

[0112] FIG. 39 is side view illustrating focusing behavior for exemplary channels of various widths within an exemplary focusing system;

[0113] FIG. 40 is a side view illustrating focusing behavior for exemplary channels of various widths within an exemplary focusing system;

[0114] FIG. 41 is a representation of focusing behavior for exemplary channels of various widths within an exemplary focusing system;

[0115] FIG. 42 is a representation of focusing behavior for exemplary channels of various widths within an exemplary focusing system;

[0116] FIG. 43 is a side view illustrating  $R_e$  dependent focusing for separation within an exemplary focusing system;

[0117] FIG. 44 is a top view illustrating the focusing of blood cells within an exemplary focusing system;

[0118] FIG. 45A is a side view of streak images of cells focusing for various  $R_e$ ; and

[0119] FIG. 45B is a representation of intensity cross-sections of cultured cells at various turns within an asymmetric channel.

#### DETAILED DESCRIPTION OF THE INVENTION

[0120] Certain exemplary embodiments will now be described to provide an overall understanding of the principles of the structure, function, manufacture, and use of the devices and methods disclosed herein. One or more examples of these embodiments are illustrated in the accompanying drawings. Those skilled in the art will understand that the devices and methods specifically described herein and illustrated in the accompanying drawings are non-limiting exemplary embodiments and that the scope of the present invention is defined solely by the claims. The features illustrated or described in connection with one exemplary embodiment may be combined with the features of other embodiments. Such modifications and variations are intended to be included within the scope of the present invention.

[0121] The invention relates to the fields of microfluidics and analyte separation. Various embodiments of the invention described below are based upon the notion that laminar flow of a fluid through microfluidic channels can result in the continuous and accurate self-ordering of particles suspended within the fluid. A variety of specific channel geometries are illustrated that take advantage of this effect to create continuous streams of ordered particles constrained in three spatial dimensions. Particles order laterally within the x-y plane (or cross-sectional plane) of the channel and can also order longitudinally along the direction of flow. An additional dimension of rotational ordering can occur for asymmetrically shaped particles.

[0122] In general, the invention features methods and devices that separate and focus streams of particles to equilibrium positions within a channel flow field based, at least in part, on inertial lift forces. In rectangular channels, this can lead, for example, to four streams of focused particles spaced a distance apart from a center of each of the four rectangular faces. For certain rectangular geometries, this four-fold symmetry can be reduced to a two-fold symmetry, with streams of particles spaced apart from each of two opposed faces of the channel.

[0123] The invention can also include methods and structures that decrease the symmetry of the system using a variety of forces, including, for example, electromagnetic, magnetic, centrifugal, hydrodynamic, thermal, sonic, optical, and/or

dielectrophoretic forces or combinations thereof. Although any force may be used to bias a particular potential minimum within the channel flow field, utilizing centrifugal forces with a curved channel structure has certain advantages. In this case, the force will increase with the square of the flow rate based only on a minor geometric change with no additional mechanical or electrical parts required. For example, the symmetry may be reduced by using inertial forces inherent in the flow through an S-shaped rectangular channel to result in a two-fold symmetry (down from four-fold) with a majority of the particles aligned with the flow in a periodic manner not corresponding to the period of the underlying channel. The geometry of the channel may also be used to change symmetry either by changing the radius of curvature or the width of the channel in a periodic manner (the channels thus curving asymmetrically) to create a single focused particle stream.

[0124] Embodiments of the invention may be advantageous in that they may employ a single stream input and require no moving parts or separate pressure control. Embodiments of the invention can also provide methods that are low cost and employ devices requiring simple, fault tolerant manufacture that may also be miniaturized. Embodiments of the invention may be operated continuously and at high volumetric flow rates with cascading outputs. The invention also requires no interactions with mechanical filters or obstacles and requires very low maintenance.

[0125] The principles relating to suspended particles are also applicable to a variety of biological materials, particularly to cells. The ability to rapidly analyze and extract information from whole blood, for example, and its component cells is of great importance for medical diagnostics and applications in basic science. Blood cells themselves contain an abundance of information relevant to disease, infection, malignancy, or allergy diagnosis. Systems and principles are presented herein related to inertial microfluidic technology as a solution for high-throughput and precise microscale control of cell and particle motion. Systems of the invention are ideally suited for applications in blood cell subtype or rare cell enumeration, sorting, and analysis. Identification and analysis of rare cells, in particular, requires large sample sizes and high-throughput. Rapid and simple microfluidic techniques presented herein can surpass the limitations of conventional sorting techniques that limit the size of samples that can be analyzed. The ability to sort, order, enumerate, and analyze particles continuously, differentially, and at high rates in a simple channel will be broadly applicable in a range of applications in continuous bio-particle separation, high-throughput cytometry, and large scale filtration systems.

[0126] While there are many configurations possible in a system for the self-ordering of particles within microfluidic channels, one embodiment of such a system 10 is illustrated in FIGS. 1A and 1B. As shown, the system 10 generally includes an inlet 12 that can be configured for introducing a sample 24 having suspended particles 22 into the system. A microfabricated chip 14 can be provided and can have at least one microfluidic channel 16 formed therein for receiving the sample 24 and for ordering and focusing the particles 22 suspended in the sample 24, as shown in FIG. 1B. A plurality of such channels 16 situated in parallel are formed in the exemplary chip 14 illustrated in FIG. 1A.

[0127] The plurality of channels 16 formed in the chip 14 can have numerous configurations which will be described in detail below. In general, however, the plurality of channels 16 can have a specified geometry configured to separate, order,