(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
11 October 2012 (11.10.2012)

WIPO | PCT

(10) International Publication Number
WO 2012/136695 A1

(51) **International Patent Classification**:
B01L 3/00 (2006.01) *G01N 33/543* (2006.01)
B01L 9/00 (2006.01)

(21) **International Application Number**:
PCT/EP2012/056143

(22) **International Filing Date**:
4 April 2012 (04.04.2012)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:
1150304-2 6 April 2011 (06.04.2011) SE
61/472,302 6 April 2011 (06.04.2011) US

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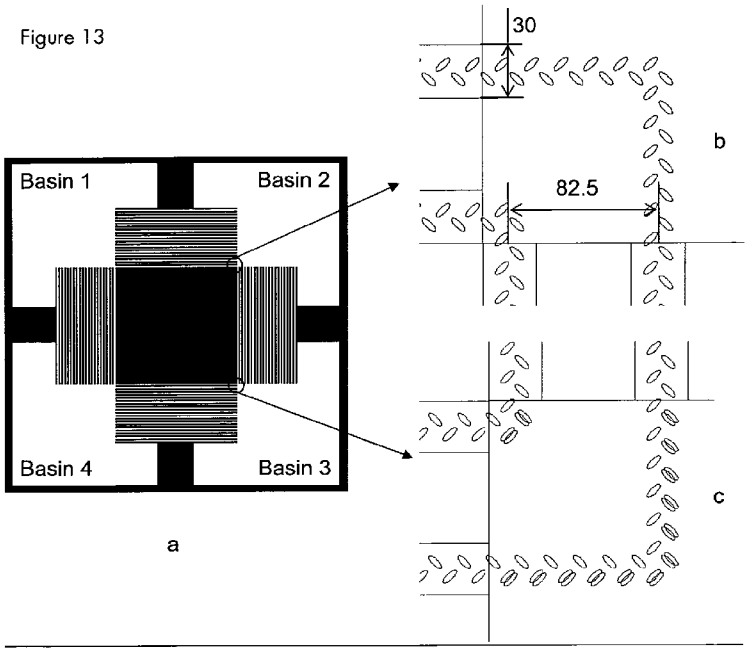
(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) **Title**: DEVICE COMPRISING ROWS OF MAGNETIC ELEMENTS IN CHANNELS AND COMPARTMENTS

Figure 13



(57) **Abstract**: A device comprises at least two compartments, said at least two compartments being in fluid connection via at least one channel, each channel comprising at least one row of magnetic elements, wherein each row of magnetic elements form a transportation line from one compartment through the at least one channel and further into at least one another compartment, wherein each magnetic element is a magnetic field source with directional properties determined by the direction of the element magnetization with respect to the element geometry, and wherein said magnetic elements comprise at least one ferromagnetic material, wherein said transportation line is adapted to transport magnetic beads when an external changing magnetic field is applied. Advantages of the invention include that it provides the possibility to move beads and/or a sample attached to beads and to analyze or separate molecules in separate compartments in a controlled way.

WO 2012/136695 A1

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

DEVICE COMPRISING ROWS OF MAGNETIC ELEMENTS IN CHANNELS AND COMPARTMENTS

Technical field

[0001] The present invention relates generally to an analysis, separation and purification device characterized by having channels and compartments in which rows of magnetic elements form transportation lines for magnetic beads leading through the channels and the compartments.

Background

[0002] In the prior art many devices for the analysis and purification of samples involving fluids are described. Many of the systems involve the use of microfluidic systems, capillary channels, channels with flow driven by pumps, porous materials, wells and reservoirs. Although such systems are successfully used today there is still room for improvements regarding the devices. For instance, miniaturized microfluidic systems exhibit a large surface area to volume ratio of the channels and compartments, thus favoring laminar flow that is an advantage when aiming at controlled transport of substances. However, the large surface area also results in increased unspecific binding of molecules on the inside of the microfluidic channels and the difficulty in mixing substances. It is therefore desirable to use modes of transport in which the molecules to be analyzed are prevented from interacting with the walls of the channels as much as possible. This is, however, not straightforward with pressure or electric field driven liquid flows in microfabricated channels.

[0003] Gunnarsson et al in Advanced Materials, 2005, 17, 1730-1734 disclose a device with a surface comprising magnetic elements forming transport lines, wherein an in-plane rotating magnetic field is applied. With this device it is possible to transport magnetic particles on a surface. In another article LE Johansson

et al in Lab Chip, 2010, Vol. 10, pp. 654-661 disclose a similar device with a surface comprising magnetic elements forming transportation lines, wherein an in-plane rotating magnetic field is applied. Both those articles report results of basic studies where the primary intention was to investigate if magnetic particles could be transported on a surface and also to investigate the forces involved during the transportation of beads on different kinds of support surfaces where different kinds of biomolecules are covalently attached to the beads.

[0004] In the article by LE Johansson et al potential applications for this kind of device are discussed in more general terms for protein separation and real time based sensor systems but no details or even suggestions of how such devices could be constructed were given.

Summary

[0001] It is an object of the present invention to alleviate at least some of the disadvantages of the prior art and to provide an analysis, separation and concentration device which can be used for detection and purification of analytes.

[0002] One important advantage of the present invention in relation to prior art (eg. by K. Gunnarsson et al. Advanced Materials, 2005, 17, 1730-1734 and L.E Johansson et al. Lab Chip, 2010, Vol. 10, pp. 654-661) is that magnetic elements in combination with microfluidic channels (see figure 1 a-b) are introduced together with compartments. Such a device makes it possible to move samples or beads and to analyze or separate molecules in separate compartments in a controlled way. The compartments with the micro channels in between are constructed with magnetic elements at the bottom of the micro-channels which allows transport of magnetic beads into different compartments. One important consequence of this approach is that the diffusion of molecules between the compartments can be minimized as is verified in the experimental section of this

application. This point, which is very important to avoid dilution of the samples, cannot easily be accomplished with devices in which flows of liquids are used to transport the analytes.

[0003] Another advantage with this invention is that the sample will not be lost due to immobilization on the walls of the micro-channels during the transportation process since the sample is transported on the surface of the beads and therefore will not come in direct contact with the walls.

[0004] This inventive step makes it possible to apply this device both for separation and purification of e.g. biomolecules but also to separate and analyze several analytes in the same e.g. blood sample, so called multiplexing

[0005] In the prior art only one kind of analyte could be isolated or detected in a blood sample using one single operation step. In the present invention several different kinds of beads can be added to the same sample. The beads are different with respect to the molecules immobilised and can then be identified since only one kind of specific interaction is allowed between the bead molecules and its counterpart in the sample. This enables multiplexed analyses and separations by use of different analyze/separation compartments. In each analytic/separation compartment, only one kind of specific interaction is allowed by using the corresponding counterpart immobilized to the walls of this compartment.

[0006] The present invention also includes the possibility to administer a flow of liquid either in the opposite or perpendicular direction to the transportation direction of beads which makes it possible to attain a separation process during the transportation of the beads where specifically bound analytes are separated from unspecifically bound molecules.

[0007] Another advantage of this invention is that compartments to which beads are transported continuously will be provided with new beads which will

improve the separation capacity since the binding capacity as defined by the number of unoccupied binding sites on the beads (a continuous supply of new beads to the compartments also imply a continuous supply of new binding sites) will be constant and the binding rate constant to the surface will not be the limiting step during the adsorption process which makes the present invention more effective for separation and purification compared to prior art.

Brief description of the drawings

[0008] The invention is described with reference to the following drawings in which :

[0009] Figure 1 shows an embodiment of a device according to the invention as described in example series 1,

[00010] Figure 2 shows the a circular 10 nm thin gold layer with 10 nm titanium as adhesion on a device as described in the experimental section,

[00011] Figure 3 shows in schematic form functionalization of beads with LALBA, lysozyme, biotin, or avidin,

[00012] Figure 4 shows a fluid cell designed to fit in an optical microscope,

[00013] Figure 5 A-C show beads immobilized to different degrees on a gold circle as detailed in the experimental section,

[00014] Figure 6 shows another view of the fluid cell designed to fit in an optical microscope,

[00015] Figure 7 A-C show beads immobilized to different degrees on a gold circle as detailed in the experimental section,

[00016] Figure 8 A-B show beads immobilized to different degrees on a gold circle as detailed in the experimental section,

[00017] Figure 9 shows parallel rows of magnetic elements on a device, each row of magnetic elements form a transportation line,

[00018] Figure 10 shows an embodiment with two basins in fluid connection via a plurality of channels, each channel comprising one row of magnetic elements forming a transport line in each channel. 10b shows a detail where the channels exit in a basin,

[00019] Figure 11 shows a micrograph of the embodiment depicted in figure 10b including beads,

[00020] Figure 12 shows parallel transportation lines comprising elliptical elements, and a 90degree turn for four of the transportation lines,

[00021] Figure 13 shows an embodiment with four basins in fluid connection via a plurality of channels, Figure 13b and c show details where the channels exit in the basins, and

[00022] Figure 14 shows a schematic overview of a device with 5 basins and a loop of transportation lines.

Detailed description

[00023] Before the invention is disclosed and described in detail, it is to be understood that this invention is not limited to particular compounds, configurations, method steps, substrates, and materials disclosed herein as such compounds, configurations, method steps, substrates, and materials may vary somewhat. It is also to be understood that the terminology employed herein is used for the purpose of describing particular embodiments only and is not intended to be limiting since the scope of the present invention is limited only by the appended claims and equivalents thereof.

[00024] It must be noted that, as used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise.

[00025] If nothing else is defined, any terms and scientific terminology used herein are intended to have the meanings commonly understood by those of skill in the art to which this invention pertains.

[00026] The term "about" as used in connection with a numerical value throughout the description and the claims denotes an interval of accuracy, familiar and acceptable to a person skilled in the art. Said interval is ± 10 %.

[00027] In a first aspect there is provided a device comprising at least two compartments, said at least two compartments being in fluid connection via at least one channel, each channel comprising at least one row of magnetic elements, wherein each row of magnetic elements form a transportation line from one compartment through the at least one channel and further into at least one other compartment, wherein each magnetic element is a magnetic field source with directional properties determined by the direction of the element magnetization with respect to the element geometry, and wherein said magnetic elements comprise at least one ferromagnetic material, wherein said transportation line is adapted to transport magnetic beads when an external changing magnetic field is applied.

[00028] In one embodiment the compartments are open basins on a surface of the device. An open basin does not have a cover or lid, whereas a compartment may have a cover or a lid.

[00029] In one embodiment the geometrical shape of the compartments is selected from the group consisting of a rectangular cuboid and a cylinder.

[00030] In one embodiment the compartments have a length, width, and height in the range from 10 μm up to 2000 μm.

[00031] In one embodiment the rows of magnetic elements are adapted to transport magnetic beads having a diameter of at least 20 nm, when en external changing magnetic field is applied.

[00032] In one embodiment the rows of magnetic elements are adapted to transport magnetic beads having a diameter in the interval 1-20 μm, when en external changing magnetic field is applied.

[00033] In one embodiment the device comprises at least four compartment, wherein one compartment is used for supply of magnetic beads, one for supply of analyte, one for rinsing of unspecifically bound molecules and at least one for analysis of molecular reactions.

[00034] In one embodiment the magnetic elements have a lateral size from 0.05 μm to 100 μm.

[00035] In one embodiment the magnetic elements have a lateral size from 0.1 μm to 50 μm.

[00036] In one embodiment the magnetic elements are arranged in more than one parallel row, wherein the distance between adjacent magnetic elements in one row is shorter compared to the distance between adjacent magnetic elements in two adjacent rows.

[00037] In one embodiment each compartment contains multiple transportation lines and wherein each transportation line extends between all compartments in the device.

[00038] In one embodiment at least one compartment is used for analysis, wherein there are binding areas in the at least one compartment used for analysis, and wherein the binding areas have a shape selected from the group consisting of a circle, a triangle, an ellipse, and an oval.

[00039] In one embodiment the binding areas have rectangular or circular shape with length and width in the range 10 μm - 2000 μm.

[00040] In one embodiment the outer surface of the device except for the binding areas at least partially comprises SiO_2 or grafted SiO_2.

[00041] In one embodiment the outer surface of the device except for the binding areas at least partially comprises polyethylene glycol (PEG) or any other molecular repellent surface.

[00042] In one embodiment the device comprises a silicon wafer.

[00043] In one embodiment the analysis device is adapted to be manufactured at least partially with lithographical techniques.

[00044] In one embodiment the transportation lines formed by the magnetic elements lead from a first compartment to a second compartment, wherein the first compartment is adapted for addition of magnetic beads, and wherein the second compartment is adapted to receive magnetic beads transported along the transportation lines.

[00045] In one embodiment the transportation lines formed by the magnetic elements form a closed loop.

[00046] In one embodiment the holder is adapted to generate a magnetic field which changes direction with time.

[00047] In one embodiment the holder is adapted to generate a magnetic field, wherein the direction of the magnetic field is adapted to rotate around an axis at some angle with respect to the rows of magnetic elements in the device.

[00048] In one embodiment the holder comprises at least one selected from the group consisting of a rotating magnet, and a coil.

[00049] In one embodiment the holder further comprises at least one chamber for fluids.

[00050] In one embodiment the holder further comprises an optical system.

[00051] In a second aspect there is provided a method of detecting at least one molecule in a sample comprising the steps:

a) providing a device as described above,

b) adding ferro- or ferrimagnetic beads to at least one compartment of the device,

c) applying a magnetic field which changes direction with time, and

d) detecting beads in at least one compartment.

[00052] In one embodiment the beads have molecules bound to their surface.

[00053] In one embodiment the magnetic beads are used for transportation of molecular complexes between different compartments.

[00054] In one embodiment the at least one compartment is used for analysis, wherein there are binding areas in the at least one compartment used for analysis, and wherein only one type of molecule is bound to the binding area in each compartment used for analysis, and wherein said at least only one type of molecule has the ability to specifically bind to only other molecular entity.

[00055] In one embodiment separation of target molecules is achieved in a sequential manner by having a group of compartments used for analysis where different compartments are accessed in a predetermined, ordered sequence.

[00056] In one embodiment said magnetic field rotates around an axis essentially in the plane of the transportation lines.

[00057] In one embodiment the diameter of the beads ranges from 50 to 200% of the distance between the magnetic elements.

[00058] In one embodiment the magnetic beads functionalized with different probe molecules are mixed in a compartment.

[00059] In one embodiment the binding area in a compartment used for analysis has only one type of molecule bound to the surface, and wherein said only one type of molecule has the ability to specifically bind to only one other molecular entity.

[00060] In one embodiment analysis of molecules is achieved in a sequential manner by having a plurality of compartments used for analysis where different compartments are accessed in a predetermined serial ordered sequence.

[00061] In one embodiment analysis of molecules is achieved by having a group of compartments used for analysis where different compartments are accessed in a predetermined, parallel ordered sequence.

[00062] In one embodiment the change of the external magnetic field is varied in order to adjust the time during which the beads are in different compartments.

[00063] In one embodiment the magnetic field could be momentarily paused in order to adjust the time during which the beads are in different compartments.

[00064] In one embodiment all compartments are situated on the same wafer and magnetic beads are transported in between the different compartments by use of magnetic elements and an external magnetic field.

[00065] In figure 2, five compartments are schematically visualized. Functionalized magnetic beads are stored in the bead compartment. To the analyte compartment, a complex sample consisting of several molecules is added. When the beads are transported through this compartment they are able to pick up the molecules which they have been functionalized for. In the rinsing compartment, the beads are transported through a buffer in order to remove any un-specifically bound molecules. The bottom surfaces of the subsequent compartments are covered with molecules which matches the molecules picked up by the beads. When a bead with a molecule matching the molecule of the compartment is transported through, it will be immobilized to the surface.

[00066] The magnetic field created by a magnetic element, exhibiting directional properties determined by the element geometry, is in one embodiment the result of an element with planar surface and with a magnetization restricted to the plane of the element. A directionally dependent magnetic field around a magnetic element is achieved by manufacturing a magnetic element with a shape that leads to a magnetic field pattern around the magnetic element that change as the magnetization of the element changes direction. Examples of shapes leading to a directional dependent magnetic field include but are not limited to triangles, ellipses, and ovals. In one embodiment the binding areas are selected from the group consisting of circles, triangles, ellipses and ovals.

[00067] Magnetic elements made of a homogenous ferromagnetic material and shaped as circular discs are not suitable, since the magnetic field pattern around such a magnetic element is not directionally dependent in the sense that its characteristics will not change as the element magnetization changes direction.

[00068] Examples of manufacturing methods for the magnetic elements include but are not limited to optical lithography where the desired pattern is transferred in a photo resist, followed by pattern development and thin film deposition of the ferromagnetic material. The method ends by photo resist removal.

[00069] In one embodiment the binding areas are circular discs. In one embodiment the binding areas have a size so that each binding area covers at least 10 magnetic elements, preferably at least 50 magnetic elements. In one embodiment the binding areas are circular with a diameter from 100 to 1000 μm.

[00070] In one embodiment the outer surface of the device, except for the binding areas, comprises SiO_2. This has the advantage that analytes such as but not limited to proteins are less likely to adsorb to the surface. In an alternative embodiment the outer surface of the device, except for the binding areas, comprises polyethylene glycol (PEG) to form a molecule repellent surface.

[00071] In one embodiment the analysis device comprises a silicon wafer. The analysis device is in one embodiment manufactured with a silicon wafer as a base.

[00072] In one embodiment the analysis device is adapted to be manufactured at least partially with a patterned photo resist. This has the advantage that an exact pattern can be applied to the surface. In one embodiment the magnetic elements are defined by a pattern transferred in a photo resist.

[00073] In one embodiment at least one binding area comprises gold. Examples of surfaces include but are not limited to gold, silanized silicon and a plastic surface provided with functional groups.

[00074] In one embodiment the holder comprises at least one selected from the group consisting of a rotating magnet and a fixed coil. These can generate rotating magnetic fields. In one embodiment several coils such as but not limited to 2, 3, 4,

5, and 6 coils, are arranged on the holder to generate a rotating magnetic field. In one embodiment the coils are connected to an electronic control unit in order to control the magnetic field generated by the coil(s).

[00075] In an alternative embodiment the device(s) generating the rotating magnetic field is instead arranged on the analysis device and not on the holder.

[00076] In one embodiment the holder further comprises an optical system. Examples of an optical system include but are not limited to a microscope adapted to observe beads on the surface, and a system to detect radiation from a sample on the device.

[00077] When using the method magnetic beads are attracted to the magnetic elements. When the applied magnetic field changes direction with time, the beads move and will be attracted by the magnetic field from an adjacent magnetic element. The beads will move to an adjacent magnetic element and thereby a transport of magnetic beads is achieved. In one embodiment the applied magnetic field rotates and the beads rotate around the magnetic elements and because of the directionally dependent magnetic field from the magnetic elements the beads can move to adjacent magnetic elements.

[00078] When the magnetic beads are moved along the magnetic elements on the surface, molecules which are bound to the magnetic particles may bind to the at least one binding area on the surface, if the specific binding capability of the molecules in the binding area has the ability to bind to the molecules on the surface of the particles.

[00079] In one embodiment it is contemplated that an analysis will be made by binding molecules from a sample on the surface of the magnetic beads. Thereafter the magnetic beads will be moved for instance to a compartment where it is analyzed. Such a compartment comprises binding areas. If molecules on the surface

of the magnetic particle have the specific binding capability to the molecules on the binding area the magnetic particle will stay in the binding area. In one embodiment the number of magnetic particles in different binding areas is read and analyzed with an optical system. In an alternative embodiment magnetic particles stuck in a binding area are read with a naked eye.

[00080] In one embodiment a possible analysis system comprising magnetic particles covered with bio-specific antibodies to a particular analyte are added to a sample liquid, e.g. urine. A bio-specific reaction between the analyte in the sample liquid and the particles take place during a time period of some tens of minutes. The particles, on the magnetic bio-chip, are transported through a flow of buffer in the opposite direction to that of the magnetic transport. In this way the particles are washed clean of non-bio-specifically bound molecules. After washing, the particles are transported onto the detector area, which consists of gold circles. When particles enter the detector they may bind by an auto bio-specific interaction to antibodies that are bound to the detector area and the particles will in this case become immobilized, or if the analyte in question is not present in the sample, the particles will pass through the particle detector area without becoming immobilized. Depending on the amount of analyte present in the sample liquid different, number of particles will stick to the detector area, whereby both a quantitative and a qualitative evaluation can be performed.

[00081] In one embodiment the analytes in the analyte compartment react with the particles moving through and are thus transported without contact with the walls of the microfluidic channel into the subsequent compartment. The amount of analyte on each particle can also be increased by allowing the particle to remain in the analyte compartment for a longer period of time. The particles are then washed during their transport though a compartment filled with a suitable washing liquid. This washing step can be made to include several washing steps by use of several

successive washing compartments. Due to the fact that the analytes are immobilized on the surface of the particle, the risk of losing the analyte is minimized. This means that the amount of the analyte on the particle stays constant irrespective of the number of washing steps used. As a result of this, it is possible to wash the particles very efficiently and straightforwardly. Moreover, the particles can be held at a certain position as long as desired before being moved to the next compartment. This is not possible in other systems due to the influence of diffusion of the particles. Finally, the particles are moved into a series of analytic compartments in which the particles bind to different positions depending on the surfaces used in the respective compartments. The detection of the analytes on the particles may then be carried out merely by counting the number of particles bound to the surface in each compartment.

Examples

Experimental series 1: Full analyses using triangular transport elements in fluid cell

[00082] A microchip was made out of patterned thin films on a silicon wafer.

[00083] The (100)-silicon wafer was covered by a photo resist. The pattern was transferred using photo lithography and consists of rows of equilateral triangles (each side 6.5μm in length) with a spacing, center-to-center, of 8.9μm, see figure 1 below. The distance between two of the parallel rows was 22μm.

[00084] A 75 nm thick film of the soft magnetic material Permalloy (80% Ni + 20% Fe) with 10 nm titanium as adhesion layer was evaporated on to the wafer and the pattern was transferred to the film through a lift-off process using acetone.

[00085] To improve the surface properties of the chip and thereby to decrease sticking of proteins to the surface, the patterned wafer was covered with a 10 nm thick film of evaporated silicon dioxide.

[00086] The silicon dioxide was covered with a second layer of photo resist. The pattern was transferred using photo lithography and the pattern consists of circles, 1200µm wide, with a center-to-center spacing of 2500µm.

[00087] A 10 nm thin gold layer with 10 nm titanium as adhesion layer was evaporated on to the patterned photo resist. The pattern was transferred to the film through a lift-off process using acetone, see figure 2 below.

[00088] The wafer was finally diced into 0.5 by 0.5 cm sized chips.

[00089] The gold circles on the individual chips were functionalized through pipetting a droplet of either anti-LALBA (anti-alpha-lactalbumin), anti-lysozyme, or biotin onto the gold and let it react for about 24 hours. The anti-LALBA, anti-lysozyme, or biotin will bind to the gold circles through a sulfur-gold bond. Before conjugation to gold, the antibodies were thiolated.

[00090] The functionalized chips were washed with PBS to remove any un-specifically bound antibodies.

[00091] The 4.9 µm (diameter) sized beads used in the experiments were Micromer-M from Micromod Partikeltechnologie GmbH. They consist of a core of maghemite (γ-Fe_2O_3) in a styrene maleic acid copolymer matrix coated with a cross-linked poly(methylmethacrylate-co-methacrylic acid) modified with bifunctional PEG with amino function (-NH-$(CH_2$-O-$CH_2)_{200}$-NH_2).

[00092] The beads were further functionalized with LALBA, lysozyme, biotin, or avidin, according to the schematics shown in figure 3 below. Biotin coated beads were mixed with avidin in order to generate avidin functionalized beads.

[00093] Figure 3 shows: (A) Thiolation of lysozyme and alpha-lactalbumin via i) reaction of amino groups with SPDP followed by ii) DTT treatment. (B) Functionalization of the beads with thiolated ligands. i) SPDP conjugation to the

amino groups on beads followed by ii) ligand coupling to beads by reaction of thiols to disulphides. (C) Biotin functionalization of beads by reaction of amino groups on beads with N-hydroxysuccinimide (NHS) biotin.

[00094] The experiments were done using a fluid cell incorporating a magnet and a tubing system. The fluid cell was designed to fit in an optical microscope. See figure 4 below.

[00095] Figure 4 shows: A) The fluid cell. B) Tubing system. C) Chip position. D) Electromagnets. E) Optical microscope.

[00096] In order to move the beads along the rows of triangles and across the gold circles a rotating in-plane magnetic field was applied. This field was generated by two perpendicular iron-core electromagnets. The chip was placed in the center of the magnet system where the magnetic field was homogenous.

[00097] The beads, suspended in PBS-Tween 20, were led onto the chip through the tubing system.

[00098] Six different experiments were performed.

1. LALBA-beads on anti-LALBA chip
2. Lysozyme-beads on anti-Lysozyme chip
3. Avidin beads on biotin chip
4. LALBA beads on anti-lysozyme
5. Lysozyme beads on anti-LALBA chip using PBS with increased ionic strength
6. Biotin beads on biotin chip

Detailed description, experimental series 1

[00099] For this experimental series the beads were functionalized with LALBA, lysozyme, biotin, or avidin. The chips were functionalized with anti-LALBA, anti-lysozyme, or biotin. Anti-LALBA only has binding sites for LALBA, anti-lysozyme only

has binding sites for lysozyme, and biotin only has binding sites for avidin. Because of this the LALBA-transporting beads will be immobilized once they reach the gold circles with anti-LALBA, anti-lysozyme gold will trap lysozyme carrying beads, and biotin gold will trap the avidin carrying beads. The beads were fed onto the chip through the tubing system of the fluid cell. When they reach the rows of triangles the rotating magnetic field was turned on and the beads start to wander towards the gold circles. When all the beads have been transported over the gold the chip is rinsed with PBS. The rinsing with PBS is expected to remove beads unspecifically bound to the gold surface.

[000100] For experiment 1-3 most of the beads are immobilized by the gold circle, see Figure 5. This is expected since the beads carry biomolecules complementary to the biomolecules on the chip and the fact that the beads stick to the gold surface even after rinsing with PBS give evidence of a strong binding force.

[000101] For experiment 4-6 the beads are transported un-effected over the gold since the biomolecules on the chip and beads are not complementary to each other. The photographs in Figure 5 show the situation after rinsing.

[000102] Figure 5 shows: A) Anti-LALBA chip with LALBA beads after 7 minutes of transport. B) Biotin chip with avidin beads after 25 minutes of transport. C) Anti-lysozyme chip with lysozyme beads after 5 minutes of transport. D) Anti-lysozyme chip with lysozyme beads after rinsing.

Experimental series 2: Full analyses using triangular transport elements in fluid cell with opposing flow

[000103] A microchip was made out of patterned thin films on a silicon wafer.

[000104] The (100)-silicon wafer was covered by 100 nm thermally grown Silicon Dioxide (SiO_2).

[000105] The Si(100) wafer was covered by double layer of photo resist. First layer was 200 nm of LOL 2000 and second layer was 1µm of positive photo resist S1813. The pattern was transferred using photo lithography and consists of equilateral ellipses (long axis 10 µm and short axis 3.3 µm in length) with a spacing, center-to-center, of 16, 5 µm. The distance between two parallel rows was 82,5 µm.

[000106] A 75 nm thick film of the soft magnetic material Permalloy (80% Ni + 20% Fe) with 10 nm titanium as adhesion layer was evaporated on to the wafer and the pattern was transferred to the film through a lift-off process using acetone.

[000107] To improve the surface properties of the chip and thereby to decrease sticking of proteins to the surface, the patterned wafer was covered with a 10 nm thick film of evaporated silicon dioxide.

[000108] The silicon dioxide was covered with a second layer of photo resist. The pattern was transferred using photo lithography and the pattern consists of circles, 1200µm wide, with a center-to-center spacing of 2500µm.

[000109] A 10 nm thin gold layer with 10 nm titanium as adhesion layer was evaporated on to the patterned photo resist. The pattern was transferred to the film through a lift-off process using acetone.

[000110] The wafer was finally diced into 0.5 by 0.5 cm sized chips.

[000111] The gold circles on the individual chips were functionalized through pipetting a droplet of biotin onto the gold and let it react for about 24 hours.

[000112] The functionalized chips were washed with PBS to remove any un-specifically bound antibodies.

[000113] The beads used in the experiments were the same as in [00010].

[000114] The beads were further functionalized firstly with biotin.

[000115] The beads were secondly further functionalized with avidin of different concentrations.

[000116] The experiments were done using a fluid cell incorporating a magnet, a tubing system, and a pump. The fluid cell was designed to fit in an optical microscope. See figure 6 below.

[000117] In order to move the beads along the rows of triangles and across the gold circles a rotating in-plane magnetic field was applied. This field was generated by two perpendicular iron-core electromagnets. The chip was placed in the center of the magnet system where the magnetic field was homogenous.

[000118] The beads, suspended in PBS-Tween 20, were led onto the chip through the tubing system.

[000119] Four different experiments were performed.

1. Avidin 1mg/ml beads on biotin chip
2. Avidin 100µg/ml beads on biotin chip
3. Avidin 1µg/ml beads on biotin chip
4. Avidin 100ng/ml beads on biotin chip

Detailed description, experimental series 2

[000120] For this experimental series the beads were functionalized with avidin of different concentrations. The chips were functionalized with biotin. Biotin only has binding sites for avidin. Because of this the avidin-transporting beads will be immobilized once they reach the gold circles with biotin. The beads were fed onto the chip through the tubing system of the fluid cell. When they reach the rows of triangles the rotating magnetic field was turned on and the beads start to wander towards the gold circles. Once the beads have entered onto the chip an opposing

flow of 100µl/min of PBS buffer was turned on. When all the beads have been transported through the flow of buffer and over the gold the chip is rinsed with PBS at a speed of 1000µl/min. The rinsing with PBS is expected to remove beads unspecifically bound to the gold surface.

[000121] For all the four experiments, beads were immobilized by the gold circle to different degrees, see Figure 7. This is expected since the beads carry fewer and fewer biomolecules complementary to the biomolecules on the chip for every time the avidin solution was diluted. The fact that the beads stick to the gold surface even after rinsing with PBS give evidence of a strong binding force.

[000122] Figure 7 shows: A) Avidin 1mg/ml beads on biotin chip, after rinsing. B) Avidin 1µg/ml beads on biotin chip, after rinsing. C) Avidin 100ng/ml beads on biotin chip, after rinsing.

Experimental series 3: Full analyses using triangular transport elements in fluid cell

[000123] The microchip was fabricated according to experimental series 1.

[000124] The gold circles on the individual chips were functionalized through pipetting a droplet of biotin onto the gold and let it react for about 24 hours.

[000125] The functionalized chips were washed with PBS to remove any un-specifically bound antibodies.

[000126] The 4.9 µm (diameter) sized beads used in the experiments were Micromer-M from Micromod Partikeltechnologie GmbH. They consist of a core of maghemite (γ-Fe_2O_3) in a styrene maleic acid copolymer matrix coated with protein A.

[000127] The beads were further functionalized firstly with anti-avidin.

[000128] The beads were secondly further functionalized with avidin of different concentrations.

[000129] The experiments were done using the fluid cell from experimental series 1.

[000130] In order to move the beads along the rows of triangles and across the gold circles a rotating in-plane magnetic field was applied. This field was generated by two perpendicular iron-core electromagnets. The chip was placed in the center of the magnet system where the magnetic field was homogenous.

[000131] The beads, suspended in PBS-Tween 20, were led onto the chip through the tubing system.

[000132] Three different experiments were performed.
1. Avidin 100µg/ml beads on biotin chip
2. Avidin 10µg/ml beads on biotin chip
3. Avidin 1µg/ml beads on biotin chip

Detailed description, experimental series 3

[000133] For this experimental series the beads were functionalized with anti-avidin and avidin of different concentrations. The reason for the change of chemistry was to avoid clustering of beads. For experimental series 2 the beads were functionalized with biotin and avidin. Any free biotin on a bead could bind to an avidin on another bead since one avidin can bind four biotin molecules. This could cause clustering of beads. In experimental series 3, the beads are functionalized with anti-avidin and avidin. Anti-avidin and avidin only have one binding site for each other which mean that one avidin no longer could connect two beads. The chips were functionalized with biotin. The avidin-transporting beads will be immobilized once they reach the gold circles with biotin. The beads were fed onto

the chip through the tubing system of the fluid cell. When they reach the rows of triangles the rotating magnetic field was turned on and the beads start to wander towards the gold circles. When all the beads have been transported over the gold the chip is rinsed with PBS at a speed of 1000µl/min. The rinsing with PBS is expected to remove beads unspecifically bound to the gold surface.

[000134] For all the three experiments, beads were immobilized by the gold circle to different degrees. This is expected since the beads carry fewer and fewer biomolecules complementary to the biomolecules on the chip for every time the avidin solution was diluted. The fact that the beads stick to the gold surface even after rinsing with PBS give evidence of a strong binding force, see Figure 8.

[000135] Figure 8 shows: A) Avidin 10µg/ml beads on biotin chip, after rinsing. B) Avidin 1µg/ml beads on biotin chip, after rinsing.

Experimental series 4: Multiplexing

[000136] A microchip was made out of patterned thin films on a silicon wafer.

[000137] The (100)-silicon wafer was covered by a photo resist. The pattern was transferred using photo lithography and consists of rows of equilateral triangles with varying side lengths. The triangles were fabricated with sides ranging from 2µm to 10µm in steps of 1µm. See figure 9 below.

[000138] Figure 9 shows triangles with sides ranging from 2µm to 10µm.

[000139] A 75 nm thick film of the soft magnetic material Permalloy (80% Ni + 20% Fe) with 10 nm titanium as adhesion layer was evaporated on to the wafer and the pattern was transferred to the film through a lift-off process using acetone.

[000140] To improve the surface properties of the chip, the patterned wafer was covered with a 10 nm thick film of evaporated silicon dioxide.

[000141] The wafer was finally diced into 0.5 by 0.5 cm sized chips.

[000142] Non-functionalized Micromer-M beads from Micromod Partikeltechnologie GmbH with diameters of 2, 3, 4, 5, 8, and 10μm were used for the experiment.

[000143] The experiments were done using the fluid cell from experimental series 1.

[000144] In order to move the beads along the rows of triangles a rotating in-plane magnetic field was applied. This field was generated by two perpendicular iron-core electromagnets. The chip was placed in the center of the magnet system where the magnetic field was homogenous.

[000145] The beads, suspended in PBS-Tween 20, were led onto the chip through the tubing system.

Detailed description, experimental series 4

[000146] The beads were transported from one end of the chip to the other while being monitored through the microscope. The results are summarized in table 1 below. The rows of the table represent different bead sizes in μm and the columns represent different triangle sizes, also in μm. X meaning good transport, - meaning poor transport, and "blank" meaning no or little interaction between beads and triangles. It should be noted that for all the different triangle sizes the distance between two adjacent triangles has the same length as the side of the triangles in question. No experiment using the same triangle size but different distance between the elements has been performed.

	10	9	8	7	6	5	4	3	2
10	X	X	X						
8	X	X	X	X	-				
5	-	-	-	X	X	X			
4		-	-	X	X	X	X	-	
3			-	-	-	X	X	X	
2							-	-	

Experimental series 5: Liquid cell, two basins

[000147] A microchip was made out of patterned thin films and epoxy resin on a silicon wafer.

[000148] The (100)-silicon wafer was covered by 100 nm thermally grown Silicon Dioxide (SiO2).

[000149] The Si(100) wafer was covered by double layer of photo resist. First layer was 200 nm of LOL 2000 and second layer was 1μm of positive photo resist S1813. The pattern was transferred using photo lithography and consists of equilateral triangles (each side 6.5 μm in length) with a spacing, center-to-center, of 8.9 μm, see figure 10 below. The distance between two parallel rows was 70 μm.

[000150] A 75 nm thick film of the soft magnetic material Permalloy (80%Ni+20%Fe) with 10 nm Titanium as an adhesion layer and 10 nm of SiO2 as a capping layer was evaporated on to wafer and the pattern was transferred to the film through a lift-off process using Acetone.

[000151] The Si wafer was covered with a 100 μm photosensitive epoxy resin SU-8. The pattern was transferred using photolithography and consists of two

basins, 2x8 mm wide, separated by 100 narrow 20 μm channels aligned onto rows of magnetic triangular elements, see figure 10.

[000152] The wafer was finally diced into 1.0 by 1.0 cm sized chips.

[000153] Non-functionalized Micromer-M beads from Micromod Partikeltechnologie GmbH were used for the experiment.

[000154] The experiments were done using the fluid cell from experimental series 1 but without the tubing system or the pump.

[000155] In order to move the beads along the rows of triangles a rotating in-plane magnetic field was applied. This field was generated by two perpendicular iron-core electromagnets. The chip was placed in the center of the magnet system where the magnetic field was homogenous.

[000156] The beads, suspended in PBS-Tween 20, were pipetted into one of the two basins. The other basin was filled with PBS buffer, through pipetting.

[000157] The magnetic field was turned on and the beads were, one by one, transported from one basin to the other through the connecting channels. See figure 11below.

Experimental series 6: Liquid cell, only transport elements

[000158] A microchip was made out of patterned thin films and epoxy resin on a silicon wafer.

[000159] The (100)-silicon wafer was covered by 100 nm thermally grown Silicon Dioxide (SiO2).

[000160] The Si(100) wafer was covered by double layer of photo resist. First layer was 200 nm of LOL 2000 and second layer was 1μm of positive photo resist

S1813. The pattern was transferred using photo lithography and consists of equilateral ellipses (long axis 10 µm and short axis 3.3 µm in length) with a spacing, center-to-center, of 16.5 µm, see figure 12 below. The distance between two parallel rows was 82.5 µm.

[000161] A 75 nm thick film of the soft magnetic material Permalloy (80%Ni+20%Fe) with 10 nm Titanium as an adhesion layer and 10 nm of SiO2 as a capping layer was evaporated on to wafer and the pattern was transferred to the film through a lift-off process using Acetone.

[000162] The wafer was finally diced into 1.0 by 1.0 cm sized chips.

[000163] Non-functionalized Micromer-M beads from Micromod Partikeltechnologie GmbH were used for the experiment.

[000164] The experiments were done using the fluid cell from experimental series 1 but without the tubing system or the pump.

[000165] In order to move the beads along the rows of ellipses a rotating in-plane magnetic field was applied. This field was generated by two perpendicular iron-core electromagnets. The chip was placed in the center of the magnet system where the magnetic field was homogenous.

[000166] The beads, suspended in PBS-Tween 20, were pipetted onto the chip.

[000167] The magnetic field was turned on and the beads were transported around and around on the chip. Since the lines of ellipses is continuous around the chip there is no starting or stopping zone. See figure 12 below.

Experimental series 7: Liquid cell, four basins

[000168] A microchip was made out of patterned thin films and epoxy resin on a silicon wafer.

[000169] The (100)-silicon wafer was covered by 100 nm thermally grown Silicon Dioxide (SiO2).

[000170] The Si(100) wafer was covered by double layer of photo resist. First layer was 200nm of LOL2000 and the second layer was 1mm of photo resist S1813. The pattern was transferred using photo lithography and consist of equilateral ellipses (long axis 10 μm and short axis 3.3 μm in length) with a spacing, center-to-center, of 16.5 μm, see figure 13 a below. The distance between two parallel rows was 82.5 μm.

[000171] A 75 nm thick film of the soft magnetic material Permalloy (80%Ni+20%Fe) with 10 nm Titanium as an adhesion layer and 10 nm of SiO2 as a capping layer was evaporated on to wafer and the pattern was transferred to the film through a lift-off process using Acetone.

[000172] The Si(100) wafer was covered by double layer of photo resist. First layer was 200nm of LOL2000 and the second layer was 1 μm of photo resist S1813. The pattern was transferred using photo lithography and consists of equilateral ellipses shifted by 2 μm with respect to magnetic elements, see figure 13 b below.

[000173] A 10 nm thin gold layer with 10 nm titanium as adhesion layer was evaporated on to the patterned photo resist. The pattern was transferred to the film through a lift-off process using acetone.

[000174] The Si wafer was covered with a 100 μm photosensitive epoxy resin SU-8. The pattern was transferred using photolithography and consists of four basins, 4x4 mm wide, separated by 20 narrow 30 μm channels aligned onto rows of magnetic triangular elements, see figure 13 below. The distance between two parallel channels was 82.5.

[000175] The wafer was finally diced into 1.0 by 1.0 cm sized chips.

Claims

1. A device comprising at least two compartments, said at least two compartments being in fluid connection via at least one channel, each channel comprising at least one row of magnetic elements, wherein each row of magnetic elements form a transportation line from one compartment through the at least one channel and further into at least one another compartment, wherein each magnetic element is a magnetic field source with directional properties determined by the direction of the element magnetization with respect to the element geometry, and wherein said magnetic elements comprise at least one ferromagnetic material, wherein said transportation line is adapted to transport magnetic beads when an external changing magnetic field is applied.

2. The device according to claim 1, wherein the compartments are open basins on a surface of the device.

3. The device according to any one of claims 1-2, wherein the geometrical shape of the compartments is selected from the group consisting of a rectangular cuboid and a cylinder.

4. The device according to any one of claims 1-3, wherein the compartments have a length, width, and height in the range from 10 μm up to 2000 μm.

5. The device according to claims any one of claims 1-4, wherein the rows of magnetic elements are adapted to transport magnetic beads having a diameter of at least 20 nm, when en external changing magnetic field is applied.

6. The device according to claims any one of claims 1-4, wherein the rows of magnetic elements are adapted to transport magnetic beads having a

diameter in the interval 1-20 μm, when en external changing magnetic field is applied.

7. The device according to claims 1-6, wherein the device comprises at least four compartment, wherein one compartment is used for supply of magnetic beads, one for supply of analyte, one for rinsing of unspecifically bound molecules and at least one for analysis of molecular reactions.

8. The device according to any one of claims 1- 7, wherein the magnetic elements have a lateral size from 0.05 μm to 100 μm.

9. The device according to any one of claims 1- 7, wherein the magnetic elements have a lateral size from 0.1 μm to 50 μm.

10. The device according to any one of claims 1-9, wherein the magnetic elements are arranged in more than one parallel row, wherein the distance between adjacent magnetic elements in one row is shorter compared to the distance between adjacent magnetic elements in two adjacent rows.

11. The device according to claims 1-10, wherein each compartment contains multiple transportation lines and wherein each transportation line extends between all compartments in the device.

12. The device according to any one of claims 1-11, wherein at least one compartment is used for analysis, wherein there are binding areas in the at least one compartment used for analysis, and wherein the binding areas have a shape are selected from the group consisting of a circle, a triangle, an ellipse, and an oval.

13. The device according to claim 12, wherein the binding areas have rectangular or circular shape with length and width in the range 10 μm - 2000 μm.

14. The device according to any one of claims 12-13, wherein the outer surface of the device except for the binding areas at least partially comprises SiO_2 or grafted SiO_2.

15. The device according to any one of claims 12-14, wherein the outer surface of the device except for the binding areas at least partially comprises polyethylene glycol (PEG) or any other molecular repellent surface.

16. The device according to any one of claims 1-15, wherein the device comprises a silicon wafer.

17. The device according to any one of claims 1-16, wherein the analysis device is adapted to be manufactured at least partially with lithographical techniques.

18. The device according to any one of claims 1-17, wherein the transportation lines formed by the magnetic elements lead from a first compartment to a second compartment, wherein the first compartment is adapted for addition of magnetic beads, and wherein the second compartment is adapted to receive magnetic beads transported along the transportation lines.

19. The device according to any one of claims 1-17, wherein the transportation lines formed by the magnetic elements for a closed loop.

20. A holder adapted to hold the device according to any one of claims 1-19, wherein the holder is adapted to generate a magnetic field which changes direction with time.

21. The holder according to claim 20, wherein the holder is adapted to generate a magnetic field, wherein the direction of the magnetic field is adapted to rotate around an axis at some angle with respect to the rows of magnetic elements in the device.

22. The holder according to any one of claims 20-21, wherein the holder comprises at least one selected from the group consisting of a rotating magnet, and a coil.

23. The holder according to any one of claims 20-22, wherein the holder further comprises at least one chamber for fluids.

24. The holder according to any one of claims 20-23, wherein the holder further comprises an optical system.

25. A method of detecting at least one molecule in a sample comprising the steps:

a) providing a device according to any one of claims 1-19,

b) adding ferro- or ferrimagnetic beads to at least one compartment of the device,

c) applying a magnetic field which changes direction with time, and

d) detecting beads in at least one compartment.

26. The method according to claim 25, wherein said beads have molecules bound to their surface.

27. The method according to any one of claims 25-26, wherein magnetic beads are used for transportation of molecular complexes between different compartments.

28. The method according to any one of claims 25-27, wherein at least one compartment is used for analysis, wherein there are binding areas in the at least one compartment used for analysis, and wherein only one type of molecule is bound to the binding area in each compartment used for analysis, and wherein said

at least only one type of molecule has the ability to specifically bind to only other molecular entity.

29. The method according to any one of claims 25-28, wherein separation of target molecules is achieved in a sequential manner by having a group of compartments used for analysis where different compartments are accessed in a predetermined, ordered sequence.

30. The method according to any one of claims 25-29, wherein said magnetic field rotates around an axis essentially in the plane of the transportation lines.

31. The method according to any one of claims 25-30, wherein the diameter of the beads ranges from 50 to 200 % of the distance between the magnetic elements.

32. The method according to any one of claims 25-31, wherein magnetic beads functionalized with different probe molecules are mixed in a compartment.

33. The method according to any one of claims 25-32, wherein the binding area in a compartment used for analysis has only one type of molecule bound to the surface, and wherein said only one type of molecule has the ability to specifically bind to only one other molecular entity.

34. The method according to any one of claims 25-33, wherein analysis of molecules is achieved in a sequential manner by having a plurality of compartments used for analysis where different compartments are accessed in a predetermined serial ordered sequence.

35. The method according to any one of claims 25-33, wherein analysis of molecules is achieved by having a group of compartments used for analysis

where different compartments are accessed in a predetermined, parallel ordered sequence.

36. The method according to any one of claims 25-35, wherein the change of the external magnetic field is varied or turned off in order to adjust the time during which the beads are in different compartments.

Drawings

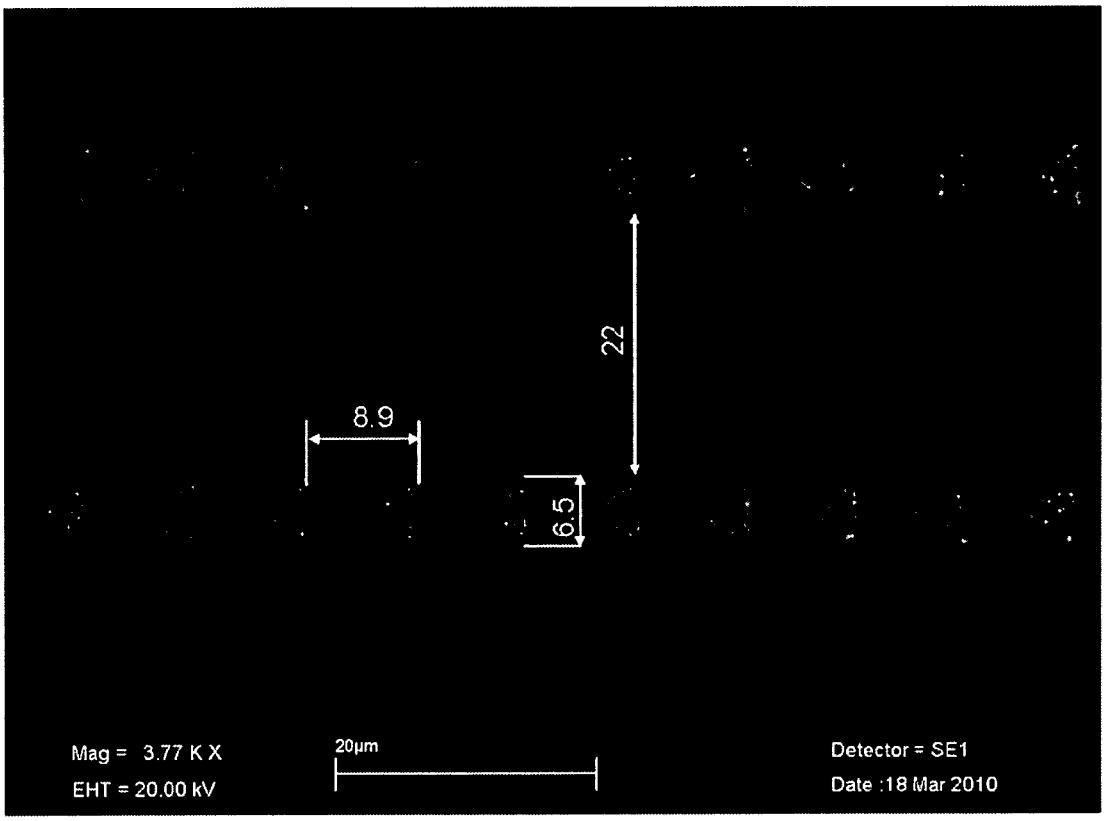


Figure 1

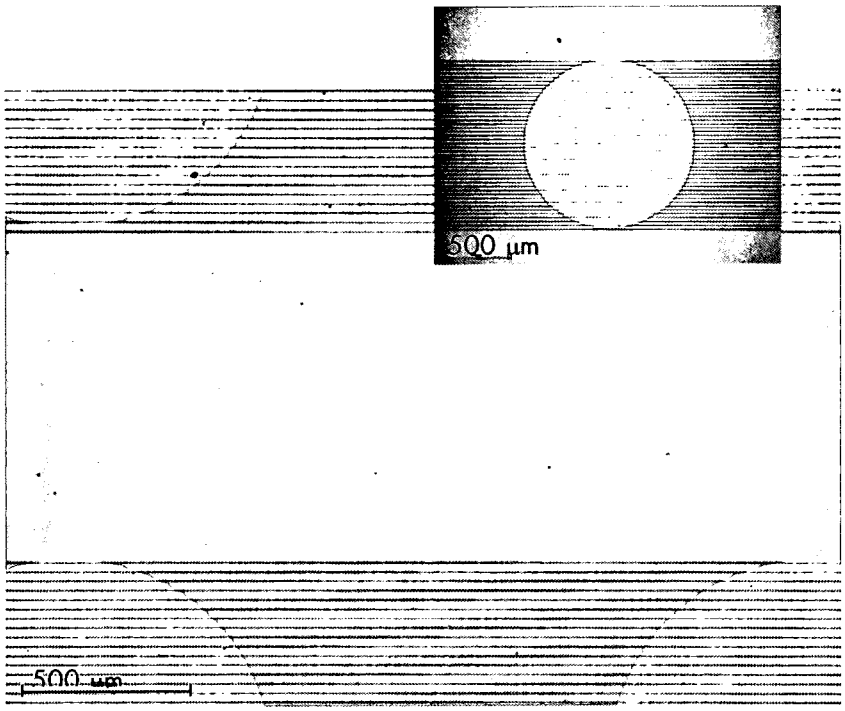


Figure 2

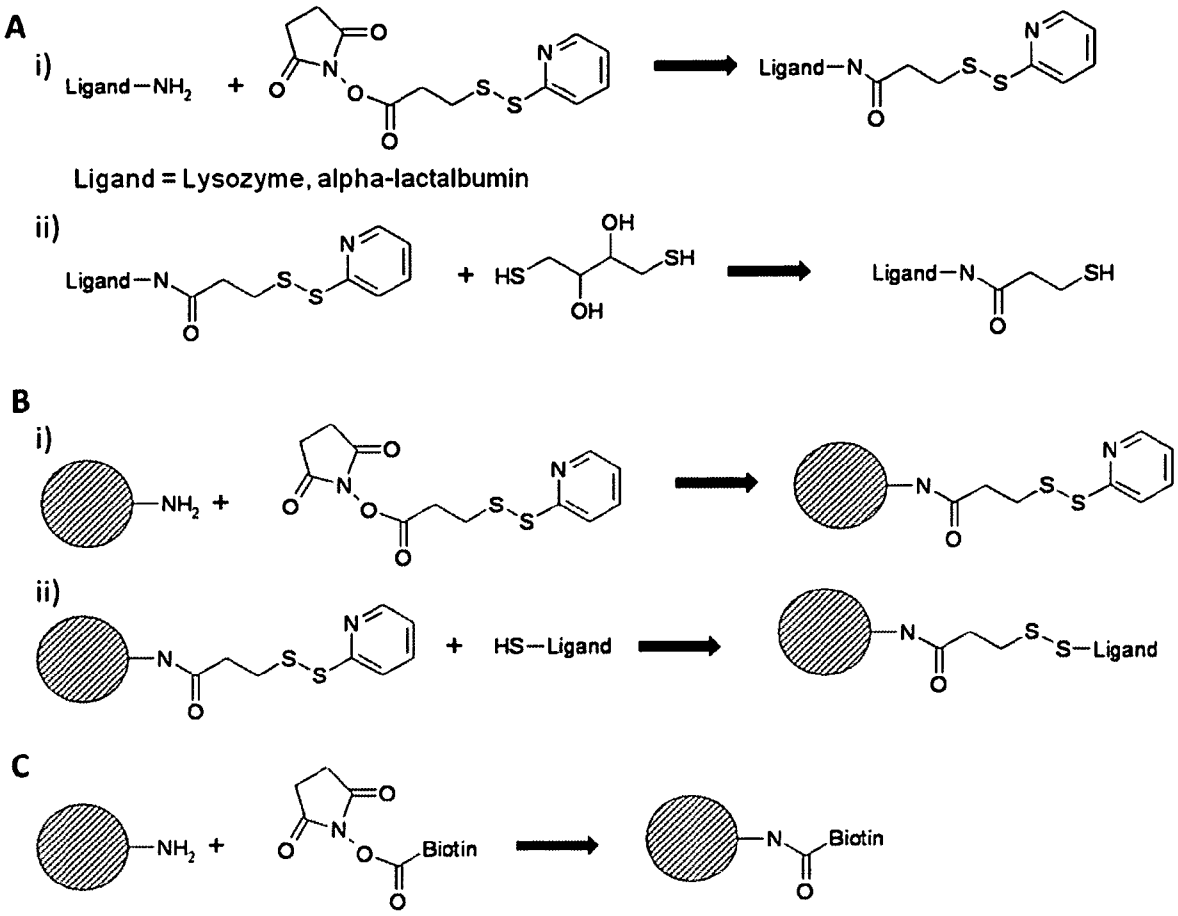

A
i)
Ligand—NH2
Ligand = Lysozyme, alpha-lactalbumin
ii)
HS
SH
OH
B
i)
ii)
HS—Ligand
C
Biotin

Figure 3

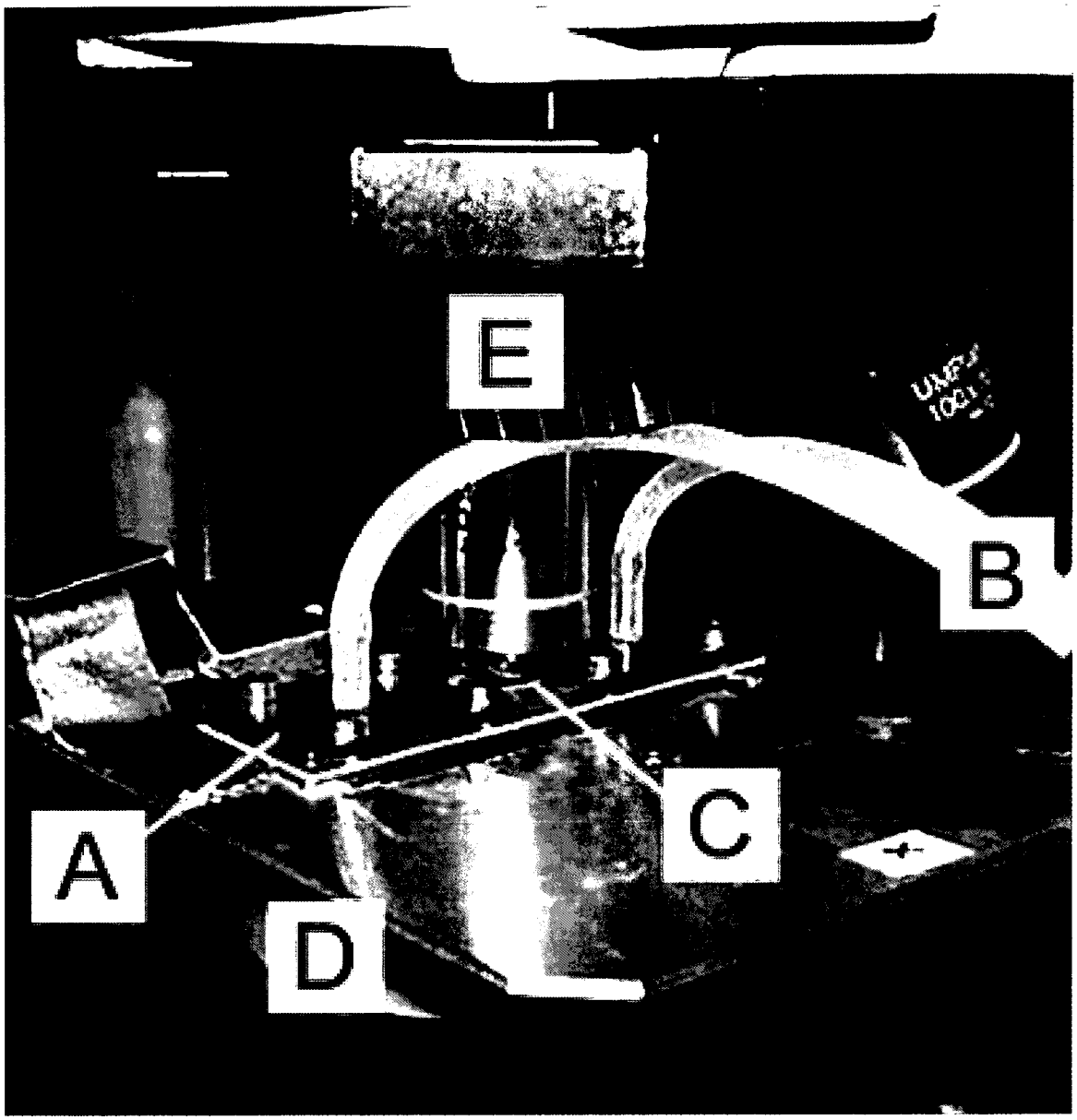


Figure 4

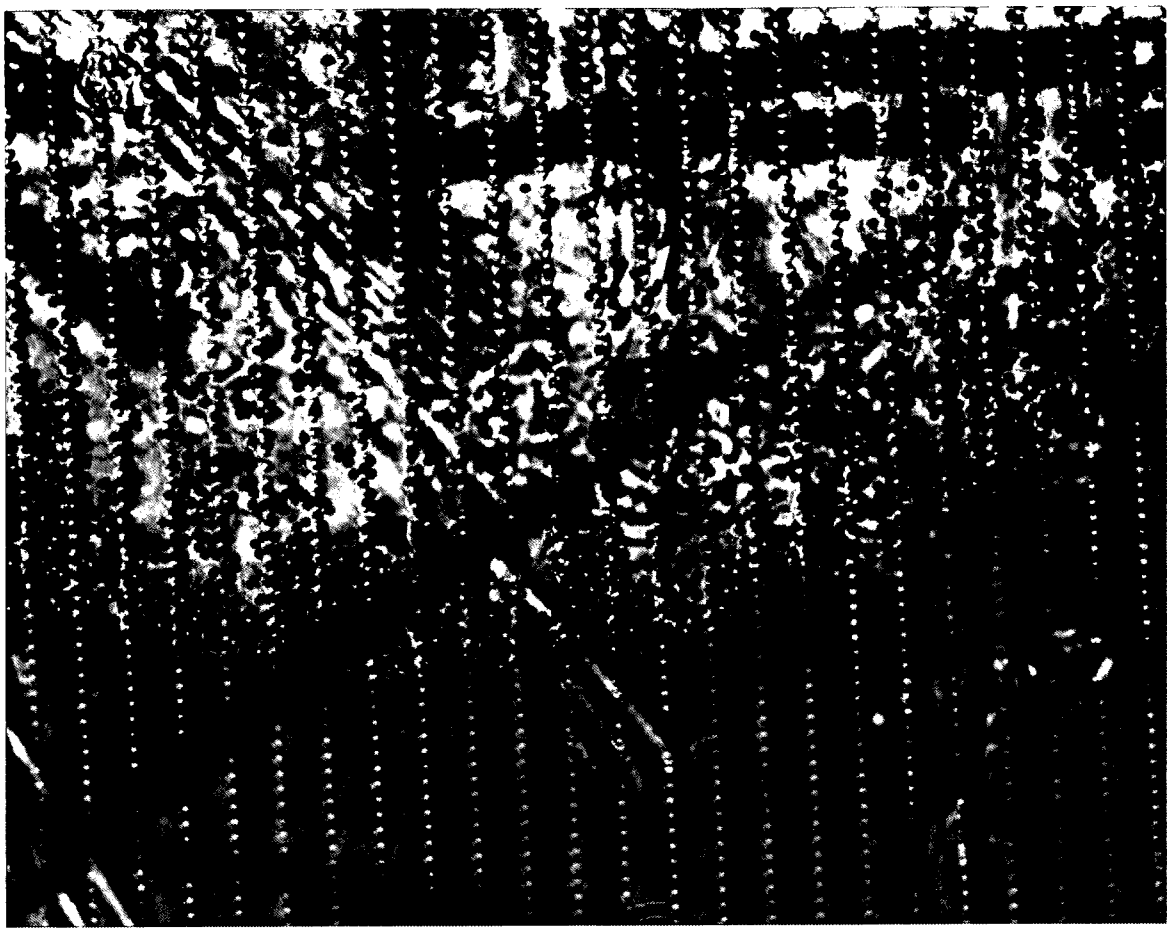


Figure 5A

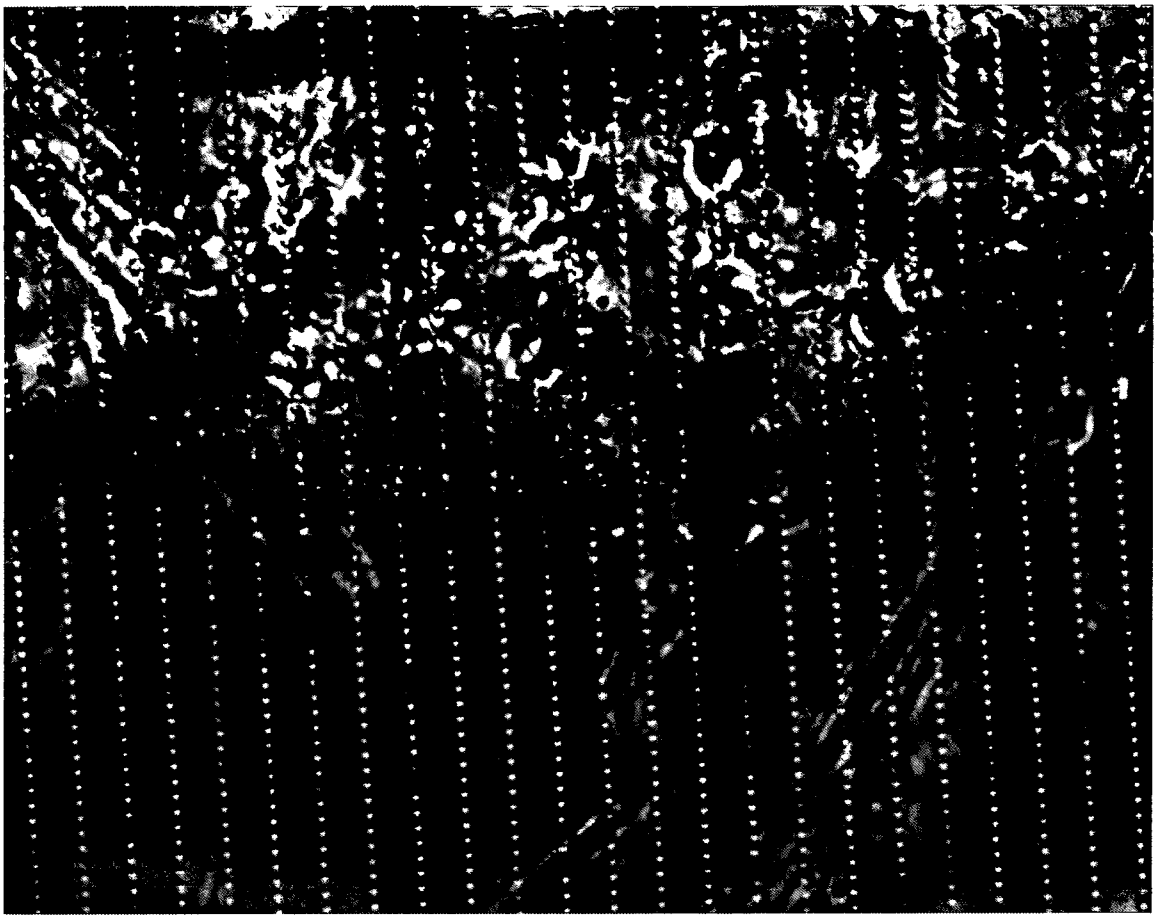


Figure 5B

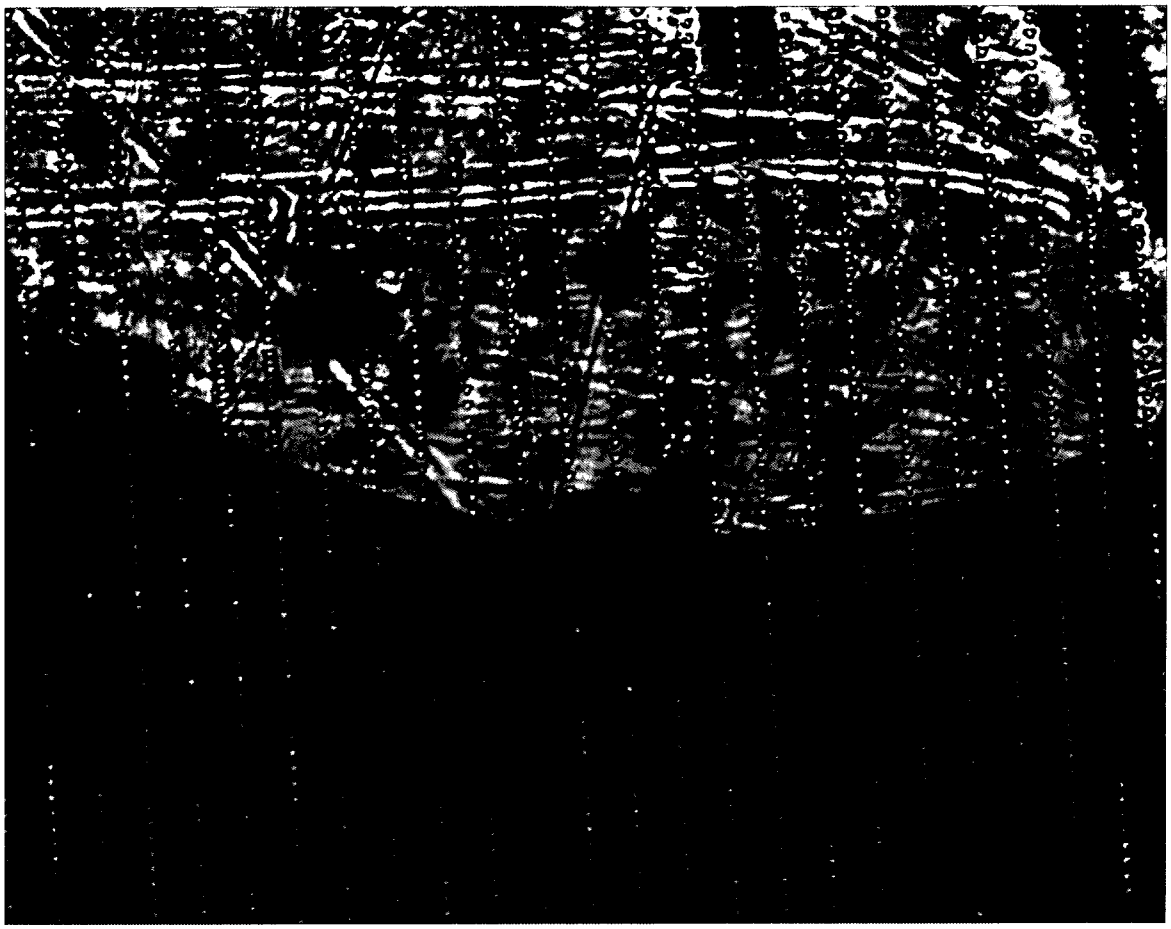


Figure 5C

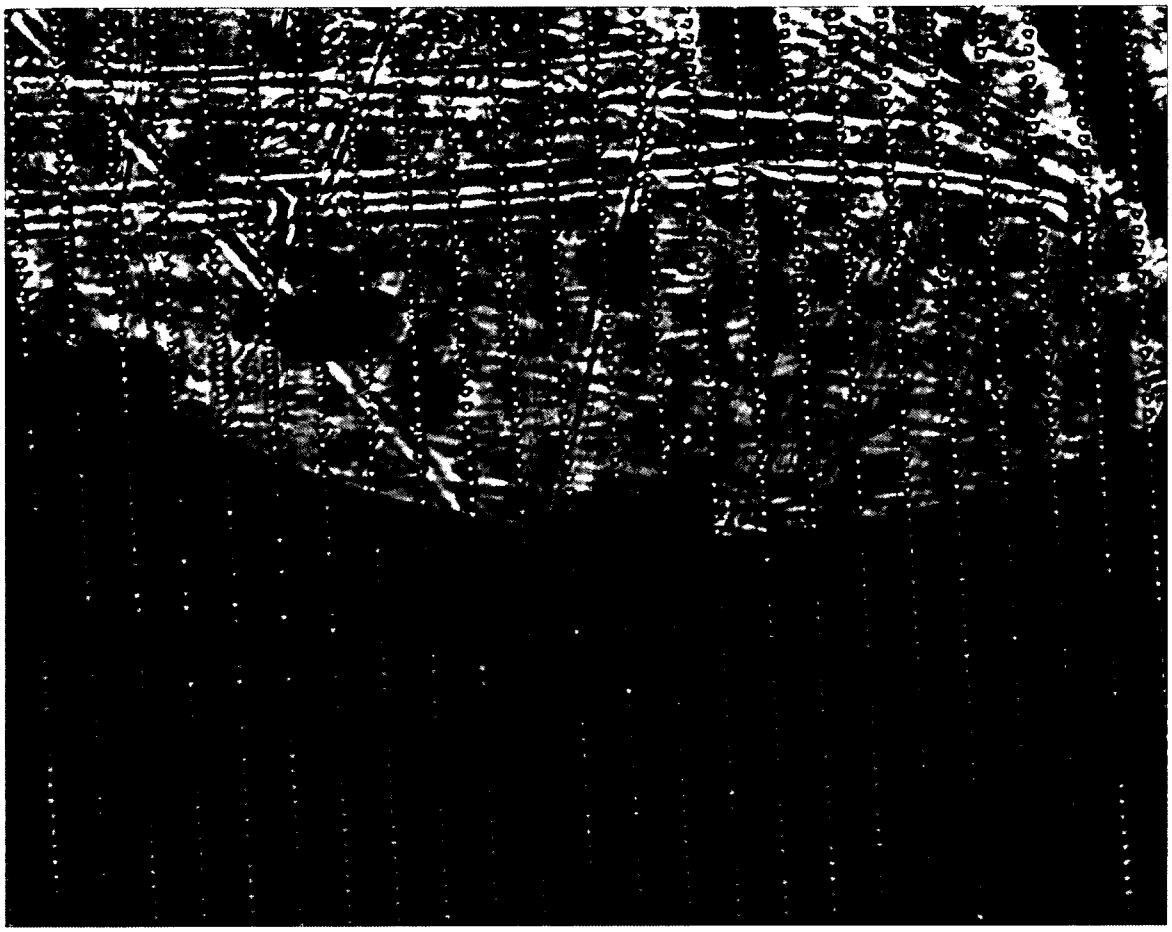


Figure 5D

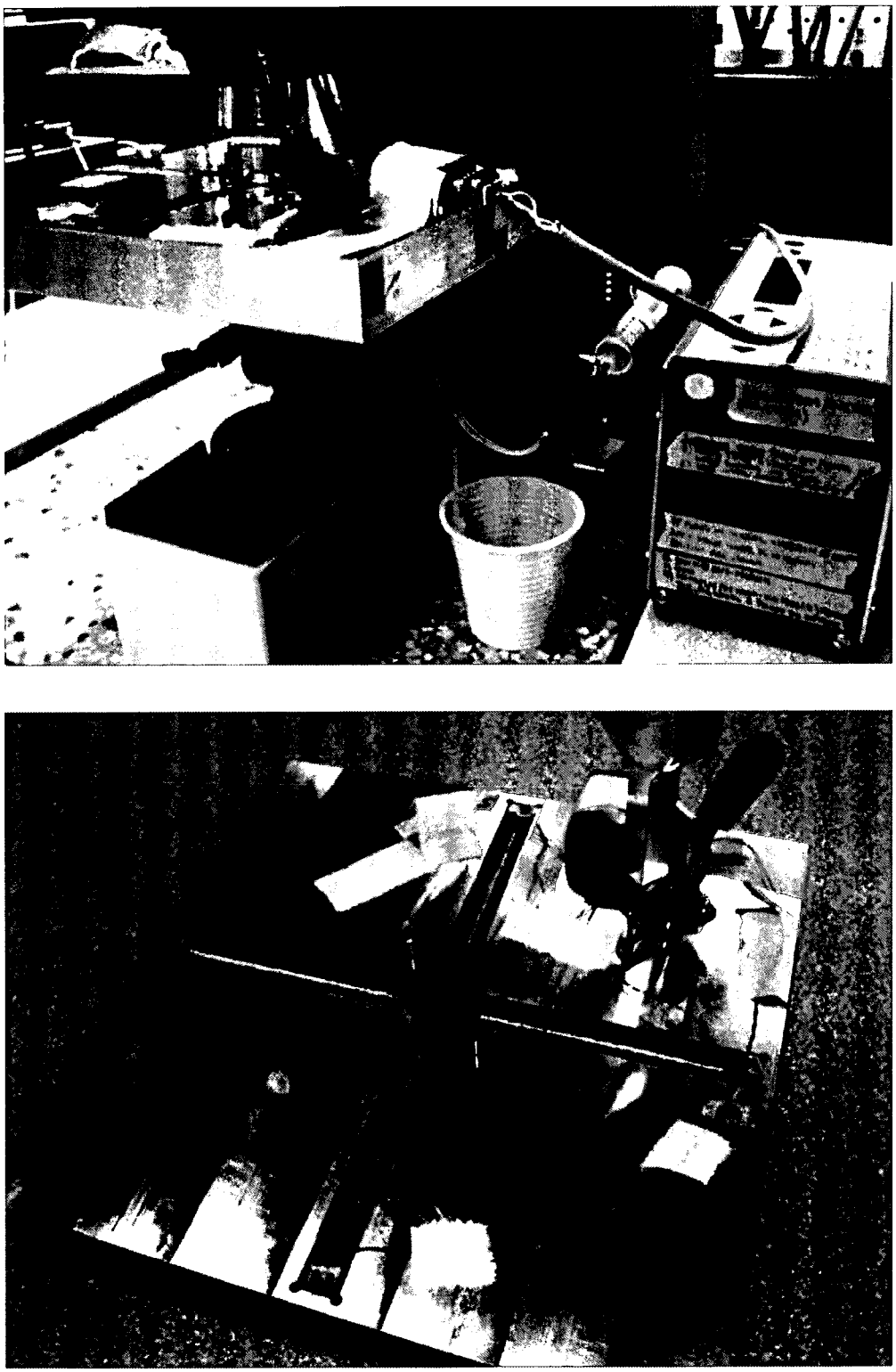


Figure 6 A and B

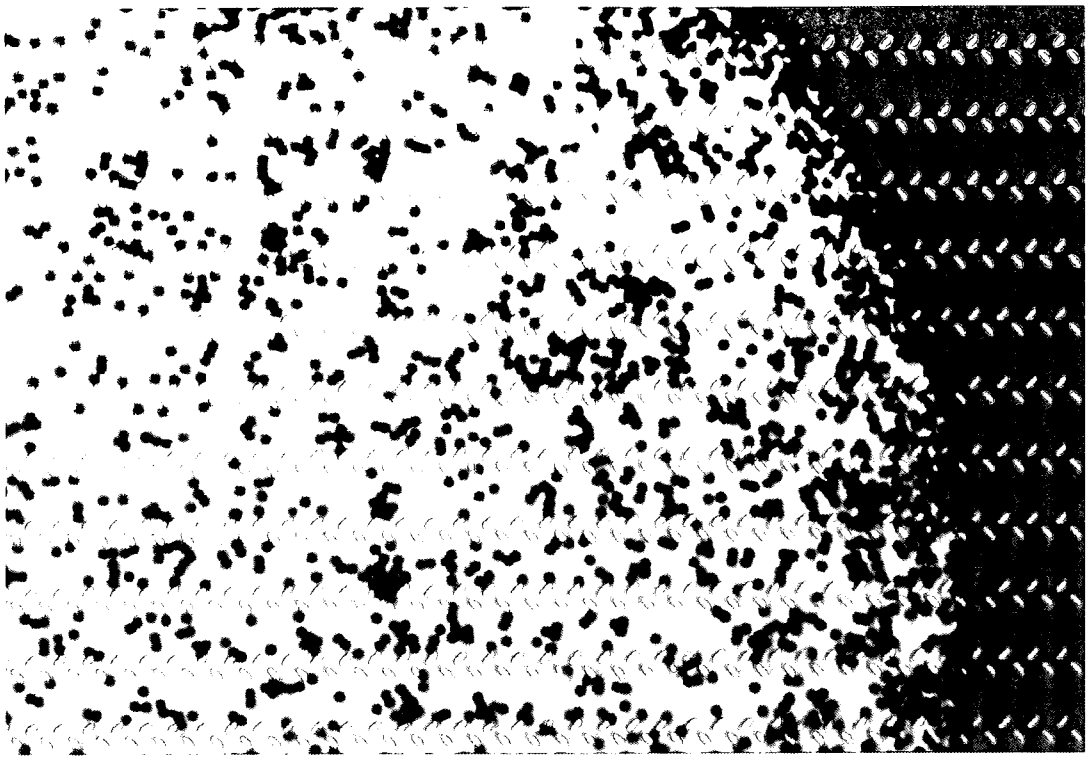


Figure 7A

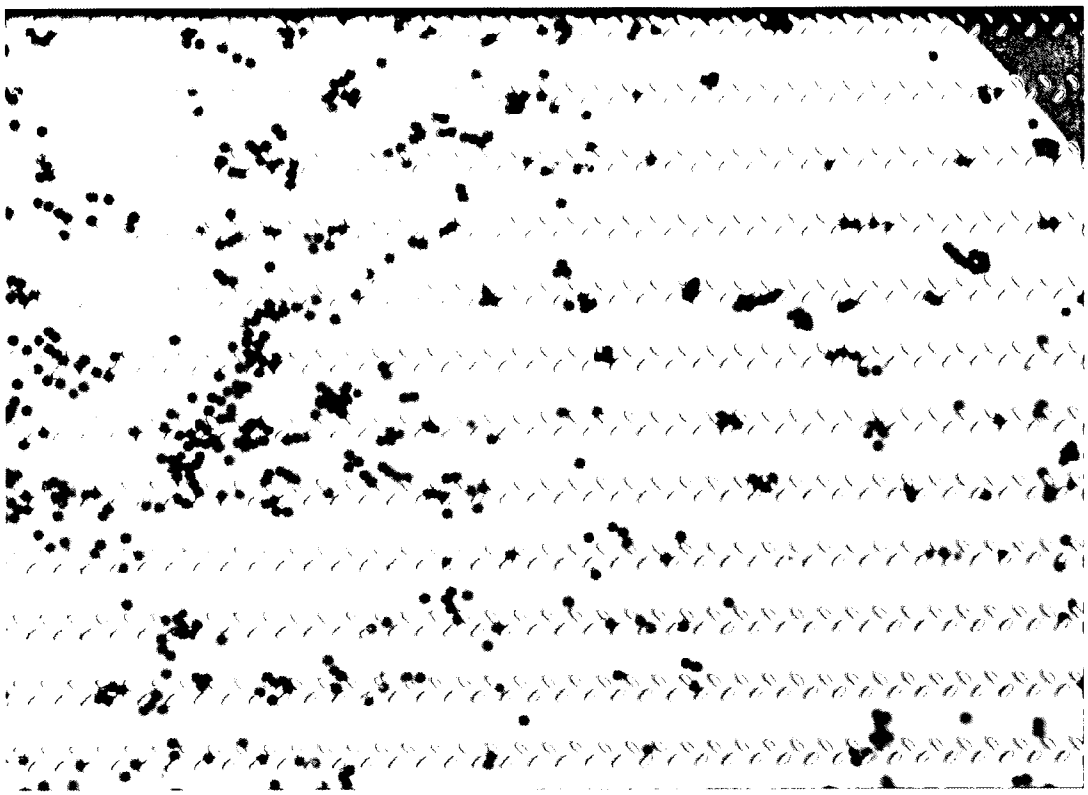


Figure 7B

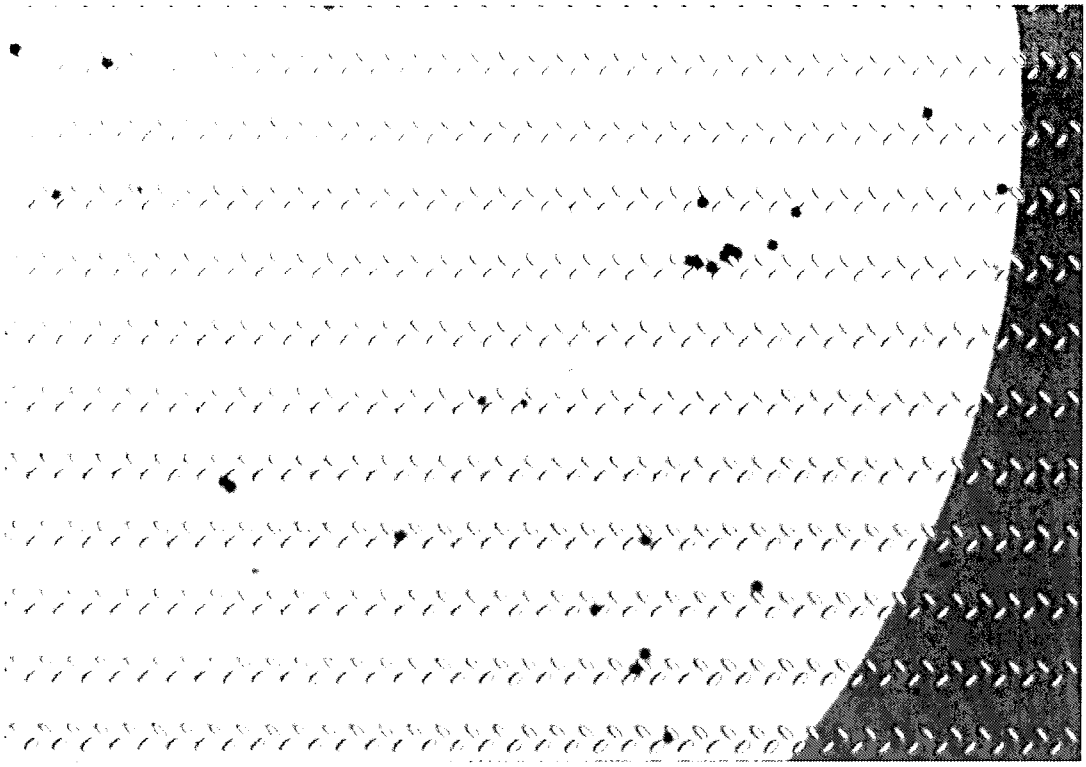


Figure 7C

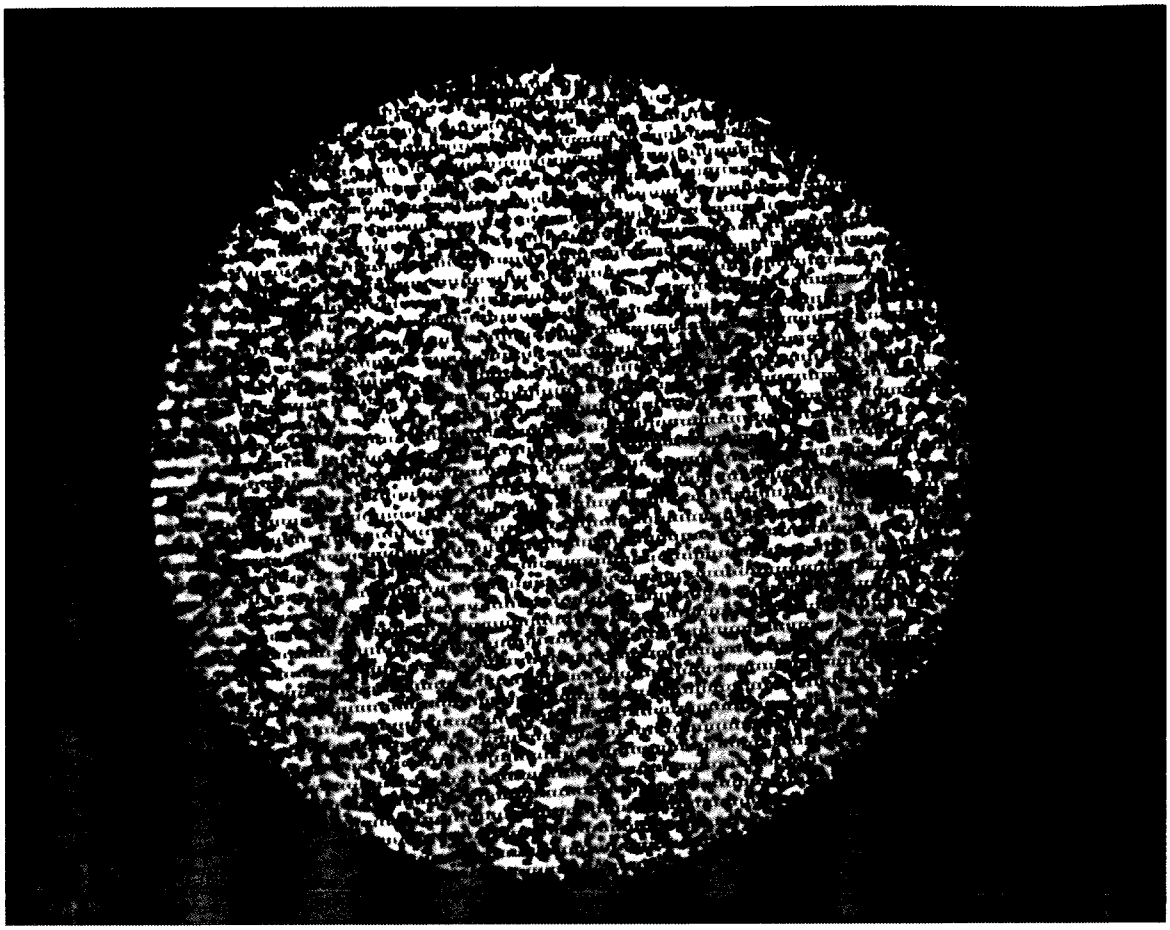


Figure 8A

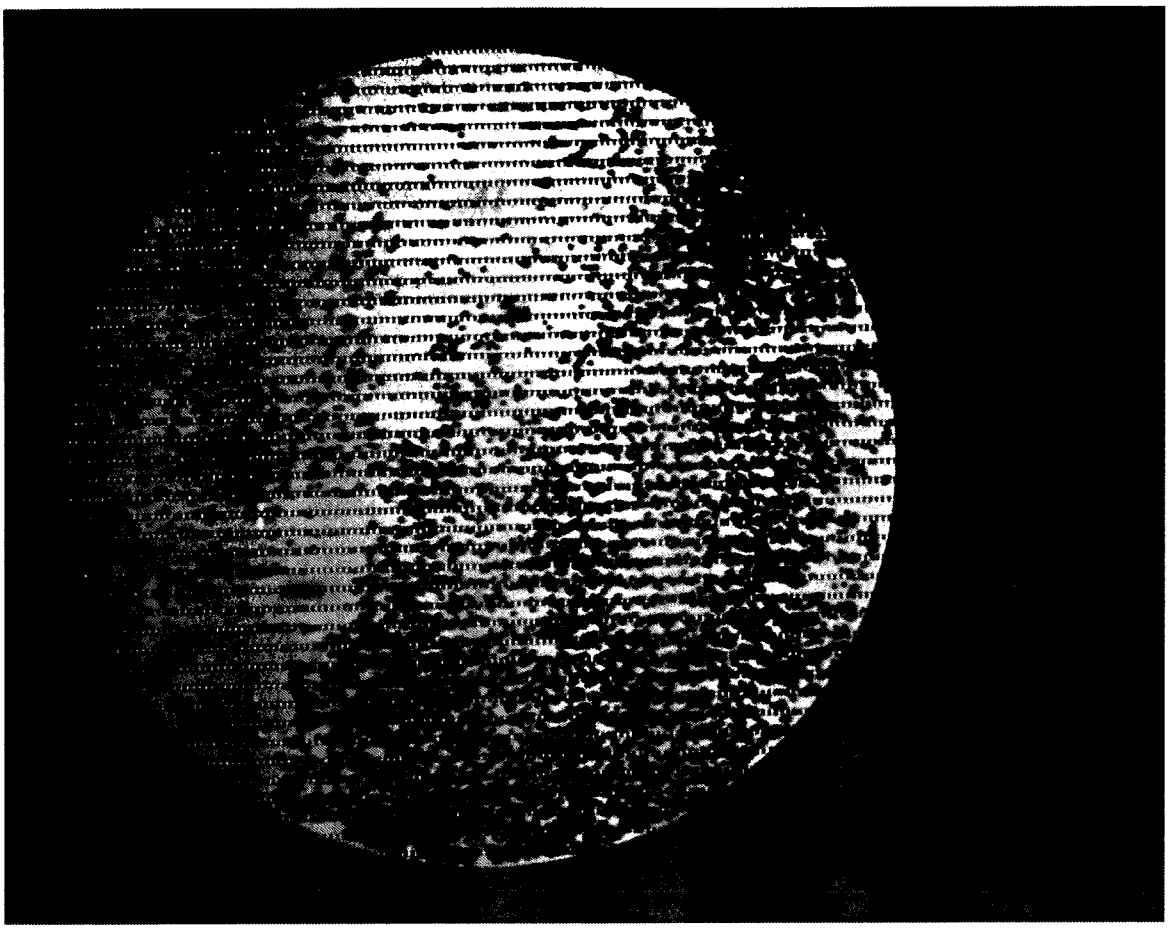


Figure 8B

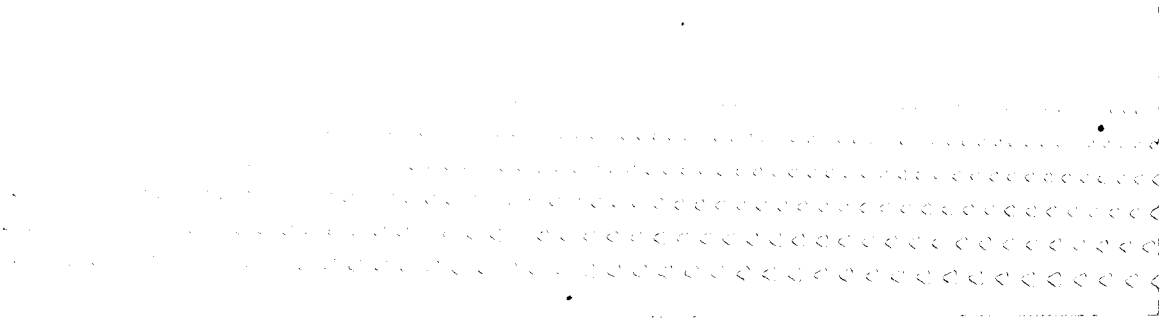


Figure 9

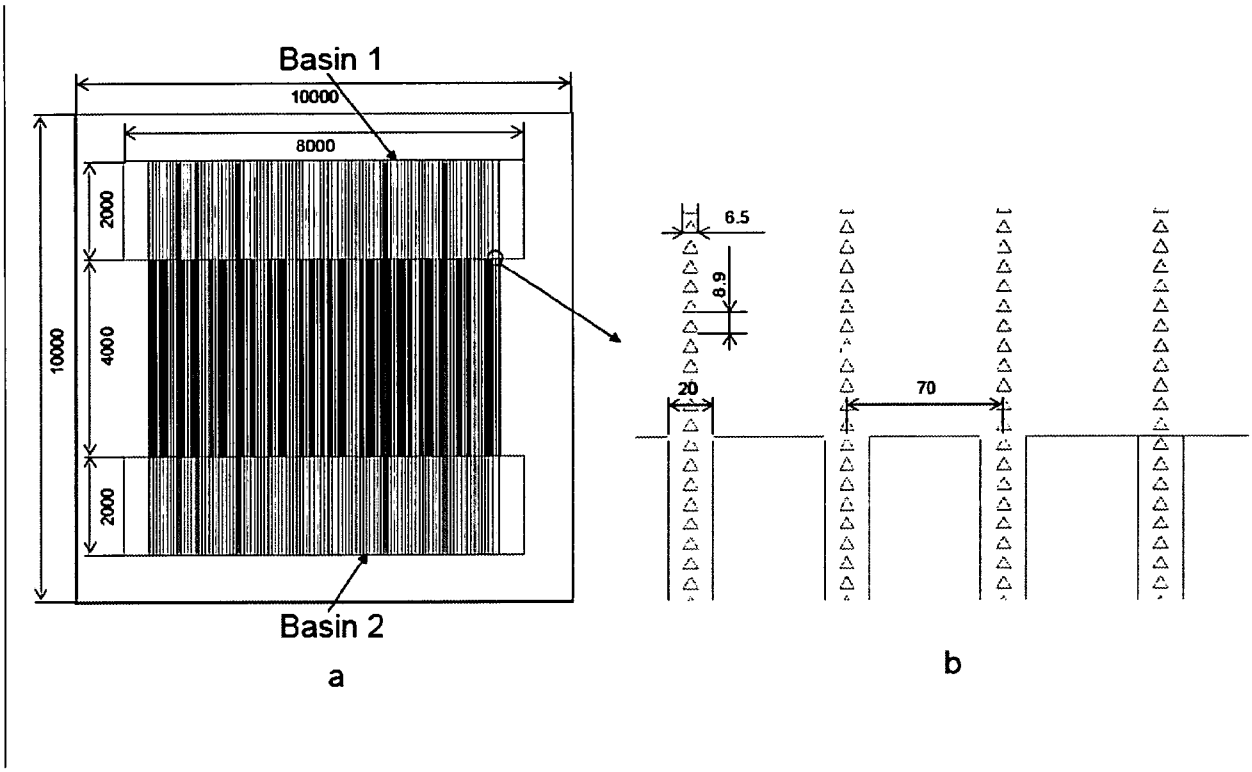


Figure 10

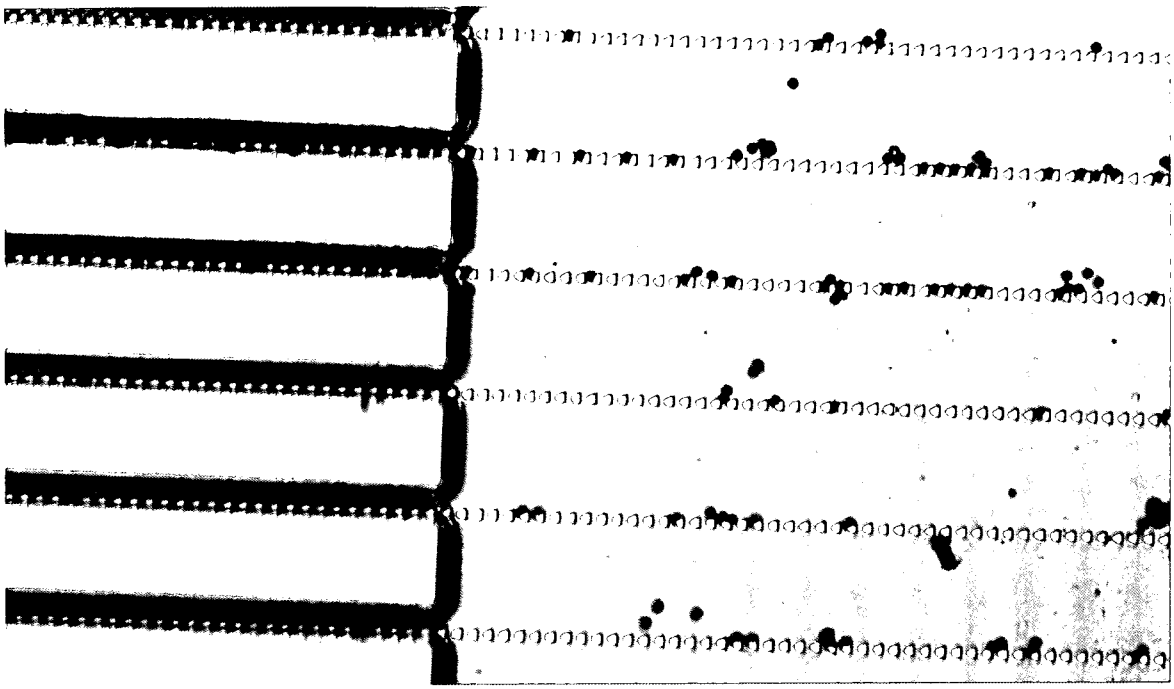


Figure 11

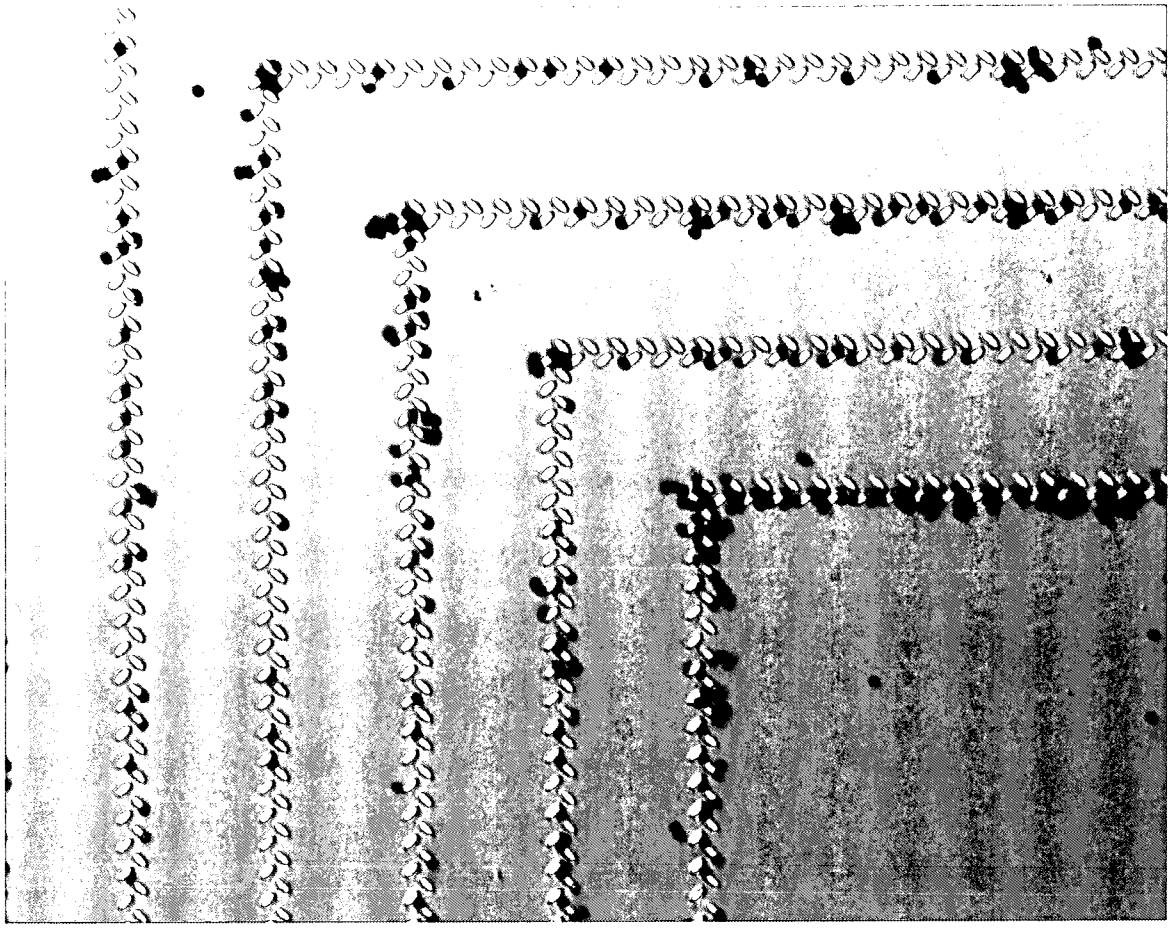


Figure 12

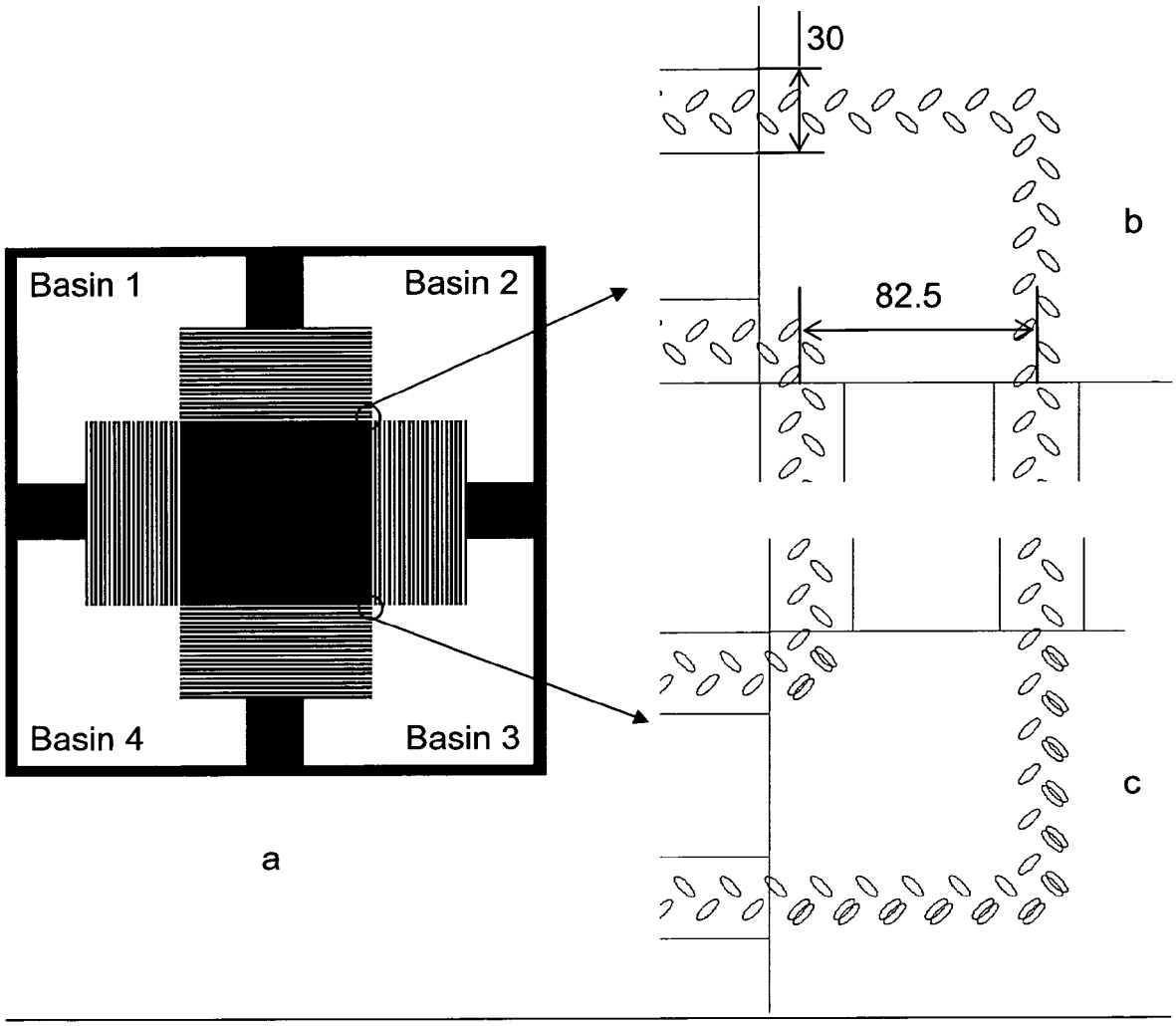


Figure 13

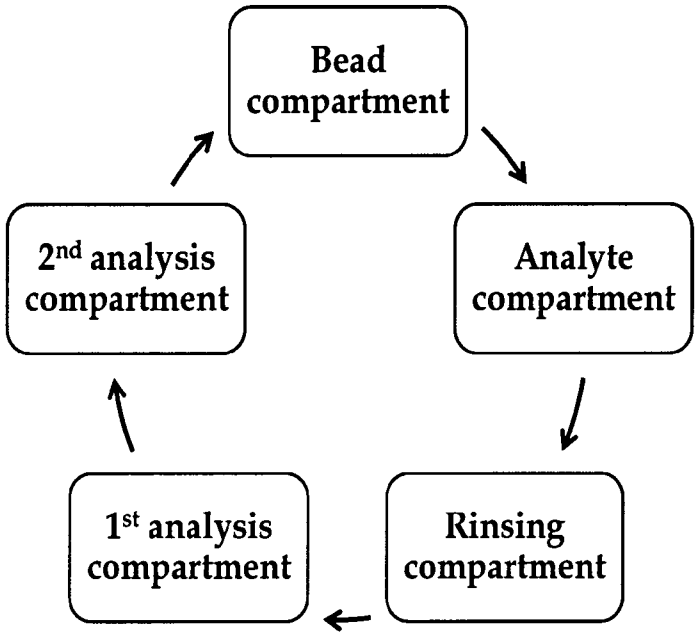

Bead compartment
Analyte compartment
Rinsing compartment
1st analysis compartment
2nd analysis compartment

Figure 14

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/056143

A. CLASSIFICATION OF SUBJECT MATTER
INV. B01L3/00 B01L9/00 G01N33/543
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
B01L G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2008/160630 A1 (LIU DAVID J [US] ET AL) 3 July 2008 (2008-07-03) paragraphs [0028] - [0033], [0047], [0049], [0057], [0084], [0085], [0110], [0115] - [0121], [0131], [0144], [146195]; figures 1-5,9,10,16-20 -----	1-36
X	US 2010/255556 A1 (HUNT THOMAS [US] ET AL) 7 October 2010 (2010-10-07) paragraphs [0042] - [0044], [0059] - [0061]; figures 1-7, 14, 21 ----- -/--	1-36

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
20 July 2012	01/08/2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Viskanic, Martino

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ANANDAKUMAR S ET AL: "Translocation of magnetic beads using patterned magnetic pathways for biosensing applications", JOURNAL OF APPLIED PHYSICS, AMERICAN INSTITUTE OF PHYSICS. NEW YORK, US, vol. 105, no. 7, 17 March 2009 (2009-03-17), pages 7B312-7B312, XP012124398, ISSN: 0021-8979, DOI: 10.1063/1.3073965 the whole document	1-36
X	US 6 716 642 B1 (WU LEI [US] ET AL) 6 April 2004 (2004-04-06) column 29, line 62 - column 32, line 8; figures 1-19, 36-38 column 46, line 50 - column 47, line 38	1,25
A	NICOLE PAMME: "Magnetism and microfluidics", LAB ON A CHIP, ROYAL SOCIETY OF CHEMISTRY, vol. 6, no. 1, 1 January 2006 (2006-01-01) , pages 24-38, XP002591314, ISSN: 1473-0197, DOI: 10.1039/B513005K [retrieved on 2005-11-28] the whole document	1-36

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2012/056143

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2008160630 A1	03-07-2008	NONE	
US 2010255556 A1	07-10-2010	US 2010255556 A1	07-10-2010
		WO 2009005680 A1	08-01-2009
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		US 2004077105 A1	22-04-2004