

lanes were used for cross-talk validation and 6 lanes were used for dynamic range studies.

[0180] The results are illustrated in FIG. 17 which shows cross-reactivity check and dilution curves for all 12 proteins. In particular, the DEAL barcode images and line profiles from a single device of panel (a) show minimal cross-talk and a series of standard antigens ranging from 1 nM to 1 pM for all 12 proteins. In the experiments shown in panel (a), 2 proteins were combined in each assay lane (FIG. 17 panel (a)).

[0181] All proteins were assayed on the same chip over the concentration range of 1 nM down to 1 pM (except PSA and TGF- β : 5 nM to 5 pM), and quantified the fluorescence signal vs. concentration for all 12 antigens as illustrated in FIG. 17 panel (b), where dilution curves for all 12 proteins are shown.

[0182] In this experiment, all the concentrations were imaged using the Genepix scanner at the same laser power (55 for 635 nm, 15 for 532 nm), optical gain (500 for 635 nm and 400 for 532 nm), and brightness/contrast (92/90) in order for quantitative comparison. Apparently, the estimated sensitivity varies a lot from ~0.3 pM (e.g. IL-1 β and IL-12) to 30 pM (TGF- β) largely depending on the antibodies being used. For example, the TGF- β antibody pair has a relatively lower binding affinity and a poorer detection limit in ELISA (according to the spec sheet, it is ~70 pg/mL compared to 5-10 pg/mL for most other cytokines). Predictably, this gave rise to a poorer performance in the DEAL assay. Although these curves clearly show a dynamic response of DEAL signals with respect to antigen concentrations, the variation remains pretty large as compared to bulk-scale immuno-assay such as ELISA.

[0183] Detection probes are not limited to fluorescent dyes, but can be any others that are capable to transduce signal from captured targets to optical, magnetic or electrical read out.

[0184] In particular, an alternative method of detection is provided by use of gold nanoparticles as probes. An exemplary illustration of detection performed using gold nanoparticles is shown in FIG. 18, wherein detection of target protein IL-1 β using gold nanoparticles as the probe is shown.

[0185] In particular, in the example of FIG. 18, 40-nm gold nanoparticles were used to visualize the captured protein (e.g. IL-1 β) of interest from human serum).

[0186] Additional examples of labels and method of detections are illustrated the U.S. Application entitled "Methods and Systems for Detecting and/or Sorting Targets" Ser. No. 11/888,502 filed on Aug. 1, 2007, incorporated herein by reference in its entirety.

Example 6

Comparative Example Related to Use of a Barcoded Array and a Conventional Microarray for Protein Detection

[0187] Comparative experiments were performed on the barcode array of example 3 and a conventional microarray printed using pin-spotting technique. The results illustrated in FIG. 15 panel d, show how apparently, the conventional microarray only achieved sensitivity 1-2 orders of magnitude worse than the DEAL barcoded chips.

[0188] A side-by-side comparison study was performed by running DEAL assays on three cytokines under identical conditions on a barcoded and a pin spotted microarrays under the experimental conditions illustrated in Example 3. The pin-spotted array was printed at the Institute for Systems Biology at 100 μ M concentration. The typical spot size was

150-200 μ m. Six sets of spots were printed corresponding to oligomers A, B, C, D, E, and F. Poly-l-lysine coated slides were used for both types of arrays. Further details are illustrated in Example 3.

[0189] The results illustrated in FIG. 15 panel e show that barcoded array exhibits greater performance with higher sensitivity than does the conventional array.

[0190] In particular, these results demonstrate that the detection sensitivity of the DEAL barcode arrays was higher and the projected sensitivity limit was better than 1 pM, as compared to 10-100 pM for conventional microarrays (FIG. 15 panel e).

[0191] The only difference between the barcoded and conventional pin-spotted platforms used in the experiment shown in FIG. 15 is the feature size. The barcode array has a line-width of 20 μ m, whereas the spot size in conventional arrays is more than 150 μ m. The mechanism for improved sensitivity in the DEAL barcode assay is not completely understood. A possible explanation which is not intended to be limited is that the improved sensitivity could be attributed to a reduced kinetic barrier and decreased diffusion time. These results are consistent with a recent report which demonstrated that DNA microarrays with smaller spot sizes could detect DNA with increased sensitivity.

Example 7

Use of a Barcoded Array for Detection of Multiple Different Targets

[0192] A barcoded array integrated with DEAL technology was used to detect multiple proteins as illustrated in FIG. 19. In particular FIG. 19 shows the use of DEAL bar-code immunoassay for the detection of five different proteins. The proteins are detected within an area that is less than would be required for the detection of a single protein using a conventional spotted microarray.

[0193] The results illustrated in FIG. 19 show in particular multiple proteins simultaneously detected using a DEAL bio-barcode. Panel A shows a schematic illustration of DEAL bar-code array for co-detection of a variety of proteins at the same time, including cytokines, chemokines, growth factors, intracellular signaling molecules and cancer markers. Panel B shows a multiparameter DEAL Bar-code immunoassays of 5 proteins at the same time, detected from human reference serum that was spiked with the five proteins: hCG, TNF- α , IL-2, IL- α , and IL-1 β . In principle, bar-code array can provide high density assay of a much greater number of protein s simply by increasing the number of microchannel s used in flow patterning.

[0194] The detection of multiple targets was performed according to the schematic representation of FIG. 20 that shows the microfluidic device used in patient serum measurement. In particular, FIG. 20 panel A shows. the schematic of the operation of a microfluidic device that is bonded onto a barcode array glass slide.

[0195] FIG. 20 Panel B shows a schematic illustrating the method to introduce fluid into microfluidic devices for molecular detection and in particular interfacing the outside sample loading/injection systems to the microfluidic device using plastic tubing and metal pins.)

Example 8

Use of a Barcoded Array to Detect Proteins Over a Broad Dynamic Concentration Range

[0196] A bio-barcode integrated with DEAL technology was used to detect biomarkers as illustrated in FIG. 21. In