

CMOS/microfluidic hybrid system with an electrical board (e.g., package substrate) and a temperature controller (e.g., thermoelectric cooler).

[0189] VI. Sample Counting and Sorting

[0190] According to another embodiment, a hybrid system 100 including sample detection and imaging components as discussed above in Section III, and various configurations of a microfluidic system as discussed above in Section V, may be employed in a number of cell counting, sorting and identification applications.

[0191] For example, FIGS. 39(a)-(d) illustrate various exemplary implementations of cell detection via RF sensing techniques as discussed above in connection with FIGS. 17-20. In FIG. 39(a), a single narrow microfluidic channel may allow only one bead-bound sample to pass over a given microcoil at a time (i.e., a fluid suspension contains magnetic beads 112 bound to samples of interest flowing through the channel 300 over a microcoil 212 (Coil 3) coupled to RF/detection components 480, which senses the magnetic beads individually). Cell counting may be accomplished based on varying fluid flow rates and characteristics of the magnetic beads suspended in the fluid. In some cases, if the fluid flows too fast, the magnetic bead may not have enough time to magnetize in the sensing coil (Coil 3) and hence may not be appropriately detected by the RF/detection components 480. In such cases, other microcoils in the linear microcoil array shown in FIG. 39(a) may be employed (e.g., Coils 1 and 2 in addition to Coil 3) to generate DC magnetic fields to magnetize beads before their arrival to the sensing coil (Coil 3), thereby facilitating detection of the beads.

[0192] In another example, as shown in FIG. 39(b), a wide microfluidic channel 300 may be implemented that passes several beads 112 at a time over a single microcoil 212 coupled to the RF/detection components 480, during which the microcoil 212 can sense multiple beads simultaneously with the counting resolution of one bead. Other counting examples are given in FIG. 39(c), in which multiplexed fluidic channels 300A, 300B and 300C are employed, and in FIG. 39(d), which illustrates two-dimensional imaging of a magnetic bead distribution via a microcoil array 200B and a single reservoir microfluidic system 300. As discussed above, with RF/detection components 480 capable of exciting each microcoil of a two-dimensional array, the microcoil array 200B is analogous to the pixel arrangement of a conventional CCD imaging system.

[0193] Another embodiment according to the present disclosure is directed to precision cell sorting methods and apparatus based on a CMOS/microfluidic hybrid system including RF/detection components, pursuant to various embodiments discussed above. Isolating a homogeneous cell population with high accuracy from a dissolved organ or tissue or from batches of pooled blood is important for conducting gene expression analysis, for cell and tissue engineering assays requiring a pure cell line, or for clinical applications (e.g., stem cell separation for bone marrow reconstitution procedures in cancer patients.). Many cells can be recognized due to the expression of unique cell surface receptors. In conventional approaches, magnetic beads coated with the ligand for these receptors have been used to engage the cells with magnetic tweezers and magnetic twisting cytometry. This technique has been used for cell sorting/separation as well, but the conventional mag-

netic separation technique employs a simple stationary magnet that statistically sorts a large group of bead-bound cells all at once, lacking controllability and precision. In contrast to conventional approaches, one embodiment of the present disclosure combines the high controllability of CMOS electronics with micro-scale manipulation and detection capabilities of the microcoil array to realize ultra-precise, high-throughput, and automated cell sorting methods and apparatus for individual biological cells attached to magnetic beads within heterogeneous suspensions.

[0194] In one exemplary implementation of this embodiment, as illustrated in FIG. 40, a solution of cells including both bead-bound cells 116 and non-magnetized cells 114 are suspended in a fluid that flows through the microfluidic channel 300 (e.g., in one exemplary implementation, capillary endothelial cells and NIH 3T3 fibroblasts may be suspended in media with 2.8 micrometer magnetic beads coated with the antibody to platelet endothelial cell adhesion molecule (PECAM), a cell surface receptor exclusive to endothelial cells; ligand-coated beads attach to endothelial cells only). The microfluidic channel passes over an RF sensor 212-1 or 212-2 (i.e., a microcoil coupled to RF/detection components 480), and whenever a bead-bound cell 116 passes over the sensor, the sensor registers and counts the bead-bound cell. In one aspect of the embodiment shown in FIG. 40, whenever the first RF sensor 212-1 detects a bead-bound cell 116, the microcoils in the first linear microcoil array 2000-1 activate sequentially to pull the bead-bound cell 116 like a "conveyor belt," thereby removing it from the combined cell fluid flow and effectively separating bead-bound cells from the general cell population. In one implementation, the linear microcoil array 2000-1 need not always be on, so as to minimize power consumption, and may be turned on with a signal of the preceding RF sensor 212-1 indicating the presence of a bead-bound cell 116.

[0195] In FIG. 40, in some cases some bead-bound cells 116 might pass the first linear microcoil array 2000-1 without being pulled out of the mainstream of flow. However, in one aspect of the embodiment of FIG. 40, multiple sensor-linear microcoil array blocks may be sequentially employed, each with the same operating protocol (e.g., note the microcoil 212-2 serving as a second "RF sensor" and the second linear microcoil array 2000-1). Such a redundant system with individual cell selection substantially increases cell sorting yield and accuracy without compromising speed. The RF sensors 212-1 and 212-2 quantitatively monitor sorting accuracy in real time by sensing the presence of magnetic bead-bound samples 116. After passing thru this system, the segregated bead-bound and unbound cells are respectively collected, with the unbound cell population available for further sorting by the same protocol to remove any bead-bound cells (presumably few) that may remain in this population.

[0196] The cell sorting methods and apparatus exemplified in the arrangement of FIG. 40 offer several important advantages over prior techniques. For example, in one aspect, individual bead-bound-cells may be separated from heterogeneous cell populations at a very low error rate, where accuracy is monitored quantitatively using RF/detection components in real time. Furthermore, the accuracy of the cell sorting methods and apparatus discussed in connection with FIG. 40 is much higher than that of the conventional magnetic separation techniques developed and used