tive ultrasonic drilling, or selective abrasive particle blasting. Care has to be taken to maintain mechanically intergrity of the heater while reducing as much material as possible.

[0174] FIG. **22**C shows a combination of cutouts and use of ambient air cooling to increase the cooling rate during the cooling stage of thermocycling. A substantial amount of cooling happens by convective loss from the bottom surface of the heater surface to ambient air. The driving force for this convective loss is the differential in temperatures between the glass surface and the air temperature. By decreasing the ambient air temperature by use of, for example, a peltier cooler, the rate of cooling can be increased. The convective heat loss may also be increased by keeping the air at a velocity higher than zero.

[0175] An example of thermal cycling performance in a PCR reaction chamber obtained with a configuration as described herein, is shown in FIG. **23** for a protocol that is set to heat up the reaction mixture to 92° C., and maintain the temperature for 1 second, then cool to 62° C., and stay for 10 seconds. The cycle time shown is about 29 seconds, with 8 seconds required to heat from 62° C. and stabilize at 92° C., and 10 seconds required to cool from 92° C., and stabilize at 62° C. To minimize the overall time required for a PCR effective to produce detectable quantities of amplified material, it is important to minimize the time required for each cycle. Cycle times in the range 15-30 s, such as 18-25 s, and 20-22 s, are desirable. In general, an average PCR cycle time of 25 seconds as well as cycle times as low as 20 seconds are typical with the technology described herein. Using reaction volumes less than a microliter (such as a few hundred nanoliters or less) permits use of an associated smaller PCR chamber, and enables cycle times as low as 15 seconds. An average cycle time of 25 seconds and as low as 20 seconds can be achieved by technology described herein, even without any forced cooling or implementing any thermal mass reductions described elsewhere herein.

Manufacturing Process for Cartridge

[0176] FIG. **24** shows a flow-chart **1800** for an embodiment of an assembly process for an exemplary cartridge as shown in FIG. **11**A herein. It would be understood by one of ordinary skill in the art, both that various steps may be performed in a different order from the order set forth in FIG. **24**, and additionally that any given step may be carried out by alternative methods to those described in the figure. It would also be understood that, where separate serial steps are illustrated for carrying out two or more functions, such functions may be performed synchronously and combined into single steps and remain consistent with the overall process described herein.

[0177] At **1802**, a laminate layer is applied to a microfluidic substrate that has previously been engineered, for example by injection molding, to have a microfluidic network constructed in it; edges are trimmed from the laminate where they spill over the bounds of the substrate.

[0178] At **1804**, wax is dispensed and loaded into the microvalves of the microfluidic network in the microfluidic substrate. An exemplary process for carrying this out is further described herein.

[0179] At **1806**, the substrate is inspected to ensure that wax from step **1804** is loaded properly and that the laminate from step **1802** adheres properly to it. If a substrate does not satisfy either or both of these tests, it is usually discarded. If substrates repeatedly fail either or both of these tests, then the wax dispensing, or laminate application steps, as applicable, are reviewed.

[0180] At **1808**, a hydrophobic vent membrane is applied to, and heat bonded to, the top of the microfluidic substrate covering at least the one or more vent holes, and on the opposite face of the substrate from the laminate. Edges of the membrane that are in excess of the boundary of the substrate are trimmed.

[0181] At **1810**, the assembly is inspected to ensure that the hydrophobic vent membrane is bonded well to the microfluidic substrate without heat-clogging the microfluidic channels. If any of the channels is blocked, or if the bond between the membrane and the substrate is imperfect, the assembly is discarded, and, in the case of repeated discard events, the foregoing process step **1808** is reviewed.

[0182] At **1812**, optionally, a thermally conductive pad layer is applied to the bottom laminate of the cartridge.

[0183] At **1814**, two label strips are applied to the top of the microfluidic substrate, one to cover the valves, and a second to protect the vent membranes. It would be understood that a single label strip may be devised to fulfill both of these roles.

[0184] At **1816**, additional labels are printed or applied to show identifying characteristics, such as a barcode #, lot # and expiry date on the cartridge. Preferably one or more of these labels has a space and a writable surface that permits a user to make an identifying annotation on the label, by hand.

[0185] Optionally, at **1818**, to facilitate transport and delivery to a customer, assembled and labeled cartridges are stacked, and cartridges packed into groups, such as groups of 25, or groups of 10, or groups of 20, or groups of 48 or 50. Preferably the packaging is via an inert and/or moisture-free medium.

Wax Loading in Valves

[0186] In general, a valve as shown in, e.g., FIGS. **25**A-C, is constructed by depositing a precisely controlled amount of a TRS (such as wax) into a loading inlet machined in the microfluidic substrate. FIGS. **25**A and **25**B show how a combination of controlled hot drop dispensing into a heated microchannel device of the right dimensions and geometry is used to accurately load wax into a microchannel of a microfluidic cartridge to form a valve. The top of FIG. **25**A shows a plan view of a valve inlet **190** and loading channel **1902**, connecting to a flow channel **1904**. The lower portions of FIG. **25**A show the progression of a dispensed wax droplet **1906** (having a volume of 75 nl±15 nl) through the inlet **1901** and into the loading channel **1902**.

[0187] To accomplish those steps, a heated dispenser head can be accurately positioned over the inlet hole of the microchannel in the microfluidic device, and can dispense molten wax drops in volumes as small as 75 nanoliters with an accuracy of 20%. A suitable dispenser is also one that can deposit amounts smaller than 100 nl with a precision of +/−20%. The dispenser should also be capable of heating and maintaining the dispensing temperature of the TRS to be dispensed. For example, it may have a reservoir to hold the solution of TRS. It is also desirable that the dispense head can have freedom of movement at least in a horizontal (x−y) plane so that it can easily move to various locations of a microfluidic substrate and dispense volumes of TRS into valve inlets at such locations without having to be re-set, repositioned manually, or recalibrated in between each dispense operation.