

The selected cells from the first stage (location G) are then passed through the second sorting stage (station 505) having MFGs 521a and 521b, thereby further purging non-target components to provide a relatively high purity solution of target to a collection channel 523 (location C).

[0103] In one embodiment, the CMACS device of FIG. 5A is designed to prevent any backflow of fluid under the operating condition by ensuring that $P_{A,B} > P_E$, $P_F > P_G$, and $P_H > P_I$. Due to the fact that the device operates under laminar flow conditions—a regime well described by classical Poiseuille equations—the pressure drop Δp in the channel is related to the volumetric flow rate Q where

$$Q = \frac{wh^3 \Delta p}{12\eta L} [1 - O(h/w)] \approx \frac{wh^3 \Delta p}{12\eta L}$$

and the flow resistance is given by $R = \Delta p / Q = 12 \eta L / wh^3$ where w , h and L are the width, height and length of the channel, respectively. As a first order approximation, a fluidic circuit model was created wherein each channel is represented by a resistor with unitless resistance $R^* = L/w$. Subsequently the volumetric flow rate in each channel and pressure at each node may be solved using a commercial circuit simulator (e.g., PSpice, Cadence Design Systems, San Jose, Calif.) and the model may be refined by solving the Navier-Stokes equations for incompressible fluid with no-slip boundary conditions (e.g., FemLab, Comsol Ltd, Los Angeles, Calif.). The models may be used to simulate the pressure distribution in the microchannels to ensure that the particles will follow the streamline into the waste channel in the absence of magnetophoretic forces.

[0104] Fractionation

[0105] Fractionating cells based on their differences in surface protein expression level allows quantitative and/or qualitative characterization of cells based on surface protein expression level. In one application, one can detect and separate tumor cells from a heterogeneous cell population using certain defined prognostic markers for cancer. Fractionation may be used more generally to sort any sample based on degree of magnetization of various sample components. The central concept is that sorting does not have to be a “binary” undertaking. Rather, it can be a ternary or higher degree separation process.

[0106] Fractionating using magnetophoretic techniques can be understood in terms of the following cell-based example. The resultant magnetic force \vec{F}_M on a cell depends on the expression level of target cells. This is because cells with more target expressed generally have greater numbers of magnetic particles coupled to them. The direction of the cells in flow is determined by a combination of the resultant magnetic force and the hydrodynamic viscous drag \vec{F}_{VD} . Using the design of an MFG, one can determine the deflection and average flow path of cells having differing levels of target expression. This allows the device design to precisely fractionate the cells by delivering different cells to multiple outlets.

[0107] A fractionating sorting station will employ one or more MFGs to generate the magnetic force, and multiple outlets to collect fractionated samples. FIG. 5B shows a fractionating sorting station 531. It includes, at the lower left side of the diagram, an inlet channel 533 for receiving magneti-

cally tagged cells 535 with different levels of expression. The varying levels of expression are indicated by different numbers of coupled antibody-magnetic particle conjugates 537. Sorting station 531 includes multiple strip-type MFGs 539, each having a different angle with respect to the direction of flow. In the depicted example, MFGs located upstream have steeper angles than MFGs located downstream. As shown, the MFGs possess a steady progression of decreasing angle in moving from the most upstream position to the most downstream position. A collection of parallel outlet channels 541 is positioned at the downstream side of fractionating sorting station 531. Cells deflected the most by the MFGs exit the “top” outlet channel 541a. Cells deflected the least exit the “bottom” outlet channel 541c, and cells deflected by an intermediate amount exit the “middle” outlet channel 541b. As can be seen in the figure, cells with a high level of expression can be collected from outlet channel 541a, cells with an intermediate level of expression can be collected from outlet channel 541b, and cells with a low level of expression can be collected from outlet channel 541c.

[0108] To verify the design of FIG. 5B, a numerical simulation was performed using COMSOL Multiphysics. In the Magnetostatics Model, nickel strips with 0.2 μm thickness, 40 μm width, and 40 μm separation distance are placed at the bottom of the device, and the magnetic field distribution and magnetic force are calculated. The simulation showed that magnetic field distribution is strongest at the edges of nickel strips.

[0109] A prototype fractionating sorting station was produced in which the MFGs were fabricated by electrom-beam evaporation of 0.2- μm nickel thin film on borosilicate glass wafers after lithography and a lift-off process. Microfluidic ports were drilled into the glass substrates using a computer-controlled milling machine. Microfluidic channels were fabricated on a silicon wafer using a depp reactive-ion-etcher, which produced 35 μm deep channels. Polydimethylsiloxane (PDMS) replicas of the silicon master mold were fabricated by applying a precursor to the silicon master, followed by curing at 70° C. for 3 hours.

[0110] To fractionate cell by surface protein expression level, a sequence of steps may be performed as shown in FIG. 5C. First, cells are labeled with magnetic beads (block 551). Second, the labeled cells enter a fractionation sorting station where they are sorted/fractionated (block 553). Next, the sorted cells are labeled with a secondary antibody-fluorochrome conjugate (block 555). Finally, the cells are analyzed using flow cytometry for quantitative data (block 557). Using the sorting station, the cells are fractionated based on their expression level and collected at multiple outlets.

[0111] In another example, cells or other species of interest may have two or more different types of markers (e.g., two different surface proteins or an antigen having two discrete epitopes). A sample suspected of harboring such species is treated with multiple different types of magnetic particles, one having an affinity for a first marker and another have an affinity for a second marker. Species having no markers will not be labeled. Species having only one marker will be labeled, but with only one type magnetic particle. Species having two markers will be labeled with two or more different types of magnetic particles. In a sorting station, the species having two more distinct markers will deflect to a greater degree than species having only one marker. Thus, a fractionating sorting station will be able to separately collect species with no markers, species with only marker, and species hav-