

[0146] Many other detection techniques useful in a microfluidics environment are known to those of skill in the art. Examples include capacitive techniques, electrochemical techniques, mass detection techniques, and the like.

EXAMPLES

Example 1—Calculating a Balance of Forces on Magnetic Particles in the Device

[0147] To quantify the magnetic field gradient, Mathematica (Wolfram Research, IL) was utilized to obtain the magnetic field distribution around nickel stripes using an integral formula:

$$B(\vec{r}) = -\frac{\mu_0}{4\pi} \int \frac{\vec{M}(\vec{r}') \cdot \vec{n}}{|\vec{r} - \vec{r}'_0|^3} (\vec{r} - \vec{r}'_0) dA$$

[0148] where $\vec{M}(\vec{r})$ is the magnetization of the nickel strip, and \vec{n} is the unit normal vector on the surface. In our device, the width and thickness of each stripe was 20 μm and 0.2 μm , respectively, with the gap between adjacent stripes of 20 μm . It was assumed that the external magnet (NdFeB) magnetized the nickel stripes to saturation, at the internal magnetization of 6,000 Gauss for nickel, along the horizontal direction. Although the magnetic flux density from the MFGs is not as strong compared to the surface of the external magnet, the gradient of the magnetic field is very large within a few microns of the line edges (see FIG. 2). As a result, the MFGs allow precise shaping of the field distribution in a reproducible manner inside microfluidic channels. Once the B-field gradient is established, the physical separation between the labeled and unlabeled bacterial cells (or other sample component) occurs through the balance between hydrodynamic and magnetophoretic forces. The simulation results indicated a magnetic field gradient >5000 T/m within 1 μm from the edge of the MFGs.

Example 2—Application of a CMACS Device to Screen a Molecular Library

[0149] In this example, a CMACS device was employed to perform magnetophoretic screening of a molecular library in a microfluidic device. Specifically, a 10^8 -member peptide library was screened to identify a consensus sequence of amino acids with affinity towards the target protein (α -FLAG M2 monoclonal antibody).

[0150] The bacterial strains used in this work displayed peptides as insertional fusions into the second extracellular loop of outer membrane protein OmpX of *E. coli*. Streptavidin-coated superparamagnetic microbeads were purchased from Dynal Biotech (M280, Carlsbad, Calif.). Streptavidin R-phycoerythrin was obtained from Molecular Probes (Carlsbad, Calif.), and the biotinylated anti-FLAG M2 antibody was obtained from Sigma.

[0151] Micro-magnetic field gradient generators (MFG) were fabricated by electron-beam evaporation of 200-nm nickel on borosilicate glass wafers after an optical lithography and a lift-off process. This involved a blanket deposition a photoresist on the glass wafer, followed by optical exposure to the MFG pattern, development of the resist, and deposition of the nickel by evaporation from a nickel target. Microfluidic vias of diameter approximately a few hundred micrometers

were drilled into the glass substrates using a computer-controlled CNC mill (Flashcut CNC, Menlo Park, Calif.). The negative-tone master mold of the microfluidic channels was fabricated on a 4-inch silicon wafer using a deep reactive-ion-etcher (SLR-770, Plasmatherm, St. Petersburg, Fla.), which produced 50 μm deep channels. Subsequently, the PDMS replicas of the silicon master mold were fabricated by applying a precursor (Sylgard 184, Dow-Corning Inc., Midland, Mich.; 10 part base resin: 1 part curing agent) to the silicon master, followed by curing at 70° C. for 3 hours. After dicing the borosilicate glass wafers, the MFC substrate and the PDMS channel were cleaned in acetone and oxidized in a UV-ozone chamber prior to their covalent bonding in a flip-chip aligner (Research Devices M8A, Piscataway, N.J.). Microfluidic inlets and outlets were attached to the device with epoxy. Each CMACS device was only used once and discarded after each usage to eliminate contamination.

[0152] A two stage CMACS device was utilized to screen a peptide library displayed on the surface of *E. coli* to isolate the consensus sequence of amino acids that exhibit high affinity binding towards the target molecule (anti-FLAG BioM2 mAb, Sigma). Since the target antibody is biotinylated it binds strongly to streptavidin, and as such, the *E. coli* clones displaying peptides with affinity for the antibody binding pocket (or affinity for streptavidin directly) become bound to the streptavidin-coated magnetic beads.

[0153] In this example, the bead-captured clones were sorted from the non-binding cells using a two-stage CMACS device **805** as depicted in FIG. 8. A bacterial peptide library **807**, antibodies **809**, and superparamagnetic beads **811** were all provided to a sample inlet port **813** in device **805**. Buffer was provided through an inlet port **815**. A waste stream containing non-binding library members **817** exited via a port **819**. Target cells **821** labeled with the magnetic beads were provided via a collection stream from a port **823**.

[0154] Prior to delivering the library to CMACS device **805** for positive screening, the initial peptide library (500 μL of cells at 2×10^9 cells/mL) was de-enriched for streptavidin (SA) binders by incubating with SA-coated magnetic beads (4×10^7 beads/mL) and negative CMACS selection. Next, the remaining cells were incubated with biotinylated target protein (α -FLAG M2 monoclonal antibody) at a final concentration of 5 nM at 4 C for 1 hour, washed twice in PBS, and incubated with magnetic beads at a concentration of 4×10^7 beads/mL for positive CMACS screening.

[0155] To reduce settling of the beads during CMACS screening, the density of the solution was adjusted to that of polystyrene beads (1.06 g/ml) by adding glycerol at a final concentration of 20% (vol/vol). Microfluidic interconnections were provided by Tygon tubing (inner diameter of 0.02 inches, Fisher Scientific), which was attached to the inlets and outlets of the device. A pair of NdFeB magnets (5 mm in diameter, K&J magnetics, Jamison, Pa.) was attached to the top and bottom side of the device to externally magnetize the MFGs. The locations of the paired magnets with respect to MFGs were adjusted under a microscope to optimize the sorting performance.

[0156] A dual-track programmable syringe pump setup (Harvard Apparatus Ph.D. 2000, Holliston, Mass.) delivered both the cell mixture and the sorting buffer into the device at a constant flow rate. The device and the tubing were filled with sorting buffer (1xPBS/20% glycerol/1% BSA) to drive out air bubbles before pumping. The volumetric sample flow rate during sorting was 500-1000 μL /hour, and the buffer flow