

be performed with methods other than fluorescence based techniques. Exemplary suitable techniques include, calorimetric detection, enzyme-catalyzed production of different colored or fluorescent dyes (with different colors being associated with distinct analytes), microparticle/nanoparticle based detection using electron microscopy, AFM, or dark-field microscopy, magnetic detection using magnetic micro/nanoparticles, electrical detection methods.

**[0092]** In some embodiments, detection can be performed by methods that use signal amplification such as gold nanoparticle based detection followed by gold or silver amplification. In particular, in some embodiments, in any of the methods and systems herein disclosed, detection can be carried out on gold nanoparticle-labeled secondary detection systems in which a common photographic development solution can amplify the gold nanoparticles as further described below. Also, if the readout comes from dark field scattering of gold particles, single molecule digital proteomics is enabled.

**[0093]** The detection can be performed with the aid of suitable equipments. In particular any equipment configured to read barcoded pattern can be used as long as the relevant sensitivity is applicable to the detection of choice.

**[0094]** For example, in some embodiments, reading the information of the arrays herein disclosed can be performed using a simple line-scan reader such as the laser line scanner schematically illustrated in FIG. 2. The one-dimensional layout of the arrays renders a higher reliability as compared to the conventional circular spot arrays as schematically illustrated in FIG. 3. In the illustration of FIG. 3, is shown how a scan reading from a same line scanner (scan b) provides a higher reliability for a barcoded pattern (panel B) if compared with a spotted array (Panel A).

**[0095]** Additional equipment suitable to detect the array herein described can be identified by a skilled person upon reading of the present disclosure. For example, when fluorescent probes are used for signal readout, laser microarray scanner (such as Axon Genepix 4000 series scanner, Affymetrix 300 scanner, etc), scanning laser confocal microscope (e.g. Nikon Eclipse C1si microscope) can be used to visualize the pattern. In particular, the parallel-stripe pattern allows a single scan of laser to read out full information with high fidelity and reliability as illustrated in FIGS. 2 and 3. This feature opens the possibility of implementing a simple laser line scanner similar as the barcode reader in supermarket for reading the barcode array described herein.

**[0096]** In other embodiments, wherein gold nanoparticles are used, light scattering microscope (such as Nikon® Eclipse LV100) can be used. In other embodiments, wherein electroless metal plating is used to enhance the nanoparticle signal, a flat bed scanner (such as Nanosphere Verigene® reader) can be used besides light scattering microscopes. In still other embodiments, wherein magnetic particles are used as probes, a magnetoresistive sensor similar to a scan head in a hard disk can be used to read out the barcode information.

**[0097]** Additional techniques are identifiable by a skilled person upon reading of the present disclosure and will not be further discussed in details.

**[0098]** Arrays and substrates herein disclosed can be manufactured using methods and systems to attach a material to a support along a predetermined pattern herein also disclosed (herein also indicated as patterning methods and systems). The methods and systems to attach material can be used to manufacture arrays and substrate according to any predetermined pattern. In embodiments, wherein the patterned mate-

rial is configured along substantially parallel lines forming a barcoded pattern, the methods and systems herein disclosed can be used to manufacture barcoded arrays and substrates.

**[0099]** In some embodiments, the barcoded surface patterning can be performed as described below in the exemplary procedure illustrated with reference to microfluidics channels patterned from polydimethylsiloxane (PDMS) that are weakly or strongly bonded to glass substrates. A skilled person would understand that the patterning method is not limited the specific microfluidic features and materials used and that a different number of channels with different dimensions as well other materials, such as injection molded micro fluidics channels, semiconductor wafers, etc., all identifiable by a skilled person upon reading of the present disclosure, may all be utilized.

**[0100]** In some embodiments, a mold can be fabricated by molding a polymer such as a PDMS elastomer from a master template, to include microchannels each having an inlet and an outlet and each of the outlets is such that it forms a portion of the desired pattern (in particular a barcoded pattern). In some embodiments, the polymer is molded using photolithography to create a photoresist pattern on a silicon wafer. Those embodiments, allow a particularly rapid prototyping. An exemplary illustration of a mold fabrication for the patterning methods and systems herein disclosed is illustrated in FIG. 4 wherein fabrication of a PDMS microchannel stamp for flow patterning of a barcode array is disclosed.

**[0101]** In another embodiment, the mold can be manufactured by providing a silicon "hard" master and by transferring the photolithographically-defined pattern into the underlying silicon wafer using a deep reactive ion etching (DRIE) process. Those embodiments allow a robust and reusable mold for higher throughput chip fabrication.

**[0102]** In some embodiments, the molded polymer can then be coupled and in particular bonded onto a support, such as a glass surface, which provides the floor for the channels of barcoded pattern. An exemplary illustration of a design two-layer PDMS fluidic channel device used for creating a multiple ring pattern (bull's eye) on a glass slide is shown in FIG. 5.

**[0103]** In some of embodiments, the substrate can be pre-coated with a material of interest. For example in embodiments wherein a barcode is manufacture using the DEAL technology further illustrate below, a polyamine polymer or poly-L-lysine polymer (Sigma-Aldrich), can be pre-coated prior to bonding to increase DNA loading of the final barcoded pattern (see below and in particular Example 2).

**[0104]** The number of microfluidic channels determines the size of the barcode array. In some exemplary embodiments the barcoded array comprises 13 to 20 parallel microchannels that wind back and forth to cover a large area (3 cm×2 cm) of the support with the DNA barcode microarray.

**[0105]** In some embodiments, patterning can be performed by contacting the capture agent or molecule of choice on the support for a time and under conditions to allow attachment on the support. More particularly, in some embodiments patterning can be performed by providing solutions, each containing the molecule of choice (e.g. a different strand of primary DNA oligomers prepared in 1×PBS buffer in embodiments wherein the array is coupled with DEAL technology), can be flowed into each of the microfluidic channels. Then, the solvent of the solution can be allowed to evaporate, e.g. by placing the solution-filled chip in a dessicator to allow solvent (e.g. water) to evaporate completely through the gas-