

particle B are then subjected to the significantly higher magnetic field gradients of the second sifter **606**, and are therefore captured on the second sifter. The sifter holder can allow the two sifters to be detached and washed separately, yielding two eluted solutions containing enriched cells labeled with either A particles or B particles.

**[0031]** In the case of cell separation, one issue is variation in surface antigen expression which could lead to variations in magnetic particle loading. For example, EpCAM expression can vary between approximately a few thousand to several hundred thousand antigens per cell across tumor types. If cells are saturated by magnetic particles, a cell labeled with 2000 of particle A will be harder to capture than a cell labeled with 500,000 of particle B. This may be addressed by functionalizing particles appropriately based on known or suspected levels of antigen expression (i.e. labeling cells with low antigen expression with high-saturation field magnetic particles), or varying the amounts of each magnetic nanoparticle type added. It may also be helpful to further tune the saturation fields of the magnetic particles as well as the applied external fields experienced by each sifter to generate maximum differences in particle moment. The difference in average magnetic field gradient can also be increased by a factor of 100 or more, by tuning both the magnetization of the sifter film, film thickness, and pore width.

**[0032]** This approach could enable multi-target CTC selection. In multi-target CTC separation, tumor cells are targeted for multiple antigens, such as EpCAM, cytokeratin, Her-2/neu, and/or Mucin1. One approach would be to employ the two-plex separation using magnetic particles with different magnetic saturation fields described above. Here, particles which are difficult to capture (e.g. Particle B) are coated with an antibody, such as anti-Mucin1, anti-Her-2/neu, or anti-cytokeratin, specific to an antigen that has generally low expression levels by CTCs. Particle A, which are easy to capture, are coated with an antibody, such as anti-EpCAM, which generally has high expression by CTCs. Cells with high EpCAM expression, with varying expressions of the alternative target antigen, will have large amounts of Particle A loading, and will thus be captured on the first of two sifters in series. Cells with weak or absent EpCAM expression, but expression of the alternative target antigens, and hence particle B, will be captured on the second sifter with higher average magnetic field gradients.

**[0033]** In order to facilitate release of captured cells, a non-magnetic spacer layer can be incorporated into the sifter. FIGS. **7a-b** show an example. Here, FIG. **7a** shows a close up side view of the soft magnetic layer (**702** and **704**) on either side of a sifter pore. FIG. **7b** shows an alternate configuration, where two soft magnetic layers (**712a**, **714a**) and (**712b**, **714b**) on either side of a sifter pore are vertically separated by a non-magnetic spacer layer (**712c**, **714c**). The arrangement of FIG. **7b** facilitates the formation of oppositely directed magnetization in the two soft magnetic layers in a demagnetized state resulting in flux closure and reduced leakage flux at the magnetic film's edges when unmagnetized. This reduction in leakage flux can prevent stray magnetic field gradients from trapping magnetically-tagged capture probes or cells at the sifter surface when the external applied magnetic field is removed. This effect is schematically shown with field distributions **716** and **706**.

**[0034]** Capture probe and cell release can also be expedited by the use of agitation of the sifter surface through ultrasonic vibration or other mechanical stirring. This can be accom-

plished by inserting the sifter into a small container containing buffer solution, and placing the container into an ultrasonic bath for 1-5 minutes. Also, mechanical stirring can be incorporated directly into the sifter device by bonding piezoelectric plates onto the top and bottom surfaces of the magnetic sifter. The piezoelectric plates can be actuated by applying an alternating current voltage. With proper amplitude and frequency of the applied signal, mechanical vibration of the plates can be transferred to the magnetic sifter substrate and membrane, resulting in release of any attached capture probes and cells.

**[0035]** Capture probe and cell release can also be expedited by switching the direction of the applied field while flushing the sifter with buffer solution. Switching the direction of the applied field 90 degrees changes the orientation of the fringing field across a pore, thus switching the locations of highest field gradients to the adjacent pore edges. Capture probes and magnetically tagged cells will then follow the magnetic field gradients. If fluid is flushed through the sifter at sufficiently high velocity, capture probes and cells can be flushed through the pores during the switch in applied magnetic field direction.

**[0036]** Capture probe and cell release can also be improved by demagnetizing the sifter using an externally applied AC field applied by an electromagnetic source, such as a solenoid magnet with an alternating current through it. Although a magnetically soft material is chosen for the sifter surface, some magnetic remanence results in non-zero magnetization at zero field. The remanent magnetization can result in field gradients capable of capturing, or holding onto, magnetically tagged capture probes and cells. The effect of this remanence can be reduced or removed by sweeping a small applied field near zero field strength during release.

**[0037]** As indicated above, anti-fouling layers can be employed to improve sifter performance. The sifter surface(s) can be treated with an anti-adhesion layer, such as Tridecafluoro 1,1,2,2-tetrahydrooctyl-trichlorosilane (FOTS). FOTS is a commonly employed antiadhesion layer for nanoimprint lithography stamps due to its low-energy surface. The sifter surface can also be treated with antifouling layers such as poly (ethylene glycol), phosphotidylcholine, or carboxybetaine based polymers, or Pluronic surfactants. These layers are commonly employed on the surfaces of biosensors and medical implants to prevent non-specific protein adsorption and cell attachment to the surface. To allow for chemical coupling of an antiadhesion or antifouling layer, the magnetic film on the sifter surface can be coated with a chemically convenient layer, such as a thin layer of gold or silicon dioxide, for performing surface modification. To attach a surfactant such as Pluronic, the sifter can simply be incubated in an aqueous pluronic solution prior to use.

**[0038]** In preferred embodiments of the invention, cell capture at a magnetic sifter is combined with quantification of the captured cells. Tagging of captured cells with fluorescent, colorimetric, radiological, plasmonic, chemiluminescent, and/or Raman tags can be employed in connection with this embodiment to provide quantitative results for captured cells. Tagged cells can be counted and distinguished based on observed color, radiation, plasmonic emission, Raman scattering, etc. Multiplex cell quantification by utilizing fluorophore-antibody conjugates with distinct emission spectra can also be performed in connection with this embodiment.

**[0039]** For example, one or more fluorescent markers can be employed that selectively bind to specific cell types.