

[0122] Fluorogenic ELF-97 phosphate and acetate (both from Molecular Probes, Eugene, Oreg.) were used to detect enzymatic activity of alkaline phosphatase (AP) and esterase (Sigma-Aldrich, St. Louis, Mo.). When an enzyme cleaved the O—R bond of the ELF-97 substrate, an intensely fluorescent precipitate of ELF-97 alcohol formed at the contact region of the fluid paths. This assay was especially suited for parallel screening because all substrates generated the same fluorescent compound upon cleavage. FIGS. 14 and 15 show detection of esterase, β -galactosidase, and alkaline phosphatase (the latter by two independent assays).

[0123] This experiment demonstrated the viability of a fluidic system incorporating a functional gel. Furthermore, several samples were able to be analyzed simultaneously and in a small volume, and the results of the analyses were able to be compared directly. Each sample analyzed in this system left a pattern of signals that corresponded to its enzymatic activity. It is believed that this method will be useful for analysis and identification of complex mixtures of enzymes in biological samples, and as a tool in biomedical assays.

Example 4

[0124] In order to demonstrate the utility of a fluidic device and related methods of the invention incorporating both a gradient generator, and patterned interaction material, an FIA (serial-dilution fluorescent immunoassay) device was constructed and operated. The FIA was performed for or the presence of HIV antibodies in HIV-positive human serum (Golden West Biological, Inc.). In traditional immunoassays for HIV, viral proteins or peptides are immobilized on microtiter plates, and a serum sample containing antibodies against these proteins is serially diluted and incubated with the plates; the antibodies immobilize on the adsorbed viral proteins. A secondary antibody conjugated to either an enzyme or a fluorophore is used to quantify these antibodies. The most common formats for immunoassays for HIV test for the presence for only one antibody; embodiments of the method and apparatus of the present invention allow testing for multiple HIV antibodies simultaneously. The present example is performed using two antibodies, but the method is generalizable to 10-100 antibodies. These antibodies are directed against the HIV envelope glycoproteins GP 41 and GP 120 (referred to herein as anti-GP41 and anti-GP 120) from human serum (Protein Sciences, Inc.). A fluorescent mouse anti-human IgG (secondary antibody derived from mouse that recognized both anti-GP 41 and anti-GP 120) (Sigma) is used for readout of the concentrations of anti-GP 41 and anti-GP 120.

[0125] This example uses a microfluidic device according to the present invention with two components to perform the miniaturized FIA. The first component is a gradient generator that makes use of a CAM to mix fluid streams (see FIGS. 17 and 20). The gradient generator mixes and dilutes a fluid containing an interaction material—here a sample of analytes (the antibodies contained in the serum)—with a dilutant—here buffer—into a series of solutions containing exponentially decreasing concentrations of antibodies. The microfluidic network has two inlets, one for the serum; the other for the dilution buffer (5% bovine serum albumin, BSA, used to block non-specific interactions between antibodies and antigens). In each fluid path where two streams meet, the CAM causes efficient mixing, resulting in equal

redistribution of the solutions between the left and right halves of the fluid path. This mixing is illustrated in FIG. 20, section C, parts 0-5, which show the mixing within the channel (looking into the direction of flow) at 0, 1, 2, 3 and 4 mm (points 0 to 4) and at the point where the stream splits in two (5). FIG. 21 shows the equal splitting index at each of points 0-5. The equal splitting index is defined as the ratio between the average intensities of the two halves of the fluid path with the higher intensity as the numerator and the lower intensity as the denominator. Accordingly, an index of one indicates complete mixing. FIG. 21 also demonstrates that mixing occurs relatively rapidly, and is nearly complete at point 3.

[0126] Using the above-described design, it was possible to achieve substantially exactly 1:1 mixing at each of the mixing sites. When the serum solution and the BSA solution were initially mixed, the concentration of the antibody was decreased by half. The fluid path with the mixed stream was then split into two halves; one of the streams was maintained in a separate fluid path and the other was again mixed 1:1 with BSA. Each successive mixing decreased the antibody concentration by half. Using 1:1 dilutions (i.e., a mixing factor of 2) in 10 sequential repetitive mixings, it was possible to achieve a dynamic range of $2^{10} \approx 10^3$ for a fluid path network with 10 dilutions using (a 1:3 dilution would achieve a dynamic range of 10^6 , with the same repetition of mixing). The design of the microfluidic system illustrated in FIG. 17 generates a series of streams with exponentially-decreasing concentrations, as long as proper division and redistribution of the analyte is ensured (when the mixing fluid path is split, each succeeding fluid path inherits half of the original concentration). One of the ways to achieve proper division is to generate a homogeneous solution after mixing, for instance, in our case with the CAM. As described in the detailed description, incorporation of any other types of components into the microfluidic system that provide substantial homogenization (i.e. any mixer) or even the proper splitting of the analyte will work in this design. (See FIG. 20 for additional information.) To test for mixing ratios generated by the gradient generator, BSA-FITC (bovine serum albumin conjugated to fluorescein) was diluted against phosphate buffer saline (PBS). FIGS. 22 and 23 show the result obtained with a microfluidic system that makes eight consecutive dilutions. The dots indicate theoretical values calculated from the fluorescence intensity of the first channel and reducing it by 50% for each dilution. Agreement between observed and expected values is good (FIG. 23, section b).

[0127] The second important component of the embodiment of the microfluidic system of this example is substrate—here a polycarbonate membrane (Osmonics, Inc.)—presenting interaction material—here the antigens—patterned in microstripes. Recombinant HIV coating glycoproteins GP 120 and GP 41 were adsorbed onto separate arrays of microstripes on a hydrophilic, polycarbonate membrane. This patterning step used an array of microfluid paths to deliver the antigens to the membrane, as described previously herein. The hydrophilic polycarbonate membrane contains nanoscale pores (~200 nm); these pores have large hydrophilic surfaces that both adsorb sufficient amount of proteins to give good sensitivity, and also maintain the proteins in hydrated and active forms when the membrane is temporarily dried during assembly of the system. When serially diluted serum containing anti-GP 120 and anti-GP