

CLAIMS

1) A kit comprising:

- A tip (20);
- A microfluidic device (1) which is a reversed open microwell system which includes an array of open microwells (2), at least one microchannel (3), at least one input port (8) for reagents and/or for one or more biological samples and at least one output port (10) for them, said input and output ports being in microfluidic communication with one or more of said microchannels (3), wherein said microchannel (3) has a cross-section area of micrometric dimensions and provides fluid to said microwells (2);

wherein said tip (20) comprises a proximal portion (22) intended to cooperate with a fluid dispensing system and a distal portion (21), said proximal portion (22) of generally tubular configuration and said distal portion (21) open tapered wherein the terminal base (25) of said distal portion (21) has an outer diameter of dimensions d_3 , and the upper base (24) of said distal portion (21) has an outer diameter of dimensions d_4 , wherein the height of said distal portion (21) of said tip (20), i.e. the distance between said upper base (24) and said terminal base (25) of said distal portion (21) is h_2 , the half-opening of the truncated cone formed by said distal portion (21) is $(90^\circ - \beta)$ and the height of said proximal portion (22) is h_3 ; wherein said input region (8) comprises a vertical channel (18) leading into said at least one microchannel (3), the upward opening of said vertical channel (18) having a diameter of d_2 , where $d_3 < d_2$, said

vertical channel (18) is a channel preferably tapered downwards having an upper base (9) and a lower base (12), said lower base (12) having a diameter of dimensions d_1 , said vertical channel (18) having a height h_1 and the half-opening of the truncated cone formed by said tapered channel being $(90^\circ - \alpha_1)$; said tip (20) and said vertical channel (18) being dimensioned so as to produce an interference coupling therebetween.

2) A kit according to claim 1, wherein said angles β and α_1 differ from each other by a maximum of 15° , preferably of 10° or 8° , even more preferably have a difference of between 4 and 5° .

3) A kit according to one of claims 1 or 2, wherein said distal portion (21) of said tip (20) has a diameter section of dimensions d_2 at a point (28) positioned along said distal portion at a height h_x relative to the terminal base (25) of said distal portion (21), said height h_x being smaller than the distance between said upper base (9) of said vertical channel (18) and the input point in said microchannel (3), wherein said distance is h_1 in the absence of any connector, where $d_3 < d_2 < d_4$ and $(90^\circ - \alpha_1) < (90^\circ - \beta)$, preferably α_1 is of between 80° and 90° , even more preferably is equal to 90° .

4) A kit according to one of claims 1 or 2, where $d_1 < d_3 < d_2$ and $(90^\circ - \alpha_1) > (90^\circ - \beta)$ and said tip (20) is inserted in said vertical channel (18) comprised in said input region (8) by a portion (27) of length h_x .

5) A kit according to one of claims 1 to 4, wherein said input region (8) further comprises a flare portion (30) hollow truncated conical in shape, having a height h_4 and an upper base

(33) and a lower base (9) which coincides with the upper base (9) of said vertical channel (18), said upper base (33) having a diameter d_5 greater than diameter d_2 of said lower base (9), wherein the half-opening of the truncated cone forming said flare portion (30) is $(90^\circ - \alpha_2)$ where $(90^\circ - \alpha_2) > (90^\circ - \beta)$.

6) A kit according to claim 5, wherein said input region (8) further comprises, above said flare portion (30), a storage region (40) which comprises a lower portion (41) and, optionally, an upper portion (42), wherein said optional upper portion (42) has a generally tubular shape having an upper base (43) and a lower base (44) of diameter d_6 and said lower portion (41) is tapered downwards and has an upper base (44) which coincides with said lower base (44) of said optional upper portion (42) and a lower base (45) of diameter d_5 , said storage region (40) has a height h_5 and the half-opening of the truncated cone which forms said lower portion (41) is $(90^\circ - \alpha_3)$, where α_3 is smaller than or equal to 90° , preferably α_3 is 0° .

7) A kit according to one of claims 1 to 4, wherein said input region (8) further comprises a storage portion (50) which comprises a lower portion (51) and, optionally, an upper portion (52), wherein said optional upper portion (52) has a generally tubular shape having an upper base (53) and a lower base (54) of diameter d_6 and said lower portion (51) is tapered downwards and has an upper base (54) which coincides with said lower base (54) of said optional upper portion (52) and a lower base (55) of diameter d_5 , said storage region (50) has a height h_5 and the half-opening of the truncated cone which forms said storage

portion (50) is $(90^\circ - \alpha_3)$, where $(90^\circ - \alpha_3) > (90^\circ - \beta)$.

8) A kit according to one of claims 1 to 7, wherein said inlet region (8) comprises at least one connector (60), having an upper base (12) coinciding with the lower base (12) of said vertical channel (18).

9) A microfluidic device (1) which is an inverted open microwells system comprising an open microwells matrix (2), at least one microchannel (3), at least one inlet door (8) for reagents and/or for one or more biological samples and at least one outlet door (10) for the same, said inlet and outlet doors being in microfluidic communication with one or more of said microchannels (3), wherein said microchannel (3) has a cross section area of micrometric dimensions and supplies fluid to said microwells (2).

10) An **unloading region (70)** comprised in a microfluidic device (1) comprising: an unloading container (12) in fluid connection with said microfluidic device (1) by means of at least one unloading channel (22) and one outlet door (10).

11) The unloading region (70) according to claim 10, wherein said microfluidic device (1) comprises at least one microchannel (3) and said unloading channel (22) emerges from said at least one microchannel (3) and is a siphon.

12) An unloading region (70) according to claim 10 or 11, characterized in that the diameter of said unloading channel (22) is such that the siphon exerts a capillary force on the fluid contained in said microchannel (3).

13) An unloading region (70) according to one of claims 10 to 12, wherein said unloading channel (22) is placed on the bottom

of said at least one microchannel (3) and is fairly orthogonal thereto and connects said at least one microchannel (3) with said unloading container (12) which is positioned below the microchannel (3) itself.

14) An unloading region (70) according to one of claims 10 to 12, wherein said outlet door (10) is placed on the bottom of said at least one microchannel (3) and enters into a first unloading container (12a), positioned below said microchannel (3), and from said first unloading container (12a) said unloading channel (22) emerges, which enters into said unloading container (12).

15) An unloading region (70) according to one of claims 10 to 14, wherein said unloading channel (22) has a cross section area of micrometric dimensions, said dimensions being comprised between 100 μm and 5 mm, preferably between 500 μm and 2 mm.

16) A method for loading/unloading fluids from a microfluidic device wherein said method comprises:

- a) making available a microfluidic device (1) comprising an inlet region (8) and an unloading region (70) according to one of claims 10 to 15, wherein said microfluidic device (1) is loaded with at least one first fluid;
- b) optionally, exerting a pressure on said at least one first fluid inlet in said microfluidic device (1);
- c) alternatively, having available an unloading region (70) wherein said unloading channel (22) has a diameter such as to ensure that said fluid can pass from said microfluidic device to said unloading channel by capillarity;

characterized in that, where the volume V of said at least one first fluid is inferior or equal to the volume of said unloading container (12), said at least one fluid reaches said unloading container (12) in a unidirectional manner.

17) A method for loading/unloading fluids according to claim 16, wherein said method further comprises:

- entering a second or further fluid by means of said inlet region (8) into said microfluidic device (1), where said first, second and/or further fluids are independently equal or different one another and are selected from the group comprising liquids and gases;
- optionally, said second and/or further fluid completely replace in said microchannel (3) or in said microchannel (3) and in said inverted open microwells (2), where present in said microfluidic device (1), the fluid entered earlier;

characterized in that said first, second and/or further fluid do not mix one another.

18) A method for the large-scale high-content analysis of biological samples, comprising the following steps, not necessarily in this order:

- a) Providing a reversed open microwell system (1) which includes an array of open microwells (2), at least one microchannel (3), at least one input port (8) for reagents and/or for one or more biological samples and at least one output port (10) for them, said input and output ports being in microfluidic communication with one or more of said microchannels (3), wherein said microchannel (3) has a

cross-section area of micrometric dimensions and provides fluid to said microwells (2);

b) Providing an automated management system of said reversed open microwell system which comprises the following features: incubator at controlled temperature, humidity and CO₂, fluid dispensing system, phase-contrast and fluorescence image acquisition;

c) Placing said reversed open microwell system (1) in said automated system;

d) Charging reagents through one or more of said input ports (8), wherein said reagents comprise: filling buffer and/or washing solution and/or one or more drugs and/or one or more dyes, and/or one or more labeled antibodies and or one or more cell viability markers;

e) Charging said one or more biological samples through one or more of said input ports (8);

i) Optionally, dyeing said cells with one or more dyes and/or one or more labeled antibodies and or one or more cell viability markers;

j) Acquiring images from one or more of said microwells (2), wherein said images are defined images T0;

p) Classifying the cells displayed, wherein said classification is made with morphological and/or functional parameters detected from the images T0.

19) A high-content analysis method according to claim 18, wherein said reagents and said at least one biological sample are contained into reservoirs, reservoirs (6) for reagents and reservoirs (7) for biological sample, respectively.

20) A high-content analysis method according to claim 19, wherein said reservoirs for reagents (6) and for biological sample (7) are integrated in said open reversed microwell system (1) and said reagents and said one or more biological sample are preloaded into said reservoirs (6) for reagents and (7) for biological sample, respectively, before of said step c) of introduction and one or more of said step d) and e) of loading of said reagents and of said one or more biological sample through one or more of said input ports (8) are made after said step c) of introduction of said reversed open microwell system (1) in said automated system, by automated pick up of the reagents from said reservoirs (6) and of said biological sample from said reservoirs (7), and consequent arrangement of the cells contained therein in one or more of said microwells (2).

21) A high-content analysis method according to claim 19, wherein said reservoirs for reagents (6) and for biological sample (7) are external to said open reversed microwell system (1) and said reagents and said one or more biological sample are preloaded into said reservoirs (6) and (7) and said reservoirs (6) and (7) are inserted into said step c) of introduction of said reversed open microwell system (1) and one or more of said step d) and e) of loading of said reagents and of said one or more biological sample through one or more of said input ports (8) are made after said step c) of introduction of said reversed open microwell system (1) in said automated system, by automated pick up of the reagents from said reservoirs (6) and of said biological sample from said reservoirs (7), and consequent arrangement of the cells contained therein in one or more of

said microwells (2).

22) High-content analysis method according to one of claims 18 to 21, also comprising a biological sample preparation prior to loading the same in one or more of said biological sample reservoirs (7).

23) High-content analysis method according to one of claims 18 to 22, also comprising, after the introduction of said reversed open microwell system in said automated system of step c):

f) Filling at least one said microchannel (3) with filling buffer;

g) Acquiring images from one or more of said microwells (2), either individually or by subgroups, wherein said images are defined baseline images;

and, in said classification step p), said classification is made with morphological and/or functional parameters detected by the comparison of images T0 with baseline images.

24) Method according to one of claims 18 to 23, wherein said method also comprises:

k) Dispensing one or more of said drugs, through said ports (8), in one or more of said microwells (2);

l) Incubating.

25) Method according to one of claims 18 to 24, wherein said method also comprises, after said step l):

m) Acquiring at least two images from said one or more microwells (2), at different times during said incubation, wherein said images are defined images T1, T2, Tn, wherein n is any number equal to or greater than 2, preferably 1,000 or 100, even more preferably 25, in a preferred embodiment is 9;

n) Optionally, between said acquisitions of said images T1, T2, Tn, further dyeing said cells with one or more dyes and/or one or more labeled antibodies and or one or more cell viability markers;

o) Optionally, between said acquisitions of said images T1, T2, Tn, further dispensing of one or more of said drugs in a microwell (2) or in one or more subgroups of microwells (2), individually or in combination;

and said classification of step p) comprises the comparison of morphological and/or functional parameters detected from images T0, T1, Tn and optionally Tbaseline.

26) Method according to one of claims 18 to 25, wherein said method also comprises, after said classification step p):

q) Analysis of the cell viability, where said analysis is cell-specific, or is carried out at the level of cell aggregate, or takes into account the whole cell population contained in one of said microwells (2).

27) Method according to claim 24, wherein in said step k), said one or more drugs are dispensed in at least two different concentrations, and when the cell viability is analyzed according to step q), a dose/response curve is obtained that is cell-specific for one or more drugs tested.

28) Method according to one of claims 24 to 27, wherein the cells that survive the exposure to one or more drugs in said step k) are exposed again to a further treatment with one or more drugs, wherein said one or more drugs are the same as already used in said step k) at different concentration, or are different from those used in said step k).

29) Method according to one of claims 18 to 28, wherein said biological sample comprises about 10-20 cells.

30) Method according to one of claims 18 to 29, wherein said biological sample is obtained from an animal and/or a human suffering from cancer and said biological sample comprises healthy cells and/or cancer cells.

31) Method according to one of claims 18 to 30, wherein said biological sample is loaded through said input ports (8), or preloaded in said reservoirs (7) in the manner in which it is collected/removed.

32) Method according to one of claims 18 to 31, wherein said biological sample is blood and is loaded through these said input ports (8), or preloaded in said reservoirs (7), immediately after collection, wherein said blood is treated with a lysis buffer once introduced in said microwells (2).

33) Method according to one of claims 18 to 32, wherein, at the end of said method, the surviving cells are harvested and used in subsequent analysis.

34) Method according to one of claims 18 to 33, comprising the following steps, in the order shown:

a) Providing a reversed open microwell system (1) which includes an array of open microwells (2), at least one microchannel (3), at least one input port (8) for reagents and/or one or more biological samples and at least one output port (10) for the same, said input and output ports being in microfluidic communication with one or more of said microchannels (3), wherein said microchannel (3) has an area in cross-section of micrometric dimensions and provides fluid

to said microwells (2);

b) Providing an automated management system of said reversed open microwell system comprising the following features: incubator at controlled temperature, humidity and CO₂, fluid dispensing system, phase contrast and fluorescence image acquisition;

c) Placing said reversed open microwell system (1) in said automated system;

f) Optionally, filling at least one of said microchannel (3) with filling buffer;

g) Optionally, acquiring images from one or more of said microwells (2), either individually or by subgroups, wherein said images are defined baseline images;

d) Loading the reagents through one or more of said input ports (8), wherein said reagents comprise: filling buffer and/or washing solution and/or one or more drugs and/or one or more dyes, and/or one or more labeled antibodies and or one or more cell viability markers and wherein said reagents are contained in reagent reservoirs (6);

e) Loading said one or more biological samples through one or more of those said input ports (8), wherein said one or more biological samples are contained in biological sample reservoirs (7);

i) Optionally, dyeing said cells with one or more dyes and/or one or more labeled antibodies and or one or more cell viability markers;

j) Acquiring images from one or more of said microwells (2), wherein said images are defined images T₀;

- k) Dispensing one or more of said drugs, through said ports (8), in one or more of said microwells (2);
- l) Incubating;
- m) Acquiring at least two images from said one or more microwells (2), at different times during said incubation, wherein said images are defined images T1, T2, Tn, wherein n is any number equal to or greater than 2, preferably 1,000 or 100, even more preferably 25, in a preferred embodiment is 9;
- n) Optionally, between said acquisitions of said images T1, T2, Tn, further dyeing said cells with one or more dyes and/or one or more labeled antibodies and or one or more cell viability markers;
- o) Optionally, between said acquisitions of said images T1, T2, Tn, further dispensing of one or more of said drugs in a microwell (2) or in one or more subgroups of microwells (2), individually or in combination;
- p) Classifying the cells displayed, wherein said classification is made with morphological and/or functional parameters detected from the images T0, T1, Tn and, optionally, Tbaseline.

35) The microfluidic device according to claim 9 comprising also an unloading region (70) according to claim 10.

36) Kit according to one of the claims 1 to 8, wherein said microfluidic device (19) is the microfluidic device according to one of the claims 9 or 35.

37) Method for uploading/unloading fluids wherein said method comprises:

- a) Making a kit available according to one of claims 1 to 8 or

36;

- b) Optionally, loading in said tip (20) a fluid;
- c) Positioning said tip (20) above said inlet region (8) and insert it until reaching an interference fitting position between said distal region (21) of said tip (20) and said vertical channel (18) in said inlet region (8);
- d) Releasing the fluid contained in said tip (20) in said microfluidic device (1) by means of said inlet region (8) or, alternatively, sucking with the tip itself the fluid already contained in said microfluidic device.

38) A method according to claim 37, wherein said kit is the kit according to claim 36 and said method further comprises that said fluid, driven by an applied pressure in said inlet region (8) in said microfluidic device (1), reaches in a unidirectional manner said unloading container (12) where the volume V of said fluid is less than or equal to the volume of said unloading container (12).

39) A high-content analysis method according to one of claims 18 to 34, wherein said method also comprises:

- Providing a kit according to one of claims 1 to 8 or 36;
- Optionally, charging a fluid into said tip (20);
- Positioning said tip (20) above said input region (8) and inserting it up to reaching an interference coupling position between said distal region (21) of said tip (20) and said vertical channel (18) in said input region (8);
- Releasing the fluid contained in said tip (20) in said microfluidic device (1) through said input region (8) or, alternatively, with the same tip suctioning fluid already

contained in said microfluidic device.

40) A high-content analysis method according to claim 39, wherein said kit is the kit according to claim 36 and said method further comprises said fluid, pushed by a pressure applied in said input region (8) in said microfluidic device (1), unidirectionally reaches said discharge container (12) where volume V of said fluid is smaller than or equal to the volume of said discharge container (12).