

eral, a maximum particle to volume ratio for a specified particle size and channel geometry can be determined using the formula:

$$\text{MaxVolumeFraction} = \frac{2N\pi a^2}{3hw},$$

**[0135]** where N is the number of focusing positions in a channel, a is the focused particle diameter, h is the channel height, and w is the channel width. Thus, samples can be diluted or concentrated to attain a predetermined ratio before and/or during introduction of the sample into the system. Additionally, certain exemplary systems may require the ratio to be adjusted after the sample is introduced into the channels.

**[0136]** Particle to volume ratios of a sample within the channels described herein can have any value sufficient to enable ordering and focusing of particles. In general, the particle to volume ratio can be less than about 50%. In other embodiments, particle to volume ratios can be less than about 40%, 30%, 20%, 10%, 8%, or 6%. More particularly, in some embodiments, particle to volume ratios can be in a range of about 0.001% to about 5%, and can preferably be in a range of about 0.01% to about 4%. More preferably the ratio can be in the range of about 0.1% to about 3%, and most preferably in the range of about 0.5% to about 2%. As will be appreciated by those skilled in the art, the particle to volume ratio of additional or extraneous particles within the sample, apart from the particle to be focused, need not necessarily be considered or adjusted. As will be further appreciated by those skilled in the art, any number of samples may not require any adjustment to the particle to volume ratio of the particle to be focused before, during, and/or after introduction into the system.

**[0137]** Various commonly used techniques for diluting or concentrating samples for adjusting a particle to volume ratio can be used in the embodiments disclosed herein. For example, a sample can be diluted or concentrated in batches before introduction into the system such that the sample ultimately introduced into the system has the required ratio before being introduced through the inlet. In other embodiments, the system can include two or more inlets for introducing the sample simultaneously with a diluent or concentrate to effect dilution or concentration. In this way, the particle to volume ratio can be adjusted within the system, whether adjustment occurs within a chamber before the sample and diluent or concentrate enter the channels or whether adjustment occurs through mixing of the sample and the diluent or concentrate within the channels. In another embodiment, the diluent or concentrate can be introduced into a center portion, fork, or branch of a channel as may be required by various applications after the unadjusted sample has traveled within the channel for some distance. A person skilled in the art will appreciate the variations possible for adjusting the particle to volume ratio of a sample within the embodiments described herein.

**[0138]** Referring again to FIGS. 1A and 1B, one or more microfluidic channels 16 can be formed in the microfabricated chip 14 and can be configured for receiving the sample 24 via one or more inlets 12 in communication with the channels 16. The channels 16 can be further configured for ordering and focusing particles of a predetermined size suspended within the sample into one or more localized streams or fluxes of particles 22 that is directed into one or more

outlets 26. In this way, particles in a dilute solution can be concentrated as illustrated in the figure. As illustrated in FIG. 1B, the localized flux 22 can include three or more particles 20 disposed longitudinally adjacent to one another and can be separated by a substantially constant longitudinal distance. Particles 20 within the flux 22 can also align rotationally relative to the channel 16.

**[0139]** In general, “localization” refers to a reduction in the area of a cross-section of a channel through which a flux of particles passes. In some preferred embodiments, particles can be localized within an area having a width of, at most, 1.05, 2, 3, 4, or 5 times the width of the particles. Localization can occur at any location within the channel, but preferably occurs within an unobstructed portion of the channel. For example, localization can occur in a portion of the channel having less than 50%, 40%, 30%, 20%, 10%, 5%, 2%, 1%, or 0.1% reduction in cross-sectional area. In certain embodiments, localization can occur in a channel having a substantially constant cross-sectional area.

**[0140]** Any number of microfluidic channels can be formed in the chip in any number of ways, described in detail below. In one exemplary embodiment, a single channel is formed on the chip for focusing particles therein. In other exemplary embodiments, a plurality of channels can be formed in the chip in various configurations of networks for focusing particles. For example, 2, 4, 6, 8, 10, 12, and more channels can be formed in the chip. As shown in FIG. 1A, a tree configuration is particularly convenient for a multiple channel system. Any number of layers can also be included within a microfabricated chip of the system, each layer having multiple channels formed therein.

**[0141]** Various channel geometries can be included on a single chip. As shown in FIG. 1A, straight sections of channels are formed in the chip near the inlet for transporting and dividing flow lines as the sample is introduced into the system. The straight sections of channel can transition to any number of symmetric and/or asymmetric curved channels for focusing particles of a predetermined size as needed. As further shown in FIG. 1A, the chip can also include straight sections of channels at an output region for analysis of focused particles, collection of focused particles, and/or for recombining stream lines. As will be appreciated by those skilled in the art, any number of curves or straight sections can be included as needed within the chip. Additional curved sections of channels can serve as “off-ramps” for focused particle streams to facilitate additional separation based on labels or tags associated with the particles. Channel forks or splits can be included at any positions within the channels to further facilitate manipulation of focused particles as needed for various applications.

**[0142]** Various channel dimensions can also be included within a single chip. Channel dimensions can decrease over the length of the chip to facilitate filtering of the sample, or for other reasons specific to an application. Channel dimensions can be larger at the input area or at the output area to enable forks or valve systems to be positioned within the channels, or to enable multiple stream lines to be separated and directed to different locations for analysis or collection. In a similar way, cross-sections of various channels can also be changed as needed within a single chip to manipulate stream lines of focused particles for particular applications. In general, any combination of channel geometries, channel cross-sections, and channel dimensions can be included on a single chip as