

[0199] Magnetic labels, tags, markers 264, or a reagent to render particles of interest magnetic, can be introduced into the system 250 and mixed with the sample 260 before its introduction into the channels 252 and/or after the particles 262 have been focused and before the particles 262 enter the analysis region. As will be appreciated by those skilled in the art, any and all conventional MACS methods and techniques can be used with the system 250 of the invention as noted above and as further described in connection with the illustrated embodiment. For example, the particles 262 can be cells incubated with magnetic markers 264 in the form of magnetic beads coated with antibodies against a particular surface antigen of the cell. This causes the cells expressing this antigen to attach to the magnetic beads. In other embodiments, certain cells, such as nucleated red blood cells, could be rendered magnetic by altering the oxidation state of the cytoplasmic Hemoglobin with a reducing agent. In addition, a cell can be sorted based on intrinsic magnetic properties. Cells having internalized ferrous containing particles, for example cells with saturated transferrin receptors, could be separated from other cells based on their higher magnetic moment. In still another example, macrophages with ingested red blood cells can be separated from other macrophages and white blood cells by virtue of the magnetic properties of the Hemoglobin in the ingested red blood cell. Regardless of the type of magnetic marker 264, the magnetic property used to identify a particle, or of where the magnetic markers 264 are introduced, however, the markers 264 will ultimately be attached to the target particles 262 of a predetermined type within the focused stream of particles as they enter the analysis region.

[0200] As shown in FIG. 15A, the channels 252 can be configured such that the focused stream of marked particles 262 and unmarked particles 266 leaving the asymmetrically shaped portion of the channels 252 will naturally flow into the second output channel 256. A magnetic biasing element, such as a magnetic field gradient and/or a magnetic field 268, can be applied across the analysis region 254 near the channel branch point such that magnetically marked particles 262 will be deflected a distance away from the focused stream in response to the magnetic field 268 and will enter the first channel output 258 instead of the second channel output 256. The tightly focused stream(s) of particles provided by any channel geometry, for example, straight, symmetric, and/or the asymmetric curvature of the channels 252 allows such a configuration as only a relatively small amount of deflection by the magnetic field 268 is required to direct the marked particles 262 into the first channel output 258. This allows systems of the invention to operate with lower noise, better accuracy, and with higher throughput as the smaller deflections required with focused particle streams allow for higher flow conditions. In one embodiment, separating cells with a weak magnetic moment is allowable because of the bare minimum deflection needed to deflect flow trajectory in a tightly focused stream. So directed, the marked and unmarked particles 262, 266 can be identified, sorted, counted, collected, and otherwise analyzed further as needed. A person skilled in the art will appreciate that any channel geometry can be used in this configuration, and any number of channels and channel branch points can be included to separate particle streams and perform sorting in parallel configurations.

[0201] Particle stream precision is essential for magnetic sorting applications of the sort described above, as increased

precision of initial particle position leads to reduced false positives after magnetic deflection and increased throughput. The lowest inertial force necessary can be calculated and used to produce single ordered streams of particles with variation in center position < 100 nm. Weaker inertial focusing equilibrium positions can facilitate magnetic deflection of labeled particles. This value can be measured by analyzing images from high-speed camera data and channel length can be adjusted as needed to compensate for lower inertial forces. In one embodiment, a design that initially produces strong equilibrium focusing forces and then changes gradually to the smaller magnitude forces by increasing the channel width gradually can reduce the effective channel length.

[0202] FIG. 15B illustrates another embodiment of a particle sorting configuration for the systems described herein. In general, an active sorting mechanism is provided in the form of a fluorescence-activated particle sorting configuration 280. Similar to FIG. 15A, a microfabricated chip can be provided having one or more asymmetric channels 282 formed therein. The chip can include an analysis or detection region 284 in proximity to an output region of each main channel 282. Each channel 282 can include a fork or channel branch point that transitions the main channel 282 into first and second output channels 288, 286.

[0203] A sample 290 can be prepared for introduction into the system 280 by tagging particles 292 of a predetermined type with an optically sensitive tag that is detectable in response to a light source 294, as is done in conventional FACS systems. In general, a tag will associate with a particle or with a characteristic of the particle, for example with a marker associated with the particle. The tag can be a dye, fluorescent, ultraviolet, or chemiluminescent agent, chromophore, and/or radio-label, any of which can be detected with or without a stimulatory event to enable fluorescence. In some embodiments, certain particles may be naturally optically detectable without requiring a tag and in other embodiments, a tagged particle may be optically detectable without the use of a light source to stimulate a scatter response. The optically sensitive tag can be prepared with the sample 290 before introduction into the system 280, or the tag can be introduced some time after the sample 290 is introduced into the channels 282 and before the particles 292 reach the detection region 284 of the chip. A person skilled in the art will appreciate that any and all conventional FACS methods and techniques can be used with the system of the invention as noted above and as further described in connection with the illustrated embodiment. Once the sample 290 is introduced into the asymmetric channels 282, whether or not particles have been optically tagged, particles of a predetermined size can be focused into a single, localized and ordered stream of particles which will naturally flow into the second output channel 286 upon reaching the branch point.

[0204] An optical assembly 296 can be positioned in proximity to the detection region 284 of the chip and can generally include the light source 294, filters 298, optics 300, and a detector 302 positioned around the channel output, a distance before the branch point, for detecting optically sensitive tagged particles 292. The light source 294 can illuminate each individual particle in the stream of focused and ordered particles as they pass through the detection region 284 of the channel 280. As the particle is illuminated, the detector 302 can detect light scattered by the particle 292 and/or the tag associated with the particle 292, thereby identifying the particle as a predetermined type. Based on certain preset param-