

light in kohler illumination with the object plane was utilized. All neutral density filters were removed and the highest power on the lamp allowed imaging with 2 μ s exposures using a Phantom v4.2 camera (Vision Research, New Jersey, USA). For flow cytometry applications, images were collected at an interval of 10 μ s using a collection window that was 32 \times 32 pixels. For larger single images and movies intervals from 20-70 μ s were used.

[0274] After separating particle solutions into fractions, individual fractions were analyzed using a Coulter counter (Beckman Coulter Z2). The coulter aperture size was 100 μ m and gain and current were set to observe particles in the size range of 3-9 μ m. Collected samples were diluted between 400 and 800 times to allow sufficient dilution for successful counting.

[0275] Blood cells were analyzed using a flow cytometer (Becton Dickinson FACSCalibur). Forward and side scatter were observed over a log scale to differentiate between platelets and other blood components. Detector voltages were turned to obtain the correct gain to observe both the scatter of the larger and smaller particles. Samples were generally diluted 100 times for measurements. 25,000-100,000 counts were observed for each sample.

[0276] Emulsions

[0277] Silicone oil in water emulsions were generated by mixing of these two immiscible phases with emulsifier present in the aqueous (continuous) phase for stabilization from coalescence of the resulting oil droplets. Silicone oil with dynamic viscosity of 9.35 cP and density of 0.935 g/cm³ was employed as the disperse phase (Dow Corning, Midland, Mich.; 200 fluid 10 cst), while the continuous phase was composed of de-ionized water containing 2% w/v poly(ethylene glycol) monooleate (Sigma-Aldrich, St. Louis, Mo.; M_n ~860) to stabilize the emulsion. After vigorous mixing of 5% v/v silicone oil with the aqueous phase, samples free of droplets larger than around 20- μ m in diameter were obtained via sedimentation for subsequent experimentation. Specifically, emulsion was extracted 1 cm from the bottom of evenly mixed emulsion that had been allowed to stand for 20 minutes so that large droplets completely evacuated the lower 2 cm of emulsion, as deduced from stokes drag on a buoyant spherical particle ($v=D^2 (\rho_{aqu}-\rho_{oil})g/(18 \eta_{aqu})\sim(3.55\times 10^4 \text{ m}^{-2}\text{s}^{-1}) D^2$).

[0278] PDMS Beads

[0279] PDMS (Polydimethylsiloxane) beads with a wide range in diameter were made in a fashion quite similar to silicone emulsions. PDMS was mixed with the standard 10:1 ratio of resin to crosslinker (Dow Corning; Sylgard 184), but prior to curing, degassed resin-crosslinker mixture was added to the same 2% w/v poly(ethylene glycol) monooleate aqueous solution at 10% w/v PDMS. After vortex-mixing until the desired size range was achieved, the tube of uncured PDMS emulsion was placed in a water bath at 70-90° C. for at least three hours to allow hardening of the liquid droplets into solid beads of PDMS. Beads larger than about 20- μ m were removed from extracted solutions of beads prior to experiment via filtration through a duplicate filter of a device as in FIG. 33.

[0280] In general, the embodiments disclosed herein present a nonintuitive phenomena associated with particles moving in a laminar flow that yields different levels of ordering within microchannel systems. Ranges of parameters are disclosed for utilization of the phenomena and key principles and forces that may responsible for the ordering are also

suggested. There are many advantages associated with the system of the invention including rapid continuous processing of samples without the need for filters or mechanical or electrical parts, high throughput applications, low noise results, and an independence in focusing for particle shape and density. Inertial focusing of the systems and methods described herein is ideal for particle sorting applications because of the precision of particle positioning into a single stream and the controlled longitudinal spacing between particles. Precise control of particle streamlines (i.e. small standard deviation of particle position) allows sorting with small induced changes in particle position. A slight induced movement of a particle away from the equilibrium streamline will yield a large difference over the background standard deviation of particle position and can allow the target particle to be extracted at a bifurcation in the channel without high levels of false positives and at high speeds. Additionally, the single file nature of the ordering and the regular longitudinal spacing insures that a deflected particle would not interact significantly with other particles in the flow. The particular geometries presented can be used in any number of applications to direct interactions of particles in inertial flows, and the system of the invention is applicable on a microscale as well as on a macroscale. It is appreciated that any and all channel geometries, system embodiments, and experimental parameters described herein can be combined in a multitude of ways to achieve specific results in various applications.

[0281] Applications of the system of the invention are widely diverse and will be useful in a wide range of industries, both commercial and academic. For example, in the biomedical field, applications of the system can be used in conventional techniques such as FACS, MACS, impedance-based particle counting, blood filtration, rare cell identification and filtration, hetero/homogenous cell signaling, among many others. For example, the properties of the particle motion induced by inertial focusing are ideally suited to cell separation and enumeration technologies. The extreme alignment and discrete spacing of each cell can be exploited to enumerate the cells individually as they flow through a microfluidic channel at high speeds by, for example, labeling cells with fluorescent tags or magnetic particles. The systems and methods described herein have many advantages over current rare-cell separation and enumeration techniques. Immunomagnetic techniques—where cells of interest are tagged with antibody coated magnetic beads—are often employed, however, cell losses occur in the processing of these samples because of its complexity and manual handling steps. A further advantage of the systems and methods is the ability to perform the cell ordering and separation at the point of care without the need for bulky equipment that is only suitable for the laboratory setting. Particle focusing techniques such as those described here can be combined with established immunomagnetic labeling and microelectronics technology to design and construct an cell separation microchip, for example, capable of handling whole blood samples that will not suffer from the problems of the current technologies. Microelectronic components can be integrated into microfluidic devices and therefore combine fluid flow and electronic manipulation or detection in a single device. The systems and methods described herein will create opportunities for the rapid screening of patients for a number of diseases and allow clinicians to follow the treatment progress of their patients.

[0282] In other applications, for example in industry, possible applications of the systems and methods of the invention