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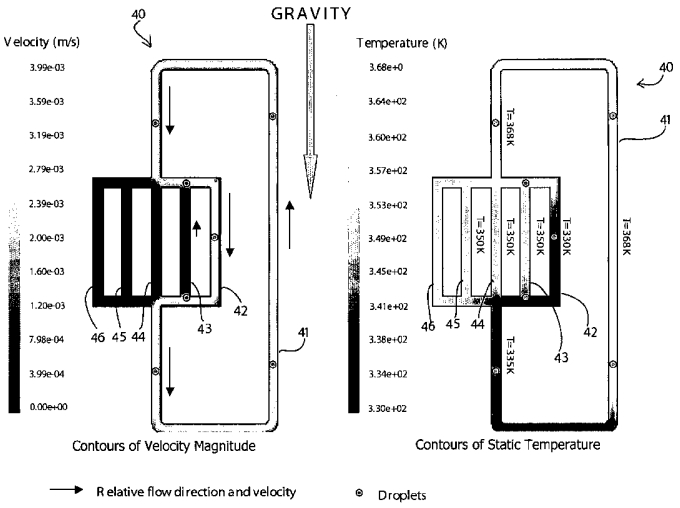

(57) Abstract: A microfluidic system (40) achieves fluid flow control and manipulation without moving parts. It has a channel in a circuit in a vertical plane having a gravity-opposed leg (41) and a gravity-assisted side having five branches (42-46). A carrier fluid conveying small sample reactors flows in the circuit. Overall flowrate in the circuit is controlled by varying the temperature differential between the right and left sides to vary buoyancy. By selectively controlling temperatures in branches (42-46) flow can be routed into one or more as desired. For example, if the temperature is lowest in one branch (42) flow will divert through this. This arrangement provides a multi-port valve. Also, the circuit may have a residency zone (61) and flowrate (and hence residency time) is this zone is controlled by varying temperature of a pair of control zones (62) on either side. The microfluidic device of the invention achieves comprehensive flow control without moving parts by virtue of temperature control and buoyancy and fluid properties.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

BUOYANCY-DRIVEN MICROFLUIDICS

INTRODUCTION

Field of the Invention

The invention relates to microfluidics such as for the pumping, control and manipulation of samples in a micro total analysis system (μTAS) device to detect the population of rare mutated cells.

Prior Art Discussion

It is known for some time that cancers have a genetic cause. With the emergence of fast methods of sequencing and the publication of the human genome, the motivation and methods are available to find the genetic causes, both germline and somatic, of the most prevalent cancers. Contemporary oncological research suggests that there is a sequence of mutations that must occur for a cancer to be life threatening, called the multistage model. Cancer could therefore be diagnosed earlier by detecting these genetic markers, thereby increasing the probability of cure.

The primary method to identify rare cells in a sample is to probe the sample using known genetic markers, the markers being specific to the type of mutation being sought, and then amplify the same sample. If the mutations are present then the amplification can be detected, usually using optical techniques. It is also possible, depending on the amplification used, to detect the number of mutated cells in the original sample; a number extremely important as firstly, it can be linked to the progress of the cancer and secondly, it provides a quantitative measure with which to diagnose remission.

The enzyme-catalysed reaction used to amplify the sample is the Polymerase Chain Reaction (PCR), which entails taking a small quantity of the strand and producing many identical copies of it *in vitro*. The most popular technology to achieve a PCR is

to batch process the reagents by thermally cycling them in a well. The state of the art comprises the following biochemical techniques:

- Amplification of target DNA using the PCR. Because of small samples and expensive reagents, the process must be able to handle very small volumes. It is also advantageous to segment the DNA-carrying sample to improve the efficiency of the PCR.
- Optical detection of the amplified strands using fluorescent markers.
- Real-time and digital PCR to establish the number of mutated cells in the original population.
- Finally, multiplexing to enable many DNA targets to be analysed simultaneously. This improves the probability of correct diagnosis.

US5270183 describes PCR in which the sample is carried in a stream of carrier fluid.

The invention is therefore directed towards achieving improved methods of control, manipulation and pumping in a microfluidic system for applications such as the above.

SUMMARY OF THE INVENTION

According to the invention, there is provided a microfluidic system comprising:

a microfluidic device comprising a channel forming a circuit;

means for delivering a carrier fluid and sample reactors into the channel;

a support for supporting the microfluidic device in a non-horizontal plane; and

a controller for applying different temperatures to the carrier fluid at different positions of the channel according to a control scheme to control carrier fluid and reactor flow by buoyancy and fluid properties.

In one embodiment, the channel is differentially heated by heating the channel walls, which in turn heat the carrier fluid, and which in turn heat the reactors.

In another embodiment, the channel comprises a plurality of branches, and flow is directed into the branches according to heating control to provide a multi-port valve.

In one embodiment, channel temperature is dynamically controlled so that at any time one or more selected branches are at a common lower temperature on a gravity-assisted side or at a higher temperature on a gravity-opposed side.

In one embodiment, the channel comprises a residency zone and at least one control zone forming a loop with the residency zone, and the controller varies temperature of the control zone to vary fluid residency time is the residency zone.

In one embodiment, the residency zone receives gravity-assisted flow, and the controller increases control zone temperature to decrease residency time, and vice versa.

In another embodiment, the residency zone receives gravity-opposed flow, and the controller decreases control zone temperature to decrease residency time and *vice versa.*

In one embodiment, the microfluidic device comprises a plurality of residency zones, each independently controlled.

In one embodiment, there are a plurality of control zones for the residency zone.

In one embodiment, there are two control zones, and they are on opposed sides of the residency zone.

In one embodiment, the system further comprises means for applying a standing wave in the channel to maintain the reactors substantially centrally in the channel.

In one embodiment, said means comprises a piezo electric device.

In a further embodiment, the controller varies the plane of the channel to vary flow in the channel according to varying gravitational effects.

In one embodiment, the controller directs thermal fluid into the channel to heat or cool the carrier fluid.

In one embodiment, the thermal fluid enters and exits from opposed sides of the channel.

In one embodiment, the controller controls thermal fluid flowrate to contribute to control of the flowrate of the carrier fluid and reactors.

The invention also provides an analysis system comprising any microfluidic system as described above, means for delivering samples as the reactors into the carrier fluid, means in the controller for controlling the carrier fluid temperature according to required reaction temperatures in addition to flow control criteria, and a detector for monitoring the reactors.

In another aspect, the invention provides a microfluidic system comprising:

a microfluidic device comprising a channel forming a circuit;

means for delivering a fluid into the channel;

a support for supporting the microfluidic device in a non-horizontal plane;

a controller for applying different temperatures to the fluid at different positions of the channel according to a control scheme to control fluid and reactor flow by buoyancy and fluid properties, and

wherein the channel comprises a plurality of parallel branches, and flow is directed into the branches according to temperature control of fluid in the branches to provide a multi-port valve.

In one embodiment, channel temperature is dynamically controlled so that at any time one or more selected branches are at a lower temperature on a gravity-assisted side or at a higher temperature on a gravity-opposed side.

In a further aspect, the invention provides a microfluidic system comprising:

a microfluidic device comprising a channel forming a circuit;

means for delivering a fluid into the channel;

a support for supporting the microfluidic device in a non-horizontal plane;

a controller for applying different temperatures to the fluid at different positions of the channel according to a control scheme to control carrier fluid and reactor flow by buoyancy and fluid properties, and

wherein the channel comprises a residency zone and at least one parallel control zone forming a loop with the residency zone, and the controller varies temperature of the control zone to vary fluid residency time is the residency zone.

DETAILED DESCRIPTION OF THE INVENTION

Brief Description of the Drawings

The invention will be more clearly understood from the following description of some embodiments thereof, given by way of example only with reference to the accompanying drawings in which:-

Fig. 1 is a diagram of a μ-TAS system incorporating microfluidic devices of the invention;

Fig. 2 is a plan view and a perspective view of a microfluidic device;

Fig. 3 shows a simple microfluidic channel for conveying sample reactors in a carrier fluid in which pumping control is achieved without moving parts;

Fig. 4 shows two representations of a microfluidic circuit incorporating a multi-port valve, again without need for moving parts;

Fig. 5 shows two representations of a microfluidic circuit having three PCR stages, with control of residency time without need for moving parts;

Fig. 6 is a representation of a simple microfluidic circuit used for tests, the results of which are shown in Fig. 6;

Fig. 7 is a velocity and temperature plot of flows;

Fig. 8 is a set of diagrams illustrating velocity vectors for flow in the circuit of Fig. 6;

Fig. 9 is a set of diagrams showing an apparatus used to experimentally demonstrate the pumping, manipulation and control of microfluidic flows without moving parts using buoyancy; and

Fig. 10 is a diagram showing piezo control of the reactors in a conduit.

Description of the Embodiments

Referring to Fig. 1 a μ-TAS system 1 comprises an electronic controller 2 and:

a mixing/sipping stage 3,

a microfluidic device 4 providing a PCR reactor, and
an optical detection system 5.

This diagram also illustrates systems for interfacing 10 with a patient to obtain a relevant sample, sample preparation 11, and bio-informatics 12.

Referring to Fig. 2, the microfluidic device 4 has a circular enclosed path 20 for droplets (also "bubbles" or "reactors") of reagent enveloped by a carrier fluid. The term "reactor" is used because each bubble or droplet is an individual micro reactor in its own environment, within which the chemical processes of amplification take place under thermal conditions imposed by the carrier fluid.

There are three thermal zones, each comprising an outer channel 21 and an inner channel 22 the inlets and outlets of which are below the plane of the path 20. The channels 21 and 22 direct thermal fluid at selected temperatures.

The carrier fluid which circulates with the reactors and the thermal fluid which enters and exits at the three thermal zones prevent contact between the reactors and the device surfaces.

The thermal fluid also controls the residency time within the thermal zones by virtue of viscous drag applied to the carrier fluid, and heating or cooling of the fluids. Pumping of the carrier fluid arises because the path 20 is in a non-horizontal plane in the gravitational field and there are temperature differences between the thermal zones. Thus flow-rates and temperatures of the reagents are controlled by:

(a) flowrate of the carrier fluid; and/or
(b) flowrates of the thermal fluids (individually controlled in each thermal zone); and/or
(c) temperatures of the thermal fluids (individually controlled in each thermal zone); and/or
(d) orientation of the path 20 from horizontal to vertical with infinite adjustment within this range, and/or
(e) Rotation about the central axis of the device

The factors (c), (d), and (e) above contribute to buoyancy of the reactors conveyed in the carrier fluid. Thus, flow control is achieved without moving parts through using buoyancy in different geometrical arrangements.

In the embodiments which follow buoyancy is used to control flow in terms of flowrate, routing through channels, and residency times in which the buoyancy is controlled by temperature variations between channel walls which heat or cool the carrier fluid. There is therefore no need for thermal fluids in the following embodiments. Also, the plane of the microfluidic circuit is not varied in these embodiments, sufficient control being achieved by virtue of the temperature control while maintaining the circuit in a vertical plane.

Buoyancy may be utilized at several levels for pumping, manipulation and control of samples. At a fundamental level the velocity in a closed loop channel due to buoyancy is obtained by letting the buoyancy forces equal the pressure drop forces. The equation to represent this may be found by substituting the Boussinesq approximation, $\rho = \rho_\infty[1 - \beta(T - T_\infty)]$, into the momentum equation and allowing it to equal the pressure drop in the channel obtained from laminar solutions. The resultant equation for a circular cross section channels is;

$$gh\rho\beta\Delta T = \frac{12\mu ul}{c^2}$$

where, g – gravity; h – vertical height, ρ - density, β – thermal expansion coefficient of fluid, T – temperature, μ – dynamic viscosity, u – velocity, l – channel length, c – channel diameter. Fluid properties, geometry and temperature difference can all vary to obtain better designs depending on the application. Fluid property variations with temperature are also important.

Referring to Figs. 3 to 6 numerical solutions were used to demonstrate the flow, and hence reactor, pumping, manipulation and control without moving parts. There is a valve for inlet and outlet of fluid and reactors, but this is closed during use. The black circles represent the reactors. Arrows represent the direction of the fluid in the channel to the left, with the velocity of the fluid being proportional to the length of the arrow.

Temperature values represent the temperature in the channel to the left. These numerical solutions were done for the properties of water in a channel of 1mm diameter and height of 38mm and are shown to scale. Wall temperatures are applied as the boundary conditions.

Fig. 3 shows a buoyancy-driven pump in microfluidic device 30 having a single closed loop channel with a right leg 31 and a left leg 32. The reactors are immersed into an immiscible bio-compatible carrier fluid. Pumping, control, and manipulation of the carrier fluid results in pumping, control and manipulation of the reactors without moving parts. The carrier fluid is heated through the walls, which in turn heats the reactors efficiently due to their small size, typically between 100 and 300 microns in diameter. The reactors never come in contact with each other or a solid wall, thus avoiding possible contamination problems. The fluid moves with a velocity so as to satisfy Equation 1, carrying with it the reactors. With control of the carrier fluid, there is continuous pumping of many samples in the same device without the need for moving parts, while also removing contamination risk, as is necessary in cell culturing and PCR for example.

The greater the temperature difference between channel legs 31 & 32 (in the horizontal direction) the greater the flowrate. This is because of the buoyancy effect. In this example the temperatures are 368K and 340K.

Fig. 4 shows a circuit 40 with buoyancy-driven pumping and manipulation in parallel channels. The representations are velocity (left) and temperature (right). The right channel is 41, and there are five parallel channels 42-46 on the left. A convection loop is created as in Fig. 3. Then the fluid is controlled and manipulated, without moving parts, to flow through any single or combination of the five parallel channels by varying wall temperatures in these channels. In the arrangement illustrated, all the fluid is controlled to flow down channel number 42, while channels 44, 45, and 46 have almost zero velocity fluid, and channel 43 has reversed fluid which further accelerates the fluid velocity in channel 42. The velocity in channel 42 has a magnitude which is approximately the sum of the magnitudes of the velocities for

channels 41 and 43. Less or more than five channels can be used, thereby allowing the system to meet high throughput requirements.

The reactors are immersed in the fluid as shown by the black circles in Fig. 4. Thus, each sample can go through a different process depending on what channel they are diverted into (the channel currently at lowest temperature), and each channel could contain a PCR reaction with different temperatures and residency times. Effectively, this creates a 5-port valve system without moving parts using buoyancy and temperature-dependent properties of the carrier fluid for use in cell sorting, sample preparation and detection for example.It will be understood that two or more of the channels 42-46 may be at the same (lower) temperature for approximately equally divided flow through them. Of course, if the multi-port valve were on the right side then the temperature control would be in the opposite sense as the flow would become gravity-opposed..

Fig. 5 shows a buoyancy-driven pumping and control PCR microfluidic device 50 with velocity (left) and temperature (right) representations. Again, black circles= represent the reactors. There is a main circuit 51 with three PCR stages 52, 60, and 70. The stage 52 has a residency zone 53 and a pair of control zones 54. The stage 60 has a residency zone 61 and a pair of control zones 62, while the stage 70 has a residency zone 71 and a pair of control zones 72.

Each residency zone represents one of the thermal zones of a PCR cycle. The embodiment gives the flexibility to vary the temperatures and times depending on sample to be processed. The embodiment shown in Fig. 5 uses two control zones for each stage, to vary the residency time of samples in each residency zone using buoyancy and fluid properties. On the gravity-assisted side, by setting the temperature of the control zones below that of the residency zone, the flow is predominately diverted through the control zones and thus the flow velocity in the residency zone is decreased. The opposite is the case on the gravity opposed side. Thus the residency time of each sample as it progresses through the required process may be controlled as described above without moving parts. The control zones effectively behave as a

controlled micro valve or regulator. A device such as the device 50 may be in any of the parallel channels of a larger device such as shown in Fig. 4.

Fig. 6 shows the boundary conditions used in a numerical model which was used to quantify the control of residency times using buoyancy as discussed in the preceding section. Here, just one thermal zone of the PCR cycle is modelled. The overall circuit has the numeral 100, and it comprises a main channel 101 and a stage having a residency zone 102 and control zones 103. The geometry is 2D and was meshed using a structured grid of 17,004 cells. The channel width is 3.5mm for all channels. The centreline to centreline width of the main loop was 42mm and of the control loop is 21mm. The centreline to centreline height of the main loop is 129.5mm and of the control loop is 42mm. The walls of the main loop are set to constant temperatures, representative of a PCR thermal cycle. The wall temperatures were varied, which in turn varied the fluid temperatures in as per Fig. 7.

Fig. 8 shows velocity vectors showing control wall temperatures of: (a) 340K (b) 380K (c) 435K. Fig. 8(d) shows the overall geometry of the CFD model indicating the area of interest shown in (a), (b) and (c).

Velocity and temperature data was extracted from the calculations and has been plotted in Fig. 7. All the velocity data given in the graph has been area averaged across the channel width. From Fig. 7, it is seen that at low control temperatures, < 355K, the velocity in the residency zone was extremely small, of the order 0.01mm/s. This corresponded to the flow regime shown in Fig. 8(a), where the majority of the fluid flows through the control zones, resulting in a large residency time in the residency zone. As the control wall temperatures are increased, a switch occurred, as the density of the fluid in the control zones decreased sufficiently for the flow in the control zones to be reversed before flowing down the residency zone, accelerating the flow in the residency zone. This corresponds to the flow regime shown in Fig. 8(b). For comparison, the average velocity in the device without the control zones was 26.9mm/s.

Although not shown in Fig. 7, if the temperature of the control zones were increased significantly, the flow within the whole device reverses direction as shown in Fig. 8(c).

Fig. 9 shows an apparatus used to experimentally demonstrate the flow regimes. The arrows illustrate heat addition and removal, Q1 and Q2 respectively from the channels for each of the flow regimes. The channels are initially in an isothermal state. To demonstrate the switching effect between the flow regimes illustrated in Figs. 8(a) and (b) above the heat transfer conditions were applied to the apparatus which is shown in Fig. 9. The time taken for dye to travel through the residency zone was recorded in Table 1 below. It is seen that there is a large change in velocity representing the regulation and switching effect.

For all the regimes of Fig. 9, the heat transfer conditions Q_1 and Q_2 were maintained at a constant value. Regime 1 in Fig. 9 corresponds to no applied heat transfer conditions in the control channels. This resulted in a residency time of 20 seconds in the central channel, demonstrating the flow regime shown in Fig. 8(a). When a small amount of heat was added to the control channels, as per regime 2, the velocity in the central channel increased dramatically, giving a residency time of 5 seconds, indicative of the flow regime shown in Fig. 8(b). Given the small difference in the applied heat gradients between these two flow regimes, this demonstrated the switching effect shown in Fig. 7 at approximately 355K. Conversely, when a small amount of heat was extracted from the control channels, as per regime 3, the velocity in the central channel decreased, resulting in a residency time of 60 seconds, indicative of the flow regime demonstrated in Fig. 8(a).

The central channel residency times for the regimes of Fig. 9 are:

Table 1

Regime 1, 20s,

Regime 2, 5s,

Regime 3, 60s.

The system of the invention may comprise a piezo-electric device mounted to apply a standing wave in the channel to maintain the reactors substantially centrally in the channel. Referring to Fig. 10 at a channel 50 piezoelectric transducers 51 are excited using an AC voltage source, this makes them oscillate in the z plane. At the correct frequency a standing wave is created within the channel. This frequency is given by;

$$\omega = \pi n\left(\frac{c+c'}{L}\right)$$

where n is the mode, c and c' the velocity of sound in the carrier fluid and channel walls, L the distance between the two transducers. This creates a force towards the node plane as shown in Fig. 10, known as Bjerknes force, see equation.

$$F = -(4/3)\pi R^3 \nabla P$$

where R is the reactor radius and P is a time-varying pressure field. This force counteracts the buoyancy force felt by the reactors in the carrier fluid, in effect holding them at a fixed plane.

This maintains the reactors centrally in the channel, effectively removing the need to buoyancy match the carrier fluid and reactors across the channel cross-section. This is very advantageous as it allows use of a single type of carrier fluid for a variety of types of sample.

The avoidance of need for moving parts provides the benefits of cost reduction, increased reliability, easier control, and reduction in cross contamination between biological samples.

It will be appreciated that the invention achieves comprehensive pumping, control and manipulation over rector flowrate and temperature, with no risk of contamination from device surfaces. The following summaries the major advantages:

Integrated pumping, manipulation, control and thermal cycling of the reactors without moving parts, and without cross-contamination;

Very high throughputs as measured by:

processing time for one sample.

- number of samples processed in series.
- number of samples processed in parallel.

Low cost and high reliability, due to the absence of moving parts.

Facility to independently control and vary all PCR parameters for process optimisation.

In the context of a μ-TAS system, a multi-port valve such as shown in Fig. 4 is particularly suitable for the sample preparation 11, mixing and sipping, and PCR reactor stages. The device of Fig. 5 is particularly suitable for the PCR stage.

The invention is not limited to the embodiments described but may be varied in construction and detail. For examples the reactors may be cells which are cultured in the carrier fluid giving the advantage that they do not come in contact with a surface and are easily nurtured for screening of these cells. Also, the reactors may be slugs which are conveyed by the carrier fluid without contacting each other but in contact with the channel walls. However, the channel walls may in this embodiment have a repellent coating to avoid contamination. Also, it is envisaged that the multi-port valve and residency time aspects of the invention may be applied to applications other than analysis systems with reactors. For example, they may control microfluidic flow of fluid for chemical reactions, without conveying reactors.

Claims

1. A microfluidic system comprising:

a microfluidic device comprising a channel forming a circuit;

means for delivering a carrier fluid and sample reactors into the channel;

a support for supporting the microfluidic device in a non-horizontal plane; and

a controller for applying different temperatures to the carrier fluid at different positions of the channel according to a control scheme to control carrier fluid and reactor flow by buoyancy and fluid properties.

2. A microfluidic system as claimed in claim 1, wherein the channel is differentially heated by heating the channel walls, which in turn heat the carrier fluid, and which in turn heat the reactors.

3. A microfluidic system as claimed in claims 1 or 2, wherein the channel comprises a plurality of branches, and flow is directed into the branches according to heating control to provide a multi-port valve.

4. A microfluidic system as claimed in claim 3, wherein channel temperature is dynamically controlled so that at any time one or more selected branches are at a common lower temperature on a gravity-assisted side or at a higher temperature on a gravity-opposed side.

5. A microfluidic system as claimed in any preceding claim, wherein the channel comprises a residency zone and at least one control zone forming a loop with the residency zone, and the controller varies temperature of the control zone to vary fluid residency time is the residency zone.

6. A microfluidic system as claimed in claim 5, wherein the residency zone receives gravity-assisted flow, and the controller increases control zone temperature to decrease residency time, and vice versa.

7. A microfluidic system as claimed in claim 5, wherein the residency zone receives gravity-opposed flow, and the controller decreases control zone temperature to decrease residency time and vice verse.

8. A microfluidic system as claimed in any of claims 5 to 7, wherein the microfluidic device comprises a plurality of residency zones, each independently controlled.

9. A microfluidic system as claimed in any of claims 5 to 8, wherein there are a plurality of control zones for the residency zone.

10. A microfluidic system as claimed in claims 9, wherein there are two control zones, and they are on opposed sides of the residency zone.

11. A microfluidic system as claimed in any preceding claim, wherein the system further comprises means for applying a standing wave in the channel to maintain the reactors substantially centrally in the channel.

12. A microfluidic system as claimed in claim 11, wherein said means comprises a piezo electric device.

13. A microfluidic system as claimed in any preceding claim, wherein the controller varies the plane of the channel to vary flow in the channel according to varying gravitational effects.

14. A microfluidic system as claimed in claim 1, wherein the controller directs thermal fluid into the channel to heat or cool the carrier fluid.

15. A microfluidic system as claimed in claim 14, wherein the thermal fluid enters and exits from opposed sides of the channel.

16. A microfluidic system as claimed in claims 14 or 15, wherein the controller controls thermal fluid flowrate to contribute to control of the flowrate of the carrier fluid and reactors.

17. An analysis system comprising a microfluidic system of any preceding claim, and means for delivering samples as the reactors into the carrier fluid, means in the controller for controlling the carrier fluid temperature according to required reaction temperatures in addition to flow control criteria, and a detector for monitoring the reactors.

18. A microfluidic system comprising:

a microfluidic device comprising a channel forming a circuit;

means for delivering a fluid into the channel;

a support for supporting the microfluidic device in a non-horizontal plane;

a controller for applying different temperatures to the fluid at different positions of the channel according to a control scheme to control fluid and reactor flow by buoyancy and fluid properties, and

wherein the channel comprises a plurality of parallel branches, and flow is directed into the branches according to temperature control of fluid in the branches to provide a multi-port valve.

19. A microfluidic system as claimed in claim 18, wherein channel temperature is dynamically controlled so that at any time one or more selected branches are at a lower temperature on a gravity-assisted side or at a higher temperature on a gravity-opposed side.

20. A microfluidic system comprising:

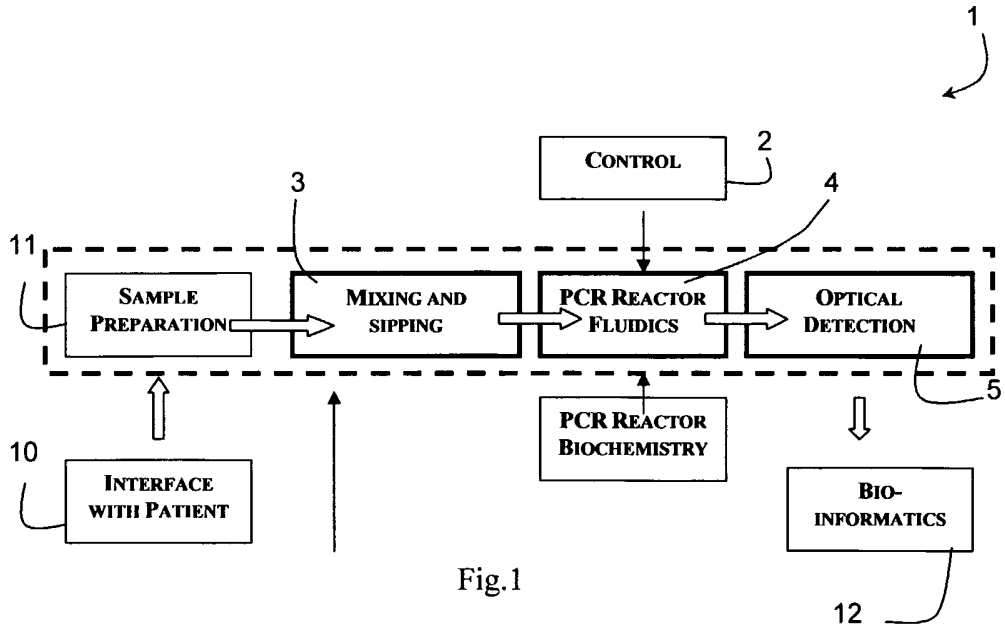
a microfluidic device comprising a channel forming a circuit;

means for delivering a fluid into the channel;

a support for supporting the microfluidic device in a non-horizontal plane;

a controller for applying different temperatures to the fluid at different positions of the channel according to a control scheme to control carrier fluid and reactor flow by buoyancy and fluid properties, and

wherein the channel comprises a residency zone and at least one parallel control zone forming a loop with the residency zone, and the controller varies temperature of the control zone to vary fluid residency time is the residency zone.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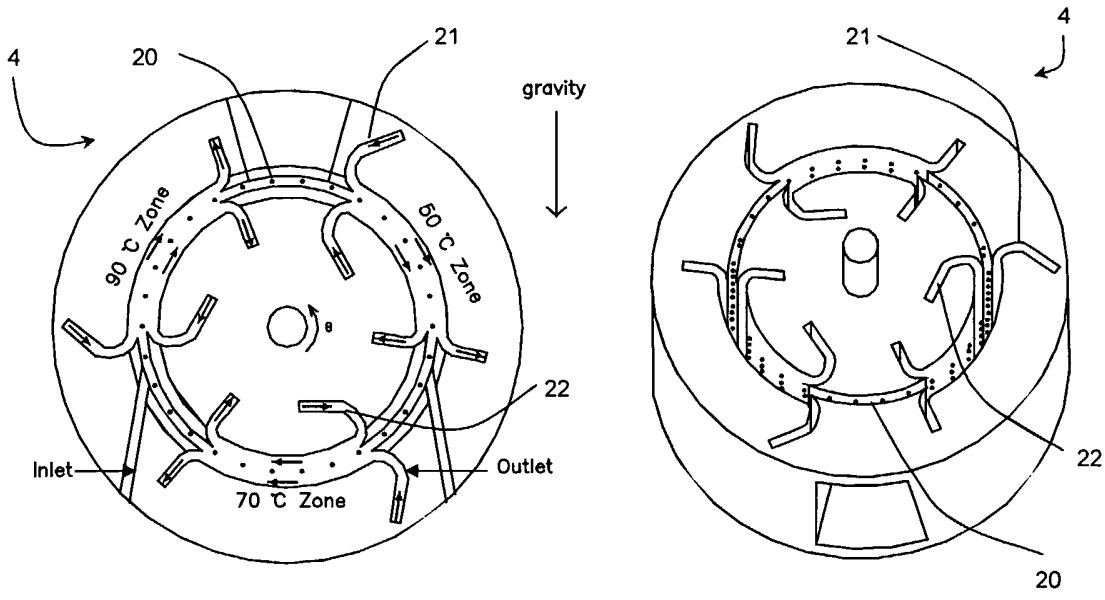
CONTROL
2
3
4
11
SAMPLE PREPARATION
MIXING AND SIPPING
PCR REACTOR FLUIDICS
OPTICAL DETECTION
5
PCR REACTOR BIOCHEMISTRY
10
INTERFACE WITH PATIENT
BIO-INFORMATICS
12

Fig.1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20
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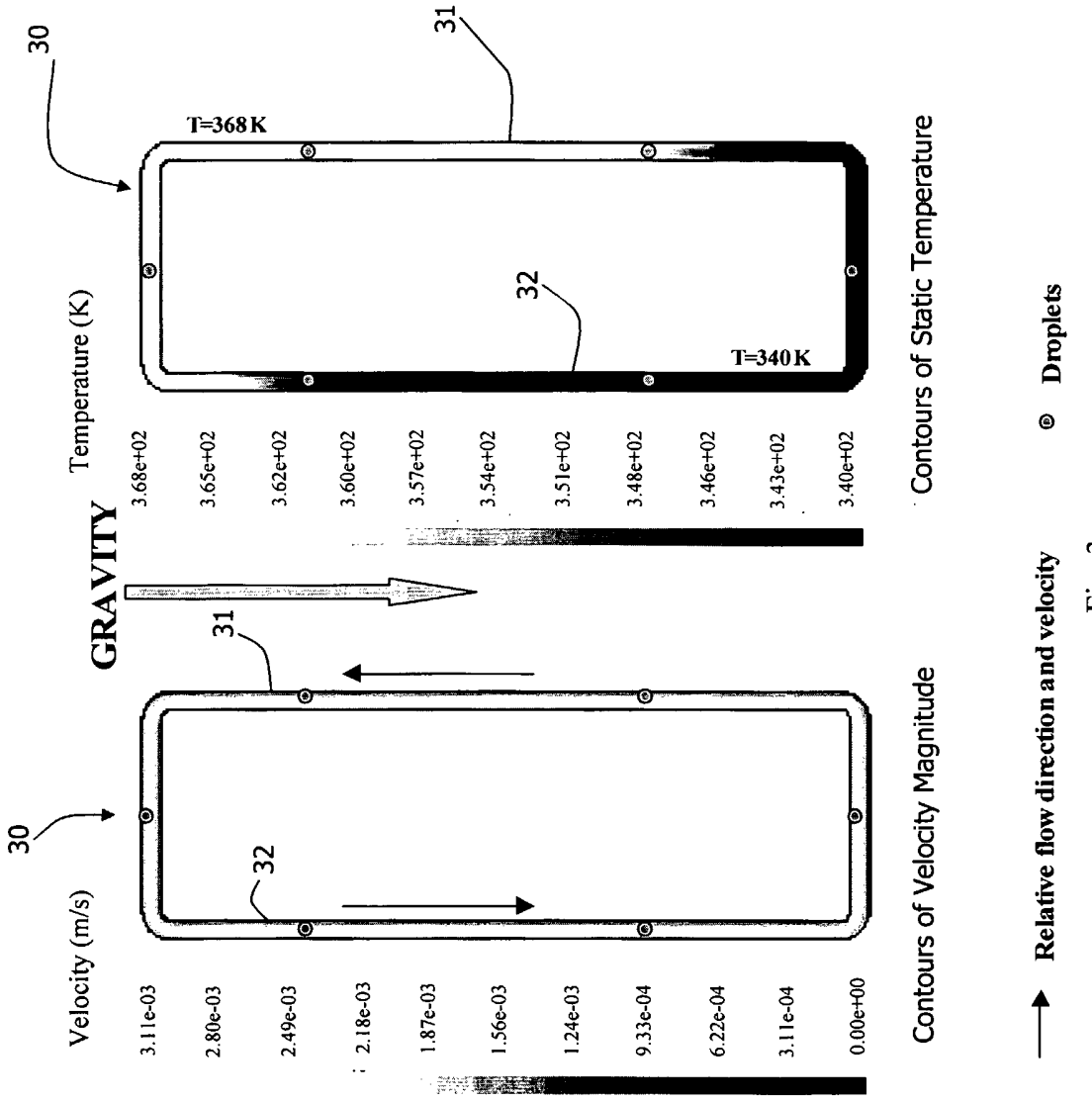
gravity
90 °C Zone
60 °C Zone
22
Inlet
Outlet
70 °C Zone
4
21
22
20

Fig. 2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Contours of Static Temperature
GRAVITY
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31
32
Velocity (m/s)
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2.49e-03
2.18e-03
1.87e-03
1.56e-03
1.24e-03
9.33e-04
6.22e-04
3.11e-04
0.00e+00
Contours of Velocity Magnitude
Relative flow direction and velocity
Droplets

Fig. 3

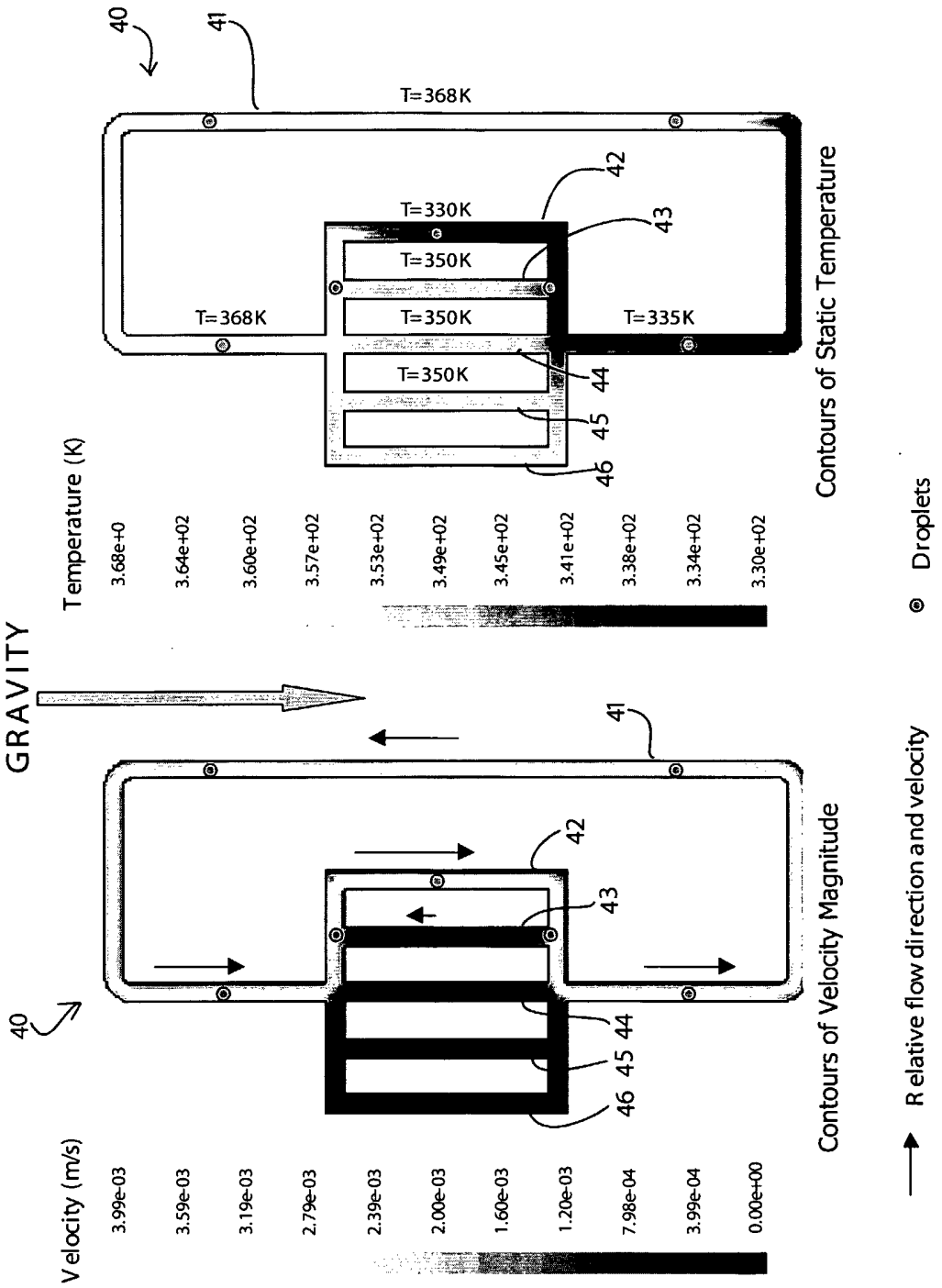
40
41
T=368K
42
T=330K
43
T=350K
T=368K
T=350K
T=335K
T=350K
44
45
46
Contours of Static Temperature
Temperature (K)
3.68e+0
3.64e+02
3.60e+02
3.57e+02
3.53e+02
3.49e+02
3.45e+02
3.41e+02
3.38e+02
3.34e+02
3.30e+02
GRAVITY
41
42
43
40
44
45
46
Contours of Velocity Magnitude
Velocity (m/s)
3.99e-03
3.59e-03
3.19e-03
2.79e-03
2.39e-03
2.00e-03
1.60e-03
1.20e-03
7.98e-04
3.99e-04
0.00e+00
Droplets
Relative flow direction and velocity

Fig4

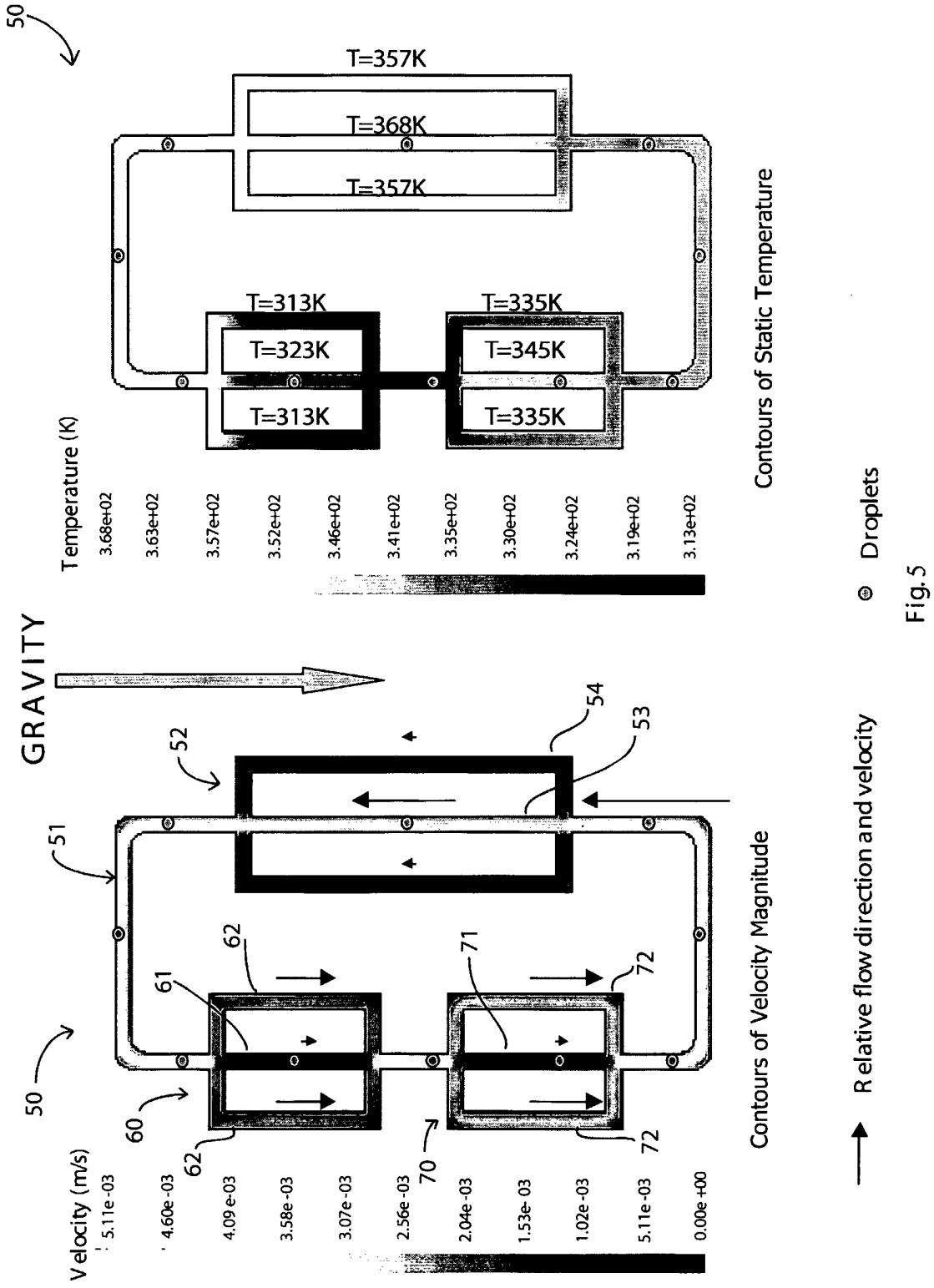
50
T=357K
T=368K
T=357K
T=313K
T=323K
T=313K
T=335K
T=345K
T=335K
Temperature (K)
3.68e+02
3.63e+02
3.57e+02
3.52e+02
3.46e+02
3.41e+02
3.35e+02
3.30e+02
3.24e+02
3.19e+02
3.13e+02
Contours of Static Temperature
GRAVITY
52
54
53
51
62
61
71
72
50
60
62
70
72
Velocity (m/s)
5.11e-03
4.60e-03
4.09e-03
3.58e-03
3.07e-03
2.56e-03
2.04e-03
1.53e-03
1.02e-03
5.11e-03
0.00e+00
Contours of Velocity Magnitude
Droplets
Relative flow direction and velocity

Fig. 5

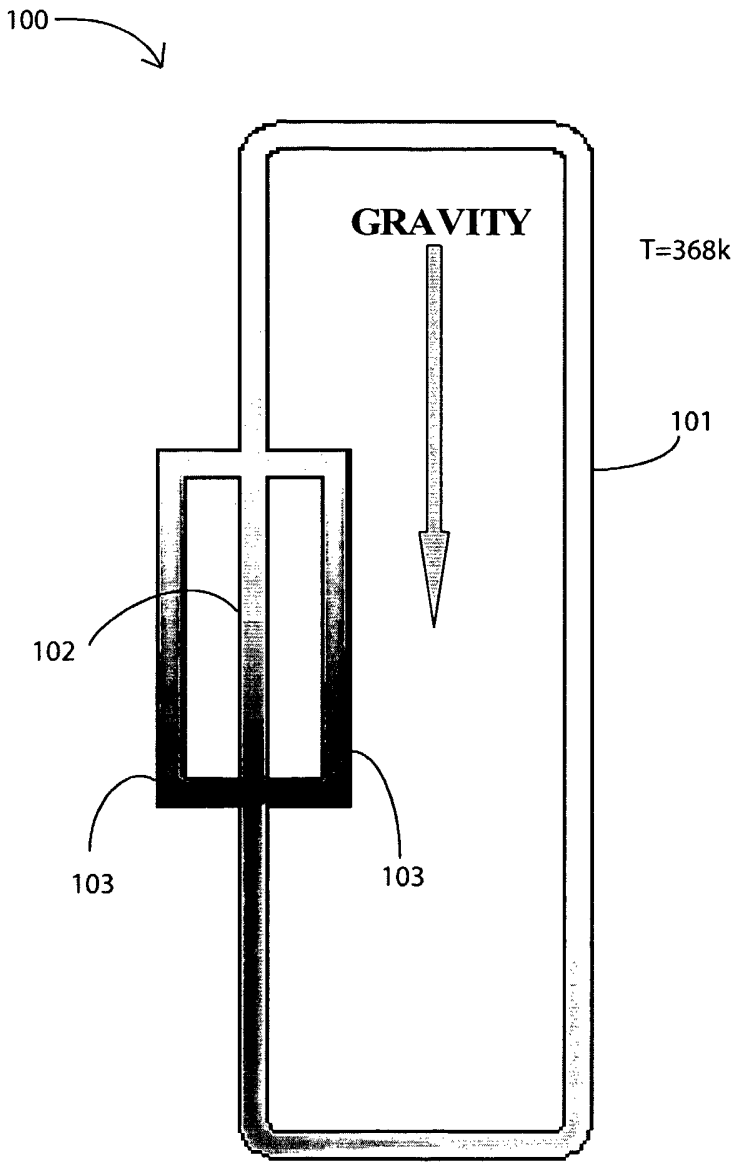

Fig. 6

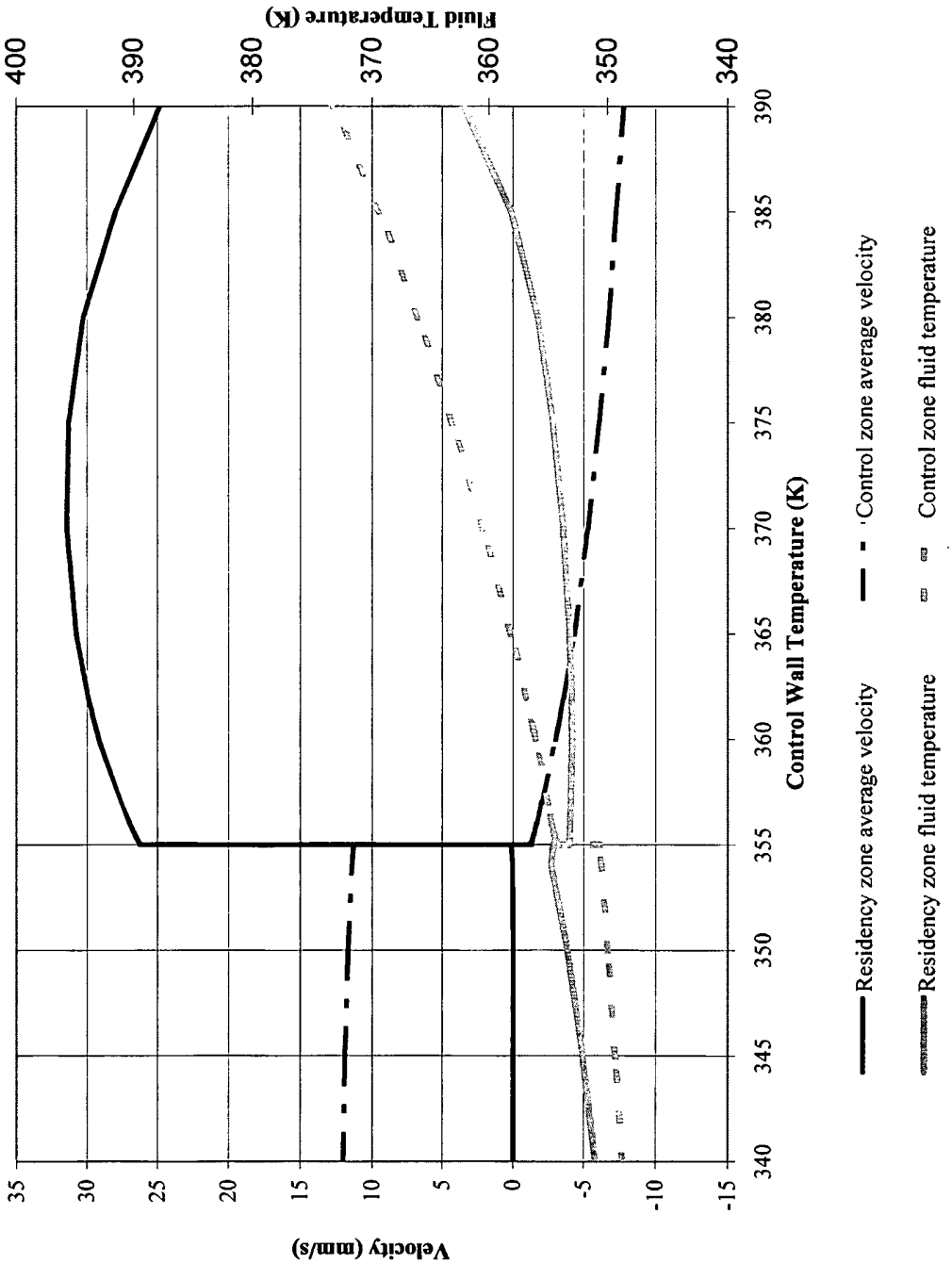
Fluid Temperature (K)
400
390
380
370
360
350
340
Velocity (mm/s)
35
30
25
20
15
10
5
0
-5
-10
-15
340
345
350
355
360
365
370
375
380
385
390
Control Wall Temperature (K)
Residency zone average velocity
Control zone average velocity
Residency zone fluid temperature
Control zone fluid temperature

Fig. 7

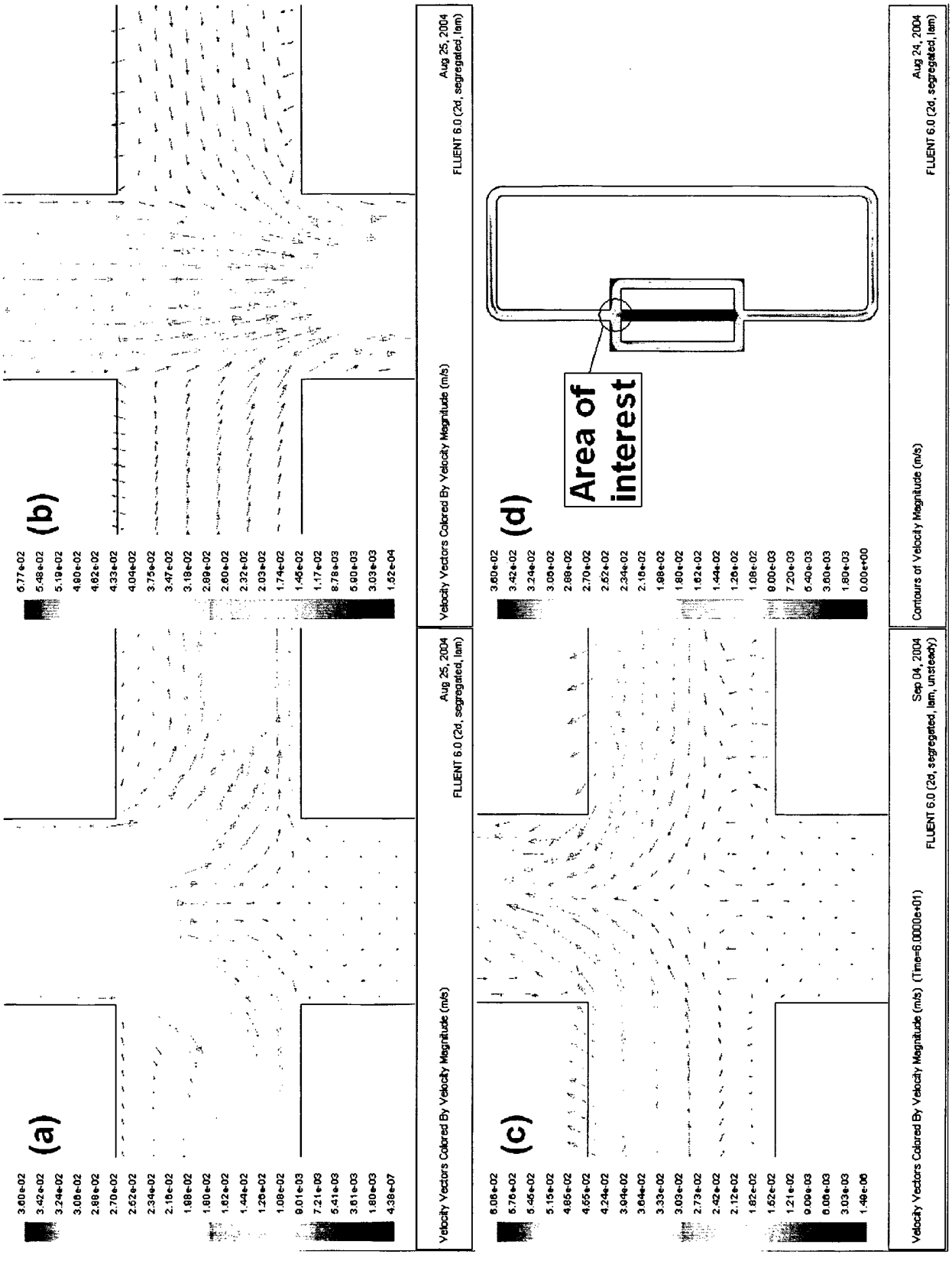
(a)
(b)
(c)
(d)
Area of interest
Velocity Vectors Colored By Velocity Magnitude (m/s)
Aug 25, 2004
FLUENT 6.0 (2d, segregated, lam)
Contours of Velocity Magnitude (m/s)
Aug 24, 2004
Sep 04, 2004
FLUENT 6.0 (2d, segregated, lam, unsteady)

Fig. 8

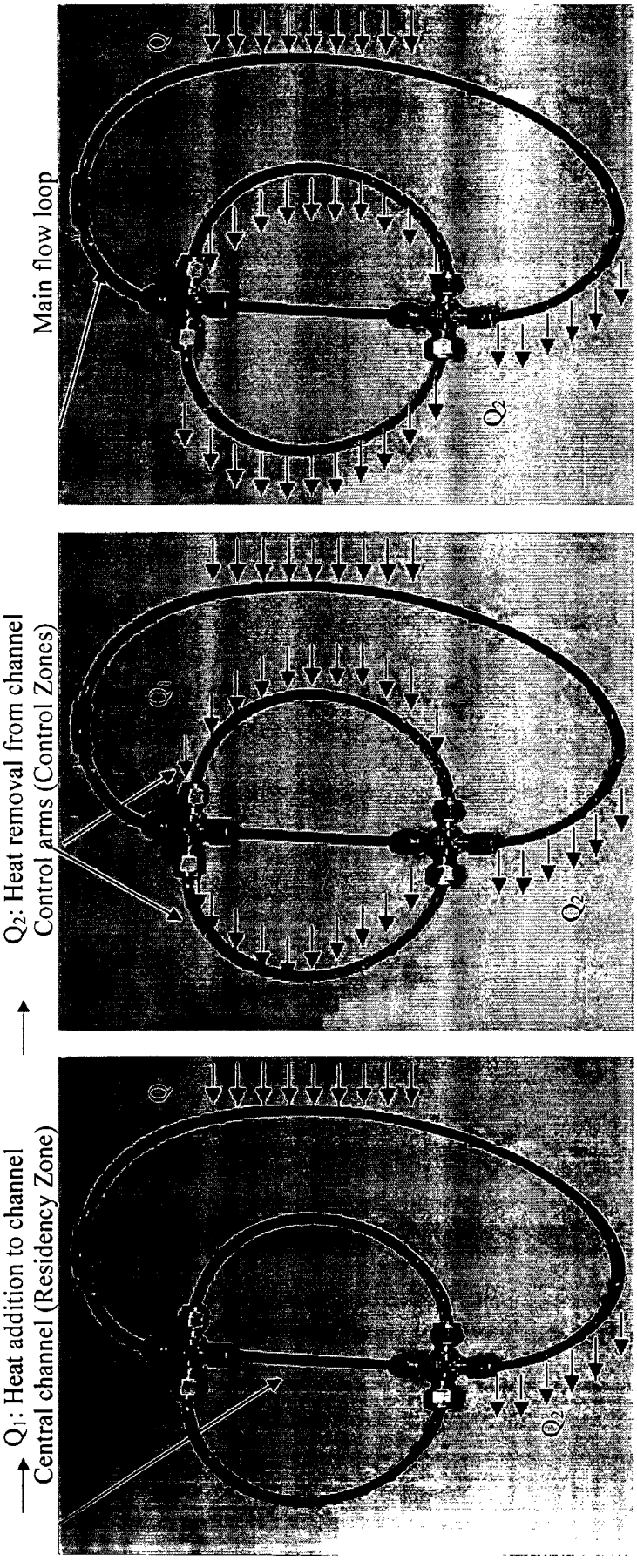
Q1: Heat addition to channel
Central channel (Residency Zone)
Q2: Heat removal from channel
Control arms (Control Zones)
Main flow loop
Q2
Q2
Q2
Flow regime 1
Flow regime 2
Flow regime 3

Fig. 9

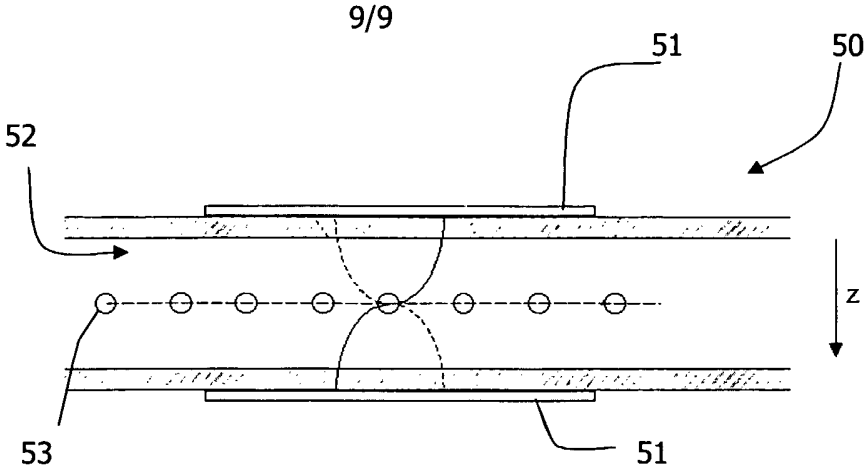

Fig.10

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IE2005/000019

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B01L3/00 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 B01L C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 270 183 A (CORBETT ET AL) 14 December 1993 (1993-12-14) abstract; figure 1 column 3, line 20 - line 51 column 6, lines 32-37	1,2, 14-17
X	WO 99/41015 A (INSTITUT FUER PHYSIKALISCHE HOCHTECHNOLOGIE E.V; KOEHLER, JOHANN, MICH) 19 August 1999 (1999-08-19) abstract	1,2
A	US 2002/037499 A1 (QUAKE STEPHEN R ET AL) 28 March 2002 (2002-03-28) figures 1,3,5,6,14 paragraphs '0313! - '0319!	1-20

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
3 June 2005	14/06/2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Wyplosz, N

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	AGRAWAL, N., UGAZ, V.: "Pumping and Thermocycling in Closed-loop convective flow systems"'Online! March 2004 (2004-03), XP002330395 ADS ABSTRACT SERVICE Retrieved from the Internet: URL:http://adsabs.harvard.edu/cgi-bin/nph-bib_query?bibcode=2004APS..MAR.R1099A& db_key=PHY> abstract ----	1-20
T	ANALYTICAL CHEMISTRY, vol. 76, 1 July 2004 (2004-07-01), pages 3707-3715, XP002330396 THERMOSIPHON-BASED PCR REACTOR abstract; figures 6,7 ----	1-20
T	AGRAWAL, N., UGAZ, V.M.: "Rapid DNA Amplification in Buoyancy Driven Closed Loop Microfluidic Systems"'Online! May 2005 (2005-05), XP002330397 NSTI CONFERENCE NANOTECH 2005 Retrieved from the Internet: URL:http://www.nsti.org/Nanotech2005/showabstract.html?absno=876> 'retrieved on 2005-06-02! abstract ----	1-20

INTERNATIONAL SEARCH REPORT

formation on patent family members

Int[redacted]ional Application No
PCT/IE2005/000019

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US 2002037499 A1	28-03-2002	US 2004248167 A1	09-12-2004
		AU 7534001 A	17-12-2001
		EP 1290226 A2	12-03-2003
		JP 2003536058 T	02-12-2003
		WO 0194635 A2	13-12-2001