



US009387478B2

(12) **United States Patent**
Bergstedt et al.

(10) **Patent No.:** **US 9,387,478 B2**

(45) **Date of Patent:** **Jul. 12, 2016**

(54) **MICRO-FLUIDIC MODULES ON A CHIP FOR DIAGNOSTIC APPLICATIONS**

B01L 2300/087; B01L 2300/0883; B01L 2300/1827; B01L 2300/1883; B01L 2400/0442; B01L 3/50273; B01L 7/525

(71) Applicant: **Lexmark International, Inc.**,
Lexington, KY (US)

USPC 435/303.1
See application file for complete search history.

(72) Inventors: **Steven W Bergstedt**, Winchester, KY (US); **Stephen J DeMoor**, Georgetown, KY (US); **Yimin Guan**, Lexington, KY (US)

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,318,114 A 3/1982 Huliba
6,283,718 B1 9/2001 Prosperetti
6,431,694 B1 8/2002 Ross
6,655,924 B2 12/2003 Ma
6,685,303 B1 2/2004 Trauernicht

(Continued)

FOREIGN PATENT DOCUMENTS

WO WO 2005094981 A1 * 10/2005

OTHER PUBLICATIONS

Jr-Hung Tsai; Liwei Lin, A Thermal-Bubble-Actuated Micronozzle-Diffuser Pump, Journal of Microelectromechanical Systems, Vo. 11, No. 6, Dec. 2002.

(Continued)

Primary Examiner — Nathan Bowers
Assistant Examiner — Lydia Edwards

(57) **ABSTRACT**

A micro-fluidic device includes a plurality of heaters on a substrate for heating the substrate. The plurality of heaters define a plurality of temperature regions having distinct temperatures on the substrate. A flow feature layer is formed above the substrate to define a channel extending across the substrate through each temperature region. As fluid is repeatedly pumped within the channel, it flows from one temperature region to a next temperature region to undergo thermal cycling.

3 Claims, 10 Drawing Sheets

(73) Assignee: **LEXMARK INTERNATIONAL, INC.**, Lexington, KY (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 107 days.

(21) Appl. No.: **13/967,838**

(22) Filed: **Aug. 15, 2013**

(65) **Prior Publication Data**

US 2014/0051161 A1 Feb. 20, 2014

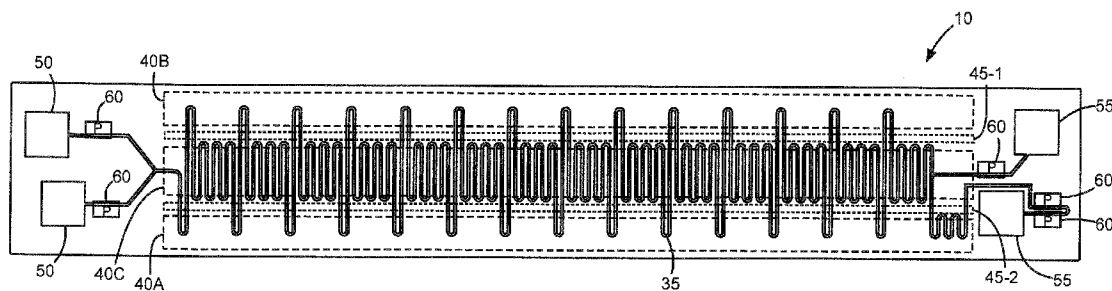
Related U.S. Application Data

(60) Provisional application No. 61/684,599, filed on Aug. 17, 2012.

(51) **Int. Cl.**
B01L 7/00 (2006.01)
B01L 3/00 (2006.01)

(52) **U.S. Cl.**
CPC **B01L 7/525** (2013.01); **B01L 3/50273** (2013.01); **B01L 2200/147** (2013.01); **B01L 2300/087** (2013.01); **B01L 2300/0816** (2013.01); **B01L 2300/0883** (2013.01); **B01L 2300/1827** (2013.01); **B01L 2300/1883** (2013.01); **B01L 2400/0442** (2013.01)

(58) **Field of Classification Search**
CPC B01L 2200/147; B01L 2300/0816;



(56)

References Cited

U.S. PATENT DOCUMENTS

6,869,273	B2	3/2005	Crivelli	
7,374,274	B2	5/2008	Cornell	
2005/0009101	A1*	1/2005	Blackburn	435/7.1
2009/0311713	A1*	12/2009	Pollack	B01L 3/502792 435/287.2
2012/0007921	A1	1/2012	Govyadinov	

OTHER PUBLICATIONS

Jung-Yeul Jung; Ho-Young Kwak, Fabrication and Testing of Bubble Powered Micropumps using Embedded Microheater, Research Paper, Department of Mechanical Engineering, Chung-Ang University, Seoul, South Korea, Received Jun. 16, 2006, Accepted Aug. 1, 2006, Published online Sep. 16, 2006, Springer-Verlag.

D.J. Laser; J.G. Santiago, A review of Micropumps, *Journal of Micromechanics and Microengineering*, 14 (2004) p. R-35-R-64, Institute of Physics Publishing.

Jun et al., Microscale Pumping with Traversing Bubbles in Microchannels, Solid-State Sensor and Actuator Workshop, Hilton Head Island, SC, Jun. 3-6, 1996.

Chen, Zongyuan; Mauk, Michael G.; Wang, Jing; Abrams, William R.; Corstjens, Paul, L.A.M.; Niedbala, R. Sam; Malamud, Daniel; Bau, Haim H., A Microfluidic System for Saliva-Based Detection of Infectious Diseases, *Ann. N. Y. Acad. Sci* 1098: 429-436 (2007) © 2007 New York Academy of Sciences doi: 10.1196/annals.1384.024.

Zhang, Chunsun; Xing, Da; Li, Yuyuan; Micropumps, microvalves and micromixers within PCR microfluidic chips: Advances and trends, Research review paper, pp. 483-514, MOE Key Laboratory of Laser Life Science & Institute of Laser Life Science, South China Normal University, No. 55, Zhongshan Avenue West, Tianhe District, Guangzhou 510631, PR China, Received Mar. 26, 2007; received in revised form May 6, 2007, accepted May 17, 2007, Available online May 23, 2007.

Zhang, Yonghao; Ozdemir, Pinar; Microfluidic DNA Amplification—a Review; Department of Mechanical Engineering, University of Strathclyde, Glasgow, G1 1XJ UK, pp. 1-26.

STMICROELECTRONICS—Microfluidics Division; Lab-on-Chip R&D; A highly integrated Lab on Chip device for rapid DNA testing in diagnostic applications; powerpoint slides.

Mastrangelo, Carlos H.; Burns, Mark A.; Burke, David T.; Microfabricated Devices for Genetic Diagnostics; Proceedings of the IEEE, vol. 86, No. 8, Aug. 1998, pp. 1769-1787.

Roper, Michael G.; Legendre, Lindsay A.; Bienvenue, Joan M.; Ferrance, Jerome P.; Landers, James P.; Toward an Integrated Microdevice for DNA Extraction and PCR Amplification in the Submicroliter Regime for Forensic DNA Analysis; pp. 1-5.

Joung, Seung-Ryong; Kim, Jaewan; Choi, Y.J.; Kim, Kang and Yong-Sang; ITO-coated glass/polydimethylsiloxane continuous-flow PCR chip; Proceedings of the 2nd IEEE International Conference on Nano/Micro Engineered and Molecular Systems; Jan. 16-19, 2007, Bangkok, Thailand, pp. 691-694.

Chen, Lin; Manz, Andreas; Day, Philip J.R.; Total nucleic acid analysis integrated on microfluidic devices; Critical Review, The Royal Society of Chemistry; Lab Chip, 2007, 7, pp. 1413-1423.

Erickson, David; Li, Dongqing; Integrated microfluidic devices; A review, Department of Mechanical and Industrial Engineering, University of Toronto, 5 King's College Road, Toronto, Ontario, Canada M5S 3G8; Received Aug. 6, 2003, accepted Sep. 3, 2003; pp. 11-26.

Day, Philip J.R.; Miniaturized PCR systems for cancer diagnosis; Biochemical Society Transactions (2009) vol. 37. part 2 pp. 424-426.

Foglieni, Barbara; Brisci, Angela; San Biagio, Floriana; Di Pietro, Patrizia; Petralia, Salvatore; Conoci, Sabrina; Ferrari, Maurizio; Cremonesi, Laura; Integrated PCR amplification and detection processes on a Lab-on-Chip platform: a new advanced solution of molecular diagnostics; *Clin Chem Lab Med* 2010; 48(3); pp. 329-336.

Yoon, Dae Sung; Lee, You-Seop; Lee, Youngsun; Cho, Hye Jung; Sung, Su Whan; Oh, Kwang W.; Cha, Junhoe; Lim, Geunbae; Precise temperature control and rapid thermal cycling in a micromachined DNA polymerase chain reaction chip; Institute of Physics Publishing, *Journal of Micromechanics and Microengineering*; Received Mar. 28, 2002, in final form Jul. 23, 2002, Published Oct. 3, 2002; pp. 813-823.

* cited by examiner

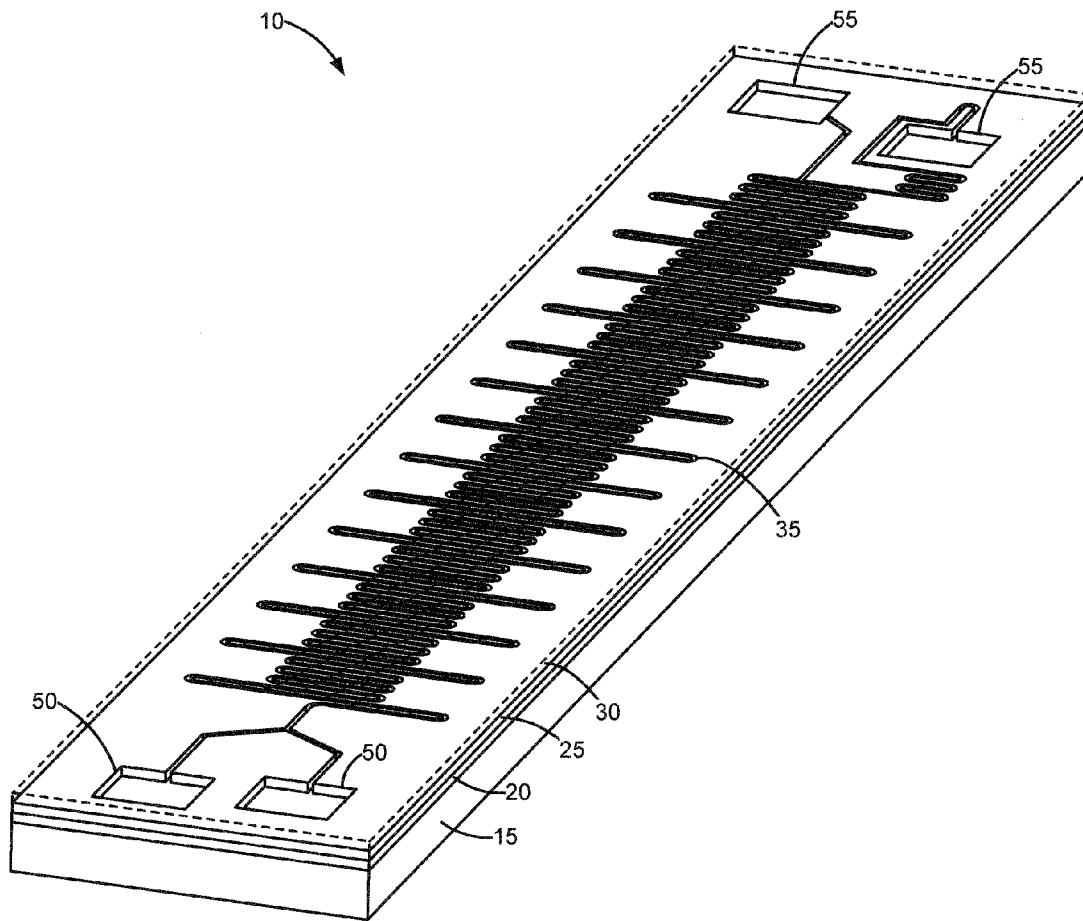


Figure 1

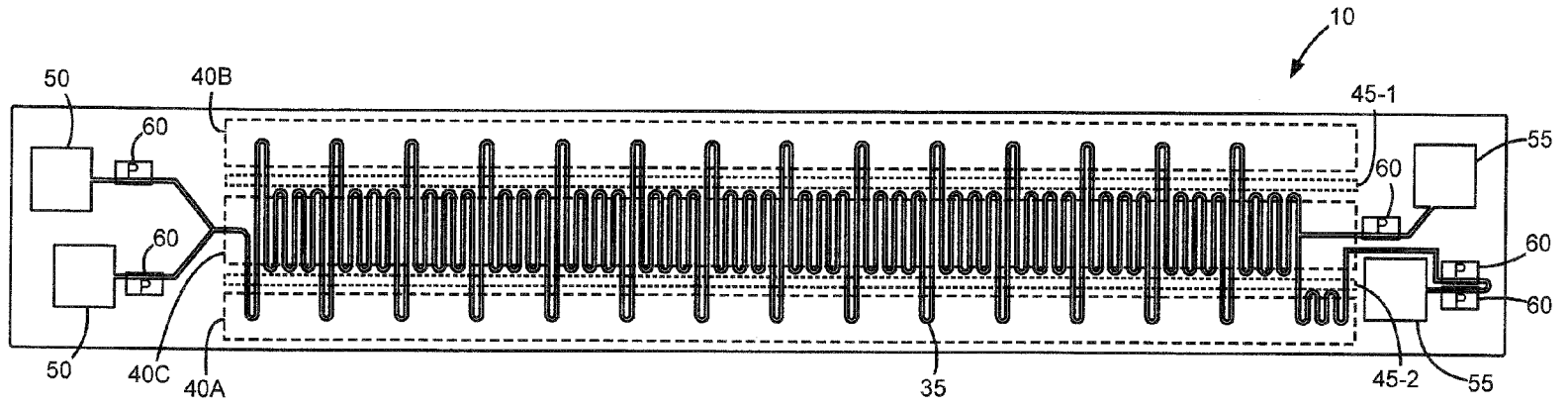


Figure 2

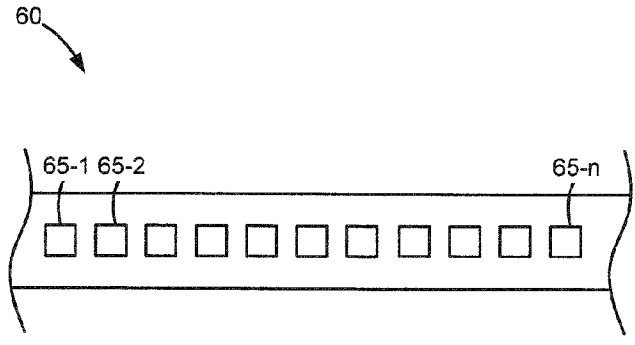


Figure 3

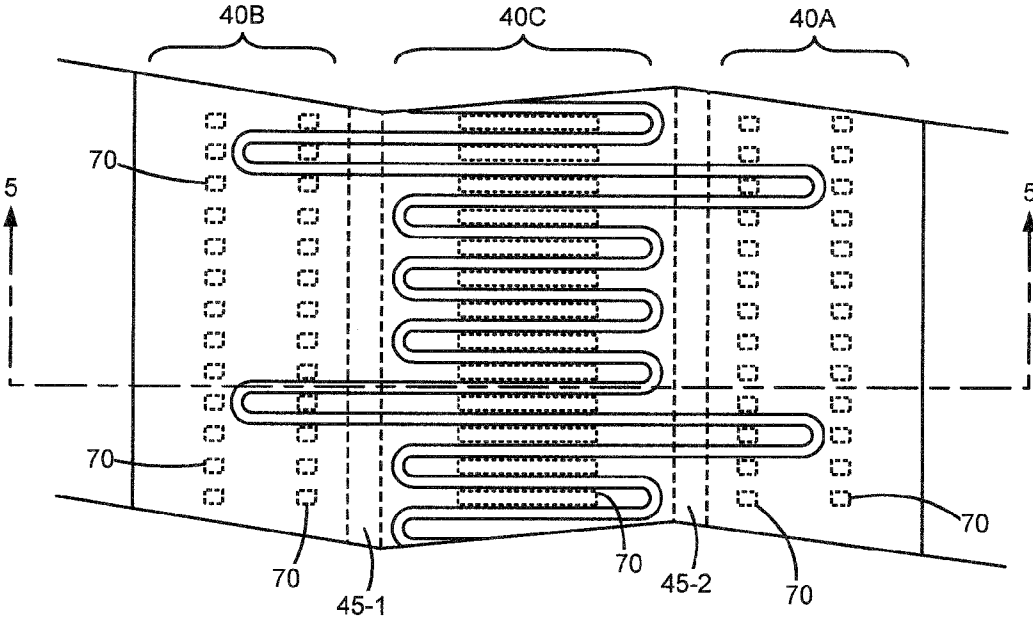


Figure 4

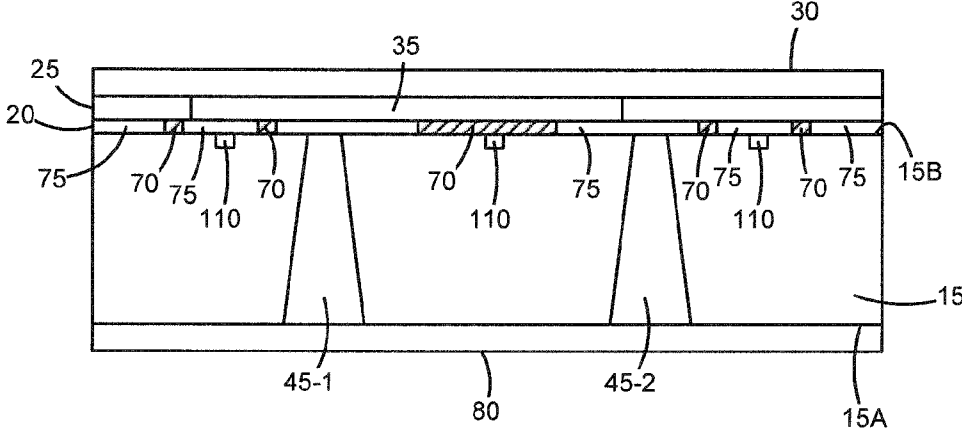


Figure 5

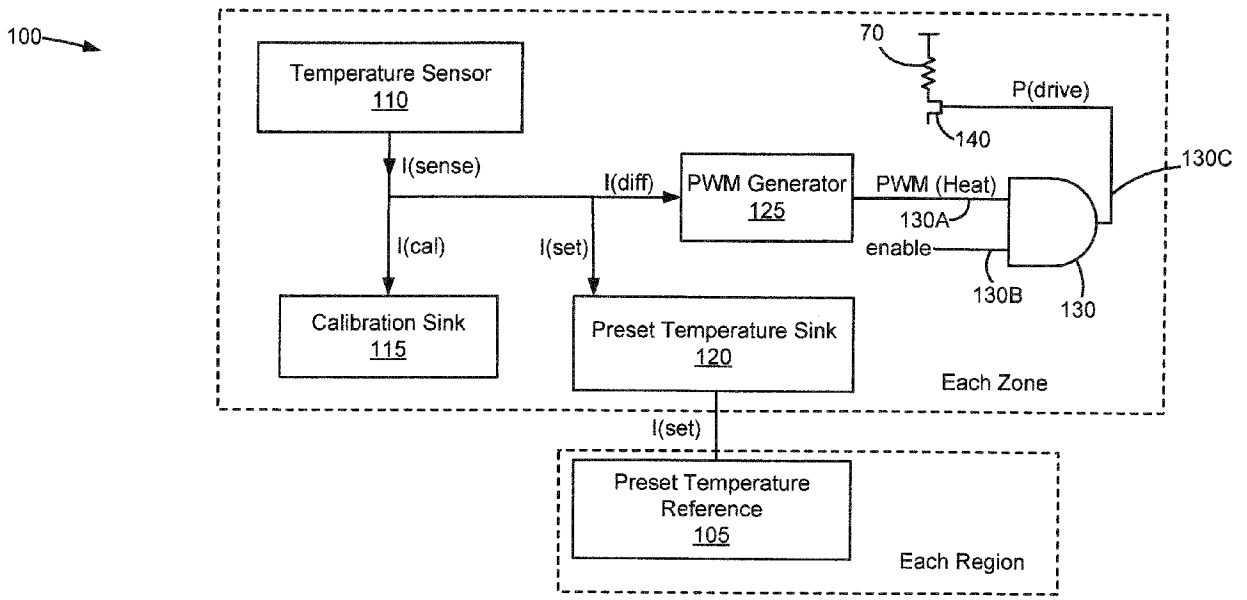


Figure 6

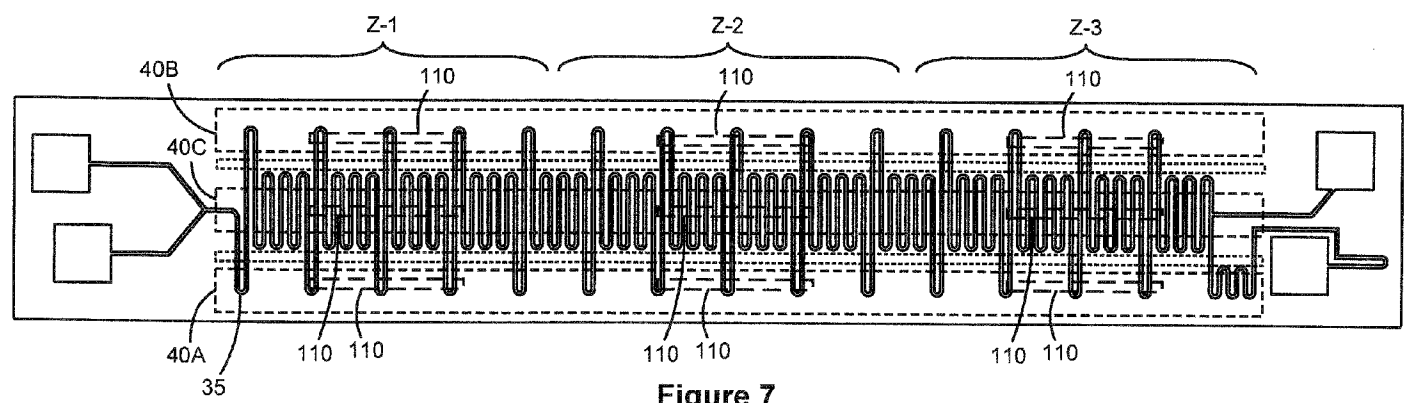


Figure 7

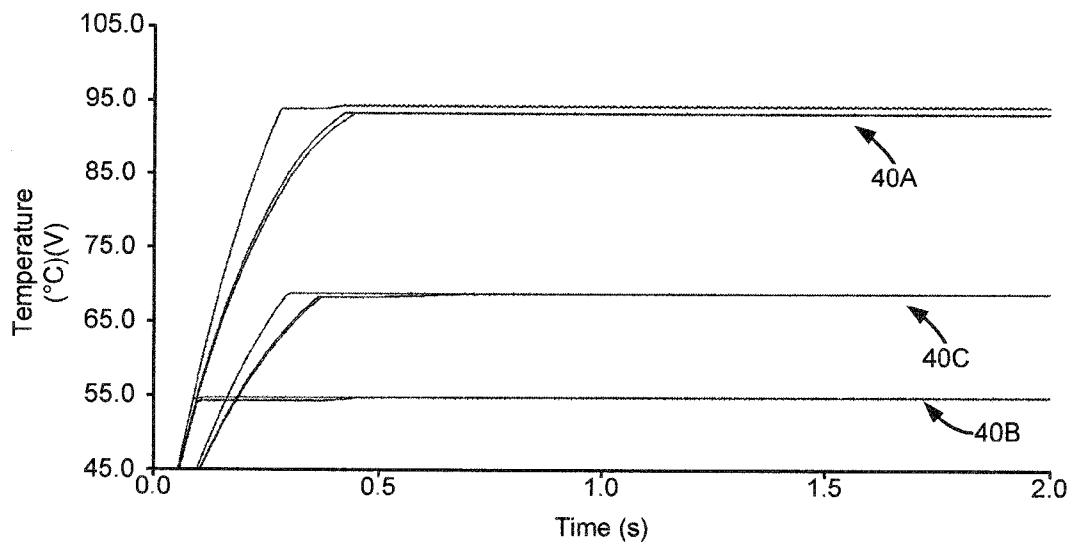


Figure 8

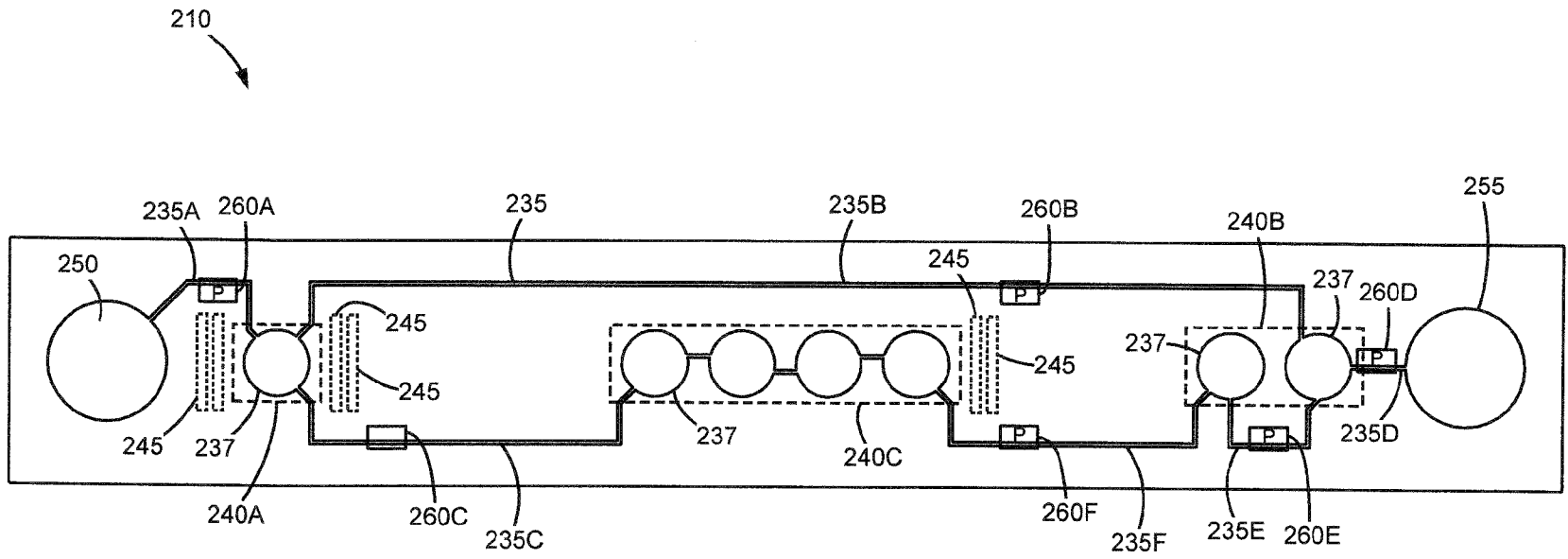


Figure 9

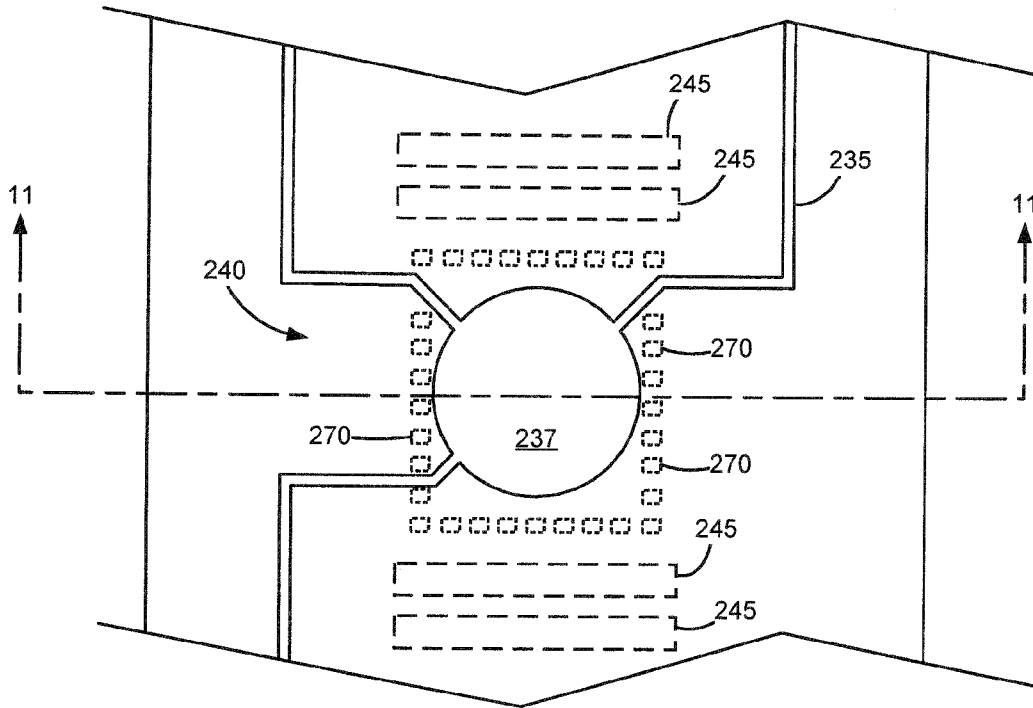


Figure 10

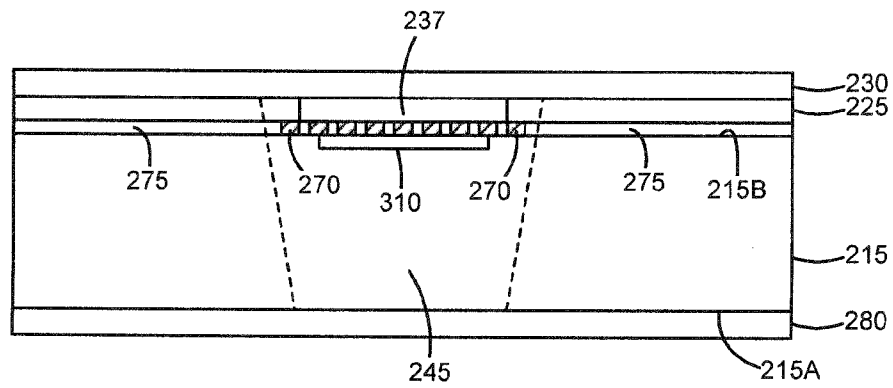


Figure 11

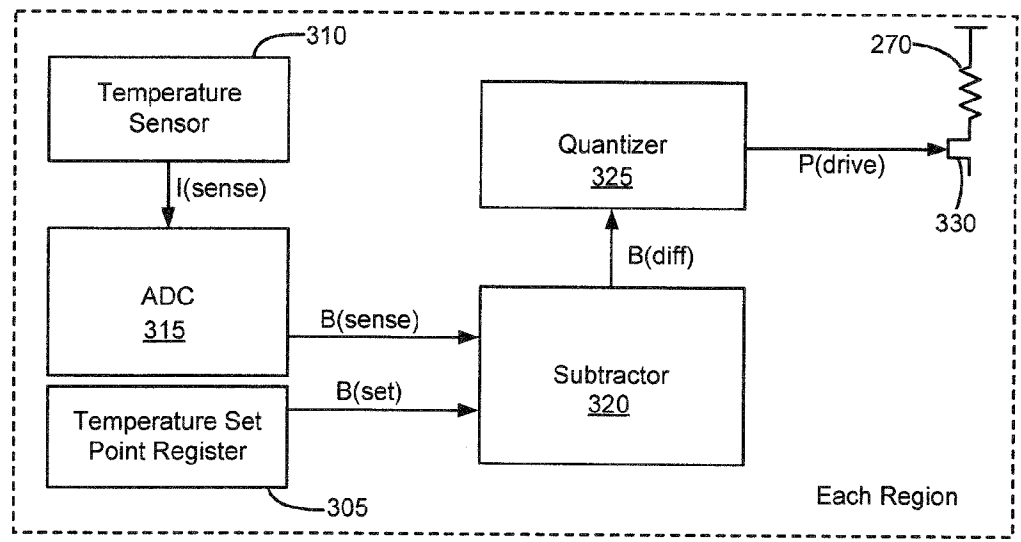


Figure 12

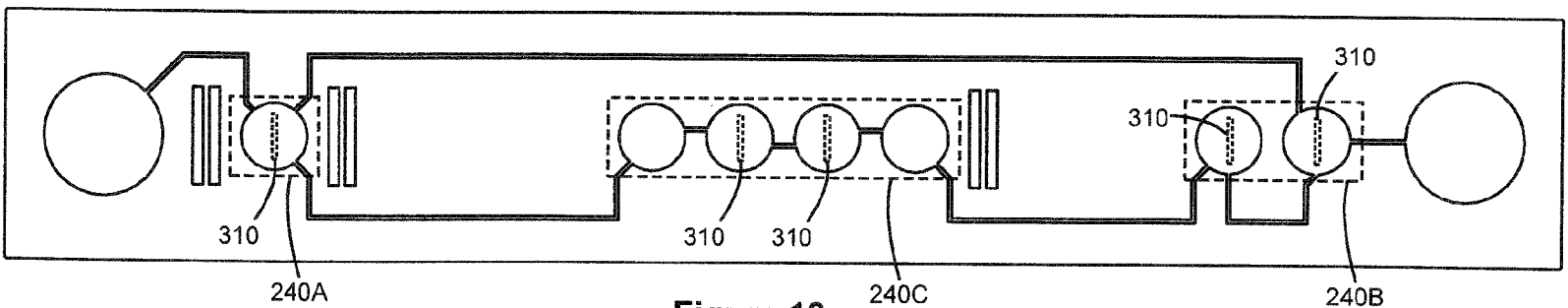


Figure 13

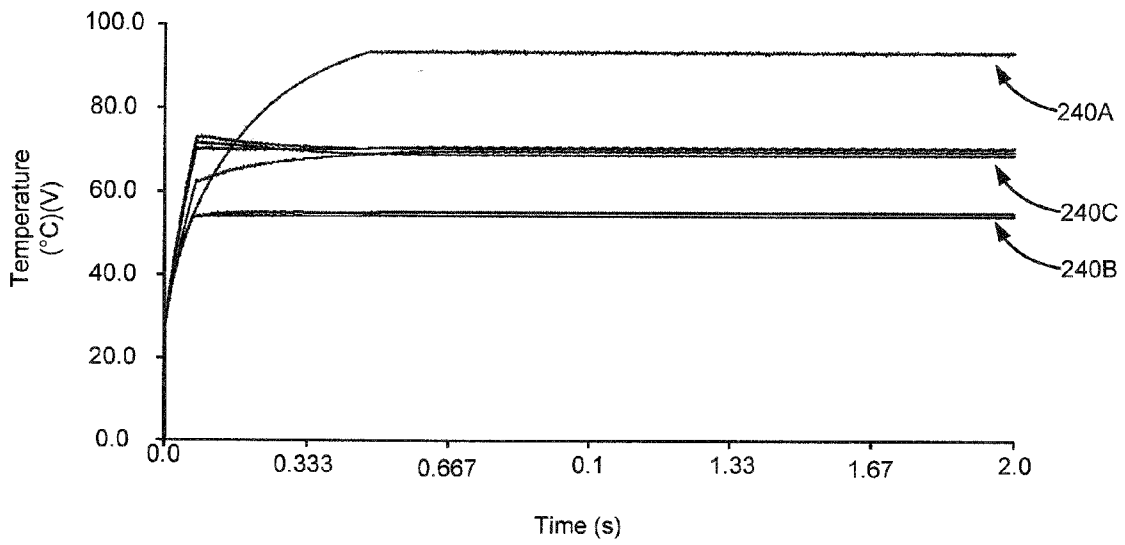


Figure 14

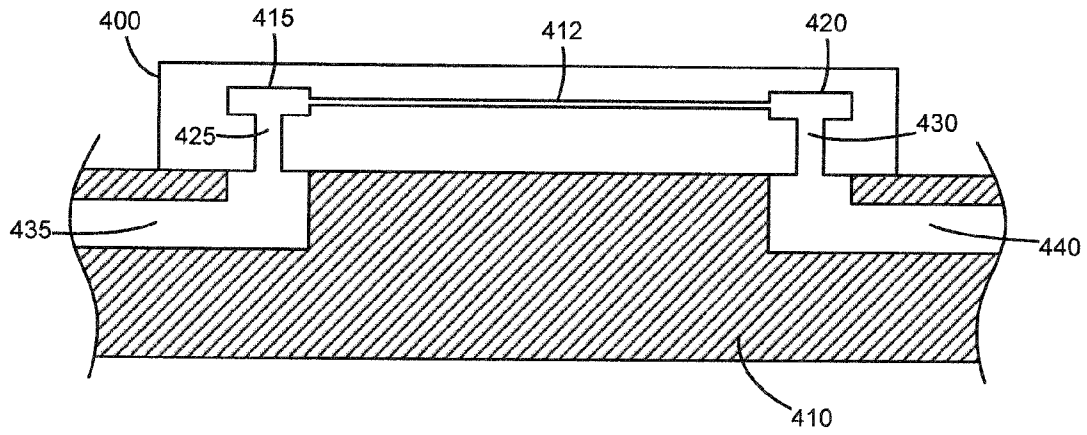


Figure 15

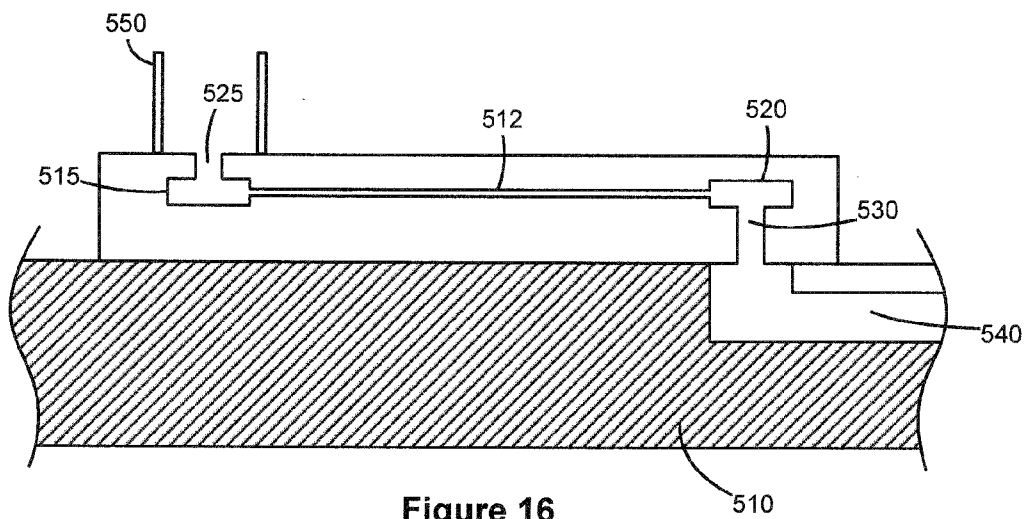


Figure 16

1

MICRO-FLUIDIC MODULES ON A CHIP FOR DIAGNOSTIC APPLICATIONS

FIELD OF THE INVENTION

The present invention relates to micro-fluid applications. More particularly, it relates to point-of-care and lab-on-a-chip devices having functional modules that enable diagnostic solutions.

BACKGROUND

Various diagnostic platforms utilize thermal cycling processes which involve heating of reagents at different temperatures to alter certain properties of the reagents. An example application is Polymerase Chain Reaction (PCR) which is a method used to amplify genetic material for detection and analysis. Analysis speed is especially important in diagnostic applications. For example, shorter analysis time would allow faster turnaround time in identifying infectious diseases, or enable the analysis to take place in the time it takes for a physician's appointment.

Thermal cycling methods generally fall under two categories: stationary and continuous flow. Stationary systems conduct thermal cycling by holding a fixed volume of sample fluid and/or reagents stationary in a chamber while varying the temperature of the chamber to alternately heat and cool the reagents. A disadvantage of this kind of thermal cycling is reduced amplification efficiency due to heating and cooling ramping rates associated with varying the chamber temperature during each cycle. Continuous flow systems, on the other hand, conduct thermal cycling by allowing fluid samples to flow through different temperature regions. In particular, each temperature region maintains a distinct temperature and reagents are allowed to pass through the temperature regions for a number of cycles by propelling them, using pumps, to flow through a long channel having sections formed on each temperature region. Delay in inter-temperature transition time can be reduced by controlling the flow rate of fluids within the channel. As a result, continuous flow systems can shorten analysis times compared to stationary thermal cycling.

A number of micro-fluidic approaches to diagnostic applications utilizing continuous flow thermal cycling have been developed for lab-on-a-chip and point-of-care devices. Micro-fluidic devices manipulate microscopic volumes of liquid inside micro-sized structures. As such, it can provide advantages over conventional and non-micro-fluidic based techniques such as smaller sample volumes, greater efficiency of chemical reagents, high speed analysis, high throughput, portability and low production costs per device allowing for disposability.

Micro-fluidic modules can be built by combining several components like channels, connectors, filters, mixers, heaters, sensors, micro-valves, micro-fluidic pumps, and etc. Among these components, it is well known to be difficult to attain micro-fluidic pumps which are ready to be assembled with micro-fluidic devices at low costs. For example, while a range of micro-fluidic devices have been miniaturized to the size of a postage stamp, these devices have often required large external pumping systems for fluid transport through channels. Unfortunately, the inclusion of these external pumps presents added complexity in coupling with fluidic channels, and also often increases the overall size of the micro-fluidic system.

Thus, there is a need for a micro-fluidic system which integrates together functional modules, such as pumps and

2

micro-fluidic structures, to provide reliable and even smaller device footprint for point-of-care diagnostic and lab-on-a-chip applications. Additional benefits and alternatives are also sought when devising solutions.

SUMMARY

The above-mentioned and other problems become solved by forming pump modules and fluidic structures monolithically on a substrate to provide a micro-fluidic system on a chip. Example embodiments utilize heater chip fabrication methods to achieve results.

In a representative embodiment, a micro-fluidic device includes a substrate and a plurality of heaters on the substrate for heating the substrate. The plurality of heaters define a plurality of temperature regions on the substrate, each temperature region having a distinct temperature. A flow feature layer formed above the substrate defines a channel that extends across the substrate through each temperature region so that when fluid is pumped within the channel, it flows from one temperature region to a next temperature region to undergo repeated heating and cooling. Repeated heating and cooling of fluids as pumping is continued thermally cycles the fluid.

In an example aspect, at least one pump is disposed along the channel for pumping fluid in the channel. In another example aspect, at least one trench is formed from a backside to a top surface of the substrate, and extended between adjacent temperature regions to thermally isolate the temperature regions from each other. In yet another example aspect, a heat sink is mounted beneath the substrate to collect heat residue between adjacent temperature regions so as to reduce temperature gradients therebetween.

These and other embodiments are set forth in the description below. Their advantages and features will become readily apparent to skilled artisans. The claims set forth particular limitations.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings incorporated in and forming a part of the specification, illustrate several aspects of the present invention, and together with the description serve to explain the principles of the invention. In the drawings:

FIG. 1 is a perspective view of a micro-fluidic continuous flow PCR device, according to an example embodiment;

FIG. 2 is a top view of the PCR device in FIG. 1;

FIG. 3 is a schematic view of a micro-fluidic pump;

FIG. 4 illustrates a top view of a portion of the PCR device shown in FIG. 2;

FIG. 5 shows a stack structure of the PCR device in FIG. 1 taken along line 5-5 of FIG. 4;

FIG. 6 is a schematic diagram of an on-chip thermal control system on the PCR device of FIG. 1, according to an example embodiment;

FIG. 7 is a top view of the PCR device in FIG. 1 showing temperature sensors disposed on different temperature regions and zones of the PCR device;

FIG. 8 is a simulation chart illustrating temperatures of each temperature region of the PCR device in FIG. 1, relative to time;

FIG. 9 is schematic view of a PCR device, according to another example embodiment;

FIG. 10 illustrates a top view of a portion of the PCR device in FIG. 9;

FIG. 11 shows a stack structure of the PCR device taken along line 11-11 of FIG. 10;

FIG. 12 is a schematic diagram of an on-chip thermal control system on the PCR device of FIG. 9, according to an example embodiment;

FIG. 13 is a top view of the PCR device in FIG. 9 showing temperature sensors disposed on different thermal regions;

FIG. 14 is a simulation chart illustrating temperatures of each thermal region of the PCR device in FIG. 9, relative to time.

FIG. 15 is a diagram of a PCR device mounted as a lab-on-a-chip device, according to an example embodiment; and

FIG. 16 is a diagram of a PCR device mounted as a lab-on-a-chip device, according to another example embodiment.

DETAILED DESCRIPTION OF THE ILLUSTRATED EMBODIMENTS

In the following detailed description, reference is made to the accompanying drawings where like numerals represent like details. The embodiments are described in sufficient detail to enable those skilled in the art to practice the invention. It is to be understood that other embodiments may be utilized and that process, electrical, and mechanical changes, etc., may be made without departing from the scope of the invention. The following detailed description, therefore, is not to be taken in a limiting sense and the scope of the invention is defined only by the appended claims and their equivalents. In accordance with the features of the invention, a micro-fluidic system for thermal cycling integrates pump modules and fluidic structures on a single chip to enable diagnostic solutions. The modules are monolithically fabricated on a substrate using inkjet technology and heater chip fabrication techniques. In the following embodiments, representative diagnostic solutions contemplate a case study for polymerase chain reaction (PCR).

PCR is a process by which genetic material, such as DNA, is amplified exponentially for detection and analysis. PCR relies on cycling a mixture of ingredients including DNA sample, primers, and enzymes used for DNA synthesis, among others, through a series of repeated temperature changes, called cycles, to repeatedly heat and cool the mixture of ingredients. Typically, PCR has three thermal control steps/points for each cycle: denaturing, hybridization/annealing, and extension. During denaturing, a heating temperature above 90° C., such as from about 94° C. to about 98° C., breaks a double-stranded DNA molecule into two complementary single-stranded DNA molecules. In the annealing step, the single-stranded DNA molecules are cooled at a lower temperature from about 50° C. to about 65° C., such as about 60° C., to allow DNA synthesis whereby the single-stranded DNA molecules seek their complementary strands (designed primer) to create incomplete double-stranded DNA molecules. During the extension step, reactions are heated at a heating temperature above 65° C., such as from about 70° C. to about 75° C., so that the incomplete double-stranded DNA molecules are extended with the help of an enzyme called DNA polymerase. The cycle is then repeated a number of times to achieve a desired amount of amplification of the DNA material.

Referring now to FIGS. 1 and 2, there is illustrated an example embodiment of a micro-fluidic device and, more particularly, a continuous flow PCR (CF-PCR) device 10. CF-PCR device 10 includes a substrate 15, a heater layer 20, a flow feature layer 25 defining fluidic structures above the heater layer 20, and a cover layer 30 over the flow feature layer 25. The flow feature layer 25 defines walls and together with the cover layer 30 forms a channel 35 in which fluid flows. The channel 35 defines a serpentine channel having a

plurality of cycles 35-1, 35-2, . . . , 35-n that extend along the length of CF-PCR device 10 through three distinct temperature regions 40A, 40B, and 40C, each region 40 for denaturation, annealing, and extension thermal control points of a PCR process, respectively. The temperature regions 40 are heated using heater elements formed and emplaced variously on heater layer 20 within each region 40, and are thermally isolated from each other by trenches 45 formed on the substrate 20 between each region 40, as will be explained in more detail below. Inlets 50 and outlets 55 formed at the ends of channel 35 serve to introduce and remove fluid from the channel 35. Any number of inlets and outlets can be formed.

Pumps 60 are disposed along portions of channel 35 to propel fluid to flow the channel. In an example embodiment, a set of pumps 60 includes a plurality of resistive heaters 65 (65-1, 65-2, . . . , 65-n) formed on heater layer 20 and along a corresponding channel portion, as shown in FIG. 3, to form a thermal bubble pump. In operation, by applying a voltage pulse to each of the heaters 65 of pumps 60, thermal bubbles are formed in a predetermined manner. For example, every heater 65 of pump 60 can form a bubble from the left to the right of the channel in sequence to push the liquid in the same direction. This cycle is then repeated to continue the pumping. Pumps of this type are the ones disclosed in more detail in U.S. patent application Ser. No. 13/556,495, filed Jul. 24, 2012, entitled "Micro-Fluidic Pump" and assigned to the assignee of the present invention. The disclosure of this patent is hereby incorporated herein by reference. As liquid is pumped through the channel 35, it repeatedly passes through the different temperature regions 40 for a number of cycles n to undergo thermal cycling. The residence time of a PCR reaction mixture within a temperature region 40 depends on the length of the channel within the region and the flow rate of the reaction within the channel 35. Accordingly, pumps 60 can be controlled to adjust the flow rate to achieve desired residence time.

FIG. 4 illustrates a top view of a portion of CF-PCR device 10 with the cover layer 30 removed to show at least a cycle of the underlying channel 35, and FIG. 5 shows a stack structure of the CF-PCR device 10 taken along line 5-5 of FIG. 4. In FIG. 4, a plurality of heating elements 70 associated with each temperature region 40 are disposed at areas surrounding the channel 35, and trenches 45 are formed between temperature regions 40 for thermal isolation. By not disposing heating elements 70 along the channel 35, unequal heating of fluids within the channel can be avoided. Instead, fluids within each region 40 can be heated substantially equivalently or uniformly by allowing heat of the substrate to act on them, as will be explained in detail below. It will be appreciated that the depicted arrangement of heating elements 70 is only for purposes of illustration and thus should not be considered limiting, and that any number of heater elements and arrangement thereof on the substrate 15 can be used.

In FIG. 5, the stack shows heating elements 70 formed on the heater layer 20 above the substrate 15, a support material 75 surrounding the heating elements 70 to serve as underlying support for the channel 35, flow feature layer 25 formed above the heating elements 70 and support material 75, cover layer 30 formed above flow feature layer 25, trenches 45 etched from a backside 15A of the substrate 15 to a top surface 15B thereof, and a heat sink 80 beneath the substrate 15. (It should be noted that FIG. 5 is not an exact cross sectional view of FIG. 4. That is, cover layer 30 is present in FIG. 5 and the heating elements 70 are projected towards the cross sectional area such that they appear directly below the channel 35. Additionally, heat sink 80 which is not shown in FIG. 1 is shown in FIG. 5.)

The heating elements **70** are in thermal contact with the substrate **15** and the substrate **15** should be thermally conductive to dissipate heat from the heating elements **70**. In operation, heat generated by the heater elements **70** travel through the substrate **15** and spreads out to each corresponding temperature region **40**. Vertical heat flow from the substrate **15** dominates the temperature of the regions **40** and is used to heat fluids flowing through corresponding channels above the substrate **15**. Trenches **45** serve to interrupt the travel of heat between temperature regions **40** to provide thermal stability. Additionally, heat sink **80** helps hold the lateral region to region heat flow to a minimum. In particular, with differing temperatures applied to each temperature region **40**, a temperature gradient can be formed between adjacent temperature regions. Heat sink **80** collects heat residue between adjacent temperature regions **40** in order to substantially minimize the temperature gradient between regions.

Referring now to FIG. **6**, there is illustrated an on-chip thermal control system **100** that can be implemented integrally with CF-PCR device **10** and used to control each temperature region **40** to provide specific temperatures required for each thermal control point for PCR, in accordance with an example embodiment of the present invention. Generally, thermal control system **100** is used to sense the temperature of a temperature region **40** and limit temperature variations at desired levels. A number of the thermal control system **100** can be provided on CF-PCR device **10** to provide coverage for each of the temperature regions **40**.

More particularly, for each temperature region **40**, a thermal control system **100** includes a preset temperature reference source **105**. Preset temperature reference source **105** can be an 8 bit digital to analog converter (DAC) connected to a serial peripheral interface, for example, that provides a desired temperature set point current input $I(\text{set})$ used to set the target temperature for a corresponding temperature region **40**. Thermal control system **100** also includes at least one temperature sensor **110** that senses and provides actual substrate temperature readings in the form of a current input $I(\text{sense})$. Temperature sensor **110** can be an active circuit composed of CMOS transistors and substrate PNP which produces an output current proportional to absolute temperature sensed. In FIG. **5**, temperature sensors **110** are shown as being formed on substrate **15** for each temperature region **40**.

To provide more accurate temperature coverage of an entire length of a temperature region **40**, each temperature region **40** may be divided into a plurality of zones with each zone having an associated temperature sensor. For example, as shown in FIG. **7**, each temperature region **40** is divided into three zones Z-1, Z-2, Z-3 sensed by corresponding temperature sensors **110**. Of course, each region can be divided into any number of zones. A calibration sink **115** may be provided to adjust for variations between zone temperature sensors **110** of each temperature region **40**. In particular, calibration sink **115** may generate a calibration source current $I(\text{cal})$ from which each of the temperature sensors **110** of a region **40** may be calibrated. Accordingly, temperature sensors **110** associated with each temperature region **40** may read the same value for the same temperature after calibration.

Once the temperature sensors **110** are calibrated, the preset temperature reference current input $I(\text{set})$ is used to set the target temperature of each zone Z via a preset temperature sink **120**. In particular, current input $I(\text{sense})$ from temperature sensor **110** and current input $I(\text{set})$ from preset temperature sink **120** combine to produce a current output $I(\text{diff})$ which is the difference between the current inputs. Essentially, current output $I(\text{diff})$ represents a difference between the target temperature and the temperature sensed by the

temperature sensor **110**. A pulse width modulation (PWM) generator **125** receives the current output $I(\text{diff})$ and outputs a pulse width modulated heat pulse PWM(heat) that is proportional to the current output $I(\text{diff})$. An AND gate **130** receives the heat pulse PWM(heat) at its input **130A**. The other input **130B** of AND gate **130** can be an enable signal for heating.

In an example embodiment, PWM generator **125** can be controlled to provide a set of quantized PWM signals during an initial thermal ramp up to a set point temperature. For example, PWM generator **125** could have a 5 phase quantized PWM signal having pulses with duty cycles of 100%, 75%, 50%, 25%, and 0%, during the initial ramp. The duty cycle is proportional to the current output $I(\text{diff})$ as discussed above. In this way, a smaller delay before reaching the set point temperature can be achieved.

Thermal control system **100** further includes a switch **140** connected to an output **130C** of AND gate **130**. The gates of switch **140** are driven by drive pulses $P(\text{drive})$ so that it periodically activates a connected heater element **70** to produce heat pulses that are delivered to the substrate **15**. Heat then spreads throughout the substrate temperature region. Process then loops until the current $I(\text{sense})$ at each temperature sensor **110** is equal to the desired set current $I(\text{set})$ of the preset temperature sink **120** which corresponds to the required temperature for the temperature region **40**. Accordingly, when the current input $I(\text{sense})$ from the temperature sensor **110** substantially equals the current $I(\text{set})$ from the preset temperature sink **120**, the region is at its target temperature.

Thermal isolation structures, i.e., trenches **45**, and heat sink **80** provide added stability in the thermal control. FIG. **8** shows a simulation chart illustrating temperatures of each of the regions **40** with respect to time. As shown, temperatures for each temperature region **40** are effectively maintained substantially linear after initial ramp. Additionally, temperature gradient along each temperature region **40** is kept minimal as indicated by the marginal deviation of thermal zone temperatures along each temperature region **40**. Thus, the thermal control system **100** allows each temperature region **40** to be thermally stable within the range of its corresponding set point temperature.

CF-PCR device **10** is fabricated on substrate **15**. The preferred substrate is silicon, which allows forming logic circuits together with the pumps and micro-fluidic structures. In addition, silicon provides high thermal conductivity to conduct heat from the heaters and heat fluids above it. The heating elements **70** associated with each region **40** and resistive heaters **65** associated with each pump **60** are formed by layers or films of semiconductor and other suitable materials formed or deposited, by using known micro-electronic fabrication techniques, on the substrate **15**. For example, such heater elements **70** can be constructed in a similar fashion as the resistive heaters **65** as disclosed in U.S. Pat. No. 8,172,369, the contents of which are hereby incorporated by reference. Logic circuits to control heaters are formed on the substrate **15** by silicon processing. The heaters are then formed with the fluidic structures. A silicon dioxide is grown or deposited as the support material **75** on top of the substrate **15** (and alternatively over the heaters). A photoimageable polymer, for example, SU-8 (MicroChem, Newton, Mass.), is used to form the flow feature layer **25**. For the cover layer **30**, a photoimageable dry film, for example, VACREL™ (DuPont) is used and applied onto the flow feature by a lamination process. Inlet and outlet ports that align with inlets **50** and outlets **55** can be formed by either deep reactive ion etching (DRIE) or a photolithography process. By DRIE, an inlet port and an outlet port can be formed by etching holes through the sub-

strate. In this case, liquid is fed into inlets **55** and the channel **35** from the backside of the substrate **15**. An inlet port and an outlet port can be formed on the top side of the CF-PCR device **10** by patterning the flow feature **25** and cover layer **30**. In addition, both DRIE and photolithography processes can be used to make an inlet port on the top side and outlet port on the backside of the CF-PCR device **10**. A highly thermal conductive material, such as Al, aluminum alloys, Cu, diamond or composite materials like copper-tungsten, can be used for heat sink **80** beneath substrate **15**.

Referring now to FIG. 9, another example embodiment of a micro-fluidic PCR device **210** is illustrated. Generally, PCR device **210** is fabricated using the same fabrication techniques as the CF-PCR device **10** discussed above, but with fluidic structures patterned to form a looped channel **235** containing a plurality of wells **237** formed on three thermal regions **240A**, **240B**, **240C**, each region for denaturation, annealing, and extension thermal control points of a PCR process, respectively. Any number of wells for each region **240** can be utilized, depending on the design contemplated. The thermal regions **240** are heated using heater elements formed and emplaced variously within each region **240** and are thermally isolated from each other by trenches **245** formed between each thermal region **240**. Inlet **250** and outlet **255** formed at the ends of looped channel **235** serve to introduce and remove fluid from the looped channel **35**. A plurality of pumps **260** are disposed along portions of the fluidic structures of channel **235** to propel fluid.

In operation, fluids are propelled by pumps **260** through the looped channel **235** to pass through each of the thermal regions **240** to complete one thermal cycle. Depending on the required heating time, PCR reaction mixtures can be allowed to dwell within wells **237** of a thermal region **240** by deactivating pumps **260**. Pumping can be continued to allow PCR reaction mixtures to repeatedly pass through the different thermal regions **240** for a required number of cycles depending on the amount of amplification desired. Pumps **260** can be controlled to pump fluids in a coordinated manner to properly direct fluid flow to desired fluid paths. For example, pumps **260A** and **260B** can be controlled to push fluids from left to right along channel portions **235A** and **235B**, respectively, while pump **260C** is controlled to push fluids from right to left along a channel portion **235C** to compel fluids within well **237** of thermal region **240A** to flow towards channel portion **235B**. Meanwhile, as pump **260B** along channel portion **235B** is controlled to push fluids from left to right, pumps **260D** and **260E** along channel portions **235D** and **235E**, respectively, can be controlled to push fluids from right to left to prevent fluids from flowing into outlet **255** and instead compel fluids within the wells **237** of thermal region **240B** to flow towards channel portion **235F**. To allow fluid flow into outlet **255**, at least each of pumps **260B**, **260D**, and **260E** can be controlled to push fluid from left to right to compel fluid within wells **237** of thermal region **240B** to flow into outlet **255**. As will be appreciated, other techniques for controlling the pumps to convey fluids within the channel can be implemented.

FIG. 10 illustrates a top view of a portion of PCR device **210** and FIG. 11 shows a stack structure of PCR device **210** taken along line 11-11 of FIG. 10. In FIG. 10, a plurality of heating elements **270** are disposed at areas surrounding the well **237** to define a thermal region **240**. Different arrangements of heating elements can be utilized. Trenches **245** are also formed to thermally isolate the thermal regions **240**. In FIG. 11, the stack shows heating elements **270** formed above and in thermal contact with a substrate **215**, a support material **275** surrounding the heating elements **270** to serve as under-

lying support for the channels **235** and wells **237**, a flow feature layer **225** formed above the heating elements **270** and support material **275** to provide upstanding walls for the channel, cover layer **230** formed above flow feature layer **225**, trenches **245** etched from a backside **215A** to a top surface **215B** of the substrate **215** (and alternatively further through the support material **275** and the flow feature layer **225**), and a heat sink **280** beneath the substrate **215**. (It should be noted that FIG. 11 is not an exact cross sectional view of FIG. 10. That is, cover layer **230** above the flow feature layer **225** if illustrated in FIG. 10 would prevent the illustration of the underlying well **237**. Hence, the cover layer **230** is not present in FIG. 10. In addition the heating elements **270** are projected towards the cross sectional area such that they appear directly below the well **237**.) As previously mentioned, the heating elements **270**, substrate **215**, trenches **245**, heat sink **280**, and other similar components may be constructed in the same fashion and serve to provide the same functions as in the CF-PCR device **10** embodiment, the differences only being the pattern of the fluidic structures, location of the thermal regions and/or trenches, and specific pump functions.

Referring now to FIG. 12, there is illustrated an on-chip thermal control system **300** that can be implemented integrally with PCR device **210** for each thermal region **240** to provide specific temperatures required for each thermal control point for PCR, in accordance with an example embodiment of the present invention. It should be noted, however, that the thermal control system **100** previously described can also be used for PCR device **210**. Conversely, thermal control system **300** can also be used for CF-PCR device **10**.

Thermal control system **300** includes a temperature set point register **305** for holding a binary value B(set) corresponding to a target temperature. Thermal control system **305** also includes at least one temperature sensor **310** that senses and provides actual substrate temperature readings of a thermal region. Temperature sensor **310** can be an active circuit composed of CMOS transistors and substrate PNP which produces an output current I(sense) proportional to absolute temperature sensed. In FIG. 11, a temperature sensor **310** is shown as being formed on substrate **215**. Any number of temperature sensors can be used for each thermal region to provide more accurate coverage. For example, as shown in FIG. 13, each of thermal regions **240B** and **240C** has two associated temperature sensors **310** defining thermal zones within each region.

Thermal control system **300** further includes an analog to digital converter (ADC) **315** that receives I(sense) from temperature sensor **310**. ADC **315** can be an 8 bit ADC that samples and cycles through each thermal zone, sampling the output current I(sense) of temperature sensor **310** and converting it to an 8-bit binary value B(sense). A subtractor **320**, which can be a 2's complement subtractor, receives as inputs the target temperature binary value B(set) from the temperature set point register **305** and the binary value B(sense) from ADC **315**, and returns a value B(diff) that corresponds to the difference between the two inputs to a quantizer **325**. Essentially, output B(diff) represents a difference between the target temperature and the temperature sensed by the temperature sensor **310**. Quantizer **325** converts the difference output B(diff) of subtractor **320** to a quantized PWM output P(drive) whose width is proportional to the difference output B(diff). The quantized PWM output P(drive) of quantizer **325** is then used to drive a switch **330** so that it periodically activates a connected heater element **270** to produce heat pulses that are delivered to the substrate **215**. Heat spreads through the substrate thermal regions and the process loops until the binary value corresponding to the current I(sense) at

temperature sensor **310** is equal to the binary value of the register **305** which corresponds to the target temperature for the thermal region. When B(sense) substantially equals B(set), the thermal region is at its target temperature.

FIG. **14** shows a simulation chart illustrating temperatures of each of the thermal regions **240** with respect to time. As shown, temperatures for each thermal region **240** are effectively maintained substantially linear after initial ramp. Additionally, temperature gradient along each thermal region **240** is kept minimal as indicated by the marginal deviation of temperatures at arbitrary points selected in the top, bottom, and middle areas of a well for each thermal region **240**.

FIG. **15** shows an embodiment of a micro-fluidic PCR device **400**, which can either be CF-PCR device **10** or PCR device **210**, mounted on a lab-on-a-chip device **410**. PCR device **400** has a channel **412**, and inlet **415** and outlet **420** aligned with an inlet port **425** and an outlet port **430**, respectively, formed through the substrate. Inlet port **425** and outlet port **430** are aligned and disposed on port holes **435** and **440**, respectively, of the lab-on-a-chip device **410**. A pressure sensitive adhesive or an epoxy adhesive can be used to bond the PCR device **400** on the chip **410**.

In another embodiment, a micro-fluidic PCR device **500** can have a top-side inlet **515** and bottom-side outlet **520**, as shown in FIG. **16**. The top-side inlet **515** aligns with an inlet port **525** formed by opening up the cover layer. On the other hand, DRIE of silicon can be used to form bottom-side outlet port **530** aligned with the outlet **520**. PCR device **500** can be mounted on a lab-on-a-chip device **510**. The outlet port **530** of PCR device **500** are aligned and disposed on a port hole **535** of the lab-on-a-chip **510**. A liquid container **540** adjacent the inlet port **525** can be attached to introduce fluid into the inlet **515** and the channel **412**.

Thus, micro-fluidic devices for point-of-care diagnostic and lab-on-a-chip applications are disclosed. The foregoing illustrates various aspects of the invention. It is not intended to be exhaustive. Rather, it is chosen to provide the best illustration of the principles of the invention and its practical application to enable one of ordinary skill in the art to utilize the invention, including its various modifications that natu-

rally follow. All modifications and variations are contemplated within the scope of the invention as determined by the appended claims. Relatively apparent modifications include combining one or more features of various embodiments with features of other embodiments.

The invention claimed is:

1. A micro-fluidic device, comprising:

a substrate having a top surface and a backside defining a thickness thereof;

a trench extending through an entirety of the thickness of the substrate;

a plurality of heaters on the substrate for heating the substrate, the plurality of heaters defining a plurality of temperature regions having distinct temperatures, wherein the trench exists between the temperature regions thereby thermally isolating the plurality of heaters on either sides of the trench along a length of the substrate;

a flow feature layer defining a channel above the substrate between a fluid inlet and outlet disposed at ends of the length of the substrate and having an intermediate portion, the channel extending across the substrate through each temperature region, wherein the channel resides under a cover defining a serpentine path in the intermediate portion having a plurality of cycles along an extent of the serpentine path, wherein the cycles pass through each of the disposed in the cycles along the extent of the serpentine path in the intermediate portion; and at least one pump between the fluid inlet and the intermediate portion of the channel but not disposed in any of the temperature regions for pumping fluid in the channel to flow from one temperature region to a next temperature region to allow the fluid to undergo thermal cycling.

2. The device of claim **1**, further comprising a heat sink mounted beneath the substrate to collect heat residue between adjacent temperature regions so as to reduce a temperature gradient therebetween.

3. The device of claim **1**, further including one or more wells disposed in said each of the temperature regions.

* * * * *