

saline ("PBS")+1% bovine serum albumin ("BSA")+0.05% Tween 20 (Poly(oxyethylene)x-sorbitane-monolaurate, purified for membrane research and available from Roche)) was formulated.

[0192] Assay Procedure: 10 microliters of human serum was mixed with 10 microliters of 2x labeled antigen mix in a microcentrifuge tube. The full volume was introduced to a fluidic sample chamber and allowed to incubate for 20 minutes at room temperature. The incubated sample was imaged with imaging device **2650** without any further processing. The signal-to-noise ratio ("SNR") value for the captured fluorescence signal was derived according to the following formula:

$$SNR = \frac{(SN - BK)}{sdBK}, \quad (\text{Eq. 1})$$

where SN is scaled signal with reader instrument noise removed, BK is scaled signal from negative control sites flanking the feature, and sdBK is standard deviation of the BK signals.

[0193] FIG. 41 shows an example of a series of digital images captured using the labeled antigen assay protocol described above. The array as shown was formed of a series of duplicated features, including fluorescently-labeled BSA as fiducial markers (BSA647), antigens associated with HIV (p24 and gp41), antigens associated with *T. pallidum* (p17 and p47), and negative sites composed of print buffer only (Neg). The arrays were incubated with 20 microliters of a 1:1 mixture of labeled antigen mix and a human serum sample for 20 minutes. Three human serum samples were used in the demonstration: 1) Seracare 9148134 is a known positive for antibodies against HIV-1; 2) Seracare BM217820 is a known positive for antibodies against *T. pallidum*; and 3) Sigma H4522 is a pooled human serum sample negative for antibodies against HIV-1 and *T. pallidum*. The resulting fluorescence signal was recorded at imaging device **2650** without a wash step. Custom reaction site-finding algorithms, implemented at computer **2670**, define feature locations and record fluorescence intensity as numerical values corresponding to average pixel intensities within defined regions, and SNR values are calculated according to Equation 1. As may be seen in FIG. 41, the appropriate reaction sites indicative of the presence of target antibodies indicative of HIV-1 and syphilis produce fluorescence signal in accordance with the known sample serostatus, thereby confirming the accuracy of the labeled antigen assay in identifying the disease state of the given biological sample.

Example 11

Real Time Signal Acquisition for a Kinetic Analysis

[0194] In another embodiment, assay system **2600** may be combined with the labeled antigen assay described herein to generate kinetic assay data rather than the more common end point assay approach. Due to the fact that the detection system is relatively insensitive to fluorescent dye in the bulk solution, assay system **2600** may be used to collect real time data as the labeled antigen-target antibody complexes bind to the immobilized antigens on the assay surface. Real time data collection allows collection of kinetic parameters that offer several potential advantages. For example, initial binding rate information may be used for very rapid assays. Because initial

binding rate is directly related to the concentration of target analyte in solution, kinetic assays may potentially provide quantitative data, with initial binding rate linked to bulk solution concentration. For qualitative assays, a biological sample with a large concentration of target analyte will show signal very quickly relative to a negative control. Example kinetic data are provided in FIG. 42 from an experiment described in detail below. In this example, two target analyte concentrations are distinguished from each other and from the negative control within 5 minutes of biological sample addition. The labeled antigen assay allows real time acquisition of signal from the assay surface in the presence of the sample mixture. Therefore, the rate of accumulation of signal on a specific antigen feature may be tracked in real time as a kinetic assay. As an example, a labeled antigen assay was performed as in Example 1, except fluorescence images were acquired at time points over fixed intervals for two biological samples varying in concentration of human serum (50% dilution and 0.58% dilution, respectively), and one biological sample of known negative HIV status (VBMA90015-01) (See FIG. 42). Signal intensity varied in both rate of increase and final intensity as a function of concentration of serum sample, with both the HIV positive samples showing more and faster accumulation of signal than the negative control sample. This result suggests the possibility of a diagnostic assay for analyzing the presence and concentration of targets by the rate of signal acquisition.

Example 12

Rapid Assay Demonstration

[0195] The experiment and data in EXAMPLE 11 may also be used as a demonstration of an extremely rapid HIV-1 antibody detection assay. FIG. 42 illustrates the collection of kinetic data. Alternatively, one may define an end point assay at, for example 5 minutes. Reviewing the data shown in FIG. 42, one sees that a high concentration HIV sample, a lower concentration sample, and a negative control are all readily distinguished in terms of quantitative fluorescence output. FIG. 42 is thus a demonstration of a very rapid (i.e., in minutes) HIV-1 antibody detection assay.

Example 13

Labeled Antigen Assay with Whole Blood Results

[0196] In another embodiment, system **2600** may be combined with the labeled antigen assay described herein to perform assays in complex sample matrices. Because of the evanescent illumination approach, the assay is relatively insensitive to various components encountered in the bulk solution. For example, many immunoassays require serum or plasma specimens, as the cellular components of whole blood may interfere with assay performance. Whole blood assay devices, such as immunochromatographic strip assays, typically require a cell separation membrane upstream from the readout zone, as red blood cells and hemolytic products can interfere with readout on these devices. Example whole blood assay results are provided in FIGS. 43-44 from an experiment described immediately below.

[0197] In an embodiment, whole blood is assayed using the labeled antigen assay described above. The ability to analyze whole blood may extend the utility of a point-of-care assay by reducing the need for biological sample preparation and the inherently necessary laboratory infrastructure. For example,