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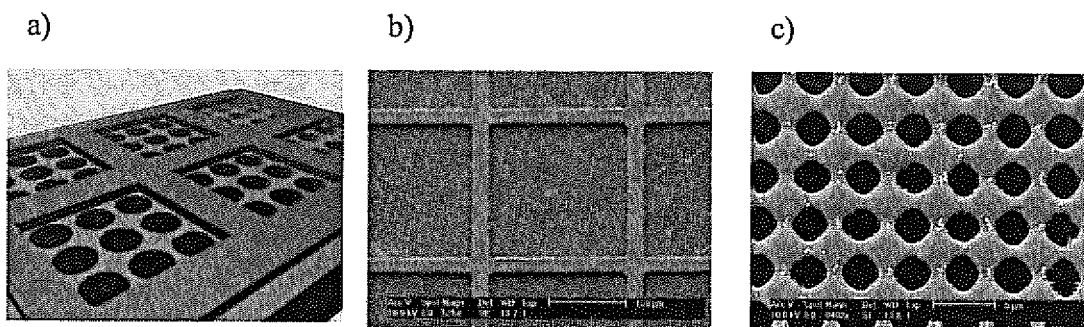
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(54) **Membranes suited for immobilizing biomolecules**

(57) The present invention relates to flow-through membranes suitable for the immobilization of biomolecules, methods for the preparation of such membranes and the use of such membranes for the immobilization of biomolecules and subsequent detection of immobi-

lized biomolecules. The invention concerns a flow-through membrane for the immobilization of biomolecules on spots on said membrane, comprising polymeric material and an internal support, wherein said membrane has pores and wherein the spots of the membrane are separated by said internal support.

FIG. 1:



Description

FIELD OF THE INVENTION

[0001] Subject of the present invention are flow-through membranes suitable for the immobilization of biomolecules, methods for the preparation of such membranes and the use of such membranes for the immobilization of biomolecules and subsequent detection of immobilized biomolecules.

BACKGROUND OF THE INVENTION

[0002] The immobilization of biomolecules is a prerequisite in many standard assays for the detection of biomolecules. Such assays are used in a variety of fields such as molecular biology, immunology, cell biology, biochemistry in clinical situations, where they serve in diagnostics and also in food science. Receptor molecules that are able to specifically bind to biomolecules with high affinity are immobilized on defined spots of a substrate and then contacted with a solution in order to detect biomolecules in that solution. Upon binding to their receptor, biomolecules (i.e. the analytes) are detected, e.g. by the use of specific probes. Most commonly used detection systems are optically and based on fluorescence, refractive index changes or spectral changes. For example, the fluorescence of the bound biomolecules itself or of fluorescently labeled probes is detected. For high-throughput screening applications, a high number of receptor molecules that specifically bind to different biomolecules have to be present on one substrate. Most often biochips, gene chips or other microarrays are used to which the receptor molecules have been attached. For detection of specific biomolecules, the position of specific receptor molecules on defined spots of such an array needs to be known in order to interpret the results. The position of specific receptor molecules encode for specific biomolecules to be detected.

[0003] Biomolecules detected in standard immobilization assays include DNA, RNA, proteins, cells, small molecules and drugs present within a sample. Accordingly, the receptor molecules ("capture probes") most often used are oligonucleotides, DNA, RNA or antibodies to which the biomolecules to be detected ("capture targets") hybridize to (in case of nucleic acids and oligonucleotides) or bind to (in case of e.g. proteins).

[0004] The ultimate goal of an array technology is to achieve high sensitivity, high density packing of spots and fast screening. Currently there are two types of micro arrays available which differ in the flow direction of the analyte towards the capture sites: flow over and flow-through. The later is the most sensitive. This technique is based on a perpendicular flow of the sample of interest through a micro porous substrate, e.g. a membrane. The high specific surface area causes a large number of specifically bonded probe molecules on the capture spots, and as a result a high sensitivity for the detection of bonded molecules. Examples of membranes which are currently used for flow-through processing are Nytran® and Pamgene® (WO 03/102585). The former is nylon membrane consisting of randomly distributed pores. Due to the large capillary forces and the interconnectivity of the pores the spot size of printed capture molecules is relatively large.

[0005] The Pamgene® membrane exhibits a mono-disperse pore distribution of non interconnected pores and is based on aluminum oxide.

SUMMARY OF THE INVENTION

[0006] This invention concerns a flow-through membrane for the immobilization of biomolecules on spots on said membrane, comprising polymeric material and an internal support, wherein said membrane has pores and wherein the spots of the membrane are separated by said internal support.

[0007] Also an object of the present invention is a method for the preparation of a membrane for the immobilization of biomolecules using photo-lithography and/or holography on a negative photo-resist.

[0008] Also within the scope of the present invention is the use of the described membranes for the immobilization and detection of biomolecules.

[0009] The basic idea of the present invention is the presence of an internal support on highly-symmetric flow-through membranes. This internal support is created by photo-lithography and/or holography of organic polymer materials.

[0010] The advantages of the membranes according to the present invention and the use of these membranes as compared to the prior art are for example a relatively small pressure drop over the membrane, a relatively small non-active volume, a well defined size of the spots of capture probes, no optical cross-talk and an even distribution of the spots of capture probes. Together these advantages result in the possibility of a dense packing of spots without interference between the spots.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011]

Fig. 1: a) Schematic representation of the flow-through membrane with incorporated support. b) Scanning electron microscope (SEM) image of flow-through membrane with incorporated support. c) SEM image Close-up of membrane.

Fig. 2: Antibody (Ab) immobilized on substrate for a one step assay

Fig. 3: Antibody immobilized on the substrate for a sandwich immunoassay

Fig. 4: Antibody immobilized on a substrate for a competitive assay

DETAILED DESCRIPTION OF EMBODIMENTS

[0012] The present invention relates to a flow-through membrane for the immobilization of biomolecules on spots on said membrane, comprising polymeric material and an internal support, wherein said membrane has pores and wherein the spots of the membrane are separated by said internal support.

[0013] The terms "internal support(s)", "internal support structure(s)" and "support structure(s)" in the context of the invention relate to structures on the surface of the membrane that separate defined areas ("spots") from each other and additionally may contribute to the overall stability of the membrane. According to the present invention a spot is a well-defined area that consists of many small pores. Preferably the spot area is enclosed by the integrated supports. The spots of the printed capture probes may be in the form of a circle (may be round) or square. The internal support structures have defined heights over the membrane surface and a defined thickness. Preferably, the internal support is arranged in a defined symmetrical geometry, e.g. a grid. The internal support prevents diffusion and optical crosstalk between adjacent spots so that for example fluorescence emissions of dyes in one spot are not absorbed by the dyes in another spot.

[0014] Preferably, the distance between the spots is from 0.1 to 100 μm , more preferably from 1 to 30 μm .

[0015] Preferably, the spots have a surface area of 1 μm^2 to 250000 μm^2 . More preferably the spot size is 25 μm^2 to 40000 μm^2 . Or even more preferably the spot size is 100 μm^2 to 22500 μm^2 . "Spot" diameters may be 1-500 μm , preferably 5-200 μm , more preferably 10-150 μm . The advantage of a small spot diameter is the achievable high signal density.

[0016] In preferred embodiments of the invention the polymer membrane layer further comprises functional groups in said defined spots for the attachment of capture probes.

[0017] In particular embodiments of the invention, said functional groups are selected from the group consisting of vinyl, epoxide, thiol, aldehydes, carboxyl, hydroxyl, amine, succinimide and lysine groups. Particularly, said capture probes are covalently attached to said functional groups. A preferred way of covalently coupling is via the standard NHS/EDC chemistry which is known to a person skilled in the art. According to the present invention it is however not necessary to couple capture probes covalently. It is also possible that immobilizing of capture probes as achieved by physical adsorption via hydrophobic interactions; e.g. in case of antibodies.

[0018] In other particular embodiments of the invention said functional groups are selected from the group consisting of amides, peptides, hydroxyl or other groups where proteins non-covalently bind. Particularly, in this case said capture probes are non-covalently attached to said functional groups. Hereto the surface of the cured epoxide group can be either coated with a thin film a polymer containing said groups or the free epoxide groups that are still present at the surface of cured epoxide resin can be reacted with molecules that contain a moiety enabling reaction with epoxide (e.g. an amine group or a carboxylic acid group) and a group that non-covalently bind the protein. The surfaces can be made charged such that there is van-der-Waals and hydrophobic interactions present allowing physical adsorption of proteins.

[0019] The capture probes according to the present invention are selected from the group consisting of oligonucleotides, DNA, RNA, aptamers, amplicons, antibodies or functional fragments of antibodies.

[0020] Preferably, the membrane and the internal support are made from the same or a different negative photo-resist selected from the group consisting of epoxide resins, acrylates, methacrylates, vinyl ethers and cinnamates. More preferably, the membrane and the internal support are made from a multivalent epoxide resin that is photocured via a cationic polymerization mechanism. It is preferred to use a resist that is able to form stable structures and is capable to form high aspect ratios. The aspect ratio, defined by the thickness of the film that forms the narrow grid divided by the diameter of the pore, can vary between 0.1 and 50, but preferably is between 1 and 10. Even more preferably the membrane and support are made of SU-8, a commercial multivalent epoxide resin.

[0021] Most preferably, the support material is the same as the materials in which the pores are being formed.

[0022] In the most preferred embodiment of the support material and the materials in which the pores are being formed is epoxide. It is most preferred that the capturing probes are present in these pores.

[0023] The pores of the membranes according to this invention may have diameters of 20 nm to 20 μm . More preferably the pores have diameters from 50 nm to 5 μm . Preferably the pores are substantially cylindrical, tapered, diabolic, spiral, oval or zigzag shaped. When the pores are made by mask-based lithographic techniques the shapes are prescribed by the shape of the transmissive areas of the mask. In a preferred embodiment the pores are made by a double holographic

exposure where two interference patterns are superimposed. The angle between the interference patterns, the periodicity of the pattern and the ratio between the mutual intensities determine the shape of the pores. Also modulation of the pore shape into the third dimension is possible, which may lead to improvement on pressure drop, flow patterns within the pores or efficiency of out coupling of fluorescent light when this is used for detection. Modulation in depth of the film is for instance achieved by adding an absorbing agent in the negative resist formulation that modulates the intensity of light in the depth of the film such that the top of the film receives a higher dose than the bottom of the film. This for instance leads to tapered pores after development. Alternatively one can make use of a standing wave of the light over the thickness of the membrane film such that a periodic modulation is achieved leading to several contraction areas within the pores. Yet, alternatively several layers can be coated on top of each other where each layer has a different photo-initiator concentration and hence another sensitivity. Higher initiator concentration leads to a larger sensitivity and thus to locally smaller pores. The pores may be perpendicular to the surface of said membrane or have an "oblique" angle. The oblique angle, defined by the angle of the pore axis with the normal of the film may range from 0° to 75°. The angle of the pores is determined by placing the substrates with the membrane coating under an angle with respect to the exposure beam in the case of mask exposure or with respect to the average of the two interference beams in case of holographic exposure. At larger angles total reflection of light start to play a role and special means to avoid this might be necessary during the exposure step, such as the use of prismatic optical elements. Preferably the angles are between 0° and 30° with respect to the surface of said membrane. Preferably, the pores are symmetrically arranged on said membrane.

[0024] The membrane of the present invention may have a thickness of 0.5 μm to 500 μm , preferably from 1 μm to 100 μm or even more preferably from 2 μm to 20 μm .

[0025] The internal support according to the present invention may preferably have a height from 1 μm to 100 μm and a width of from 1 to 100 μm , more preferably, a height of from 2 μm to 50 μm and a width of from 2 μm to 50 μm .

[0026] Preferably, said membrane is transparent or translucent. Transparent is defined as that the transmission of a transmitting beam of visible light, perpendicular to the surface and at the location outside the supports, while the membrane is filled with water must be higher than 70 %. With "translucent" a lower transmission as just defined is accepted but the transmitted light as collected by an integrated sphere must be higher than 70 %.

[0027] In some particular embodiments of the invention said internal support is transparent or translucent.

[0028] Preferably, the membrane additionally comprises one or more metallic layers. The metallic layers are highly reflective with a reflection coefficient >0.8 . The preferred materials are silver, gold and aluminum. The metal layers may be used to limit cross-talk between neighboring areas and/or to direct light efficiently to the detector.

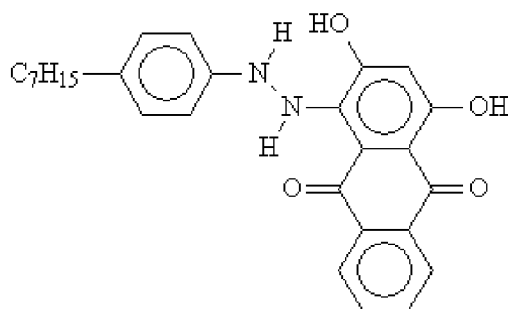
[0029] In some embodiments the membrane comprises two or more polymeric membrane layers, and capture probes are attached to the uppermost membrane and the lower membrane layer(s) are used for temperature regulation or light management. Light management in the case of fluorescent detection is a means to couple light needed for excitation effectively into the dye molecules and the emitted light efficiently to the detector. Temperature control is desirable for instance in order to control specific and non-specific binding during hybridization.

[0030] Proteins may be bound at temperatures in the range of from around 20°C to around 40°C, preferably at around room temperature or at around 37°C.

[0031] DNA hybridizations may be conducted as follows: denaturing of ddDNA /PCR (around 95 °C), annealing temperatures around 50-70 °C. The annealing temperature of a specific DNA fragment depends on the sequence. If one wishes to use NASBA technology (non-PCR) the isothermal temperature might be around 42 °C. Furthermore, QPCR may be performed on the membranes

[0032] The membrane and/or the internal support may be colored with a dye selected from the group consisting of acridine dyes, anthraquinone dyes, arylmethane dyes, azo dyes, cyanine dyes, diazonium dyes, nitro dyes, nitroso dyes, phthalocyanine dyes, quinone-imine dyes, thiazole dyes, xanthene dyes, and rhodamine dyes. Alternatively pigments can be used from the group consisting of arsenic pigments, carbon pigments, cadmium pigments, iron oxide pigments, Prussian blue, chromium pigments, cobalt pigments, lead pigments, copper pigments, titanium pigments, ultramarine pigments, mercury pigments, zinc pigments, and clay earth pigments. Also pigments from biological origins like Alizarin, Alizarin Crimson, Gamboge, Indigo, Indian Yellow, Cochineal Red, Tyrian Purple, Rose madder, and organic pigments like Pigment Red 170, Phthalo Green, Phthalo Blue, Quinacridone Magenta can be used. Preferably a dye or pigment is selected that does not hinder the photochemical process for the structuring of the resists material. E.g. the dye must have a low absorption in the wavelength region between 350 and 400 nm. But the dye has a high absorption in the wavelength region of the emission of the fluorescent dye i.e. between 450 and 800 nm. Preferably, the concentration of the dye is chosen such that the absorbance is above 0.5 and preferably above 1 and even more preferably is above 2.

[0033] An example of a suited dye is the following quinone dye:



[0034] The internal support may be colored with a fluorescence absorbing dye absorbing at wavelengths of from 450 to 800 nm, such that fluorescence from neighboring spots is absorbed. Not only fluorescence techniques can be applied on the membranes, one can also apply enzymatic amplifications, and chemiluminescence techniques. Standard biology optical detection systems are known to a person skilled in the art.

[0035] In some particular embodiments the polarity of the surface of the spots is different from the polarity and /or hydrophobicity of the inner surface of the pores

[0036] One of the advantages of the membranes of the present invention is the minimal pressure drop over the membrane which is partly achieved because of the uniform and highly symmetrical distribution of the pores of the membranes according to the present invention. The skilled person knows that the pressure drop over the membrane depends for example on aperture (total open area), membrane thickness and pore shape.

[0037] The homogeneous membranes of the present invention exhibit low inter and intra-assay variations. A further advantage of the present invention is the transparency of the membrane, improving the detection sensitivity of the device.

[0038] The membranes of the present invention exhibit reduced or minimal fluorescent crosstalk. In fluorescent cross-talk the fluorescence from neighboring spots interferes with the detection of the signal from a single spot. Furthermore, optical cross talk can also lead to lower signal/noise ratio decreasing the assay sensitivity. As there is almost no or minimal fluorescent crosstalk for the inventive membranes the assay sensitivity is high.

[0039] Also within the scope of the invention are methods for the preparation of any of the membranes described herein above for the immobilization of biomolecules using photo-lithography and/or holography on a negative photo-resist.

[0040] The method comprises in one embodiment the steps of

- applying a negative photo-resist on a substrate;
- exposing the negative photo-resist to a holographic interference pattern or exposing the negative photo-resist to radiation through a mask, thereby creating the latent image of the membrane in the negative photo-resist;
- exposing the negative photo-resist to radiation through a mask or to a holographic interference pattern, thereby creating the latent image of a support structure, wherein the support structure separates said spots on said membrane;
- post-baking and subsequently cooling the negative photo-resist; and
- developing the negative photo-resist.

[0041] In an alternative embodiment the method comprises the steps of:

- applying a first layer of negative photo-resist on a substrate;
- exposing the negative photo-resist to a holographic interference pattern or exposing the negative photo-resist to radiation through a mask, thereby creating the latent image of the membrane in the negative photo-resist;
- applying a second layer of negative photo-resist on said first layer of negative photo-resist;
- exposing the negative photo-resist to radiation through a mask or to a holographic interference pattern, thereby creating the latent image of a support structure, wherein the support structure separates said spots on said membrane;
- post-baking and subsequently cooling the negative photo-resist; and
- developing the negative photo-resist.

[0042] In another alternative embodiment the method comprises the steps of:

- applying a first layer of negative photo-resist on a substrate;
- exposing the first layer of negative photo-resist to a holographic interference pattern or exposing the negative photo-resist to radiation through a mask, thereby creating the latent image of the membrane in the negative photo-resist;
- post-baking and subsequently cooling the negative photo-resist;

- developing the first layer of negative photo-resist;
- applying a second layer of negative photo-resist on said first layer of negative photo-resist;
- exposing the second layer of negative photo-resist to radiation through a mask or to a holographic interference pattern, thereby creating the latent image of a support structure, wherein the support structure separates said spots on said membrane;
- post-baking and subsequently cooling the negative photo-resist; and
- developing the second layer of negative photo-resist.

[0043] Preferably, the methods may additionally comprise the step of pre-baking and subsequently cooling said negative photo-resist after any of the application steps and before exposing said negative photo-resist to a holographic interference pattern or to radiation.

[0044] Additionally, the methods may comprise the step of pre-heating said negative photo-resist before any or all of the post-baking steps, preferably between 60 to 70 °C, more preferably around 65 °C.

[0045] Preferably, the first exposing step is exposing to a holographic interference pattern and the second exposing step is exposing to radiation through a mask.

[0046] Alternatively to holographic interference patterns in the first exposing step, this step may be conducted by exposing the negative photo-resist to radiation through a mask. Depending on the pore dimension and the membrane thickness diffraction problems may arise. Thus, holography may be used especially for smaller pores. Therefore, the use of holographic interference patterns in the first exposing step is preferred according to the present invention.

[0047] For the making of larger dimensions e.g. the integrated support as mentioned in the second exposing step, masks may be used. Holographic interference patterns may be also an alternative for the second exposing step.

[0048] In order to make pores according to the present invention two holographic exposures are needed. This is explained in detail above.

[0049] This radiation of the exposing steps may be light and preferably UV-light. The wavelength of the radiation used is dependent on the initiator system of the photo-resist material. The initiator may be sensitized to a higher wavelength when molecules are added with absorption bands in that region and energy levels such that energy transfer takes place to the initiator. For most epoxide resists UV is used because of the initiator system used.

[0050] Preferably, the substrate is a glass substrate. In a preferred embodiment the glass is coated with an intermediate layer that promotes wetting but allows an easy release later on. Examples are monolayers of specific silanes such that the surface contains polar groups like those of hydroxyl containing silanes. Also an intermediate polymeric layer may be used that later can be easily removed e.g. by washing in water. Suited intermediate layers are polyvinylalcohol or polypyrrolidone. A person skilled in that art knows further examples for substrates e.g. polyester.

[0051] The mask used in the exposing steps of the inventive method may be a contact mask or a proximity mask or a well projection mask. It is preferred to use a contact mask. The exposing with a holographic interference pattern may for example be a double holographic exposure (double-exposure holography). A single holographic exposure creates an image of the interference pattern formed by the two interfering laser beams. For the second exposure the substrate is rotated giving a second interference pattern that is at an angle with the interference pattern created by the first holographic exposure. The angle between the two holographic exposure steps may be 90 degrees but can be any other angle between 5 degrees and 175 degrees. Instead of a double holographic exposure with a two-beam holographic exposure also three beam or four beams exposures are possible which creates the desired image in a single exposure step.

[0052] A pre-baking step may be used to evaporate the solvent without formation of bubbles or voids by excessive evaporation of the solvent. This pre-baking is preferred and desirable but not always mandatory. For instance, if the membrane film is stored at room temperature for a while the solvent evaporates. For some epoxide based resists it is very advisable to introduce the pre-baking step, e.g. the epoxy based SU8 resist. For other resists it may be advisable or preferred. Pre-baking may be conducted between 60 to 180 °C. The procedure may be optimized according to the resist used. A pre-baking step is preferred wherein the temperature is between 60 to 70 °C, preferably around 65 °C for up to 3 minutes, preferably around one minute and subsequently ramping the temperature to 90 to 100 °C, preferably around 95 °C and holding the temperature there for up to 4 minutes, preferably for around 2 minutes.

[0053] The subsequent cooling step comprises cooling to below 30°C, preferably between 18 to 22°C, more preferably room temperature.

[0054] The post-baking may be conducted by a temperature between 60 to 180 °C, preferably between 65 °C and 95 °C. The post-baking step may preferably be conducted with a brief pre-heating step at temperature between 60 to 70 °C, preferably around 65 °C. Subsequently, temperature is raised to 90 to 100 °C, preferably at around 95 °C.

[0055] The subsequent cooling step comprises cooling to below 30°C, preferably between 18 to 22°C, more preferably room temperature.

[0056] Any of the steps of the above described methods may in some embodiments be repeated for the addition of further layers of negative photo-resist.

[0057] According to the method of the invention washing and rinsing steps may be conducted between and after the respective method steps. Washing and/ or rinsing may especially be applied after the developing steps for instance.

[0058] In a further embodiment the methods comprise the steps for the addition of metallic layers to the membrane as described above.

[0059] The methods may also comprise additional steps for the addition of capture probes to spots on said membrane. Inkjet printing is a preferred method according to the present invention. Other means can also be applied as hand pipetting or complex offset printing.

[0060] The present invention also relates to the use of any of the membranes described above for the immobilization and detection of biomolecules.

[0061] Preferably, the membranes according to this invention are used for the detection of biomolecules bound to said capture probes.

[0062] The biomolecules bound to said capture probes are for example detected by fluorescently labeled probes. Other labels may be chemiluminescent, enzymatic, or conductivity labels.

[0063] For example the membranes according to the present invention may be used for ELISA assays, bioassays, binding and interaction assays, nucleic acid hybridization assays.

[0064] "Biomolecules" in the context of this invention are molecules naturally occurring in living organisms or molecules that are otherwise biologically relevant or molecules derived therefrom. Particularly relevant biomolecules in the context of this invention are macromolecules such as peptides, proteins, oligosaccharides, oligonucleotides and nucleic acids, like DNA, RNA, LNA and PNA.

[0065] "Oligonucleotides" in the context of this invention are short sequences of nucleotides with 2 to 200 nucleotides, particularly sequences with 2 to 20 nucleotides.

[0066] Functional groups in the context of this invention are groups on the surface of the membrane spots to which capture probes can be attached, e.g. covalently attached or non-covalently attached. In case of a covalent attachment of the capture probes, the functional groups may be for example acrylate, epoxide, thiol, carboxyl, hydroxyl and amine groups. Other groups are described above.

[0067] The term "antibody" in the context of the present invention comprises monoclonal and polyclonal antibodies and binding fragments thereof, in particular Fc-fragments as well as so called "single-chain-antibodies" (Bird R. E. et al (1988) Science 242:423-6), chimeric, humanized, in particular CDR-grafted antibodies, and dia- or tetra-antibodies (Holliger P. et al (1993) Proc. Natl. Acad. Sci. U.S.A. 90:6444-8) and "lama antibodies". Also comprised are immunoglobulin like proteins that are selected through techniques including, for example, phage display to specifically bind to the polypeptides of the present invention.

[0068] A photo-resist is a light-sensitive material used for example in photolithography and photoengraving to form a patterned coating on a surface. During photo-lithography the photo-resist is exposed to light and subsequently a developer is added. A negative photo-resist is a photo-resist in which the portion of the photo-resist that is exposed to light becomes relatively insoluble to the photo-resist developer whereas the unexposed portion of the photo-resist is dissolved by the photo-resist developer. Examples for negative photo-resists include epoxide resins, acrylate resins, methacrylate resins, polyvinylcinnamate, bisazide modified polymers such as polyvinylalcohol, polyvinylacetate.

[0069] A pre-baking step according to the methods of the present invention ensures that all solvent has evaporated and that the photo-resist is in the glassy state (enabling the use of multiple exposure steps) is described above. In a preferred embodiment it comprises heating the negative photo resist material to 65°C for 1 minute, ramping to 95°C, and leaving at 95°C for 2 minutes. Subsequently, the photo resist is slowly cooled to room temperature to avoid building up unwanted stresses in the film.

[0070] A post-baking step according to the methods of the present invention has been described above and in a preferred embodiment. It comprises heating the negative photo resist material to 65°C for 1 minute, ramping to 95°C, and leaving at 95°C for 2 minutes to induce cross-linking of the photo-resist, rendering the exposed areas insoluble to the developer liquid. Subsequently, the photo resist is cooled to room temperature.

[0071] Developers used according to the methods of the present invention are for example mr-Dev 600 (MicroChem. Corporation). Further examples for developers are known to a person skilled in the art.

[0072] The holographic interference pattern according to particular embodiments of the present invention is created by preferentially using an energy density of around 140mJ/cm² at a wavelength of ca. 351 nm.

[0073] The pore geometry of the basic membrane can be adjusted by changing the holographic interference pattern and the rotation angle between the exposure steps. The design of the contact mask determines the structure of the support, which determines the final shape of the micro-array.

[0074] Inkjet printing to attach the capture probes to defined spots on the membrane can be performed.

[0075] Subject of the present invention is the use of any of the membranes according to the invention for the immobilization and detection of biomolecules.

[0076] The biomolecules are selected from the group consisting of proteins, peptides, oligonucleotides, RNA, DNA, antibodies, tissue, cells, drugs, chemical compounds. Biomolecules may be detected which may be bound to capture

probes.

[0077] The biomolecules may be detected by fluorescently labeled probes. Further labels were described above.

[0078] Subject of the present invention is the use of any of the membranes according to the invention for ELISA assays, bioassays, binding and interaction assays, nucleic acid hybridization assays.

Example 1: Preparation of a flow-through membrane

[0079] The membrane is produced by applying a thin layer of negative photo-resist material (SU-8, commercially available, MicroChem. Corporation) on a glass substrate that is coated with an adhesion promoter. A pre-bake step (1 minute at 65°C, ramping to 95 °C and leave at 95°C for 2 minutes) ensures that all solvent has evaporated and that the SU-8 is in the glassy state - enabling the use of multiple exposure steps. The cooling is performed slowly to avoid building up unwanted stresses in the film. The sample is then exposed to a holographic interference pattern to create the latent image of the membrane in the photo-resist (140mJ/cm², 351 nm). Subsequently, the post-bake step is performed. The sample is heated to 65°C for 1 minute, 95°C for 2 minutes to induce cross-linking of the photo-resist, rendering the exposed areas insoluble to the developer liquid. The non cross-linked areas are washed away with developer (mr-Dev 600, MicroChem. Corporation), followed by rinsing with isopropanol. This way, the basic membrane is created. Another layer of photo-resist is applied on top of the membrane. The pre-bake step is repeated. Hereafter, the sample is exposed to UV-light through a contact mask to create the latent image of the support structure. Again, the exposed areas are cross-linked in a post-bake step. Optionally, the pore geometry of the basic membrane can be adjusted by changing the holographic interference pattern and the rotation angle between the exposure steps. The design of the contact mask determines the structure of the support, which determines the final shape of the micro-array.

Example 2: Antibody (Ab) immobilized on substrate; one-step assay

[0080] In this exemplary embodiment, an antibody is immobilized on the flow-through membrane (Fig. 1). The binding of a fluorescent labeled analyte of interest to the antibody is detected by fluorescence with an optical set-up, e.g. with a CCD camera. A sample containing the analyte to be determined is exposed to the flow-through membrane. Before detection, the membrane is washed to remove unspecific binding. The analytes of the sample are fluorescently labeled, e.g. with the dyes CyDye, Alexa Fluor etc.

Example 3: Antibody (Ab) immobilized on the substrate; sandwich immunoassay

[0081] In this exemplary embodiment, an antibody (Ab1) is immobilized on the flow-through membrane (see Fig. 2). A sample containing the analyte to be determined is exposed to the flow-through membrane. Then, the membrane is washed to remove excess analytes. A secondary antibody (Ab2) which is biotinylated is added, then the membrane is washed again to remove unspecific binding. AF633- or Cy5-labelled streptavidin is added. The Ab1 :analyte:Ab2-streptavidin complex is detected by fluorescence with an optical set-up, e.g. with a CCD camera.

Example 4: Antigen (Ag) on substrate; competitive assay

[0082] In this exemplary embodiment, an antigen (Ag) is immobilized on the flow-through membrane (see Fig. 3). A sample containing the unlabelled analyte antibody to be determined is exposed to the flow-through membrane together with a labeled antibody (e.g. biotinylated) of known concentration. Then, the membrane is washed to remove excess antibodies. AF633- or Cy5-labelled streptavidin is added, then the membrane is washed again to remove unspecific binding. The Ag:labeled antibody:labelled streptavidin complex is detected by fluorescence with an optical set-up, e.g. with a CCD camera. In this assay there is an inverse relationship between signal intensity and the amount of analyte present. Higher signal intensity would mean that the analyte of interest is present in minute quantities.

Claims

1. Flow-through membrane for the immobilization of biomolecules on spots on said membrane, comprising polymeric material and an internal support, wherein said membrane has pores and wherein the spots of the membrane are separated by said internal support.
2. The flow-through membrane according to claim 1, wherein said polymer membrane further comprises functional groups in said defined spots for the attachment of capture probes.

3. The flow-through membrane according to claim 2, wherein said capture probes are covalently attached to said functional groups.
4. The flow-through membrane according to any one of the claims 2 to 3, wherein the capture probes are selected from the group consisting of oligonucleotides, DNA, RNA, aptamers, amplicons, antibodies or functional fragments of antibodies.
5. The flow-through membrane according to any one of the claims 1 to 4, wherein the pores are symmetrically arranged on said membrane.
6. The flow-through membrane according to any one of the claims 1 to 5, wherein said membrane is transparent or translucent.
7. The flow-through membrane according to any one of the claims 1 to 6, wherein the membrane additionally comprises one or more metallic layers.
8. The flow-through membrane according to any one of the claims 1 to 7, wherein the membrane comprises two or more polymeric membrane layers, and wherein capture probes are attached to the uppermost membrane.
9. The flow-through membrane according to any one of the claims 1 to 8, wherein the membrane and/or the internal support are colored with a dye having low absorption in the region between 350 and 450 nm and high absorption in the region between 450 and 850 nm.
10. Method for the preparation of a membrane according to any one of the claims 1 to 9, for the immobilization of biomolecules using photo-lithography and / or holography on a negative photo-resist.
11. The method according to claim 10 comprising the steps of
 - applying a negative photo-resist on a substrate;
 - exposing the negative photo-resist to a holographic interference pattern or exposing the negative photo-resist to radiation through a mask, thereby creating the latent image of the membrane in the negative photo-resist;
 - exposing the negative photo-resist to radiation through a mask or to a holographic interference pattern, thereby creating the latent image of a support structure, wherein the support structure separates said spots on said membrane;
 - post-baking and subsequently cooling the negative photo-resist; and
 - developing the negative photo-resist.
12. The method according to any one of the claims 10-11, wherein the first exposing step is exposing to a holographic interference pattern and the second exposing step is exposing to radiation through a mask.
13. The method according to any one of the claims 10-12, wherein the substrate is a glass substrate.
14. The use of any of the membranes according to claims 1 to 9 for the immobilization and detection of biomolecules.

FIG. 1:

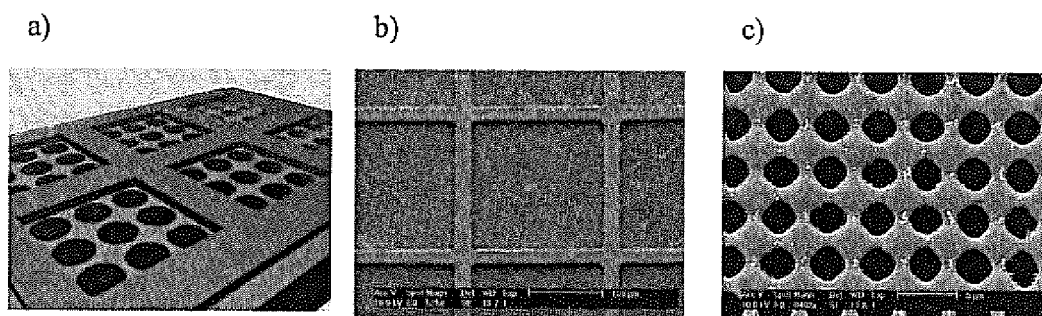


FIG. 2:

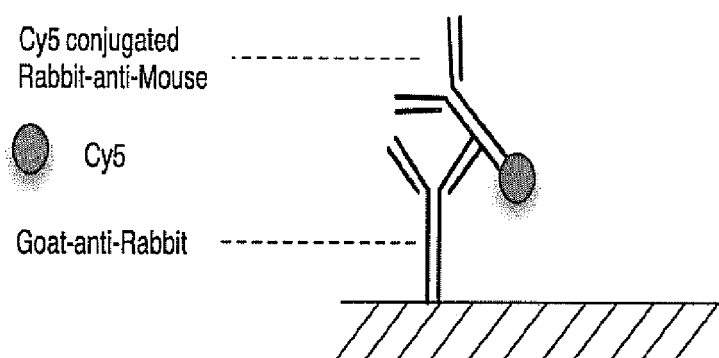


FIG. 3:

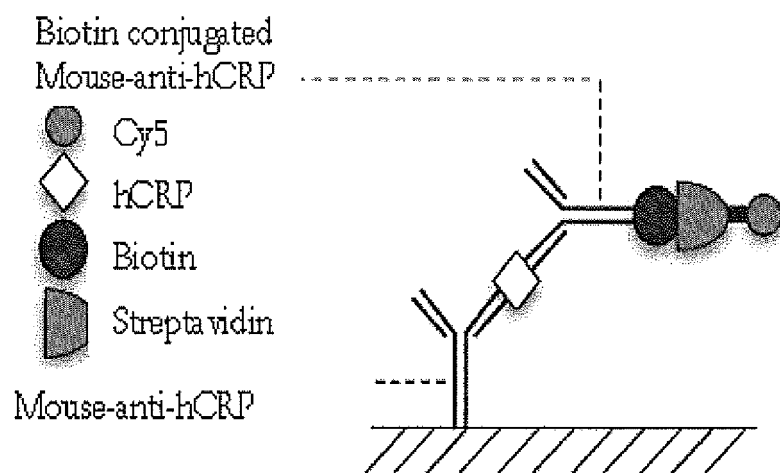
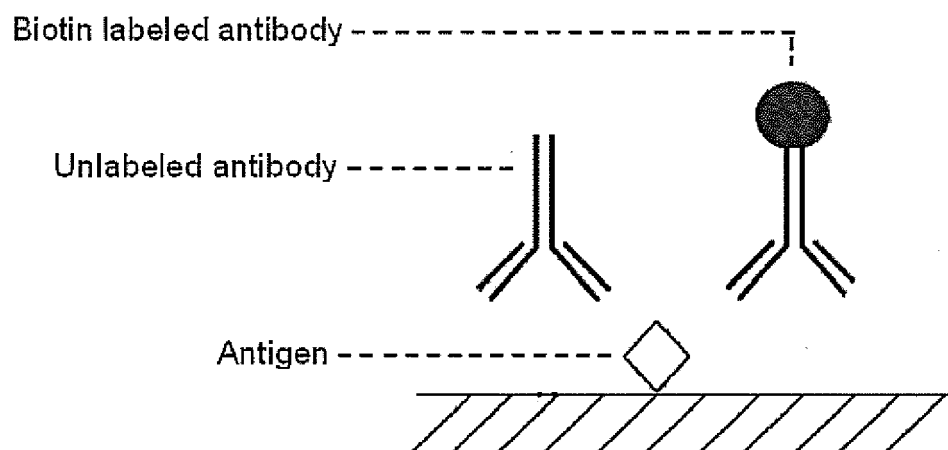


FIG. 4:





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