CLAIMS

1. A method of processing biological micro-objects in a micro-fluidic device comprising a housing disposed on a base, said housing comprising a flow path configured to hold a first liquid medium and a plurality of holding pens, each holding pen of said plurality comprising a physical enclosure enclosing an interior space and having an opening adjacent to said flow path configured to permit biological micro-objects to be reversibly moved into and/or out of said interior space, wherein said enclosure is structured to (i) hold one or more biological micro-objects suspended in a second liquid medium and (ii) impede a direct flow of said first liquid medium into said interior space while allowing diffusive exchange between said second liquid medium and said first liquid medium, said method comprising:

deterministically selecting and moving a droplet of the second liquid medium including one or more biological micro-objects suspended therein into the interior space of a first holding pen of the plurality of holding pens, wherein:

the micro-fluidic device further comprises an electrode mechanism comprising an electrowetting device configured to create electrokinetic forces; and

moving the droplet of second liquid medium into the interior of the first holding pen comprises selectively activating the electrowetting electrode mechanism to apply electrokinetic forces to the droplet of second liquid medium, whereby the biological micro-object is disposed in the holding pen outside the flow of the first liquid medium; and

providing to said flow path a flow of the first liquid medium over a time period, wherein said first liquid medium is a different liquid medium from said second liquid medium of said droplet.

2. The method of claim 1, further comprising a step of monitoring during said time period a characteristic of said one or more biological micro-objects in said droplet in said first holding pen.

3. The method of claim 2, wherein said characteristic is a biological activity of said one or more biological micro-objects in said droplet in said first holding pen.

4. The method of claim 3, wherein said biological activity comprises cloning, and said step of monitoring comprises monitoring said cloning of said one biological micro-object in said droplet in said first holding pen.

5. The method of claim 3, wherein said biological activity comprises secreting secretions, and said step of monitoring comprises monitoring said secretions of said one or more biological micro-objects in said droplet in said first holding pen.

6. The method of claim 1, wherein said step of moving said droplet comprises:

(i) moving a droplet containing one and only one of said one or more biological micro-objects into said first holding pen; or

(ii) moving a droplet containing a biological micro-object into each of a plurality of holding spaces in said first holding pen; or

(iii) moving a droplet containing a different type of biological micro-object into each holding pen of the plurality of holding pens.

7. The method of claim 1, wherein said electrowetting electrode mechanism is an optoelectronic wetting (OEW) device.

8. The method of claim 2, further comprising a step of selecting a set of biological micro-objects in said droplet in said first holding pen, wherein said set of biological micro-objects meet a predetermined criteria, threshold or condition associated with said biological activity or said biological state.

9. The method of claim 8, further comprising a step of processing or using said set of biological micro-objects in said droplet in said first holding pen.

10. The method of claim 1, further comprising a step of removing said set of biological micro-objects in said droplet from said first holding pen.

11. The method of claim 10, wherein said step of removing comprises activating said electrode mechanism comprising said electro-wetting device.

12. The method of claim 11, wherein said step of activating said electrode mechanism is optically controlled.

13. A micro-fluidic device comprising:

an electrode mechanism comprising an electrowetting device for moving a droplet of a second liquid medium in which one or more biological micro-objects are suspended;

a housing disposed on a base, said housing comprising a flow path configured to hold a first liquid medium; and

a plurality of holding pens disposed within said housing, each of said holding pens comprising:

a physical enclosure enclosing an interior space, said enclosure structured to (i) hold one or more biological micro-objects suspended in said second liquid medium of said droplet and (ii) impede a direct flow of said first liquid medium into said interior space, while allowing diffusive exchange between said first liquid medium and said second liquid medium; and

an opening in said enclosure disposed adjacent to said flow path configured to permit biological micro-objects to be moved into and/or out of said interior space upon activation of the electrode mechanism.

14. The micro-fluidic device of claim 13, wherein said electrowetting electrode mechanism comprises an optoelectronic wetting (OEW) device.

15. The micro-fluidic device of claim 13, wherein said holding pens of said plurality are disposed in said housing such that said openings of said holding pens are immediately adjacent said flow path.

16. The micro-fluidic device of claim 15, wherein:

said flow path comprises a fluidic channel in said housing, and

said openings of said holding pens are directly connected to said fluidic channel.

17. The micro-fluidic device of claim 16, wherein said micro-fluidic device comprises a plurality of fluidic channels.

18. The micro-fluidic device of claim 13, wherein:

one of said plurality of holding pens comprises an inner wall extending from said opening into said enclosure.

19. The micro-fluidic device of claim 18, wherein said inner wall comprises a barrier between said opening of said holding pen and an inner containment space within said enclosure of said holding pen.

20. The micro-fluidic device of claim 13, wherein one of said plurality of holding pens further comprises an inner pen comprising an enclosure and an opening, wherein said inner pen is disposed inside said enclosure of said holding pen.

21. The micro-fluidic device of claim 13, wherein:

one of said plurality of holding pens comprises holding spaces separated by interior walls disposed inside said enclosure of said one of said plurality of holding pens, and

each said holding space is configured to hold a droplet of said liquid medium containing one of said one or more biological micro-objects.

22. The micro-fluidic device of claim 13, further comprising:
an inlet into said housing to said flow path, and
an outlet from said housing from said flow path.

23. The micro-fluidic device of claim 22, wherein said plurality of holding pens of said micro-fluidic device are disposed in said flow path between said inlet and said outlet.

24. The micro-fluidic device of claim 23, further comprising a gas exchanger disposed in said flow path between said inlet and said plurality of holding pens.

25. The micro-fluidic device of claim 13, further comprising a gas exchanger disposed in said housing.

26. The micro-fluidic device of claim 13, wherein all or part of said housing is gas permeable.

27. The micro-fluidic device of claim 26, wherein said housing comprises a flexible material.

28. The micro-fluidic device of claim 13, further comprising means for monitoring a biological activity of said one or more biological micro-objects in said droplet of said liquid medium in said holding pen.

29. The micro-fluidic device of claim 28, wherein said means comprises sensors configured to detect relevant conditions of a holding pen of said plurality of holding pens within said housing and/or media within said housing.