

WHAT IS CLAIMED IS:

1. A method for capturing cells of interest in a digital microfluidic system, the method comprising:

5 (a) utilizing a droplet actuator of the digital microfluidic system to transport a sample droplet to a microwell device, the microwell device including a substrate having a plurality of microwells that open onto a droplet operations surface of the microwell device, the sample droplet including cells of interest that enter the microwells;

10 (b) introducing capture beads to the microwells, wherein capture elements are immobilized on the capture beads; and

(c) utilizing the droplet actuator to transport a cell lysis reagent droplet to the microwell device, wherein portions of the cell lysis reagent droplet enter the microwells and, during an incubation period, cause the cells of interest to release analyte that is captured by the capture elements on the capture beads.

15 2. The method of claim 1, further comprising utilizing the droplet actuator to transport the sample droplet away from the microwell device while leaving at least a portion of the cells of interest captured in the microwells.

20 3. The method of claim 1, further comprising permitting at least a portion of the cells of interest to settle into the microwells.

25 4. The method of claim 1, wherein the droplet operations surface of the microwell device includes interstitial areas between microwells that are hydrophobic such that substantially no residue of the cells of interest or capture beads remains on the droplet operations surface of the microwell device.

5. The method of claim 1, further comprising removing the capture beads with the analyte captured thereon from the microwells.

30 6. The method of claim 5, wherein the removing operation includes positioning a

magnet proximate to the microwells to form a magnetic field that pulls the capture beads from the microwells.

7. The method of claim 1, further comprising utilizing a magnetic field to move the capture beads to and away from the microwells.
8. The method of claim 1, wherein each of the capture beads includes a plurality of the capture elements.
9. The method of claim 8, wherein the plurality of capture elements include a capture sequence and a unique barcode sequence.
10. The method of claim 9, wherein the capture sequence is one of i) a poly-T sequence for capture of total mRNA, or ii) a plurality of transcript-specific capture sequences that target a panel of genes of interest.
11. The method of claim 1, wherein the capture beads are sized such that only one of the capture beads fits in one of the microwells.
12. The method of claim 1, wherein the capture beads are deposited in the microwells by gravity.
13. The method of claim 1, wherein the capture beads are magnetic and are deposited in the microwells utilizing a magnetic attraction.
14. A digital fluidics system for capturing cells of interest, the system comprising:
 - (a) a droplet actuator including a droplet operations gap, the droplet actuator including droplet operations electrodes arranged proximate to the droplet operations gap;
 - (b) a microwell device including a substrate, the microwell device including microwells formed in the substrate, the microwells opening onto a droplet operations surface of the microwell device, the microwell device coupled to

the droplet actuator and positioned such that the microwells face the droplet operations gap; and

(c) a controller configured to execute program instructions to:

- 5 (1) direct the droplet actuator to transport a sample droplet to a microwell device, the sample droplet including cells of interest that enter the microwells;
- (2) introduce capture beads to the microwells, wherein capture elements are immobilized on the capture beads; and
- 10 (3) direct the droplet actuator to transport a cell lysis reagent droplet to the microwell device, wherein portions of the lysis reagent droplet enter the microwells and, during an incubation period, cause the cells of interest to release analyte that is captured by the capture elements on the capture beads.

15 15. The system of claim 14, wherein the substrate includes a hydrophobic layer disposed thereon, the hydrophobic layer forming the droplet operations surface.

 16. The system of claim 14, wherein the droplet operations gap is configured to retain a filler fluid including the sample droplet containing the cells of interest.

20 17. The system of claim 14, wherein the microwells have a size and pitch dimensioned to receive only a single cell from the cells of interest in the sample droplet and to receive only a single one of the capture beads.

25 18. The system of claim 14, wherein the microwells have a depth of between 30 μm and 50 μm .

 19. The system of claim 14, wherein the microwells have a diameter of between 3 μm and 60 μm .

30 20. The system of claim 14, wherein the microwells are spaced from one another by a pitch no greater than 80 μm .

21. The system of claim 14, wherein the controller is further configured to execute program instructions to, during and/or prior to the incubation period, direct the droplet actuator to transport a fluid immiscible with the cell lysis reagent droplet to the microwell device, wherein the immiscible fluid does not enter the microwells and cell traps, thereby encapsulating single beads with single cells with cell lysis reagent.
22. A method for capturing cells of interest in a digital microfluidic system, the method comprising:
- (a) utilizing a droplet actuator of the digital microfluidic system to transport a sample droplet to a microwell device, the microwell device including a first substrate having a plurality of microwells that open onto a droplet operations surface of the microwell device, and a plurality of cell traps that open onto the droplet operations surface of the microwell device, wherein each cell trap is positioned above a microwell, the sample droplet including cells of interest that enter the cell traps;
 - (b) introducing capture beads to the microwells, wherein capture elements are immobilized on the capture beads; and
 - (c) utilizing the droplet actuator of the digital microfluidic system to transport a cell lysis reagent droplet to the microwell device, wherein portions of the cell lysis reagent droplet enter the microwells and cell traps and, during an incubation period, cause the cells of interest to release analyte that is captured by the capture elements on the capture beads.
23. The method of claim 22, wherein the capture beads are sized such that only one of the capture beads fits in one of the microwells.
24. The method of claim 22 or 23, wherein the cell traps are sized such that only one of the cells of interest fits in one of the cell traps.
25. The method of any one of claims 22-24, further comprising:

(d) during and/or prior to the incubation period, utilizing the droplet actuator to transport a fluid immiscible with the cell lysis reagent droplet to the microwell device, wherein the immiscible fluid does not enter the microwells and cell traps, thereby encapsulating single beads with single cells with cell lysis reagent.

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26. The method of claim 22, further comprising removing the capture beads with the analyte captured thereon from the microwells.

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27. The method of claim 26, wherein the removing operation includes positioning a magnet proximate to the microwells to form a magnetic field that pulls the capture beads from the microwells.

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28. The method of claim 22, further comprising utilizing a magnetic field to move the capture beads to and away from the microwells.

29. The method of claim 22, wherein each of the capture beads includes a plurality of the capture elements.

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30. The method of claim 29, wherein the plurality of capture elements include a capture sequence and a unique barcode sequence, wherein the capture sequence is optionally one of i) a poly-T sequence for capture of total mRNA, or ii) a plurality of transcript-specific capture sequences that target a panel of genes of interest.

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31. A digital fluidics system for capturing cells of interest, the system comprising:

(a) a droplet actuator including a droplet operations gap, the droplet actuator including droplet operations electrodes arranged proximate to the droplet operations gap;

(b) a microwell device including:

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(1) a first substrate, the microwell device including microwells formed in the first substrate, the microwells opening onto a droplet operations surface of the microwell device, the microwell device coupled to the droplet actuator and positioned such that the microwells face the droplet operations gap;

and

- (2) a second substrate, the microwell device including cell traps formed on the second substrate, wherein each cell trap is positioned above a microwell, the cell traps opening onto the droplet operations surface of the microwell device;

(c) a controller configured to execute program instructions to:

- (1) direct the droplet actuator to transport a sample droplet to a microwell device, the sample droplet including cells of interest that enter the cell traps;

- (2) introduce capture beads to the microwells, wherein capture elements are immobilized on the capture beads; and

- (3) direct the droplet actuator to transport a cell lysis reagent droplet to the microwell device, wherein portions of the lysis reagent droplet enter the cell traps and microwells and, during an incubation period, cause the cells of interest to release analyte that is captured by the capture elements on the capture beads.

32. The system of claim 31, wherein the first substrate includes a hydrophobic layer disposed thereon, the hydrophobic layer forming the droplet operations surface.

33. The system of claim 31, wherein the microwells have a size and pitch dimensioned to receive only a single one of the capture beads.