Figure 1

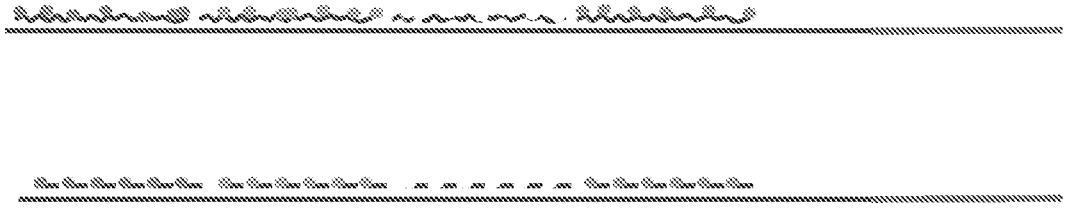


Figure 2

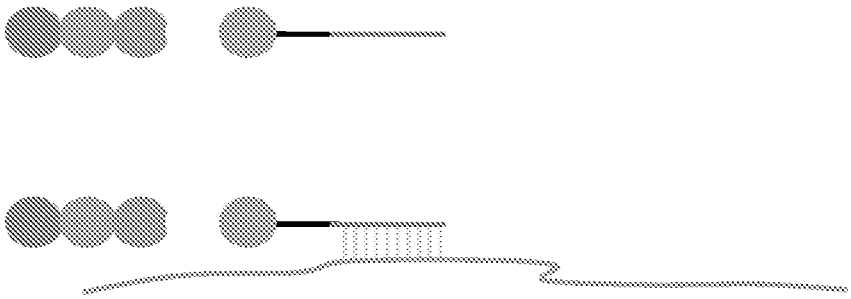


Figure 3

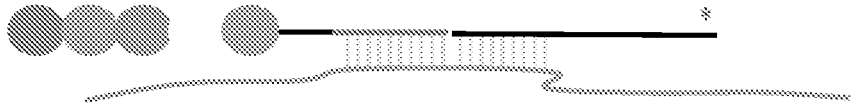


Figure 4

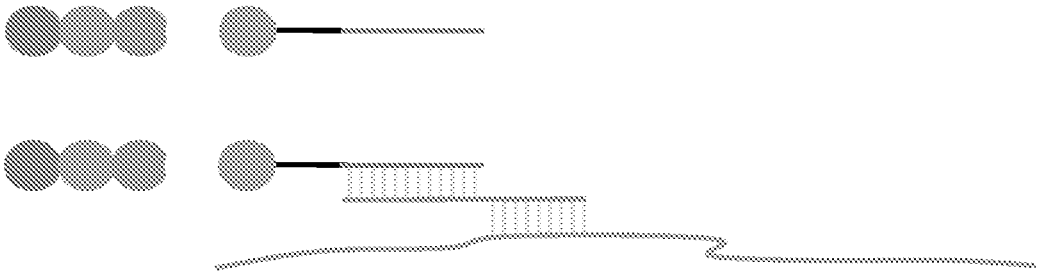


Figure 5

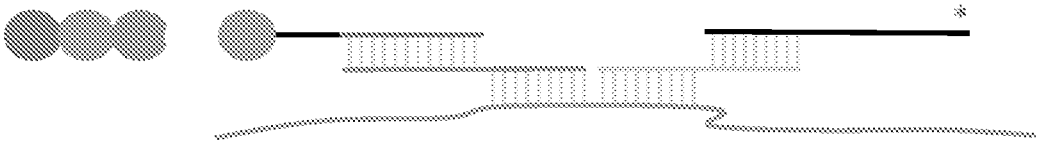


Figure 6

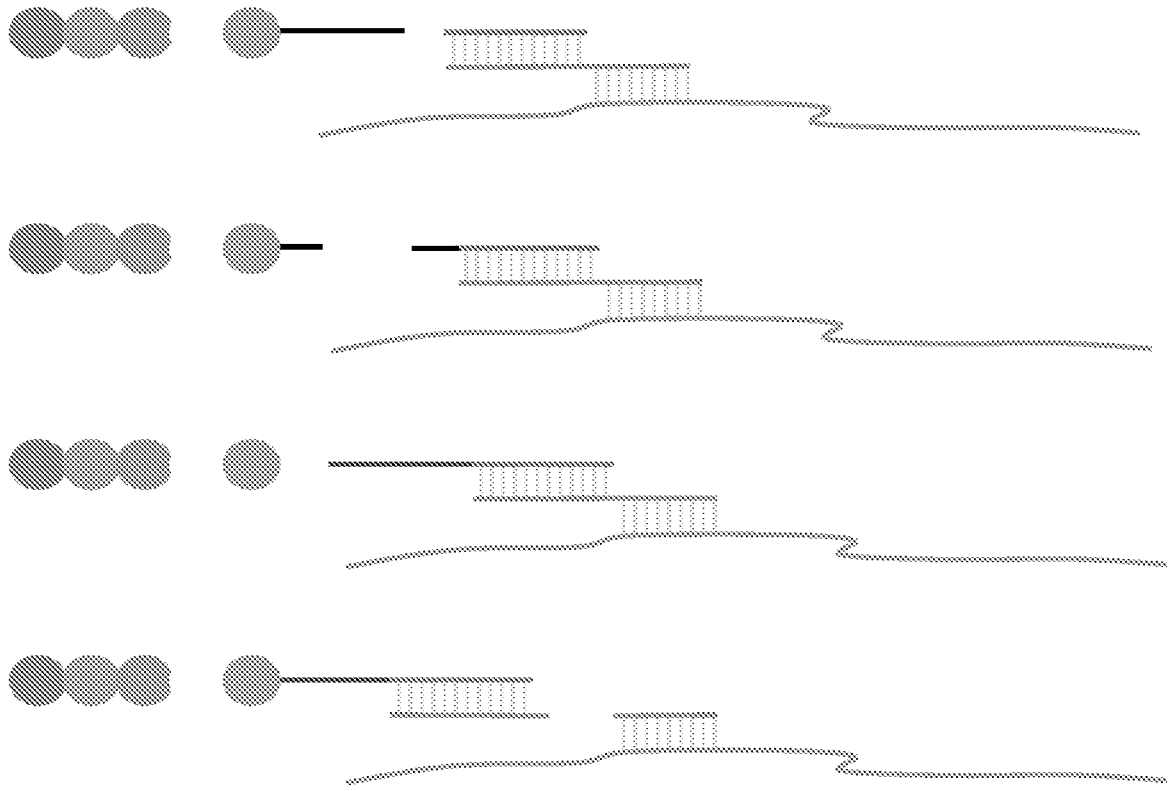


Figure 7

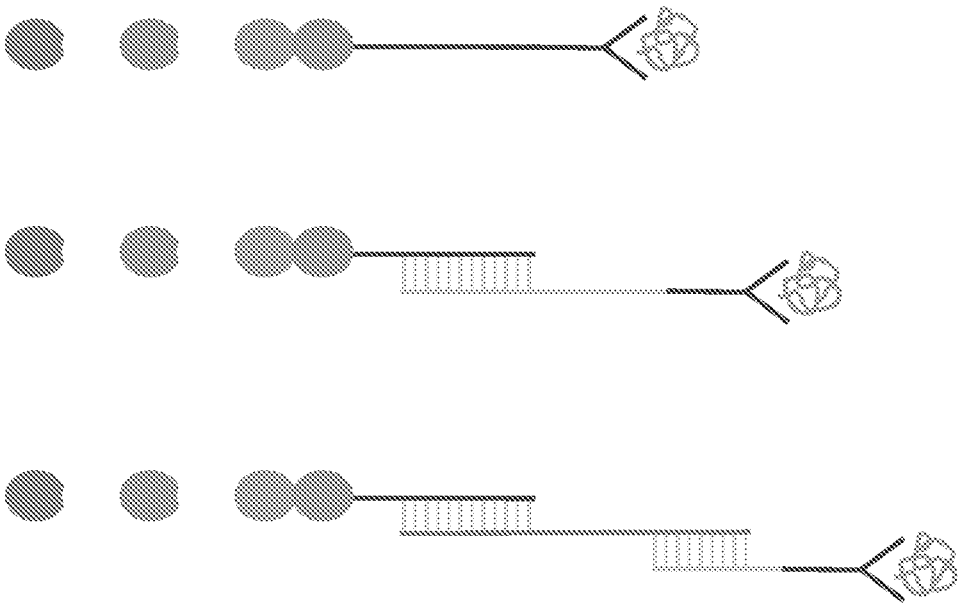


Figure 8

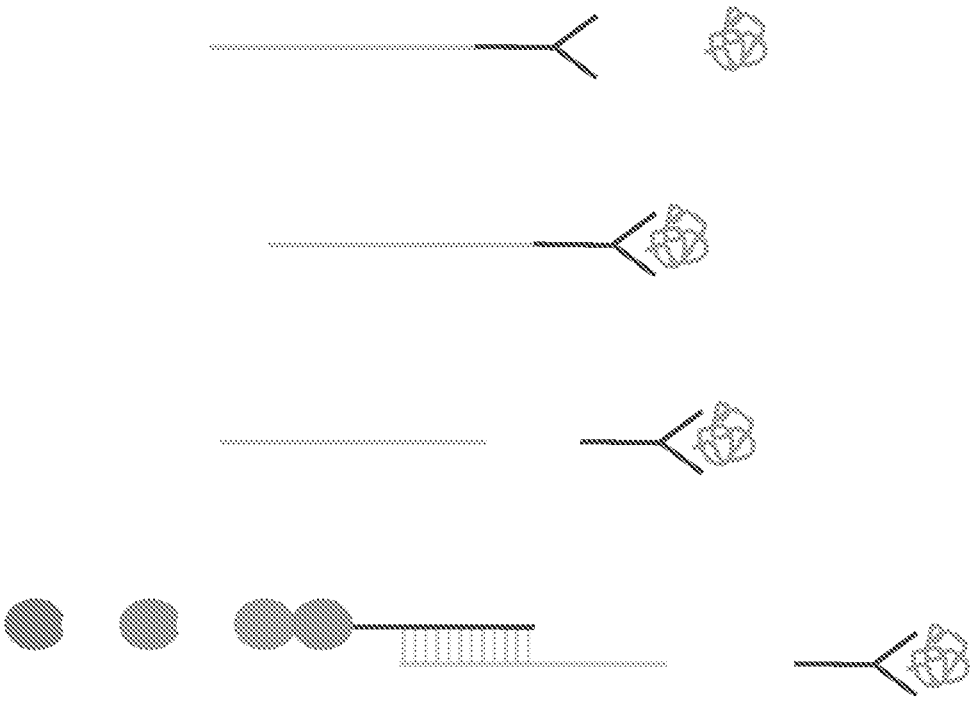


Figure 9

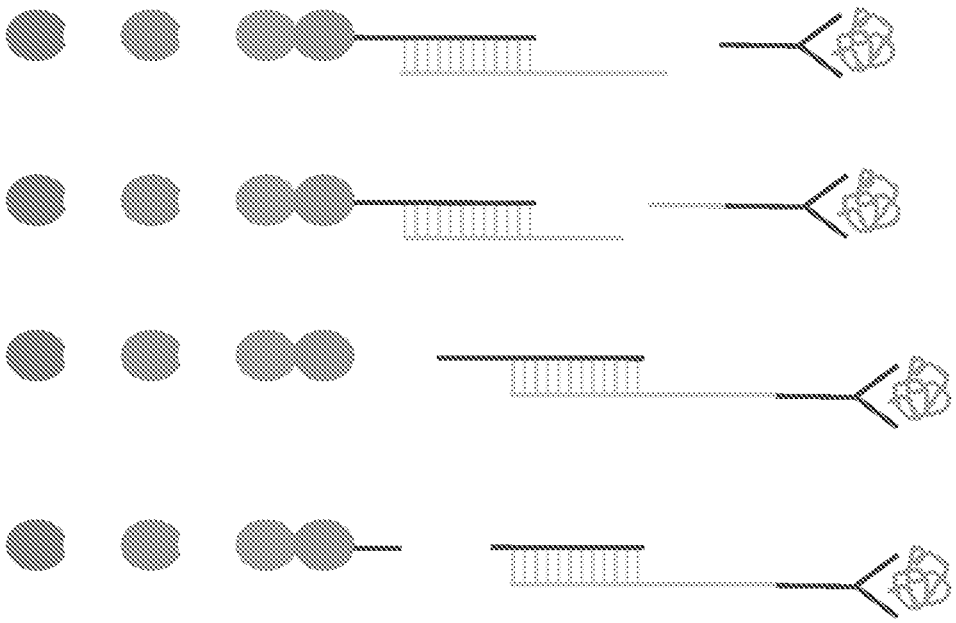


Figure 10

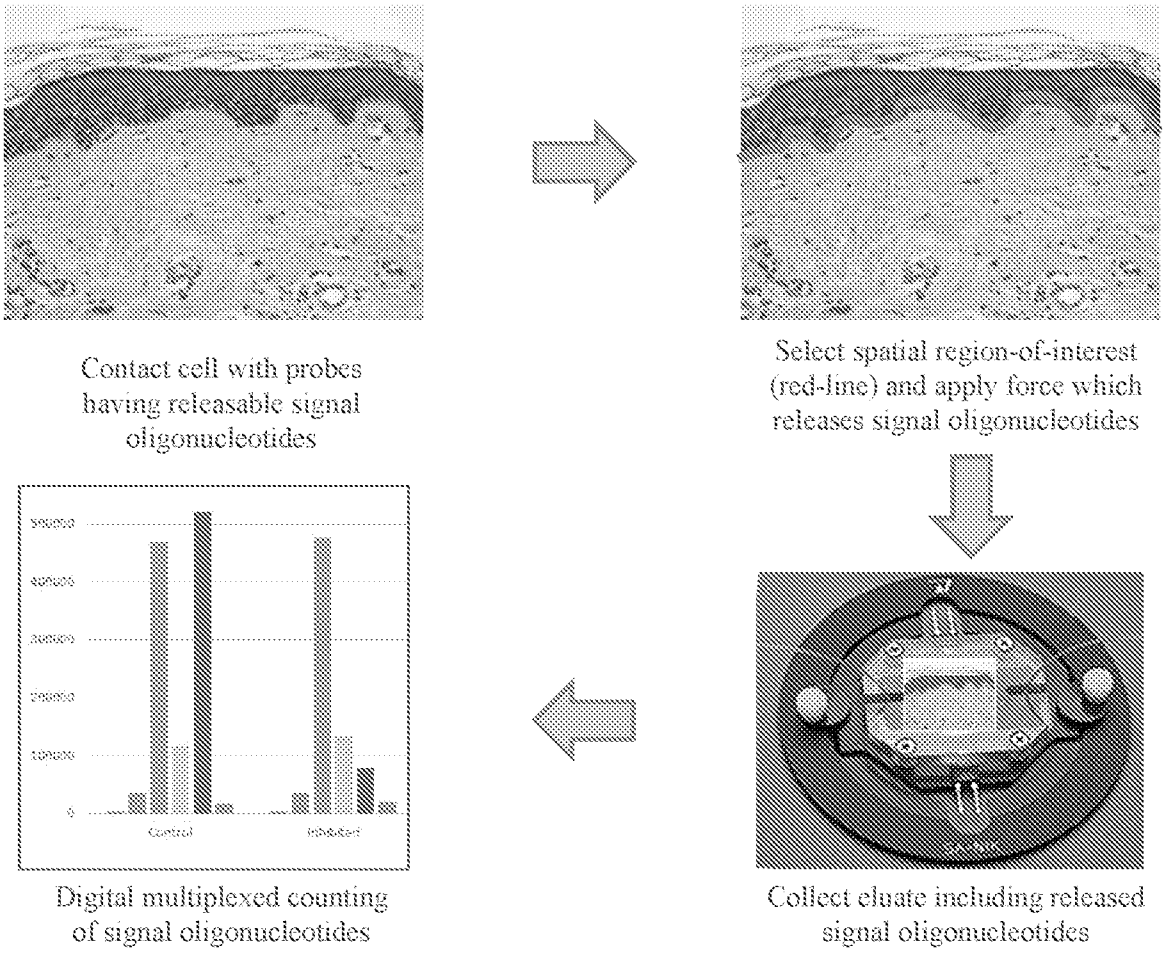

Contact cell with probes having releasable signal oligonucleotides
Select spatial region-of-interest (red-line) and apply force which releases signal oligonucleotides
Collect eluate including released signal oligonucleotides
Digital multiplexed counting of signal oligonucleotides

Figure 11

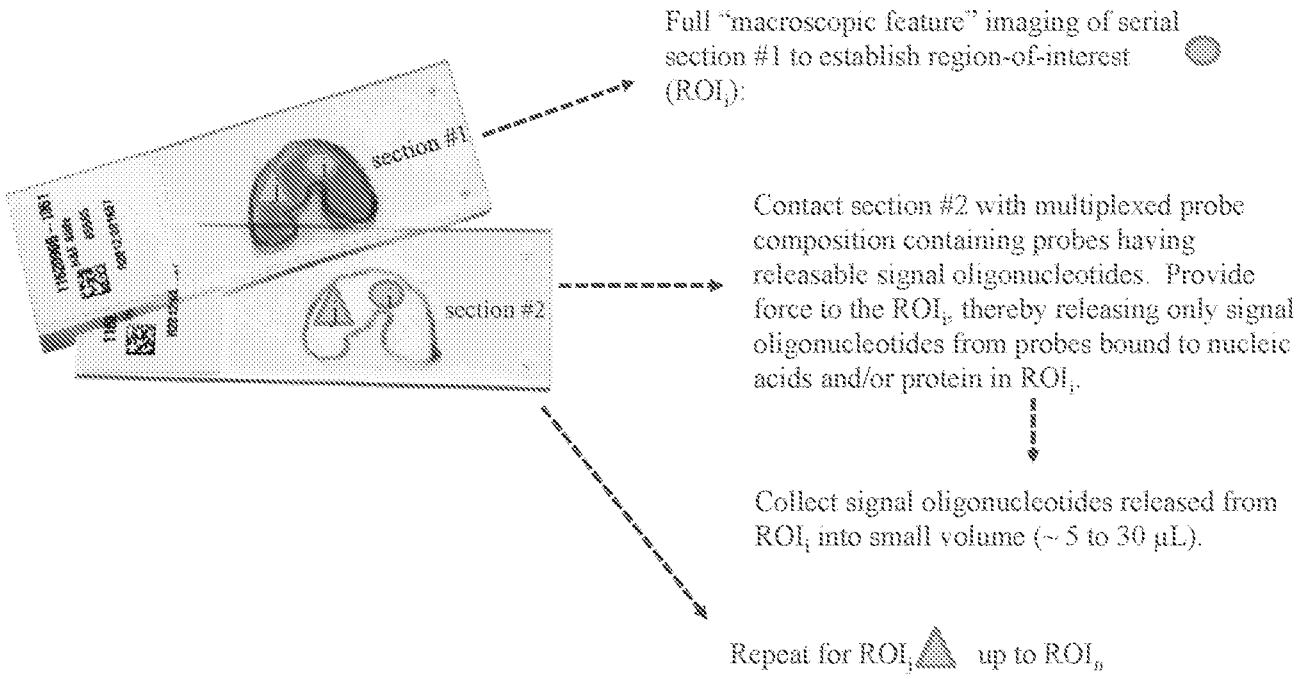
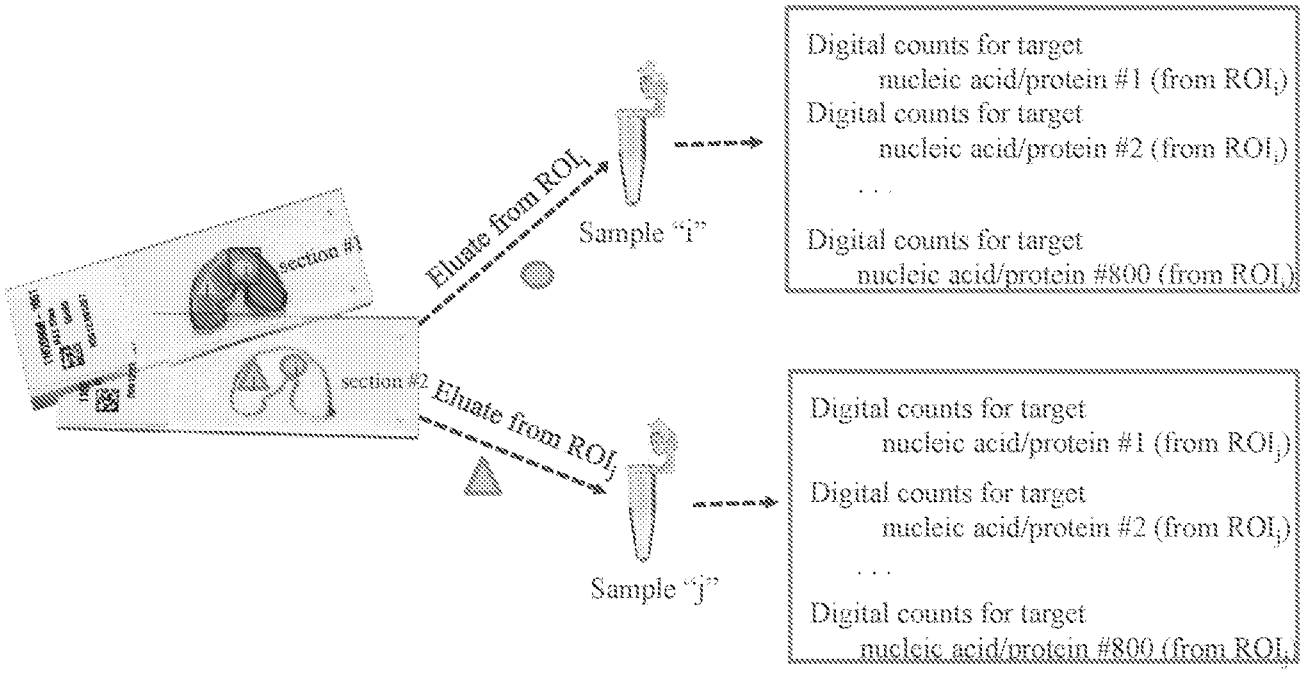

Full "macroscopic feature" imaging of serial section #1 to establish region-of-interest (ROI_1):
section #1
section #2
Contact section #2 with multiplexed probe composition containing probes having releasable signal oligonucleotides. Provide force to the ROI_1, thereby releasing only signal oligonucleotides from probes bound to nucleic acids and/or protein in ROI_1.
Collect signal oligonucleotides released from ROI_1 into small volume (~ 5 to 30 µL).
Repeat for ROI_1 up to ROI_n

Figure 12


section #1
section #2
Eluate from ROI$_i$
Eluate from ROI$_j$
Sample "i"
Sample "j"
Digital counts for target nucleic acid/protein #1 (from ROI$_i$)
Digital counts for target nucleic acid/protein #2 (from ROI$_i$)
. . .
Digital counts for target nucleic acid/protein #800 (from ROI$_i$)
Digital counts for target nucleic acid/protein #1 (from ROI$_j$)
Digital counts for target nucleic acid/protein #2 (from ROI$_j$)
. . .
Digital counts for target nucleic acid/protein #800 (from ROI$_j$)

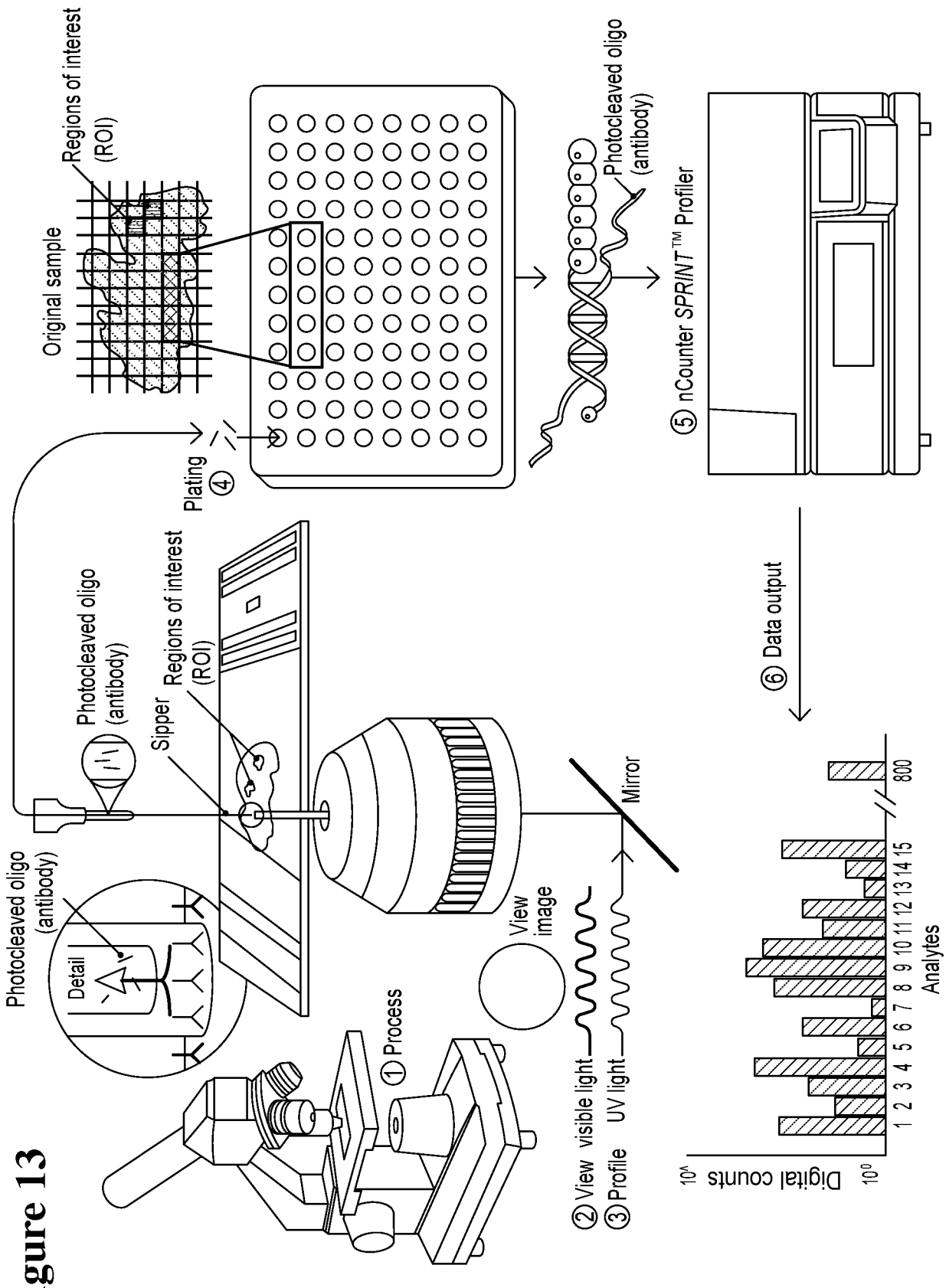

Original sample
Regions of interest (ROI)
Plating ④
Photocleaved oligo (antibody)
⑤ nCounter SPRINT™ Profiler
⑥ Data output
Digital counts
Analytes
Photocleaved oligo (antibody)
Sipper
Regions of interest (ROI)
Photocleaved oligo (antibody)
Detail
① Process
② View visible light
③ Profile UV light
View image
Mirror

Figure 13

Figure 14

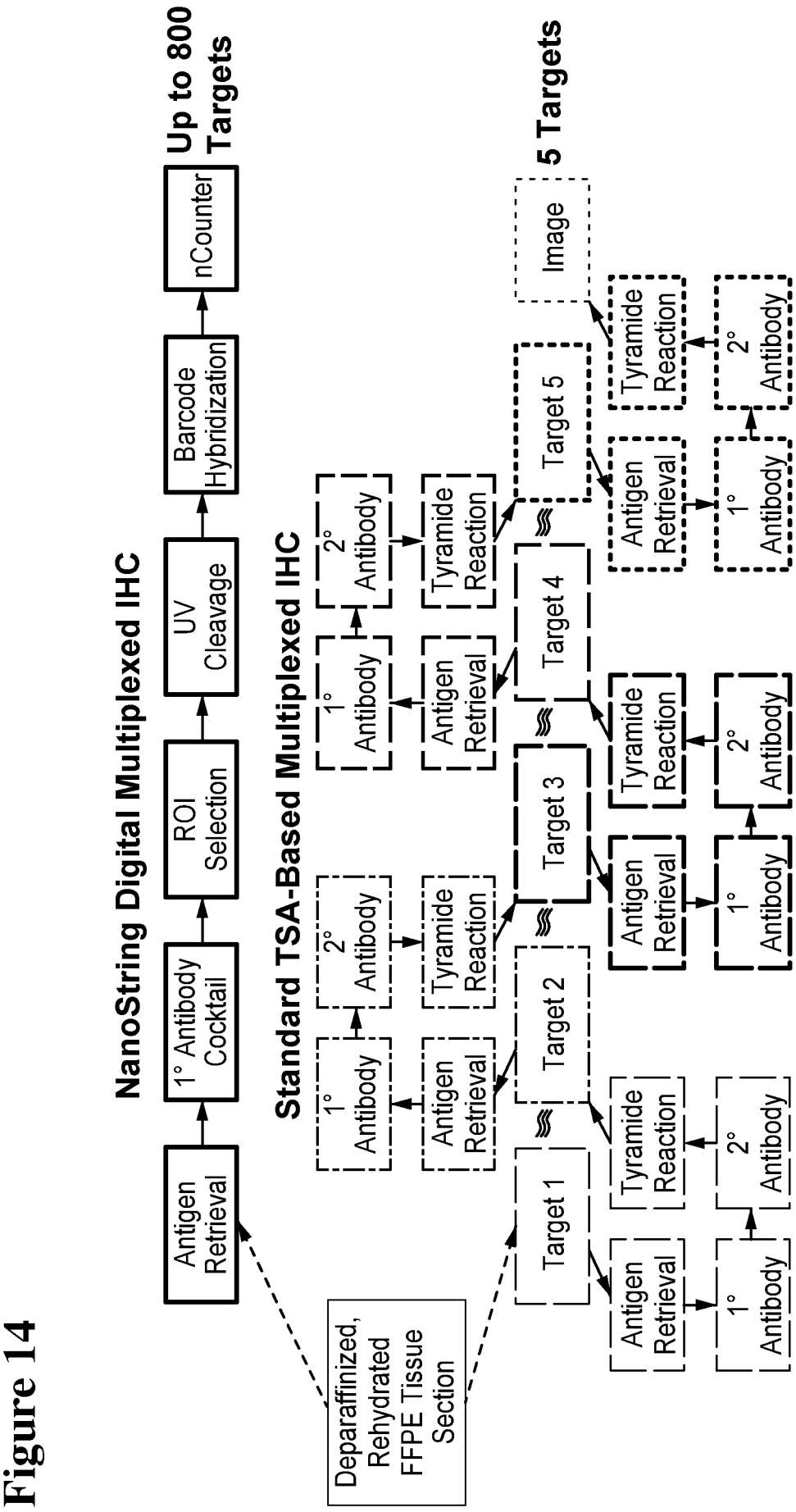

NanoString Digital Multiplexed IHC
Antigen Retrieval
1° Antibody Cocktail
ROI Selection
UV Cleavage
Barcode Hybridization
nCounter
Up to 800 Targets
Standard TSA-Based Multiplexed IHC
Deparaffinized, Rehydrated FFPE Tissue Section
Antigen Retrieval
1° Antibody
2° Antibody
Tyramide Reaction
Target 1
Target 2
Target 3
Target 4
Target 5
5 Targets
Image

Figure 15

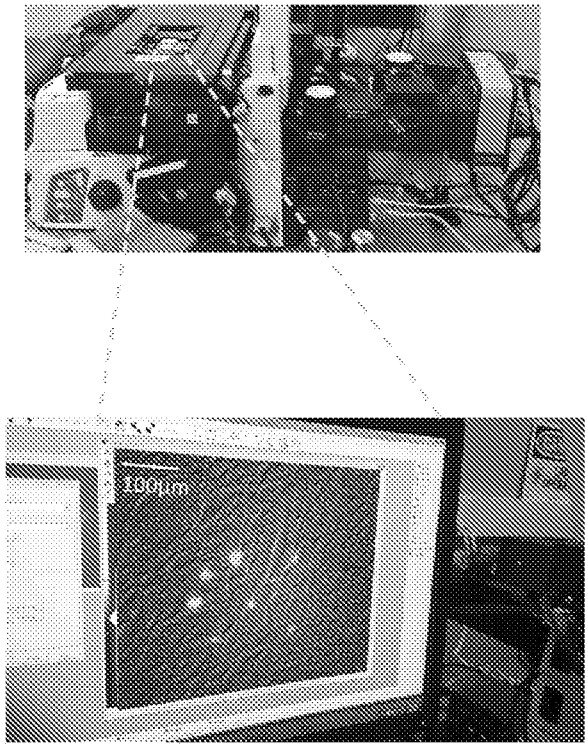
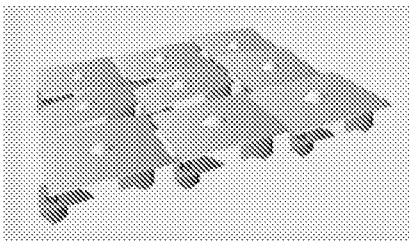


Figure 16



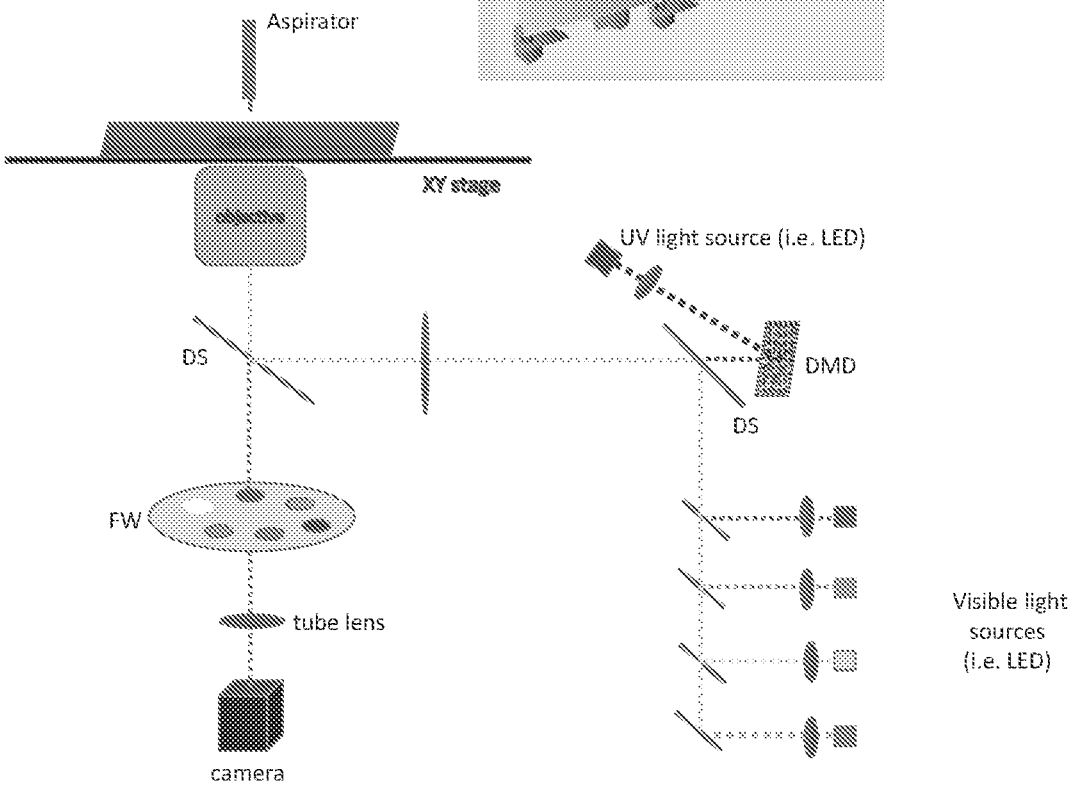

Aspirator
XY stage
UV light source (i.e. LED)
DS
DMD
DS
FW
tube lens
camera
Visible light sources (i.e. LED)

Figure 17

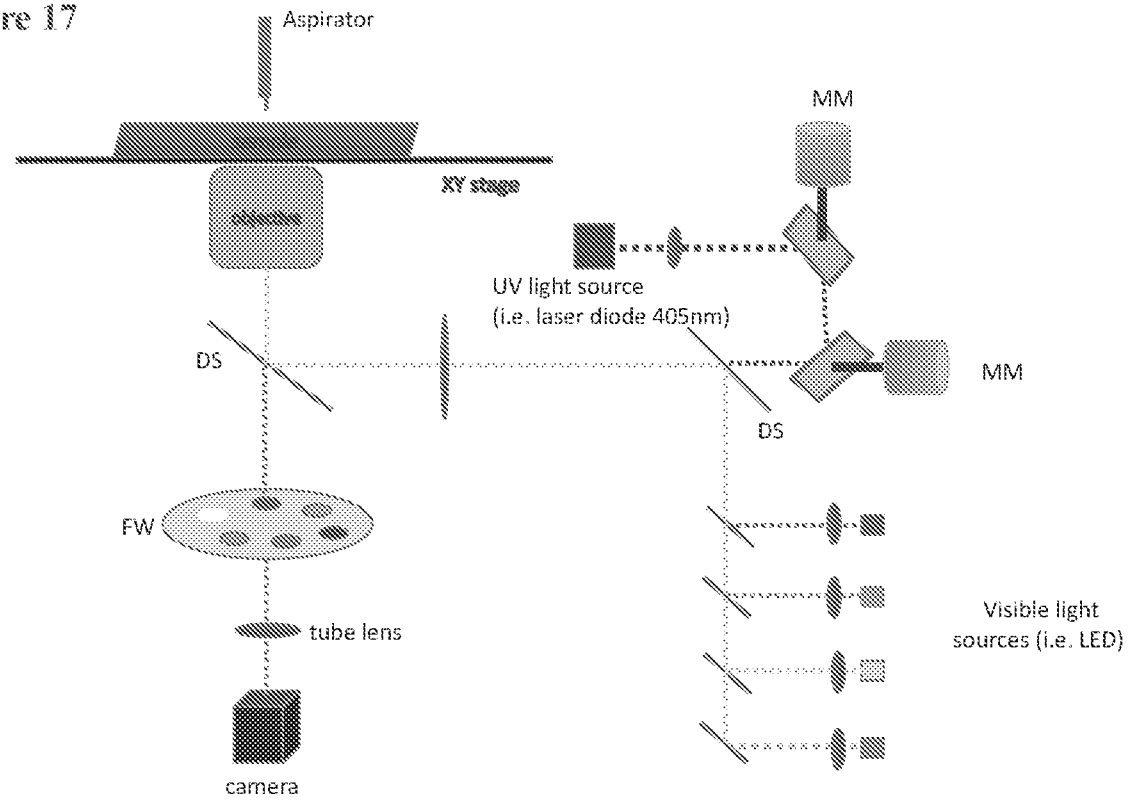

Aspirator
XY stage
Objective
MM
UV light source
(i.e. laser diode 405nm)
DS
MM
DS
FW
tube lens
camera
Visible light
sources (i.e. LED)

Figure 18

Figure 19

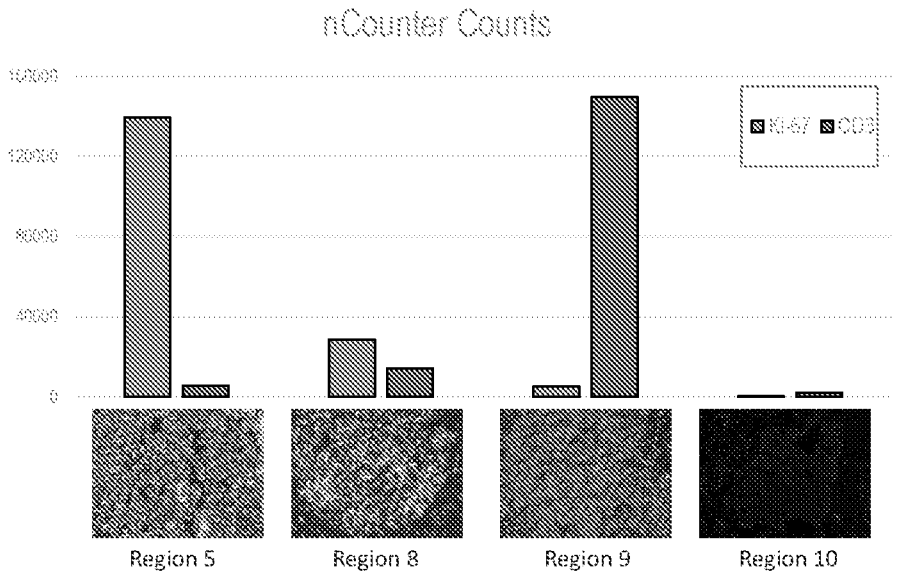

nCounter Counts
Ki-67
CD3
Region 5
Region 8
Region 9
Region 10

Figure 20

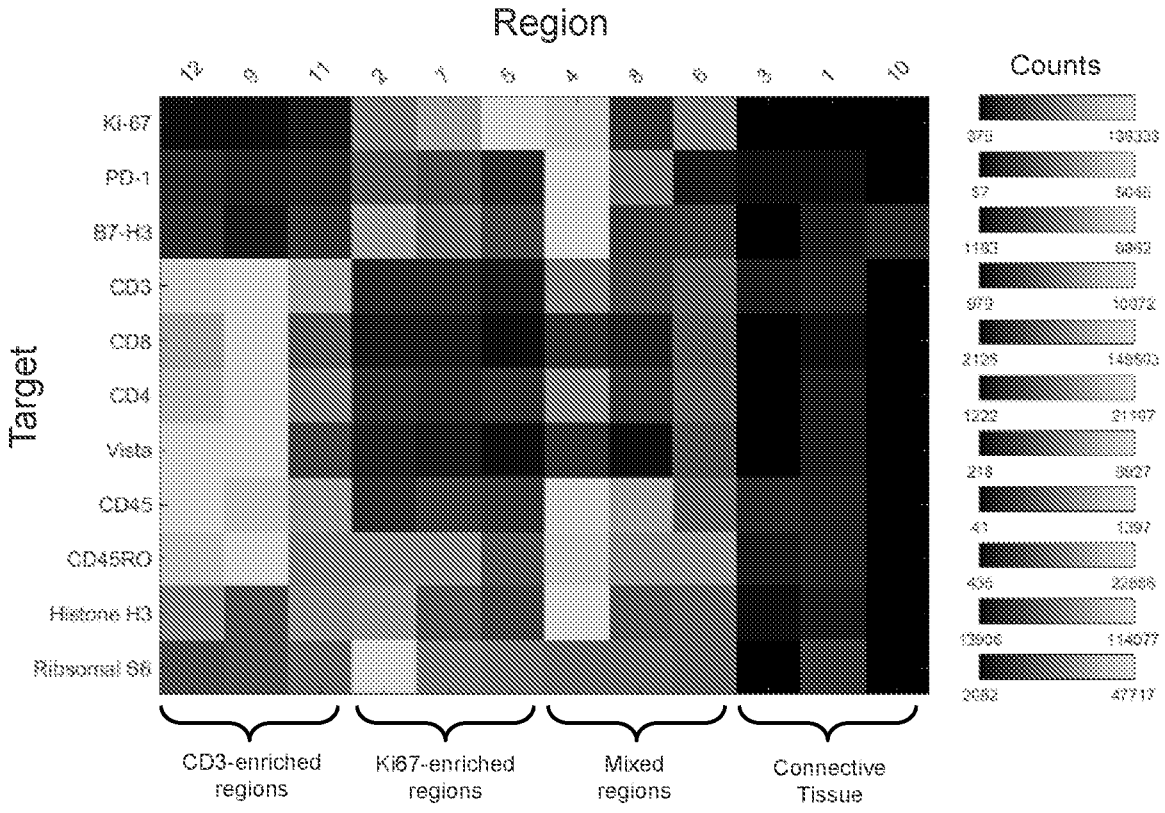

Region
Counts
Target
Ki-67
PD-1
B7-H3
CD3
CD8
CD4
Vista
CD45
CD45RO
Histone H3
Ribsomal S6
CD3-enriched regions
Ki67-enriched regions
Mixed regions
Connective Tissue

Figure 21

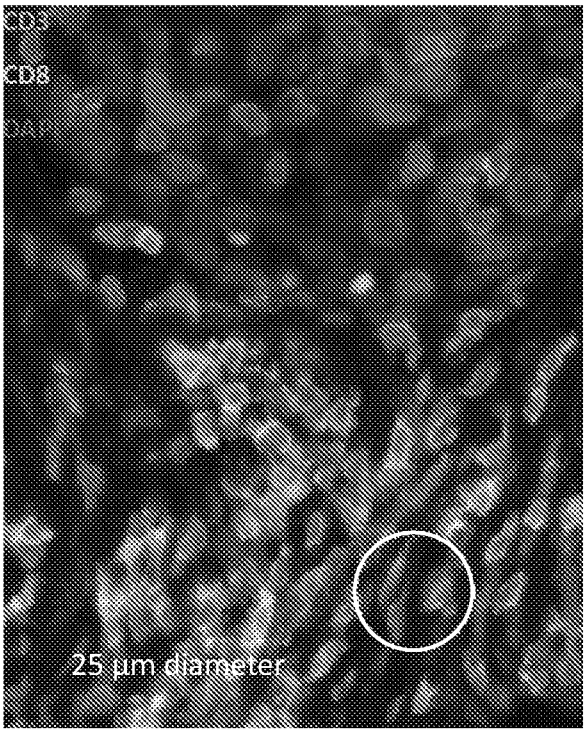


Figure 22

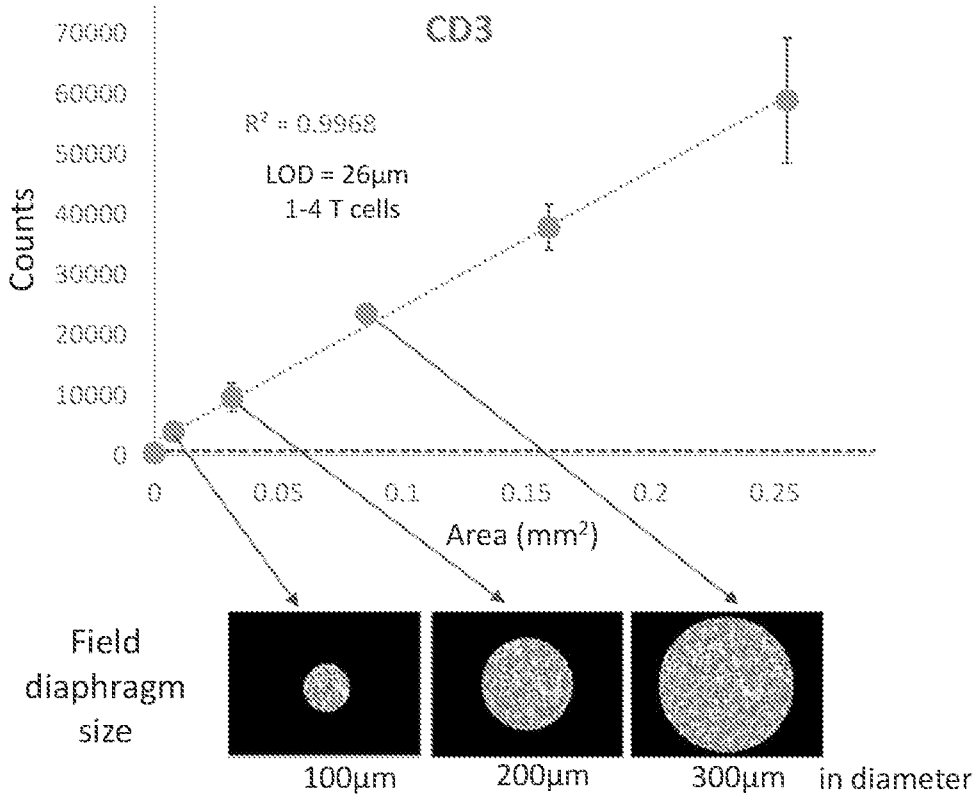

CD3
R^2 = 0.9968
LOD = 26µm
1-4 T cells
Counts
70000
60000
50000
40000
30000
20000
10000
0
0
0.05
0.1
0.15
0.2
0.25
Area (mm^2)
Field diaphragm size
100µm
200µm
300µm
in diameter

Figure 23

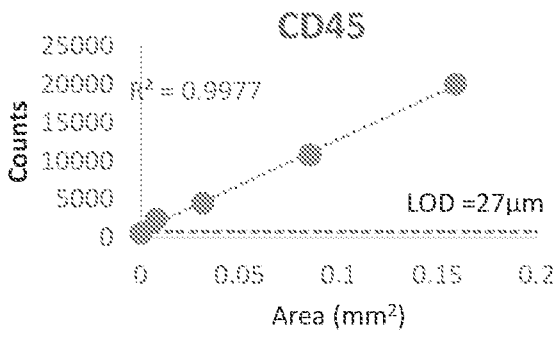


Figure 24

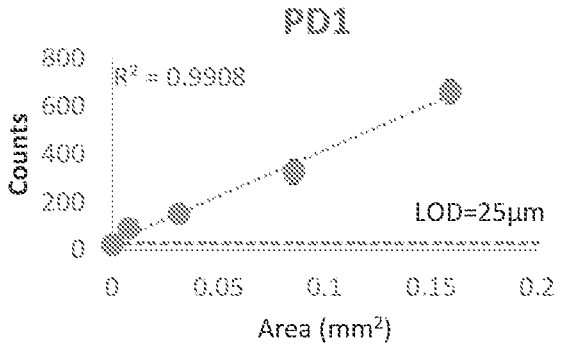


Figure 25

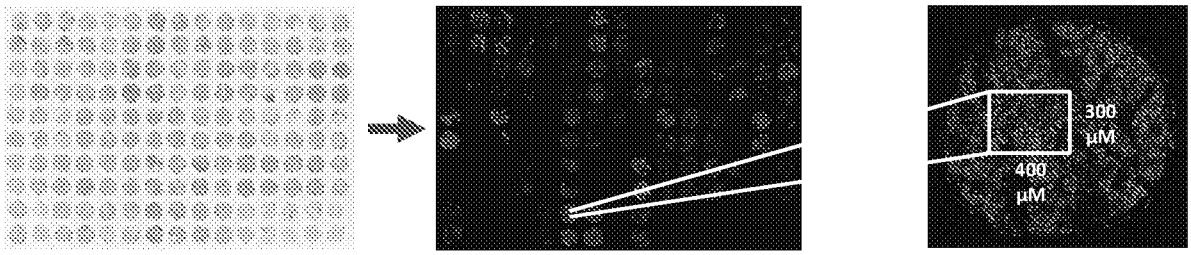


Figure 26

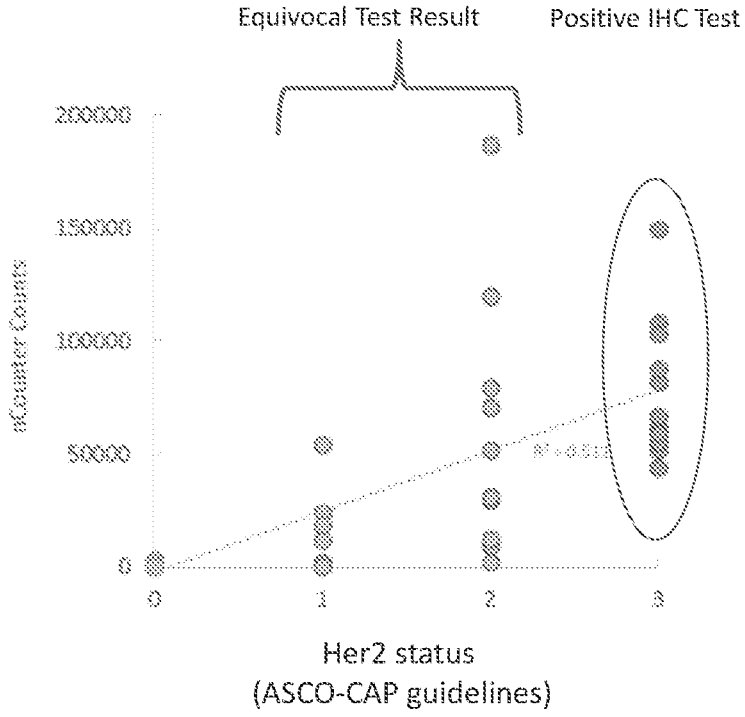

Equivocal Test Result
Positive IHC Test
200000
150000
100000
50000
0
Her2 status
(ASCO-CAP guidelines)

Figure 27

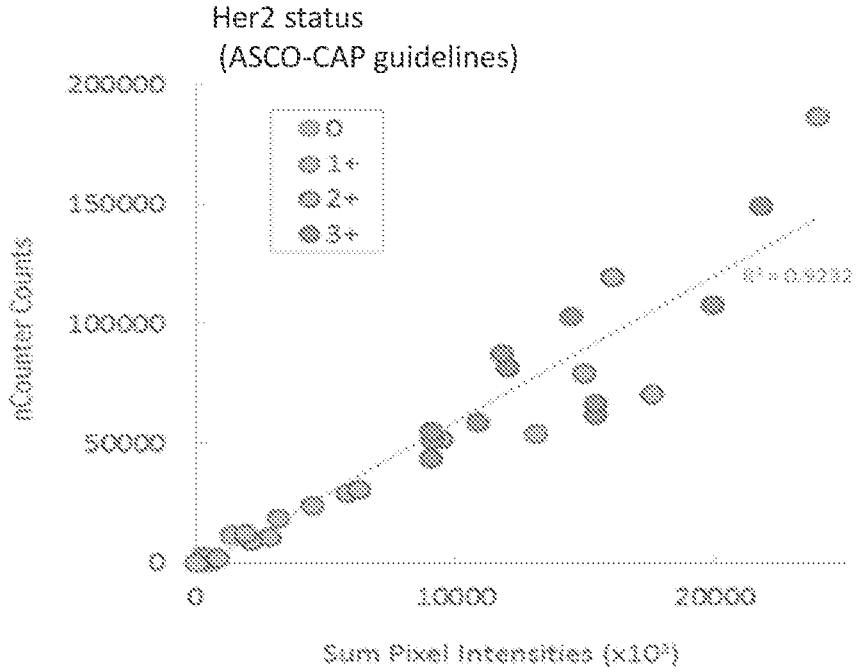

Her2 status
(ASCO-CAP guidelines)
0
1+
2+
3+
nCounter Counts
200000
150000
100000
50000
0
0
10000
20000
Sum Pixel Intensities (x10³)

Figure 28

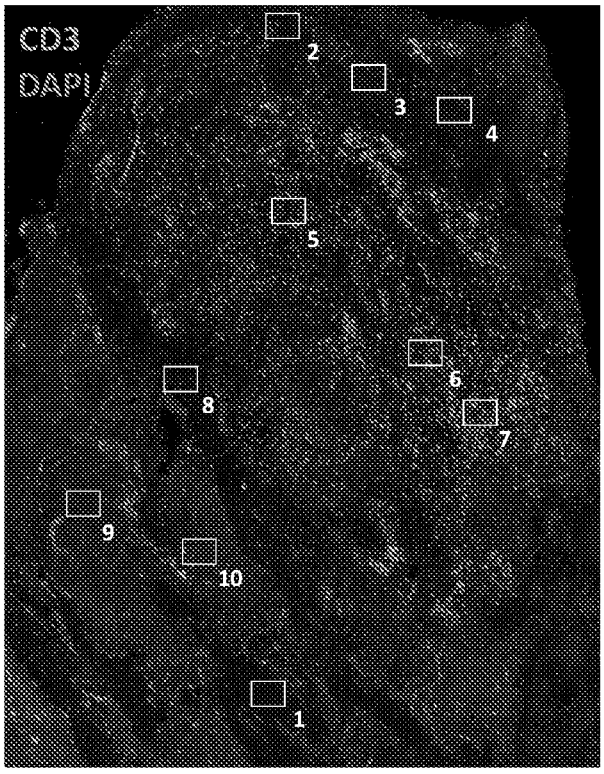


Figure 29

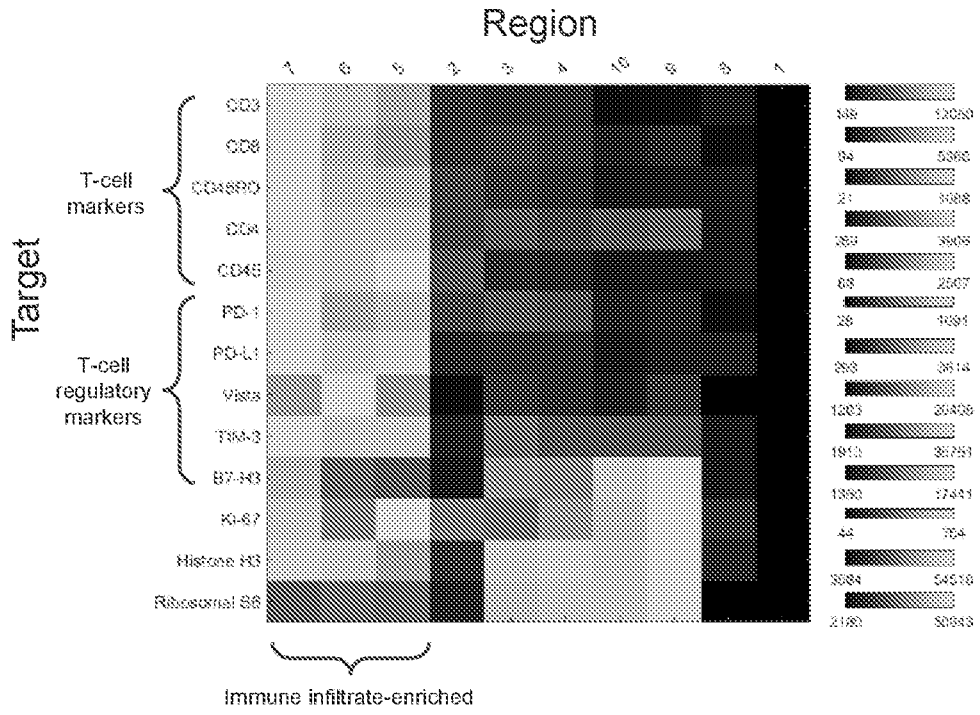

Region
Target
T-cell markers
T-cell regulatory markers
CD3
CD8
CD45RO
CD4
CD45
PD-1
PD-L1
Vista
TIM-3
B7-H3
KI-67
Histone H3
Ribosomal S6
Immune infiltrate-enriched

Figure 30A

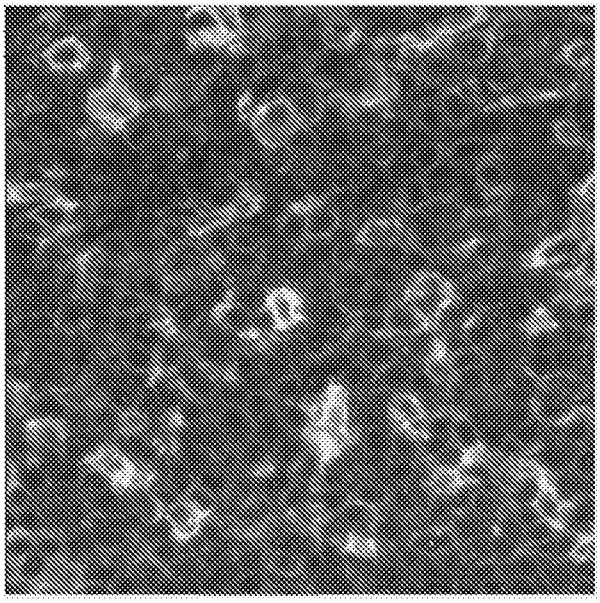


Figure 30B

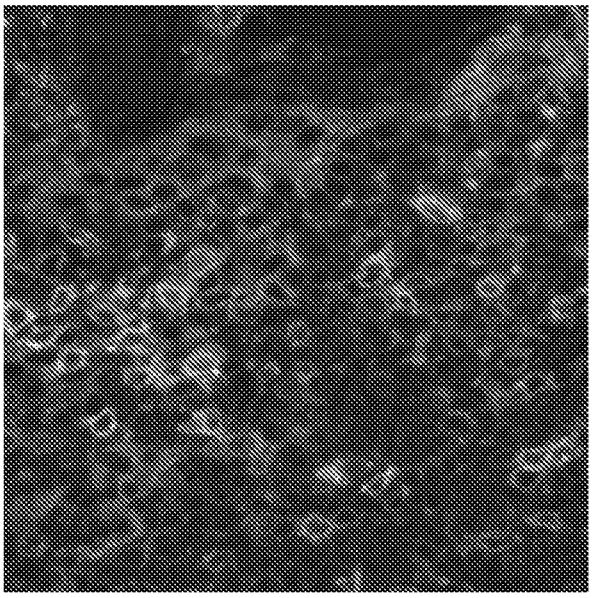


Figure 31B

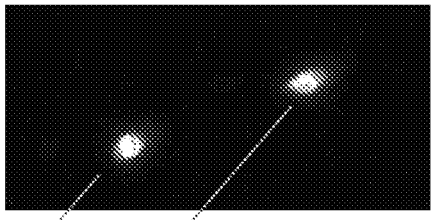
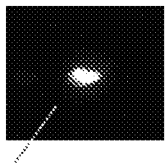
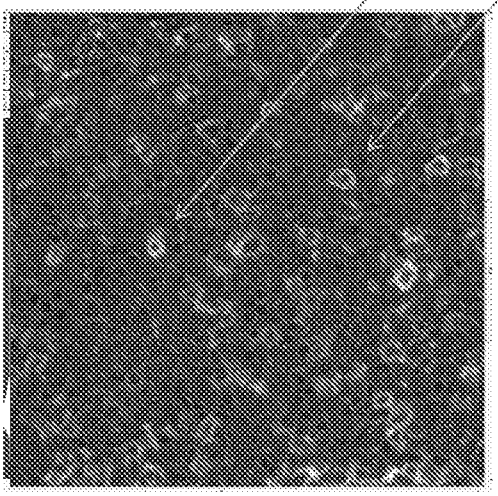


Figure 31D





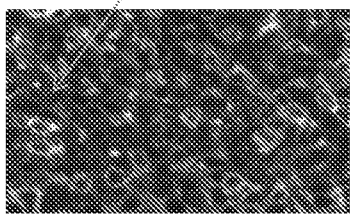


Figure 31C

Figure 31A

Figure 32

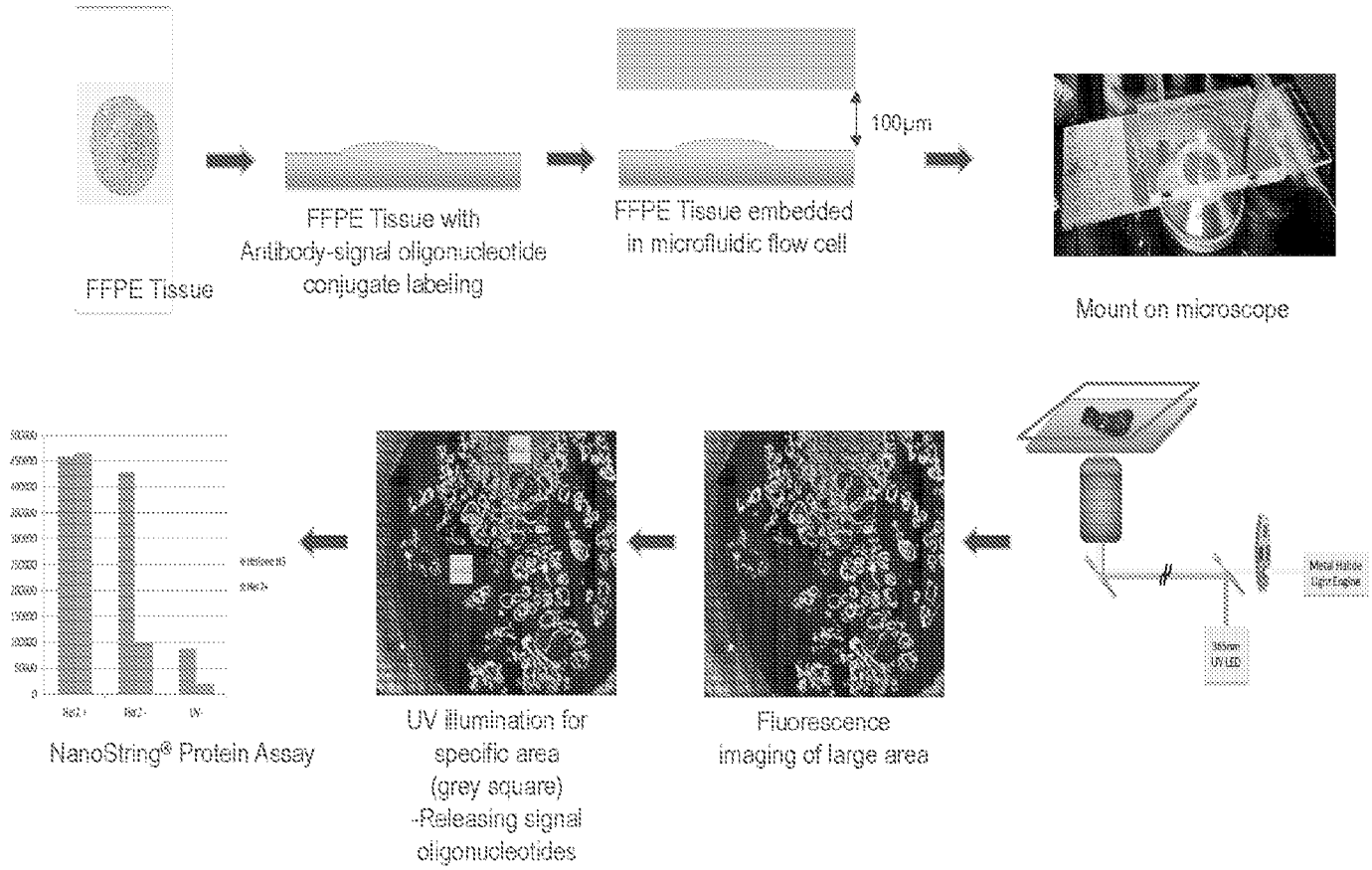

FFPE Tissue
FFPE Tissue with Antibody-signal oligonucleotide conjugate labeling
100µm
FFPE Tissue embedded in microfluidic flow cell
Mount on microscope
Metal Halide Light Source
365nm UV LED
Fluorescence imaging of large area
UV illumination for specific area (grey square) -Releasing signal oligonucleotides
NanoString® Protein Assay

Figure 33

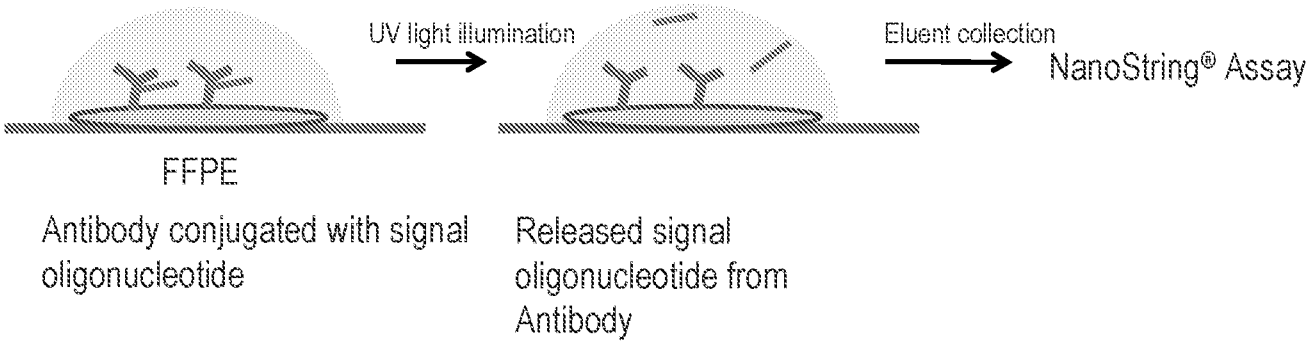


Figure 34

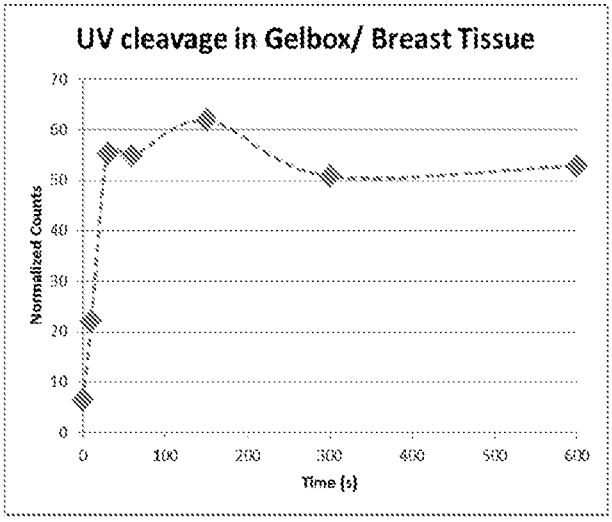
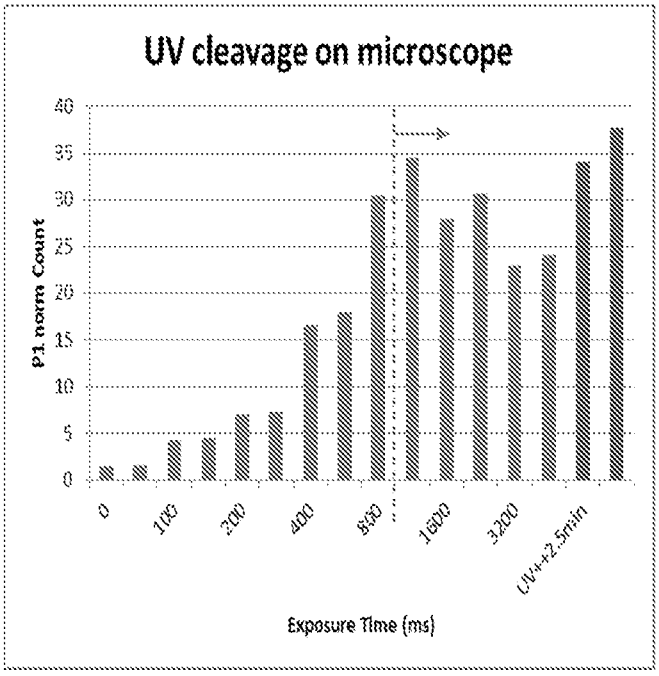
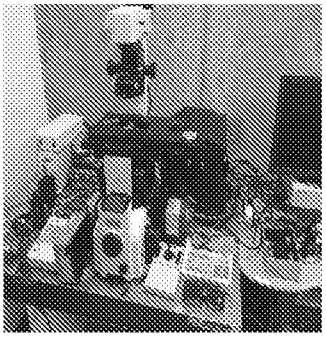
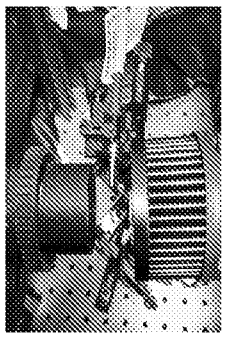


Figure 35


UV cleavage on microscope
P1 norm Count
40
35
30
25
20
15
10
5
0
0
100
200
400
800
1600
3200
UV+2.5min
Exposure Time (ms)





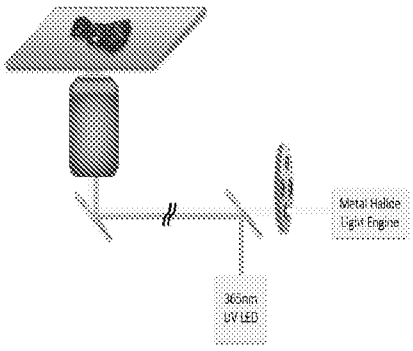

Metal Halide
Light Engine
365nm
UV LED

Figure 36

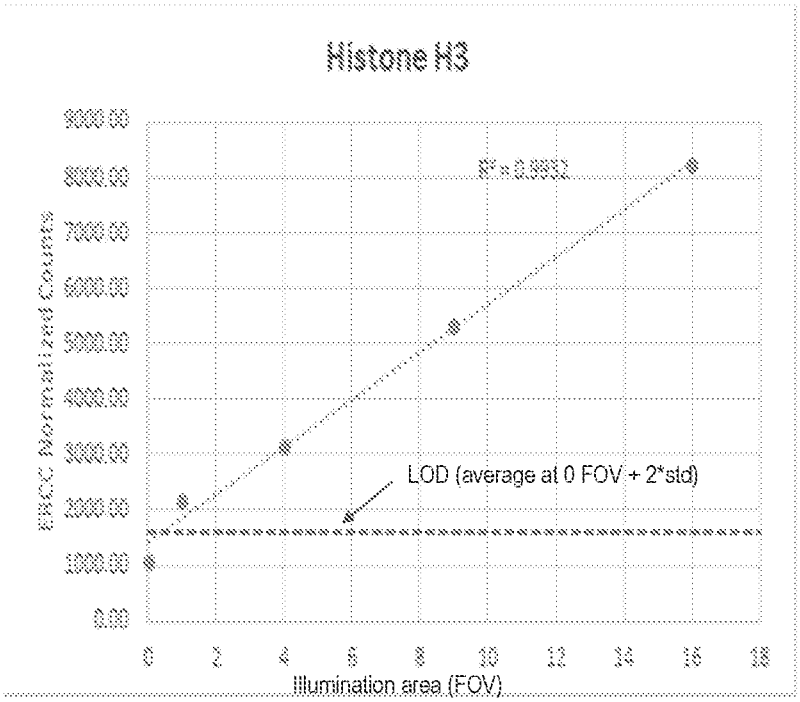

Histone H3
9000.00
8000.00
7000.00
6000.00
5000.00
4000.00
3000.00
2000.00
1000.00
0.00
ERCC Normalized Counts
R² = 0.9952
LOD (average at 0 FOV + 2*std)
0
2
4
6
8
10
12
14
16
18
Illumination area (FOV)

Figure 37

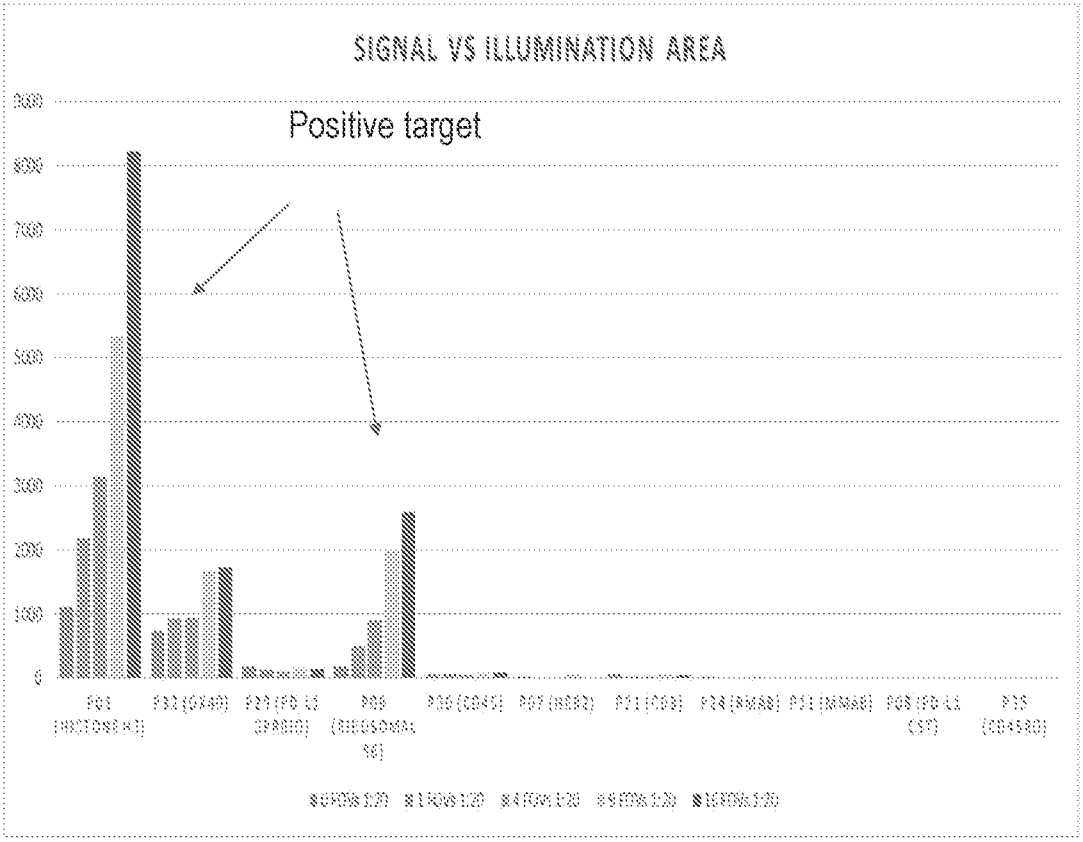
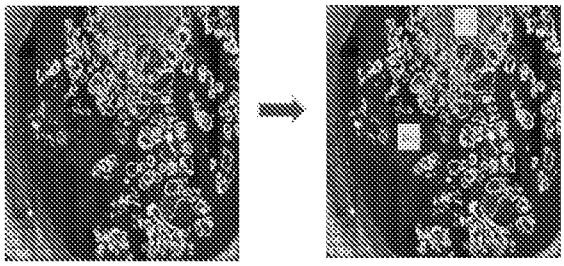

SIGNAL VS ILLUMINATION AREA
Positive target
9000
8000
7000
6000
5000
4000
3000
2000
1000
0

Figure 38



Fluorescence imaging of large area

UV illumination for specific area (grey square)

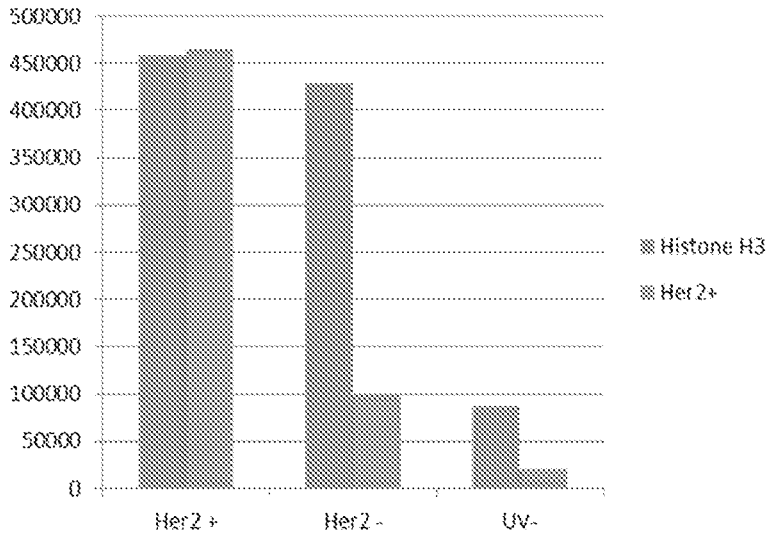
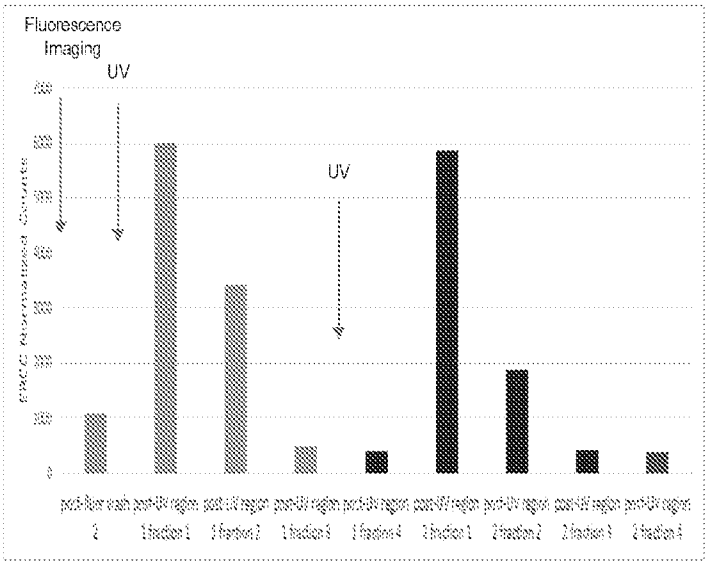
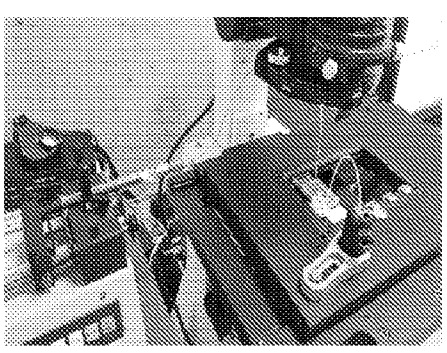
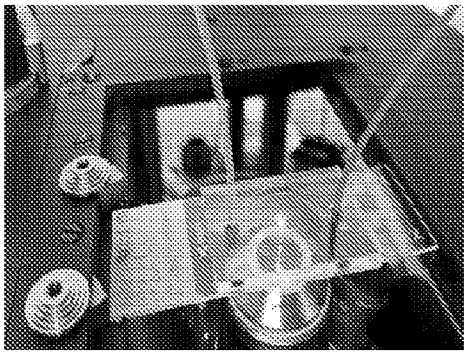


Figure 39


Fluorescence Imaging
UV
UV





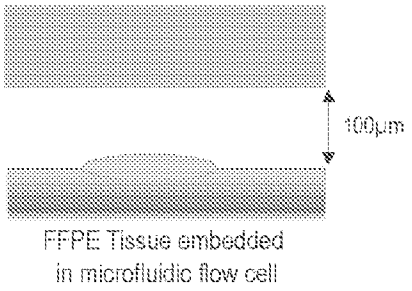

100µm
FFPE Tissue embedded
in microfluidic flow cell

Figure 40

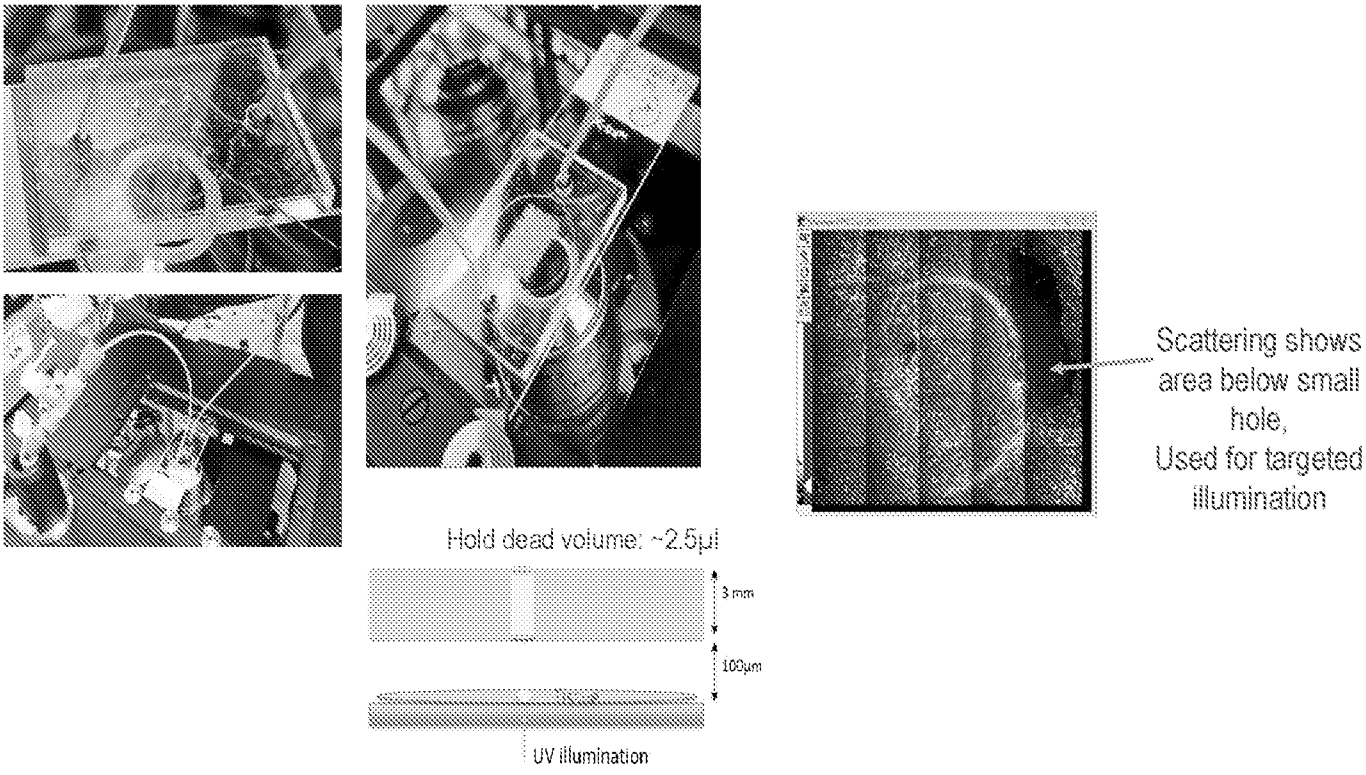

Scattering shows area below small hole,
Used for targeted illumination
Hold dead volume: ~2.5µl
3 mm
100µm
UV illumination

Figure 41A

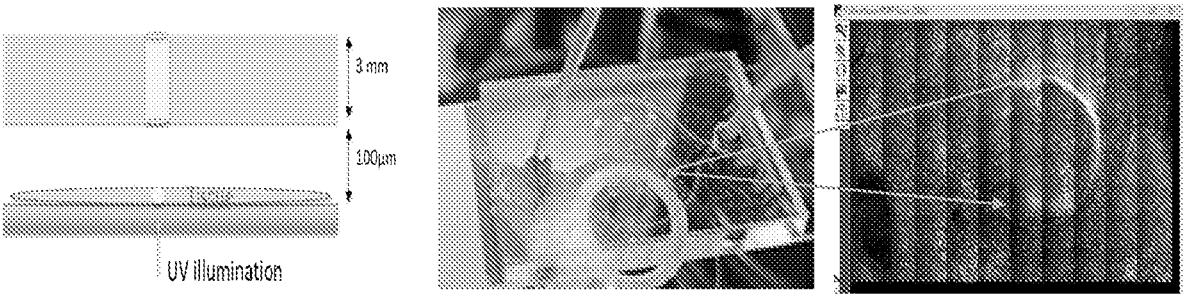


Figure 41B

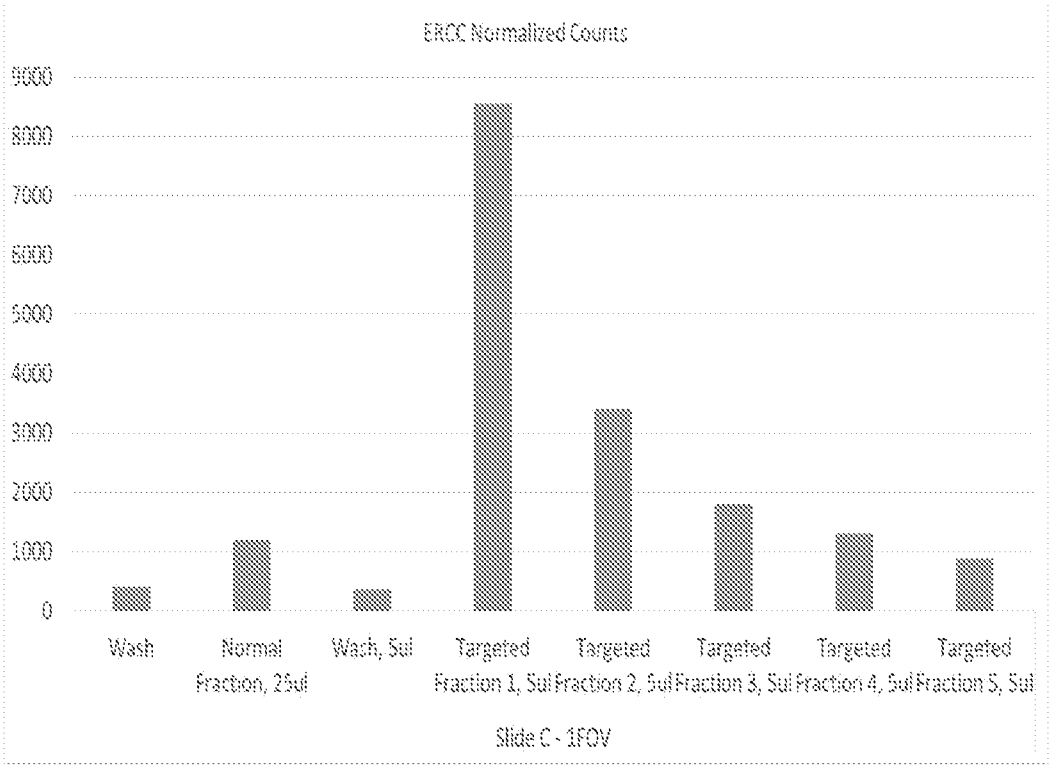

ERCC Normalized Counts
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Wash
Normal Fraction, 25ul
Wash, 5ul
Targeted Fraction 1, 5ul
Targeted Fraction 2, 5ul
Targeted Fraction 3, 5ul
Targeted Fraction 4, 5ul
Targeted Fraction 5, 5ul
Slide C - 1FOV

Figure 41C

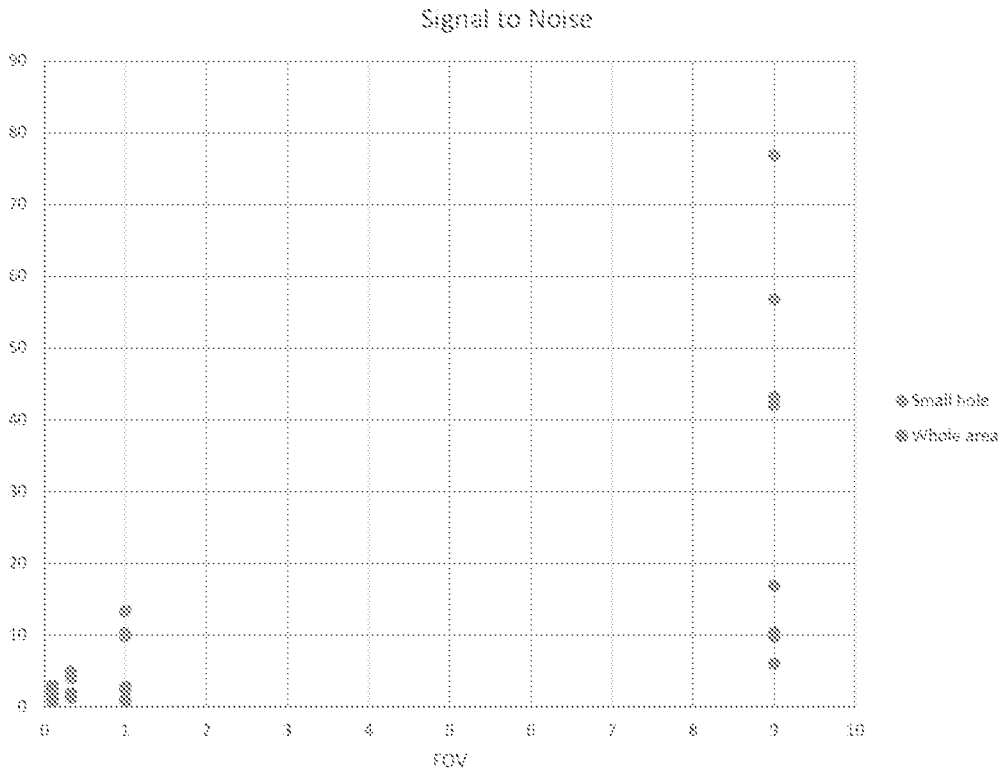

Signal to Noise
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
8
9
10
FOV
Small hole
Whole area

Figure 42A

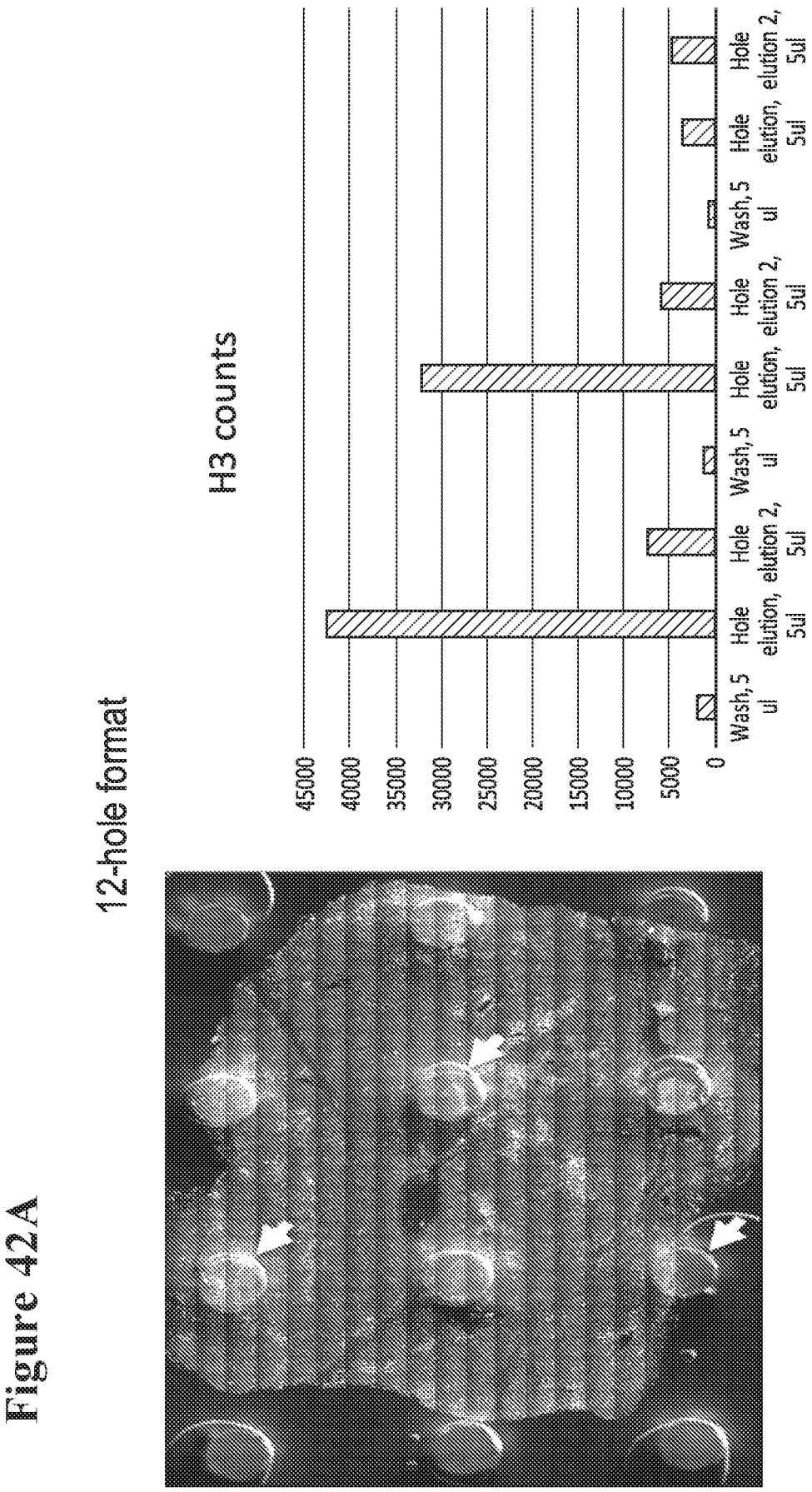
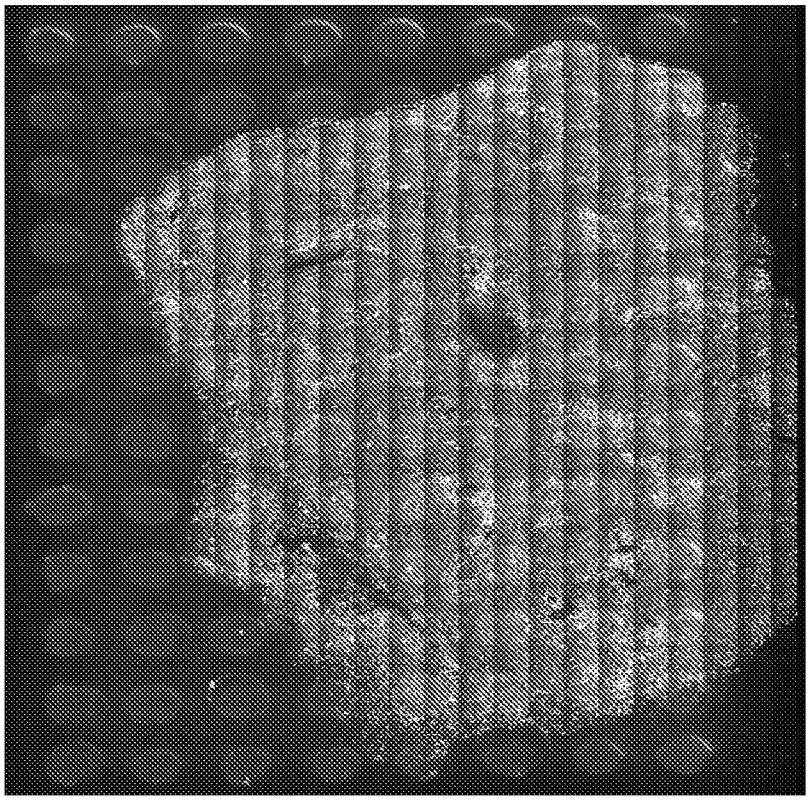
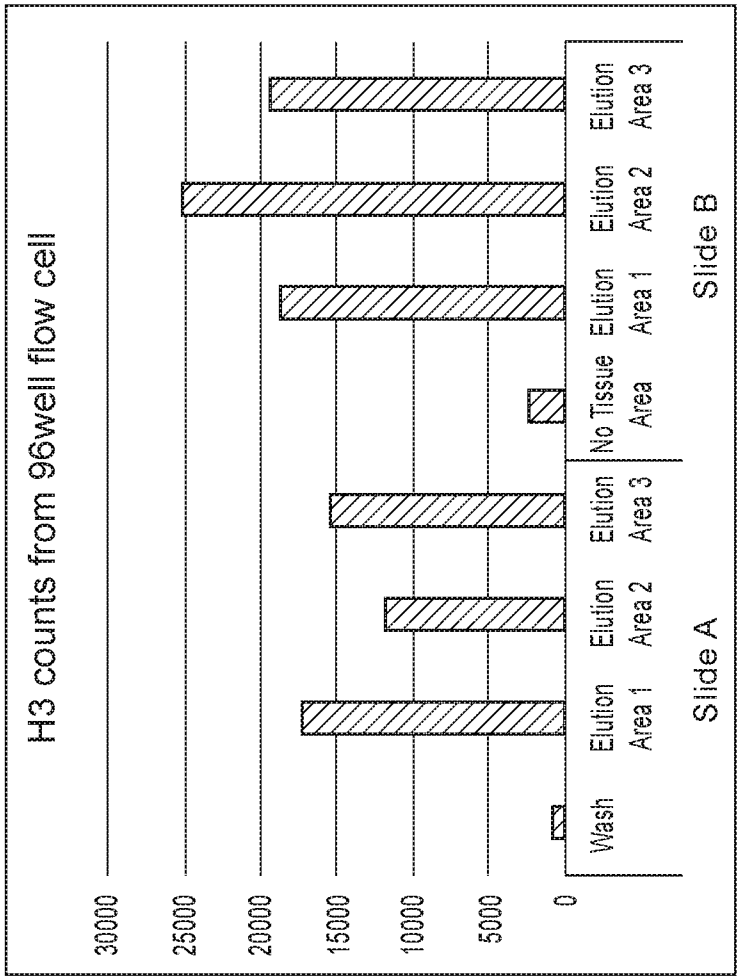

12-hole format
H3 counts
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
Wash, 5 ul
Hole elution, elution 2, 5ul 5ul
Hole Wash, 5 ul
Hole elution, elution 2, 5ul 5ul
Hole Wash, 5 ul
Hole elution, elution 2, 5ul 5ul

Figure 42B

96-hole format





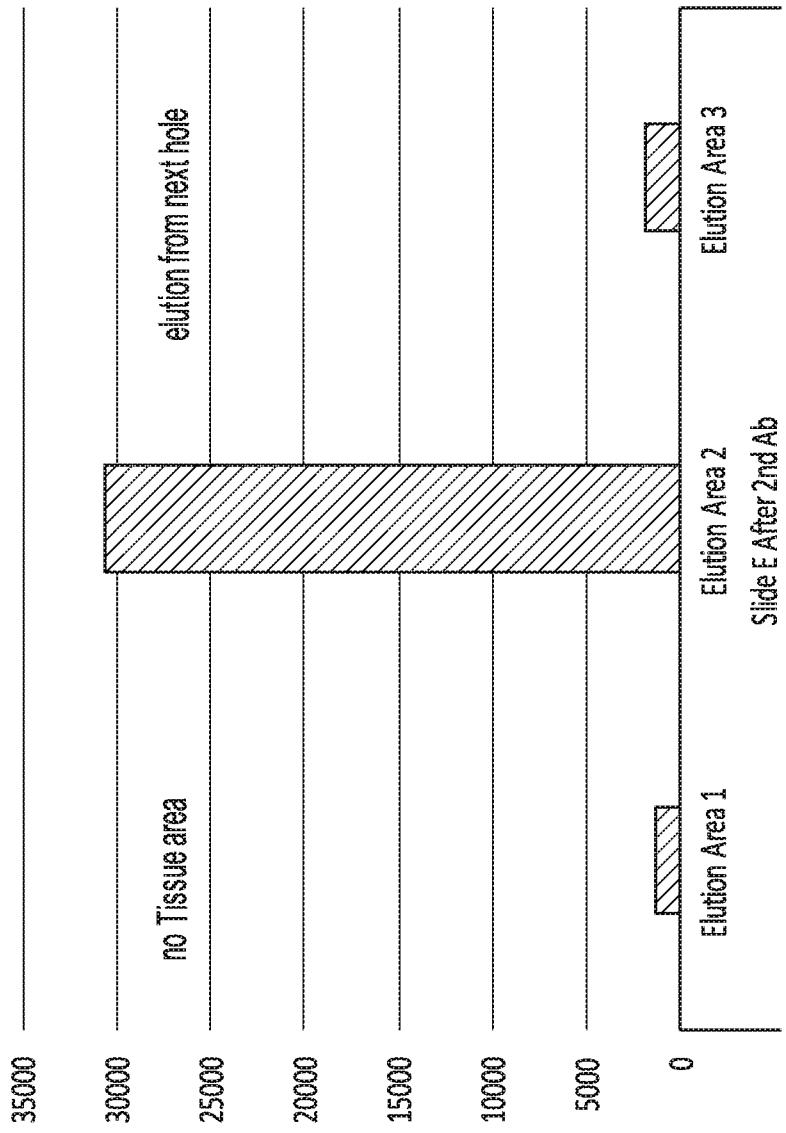

H3 counts
35000
30000
25000
20000
15000
10000
5000
0
no Tissue area
elution from next hole
Elution Area 1
Elution Area 2
Slide E After 2nd Ab
Elution Area 3

Figure 42C

Figure 43A

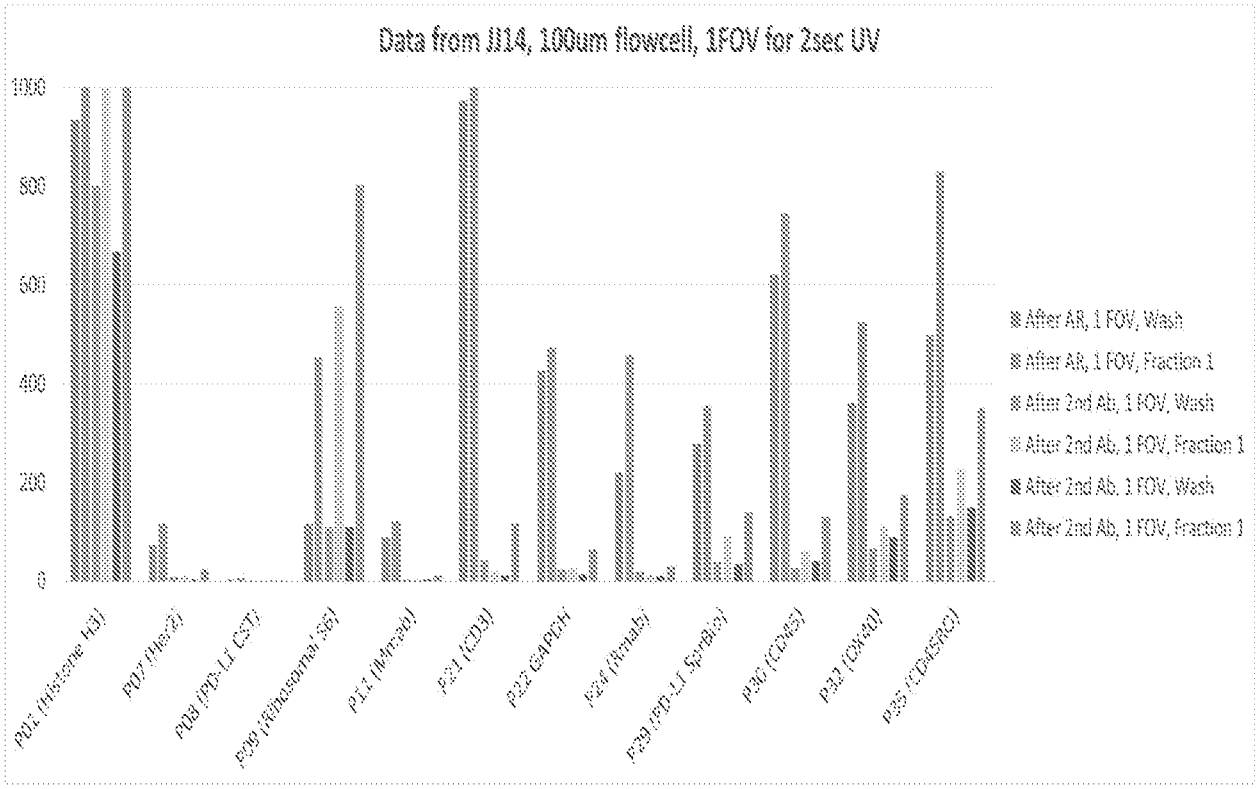

Data from JJ14, 100um flowcell, 1FOV for 2sec UV
1000
800
600
400
200
0
P01 (Histone H3)
P07 (Her2)
P08 (PD-L1 CST)
P09 (Ribosomal S6)
P11 (Mmab)
P21 (CD3)
P22 GAPDH
P24 (Rmab)
P29 (PD-L1 SprBio)
P30 (CD45)
P32 (CK40)
P35 (CD45RO)
After AR, 1 FOV, Wash
After AR, 1 FOV, Fraction 1
After 2nd Ab, 1 FOV, Wash
After 2nd Ab, 1 FOV, Fraction 1
After 2nd Ab, 1 FOV, Wash
After 2nd Ab, 1 FOV, Fraction 1

Figure 43B

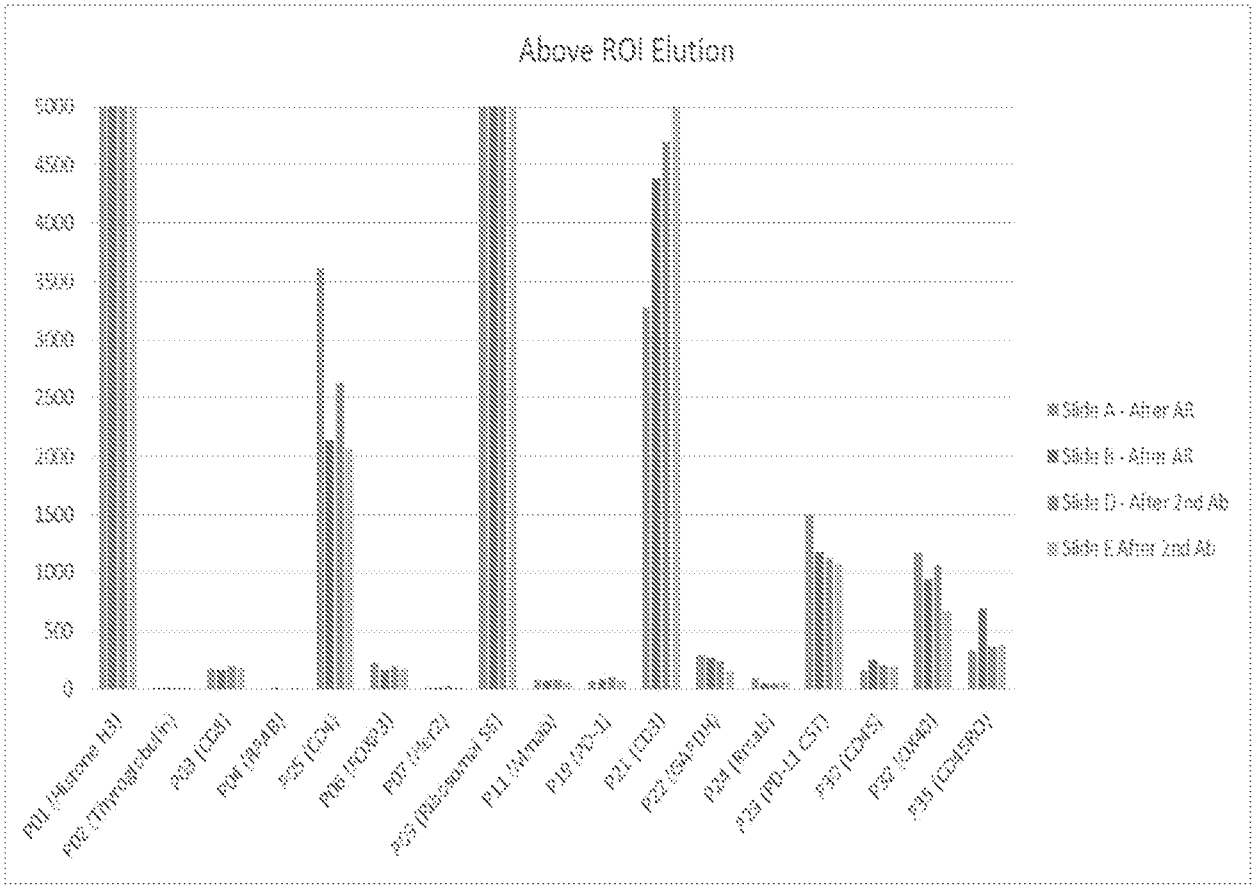

Above ROI Elution
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Slide A - After AR
Slide B - After AR
Slide D - After 2nd Ab
Slide E After 2nd Ab

Figure 44

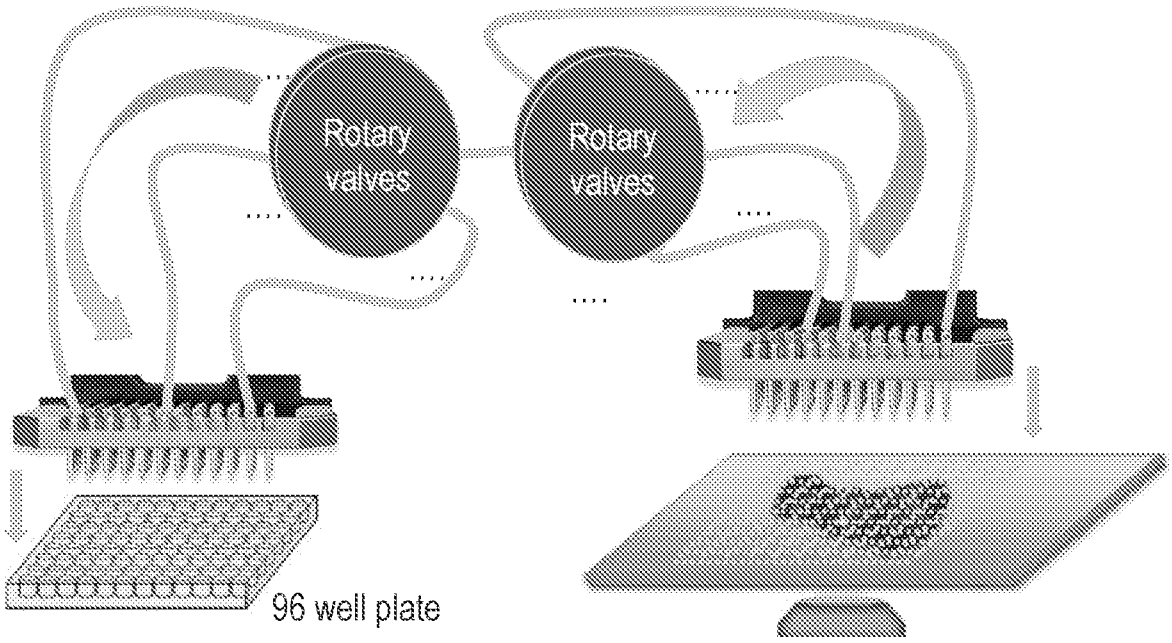


Figure 45

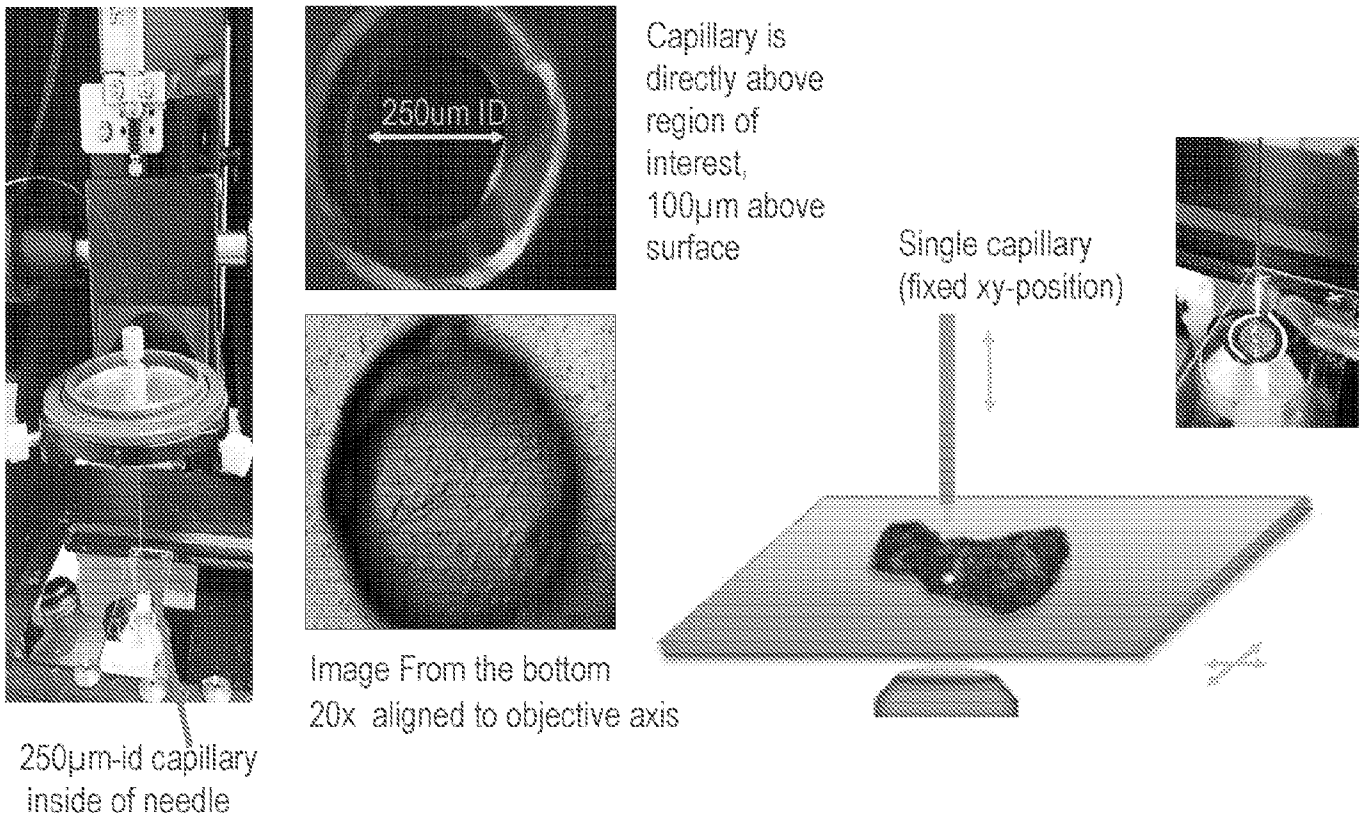

250µm ID
Capillary is directly above region of interest, 100µm above surface
Single capillary (fixed xy-position)
Image From the bottom 20x aligned to objective axis
250µm-id capillary inside of needle

Figure 46A

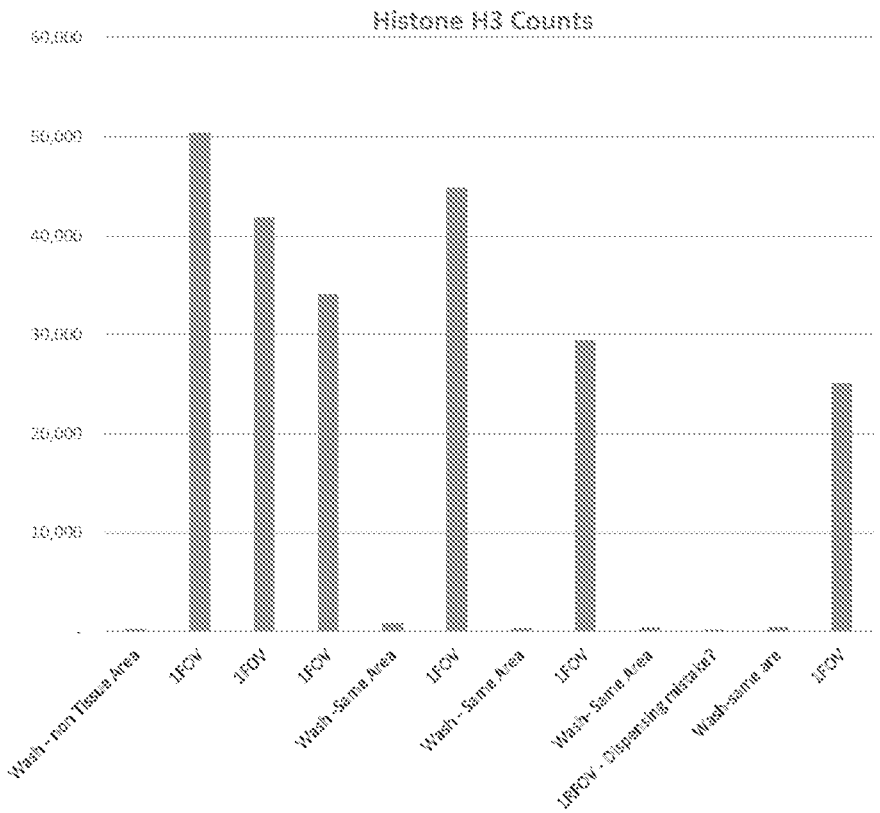

Histone H3 Counts
60,000
50,000
40,000
30,000
20,000
10,000
-
Wash - non Tissue Area
1FOV
1FOV
1FOV
Wash -Same Area
1FOV
Wash - Same Area
1FOV
Wash- Same Area
1FOV - Dispersing mixture?
Wash-same are
1FOV

Figure 46B

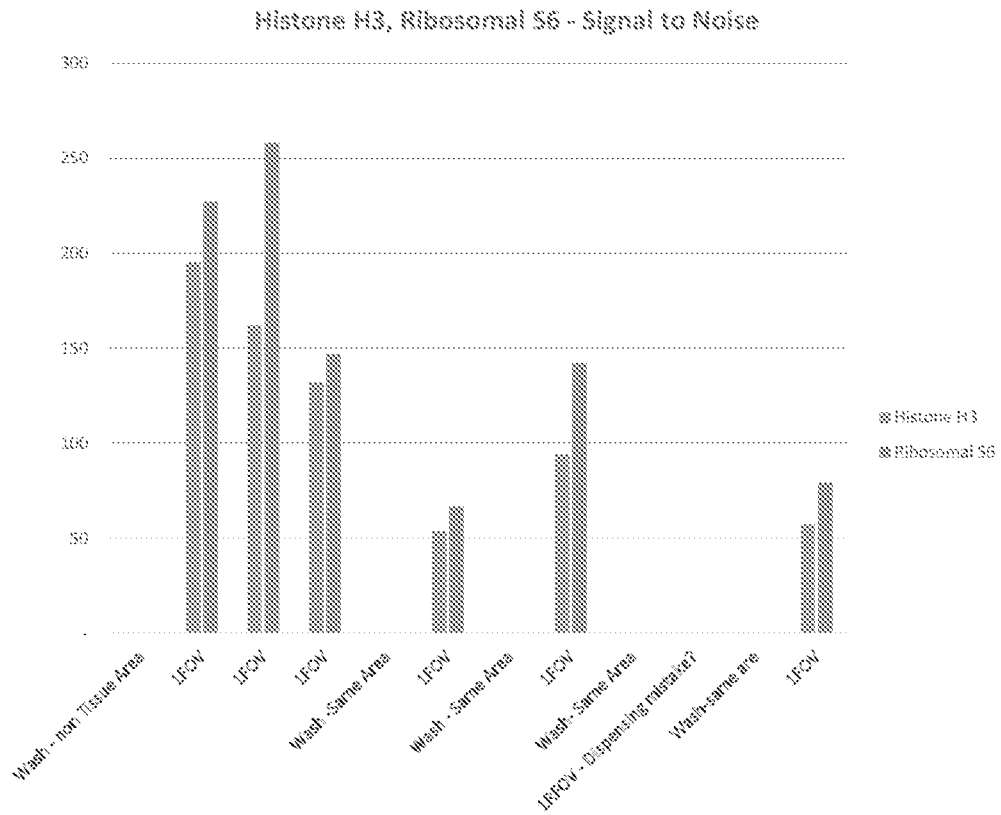

Histone H3, Ribosomal S6 - Signal to Noise
300
250
200
150
100
50
-
Histone H3
Ribosomal S6
Wash - non Tissue Area
1FOV
1FOV
1FOV
Wash -Same Area
1FOV
Wash - Same Area
1FOV
Wash - Same Area
1FOV - Dispensing mistake?
Wash-same are
1FOV

Figure 47

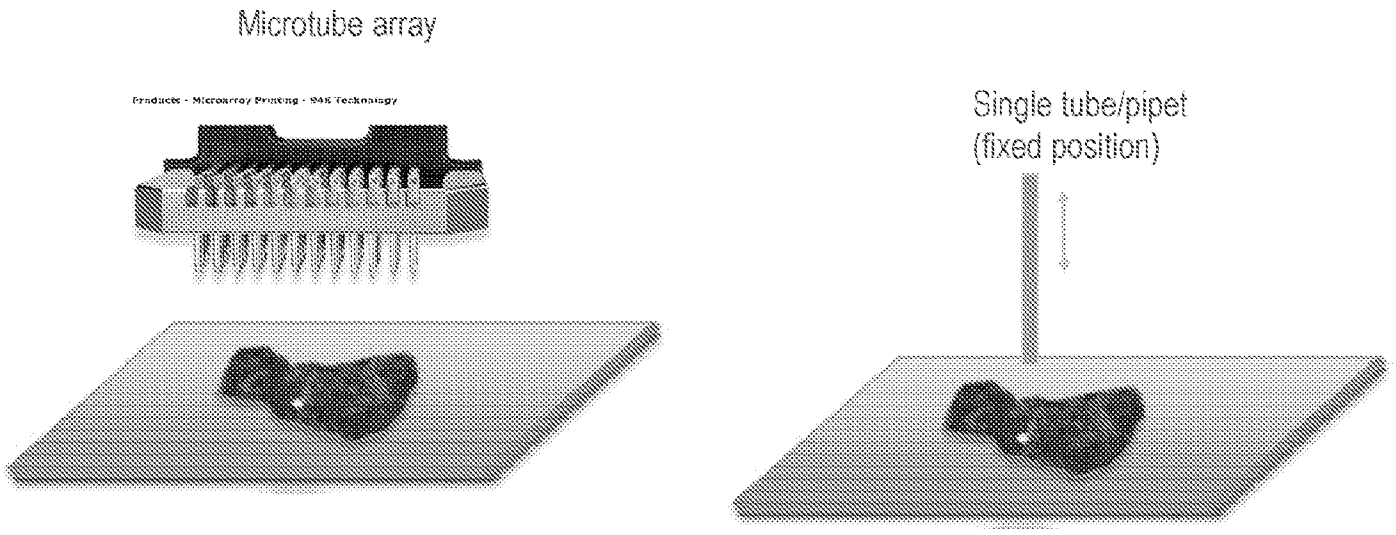


Figure 48

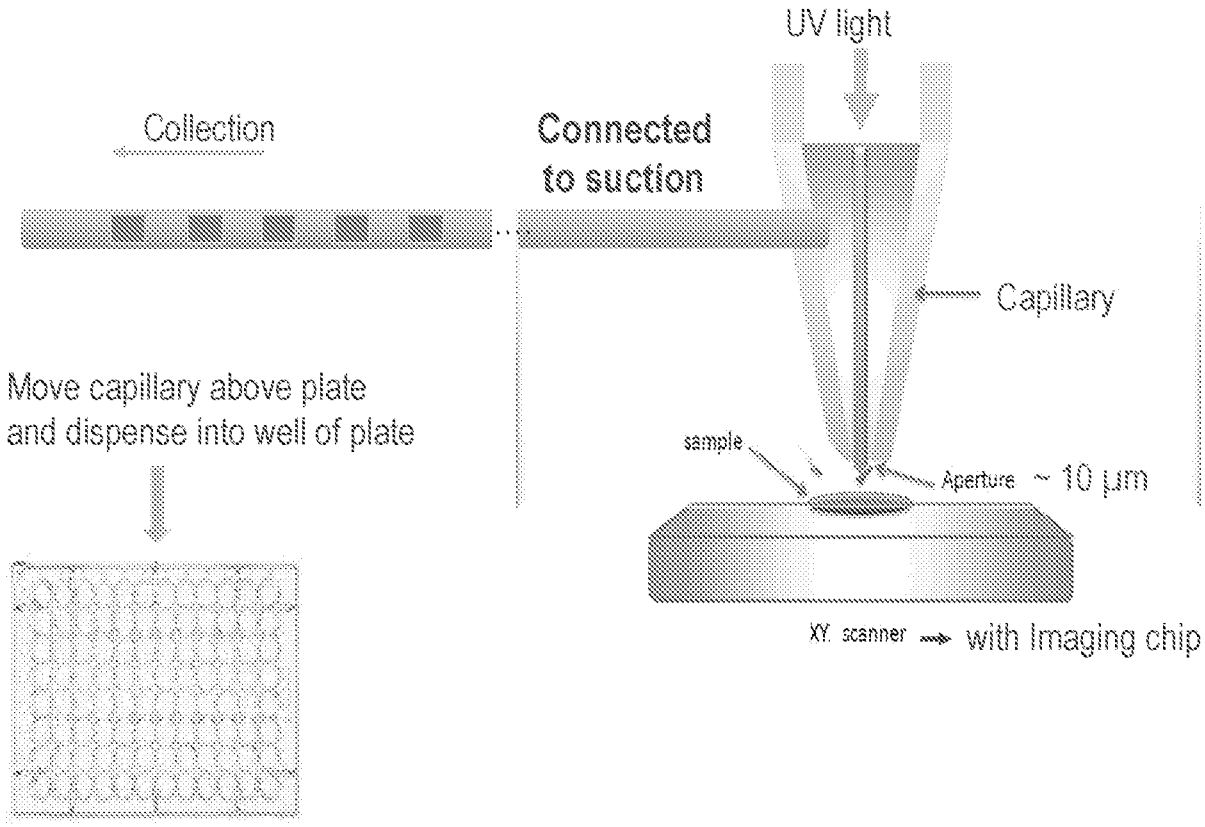

UV light
Collection
Connected to suction
Capillary
Move capillary above plate and dispense into well of plate
sample
Aperture ~ 10 µm
XY scanner
with Imaging chip

Figure 49

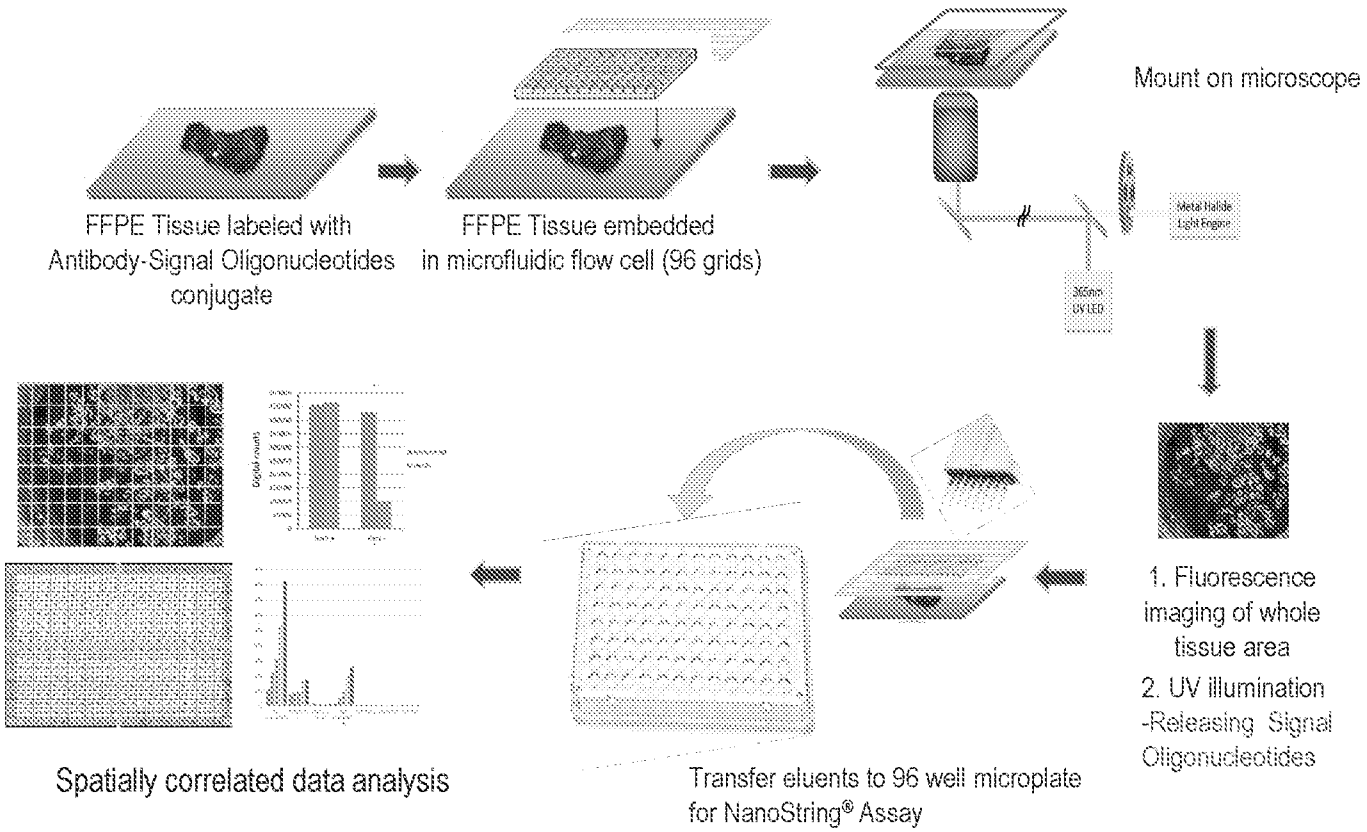

FFPE Tissue labeled with Antibody-Signal Oligonucleotides conjugate
FFPE Tissue embedded in microfluidic flow cell (96 grids)
Mount on microscope
Metal Halide Light Engine
365nm UV LED
1. Fluorescence imaging of whole tissue area
2. UV illumination -Releasing Signal Oligonucleotides
Transfer eluents to 96 well microplate for NanoString® Assay
Spatially correlated data analysis

Figure 50

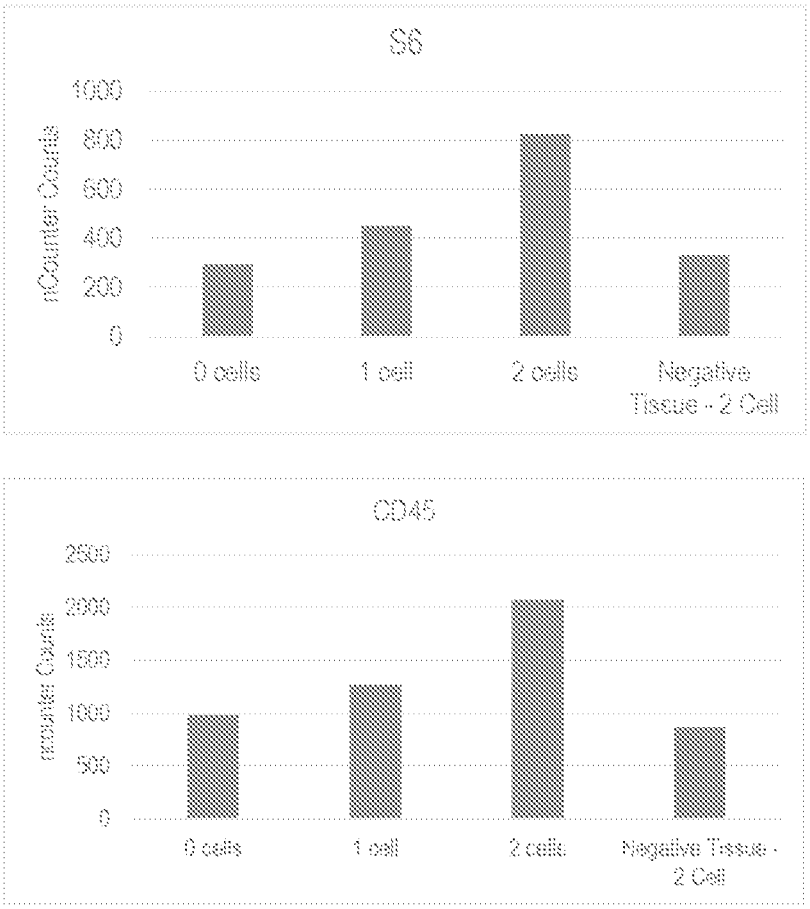

S6
nCounter Counts
1000
800
600
400
200
0
0 cells
1 cell
2 cells
Negative Tissue - 2 Cell
CD45
nCounter Counts
2500
2000
1500
1000
500
0
0 cells
1 cell
2 cells
Negative Tissue - 2 Cell

Figure 51

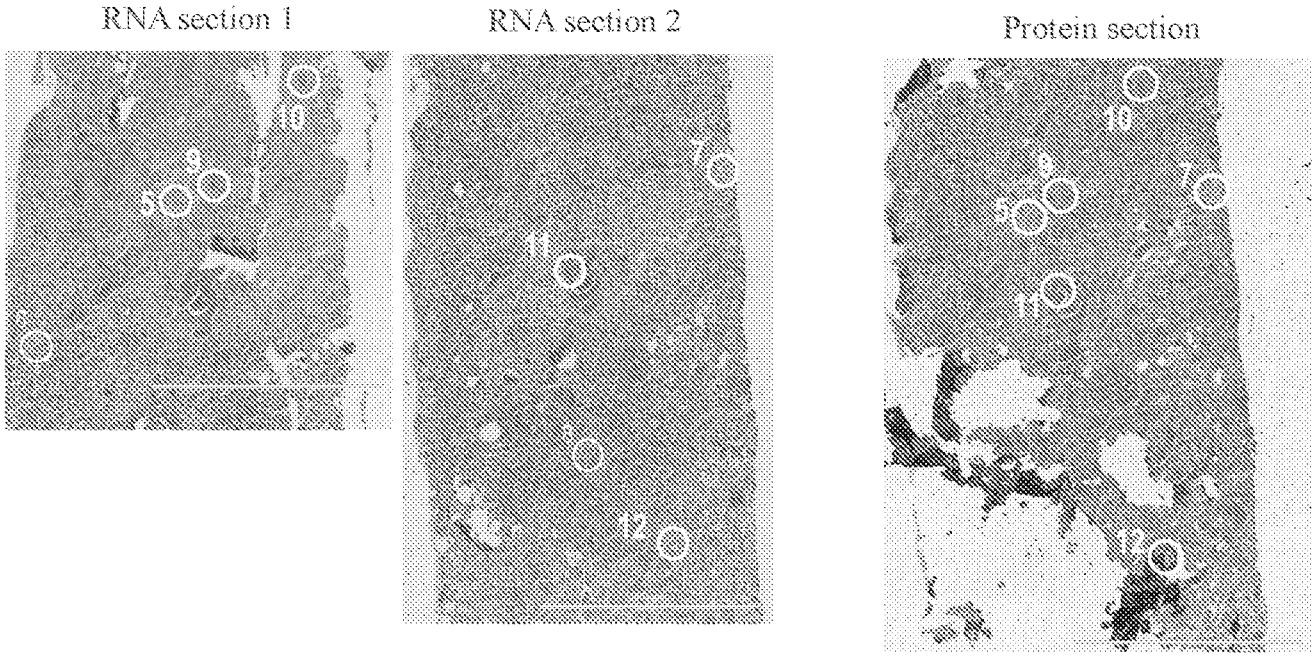


Figure 52

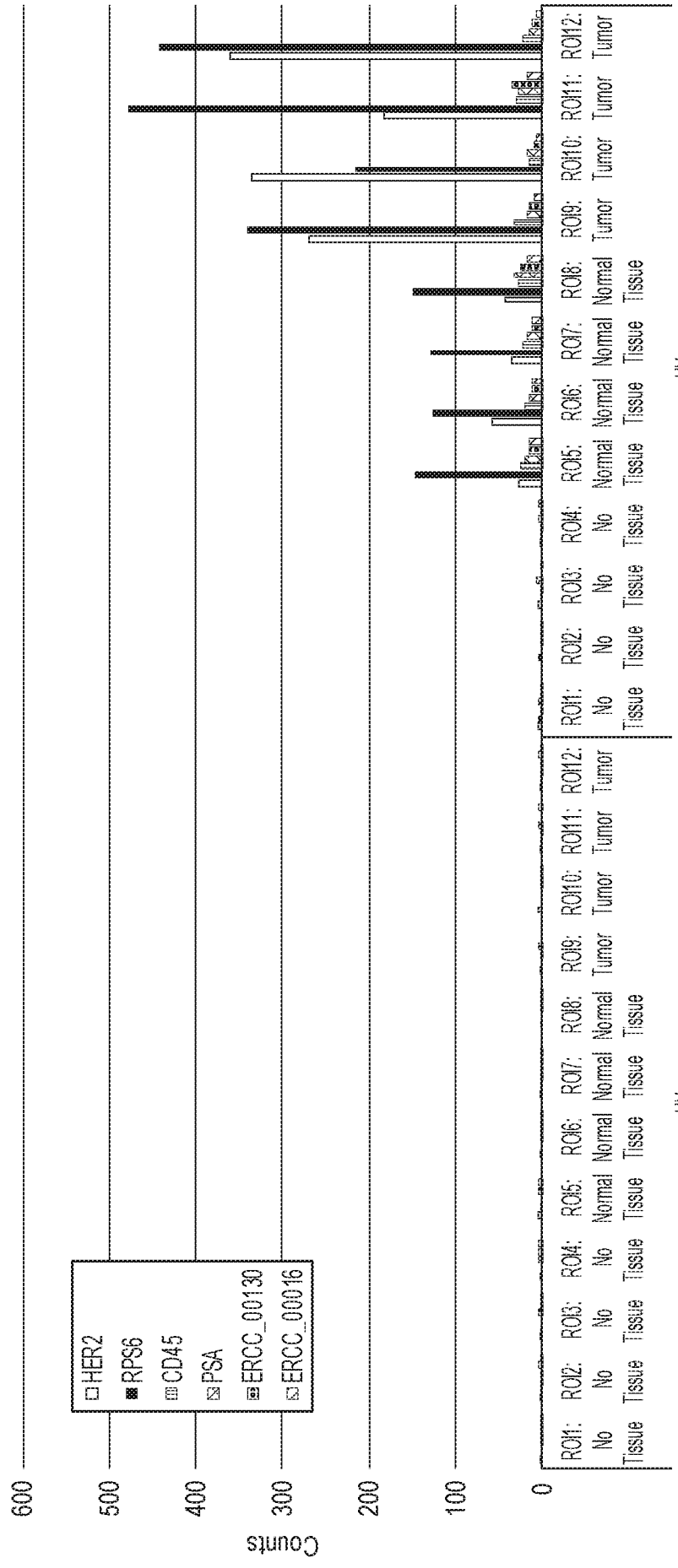

RNA Probe Counts in HER2 3+ Breast Tissue
Counts
600
500
400
300
200
100
0
HER2
RPS6
CD45
PSA
ERCC_00130
ERCC_00016
ROI1: No Tissue
ROI2: No Tissue
ROI3: No Tissue
ROI4: No Tissue
ROI5: Normal Tissue
ROI6: Normal Tissue
ROI7: Normal Tissue
ROI8: Normal Tissue
ROI9: Tumor
ROI10: Tumor
ROI11: Tumor
ROI12: Tumor
-UV
+UV

Figure 53

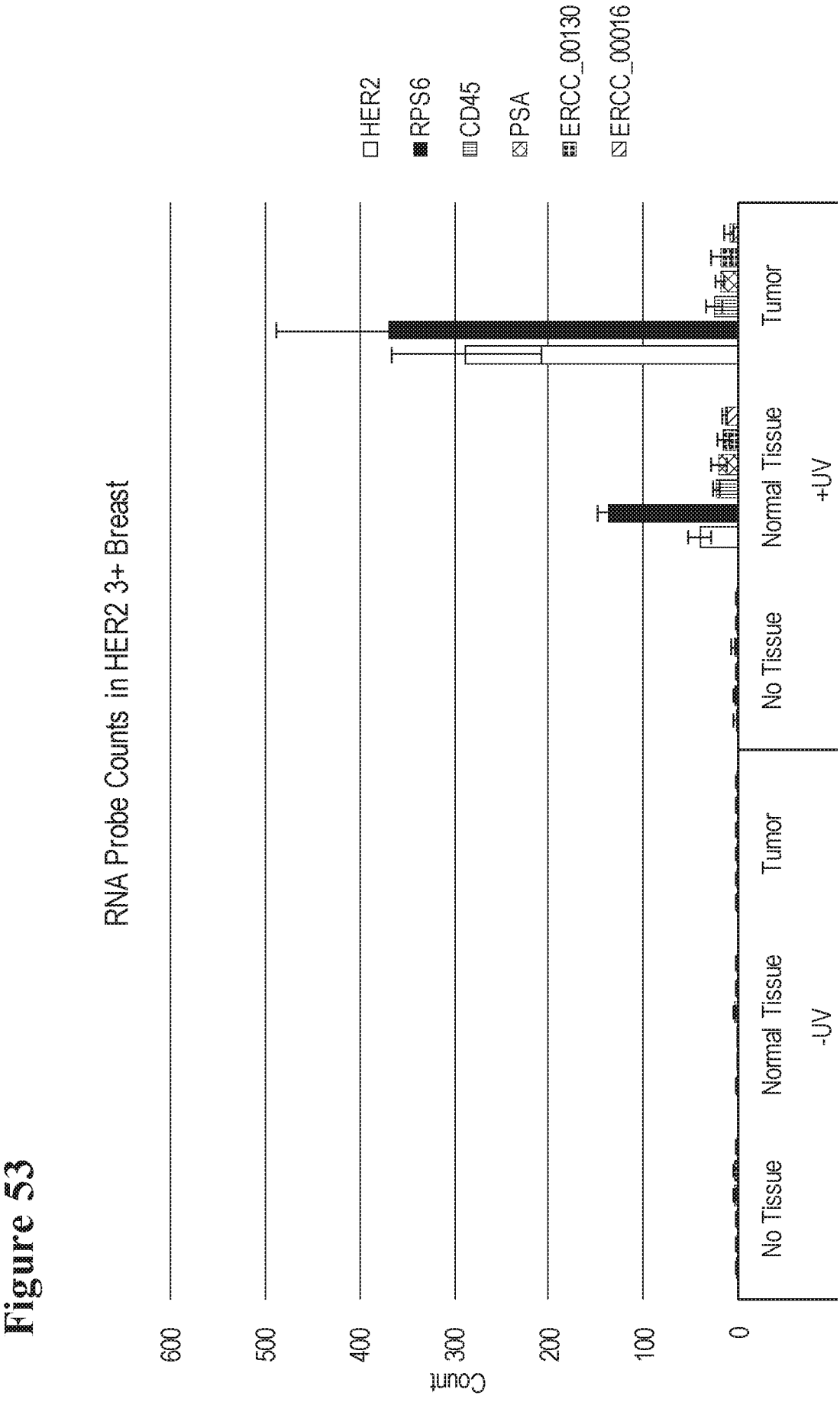

RNA Probe Counts in HER2 3+ Breast
HER2
RPS6
CD45
PSA
ERCC_00130
ERCC_00016
Count
600
500
400
300
200
100
0
No Tissue
Normal Tissue
Tumor
-UV
No Tissue
Normal Tissue
Tumor
+UV

Figure 54

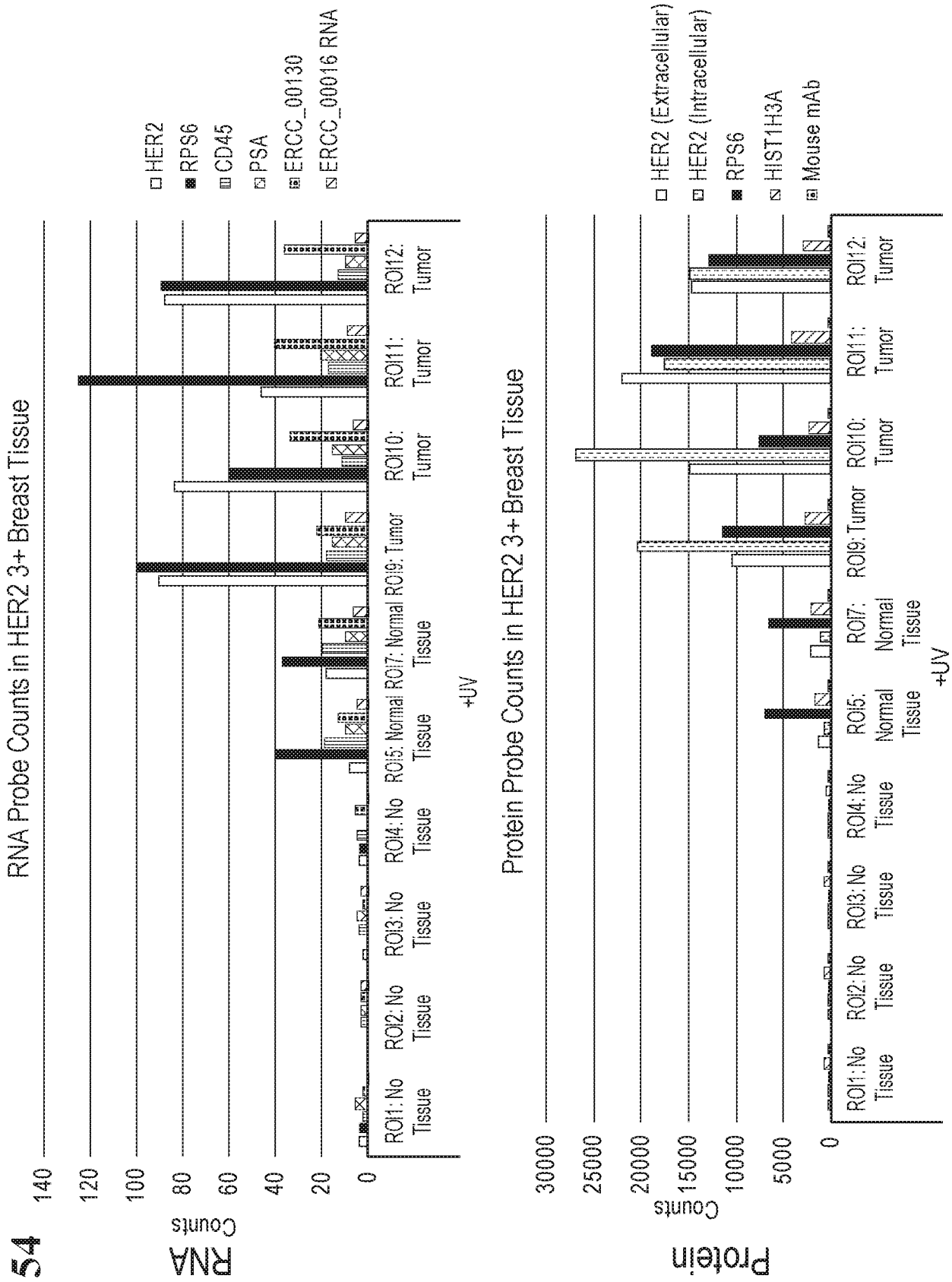

RNA Probe Counts in HER2 3+ Breast Tissue
RNA
Counts
140
120
100
80
60
40
20
0
HER2
RPS6
CD45
PSA
ERCC_00130
ERCC_00016 RNA
ROI1: No Tissue
ROI2: No Tissue
ROI3: No Tissue
ROI4: No Tissue
ROI5: Normal Tissue
ROI7: Normal Tissue
ROI9: Tumor
ROI10: Tumor
ROI11: Tumor
ROI12: Tumor
+UV
Protein Probe Counts in HER2 3+ Breast Tissue
Protein
Counts
30000
25000
20000
15000
10000
5000
0
HER2 (Extracellular)
HER2 (Intracellular)
RPS6
HIST1H3A
Mouse mAb
ROI1: No Tissue
ROI2: No Tissue
ROI3: No Tissue
ROI4: No Tissue
ROI5: Normal Tissue
ROI7: Normal Tissue
ROI9: Tumor
ROI10: Tumor
ROI11: Tumor
ROI12: Tumor
+UV

Figure 55

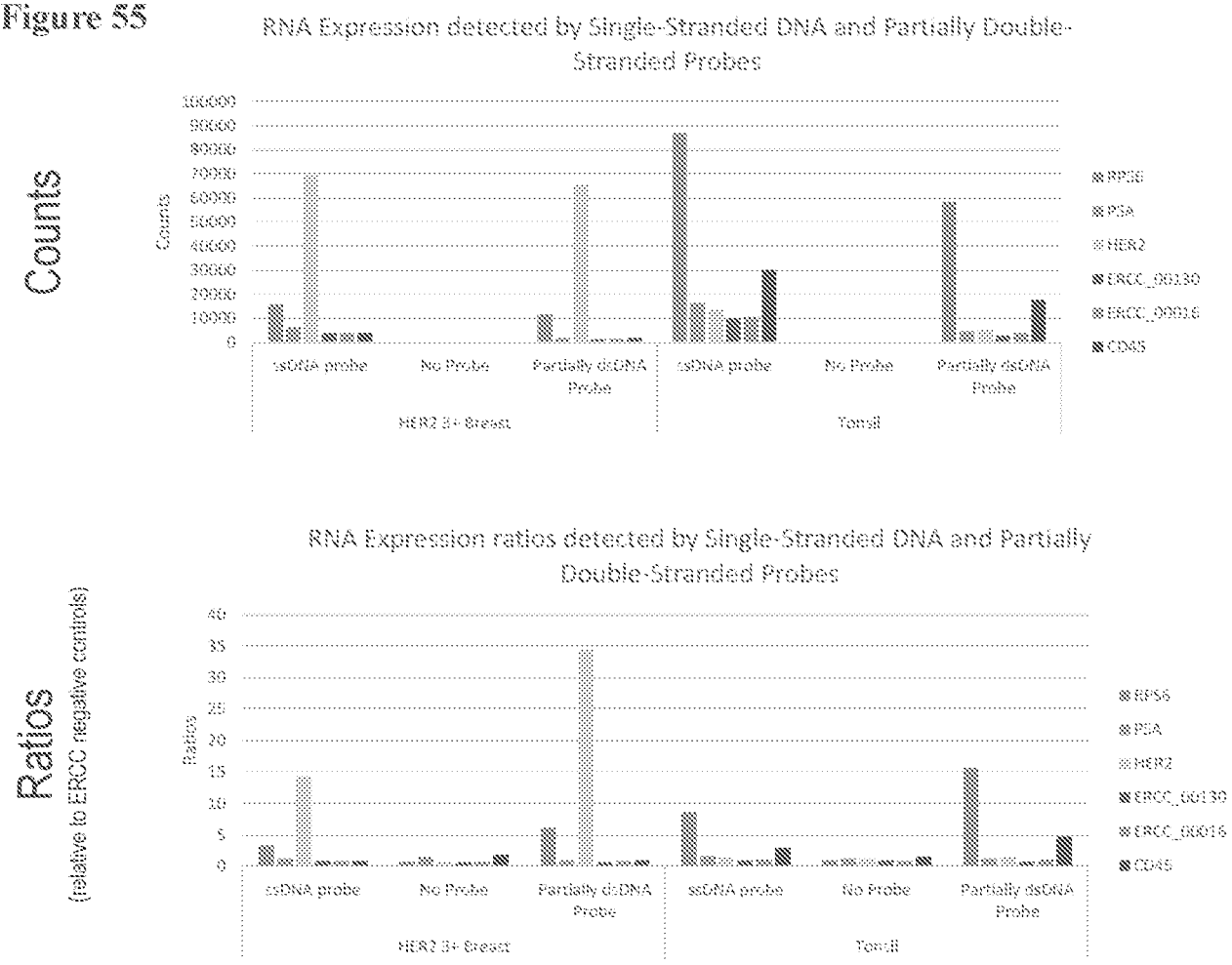

RNA Expression detected by Single-Stranded DNA and Partially Double-Stranded Probes
Counts
Counts
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
ssDNA probe
No Probe
Partially dsDNA Probe
ssDNA probe
No Probe
Partially dsDNA Probe
HER2 3+ Breast
Tonsil
RPS6
PSA
HER2
ERCC_00130
ERCC_00016
CD45
RNA Expression ratios detected by Single-Stranded DNA and Partially Double-Stranded Probes
Ratios
(relative to ERCC negative controls)
Ratios
40
35
30
25
20
15
10
5
0
ssDNA probe
No Probe
Partially dsDNA Probe
ssDNA probe
No Probe
Partially dsDNA Probe
HER2 3+ Breast
Tonsil
RPS6
PSA
HER2
ERCC_00130
ERCC_00016
CD45

Figure 56

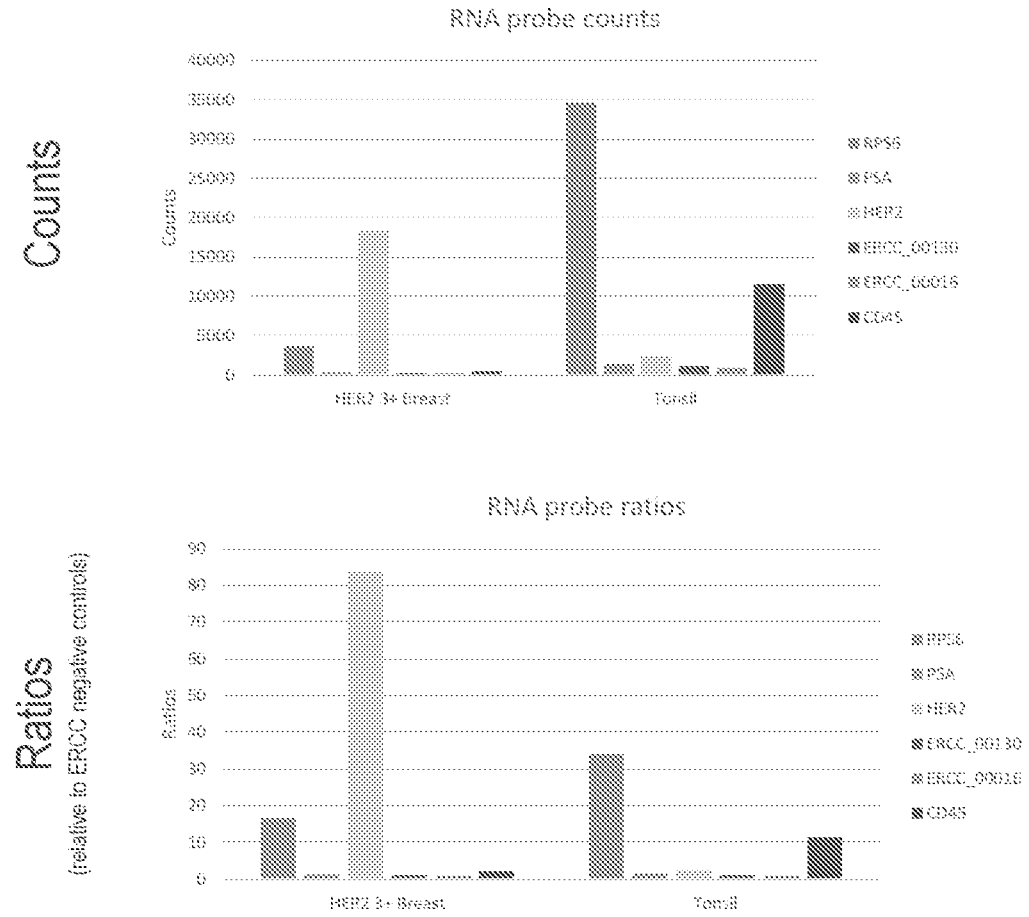

RNA probe counts
Counts
40000
35000
30000
25000
20000
15000
10000
5000
0
HER2 3+ Breast
Tonsil
RPS6
PSA
HER2
ERCC_00130
ERCC_00018
CD45
RNA probe ratios
Ratios
(relative to ERCC negative controls)
90
80
70
60
50
40
30
20
10
0
HER2 3+ Breast
Tonsil
RPL6
PSA
HER2
ERCC_00130
ERCC_00018
CD45

Figure 57

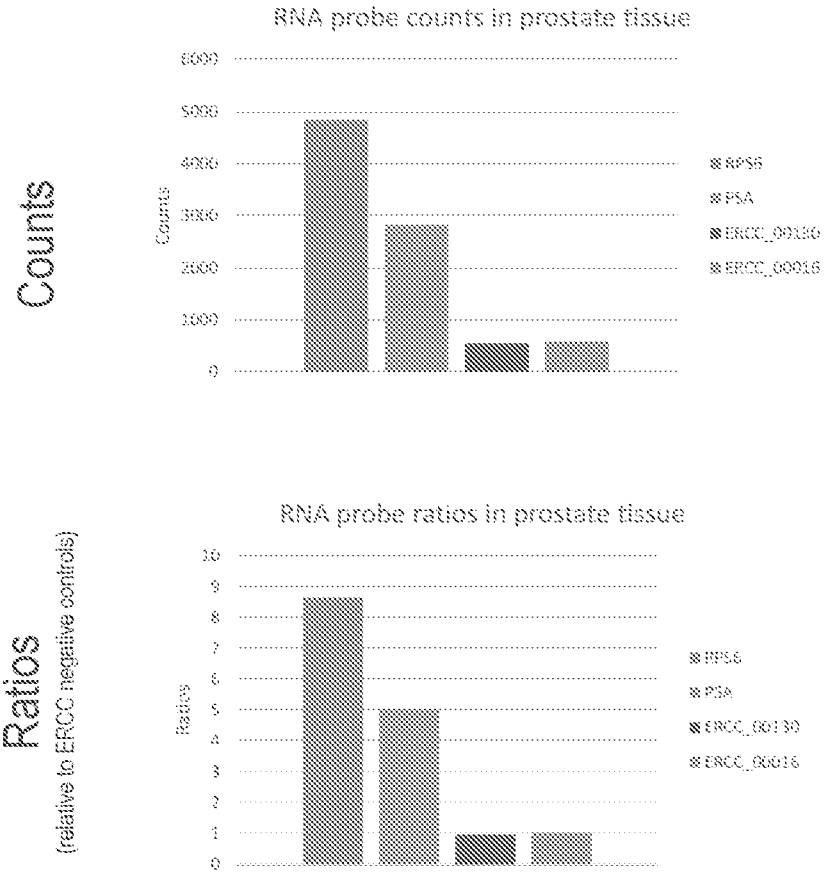
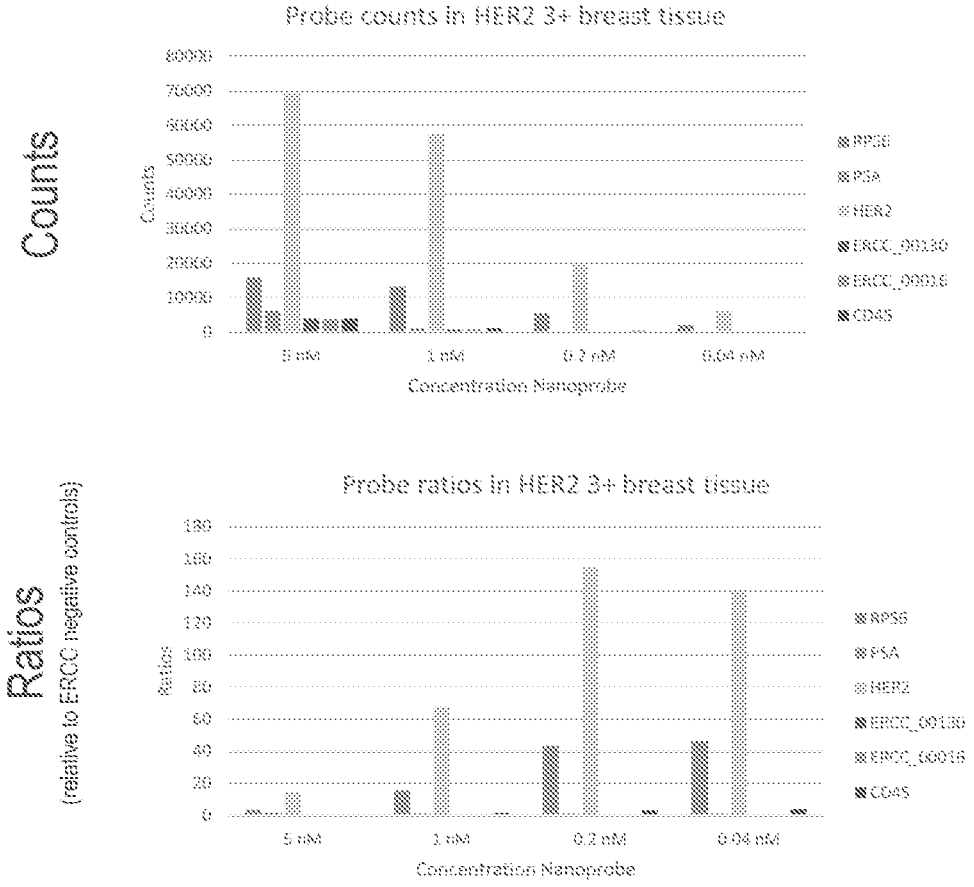

RNA probe counts in prostate tissue
Counts
6000
5000
4000
3000
2000
1000
0
RPS6
PSA
ERCC_00130
ERCC_00016
RNA probe ratios in prostate tissue
Ratios
(relative to ERCC negative controls)
10
9
8
7
6
5
4
3
2
1
0
RPS6
PSA
ERCC_00130
ERCC_00016

Figure 58


Counts
Probe counts in HER2 3+ breast tissue
Counts
80000
70000
60000
50000
40000
30000
20000
10000
0
5 nM
1 nM
0.2 nM
0.04 nM
Concentration Nanoprobe
RPS6
PSA
HER2
ERCC_00130
ERCC_00016
CD45
Ratios
(relative to ERCC negative controls)
Probe ratios in HER2 3+ breast tissue
Ratios
180
160
140
120
100
80
60
40
20
0
5 nM
1 nM
0.2 nM
0.04 nM
Concentration Nanoprobe
RPS6
PSA
HER2
ERCC_00130
ERCC_00016
CD45