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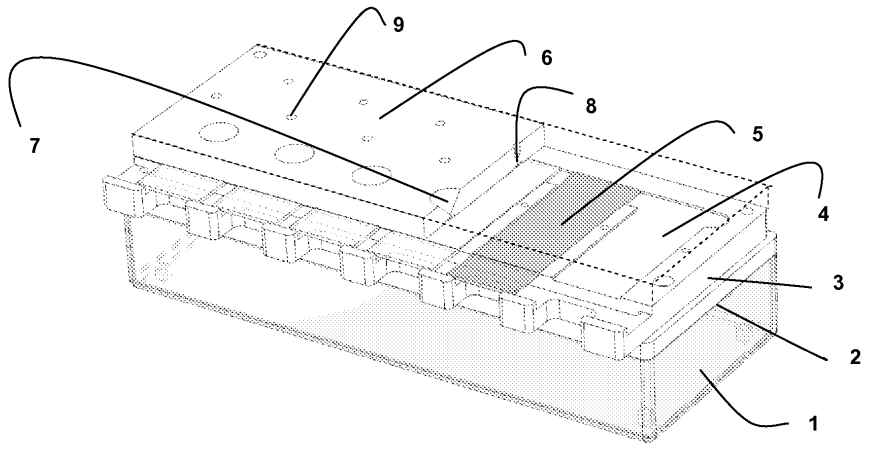
(54) Apparatus and platform for multiplex analysis

(57) The design and performance of an analytical apparatus and correspondingly designed microarray platform devices for facilitated multiplex analysis of analytes in liquids are disclosed. The apparatus in combination with the microarray platform devices are designed for performing bioanalytical assays and for detecting the presence of organic molecular analytes, optionally in microarray format. Several microarray platform devices, including one or several channel-type reaction chambers can be simultaneously analyzed. In preferred aspects, microarray platform devices are laminated microstructured microarray platforms, providing integrated channel-based reaction chambers having capture molecules printed and immobilized in open channel structures prior to lamination. Said microstructured microarray platforms are designed for use in conjunction with the apparatus leading to short assay times, low reagent volumes and performance at temperatures above, and differing from ambient.

FIG. 1.



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Description

FIELD OF THE INVENTION

[0001] The present invention relates to an apparatus and devices for the manufacturing and development of microarrays, as well as their application and use in the field of high throughput analytics including drug development and quality control, or as part of prognostic and diagnostic test systems.

BACKGROUND OF THE INVENTION

[0002] A variety of biological and chemical assays have been developed for detecting the presence of compounds of interest in complex multicomponent samples. Microarray devices in general and, in particular microarrays containing biopolymers as capture ligands such as proteins, polysaccharides, nucleic acids as well as low molecular compounds, have a wide range of research, diagnostic and analytical applications. Microarray devices measure and quantitate molecular interactions upon chemical biochemical or immunological interaction of surface immobilized capture ligands with their corresponding target molecules. Such biological, biochemical or chemical interaction assays are based on exposing an unknown sample to one or more known reactant and can register the progress or measure the outcome of the reaction. It is often desirable to expose a sample to multiple reactants, to react multiple reactants with dilutions of a single sample, or to perform a particular assay with a given sample at a specific time and location.

[0003] The in-vitro diagnostic industry has envisioned, for example, a diagnostic "protein chip" in which a microarray of antibodies or capture ligands are printed on a test piece. In theory, after such a chip has been inoculated with a patient sample (for instance a drop of blood, urine or saliva), proteins would bind to highly specific antibodies or capture ligands and would be quickly measured to determine whether they are at normal or abnormal levels. Such a technology would enable healthcare practitioners to early prognose or diagnose the health status, and administer appropriate medication and/or therapies rapidly and with greater confidence.

[0004] Many of the technical hurdles associated with the microarray technology including arrays of nucleic acids, proteins, carbohydrates or cell arrays, have been addressed and solutions have been proposed or provided to effect capture ligand immobilization, suppression of non specific binding, preservation of biological activity, fluid transport, timed reagent supply, time-effective analytical processing and signal amplification.

[0005] Current limitations of microarrays are the time and the effort required to develop appropriately designed analytical assays, and to obtain in a short time, robust and reliable high quality results, in particular with ad hoc performed, diagnostic point-of-use assays.

[0006] The current microarray technology uses apparatuses and microarray platforms that are customized as product-specific formats (for instance Affymetrix gene chip, or the Nanogen gene chip, Randox analytical platform) or open platforms, designed for general use, on the basis of glass slides or plastic slides in standard dimensions. Whereas product-specific formats have overcome some limitations of reproducible processing with large and expensive machines, capable of processing large numbers of platforms simultaneously simple and effective systems designed for rapid and addressabke single platform processing or for rapid multiplex platform analysis are not available.

[0007] Loeffler et al. (EP1171761), Tanaami Takeo (JP2004226068) and Sigrist et al. (EP1300194) describe multicomponent slide processing chambers designed for microarray slides. Fluidic systems and fluidic arrays and methods for using them to promote bio-interactions have been described by Lee et al. (US Pat. App. 20040258571). McNeely et al (US 20040037739) disclose a method and a system for providing a fluidic interface to slides bearing microarrays of biomolecules or other samples immobilized thereon. US Pat. No 5,797,898 and US Pat. No. 6,123,861 describe microchip devices that release drug molecules from reservoirs having reservoir caps that actively or passively disintegrate. Kroy et al (US Pat. No. 5,252,294) discloses micromechanical structures having closed cavities for use in storage and handling of substances. US Pat. No. 5,948,673 to Cottingham describes a self-contained multichamber reactor for performing hybridization assays in a sealed unit wherein some reactants are provided by coating the walls of the chambers and other reactants are introduced into the open chambers prior to starting the reaction.

[0008] In view of the foregoing, a need exists for apparatuses and corresponding devices enabling diagnostic and analytical use in initiating and controlling biochemical, immunological or chemical reactions, for performing fast bioanalytical sensing, for measurements in a microscale area or volume compatible with microarray technologies. It would also be desirable to provide methods of making and using such devices and the corresponding easy-to-handle apparatus.

DEFINITIONS

[0009] In this specification, the following terms should be interpreted according to the corresponding definitions provided hereafter.

"Apparatus". The expression apparatus relates to a contrivance for analytical use which allows to house and to process

analytically a selected amount of microarray platform devices, whereby the microarray platform devices are placed and screw-tightened between a structured shelf plate and a cover plate, the later giving access and connection to an entry port and exit port. Underlying to the shelf plate is a heating pad mounted on a plate, ultimately allowing temperature adjustment in the microarray platform reaction chamber at and above ambient temperature. The entire assembly of heating pad and -plate, shelf plate and cover plate, with inserted microarray platform devices, is mounted on a socket which houses the electrical wiring and connections to the heating pad and an electrical controller unit, the latter is not part of the invention.

[0010] "Device". In the following, the expression device is used in the context of the microarray platform, said microarray platform device consists of a first microarray substrate and a second microarray substrate, each substrate having a first and a second surface. The microarray platform device is applicable as analytical tool in combination with the apparatus disclosed here. The microarray platform and the corresponding apparatus form an ensemble to be used for analytical and bioanalytical purposes.

[0011] "Microarray platform". Microarray platforms, in general, refer to 2D arrays, typically at the surface of a flat glass or a polymer material, a filter, or silicon wafer, upon which chemical or biochemical molecular species are deposited or synthesized in a predetermined spatial order allowing them to be made available as probes in parallel manner. The present invention refers to such microarrays where the chemical, biochemical or immunological molecular species are deposited as ligands on top of substructures, the latter being arranged within preformed open channels.

[0012] A "channel", as used herein, means a 3D feature in a microarray platform substrate material which, in its open form ("open channel") provides physical external access from at least one direction and which, in its closed form ("closed channel"), is capable of conducting fluids such as liquids or gas, in controlled, directional manner. The expression "closed channel" as used here, refers to channels that are closed along the entire length with the exception of openings at channel entry and exit. Further to this, closed channels can have any cross-sectional shape and cross-sections along one channel may have different types of cross-sections.

[0013] "Substructures". The term "substructure" in the context of "substructured channels", refers to differences in channel cross-sections along the length of open channels. Microchannel substructuration includes micro- and nanostructuration of the bottom of open channels and/or their side walls, as attained by any type of material structuration process, such as for instance by wet-etching or dry-etching, micromachining, e-beam treatment, abrasion, hot embossing or injection moulding.

[0014] The term "in-channel printing" relates to the process of depositing liquids containing the ligand, eventually in combination with chemical reagents that effect ligand immobilization, into open channels. Such liquid deposition can be attained with any device or instrument capable of making available and placing small sample volumes, in precise locally addressable fashion.

[0015] The term "reactant" is used for any type of chemical species that participates in a chemical, biochemical or immunological interaction, with or without molecular change of either species involved.

[0016] "Ligand", as used herein, refers to chemical biochemical or immunological species that are immobilized on the first surface of the microarray platform and participate as reactant. Ligand immobilization includes all processes of adsorptive binding (e.g. surface precipitation, hydrophobic interactions, ionic interactions as well as combinations thereof) and all processes involving covalent ligand binding.

[0017] "Target probe". Target probes are solute components interacting with said ligands or ligand - target complexes in reaction chambers.

[0018] The "reaction chamber", as used in the present invention is the physical space and volume where the ligands and target probes undergo chemical, biochemical or immunological interactions, or participate as reactants in chemical, biochemical or immunological interactions.

SUMMARY OF THE INVENTION

[0019] The present invention introduces an apparatus and corresponding devices to be used with the apparatus for performing biochemical, immunological or chemical reactions on a novel microarray platform, the ensemble consisting of one or several microarray platform devices and the ensemble consisting of the apparatus and the platforms being appropriate for performing microarray-based analyses. It is a further object of the invention to provide methods to use said apparatus and microarray platform devices for analytical purposes.

[0020] The invention further comprises an apparatus for analytical use which allows to hold and simultaneously develop a selected amount of microarray platform devices, individually or up to six items in parallel, whereby the microarray platform devices are placed and screw- tightened between a structured shelf plate and a cover plate, the later giving access and connection to an entry port and an exit port. Underlying to the shelf plate is a heating plate allowing temperature adjustment in the reaction chamber at, and above ambient temperature. A preferred embodiment of the present invention is the fact that the second substrate of the microarray platform is contacting the shelf plate, thus providing efficient temperature transfer to the reaction chamber. The entire assembly of heating plate, shelf plate and cover plate, with

inserted microarray platform devices, are mounted on a socket which houses the electrical wiring and connections to the heating plate and a temperature controller unit (the latter is not part of the invention). A further external instrument is required to actuate the transport of liquids from the entry compartment of the apparatus, leading said liquids into and through the reaction chamber of the microarray platform device. Such instrumentation is not part of the present invention.

[0021] The invention broadly comprises a microarray platform device that can be as large as or larger than a microscope slide having a first and a second surface and one or more engraved microstructure in the said first surface, wherein said microstructure in the first substrate comprises one or more substructures and one or more biologically reactive sites disposed within the said microstructure of the second surface or on top of said substructures, and a microarray platform second substrate forming low volume reaction chambers when assembled with the first surface of the second substrate, said microarray first and second substrate forming the microarray platform.

[0022] A preferred embodiment of the invention concerns the microarray platform device which includes an in-channel printed array (array-printed into an open channel) of one or more ligand (bio)molecules, whereby the ligand (bio)molecules are deposited and preferably covalently immobilized within the channel forming cavities prior to channel closure, said channel closure being effected by sealing the open channels with a second microarray platform substrate. These microarray devices may be used in combination with the apparatus for single sample analysis, or for serial analysis in conjunction with, for instance, robot driven multi-pipetting systems.

[0023] In further preferred embodiments of the present invention, the inner surfaces of the open channel (of the first microarray substrate' s second surface) are substructured whereby the substructures are designed to effect liquid flow perturbation. In additional preferred embodiments of the present invention, said substructures are designed for confined deposition of ligand molecules at elevations, other than the channel bottom, thus enabling distinctive ligand placement and elevation-selective scanning of the platform by optical means, for instance by confocal microscopy. Ultimately preferred substructuration elements of the present invention ascertain fast perturbed liquid flow within height-structured microchannels.

[0024] In further preferred embodiments of the present invention, the reaction chamber forming open channels are engraved on the second surface of the first microarray platform substrate, and the entry and exit connections are feed-through connections (wholes) with pressure seal structures on the first surface of the first microarray substrate, which is opposite to the reaction chamber (assembled on the second surface of the first microarray substrate.

[0025] In still further preferred embodiments, the fluid entry ports of the microarray platforms, located at the first surface of the first microarray substrate, connect to fluid entries of the cover plate, and the overall fluid transport is actuated by either a mechanical liquid displacement mechanisms or by applying a positive or negative gas pressure (vacuum or suction). Such fluid actuation is effected by an appropriate instrument, said instrument is not part of the present invention.

[0026] In a further preferred embodiment of the present invention, the apparatus shown in FIG. 1 ensures close contact fitting of the microarray platform to the shelf plate by screws and enables fast and efficient temperature transport from the heating plate, passing via the shelf plate to the first surface of the second microarray substrate and the reaction chamber, which is separated by said second substrate.

[0027] Specific preferred embodiments of the present invention will become evident from the following more detailed description of certain embodiments and the claims.

BRIEF DESCRIPTIONS OF THE DRAWINGS

[0028] FIG. 1 depicts the apparatus and its constituting components; the socket 1, the heating plate 2, the shelf plate 3, the microarray platform device 5 and the cover plate 6. The latter is shown here shown as partial section for better demonstration of the positioning of the microarray platform devices and the entry 7 and exit 8 connections.

[0029] FIG. 2 depicts is an overview in part (FIG. 2A), a longitudinal section (FIG. 2B) and a cross section (FIG. 2C) of an assembly consisting of the shelf plate 3, microarray platform devices 5 and cover plate 6.

[0030] FIG. 3 is a drawing of the heating plate 2 and the heating pad 11.

[0031] FIG. 4 is an perspective view of the first surface of the microarray platform substrate 12.

[0032] FIG. 5 is a perspective view of the second surface of the first microarray platform substrate 13 with an engraved meander-type open channel 14. The second microarray substrate with its first 15 surface and second surface 16, is shown above, as laminate for illustration purposes.

[0033] FIG. 6 depicts the preferred option of sub-structures in the reaction chamber forming open channels.

[0034] FIG. 7 is a fluorescence scan image of in-channel printed fluorescent ligand molecules.

DETAILED DESCRIPTION OF THE INVENTION

[0035] The design of the apparatus according to the present invention is depicted in FIG. 1. The apparatus consists of a socket 1, a heating plate 2 carrying a heating pad 11, a structured shelf plate 3 for placing up to 6 microarray platform devices 6 and a cover plate. In a preferred embodiment metallic materials are used for the socket, the heating base

plate and the shelf plate, whereas the material of the cover plate is a transparent synthetic polymer. For standard use, the socket, the heating base plate, the heating pad and the overlaying shelf plate are assembled and jointed by screws, the heating pad being electrically connected to an electrical controller unit which is not part of the present invention.

[0036] In a further aspect, the invention provides an apparatus, sketched in FIG. 1, for processing a sample, in particular a chemical, biochemical or biochemical sample, the apparatus comprising in addition computer control means and temperature control means; whereby fluid activation means, computer control means and temperature control means are not part of the present invention.

[0037] The apparatus shown in FIG 1. is used in conjunction with microarray platform devices. Conveniently, the holding means comprises screw-tightening means (wholes 9 for placing such screws) suitable for "sandwiching" the microarray platform devices between the shelf-plate and the cover plate. Such holding mechanism enables tight fitting of the entry port of the cover plate 17 and the entry port of the microarray platform 18, and the exit port of the microarray platform 19 and the exit port of the cover plate 20. Tight fitting between the two parts mentioned may be achieved by guiding structures that allow pressure sealing or any other type of liquid seal known to those skilled in the art. Precise alignment of the microarray platform device and cover plate may further be ascertained by recipient cavities and corresponding pins on the contacting surfaces of the ensemble components.

[0038] Details of the heating means are shown in FIG. 3. The metallic heating plate 2 with the heating pad 11 assembled on top, is fixed on the upper part of the socket. As part of this invention, the shelf plate is individually tightened by screws on top of the heating pad. Such a configuration allows easy removal of the shelf plate without disconnecting electrical connections, in case decontamination of the shelf-plate is required.

[0039] FIG. 2A shows an assembly of the shelf plate and the cover plate with 5 microarray platform devices set in place. For loading a desired number of microarray platforms 5, said microarray platforms are placed in the recesses 4 and the cover plate is placed on top forming a "sandwich-type" assembly. FIG. 2B and FIG. 2C depict cross-sections of such an assembly including microarray platform devices. It is important to note, that the entry 17 and the exit 20 of the cover plate spatially connect to the entry port 18 and exit port 19 of the microarray platform on the first surface of the microarray platform substrate. Liquid tight connections are attained by properly placed 0-rings or equivalent state-of-the-art fixations at either connection. Tight assembly of the microarray platforms, "sandwiched" between the shelf plate and the cover plate is enforced by thumb tighten screws, one placed on each side of any microarray platform used in the analytical process. This allows to process in parallel any selected number of microarray platforms between one and six.

[0040] Details on the analytical platform according to the present invention are depicted in FIG. 4. and FIG. 5. Microarray platforms having conventional microscope slide dimensions (US standard: 1" x 3"; EU standard: 75 x 25 mm) are preferred due to the many commercial available analytical instruments (slide format based microarray printer, microarray reader, hybridization instruments, microarray development devices) for efficient processing and microarray development of said format. Although preferred, the present invention is not restricted to the slide format and the principle of in-channel on top of substructure printing may be applied to many different formats as well as for different materials.

[0041] Any type of material that allows microstructuring of the first microarray substrate and substantial sealing with the second microarray substrate is considered appropriate for manufacturing microarray platforms according to the invention. Preferred first substrate materials are synthetic polymers with low fluorescence background which can be structured by, for instance, injection moulding or hot embossing and which can be produced in high volumes. Such first substrate can be homopolymers or copolymers, for example polyurethanes, polyesters, cyclic olefins, polypropylene, polycarbonate, polyethylene, polyesters, acrylates, polyamides, polyureas or other organic polymers known to those skilled in the art. Alternatively, materials as first microarray substrate from the section of non-plastic materials include glass, silicon-based materials, noble metals, quartz, composites, generally materials that can be structured in micrometer and nanometer dimensions by commonly known processes. First microarray substrates may be microstructured in one type of material and secondarily treated with a local or full surface coating to attain appropriate substrate properties as required for detection (e.g. direct or fluorophore dependent optical measurement, gold coating for the detection of molecular interactions by surface plasmon resonance, appropriate waveguide coatings combined with diffraction gratings for refractometric measurements, interdigitated electrodes combined with microelectronic connections to register locally generated electrochemical signals). Materials that are per se expensive and difficult to microstructure are the least recommended for use.

[0042] In the preferred embodiment, microstructuration of the microarray platform may conform with, or are similar to the drawing in FIG. 4 and FIG. 5. The first substrate of the microarray platform is structured on both sides, the first surface of the first platform substrate 12 is shown in FIG.4, and the second surface of the first substrate 13 is depicted in FIG.5. The first surface of the first microarray substrate in slide format consists of an area reserved for slide marking 23 and the surface of the microarry platform 12 that is in contact with the cover plate. The entry port 18 establishes the trans-platform connection 22 to the microstructures on the second surface of the microarray platform first substrate (see FIG. 4). The area surrounding the port 24 includes the recipient part of the pressure seal structure on the cover plate 10. The exit port 19 on the first surface of the first microarray platform surface establishes direct contact to the exit port of the cover plate 20, said exit being connected to the actuation system which may be either any type of gas-based

pump mechanism, any type of system generating suction by vacuum or flux driven device by positive pressure exerted on the conical entry compartment on the cover plate. If the microarray platform device is used for serial analysis manually or in conjunction with, for instance, robot driven multipipetting systems, the area surrounding the entry port 7 is shaped as liquid recipient open compartment.

[0043] FIG. 6 shows an exploded view of the second surface 13 of the first microarray substrate with a meander-type structure 14 representing one of many possible open channel structure design and arrangements. Other arrangements of the open channel structures may adopt the form of straight lines, a spiral with for example an exit port at the centre, or a reversed spiral arrangement. In a further arrangement, individual open channel structures may be multiplexed on the second surface of the first microarray platform substrate platform. The multiplicity of said structural arrangements implies that there is a plurality of entry ports and exit ports corresponding to the number of microstructures on said microarray platform.

[0044] Independent on the type of the 2D arrangement, the open channels 25 in the second surface of the first microarray platform substrate 14 are converted into closed channels by overlaying the second microarray platform substrate. In a preferred arrangement, said overlaying second substrate is a thin film, flexible material generally known as laminate, 15 & 16, able to close the open channels of the microarray platform upon application to the solid structured first microarray platform substrate 13. Liquid proof closure of the channels is attained with for instance laminates which reversibly or irreversibly interact with the surface 13. Liquid proof closure can be attained by gluing at ambient temperature using commercial laminates or by thermosealing or welding of laminates, either one of the procedures mentioned corresponds to the state of the art currently used for reversible or irreversible microplate sealing. The sealing laminates 15 & 16 are preferably about 100 micrometer thick, nonpermeable to liquids and gases, temperature stable in the range of - 40°C to +100 °C, optically transparent down to 250 nm, and resistant to chemicals and solvents. Such laminates are generally provided with a layer of pressure sensitive adhesive on their lower surfaces 15. In practice, the laminates are applied in a manner similar to adhesive tapes, and serve to permanently seal the open channels, by covering the entire surface 13.

[0045] The invention is not limited to lamination, but liquid proof channel closure can as well be attained by material fusion. This applies to the particular case when hard materials are used as second microarray platform substrates (e.g. of glasses or silicon-based materials), or by welding of hard plastics and other procedures known to those skilled in the art.

[0046] FIG. 6 depicts a preferred option of open channel substructures. The cross-sectional forms of the open channels 25 may be U-shaped, rectangular, with or without rounded bottom features, V-shaped, oval or round. In a preferred embodiment, the second surface of the first microarray platform substrate 13 and the first surface of the second microarray platform substrate 15, are flat, leading to channel cross-sections of the mentioned forms with a flat cover. The height of the so formed closed channels is set by the manufacturing step of the second surface of the first substrate 13. The depth of the channel is at least 30 μm and at most 400 μm deep, more preferably between 50 μm and 150 μm deep. The width of the open channel 25 is at least 200μ m and at most 1000 μm, more preferably between 400 μm and 800 μm. The width of the bridges 26 between the individual channels are at least 200 μm and at most about 1000 μm, more preferably between 400 μm and 600 μm. Microstructuration, complementary to the second surface of the first substrate 14, may be applied to the first surface of the second microarray platform substrate 15, in particular when hard materials are used as second substrates. Such structuration may ultimately yield round, oval, diamond-shaped, rectangular or square cross-sections of the closed channels.

[0047] Depending on the form and size of the closed channels, the total volume of the reaction chamber is between at least 4 μl and at most 40μl, more preferably between 15μl and 25 μl.

[0048] The inventive in-channel printing, and in-channel detection of chemical and biochemical reactions may include substructured open channel topographies, whereby said substructuration ameliorates molecular interactions due to local changes of the fluid flux conditions. Said substructuration further improves the exposure of the immobilized ligand to reagents, to rinsing buffers and solvents, and - in certain configurations - places the capture ligands at distinctive topical elevations allowing not only confined capture ligand deposition but also distinctive addressable reading of generated signals by, for instance, confocal microscopy or mass sensitive optical detection such as refractometry or surface plasmon resonance. FIG. 6 depicts enlarged partial views of exemplary substructures which may be integrated parts of the reaction chambers. Said substructures are generated during manufacturing of the platform by either hot-embossing, injection moulding, ablation technologies common in the art (for instance micromachining, laser ablation, e-beam ablation), by etching procedures (wet etching, dry etching) or positive imprint moulding. The capture ligands are printed at specific sites within the open channel using contact or non-contact microarray printer systems, known to those skilled in the art.

[0049] Selected topographies of preferred substructures are further detailed in FIG. 6. The most simplistic version of in-channel surface substructure consists of an arrangement of protruding disk-shaped structures 27 at the bottom of the open channels. The top surface of said disk-shaped structures may be minimally concave which allows efficient local deposition and confinement of printed ligand solutions. In some further embodiments, wall-to-wall extended wave-like structures with smooth transitions between alternating elevations are introduced into the open channels, the ligands being printed on top of the elevations, or in another embodiment at the intermittent depressions. Such substructures

effect efficient fluid flux distortion and favour the reaction kinetics at the site of molecular interaction.

[0050] The present invention requests deposition of the capture ligands into open channel structures. Ligand deposition is achieved by contact or non-contact bioprinters with preference for instruments allowing precise 2D positioning of the printing robot and precise aliquotation of small volumes per deposition event. Current commercial instruments deliver liquid volumes in the range between 5 μl and several μl (picoliters).

[0051] The depostition of ligands can be carried out with any type of instrumentation, generally known as microarraying systems, bioprinter, arrayer, nanoplotter or nanoprinter, that allow precise local deposition of liquids in controlled quantities. Said microarraying systems are commercially available and their specifications are generally provided by the producer. Piezo activated printing and pin mediated deposition of ligand liquids are among the preferred print systems and their application confers to state-of-the-art procedures. In relation to the present invention it is essential, that fluid deposition occur from the open face of the "open channel" 25. The 2D alignment of the instrument must be sufficiently adjustable and precise to allow printing into the channels and, in the case of channel substructuration, to print onto or into the substructures in question. The sum of all print features may yield a 2D array of deposited ligands, eventually including sample redundancies. A preferred type of print feature is local spotting of individual ligand solutions. Besides spotted features, the microarray instrumentation may also be used to fully or partially deposit ligand solutions, within or along a preset structure, such as extended areas within a channel or as described in Example 2, a side-to-side traverse section of a meander-type channel arrangement, whereby the result may finally resolve in a type of line barcode. Similarly, the processed signal image of an in-channel printed array of a plurality of features may result in a 2D barcode.

[0052] In some embodiments, mere deposition of the ligands into the open channels may lead to physical adsorption, (also known as physisorption) or to chemisorption of the ligands. Said sorption processes depend on the type of material used as first microarray platform substrate, the physicochemical nature of the ligand, and the solvent. In some cases, microarray-based assays may be performed on the basis of ligand adsorption. For example, molecular interactions based on forces such as bioaffinity and hydrophilicity or hydrophobicity may be sufficiently strong and lead to stable target probe binding.

[0053] In some embodiments it may be desired to provide relatively strong interaction of the ligand with the channel forming material. Such interactions can be attained through high affinity binding of ligand molecules or by covalent attachment of the ligands to the material. Some of the processes leading to covalent ligand binding require pre-activation of the material or the attachment of reactive chemical species prior to the deposition of the ligand. Chemical processes leading to strong, and in particular to covalent binding of ligands are numerous, well described in the literature on the subject, and are known to those skilled in the art. Covalent target ligand binding may be attained according to the methods described in the monography entitled Bioconjugate Techniques, ed. G. T. Hermanson, 1996, Academic Press Inc., and in a review "Surface immobilization of biomolecules by light" H. Sigrist; A. Collioud; J.-F. Clemence; H. Gao; R. Luginbuehl; M. Saenger; G. Sundarababu, Optical Engineering 1995, 34, 2339-2348, which are hereby incorporated by reference in their entirety. The method described in the mentioned references may be modified where required to accommodate the (first microarray platform substrate) material and the target ligand. In view of the simplicity of the process, covalent ligand binding is preferably effected by photolinker polymer mediated processes, said processes imply the widespread reactivity of photogenerated intermediates (in particular carbenes and nitrenes or ketyl radicals) with a large variety of materials including plastic polymers and elastomers.

Example 1

[0054] A polycarbonate plastic substrate (7.5 x 2.5 x 1mm) was structured on the second surface with a meander-type open channel in accordance with the present invention, a recipient area was engraved on first surface of the plastic slide and the connecting entry and exit wholes were drilled. The second surface of the plastic substrate which exposes the meander-type open channel was laminated with a multipurpose adhesive polyethylene tape (for instance Simport T329-1). Microarray platform device function was explored by applying 10 to 50 μltest solution on the recipient area and by subsequent applying negative pressure at the exit port, after connecting the port to a peristaltic pump. The fluid samples tested for seal-proof conduct included, among others, phosphate buffered saline, water, aqueous solutions of artificial colours (food colours), bovine serum albumin in phosphate buffered saline, as well as samples of freshly drawn blood. The results confirmed that the microarray platform device is liquid proof, and that fluids of different composition and density can be passed through the reaction chamber by applying negative pressure (e.g. vacuum generated by peristaltic pump action).

Example 2

[0055] A microstructured microarray platform with open channels as detailed in Example 1 was treated with the photolinker polymer OptoDex® (a product of arrayon biotechnology SA, Neuchatel, Switzerland) and dried at ambient temperature for 2 h at 5 x 10-2 mbar to yield a photoactivatable surface. The following ligands were dissolved in 0.5 mM

sodium phosphate and 1.5 mM NaCl, pH 7.4: mouse immunoglobulin 0.5 mg/ml (mIgG); human immunoglobulin 0.5 mg/ml (hIgG). 2 μ| of each sample was pipetted into separated side-to-side segments of a open channel meander-type structure. After deposition, the samples were dried at ambient temperature for 1 h at 5 x 10-3 mbar and the printed surfaces were irradiated for 4 min with an Oriel Lamp (350 nm, 11 mW/cm2). After photoimmobilization, the open channels were laminated and the reaction chamber was rinsed first with 20 μl phosphate buffered saline (PBS) containing 1% bovine serum albumin, 20 μl PBS/Tween® 20, 20 μl PBS and 20 μl deionized water. For control purposes, the microarray platform was scanned for Cy5 fluorescence with the Affymetrix Array Scanner 428 before further treatment with a fluorescent target.

[0056] Immunostaining of the immobilized antigen was carried out by introducing 50 μl Cy5-fluorescently labelled anti-mouse antibody, fluid flux being driven by applying negative pressure with a peristaltic pump. The incubation with the antigen was carried out for 5 min including reverse flow agitation of the antigen solution. After the incubation, the reaction chamber was rinsed first with 50 μl phosphate buffered saline (PBS) containing 1% bovine serum albumin, followed by 50 μl PBS/Tween® 20, 50 μl PBS and 20 μl deionized water. The laminate was removed and the microarray platform was scanned with the Affymetrix Array Scanner 428. The results obtained are summarized in the following Table 1:

TABLE I

Photoimmobilized ligand	Cy5 fluorescence recovery before immunostaining (arbitrary units)	Cy5 fluorescence recovery after immunostaining (arbitrary units)
Channel segment 1: mIgG	273 ± 96	54585 ± 2594
Channel segment 2: mIgG	378 ± 109	47877 ± 3647
Channel segment 3: hIgG	283 ± 58	9538 ± 471

Example 3

[0057] Example 3 documents the procedures for addressable in-channel deposition of ligand molecules and establishes the signal detection process by confocal microscopy. FIG. 7 shows a fluorescence scan image of a microarray platform with meander-type microstructures after deposition of fluorescently labelled ligand (Cy5 labelled OptoDex). Using the Nanoplotter NP2, a product of GeSiM Ltd, Germany, which allows for precise liquid sample deposition in non-contact mode, the fluorescent ligand samples were injected into and placed within the open channels. The instruments software enables appropriate positioning of the piezo pipettes, their activation allowed the deposition of single droplets having a volume of about 0.4 nl each. FIG. 7 shows that the droplets deposit as individual spots without contacting the walls of the open channel. Spots having different ligand concentrations are distinctly revealed by fluorescence scanning with the Affymetrix Array Scanner 428, (a product of Affymetrix Ltd, USA). Further to this observation, it has been found that the fluorescence signal can be detected by confocal microscopy without removal of the laminate. Spot size and optical resolution are comparable yet the signal intensity is reduced as expected due to the presence of the laminate. The results are summarized in Table II.

TABLE 11

Detection of in-channel printed ligands	Recovery of Cy5-OptoDex (arbitraty units 2 fields x 2 columns x 6 rows: 24 spots)
Without laminate	18753 ± 3013
With laminate	10446 ± 3409

Example 4

[0058] The performance of the microarray platform device and the apparatus, both subject of the present invention, was investigated by analysing the content of anti-tetanus antibodies in human blood serum. The results of the comparative investigation, presented in TABLE III, were obtained by analysing blood sera of 16 donors for the presence of anti-tetanus toxoid antibodies by either standard ELISA (Enzyme Linked Immuno-Sorbent Assay) procedures, a diagnostic procedure known to those skilled in the art, and by microarray procedures. Microarray procedures were performed as follows: Prior to the local deposition of the antigen tetanus toxoid to the microarray platform, the microarray platform was coated with a thin-film layer of the photolinker polymer OptoDex® and dried. Tetanus toxoid was dissolved in phosphate buffer and the solution was printed with a robotic system into the open channels, whereby a single droplet of such antigen solution (400 pL volume each) was deposited on top of each protruding disk-shaped structure. Positive

and negative features containing either human immunoglobulin IgG or mouse immunoglobulin, respectively were also printed analogously onto other disc-shape substructures of the microarray platform together with fluorescence calibration standard solutions. Following printing, the microarray devices were first dried by evacuation and exposed to light (350 nm, 4 min, 10 mW/cm2) effecting covalent (photo-induced) immobilization of the printed molecules; then the microarray platforms were laminated. After mounting the printed microarray in the apparatus described in FIG 1., the reaction chamber was perfused first with buffer, then a sample of 2 microliter serum, diluted in 100 microliter diluent buffer, was introduced in the reaction chamber by actuation with a peristaltic pump and incubated during 16 min by alternating cyclic movement of the sample solution. Upon completion of the incubation, the reaction chamber of the microarray platform was rinsed with buffer, and subsequently perfused with fluorophore labelled antihuman immunoglobulin. After 16 min. the microarray was rinsed and the microarray platform was scanned for fluorescence. Relative to calibration standards, the fluorescence intensity was related to the immunoglobin content in the original test sample and the resulting anti-tetanus toxoid antibody concentration was expressed as International Units (IU/L)

TABLE III

	Anti-Tetanus Toxoid Antibody Concentration in Patient Serum [International Units/Liter Serum]	
Serum ID	*Present microarray procedure*	*ELISA procedure*
60052	12	9
71246	20	30
82284	51	57
60122	192	212
82106	307	401
82300	507	518
62103	628	537
61180	903	790
60394	1058	1403
82236	1389	1501
82303	1592	1657
60284	1626	1995
82316	1532	2050
82299	2354	2697
82424	4591	6106
81109	5761	8120

Claims

1. A dedicated apparatus for performing multiplex analytical assays on micro-structured analytical platform devices consisting of the following parts assembled on a socket:

a) a heating unit connected to an external electrical controller unit,
b) a structured shelf plate for placing microarray platform devices,
c) one or more microstructured analytical devices, with individually addressable reaction chambers, at least one of said devices comprising a microstructured first substrate with one or more grafted ligand (bio)molecule and a second substrate, which transforms the microstructures in the first microarray platform substrate into a channel-based reaction chamber, wherein one or a plurality of different capture molecules are deposited and immobilized within the microstructures of the first substrate, and the second substrate is applied thereafter, forming a part of a microfluidic system,
d) a cover plate providing feed-through entry and exit connections to the analytical platform devices and a fluid activation system, respectively.

2. An analytical apparatus according to claims 1, wherein a multiplex actuation system allows simultaneous perfusion of up to six microarray platform devices with reagents required to perform chemical, biochemical or immunological reactions within individually addressed reaction chambers, whereby liquids are agitated by cyclic forward/reverse liquid movement.

3. An apparatus according to claims 1 to 2 wherein integrated heating allows adjustment of the temperature of the fluids in the reaction chamber of the microarray platform devices to a preset temperature in the range of 20°C to 92°C, preferably in the temperature range of ambient temperature to 40°C.

4. A microarray platform device comprising a first substrate comprising a microstructured first microarray platform with one or more grafted ligand (bio)molecule and a second substrate, which transforms the microstructures in the first microarray platform substrate into a channel-based reaction chamber, wherein one or a plurality of different capture molecules are deposited and immobilized within the microstructures of the first substrate, and the second substrate is applied thereafter, forming a part of a microfluidic system.

5. A device according to claim 4, where the depth of the microstructures is between 15 and 400 micrometer, preferably 100 micrometer.

6. A device according to claims 4 and 5, where the bridges between the microstructures are between 200 and 1000 micrometer, preferably 500 micrometer.

7. A device according to claims 4 to 6, where the bottom of the microstructured surfaces present flow-distorting disc-shaped substructures.

8. A device according to claims 4 to 7 where the elevations in the channels are disc-shaped substructures having a radius of 150 micrometer and a height of 25 to 45 micrometer.

9. A device according to claims 4 to 8, where the second microarray platform substrate is a transparent hard plastic, an elastomer or a glass.

10. A device according to claims 4 to 9 where the capture molecules are printed and immobilized into the open channels of a microstructured first microarray platform, whereby the total feature of deposited (bio)molecules forms an array.

11. A device according to claims 4 to 10 where the capture molecules are immobilized at the flat bottom of the microstructured first microarray platform prior to application of the second microarray platform substrate.

12. A device according to claim 4 to 11 where the capture molecules are immobilized on the elevations of the microstructured first microarray platform prior to application of the second microarray platform substrate.

13. A device according to claim 4 to 12 where reagent binding to the probing molecule is recorded by fluorescence detection of an assay reagent after removal of the second microarray platform substrate.

14. A device according to claims 4 to 12 where reagent binding to the probe molecule is recorded by fluorescence detection of an assay component without prior removal of the second microarray platform substrate.

15. Use of the microarray platform device according to claims 4 to 14, with the apparatus according to claims 1 to 3, for microanalytical investigations in particular microarray based analyses and for microarray based environmental or food analyses.

FIG. 1.

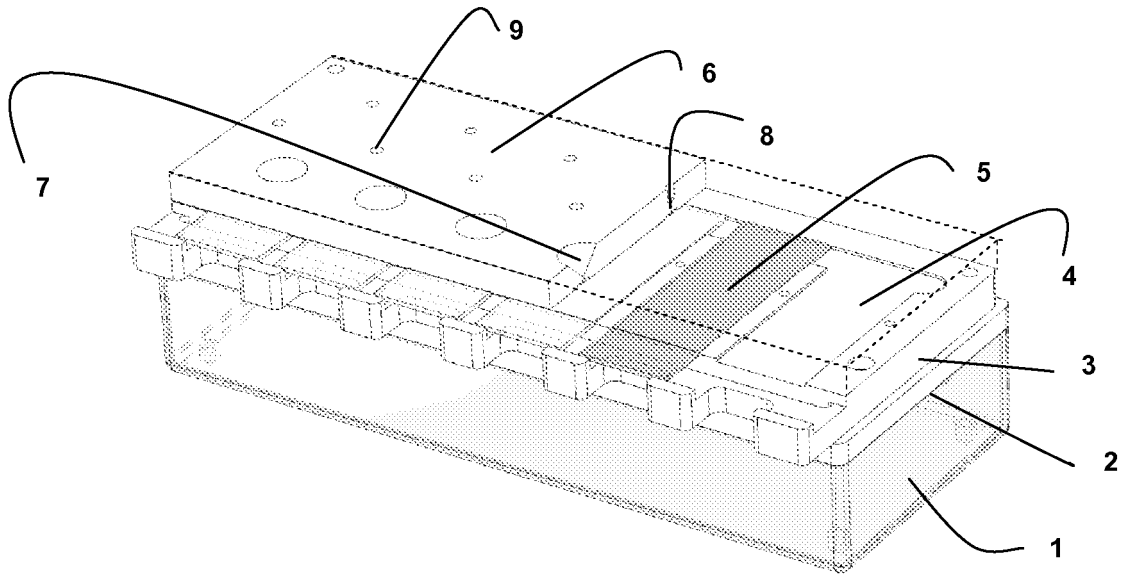


FIG. 2A

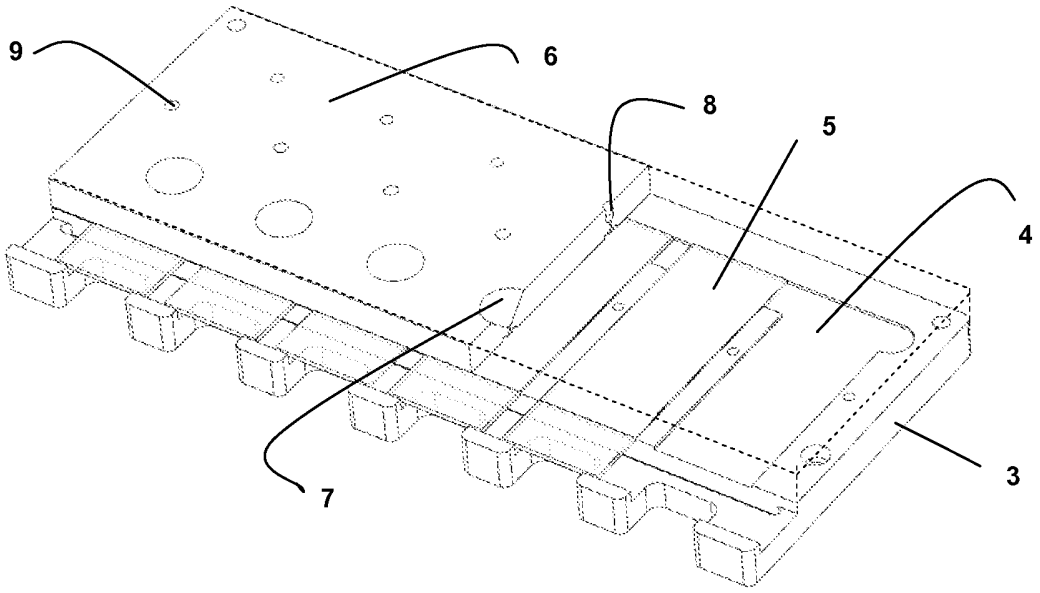


FIG. 2B

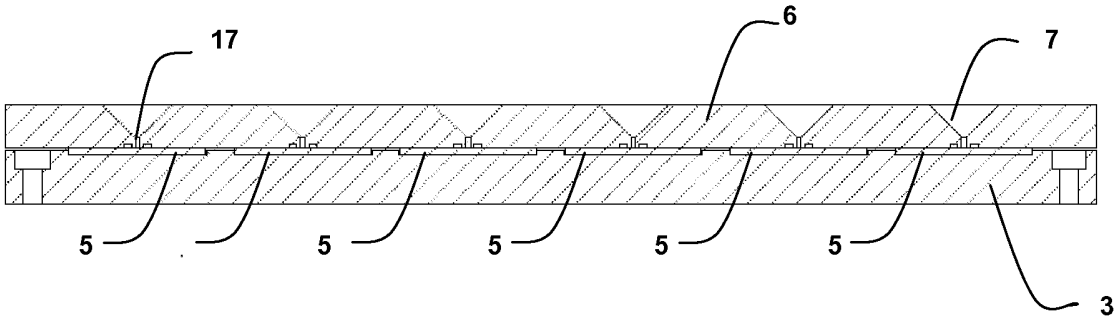


FIG. 2C

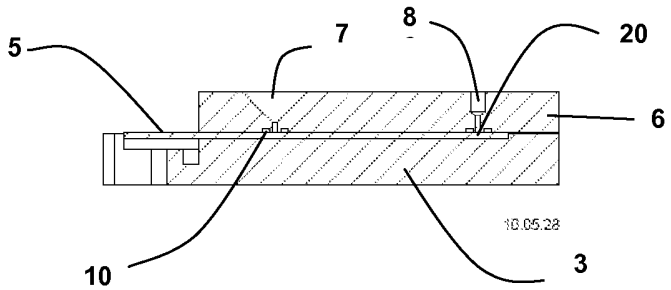


FIG. 3

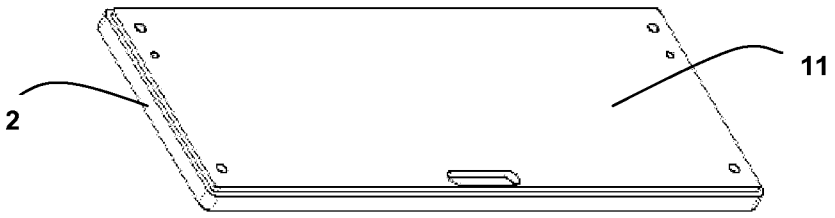


FIG. 4

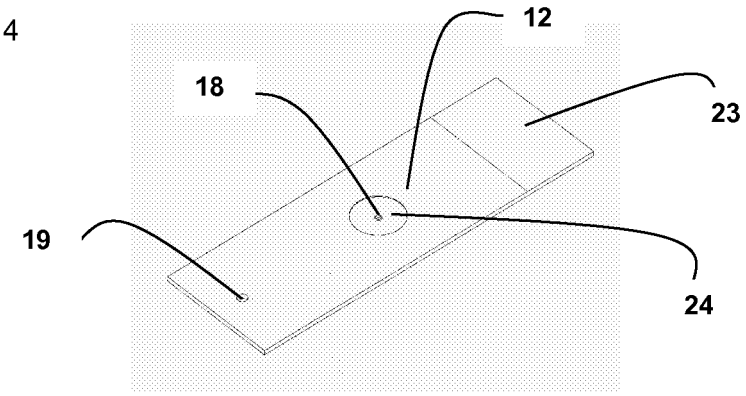


FIG. 5

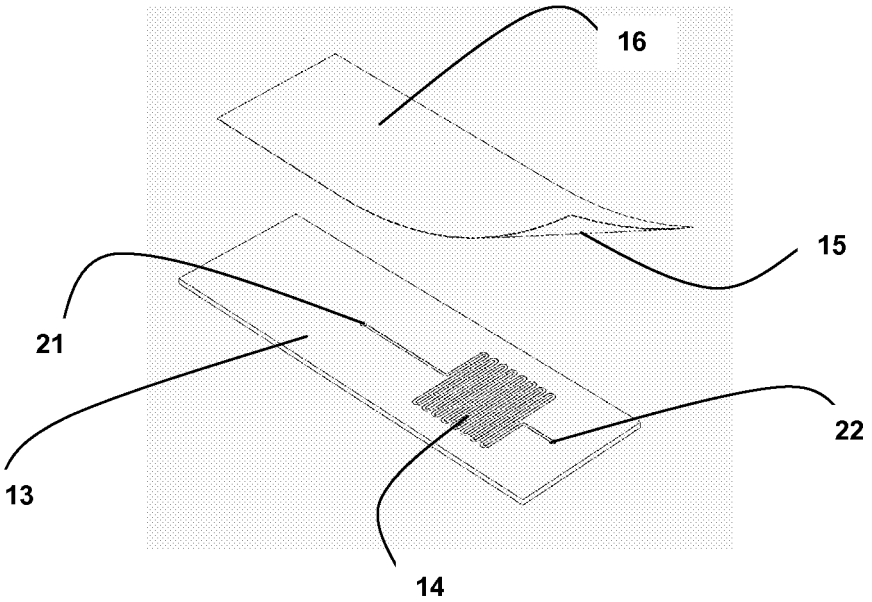


FIG. 6

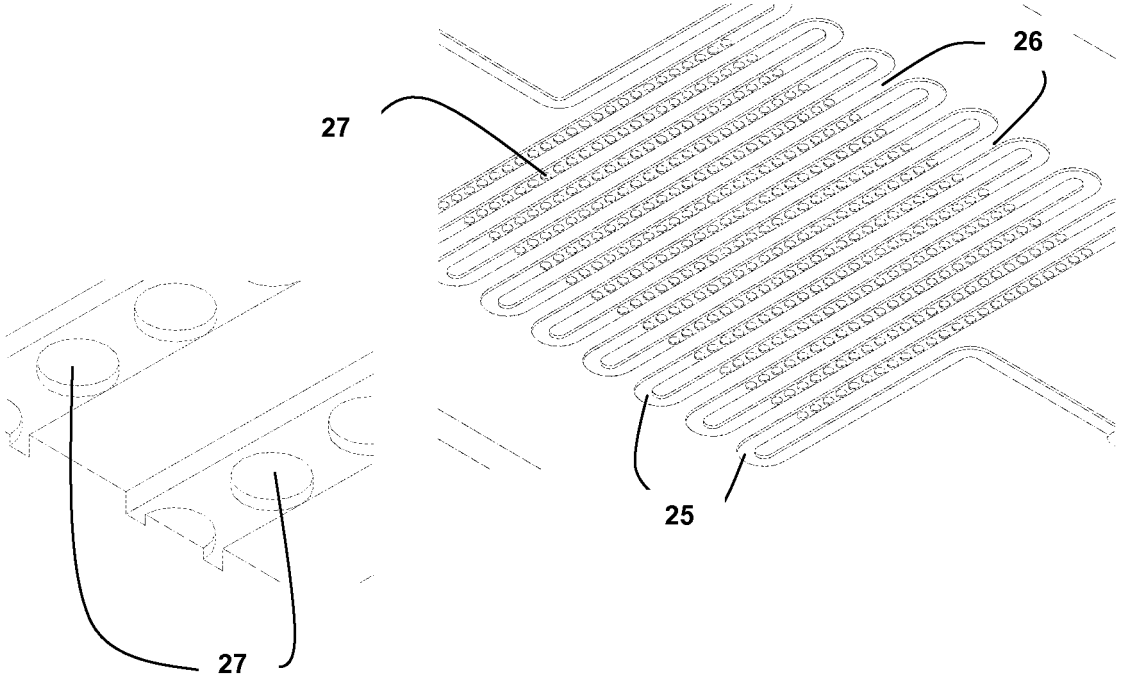
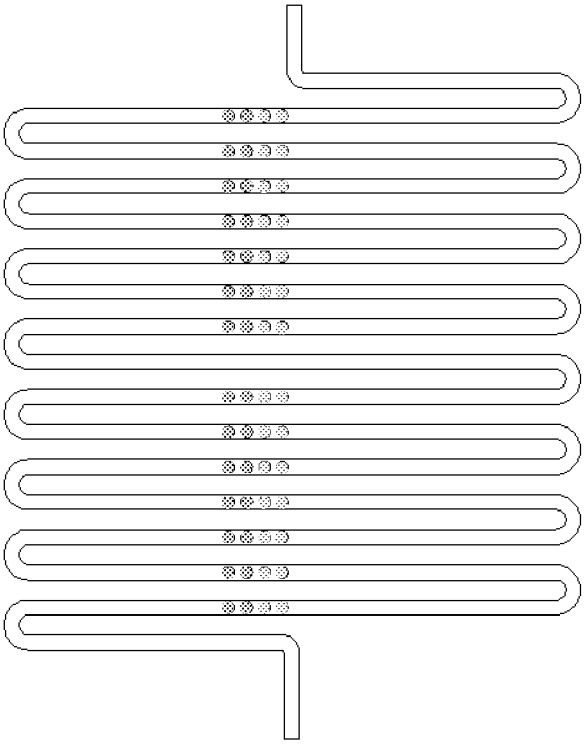


FIG. 7



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EUROPEAN SEARCH REPORT

Application Number
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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	WO 2005/016529 A1 (THINXXS GMBH [DE]; WEBER LUTZ [DE]) 24 February 2005 (2005-02-24) * abstract * * page 6, line 20 - page 9, line 18 * * page 21, line 25 - page 22, line 10 * * figures 1-3,11 *	1-15	INV. B01L3/00 B01L9/00
X	US 2005/009101 A1 (BLACKBURN GARY [US]) 13 January 2005 (2005-01-13)	4-14	
A	* abstract * * paragraphs [0045] - [0048], [0107], [0176] - [0178], [0373] - [0402]; figures 20-31 *	1-3	
X	US 2003/129671 A1 (WILDING PETER [US] ET AL) 10 July 2003 (2003-07-10)	4-14	
A	* abstract * * paragraphs [0009] - [0018], [0045], [0049], [0061] - [0071]; figures 1,5,10, *	1-3	
			TECHNICAL FIELDS SEARCHED (IPC)
X	DE 203 12 088 U1 (IBIDI GMBH [DE]) 16 December 2004 (2004-12-16) * the whole document *	4-14	B01L
X	WO 03/036298 A2 (EXIQON AS [DK]; NORHOLM MIKKEL [DK]; MOLLER SOREN [DK]; KONGSBAK LARS) 1 May 2003 (2003-05-01)	4-14	
A	* abstract * * page 2, line 19 - page 6, line 2 * * page 7, line 15 - page 10, line 22 * * page 31, line 1 - page 34, line 41 * * figures 2A,3A,4 *	1-3	
	-/--		

The present search report has been drawn up for all claims

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A	US 2004/219661 A1 (CHEN CHIEN-AN [TW] ET AL) 4 November 2004 (2004-11-04) * abstract * * paragraphs [0023] - [0027]; figures 3,6 * -----	1-15	
			TECHNICAL FIELDS SEARCHED (IPC)

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2005016529 A1	24-02-2005	DE 10336849 A1	10-03-2005
		EP 1654065 A1	10-05-2006
		JP 4640549 B2	02-03-2011
		JP 2007501940 A	01-02-2007
		US 2006215155 A1	28-09-2006
US 2005009101 A1	13-01-2005	NONE	
US 2003129671 A1	10-07-2003	NONE	
DE 20312088 U1	16-12-2004	NONE	
WO 03036298 A2	01-05-2003	AU 2002348982 A1	06-05-2003
US 2004219661 A1	04-11-2004	TW 579430 B	11-03-2004

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Patent documents cited in the description

- EP 1171761 A, Loeffler **[0007]**
- JP 2004226068 B, Tanaami Takeo **[0007]**
- EP 1300194 A, Sigrist **[0007]**
- US 20040258571 A, Lee **[0007]**
- US 20040037739 A, McNeely **[0007]**
- US 5797898 A **[0007]**
- US 6123861 A **[0007]**
- US 5252294 A, Kroy **[0007]**
- US 5948673 A **[0007]**

Non-patent literature cited in the description

- Bioconjugate Techniques. Academic Press Inc, 1996 **[0053]**
- **H. SIGRIST ; A. COLLIOUD ; J.-F. CLEMENCE ; H. GAO ; R. LUGINBUEHL ; M. SAENGER ; G. SUNDARABABU.** Surface immobilization of biomolecules by light. *Optical Engineering,* 1995, vol. 34, 2339-2348 **[0053]**