

3-color marking schemes can be employed. For example, a first fluorescent marker can be used that binds specifically to a first cell type, a second fluorescent marker can be used that binds specifically to a second cell type, and a third fluorescent marker that labels all cell types (e.g., nuclear stain 4',6-diamidino-2-phenylindole (DAPI)) can be used to help distinguish cells from fluorescent debris. Use of these three fluorescent markers simultaneously will provide improved specificity for quantifying the first and second cell types.

[0040] This approach is suitable for clinical evaluation using patient blood samples. For example, cancer can be clinically evaluated in this manner by magnetically sifting and quantifying circulating tumor cells in a patient blood sample. Similarly, HIV or other disease states can be clinically evaluated in this manner by magnetically sifting and quantifying lymphocytes in a patient blood sample.

EXPERIMENTS

[0041] Several experiments have been performed using magnetic sifters for capturing and releasing biological cells. The magnetic sifter configuration in these experiments is similar to the above-described magnetic sifters, except that the sifters used in these experiments did not have a higher pore concentration at the sifter edge than at the sifter center. Instead, the pore density was the same at the center and the edge of the sifter. Despite this difference, the encouraging results of the following experiments are expected to also apply to sifters having higher pore concentration at the edge than at the center. These experiments generally relate to analysis of blood samples, where it is important to distinguish abnormal cells (e.g., circulating tumor cells (CTCs)) from normal cells such as red blood cells (RBCs) and white blood cells (WBCs).

Experiment 1

H1650 Separations from Whole Blood, Pretagged

[0042] There are several key advantages to working with whole blood, as opposed to a lysed blood sample with the RBCs removed. First, the time between blood sample acquisition and processing with the sifter is reduced. Although lysing the blood is fairly straightforward, the 15 minutes of incubation with the lysis buffer and 2 washing steps requiring 10 minutes of centrifuging each, typically results in an additional hour of sample preparation prior to the capture step. Second, RBC lysis can result in nucleated cell loss (i.e. non-RBCs), either due to non-specific lysis or loss due to sample handling during the washing steps. Third, the activation of WBC cells may be reduced if the WBC are left in their native environment prior to processing with the sifter, leading to less adhesion to the sifter surface and higher and more accurate purity measurements.

[0043] Differences in WBC adhesion between lysed and whole blood samples were observed by passing whole blood through a sifter and imaging the sifter before and after washing steps. Following passage of 200 μ L of whole blood, the sifter surface appeared clean in reflectance mode. When imaging transmitted light, the pores appeared filled with a residual solution of RBCs, as indicated by the red color of pores. Following a rinsing step of flowing 200 μ L of phosphate buffered saline (PBS) through the sifter at 2 ml/hr, the sifter appeared clean in both reflectance and transmittance mode. This differs significantly from the case of passing lysed blood through the sifter, where the sifter surface is decorated

with remaining white blood cells even after repeated washing with pluronic solution and PBS buffer.

[0044] In addition to working with whole blood, a method for spiking low, clinically relevant concentrations of H1650 into blood samples was developed. Here, H1650 refers to a non-small-cell lung cancer (NSCLC) cells (NCI-H1650). Previous experiments relied on serial dilutions and verification of concentration with the hemocytometer to prepare H1650 samples with known concentrations. The hemocytometer is only accurate for cell concentrations greater than a few thousand cells per ml, since the volume counted with the hemocytometer is contained within a square region 9 mm² in area and a height of 100 μ m. For a sample with an actual concentration of 1000 cells/ml, only one cell is expected to be found in this entire volume (9×10^{-4} ml). To prepare low concentration samples, a method was developed in which a small (~ 5 μ L) droplet of H1650 cells was pipetted onto the inside of a centrifuge cap. The H1650 were then allowed to settle to the surface, which took approximately 30 seconds for magnetically labeled cells using a magnetic field to accelerate sedimentation, and a few minutes for non-magnetically labeled cells by gravity. The droplet was then imaged with fluorescence microscopy, and the exact number of H1650 cells was counted. Samples ranging from 1-200 cells are readily prepared. Beyond that, counting becomes tedious without the aid of counting grids on the inside of the centrifuge tube. Whole, untreated blood was then added to the centrifuge tube, the cap was closed, and the H1650 and blood sample were mixed by gentle inversion.

[0045] In these experiments, H1650 were fluorescently and magnetically labeled prior to spiking into whole blood. Quantification of the H1650 was performed by counting captured cells on the sifter surface, since the background concentration of RBCs prohibited the use of the hemocytometer or flow cytometry. In these experiments, 0.5 mL of whole, untreated blood samples containing $\sim 100 \pm 20$ H1650 cells/ml were processed with sifters containing 40 μ m pores. In this work, it was important to ensure that magnetic field gradients at locations other than the sifter pores were sufficiently small to prevent capture at locations other than the sifter pores.

[0046] Separations were performed on 0.5 mL whole blood samples containing pre-fluorescently and pre-magnetically labeled H1650 at concentrations of 50-100 H1650/mL. Following separation, the sifters were removed from the flow apparatus and imaged by fluorescent microscopy, and the number of H1650 cells on the sifter surface was compared with the known number of H1650 spiked into the blood sample. H1650 were then eluted by reinserting the sifter into the flow apparatus and flushing with 500 μ L of PBS buffer in the absence of an applied magnetic field. The sifter was again reimaged to confirm the detachment of captured H1650.

[0047] H1650 cells labeled with Green CellTracker™ dye were clearly observed in the sifter pores. The cells were counted manually, requiring approximately one minute to scan over the entire sifter surface.

[0048] Capture efficiency as a function of sample flow rate was measured by varying the inlet flow velocity during the capture step from 1-5 ml/hr, and the results are shown in FIG. 8. There is a clearly linear relationship between the flow velocity and the capture yield of the separation process. The capture efficiency decreases with increasing flow rate, due to the increased linear velocity of the cells through the pores, resulting in a larger drag force. Capture yield did not increase significantly at flow rates slower than 1 ml/hr, and in fact