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(54) **Title**: METHOD FOR SPATIALLY MANIPULATING A MICROSCOPIC OBJECT AND DEVICE FOR CONDUCTING SAID METHOD

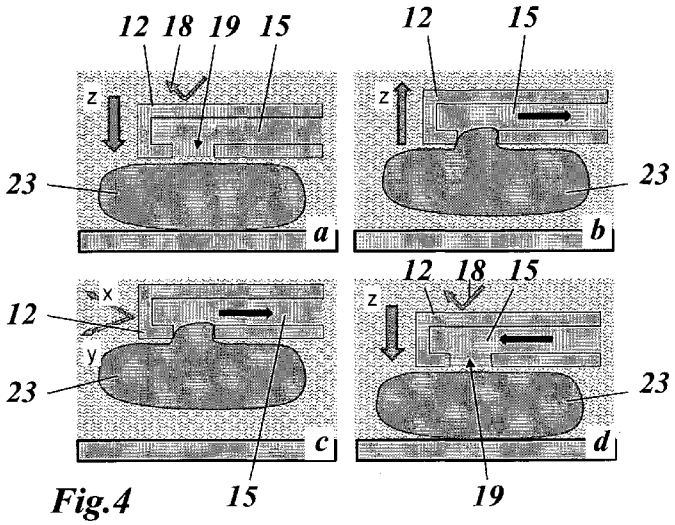

Fig.4

(57) **Abstract**: The invention relates to a method for spatially manipulating a microscopic object (20,23,33), said method comprising the steps of: providing a cantilever (12) having a tip with an opening (19) and a microchannel (15) extending through the cantilever (12) in its longitudinal direction, said microchannel (15) being fluidly connected to the opening (19) at the tip of the cantilever; providing suspension means for holding the cantilever (12) and spatially moving the cantilever along a predetermined spatial path; providing pressurizing means for applying a predetermined pressure to the microchannel (15) within the cantilever; moving the cantilever (12) with its tip to the microscopic object (20,23,33) to be spatially manipulated, such that the opening (19) of the tip is adjacent to the microscopic object (20,23,33); picking up, with said cantilever (12), a part of the microscopic object (20,23,33) or the microscopic object (20,23,33) as a whole by reducing the pressure within the microchannel (15) relative to the pressure outside the tip of the cantilever (12); and moving said part of the microscopic object (20,23,33) or said microscopic object (20,23,33) as a whole along a predetermined spatial path by means of the cantilever (12).

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Method for spatially manipulating a microscopic object and device for conducting said method

The present invention pertains to the field of manipulating microscopic objects such as bacteria, biological cells, neurons, or submicron objects such as virus or nanoparticles, or the like.

The ability to obtain information at the single-cell level is becoming of central importance for numerous biological questions and represents a major challenge. There is an increasing awareness that individual cells, although genetically identical to sister cells, show different phenotypes and expression profiles of genes (transcripts) and in consequence levels of proteins and metabolites. New innovative technologies are required to address individual cells, whereby "to address" has a broad meaning ranging from displacement, injection up to analysis. For the following the focus shall be on the controlled spatial displacement of single cells.

Several years ago A. Ashkin and co-workers pioneered the development of the optical tweezers as mean to manipulate biological objects (see for example EP 307 940). Exploiting the trapping forces due to the radiation pressure through intense and collimated lasers, they showed that one could handle viruses, bacteria, cells up to cellular organellae. Yet, it is still debated at which extent optical tweezers damage the trapped organisms.

Glass micropipettes, the oldest instrument to manipulate single organisms, are not truly apt for displacement experiments. Operated by means of micromanipulators combined with a pressure controller for the suction of the biological objects, their positioning is still followed with optical microscopy which is limited by its intrinsic resolution of the order of 1µm. Consequently, the approach toward an organism without damaging it is a hit-and-miss procedure, while if successfully sucked, it is practically impossible to safely reposition it onto another location of the substrate surface.

The atomic force microscope AFM (G. Binnig, C. F. Quate, and C. Gerber, Phys Rev Lett 56 (9), 930 (1986)) has the required force feedback in the pN range. With respect of organism manipulation, it is employed for a bright spectrum of adhesion experiments but it is not conceived for displacement experiments because organisms are stably attached to the underside of the cantilever preventing their release on a another position of the substrate.

The development of the "FluidFM" (A. Meister, M. Gabi, P. Behr, P. Studer, J. Voros, P. Niedermann, J. Bitterli, J. Polesel-Maris, M. Liley, H. Heinzelmann, and T. Zambelli, Nano Lett 9 (6), 2501 (2009)) combining the precise AFM force feedback with nanofluidics via an incorporated microchannel directly in the cantilever (see EP-A1-1 990 626) opens novel strategies for the spatial manipulation of biological objects. The microchannel in the cantilever ends with a submicron aperture at the apex of the pyramidal tip and on the other side with a reser-

voir. A channel is also machined in the AFM probeholder. By conveniently fixing the chip against the probeholder, a continuous fluidic pipeline is obtained connecting the tip aperture with a syringe or a pressure controller. Therefore, the "FluidFM" can be immersed in liquid environment while a pressure can be applied to the solution inside the channel.

The probing area of the "FluidFM" is shown in Fig. 1: The atomic force microscope (AFM) 10 of Fig. 1 comprises a probeholder 11, which is attached to a micro-manipulating mechanism of the AFM not shown. Attached to the probeholder 11 is an elongated cantilever 12, which is fabricated by means of an MEMS (Micro-Electro-Mechanical-Systems) technology. The cantilever 12 is provided with an internal microchannel 15, which extends in the longitudinal direction of the cantilever 12. The microchannel 15 is, with its outer end, in hydraulic connection with an opening 19 a pyramidal tip 13 of the cantilever 12. At the other end, the microchannel 15 connects to a supply channel 14 running through the probeholder 11 to receive a predetermined fluid pressure from an external controllable pressure source 17 via a tubing 16. Fig. 1 further shows an impedance measuring means 34, which is connected to the liquid path. This impedance measuring means 34 is not part of the well-known "FluidFM" device, but is a novel feature of the device according to the invention, which will be explained in detail below.

The "FluidFM" is used for local liquid dispensing and stimulation of single living cells under physiological conditions. The nanofluidic microchannel 15 in the cantilever 12 allows soluble molecules to be dispensed through the submicrometer aperture or opening 19 in the AFM tip. The sensitive AFM force feedback, which is established by means of a laser beam 18, allows controlled approach of the tip to a sample for extremely local modification of surfaces in liquid environments. It also allows reliable discrimination between gentle contact with a cell membrane or its perforation. Using these two procedures, dyes have been introduced into individual living cells and even selected subcellular structures of these cells.

Now, it is a central object of the invention to establish a method for spatially manipulating a microscopic object by using device based on the principles of the "FluidFM" described above.

It is a further object of the invention to provide a device for spatially manipulating a microscopic object according to the inventive method.

The method according to the present invention comprises the steps of: providing a cantilever having a tip with an opening and a microchannel extending through the cantilever in its longitudinal direction, said microchannel being fluidly connected to the opening at the tip of the cantilever; providing suspension means for holding the cantilever and spatially moving the cantilever along a predetermined spatial path; providing pressurizing means for applying a prede-

termined pressure to the microchannel within the cantilever; moving the cantilever with its tip to the microscopic object to be spatially manipulated, such that the opening of the tip is adjacent to the microscopic object; picking up, with said cantilever, a part of the microscopic object or the microscopic object as a whole by reducing the pressure within the microchannel relative to the pressure outside the tip of the cantilever; and moving said part of the microscopic object or said microscopic object as a whole along a predetermined spatial path by means of the cantilever.

An embodiment of the inventive method is characterized in that, in a further step, said part of the microscopic object or said microscopic object as a whole is released from the cantilever by increasing the pressure within the microchannel relative to the pressure outside the tip of the cantilever.

According to another embodiment of the inventive method, said part of the microscopic object or said microscopic object as a whole is picked up by introducing it into the interior of the cantilever, especially into the microchannel of the cantilever.

According to another embodiment of the inventive method, said part of the microscopic object or said microscopic object as a whole is released into a recess or into an opening of a processing means for being processed.

Another embodiment of the inventive method is characterized in that a cantilever with an elongated tip of high aspect ratio extending perpendicular to the longitudinal direction of the cantilever is used to pick up one specific microscopic object from an aggregation of several microscopic objects.

Another embodiment of the inventive method is characterized in that a cantilever with a sharpened tip extending perpendicular to the longitudinal direction of the cantilever is used to tear off a part of a microscopic object.

Another embodiment of the inventive method is characterized in that the pressurizing means comprises a pressure source, which produces by means of a pump or by capillary, osmotic or electro-osmotic forces.

Another embodiment of the inventive method is characterized in that a cantilever is used, the surface of which is modified to avoid an adhesion of the microscopic object, and especially by either having hydrophilic or hydrophobic properties.

According to another embodiment of the inventive method, during picking up the a microscopic object, the corresponding deflection of the cantilever, which is due to an adhesion force acting between said microscopic object and the surface supporting said microscopic object, is used to calculate said adhesion force.

According to another embodiment of the inventive method, during picking up, the impedance through the opening of the cantilever is measured in order to characterize the tightness of the microscopic object closing the opening.

According to another embodiment of the inventive method, the impedance through the microchannel of the cantilever is measured in order to determine the number and/or mass of microscopic objects collected in said microchannel after being picked up by said cantilever.

Just another embodiment of the inventive method is characterized in that the cantilever is sensed by means of a laser beam in order to determine the number and/or mass of microscopic objects collected in said microchannel after being picked up by said cantilever. Instead of a laser beam, a tuning fork or piezo resistive element, or another suitable sensing means may be used.

The device according to the invention comprises: a cantilever having a tip with an opening and a microchannel extending through the cantilever in its longitudinal direction, said microchannel being fluidly connected to the opening at the tip of the cantilever; suspension means for holding the cantilever and spatially moving the cantilever along a predetermined spatial path; and pressurizing means for applying a predetermined pressure to the microchannel within the cantilever; whereby said pressurizing means is configured to generate a pressure within the microchannel which is lower than the pressure outside the tip of

the cantilever, and whereby an hydraulic impedance measuring means is provided for measuring the hydraulic impedance within the cantilever.

According to an embodiment of the inventive device, the opening of the cantilever is flush with the outer surface of the cantilever.

According to another embodiment of the inventive device, the cantilever has an elongated, especially cylindrical, tip of high aspect ratio extending perpendicular to the longitudinal direction of the cantilever.

According to another embodiment of the inventive device, the cantilever has a sharpened tip extending perpendicular to the longitudinal direction of the cantilever.

According to another embodiment of the inventive device, the cantilever has a pyramidal tip extending perpendicular to the longitudinal direction of the cantilever. Other geometric shapes of the tip are possible but perhaps not easy to integrate into the cantilever.

According to just another embodiment of the inventive device, the microchannel of the cantilever is configured to receive one or more of said microscopic objects.

The invention will now be explained in detail with respect to the drawings.

Fig. 1 shows the probing area of a device according to an embodiment of the invention, which is based on the "FluidFM" design and comprises means for measuring the hydraulic impedance within the channelled cantilever;

Fig. 2 shows a magnification of the hollow cantilever picking up a microscopic object by applying a negative pressure inside the cantilever in accordance with the method according to the invention;

Fig. 3 shows the cantilever picking up a microscopic object, thereby measuring the adhesive force by means of the deflection of the cantilever in accordance with an embodiment of the inventive method;

Fig. 4 shows various steps of the manipulation procedure according to the invention, whereby (a) relates to a force-controlled approach of the cantilever, (b) relates to applying a negative pressure and picking up the microscopic object, (c) relates to a displacement in an x-y-plane, and (d) relates to the force-controlled landing at a desired position and release of the microscopic object by means of an overpressure pulse;

Fig. 5 shows the use of a cantilever with a high aspect ratio tip, e.g. in form of a hollow cylinder, to pick up a microscopic object embedded in a

hollow of an uneven surface, according to an embodiment of the invention;

Fig. 6 shows the use of a cantilever with a high aspect ratio tip, e.g. in form of a hollow cylinder, to pick up a single microscopic object from inside an aggregation of objects, e.g. tissue, according to an embodiment of the invention;

Fig. 7 shows the use of a cantilever with a sharpened tip to cut out a part of an object by applying negative pressure or pushing forces (a) and removing said part to either gain access to the inside of the object or use said part for further analysis (b), according to another embodiment of the invention;

Fig. 8 shows the process of sucking microscopic objects into the microchannel of the cantilever, according to an embodiment of the invention;

Fig. 9 shows the process of placing a microscopic object, which is released from the microchannel of the cantilever, into a recess, which may serve as a reacting tube, according to another embodiment of the invention;

Fig. 10 shows the process of placing a microscopic object, which is released from the microchannel of the cantilever, into an opening, which may be part of an aspiration nozzle of an analytical device, according to another embodiment of the invention;

Fig. 11 shows, in a perspective view, a cantilever with a flat/blunt tip, according to an embodiment of the invention;

Fig. 12 shows, in a perspective view, a cantilever with a cylindrical tip with a high aspect ratio, according to another embodiment of the invention;

Fig. 13 shows, in a perspective view, a cantilever with a pyramidal tip, according to another embodiment of the invention;

Fig. 14 shows a photograph of various yeast cells (circled) before being spatially manipulated; and

Fig. 15 shows the yeast cells of Fig. 14 after being aligned in a row by means of the method according to the invention.

So far, only pulled glass micropipettes or optical tweezers can be used to displace microscopic objects with the disadvantage of not having control over the applied force to the object. Another kind of sample manipulating apparatus, which is based on an atomic force microscope (AFM), and which uses a mechanical tweezer, is disclosed in the document US-A1 2008/0314131.

The adhesion force of cells is measured nowadays by a laborious process. Cells or bacteria are grown on a cantilever bar for about 30 min and then brought in contact with a surface. There the cell has to adhere for another 30 min before the cantilever can be retracted and the adhesion force can be measured. Or vice versa, the cell is attached first to the surface. This is also a limitation of this method since the attachment of the cell to the cantilever may induce cellular changes or surface property changes, since additional adhesive molecules have to be introduced or the cells undergo already other biological processes changing the cell adhesive properties.

With the present invention this whole measurement can be performed in less than a minute. As shown in Fig. 2, the hollow cantilever 12 approaches with its tip opening 19 a microscopic object or cell 20 lying on a surface 22, under force control without risking cellular damage. By applying a negative pressure (<1bar) with respect to the fluid environment of the cantilever 12 the object or cell 20 is partly sucked into the opening 19 and is kept there, like with a suction cup. The quality of the sealing between the object 20 and the inner rim of the opening 19 can be controlled and monitored by measuring simultaneously the impedance through the cantilever tip opening 19 with the impedance measuring means 34 (see Fig. 1). To do this electrically, electrodes 35 may be placed at suitable positions in and outside the cantilever 12 (see Fig. 3).

The object or cell 20 now can be simply pulled away from the surface by retracting the cantilever 12 and measuring the adhesion force 21 (symbolized by a spring) at the same time (Fig. 3). The adhesion force 21 is measured by the deflection d of the cantilever 12.

The object or cell 20 can then be unloaded from the tip by either releasing the negative pressure or apply a small overpressure, so that the cantilever 12 can be reused immediately for the next adhesion force measurement. This allows fast and fully automated serial measurement of adhesion forces. Moreover the system can simply be used to spatially manipulate cells or other microscopic objects 20

The opening 19 of the hollow cantilever 12 can be only a simple hole (flat/blunt tip, see Fig. 11), or at the end of cylindrical or pyramidal tip structure (24 in Fig. 12 and 32 in Fig. 13). The applied force is detected by measuring the bending of the cantilever with a deflected laser beam (18 in Fig. 1), or a tuning fork or piezoresistive material attached to the cantilever 12.

A complete sequence of spatial manipulating steps is shown in Fig. 4: In a first step, the cantilever 12 approaches the object 23 to be manipulated in the z-direction. During this step, the force exerted on the object 20 by the tip is monitored by means of a laser beam 18 (reflected light beam in Fig. 4(a)). When the object 23 adheres to the tip by means of a negative pressure within the microchannel 15 of the cantilever 12, the object 23 is lifted in z-direction to separate

it from the surface (Fig. 4(b)). After lift-off the object can be moved in the x-y-plane by moving the cantilever 12 with the cantilever translating means of the AFM, accordingly (Fig. 4(c)). During this step the negative pressure in the microchannel 15 is maintained. Finally, the object 20 may be placed elsewhere by approaching the surface in z-direction and applying a positive pressure to the microchannel 15 (Fig. 4(d)). Again, the force exerted on the object 20 by the tip during this step is monitored by means of the reflected laser beam 18.

When the microscopic object 20 to be picked up is embedded in a hollow of an uneven surface 25 (Fig. 5), a cantilever 12 may be used, which comprises a high aspect ratio tip 24, e.g. in form of a hollow cylinder. The same cantilever configuration may be used to

Such tips are also able to pick up a single object from inside an object aggregation 26 (Fig. 6), e.g. tissue.

The microscopic objects 20 that can be spatially manipulated in the way described above can be living biological cells such as eukaryotic cells, yeast, bacteria or vesicles, viruses or solid material such as nanoparticles, crystals, fibers or polymeric material.

The same procedure can be applied to measure the adhesion force of any object to the surface by choosing the proper tip geometry, hole size and measur-

ing the bending of the cantilever 12 when pulling the object away from the surface, e.g. an object-surface interaction or an object-object interaction.

The same procedure can be used to remove parts of the object when the attachment force is stronger than the pulling force.

When a cantilever 12 with a sharpened tip 27 is attached to the probeholder 11 (Fig. 7), the procedure can be used to cut into an object 20 using the sharp edges of the opening either by the force produced by the negative pressure or by pushing the cantilever 12 into the object 20 (Fig. 7(a)). The cutting 28 may be removed to either gain access to the inside of the object 20 through the cut-out 29, or to use said cutting 28 for further analysis (Fig. 7(b)).

If the opening 19 (and the cross section of the microchannel 15) is larger than the object 20, one or more object(s) can be collected inside of the hollow cantilever 12 and be dispensed somewhere else (Fig. 8, Fig. 9 and Fig. 10). For example, the objects 20 can be displaced/dispensed on a surface 22 or into a recess or hole 30 (Fig. 9). Especially, the hole can be an aspiration hole or nozzle 31 connected to an analytical tool such as a mass spectrometer, PCR machine or UV/VIS/IR spectrometer, or the hole can be a reaction tube for further analysis, e.g. PCR, ELISA (Fig. 10).

Fig. 14 and 15 show photographs (differential interference contrast images) of an exemplary spatial manipulation of single viable cells. Fig. 14 exhibits five

yeast cells 33 (encircled) in an irregular configuration before the spatial manipulation with the cantilever 12. In Fig. 15, these five yeast cells 33 form a straight line after being manipulated in accordance with the present invention.

The „FluidFM" technology uses microchanneled AFM cantilevers which are fixed to an AFM probeholder. A continuous fluidic circuit is achieved connecting an external controlled pressure source with an aperture at the tip of the hollow cantilever. In this way, both an overpressure and an underpressure can be applied to the liquid inside the fluidic circuit. The invention combines a force-controlled approach with applied pressure to grasp living organisms cultured on a glass slide and displaces them with micrometric precision in a simple and reproducible way. In this way, myblasts, neurons, yeast and bacteria or other microscopic objects may be manipulated with high precision.

List of reference numerals

10	atomic force microscope (AFM)
11	probeholder
12	cantilever
13,24,27,32	tip
14	supply channel
15	microchannel
16	tubing
17	pressure source (controlled)

18	laser beam
19	opening
20,23	object (microscopic)
21	adhesion force
22,25	surface (substrate)
26	aggregation (of objects)
28	cutting
29	cut-out
30	recess
31	aspiration nozzle
33	yeast cell
34	impedance measuring means
35	electrode
d	deflection

Claims:

1. Method for spatially manipulating a microscopic object (20, 23, 33), said method comprising the steps of: providing a cantilever (12) having a tip (13, 24, 27, 32) with an opening (19) and a microchannel (15) extending through the cantilever (12) in its longitudinal direction, said microchannel (15) being fluidly connected to the opening (19) at the tip (13, 24, 27, 32) of the cantilever (12); providing suspension means (11) for holding the cantilever (12) and spatially moving the cantilever (12) along a predetermined spatial path; providing pressurizing means (14, 16, 17) for applying a predetermined pressure to the microchannel (15) within the cantilever; moving the cantilever (12) with its tip (13, 24, 27, 32) to the microscopic object (20, 23, 33) to be spatially manipulated, such that the opening (19) of the tip (13, 24, 27, 32) is adjacent to the microscopic object (20, 23, 33); picking up, with said cantilever (12), a part (28) of the microscopic object (20, 23, 33) or the microscopic object (20, 23, 33) as a whole by reducing the pressure within the microchannel (15) relative to the pressure outside the tip (13, 24, 27, 32) of the cantilever (12); and moving said part (28) of the microscopic object (20, 23, 33) or said microscopic object (20, 23, 33) as a whole along a predetermined spatial path by means of the cantilever (12).

2. Method according to claim 1, characterized in that, in a further step, said part (28) of the microscopic object (20, 23, 33) or said microscopic object (20, 23, 33) as a whole is released from the cantilever (12) by increasing the pressure within the microchannel (15) relative to the pressure outside the tip (13, 24, 27, 32) of the cantilever (12).

3. Method according to claim 1 or 2, characterized in that said part (28) of the microscopic object (20, 23, 33) or said microscopic object (20, 23, 33) as a whole is picked up by introducing it into the interior of the cantilever (12), especially into the microchannel (15) of the cantilever (12).

4. Method according to claim 2, characterized in that said part (28) of the microscopic object (20, 23, 33) or said microscopic object (20, 23, 33) as a whole is released into a recess (30) or into an opening (31) of a processing means for being processed.

5. Method according to one of the claims 1 to 4, characterized in that a cantilever (12) with an elongated tip (24) of high aspect ratio extending perpendicular to the longitudinal direction of the cantilever (12) is used to pick up one specific microscopic object (20) from an aggregation (26) of several microscopic objects (20).

6. Method according to one of the claims 1 to 4, characterized in that a cantilever (12) with a sharpened tip (27) extending perpendicular to the longi-

tudinal direction of the cantilever (12) is used to tear off a part (28) of a microscopic object (20, 23, 33).

7. Method according to one of the claims 1 to 6, characterized in that the pressurizing means (14, 16, 17) comprises a pressure source (17), which produces by means of a pump or by capillary, osmotic or electro-osmotic forces.

8. Method according to one of the claims 1 to 7, characterized in that a cantilever (12) is used, the surface of which is modified to avoid an adhesion of the microscopic object (20, 23, 33), and especially by either having hydrophilic or hydrophobic properties.

9. Method according to one of the claims 1 to 7, characterized in that, during picking up the a microscopic object (20, 23, 33), the corresponding deflection (d) of the cantilever (12), which is due to an adhesion force (21) acting between said microscopic object (20, 23, 33) and the surface (22) supporting said microscopic object (20, 23, 33), is used to calculate said adhesion force (21).

10. Method according to one of the claims 1 to 9, characterized in that, during picking up, the impedance through the opening (19) of the cantilever is measured in order to characterize the tightness of the microscopic object (20, 23, 33) closing the opening (19).

11. Method according to one of the claims 1 to 9, characterized in that the impedance through the microchannel (15) of the cantilever is measured in order to determine the number and/or mass of microscopic objects (20, 23, 33) collected in said microchannel (15) after being picked up by said cantilever (12).

12. Method according to one of the claims 1 to 9, characterized in that the cantilever (12) is sensed by means of a laser beam (18) in order to determine the number and/or mass of microscopic objects (20, 23, 33) collected in said microchannel (15) after being picked up by said cantilever (12).

13. Device for spatially manipulating a microscopic object (20, 23, 33) according to one of the claims 1 to 9, comprising: a cantilever (12) having a tip (13, 24, 27, 32) with an opening (19) and a microchannel (15) extending through the cantilever (12) in its longitudinal direction, said microchannel (15) being fluidly connected to the opening (19) at the tip (13, 24, 27, 32) of the cantilever (12); suspension means (11) for holding the cantilever (12) and spatially moving the cantilever (12) along a predetermined spatial path; and pressurizing means (14, 16, 17) for applying a predetermined pressure to the microchannel (15) within the cantilever; characterized in that said pressurizing means (14, 16, 17) is configured to generate a pressure within the microchannel (15) which is lower than the pressure outside the tip (13, 24, 27, 32) of the cantilever (12), and that an impedance measuring means

(34, 35) is provided for measuring the hydraulic impedance within the cantilever (12).

14. Device according to claim 13, characterized in that the opening (19) of the cantilever (12) is flush with the outer surface of the cantilever (12).

15. Device according to claim 13, characterized in that the cantilever (12) has an elongated, especially cylindrical, tip (24) of high aspect ratio extending perpendicular to the longitudinal direction of the cantilever (12).

16. Device according to claim 13, characterized in that the cantilever (12) has a sharpened tip (27) extending perpendicular to the longitudinal direction of the cantilever (12).

17. Device according to claim 13, characterized in that the cantilever (12) has a pyramidal tip (32) extending perpendicular to the longitudinal direction of the cantilever (12).

18. Device according to claim 13 or 14, characterized in that the microchannel (15) of the cantilever (12) is configured to receive one or more of said microscopic objects (20, 23, 33).

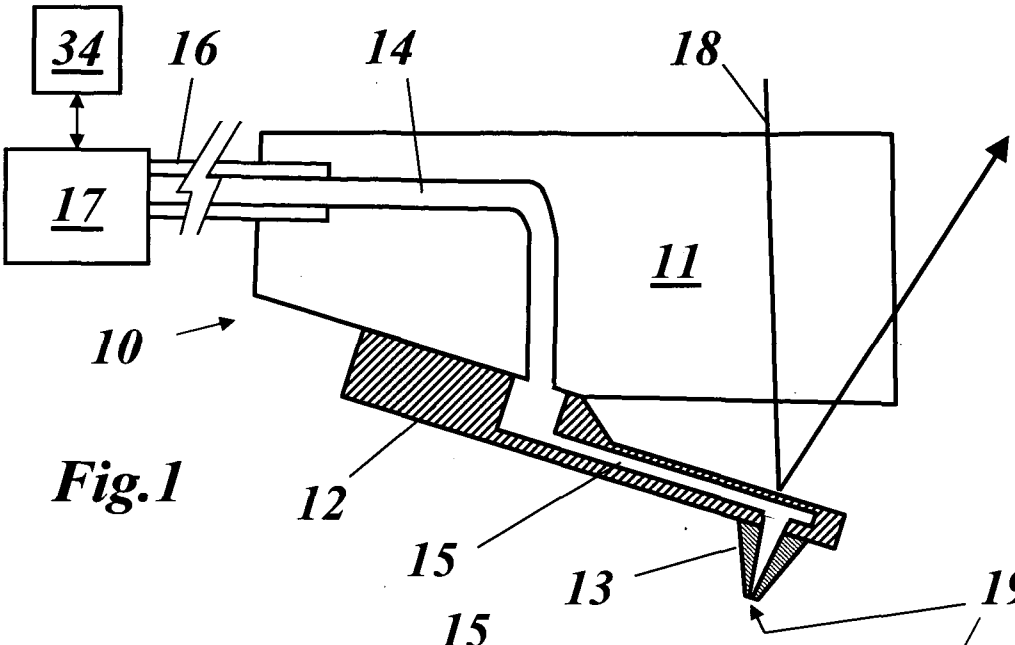


Fig.1

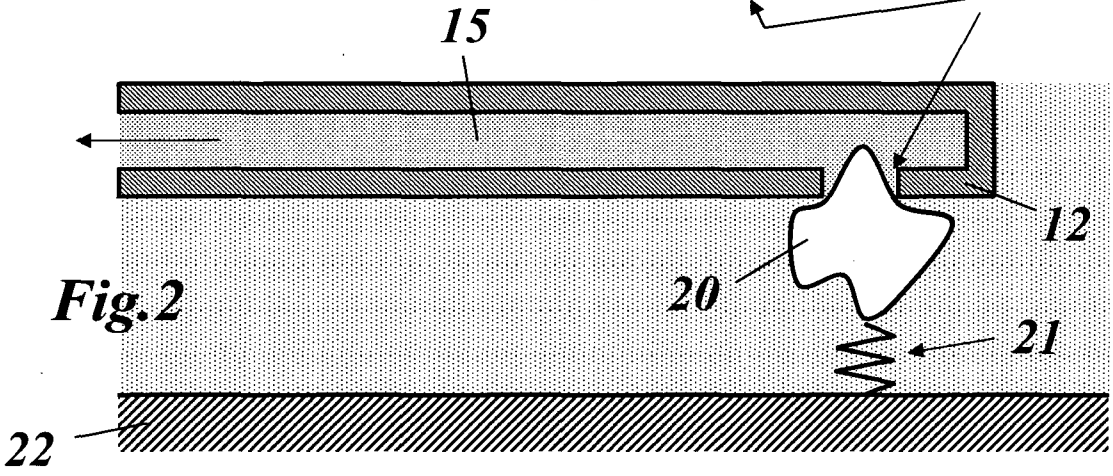


Fig.2

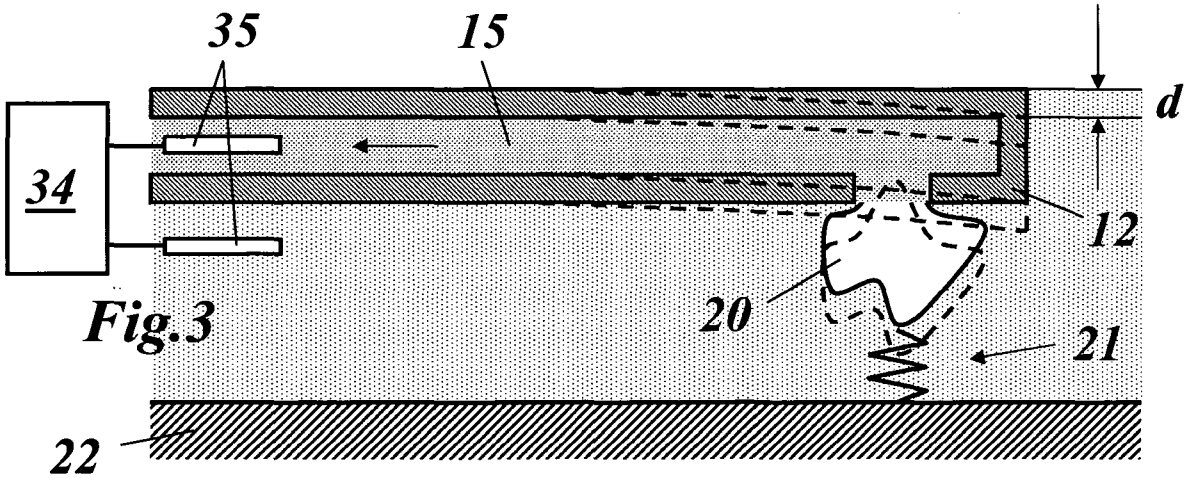
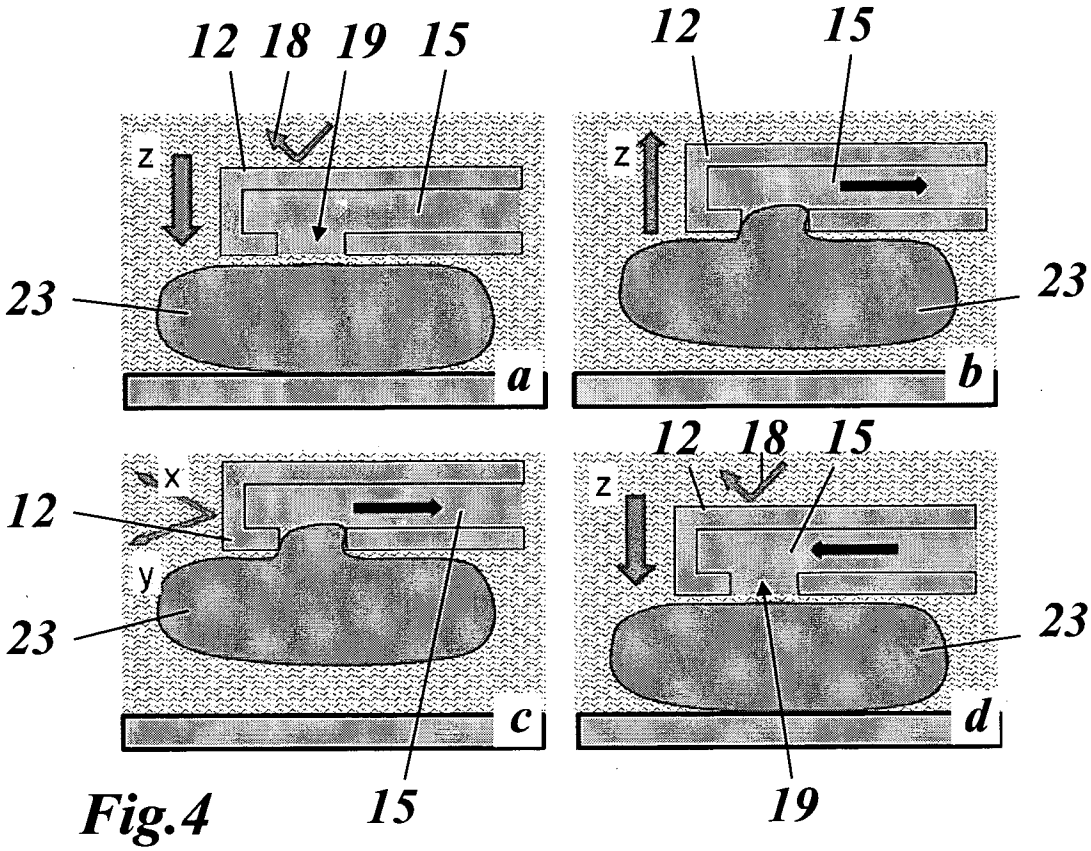


Fig.3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z
23
d
19

Fig.4

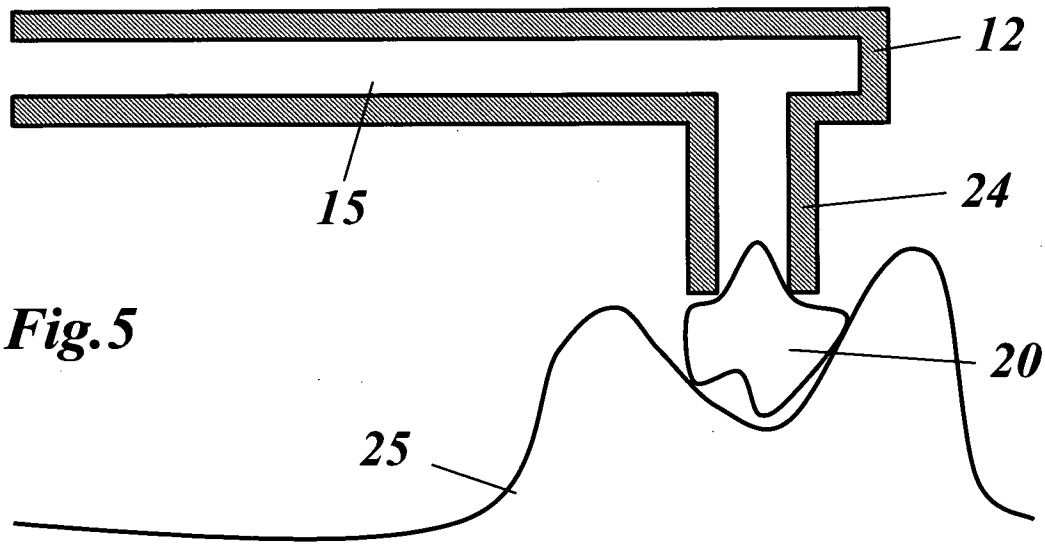
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Fig.5

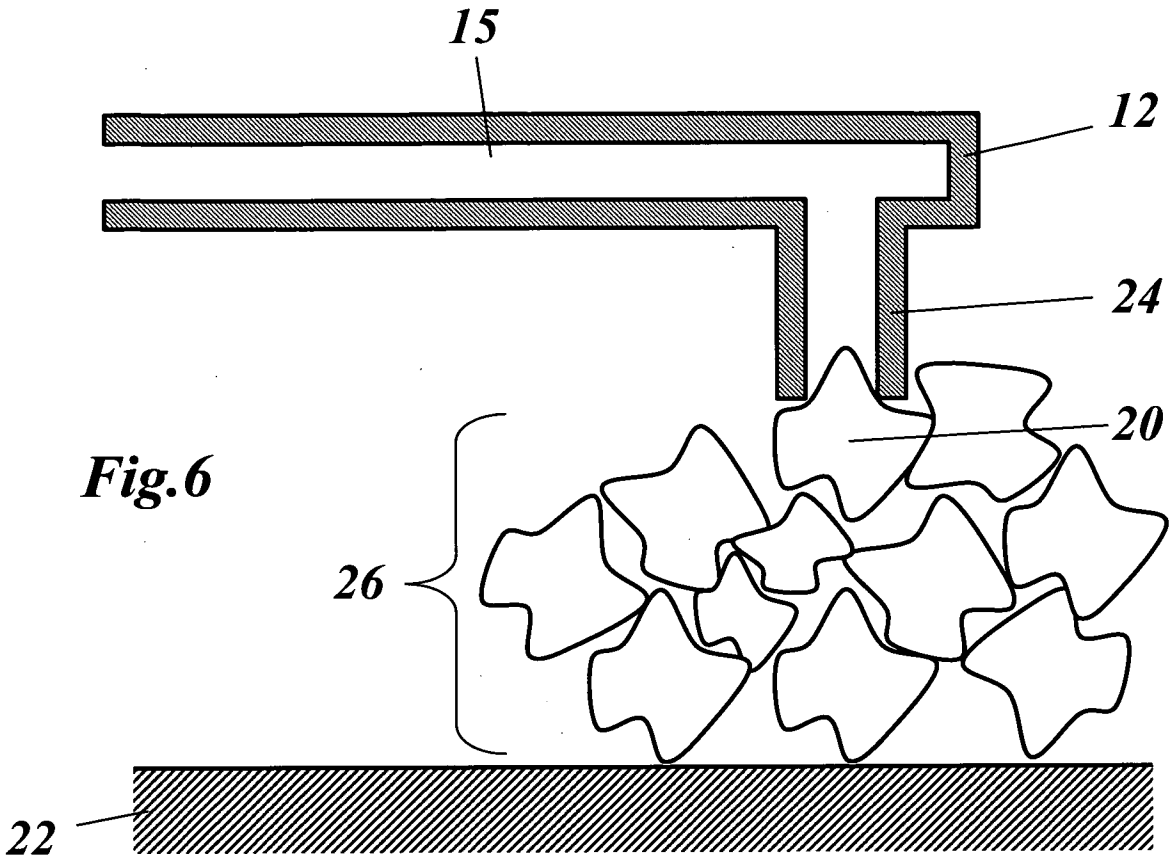

Fig.6

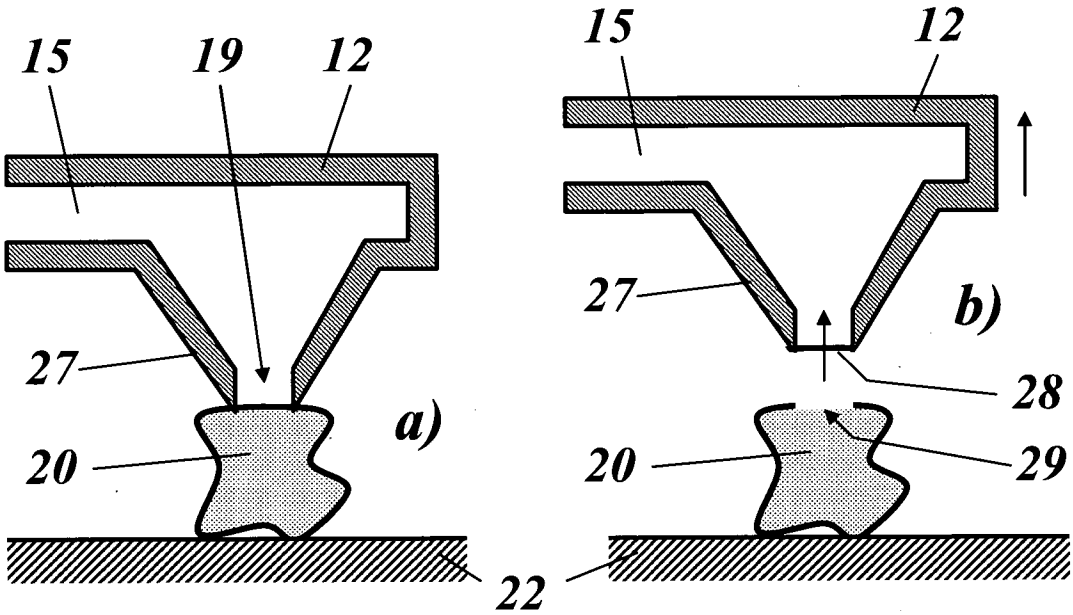

Fig.7

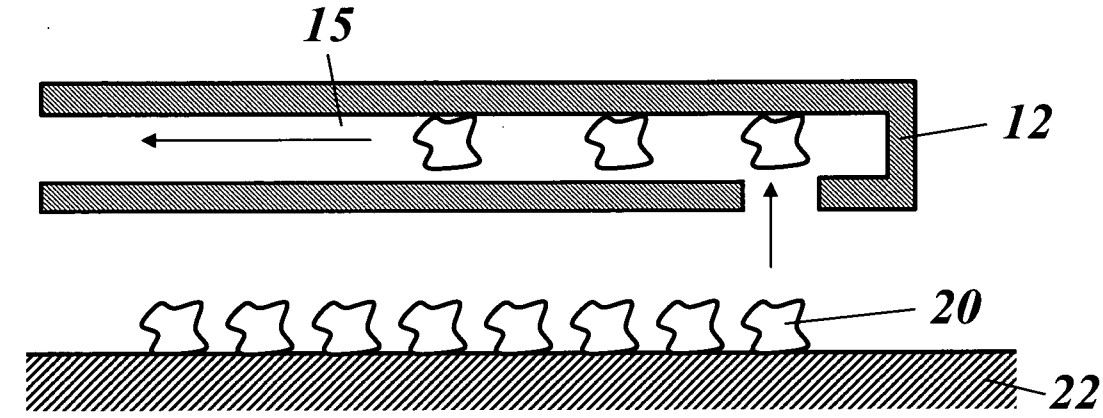

Fig.8

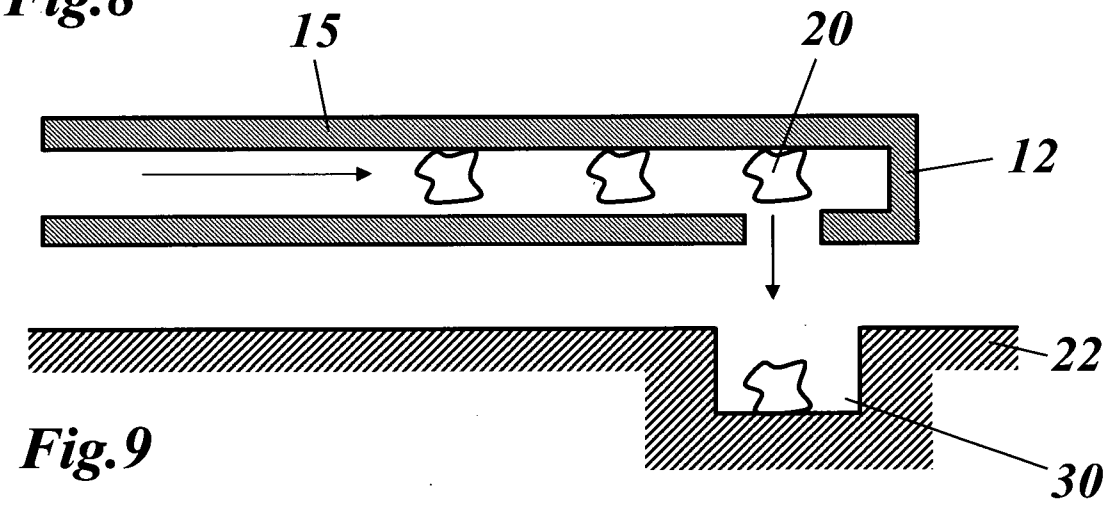

Fig.9

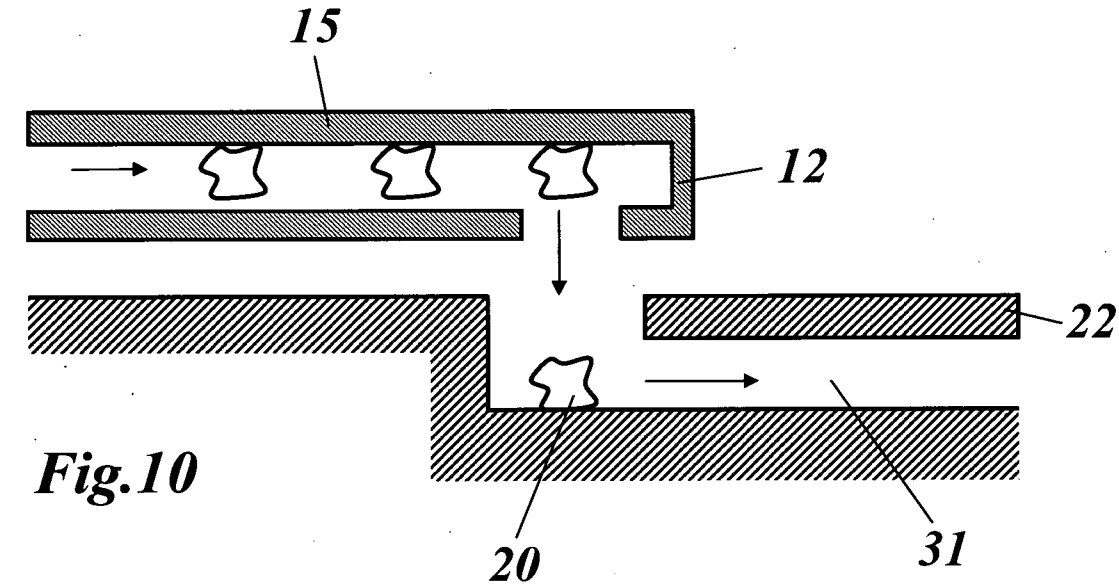

Fig.10

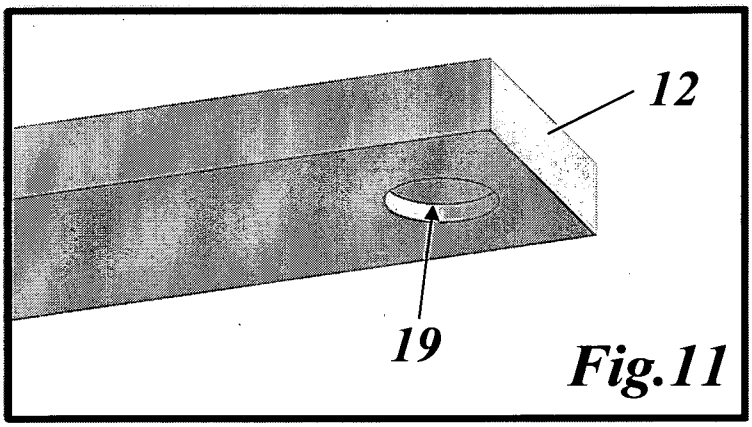

Fig.11

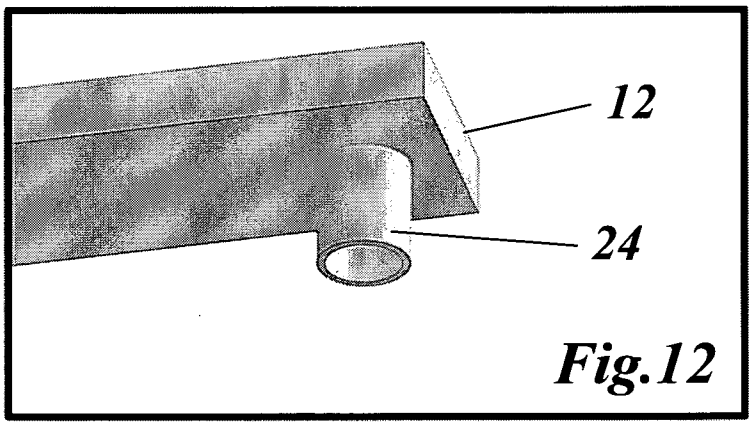

Fig.12

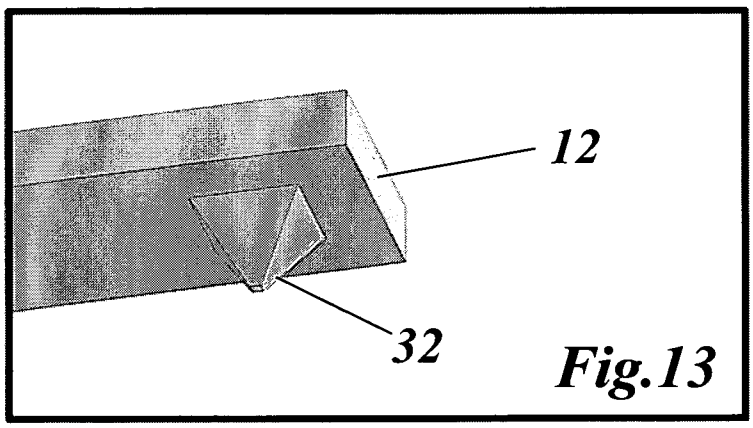

Fig.13

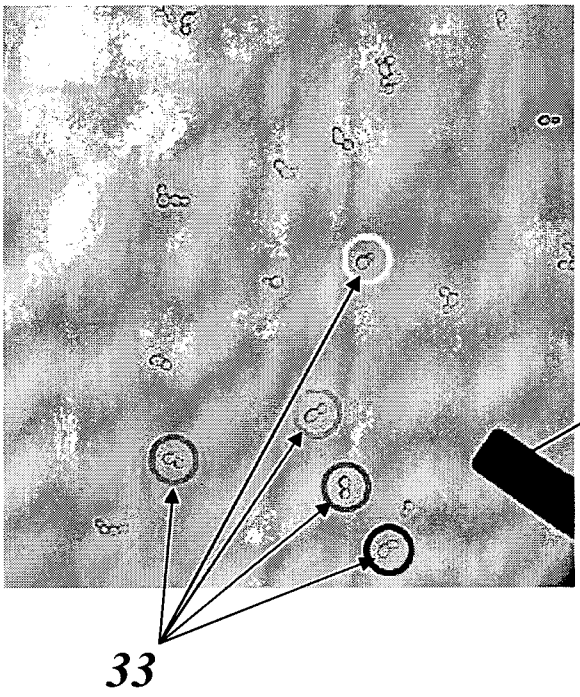

Fig.14

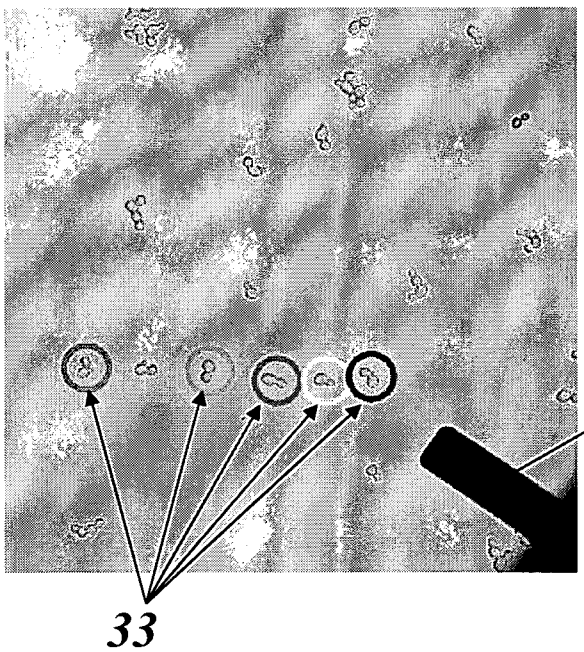

Fig.15

INTERNATIONAL SEARCH REPORT

International application No
PCT/CH2011/000020

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01Q60/38 G01Q80/00 B01L3/02 C12M1/26 C12M3/00
ADD.

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)
G01Q B01L C12M

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

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

EPO-Internal, WPI Data, IBM-TDB

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 2 083 257 A1 (HELMHOLTZ ZENTRUM MUENCHEN DEU [DE]) 29 July 2009 (2009-07-29)	1,2,4,5, 7,13,15
Y	figure 1 paragraph [0016] - paragraph [0047]	3,6,8,9, 12,14, 16-18
A		10,11

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

[X] See patent family annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 March 2011	22/03/2011

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Polesello, Paolo

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INTERNATIONAL SEARCH REPORT

International application No
PCT/CH2011/000020

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MEISTER ANDRÉ ET AL: "FluidFM: combining atomic force microscopy and nanofluidics in a universal liquid delivery system for single cell applications and beyond", NANO LETTERS, ACS, WASHINGTON, DC, US LNKD- DOI:10.1021/NL901384X, vol. 9, no. 6, 1 June 2009 (2009-06-01), pages 2501-2507, XP007910125, ISSN: 1530-6984 cited in the application figures 1,3	3,6,8,9, 12,14, 16-18

X	KATSUSHI FURUTANI ET AL: "Application of AZARASHI (seal) positioning mechanism to micromanipulation by vacuum suction", OPTOMECHATRONIC TECHNOLOGIES, 2009. ISOT 2009. INTERNATIONAL SYMPOSIUM ON, IEEE, PISCATAWAY, NJ, USA LNKD- DOI:10.1109/ISOT.2009.5326100, 21 September 2009 (2009-09-21), pages 65-70, XP031563496, ISBN: 978-1-4244-4209-6	1,2,4,7, 13,14, 16-18
A	figures 3,6 paragraphs [0III], [0IVA]	3,5,6, 8-12,15

X	WO 2010/012423 A1 (ETH ZURICH [CH]; GABI MICHAEL [CH]; VOEROES JANOS [CH]; ZAMBELLI TOMAS) 4 February 2010 (2010-02-04)	13,14, 16-18
A	figure 8 page 10, line 19 - page 11, line 11	1-12,15

X	DE 198 06 639 A1 (CREAVIS TECH & INNOVATION GMBH [DE]) 19 August 1999 (1999-08-19)	13
A	figures 3-5 column 2, line 51 - column 5, line 10	1-12, 14-16

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/CH2011/000020

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		WO 2009092495 A1	30-07-2009
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