

from when a sample is introduced to the system to the time when a titration result is realized.

[0113] Sample fluids and titrating fluids may be flowed through a microfluidic system using any of the methods that are known to those skilled in the art or are described herein. In one embodiment, a sample, a titrant, or both, may be flowed through a microfluidic device without a pump. For example, gravity may be used to push fluids through the device. Flow pressure may be provided by raising sample and/or titrant reservoirs above a microfluidic device and allowing the respective fluids to flow through tubing to inlets of the device. Flow rates may be adjusted by varying the height of the reservoirs.

## EXAMPLES

### Example 1

[0114] To demonstrate that individual contact regions in an array of contact regions may be addressed independently, calcium(II) ions were detected in a fluid path of one layer of a 5x5 array using a fluorescent probe, fluo-3 (Molecular Probes, Eugene, Oreg.), in a fluid path in the other layer; the other eight fluid paths were filled with water, as illustrated in FIGS. 8 and 9. The fluid paths were 200 microns wide. Fluorescence was observed only at the contact regions of the fluid paths containing calcium(II) ions and fluo-3. FIG. 8 illustrates that where the convection controller comprised a single polycarbonate membrane 10 micrometers thick with 0.1 micrometer vertical through-holes there was weak flow through the contact region due to unequal pressures and diffusion that led to leakage of fluorescent calcium(II)/fluo-3 complex into the fluid path. However, because the signal is stronger at the contact region, convection through the membrane is demonstrated to be much lower than diffusion (otherwise convection would have flushed the fluorescent product out of the membrane at the contact region). It takes 0.1 second (s) for a small molecule with the diffusion constant  $D \approx 10^{-5} \text{ cm}^2/\text{s}$  to diffuse 10  $\mu\text{m}$  (the thickness of the membrane). Convection through the membrane was therefore much lower than 0.01 cm/s.

[0115] Leakage was not observed in a system where the convection controller included two membranes with a 200 micrometer thick microwell between them, resulting in a  $8 \times 10^{-12} \text{ m}^3$  space. Because it takes 10 s for a small molecule to diffuse 200  $\mu\text{m}$  (the size of the microwell), the convective flow rate through the microwell may be deduced to be much lower than 0.002 cm/s. The lack of leakage in this system is illustrated in FIG. 9.

[0116] The fluidic systems of both FIGS. 8 and 9 were designed to balance fluid pressure, and thus to prevent convection, across each contact region of the fluid paths. The distances from any contact region to the inlet was designed to be equal in the two layers of fluid paths, as was the distance from the contact region to the outlets. Assuming that all fluid paths were of uniform dimensions, and the pressure was equal at all inlets and at all outlets, this design ensured that there was no pressure differential across the contact regions. However, variations in the pressures, and imperfections in the dimensions of the fluid paths, as well as diffusion of calcium ions through the membrane, were sufficient to generate observable flow through the convection controller.

[0117] This experiment demonstrates that specific contact regions were addressable in fluidic systems having convection controllers comprising both a single membrane and a pair of membranes separated by a space.

### Example 2

[0118] A fluidic system with a convection controller including a space in the form of a microwell was used to demonstrate that detection of bacteria by agglutination of test beads was possible, as illustrated in FIG. 10. The convection controllers included two polycarbonate membranes 52 with approximately 1.0- $\mu\text{m}$  pores separating a microwell from two upper fluid paths 20, containing Staphyloslide™ Latex beads (VWR Pittsburgh, Pa.), and a bottom fluid path 30, containing *Staphylococcus aureus*. The membranes were permeable to both beads and bacteria. The upper fluid paths were filled with two types of blue Staphyloslide™ Latex beads. The first fluid path was filled with a suspension of test beads coated with human fibrinogen and immunoglobulin G (IgG) on their surfaces.

[0119] The second fluid path was filled with a suspension of control beads without fibrinogen or IgG on their surfaces. The bottom fluid path was filled with a suspension of *Staphylococcus aureus* (ca.  $10^9$  bacteria/mL). The pressure was made slightly higher in the fluid path with bacteria relative to the fluid paths with beads to maintain weak flow of bacteria into the fluid paths containing the beads. The fluid flow was maintained by gravity, and the pressure in the fluid paths was regulated by controlling the height of fluid in reservoirs connected to the inlets. When bacteria came into contact with the test beads, protein A on the surface of the bacterial cell wall bound to the Fc region of IgG on multiple beads, and the beads agglutinated. Agglutination was visible only at the contact region 54 where the test solution and the bacteria came into contact. Agglutination occurred on the surface of the membrane in the fluid path containing the beads.

[0120] This experiment demonstrates that it is possible to detect the presence of bacteria by the agglutination of test beads and that embodiments of the present invention are useful as biological assays.

### Example 3

[0121] To demonstrate the use of a system that incorporated a functional gel, a combination of colorimetric and fluorometric assays in a 5x5 fluid path array were used, as illustrated in FIGS. 12-15. The substrates were first immobilized in agarose gels 34 in one set of parallel fluid paths, then the solutions of enzymes were injected into the other set of fluid paths. The agarose did not flow and, therefore, balancing of pressures within the fluid paths was not required. In this experiment, the enzymes diffused from their respective fluid paths, through a membrane convection controller, as described in Example 1, and into the fluid paths that contained the gels with the substrates. Chromogenic substrates 5-bromo-4-chloro-3-indolyl galactoside (X-Gal) and a combination nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (NBT/BCIP) (all from Molecular Probes, Eugene, Oreg.) were used to detect the activities of  $\beta$ -galactosidase ( $\beta$ -gal) and alkaline phosphatase (AP), respectively (both from Sigma-Aldrich, St. Louis, Mo.). Cleavage of these substrates generated a dark blue precipitate localized at the contact region of the fluid paths.