

move toward the channel outer edge, and recirculate along the top and bottom of the channel until they reach an equilibrium position. In other words, the lift forces contribute to focusing the particles in two positions, above and below the plane of symmetry of the channel, along the height while the dean forces affect the lateral position. In accordance with  $R_p$ , the lateral equilibrium position can be manipulated simply by changing particle diameter ( $a$ ), geometry ( $D_h$ ), and curvature ratio ( $\delta$ ).

**[0167]** In accordance with the above-described theory, which is generally applicable to all channel geometries, various combinations of parameters will result in localization of a flux of particles in a channel with a given geometry. In general, in certain embodiments, the Reynolds number of the flowing sample can be between about 1 and about 250, the Dean number of the flowing sample can be less than about 20, and/or the ratio of particle diameter to hydraulic diameter can be less than about 0.5. Properties more particularly related to certain channel geometries in view of the above described theory will be discussed below.

**[0168]** As previously noted, FIGS. 2A and 2B illustrate one embodiment of a straight channel having a rectangular cross-section showing force vectors acting on particles therein. Referring now to FIGS. 8A-8C, the separation, ordering, and focusing of particles within these exemplary straight channels will be discussed in more detail. In general, at low flow rates, particles flowing within these exemplary channels distribute uniformly across the cross-section of the channel. As  $R_p$  is increased with increasing fluid velocity, patterns of particle segregation in laminar flow become observable that depend significantly on channel scale and symmetry. In general, uniformly distributed particles in rectangular channels migrate across streamlines to four symmetric equilibrium positions **82a**, **82b**, **82c**, **82d** at the centers of the faces of the channel and toward the channel edge of a rectangular channel **80** having an aspect ratio of 1:1, as shown in FIGS. 8A-8C. In the illustrated embodiment, particles 9  $\mu$ m in diameter suspended in water were observed in 50  $\mu$ m-wide square channels, providing a particle diameter to channel diameter of 0.18. An inlet region **84** is shown where the particles are initially uniformly distributed within the fluid but start to focus shortly thereafter to the four channel faces, as shown in FIG. 8B. FIG. 8C illustrates that the degree of focusing increases with  $R_p$  at a given distance along the channel and also increases with the distance traveled along the channel. For  $R_p=2.9$  ( $R_c=90$ ), complete focusing is observed after a distance of  $\sim 1$  cm.

**[0169]** In general, for a given particle size, focusing occurs at a specific distance to the channel wall. The equilibrium position for particles is  $\sim 9$   $\mu$ m from the channel edge for  $R_c=90$  and agrees with theoretical predictions of  $\sim 8$   $\mu$ m in an infinite plane system ( $R_c=100$ ). This distance is also predicted to move closer to the wall for a given particle size as  $R_c$  increases. Focusing occurs at channel faces as opposed to corners despite the symmetric features of corners. Presumably, the dominant wall effect acts from two directions on a particle within a corner, and creates an unstable equilibrium point, as shown in FIGS. 8A and 8B. Inertial lift forces alone allow two-dimensional focusing to four precise positions within the lateral face of a rectangular channel.

**[0170]** Referring now to FIGS. 9A-9D, an alternative rectangular geometry for straight channels is provided. In one embodiment, the rectangular cross-section of a straight channel **90** can be adjusted to produced specific and/or optimized

focusing results. In particular, the aspect ratio of the channel **90** cross-section can be changed from about 1 to 1 to about 2 to 1 as shown in FIG. 9A. In addition, particle diameter to channel diameter ratios greater than 0.3 can be employed. When the aspect ratio and the particle diameter to channel diameter are adjusted in this way, particle focusing and ordering can become much more robust (i.e. less deviation in position). In addition, ordering positions reduce from four in the 1 to 1 rectangular channels described above to two in the optimized channels, as shown in FIG. 9A, and particles in the two ordering sites **94a**, **94b** are observed to interact and order across the channel **90**. Ordering occurs for low to high particle concentrations, where only the particle-particle distance is affected by concentration. Importantly, particles become evenly spaced in the direction of flow even to high particle concentration ( $\sim 50 \times 10^6$ /ml).

**[0171]** The new ordering provides a tighter distribution in particle lateral position in the flow as well as improved particle-particle interactions leading to long regular chains **92** of particles with uniform spacing in the direction of flow, as shown in FIGS. 9B and 9C. Precision ordering of cells and particles of 5-15  $\mu$ m in size can be demonstrated for a variety of particle/cell densities ( $<5\%$ ) at continuous flow, most clearly illustrated in FIG. 9B. As noted above, the geometry change reduces the four ordering positions observed within square or 1 to 1 ratio channels almost entirely to the two ordering positions **94a**, **94b**. Further, particles ordered in positions across the channel **90** also interact to create a uniform fluid buffer between them.

**[0172]** In one exemplary system having a 2:1 rectangular geometry, particles all travel with a speed of 13.2-13.8 cm/s (mean fluid velocity being 11.9 cm/s) and exhibit a center-center spacing of 42-45  $\mu$ m between adjacent particles when they are focused to the same side of the channel **90**, but are separated by only 23-25  $\mu$ m in the direction of flow when the alternating pattern is present. These two patterns can also be found in combination, the particular ratio of one to the other depending most on the local concentration of particles; if the concentration is low, the particle-particle spacing present within the linear array is allowed, as shown in FIG. 9C. As the local concentration increases, however, particles are found more frequently in the interstitial sites on the other side of the channel **90**, as illustrated in FIG. 9B. Equilibrium particle spacing at the end of a 6 cm channel is generally linearly dependent on the particle diameter and channel diameter.

**[0173]** In another embodiment shown in FIG. 9D, the conditions described with respect to FIGS. 9A-C are applied to particles of two different predetermined types. The particles **92** of a first type (illustrated as open circles) can be introduced into the channel through a first input branch (the lower branch in the figure as illustrated), while a second particle type (illustrated as closed circles) can be introduced into the channel through a second separate channel branch, the upper input branch as shown in the figure. As shown, the two types of particles move from separate input branches into a single channel and are ordered and focused into two streams corresponding to two equilibrium positions on opposite sides of the channel. Where the first and second particles are differing cell types, particles having differing chemistries, or some combination thereof, having the particles focused and ordered such that the particles generally alternate between particles of the first type and particles of the second type as