

in some biomaterial, such as blood, serum, biological tissue, or as a component of a cell culture (herein also indicated as bio-barcode assay).

**[0130]** The biological material can be pretreated so as to release the biomolecules of interest, to remove biological material that can interfere with binding of the biomolecules in the surface bound bioassay. An exemplary pretreatment procedure includes separating blood cells from blood plasma (or serum), and then measuring the proteins from the plasma. In other procedures the separated cells could be further separated into white and red blood cells, which can be therefore subjected to further analysis. An exemplary surface bound bioassay can be carried out as follows: The biomolecule of interest is bound to a (primary or 1<sup>o</sup>) surface-bound capture agent molecule (e.g. an antibody or complementary single-stranded DNA oligomer) that specifically recognizes and binds to the biomolecule of interest. Typically, a secondary (or 2<sup>o</sup>) capture agent containing some label for detection, such as a fluorescent molecule, is introduced to bind to the surface-bound biomolecule.

**[0131]** The bio-barcode can be manufactured patterning the capture agents of choice on a substrate along substantially parallel lines. In certain microfluidic applications the substantially parallel lines can be formed by channels or channel portions. Exemplary illustration of different embodiments wherein capture agents are attached to a surface in a bio-barcode are shown in FIG. 10 (capture agents DNA molecules for detection of polynucleotide (e.g. mRNA and microRNA) to be configured in a barcoded array), FIG. 11 (DNA-encoding antibodies to enable immuno-sandwich assay on barcode array allowing detection of proteins, cell surface markers, glycoproteins, virus and bacteria in multiplex) and FIG. 12 (schematic illustration showing how increased DNA loading helps to enhance detection sensitivity in application wherein the bio-barcode is coupled with DEAL technology see below).

**[0132]** Patterning of capture agents, for example, antibody arrays for detecting proteins or complementary DNA arrays for detecting polynucleotides, results in an increased sensitivity of molecules such as polynucleotide, nucleic acid (mRNA, miRNA, DNA etc). An increased sensitivity could be in particular associated with two factors: (1) the increased loading of capture DNA using poly-amine to coat substrate surface (for embodiments wherein the capture agent is a polynucleotide and in particular DNA) and (2) the reduced feature size with respect to conventional pin spotted arrays (e.g. 20  $\mu\text{m}$  in barcoded array vs. 200  $\mu\text{m}$  in conventional pin-spotted array) lowers the diffusion barrier and leads to high binding efficiency.

**[0133]** In some embodiments the capture agents include one or more component. In particular, in some embodiments the capture agents can be formed by a substrate polynucleotide and a polynucleotide encoded-protein in application of the technology (herein also identified as DEAL) described in U.S. patent application Ser. No. 11/888,502 herein incorporated by reference in its entirety.

**[0134]** Accordingly, the wording "substrate polynucleotide" as used herein refers to a polynucleotide that is attached to a substrate so to maintain the ability to bind to its complementary polynucleotide. A substrate polynucleotide can be in particular comprised of a sequence that specifically binds and is thereby defined as complementary with an encoding-polynucleotide of a polynucleotide encoded protein.

**[0135]** The wording "polynucleotide-encoded protein" refers to a polynucleotide-protein complex comprising a protein component that specifically binds to, and is thereby defined as complementary to, a target and an encoding polynucleotide attached to the protein component. In some embodiments, the encoding polynucleotide attached to the protein is protein-specific. Those embodiments can be used to perform assays that exploit the protein-specific interaction to detect other proteins, cytokines, chemokines, small molecules, DNA, RNA, lipids, etc., whenever a target is known, and sensitive detection of that target is required. The term "polynucleotide-encoded antibody" as used herein refers to a polynucleotide-encoded protein wherein the protein component is an antibody.

**[0136]** In the polynucleotide-encoded proteins herein disclosed each protein specifically binds to, and is thereby defined as complementary to, a pre-determined target, and each encoding polynucleotide-specifically binds to, and is thereby defined as complementary to, a pre-determined substrate polynucleotide.

**[0137]** In embodiments wherein the protein is an antibody, the protein-target interaction is an antibody-antigen interaction. In embodiments wherein the protein is other than an antibody, the interaction can be receptor-ligand, enzyme-substrate and additional protein-protein interactions identifiable by a skilled person upon reading of the present disclosure. For example, in embodiments where the protein is streptavidin, the protein-target interaction is a receptor-ligand interaction, where the receptor is streptavidin and the ligand is biotin, free or attached to any biomolecules. An exemplary schematic illustration is shown in FIG. 12.

**[0138]** When coupled with the DEAL technique, the amount of polynucleotides that is deposited onto a given spatial location within the bio-barcode array can be controlled in view of the desired sensitivity and concentration range over which the biomolecule of interest can be detected. By using two or more stripes within the same bio-barcode array, each optimized to detect the same biomolecule but over different concentration ranges, the concentration range over which that protein can be detected, as compared to a conventional assay, can be dramatically increased.

**[0139]** The concentration range of DNA detectable with a Bio-Barcode array coupled with DEAL can be as low as 1 pM to 100 nM using 200  $\mu\text{M}$  loading of capture DNA on 20  $\mu\text{m}$  barcode stripes. Target molecules suitable for this technique include messenger RNAs, micro RNAs, the fragments of genomic DNAs, viral DNA, bacterial DNA, and synthesized polynucleotides.

**[0140]** Some embodiments wherein the Bio-Barcode is coupled with DEAL shows an increased sensitivity if compared with embodiments wherein protein capture agents are patterned directly on a substrate. In particular, in some embodiments wherein antibodies are patterned directly into barcoded array with fabrication methods that require application of high temperatures when the antibodies are attached to the substrate, all the target molecules that can be detected by DEAL are in principle detectable, but a lower sensitivity might be seen due to the poor stability of the antibody in a dry state.

**[0141]** When coupled with the DEAL technique, the bio-barcode array withstands the processing conditions associated with micro fluidics chip fabrication. As a consequence, the Bio Bar .Bar Code array can be advantageously manufactured as illustrated in the exemplary procedure outlined below