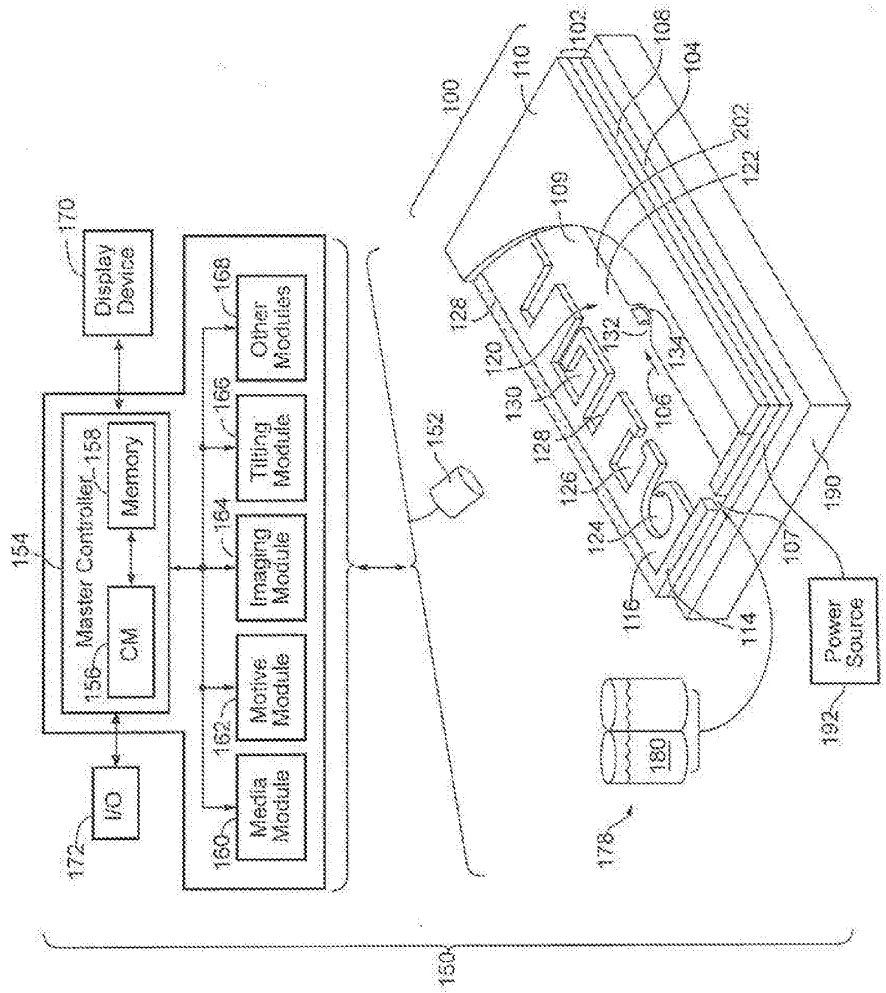
Display Device
170
Other Modules
168
Tilting Module
166
Imaging Module
164
Motive Module
162
Media Module
160
Master Controller
158
Memory
CM
156
154
I/O
172
152
100
110
102
106
104
202
122
109
128
120
132
134
130
106
128
126
124
116
114
107
130
180
178
Power Source
192
150

FIG. 1A

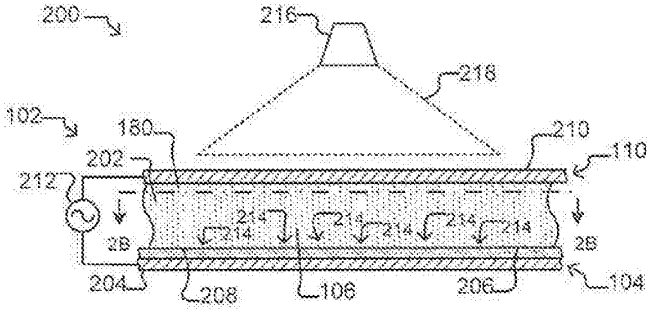
200
216
218
102
160
210
110
202
212
214
214
214
214
214
214
2B
2B
204
208
106
206
104

FIG. 1B

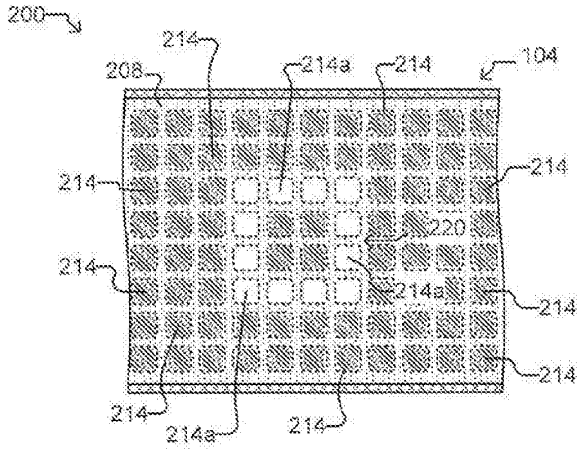
200
214
208
214a
214
104
214
214
220
214a
214
214
214
214a
214

FIG. 1C

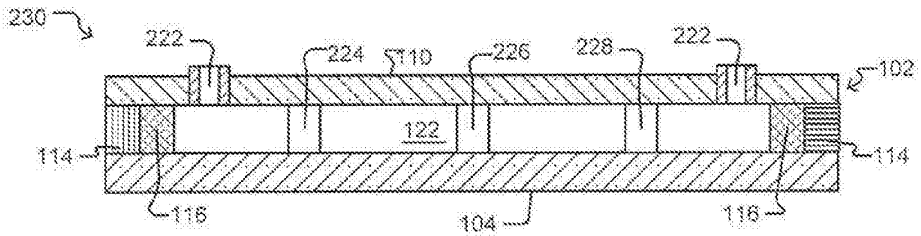
230
222
224
110
226
228
222
102
122
114
114
116
104
116

FIG. 2A

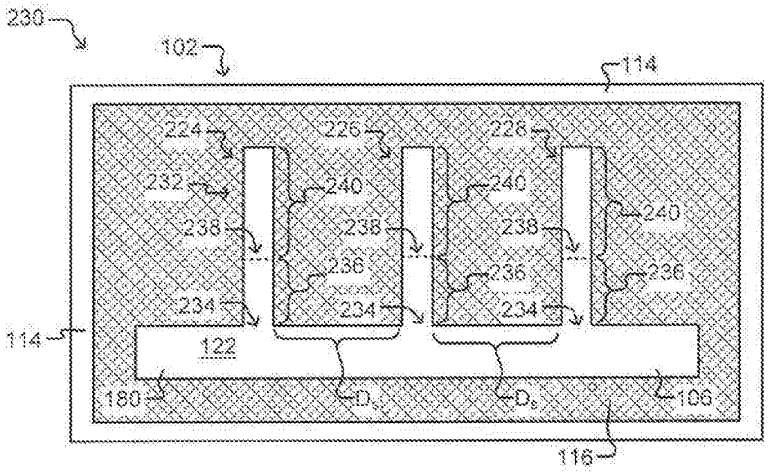
230
102
114
224
226
226
232
240
240
240
238
238
238
236
236
236
234
234
234
114
122
180
D_s
D_s
106
116

FIG. 2B

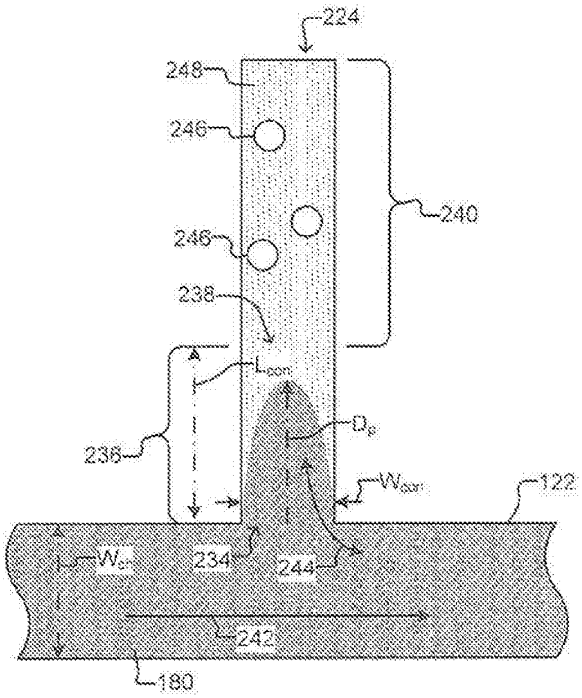
224
248
246
240
246
238
L_{corr}
D_p
236
W_{corr}
122
W_{gr}
234
244
242
180

FIG. 2C

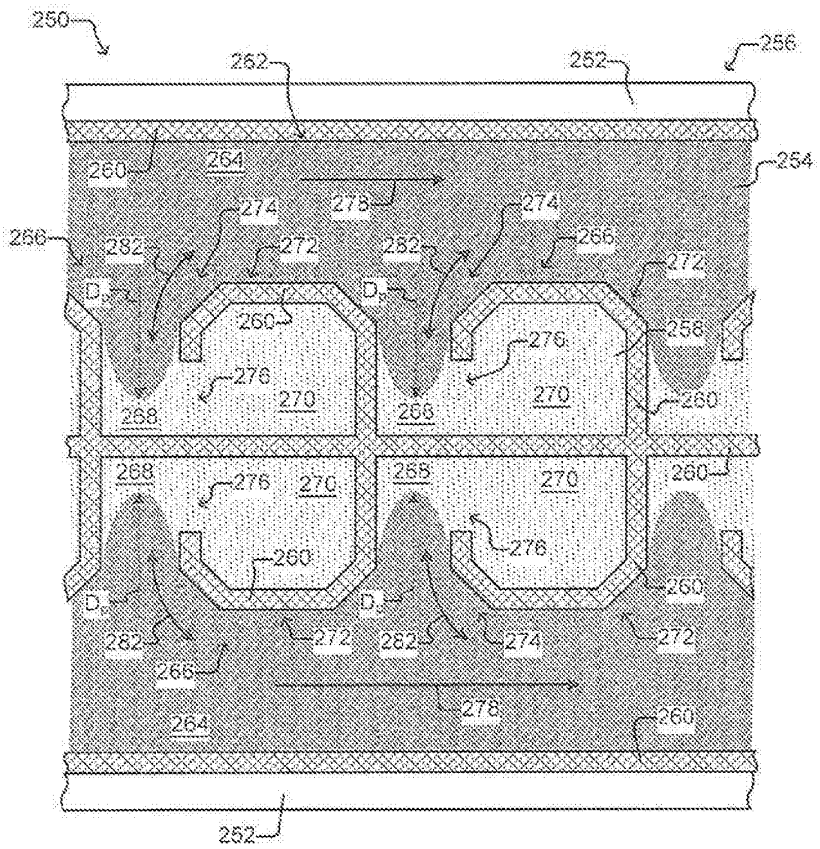
250
262
252
256
260
264
254
278
274
266
282
272
D_s
276
270
268
258

FIG. 2D

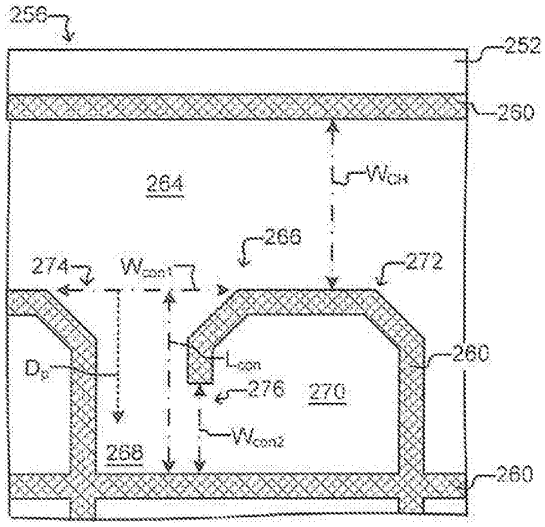
256
252
260
264
W_{CH}
266
274
W_{con1}
272
260
D_p
L_{con}
276
270
W_{con2}
268
260

FIG. 2E

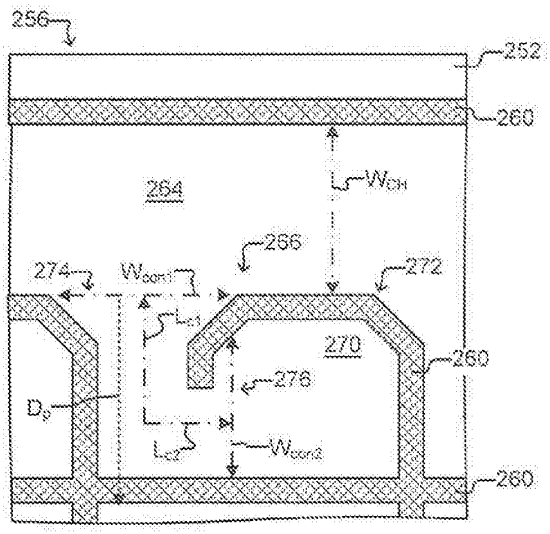
256
252
260
264
W_{CH}
266
274
W_{con1}
272
L_{c1}
270
260
276
D_p
L_{c2}
W_{con2}
260

FIG. 2F

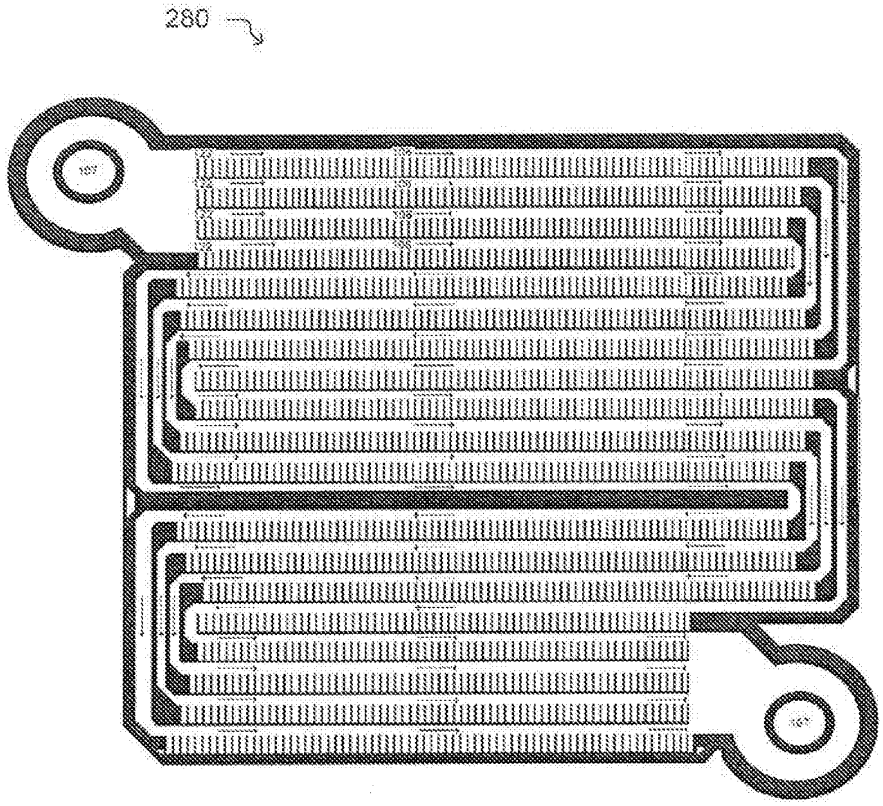

FIG. 2G

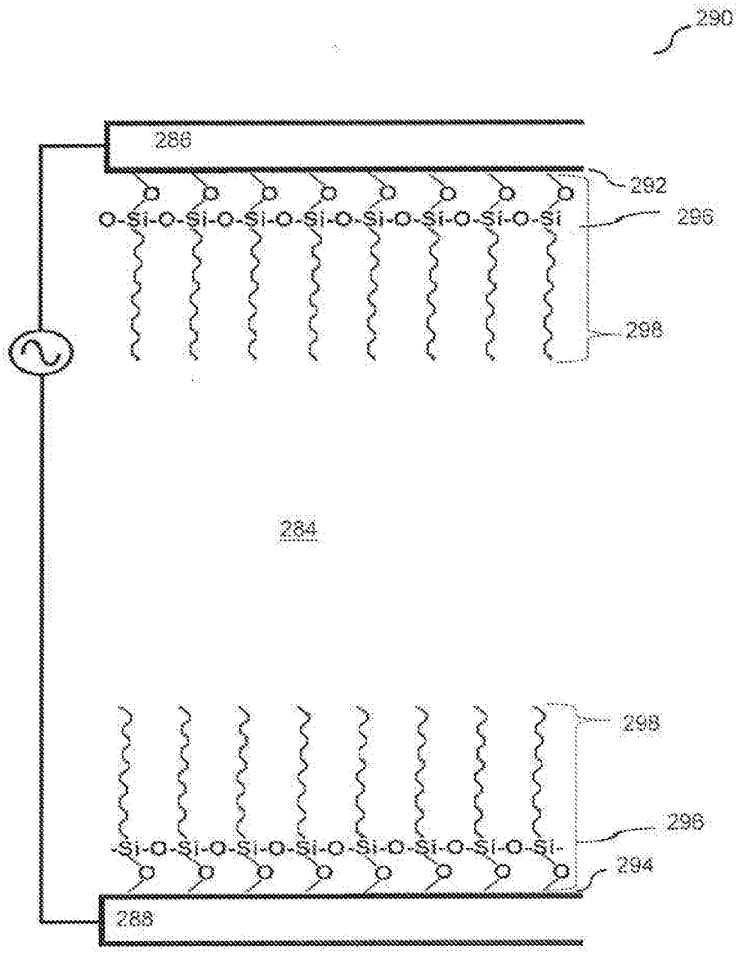

FIG. 2H

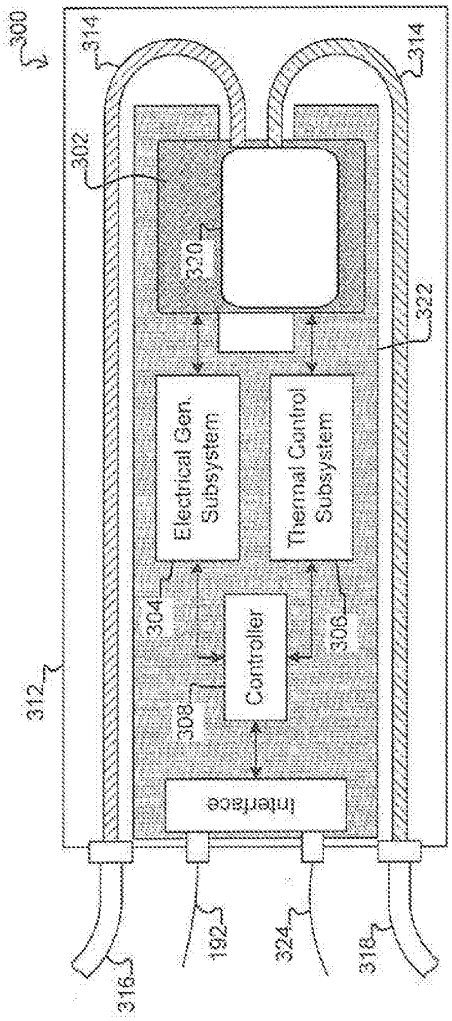
300
314
314
302
320
322
Electrical Gen. Subsystem
Thermal Control Subsystem
304
306
Controller
308
312
Interface
316
192
324
318

FIG. 3A

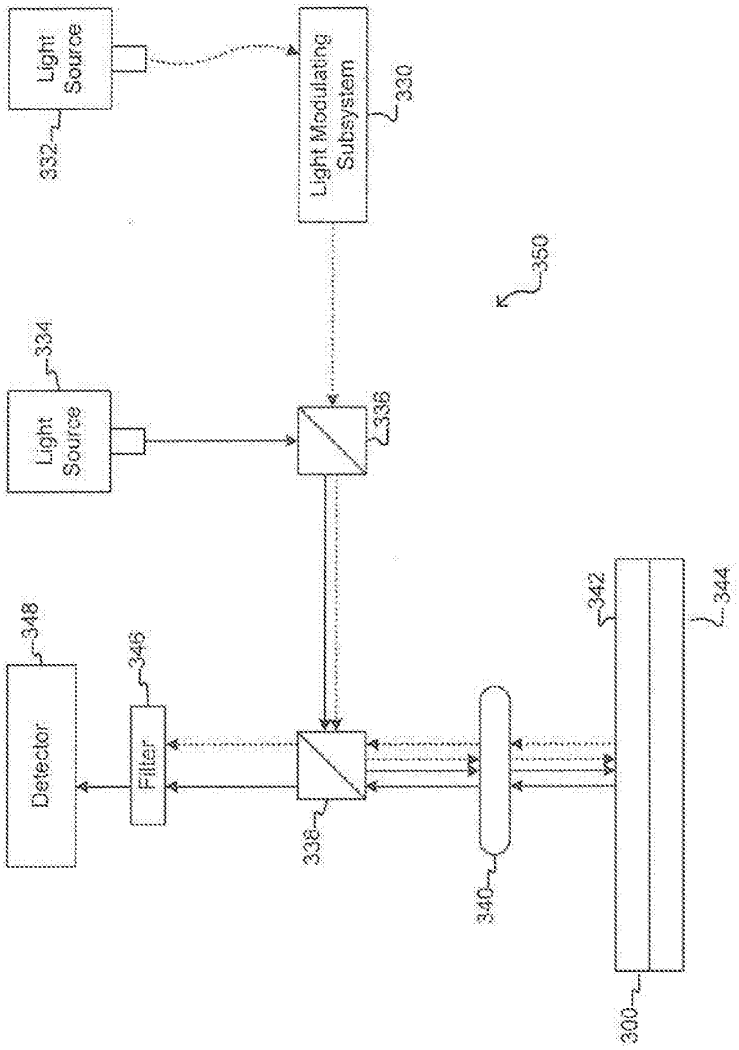
Light Source
332
Light Modulating Subsystem
330
350
Light Source
334
336
Detector
348
Filter
346
338
340
342
344
300

FIG. 3B

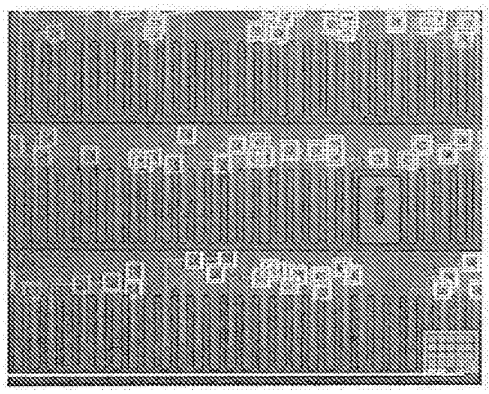

FIG. 4A

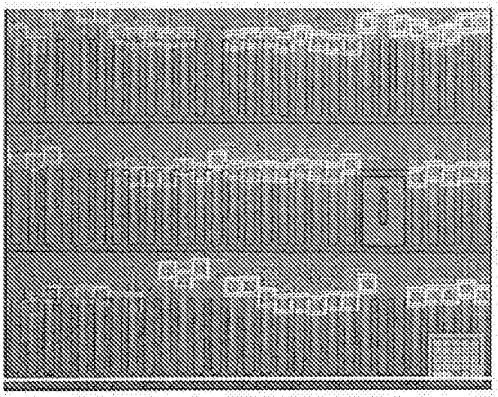

FIG. 4B

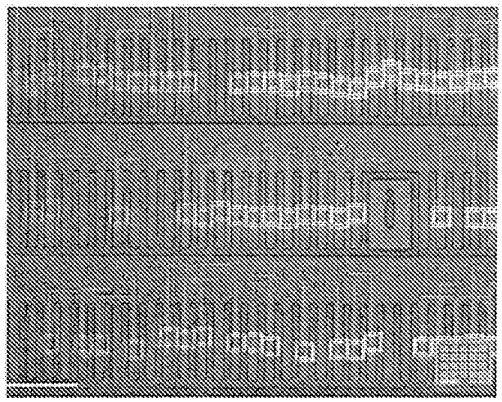

FIG. 4C

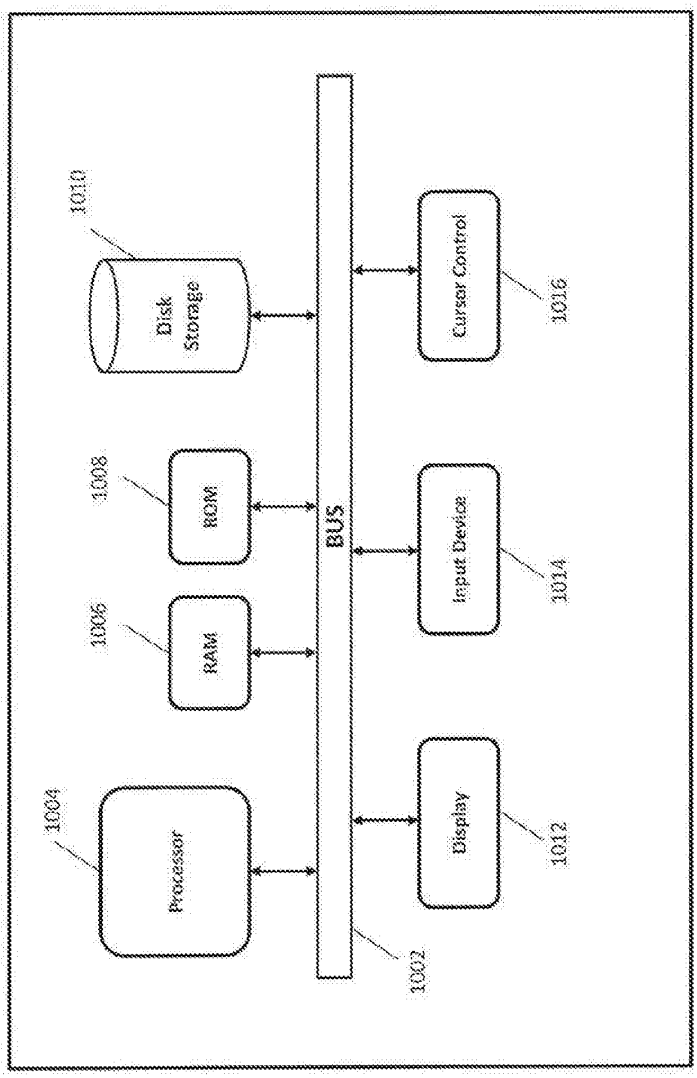
1000
1002
BUS
1004
Processor
1006
RAM
1008
ROM
1010
Disk Storage
1012
Display
1014
Input Device
1016
Cursor Control

FIG. 5

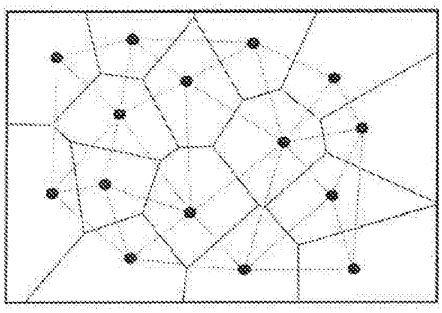

FIG. 6A

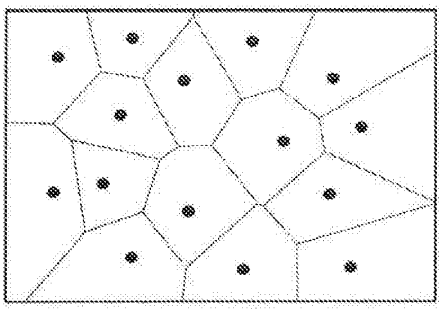

FIG. 6B

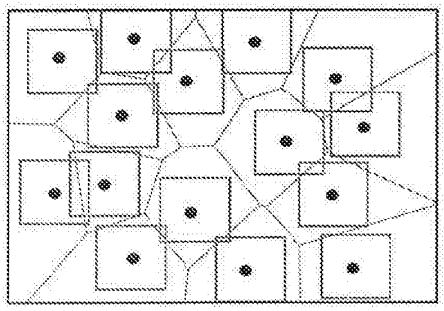

FIG. 6C

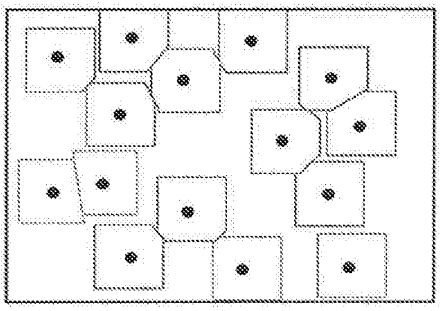

FIG. 6D

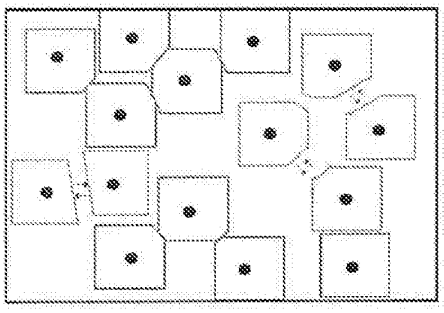

FIG. 6E

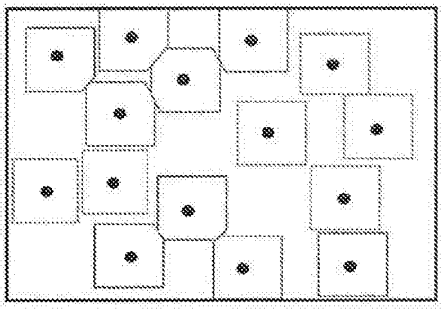

FIG. 6F

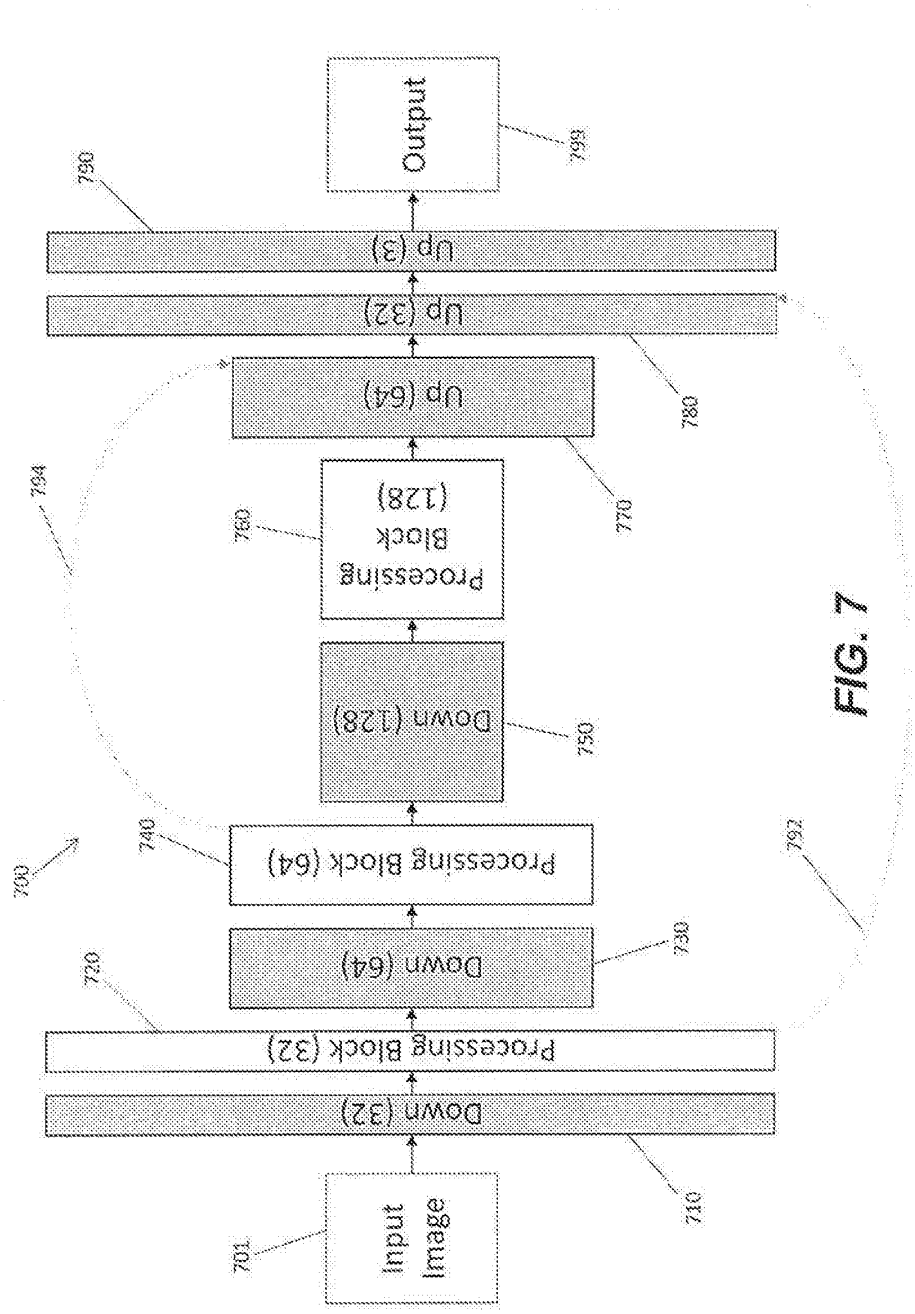
Input Image
701
Down (32)
710
Processing Block (32)
720
Down (64)
730
Processing Block (64)
740
Down (128)
750
Processing Block (128)
760
Up (64)
770
Up (32)
780
Up (3)
790
Output
799
700
792
794

FIG. 7

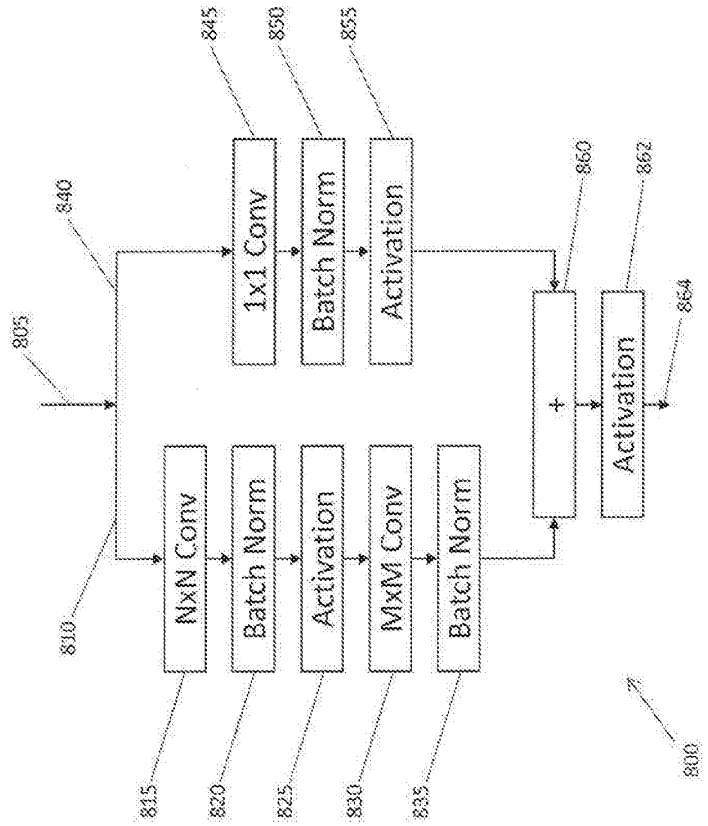
805
810
815
NxN Conv
820
Batch Norm
825
Activation
830
MxM Conv
835
Batch Norm
840
845
1x1 Conv
850
Batch Norm
855
Activation
860
+
862
Activation
864
800

FIG. 8A

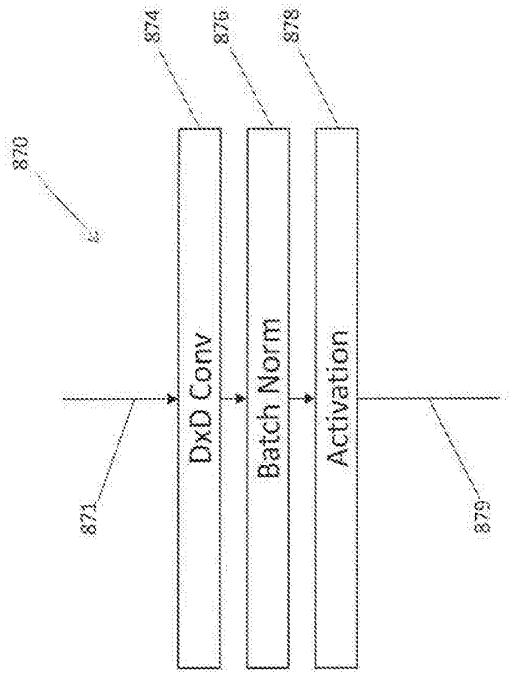

FIG. 8B

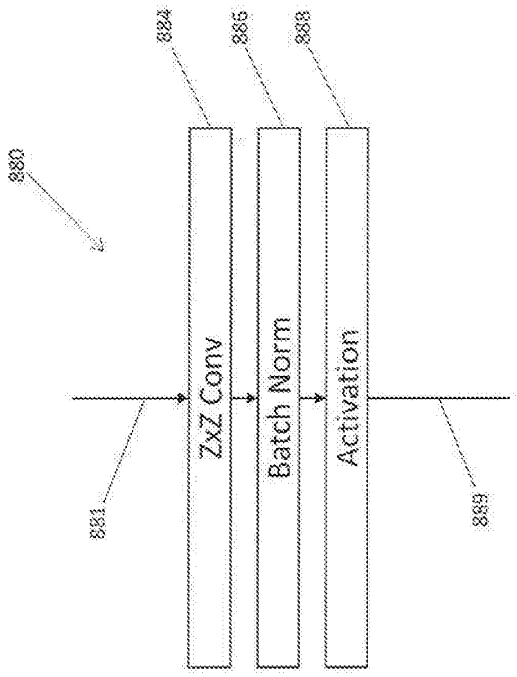

FIG. 8C

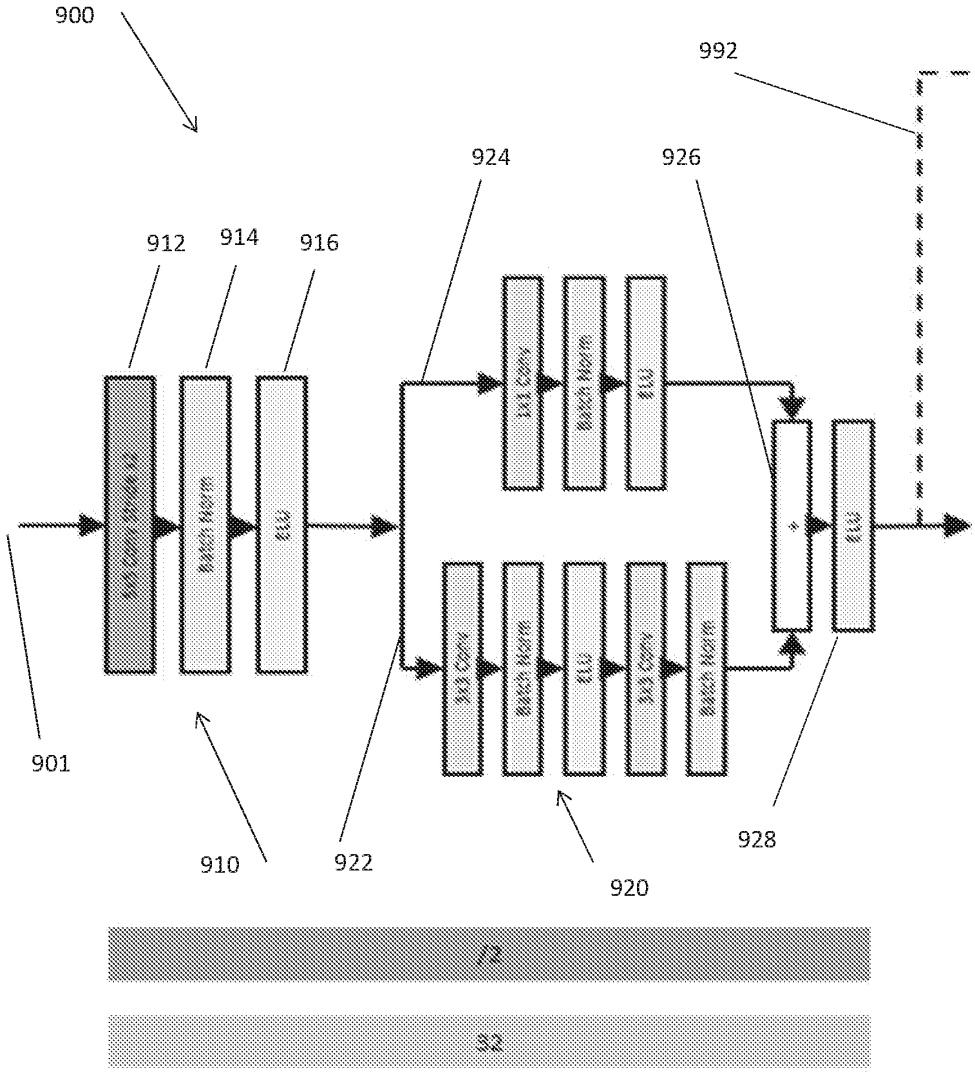
900
992
924
926
912
914
916
3x3 Conv, Stride 2
Batch Norm
ELU
1x1 Conv
Batch Norm
ELU
3x3 Conv
Batch Norm
ELU
3x3 Conv
Batch Norm
+
ELU
901
910
922
920
928
72
32

FIG. 9A

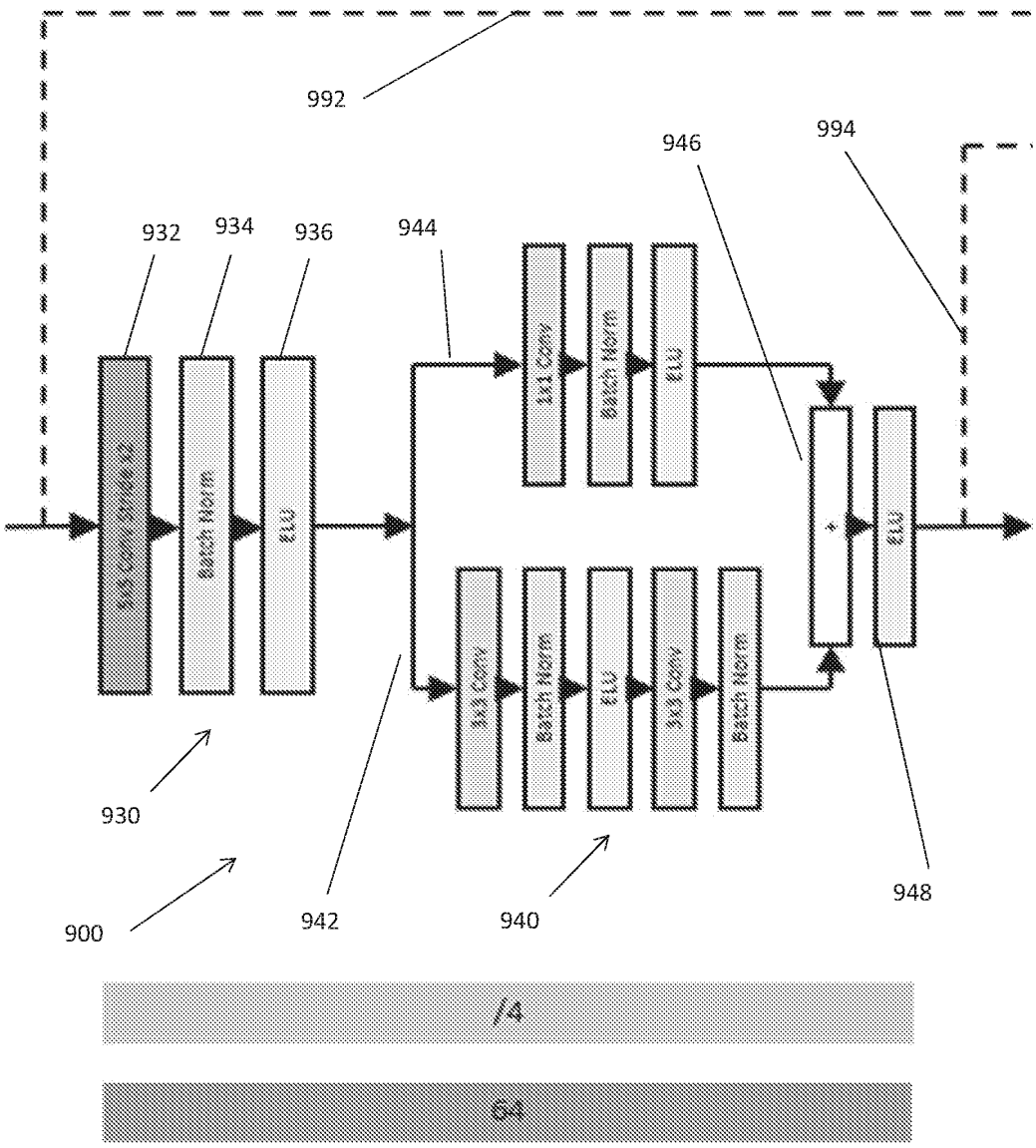
992
994
946
932
934
936
944
3x3 Conv /2
Batch Norm
ELU
1x1 Conv
Batch Norm
ELU
3x3 Conv
Batch Norm
ELU
3x3 Conv
Batch Norm
+
ELU
930
900
942
940
948
/4
64

FIG. 9B

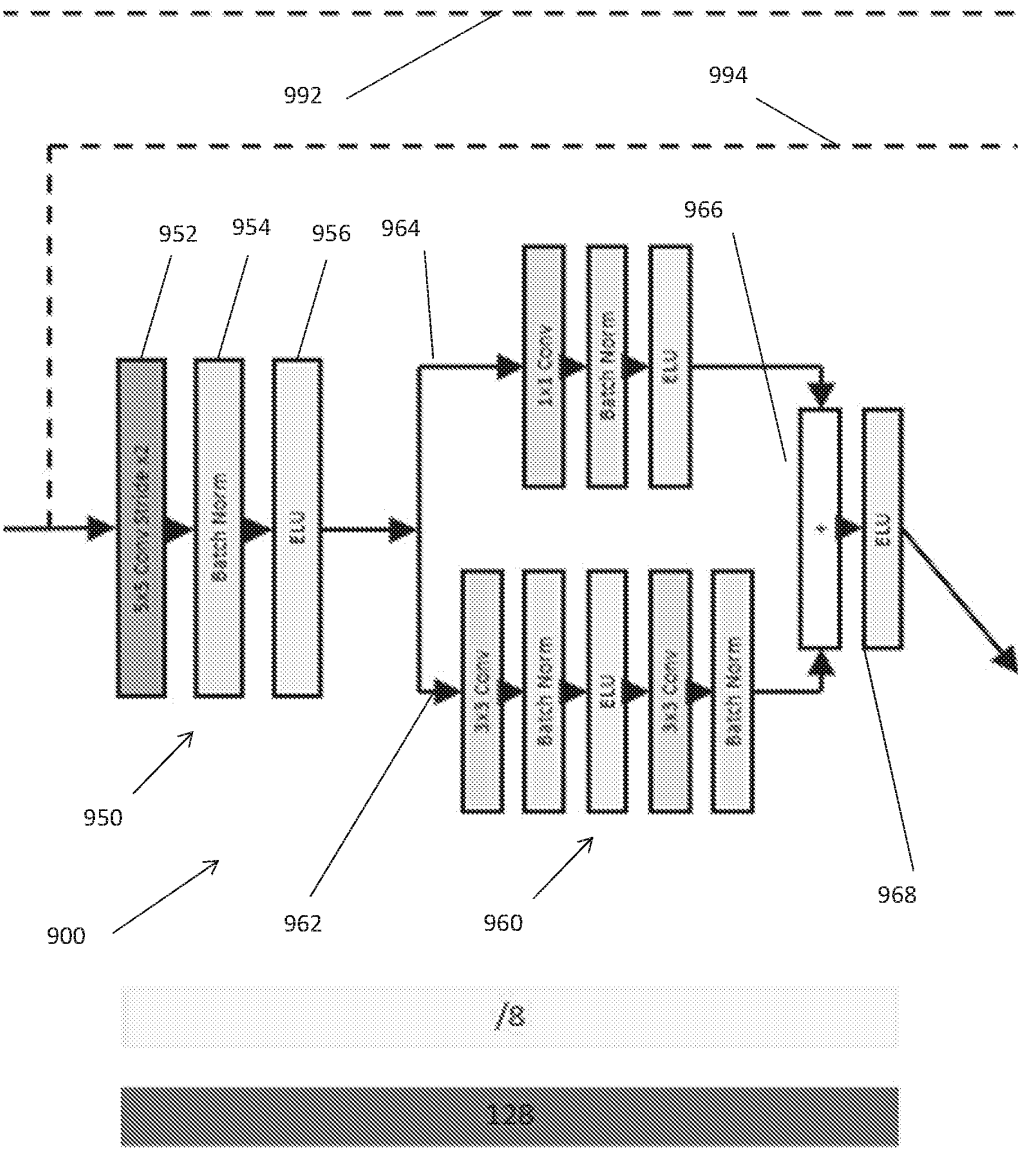
992
994
952
954
956
964
966
3x3 Conv Stride 2
Batch Norm
ELU
3x3 Conv
Batch Norm
ELU
3x3 Conv
Batch Norm
ELU
3x3 Conv
Batch Norm
+
ELU
950
900
962
960
968
/8
128

FIG. 9C

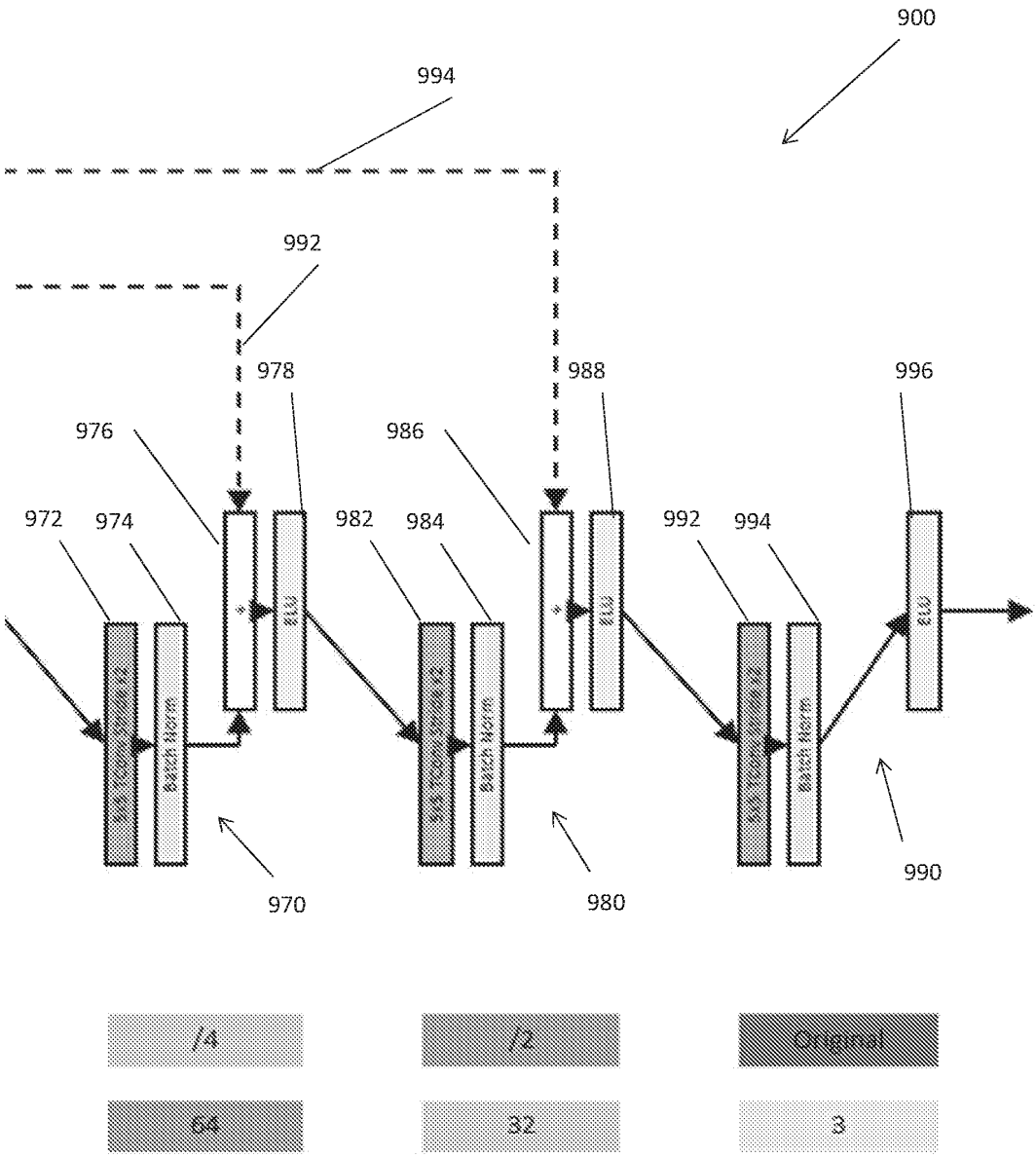
900
994
992
978
988
996
976
986
972
974
982
984
992
994
ELU
Batch Norm
970
980
990
/4
/2
Original
64
32
3

FIG. 9D

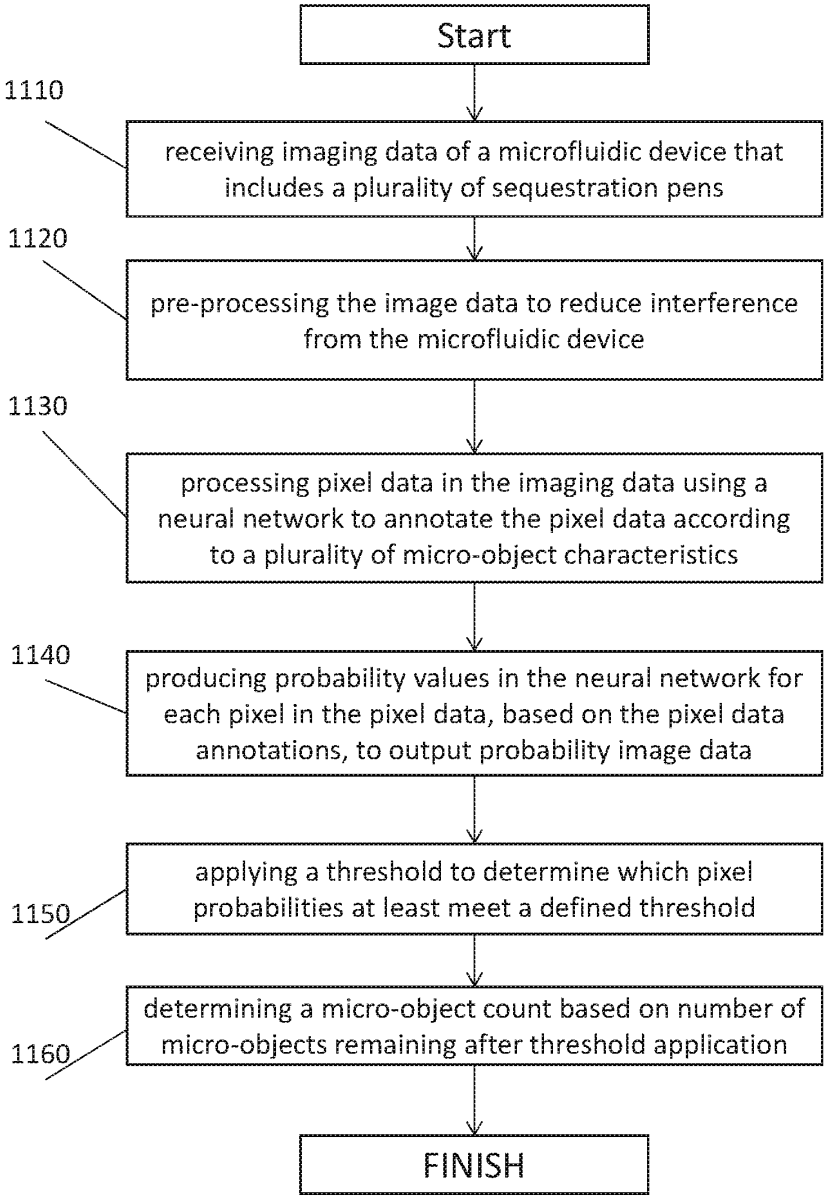
Start
1110
receiving imaging data of a microfluidic device that includes a plurality of sequestration pens
1120
pre-processing the image data to reduce interference from the microfluidic device
1130
processing pixel data in the imaging data using a neural network to annotate the pixel data according to a plurality of micro-object characteristics
1140
producing probability values in the neural network for each pixel in the pixel data, based on the pixel data annotations, to output probability image data
applying a threshold to determine which pixel probabilities at least meet a defined threshold
1150
determining a micro-object count based on number of micro-objects remaining after threshold application
1160
FINISH

FIG. 10

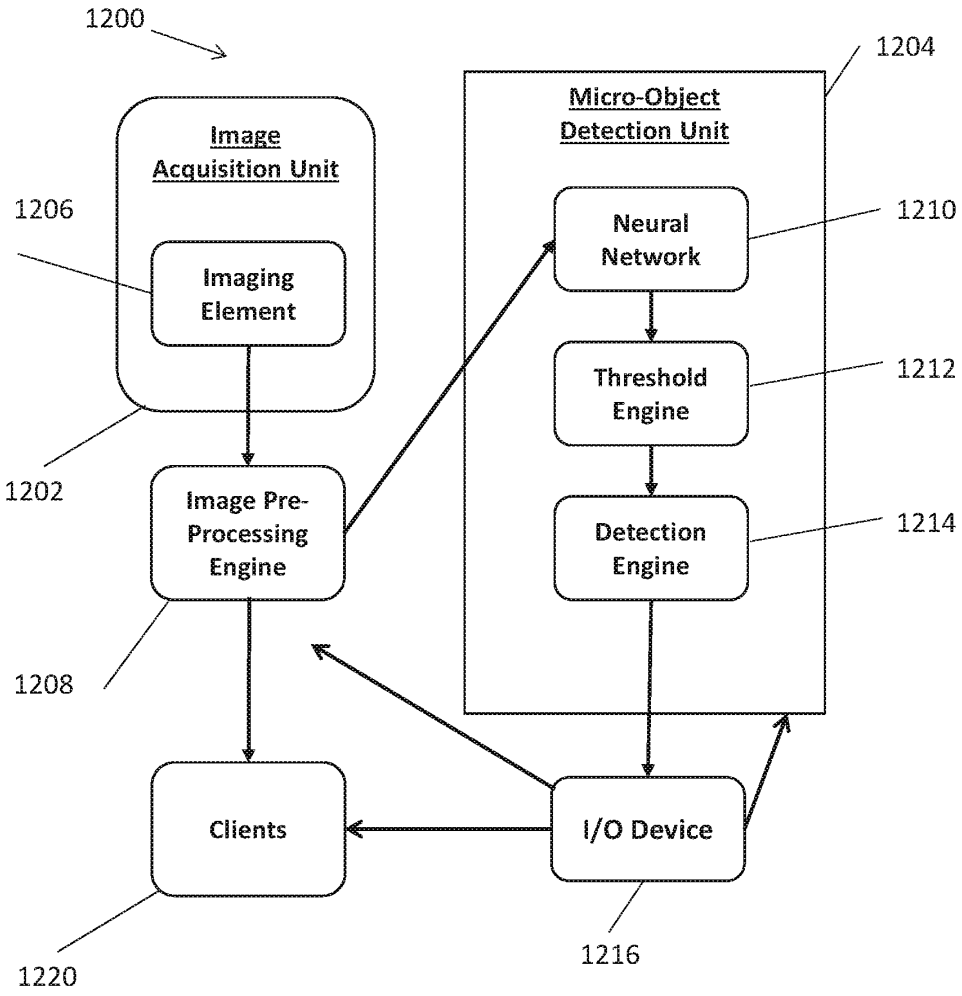
1200
1204
Micro-Object Detection Unit
Image Acquisition Unit
1206
Imaging Element
Neural Network
1210
Threshold Engine
1212
Detection Engine
1214
1202
Image Pre-Processing Engine
1208
Clients
I/O Device
1220
1216

FIG. 11

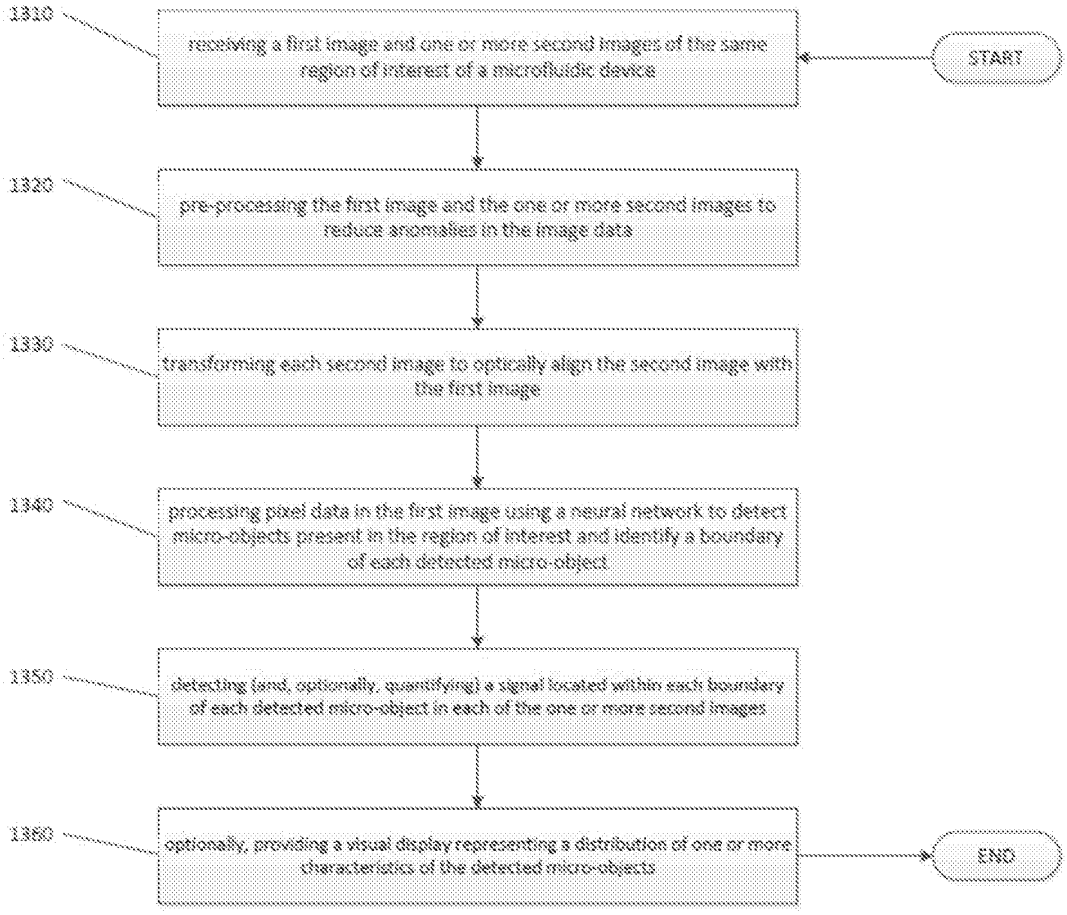
1310
receiving a first image and one or more second images of the same region of interest of a microfluidic device
START
1320
pre-processing the first image and the one or more second images to reduce anomalies in the image data
1330
transforming each second image to optically align the second image with the first image
1340
processing pixel data in the first image using a neural network to detect micro-objects present in the region of interest and identify a boundary of each detected micro-object
1350
detecting (and, optionally, quantifying) a signal located within each boundary of each detected micro-object in each of the one or more second images
1360
optionally, providing a visual display representing a distribution of one or more characteristics of the detected micro-objects
END

FIG. 12

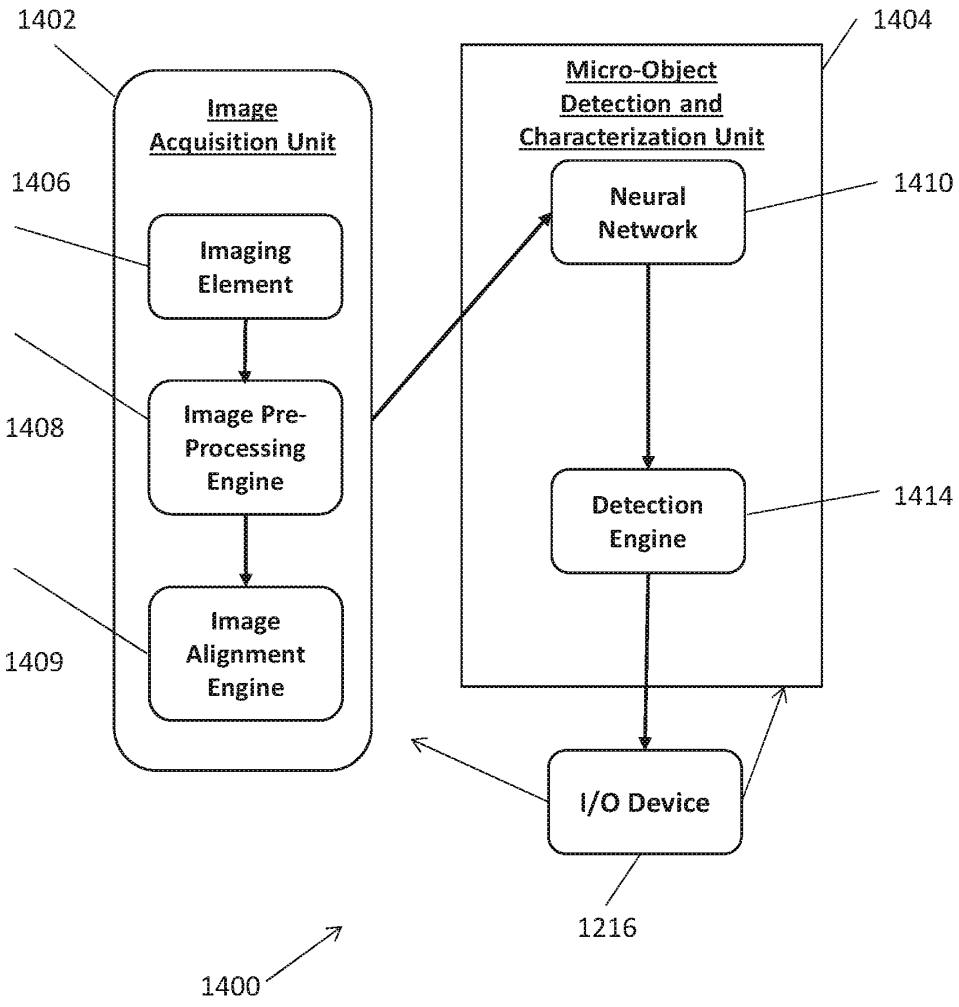
1402
Image Acquisition Unit
1406
Imaging Element
1408
Image Pre-Processing Engine
1409
Image Alignment Engine
1404
Micro-Object Detection and Characterization Unit
Neural Network
1410
Detection Engine
1414
I/O Device
1216
1400

FIG. 13

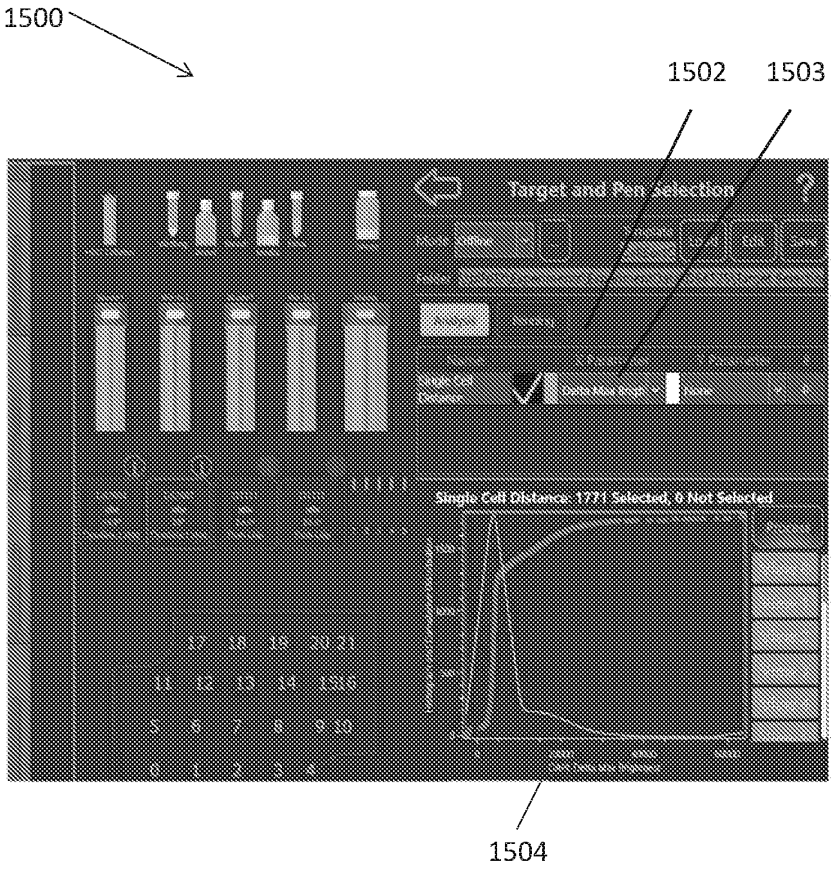
1500
1502
1503
Target and Pen Selection
Single Cell Distance: 1771 Selected, 0 Not Selected
1504

FIG. 14

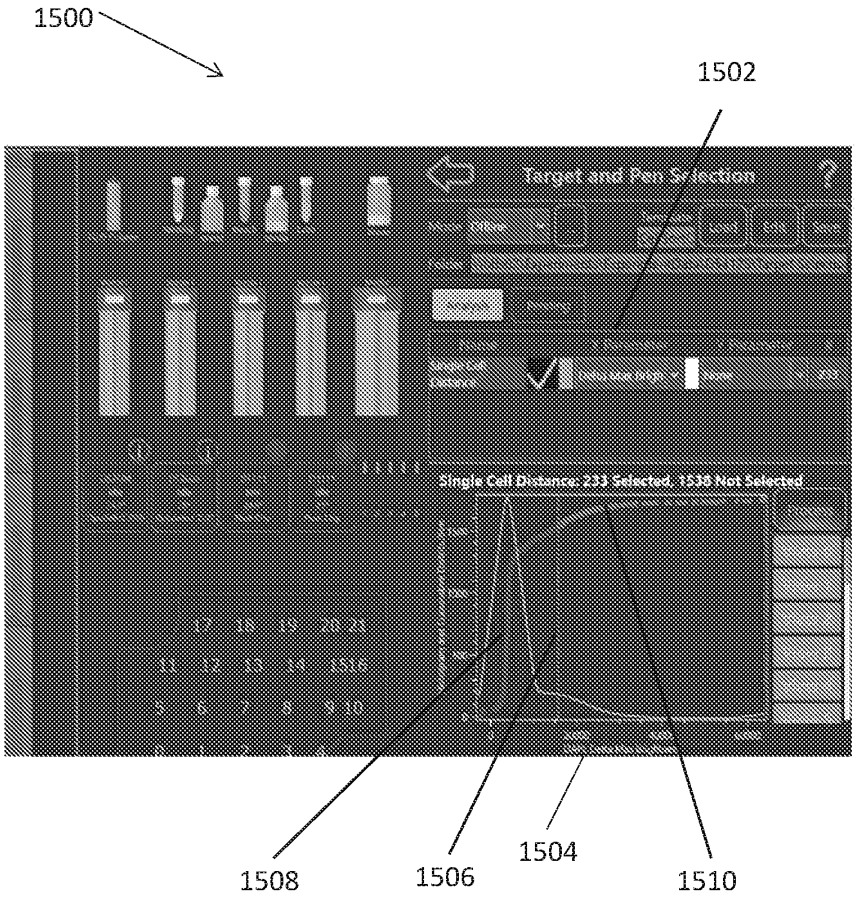
1500
1502
Target and Pen Selection
Single Cell Distance: 233 Selected, 1530 Not Selected
1508
1506
1504
1510

FIG. 15

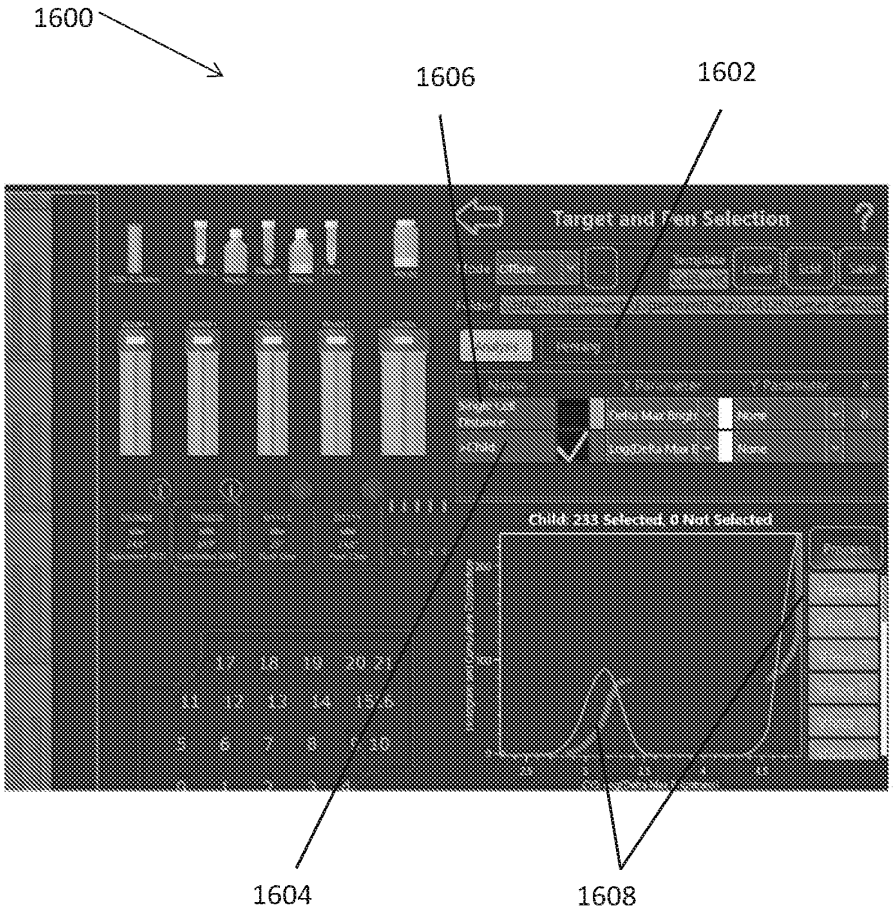
1600
1606
1602
Target and Fan Selection
Child: 233 Selected, 0 Not Selected
1604
1608

FIG. 16

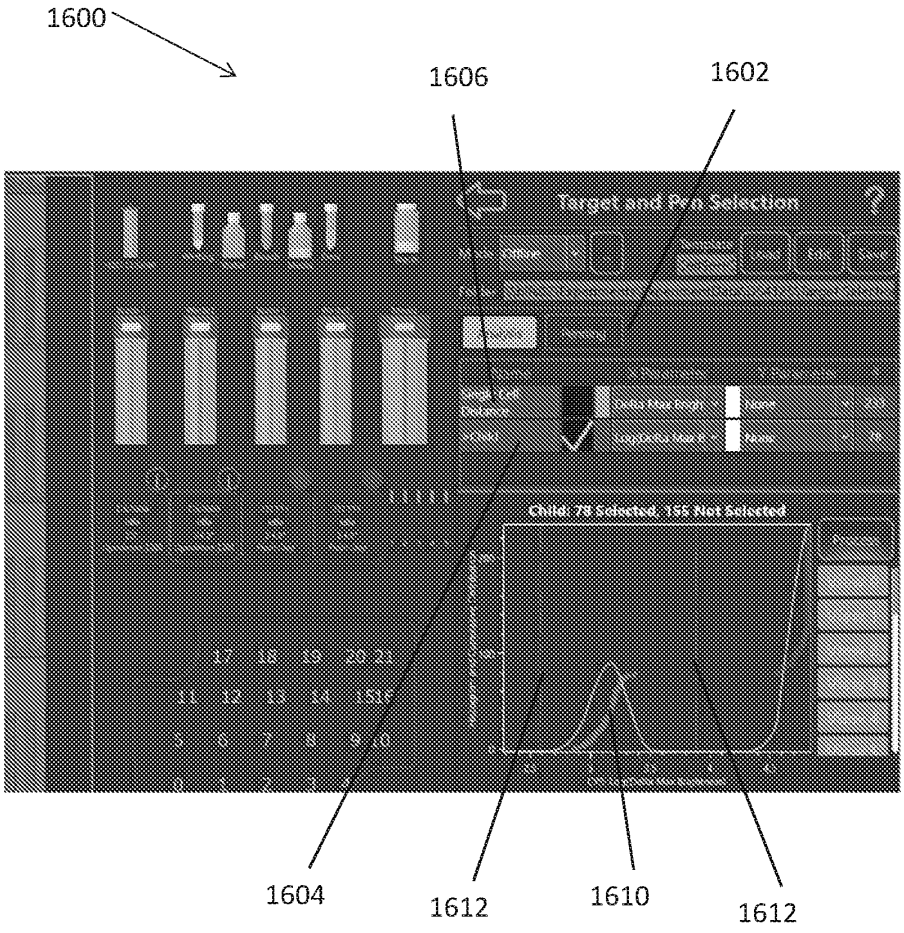
1600
1606
1602
Target and Pro Selection
Child: 78 Selected, 155 Not Selected
1604
1612
1610
1612

FIG. 17

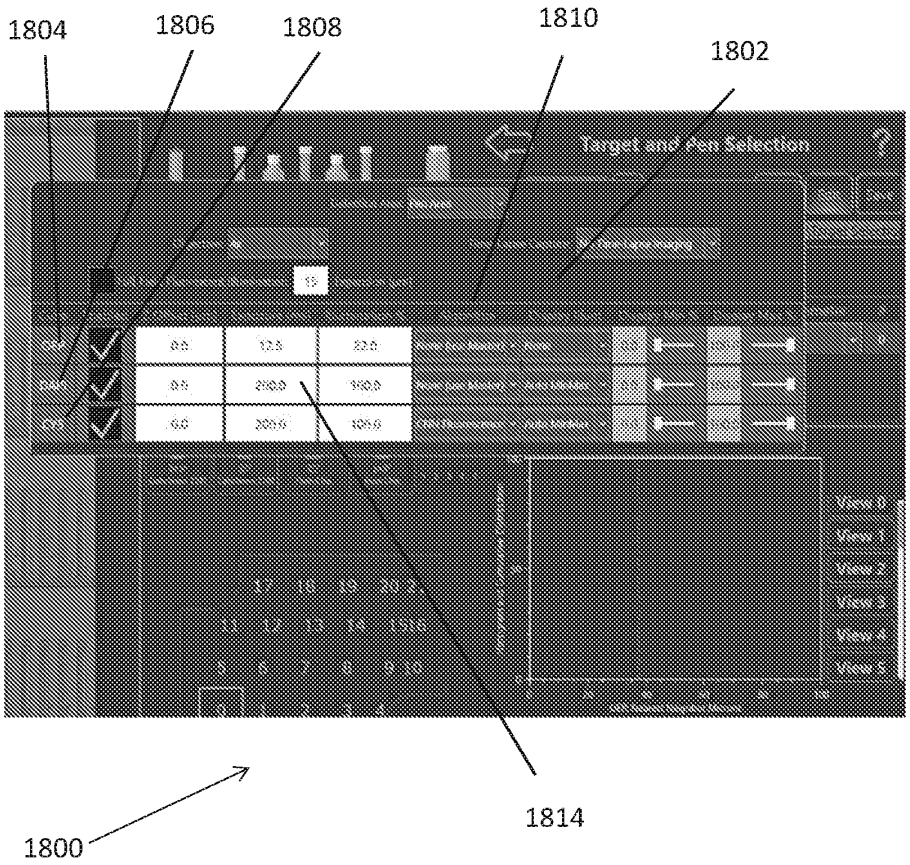

FIG. 18

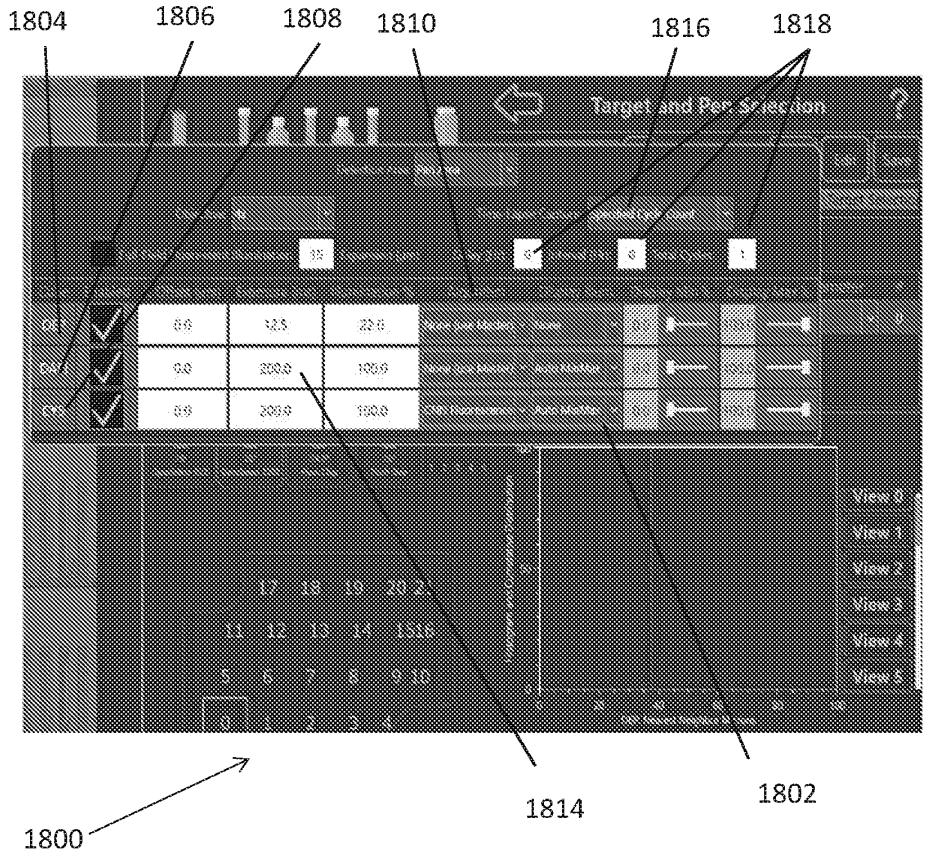

FIG. 19

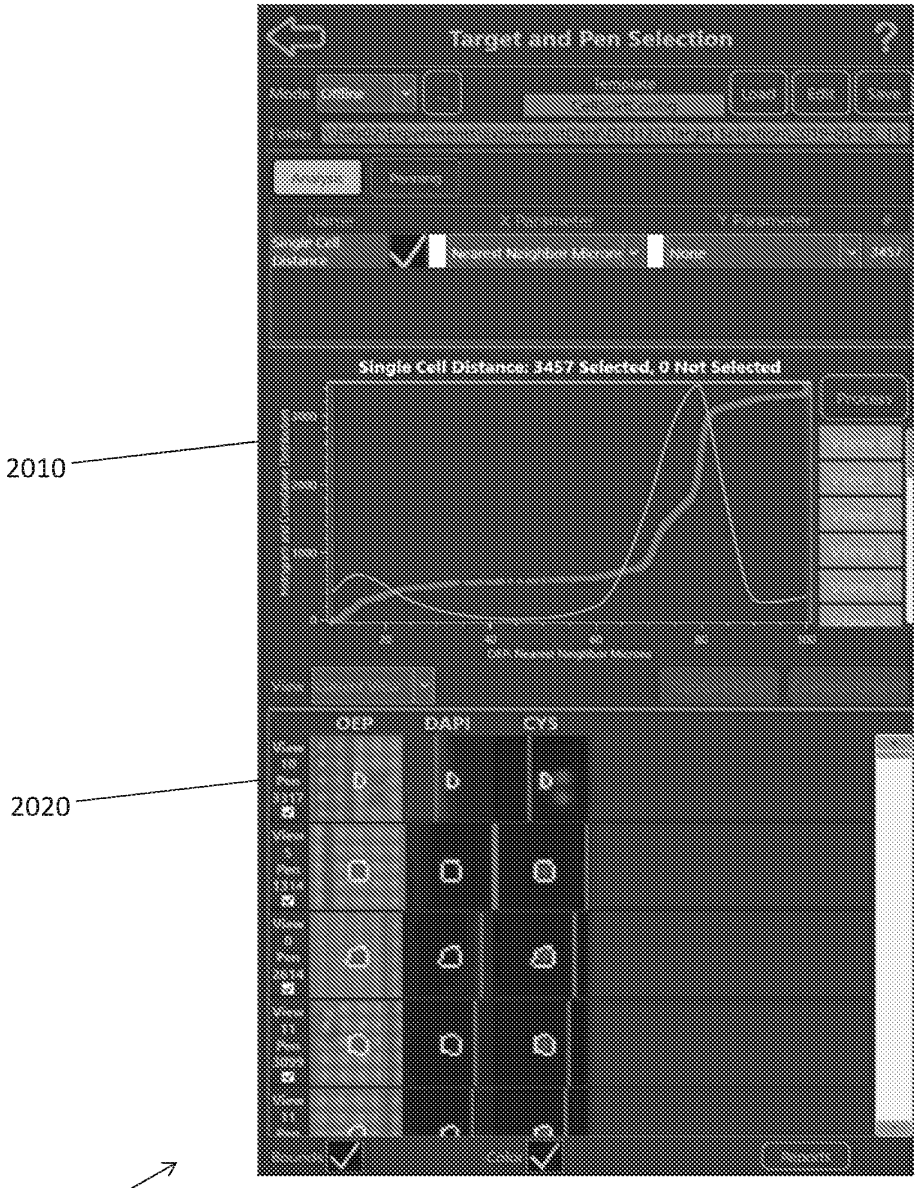
Target and Pen Selection
Single Cell Distance: 3457 Selected, 0 Not Selected
DSP
DAPI
CY5
2010
2020
2000

FIG. 20

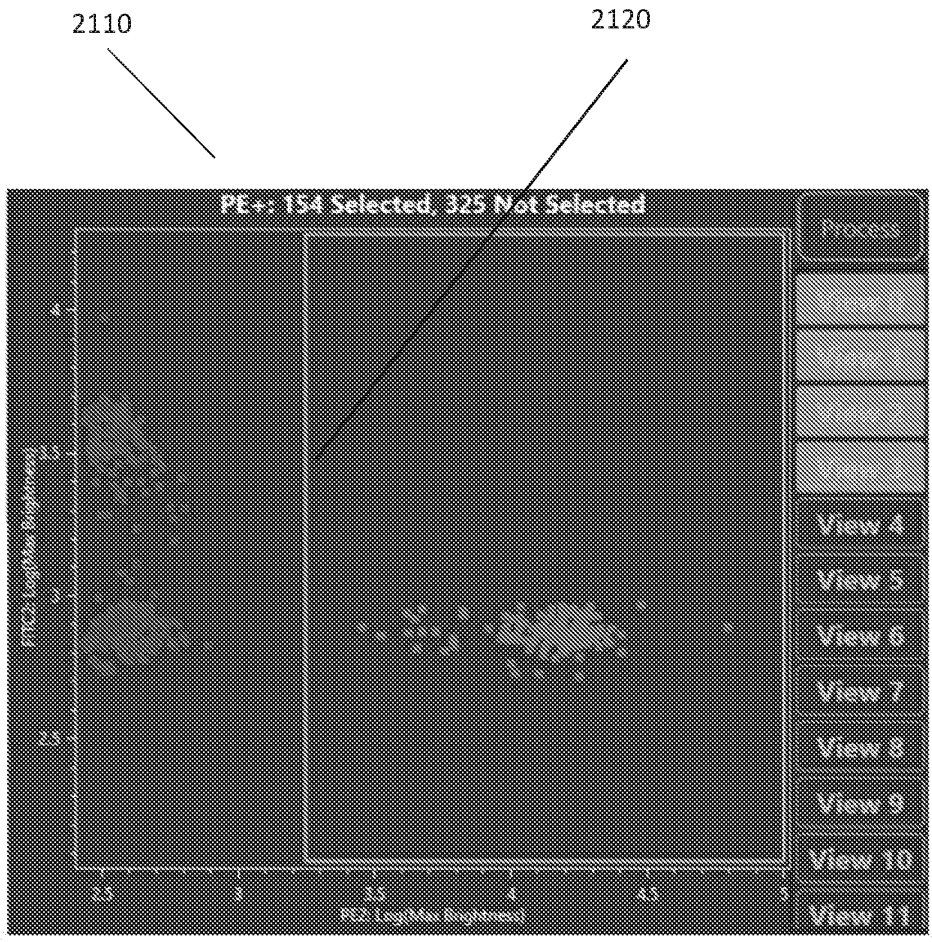
2110
2120
PE+: 154 Selected, 325 Not Selected
View 4
View 5
View 6
View 7
View 8
View 9
View 10
View 11
2000

FIG. 21

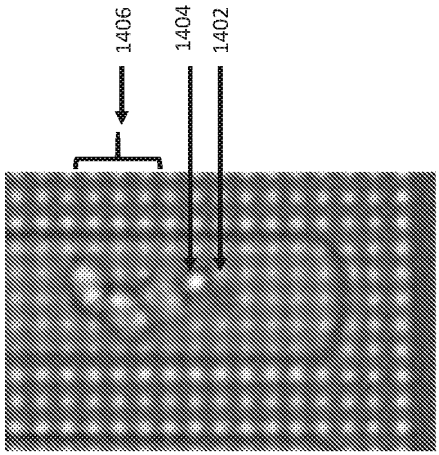

FIG. 23B
Enlarged area from FIG. 15A

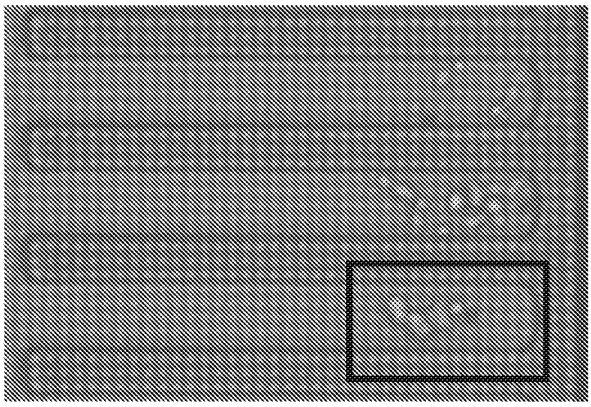

FIG. 23A
T cells and TCL 1 Tumor cells

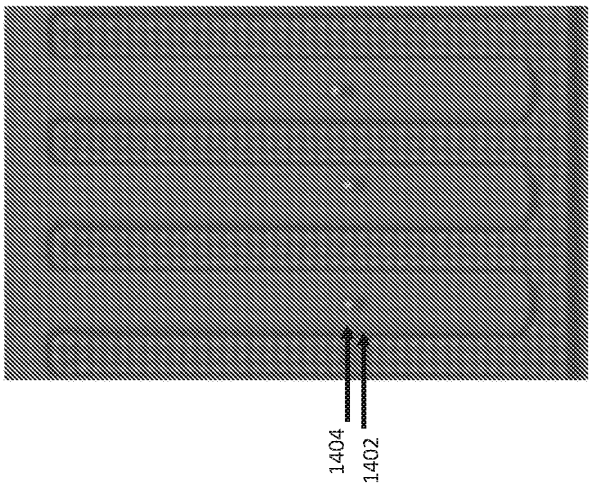

FIG. 22
T cells and Beads

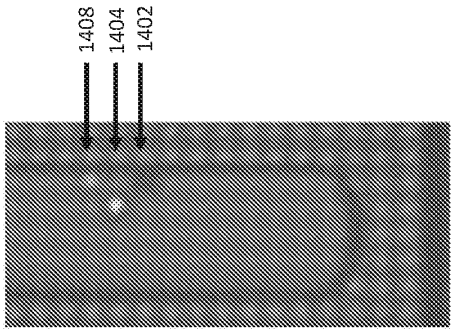

FIG. 24B

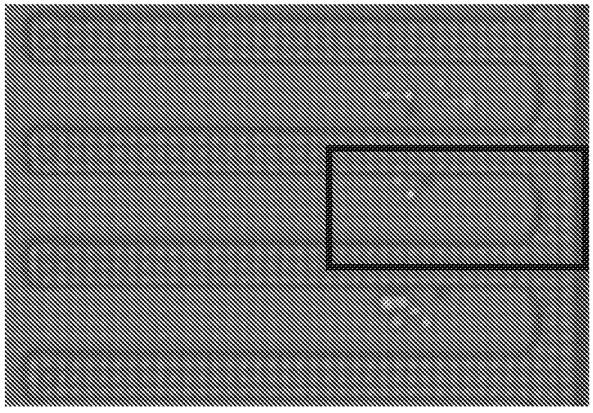

FIG. 24A
T cells plus SLC45 Tumor cells

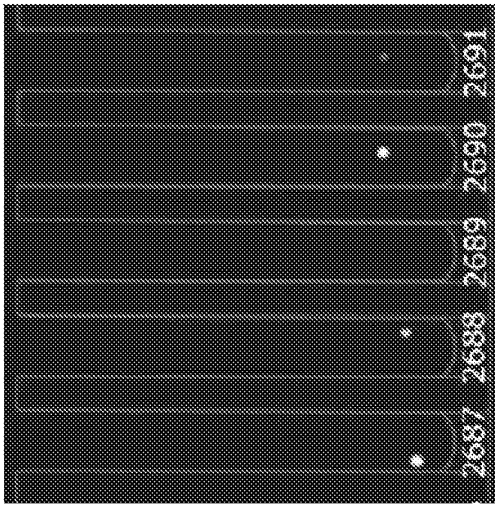

FIG. 25B
Assay Results TxRed

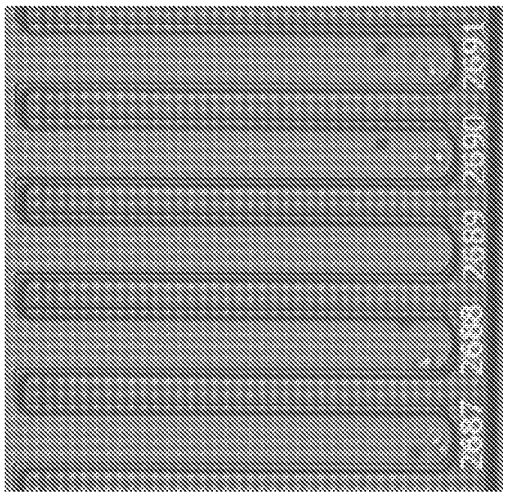

FIG. 25A
Assay result brightfield

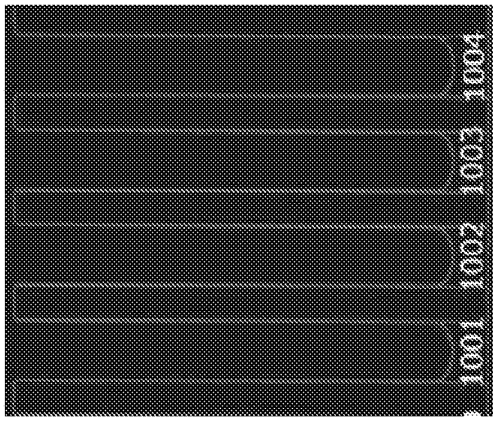

FIG. 26B
assay Result TxRed

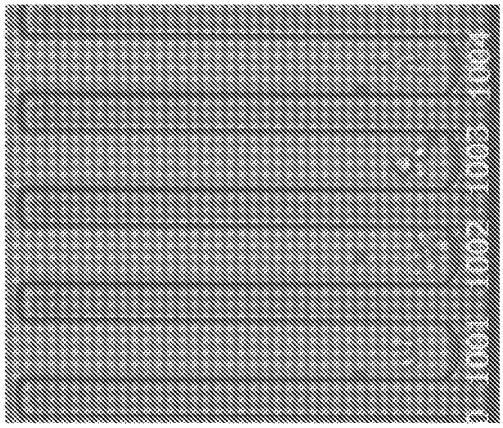

FIG. 26A
assay results brightfield

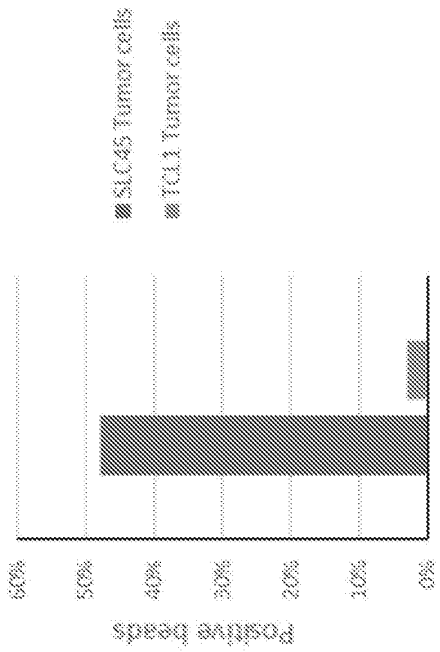

FIG. 27
Quantification
of assay results

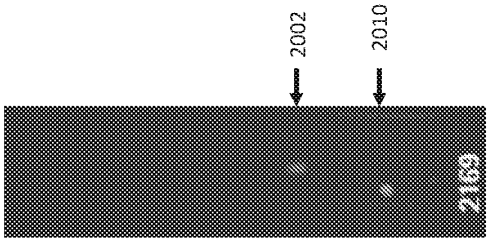

FIG. 28D
Assay Result FITC

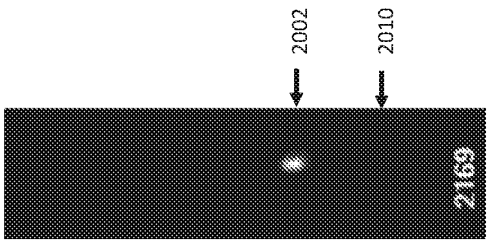

FIG. 28C
Assay Result TXRED

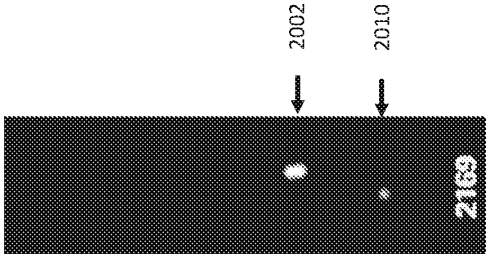

FIG.28B
Assay Result Cy5

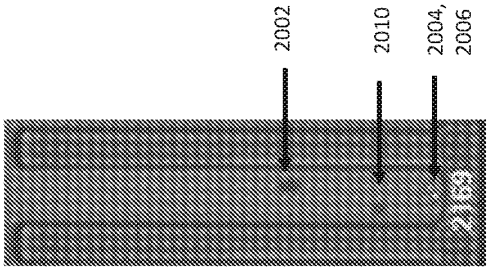

FIG.28A
Assay Result Brightfield

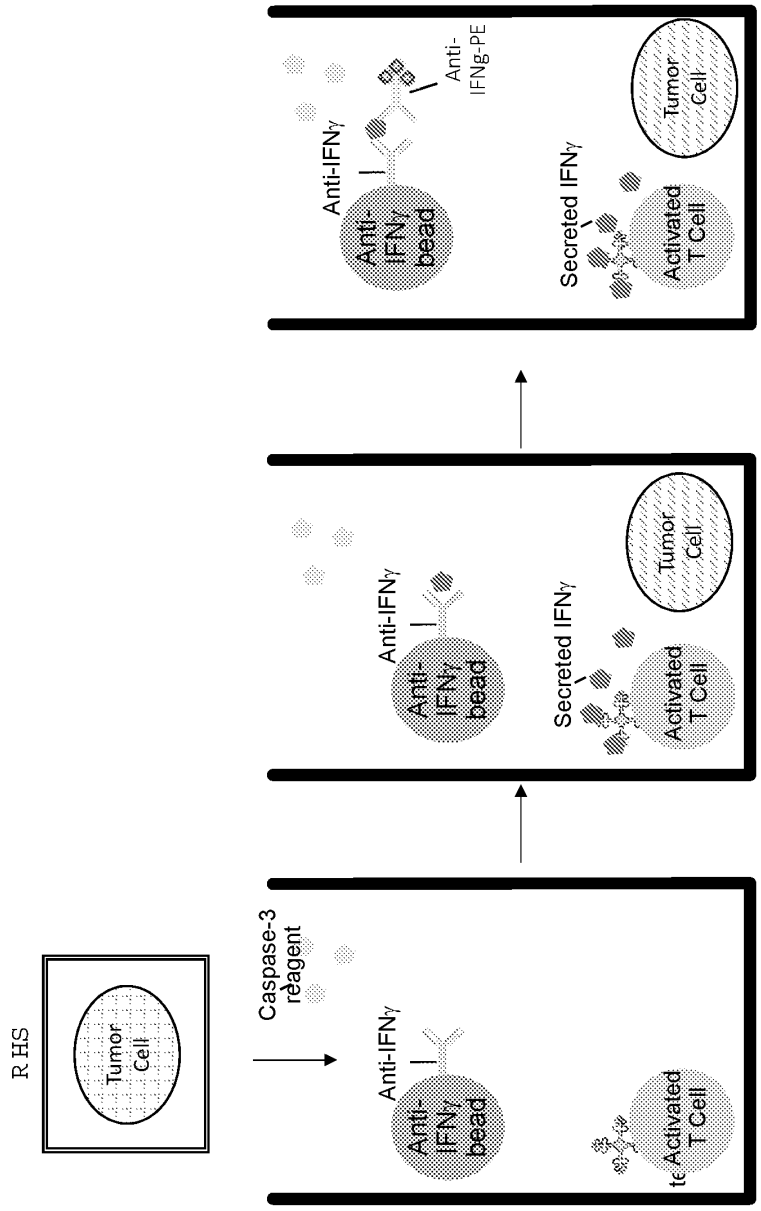
R HS
Tumor Cell
Caspase-3 reagent
Anti-IFNγ
Anti-IFNγ bead
Activated T Cell
Secreted IFNγ
Tumor Cell
Anti-IFNg-PE

FIG. 29

FIG. 30E

FIG. 30D

FIG. 30C

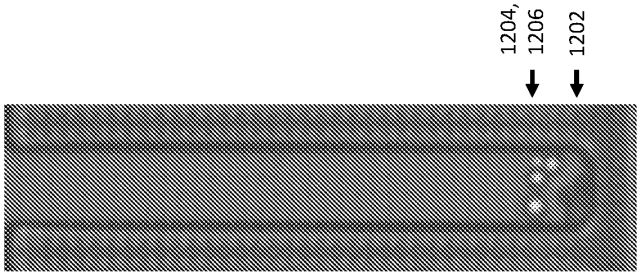

FIG. 30B

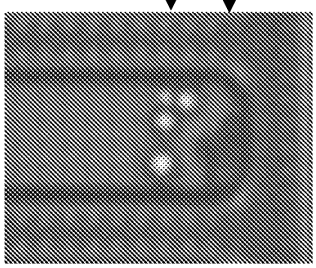

FIG. 30A

100%
80%
60%
40%
20%
0%
% T cell killing
100%
80%
60%
40%
20%
0%
% Non-specific lysis
TCL1
SUDHL2

FIG. 31

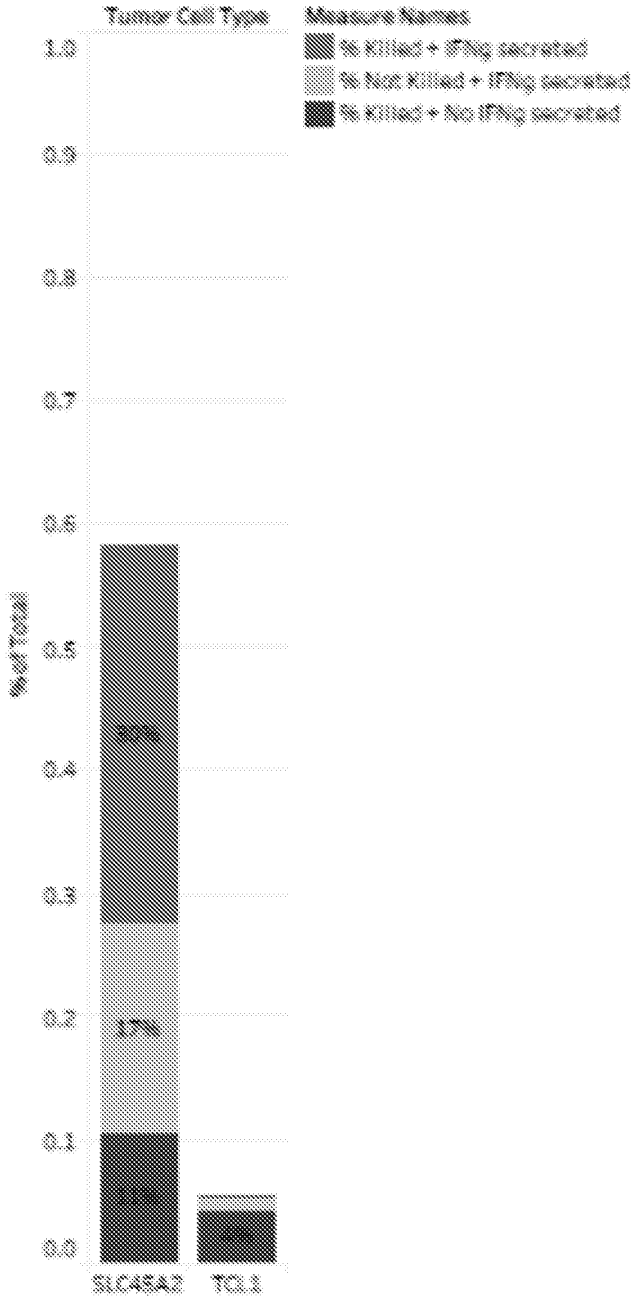
Tumor Cell Type
Measure Names
% Killed + IFNg secreted
% Not Killed + IFNg secreted
% Killed + No IFNg secreted
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
% of Total
39%
47%
SLC45A2
TCL1

FIG. 32

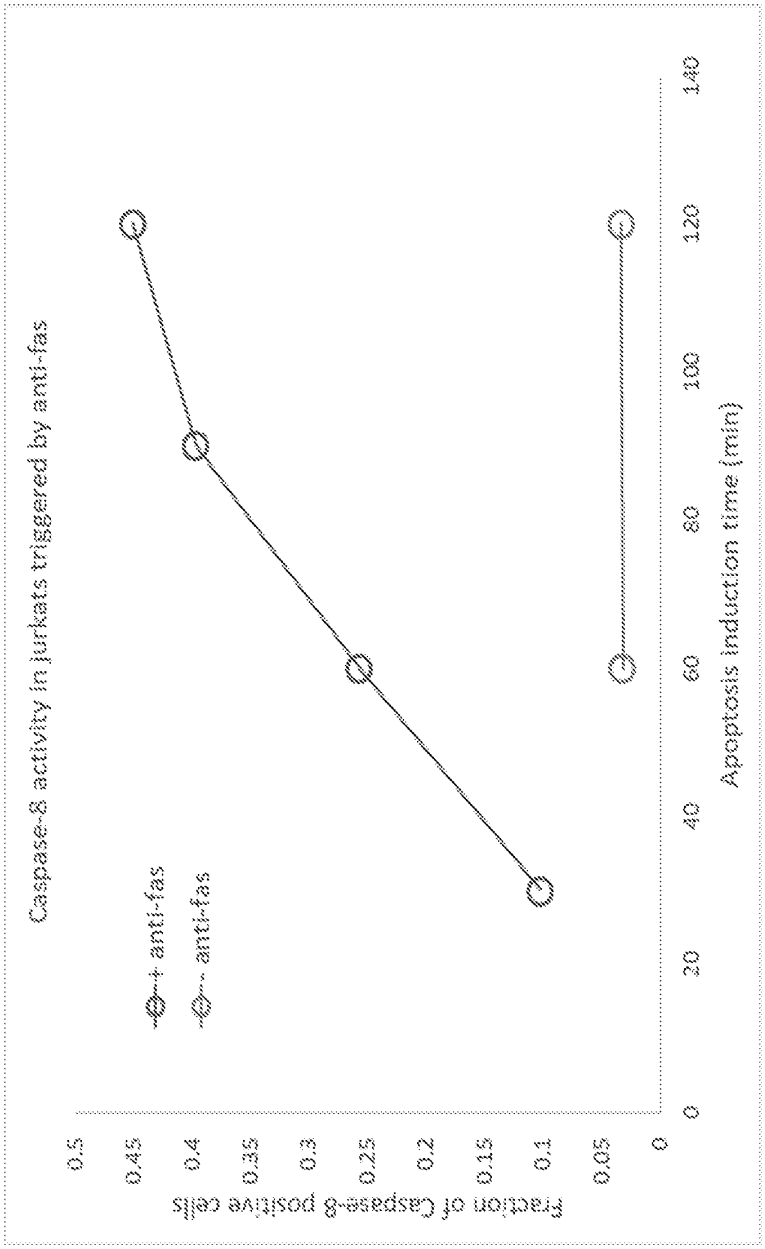
Caspase-8 activity in jurkats triggered by anti-fas
+ anti-fas
- anti-fas
Fraction of Caspase-8 positive cells
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
20
40
60
80
100
120
140
Apoptosis induction time (min)

FIG. 33

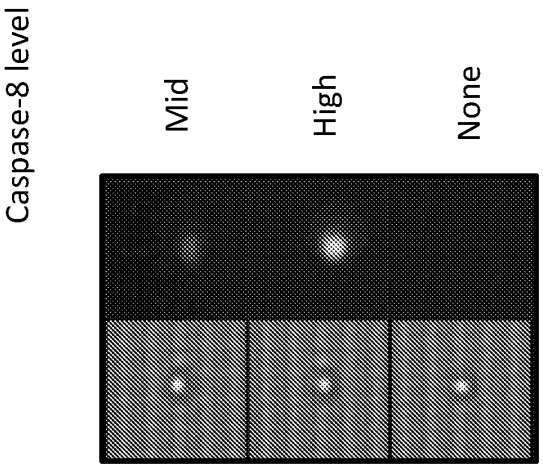

FIG. 34B

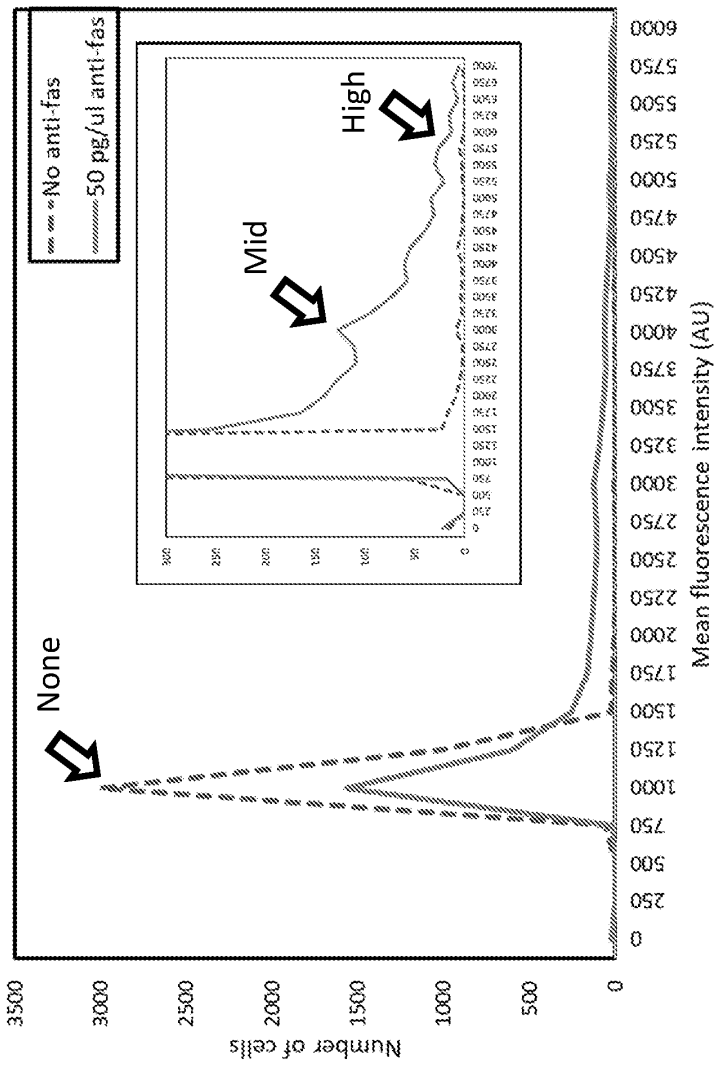

FIG. 34A