HYBRIDIZATION APPARATUS

Field of the Invention

[0001] The present invention generally relates to microarray technology, and more particularly to hybridization apparatus and method of allowing chemical or biochemical reactions with small volume samples on microarray slides.

Background of the Invention

[0002] High throughput methods for performing multiple biological and chemical analyses provide simultaneousness, quickness and convenience. One of the methods is microarray technology in which multiple samples are arrayed on a planar substrate such as a glass slide so that the samples can be analyzed simultaneously. The samples to be analyzed in a microarray format include DNA, RNA, oligonucleotides, proteins, peptides, carbohydrates, lipids, drugs, co-factors, and small molecules.

[0003] In microarrrays, the targets or probes are usually immobilized on a solid support having a surface area that can be smaller than a few square centimeters. A liquid containing the reactants is then brought into contact with the immobilized targets or probes on the solid support. In a typical procedure, the fluid is placed on the support, and the fluid and solid support are covered with another slide and placed in an environmentally controlled chamber such as an incubator for several hours. Typically, the reactants in the liquid diffuse to the interface where they react with the immobilized probes or targets, and a reaction, such as hybridization reaction, occurs.

[0004] However, some of the current limitations of microarrays include the time and care required for processing of slides, and the difficulty in obtaining consistent and high quality results. One particular problem in chemical and biological assays is inadequate mixing where very small samples of chemical, biochemical, or biological fluids are typically involved. For example, when sample probes are taken from patient tissues such as cancer for analysis, usually the sample probes are scarce and can be used once. Inhomogeneous solutions resulting from inadequate mixing can lead to poor hybridization kinetics, low efficiency, low sensitivity, and low yield.

[0005] A number of approached have been provided for mixing reaction solutions on microarray slides. For example, WO 03/015923 discloses a mixing method by inflation and deflation of mixing bladders located adjacent to the microfluidic chamber or channel causing inward or outward deflection of the chamber wall, and consequently displacement of fluid within the chamber. Another example is providing a bubble within the fluid film, so that as the chamber moves by mechanical, manual or other means, the bubble therein moves and fluid is displaced thereby resulting in mixing. See, U.S. Patents 6,613,529 and 6,258,593. However, the methods disclosed in the prior arts have one or more limitations including requirements of relatively large sample volumes and/or large hybridization chambers, and inconvenient or complicated equipment.

[0006] In addition, minimizing of the sample probe volume is very desirable in situations where the sample is hard to come by as mentioned above. The labor-intensive manual coverslip method uses excellent small amount of sample probes. However, it requires skill and experience. Moreover, it is not possible to ensure even concentration of samples across the entire covered area on the slide by any available aiding or agitation method.

[0007] Therefore, there is an imperative need to have a hybridization apparatus and method that can load the coverslip automatically and ensure even concentration of sample probes across the entire covered area. This invention satisfies this need by disclosing a hybridization apparatus and method that employs vacuum suction for automatically loading the coverslip and mixing the sample probes. Other advantages of this invention will be apparent with reference to the detailed description.

Summary of the Invention

[0008] The present invention provides a uniquely designed hybridization apparatus that enables reproducible hybridizations with small amount of sample probes. In one preferred embodiment, the hybridization apparatus for effecting a reaction on a solid surface of a substrate within an enclosed space comprises a hybridization chamber for providing the enclosed space within which the reaction on the solid surface of the substrate can be performed, wherein the substrate is disposed one or more spots of probes; and an applicator module for automatically loading a coverslip over the substrate and mixing a sample fluid disposed between the coverslip and the substrate by vacuum suction.

[0009] Accordingly, one object of the invention is to enable the integration of automated pre-hybridization, hybridization and post-hybridization processing for microarrayer slide processor.

[0010] Another object of the invention is that pre- and post-hybridization processes can be carried out manually or automatically via the uniquely designed gate for the removal of coverslip and washing operation.

[0011] Yet another object of the invention is that the unique automated coverslip applicator ensures reproducibility of bubble-free uniform coverage of sample probes over the substrate slide.

[0012] Another object of the invention is that the unique vacuum method provides full agitation of coverslip to promote better uniform concentration of sample probes over the substrate slide.

[0013] Still another object is that the modular design of chamber allows it to be set up for a multiple independent slide processor.

[0014] The objectives and advantages of the invention will become apparent from the following detailed description of preferred embodiments thereof in connection with the accompanying drawings.

Brief Description of the Drawings

[0015] Preferred embodiments according to the present invention will now be described with reference to the Figures, in which like reference numerals denote like elements.

[0016] FIG 1 is the front view from the gate **9** of the hybridization chamber.

[0017] FIG 1A is the entire sectional view through A-A of the hybridization chamber.

[0018] FIG 2 shows an exploded view of the applicator module that comprises the check valve unit **1**, applicator cylinder **2**, bushing **15** and applicator head **4**.

[0019] FIG 3 shows an exploded view of the gate module that comprises the heater base **8**, gate **9**, gate cylinder **10**, guide rod **11** and flipper guide **13**.

[0020] FIG 4 is an illustration of the moment when the vacuum actuated applicator head **4** is brought up with coverslip **5** above the DNA sample probe deposited on the sample slide **6**.

[0021] FIG 5 is an illustration of the moment when the coverslip **5** is lowered onto the DNA sample probe deposited on the slide **6** and the vacuum agitation cycle is to going to get started.

[0022] FIG 6 is an illustration of the loading of a glass slide into the hybridization chamber.

Detailed Description of the Invention

[0023] The present invention may be understood more readily by reference to the following detailed description of certain embodiments of the invention.

[0024] Throughout this application, where publications are referenced, the disclosures of these publications are hereby incorporated by reference, in their entireties, into this application in order to more fully describe the state of art to which this invention pertains.

[0025] Prior to detailed description the preferred embodiments of the present invention, it is noted that the microarray technology has broad applications as discussed above. Therefore, the exemplary DNA/RNA samples in the preferred embodiments are used for the purpose of illustration only. Any application of the present invention to other samples is well within the scope of the present invention.

[0026] The term "hybridization" as used herein means a process by which a stable complex of two or more molecules is formed. Any of the two or more molecules may be deposited on a substrate, while the other dissolved in a hybridization solution. The typical example of hybridization is the binding between the sense and anti-sense strands of double stranded DNA. The other examples include, but not be limited to, DNA:RNA, RNA:RNA, antigen:antibody, enzyme:substrate, and receptor:ligand. The term "hybridization solution" as used herein means a solution suitable for use in a hybridization reaction.

[0027] The terms "mix" and "mixing" as used herein mean to cause fluids to motion within a volume so as to more uniformly distribute solution components.

[0028] The term "substrate" as used herein means a surface upon which probe molecules may be adhered. The substrate may be composed of any material that is compatible with the fluids with which the surface comes in contact, such as, for example, glass, gel, silicon, fused silica, paper, metal, ceramic, plastic, or other polymers such as nylon. The substrate surfaces may be functionalized with, e.g., silane mixtures, so that the

background signals can be reduced. See, U.S. Patent 6,258,593. The term "functionalization" as used herein relates to modification of a solid substrate to provide a plurality of functional groups on the substrate surface. The substrate may be rigid or flexible, and may define a shape that is substantially planar in the shape of a square, a rectangle, or any other convenient substantially planar shape. The production of microarray slides with different samples is well known to those skilled in the art. Thus, there is no further detailed description of microarray slides and their making.

[0029] Now referring to FIG 1, there is provided a hybridization chamber in accordance with one preferred embodiment of the present invention. FIG 1 is the front view from the gate **9** of the hybridization chamber. It will be understood that all components may take any suitable form, and are not limited to the shapes and dimensions illustrated in these figures.

[0030] As shown in FIG 1A, the hybridization chamber comprises Check valve unit **1** connected to an external Vacuum agitator (for vacuum suction/agitation); Applicator cylinder (up/down) **2**, Chamber cover **3**; Applicator head **4**; Coverslip **5**; Slide **6**; Slide base **7**; Heater base **8**; Gate **9**; Gate cylinder **10**; Guide rod **11**; Hinge **12**; Flipper guide **13**; Pin **14**; Bushing **15**; Dampers **16**; Stoppers **17**; Inlet port **18**; Opening **19**; Chamber **20**, knob **21**, and holding pins **22**. FIG 1A is the entire sectional view through A-A of the hybridization chamber. For the convenience of description, the hybridization chamber may be divided into three main modules: applicator module as shown in detail in FIG 2; gate module as shown in detail in FIG 3; and chamber module including the chamber cover **3**, glass slide **6**, and slide base **7**.

[0031] The chamber **20** of the chamber module is formed between the chamber cover **3** and the slide base **7**, wherein the glass slide **6** is disposed upon the slide base **7** within the chamber **20**. The chamber **20** is sealed with two embedded O-rings and the gate **9**. The smaller O-ring for sealing, e.g., the bar code label of the glass slide **6** is embedded in the middle step of the bottom surface of the chamber cover **3** nearer to the hinge **12** and is made compressed against the top surface of the slide **6** upon closing the chamber cover **3** over the slide base **7**. The bigger O-ring for sealing the entire hybridization area of the glass slide **6** is embedded in the lower step of top surface of the slide base **7** and is made compressed against the bottom surface of the chamber cover **3** upon closing the chamber cover **3** over the slide base **7**. The chamber **20** is fully enclosed when the gate **9** is at a

retracted position and the embedded O-ring is made compressed against the opening **19** at the front surface of the chamber cover **3**. Once the chamber **20** is fully enclosed, the proximal end of the applicator head **4**, coverslip **5**, DNA sample probe and glass slide **6** are closed up in the enclosed chamber **20** for the subsequent automated DNA/RNA hybridization process to be carried out.

[0032] FIG 4 is an illustration of the moment when the vacuum actuated applicator head **4** is brought up with coverslip **5** above the DNA sample probe deposited on the sample slide **6**. FIG 5 is an illustration of the moment when the coverslip **5** is lowered onto the DNA sample probe deposited on the slide **6** and the vacuum agitation cycle is to going to get started. Referring now to FIG 4 and FIG 5, the applicator module is so uniquely designed that it performs dual functions. One is applying the coverslip **5** over the DNA sample probe deposited on the slide **6**. Another is agitating the DNA sample probe via the coverslip **5**. The vacuum suction is transmitted through an inner channel in the stem **4a** of the applicator head **4**, and the applicator cylinder 2 moves the applicator head **4** with the coverslip **5** downwards when the applicator module is performing the dual functions. This provides efficiency, convenience and simplicity.

[0033] FIG 2 shows an exploded view of the applicator module that comprises the check valve unit **1**, applicator cylinder **2**, bushing **15** and applicator head **4**. The busing **15** is mounted on top of the chamber cover **3** with an O-ring sandwiched between them. The inner circumference of the O-ring forms an air tight sealing to the outer circumference of the stem **4a** of the applicator head **4** when it is inserted into the bushing **15** from the hole at the bottom of the chamber cover **3**. The distal end of the stem **4a** is connected to the check valve **1** via a hole in the tie plate of the applicator cylinder **2** with a threaded union. The check valve **1** acts as a seal to the enclosed chamber **20** when vacuum suction is deactivated. The tie plate is connected to the piston rod of the applicator cylinder **2** which together with two dampers **16** are mounted on top of the chamber cover **3**. The surface of the proximal end of the applicator head **4** is designed with grooves to allow vacuum suction acting on top of the coverslip **5** to hold it above the DNA/RNA printed slide **6**.

[0034] FIG 3 shows an exploded view of the gate module that comprises the heater base **8**, gate **9**, gate cylinder **10**, guide rod **11** and flipper guide **13**. The heater base **8** is fastened with its top surface towards the bottom surface of the slide base **7**. A thin heater pad (not shown) is sandwiched between them to heat up the enclosed chamber **20** by heat

conduction pathway. The gate cylinder **10** with the tie plate attached to the piston rod is fastened to the bottom surface of the heater base **8**. The flipper guide **13** is fastened to the back surface of the heater base **8** with its slanting edge facing up at a location just below the front opening **19** of the chamber cover **3**. One ends of the guide rods **11** are free while the other ends are attached to the U-shaped tie plate. The gate **9** is hinged in between the two ends of the U-shape tie plate by a hinge **12** at the upper ends of the tie plate. The free ends of the guide rods **11** are inserted into the guiding holes from the back surface of the heater base **8** and they are fastened to the tie plate of the gate cylinder **10**. The pin **14** which is inserted into one side of the gate **9** nearer to the lower corner is brought into contact and rest on the slant edge of the flipper guide **13** when the gate **9** is in the open position as shown in FIG. 1A.

[0035] Now referring to FIGS 1-6, the operation of the hybridization apparatus provided in the present application is illustrated by using a DNA hybridization experiment.

[0036] Pre-hybridization setup

[0037] The chamber cover **3** is opened to manually load the pre-processed DNA printed glass slide **6** into the nested area at the top surface of the slide base **7**. In certain preferred embodiments, the chamber cover **3** is opened by first manually activating the gate **9** open and then by unscrewing the knob **21** in anticlockwise directions.

[0038] Now referring to FIG 6, it is an illustration of loading a glass slide, DNA sample probe, and coverslip just before closing of the chamber cover **3**. The glass slide is placed onto the raised platform with guide pins for nesting. Upon placing the glass slide, the vacuum suction of the applicator module is activated to hold the coverslip **5** by the grooved surface at the proximal end of the applicator head **4**. In the meantime, the DNA sample probe of 72 microliters is dispensed onto the center of the glass slide **6** with a micro pipette. Then, the chamber cover **3** is closed against the O-ring that is embedded in the groove **7a** around the platform of the slide base **7**. The chamber cover **3** is locked against the holding pins **22** by screwing the knob **21** in clockwise direction and then by manually activating the gate **9** close. It is appreciated that after the pre-hybridization setup, all the subsequent operations described hereinafter are automatic.

[0039] Hybridization

[0040] Upon the start of hybridization process, the coverslip **5** is gradually lowered down by the applicator cylinder **2** against the two dampers **16** until the tie plate of the

applicator cylinder **2** touches the stoppers **17** of the dampers **16**. The speed of lowering the coverslip **5** is preferably at 1mm/s or less that is achievable by the dampers **16**, pneumatic flow control valves and pneumatic pressure supply between 1 to 10 bars. The predetermined travel distance of the tie plate of applicator cylinder **2** is accurate and repeatable by the stoppers **17** of dampers **16**. One advantage of the applicator module provided in the present application is the automated handling of the coverslip **5** for a reproducible spreading of sample probes as small as 72 micro liters. It also produces uniform and bubbleless spreading of sample probes across the entire area under the coverslip **5**.

[0041] After the coverslip **5** is lowered onto the DNA sample probe deposited on the glass slide **6** by the applicator cylinder **2** as shown in FIG 5, the vacuum agitation cycle is started immediately with a pre-programmed ON and OFF durations and number of cycles. The ON and OFF durations each can be set from 0ms to 10s or longer. Similarly, the number of vacuum agitation cycles can be set from 0 to 100 or more, depending on the desired performance result of the uniform distribution of sample probe concentration under the entire coverslip. The vacuum agitation cycle can be repeated with programmable intervals throughout the entire automatic hybridization process that could be as long as 8 hours at 45 to 60°C used by any standard hybridization protocol.

[0042] Post-hybridization

[0043] Upon completion of the hybridization process, while the gate **9** is still closed, wash solution (not shown) is dispensed by a syringe pump (not shown) into the enclosed chamber **20** via the inlet port **18** of the chamber cover **3** near the hinge **12**. The wash solution is used to dislodge the coverslip **5** from the glass slide **6** by combining the aspirate and dispense actions of the syringe pump for a predetermined duration. At the end of the dislodging, the gate **9** is opened by the guide rod **11** when the gate cylinder **10** is retracted. In the process of gate opening, the gate **9** is tilted upwards about the gate hinge **12** as the pin **14** is glided along the slanting edge of the flipper guide **13**. The tilting allows a greater space in front of the opening **19** of chamber cover **3** after the gate **9** is fully opened. The wash solution is pushed out from the opening **19** of chamber cover **3** by the continuous dispensing of more wash solution with the syringe pump. The dispensing is continuing until a predetermined time when the coverslip **5** is flushed out of the chamber **20** and the unbound samples probe is washed away. The post hybridization process is

completed and the chamber cover **3** is opened to unload the glass slide **6** for drying and subsequent scanning operation.

[0044] In a preferred embodiment, the aforementioned apparatus is used in hybridization. In such a case, the reaction area of the substrate surface has a plurality of molecular probes bound thereto, and serves as a hybridization region. Preferably, the molecular probes are arranged in a spatially defined and physically addressable manner, i.e., are present in one or more "arrays." In a most preferred embodiment, the probes are oligonucleotide probes (including cDNA molecules or PCR products), although other biomolecules, e.g., oligopeptides and the like, may serve as probes as well.

[0045] It is to be appreciated that the operation of the apparatus of the present invention requires other components that are not shown in FIGS 1-6 or described herein. For example, the components include power supply, vacuum supply and solution supply. All the components are well within the knowledge known to those skilled in the art. Thus, there is no detailed description provided herein for the sake of brevity.

[0046] While the present invention has been described with reference to particular embodiments, it will be understood that the embodiments are illustrative and that the invention scope is not so limited. Alternative embodiments of the present invention will become apparent to those having ordinary skill in the art to which the present invention pertains. Such alternate embodiments are considered to be encompassed within the spirit and scope of the present invention. Accordingly, the scope of the present invention is described by the appended claims and is supported by the foregoing description.

Claims

What is claimed is:

1. A hybridization apparatus for effecting a reaction on a solid surface of a substrate within an enclosed space, comprising:

a hybridization chamber for providing the enclosed space within which the reaction on the solid surface of the substrate can be performed, wherein the substrate is disposed one or more spots of probes; and

an applicator module for automatically loading a coverslip over the substrate and mixing a sample fluid disposed between the coverslip and the substrate by vacuum suction.

2. The hybridization apparatus of claim 1, wherein the substrate is a microarray slide.

3. The hybridization apparatus of claim 2, wherein the microarray slide is made of a material selected from the group consisting of glass, gel, silicon, fused silica, paper, metal, ceramics, plastic, or nylon.

4. The hybridization apparatus of claim 1, wherein the probes disposed onto the substrate are selected from the group consisting of DNA, RNA, oligonucleotides, proteins, peptides, carbohydrates, lipids, drugs, and small molecules.

5. The hybridization apparatus of claim 1, wherein the hybridization chamber is formed by a chamber cover, a substrate base and a gate; wherein the substrate base is provided for the substrate so that the substrate is secured within the hybridization chamber; and wherein the chamber cover provides connections between the hybridization chamber and other components of the hybridization apparatus.

6. The hybridization apparatus of claim 5, wherein the chamber cover has one or more inlet ports for allowing reaction solutions be supplied into the hybridization chamber.

7. The hybridization apparatus of claim 1, wherein the applicator module comprises a check valve unit, an applicator cylinder, a bushing and an applicator head; wherein the applicator head has a stem that is connected to the check valve unit through the applicator cylinder and bushing, and an applicator head that is designed to hold the coverslip up before the sample fluid is disposed onto the substrate and hold onto the coverslip the sample fluid thereafter; and wherein the applicator cylinder and the bushing are disposed onto the top of the hybridization chamber.

8. The hybridization apparatus of claim 1, further comprising a gate module for providing an outlet port from which any liquid solution can be discharged easily.

9. The hybridization apparatus of claim 8, wherein the gate module comprises a heater base, a gate, a gate cylinder, a guide rod, and a flipper guide; wherein the heater base is disposed with its top surface towards the bottom surface of the substrate base; wherein the gate cylinder with an U-shaped tie plate is disposed to the bottom surface of the heater base; wherein the flipper guide is disposed to the back surface of the heater base; wherein one end of the guide rod is attached to the U-shaped tie plate; wherein the gate is hinged in between the two ends of the U-shaped tie plate; and wherein the free ends of the guide rod are inserted into the guiding holes from the back surface of the heater base.

10. The hybridization apparatus of claim 9, wherein the gate module further comprises a pin; wherein the pin is inserted into one side of the gate nearer to the lower corner and brought into contact and rest on the slant edge of the flipper guide when the gate is in the open position.

11. A method of performing a hybridization by using a hybridization apparatus that includes a hybridization chamber, an applicator module and a gate module, the method comprising steps of:

manually loading a substrate onto a substrate base;

manually disposing a sample probe onto the substrate;

disposing a coverslip onto an applicator head in a correct position so that the coverslip may properly cover the sample probe on the substrate when they come into contact; and

starting an automated hybridization protocol; thereby the applicator head applies the coverslip onto the sample probe on the substrate and mixing the sample probe during hybridization.