

magnetic particles and/or the sample materials in suspension. In certain embodiments, the density of buffer is between about 1 and 1.2 g/ml.

[0091] Some commonly used sorting buffers include phosphate buffered saline, deionized water, etc. Obviously, the actual buffer composition depends on the application and the nature of the sample and target. In a specific embodiment used to sort bacteria, the buffer comprises 1×PBS (phosphate buffered saline)/20% glycerol/1% BSA (bovine serum albumin) (all by volume) and has a density of 1.06 g/ml.

[0092] In certain embodiments, a sorting stage operates in constant flow processes to effect sorting. This does not mean that certain sorting operations cannot be performed without interruption of fluid flow. For example, in certain embodiments it may be necessary to intermittently pause the flow for process tuning or for certain designated operations such as detection, amplification, and/or lysis.

[0093] During constant flow conditions, the overall flow rate within the magnetophoretic sorting region of a microfluidic device will depend upon throughput goals as well as the total area of the channels and the resistance of the channels within the device. In certain embodiments, the process is performed with a volumetric sample flow rate of between about 10 $\mu\text{L}/\text{hour}$ and 500 ml/hour. Typically, the high end of this range is attained with a multi-station parallel flow device or system. For a single sorting station, the sample flow rate may be between about 10 and 5000 $\mu\text{L}/\text{hour}$ (preferably between about 50-1000 $\mu\text{L}/\text{hour}$), and the buffer flow rate of about 1-10 times that of the sample flow (preferably 2-4 times the sample flow). In a typical CMACS device, the fluid velocity in the sorting region is between about 100 $\mu\text{m}/\text{s}$ and 50 cm/s, typically in the range of about 1-10 mm/s (e.g., approximately 2-5 mm/s).

[0094] Generally, sorting stages should be designed so that little if any unlabeled components cross the stream boundaries by diffusion. This may be accomplished by designing the device to have a relatively fast flow rate in the sorting region, and/or a relatively large distance for sample to traverse from a sample stream to a collection outlet channel. As an example, the typical diffusivity of a 1 μm -sized cell in an aqueous buffer at room temperature is $D=0.2 \mu\text{m}^2/\text{s}$. At a velocity of few mm/s, the dwelling time of each cell in the channel is typically less than a second, during which the cell can diffuse by only a few microns. If the device is operated such that a portion of the buffer stream is bled into the waste channel with a width $>10 \mu\text{m}$, which ensures that non-target cells that are able to cross the stream boundary through diffusion are unable to enter the collection channel.

[0095] Typical dimensions suitable for use with a single sorting region having at least one buffer inlet channel, at least one sample inlet channel, at least one outlet collection channel and at least one waste channel will now be presented. Depending on the relative amounts of target and non-target species, the ratio of cross sectional area of the collection channel(s) to cross sectional area of the waste outlet channel (s) may be about 100:1 to 1:100. Further, the ratio of cross sectional area of the buffer inlet channel to cross sectional area of the sample inlet channels may be about 100:1 to 1:100.

[0096] For further context, typical channel dimensions suitable for use with the microfluidic device of FIG. 1 will now be presented. In this example, all channels may have the same depth, e.g., approximately 1 micrometers to 10 millimeters depending on the magnetic field gradient size, although typically not greater than about 100 micrometers (e.g., 50

micrometers). In certain embodiments, the width of a waste outlet channel is in the range about 10 micrometers to 5 centimeters, although it is typically at least about 500 micrometers. The width of a collection channel may be between about 1 micrometer to about 5 centimeters (e.g., 80 micrometers). The width of a sample or buffer inlet channel may be approximately that of a waste outlet channel (e.g., about 10 micrometers to 5 centimeters, although typically at least about 500 micrometers). Additionally, the total width of the separation region may be, in certain embodiments, the sum of the widths of all inlet channels or all outlet channels.

[0097] The channels, inlets, vias, pumps, etc. required for a microfluidic sorting station of this invention may be fabricated using well know fabrication techniques (e.g., various microfabrication procedures) or purchased as necessary. In a specific example, borosilicate glass wafers may be affixed to PDMS replicas of a silicon master mold fabricated by applying a precursor to the silicon master, followed by curing. A binding agent such as epoxy may be used to bond the glass and PDMS layers.

[0098] Fluid flow may be either pressure-driven or electrokinetic-driven. Pressure-driven flows are created by pumps (e.g., peristaltic pumps), syringes, etc. that are readily available for small volume microfluidics applications. In a specific embodiment, a dual-track programmable syringe pump (Harvard Apparatus Ph.D. 2000, Holliston, Mass.) is employed to deliver both the sample mixture and the sorting buffer into the device at constant flow rates.

[0099] The flow of sample in the microchannel may be monitored through a suitable detector such as a bright-field microscope (e.g., the DM 4000, LEICA Microsystems AG, Wetzlar, Germany) and a cooled CCD camera (e.g., the ORCA-AG, Hamamatsu Corporation, Bridgewater, N.J.).

[0100] Multi-Stage Sorting

[0101] Two or more sorting stages may be integrated on a single microfluidic system or even a single microfluidics chip in a sequential manner to improve purity. Further, in certain embodiments, at least two sorting stations are provided in parallel to improve throughput. In certain embodiments, at least three sorting stations are provided in parallel, and in certain embodiments at least four sorting stations are provided in parallel. Likewise, in certain embodiments, at least two, three, or four sorting stations (or stages) are provided in series. Frequently, when multiple stages are provided in series at least two of the upstream stations are provided in parallel. Their outputs may combine to feed a downstream station.

[0102] FIG. 5A presents one example of a microfluidics device having at three MFG-based sorting stations: two parallel stations **503a** and **503b** being provided upstream of a third station **505** fed by both the parallel upstream stages. The hydrodynamics of the three-stage device is designed such that an inlet mixture of the sample is partitioned equally into the upper and lower inlet sorting channels **507a** and **507b** of the first stage, while the buffer solution is divided into three streams provided by channels **509a**, **509b**, and **509c**. The streams remain laminar throughout the device due to their low Reynolds number. In the first stage, all cells flow through sorting stations **503a** and **503b** having MFGs **513a** and **513b** (location E), and flow pattern is designed such that, when the MFGs are not magnetized by an external field, all cells transported to waste outlet channels **515a** and **515b** (location D). When the MFGs are magnetized by an external field, the magnetically-labeled sample components are selectively deflected into the buffer stream via channels **517a** and **517b**.