

[0132] A few specific examples of integrated microfluidic systems will now be presented. In FIG. 7B, an integrated microfluidic system 750 is useful for identifying cells or other analyte components having at least two accessible target proteins. In this embodiment, a sample suspected of having a particular type of cell (e.g., a tumor cell) is provided to a sorting station 751 along with magnetic beads coated with an antibody to a first target on the tumor cell. These may be provided via an inlet channel 753. A separate buffer inlet may also be provided. After sorting the magnetically labeled tumor cells, they flow to a binding station 753 where fluorescently labeled antibodies to a second target on the tumor cells are delivered via an inlet channel 755. There, the antibodies come in contact with and bind to surface antigens on the tumor cells. The cells then flow to a detection module 759, where they are exposed to light of an excitation frequency for the fluorophore of the second antibody. Fluorescence detected on trapped cells indicates that the cells harbor both the first and second targets. This may be conclusive evidence that a particular type of tumor cell is present in the sample.

[0133] In FIG. 7C, a biological sample suspected of having a particular bacterial pathogen is introduced to a sorting station 761 via an inlet channel 763. In this example, it is assumed that the sample has been pre-labeled with magnetic beads coated with an antibody to a surface protein of the suspected pathogen. Such labeling may be accomplished off-chip or on-chip in a pre-processing module or station as indicated above. Sorting in station 761 separates the bacteria in question from other sample components. In the depicted embodiment, the magnetic beads (with attached bacteria if present) are delivered via a collection channel 762 to a lysis station 765 where a strong magnetic field is temporarily applied via a magnet 767 (permanent or electromagnet) to hold the magnetic beads stationary. Then a chemical lysing agent is introduced to lysing station 765 via an inlet 769. The lysing agent disrupts the bacterial membranes to release the genetic material, which is then free to pass out of the lysis chamber in a flow field to an amplification module 771 through a channel 772. In this module, nucleotide building blocks, primers, Taq polymerase, and buffer are provided via an inlet channel 773. Thermal cycling to drive a polymerase chain reaction in module 771 is controlled using a heating element 775. The bacterial DNA is thereby amplified while passing through module 771. It then passes out of the amplification module and enters a detection module 777 (e.g., a microarray), where it may be detected via a fluorescent signature. Alternatively, PCR may be conducted using a Taq-Man™ oligonucleotide probe to enable fluorescent detection in detection module 777. Note that a controller 779 may be employed to control the timing of thermal cycling, the application of a magnetic field, etc. during operation.

[0134] Certain embodiments employ two levels of detection, one for a first target species located on the surface of a cell or virus and a second for a second target associated with a component of the cell or virus. One example of an integrated device or system that may be employed for this purpose is depicted in FIG. 7D. In the depicted example, a first section of the device/system labels, separates and detects target cells or viruses from a sample. A second section then releases components of the cells or virus, which components are further manipulated by, e.g., amplification, and ultimately detected. Hence whole cells or viruses are first detected and then one or more components of the cells or viruses are separately detected. In some embodiments, the first target on the cell or

virus is a surface protein, saccharide, or lipid. In some embodiments, the second target of the cell or virus is a nucleic acid or intracellular protein, saccharide, or lipid.

[0135] Turning now to FIG. 7D, a device or system 780 includes a first detection section 781 for detecting a cell or virus and a second detection section 783 for detecting a cell or virus component. Sections 781 and 783 are in fluid communication with one another. In detection section 781, a sample is provided a labeling station 785 via a sample inlet 787. In this station, the sample is contacted with magnetic particles which label cells or viruses having a first target on their membranes or protein coats. Labeled cells or viruses then flow to a sorting station 789 via an inlet 791. In station 789, buffer switching takes place under the influence of a magnetic field gradient in the manner described above. Cells or viruses harboring a surface target are thereby separated from other components of the sample and selectively delivered to a first detection station 793 via a channel 795. The cells or viruses are detected using fluorescence or other signature.

[0136] At this point in the device or system, the first level of detection has been completed and the cells or viruses are ready for the second level of detection, which is implemented in section 783. Initially, cells or viruses leave detection station 793 and flow via a channel 795 to a station 797 where the cells or viruses are disrupted in a manner that releases at least some of their components for further analysis. As explained elsewhere, the necessary disruption may be chemical, thermal, mechanical, acoustic, etc. as appropriate for the species of sample under analysis. In the depicted example, a separate inlet channel 799 provides reagent for disrupting the cell membrane or viral protein coat to release genetic material or other contents. In some embodiments, the cells or viruses are held stationary (at least temporarily) during treatment to release their components. The components released from the cell or membrane travel via a channel 784 from station 797 to a station 782, where the components are “manipulated” to facilitate further detection. The type of manipulation employed depends upon the type of component under consideration. For example, nucleic acids may be amplified in station 782 as described elsewhere herein. In other examples, subcellular components such as Golgi, cytoskeletal components, histones, mitochondria, etc. may be labeled with markers specific for those components (typically a biomolecule found within the component) in station 782. The markers, amplification reagents, or other component manipulation agent may flow into station 782 via an inlet channel 786. After appropriate manipulation in station 782, the components flow to a component detection station via a channel 790. There the component itself is detected by fluorescence, etc. as understood by those of skill in the art.

[0137] In some applications, loss of target species in a sample can lead to lack of commercial acceptance. Losses may be particularly problematic when the target is a rare cell species such as certain pathogens, tumor cells, stem cells, etc. As indicated, near 100% recovery of target species is sometimes desirable, even in cases where the initial concentration target in sample is extremely low (e.g., no greater than about 10^{-5} or even 10^{-7}).

[0138] It has been found that often the most significant losses in microfluidic devices such as those described herein are in the delivery of the sample to the microfluidic devices. Particular problems occur when the sample passes through a pump or syringe when delivered to the device. In certain embodiments, losses are minimized by using designs in