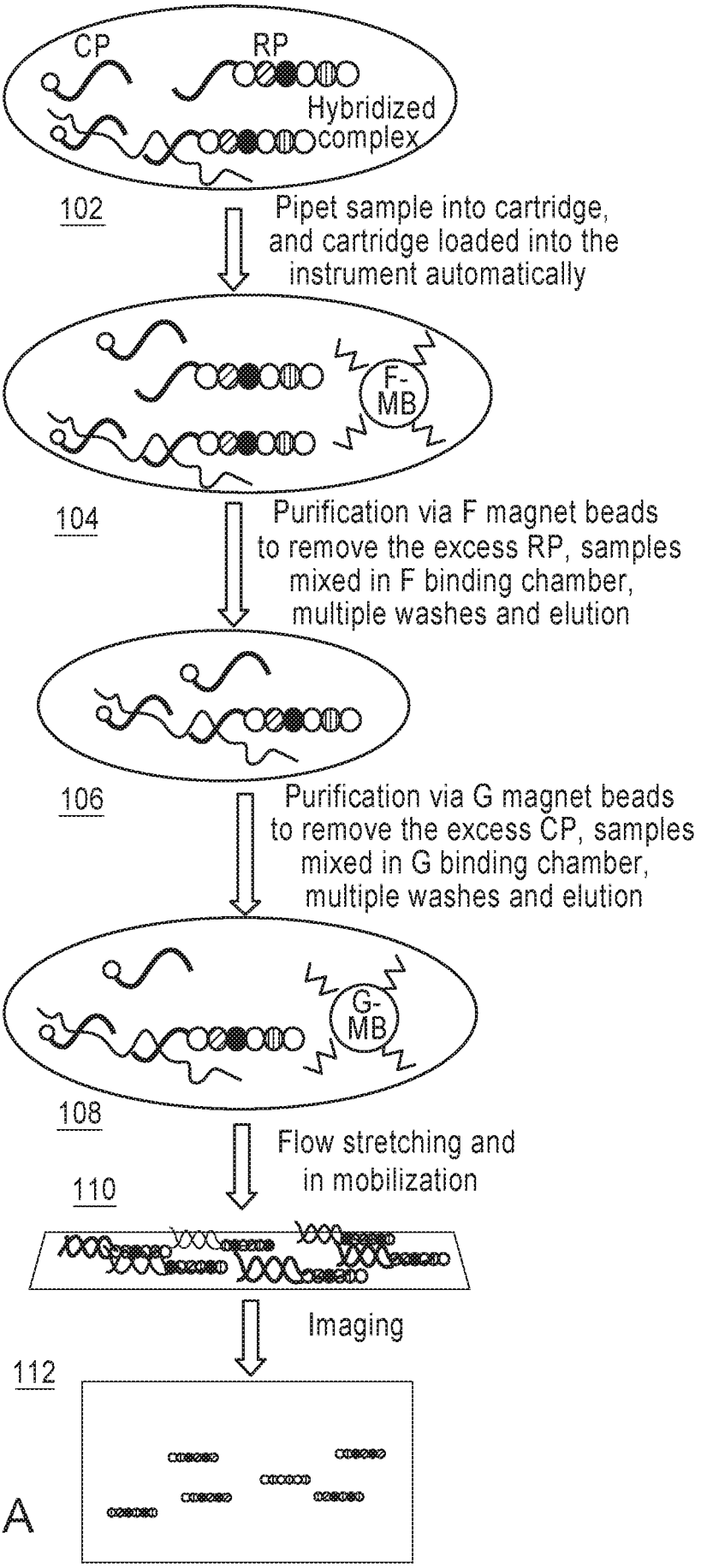

CP
RP
Hybridized complex
102
Pipet sample into cartridge, and cartridge loaded into the instrument automatically
F-MB
104
Purification via F magnet beads to remove the excess RP, samples mixed in F binding chamber, multiple washes and elution
106
Purification via G magnet beads to remove the excess CP, samples mixed in G binding chamber, multiple washes and elution
G-MB
108
Flow stretching and in mobilization
110
Imaging
112

FIGURE 1A

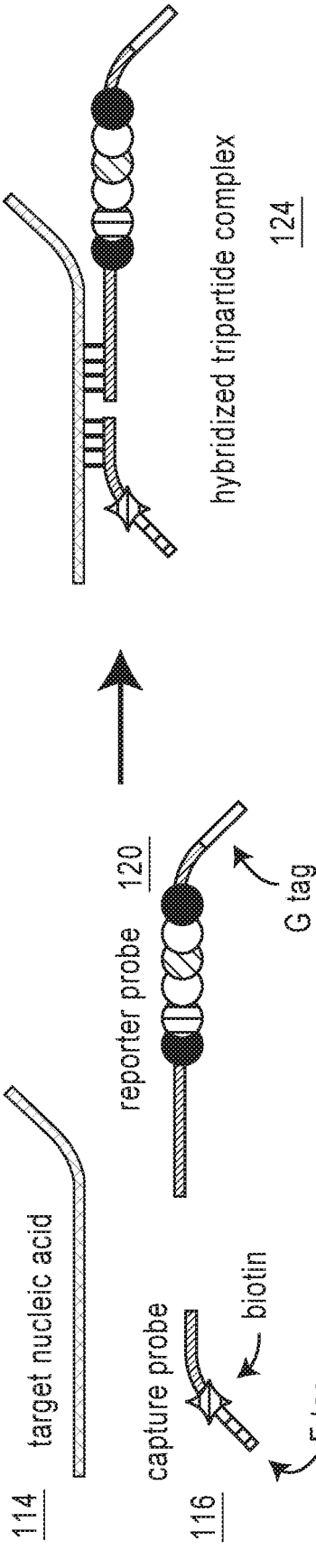

hybridized tripartide complex
124
reporter probe 120
G tag
122
target nucleic acid
114
capture probe
biotin
F tag
118
116

FIGURE 1B

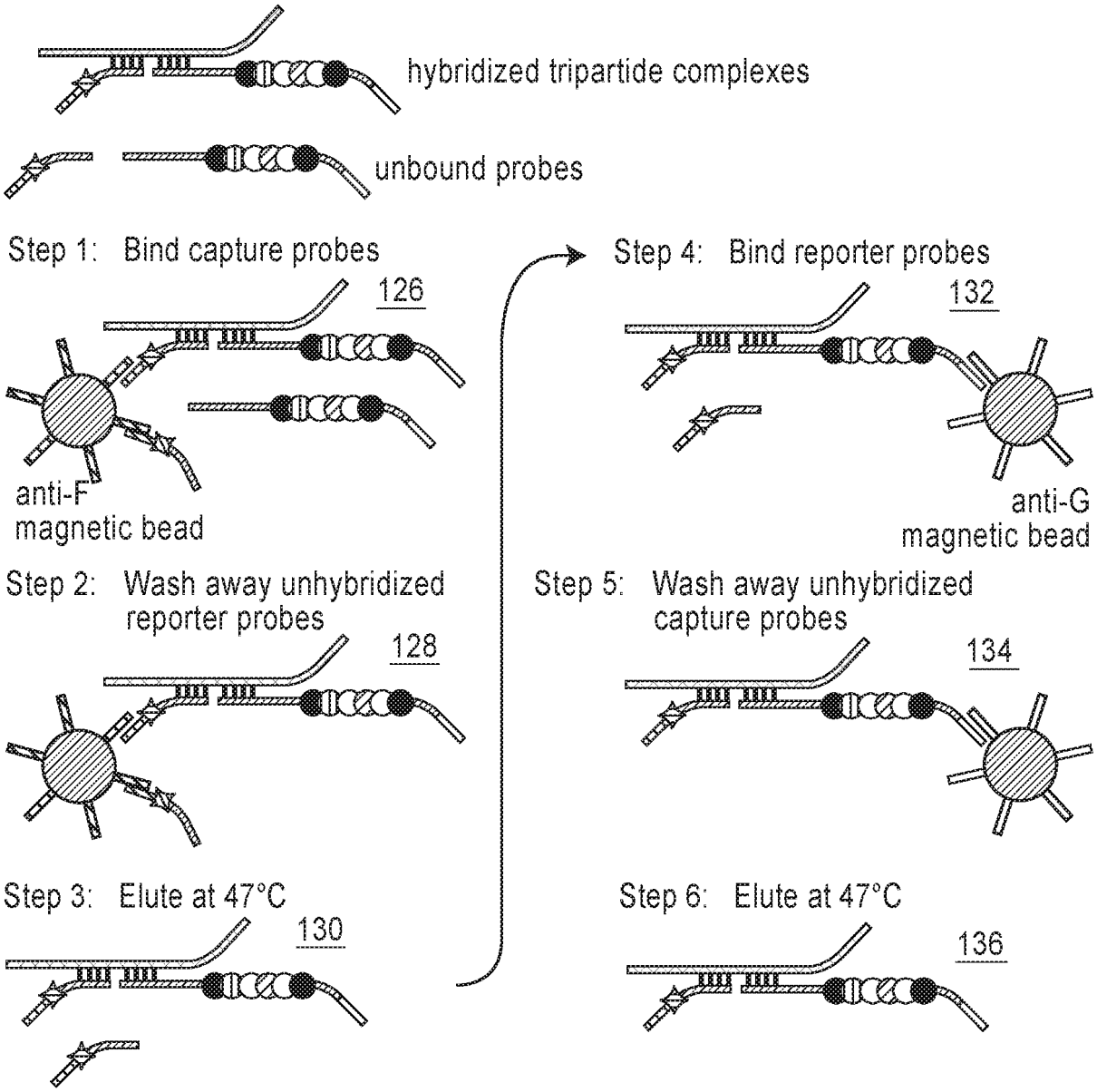

hybridized tripartide complexes
unbound probes
Step 1: Bind capture probes
126
anti-F
magnetic bead
Step 2: Wash away unhybridized reporter probes
128
Step 3: Elute at 47°C
130
Step 4: Bind reporter probes
132
anti-G
magnetic bead
Step 5: Wash away unhybridized capture probes
134
Step 6: Elute at 47°C
136

FIGURE 1C

Biotinylated capture probes bind randomly to the streptavidin-coated surface of the cartridge.
138
Microfluidic flow elongates and aligns the reporter barcodes along the surface.
Direction of flow
140
Immobilization buffer contains biotinylated, anti-G oligonucleotides that anchor the reporter probes to the imaging surface.
Direction of flow
142

FIGURE 1D

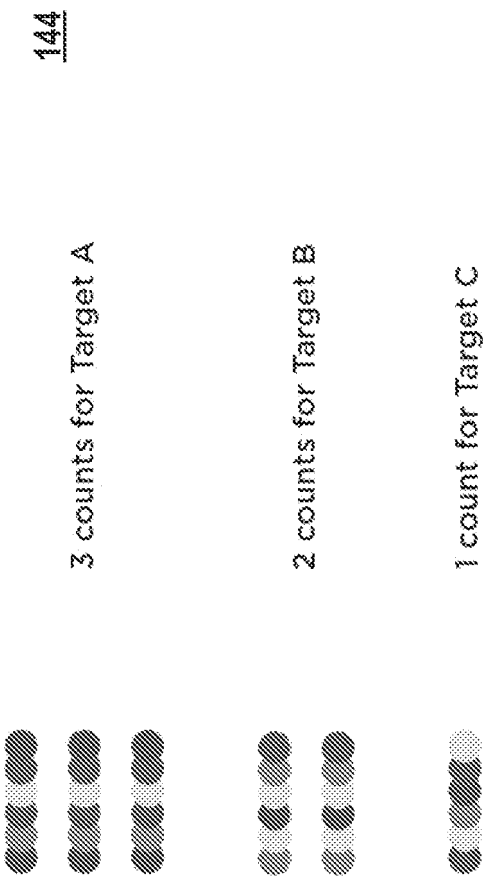

144
3 counts for Target A
2 counts for Target B
1 count for Target C

FIGURE 1E

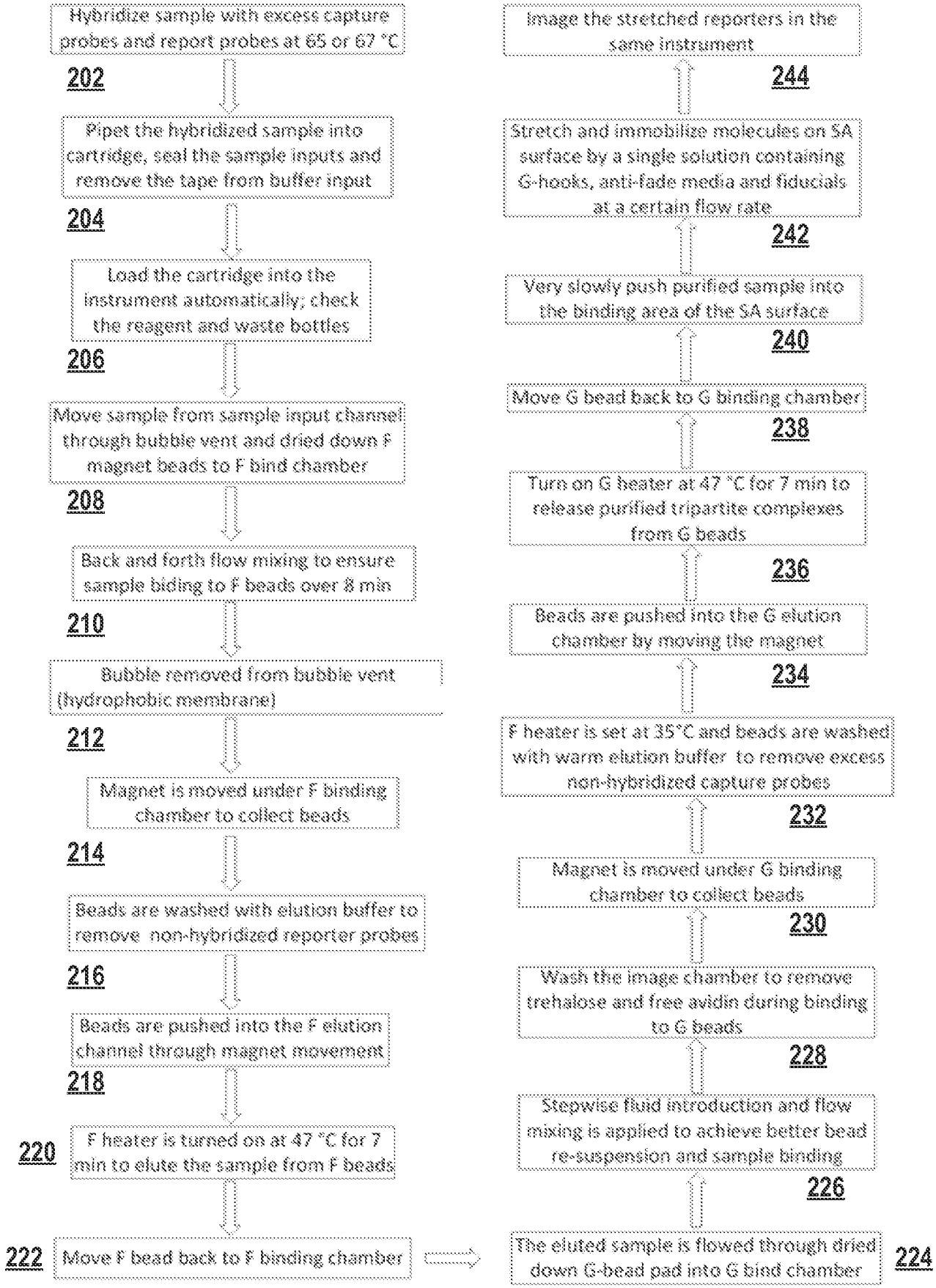

Hybridize sample with excess capture probes and report probes at 65 or 67 °C
202
Pipet the hybridized sample into cartridge, seal the sample inputs and remove the tape from buffer input
204
Load the cartridge into the instrument automatically; check the reagent and waste bottles
206
Move sample from sample input channel through bubble vent and dried down F magnet beads to F bind chamber
208
Back and forth flow mixing to ensure sample biding to F beads over 8 min
210
Bubble removed from bubble vent (hydrophobic membrane)
212
Magnet is moved under F binding chamber to collect beads
214
Beads are washed with elution buffer to remove non-hybridized reporter probes
216
Beads are pushed into the F elution channel through magnet movement
218
220
F heater is turned on at 47 °C for 7 min to elute the sample from F beads
222
Move F bead back to F binding chamber
The eluted sample is flowed through dried down G-bead pad into G bind chamber
224
Stepwise fluid introduction and flow mixing is applied to achieve better bead re-suspension and sample binding
226
Wash the image chamber to remove trehalose and free avidin during binding to G beads
228
Magnet is moved under G binding chamber to collect beads
230
F heater is set at 35°C and beads are washed with warm elution buffer to remove excess non-hybridized capture probes
232
Beads are pushed into the G elution chamber by moving the magnet
234
Turn on G heater at 47 °C for 7 min to release purified tripartite complexes from G beads
236
Move G bead back to G binding chamber
238
Very slowly push purified sample into the binding area of the SA surface
240
Stretch and immobilize molecules on SA surface by a single solution containing G-hooks, anti-fade media and fiducials at a certain flow rate
242
Image the stretched reporters in the same instrument
244

FIGURE 2

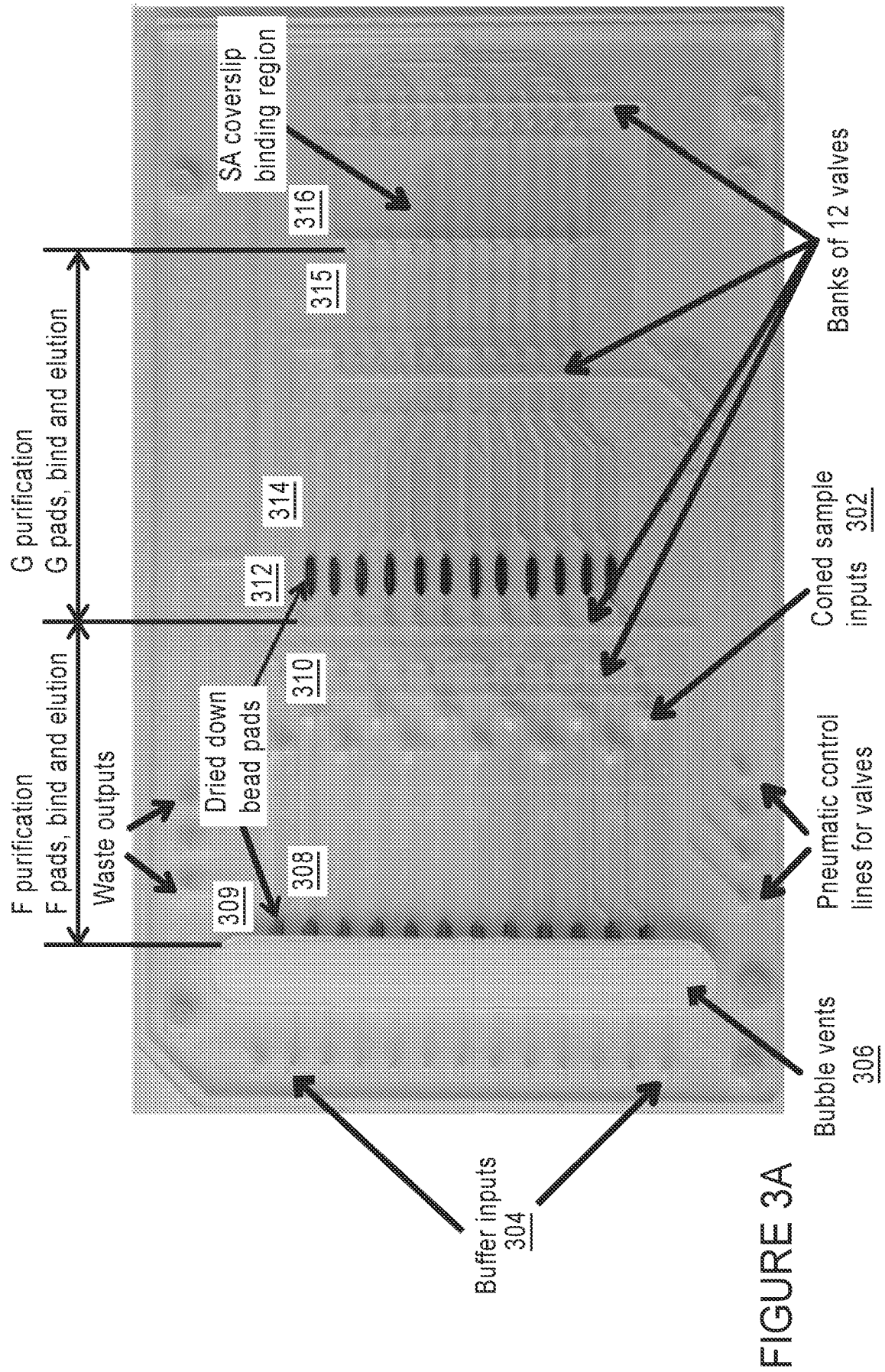

F purification
F pads, bind and elution
Waste outputs
309
Dried down
bead pads
308
310
312
314
G purification
G pads, bind and elution
315
SA coverslip
binding region
316
Banks of 12 valves
Coned sample
inputs 302
Pneumatic control
lines for valves
Bubble vents
306
Buffer inputs
304

FIGURE 3A

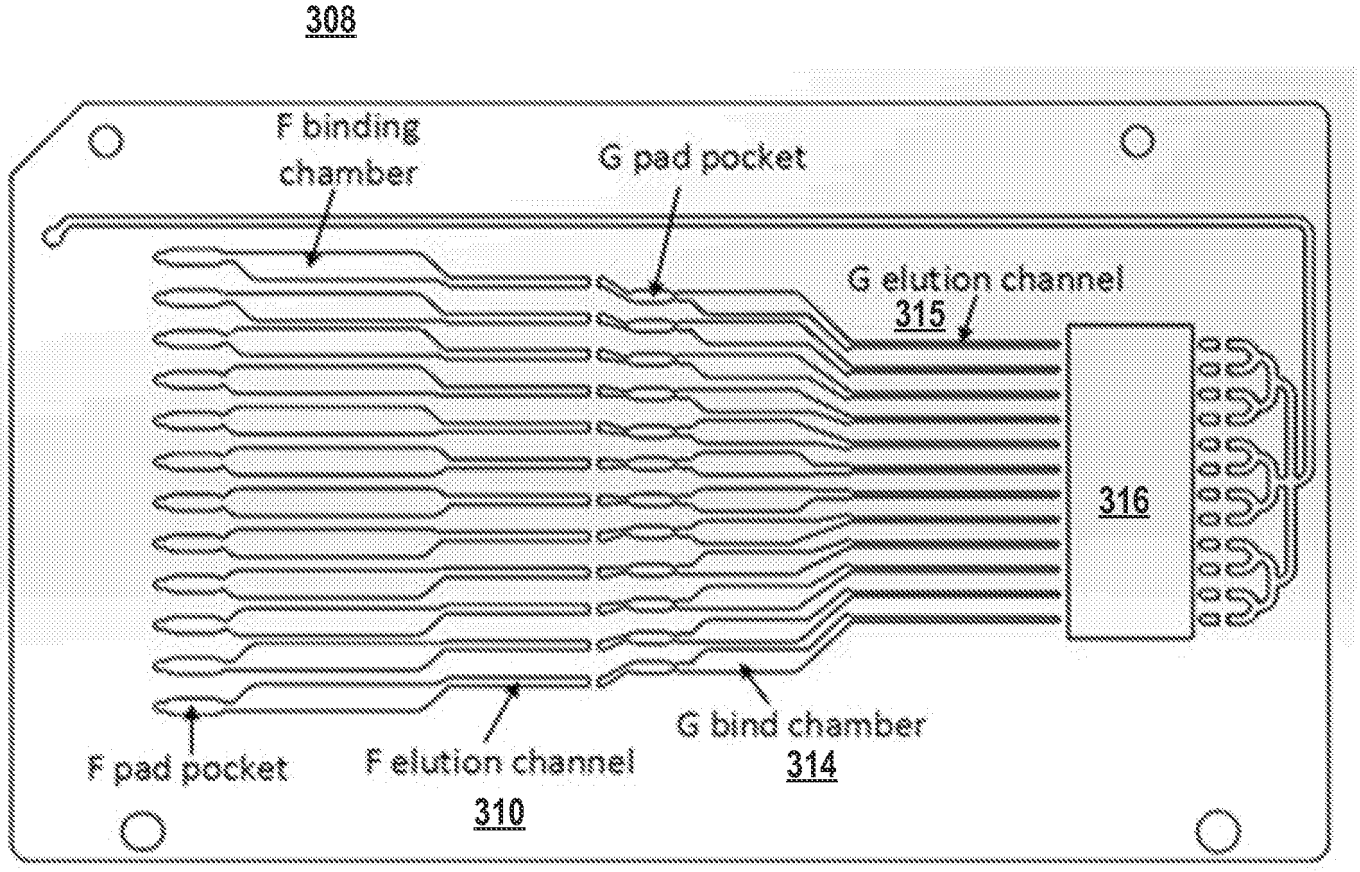
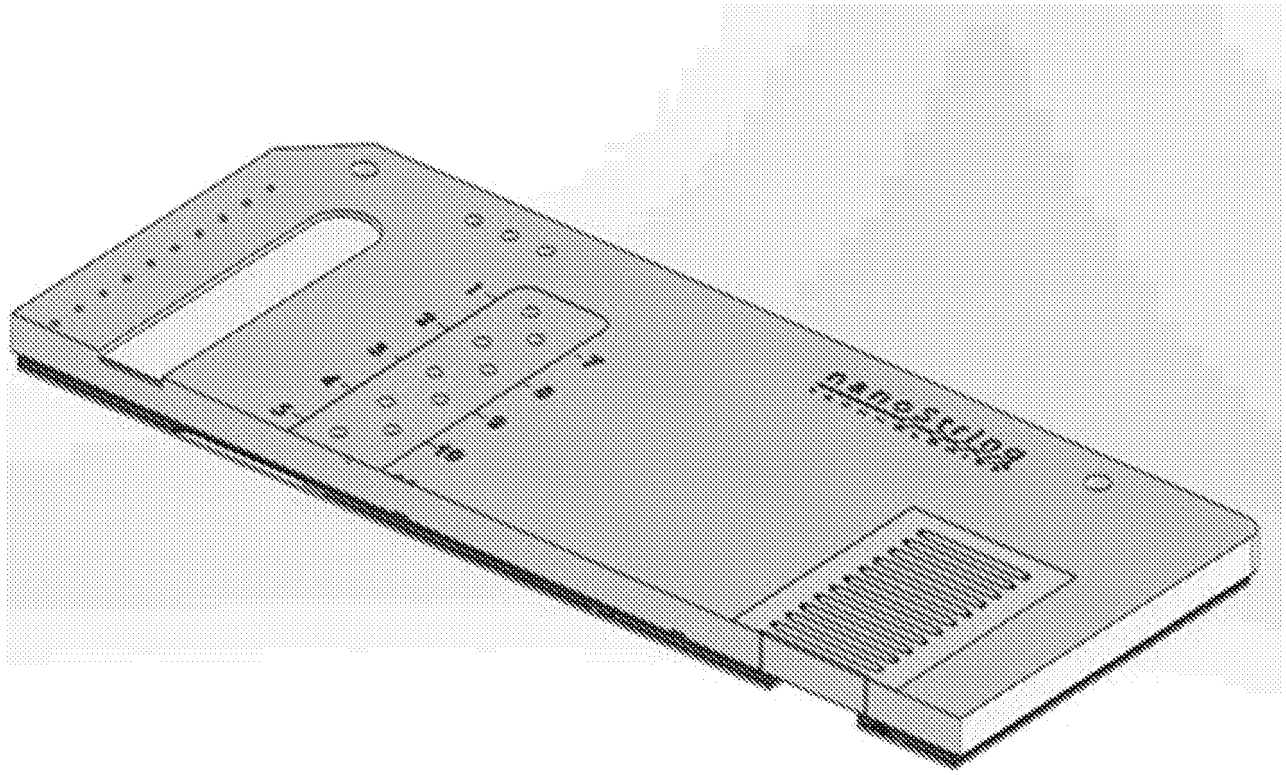

308
F binding chamber
G pad pocket
G elution channel
315
316
F pad pocket
F elution channel
310
G bind chamber
314

FIGURE 3B



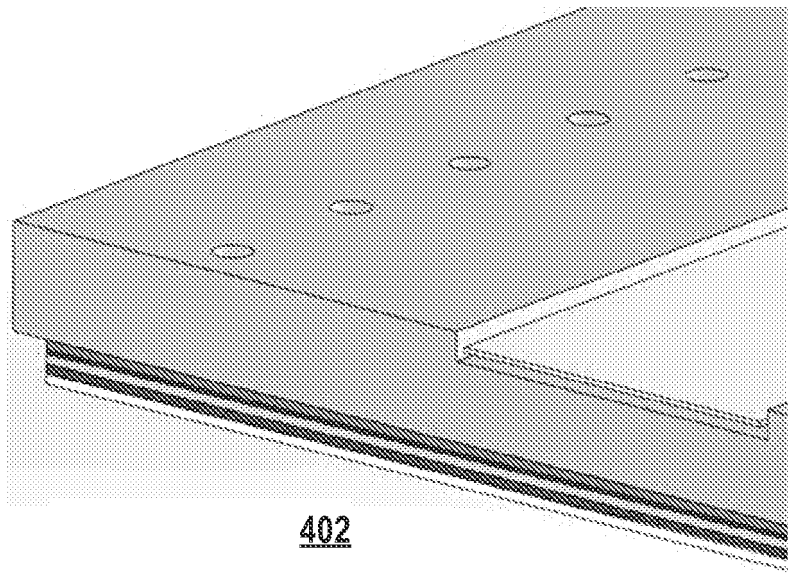


FIGURE 4

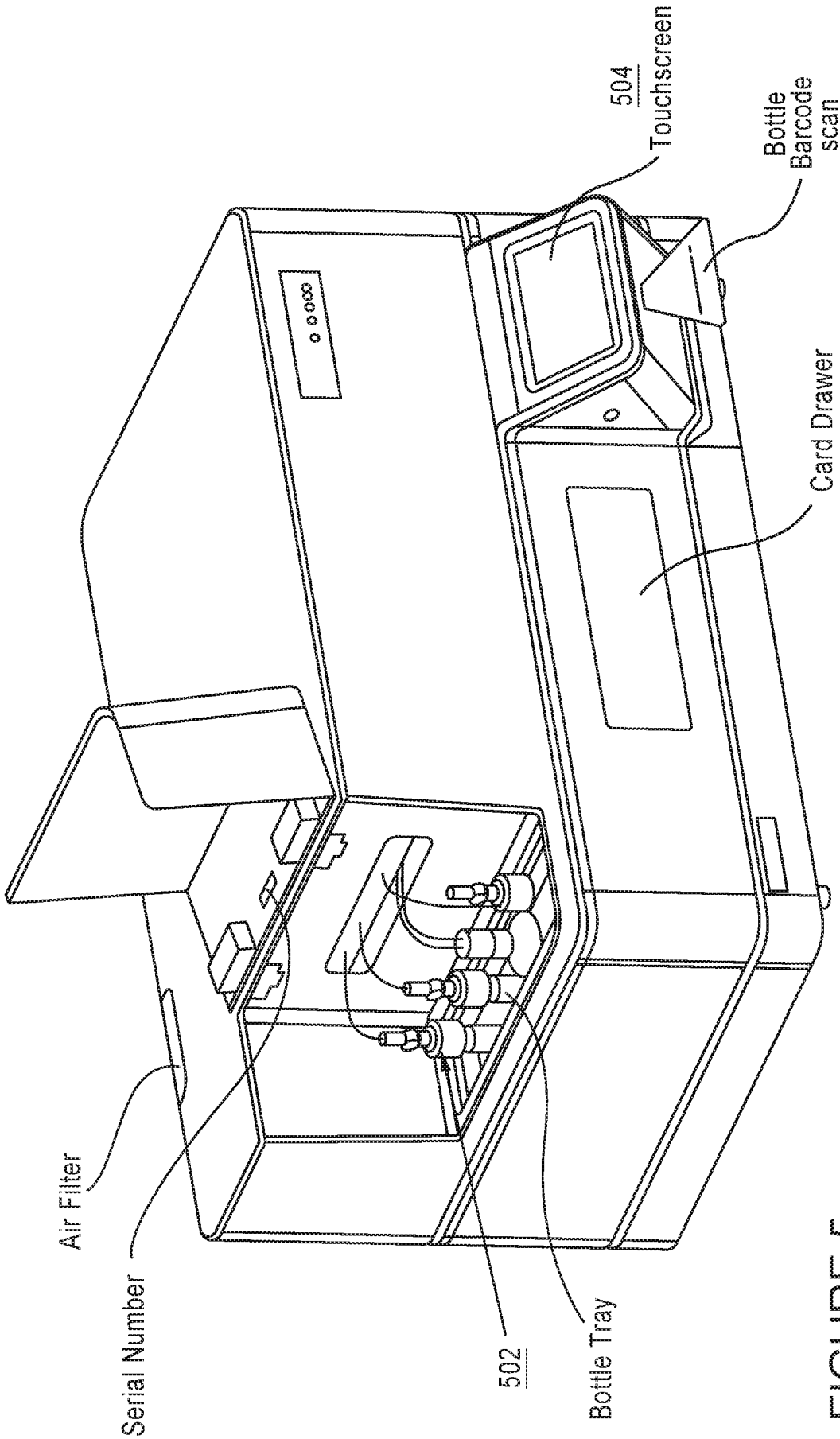

504 Touchscreen
Bottle Barcode scan
Card Drawer
Air Filter
Serial Number
502
Bottle Tray

FIGURE 5

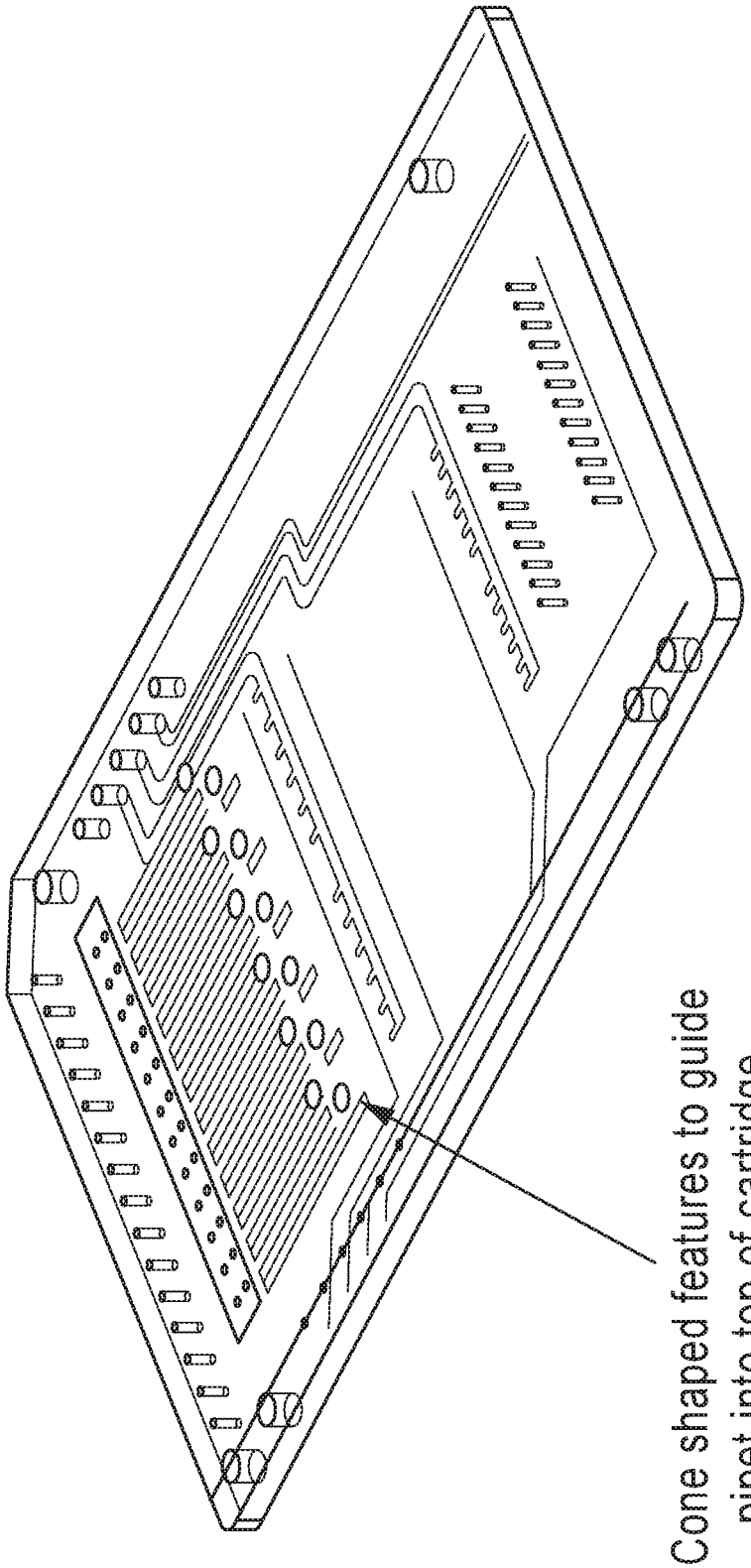

Cone shaped features to guide pipet into top of cartridge
602

FIGURE 6A

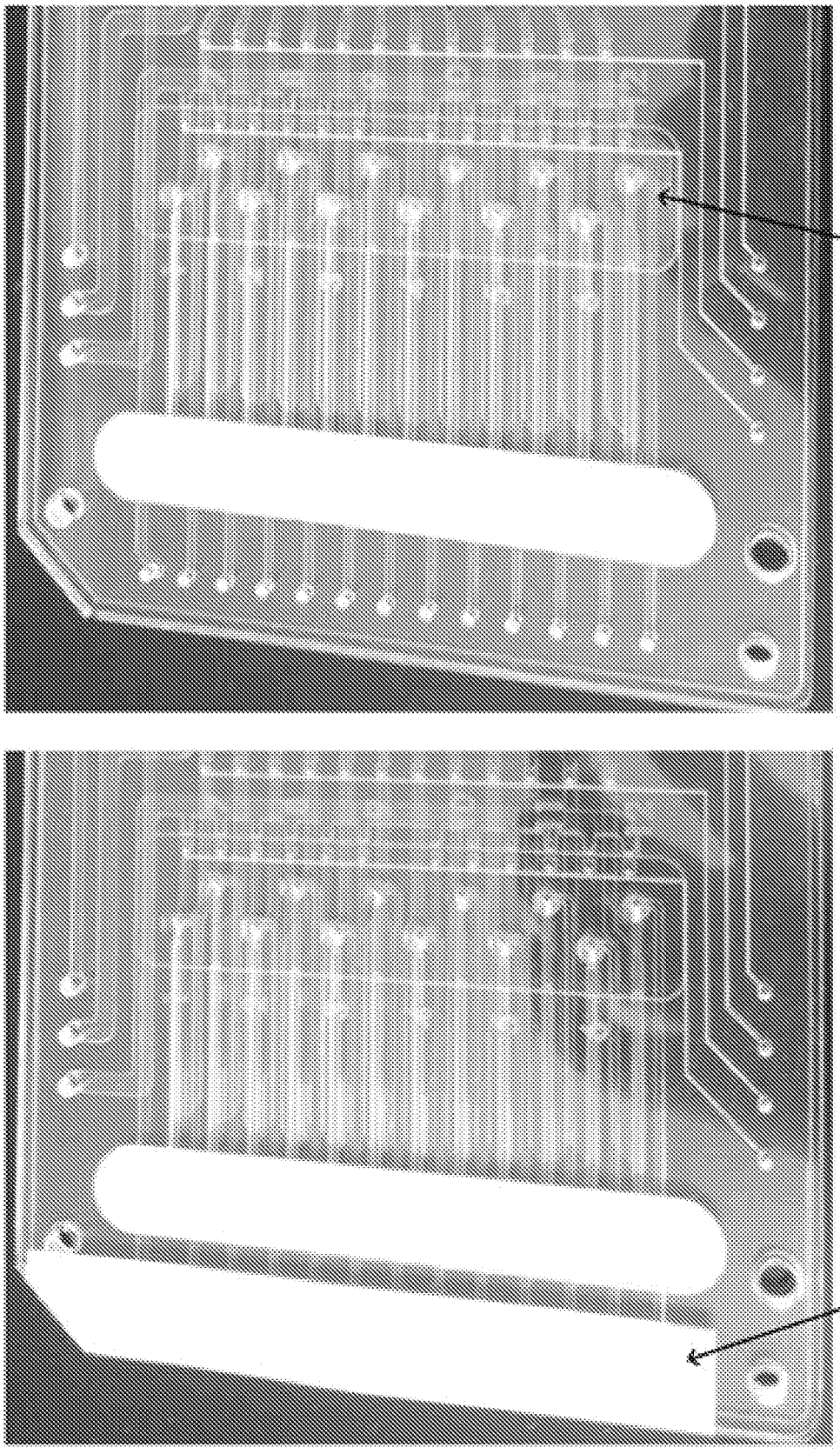


FIGURE 6B

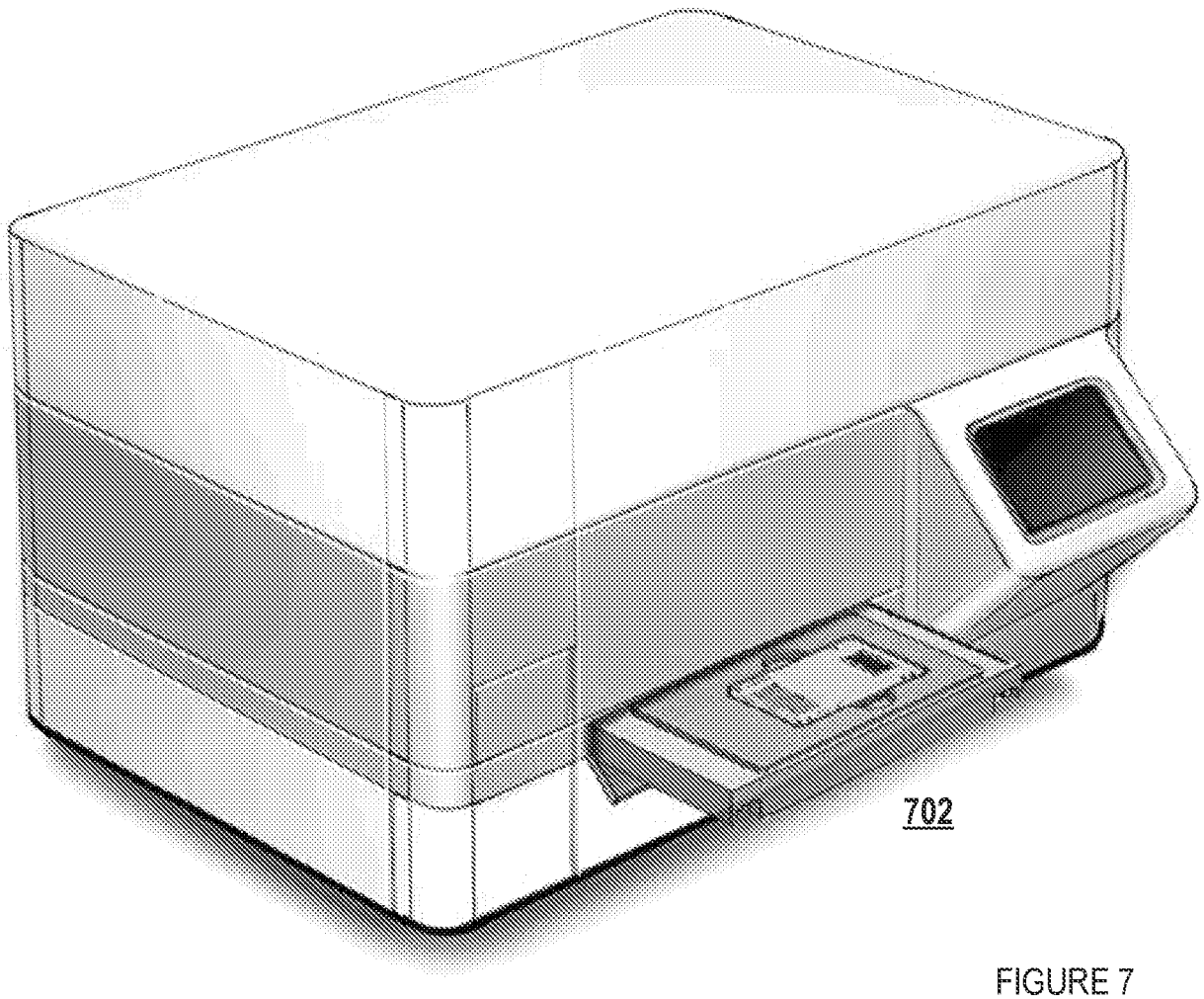


FIGURE 7

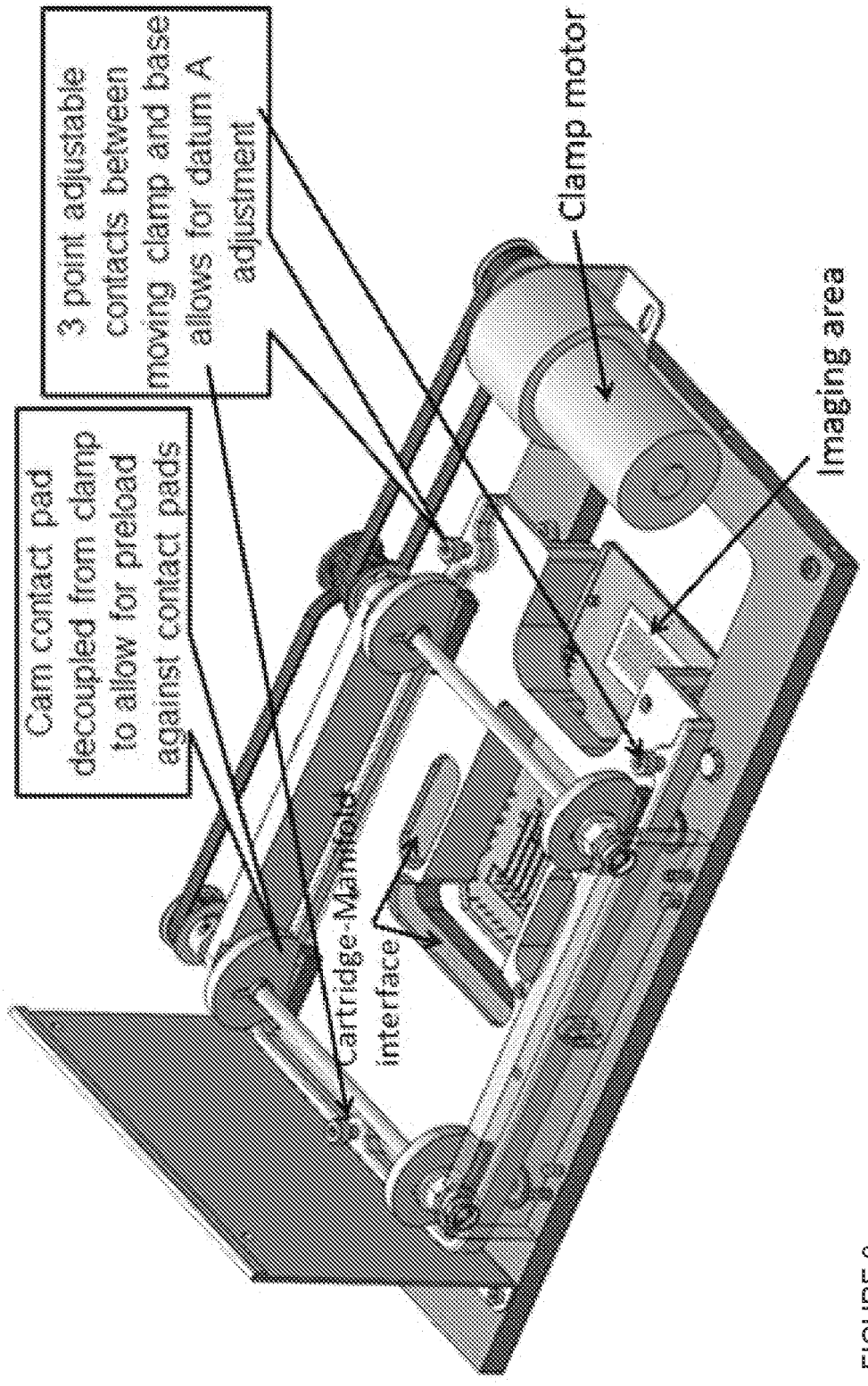

3 point adjustable contacts between moving clamp and base allows for datum A adjustment
Cam contact pad decoupled from clamp to allow for preload against contact pads
Clamp motor
Imaging area
Cartridge-Mamtolic interface

FIGURE 8

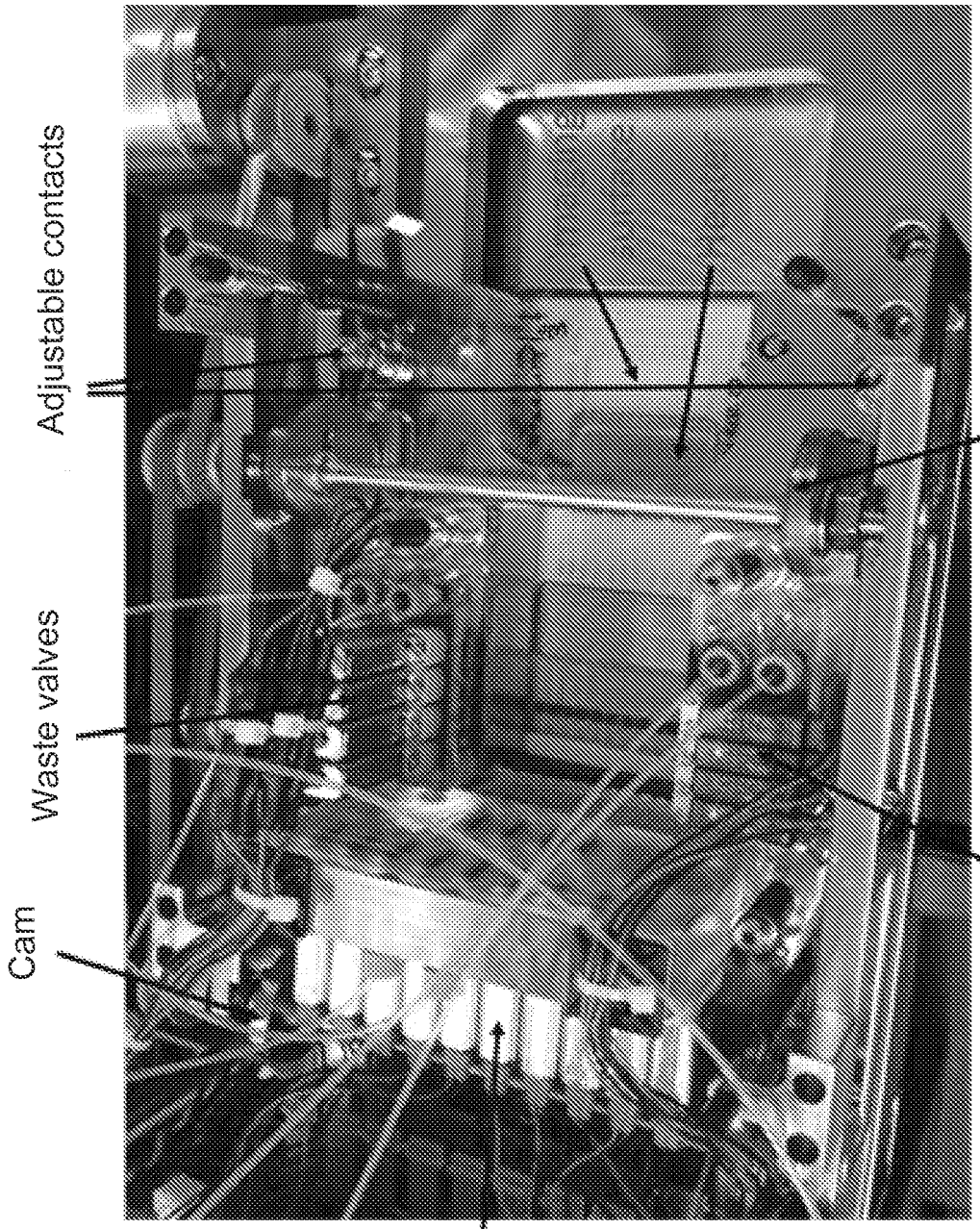

904
Cam
Waste valves
Adjustable contacts
Off card buffer input valves
902
Pneumatic valves
Cam

FIGURE 9A

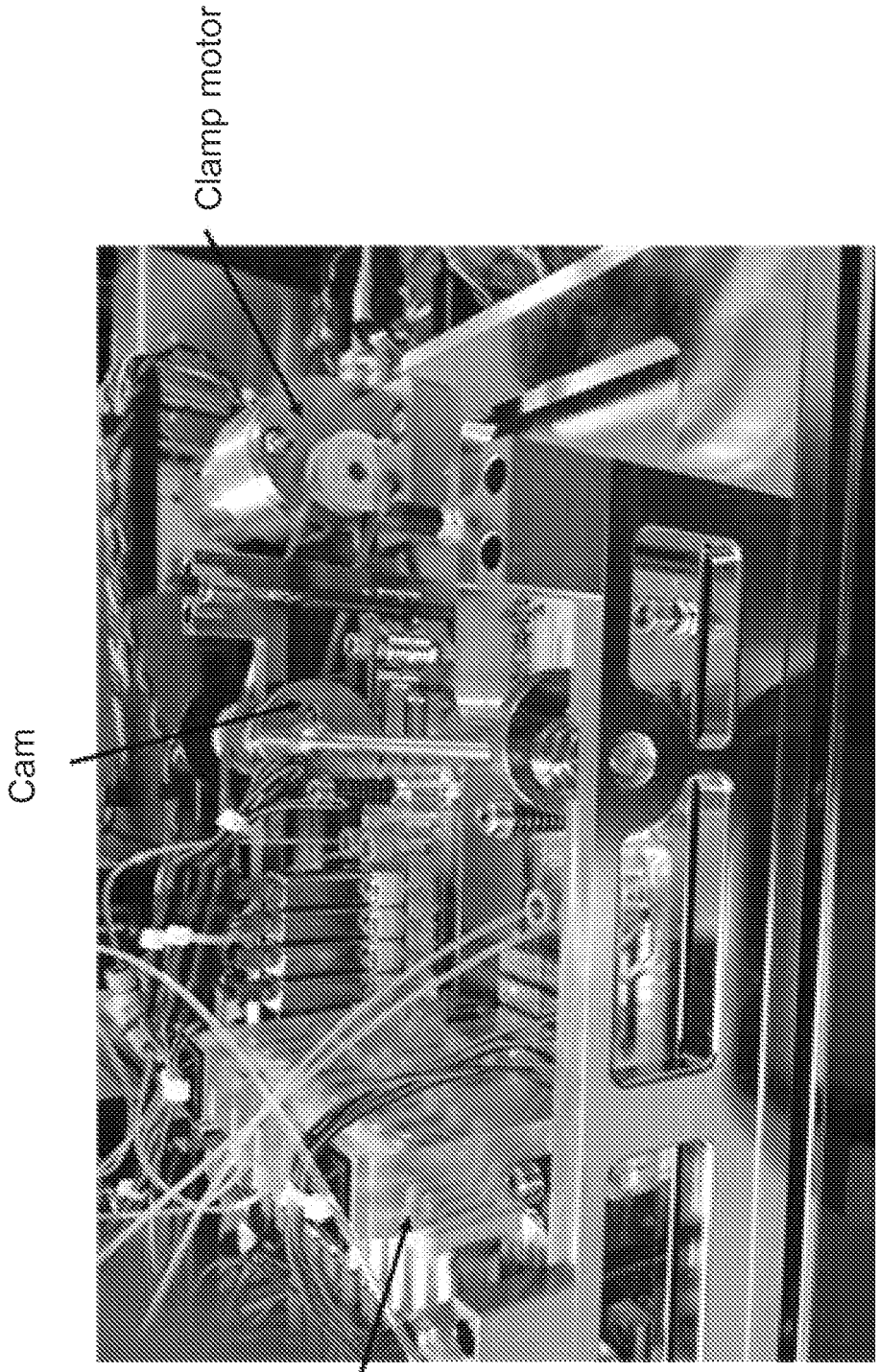


FIGURE 9B

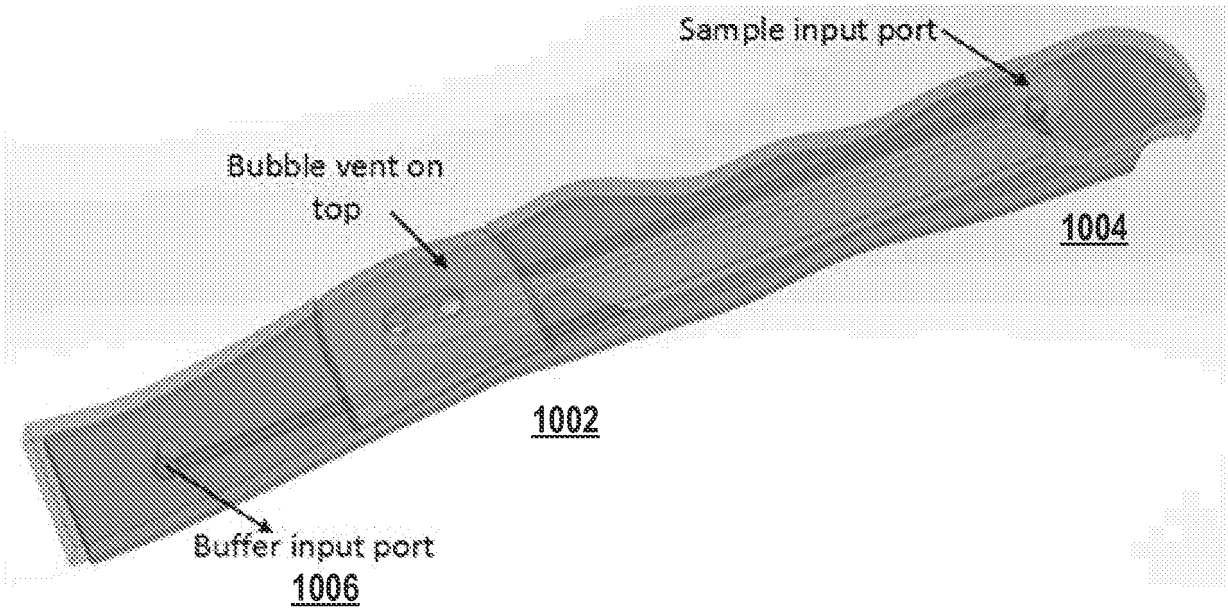

Sample input port
Bubble vent on top
1004
1002
Buffer input port
1006

FIGURE 10

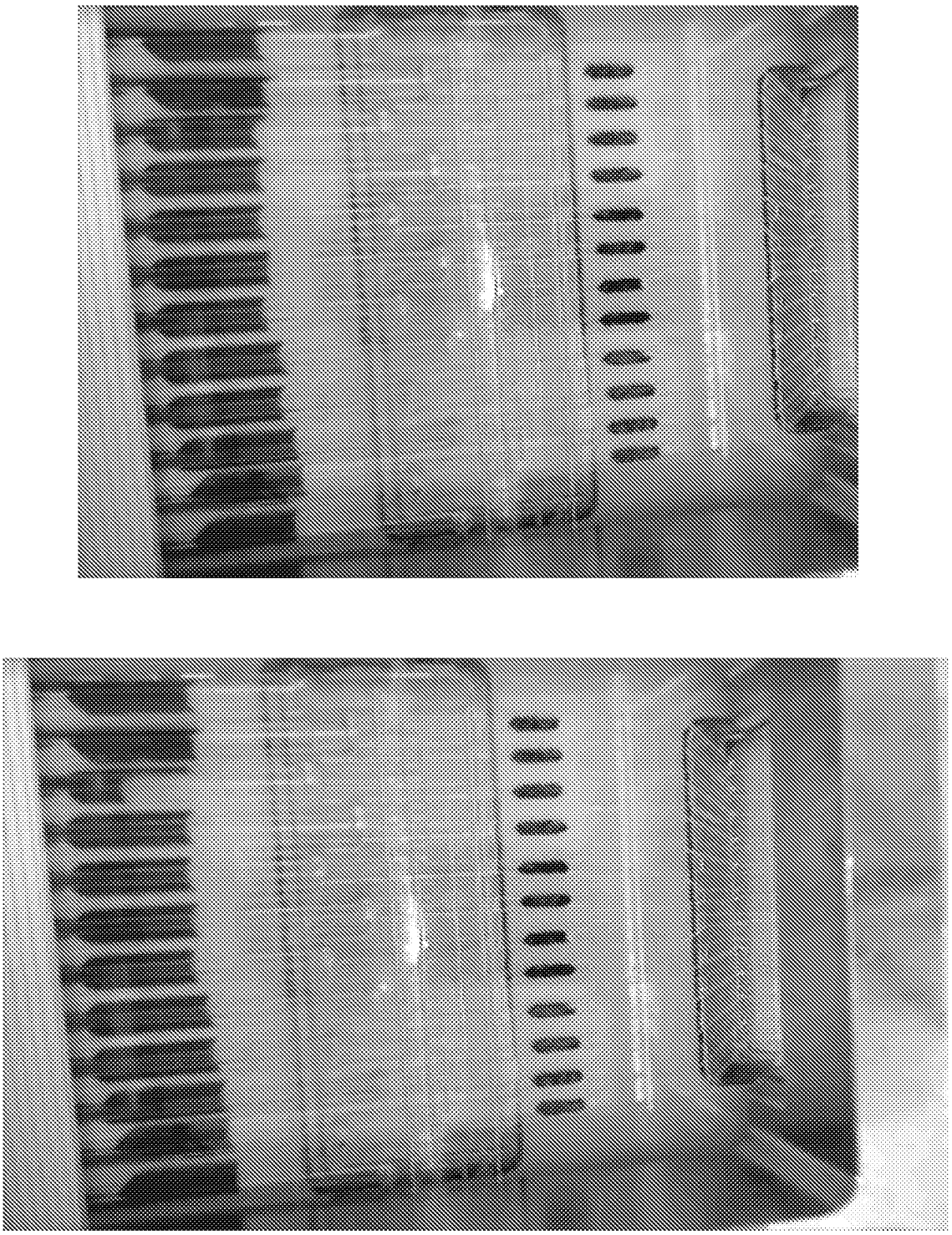


FIGURE 11

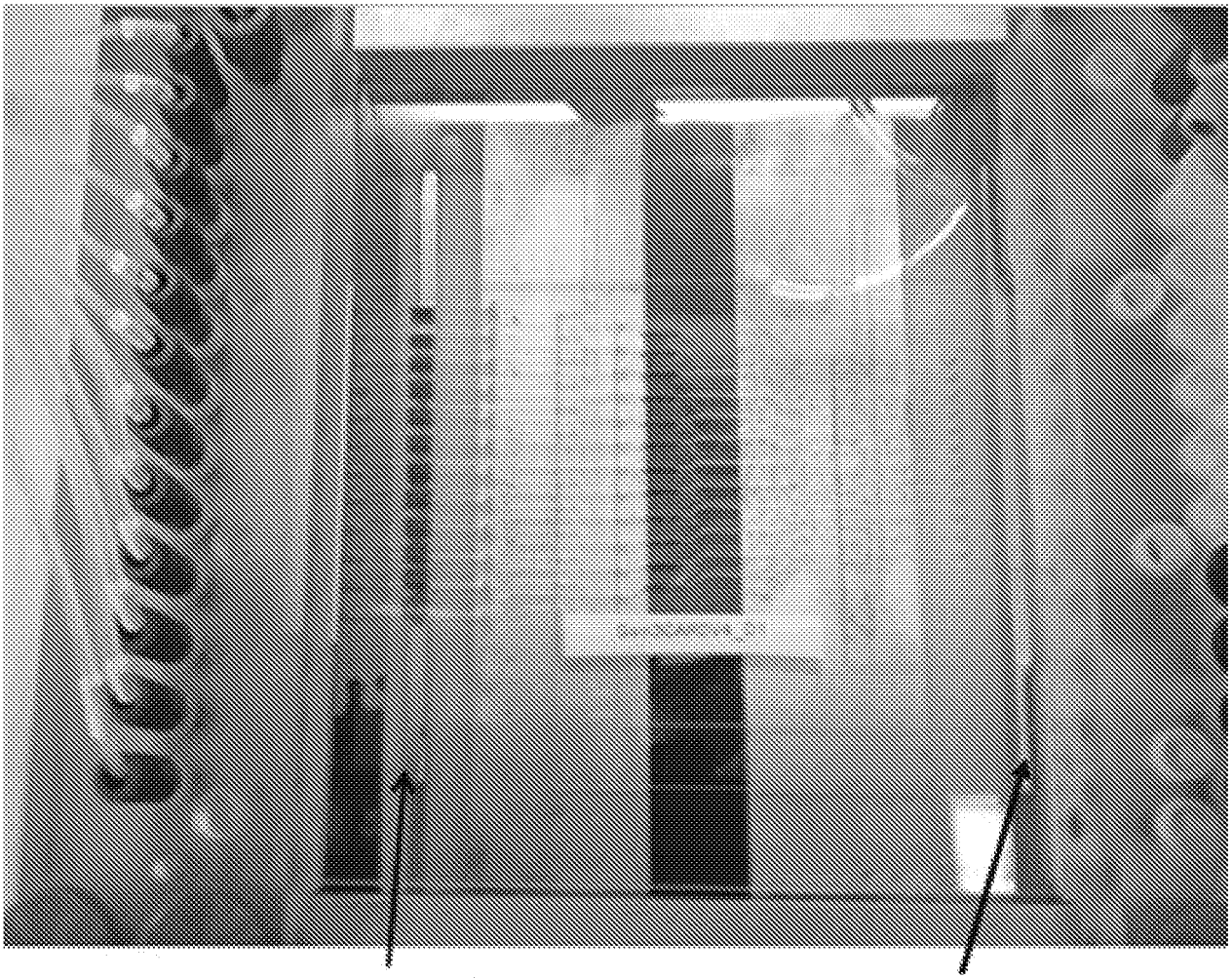
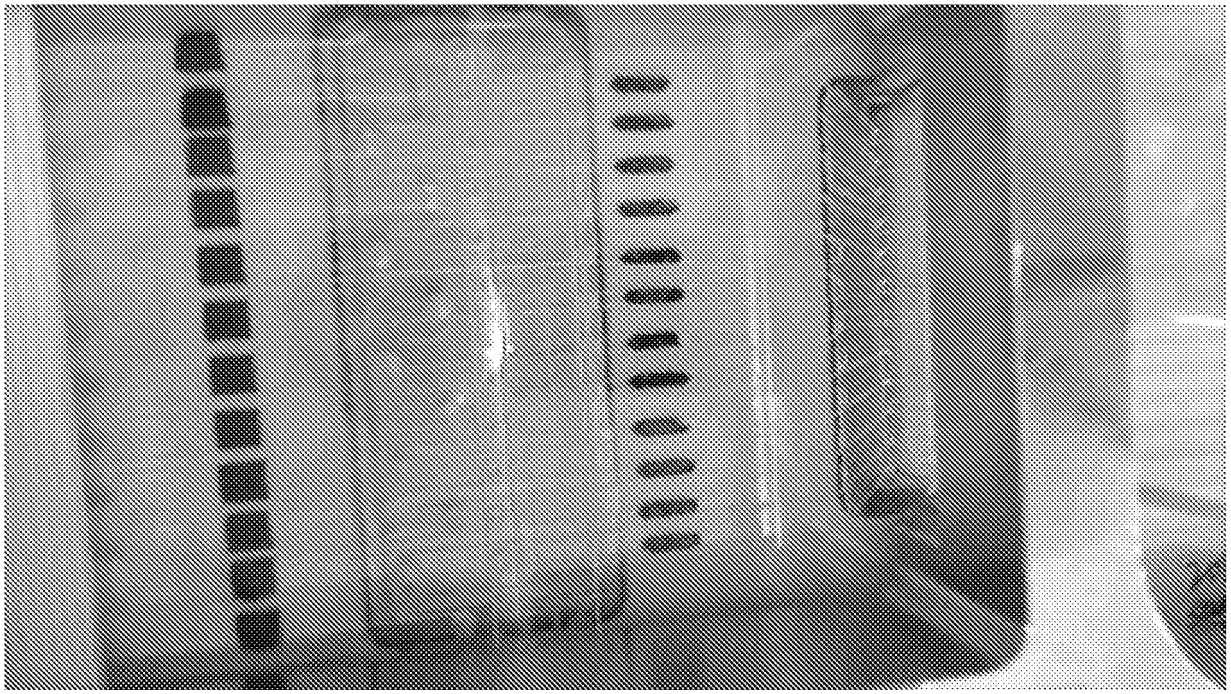


FIGURE 12



1302

FIGURE 13A

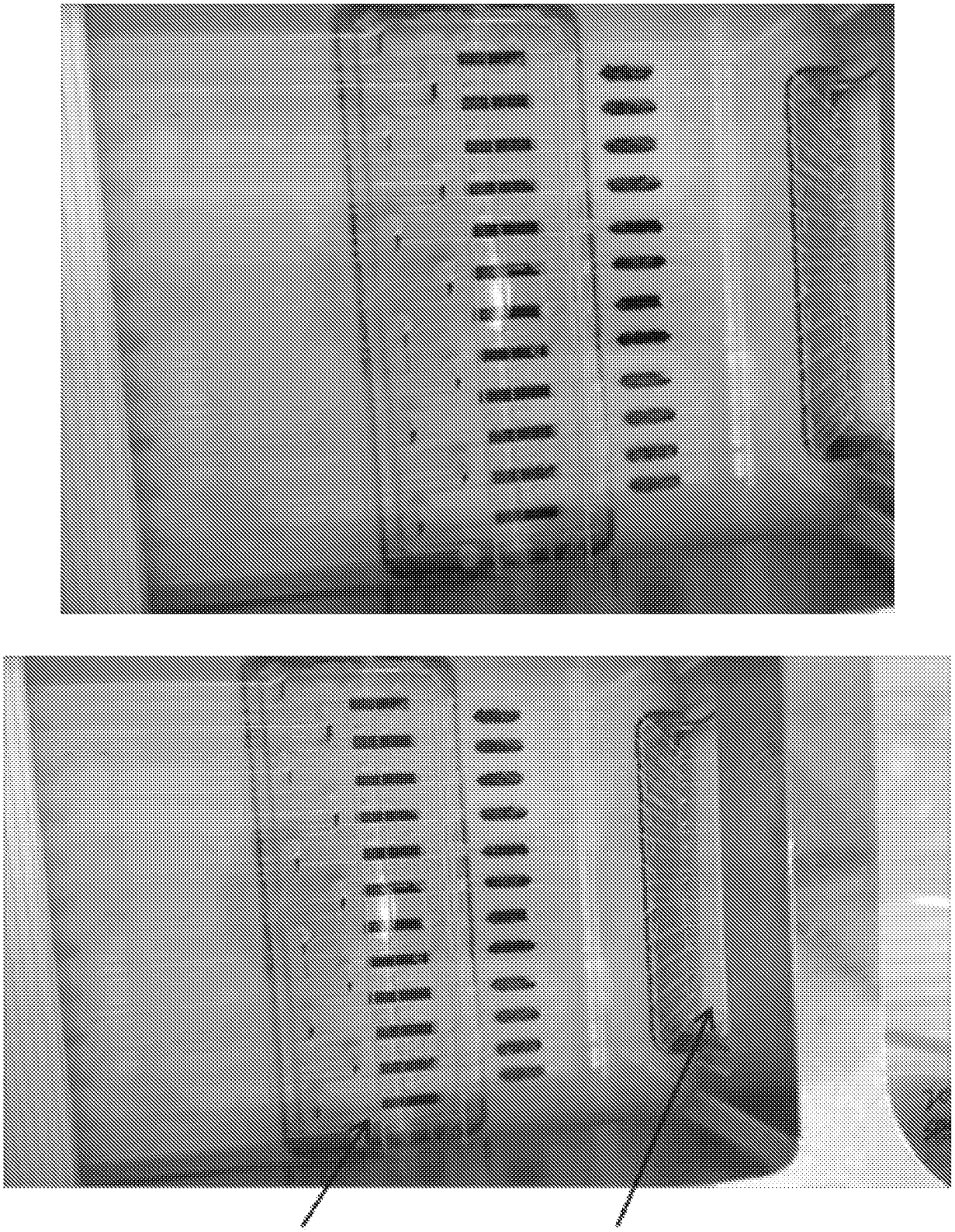
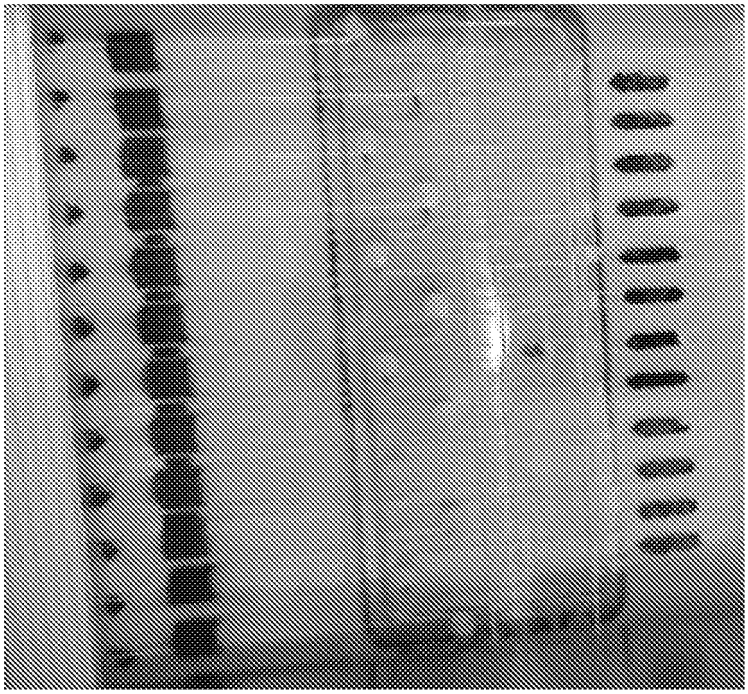


FIGURE 13B



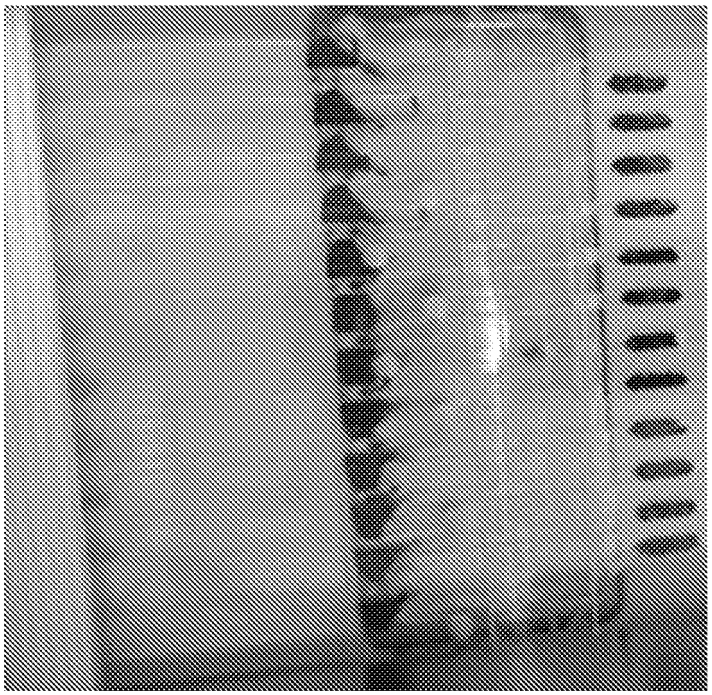
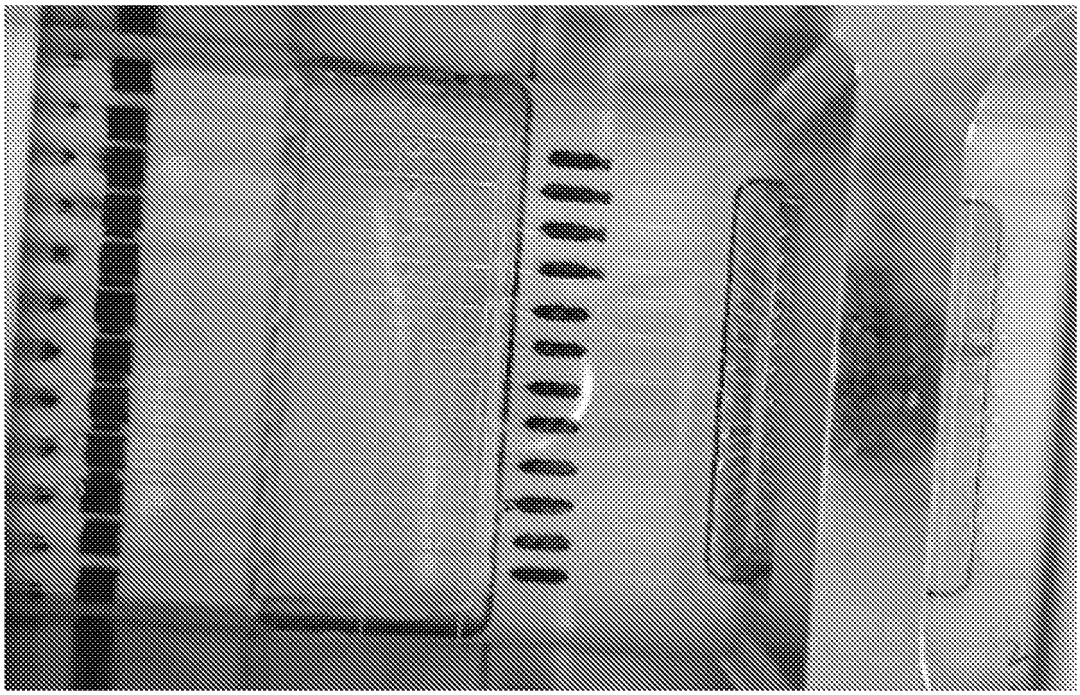
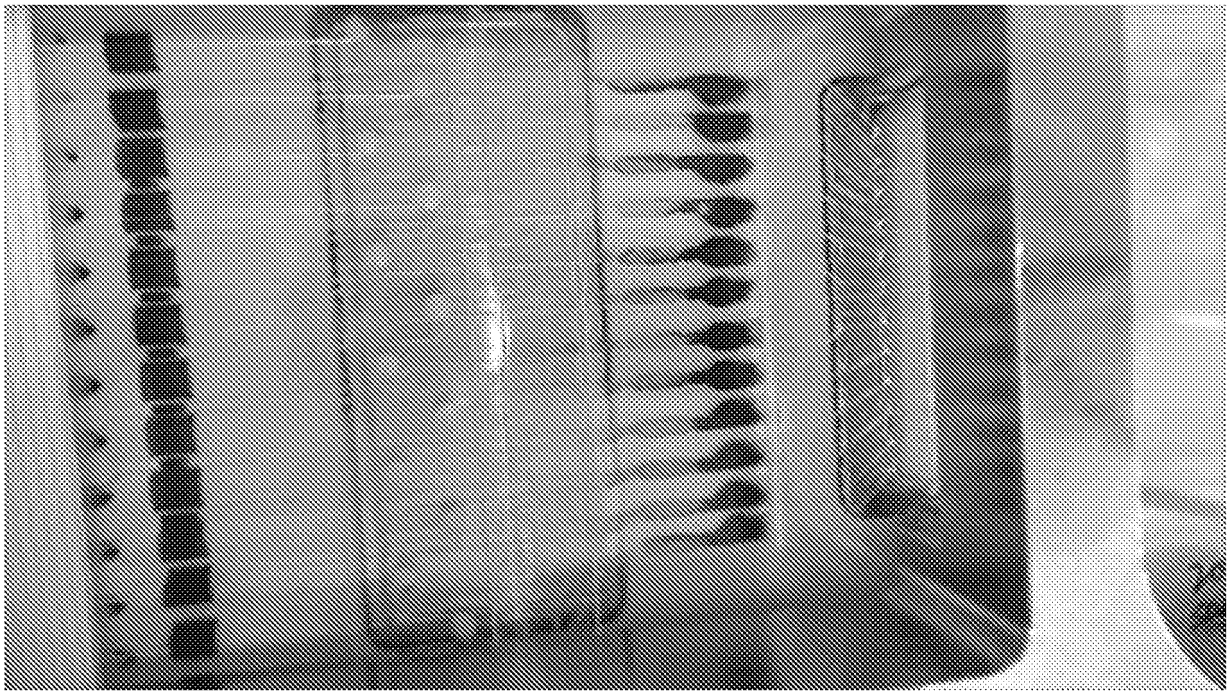


FIGURE 14

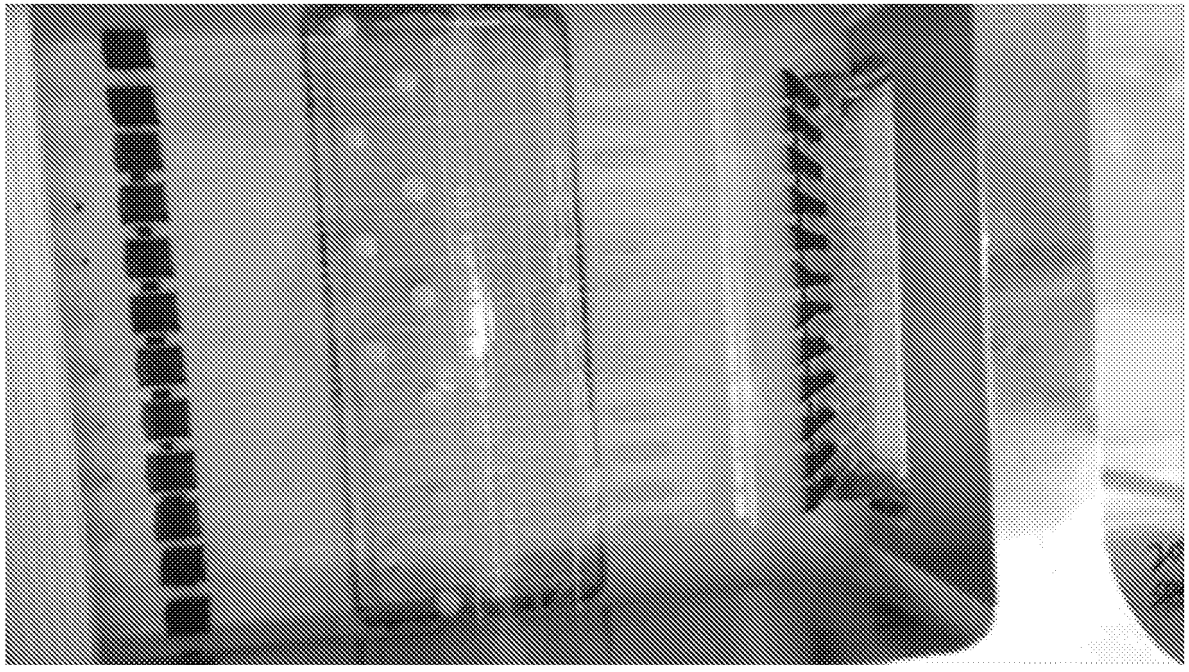


1502

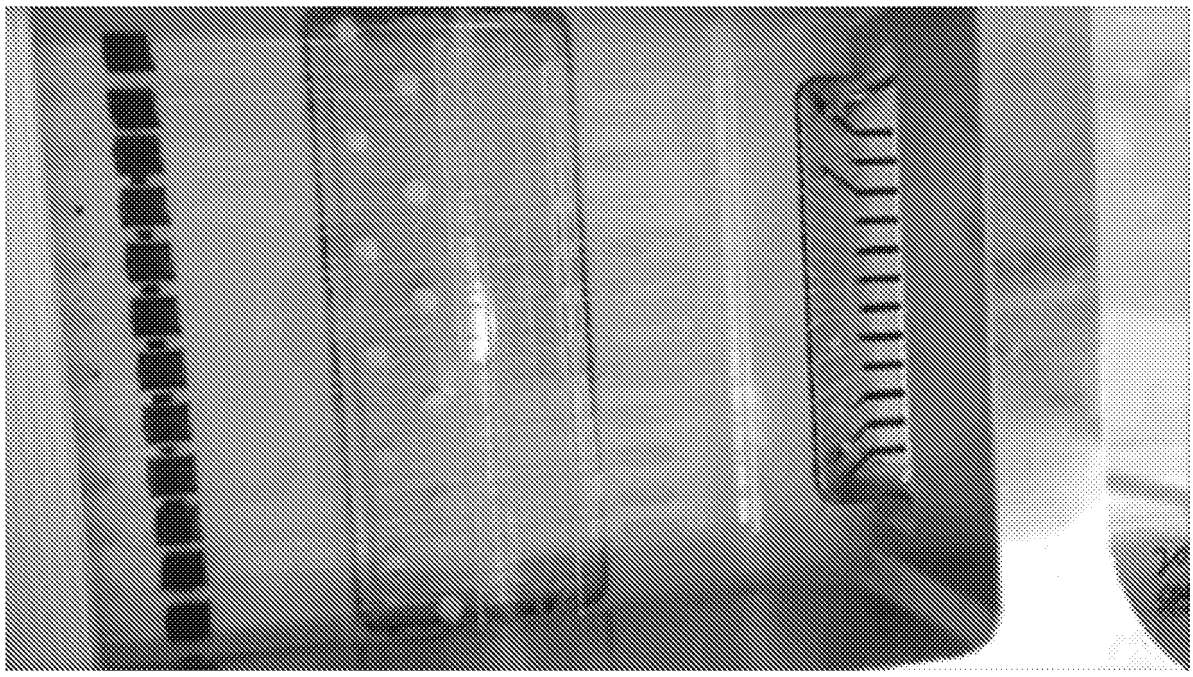


1504

FIGURE 15



1602



1604

FIGURE 16

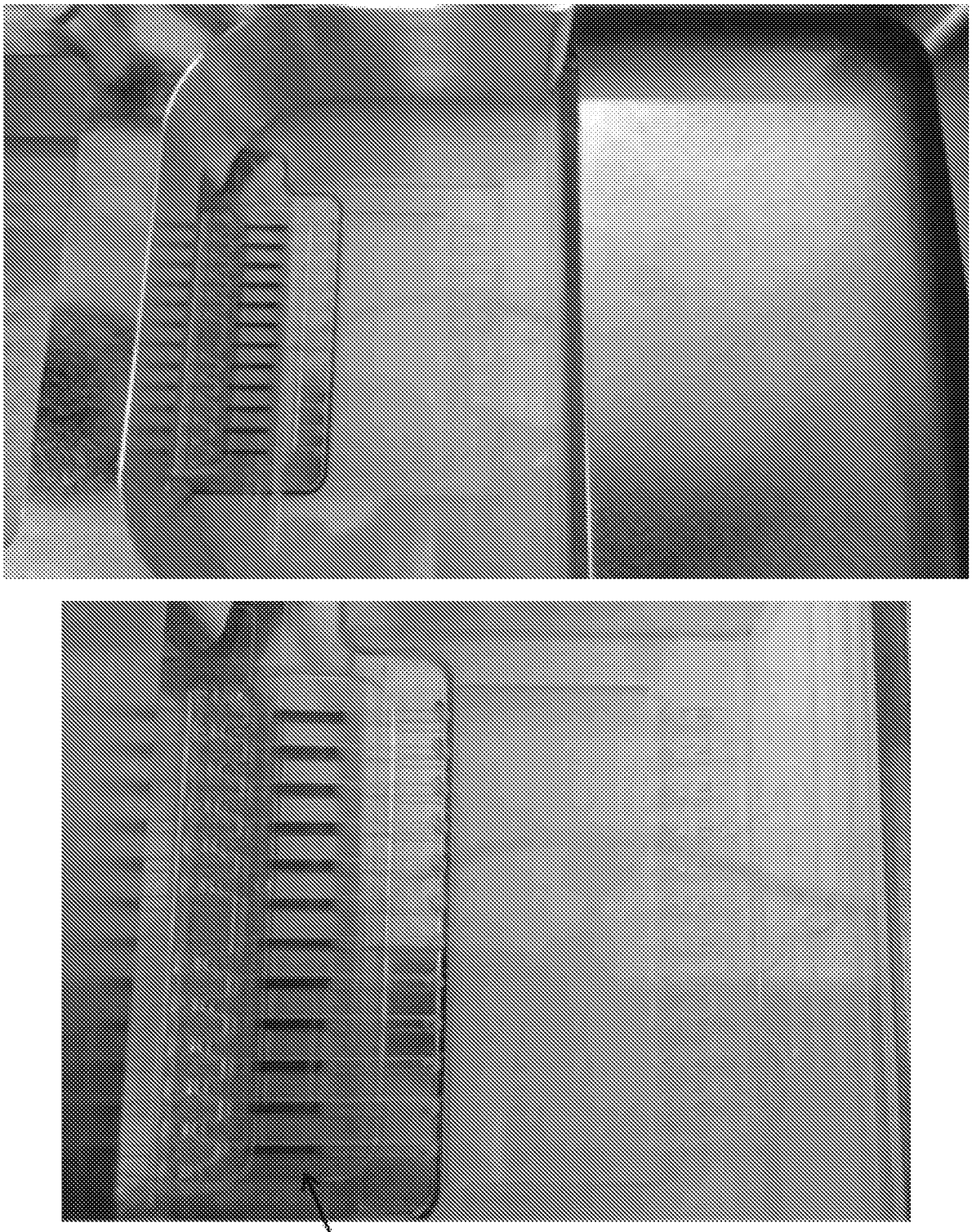


FIGURE 17

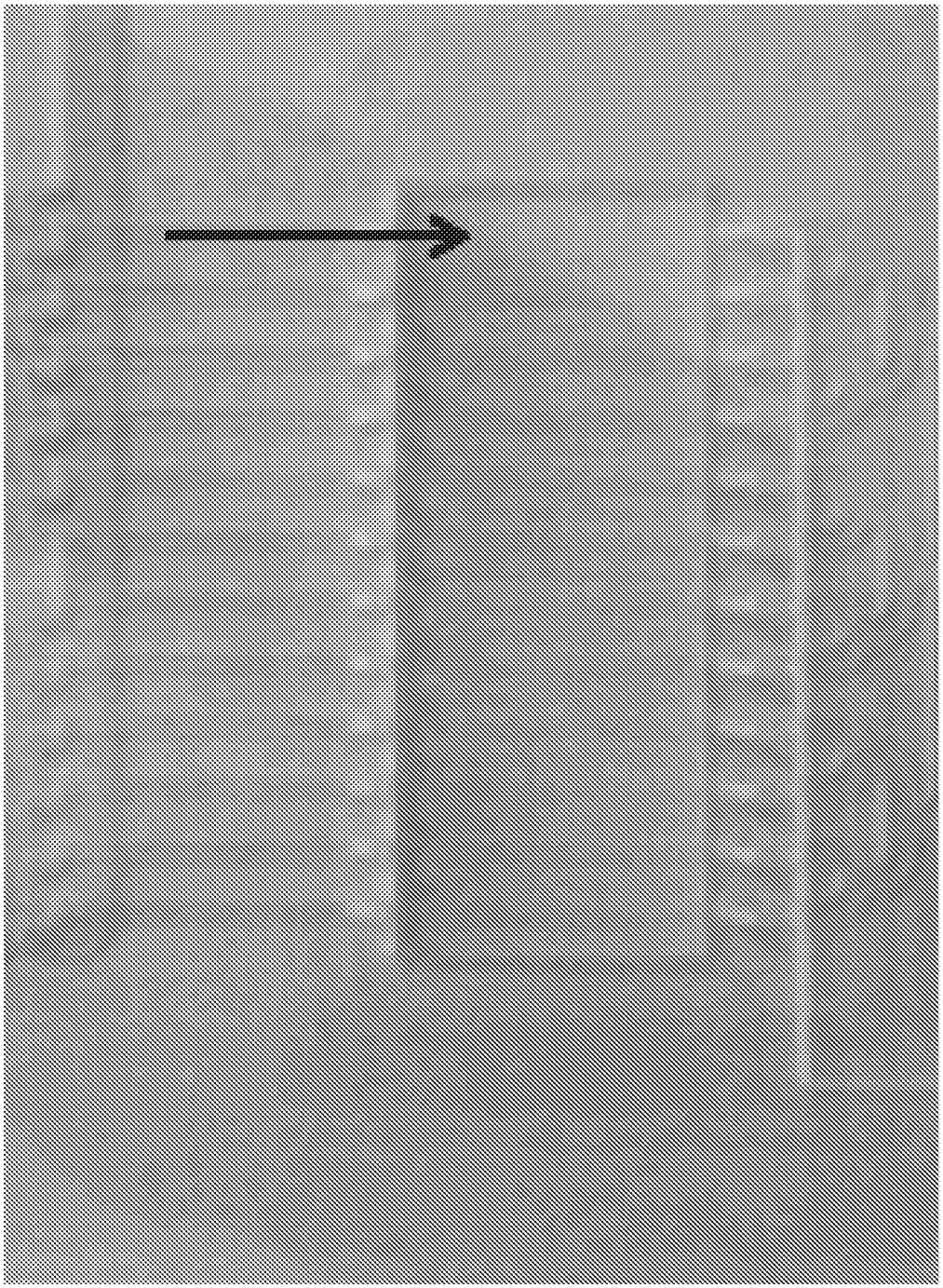


FIGURE 18

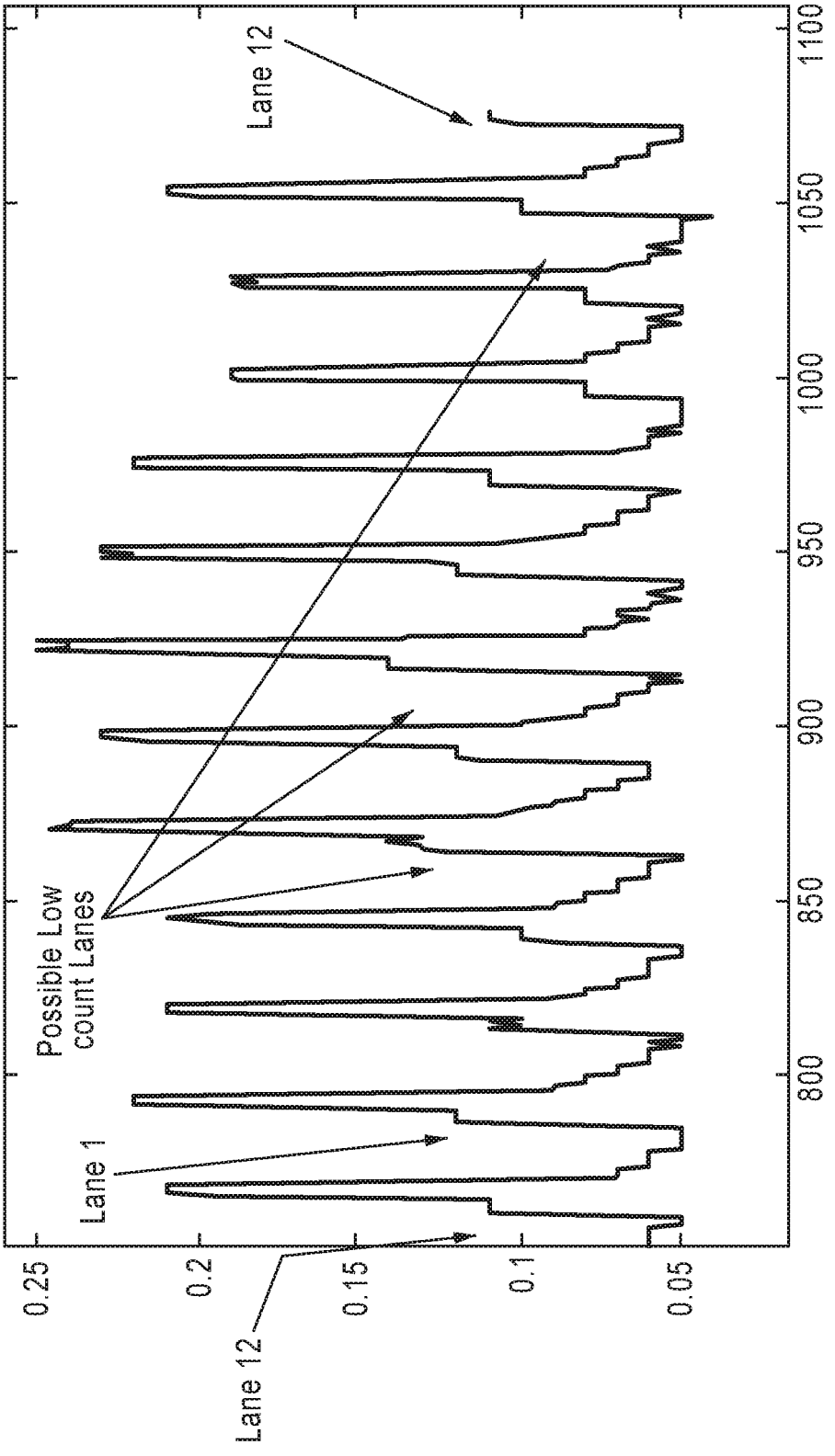

Lane 12
Possible Low count Lanes
Lane 1
Lane 12
0.25
0.2
0.15
0.1
0.05
800
850
900
950
1000
1050
1100
1902

FIGURE 19A

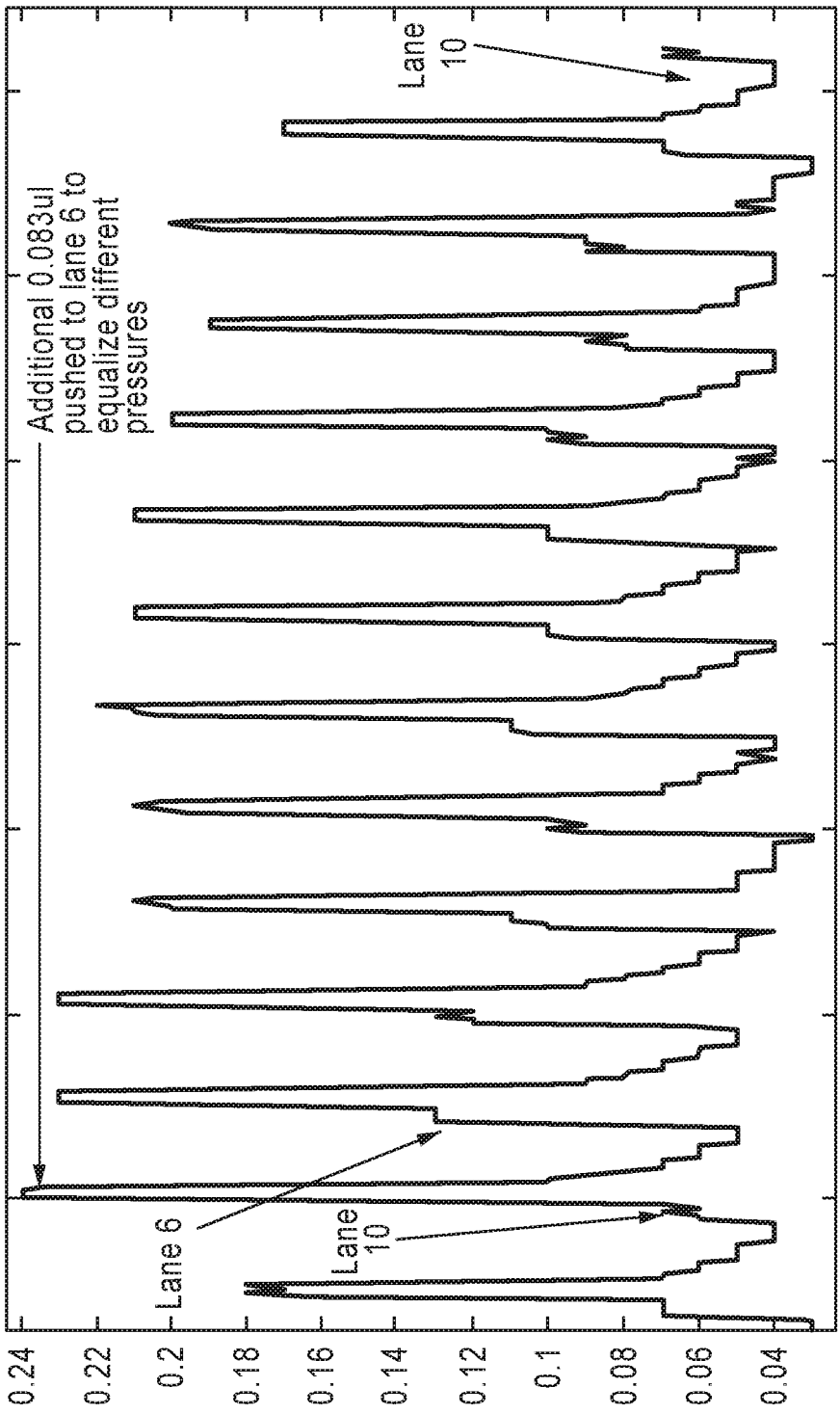

Additional 0.083ul pushed to lane 6 to equalize different pressures
Lane 6
Lane 10
Lane 10
0.24
0.22
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
4850
4900
4950
5000
5050
5100
5150
1904

FIGURE 19B

Surface of cartridge is coated with streptavidin
Hybridized Probes Bind to Cartridge
2002

FIGURE 20

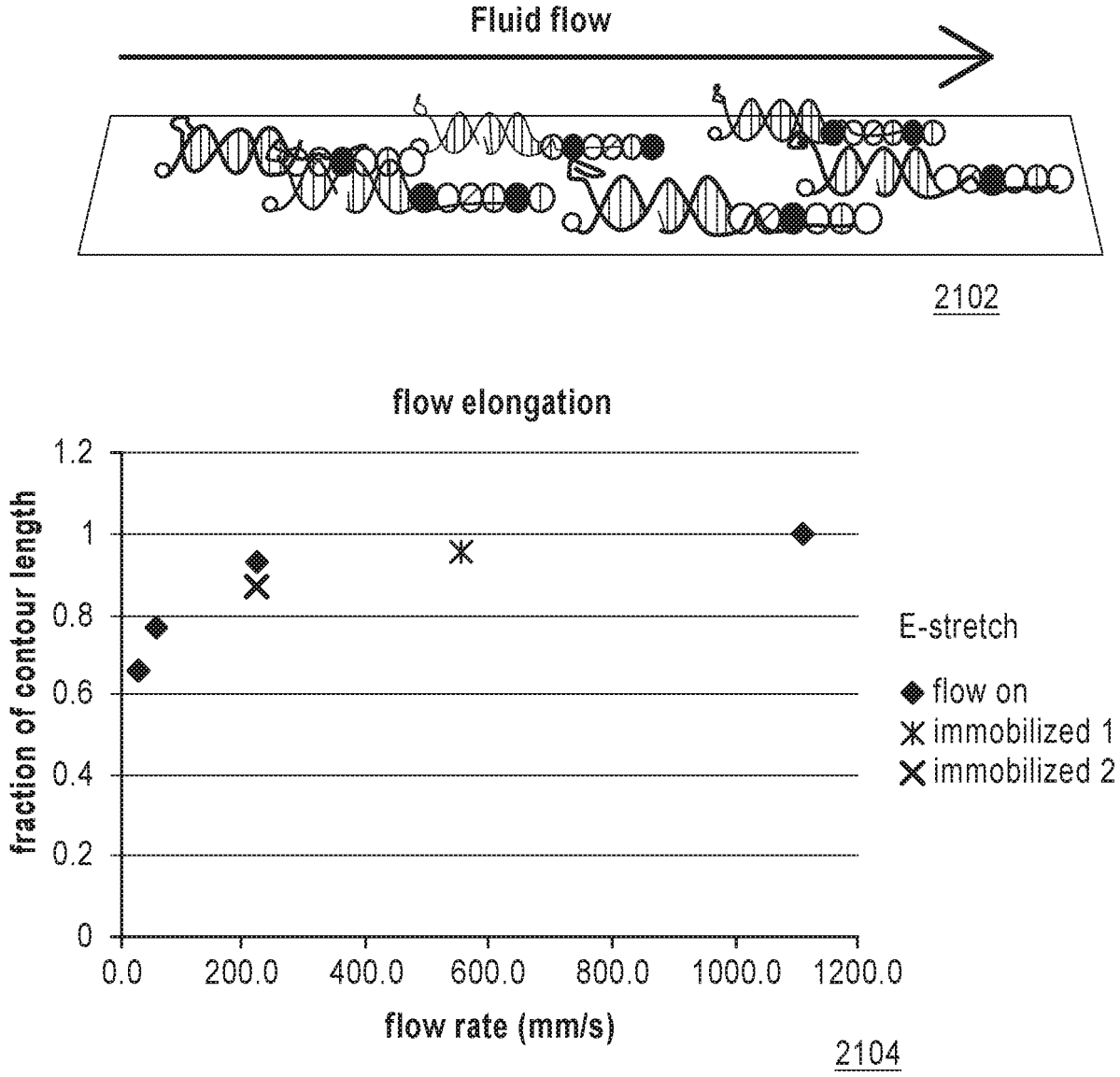

Fluid flow
2102
flow elongation
fraction of contour length
1.2
1
0.8
0.6
0.4
0.2
0
0.0
200.0
400.0
600.0
800.0
1000.0
1200.0
flow rate (mm/s)
E-stretch
flow on
immobilized 1
immobilized 2
2104

FIGURE 21

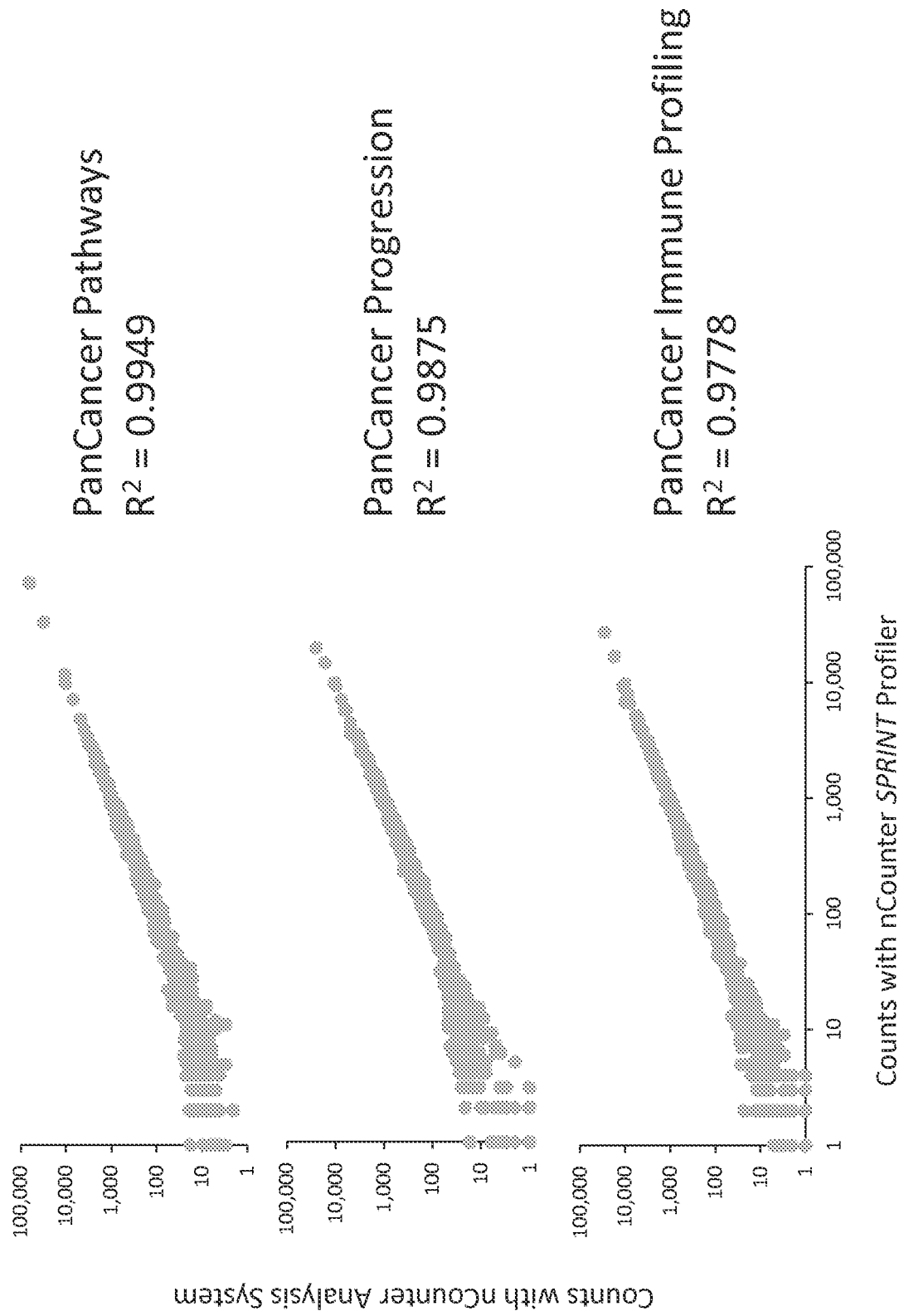

PanCancer Pathways
R^2 = 0.9949
PanCancer Progression
R^2 = 0.9875
PanCancer Immune Profiling
R^2 = 0.9778
100,000
10,000
1,000
100
10
1
Counts with nCounter SPRINT Profiler
Counts with nCounter Analysis System

FIGURE 22

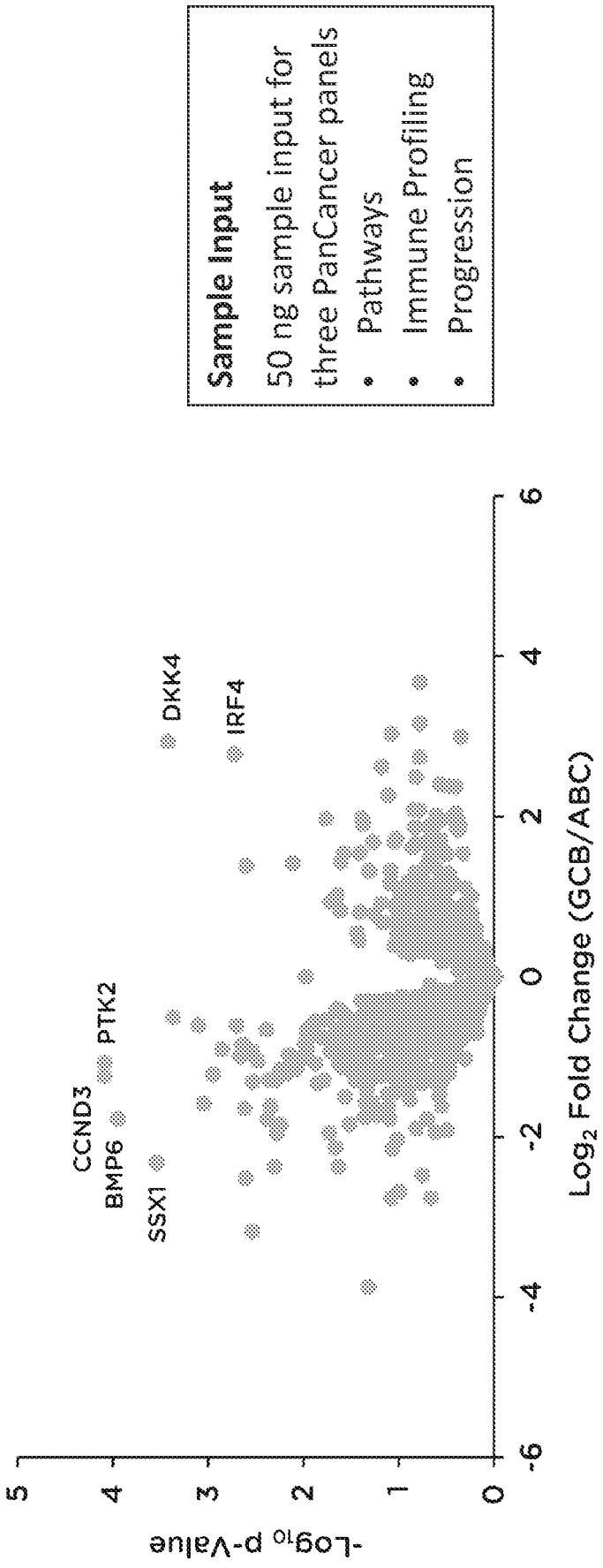

Sample Input
50 ng sample input for three PanCancer panels
• Pathways
• Immune Profiling
• Progression
DKK4
IRF4
CCND3
PTK2
BMP6
SSX1
-Log10 p-Value
Log2 Fold Change (GCB/ABC)
5
4
3
2
1
0
-6
-4
-2
0
2
4
6

FIGURE 23

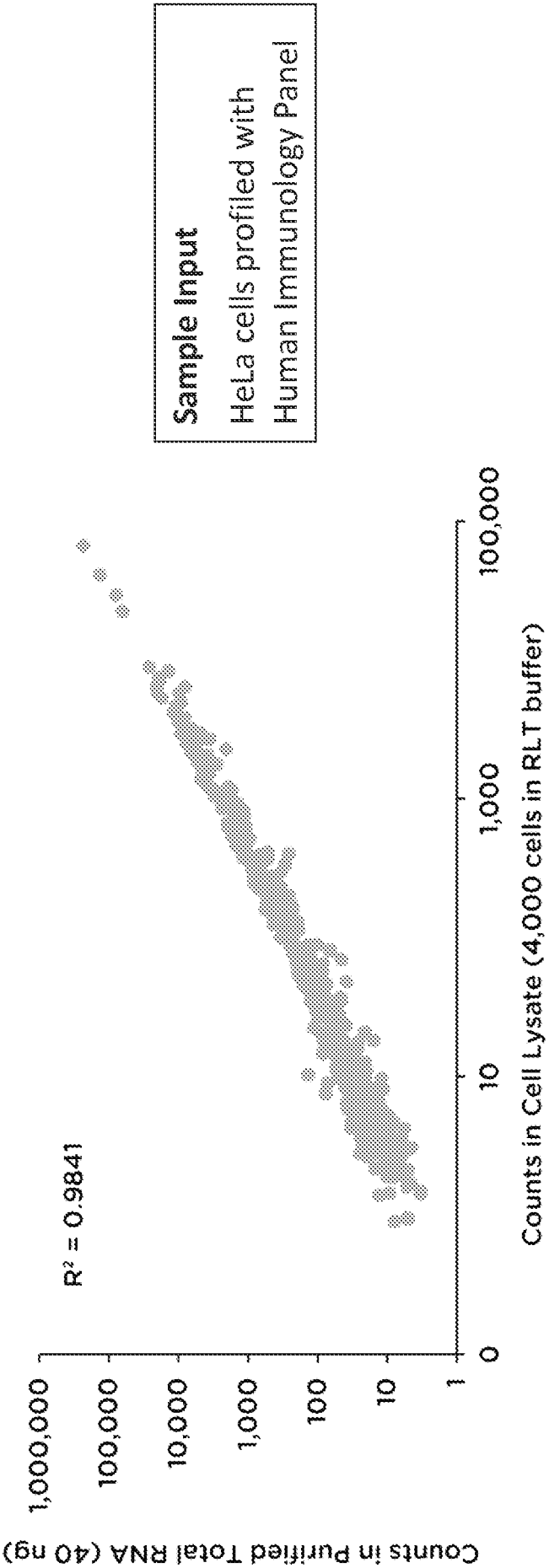

Sample Input
HeLa cells profiled with
Human Immunology Panel
R² = 0.9841
Counts in Purified Total RNA (40 ng)
1,000,000
100,000
10,000
1,000
100
10
1
0
10
1,000
100,000
Counts in Cell Lysate (4,000 cells in RLT buffer)

FIGURE 24

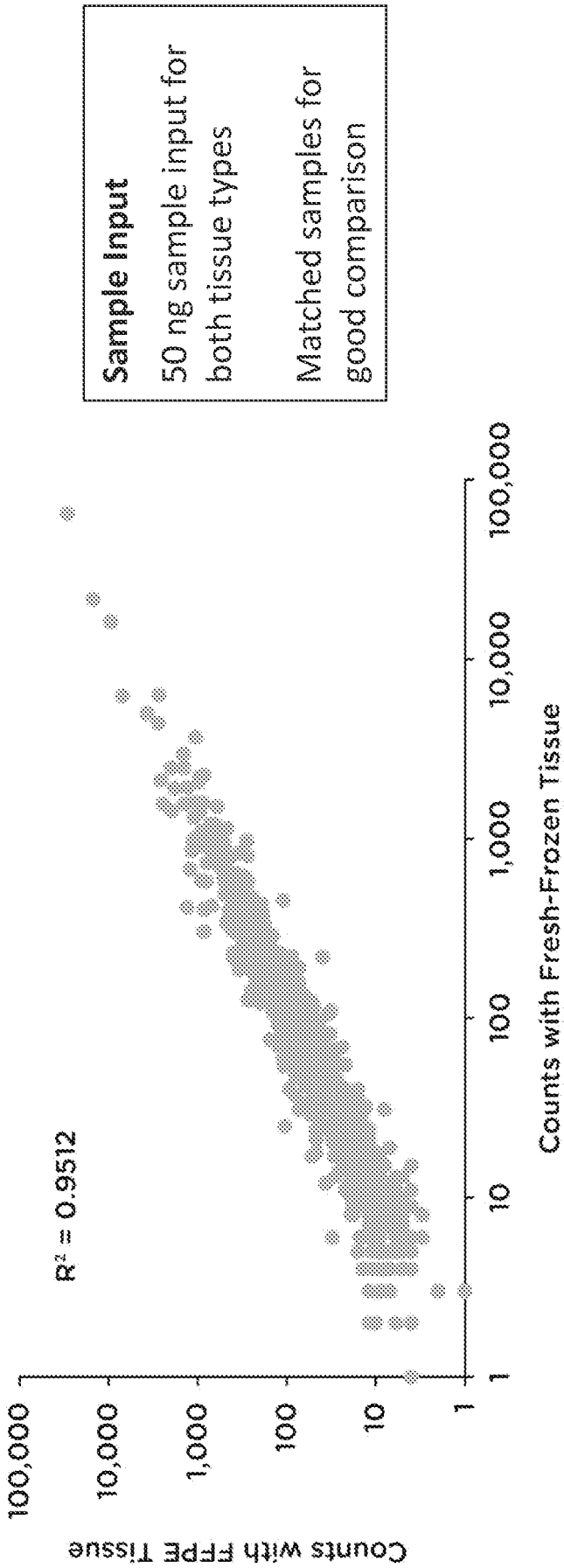

Sample Input
50 ng sample input for both tissue types
Matched samples for good comparison
R² = 0.9512
Counts with FFPE Tissue
Counts with Fresh-Frozen Tissue
100,000
10,000
1,000
100
10
1
1
10
100
1,000
10,000
100,000

FIGURE 25

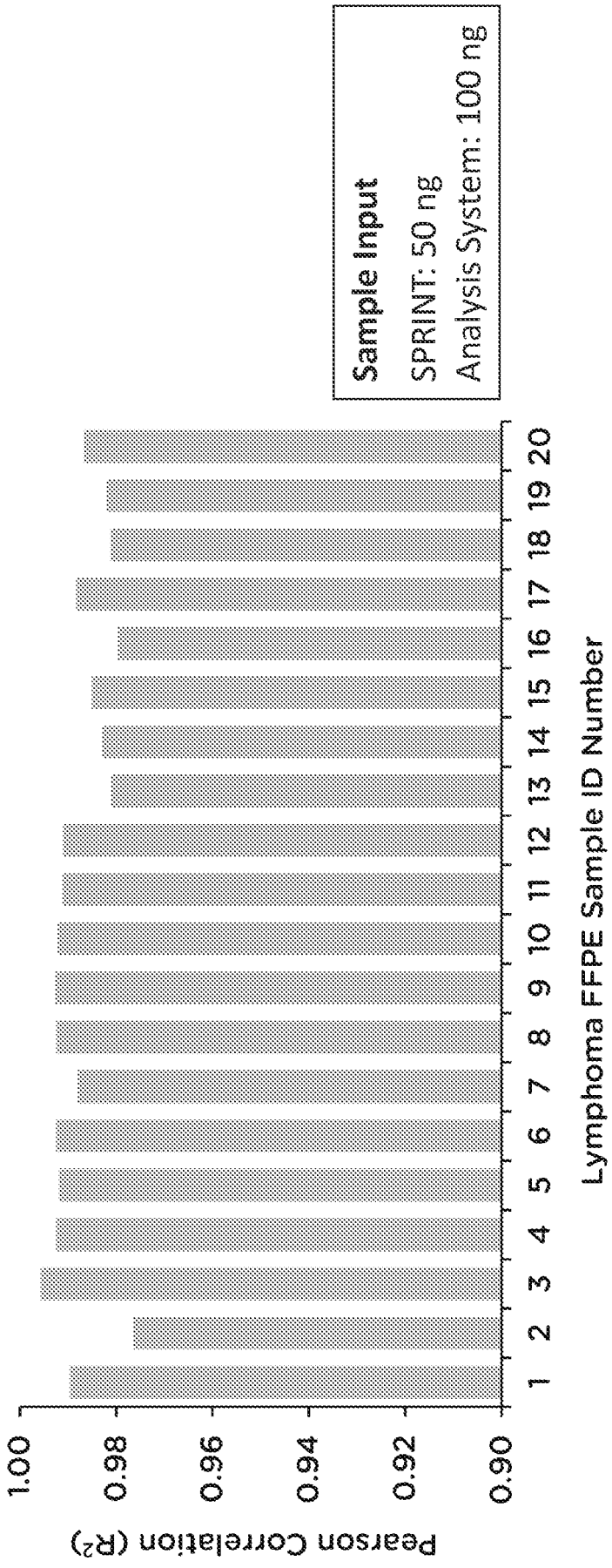

Sample Input
SPRINT: 50 ng
Analysis System: 100 ng
Pearson Correlation (R^2)
1.00
0.98
0.96
0.94
0.92
0.90
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Lymphoma FFPE Sample ID Number

FIGURE 26

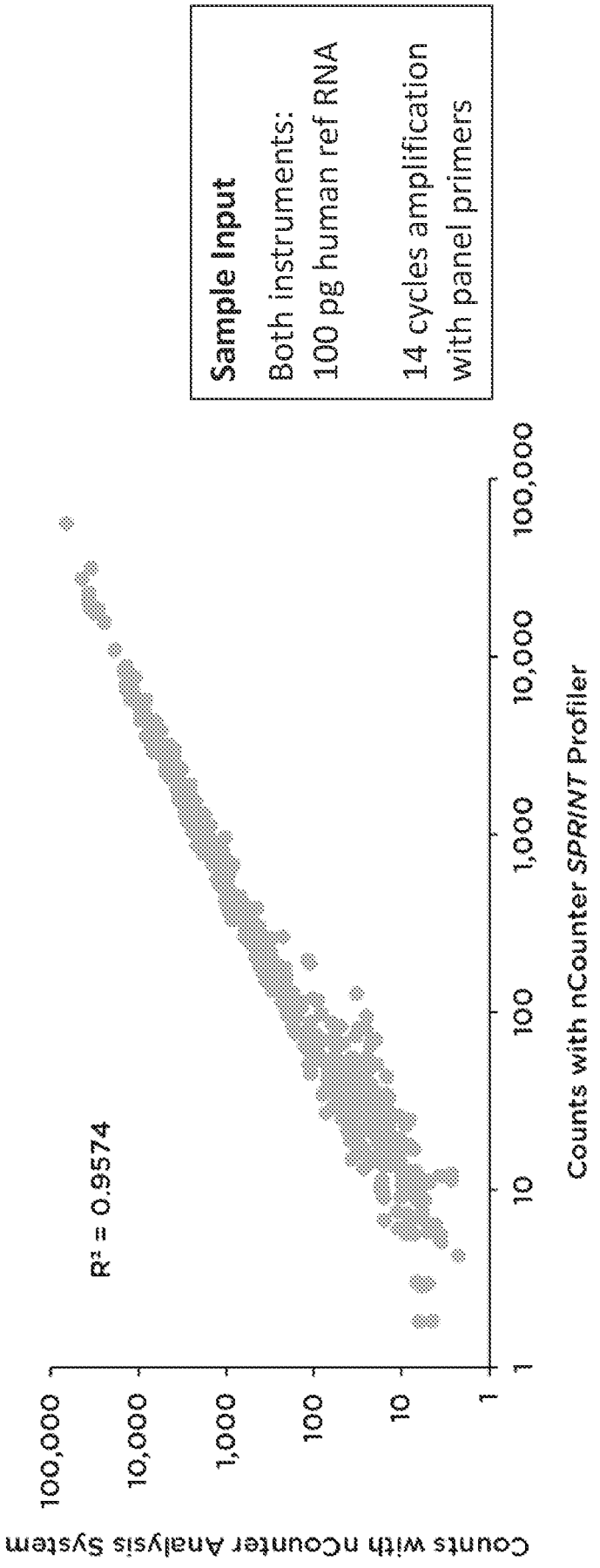

Sample Input
Both instruments:
100 pg human ref RNA
14 cycles amplification
with panel primers
R² = 0.9574
Counts with nCounter Analysis System
Counts with nCounter SPRINT Profiler
1
10
100
1,000
10,000
100,000

FIGURE 27

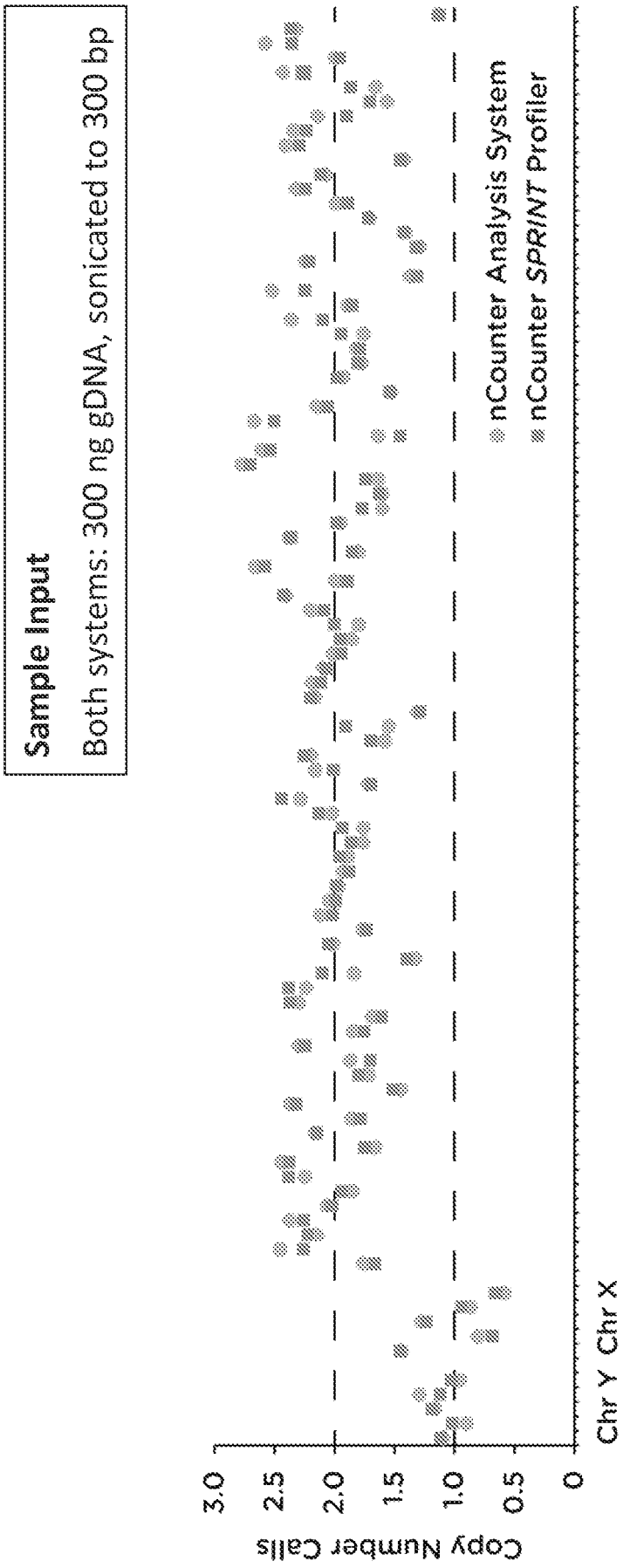

Sample Input
Both systems: 300 ng gDNA, sonicated to 300 bp
nCounter Analysis System
nCounter SPRINT Profiler
Copy Number Calls
3.0
2.5
2.0
1.5
1.0
0.5
0
Chr Y Chr X

FIGURE 28

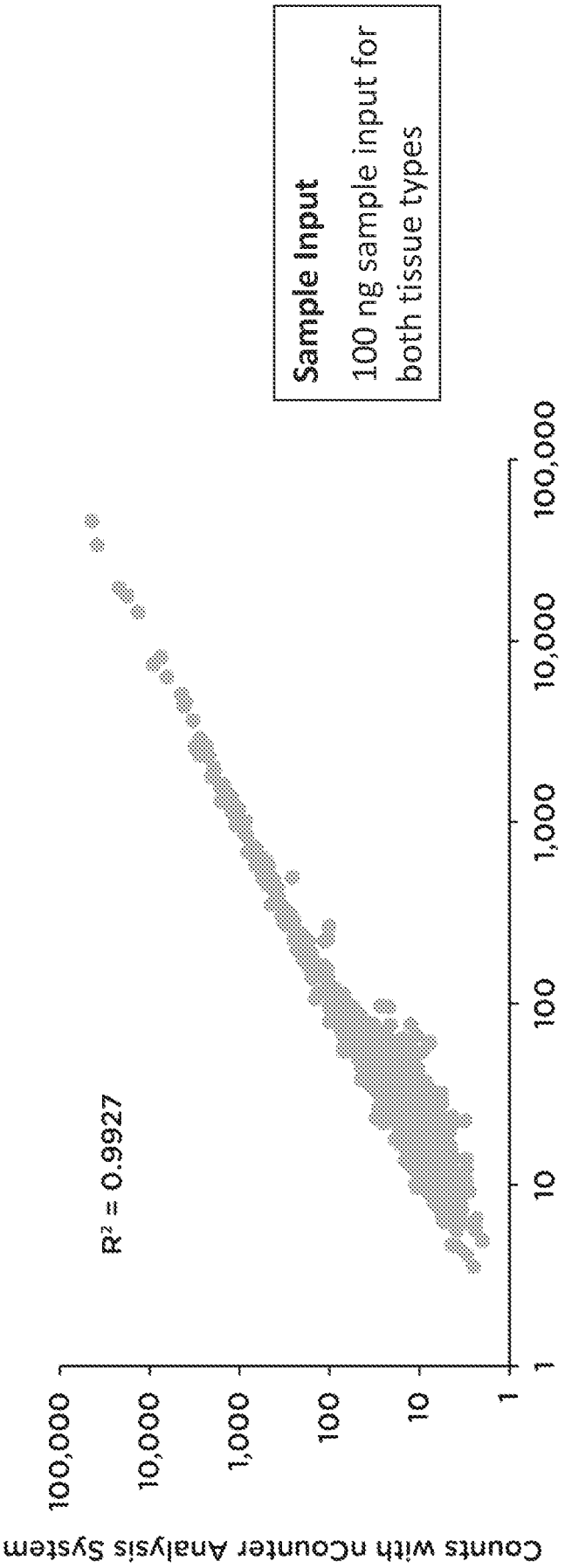

Sample Input
100 ng sample input for both tissue types
R^2 = 0.9927
Counts with nCounter SPRINT Profiler
Counts with nCounter Analysis System
1
10
100
1,000
10,000
100,000

FIGURE 29

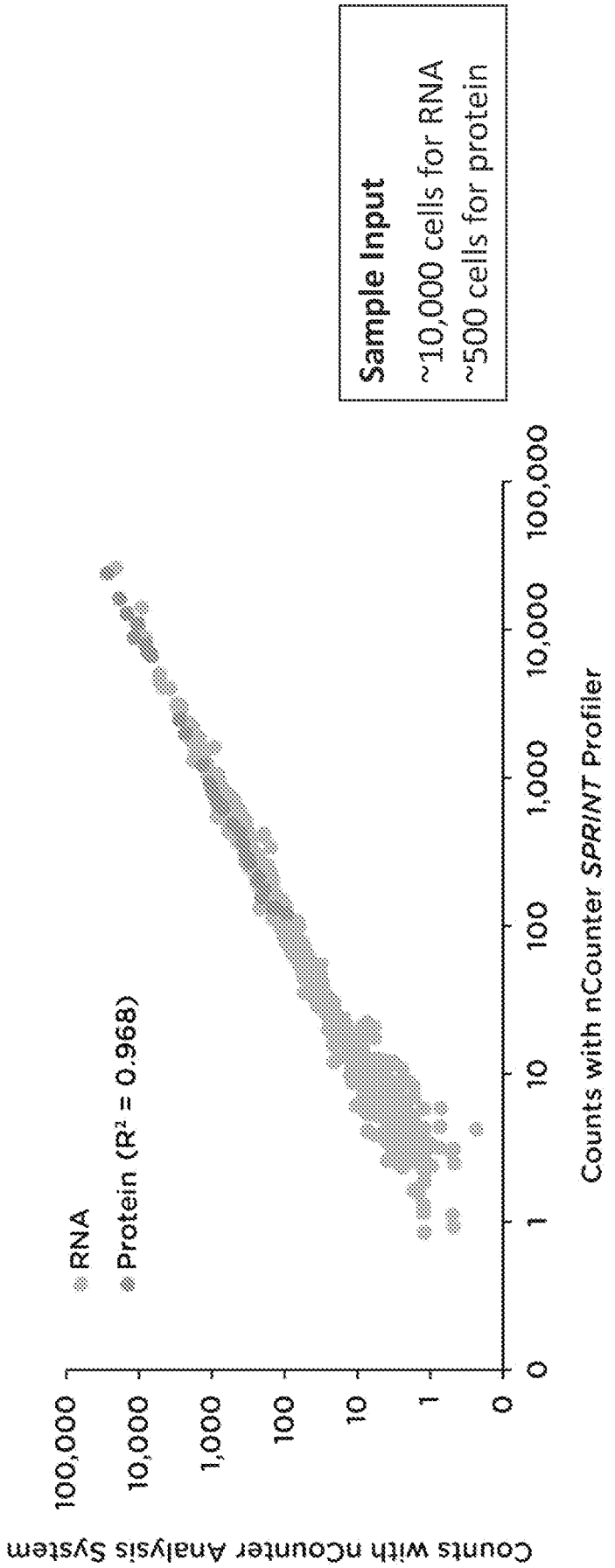

Sample Input
~10,000 cells for RNA
~500 cells for protein
RNA
Protein (R^2 = 0.968)
Counts with nCounter Analysis System
Counts with nCounter SPRINT Profiler
0
1
10
100
1,000
10,000
100,000

FIGURE 30