

gous sequence thereof. In some embodiments, fluorescence-labeled or biotin-labeled antibodies are then used for signal readout.

[0118] In some embodiments, the lines are formed by one or more channels configured to host the material to be patterned. In particular, in some embodiments the fluidic channel width can be made ranging from 0.5 μm to 1 cm. The height can be typically $>1/10$ of the channel width when a soft materials such as PDMS is employed, and can be less if a harder material (e.g. glass, silicon, polystyrene, PMMA, polycarbonate or epoxy) is used to make the fluidic channels.

[0119] In embodiments when a two-layer device is used for patterning arrays, the channel can be as short as 1 mm and up to meters when the channel is shaped to cover the entire substrate (e.g. a glass slide 1"×3") for example by turning back and forth on the substrate. In embodiments where a larger substrate is used, the channel length can be longer since the length is defined by the substrate and the application of interest.

[0120] The array can be in principle made into any custom-designed shapes such as stripes, rings, concentric rings (see for example the illustration of FIGS. 5 and 6), triangles, rectangles, polyhedrons, stars, cross-bars, letters, pictures on flat, convex, concave or irregular substrates. In particular in FIG. 6 a multiple ring pattern suitable to application such as a bio-assay for detection of targets secreted by a sample such as a cell placed in the middle, is shown. In particular the images of FIG. 6 show the detection of proteins IL-2 and TNF- α visualized by Cy3 and Cy5 fluorescent probes.

[0121] In embodiments, wherein the channels are used to pattern polynucleotides (e.g. DNA) or proteins (e.g. antibodies), the channels width can be anywhere from 0.5 μm to 1 cm and the height can range from 1 μm to 1 cm, and the length can any that is allowed by the area of the given substrate. An exemplary 2- μm barcode array is shown in FIG. 7, wherein a barcoded array of fluorescent DNA molecules manufactured according to the teaching of the present disclosure, is illustrated. For optimum demonstrated performance of polynucleotide detection using a complementary DNA barcoded array, a channel width of 20 μm and height of 20 μm are preferred when a 200- μM capture DNA solution is used and the developed array is visualized using fluorescence scanner. In embodiments, wherein a DNA barcoded array is used to immobilize DNA encoded antibodies and subsequent immuno-sandwich assay, the same channel width and height are preferred (see below description of DEAL technology).

[0122] In some embodiments, some or all of the substantially parallel lines are connected to one another through at least one of the ends. More particularly, in applications wherein the lines are formed by channels the substantially parallel lines can be connected to one another to form a single channels configured in a serpentine-like shape. Serpentine-like channels allow the fabrication of repeated barcode arrays over a large area, e.g. the entire glass slide (1"×3"), in a single step of flowing capture agents. It represents a significant advantage in large-scale, low cost manufacture of barcoded arrays for detection applications. In addition, it allows an assay to be executed in multiple repeats at the same thus reduce the statistic errors. An exemplary illustration of a serpentine-like channel is shown in FIG. 8. Additional connections between the substantially parallel lines of a pattern or multiple patterns (for example multiple barcoded patterns

connected to form a pyramid to increase DNA loading in application wherein barcode is manufactured in connection with DEAL technology).

[0123] The material to be patterned can be disposed along the parallel lines according to a specific experimental design of choice. For example, in embodiments where a plurality of capture agents are patterned, the capture agents can be disposed with each capture agent disposed along one line, or with two or more capture agents located disposed along portions of a single channel. In other embodiments, the material to be tested (and in particular detected) can be patterned along one line or portion of a line of the barcode. Exemplary illustrations of those embodiments are shown in FIGS. 1 and 7.

[0124] In some embodiments, the patterned material can be used for target detection. In those embodiments, typically capture agents are patterned on the substrate, to form detectable capture agent target complexes. In other embodiments, detectable targets are patterned directly on the material. For example, a number of serum samples from multiple patients can be patterned into a barcoded array. In such array, each stripe contains the biomolecules in the entire plasma proteome of that patient. This array can be exploited to screen for antibodies, ligands, drug candidates, and comparison of biological profiles among patients. Those embodiments are exemplified for the barcoded arrays, substrates, methods and systems of Examples 3-14 and illustrated in the related figures and further described below.

[0125] In some embodiments, assays are performed in a non-microfluidic environment. An exemplary illustration of those embodiments is shown in FIG. 9, wherein execution of multiple assays in twelve isolated wells using a barcoded array is illustrated. In particular, the barcoded array illustrated in FIG. 9 is manufactured on a supporting glass slide including wells, wherein each well contains a different sample such as human serum. In the experiments illustrated in FIG. 9, protein detection from the different samples is visualized by fluorescence imaging.

[0126] In some embodiment, assays are performed in microfluidics which allows handling particularly small amounts of biospecimens (such as a finger prick of blood, tissue from skinny needle biopsy, etc).

[0127] In some embodiments, the barcode array can be used to detect multiple proteins and/or genes from a single cell via on-chip single cell culture, lysis, mRNA and protein isolation/purification, in particular using an integrated microfluidic device such as the one described in the U.S. Application entitled "Microfluidic Devices, Methods and Systems for Detecting Target Molecules" Serial No. to be assigned filed on Jul. 16, 2008, Docket Number P235-US, incorporated herein by reference in its entirety.

[0128] A further description of the arrays, substrates, devices methods and systems of the present disclosure is provided with reference to microfluidic applications wherein the sample is a material of biological origin (bio sample) and the targets are biomarkers. A person skilled in the art will appreciate the applicability of the features described in detail for microfluidics and biomarkers for non-microfluidic applications and/or for other biologic, organic and inorganic samples and targets.

[0129] In some embodiments, the arrays, devices methods and systems herein disclosed can be used to perform a surface bound bioassay based on detection a biomolecule of interest