US 20100213063A1

(19) **United States**

(12) **Patent Application Publication**

(10) **Pub. No.: US 2010/0213063 A1**

(43) **Pub. Date: Aug. 26, 2010**

ZENHAUSERN et al.

(54) **ANALYSIS**

(76) Inventors: **Frederic ZENHAUSERN**, Phoenix, AZ (US); **Alan Nordquist**, Payson, AZ (US); **Ralf Lenigk**, Chandler, AZ (US); **Cedric Hurth**, Tempe, AZ (US); **Jianing Yang**, Phoenix, AZ (US)

Correspondence Address:
MERCHANT & GOULD PC
P.O. BOX 2903
MINNEAPOLIS, MN 55402-0903 (US)

(21) Appl. No.: **12/703,010**

(22) Filed: **Feb. 9, 2010**

Related U.S. Application Data

(60) Provisional application No. 61/151,104, filed on Feb. 9, 2009, provisional application No. 61/151,107, filed on Feb. 9, 2009, provisional application No. 61/151,111, filed on Feb. 9, 2009, provisional application No. 61/151,117, filed on Feb. 9, 2009.

Publication Classification

(51) **Int. Cl.**
B01D 59/42 (2006.01)
G01N 27/26 (2006.01)

(52) **U.S. Cl.** **204/452**; 204/603

(57) **ABSTRACT**

Analysis methods and apparatus are provided for inspecting a channel, such as a capillary electrophoresis channel, in a device. Configuration and alignment systems are provided, together with optical systems and temperature control.

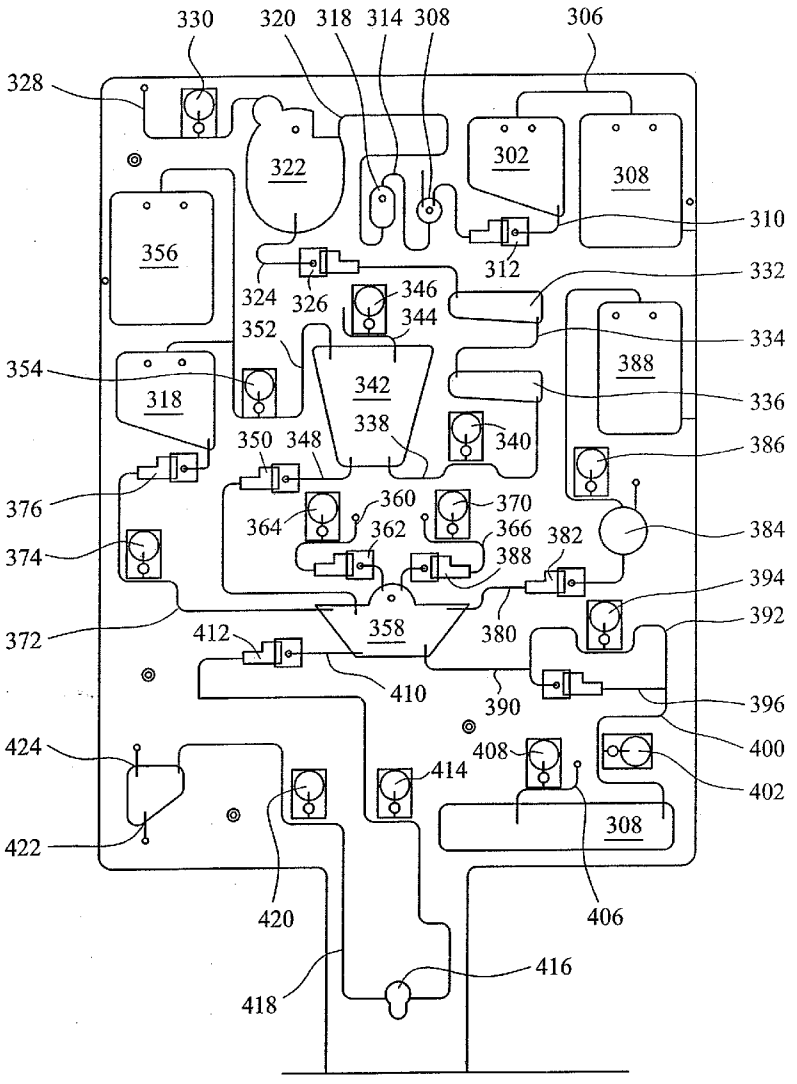
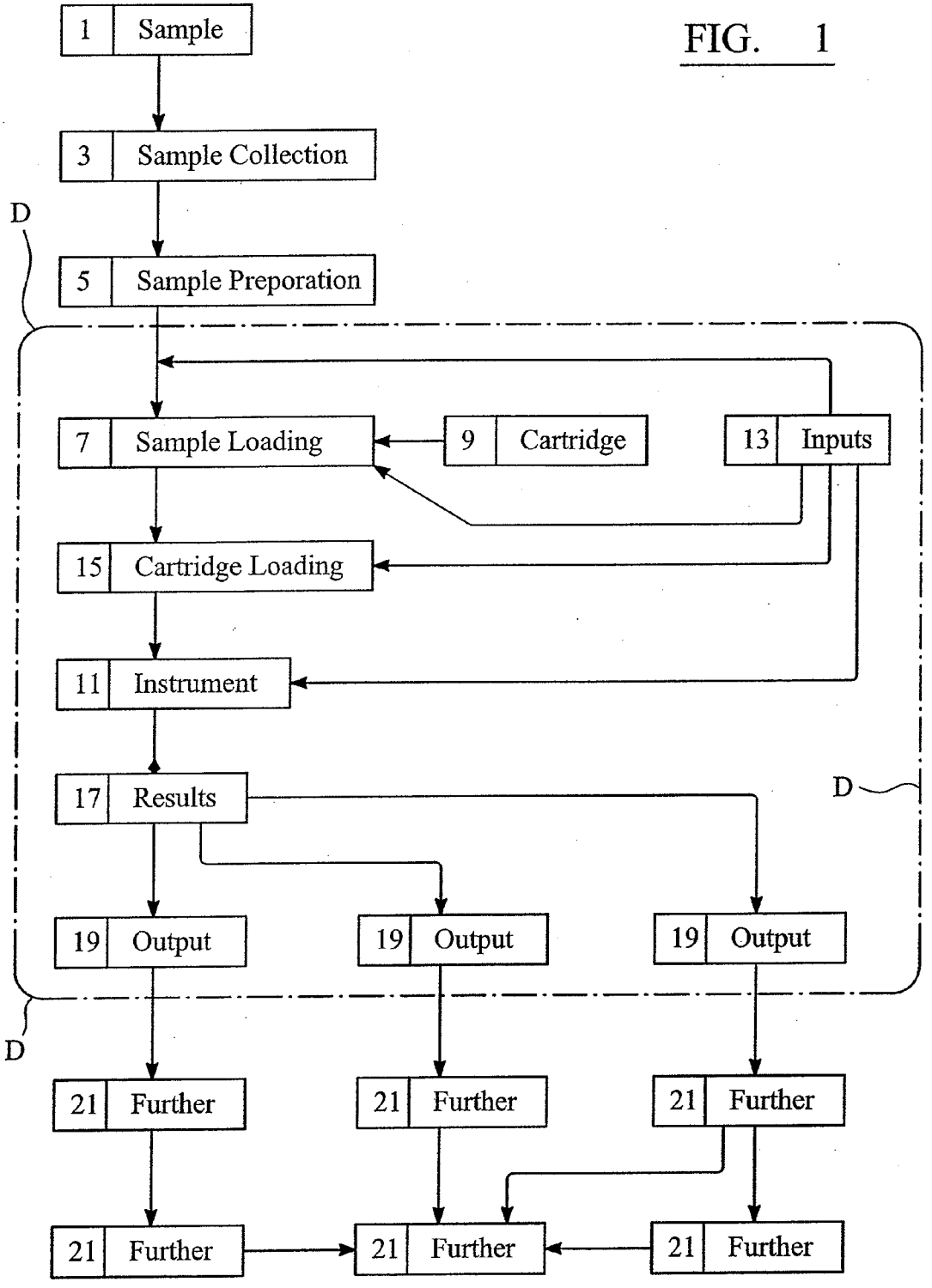


FIG. 1


1 Sample
3 Sample Collection
D
5 Sample Preporation
7 Sample Loading
9 Cartridge
13 Inputs
15 Cartridge Loading
11 Instrument
17 Results
D
19 Output
19 Output
19 Output
D
21 Further
21 Further
21 Further
21 Further
21 Further
21 Further

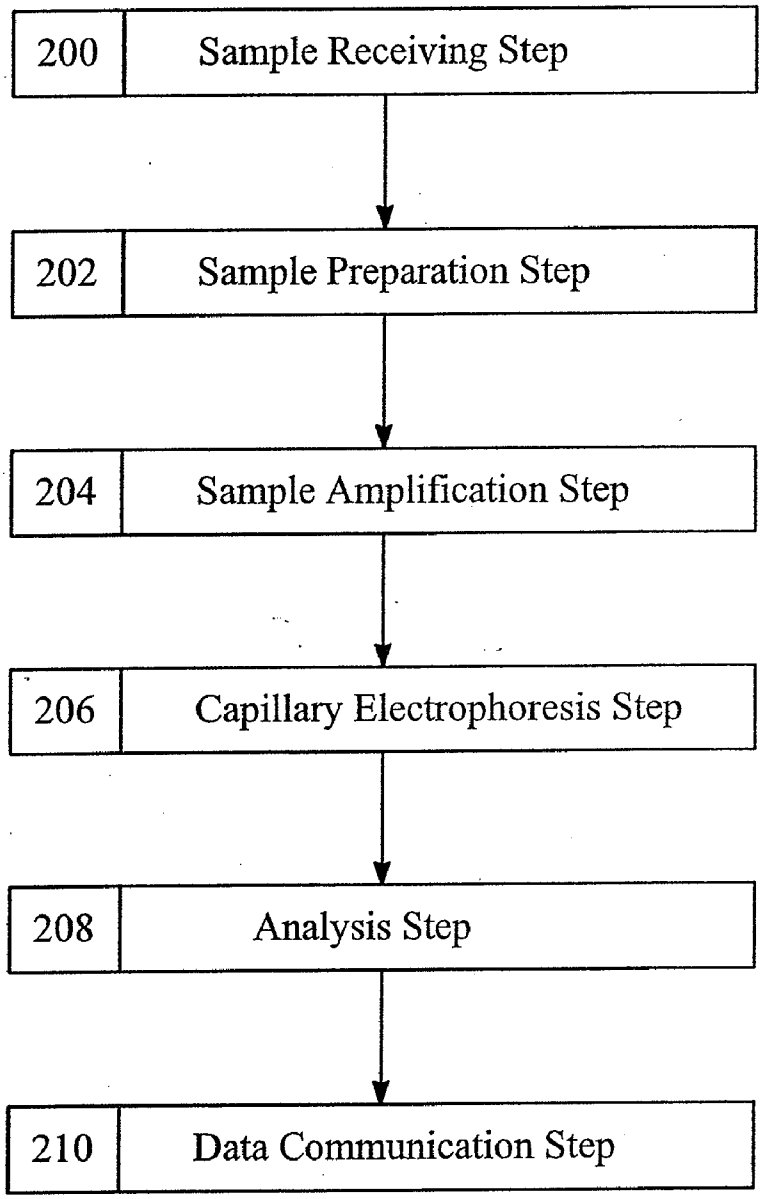
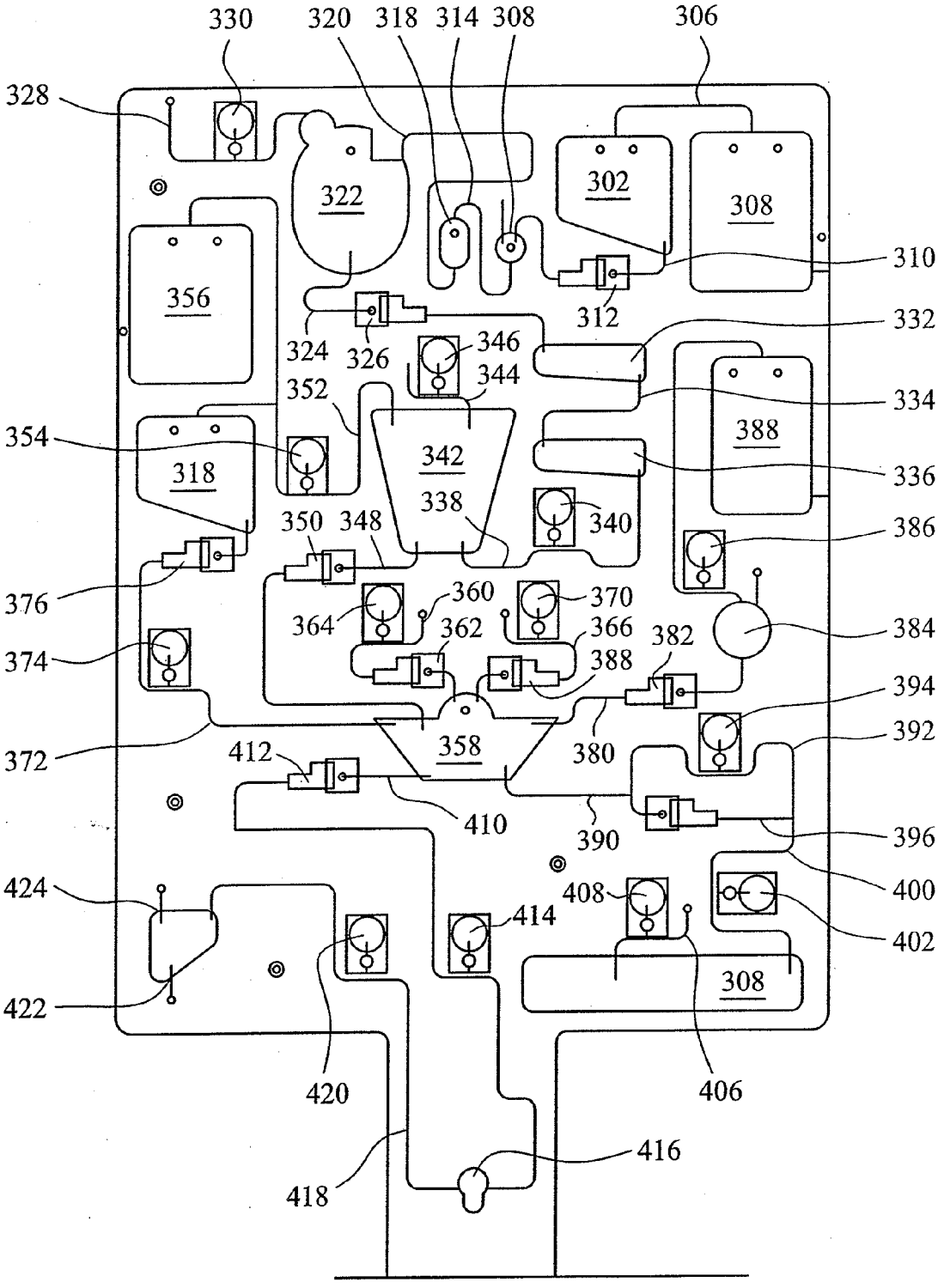

200
Sample Receiving Step
202
Sample Preparation Step
204
Sample Amplification Step
206
Capillary Electrophoresis Step
208
Analysis Step
210
Data Communication Step

FIG. 2


330
320
318
314
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328
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310
356
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342
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360
370
364
362
366
382
384
374
388
394
372
358
380
392
412
410
390
396
424
408
400
414
402
422
308
420
406
416
418

FIG. 3a

Design Specifications

Design Specification : fSS Buccal					
Functional Chambers	Volume	Depth	Tool	Vent	Note
C1 (Lysis)	300ul total	1mm			
C2 (Purification/Beads)	311ul total	0.75mm		Yes	
Expansion Chamber	67ul total	0.75mm			
C3 (Dwell)	250ul total	0.5mm		Yes	
C4 (Heating/Magnet)	250ul total	1.0mm		Yes	
Bead Storage Chamber	30ul total	1.0mm		Yes	
PCR Chamber	23ul	1.0mm			
Binding Buffer Chamber	40ul total	2.0mm		Yes	
Auxiliary Chambers					
Elution	150ul total	2.0mm		Yes	
Wash Buffer	250ul total	1.0mm		No	
Recovery	200ul	2.0mm		Yes	
EC Pump	1124ul total	2.0mm			
Waste	1000ul total	2.5mm		Yes	
Channel					
Flow Channel		0.5mm	0.5mm BEM		
Pump Channel		0.5mm	0.5mm BEM		See A.N.
PCR Entrance Channel		1.0mm	1.5mm BEM		
Paraffin Valve	Diameter				
Open Valve	1.0mm	0.5mm			
Close Valve	2.0mm	1.0mm			
Close Valve 12/13	3.0mm	1.0mm			PCR Close valves enlarged
Cartridge Specification					
Height	160mm				
Width	125mm				
Thickness	3.0mm				
Electrode Glue	UV Glue				
Alignment Pin	2.3mm				
Bonding Tape	90106				
Fabrication Note					
Tape trimmed in chambers					

FIG. 3b

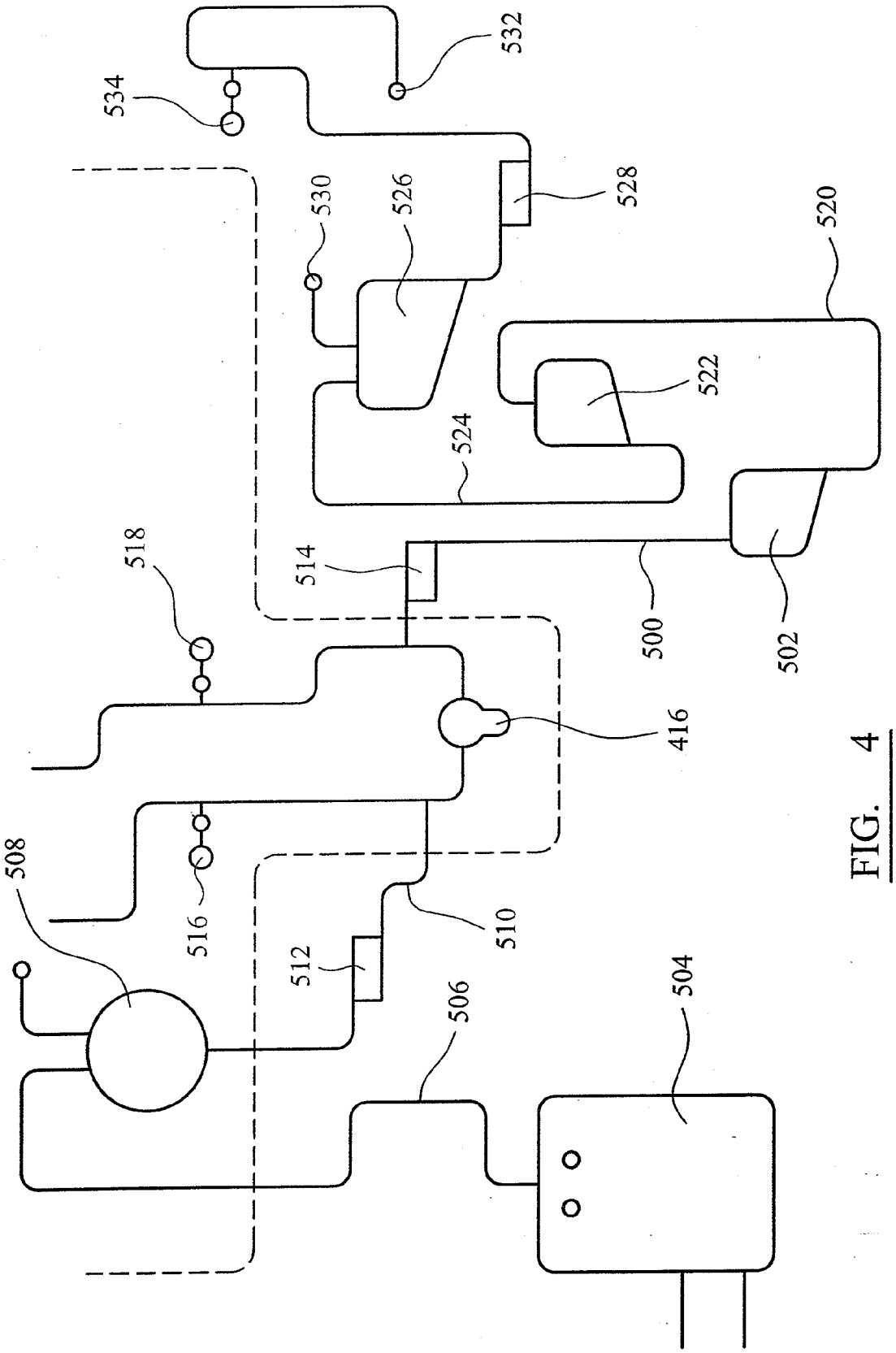

532
534
530
526
528
520
522
524
518
514
500
502
416
508
516
512
510
506
504

FIG. 4

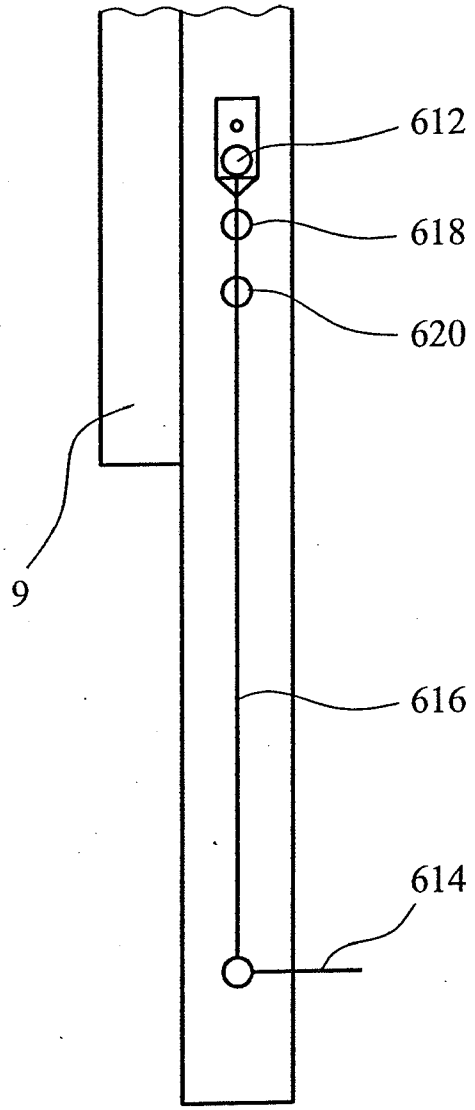

612
618
620
9
616
614

FIG. 5a

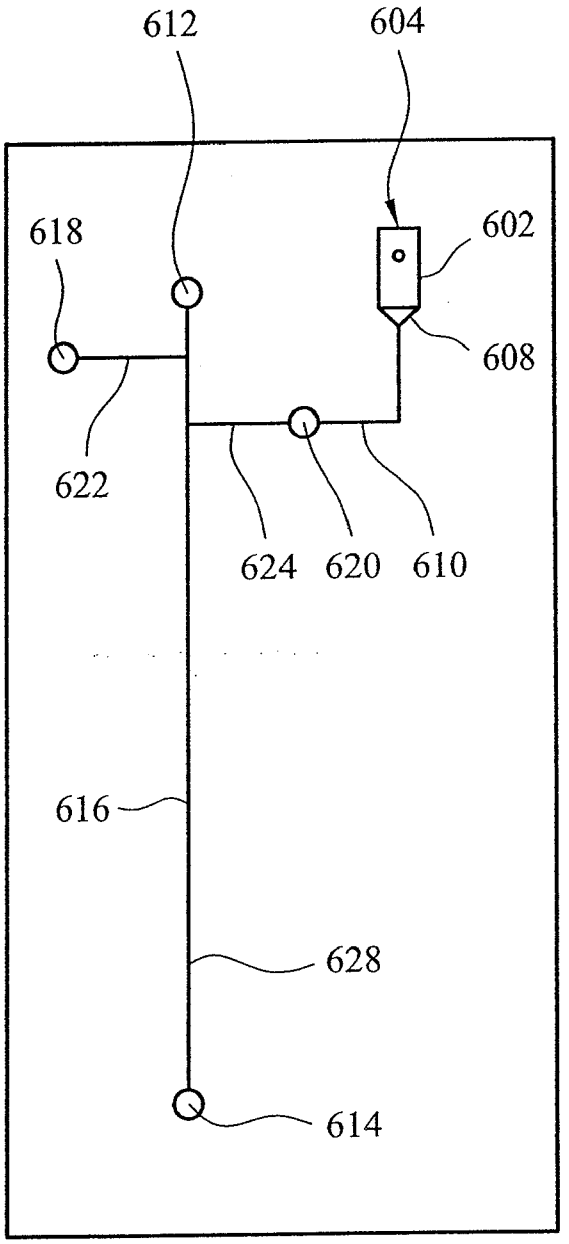

612
604
618
602
608
622
624
620
610
616
628
614

FIG. 5b

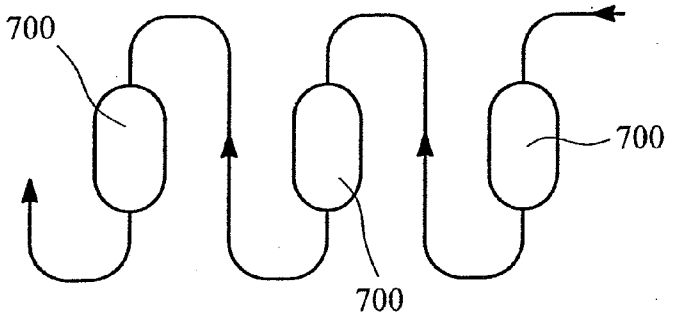


FIG. 6a

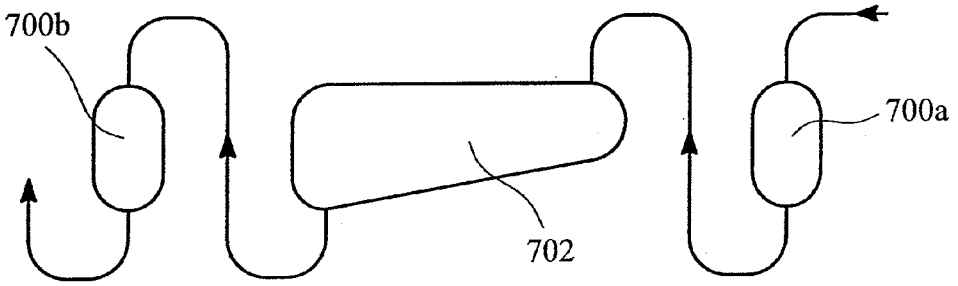


FIG. 6b

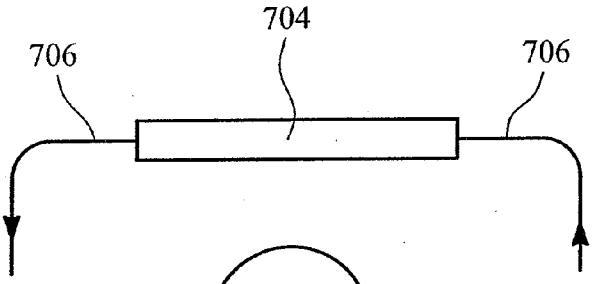


FIG. 6c

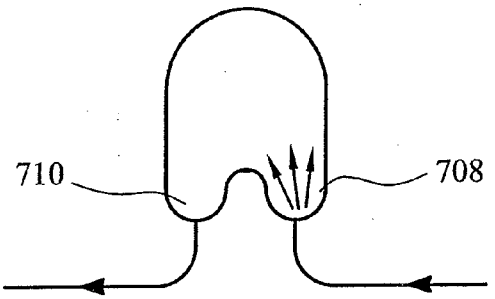


FIG. 6d

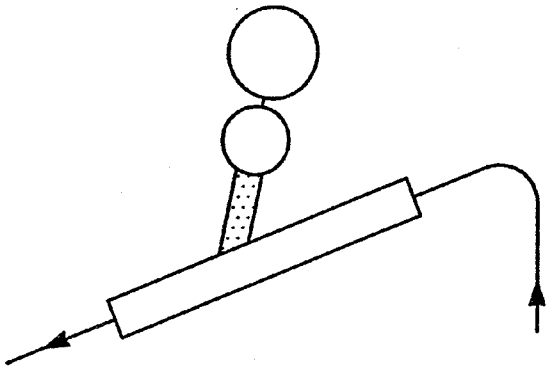


FIG. 6e

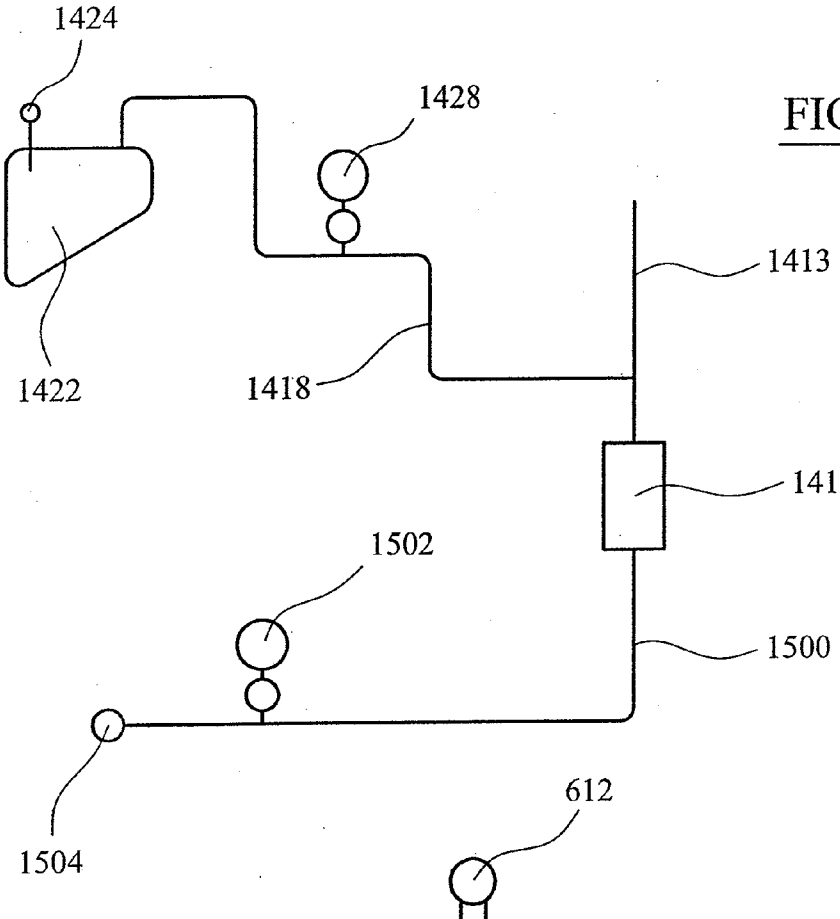

1424
1428
1413
1422
1418
1416
1502
1500
1504

FIG. 7

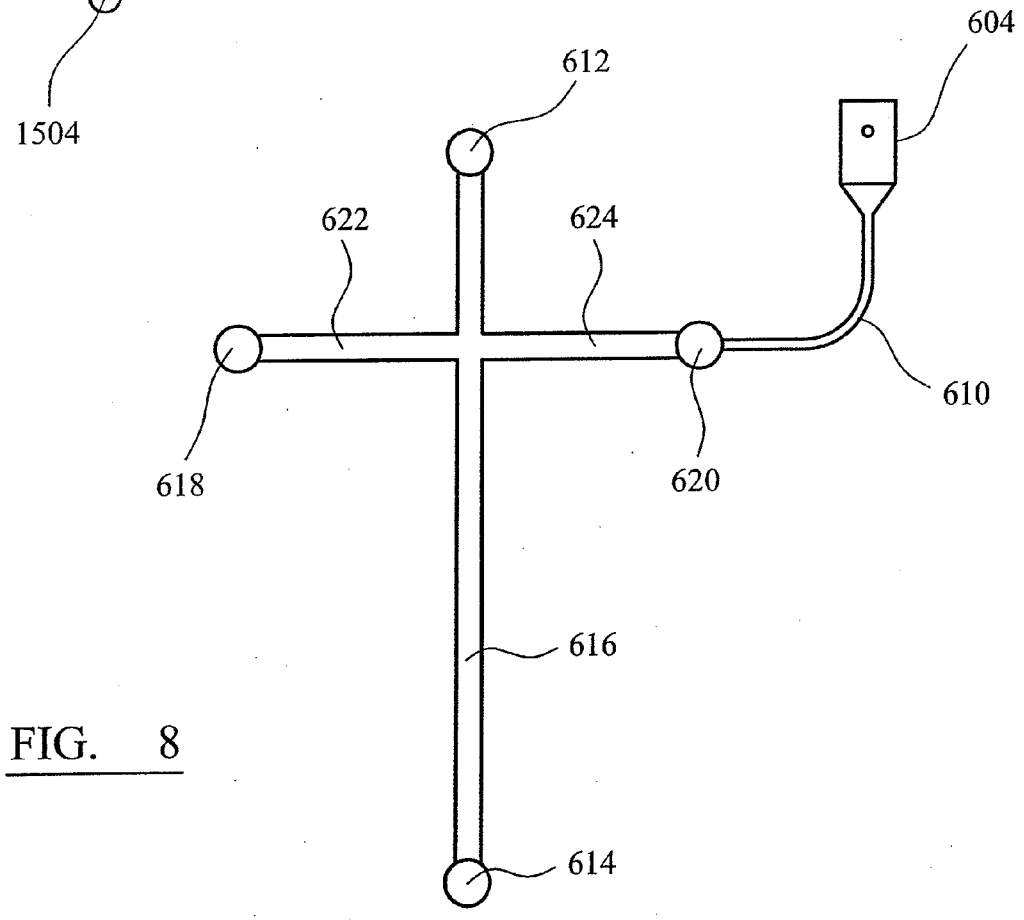

604
612
622
624
610
618
620
616
614

FIG. 8

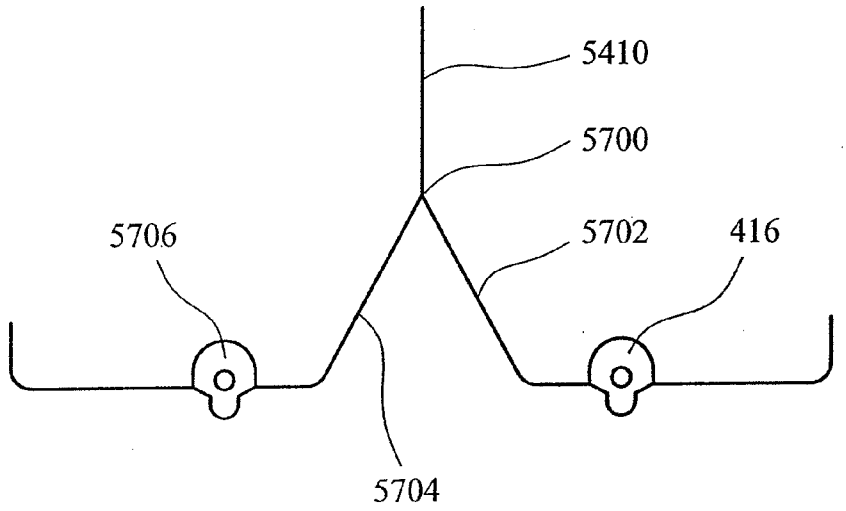

5410
5700
5706
5702
416
5704

FIG. 9

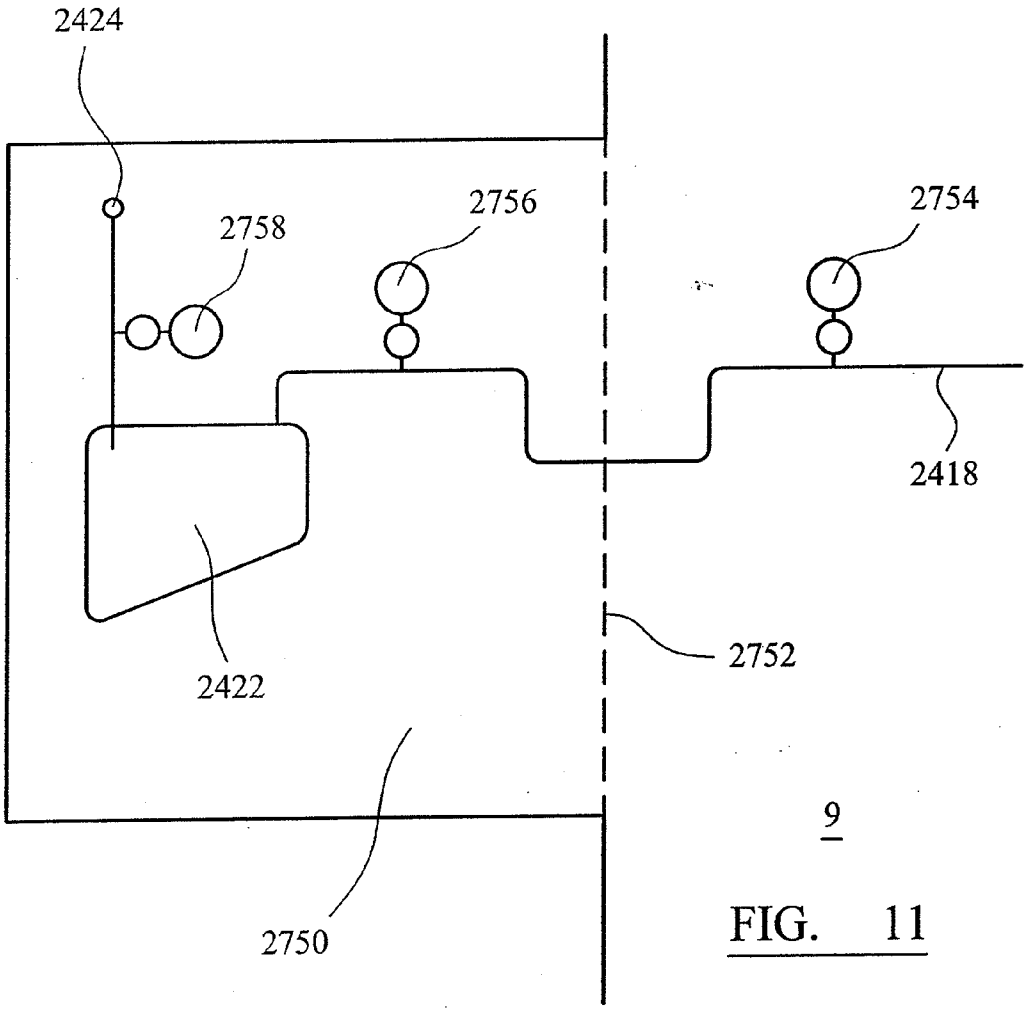

2424
2758
2756
2754
2418
2422
2752
2750
9

FIG. 11

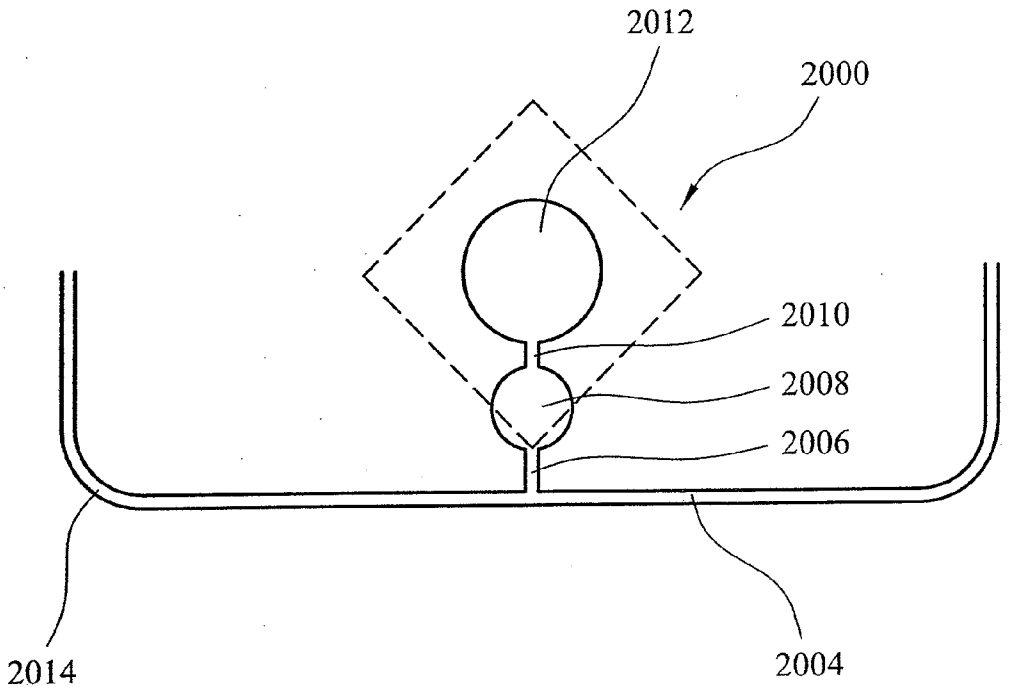


FIG. 10a

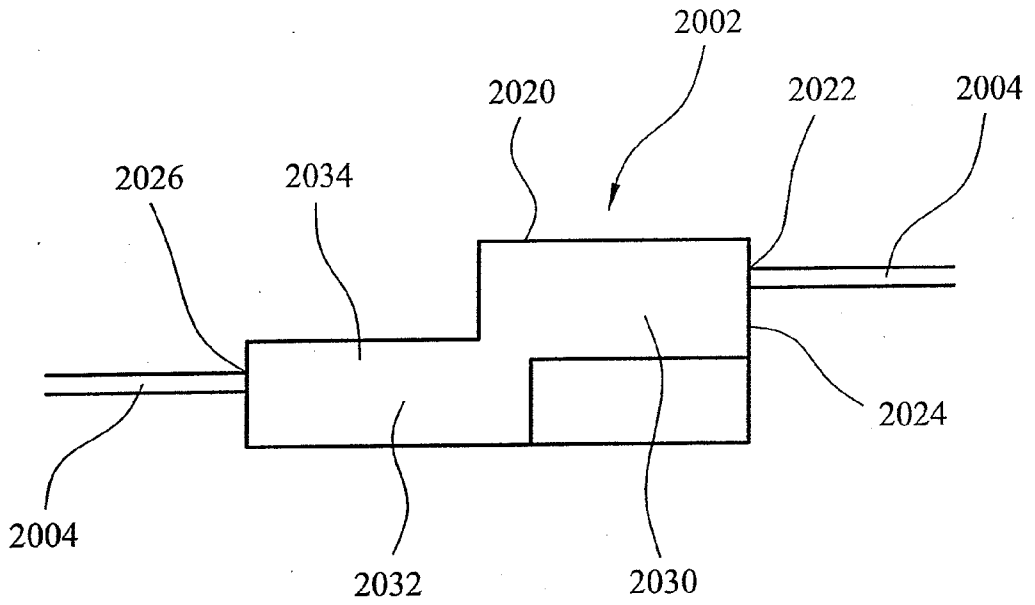


FIG. 10b

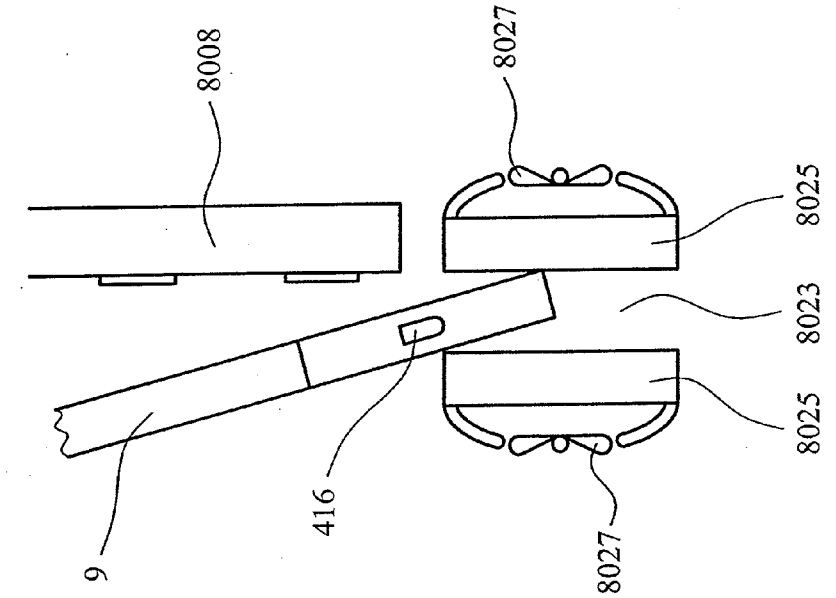

8008
8027
8025
8023
8025
9
416
8027

FIG. 13

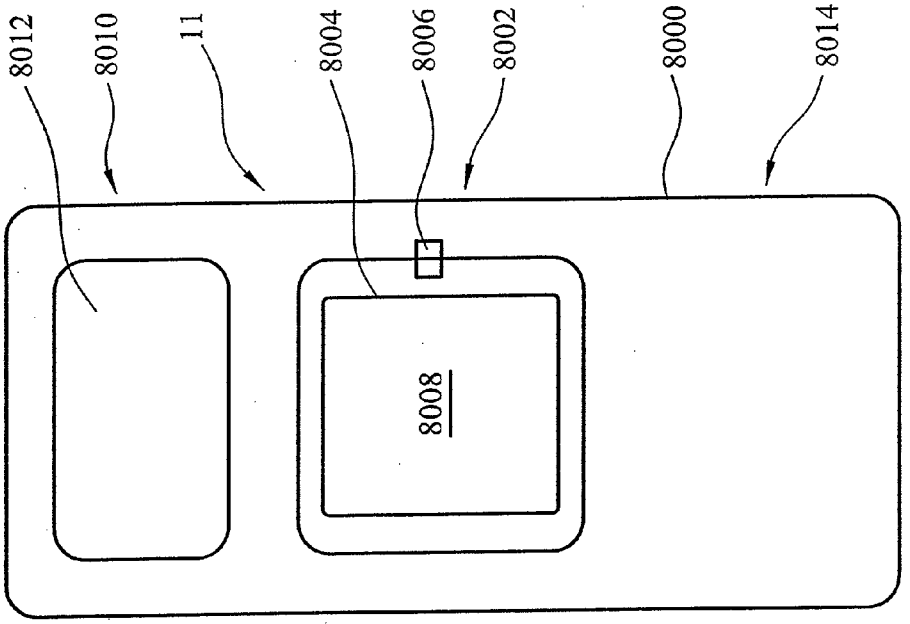
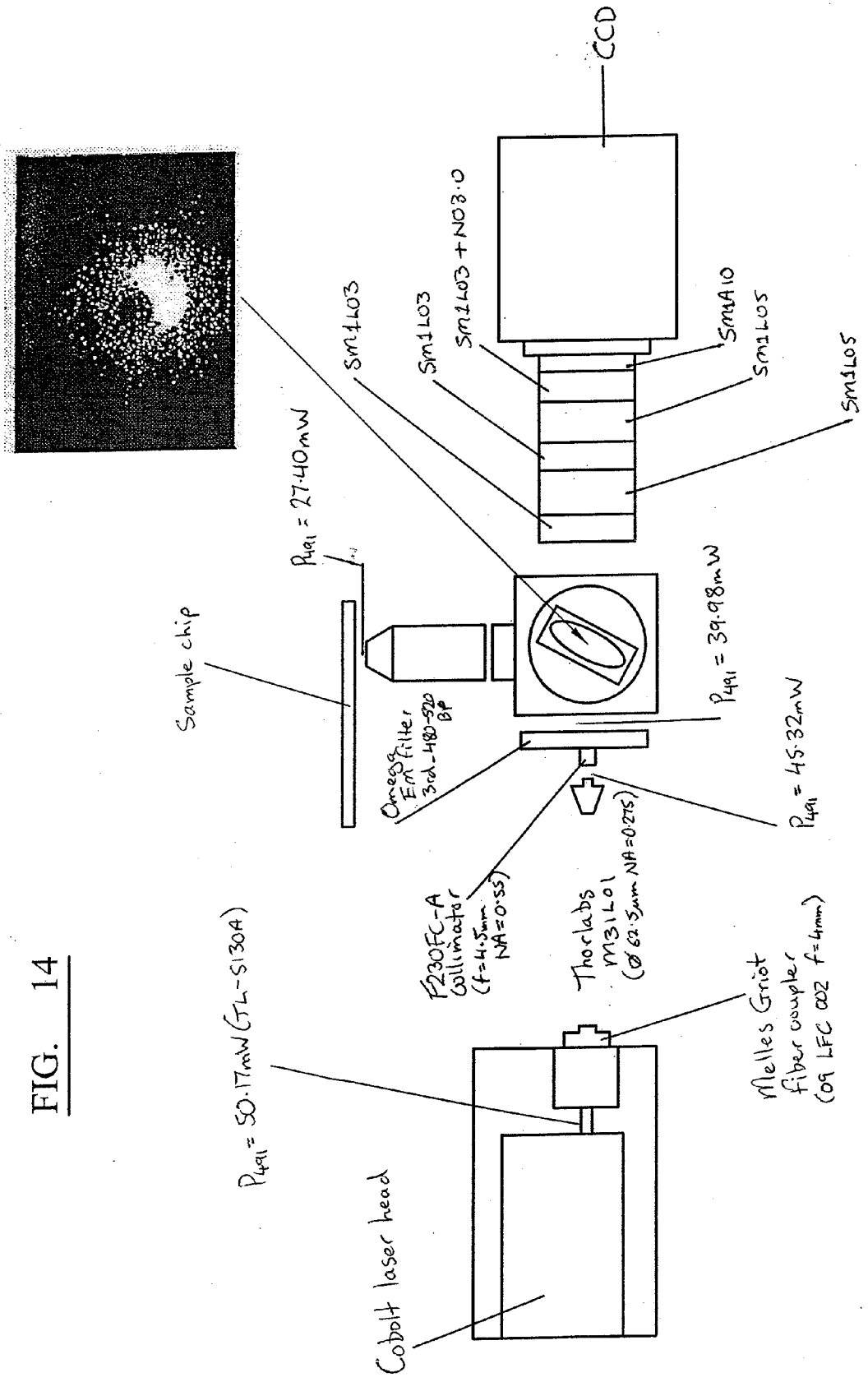

8012
8010
11
8004
8006
8002
8000
8014
8008

FIG. 12

FIG. 14


CCD
SM1L03 + NO8.0
SM1L03
SM1L03
SMA10
SM1L05
SM1L05
P_{491} = 27.40mW
Sample chip
Omega EM Filter 3rd-480-520 BP
P_{491} = 39.98mW
P_{491} = 45.32mW
P230FC-A Collimator (f=4.5mm NA=0.55)
Thorlabs M31L01 (Ø 62.5μm NA=0.275)
Melles Griot Fiber coupler (09 LFC 002 f=4mm)
P_{491} = 50.17mW (TL-5130A)
Cobolt laser head

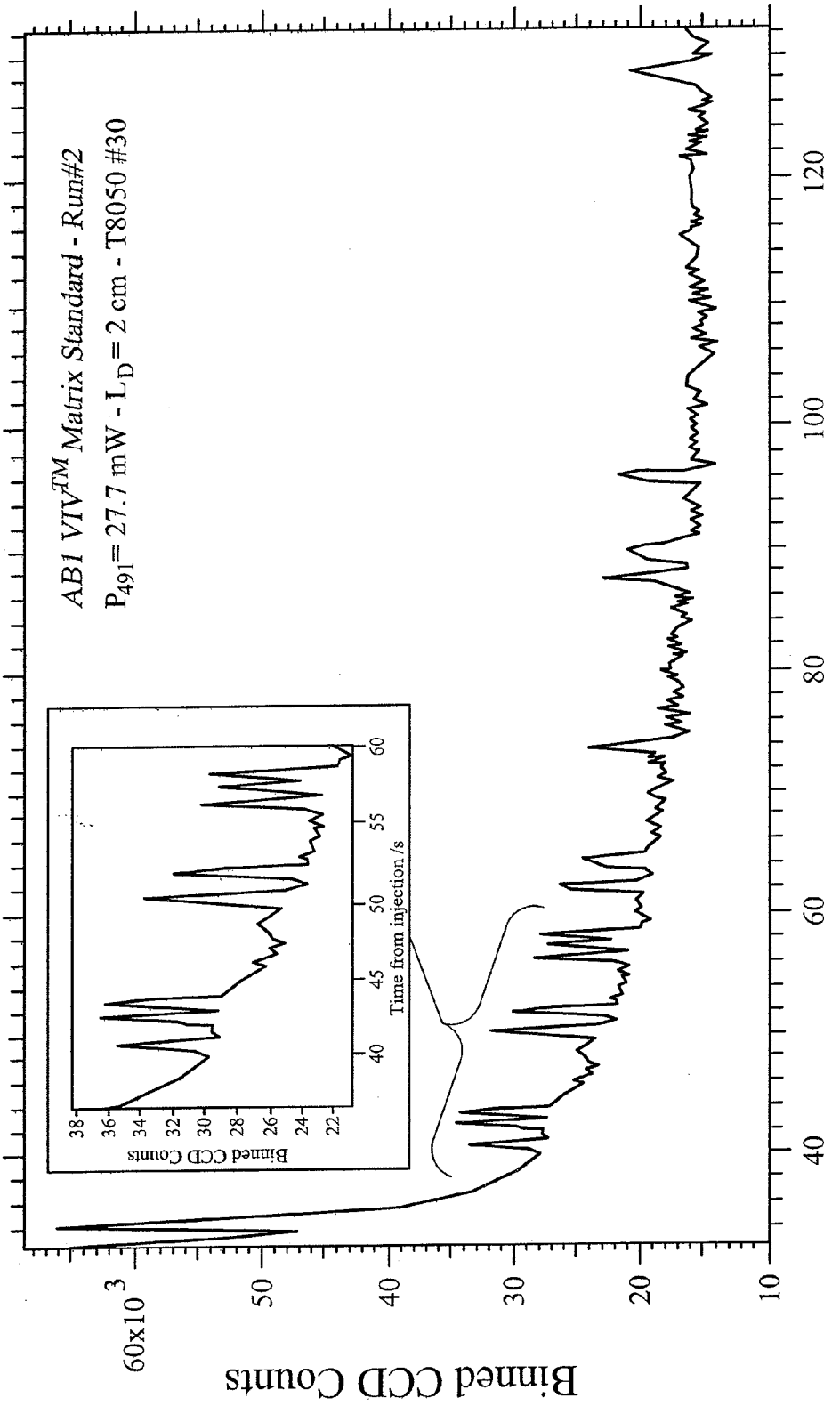

AB1 VIV™ Matrix Standard - Run#2
P491= 27.7 mW - LD = 2 cm - T8050 #30
Binned CCD Counts
60x10 3
50
40
30
20
10
40
60
80
100
120
Time from injection /s
Binned CCD Counts
38
36
34
32
30
28
26
24
22
40
45
50
55
60
Time from injection /s

FIG. 15

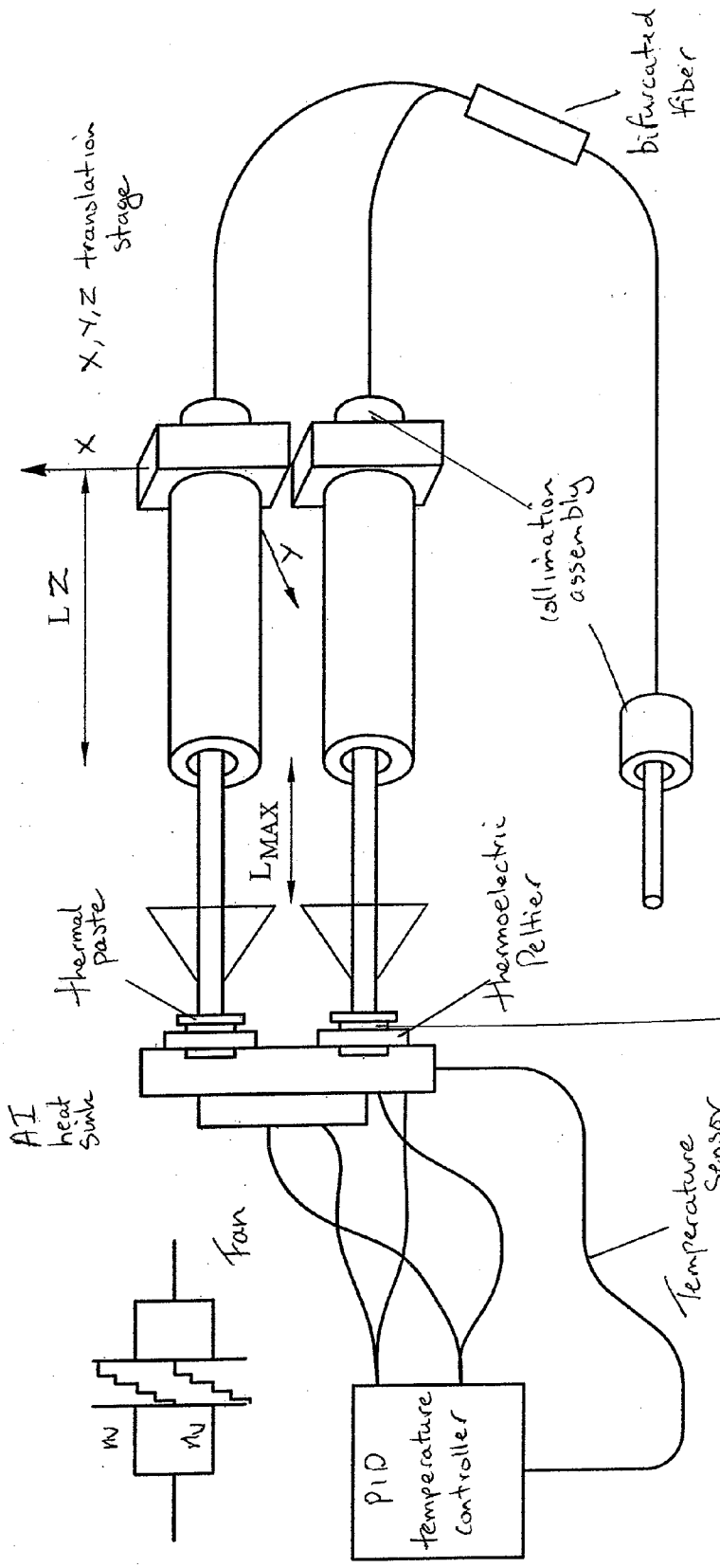

bifurcated fiber
X,Y,Z translation stage
X
Y
LZ
collimation assembly
LMAX
Thermal paste
Thermoelectric Peltier
acrylic collimator
Al heat sink
fan
Temperature Sensor
PID temperature controller

FIG. 16

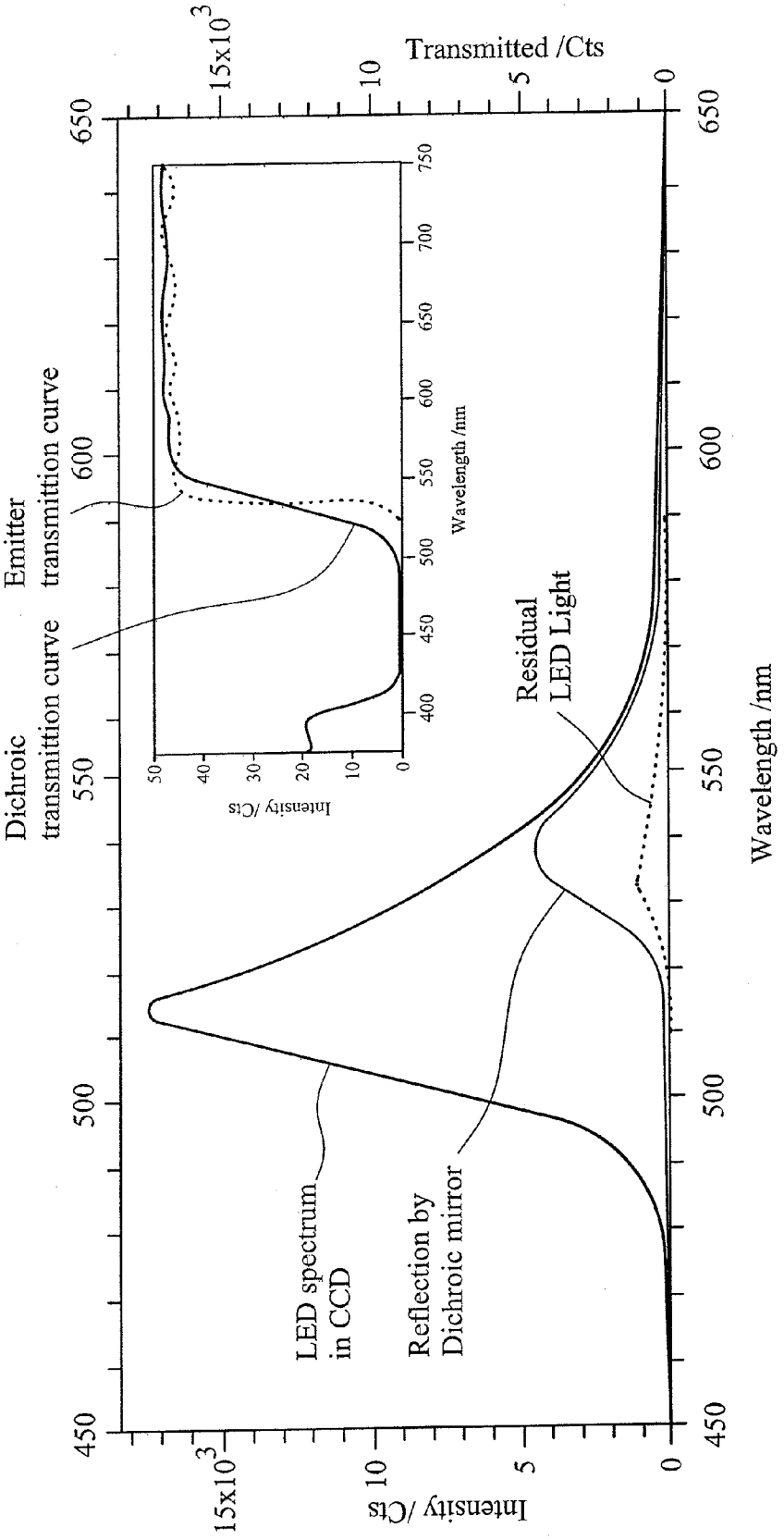

Transmitted /Cts
15x10^3
10
5
0
650
600
550
500
450
Wavelength /nm
Intensity /Cts
Dichroic transmittion curve
Emitter transmittion curve
Residual LED Light
LED spectrum in CCD
Reflection by Dichroic mirror
Wavelength /nm
Intensity /Cts

FIG. 17

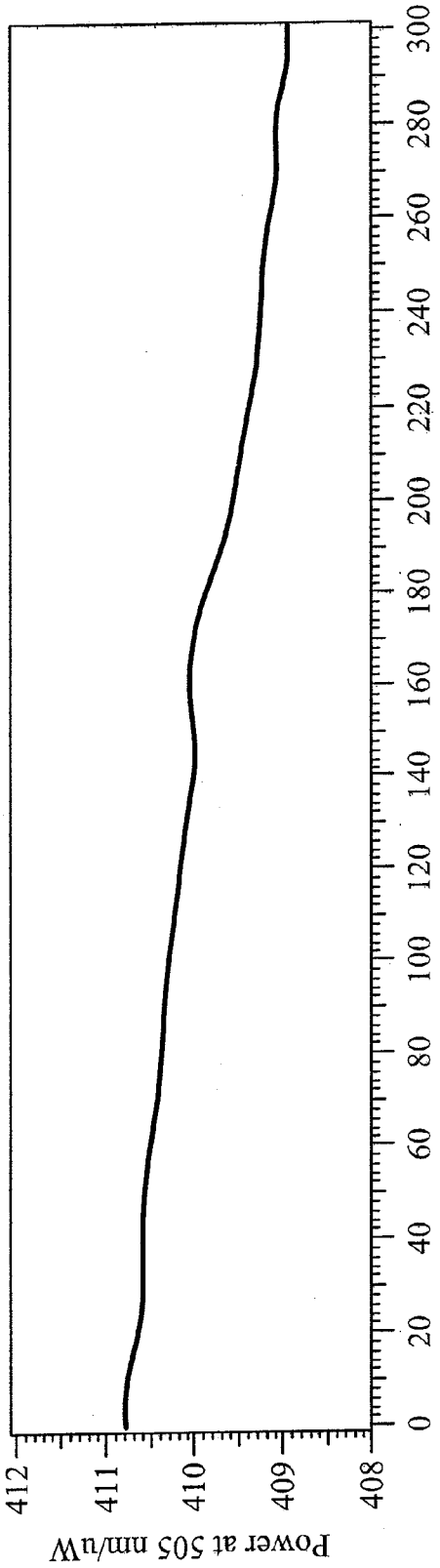

Power at 505 nm/uW
412
411
410
409
408
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
Time /m

FIG. 18

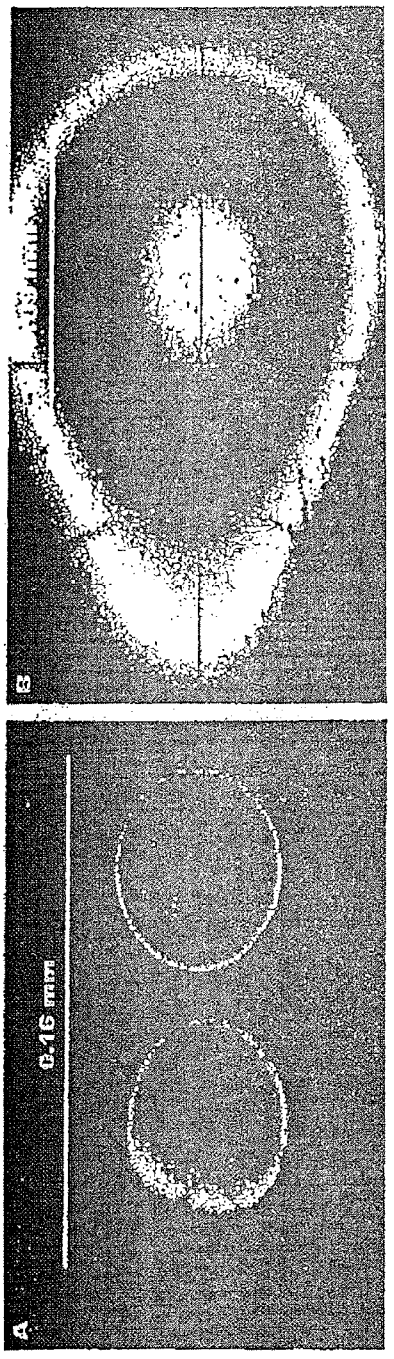

A
0.16 mm
B

FIG. 19

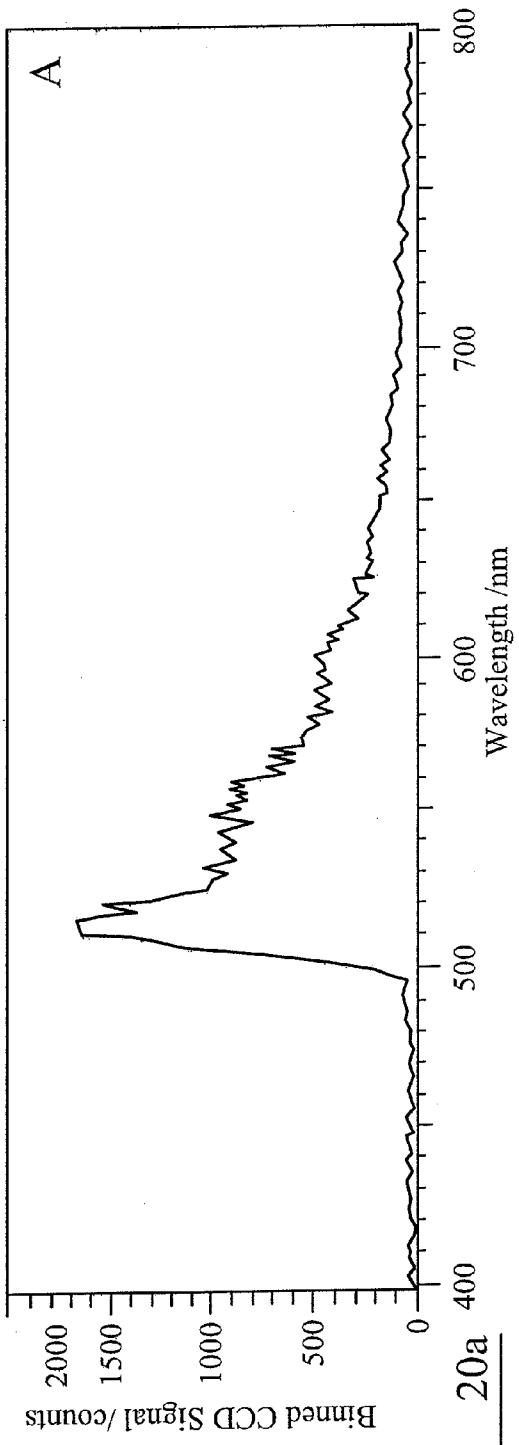


FIG. 20a

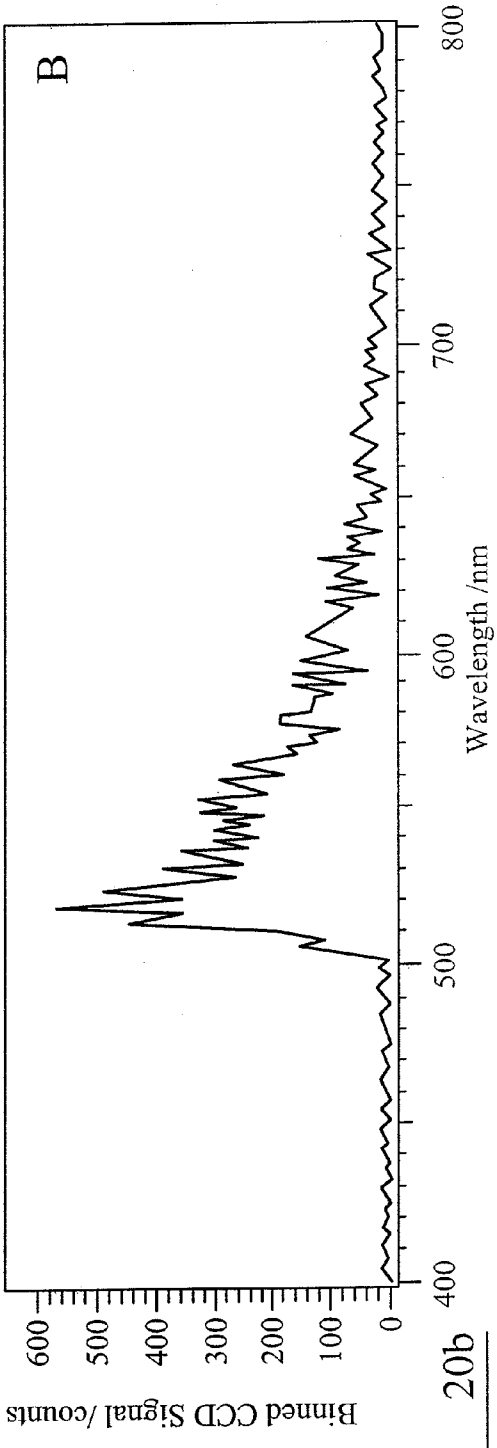


FIG. 20b

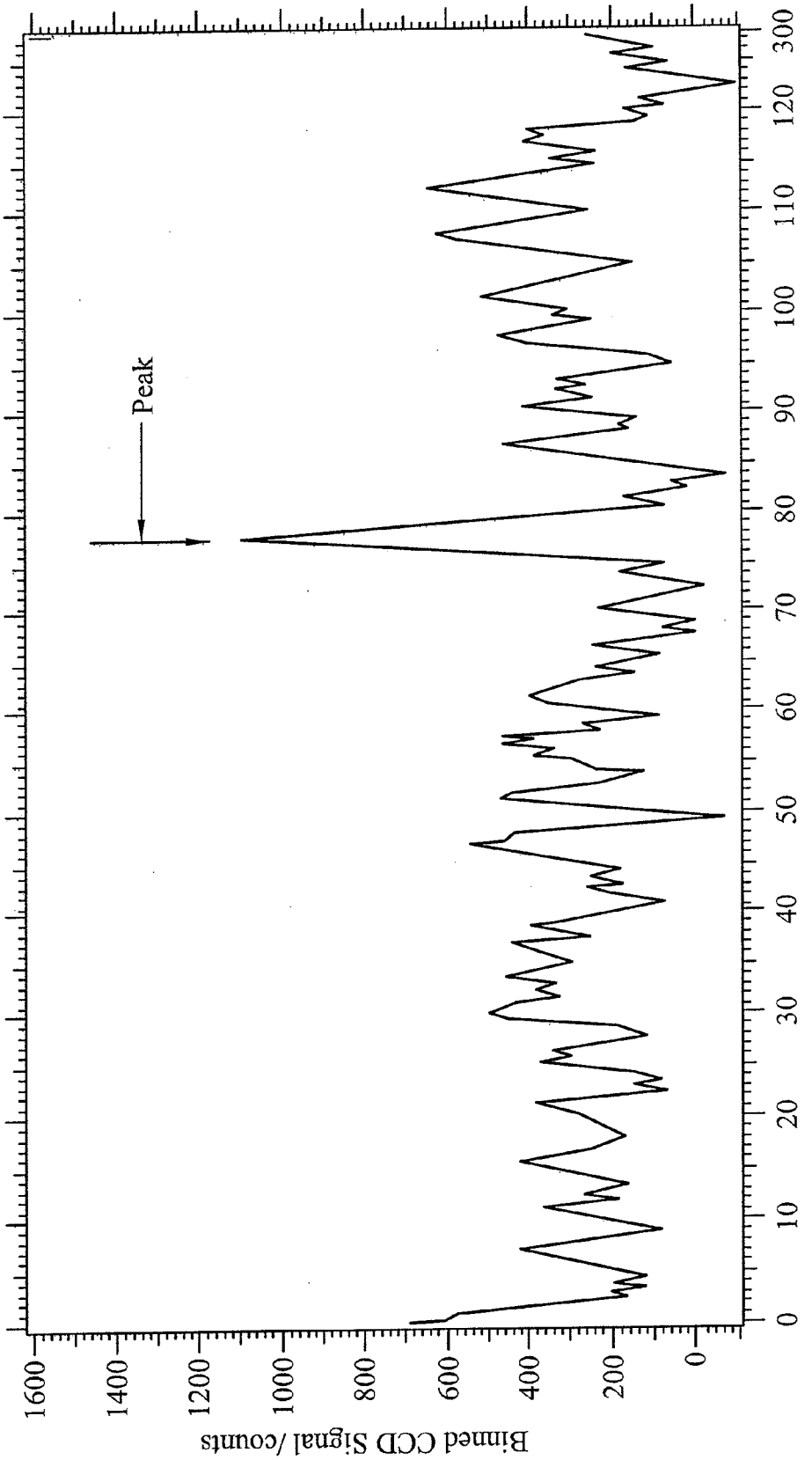

Binned CCD Signal /counts
1600
1400
1200
1000
800
600
400
200
0
Peak
0
10
20
30
40
50
60
70
80
90
100
110
120
300
Time /m

FIG. 21

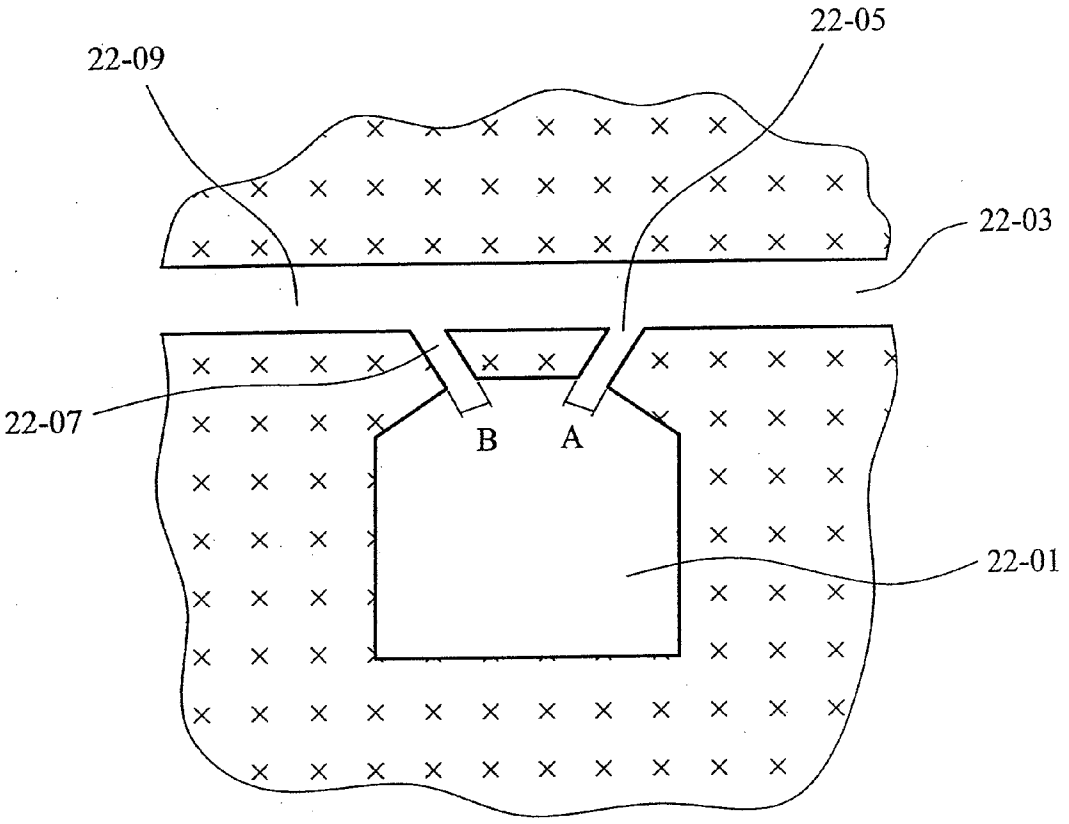


FIG. 22

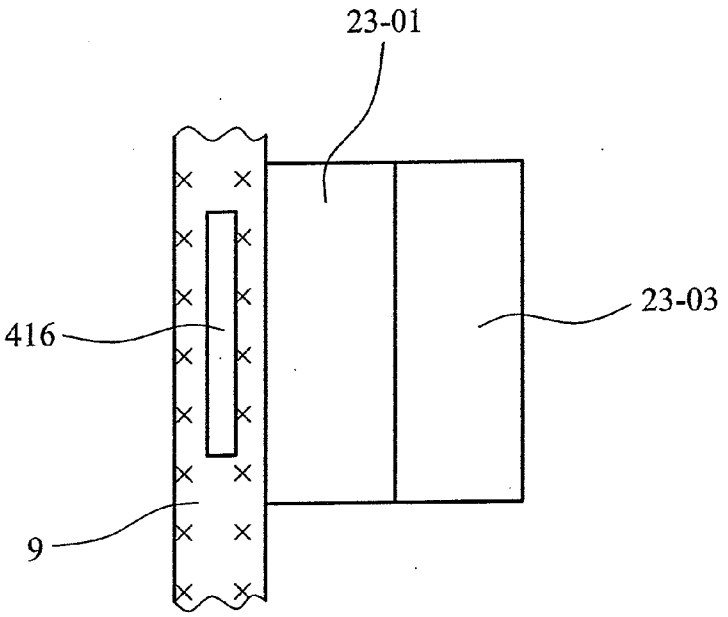


FIG. 23

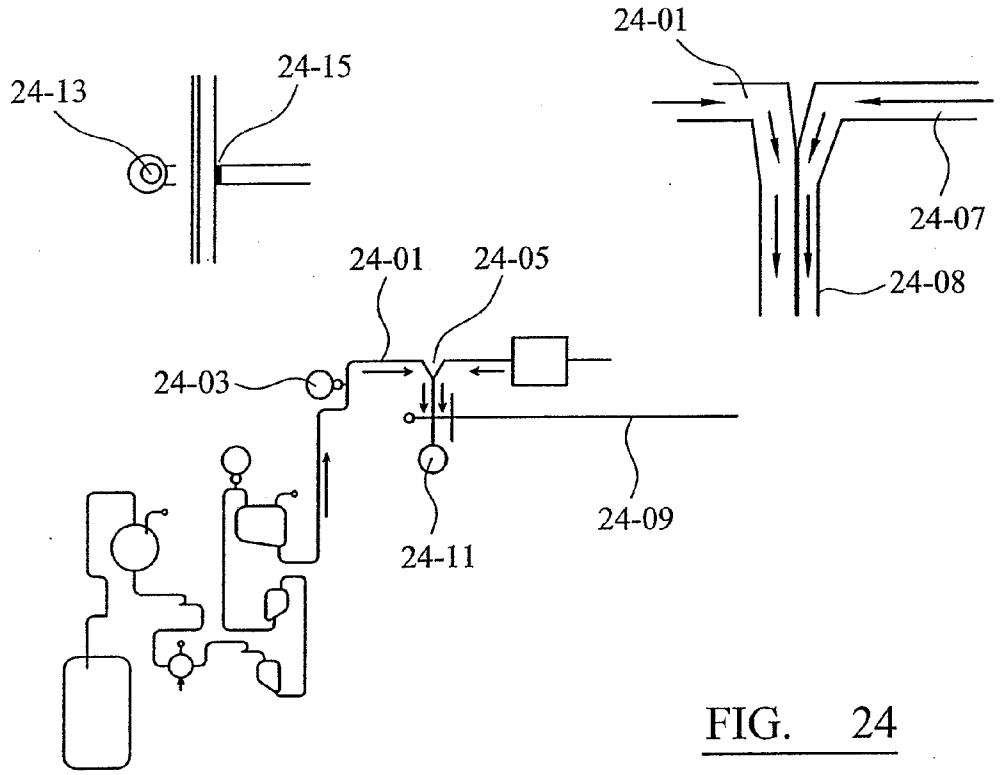

24-01
24-15
24-13
24-07
24-08
24-01
24-05
24-03
24-09
24-11

FIG. 24

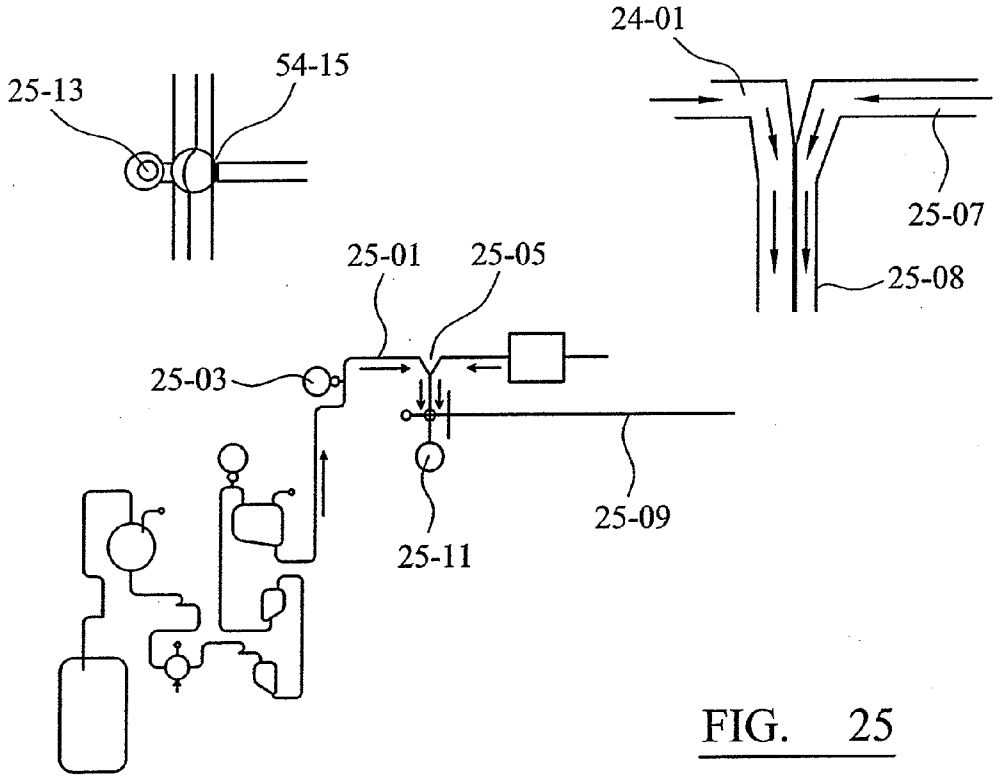

24-01
54-15
25-13
25-07
25-08
25-01
25-05
25-03
25-09
25-11

FIG. 25

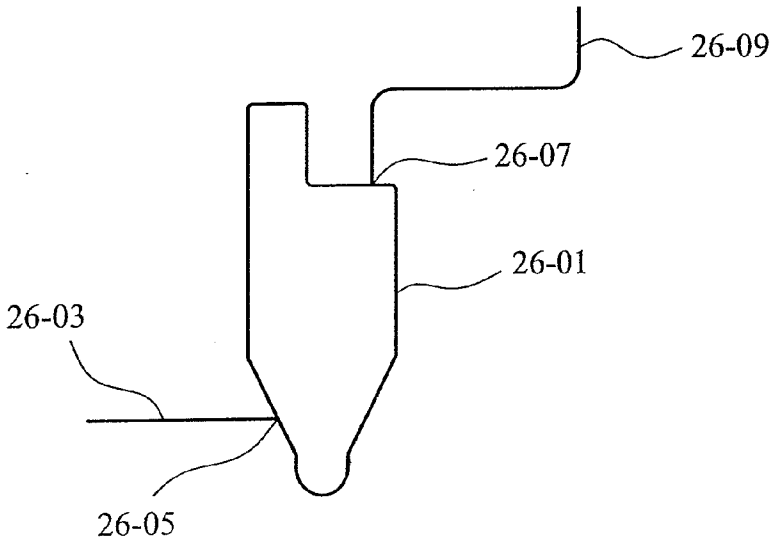

26-09
26-07
26-01
26-03
26-05

FIG. 26

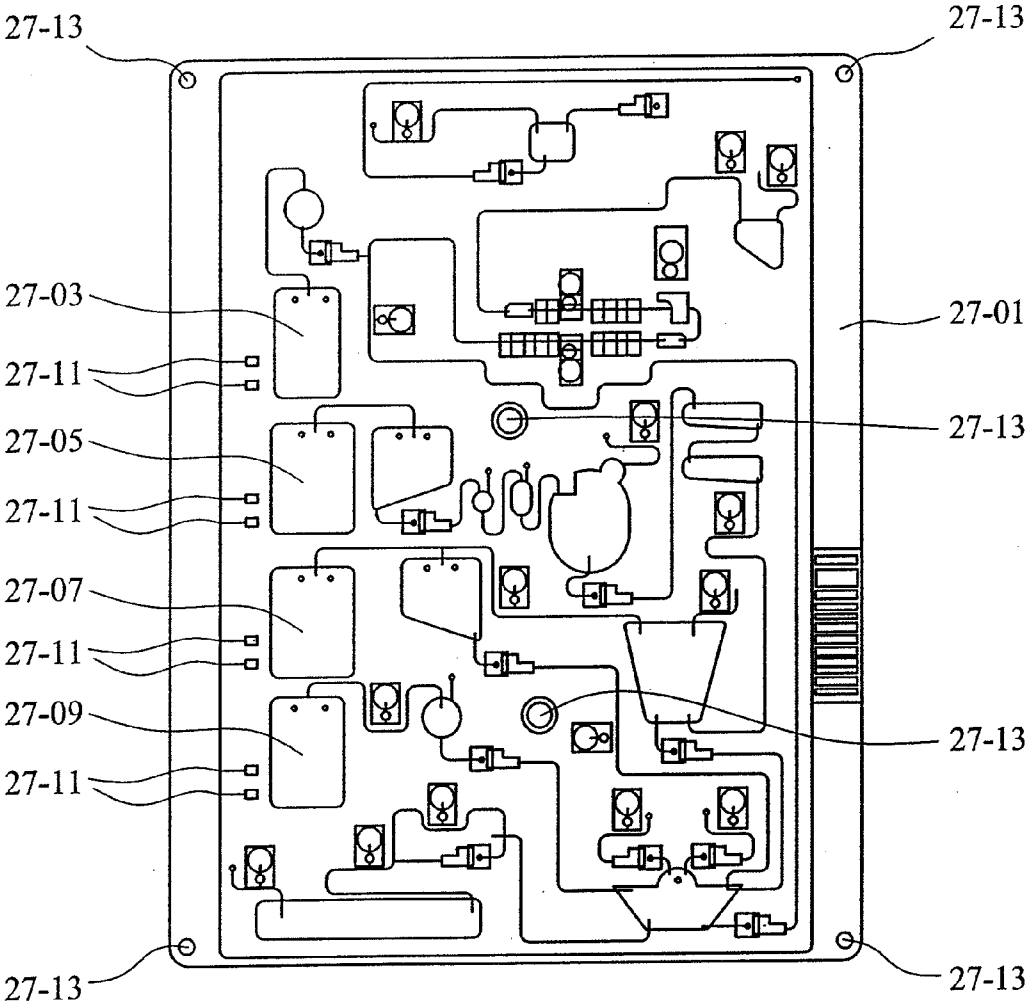
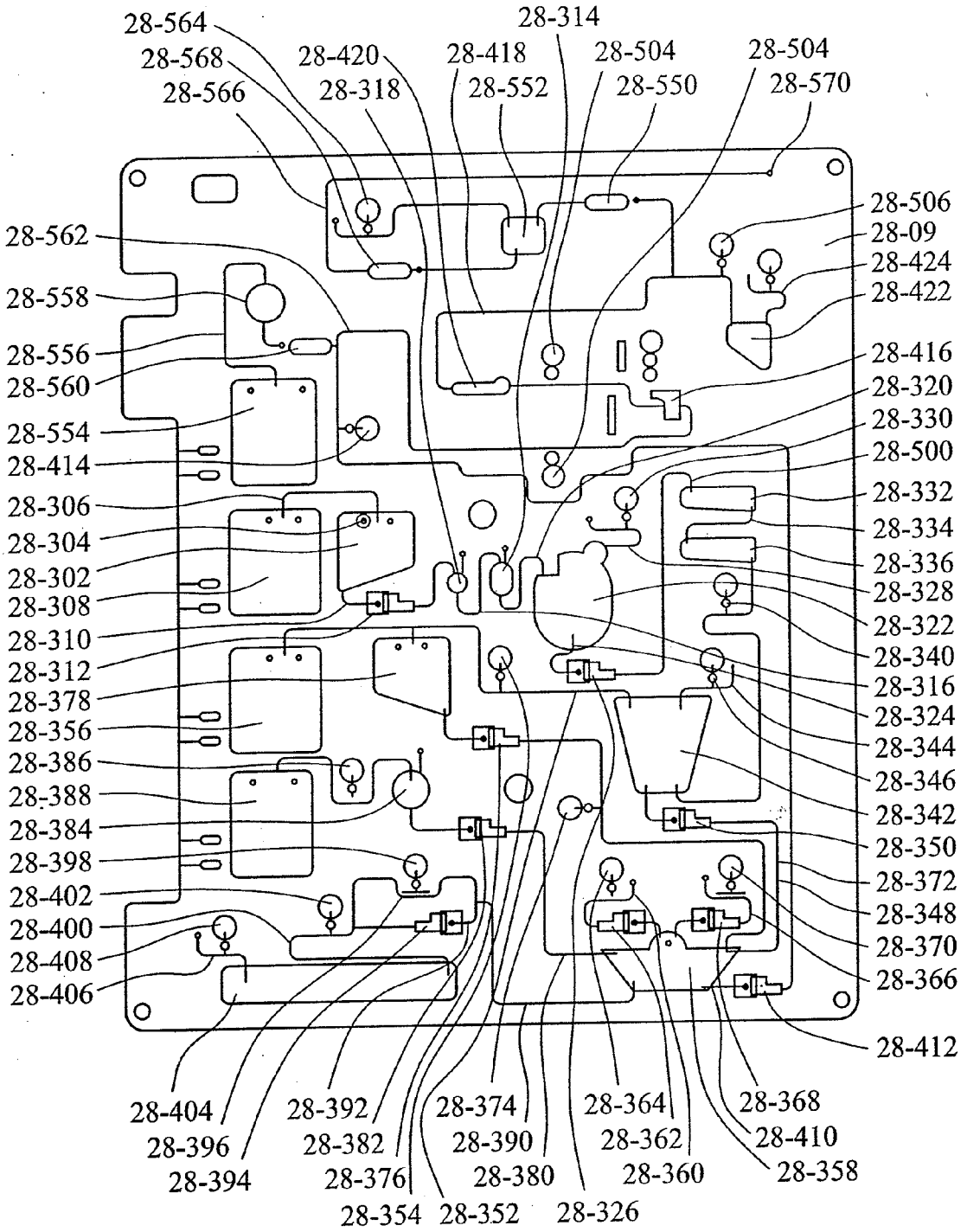

27-13
27-13
27-03
27-01
27-11
27-13
27-05
27-11
27-07
27-11
27-09
27-13
27-11
27-13
27-13

FIG. 27


28-564
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28-562
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28-406
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28-09
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28-330
28-500
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28-336
28-328
28-322
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28-316
28-324
28-344
28-346
28-342
28-350
28-372
28-348
28-370
28-366
28-412
28-404
28-396
28-394
28-392
28-382
28-376
28-354
28-374
28-390
28-380
28-352
28-326
28-364
28-362
28-360
28-368
28-410
28-358

FIG. 28a

Functional Chambers	Volume	Depth	Tool	Vent	Note
C1 (Lysis)	300ul	1mm	Std Cutter	No	
C2 (Purification)	311ul	0.75mm	Std Cutter	Yes	
Expansion/Mixing Chambers	67ul	0.75mm	Std Cutter	No	
C3 (Dwell or Initial Binding))	250ul	0.5mm	Std Cutter	Yes	
C4 (PCR washing and release)	250ul	1.0mm	Std Cutter	Yes	
Bead Storage (BSC)	30ul	1.0mm	Std Cutter	No	
Binding Buffer (BBC)	40ul	2.0mm	Std Cutter	No	
Elution	150ul	2.0mm	Std Cutter	No	
Wash Buffer	250ul	2.0mm	Std Cutter	No	
Archive	200ul	2.0mm	Std Cutter	No	
Waste	1000ul	2.5mm	Std Cutter	Yes	
PCR	10ul	1.0mm	Std Cutter	No	
Formamide	105ul	1.0mm	Std Cutter	No	
Denaturing	105ul	1.0mm	Std Cutter	No	
Channel					
PCR Red		0.25mm	0.5mm BEM		Sigma-Aldrich 411663
PCR Cyan		1.0mm	1.0mm BEM		Sigma-Aldrich 411663
PCR White/Black		0.35mm	0.5mm BEM		Sasolwax H1
Magenta		0.5mm	1.0mm BEM		
EC PUMP Yellow		2.0mm	1.0mm BEM		
Paraffin Valve	Volume	Depth	Tool		
LMW OV's 1.5mm diameter	1.77ul	0.5mm	Std Cutter		
LMW OV's 3.0mm diameter	7.1ul	1.0mm	Std Cutter		
HMWV CV15	7.1ul	1.0mm	Std Cutter		
Cartridge Specification					
Substrate width - 175mm					
Substrate length - 228mm					
Substrate 3.0mm PC					
Capping Layer 0.5mm PC					
PSA 90106 cold bond assy					Adhesives Research
EC pump UV glue 1180-M					
Bar Code pocket	N/A	0.2mm	Std Cutter		May opt for flduclai:marks instead

FIG. 28b

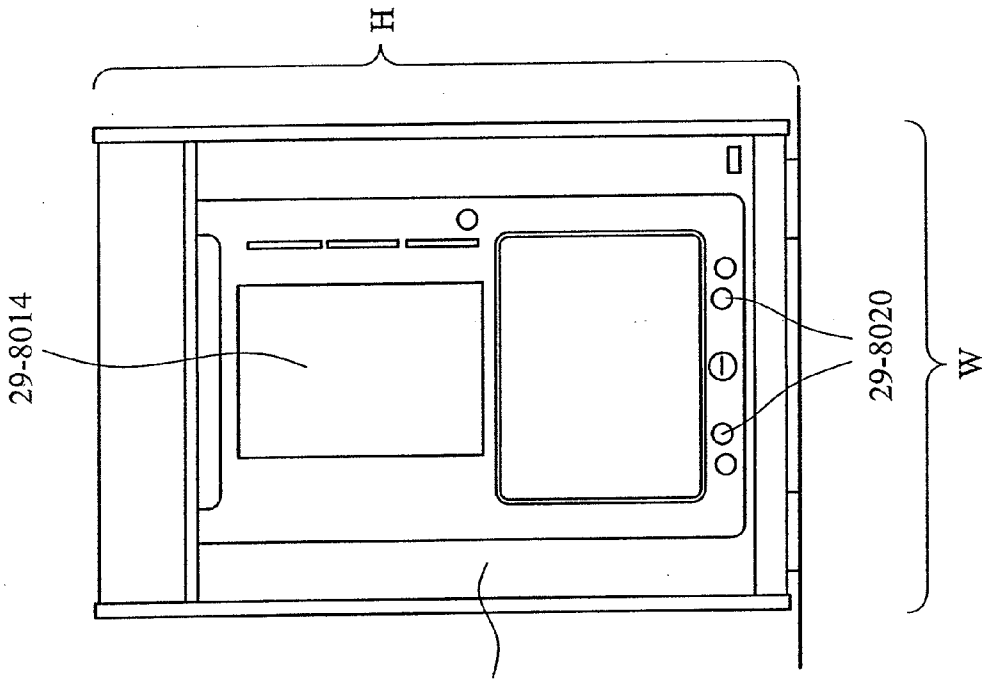


FIG. 29b

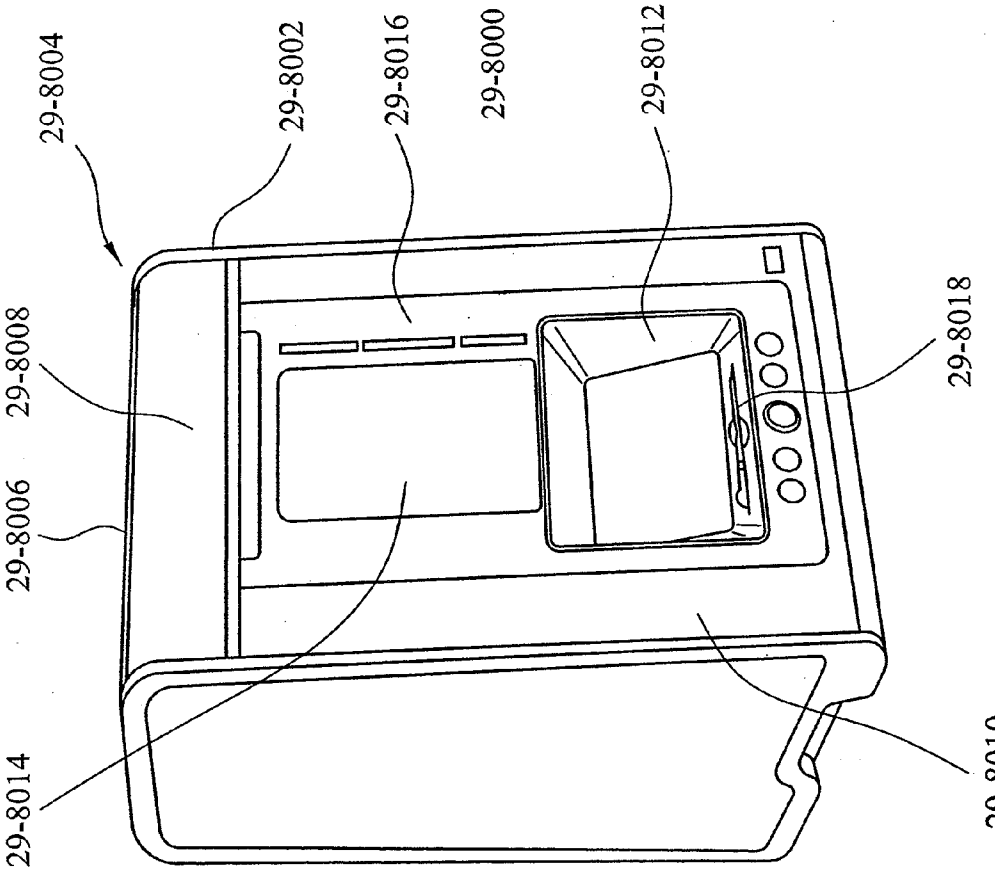


FIG. 29a

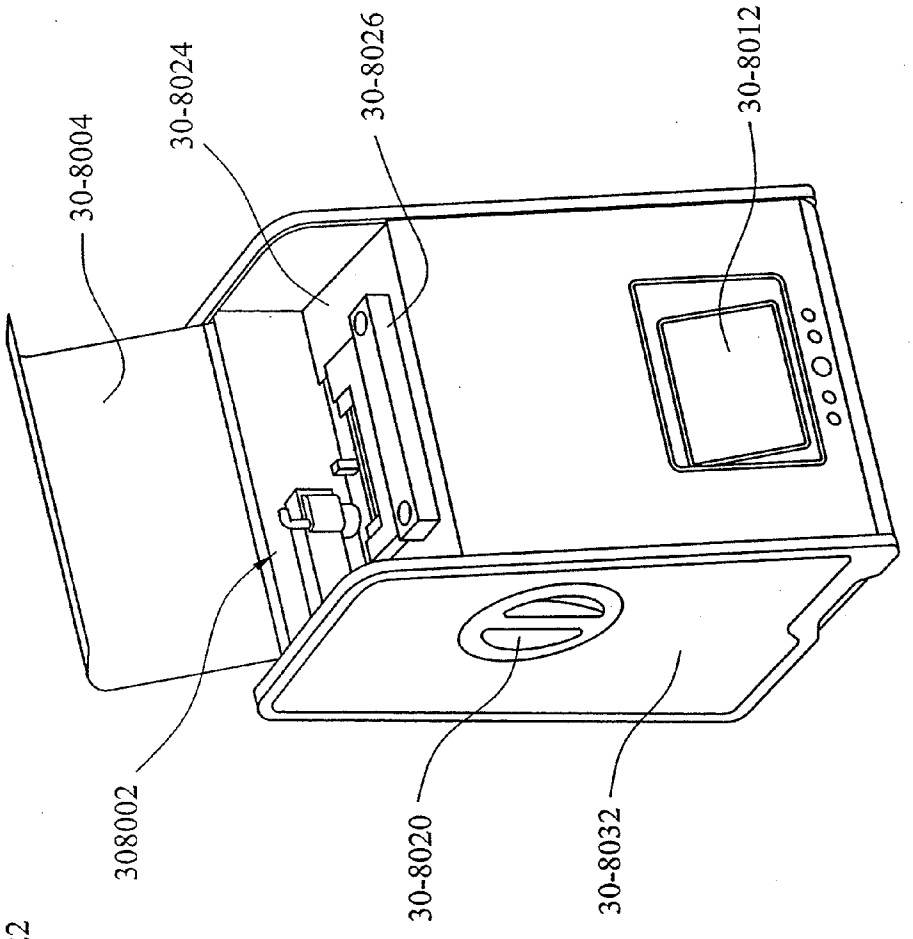


FIG. 30

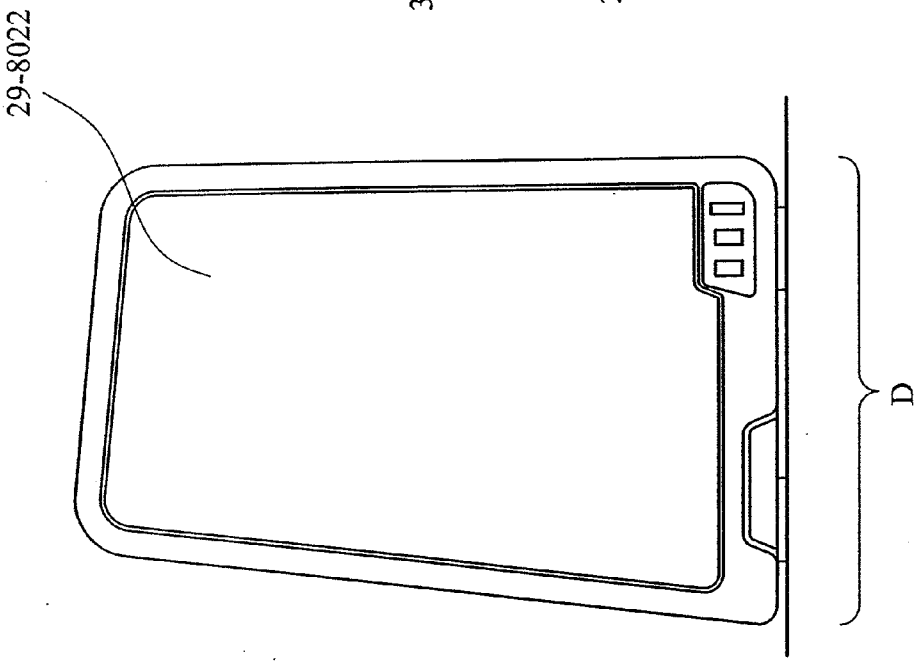


FIG. 29c

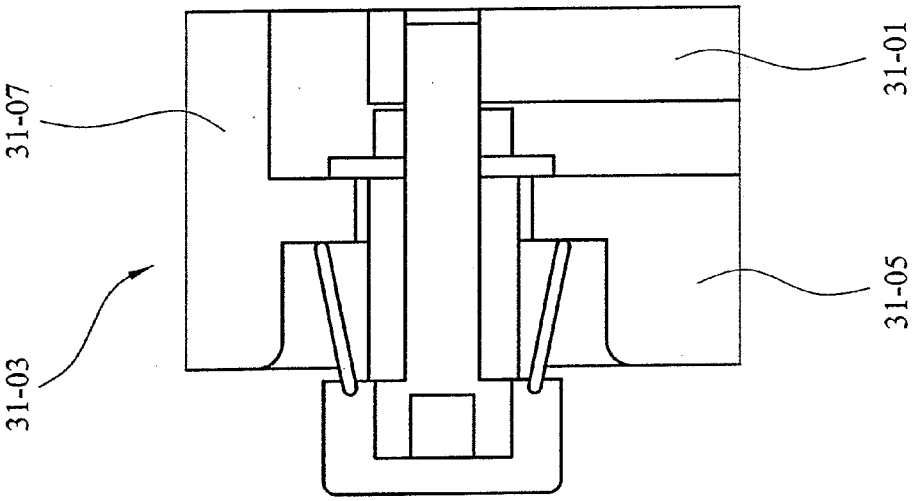


FIG. 31b

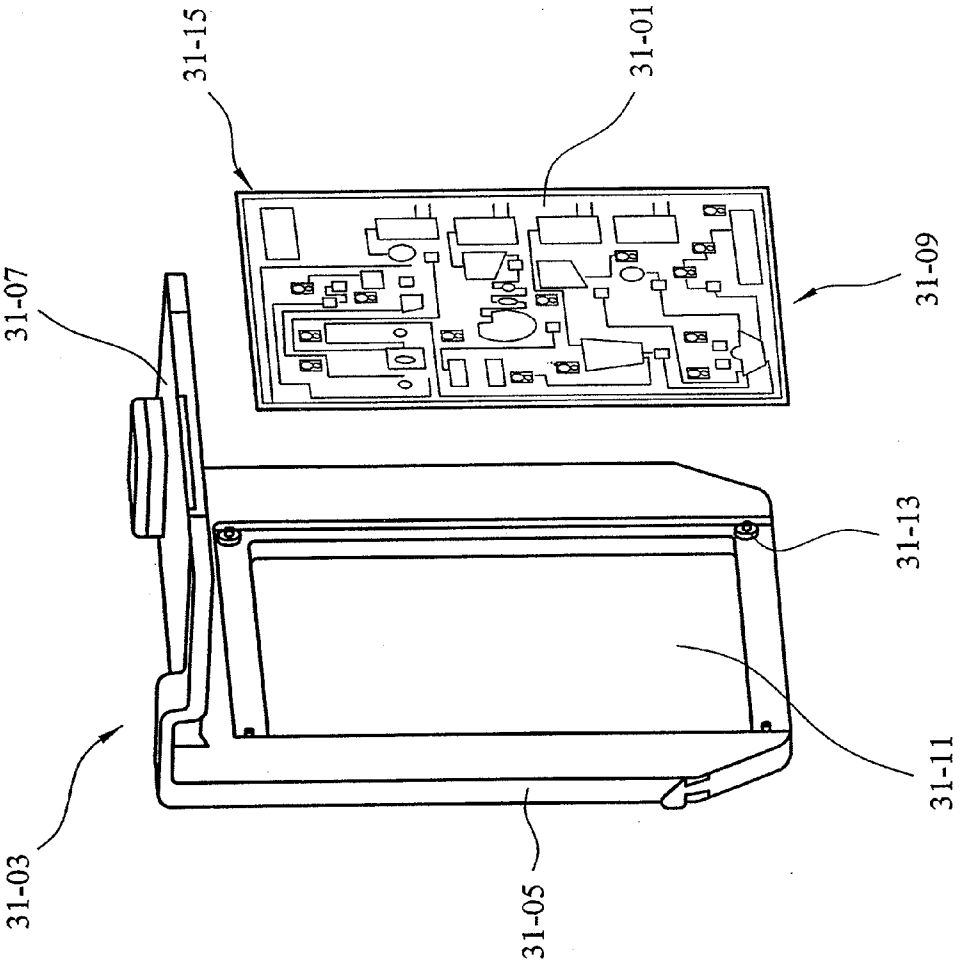


FIG. 31a

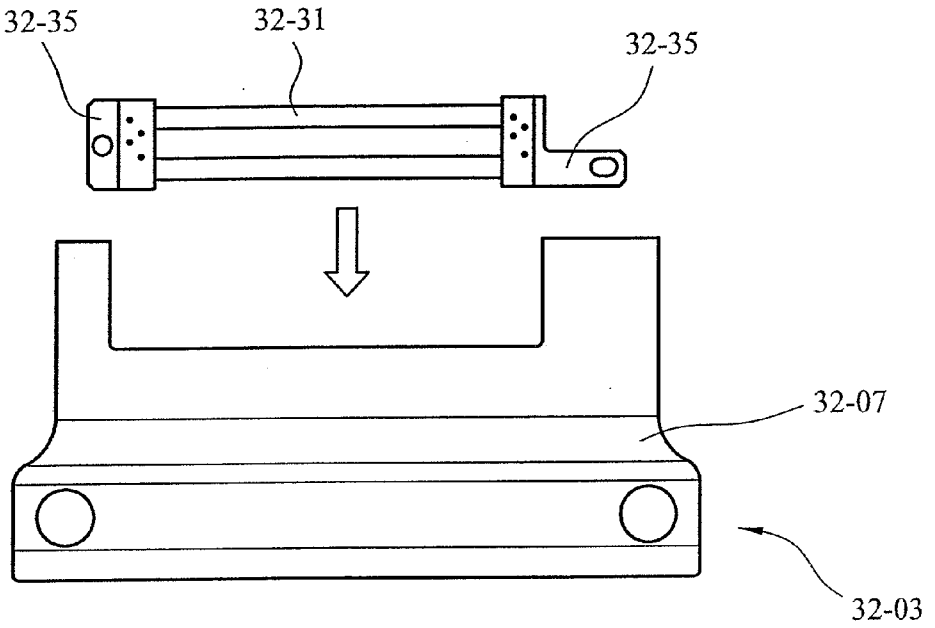


FIG. 32a

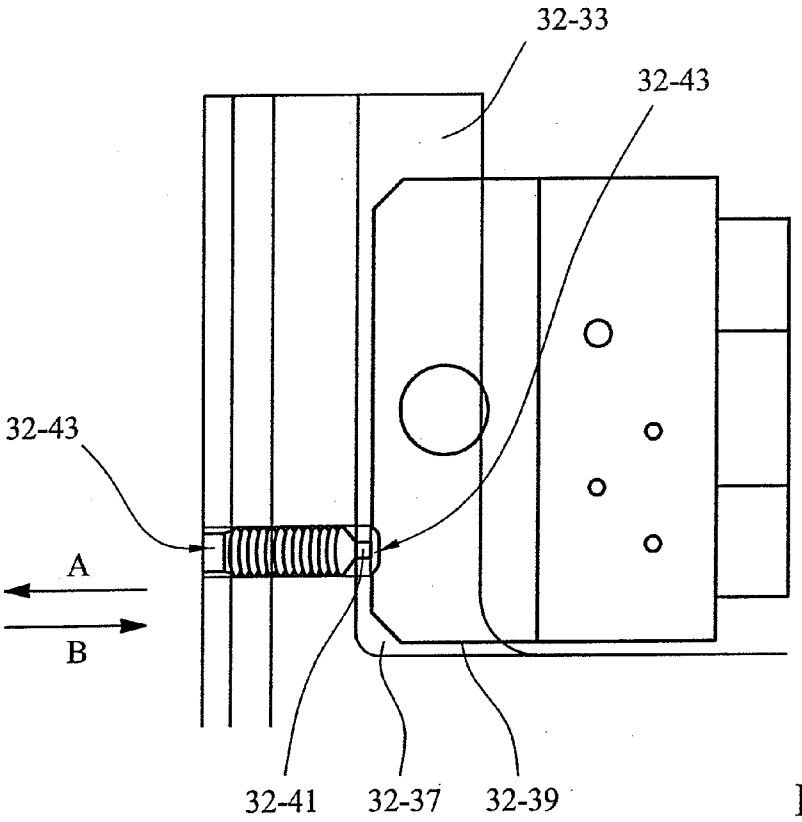


FIG. 32b

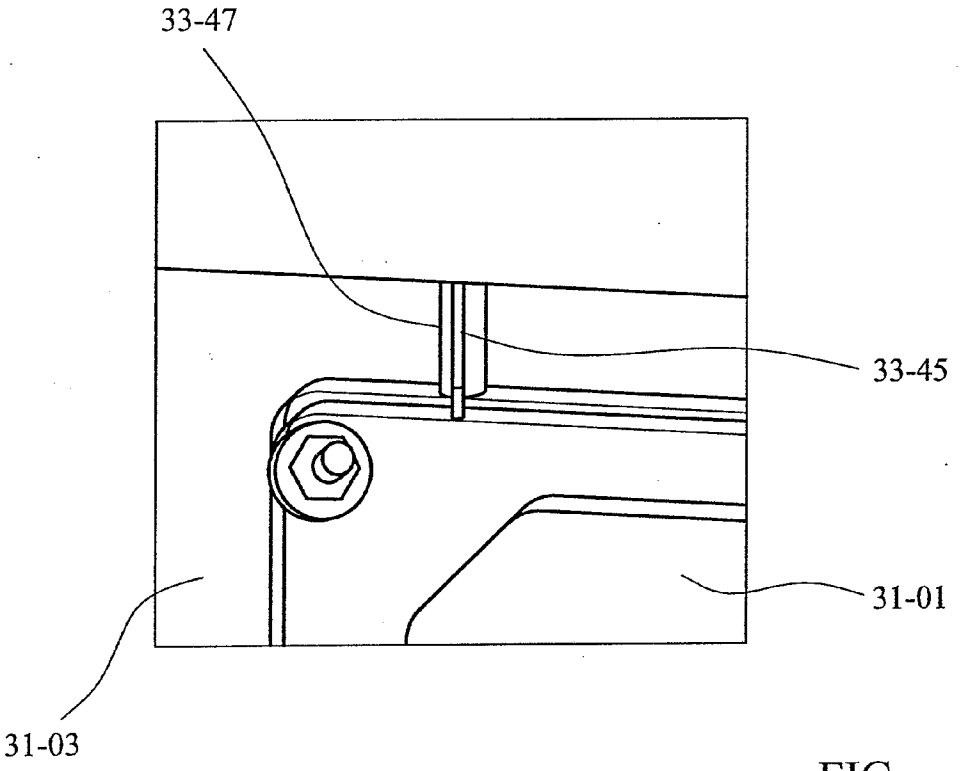


FIG. 33a

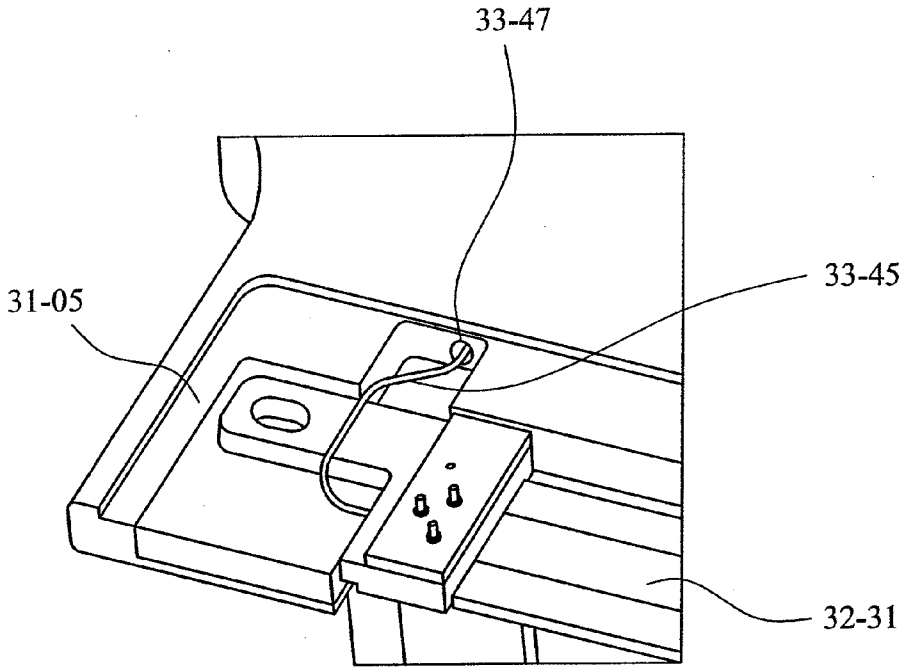


FIG. 33b

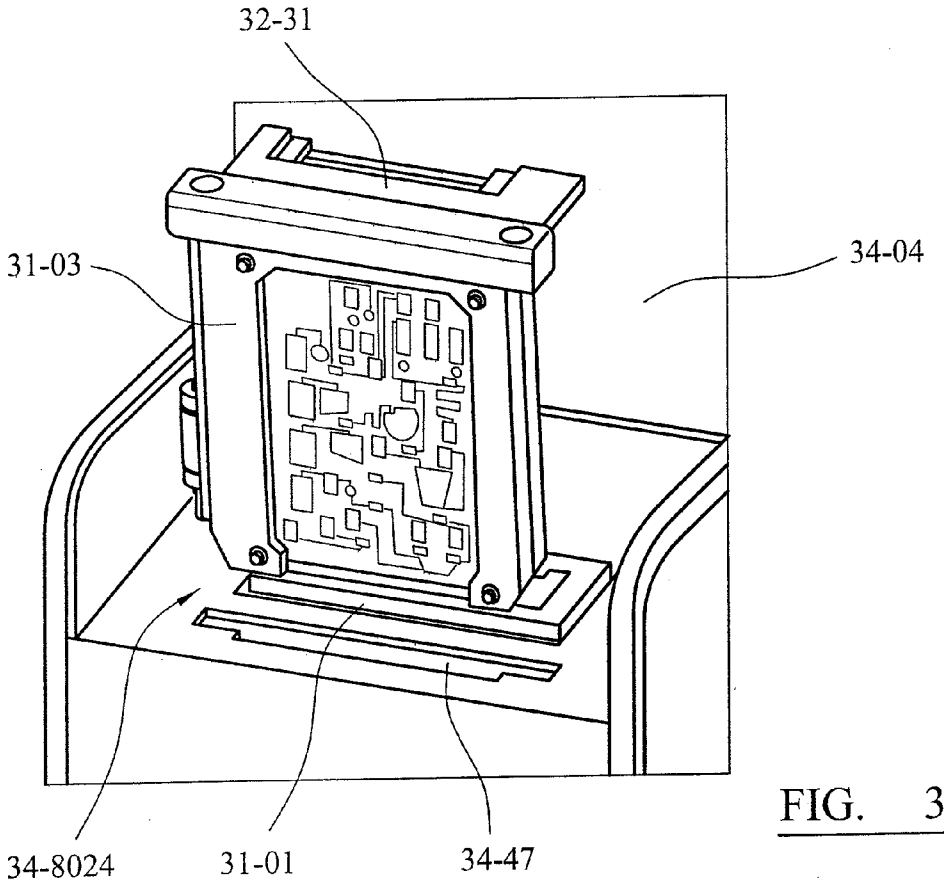


FIG. 34a

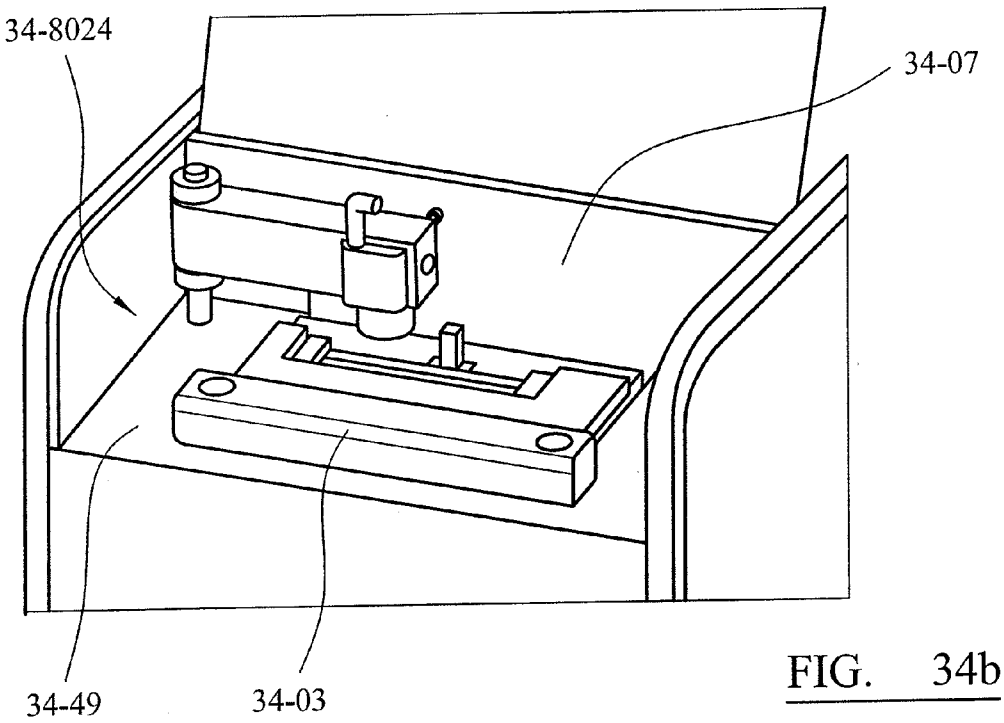


FIG. 34b

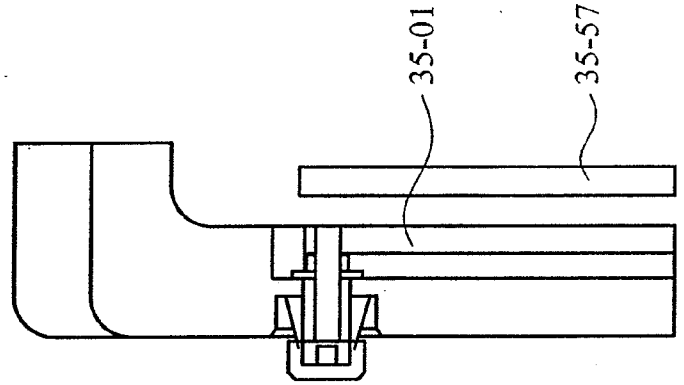


FIG. 35c

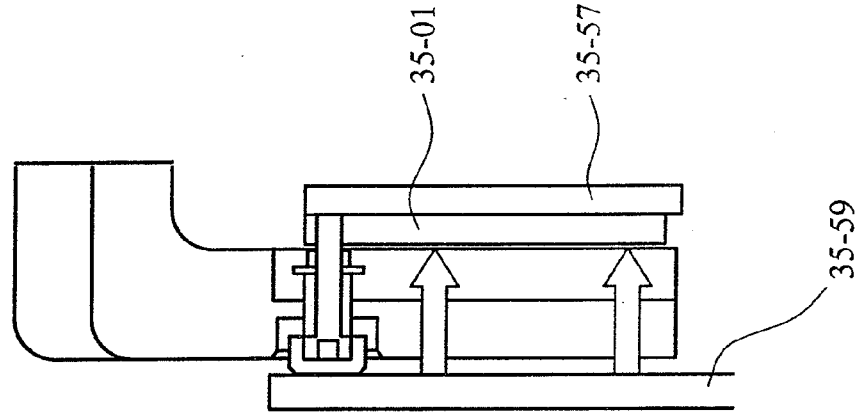


FIG. 35b

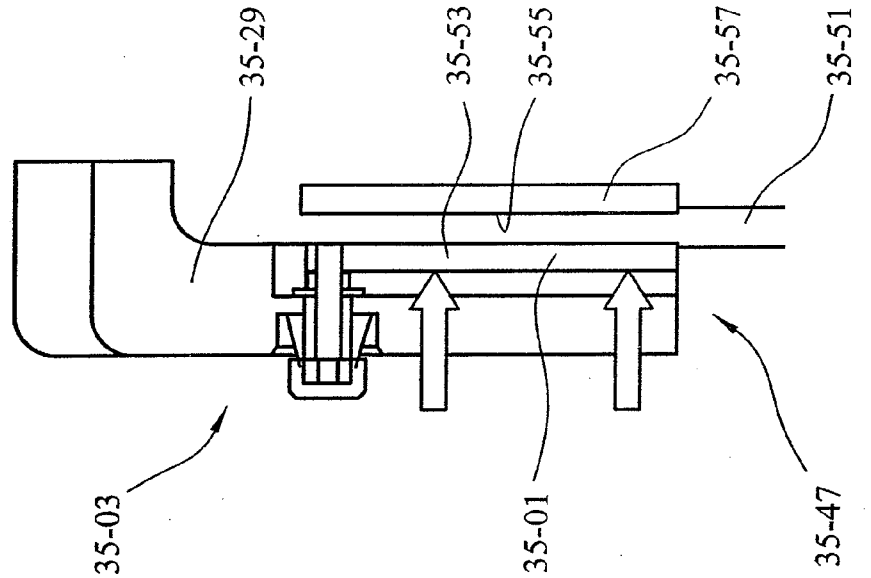


FIG. 35a

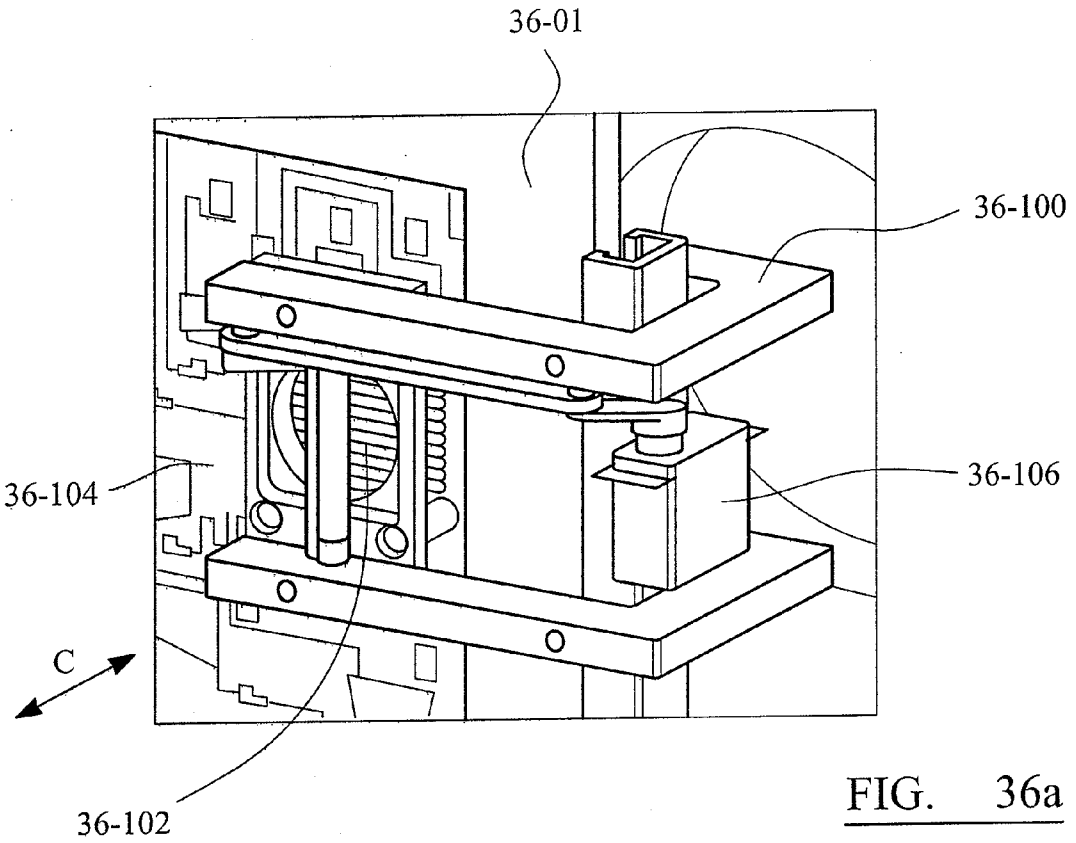


FIG. 36a

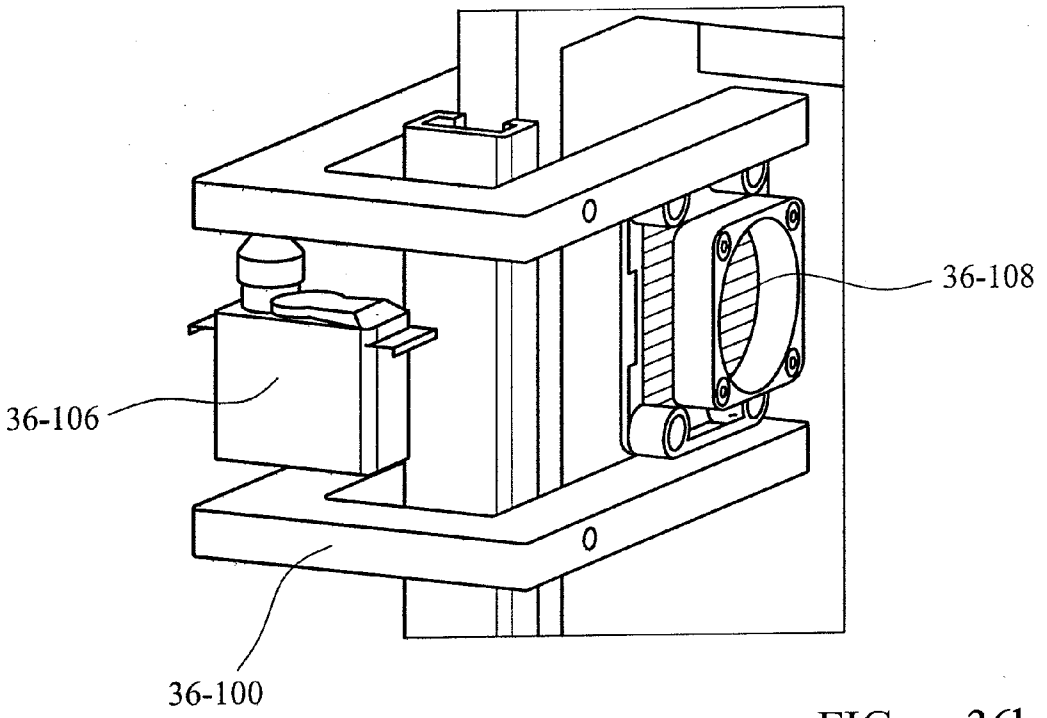


FIG. 36b

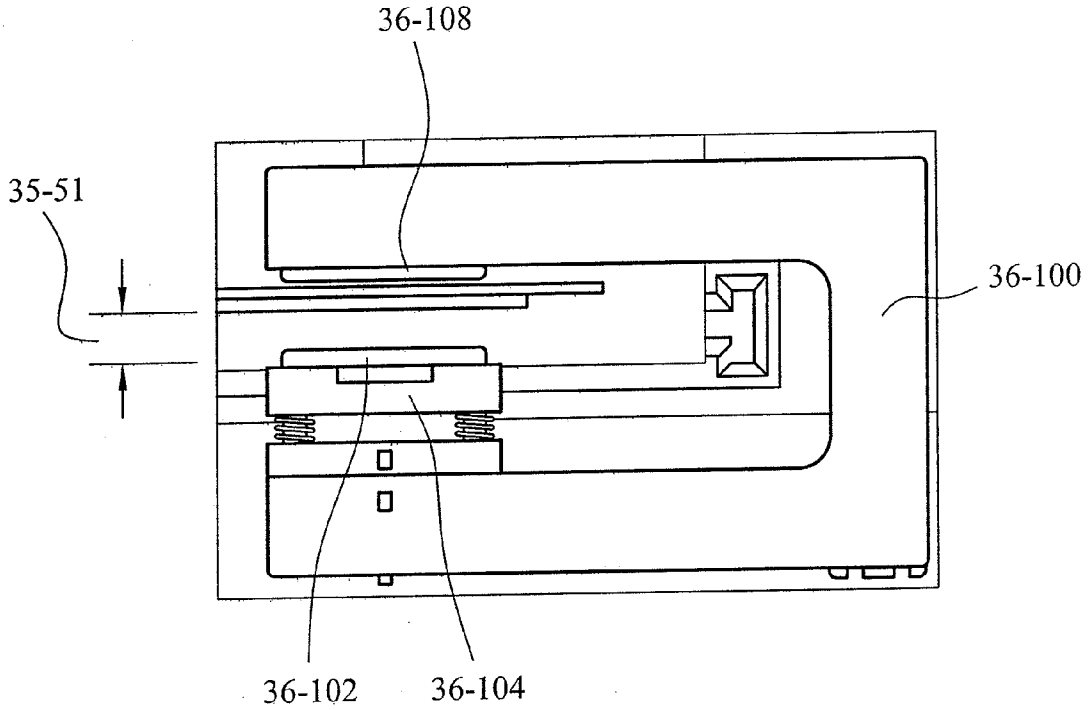


FIG. 36c

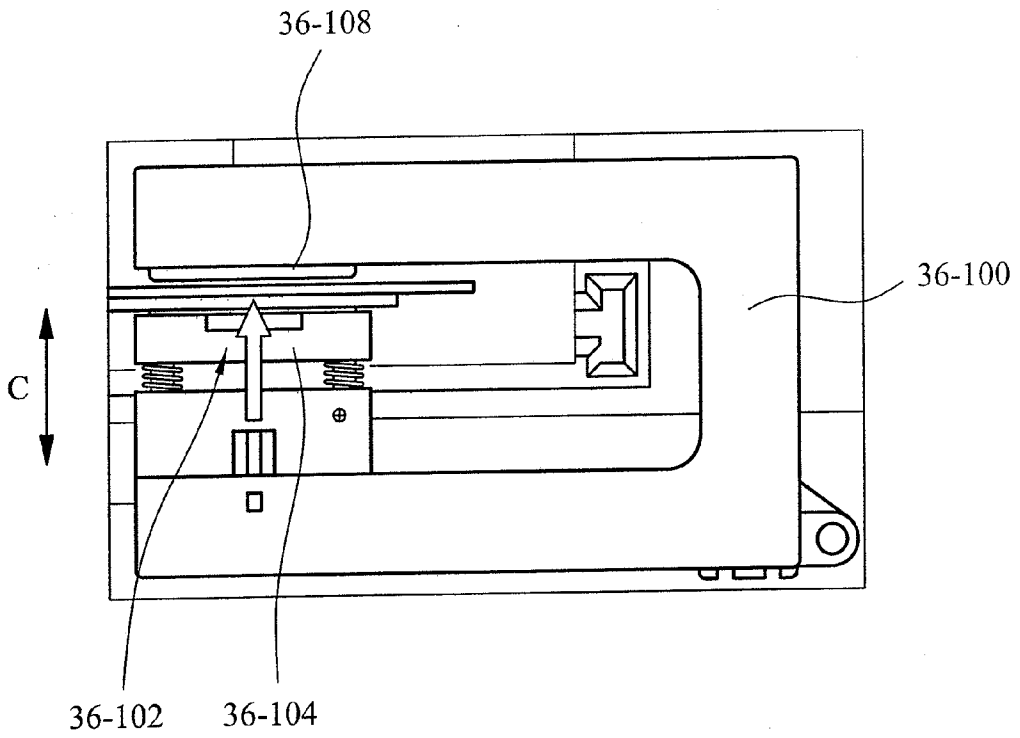


FIG. 36d

FIG. 37a

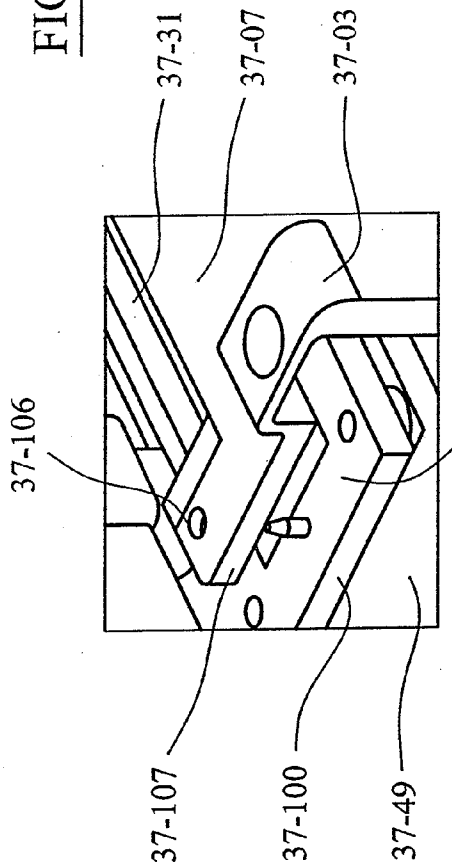
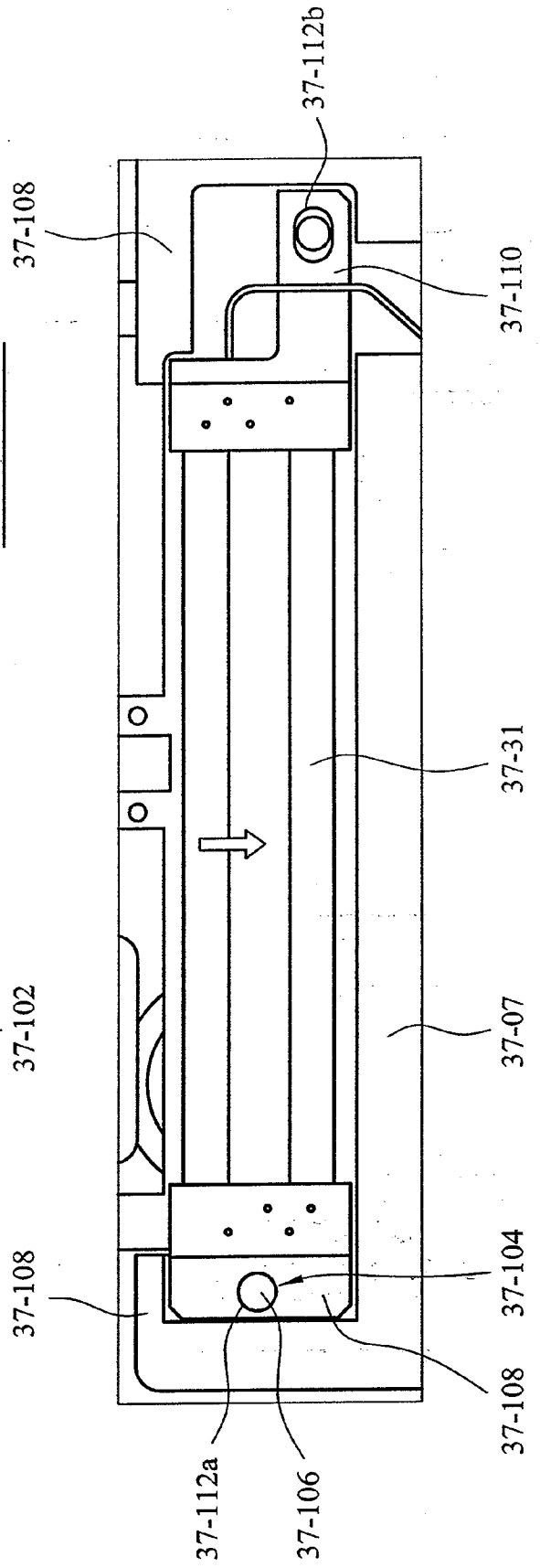


FIG. 37b



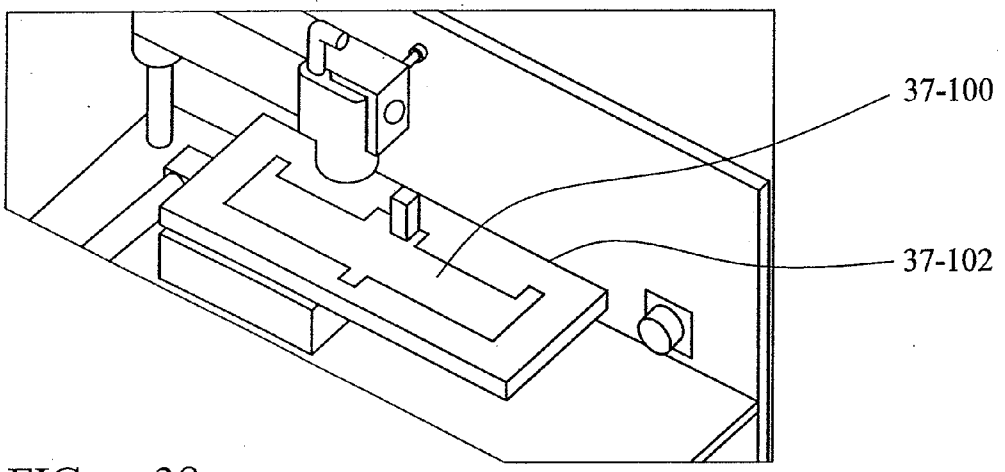


FIG. 38

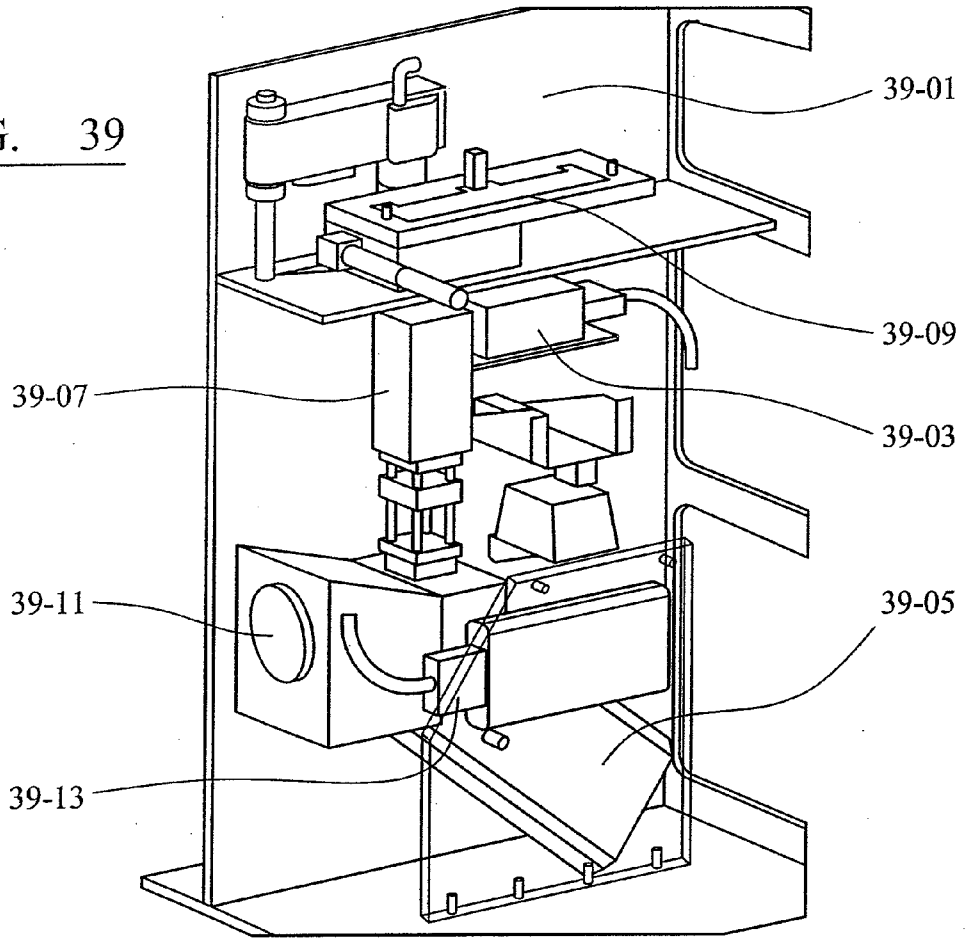


FIG. 39

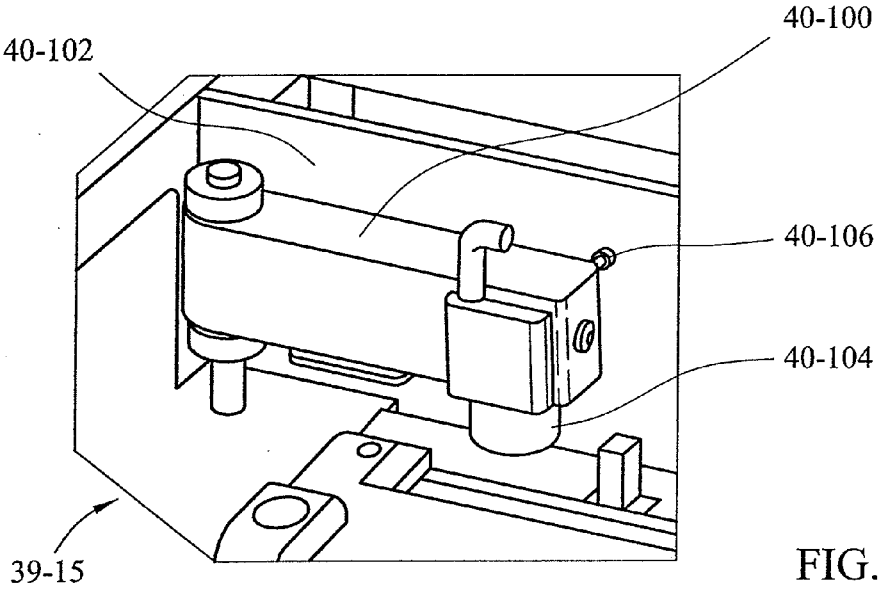


FIG. 40a

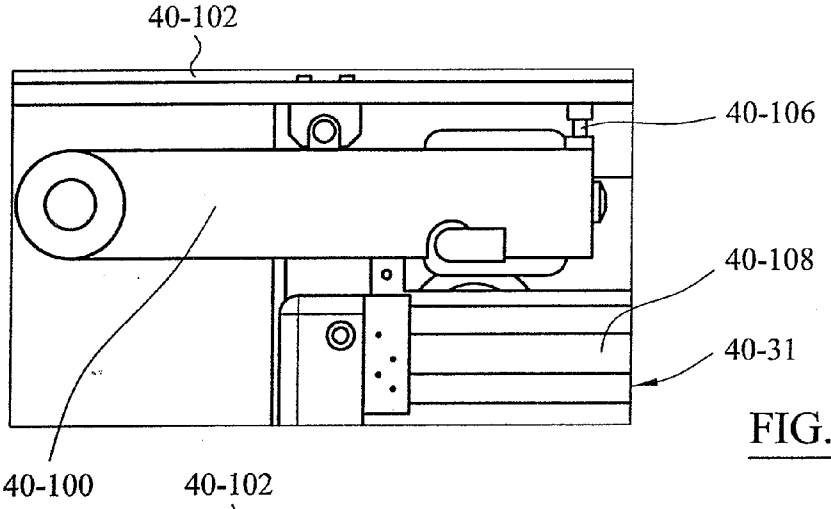


FIG. 40b

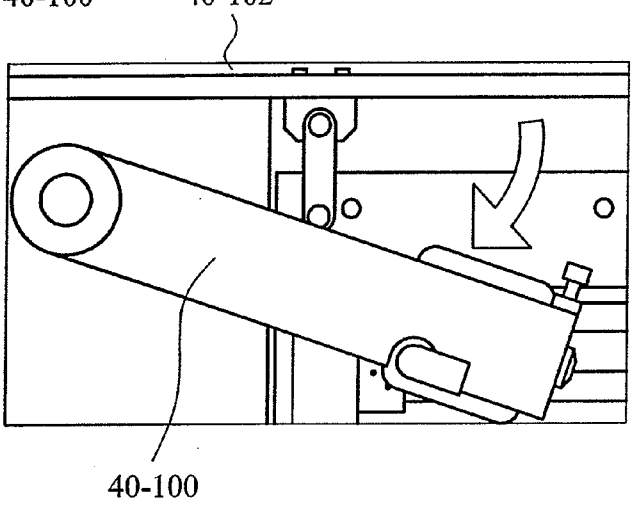


FIG. 40c

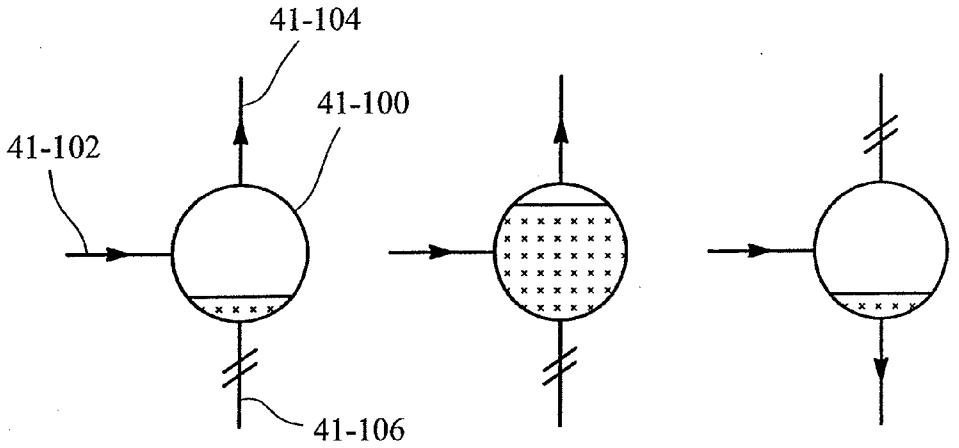


FIG. 41a

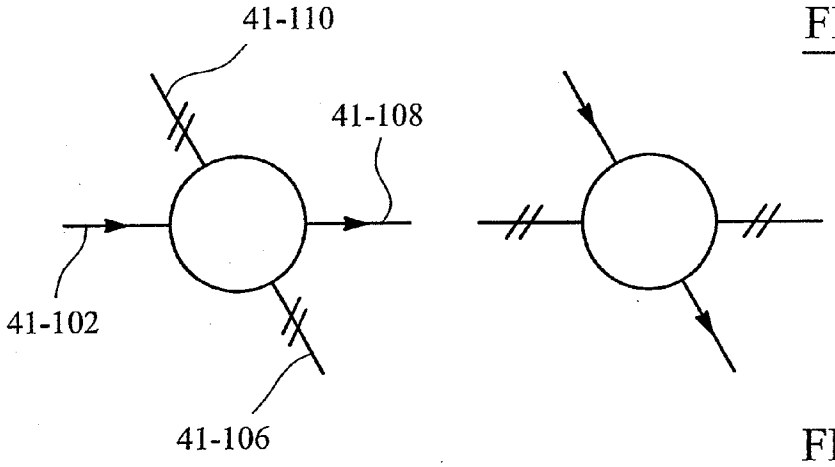


FIG. 41b

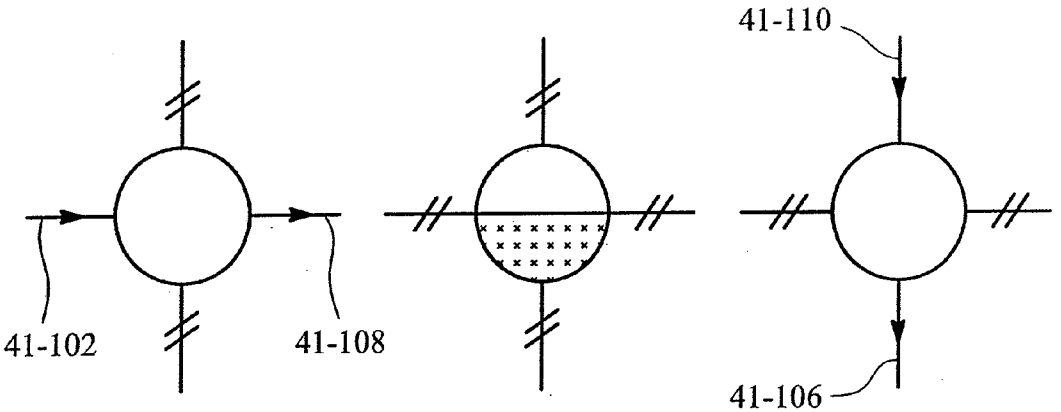


FIG. 41c

FIG. 42a

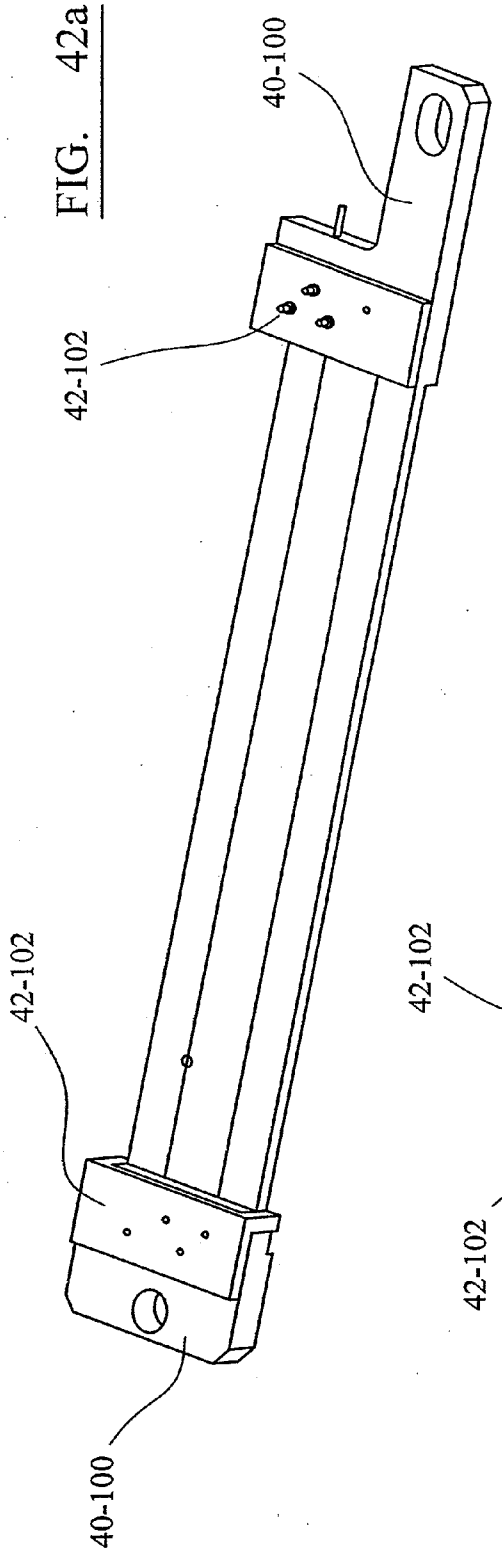
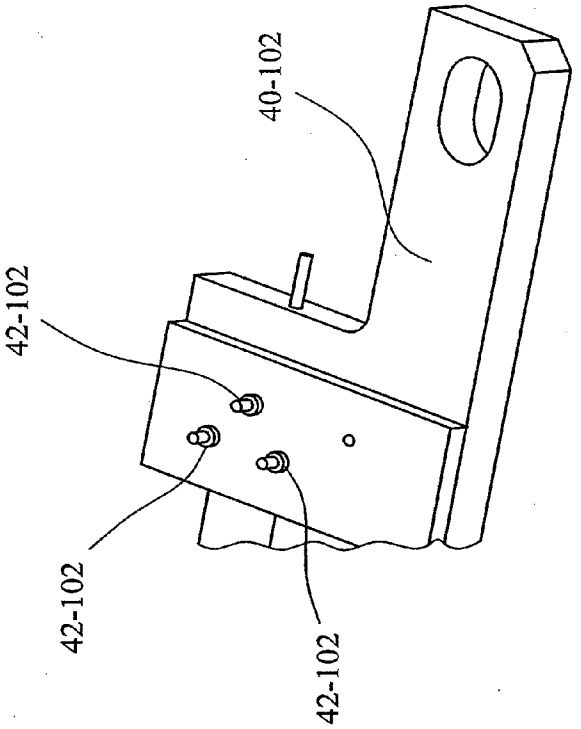


FIG. 42b



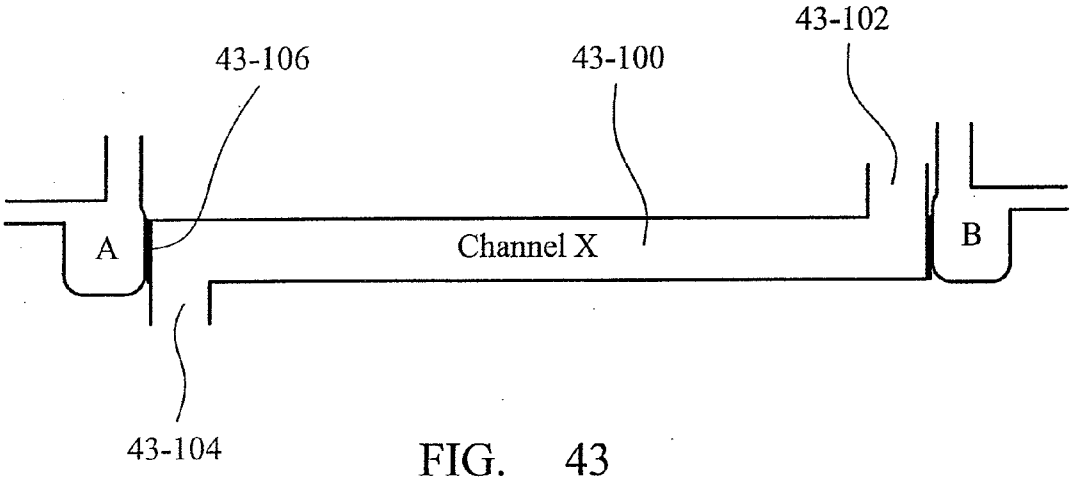


FIG. 43

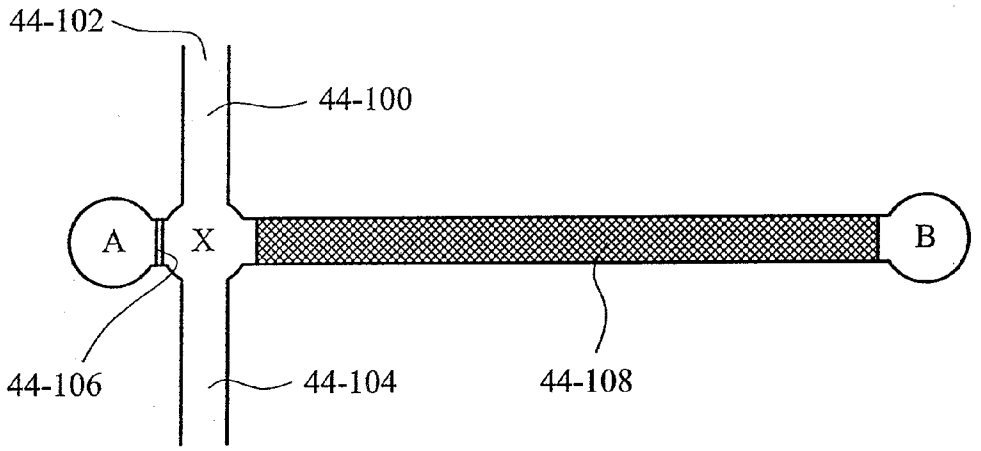


FIG. 44

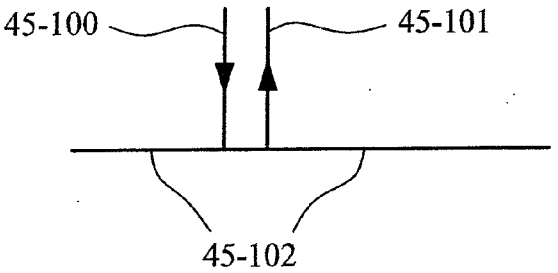


FIG. 45

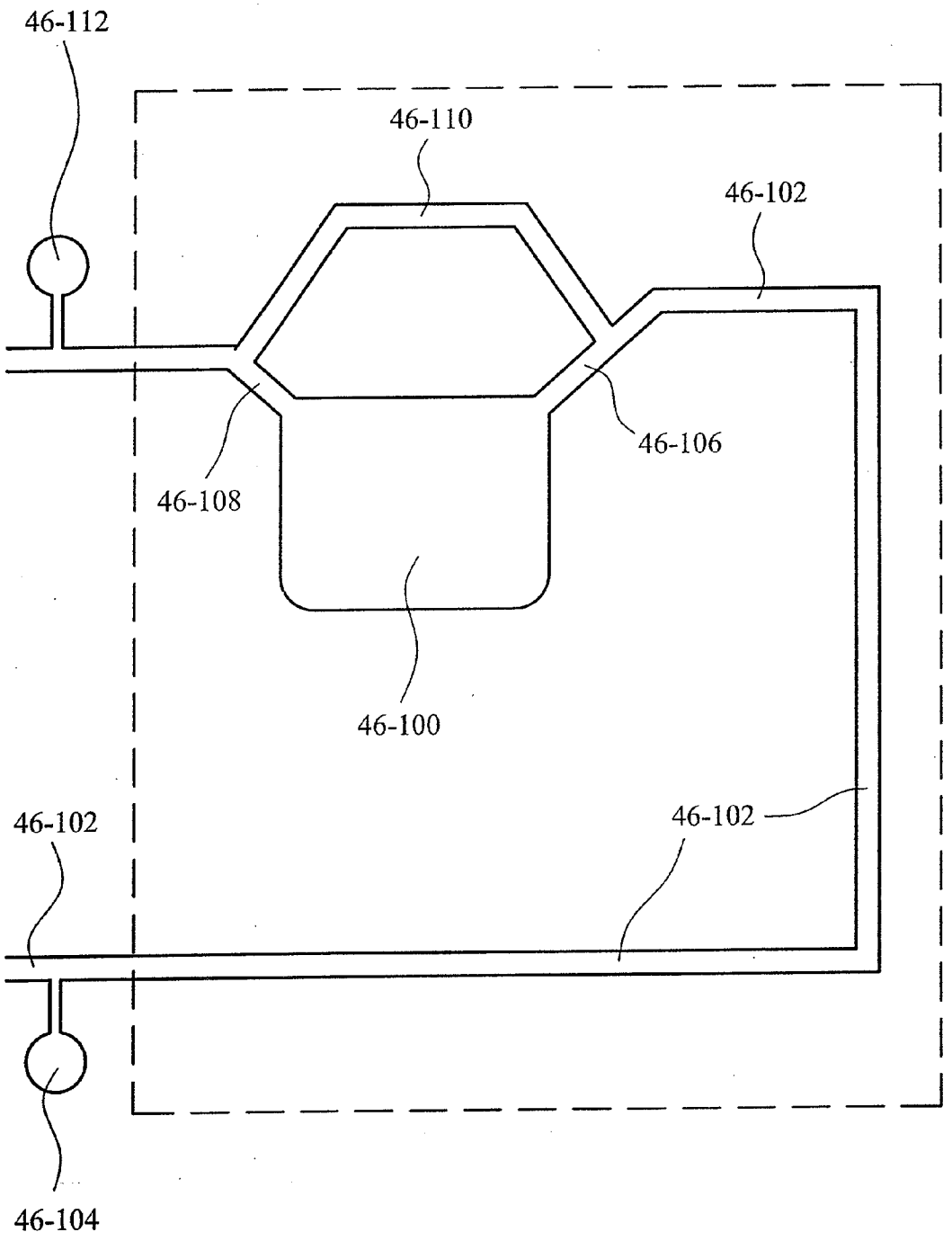


FIG. 46

ANALYSIS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a Utility application which claims benefit of Ser. No. 61/151,104, filed Feb. 9, 2009 in the United States, and of Ser. No. 61/151,107, filed Feb. 9, 2009 in the United States, and of Ser. No. 61/151,111, filed Feb. 9, 2009 in the United States, and of Ser. No. 61/151,117, filed Feb. 9, 2009 in the United States, and which application(s) are incorporated herein by reference. A claim of priority to all, to the extent appropriate is made.

BACKGROUND OF THE INVENTION

[0002] The invention concerns improvements in and relating to analysis, particularly, but not exclusively, in relation to biological samples, such as DNA.

SUMMARY OF THE INVENTION

[0003] According to a first aspect of the invention, there is provided an instrument for analysing a sample, the instrument including:

[0004] one or more sample processors;

[0005] a channel for performing an electrophoretic separation on at least a part of the sample;

[0006] a detection location in the channel;

[0007] an optical system for considering the detection location.

[0008] The optical system may include a light source.

[0009] The light source may be a laser. The laser may be a solid state laser. The laser may be a diode pumped laser.

[0010] The laser may provide a power output of at least 25 mW, preferably at least 35 mW and more preferably at least 45 mW. The power output may be considered at 491 nm. The power output may be considered at 505 nm. The power output may be considered at a given 1 nm wavelength increment at between 480 and 525 nm, preferably 485 and 515 nm.

[0011] The laser may have a power consumption of less than 100 W, preferably less than 75 W and ideally less than 50 W. The power consumption may be between 20 and 45 W.

[0012] The laser may be mounted on a heat sink.

[0013] The light source may be one or more light emitting diodes. The light emitting diode may be a cyan light emitting diode. Two light emitting diodes may be provided in parallel.

[0014] The light may be provided as an open beam between one or more components, for instance between the light source and a collimator and/or light source and a filter and/or light source and a mirror and/or light source and a detection location and/or light source and a detector unit and/or light source and spectrometer and/or light source and camera.

[0015] The light may pass through a fibre coupler. The fibre coupler may have a lens. The lens may be an achromatic lens. The lens may have a focal length of between 3 and 4 mm.

[0016] The light may pass through a patch cable assembly.

[0017] The light may pass through an optical fibre. The optical fibre may have a diameter of between 25 and 75:m, preferably 62.5:m+/−5:m. The optical fibre may convey the light from a location within 50 cm of the laser to a location within 50 cm of the detection location. The distance to one or both locations may be less than 30 cm, preferably less than 15 cm.

[0018] The light may pass through a collimator. The collimator may have a focal length of between 4 and 5 mm.

[0019] The light may pass through a filter. The filter may be a logpass filter. The filter may provide a cut-off wavelength. The filter may have a T50 at between 500 and 530 nm, for instance 515 nm.

[0020] The light may be provided to a mirror. The mirror may be angled at 45° to the beam of light. The mirror may direct the light to the detection location. The mirror may be a glass disc. The mirror may be circular. The mirror may have a maximum dimension, preferably diameter, of between 10 mm and 40 mm, preferably 25 mm+/−5 mm. The mirror may be provided with a mirror at its centre. The mirror may be an ellipse. The ellipse may have a maximum dimension of between 1.5 mm and 2.5 mm, preferably 2 mm+/−5%. The ellipse may have a minimum dimension of between 0.75 and 1.25 mm, preferably 1+/−5%.

[0021] The light may be focussed on the detection location. The light may be focussed by one or more lenses. The lens or lenses may have an adjustable focus.

[0022] The light power output may be at least 10 mW at the detection location, preferably at least 20 mW and more preferably at least 25 mW. The light power output may be less than 100 mW at the detection location, possibly less than 80 mW and possibly less than 60 mW.

[0023] The light may produce fluorescent light at the detection location, particularly in the presence of a fluorescent dye at the detection location.

[0024] Fluorescent light which is incident upon the mirror may be transmitted to the detector unit. The mirror may transmit, preferably anisotropically, the light to the detector unit. The light is preferably not filtered.

[0025] The detector unit may include a slit. The slit may be positioned in front of a spectrometer. The slit may provide diffraction-limited incident light to the spectrometer. The spectrometer may be provided with a diffraction grating. The spectrometer may be provided with a lens. The lens may be a plano-convex lens. The lens may have a focal length of between 40 mm and 60 mm, preferably 50 mm+/−5%. The lens may be provided with an anti-reflective coating. The coating may be anti reflective at between 300 nm and 700 nm, more preferably for the range 350-650 nm.

[0026] The detector unit may include a charge coupled device. The charge couple device may be cooled. The charge coupled device may generate signals. Some or all of the signals may be binned. The signals may form or may be used to produce the results of the analysis.

[0027] The instrument may be sensitive to the presence of greater than 1.5 pM, preferably 2 pM and ideally 2.5 pM of fluorescein dye at pH 7.

[0028] In a preferred form, the optical system may include a laser, directing and focussing optics, return light collecting optics, spectrometer and camera.

[0029] One or more parts of the optical system may be provided within an enclosure. The enclosure may separate those parts of the optical system from the environment and/or from one or more other parts of the instrument.

[0030] The light detector may be thermally isolated from the detection location and/or channel. The light detector may be in a separate enclosure to the detection location and/or channel.

[0031] The channel may be maintained at a predetermined temperature +/−0.7° C., preferably +/−0.5° C., and more preferably +/−0.3° C. The predetermined temperature may be 15° C.

[0032] The channel temperature may be maintained by maintaining the temperature of the electrophoresis cartridge section, such as that section containing the channel and/or by maintaining the temperature within the enclosure holding the electrophoresis cartridge section, such as that section containing the channel. The enclosure may be thermostatically controlled.

[0033] The channel and/or electrophoresis cartridge section, such as that section containing the channel, may be in contact with a thermally conductive block or heat sink. One or more heaters may be provided in contact with the block or heat sink. One or more controllers may be provided for a heater, potentially linked to a temperature sensor.

[0034] The channel may be provided in contact with a heating location. The heating location may be in the form of a thermally conductive block or heat sink, preferably provided in contact with one or more heaters. The heating location may provide a planar surface portion. The heating location may contact a planar surface by the channel, for instance by the electrophoresis cartridge section. The heating location may have a planar surface portion whose boundaries match those of the planar surface of the channel, for instance as provided by the electrophoresis cartridge section.

[0035] The heating location may be bounded by one or more protruding or elevated sections. The protruding or elevated section(s) may surround the heating location and/or the planar surface of the channel.

[0036] The heating location may be recessed compared with one or more sections provided in proximity thereto. The section(s) may surround the heating location and/or the planar surface of the channel.

The channel or electrophoresis cartridge section containing the channel may fit into the heating location. A snug fit may be provided. Particularly in respect of the light emitting diode light source, the following features and options may be provided.

[0037] The light may pass to a bifurcated optical fibre assembly.

[0038] The power supply may be a DC power supply, preferably a stabilised voltage. The voltage may be 3.6V+/−20%, preferably +/−10%. The current may be 1.8 A+/−20%, more preferably +/−10%.

[0039] The light emitting diodes may be maintained at 15° C.+/−2° C., preferably +/−1° C.

[0040] The light emitting diodes may be cooled. The cooling may include a heat sink, for instance an aluminium heat sink. The cooling may include one or more Peltier devices. The cooling may include one or more fans. A temperature sensor may be provided to monitor the temperature.

[0041] The light may pass to a collimator, for instance an acrylic moulded lens. The light may be collimated to a cone angle of between 10° and 20°, preferably 15°+/−1°.

[0042] The light may be passed to a plano-convex lens. The plano-convex lens may have a focal length of between 30 and 40 mm, for instance 35 mm+/−5%

[0043] The light may be passed to a collimator including an aspheric lens, for instance with a focal length of between 8 and 12 mm, for instance 10 mm+/−5%.

[0044] The light may pass to an arm of a multi-strand core. Where two light sources are used, the light may pass to a bifurcated core, for instance a silica core.

According to a second aspect of the invention, there is provided a method for analysing a sample, the method including:

[0045] performing one or more sample processes;

[0046] introducing at least a part of the sample into a channel;

[0047] performing an electrophoretic separation on at least a part of the sample;

[0048] considering the characteristics present at a detection location in the channel using an optical system.

According to a third aspect of the invention, there is provided a method of producing an instrument for analysing a sample, the method including:

[0049] forming one or more sample processors;

[0050] forming a channel for performing an electrophoretic separation on at least a part of the sample;

[0051] positioning an optical system relative to a detection location in the channel.

[0052] The first and/or second and/or third aspects of the invention may include any of the features, options or possibilities set out elsewhere in this application, including in the other aspects of the invention, the specific description of the embodiments and the drawings.

According to a fourth aspect, the invention provides a method of configuring an optical system for a detection location, the method including:

[0053] providing a channel having a detection location for performing an electrophoretic separation on at least a part of a sample;

[0054] considering one or more characteristics of the channel and/or optical system;

[0055] configuring the optical system relative to the detection location.

According to a fifth aspect, the invention provides apparatus for configuring an optical system for a detection location, the apparatus including:

[0056] a channel having a detection location;

[0057] an optical system;

[0058] one or more elements for adjusting the configuration of the optical system.

[0059] The configuration may be with respect to the position of one or more parts of the optical system relative to the channel and/or detection location. The position may be the position along the axis of the channel. The position may be the position perpendicular to the axis of the channel, parallel to the axis along which light is applied to the detection location. The position may be the position perpendicular to the axis of the channel, perpendicular to the axis along which light is applied to the detection location.

[0060] The configuration may be with respect to the focus of the light applied to the detection location.

[0061] The configuring and/or adjustment may be made automatically by the instrument.

[0062] The configuring and/or adjustment may be made periodically. The configuring and/or adjustment may be made before each device and/or cartridge is processed.

[0063] The configuring may be with reference to a marker. The marker may be provided at a known position relative to the channel and/or on the electrophoresis cartridge. The known position may be along the axis of the channel. The

known position may be perpendicular to the axis of the channel. The known position may be known relative to the detection location.

[0064] The marker may be responsive to light, particularly light from the light source. The marker may be a physical mark. The physical mark may enhance and/or alter the response to the incident light at that location. The marker may be a dye which fluoresces in response to light from the light source. Preferably the dye fluoresces at a wavelength within the range of wavelengths encountered during an analysis. The output from the marker is preferably detected by the detection unit.

[0065] The configuring and/or adjustment may include a stage in which one or more components of the optical system and/or the channel are moved until the marker is detected. The stage may end with the optical system centred on the marker.

[0066] The configuring and/or adjustment may include a stage in which one or more components of the optical system and/or channel are moved a given distance from the marker. This movement may be in one or more directions and may be so as to align with the detection location. This may be a second stage provided after the first stage.

[0067] The first and/or second stages may be used to provide X axis and/or Y axis alignment of the incident light with the detection position or to confirm X axis and/or Y axis alignment of the incident light with the detection position.

[0068] The configuration may be with reference to the channel. The channel may be responsive to light, particularly light from the light source. The incident light may be moved across the channel. The incident light may be moved from the cartridge surface on one side of the channel, at least part way across the channel, particularly more than half way across the channel. The incident light may be moved from the cartridge surface on one side of the channel to the cartridge surface on the other side of the channel. The configuration with reference to the channel may use variation in the detected light with position across the channel. The variation may be detected by the detection unit. One or more components of the optical system may be moved and/or the channel may be moved. The channel and/or the content of the channel may be modified to enhance the variation. The optical system and/or light may be modified to enhance the variation. For instance, the light may be polarised, potentially by a polariser inserted into the path of the incident light. The polariser is preferably removed before a sample is separated in the channel.

[0069] The configuring and/or adjustment may include a stage in which one or more components of the optical system and/or the channel are moved along a direction parallel, for instance +/−5°, to the axis of the channel, for instance until a marker is detected. The stage may end with the optical system centred on the marker.

[0070] The configuring and/or adjustment may include a stage in which one or more components of the optical system and/or channel are moved along a direction perpendicular, for instance +/−5°, to the channel. The movement may continue until the variation has reached and/or passed a maximum and/or minimum. One or more components of the optical system and/or channel may be moved to return the incident light to the minimum or maximum value. This may be a second stage provided after the first stage.

[0071] The configuration may be with respect to the distance between a focusing lens and the channel and/or in respect of the working distance. The configuration may be with respect to the Z axis.

[0072] The configuration may be made with respect to a source or location. The source and/or location may respond to incident light to give emitted light. The light may be detected by the detector unit. The source and/or location may provide a known response, either in itself or in response to incident light.

[0073] The source or location may be in the same plane, perpendicular to the incident light, as a known part of the channel. The source or location may be in a plane, perpendicular to the incident light, having a known separation from the plane in which a known part of the channel is provided. The known part of the channel may be the centre of the channel.

[0074] The focus of the incident light may be adjusted, preferably automatically. The response of the detector unit to the variation in focus may be considered. The focus may be adjusted to maximise the response. This configuration may be used in the consideration of a sample. The configuration may be subject to a further known adjustment where the plane of the source and/or location and the plane of the know part of the channel differ by a known distance.

[0075] The configuration along the axis of the channel may be provided followed by the configuration perpendicular to the axis of the channel, parallel to the axis along which light is applied to the detection location and/or followed by the configuration perpendicular to the axis of the channel, perpendicular to the axis along which light is applied to the detection location. Preferably, the configuration along the axis of the channel is provided followed by the configuration perpendicular to the axis of the channel, parallel to the axis along which light is applied to the detection location, followed by the configuration perpendicular to the axis of the channel, perpendicular to the axis along which light is applied to the detection location.

[0076] The configuration and/or adjustment may be provided using the optical system used in detecting the results from the channel and/or may be provided using a separate alignment system. The separate alignment system may be separate from the optical system in using one or more components which are not used by the optical system. The one or more components may be: a variable position support and/or an actuator and/or a camera and/or a light detector and/or a light source. One or more components of the separate alignment system may also be a component of the optical system, for instance a light source, such as a laser.

[0077] An alignment system may be provided, particularly a separate alignment system. The alignment system may include one or more of: a variable position support and/or an actuator and/or a camera and/or a light detector and/or a light source.

[0078] The alignment system may include a variable position support. The variable position support may be mounted on the instrument casing and/or on one or more intermediate supports. The variable position support may be an arm. The variable position support may be pivotally mounted. The variable position support may be moved by an actuator. The actuator may be provided on the variable position support, for instance within that support.

[0079] The alignment system and/or variable position support may have a first position, for instance a stowed position. The alignment system and/or variable position support may have a second position, for instance a use position.

[0080] In the first position, the camera may be further from the channel than in a second position. In the first position, the

variable position support both ends of the support may be in proximity with the casing of the instrument.

[0081] In the second position, the camera may be closer to the channel than in the first position. In the second position, the variable position support may have one end in proximity with the casing of the instrument and the other end distal to the casing of the instrument.

[0082] In the second position, the camera/light detector, and particularly the optical axis thereof, may be positioned in an alignment with the channel. The alignment may be a less accurate alignment than a second alignment. The second alignment may be obtained by varying the position of the variable position support and/or camera./light detector. The varying of the position may be through rotation. The varying of the position may be controlled by an operator of the instrument and/or by the instrument. The varying of the position may be in response to the form of diffraction pattern observed by the camera/light detector. The position may be varied to give a different and/or particular form of diffraction pattern. The position may be varied to provide alignment with the centre of the channel. The second alignment may give a different and/or particular form of diffraction pattern. The second alignment may provide alignment with the centre of the channel.

[0083] The alignment system may be used to control one or more components of the optical system so as to provide alignment between the channel and the optical system.

[0084] The alignment system may be used to control the position of the channel and/or electrophoresis cartridge section containing the channel, preferably to provide alignment between the channel and the optical system.

[0085] The alignment system may be used to provide alignment of the optical system with the centre of the channel. The alignment system may be used to provide alignment of the system with a position along the length of the channel. The alignment system may be used to focus the optical system with respect to the channel.

[0086] The fourth and/or fifth aspects of the invention may include any of the features, options or possibilities set out elsewhere in this application, including in the other aspects of the invention, the specific description of the embodiments and the drawings.

[0087] Any of the aspects of the invention may include any of the following options, features or possibilities.

[0088] The sample may be received from one or more of: a swab, a buccal swab, a cotton swab, a soft swab, a solution, a suspension, an item of clothing, an item placed in the mouth, a cigarette or piece thereof, chewing gum or saliva.

[0089] The sample may be a skin sample, blood sample, cell sample, bodily fluid sample, hair sample, saliva sample or sample containing one or more of these.

[0090] The sample may be a forensic sample. The sample may be a medical sample.

[0091] The analysis may be for diagnostic purposes. The analysis may be for forensic purposes.

[0092] The analysis may be for use in the consideration of marker targets, diagnostic assays, disease markers, biobanking applications, STR based targets in transplants, identification of drug resistant microorganisms, blood testing, mutation detection, DNA sequencing, food analysis, pharmogenetics and pharmogenomics, medical fields, biotech fields, in determining familial relationships, paternity testing and pedigree testing in animals.

[0093] The analysis may be for use in border control, security or customs situations and/or uses.

[0094] The device may be a microfluidic device. The instrument may incorporate a microfluidic device. The device may be a device processing a sample of less than 50:1, preferably less than 30:1, more preferably less than 20:1, potentially less than 10:1 in one or more steps. The device may be a device processing a fluid, particularly a liquid, of less than 50:1, preferably less than 30:1, more preferably less than 20:1, potentially less than 10:1 in one or more steps.

[0095] The device may process and/or contain a fluid, particularly a liquid, of less than 50:1, preferably less than 30:1, more preferably less than 20:1, potentially less than 10:1 in one or more of the following steps: a sample receiving step and/or sample preparation step and/or sample extraction step and/or sample retention step and/or purification step and/or washing step and/or elution step and/or amplification step and/or PCR step and/or denaturing step and/or investigation step and/or electrophoresis step and/or analysis step and/or results output step.

[0096] The device may incorporate one or more channels or chambers with a maximum dimension of less than 1000:m, possible less than 750:m and preferably less than 550:m.

[0097] The device may incorporate one or more channels or chambers with a maximum dimension of less than 500:m, possible less than 250:m and preferably less than 100:m.

[0098] The device may include a chambers provided with one or more reagents. One or more chambers may be so provided. The reagents may be different. The reagents may be in liquid form. The reagents may be provided on and/or in the surface of a solid. The solid may be one or more beads. The solid may be magnetic.

[0099] One or more reagents may be provided for cell lysis. One of more reagents may be provided for a selective extraction of DNA containing material from other material. One or more reagents may be provided for washing. One or more reagents may be provided for elution, particularly from the surface of a solid. One or more reagents may be provided for amplification, particularly PCR based amplification. One or more reagents may be provided for denaturing. One or more reagents may be provided for electrophoresis.

[0100] Preferably the device has a stored form and a use form. In the use form, the sample to be processed may be loaded into the device. Preferably one or more reagents are pre-loaded into the device and/or are present in the device when in the stored form. One or more reagents may be loaded into the device in the use form.

[0101] The device and/or method may include one or more pumps. Preferably the device only includes pumps of a single type. Preferably the pumps of the single type are identical with respect to chamber shape and/or electrode positions and/or electrode materials and/or orientation and/or chamber volume and/or pump electrolyte and/or pump electrolyte concentration.

[0102] One or more, preferably all, of the pumps may be electrochemical pumps.

[0103] The device may have an orientation of use, preferably one electrode in the pump chamber is provided above the other. The pump chamber may have a height greater than its width. The pump chamber may have a width greater than its depth.

[0104] The pump chamber may have an outlet. Preferably the outlet is provided in the upper section of the pump chamber. The upper section may be the upper 20%, preferably

10%, and more preferably 5% of the height of the chamber. The outlet may be in the top wall of the chamber.

[0105] The pump chamber may contain NaCl. The molarity of the electrolyte in the pump chamber may be between 0.2M and 3M, preferably 1M+/−15%.

[0106] The electrophoresis step and/or electrophoresis cartridge section may be provided with a channel, for instance a capillary for electrophoresis.

[0107] The channel may be provided with a matrix. Preferably the matrix resists the passage of elements, the resistance being related to the size of the element. Preferably different size elements migrate through the matrix at different rates, the larger migrating slower.

[0108] The channel may be provided with an inert bed of particulate material to form the matrix.

[0109] The channel may be provided with a gel, particularly a polymer gel. The channel may be provided with polyhydroacrylamide, polydimethylacrylamide or mixtures there of. The channel may be provided with a cross-linked polymer. The cross-linking of the polymer may be provided in situ.

[0110] One or more surfaces of the channel may be treated, for instance with a hydrophilic coating, for instance poly (hydroxyethlacrylamide).

[0111] The channel may be provided with a matrix during electrophoresis. The channel may be provided without a matrix prior to electrophoresis, with the matrix being introduced before electrophoresis commences. The matrix or a material for forming the matrix may be stored at a location removed from the channel in which electrophoresis is provided. The matrix or material for forming the matrix may be stored in a chamber. The chamber may be connected by a channel to the channel in which electrophoresis is provided.

[0112] The matrix and/or material for forming the matrix may be altered before use in the electrophoresis step. The alteration may be provided before and/or during and/or after the matrix and/or material for forming the matrix is provided in the channel. The alteration may be polymerisation. The alteration may be caused and/or triggered by heating and/or the application of light, such as U/V light. The alteration may be applied to all of the matrix and/or material for forming the matrix or only a part thereof. One or more parts of the matrix may be prevented from alteration, for instance by masking those parts and/or excluding heat and/or excluding light from them.

[0113] The sample receiving step may include the transfer of a sample from outside the device and/or instrument, to inside the device and/or instrument. The sample receiving step may receive the sample from a collection device or from a storage device. The sample receiving step may include the transfer of the sample to a channel or chamber within the device.

[0114] The sample preparation step may include contacting the sample with one or more reagents and/or one or more other components. The reagents and/or other component may be used to prepare the sample for one or more of the subsequent steps.

[0115] The sample extraction step may be part of or separate from the sample preparation step. The sample extraction step may include contacting the sample with one or more reagents and/or components which select the sample component(s) relative to one or more waste components in the sample. The selected sample component(s) may be removed from the waste component(s) and/or the waste component(s) may be removed from the selected sample components. The waste component(s) may flow away from the extraction step. The waste component(s) may be washed away from the extraction step using one or more further reagents and/or components.

[0116] The sample retention step may be a part of or may be separate from the sample preparation step and/or sample extraction step. The sample retention step may include contacting the sample with one or more reagents and/or components which retain the sample component(s) relative to one or more waste components in the sample. The sample component(s) may be retained on one or more beads. The beads may be magnetic. The retained sample component(s) may be removed from the waste component(s) and/or the waste component(s) may be removed from the retained sample components. The waste component(s) may flow away from the retention step. The waste component(s) may be washed away from the retention step using one or more further reagents and/or components. The waste component(s) may flow past the location of retention. The waste component(s) may be washed away using one or more further reagents and/or components which flow past the location of retention.

[0117] The retained and/or selected sample may be eluted, preferably with the eluent conveying the retained and/or selected sample to the next step.

[0118] The purification step may be a part of or may be separate from the sample preparation step and/or sample extraction step and/or sample retention step. The purification step may separate the selected sample components, for instance DNA, from one or more waste components of the sample, for instance cellular material, PCR inhibitors and chemical inhibitors.

[0119] The washing step may be a part of or may be separate from the sample preparation step and/or sample extraction step and/or sample retention step and/or purification step. The washing step may remove one or more components of the sample from the location of one or more other components of the sample.

[0120] The elution step may be a part of or may be separate from the sample preparation step and/or sample extraction step and/or sample retention step and/or purification step and/or washing step. The elution step may remove one or more components of the sample from a first form into a second form. The first form may be bound to a surface or substrate, for instance on a bead. The second form may be in a liquid, for instance the eluent.

[0121] The amplification step may include contacting the sample with one or more reagents and/or components to cause amplification. The amplification step may include contacting the sample with conditions, preferably of a cyclic nature, to cause amplification. The amplification may be provided by a PCR step.

[0122] The denaturing step may prepare the sample for electrophoresis. The denaturing step may include contacting the sample with one or more reagents and/or components. The denaturing step may include contacting the sample with conditions, preferably of a cyclic nature, to cause denaturing.

[0123] The investigation step may provide a characteristic for one component of the sample which differs from the characteristic for one or more other components of the sample. The characteristic may be one or more detectable positions and/or one or more signals and/or one or more intensities and/or one or more colours and/or one or more concentrations and/or presence of one or more characteristics and/or absence of one or more characteristics.

[0124] The electrophoresis step may be part of or may be separate from the investigation step. The electrophoresis step may include transferring the sample to a start location for electrophoresis and/or a mobility based separation and/or a size based separation. The start location may be in a channel. The electrophoresis step may include one or more voltage conditions. One or more voltage conditions may be used to transfer the sample to the start location. One or more voltage conditions may be used to provide the separation.

[0125] The analysis step may establish one or more of the characteristics of the sample. The analysis may interrogate the instrument, particularly the device, and/or may seek a response from the instrument, particularly the device. The analysis may subject the instrument, particularly the device, to an operation, for instance the application of light. The analysis may consider the response to the operation, for instance the light returning.

[0126] The analysis step may include one or more operations involving an interaction with the device. The analysis step may include one or more operations not involving an interaction with the device. One or more of the interactions may be electromagnetic interactions.

The analysis step may apply light to the device. The analysis step may receive light from the device. The analysis step may establish the relative position of the elements having a characteristic, for instance an allele having a fluorescent dye. The analysis step may establish the relative size of the elements having a characteristic, for instance an allele having a fluorescent dye. The analysis step may generate one or more results. The light may be of visible and/or non-visible wavelengths. The results output step may display the one or more results from the analysis step and/or a processed form thereof.

[0127] The results output step may transmit the one or more results from the analysis step and/or a processed form thereof to a remote location. The results output step may compile the one or more results into a transmission form. The transmission may be via a telecommunications network. The results may be provided in a format compatible with one or more software applications.

[0128] The results output step may be followed by a further processing step. The further processing may interpret the results to provide further results. The further processing step may analyse the results to provide a DNA profile for the sample. The further processing step may provide an indication of a match between the sample and a database record of a sample. The further processing step may be provided at a location remote from the instrument. The further processing step may be provided at a location connected to the instrument, at least part of the time, by a telecommunications network. The further processing step may return to the instrument and/or a computer, preferably within 200 m of the site of the instrument, the further processed results.

[0129] The results may be processed on the instrument to give processed results. The processed results may extract from the results the signals, sections of signals or positions attributable to a characteristic being analysed for, such as an allele. The results and/or processed results may be provided to the results output step.

BRIEF DESCRIPTION OF THE DRAWINGS

[0130] Various embodiments of the present invention will now be described, by way of example only, and with reference to the accompanying drawings in which:

[0131] FIG. **1** is a schematic illustration of the stages involved in the consideration of a sample from collection to results and illustrates the positioning of the embodiments of the present invention in that context;

[0132] FIG. **2** is a schematic illustration of the key steps provided on or by an instrument embodying the present invention;

[0133] FIG. **3***a* is a front face view of part of a cartridge embodying the present invention;

[0134] FIG. **3***b* is a table of dimensions and volumes for a cartridge according to the present invention, and components thereof;

[0135] FIG. **4** is a front face view of a further part of the cartridge of FIG. **3***a* and embodying further features of the present invention;

[0136] FIG. **5***a* is a side view of the section of the cartridge of FIGS. **3***a* and **4** where in joins the electrophoresis cartridge section;

[0137] FIG. **5***b* is a front view of the electrophoresis cartridge section shown in FIG. **5***a*, with the section of the cartridge omitted;

[0138] FIGS. **6***a* to **6***e* are schematic illustrations of alternative arrangements for contacting the fluid and beads;

[0139] FIG. **7** is an illustration of an alternative structure for providing sample to the PCR chamber;

[0140] FIG. **8** is a front view of the electrophoresis cartridge section showing an alternative form of injector;

[0141] FIG. **9** is a schematic illustration of the parallel PCR chamber arrangement used in providing real time PCR and feedback of the results;

[0142] FIG. **10***a* is an illustration of a closing valve used in the present invention;

[0143] FIG. **10***b* is an illustration of an opening valve used in the present invention;

[0144] FIG. **11** shows an option for the archiving of a part of the sample handled;

[0145] FIG. **12** is a schematic front view of one embodiment of the instrument;

[0146] FIG. **13** is a side view showing the insertion of the cartridge into the instrument;

[0147] FIG. **14** is a schematic of the light source, optics and detector setup for the electrophoresis section of the instrument;

[0148] FIG. **15** is an electropherogram showing the variation in signal from the detector setup with time;

[0149] FIG. **16** is a schematic of an example of a system for detecting fluorescence;

[0150] FIG. **17** is a plot of LED spectrum, light reflected, and residual LED light over a range of wavelengths;

[0151] FIG. **18** is a plot of power of the LED-module over time;

[0152] FIG. **19** is an illustration showing beam shape and size as measured by the laser camera;

[0153] FIGS. **20***a* and **20***b* are plots of CCD signal v/s wavelengths for static fluorescence measurements; and

[0154] FIG. **21** is a plot of CCD signal v/s time for dynamic fluorescence measurements;

[0155] FIG. **22** is an illustration of a PCR chamber according to a further embodiment;

[0156] FIG. **23** is an illustration of the position of stacked Peltier effect devices;

[0157] FIG. **24** is an illustration of an embodiment for loading a CE channel

[0158] FIG. **25** is an illustration of a further embodiment for loading a CE channel;
[0159] FIG. **26** is an illustration of a further embodiment of a PCR chamber;
[0160] FIG. **27** is a front face view of a cartridge according to an embodiment;
[0161] FIG. **28***a* is a front face view of a cartridge according to a different embodiment;
[0162] FIG. **28***b* is a table of dimensions and volumes for the FIG. **28***a* cartridge;
[0163] FIG. **29***a* is a perspective view of an embodiment of the instrument;
[0164] FIG. **29***b* is a front view of the instrument of FIG. **29***a;*
[0165] FIG. **29***c* is a side view of the instrument of FIG. **29***a;*
[0166] FIG. **30** is a perspective view of another instrument embodiment;
[0167] FIG. **31***a* is an illustration of a carrier, cartridge and CE chip embodiment;
[0168] FIG. **31***b* is an illustration of a detail of the carrier to cartridge engagement;
[0169] FIG. **32***a* is an illustration of a carrier to CE chip engagement;
[0170] FIG. **32***b* is a cut away illustration of a part of the FIG. **32***a* engagement;
[0171] FIG. **33***a* is an illustration of the tube and cartridge connection;
[0172] FIG. **33***b* is an illustration of the tube to CE chip connection;
[0173] FIG. **34***a* is an illustration of the carrier being inserted into the instrument;
[0174] FIG. **34***b* is an illustration of the inserted carrier;
[0175] FIG. **35***a* is an illustration of the cartridge and carrier in the insertion form;
[0176] FIG. **35***b* is an illustration of the cartridge and carrier in the use form;
[0177] FIG. **35***c* is an illustration of the cartridge returned to the carrier;
[0178] FIG. **36***a* is a perspective view of the position of the pair of calipers;
[0179] FIG. **36***b* is a perspective view of the back of the pair of calipers;
[0180] FIG. **36***c* is a plan view of the caliper structure in the open form;
[0181] FIG. **36***d* is a plan view of the caliper structure in the closed form;
[0182] FIG. **37***a* is a perspective view of the second support of the carrier and CE chip;
[0183] FIG. **37***b* is a partial cut away illustration of the second support and CE chip;
[0184] FIG. **38** is a perspective view of the CE chip heater board;
[0185] FIG. **39** is a perspective view of an embodiment of the optics;
[0186] FIG. **40***a* is a perspective view of the alignment structure;
[0187] FIG. **40***b* shows the alignment structure of FIG. **40***a* in the stowed position;
[0188] FIG. **40***c* shows the alignment structure of FIG. **40***a* in the use position;
[0189] FIG. **41***a* shows three positions for an alternative PCR chamber embodiment;
[0190] FIG. **41***b* shows two positions for a further PCR chamber embodiment;
[0191] FIG. **41***c* shows three positions for a still further PCR chamber embodiment;
[0192] FIG. **42***a* shows a CE chip embodiment;
[0193] FIG. **42***b* shows a detail of the CE chip of FIG. **42***a;*
[0194] FIG. **43** shows an approach to loading sample to the CE step;
[0195] FIG. **44** shows an alternate approach to loading sample to the CE step;
[0196] FIG. **45** shows a further alternative for loading sample to the CE step
[0197] FIG. **46** shows a further embodiment of a PCR chamber.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0198] In a variety of cases it is desirable to be able to analyse a biological sample to obtain information on the sample and/or one or more components of the sample. Such cases include medical diagnostics, for instance to look for disease markers, and forensic science, for instance to establish a DNA profile.
[0199] At present, such analyses are conducted by highly trained scientists in a laboratory environment. This means that a significant amount of effort and experience goes into the handling of the samples, the use of the analysis equipment and the formulation of the conclusions reached. However, the need to convey the sample to a laboratory environment and then receive the results back from the laboratory environment introduces a potential time delay between obtaining the sample and obtaining the results thereon. The need to use a laboratory environment and highly trained scientists potentially adds to the time required, as the supply of such people and resources is limited. The need to use a laboratory environment and highly trained scientists potentially adds to the cost as there are capital and running costs associated with such facilities and the scientists.
[0200] If fewer laboratory style environments are to be used for the analysis or the staff used are less specialised, then there is the potential for problems with the analysis, unless a proper and reliable system is provided.
[0201] The present invention has amongst its potential aims to enable analysis of samples at a greater variety of locations and/or non-laboratory type locations. The present invention has amongst its potential aims to enable analysis by personnel having a lower level of training and/or experience. The present invention has amongst its potential aims to enable lower cost and/or faster analysis of samples. The present invention has amongst its potential aims to enable greater use and/or more successful use of analysis by law enforcement authorities.
[0202] Many of the concepts and issues to be addressed by the invention are best understood by way of the following examples. It should be noted, however, that these examples are by their very nature detailed and exhaustive, and that benefits from the present invention arise even when only small sections of the examples are implemented in other embodiments of the present invention.
[0203] The various embodiments and examples explain the invention initially in the context of a reference sample; that is a sample collected from a known individual under controlled conditions. An example of a reference sample would be a sample collected by a swab from the buccal cavity of a person

who has been arrested, the sample being collected at a police station. The invention is also suited to casework samples; that is a sample collected from a location from an unknown individual under non-controlled conditions. An example would be a spot of blood collected by a swab from a crime scene, with the source of the blood unknown. Where the differences between reference samples and casework samples have an impact on the preferred forms of the instrument, cartridge and methods, the casework sample embodiments are separately described.

[0204] The substitution of one or more components by one or more different components or different arrangements of components is also envisaged where particular conditions or issues arise. Again, after the discussion of the reference sample and casework sample contexts for the instrument, these alternatives are described.

[0205] As a starting point, it is useful to establish the context of the instrument, cartridge and methods of use in the overall context in which they may be used, by way of example. Thus in FIG. **1** there is a schematic of the overall process into which the present invention fits. This overall process includes a sample **1** which is gathered in a sample collection stage **3**. This is followed by a sample preparation stage **5**. In the subsequent sample loading stage **7**, a prepared cartridge **9** is loaded with the collected and prepared sample **1**. The next stage is the cartridge installation stage **15** in which the cartridge **9** is introduced to the instrument **11**. The instrument **11** also receives various inputs **13** at the sample loading stage **7** and/or at the cartridge installation stage **15** and/or subsequently.

[0206] The structure and processes performed within the instrument **11** and cartridge **9** are described further below in the context of FIG. **2**.

[0207] Once the instrument **11** has completed these stages and achieved the analysis, the next stage is the results stage **17**. This is followed by one or more output stages **19**, and potential further stages **21** which integrate the analysis into the criminal justice system of that jurisdiction. A wide range of possible links between the various output stages **19** and further stages **21** may be possible, with some being linked to just one stage and others be the result of multiple such stages and/or combinations thereof.

[0208] An output stage **19** may include the transmission of the results from the instrument to a remote location for processing. The processing may be performed using complex software and/or hardware tools, before the final results are returned to the instrument **11** or to another computer. Processing the results at a remote location may be preferably in terms of the size, cost or complexity of the software/hardware needed to perform the processing thus only being provided at a limited number of locations, rather than a part of each instrument.

[0209] The following description of the operation of the instrument **11**, in a generally sequential manner, provides full details of the key instrument stages and their interrelationship.

[0210] Referring to FIG. **2**, the instrument has a sample receiving step **200**, sample preparation step **202**, sample amplification step **204**, electrophoresis step **206** and analysis step **208** and data communication step **210**.

[0211] In the sample receiving step **200**, the sample **1** is transferred from a sample storage and/or processing stage **5**, which is outside of the cartridge **9** and instrument **11**, to a location on the cartridge **9**.

[0212] The initial collection device is frequently a swab. The swab is used to pick up the sample **1** from an article or substrate.

[0213] In the sample preparation step **202**, the key components within the sample are contacted with the reagents and/or components intended to prepare the sample for the subsequent steps. In this embodiment, the sample preparation step **202** contacts the sample with beads to retain the DNA and recover it, whilst the other components which are not to be recovered flow through and away. The sample preparation step **202** also includes contact with a wash agent to improve the separation of the DNA from the other components. The wash agent flows through the chamber holding the beads and retained DNA and flows to a further chamber, a waste chamber. The wash agent is followed by an elution agent to release the DNA from the beads for the subsequent steps.

[0214] In the sample amplification step **204**, the DNA is contacted with amplification reagents and provided with the conditions necessary to achieve amplification through PCR.

[0215] In the electrophoresis step **206**, the amplified DNA is conveyed to a start point for a mobility based separation within a capillary. An electric field is then used to separate the complex DNA amplicons into different size clusters.

[0216] In the analysis step **208**, the channel is inspected to establish the relative position and hence size of elements detected in the capillary. This is achieved by an excitation light source, fluorescent markers associated with the elements to be detected and suitable optics to detect the fluorescent light resulting.

[0217] In the data communication step **210**, the instrument compiles the necessary data packet for transmission and transmits it to a remote location for consideration. The data packet includes information on the electrophoresis results, sample identity and other information. The analysed results may be received by the instrument as part of the data communication step **210**.

[0218] Some data processing may be performed on the instrument itself, for instance to deconvolute the analysis results to indicate the peaks indicative of alleles present.

[0219] The instrument can be provided in a format which considers a single sample at a time, or can be provided in a format which considers multiple samples at a time. The multiple samples may each be run on separate cartridges, but modified cartridges which handle multiple samples are possible. The handling of multiple cartridges is beneficial in allowing a single set of controllers, power supplies, optics and the like to consider multiple samples, with reduced capital costs.

Cartridge

[0220] Key to the operation of the instrument is a disposable, single use cartridge **9**. This cartridge **9** is intended to only process and provide the results for analysis on a single occasion. The disposable nature of the cartridge **9** places a number of constraints on the cartridge **9** in terms of the materials which can be used, because of the need to keep manufacturing, assembly or purchase costs low.

[0221] The detailed layout of the cartridge **9** is now described. Later, a description of the sequence of operation of the elements which make up the cartridge is provided.

[0222] FIG. **3***a* is an illustration of that part of the sample receiving step **200** provided on the cartridge **9**, the whole sample preparation step **202** and the whole sample amplifi-

cation step **204**. The subsequent steps and their respective pasts of the cartridge **9** are illustrated separately.

[0223] FIG. **3***b* provides details of the volumes of the various chambers used, the depths (into the page in effect) for the various components and the overall dimensions of this part of the cartridge **9**.

[0224] The cartridge **9** is provided with a sample introduction chamber **302** connected to a channel **304** leading to the outside of the cartridge **300**. This forms those parts of the sample receiving step **200** provided on the cartridge **9**.

[0225] The sample preparation step **204** follows. To provide this, the sample introduction chamber **302** is connected to a pumping fluid channel **306** and hence to a first electrochemical pump **308**. The sample introduction chamber **302** has an outlet channel **310** which passes valve **312** and provides an inlet to purification buffer chamber **314**. Valve **312** is initially open.

[0226] Purification buffer chamber **314** is connected via channel **316** to bead storage chamber **318**. The bead storage chamber **318** is connected via channel **320** to initial mixing chamber **322**. The outlet channel **324** from initial mixing chamber **322** is blocked by closed valve **326**, but a vent channel **328** is open because valve **330** is open initially.

[0227] The outlet channel **324** leads past valve **326** to a first further mixing chamber **332** and then through channel **334** to second further mixing chamber **336**. The outlet **338** from the second further mixing chamber **336** leads past valve **340** to incubation chamber **342**, where bubble mixing assists the DNA to bead binding process.

[0228] The incubation chamber **342** has a vent channel **344** provided with valve **346** and an outlet channel **348** which is initially closed by valve **350**. The incubation chamber **342** is also provided with a pumping fluid inlet channel **352** which passes valve **354** and is connected to second electrochemical pump **356**.

[0229] The outlet channel **348** from the incubation chamber **342** leads to capture chamber **358** where the beads and hence bound DNA are collected. The capture chamber **358** is provided with a first vent channel **360** which passes first valve **362** and second valve **364**. The capture chamber **358** is also provided with a second vent channel **366** which passes first valve **368** and second valve **370**.

[0230] Also connected to capture chamber **358** is wash buffer channel **372**. The wash buffer channel is connected to first valve **374** and second valve **376** and leads from second electrochemical pump **356** through wash buffer chamber **378** to the capture chamber **358**.

[0231] Also connected to capture chamber **358** is an elution liquid channel **380**. The elution liquid channel **380** is connected to first valve **382**, elution liquid storage chamber **384**, second valve **386** and back to third electrochemical pump **388**.

[0232] The capture chamber **358** has a wash outlet channel **390** which splits into a first wash outlet channel section **392** which passes valve **394**, and into a second wash outlet channel section **396** which passes valve **398**. After passing their respective valves **394**, **398**, the first wash outlet channel section **392** and second wash outlet channel section **396** rejoin one another to form further wash channel **400**. The further wash channel **400** leads past valve **402** into waste chamber **404**. The waste chamber **404** is vented along vent channel **406** past valve **408**. These elements provide the sample preparation step **202**.

[0233] To provide the sample amplification step **204**, capture chamber **358** is also provided with elution outlet channel **410** which leads past valve **412** and past valve **414** and into PCR chamber **416**. The outlet channel **418** from the PCR chamber **416** leads past valve **420** into archive chamber **422**. The archive chamber **422** is vented through vent channel **424**. The role of the archive chamber **422** is described further below.

[0234] Provided within the PCR chamber **416** is a bead loaded with the reagents, a multimix, needed for the PCR process. The reagents/multimix include primers dNTPs and PCR reaction mix, including Tris buffer, $MgCl_2$, NaCl and BSA. These reagents are released into the sample once it contacts the bead in the PCR chamber **416** and the temperature is raised above ambient temperature.

[0235] The above circuit overall, is sufficient to receive, retain, wash, elute and perform PCR on the sample, as well as storing the waste from the process and an archive of the PCR product.

[0236] Subsequently, the arrangement shown in FIG. **4** can be used to transfer the now amplified DNA from the PCR chamber **416** into the electrophoresis step **206**.

[0237] In FIG. **4**, the PCR chamber **416** is the same PCR chamber **416** which was illustrated in FIG. **3** and described above. Other features were omitted from FIG. **3** to improve the clarity of that Figure.

[0238] Leading from the PCR chamber **416** is a denaturing feed channel **500** which is connected to an amplified material mixing chamber **502**. The amplified material is pumped from PCR chamber **416** by the action of fourth electrochemical pump **504** which is connected to channel **506**, hence to denaturing reagent storage chamber **508** and through channel **510** to the PCR chamber **416**. Formamide is provided in the denaturing reagent storage chamber in the preferred form.

[0239] These components are isolated from the PCR chamber **416** during the sample amplification step **204** by closed valve **512** and closed valve **514**. Both valve **512** and **514** are opened and valves **516** and **518** are closed to convey the amplified material away from the PCR chamber **416**.

[0240] From the denaturing feed channel **500**, the amplified material and denaturing reagents enter the first amplified material mixing chamber **502**, pass through channel **520**, into second amplified material mixing chamber **522**, through channel **524** and into third amplified material mixing chamber **526**. Whilst the third amplified material mixing chamber **526** fills, valve **528** is shut and vent **530** is open. An overall volume of 45:1 is provided, 5:1 from the PCR chamber and 40:1 from the denaturing reagent storage chamber **508**.

[0241] The amplified material is held in the third mixing chamber **526** for the necessary time and at the necessary temperature to complete the denaturing process. Once this has been achieved, the valve **528** is opened and further pumping by the fourth electrochemical pump **504** pumps the denatured material to the electrophoresis step inlet **532**. At the inlet **532**, the denatured material passes out of the plane of the cartridge **9** and to the electrophoresis cartridge section behind. Once past through the inlet **532**, valve **534** is shut to isolate the cartridge **9** from the electrophoresis cartridge section **600**.

[0242] The overall result of this structure is the pumping of the amplified DNA to a start point for the electrophoresis step **206**.

[0243] The transfer from PCR to CE steps is provided in a way which allows easy integration of the steps, does not

impact upon the temperature and pressure stability required in PCR and achieves minimal sample loss during transfer. Automated mixing of the sample and size standards during transfer and possibilities for pre-concentrating the sample before CE are also rendered possible.

[0244] The overall configuration of the electrophoresis step **206** can be seen in the side view of FIG. **5***a* and front view of FIG. **5***b*.

[0245] The inlet **532** leads from the plane of the cartridge **9**, through into the plane of the electrophoresis cartridge section **600**. Here, the inlet **532** leads into the top section **602** of an electrophoresis feed reservoir **604**. The top section **602** is empty, but the lower section **606** is provided with the gel **608** which also fills the capillary **610**. The sample is pumped into the electrophoresis feed reservoir **604** by a fourth electrochemical pump, not shown.

[0246] Sample flow from the reservoir **604** into the correct position within the capillary **610** is achieved using electrophoresis as the transport mechanism.

[0247] In this embodiment, the injector structure provided within the capillary cartridge section **600** is a double T injector. This includes a first electrode location **612**, second electrode location **614** provided at the other end of the long capillary **616** in which the size based separation is achieved. A third electrode location **618** and fourth electrode location **620** are provided in side arms **622** and **624** respectively. The side arms are offset relative to one another, with side arm **624** further towards the second electrode location **614**, than the side arm **622**.

[0248] Initially, sample is drawn from the liquid phase in the reservoir **604** through the interface with the gel provided in the reservoir **604** and hence into the gel by a voltage applied to the electrode present at the third electrode location **618**. Once the sample has been drawn past the fourth electrode location **620**, a voltage is also applied to the electrode at the fourth electrode location. Generally, the electrode at the third electrode location may be at a voltage of 600V and the electrode at the fourth electrode location may be at a voltage of 200V. The voltage may be floating for the electrodes at the first **612** and second **614** electrode locations.

[0249] This situation results in sample being drawn along side arm **624**, along the section **626** and into side arm **622**, such that sample is present in the two side arms **622** and **624** and the section **626** of the capillary **616**.

[0250] This gives the plug of sample upon which the electrophoresis's to act in the section **626**.

[0251] To reduce the cost of the electrodes used, consistent with the cartridge being single use, platinum coated, gold coated, carbon, nickel and other lower cost electrodes may be used.

[0252] Once positioned, the separation voltages are applied: 1500V at the electrode at the second electrode location **614**; 0V at the electrode at the first electrode location **612**; and 200V at the electrodes present at the third electrode position **618** and fourth electrode positions **620**.

[0253] The capillary **616** is filled with a gel matrix which preferentially retards the speed of progress of elements within the DNA as their size increases. The result is a size based separation of the elements, with the faster elements reaching the detection location **626** first and the slowest reaching the detection location **628** last. The different times at which the signals are generated and form the electropherogram indicate the size of the element behind that signal.

[0254] It is possible to assist in the interpretation of the unknown element sizes by using a size standard within the capillary. This is provided with a different dye colour or otherwise rendered distinct. The method set out in U.S. patent application No. 61/096,424, the contents of which are hereby incorporated by reference, offers approaches for determining the sizes of the unknowns from the size standard.

[0255] The setup and operation of the light source, optics and detector is described in detail below.

[0256] Other embodiments of the cartridge have also been developed.

[0257] As shown in FIG. **27**, the cartridge **27-01** has been modified by providing the electrochemical pumps **27-03**, **27-05**, **27-07**, **27-09** with connections between the wires leading to the electrodes in the pumps and the power source not shown of the Pogo™ pin type. The pins **27-11** are spring loaded in the recesses of the cartridge **27-01** and in use contact similar spring loaded pins (not shown) on the other side of the cartridge to instrument interface. A reliable electrical contact is thus provided and the cartridge is more robust against damage during storage, installation and use than designs in which the wires for the electrochemical pumps protruded from the side of the cartridge.

[0258] The form shown in FIG. **27** also features guide holes **27-13** which are used in the alignment of the cartridge and instrument, as described in more detail below.

[0259] A preferred embodiment of the cartridge is shown in FIG. **28***a*. This is an illustration of that part of the sample receiving step **200** provided on the cartridge **28-09**, the whole sample preparation step **202**, the whole sample amplification step **204**, the whole sample denaturation step and the feed to the capillary electrophoresis step **206**.

[0260] FIG. **28***b* provides details of the volumes of the various chambers used, the depths (into the page in effect) for the various components and the overall dimensions of this part of the cartridge **28-09**.

[0261] The cartridge **28-09** is provided with a sample introduction chamber **28-302** connected to a channel **28-304** leading to the outside of the cartridge **28-09**. This forms those parts of the sample receiving step **200** provided on the cartridge **28-09**.

[0262] The sample preparation step **204** follows. To provide this, the sample introduction chamber **28-302** is connected to a pumping fluid channel **28-306** and hence to a first electrochemical pump **28-308**. The sample introduction chamber **28-302** has an outlet channel **28-310** which passes valve **28-312** and provides an inlet to bead storage chamber **28-318**. Valve **28-312** is initially open.

[0263] The bead storage chamber **28-318** has an outlet channel **28-316** leading to binding buffer storage chamber **28-314**. This sequence of chambers is reversed compared with the FIG. **3***a* embodiment. The binding buffer storage chamber **28-314** has an outlet channel **28-320** which leads to mixing/purification chamber **28-322**.

[0264] Mixing/purification chamber **28-322** is connected via channel **28-324** through valve **28-326** and via channel **28-500** to first further mixing chamber **28-332**. The outlet channel **28-324** from mixing/purification chamber **28-322** is blocked by closed valve **28-326**, but a vent channel **28-328** is open because valve **28-330** is open initially.

[0265] The outlet channel **28-324** leads past valve **28-326** to a first further mixing chamber **28-332** and then through channel **28-334** to second further mixing chamber **28-336**. The outlet **28-338** from the second further mixing chamber

28-336 leads past valve **28-340** to incubation chamber **28-342**, where bubble mixing assists the DNA to bead binding process. The incubation chamber **28-342** may be actively heated or may simply provide the necessary dwell time and/or other binding conditions needed.

[0266] The incubation chamber **28-342** has a vent channel **28-344** provided with valve **28-346** and an outlet channel **28-348** which is initially closed by valve **28-350**. The incubation chamber **28-342** is also provided with a pumping fluid inlet channel **28-352** which passes valve **28-354** and is connected to second electrochemical pump **28-356**.

[0267] The outlet channel **28-348** from the incubation chamber **28-342** leads to capture chamber **28-358** where the beads and hence bound DNA are collected. The capture chamber **28-358** is provided with a first vent channel **28-360** which passes first valve **28-362** and second valve **28-364**. The capture chamber **28-358** is also provided with a second vent channel **28-366** which passes first valve **28-368** and second valve **28-370**.

[0268] Also connected to capture chamber **28-358** is wash buffer channel **28-372**. The wash buffer channel is connected to first valve **28-374** and second valve **28-376** and leads from second electrochemical pump **28-356** through wash buffer chamber **28-378** to the capture chamber **28-358**.

[0269] Also connected to capture chamber **28-358** is an elution liquid channel **28-380**. The elution liquid channel **28-380** is connected to first valve **28-382**, elution liquid storage chamber **28-384**, second valve **28-386** and back to third electrochemical pump **28-388**.

[0270] The capture chamber **28-358** has a wash outlet channel **28-390** which splits into a first wash outlet channel section **28-392** which passes valve **28-394**, and into a second wash outlet channel section **28-396** which passes valve **28-398**. After passing their respective valves **28-394**, **28-398**, the first wash outlet channel section **28-392** and second wash outlet channel section **28-396** rejoin one another to form further wash channel **28-400**. The further wash channel **28-400** leads past valve **28-402** into waste chamber **28-404**. The waste chamber **28-404** is vented along vent channel **28-406** past valve **28-408**. These elements provide the sample preparation step **202**.

[0271] To provide the sample amplification step **204**, capture chamber **28-358** is also provided with elution outlet channel **28-410** which leads past valve **28-412** and past valve **28-414** and past valve **28-502** and into PCR chamber **28-416**. The outlet channel **28-418** from the PCR chamber **28-416** leads past valve **28-420** and past valve **28-504** and past valve **28-506** into archive chamber **28-422**. The archive chamber **28-422** is vented through vent channel **28-424**. The role of the archive chamber **28-422** is as described further above.

[0272] Provided within the PCR chamber **28-416** is a bead loaded with the reagents, a multimix, needed for the PCR process. The reagents/multimix include primers dNTPs and PCR reaction mix, including Tris buffer, $MgCl_2$, NaCl and BSA. These reagents are released into the sample once it contacts the bead in the PCR chamber **28-416** and the temperature is raised above ambient temperature.

[0273] The above circuit overall, is sufficient to receive, retain, wash, elute and perform PCR on the sample, as well as storing the waste from the process and an archive of the PCR product.

[0274] The PCR part of the circuit has been moved to the upper section of the cartridge compared with the previous embodiments so as to present it physically closer to the CE chip.

[0275] Subsequently, the further arrangement shown in FIG. **28***a* can be used to prepare, denaturation step, and transfer the now amplified DNA from the PCR chamber **28-416** into the electrophoresis step **206**.

[0276] Leading from the PCR chamber **28-416** is outlet channel **28-418**. This splits after valves **28-420** and **28-504** into a denaturing feed channel **28-550** and the channel leading to the archive chamber **28-422**. The denaturing feed channel **28-550** is connected to a denaturation chamber **28-552**. The amplified material is pumped from PCR chamber **28-416** by the action of fourth electrochemical pump **28-554** which is connected to channel **28-556**, hence to denaturing reagent storage chamber **28-558** and through valve **28-560** and channel **28-562** to the PCR chamber **28-416**. Formamide is provided in the denaturing reagent storage chamber **28-558** in combination with the size standards to be used in the capillary electrophoresis step.

[0277] These components are isolated from the PCR chamber **28-416** during the sample amplification step **204** by closed valve **28-502** and closed valve **28-420**. Both valve **28-502** and **28-420** are opened and valves **28-414** and **28-506** are closed to convey the amplified material away from the PCR chamber **28-416** to the denaturation chamber **28-552**. This is vented through valve **28-564**, with exit channel **28-566** closed by valve **28-568**.

[0278] The amplified material is held in the denaturation chamber **28-552** for the necessary time and at the necessary temperature to complete the denaturing process. Once this has been achieved, the valve **28-568** is opened and further pumping by the fourth electrochemical pump **28-554** pumps the denatured material to the electrophoresis step inlet **28-570**.

[0279] At the inlet **28-570**, the denatured material passes out of the plane of the cartridge **9** and through a tube to the electrophoresis cartridge section behind. The overall result of this structure is the pumping of the amplified DNA to a start point for the electrophoresis step **206**.

[0280] Details of the connection of the inlet **28-570** to the CE chip are provided below.

[0281] Throughout the operations described above and in the sections that follow, various checks are made on operating conditions, component performance and successful operation so as to ensure the processing is correctly provided from start to finish. Errors or problems are indicated to the operator.

[0282] Cartridge Sequence of Operation

[0283] The sequence of operation, purely by way of example, applied to the cartridge shown in and described in relation to FIGS. **3***a* and *b* is as follows, with sample timings also given.

Time since start (sec)	Change	Purpose and notes
0.0	Incubation chamber 358 - adjust temperature to 25° C.	
0.9	Valve 312 - opening valve - heat on	
31.5	First electrochemical pump 308 - on	
73.3	Valve 330 - closing valve - heat off	
121.1	Valve 312 - opening valve - heat off	

-continued

Time since start (sec)	Change	Purpose and notes
138.7	First electrochemical pump 308 - off	
187.8	Valve 326 - opening valve - heat on	
212.3	Valve 312 - opening valve - heat on	
233.9	Valve 330 - closing valve - heat off	
236.0	First electrochemical pump 308 - on	
324.3	Valve 312 - opening valve - heat off	
368.6	Valve 326 - opening valve - heat off	
370.4	Valve 346 - closing valve - heat on	
401.0	First electrochemical pump 308 - off	
461.4	Valve 346 - closing valve - heat off	
653.4	Valve 350 - opening valve - heat on	
655.1	Magnet - field applied to chamber	
656.4	Valve 326 - opening valve - heat on	
684.5	First electrochemical pump 308 - on	
783.4	Valve 326 - opening valve - heat off	
804.1	Valve 394 - closing valve - heat on	
815.4	Valve 340 - closing valve - heat on	
829.6	Valve 350 - opening valve - heat off	
840.8	Magnet - field removed from chamber	
867.5	First electrochemical pump 308 - off	
894.2	Valve 394 - closing valve - heat off	
944.5	Valve 368 - opening valve - heat on	
975.5	Valve 340 - closing valve - heat off	
977.2	Second electrochemical pump 356 - on	
1025.8	Valve 354 - closing valve - heat on	
1036.2	Valve 368 - opening valve - heat off	
1050.8	Second electrochemical pump 356 - off	
1079.7	Valve 324 - opening valve - heat on	
1080.6	Valve 368 - opening valve - heat on	
1116.3	Valve 354 - closing valve - heat off	
1118.0	Second electrochemical pump 356 - on	
1181.3	Valve 370 - closing valve - heat on	
1196.4	Valve 368 - opening valve - heat off	
1228.3	Valve 324 - opening valve - heat off	
1233.9	Second electrochemical pump 356 - off	
1244.2	Valve 398 - opening valve - heat on	
1249.4	Valve 324 - opening valve - heat on	
1271.8	Valve 370 - closing valve - heat off	
1273.1	Magnet - field applied to chamber	
1284.7	Second electrochemical pump 356 - on	
1328.6	Valve 324 - opening valve - heat off	
1333.8	Valve 402 - closing valve - heat on	
1334.7	Valve 408 - closing valve - heat on	
1379.9	Valve 398 - opening valve - heat off	
1383.8	Magnet - field removed from chamber	
1393.9	Second electrochemical pump 356 - off	
1419.5	Valve 362 - opening valve - heat on	
1435.4	Valve 402 - closing valve - heat off	
1465.1	Valve 408 - closing valve - heat off	
1466.0	Second electrochemical pump 356 - on	
1474.6	Valve 374 - closing valve - heat on	
1493.6	Valve 362 - opening valve - heat off	
1501.8	Valve 382 - opening valve - heat on	
1504.8	Valve 362 - opening valve - heat on	
1508.7	Second electrochemical pump 356 - off	
1531.9	Third electrochemical pump 388 - on	
1578.8	Incubation chamber 358 - adjust temperature to 60° C.	
1585.0	Valve 374 - closing valve - heat off	
1586.6	Valve 362 - opening valve - heat off	
1588.5	Valve 364 - closing valve - heat on	
1633.3	Valve 382 - opening valve - heat off	
1640.4	Third electrochemical pump 388 - off	
1679.0	Valve 364 - closing valve - heat off	
1881.0	Valve 412 - opening valve - heat on	
1882.9	Valve 382 - opening valve - heat on	
1906.2	Magnet - field applied to chamber	
1914.9	Third electrochemical pump 388 - on	
1952.3	Incubation chamber 358 - adjust t to 25° C.	
2010.0	Third electrochemical pump 388 - off Magnet - field removed from chamber Valve 382 - opening valve - heat off Valve 412 - opening valve - heat off	

-continued

Time since start (sec)	Change	Purpose and notes
2017.3	Valve 420 - closing valve - heat on Valve 414 - closing valve - heat on	Isolate PCR chamber
2173.3	Valve 420 - closing valve - heat off Valve 414 - closing valve - heat off	
2185.0	Incubation chamber temperature control - off	

[0284] Cartridge Alternatives

[0285] There are a variety of alternatives for the various components within the cartridge and/or their operation. Some of these are now described, by way of example only.

[0286] 1) Bead Handling

[0287] As described above, the cartridge makes use of a bead storage chamber **318** from which the beads are washed in operation. This washing action provides contact between the sample, reagents and the beads. Mixing results in the beads taking up the DNA in the sample and retaining it. Subsequent retention of the beads allows the DNA to be separated from the rest of the sample and allows washing stages to improve further this separation.

[0288] It is important to ensure that the beads are displaced from their storage location, such that the beads are available, in contact with the relevant liquids, to perform their task. Modifications to the manner in which the beads are stored and/or dispensed can assist in this. The beads may be stored away from the cartridge. They may be introduced to the cartridge to prepare it for use.

[0289] Firstly, it is possible to provide a dispersant together with the beads so as to keep them dispersed and hence more easily collected and carried by the fluid flow. This can help prevent blockages and/or agglomerations of beads. Different dispersants and/or variations in the amount provided can be used to tailor this.

[0290] Secondly, it is possible to provide the beads in a series of bead storage chambers, rather than in a single chamber. FIG. **6***a* illustrates one such arrangement, where the beads are split into three groups, each in its own chamber **700**. In this way, the contact between the fluid and the beads is staggered and a compacted mass of beads is avoided on the lead edge of the fluid. A variation on this is provided in FIG. **6***b*, where a first bead storage chamber **700***a* is separated from the second bead storage chamber **700***b* by a mixing chamber **702**.

[0291] Thirdly, the contact can be provided with a thin chamber **704** whereby the transition of the fluid from the thing channel **706** into the chamber causes non-laminar flow and hence improved mixing. The provision of the beads spread along the length of the chamber **704** also means that they do not contact the fluid all at the same time.

[0292] Fourthly, the flow direction and/or chamber design can be modified to encourage displacement of the beads from their storage position into a mixed form with the fluid. Thus in the FIG. **6***d* form, the fluid enters the chamber **700** in one bottom corner **708** and displaces, arrows, the beads resting in that part. A swirling flow within the chamber **700** gives mixing, before the fluid and bead mixture exits the chamber **700** through the other bottom corner **710**.

[0293] Fifthly, the beads can be stored in a side arm **712** or other form of passage. As the flow of fluid passes through thin chamber **714** and past the junction **716** with the arm **712**, a

force is applied behind the mass of stored beads in the side arm **712**. This forces the mass of stored beads towards and into the junction **716** where they gradually contact and are swept away by the fluid flow. Gradual dispersal of the beads into the fluid is provided. The motive force behind the beads can be provided by a similar structure to that used to move material in the context of the closing valves described herein.

[0294] 2) PCR Chamber Filling

[0295] In the above system, the amount of the processed sample which is made available to the PCR stage is controlled by the relative height of the outlet from the PCR chamber to the archive chamber leading to overflow of excess sample into the archive chamber. This results in a PCR chamber which is not completely full of sample during PCR. As PCR involves heating of the sample, evaporation and/or condensation of part of the sample may occur at a location outside of the PCR chamber. This can reduce the reagents present in the PCR chamber and hence reduce the efficiency of the PCR stage.

[0296] In an alternative form, the PCR chamber is entirely filled with the sample before PCR is started. This is achieved using the arrangement of FIG. **7** where the majority of the components have the same structure and function as shown in the FIG. **3** and FIG. **4** description. The differences are in the section around the PCR chamber **416**.

[0297] In this alternate form, the PCR chamber **1416** is fed material along channel **1413**. Initially, the path of least resistance to this fluid flow is through the PCR chamber **1416**, along channel **1500**, past opened valve **1502** and onto vent **1504**. The vent **1504** is hydrophobic and so allows the passage of the air displaced from the PCR chamber **1416** and channel **1500** by the material's advance. Once the fluid reaches the vent **1504**, however, the path of least resistance changes and further flow occurs along channel **1418** past valve **1428** and into archive chamber **1422**, which is provided with vent **1424**. By this time, the PCR chamber **1416** is completely full of liquid and hence the volume of the liquid subjected to PCR is guaranteed.

[0298] As before, the valves around the PCR chamber **1416** are closed during the amplification itself, so as to isolate the PCR chamber **1416**.

[0299] In a third alternative, the configuration shown in FIG. **22**, the PCR chamber **22-01** is along channel **22-03**. Initially, the path of least resistance to this fluid flow is through the inlet **22-05** to the PCR chamber **22-01**. Once the PCR chamber **22-01** has filled, the liquid overflows through exit **22-07** into channel **22-09** which is a continuation of channel **22-03**. Further fluid flow simply by-passes the PCR chamber **22-01** and flows through channel **22-03** and then channel **22-09**. To control the flow correctly, the dimension A of the inlet **22-05** is greater than the dimension B of the outlet **22-07**. The dimension is preferably greater in terms of the cross-sectional area, perpendicular to the direction of flow. The complete filling of the PCR chamber **22-01** ensure the volume of the liquid subjected to PCR is guaranteed.

[0300] Various shapes are possible for the PCR chamber. FIG. **26** provides an example in which the PCR chamber **26-01** is formed as smooth as possible. This assists with full fluid contact with the surfaces and hence complete and accurate filling of the PCR chamber **26-01**. The sample flows along channel **26-03** and enters the PCR chamber **26-01** via inlet **26-05** provided towards the bottom of the PCR chamber **26-01**. The sample fills the PCR chamber **26-01** before overflowing through outlet **26-07** provided towards the top of the PCR chamber **26-01** and into channel **26-09**.

[0301] In the embodiment of FIG. **46**, a variation on the above principle is provided. The flow to the PCR chamber **46-100** passes along channel **46-102** and past valve **46-104**. The channel **46-102** turns as it approaches the chamber **46-100** and provides inlet channel **46-106**. The natural flow is along this route. As the flow progresses, the PCR chamber **46-100** fills, with the gas exiting through outlet channel **46-108**. The outlet channel **46-108** has a similar configuration to inlet channel **46-106**, but the cross-sectional area of the outlet channel **46-108** is much smaller than that of the inlet channel **46-106**. As a result, when the liquid reaches the outlet channel **46-108**, the flow resistance increases greatly and flow is redirected along the by-pass channel **46-110** in preference. Both the outlet channel **46-108** and the by-pass channel **46-110** lead past valve **46-112** to exit channel **46-114**. The Peltier effect device heats the area within the dotted lines and so ensures that as much of the space between the two valves, **46-104** and **46-112** is heated so as to minimise any condensation within that space.

[0302] 3) Sample Concentration Before Capillary Electrophoresis

[0303] In some instances, it may be helpful to increase the concentration of the sample prior to its use in the electrophoresis step and/or to reduce the size of the sample as it is injected.

[0304] Once suitable approach for doing so is set out in European patent publication no 1514100, the contents of which are incorporated herein by reference. This technique uses careful balancing of the electrophoretic velocity of the DNA and the opposing electroosmotic velocity to concentrate the DNA at the liquid to gel interface. A change in conditions can then be used to drawn the concentrated DNA into the electrophoresis step as a concentrated and small sample.

[0305] Another option is hydrodynamic stacking. This is based upon the variation in the flow velocity between sample and the location from which the size based separation starts, for instance through the use of adjustments to conductivity, buffer components, pH and the like. An example of such an approach is field amplified sample stacking, FASS. This provides higher electric fields in the lower conductivity zones than in the higher conductivity zones. The sudden potential drop at the interface between the two zones causes sample stacking there.

[0306] Mechanical pre-concentration is also a possibility. Packed beds, nanochannels, immobilised polymers and membranes all offer the possibility of trapping and concentrating the sample. Electro-elution, where by the release of the sample is caused by the application of an electric potential to a membrane, is one possibility.

[0307] A combined technique approach to pre-concentration may be particularly beneficial. Such an approach is shown in FIG. **24**, in the case of CE channel being in the same plane as the rest of the cartridge, and FIG. **25**, in the case of the CE channel not being in the same plane as the rest of the cartridge.

[0308] As illustrated, the combined flow **24-01**, **25-01** of DNA containing sample and formamide pass valve **24-03**, **25-03** and then reach a junction **24-05**, **25-05**. The Y-shaped junction brings the combined flow **24-01**, **25-01** into proximity with the running buffer flow **24-07**, **25-07** in channel **24-08**, **25-08**. These flows cross the CE channel **24-09**, **25-09** and any excess passes to chamber **24-11**, **25-11**. The left-hand detail shows the construction present at the intersection of the CE channel **24-09**, **25-09** and the channel **24-08**, **25-08**.

[0309] In the FIG. **24** form, the stacking interface **24-11** is provided between the combined flow **24-01** and buffer flow **24-07**. The electric potential is provided by electrode **24-13**. The second stacking function is provided by the membrane **24-15** provided between the buffer flow **24-07** and the CE channel **24-09**.

[0310] In the FIG. **25** form, the stacking interface is similarly provided.

[0311] 4) Alternative Electrophoresis Channel Configuration

[0312] In the embodiment described above, the injector is of the double T type. As an alternative, it is possible to use a cross-channel injector, as shown in FIG. **8**.

[0313] In this case, the reservoir **604**, channel **610** and other parts leading to the fourth electrode location **620** are the same. The arm **624** provided with the fourth electrode location **620** and the arm **622** provided with the third electrode location **618** are aligned on a common axis and at 90° to the main capillary **616**.

[0314] The sample is drawn towards the electrode at the third electrode position **618** by the application of a voltage. To prevent dispersion of the sample into the main capillary, towards the first **612** and/or second **614** electrode locations, a voltage is applied to the electrode at the first electrode location **612** and to the electrode at the second electrode location **614**. This has the effect of pinching the part of the sample at the intersection of the main capillary **616** and the arms **622**, **624**, and maintaining the minimal size of the plug which is then used in the capillary electrophoresis.

[0315] A further electrophoresis channel configuration is shown in FIG. **43**. In this case, the sample flows along channel **43-100** from inlet **43-102** to outlet **43-104**. A potential difference is applied between locations A and B. This draws the DNA in the sample towards the membrane **43-106**. The membrane is sized, 10-14 kDa cutoff, to retain the DNA. The separation matrix is then flowed into the channel **43-100**; UV activation may be provided, as discussed elsewhere. The same buffers at location A, B and in the matrix are then provided for the electrophoretic separation to be provided through the application of a potential difference between A and B.

[0316] The polarity may be provided in the reverse direction before the CE run, for instance to ensure the buffer extends from A to B. DNA is not lost as the flow will maintain it on the membrane **43-106**.

[0317] Between loading to the membrane **43-106** and the CE separation, it is possible to introduce a variety of reagents/buffers into locations A and/or B and/or the channel **43-100** to assist in purifying the DNA and/or to optimise CE conditions, for instance through removal of excess salts and/or unincorporated PCR primers. Both locations A and B have their own inlets and outlets for this purpose.

[0318] A still further configuration is shown in FIG. **44**. In this case, again the sample flows through channel **44-100** from inlet **44-102** to outlet **44-104**. A potential difference between A and B is used to attract and retain the DNA on a membrane **44-106**. By swapping to an electrolyte flow through channel **44-100** and changing the potential difference it is possible to load the DNA to the matrix in main channel **44-108**. The CE can then be performed.

[0319] Again one or more cleaning or condition controlling steps may be provided before CE is conducted.

[0320] A yet further configuration is shown in FIG. **45**. In this case, the arm **45-100** leading the sample into the main channel **45-102** where CE is performed extends downwards, at least partially aligned with gravity. The arm **45-104** leading away from the main channel **45-102** extends upward, at least partially aligned with gravity. In this way gravitation effects promote retention within the main channel **45-102**, rather than encouraging flow away from it and into another arm.

[0321] 5) Cartridge Variant for Real Time PCR Performance

[0322] In the cartridge **9** described above, the cartridge **9** is being used to consider a reference sample. In this alternative embodiment, the changes to the cartridge **5009** beneficial to the consideration of a casework sample are considered.

[0323] A major difference between a casework sample and a reference sample is that whilst the amount of DNA recovered in a reference sample has a degree of consistency, and is of a high level, this is not the case for a casework sample. The manner in which the sample is left, the passage of time, the collection process and other factors can all result in the amount of DNA in a casework sample being unpredictable, and often lower, than desired.

[0324] To counteract this, the casework sample processing seeks to ensure that the amount of DNA arising from the amplification process is within certain bounds.

[0325] To do this, the casework sample provides for parallel processing of the sample, particularly in terms of the sample amplification step **204**.

[0326] The sample receiving step **200** and sample preparation step **202** are basically the same as previously described. The difference comes in the sample amplification step **206**.

[0327] The channel **5410** containing the eluted DNA from the beads held in the incubation chamber **5358** leads to a junction **5700** where the flow is split into two separate streams **5702**, **5704**.

[0328] The first stream **5702** passes into a PCR chamber **416** of the type previously described (and is not illustrated further). The subsequent handling of this by the cartridge **9** is as described above, save for the possible changes in the sample amplification conditions/duration described shortly.

[0329] The second stream **5704** passes into a second separate PCR chamber **5706**. This second PCR chamber **5706** contains a bead provided with a coating containing the necessary regents for PCR and for a quantification analysis.

[0330] During processing, PCR is advanced in the PCR chamber **416** and in the second PCR chamber **5706**, in parallel. After a given number of PCR cycles for the second PCR chamber **5706**, the contents of the second PCR chamber **5706** are considered to establish the quantity of DNA which has been generated by the PCR cycles up to that point. This can be equated to the amount of DNA present within the original sample and hence the amount of DNA the PCR chamber **416** is working on. As a result of the quantification, the PCR conditions and/or cycle number for the PCR chamber **416** can be varied to optimise the quality of amplification product.

[0331] Further details on the operation of such a system and the use of this feed back are to be found in 61/026,869, the contents of which are incorporated herein by reference, particularly as they relate to the parallel conduct of PCR and the use of the results from one PCR to control and/or modify the conduct of the other PCR.

[0332] Suitable reagents include the Plexor HY kit available from Promega Inc, 2800 Woods Hollow Road, Madson, Wis. 53711, USA and Quantifiler® Duo DNA quantification kit available from Applied Biosystems, Foster City, Calif., 944404, USA.

[0333] To establish the quantity of DNA present, it is necessary to interrogate the sample using an excitation light source and then quantify the amount of light arising. To do this, light from a light source is conveyed to the second PCR chamber **5706** and focussed thereon using a lens system. The excitation light interacts with the dye(s) associated with the sample. The fluorescent light generated is detected and is proportional to the quantity of DNA present.

[0334] The light source used could be the same light source as is used for the electrophoresis step **206**, and described in detail below. The light would be conveyed to the second PCR chamber **5706** by an optical fibre. Because the Peltier heater/coolers are positioned in front of and behind the second PCR chamber **5706**, the light for the detection is introduced from the side of the cartridge **9**. The light source may be a laser, for instance of the type and/or with the set up discussed further below in the electrophoresis step **206**. As an alternative, however, it is possible to use a light emitting diode based light source, as described below.

[0335] Depending upon the quantity, the number of cycles used in the PCR chamber **416** may be increased, decreased or kept at the normal level, so as to provide a quantity of DNA within the desired range after PCR has been completed in PCR chamber **416**.

[0336] In the context of real time quantification and/or the handling of samples from crime scenes (rather than those taken under controlled conditions from individuals), differences in the implementation of the invention may be provided. These may include:

[0337] 1) The parallel processing of the sample so as to allow the results from a first processing of the sample to inform on the optimum conditions etc to be used in the main processing of the sample. Further details of such an approach are to be found in WO2009/098485, the contents of which are incorporated herein by reference with respect to the parallel processing and consideration of samples and the feedback of information from one processing to the other.

[0338] 2) The efficiency of the extraction should be as high as possible, for instance through optimised sample recovery, lysis and amplification. The use of various processes and/or reagents to separate the DNA of interest from problematic components, such as PCR inhibitors, is beneficial in this respect.

[0339] 3) The cartridge used will feature many of the steps and components exemplified above, but with the incorporation of the parallel PCR circuit and the ability to analyse the results therefrom, for instance using a laser or LED to apply light to the liquid, with the return light being detected to inform on the PCR process. Photo diodes and/or cameras can be used in the light detection. A control material may be provided within the sample to provide a reference value with respect to the light detected.

[0340] 4) The instrument would benefit from being able to run positive and/or negative controls. These could be run in the same cartridge as the sample. The controls may be handled by the operator in the same manner as the sample of interest so as to inform on contamination risks. The controls may just be run periodically so as to check on the instrument, for instance in the form of a calibration check.

Cartridge Components

[0341] Within the cartridge are a significant number of components, with each being optimised with respect to its role and its role in combination with the other components.

1) Valves

[0342] To minimise manufacturing costs and give consistent operation, all of the valves in the cartridge are one of two types. The two types are a closing valve **2000**; FIG. **10***a*; and an opening valve **2002**; FIG. **10***b*.

[0343] The closing valve **2000** is shown schematically in FIG. **10***a*. The closing valve **2000** is positioned above, relative to the direction of gravity, the channel **2004** to be closed. The closing valve **2000** is formed by a conduit **2006** which is in fluid communication with the channel **2004** and is in fluid communication with the bottom of a valve reservoir **2008**. The valve reservoir **2008** is filled with paraffin wax and is 3 mm in diameter and is provided with the conduit **2006**. On the top of the valve reservoir **2008**, a gas passage **2010** provides fluid communication with a valve gas reservoir **2012**. The valve gas reservoir **2012** is full of air.

[0344] The dotted line in FIG. **10***a* shows that part of the location of the closing valve **2000** which is in contact with a heater element, not shown, provided on the adjoining printed circuit board of the instrument.

[0345] When the closing valve **2000** is to be activated, the heater element is caused to heat up. This both melts the paraffin wax in the valve reservoir **2008** and causes the air in the valve gas reservoir **2012** to expand. The expansion of the air provides the driving force to displace the melted paraffin wax from the valve reservoir **2008** into the conduit **2006** and then into the channel **2004**.

[0346] The volume of paraffin wax displaced is controlled by the temperature to which the valve gas reservoir **2012** is heated (variation in pressure) and the duration of the heating applied (as the paraffin wax soon solidified once the heating is switched off).

[0347] Continued displacement of the paraffin wax into the channel **2004** causes the paraffin wax to expand in each direction along the channel **2004**.

[0348] In some cases, the fluid in the channel will not compress or move in one direction (or is limited in the extent possible) and so the flow of the paraffin wax within the channel **2004** occurs preferentially in the other direction. Normally, the paraffin wax is displaced into the channel **2004** until a 2 mm to 10 mm length of the channel **2004** is filled. With the heat removed, the paraffin wax sets in this new position and the channel **2004** is reliably sealed.

[0349] The section where the channel **2004** is to be shut, is deliberately chosen to be horizontal, relative to the direction of gravity, as this assists the retention of the paraffin wax at the location to be sealed.

[0350] To assist further in the formation of the seal, it is beneficial to arrange the closing valve so that it is between one or two upward, relative to the direction of gravity, bends. As shown in FIG. **10***a* the bend **2014** provides assistance in the accurate formation of the seal within the channel **2004**.

[0351] The opening valve **2002** is shown schematically in FIG. **10***b*. The opening valve **2002** is positioned as a part of the channel **2004** the fluid flows through. The opening valve **2004** is formed by a valve chamber **2020** which has an inlet **2022** from the channel **2004** in a first side wall **2024** and an outlet **2026** leading to the continuation of the channel **2004** in the opposing side wall **2028**.

[0352] The paraffin wax is positioned in the initial section **2030** of the valve chamber **2020**. Downstream of this initial section **2030**, is a trap section **2032**. The dotted line in FIG. **10***b* shows that part of the opening valve **2002** which is in contact with a heater element, not shown, provided on the adjoining printed circuit board of the instrument.

[0353] When the opening valve **2002** is to be activated, the heater element is caused to heat up. This melts the paraffin

wax in the initial section **2030**. By the time the paraffin is melted, or shortly thereafter, an electrochemical pump upstream of the opening valve **2002** has been activated for sufficient time to cause a pressure build up, upstream of the opening valve **2002**. This pressure causes the driving force to displace the melted paraffin wax from the initial section **2030** and downstream into the trap section **2032**. Once in the trap section **2032**, the passage **2034** above the paraffin wax is clear allowing fluid communication through the opening valve.

[0354] With the heat removed, the paraffin wax sets in this new position and the channel **2004** and passageway **2034** is reliably opened.

[0355] The section where the channel **2004** is to be opened is deliberately chosen to be horizontal, relative to the direction of gravity, as this assists the retention of the paraffin wax in the trap section **2032**.

[0356] In some applications, particularly those close to the high temperatures used in the PCR chamber, the valves benefit from using a high melting point wax. This melts at greater than 95° C. and so does not melt under PCR conditions. In some cases, the valve performance can be improved further by using a high melting point and lower melting point mixture; with the lower melting point wax tending to fill any cracks which form in the higher melting point wax.

[0357] A further valve embodiment is shown in FIG. **47**. The channel **47-100** is connected to the valve by a side channel **47-102** as usual. The side channel **47-102** leads to a first chamber **47-104**. This is connected via a short channel **47-106** to a larger second chamber **47-108**.

2) Chambers

[0358] Within the cartridge, a variety of chambers are provided for a variety of purposes. To achieve those purposes efficiently and effectively, the chamber designs are optimised in various ways.

[0359] With respect to the incubation chamber **358**, this is provided with a broad base which is generally horizontal. In operation, the offset magnet (not shown) is used to restrain the magnetic beads in position during washing and during elution. The broad base provides a suitable location to which the beads can be drawn and secured, whilst exposing them to the wash flow or to the elution flow.

[0360] The sloping walls within the incubation chamber **358** and the bubble mixing chamber **342** are provided to promote the flow of eluent, introduced into the chambers at the top, to the outlet at the bottom of the chamber.

[0361] The angular corners are used to generate improved pressure gradients from the inlet for a part of the process to the outlet in that respective part of the process.

[0362] The first further mixing chamber **332** and second further mixing chamber **336** are provided to encourage non-laminar flow within the flow route. As the fluid transitions from the channel, with its cross-section, to the chambers, with their increased cross-section, non-laminar flow arises. This gives good mixing for the different density fluids and particles which are all to be mixed. Such mixing forms are significantly better in this respect than bubble mixing alone or piezoelectric based mixing.

[0363] The PCR chamber **416** has two principle embodiments; as described above. In each, the PCR regents are provided within the degradable shell of a bead located within the PCR chamber **416**. To ensure proper flow of the liquids around and past the bead, the bead is provided with a bead seat. This provides a defined rest position for the bead, but as the bead is only contacted at discrete locations when in the seat, fluid is still able to flow past the bead. The seat ensures that the bead does not block at inlet to and/or outlet from the PCR chamber **416**. The seat ensures that there are no large areas of the bead surface, and hence of the reagents, which are isolated for fluid contact.

[0364] In the second of the PCR chamber **416** embodiments, described in the alternatives for the cartridge section, the PCR chamber **416** is completely filled with fluid. This gives a reproducible volume of fluid in the PCR process. The same position arises with the third embodiment, FIG. **22**.

[0365] In the first of the PCR chamber **416** embodiments, the maximum level of fluid within the PCR chamber **416** is controlled by the relative height of the outlet within the chamber. The outlet in effect acts as an overflow for the fluid, once the PCR chamber **416** has filled to this level. A head space remains above the fluid, within the PCR chamber **416**.

3) Vents

[0366] To allow fluid flow, air or sample, around the cartridge **9**, various vents need to be provided for various chambers.

[0367] To prevent any risk or suggestion that material can enter the cartridge **9** through such vents, each of the vents is provided with a filter element to exclude particulate material. In addition, when a vent is part of the active processing on the cartridge **9**, the vent is under positive pressure and so air is flowing out through the vent. This too assists in preventing any risk of particulate material entering the cartridge **9**.

[0368] In some situations, it is desirable to be able to allow air to pass through the vent freely, but for the vent to resist the passage of any subsequent liquid. An example is to be found in the alternative PCR chamber **416** filling embodiment. To provide this, those vents are hydrophobic. The vent may be hydrophobic because of the base material forming the vent and/or because of a treatment applied to the material of the vent. Such a treatment can be provided, for instance, by using polypropylene material and/or by providing a polysulphone coating.

4) Archive

[0369] As described above, the fluid not needed in the PCR chamber **416**, is pumped onward to an archive chamber **422**.

[0370] The purpose of the archive chamber **422** is to provide a storable record of the sample supplied to the sample amplification stage **204**, and the PCR chamber **416** in particular.

[0371] If needed, the sample in the archive chamber **422** can be accessed at a later date to enable a further amplification and analysis to be performed. Further processing in this way is useful where it is necessary to repeat the analysis, for instance by way of verification. Alternatively, further processing enables a different amplification and analysis protocol to be applied, for instance, a protocol suitable for low levels of DNA within the sample.

[0372] In the form shown in FIG. **3**, the archive chamber **422** is an integral part of the overall cartridge **9**.

[0373] In an alternative, form shown in FIG. **11**, the archive chamber **2422** is still fed the surplus sample through a channel **2418** leading away from the PCR chamber, not shown.

[0374] The archive chamber **2422** is positioned on a stub **2750** which extends from the side of the cartridge **9**. The stub **2750** is connected to the cartridge **9** during normal use, but a

line of weakness **2752** is provided. This allows the stub to be snapped off the cartridge **9** after the completion of the processing. This means the archive function can be provided by only storing the stub **2750**, rather than have to store the far larger overall cartridge **9**. Given the number of samples which may be considered, and the time for which they have to be stored, saving of storage space is a significant issue.

[0375] To seal the archive chamber **2422**, once it has been loaded, a closing valve **2754** is provided on the cartridge **9** side of the line of weakness **2752** and a further closing valve **2756** is provided on the stub **2750** side of the line of weakness **2752**. These valves are activated to place paraffin wax in the channel **2418** on either side of the line of weakness **2752**. To provide for long term storage, a further closing valve **2758** is provided on the channel leading from the archive chamber **2422** to the vent **2424**.

[0376] Just as the cartridge **9** is provided with an identifier, which is used to link it in the records to the sample loaded upon it, then the stub **2750** is also provided with a common identifier so as to maintain the link after the stub **2750** is broken off the cartridge **9**.

5) Reagents

[0377] Various options exist for the provision of the reagents needed in the various steps of the processing. As far as possible, so as to keep the processing as simple as possible for the user, the cartridge **9** is provided with pre-loaded reagents. Examples of such pre-loaded reagents would include the bead provided in the PCR chamber **416**; with the bead carrying the PCR regents inside. Other pre-loaded regents include the various wash liquids and elution liquids described in the methodology above.

[0378] If necessary, one or more reagents can be provided separate from the cartridge **9**, and be loaded onto the cartridge at or close to the time of use. This may be necessary where the reagent is unable to withstand prolonged storage under the conditions to which the cartridge **9** is exposed. These may be conditions of temperature and/or mechanical conditions such as vibration or orientation.

[0379] A preferred form of reagent provision is provided where the reagent(s) are provided as part of a solid phase reagent or solid phase reagent storage component, with release of the reagent being triggered by an increased temperature. Gel forms of reagent and/or reagent storage component, preferably triggered to release by the application of higher temperatures are also a useful option.

6) Electrochemical Pumps

[0380] To simplify the construction and costs of the cartridge, a common approach is used to providing the motive power to the various operations on the cartridge; electrochemical pumps. Each of the electrochemical pumps consists of a pair of electrodes immersed in the electrolyte. The flow of a current results in off gassing. The off gas collects in the top of the electrochemical pump, increases in pressure and leaves the pump via the outlet in the top of the pump. This off gas pushes ahead of itself other fluids encountered in the channels and chambers. The off gas contributes to bubble mixing in some of the stages.

[0381] To give a desired extent of pumping, the volume of the electrochemical pump can be varied. The extent of pumping can be delivered in one, two or more goes, as turning off the current stops the pumping action.

[0382] The rate of pumping and/or pressure delivered can be varied by varying the molarity of the electrolyte. Sodium chloride is the preferred electrolyte; used at 1M; and used in conjunction with aluminium electrodes.

7) Electrophoresis Matrix

[0383] The material provided within the capillary of the electrophoresis stage is important to the reliability and resolution of the analysis obtained.

[0384] Various possible materials can be used in the capillary. These include the use of polymer matrix, for instance a polyhydroacrylamide, a polydimethylacrylamide or mixtures there of. The polymers may be cross-linked to give the desired properties and/or formed into their state of use within the capillary, after loading. It is also possible to use an inert bed of particulate material to form the matrix in which the size based separation is achieved.

[0385] As well as optimising the performance through the properties of the gel, it is also possible to treat the capillary walls to improve properties. For instance it is possible to apply hydrophilic coatings, such as poly(hydroxyethlacrylamide).

[0386] A potential methodology for the electrophoresis matrix is to store that material in a chamber which is a part of the CE chip, but not use that chamber for the CE separation. Instead, when required for use, the stored matrix is moved from the chamber into the capillary so as to fill it to the desired degree. As a result of loading just before use, the matrix is no subject to sedimentation effects; these can have a detrimental effect on the analysis. Pressure loading can be used for this purpose.

[0387] Another potential methodology is to fill the main channel and arms of the CE chip with the matrix. Those parts of the CE chip where the matrix is not needed, for instance aside from the main channel, may be masked. In this way, when UV light is applied the parts where the matrix is not needed retain the matrix unaltered. The unaltered matrix can be washed away. Where the matrix is exposed to UV light it is altered and resists washing away.

8) CE Chip Design

[0388] A preferred configuration for the CE chip is shown in FIG. **42***a* and the detailed partial view of FIG. **42***b*.

[0389] The end portions **42-100** cooperate with the carrier when the chip is mounted within it. The external profile of the base of the CE chip is designed to match with that defined by the raised surface around the CE chip heater board, described elsewhere in this document.

[0390] As described below, a number of electrodes are required in different parts of the channels provided within the CE chip so as to load the sample and then perform the necessary separation to give the analysis. These electrodes within the channels are connected to pins **42-102** which extend above the plane of the CE chip. These pins **42-102** are positioned so that they are within the cut away portion of the second support and so are exposed. This allows suitable connections to be made to these pins **42-102** so as to apply the necessary voltages to them and to the electrodes connected to them.

[0391] The CE chip is shown with a single channel in which CE is performed, but channels suitable to perform separations on multiple samples could be provided.

9) PCR Chamber Sealing

[0392] In the embodiments described elsewhere, the chambers and the valves which are used to seal the channels leading to and from them are separate. In the following embodiment, the chambers and the valves are integrated as a single component.

[0393] As shown in FIG. **41***a*, the PCR chamber **41-100** is provided in the cartridge. However, the walls defining the circumference, at least, of the chamber **41-100** are rotatable within the body of material forming the cartridge. In the lefthand form, the rotatable wall is positioned such that the holes therein are aligned with the inlet channel **41-102** and the loading outlet channel **41-104**. As a result, liquid can enter and gas leaves the chamber **41-100** until the chamber is full, centre form. The rotatable wall can then be rotated to align the holes therein with the inlet channel **41-102** and the dispense outlet **41-106**, right hand form, to allow the contents to be emptied.

[0394] A variant of this approach is shown in FIG. **41***b*, where inlet channel **41-100** is connected to outlet channel **41-108**. Rotation aligns the holes with dispense inlet **41-110** and dispense outlet **41-106**.

[0395] The variant in FIG. **41***c* uses the arrangement to seal the chamber during PCR. In the left hand form, the inlet channel **41-102** is connected to and fills the chamber up to the level of the outlet channel **41-108**. Partial rotation offsets the holes in the rotating wall from alignment with any of the inlets/outlets, centre form. After PCR, further rotation aligns the holes with the dispense inlet **41-110** and dispense outlet **41-106**.

[0396] The extent of rotation may be limited by abutment surfaces provide in the cartridge wall which abut surfaces on the rotating walls or vice versa. Partially circular forms for the hole in the cartridge which receives the rotating walls and/or vice versa may also be used to control or limit rotation in one or both directions.

[0397] Rotation may be provided by cooperation between an actuator and a slot in the circular wall.

[0398] Rotation may cause pads or other pliable material to be compressed or otherwise deformed to give sealing.

[0399] One or more of the channels may serve as a light path, rather than or in addition to being a fluid flowpath, so as to allow an investigatory instrument to shine light into the liquid contained within the chamber. Such an embodiment is useful in the context of the cartridge variant for real time PCR discussed above.

Instrument Configuration and Appearance

[0400] The instrument **11** is illustrated in FIG. **12** and is provided within a casing **8000**. The mid section **8002** of the instrument **11** is provided with a door **8004** provided with a latch **8006**. Behind the door **8004** is the location at which the cartridge **9** is mounted in use. This location is a position in which the plane of the cartridge **9** is parallel to the plane of a printed circuit board **8008**. At the location, the cartridge **9** and components on the printed circuit board **8008** contact one another.

[0401] Behind the printed circuit board **8008** are the electronics for operating and controlling the components provided on the printed circuit board **8008**. These include the power supplies, voltage controllers, temperature controllers and the like.

[0402] The upper section **8010** of the instrument **11** provides the display **8012** by means of which the user inputs information into the instrument **11** and receives visual information from the instrument. The software and hardware for operation of the display **8012** are provided on a computer positioned behind the display screen **8012** in the upper section **8010**.

[0403] The lower section **8014** of the instrument **11** contains the high voltage power supply and controller for the laser used in the inspection of the capillary electrophoresis. Also in this lower section **8014** are the charge couple device used to sensor the fluorescence and the optics for conveying the light to and from the capillary.

[0404] Another embodiment of the instrument is shown in FIGS. **29***a*, **29***b* and **29***c*. The instrument **29-11** is provided within a casing **29-8000**. The upper section **29-8002** of the instrument **11** is provided with a door **29-8004**. The door **29-8004** is a combination of a top section **29-8006** and front section **29-8008** of the casing **29-8000**.

[0405] The lower section **29-8010** of the instrument **11** provides the display **29-8012** by means of which the user inputs information into the instrument **11** and receives visual information from the instrument **11**.

[0406] The window **29-8014** allows for visual inspection of the cartridge used. A series of light bars **29-8016** are used to indicate the extent of progress through the steps involved; the more of the bar which is lit the greater the extent of the step performed.

[0407] A stylus **29-8018** is used by the operator to interact with the display **29-8012**.

[0408] Various control buttons **29-8020** are provided below the screen **29-8012**.

[0409] The overall dimensions of the instrument are width, W, 419 mm, overall height, OH, 621 mm, depth, D, 405 mm.

[0410] The side panel **29-8022** is removable for maintenance purposes.

[0411] The embodiment of FIG. **30** shows the door **30-8004** structure more clearly, together with the workspace **30-8024** that is accessed through it. The workspace **30-8024** includes the slot into which the cartridge carrier **30-8026** is inserted. The cartridge carrier **30-8026** is as described elsewhere in this document. The workspace **30-8024** also includes the lane finding apparatus **30-8028**.

[0412] The cover **30-8030** in the side panel **30-8032** is opened by rotation to allow access to the optics for maintenance purposes.

Cartridge to Instrument Interface

[0413] As described above, once the cartridge **9** is loaded with the sample, the cartridge **9** is loaded into the instrument **11** for the processing to be conducted.

[0414] As a first step, the latch **8004** is released and the door **8002** is opened.

[0415] To insert the cartridge **9**, FIG. **13**, the section of the cartridge **9** which bears the PCR chamber **416** is inserted into a slot **8023** between the components which will control the PCR process. These components include the thermoelectric heaters/coolers, Peltier devices **8025**, and fans **8027** there for. These components are free to travel to a limited extent to help with the locating of the cartridge **9** within the slot **8023**, whilst

being forcibly returned to the optimum position after insertion so as to give effective heating/cooling.

[0416] The cartridge **9** is provided with a series of recesses which cooperate with dowels extending through the printed circuit board **8008** to accurately register the cartridge **9** relative to the printed circuit board **8008**. The dowel arrangement is such that the cartridge **9** cannot be fitted the wrong way round.

[0417] Once positioned, the cartridge **9** is provided in a plane which is parallel to the plane of the printed circuit board **8008**. Both components have flat surfaces facing one another so as to assist with the good contact needed between them.

[0418] The closing of the door **8002** and operation of the latch **8004** applies a compressive force to the cartridge **9** by way of a series of spring loaded pins mounted on the inside surface of the door **8002**. This helps hold the cartridge **9** in firm contact with the printed circuit board **8008**.

[0419] The printed circuit board **8008** is important to the successful operation of the invention. It provides the energy sources for the various components to be driven on the cartridge **9**. In effect, the drivers are all provided in the cartridge **9**, but the energy sources are provided on the printed circuit board **8008**. In this way, the precision operation needed is ensured by the expensive and bespoke electronics and arrangement of the printed circuit board **8008**; a reusable component of the instrument. In this way, the cartridge **9** is simple and self-contained. This reduces the complexity of the interface between the two and also removes the risk of contamination of the contents of the cartridge **9**. The only transfer between the printed circuit board **8008** and the cartridge **9** is conducted and radiated heat from the heaters and the magnetic field provided by the magnet.

[0420] The components provided on the printed circuit board include:

[0421] a) The electrical contacts **9000** which connect to the pins of the electrochemical pump electrodes on the cartridge **9**. These provide the electrical power, when needed, to operate the electrochemical pumps.

[0422] b) The electrical heaters **9002** which are used to apply heat to the valves on the cartridge so as to open or close the valves depending upon their type. These are square areas of resistance heating material which is applied by printing a paste to the desired location. The heating effect is improved if the square block is rotated through 45° relative to the axis of the channel subject to the valve.

[0423] c) The magnet **9004** which is advanced into proximity with the cartridge **9** when it is desired to retain the beads and prevent them from moving. The magnet **9004** is retracted away from the cartridge **9** when it is desired to release the beads within the chamber **358**.

[0424] d) The sensors **9006** are providing feed back and/or verification of the conditions induced by the heaters etc.

Alternatives for Cartridge to Instrument Interface

[0425] If it is necessary to alter or improve the contact between the cartridge and the printed circuit board, there are various options for doing so, including the following:

[0426] a) The loading provided by the sprung pins mounted on the door **8002** can be increased. This applies a force to the cartridge **9** and pushes it against the printed circuit board **8008**.

[0427] b) The cartridge **9** can be mechanically clipped to the printed circuit board **8008**, with the clip(s) applying a compressive force.

[0428] c) The cartridge **9** can be provided with a compressible substrate mounted on the surface which is intended to contact the printed circuit board. In this way, when then cartridge **9** and printed circuit board **8008** are pushed together, the substrate will provide good all over contact. The substrate can be a solid material, paste or even a liquid. The materials of the substrate, or parts there of, are selected so as to provide maximum thermal conductivity, for instance. Particles, nanoparticles or other materials may be added to alter the properties. The substrate may be protected, prior to use, by a peelable backing.

[0429] d) As described above, the components (such as heaters etc) are provided in a fixed position on the printed circuit board **8008**. This means they move with the printed circuit board **8008**. It is possible to provide one or more, and even each of these components with a degree of independent movement. For instance, they may be provided with a sprung mounting on the printed circuit board. In this way, each is able to independently adjust its position, forward and backwards, relative to the cartridge.

[0430] e) As shown in FIG. **23**, it is possible to provide the section of the cartridge **9** which bears the PCR chamber **416** in opposition to stacked components which will control the PCR process. In this example, the stack includes a first Peltier device **23-01** in contact with the cartridge **9** and in contact with and aligned with a second Peltier device **23-03**. The stacking of the devices allows high temperatures, for instance greater than 150° C. to be obtained within the PCR chamber. Such temperatures are beneficial in terms of melting the high melting point wax seals described elsewhere within this document.

[0431] f) Alternative forms of heater may be used instead of Peltier effect device. For instance infra red heating devices may be used. The material around the PCR chamber, or a part of that material, may be capable of resistance heating to give the necessary heating for the chamber. Resistance heaters positioned against the cartridge may be used. Microwave heating may be used.

Alternative Cartridge to Instrument Interface

[0432] In the alternative embodiments of the instrument described above in relation to FIGS. **29***a, b, c* and FIG. **30**, the cartridge is not loaded directly into the instrument. Instead, once loaded with the sample, the cartridge **31-01** is loaded into a cartridge carrier **31-03**.

[0433] The use of the carrier **31-03** means that the cartridge **31-01** and the CE chip can be constructed separately. This allows different material and/or different production tolerances to be used for the different components; a beneficial effect on cost and/or performance and/or the balance between those can thus be provided.

[0434] The carrier **31-03** also allows for easy assembly of the required components and their insertion into the instrument in a unitary form. At the same time, the carrier is designed so as to allow separate alignment checking and adjustment for the cartridge and the CE chip so that both are in their correct, optimised position within the instrument.

[0435] If desired, the cartridge position can be checked and any alignment adjustment necessary can be made. Before CE

starts, a separate check can be made on the alignment of the CE chip, within any adjustments it needs being made before CE starts.

[0436] The cartridge carrier **31-03** is illustrated in FIG. **31***a*. The cartridge carrier **31-03** includes a first support **31-05** and a second support **31-07** which is perpendicular to the first support **31-05**.

[0437] The first support **31-05** is used to carry the cartridge **31-01**. The second support **31-07** is used to carry the capillary electrophoresis, CE, chip; this interaction is described further below.

[0438] The prepared cartridge **31-01** is presented with its face **31-09** to the face **31-11** defined by the first support **31-05**. An externally threaded screw **31-13** provided at each corner of the first support **31-05** is received into an opposing aperture **31-15** provided at each corner of the cartridge **31-01**. Rotation of the screws **31-13** causes them to engage with and enter an internal screw thread provided in the apertures **31-15**. Further tightening mounts the cartridge **31-01** on the first support **31-05** and hence the carrier **31-03** in a secure and known position.

[0439] The interaction between the cartridge **31-01** and the carrier **31-03** is shown in more detail in FIG. **31***b* in relation to one of the screws **31-13**.

[0440] The screw **31-13** is provided with a knurled head **31-17**. The threaded engagement occurs between the end **31-19** of the screw **31-13** and the aperture **31-21** in the cartridge **31-01**. A jam nut **31-23** in cooperation with a washer **31-25** serves to hold the screw **31-13** on the carrier when not engaged with a cartridge **31-01** The jam nut **31-23**. washer **31-25** and sleeve **31-27** serve to prevent over tightening between the carrier **31-03** and the cartridge **31-01**.

[0441] Rotation of the screw **31-13** pulls the knurled head **31-17** and the cartridge **31-01** closer together. This causes compression of the conical spring **31-29** between the knurled head **31-17** and an abutment surface **31-31** on the first support **31-05**. The spring **31-29** assists in ensuring correct alignment during tightening. Once rotation is finished, the first support **31-05** and hence carrier **31-03** is in a known position relative to the cartridge **31-01**.

[0442] The CE chip **32-31** is inserted into the carrier **32-03** as shown in FIG. **32***a*. The CE chip **32-31** is slid into a slot. As shown in FIG. **32***b*, the second support **32-07** provides such a slot **32-33** at either end for receiving the end portions **32-35** of the CE chip **32-31**. An incline **32-37** on the lead edge **32-39** of the CE chip **32-31** engages with the end **32-41** of a spring loaded plunger **32-43** and causes it to displace outward, arrow A. Once the recess **32-43** is presented to the end **32-41** of the plunger **32-43**, the plunger **32-43** returns, arrow B, and so prevents onward movement of the CE chip **32-31** past the desired position.

[0443] Once the cartridge **31-01** and the CE chip **32-31** are inserted into the carrier **31-03**, **32-03**, the fluid connection between the two is provided by a tube **33-45**. The insertion of the cartridge **31-01** into the carrier **31-01** causes the electrophoresis step inlet **28-570** on the cartridge **31-03** (see FIG. **28***a*) to become connected to the tube **33-45**. As shown in FIG. **33***a*, the tube **33-45** extends upward, parallel to the plane of the cartridge **31-01** and the first support **31-05** through an opening **33-47** in the carrier **31-03**. As shown in FIG. **33***b*, once through the opening **33-47**, the tube **33-45** makes a 90° turn into the plane of the second support **31-07** and the CE chip **32-31**. The tube **33-45** is accommodated within the second support **31-07** above the CE chip **32-31**. A further 90° turn leads the tube **33-45** into the CE chip **32-31**. The remaining fluid transport is handled within the CE chip **32-31** itself, as described elsewhere in this document.

[0444] After insertion of the cartridge **31-01** and the CE chip **32-31** into the carrier **31-03**, as described above, the carrier **31-03** is ready for insertion.

[0445] As a first step, the door **34-8004** is opened, FIG. **34***a*, to expose the workspace **34-8024**. The work space **34-8024** includes the slot **34-47** that the carrier **34-03** is inserted into.

[0446] The carrier **34-03** is inserted into the slot **34-47** until the second support **34-07** comes to rest on the surface **34-49** of the workspace **34-8024**. The cooperation of the carrier **34-03** with the slot **34-47** ensures the correct general positioning of the cartridge **34-01** with respect to the instrument, both in terms of lateral and vertical positioning; FIG. **34***b*.

[0447] Insertion in this way provides the section of the cartridge which bears the PCR chamber between the components which will control the PCR process; as described further below.

[0448] Once inserted, the door **34-8004** is closed. The closing of the door **34-8004** triggers various actions based upon contact between the closed door **34-8004** and casing. The clamping of the cartridge to the PCB, the positioning of the CE chip on the CE chip heater board, the introduction of the electrical contacts to the pins provided on the CE chip, the introduction of the electrical contacts to the pins providing the conduction path to the electrodes in the electrochemical pumps are all triggered in this way. The closure of the door **34-8004** is also used to turnoff the interlock for various safety systems within the instrument. The interlock prevents, for instance, the laser being active with the door or any other opening in the instrument's casing being open. a similar principle applies to the power supplies within the instrument.

[0449] As with other embodiments, it is important to provide effective and accurate contact between the cartridge and the instrument interface. In FIGS. **35***a, b* and *c* the provision of the contact is illustrated.

[0450] FIG. **35***a* shows the carrier **35-03** in position in the slot **35-47**. In the insertion position, as shown, the arrangement provides for a gap **35-51** between the face **35-53** of the cartridge **35-01** which opposes the face **35-55** of the printed circuit board **35-57** of the instrument.

[0451] In the next step, FIG. **35***b*, the cartridge **35-01** is moved into the use position. A platen **35-59** is moved, direction of arrows, by an actuator, not shown. This causes the cartridge **35-01** to be brought into full contact with the PCB **35-57**. The movement is such that the conical spring **35-29** is further compressed. During this movement, a series of rods which extend through the PCB **35-37** enter various holes (**27-13** in FIG. **27**) and so ensure that the alignment between the cartridge and the PCB is correct in that orientation too.

[0452] When the use of the cartridge **35-01** has finished, then the force applied to the platen **35-59** by the actuator is released. As a result, the carrier **35-03** is returned to the insertion position by return springs, not shown. The release causes the conical springs **35-29** to pull the cartridge **35-01** back into position inside the carrier **35-03**, FIG. **35***c*. The carrier **35-03** can then be removed by lifting it out of the slot **35-47**, taking with it the cartridge **35-01**.

[0453] The face to face contact between the cartridge and the PCB provides the majority of the interactions between the cartridge and the instrument, for instance, heating for valve control, sensor etc. The contact between the PCR chamber

and its temperature cyclers are provided through further components, however; see FIGS. **36***a, b, c* and *d*.

[0454] In FIG. **36***a*, the cartridge **36-01** is shown inserted into the slot provided in the instrument. Once inserted, the section of the cartridge **36-01** bearing the PCR chamber is positioned between a pair of calipers **36-100**. The PCB is cut away at this location so as to not be in the way of the Peltier effect devices **36-102**, **36-108** and pair of calipers **36-100**. The calipers **36-100** are floating such that they do no interfere with the contact sought between the cartridge **36-01** and the PCB during the movement from the insertion position to the use position.

[0455] The front caliper **36-100***a* is provided with a Peltier effect device **36-102** mounted on a support **36-104** which is capable of reciprocating movement, arrow C, under the control of actuator **36-106**. The actuator **36-106** is also mounted on the pair of calipers **36-100**.

[0456] The back caliper **36-100***b* is provided with a second Peltier effect device **36-108** mounted fixedly on the caliper **36-100***b*. The second Peltier effect device **36-108** is provided in opposition to the Peltier effect device **36-102**.

[0457] In the open position shown in FIG. **36***c*, such as is provided with the cartridge in the insertion position, the distance between the opposing faces **36-110**, **36-112** of the Peltier effect device **36-102** and the second Peltier effect device **36-108** is more than the thickness of that section of the cartridge **36-01** and more than the thickness of the carrier **36-03** which passes between the pair of calipers **36-100** during insertion of the carrier **36-03**.

[0458] In the closed position shown in FIG. **36***d*, such as is provided during the amplification step, the distance is reduced. This is achieved by the actuator **36-106** moving the Peltier effect device **36-102** on the front caliper **36-100***a* towards the cartridge **36-01** and towards the opposing second Peltier effect device **36-100***b*. This actuation, combined with the floating nature of the pair of calipers **36-100** brings both of the Peltier effect devices into firm contact with the cartridge **36-01** on opposing sides thereof. They are now in position to provide the necessary heating and/or cooling for the PCR step.

[0459] Thermocouples to sense the temperatures applied, and potentially to be used to control the temperatures applied, are provided in close proximity with the Peltier effect devices, embedded in copper shims, bonded to the Peltier effect devices.

[0460] Before the carrier **36-03** is removed, the actuator **36-106** returns the Peltier effect devices **36-100** to the open position.

[0461] In addition to the carrier allowing for relative movement of the cartridge to ensure correct positioning with respect to the PCB, the carrier also allows for totally independent relative movement of the CE chip. This is importing in ensuring correct positioning of the CE chip for the CE step. This is achieved by the structure and operation shown in FIGS. **37***a* and *b*.

[0462] As the carrier **37-03** with the CE chip **37-31** in it is inserted into the slot in the instrument, the second support **37-07** approaches the work surface **37-49**. The work surface **37-49** carries a CE chip board heater **37-100** in the form of a planar surface. this is surrounded by a raised surface **37-102** which provides a nest for the CE chip **37-31** once positioned.

[0463] Projecting pins **37-104** on the work surface **37-49** enter apertures **37-106** provided in the second support **37-07** of the carrier **37-03**; FIG. **37***a*. In FIG. **37***b*, the top part of the second support **37-07** is shown cut away so that the full extent of the CE chip **37-31** can be seen. The apertures **37-106** in the second support **37-07** align with the slot **37-108** which receives the end portions **37-108**, **37-110** of the CE chip **37-31**. As a result, the end portions **37-108**, **37-110** are also provided with through apertures **37-112***a*, **37-112***b*. The projecting pins **37-104** thus pass through these apertures **36-112***a*, **36-112***b* too as the carrier **37-03** approaches the work surface **37-49**.

[0464] The conical ends of the pins **37-104** mean that they enter the apertures **37-106**, **37-112***a, b*, even where there is potential misalignment. The fuller diameter parts of the pins **37-104** encourage the CE chip **37-31** into the correct position. The CE chip **37-31** is centred to the CE chip board heater **37-100** as a result. The CE chip heater board **37-100** and raised surface **37-102** can be seen clearly in FIG. **38**.

Electrophoresis Components

1) Optics

[0465] In the electrophoresis step **206**, at the detection location **628**, light from a laser **800** is focussed to be incident upon the fluorescent dye associated with a DNA element to make it detectable.

[0466] A different dye is used for each different DNA element type; a type is generally associated with a given locus.

[0467] To get good sensitivity, it is important for the incident light to be of sufficient intensity for the detectors to receive sufficient light to be sensitive to the emitted fluorescent light, but for the intensity not to be so high as to give rise to photobleaching of the dyes. To provide for this, the following arrangement is used; FIG. **14**.

[0468] The light source is a compact laser **900** which is mounted on a heat sink **902**. The laser **900** is a Cobolt Calypso laser (from Cobolt AB, Kraftriken **8**, SE-104 05, Stockholm, Sweden) and emits at 491 nm with a maximum power of 50 mW. The light emitted by the laser **900** is fed to a fibre coupler **904** (09 LFC 001, f=3.5 mm from Melles Griot, 2051 Palomar Airport Road, 200, Carlsbad, Calif. 92011, USA) and hence into an patch cable assembly (M31L01, from Thorlabs, 435 Route 206 North, Newton, N.J., 07860, USA) and optical fibre **906** (GIF625, dia 62.5:m, NA=0.275 from Thorlabs, 435 Route 206 North, Newton, N.J., 07860, USA).

[0469] The use of the optical fibre **906** is beneficial as it safely controls the laser light direction, enables the laser light to be easily conveyed to the position of use and enables mechanical stability to be provided within the overall system. At the end of the optical fibre **906** a power of up to 45.32 mW is still observed.

[0470] The laser light then passes through a collimator **908** (F230FC-A, F=4.5 mm, NA=0.55, from Thorlabs) and a log-pass filter with a sharp cut-off wavelength, EM filter (Omega Optical XF3093, T50=515 nm) before reaching the spot mirror **910**.

[0471] The spot mirror **910** is used to both direct the laser light to the detection location **628** of the capillary and to transmit, anisotropically and without filtering, the fluorescent light received there from to the detector unit. It is angled at 45° to the beam of laser light. To do this, the reflector **910** consists of a 25 mm round glass disc which transmits all light from <80 above 380 nm. An ellipse, 2 mm long by 1 mm wide, is provided at the centre of the reflector **910** (so as to present an effective 1 mm circular mirror), formed of a highly reflective mirror layer deposited there (reflectivity of 99.99%).

[0472] Before reaching the detection location **628**, the laser light passes through a focussing lens **912**. This can be a microscope optic or other such adjustable focussing lens. Such optics are useful as they introduce no optical aberrations to the light, shape the beam for application to the detection location **628** and don't give any selective loss of light colours. The power reaching the detection location **628** is over 27.40 mW.

[0473] The fluorescent light is effectively scattered from the dye in the capillary **616** in all directions. For the fluorescence light to reach the detector unit, that light needs to hit the spot mirror **910** at a location outside of the glass spot. If it does so, the light is transmitted into the detector unit **914**.

[0474] The detector unit **914** includes a slit in front of a spectrometer to obtain diffraction-limited incident light, the spectrometer provided with a diffraction grating and a lens **918** (LA1608A plano convex, f=50 mm, D=25 mm, with anti-reflective coating within 350-650 nm, made of BK7 glass, Thorlabs Inc), to direct the light to the charge coupled device **916**. The CCD **916** has spectroscopic abilities.

[0475] The CCD **916** generates the signals which are then used to generate the electropherogram, an example of which is shown in FIG. **15**

[0476] Using such an approach, a sensitivity approaching that of laboratory style electrophoresis instruments can be reached. The instrument is able to detect down to the presence of 2.5 pM of fluorescein dye at pH 7.

[0477] In an alternative approach, certain problems with the stability of the fibre optics can be avoided by providing an open beam approach to delivering the light from the laser to the channel.

[0478] An alternative embodiment of the optics is shown in the cut away perspective view of FIG. **39**. The instrument casing **39-01** provides various mounts for the optics. The light is generated by the laser head **39-03** operated under control by the laser controller **39-05**. The light enters the optics **39-07** and is directed at the channel in the CE chip, not shown, mounted in the CE chip heater board **39-09**.

[0479] The return light enters the optics **39-07** and is directed back to the spectrometer **39-11** and CCD camera **39-13**. Above the CE chip heater board **39-09** is the chip alignment structure **39-15** which is described further below.

2) Calibration and Verification for Optics

[0480] When first using the optics for detecting the electrophoresis results, and periodically thereafter, it is beneficial to ensure that the optics are properly calibrated to the capillary **616** at the detection location **628** in the electrophoresis cartridge section. This ensures best transmission of the excitation light into the detection location **628**, best recovery of the fluorescence light from the dyes encountered at the detection location **628** and the performance of the detection at the detection location **628** (and hence at the correct distance from the point at which the sample is injected).

[0481] To achieve these aims, the electrophoresis cartridge section is provided with various aids. These are intended to allow automated verification and calibration of the position by the instrument **11**.

[0482] Firstly, a fixed marker is provided on the electrophoresis cartridge section, a known distance along the capillary **616** and a known distance perpendicular to the capillary **616**, from the detection location **628**. When the laser light is incident upon the fixed marker, a response is detected by the CCD **916**. The position of the incident laser light is thus known. The incident position of the laser light along the capillary is thus correct. The known distance of the fixed marker from the detection location **628**, perpendicular to the capillary **616** can then be used to adjust the position at which the laser light is incident so as to correspond with the detection location **628**. X and Y axis verification of the incident laser light position corresponding with the detection location **628** is thus provided. The marker could be a physical mark (for instance etched) on the cartridge and/or a coloured mark (for instance a dye) and/or a quantum dot.

[0483] To provide for the verification on the Z axis, the working distance between the lens and the capillary **616**, a known source, with a known characteristic is provided on the electrophoresis cartridge section at a known Z axis distance relative to the correct Z axis distance of the capillary **616**. By adjusting the focus of the lens so as to maximise the response by the CCD **916**, the correct working distance for the known source is established. An adjustment can then be made to reflect the relative working distance for the known source relative to the capillary **616**. Ideally, these are in the same plane at the same working distance so as to allow the known to provide direct verification for the Z axis position relative to the capillary **616**.

[0484] As an alternative means of verification on the position, it is possible to use the marker for the X axis and then use variation in transmission to check the Y axis position. Thus a marker is used to determine the correction position along the axis of the capillary **616**. The adjustment can then scan in the Y axial direction are use the CCD (or another detector) to consider the variation with position. The reflected signal will be constant at a level when the laser light is incident on the cartridge away from the capillary. When incident light traverses the capillary **616**, then the signal will vary in a predictable manner, so allowing the position to be set subsequently at the position corresponding to the middle of the capillary **616** in the signal. To assist in this, it is possible to introduce a polariser insert for the calibration part of the process so as to increase the observed variation in the signal. The polariser is removed before the actual electrophoresis results collection starts. The effect whose variation is detected can arise from the capillary **616** itself, a marker at a known distance from the capillary **616** or a material present in the capillary **616** (for instance, a dye labelled component provided as part of a sizing standard, whose mobility is higher than the other elements of the size standard or unknown elements).

[0485] The FIG. **39** and FIGS. **40***a, b* and *c* embodiment shows the alignment structure **39-15** and its operation.

[0486] The alignment structure **39-15** is in the form of a swing arm **40-100** which can be pivoted relative to the casing **40-102** under the power of an actuator contained within the swing arm **40-100**. The other end of the swing arm **40-100** is provided with a camera **40-104**.

[0487] In the stowed position, FIG. **40***b*, the swing arm is positioned in contact with a hard stop **40-106** mounted on the casing **40-102** too. In the check position, FIG. **40***c*, the actuator has caused the swing arm **40-100** to swing away from the casing **40-102** and so position the camera **40-106** over the channel **40-108** in the CE chip **40-31**.

[0488] In the use position, triggered by the operator, a laser is activated and this creates a diffraction pattern which can be seen on the camera display. The adjustment for the CE chip position is used to move the CE chip until the diffraction pattern indicates that the middle of the channel has been

located. The alignment of the channel with the optics used in the analysis is thus provided. The camera can also be used to achieve focussing of the system in the Z axis adjustment.

3) Electrophoresis Environment Control

[0489] For the necessary resolution to be obtained in the electrophoresis step **206**, the temperature of the capillary **616** and its contents need to be carefully controlled at the optimum temperature. In the present embodiment, the electrophoresis cartridge section is in contact with a thermally conductive block, with a series of resistance heaters provided on the opposing side of the block. These are provided with controllers and are capable of maintaining the temperature of the electrophoresis cartridge section at the optimum temperature +/−0.3° C.

[0490] In addition, the cavity that the electrophoresis cartridge section is provided in is thermostatically controlled at the optimum temperature. This reduces still further temperature variation before, during and after use.

[0491] The use of a CE chip heating bed, and raised surface around it, is beneficial in controlling the temperature within the CE chip. The nest so formed ensures consistent positioning and good contact.

4) Use of LED's as Light Source

[0492] FIG. **16** depicts a schematic of an example of a system for detecting fluorescence. The system includes light emitting diodes (LEDs), e.g., high power cyan LEDs, to provide excitation wavelength light to detect dyes combined with biological samples. The system also includes a bifurcated optical fibre assembly made, e.g., from high transmission fused-silica cores with high numerical apertures (NAs), e.g., NA=0.22. The LED excitation system described herein can be applied for DNA detection in capillary electrophoresis systems in mobile analytical units. The compactness and light weight of the LED system enables automating assays for nucleic acid studies. Using the compact and light weight system allows creating bench-top analysis systems that can be used both in the laboratory and in the field.

[0493] In some implementations, two LEDs are assembled in parallel and supplied with a stabilized DC voltage of 3.6 V. The current passing through the LED assembly is 1.8 A. The junction is maintained at 15±1° C. by a Proportional-Integrative-Derivative (PID) control loop (Model TE-36-25 from T.E. Technology, Inc.) acting on two 13×13 mm thermoelectric modules. To save power, and space, two Peltiers modules are controlled in parallel and the thermocouple sensor is placed on only one of them assuming that, by construction symmetry, they both behave similarly. An aluminum heat sink and a fan (12 V DC) complete the cooling module. This module extends the lifetime of the LEDs by two orders of magnitude. Without cooling the junction, the supplied current is 2.7 A.

[0494] The first step of collimation is the use of an acrylic-molded lens from Lumiled, which collimates the emitted light to a 15° cone half-angle (NA~n $\sin(2_{1/2})$~0.26). The light is then focused onto a plano-convex lens (f=35 mm, D=25 mm; NA~D/2f~0.36). NA_{LED}<NA_{lens} or the numerical apertures are matched. The distance between the apex of the lens and the plane of the collimator, L_{max}, is adjusted by a micrometer screw to maximize the power read by a calibrated silicon photodiode sensor. The value obtained (25 mm) is only close to the focal length f since the collimated LED is not a point source. The light beam is then refocused onto a collimation package assembled around an aspheric lens (f=10 mm, D=5 mm; NA~D/2f~0.25, Ocean Optics Ltd) within an anodized aluminum lens tube of length l=30 mm Each LED is thus coupled into one arm of a 2 m-long bifurcated silica core (Ø=600 μm, NA=0.22) optical fibre assembly (attenuation: 0.013 dB/m at 505 nm—relative transmission: 82% (arm **1**) and 87% (arm **2**)).

[0495] Table 1 illustrates a power optimization of the system depicted in FIG. **16**. The power at 505 nm, P505, is read by the silicon photodiode while the distance between the LED collimator and the lens surface (L_{max}), the lens geometry, and the lens tube length (l) are changed. Only one arm of the bifurcated fibre is used.

TABLE 1

Lens	I	Lmax	Psos
Hemispherical	3 cm	20 mm	225.2 μW
Hemispherical	5 cm	18 mm	200.4 μW
Hemispherical	8 cm	19 mm	222.8 μW
Cylindrical	3 cm	9 mm	170.9 μW
Cylindrical	5 cm	9 mm	164.1 μW
Plano-convex	3 cm	16 mm	220.9 μW
Plano-convex	5 cm	15 mm	204.1 μW
Plano-convex	8 cm	15 mm	173.7 μW
None	None	12 mm	187.4 μW

[0496] For the bias values described above, when both arms of the fibre are used, the power at 505 nm read by the photodiode is 820 μW.

[0497] FIG. **17** is a plot of LED spectrum, light reflected, and residual LED light over a range of wavelengths (nm). FIG. **17** illustrates an LED spectrum obtained in the cooled CCD (diodes: Ug=2.0 V; I=0.3 A; T=15° C.), calculated light reflected by the dichroic mirror, and residual LED light after the emitter. The insert shows the transmission curves of the dichroic and emitter. The plot indicates that there is a loss of power when the incident light is reflected onto the sample. Additionally, light is red-shifted by 20 nm, which causes some of the LED light to interfere with the carboxyfluorescein dyes. The choice of available emitters and dichroic mirrors is limited by the dyes chosen to label the migrating DNA strands.

[0498] FIG. **18** is a plot of power of the LED-module over time. During a CE experiment, it is crucial to reduce the fluctuations of the power of the light source within less than 1%. FIG. **18** shows an example of the power recorded by the silicon photodiode (Probe S130A, Thorlabs) using the internal calibration function to record the power emitted by the fiber-LED assembly at 505 nm over time. The diodes are supplied with a 3.4 V DC voltage corresponding to a current of 1.4 A while the junction is maintained at 15±1° C. The room is maintained at a temperature of 22° C. (R.H.=24%). The plot illustrates a temporal power evolution of the LED-module. The lines mark regimes where the power drops, e.g., by 4.8 nW/s, 11.6 nW/s, and 5.0 nW/s. Overall, the power drops by about 1.95 μW over 5 min, i.e. 0.48%.

FIG. **19** is an illustration showing beam shape and size after the sample objective as measured by the laser camera. The asymmetry observed is due to imperfections occurring when the two fibre arms are fused because of the large core diameter of the fibre, mismatches between the LED-to-LED and the fiber-to-fibre distances, and tilt in the optical elements. In the results reported in the next section, the situation correspond-

ing to the single-spot will be used. One method includes adjusting all the optics to obtain the maximum power at the merged end of the bifurcated fibre. This can yield a misshapen light beam as the core size of each arm is large (multimode fibre). To characterize the beam shape and size after the microscope objective, i.e. at the entrance of the microchip, a Coherent Lasercam II ½ camera was placed on an {x,y,z} translation stage equipped with micrometer precision positioners and equipped with a Leica HCX PL FLUOTAR (40×, NA=0.75, WD=0.40 mm) and adjustable filters. The objective was brought within ~8 mm of the Olympus LUCPLFLN (20×, NA=0.45, WD=6.6-7.8 mm) mounted on the CE setup. This allowed directly imaging the beam coming out of the fiber-LED assembly via the CE setup. The micrometer positioners allowed measuring the dimension of the beam with a precision of 10 μm by moving the camera from one spot of the obtained beam profile image to another and reporting the traveled distance. The power can be maximized by adjusting each optical collimation element (P=1.6 mW at 505 nm) (A) or the collimation elements can be adjusted to give one single spot (P=1.0 mW at 505 nm) (B).

[0499] The system was employed for both static and dynamic fluorescence measurements. For the static fluorescence measurements, a 1 μM fluorescein, 6-FAM or rhodamine B solution is loaded into the microchannel by using a standard laboratory vacuum line (13 PSI (0.88 atm) depression) to pull the solution through the channel via 2-mm-diameter access holes. The glass microchannel is anisotropically etched with fluorhydric acid (HF) in Schott Borofloat® low-fluorescence glass (CE chip X8050, Micronit, B.V., The Netherlands). It is semi-elliptic with a width of 50 μm, a depth of 20 μm and a length of 85 mm. The plastic microchannels are hot-embossed into a 1.1-mm-thick cyclic olefin copolymer (COC) sheet at ~160° C. from a reactive-ion etched Si(100) master. The channel section is tapered with a 25° taper angle and has a width of 60 μm (top) and 39 μm (bottom), a depth of 20μ and a length of 85 mm. Glass capillaries that are 1-cm-long (inner diameter 4 mm) borosilicate are epoxy-glued onto the access holes to act as reservoirs (or wells). All solutions are filtered with a nylon membrane (pore diameter: 0.2-μm) to remove small particles that will clog the channel.

[0500] The loaded chip is placed on the CE setup and the focus of the 63× sample objective is aligned with the bottom of the channel. The emitted fluorescent light is gathered onto the 26.6 mm×6.7 mm (1024×255 pixels) array of the thermoelectrically cooled Andor CCD. The processed signal is vertically binned from the software-restricted central rows irradiated by the light focused onto the spectrometer entrance slit. The CCD is cooled down to −50° C. to reduce the binned dark counts to 270 while the exposure time is 0.05 s.

[0501] FIGS. **21**A and **21**B are plots of CCD signal v/s wavelengths. The plots indicate the vertically-binned signal from a 1 μM 6-FAM solution loaded into a glass microchannel (A) and a 1 μM fluorescein solution loaded into a plastic COC channel (B). The counts from the same microchannel filled with water are subtracted to take into account the autofluorescence of the glass or plastic microdevice. The power emitted from the system is 0.98 mW and 1.03 mW at 505 nm for glass and COC, respectively. This is obtained by supplying the two LEDs (placed in series) with a constant current of 0.74 A, which corresponds to a voltage of 7.0 V. Due to the choice of filters (emitter cut-on: T50 at 535 nm), only the tail of the fluorophore emission is observed (fluorescein: $8.^{em}{}_{max}$=513 at pH=13, 6-FAM: $8^{em}{}_{max}$=517 at pH=9. The signal-to-noise ratio is 87 for 1 μM 6-FAM in glass and 36 for 1 μM fluorescein in COC. The SNR is lower in glass because 6-FAM is known to photobleach faster than fluorescein. The detection limit parameters for glass and plastic CE microdevices are summarized in Table 2.

TABLE 2

Device material	Fluorophore	Power at 505 nm	Maximum counts	signal-to-noise ratio
Glass	1 uM 6-FAM	0.98 mW	720	36
COC	1 uM fluorescein	1.03 mW	1750	87

For dynamic fluorescence measurements, glass microchannels are loaded with reagents similar to the reagents for the static measurement testing, but a first sequence of reagents are flushed through the microdevice to reduce the effect of the electroosmotic flow (EOF) that opposes the electrophoretic flow and results in peak distortion from a Gaussian shape and therefore loss of resolution. EOF arises from the re-equilibration of the electrical double layer arising from the surface charge of the microchannel walls after the perturbation caused by the migrating charges under the electric field. The EOF can be efficiently controlled by using a coating polymer matrix such-as poly-N-hydroxyethylacrylamide (pHEA) dissolved in water at 0.1% w/v.

[0502] The DNA fragments are separated by electrophoretically migrating within a sieving polymer matrix such as POP-5™ (Applied Biosystems, Inc.), a mixture of polyacrylamides in an appropriate buffer, according to their size and interactions with the polymer network. After the pHEA coating has been applied, 1×A.C.E.™ buffer (Amresco, Inc.) is flushed into the channel by vacuum followed by POP-5™. A 1 μM solution of a poly-adenine oligonucleotide labeled with 6-FAM is placed in the sample well and will be electrokinetically injected in the separation channel via a cross-injection geometry. 1× A.C.E.™ buffer is placed in the sample waste, buffer waste, and waste wells to ensure ionic conductivity in the whole device.

[0503] FIG. **21** is a plot of CCD signal v/s time for dynamic fluorescence measurements. The plot indicates fully binned CCD signal showing the peak corresponding to the elution of the 1 μM oligonucleotide (elution time, t_{el}=77 s) detected by the optical module. The nature of the peak is confirmed by the spectrum obtained in the CCD at t=77 s. It is similar to the peak shown in FIG. **20***a*. The signal-to-noise ratio of 10 can be improved by uniformly heating the chip to 50° C. The plot shows the result of the migration of the oligonucleotide while the LED-fibre assembly delivers about 980 μW at 505 nm. The two LEDs, placed in parallel, are supplied with 3.9 V (I=1.9 A) while the junction is kept at 15° C. The migration field in the separation channel is 110 V/cm.

[0504] In this manner, an optical excitation module capable of visualizing a 1 μM oligonucleotide migrating in a glass microchannel loaded with a sieving matrix is assembled and tested. The output fibre beam size and divergence, the power distribution in the beam exiting the fibre assembly as well as the output power stability over time approach the specifications of existing LIF setups. A modified epifluorescence microscope arrangement is used in conjunction with a lightweight compact fixed spectrograph built around ion-etched grating and aligned with a cooled Charge-Coupled Device (CCD) camera for added sensitivity. Fluorescent dyes such as

fluorescein, 6-carboxyfluorescein (6-FAM) and rhodamine B can be detected in conventional plastic (cyclic olefin copolymer) and glass microchannels at submicromolar levels. A migrating single-stranded oligonucleotide DNA fragment (10-mer) labeled with 6-FAM can also be detected with high signal-to-noise ratio when electrophoretically migrated in the microchannels at 100 V/cm. LEDs operated in conjunction with Peltier elements controlled by a Proportional Integrative Derivative (PID) module can be used to replace bulky, expensive and power-consuming Argon ion lasers conventionally used in Laser Induced Fluorescence (LIF) Capillary Electrophoresis (CE) experiments. The LEDs in the system can be HP803-CN obtained from Roithner LaserTechnik GmBH or Luxeon Star series from Philips Lumiled Lighting Company that offer LEDs emitting at 505±15 nm with a full-width at half maximum of 20 nm. The LEDs are available with a Lambertian profile with a half-cone angle of 75°, which is not suited for microchip applications. However, these are high power LEDs with a nominal radiometric output power of 45 or 80 mW. When properly collimated, the available power becomes relevant to applications of DNA detection by CE.

[0505] While this specification contains many specifics, these should not be construed as limitations on the scope of the disclosure or of what may be claimed, but rather as descriptions of features specific to particular implementations of the disclosure. Certain features that are described in this specification in the context of separate implementations can also be implemented in combination in a single implementation. Conversely, various features that are described in the context of a single implementation can also be implemented in multiple implementations separately or in any suitable subcombination. Moreover, although features may be described above as acting in certain combinations and even initially claimed as such, one or more features from a claimed combination can in some cases be excised from the combination, and the claimed combination may be directed to a subcombination or variation of a subcombination.

Similarly, while operations are depicted in the drawings in a particular order, this should not be understood as requiring that such operations be performed in the particular order shown or in sequential order, or that all illustrated operations be performed, to achieve desirable results. In certain circumstances, multitasking and parallel processing may be advantageous. Moreover, the separation of various system components in the implementations described above should not be understood as requiring such separation in all implementations, and it should be understood that the described program components and systems can generally be integrated together in a single software product or packaged into multiple software products.

[0506] Thus, particular implementations of the disclosure have been described. Other implementations are within the scope of the following claims. For example, the actions recited in the claims can be performed in a different order and still achieve desirable results. In some implementations, the sharpness of the cut-on edge of the dichroic mirror can be improved and the lower wavelength T_{50} can be shifted to a lower wavelength to improve the signal-to-noise ratio. In some implementations, the diodes can be operated in a pulsed AC mode where the "on" time is synchronized with the frame acquisition of the CCD camera, thereby also extending the lifetime of the LEDs. In some implementation, a customized LED array can be used that does not have the mold that yields divergent light. In some implementations, the collimation parts can be embedded in a rigid casing made, e.g., from black anodized aluminum.

[0507] In some implementations, the LED-based detection system described in this disclosure can be used as the microfluidic electrophoresis system that is described in the attachment, which is enclosed as part of the present disclosure.

5) Size Standards

[0508] The size standards used in the invention are beneficially stored within the formamide pump liquid.

[0509] The size standards may be provided according to the form detailed in International Patent Application no PCT/GB2009/002186, the contents of which are incorporated herein by reference, particularly with respect to the provision of and use of size standards which operate within a single CE channel, together with the sample being considered.

Instrument Performance

[0510] The result of the above embodiment is the provision of an instrument, cartridge and operating method which provides quick, reliable sample analysis, whilst doing so at a wide variety of locations and when operated by a wide variety of people.

[0511] By way of abilities are performance, the invention provides a fully integrated instrument capable of performing extraction, PCR, electrophoresis and analysis, whilst requiring minimal training and/or intervention by the user. In its optimum form, a fully automated system from start to finish is provided, the user simply needing to load the cartridge into the instrument and start it.

[0512] The modular nature of the instrument allows for upgrading of one or more modules without impact on the other modules. The data output format has been carefully selected to allow the analysis of the data outputted by a variety of existing analysis software applications, such is I^3 of Forensic Science Service Limited, and future software applications.

[0513] The end result of the analysis may be a profile for the sample and/or an indication of a match between the sample and a database recorded sample and/or other interpretation based data.

[0514] The use of a single cartridge type to handle a wide variety of sample from a wide variety of sources is beneficial. The methodology is able to handle samples originating from buccal swabs, cotton and other soft swabs, aqueous samples, clothing samples, cigarette butts, chewing gum and the like.

[0515] The methodology is also able to separate the useful DNA from residual cellular material, PCR inhibitors (such as ethanol, indigo etc) and chemical inhibitors.

[0516] The instrument is fully portable and so can be used in a wide variety of locations. The fully sealed and protected nature of the cartridge means that contamination is not a risk, even where the instrument is used outside of laboratory standard conditions. The instrument operates off a standard mains power supply, 110-240V, 50 Hz, using a conventional electric plug.

[0517] With respect to the overall time, from the sample receiving step **202**, to the transmission away from the instrument in the data communication step **210**, the embodiment described provides this process in a time period of 141 minutes. That time period can be reduced, including by the options and variables set out in the following paragraphs.

[0518] With respect to the sample receiving step **2002**, the embodiment described provides this step in a time period of 2 minutes. Time periods of between 20 seconds and 5 minutes are easily achievable, depending upon the loading methodology used and the number of reagents or samples that need to be loaded.

[0519] With respect to the sample preparation step **202**, the embodiment described provides this step in a time period of 24 minutes. That time period can be reduced by shortening the residence in one or more of the chambers, for instance the incubation chamber **358**, and/or by reducing the time separation between a valve being activated and reliance on the outcome of the activation and/or by reducing the washing and/or elution volumes used. Time periods of between 15 to 30 minutes are easily achievable.

[0520] With respect to the sample amplification step **204**, the embodiment described provides this step in a time period of 80 minutes. That time period can be reduced by shortening the number of cycles used, the duration of one or more parts of a cycle and the time period after introduction to the chamber and before PCR starts and/or after PCR finishes and before the sample is removed to the next stage. Again, the time separation between a valve being activated and reliance on the outcome of the activation is of significance. Time periods of between 60 to 120 minutes are easily achievable.

[0521] With respect to the electrophoresis step **206**, the embodiment described provides this step in a time period of 15 minutes. That time period can be reduced by the use of higher voltages and/or faster migration media in the capillary and/or reductions in the sample introduction time. Time periods of between 1 to 60 minutes are easily achievable. This functionality is achieved in an instrument weighing less than 10 kg and occupying a footprint of less than 0.1 m^2.

Instrument Fields of Use

[0522] The structures and method discussed above are useful in the consideration of a wide variety of samples, over and above forensic samples. For instance, they can be used: the consideration of marker targets, diagnostic assays, disease markers, biobanking applications, STR based targets in transplants, identification of drug resistant microorganisms, blood testing, mutation detection, DNA sequencing and the like. Food analysis, pharmogenetics and pharmogenomics are also areas of use. A wide variety of uses in the medical and/or biotech field can make use of the invention.

[0523] The invention is also applicable in situations where familial relationships need to be determined from DNA, for instance paternity testing. Pedigree testing in animals is a further example.

[0524] The use of the invention in border control, security, customs situations and other governmental type uses is beneficial.

1. A method of configuring an optical system for a detection location, the method including:

providing a channel having a detection location for performing an electrophoretic separation on at least a part of a sample;

considering one or more characteristics of the channel and/or optical system; configuring the optical system relative to the detection location.

2. A method according to claim **1** in which the optical system is provided in an instrument, the channel is provided in a device which can be inserted into and removed from the instrument and the configuring of the optical system is made before the electrophoretic separation.

3. A method according to claim **1** in which the configuring of the optical system is made after one or more other steps have been performed on the device.

4. A method according to claim **1** in which the configuring is with respect to the position of one or more parts of the optical system relative to the channel.

5. A method according to claim **4** in which the position is the position perpendicular to the axis of the channel, parallel to the axis along which light is applied to the detection location.

6. A method according to claim **4** in which the position is the position perpendicular to the axis of the channel, perpendicular to the axis along which light is applied to the detection location.

7. A method according to claim **4** in which the configuring is with respect to the focus of the light applied to the detection location.

8. A method according to claim **1** in which the configuring is made with reference to the channel, the channel is responsive to light and the variation in the detected light with position across the channel is considered.

9. A method according to claim **1** in which one or more components of the optical system are moved and/or the channel is moved to configure the optical system.

10. A method according to claim **1** in which the configuring is provided using the optical system used in detecting the results from the channel.

11. A method according to claim **1** in which the configuring is provided using a separate alignment system.

12. A method according to claim **11** in which the separate alignment system includes one or more of: a variable position support and/or an actuator and/or a camera and/or a light detector and/or a light source.

13. A method according to claim **11** in which the alignment system is used to control one or more components of the optical system so as to provide alignment between the channel and the optical system.

14. A method according to claim **11** in which the alignment system includes a variable position support, the variable position support having a first position, the variable position support having a second position, in the second position, the camera/light detector provided on the variable position support being positioned in an alignment with the channel.

15. A method according to claim **14** in which the alignment is less accurate than a second alignment and the second alignment is obtained by varying the position of the variable position support.

16. A method according to claim **15** in which the varying of the position is in response to the form of diffraction pattern observed by the camera/light detector.

17. Apparatus for configuring an optical system for a detection location, the apparatus including:

a channel having a detection location;

an optical system;

one or more elements for adjusting the configuration of the optical system.

18. Apparatus according to claim **17** in which the optical system is provided in an instrument, the channel is provided in a device which can be inserted into and removed from the instrument.

19. Apparatus according to claim **17** in which one or more components of the optical system are moveable and/or the channel is moveable to configure the optical system.

20. Apparatus according to claim **17** in which a separate alignment system to the optical system is provided.

21. Apparatus according to claim **20** in which the separate alignment system includes one or more of: a variable position support and/or an actuator and/or a camera and/or a light detector and/or a light source.

22. Apparatus according to claim **20** in which the alignment system includes a variable position support, the variable position support having a first position, the variable position support having a second position, in the second position, the camera/light detector provided on the variable position support being positioned in an alignment with the channel.

23. An instrument for analysing a sample, the instrument including:

one or more sample processors;

a channel for performing an electrophoretic separation on at least a part of the sample;

a detection location in the channel;

an optical system for considering the detection location.

24. An instrument according to claim **23**, wherein the optical system includes one or more of a light source, a collimator, a filter, a mirror, a spectrometer and camera.

25. An instrument according to claim **23** in which the channel is maintained at a predetermined temperature +/−0.7° C.

26. An instrument according to claim **23** in which the channel is provided in contact with a heating location.

27. An instrument according to claim **26** in which the heating location provides a planar surface portion.

28. An instrument according to claim **27** in which the heating location has a planar surface portion whose boundaries match those of the element containing the channel.

29. An instrument according to claim **28** in which the heating location is bounded by one or more protruding or elevated sections.

30. A method for analysing a sample, the method including:

performing one or more sample processes;

introducing at least a part of the sample into a channel;

performing an electrophoretic separation on at least a part of the sample;

considering the characteristics present at a detection location in the channel using an optical system.

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