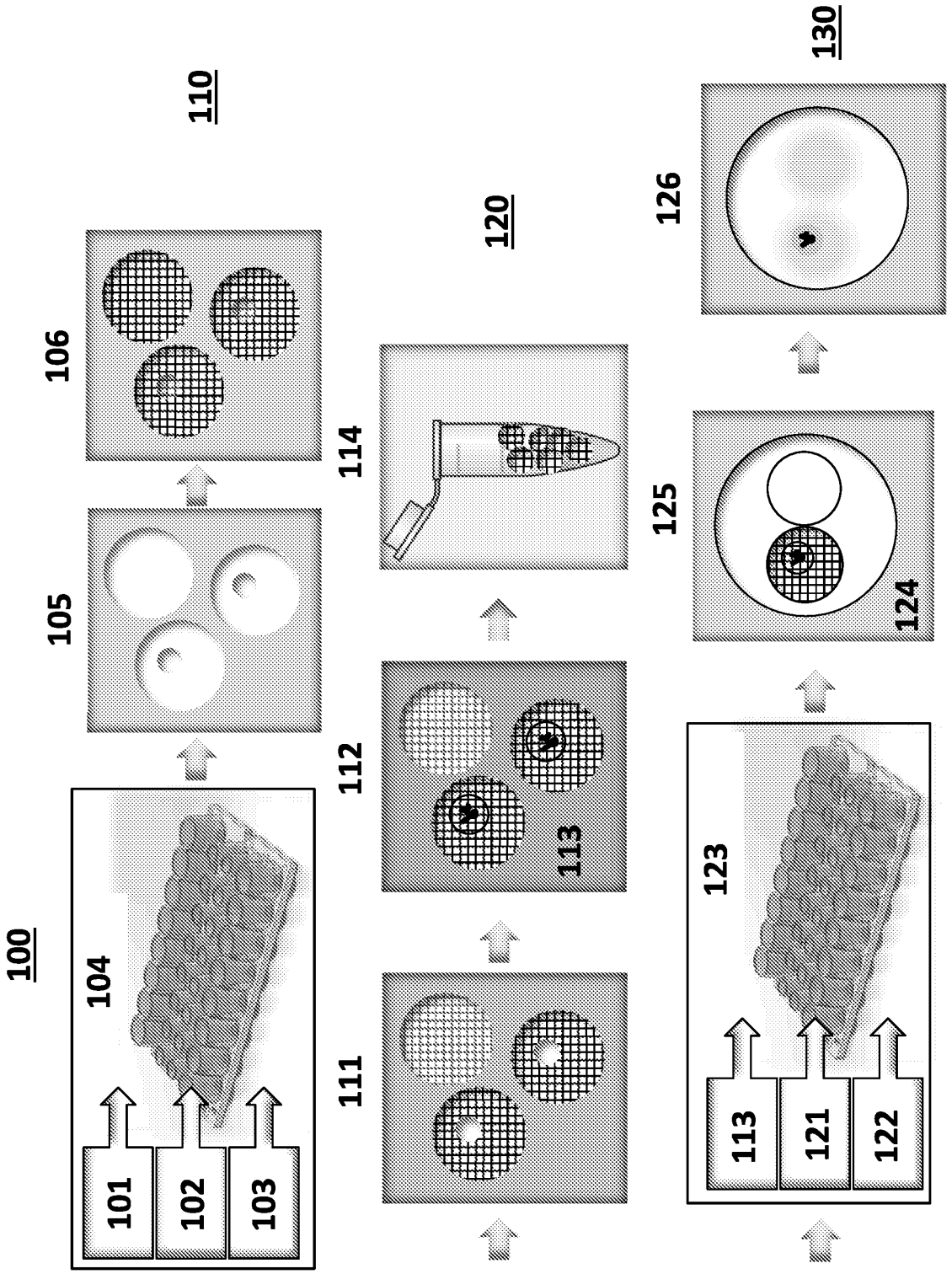

100
104
101
102
103
105
106
110
111
112
113
114
120
123
113
121
122
125
124
126
130

FIG. 1A

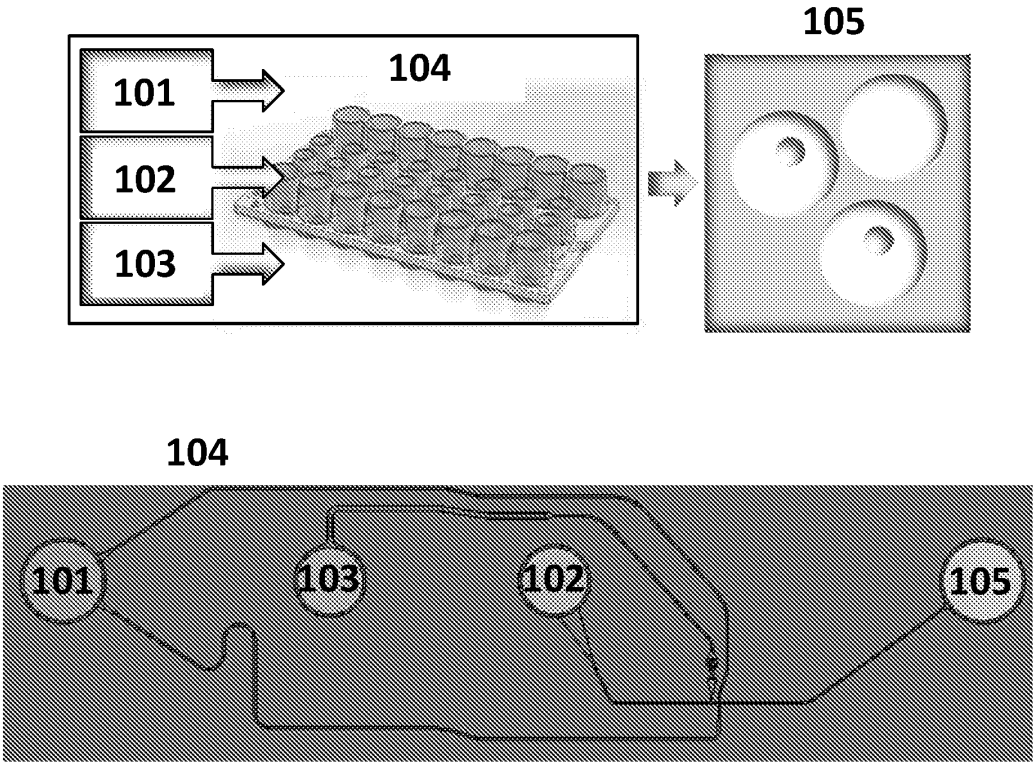
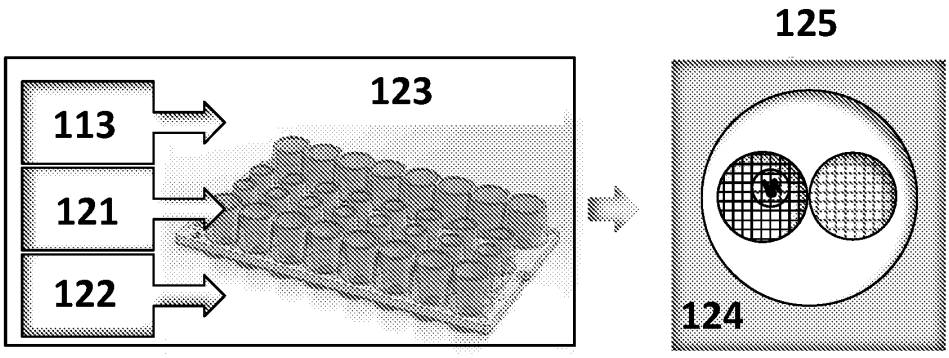


FIG. 1B



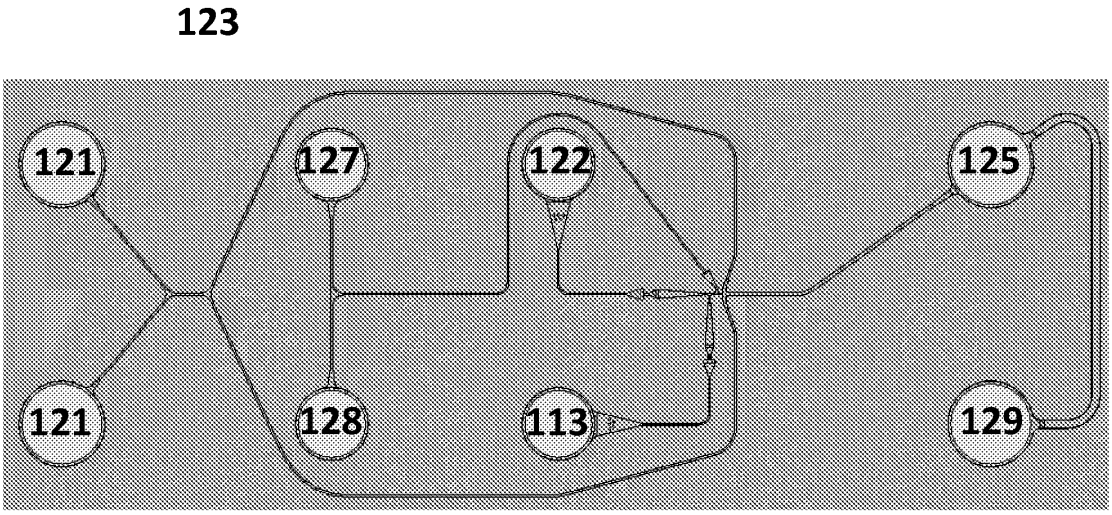


FIG. 1C

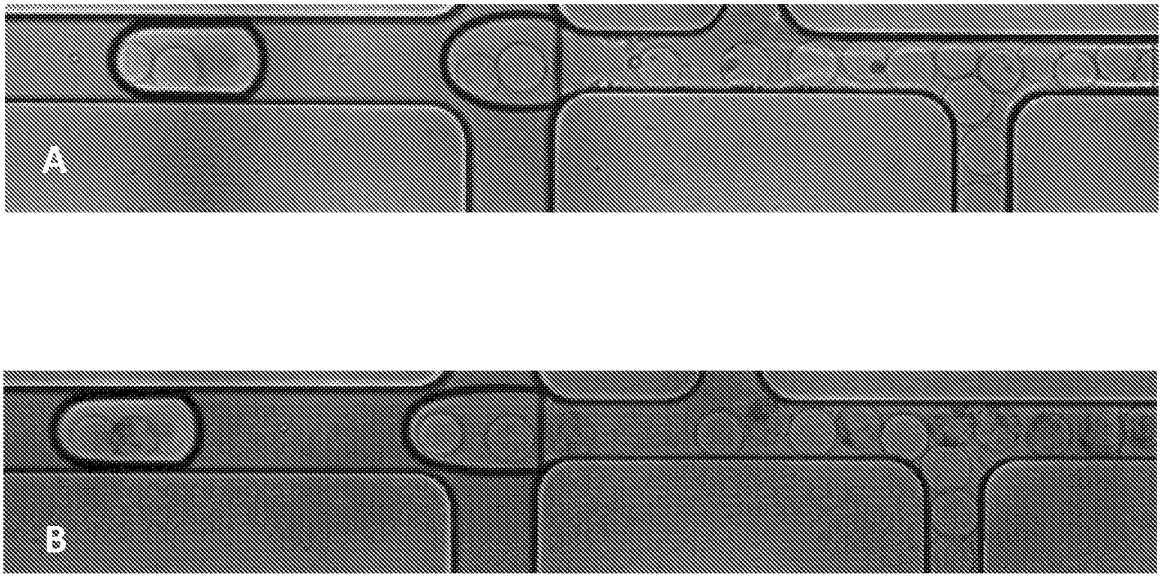


FIG. 1D

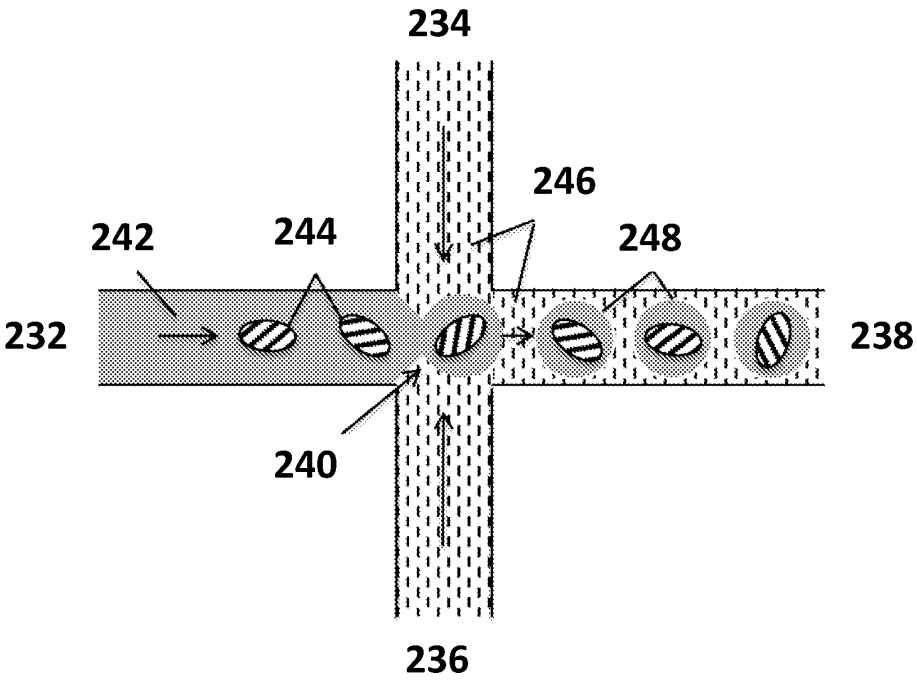


FIG. 2

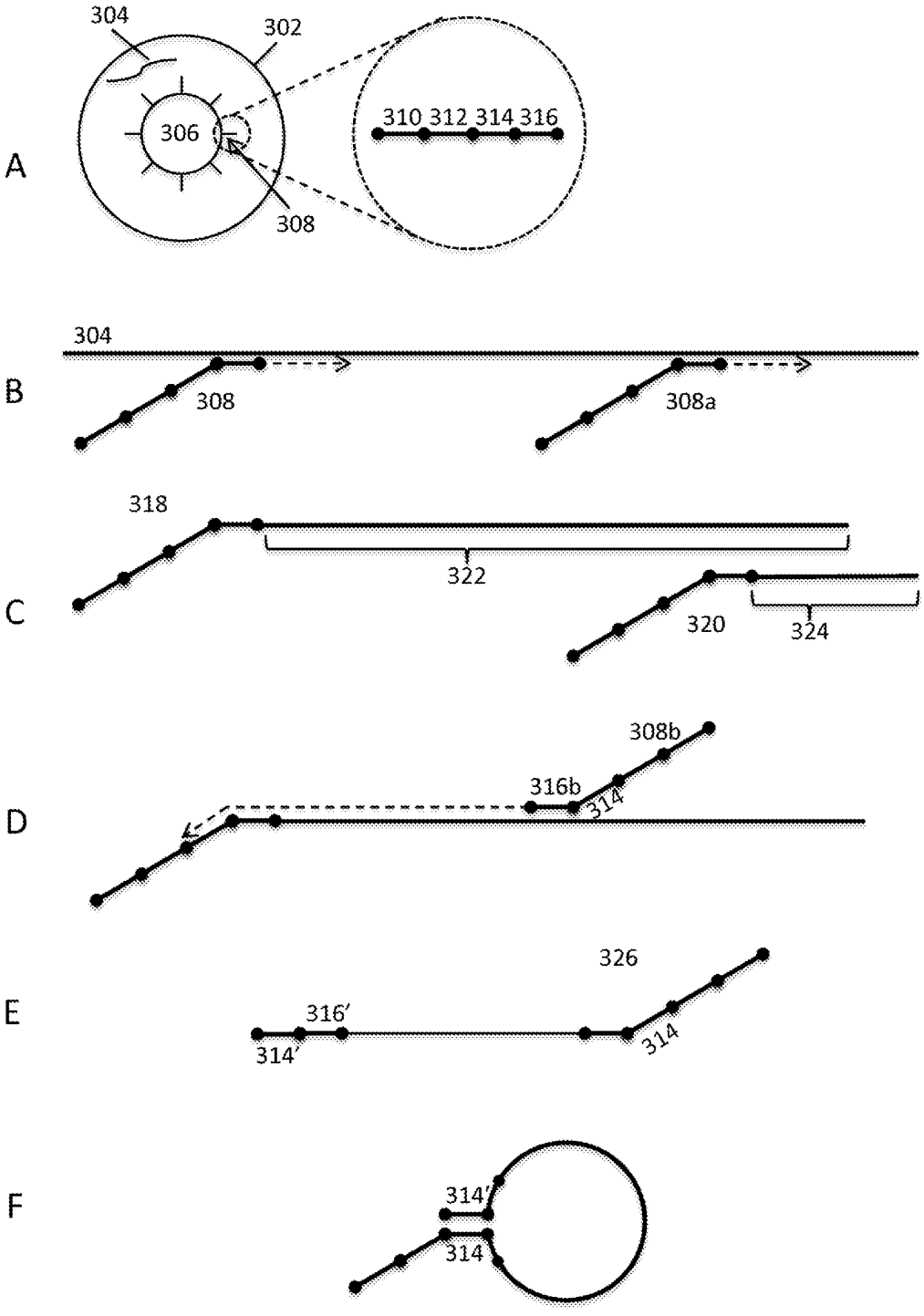

A
304
302
306
308
310 312 314 316
B
304
308
308a
C
318
322
320
324
D
308b
316b
314
E
326
316'
314'
314
F
314'
314

FIG. 3

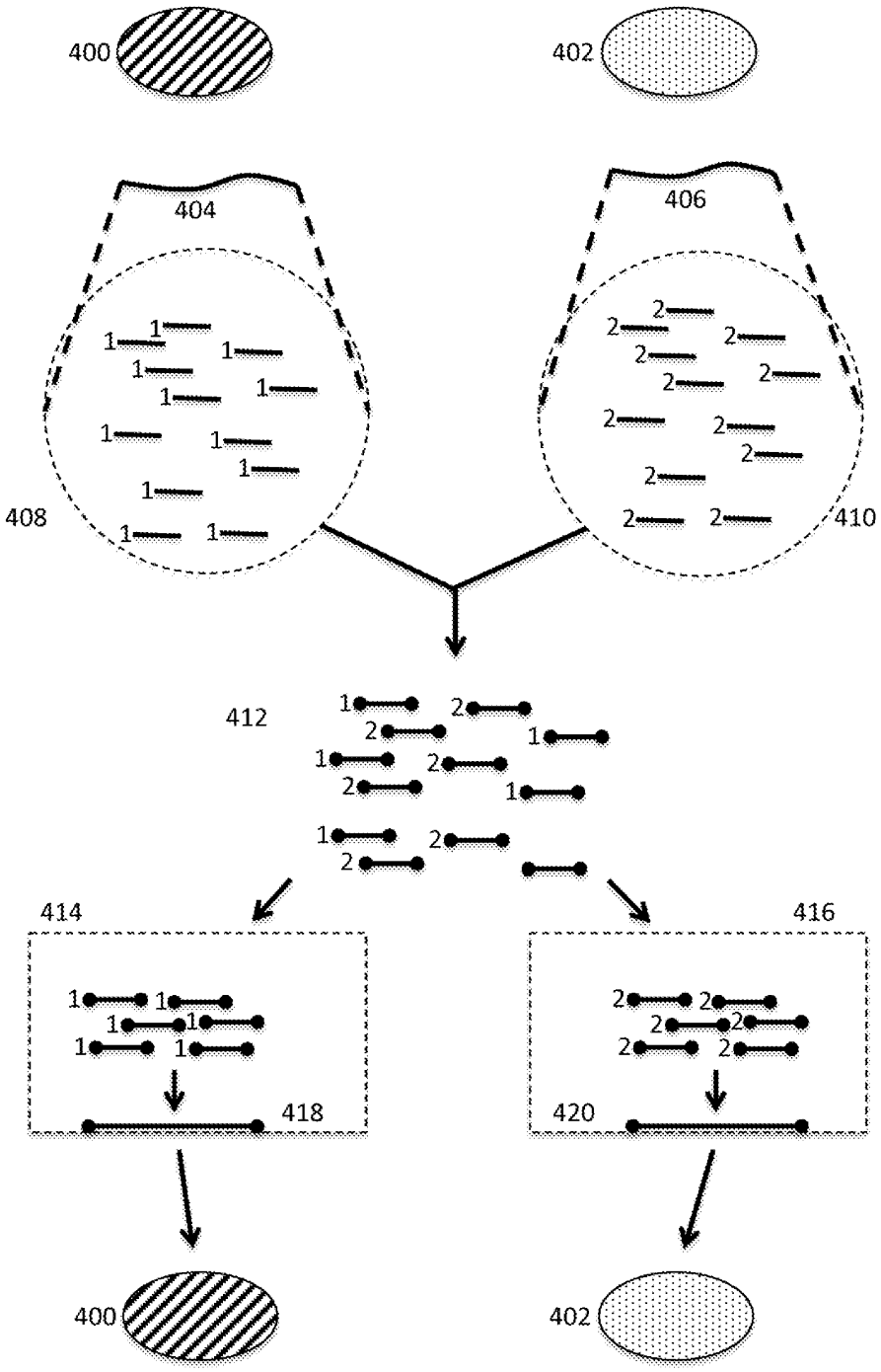


FIG. 4

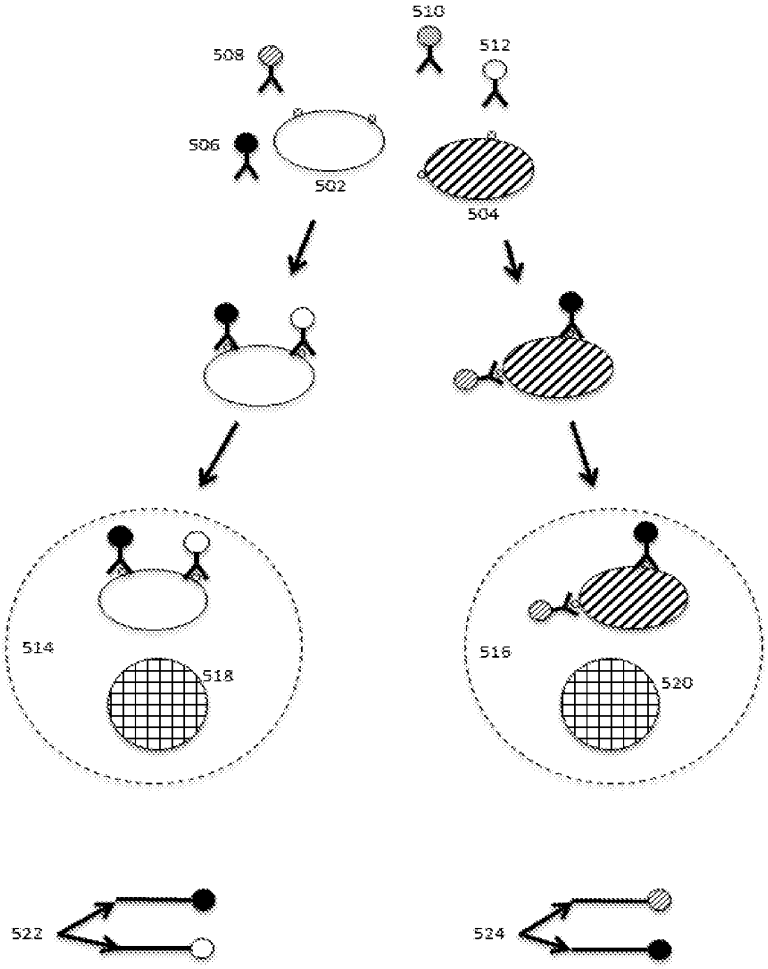


FIG. 5

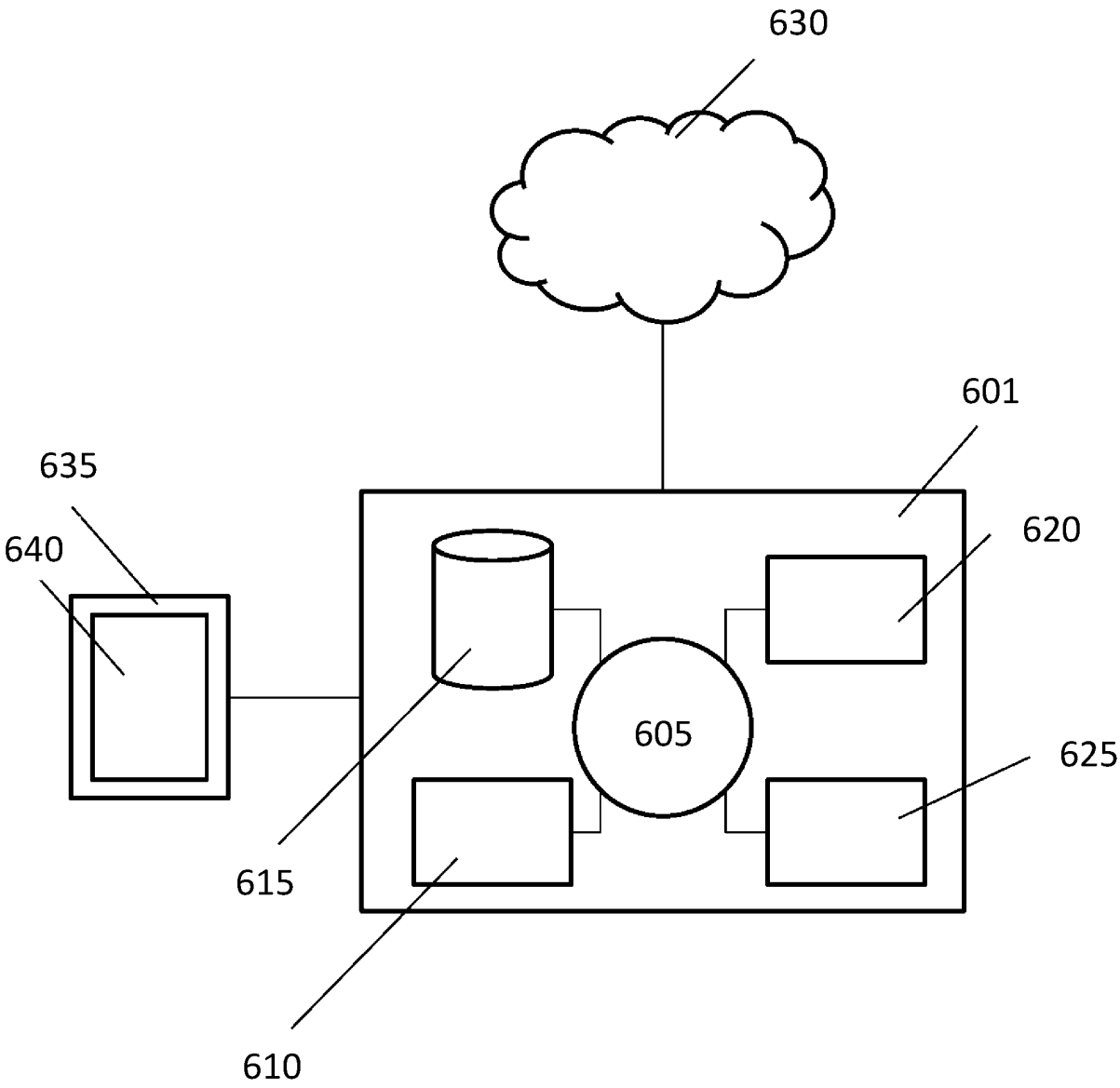


FIG. 6

700

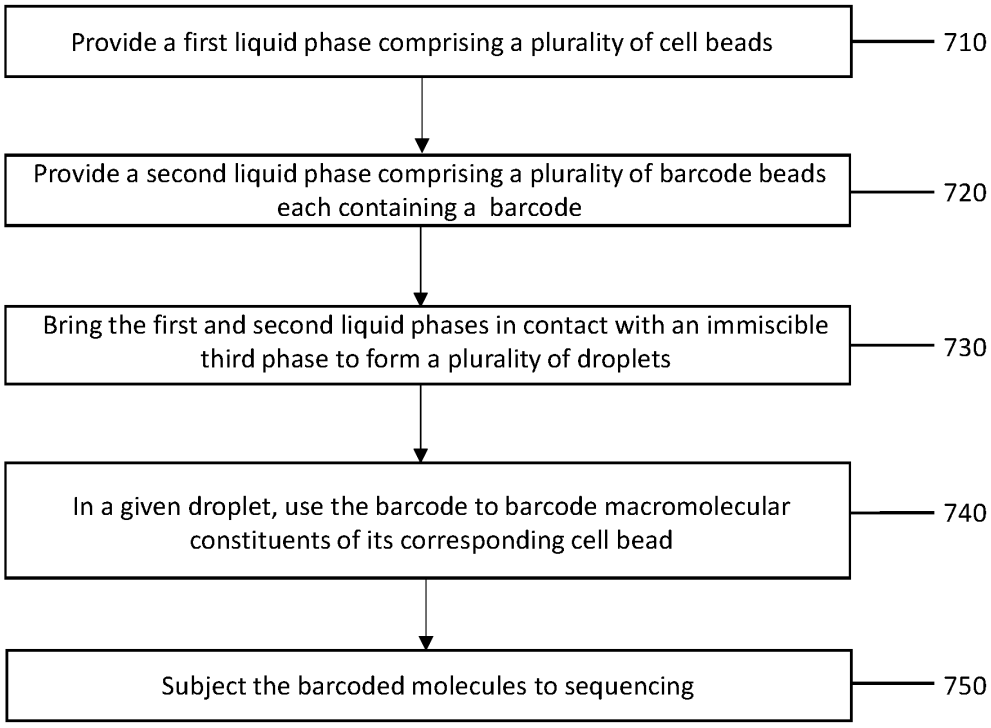

Provide a first liquid phase comprising a plurality of cell beads
710
Provide a second liquid phase comprising a plurality of barcode beads each containing a barcode
720
Bring the first and second liquid phases in contact with an immiscible third phase to form a plurality of droplets
730
In a given droplet, use the barcode to barcode macromolecular constituents of its corresponding cell bead
740
Subject the barcoded molecules to sequencing
750

FIG. 7

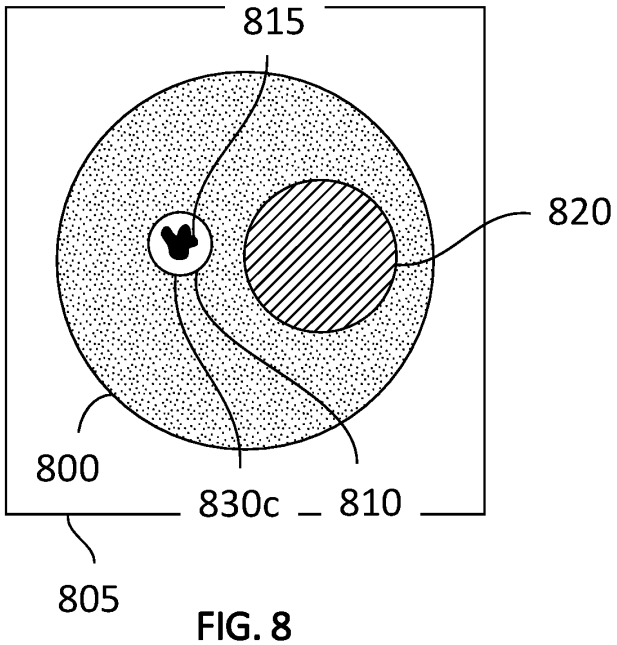


FIG. 8

900

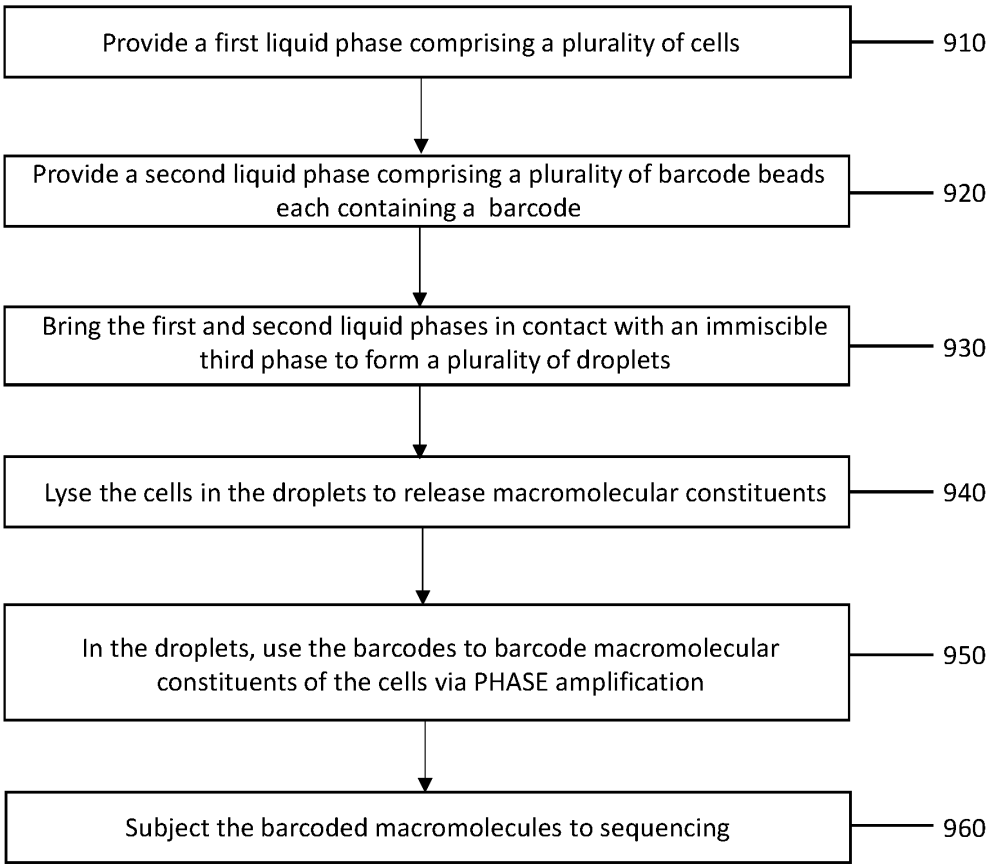

Provide a first liquid phase comprising a plurality of cells
910
Provide a second liquid phase comprising a plurality of barcode beads each containing a barcode
920
Bring the first and second liquid phases in contact with an immiscible third phase to form a plurality of droplets
930
Lyse the cells in the droplets to release macromolecular constituents
940
In the droplets, use the barcodes to barcode macromolecular constituents of the cells via PHASE amplification
950
Subject the barcoded macromolecules to sequencing
960

FIG. 9

1000

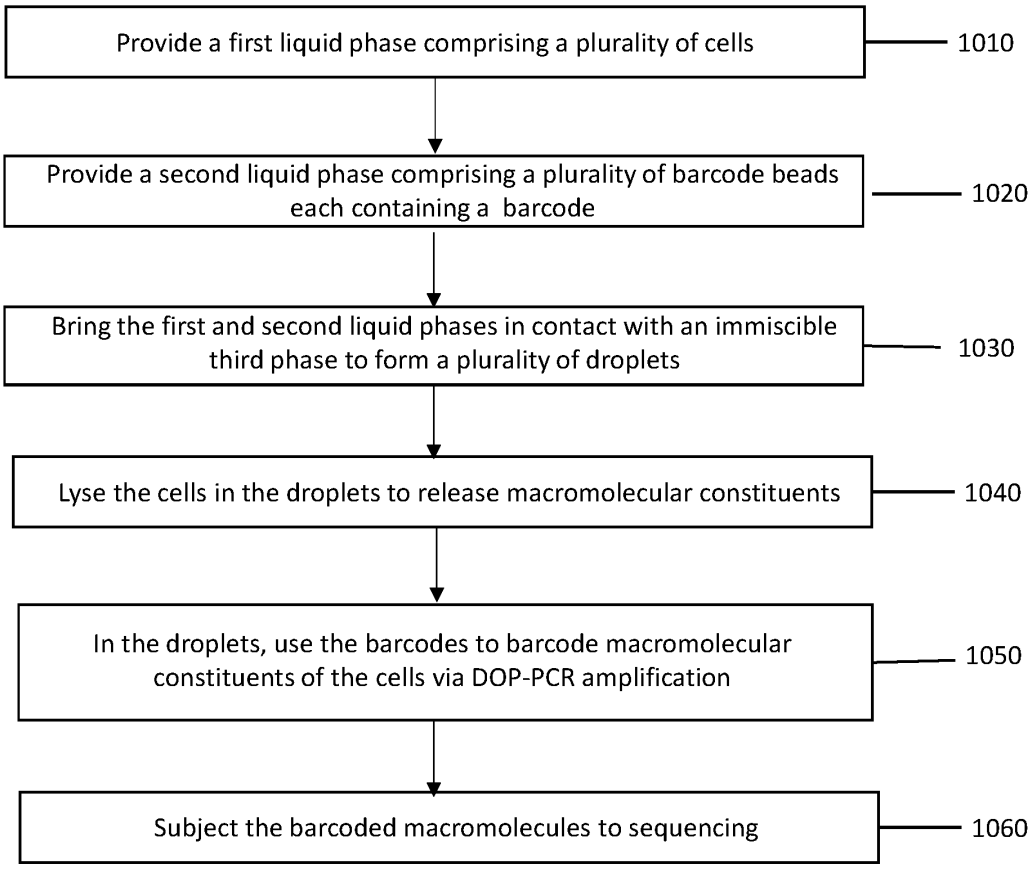

Provide a first liquid phase comprising a plurality of cells
1010
Provide a second liquid phase comprising a plurality of barcode beads each containing a barcode
1020
Bring the first and second liquid phases in contact with an immiscible third phase to form a plurality of droplets
1030
Lyse the cells in the droplets to release macromolecular constituents
1040
In the droplets, use the barcodes to barcode macromolecular constituents of the cells via DOP-PCR amplification
1050
Subject the barcoded macromolecules to sequencing
1060

FIG. 10

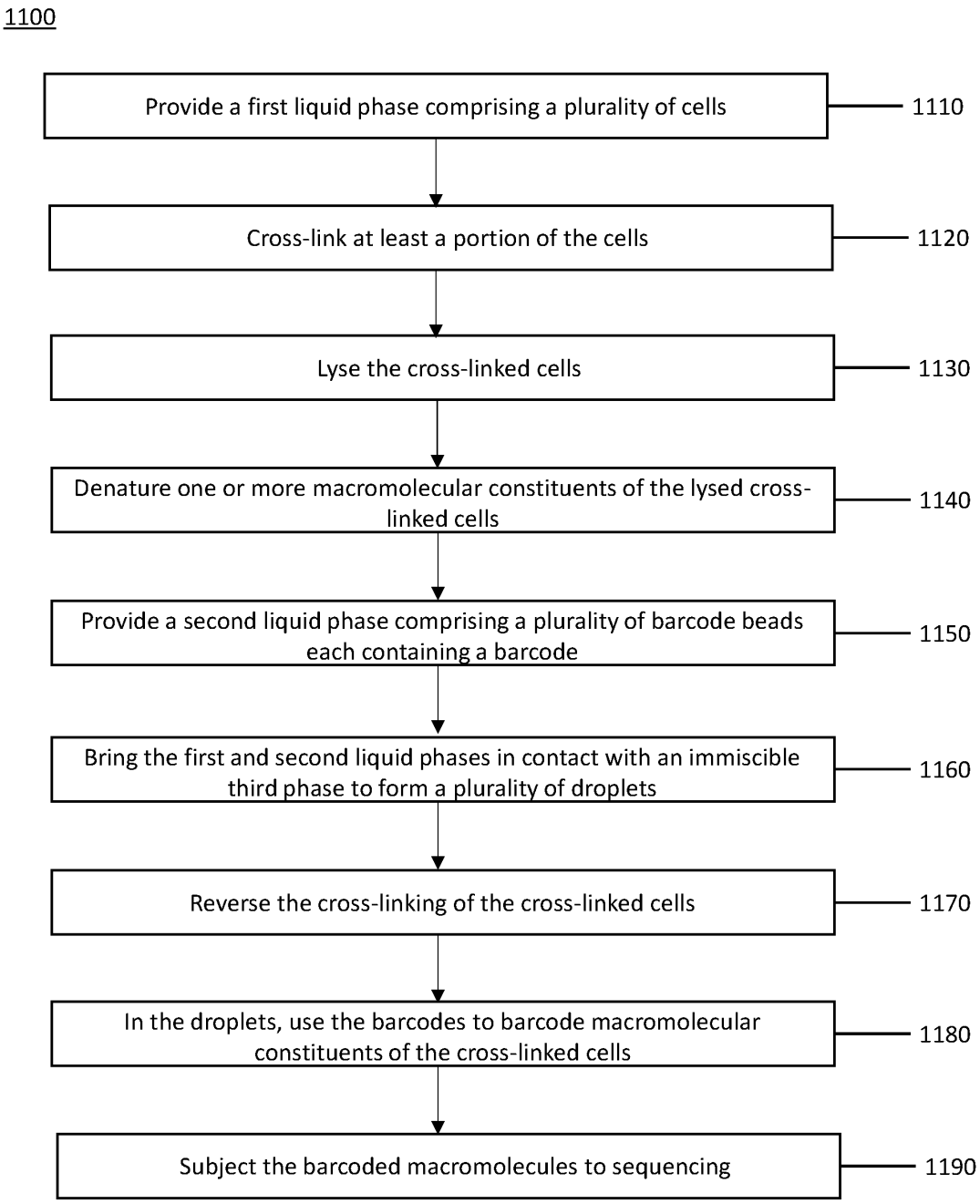

1100
Provide a first liquid phase comprising a plurality of cells
1110
Cross-link at least a portion of the cells
1120
Lyse the cross-linked cells
1130
Denature one or more macromolecular constituents of the lysed cross-linked cells
1140
Provide a second liquid phase comprising a plurality of barcode beads each containing a barcode
1150
Bring the first and second liquid phases in contact with an immiscible third phase to form a plurality of droplets
1160
Reverse the cross-linking of the cross-linked cells
1170
In the droplets, use the barcodes to barcode macromolecular constituents of the cross-linked cells
1180
Subject the barcoded macromolecules to sequencing
1190

FIG. 11

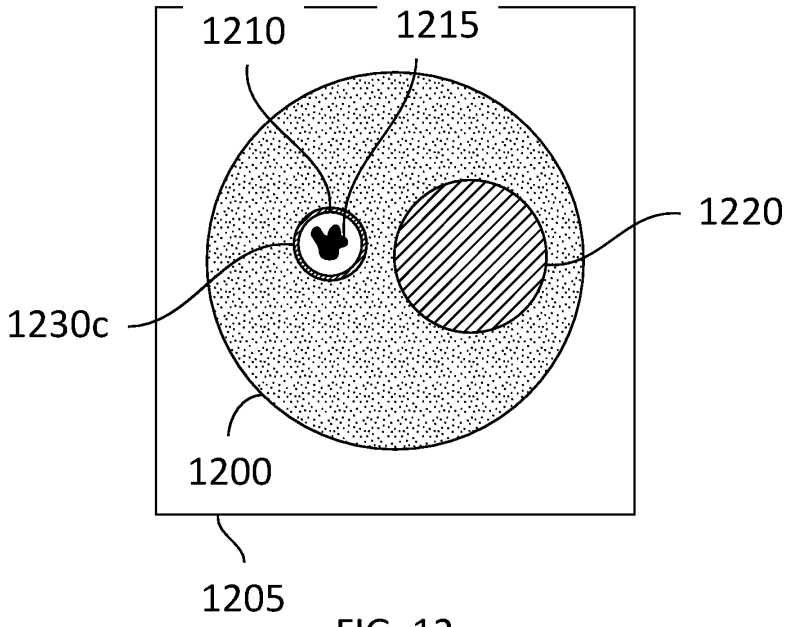


FIG. 12

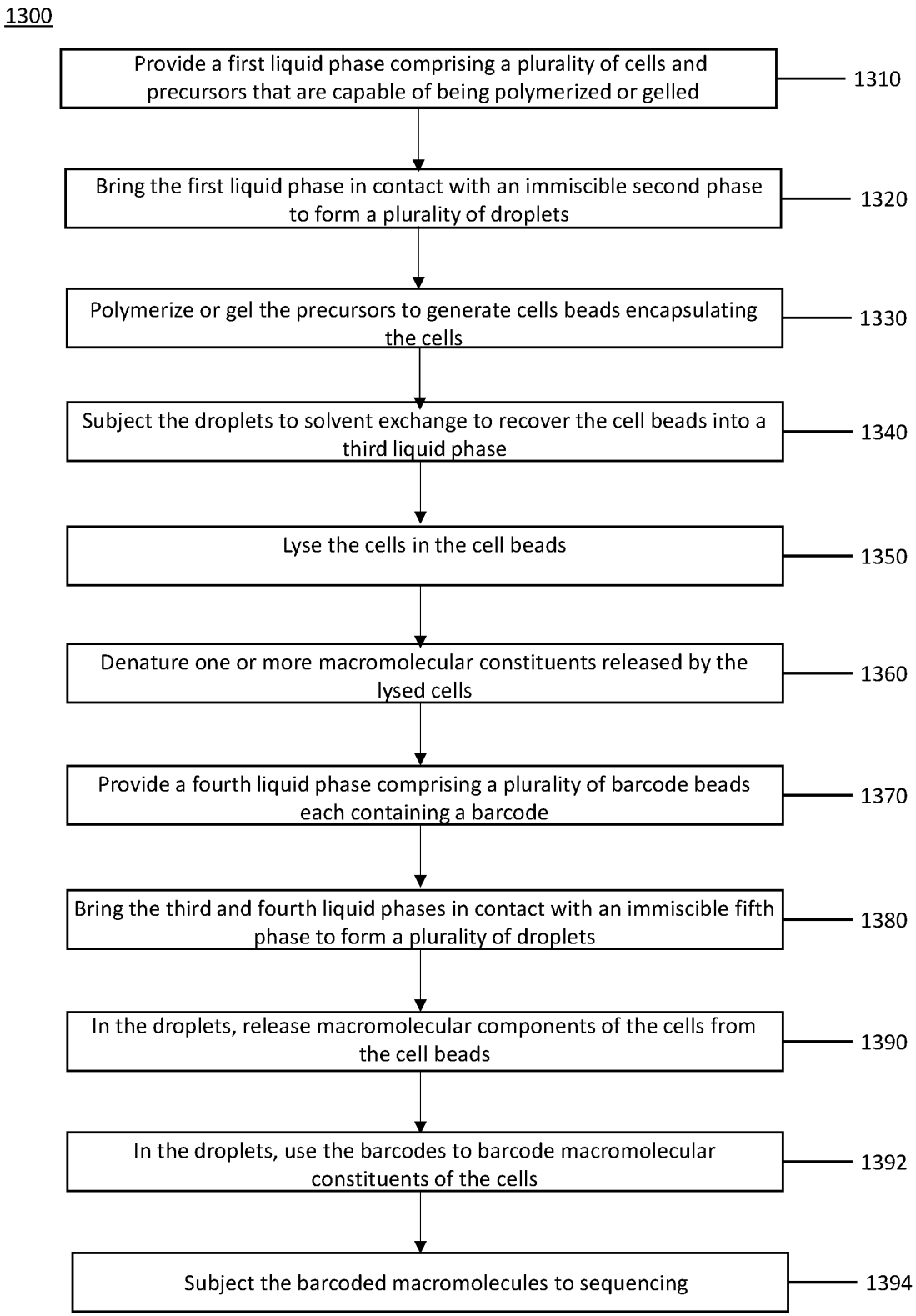

1300
Provide a first liquid phase comprising a plurality of cells and precursors that are capable of being polymerized or gelled
1310
Bring the first liquid phase in contact with an immiscible second phase to form a plurality of droplets
1320
Polymerize or gel the precursors to generate cells beads encapsulating the cells
1330
Subject the droplets to solvent exchange to recover the cell beads into a third liquid phase
1340
Lyse the cells in the cell beads
1350
Denature one or more macromolecular constituents released by the lysed cells
1360
Provide a fourth liquid phase comprising a plurality of barcode beads each containing a barcode
1370
Bring the third and fourth liquid phases in contact with an immiscible fifth phase to form a plurality of droplets
1380
In the droplets, release macromolecular components of the cells from the cell beads
1390
In the droplets, use the barcodes to barcode macromolecular constituents of the cells
1392
Subject the barcoded macromolecules to sequencing
1394

FIG. 13

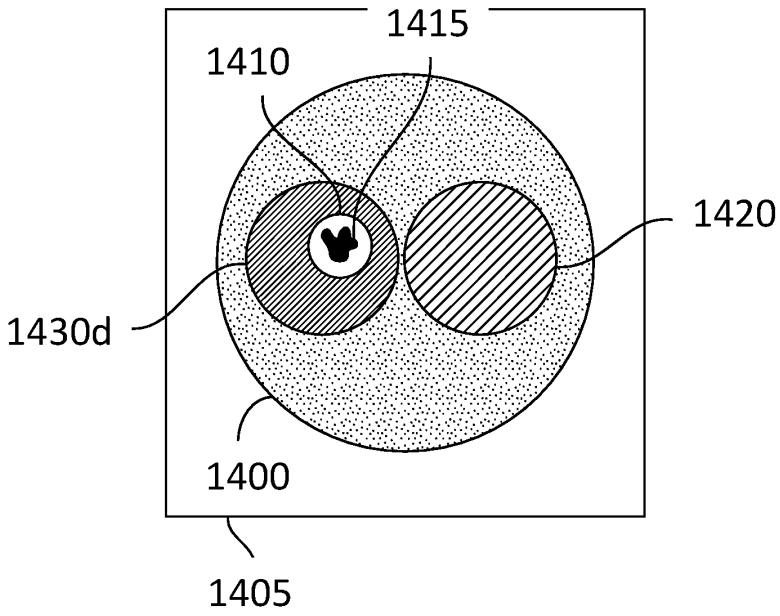


FIG. 14

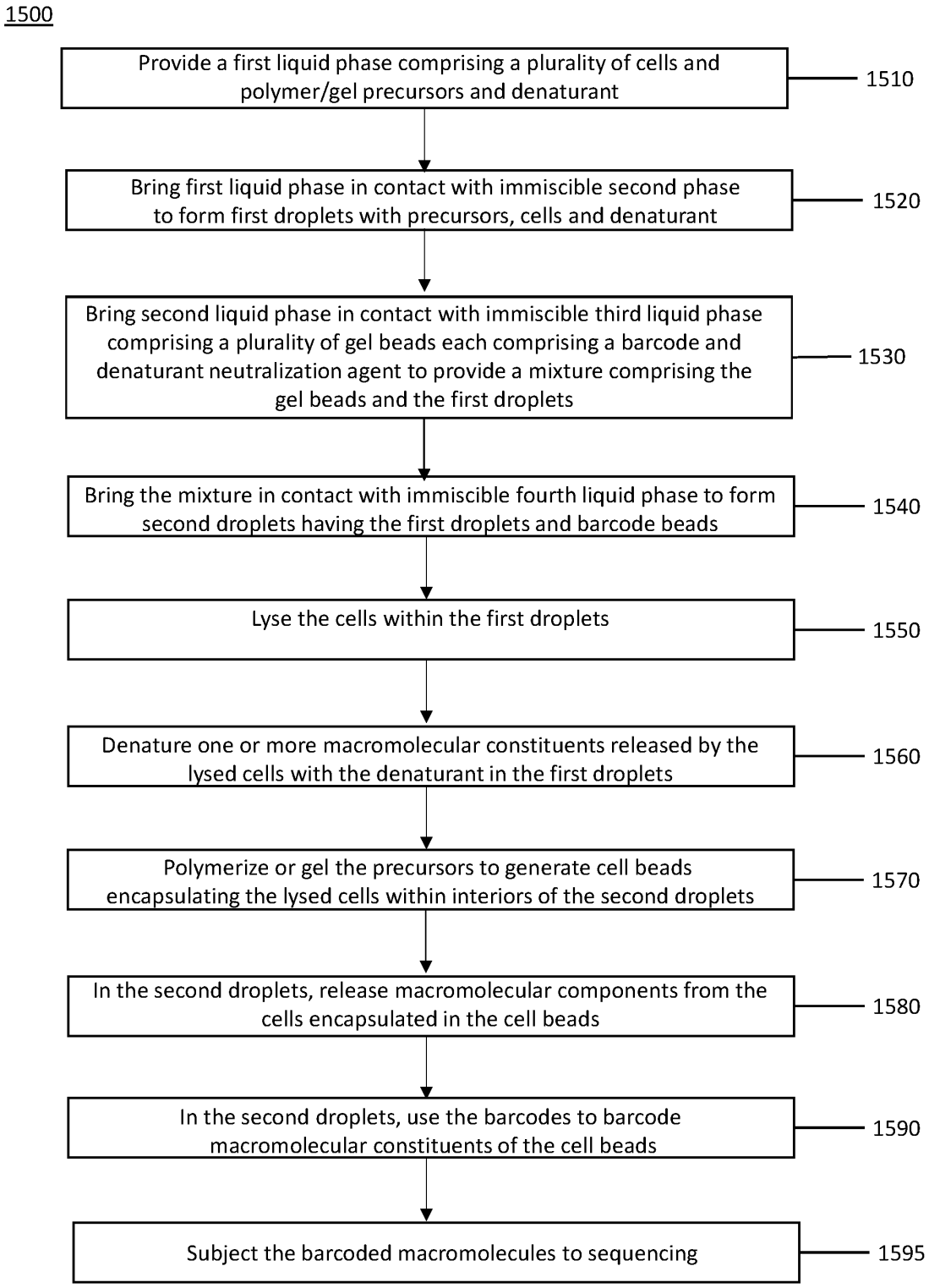

1500
Provide a first liquid phase comprising a plurality of cells and polymer/gel precursors and denaturant
1510
Bring first liquid phase in contact with immiscible second phase to form first droplets with precursors, cells and denaturant
1520
Bring second liquid phase in contact with immiscible third liquid phase comprising a plurality of gel beads each comprising a barcode and denaturant neutralization agent to provide a mixture comprising the gel beads and the first droplets
1530
Bring the mixture in contact with immiscible fourth liquid phase to form second droplets having the first droplets and barcode beads
1540
Lyse the cells within the first droplets
1550
Denature one or more macromolecular constituents released by the lysed cells with the denaturant in the first droplets
1560
Polymerize or gel the precursors to generate cell beads encapsulating the lysed cells within interiors of the second droplets
1570
In the second droplets, release macromolecular components from the cells encapsulated in the cell beads
1580
In the second droplets, use the barcodes to barcode macromolecular constituents of the cell beads
1590
Subject the barcoded macromolecules to sequencing
1595

FIG. 15

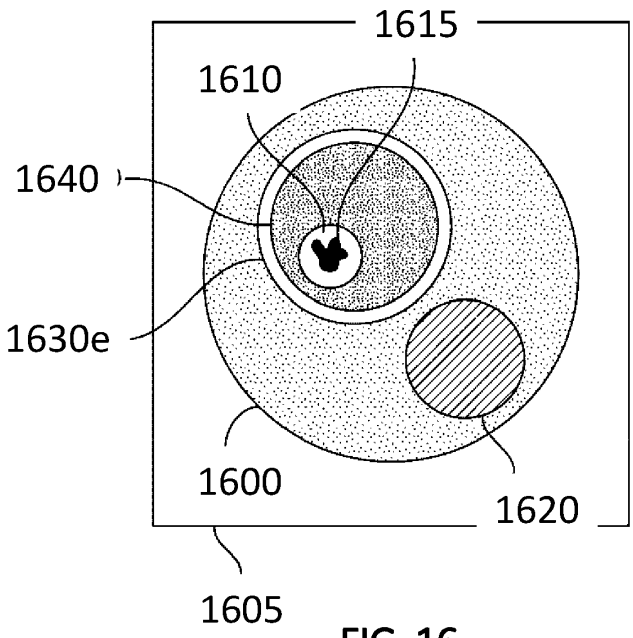


FIG. 16

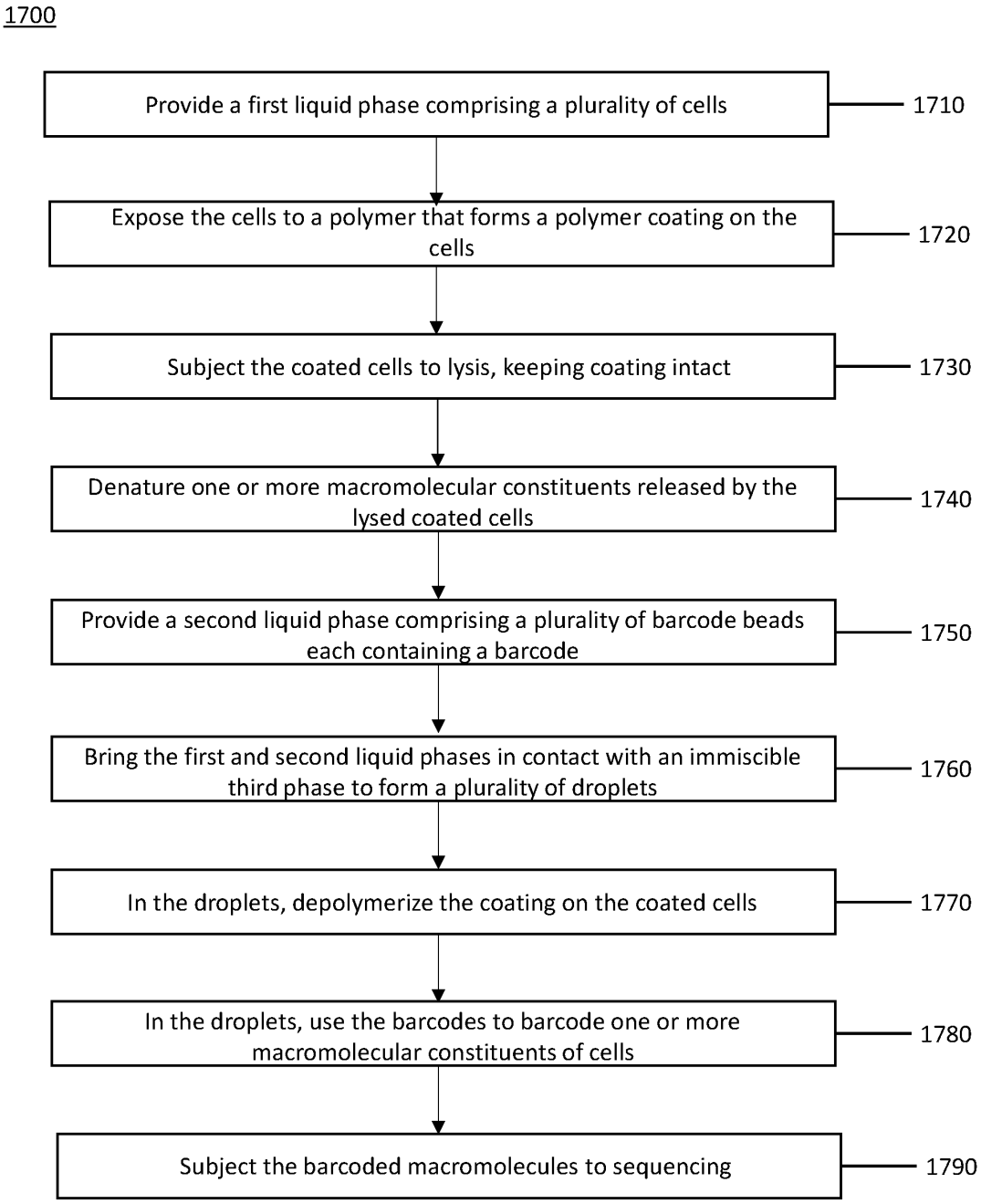

1700
Provide a first liquid phase comprising a plurality of cells
1710
Expose the cells to a polymer that forms a polymer coating on the cells
1720
Subject the coated cells to lysis, keeping coating intact
1730
Denature one or more macromolecular constituents released by the lysed coated cells
1740
Provide a second liquid phase comprising a plurality of barcode beads each containing a barcode
1750
Bring the first and second liquid phases in contact with an immiscible third phase to form a plurality of droplets
1760
In the droplets, depolymerize the coating on the coated cells
1770
In the droplets, use the barcodes to barcode one or more macromolecular constituents of cells
1780
Subject the barcoded macromolecules to sequencing
1790

FIG. 17

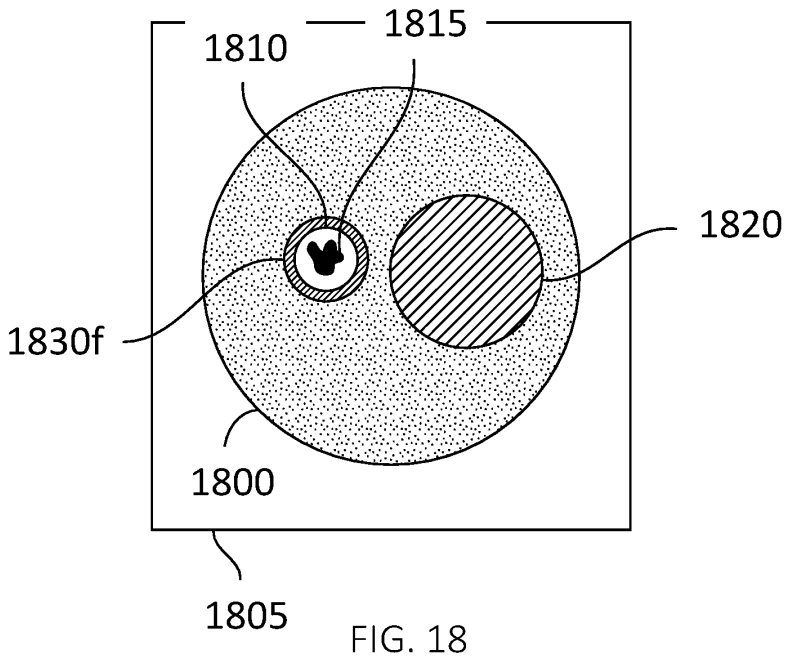


FIG. 18

1900

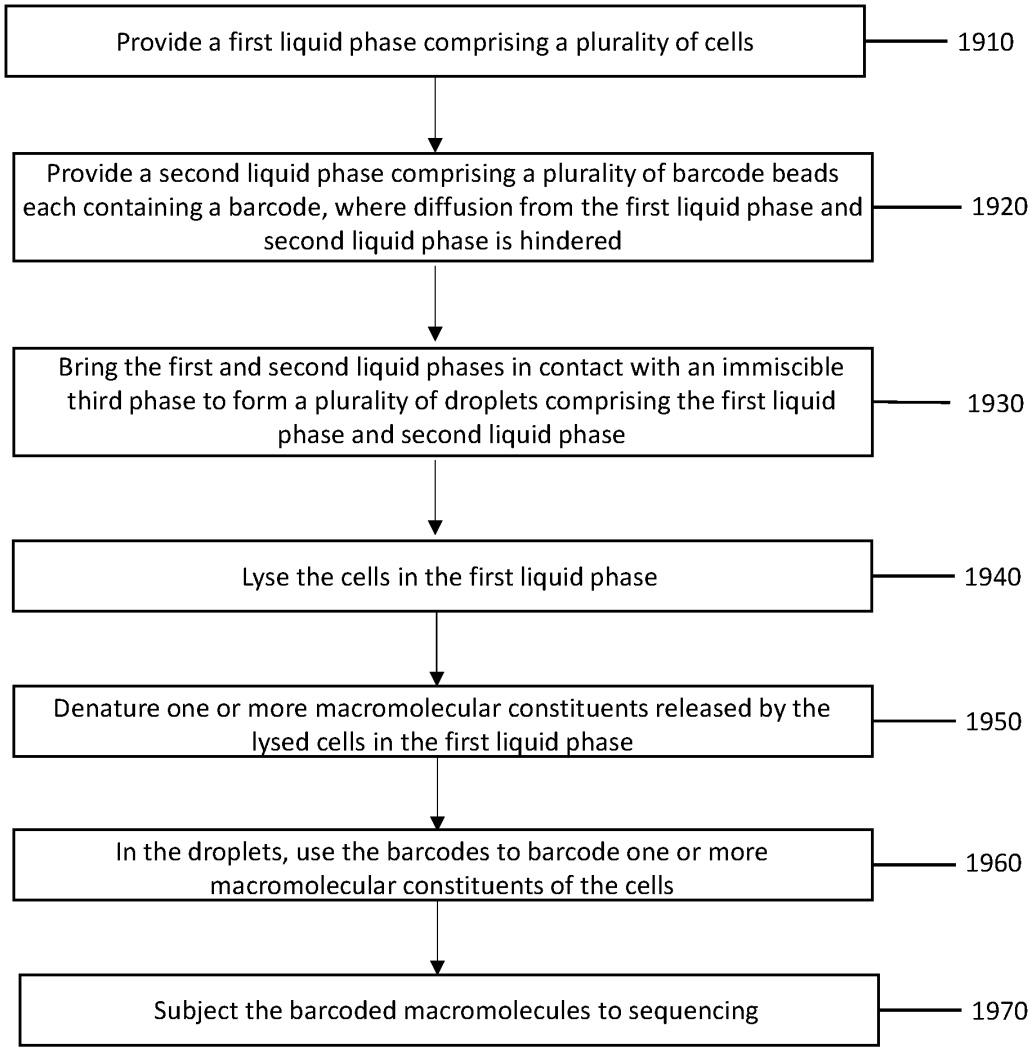

Provide a first liquid phase comprising a plurality of cells
1910
Provide a second liquid phase comprising a plurality of barcode beads each containing a barcode, where diffusion from the first liquid phase and second liquid phase is hindered
1920
Bring the first and second liquid phases in contact with an immiscible third phase to form a plurality of droplets comprising the first liquid phase and second liquid phase
1930
Lyse the cells in the first liquid phase
1940
Denature one or more macromolecular constituents released by the lysed cells in the first liquid phase
1950
In the droplets, use the barcodes to barcode one or more macromolecular constituents of the cells
1960
Subject the barcoded macromolecules to sequencing
1970

FIG. 19

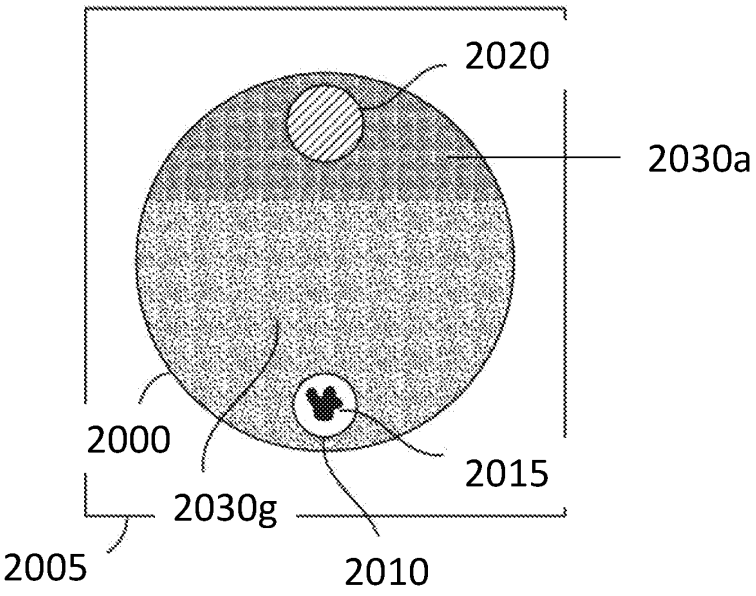


FIG. 20

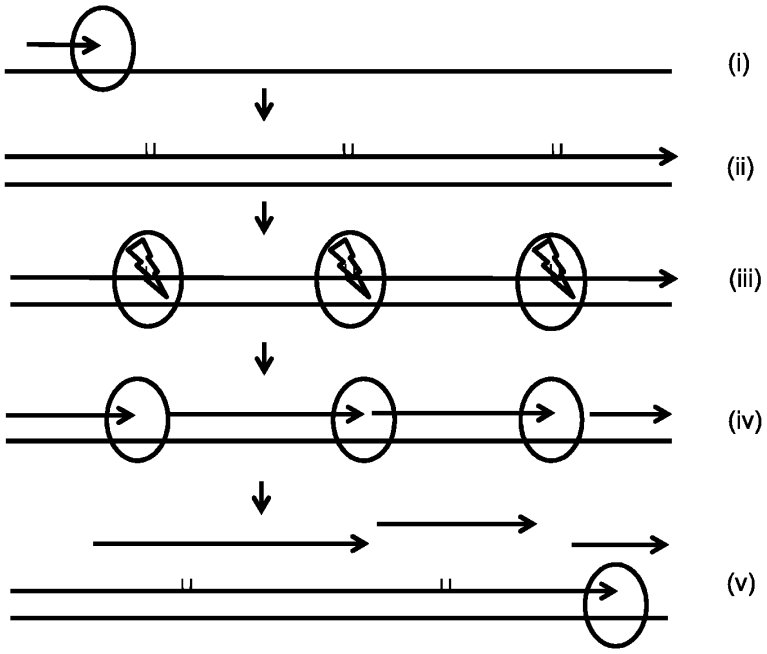


FIG. 21

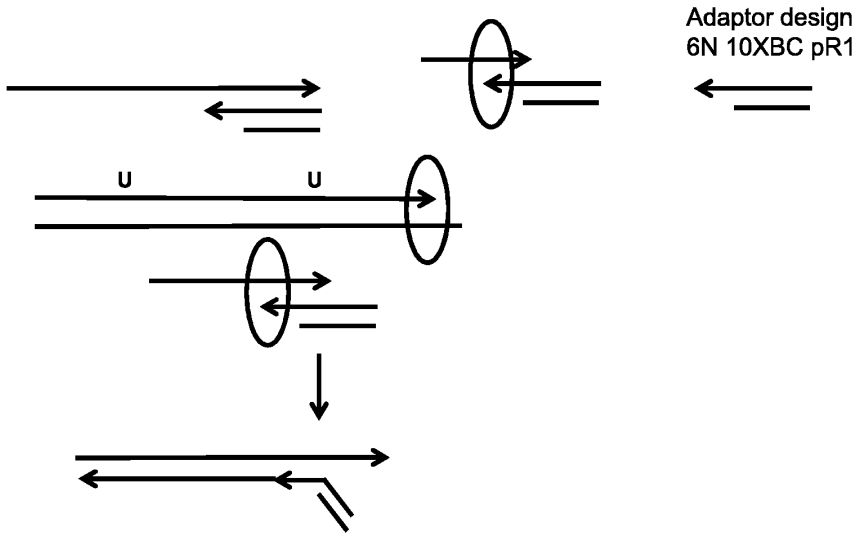


FIG. 22A

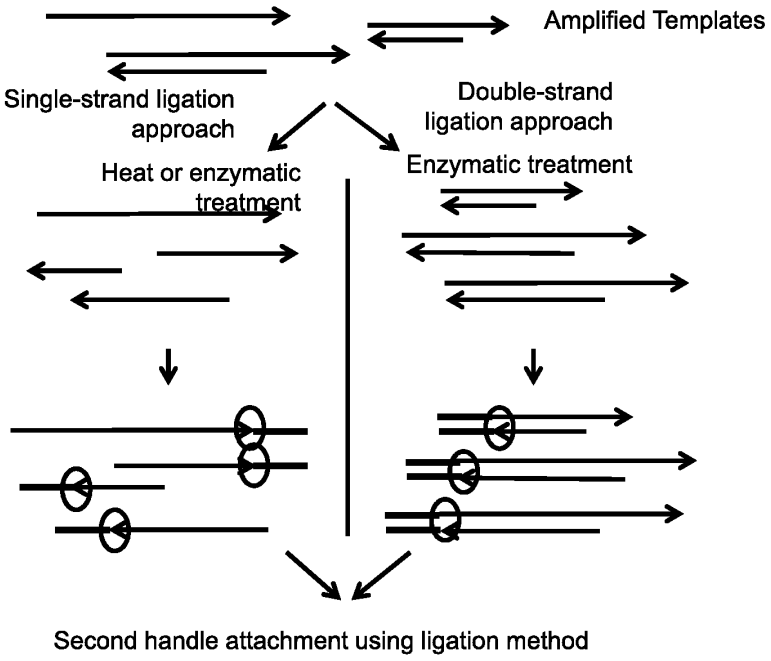

Amplified Templates
Single-strand ligation approach
Double-strand ligation approach
Heat or enzymatic treatment
Enzymatic treatment
Second handle attachment using ligation method

FIG. 22B

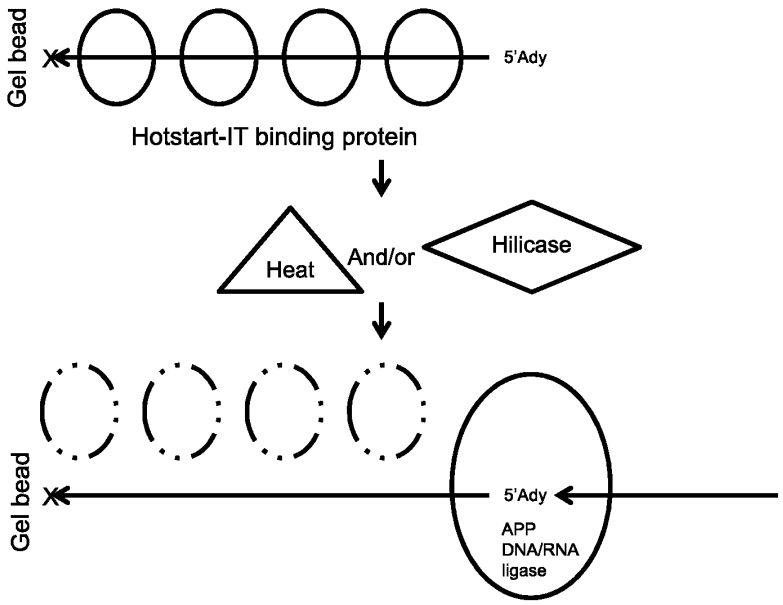

Gel bead
5'Ady
Hotstart-IT binding protein
Heat
And/or
Hilicase
Gel bead
5'Ady
APP
DNA/RNA
ligase

FIG. 22C

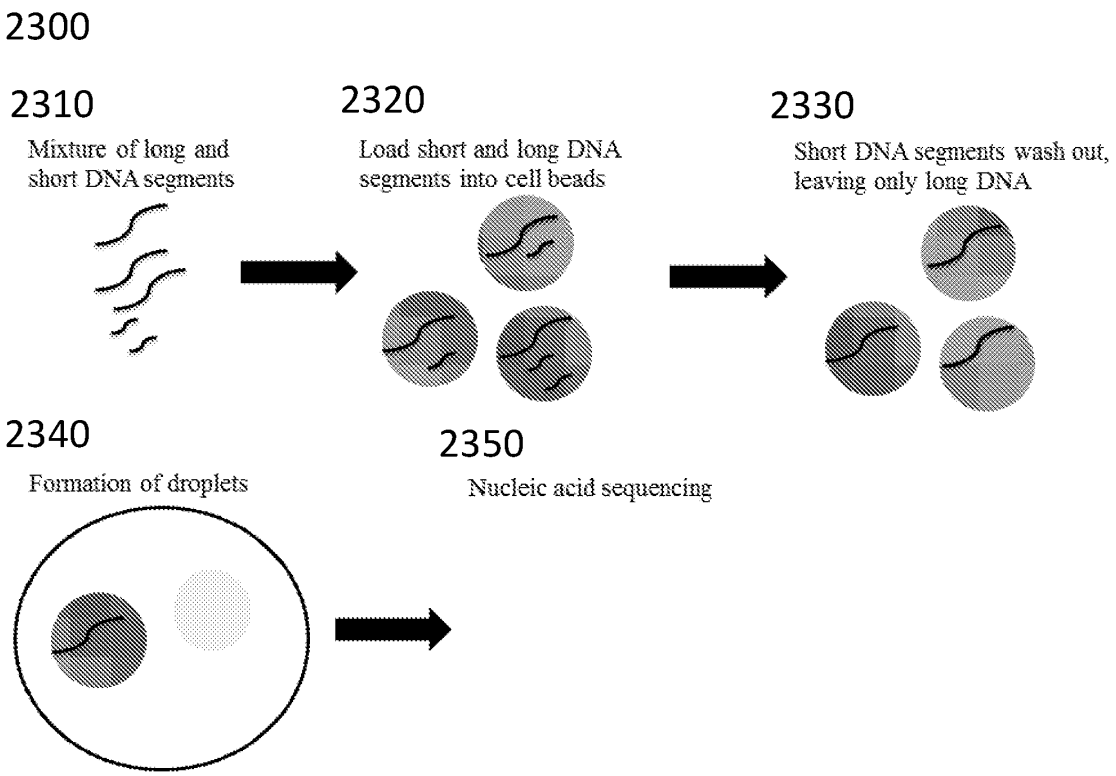

2300
2310
Mixture of long and short DNA segments
2320
Load short and long DNA segments into cell beads
2330
Short DNA segments wash out, leaving only long DNA
2340
Formation of droplets
2350
Nucleic acid sequencing

FIG. 23

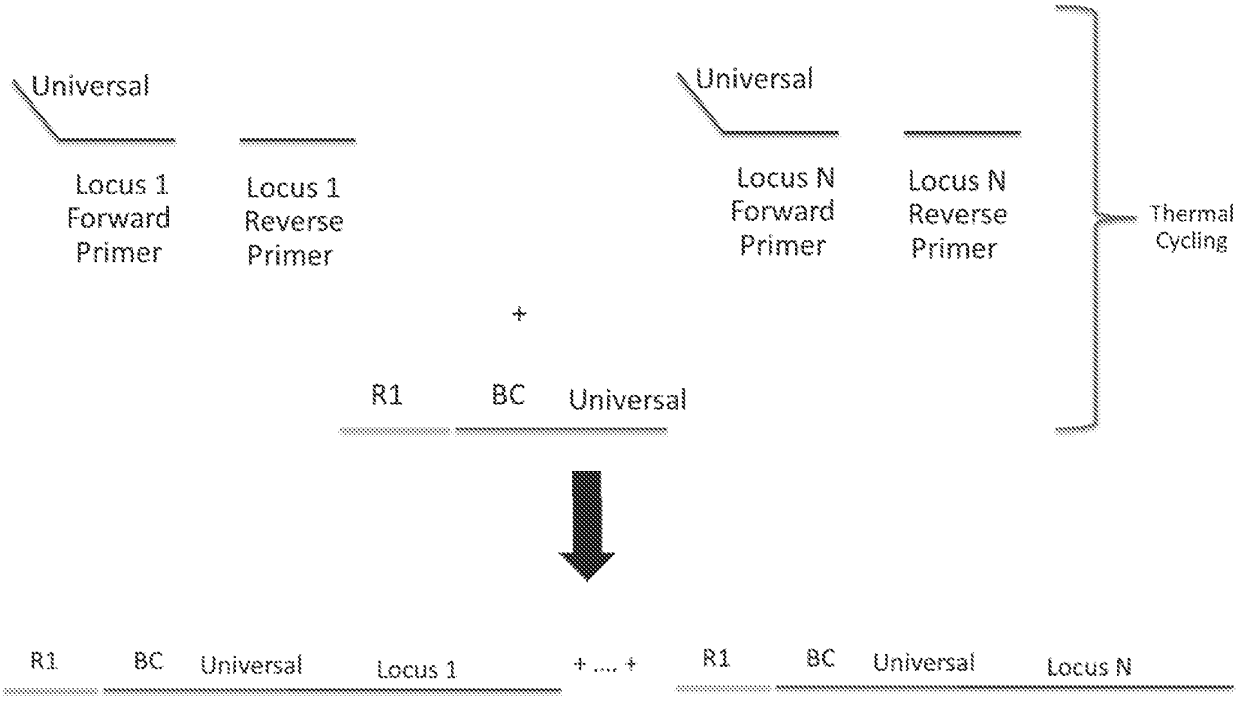

Universal
Locus 1 Forward Primer
Locus 1 Reverse Primer
Universal
Locus N Forward Primer
Locus N Reverse Primer
Thermal Cycling
+
R1
BC
Universal
R1
BC
Universal
Locus 1
+ .... +
R1
BC
Universal
Locus N

FIG. 24

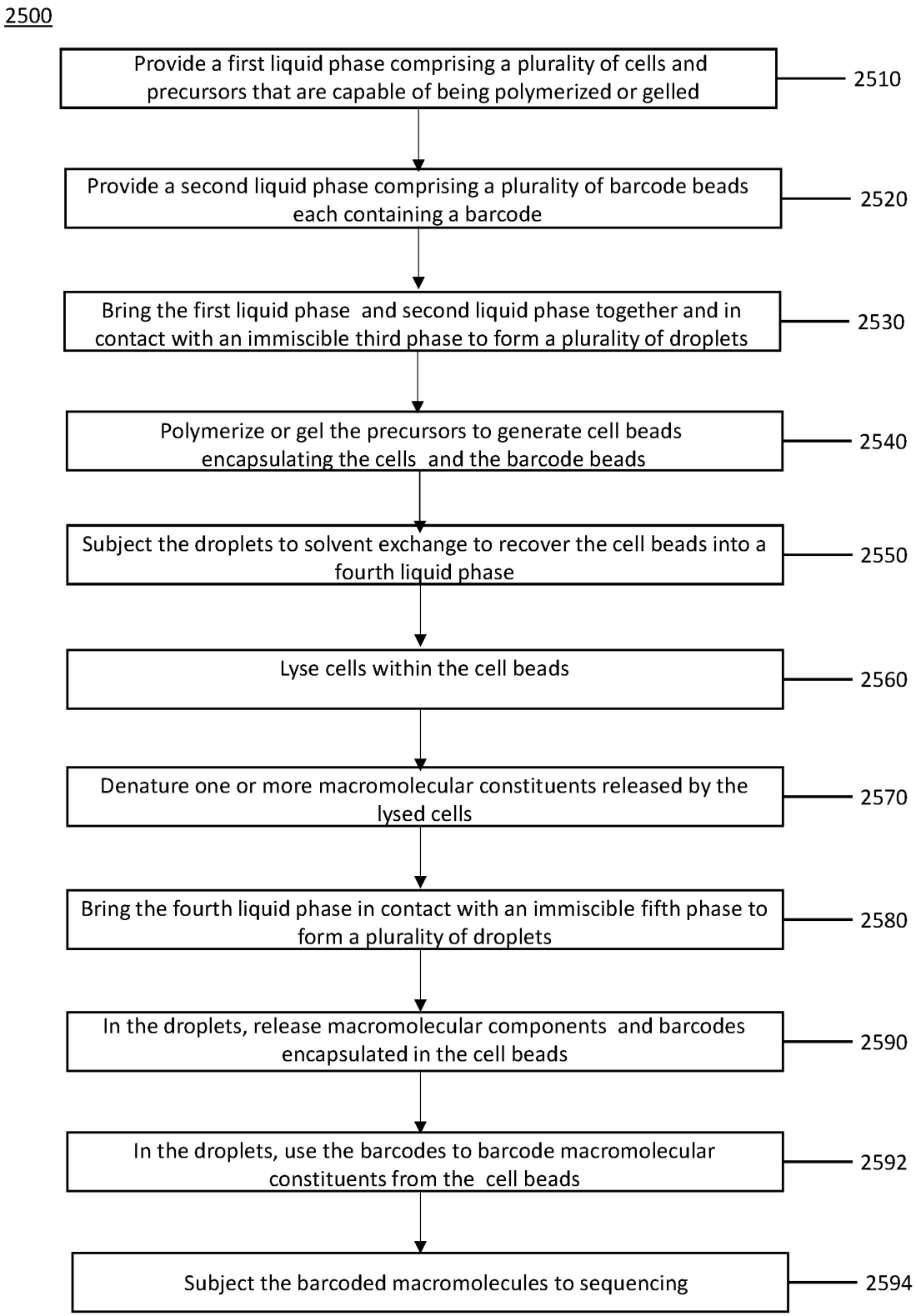

2500
Provide a first liquid phase comprising a plurality of cells and precursors that are capable of being polymerized or gelled
2510
Provide a second liquid phase comprising a plurality of barcode beads each containing a barcode
2520
Bring the first liquid phase and second liquid phase together and in contact with an immiscible third phase to form a plurality of droplets
2530
Polymerize or gel the precursors to generate cell beads encapsulating the cells and the barcode beads
2540
Subject the droplets to solvent exchange to recover the cell beads into a fourth liquid phase
2550
Lyse cells within the cell beads
2560
Denature one or more macromolecular constituents released by the lysed cells
2570
Bring the fourth liquid phase in contact with an immiscible fifth phase to form a plurality of droplets
2580
In the droplets, release macromolecular components and barcodes encapsulated in the cell beads
2590
In the droplets, use the barcodes to barcode macromolecular constituents from the cell beads
2592
Subject the barcoded macromolecules to sequencing
2594

FIG. 25

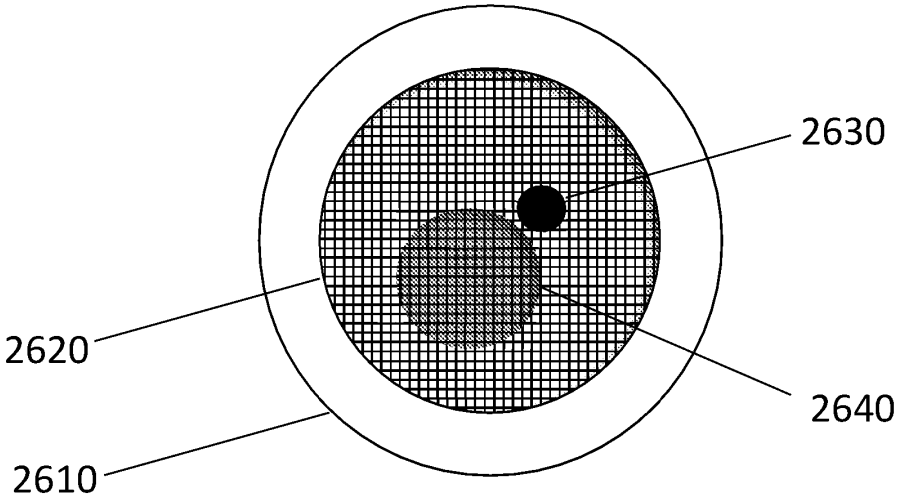


FIG. 26A

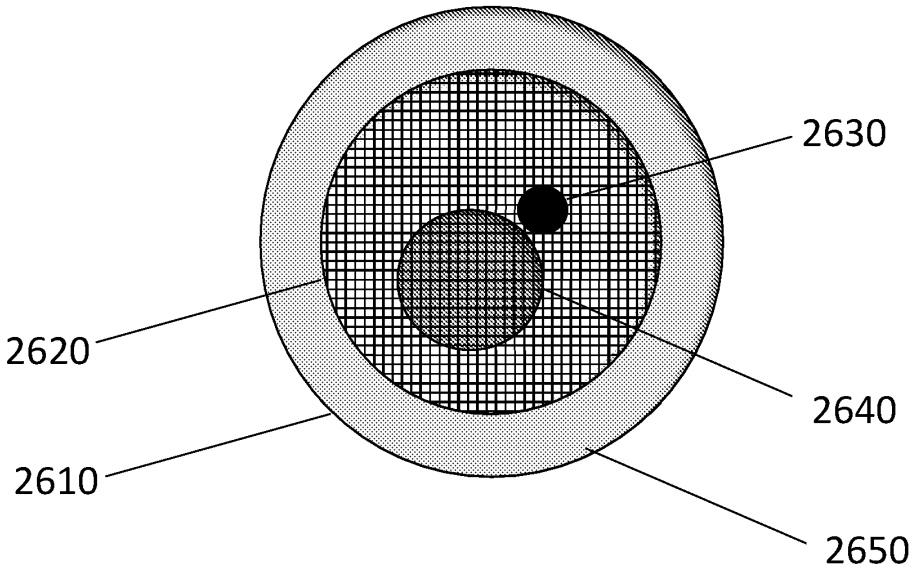


FIG. 26B

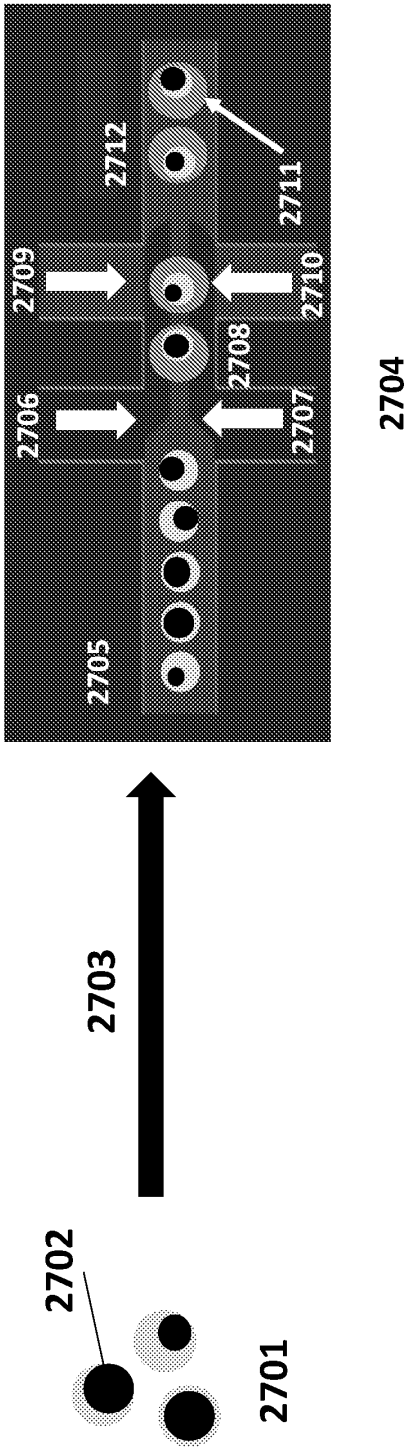


FIG. 27

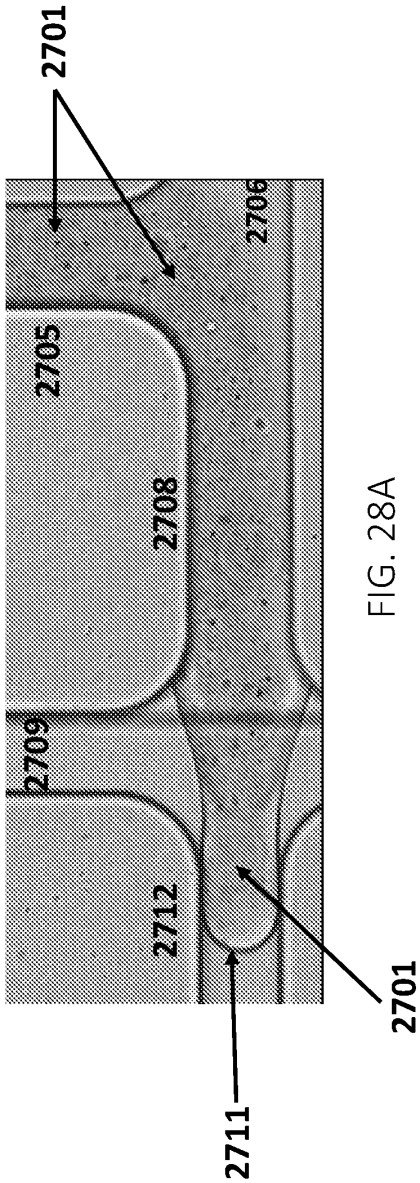


FIG. 28A

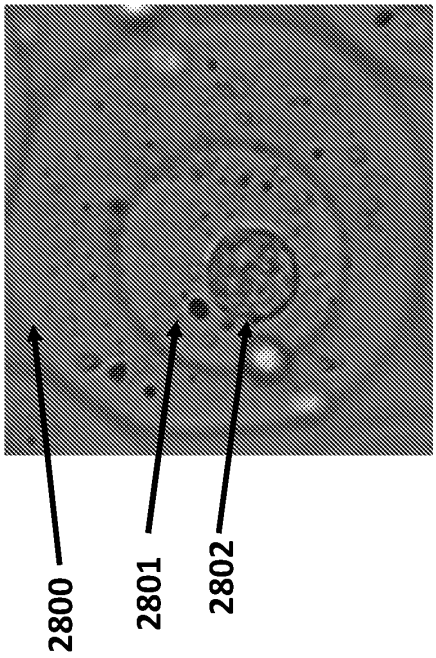


FIG. 28B

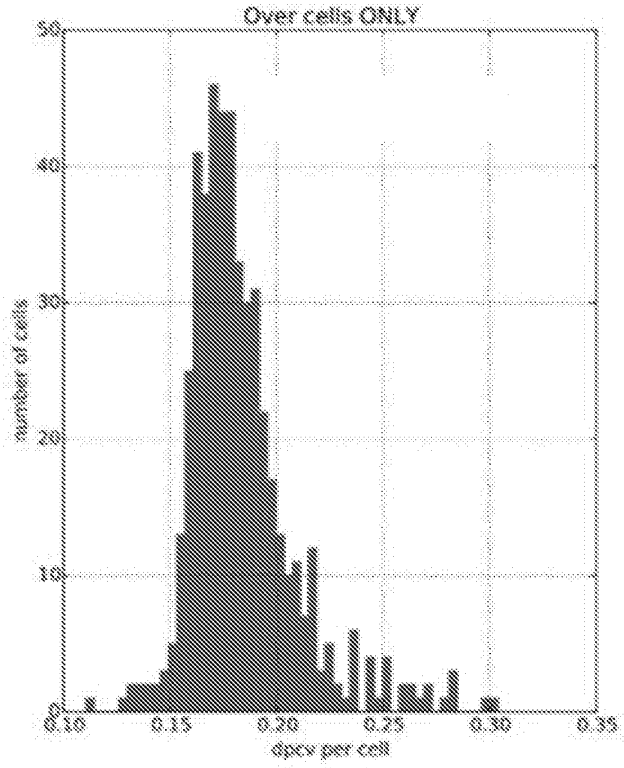

Over cells ONLY
50
40
30
20
10
0
number of cells
0.10
0.15
0.20
0.25
0.30
0.35
dpcv per cell

FIG. 29

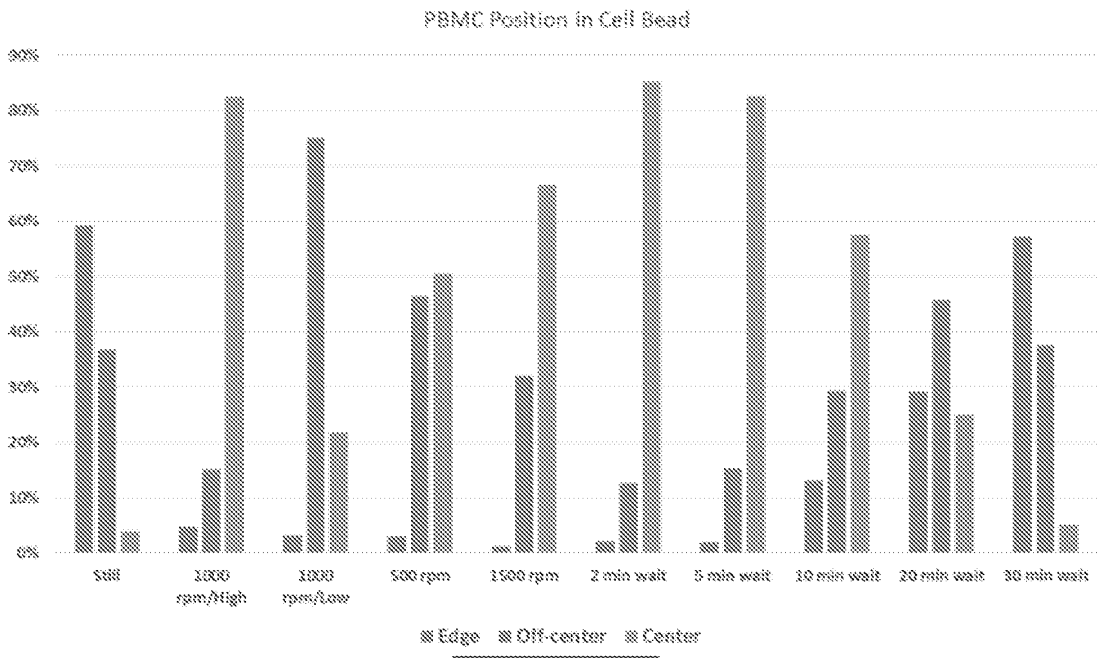

PBMC Position in Cell Bead
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Still
1000 rpm/High
1000 rpm/Low
500 rpm
1500 rpm
2 min wait
5 min wait
10 min wait
20 min wait
30 min wait
Edge
Off-center
Center

FIG. 30

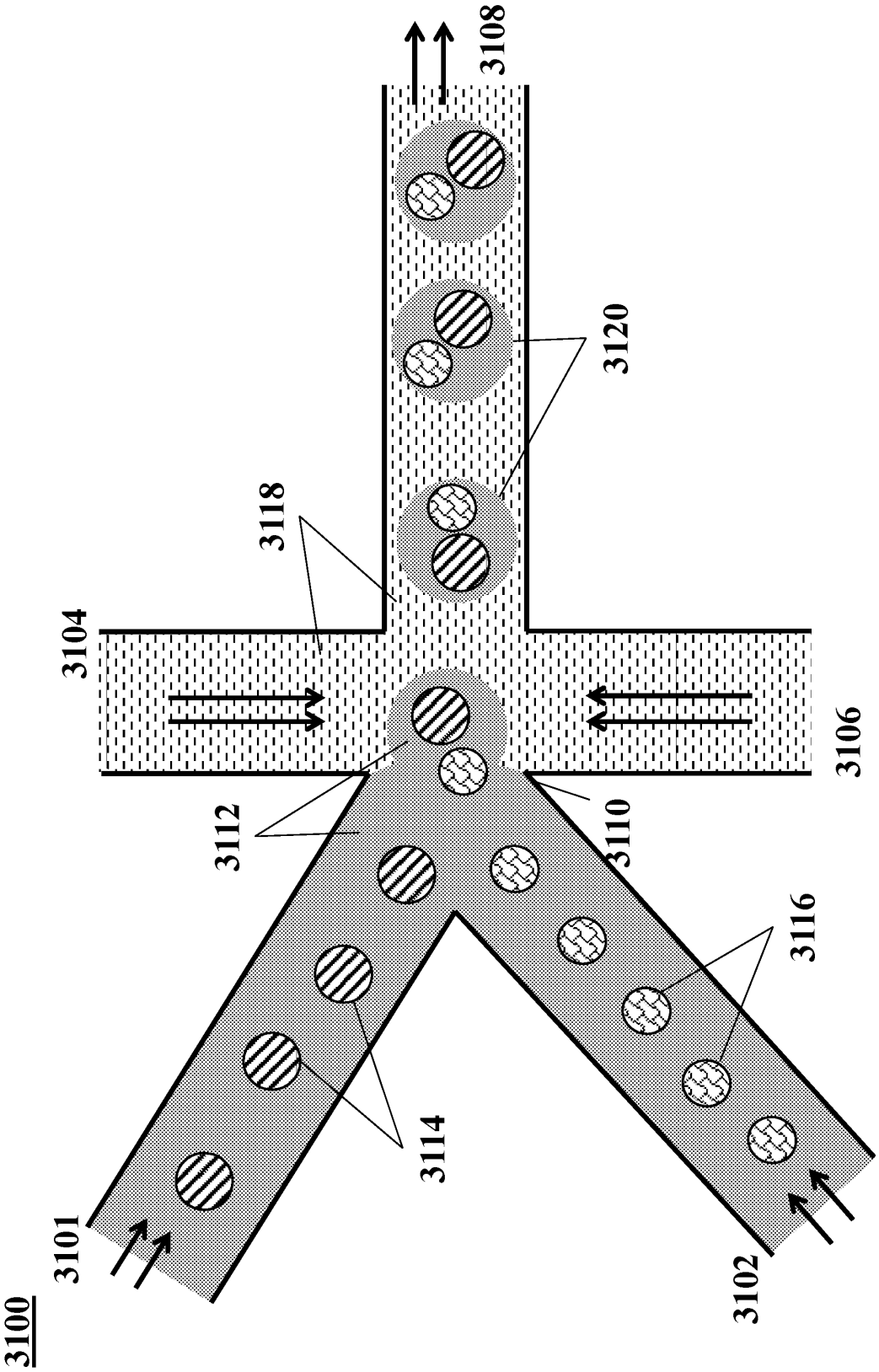


FIG. 31