 can push the membrane open 55 and the liquid or air can stream towards the outlet port 46 and to the vessel holder (not shown).

[0059] In figure 8B, the control valve 45 pressurizes the membrane chamber 54 and so the membrane from the rear. The membrane closes 56 the liquid / air inlet port 44 and the inlet medium is no longer able to stream towards the outlet port 46 and to the vessel holder (not shown).

[0060] The vessel holders 1 are applied with their stands 15 at the bottom to control unit printed circuit board assemblies (CU PCBA) 60 as can be seen in figure 9. It is intended that a CU PCBA 60 is designed to control one or two units each comprising four vessel holders 1. For each additional unit of four vessel holder 1, a further CU PCBA 60 has to be connected to a control unit for the CU PCBAs.

[0061] Figure 9 shows an assembly of CU PCBA 60, vessel holder 1 with reaction vessel 5 and electrical heating element 20. It is to be noted that it is also within the scope of the present invention that the heating element 20 may be printed onto the vessel holder 1 instead of using a separate heating element 20. The CU PCBA 60 contains the power routing, and as the name indicates, carries the vessel holders, the thermal sensors and the electrical heater devices and connectors.

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[0062] Figure 10 shows in its top right part a partial crosssectional view of the smallest scalable unit of four vessel holders 1 on a CU PCBA 60 with fluidics connection and control valves 45 and a reaction vessel 5.

[0063] For the scalable and potentially high number of PCR reactions running in different vessels at the same time it is necessary to find a way to best support real-time optical measurement of the fluorescence emissions.

[0064] As with all PCR reactions in all vessels, starting points, ramping speeds, durations and assay profiles can minimally to widely vary, it is nearly impossible to do a scheduled timing to allow for valid measurements. As for example with 32 vessels and 6 fluorescent dyes this could be 6 x 32 = 192 fluorescent excitations / emission readings running in parallel.

[0065] For this reason, the designed solution provides a measurement speed that allows for gathering enough measurement points under worst case timing considerations. A following software based interpretation distinguishes between valid and invalid measurement points.

[0066] A camera or photo diode is used for emission detection. A camera allows for measuring multiple up to all reaction vessels at one particular fluorescence wavelength at a time. A fast as possible sequence of camera based readings through all fluorescence wavelengths (up to 6) gives the opportunity to measure all vessels in the camera field of view at all different wavelengths within a < 4 seconds interval. With the demand that a valid reading can only be performed during the end of an elongation phase of a PCR cycle and under the assumption that the shortest valid reading interval in an elongation phase is considered to be >= 4 seconds, at least one valid reading can be performed in every cycle.

[0067] Figure 11 shows an embodiment with a single fiber concept, a schematized wireless power supply for individual excitation diodes per channel. The excitation light path is rotating on a carrousel for positioning purposes under the optical fibers 81.

[0068] In the design of figure 11, one optical fiber 81 per reaction vessel (not shown) is used to:

- maximize the number of reaction vessels monitored in the field of view of camera 70
- allow for a higher number of camera pixels per monitored vessel
- decrease the necessary camera resolution

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- eliminate spatial constraints for positioning of camera and vessels
- position the fibers in the center of the fov, etc.

[0069] Each wavelength specific optical channel is built as a subassembly comparable to the following schematics. A slight emitting laser diode 72 is arranged next to an input lens array 73. The light passes a condenser 74, homogenizer 75, a collimating lens 76 and an excitation filter 77. A fiber lens array 79 is located directly below a vial or is accessing the side of the vial at a tilted angle. Excitation and emission optical paths are separated via a channel specific dichroic mirror 78 as shown in figure 12. A camera lens 71 is arranged between camera and emission filter 80

[0070] Each of these subassemblies is mounted on a carrousel 81 so it can be positioned in the optical path between fiber lens array 79 and camera 70 by the carrousel drive. The overall number of subassemblies (optical channels) in the module is scalable between one and a maximum number that is limited due to measurement timing restrictions. The described design is heading for a maximum number of six optical channels.

[0071] The wireless power supply for the excitation diodes comprises an energy transmitter PCB and a receiver PCB that is attached to the rotating wheel of the carrousel. Wireless power and a communication and control channel are transmitted via inductive coupling.

[0072] In case photo diodes are used instead of a camera for this approach, one or more photo diodes need to be mechanically moved to read the emission rates of the bundled fibers in the array one after another. In this case the orientation of the fiber array pattern and diode movement pattern need to match to perform all readings. The number of photo diodes necessary depends on the number of reagent vessels to be read and the reading speeds that can be achieved.

[0073] Figure 13 shows an embodiment with a dual fiber concept of two optical fibers 82, 82' per reaction vessel (not shown) to achieve the benefits of the single fiber version plus generate additional advantages:

- dichroic mirrors can be avoided.
- the parts that need to be mechanically moved per channel switch can be reduced

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[0074] The optical fibers 82 are located directly below a vial or at a tilted angle below the vial (figure 14). Excitation 63 and emission 64 paths use different fibers. At the reagent vessel position excitation and emission fibers can be placed in line or at a defined angle towards each other

[0075] An installation with a defined angle between the fibers does lead to a controlled reagent vessel volume 65 for excitation and emission as illustrated in figure 14. In case photo diodes are used instead of a camera the aspects are comparable to the single fiber solution

[0076] The vessel design according to the present disclosure and as shown in figure 15 has to serve multiple functions:

[0077] The core functionality is to safely contain the desired volume of sample and reaction mix, which in the described case is up to 50 µl.

[0078] The vessel design further needs to provide the best possible thermal contact between this amount of liquid and surrounding means for heating and cooling by a thermal contact section 501, which can be provided as a thin separation wall with a big contact surface and a good and reliable thermal conductivity.

[0079] A transportation interface 502 for the transportation system can be provided for handling the vessel safely to ensure secure application in and take it out of a vessel holder. Additionally, the design may contain a mechanical retention section 503 to maintain a proper seating in the vessel holder and allow for a reliable and reproducible thermal contact between holder and vessel.

[0080] The design of the vessel shall prevent evaporation of sample and reaction mix during the thermal cycling by top cover 504. Therefore, it has to be able to contain an additional oil layer on top of sample and reaction mix and has to have a cap / top cover 504 to close the inner vial chamber after all liquid is dispensed into it.

[0081] Finally, the vessel design needs to provide optical paths and optical access for the optical measurement during and at the end of thermal cycling. Thin and transparent material at the optical access bottom 505 with a specific shape to best support and optimize the optical paths in this specific area of the reagent vessel is implemented.

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[0082] In an alternative design, the liquid cooling may be achieved by a direct contact between the fluid and the reaction vessel. Figure 16A, B show two different embodiments for allowing the fluid to directly heat and cool the reaction vessel. It is also conceivable that only the fluid for cooling or heating will be used adjusting the temperature of the reaction vessel. Mixing cool and hot fluids is another example of tempering the reaction vessel to an intended temperature.

[0083] In figure 16A a rotary valve is shown that can open and close access ports for hot and cool liquids, compressed air. A rotary valve consists of an inner rotating PCR vial carrier 100 and stationary outer valve housing 101. By rotating the vial carrier 100 contact to a respective liquid port 102 established so that hot, cold or both can flow around the reaction vessel 5. A drainage channel 103 may be permanently open or opened and closed if necessary. The fluid being in contact with the reaction vessel may be mixed with hot or cool fluid in order to increase or lower the temperature. Alternatively, the liquid around the reaction vessel may be flushed out and replaced by hot or cool fluid.

[0084] Figure 16B shows an embodiment with a vessel having inbuilt fluidic channels for a heating and cooling fluid. Such an embodiment may be used for quantitative OCR analysis. Various fluids or mixtures of fluids different from water may be used for thermal ramping (heating and cooling) or for maintaining stable temperatures. Various additives may be used for improving specific parameters of the fluids or the process per se.

[0085] In the design shown in figure 16B, one valve indirectly switches between air and cooling fluid supply for the vessel holder. This valve may alternatively operate directly. It is possible that the valve operates with any linear or rotary movement. The vessel holder itself may linearly shift or rotate the vessel or an outer housing for changing the liquid supply and thus obtaining a mixture of liquids.

[0086] It is within the scope of such alternatives to use more than one valve for supplying liquids for thermal ramping. More than one reaction vessels can be accommodated in a multi reaction vessel holder to allow for parallel processing of reaction vessels with lower flexibility but less hardware and software effort for obtaining an equivalent throughput in the module.

[0087] It is also conceivable to have more than one reaction vessels as part of a one piece multi-vial vessel to achieve the described alternative solutions. A potential liquid flow

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through the multi-vial vessel could run for instance in parallel around the multi-vials. The described approaches for combining or having inbuilt multi vessels are not limited to any numbers of reactions vessels.

[0088] The same applies for the number of vessel holders as shown in figure 10. The present invention is not limited to have four vessel holders connected, but also refers to have one of the vessel holder as shown in figure 10 to be the smallest unit.

[0089] Figure 187 to 24 show alternative embodiments for the optical design. The alternatives differ in their implementation of excitation and emission technology as well as in reading out emission.

[0090] Figure 17 shows an embodiment, with a a light emitting / laser diode array 72 being implemented for excitation. Each vessel and every fluorophore would require an individual diode. The number of diodes would be equivalent to the number of vessels multiplied with the number of fluorophores. Similarly, an array of photo diodes 85, one per fluorophore per vessel can be implemented for emission detection. Bifurcated optical fibers can used to couple excitation and emission paths with the vessels

[0091] Figure 18 shows an embodiment using broad band photo diodes for emission detection 64. One optical filter per fluorophore per vessel is used to allow read out of the specific wavelength. The filters are arranged in a filter array 86. Bifurcated optical fibers may be used to couple excitation and emission paths with the vessels.

[0092] Figure 19 shows an embodiment implementing a broad band light source in combination with a filter wheel 84 for excitation of all channel fluorophores. Bifurcated optical fibers can be used to couple excitation and emission paths with the vessels.

[0093] Figure 20 depicts an embodiment implementing variant filter wheels 84 and a camera into above described designs to perform emission 64 measurement. Any combination of the introduced alternatives for excitation and emission paths is of course also possible.

[0094] Figure 21 shows an embodiment using a dichroic mirror 78 instead of bifurcated fibers to split optical paths for excitation 63 and emission 64. An optical channel subassembly can be built in combination with excitation diodes, filters, lenses and emission detection diodes. Channel subassemblies can be positioned in various orientations (e.g. linear, circular) and coupled with vessels by optical fibers.

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[0095] Figure 22 shows an embodiment, wherein optical paths 88 directly end at the reaction vessel and 'look' at it from two different directions, so that a split of the optical paths is not necessary. The reaction vessels can be positioned with respect to the optical channels or vice versa e.g. via a carrousel or linear drive. In this embodiment, a camera may be used to measure more than one or all reaction vessels at a time (as with the described design).

[0096] Figure 23 depicts an embodiment, where optical paths 88 for excitation or emission detection are folded by using moving mirrors 89 to reach various vessel positions. Moving multi-facet mirror arrangements may reach any type of positional arrangement of reaction vessels for excitation and emission measurement.

[0097] Figure 24 shows a further embodiment for emission detection employing a camera with a direct view at a number of reactions / reaction vessels as illustrated on the left side of the figure.

[0098] The advantages of the invention can be summarized as follows:

- With a scalable thermal cycler module integrated in an analyzer system all preparation steps prior to the PCR process and the real-time measurement and all loading and unloading of vessels and other consumables, of supplies and waste can be fully automated.
- Widely spread throughput requirements and needs of diverse analyzer systems can perfectly be addressed by the overall scalability of the design.
- The various demands for optical measurement, meaning a wide diversity in number and type of fluorophores and dyes can be addressed similarly.
- Non-standard vessels are implemented and can be used to achieve better and more homogeneous thermal and optical performance and do provide all necessary robotic handling interfaces.
- Individual PCR vessels and vessel compartments allow for individual assay processing in terms of assay protocols, timing, and temperature profiles.
- Ramping speeds can be improved, to allow for shorter PCR cycles, reduced overall processing times and maximized throughput.
- Typical spatial restrictions can be avoided as with transport of cooling and heating fluid the generation of energy is separated from the application area.

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Reference Numerals

1	reaction vessel holder
2	contact surface reaction vessel
3	optical path access area
4	indications for thermal sensor placement
5	reaction vessel
6	ventilation channel
10	liquid channel
11	cooling fluid supply
15	stands
20	electrical heater
30	multilayer liquid port
35	sealing contact surface vessel holder
40	membrane chamber
44	inlet port
45	control valve
46	outlet port
47	liquid/air from supply
48	liquid/air to holder
50	water
51	air
52	reflow
53	membrane chamber to ambient
54	membrane chamber pressurized
55	open membrane
56	closed membrane
60	control unit printed circuit board (CU PCB)
70	camera
71	camera lens
72	light emitting diode/array
73	input lens array
74	condenser
75	homogenizer
76	collimating lens
77	filter
78	dichronic mirror
79	fiber lens array
80	emission filter
81	caroussel
82, 82'	optical fiber
83	optical fiber array
84	filter wheel
85	photo diode array
86	broad band photo diodes
87	broad band light source
88	optical path
100	rotating valve carrier
101	outer valve housing
102	liquid port
103	drainage port
501	thermal contact section
502	transportation interface
503	mechanical retention section
504	top cover
505	optical access bottom

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ANSPRÜCHE

1. Ein System zum thermischen Cycling, umfassend
   - mindestens einen Gefäßhalter zum Aufnehmen eines Reaktionsgefäßes, der eine Kontaktfläche für das Reaktionsgefäß bereitstellt, wobei der Gefäßhalter von Flüssigkeitskanälen zum Kühlen umgeben ist und mindestens eine elektrische Heizung an dem Gefäßhalter angebracht ist; und
   - einen Mehrschichtanschluss zum Anbringen des mindestens einen Gefäßhalters zum Bereitstellen der Kühlflüssigkeit für den mindestens einen Reaktionsgefäßhalter; und
   - mindestens eine Steuereinheit zum Anschließen an die mindestens eine elektrische Heizung.

2. Das System nach Anspruch 1, wobei der mindestens eine Gefäßhalter an seiner unteren Seite eine Öffnung für einen optischen Strahlgang aufweist.

3. Das System nach Anspruch 2, wobei der Strahlgang für den optischen Weg des mindestens einen Gefäßhalters einen Ventilationskanal für dessen Reinigung aufweist.

4. Das System nach einem der Ansprüche 1 bis 3, wobei der mindestens eine Gefäßhalter einen Einlass und einen Auslass für die Flüssigkeit zum Kühlen an der oberen Seite des mindestens einen Gefäßhalters aufweist

5. Das System nach einem der Ansprüche 1 bis 4, wobei der mindestens eine Gefäßhalter einen internen Fluidweg aufweist, der die Flüssigkeit zum Kühlen auf einer Seite des Gefäßhalters von seiner oberen zu seiner unteren Seite und auf seiner gegenüberliegenden Seite von der unteren zu seiner oberen Seite transportiert.

6. Das System nach einem der Ansprüche 1 bis 5, wobei der mindestens eine Gefäßhalter auf gegenüberliegenden Seiten eine Aufnahme für maximal zwei der mindestens einen elektrischen Heizung aufweist.

7. Das System nach einem der Ansprüche 1 bis 6, wobei die mindestens eine Steuereinheit eine gedruckte Leiterplatte (PCB) ist.

8. Das System nach einem der Ansprüche 1 bis 7, wobei die gedruckte Leiterplatte mit mindestens einem und maximal acht der mindestens einen Gefäßhalterung und mindestens einer elektrischen Heizung verbunden ist, wobei die vier der mindestens einen Gefäßhalterung und befestigten mindestens einen elektrische Heizung eine Einheit bilden.

9. Das System nach einem der Ansprüche 1 bis 8, wobei der Mehrschichtanschluss mindestens ein Steuerventil für jeden der mindestens einen Gefäßhalter aufweist.

10. Das System nach Anspruch 9, wobei das mindestens eine Steuerventil ein 3/2-Wegeventil ist.

11. Das System nach Anspruch 9 oder 10, wobei das mindestens eine Steuerventil mit zwei Membranventilen verbunden ist.

12. Das System nach Anspruch 11, wobei die Membranventile 2-port / 2-Wegeventile sind

13. Das System nach einem der Ansprüche 9 bis 12, wobei das mindestens eine Steuerventil mit Druckluft verbunden ist, um die Membranen zwischen den Anschlüssen umzuschalten.

14. Das System nach einem der Ansprüche 1 bis 13, wobei der mindestens eine Gefäßhalter Stative zur Befestigung an der PCB aufweist..

15. Das System nach einem der Ansprüche 1 bis 14, wobei die mindestens eine elektrische Heizung elektrische Verbindungen zur Befestigung an elektrischen Verbindungen auf der PCB zur Übertragung von Elektrizität und zur Steuerung der mindestens einen elektrischen Heizung aufweist.

16. Das System nach einem der Ansprüche 1 bis 15, wobei vier Gefäßhalter, ein Mehrschichtanschluss und eine PCB eine Einheit bilden.

17. System nach einem der Ansprüche 1 bis 16, ferner umfassend eine optische Detektionsvorrichtung.

18. Das System nach einem der Ansprüche 1 bis 17, wobei die optische Detektionsvorrichtung für jeden der Gefäßhalter mindestens eine optische Faser und einen dichroitischen Spiegel für den Erregungs- und Emissionspfad umfasst.

Figure 1

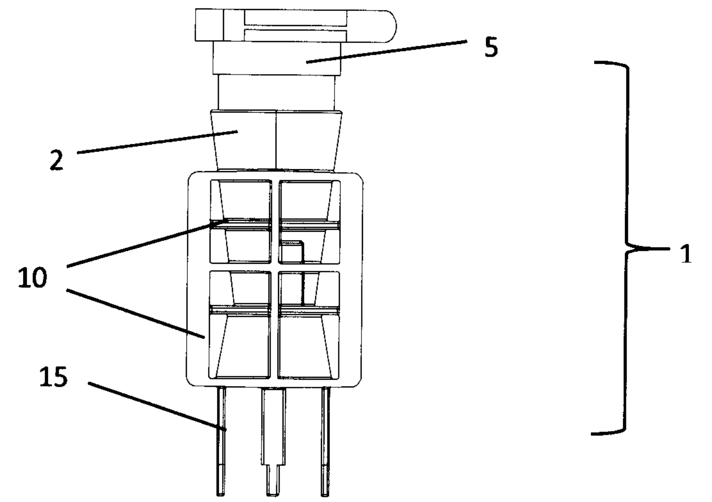

Figure 2

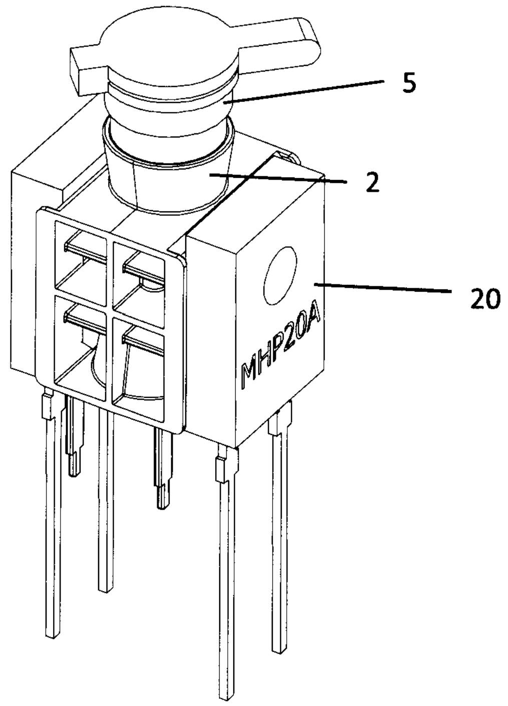

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Figure 3

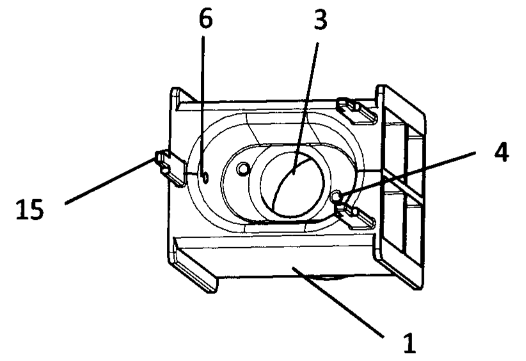
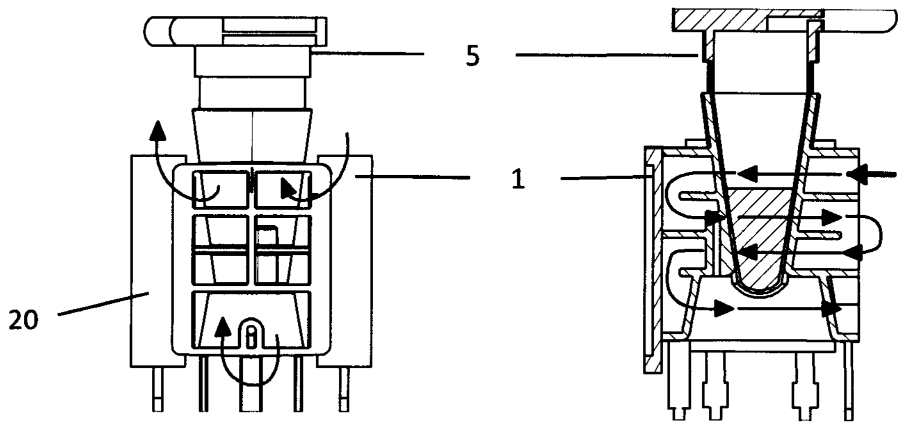


Figure 4



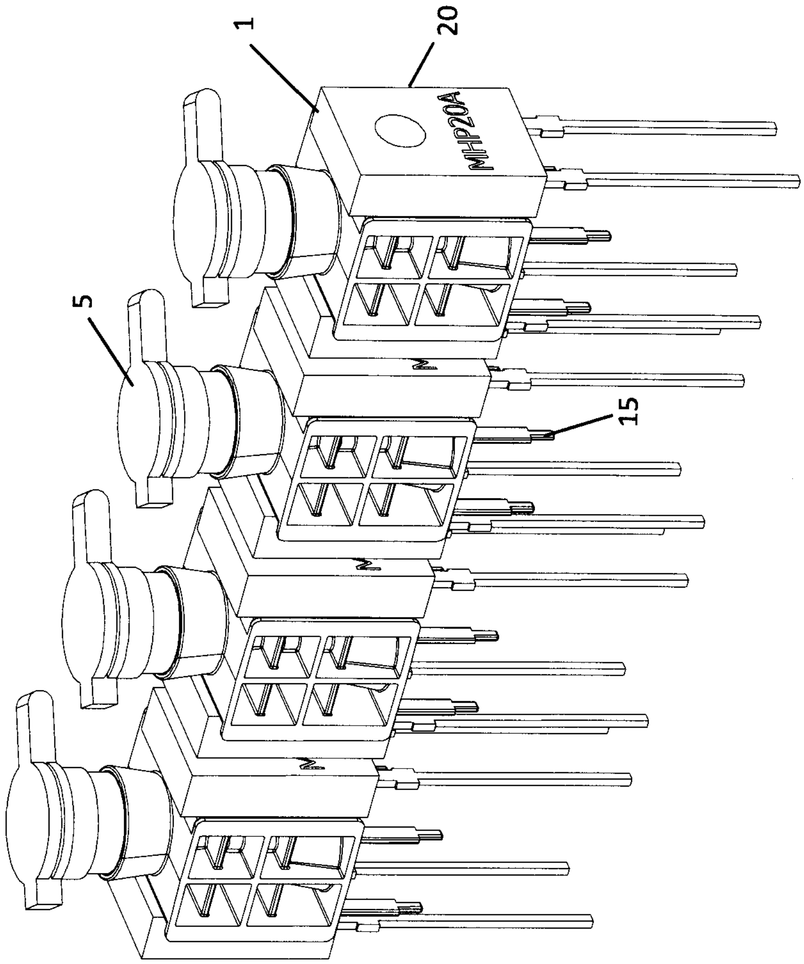


Figure 5

Figure 6A

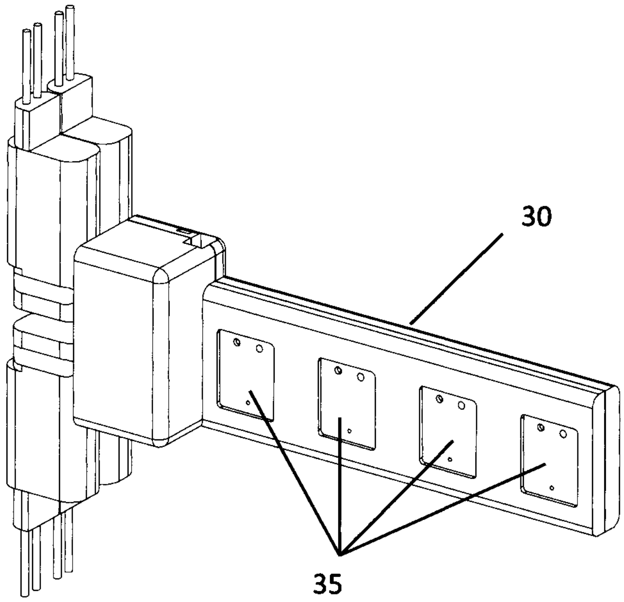


Figure 6B

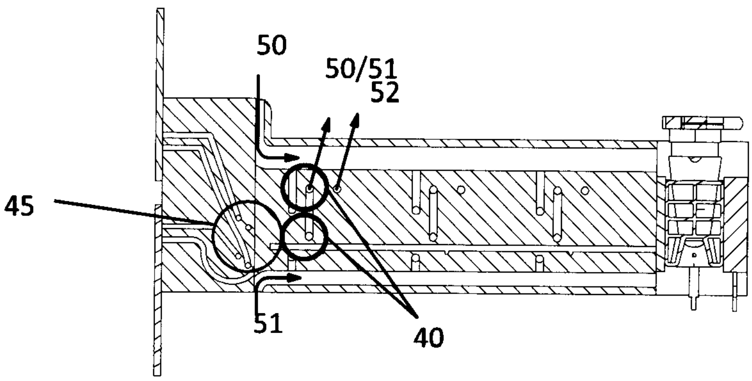


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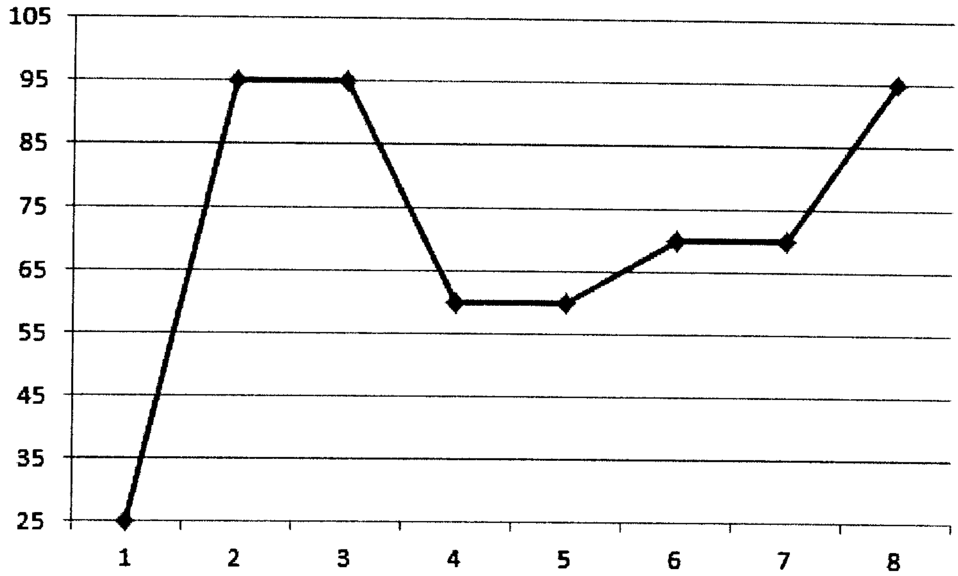
Figure 7



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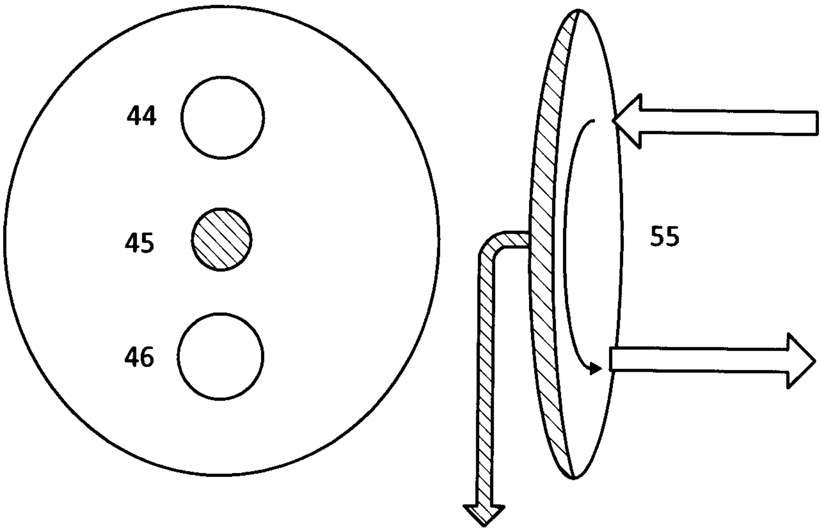


Figure 8A

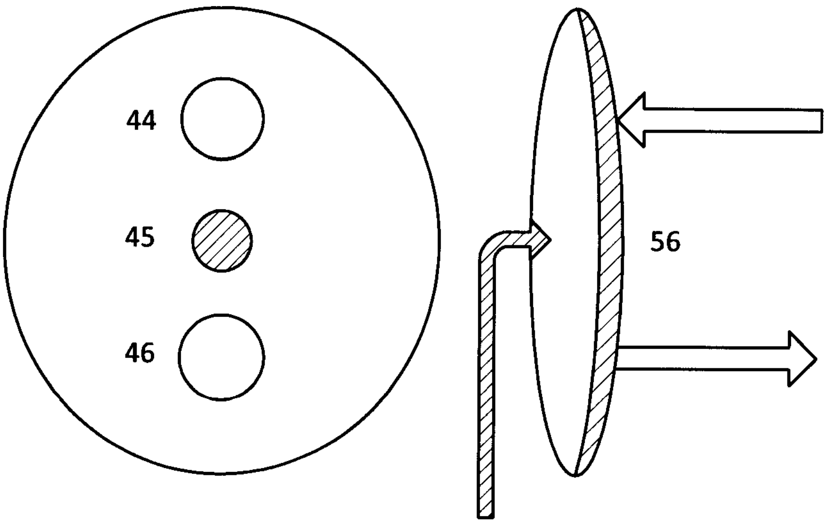


Figure 8B

Figure 9

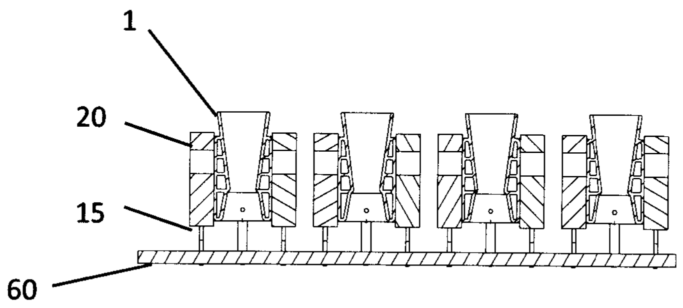
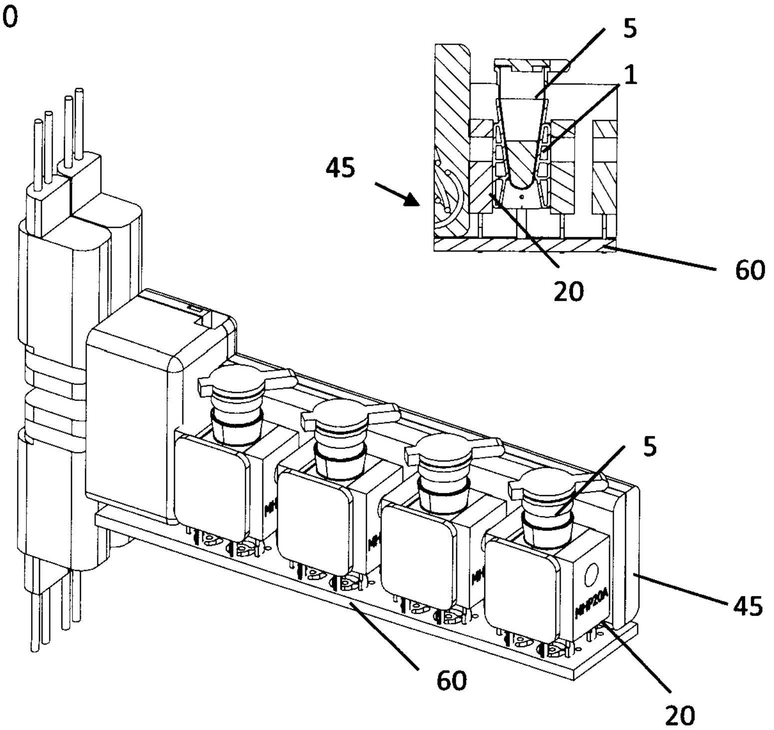


Figure 10



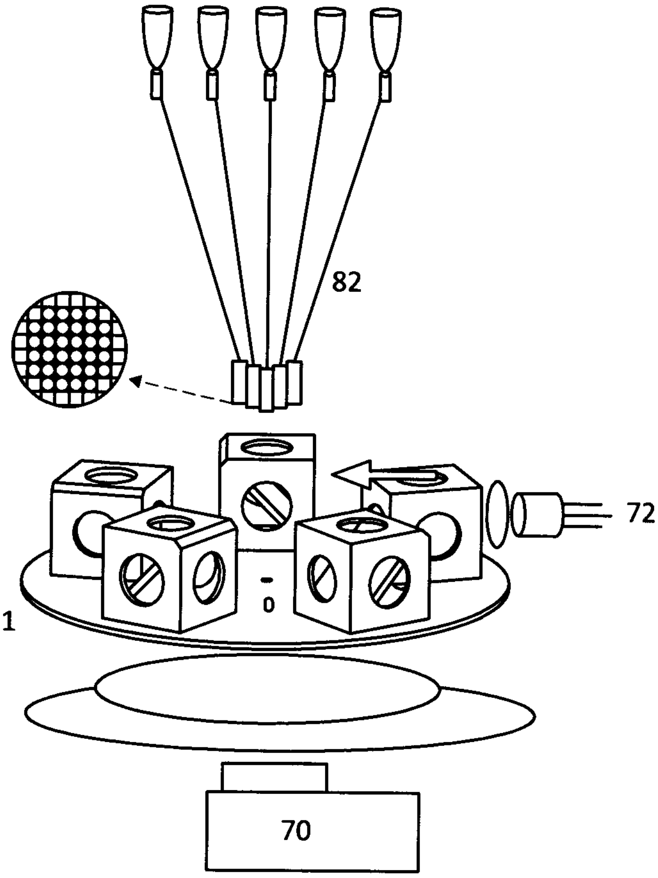


Figure 11

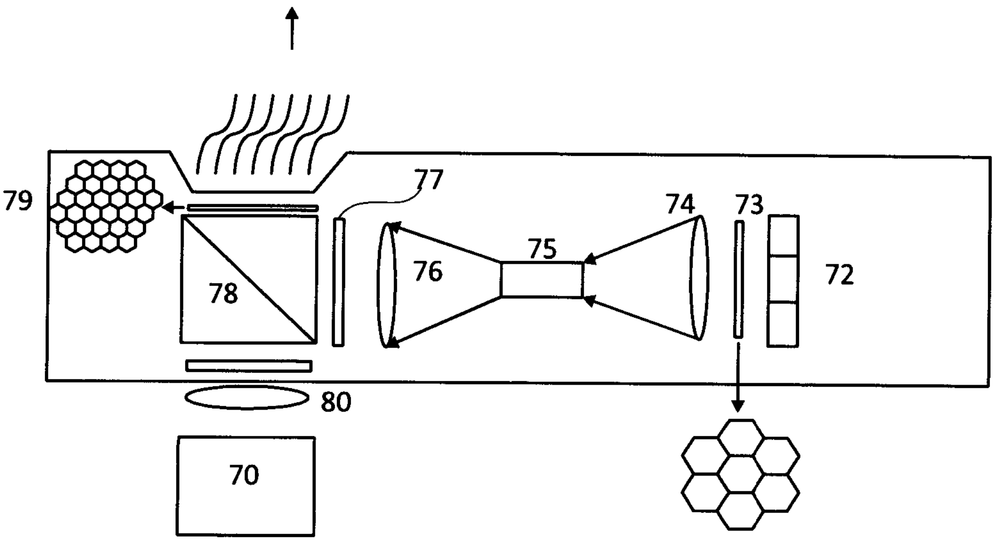


Figure 12

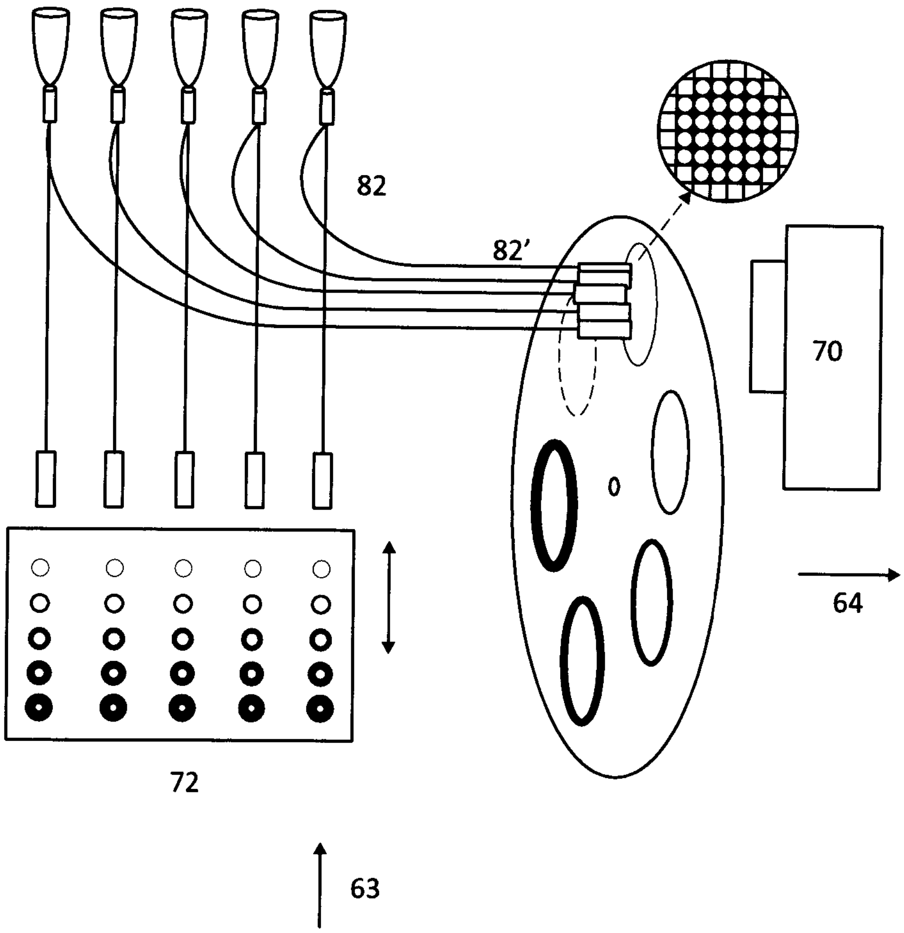


Figure 13

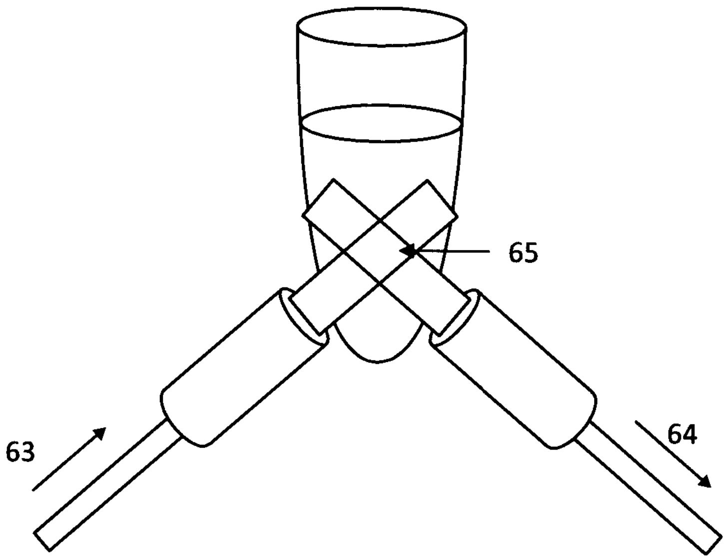


Figure 14

Figure 15

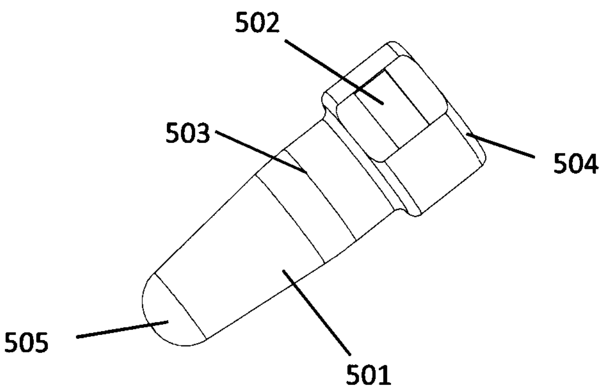


Figure 16A

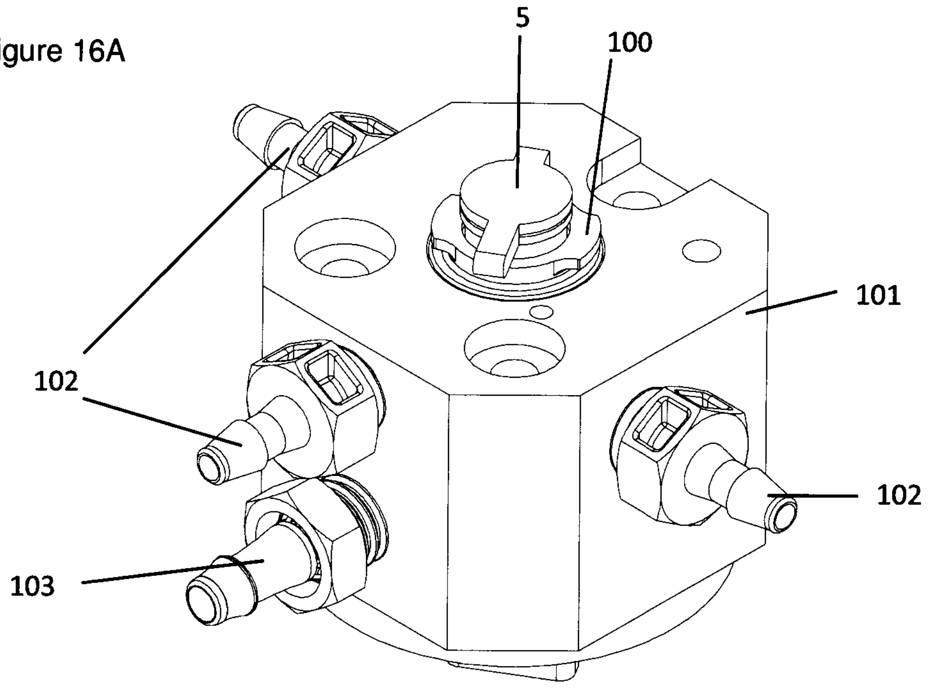
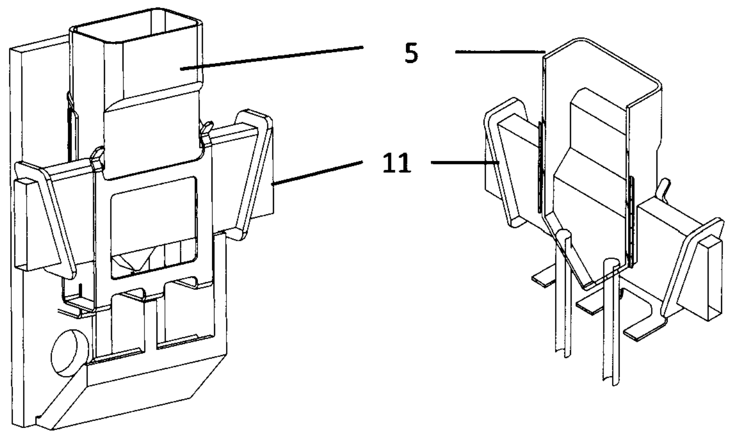


Figure 16B



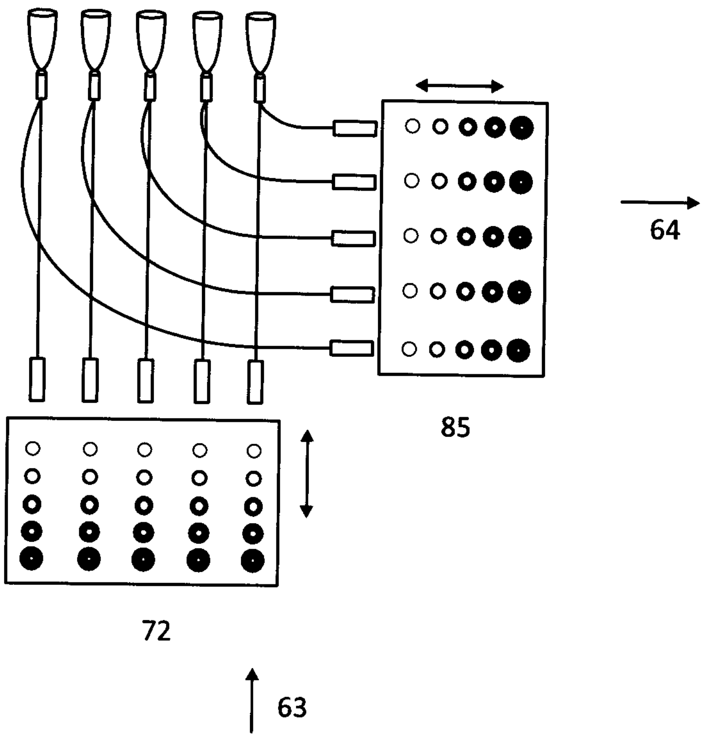


Figure 17

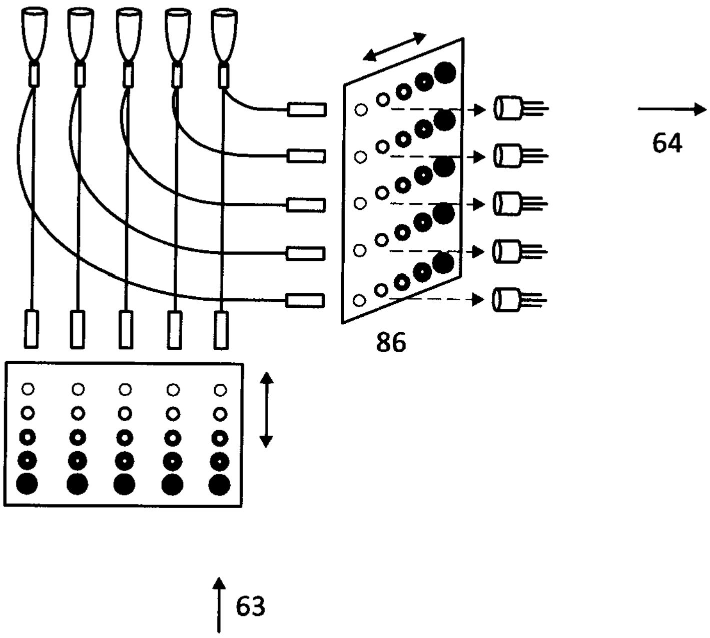


Figure 18

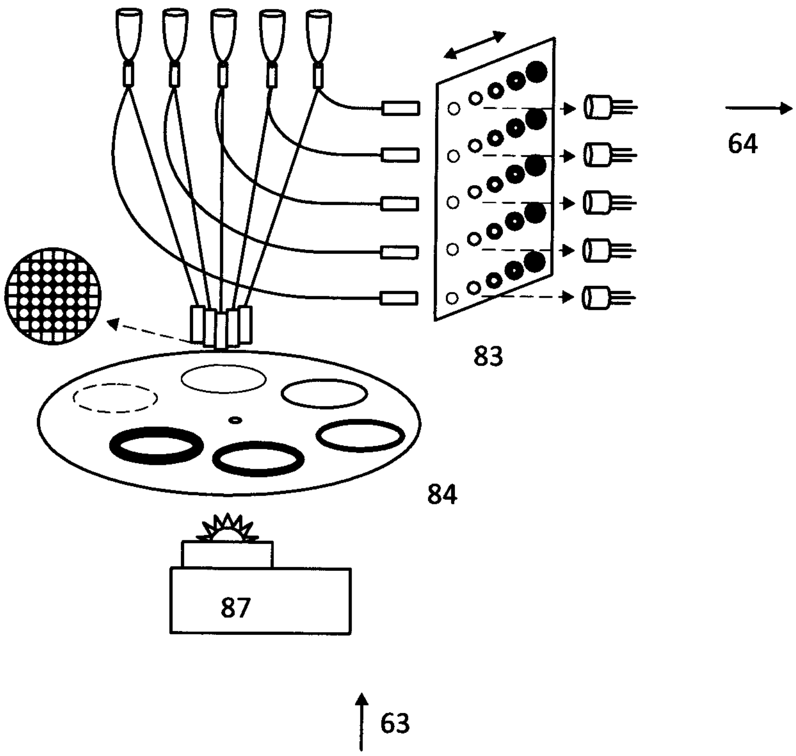


Figure 19

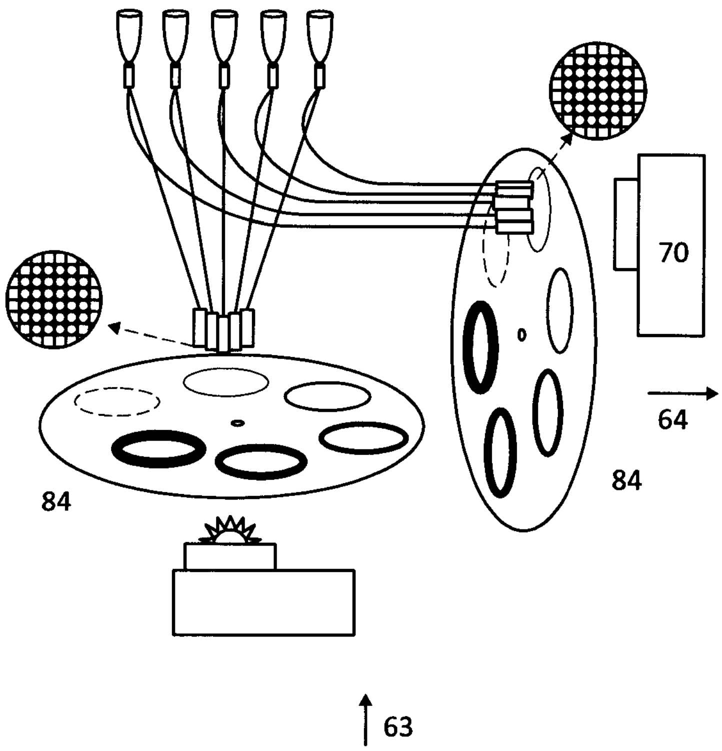


Figure 20

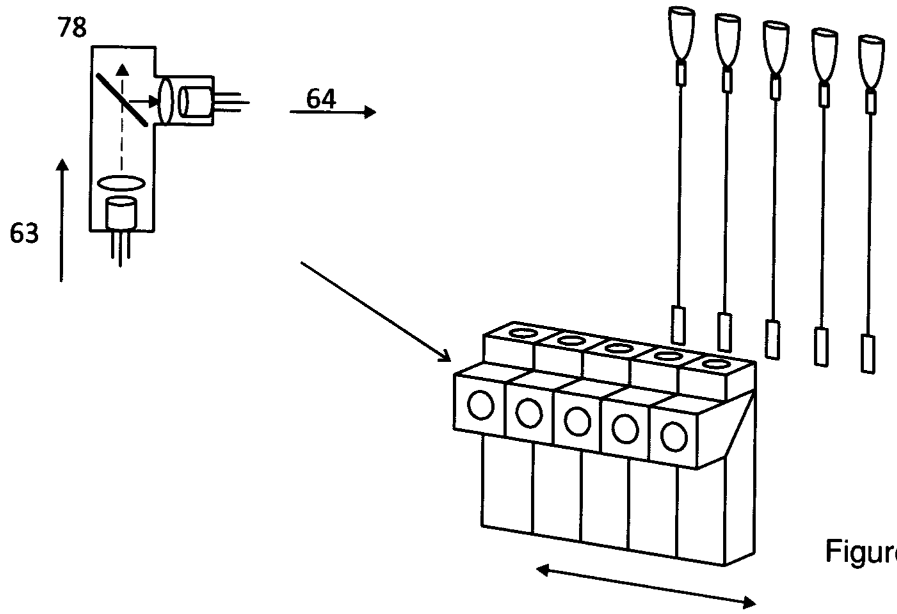


Figure 21

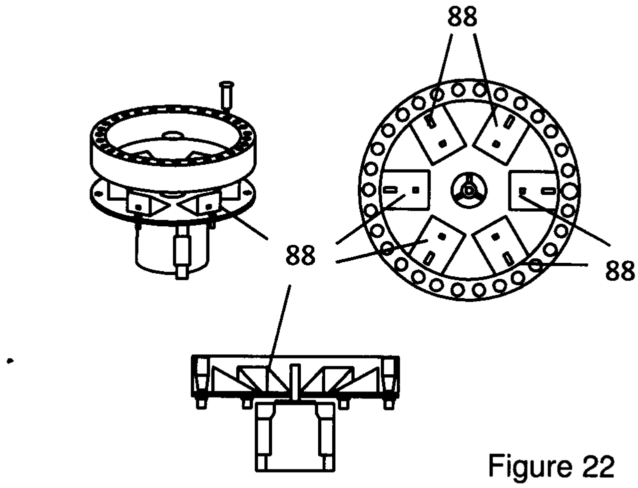


Figure 22

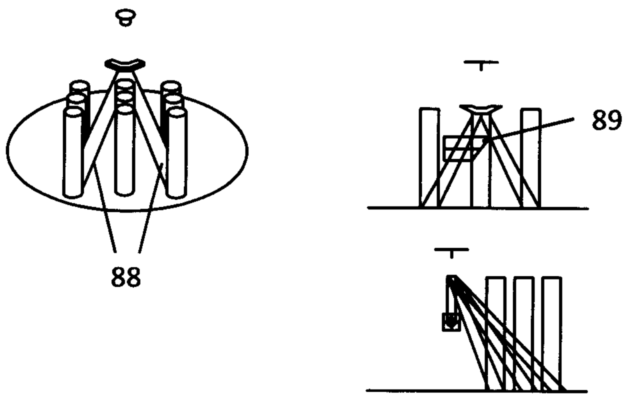


Figure 23

24IP LAW GROUP

LU100593

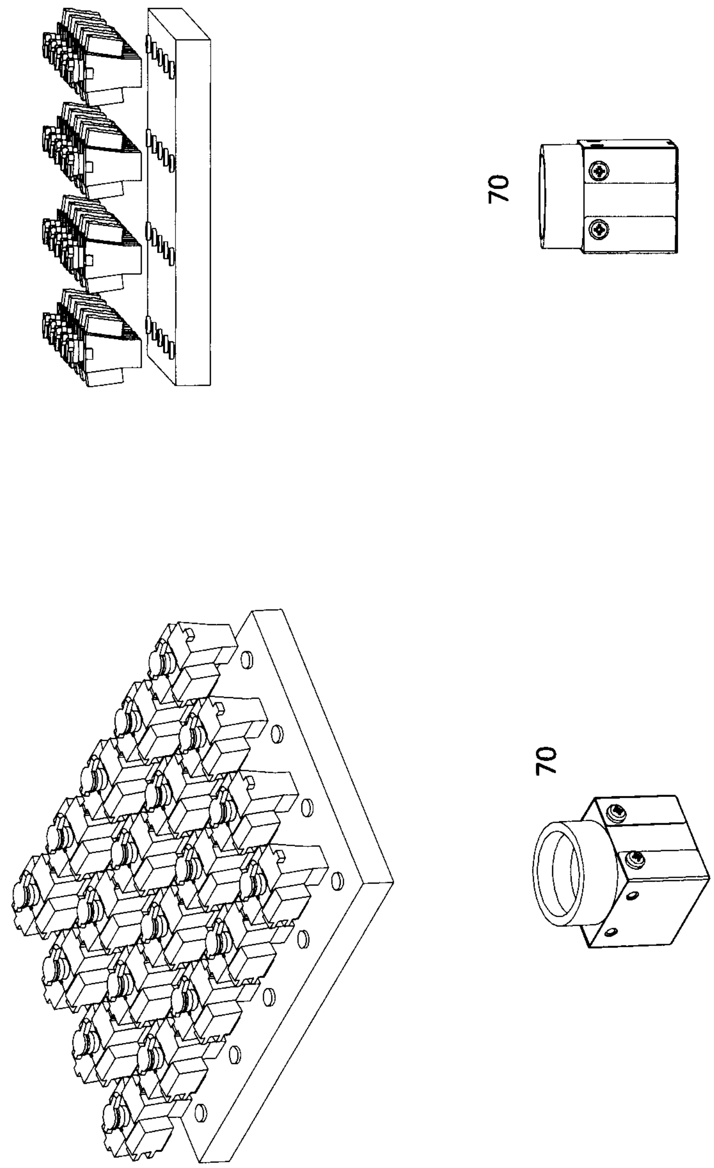


Figure 24

22/12/2012

ABSTRACT

The invention refers to a system for thermal cycling, comprising at least one vessel holder for taking up a reaction vessel providing a contact surface for the reaction vessel, wherein the vessel holder is surrounded by liquid channels for cooling and at least one electrical heater is attached to the vessel holder; and a multilayer liquid port for attachment of at least one vessel holder for providing the cooling liquid to the at least one reaction vessel holder.; and at least one control unit for connecting with the at least one electrical heater.

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LE GOUVERNEMENT
DU GRAND-DUCHÉ DE LUXEMBOURG
Ministère de l'Économie

National Application Number

SEARCH REPORT

in accordance with Article 35.1 a)
of the Luxembourg law on patents
dated 20 July 1992

LO 1853
LU 100593

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X	US 2011/312102 A1 (JO SUNG-HO [KR]) 22 December 2011 (2011-12-22) * paragraphs [0003], [0084] - [0200]; figures 1-5 *	1-18	INV. B01L7/00 C12Q1/68
X	US 2009/263782 A1 (WARD DAVID [GB] ET AL) 22 October 2009 (2009-10-22) * paragraphs [0008] - [0076]; figures 1-8 *	1-18	
A	EP 2 415 855 A1 (KANAGAWA KAGAKU GIJUTSU AKAD [JP]; NAT UNIV CORP TOKYO MED & DENT [JP]) 8 February 2012 (2012-02-08) * paragraphs [0037] - [0041]; figures 10-13 *	4-6,9-12	
A	EP 2 404 676 A1 (UNIV CALIFORNIA [US]) 11 January 2012 (2012-01-11) * paragraphs [0059] - [0064]; figures 1-4 *	9-13	
			TECHNICAL FIELDS SEARCHED (IPC)
			B01L C12Q

The present search report has been drawn up for all claims

	Date of completion of the search	Examiner
	7 May 2018	Viskanic, Martino

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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EPO FORM 1503 03.82 (P04C30)

ANNEX TO THE SEARCH REPORT ON LUXEMBOURG PATENT APPLICATION NO.

LO 1853
LU 100593

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

07-05-2018

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		WO 2004061085 A2	22-07-2004

LE GOUVERNEMENT
DU GRAND-DUCHÉ DE LUXEMBOURG
Ministère de l'Économie

WRITTEN OPINION

File No.	Filing date *(day/month/year)*	Priority date *(day/month/year)*	Application No.
LO1853	22.12.2017		LU100593
International Patent Classification (IPC) INV. B01L7/00 C12Q1/68			
Applicant Stratec Biomedical AG			

This report contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the application
- ☒ Box No. VIII Certain observations on the application

Examiner
Viskanic, Martino

WRITTEN OPINION

Box No. I Basis of the opinion

1. This opinion has been established on the basis of the latest set of claims filed before the start of the search.
2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ on paper

   ☐ in electronic form

   c. time of filing/furnishing:

   ☐ contained in the application as filed.

   ☐ filed together with the application in electronic form.

   ☐ furnished subsequently.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

Box No. V Reasoned statement with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty	Yes:	Claims	9-13
	No:	Claims	1-8, 14-18
Inventive step	Yes:	Claims	
	No:	Claims	1-18
Industrial applicability	Yes:	Claims	1-18
	No:	Claims	

2. Citations and explanations

   see separate sheet

Application No.
LU100593

WRITTEN OPINION

Box No. VIII Certain observations on the application

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1 US 2011/312102 A1 (JO SUNG-HO [KR]) 22 December 2011 (2011-12-22)

D2 US 2009/263782 A1 (WARD DAVID [GB] ET AL) 22 October 2009 (2009-10-22)

1 Document D1 anticipates in fig. 5 the following features of D1:
a system for thermal cycling (cf. [0003]) suitable for taking up reaction vessels (410), wherein the vessel holder (421a) is surrounded by liquid channels (450) for cooling (cf. [0119]) and electrical heaters (425) for heating the vessels (cf. [0113]) and comprises liquid ports (cf. fig. 10 (1141a-b) and [0200]) for the cooling fluid and a control unit (420) for controlling the heaters.
The disclosure of D1 furthermore describes optical paths access areas at the lower side of the vessels (cf. fig. 1), the optical paths having a ventilation channel (cf. [0109] referring to through-holes 331).
Additionally the controller is described to comprise a PCB (cf. [0183]) and connections to the heaters (322b).
The disclosure of D1 is thus considered to be novelty-destroying for the subject matter of claims 1-3, 7, 8, 15, 17, 18.

1.1 Also D2 discloses in fig. 5 the subject matter of claim 1, whereby the vessel-holder (22) comprises resistive heaters and a controller on a PCB (cf. [0076]). Additionally D2 discloses a plurality of channels (64) surrounding the vessels and carrying a coolant fluid (cf. [0073]).

1.2 The remaining dependent claims do not appear to contain any additional features which, in combination with the features of any claim to which they refer, meet the requirements of novelty and/or inventive step, see therefore the passages cited in the search report.

Re Item VIII

Certain observations on the application

2 It is not clear from claim 1 whata a "multilayer liquid port" is.

2.1 In claim 3 the term "restriction vessel" is unclear.