

casian and the remaining three were Asian, Hispanic and African-American. The medical history is summarized in the supplementary materials.

[0212] Finger pricks were performed using BD Microtainer Contact-Activated Lancets. Blood was collected with SAFE-T-FILL capillary blood collection tubes (RAM Scientific), which we pre-filled with 80 μ L of 25 mM EDTA solution. A 10 μ L volume of fresh human blood from a healthy volunteer was collected in this EDTA-coated capillary, dispensed into the tube, and rapidly mixed by inverting a few times. The spiked blood sample was prepared in a similar means except that 40 μ L of 25 mM EDTA solution and 40 μ L of recombinant solution were mixed and pre-added in the collection tube. Then 2 μ L of 0.5 M EDTA was added to bring the total EDTA concentration up to 25 mM.

[0213] Execution of blood separation and plasma protein measurement was performed using an integrated platform extensively described in U.S. entitled "Microfluidic Devices, Methods and Systems for Detecting Target Molecules" Serial No. to be assigned filed on Jul. 16, 2008, Docket Number P235-US herein incorporated by reference in its entirety.

[0214] The integrated platforms were first blocked with the buffer solution for 30-60 minutes. The buffer solution prepared was 1% w/v Bovine Serum Albumin Fraction V (Sigma) in 150 mM 1 \times PBS without calcium/magnesium salts (Irvine Scientific). Then DNA-antibody conjugates (~50-100 nM) were flowed through the plasma assay channels for ~30-45 min. This step transformed the DNA arrays into capture-antibody arrays. Unbound conjugates were washed off by flowing buffer solution through the channels. At this step, the integrated platform was ready for the blood test. Two blood samples prepared as mentioned above were flowed into the integrated platforms within 1 minute of collection. The integrated platform quickly separated plasma from whole blood, and the plasma proteins of interest were captured in the assay zone where DEAL barcode arrays were placed. This whole process from finger-prick to plasma protein capture took <10 minutes. In the cancer-patient serum experiment, the as-received serum samples were flowed into the integrated platforms without any pre-treatment (i.e. no purification or dilution). Afterwards, a mixture of biotin-labeled detection antibodies (~50-100 nM) for the entire protein panel and the fluorescence Cy5-strapavidin conjugates (~100 nM) were flowed sequentially into the integrated platforms to complete the DEAL immunoassay. The unbound fluorescence probes were rinsed off by flowing the buffer solution for 10 minutes. At last, the PDMS chip was removed from the glass slide. The slide was immediately rinsed in 1/2 \times PBS solution and deionized water, and then dried with a nitrogen gun. Finally, the DEAL barcode slide was scanned by an Axon Instruments Genepix Scanner.

[0215] The serum samples from 24 cancer patients were assayed using two chips, each containing 12 separate assay units that were operated in parallel. In every assay unit, 50 sets of DEAL barcodes were placed in the detection channel for statistical sampling of the serum. In all experiments, 25 μ L of patient serum, or 500 nanoliters per barcode, was used for each assay. The white-blood cell secreted proteins included inflammatory molecules and cytokines. These proteins are employed by immune cells for intracellular communication, and have significant implications in tumor microenvironment formation, and in tumor progression and metastasis. Thus, this panel provides information on both cancer and the immune system.

[0216] Experiments were repeated at least 2-3 times. In every integrated platform, multiple sets of barcode arrays were patterned in a single assay channel to allow simultaneous parallel measurements. For example, 50 sets of barcode were used in assaying a cancer patient serum sample, with each barcode detecting the full panel of proteins. Quantitation of fluorescence signal was performed using either the Genepix software or imageJ (NIH). In processing the cancer-patient data, the background intensity for each channel was individually identified, and then re-assigned to a common background level of 20 arbitrary units. The intensities of all "bars" in a given channel are normalized to that channel's background. Therefore, the data in FIG. 10 corresponds to the bar's fluorescence intensity differences relative to its own channel's background, plus the common background level of 20. This treatment minimizes interference from non-specific background signal, but could make it indistinguishable between the positive results with high background (e.g. B10) and the true negative results (e.g. B9 and B11).

[0217] The results are illustrated in FIGS. 24 and 25, which show the related profile of cancer patients (FIG. 24) together with their medical history (FIG. 25).

[0218] In particular, fluorescence images each showing four sets of representative barcodes obtained from the 24 patient samples are shown in FIG. 24. The proteins measured include cancer marker PSA and eleven cytokines also indicated in details in FIG. 25. In the barcode image panel, the left two columns were performed on the same chip while the right two were from the other. The samples were randomly picked in the assay to minimize arbitrary bias. B01-B11 denote 11 samples from breast cancer patients, P01-P11 denote those from prostate cancer patients, whereas the S01 and S02 are unknown samples from a different supplier.

[0219] The medical records for all patients are summarized in FIG. 24 which shows a brief summary of cancer patient medical records. The two unknowns are not included in the table.

[0220] A more detailed medical history of the patients is included in the following table 1.

TABLE 1

Medical Record of cancer patients.						
PATIENT	CANCER	GENDER/AGE	RACE	UICC STAGE	GLEASONS SCORE	OTHERS
B01	Breast	Female/62	Caucasian	T2N0M0		wine 200 mL/day
B02	Breast	Female/79	Caucasian	T4N2M0		
B03	Breast	Female/71	Caucasian	T1cNXM0		1-2 drinks/day
B04	Breast	Female/72	Caucasian	T2NXM0		hypertension
B05	Breast	Female/89	Caucasian	T3N0MX		arthritis
B06	Breast	Female/56	Asian	T1NXM0		