Abstract: An electrophoresis microchip (200) comprising a substrate having at least one outer surface; at least one main channel (210) extending a first distance within the substrate, the main channel having first and second channel ends; at least one cross channel (220) extending for a second distance within the substrate, the cross channel having first and second ends, wherein the cross channel intersects the main channel; and a primary passage (230) provided in the substrate for each of the first and second main channel ends and each of the first and second cross channel ends, said primary passage connecting the outer surface of the substrate to corresponding one of the first and second main channel ends and the second cross channel ends, wherein at least one of said primary passages is provided with a flared opening.
ELECTROPHORESIS MICROCHIP AND SYSTEM

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/190,060, filed on March 17, 2000, the contents of which are incorporated by reference.

FIELD OF THE INVENTION

The present invention relates to the interface between an electrophoresis microchip and the supporting systems for the microchip during the electrophoretic operations. Specifically, the invention relates to the connections between the microchip and its supporting systems for sample and buffer delivery, washing and reconditioning the microchip, and detection of the electrophoretic operations.

BACKGROUND OF THE INVENTION

Electrophoresis has become an indispensable tool for the biotechnology and other industries, as it is used extensively in a variety of applications, including the separation, identification and preparation of pure samples of nucleic acids, proteins and carbohydrates. Of increasing interest in the broader field of electrophoresis is capillary electrophoresis (CE), where particular entities of species are moved through a medium in an electrophoretic channel of capillary dimensions under the influence of an applied electric field.

Capillary electrophoresis is generally employed to analyze an extremely small quantity of samples, such as proteins or nucleic acids. Other benefits of CE include rapid run time and high separation efficiency. A capillary electrophoresis system or apparatus usually charges a glass capillary having an inner diameter of not more than 100 μm with a migration medium such as buffer or gel, introduces a sample into an end of the capillary, and applies a high voltage across the capillary to separate molecules based on
differences in size or charge-to-size ratio. Since capillaries have large surface area relative to their small volume, resulting in high cooling efficiency, high voltages can be applied in analyzing small quantities of samples at high speed and in high resolution.

However, due to their small outer diameters, capillaries are very fragile, even though they are usually protected by a polyimide coating. Extreme care must be used during the electrophoretic operation. Further, accurate measurement of the samples and their injection into the capillaries are difficult. These difficulties have led to the development of using micro-channels or trenches of capillary dimension in a planar substrate, known as a microchip or an electrophoresis microchip. Electrophoresis operations conducted using such a device are sometimes called micro-channel electrophoresis (MCE).

As described in D. Harrison et al., Anal. Chem. Acta, 283: 361-366 (1993), such an electrophoresis microchip may consist of a pair of transparent substrates. As illustrated in Figure 1A-1D, one of the substrates, substrate 100, is provided with migration grooves/channels 101, 102 and 103 of capillary dimension, which can be formed by etching, while the other substrate 110 is provided with holes 111-114, corresponding to the ends of grooves 101 and 102. Substrates 100 and 110 are then super-imposed over each to form microchip 120, which is shown in Figure 1C and 1D.

During a typical microchip electrophoretic operation, the migration channels 101-103 are first filled with suitable buffer. A sample is then injected into channel 102 through hole 113. A voltage is applied across channel 102 between holes 113 and 114 for a specified period of time, which allows dispersion of the sample across channel 102 and, especially, into channel 103. A higher voltage is then applied across the main separation channel 101 between holes 111 and 112. The sample will form bands along channel 101 due to different mobility of the different types of molecules in the sample. The bands
will be detected at a detection position (not shown) near the end of channel 101/hole 112.

In traditional capillary electrophoretic operations, a replaceable gel may be used that allows the capillaries to be used many times. After each electrophoresis separation is completed, the replaceable gel, which usually is a linear, rather than cross-linked, polymer, is pushed out of the capillaries by high pressure (about 2000 psi). The capillaries are then washed and preconditioned before filling with new gel by high pressure.

When microchip electrophoretic operations are used for DNA sequencing or genotyping, a high viscosity gel is usually used as the separation medium. This is usually accomplished by filling the microchannels with a cross-linked polymer before the top plate is added to the lower microchannel plate and fused together with the lower plate. Once several DNA sequencing or genotyping separations have been performed through the medium, the entire electrophoresis microchip assembly is usually discarded.

Various attempts have been made to further improve the operation of electrophoresis using microchips. For example, U.S. Patent No. 5,858,195 discloses a microchip laboratory system and method providing fluid manipulations for a variety of applications, including sample injection for microchip chemical separations. The microchip is fabricated using standard photolithographic procedures and chemical wet etching, with the substrate and cover plate joined using direct bonding. Capillary electrophoresis and electro-chromatography are performed in channels formed in the substrate. Analytes are loaded into a four-way intersection of channels by electrokinetically pumping the analyte through the intersection, followed by switching of the potentials to force an analyte plug into the separation channel.

U.S. Patent No. 6,007,690 discloses integrated microfluidic devices comprising at least an enrichment channel and a main electrophoretic flowpath. In the integrated devices, the enrichment channel and the main
electrophoretic flowpath are positioned so that waste fluid flows away from the main electrophoretic flowpath through a discharge outlet. The devices claim to be applicable in a variety of electrophoretic applications, including clinical assays, high throughput screening for genomic and pharmaceutical applications, point-of-care in vitro diagnostics, molecular genetic analysis and nucleic acid diagnostics, cell separations, and bioresearch generally.

U.S. Patent No. 6,013,168 discloses a microchip electrophoresis apparatus in which, when a microchip is set on a tray and operation of an apparatus is begun, the microchip moves to feed position in order to be filled with a buffer solution, whereupon a sample is injected into this microchip. Thereafter the tray is located on a detecting position, so that a sample introduction voltage is applied between a sample reservoir and a sample waste reservoir for introducing the sample into a separation passage. Subsequently, the operation is switched to application of a separation voltage between a buffer reservoir and a drain reservoir, for beginning analysis. When the analysis has begun, a detector detects a migration pattern in the separation passage, so that a signal processing board data-processes the detected pattern.

U.S. Patent No. 6,042,708 discloses microchip electrophoretic method and apparatus, in which, in order to irradiate a constant range of a separation passage of a microchip, light from a light source linearly extending along the separation passage is transmitted through a cylindrical lens and a bandpass filter and introduced into the separation passage. The light transmitted through the separation passage of the microchip is introduced into a photocell array through a cylindrical lens and detected. Measurement is repetitively performed and accumulated to determine migration patterns.

U.S. Patent No. 6,045,676 discloses a microfabricated capillary electrophoresis chip that includes an integral thin film electrochemical detector for molecules separated in the capillary.
U.S. Patent No. 6,046,056 discloses novel microfluidic devices and methods that are useful for performing high-throughput screening assays. In particular, the devices and methods of the invention are useful in screening large numbers of different compounds for their effects on a variety of chemical, and preferably, biochemical systems.

As illustrated by these patents, the contents of which are herein incorporated by reference, much progress has been made in improving the operations of electrophoresis using microchips. However, in order to effectively use a microchip as a platform for high throughput analysis, especially in an automated system, a number of problems will have to be resolved. One of these problems is the macro-to-micro interface. This includes the loading/filling system, injection system, detection system, washing/reconditioning system and electrical connections to proper microchip regions with proper parameters. Improvement in any of these areas of the interface, especially the ability to accommodate high pressure, will increase the overall efficiency of the microchip electrophoretic operations.

**SUMMARY OF THE INVENTION**

The present invention provides a novel electrophoresis microchip and system for performing electrophoretic operations. In one aspect of the invention, a novel mechanism is provided for the connection between a microchip and capillaries for transferring fluids such as samples, buffer, gel, and washing solutions and between the microchip and optical fiber for detection purposes. This mechanism provides quick connections between the electrophoresis microchip substrate and the capillaries so as to allow transfers of liquid, including highly viscous gel, between the substrate and the capillaries under high pressure. In another aspect of the invention, a novel mechanism is provided for continued supply of buffer during the electrophoretic operation process such that used buffer and agents can be replaced.
with fresh ones. This mechanism alleviates or eliminates the ion depletion problem associated with conventional microchip electrophoresis.

In one embodiment, the present invention provides an electrophoresis microchip. The microchip comprises (1) a substrate having at least one outer surface; (2) at least one main channel extending for a first distance within the substrate and having first and second main channel ends; (3) at least one cross channel extending for a second distance within the substrate and having first and second cross channel ends, wherein the cross channel intersects the main channel; and (4) at least one primary passage provided in the substrate for each of the first and second main channel ends and each of the first and second cross channel ends, the passage connecting the outer surface of the substrate to a corresponding one of the first and second main channel ends and the first and second cross channel ends, wherein at least one of said primary passages is provided with a flared opening.

In a preferred embodiment, the flared opening comprises a tapered cylindrical shape having a first cross section formed on the outer surface of the substrate and a second cross section formed inside the substrate at the end of the tapered cylindrical shape, said first cross section being larger than said second cross section. In a more preferred embodiment, the first cross section and the second cross section are substantially circular and, most preferably, the diameter of the first cross section ranges from about 0.2 mm to about 20 mm and the diameter of the second cross section ranges from about 10 μm to about 5 mm. As to the length of the tapered cylindrical shape, it preferably has an axis length ranging from about 0.1 mm to about 20 mm.

In another preferred embodiment, the main channel and the cross channel are oriented substantially parallel to the outer surface and the primary passages are oriented substantially transverse to the main channel and the cross channel. The substrate preferably comprises a pair of plate members having grooves formed on a surface of at
least one plate member to form the main channel and the cross channel.

In yet another preferred embodiment, the substrate comprises a plurality of main channels and a plurality of cross channels, wherein each of the cross channels intersects one of the main channels. More preferably, two or more channels may share a common first channel end and a common second channel end.

The microchip of the present invention preferably comprises an integrated electrode deposited on the walls of the primary passages.

In another preferred embodiment of the present invention, the substrate further comprises secondary passage that connects the outer surface of the substrate and the main channel at a point between the second main channels end and the intersection of the main channel and the cross channel. In certain applications, such as DNA sequencing, the secondary passage may connect to a capillary that actually performs the separation. Thus the microchip, in this situation, effectively serves as an injector, which preferably provides a non-biased sample, for the electrophoresis capillary.

The secondary passage may also serve as a detection passage. Preferably, the detection passage connects the outer surface of the substrate to the main channel at a point between the second main channel end and the intersection of the main and cross channel. More preferably, the detection passage is substantially transverse to the main channel and comprises a tapered cylindrical shape having a first cross section formed on the outer surface of the substrate and a second cross section formed inside the substrate at the end of the tapered cylindrical shape, said first cross section being larger than said second cross section. Most preferably, the diameter of the first cross section ranges from about 0.2 mm to about 10 mm and the diameter of the section cross section ranges from about 20 μm to about 5 mm.

In another preferred embodiment of the present invention, the cross channel comprises first sub-cross
channel, which intersects the main channel at position A along the main channel and second sub-cross channel, which intersects the main channel at position B along the main channel. Further, the first sub-cross channel extends from the first cross channel end to position A and the second sub-cross channel extends from the second channel end to position B. Thus, in this embodiment the cross channel intersects the main channel at two different points and that the total length of the two sub-cross channels equals to the second distance. Points A and B are preferably close to each other and are both closer to the first main channel end then the second main channel end. In this embodiment, the microchip is able to provide a non-biased sample.

In another embodiment, the present invention provides an electrophoresis microchip which comprises (1) a substrate having at least one outer surface; (2) at least one main channel extending for a first distance within the substrate, the main channel having first and second main channel ends; (3) at least one cross channel extending for a second distance within the substrate, the cross channel having first and second cross channel ends, wherein the cross channel intersects the main channel; (4) a primary passage provided in the substrate for each of the first and second main channel ends and each of the first and second cross channel ends, the primary passage connecting the outer surface of the substrate to a corresponding one of the first and second main channel ends and the first and second cross channel ends; and (5) a secondary passage provided in the substrate for each of the first and second main channel ends, the secondary passage connecting an outer surface of the substrate to a corresponding one of the first and second main channel ends.

In a preferred embodiment, the substrate comprises first and second outer surfaces parallel to each other, wherein the at least one main channel is parallel to the first and second outer surfaces. More preferably, the primary and secondary passages to the first and second main channel ends are substantially transverse to the at least
one main channel, said primary passages connecting said first outer surface to said first and second main channel ends and said secondary passages connecting said second outer surface to said first and second main channel ends. In another preferred embodiment, at least one of the primary and the secondary passages is provided with a flared opening.

In a third embodiment, the present invention provides a system for performing electrophoretic operations. The system comprises (a) at least one container configured to hold a liquid; (b) an electrophoresis microchip comprising (1) a substrate having at least one outer surface; (2) at least one main channel extending for a first distance within the substrate, the main channel having first and second main channel ends; (3) at least one cross channel extending for a second distance within the substrate, the cross channel having first and second cross channel ends, wherein the cross channel intersects the main channel; and (4) at least one primary passage provided in the substrate for each of the first and second main channel ends and each of the first and second cross channel ends, the primary passage connecting the outer surface of the substrate to a corresponding one of the first and second main channel ends and the first and second cross channel ends, wherein at least one of said passages is provided with a flared opening; and (c) at least one capillary having first and second ends, said first capillary end connecting said at least one container and said second capillary end being inserted into said at least one flared opening of said primary passages.

In a preferred embodiment, the container comprises a chamber containing a micro-titer plate having a plurality of wells, said wells connecting to said first capillary end. More preferably, the chamber is sealed and provided with pump means for increasing pressure inside the chamber such that a liquid contained in said wells is forced into said at least one capillary.

In another preferred embodiment, the second end of said at least one capillary is secured to said flared
opening of said at least one primary passages by an adhesive.

In yet another preferred embodiment, the microchip further comprises a detection passage connecting the outer surface of the substrate to the main channel at an area between the first and second main channel ends. More preferably, the detection passage is substantially transverse to the main channel and comprises a tapered cylindrical shape having a first cross section formed on the outer surface of the substrate and a second cross section formed inside the substrate at the end of the tapered cylindrical shape, said first cross section being larger than said second cross section. Most preferably, an optical fiber is connected to the detection passage.

In a fourth embodiment, the present invention provides a system for performing electrophoretic operations. The system comprises (a) at least one container configured to hold a liquid; (b) an electrophoresis microchip comprising (1) a substrate having at least one outer surface; (2) at least one main channel extending for a first distance within the substrate, the main channel having first and second main channel ends; (3) at least one cross channel extending for a second distance within the substrate, the cross channel having first and second cross channel ends, wherein the cross channel intersects the main channel; (4) a primary passage provided in the substrate for each of the first and second main channel ends and each of the first and second cross channel ends, the primary passage connecting the outer surface of the substrate to a corresponding one of the first and second main channel ends and the first and second cross channel ends; and (5) a secondary passage provided in the substrate for each of the first and second main channel ends, the secondary passage connects an outer surface of the substrate to a corresponding one of the first and second main channel ends; (c) at least one primary capillary having first and second ends, said first end of said primary capillary connecting said at least one container and said second end of said primary capillary
connecting said primary passages; and (d) at least one secondary capillary having first and second ends, said first end of said secondary capillary connecting said secondary passages.

Preferably, the system further comprises pump means to force said liquid in said container through said primary capillary, said primary passage, said first and second main channel ends, said secondary passage, and said secondary capillary. More preferably, the pump means transfers said liquid through said first main channel end and said second main channel end at a substantially equal rate.

In a fifth embodiment, the present invention provides a system for performing electrophoretic operations that comprises (a) a microchip comprising (1) a substrate having at least one outer surface, (2) at least one main channel that extends for a first distance within the substrate and has first and second main channel ends, (3) at least one cross channel that extends for a second distance within the substrate and has first and second cross channel ends, wherein the cross channel intersects the main channel, (4) at least one primary passage provided in the substrate for each of the first and second main channel ends and each of the first and second cross channel ends, the primary passage connecting the outer surface of the substrate to a corresponding one the first and second main channel ends and the first and second cross channel ends, wherein at least one of said passages is provided with a flared opening; and (b) at least one capillary that connects to the second main channel end through the corresponding primary passage. Thus, in this embodiment, the sample may be first introduced through the cross channel into the main channel and then, through the passage connecting the main channel and the outer surface of the substrate, to the capillary, which performs the separation. Thus, the microchip, in effect, serves as an injector for the capillary. This embodiment is especially suitable for electrophoresis separations that require a long separation length.
In a preferred embodiment, the cross channel comprises first sub-cross channel, which intersects the main channel at position A and second sub-cross channel, which intersects the main channel at position B. The first sub-cross channel extends from the first cross channel end to position A and the second sub-cross channel extends from the second channel end to position B. Thus, in this preferred embodiment, the cross channel intersects the main channel at two different points and that the total length of the two sub-cross channels equals to the second distance. The microchip of this embodiment may serve as an injector, preferably providing a non-biased sample, for an electrophoresis capillary, which performs the separation.

In a sixth embodiment, the present invention provides a system for performing electrophoretic operations. The system comprises (a) a microchip comprising: a substrate having at least one outer surface, at least one main channel that extends for a first distance within the substrate and has first and second main channel ends, at least one cross channel that extends for a second distance within the substrate and has first and second cross channel ends, wherein the cross channel intersects the main channel, at least one primary passage provided in the substrate for each of the first and second main channel ends and each of the first and second cross channel ends, where each of the primary passage connects the outer surface of the substrate to a corresponding one of the first and second main channel ends and the first and second cross channel ends; (b) at least one secondary passage provided in the substrate that connects the outer surface of the substrate and the main channel at a point between the second main channel end and the intersection of the main and cross channels; and (c) at least one capillary that connects to the main channel through the secondary passage; wherein at least one of the primary and secondary passages is provided with a flared opening. Thus, in this embodiment, the sample is first introduced through the cross channel into the main channel and then, through the secondary passage, to the capillary, which usually performs
the separation. Thus, the microchip, in effect, serves as an injector, preferably providing a non-biased sample, for the electrophoresis capillary.

In a preferred embodiment, the cross channel comprises first sub-cross channel, which intersects the main channel at position A and second sub-cross channel, which intersects the main channel at position B. The first sub-cross channel extends from the first cross channel end to position A and second sub-cross channel extends from the second channel end to position B. Further, the secondary passage connects the main channel at a position between the second main channel end and both positions A and B. Thus, in this preferred embodiment, the cross channel intersects the main channel at two different points and that the total length of the two sub-cross channels equals to the second distance. Further, the sample is usually drawn first from the cross channel into the main channel between positions A and B. The sample, traveling in the direction of the second main channel end, is then drawn into the capillary through the secondary passage.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates a conventional microchip for performing electrophoretic operations. Fig. 1A is a plan view showing the first of a pair of substrates provided with groves to form capillary channels. Fig. 1B is a plan view showing the other substrate provided with through holes corresponding to the capillary channel ends in the first substrate. Fig. 1C is an elevation view showing a microchip formed by superimposing the two substrates in Figs. 1A and 1B. Fig. 1D is a perspective view showing the microchip in Fig. 1C.

Figure 2 illustrates an electrophoresis microchip of the present invention. Fig. 2A is a perspective view of a microchip having a plurality of main channels and cross channels and tapered cylindrical openings to the channel ends. Fig. 2B is an elevation view showing passages provided with tapered cylindrical openings and capillaries fitting into the openings. Fig. 2C is a perspective view
showing the microchip with a plurality of capillaries fitting into the tapered cylindrical openings to a plurality of cross channel ends.

Figure 3 illustrates cross-sectional view showing a main channel of the microchip having capillaries fitting into the tapered cylindrical openings and connecting to the main channel ends, electrodes at the main channel ends, and a detection passage having a tapered cylindrical opening with an optical fiber.

Figure 4 illustrates a system of the present invention provided with a running buffer system.

Figure 5 illustrates another system of the present invention. Fig. 5A is a perspective view showing the microchip, the container, and a capillary connecting the microchip and the container. Fig. 5B is a perspective view showing a plurality of samples being delivered from the container to the microchip.

Figure 6 illustrates another system of the present invention, wherein the microchip serves as an injector for a longer electrophoresis capillary separation system. Fig. 6 is a perspective view showing the microchip and a capillary connecting to the microchip at a main channel end.

**DETAILED DESCRIPTION OF THE INVENTION**

The present invention provides a novel connection mechanism for the interface between the micro-channels of an electrophoresis microchip and various systems to and from the channels to perform the electrophoretic operation, including the loading/filling system, sample injection system, channel flushing/washing system, detection system and electrical connections such as electrodes for the separation channels. The invention provides passages having flared openings in the microchip substrate that facilitate the connection between the micro-channels, such as sample and separation channels, of the microchip and the capillaries and optical fibers supporting the microchip electrophoretic operation. These flared openings provide effective and easy to operate connections between the
micro-channels and capillaries that supply or transfer out liquids to and from the channels and optical fibers for detection of the electrophoretic operations. The invention further provides a novel mechanism for supplying buffer to the microchip. In particular, the mechanism allows constant supply of buffer to the ends of separation channels without disrupting the on-going electrophoretic operations (a "running buffer system"). Preferably, the running buffer system is used in connection with the novel interface mechanism.

As used in the present invention, "liquid" or "buffer" includes gels that are used as separation media for electrophoresis operations. As understood by one of ordinary skill in the art, these gels may comprise linear or cross-linked polymers. One example of a cross-linked polymer used as electrophoresis gel is polyacrylamide. Further, some gels used as separation media in electrophoretic operations have high viscosities, e.g., 100 - 50000 cp.

As used herein, an "injector" includes an apparatus, system, or any part thereof that provides an amount of liquid or sample to another apparatus, system or another part thereof.

In one embodiment, the present invention provides an electrophoresis microchip which comprises (1) a substrate that has at least one outer surface; (2) at least one main channel that extends within the substrate between first and second main channel ends; (3) at least one cross channel that extends within the substrate between first and second cross channel ends and intersects the main channel; and (4) at least one primary passage provided in the substrate for each of the first and second main channel ends and each of the first and second cross channel ends. The primary passage connects the outer surface of the substrate to a corresponding one of the first and second main channel ends and the first and second cross channel ends. Further, at least one of the primary passages is provided with a flared opening.
The microchip substrate of the present invention can be in any shape that is suitable for electrophoretic operation, although it usually has a rectangular block shape. Suitable substrate materials are generally selected based on their compatibility with the conditions present in the electrophoretic operation to be performed. Such conditions include extremes of pH, temperature, salt concentration, inertness to the sample and application of electrical fields. Examples of suitable substrate materials include glass, quartz, silicon and polymeric materials. Persons skilled in the art would be able to select suitable substrate materials for particular electrophoretic operations.

The main and cross channels in the microchip and the primary and secondary passages connecting these channels to the substrate surface can be created by any method known to persons skilled in the art, including, but not limited to, etching, photolithography, and standard mechanical methods such as drilling. In this regard, the microchip substrate of the present invention can be formed from one piece of material or from the combination of a plurality of pieces. Preferably, the substrate is formed by combining two pieces of substrates, wherein the channels and the passages have been formed on either or both of the pieces before their combination. Further, the primary passages may connect the main and cross channels to any outer surface of the microchip substrate, although the passages are preferably substantially transverse to the channels.

According to the present invention, at least one of the passages is provided with a flared opening, which, as used in the present invention, refers to an opening of the passage that has a bigger cross-section area at the surface of the substrate than at the connection between the opening and the passage. Such flared openings enable capillaries or optical fibers to be connected with the passages through press fitting. It also allows easy assembling and dis-assembling of the electrophoretic system. Through such connections between capillaries and
optical fibers and the micro-channels of the electrophoresis microchip, buffer/gel loading, sample injection, detection and channel washing and reconditioning can be effectively and efficiently carried out. The connection mechanism of the present invention allows transfers of liquid, including high viscous gel, between the substrate and the capillaries under high pressure. Thus, buffer and gel can be flushed out, making repeated uses of the microchip possible even when high viscous gel is used as the separation medium.

Although the flared opening may be in any shape that provides a tight fitting for the particular capillaries or optical fibers, according to the present invention, it preferably comprises a tapered cylindrical shape having a bigger cross section formed on the outer surface of the substrate and a smaller cross section formed inside the substrate at the end of the tapered cylindrical shape where it connects to the passage. In a more preferred embodiment, the first cross section and the second cross section are substantially circular and, most preferably, the diameter of the bigger cross section ranges from about 0.2 mm to about 20 mm and the diameter of the smaller cross section ranges from about 10 μm to about 5 mm. The exact dimensions of the flared openings for a particular microchip depend on the capillaries and optical fibers to be connected to the microchip for a particular electrophoretic operation. One of the functions of the flared openings is to “catch” the capillaries and the optical fibers easily. Therefore, the bigger cross-section of the opening at the surface of the substrate should be dimensioned according to the outer diameters of the capillaries or the optical fibers to be connected through the opening. As to the length of the tapered cylindrical shape, it preferably has an axis length which ranges from about 0.1 mm to about 20 mm.

The flared openings provided by the present invention have demonstrated the ability to facilitate connections between the microchip and the capillaries that can withstand pressure of about 2000 psi, required to flush
high viscosity (up to 50,000 cp) gel into the capillary tubes and the microchannels in electrophoresis microchips.

In a preferred embodiment, the main channel and the cross channel are oriented substantially parallel to the outer surface, which is preferably a flat surface. The passages are oriented substantially transverse to the main channel and the cross channel.

In another preferred embodiment, the substrate comprises a plurality of main channels and a plurality of cross channels with each of the cross channels intersecting one of the main channels.

Figs. 2A-2C illustrate one embodiment of the present invention's microchip. Fig. 2A is a perspective view of the microchip 200 having a plurality of main channels 210 and a plurality of cross channels 220. Each main channel 210 intersects a cross channel at a substantially right angle and both the main channels and the cross channels are substantially parallel to the outer surface of the substrate. A passage 230 is provided for each of the two ends of the main channel 210 and cross channel 220 that connects the channels to the outer surface of the microchip. The passages 230 are oriented substantially transverse to the main channels 210 and the cross channels 220 and each of the passages is provided with a tapered cylindrical opening.

Fig. 2B is a cross-sectional view of the microchip showing the main channel 210, the passage 230 and the capillary 240 fitting into the tapered cylindrical opening of the passage 230. As illustrated, microchip 200 is formed by substrates 201 and 202. A groove is provided in substrate 202 which, after the combination of substrates 201 and 202, forms main channel 210. Passages 230 are provided in substrate 201 which, after the combination of substrates 201 and 202, connect main channel 210 to the outer surface 203 of the substrate. Capillary 240 can be press fit into the tapered cylindrical opening 204 of passage 230. The tapered cylindrical opening 204 has the biggest cross-section area at the outer surface 203 of the substrate that has a diameter of Da and the smallest cross-
section area at the end of the opening 304 that has a
diameter of Db.

Fig. 2C is a perspective view showing microchip
200 and a plurality of capillaries 240 connected to the
channels through passages having tapered cylindrical
openings.

In another preferred embodiment of the present
invention, the main channels and the cross channels may be
arranged in such ways that they are suitable for multiple
electrophoretic operations at the same time or occupy the
smallest space possible. Therefore, the channels are not
necessarily laid out straightly. Furthermore, two or more
channels may share a common first channel end or a common
second channel end to suit a particular design and
requirements of an electrophoretic operation. For example,
a plurality of main channels may share a common end which
connects to a common outlet passage, or two or more cross
channels may share a common channel end connected to a
passage which serves as a common sample waste outlet.

For high throughput analysis, the prior art has
generally focused on the virtue of densely packing a large
number of separation channels into a relatively small
space. The present invention, however, recognizes that
more emphasis should be put on reducing the total-analysis-
time, including cleaning, filling, sample loading and
separation time, and reducing the total number of
reservoirs. The present invention’s novel connection
mechanism reduces both the total-analysis-time and the
total number of reservoirs, thus facilitating more
efficient electrophoretic operations.

Electrodes are an important part of any
electrophoretic system. Voltages in the kilovolt range are
commonly used in these separations, but currents are
normally in the microamp range. The three requirements for
the electrodes are: 1) that they are rugged enough for
repeated use, 2) that they are in intimate electrical
contact with the sample reservoirs, and 3) that they are
non-reactive under the conditions experienced during use -
this includes not adsorbing sample components which might
foul the electrodes or contaminate subsequent separations. Two types of electrodes, integrated electrodes and external electrodes, can be used in the present invention, with the ultimate design of electrophoretic operation and the reliability of the electrodes being the deciding factors.

For integrated electrodes, gold electrodes can be deposited on the surface of the microchip in the appropriate reservoirs. While gold itself does not adhere adequately to glass, a layer of chrome can be used effectively as an adhesive layer between the glass and the gold. The use of integrated electrodes has been successful in some of the applications, but it has been shown that the stability of the electrodes is relatively poor, with loss of gold from the surface over time (20-50 runs). Use of these electrodes in high throughput screening (HTS) analyses will require better bonding of the electrode to the surface, or frequent replacement of the microchip; the increased cost of fabricating chips with integrated electrodes must also be taken into account. These integrated electrodes would have to be brought into contact with the electrical connections on the instrument itself. This is possible by extending the integrated electrodes to one edge of the microchip and using a zero insertion force (zif) edge connector that would be in contact with the electrodes on the microchip domain in which separation is taking place. These integrated electrodes could be separate for each channel or could be combined, i.e., all the inlet electrodes brought to the same edge connection, to decrease the number of contact points needed.

External electrodes can also be used in the present invention. Small platinum or gold electrodes can be put directly into the appropriate reservoirs (passages) on the microchip. Sufficient contact between the buffer and the electrodes must be maintained during separation. Washing of the electrodes between runs is desirable to prevent sample contamination. The microchip of the present invention may use any type of electrodes that are suitable for micro-channel electrophoretic operations.
In another preferred embodiment of the present invention, the microchip substrate further comprises a secondary passage that connects the outer surface of the substrate and the main channel at a point between the second main channel end and the intersection. This secondary passage may serve a number of purposes. For example, it may facilitate a connection between the microchip and a capillary that is part of a capillary electrophoresis system. Thus, in this situation, the microchip serves as an injector for the capillary, which actually performs the separation.

The secondary passage may also serve as a detection passage. Thus, the detection passage connects the outer surface of the substrate to the main channel at an area between the second main channel end and the intersection of the main and cross channels, but usually closer to the second main channel end. Preferably, the detection passage is substantially transverse to the main channel and comprises a tapered cylindrical shape having a bigger cross section formed on the outer surface of the substrate and a smaller cross section formed inside the substrate at the end of the tapered cylindrical shape.

Most preferably, the diameter of the first cross section ranges from about 0.2 mm to about 10 mm and the diameter of the section cross section ranges from about 20 μm to about 5 mm.

Various detection systems suitable for micro-channel electrophoretic operations and known to persons skilled in the art can be used with the present invention. Preferably, the detection system uses optical fibers as probes, in association with laser induced fluorescence detection, to deliver laser beam and collect fluorescence. These optical fibers usually have high refractive index cores with diameters ranging from about 2 μm to about 300 μm. The outer diameters of the fibers usually range from about 50 μm to about 1000 μm. Preferably, optical fibers having numerical aperture (N.A.) Larger than 0.25 are used in the present invention. The optical fibers used in the present invention can be obtained from Corning, Inc.
(Corning, NY), 3M, Inc. (New Haven, CT), and SpectraTrans Communication Fiber Technologies, Inc. (Sturbridge, MA).

Fig. 3 is a cross-sectional view of a microchip of the present invention with an optical fiber detector. Microchip 300 comprises a main separation channel 310 and two primary passages 320 connecting main channel 310 at its two ends to the surface of the microchip 300. Primary passages 320 are provided with tapered cylindrical openings. A detection passage 330 is provided between primary passages 320 and connecting main channel 310 to the surface of microchip 300. Optical fiber 340 is press fit into detection passage 330. Fig. 3 further illustrates capillaries 350 press fit into primary passages 320 and electrodes 360 provided for the main channel 310.

In another preferred embodiment of the present invention, the cross channel comprises sub-cross channel one, which intersects the main channel at position A on the main channel and sub-cross channel two, which intersects the main channel at position B on the main channel. Further, the sub-cross channel one contains the first cross channel end and extends from the first cross channel end to position A and the sub-cross channel two contains the second cross channel end and extends from the second channel end to position B. Thus, in this embodiment the cross channel intersects the main channel at two different points and that the total length of the two sub-cross channels equals to the second distance. As understood by those skilled in the art, the configuration of the main and cross channels in this embodiment provides a non-biased sample introduction system. Thus, when the sample is introduced through one of the cross channel ends, a non-biased portion of the sample will be present, e.g., through an electrical potential across the first and second cross channel ends, in the main channel between points A and B. This non-biased portion of the sample will then be subject to separation across the main channel.

In another embodiment, the present invention provides an electrophoresis microchip with a running buffer system. The microchip comprises (1) a substrate; (2) at
least one main channel extending within the substrate and having first and second main channel ends; (3) at least one cross channel extending within the substrate between first and second cross channel ends and intersecting the main channel; (4) a primary passage provided in the substrate for each of the first and second main channel ends and each of the first and second cross channel ends and connecting the outer surface of the substrate to the corresponding channel ends; and (5) a secondary passage provided in the substrate for each of the first and second main channel ends and connecting the same or a different outer surface of the substrate to the corresponding first and second main channel ends. Therefore, in this embodiment, the present invention provides two separate passages to each end of the main channel. This allows liquids, especially buffer, to be continuously supplied to the two ends of the main channel.

During electrophoresis, ions in the buffer reservoirs, or passages in the present invention, are transferred as required by the imposed electric field. Buffer depletion becomes a problem if the size of the reservoir is too small; or if the electrophoretic separation is too long. The size of the reservoirs which can be prepared in the microchip itself is limited by the space available. Limitations on spacing is also imposed by the voltages applied between reservoirs. The present invention encompasses two methods for increasing the volume of buffer available at each reservoir: on-chip reservoirs and external reservoirs.

In a preferred embodiment, passages connecting the main and cross channels to the outer surface of the microchip serve as on-chip reservoirs for samples and buffer. The volume of the on-chip reservoirs on the microchips can be increased by addition of a third substrate layer to the chips. This layer, with the appropriately drilled holes, would cover only the top part of the micro-channel design such that it would not interfere with detection. Volumes in the reservoirs would be increased by the thickness of the glass used for this
third layer, with the needed volume of these reservoirs
determining the final design thickness. This substrate
layer could be annealed to the microchip or could be bonded
using a UV-curable adhesive. This third layer would allow
easier integration of the electrodes since there would be a
larger reservoir surface on which to deposit metal. In
addition, this would provide two layers on which
connections between like electrodes could be fashioned.

An alternative approach would be to have
reservoirs that were remote from the microchip, external
reservoirs, either as part of the interface or connected to
the on-chip reservoirs through the interface. In either
case, external electrodes could be used in a design that
avoids problems with washing of the electrodes between
runs. Problems associated with providing sufficient
contact between the electrodes and buffer would also be
eliminated. According to the present invention, buffer
flows through capillaries connected to the end of the main
separation channels via the primary passages. After
passing through the ends of the main channel, the buffer
then flows through the secondary passages into outlet
capillaries. Since the buffer flow rates at both ends of
the main channels are maintained substantially the same,
the acting force at both ends of the main channel are equal
and, therefore, no counter flow is created in the channel.
Further, the primary passages and the secondary passages
may be arranged in various configurations as long as both
of the passages connect the ends of the main channels to an
outer surface of the microchip substrate.

In a preferred embodiment associated with the
running buffer system, the substrate of the electrophoresis
microchip comprises first and second outer surfaces
parallel to each other and the main channel is parallel to
the first and second outer surfaces. More preferably, the
primary and secondary passages to the first and second main
channel ends are substantially transverse to the at least
one main channel. The primary passages connect the first
outer surface to the first and second main channel ends and
the secondary passages connect the second outer surface to
the first and second main channel ends. Most preferably, at least one of the primary and the secondary passages is provided with a flared opening.

Fig. 4 illustrates a microchip with a running buffer system. Microchip 400 is provided with a main separation channel 401. Two primary passages 420 connect main channel 410 at its two ends, which also connect to two secondary passages 430. The primary passages and the secondary passages have openings on the opposite sides of microchip 400. Inlet capillaries 440 are connected to the primary passages and provide inlet buffer during the electrophoretic operation, while outlet capillaries 450 are connected to the secondary passages and provide an outlet for the buffer.

The present invention further provides a system for performing electrophoretic operations. The system comprises (1) at least one container configured to hold a liquid; (2) an electrophoresis microchip comprising (a) a substrate having at least one outer surface (b) at least one main channel extending within the substrate between first and second main channel ends, (c) at least one cross channel extending within the substrate between first and second cross channel ends and intersecting the main channel, and (d) a primary passage provided in the substrate for each of the first and second main channel ends and each of the first and second cross channel ends and connecting the outer surface of the substrate to a corresponding one of the main channel and cross channel ends, wherein at least one of the primary passages is provided with a flared opening; and (3) at least one capillary connecting the container and the flared opening of the primary passages. Therefore, according to the present invention, liquids such as samples, buffer and washing/reconditioning media can be transferred from a container to the microchip through capillaries. Advantageously, the flared opening on the microchip and the capillary press fit into it provide a tight and easy to operate mechanism for the connection between the micro-

- 25 -
channels in the microchip and the liquids to and from the channels.

In a preferred embodiment, the container comprises a chamber containing a micro-titer tray having a plurality of wells, which are connected to the capillaries. Although various suitable liquid delivery means, such as vacuum means, may be used to transfer the liquid from the container to the microchip, the preferred embodiment is that the chamber is sealed and provided with pump means for increasing pressure inside the chamber such that a liquid contained in the wells is forced into the capillary.

Fig. 5A and 5B illustrate an embodiment of the present invention's system. Microchip 500 is provided with a main channel 501 and a cross channel 502. Each of the channels is provided with two passages 503 connected to the two ends of each channel. The main channel 501 serves as the separation channel and is provided with electrodes at its two ends. The cross channel 502 serves as sample loading channel. Container 510 comprises a sealed chamber containing a micro-titer tray 511 having a plurality of sample wells 512. Container 510 is further provided with pump means 513 that can increase the pressure inside the container under controlled conditions. Capillary 520 connects one of the sample wells 512 to one of the passages 503 for the cross channel. As the pressure inside the sealed chamber 510 is increased by pump means 513, sample is pushed well 512 into capillary 520, passage 503, and the cross channel 502 for the electrophoretic operation. Fig. 5B illustrates a system in which a plurality of samples can be delivered simultaneously to a microchip having a plurality of separation channels and sample loading channels.

It should be noted that, although Fig. 5 demonstrates only the capability of the present invention's system to deliver samples, the system can also be used for buffer filling and channel washing/reconditioning purposes. Flushing and reconditioning microchip channels are indispensable steps for microchip capillary electrophoresis separations as is the case for the conventional capillary
format. The purpose of flushing and reconditioning is to facilitate the regeneration of the initial separation environment such that reproducible separations can be obtained. For un-coated microchip channels, flushing and reconditioning with reagent such as NaOH is sufficient to ensure appropriate regeneration of the initial separation environment for the analysis of small molecules. Therefore, the flushing and reconditioning process of the present invention may be conducted as follows: (1) empty all reservoirs/passes; (2) rinse reservoirs/passes with \( H_2O \); (3) flush channels with \( H_2O \); (4) flush channels with either NaOH or buffer; (5) fill channels and reservoirs/passes with buffer, and (6) load samples. The procedure can be accomplished either manually or in combination with an automated process.

According the present invention, the connection between the capillary and the microchip can be either retractable or permanent. The unique shape of the flared opening, especially the tapered cylindrical shape, allows capillaries to be easily and tightly connected to the microchip by using little force. For example, a force of 0.2 pound acting on a capillary having a diameter of 150 \( \mu \text{m} \) (or area of 27x10^{-6} square inches) would create a pressure of 7500 psi on the walls of the opening. The tight fitting between the capillary and the microchip make it possible to use high pressure in sample and buffer delivery and, especially, in delivery of washing media to clean and recondition the channels. At the same time, the flared opening also allows easy dis-assembly of the capillary from the microchip. For example, a force of 0.5 pound would easily remove the capillary from the flared opening. This allows for repeated and quick assembly of different systems for different electrophoretic operations.

In another preferred embodiment of the present invention, the connection between the capillary and the microchip through the flared opening is made permanent through the use of adhesives. Any adhesive suitable for connecting the particular capillary and the particular material of the microchip can be used. Factors such as
pressure, pH, salt concentration, the samples being analyzed should also be considered in choosing the adhesive. In many applications, the preferred adhesive for the present invention is polyimide, which, when applied to the capillary, also provides extra flexibility to the capillary.

In another embodiment, the present invention provides a system for performing electrophoretic operations with running buffer. The system comprises (1) at least one container configured to hold a liquid; (2) an electrophoresis microchip comprising (a) a substrate having at least one outer surface, (b) at least one main channel extending within the substrate between first and second main channel ends, (c) at least one cross channel extending within the substrate between first and second cross channel ends and intersecting the main channel, (d) a primary passage provided in the substrate for each of the first and second main channel ends and each of the first and second cross channel ends and connecting the outer surface of the substrate to corresponding one of the channel ends, and (e) a secondary passage provided in the substrate for each of the first and second main channel ends and connecting an outer surface of the substrate to a corresponding one of the main channel ends; (3) at least one primary capillary connecting the container and the primary passage; and (4) at least one secondary capillary connecting to the secondary passage.

Therefore, in this embodiment of the invention, the system combines the electrophoresis microchip with running buffer mechanism, as illustrated in Fig. 4, and the system with liquid delivery mechanism, as illustrated in Fig. 5. Although any suitable liquid transfer means may be used in the system, the system preferably comprises pump means for transferring liquid in the container through the primary capillary to the primary passage, the first and second main channel ends, the secondary passage, and the secondary capillary. More preferably, the pump means transfers the liquid through the first main channel end and the second main channel ends at a substantially equal rate.
This allows the buffer inside the main channel to stay undisturbed, while fresh buffer is continuously provided to the channel ends. Many types of pumps can be used to serve as the pump means of the present invention, for example, a high pressure liquid chromatography pump (HPLC pump) from Alltech Corporation (Model No. 301300).

The present invention further provides a system for performing electrophoretic operations whereby the microchips described above serve as injectors for capillary and other means of separations ("injector system"). Thus, the system comprises a microchip herein described and a capillary, which may be part of an electrophoresis capillary separation system, provided that one end of the capillary is connected to the microchip either through one of the primary passages or through a secondary passage that connects the main channel, at a different position from the primary passage, to the outer surface of the microchip. Therefore, in this system, samples may first loaded onto the cross channel and/or the main channel of the microchip and then injected, through either the primary or the secondary passage, into the capillary for separation.

Preferably in the injector system, a separation voltage is provided between the microchip, preferably the first main channel end, and the electrophoresis capillary separation system, preferably the other end of the capillary. The separation voltage may also be provided between the two ends of the capillary. A sample loading voltage may be provided between the two cross channel ends, between the two main channel ends, or between any of the cross channel ends and any of the main channel ends.

The injector system is applicable for any capillary electrophoresis and is especially suitable for DNA sequencing or genotyping by electro-kinetic injection. It has the advantage of introducing non-biased samples through the microchip into the capillary. Therefore, the high-pressured capillary to microchip plate junction of the present invention can be used in this particular system to take advantage of both the superior electro-kinetic properties of the microchip/microchannel plate.
electrophoresis system and the longer electrophoresis length offered by capillaries. Furthermore, this system can be adapted to multiple capillary array system using multiple microchannel plate design.

Figure 6 illustrates an embodiment of the injector system. In this embodiment, a microchip 600 is provided with a main channel 610, which is connected to the outer surface of the microchip at first main channel end via primary passage 611 and at second main channel end via primary passage 612. Microchip 600 is further provided with a cross channel that comprises two sub-cross channels. First sub-cross channel 620 connects to the outer surface of the microchip via primary passage 621 and intersects main channel 610 at position A. Second sub-cross channel 630 connects to the outer surface of the microchip via primary passage 631 and intersects main channel 610 at position B which is spaced apart from position A along the main channel 610 and is closer to primary passage 612 than is position A. A capillary 640 is connected to the microchip via primary passage 612.

In the arrangement of Fig. 6, one may further provide a secondary passage, such as detection passage 330 seen in Fig. 3, connecting the outer surface of the substrate to the main channel 610 at a point between the primary passage 611 and the primary passage 612. Preferably, such a secondary passage would be between position B and primary passage 612 at the second main channel end. Capillary 640 may connect to the microchip via such a secondary passage, rather than primary passage 612. As described earlier, an optical detector fiber, or the like, may be used to detect the presence of samples through such a secondary passage.

In operation, a sample is first loaded into the microchip through primary passages 621 or 631. A sample loading voltage (not shown) is then applied to draw the sample into main channel 610, especially into the area between positions A and B. A separation voltage 650 and 660 is then applied between primary passage 611 and the other end of capillary 640 and separation is mainly
performed within capillary 640. It should be noted that capillary 640 is usually part of an electrophoresis separation system, which may perform functions such as filling, flushing, and detection.

While the present invention has been described with reference to the above specific and preferred embodiments, it should be noted that the scope of the invention is not limited to these examples. One skilled in the art may find variations of these embodiments that fall within the spirit of the present invention, the scope of which is defined by the claims set forth below.
What is claimed is:

1. An electrophoresis microchip comprising:
   a substrate having at least one outer surface;
   at least one main channel extending for a first
distance within the substrate, the main channel having
first and second main channel ends;
   at least one cross channel extending for a second
distance within the substrate, the cross channel having
first and second cross channel ends, wherein the cross
channel intersects the main channel; and
   a primary passage provided in the substrate for
each of the first and second main channel ends and each of
the first and second cross channel ends, said primary
passage connecting the outer surface of the substrate to a
corresponding one of the first and second main channel ends
and the first and second cross channel ends, wherein at
least one of said primary passages is provided with a
flared opening.

2. The microchip of claim 1, wherein the flared
opening comprises a tapered cylindrical shape having a
first cross section formed on the outer surface of the
substrate and a second cross section formed inside the
substrate at the end of the tapered cylindrical shape, said
first cross section being larger than said second cross
section.

3. The microchip of claim 2, wherein the first cross
section and the second cross section are substantially
circular.

4. The microchip of claim 3, wherein the diameter of
the first cross section ranges from about 0.2 mm to about
10 mm and the diameter of the section cross section ranges
from about 10 μm to about 5 mm.
5. The microchip of claim 3, wherein the axis of the tapered cylindrical shape has a length ranging from about 0.1 mm to about 10 mm.

6. The microchip of claim 1, wherein the main channel and the cross channel are oriented substantially parallel to said outer surface and said primary passages are oriented substantially transverse to the main channel and to the cross channel.

7. The microchip of claim 1, wherein said substrate comprises a pair of plate members having grooves formed on a surface of at least one plate member to form said main channel and said cross channel.

8. The microchip of claim 1, comprising a plurality of main channels and cross channels, wherein each of the cross channels intersects one of the main channels.

9. The microchip of claim 8, wherein two or more channels share a common first channel end or a common second channel end.

10. The microchip of claim 1, wherein at least one of said primary passages further comprise electrodes deposited on the walls of the passages.

11. The microchip of claim 1, further comprising a secondary passage connecting the outer surface of the substrate to the main channel at a point between the second main channel end and the intersection of said main channel and said cross channel.

12. The microchip of claim 11, wherein the secondary passage is a detection passage substantially transverse to the main channel and comprises a tapered cylindrical shape having a first cross section formed on the outer surface of the substrate and a second cross section formed inside the substrate at the end of the tapered cylindrical shape, said
first cross section being larger than said second cross section.

13. The microchip of claim 12, wherein the diameter of the first cross section ranges from about 1 mm to about 10 mm and the diameter of the section cross section ranges from about 20 µm to about 5 mm.

14. The microchip of claim 1, wherein the cross channel comprises first sub-cross channel intersecting the main channel at position A along the main channel and second sub-cross channel intersecting the main channel at position B along the main channel, said first sub-cross channel extending from the first cross channel end to said position A and said second sub-cross channel extending from the second channel end to said position B.

15. In an electrophoresis microchip having a plurality of primary passages connecting an outer surface thereof to main and cross channels therein, the improvement comprises providing a flared opening for at least one of the primary passages so as to facilitate insertion of a capillary into said primary passage.

16. An electrophoresis microchip comprising:
a substrate having at least one outer surface;
at least one main channel extending for a first distance within the substrate, the main channel having first and second main channel ends;
at least one cross channel extending for a second distance within the substrate, the cross channel having first and second cross channel ends, wherein the cross channel intersects the main channel;
a primary passage provided in the substrate for each of the first and second main channel ends and each of the first and second cross channel ends, said primary passage connecting the outer surface of the substrate to a corresponding one of the first and second main channel ends and the first and second cross channel ends; and
a secondary passage provided in the substrate for each of the first and second main channel ends, said secondary passage connecting an outer surface of the substrate to a corresponding one of the first and second main channel ends.

17. The microchip of claim 16, comprising first and second outer surfaces parallel to each other, wherein the main channel is parallel to the first and second outer surfaces.

18. The microchip of claim 17, wherein said primary and secondary passages to the first and second main channel ends are substantially transverse to the at least one main channel, said primary passages connecting said first outer surface to said first and second main channel ends and said secondary passages connecting said second outer surface to said first and second main channel ends.

19. The microchip of claim 16, wherein at least one of the primary and the secondary passages is provided with a flared opening.

20. In an electrophoresis microchip having a plurality of primary passages connecting an outer surface thereof to main and cross channels therein, the improvement comprises providing a secondary passage in the substrate connecting an outer surface of the microchip to an end of a main channel therein so as to provide an exit for an liquid flown into said end of said main channel via a corresponding primary passage.

21. A system for performing electrophoretic operations comprising:
   (a) at least one container configured to hold a liquid;
   (b) an electrophoresis microchip comprising:
       a substrate having at least one outer surface;
at least one main channel extending for a first distance within the substrate, the main channel having first and second main channel ends;

at least one cross channel extending for a second distance within the substrate, the cross channel having first and second cross channel ends, wherein the cross channel intersects the main channel; and

a primary passage provided in the substrate for each of the first and second main channel ends and each of the first and second cross channel ends, said passage connecting the outer surface of the substrate to a corresponding one of the first and second main channel ends and the first and second cross channel ends, wherein at least one of said passages is provided with a flared opening; and

(c) at least one capillary having first and second capillary ends, said first capillary end connecting said at least one container and said second capillary end being inserted into said at least one flared opening of said primary passages.

22. The system of claim 21, wherein the at least one container comprises a chamber containing a micro-titer plate having a plurality of wells, said wells connecting to said first capillary end.

23. The system of claim 22, wherein the chamber is sealed and provided with pump means for increasing pressure inside the chamber such that a liquid contained in said wells is forced into said at least one capillary.

24. The system of claim 21, wherein said second end of said at least one capillary is secured to said flared opening of said at least one primary passages by an adhesive.

25. The system of claim 21, wherein the microchip further comprises a detection passage connecting the outer
surface of the substrate to the main channel at an area between the first and second main channel ends.

26. The system of claim 25, wherein said detection passage is substantially transverse to the main channel and comprises a tapered cylindrical shape having a first cross section formed on the outer surface of the substrate and a second cross section formed inside the substrate at the end of the tapered cylindrical shape, said first cross section being larger than said second cross section.

27. The system of claim 25, further comprising an optical fiber, said optical fiber connecting to said detection passage.

28. A system for performing electrophoretic operations comprising:
   (a) at least one container configured to hold a liquid;
   (b) an electrophoresis microchip comprising:
       A substrate having at least one outer surface;
       at least one main channel extending for a first
       distance within the substrate, the main channel having
       first and second main channel ends;
       at least one cross channel extending for a second
       distance within the substrate, the cross channel having
       first and second cross channel ends, wherein the cross
       channel intersects the main channel;
       a primary passage provided in the microchip for
       each of the first and second main channel ends and each of
       the first and second cross channel ends, said primary
       passage connecting the at least one outer surface of the
       substrate to a corresponding one of the first and second
       main channel ends and the first and second cross channel
       ends; and
       a secondary passage provided in the substrate for
       each of the first and second main channel ends, said
       secondary passage connects an outer surface of the

-37-
substrate to a corresponding one of the first and second main channel ends;
(c) at least one primary capillary having first and second ends, said first end of said primary capillary connecting said at least one container and said second end of said primary capillary connecting said primary passages; and
(d) at least one secondary capillary having first and second ends, said first end of said secondary capillary connecting said secondary passages

29. The system of claim 28, further comprising pump means for transferring said liquid in said container through said primary capillary, said primary passage, said first and second main channel ends, said secondary passage, and said secondary capillary.

30. The system of claim 29, wherein the pump means transfers said liquid through said first main channel end and said second main channel ends at a substantially equal rate.

31. A system for performing electrophoretic operations comprising:
(a) a microchip comprising:
a substrate having at least one outer surface;
at least one main channel extending for a first distance within the substrate, the main channel having first and second main channel ends;
at least one cross channel extending for a second distance within the substrate, the cross channel having first and second cross channel ends, wherein the cross channel intersects the main channel; and
(a primary passage provided in the substrate for each of the first and second main channel ends and each of the first and second cross channel ends, each passage connecting the outer surface of the substrate to a corresponding one of the first and second main channel ends and the first and second cross channel ends, wherein at
least one of the passages is provided with a flared opening; and

(b) at least one capillary connecting to the main channel at one of the two main channel ends via the corresponding primary passage.

32. The system of claim 31, wherein the cross channel comprises:

a first sub-cross channel extending from said first cross channel end to a first position along the main channel, and

a second sub-cross channel extending from said second cross channel end to a second position along the main channel,

wherein the first and second positions are spaced apart along the main channel.

33. A system for performing electrophoretic operations comprising:

(a) a microchip comprising:

a substrate having at least one outer surface;

at least one main channel extending for a first distance within the substrate, the main channel having first and second main channel ends;

at least one cross channel extending for a second distance within the substrate, the cross channel having first and second cross channel ends, wherein the cross channel intersects the main channel;

a primary passage provided in the substrate for each of the first and second main channel ends and each of the first and second cross channel ends, each primary passage connecting the outer surface of the substrate to a corresponding one of the first and second main channel ends and the first and second cross channel ends; and

a secondary passage provided in the substrate, said secondary passage connecting the outer surface of the substrate and the main channel at a point between said second main channel end and the intersection of the main channel and the cross channel; and
(b) at least one capillary connecting to the main channel at one of the two main channel ends via the corresponding primary passage;

wherein at least one of said primary and secondary passages is provided with a flared opening.

34. The system of claim 33, wherein the cross channel comprises:

a first sub-cross channel extending from said first cross channel end to a first position along the main channel, and

a second sub-cross channel extending from said second cross channel end to a second position along the main channel, wherein

the first and second positions are spaced apart along the main channel, and

the secondary passage connects to the main channel at a point between said second main channel end and both the first and second positions.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : G01N 27/26, 27/447
US CL. : 304/450, 451,600,601; 422/99,102

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 204/450, 451, 600, 601; 422/99, 102

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

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

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>US 5,890,745 A (KOVACS) 06 April 1999, fig. 15 &amp; col. 7, lines 37-50</td>
<td>1, 10, 14, 15, 21, 22, 24, 31, 32</td>
</tr>
<tr>
<td>Y</td>
<td>US 5,989,402 A (CHOW et al) 23 November 1999, fig. 1</td>
<td>1, 10, 14, 15, 21, 22, 24, 31, 32</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C.  See patent family annex.

Date of actual completion of the international search: 29 MAY 2001

Date of mailing of this international search report: 13 JUN 2001
B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

USPAT, JPOABS, EPOABS, DERWENT, CAPLUS
search terms: microchip, microchannel, microconduit, microfluidic, microfabricat?, microanaly?, opening, aperture, hole, passage, taper?, flare?, conical