MINIATURIZED THERMAL CYCLER

FIELD OF THE INVENTION

The invention relates to the general field of MEMS with particular reference to thermal cycling chambers for use in, for example, polymerase chain reactions as well as other reactions that involve thermal cycling.

BACKGROUND OF THE INVENTION

PCR (Polymerase Chain Reaction) is a molecular biological method for the in-vitro amplification of nucleic acid molecules. The PCR technique is rapidly replacing many other time-consuming and less sensitive techniques for the identification of biological species and pathogens in forensic, environmental, clinical and industrial samples. PCR using microfabricated structures promises improved temperature uniformity and cycling time together with decreased sample and reagent volume consumption.

An efficient thermal cycler particularly depends on fast heating and cooling processes and high temperature uniformity. Presently, microfabricated PCR is preferably carried out on a number of samples during a single thermal protocol run. It is a great

advantage if each reaction chamber can be controlled to have an independent thermal cycle. This makes it possible to run a number of samples with independent thermal cycles simultaneously (parallel processing). The first work on multi-chamber thermal cyclers fabricated multiple reaction chambers by silicon etching. Although separate heating elements for every reaction chamber can be realized, it was impossible in these designs to eliminate thermal cross-talk between adjacent reaction chambers during parallel processing because of limited thermal isolation between reaction chambers. As a result, multiple chambers having independent temperature protocols could not be used. Additionally, temperature uniformity achieved inside the reaction chamber was ±5K in this thermal isolation and heating scheme.

Integration of the reaction chamber with micro capillary electrophoresis (CE) is also an interesting subject, in which small volumes of samples/reagents will be required both for PCR and CE. Again, a high degree of thermal isolation is very important particularly where various driving/detection mechanisms prefer a constant room temperature substrate.

A number of microfabricated PCR devices have been demonstrated in the literature. Most of them were made of silicon and glass, while a few others were using silicon bonded to silicon. On-chip integrated heaters and temperature sensors become important in the accurate control of the temperature inside these small reaction chambers. Good thermal

isolations have been proved promising for quick thermal response. Micro reaction chamber integrated with micro CE was only demonstrated where no PCR thermal cycling was performed (only slowly heated to 50 °C in 10-20 seconds and held for 17 minutes). Parallel processing microfabricated thermal cyclers with multi-chamber and independent thermal controls have not yet been reported.

A routine search of the prior art was performed with the following references of interest being found: Northrup et al. (US 5,589,136 Dec. 1996), Northrup et al. (US 5,639,423, US 5,646, 039, and US 5,674,742), and Baier Volker et al, in US 5,716,842 (Feb. 1998), did early work on multi-chamber thermal cyclers fabricated by silicon etching. Baier et al. (US 5,939,312 Aug.1999) describe a miniaturized multi-chamber thermal cycler. This latter reference includes the following features — 1. multiple chambers placed together within a silicon block from which they are thermally isolated. This approach works against fast cycling because of slow cooling by the chambers. 2. The chambers are packed together very closely, with minimal thermal isolation from one another, so all chambers must always to be thermally cycled with the same thermal protocol. The individual chambers were not subject to independent thermal control of multi-chambers. 3. Baier's units have thin-film heaters that cover the whole bottom of the chamber (as in conventional heating designs). 4. Baier's apparatus is limited to the chambers, no micro-fluidic components (valves, fluidic manipulation, flow control, etc.) being included.

Micro-fabricated PCR reaction chambers (or thermal cyclers) have been reported in the technical literature by a number of experimenters, including:

(1). Adam T. Woolley, et al, (UC Berkeley), "Functional Integration of PCR Amplification and Capillary Electrophoresis in a Microfabricated DNA Analysis Device", Analytical Chemistry, Vol. 68, pp. 4081-4086, (2). M. Allen Northrup, et al, (Lawrence Livermore National Lab, UC Berkeley, Roche Molecular Systems), "DNA Amplification with a microfabricated reaction chamber", 7th Intl. Conf. Solid-State Sensors and Actuators, pp. 924-926, (3). Sundaresh N. Brahmasandra, et al, (U. Michigan), "On-Chip DNA Band Detection in Microfabricated Separation Systems", SPIE Conf. Microfuidic Devices and Systems, Santa Clara, California, Sep. 1998, SPIE Vol. 3515, pp. 242-251, (4). S. Poser, et al, "Chip Elements for Fast Thermocycling", Eurosensors X, Leuven, Belgium, Sep 96, pp. 11971199. The latter showed promising results for use of well thermal isolation as a means for achieving quick thermal response.

Also of interest, we may mention: (5). Ajit M. Chaudhari, et al, (Stanford Univ. and PE Applied Biosystems), "Transient Liquid Crystal Thermometry of Microfabricated PCR Vessel Arrays", J. Microelectromech. Systems, Vol. 7, No. 4, 1998, pp. 345-355, (6). Mark A Burns, et al, (U Michigan), "An Integrated Nanoliter DNA Analysis Device", Science 16, Oct 1998, Vol. 282, pp. 484-486, and (7). P.F. Man, et al, (U. Michigan), "Microfabricated Capillary-Driven Stop Valve and Sample Injector", IEEE MEMS'98 (provisional), pp. 45-50.

SUMMARY OF THE INVENTION

It has been an object of the present invention to provide a microfabricated thermal cycler which permits simultaneous treatment of multiple individual samples in independent thermal protocols, so as to implement large numbers of DNA experiments simultaneously in a short time.

A further object of the invention has been to provide a high degree of thermal isolation for the reaction chamber, where there is no cross talk not only between reaction chambers, but also between the reaction chamber and the substrate where detection circuits and/or micro fabricated Capillary Electrophoresis units could be integrated.

Another object has been to achieve temperature uniformity inside each reaction chamber of less than ±0.5K together with fast heating and cooling rates in a range of 10 to 60 K/s range.

These objects have been achieved by use of a thermal isolation scheme realized by silicon etch-through slots in a supporting silicon substrate frame. Each reaction chamber is thermally isolated from the silicon substrate (which is also a heat sink) through one or more silicon beams with fluid-bearing channels that connect the reaction chamber to both a sample reservoir and a common manifold. Each reaction chamber has a silicon

membrane as its floor and a glass sheet as its roof. This reduces the parasitic thermal capacitance and meets the requirement of low chamber volume. The advantage of using glass is that it is transparent so that sample filling and flowing can be seen clearly. Glass can also be replaced by any kind of rigid plastic which is bio- and temperature- compatible.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1a shows a plan view of a first embodiment of the invention.

FIGs. 1b and 1c are orthogonal cross-sections taken through FIG. 1a.

FIG. 2 is a closeup view of a portion of FIG. 1a.

FIG. 3 illustrates the air injector and pressure valve part of the structure.

FIG. 4 shows a group of three cycling chambers integrated within a single unit.

FIG. 5 shows a full population of cycling chambers covering an entire wafer.

FIG. 6 illustrates how the resistor strips may be located inside slots in a conductive silicon beam.

FIG. 7a shows a plan view of a second embodiment of the invention.

FIGs. 7b and 7c are orthogonal cross-sections taken through FIG. 7a.

FIG. 8 is a closeup view of a portion of FIG. 7a.

FIG. 9 is the equivalent of FIG. 1 for the second embodiment.

FIG. 10 shows the starting point for the process of the present invention.

FIGs. 11 and 12 illustrate formation of resistive heaters and temperature sensors.

FIGs. 13 and 14 illustrate the formation of the silicon membrane and etch-through slots that are needed to achieve a high level of thermal isolation for the chamber.

FIG. 15 shows how a sheet of dielectric material is bonded to the top surface to form the chamber.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The basic principle that governs the present invention is that the thermally conductive cycler chamber is thermally isolated from its surroundings except for one or more heat transfer members through which all heat that flows in and out of the chamber passes. Consequently, by placing at least one heating element in each transfer area, heat lost from the chamber can be continuously and precisely replaced, as needed. This is achieved by placing, within the chamber, at least one temperature sensor per heating element and locating this sensor close to the heating elements. Additionally, by connecting the heat transfer areas to a heat sink through a high thermal conductance path, the chamber can also be very rapidly cooled, when so desired.

Also included as part of the structure of the present invention is a fully integrated fluid dispensing and retrieval system. This allows multiple chambers to share both a common heat sink as well as an inlet fluid source reservoir with both fluid flow and temperature being separately and independently controllable. As a result, thermal cross-talk between chambers can be kept to less than about 0.5 °C at a temperature of about 95 °C while temperature uniformity within an individual chamber can be reliably maintained, both theoretically and experimentally, to a level of less than ± 0.3K.

We now disclose two embodiments of the present invention as well as a process

for manufacturing part of the structure.

First embodiment

Referring now to FIG. 1a, the top-left portion is a plan view of the structure. Seen there is chamber 11 which is connected at both ends to silicon frame 1 through monocrystalline silicon beams 10. Heaters 5 are at each end inside the heat transfer areas. The latter are discussed above but are not explicitly shown since they have been introduced into the description primarily for pedagogical purposes. In addition to the heaters, each chamber contains at least one temperature sensor 4 for each heating element 5. They are located close to the heating elements, as shown.

Fluid bearing channels dispense fluid into and remove fluid from the chamber 11. They are brought into the chamber through the silicon beams 10. As can be more clearly seen in the closeup shown in FIG. 2, unprocessed fluid is stored in common reservoir 7 and is directed to chamber 11 through fluid-bearing channel 31. Control of fluid flow is achieved by use of compressed gas (usually, but not necessarily, air), or hydraulic/pneumatic pressure with a gas-liquid interface at the valve, that connects gas source 25 to channel 31 through air injector 19. Since the capillary force drives the fluid from reservoir 7 to valve 8 (FIG. 3), stopping there, an additional pressure impulse will help the

fluid to pass through valve 8 and, after that, no more external pressure is needed as the fluid will continue to flow, being driven by capillary forces.

To prevent unintended entry of fluid into the chamber, pressure valves 8, as seen in FIG. 1c, are placed at both ends of the chamber. A closeup of the area contained within circle 33 of FIG. 2 is shown in FIG. 3 to illustrate how the valves operate. A short length 16 of the fluid-bearing channel is made narrower than the rest of the channel. When fluid coming from the right side reaches point 15 it will be drawn into 16 through surface tension (capillary action) if it wets the inside of the channel (i.e. channel walls are hydrophilic). Then, when the fluid reaches point 17, the same surface tension forces that drew the fluid into 16 will act to hold it inside 16 and prevent it from proceeding down channel 13. If the fluid finds the channel walls to be hydrophobic, then surface tension will act to keep it from entering 16. Either way, additional pressure is needed to make the fluid pass through valve 8. The recorded pressure barriers for water (about 6kPa for valves, > 10kPa for the air injector) are enough to allow on-chip automatic control of fluid flow.

Returning now to FIG. 1a, the fluid-bearing channel on the far side of chamber 11 is seen to terminate at local reservoir 9. When fluid is forced into chamber 11, the air that is already in the chamber is forced out and passes into local (sample) reservoir 9 where it is allowed to escape but without allowing any liquid to enter it. When temperature

cycling has been completed, pressure for the air injector is used to transfer the sample from the chamber into reservoir 9 where it can be collected into a pipette/tube or other collector..

Referring now to FIG. 4, shown there is an example of several chambers integrated to form a single multi-sample recycling unit. As can be seen, the individual chambers 11 are positioned inside the interior open area of silicon frame 1 and are connected to it through silicon beams 10. It is important to note that, except for these beams, the chamber is always thermally isolated from the frame by open space 3 (shown as a thin slot in FIG. 2). Fig. 5 shows how the sub-structure seen in FIG. 4 appears when full wafer 66 of silicon has been used to form multiple chambers.

Returning once more to FIG. 1c, as can be seen, the part of the chamber between valves 8 (where the actual temperature cycling occurs) is effectively a sandwich between glass plate 2 and silicon membrane 12 which is only between about 30 and 100 microns thick. This arrangement enables the physical volume (less than about 100 micro-liters) and thermal capacitance of the chamber to be kept to a minimum.

Also seen in FIG. 1b are bonding pads 6. These facilitate the bonding of glass sheet 2 to the silicon. As a feature of the present invention these pads are placed inside trench 18 as illustrated in FIG. 6. These facilitate the application of anodic bonding to our

structure. Anodic bonding is an excellent bonding technique that allows high stability at high temperature in various chemical environments as no polymer is used. The silicon and glass wafers are heated to a temperature (typically in the range 300-500°C depending on the glass type) at which the alkali metal ions in the glass become mobile. The components are brought into contact and a high voltage applied across them. This causes the alkali cations to migrate from the interface resulting in a depletion layer with high electric field strength. The resulting electrostatic attraction brings the silicon and glass into intimate contact. Further current flow of the oxygen anions from the glass to the silicon results in an anodic reaction at the interface and the result is that the glass becomes bonded to the silicon with a permanent chemical bond.

Note that although we exemplify sheet 2 as being made of glass, other materials such as rigid plastics, fused quartz, silicon, elastomers, or ceramics could also have been used. In such cases, appropriate bonding techniques such as glue or epoxy would be used in place of anodic bonding.

Finally, in FIGs. 1b and 1c we note the presence of heat sink 14 to which the silicon frame 1 is thermally connected. An important advantage of this arrangement is that silicon substrate 1 can be kept close to room temperature rather than near the temperature of the reaction chamber during heating. This facilitates integration of the PCR thermocycler with other parts of micro total-analysis-system (μTAS) on a single chip, as well as for

multi-chamber reaction with independent thermal control, as discussed earlier.

Second embodiment

The second embodiment of the invention is generally similar to the first embodiment except that, instead of being connected to the silicon frame through two silicon beams, only a single cantilever beam is used. This has the advantage over the first embodiment that elimination of asymmetry due to fabrication/packaging and heating is achieved, resulting in easier control and uniformity of temperature. It is illustrated in FIGs. 7a-c and, as just noted, most parts marked there are the same as those shown in FIG. 1a-c.

Since there is only one silicon beam available, it has to be used for both introducing as well as removing liquid to and from the chamber. This has been achieved by the introduction of baffle 76 that is parallel to the surface of the chamber (at the transfer area) and that is orthogonally connected to the transfer area by a sheet of material 84 that serves to separate incoming from outgoing liquid. Its action can be better seen in the closeup provided by FIG. 8. As in the first embodiment, liquid from common reservoir 7 is sent along channel 31 into the chamber. An air injector is also used to accomplish this

although it is not shown in this figure. When the incoming liquid enters the chamber it is directed by baffle 76 to flow in direction 81.

Emptying of the chamber is accomplished in a similar manner to that of the first embodiment except that local sample reservoir 9 is on the same side as the inlet reservoir 7. When the chamber is to be emptied, baffle 76 again directs the flow of liquid, this time in direction 82. Seen in FIG. 7c, but not shown in FIG. 8, is valve 8. There are, of course, two such valves, as in the first embodiment, but the one that can be seen is blocking a view of the other one.

FIG. 9 is analogous to FIG. 4 and illustrates a group of three cycling chambers 11 suspended within the interior open area of silicon frame 1 which is itself part of a full silicon wafer.

Process for manufacturing the invention

We now describe a process for manufacturing the frame portion of the structure of the invention. Before proceeding we note that all figures that follow (FIGs. 10-15) show only the right hand side of the chamber but, since the left side is a mirror reflection of the

right side, the process for manufacturing the entire chamber is readily envisaged.

Referring now to FIG. 10, process begins with the provision of silicon wafer 101, between about 350 and 700 microns thick, in whose upper surface, two inner trenches 103 and two outer trenches 104 are etched to a depth of between about 0.1 and 1 microns. The width of inner trenches 103 is between about 20 and 500 microns while that of outer trenches 104 is between about 50 and 500 microns.

Next , dielectric layer 102 is formed over the entire surface. Its thickness is between about 0.02 and 0.5 microns. Our preferred material for dielectric layer 102 has been silicon oxide formed by thermal oxidation or CVD (chemical vapor deposition) but other materials such as phosphosilicate glass (PSG), silicon nitride, polymers, and plastics could also have been used.

Next, as seen in FIG. 11, a layer of a material that is suitable for use as a temperature sensor (thermistor) 105 and also as a resistive heater is deposited to a thickness between about 1,000 and 10,000 Angstroms. Our preferred material for this has been aluminum but other materials such as gold, chromium, titanium, or polysilicon could also have been selected. This layer is then patterned and etched to form temperature sensors and the heater element. Bonding strips 106 are also shown.

Moving on to FIG. 12, two top preliminary trenches 112 are then etched into the top surface to a depth of between about 30 and 100 microns and a width of between about 20 and 100 microns. The trenches 112 are located between inner trenches 103 and outer trenches 104, each about 100 microns from the inner trench.

Next, as seen in FIG. 13, the upper surface of the wafer is patterned and etched to form chamber trench 113. This is centrally located between the inner trenches 103 and is given a depth between about 30 and 500 microns and a width between about 100 and 10,000 microns. Trench 112 is not protected while trench 113 is being formed so that at the end of this step in the process, its depth will have increased. Also at this stage, second dielectric layer 132 is formed on all surfaces that don't already have a dielectric layer on them. Its thickness is between about 1,000 and 5,000 Angstroms. In FIG. 13, the newly extended and lined trench 112 is now designated as trench 131. Its depth is between about 60 and 600 microns.

Referring now to FIG. 14, the lower surface of the wafer is patterned and etched to form under-trench 141. This is wide enough to slightly overlap the top preliminary trenches 131 and it is deep enough so that, at the completion of this step, trench 131 will be penetrating all the way through to the wafer's under-side and the wafer thickness (under trench 113) will have been reduced to between about 30 and 100 microns. In this way, silicon membrane 12 and frame 1, as shown in earlier figures, will have been formed.

The final step in the process is illustrated in FIG. 15. Sheet of dielectric material 152 is micro-machined to form holes in selected locations (as an example, see 9 in FIG. 1) and then bonded to the wafer to form a hermetically sealed chamber that is thermally isolated from the wafer by slot 3. For sheet 152, our preferred material has been glass which we then bonded to the wafer by means of anodic bonding. However, as noted earlier, other materials such as rigid plastics, fused quartz, silicon, elastomers, or ceramics could also have been used. In such cases, appropriate bonding techniques such as glue or epoxy would be used in place of anodic bonding. Finally, an etching step is used to remove the second dielectric layer 132 in the open areas that contain bond-pads for electrical connections.

Results

By using the above described structures and manufacturing process, we have been able to both build and simulate units that meet the following specifications:

Heating power: < 1.7 Watt

Heating voltage: 8 volts

Ramp rate: 15-100 °C/s

Cooling rate: 10-70 °C/s

Temperature uniformity: < ± 0.3 °C (accuracy ± 0.2 °C)

Cross-talk: < 0.4 °C at 95 °C

The effectiveness of the units for Micro PCR use reaction was verified with the Plasmid/Genomic DNA reaction and agarose gel electrophoresis. The result was adequate amplification in a reduced reaction time relative to existing commercial PCR machines. It was also confirmed that the units may be reused after cleaning.

While the invention has been particularly shown and described with reference to the preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made without departing from the spirit and scope of the invention. The miniaturized thermal cycler of the present invention may, for example, be used as a thermal cycling chamber for various types of biological and/or chemical reactions.

What is claimed is:

CLAIMS

1. A thermal cycling unit, comprising:

a chamber, thermally isolated from its surroundings except for one or more heat transfer areas through which all heat that flows in and out of the chamber passes;

within the chamber, at least one heating element per transfer area, each such heating element being located within a transfer area;

a first fluid bearing channel that connects to the chamber through a first orifice located within a transfer area;

a second fluid bearing channel that connects to the chamber through a collection orifice located within a transfer area;

within the chamber, at least one temperature sensor per heating element located close to that heating element; and

means for sending fluid into, and removing fluid from, the chamber through said channels and orifices.

2. The thermal cycling unit described in claim 1 wherein there is a plurality of chambers each of which can be independently heated and cooled.

3. The thermal cycling unit described in claim 1 wherein there is a plurality of chambers and thermal cross-talk between the chambers is less than about 0.5 °C at temperatures ranging from about 20 to 95 °C.

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4. A structure for thermal cycling, comprising:

a frame made of a thermally conductive material and having an open interior area;

suspended within said open area, a plurality of chambers in the form hollow bodies having first and second opposing ends each of which is connected to the frame through a thermally conductive beam;

within each chamber, at each end, a heat transfer area through which all heat that flows in and out of the chamber passes;

within each chamber, two heating elements, one each symmetrically disposed around one each of said transfer areas;

for each chamber, a first fluid bearing channel that enters the chamber at its first end through a first orifice located within the transfer area at that end;

for each chamber, a second fluid bearing channel that enters the chamber at its second end through a second orifice located within the transfer area at that end;

within each chamber, at least one temperature sensor per heating element, said sensor being located close to said heating element; and

means for sending fluid into, and removing fluid from, each chamber through said channels and orifices whereby fluid flow into and out of each chamber is individually controllable.

5. The structure described in claim 4 wherein the frame is thermally connected to a heat sink.

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6. The structure described in claim 4 wherein the frame and the beams are thermally conductive materials selected from the group consisting of monocrystalline silicon germanium and gallium arsenide, metals, and ceramics.

7. The structure described in claim 4 wherein each chamber further comprises a silicon membrane between about 30 and 100 microns thick surrounded by sidewalls that have been anodically bonded to a sheet of glass, whereby the chamber has low thermal capacitance.

8. The structure described in claim 4 wherein the heating elements in each chamber are independently controllable.

9. The structure described in claim 4 wherein the said fluid bearing channels are located inside the beams.

10. The structure described in claim 4 wherein said means for sending fluid into each chamber further comprises a source of compressed gas connected to the first channel, said compressed gas causing liquid to flow into the chamber from a common reservoir..

11. The structure described in claim 10 wherein said means for removing fluid from each chamber further comprises a local reservoir into which gas from the chamber is

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forced when, under pressure from said compressed gas or from hydraulic or pneumatic interaction with a gas-liquid interface at the valve, the liquid fills the chamber.

12. The structure described in claim 4 wherein the fluid bearing channels include at least one pressure barrier capable of stopping both hydrophillic and hydrophobic liquid flow.

13. The structure described in claim 12 wherein said pressure barrier further comprises a section of the fluid-bearing channel that is narrower than other parts of the channel.

14. The structure described in claim 4 wherein each chamber has an interior volume that is less than about 100 micro-liters.

15. The structure described in claim 4 wherein thermal cross-talk between the chambers is less than about 0.5 °C at temperatures ranging from about 20 to 95 °C.

16. A structure for thermal cycling, comprising:

a frame made of a thermally conductive material and having an open interior area;

suspended within said open area, a plurality of chambers in the form hollow bodies each being connected to the frame through a single thermally conductive beam;

within each chamber a heat transfer area, having an interior surface, through which

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all heat that flows in and out of the chamber passes;

within each chamber, a heating element symmetrically disposed inside the transfer area;

for each chamber, a first fluid bearing channel that enters the chamber through a first orifice located within the transfer area;

for each chamber, a second fluid bearing channel that enters the chamber through a second orifice located within the transfer area;

within each chamber, a baffle that is parallel to the surface of the transfer area and that is orthogonally connected to the transfer area by a sheet of material that comes between the first and second orifices;

within each chamber, at least one temperature sensor per heating element, said sensor being located close to said heating element; and

means for sending fluid into, and removing fluid from, each chamber through said channels and orifices whereby fluid flow into and out of each chamber is individually controllable.

17. The structure described in claim 16 wherein the frame is thermally connected to a heat sink.

18. The structure described in claim 16 wherein the frame and the beam are monocrystalline silicon.

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19. The structure described in claim 16 wherein each chamber further comprises a silicon membrane between about 30 and 100 microns thick, surrounded by sidewalls that have been anodically bonded to a sheet of glass, whereby the chamber has low thermal capacitance.

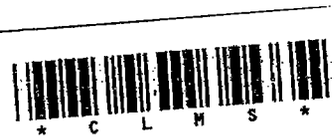
20. The structure described in claim 16 wherein the heating elements in each chamber are independently controllable.

21. The structure described in claim 16 wherein the said fluid bearing channels are located inside the beam.

22. The structure described in claim 16 wherein said means for sending fluid into each chamber further comprises a source of compressed gas connected to the first channel, said compressed gas causing liquid to flow into the chamber from a common reservoir.

23. The structure described in claim 22 wherein said means for removing fluid from each chamber further comprises a local reservoir into which gas from the chamber is forced when, under pressure from said compressed gas, the liquid fills the chamber.

24. The structure described in claim 16 wherein the fluid bearing channels include at least one pressure barrier capable of stopping both hydrophillic and hydrophobic liquid



flow.

25. The structure described in claim 24 wherein said pressure barrier further comprises a section of the fluid-bearing channel that is narrower than other parts of the channel.

26. The structure described in claim 16 wherein each chamber has an interior volume that is less than about 100 micro-liters.

27. The structure described in claim 16 wherein thermal cross-talk between the chambers is less than about 0.5 °C at temperatures ranging from about 20 to 95 °C.

28. A process for manufacturing a thermal cycler, comprising the sequential steps of:

providing a silicon wafer having upper and lower surfaces;

in said upper surface, etching two inner and two outer trenches to a first depth, said inner tenches having a first width and said outer trenches having second width;

forming a first dielectric layer on said upper surface, including said trenches;

depositing a layer of material suitable for use as a sensor and as a resistive heater;

patterning and etching the material layer to form temperature sensors and heater elements;

in said upper surface etching, to a second depth, two top preliminary trenches having a third width, each being located between an inner trench and an outer trench;

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patterning and etching said upper surface whereby a chamber trench, having a fourth width and located between said inner trenches, is formed to a third depth and the top preliminary trenches have their depth increased to a fourth depth;

forming a second dielectric layer on said upper surface, including all trenches;

patterning and etching the lower surface of the wafer to form an under-trench that is wide enough to slightly overlap the top preliminary trenches, to a depth such that the top preliminary trenches extend through said lower surface and, within the chamber trench, the wafer has a thickness that is between about 30 and 100 microns;

providing a sheet of dielectric material and micro-machining said sheet to form holes in selected locations; and

bonding the sheet to the wafer thereby forming a hermetically sealed chamber that is thermally isolated from the wafer.

29. The process described in claim 28 wherein, at the start of the process the silicon wafer has a thickness between about 350 and 700 microns.

30. The process described in claim 28 wherein said first trench depth is between about 0.1 and 1 microns, said inner trenches' first width is between about 20 and 500 microns and said outer trenches' second width is between about 50 and 500 microns.

31. The process described in claim 28 wherein the first dielectric layer is selected from

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the group consisting of silicon oxide, phosphosilicate glass, silicon nitride, polymers, and plastics.

32. The process described in claim 28 wherein the layer of material suitable for use as a sensor and as a resistive heater is selected from the group consisting of monocrystalline silicon germanium and gallium arsenide, metals, and ceramics.

33. The process described in claim 28 wherein said second depth of the two top preliminary trenches is between about 30 and 100 microns and their third width is between about 20 and 100 microns and each top preliminary trench is about 100 microns from an inner trench

34. The process described in claim 28 wherein said fourth width of the chamber trench is between about 100 and 10,000 microns and said increased fourth depth of the top preliminary trenches is between about 60 and 600 microns.

35. The process described in claim 28 wherein the second dielectric layer is formed to a thickness between about 0.1 and 0.5 microns.

36. The process described in claim 28 wherein said under-trench has a width between about 200 and 12,000 microns and a depth between about 50 and 500 microns.

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37. The process described in claim 28 wherein said sheet of dielectric material is glass and bonding of the sheet to the wafer is achieved by means of anodic bonding.

38. The process described in claim 28 wherein said sheet of dielectric material is selected from the group consisting of rigid plastics, fused quartz, silicon, elastomers, and ceramics, and bonding is by means of glue or epoxy.

