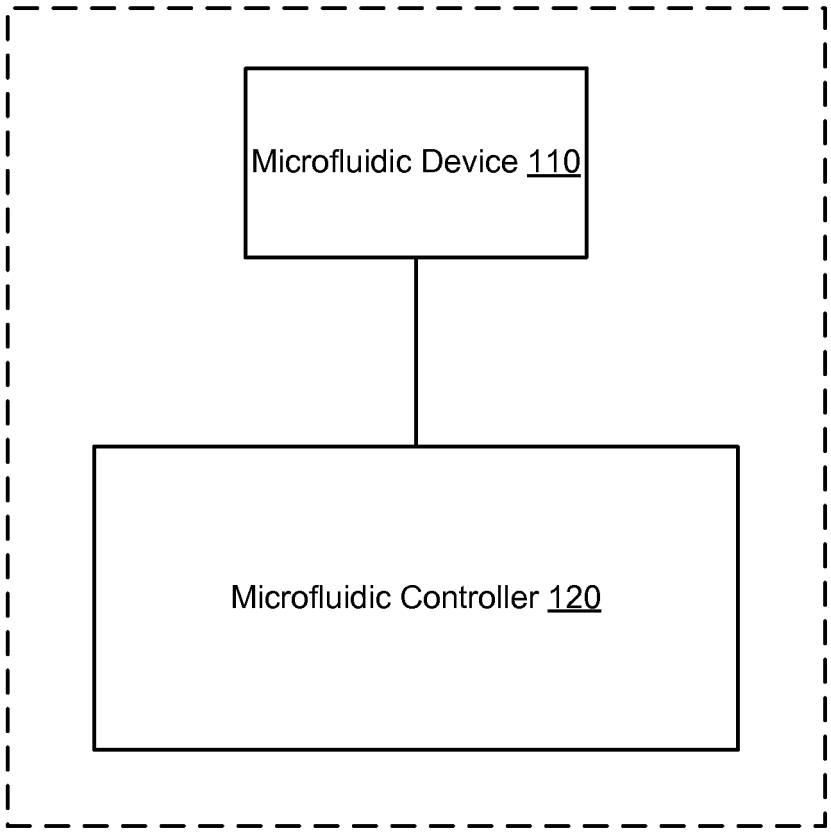


FIG. 1

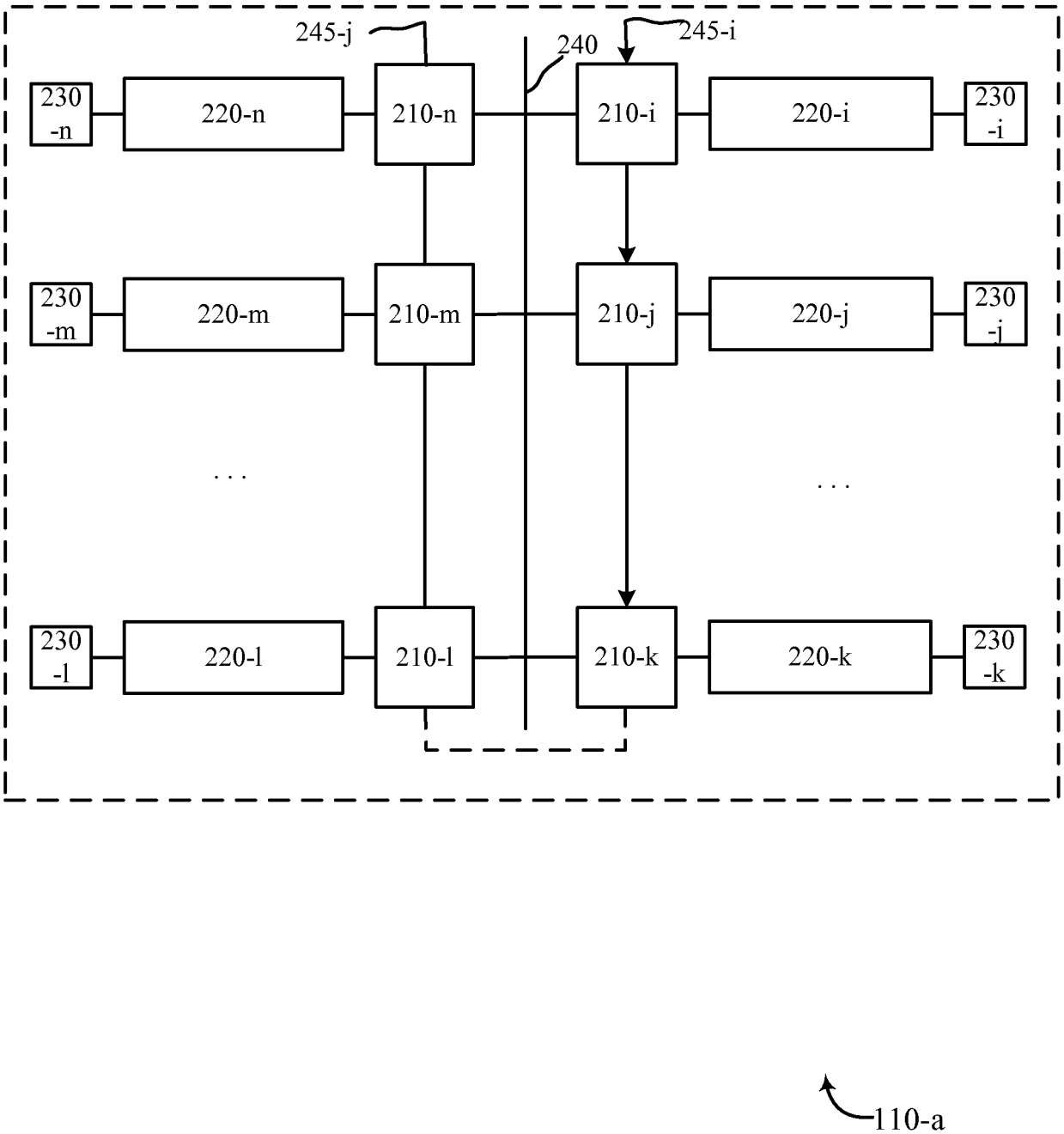

245-j
240
245-i
230 -n
220-n
210-n
210-i
220-i
230 -i
230 -m
220-m
210-m
210-j
220-j
230 -j
. . .
. . .
230 -l
220-l
210-l
210-k
220-k
230 -k
110-a

FIG. 2

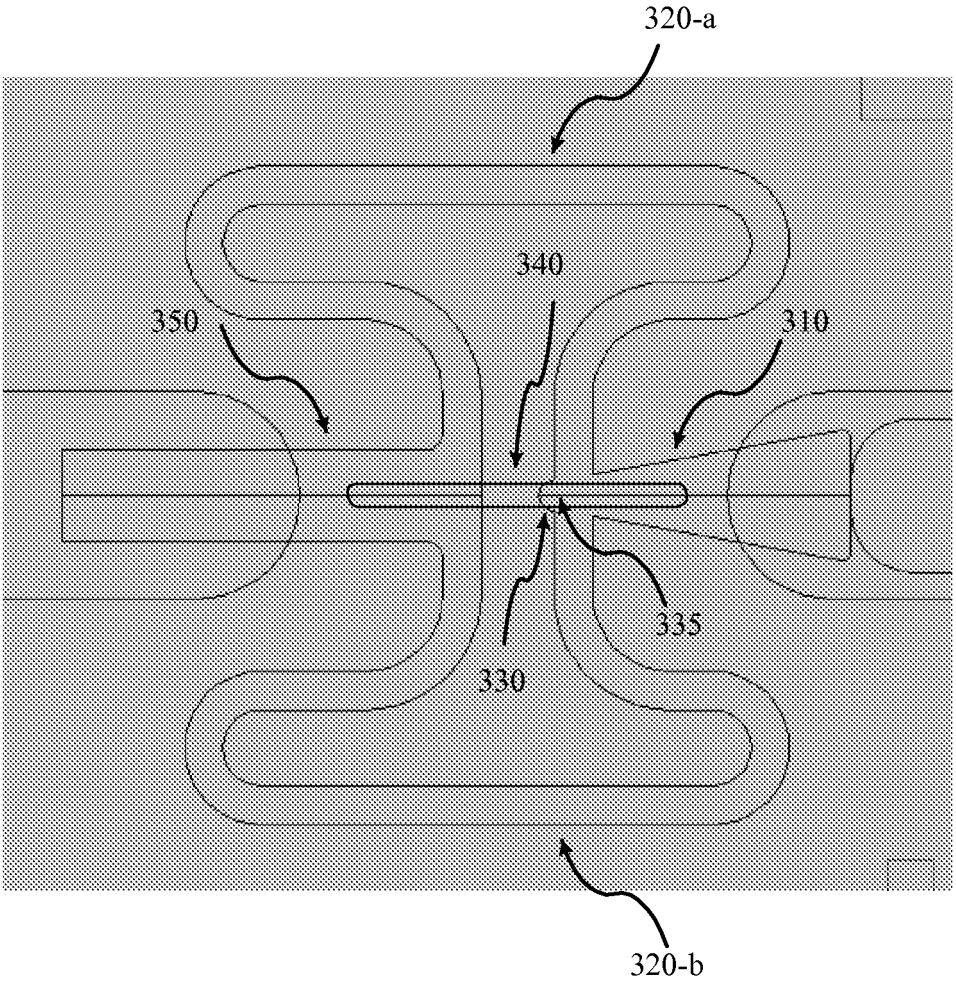
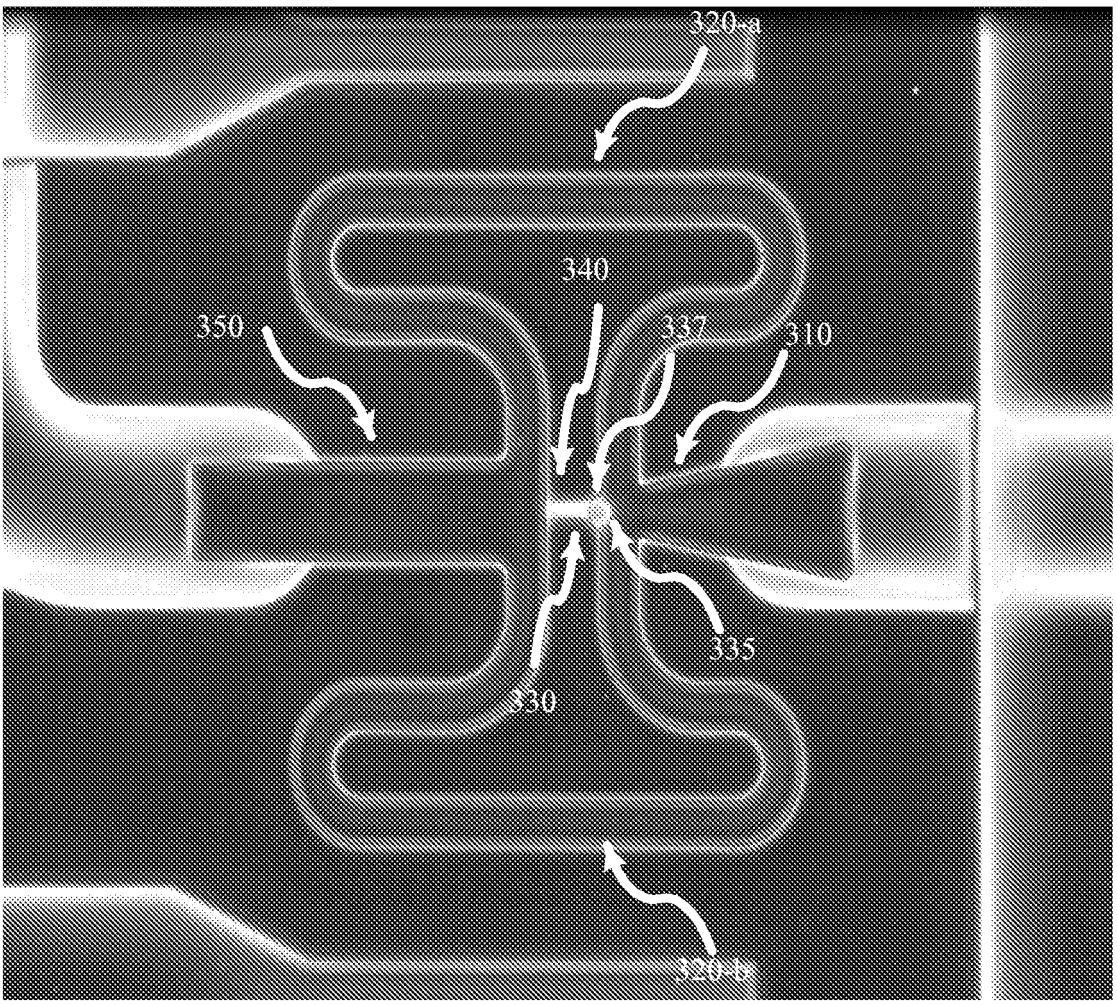


FIG. 3A



210-b

FIG. 3B

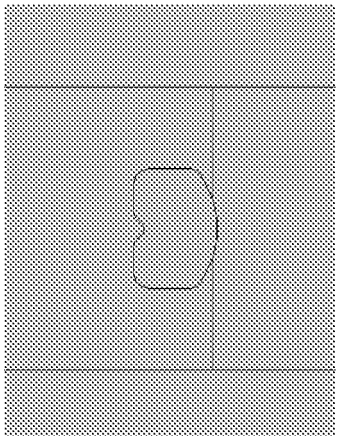


FIG. 3C

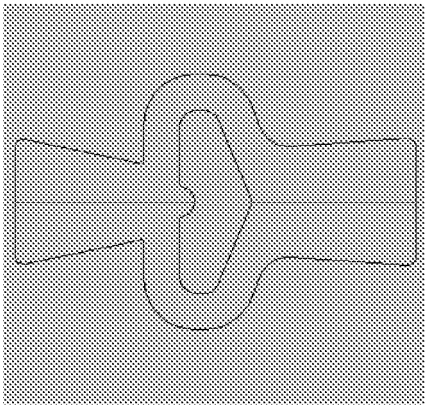


FIG. 3D

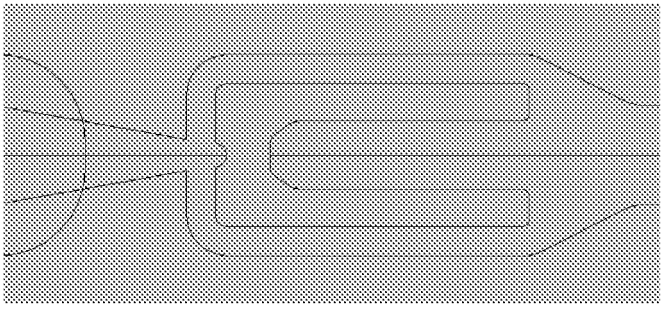
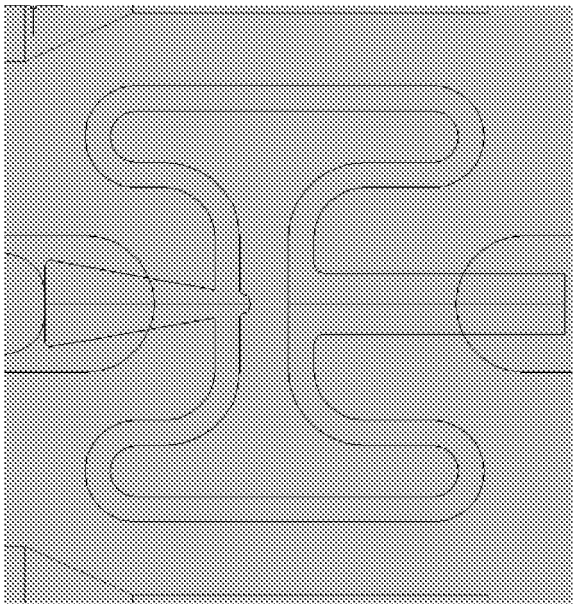
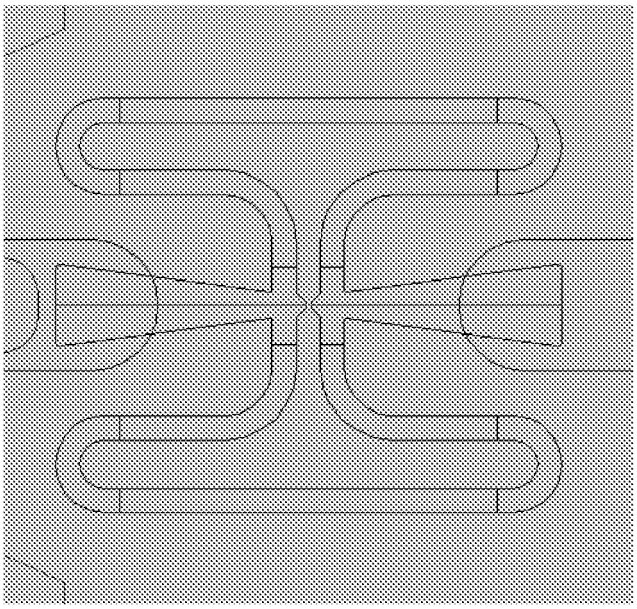


FIG. 3E



210-k-f

FIG. 3F



210-k-g

FIG. 3G

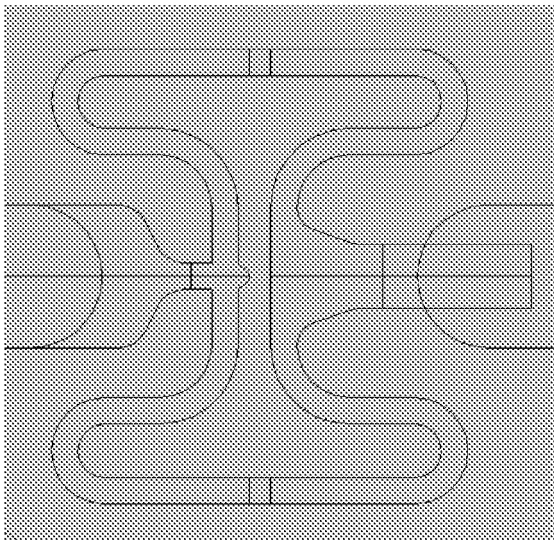


FIG. 3H

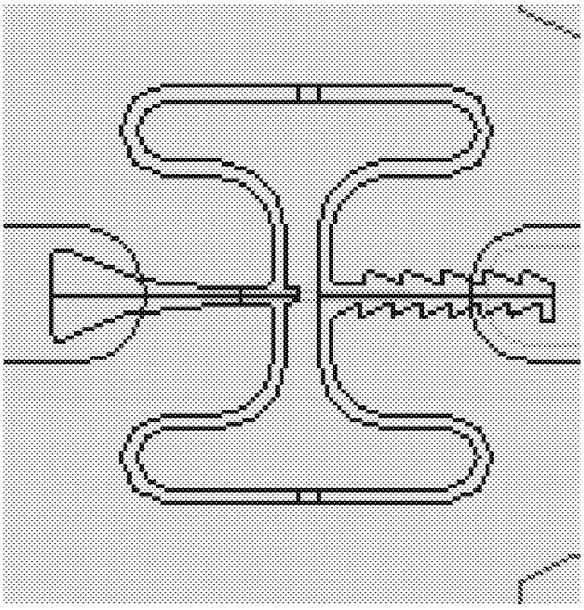


FIG. 3I

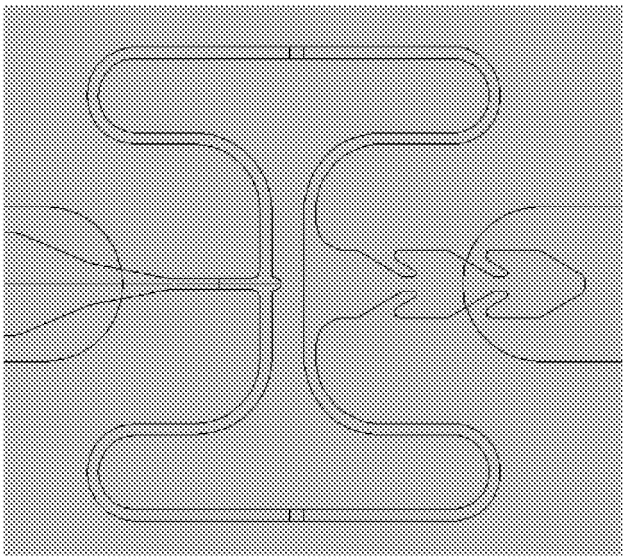


FIG. 3J

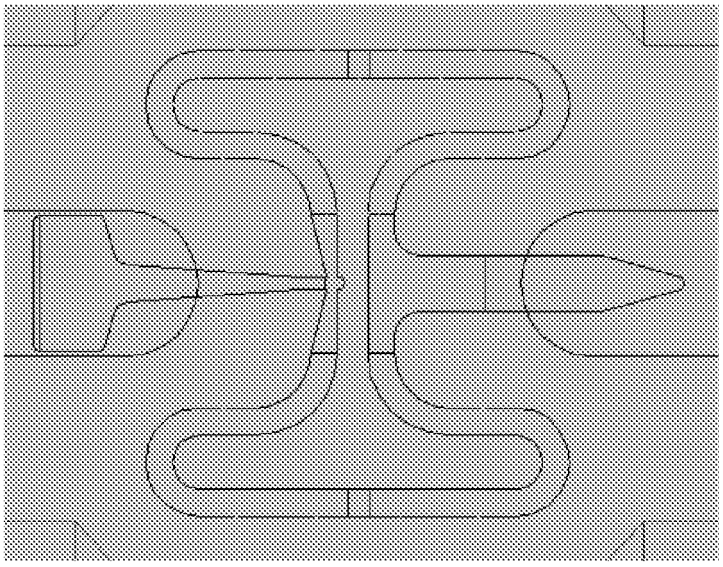


FIG. 3K

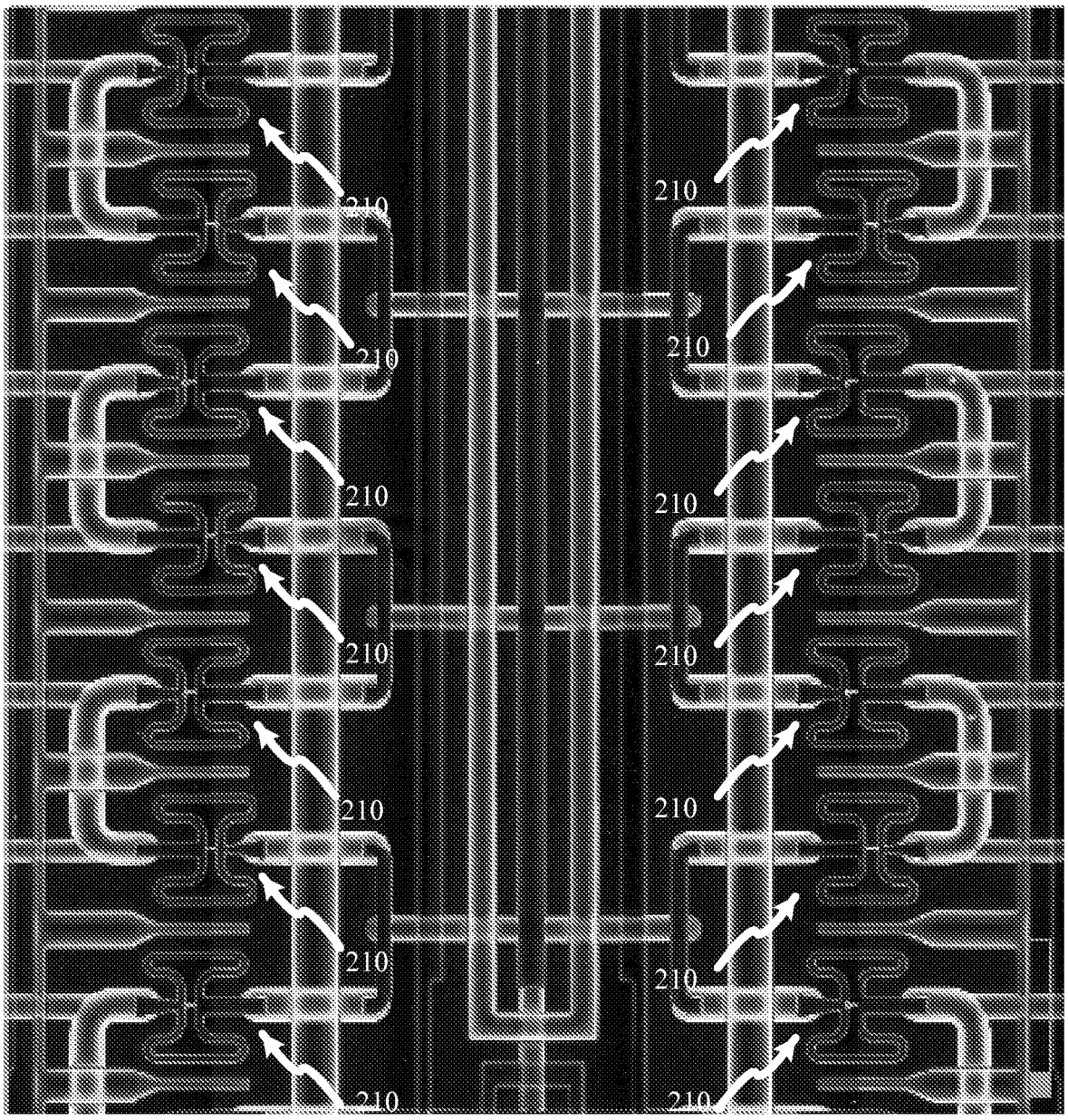


FIG. 4

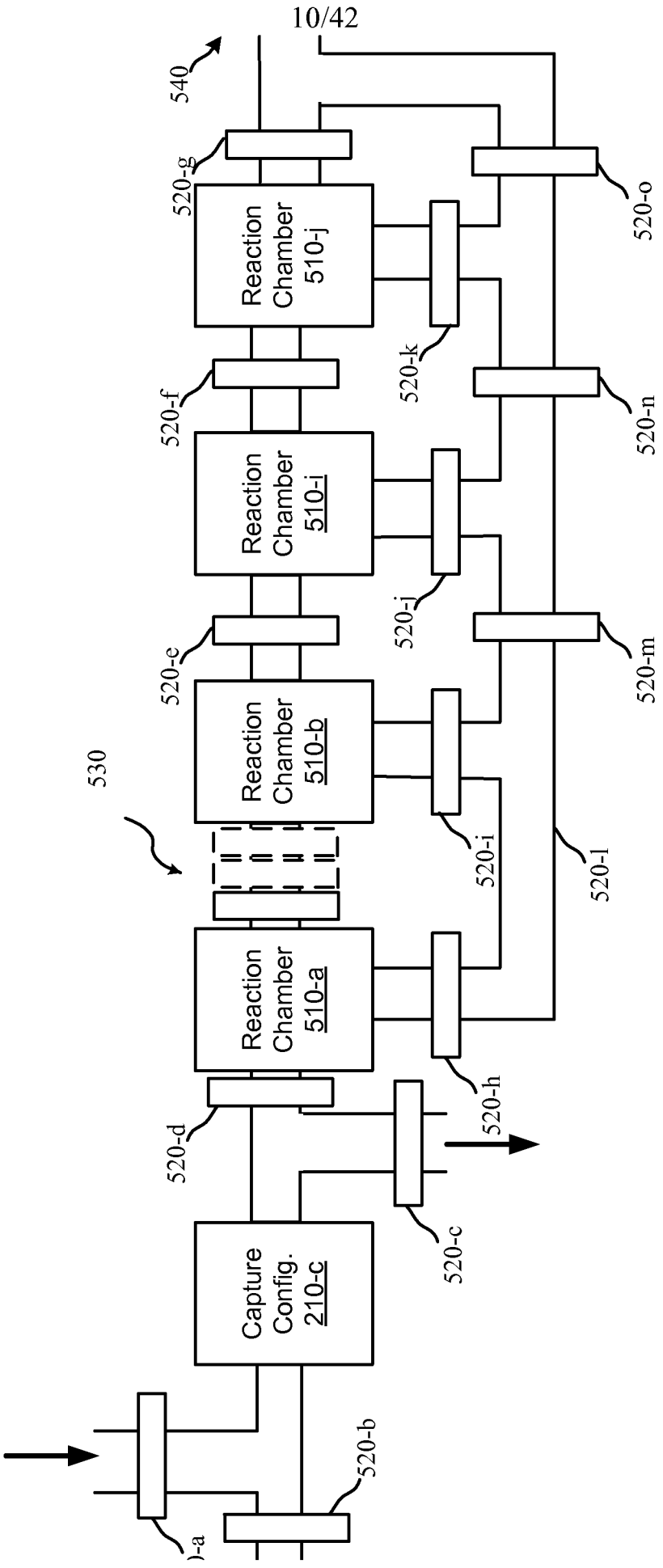

540
520-g
Reaction Chamber 510-j
520-o
220-c
520-k
520-f
Reaction Chamber 510-i
520-n
520-j
520-e
Reaction Chamber 510-b
520-m
530
520-i
520-l
Reaction Chamber 510-a
520-h
520-d
520-c
Capture Config. 210-c
520-b
)-a

FIG. 5A

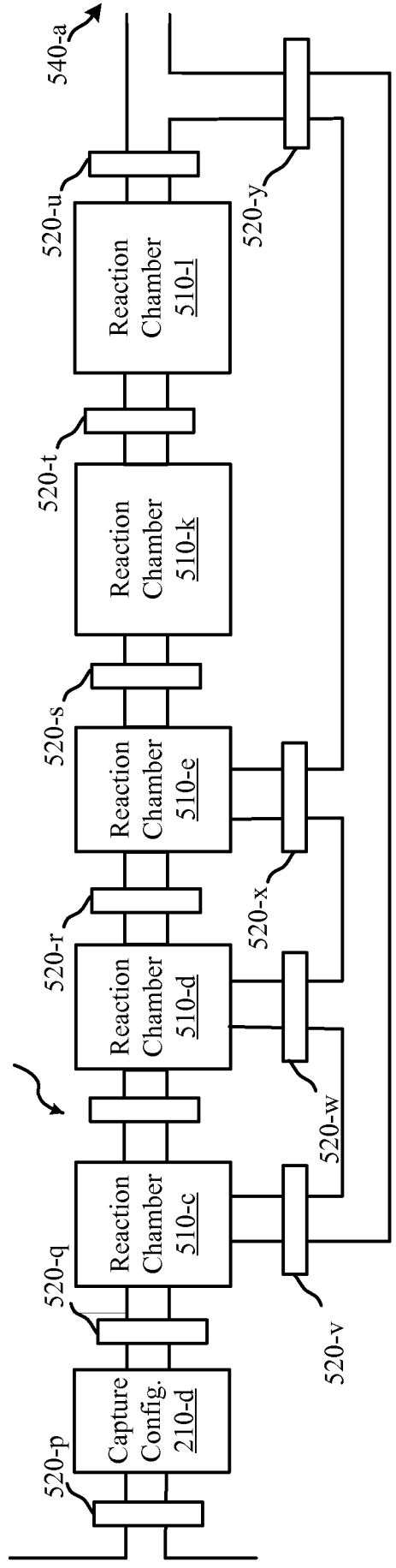

540-a
520-u
Reaction Chamber 510-l
520-y
220-d
520-t
Reaction Chamber 510-k
520-s
Reaction Chamber 510-e
520-x
520-r
Reaction Chamber 510-d
520-z
520-w
520-q
Reaction Chamber 510-c
520-v
520-p
Capture Config. 210-d

FIG. 5B

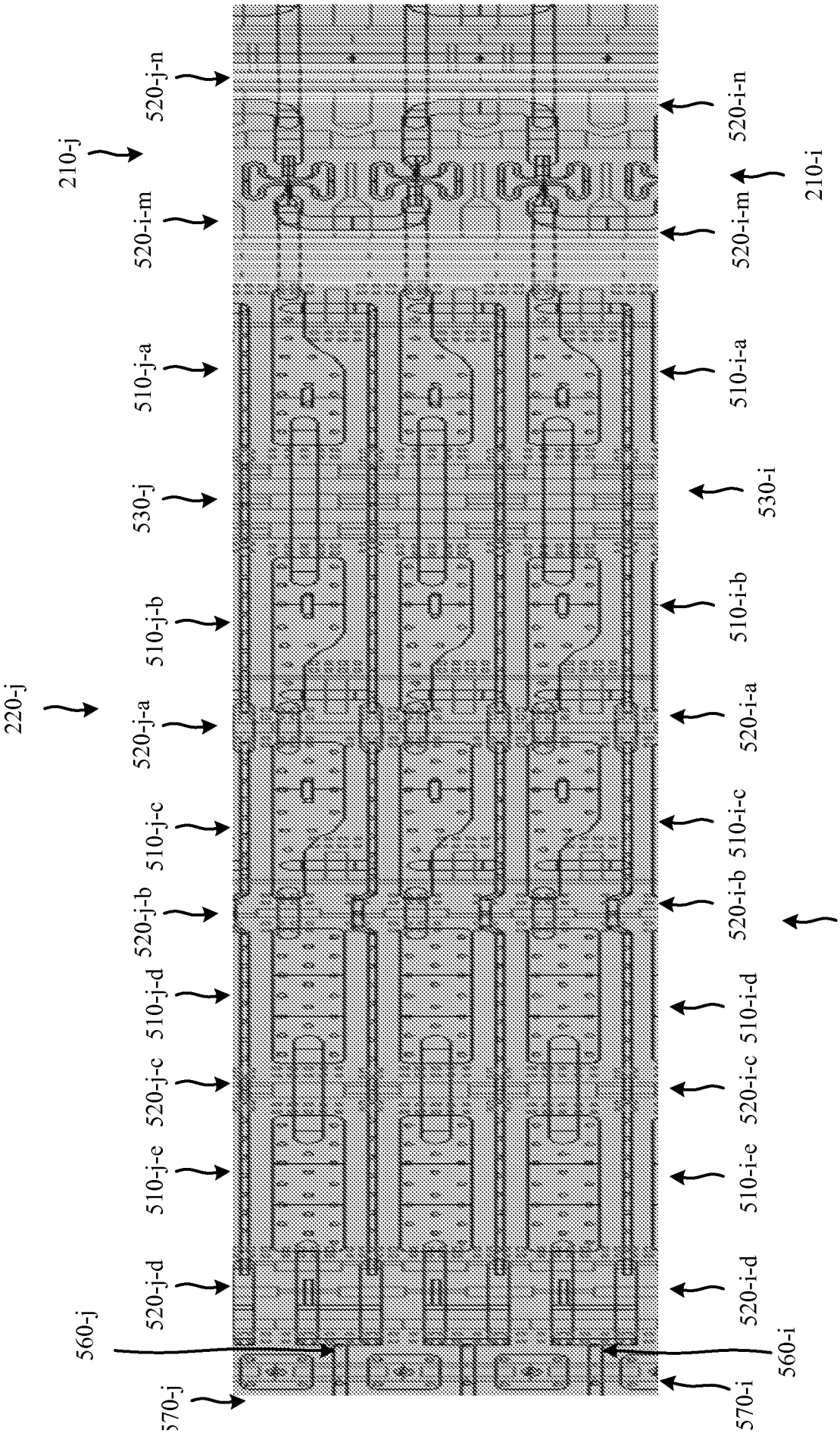
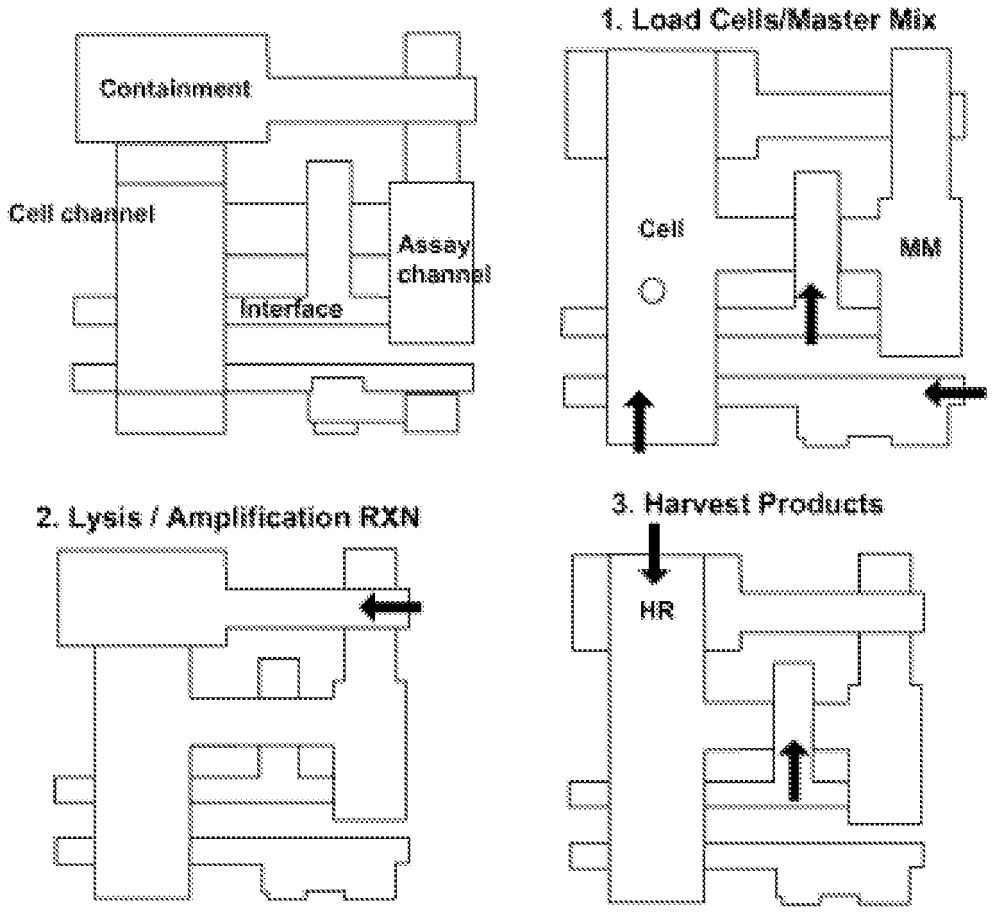
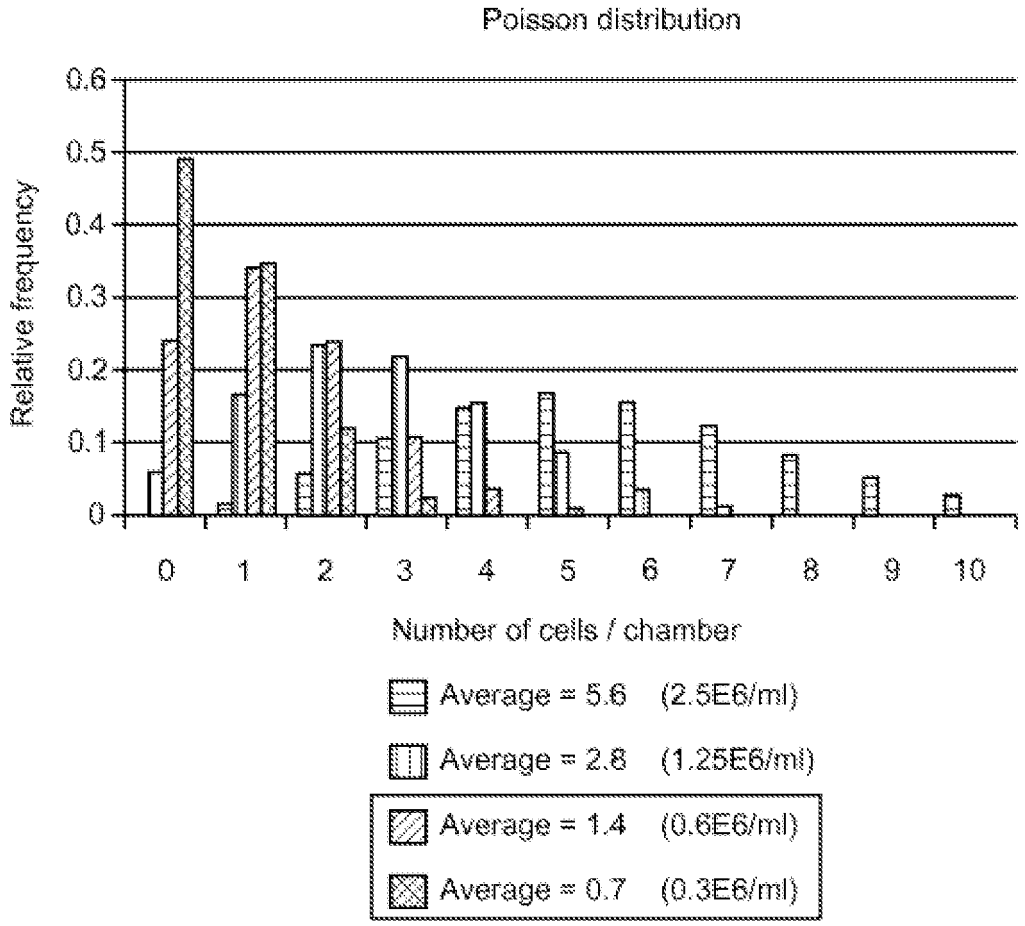

210-j
520-j-n
520-i-m
520-i-n
210-i
510-j-a
510-i-a
530-j
530-i
510-j-b
510-i-b
220-j
520-j-a
520-i-a
510-j-c
510-i-c
520-j-b
520-i-b
510-j-d
510-i-d
520-j-c
520-i-c
510-j-e
510-i-e
520-j-d
520-i-d
560-j
560-i
570-j
570-i
220-i

FIG. 5C



	(nl)	Ratio
Cell Channel	2.4	
Assay Channel	8.9	~4:1 Chamber 1: Cell Channel

FIG. 6



The "best" theoretical cell density for single cells:
~1 per chamber on average = 1 / 2.4 nl = ~ 0.5E6/ml

FIG. 7

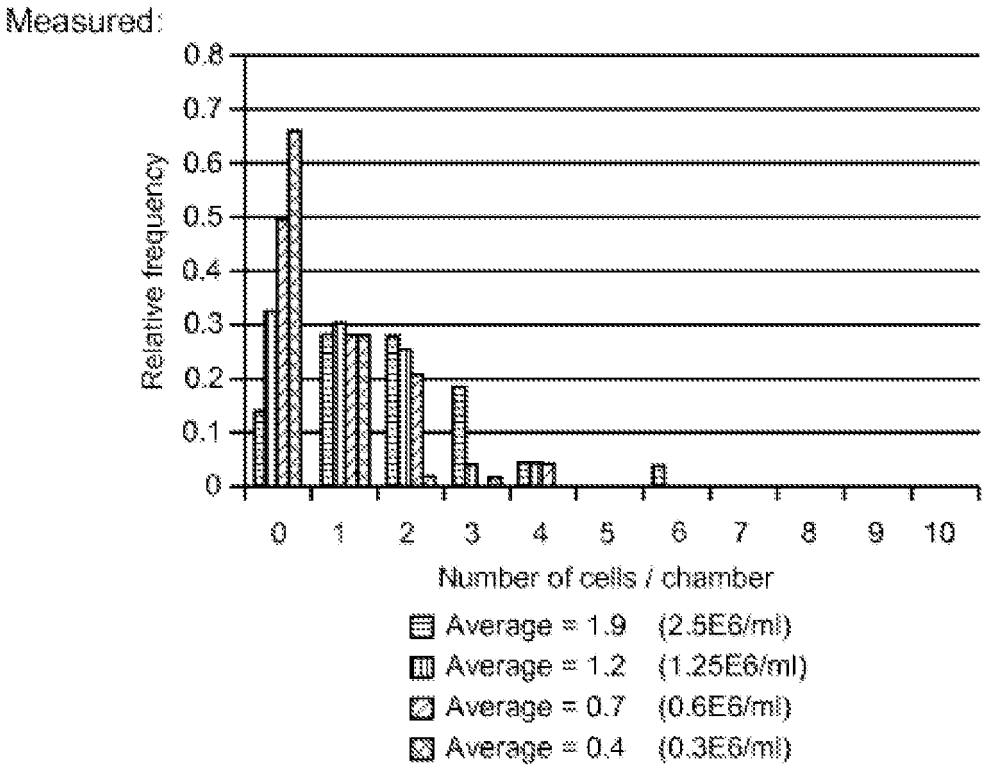

Measured:
Relative frequency
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0 1 2 3 4 5 6 7 8 9 10
Number of cells / chamber
Average = 1.9 (2.5E6/ml)
Average = 1.2 (1.25E6/ml)
Average = 0.7 (0.6E6/ml)
Average = 0.4 (0.3E6/ml)

FIG. 8A

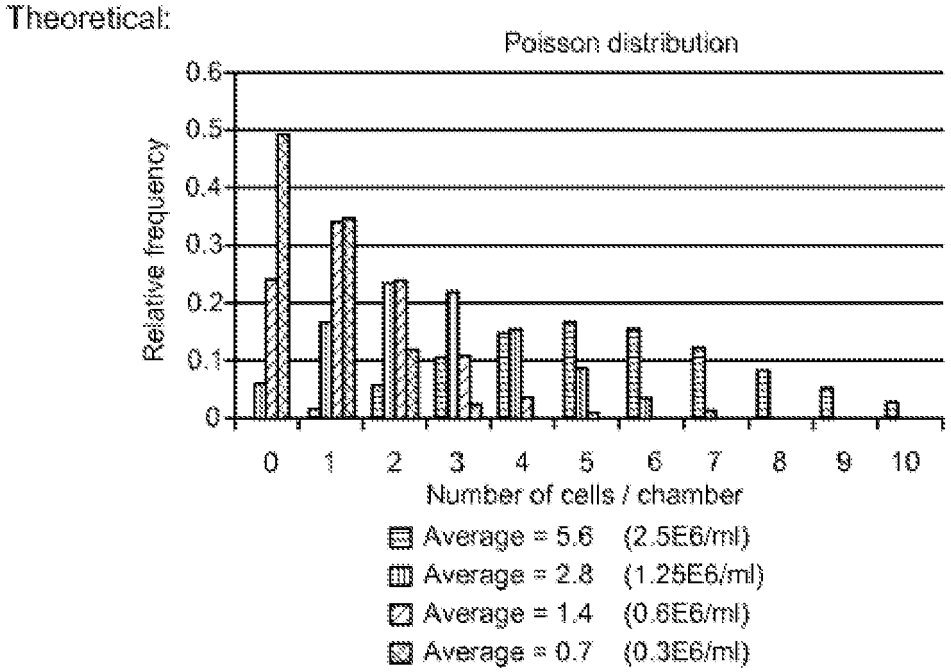
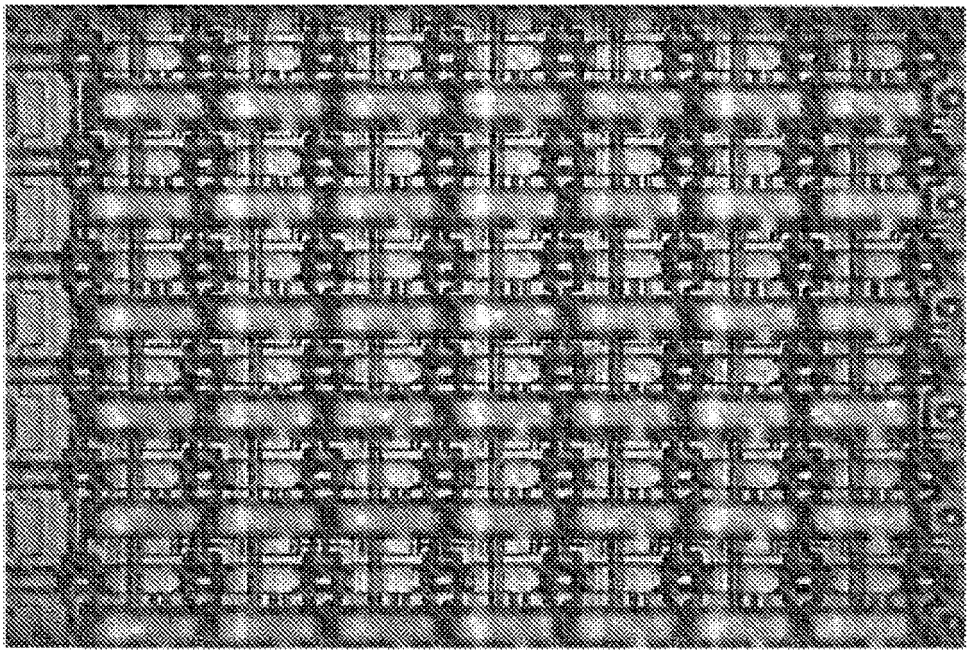

Theoretical:
Poisson distribution
Relative frequency
0.6
0.5
0.4
0.3
0.2
0.1
0
0 1 2 3 4 5 6 7 8 9 10
Number of cells / chamber
Average = 5.6 (2.5E6/ml)
Average = 2.8 (1.25E6/ml)
Average = 1.4 (0.6E6/ml)
Average = 0.7 (0.3E6/ml)

FIG. 8B

0.15E6 /ml

Expect
0-1
chamber



	Brightfield	Fluorescence
Average cells/ chamber	0.29	0.52

FIG. 9

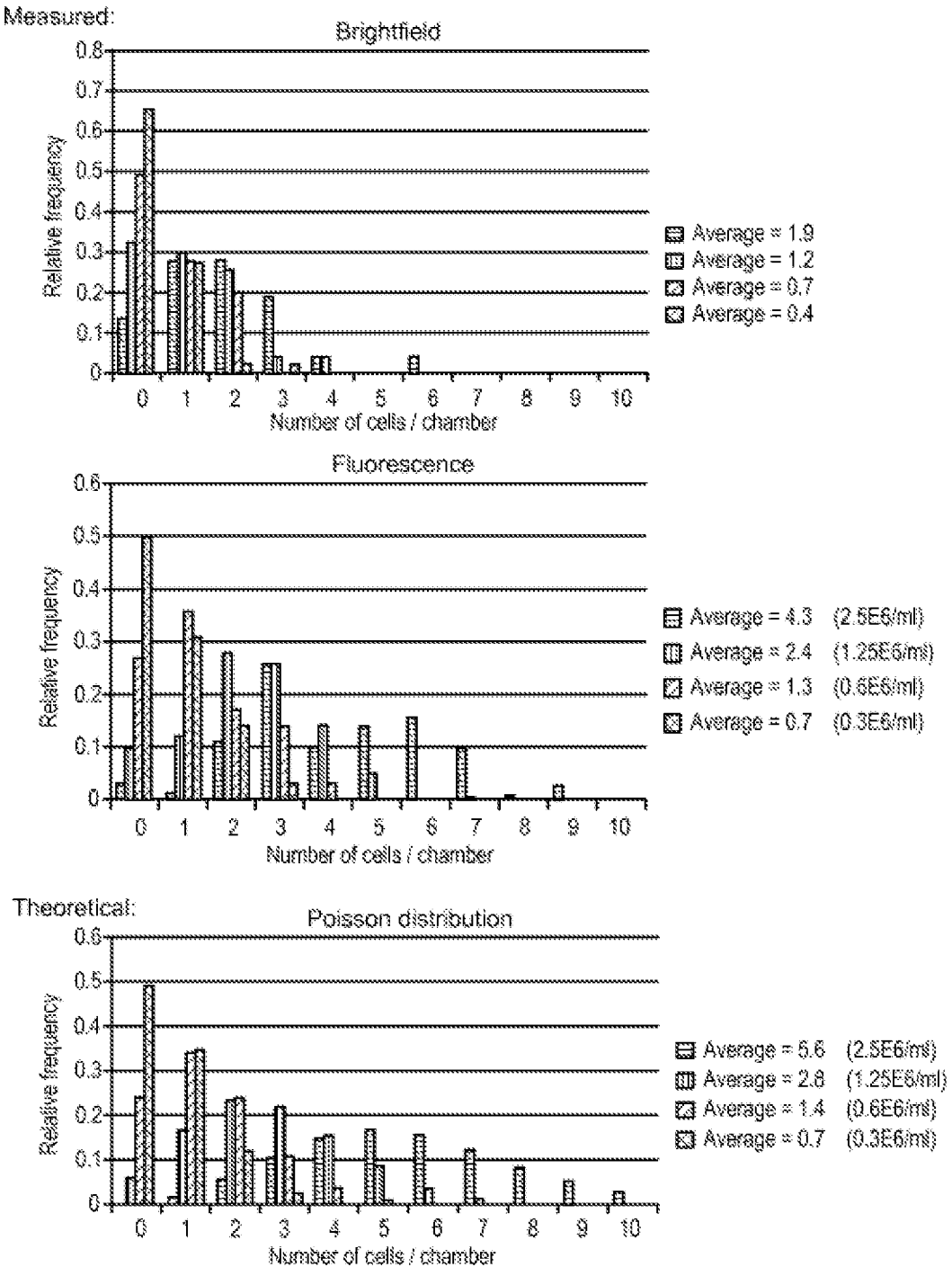

Measured:
Brightfield
Relative frequency
Number of cells / chamber
Average = 1.9
Average = 1.2
Average = 0.7
Average = 0.4
Fluorescence
Relative frequency
Number of cells / chamber
Average = 4.3 (2.5E6/ml)
Average = 2.4 (1.25E6/ml)
Average = 1.3 (0.6E6/ml)
Average = 0.7 (0.3E6/ml)
Theoretical:
Poisson distribution
Relative frequency
Number of cells / chamber
Average = 5.6 (2.5E6/ml)
Average = 2.8 (1.25E6/ml)
Average = 1.4 (0.6E6/ml)
Average = 0.7 (0.3E6/ml)

FIG. 10

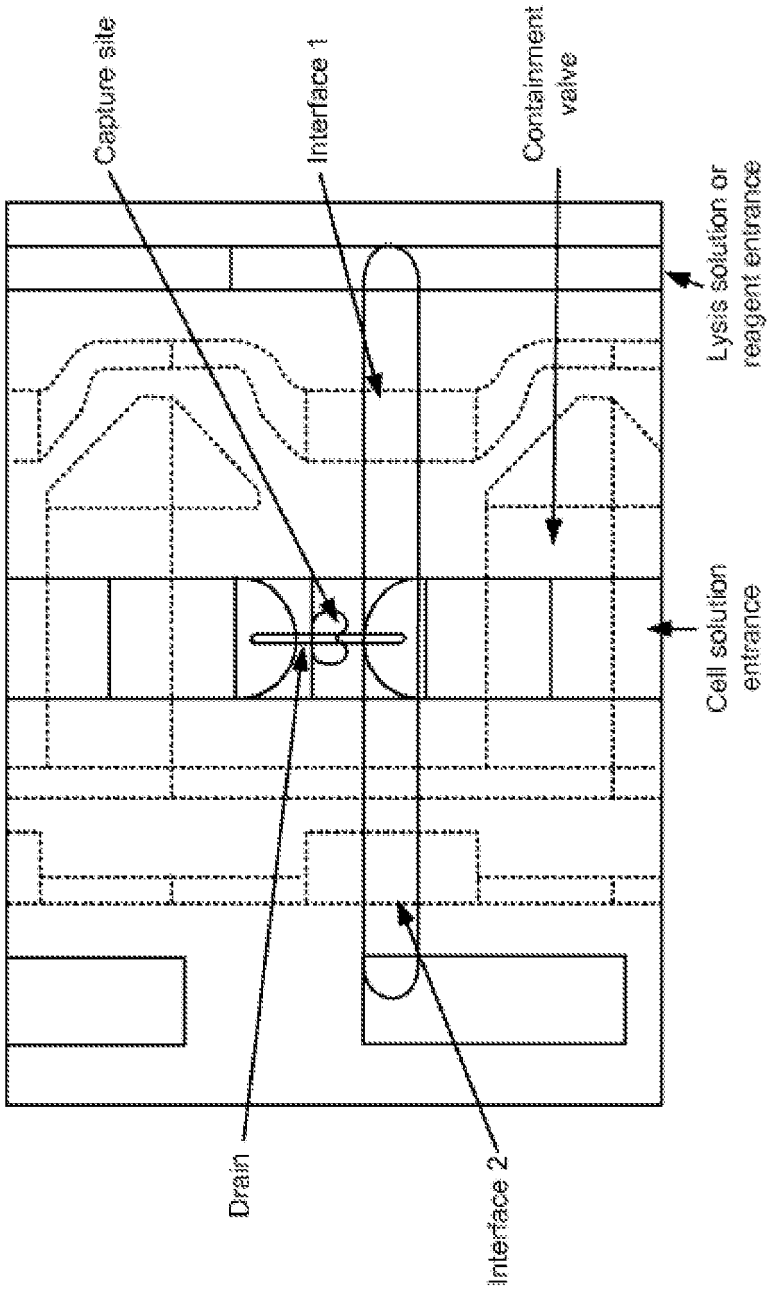

Capture site
Interface 1
Containment valve
Lysis solution or reagent entrance
Cell solution entrance
Drain
Interface 2

FIG. 11A

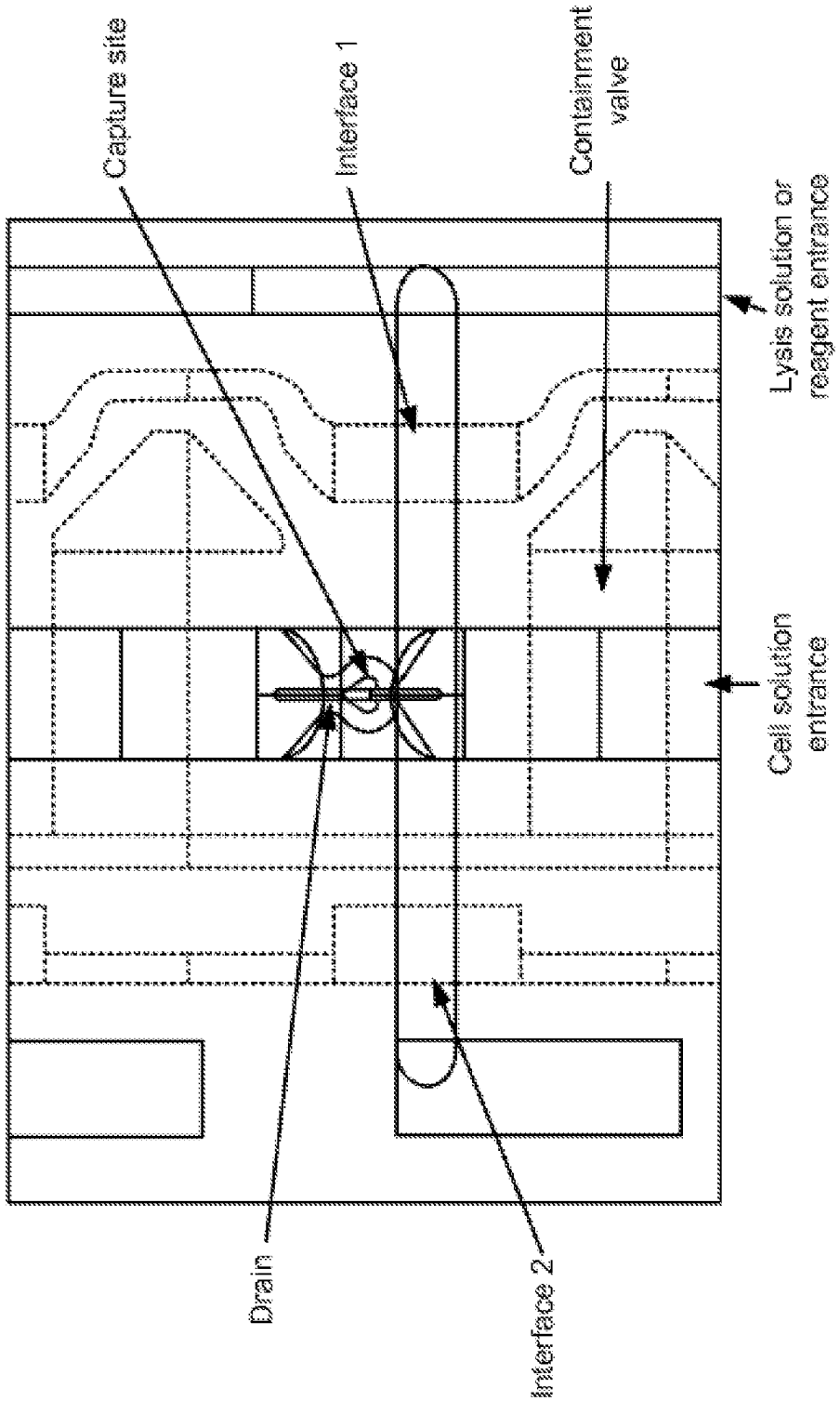

Capture site
Interface 1
Containment valve
Lysis solution or reagent entrance
Cell solution entrance
Drain
Interface 2

FIG. 11B

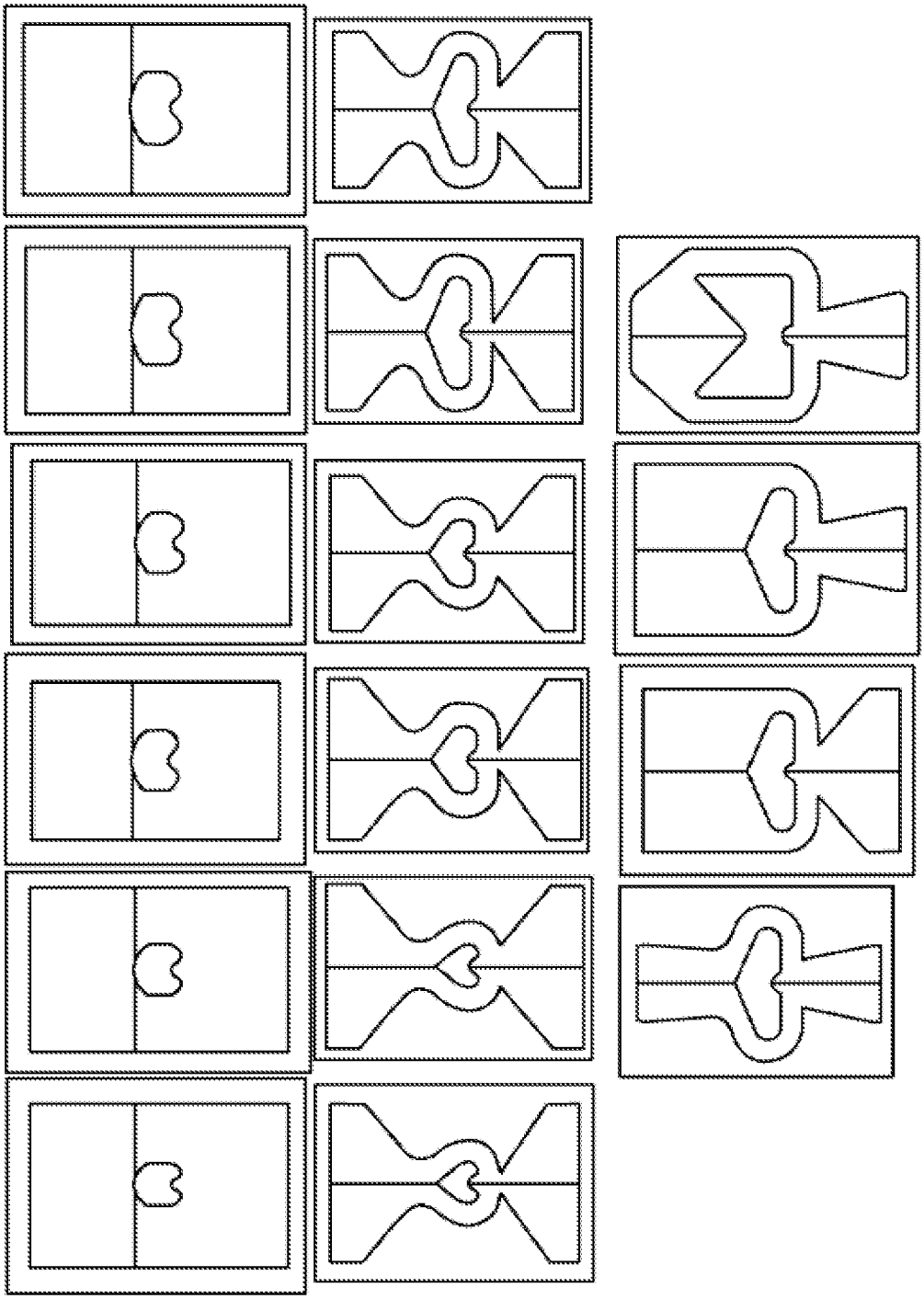


FIG. 12

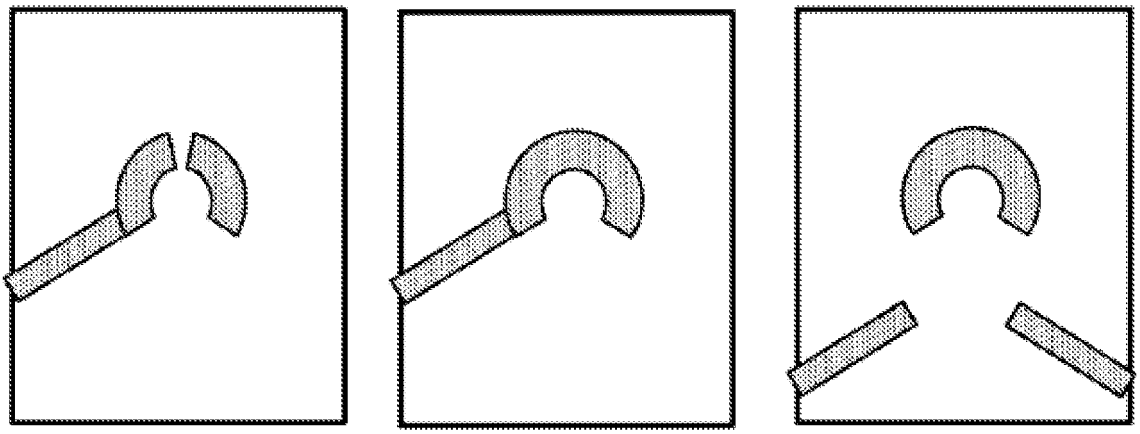


FIG. 13A

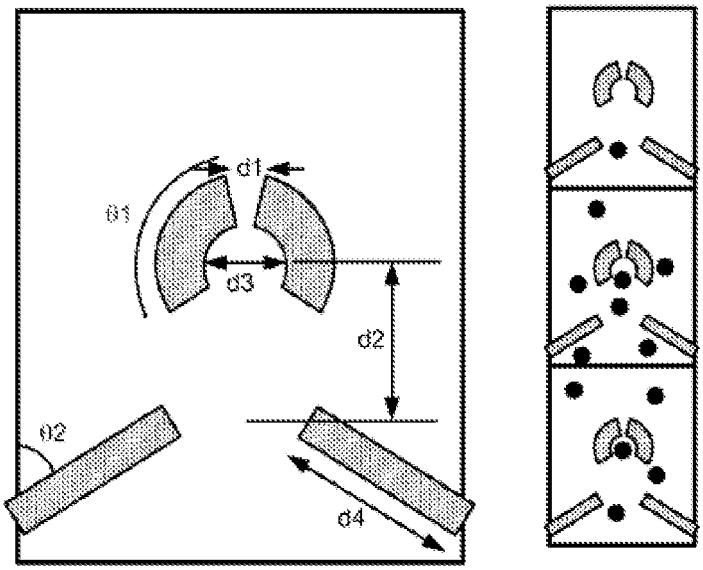


FIG. 13B

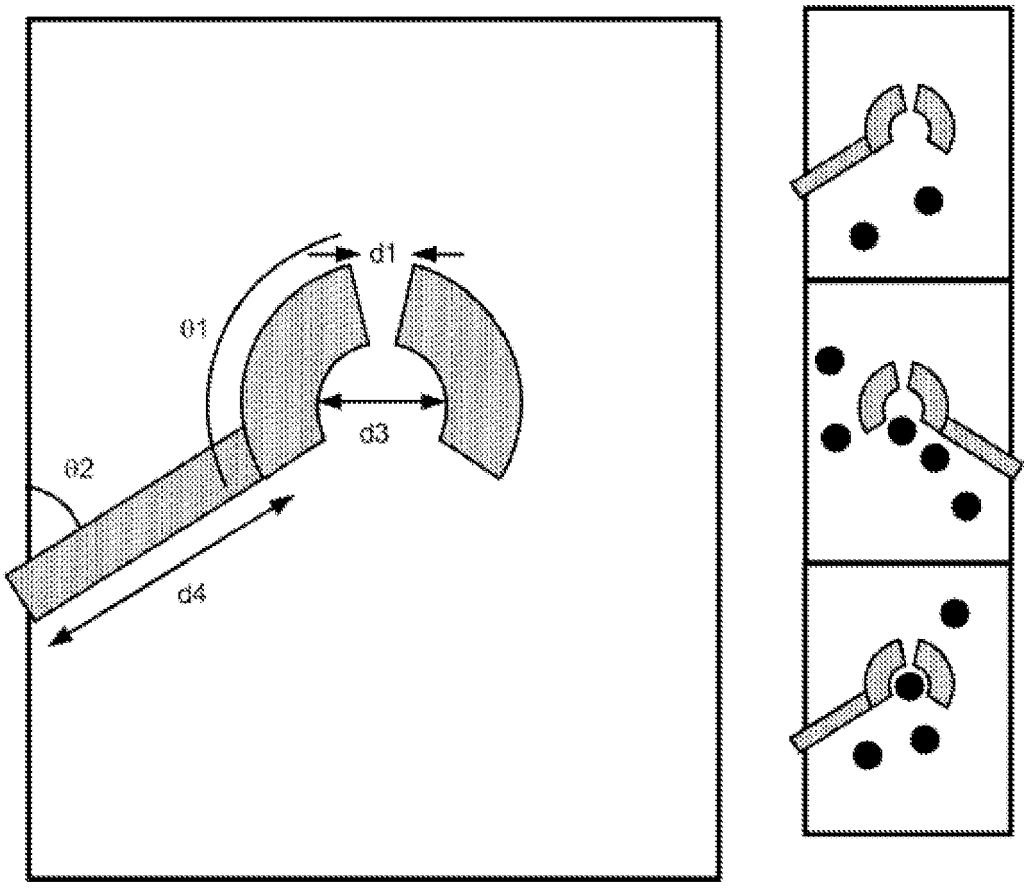


FIG. 13C

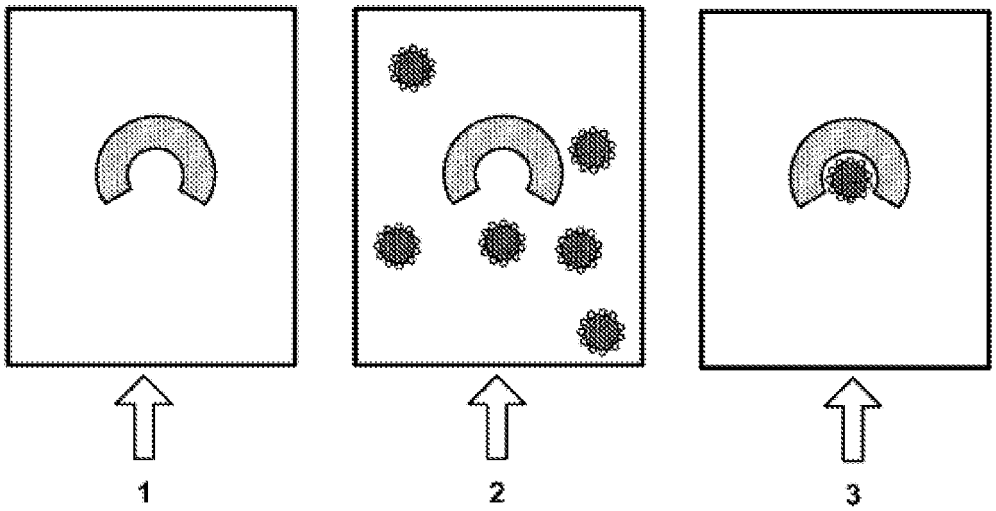


FIG. 14A

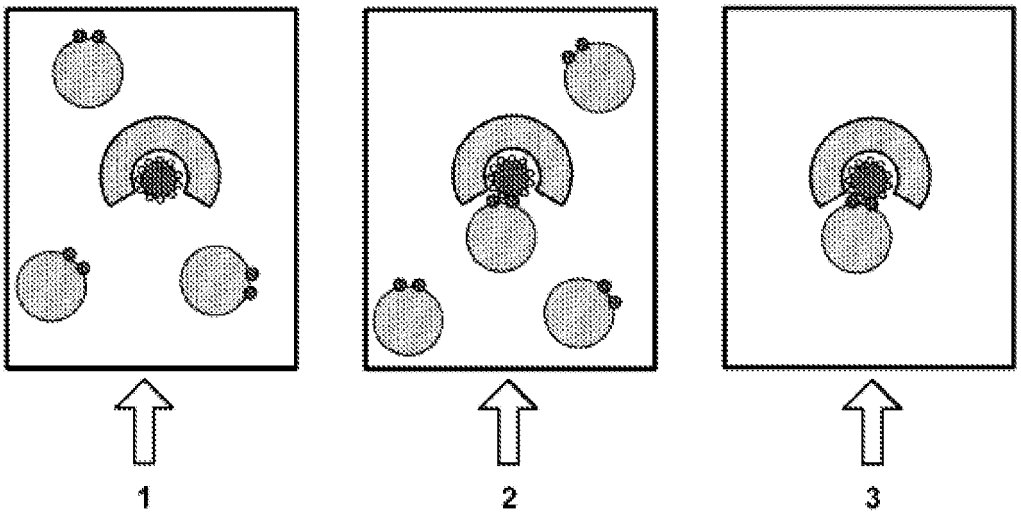


FIG. 14B

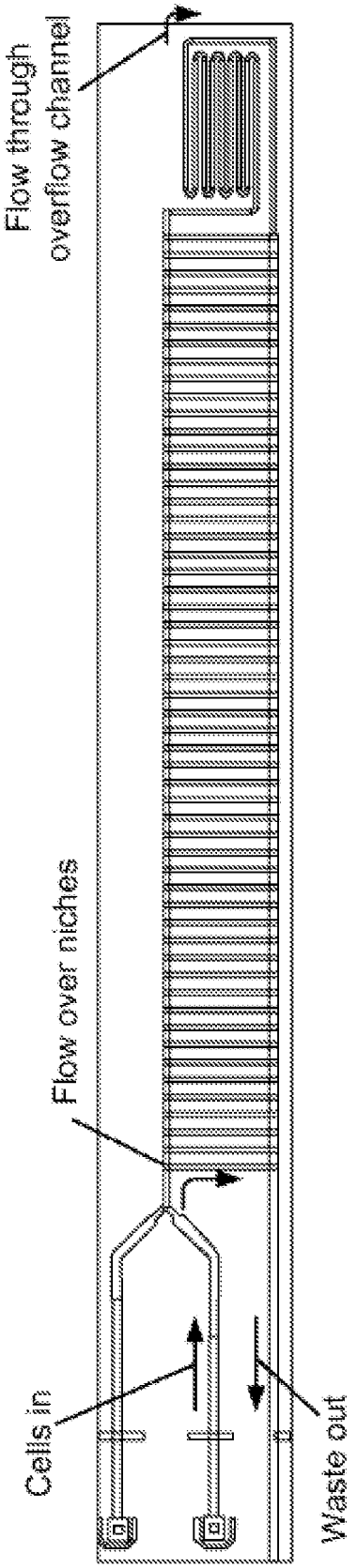

Flow through overflow channel
Flow over niches
Cells in
Waste out

FIG. 15A

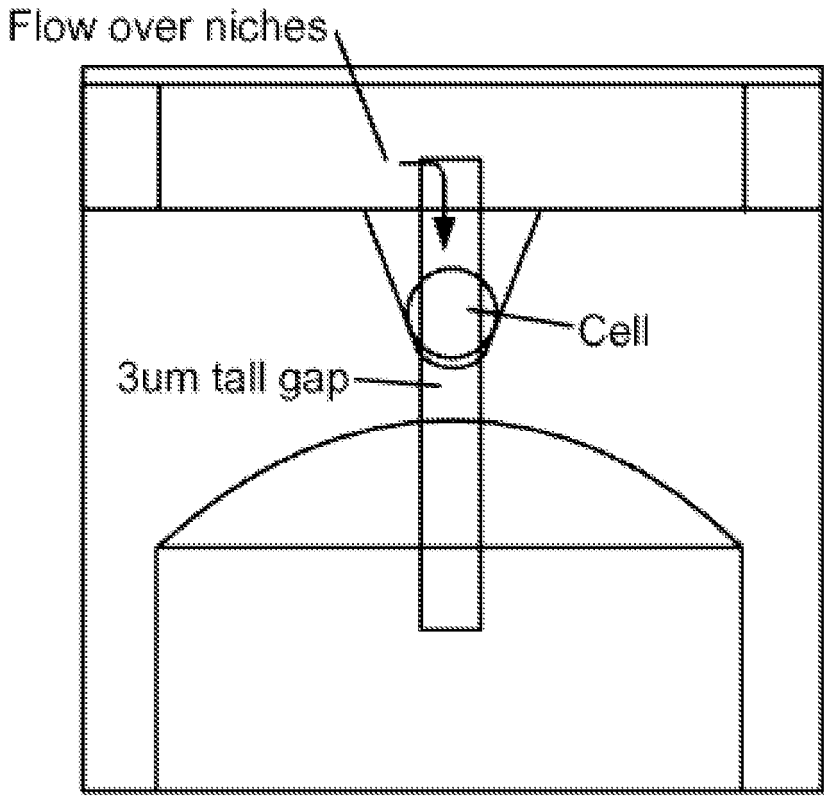


FIG. 15B

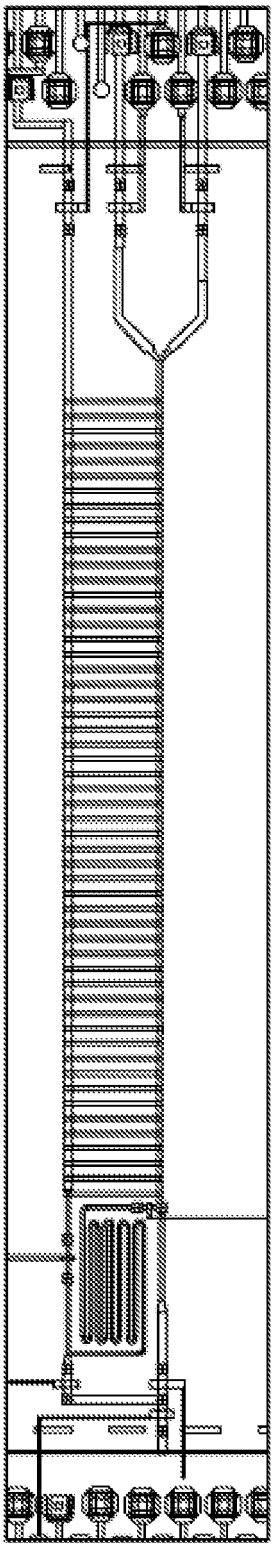


FIG. 15C

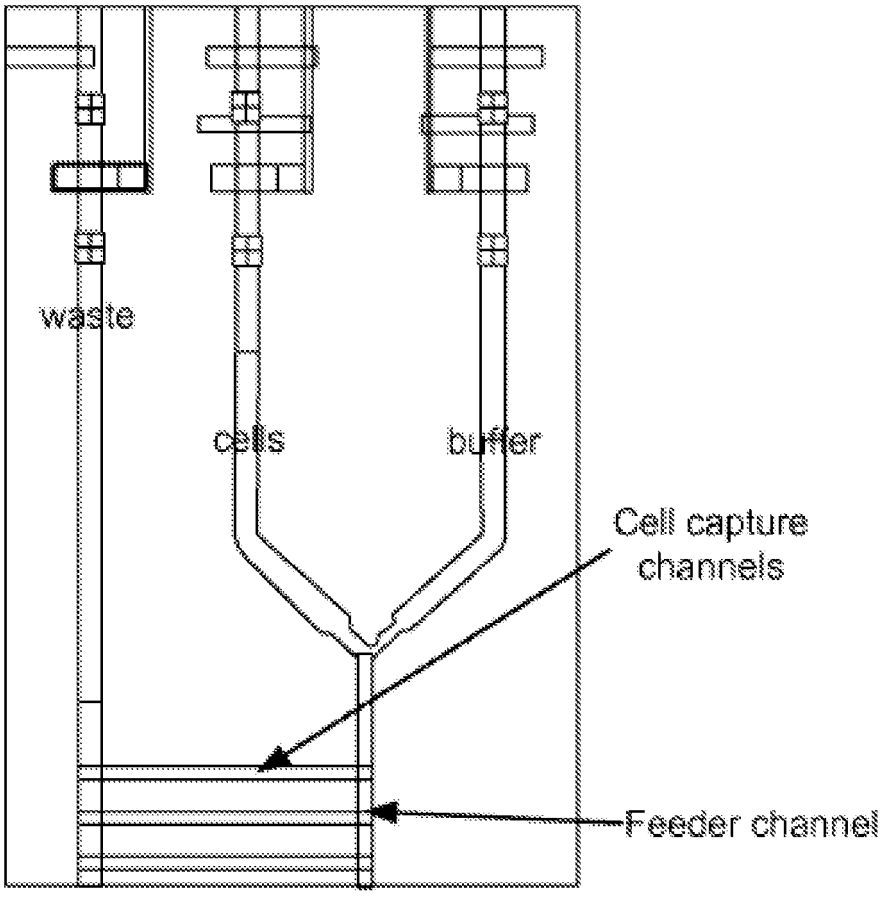


FIG. 15D

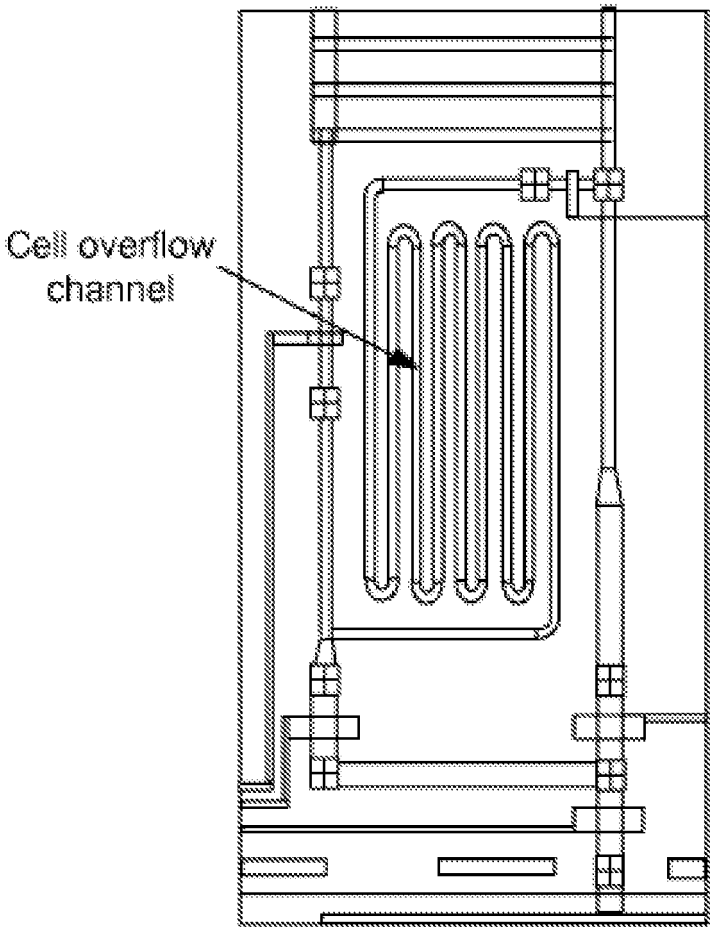


FIG. 15E

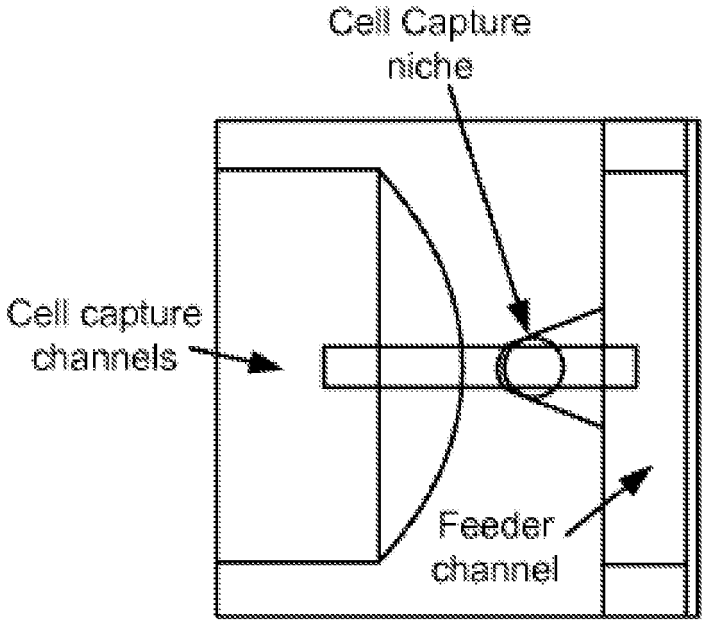


FIG. 15F

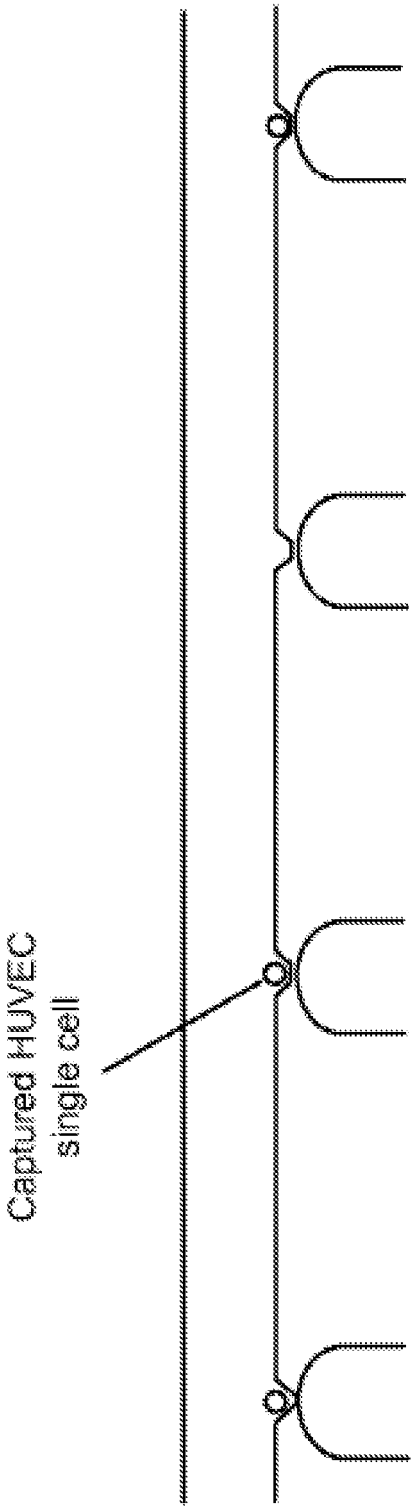


FIG. 15G

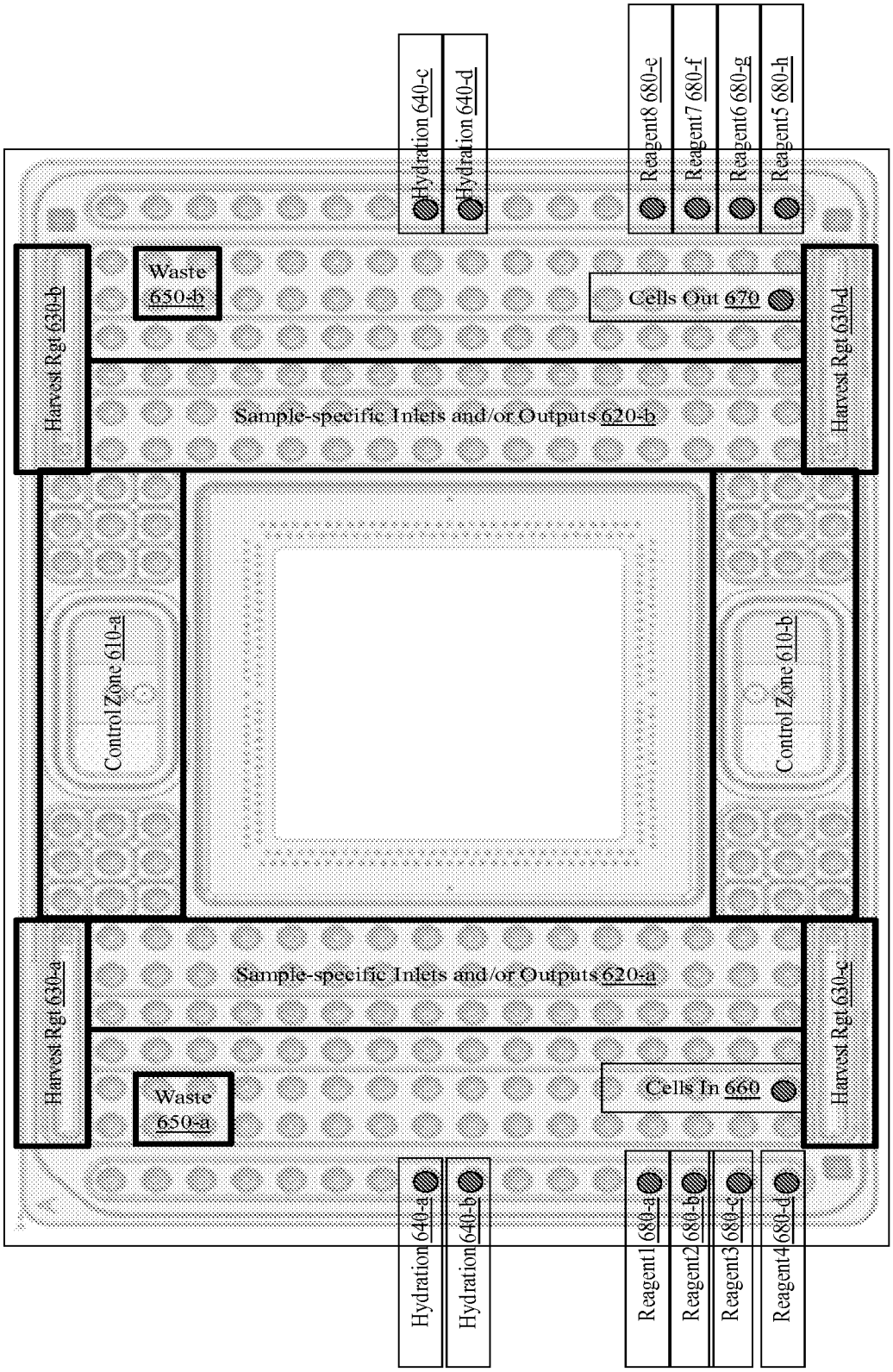

1600
Hydration 640-c
Hydration 640-d
Reagent8 680-e
Reagent7 680-f
Reagent6 680-g
Reagent5 680-h
Waste 650-b
Cells Out 670
Harvest Rgt 630-b
Harvest Rgt 630-d
Sample-specific Inlets and/or Outputs 620-b
Control Zone 610-a
Control Zone 610-b
Harvest Rgt 630-a
Harvest Rgt 630-c
Sample-specific Inlets and/or Outputs 620-a
Waste 650-a
Cells In 660
Hydration 640-a
Hydration 640-b
Reagent1 680-a
Reagent2 680-b
Reagent3 680-c
Reagent4 680-d

FIG. 16

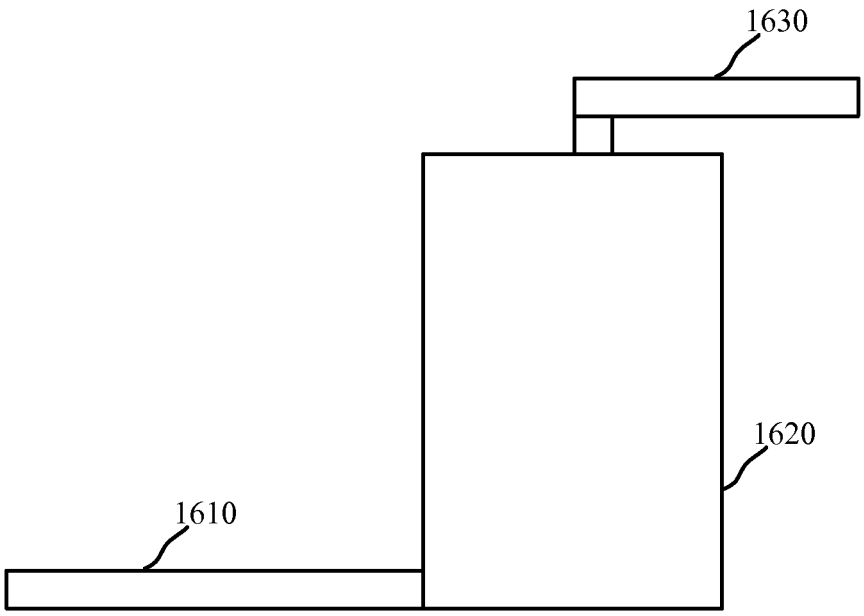


FIG. 16A

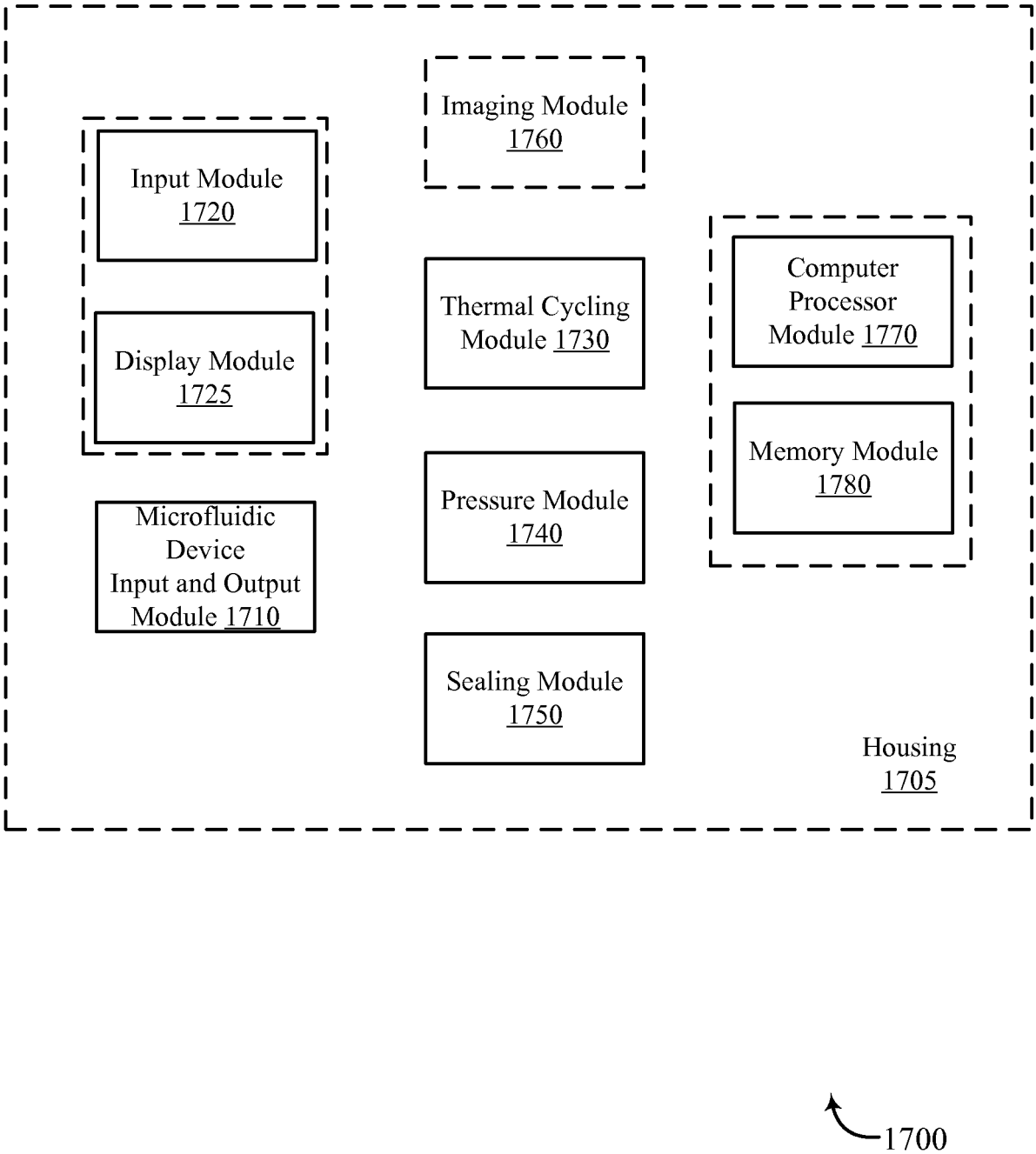

Imaging Module 1760
Input Module 1720
Display Module 1725
Thermal Cycling Module 1730
Computer Processor Module 1770
Memory Module 1780
Microfluidic Device Input and Output Module 1710
Pressure Module 1740
Sealing Module 1750
Housing 1705
1700

FIG. 17

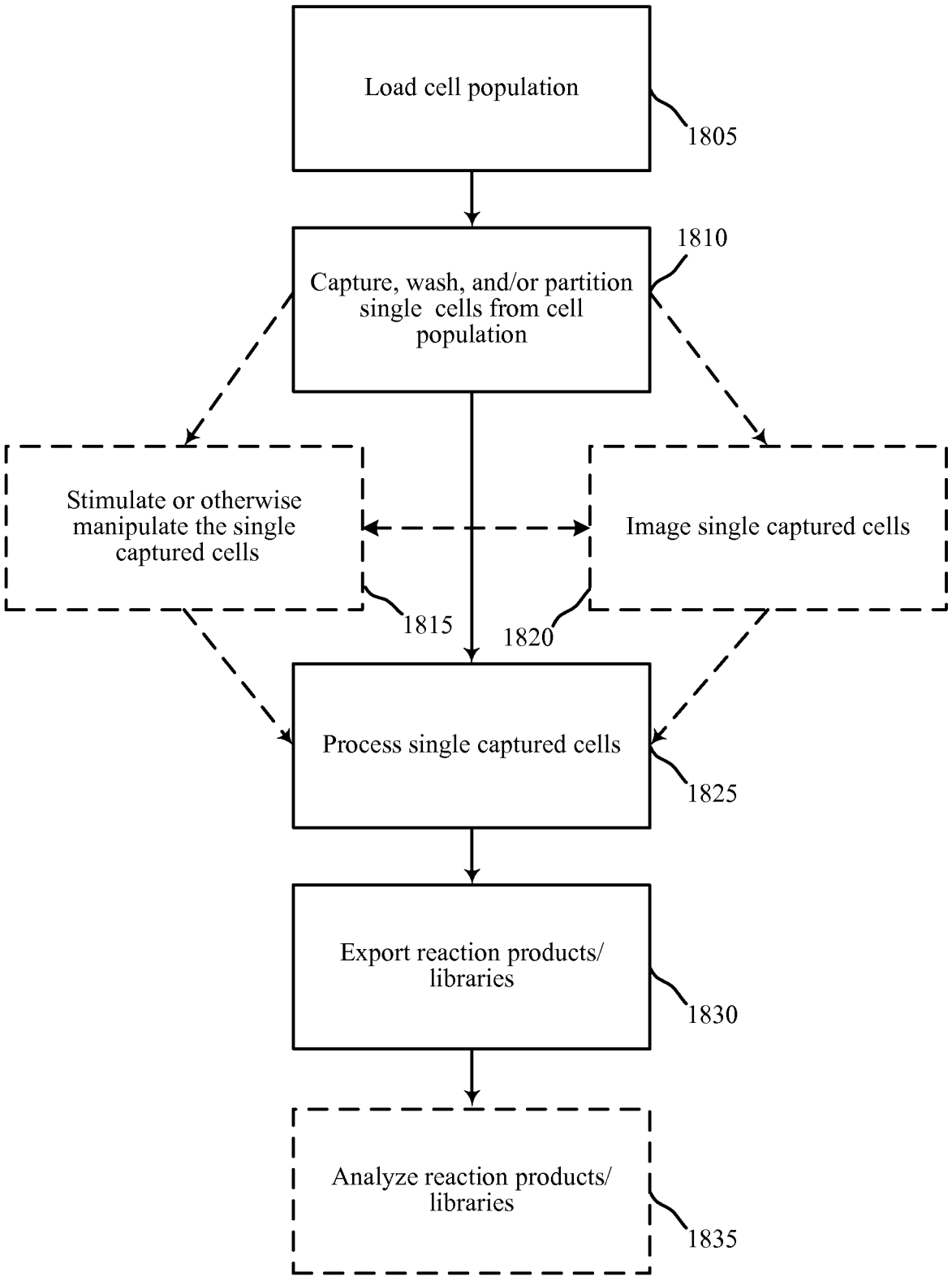

Load cell population
1805
Capture, wash, and/or partition single cells from cell population
1810
Stimulate or otherwise manipulate the single captured cells
1815
Image single captured cells
1820
Process single captured cells
1825
Export reaction products/ libraries
1830
Analyze reaction products/ libraries
1835
1800

FIG. 18

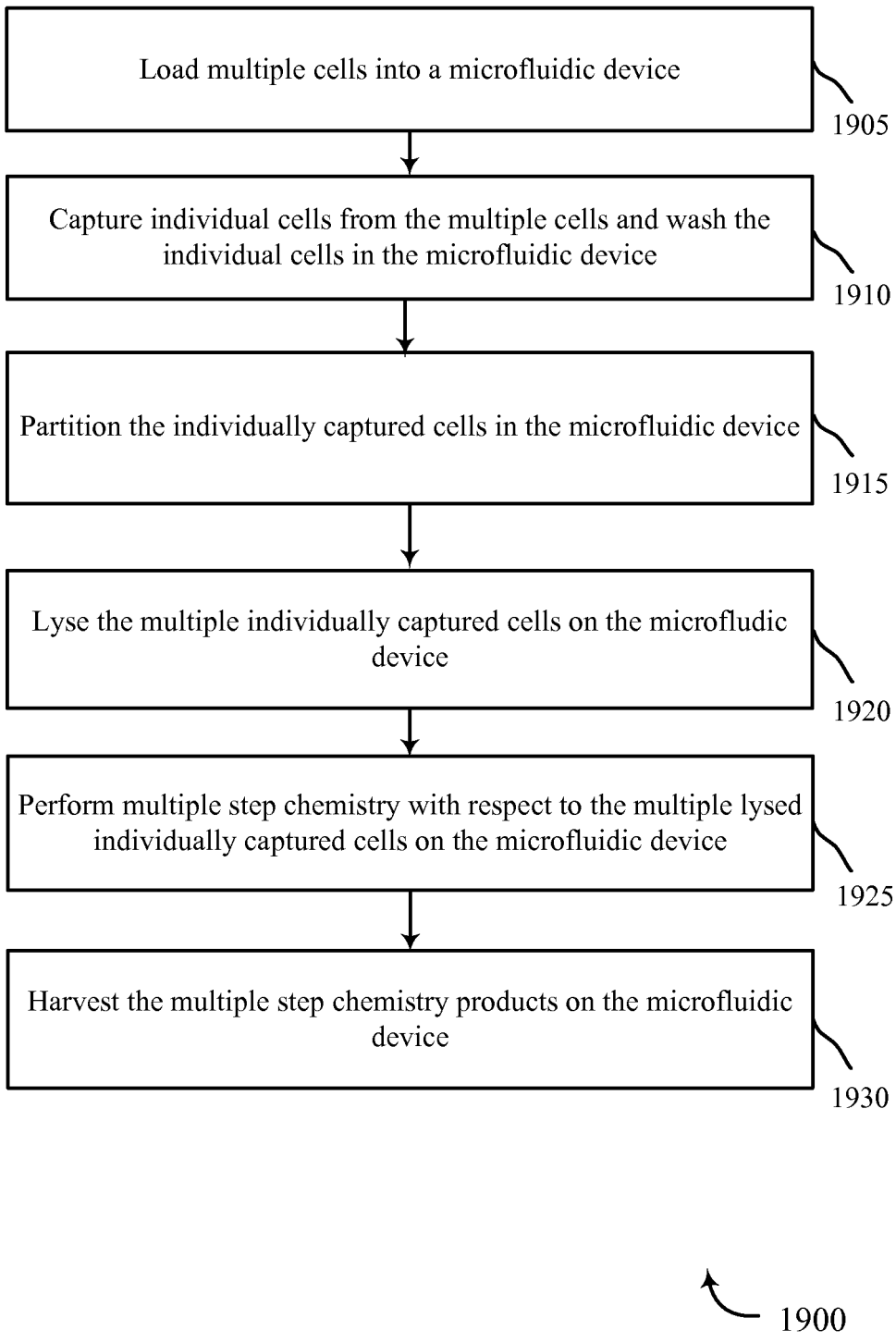

Load multiple cells into a microfluidic device
1905
Capture individual cells from the multiple cells and wash the individual cells in the microfluidic device
1910
Partition the individually captured cells in the microfluidic device
1915
Lyse the multiple individually captured cells on the microfluidic device
1920
Perform multiple step chemistry with respect to the multiple lysed individually captured cells on the microfluidic device
1925
Harvest the multiple step chemistry products on the microfluidic device
1930
1900

FIG. 19

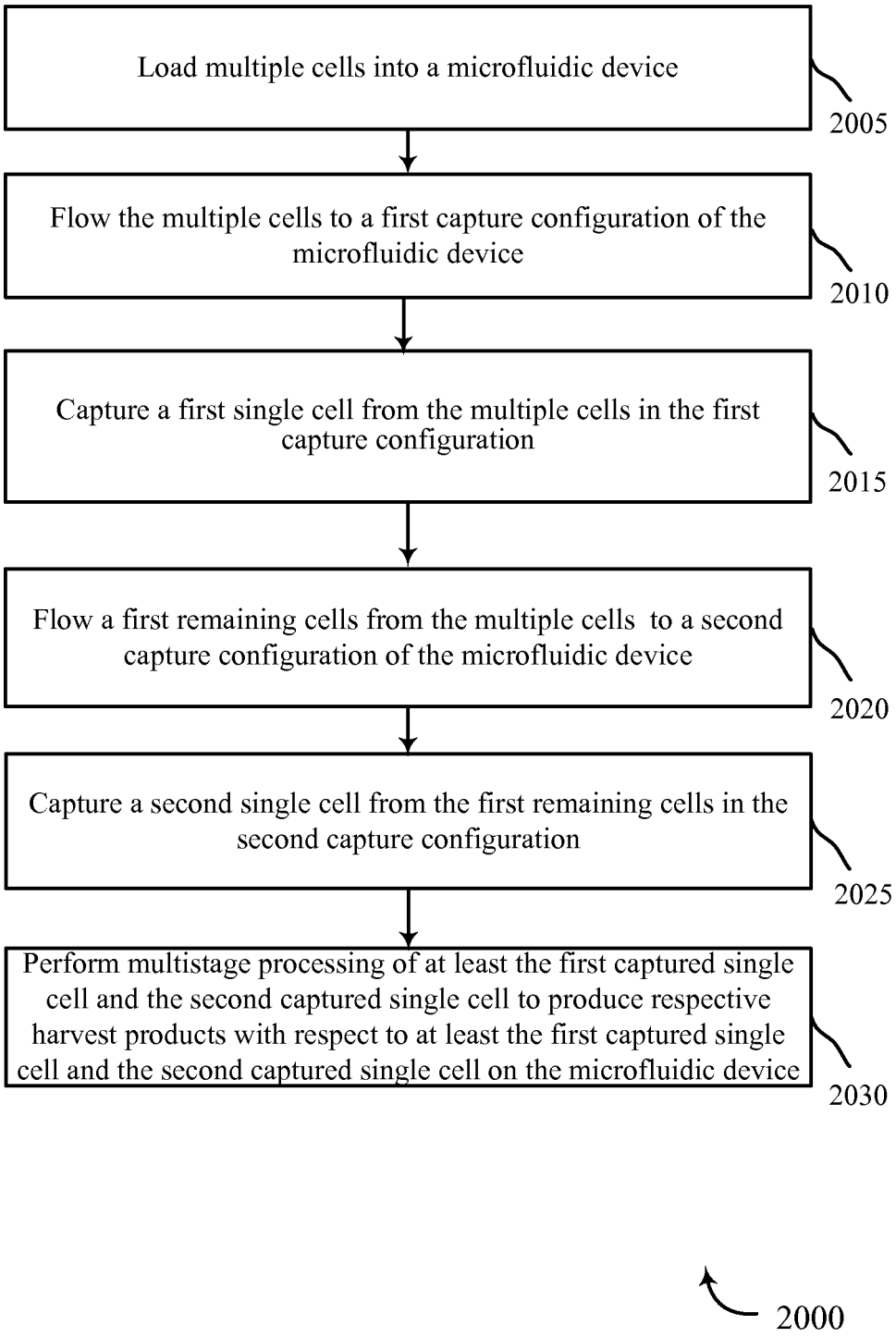

Load multiple cells into a microfluidic device
2005
Flow the multiple cells to a first capture configuration of the microfluidic device
2010
Capture a first single cell from the multiple cells in the first capture configuration
2015
Flow a first remaining cells from the multiple cells to a second capture configuration of the microfluidic device
2020
Capture a second single cell from the first remaining cells in the second capture configuration
2025
Perform multistage processing of at least the first captured single cell and the second captured single cell to produce respective harvest products with respect to at least the first captured single cell and the second captured single cell on the microfluidic device
2030
2000

FIG. 20

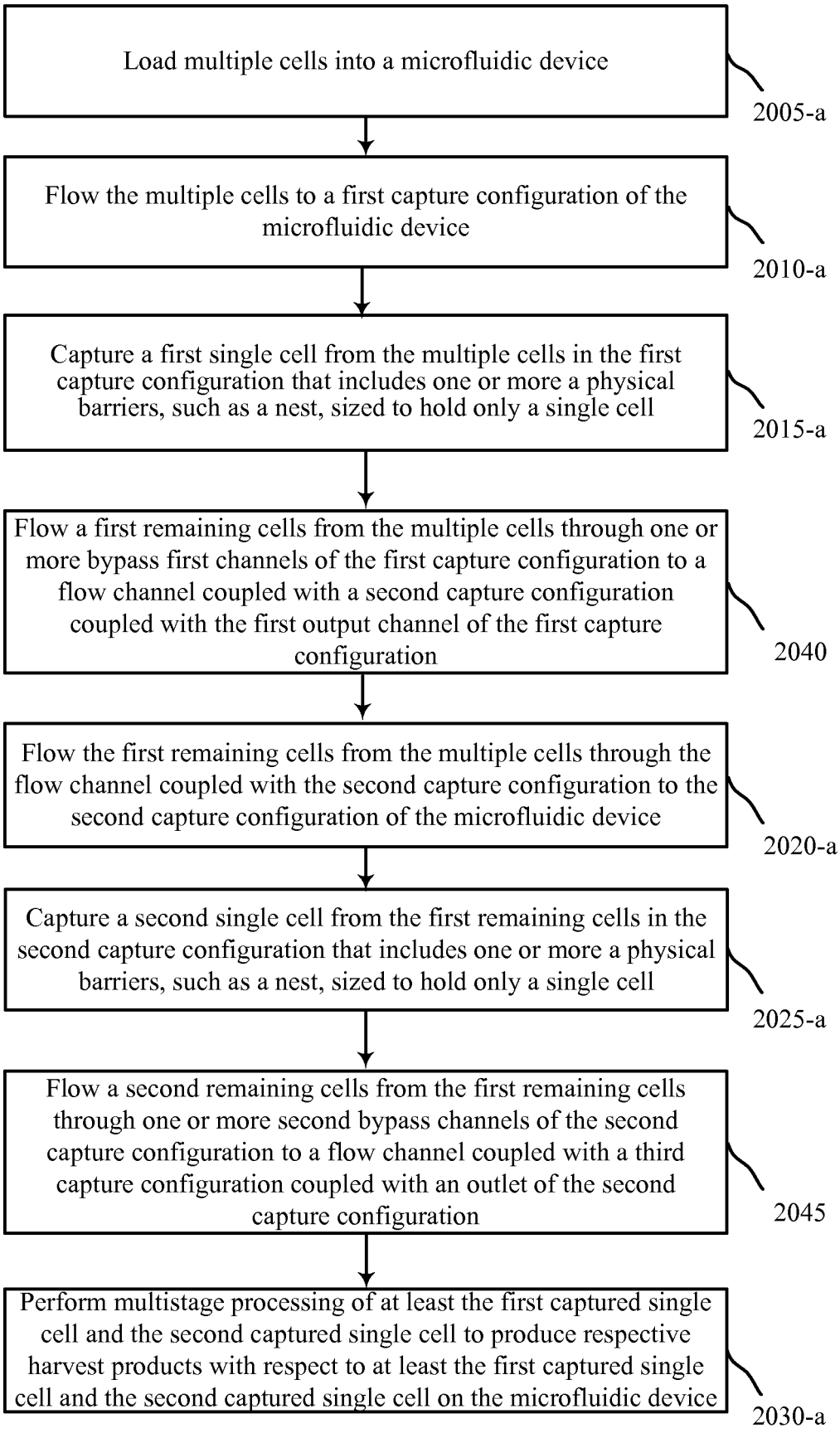

Load multiple cells into a microfluidic device
2005-a
Flow the multiple cells to a first capture configuration of the microfluidic device
2010-a
Capture a first single cell from the multiple cells in the first capture configuration that includes one or more a physical barriers, such as a nest, sized to hold only a single cell
2015-a
Flow a first remaining cells from the multiple cells through one or more bypass first channels of the first capture configuration to a flow channel coupled with a second capture configuration coupled with the first output channel of the first capture configuration
2040
Flow the first remaining cells from the multiple cells through the flow channel coupled with the second capture configuration to the second capture configuration of the microfluidic device
2020-a
Capture a second single cell from the first remaining cells in the second capture configuration that includes one or more a physical barriers, such as a nest, sized to hold only a single cell
2025-a
Flow a second remaining cells from the first remaining cells through one or more second bypass channels of the second capture configuration to a flow channel coupled with a third capture configuration coupled with an outlet of the second capture configuration
2045
Perform multistage processing of at least the first captured single cell and the second captured single cell to produce respective harvest products with respect to at least the first captured single cell and the second captured single cell on the microfluidic device
2030-a
2000-a

FIG. 20A

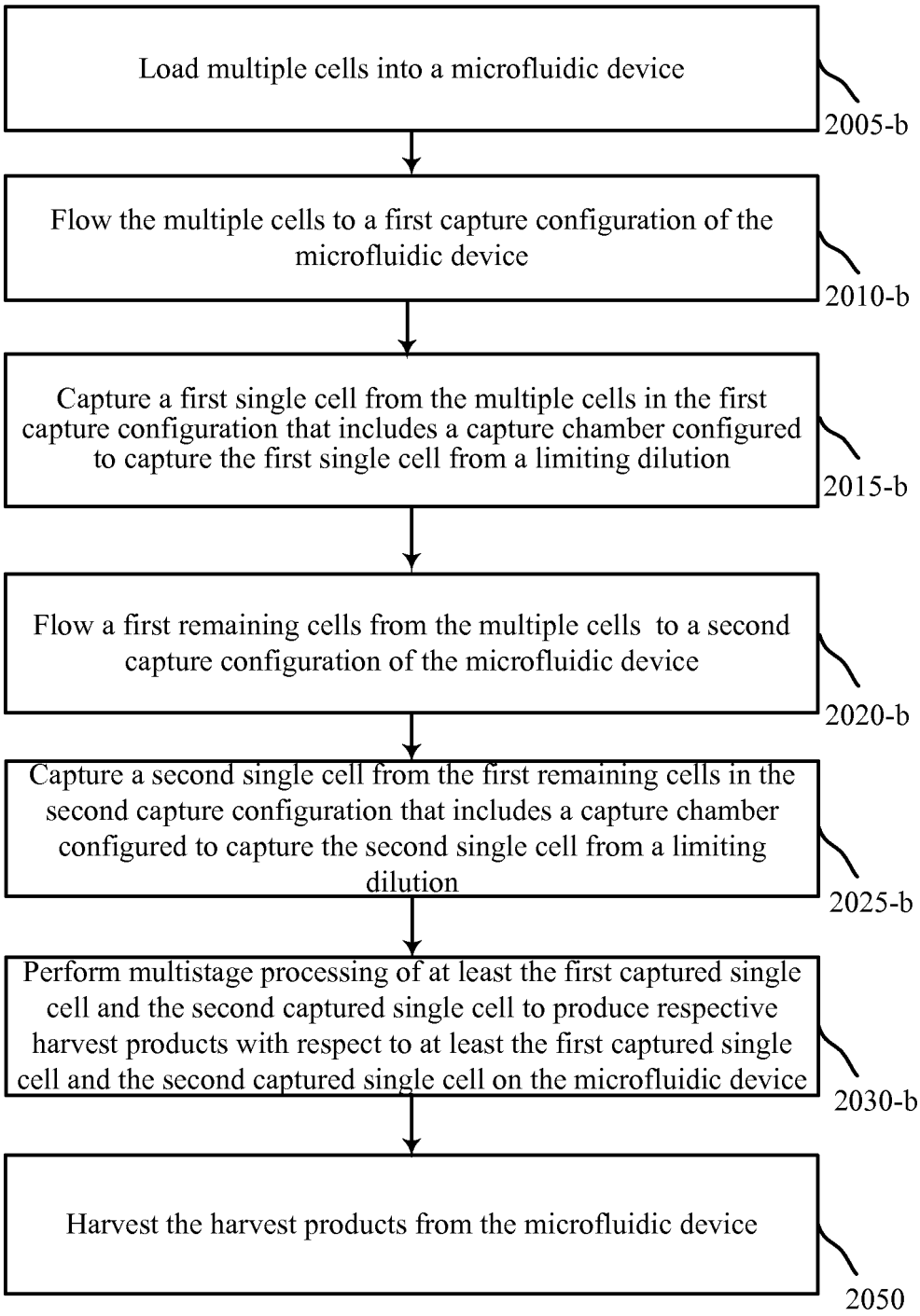

Load multiple cells into a microfluidic device
2005-b
Flow the multiple cells to a first capture configuration of the microfluidic device
2010-b
Capture a first single cell from the multiple cells in the first capture configuration that includes a capture chamber configured to capture the first single cell from a limiting dilution
2015-b
Flow a first remaining cells from the multiple cells to a second capture configuration of the microfluidic device
2020-b
Capture a second single cell from the first remaining cells in the second capture configuration that includes a capture chamber configured to capture the second single cell from a limiting dilution
2025-b
Perform multistage processing of at least the first captured single cell and the second captured single cell to produce respective harvest products with respect to at least the first captured single cell and the second captured single cell on the microfluidic device
2030-b
Harvest the harvest products from the microfluidic device
2050
2000-b

FIG. 20B

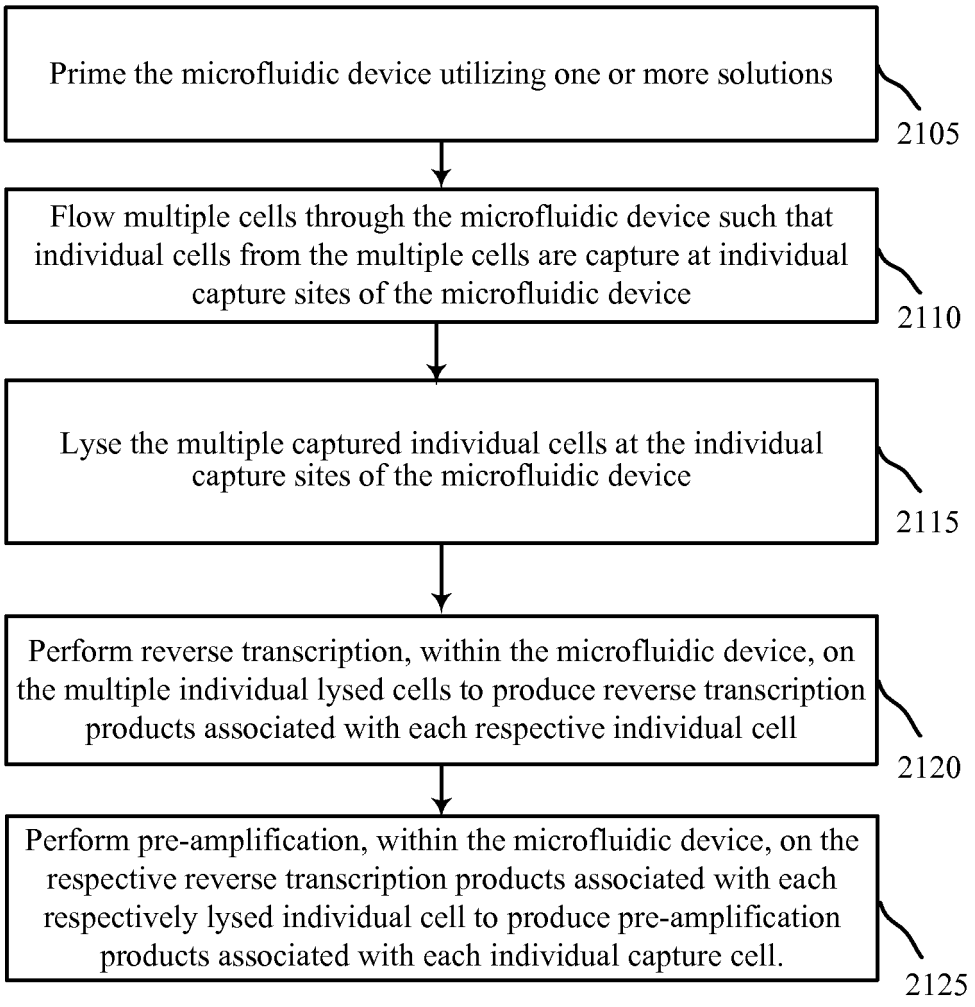

Prime the microfluidic device utilizing one or more solutions
2105
Flow multiple cells through the microfluidic device such that individual cells from the multiple cells are capture at individual capture sites of the microfluidic device
2110
Lyse the multiple captured individual cells at the individual capture sites of the microfluidic device
2115
Perform reverse transcription, within the microfluidic device, on the multiple individual lysed cells to produce reverse transcription products associated with each respective individual cell
2120
Perform pre-amplification, within the microfluidic device, on the respective reverse transcription products associated with each respectively lysed individual cell to produce pre-amplification products associated with each individual capture cell.
2125
2100

FIG. 21

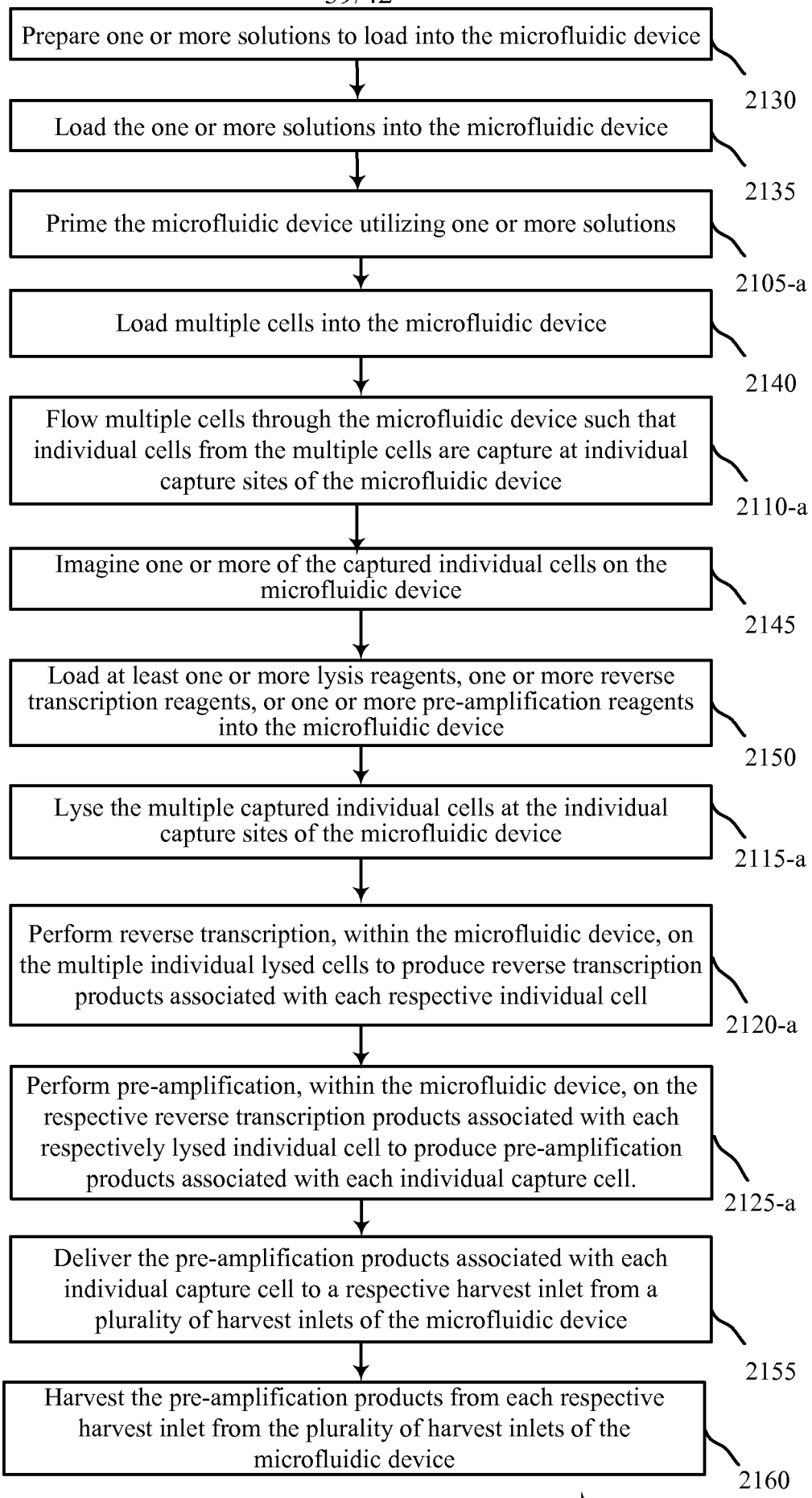

Prepare one or more solutions to load into the microfluidic device
2130
Load the one or more solutions into the microfluidic device
2135
Prime the microfluidic device utilizing one or more solutions
2105-a
Load multiple cells into the microfluidic device
2140
Flow multiple cells through the microfluidic device such that individual cells from the multiple cells are capture at individual capture sites of the microfluidic device
2110-a
Imagine one or more of the captured individual cells on the microfluidic device
2145
Load at least one or more lysis reagents, one or more reverse transcription reagents, or one or more pre-amplification reagents into the microfluidic device
2150
Lyse the multiple captured individual cells at the individual capture sites of the microfluidic device
2115-a
Perform reverse transcription, within the microfluidic device, on the multiple individual lysed cells to produce reverse transcription products associated with each respective individual cell
2120-a
Perform pre-amplification, within the microfluidic device, on the respective reverse transcription products associated with each respectively lysed individual cell to produce pre-amplification products associated with each individual capture cell.
2125-a
Deliver the pre-amplification products associated with each individual capture cell to a respective harvest inlet from a plurality of harvest inlets of the microfluidic device
2155
Harvest the pre-amplification products from each respective harvest inlet from the plurality of harvest inlets of the microfluidic device
2160

FIG. 21A

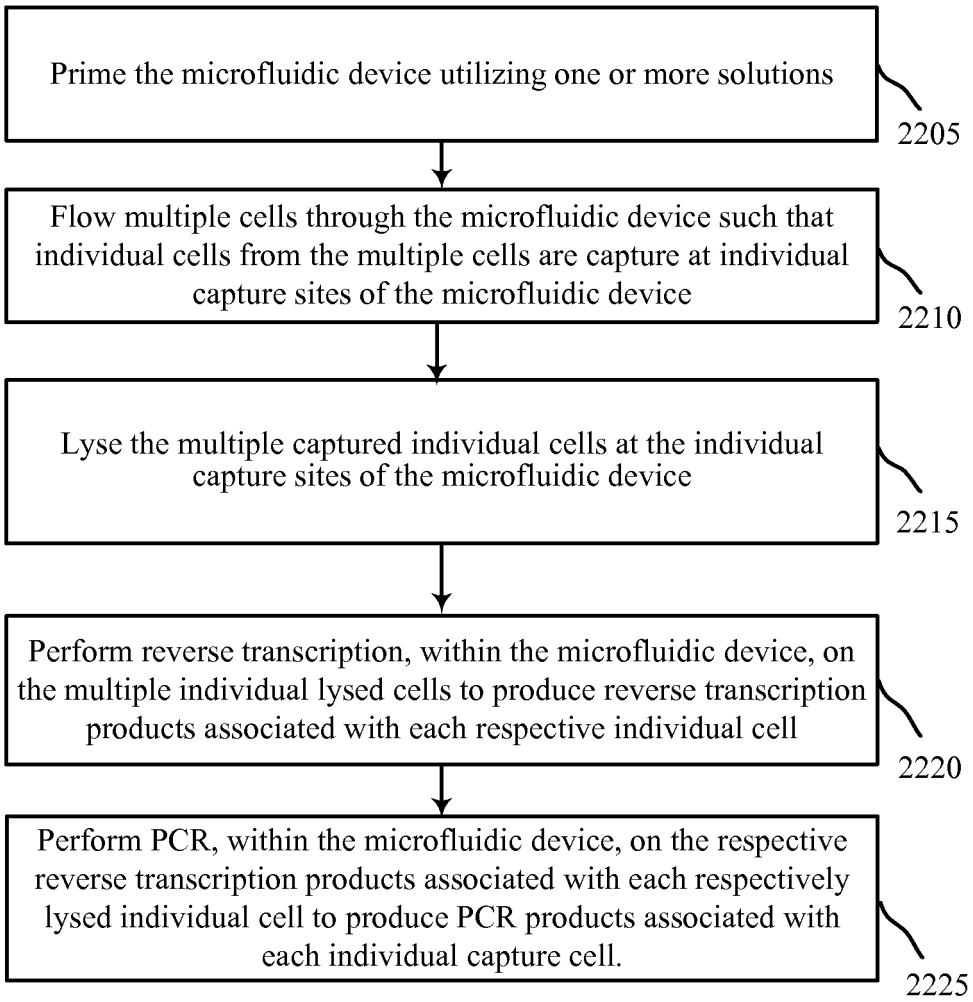

Prime the microfluidic device utilizing one or more solutions
2205
Flow multiple cells through the microfluidic device such that individual cells from the multiple cells are capture at individual capture sites of the microfluidic device
2210
Lyse the multiple captured individual cells at the individual capture sites of the microfluidic device
2215
Perform reverse transcription, within the microfluidic device, on the multiple individual lysed cells to produce reverse transcription products associated with each respective individual cell
2220
Perform PCR, within the microfluidic device, on the respective reverse transcription products associated with each respectively lysed individual cell to produce PCR products associated with each individual capture cell.
2225
2200

FIG. 22

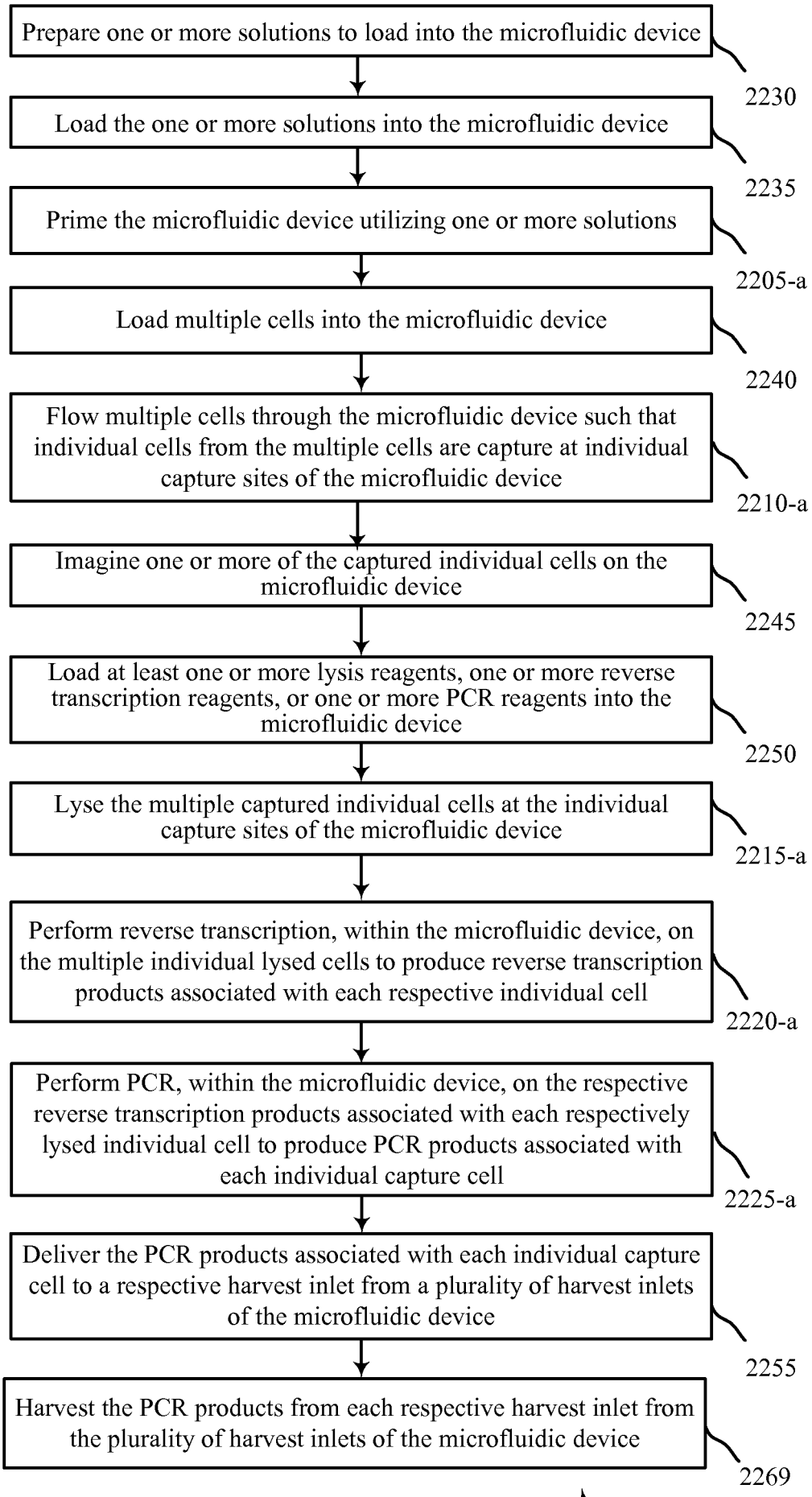

Prepare one or more solutions to load into the microfluidic device
2230
Load the one or more solutions into the microfluidic device
2235
Prime the microfluidic device utilizing one or more solutions
2205-a
Load multiple cells into the microfluidic device
2240
Flow multiple cells through the microfluidic device such that individual cells from the multiple cells are capture at individual capture sites of the microfluidic device
2210-a
Imagine one or more of the captured individual cells on the microfluidic device
2245
Load at least one or more lysis reagents, one or more reverse transcription reagents, or one or more PCR reagents into the microfluidic device
2250
Lyse the multiple captured individual cells at the individual capture sites of the microfluidic device
2215-a
Perform reverse transcription, within the microfluidic device, on the multiple individual lysed cells to produce reverse transcription products associated with each respective individual cell
2220-a
Perform PCR, within the microfluidic device, on the respective reverse transcription products associated with each respectively lysed individual cell to produce PCR products associated with each individual capture cell
2225-a
Deliver the PCR products associated with each individual capture cell to a respective harvest inlet from a plurality of harvest inlets of the microfluidic device
2255
Harvest the PCR products from each respective harvest inlet from the plurality of harvest inlets of the microfluidic device
2269

FIG. 22A

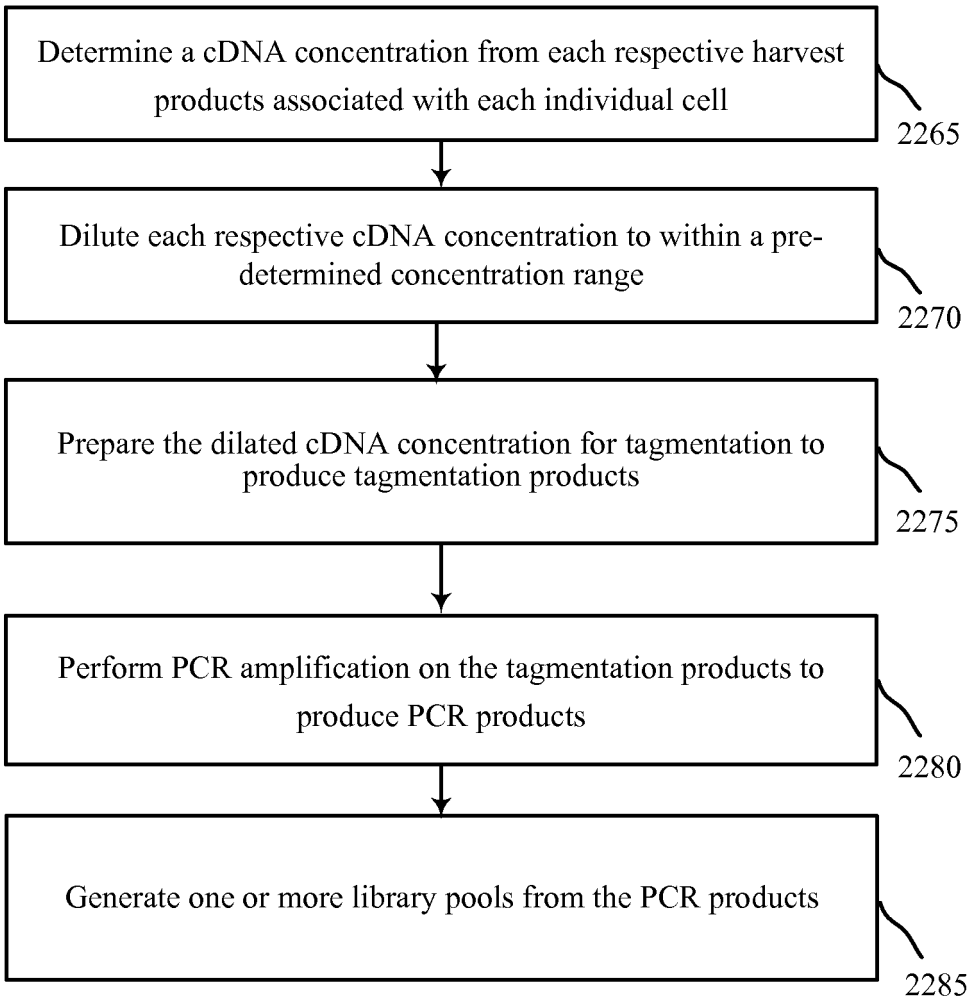

Determine a cDNA concentration from each respective harvest products associated with each individual cell
2265
Dilute each respective cDNA concentration to within a pre-determined concentration range
2270
Prepare the dilated cDNA concentration for tagmentation to produce tagmentation products
2275
Perform PCR amplification on the tagmentation products to produce PCR products
2280
Generate one or more library pools from the PCR products
2285
2200-b

FIG. 22B