

[0194] Any and all components of the system can be reconditioned for reuse after at least one use. Reconditioning can include any combination of the steps of disassembly of the a system of the invention or system components, followed by cleaning and/or replacement of particular pieces, and subsequent reassembly. In particular, a system of the invention can be disassembled, and any number of the particular pieces or parts of the device can be selectively replaced or removed in any combination. Upon cleaning and/or replacement of particular parts, the device can be reassembled for subsequent use either at a reconditioning facility, or by a surgical or research team immediately prior to a procedure or test. Those skilled in the art will appreciate that reconditioning of a device can utilize a variety of techniques for disassembly, cleaning/replacement, and reassembly. Use of such techniques, and the resulting reconditioned device, are all within the scope of the present application.

[0195] The systems described herein can be used in a wide range of conventional enumerating, sorting, concentrating and ordering techniques. There is an ever increasing need in biological research, for example, for more accurate and efficient methods to manipulate and separate target particle and cell populations. Disciplines ranging from immunology and cancer medicine to stem cell biology are highly dependent on the identification of uncontaminated populations of particular particle and cell subsets for detailed characterization. Clinically, microbiologists routinely isolate bacterial cells and white blood cell subsets for diagnostic purposes. Tumor antigen-specific regulatory T cells can be discovered in the circulating blood of cancer patients, presenting a new potential target for immunotherapy of metastatic melanoma. Environmental sensing requires surveillance of water, food and beverage processing for specific bacterial cell contamination. Vaccine developers work largely with antigen-specific T lymphocytes, rare cells which may differ from one another by no more than a single amino acid in a peptide fragment presented on the cell surface. In these different applications a common problem is presented: the need to isolate, separate and characterize subpopulations of cells present within heterogeneous, complex fluids. During the processing of these samples, the target cell population must be handled with gentle care, preventing alteration of the cell's physiological state to allow for subsequent expression profiling and molecular studies. Moreover, the cells of interest may be present at extremely low frequencies—often less than 1 cell in 10,000,000 cells, for circulating tumor cells or disease-specific T lymphocytes, increasing the complexity of the challenge. As shown in FIG. 15C, the frequency of the target cell population in whole blood, for example, varies greatly depending on the application, illustrating the necessity for a dynamic sorting device that can process both small (10 μ l) and large (10 ml) amounts of whole blood with equal specificity and efficiency without altering the integrity of the cells.

[0196] Applications for a sensitive, high throughput, point-of-care particle and blood cell manipulator are far reaching. In the area of prenatal diagnosis of genetic abnormalities, for example, fetal nucleated red blood cells are a promising candidate for non-invasive diagnosis. However, the concentration of nucleated red blood cells in maternal blood is very low (1 per 10^6 cells), current cell sorting techniques are not suitable for analysis. In the field of cancer research, the ability to selectively isolate and characterize extremely rare (1 in 10^9 cells) circulating tumor cells (CTCs) could transform patient diagnosis, prognosis and treatment. With increased through-

put provided by systems of the invention described herein, the potential exists to isolate circulating tumor cells in very early stage cancer patients where the frequency of cells is proposed to be even lower. Fundamental to self/non-self recognition, a T cell contains a unique surface receptor that recognizes a specific peptide sequence, or antigen; although the exact diversity of T cells in the body is unknown, estimates suggest that there are at least 2.5×10^7 unique T cells in human blood. Isolating these cells becomes a significant challenge when their frequency in blood is quite low, thus requiring a large sample volume to be processed in order to isolate a statistically significant number of these cells. For example, in individuals latently infected with tuberculosis, the frequency of CD8+ T cells specific for a particular T8 antigen may be less than 1 in 200,000 peripheral blood mononuclear cells, which is the limit of sensitivity with existing sorting and ordering systems. The ability to measure even lower frequencies would be beneficial to vaccine development and diagnostics. Nonetheless, given 1 ml of whole blood, fewer than five specific antigen-specific T cells (ATGs) might be present, meaning that it might be necessary to process as much as 5-10 ml of whole blood samples in order to obtain an ATG population of a reasonable size, which conventional systems are incapable of doing in any time-sensitive manner, if at all.

[0197] The systems and methods described herein thus provide a manner in which rare cells can be sorted, separated, enumerated, and analyzed continuously and at high rates. Whether a particular cell is a rare cell can be viewed in at least two different ways. In a first manner of characterizing a cell as rare, the rare cell can be said to be any cell that does not naturally occur as a significant fraction of a given sample. For example, for human or mammalian blood, a rare cell may be any cell other than a subject's blood cell (such as a red blood cell and a white blood cell). In this view, cancer or other cells present in the blood would be considered rare cells. In addition, fetal cells (including fetal blood cells) present in a sample of the mother's blood should be considered rare cells. In a second manner of characterizing a cell as rare might take into account the frequency with which that cell appears in a sample or with respect to other cells. For example, a rare cell may be a cell that appears at a frequency of approximately 1 to 50 cells per ml of blood. Alternatively, rare cell frequency within a given population containing non-rare cells can include, but is not limited to, frequencies of less than about 1 cell in 100 cells; 1 cell in 1,000 cells; 1 cell in 10,000 cells; 1 cell in 100,000 cells; 1 cell in 1,000,000 cells; 1 cell in 10,000,000 cells; 1 cell in 100,000,000 cells; or 1 cell in 1,000,000,000 cells.

[0198] Referring now to FIG. 15A, one embodiment of a particle sorting configuration for the systems described herein is provided. In particular, FIG. 15A illustrates a passive sorting mechanism in the form of a magnetic-activated particle sorting configuration 250. A microfabricated chip can generally be provided having one or more asymmetric channels 252 formed therein. An analysis region of the chip can also be provided in which an output region 254 of each main channel 252 can include a fork or channel branch point that transitions the main channel 252 into first and second output channels 258, 256. A sample 260 can be prepared for introduction into the system 250 and target particles 262 of a predetermined size can be directed into the asymmetrically curving channels 252 to be focused into a single, tightly localized stream.