

integrated into the cover slip. The voltage differential between each pad on the cover slip and the electrode under the substrate can be individually controlled by computer. If the target molecule is negative charged, the target will be propelled by a negative electrode and attracted to the positive electrode.

[0177] The adjacent electrode pads on the cover slip can be turned positive or negative with reference to the electrode under the substrate in a programmed sequence. For example, as illustrated in FIG. 27, a target molecule with a negative charge is initially positioned under Pad 1 on top of a first probe. When Pad 2 is given a positive charge, the negatively-charged target molecule is pulled towards Pad 2. Next, a negative charge is applied to all of the Pads 1-7 for a period of time. This causes the target molecule to be driven towards the substrate surface under Pad 2, where a second probe is located. By this process, the target molecule is moved laterally by one pad-distance. When Pad 3 is turned positive and then negative, the target molecule is moved one step further to be positioned next to a third probe.

[0178] In this way, charged target molecules in the sample can be driven up and down between the cover slip and the substrate slide and are transported along the pads in a "zig-zag" fashion as illustrated in FIG. 27. This "zig-zag" movement is characterized by a change in direction of the moving charged particles of less than 180°.

[0179] The lower half of FIG. 27 illustrates the voltage distributions across the electrode pads in time sequence for achieving such transport effect. The frequency of the positive-negative change on the electrode pad is adjusted so that the target molecule can associate or hybridize with its complementary probe for a desired time before it is pulled away from the substrate surface. By programming the timing and/or voltages of the pad array across the entire cover slip, the system can drive target molecules to move along a predetermined route to contact each probe in a speedy and orderly fashion, as illustrated in FIG. 27.

[0180] An alternative voltage sequence is illustrated in FIG. 28. Pad 1 is given a positive charge first, which lifts the target molecule up (if it is not specifically hybridized to the probe). Then Pad 2 is turned positive and Pad 1 is turned negative. This moves the molecule to a new position just under Pad 2. When the entire pad array is then turned negative, the molecule is pushed towards the substrate surface under Pad 2. Now the molecule has advanced by one pad-position laterally. By repeating in this fashion, the target molecule in the liquid sample can be transported along a predetermined route under the electrode pads to contact each of the probes in the probe array.

[0181] This hybridization apparatus can significantly improve the rate and the sensitivity of microarray hybridization. First, the rate of hybridization is increased by increasing the chance that the target molecule collides with its complementary sequence because the target molecule is moved along the surface of the substrate in the hybridization chamber. Second, when the electrodes on the cover slip are positive, target molecules that are not specifically hybridized to a specific probe can be forced by the electric field to move away from the microarray. The voltage used is high enough to pull the unhybridized target molecules away from the probe without pulling away hybridized target molecules or any probe on the substrate slide. This action can enhance the hybridization specificity.

[0182] In FIGS. 27-29, all electrodes are isolated from the liquid sample. The transportation process can therefore be defined as a "dielectrophoresis" mechanism. This kind of electric transport system may utilize a relatively large voltage to transport charged particles. This is because the buffer solutions are relatively good conductors in comparison with conventional microarray substrates and cover slips, which are made of glass or other dielectric materials.

[0183] It is also possible to submerge a set of electrodes in the sample solution and make use of an electrophoresis mechanism to transport the target molecules. The spatial pattern of electrode pads can be the same as the system shown in FIG. 26 except the electrode pads are now provided on the surface of the cover slip that faces the substrate. An advantage of such a pad array configuration is that it is easier to set up a continuous circulating transport route and while utilizing a relatively lower voltage.

[0184] It is noted that increasing the density of pad arrays increases the number of electronic connections used and can increase the complexity of the flow control algorithm. FIG. 29 shows a simplified electrode configuration in which the electrodes are positioned near the sides of the hybridization chamber. It is possible to fabricate these electrodes by electric plating methods and combine the electrode pads with the risers on the cover slip. In this configuration, the electrodes are substantially thick such that they also function as spacers between the substrate and the cover slip.

[0185] In yet another embodiment, the upper electrode pads can be provided on the inner surface of the cover slip, as shown in FIG. 30. This can enable the target molecules to be transported in a lateral direction using a relatively smaller voltage. The electrode pads can be in direct contact with the sample solution (electrophoresis) or a very thin layer of dielectric material can be coated on the pads to provide isolation (dielectrophoresis). To create more vertical movement of the target molecules towards the probes on the substrate surface, the gap between the cover slip and the substrate can be formed as small as possible also shown in FIG. 30. Using, for example, precision etching as is found in semiconductor manufacturing, it is possible to form a gap having a height in the sub-micrometer range. Because of the small gap, the target molecules can reach the probes by diffusion relatively quickly.

[0186] Another way to create more movement of the target molecules towards the probes on the substrate surface is to coat a layer of a conductor, such as metal, on a conventional substrate to serve as the lower electrode, as shown in FIG. 31. If the selected conductive layer is not compatible with the probe or target liquid, a thin biocompatible layer can be coated on top of the conductive layer to provide a base for probe bonding and target hybridization. The biocompatible layer can be, for example, silicon dioxide, silicon, or any other suitable material. Alternatively, a suitable conductive material can be used as the microarray substrate so that the substrate itself can be used as the lower electrode. Examples of such materials include p or n type doped silicon. Alternatively, the substrate can be intrinsic silicon having an upper surface doped to become p or n type conductive layer to serve as the lower electrode.

[0187] FIG. 32 shows an alternative approach. In an electric field, there exists field lines which plot the direction of dielectric force in the field. Charged molecules are