Claims:

1. A device for manipulating and/or determining one or more characteristics of cells contained within a biological sample, the device comprising:

a sorting component configured to separate cell-containing microdroplets from empty ones into a population of cell-containing first microdroplets;

a microdroplet manipulation component configured to manipulate the first microdroplets using real or virtual electrowetting electrodes, the microdroplet manipulation component including:

a first zone configured to arrange the first microdroplets into an array for optical inspection and, optionally, to introduce a reporter system into each first microdroplet by means of microdroplet merging;

a second zone located within or adjacent the first zone and configured to detect merged microdroplets in one or more detection windows; and

optionally a third zone in which microdroplets can be sub-divided and isolated for later recovery from the instrument; and

an optical detection system configured to detect an optical signal from the microdroplets via the one or more detection windows, wherein, for merged microdroplets, the signal arises from an interaction between the reporter system and the cells or an expressed product thereof.

2. A device as claimed in claim 1, wherein the optical detection system is selected from: a brightfield microscope, a darkfield microscope, a means for detecting chemiluminescence, a means for detecting Förster resonance energy transfer, and a means for detecting fluorescence

3. A device as claimed in claim 1 or 2, further comprising a cell-culturing component, either integral with the device or separately located before or after the sorting component, configured to hold the microdroplets whilst any cells contained within are cultured.

4. A device as claimed in claim 3, wherein the cell-culturing component is further configured to agitate the contents of each microdroplet using optically-mediated electrowetting forces.

5. A device as claimed in any one of claims 3 or 4, wherein the device further comprises a heater and temperature controller configured to control the temperature of the microdroplets in the cell-culturing component within the range 25 to 40^{0}C.
6. A device as claimed in any preceding claim, further comprising a sample preparation component, either integral with the device or separately located before the sorting component, configured to create an emulsion of the microdroplets in an immiscible carrier fluid from the biological sample.
7. A device as claimed in any one claims 3 to 6, wherein at least one of the sorting component, the cell-culturing component and the sample preparation component is configured with electrowetting electrode locations to enable the droplets to be manipulated therein and/or therebetween.
8. A device as claimed in claim 6, wherein the sample preparation component includes at least one location where an electrowetting stretching force is applied to the biological sample, and is configured to sever microdroplets from the biological sample into the carrier fluid.
9. A device as claimed in any one of the preceding claims, wherein the optical detection means comprises a source of first electromagnetic radiation adapted to impinge on the first microdroplets in the one or more detection windows, and further comprises a detector for detecting fluorescence emitted from the microdroplets.
10. A device as claimed in any one of the preceding claims wherein the microdroplet manipulation component includes one or more OEWOD structures comprised of:

a first composite wall comprised of:

a first substrate

a first transparent conductor layer on the substrate, the first transparent conductor layer having a thickness in the range 70 to 250nm;

a photoactive layer activated by electromagnetic radiation in the wavelength range 400-850nm on the conductor layer, the photoactive layer having a thickness in the range 300-1500nm and

a first dielectric layer on the photoactive layer, the first dielectric layer having a thickness in the range 30 to 160nm;

a second composite wall comprised of:

a second substrate;

a second conductor layer on the substrate, the second conductor layer having a thickness in the range 70 to 250nm and

optionally a second dielectric layer on the second conductor layer, the second dielectric layer having a thickness in the range 30 to 160nm

wherein the exposed surfaces of the first and second dielectric layers are disposed 20-180μm apart to define a microfluidic space adapted to contain microdroplets;

an A/C source to provide a voltage across the first and second composite walls connecting the first and second conductor layers;

at least one source of electromagnetic radiation having an energy higher than the bandgap of the photoactive layer adapted to impinge on the photoactive layer to induce corresponding virtual electrowetting locations on the surface of the first dielectric layer; and

means for manipulating the points of impingement of the electromagnetic radiation on the photoactive layer so as to vary the disposition of the virtual electrowetting locations thereby creating at least one electrowetting pathway along which the microdroplets may be caused to move.

11. A device as claimed in any one of the preceding claims, wherein the first zone includes a reservoir comprising an array of first microdroplet-holding sites and a port for introducing second microdroplets containing the reporter system, the first zone being further configured to drive the second microdroplets across the first microdroplet-holding sites so that first and second microdroplets are caused to merge.
12. A device as claimed in claim 11, wherein the device is configured to drive the second microdroplets across the first microdroplet-holding sites via one or more pathways of virtual electrowetting electrodes.
13. A device as claimed in claim 11, wherein the device is configured to introduce microdroplets to a first region of the device, connected to the port, in a continuous hydraulic flow, and wherein a second region of the device, overlapping the first region, is configured to channel microdroplets from the first region into a third region of the device for further operations
14. A device as claimed in claim 10, wherein at least the surface of the first dielectric layer is provided with an anti-fouling coating.

15. A device as claimed in any one of the preceding claims, further comprising a microprocessor adapted by means of a feedback loop to control one or more of the performance of the sorting component; the rate of introduction of the first microdroplets into the first zone and the rate of merging of the first and second microdroplets in response to a signal supplied by the detection system.
16. A device as claimed in any one of the preceding claims, further comprising an optical assembly configured to illuminate the device and to detect signals characterising cells within the device.
17. A method for using the device of any preceding claim to manipulate and/or determine one or more characteristics of cell types in a biological sample, the method comprising the steps of:

creating from the biological sample aqueous first microdroplets in an immiscible carrier fluid, at least some of which are believed to contain cells of a particular cell type;

moving the first microdroplets along a pathway using real or virtual electrowetting electrodes to at least one microdroplet-merging location;

moving aqueous second microdroplets containing a reporter system characteristic of the cell type whose characteristics are being investigated along a pathway using real or virtual electrowetting electrodes to the microdroplet merging location;

merging the first and second microdroplets at the merging location to produce merged microdroplets; and

analysing the contents of each merged microdroplet with an optical detection system and detecting an optical signal characteristic of an interaction between the cell and the reporter system.

18. A method as claimed in claim 17, wherein the step of creating the first microdroplets further comprises separating cell-containing first microdroplets from a population of microdroplets including both cell-containing and empty microdroplets.
19. A method as claimed in claim 17 or claim 18, wherein the step of creating the first microdroplets further comprises culturing the population of microdroplets under conditions which cause cell growth and division.
20. A method as claimed in any one of claims 17 to 19, wherein the cell-containing first microdroplets are sorted by the output of measuring a state of the cells with an optical detection system.

21. A method as claimed in any one of claims 17 to 20, wherein the step of creating the first microdroplets further comprises severing microdroplets from the biological sample by application of an electrowetting stretching force.
22. A method as claimed in claim 21, wherein the microdroplets are severed into the immiscible carrier fluid, the immiscible carrier fluid comprising a hydrocarbon or silicone oil.
23. A method as claimed in any one of claims 17 to 21, wherein the immiscible carrier fluid is a fluorocarbon oil which has been optionally hydrated with aqueous micelles or secondary microdroplets.
24. A method as claimed in claim 19, wherein culturing the population of microdroplets comprises contacting the population of microdroplets in the immiscible carrier fluid with a flow of carrier fluid containing cell-culturing nutrients and/or dissolved gas.
25. A method as claimed in claim 24, wherein the dissolved gases consist of one or more of oxygen, nitrogen and carbon dioxide.
26. A method as claimed in claim 24, wherein the immiscible carrier fluid is periodically purged of gases detrimental to the culturing of the cells.
27. A method as claimed in any one of claims 19 to 26, wherein culturing the population of microdroplets comprises stirring or agitating the microdroplets by application of an optically-mediated electrowetting force at virtual electrowetting electrode locations where the microdroplets are held.
28. A method as claimed in any one of claims 17 to 27, wherein the reporter system is a reporter gene, cell-surface biomarker or a reporter molecule selective for an enzyme or antibody expressed by cells of the cell type being sought.
29. A method as claimed in any one of claims 17 to 27, wherein the reporter system is a luminescent reporter cell which reacts selectively to the presence of an enzyme or antibody expressed by cells of the cell type being sought.
30. A method as claimed in any one of claims 17 to 27, wherein the optical detection system is one of a brightfield microscope, a darkfield microscope, a means for detecting chemiluminescence, a means for detecting Förster resonance energy transfer or a means for detecting fluorescence.
31. A method as claimed in any one of claims 17 to 30, wherein microdroplets are transported between locations on the device using an OEWOD structure adapted to

generate a pathway of virtual electrowetting electrodes using electromagnetic radiation, and optionally provided with an anti-fouling and/or biocompatible coating.

32. A method for using the device of any of claims 1 to 16 to manipulate and/or determine one or more characteristics of cell types in a biological sample, the method comprising the steps of:

creating from the biological sample aqueous first microdroplets in an immiscible carrier fluid, at least some of which are believed to contain cells of a particular cell type;

moving the first microdroplets along a pathway using real or virtual electrowetting electrodes to at least one microdroplet inspection location; and

analysing the contents of each microdroplet with an optical detection system to determine one or more characteristics of a cell contained in that microdroplet, the one or more characteristics comprising at least one of: cell morphology, cell motility, or cell membrane integrity.

CLAIMS

1. A device for manipulating and/or determining one or more characteristics of cells contained within a biological sample, the device comprising:

a sorting component (3) configured to separate cell-containing microdroplets from empty ones (4) into a population of cell-containing first microdroplets (5);

a microdroplet manipulation component configured to manipulate the first microdroplets using real or virtual electrowetting electrodes, the microdroplet manipulation component including:

a first zone (8) configured to arrange the first microdroplets into an array for optical inspection and to introduce a reporter system (7) into each first microdroplet by means of microdroplet merging;

a second zone located within or adjacent the first zone and configured to detect microdroplets in one or more detection windows (10); and

optionally a third zone in which microdroplets can be sub-divided and isolated for later recovery from the device; and

an optical detection system (11) configured to detect an optical signal from the microdroplets via the one or more detection windows, in which, for merged microdroplets (9), the signal arises from an interaction between the reporter system and the cells or an expressed product thereof.

2. A device according to Claim 1, in which the optical detection system (11) is selected from: a brightfield microscope, a darkfield microscope, a means for detecting chemiluminescence, a means for detecting Förster resonance energy transfer, and a means for detecting fluorescence.

3. A device according to Claim 1 or 2, further comprising a cell-culturing component, either integral with the device or separately located before or after the sorting component (3), configured to hold the microdroplets whilst any cells contained within are cultured.

4. A device according to Claim 3, in which the cell-culturing component is further configured to agitate the contents of each microdroplet using optically-mediated electrowetting forces.

5. A device according to Claim 3 or 4, in which the device further comprises a heater and temperature controller configured to control the temperature of the microdroplets in the cell-culturing component within the range 25 to 400C.

6. A device according to any preceding claim, further comprising a sample preparation component, either integral with the device or separately located before the sorting component (3), configured to create an emulsion of the microdroplets (2) in an immiscible carrier fluid from the biological sample.

7. A device according to any of Claims 3 to 6, in which at least one of the sorting component (3), the cell-culturing component and the sample preparation component is configured with electrowetting electrode locations to enable the droplets to be manipulated therein and/or therebetween.

8. A device according to Claim 6, in which the sample preparation component includes at least one location where an electrowetting stretching force is applied to the biological sample, and is configured to sever microdroplets from the biological sample into the carrier fluid.

9. A device according to any preceding claim, in which the optical detection means (11) comprises a source of first electromagnetic radiation adapted to impinge on the first microdroplets in the one or more detection windows (10), and further comprises a detector for detecting fluorescence emitted from the microdroplets.

10. A device according to any preceding claim, in which the microdroplet manipulation component includes one or more OEWOD structures comprised of:

a first composite wall comprised of:

a first substrate (14)

a first transparent conductor layer on the substrate (15), the first transparent conductor layer having a thickness in the range 70 to 250nm;

a photoactive layer (17) activated by electromagnetic radiation in the wavelength range 400-850nm on the conductor layer, the photoactive layer having a thickness in the range 300-1500nm and

a first dielectric layer (18) on the photoactive layer, the first dielectric layer having a thickness in the range 30 to 160nm;

a second composite wall comprised of:

a second substrate (13);

a second conductor layer on the substrate (15), the second conductor layer having a thickness in the range 70 to 250nm and

optionally a second dielectric layer (18) on the second conductor layer, the second dielectric layer having a thickness in the range 30 to 160nm

in which the exposed surfaces of the first and second dielectric layers are disposed 20-180μm apart to define a microfluidic space adapted to contain microdroplets;

an A/C source (16) to provide a voltage across the first and second composite walls connecting the first and second conductor layers;

at least one source of electromagnetic radiation (20) having an energy higher than the bandgap of the photoactive layer adapted to impinge on the photoactive layer to induce corresponding virtual electrowetting locations on the surface of the first dielectric layer; and

means for manipulating the points of impingement of the electromagnetic radiation on the photoactive layer so as to vary the disposition of the virtual electrowetting locations thereby creating at least one electrowetting pathway along which the microdroplets may be caused to move.

11. A device according to any preceding claim, in which the first zone (8) includes a reservoir comprising an array of first microdroplet-holding sites and a port (6) for introducing second microdroplets containing the reporter system (7), the first zone being further configured to drive the second microdroplets across the first microdroplet-holding sites via one or more pathways of virtual electrowetting electrodes so that first and second microdroplets are caused to merge.

12. A device according to Claim 11, in which the device is configured to introduce second microdroplets to a first region (8) of the device, connected to the port (6), in a continuous hydraulic flow, and in which a second region of the device, overlapping the first region, is configured to channel microdroplets from the first region (8) into a third region of the device for further operations.

13. A device according to Claim 10, in which at least the surface of the first dielectric layer (18) is provided with an anti-fouling coating (19).

14. A device according to any preceding claim, further comprising a microprocessor (24) adapted by means of a feedback loop to control one or more of the performance of the sorting component (3); the rate of introduction of the first microdroplets into the first zone (8) and the rate of merging of the first and second microdroplets in response to a signal supplied by the detection system.

15. A method for using a device according to any preceding claim to manipulate and/or determine one or more characteristics of cell types in a biological sample, the method comprising the steps of:

creating from the biological sample aqueous first microdroplets (2) in an immiscible carrier fluid, at least some of which are believed to contain cells of a particular cell type;

moving the first microdroplets along a pathway using the real or virtual electrowetting electrodes to at least one microdroplet-merging location (8);

moving aqueous second microdroplets containing a reporter system (7) characteristic of the cell type whose characteristics are being investigated along a pathway using real or virtual electrowetting electrodes to the microdroplet merging location (8);

merging the first and second microdroplets at the merging location (8) to produce merged microdroplets (9); and

analysing the contents of each merged microdroplet (9) with the optical detection system (11) and detecting an optical signal characteristic of an interaction between the cell and the reporter system;

in which the interaction between the cell and the reporter system which is characteristic of the cell type, identifies the nature of the cells in the biological sample.

16. A method according to Claim 15, in which the step of creating the first microdroplets further comprises separating cell-containing first microdroplets (5) from a population of microdroplets (2) including both cell-containing and empty microdroplets (4).

17. A method according to Claim 15 or 16, in which the step of creating the first microdroplets further comprises culturing the population of microdroplets under conditions which cause cell growth and division.

18. A method according to Claim 15, 16 or 17, in which the step of creating the first microdroplets further comprises severing microdroplets from the biological sample by application of an electrowetting stretching force.

19. A method according to any of Claims 15 to 18, in which the immiscible carrier fluid is a hydrocarbon, or silicone, or fluorocarbon oil which has been optionally hydrated with aqueous micelles or secondary microdroplets.

20. A method according to Claim 17, in which culturing the population of microdroplets comprises contacting the population of microdroplets in the immiscible carrier fluid with a flow of carrier fluid containing cell-culturing nutrients and/or dissolved gas consisting of one or more of oxygen, nitrogen and carbon dioxide.

21. A method according to Claim 20, in which the immiscible carrier fluid is periodically purged of gases detrimental to the culturing of the cells.

22. A method according to any of Claims 17 to 21, in which culturing the population of microdroplets comprises stirring or agitating the microdroplets by application of an optically-mediated electrowetting force at virtual electrowetting electrode locations where the microdroplets are held.

23. A method according to any of Claims 15 to 22, in which the reporter system (7) is a reporter gene, cell-surface biomarker or a reporter molecule selective for an enzyme or antibody expressed by cells of the cell type being sought, or is a luminescent reporter cell which reacts selectively to the presence of an enzyme or antibody expressed by cells of the cell type being sought.

24. A method according to any of Claims 15 to 23, in which microdroplets are transported between locations on the device using an OEWOD structure adapted to generate a pathway of virtual electrowetting electrodes using electromagnetic radiation, and optionally provided with an anti-fouling and/or biocompatible coating.