A microfluidic chip (100) comprises a layer (10, 60) with an array (30) of bead trapping cavities (20) provided in that layer (10, 60), wherein each of the cavities (20) has a conic shape, defined by one or more lateral walls (21-24) that are, each, hydrophilic, and wherein each of the cavities (20) extends as a blind hole in the thickness of said layer (10, 60). Methods of fabrication of such a chip are further provided.
MICROFLUIDIC CHIP WITH CONIC BEAD TRAPPING CAVITIES AND FABRICATION THEREOF

FIELD OF THE INVENTION

The invention relates in general to the field of microfluidic chips, and in particular to microfluidic chips equipped with bead trapping cavities, e.g., for bioanalysis applications, as well as related fabrication methods.

BACKGROUND OF THE INVENTION

Microfluidics generally refers to microfabricated devices, which are used for pumping, sampling, mixing, analyzing and dosing liquids. Prominent features thereof originate from the peculiar behavior that liquids exhibit at the micrometer length scale. Flow of liquids in microfluidics may typically be laminar. Volumes well below one nanoliter can be reached by fabricating structures with lateral dimensions in the micrometer range. Reactions, which are limited at large scales (by diffusion of reactants) can thus be accelerated. Microfluidics are accordingly used for various applications.

Many microfluidic devices have user chip interfaces and closed flowpaths. Closed flowpaths facilitate the integration of functional elements (e.g. heaters, mixers, pumps, UV detector, valves, etc.) into one device while minimizing problems related to leaks and evaporation.

Often, receptors on surfaces are used to bind specific analytes that need to be detected in samples. After binding, the sample and interfering species can be rinsed off. The receptor-analyte complex can then be detected directly (via e.g. change in mass, refractive index, etc.) or indirectly (fluorescence immunoassays, etc.). While microfluidics are promising devices for analysis, it is currently very challenging to integrate receptors inside microfluidic chips.

Solutions have been proposed, where receptors are patterned on a microfluidic chip's surface. In more detail, a microfluidic chip can be sealed with a layer of PDMS onto which lines of capture antibodies are patterned. In that case, patterning the capture antibodies is done by adsorbing the antibodies from a solution using a stencil. However, such an approach is cumbersome, has low throughput, requires a stencil, and is otherwise slow due to the adsorption process. In addition, PDMS is expensive and contaminates surfaces (makes hydrophilic surfaces hydrophobic after ~20 minutes contact time).

Other solutions, which have been known for a long time, rely on microbeads, which are used for assays. Here, the beads are typically coated with a receptor. The beads can be used in solution (e.g., magnetic beads,
or single bead in capillary) or after deposition/localization in a specific area of a microfluidic chip. Two cases can be distinguished:

- Magnetic beads: the separation of the beads from interfering species and sample is achieved by using a magnet and rinsing. Magnetic beads are, however, more expensive and difficult to prepare than non-magnetic beads. Moreover, these beads are opaque and not well suited for optical/fluorescence-based assays; and
- Non-magnetic beads, for which many techniques have been developed for localizing/manipulating beads in microfluidics.

However, such techniques have the following drawbacks. They require either:

- Specific actuation means (electrodes, magnetic structures, focused light, transducers, piezoelectric structures, etc.), and are therefore complicated and expensive; or
- Specific geometries of the microfluidic flow paths (special radii of curvature, constrictions), it being noted that the hydraulic resistance of the chip with and without beads can dramatically differ, and that the stability of trapped beads systematically is an issue. Also, the viscosity of specific samples/liquids can be a problem.

As noted above, some solutions use constrictions or “filters”, which are directly part of the flow path in a microfluidic chip, for trapping beads. However, such solutions lead to a trade-off between the signal intensity and signal quality. All the more, the bead stability is an issue.

The present invention proposes a definitive solution to the problem of bead stability.

**BRIEF SUMMARY OF THE INVENTION**

According to a first aspect, the present invention is embodied as a microfluidic chip comprising a layer with an array of bead trapping cavities provided in that layer, wherein each of the cavities has a conic shape, defined by one or more lateral walls that are, each, hydrophilic, and wherein each of the cavities extends as a blind hole in the thickness of said layer.

In embodiments, at least some of the cavities have a pyramidal shape formed by lateral walls that are, each, hydrophilic, and wherein, preferably, this pyramidal shape is essentially defined by at least four lateral walls.

Preferably, the said layer comprises one or more semiconductor element such as silicon, and the pyramidal shapes of the cavities exhibit a geometry consistent with an anisotropic etching process of fabrication of the cavities in the layer.
In preferred embodiments, at least one of, and preferably most of, the cavities are, each, filled with a bead, preferably a microbead, having, on average, a diameter between 1 and 40 \( \mu \text{m} \), preferably 2 and 20 \( \mu \text{m} \), and more preferably 2 and 10 \( \mu \text{m} \).

Preferably, a majority of the cavities of said array are filled, each, with only one bead, which preferably is a microbead having, on average, a diameter between 1 and 40 \( \mu \text{m} \), preferably 2 and 20 \( \mu \text{m} \), and more preferably 2 and 10 \( \mu \text{m} \).

In embodiments, the ratio of an average dimension of an opening of the cavities to an average diameter of beads in the cavities is between 1.0 and 2.4, and preferably between 1.4 and 2.0.

Preferably, the ratio of an average depth of the cavities to an average diameter of beads in the cavities is at least of 0.5, preferably 1.0 and more preferably 1.3.

In preferred embodiments, two or more subgroups of at least one of the cavities, which subgroups preferably are rows or columns of the array of cavities, are connected by at least one microchannel, and wherein, more preferably, said one or more of the subgroups is defined in a microchannel portion, whose bottom wall or top wall is formed by a surface of said layer.

Preferably, the array is sealed by a cover layer extending vis-a-vis the array of cavities.

In embodiments, the chip comprises several arrays of one or more bead trapping cavities, wherein said arrays are preferably inserted between distinct pairs of microchannel portions.

Preferably, the chip comprises at least two different types of beads, located in one or more cavities of at least two of said several arrays, respectively, wherein the beads of different types preferably differ in terms of size, coating, material and/or color.

According to another aspect, the invention is embodied as a method of fabrication of a microfluidic chip according to any one of the above embodiments, which method comprises:

providing a microfluidic chip body with a layer; and

fabricating an array of bead trapping cavities in that layer, wherein each of the cavities has a conic shape, defined by one or more lateral walls that are, each, hydrophilic, and wherein each of the cavities extends as a blind hole in the thickness of said layer.

Preferably, fabricating the array comprises anisotropically etching said layer to obtain the cavities, preferably using a self-limited anisotropic etching process.
In embodiments, the method further comprises: depositing beads into the cavities of the array by spotting a droplet of a bead solution; and sealing the array with a cover layer placed such as to extend vis-a-vis the array of cavities, and wherein sealing the array preferably comprises laminating the cover layer.

Preferably, the method further comprises, after depositing beads and prior to sealing, in that order: drying the array of cavities with beads therein; removing excess beads not trapped inside cavities, preferably by rinsing the array with a rinsing solution and/or by applying a tape to which excess beads stick; and drying again the array of cavities with beads therein, if necessary.

Devices and methods embodying the present invention will now be described, by way of non-limiting examples, and in reference to the accompanying drawings. Technical features depicted in the drawings are not necessarily to scale.

BRIEF DESCRIPTION OF SEVERAL VIEWS OF THE DRAWINGS

- FIGS. 1, 2, 12 and 13 show, each, a 2D (top) view of a simplified representation of a microfluidic chip, according to distinct embodiments of the invention;

- FIG. 3 shows: a section view (upper figure); and a top view (lower figure) of a simplified representation of a bead trapped in a pyramidal cavity, as involved in embodiments;

- FIG. 4 is a scanning electron microscope image of beads trapped in pyramidal cavities, as involved in embodiments;

- FIGS. 5A - 51 schematically illustrates detailed steps of a method of fabrication of a microfluidic chip, according to embodiments of the invention;

- FIG. 6 schematically illustrate selected steps of the fabrication method of FIG. 5, where the upper row shows section views of microfluidic chips while the lower row shows the corresponding top views;

- Similarly, FIGS. 7 - 9 schematically illustrate selected steps of variants to the fabrication method of FIG. 5;

- FIG. 10 is a flowchart corresponding to FIG. 5;

- FIG. 11 shows 2D views of simplified representations of improved pyramidal cavities, as involved in embodiments. The leftmost figure is a top view while the other figures are section views;

- FIG. 14 shows a negative of a fluorescence image of beads trapped in arrays of cavities obtained according to embodiments of the invention; and
FIG. 15 is a section view of a simplified representation of bead trapped in a truncated conic cavity, as involved in embodiments.

DETAILED DESCRIPTION OF THE INVENTION

First, and referring generally to FIGS. 1 - 4, and 11 - 13, an aspect of the invention is described, which concerns a microfluidic chip 100. The chip notably comprises a working layer 10, 60, which layer comprises an array 30 of bead trapping cavities 20 (also referred to as traps) provided therein. Remarkably, each of the cavities 20 has a conic shape, subtended by one or more lateral walls 21 - 24. Importantly, this or these lateral walls are hydrophilic and each of the cavities 20 extends as a blind hole in the thickness of the working layer 10, 60.

The working layer 10, 60 can be a substrate layer 10 or a cover lid 60. The cavities are preferably provided in the thickness of a layer, whose surface is the bottom wall or the top wall of a microfluidic channel or a channel portion 12a, as illustrated in FIG. 2.

In the present application: "conic" means also "conical", i.e., having the form of or resembling a cone. By "conic shape", and consistently with one general definition of a cone (http://en.wikipedia.org/wiki/Cone), it is meant a three-dimensional geometric shape that tapers smoothly from a flat base (corresponding to the opening 28 of the cavity), which flat base is not necessarily circular, to an opposite end that has a sectional area smaller than that of the base 28, e.g., an apex or a truncation surface. Other definitions assume that a cone is a particular case of a pyramid, i.e., defining a cone as a pyramid with a circular cross section, see e.g., http://mathworld.wolfram.com/Conic.html. Here, it is assumed that conic shapes include pyramids (with polygonal bases) as a particular case. That is, present conic shapes do not necessarily have a circular cross section.

Due to fabrication constraints, the conic shape shall nevertheless be likely truncated or, at least, not terminate as a perfect, point-like, apex. In fact, advantage can even be taken of a truncated cone, as to be discussed later. As evoked just above and further illustrated in embodiments below, the conic hole shall preferably have a polygonal base (the base corresponding to the aperture of the cavity 20), and therefore be pyramidal (with a polygonal base). The conic shape may, in such a case, be referred to as an inverse pyramidal shape since the beads 50 are likely captured from liquid droplets spotted directly (by liquid spotting techniques) on top of a lower array 30 of bead traps, where the traps are the cavities 20, which are open on the top, i.e., hollowed inward. However, this configuration can be reversed, such as to have cavities provided in a cover lid, see FIG. 7.
Using blind hole-like conic cavities for the bead traps turned out to be surprisingly advantageous, in terms of stability of the beads, compared to other solutions that present inventors also tested, e.g., using pillars or through hole-like cavities for the traps. Namely, the resulting traps have shown an unprecedented propensity to keep the beads 50, after rinsing. Embodiments of the present invention apply to microbeads (e.g., beads with receptors on their surfaces, where such receptors can be used to bind ligands in solutions as conventionally done in biological assays), have moderate complexity and do not require additional means (like vacuum, magnetic field, or electric field) to keep the beads in place.

The origin of the remarkable improvement in the beads' stability is not clear yet; the improved stability may be due to interfacial and/or mechanical phenomenon occurring laterally (conic cavities better protect beads from lateral, in-plane motion of fluids (gases or liquids) when rinsing) and/or below the bead, when the latter is trapped in a cavity, i.e., at the level of the pointed end of the conic hole. Still, the lateral wall(s) of the conic cavities need be hydrophilic for it to work.

The present designs and corresponding fabrication methods furthermore allow for placing the beads before covering the traps, at variance with most, if not all the known solutions.

In terms of fabrication, present inventors have noted that drying the beads 50 and traps 20, after spotting the bead solution and before rinsing the excess beads, still substantially improves the stability of the trapped beads. This shall be commented later in detail.

In some known prior art solutions (see, e.g., Sohn et al. biosensors and bioelectronics, vol. 21, 2005, pp. 303 - 312), through hole-like pyramidal cavities have been fabricated, in order to trap "giant" beads, i.e., beads that are at least one order of magnitude larger, in diameter, than beads as contemplated herein. However, since the pyramidal cavities fabricated are through holes in that case, they have to have a minimal depth that is by far too large for the present applications. Also, such solutions require pick-and-place tools and application of a backside vacuum, which cannot be applied to smaller beads such as microbeads, as contemplated here, which typically are polymer beads that have a diameter in the range of 1 - 40 µm (such beads are typically obtained from a liquid suspension).

Although the present invention allows to achieve stable beads in the cavities 20, it does, however, not preclude additional use of dielectrophoretic DEP electrodes or the like. DEP electrodes can indeed be located in or near the traps, e.g., to levitate the beads 50, for example to recover beads, if desired, or expose the beads more to a solution flowing in the microchannel where an array 30 of traps is located. In addition, in embodiments, the surface of the cavities can be metalized, e.g., using Al in order to amplify the fluorescence signal by reflection.

Referring now more particularly to FIGS. 3, 4, 11 and 15, some or all of the cavities 20 may be given a pyramidal shape, i.e., a conic volume with polygonal base, that is, a cavity formed by several, distinct lateral
walls 21 - 24, which walls, in the present case, are, each, hydrophilic. The pyramidal shape could for instance be a tetrahedron, a square pyramid, or be more complex. Preferably though, this shape is essentially defined by (at least) four lateral walls 21 - 24. Thus, such a cavity 20 typically has triangular walls 21 - 24 that converge, ideally, to a point but, more likely, to a truncation surface 29. The flat base that defines the opening 28 of the cavity has a polygon shape, which is at least trilateral, and preferably is quadrilateral. In the latter case, the pyramidal shape is essentially defined by at least four lateral walls 21 - 24.

Note that the walls need not be perfectly flat walls, be it due to hazards in the fabrication processes. However, it is noted that preferred fabrication techniques disclosed herein (see below) essentially prevent this. Some residual defects may be present, e.g., due to the photolithography processes but this is actually rare. Rather, the lateral walls are typically flat since anisotropic etching follows crystallographic planes. It is for instance possible to control shapes such as depicted in FIG. 11 by modifying the mask (layout), therefore the final shapes can be controllable and predictable, at least in embodiments. One or more of the lateral walls may for instance be structured or shaped, as illustrated in FIGS. 11 (where one of the wall is structured 23a - d, such as to allow for local liquid injection, as well as bead release and aspiration, especially for those embodiments where no cover layer is used.

Using a pyramidal shape has been found to result in even better results, compared to "more" circular conic cavities, in terms of stability of the beads. The reason for it is not fully understood yet. This might be due to the free spaces left in the corners around a spherical bead, which allows for the residual liquid to evaporate, see FIG. 3, at variance with circular conic shapes.

FIG. 4 is a scanning electron microscope image showing beads trapped in pyramidal cavities. In this image:

- "EHT" stands for Electron High Tension in kilo-Volt, kV;
- "WD" denotes the working distance between the sample surface and the low portion of the lens;
- "Mag" is for magnification;
- "Tilt Angle" denotes the angle of the normal of the sample stage with respect to the axis of the electron gun; and
- "Signal A = SE2" indicates that a detector of secondary electrons is used.

Referring to FIG. 4: in embodiments, the working layer 10 comprises one or more semiconductor element such as silicon, and the pyramidal shapes of the cavities 20 exhibit a geometry that is consistent with the fabrication process used to obtain the cavities, which process advantageously uses an anisotropic etching process (one typically use silicon wafers that exhibit <100> crystallographic orientation, since other orientations, e.g. <111>, would not yield the desired pyramidal shapes). Now, although silicon is preferred, the following considerations apply to other semiconductors, e.g., Group IV elements e.g., Ge or compound semiconductors e.g., SiGe, other compound III-V or II-VI materials, and their respective oxides or nitrides.
For instance, GaAs and Ge can be etched anisotropically. Also, principles underlying present methods can be applied to some metallic layers and respective oxide. However, metallic layers are less practical. In particular, they may not exhibit comparable crystallographic uniformity in the layer thickness. Still, one may for instance contemplate using A1203 surfaces. A1203 can be used as a thin-film dielectric up to 100 - 200 nm, and deposited either by sputtering, or by atomic layer deposition ALD. The latter is an expensive but high quality technique. Still, the thickness of the layer required for microbead trapping makes it challenging to use ALD.

Pyramidal shapes are fortuitously compatible with anisotropic etching processes. An anisotropic etching process of fabrication of the cavities shall result, for Si or similar semiconductor elements, in an angle of ideally 54.7° between the flat base of the pyramid (i.e., the opening 28) and a contiguous wall, which, in turn, determines a depth-to-width ratio of the pyramid. Such processes allows for an easy and clean fabrication of the cavities, as can be seen in FIG. 4. This ideal angle may, however, slightly vary, due to small defect.

In variants, dry-film resists can be used and more generally some polymers, plastics and metals can be contemplated. Preferably, epoxy-based dry-film resists are used, which can be patterned by hot embossing. Hot embossing can be applied to other plastic materials as well (e.g., PMMA, COC, polycarbonate), for which the usual plastic patterning methods are likely not suitable for creating pyramidal microcavities. For example, after having fabricated an array of cavities using anisotropic etching of Si, the cavities can be filled by Ni electroplating. The electroplated Ni layer is then released, forming a mold or stamp to be used in a subsequent hot embossing process. The exact replica of cavities in Si can be created on the plastic layer by applying pressure and temperature to the stamp in contact with the plastic, and then cooling and demolding.

Referring now more specifically to FIG. 14, in embodiments, the chip is prepared such that its cavities (i.e., at least some of, and preferably most of the cavities 20) are, each, filled with a bead 50, 51, 52. As noted earlier, such beads are preferably microbeads, i.e., having, on average, a diameter between 1 and 40 μm. For applications as contemplated herein, a diameter between 2 and 20 μm, and more preferably between 2 and 10 μm, is preferred. The beads are preferably polymer beads, e.g., polystyrene, although silica or latex beads could also be used, in principle.

As illustrated in the accompanying figures, the dimensions of the cross-sectional areas of the cavities are comparable to the dimensions of the beads. Bead trapping cavities are traps specifically designed to trap single beads. As a result, a bead may typically contact the cavity walls in two (or more) points, or even be stuck at the level of a pointed end of a cavity. "Cavities" are contemplated herein are objects distinct from the microchannels or other microfluidic features. Owing to the solutions proposed herein, the majority of the cavities 20 of the array 30 may be filled, each, with only one bead 50, 51, 52, as seen in FIG. 14.
At present, details as to the relative dimensions of the cavities vs. beads are given, which allows for optimizing the stability of the trapped beads and the occupancy of the traps. Referring more particularly to FIGS. 3, 11 and 15: the ratio of an average (linear) dimension of a cavity opening 28, to the average bead diameter, should preferably be between 1.0 and 2.4. Still, best results (in terms of occupancy) have been observed for a ratio between 1.4 and 2.4 (namely, 40 - 60% occupancy, on average). These results still improve when the ratio is within 1.4-2.0 (up to 60% occupancy, on average, can be observed after rinsing). In some cases, occupancy of up to 90% could be achieved. Yet, keeping the above ratio under 2.0, or preferably under 1.8, allows to essentially prevent multiple occupancy of a same cavity.

For example, and according to the various results compiled by the inventors, for a 10 µm (diameter) bead, the maximal dimension of the flat base that defines the opening 28 of the cavities 20 should preferably be less than 24 µm, and more preferably less than 18 µm. These preferred figures are for instance confirmed by the fluorescence image shown in FIG. 14. The latter actually shows a negative of a fluorescence image of beads trapped in arrays of cavities obtained according to the method described later, in reference to FIG. 5. The cavities of the chip were actually obtained by performing a 13 µm deep TMAH etching of a Si substrate; a 200 µm x 500 µm array was used. Beads were integrated by pipetting approximately 200 nL of 10 µm Fluoro-MAX (from Thermo Scientific™) bead solution (not diluted) on each array. Thus, in each of the images of FIG. 14, 10 µm diameter beads are used. The lateral dimensions of the openings of the cavities (not visible in the images) vary from 8 to 24 µm, while the corresponding final occupancies rise from 5 to 63 %, with a peak at 80 - 90 % seen for 18 - 20 µm apertures. For this particular case, the exact percentages obtained are: 8 µm: 5.4%; 10 µm: 24.4%; 12 µm: 32.5%; 14 µm: 46.3%; 16 µm: 51.6%; 18 µm: 80.1%; 20 µm: 89.9%; 22 µm: 72.2%; and 24 µm: 63.2%. However, multiple occupancies appear at 18 µm and increase above. Thus, the range of apertures’ dimensions may accordingly be restricted (for 10 µm diameter beads) to, e.g., 14 - 18 µm, or even 16 - 18 µm. Consistently, the ratio of the average dimension of a cavity opening 28 to the average bead diameter could restrict to 1.4 - 1.8 or 1.6 - 1.8. Note that the percentage values arising from the particular case above differ from the average values mentioned earlier, since the latter which were compiled from various experiences.

Referring now to FIGS. 3 and 15: the ratio of an average depth of the cavities 20 to an average diameter of beads 50 is preferably of at least 0.5, more preferably 1.0 and even more preferably 1.3. For instance, experiments conducted by the present inventors have shown that 10 µm diameter-beads were able to stay, stable, in 8 µm x 8 µm openings, although the final occupancy/stability by the beads was found suboptimal in that case. Such dimensions imply a depth of approximately 5 µm, when using a self-limited anisotropic etching process. Accordingly, the ratio of the average cavity depth to the average bead diameter can be as low as 0.5. Now, for an anisotropically etched pyramid, a depth of at least (approximately) 1.0 d (where d is the diameter of the bead) is necessary when willing to obtain beads that are fully buried (i.e., embedded) in the cavity, provided that the conic cavity is truncated and that the truncation surface 29 is large enough
to accommodate the bead, as illustrated in FIG. 15. For example, a 10 µm bead can be fully buried into the
cavity if the opening is at least of 19.33 µm x 19.33 µm, and the depth is at least of 10 µm minimum, using
a time-based etching, where the etching is stopped before all planes merge at the apex. If cavities are not
truncated (they have a point-like apex, i.e. self-limited etching), then the ratio needs be of approximately
1.3, at least, as illustrated in FIG. 3.

Yet, present inventors have further realized that the beads need not necessarily be entirely buried in the
cavities. That is, a fraction of the beads can emerge above, i.e., protrude from, the plane of the opening 28,
to maintain a satisfactory stability, as illustrated in FIG. 5 (not to scale). The above ratio can therefore be
accordingly decreased.

Referring now to FIGS. 1, 2, 6 - 9, and 12 - 13, in embodiments, the microfluidic chip 100 may exhibit
two or more subgroups 32 of cavities 20, e.g., rows or columns of an array 30, where two subgroups are
connected by at least one microchannel 14. Preferably, one or more of these subgroups are defined in a
microchannel portion 12a, whose bottom wall or top wall is defined by the working layer 10. Such
configuration allows for multiplexing, while preventing cross-contamination among the subgroups 32.
Therefore, it is preferred to split the array 30 in subgroups 32 of beads 50, in order to partly isolate the
subgroups. The array of cavities can for instance be arranged so as to match typical coordinates of microtiter
wells: on a microtiter plate, wells are positioned on a quadratic lattice with a spacing of 9, 4.5, or 2.25 mm.
This can facilitate the dispensing of solutions of beads using robotic instruments. For an increased
integration of the arrays of cavities, an hexagonal lattice can also be selected. For instance, the chip design
used in the experiments shown in FIG. 14 comprises arrays, which are distributed along a serpentine-shape
microfluidic channel (200 µm wide, 14 µm deep) in an hexagonal lattice arrangement, where corner-to-
corner distance from one array to the other is about 1.1 mm. This arrangement permits the placement of 10
individual arrays (each occupies 200 µm x 500 µm area) within a total area of 3 mm x 5 mm, and permits
dispensing 200 nL droplets of bead solution to each array without merging of droplets or cross-
contamination.

Next, as seen in the “final” devices shown in FIGS. 5 - 9, the array 30 of cavities 20 of the microfluidic
chip 100 is preferably sealed by a cover layer 60, extending vis-a-vis the array 30. The cover layer protects
and seals the cavities and their contents. Beyond the sole cavities 20, the cover layer 60 shall likely seal
other microfluidic structures typically present on the chip 100. Examples of such microstructures are: a
loading pad 11, a detection antibody (or dAb) deposition zone 70, a capillary pump 16 or air vents 18, as
illustrated in FIGS. 1 and 2.

Note that although the cover layer 60 extends vis-a-vis the array 30, it does not necessarily contact the
cavities, since a gap is usually needed to form the microfluidic channel. In the fabrication process of FIG.9,
the cavities are sealed by the cover layer because the microchannels are arranged between the cavities. Yet,
in the other fabrication techniques discussed herein, the cover film 60 does actually not directly contact the cavities, there is always a gap introduced by the channel layer deposition (FIG. 6) or etching (FIGS. 7 and 8). Typically, the channel depth is between 1 and 20 \( \mu \)m. Shallower channels would yield higher hydraulic resistance and less thickness uniformity, while the fabrication of deeper channels would be more difficult and/or time-consuming. In general, the minimal lateral dimensions of the features that can be fabricated increases with the depth of the channel due to aspect-ratio limitations of the channel fabrication techniques. Current fabrication techniques (wet or dry etching or dry-film resist patterning) would easily provide structures with aspect-ratio higher than 1 (e.g., 20 \( \mu \)m wide features in 20 \( \mu \)m deep channels) without extensive parameter optimization effort.

In that respect, the channel height (or depth) can be adjusted to make sure that the beads do not escape during the flow. Owing to the present solutions, the stability of beads is markedly improved. Still, there might always be a residual a risk of loosing some beads in the presence of a strong fluid flow in the microchannel. Now, if the microfluidic channel height is slightly less than the diameter of the bead (e.g., less than 10 \( \mu \)m in the previous examples), the beads can never escape during the flow. Too small channel depths (e.g., 1 \( \mu \)m) would, however, increase the hydraulic resistance and decrease the total liquid capacity (volume) of the chip; too large depths (e.g., more than 10 \( \mu \)m) would increase the probability of loosing some beads. Having a channel depth slightly less than the diameter of the bead would also be beneficial in case the excess of beads are removed using a tape (i.e., the beads inside the cavities would stay and the excess inside the channel would stick to the tape).

Referring now more specifically to FIGS. 12 and 13, the chip 100 may actually comprise several arrays 30 (each comprising one or more bead trapping cavities 20). These arrays 30 are preferably inserted between distinct pairs of microchannel portions, i.e., in parallel or in series, in a given flow path. For example, these channel portions may notably consist of parallel split channels, as shown in FIGS. 13, to avoid cross-contamination from one portion to the other, or may be arranged in series (as in FIG. 12). They could also be arranged in channel portions of a serpentine channel (not shown).

Next, one may also advantageously use different types 51, 52 of beads, located in the cavities 20 of the array(s) 30. Owing to the preferred deposition techniques, the beads 51, 52 are typically deposited in distinct arrays 30. For example, two different types of beads can be used: one type 51 of bead is used for analyte detection, while the other type 52 of bead is used for control, as illustrated in FIGS. 12, 13. As said earlier, the arrays 30 may be in series or in parallel, depending on the actual application sought. In most applications contemplated herein (e.g., bioanalyses), the beads differ in terms of coating. The beads may nevertheless differ, more generally, in terms of size, coating, material and/or color.

Note that the prior art solutions mostly impose sealing the device before loading the beads (via a bead solution flow). This makes it difficult, if not impossible, to have different types of beads located at
predetermined positions in the device. As a consequence, this implies more steps, in practice, for bioanalysis.

According to another aspect, the present invention can be embodied as a method of fabrication of a chip 100 as described above. Fabrication methods are now described in reference to FIGS. 5 - 10. Most generally, such methods revolve around a basic step of fabrication (step S20) of an array 30 of bead trapping cavities 20, in a working layer 10, 60 of the device 100. Consistently with the devices 100 described earlier, the fabrication ensures that each of the cavities 20 has a conic shape, defined by one or more lateral walls 21 - 24 that are, each, hydrophilic. The cavities 20 extend, each, as a blind hole in the thickness of the working layer 10, 60.

Different approaches are possible, depending on the materials 10, 60 chosen. The cavities may be provided in a substrate layer 10 or in a cover layer or film 60. As further evoked earlier, the fabrication methods may involve anisotropic etching processes, hot embossing processes, or any other suitable process to obtain the cavities 20. So far, anisotropic etching processes seem to be the most promising, in terms of quality achieved for the cavities. Self-limited anisotropic etching processes are preferred to time-based etching processes because the former is less susceptible to variations in the etch rate and allows the depth stay almost constant in the case of over-etching (the final depth is defined by the dimension of the cavity opening 28).

As evoked earlier, the anisotropic etching process is preferably carried out on <100> wafers, having a flat in the <110> direction; thus the top surface has a normal in <100> direction. The exposed face of the wafer is accordingly parallel to (100) planes, i.e., orthogonal to the (100) direction in the basis of the reciprocal lattice vectors (diamond structure for Si). Besides the fabrication of cavities, anisotropic etching processes can also be used to fabricate microfluidic structures (e.g. FIG 8 and 9). If it is not detrimental for the microfluidic structures, e.g., channels, to have slanted sidewalls, then wet etching of Si wafer with a <100> crystal orientation is preferred over dry etching techniques because wet etching is compatible with batch processing and therefore can be overall faster, depending on the number of wafers processed. Note that, wet etching is usually slower than dry etching, which can be much faster per wafer. The overall throughput thus depends on the number of wafers processed altogether.

If necessary, the array(s) 30 can be cleaned (e.g., using ethanol, water, etc.) and/or treated with a plasma (e.g., air, oxygen or helium). In all cases, beads 50, 51, 52 can be deposited S30 into the cavities 20 by merely spotting S32 a droplet of a bead solution 55, e.g., on top of the array(s) 30. For example, one may apply approximately 2 μL of stock bead solution (typically provided as a 1% solid suspension) to the array(s) 30.

After depositing S30 the beads (and prior to sealing S40 the chip), it is recommended to:

- Dry S34 the cavities 20 (the beads 50 remain in the cavity or nearby); and then
- Remove S36 excess beads (i.e., those beads not trapped inside the cavities 20), e.g., by rinsing S36a the array(s) 30 with a rinsing solution and/or by applying a tape to which excess beads stick S36b; prior to
- Drying again S38 the array 30 of cavities 20 with remaining beads therein, if necessary, i.e., the last drying step is not always required, e.g., when only applying a tape to remove excess beads.

Present inventors have realized that drying the array 30 before removing the excess beads resulted in surprisingly more stable beads 50. The impact on final occupancies was accordingly found to be substantial: occupancy can be improved by 20 to 60%, or even more, depending on other conditions, thanks to the prior step of drying S34. It can be speculated that conic cavities better protect beads from lateral, in-plane motion of fluids (gases or liquids) when rinsing and drying (e.g., N2 flow). Preferred rinsing solutions are, e.g., buffer solutions or deionized water. More generally, it can be any solution that do not adversely affect the beads or proteins coated thereon.

Finally, the array 30, and more generally part or all of the chip 100 can be sealed S40 with a cover layer 60, e.g., a dry film, that is preferably laminated, for ensuring a good seal. When the cavities are provided in the layer 10, the cover 60 is placed such as to extend vis-a-vis the array 30 of cavities 20, thereby forming closed microfluidic channels 12, 12a, 14 and structures 16, 18.

Four different fabrication examples are now discussed in detail. A first fabrication example is illustrated in detail in FIG. 5. The detailed steps of FIG. 5 are otherwise captured in the flowchart of FIG. 10. FIG. 6 only illustrates selected steps of this first fabrication method, whereas FIGS. 7 - 9 illustrate selected steps of other possible fabrication methods. In each of FIGS. 6 - 9, the upper row of figures are section views of the microfluidic chip, at different fabrication stage, whereas the lower row shows the corresponding top views.

Referring first to FIGS. 5, 6 and 10, the first fabrication example uses anisotropic cavity etching followed by a dry-film resist channel fabrication. In detail:

- Step S10: A microfluidic chip body is provided, which comprises a layer (or substrate) 10.
- Block S20: fabrication of the cavities and channels:
  - S21, FIG. 5A: The layer 10 is oxidized, e.g., silicon is oxidized by thermal oxidation to obtain a SiO2 layer 100. The electrically insulating layer 100 obtained typically covers the whole substrate 10. Instead of an oxide, one may also seek to obtain nitride such as Si3N4.
  - At step S22, FIG. 5B: The oxide is patterned, e.g., using dry or wet etching. To that aim, a photoresist is typically used as a mask (FIG. 5B schematically depicts the chip as of after etching of the oxide and stripping of the photoresist);
Then, the substrate layer 10 is anisotropically etched, step S24, FIG. 5C, to obtain cavities 20 and other deposition zones 70. Preferably, a wet etchant is used, which typically is TMAH or KOH. A short oxide etch (e.g., BHF) is typically done prior to Si etching in order to remove the native oxide on the Si surface;

Next: the oxide is stripped, step S25, FIG. 5D. This, however, is optional as the oxide could be left on the surface if desired. Typically, a buffered oxide etch (e.g., BHF) is used to that aim; and the channels’ lateral walls 62 are patterned, preferably by depositing, exposing and then developing a dry-film resist (negative photoresist) or an epoxy-based negative photoresist (e.g., SU-8), step S26, FIG. 5D;

- Block S30: bead deposition (also called reagent integration):
  - Step S32, FIG. 5E: A droplet of a bead solution is deposited on a cavity array 20 (different types of beads could be deposited on respective arrays, as illustrated in FIGS. 12 - 13);
  - Step S34, FIG. 5F: The arrays 30 and beads are dried (preferably by natural evaporation, by a stream of N2 or by placing the chip in a controlled environment or on a warm plate, etc.);
  - Step S36: Excess beads are removed, preferably by: (i) rinsing them, e.g., using a stream of deionized water or a buffered solution, step S36a, FIG. 5G; and/or (ii) applying a tape such as an adhesive tape or PDMS, to which excess beads will stick, step S36b, FIG. 5H.

If necessary, the tape is applied several times; and

- S40, FIG. 5I: Finally, the chip 100 (and in particular the array(s) 30 of cavities) is sealed with a cover layer 60 (e.g., a dry-film resist) that is preferably laminated, step S42. To that aim, the cover layer 60 is typically moderately heated, e.g., to 45 - 50°C. The cover film can have an opening at the level of a loading pad to pipette liquid (as seen in step S40, FIG. 6). Such an opening can be patterned by cutting or punching.

The above example of fabrication method allows for flexible designs, as the fabrications of cavities and channels are decoupled. It can further be realized that such a method enables circular channel structures. The drawbacks of such a method, however, are that it requires two masks and the rinsed beads might stay inside the channels.

Referring now to FIG. 7, a second fabrication example is described, where the beads are integrated to the cover layer 60 (e.g., a dry-film resist), instead of the layer 10. Briefly:

- Step S20a: a mold 65 is used to pattern cavities in the layer 60 (e.g., by hot embossing);
- Step S20b: the patterned layer 60 is detached;
- Step S30: beads are deposited in the cavities of the patterned layer 60, again by pipetting a droplet of bead solution, drying, rinsing and again drying;
- Step S40: Finally, the MF chip is sealed by placing the cover layer 60 on the chip, preferably by laminating the cover layer 60 thereon.

In this method, the beads' integration is independent from the substrate and the channels, which allows for more flexibility in the design of the substrate 10 and the channels. The MF chip can have microfluidic structures, which are etched (anisotropic etching, or deep reactive etching), or deposited (dry-film resist, SU-8, etc.), or patterned by hot-embossing or molding. The cover film can be laminated on any compatible substrate with or without prior surface modification or treatment. However, this method may require a transparent substrate for more efficient fluorescent detection. Also, the beads may perhaps more easily detach from the film, compared to beads integrated in a substrate.

Next, referring to FIG. 8, a third fabrication example is briefly described, which uses a two-step photolithography and anisotropic Si etching:

- Step S20c: Channels are etched by anisotropic Si etching. A thermal oxidation follows (not shown); then
- Step S20d: Cavities are etched by anisotropic Si etching after patterning of the oxide;
- Step S30: Beads are integrated; and
- Step S40: The chip is sealed.

Such a fabrication method allows for a flexible design, as trap and channel fabrications are decoupled. However, it requires more fabrication steps (two masks are needed), as well as an additional thermal oxidation between the two etching steps. In addition, in this method, rinsed beads might stay inside the channels.

Finally, and referring to FIG. 9, a last fabrication example is described, which relies on a single-step photolithography and anisotropic Si etching. At step S20e: Channels and cavities are etched at the same time, using anisotropic etching, in a single photolithographic step. Then, the beads are integrated at step S30 and the chip is sealed, step S40. With such a method, both the channels and traps are fabricated in one etching step, which is more cost effective. Also, the channel depth can be defined by adjusting the width of the channel because wider openings result in deeper channels and vice versa in the self-limited etching process. Each cavity can be connected to each other and to the microfluidic network via microfluidic channels whose widths are smaller than those of the cavities, as illustrated in S20e (FIG.9). However, such a fabrication method implies stricter design rules, leads to higher hydraulic resistances due to the small dimensions of the microfluidic channels interconnecting the cavities. There is also a risk of air bubble formation inside the cavities.
At present, an example of application of embodiments of the present invention is discussed. Namely, a simple ligand-receptor assay is demonstrated in an open-channel system (the chip is not sealed with a cover film for the sake of simplicity):

- "Superavidin"-coated beads (10 μm diameter) in polystyrene from Bangs Laboratories Inc. are used: 10 μm polystyrene beads are coated with avidin, a protein known to bind very strongly to biotin. The commercial stock solution contains the beads as 1% solids;
- The stock solution is diluted to 1/5 with PBS + 0.5% Tween 20 (larger dilutions can also be used);
- An array of cavities as obtained according to FIGS. 5A - 5C (steps S10 to S24 in FIG. 10) is used as is: Approximately 2 μL of the bead solution is spotted on the array and let dry (1 to 2 minutes suffice for complete drying);
- The array and beads are rinsed under a stream of PBS + 0.5% Tween 20 (approximately 30 mL for 10 s);
- The array and beads are then rinsed under a stream of DI water (approximately 30 mL for 10 s);
- The array is then dried under a stream of nitrogen;
- The array and beads are covered with a droplet of 1% BSA in PBS + 0.5% Tween 20 for 15 min;
- The array and beads are rinsed with PBS + 0.5% Tween 20, water and dried under a stream of nitrogen.
- Finally, the chip can be sealed and stored.

For a typical assay, a user would do the following next three steps, wherein an analyte is represented by a fluorescently-labeled biotin molecule.

- The array and beads are exposed to a 50 μg/mL solution of biotin-590-Atto (Sigma-Aldrich®) for 15 min while being protected from light. During this step, biotin-590-Atto binds to avidin on the beads;
- The array and beads are rinsed with PBS + 0.5% Tween 20 and water. These rinsing steps are optional but can be applied for increasing the sensitivity of the assay.
- The binding of biotin-Atto-590 (ligand) to avidin on the beads (receptor) is monitored using a fluorescence microscope. The single bead cavities allowed for simple signal reading and interpretation, without any optical artifact.

The methods and devices described herein can be used in the fabrication of microfluidic chips. The resulting chips can be distributed by the fabricator in raw form (e.g., as a structured bilayer device) or in a packaged form. In the latter case the chip can be mounted in a single chip package. In any case the chip can then be integrated with other elements, as part of either (a) an intermediate product or (b) an end product.
To conclude, embodiments of the present invention provide various advantages. For example:

- Embodiments of the invention allows to keep the beads in a particularly and surprisingly stable manner, especially if the beads and cavity arrays are dried before removing excess beads. Only a few beads are lost during subsequent exposure to liquids and drying. After many years of global researches on microfluidics and patterning receptors for assays, a very efficient method for integrating beads (or receptors) to microfluidics was found at last;

- Present designs and corresponding fabrication methods furthermore allow for placing the beads before covering the traps;

- The arrays of cavities may be dimensioned relatively to the beads to trap a single bead per cavity. Single bead cavities allows simpler signal reading and interpretation, requires a single layer to accommodate the beads, and give rise to less (or even no) optical artifact; and

- Fabrication methods proposed herein enable high-throughput manufacture. Various bead integration strategies were proposed, which allow flexible designs of microfluidics and flow paths. The concepts underlying embodiments of the present invention are incidentally compatible with several microfluidic features (mirrors, plastic chip fabrication, etc.).

While the present invention has been described with reference to a limited number of embodiments, variants and the accompanying drawings, it will be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the scope of the present invention. In particular, a feature (device-like or method-like) recited in a given embodiment, variant or shown in a drawing may be combined with or replace another feature in another embodiment, variant or drawing, without departing from the scope of the present invention. Various combinations of the features described in respect of any of the above embodiments or variants may accordingly be contemplated, that remain within the scope of the appended claims. In addition, many minor modifications may be made to adapt a particular situation or material to the teachings of the present invention without departing from its scope. Therefore, it is intended that the present invention not be limited to the particular embodiments disclosed, but that the present invention will include all embodiments falling within the scope of the appended claims. In addition, many other variants than explicitly touched above can be contemplated. For example, other materials than those explicitly mentioned herein could be used for each of the layers 10, 60. In addition, the channels, loading pads, air vents, cavities, etc., could be provided with various dimensions.
REFERENCE LIST

10 Basis layer
100 Oxide layer
5 11 Loading pad
12 Microfluidic channels (microchannels)
12a MicroChannel portion
14 Multiplexing microchannels
16 Capillary pump
10 18 Air vents
20 Bead trapping cavities
21 - 24 Lateral walls of cavities
24a - d Structured (multi-faceted) wall
28 Cavity opening/conic base
15 29 Cavity bottom surface
30 Array of bead trapping cavities
32 Cavity subgroup (row/column)
50 Beads
51 Analyte detection beads
20 52 Control beads
55 Bead solution (liquid droplets)
60 Cover layer (dry-film), lid
62 Channel walls
65 Mold for embossing cavities in cover layer
25 70 Cavity for detection anti-body (dAb) deposition
72 deposited detection anti-body (dAb)
100 Microfluidic chip
1. A microfluidic chip (100) comprising a layer (10, 60) with an array (30) of bead trapping cavities (20) provided in that layer (10, 60), wherein each of the cavities (20) has a conic shape, defined by one or more lateral walls (21 - 24) that are, each, hydrophilic, and wherein each of the cavities (20) extends as a blind hole in the thickness of said layer (10, 60).

2. The microfluidic chip (100) of claim 1, wherein at least some of the cavities (20) have apyramidal shape formed by lateral walls (21 - 24) that are, each, hydrophilic, and wherein, preferably, this pyramidal shape is essentially defined by at least four lateral walls (21 - 24).

3. The microfluidic chip (100) of claim 1 or 2, wherein said layer (10) comprises one or more semiconductor element such as silicon, and the pyramidal shapes of the cavities (20) exhibit a geometry consistent with an anisotropic etching process of fabrication of the cavities in the layer (10).

4. The microfluidic chip (100) of any one of claims 1 to 3, wherein at least one of, and preferably most of, the cavities (20) are, each, filled with a bead (50, 51, 52), preferably a microbead, having, on average, a diameter between 1 and 40 µm, preferably 2 and 20 µm, and more preferably 2 and 10 µm.

5. The microfluidic chip (100) of claim 4, wherein a majority of the cavities (20) of said array (30) are filled, each, with only one bead (50, 51, 52), which preferably is a microbead having, on average, a diameter between 1 and 40 µm, preferably 2 and 20 µm, and more preferably 2 and 10 µm.

6. The microfluidic chip (100) of claim 4 or 5, wherein the ratio of an average dimension of an opening (28) of the cavities (20) to an average diameter of beads (50, 51, 52) in the cavities (20) is between 1.0 and 2.4, and preferably between 1.4 and 2.0.

7. The microfluidic chip (100) of any one of claims 4 to 6, wherein the ratio of an average depth of the cavities (20) to an average diameter of beads (50) in the cavities (20) is at least of 0.5, preferably 1.0 and more preferably 1.3.

8. The microfluidic chip (100) of any one of claims 1 to 7, wherein two or more subgroups (32) of at least one of the cavities (20), which subgroups preferably are rows or columns of the array (30) of cavities (20), are connected by at least one microchannel (14), and wherein, more preferably, said one or more of the subgroups is defined in a microchannel portion (12a), whose bottom wall or top wall is formed by a surface of said layer (10).

9. The microfluidic chip (100) of any one of claims 1 to 8, wherein the array (30) is sealed by a cover layer (60) extending vis-a-vis the array (30) of cavities (20).
10. The microfluidic chip (100) of any one of claims 1 to 10, comprising several arrays (30) of one or more bead trapping cavities (20), wherein said arrays are preferably inserted between distinct pairs of microchannel portions (14).

11. The microfluidic chip (100) of claim 10, comprising at least two different types (51, 52) of beads, located in one or more cavities of at least two of said several arrays (30), respectively, wherein the beads of different types preferably differ in terms of size, coating, material and/or color.

12. A method of fabrication of a microfluidic chip (100) according to any one of claims 1 to 11, comprising:

   providing (S10) a microfluidic chip body with a layer (10, 60); and

   fabricating (S20) an array (30) of bead trapping cavities (20) in that layer (10, 60), wherein each of the cavities (20) has a conic shape, defined by one or more lateral walls (21 - 24) that are, each, hydrophilic, and wherein each of the cavities (20) extends as a blind hole in the thickness of said layer (10, 60).

13. The method of claim 12, wherein fabricating (S20) the array (30) comprises anisotropically etching (S24) said layer (10) to obtain the cavities (20), preferably using a self-limited anisotropic etching process.

14. The method of claim 12 or 13, further comprising

   depositing (S30) beads (50) into the cavities (20) of the array (30) by spotting (S32) a droplet of a bead solution (55); and

   sealing (S40) the array (30) with a cover layer (60) placed such as to extend vis-a-vis the array (30) of cavities (20), and wherein sealing (S40) the array (30) preferably comprises laminating (S42) the cover layer (60).

15. The method of claim 14, wherein the method further comprises, after depositing (S30) beads (50) and prior to sealing (S40), in that order:

   drying (S34) the array (30) of cavities (20) with beads (50) therein,

   removing (S36) excess beads not trapped inside cavities (20), preferably by rinsing (S36a) the array (30) with a rinsing solution and/or by applying a tape to which excess beads stick (S36b); and

   drying again (S38) the array (30) of cavities (20) with beads therein, if necessary.
S10: Provide MF chip body with layer 10

S21: Oxidize layer 10

S22: Pattern oxide layer 10

S24: Anisotropic etching of layer 10

S25: Strip oxide layer 10

S26: Pattern channel's lateral walls 62

S20: Fabricate cavities 20 and channels 12, 14

S32: Spotting bead solution on array 30

S34: Dry array 30 with beads 50

S36: Remove excess beads, e.g., by rinsing

S38: Dry array with trapped beads

S42: Laminate cover to seal MF chip

S40: Seal array 30 with cover layer 60

FIG. 10
INTERNATIONAL SEARCH REPORT
International application No. PCT/IB2015/051579

A. CLASSIFICATION OF SUBJECT MATTER
G01N 33/53(2006.01)i; G01N 33/554(2006.01)i; G01N 33/543(2006.01)i; H01L 21/00(2006.01)i

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)
G01N, H01L

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)
CNPAT, CNKI, WPI, EPDOC, IEEE: IBM, microfluidic, chip, array, bead?, particle?, trap+, cavity+, recess, groove, trench, microchannel, hydrophilic, conic, hold, subgroup, dry

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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

Date of the actual completion of the international search 18 June 2015
Date of mailing of the international search report 26 June 2015

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