

"METHOD AND DEVICE FOR OPTICAL ANALYSIS OF PARTICLES AT LOW TEMPERATURES"

5 Technical field

The present invention concerns methods and devices for the manipulation of particles in suspension in a fluid, for example contained in conductive or highly conductive solutions, when optical analysis of the manipulated particles
10 has to be performed at temperatures below ambient temperature. The invention can be applied mainly in the implementation of biological protocols on live cells.

State of the art

15 The patent application PCT/WO 00/69565 to G. Medoro describes a device and a method for the manipulation of particles via the use of closed dielectrophoretic potential cages. The force used to maintain the particles in suspension or to move them inside the microchamber dissipates, by Joule effect, a power
20 which is proportional to the square of the amplitude of the voltages applied and grows linearly with the increase in the electrical conductivity of the liquid in suspension, causing an uncontrolled increase in temperature inside the microchamber. The manipulation operations can be individually
25 controlled by the programming of memory elements and circuits associated with each element of an array of electrodes integrated in the same substrate; said circuits contribute to the temperature increase, dissipating power in the substrate in direct contact with the suspension liquid. This results in
30 an important limitation due to the variation in the gene expression or to high levels of stress or to the death of the biological particles present in the sample for solutions with high electrical conductivity, limiting the application of these methods and devices to the use of beads or non-live
35 cells.

The limitations of the known art are overcome by the patent application EP1945368 in the name of the same Applicant, which allows the manipulation of biological particles by means of the technique described in PCT/WO 00/69565 (or by means of other techniques that develop heat) preserving the vitality and biological functions of the cells independently of the forces used and/or the conductivity of the liquid in suspension, therefore allowing the manipulation of live cells.

However, numerous applications require the suspension liquid and/or all the interior of the microchamber to be maintained, during the manipulation procedure, at temperatures far below the ambient temperature, for example at temperatures below 10°C and, more frequently, between 3 and 5°C, for example 4°C.

At such low temperatures ambient humidity condenses on the outer surface of the microchamber cover, which is made of transparent material in order to permit observation and optical analysis of the cells in suspension, either by means of devices or sensors outside the microchamber, for example an optical microscope, or by means of optical sensors inside the microchamber, integrated in the substrate, which, however, require adequate external lighting in order to function correctly.

The presence of condensed humidity on the microchamber cover causes both blurring of the image that can be acquired from the outside and alteration in the passage of the external light, preventing correct performance of the analysis protocols, unless non-optical internal sensors are relied upon completely, for example impedentiometric sensors, but this is not always possible or convenient.

Subject of the invention

The present invention concerns a method and device for performing the optical analysis of particles contained in

suspension in a fluid, typically a liquid, arranged inside a microfluidic device which maintains it at a temperature significantly lower than the ambient temperature and such as to trigger phenomena of condensation on the outer surface of the microfluidic device. Typically, the microfluidic device serves to perform manipulation and/or control of the position of the particles, for example by means of electrical force fields in electrically conductive solutions, and more generally by means of any other system, in conditions that simultaneously require a relatively low operating temperature and performance of an optical analysis of the position and/or appearance of the manipulated particles, or for the detection of morphological parameters or for the quantification of fluorescence intensity. The force fields can be dielectrophoresis (positive or negative), magnetophoresis, electrophoresis, electrohydro-dynamic or electrowetting on dielectric, or combinations of these phenomena, characterised by a set of points of stable equilibrium for the particles. Optical tweezers can also be used.

The main aspect of the invention concerns prevention of the condensation of humidity on the outer surface of the cover of a microfluidic device operating with a relatively low internal temperature either by increase in the temperature of the outer surface of the cover to above the condensation temperature (dew point), or by lowering the temperature and/or ambient humidity in the vicinity of the cover of the microfluidic device, so as to bring the condensation temperature (dew point) to below the internal operating temperature of the microfluidic device.

For said purpose, the system can benefit from the use of one or more integrated or external sensors for control of the temperature and, if necessary, of the ambient humidity and temperature of the outer surface of the cover by means of a feedback control.

The invention furthermore allows the use of external optical systems of the transmission type.

5 Brief description of the figures

Figures 1A and 1B illustrate schematically in longitudinal section two different embodiments of a microfluidic device which implements a first embodiment of the method of the invention;

10 Figure 2 illustrates schematically an overhead plan view of an embodiment of the microfluidic device of figure 1;

Figure 3 illustrates schematically on an enlarged scale a vertical section of the microfluidic device of figure 2;

15 Figure 4 is a diagram showing the variation in dew point according to the humidity and temperature of the air;

Figure 5 illustrates schematically in longitudinal section a microfluidic device which implements a second embodiment of the method of the invention; and

20 Figures 6 and 7 are diagrams showing the variation in temperature of the upper surface of the microfluidic device of figure 2 on the basis of the variation of some operating parameters.

Detailed disclosure

25 Hereinbelow, the term particles will be used to indicate micrometric or nanometric entities, natural or artificial, such as cells, subcellular components, viruses, liposomes, niosomes, microbeads and nanobeads, or also smaller entities such as macro-molecules, proteins, DNA, RNA, etc., also drops
30 of liquid immiscible in the suspension medium, for example oil in water, or water in oil, or also drops of liquid in gas (such as water in air) or bubbles of gas in liquid (such as air in water).

35 The object of the present invention is to provide a method and a device for the optical analysis of particles at temperatures

below ambient temperature and, in particular, at relatively low temperatures (3-6°C), the particles being maintained in suspension in a fluid, typically a liquid, arranged inside a microfluidic device, which also allows manipulation of the particles.

By manipulation we mean control of the position of single particles or groups of particles or the movement in space of said particles or groups of particles.

Said manipulation can be performed by any means integrated in the device according to the invention or interacting with it from the outside. Typically the manipulation is performed by means of an array of electrodes, which can be selectively activated and addressed, integrated in a substrate and facing one single counter-electrode which also acts as a cover of the microchamber, according to the description in PCT/WO 00/69565, the content of which is incorporated herein for the necessary parts.

With reference to the figures 1A,B and 2, the reference number 1a, respectively 1b, indicates a device for performing optical analysis of particles 2 contained in suspension in a fluid 3, typically a liquid, capable of operating by maintaining the particles 2 and the liquid 3 at temperatures below ambient temperature and, in particular, near to zero degrees centigrade, typically temperatures between 3 and 6°C and preferably at a temperature of approximately 4°C.

The device 1a,1b comprises, according to the outlined schematic sketches illustrated in figure 1A and 1B, where similar or identical details are indicated by the same reference numbers for the sake of simplicity, at least one microchamber 4 containing in use the fluid 3 and delimited between a first surface 5 and a second surface 6; and cooling means, indicated overall by 7, thermally coupled with the

surface 5 by means of a first thermal resistance RLW.

By the term "microchamber" we mean here and below a chamber suitable for containing a small volume of fluid 3, typically
5 between 1 nanolitre and 5000 microlitres, and preferably between 1 microlitre and 100 microlitres and having one of its three dimensions measuring less than 1 mm.

The device 1 furthermore comprises a thermal inspection
10 surface 8 thermally coupled with the surface 6 via a second thermal resistance RHI.

The cooling means 7 can be of any appropriate type adapted to subtract heat from the microchamber 4 while the device 1a/1b
15 is operative and performs manipulation of the particles 2, in a quantity such as to maintain the fluid 3 at a first pre-set temperature T_1 , below the ambient temperature, as already indicated typically 4°C.

20 In the example illustrated in figure 1B, the cooling means 7 consist of a plate 10 arranged in contact with the thermal resistance RLW and in which one or more Peltier cells 11 are integrated (only one of which is shown only schematically, since it is known per se) controlled by a device 12 for
25 controlling the internal temperature of the chamber 4, only schematically represented by a block.

In the example illustrated in figure 1A, on the other hand, the cooling means 7 consist of a second microchamber 10c, in
30 which a flow of coolant 11c runs, in contact with the thermal resistance RLW; said coolant is indicated by an arrow in figure 1A and is circulated in a closed circuit from which the heat removed from the microchamber 4 through the surface 5 is continuously eliminated by means of a pump 12c coupled with a
35 Peltier cell, 13c, indicated schematically by a block.

With reference also to figures 2 and 3, the thermal resistance RLW consists of a flat sheet 14, for example a quartz sheet, transparent if necessary, a first upper face of which consists of the surface 5 and a second lower face of which consists of a surface 15 arranged in contact with the plate 10; the thermal resistance RHI consists of a flat sheet 16, necessarily made of a transparent material, for example consisting of a sheet of mineral glass or quartz, a first face of which, facing the side opposite the microchamber 4, constitutes the optical inspection surface 8, and a second face of which, opposite the first face, constitutes the surface 6.

The two sheets 14,16 are arranged facing and are separated from each other by a perimeter spacer 18 (indicated schematically only by a line in figure 1), which delimits together with the sheets 14,16 the inner volume of the microchamber 4. Either the latter, or the whole unit consisting of the sheets 14,16 and the spacer 18, rests on a base 19 (figure 3).

The device 1a/1b according to the invention also comprises electronic means for manipulating the particles 2 before, during and after optical analysis of the same.

If dielectrophoresis potential cages, as described in WO 00/69565, are used to manipulate the particles 2, the surface 5 constitutes the substrate which supports an array 100 of microelectrodes, while the surface 6 is entirely coated by an ITO layer 101, which constitutes the counter-electrode. In addition to the microelectrodes of the array 100, one or more optical sensors can be integrated in the substrate to detect any alterations (due for example to the presence of a particle 2) of the ambient light which penetrates into the microchamber 4 through the surface 8. Alternatively or in addition, optical sensors can be provided outside the microchamber 4, for

example consisting of a simple microscope 21 of any type, indicated schematically by a block in figure 1A and 1B, which observes the inside of the microchamber 4 through the surface 8. The microscope 21 can identify images and receive any light reflected from the substrate consisting of the surface 5 for example according to the trajectories indicated by the arrows in figure 1A, 1B.

According to a first embodiment of the invention, the second thermal resistance RHI has a thermal conductivity value of at least one order of magnitude and, preferably, two orders of magnitude, below that of the first thermal resistance RLW; for example, with the materials indicated above, the sheet 14 has a conductivity of approximately $150 \text{ W/}^\circ\text{K}\cdot\text{m}$ while the sheet 16 has a thermal conductivity of only approximately $1.2 \text{ W/}^\circ\text{K}\cdot\text{m}$.

In combination with this characteristic, the device 1 furthermore comprises, according to the invention, means 24, indicated schematically by a block in figure 1, to establish a thermal flow F (indicated schematically by a double arrow in figure 1) at the optical inspection surface 8 such that the surface 8 is constantly maintained at a temperature T_2 higher than the temperature T_d of condensation of the ambient humidity (dew point) contained in the air which laps in use the optical inspection surface 8.

As illustrated in the diagram of figure 4, the temperature T_d can be easily calculated according to the temperature of the ambient air and the amount of humidity present in the ambient air. For mean values usually found in a laboratory (23°C and 50% humidity), the temperature T_d is approximately 12°C . It is evident that since the temperature T_1 in the microchamber 4 is approximately 4°C , the temperature of the surface 8 would inevitably reach, in use, by conduction, a temperature lower than T_d , producing blurring of the surface 8.

This phenomenon is avoided according to the invention by the combination of an appropriate choice of the ratio between the thermal conductivities of the thermal resistances RLW and RHI and the simultaneous presence of the means 20. This combination surprisingly allows, on the one hand, as will be seen, a temperature T2 of the surface 8 to be maintained always higher than the temperature Td of the air that laps in use the surface 8, so as to avoid and/or eliminate blurring of the surface 8 when the temperature T1 inside the microchamber 4 is very low. On the other hand, possible heating of the fluid 3 is avoided or at least limited. In fact it has been experimentally shown that even if a "hot" thermal flow reaches the microchamber 4, any increase in the temperature T1 can be easily avoided by lowering the temperature set by the control unit 12 on the Peltier cell 11 without triggering undesired convective motions inside the microchamber 4.

According to a first possible embodiment of the invention, the device 1b (or 1a) comprises means 24b (figures 2,3) to heat the optical inspection surface 8 to above the dew point of the ambient air, or above the temperature Td.

Said means 24b consist in a resistor directly applied integrally in one piece on the surface 8 of the sheet 16. According to an embodiment example illustrated only schematically and only partly in figure 1, the resistor 24b consists of a transparent conductive resistive layer 25, for example ITO, applied uniformly over the whole optical inspection surface 8.

In a second possible embodiment of the invention, the resistor 24b consists of at least one and preferably a plurality of filiform microresistors, or wires, 26 applied integrally in one piece to the optical inspection surface 8, preferably arranged in a comb shape and uniformly spaced from one another.

The filiform microresistors 26 are electrically connected, each at the same one end thereof, to a distribution frame 27 of the electrical supply current, consisting of a metal foil in a comb shape, but arranged opposite the filiform microresistors 26; in other words, respective "teeth" 28 of the comb-shaped metal foil 27 face the side opposite the wires 26 and extend outside the surface 8, being arranged on one edge 29 of the device 1 outside the operating area consisting of the microchamber 4.

The frame 27 receives the electrical supply current through the "teeth" 28, by means of a plurality of conductor bridges 30 which connect a plurality of different points of the frame 27, consisting of the "teeth" 28, to at least one common collector 31 arranged at the base element 19 of the device 1.

The conductor bridges 30 consist of deformable metal wires bent to form S-shaped frames or forks on the plane of figure 3. The electrical supply of the electrodes and any optical sensors in the substrate that may be present on the surface 5 is also provided by bridges 30, using other collectors 32,33.

To allow the device 1b (1a) to operate correctly, i.e. to maintain the temperature T_2 above the temperature T_d of the air that laps in use the surface 8 without excessive heat loss towards the microchamber 4, to maintain the temperature of the entire surface 8 as uniform as possible and not interfere with the integrated optical sensors and/or with the external sensors, like the microscope 21, the filiform microresistors 26 must have a width L (figure 3) equal to approximately one tenth of the pitch P (i.e. the spacing) between the same in a direction transverse to their longitudinal extension. In other words, the L/D ratio must be preferably equal to $1/10$ and in any case be between $1/2$ and $1/100$.

To improve the performance of the device 1b (1a), it preferably also comprises means 35 (figure 2) to continuously measure in use the temperature T_2 of the surface 8 and means 36 to operate in feedback the means 24b to heat the optical inspection surface; for example the temperature detection means 35 can consist of an electrical resistance applied on the surface 8, which varies its resistivity according to the temperature T_2 , or of an optical sensor arranged facing the surface 8; in the first case, one of the filiform microresistors 26 can be used as the electrical resistance, making the appropriate electrical connections. The means 36 consist of the microprocessor which controls all the functions of the device 1b (1a) or of a dedicated microprocessor, and they interact for example with a power supply unit 37 (figure 3) which controls the current supplied to the frame 27 via the bridges 30. There must be a large number of the latter, so that the spacing intervals there between make the temperature on the opposite side of the sheet 16 substantially uniform.

Observing the parameters indicated, the trend of the temperature gradient through the thickness of the sheet 16 for different levels of electrical power supplied to the wires or "fingers" 26 is the one shown in figure 6, which represents the worst scenario, in other words in the middle of the pitch P , i.e. the temperature at the surface area 8 without resistors comprised between two filiform microresistors or "fingers" 26, and in figure 7, which shows the best scenario, i.e. the temperature directly below a microresistor or "finger" 26. The temperature gradient that can be maintained through the sheet 16, taking account of the heat losses towards the outside, allows a temperature very close to T_1 to be maintained on the surface 6, while the temperature T_2 on the surface 8 is uniformly higher than the temperature T_d .

For this purpose, and independently of the embodiment of the heating means 24b, it is also convenient for the thickness of

the sheet 16 to be determined at the project stage using the following formula:

$$H_{lid} > \left(\frac{T_{dp} - T_0 - \Delta T_{max}}{\Delta T_{max}} \right) \left(\frac{\sigma_{lid}}{\sigma_{buf}} \right) H_c \quad (1)$$

$$\Delta T_{max} = (T_{buf} - T_0)_{max} \quad (2)$$

where H_{lid} and H_c are respectively the thickness of the sheet 16 constituting the cover of the microchamber 4 and the thickness of the microchamber 4 itself, T_{dp} is the dew point temperature of the ambient air, T_0 is the theoretical operating temperature desired inside the microchamber 4, T_{buf} coincides with the temperature T_1 , i.e. it is the temperature of the liquid 3, ΔT_{max} is the maximum increase in temperature tolerable inside the microchamber 4 with respect to the theoretical operating temperature T_0 , σ_{lid} and σ_{buf} are the thermal conductivity of the material of the sheet 16 and of the liquid (buffer) 3 respectively.

With reference to figure 5, where the details similar or identical to those already described are indicated by the same numbers, the device 1b comprises heating means 24c located outside the sheet 16 and consisting in the example illustrated of a fan 40 and a resistance 41 which, controlled by the control unit 36 connected to a sensor 35 of the temperature T_2 of the surface 8 heat the latter, sending to it a flow of ambient air.

On the basis of the description, the invention therefore also concerns a method for performing the optical analysis of particles 2 contained in suspension in a fluid 3, at temperatures below the ambient temperature, comprising the steps of:

i.- arranging the particles 2 in suspension inside at least one microchamber 4 containing the fluid 3 in a space

delimited between the surfaces 5 and 6;

ii.- thermally coupling the surface 5, via the thermal resistance RLW, with the cooling means 7 adapted to subtract heat from the fluid 3, and thermally coupling the surface 6, via the thermal resistance RHI, with the optical inspection surface 8 to be maintained clear;

iii.- bringing the fluid 3 to the temperature T1, lower than the ambient temperature, by means of the cooling means 7; and

iv.- while the particles 2 are being optically analysed, establishing at the optical inspection surface 8 a thermal flow F such that the optical inspection surface 8 is constantly maintained at the temperature T2, higher than the temperature Td of condensation of the ambient humidity (dew point) contained in the air which laps the optical inspection surface;

v.- where the thermal resistances RHI and RLW are chosen so that the thermal resistance RHI has a thermal conductivity value preferably at least one order of magnitude below that of the first thermal resistance RLW and, in any case, equal to at least half of the thermal conductivity of the thermal resistance RLW.

Typically, the thermal flow F is an input thermal flow, in the sense that step (iv) is performed by heating the optical inspection surface 8 to above the dew point of the ambient air, as previously illustrated.

Heating of the surface 8 can be obtained, preferably, by Joule effect, providing on the same, outside the microchamber 4, a resistor 24b chosen from the group consisting of: a transparent conductive resistive layer, for example of Indium Tin Oxide (ITO, or based on nano tubes of carbon, or conductive polymers such as poly(3,4-ethylenedioxythiophene) (PEDOT)) uniformly applied over the entire optical inspection surface 8; a plurality of filiform microresistors applied on the optical inspection surface 8, arranged in a

comb shape, uniformly spaced from one another transverse to the direction of longitudinal extension of the microchamber 4.

The filiform microresistors 26 which, according to an embodiment not illustrated for the sake of simplicity, can also be arranged in a grid pattern, or so as to cross one another, are in any case supplied so that the current density distribution is uniform; in the case illustrated, using the current distribution frame 27 arranged longitudinally orthogonal to the filiform microresistors 26, which in turn receives the current by means of a plurality of conductor bridges 30 which connect a plurality of different points 28 of the distribution frame 27, arranged on the side opposite the filiform microresistors 26, to at least one common collector 31.

Alternatively, as has been seen, the optical inspection surface 8 is maintained at a temperature higher than the dew point by forcing an air flow over the same by forced convection, generated for example by a fan 40. Said flow counters the lowering of the temperature of the optical inspection surface 8 due to the absorption of heat by the cooling system through the liquid in the microchamber 4, as illustrated in figure 5.

In any case, heating of the optical inspection surface 8 is preferably feedback controlled by continuously measuring the current temperature T2 of the same, preferably by means of a resistance 35 applied to the optical inspection surface 8 or by means of an infrared sensor arranged facing the optical inspection surface 8.

According to a different embodiment of the invention, however, the step (iv) can be performed equally effectively by cooling a quantity of ambient air immediately surrounding the optical inspection surface 8 which laps the same, at a temperature T3

such that the dew point of said quantity of air becomes lower than the temperature T_2 of the optical inspection surface 8 due to thermal transmission by conduction from and to the microchamber 4 through the thickness of the sheet 16.

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A solution of this kind can be implemented by a device 1b (or 1a) similar to the one illustrated schematically in figure 5, where a fan 40 is used to recirculate the same quantity of air present in the vicinity of the surface 8 through a cooling
10 element 41, for example consisting of a set of Peltier cells all arranged around the sheet 16.

The temperature T_d , especially in the implementations previously described, can be fixed beforehand, taking 12°C as
15 a fixed estimate, which is the value that occurs in the majority of cases. Vice versa, the actual temperature T_d of the ambient air and the cooled air which laps the surface 8 due to the action of the fan 40 is calculated, for example, by detecting both the temperature and ambient humidity, and those
20 of said cooled air, by means of appropriate sensors 42 connected to the control unit 36.

Obviously the cooling caused by the set of Peltier cells which establishes the required thermal flow F according to the
25 invention can be used to lower both the temperature and humidity of the ambient air, or only the temperature or only the humidity.

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CLAIMS

1. A method for optical analysis of particles (2) contained in suspension in a fluid, at temperatures lower than dew point temperature, comprising the steps of:
- i. arranging the particles in suspension within at least one microchamber (4) containing said fluid and delimited between a first and a second surface (5,6);
 - ii. thermally coupling the first surface (5), by means of a first thermal resistance (RLW), to first cooling means (7) adapted to subtract heat from the fluid, and thermally coupling the second surface (6), by means of a second thermal resistance (RHI), to an optical inspection surface (8);
 - iii. bringing said fluid to a first temperature (T1), lower than dew point temperature, by means of said first cooling means;
 - iv. while the particles (2) are being optically analysed, establishing at the optical inspection surface (8) a thermal flow (F) such that the optical inspection surface is constantly maintained at a second temperature (T2) higher than the dew point temperature (Td) of the ambient humidity contained in the air which laps on the optical inspection surface (8); said first and second thermal resistances being chosen so that the second thermal resistance (RHI) has a thermal conductivity value, preferably, at least one order of magnitude lower than that of the first thermal resistance (RLW) and, in any case, equal to at least half the thermal conductivity of the first thermal resistance (RLW).
2. A method according to claim 1, characterized in that step (iv) is carried out by heating the optical inspection surface (8) to above ambient air dew point.
3. A method according to claim 2, characterized in that the optical inspection surface is heated by Joule effect, by arranging on the same, externally to the microchamber (4), a

resistor (24b) chosen from the group consisting of: a transparent conductive resistive layer (25), e.g. ITO, applied uniformly on the entire optical inspection surface; a plurality of filiform microresistors (26) arranged on the optical inspection surface, preferably in a comb shape, uniformly spaced from one another.

4. A method according to claim 3, characterized in that said filiform microresistors (26) are supplied so that the current density distribution is homogenous by using a current distribution frame (27) arranged opposite the filiform microresistors, which in turn receives current by means of a plurality of conductor bridges (30), which connect a plurality of different points (28) of the distribution frame, arranged on the side opposite the filiform microresistors, to at least one common collector (31).

5. A method according to claim 2, characterized in that the optical inspection surface (8) is heated by forcing an air flow over the same.

6. A method according to claim 1, characterized in that step (iv) is carried out by cooling an amount of ambient air immediately surrounding the optical inspection surface (8) and so lapping the inspection surface at a temperature such that the dew point (T_d) of said amount of air is lower than said second temperature (T_2) of the optical inspection surface.

7. A method according to one of the claims from 2 to 6, characterized in that the temperature of the optical inspection surface is feedback controlled by continuously measuring the instant temperature (T_2) of the optical inspection surface, preferably by means of a resistor applied to said optical inspection surface (35), or by means of an infrared sensor arranged facing the optical inspection surface.

8. A method according to claim 7, characterized in that the temperature (T2) at which to maintain the optical inspection surface is calculated as a function of the parameters ambient
5 air temperature and ambient air humidity, which are continuously detected by means of appropriate sensors.

9. An apparatus (1a,1b) for optical analysis of particles (2) contained in suspension in a fluid (3), at temperatures lower
10 than dew point temperature, comprising:

- at least one microchamber (4) containing said fluid and delimited between a first (5) and a second (6) surface;
- first cooling means (7) which are thermally coupled with the first surface by means of a first thermal resistance (RLW) and
15 are adapted to subtract heat from the microchamber by an amount such as to maintain said fluid at a predetermined first temperature (T1), lower than the dew point temperature; and
- an optical inspection surface (8) thermally coupled to the second surface by means of a second thermal resistance (RHI);
20 characterized in that, in combination:

- the second thermal resistance (RHI) has a thermal conductivity value, preferably, of at least one order of magnitude, and even more preferably, of two orders of magnitude lower than that of the first thermal resistance
25 (RLW) and, in all cases, equal to at least half the thermal conductivity of the first thermal resistance (RLW); and

- the apparatus further comprises means (24) for establishing at the optical inspection surface (8), while the apparatus (1a/1b) is operative and manipulation and/or optical analysis
30 of the particles (2) are being performed with it, a thermal flow (F) such that the optical inspection surface is constantly maintained at a second temperature (T2), higher than the dew point temperature (Td) of the ambient humidity contained in the air which laps the optical inspection surface
35 (8) in use.

10. An apparatus according to claim 9, characterized in that it comprises means (24b;24c) for heating the optical inspection surface (8) to above the ambient air dew point.

5 11. An apparatus according to claim 10, characterized in that said means for heating the optical inspection surface consist of a resistor (24b) constituted by: a transparent conductive resistive layer (25), e.g. ITO, applied uniformly on the entire optical inspection surface; or a plurality of filiform
10 microresistors (26) applied integrally in one piece on the optical inspection surface (8) and arranged on the same, preferably in a comb shape, uniformly spaced from one another.

12. An apparatus according to claim 11, characterized in that
15 said filiform microresistors (26) are electrically connected, each on the side of the same one end thereof, to a current distribution frame (27) constituted by a metal foil which receives electric current by means of a plurality of conductor
bridges (30) which connect a plurality of different points
20 (28) of the distribution frame, arranged on the side opposite the filiform microresistors, to at least one common collector (31) arranged at a base element (19) of the apparatus which supports the microchamber; said filiform microresistors (26)
having a width equal to approximately one tenth of the pitch
25 between the same, in a direction transverse to their longitudinal extension.

13. An apparatus according to claim 9, characterized in that
said means (24) for establishing said thermal flow (F) at the
30 optical inspection surface (8) comprise forced ventilation means (40) of the optical inspection surface (8) and, preferably, heating or cooling means (41) of the forced ventilation flow.

35 14. An apparatus according to one of the claims from 9 to 13, characterized in that it comprises means (35) for measuring

said second temperature (T2) and means (36;37) for actuating in feedback said means (24) for establishing said thermal flow (F) at the optical inspection surface (8); and preferably means for calculating the ambient air dew point (Td).

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15. Apparatus as claimed in claim 9, characterised in that it comprises electronic means (100, 101) for performing the manipulation of said particles (2) in said microchamber (4).

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