

bar magnets. The angled position of the magnets can be chosen to focus the magnet field along the centerlines of the BPumps.

[0099] Pneumatic routing for control of valves and pumps is shown in FIG. 23. Solenoid blocks each carry eight two-position solenoids which route either vacuum or positive pressure to outputs 1-8 on each block. Solenoid outputs are connected to the indicated chip wells with tubing. Solenoid labels are used to address individual solenoids in DevLink code. Note that Reagent and Bead Rail valves can be identically labeled, indicating that these valves are operated simultaneously. Alternatively, these valves may be operated independently. Within the chip, however, access to the processors can be gated by two pairs of valves labeled Reagents and Beads. Other valves and pumps which share the same label may operate simultaneously, without differentiation. Thus, the two chip processors may operate simultaneously and in parallel. Alternatively, the two chip processors can be configured to operate independently. Alternative configurations can be designed by choosing appropriate valve, channel, pneumatic, and control configurations.

[0100] Vacuum and positive pressure can be generated by a small double-headed Hargraves diaphragm pump. These pumps can be capable of generating vacuums of about 21 in. Hg, and positive pressures of up to about 25 PSI. Chips can be run at maximum vacuum and 15 PSI positive pressure. For transport of viscous materials, increasing pump membrane transition times can improve pumping performance. Pump transition times can be adjusted by inserting an adjustable orifice in the pneumatic line driving chip Pumps. A range of precision orifices can be purchased from Bird Precision (<http://birdprecision.com>).

[0101] In addition, and as discussed more fully below, BPump performance can be improved with higher vacuum levels (28 in. Hg), which can be generated with a KNF UN86 pump connected in series with the vacuum side of the Hargraves pump.

[0102] In some embodiments, a base can include a support structure, one or more pneumatic manifolds, which may be pneumatic floaters, one or more pneumatic inserts, and one or more temperature controlling devices. An exploded view of a system is shown in FIG. 5. The system includes a fluidic manifold (reservoir & reservoir bottom), microfluidic chip (061 chip), floater, inserts, thermoelectric coolers (TECs), and a support structure (aluminum manifold) is shown in FIG. 5. An assembled view of FIG. 5 is shown in FIG. 6.

[0103] The heat sinking capacity for the TECs can be increased by mounting them directly on a large aluminum manifold which serves as the base plate of the system. The upper (working) surfaces of the TECs touch the Reservoir Bottom, directly beneath the serpentine incubation channels, when the system is fully assembled. Moderate force can be exerted on this interface by tightening four thumb screws (not shown).

[0104] Another feature is the use of a small Pneumatic Floater to carry magnets and provide a pneumatic interface to the bottom of the chip. The Pneumatic Floater can serve the same purpose as the previous pneumatic manifold, but it rides on springs mounted onto the Aluminum Manifold. The spring force can serve to compress the o-rings that provide gas-tight connections to the bottom surface of the chip.

[0105] The use of springs for mounting or compressing of the pneumatic floater to the microchip can facilitate assembly of the system can reduce the need for production of high-

tolerance components. In the case of the system utilizing a support structure that has mounted to it the thermoelectric cooler and the pneumatic floater, the thermoelectric cooler must interface with the cartridge and the pneumatic floater must interface with the microfluidic chip. The chip is also interfaced with the cartridge. Because the chip, the cartridge, the support structure, the thermoelectric coolers, and the pneumatic floaters may each vary in thickness from device to device, springs can allow for proper interfacing of both pairs of components without the need to produce each component in high tolerance or high accuracy or precision. This can reduce the time for manufacture of each component and the time for assembly of the system. The time for manufacture of each component can be up to about, less than about, or about 0.1, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 24, 36, or 48 hours. The time for assembly of the system can be up to about, less than about, or about 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, or 24 hours.

II. Applications

A. mRNA Amplification

[0106] Gene expression microarrays can monitor cellular messenger RNA (mRNA) levels. Messenger RNA can constitute typically only 1-3% of cellular total cellular RNA. The vast majority of cellular RNA can be ribosomal RNA (rRNA), and these molecules may interfere with mRNA analysis by competing with mRNA for hybridization to microarray probes. Any mRNA amplification method can be performed by the devices described herein, for example LAMP, TLAD (Eberwine), and MDA. In some embodiments of the invention, isothermal mRNA amplification methods can be performed using the devices described herein. In other embodiments, thermal cycling can be performed to accomplish PCR or cycle sequencing. Messenger RNA amplification procedures can specifically target polyadenylated (polyA+) mRNA for amplification, virtually eliminating rRNA interference. This characteristic can remove any need to pre-purify mRNA from total RNA, which can be an inefficient, time-consuming, and expensive process. In addition, by greatly increasing the amount of target RNA (that is, amplified mRNA or aRNA) available for microarray hybridization, mRNA amplification can allow much smaller samples (fewer numbers of cells) to be analyzed. This is, of course, generally helpful because the relatively large amount of target RNA required for microarray analysis (typically 15 ug) can be frequently difficult to obtain. Moreover, it can be relevant for many important clinical diagnostic applications analyzing samples containing few cells, for example, samples derived from fine needle aspirates (FNA) or laser capture microdissection (LCM).

[0107] As shown in FIG. 24A, the overall microarray sample prep process can begin with total cellular RNA, which may be characterized by microchip capillary electrophoresis with an Agilent Bioanalyzer to quantitate 28S/18S ratios and to generate a RNA Integrity Number (RIN). If the total RNA is of sufficient quality, the mRNA can be amplified, and the amplified RNA (aRNA) can then be fragmented and hybridized to microarrays. The methods, devices, and systems described herein can allow for execution of the mRNA amplification process on a microchip-based system. The mRNA amplification chemistry can utilize Eberwine mRNA amplification, as implemented in the Ambion Message Amp III kit. This process is outlined in FIG. 24B, which shows that the amplification process can comprise two multistep compo-