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(54) **DEVICE FOR THERMOCYCLING BIOLOGICAL SAMPLES, MONITORING INSTRUMENT COMPRISING THE SAME, AND METHOD FOR THERMOCYCLING BIOLOGICAL SAMPLES USING SUCH DEVICE**

(57) The present invention provides a device for thermocycling biological samples, the device comprising a mount for receiving the biological samples, a heat pump for heating and cooling the mount, wherein the heat pump is thermally coupled to the mount, a heat sink comprising a primary heat exchanger with an inner space perfused by a heat exchanging fluid, and a secondary heat exchanger, wherein the primary heat exchanger is thermally coupled to the heat pump, and wherein the secondary heat exchanger is thermally coupled to the heat pump through the primary heat exchanger, and a control unit for controlling the thermocycling of the biological samples. The present invention further provides an instrument for simultaneously monitoring multiple nucleic acid amplification reactions during thermocycling biological samples by means of such device, and also to a method for thermocycling biological samples using such device for thermocycling biological samples.

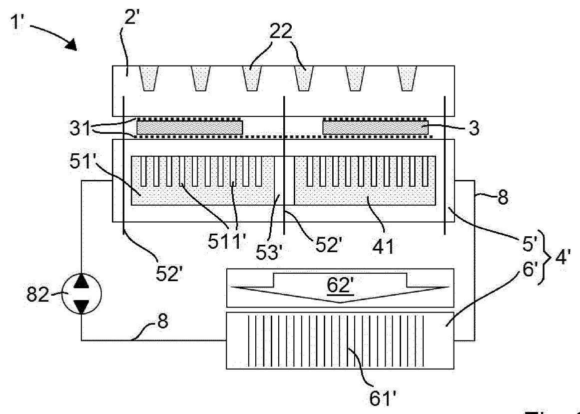

Fig. 3

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Description

TECHNICAL FIELD

[0001] Generally, the present invention relates to the technical field of sample analysis, such as the analysis of biological samples, and further to the technical field of high throughput analysis of biological samples.

[0002] In particular, the present invention is directed to a device for thermocycling biological samples, to an instrument for simultaneously monitoring multiple nucleic acid amplification reactions during thermocycling biological samples by means of such device, and also to a method for thermocycling biological samples using such device for thermocycling biological samples.

[0003] In other words, the present invention relates to a thermocycling structure or device, also referred to as thermocycler or thermal cycler, for performing chemical and/or biological reactions, such as, for example, Polymerase Chain Reactions (PCR), wherein such thermocycling device can be provided as internal part of a laboratory instrument, and wherein such thermocycling device usually includes at least a sample mount, a heat pump with a heat sink, as well as a control unit for controlling the heating and cooling of the heat pump during thermocycling. The present invention also relates to an instrument for simultaneously monitoring multiple nucleic acid amplification reactions during thermocycling biological samples by means of such thermocycling device, wherein the monitoring instrument further comprises an excitation light source for applying excitation energy to the nucleic acid amplification reactions and a sensor for simultaneously detecting light emitted from the multiple nucleic acid amplifications, and moreover to a respective thermocycling method using such a thermocycling device.

BACKGROUND

[0004] Biological samples are usually taken from patients by medical personnel in hospitals or in private practice, for laboratory analysis, e.g. for determining concentration levels of different components within the taken samples. Accordingly, the terms "sample" and "biological sample" refer to material(s) that may potentially contain an analyte of interest, wherein the biological sample can be derived from any biological source, such as a physiological fluid, including blood, saliva, ocular lens fluid, cerebrospinal fluid, sweat, urine, stool, semen, milk, ascites fluid, mucous, synovial fluid, peritoneal fluid, amniotic fluid, tissue, cultured cells, or the like, and wherein the sample can be suspected to contain a certain antigen or nucleic acid.

[0005] For many biological, biochemical, diagnostic or therapeutic applications, it is essential to be able to accurately determine the amount or concentration of a certain substance or compound in a biological sample contained in a reaction mixture, such as a certain antigen or nucleic acid as mentioned above. In order to be able to achieve this goal accurately, methods have been developed over the years, such as the widely known Polymerase Chain Reaction (PCR), for example in the form of a real-time PCR, digital PCR (dPCR) or multiplex PCR, which enable the in vitro synthesis of nucleic acids in a biological sample, through which a DNA segment can be specifically replicated, i.e. a cost-effective way to copy or amplify small segments of DNA or RNA in the sample. The development of these methods for amplifying DNA or RNA segments has generated enormous benefits in gene analysis as well as the diagnosis of many genetic diseases, or also in the detection of viral load. Usually, thermal cycling, also referred to as thermocycling, can be utilized to provide heating and cooling of reactants in a sample provided inside a reaction vessel for amplifying such DNA or RNA segments, wherein laboratory instruments including thermocyclers are commonly used in order to achieve an automatic procedure of diagnostic assays based on PCR, in which, during a PCR conduct, the liquid PCR-samples have to be heated and cooled to differing temperature levels repeatedly and have to be maintained for a certain amount of time at different temperature plateaus. In order to be able to accurately maintain such temperature plateaus during thermal cycling, thermal uniformity throughout a thermal block of a thermal cycler should be maintained, so that different sample wells can be heated and cooled uniformly to obtain uniform sample yields between samples wells.

[0006] In the course of a typical PCR conduct, a specific target nucleic acid is amplified by a series of reiterations of a cycle of steps in which nucleic acids present in the reaction mixture are (a) denatured at relatively high temperatures, for example at a denaturation temperature of more than 90° C, usually about 94°-95°C, for separation of the double-stranded DNA, then (b) the reaction mixture is cooled down to a temperature at which short oligonucleotide primers bind to the single stranded target nucleic acid, for example at an annealing temperature of about 52°-56° C for primer binding at the separated DNA strands in order to provide templates (annealing), and, thereafter, (c) the primers are extended/elongated using a polymerase enzyme, for example at an extension temperature at about 72°C for creation of new DNA strands, so that the original nucleic acid sequence has been replicated. Repeated cycles of denaturation, annealing and extension, usually about 25 to 30 repeated cycles, result in the exponential increase in the amount of target nucleic acid present in the sample, wherein the time for heating and cooling the samples has a significant influence on the overall process time. Accordingly, less time spent at non-optimum temperatures results in better or more precise chemical outcomes. In particular, a specific minimum time for holding any reaction mixture at each of the temperature plateaus is required after reaching the same, wherein such minimum holding times the minimum time it takes to complete one thermal cycle. Any time in transition between PCR temperature plateaus is time added to this minimum cycle time. Therefore, since the number of thermal cycles can be large, such additional time unnecessarily heightens the total time needed to complete PCR conduct. Thus, a decrease in heating and cooling time is essential for an efficient and cost effective process and an increase in throughput of a thermocycling device for PCR. Accordingly, there is a need to make diagnostic assays faster, cheaper and simpler to perform while achieving precision as well as efficiency.

[0007] Commonly known thermocycling devices for amplifying DNA segments by means of PCR basically consist of a mount for receiving the samples, often also referred to as a sample tempering mount, and a heat pump attached to the mount, wherein the combination of mount and heat pump can also be referred to as heat block or thermal block. The heat pump, often provided in the form of a thermoelectric device or thermoelectric cooler (TEC), for example in the form of a Peltier element, is usually used for active heating and cooling of the mount and, thus, for actively controlling the temperature provided to the samples. TECs are solid-state heat pumps usually made from semiconductor materials sandwiched between ceramic plates, wherein an amount of heat pumped is proportional to the amount of current flowing through the TEC, resulting in increased temperature control, wherein, by reversing the current, TECs can function as heaters or coolers, which is highly useful for thermocycling at different temperatures. However, due to the relatively large amount of heat being pumped over a small area, TECs in general require some kind of heat dissipation means, such as a heat sink thermally coupled to the TEC, to dissipate the heat away from the TEC and, for example, into the ambient environment, which heat sinks are often additionally provided with a fan used as air cooling means in order to facilitate heat dissipation of the heat sink to the ambient air.

[0008] However, since the use of heat sinks combined with fans can causes significant noise emission, and since such combinations for heat dissipation are usually heavy in weight, alternative solutions have been developed in the recent past, for example the heating/cooling of mounts by means of fluid carrying systems. As an example of such known prior art, WO 2006/105919 A1 discloses a device for the simultaneous thermocycling of multiple samples, which device basically comprises a mount for receiving the samples, a heat pump attached to the mount, and a heat sink, wherein a so-called thermal base in the form of a vapor chamber component using Vapor Chamber Technology ("VC-Tech") is provided in between the heat pump and the heat sink in thermal contact therewith. Such thermal base provides an improved spreading of heat across its entire cross section area in order to provide a high thermal conductivity between the heat pump and the heat sink. However, even though the transfer of heat between the heat pump and the heat sink can be improved by the provision of such thermal base, the heat dissipation capabilities of the device are limited by the heat transfer properties of the heat sink. As already mentioned above, for this purpose, fans can be provided in order to improve the heat transfer abilities of the heat sink. However, , heat sink designs which are usually cooled by air with a fan are not only noisy, large and heavy but also mandatorily determine a direction of the cooling air flow in close proximity to the mount and, thus, in close proximity to the sample, which is not desired and, thus, require counter measures to direct the air flow further away from the sample.

[0009] Also, the mounts of thermocycler are usually made of a solid material which requires a smart overall thermal design in order to achieve a superior thermal homogeneity of the mount due to the fact that already small temperature differences on the cooling side of the mount result in thermal inhomogeneity on the sample receiving side of the mount. In general, current thermocyclers use mostly forced air, extruded heat sinks and sometimes also heat spreaders between the heat sink and the bottom side of the heat pump, for example in the form of thermal interface plates, thermal interface grease, or the above mentioned vapor chamber technology, wherein such heat sinks can only provide limited heat transfer from the heat sink's fin surfaces into the cooling air, wherein a maximizing of heat transfer rates generally results in large scale heat sinks and, thus, in an increased size and weight of the thermocycler. Furthermore, such a heat exchange setup is rather stiff and inflexible, since such large-sized heat sink needs to be arranged directly underneath the heat pump and in almost direct connection with its bottom side. In further detail, since mount heating/cooling is based on thermal conductance, a homogeneous and/or at least symmetric placement of thermal heating and cooling elements, such as the generally used TECs, is usually required when implementing the solutions of the prior art. Due to these and other problems and disadvantages, the prior art suggestions as presented above can not fulfill the needs of users nowadays and, thus, do not provide satisfying solutions. Therefore, the general need exists these days in the present technical field to provide a device for simultaneous thermocycling biological samples with a further improved heating/cooling performance in order to make diagnostic assays a lot faster and, thus, cheaper to perform while maintaining or even improving precision as well as efficiency.

SUMMARY OF THE INVENTION

[0010] The present invention addresses the above described problems of improving the thermocycling of biological samples by means of improved heating/cooling performance of a mount of a thermocycling device. According to a first aspect of the present invention, a device for thermocycling biological samples is provided, which device can be a thermocycler or thermocycler unit for the simultaneous thermocycling of multiple samples to perform multiple nucleic acid amplification reactions, wherein such device can be an internal or integral part of a laboratory instrument, such as an analytical instrument or the like, and more particularly wherein such device can be provided inside a housing of such instrument.

[0011] The inventive device comprises a mount for receiving the biological samples, a heat pump for heating and cooling the mount, the heat pump being thermally coupled to the mount, a heat sink, and a control unit for controlling the thermocycling of the biological samples. In general, the combination of mount, heat pump and heat sink can also be referred to as thermal block unit of the inventive device. The heat sink of the device comprises a primary heat exchanger with an inner space perfused by a heat exchanging fluid, and a secondary heat exchanger, wherein the primary heat exchanger is thermally coupled to the heat pump, and wherein the secondary heat exchanger is thermally coupled to the heat pump through the primary heat exchanger. Here, the heat exchanging fluid can be a heat exchanging liquid, such as a water-based cooling liquid medium, for example a mixture of water and ethanol, wherein the inner space of the primary heat exchanger provides a cavity where the liquid cooling medium can flow through for heat transfer to the secondary heat exchanger. Accordingly, contrary to the commonly known heat sinks, the heat sink of the present invention, which is used for heat dissipation of the heat pump, is not implemented in the form of a commonly known heat sink with fins, but is implemented in the form of a combination of primary heat exchanger provided at the heat pump, i.e. the bottom side of the heat pump, and a secondary heat exchanger which is provided at a different location, for example spaced apart from the heat pump as well as from the primary heat exchanger, but is thermally coupled to the heat pump via the primary heat exchanger. Thereby, by means of the heat exchanging fluid, heat can be transported away from the primary heat exchanger and can be transferred into the secondary heat exchanger for dissipation to ambient, or to laboratory infrastructure. Thus, it becomes possible to rapidly transport heat away from the primary heat exchanger. By the described heat exchanging fluid flow, the mount tempering can be operated at optimum conditions, e.g. by bringing well cooled fluid into the primary heat exchanger during cooling of the mount, by bringing warm fluid into the primary heat exchanger during heating cycles of the mount, and/or by bringing adequate tempered fluid into the primary heat exchanger during plateau phases to reduce any temperature differences and, therefore, reduce thermal losses. Moreover, since the secondary heat exchanger can be arranged well away from the PCR application site and in various orientations, the secondary heat exchanger can be arranged in a flexible manner in numerous locations within, at or outside of a thermal cycler, as desired.

[0012] In accordance with the present invention, the mount for receiving the biological samples can be implemented as a mount comprising at least one flat surface or, alternatively, a surface geometry with a plurality of wells, i.e. with a structured upper side comprising recesses in order to provide a certain well geometry for receiving reaction vessels, i.e. for receiving either one or a plurality of single reaction vessels or a plurality of reaction vessels combined into a multi-well plate or the like. Also, the surface of the mount can have a curved shape, and is preferably suitable for use with thermal interface material. In case of the use of a multi-well plate, the same can be received in the well geometry of the mount, wherein the mount as well as the respective multi-well plate can provide, for example, 6, 12, 24, 48, 96, 384 or 1536 sample wells, or even more. This means that the biological samples can be received in an array of reaction vessels of a multi-well plate mounted in a respective recess structure of the mount or on top of the mount. Alternatively, the mount can be a mount with a flat upper side, i.e. the mount can substantially be a plate for receiving, on its surface, a cartridge, a slide or any other kind of consumable comprising the biological samples, or a microfluidic device comprising one or more flow channels connected to an array of sample wells for receiving the biological samples. Moreover, the mount can comprise either a flat lower side, or alternatively a structured lower side, wherein the lower side of the mount can be integrated with a heat exchanger or with an additional heating/cooling element. Furthermore, the mount of the present invention can use Vapor Chamber Technology ("VC-Tech"), for example in the form of a compact liquid heat exchanger provided within the mount, such as a vapor chamber, i.e. a heat pipe for heat spreading and isothermalizing in order to be able to absorb or provide required thermal energy, wherein a vapor chamber is generally able to transport heat from a heat source to a heat sink with a very small temperature gradient. Accordingly, the use of VC-Tech in the mount of the present invention can achieve superior thermal homogeneity, even in case of undesired uneven thermal heating and/or cooling. Also, in case of using fins within the vapor chamber, a more effective heat transfer from liquid to the solid can be achieved, and the high thermal capacity of water based cooling medium fins allows for a smaller heat transfer surface and, therefore, miniaturized vapor chamber design.

[0013] With the implementation of vapor chamber technology inside the mount, the mount is able to distribute any applied temperature much more evenly and rapidly to or from the reaction vessels, i.e. the samples undergoing PCR. Thus, a superior vessel-to-vessel uniformity can be provided, which can ensure consistent results over all reaction vessels, wherein a fast transient response of all vessels on the same temperature level right after reaching the target plateau temperature allows a reduction of the plateau time and speeding up the whole PCR process.

[0014] Furthermore, in accordance with the present invention, a heat pump is generally to be understood as a component that moves heat from one location, i.e. the heat source, to another location, usually in the form of a sink or heat sink. Thus a heat pump may be thought of as a "heater" if the objective is to warm the mount, or as a "cooler" if the objective is to cool the mount. Usually, thermoelectric heating or cooling by means of the Peltier effect is used to create a heat flux. As an example, a thermoelectric cooler "TEC", preferably in the form of a Peltier element, is used as a solid-state active heat pump with consumption of electrical energy, depending on the direction of the current, which heat pump transfers heat from its one side to the other, for example in order to provide heat to the mount during heating phases, such as during PCR denaturation, or to retract heat from the mount during cooling phases, such as during PCR annealing, and provide the heat to a sink or heat sink.

[0015] A heat sink in the sense of the present invention is to be understood as a passive heat exchanging structure that transfers heat or cold generated by an electronic or a mechanical component, such as the above described heat pump, to a fluid medium, often air or a liquid coolant, where it is dissipated away from the heat pump, thereby allowing regulation of the heat pump's temperature at optimal levels. Heat sinks are often used with components where the heat dissipation ability of the component itself is insufficient to moderate its temperature, or at least insufficient to moderate its temperature within the desired time frame, as is often the case with heat pumps in PCR applications. A heat sink in the classical sense is designed to maximize its surface area in contact with the cooling medium surrounding it, such as air. Air velocity, choice of material, protrusion design and surface treatment are factors that affect the performance of a heat sink. The heat sink in accordance with the present invention, however, comprises a plurality of heat exchangers, i.e. at least a proximate or primary heat exchanger and a remote or secondary heat exchanger, with both heat exchangers being able to transfer heat or cold to a fluid medium, such as a heat exchanging fluid, for example in the form of a liquid coolant, to dissipate the heat or cold away from the source, i.e. the heat pump. Here, the heat exchanging fluid perfuses an inside or inner space of the primary heat exchanger, wherein the "inner space" defines a space internal to the primary heat exchanger in or through which the heat exchanging fluid can flow/stream. Accordingly, the primary heat exchanger is based on a fluid heating and/or cooling system, such as a liquid cooling system, wherein the fluid heating and/or cooling system enables a low noise design of the heat sink, which is light in weight and comprises a compact design, but still allows for efficient heating/cooling. Thus, the thermal capacitance of the used fluid and/or the design of the primary heat exchanger achieve an optimized heat transfer to and from the heat pump and, thus, to and from the mount.

[0016] According to a specific embodiment of the present invention, the mount of the device for thermocycling biological samples is connected to the heat pump and/or the heat pump is connected to the primary heat exchanger, for example by means of a releasable force-fit connection, such as clamping. Here, according to a further specific embodiment, the mount, the heat pump and the primary heat exchanger are clamped together in order to form a tight connection therebetween, thereby constituting a thermal block unit as already mentioned above. By means of the clamped combination of components, homogenous contact pressure can be achieved between these components, whereby homogenous thermal contact can be established between the mount, the heat pump and the primary heat exchanger, i.e. between the lower side of the mount and the upper side of the heat pump as well as between the lower side of the heat pump and the upper side of the primary heat exchanger. For the clamping of the mentioned components, a clamping mechanism can be provided as part of the inventive device, which can be implemented in the form of a threaded connection, wherein, for example, threaded bolts or screws are guided through through-holes provided inside the primary heat exchanger and through-holes or gaps within the heat pump and are screwed into respective counterparts, such as threaded holes provided in the mount, wherein the screwing connection between bolts/screws and mount can be further fixed by means of adhesive or the like. Additionally, on the side of the primary heat exchanger facing away from the mount, the bolts/screws can be further provided with disc springs or the like, fixed by means of screw heads or additional screw nuts, in order to provide a distinctive clamping pressure on the thus connected components of the inventive device by means of a distinct torque to be applied onto the screw nuts.

[0017] According to a further specific embodiment of the present invention, the secondary heat exchanger is thermally connected to the primary heat exchanger by means of a heat exchanging fluid circulation system for circulating the heat exchanging fluid between the primary heat exchanger and the secondary heat exchanger. Thereby, it becomes possible to stream hot or cold heat exchanging fluid away from the primary heat exchanger, heat or cool the same by means of the secondary heat exchanger, and recirculate the heated or cooled heat exchanging fluid back to the primary heat exchanger. Thus, it becomes possible to adjust the temperature level of the heat exchanging fluid inside the inner space of the primary heat exchanger by means of a component provided or arranged away from the heat source, i.e. the heat pump, and, thus, to react fast to undesired temperature levels at the primary heat exchanger. As an alternative to the connection between the primary heat exchanger and the secondary heat exchanger by means of the above described heat exchanging fluid circulation system, the secondary heat exchanger can alternatively or additionally be thermally connected to the primary heat exchanger by means of a heat-transfer component such as a heat pipe or the like, which can further improve the function of the heat sink in regard to a fast control of the temperature of the heat pump. Here, the inner space or cavity of the primary heat exchanger can provide a location where the heat is collected and transferred to the heat pipe, to be transported to the secondary heat exchanger. In line with a further preferred embodiment of the present invention, the circulation system can comprise a tubing connection between the primary heat exchanger and the secondary heat exchanger for circulatory transfer of the heat exchanging fluid between the primary heat exchanger and the secondary heat exchanger. Here, the tubing connection is established by means of one or several tubes, supporting the arrangement of the secondary heat exchanger spaced apart from the primary heat exchanger, wherein the tubing connection can be implemented in the form of a circular tubing connection in order to be able to discharge the heat exchanging fluid out of the inner space of the primary heat exchanger and towards the secondary heat exchanger, pass the heat exchanging fluid along the secondary heat exchanger, or also into an inner space provided inside the secondary heat exchanger, and recirculate the same to and into the primary heat exchanger.

[0018] Accordingly, the heat exchanging fluid, for example a liquid cooling medium, can transport heat away from the primary heat exchanger and transfers it into the secondary heat exchanger for dissipation of the heat to ambient conditions at the location of the secondary heat exchanger which can be arranged outside the thermocycler, or for dissipation of the heat to adjacent laboratory infrastructure, for example a ventilation system, a cooling system or the like.

[0019] Now, the fluid volume already provided inside the tubing system may already be regarded as an initial fluid reservoir. However, and in accordance with a further specified embodiment of the circulation system of the present invention, at least one (additional) fluid reservoir accommodating heat exchanging fluid of a predetermined temperature can be provided as part of the circulation system, i.e. can be fluid-connected with the circulation system, or better with the tubing connection, which can be used, on demand, in case heat exchanging fluid of a certain temperature as stored inside the fluid reservoir can be used to heat or cool the heat exchanging fluid streaming inside the circulation system. Here, the circulation system can comprise a plurality of fluid reservoirs accommodating fluid of different predetermined temperatures. Accordingly, any such reservoir can be built as an extra volume in the liquid path provided by the circulation system. For example, one fluid reservoir can store heated heat exchanging fluid, and another fluid reservoir can store cooled heat exchanging fluid, and each fluid reservoir can add stored heat exchanging fluid into the circulation system on demand. According to a specific embodiment of the mentioned tubing connection, the used tubes can be made of an inner layer of ethylene propylene diene monomer (EPDM) rubber and an outer layer of nitrile butadiene (NBR) rubber, preferably enforced with a synthetic mesh, or can be generally made of EPDM, NBR, fluorinated ethylenepropylene polymer (FEP), Polytetrafluoroethylene (PTFE), Polyvinyl chloride (PVC), polyethersulfone (PES), fluoroelastomer (FKM), silicone, or can also generally made of metal tubes or the like, or can additionally be jacketed by means of a heat isolating material.

[0020] Moreover, according to a further specific embodiment of the tubing connection, the tubing connection can be established by flexible tubing, i.e. one or several flexible tubes or flexible pipes, in order for the secondary heat exchanger to be variably positioned in relation to the primary heat exchanger, for example far away from the primary heat exchanger and, thus, far away from the heat pump. Accordingly, since the secondary heat exchanger can be provided within a housing of the instrument accommodating the thermocycler, such as an analytical instrument, and can be placed therein independently of the primary heat exchanger, it becomes possible to position the secondary heat exchanger, for example, near a front part of the inner space of the housing. Such positioning makes it possible to unobstructedly suck cool ambient air, for example by means of a fan or the like, via a ventilation opening, a ventilation grille or the like, which is provided on the front side of the housing. Furthermore, the described variable positionability of the secondary heat exchanger independently from the primary heat exchanger results in the fact that the risk of warm air generated by neighboring laboratory appliances being sucked into the housing can be avoided due to the possibility of arranging the secondary heat exchanger in a location away from the warm air source, and the cooling efficiency of the thermal mount unit can be significantly improved. As a particular example, the presently described improved cooling concept based on the possibility of a forced airflow input from the front side of the housing can be implemented by means of a forced input of air entering through a ventilation opening provided at the top and/or bottom of a loading flap of the housing, and is further supported by a hot air outlet, e.g. another ventilation opening, provided at the back side of the thermocycler in a respective suitable position. Accordingly, (cool) ambient air can be introduced through a top and/or a bottom of the loading flap at a front side of the housing, and a hot air outlet provided at the back side of the housing can discharge the hot air to be removed from the thermocycler unit. Thus, any kind of cooling fan provided for the heat sink can now be integrated directly with the thermocycler unit, and does not necessarily have to be provided integrally with the housing or the loading flap anymore, resulting in a fan integration within the housing's inner space, and directly at the thermocycler unit, achieving a significantly reduced noise level for the benefit of a user as well as a design advantage since the ventilation openings, i.e. the air supply, can be hidden anywhere in the housing.

[0021] Basically, the presently described heating/cooling concept of a thermocycler unit within the housing of an analytical instrument allows a free arrangement of the second heat exchanger as well as its respective fan, if any, around the thermocycler unit inside the housing. For example, the fan can sit directly at or on the secondary heat exchanger, making it an integral part of the thermocycler unit and, thus, resulting in the fact that the entire thermocycler unit is a secluded and, thus, interchangeable unit within the analytical instrument. Moreover, due to the fact that the presently described solution achieves sufficient heating/cooling performance in order to omit the provision of any kind of extruded heat sink element arranged directly underneath the TECs, sufficient air flow areas exists inside the housing, so that the fan does not have to arranged directly at the ventilation openings but can be arranged in a distance away from any ventilation opening. This is advantageous since such further distance from the housing, preferably supported by a certain deflection of air flow within the housing, helps to further reduce the mentioned noise level.

[0022] According to a further specific embodiment of the present invention, at least one fluid pump, such as a liquid pump, is connected to the primary heat exchanger and the secondary heat exchanger for pumping the heat exchanging fluid and controlling the flow speed of heat exchanging fluid in the heat sink, i.e. in the previously described circulation system between the primary heat exchanger and the secondary heat exchanger. The fluid pump of the device can be used to achieve fluid flow, and can be controllable, e.g. the pump performance as well as the direction of flow can be controlled. Furthermore, the fluid pump can be arranged between the primary heat exchanger and the secondary heat exchanger, and can be connected within the circulation system, such as a connecting part within the tubing connection as described above, in order to be providing the function of pumping heat exchanging fluid from the primary heat exchanger to the secondary heat exchanger, and/or vice versa. By the regulation of the heat exchanging fluid pump, a heating or cooling capacity within the primary heat exchanger can be well controlled, wherein the addition of different temperature level fluid reservoirs as described above can further improve the controllability of the heating or cooling capacity of the heat sink, since the contents of such fluid reservoirs are very fast available and can be transported by means of the fluid pump into the primary heat exchanger.

[0023] According to a further specific embodiment of the present invention, a thermal coupling between the mount and the heat pump and/or between the heat pump and the primary heat exchanger is established by means of a thermal interface material, wherein any such thermal interface material can comprise at least one of a carbon based material, such as graphite, for example pure pyrolytic graphite, or graphene, a silicone compound, a phase change material and thermal grease, or a combination thereof. In general, any thermal coupling connection between components of the presently described device can be provided with such thermal interface material, in order to ensure a sufficient thermal conductivity at the contact surfaces of such components. Accordingly, any heating or cooling component is thermally connected to its adjacent component or components by means of using thermal interface material as mentioned before, in order to enhance the thermal coupling between any of those coupled components.

[0024] Furthermore, in accordance with the present invention, the primary heat exchanger can be a so called solid-to-liquid heat exchanger, i.e. a heat exchanger in which heat is transferred between a solid object, such as the outer hull of the primary heat exchanger comprising an inner space, and a liquid such as one example of the heat exchanging fluid provided inside the inner space of the primary heat exchanger, wherein the primary heat exchanger in the form of a solid-to-liquid heat exchanger can be a compact liquid heat exchanger providing a compact design. In this regard, the inner space of the primary heat exchanger can comprise one or more projections for surface enlargement, such as fins, provided on the inner surface of the inner space of the primary heat exchanger and surrounded by the heat exchanging fluid, wherein the fins can be pin fins or swage fins, or any other suitable kind of respective surface enlarging projection. Here, the secondary heat exchanger can comprise a similar structure as the primary heat exchanger. Additionally, the secondary heat exchanger may also be provided with external fins, such as known from usual heat sinks. Moreover, the primary heat exchanger can consist of one or more parts made from copper or other suitable thermally conducting materials, such as aluminum or silver, wherein the solid parts of the primary heat exchanger constitute the parts made from thermally conducting material. Here, the part of the primary heat exchanger which is not directly connected to any heat producing components and/or the mount can consist of a thermally conducting material, but does not have to consist of such a thermally conducting material, which can improve the design and weight of the primary heat exchanger.

[0025] Moreover, the primary heat exchanger can be designed with different layers of material, and the layers can be made of different material, for example one material for an upper layer or layers, and another material for lower layers. Also, the primary heat exchanger's side without contact to the mount or the heat pump can be made out of a plastic or polymer material without any thermal relevance. Also, a thickness of an interface side of the primary heat exchanger can be used as a thermal reservoir. Additionally or alternatively, the primary heat exchanger can also comprise at least one through hole provided for allowing an assembly of the device in the manner of a thermally sandwiched structure, meaning that all components of the inventive device can be assembled to form a thermally sandwiched structure by means of respective through holes and respective connectors, such as screws or the like, in order to force the components of the device to be sandwiched together.

[0026] According to a further specific embodiment of the present invention, with particular view on the secondary heat exchanger, the same can comprise a temperature sensor for controlling its heat exchange rate, wherein the detection values of the temperature sensor can be used by a control unit or the like to counteract any undesired measured heat exchange rate values. Furthermore, the secondary heat exchanger can comprise an inner space containing heat exchanging fluid pumped through its inner space, similar to the primary heat exchanger, wherein a heat exchange rate of the secondary heat exchanger can be controlled by means of a pump speed sensor which controls the speed of the fluid pumped through the inner space of the secondary heat exchanger, and/or a volume flow sensor, also referred to as fluid flow sensor, which controls the flow volume flowing through the secondary heat exchanger. In accordance with the necessity to change the heat exchange rate of the secondary heat exchanger based on the measured pump speed sensor values and/or volume flow sensor values, the fluid pump pumping the heat exchanging fluid through the secondary heat exchanger can be controlled to pump more or less fluid therethrough, in order to change the heat exchange rate to the desired level controlled by the pump speed sensor and/or the volume flow sensor. Moreover, the secondary heat exchanger can also interact with a working fluid for cooling the secondary heat exchanger, wherein the heat exchange rate of the secondary heat exchanger is controlled by means of a working fluid pump, meaning that the secondary heat exchanger can be connected to a working fluid by means of an interior channel, a working fluid pipe or the like, wherein the secondary heat exchanger can be heated and/or cooled not only indirectly by the heat exchanging fluid received from the primary heat exchanger or the fluid reservoirs within the circulation system, but can also be actively heated or cooled by means of the working fluid, which can be a cooling fluid or the like.

[0027] Alternatively or additionally, the secondary heat exchanger can interact with a fan and/or a radiator, in particular in case the secondary heat exchanger is provided with external fins or the like in order to enlarge the overall surface area of the secondary heat exchanger, wherein the heat exchange rate of the secondary heat exchanger is controlled by means of a fan speed sensor and/or a radiator temperature sensor. Alternatively or additionally, another kind of fluid cooling structure or mechanism can also be provided for the secondary heat exchanger, or also another element with a different temperature in thermal connection with the secondary heat exchanger, for dissipation of heat. Accordingly, in case the secondary heat exchanger dissipates heat to ambient air by e.g. a radiator or a fan, or to laboratory infrastructure, such as a cooled water circuit or a different cold/heat reservoir, its heat exchange rate is monitored and can be affected by means of a respective control based on the monitoring results, such as provided directly from the secondary heat exchanger temperature sensor, or indirectly from the pump speed sensor, the volume flow sensor, the fan speed sensor and/or the radiator temperature sensor. In general, all components of the inventive device can be equipped with sensors, such as temperature sensors, flow sensors, vibration sensors, pressure sensors, etc.. Also, the ambient air temperature can be monitored, for example by means of an ambient sensor monitoring, for example humidity and/or ambient temperature, wherein a temperature difference between ambient temperature and the temperature of the heat exchanging fluid can be monitored, in order to control heat transfer in an accurate manner.

[0028] According to a further specific embodiment of the present invention, with particular view on the mount of the inventive device, the mount can be made of solid material, such as silver or aluminum, solid ceramic, a carbon based material or any combination thereof, and can function as a heat conductor for achieving thermal homogeneity, as also already described further above. Alternatively, or also in part additionally, provided that the mount can consist of several parts and materials, the mount can be made of solid thermal conductive material, such as copper, and can comprise a vapor chamber (VCM; 3D heat pipe) for transporting and distributing heat between the heat pump and the biological samples in a uniform manner. Here, in case the mount provides for a vapor chamber, then the heat pump can be placed at almost any location of the mount, not necessarily between mount and primary heat exchanger, due to the excellent thermal conductivity of a vapor chamber. One example of such a vapor chamber mount is described in WO 2013/075839 A2.

[0029] According to a further specific embodiment of the present invention, with particular view on the heat pump, the same can be one of a thermoelectric device, a resistive heater, or a combination thereof, wherein the thermoelectric device can be a Peltier element. Alternatively or additionally, the resistive heater can be one of a ceramic heater, a heating foil, a heating cartridge or a wire wound heater. Moreover, the heat pump can be arranged between the mount and the primary heat exchanger, for example in a sandwiched manner, as already indicated above in relation to another specific embodiment of the present invention. Furthermore, the heat pump can be arranged at least in part inside a recess provided in the mount, in more detail in a recess provided in the side of the mount opposite of the side receiving the biological samples.

[0030] In general, a perimeter heater or edge heater can also be provided at the mount in order to minimize any thermal non-uniformity across the samples or any kind of sample holder. Such perimeter heater can be positioned at the side of or on top of the mount and around a sample holder, such as a multi-well plate, to counter any heat loss at its edges. Additionally, a heated cover can be provided, designed to keep the reaction vessels closed during thermal cycling and to heat the upper portion of such vessels to prevent condensation and the like.

[0031] According to a further specific embodiment of the present invention, the inventive device can further comprise at least one sensor for controlling the temperature at the biological samples received in the mount, wherein such sensor can be a temperature sensor, a fan speed sensor or a fluid flow sensor, and wherein such sensor can be provided at the mount, at the heat pump, at the primary heat exchanger, and/or at the secondary heat exchanger. Thus, by providing a respective sensor at any of the components of the inventive device, the temperature of the biological samples during PCR can be monitored closely, and the heating/cooling function of the device can be controlled based on the measured temperature values of the samples, in order to regulate the different temperature plateaus of the PCR accurately and efficiently. Accordingly, such sensor(s), for example temperature sensors, enable a precise control of the mount temperature by a control algorithm or the like, wherein sensors in the heat exchangers, such as fan speed sensors, temperature sensors, and other sensors are used to control their respective heating/cooling rate, wherein the heating/cooling power can be significantly varied by changing fan speed and/or fluid pump speed. For example, a heat sink temperature measurement can be carried out by the mentioned control algorithm, for linearizing thermal output power from the heat pump.

[0032] In other words, when summarizing the above, a device for PCR application for one or more samples is suggested, wherein the samples are placed on a mount with or without well geometry, wherein the device includes the mount, one or more heater elements at any position of the mount, a primary heat exchanger, a secondary heat exchanger for exchanging heat to ambient air or laboratory infrastructure, and a connecting element between the primary and the secondary heat exchangers, for example in the form of cooling liquid tubes or the like, or, alternatively or additionally, a heat pipe, wherein the thermal layers of the device, i.e. its components, can be thermally connected via thermal interface material, and wherein the thermal components can be equipped with sensors, such as temperature sensors, fan speed sensors, fluid flow sensors, volume flow sensors or the like, to control the temperature at the samples in or on the mount.

[0033] According to another aspect of the present invention, an instrument for simultaneously monitoring multiple nucleic acid amplification reactions during thermocycling biological samples is provided, wherein the instrument comprises a device for thermocycling the biological samples as described previously, and wherein the instrument further comprises one or several excitation light sources for applying excitation energy to the nucleic acid amplification reactions occurring inside one or several reaction vessels received by the mount, and one or more sensors for simultaneously detecting light emitted from the multiple nucleic acid amplifications. Such an instrument can be structured similar to the already known Roche LightCycler® instruments which provide high-performance, and high-throughput PCR platforms which can be used, for example, for gene detection, gene expression analysis, genetic variation analysis, and array data validation. Such benchtop instrument solutions comprise, inter alia, a plate-based real-time PCR device as a robotically controllable, automated high-throughput solution for general laboratory use.

[0034] Usually, an automated processing system, such as an analytical, pre-analytical or post-analytical processing system, which is commonly employed in state-of-the-art laboratories for automatically processing biological sample, can comprise the previously described device, or even the entire previously described instrument. Here, the term "laboratory instrument" or "instrument" of the laboratory encompasses any apparatus or apparatus component operable to execute one or more processing steps / workflow steps on one or more biological samples, and covers analytical instruments, pre-analytical instruments, and also post-analytical instruments. The expression "processing steps" thereby refers to physically executed processing steps, such as conducting the particular steps of a PCR conduct. The term "analytical" as used herein encompasses any process step carried out by one or more laboratory devices or operative units which are operable to execute an analytical test on one or more biological samples. In the context of biomedical research, analytical processing is a technical procedure to characterize the parameters of a biological sample or of an analyte. Such characterization of parameter comprises, for example, the determination of the concentration of particular proteins, nucleic acids, metabolites, ions or molecules of various sizes in biological samples derived from humans or laboratory animals, or the like. The gathered information can be used to evaluate e.g. the impact of the administration of drugs on the organism or on particular tissues. Further analyses may determine optical, electrochemical or other parameters of the samples or the analytes comprised in a sample.

[0035] According to another aspect of the present invention, a method for thermocycling biological samples using a device for thermocycling the biological samples as described previously is provided with the present invention, wherein the inventive method comprises, inter alia, the step of monitoring the temperature at the biological samples, for example by means of the sensors as described further above, and the step of controlling the output of the heat pump and/or the heat exchange rate of the primary heat exchanger and/or the output of any further heater, such as an edge heater, wherein the controlling step is carried out based on the detected and monitored temperature at the biological samples resulting from the monitoring step. With such method, a thermal performance of the inventive device can be actively influenced by controlling the heat pump or any additional heaters, by controlling the performance of the heat sink, i.e. the primary heat exchanger and/or the secondary heat exchanger, and/or by monitoring and exploiting further environmental parameters or design parameters as mentioned above.

[0036] According to a specific embodiment of the above described method, the above described step of controlling the heat exchange rate of the primary heat exchanger can be executed by the control unit of the described device, wherein the controlling step can comprise a control of the heat exchange rate of the second heat exchanger and/or a stop or reverse of a flow of heat exchanging fluid through the inner space of the primary heat exchanger. Moreover, according to a further specific embodiment of the present invention, the step of controlling the heat exchange rate of the primary heat exchanger can also include the control of fluid speed to cool the heat exchange fluid in the heat sink above ambient temperature and approximate to the predetermined lowest temperature of the thermocycles controlled by the control unit. Accordingly, a thermocycler specific method feature for the controlling step is, for example, the stop of a flow of heating or cooling liquid in the heat sink at certain PCR protocol steps, which can minimize thermal losses during the PCT temperature plateau phases. Moreover, the active control of the temperature of the heat exchanging fluid, such as cooling liquid flowing through the heat sink, can be used to control the overall thermal performance of the device. As a further example, reversing the fluid flow can be used to fill the primary heat exchanger and/or the secondary heat exchanger with a heat exchanging fluid with either a higher or lower temperature compared to the fluid currently in place inside the primary heat exchanger and/or the secondary heat exchanger. Additionally or alternatively, the inside of the already described fluid reservoirs can be refilled or replenished with fluid of a certain (high or low) temperature. Also, additionally or alternatively, the flow path as defined by the circulation system, i.e. the tubing system, optionally together with the fluid reservoirs and pumps, if any, can be modified in a certain way, for example by inclusion of additional tubing, reservoirs and pumps, in order to achieve a certain control effect, for example by prolonging the flow path, increasing the flow speed, and/or enlarging the amount of fluid provided inside the flow path of the heat exchanging fluid.

[0037] The above described method steps can be controlled by the control unit of the described device, which can also control any kind of actuation or monitoring of the above described device and its components, wherein the term "control unit" as used herein encompasses any physical or virtual processing device, such as a CPU or the like, which can also control the entire instrument or even an entire workstation comprising one or more laboratory instruments in a way that workflow(s) and workflow step(s) are conducted. The control unit may, for example, carry different kinds of application software and instruct the automated processing system or a specific instrument or device thereof to conduct pre-analytical, post analytical and analytical workflow(s)/ workflow step(s). The control unit may receive information from a data management unit regarding which steps need to be performed with a certain sample. Further, the control unit might be integral with a data management unit, may be comprised by a server computer and/or be part of one instrument or even distributed across multiple instruments of the automated processing system. The control unit may, for instance, be embodied as a programmable logic controller running a computer-readable program provided with instructions to perform operations. Here, in order to receive such instructions by a user, a user interface can additionally be provided, wherein the term "user interface" as used herein encompasses any suitable piece of application software and/or hardware for interactions between an operator and a machine, including but not limited to a graphical user interface for receiving as input a command from an operator and also to provide feedback and convey information thereto. Also, a system / device may expose several user interfaces to serve different kinds of users / operators.

[0038] As used herein and also in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. Similarly, the words "comprise", "contain" and "encompass" are to be interpreted inclusively rather than exclusively; that is to say, in the sense of "including, but not limited to". Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise. The terms "plurality", "multiple" or "multitude" refer to two or more, i.e. 2 or >2, with integer multiples, wherein the terms "single" or "sole" refer to one, i.e. =1. Furthermore, the term "at least one" is to be understood as one or more, i.e. 1 or >1, also with integer multiples. Accordingly, words using the singular or plural number also include the plural and singular number, respectively. Additionally, the words "herein," "above,", "previously" and "below" and words of similar import, when used in this application, shall refer to this application as a whole and not to any particular portions of the application.

[0039] The description of specific embodiments of the disclosure is not intended to be exhaustive or to limit the disclosure to the precise form disclosed. While the specific embodiments of, and examples for, the disclosure are described herein for illustrative purposes, various equivalent modifications are possible within the scope of the disclosure, as those skilled in the relevant art will recognize. Specific elements of any foregoing embodiments can be combined or substituted for elements in other embodiments. Also, in drawings, same reference numerals denote same elements to avoid repetition, and parts readily implemented by one of ordinary skill in the art may be omitted. Furthermore, while advantages associated with certain embodiments of the disclosure have been described in the context of these embodiments, other embodiments may also exhibit such advantages, and not all embodiments need necessarily exhibit such advantages to fall within the scope of the disclosure as defined by the appended claims.

[0040] The following examples are intended to illustrate various specific embodiments of the present invention. As such, the specific modifications as discussed hereinafter are not to be construed as limitations on the scope of the present invention. It will be apparent to the person skilled in the art that various equivalents, changes, and modifications may be made without departing from the scope of the present invention, and it is thus to be understood that such equivalent embodiments are to be included herein. Further aspects and advantages of the present invention will become apparent from the following description of particular embodiments illustrated in the figures.

BRIEF DESCRIPTION OF THE DRAWINGS

[0041]

Figure 1	is a conceptual illustration of a device for thermocycling biological samples according to an embodiment of the present invention;
Figure 2	is a schematic structural illustration of the device for thermocycling biological samples according to the embodiment as shown in fig. 1;
Figure 3	is a schematic functional illustration of a device for thermocycling biological samples according to another embodiment of the present invention;
Figure 4	is a schematic functional illustration of a modified device for thermocycling biological samples according to the embodiment as shown in fig. 3; and
Figure 5	is a flowchart of a method using the device according to an embodiment of the present invention.

LIST OF REFERENCE NUMERALS

[0042]

1	device for thermocycling biological samples
1'	device for thermocycling biological samples
2	mount / sample tempering mount
21	mount's upper flat surface
2'	mount / sample tempering mount
22'	indentation / well
3	heat pump / TEC / heater
3'	heater
31	thermal interface material TIM
4	heat sink (including primary and secondary heat exchanger)
4'	heat sink (including primary and secondary heat exchanger)
41	heat exchanging fluid / heating/cooling liquid
5	primary heat exchanger
5'	primary heat exchanger
51	primary heat exchanger's inner space
51'	primary heat exchanger's inner space
511'	fins
52'	screw
53'	through hole in primary heat exchanger
6	secondary heat exchanger
6'	secondary heat exchanger
61'	fins
62'	fan
7	control unit
8	heat exchanging fluid circulation system
81	fluid reservoir
82	fluid pump
9	sensor
91	temperature sensor
92	fluid flow sensor

100	monitoring step
200	controlling step
300	heat exchanging fluid flow controlling step
400	fluid reservoir controlling step

DETAILED DESCRIPTION

[0043] Fig.1 shows a functional concept of a device 1 for thermocycling biological samples according to an embodiment of the present invention, wherein the device 1 is intended for PCR applications, for example as one main component of an analysis instrument. As can be gathered from the schematic illustration of fig. 1, the device 1 comprises a sample tempering mount 2 and a heat pump 3, which are illustrated in fig. 1 as one functional unit for the sake of simplicity, wherein the mount 2 can also be implemented as a mount including a heat pipe in the form of a vapor chamber or the like. Here, the mount 2 can comprise a structured upper side with recesses in order to provide a certain well geometry for receiving reaction vessels, or with a flat upper side for receiving a cartridge, a slide or any other kind of consumable bearing the biological samples to be thermocycled, or a microfluidic device comprising one or more flow channels connected to an array of sample wells for receiving the biological samples. Moreover, the device 1 comprises a heat sink 4 consisting, in a functional sense, of a primary heat exchanger 5 and a secondary heat exchanger 6, wherein the mount 2, the heat pump 3 and the primary heat exchanger 5 constitute a functional unit which can be sandwiched together, for example by means of a clamping mechanism for clamping these components together in order to achieve a tight thermally-connected unit. Furthermore, the mount 2, the heat pump 3 and the primary heat exchanger 5 can comprise a thermal interface material 5 thereinbetween, wherein, in use of the device 1, thermal interface material 31 is only depicted in fig. 1 between the lower side of the mount-heat pump-combination 2, 3, i.e. the lower side of the heat pump 3, and the upper side of the primary heat exchanger 5, in order to improve the thermal conductivity between these components.

[0044] As can also be gathered from fig. 1, the primary heat exchanger 5 is connected to the secondary heat exchanger by a heat exchanging fluid circulation system 8, wherein, in fig. 1, only a single line stands for the circulation system 8 circulating heat exchanging fluid between the two heat exchangers 5, 6, i.e. within the heat sink 4. In particular, the secondary heat exchanger 6 can be arranged away from the primary heat exchanger 5 without disrupting the functional unit of the heat sink 4, since a thermal connection between the primary heat exchanger 5 and the secondary heat exchanger 6 is established by a connecting element, such as fluid-bearing tubes or a heat pipe, constituting a circulation system 8 for heat exchanging fluid. Moreover, as depicted in fig. 1, the device 1 comprises a particular monitoring system based on a plurality of sensors 9, which are for control and prediction of the overall tempering of the device 1. Here, a temperature sensor 91, or alternatively a heat flux sensor or the like, is provided at the unit of mount 2 and heat pump 3 for measuring the temperature of mount 2 and heat pump 3, another temperature sensor 91 is provided on the thermal interface material 31 for measuring its temperature, a further temperature sensor is provided at the primary heat exchanger 5 for measuring its temperature, a further temperature sensor 91 is provided at the secondary heat exchanger 6 for measuring its temperature, and a fluid flow sensor or volume flow sensor 92 is provided at the heat exchanging fluid circulation system 8 for measuring the fluid flow, i.e. the flow velocity of the heat exchanging fluid circulating inside the heat exchanging fluid circulation system 8. Here, the sensor 92 might also be able to measure the temperature of the heat exchanging fluid, or the pump speed of a pump (not shown) provided in the fluid circulation system 8, in order to improve the monitoring capability of the device 1. All sensors 9 are connected to a control element or control unit 7, for example in the form of a CPU 7, which is able to receive the measuring signals from the sensors 9 and process the same, in order to monitor the temperatures at different locations of the device 1, and to control and actively influence the temperature of the samples at the mount 2 in regard to the PCR thermocycling requirements.

[0045] As a more concrete illustration, fig. 2 depicts the concept of the device 1 of fig. 1 in a structural manner. Accordingly, it can be gathered from fig. 2 that the device 1 comprises the mount 2 with a flat surface 21, wherein several heating/cooling elements, depicted in form of heat pumps or TECs 3, such as Peltier elements, are provided in between the mount 2 and the primary heat exchanger 5 in the form of a solid-to-liquid heat exchanger. Reference sign 3 in fig. 2 can also illustrate the position of a heating element, as an alternative to a Peltier element. Furthermore, additional heating/cooling elements are depicted in fig. 2 on a side surface of the mount 2, or also on the flat surface 21 of the mount 2, which elements 3' are preferably implemented as heaters, such as multiwell plate edge heaters or the like. Thereby, a comprehensive heating/cooling of the mount 2 can be achieved, wherein any thermal connection between the heat pumps 3 and the mount 2, as well as a thermal connection between the heaters 3' and the mount 2 can be enforced by the use of thermal interface material 31. Also, a connection surface between the heat pumps 3 arranged in between the mount 2 and the primary heat exchanger 5 are provided with thermal interface material 31, in order to enhance any temperature conductivity between the mount 2, the heat pumps 3 and the heat sink 4, constituting a thermal block unit, wherein the components of the thermal block unit, i.e. the mount 2, the primary heat exchanger 5 and the heat pumps 3 arranged therebetween can be clamped together in order to establish a tight thermally conducting assembly. A respective clamping mechanism is described further below in regard to figs. 3 and 4. From fig. 2, similar to the concept as shown in fig. 1, it can be clearly taken that the remaining part of the heat sink 4, i.e. the secondary heat exchanger 6, can be arranged in a spaced-away manner distant from the primary heat exchanger 5, wherein the secondary heat exchanger 6 used to support a temperature compensation function of the primary heat exchanger 5 in regard to the mount 2 can be arranged far away from the thermal block unit in order to be thermally independent therefrom.

[0046] As can also be gathered from fig. 2, the primary heat exchanger 5 comprises an inner space 51 filled with heat exchanging fluid 41, such as a cooling liquid or the like, and the primary heat exchanger 5, or better the inner space 51 of the primary heat exchanger 5, is in fluid connection with the secondary heat exchanger 6 by means of a connecting element, such as a flexible tubing system or the like, constituting the heat exchanging fluid circulation system 8. In order to be able to circulate the heat exchanging fluid 41 from the primary heat exchanger 5 to the secondary heat exchanger 6, and back, a fluid pump 82 is provided as part of the heat exchanging fluid circulation system 8, and is, thus, arranged in between the primary heat exchanger 5 and the secondary heat exchanger 6. Accordingly, in case the temperature of the samples is too high, or in case the sample temperature needs to be decreased in order to achieve a lower temperature plateau during PCR cycling, the temperature of the mount 2 needs to be decreased rapidly by means of the heat pumps 3, and the removed heat must be removed from the heat pumps 3. In such case, the primary heat exchanger 5 acquires the heat to be removed, transfers the same to the heat exchanging fluid 41. The heated-up heat exchanging fluid 41 is then transported away, i.e. out of the primary heat exchanger 5 into the tubing of the heat exchanging fluid circulation system 8 by means of the fluid pump 82. Here, the temperatures are monitored by the control unit 7, processed, and, in line with a respective algorithm, the control unit 7 drives the fluid pump 82 in order to increase or decrease the heat exchanging fluid flow speed in the heat exchanging fluid circulation system 8. Thereby, heat can be removed from the primary heat exchanger 5 faster or slower, as desired, in order to control the heat removal from the heat pumps 3 arranged in between the mount 2 and the primary heat exchanger 5. Accordingly, the heat is removed from the primary heat exchanger 5 and transferred into the heat exchanging fluid 41 which, then, is transferred by means of the fluid pump 82 and the heat exchanging fluid circulation system 8 to the secondary heat exchanger 6, which can transfer the removed heat to ambient air or laboratory infrastructure.

[0047] As an alternative to the previously described device 1 based on fig. 2, fig. 3 depicts the concept of another embodiment of the device 1' in a structural manner. Accordingly, it can be gathered from fig. 3 that the device 1' comprises a mount 2' with a structure in its upper surface, wherein the mount's upper surface comprises indentations 2 which can be used as wells for receiving the biological samples to be thermocycled, or for receiving a multi-well plate comprising a plurality of wells comprising the biological samples therein, wherein several heat pumps 3, such as Peltier elements, are provided in between the mount 2' and the primary heat exchanger 5' in the form of a solid-to-liquid heat exchanger. Here, additionally to the respective version in the previously described embodiment, the primary heat exchanger 5' comprises an inner space 51' including fins 511', such as pin fins or swage fins, within the inner space 51', for example protruding from one large inner surface side of the inner space 51', the fins 511' providing a more effective heat transfer from the solid parts of the primary heat exchanger 5' to the heat exchanging fluid 41 perfusing the inner space 51' of the primary heat exchanger 5, i.e. flowing or being streamed through the inner space 51' of the primary heat exchanger 5'. Here, a high thermal capacity of the fins 511' allows for a smaller heat transfer surface and, therefore, for a miniaturized design of the primary heat exchanger 5'.

[0048] Similarly to the previously described embodiment, a comprehensive heating/cooling of the mount 2' can be achieved by the structure of device 1', wherein any thermal connection between the heat pumps 3 and the mount 2' as well as between the heat pumps 3 and the primary heat exchanger 5' is amplified by the use of thermal interface material 31, in order to enhance any temperature conductivity between the mount 2', the heat pumps 3 and the primary heat exchanger 5'. These components again constituting a thermal block unit, wherein the components of the thermal block unit, i.e. the mount 2', the primary heat exchanger 5' and the heat pumps 3 arranged therebetween can be mechanically connected together, for example by means of a mechanical interface mount in the form of screws 52', or alternatively threaded bolts, or the like, in combination with a respective counterpart in the mount, such as a threaded hole, as well as a spring or a nut-spring combination, connecting and pulling/pushing the primary heat exchanger 5' and the mount 2' together, thereby sandwiching the heat pumps 3 thereinbetween, i.e. clamping these components together, in order to establish a tight thermally conducting assembly. For example, the screws 52' can pass through one or several through holes 53' provided in the primary heat exchanger 5' and fit into respective threaded holes provided in the mount 2'. Further, and similar to the concept as shown in fig. 3, it can be clearly taken that a heat sink 4' consists of the primary heat exchanger 5' and a secondary heat exchanger 6', wherein the secondary heat exchanger 6', can be arranged in a spaced-away manner distant from the primary heat exchanger 5', and wherein the secondary heat exchanger 6' supporting a temperature compensation function of the primary heat exchanger 5' in regard to the mount 2' can be arranged far away from the thermal block unit in order to be thermally independent therefrom.

[0049] As can also be gathered from fig. 3, the primary heat exchanger 5' is in fluid connection with the secondary heat exchanger 6' by means of a heat exchanging fluid circulation system 8 comprising a fluid pump 82' arranged within the fluid connection between the primary heat exchanger 5' and the secondary heat exchanger 6', which achieves the same function as described in regard to the previously described embodiment. In the presently described embodiment as depicted in fig, 3, the secondary heat exchanger 6' not only allows a streaming of the heat exchanging fluid 41 there through in order to achieve a fluid-to-solid temperature transfer, but also further comprises fins 61' on its outer surface, such as pin fins or swage fins, for example protruding from an outer surface of the secondary heat exchanger 6' to the outside, which fins 61' providing a more effective heat transfer from the solid parts of the primary heat exchanger 6' to the ambient air. In order to further improve the heat transfer from the heat exchanging fluid 41 streaming through the secondary heat exchanger 6' to the solid parts of the secondary heat exchanger 6' itself, to the fins 61' and then to the ambient air, a fan 62' is provided in connection with the secondary heat exchanger 6' as depicted in fig. 3. Accordingly, in case the temperature of the heated-up heat exchanging fluid 41 inside the secondary heat exchanger 6' is too high, the fan can be activated and its speed, i.e. the fan speed, can not only be monitored by the control unit 7 but can also be increased or decreased, as desired, in order to improve a cooling performance of the secondary heat exchanger 6'.

[0050] In fig. 4, a modified or complemented embodiment of the device 1' for thermocycling the biological samples as described in regard to fig. 3 is shown, which is almost identical but with the difference that the modified device 1' comprises additional fluid reservoirs 81 within the heat exchanging fluid circulation system 8. In particular, one fluid reservoir 81 is provided in fluidic connection in between the inner space 51' of the primary heat exchanger 5' and the fluid pump 82, and another fluid reservoir 81 is provided between the inner space 51' of the primary heat exchanger 5' and the secondary heat exchanger 6' on the other side of the circulation circle. Each fluid reservoir 81 can accommodate heat exchanging fluid 41 of a predetermined temperature, which fluid can be provided as part of the circulation system 8, i.e. is fluid-connected with the circulation system 8, or better with the tubing of the circulation system 8. Thus, the additional fluid in the fluid reservoirs 81 can be used, on demand, in case heat exchanging fluid 41 of a certain temperature as stored inside the respective fluid reservoir 81 can be used to heat or cool the heat exchanging fluid 41 streaming inside the circulation system 8. For example, one of the depicted fluid reservoirs 81 can store cold heat exchanging fluid 41, and the other can store heated heat exchanging fluid 41, and the control unit 7 can activate the one or the other fluid reservoir 81 in case the heat exchanging fluid 41 inside the tubing of the system 8 must be cooled down or heated up, by adding the respective cold or hot heat exchanging fluid 41 from one of the reservoirs 81. Thus, depending on the need, the circulation system 8 can comprise multiple hot and/or cold fluid reservoirs 81 accommodating heat exchanging fluid 41 of different predetermined temperatures, thereby enabling a certain control of the temperature of the heat exchanging fluid 41 provided to the secondary heat exchanger 6' or returning to the primary heat exchanger 5'.

[0051] In fig. 5, the depicted flowchart shows the main steps of a method using the inventive device 1, 1' of the present invention. In particular, the method for thermocycling biological samples using the device 1, 1' is based on the measuring values as received from the sensors 9. Here, the method monitors in step 100 the temperature or other values as received from the sensors 9, and it is determined, in the same step 100, for example based on the values from the flow sensor 92 or the temperature sensors 91 that the temperature at the mount 2, 2', i.e. the temperature at the samples to be cycled, is too high or is in need of cooling down. Depending on the outcome of step 100, the control unit 7 proceeds to step 200, i.e. a step of controlling the output of the heat pumps 3, the output of the heaters 3', and/or the heat exchange rate of the primary heat exchanger 5, 5'. Here, for example, the control unit 7 can increase the fluid pump's performance, or can stop the pumping at all or reverse the pumping direction, and, optionally, can introduce cold heat exchanging fluid 41 from the fluid reservoirs 81. Alternatively or additionally, the control unit 7 can increase/decrease the output of the heaters 3', as desired, and/or can activate or increase the function of the fan 61' in case the temperature of the primary heat exchanger 5, 5' needs to be decreased as fast as possible. For example, in step 300, the control unit 7 can stop or reverse the flow of heat exchanging fluid 41 through the inner space 51, 51' of the primary heat exchanger 5, 5'. Moreover, in step 400, the control unit 7 can open one or several of the fluid reservoirs 81 in order to add heated or cooled heat exchanging fluid 41.

[0052] While the current invention has been described in relation to its specific embodiments, it is to be understood that this description is for illustrative purposes only. Accordingly, it is intended that the invention be limited only by the scope of the claims appended hereto.

Claims

1. A device (1; 1') for thermocycling biological samples, the device comprising the following components:

a mount (2; 2') for receiving said biological samples;
a heat pump (3) for heating and cooling the mount, wherein the heat pump (3) is thermally coupled to the mount;
a heat sink (4; 4') comprising a primary heat exchanger (5; 5') with an inner space (51; 51') perfused by a heat exchanging fluid (41), and a secondary heat exchanger (6; 6'), wherein the primary heat exchanger (5; 5') is thermally coupled to the heat pump (3), and wherein the secondary heat exchanger (6; 6') is thermally coupled to the heat pump (3) through the primary heat exchanger (5; 5'); and
a control unit (7) for controlling the thermocycling of said biological samples.

2. The device (1; 1') according to claim 1, wherein the mount (2; 2') is connected to the heat pump (3) and/or the heat pump (3) is connected to the primary heat exchanger (5; 5'), preferably by means of a releasable force-fit connection, further preferably by clamping.

3. The device (1; 1') according to claim 1 or 2, wherein the secondary heat exchanger (6; 6') is spaced apart from the first heat exchanger (5; 5').

4. The device (1; 1') according to any one of the preceding claims, wherein the secondary heat exchanger (6; 6') is thermally connected to the primary heat exchanger (5; 5') by means of a heat exchanging fluid circulation system (8) for circulating said heat exchanging fluid (41) between the primary heat exchanger (5; 5') and the secondary heat exchanger (6; 6').

5. The device (1; 1') according to any claim 4, wherein the circulation system (8) comprises
a tubing connection between the primary heat exchanger (5; 5') and the secondary heat exchanger (6; 6') for circulatory transfer of said heat exchanging fluid (41) between the primary heat exchanger (5; 5') and the secondary heat exchanger (6; 6'), preferably wherein the tubing connection is established by flexible tubing for variably positioning the secondary heat exchanger (6; 6') in relation to the primary heat exchanger (5; 5'); and/or
at least one fluid reservoir (81) accommodating heat exchanging fluid (41) of a predetermined temperature, preferably wherein the circulation system comprises a plurality of fluid reservoirs accommodating heat exchanging fluid (41) of different predetermined temperatures.

6. The device (1; 1') according to any one of the preceding claims, wherein at least one fluid pump (82) is connected to the primary heat exchanger (5; 5') and the secondary heat exchanger (6; 6') for pumping said heat exchanging fluid (41) and controlling the flow speed of heat exchanging fluid (41) in the heat sink (4; 4'), preferably wherein the fluid pump (82) is arranged between the primary heat exchanger (5; 5') and the secondary heat exchanger (6; 6').

7. The device (1; 1') according to any one of the preceding claims, wherein a thermal coupling between the mount (2; 2') and the heat pump (3) and/or between the heat pump (3) and the primary heat exchanger (5; 5') is established by means of a thermal interface material (31), preferably wherein the thermal interface material (31) comprises at least one of a carbon based material, such as graphite, preferably pyrolytic graphite, or graphene, a silicone compound, a phase change material and thermal grease.

8. The device (1; 1') according to any one of the preceding claims, wherein
the primary heat exchanger (5; 5') is a solid-to-liquid heat exchanger, preferably a compact liquid heat exchanger;
the inner space (51') of the primary heat exchanger (5') comprises fins (511'), preferably wherein the fins (511') are pin fins or swage fins;
the primary heat exchanger (5; 5') consists of one or more parts made from copper or other thermally conducting materials; and/or
the primary heat exchanger (5; 5') comprises at least one through hole allowing an assembly of the device (1; 1') in the manner of a thermally sandwiched structure.

9. The device (1; 1') according to any one of the preceding claims, wherein
the secondary heat exchanger (6; 6') comprises a temperature sensor (91) for controlling its heat exchange rate;
the secondary heat exchanger (6; 6') comprises an inner space containing heat exchanging fluid (41) pumped through its inner space, preferably wherein a heat exchange rate of the secondary heat exchanger (6; 6') is controlled by means of a pump speed sensor and/or volume flow sensor (92);
the secondary heat exchanger (6; 6') interacts with a working fluid for cooling the secondary heat exchanger (6; 6'), wherein the heat exchange rate of the secondary heat exchanger (6; 6') is controlled by means of a working fluid pump; and/or
the secondary heat exchanger (6') interacts with a fan (62') and/or a radiator, wherein the heat exchange rate of the secondary heat exchanger (6') is controlled by means of a fan speed sensor and/or a radiator temperature sensor.

10. The device (1; 1') according to any one of the preceding claims, wherein
the mount (2; 2') is made of solid material, preferably silver or aluminum, and functions as a heat conductor for achieving thermal homogeneity ; and/or
the mount is made of solid thermal conductive material, preferably copper, and comprises a vapor chamber for transporting and distributing heat between the heat pump (3) and the biological samples in a uniform manner.

11. The device (1; 1') according to any one of the preceding claims, wherein
the heat pump (3) is one of a thermoelectric device, a resistive heater, or a combination thereof, the thermoelectric device preferably being a Peltier element, and/or the resistive heater preferably being one of a ceramic heater, a heating foil, a heating cartridge or a wire wound heater;
the heat pump (3) is arranged between the mount (2; 2') and the primary heat exchanger, preferably in a sandwiched manner; and/or
the heat pump (3) is arranged at least in part inside a recess provided in the mount (2; 2').

12. The device (1; 1') according to any one of the preceding claims, further comprising at least one sensor (9) for controlling the temperature at the biological samples received in the mount (2; 2'), preferably wherein the at least one sensor (9) is one of a temperature sensor (91), a fan speed sensor, a pump speed sensor and a volume flow sensor (92), further preferably wherein the at least one sensor (9) is provided at the mount (2; 2'), the heat pump (3), the primary heat exchanger (5; 5'), and/or the secondary heat exchanger (6; 6').

13. The device (1; 1') according to any one of the preceding claims, wherein the heat exchanging fluid (41) is a heat exchanging liquid (41), preferably wherein the heat exchanging liquid (41) is a water-based cooling liquid medium.

14. The device (1; 1') according to anyone of the preceding claims, wherein
said biological samples are placed on the mount (2; 2'), preferably wherein the mount (2; 2') comprises at least one flat surface (21) or a surface geometry with a plurality of indentations (22');
said biological samples are received in an array of reaction vessels of a multi-well plate mounted in a respective recess structure of the mount (2') or on top of the mount (2); and/or
said biological samples are received in a cartridge or a slide arranged on the mount (2).

15. The device (1; 1') according to any one of the preceding claims, wherein the device (1; 1') is a thermocycler for the simultaneous thermocycling of multiple samples to perform multiple nucleic acid amplification reactions.

16. An instrument for simultaneously monitoring multiple nucleic acid amplification reactions during thermocycling biological samples, the instrument comprising

a device (1; 1') for thermocycling said biological samples according to any one of the preceding claims;
at least one excitation light source for applying excitation energy to the nucleic acid amplification reactions; and
at least one sensor for simultaneously detecting light emitted from the multiple nucleic acid amplifications.

17. A method for thermocycling biological samples using the device (1; 1') according to any one of claims 1 to 15, the method comprising the steps of

a) monitoring the temperature at the biological samples; and
b) based on the monitored temperature at the biological samples, controlling the output of the heat pump (3) and/or the heat exchange rate of the primary heat exchanger (5; 5').

18. The method according to claim 17, wherein the controlling of the heat exchange rate of the primary heat exchanger (5; 5') in step b) is executed by the control unit (7) and preferably comprises a control of the heat exchange rate of the second heat exchanger (6; 6') and/or a stop or reverse of a flow of heat exchanging fluid (41) through the inner space (51; 51') of the primary heat exchanger (5; 5').

19. The method according to claim 17 or 18, wherein the controlling of the heat exchange rate of the primary heat exchanger (5; 5') in step b) includes the control of fluid volume speed to cool the heat exchange fluid (41) in the heat sink (4; 4') above ambient temperature and approximate to the predetermined lowest temperature of the thermocycles controlled by the control unit (7).

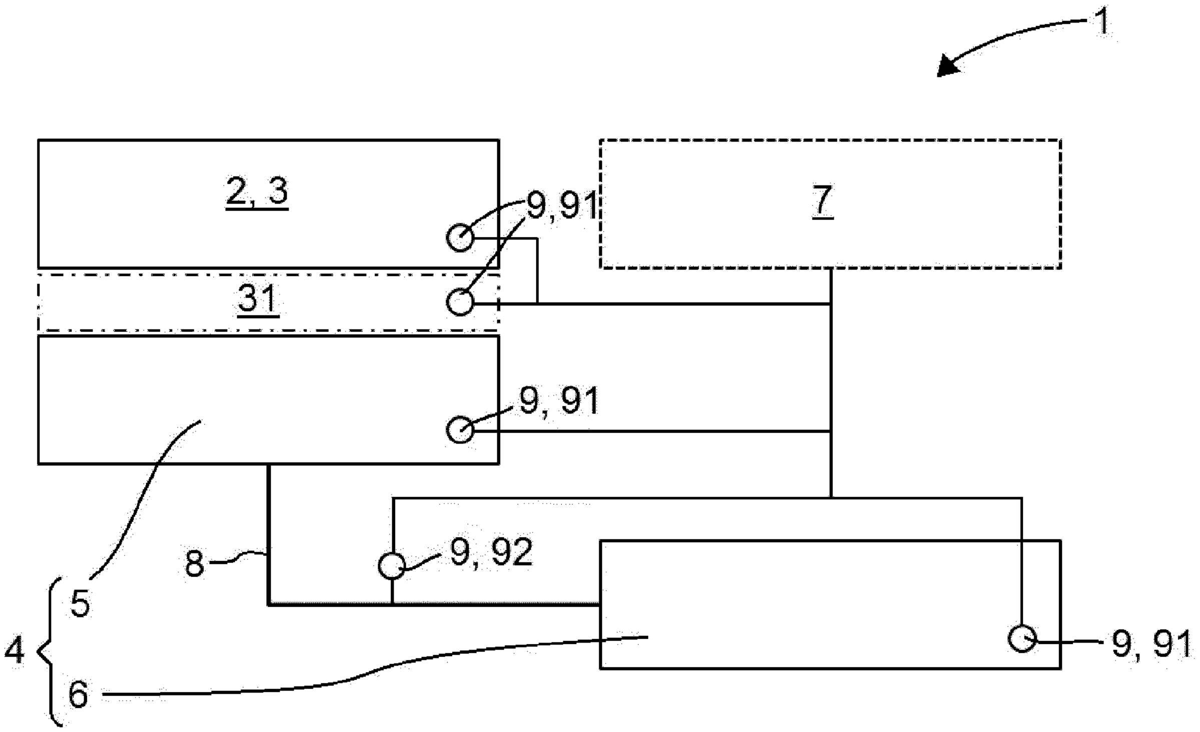


Fig. 1

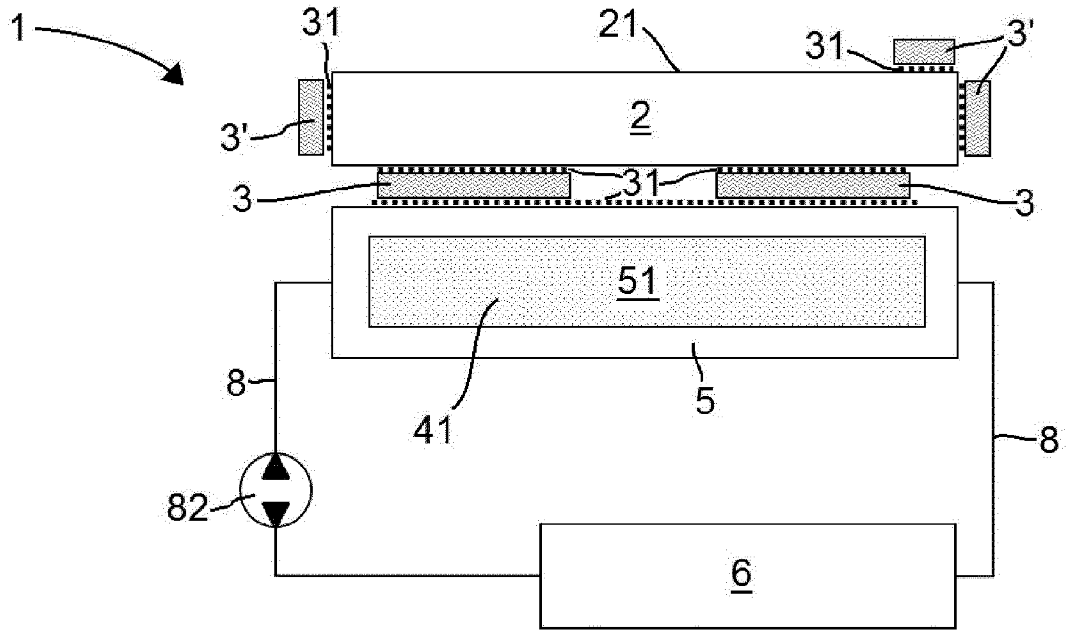
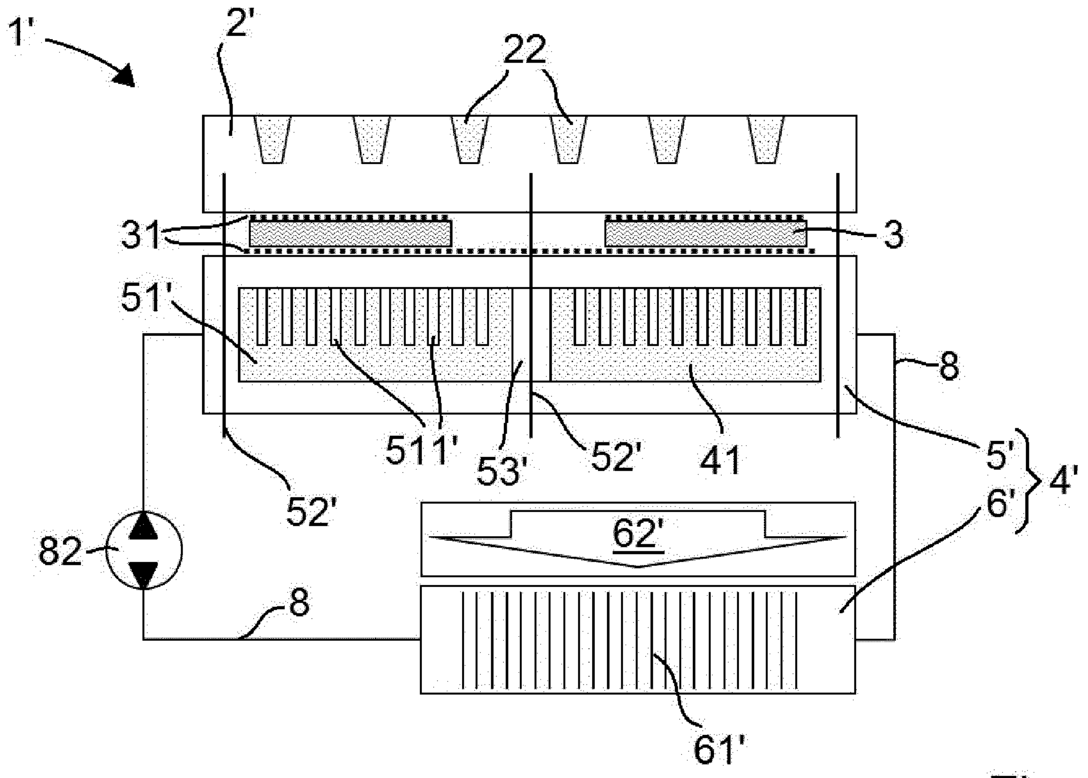


Fig. 2


1'
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31
3
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Fig. 3

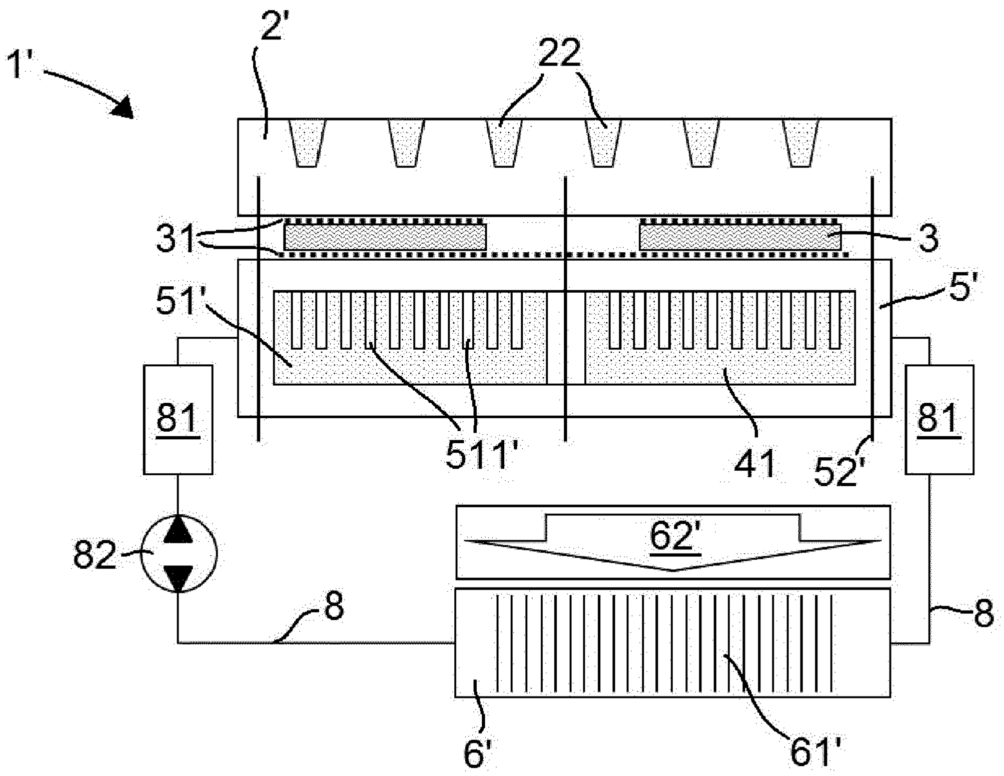
1'
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Fig. 4

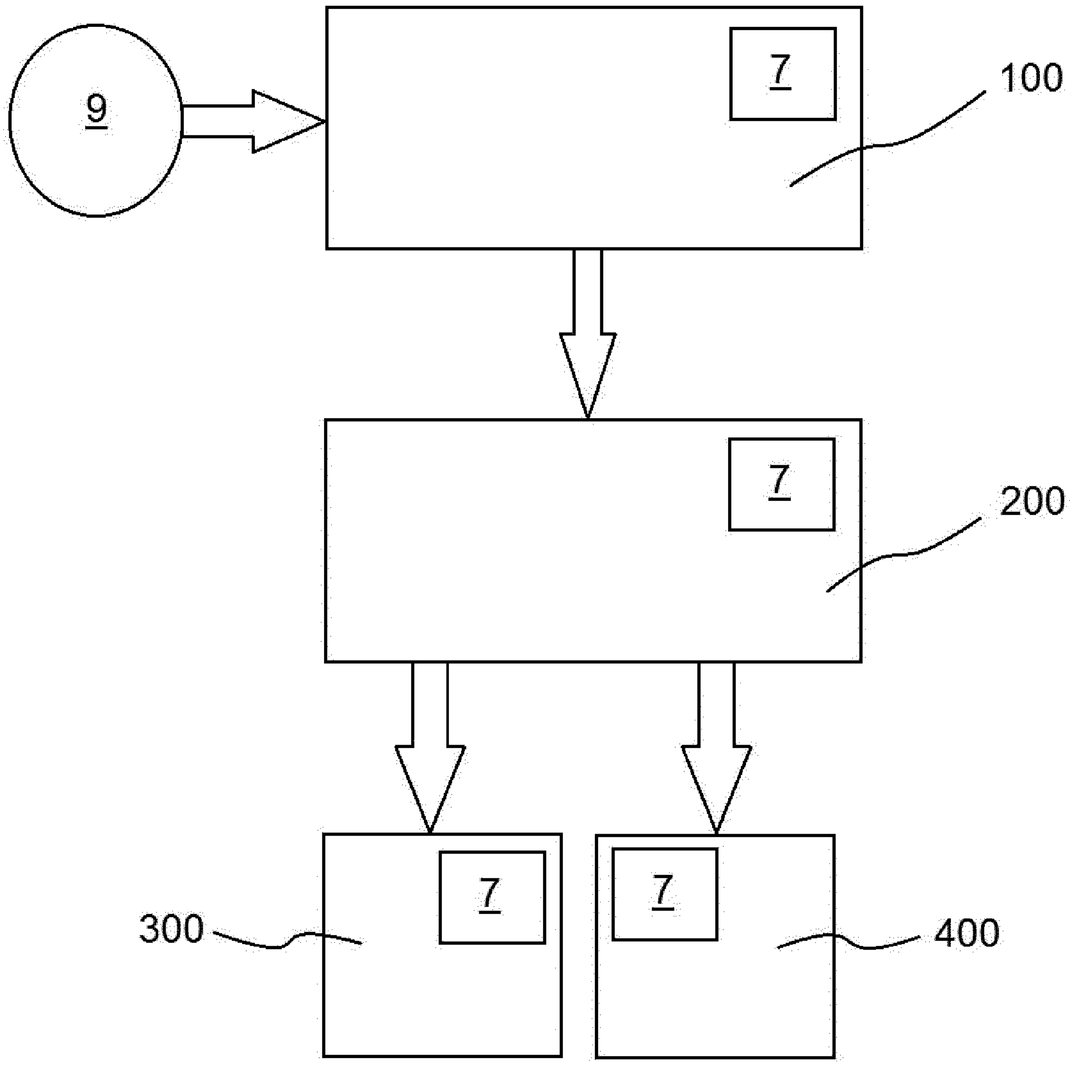

Fig. 5

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EUROPEAN SEARCH REPORT

Application Number
EP 18 15 6326

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 2002/072112 A1 (ATWOOD JOHN GIRDNER [US] ET AL) 13 June 2002 (2002-06-13) * paragraphs [0096], [0099], [0100] - [0103], [0106], [0109], [0110], [0127] - [0129], [0134], [0408]; figures 1,6,8,9,15,20,44-46,47A, * -----	1-19	INV. B01L7/00
X	WO 2014/185628 A1 (MICRO DIGITAL CO LTD [KR]) 20 November 2014 (2014-11-20) * see Enclosure 1 passages: page 1 lines 9-11, page 2 lines 1-15,lines 34-43, page 4 lines 16-42, page 6 lines 4-11; figures 1-4 * -----	1-19	
X	EP 2 520 667 A1 (APPLIED BIOSYSTEMS LLC [US]) 7 November 2012 (2012-11-07) * paragraphs [0028], [0035], [0043], [0048], [0049], [0060], [0063], [0066], [0077]; figures 6,12,17 * -----	1-19	
A	WO 2015/039022 A2 (PHONONIC DEVICES INC [US]) 19 March 2015 (2015-03-19) * paragraph [0059]; figures 5,6 * -----	1-19	TECHNICAL FIELDS SEARCHED (IPC) B01L C12Q C12M
A	EP 2 127 751 A1 (ROCHE DIAGNOSTICS GMBH [DE]; HOFFMANN LA ROCHE [CH]) 2 December 2009 (2009-12-02) * abstract * -----	1-19	

The present search report has been drawn up for all claims

1

Place of search	Date of completion of the search	Examiner
The Hague	16 May 2018	Goodman, Marco

CATEGORY OF CITED DOCUMENTS

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ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 18 15 6326

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16-05-2018

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2002072112 A1	13-06-2002	US 2002072112 A1	13-06-2002
		US 2004086927 A1	06-05-2004
		US 2005084957 A1	21-04-2005
		US 2006269641 A1	30-11-2006
		US 2006286659 A1	21-12-2006
		US 2008299651 A1	04-12-2008
		US 2009155765 A1	18-06-2009
WO 2014185628 A1	20-11-2014	KR 20140134435 A	24-11-2014
		WO 2014185628 A1	20-11-2014
EP 2520667 A1	07-11-2012	EP 2057435 A1	13-05-2009
		EP 2076605 A2	08-07-2009
		EP 2082027 A2	29-07-2009
		EP 2520667 A1	07-11-2012
		US 2008038163 A1	14-02-2008
		US 2008050781 A1	28-02-2008
		US 2008124722 A1	29-05-2008
		US 2011256616 A1	20-10-2011
		US 2013295654 A1	07-11-2013
		US 2017087556 A1	30-03-2017
		WO 2007149696 A1	27-12-2007
		WO 2007150042 A2	27-12-2007
		WO 2007150043 A2	27-12-2007
WO 2015039022 A2	19-03-2015	CN 105556222 A	04-05-2016
		CY 1119116 T1	14-02-2018
		DK 3047219 T3	14-08-2017
		EP 3047219 A2	27-07-2016
		ES 2629264 T3	08-08-2017
		HR P20171064 T1	06-10-2017
		JP 2016532073 A	13-10-2016
		KR 20160055803 A	18-05-2016
		LT 3047219 T	10-07-2017
		PT 3047219 T	14-07-2017
		SI 3047219 T1	30-10-2017
		US 2015075184 A1	19-03-2015
		WO 2015039022 A2	19-03-2015
EP 2127751 A1	02-12-2009	CA 2666033 A1	19-11-2009
		CN 101585009 A	25-11-2009
		EP 2127751 A1	02-12-2009
		HK 1137688 A1	28-11-2014
		JP 5254874 B2	07-08-2013
		JP 2009278971 A	03-12-2009
		US 2010122807 A1	20-05-2010

EPO FORM P0459

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ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 18 15 6326

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16-05-2018

Patent document cited in search report	Publication date	Patent family member(s)	Publication date

EPO FORM P0459

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

REFERENCES CITED IN THE DESCRIPTION

Patent documents cited in the description

- WO 2006105919 A1 **[0008]**
- WO 2013075839 A2 **[0028]**