[0084] The cartridge can further include a heat sealable laminate layer **222** (typically between about 100 and about 125 microns thick) attached to the bottom surface of the microfluidic substrate using, for example, heat bonding. The cartridge can further include a thermal interface material layer **220** (typically about 125 microns thick), attached to the bottom of the heat sealable laminate layer using, for example, pressure sensitive adhesive. This layer **220** can be compressible and have a higher thermal conductivity than common plastics, thereby serving to transfer heat across the membrane more efficiently to the components of the microfluidic network.

[0085] Application of minimal pressure on the cartridge: a force member on the apparatus can compress the compliant label of the cartridge. This can cause the bottom of the cartridge to be pressed down against the microheater substrate present in the heater unit. Springs, for example, present in the force member can deliver, for example approximately 50 lb of pressure to generate a minimum pressure, for example 2 psi over the entire cartridge bottom.

[0086] Thermal interface: the cartridge underside can have a layer of mechanically compliant heat transfer laminate that can enable thermal contact between the microfluidic substrate and the microheater substrate of the heater unit. A minimal pressure of 1 psi can be employed for reliable operation of the thermal valves, gate and pumps present in the microfluidic cartridge.

[0087] Table 1 outlines volumes, pumping pressures, and operation times associated with various components of a microfluidic cartridge.

TABLE 1

Operation	Pumping Pressure	Displacement Volume	Time of Operation
Moving valve wax plugs	~1-2 psi	<1 μl	5-15 seconds
Operation	**Pump Used**	**Pump Design**	**Pump Actuation**
Moving valve wax plugs	Thermopneumatic pump	1 μl of trapped air	Heat trapped air to ~70-90 C.

[0088] A valve (sometimes referred to herein as a microvalve) is a component in communication with a channel, such that the valve has a normally open state allowing material to pass along a channel from a position on one side of the valve (e.g., upstream of the valve) to a position on the other side of the valve (e.g., downstream of the valve). Upon actuation of the valve, the valve transitions to a closed state that prevents material from passing along the channel from one side of the valve to the other. For example, in one embodiment, a valve can include a mass of a thermally responsive substance (TRS) that is relatively immobile at a first temperature and more mobile at a second temperature. The first and second temperatures are insufficiently high to damage materials, such as polymer layers of a microfluidic cartridge in which the valve is situated. A mass of TRS can be an essentially solid mass or an agglomeration of smaller particles that cooperate to obstruct the passage. Examples of TRS's include a eutectic alloy (e.g., a solder), wax (e.g., an olefin), polymers, plastics, and combinations thereof. Generally, for such a valve, the second temperature is less than about 90° C. and the first temperature is less than the second temperature (e.g., about 70° C. or less). A chamber is in gaseous communication with the mass of TRS. Upon heating gas (e.g., air) in the chamber and heating the mass of TRS to the second temperature, gas pressure within the chamber moves the mass into the channel obstructing material from passing therealong. Various exemplary valves are shown in FIGS. **10A-10C**.

Highly Multiplexed Cartridge Embodiments

[0089] Embodiments of the microfluidic substrate described herein may be constructed that have high-density microfluidic circuitry on a single substrate that thereby permit processing of multiple samples in parallel, or in sequence, on a single cartridge. Preferred numbers of such multiple samples include 24, 36, 40, 48, 50, 60, 64, 72, 80, 84, 96, and 100, but it would be understood that still other numbers are consistent with the technology herein, where deemed convenient and practical.

[0090] Accordingly, different configurations of lanes, sample inlets, and associated heater networks than those explicitly depicted in the FIGs that can facilitate processing such numbers of samples on a single substrate are within the scope of the instant disclosure. Similarly, alternative configurations of detectors and heating elements for use in conjunction with such a highly multiplexed substrate are also within the scope of the description herein.

[0091] Accordingly, it is to be understood that the microfluidic substrates and cartridges described herein are not to be limited to rectangular shapes, but can include cartridges having circular, elliptical, triangular, rhombohedral, square, and other shapes. Such shapes may also be adapted to include some irregularity, such as a cut-out, to facilitate exact placement of a cartridge in a complementary apparatus as further described herein.

[0092] In an exemplary embodiment, a highly multiplexed cartridge has 48 sample lanes, and permits independent control of each valve in each lane by suitably configured heater circuitry, with 2 banks of thermocycling protocols per lane, as shown in FIG. **11**. In the embodiment in FIG. **11**, the heaters (shown superimposed on the lanes) are arranged in three arrays. The heaters are themselves disposed within one or more substrates. Heater arrays **502**, **508** in two separate glass regions only apply heat to valves in the microfluidic networks in each lane. Because of the low thermal conductivity of glass, the individual valves may be heated separately from one another. This permits samples to be loaded into the cartridge at different times, and passed to the PCR reaction chambers independently of one another. The PCR heaters **504**, **506** are mounted on a silicon substrate—and are not readily heated individually, but thereby permit batch processing of PCR samples, where multiple samples from different lanes are amplified by the same set of heating/cooling cycles. It is preferable for the PCR heaters to be arranged in 2 banks (the heater arrays on the left **506** and right **508** are not in electrical communication with one another), thereby permitting a separate degree of sample control.

[0093] FIG. **12** shows a representative 48-sample cartridge compatible with the heater arrays of FIG. **11**, and having a configuration of inlets different to that depicted on cartridges herein. The inlet configuration is exemplary and has been designed to maximize efficiency of space usage on the cartridge. The inlet configuration can be compatible with an automatic pipetting machine that has dispensing heads situated at a 9 mm spacing. For example, such a machine having 4 heads can load **4** inlets at once, in 12 discrete steps, for the