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(54) Title: IMPROVEMENTS IN AND RELATING TO DEVICES

(57) Abstract: A method of analysis, instrument for analysis and device for use in such an instrument are provided, which perform a number of processes need to reach a useful result in the context of a wide variety of samples. The sequence of those processes being optimised. A device, instrument using the device and method of use are also provided which offer reliable performance of a heating based process, with minimal condensation and/or sample loss issues.



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IMPROVEMENTS IN AND RELATING TO DEVICES

This application is being filed on 09 February 2010, as a PCT International Patent application in the name of Forensic Science Service Limited, a Great Britain national corporation, applicant for the designation of all countries except the U.S.,
5 and Frederic Zenhausern, a citizen of the U.S., Alan Nordquist, a citizen of the U.S.,
Ralf Lenigk, a citizen of Germany, Cedric Hurth, a citizen of France, Jianing Yang, a citizen of the U.S., Xiaojia Chen, a citizen of China, Matthew Estes, a citizen of the U.S., John Lee-Edgehill, a citizen of Great Britain, Nina Moran, a citizen of Great Britain, Andrew Hopwood, a citizen of Great Britain, and Pieris Koumi, a
10 citizen of Great Britain, applicants for the designation of the U.S. only, and claims priority to U.S. Provisional Patent Application Serial No. 61/151,104 filed on 09 February 2009, U.S. Provisional Patent Application No. 61/151,107 filed on 09 February 2009, U.S. Provisional Patent Application No. 61/151,111 filed on 09 February 2009, and U.S. Provisional Patent Application No. 61/151,117 filed on 09
15 February 2009.

Background of the Invention

The invention concerns improvements in and relating to analysis, particularly, but not exclusively, in relation to biological samples, such as DNA.
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Summary of the Invention

According to a first aspect of the invention, there is provided an instrument for analysing a sample, the instrument including:
a device having one or more sample processors;
25 electronics for operating the sample processors.

According to a second aspect of the invention, there is provided a device, for processing a sample, the device including:
one or more sample processors.
30

According to a third aspect of the invention, there is provided a method of producing a device, the method including:
forming one or more sample processors;
providing electronics for operating the sample processors.

The instrument may provide an integrated set of process steps and/or sample processors. The process steps and/or sample processors may include a sample receiving step and/or sample preparation step and/or sample extraction step and/or
5 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 detection step and/or electrophoresis step and/or analysis step and/or results output step.

10 The device may be a cartridge. The device is preferably a single use device. The device is preferably only used to process and/or provide the results for one sample. The device is preferably disposable.

The device may have an orientation of use, for instance in the instrument.

15 The sample receiving step may be provided on the device.

The sample receiving step may include an inlet to the device. The sample receiving step may include a chamber, preferably into which the sample is received. The chamber may have an inlet in the upper portion of the chamber, for instance the upper 20%. The chamber may have a gas outlet, for instance a vent. The gas outlet
20 may be provided in the upper portion of the chamber, for instance the upper 20%.

The chamber may be connected to a pump, for instance an electrochemical pump. The pump may be a first pump provided on the device. The first pump may provide the drive to move one or more fluids and/or liquids through the chamber and/or first chamber and/or second chamber and/or third chamber and/or fourth
25 chamber and/or into a fifth chamber. The inlet from the pump may be provided in the upper section of the chamber, for instance the upper 20%, preferably upper 5%.

The chamber may have a sample outlet. The sample outlet may be provided in the lower portion of the chamber, for instance the lower 20%, more preferably lower 10% and ideally the lowest part of the chamber. The outlet may be in the
30 bottom wall of the chamber.

The sample receiving step may have a first state in which it is isolated from one of more of the other steps in the cartridge. The one or more other steps may be a 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 sample

amplification step and/or denaturation step and/or detection step and/or electrophoresis step and/or analysis step. The sample receiving step may have the first state during loading of the sample. The sample receiving step may be provided with a valve. The valve may be provided at the sample outlet and/or on the channel leading from the sample receiving step and/or leading from the sample outlet. The valve may be a closed state to open state valve.

The outlet channel may be connected to the next step, for instance to the sample preparation step.

The sample preparation step may be provided on the device. The sample preparation step may be provided on the same device as the sample receiving step.

The sample preparation step may include an inlet, preferably a channel. The channel may be connected to the sample receiving step. The sample preparation step may include a first chamber, preferably into which the sample passes.

The first chamber may have an inlet in the upper portion of the first chamber, for instance the upper 20%.

The first chamber may have a circular cross-section. The cross-section may be relative to a horizontal axis. The first chamber may be provided with a buffer. The buffer may be provided to control conditions for a subsequent process and/or reaction, for instance in one or more further chambers and/or channels. The first chamber may be provided with a one or more particles. The particles may be beads. One or more of the particles may be magnetic. The one or more particles may have a magnetic material within a surface layer or layers. The particles may be provided with one or more reagents or materials which releasable bind and/or link and/or combine with a part of the sample, for instance DNA.

The first chamber may have a sample outlet. The sample outlet may be provided in the lower portion of the chamber, for instance the lower 20%, more preferably lower 10% and ideally the lowest part of the chamber. The outlet may be in the bottom wall of the chamber.

References to vertical within the document may mean within 25° of the vertical, preferably within 10° and ideally within 5° , and potentially be completely vertical. References to horizontal within the document may mean within 25° of the horizontal, preferably within 10° and ideally within 5° , and potentially be completely horizontal.

Reference to the number of a chamber, such as to a fourth chamber do not

mean or imply that the chamber has to be preceded by such a number of chambers. The number is merely used to clarify one chamber from another.

The sample outlet may connect to a channel. Preferably the channel has a plurality of sections. The channel may have a vertical section and/or a horizontal section and/or a second vertical section and/or a second horizontal section and/or a
5 third vertical section. The channel may have a vertical section and a horizontal section and a second vertical section and/or a second horizontal section and/or a third vertical section. The channel may connect to a second chamber.

The second chamber may have an inlet in the upper portion of the second
10 chamber, for instance the upper 20%.

The second chamber may have an elongate cross-section. The second chamber may have a cross-section formed by a semicircle at each end and a rectilinear section joining them. The cross-section may be relative to a horizontal axis. The second chamber may be provided with one or more particles. The
15 particles may be beads. One or more of the particles may be magnetic. The one or more particles may have a magnetic material within a surface layer or layers. The particles may be provided with one or more reagents or materials which releasable bind and/or link and/or combine with a part of the sample, for instance DNA. The second chamber may be provided with a buffer. The buffer may be provided to
20 control conditions for a subsequent process and/or reaction, for instance in one or more further chambers and/or channels.

The second chamber may have a sample outlet. The sample outlet may be provided in the lower portion of the second chamber, for instance the lower 20%, more preferably lower 10% and ideally the lowest part of the second chamber. The
25 outlet may be in the bottom wall of the chamber.

The sample outlet may connect to a channel. Preferably the channel has a plurality of sections. The channel may have a vertical section and/or a horizontal section and/or a second vertical section and/or a second horizontal section and/or a third vertical section and/or a third horizontal section and/or fourth vertical section.
30 The channel may have a vertical section and a horizontal section and a second vertical section and a second horizontal section and a third vertical section and/or a third horizontal section and/or fourth vertical section. The channel may connect to a third chamber.

The third chamber may have an inlet in the upper portion of the third

chamber, for instance the upper 25%.

The third chamber may have a non-linear cross-section. The third chamber may have a cross-section formed by a semicircles or part semicircles at one or both ends. A rectilinear section may join the semicircles or part semicircle together. The cross-section may be relative to a horizontal axis. The third chamber may be provided with a one or more particles. The particles may be beads. One or more of the particles may be magnetic. The one or more particles may have a magnetic material within a surface layer or layers. The particles may be provided with one or more reagents or materials which releasable and/or reversibly bind and/or link and/or combine with a part of the sample, for instance DNA.

The third chamber may have a gas outlet, for instance a vent. The gas outlet may be provided in the upper portion of the chamber, for instance the upper 20%, preferably upper 10% and ideally upper 5%. The gas outlet may be provided in the top wall of the third chamber. The gas outlet may be provided in a recess at the top of the third chamber. The gas outlet may lead to the outside of the device, for instance through a vent. A valve may be provided between the third chamber and the vent. The valve may be an open state to closed state valve.

The third chamber may have a sample outlet. The sample outlet may be provided in the lower portion of the third chamber, for instance the lower 10%, more preferably lower 5% and ideally the lowest part of the third chamber. The outlet may be in the bottom wall of the chamber.

The sample outlet may connect to a channel. Preferably the channel has a plurality of sections. The channel may have a vertical section and/or a horizontal section and/or a second vertical section and/or a second horizontal section and/or a third horizontal section and/or a third vertical section. The channel may have a vertical section and a horizontal section and a second vertical section and a second horizontal section and/or a third vertical section and/or a third horizontal section. The channel may connect to one or more further chambers, such as a fourth chamber.

The sample preparation step or a part thereof may have a first state in which it is isolated from one of more of the other steps in the cartridge and/or from one or more other parts of the sample preparation step. The one or more other steps may be a sample receiving 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 sample

amplification step and/or electrophoresis step and/or analysis step. The sample preparation step or part thereof may have the first state during contacting of the sample with the buffer and/or particles and/or first chamber and/or second chamber and/or third chamber. The sample preparation step or a part thereof may be provided with a valve. The valve may be provided at the sample outlet, preferably from the third chamber and/or on the channel leading from the sample preparation step to a further step and/or on the channel leading from the part of the sample preparation step to the next part of the sample preparation step and/or on the channel leading from the sample outlet. The valve may be a closed state to open state valve.

10 The sample preparation step and/or a part of the sample preparation step, particularly the part that follows the part described above, may include a fourth chamber.

The fourth chamber may have an inlet in the upper portion of the fourth chamber, for instance the upper 25%. The inlet may be in a corner of the fourth chamber.

15 The fourth chamber may have a non-linear cross-section. The fourth chamber may have a cross-section formed by a horizontal top wall, horizontal or inclined lower wall and transition end walls joining the top and lower walls. The transition end walls may be curved. The cross-section may be relative to a horizontal axis. The fourth chamber may be provided with a gas, such as air, preferably prior to the sample arrival.

20 The fourth chamber may have a sample outlet. The sample outlet may be provided in the lower portion of the fourth chamber, for instance the lower 10%, more preferably lower 5% and ideally the lowest part of the fourth chamber. The outlet may be in the bottom wall of the chamber or preferably in a corner of the chamber, ideally the corner opposing the inlet.

25 The sample outlet may connect to a channel. Preferably the channel has a plurality of sections. The channel may have a vertical section and/or a horizontal section and/or a second vertical section. The channel may have a vertical section and a horizontal section and a second vertical section. The channel may connect to a fifth chamber.

The fifth chamber may have an inlet in the upper portion of the fifth chamber, for instance the upper 25%. The inlet may be in a corner of the fifth

chamber.

The fifth chamber may have a non-linear cross-section. The fifth chamber may have a cross-section formed by a horizontal top wall, horizontal or inclined lower wall and transition end walls joining the top and lower walls. The transition end walls may be curved. The cross-section may be relative to a horizontal axis. The fifth chamber may be provided with a gas, such as air, preferably prior to the sample arrival.

The fifth chamber may have a sample outlet. The sample outlet may be provided in the lower portion of the fifth chamber, for instance the lower 10%, more preferably lower 5% and ideally the lowest part of the fifth chamber. The outlet may be in the bottom wall of the chamber or preferably in a corner of the chamber, ideally the corner opposing the inlet.

The sample outlet may connect to a channel. Preferably the channel has a plurality of sections. The channel may have a vertical section and/or a horizontal section and/or a second vertical section and/or second horizontal section and/or third vertical section and/or third horizontal section and/or fourth vertical section. The channel may have a vertical section and a horizontal section and a second vertical section and/or second horizontal section and/or third vertical section and/or third horizontal section and/or fourth vertical section. The channel may connect to a sixth chamber.

The sixth chamber may have an inlet in the lower portion of the sixth chamber, for instance the lower 20%, preferably lower 10% and ideally lower 5%. The inlet may be in the bottom wall of the sixth chamber.

The sixth chamber may have a non-linear cross-section. The sixth chamber may have a cross-section formed by a horizontal bottom wall, horizontal top wall and side walls that diverge between the bottom and the top. The corners may be provided with curved transition walls. The sixth chamber may be provided with air.

The sixth chamber may have a gas outlet, for instance a vent. The gas outlet may be provided in the upper portion of the chamber, for instance the upper 20%, preferably upper 10% and ideally upper 5%. The gas outlet may be provided in the top wall of the sixth chamber. The gas outlet may lead to the outside of the device, for instance through a vent. A valve may be provided between the sixth chamber and the vent. The valve may be an open state to closed state valve

The sixth chamber may be connected to a pump, for instance an

electrochemical pump. The pump may be a second pump provided on the device. The second pump may provide the drive to move one or more fluids and/or liquids through the sixth chamber and/or seventh chamber and/or into a waste chamber. The pump may provide gas to one or more of the chambers, particularly the sixth chamber. The gas may promote mixing within the one or more chambers, particularly the sixth chamber. The inlet from the pump may be provided in the upper section of the sixth chamber, for instance the upper 20%, preferably upper 5%.

The channel connecting the pump to the sixth chamber may be provided with a valve. The valve may be an open state to closed state valve. The channel may include a first vertical section and/or first horizontal section and/or second vertical section and/or second horizontal section and/or third horizontal section and/or fourth vertical section.

The sixth chamber may have a sample outlet. The sample outlet may be provided in the lower portion of the sixth chamber, for instance the lower 10%, more preferably lower 5% and ideally the lowest part of the sixth chamber. The outlet may be in the bottom wall of the chamber.

The sample outlet may connect to a channel. Preferably the channel has a plurality of sections. The channel may have a vertical section and/or a horizontal section and/or a second vertical section and/or a second horizontal section and/or a third vertical section. The channel may have a vertical section and a horizontal section and a second vertical section and a second horizontal section and/or a third vertical section. The channel may connect to one or more further chambers, such as a seventh chamber.

The sample preparation step or a part thereof may have a first state in which it is isolated from one of more of the other steps in the cartridge and/or from one or more other parts of the sample preparation step. The one or more other steps may be a sample receiving step and/or sample retention step and/or purification step and/or washing step and/or elution step and/or sample amplification step and/or electrophoresis step and/or analysis step. The sample preparation step or part thereof may have the first state during contacting of the sample with the fourth chamber and/or fifth chamber and/or sixth chamber and/or during mixing of the sample, buffer and particles and/or during heating of the sixth chamber. The sample preparation step or a part thereof may be provided with a valve. The valve may be

provided at the sample outlet, preferably from the sixth chamber and/or on the channel leading from the sample preparation step to a further step and/or on the channel leading from the part of the sample preparation step to the next part of the sample preparation step and/or on the channel leading from the sample outlet. The valve may be a closed state to open state valve.

The seventh chamber may have an inlet in the upper portion of the seventh chamber, for instance the upper 20%, preferably upper 10% and ideally upper 5%. The inlet may be in the top wall of the seventh chamber.

The seventh chamber may have a non-linear cross-section. The seventh chamber may have a cross-section formed by a horizontal bottom wall, horizontal top wall and side walls that diverge between the bottom and the top. The corners may be provided with curved transition walls. The top wall may include a recess, such as a semi-circular recess. The seventh chamber may be provided with air.

The seventh chamber may have a gas outlet, for instance a vent. The gas outlet may be provided in the upper portion of the chamber, for instance the upper 20%, preferably upper 10% and ideally upper 5%. The gas outlet may be provided in the top wall of the seventh chamber. The gas outlet may lead to the outside of the device, for instance through a vent. A valve may be provided between the seventh chamber and the vent. The valve may be an open state to closed state valve. The channel leading to the valve may connect to the seventh chamber in a recess, such as a semi-circular recess, provided in the upper section of the seventh chamber.

The seventh chamber may have a second gas outlet, for instance a vent. The second gas outlet may be provided in the upper portion of the chamber, for instance the upper 20%, preferably upper 10% and ideally upper 5%. The second gas outlet may be provided in the top wall of the seventh chamber. The second gas outlet may lead to the outside of the device, for instance through a second vent. A second valve may be provided between the seventh chamber and the second vent. The second valve may be an open state to closed state valve. The second channel leading to the second valve may connect to the seventh chamber in a recess, such as a semi-circular recess, provided in the upper section of the seventh chamber.

The seventh chamber may be connected to a pump, for instance an electrochemical pump. The pump may be a second pump provided on the device. The seventh chamber may be connected to the pump by a first route through the sixth chamber and/or by a second route through an eighth chamber and/or wash

chamber.

The sample preparation step or a part thereof may have a first state in which it is isolated from one or more of the other steps in the cartridge and/or from one or more other parts of the sample preparation step. The one or more other steps may be
5 a sample receiving 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 sample amplification step and/or electrophoresis step and/or analysis step. The sample preparation step or part thereof may have the first state during contacting of the sample with the seventh chamber. The sample preparation step or a part thereof may
10 be provided with one or more valves, preferably to provide the isolation.

A valve may be provided at the sample outlet, preferably from the seventh chamber and/or on the channel leading from the sample preparation step to a further step and/or on the channel leading from the part of the sample preparation step to the next part of the sample preparation step and/or on the channel leading from the
15 sample outlet. The valve may be a closed state to open state valve.

One or more valves may be provided on the channel connecting the second pump to the seventh chamber by a second route. The one or more valves may include an open state to closed state valve or valves and/or a closed state to open state valve or valves.

20 One or more valves may be provided on the channel connecting the third pump to the seventh chamber. The one or more valves may include an open state to closed state valve or valves and/or a closed state to open state valve or valves.

One or more valves may be provided on the channel connecting the seventh chamber to an tenth chamber and/or waste chamber. The one or more valves may
25 include an open state to closed state valve or valves and/or a closed state to open state valve or valves.

The seventh chamber may be connected to an eighth chamber and/or wash chamber, for instance by a channel.

The inlet to the eighth chamber may be provided in the upper portion of the eighth chamber, for instance the upper 20%, preferably upper 10% and ideally upper
30 5%. The inlet may be in the top wall of the eighth chamber. The outlet may be provided in the corner of the eighth chamber.

The channel connecting the eighth chamber and/or wash chamber to the seventh chamber may have a plurality of sections. The channel may have a vertical

section and/or a horizontal section and/or a second vertical section and/or a second horizontal section and/or a third vertical section and/or a third horizontal section. The channel may have a vertical section and a horizontal section and a second vertical section and a second horizontal section and/or a third vertical section and/or third horizontal section.

The channel may include one or more valves. The one or more valves may include an open state to closed state valve or valves and/or a closed state to open state valve or valves.

The eighth chamber and/or wash chamber may have an inlet in the upper portion of the eighth chamber, for instance the upper 20%.

The eighth chamber may be connected to a pump, for instance an electrochemical pump. The pump may be a second pump provided on the device. The second pump may provide the drive to move one or more fluids and/or liquids through the eighth chamber and/or seventh chamber and/or sixth chamber and/or into a tenth chamber. The inlet from the pump may be provided in the upper section of the chamber, for instance the upper 20%, preferably upper 5%.

The eighth chamber may have an outlet. The outlet may be provided in the lower portion of the chamber, for instance the lower 20%, more preferably lower 10% and ideally the lowest part of the chamber. The outlet may be in the bottom wall of the chamber.

The sample preparation step or a part thereof may have a first state in which it is isolated from one or more of the other steps in the cartridge and/or from one or more other parts of the sample preparation step. The one or more other steps may be a sample receiving step and/or sample extraction step and/or purification step and/or washing step and/or elution step and/or sample amplification step and/or electrophoresis step and/or analysis step. The sample preparation step or part thereof may have the first state during contacting of the sample with the seventh chamber and/or contact between the eighth chamber and/or wash chamber and the seventh chamber. The sample preparation step or a part thereof may be provided with one or more valves, preferably to provide the isolation.

The seventh chamber may be connected to a tenth chamber and/or waste chamber, for instance by a channel. The outlet from the seventh chamber may be provided in the lower portion of the seventh chamber, for instance the lower 20%, preferably lower 10% and ideally lower 5%. The outlet may be in the bottom wall

of the seventh chamber. The outlet may be provided in the corner of the seventh chamber.

5 The channel connecting the seventh chamber to the tenth chamber and/or waste chamber may have a plurality of sections. The channel may have a vertical section and/or a horizontal section and/or a second vertical section and/or a second horizontal section and/or third vertical section and/or third horizontal section and/or fourth vertical section and/or fourth horizontal section and/or fifth vertical section. The channel may have a vertical section and a horizontal section and a second vertical section and a second horizontal section and third vertical section and/or third horizontal section and/or fourth vertical section and/or fourth horizontal section and/or fifth vertical section.

10 The channel may include one or more valves. The one or more valves may include a closed state to open state valve or valves, preferably provided on the channel above.

15 The one or more valves may include an open state to closed state valve or valves, preferably provided on a parallel channel section. The parallel channel section may include a first vertical section and/or first horizontal section and/or second vertical section and/or second horizontal section and/or third vertical section and/or third horizontal section and/or fourth vertical section. The parallel channel section may be connected to the second vertical and/or third vertical sections of the channel it is provided as a parallel channel to.

20 The tenth chamber and/or waste chamber may have a rectilinear cross-section, potentially with rounded corners.

25 The tenth chamber and/or waste chamber may have an inlet in the upper portion of the chamber, for instance the upper 20%.

30 The tenth chamber and/or waste chamber may be connected to a pump, for instance an electrochemical pump. The pump may be a second pump provided on the device. The second pump may provide the drive to move one or more fluids and/or liquids through the eighth chamber and/or seventh chamber and/or sixth chamber and/or into a tenth chamber.

The tenth chamber and/or waste chamber may have a gas outlet, for instance a vent. The gas outlet may be provided in the upper portion of the chamber, for instance the upper 20%, preferably upper 10% and ideally upper 5%. The gas outlet may be provided in the top wall of the tenth chamber. The gas outlet may lead to the

outside of the device, for instance through a vent. A valve may be provided between the tenth chamber and the vent. The valve may be an open state to closed state valve

The sample preparation step or a part thereof may have a first state in which it is isolated from one of more of the other steps in the cartridge and/or from one or
5 more other parts of the sample preparation step. The one or more other steps may be a sample receiving step and/or sample extraction step and/or sample retention step and/or sample amplification step and/or electrophoresis step and/or analysis step. The sample preparation step or part thereof may have the first state during
10 contacting of the sample with the seventh chamber and/or contact between the ninth chamber and/or elution chamber and the seventh chamber and/or tenth chamber and/or waste chamber. The sample preparation step or a part thereof may be provided with one or more valves, preferably to provide the isolation.

The seventh chamber may be connected to the ninth chamber and/or elution chamber, for instance by a channel. The inlet to the seventh chamber may be
15 provided in the upper portion of the seventh chamber, for instance the upper 20%, preferably upper 10% and ideally upper 5%. The inlet may be in the top wall of the seventh chamber. The inlet may be provided in the corner of the seventh chamber.

The channel connecting the ninth chamber and/or elution chamber to the seventh chamber may have a plurality of sections. The channel may have a vertical
20 section and/or a horizontal section and/or a second vertical section and/or a second horizontal section. The channel may have a vertical section and a horizontal section and a second vertical section and a second horizontal section.

The channel may include one or more valves. The one or more valves may include an open state to closed state valve or valves and/or a closed state to open
25 state valve or valves.

The ninth chamber and/or elution chamber may have a circular cross-section. The cross-section may be relative to a horizontal axis. The ninth chamber and/or elution chamber may be provided with an eluent. The eluent may be provided to control conditions for a subsequent process and/or reaction, for instance in one or
30 more further chambers and/or channels, such as the seventh chamber.

The ninth chamber and/or elution chamber may have an inlet in the upper portion of the ninth chamber and/or elution chamber, for instance the upper 20%.

The ninth chamber and/or elution chamber may be connected to a pump, for instance an electrochemical pump. The pump may be a third pump provided on the

device. The third pump may provide the drive to move one or more fluids and/or liquids through the ninth chamber and/or elution chamber and/or seventh chamber and/or other step and/or amplification chamber. The inlet from the pump may be provided in the upper section of the chamber, for instance the upper 20%, preferably upper 5%.

The ninth chamber and/or elution chamber may have an outlet. The outlet may be provided in the lower portion of the chamber, for instance the lower 20%, more preferably lower 10% and ideally the lowest part of the chamber. The outlet may be in the bottom wall of the chamber.

The sample preparation step or a part thereof may have a first state in which it is isolated from one or more of the other steps in the cartridge and/or from one or more other parts of the sample preparation step. The one or more other steps may be a sample receiving step and/or sample extraction step and/or sample retention step and/or washing step and/or sample amplification step and/or electrophoresis step and/or analysis step. The sample preparation step or part thereof may have the first state during contacting of the sample with the seventh chamber and/or contact between the ninth chamber and/or elution chamber and the seventh chamber. The sample preparation step or a part thereof may be provided with one or more valves, preferably to provide the isolation.

The seventh chamber may have a sample outlet. The sample outlet may be provided in the lower portion of the seventh chamber, for instance the lower 10%, more preferably lower 5% and ideally the lowest part of the seventh chamber. The outlet may be in the bottom wall of the chamber and/or in a corner of the chamber.

The sample outlet may connect to a channel. Preferably the channel has a plurality of sections. The channel may have a horizontal section and/or a vertical section and/or a second horizontal section and/or a second vertical section and/or third horizontal section and/or third vertical section and/or fourth horizontal section and/or fourth vertical section and/or fifth horizontal section. The channel may have a horizontal section and a vertical section and a second horizontal section and a second vertical section and third horizontal section and/or third vertical section and/or fourth horizontal section and/or fourth vertical section and/or fifth horizontal section. The channel may connect to one or more further chambers, such as an eleventh chamber, and/or to an amplification step.

The sample preparation step or a part thereof may have a first state in which

it is isolated from one or more of the other steps in the cartridge and/or from one or more other parts of the sample preparation step. The one or more other steps may be a sample receiving step and/or sample retention step and/or purification step and/or washing step and/or electrophoresis step and/or analysis step. The sample preparation step or part thereof may have the first state during contacting of the sample with the seventh chamber and/or further chamber and/or amplification step. The sample preparation step or a part thereof may be provided with one or more valves. The valve may be provided at the first and/or second gas outlets for the seventh chamber and/or channel to the second pump and/or channel to the sixth chamber and/or channel to the tenth chamber.

The sample amplification step may be provided on the device. The sample amplification step may be provided on the same device as the sample receiving step and/or sample preparation step.

The sample amplification step may include a first inlet, preferably a channel. The channel may be connected to the sample receiving step and/or sample preparation step.

The sample amplification step may include a second inlet, preferably a channel. The channel may be connected to a pump, for instance the fourth pump on the device.

The sample amplification step may include a first outlet, preferably a channel. The channel may be connected to a sample storage step or location, for instance a chamber.

The sample amplification step may include a second outlet, for instance a channel. The channel may be connected to a further step, for instance a denaturing step and/or electrophoresis step and/or analysis step.

The first inlet and/or second outlet may be provided on the inlet channel for the amplification step. The first inlet and/or second outlet may share a section of channel and have separate channel sections.

The first outlet and/or second inlet may be provided on the outlet channel for the amplification step. The first outlet and/or second inlet may share a section of channel and have separate channel sections.

The sample amplification step may include a chamber, for instance an eleventh chamber. The chamber is preferably connected to the channel. The chamber preferably receives the sample. The sample may be a washed sample. The

sample may be a purified sample. The sample may be less than the whole of the sample provided to the device.

The chamber may be provided with a curved base. The base may be semi circular in cross-section. The base may be a part of a cylinder or hemisphere or proportion thereof. The chamber may be provided with a curved top. The top may be semi circular in cross-section. The top may be a part of a cylinder or hemispherical or a portion thereof.

The top may be a larger volume than the bottom. The top hemisphere or portion thereof may be larger than the lower hemisphere or portion thereof.

A transition surface may extend between the base of the chamber and the top of the chamber.

The chamber may include a support location for one or more particles, such as a bead. The one or more particles may provide one or more or all the reagents for a reaction, particularly an amplification, such as PCR. The support location may define a position of rest for the one or more particles. Preferably in the position of rest, the one or more particles do not block or obscure an inlet to and/or outlet from the chamber. Preferably in the position of rest at least 50%, preferably at least 60% and more preferably at least 70% of the surface area of the one or more particles are exposed to the chamber.

Preferably an inlet for a sample and/or an inlet from a previous chamber is provided in a side wall of the chamber. The inlet may be provided in the mid section of the height of the chamber, preferably the middle 20%, more preferably the middle 10%.

Preferably the outlet for the sample and/or outlet to a receiving location and/or other chamber is provided in a side wall of the chamber. The outlet may be provided in the mid section of the height of the chamber, preferably the middle 20%, more preferably the middle 10%.

The inlet and the outlet are preferably provided opposite one another. The inlet and the outlet are preferably provided at the same height in the chamber.

The chamber may have an orientation of use. A chamber may be provided with a horizontal base and/or a horizontal top. The base and/or top, may be horizontal $\pm 10^\circ$, preferably $\pm 5^\circ$ and more preferably $\pm 3^\circ$.

The chamber may be provided with one or more side walls. The side wall(s) may be vertical $\pm 10^\circ$, preferably $\pm 5^\circ$ and more preferably $\pm 3^\circ$.

The chamber may include a support location for one or more particles, such as a bead. The one or more particles may provide one or more or all the reagents for a reaction, particularly an amplification, such as PCR. The support location may define a position of rest for the one or more particles. Preferably in the position of rest, the one or more particles do not block or obscure an inlet to and/or outlet from the chamber. Preferably in the position of rest at least 50%, preferably at least 60% and more preferably at least 70% of the surface area of the one or more particles are exposed to the chamber.

Preferably an inlet for a sample and/or an inlet from a previous chamber is provided in the top of the chamber or in the upper section of the chamber. The upper section may be the upper 20%, more preferably the upper 10%.

Preferably the outlet for the sample and/or outlet to a receiving location and/or other chamber is provided in the top of the chamber. The upper section may be the upper 20%, more preferably the upper 10%. The inlet and the outlet may be the same.

Preferably the chamber is provided with a chamber filling outlet. Preferably fluid enters the chamber via the inlet and flows out of the chamber through the chamber filling outlet during the filling of the chamber. The chamber filling outlet is preferably provided in the base or lower section of the chamber, for instance the lower 20% or more preferably 10%.

The channel connected to the inlet to the chamber may be provided with a valve. The channel connected to the outlet from the chamber may be provided with a valve. One or more of the valves may be open state to closed state valves, particularly for the first inlet and/or second outlet channels. One or more of the valves may be closed state to open state valves, particularly for the second inlet and/or first outlet channels. One or more of the valves may be provided closer to the chamber than the split into the first inlet and second outlet and/or second inlet and first outlet sections of channel. If the valves are provided further from the chamber than the split into the first inlet and second outlet and/or second inlet and first outlet sections of channel, then a separate valve may be provided for each channel section.

The valve connected to the inlet may provide a first sealing location. The valve connected to the outlet may provide a second sealing location. One or more interconnected channels and chambers may be provided between the first sealing location and the second sealing location. Preferably the channel connected to the

inlet, the chamber and the channel connected to the outlet are provided between the first sealable location and the second sealable location.

The section of the device including the first sealable location, second sealable location and channels and chambers provided there between may have an extent, preferably in a first plane. The section of the device including the first sealable location, second sealable location and channels and chambers provided there between may have a planar form and/or planar exterior surface extending in a first plane.

A heating device may be provided to heat the chamber. The heating device may have an extent parallel to the first plane. The heating device may have an extent parallel to the first plane of the planar form of the section and/or planar exterior surface. The extent of the heating device may be greater than 75% of the extent of the channels and chambers between the first sealable location and the second sealable location. The extent of the heating device may be greater than 75% of the extent of the channels and chambers between the first sealable location and the second sealable location, considered in terms of the area those extend to in the first plane. The extent of the heating device may be greater than 80%, 90% or even 95%, possibly even 98% or 100% of such extents. The heating device may be incident with at least 75% of the extent of the channels and chambers provided between the first sealable location and the second sealable location, when the extent of those channels and chambers is projected perpendicular to the first plane. The extent of the heating device may be greater than 80%, 90% or even 95%, possibly even 98% or 100% of such extents.

The chamber may have an orientation of use. A chamber may be provided with a horizontal base and/or a horizontal top. The base and/or top, may be horizontal $\pm 10^\circ$, preferably $\pm 5^\circ$ and more preferably $\pm 3^\circ$.

The chamber may be provided with one or more side walls. The side wall(s) may be vertical $\pm 10^\circ$, preferably $\pm 5^\circ$ and more preferably $\pm 3^\circ$.

The junction between the base and the side walls may be curved. The junction between the top and the side walls may be curved. The junction between the top and the side walls may be provided by an intermediate wall. The intermediate wall may be inclined relative to the top and/or side walls.

The chamber may include a support location for one or more particles, such as a bead. The one or more particles may provide one or more or all the reagents for

a reaction, particularly an amplification, such as PCR. The support location may define a position of rest for the one or more particles. Preferably in the position of rest, the one or more particles do not block or obscure an inlet to and/or outlet from the chamber. Preferably in the position of rest at least 50%, preferably at least 60% and more preferably at least 70% of the surface area of the one or more particles are exposed to the chamber. The support location may be provided by the base of the chamber.

Preferably an inlet for a sample and/or an inlet from a previous chamber is provided in the top of the chamber or in the upper section of the chamber. The upper section may be the upper 20%, more preferably the upper 10%.

The inlet may be provided in a corner of the chamber.

Preferably the outlet for the sample and/or outlet to a receiving location and/or other chamber is provided in the top of the chamber. The upper section may be the upper 20%, more preferably the upper 10%. The inlet and the outlet may be provided at the same height.

The outlet may be provided in a corner of the chamber.

An inlet channel may be provided which leads to the inlet. An outlet channel may be provided which leads away from the outlet. A by-pass channel may be provided for the chamber. The by pass channel may connect a part of the inlet channel to a part of the outlet channel.

The by-pass channel may be a continuation of the channel from which the inlet channel and/or outlet channel branch. The by-pass channel and channel may have a common axis.

The by-pass channel may be a branch from the channel from which the inlet channel branches. The by-pass channel and/or inlet channel may be provided with an axis which is not a continuation of the axis of the channel from which they branch. Preferably, the by-pass channel is provided with an axis which is not a continuation of the axis of the channel from which it branches, with still more preferably the inlet channel being provided with on a common axis to that of the portion of the channel which adjoins it..

The by-pass channel may be a branch from the channel from which the outlet channel branches. The by-pass channel and/or outlet channel may be provided with an axis which is not a continuation of the axis of the channel from which they branch. Preferably, the by-pass channel is provided with an axis which is not a

continuation of the axis of the channel from which it branches, with still more preferably the outlet channel being provided with on a common axis to that of the portion of the channel which adjoins it.

5 Preferably one or more dimensions of the outlet channel are smaller than the corresponding dimension of the inlet channel. The value of the one or more dimensions may be considered at the location within the inlet channel and/or outlet channel where that dimension has its lowest value. The one or more dimensions may include one or more or all of the width and/or height and/or cross-sectional area. The cross-sectional area may be measured perpendicular to the direction of
10 flow in the inlet channel and/or outlet channel and/or perpendicular to the alignment or axis of the inlet channel and/or outlet channel.

The resistance to fluid flow provided by the outlet and/or outlet channel may be greater than the resistance to fluid flow provided by the inlet and/or inlet channel. The resistance to fluid flow provided by the outlet and/or outlet channel may be
15 greater than the resistance to fluid flow provided by the by-pass channel.

The path of least resistance for the fluid may be through the inlet and into the chamber until the fluid reaches the outlet and/or outlet channel. The path of least resistance for the fluid may be through the by-pass channel once the fluid has reached the outlet and/or outlet channel.

20 The fluid flow may switch from the inlet channel to the by-pass channel when a predetermined volume of fluid is provided in the chamber.

The chamber may have an orientation of use. A chamber may be provided with a curved base. The base may be semi circular. The base may be a hemisphere or proportion thereof. The chamber may be provided with a top wall, such as a
25 planar top wall. The top wall may be provided in one or more portions. The plane of one or more of those portions may be different to the plane of one or more of the other portions. Preferably the planes are parallel.

An inclined transition surface may extend between the base of the chamber and the side walls of the chamber. The side wall may connect to the top of the
30 chamber. The side walls may be vertical in the orientation of use.

The chamber may include a support location for one or more particles, such as a bead. The one or more particles may provide one or more or all the reagents for a reaction, particularly an amplification, such as PCR. The support location may define a position of rest for the one or more particles. Preferably in the position of

rest, the one or more particles do not block or obscure an inlet to and/or outlet from the chamber. Preferably in the position of rest at least 50%, preferably at least 60% and more preferably at least 70% of the surface area of the one or more particles are exposed to the chamber.

5 Preferably an inlet for a sample and/or an inlet from a previous chamber is provided in a side wall of the chamber. The inlet may be provided in the lower section of the height of the chamber, preferably the lower 30%, more preferably the lower 10%.

10 Preferably the outlet for the sample and/or outlet to a receiving location and/or other chamber is provided in a top wall of the chamber. The outlet may be provided in the top section of the height of the chamber, preferably the top 20%, more preferably the top 10%.

The inlet and the outlet are preferably provided opposite one another. The inlet and the outlet are preferably provided at different heights in the chamber.

15 The sample amplification step or a part thereof may have a first state in which it is isolated from one or more of the other steps in the cartridge and/or from one or more other parts of the sample amplification step. The one or more other steps may be a sample receiving step and/or sample preparation step and/or sample retention step and/or purification step and/or washing step and/or elution step and/or
20 electrophoresis step and/or analysis step. The sample amplification step or part thereof may have the first state during contacting of the sample with the chamber, particularly the chamber in which amplification is provided.

The sample denaturing step may be provided on the device. The sample denaturing step may be provided on the same device as the sample receiving step
25 and/or sample preparation step and/or sample amplification step. The sample denaturing step may include a chamber.

The chamber may be connected to the amplification step, preferably by a channel. The channel may be connected to the second outlet from the amplification step. The inlet to the chamber may be provided in the upper portion of the chamber,
30 for instance the upper 20%, preferably upper 10% and ideally upper 5%. The inlet may be in the top wall of the chamber. The inlet may be provided in the corner of the chamber.

The channel connecting the amplification step and/or amplification chamber to the chamber may have a plurality of sections. The channel may have a horizontal

section and/or a vertical section and/or a second horizontal section and/or a second vertical section. The channel may have a horizontal section and a vertical section and a second horizontal section and/or a second vertical section.

5 The channel may include one or more valves. The one or more valves may include an open state to closed state valve or valves and/or a closed state to open state valve or valves.

The chamber may have a non-linear cross-section. The chamber may have a cross-section formed by a horizontal top wall, inclined lower wall and end walls joining the top and lower walls. The transition end walls may be curved or linear.
10 The cross-section may be relative to a horizontal axis.

The chamber may have a sample outlet. The sample outlet may be provided in the lower portion of the chamber, for instance the lower 10%, more preferably lower 5% and ideally the lowest part of the chamber. The outlet may be in the bottom wall of the chamber or preferably in a corner of the chamber, ideally the
15 corner opposing the inlet.

The chamber may be connected to a pump, for instance an electrochemical pump. The pump may be a fourth pump provided on the device. The fourth pump may provide the drive to move one or more fluids and/or liquids through the amplification step and/or amplification chamber and/or chamber and/or one or more
20 further chambers and/or denaturing step.

The connection to the pump may be via the amplification step and/or amplification chamber.

In one embodiment, the amplification chamber is connected to the denaturing chamber, preferably with no further chambers provided there between.

25 The channel leading from the amplification chamber to the denaturing chamber may split into two channels. One of the two channels may lead, preferably past a valve, to the denaturing chamber. The denaturing chamber may have a vent channel which preferably extends past a valve. One of the two channels may lead, preferably past a valve to an archive chamber. The archive chamber may have a
30 vent channel which preferably extends past a valve.

The denaturing chamber may have an outlet channel which leads to the analysis step and/or electrophoresis step.

In a second embodiment, the pump may be connected to a channel which leads to an inlet for a further chamber. The further chamber may contain one or

more reagents or materials, for instance for denaturing the sample. The further chamber may have an outlet leading to a channel and/or to the amplification step and/or to the amplification chamber.

5 Particularly in a second embodiment, the chamber may be connected to a second chamber, preferably by a channel. The inlet to the second chamber may be provided in the upper portion of the second chamber, for instance the upper 20%, preferably upper 10% and ideally upper 5%. The inlet may be in the top wall of the second chamber. The inlet may be provided in the corner of the second chamber.

10 Particularly in a second embodiment, the channel connecting the chamber to the second chamber may have a plurality of sections. The channel may have one or more horizontal sections and/or one or more vertical sections.

15 Particularly in a second embodiment, the second chamber may have a non-linear cross-section. The chamber may have a cross-section formed by a horizontal top wall, inclined lower wall and end walls joining the top and lower walls. The transition end walls may be curved or linear. The cross-section may be relative to a horizontal axis.

20 Particularly in a second embodiment, the second chamber may have a sample outlet. The sample outlet may be provided in the lower portion of the second chamber, for instance the lower 10%, more preferably lower 5% and ideally the lowest part of the second chamber. The outlet may be in the bottom wall of the second chamber or preferably in a corner of the second chamber, ideally the corner opposing the inlet.

25 Particularly in a second embodiment, the second chamber may be connected to a third chamber, preferably by a channel. The inlet to the third chamber may be provided in the upper portion of the third chamber, for instance the upper 20%, preferably upper 10% and ideally upper 5%. The inlet may be in the top wall of the third chamber. The inlet may be provided in the corner of the third chamber.

30 Particularly in a second embodiment, the channel connecting the second chamber to the third chamber may have a plurality of sections. The channel may have one or more horizontal sections and/or one or more vertical sections.

Particularly in a second embodiment, the third chamber may have a non-linear cross-section. The chamber may have a cross-section formed by a horizontal top wall, inclined lower wall and end walls joining the top and lower walls. The transition end walls may be curved or linear. The cross-section may be relative to a

horizontal axis.

Particularly in a second embodiment, the third chamber may have a sample outlet. The sample outlet may be provided in the lower portion of the third chamber, for instance the lower 10%, more preferably lower 5% and ideally the lowest part of the third chamber. The outlet may be in the bottom wall of the third chamber or preferably in a corner of the third chamber, ideally the corner opposing the inlet.

The sample denaturation step or a part thereof may have a first state in which it is isolated from one of more of the other steps in the cartridge and/or from one or more other parts of the sample denaturation step. The one or more other steps may be a sample receiving step and/or sample extraction step and/or sample retention step and/or washing step and/or sample amplification step and/or electrophoresis step and/or analysis step. The sample denaturation step or part thereof may have the first state during contacting of the sample with the chamber and/or second chamber and/or third chamber and/or during denaturation of the sample. The sample preparation step or a part thereof may be provided with one or more valves, preferably to provide the isolation. The first inlet to the amplification step and/or amplification chamber may be provided with a valve, preferably of the open state to closed state type. The first outlet from the amplification step and/or amplification chamber may be provided with a valve, preferably of the open state to closed state type. The outlet from the denaturation step to the electrophoresis step and/or the channel connected to the outlet of the third chamber may be provided with a valve, preferably of the closed state to open state type.

The electrophoresis step may be provided on the device. The electrophoresis step may be provided on the same device as the sample receiving step and/or sample preparation step and/or sample amplification step. The electrophoresis step may include a channel.

The channel may be connected to the amplification step and/or denaturing step. The channel may extend from the plane of the device to a location behind the plane of the device.

The electrophoresis step may be provided on an element. The element may be a part of or be separate from the device. The element may be planar. The element may include one or more channels. The element may include one or more channels in which electrophoresis is provided. One or more electrodes may be

provided on the element. One or more electrodes may be provided to load the sample into the element. One or more electrodes may be provided to perform the electrophoresis step. The electrodes may be provided in portions which have a greater depth than one or more other parts of the element. The one or more other parts of the element may include the part in which the channel is provided. The electrodes may be provided in portions which adjoin end portions of the element. The end portions may provide the mounting for the element on a carrier and/or relative to the device, such as a cartridge.

The connection between the one or more electrodes and the operating electronics for the instrument may be provided by one or more pins mounted on the element. The one or more pins may be spring loaded. The one or more pins may be partially or fully recessed into a surface of the element, particularly the greater depth portion(s) thereof. The connection may be provided or may be further provided by one or more pins mounted on the instrument. The one or more pins may be spring loaded. The connection may be made when the element is put in the use position.

The element, and particularly a channel therein, may be connected to the device, such as a cartridge, by a conduit. The conduit may be flexible. The conduit may be a tube.

The channel may be connected to a chamber. The chamber may contain a liquid to matrix interface, preferably a horizontal interface. A pump, preferably an electrochemical pump, preferably the fourth pump may convey the sample to the chamber. The pump, preferably the electrochemical pump, may also convey a buffer and/or formamide to the chamber. The buffer and/or formamide may displace the content of the amplification and/or PCR chamber into the chamber. The buffer and/or formamide may include one or more components for the electrophoretic separation and/or analysis. The one or more components may include a size standard.

The sample may be concentrated before the start of electrophoresis.

The sample may be concentrated before the sample enters the matrix. The sample may be concentrated by the electrophoretic velocity on one side of the interface exceeding the opposing electroosmotic velocity and/or by the electrophoretic velocity on the other side of the interface being less than the opposing electroosmotic velocity.

The sample may be collecting and/or concentrated at a first location. The

sample may be further collected and/or concentrated at a second location.

The sample may be collecting and/or concentrated at a first location in the form of an interface. The sample may be further collected and/or concentrated at a second location in the form of an interface. The first interface may be planar. The second interface may be planar. The first interface may be provided by a series of surfaces. The second interface may be provided by a series of surfaces. The series of surfaces may be provided by particles or beads or channels or mixtures thereof

The sample may be collecting and/or concentrated at a first interface. The sample may be further collected and/or concentrated at a second interface. The sample may be stacked at a first interface. The sample may be further stacked at a second interface.

The first interface may be a liquid to liquid interface or liquid to solid or gel interface or solid or gel to solid or gel interface. The first interface may be a membrane. The second interface may be a liquid to liquid interface or liquid to solid or gel interface or solid or gel to solid or gel interface. The second interface may be a membrane. The first and second interfaces may be of the same or different types.

The sample or a part thereof may be collected and/or concentrated by flowing a first fluid past a first side of an interface. The sample or a part thereof may be collected and/or concentrated by flowing a second fluid past a second side of an interface.

The sample may be fed to one side of the interface, for instance the first side. A reagent, for instance a buffer may be fed to the other side of the interface., for instance the second side. The channel feeding the sample and/or the channel feeding the reagent to the channel containing the interface may be at least partially with the channel containing the interface. The channels may be aligned at an angle of less than 30° . Both channels may be so aligned. The channel feeding the sample and/or the channel feeding the reagent to the channel containing the interface may be curved so as to align their flow with the direction of flow within the channel containing the interface.

The channel for electrophoresis may be provided at an angle to the interface, for instance greater than 75° . The channel may be perpendicular to the interface, particularly the plane thereof. The channel for electrophoresis may be provided at an angle to the first and the second interface, for instance greater than 75° . The channel may be perpendicular to the first and the second interface, particularly the plane

thereof.

The sample or a part thereof may be collected and/or concentrated at the first interface and then at the second interface. Conditions on one or both sides of the first interface may be varied to cause collection and/or concentration at the first interface. Conditions on one or both sides of the second interface may be varied to cause collection and/or concentration at the first interface. Conditions on one or both sides of the first interface may be varied to cause collection and/or concentration at the second interface. Conditions on one or both sides of the second interface may be varied to cause collection and/or concentration at the second interface. The conditions which are varied may be one or more of reagent or reagents present, the reagent or reagents concentration, pH, temperature, conductivity of the components present or electrical potential present. The conditions may vary at or in proximity with the interface.

The electrical potential may be applied by a voltage across a first electrode and a second electrode. The first electrode may be provided to one side of the interface, particularly the first and the second interfaces. The second electrode may be provided to the other side of the interface, particularly the first and second interfaces. The first electrode may be provided in a channel or chamber connected to, but spaced from the channel through which the sample is introduced and/or in which the interface is provided. The second electrode may be provided in the channel for electrophoresis. The second electrode may be provided beyond the channel for electrophoresis, compared with the position of the first electrode.

The second interface may lead to the channel for electrophoresis. The second interface may be in contact with the matrix within the channel for electrophoresis.

The sample may be introduced to a channel, that channel being in contact with a first interface. That channel may be in contact with a second interface. The first and second interfaces may be provided at opposing ends of the channel. The first and second interfaces may be provided in opposition to one another, with a length of channel there between. The length of channel there between may include the channel in which electrophoresis is provided.

An electrode may be provided on the side of the first interface away from the channel. An electrode may be provided on the side of the second interface away from the channel. An electrical potential may be applied to one or both of the

electrodes, preferably across the electrodes.

At least a part of the sample, such as DNA in the sample, may be moved towards the first interface by the electrical potential. The first interface may be downstream of a second interface relative to the direction in which the sample flows
5 into the channel. The electrical potential may be applied as the sample flows through the channel. The flow may be from an inlet to an outlet. A waste sample chamber may be provided downstream of the channel.

The first and/or second interface may be impermeable to one or more components of the sample, such as DNA. The first and/or second interface may be
10 impermeable to components of greater than 5kDa, or even greater than 8kDa.

A further material may flow into the channel, preferably after the sample flow and/or after the at least a part of the sample is at the first interface. The further material may displace the sample flow from the channel. One or more other materials may flow through the channel between the sample flow and the further
15 material.

The further material may provide a matrix for the electrophoresis in the channel. The further material may be introduced into the channel and then altered to provide the matrix for electrophoresis. The further material may be altered by the application of light, such as UV light, and/or heating. The further material may be
20 altered by polymerisation.

The one or more other materials may include one or more buffers and/or one or more salt removal agents and/or one or more DNA purification reagents and/or one or more PCR primer removal reagents.

The sample may be introduced to a channel, that channel being in contact with a first interface. The first interface may be provided at one end of a channel,
25 with a length of channel there between. The length of channel there between may include the channel in which electrophoresis is provided.

An electrode may be provided on the side of the interface away from the channel. An electrode may be provided on the other side of the interface, with the length of the channel provided between that electrode and the other electrode. An
30 electrical potential may be applied to one or both of the electrodes, preferably across the electrodes.

At least a part of the sample, such as DNA in the sample, may be moved towards the interface by the electrical potential. The interface may be provided in a

wall of the channel through which the sample flows and/or may be across a channel extending off the channel within which the sample flows. The electrical potential may be applied as the sample flows through the channel. The flow may be from an inlet to an outlet. A waste sample chamber may be provided downstream of the channel.

The first interface may be impermeable to one or more components of the sample, such as DNA. The first interface may be impermeable to components of greater than 5kDa, or even greater than 8kDa.

The electrical potential may be used to transfer the at least a part of the sample from the interface to the matrix in which electrophoresis is conducted. The electrical potential may be reversed to provide this transfer.

One or more other materials may flow through the channel after the sample flow.

The one or more other materials may include one or more buffers and/or one or more salt removal agents and/or one or more DNA purification reagents and/or one or more PCR primer removal reagents.

The channel may be connected to the electrophoresis channel. The electrophoresis channel may be linear. The electrophoresis channel may have a side channel, preferably the side channel is connected to the channel. The electrophoresis channel may have a second side channel. The second side channel may be axially aligned with the first side channel or may be offset relative thereto. The electrophoresis channel may be provided with an electrode at one end of a separation length and a second electrode at the other end of a separation length. The first side channel and/or second side channel may be provided with an electrode. One or more of the electrodes may have a coating, for instance a platinum coating, gold coating, carbon coating, nickel coating. One or more of the electrodes may be of platinum, gold, carbon or nickel.

The channel may be provided with a first side channel through which the sample or at least a part thereof is introduced. The first side channel may provide flow in the direction of gravity to the channel. The channel may be provided with a second side channel, preferably through which the sample or a part thereof exits the channel. The second side channel may provide flow in a direction against gravity away from the channel. The junction between the first side channel and the channel may be spaced along the channel when compared with the junction between the second side channel and the channel.

A detection location may be provided at a position along the separation

length.

The sample amplification step may include a split into a first channel and a second channel. The first channel may be connected to the amplification step and/or amplification chamber as described above. The second channel may be connected to
5 a second amplification step and/or amplification channel.

The sample amplification step may include a supply of sample to a first channel and a second channel. The first channel may be connected to the amplification step and/or amplification chamber as described above. The second channel may be connected to a second amplification step and/or amplification
10 channel.

The first amplification step and/or amplification chamber may be connected in series with the second amplification step and/or amplification chamber. The first amplification step and/or amplification chamber may be connected in parallel with the second amplification step and/or amplification chamber.

The second amplification step and/or second amplification chamber may have any of the features, options and possibilities set out elsewhere, including those of the amplification step and/or amplification chamber.

The second amplification step and/or second amplification chamber may be provided with a quantification unit, for instance for the amount of sample therein, ideally the amount of DNA. The quantification unit may provide the amount of sample at one or more times before, during or after amplification in the second amplification step and/or second amplification chamber.

The quantification unit may include one or more reagents provided in or introduced to the second amplification step and/or second amplification chamber.

The quantification unit may include a device sensitive to a characteristic of the sample and the amount thereof. The characteristic may be light, particularly fluorescent light. The quantification unit may include the optical system and/or light detector used in the electrophoresis step and/or analysis step.

The sample preparation step may include one or more chambers, preferably
30 into which the sample passes.

The chamber may be provided connected to one or more further chambers. Each of the chambers may be provided with a one or more particles. The particles may be beads. One or more of the particles may be magnetic. The one or more particles may have a magnetic material within a surface layer or layers. The

particles may be provided with one or more reagents or materials which releasable bind and/or link and/or combine with a part of the sample, for instance DNA. The particles, such as beads, may be stored in the chamber before use. The particles, such as beads, may be introduced to the chamber to prepare it for use, for instance
5 within 5 hours, or even within 1 hour, of use occurring.

A plurality of chamber may be provided, connected in series. Two or more of the chambers may have an inlet in the upper portion of the chamber, for instance the upper 20%. The inlet may be in the top wall of the chamber. Two or more of the chambers may have an outlet in the lower portion of the chamber, for instance
10 the lower 20%, more preferably lower 10% and ideally the lowest part of the chamber. The outlet may be in the bottom wall of the chamber.

The chambers may be connected to each other by one or more channels. Preferably the channel has a plurality of sections. The channel may have one or more vertical sections and/or a one or more horizontal sections.

Two or more of the chambers may have the same configuration and/or shape. One or more of the chambers may have a different configuration and/or shape to one or more of the others.
15

One or more of the chambers may be a channel or passageway which is larger in respect of one or more dimensions than the channel leading to it and/or
20 from it. The one or more particles may be provided in the channel or passageway which is larger.

One or more chambers may be provided having a particulate collection and/or holding location. Flow into the chamber preferably passes through the particulate collection and/or holding location, preferably preferentially to flow
25 through other locations in the chamber. The particulate collection and/or holding location may be a recess in the bottom of the chamber.

One or more chambers, channels or passageways may be provided in which the one or more particles are provided in a channel connected to the one or more chambers, channels or passageways. The one or more particles may be displaceable
30 from the channel into the one or more chambers, channels or passageways. A material may be provided in the channel to displace the one or more particles.

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

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.

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

The sample may be a forensic sample. The sample may be a medical sample.

The analysis may be for diagnostic purposes. The analysis may be for forensic purposes.

10 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
15 pedigree testing in animals.

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

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
20 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.

The device may process and/or contain a fluid, particularly a liquid, of less
25 than 1ml, possibly less than 500:1, possibly less than 250 :1, potentially less than 200 :1, possibly less than 175 :1, possibly 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
30 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 detection step and/or analysis step and/or results output step.

The device may incorporate one or more channels or chambers with a

maximum dimension of less than 1000:μm, possible less than 750:μm and preferably less than 550:μm.

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

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.

10 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.
15 One or more reagents may be provided for denaturing. One or more reagents may be provided for electrophoresis.

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
20 stored form. One or more reagents may be loaded into the device in the use form.

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
25 electrolyte and/or pump electrolyte concentration.

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

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.

30 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.

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%.

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

5 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.

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

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

15 One or more surfaces of the channel may be treated, for instance with a hydrophilic coating, for instance poly(hydroxyethylacrylamide).

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.

25 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.

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.

5 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.

10 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.

15 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.

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

25 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.

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
5 sample from the location of one or more other components of the sample.

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
10 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.

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

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
20 conditions, preferably of a cyclic nature, to cause denaturing.

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
25 or more concentrations and/or presence of one or more characteristics and/or absence of one or more characteristics.

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
30 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.

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.

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.

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.

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 200m of the site of the instrument, the further processed results.

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

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

Figure 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;

10 Figure 2 is a schematic illustration of the key steps provided on or by an instrument embodying the present invention;

Figure 3a is a front face view of part of a cartridge embodying the present invention;

15 Figure 3b is a table of dimensions and volumes for a cartridge according to the present invention, and components thereof;

Figure 4 is a front face view of a further part of the cartridge of Figure 3a and embodying further features of the present invention;

Figure 5a is a side view of the section of the cartridge of Figure 3a and 4 where it joins the electrophoresis cartridge section;

20 Figure 5b is a front view of the electrophoresis cartridge section shown in Figure 5a, with the section of the cartridge omitted;

Figures 6a to 6e are schematic illustrations of alternative arrangements for contacting the fluid and beads;

25 Figure 7 is an illustration of an alternative structure for providing sample to the PCR chamber;

Figure 8 is a front view of the electrophoresis cartridge section showing an alternative form of injector;

Figure 9 is a schematic illustration of the parallel PCR chamber arrangement used in providing real time PCR and feedback of the results;

30 Figure 10a is an illustration of a closing valve used in the present invention;

Figure 10b is an illustration of an opening valve used in the present invention;

Figure 11 shows an option for the archiving of a part of the sample handled;

Figure 12 is a schematic front view of one embodiment of the instrument;

- Figure 13 is a side view showing the insertion of the cartridge into the instrument;
- Figure 14 is a schematic of the light source, optics and detector setup for the electrophoresis section of the instrument;
- 5 Figure 15 is an electropherogram showing the variation in signal from the detector setup with time;
- Figure 16 is a schematic of an example of a system for detecting fluorescence;
- Figure 17 is a plot of LED spectrum, light reflected, and residual LED light over a range of wavelengths;
- 10 Figure 18 is a plot of power of the LED-module over time;
- Figure 19 is an illustration showing beam shape and size as measured by the laser camera;
- Figures 20a and 20b are plots of CCD signal v/s wavelengths for static fluorescence measurements; and
- 15 Figure 21 is a plot of CCD signal v/s time for dynamic fluorescence measurements;
- Figure 22 is an illustration of a PCR chamber according to a further embodiment;
- Figure 23 is an illustration of the position of stacked Peltier effect devices;
- 20 Figure 24 is an illustration of an embodiment for loading a CE channel
- Figure 25 is an illustration of a further embodiment for loading a CE channel;
- Figure 26 is an illustration of a further embodiment of a PCR chamber;
- Figure 27 is a front face view of a cartridge according to an embodiment;
- Figure 28a is a front face view of a cartridge according to a different embodiment;
- 25 Figure 28b is a table of dimensions and volumes for the Figure 28a cartridge;
- Figure 29a is a perspective view of an embodiment of the instrument;
- Figure 29b is a front view of the instrument of Figure 29a;
- Figure 29c is a side view of the instrument of Figure 29a;
- 30 Figure 30 is a perspective view of another instrument embodiment;
- Figure 31a is an illustration of a carrier, cartridge and CE chip embodiment;
- Figure 31b is an illustration of a detail of the carrier to cartridge engagement;
- Figure 32a is an illustration of a carrier to CE chip engagement;
- Figure 32b is a cut away illustration of a part of the Figure 32a engagement;

Figure 33a is an illustration of the tube and cartridge connection;
Figure 33b is an illustration of the tube to CE chip connection;
Figure 34a is an illustration of the carrier being inserted into the instrument;
Figure 34b is an illustration of the inserted carrier;
5 Figure 35a is an illustration of the cartridge and carrier in the insertion form;
Figure 35b is an illustration of the cartridge and carrier in the use form;
Figure 35c is an illustration of the cartridge returned to the carrier;
Figure 36a is a perspective view of the position of the pair of calipers;
Figure 36b is a perspective view of the back of the pair of calipers;
10 Figure 36c is a plan view of the caliper structure in the open form;
Figure 36d is a plan view of the caliper structure in the closed form;
Figure 37a is a perspective view of the second support of the carrier and CE
chip;
Figure 37b is a partial cut away illustration of the second support and CE
15 chip;
Figure 38 is a perspective view of the CE chip heater board;
Figure 39 is a perspective view of an embodiment of the optics;
Figure 40a is a perspective view of the alignment structure;
Figure 40b shows the alignment structure of Figure 40a in the stowed
20 position;
Figure 40c shows the alignment structure of Figure 40a in the use position;
Figure 41a shows three positions for an alternative PCR chamber
embodiment;
Figure 41b shows two positions for a further PCR chamber embodiment;
25 Figure 41c shows three positions for a still further PCR chamber
embodiment;
Figure 42a shows a CE chip embodiment;
Figure 42b shows a detail of the CE chip of Figure 42a;
Figure 43 shows an approach to loading sample to the CE step;
30 Figure 44 shows an alternate approach to loading sample to the CE step;
Figure 45 shows a further alternative for loading sample to the CE step
Figure 46 shows a further embodiment of a PCR chamber..

Detailed Description of the Preferred Embodiments

Overview

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.

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.

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.

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.

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.

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.

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.

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 Figure 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.

The structure and processes performed within the instrument 11 and cartridge 9 are described further below in the context of Figure 2.

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.

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.

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.

Referring to Figure 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.

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.

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

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.

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

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.

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.

In the data communication step 210, the instrument compiles the necessary data packet for transmission and transmits it to a remote location for consideration.

5 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.

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.

10 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
15 and the like to consider multiple samples, with reduced capital costs.

Cartridge

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
20 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.

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
25 provided.

Figure 3a 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 amplification step 204. The subsequent steps and their respective parts of the cartridge 9 are illustrated separately.

30 Figure 3b 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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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, MgCl₂, 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.

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.

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

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

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.

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.

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.

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.

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

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.

The overall configuration of the electrophoresis step 206 can be seen in the side view of Figure 5a and front view of Figure 5b.

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.

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

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.

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.

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.

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

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.

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.

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.

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 US patent application no 61/096424, the contents of which are hereby incorporated by reference, offers approaches for determining the sizes of the unknowns from the size standard.

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

Other embodiments of the cartridge have also been developed.

As shown in Figure 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.

15 The form shown in Figure 27 also features guide holes 27-13 which are used in the alignment of the cartridge and instrument, as described in more detail below.

A preferred embodiment of the cartridge is shown in Figure 28a. 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.

Figure 28b 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.

25 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.

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.

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 Figure 3a embodiment. The binding buffer storage chamber 28-314 has an outlet channel 28-320 which leads to mixing/purification chamber 28-322.

5 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.

10 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
15 heated or may simply provide the necessary dwell time and/or other binding conditions needed.

 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
20 28-352 which passes valve 28-354 and is connected to second electrochemical pump 28-356.

 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
25 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.

 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
30 and leads from second electrochemical pump 28-356 through wash buffer chamber 28-378 to the capture chamber 28-358.

 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.

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
5 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
10 step 202.

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
15 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.

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
20 dNTPs and PCR reaction mix, including Tris buffer, MgCl₂, 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.

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
25 archive of the PCR product.

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.

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

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.

5 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.

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.

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.

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.

Details of the connection of the inlet 28-570 to the CE chip are provided below.

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.

Cartridge Sequence of Operation

30 The sequence of operation, purely by way of example, applied to the cartridge shown in and described in relation to Figure 3a 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	
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	
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	

Cartridge Alternatives

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.

1) Bead Handling

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.

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.

5 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.

10 Secondly, it is possible to provide the beads in a series of bead storage chambers, rather than in a single chamber. Figure 6a 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 Figure 6b, where a first bead storage chamber 700a is separated from the second bead storage chamber 700b by a mixing chamber 702.

15 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.

20 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 Figure 6d 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.

25 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
30 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.

2) PCR Chamber Filling

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.

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

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.

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

In a third alternative, the configuration shown in Figure 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.

5 Various shapes are possible for the PCR chamber. Figure 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
10 channel 26-09.

In the embodiment of Figure 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
15 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
20 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.

25

3) Sample Concentration Before Capillary Electrophoresis

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.

30 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.

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.

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.

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

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.

In the Figure 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.

In the Figure 25 form, the stacking interface is similarly provided.

30

4) Alternative Electrophoresis Channel Configuration

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 Figure 8.

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.

5 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
10 the minimal size of the plug which is then used in the capillary electrophoresis.

A further electrophoresis channel configuration is shown in Figure 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, 10-14kDa
15 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.

The polarity may be provided in the reverse direction before the CE run, for
20 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.

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,
25 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.

A still further configuration is shown in Figure 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
30 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.

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

A yet further configuration is shown in Figure 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.

5) Cartridge Variant for Real Time PCR Performance

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.

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.

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

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

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.

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.

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.

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 reagents for PCR and for a quantification analysis.

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.

Further details on the operation of such a system and the use of this feed back are to be found in 61/026869, 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.

Suitable reagents include the Plexor HY kit available from Promega Inc, 2800 Woods Hollow Road, Madison, Wisconsin 53711, USA and Quantifiler® Duo DNA quantification kit available from Applied Biosystems, Foster City, California, 944404, USA.

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.

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.

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.

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:

5 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
10 information from one processing to the other.

 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.

15 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
20 may be provided within the sample to provide a reference value with respect to the light detected.

 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
25 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

30 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

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; Figures 10a; and an opening valve 2002; Figures 10b.

5 The closing valve 2000 is shown schematically in Figure 10a. 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 3mm in diameter and is provided with the conduit 2006. On the top of
10 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.

The dotted line in Figure 10a 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.

15 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.

20 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).

Continued displacement of the paraffin wax into the channel 2004 causes the
25 paraffin wax to expand in each direction along the channel 2004.

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 2mm to 10mm length of the
30 channel 2004 is filled. With the heat removed, the paraffin wax sets in this new position and the channel 2004 is reliably sealed.

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.

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 Figure 10a the bend 2014 provides assistance in the accurate formation of the seal within the channel 2004.

5 The opening valve 2002 is shown schematically in Figure 10b. 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.

10 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 Figure 10b 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.

15 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
20 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.

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

25 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.

 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
30 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.

 A further valve embodiment is shown in Figure 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.

5 2) *Chambers*

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.

10 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.

15 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.

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.

20 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
25 based mixing.

30 The PCR chamber 416 has two principle embodiments; as described above. In each, the PCR reagents 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.

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, Figure 22.

5 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.

10

3) Vents

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

15 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.

20 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
25 polypropylene material and/or by providing a polysulphone coating.

4) Archive

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

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.

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.

In the form shown in Figure 3, the archive chamber 422 is an integral part of the overall cartridge 9.

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

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.

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.

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.

30

5) Reagents

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 reagents inside. Other pre-loaded reagents include the various wash liquids and elution liquids described in the methodology above.

5 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.

10 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.

15

6) Electrochemical Pumps

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
20 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.

25 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.

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;
30 and used in conjunction with aluminium electrodes.

7) Electrophoresis Matrix

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

Various possible materials can be used in the capillary. These include the use of polymer matrix, for instance a polyhydroacrylamide, a polydimethylacrylamide or mixtures thereof. 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.

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(hydroxyethylacrylamide).

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.

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

A preferred configuration for the CE chip is shown in Figure 42a and the detailed partial view of Figure 42b.

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.

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.

5 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*

10 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.

15 As shown in Figure 41a, 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 left hand 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
20 contents to be emptied.

 A variant of this approach is shown in Figure 41b, 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.

25 The variant in Figure 41c 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.

30 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.

 Rotation may be provided by cooperation between an actuator and a slot in

the circular wall.

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

5 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

10 The instrument 11 is illustrated in Figure 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,
15 the cartridge 9 and components on the printed circuit board 8008 contact one another.

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.

20 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.

25 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.

30 Another embodiment of the instrument is shown in Figures 29a, 29b and 29c. 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.

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.

5 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.

A stylus 29-8018 is used by the operator to interact with the display 29-8012. Various control buttons 29-8020 are provided below the screen 29-8012.

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

The side panel 29-8022 is removable for maintenance purposes.

The embodiment of Figure 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. 15 The workspace 30-8024 also includes the lane finding apparatus 30-8028.

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

20

Cartridge to Instrument Interface

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.

25 As a first step, the latch 8004 is released and the door 8002 is opened.

To insert the cartridge 9, Figure 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 30 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.

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.

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.

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.

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.

The components provided on the printed circuit board include:

- 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.
- 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.
- 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.

- 5 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

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:

- 10 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.
- b) The cartridge 9 can be mechanically clipped to the printed circuit board 8008, with the clip(s) applying a compressive force.
- 15 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.
- 20 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.
- 25 e) As shown in Figure 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
- 30

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 150oC 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.

5

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.

10

Alternative Cartridge to Instrument Interface

In the alternative embodiments of the instrument described above in relation to Figures 29a, b, c and Figure 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.

15

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.

20

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.

25

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..

30

The cartridge carrier 31-03 is illustrated in Figure 31a. 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.

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.

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.

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

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.

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.

The CE chip 32-31 is inserted into the carrier 32-03 as shown in Figure 32a. The CE chip 32-31 is slid into a slot. As shown in figure 32b, 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.

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 Figure 28a) to become connected to the tube

33-45. As shown in Figure 33a, 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 Figure 33b, 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.

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.

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

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; Figure 34b.

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.

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.

As with other embodiments, it is important to provide effective and accurate contact between the cartridge and the instrument interface. In Figures 35a, b and c

the provision of the contact is illustrated.

Figure 35a 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.

In the next step, Figure 35b, 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 figure 27) and so ensure that the alignment between the cartridge and the PCB is correct in that orientation too.

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, Figure 35c. The carrier 35-03 can then be removed by lifting it out of the slot 35-47, taking with it the cartridge 35-01.

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 Figure 36a, b, c and d.

In Figure 36a, 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 not interfere with the contact sought between the cartridge 36-01 and the PCB during the movement from the insertion position to the use position.

The front caliper 36-100a 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.

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

5 In the open position shown in Figure 36c, 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.

10 In the closed position shown in Figure 36d, 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 36-100a towards the cartridge 36-01 and towards the opposing second Peltier effect device 36-100b. This actuation, combined with the floating nature of the pair of calipers 36-100 brings
15 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.

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

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

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
25 independent relative movement of the CE chip. This is important in ensuring correct positioning of the CE chip for the CE step. This is achieved by the structure and operation shown in Figure 37a and b.

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
30 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.

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; Figure 37a. In Figure 37b,

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
5 through apertures 37-112a, 37-112b. The projecting pins 37-104 thus pass through these apertures 36-112a, 36-112b too as the carrier 37-03 approaches the work surface 37-49.

The conical ends of the pins 37-104 mean that they enter the apertures 37-106, 37-112a, b, even where there is potential misalignment. The fuller diameter
10 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 Figure 38.

Electrophoresis Components

15 **1) Optics**

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.

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

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;
25 Figure 14.

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 491nm with a maximum power of 50mW. The light emitted by the laser 900 is fed to a fibre coupler 904 (09 LFC 001, f=3.5mm
30 from Melles Griot, 2051 Palomar Airport Road, 200, Carlsbad, CA 92011, USA) and hence into an patch cable assembly (M31L01, from Thorlabs, 435 Route 206 North, Newton, New Jersey, 07860, USA) and optical fibre 906 (GIF625, dia 62.5:μm, NA = 0.275 from Thorlabs, 435 Route 206 North, Newton, New Jersey,

07860, USA).

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
5 end of the optical fibre 906 a power of up to 45.32mW is still observed.

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

The spot mirror 910 is used to both direct the laser light to the detection
10 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 25mm round glass disc which transmits all light from <80 above 380nm. An ellipse, 2mm long by 1mm wide, is provided at the centre of the reflector 910 (so as to present an effective
15 1mm circular mirror), formed of a highly reflective mirror layer deposited there (reflectivity of 99.99%).

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
20 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 27.40mW.

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
25 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.

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=50mm, D=25mm, with anti-
30 reflective coating within 350-650nm, made of BK7 glass, Thorlabs Inc), to direct the light to the charge coupled device 916. The CCD 916 has spectroscopic abilities.

The CCD 916 generates the signals which are then used to generate the electropherogram, an example of which is shown in Figure 15

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 2.5pM of fluorescein dye at pH 7.

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.

An alternative embodiment of the optics is shown in the cut away perspective view of Figure 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.

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

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).

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.

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
5 be a physical mark (for instance etched) on the cartridge and/or a coloured mark (for instance a dye) and/or a quantum dot.

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
10 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
15 provide direct verification for the Z axis position relative to the capillary 616.

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
20 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
25 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
30 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).

The Figure 39 and Figure 40a, b and c embodiment shows the alignment structure 39-15 and its operation.

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.

5 In the stowed position, Figure 40b, the swing arm is positioned in contact with a hard stop 40-106 mounted on the casing 40-102 too. In the check position, Figure 40c, 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.

10 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
15 also be used to achieve focussing of the system in the Z axis adjustment.

3) Electrophoresis Environment Control

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
20 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.

25 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.

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
30 consistent positioning and good contact.

4) Use of LED's as Light Source

Figure 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.

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 13x13 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.

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 ($NA \sim n \sin(2_{1/2}) \sim 0.26$). The light is then focused onto a plano-convex lens ($f=35$ mm, $D=25$ mm; $NA \sim D/2f \sim 0.36$). $NA_{LED} < NA_{lens}$ 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; $NA \sim D/2f \sim 0.25$, 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 ($\varnothing = 600$ μ m, NA = 0.22) optical fibre assembly (attenuation: 0.013 dB/m at 505 nm - relative transmission: 82% (arm 1) and 87% (arm 2)).

Table 1 illustrates a power optimization of the system depicted in Figure 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	l	L_{max}	P_{sos}
Hemispherical	3 cm	20 mm	225.2 μ W
Hemispherical	5 cm	18 mm	200.4 μ W
Hemispherical	8 cm	19 mm	222.8 μ W
Cylindrical	3 cm	9 mm	170.9 μ W
Cylindrical	5 cm	9 mm	164.1 μ W
Plano-convex	3 cm	16 mm	220.9 μ W
Plano-convex	5 cm	15 mm	204.1 μ W
Plano-convex	8 cm	15 mm	173.7 μ W
None	None	12 mm	187.4 μ W

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 μ W.

Figure 17 is a plot of LED spectrum, light reflected, and residual LED light over a range of wavelengths (nm). Figure 17 illustrates an LED spectrum obtained in the cooled CCD (diodes: $U_g = 2.0$ 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.

Figure 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%. Figure 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 μ W over 5min, i.e. 0.48%.

Figure 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-fiber distances, and tilt in the optical elements. In the results reported in the next section, the situation corresponding 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 (40X, NA = 0.75, WD = 0.40 mm) and adjustable filters. The objective was brought within ~ 8 mm of the Olympus LUCPLFLN (20X, 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).

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 20 µm 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.

The loaded chip is placed on the CE setup and the focus of the 63X sample objective is aligned with the bottom of the channel. The emitted fluorescent light is gathered onto the 26.6 mm x 6.7 mm (1024 x 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.

Figures 21A and 21B are plots of CCD signal v/s wavelengths. The plots indicate the vertically-binned signal from a 1 μ M 6-FAM solution loaded into a glass microchannel (A) and a 1 μ M 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: $8_{\text{max}}^{\text{em}} = 513$ at pH=13, 6-FAM: $8_{\text{max}}^{\text{em}} = 517$ at pH=9). The signal-to-noise ratio is 87 for 1 μ M 6-FAM in glass and 36 for 1 μ M 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 μ M 6-FAM	0.98 mW	720	36
COC	1 μ M 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.

The DNA fragments are separated by electrophoretically migrating within a sieving polymer matrix such as POP-5TM (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, 1X A.C.E.TM buffer (Amresco, Inc.) is flushed into the channel by vacuum followed by POP-5TM. A 1 μ M 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. 1X A.C.E.TM buffer is placed in the sample waste, buffer waste, and waste wells to ensure ionic conductivity in the whole device.

Figure 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 μ M 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 Figure 20A. 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 μ W 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.

In this manner, an optical excitation module capable of visualizing a 1 μ M 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
5 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
10 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.

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
15 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
20 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.

25 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
30 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.

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.

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

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

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

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.

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.

5 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 as I³ of Forensic Science Service Limited, and future software applications.

10 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.

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, 15 clothing samples, cigarette butts, chewing gum and the like.

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

20 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, 50Hz, using a conventional electric plug.

25 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.

30 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.

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
5 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.

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
10 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
15 minutes are easily achievable.

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
20 minutes are easily achievable.

This functionality is achieved in an instrument weighing less than 10kg and occupying a footprint of less than 0.1m².

Instrument fields of use

25 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
30 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.

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.

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

CLAIMS

1. An instrument for analysing a sample, the instrument including:
a device having one or more sample processors;
5 electronics for operating the sample processors, wherein the sample processors include a sample preparation step, sample extraction step, sample retention step, elution step, amplification step, denaturing step, detection step and results output step.
- 10 2. A device, for processing a sample, the device including:
one or more sample processors, wherein the sample processors include a sample preparation step, sample extraction step, sample retention step, elution step, amplification step, denaturing step, detection step and results output step.
- 15 3. A device for inclusion in instrument according to claim 1 and/or according to claim 2 in which the device is a cartridge.
4. A device according to claim 3 in which the device includes a sample processor for one or more of a sample receiving step, purification step, washing step, PCR
20 step, investigation step, analysis step.
5. A device according to claim 3 or claim 4 in which the sample receiving step includes the transfer of a sample from outside the device to inside the device, the sample receiving step receiving the sample from a collection device or from a
25 storage device.
6. A device according to any of claims 3 to 5 in which the sample preparation step includes contacting the sample with one or more reagents.
- 30 7. A device according to any of claims 3 to 6 the the sample retention step includes 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.

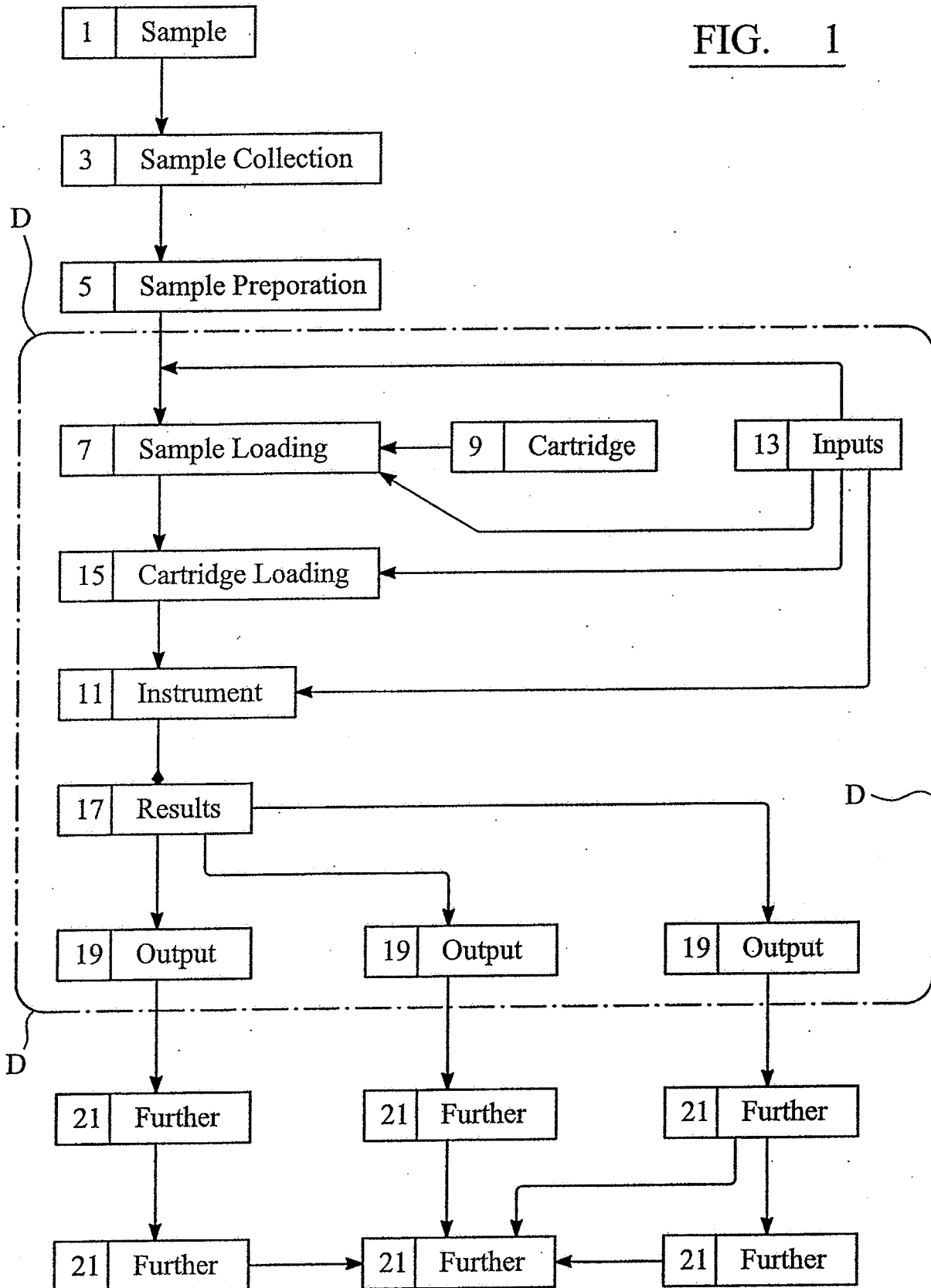
8. A device according to claim 7 in which the sample component(s) are retained on one or more magnetic beads.
9. A device according to claim 7 or claim 8 in which the waste component(s) are removed from the retained sample components by being washed away from the retention step using one or more further reagents and/or components.
10. A device according to any of claims 7 to 9 in which the retained and/or selected sample is eluted with the eluent conveying the retained and/or selected sample to the next step.
11. A device according to any of claims 7 to 10 in which the elution step removes one or more components of the sample from a first form into a second form, the first form being bound to a surface or substrate, the second form being in a liquid.
12. A device according to any of claims 3 to 11 in which the amplification step includes contacting the sample with one or more reagents and/or components to cause amplification and/or contacting the sample with conditions to cause amplification.
13. A device according to any of claims 3 to 12 in which the denaturing step prepares the sample for electrophoresis.
14. A device according to any of claims 3 to 13 in which the electrophoresis step includes transferring the sample to a start location for electrophoresis, the start location being in a channel, one or more voltage conditions being used to transfer the sample to the start location, one or more further voltage conditions may be used to provide the separation.
15. A device according to any of claims 3 to 14 in which the analysis step establishes one or more of the characteristics of the sample by seeking a response from the device to an operation, the operation being the application of light.

16. A device according to any of claims 3 to 15 the analysis step establishes the relative position of the elements having a characteristic
17. A device according to any of claims 3 to 16 in which the results output step
5 displays the one or more results from the analysis step and/or a processed form thereof.
18. A device according to any of claims 3 to 17 in which the results output step transmits the one or more results from the analysis step and/or a processed form
10 thereof to a remote location
19. A device according to any of claims 3 to 18 the results output step is followed by a further processing step, the further processing interpreting the results to provide further results, the further processing step being provided at a location remote from
15 the instrument.
20. A method for analysing a sample, the method including providing an instrument including:
a device having one or more sample processors;
20 electronics for operating the sample processors, wherein the sample processors include a sample preparation step, sample extraction step, sample retention step, elution step, amplification step, denaturing step, detection step and results output step;
operating one or more of the sample processors.
25
21. A method according to claim 20, wherein the device is a cartridge.
22. A method according to claim 20 or claim 21 wherein the device includes a sample processor for one or more of a sample receiving step, purification step,
30 washing step, PCR step, investigation step, analysis step.
23. A device, for processing a sample, the device including:
one or more sample processors, wherein one of the sample processors is provided using a chamber with an inlet and an outlet, a valve being connected to the

- inlet to provide a first sealing location, a further valve connected to the outlet to provide a second sealing location, one or more interconnected channels and chambers being provided between the first sealing location and the second sealing location, the channel or channels and chamber or chambers provided between the first sealable location and the second sealable location having a first extent, a heating device being provided to heat the chamber, the heating device having a second extent parallel to the first extent of the channel or channels and chamber or chambers, wherein the second extent is at least 75% of the first extent.
- 5
- 10 24. A device according to claim 23, wherein a channel connected to the inlet, the chamber and a channel connected to the outlet are provided between the first sealable location and the second sealable location.
- 15 25. A device according to claim 23 or claim 24, wherein the channel or channels and chamber or chambers provided between the first sealable location and the second sealable location have an extent in a first plane and the heating device has an extent parallel to the first plane.
- 20 26. A device according to any of claims 23 to 25 in which the extent of the heating device is greater than 90%.
27. An instrument including a device according to any of claims 23 to 26.
- 25 28. A method for analysing a sample, the method including providing an instrument including:
- a device having one or more sample processors;
 - electronics for operating the sample processors, wherein one of the sample processors is provided using a chamber with an inlet and an outlet, a valve being connected to the inlet to provide a first sealing location, a further valve connected to the outlet to provide a second sealing location, one or more interconnected channels and chambers being provided between the first sealing location and the second sealing location, the channel or channels and chamber or chambers provided between the first sealable location and the second sealable location having a first extent, a heating device being provided to heat the chamber, the heating device
- 30

having a second extent parallel to the first extent of the channel or channels and chamber or chambers, wherein the second extent is at least 75% of the first extent.

FIG. 1



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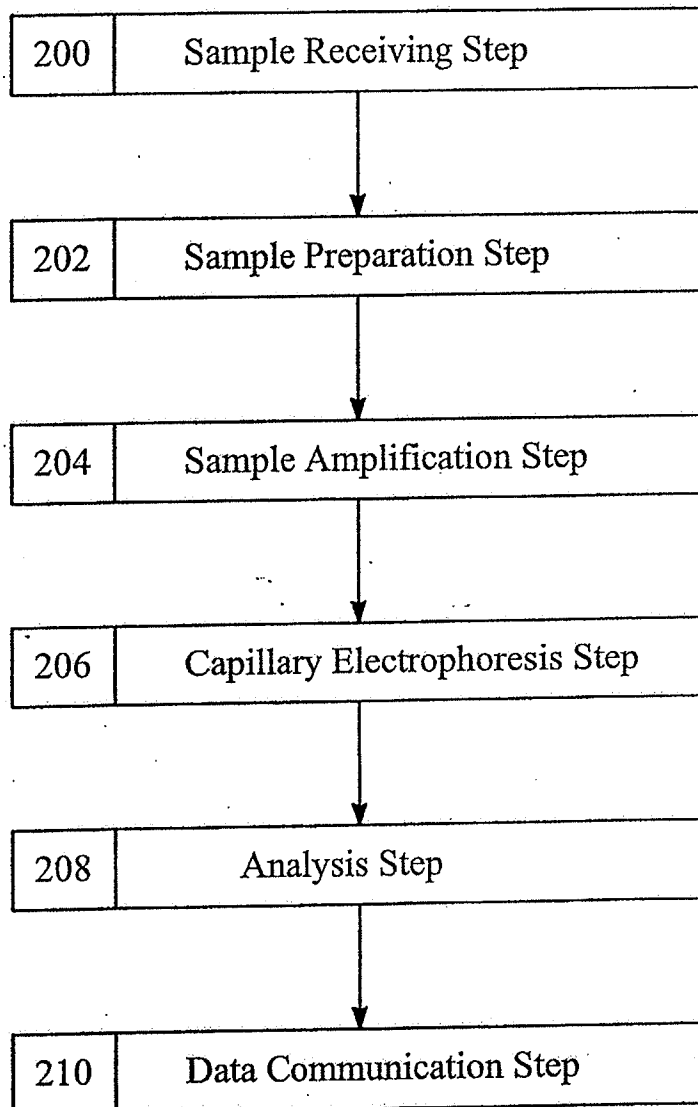
FIG. 2

FIG. 3b

Design Specifications

Design Specification : ISS 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						

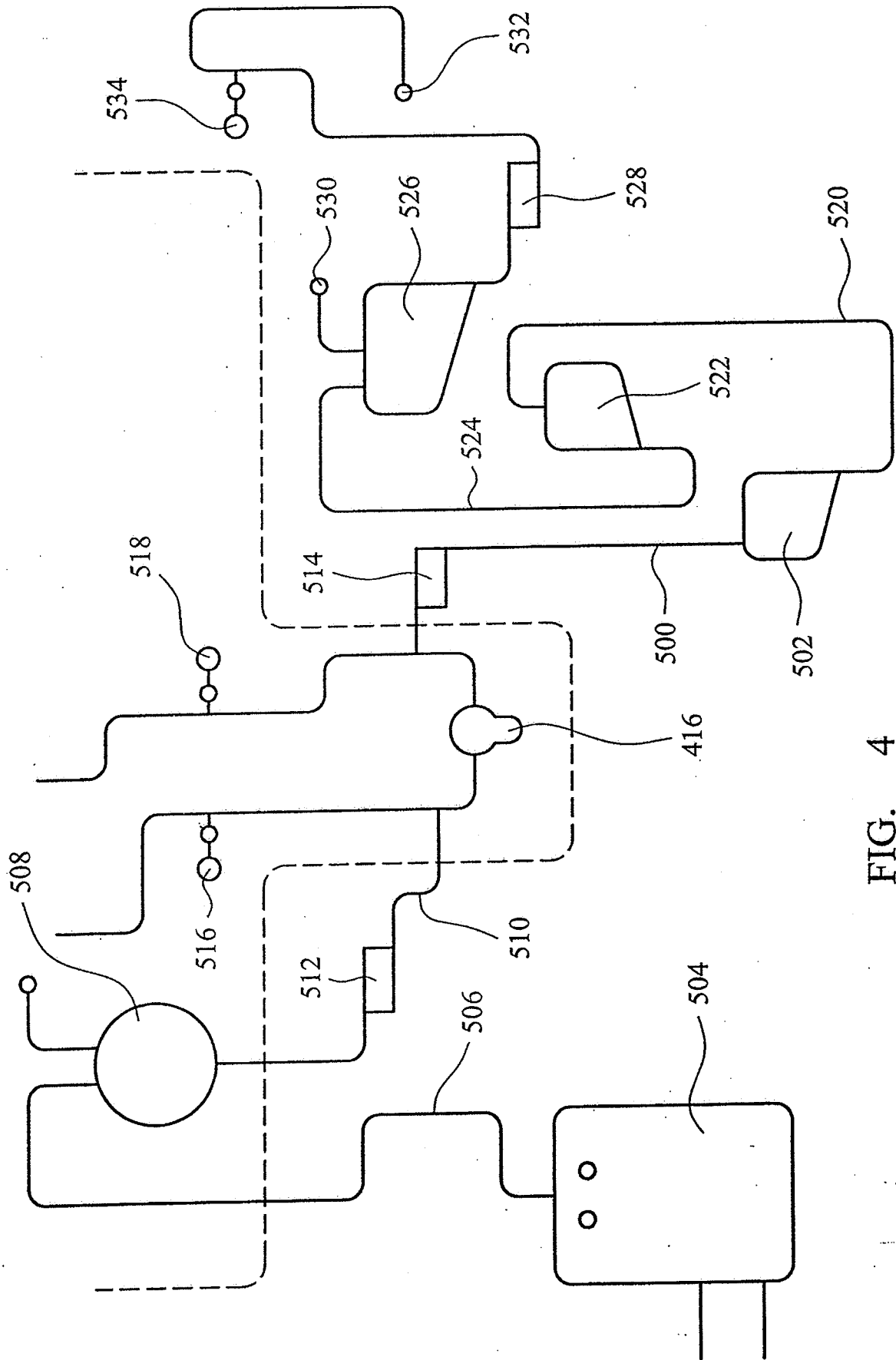


FIG. 4

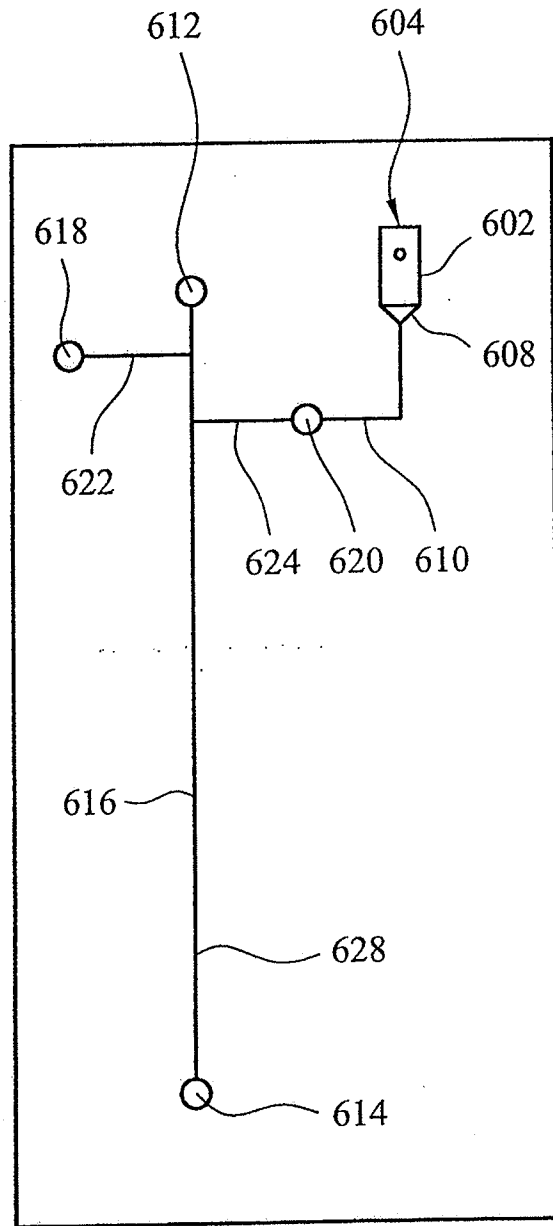
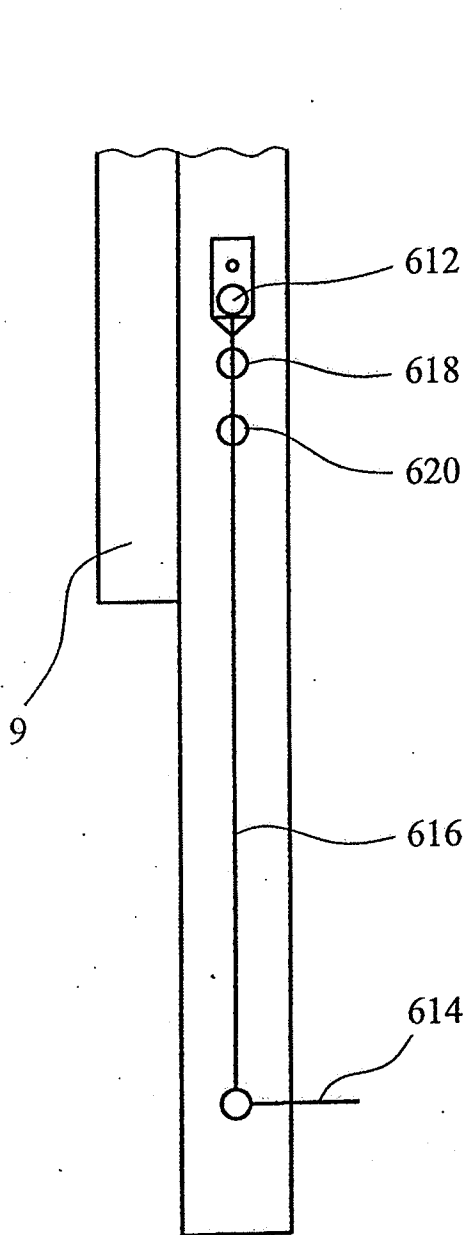


FIG. 5a

FIG. 5b

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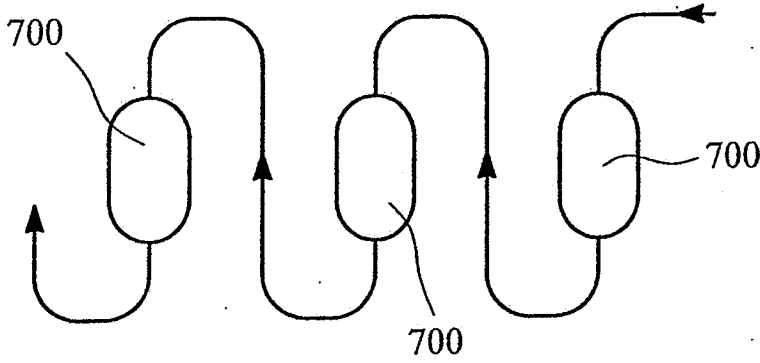


FIG. 6a

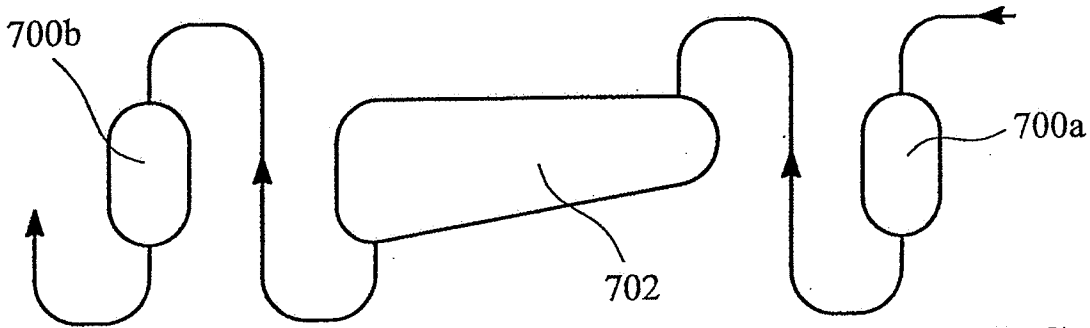


FIG. 6b

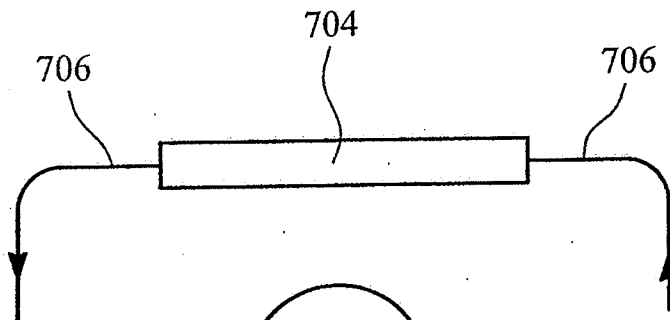


FIG. 6c

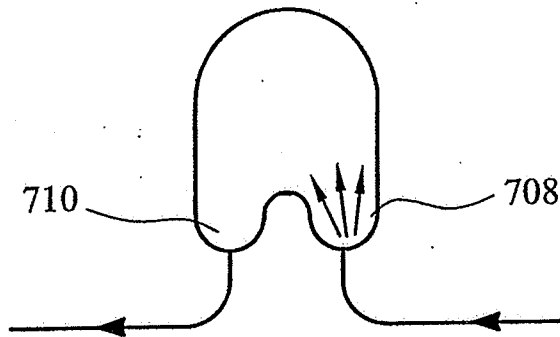


FIG. 6d

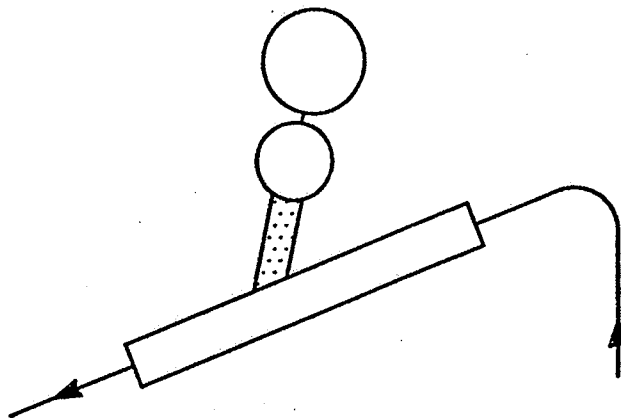


FIG. 6e

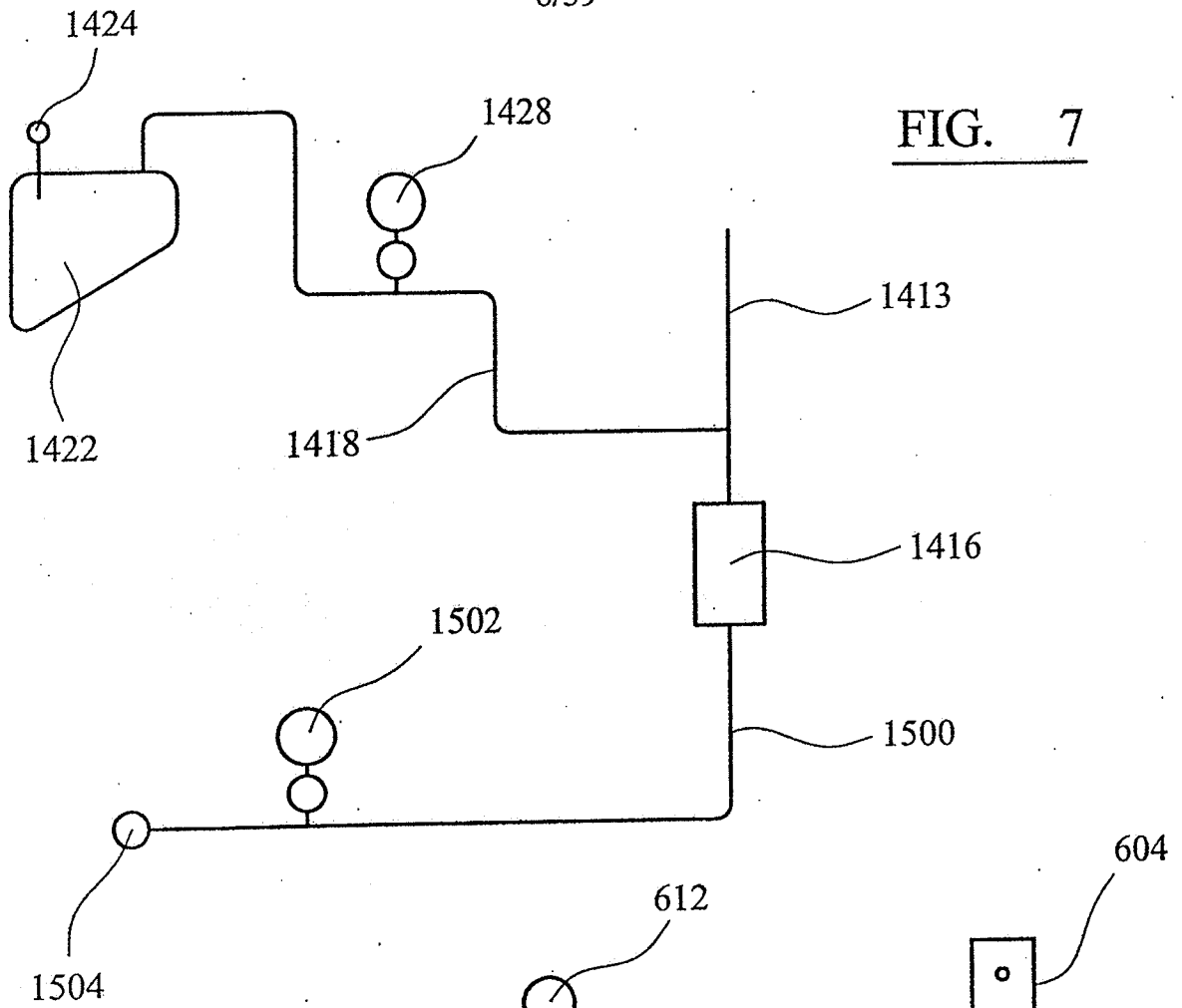


FIG. 7

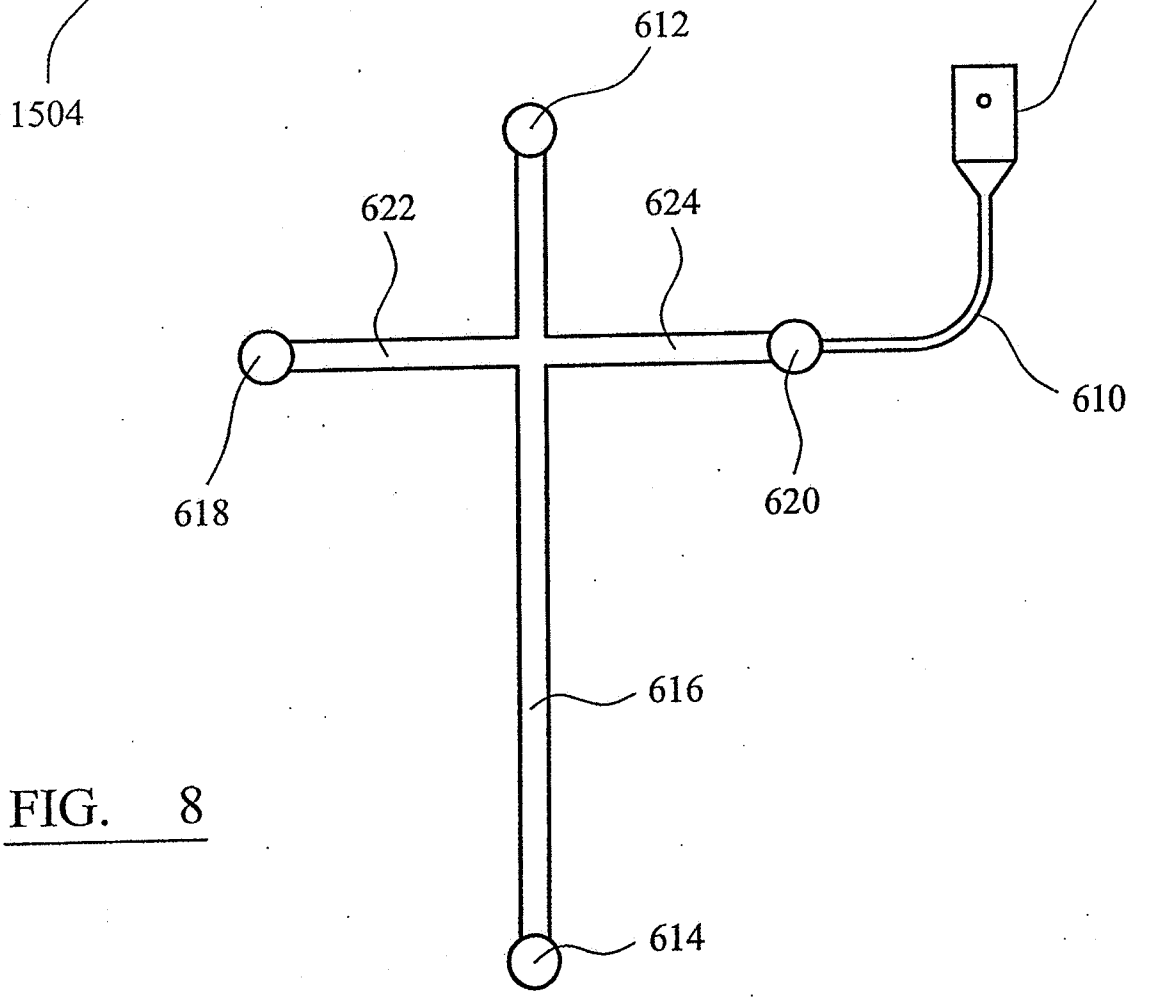


FIG. 8

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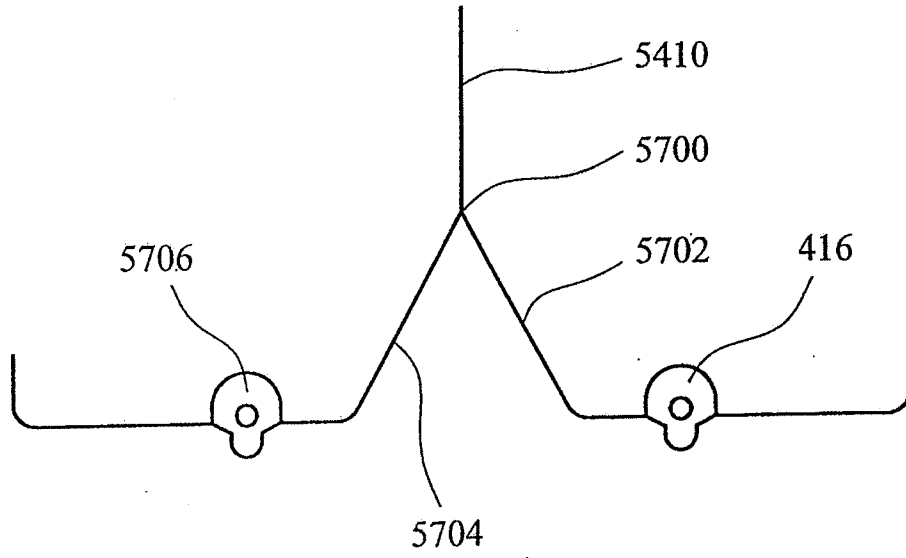


FIG. 9

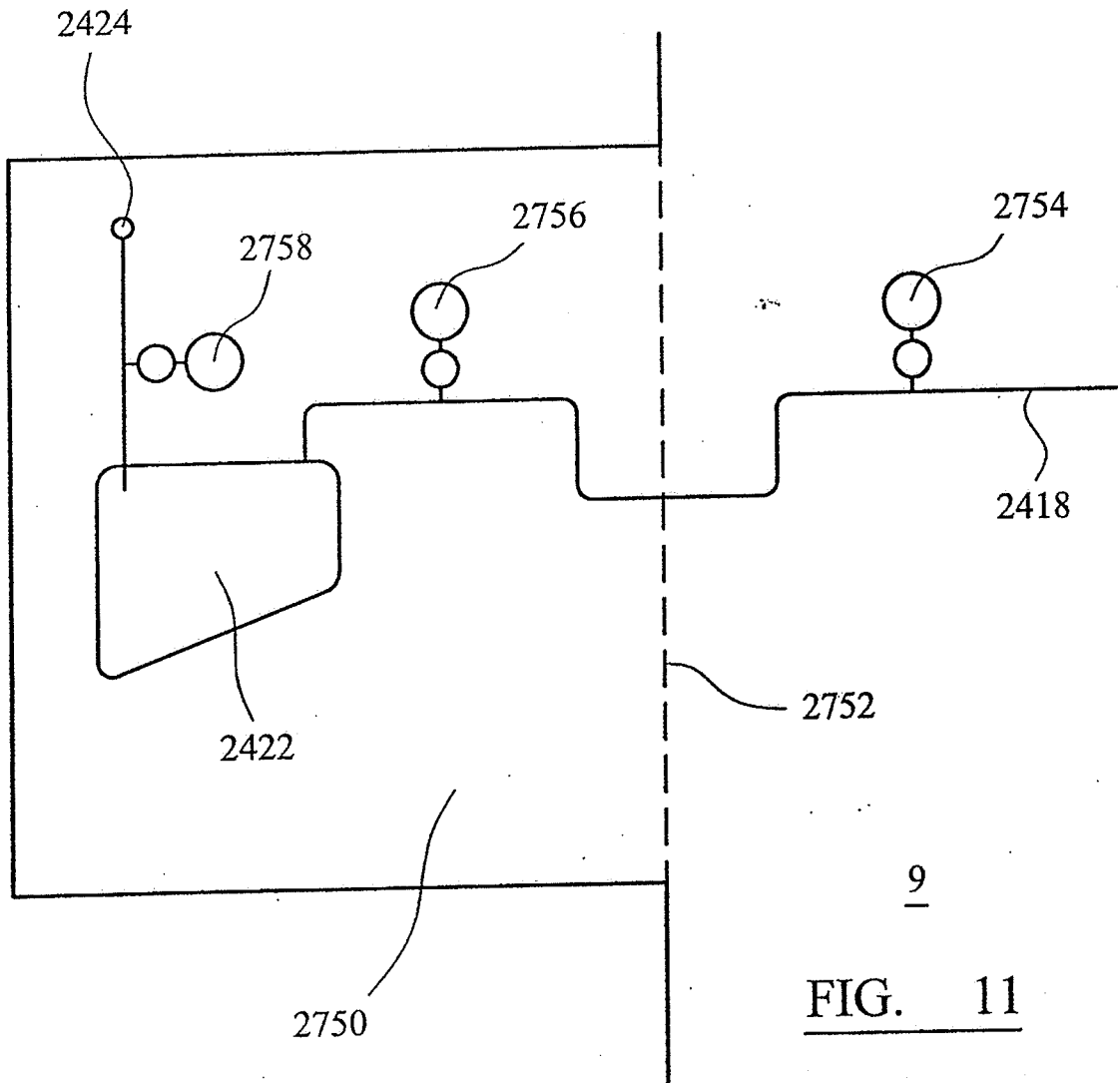


FIG. 11

-10/39-

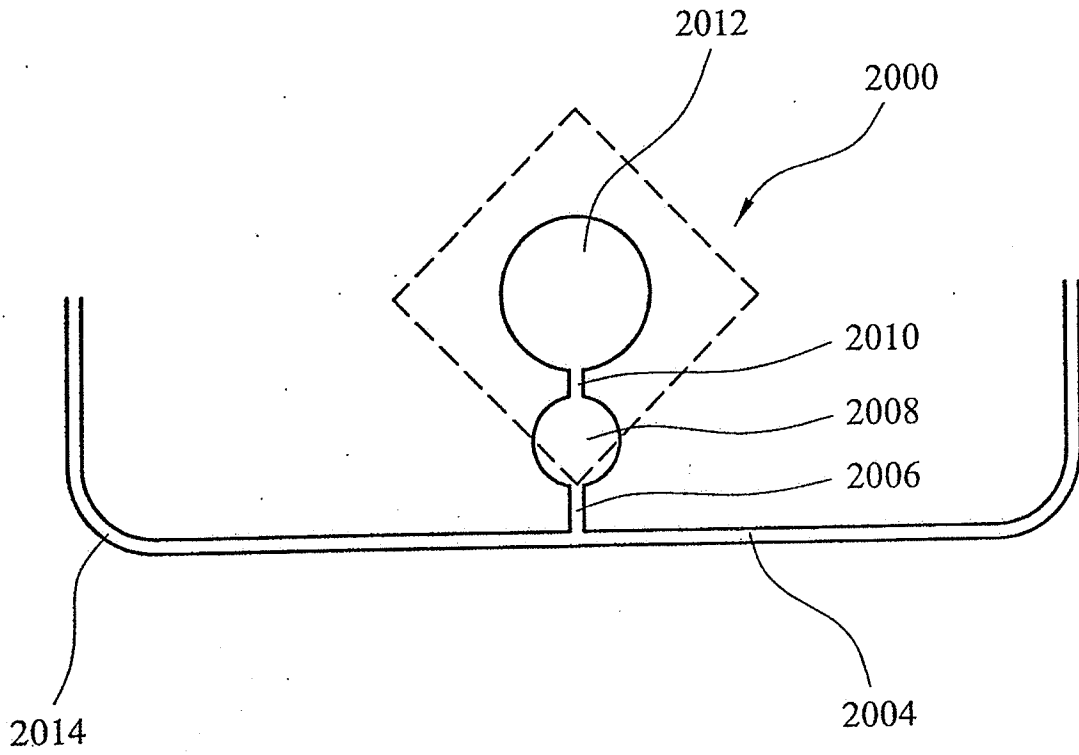


FIG. 10a

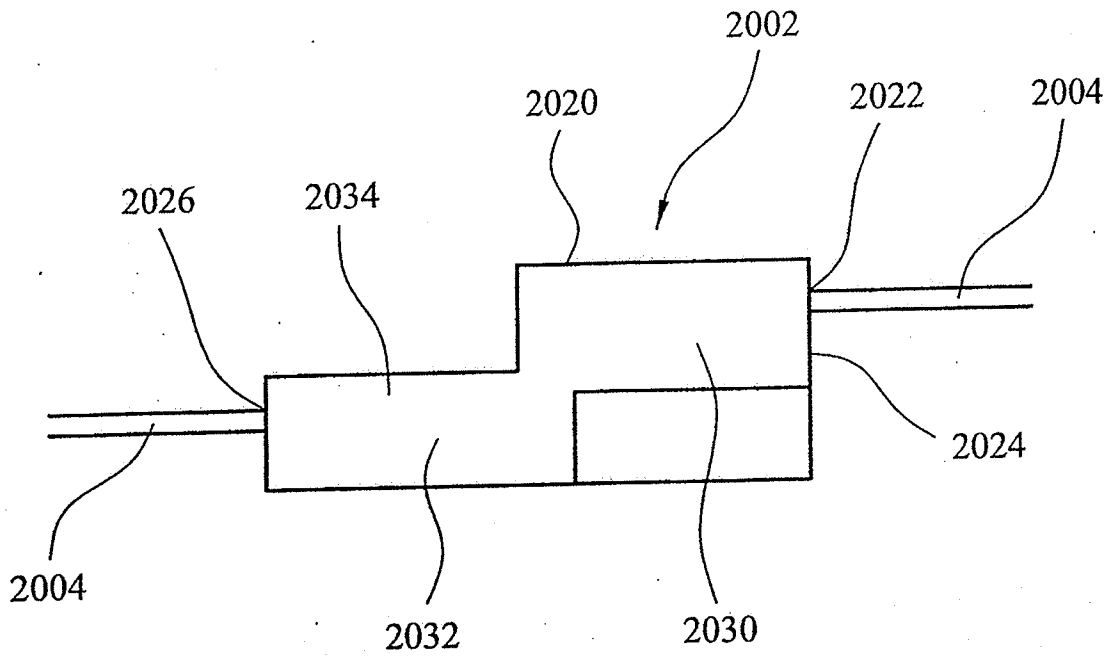


FIG. 10b

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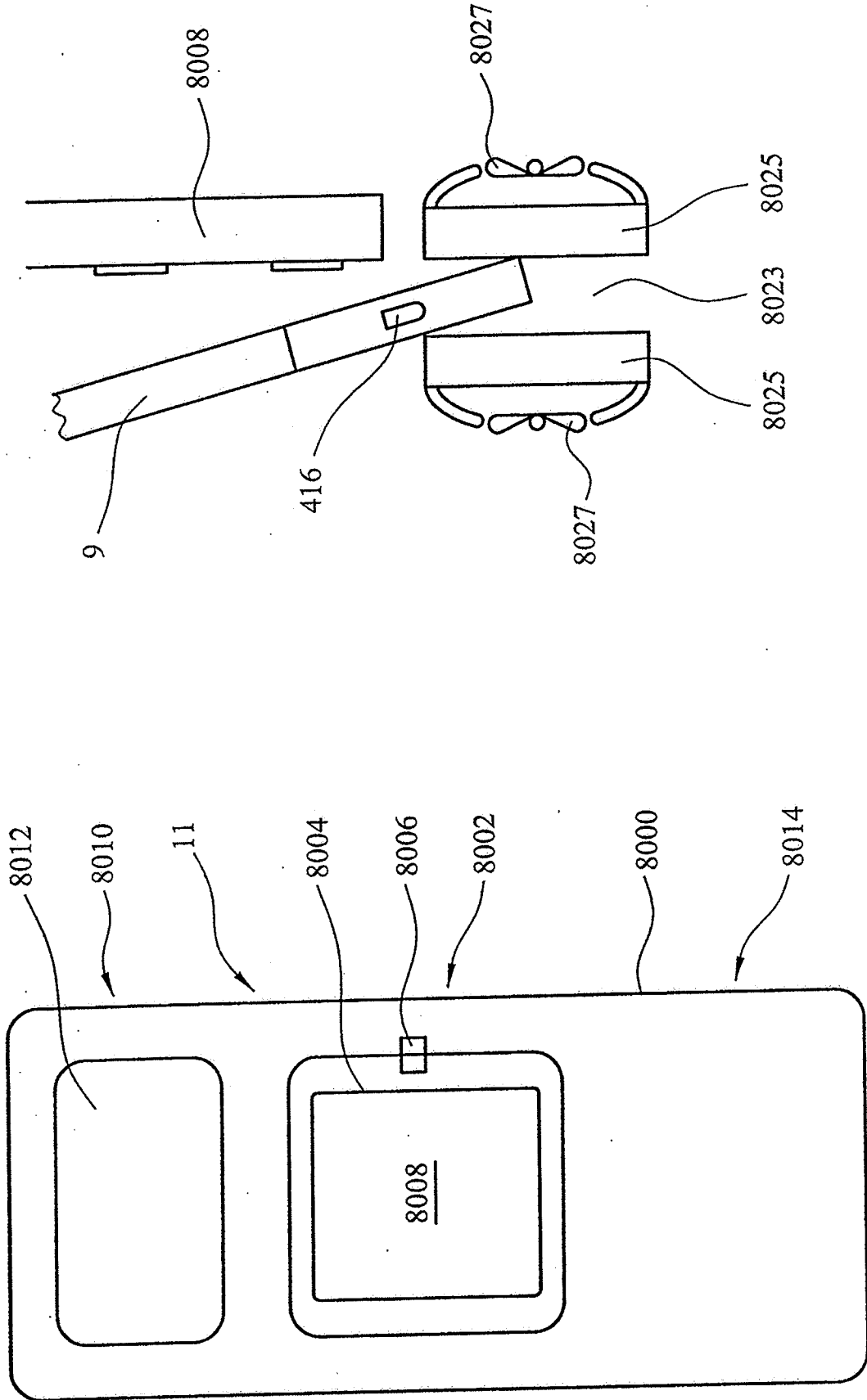


FIG. 13

FIG. 12

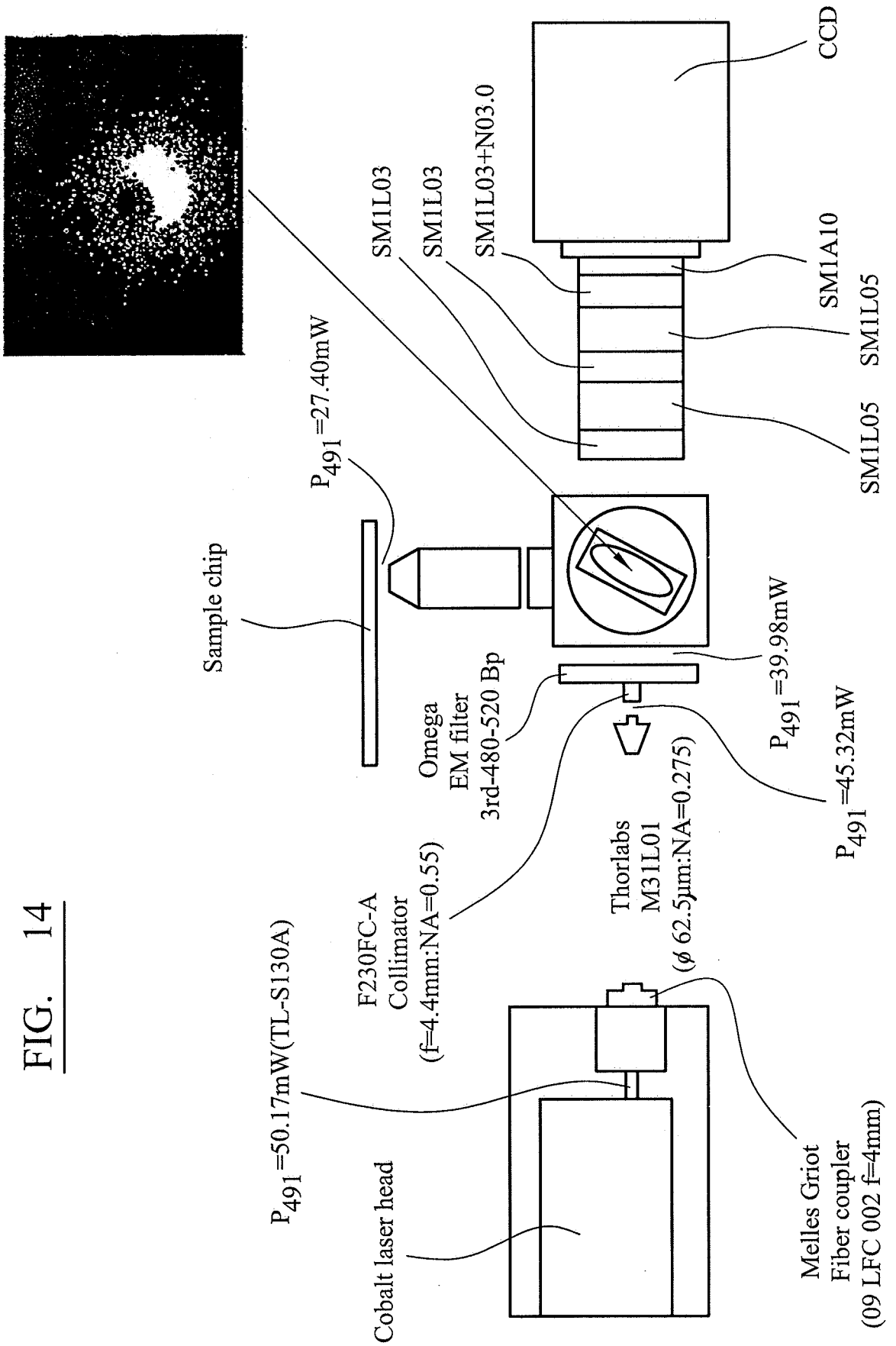


FIG. 14

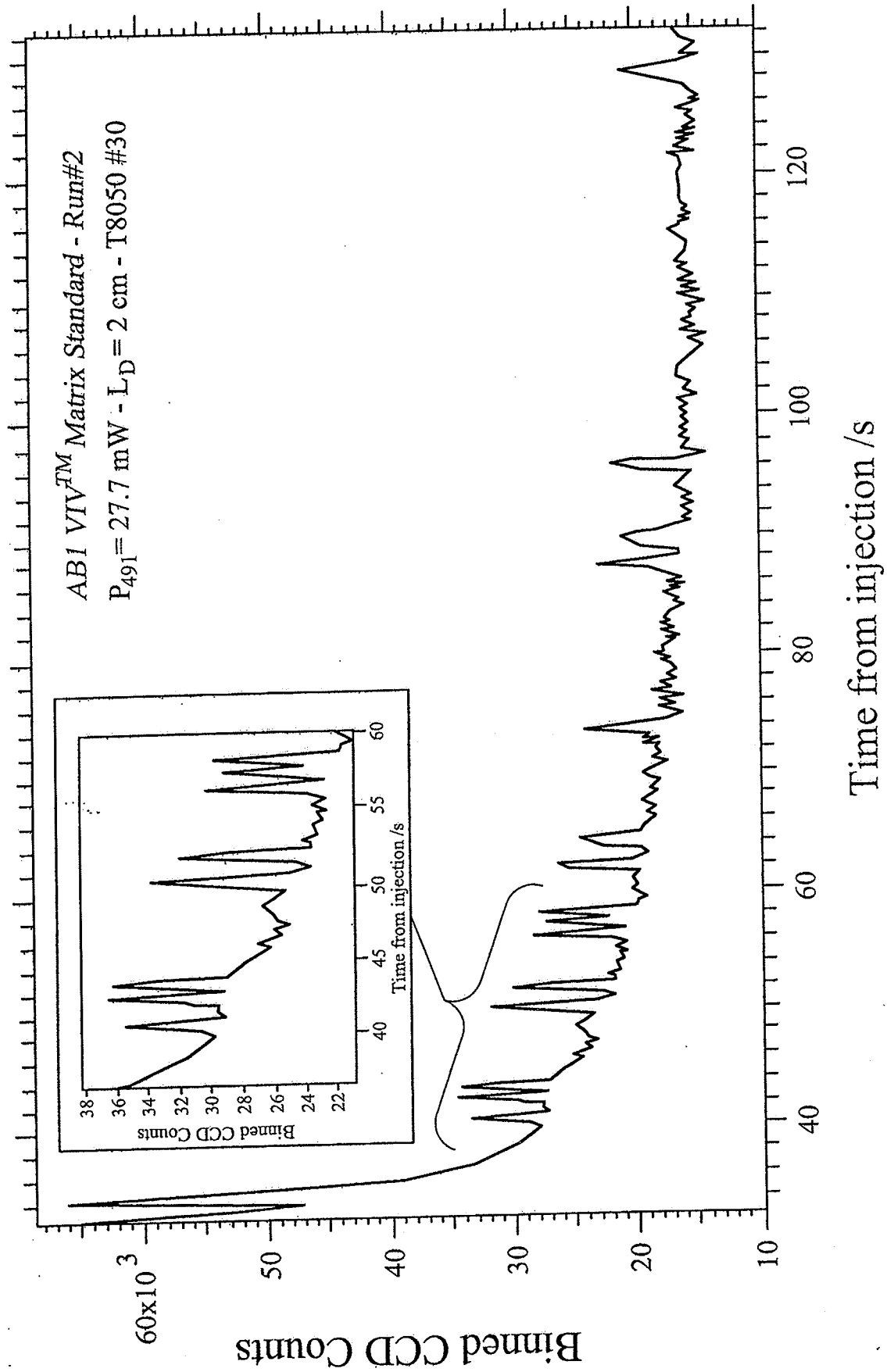


FIG. 15

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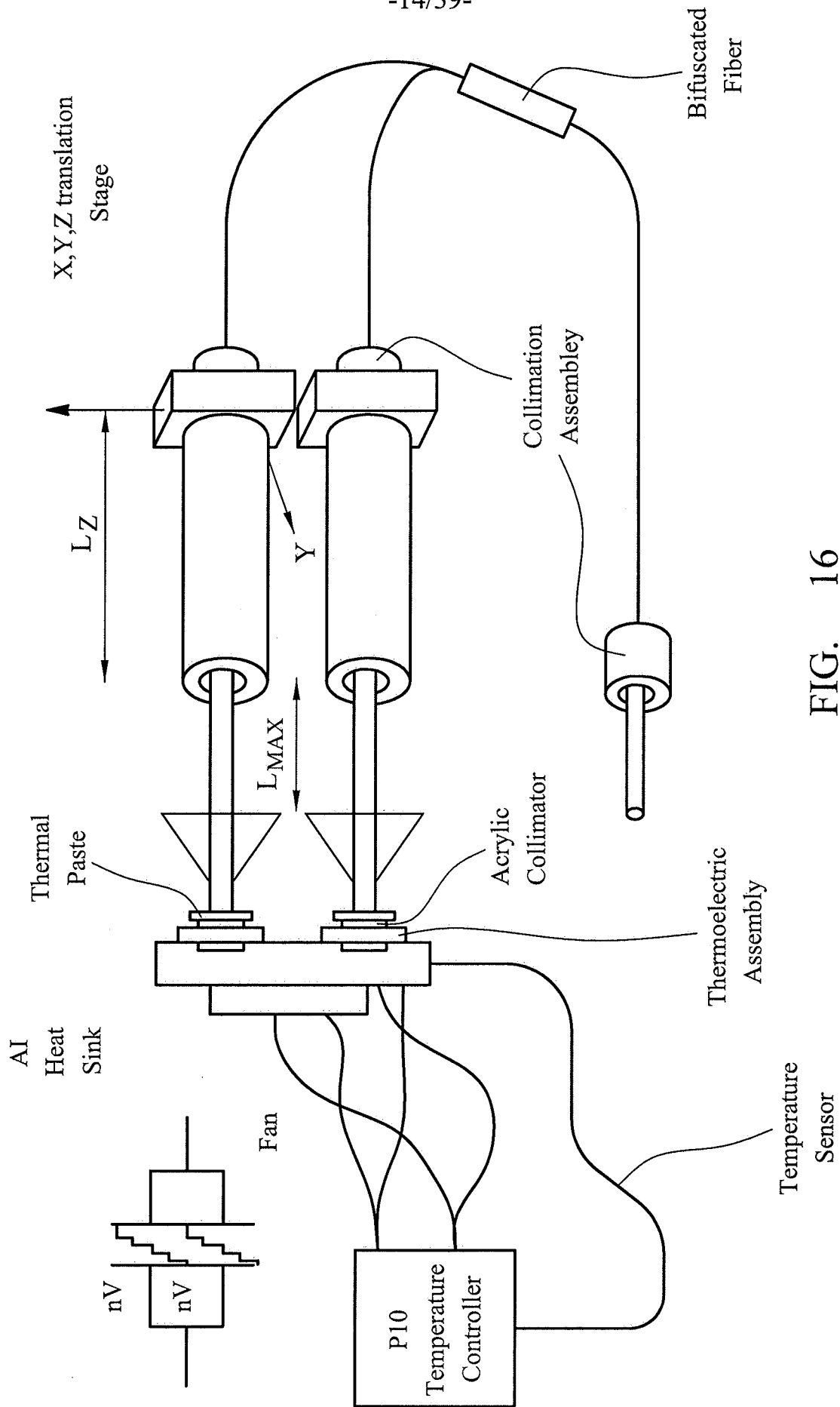


FIG. 16

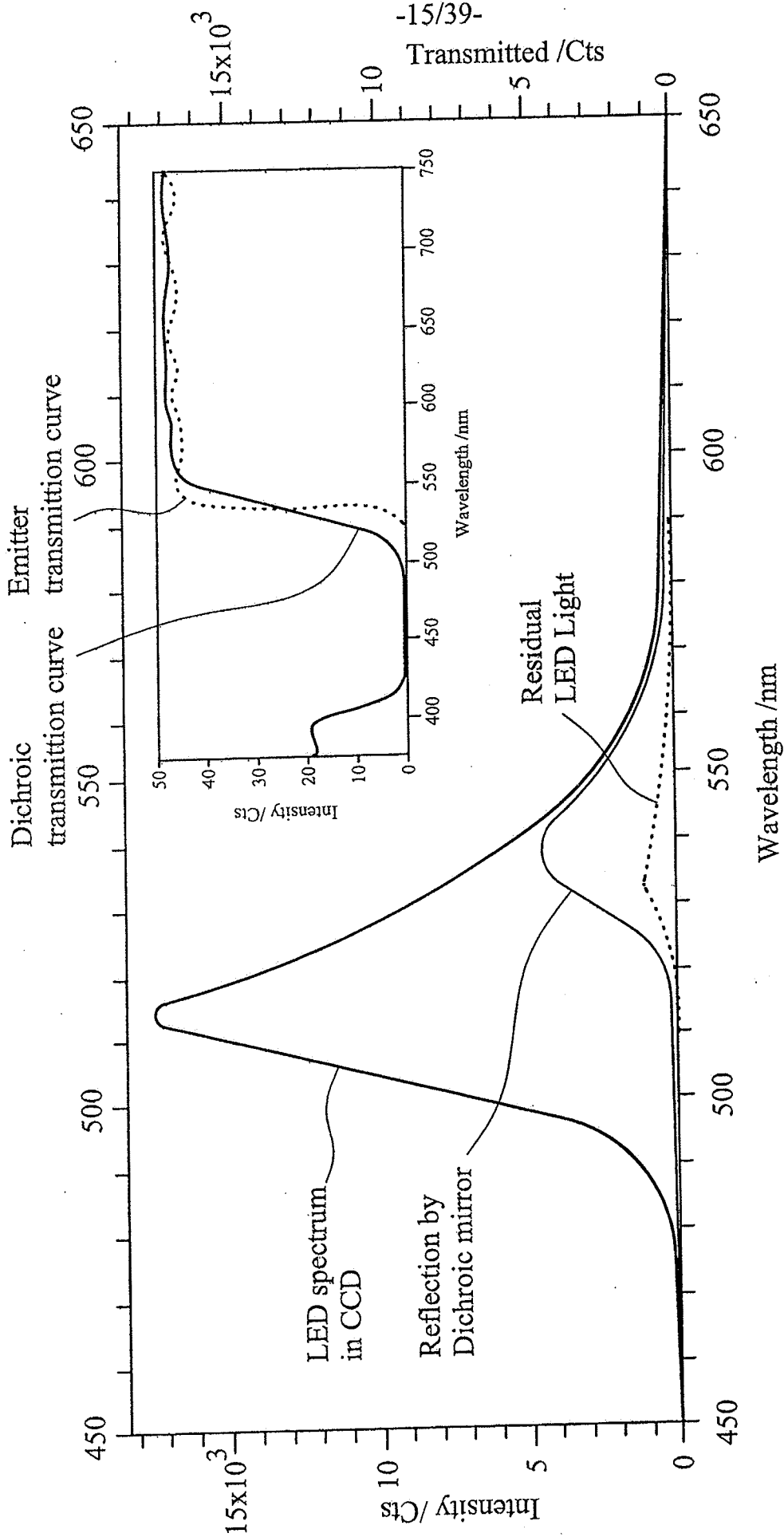


FIG. 17

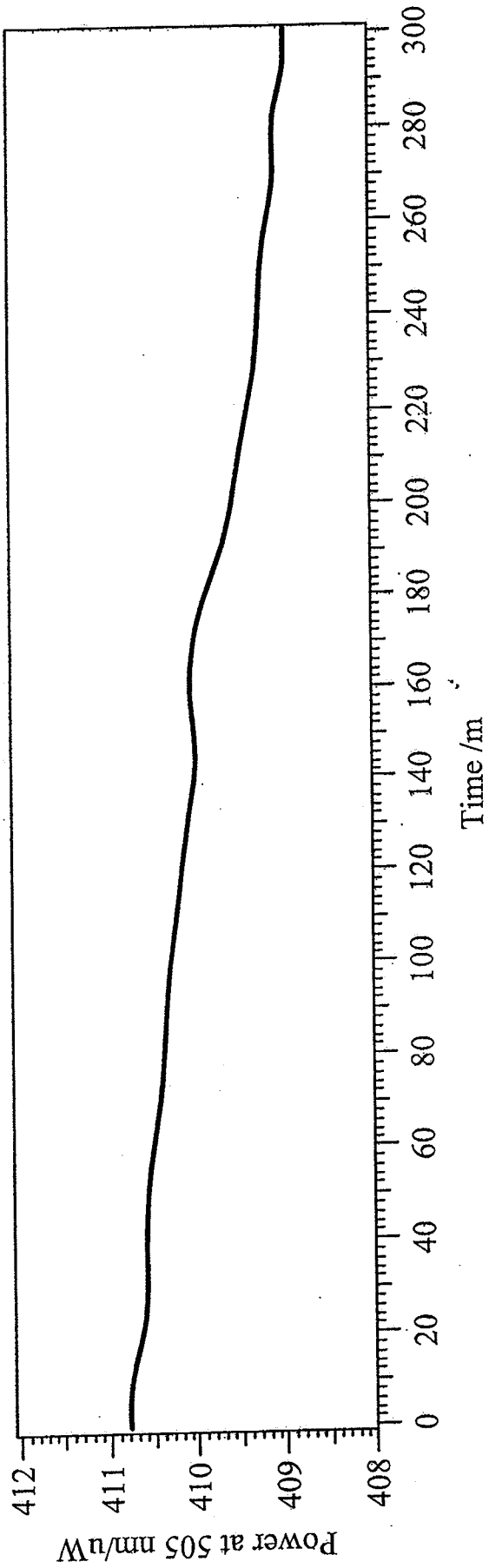


FIG. 18

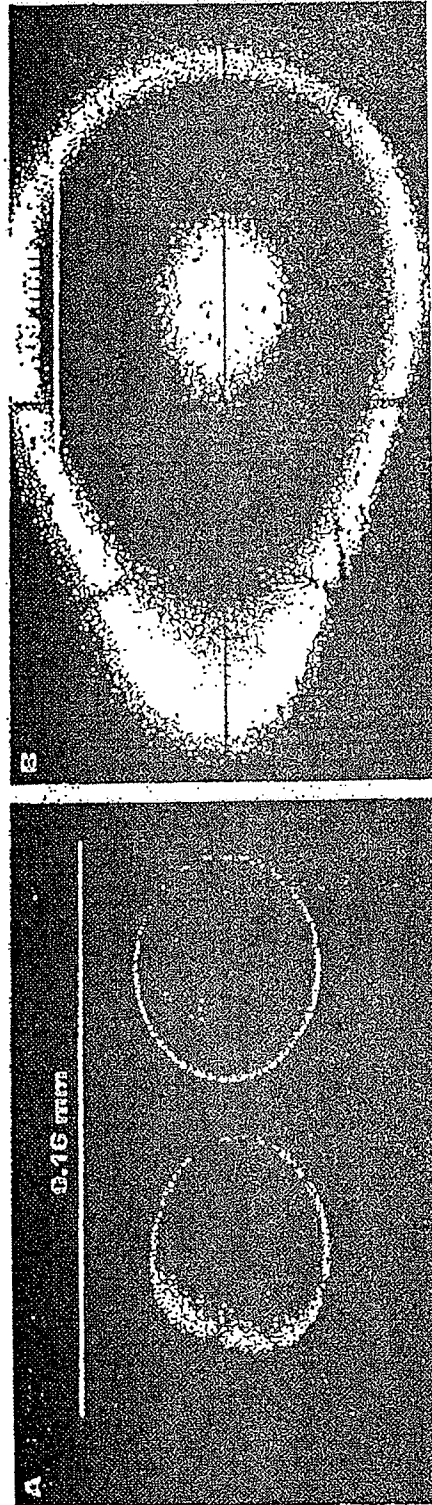


FIG. 19

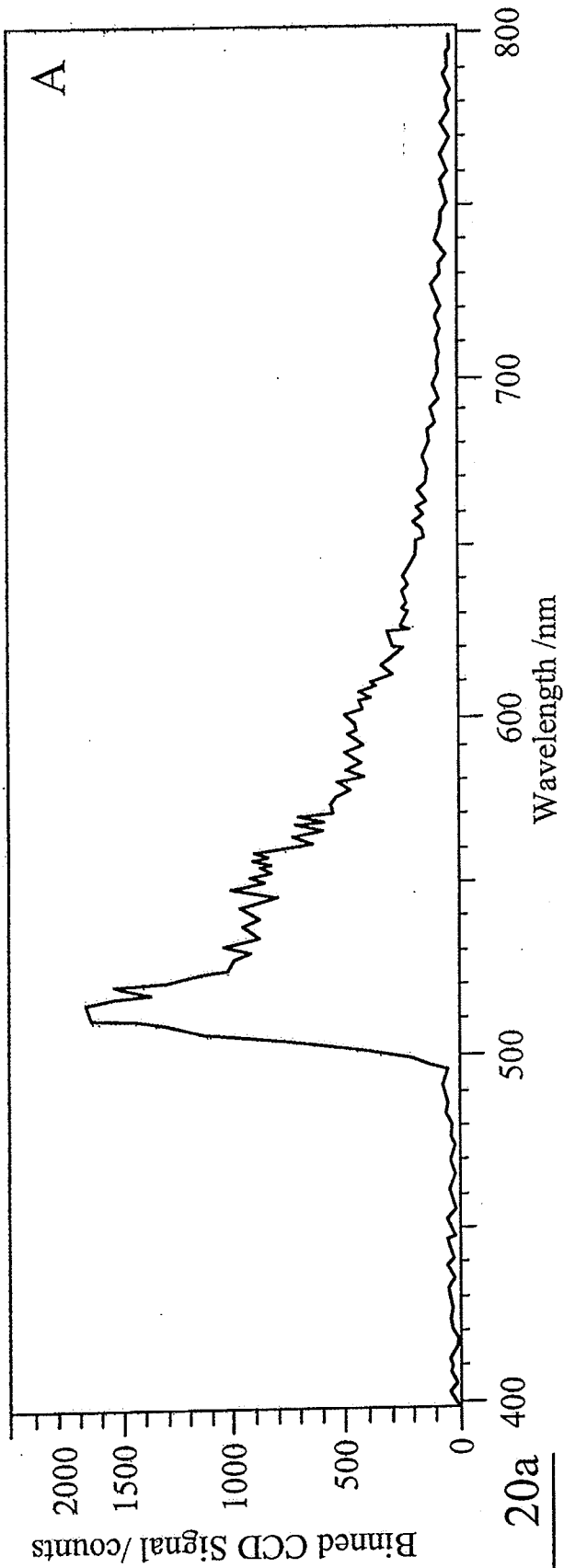


FIG. 20a

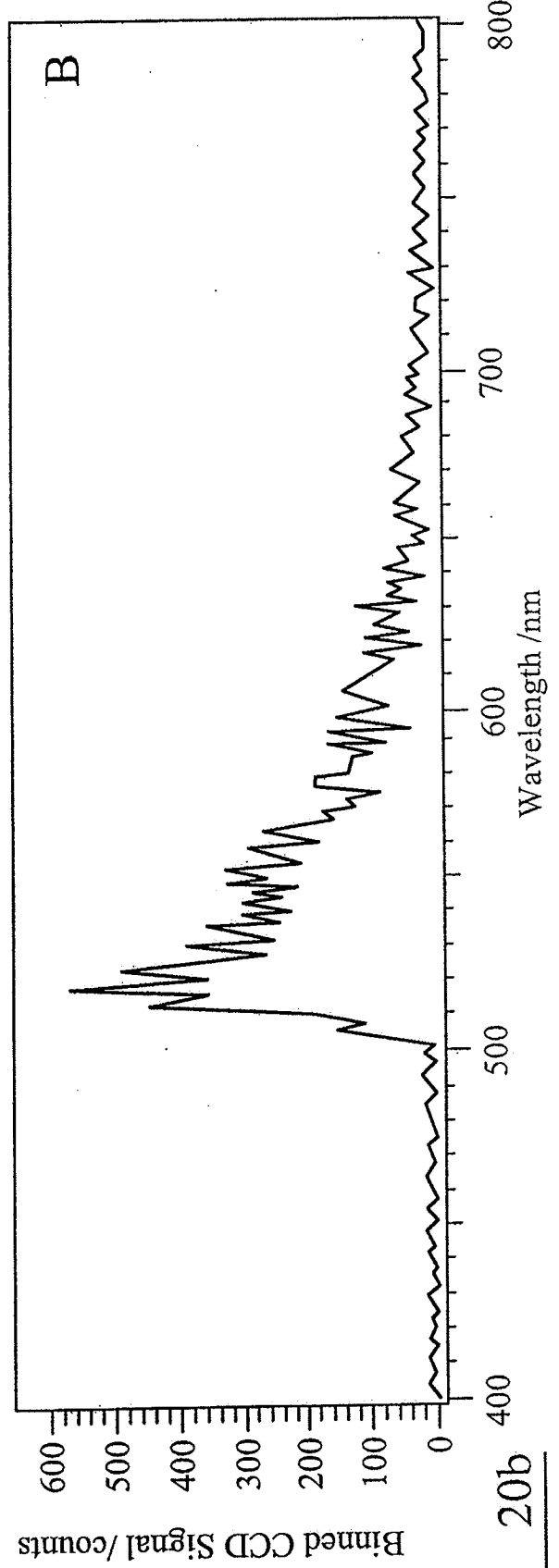
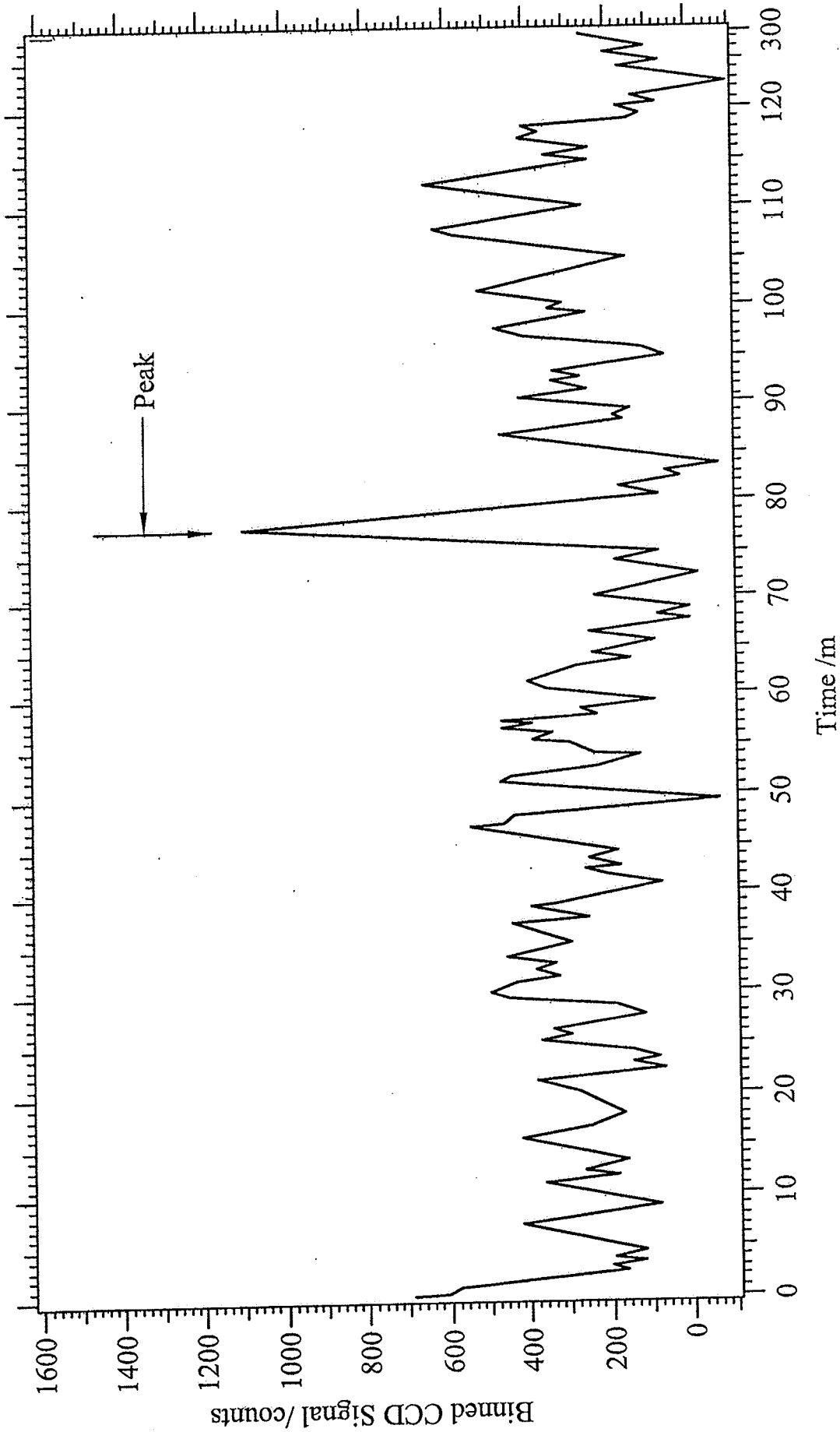


FIG. 20b

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Time /m
FIG. 21

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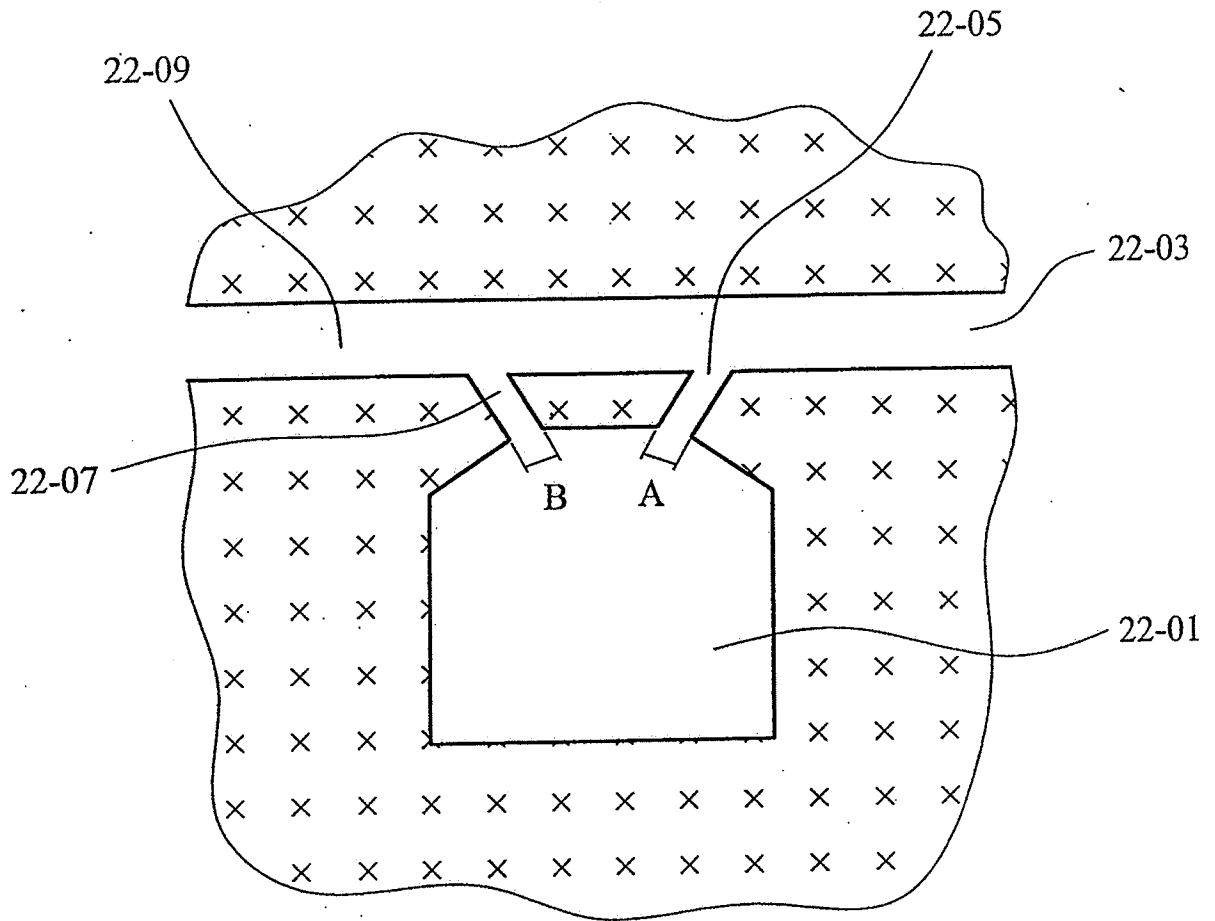


FIG. 22

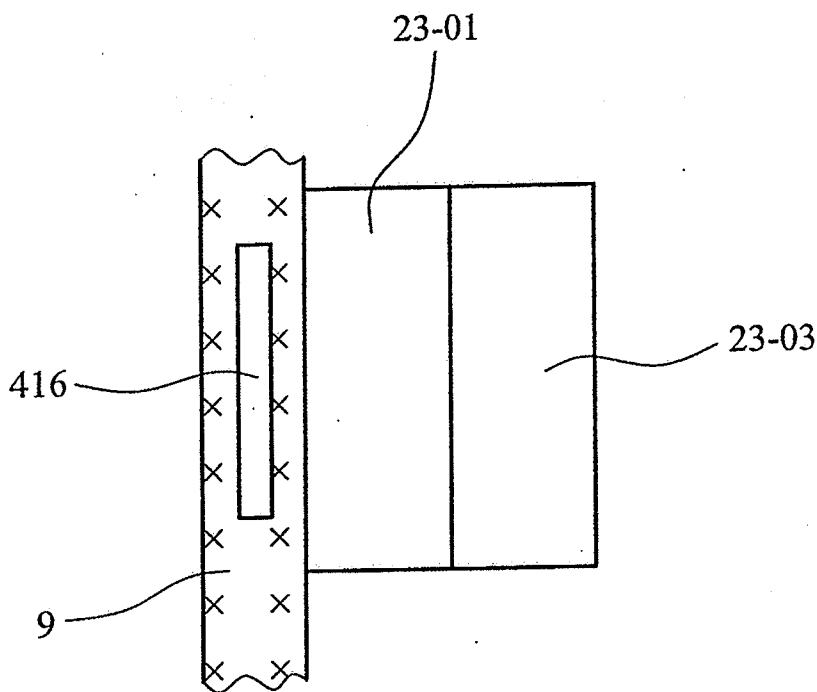


FIG. 23

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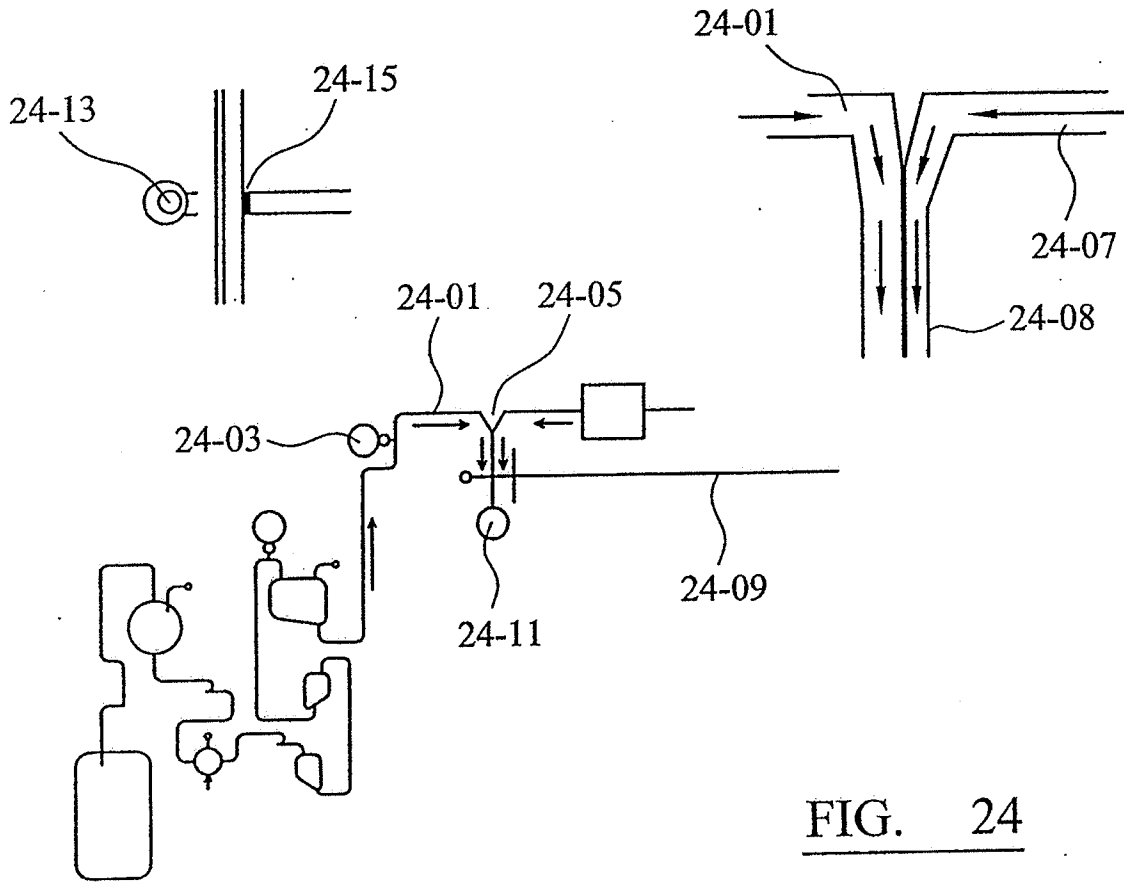


FIG. 24

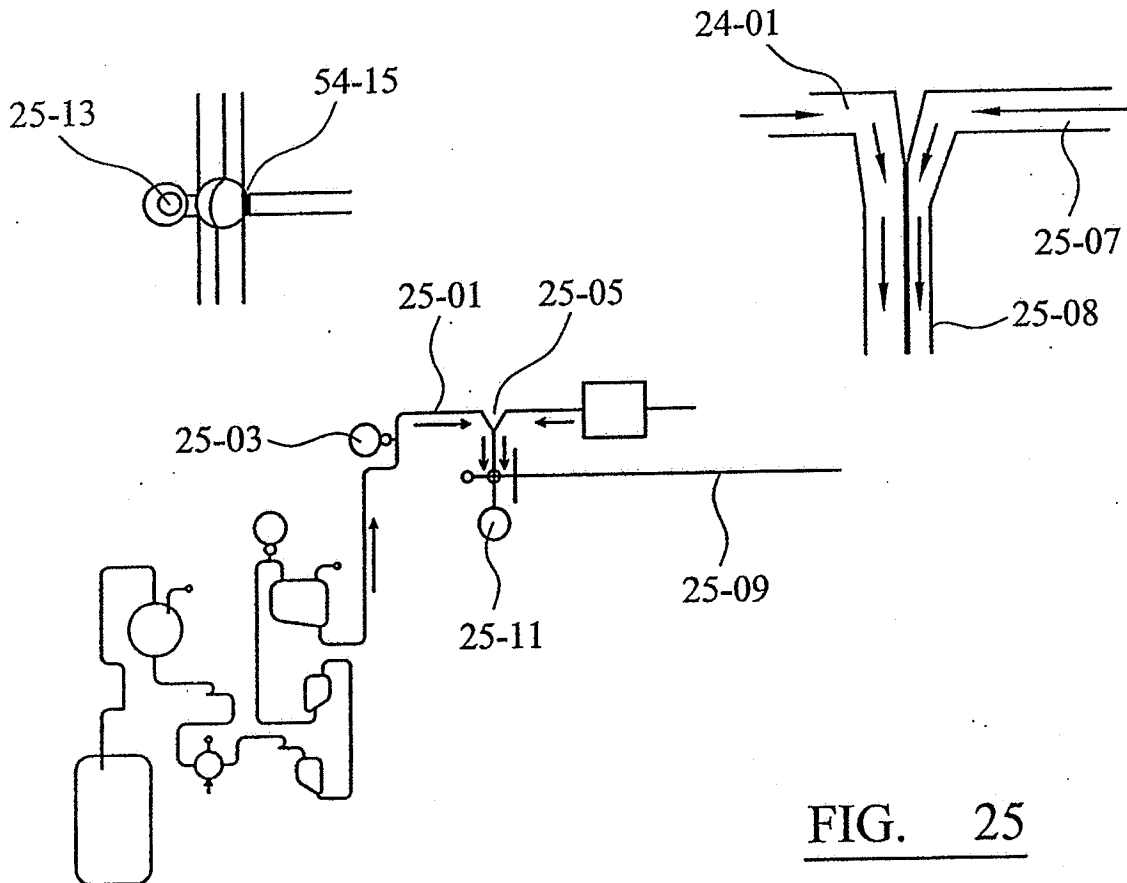


FIG. 25

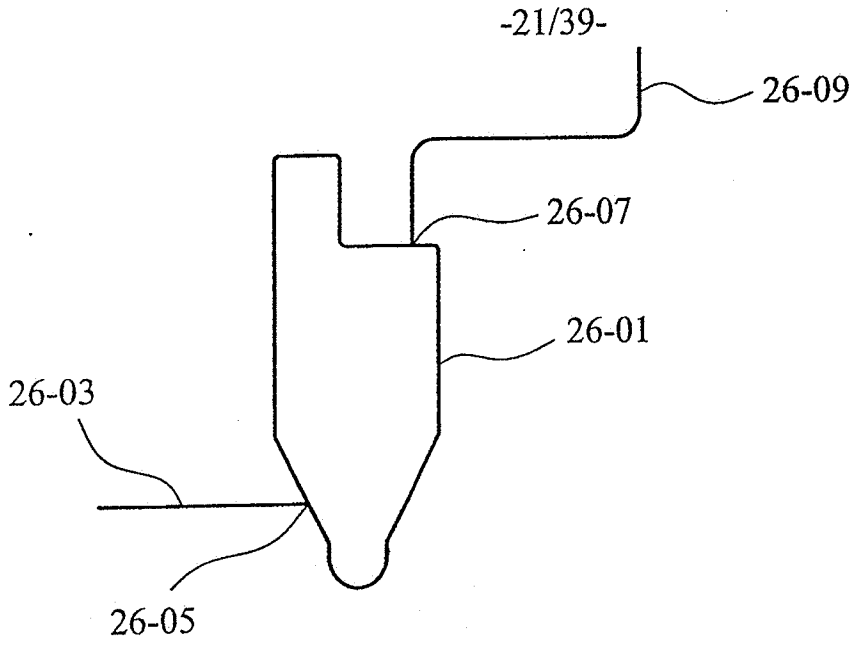


FIG. 26

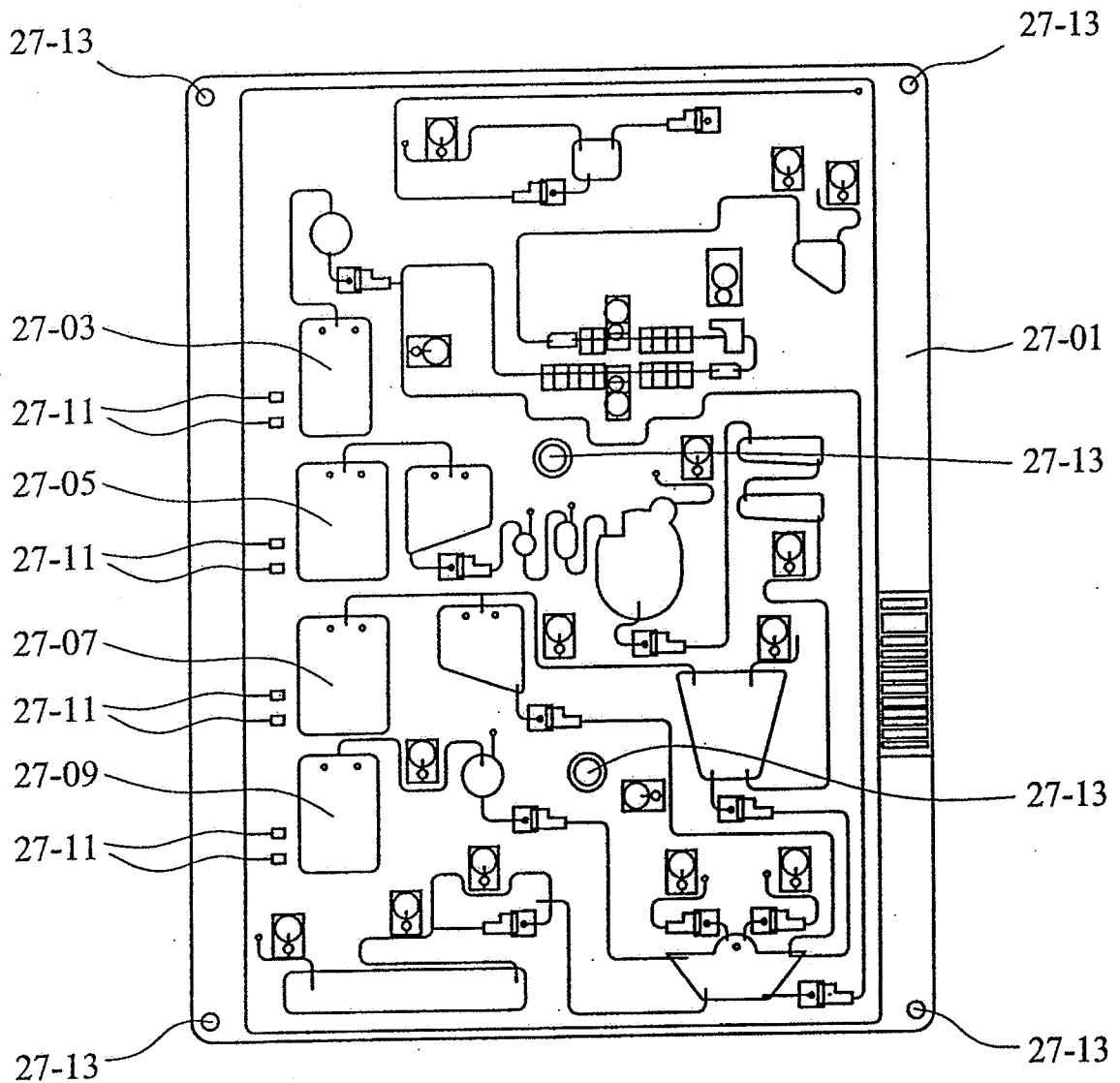


FIG. 27

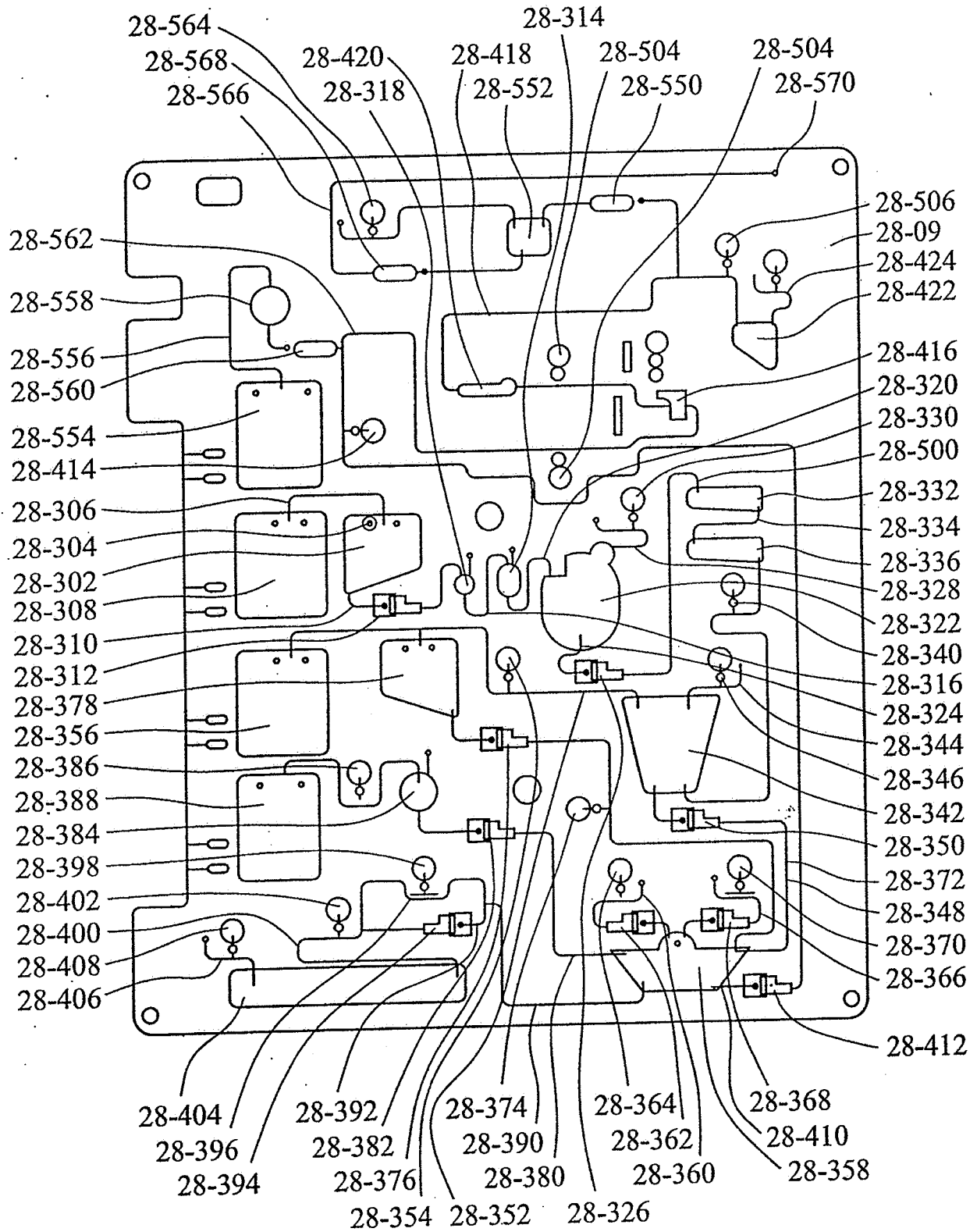


FIG. 28a

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 HI
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		
HMW 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 fluidic marks instead

FIG. 28b

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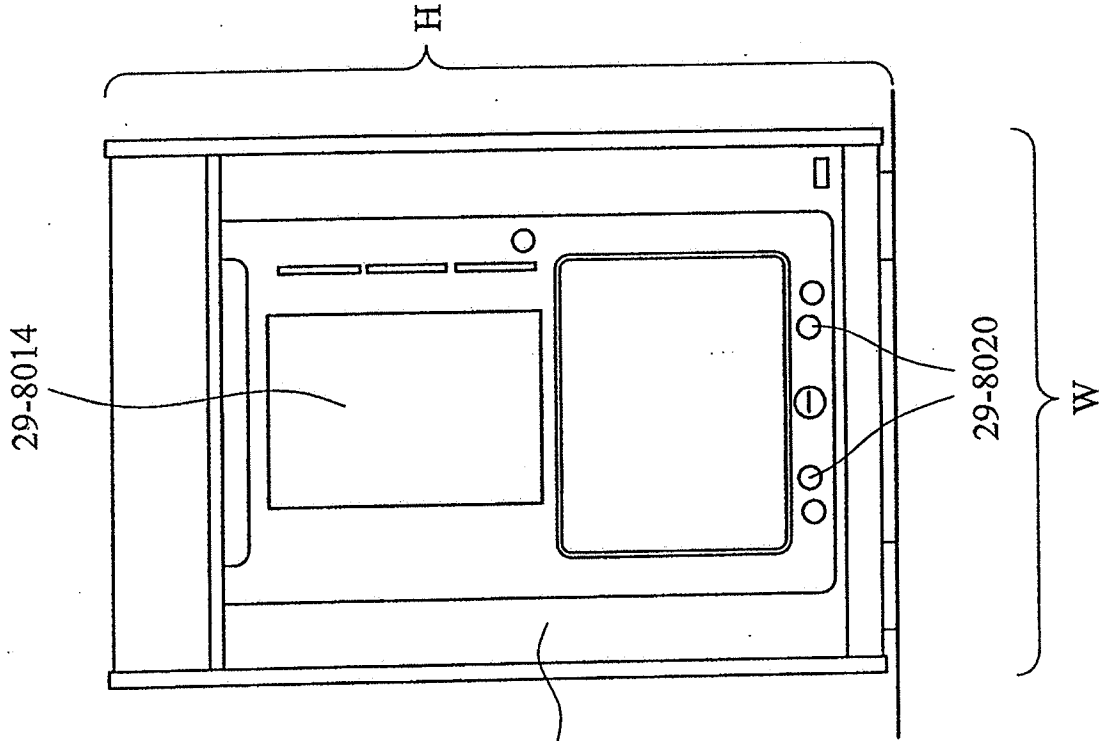


FIG. 29b

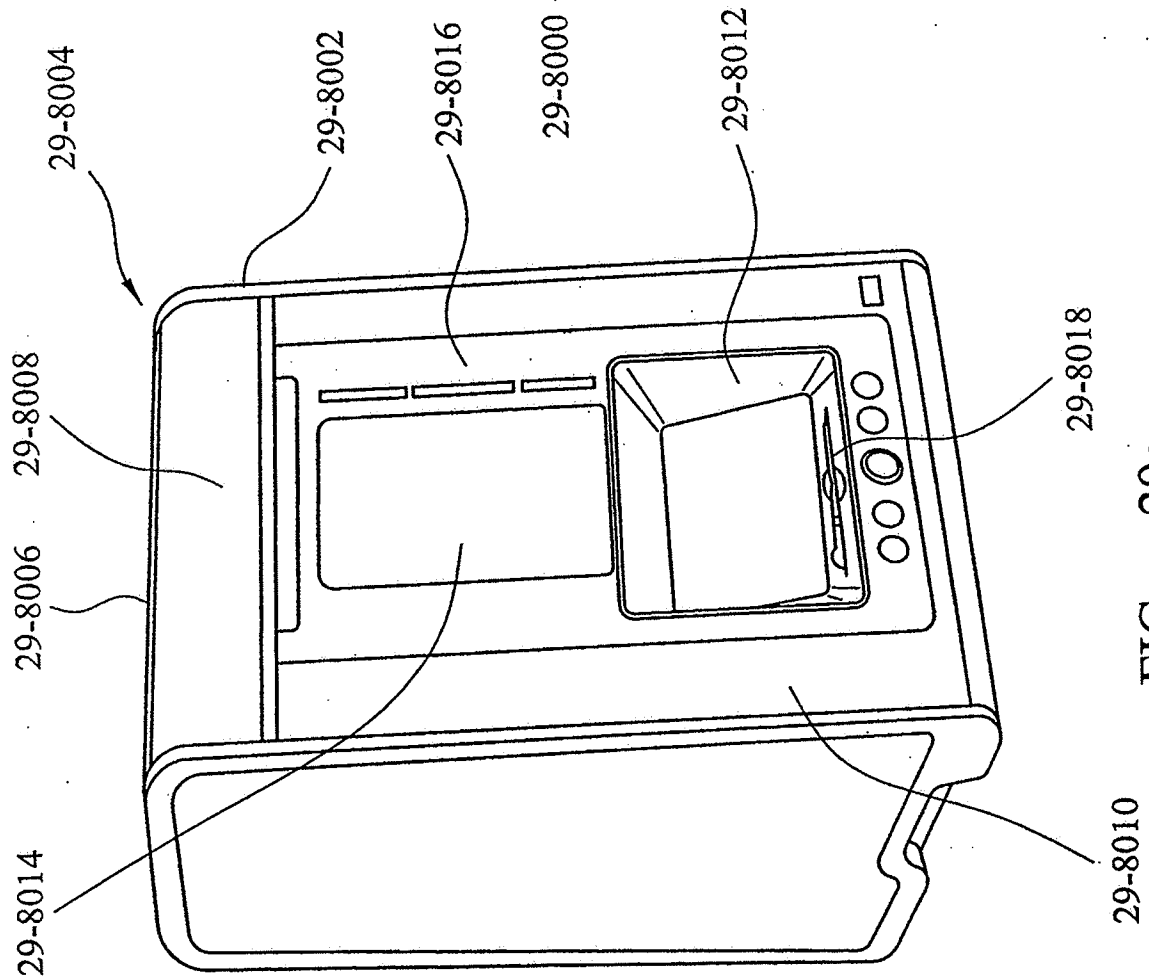


FIG. 29a

-25/39-

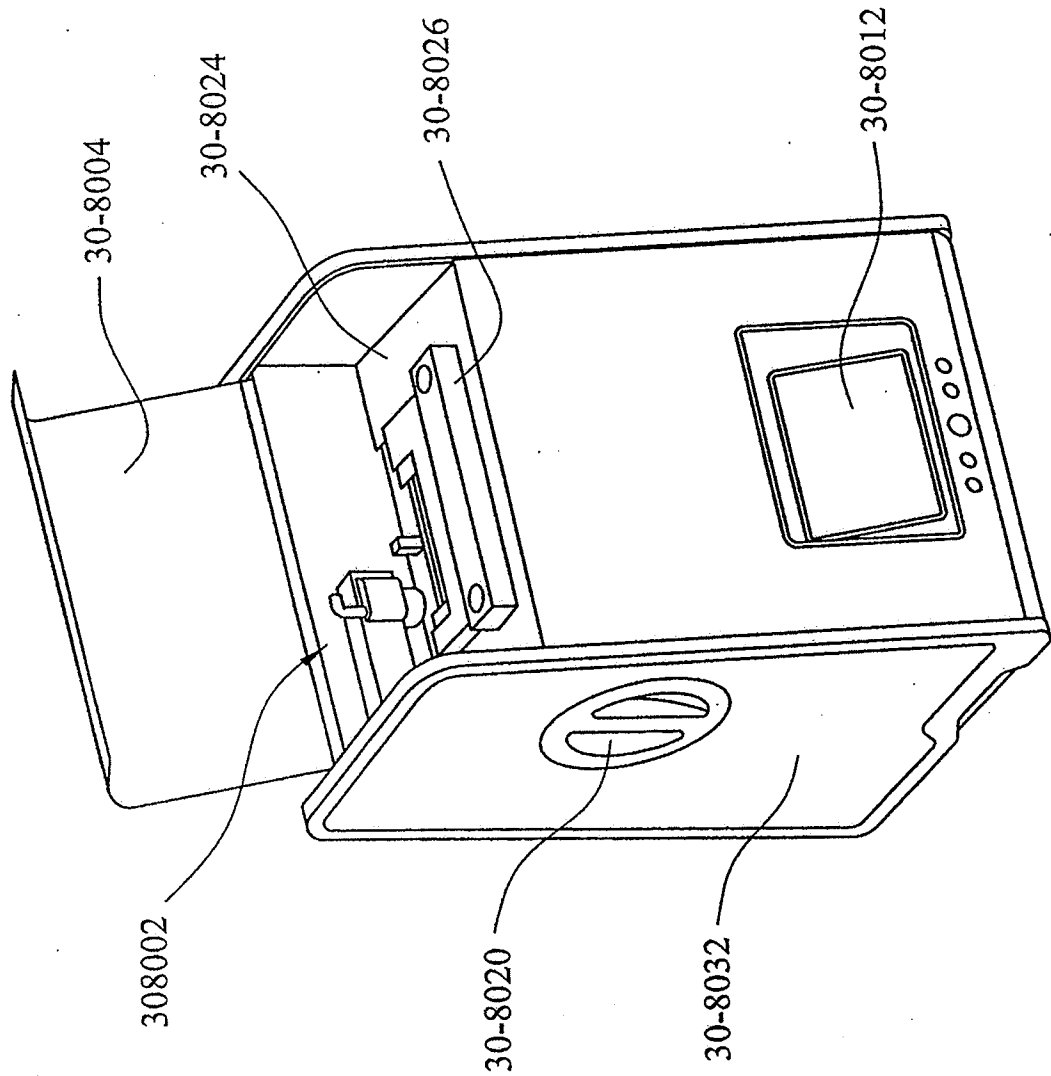


FIG. 30

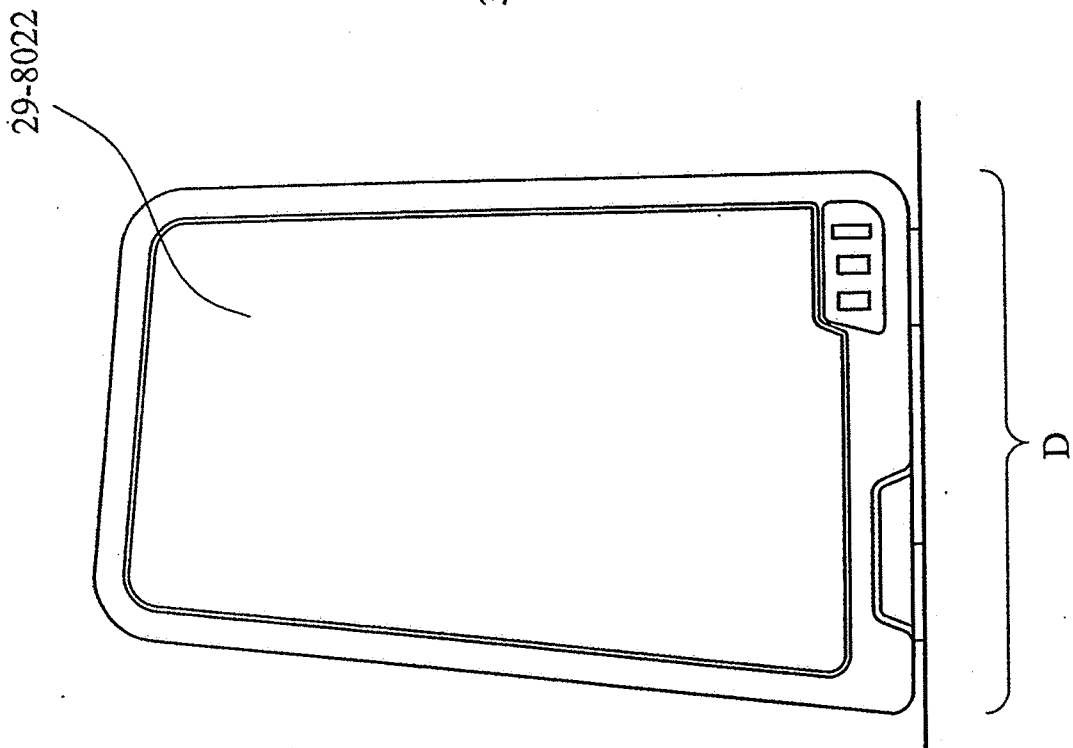


FIG. 29c

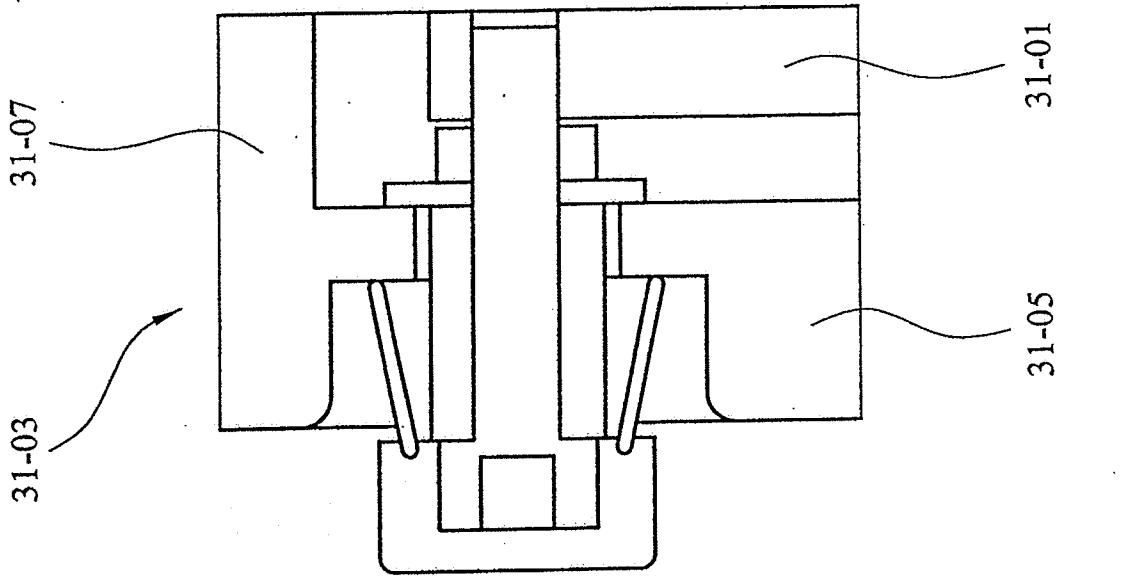


FIG. 31b

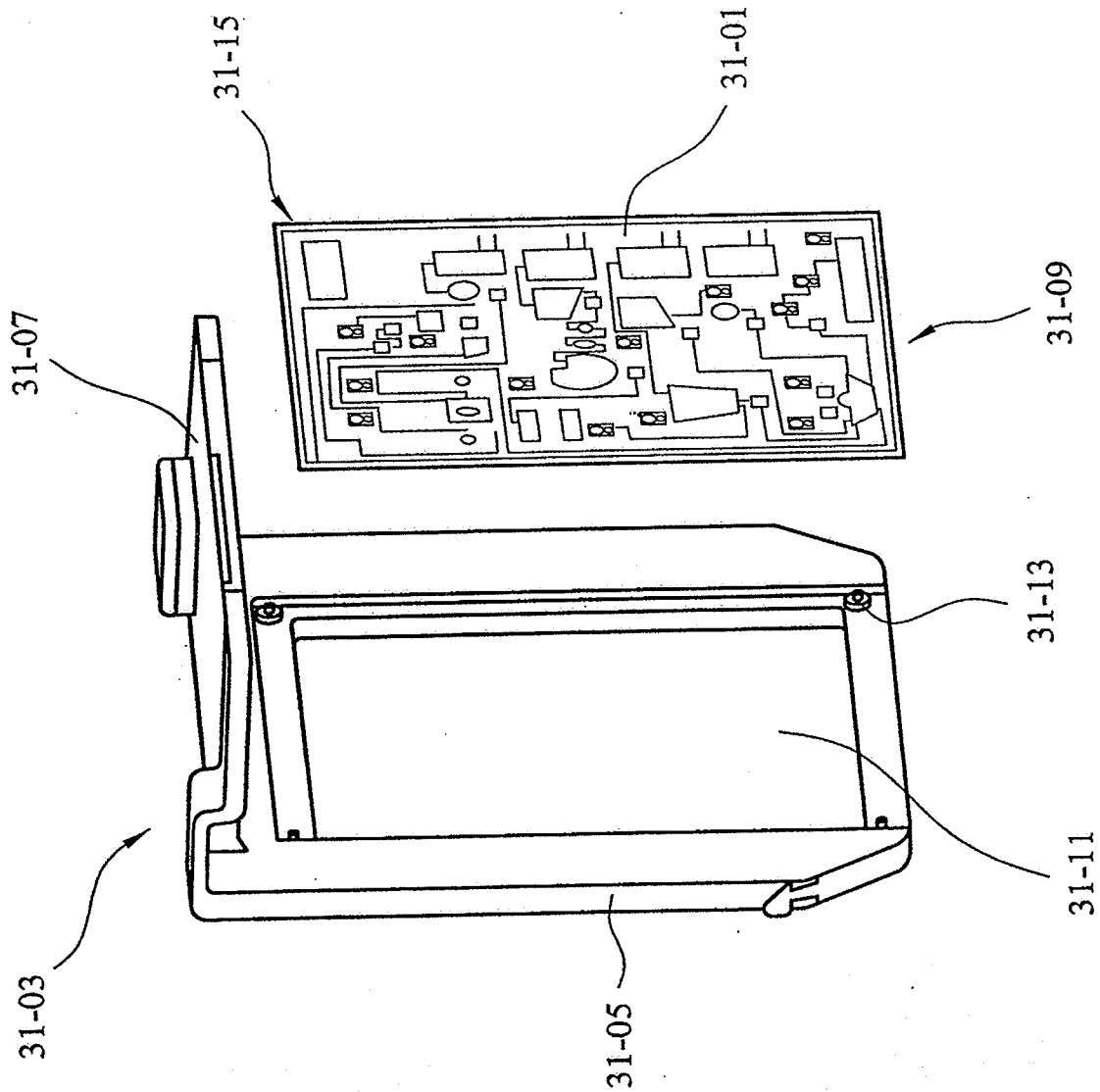


FIG. 31a

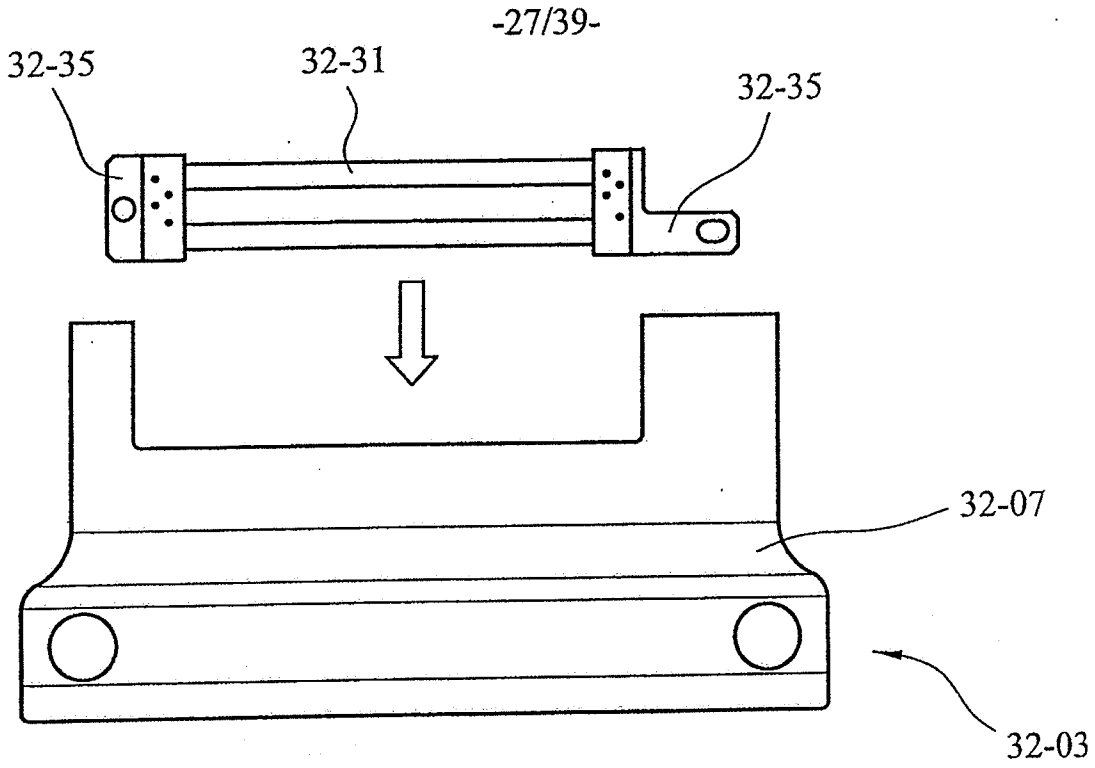


FIG. 32a

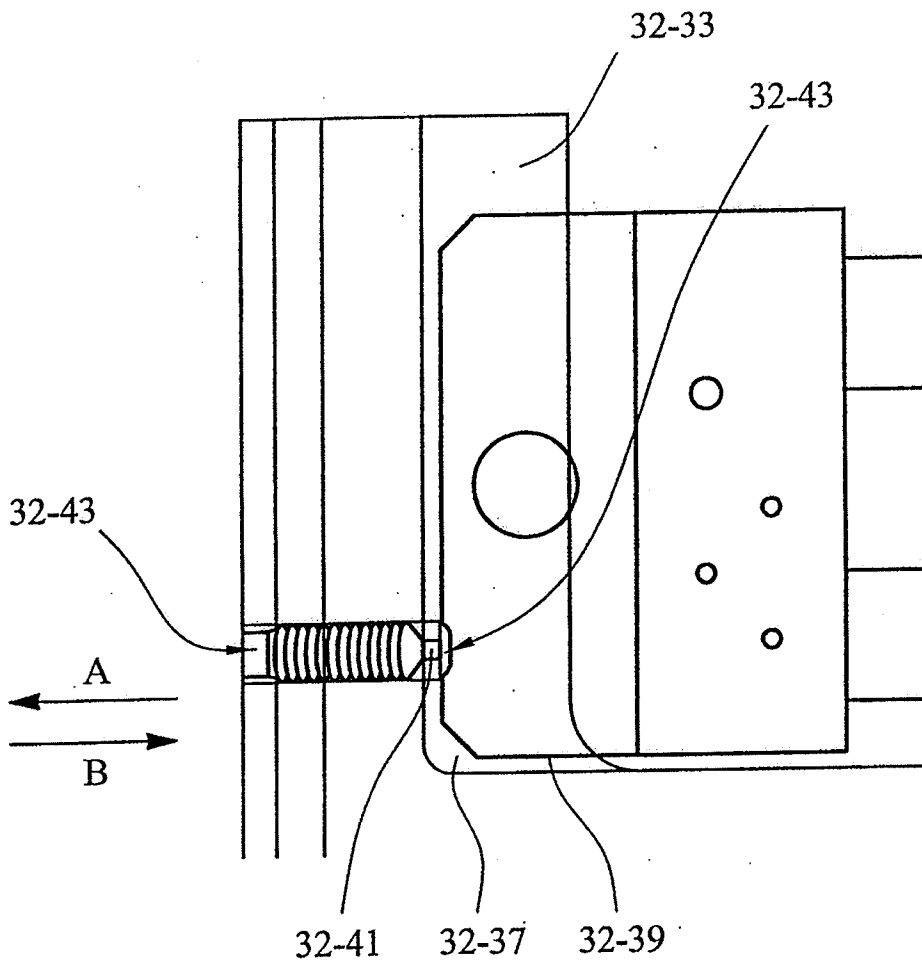


FIG. 32b

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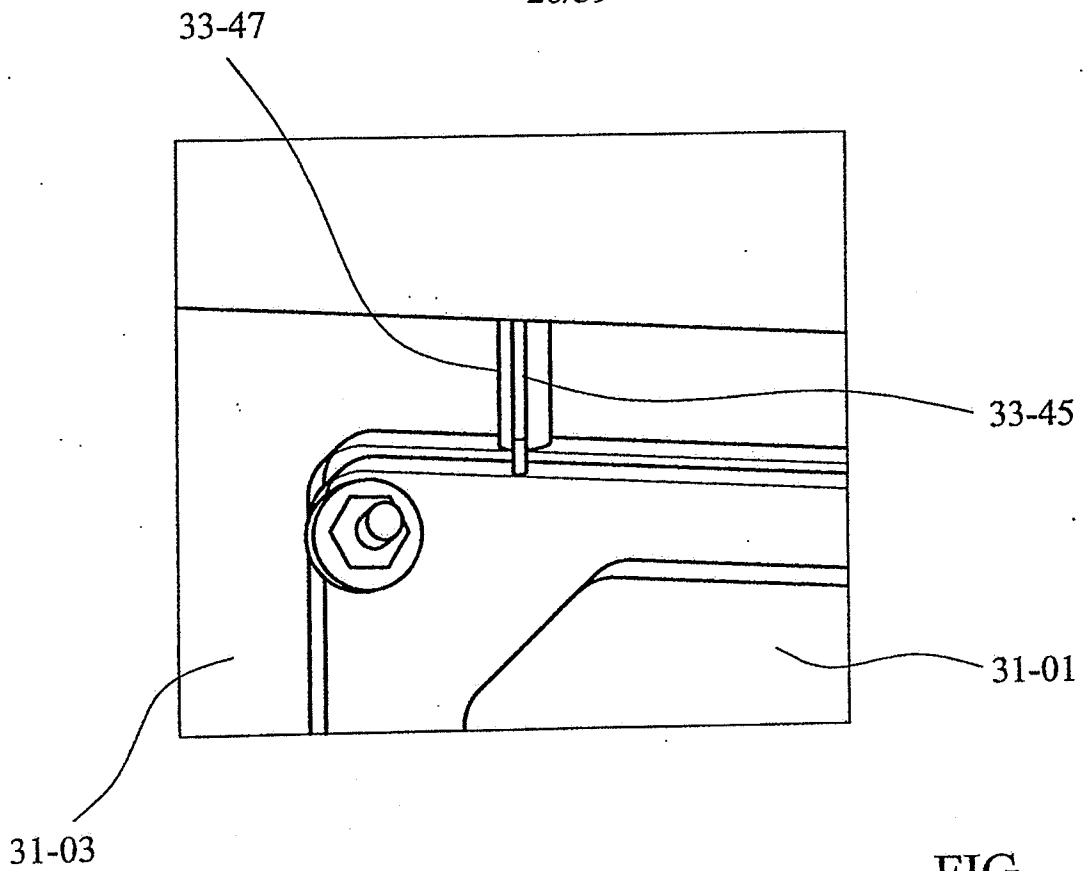


FIG. 33a

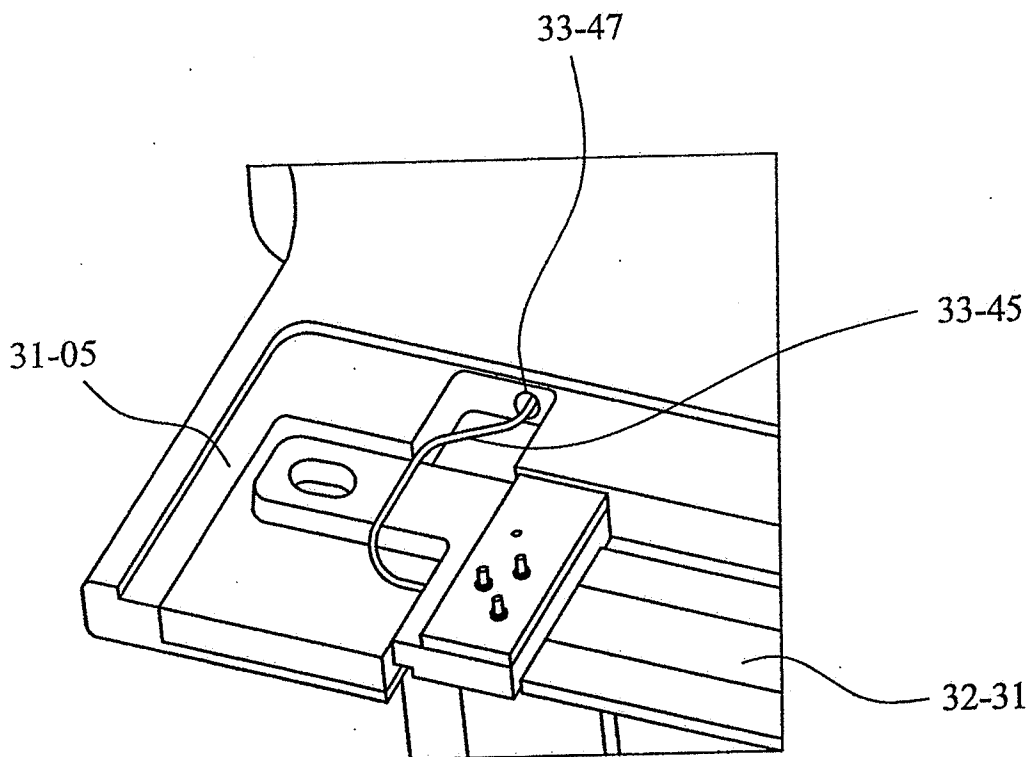


FIG. 33b

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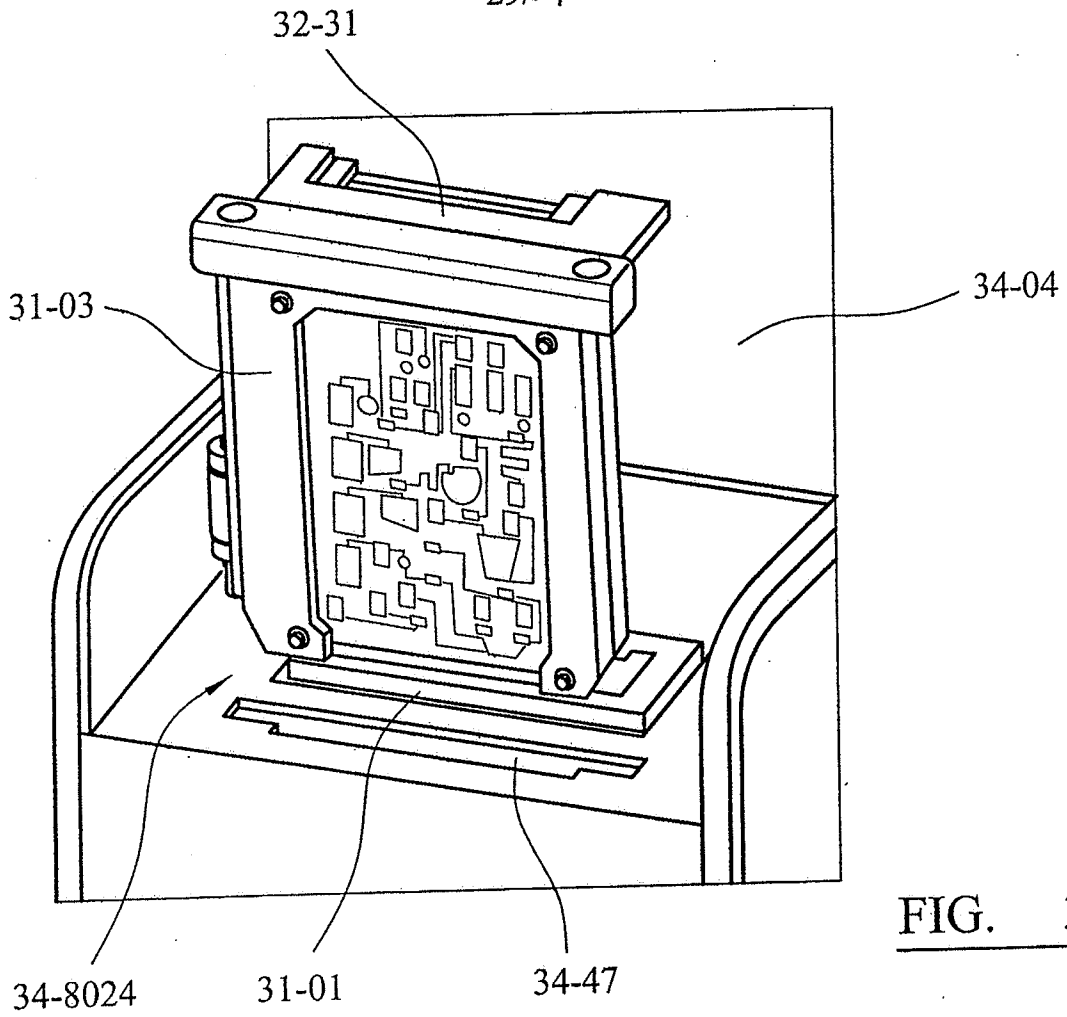


FIG. 34a

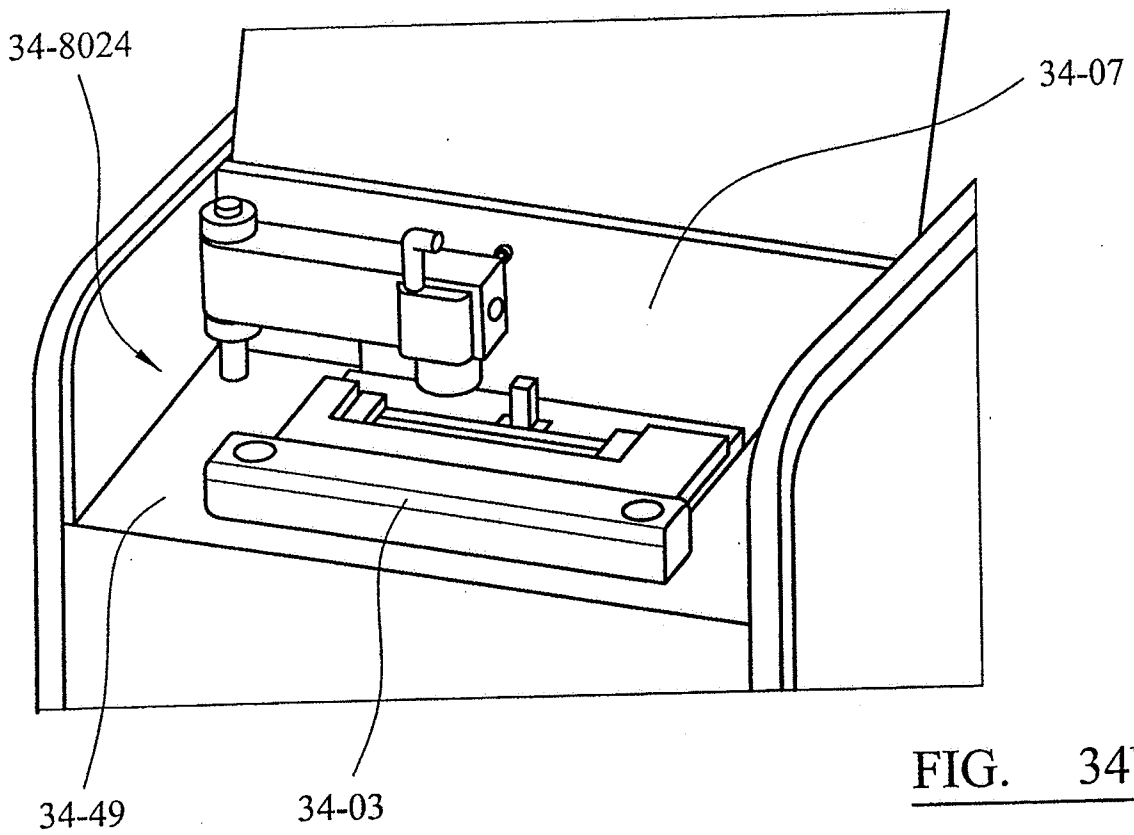


FIG. 34b

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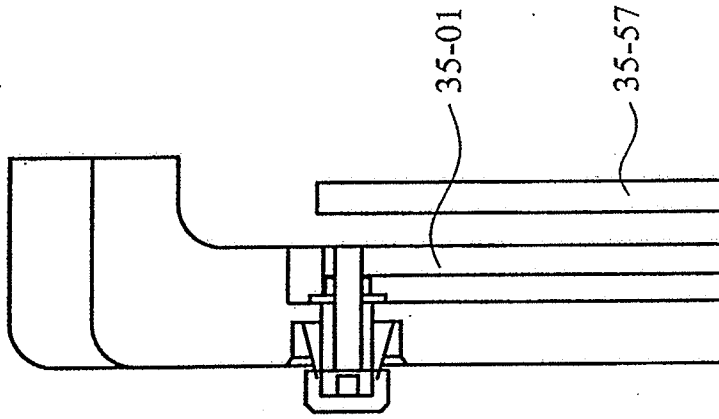


FIG. 35c

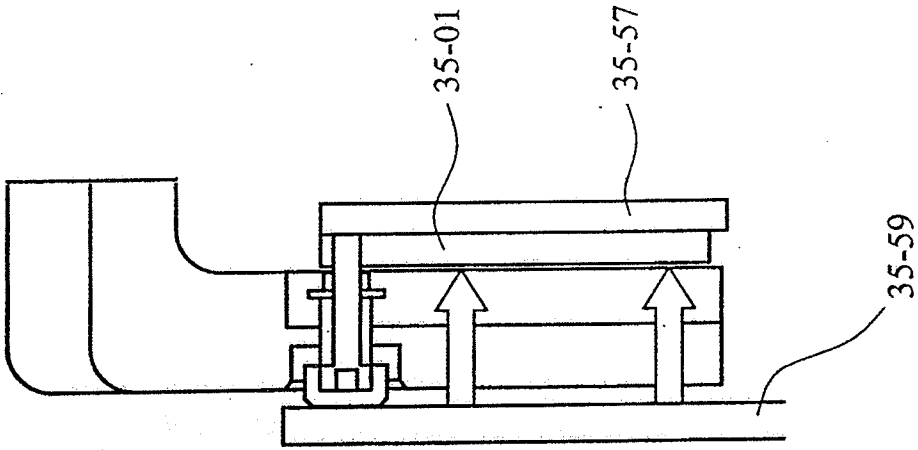


FIG. 35b

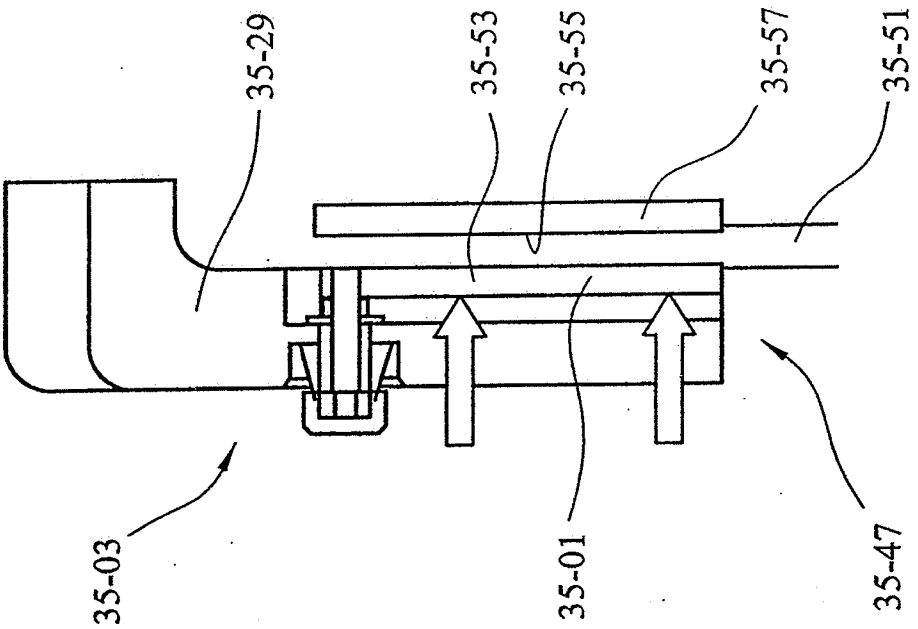
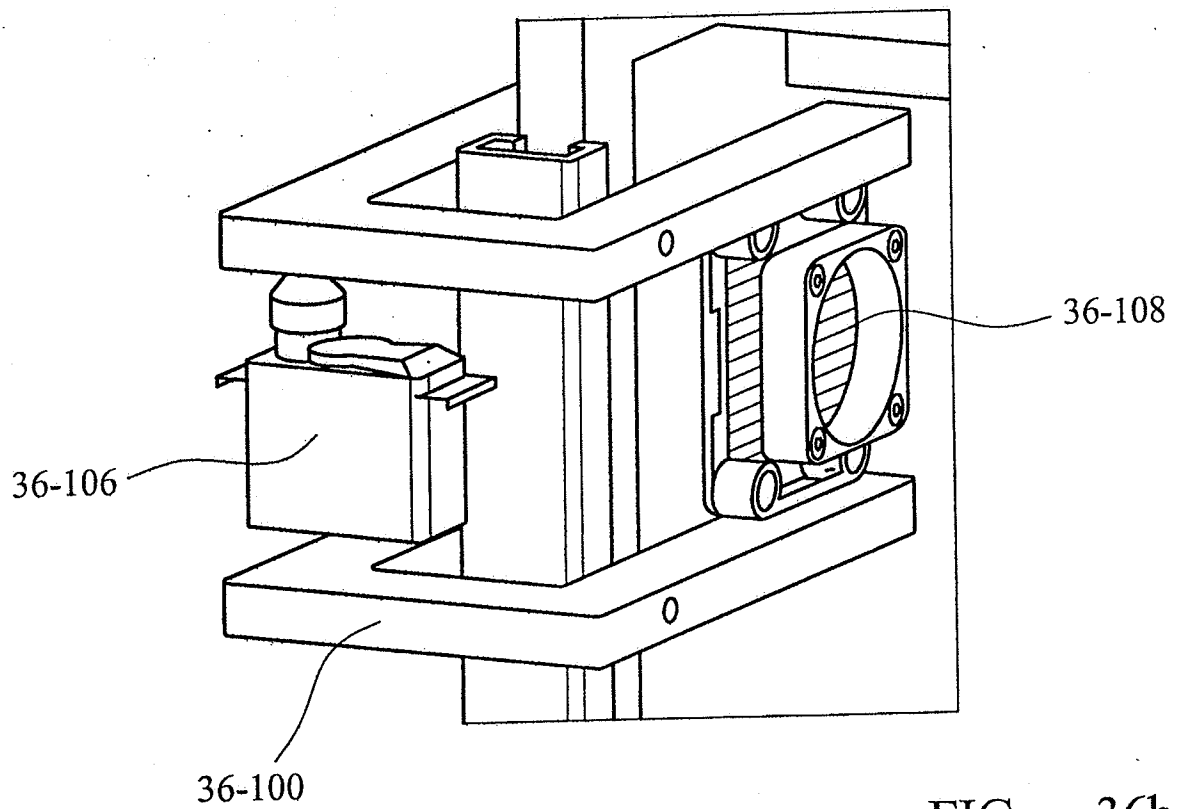
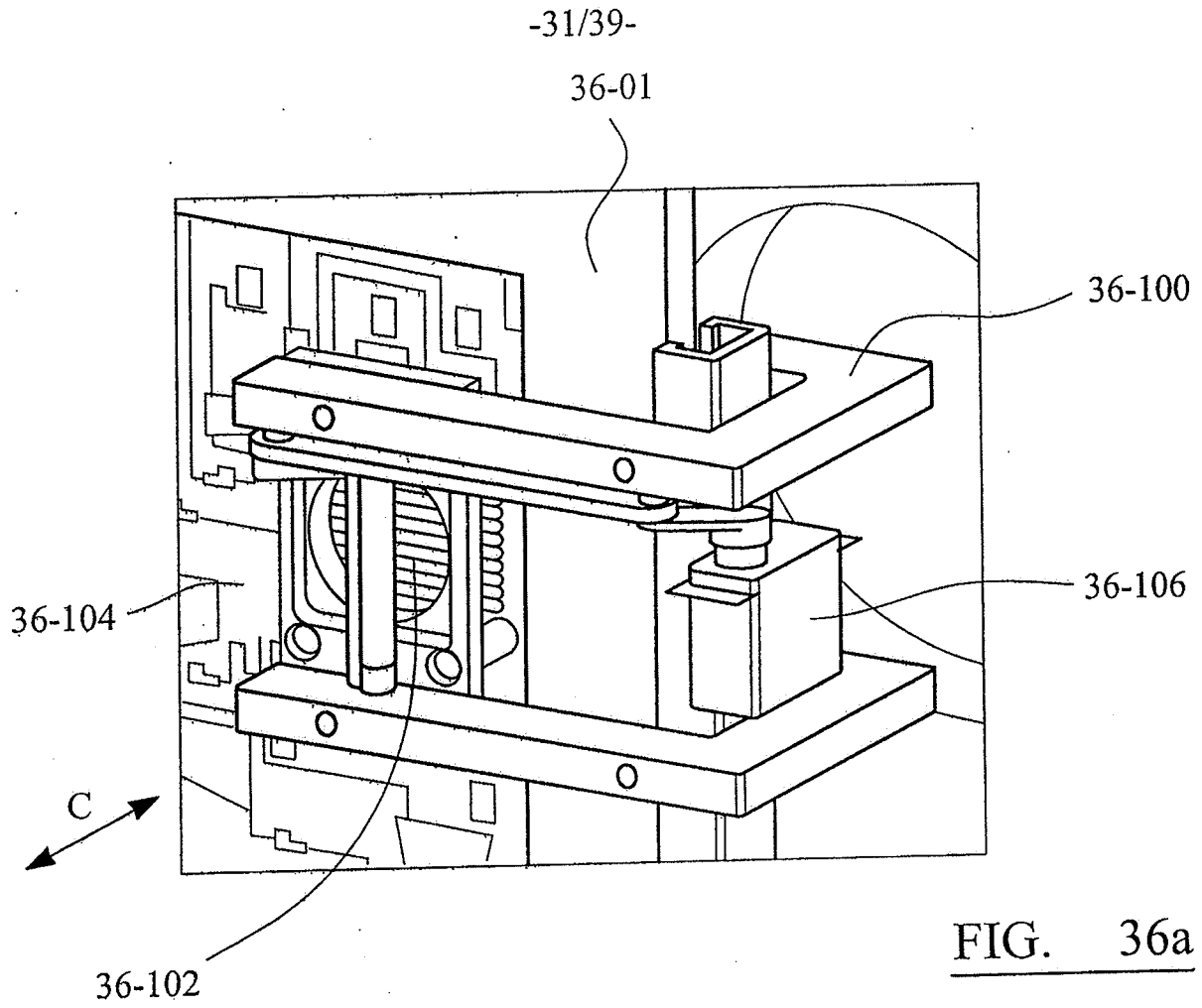


FIG. 35a



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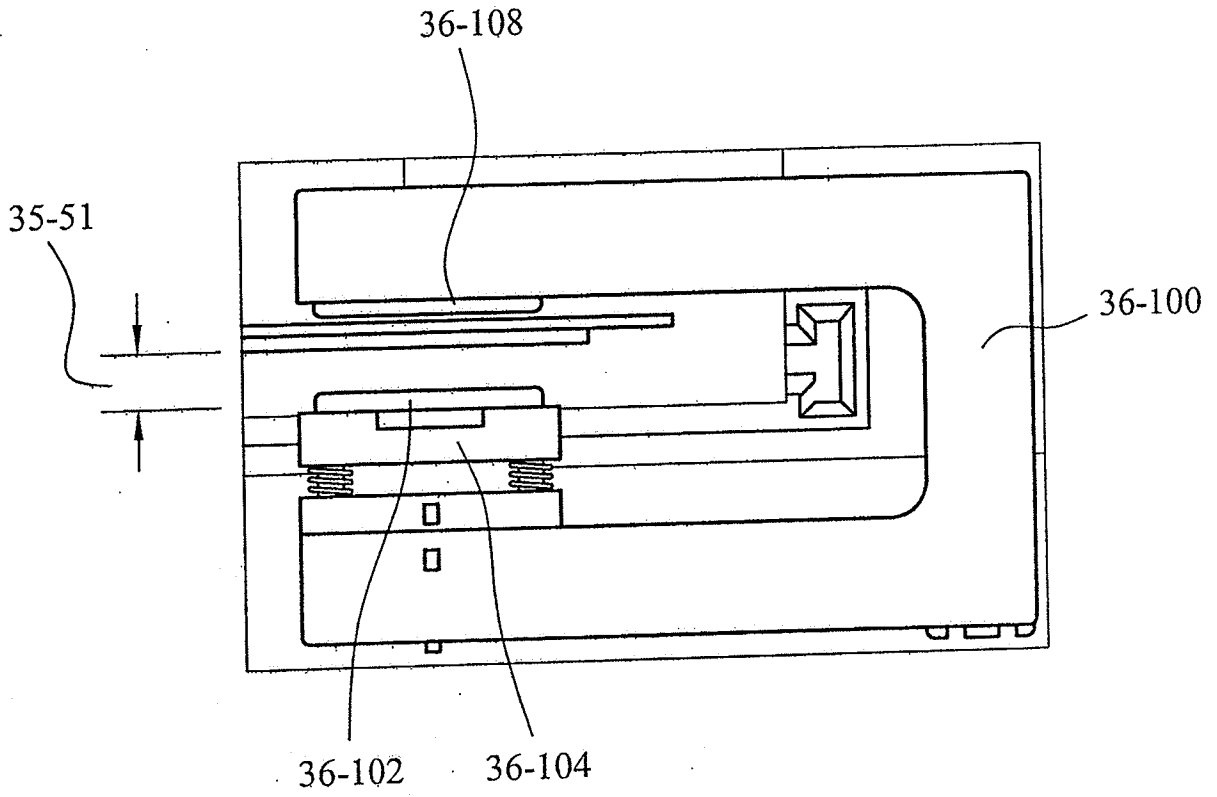


FIG. 36c

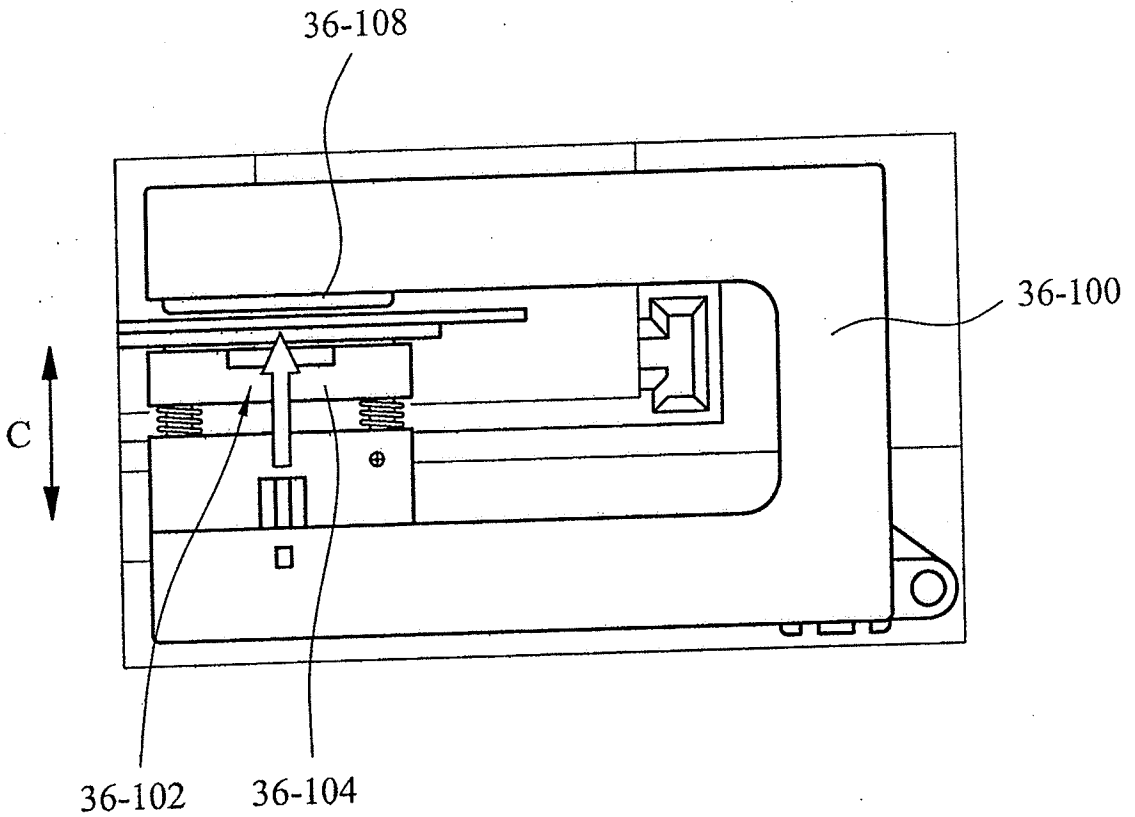


FIG. 36d

FIG. 37a

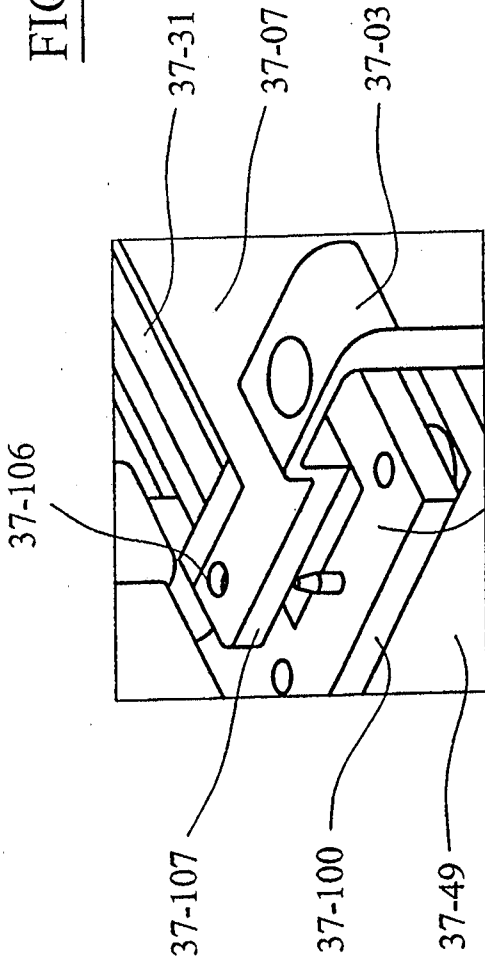
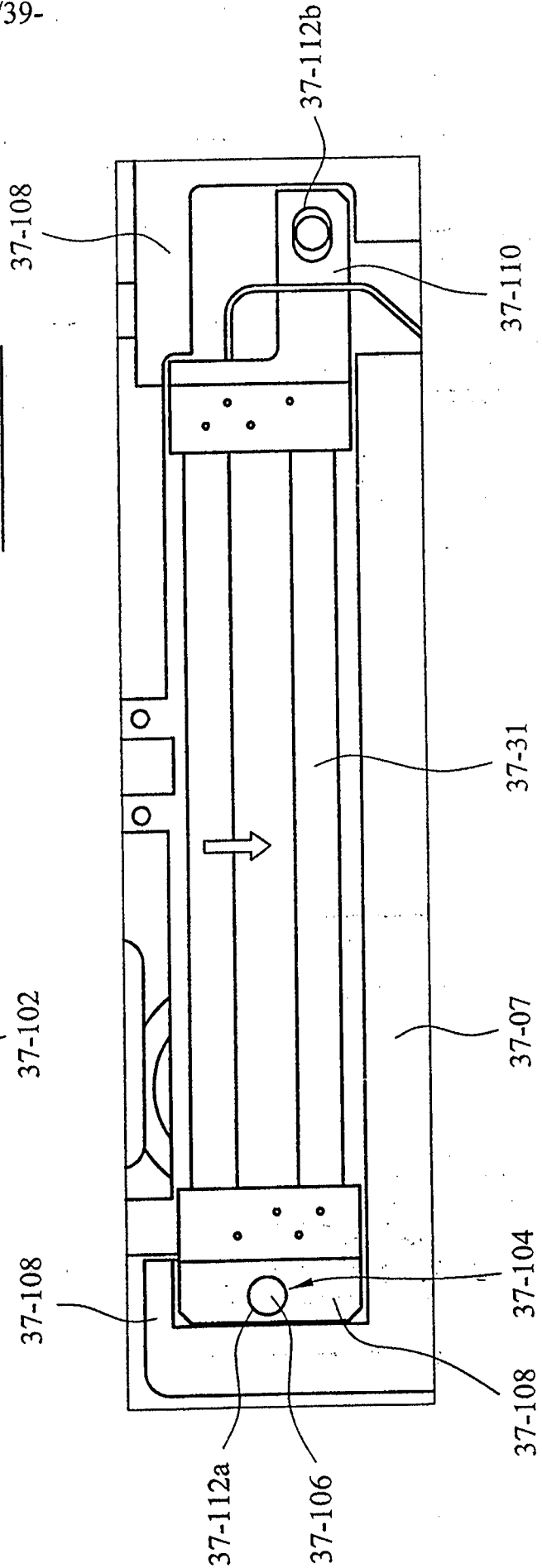


FIG. 37b



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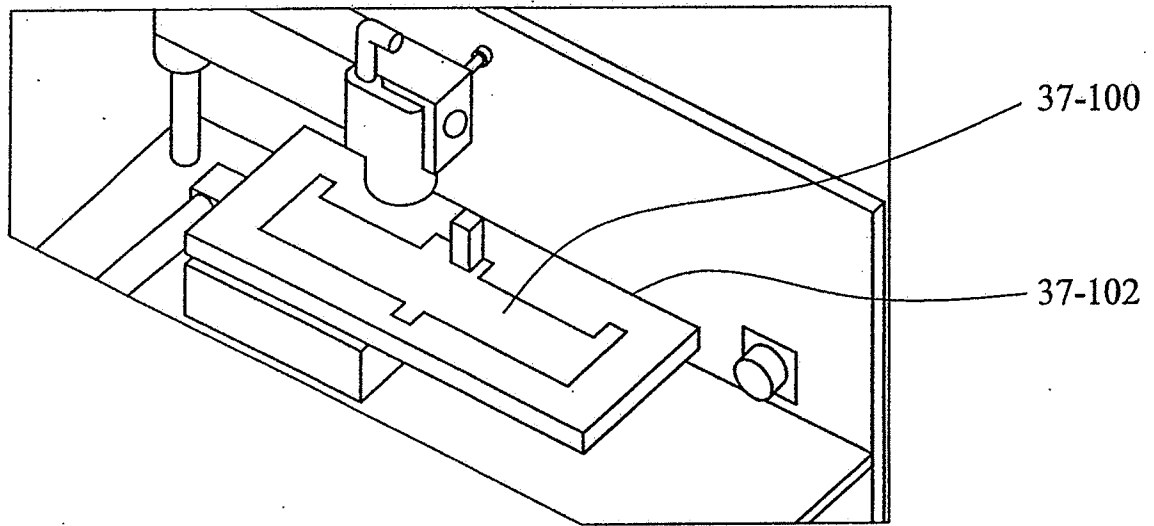


FIG. 38

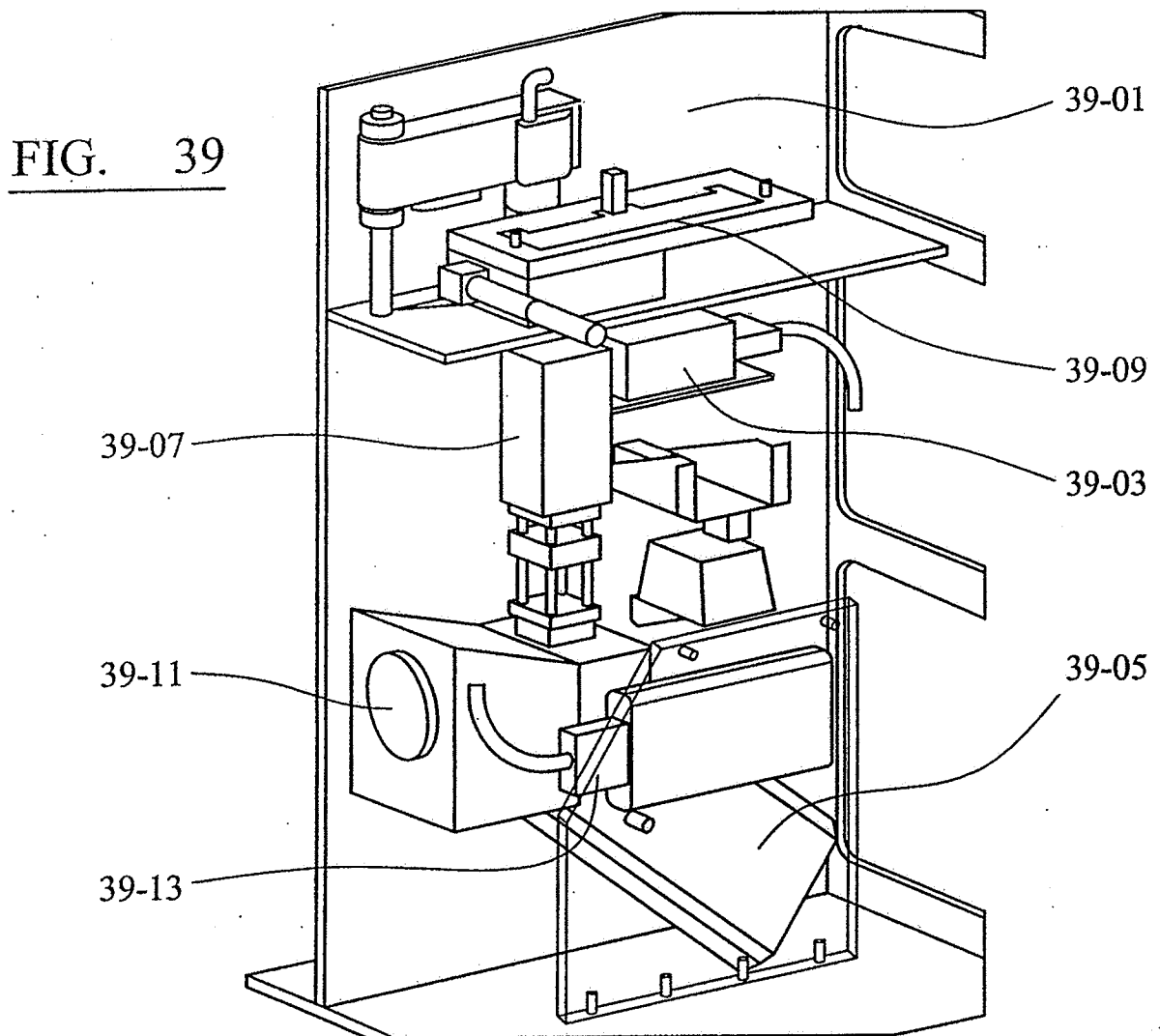


FIG. 39

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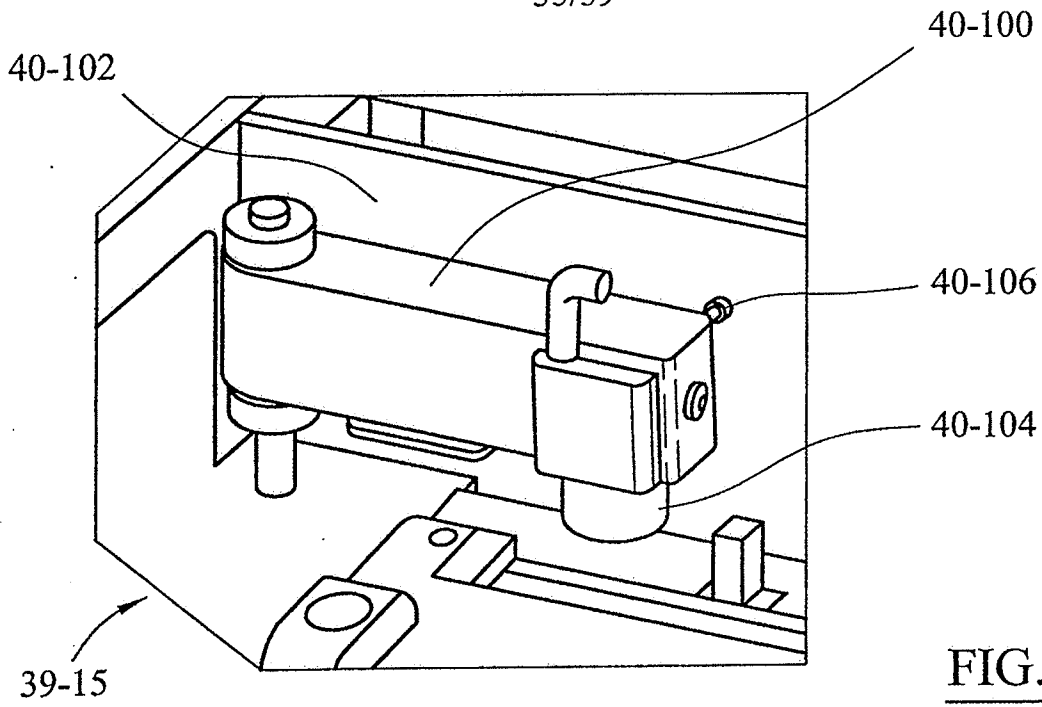


FIG. 40a

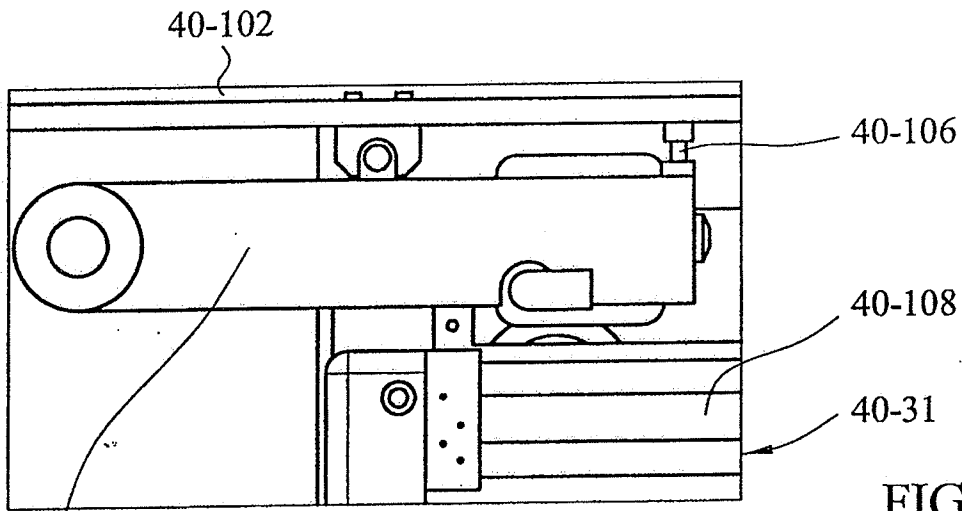


FIG. 40b

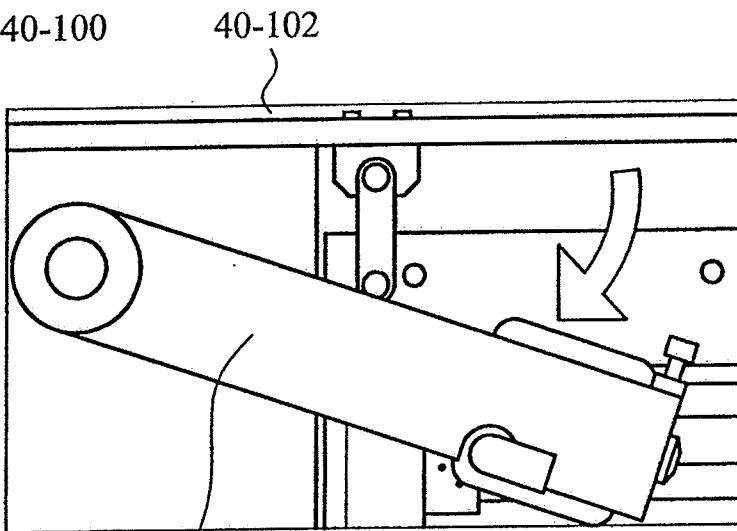


FIG. 40c

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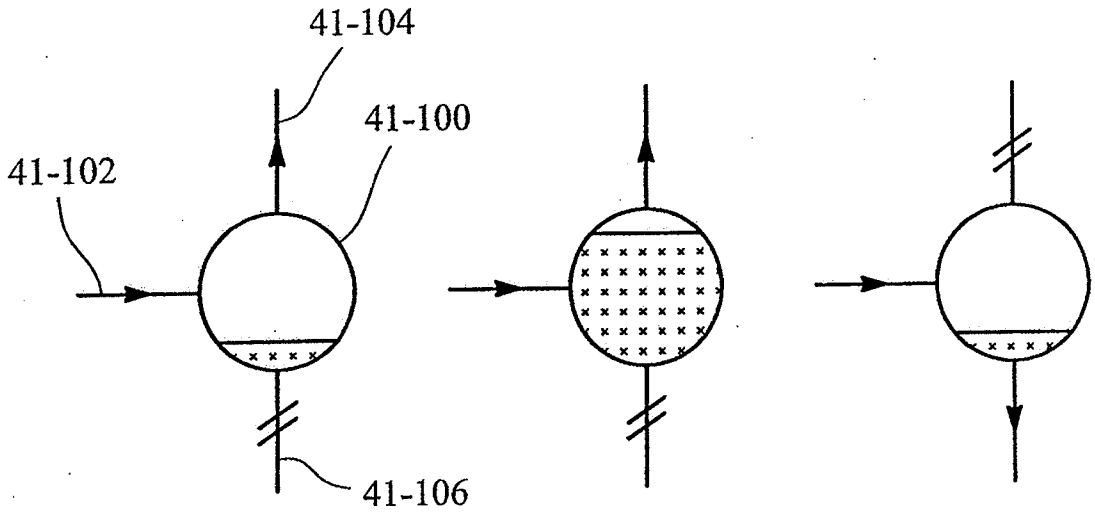


FIG. 41a

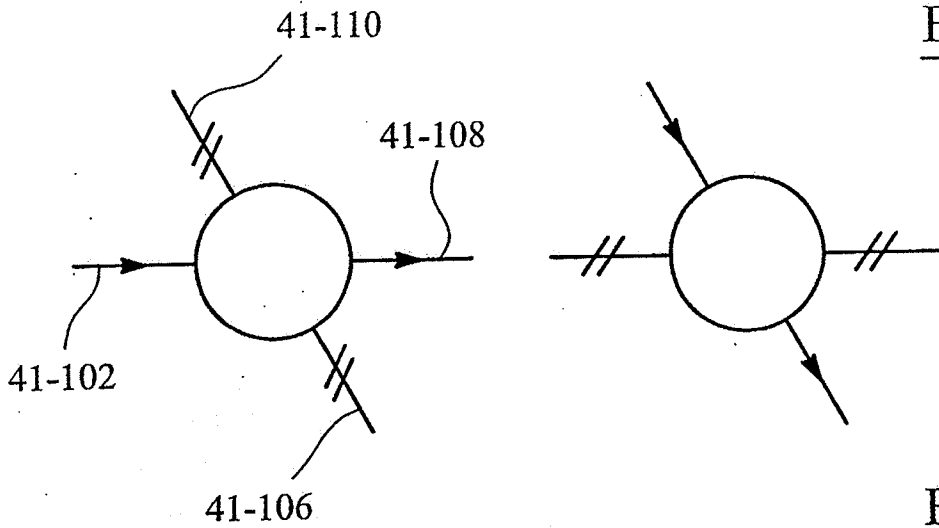


FIG. 41b

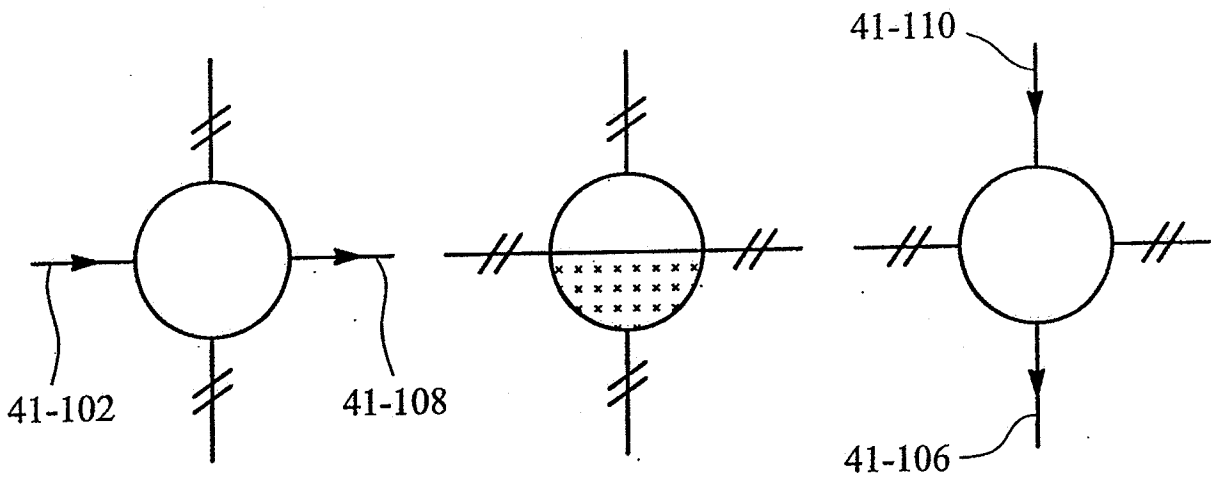
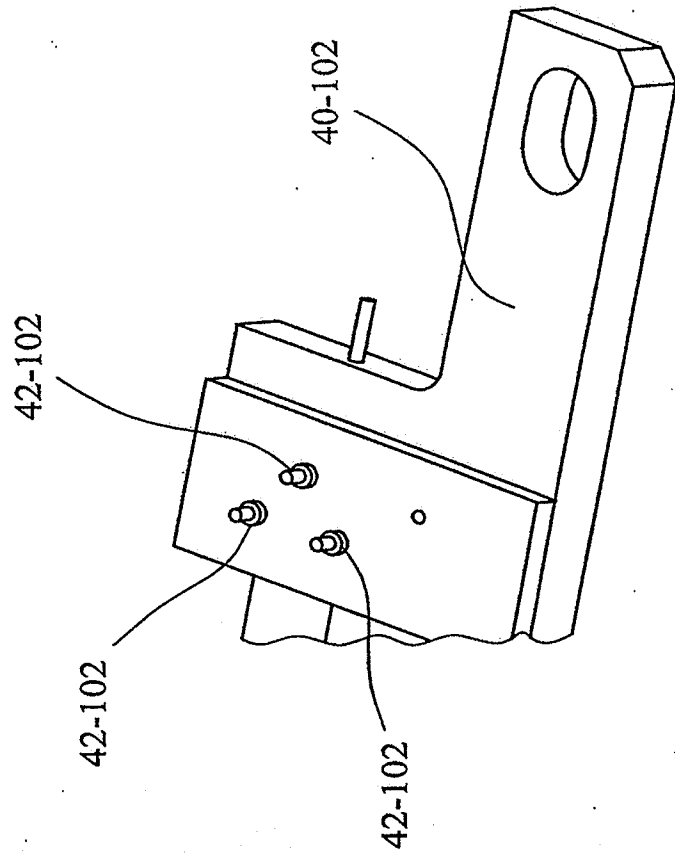
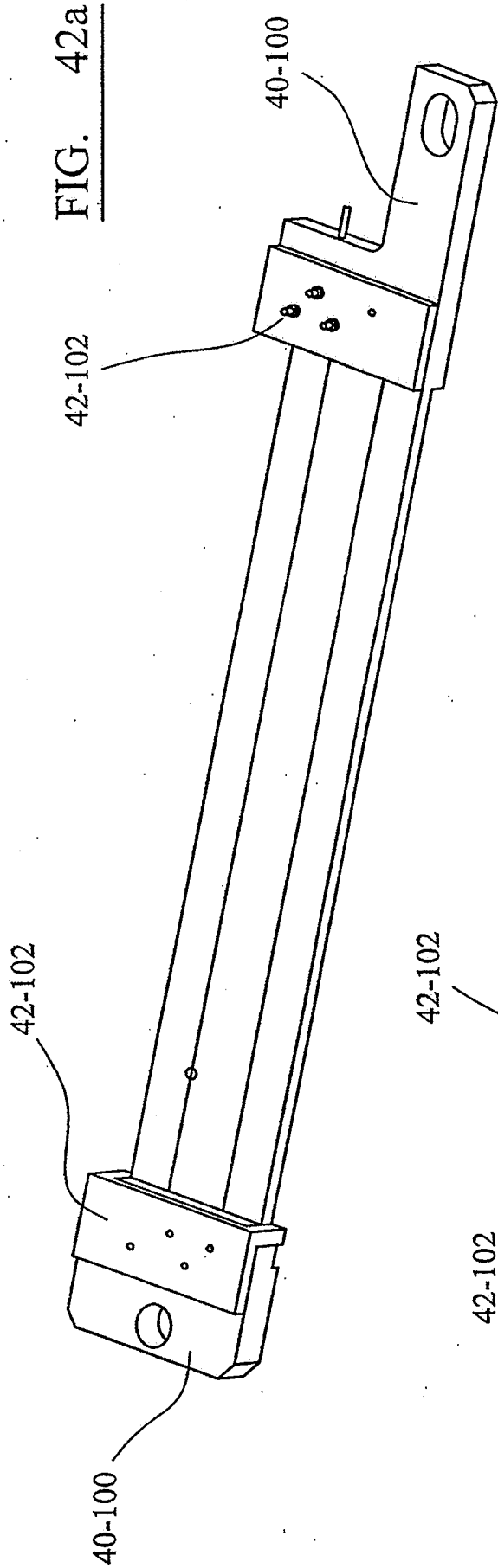


FIG. 41c



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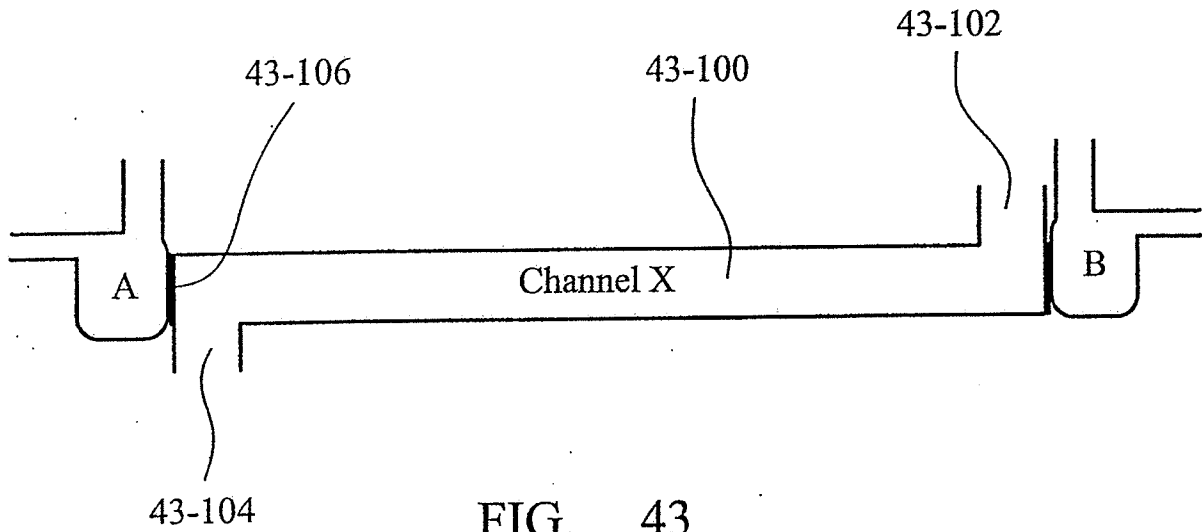


FIG. 43

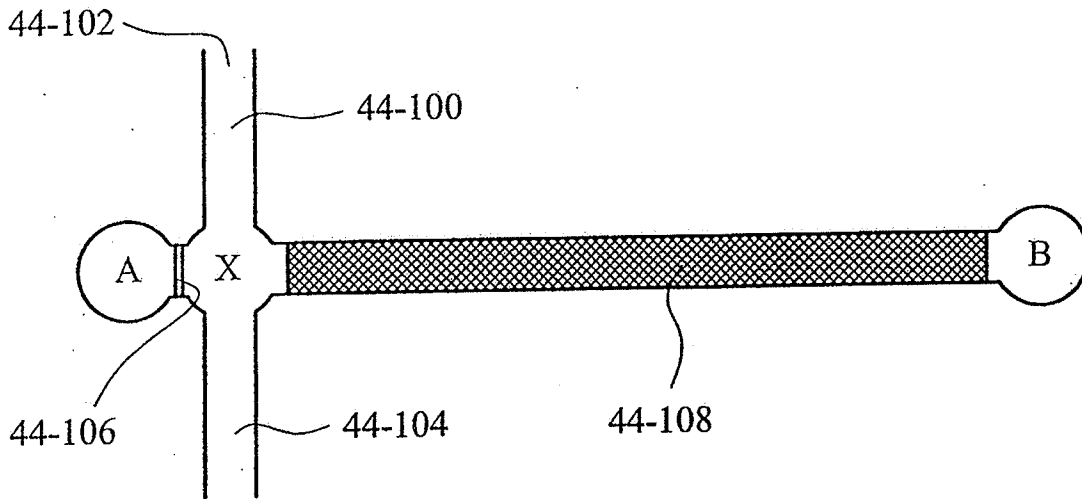


FIG. 44

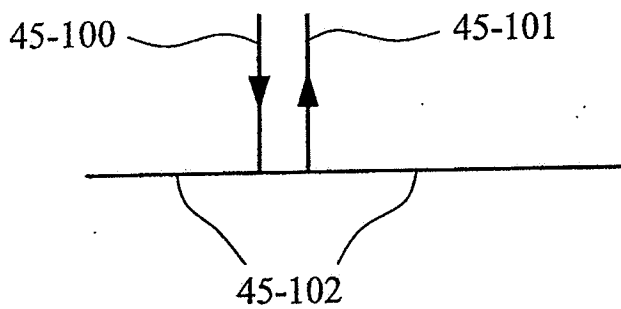


FIG. 45

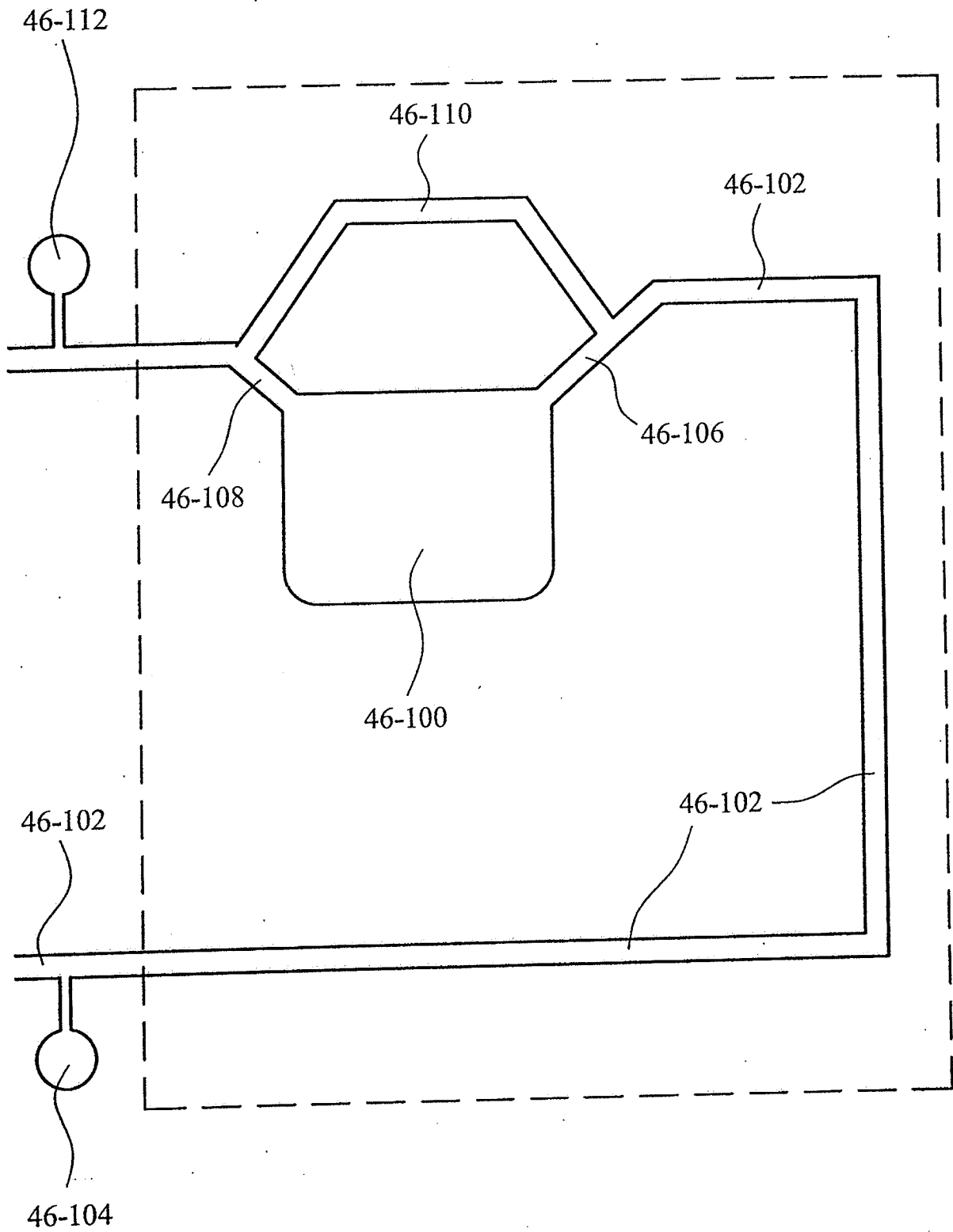


FIG. 46