

permeable PDMS, leaving the molecules to be attached (e.g. DNA molecules) behind. In some embodiments, this process can take from several hours to overnight to complete.

[0106] Following patterning of the molecules, the mold is usually decoupled from the support. In some embodiments, once the mold is removed from the support the patterned molecule can be subjected to subsequent treatments (e.g. DNA molecule can be fixed to the glass surface by thermal treatment at 80 C for 4 hours, or by UV crosslinking; removal of salts or other precipitates that might have formed during one or more of the previous operations which can be removed, for example, by rapidly dipping the slide in deionized water prior to bonding the blood-assay chip to the slide). An exemplary procedure of the patterning method herein disclosed is illustrated in Example 15.

[0107] In particular, in some specific embodiments, a series of microfluidics channels is patterned into PDMS, and those channels bonded onto a glass surface so that one out of the 4 channel walls is the glass surface itself. The numbers of micro fluidics channels determines the size of the barcoded array. In this way, a solution flowing through the micro fluidics channel will come into contact with the glass substrate. Typical dimensions of these micro fluidics channels for barcoded used for biological assays are 10 micrometers or larger. In particular, in embodiments where material is patterned to be subjected to a bio assay, the channel width defines the width of an individual bio-assay measurement area within the final bar code. In those embodiments, if the final measurement of the biomolecule is done using optical methods, then a 10 micrometer wide area constitutes a size that is readily imaged using low-cost optics. Larger and smaller bars are also possible.

[0108] A different material and in particular a different biological species (or a different concentration of the same biological species), such as DNA oligomers, can then be flowed in to each of the individual micro fluidics channels.

[0109] The biological species or other patterned material can then be attached to the glass surface areas within those microfluidics channels using electrostatic or other chemical interactions. The glass may be pre-coated with some molecular component to increase the chemical interaction between the biological species and the glass surface (see above and below in particular Example 2).

[0110] The solvent from the solution containing the patterned material (e.g. the biological species) is then removed. If that solution is water and the fluidics (e.g. microfluidics) is fabricated from PDMS, then the water can be let naturally evaporate through the PDMS, leaving the patterned material attached to the substrate thus providing a the patterned array on the substrate. In some embodiments, it may be desirable to introduce additional channel (e.g. micro fluidics channels) at this point for handling and introducing the biological sample of interest.

[0111] The microfluidic bar-code patterning chip may be made by molding silicon elastomer from a master template. The master template may be fabricated from many materials. One method is to fabricate the master by using photolithography to expose an SU8 2015 photoresist. Regions of the photoresist are removed following lithographic exposure, and the remaining material constitutes the master. Alternatively, photolithographic patterning methods, coupled with deep reactive ion etching (DRIE), can be utilized to prepare a master from a silicon wafer. These various methods for preparing microfluidics molds and microfluidics channels from

those molds are well known in the art. (Gael Thuillier and Chantal Khan Malek, *Microsys. Technol* 12, 180, 2005.)

[0112] The patterned material can comprise any substance of interest suitable to be attached to a support, including organic or inorganic substances, Exemplary inorganic material that can be patterned using the patterning methods and systems herein disclosed include but are not limited to gold nanoparticles that can attach to thiol functionalized substrate surface, iron oxide nanoparticles that can be deposited onto the substrate using magnetic field, and silica particles that can be immobilized by cationic polymer coated substrate, and so on.

[0113] Exemplary organic that can be patterned using the patterning methods and systems herein disclosed include but are not limited to living species and their mixtures such as cells, virus, bacteria and fungi, complex biospecimens and their mixtures such as tissue, tissue lysate, cell lysate, serum, saliva and joint fluid, monotypic molecule and their mixtures such as polynucleotides, proteins, antibodies, glycoproteins, polysaccharides, lipopolysaccharides, ligands, peptides, polypeptides, lipids, drugs, drug candidates, antigens and the fragments, portions, and components or any of above. The organic materials can also include non-biological materials such as polymers, oligomers, dye molecules, conducting polymers, responsive polymer, gas sensing polymers, liquid crystals and metal organic frameworks (MOFs), carbon nanotube, fullerene, grapheme, and their nano/microstructures. In some embodiments, the patterned material comprises capture agents. In some embodiments, the patterned material comprises detectable targets. In other embodiments, the patterned material comprises a material, such as cells or other biological material to be assayed. In other embodiments, the patterned material can comprise other organic or inorganic substance for which the barcoded configuration is desired (e.g. liquid crystal for LCD manufacturing, or gas selective polymers to be used as gas sensors).

[0114] According to the patterning methods and systems herein disclosed, a pattern and in particular a barcoded pattern or array can be created on very small area and patterning of magnetic ID or other material can therefore be performed onto small-sized products.

[0115] In some embodiments, wherein the pattern is used for the detection through capture agents, the capture agent is formed by a polynucleotide and in particular a DNA polynucleotide, that bind about 10 to 20 consecutive bases of a target RNA via complementary hybridization. In some of those embodiments the arrays, substrates, methods and systems herein disclosed can be used to detect messenger RNA (mRNA) and in particular mRNA from a biospecimen (e.g. tissue lysate). In some of those embodiments, another labeled DNA stand (e.g. fluorescently labeled) is designed to bind to ~10-20 different bases of the captured mRNA for signal read out. In some embodiments, a multiplexed measurement of a panel of mRNA molecules can be performed on a barcode array patterned with stripes of their capture agent DNA.

[0116] In some embodiments, wherein the pattern is used for the detection, the target is a microRNA (miRNA) a type of short RNA molecules (22 bases) that regulate gene expression at the post-transcription level

[0117] In some embodiments, wherein the pattern is used for detection, the target can be a transcription factor, and the capture agent is a polynucleotide and in particular a DNA polynucleotide having the same sequence of the binding site of the transcription factor, or a portion thereof or an homolo-