

with reference to an exemplary array including 10 antibodies used as capture agents (10 CAs) labeled with single stranded DNA used as encoding polynucleotide.

[0142] The 10 antibodies against the biomarker of interest are chemically labeled with single-stranded DNA (ssDNA) oligomers. The complementary ssDNA' oligomers can be deposited onto regions of a surface. DNA hybridization assembles the 10 CAs onto those particular regions.

[0143] The 10 CAs are patterned using microfluidics channels. The channel widths and densities are limited by what can be patterned—smaller channels and higher densities than are practical using other methods are readily achieved. Typically channels of widths of at least 10 micrometers, spaced by distances of at least 50 micrometers, are most practical for typical bioassays, such as analyzing multiple proteins from serum. This allows for large numbers of measurements to be carried out in a relatively small microfluidics channel.

[0144] Spot sizes significantly smaller than 10 micrometers are also possible with this technique, as are significantly higher spot densities. These may be useful for more specialized applications, such as would be required for measuring a panel of protein biomarkers and other biomolecules from circulating tumor cells, cancer stem cells, and other extremely rare cell types.

[0145] The bio-barcode patterned microfluidics channels are readily aligned with other microfluidics channels, such as are used for the handling of the biological specimen from which the assays are performed. For example, alignment markers that are utilized to align the bio-barcode microfluidics channels can also be utilized to assemble the microfluidics channels for handling the biological sample. This is standard fabrication practice.

[0146] The density of 1° CAs that can be deposited onto such a small spot can be significantly higher than what can be achieved using spotting methods. Repeated depositions of 10 CAs through the same microfluidics channels can achieve a very high surface loading of the 10 CAs. Conversely, the DEAL technique utilizes single-stranded DNA (ssDNA) oligomers as capture agents for the 10 CA antibodies that are, in turn, utilized to detect proteins. The DNA can be loaded at very high levels using the bio-barcode Array because of the high solubility of DNA in water. This, in turn, can lead to very high coverage of the 1° antibody CAs.

[0147] Multiple numbers and classes of capture agents can be placed on specific, microscopic locations on a surface using microfluidic patterning of the 10 capture agents. In this way, the panel of biomolecules is detected by detecting labeling signals (for example, fluorescence) from the region of the surface where the pattern of 10 capture agents was placed.

[0148] In some embodiments, wherein the arrays, substrates methods and systems herein disclosed are performed in microfluidics, the capture agents can be attached on the location with a method to attach molecule along a predetermined pattern herein disclosed. In those embodiments, using a microchannel-guided flow-patterning approach, a barcode arrays can be manufactured that are at least an order of magnitude denser than conventional microarrays. In some embodiments, this result can be accomplished by creating a mold, e.g. a polydimethylsiloxane (PDMS) mold containing the desired number of microfluidic channels, e.g. 13-20 parallel microfluidic channels, with each channel conveying a different biomolecule capture agent. A skilled person will understand that the number of channels can readily be expanded to include 100 or more different capture agents;

whereas in microcontact printing, the patterning difficulty increases exponentially as the number of proteins printed is increased, due to the challenges of aligning multiple stamps to print multiple proteins.

[0149] In some embodiments, the barcoded array is a DEAL barcoded array. In some of those embodiments polyamine coated glass surfaces can be used to allow significantly higher DNA loading than do more traditional aminated surfaces. DNA “bars” of 2 micrometers in width could be successfully patterned. In some exemplary embodiments, described herein an about 20-micrometer (μm) channel width was chosen because the fluorescence microarray scanner has a resolution of 5 μm .

[0150] In those embodiments a 10-fold increase in array density is achieved as compared to a typical pin-spotted DNA array (i.e. 150 μm spot diameters at 300 μm pitch), and greatly expands the numbers of proteins that can be measured within a microfluidic chip disclosed herein for a given sample size. In particular, in some embodiments, simultaneous detection of 12 to 20, up to 50 or even more than 50 proteins. This feature can be used in applications where detection of multiple targets is desired, for example detection of a biological profiles but also a variety of waste gases (e.g. from car engine exhaust) or pollutants in a sample.

[0151] The protein assay can be carried out on the 10 CAs array as described above. Use of DNA hybridization as an assembly strategy allows for multiple proteins to be detected within the same microenvironment, since the various 10 CA antibodies for the various proteins to be detected can be each labeled with a different ssDNA oligomer. Also use of DNA hybridization as an assembly strategy allows preparation of the substrate including ssDNA in early in the fabrication process so that a substrate including the ssDNA can be treated, dried out, heated, shipped and provided to the final user in a ready to use systems that also include complementary capture agents. Exemplary applications are described in Examples 1 to 7 and in the related figures describe the barcode array patterning technique and DEAL bar-code chips for protein detection.

[0152] A person skilled in the art would understand that the array herein disclosed can include patterning a variety of biological materials, e.g. DNA, proteins, sera and tissue lysates, using micro fluidic channels. The Bio Bar-code Array method can be applied to the fabrication of bio-chips and integrated biosensing devices for high-density, multiplexed and sensitive detection of DNA and proteins in clinic diagnostics of human diseases like cancers, and for high-throughput drug screening. In some embodiments the patterning is based upon a new, yet simple and reliable approach—micro channel guided surface patterning of a large number of different biological species to fabricate a small-size, high-density array.

[0153] The systems herein disclosed can be provided in the form of arrays or kits of parts. An array sometimes referred to as a “microarray” includes any one, two or three dimensional arrangement of addressable regions bearing a particular molecule associated to that region. Usually the characteristic feature size for microarrays is micrometers.

[0154] In a kit of parts, various components can be comprised in the kit independently. In some embodiments, a patterned substrate can be provided together with a label and/or other reagents suitable to perform detection. In some embodiments, a device suitable for detecting the pattern can also be included.