

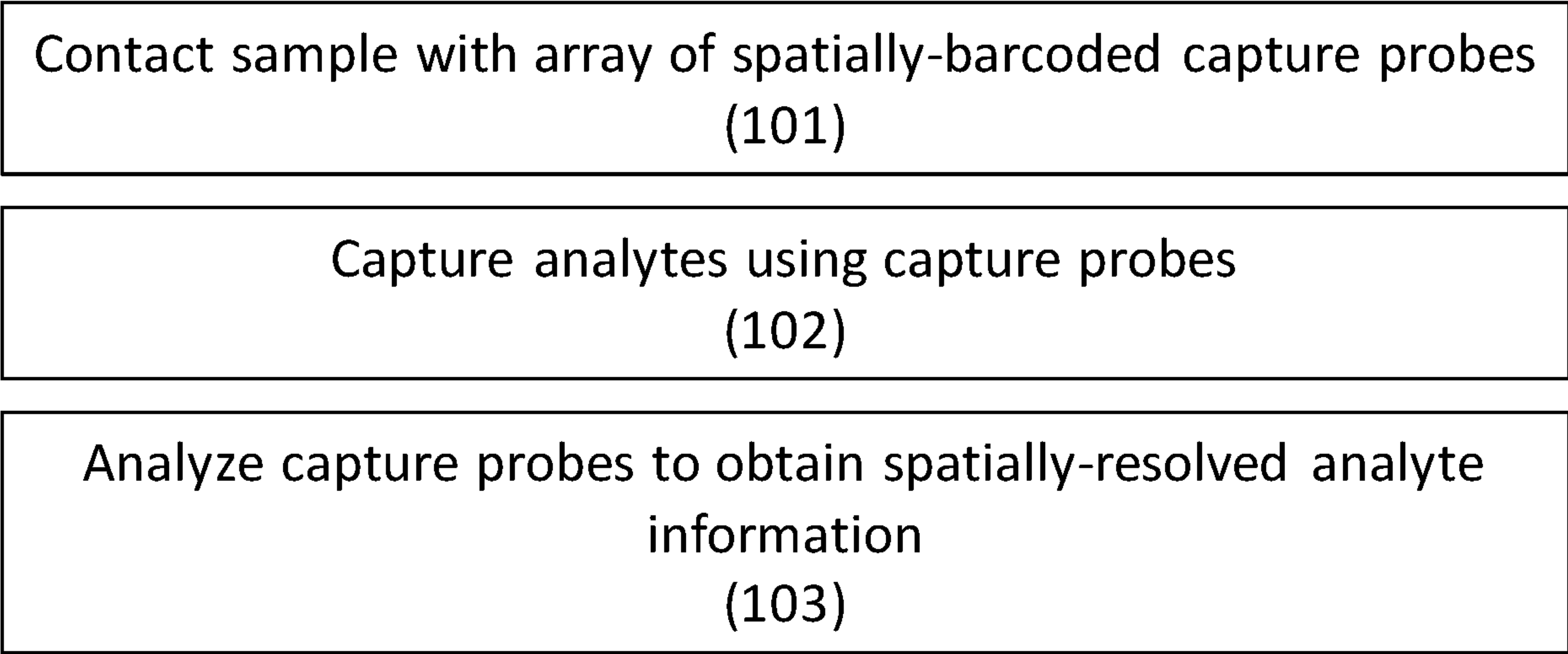


Figure 1

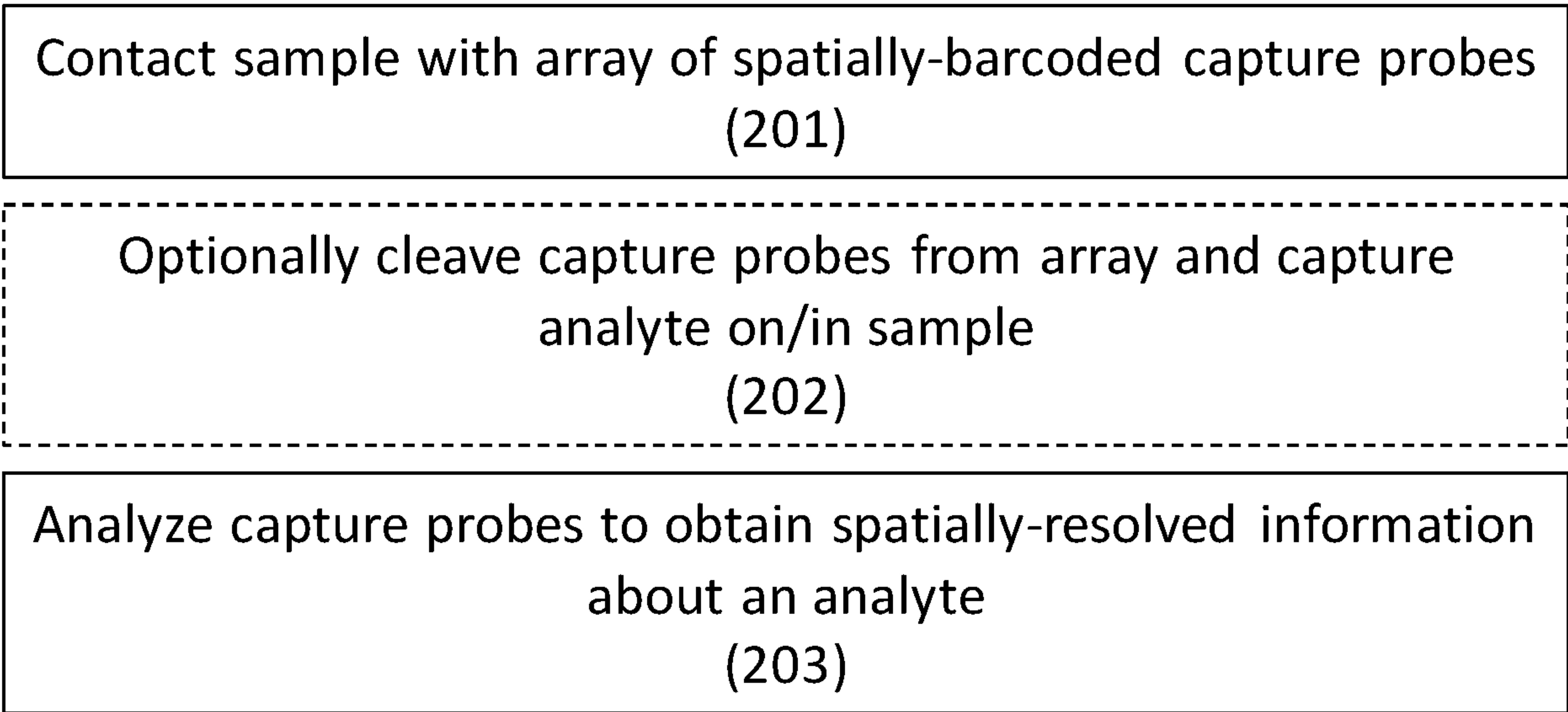


Figure 2

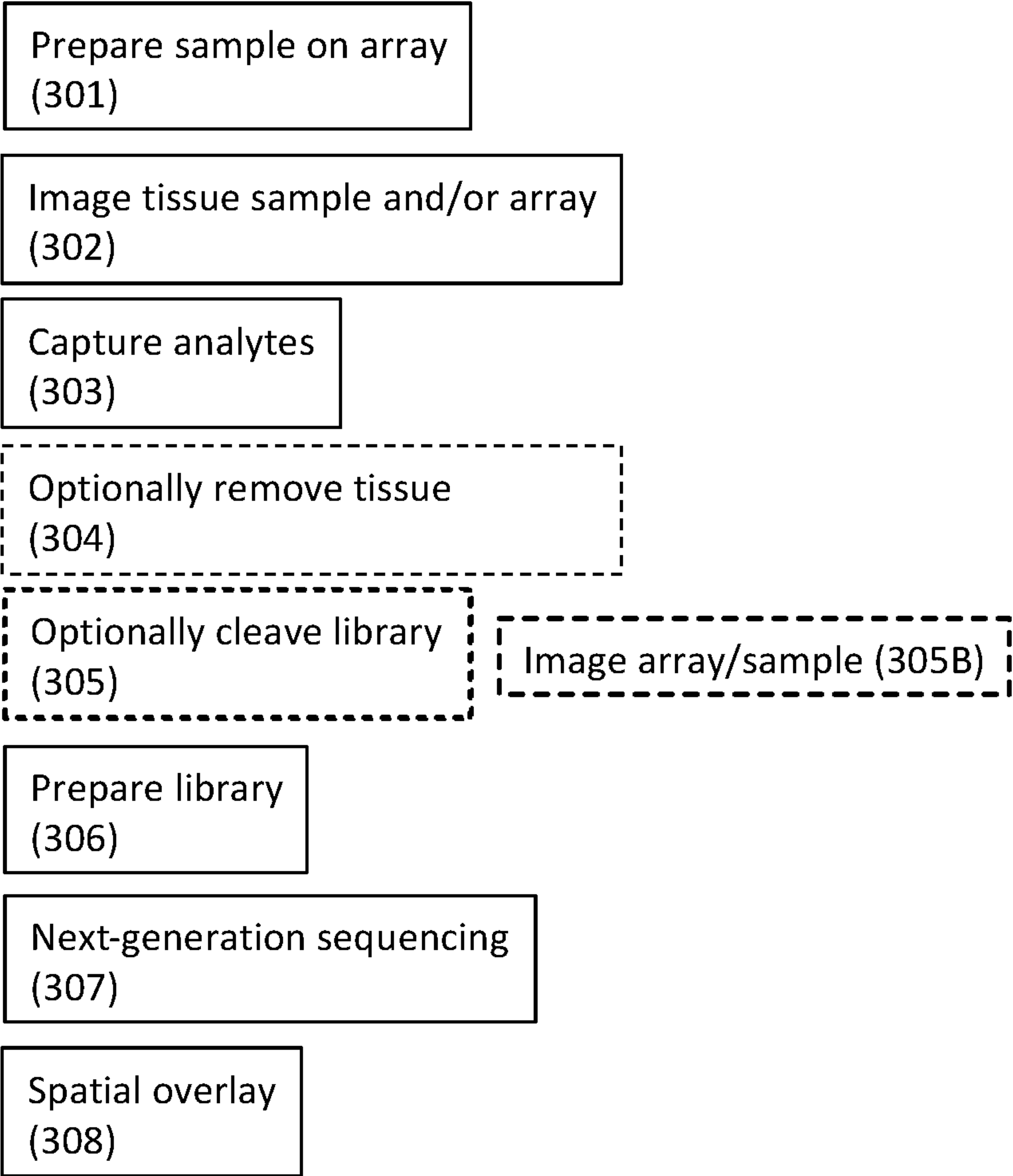
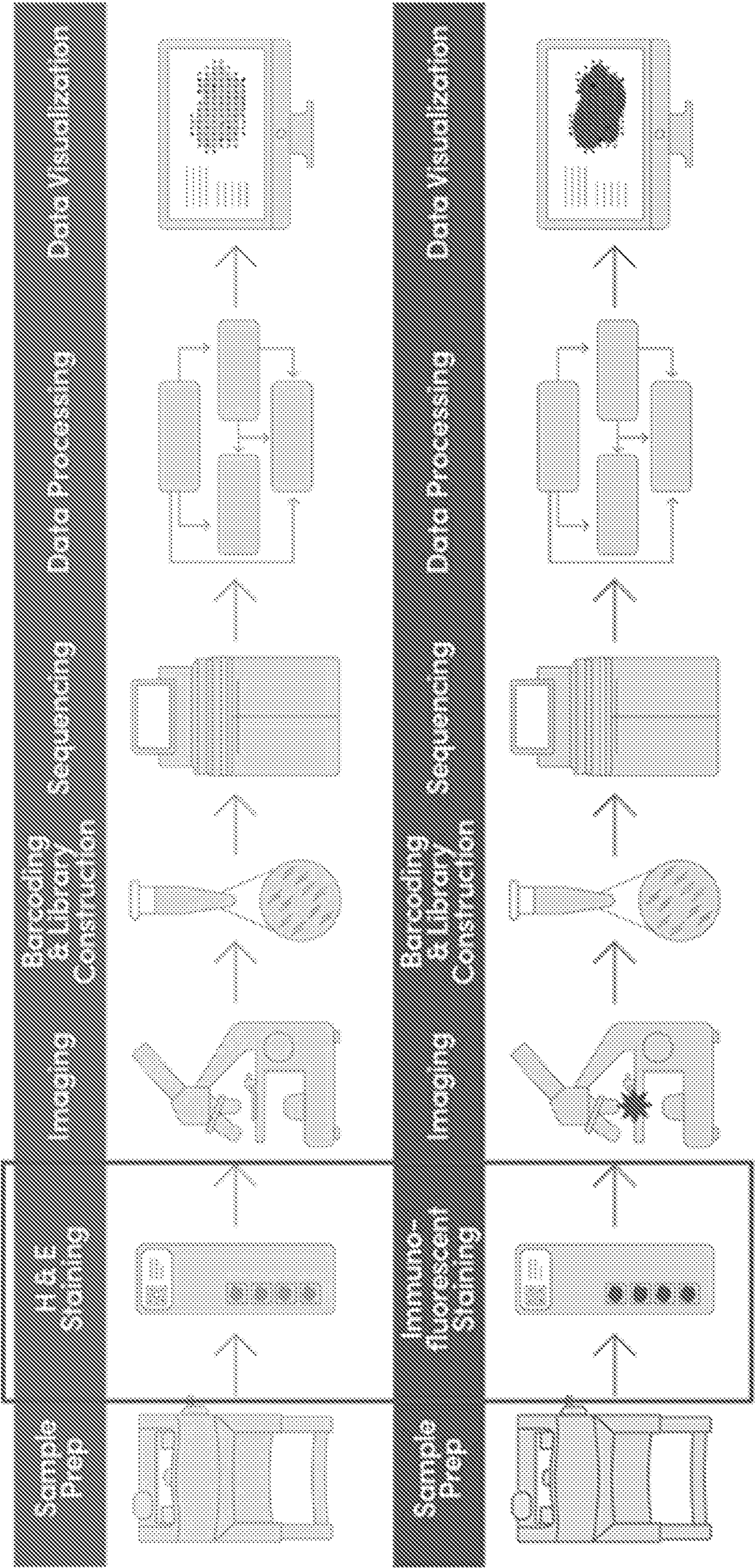


Figure 3A

302



304

Figure 3B

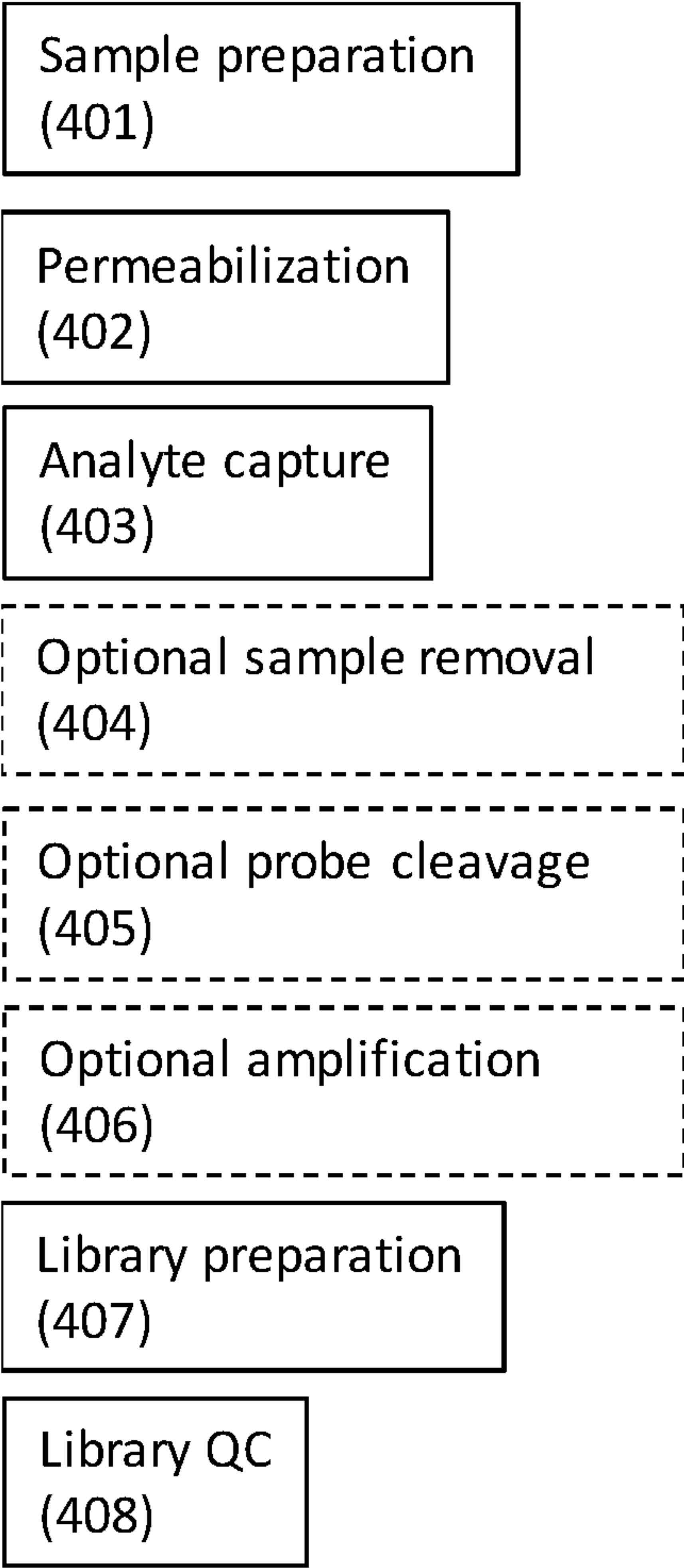


Figure 4

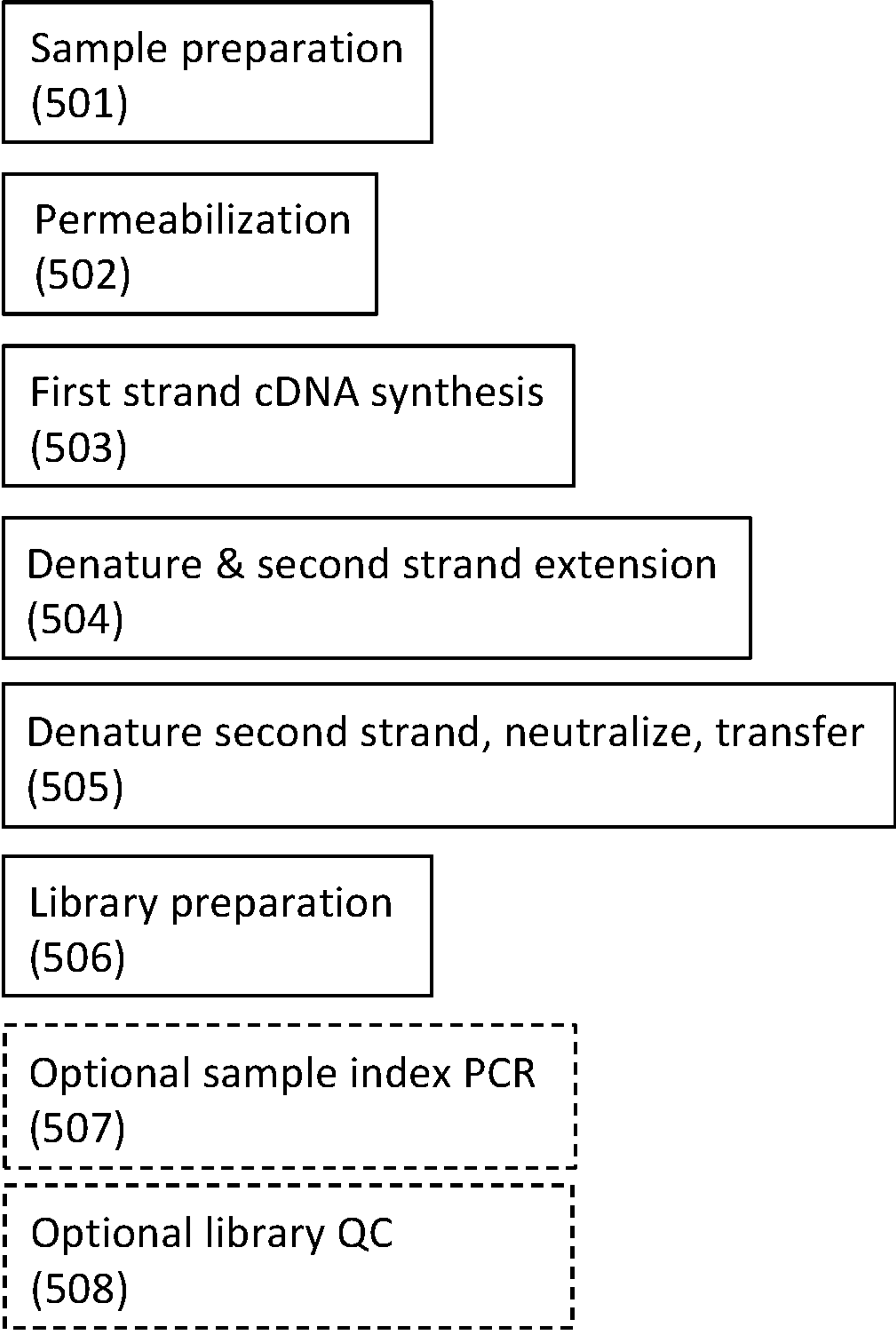


Figure 5

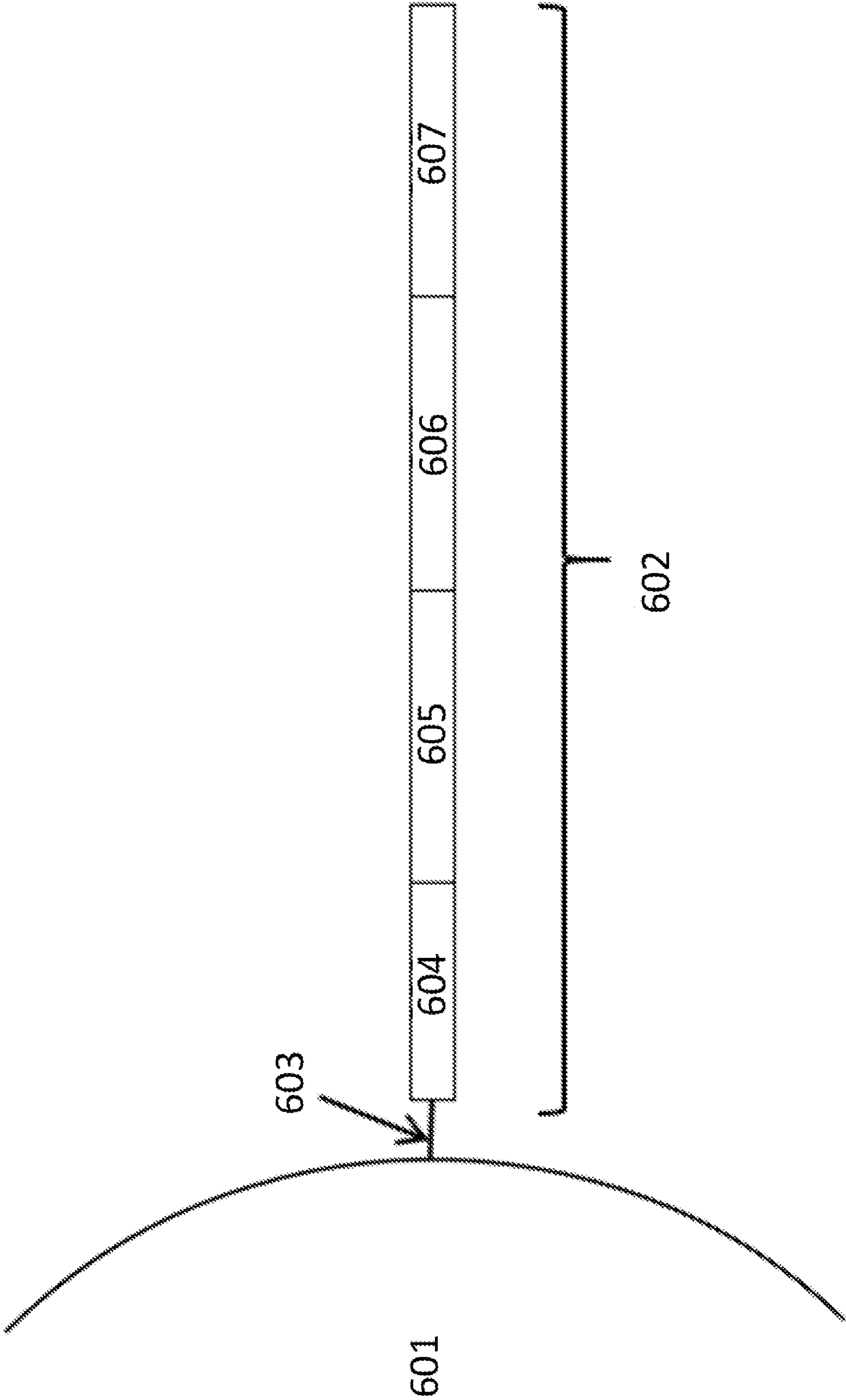


Figure 6

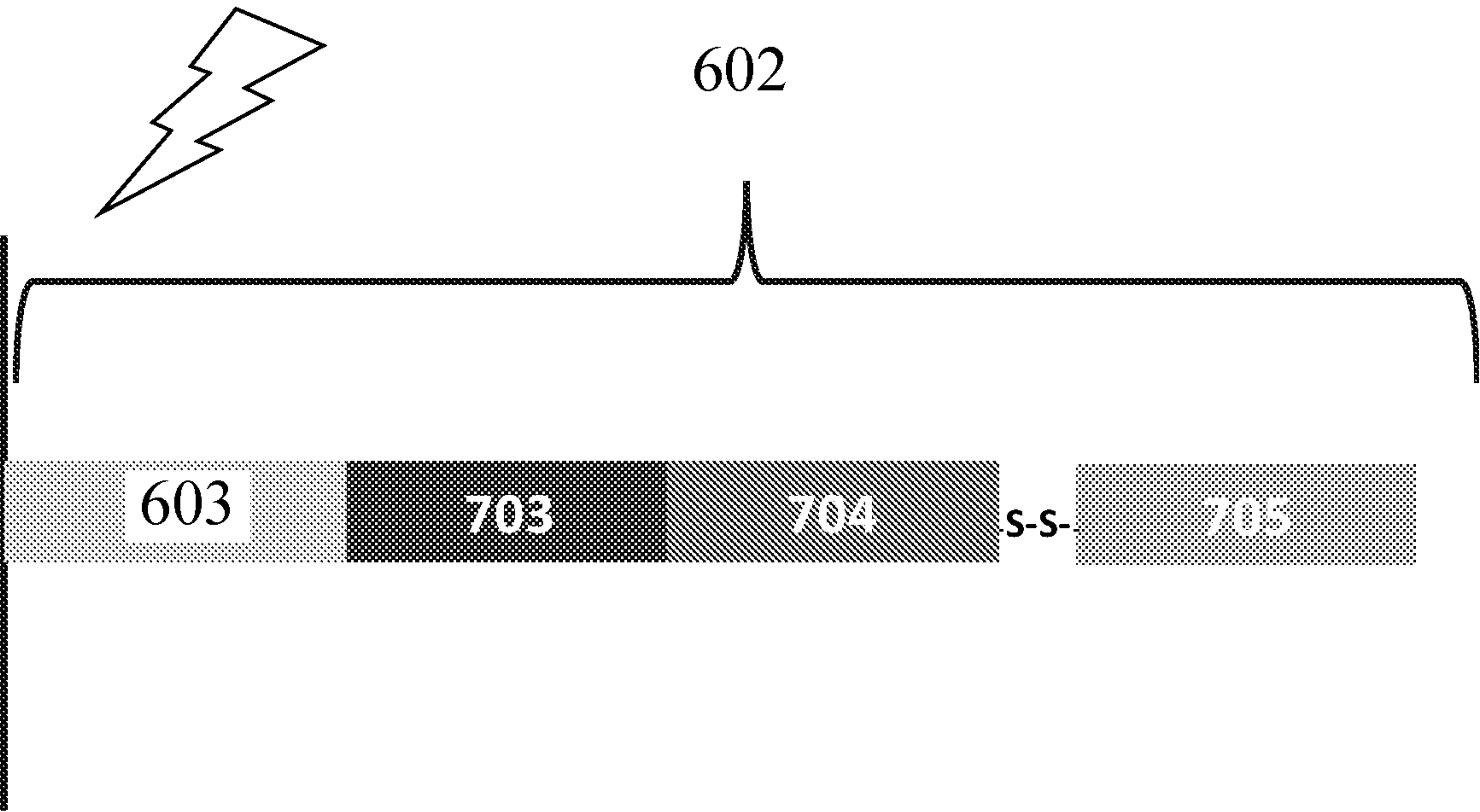


Figure 7

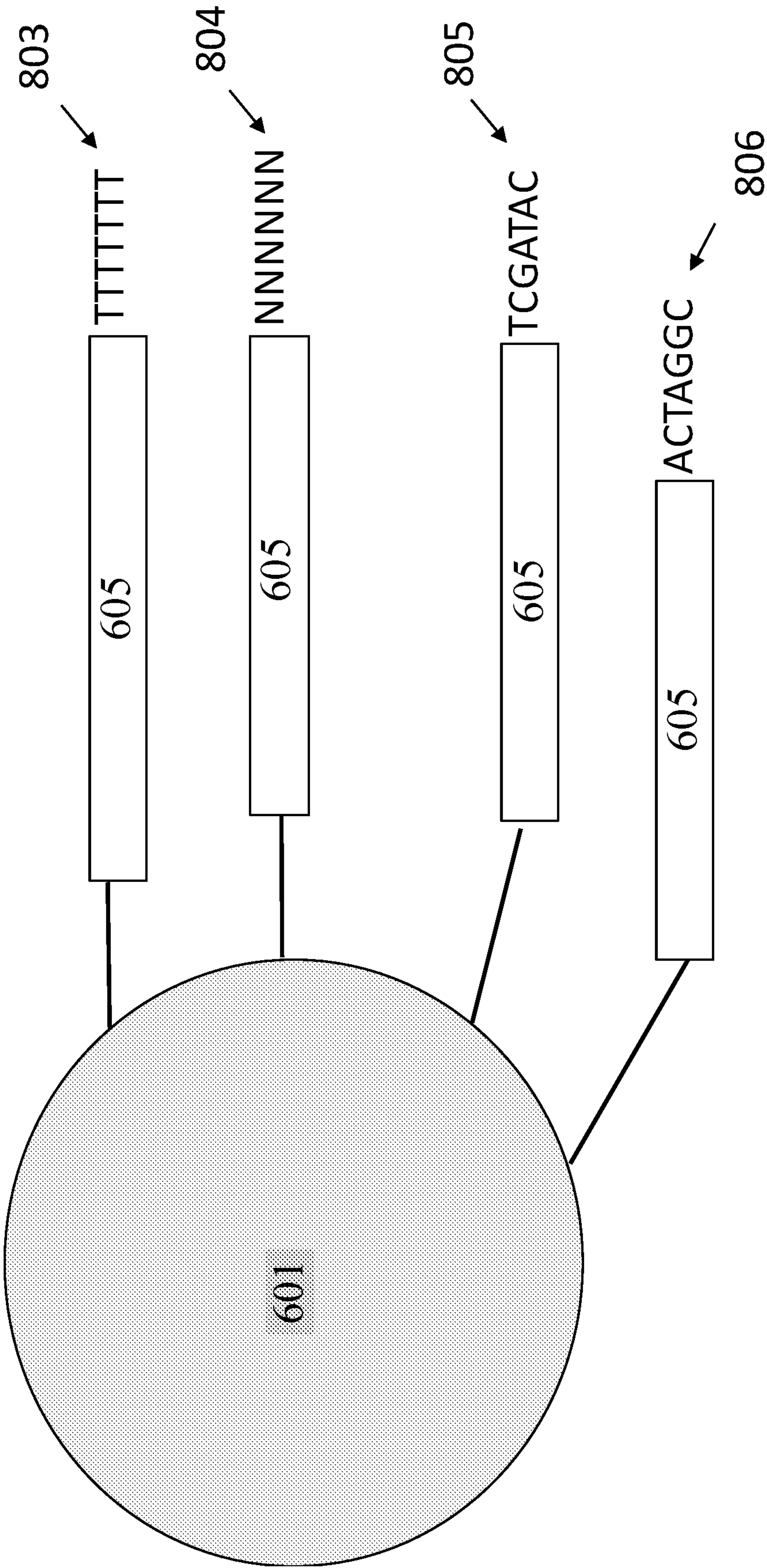


Figure 8

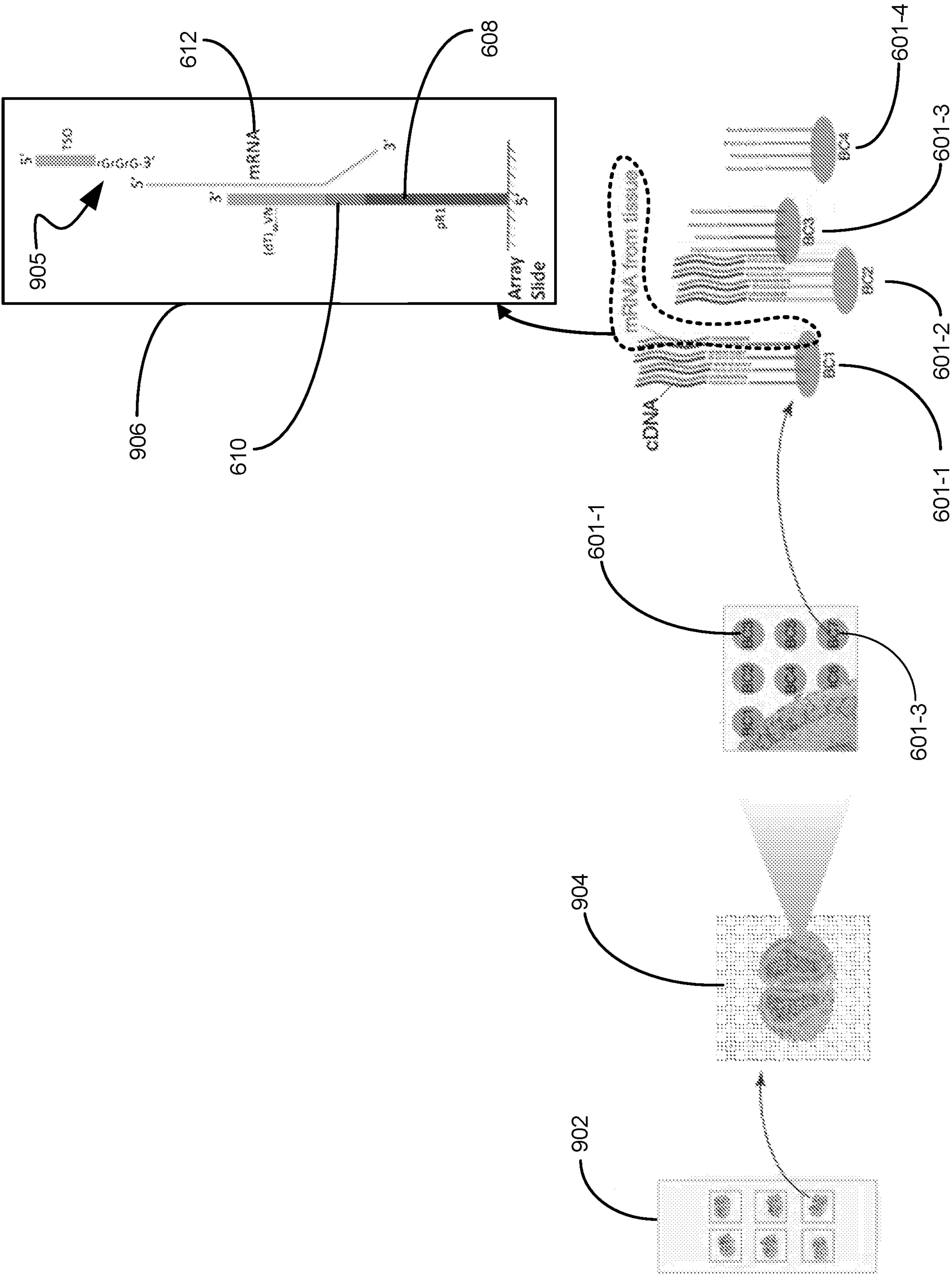


Figure 9

11/70

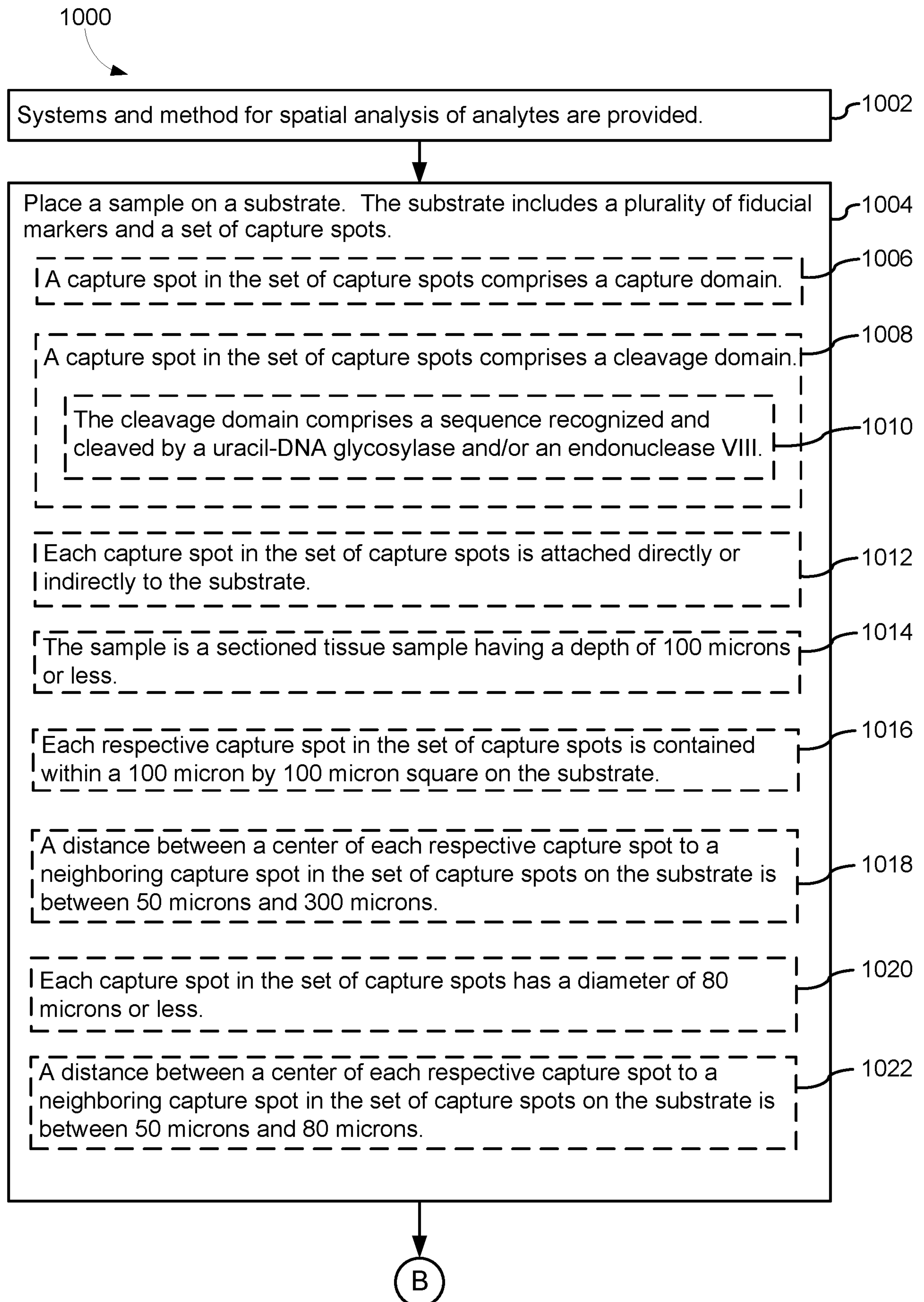
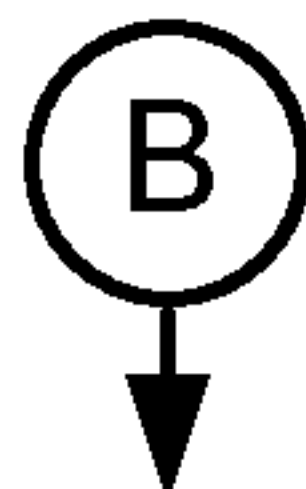


Figure 10A

12/70



Obtain one or more images 1124 of the sample on the substrate. The such image comprises a plurality of pixels in the form of an array of pixel values.

1024

For each respective image in the one or more images, obtain a corresponding plurality of sequence reads, in electronic form, from the set of capture spots (e.g., by *in-situ* sequencing of the set of capture spots on the substrate, high-throughput sequencing etc.).

1026

The corresponding plurality of sequence reads comprises 10,000 or more sequence reads, 50,000 or more sequence reads, 100,000 or more sequence reads, or 1×10^6 or more sequence reads.

1028

The corresponding plurality of sequence reads include 3'-end or 5'-end paired sequence reads.

1030

Each respective capture probe plurality in a set of capture probe pluralities is (i) at a different capture spot in the set of capture spots and (ii) directly or indirectly associates with one or more analytes from the sample.

1032

The one or more analytes (e.g., DNA, RNA) comprises 5 or more analytes, 10 or more analytes, 50 or more analytes, 100 or more analytes, 500 or more analytes, 1000 or more analytes, 2000 or more analytes, or between 2000 and 10,000 analytes.

1034

The one or more analytes is a plurality of analytes. A respective capture probe plurality in the set of capture probe pluralities includes a plurality of capture probes. Each capture probe in the plurality of capture probes includes a capture domain that is characterized by a capture domain type in a plurality of capture domain types. Each respective capture domain type in the plurality of capture domain types is configured to bind to a different analyte in the plurality of analytes.

1036

The plurality of capture domain types comprises between 5 and 15,000 capture domain types and the respective capture probe plurality includes at least five, at least 10, at least 100, or at least 1000 probes for each capture domain type in the plurality of capture domain types.

1038



Figure 10B

13/70

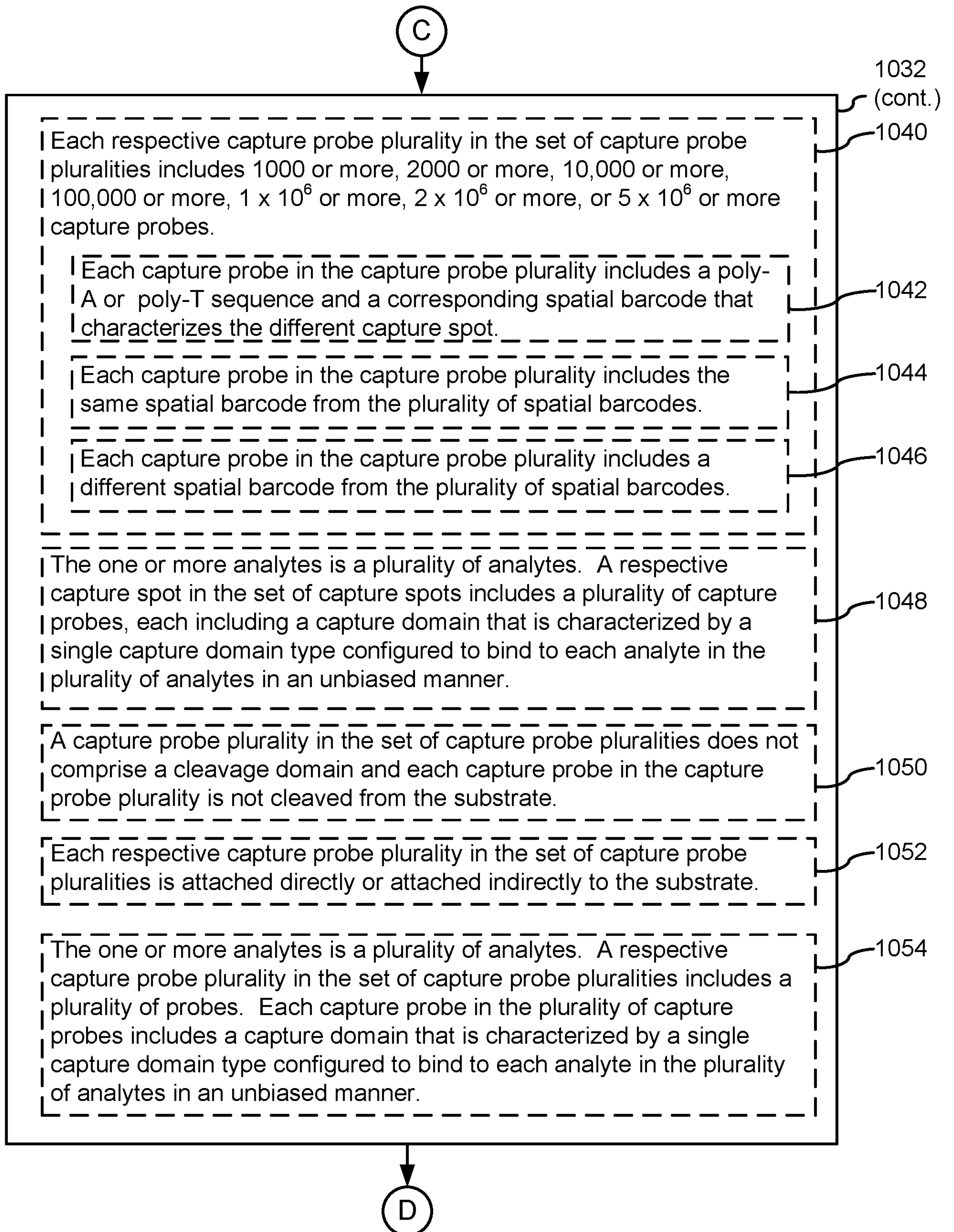


Figure 10C

14/70

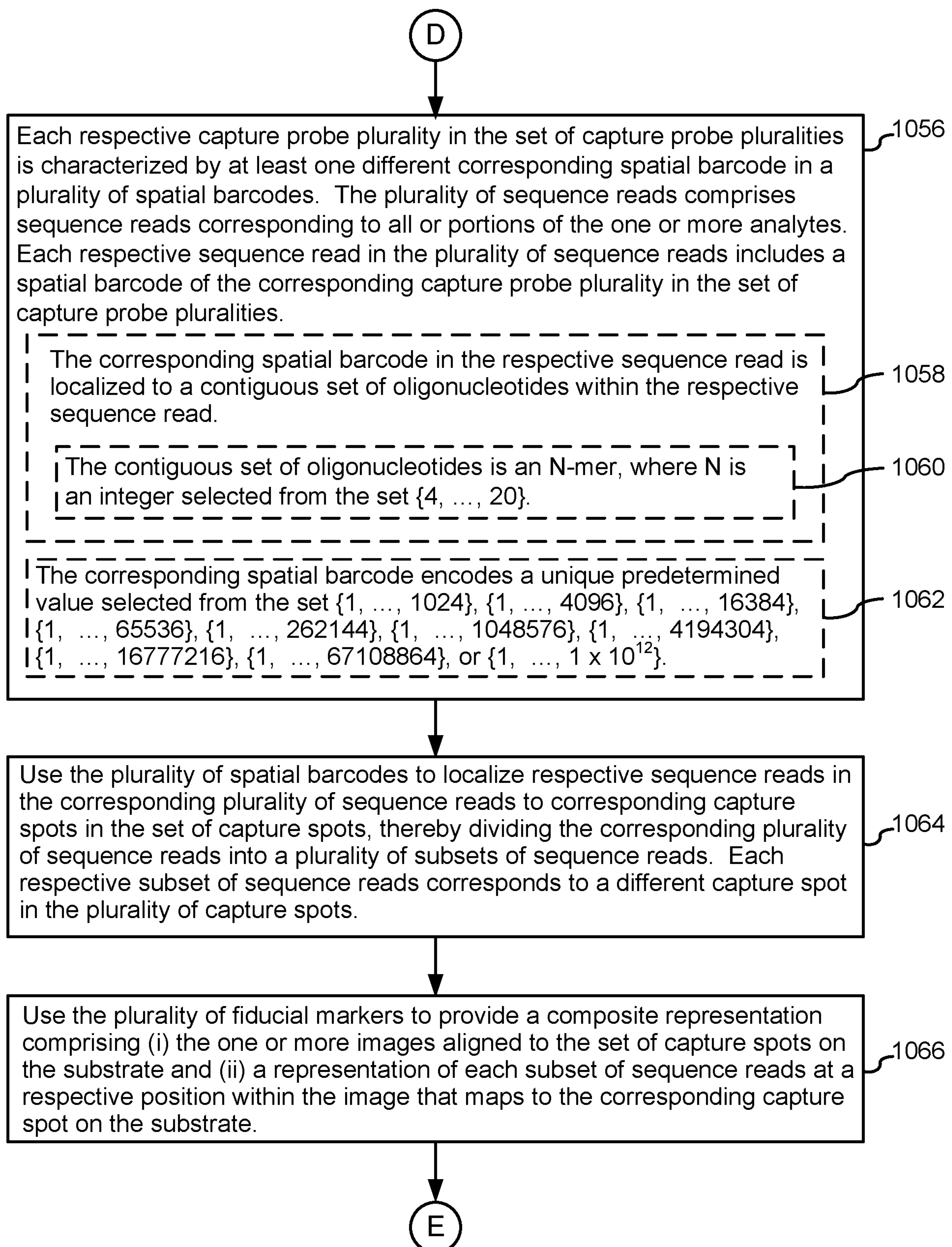


Figure 10D

15/70

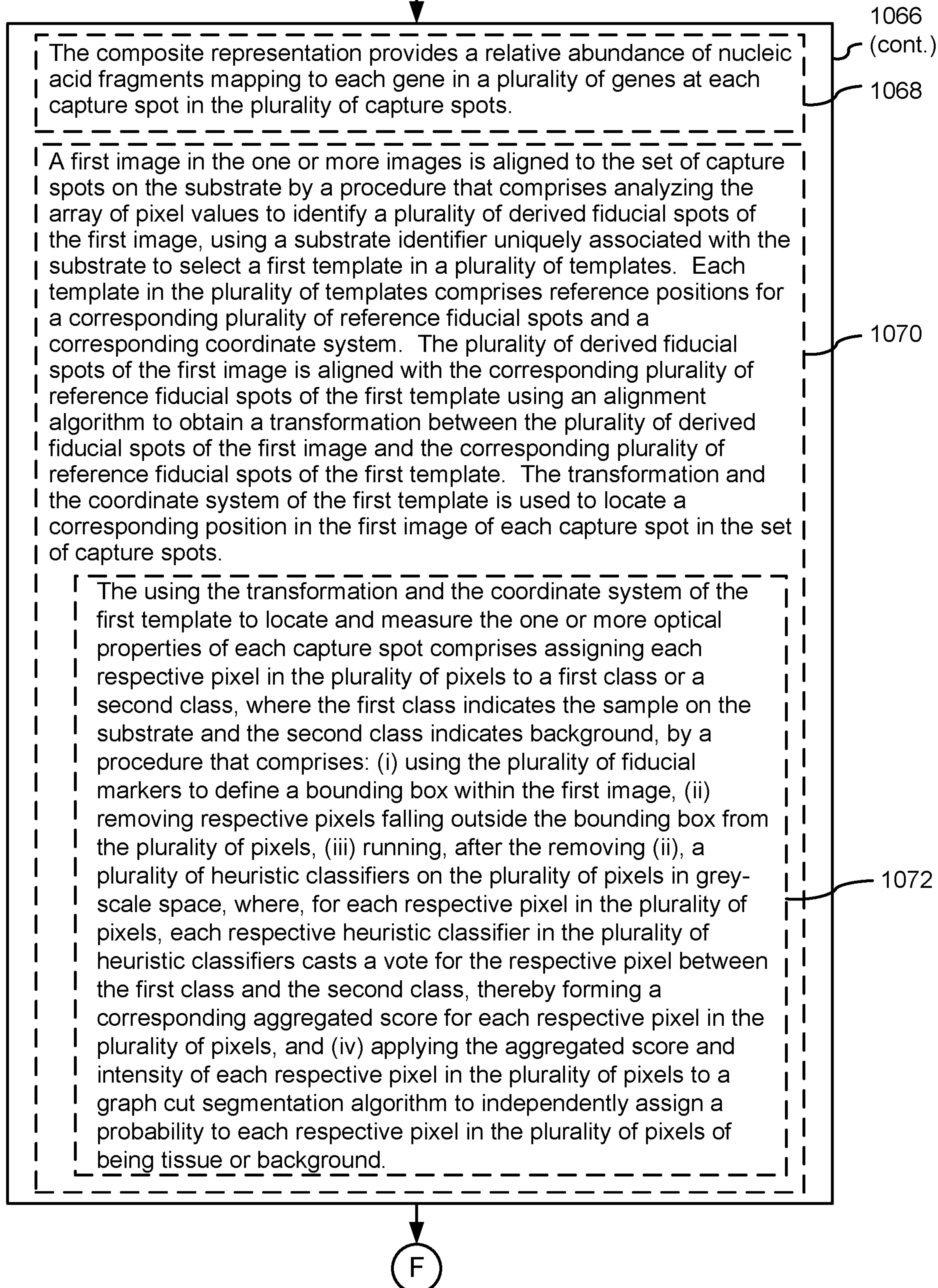


Figure 10E

16/70

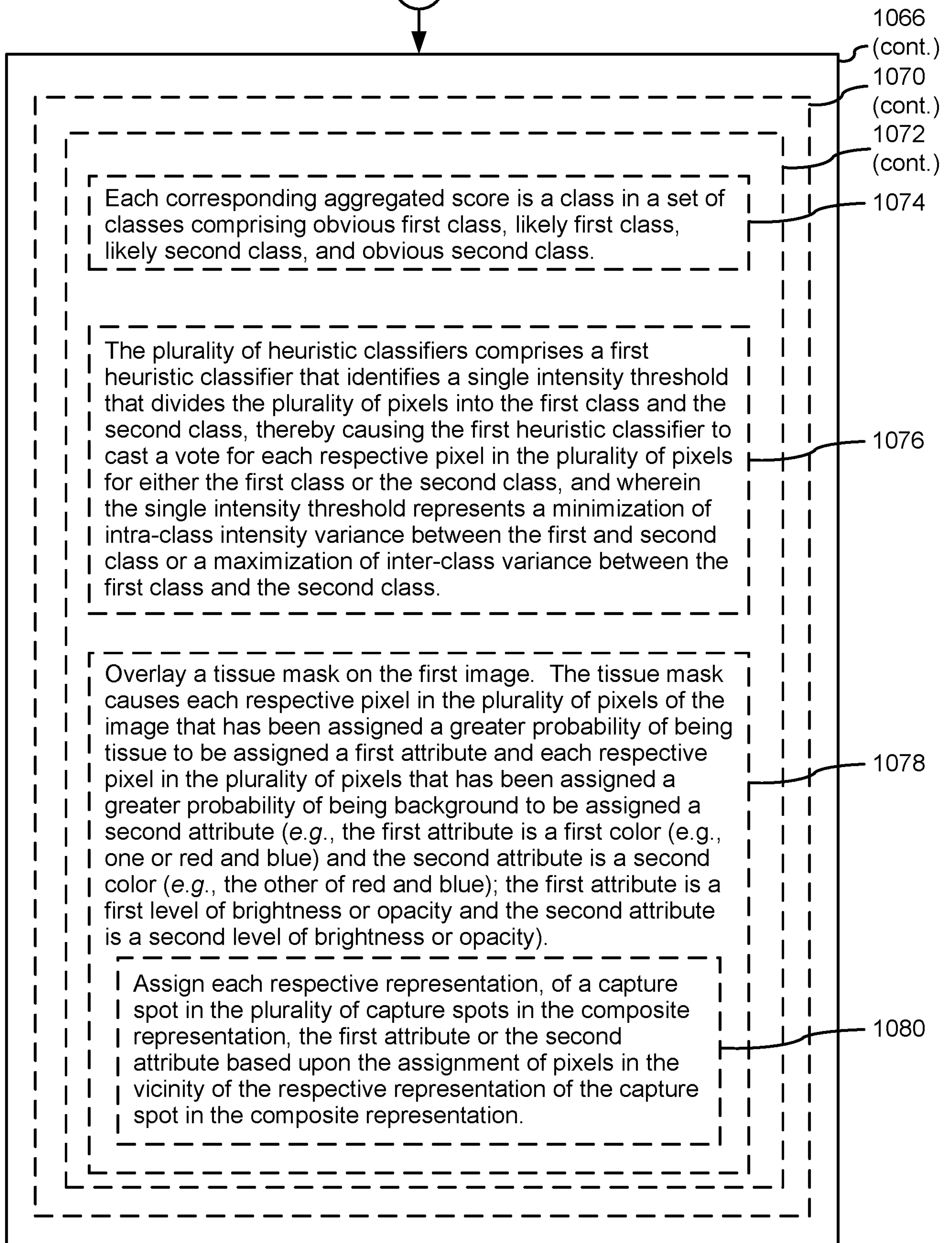


Figure 10F

17/70

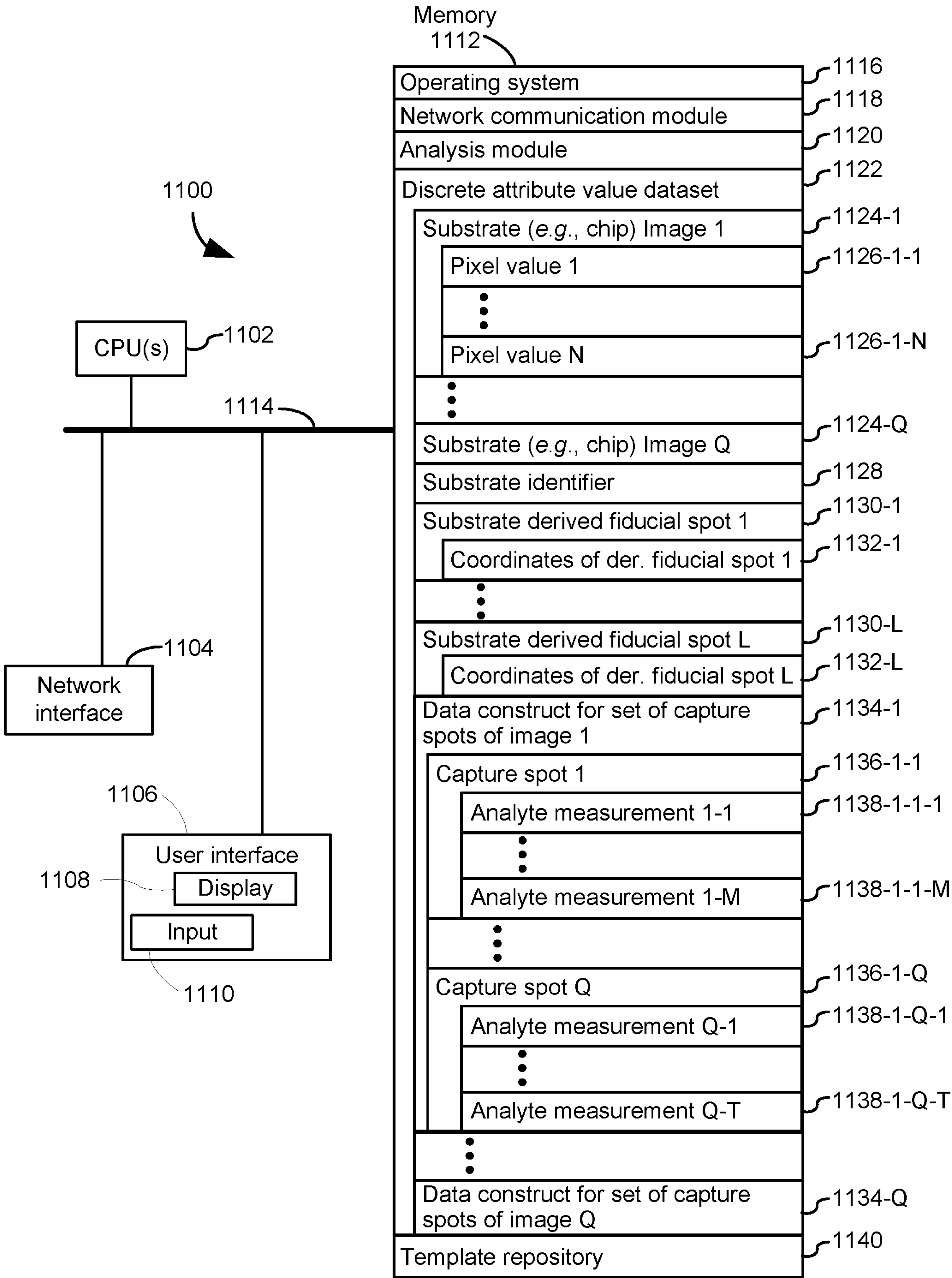


Figure 11A

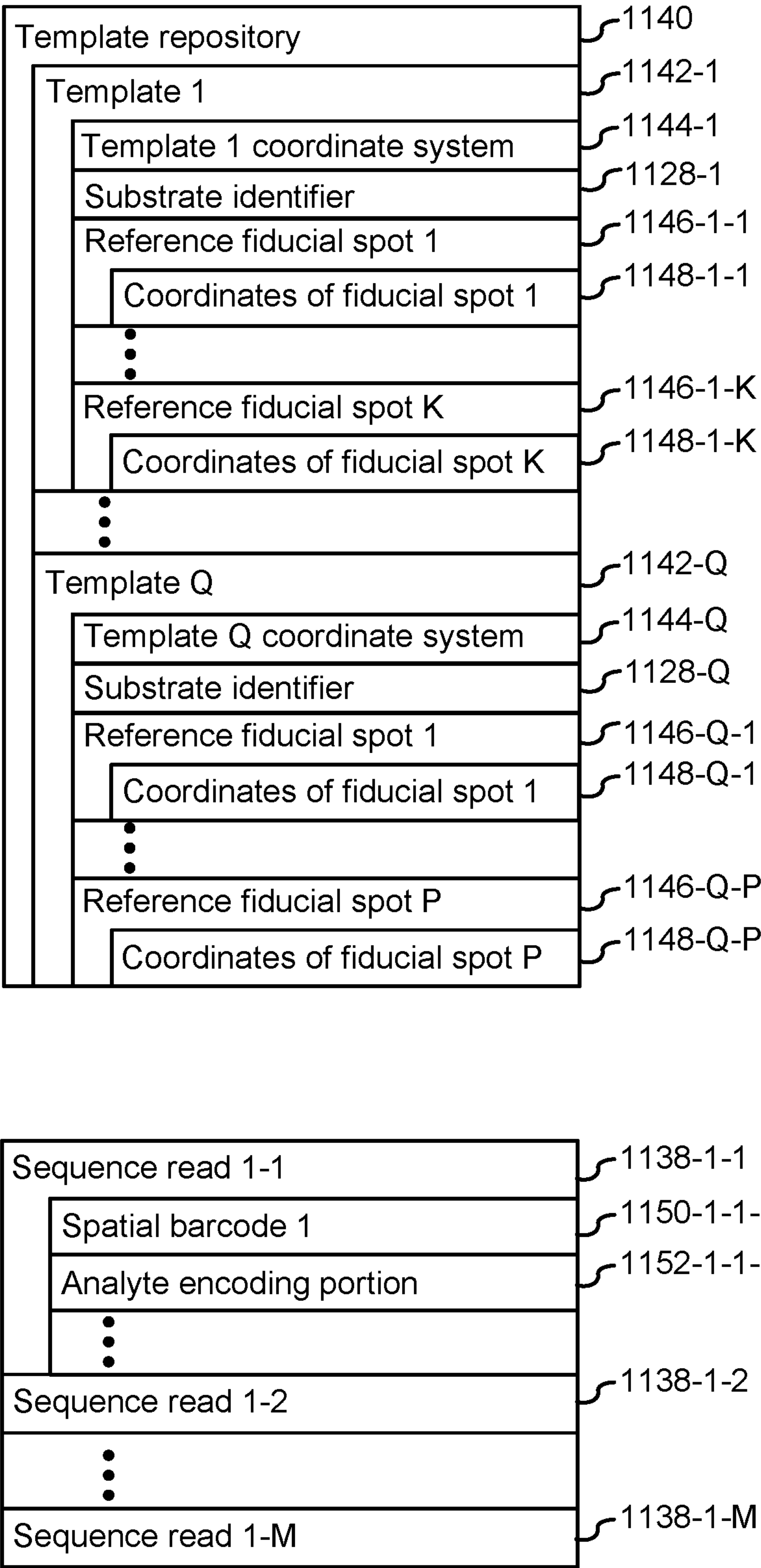


Figure 11B

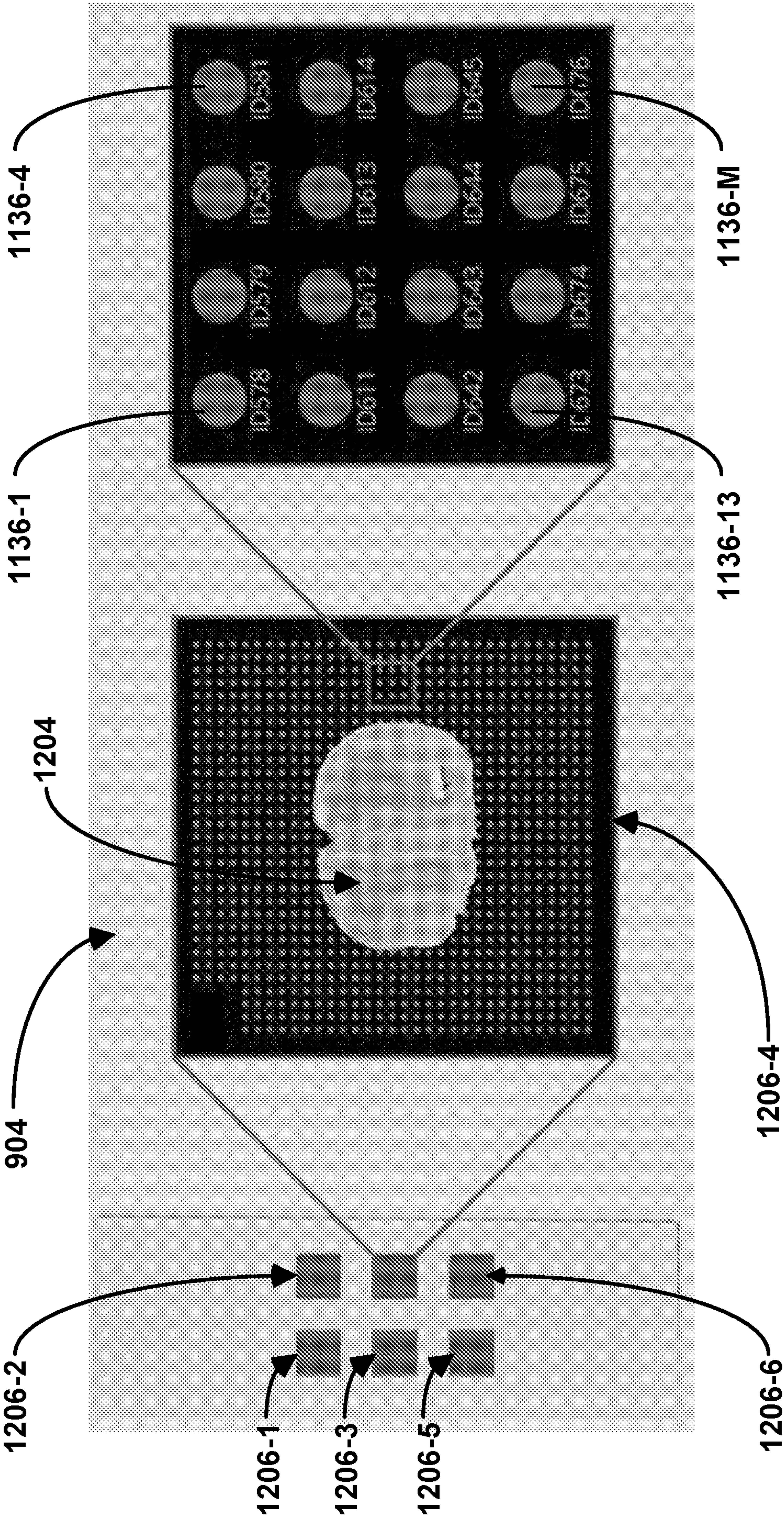


Figure 12

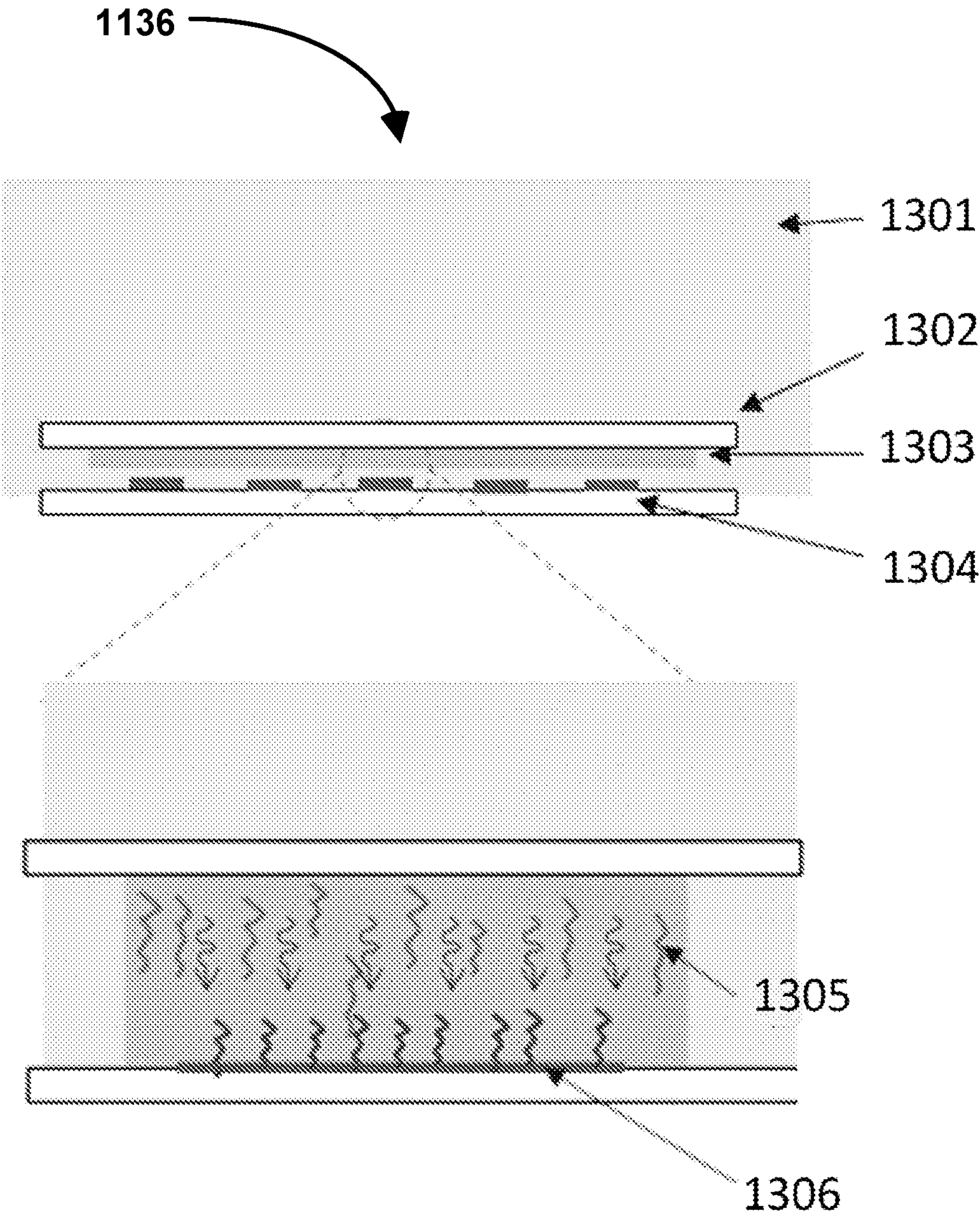


Figure 13

21/70

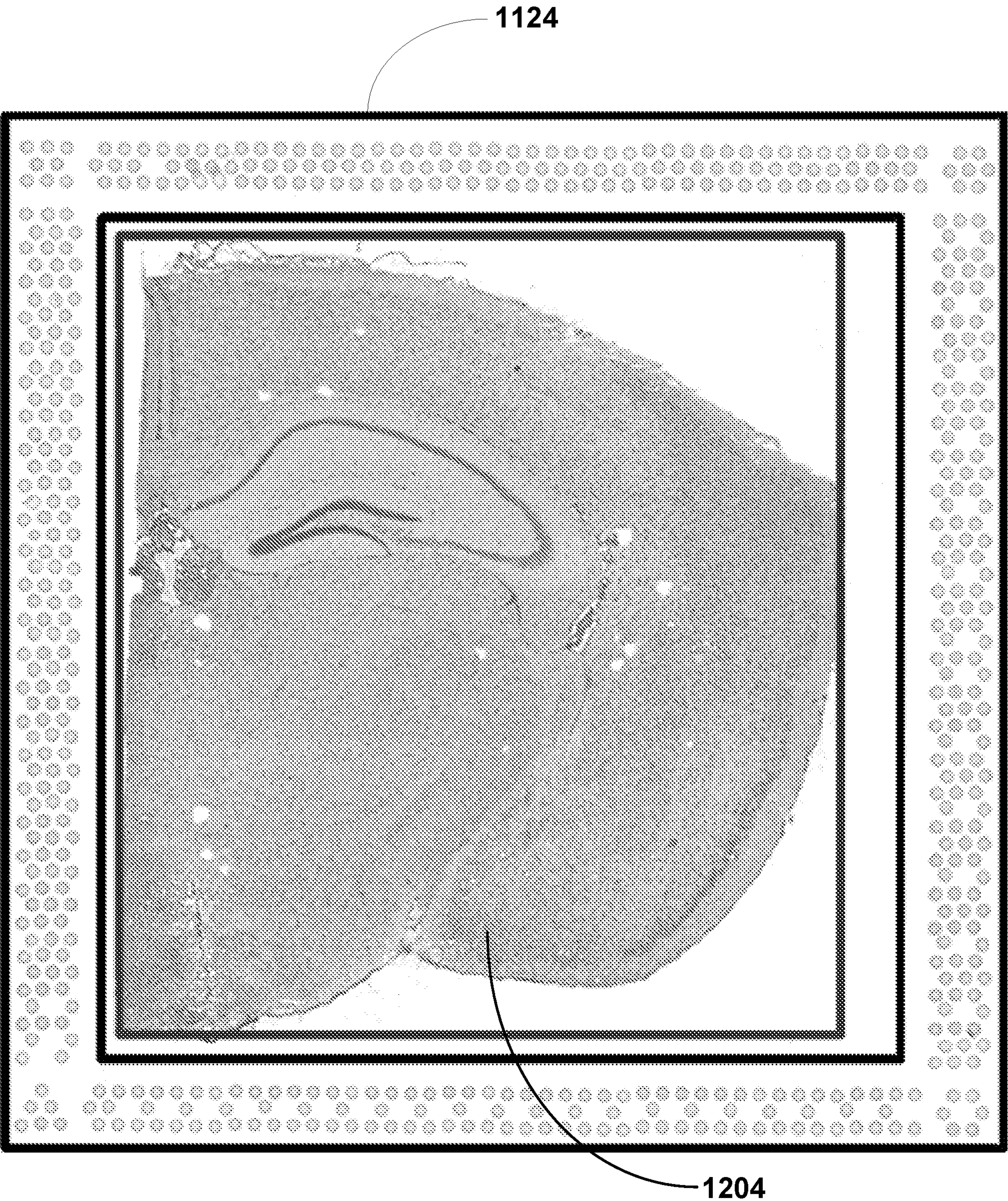


Figure 14

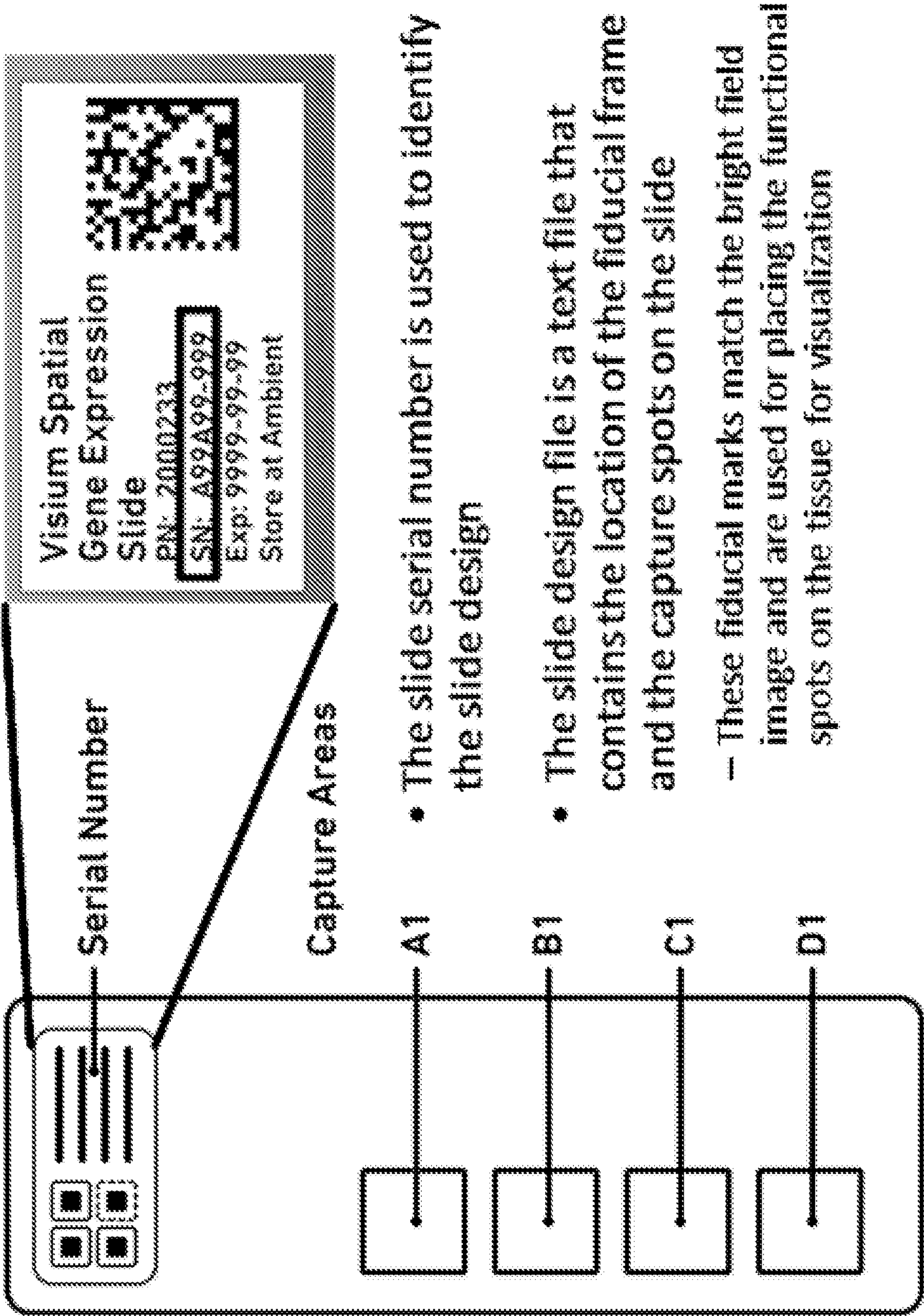


Figure 15

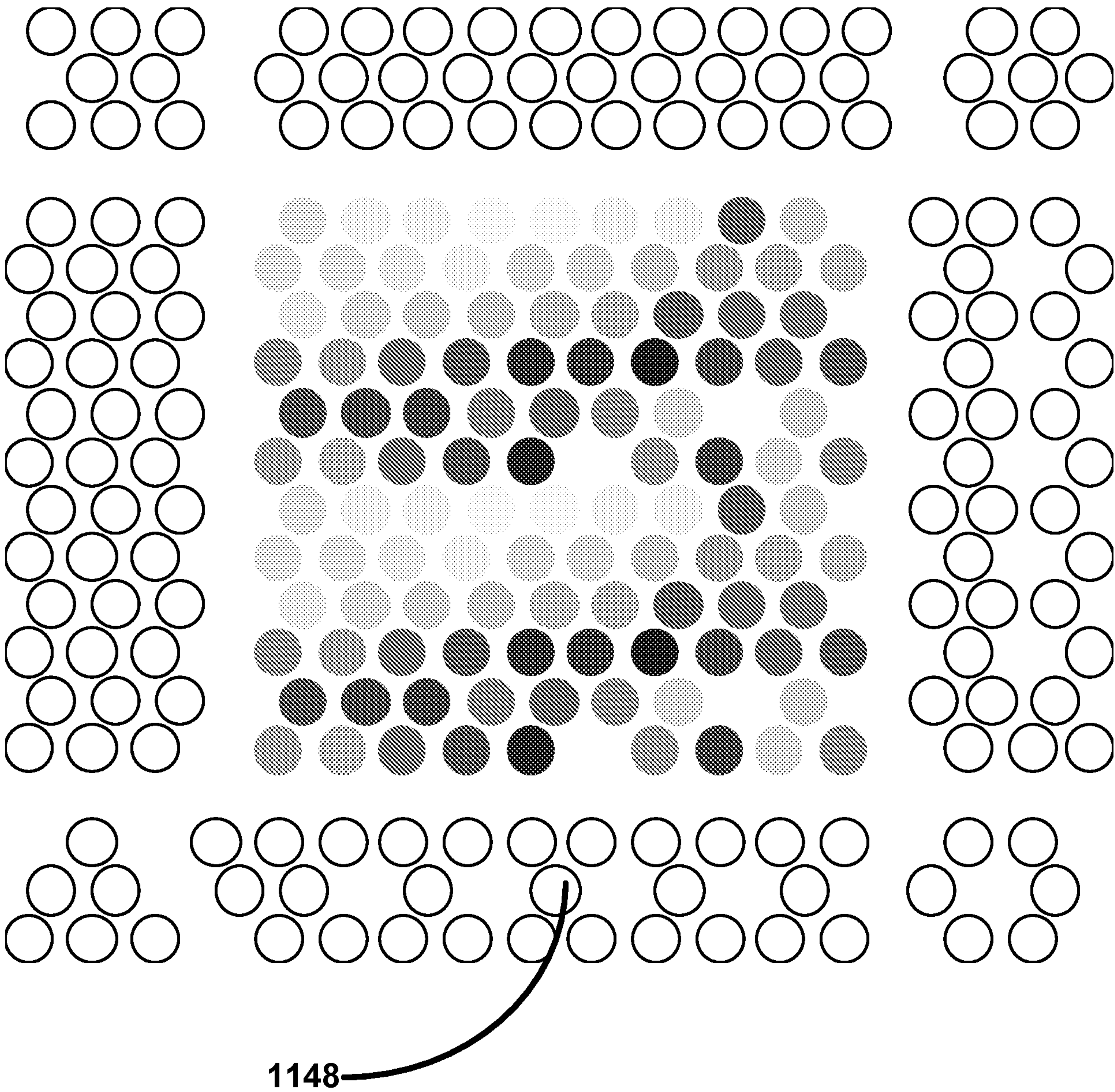


Figure 16

Align Spots
Identify Spots under Tissue
Extract Barcode, UMI
Align Reads
Correct Barcodes
Filter UMIs
Count UMIs
Secondary Analysis

- Bright field image contains the tissue in relation to the fiducial frame

1124

உயர்நீதிமன்றம்

1702-1

1702-2-

1204

1702-

Figure 17

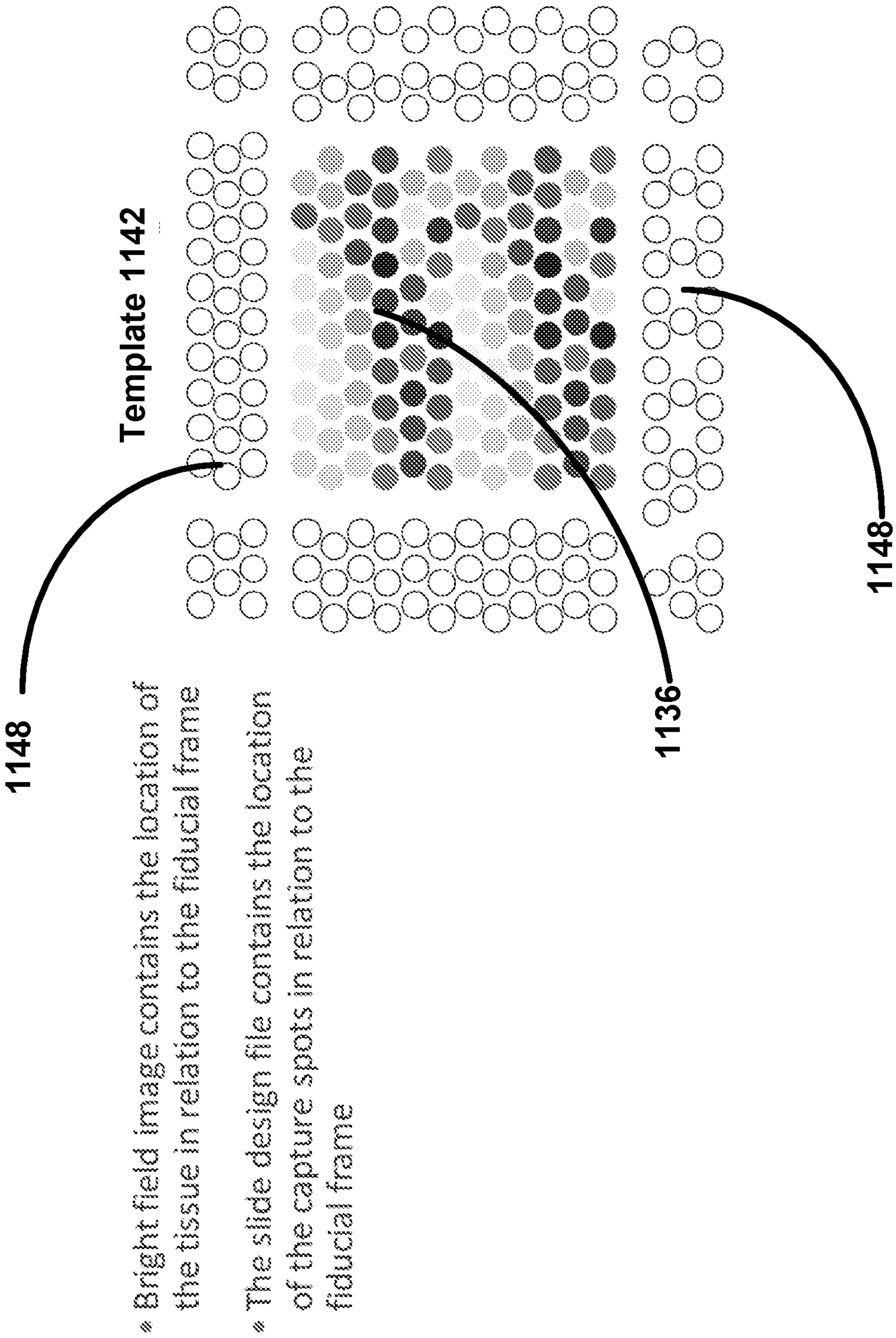
1142

Align Spots
Identify Spots under Tissue
Extract Barcode, UMI
Align Reads
Correct Barcodes
Filter UMIs
Count UMIs
Secondary Analysis

- » Bright field image contains the location of the tissue in relation to the fiducial frame
- » The slide design file (template) contains the location of the capture spots in relation to the fiducial frame

ATF	1				
20	5				
"Type=GenePix ArrayList V1.0"					
"BlockCount=16"					
"BlockType=2"					
"ArrayRevision=A"					
"Block1= 4288, 29652, 105, 100, 75, 30, 260"					
"Block2= 4825, 30073, 70, 128, 50, 39, 174"					
"Block3= 13288, 29652, 105, 100, 75, 30, 260"					
"Block4= 13825, 30073, 70, 128, 50, 39, 174"					
"Block5= 4288, 38652, 105, 100, 75, 30, 260"					
"Block6= 4825, 39073, 70, 128, 50, 39, 174"					
"Block7= 13288, 38652, 105, 100, 75, 30, 260"					
"Block8= 13825, 39073, 70, 128, 50, 39, 174"					
"Block9= 4288, 47652, 105, 100, 75, 30, 260"					
"Block10= 4825, 48073, 70, 128, 50, 39, 174"					
"Block11= 13288, 47652, 105, 100, 75, 30, 260"					
"Block12= 13825, 48073, 70, 128, 50, 39, 174"					
"Block13= 4288, 56652, 105, 100, 75, 30, 260"					
"Block14= 4825, 57073, 70, 128, 50, 39, 174"					
"Block15= 13288, 56652, 105, 100, 75, 30, 260"					
"Block16= 13825, 57073, 70, 128, 50, 39, 174"					
Block	Column	Row	ID	Name	
1	1	1	F	FRAME	
1	2	1	F	FRAME	
1	3	1	F	FRAME	
1	4	1	F	FRAME	
1	5	1	F	FRAME	
1	6	1	EMPTY	EMPTY	

Figure 18



Align Spots
Identify Spots under Tissue
Extract Barcode, UMI
Align Reads
Correct Barcodes
Filter UMIs
Count UMIs
Secondary Analysis

Figure 19

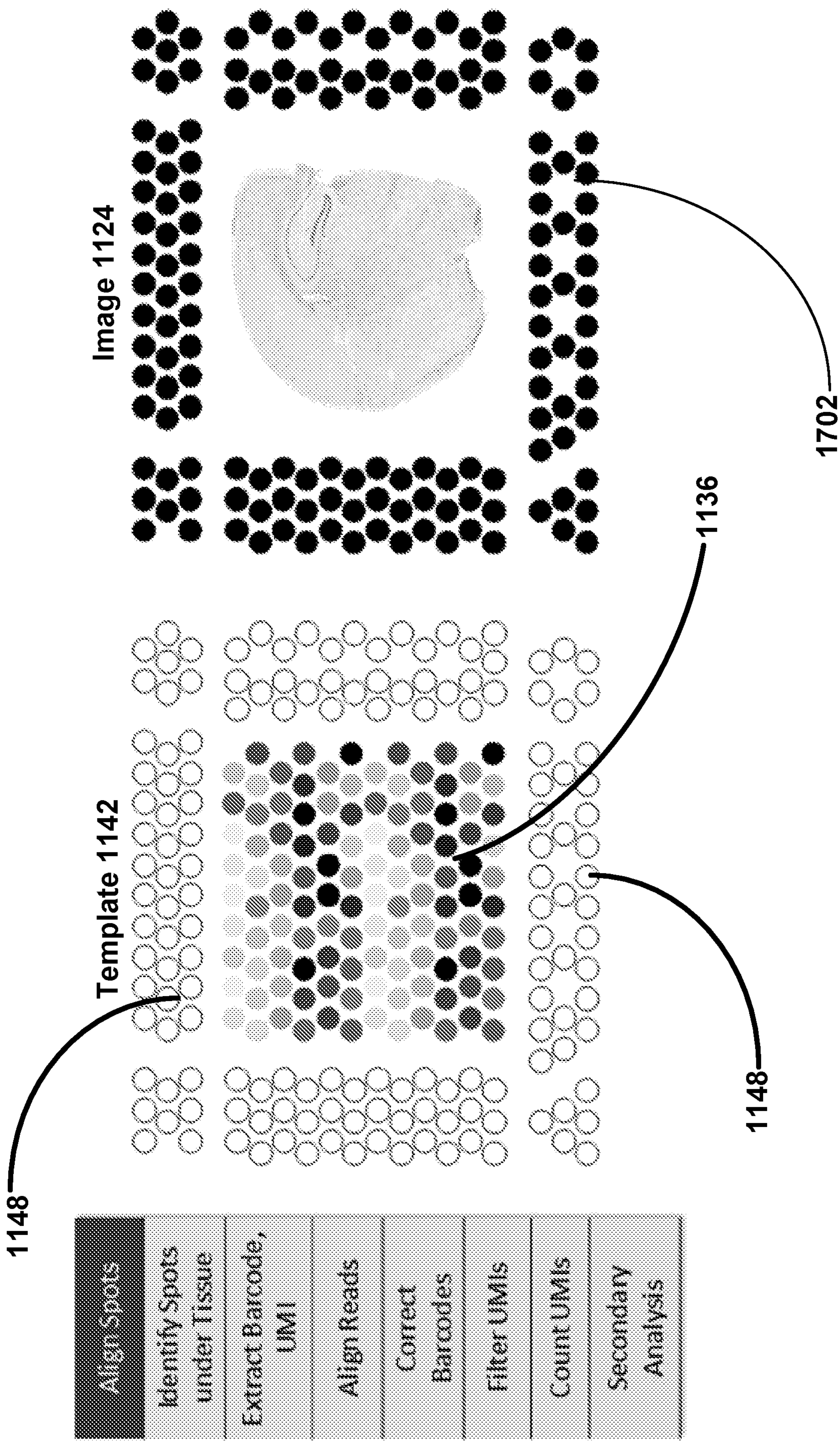
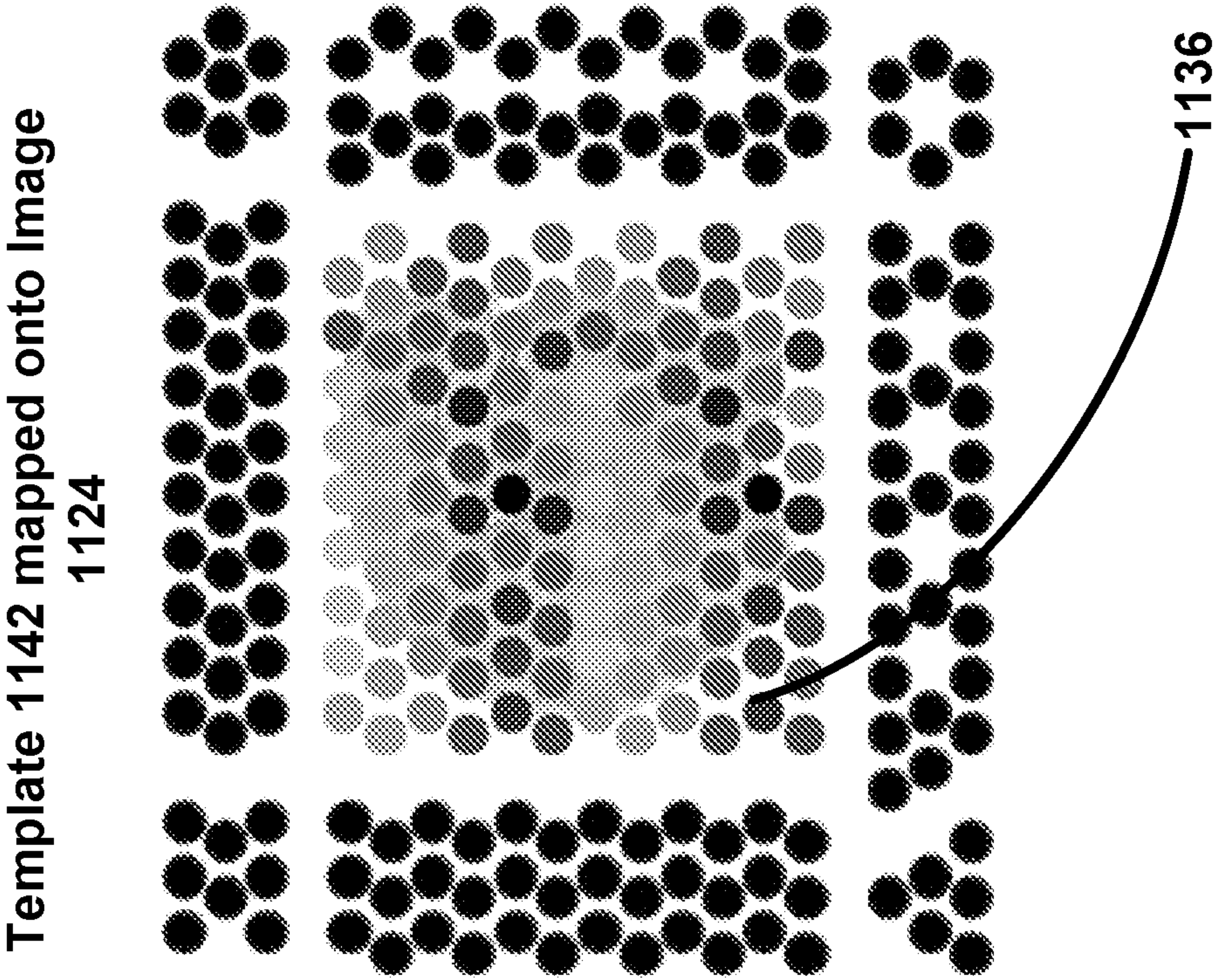


Figure 20



- Bright field image contains the location of the tissue in relation to the fiducial frame
- The slide design file contains the location of the active spots in relation to the fiducial frame
- Using the fiducial frame that is common to both inputs the pipeline can overlay the exact location of the active spots on the tissue
 - I.E Space Ranger uses the slide design to draw the active spots on the tissue

Align Spots
Identify Spots under Tissue
Extract Barcode, UMI
Align Reads
Correct Barcodes
Filter UMIs
Count UMIs
Secondary Analysis

Figure 21

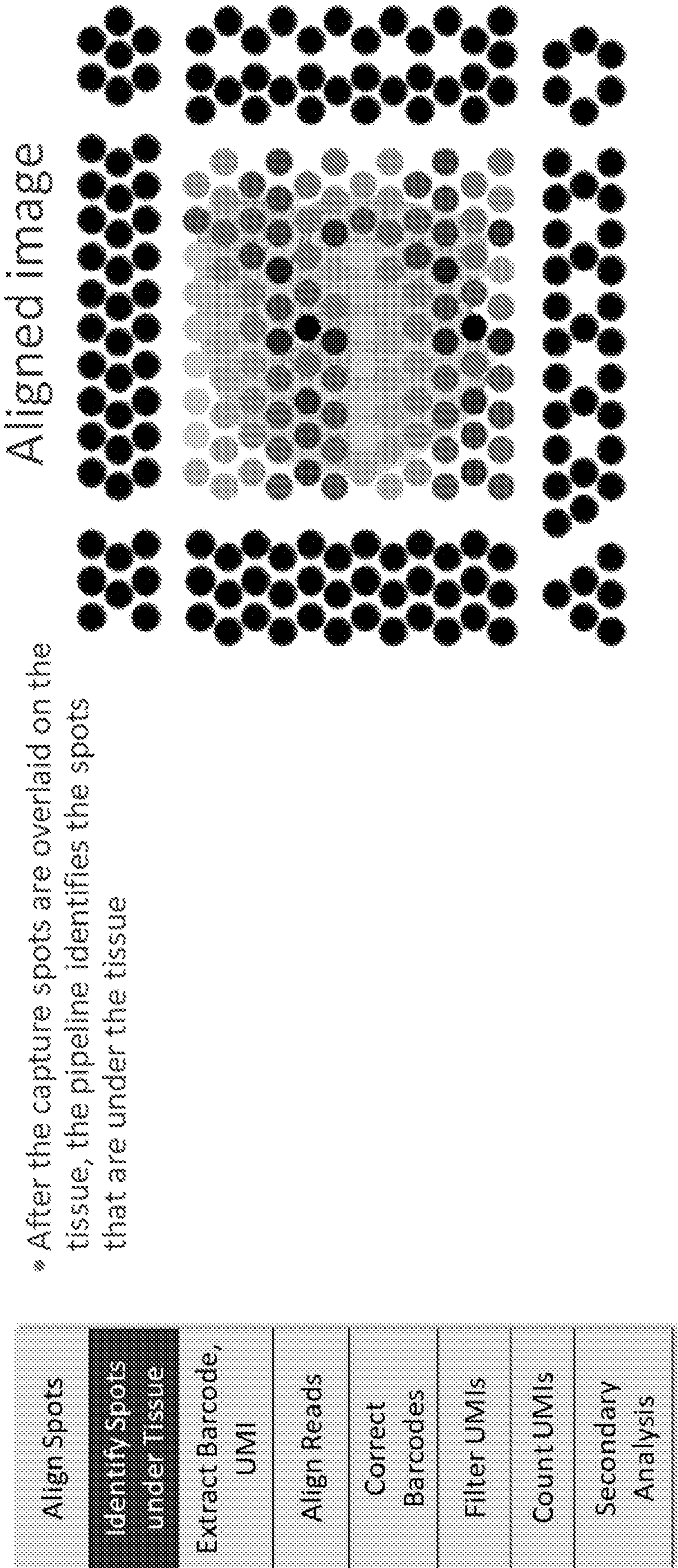
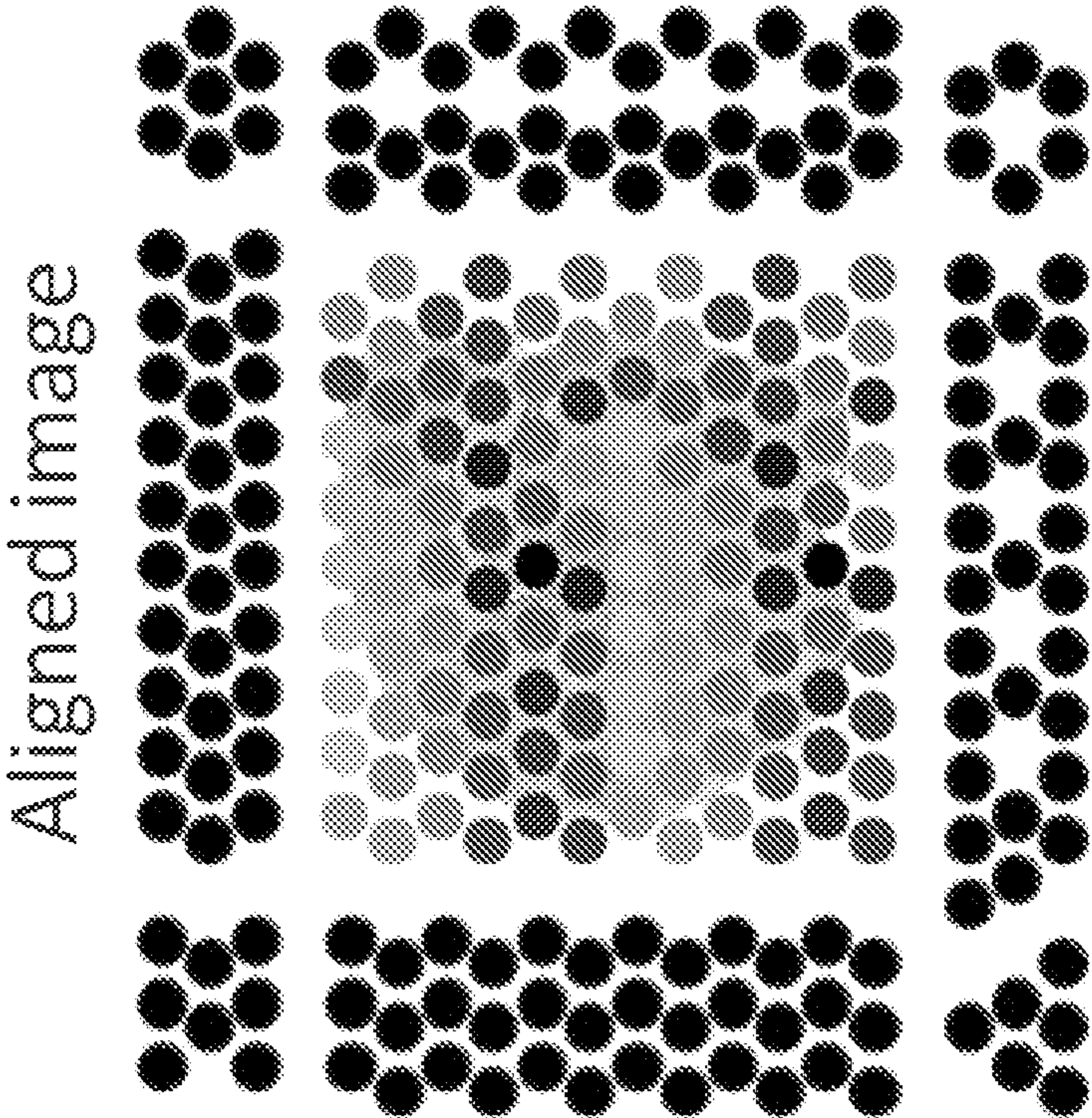


Figure 22



- » After the capture spots are overlaid on the tissue, the pipeline identifies the spots that are under the tissue
- » Once identified the spots outside of the tissue are excluded from further analysis
- » The spots under the tissue are used to generate a filtered barcode matrix used in secondary analysis
- » The input image is then broken into tiles for rapid viewing (similar to indexing a large text file)

Align Spots
Identify Spots under Tissue
Extract Barcode, UMI
Align Reads
Correct Barcodes
Filter UMIs
Count UMIs
Secondary Analysis

Figure 23

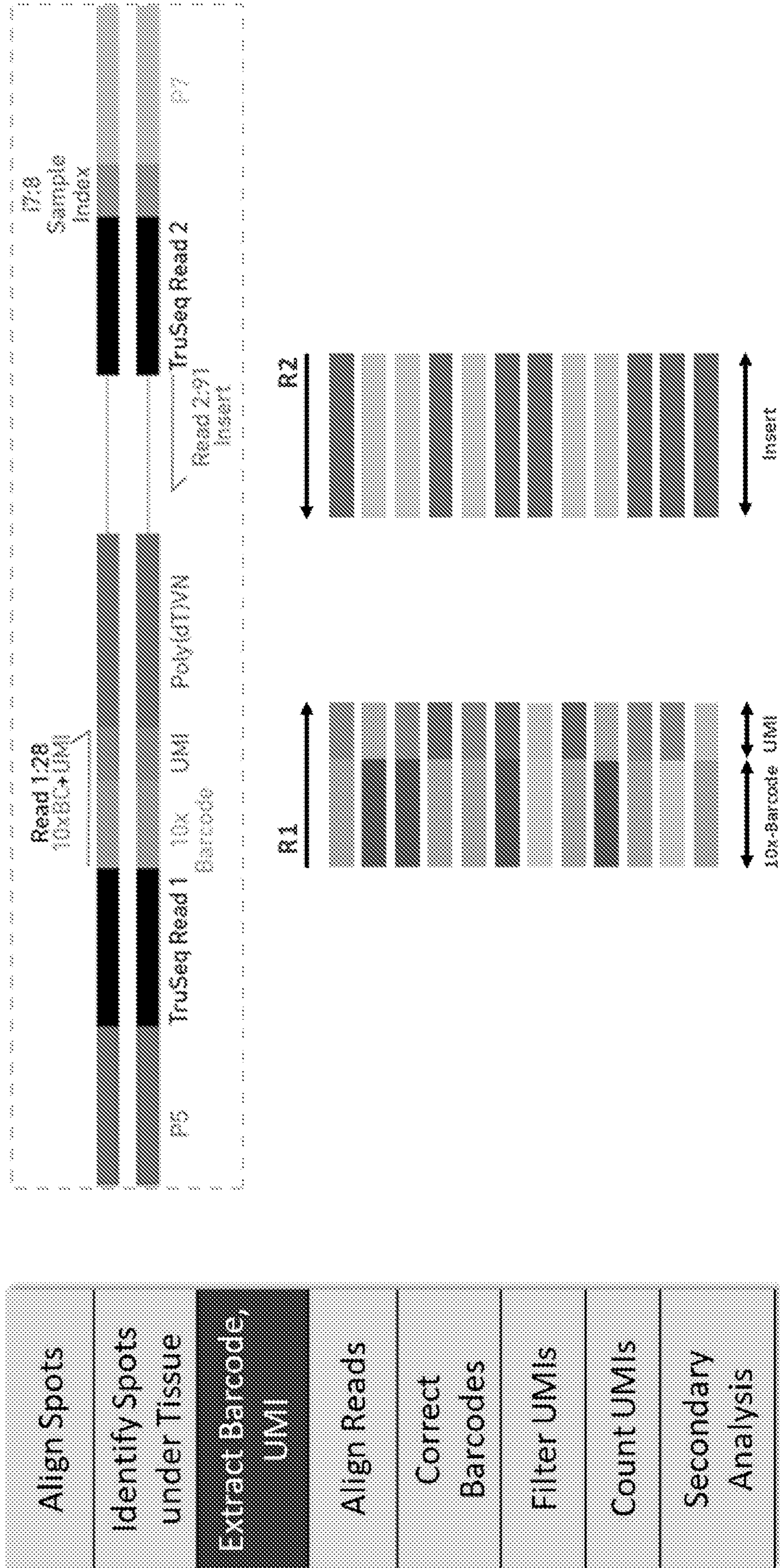


Figure 24

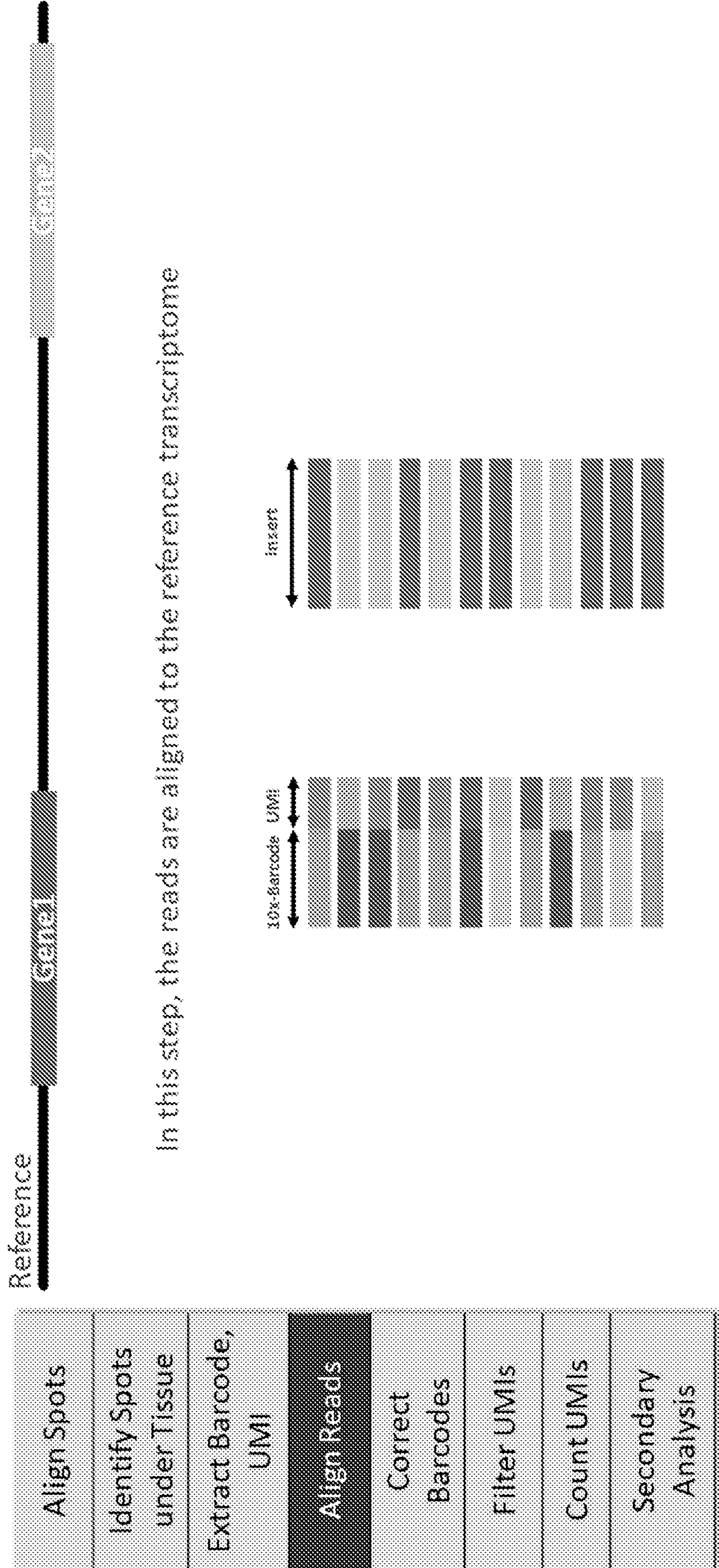
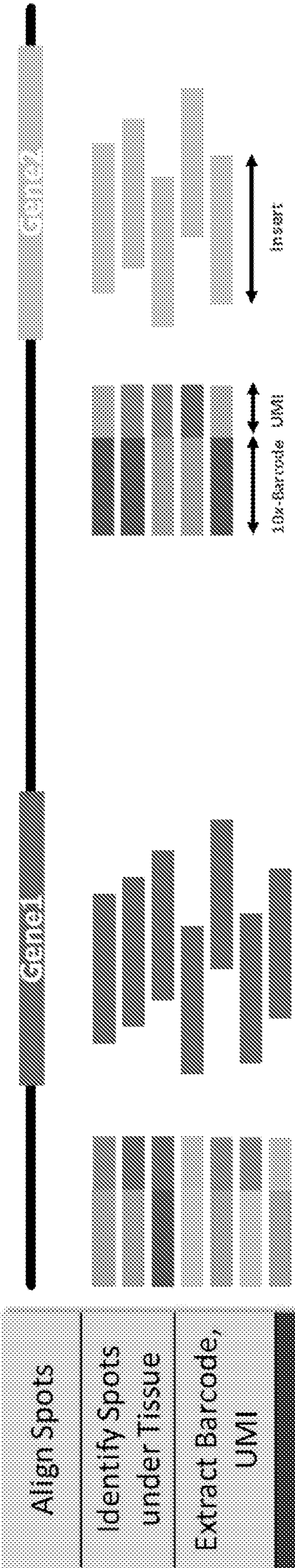


Figure 25



Alignment done via STAR (Spliced Transcripts Alignment to a Reference)

- Robust, open-source, junction-aware RNA-seq aligner
- Aligns reads to the genome and transcriptome simultaneously

Figure 26

Align Spots
Identify Spots under Tissue
Extract Barcode, UMI
Align Reads
Correct Barcodes
Filter UMIs
Count UMIs
Secondary Analysis

• Capture spot barcodes

- Must be on a list of known capture spot barcode sequences
- May allow 1 bp mismatch if the mismatch occurs at a low-quality position (the barcode is then corrected).

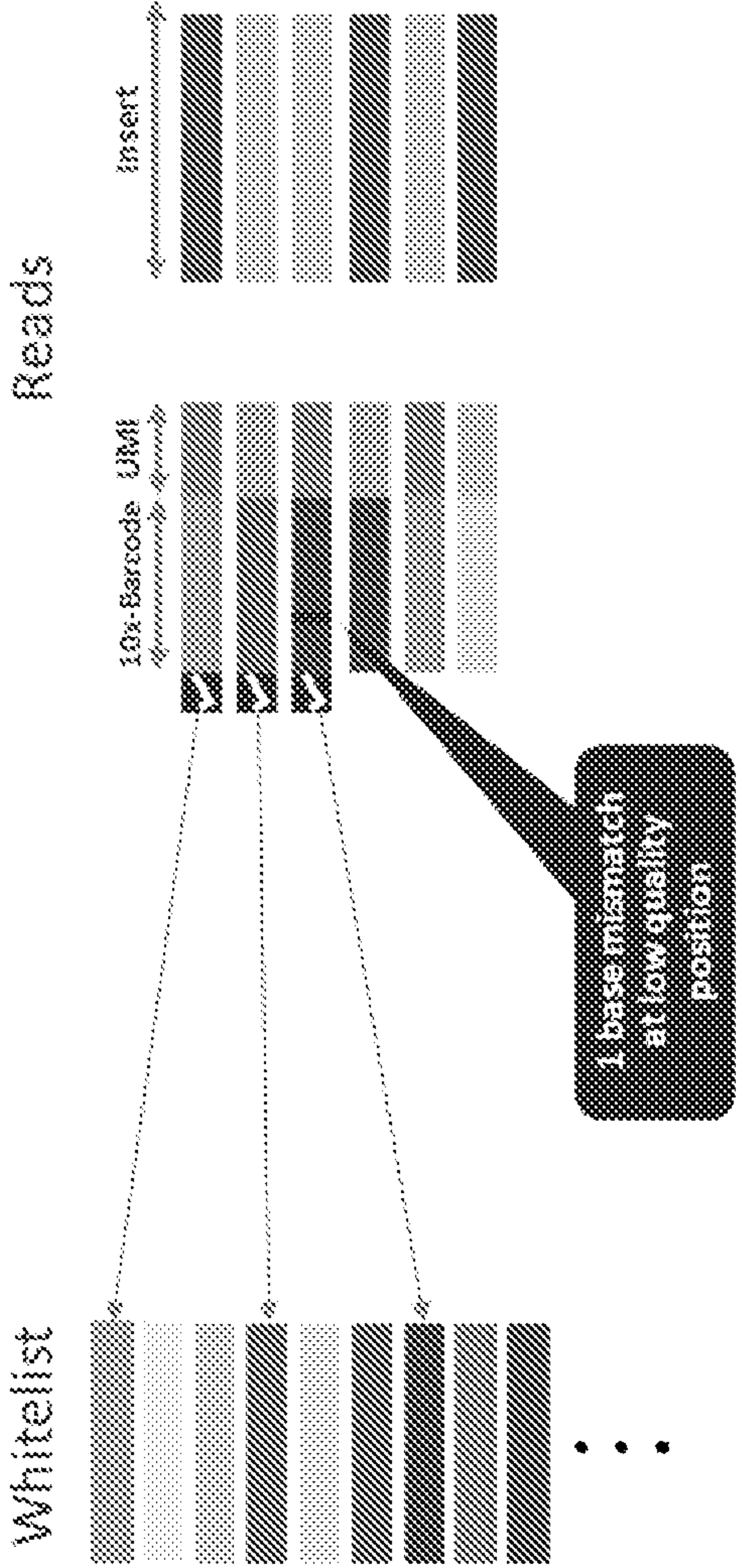


Figure 27

Align Spots
Identify Spots under Tissue
Extract Barcode, UMI
Align Reads
Correct Barcodes
Filter UMIs
Count UMIs
Secondary Analysis

- * Filter UMIs
 - Must not be a homopolymer, e.g. AAAAAAAAAA
 - Must not contain N
 - Must not contain bases with base quality < 10
- * Correct UMIs
 - UMIs that are 1 mismatch away from a higher count UMI are corrected to that UMI if they share a cell barcode and gene.

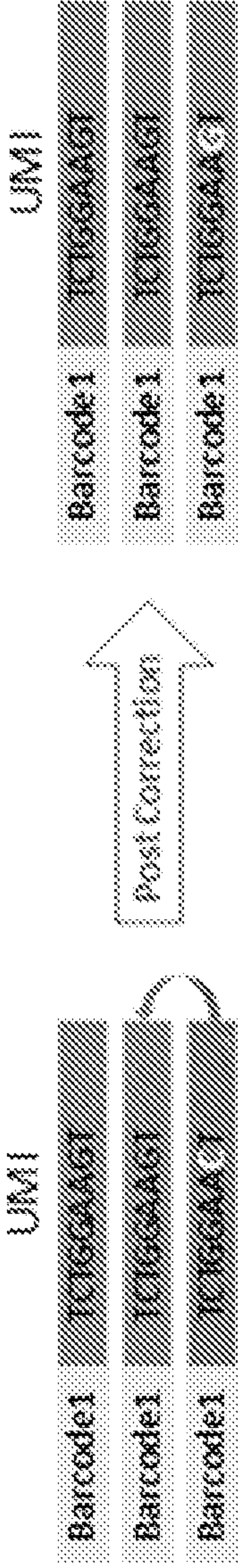
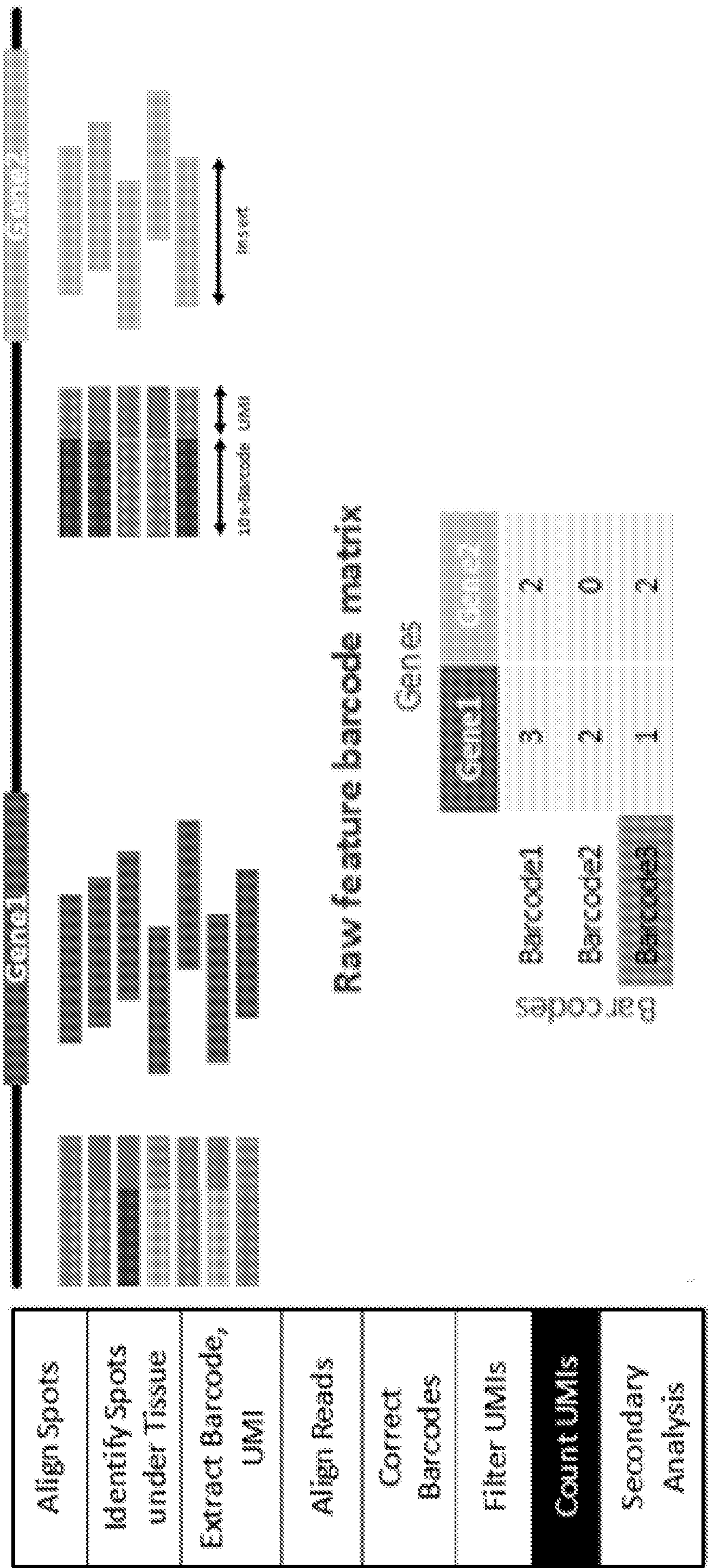


Figure 28



- * Only the confidently mapped reads with valid barcodes and UMIs are used for counting
- * Counting is done for all observed barcodes generating an unfiltered feature barcode matrix

Figure 29

Align Spots
Identify Spots under Tissue
Extract Barcode, UMI
Align Reads
Correct Barcodes
Filter UMIs
Count UMIs
Secondary Analysis

• Secondary analysis done only on spots under tissue

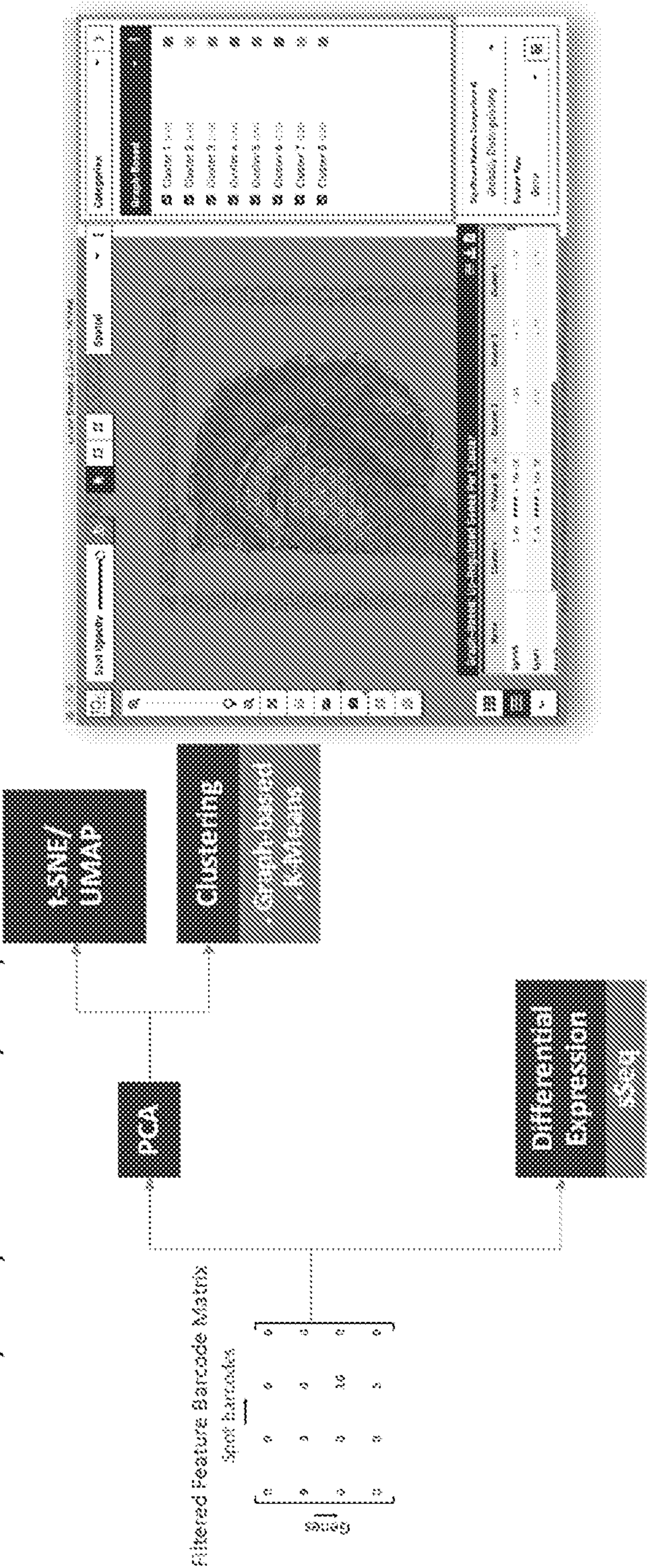


Figure 30

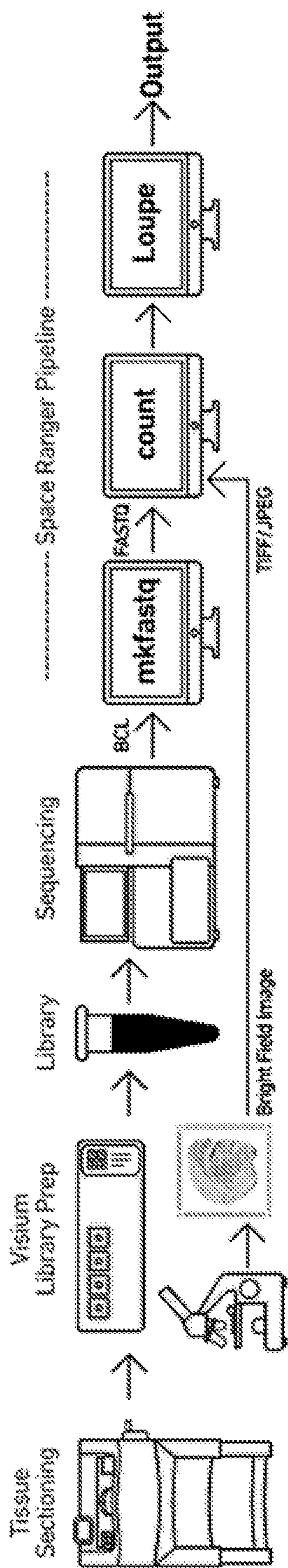


Figure 31

- View spot clusters in the context of the tissue image

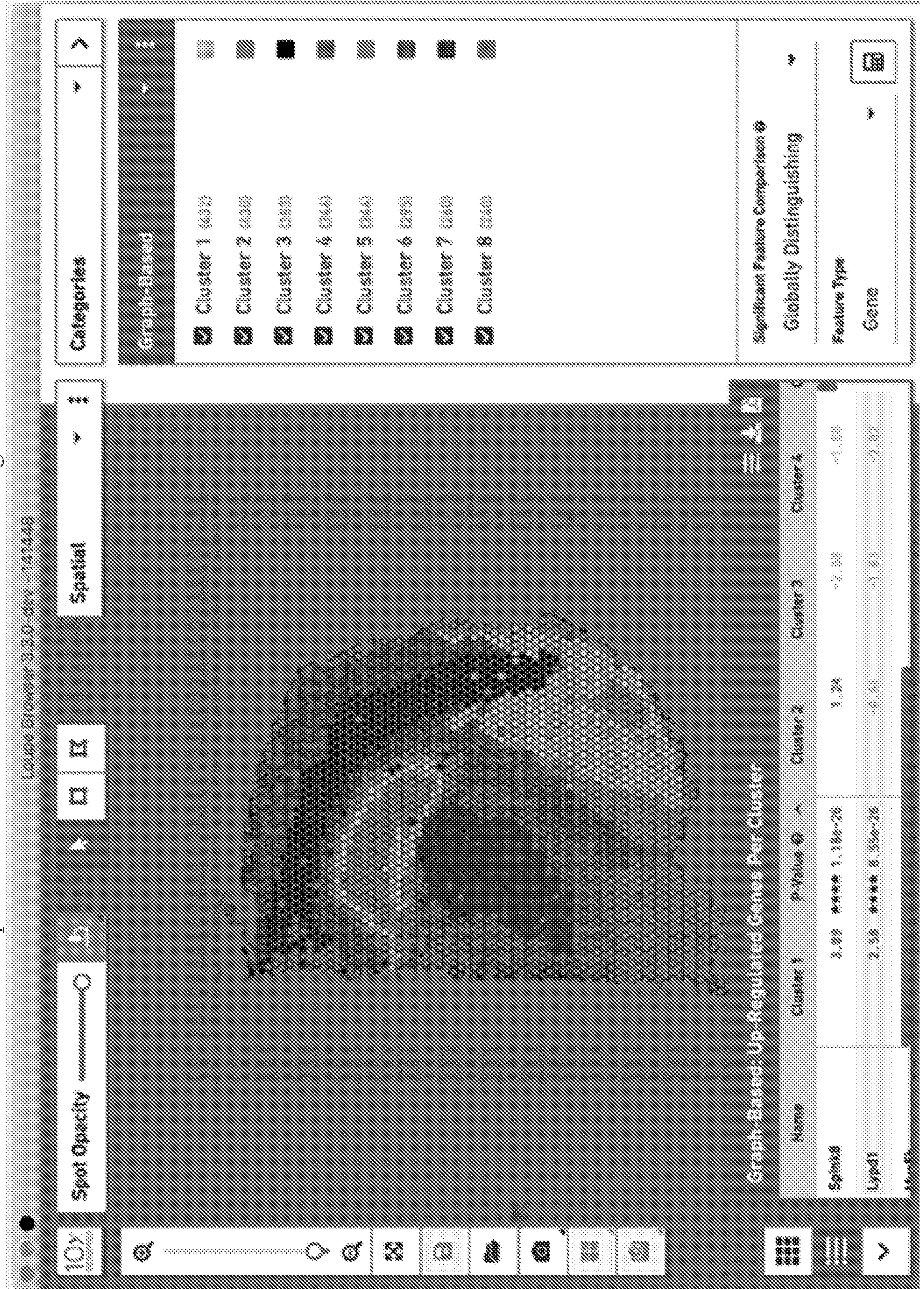


Figure 32

- Zoom in for more detail

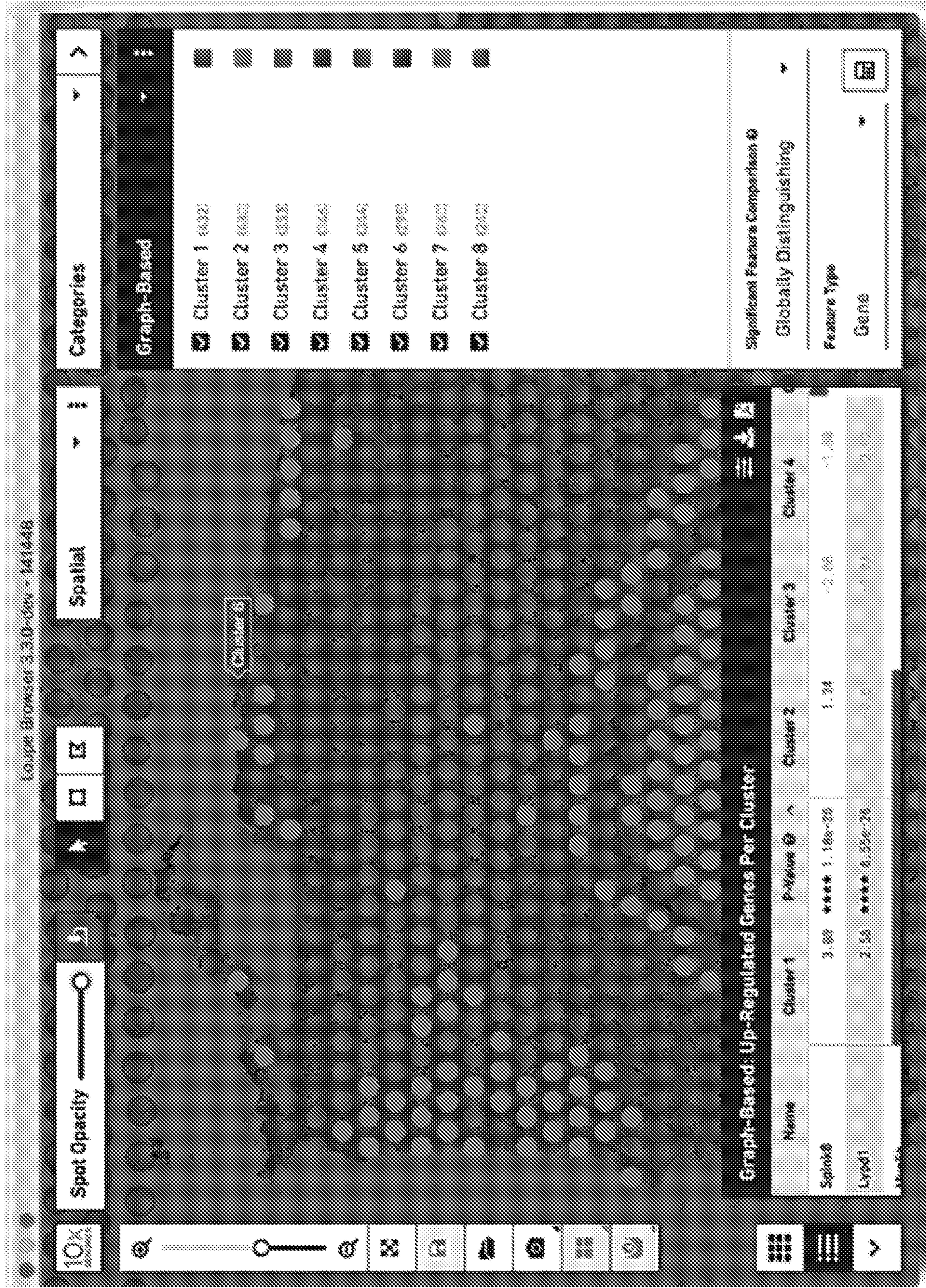


Figure 33

- Create custom categories and clusters for differential expression analysis

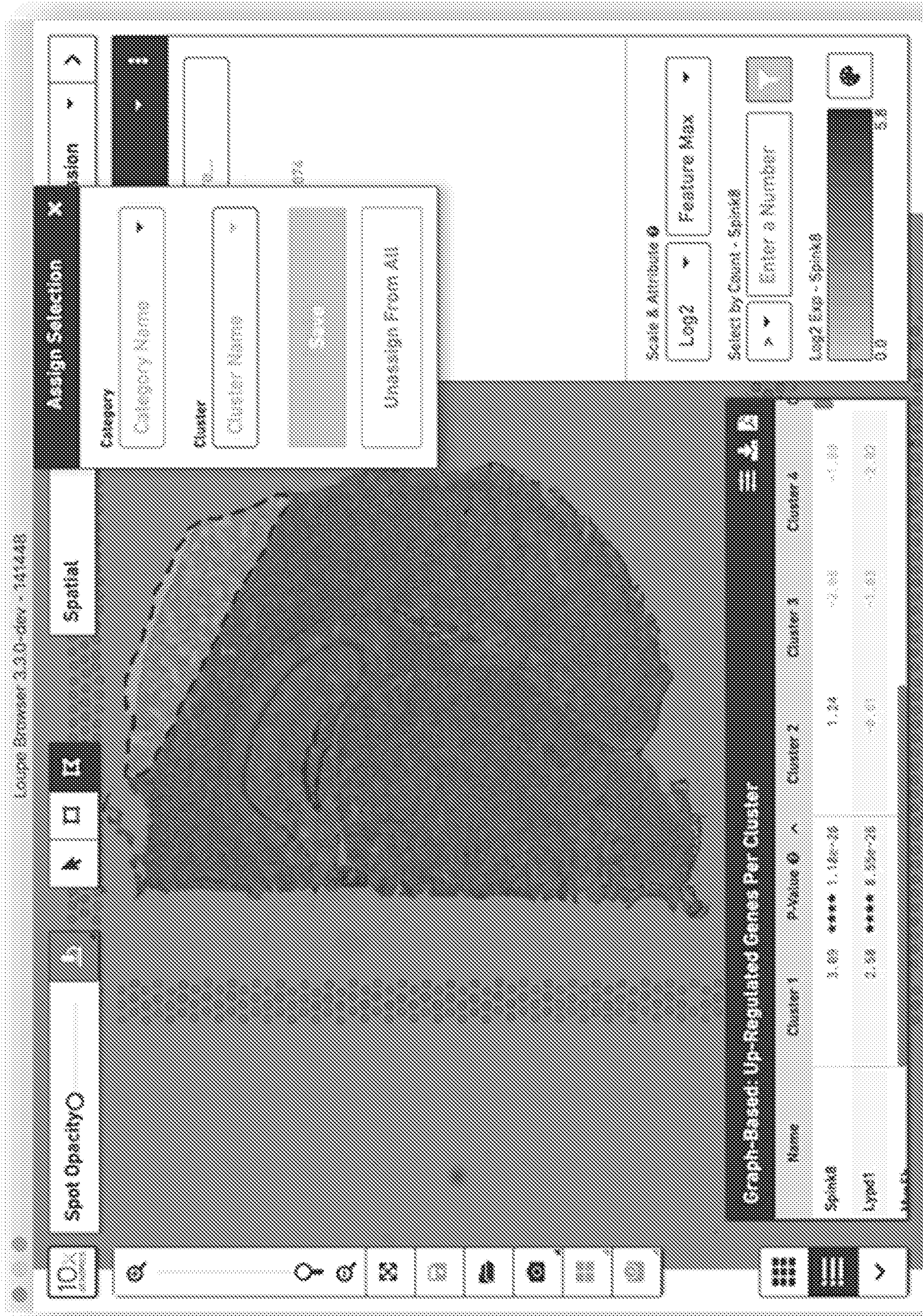


Figure 34

- View expressed genes in the context of the tissue image



Figure 35

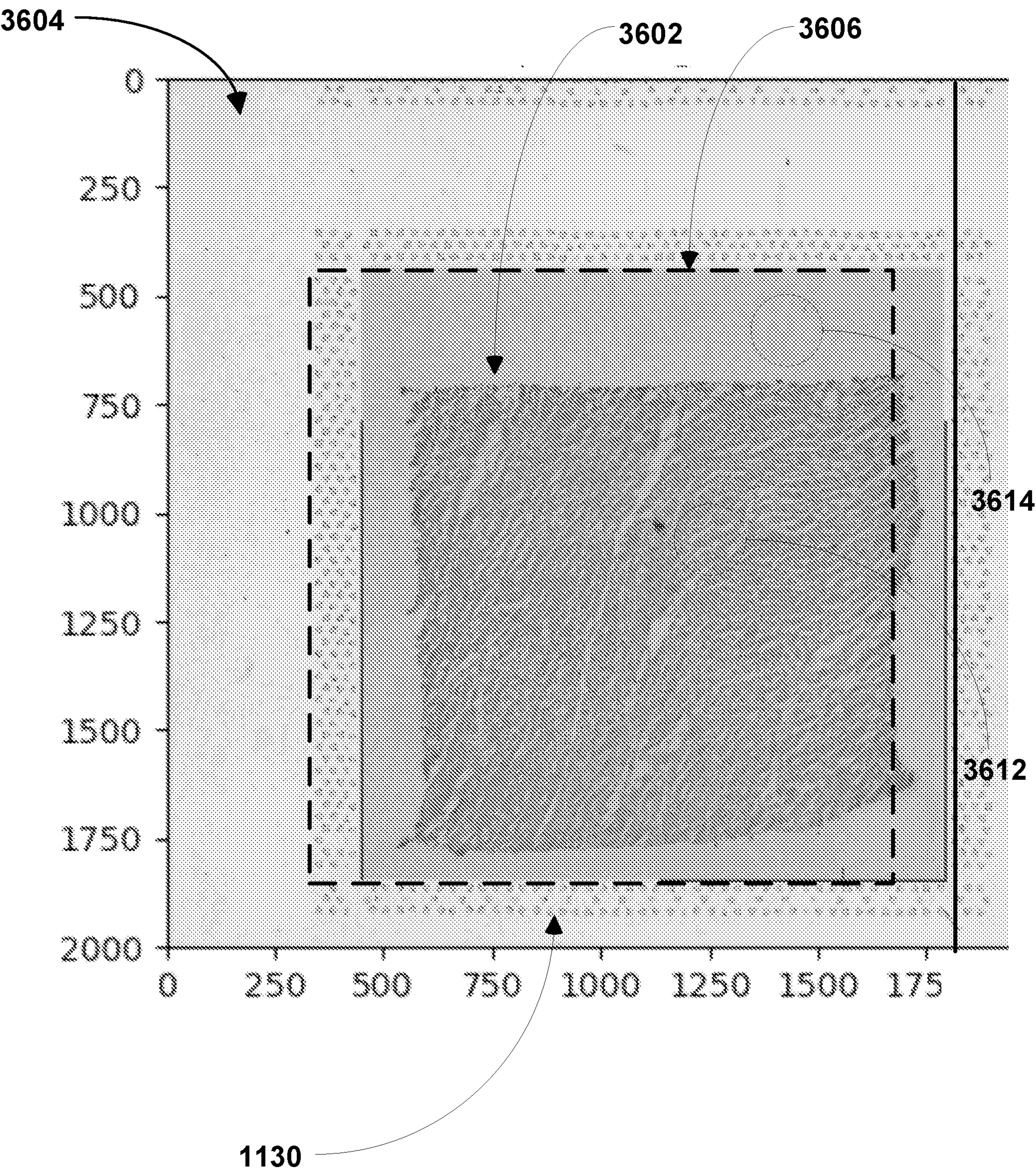


Figure 36A

44/70

3616

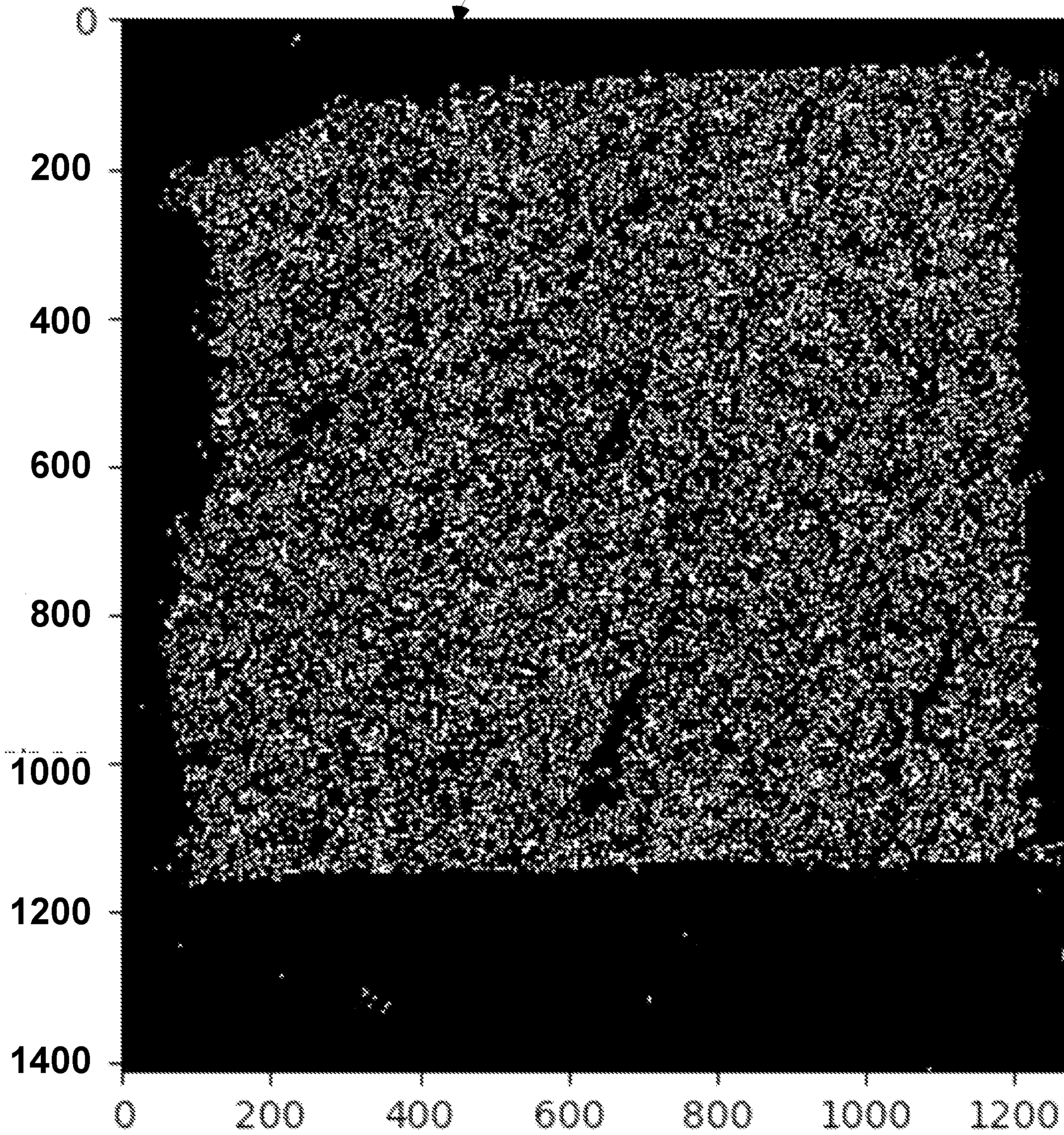


Figure 36B

45/70

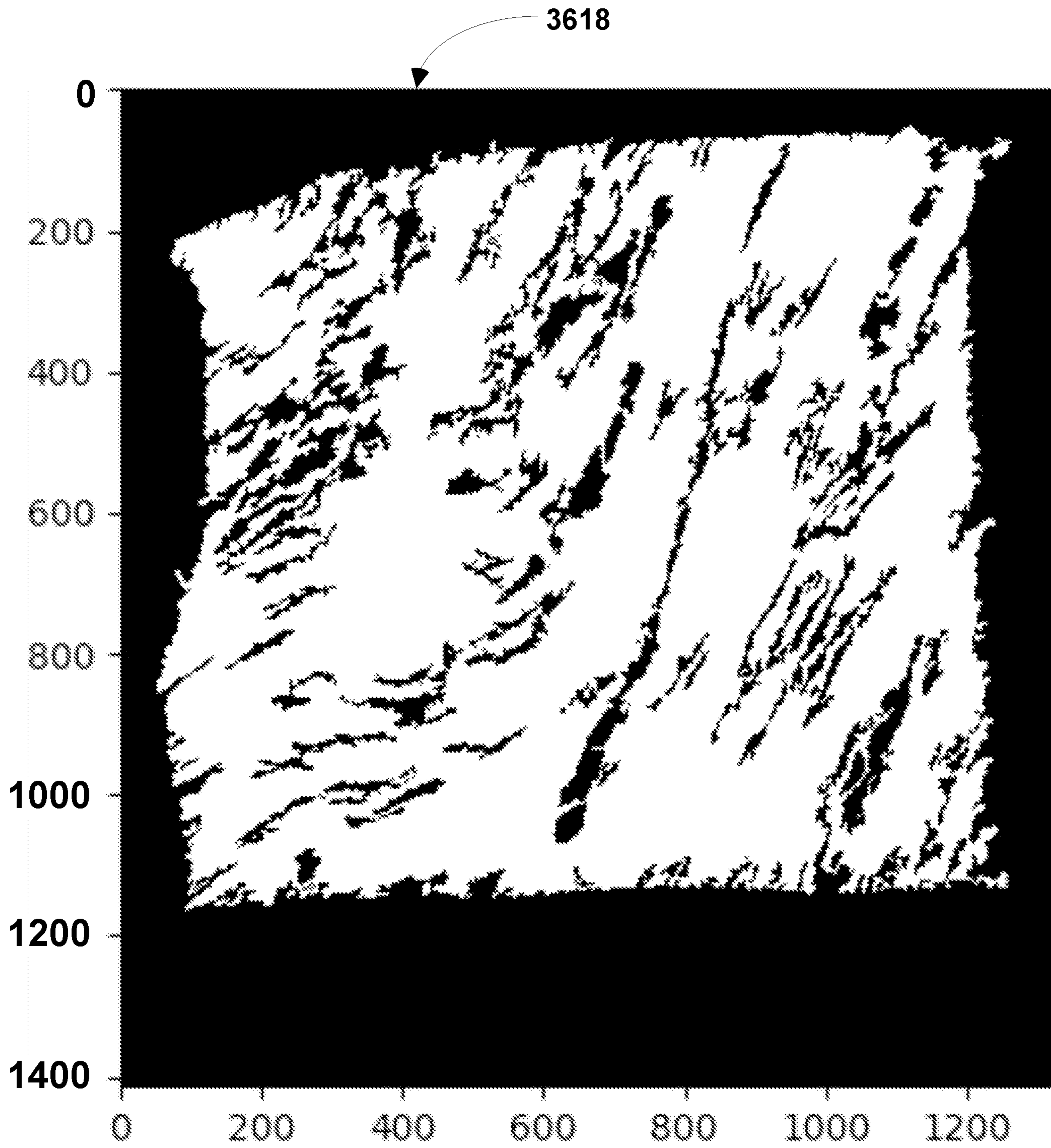


Figure 36C

46/70

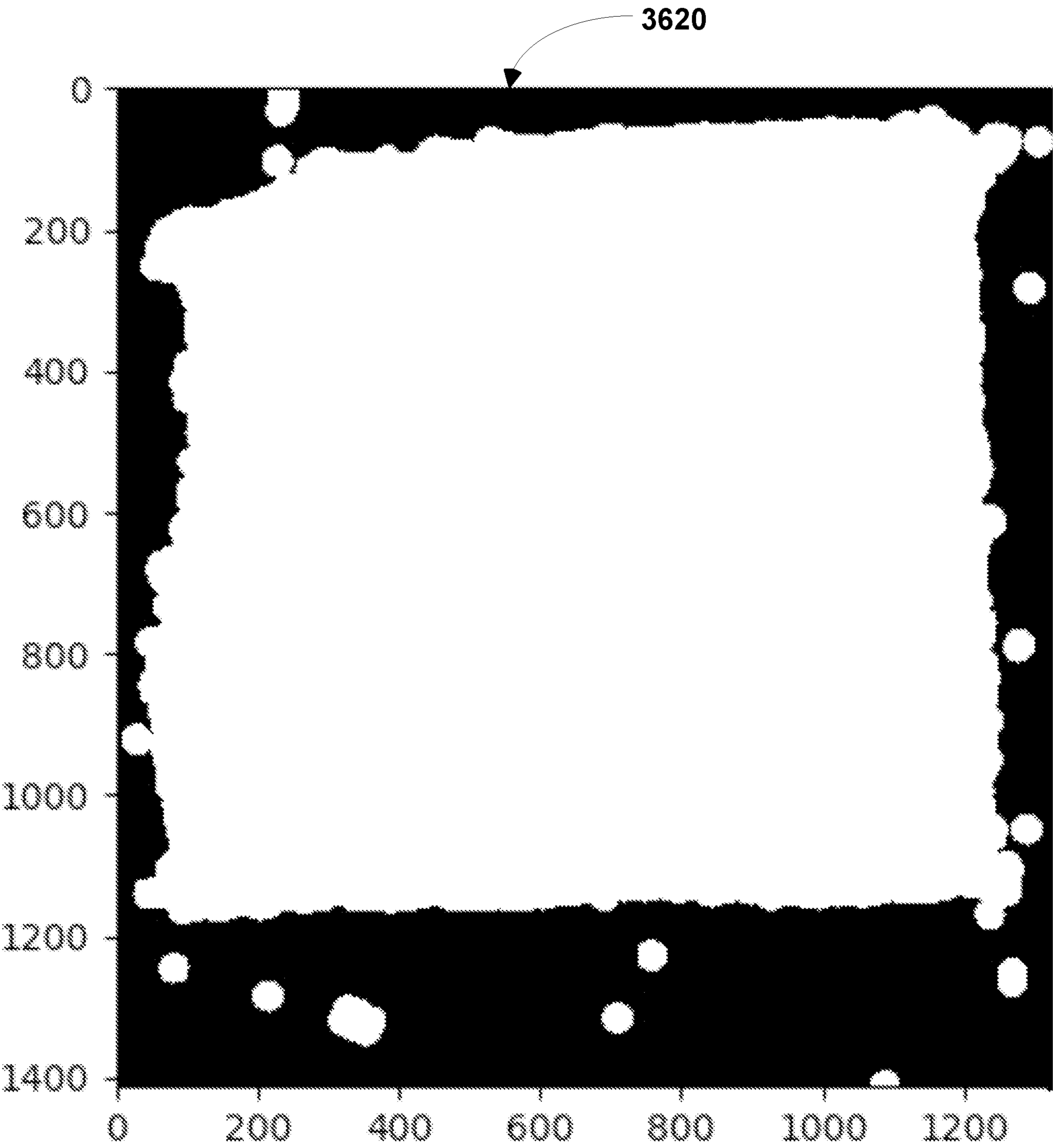


Figure 36D

47/70

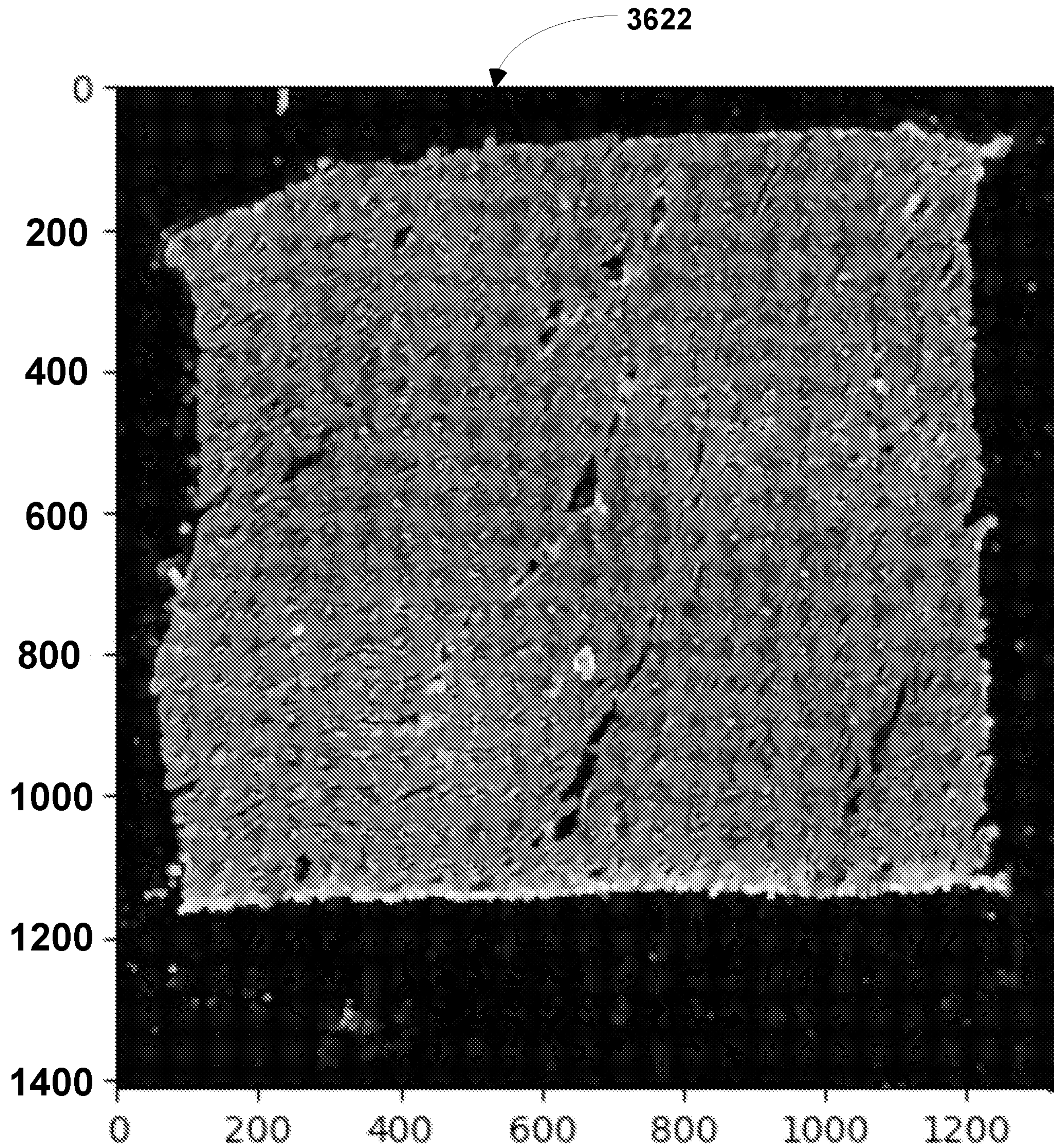


Figure 36E

48/70

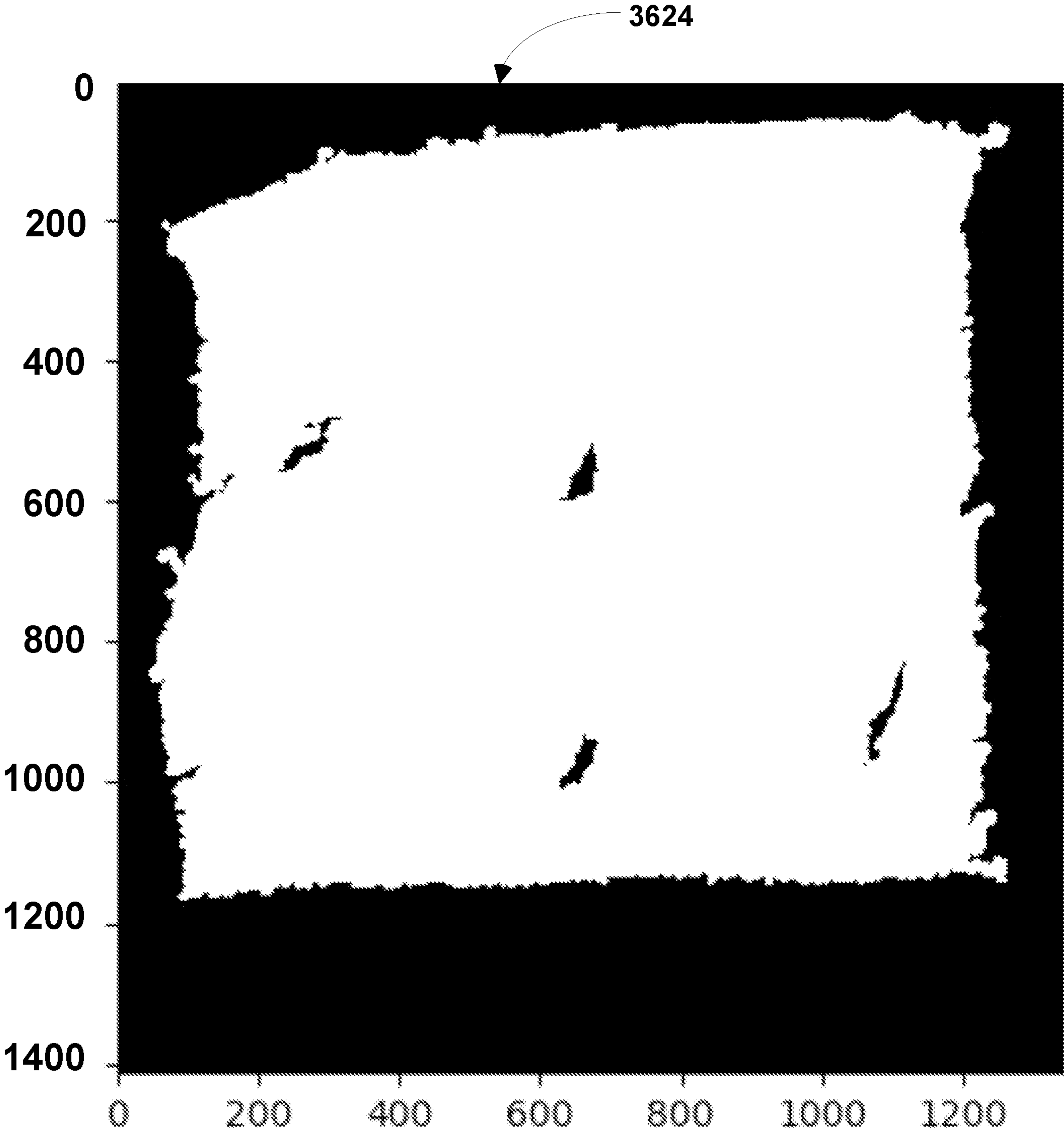


Figure 36F

49/70

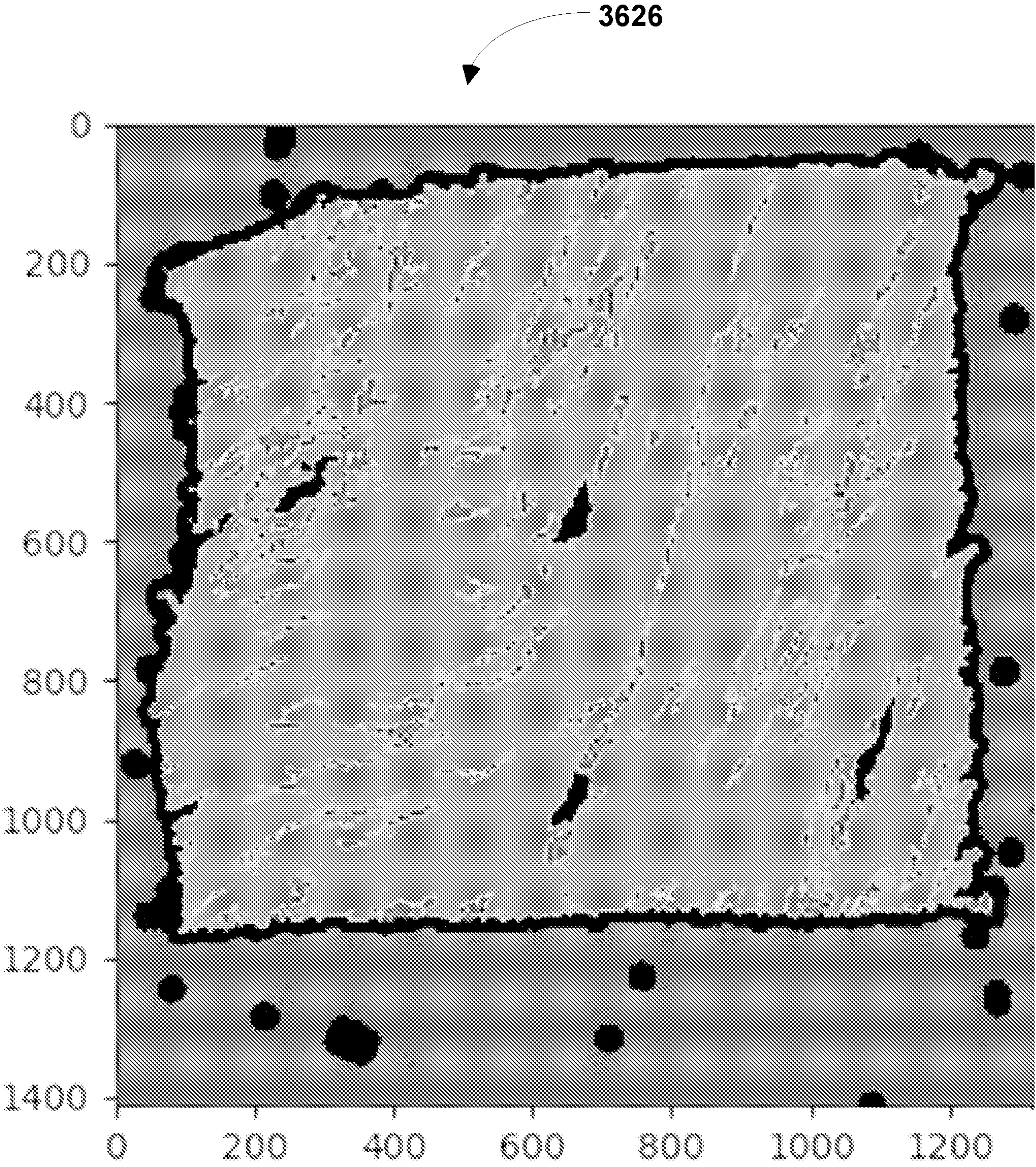


Figure 36G

50/70

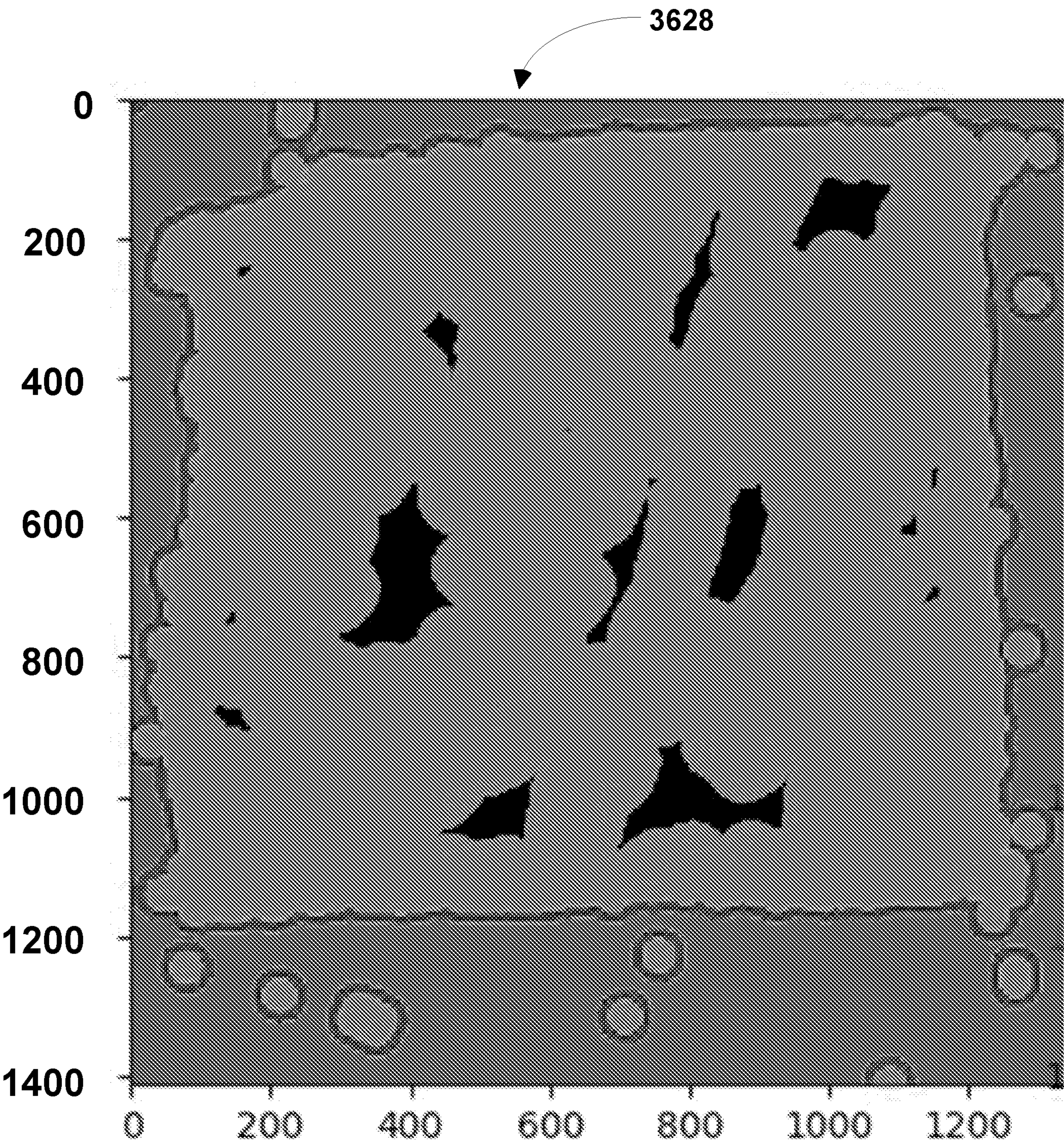


Figure 36H

51/70

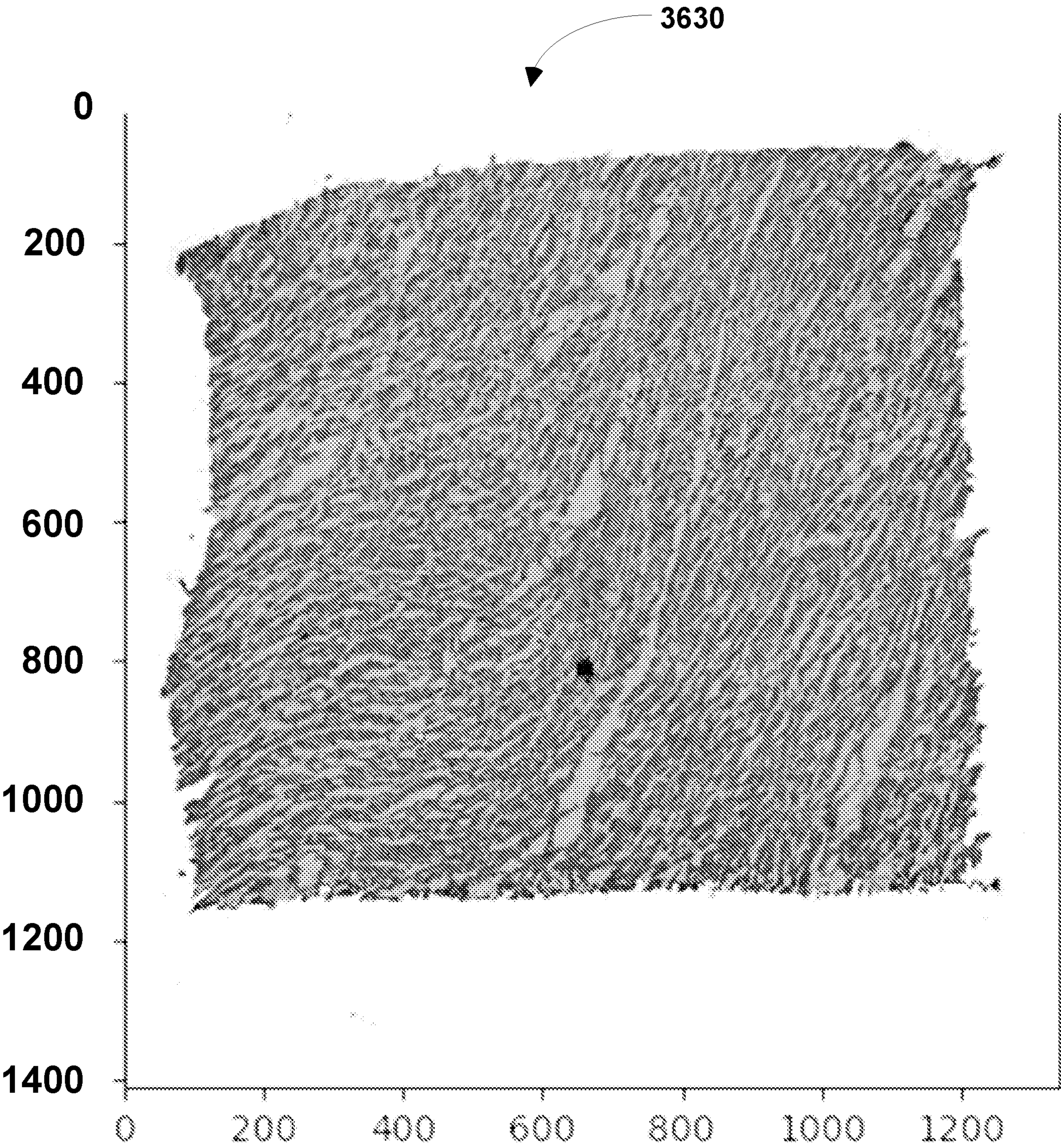


Figure 36l

On Substrate

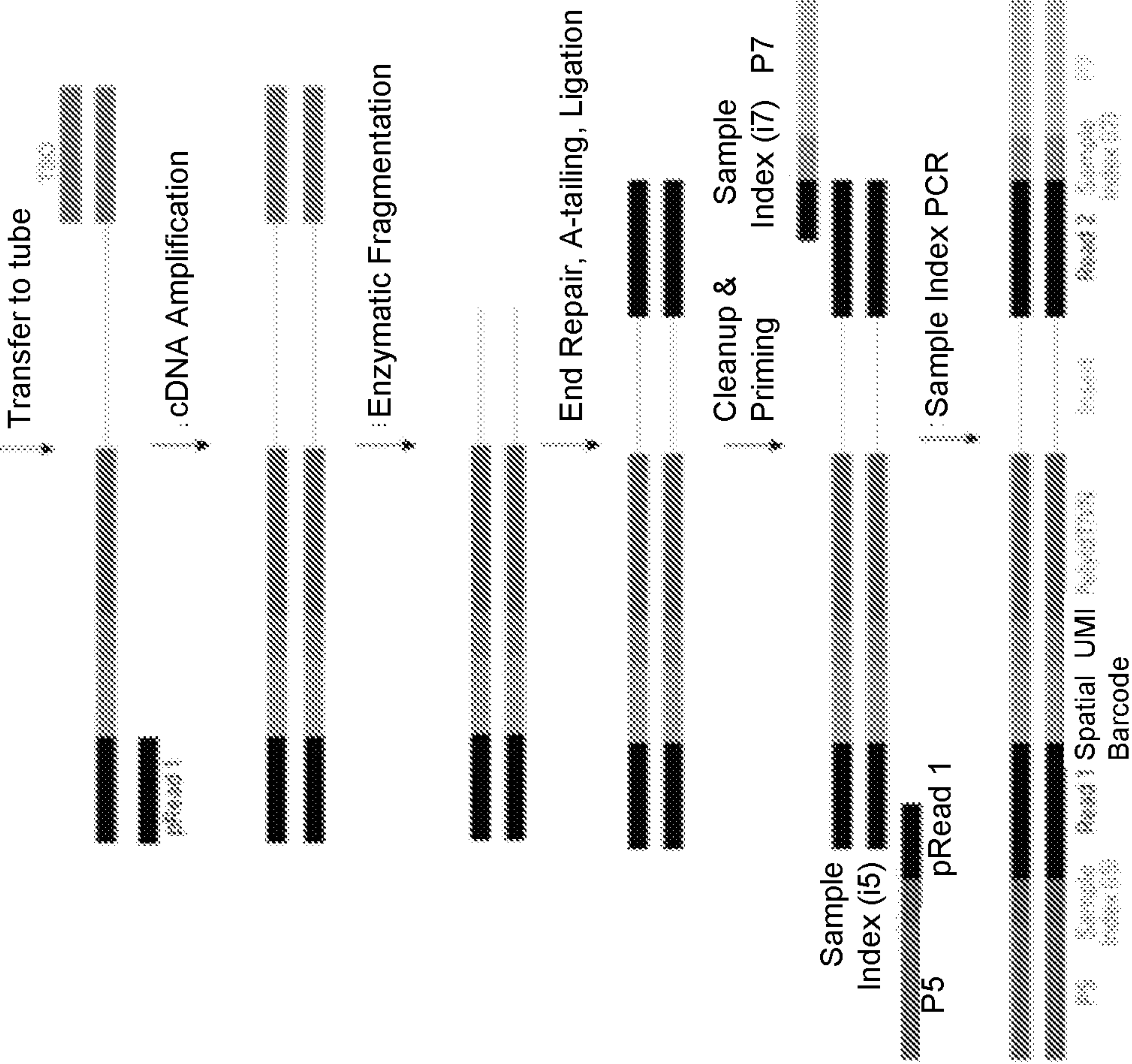
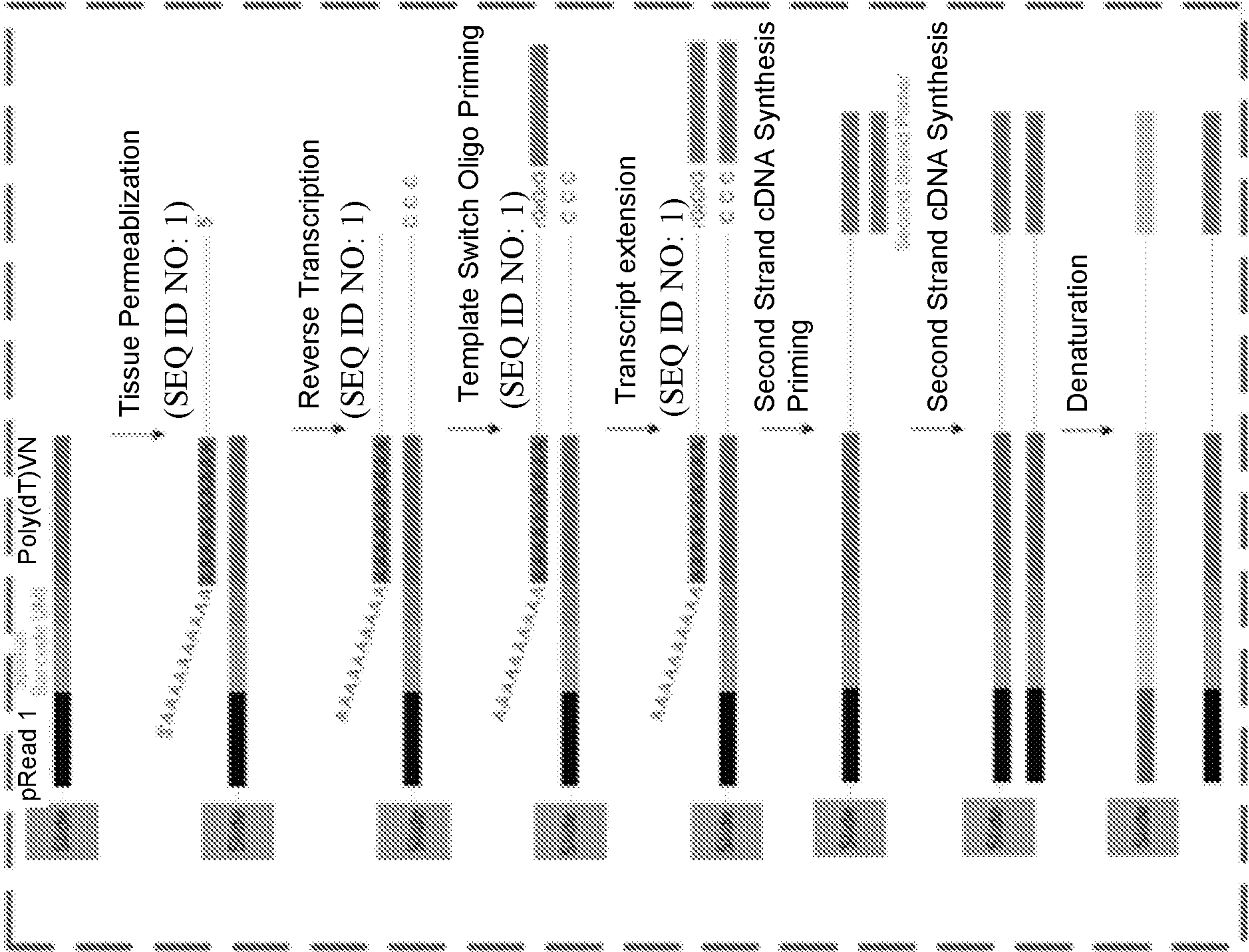


Figure 37

53/70

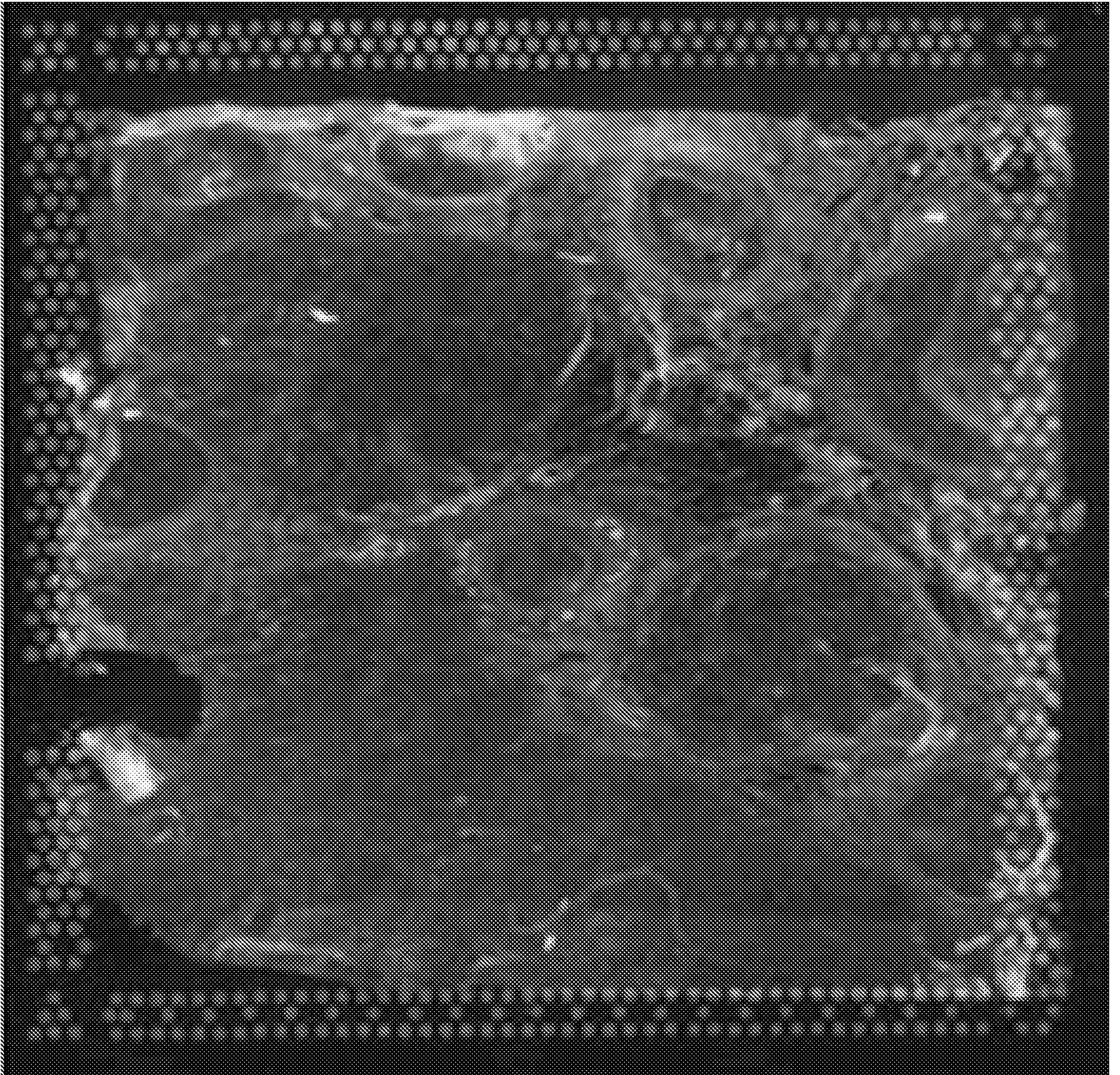


Figure 38A

DC: CD3 Channel

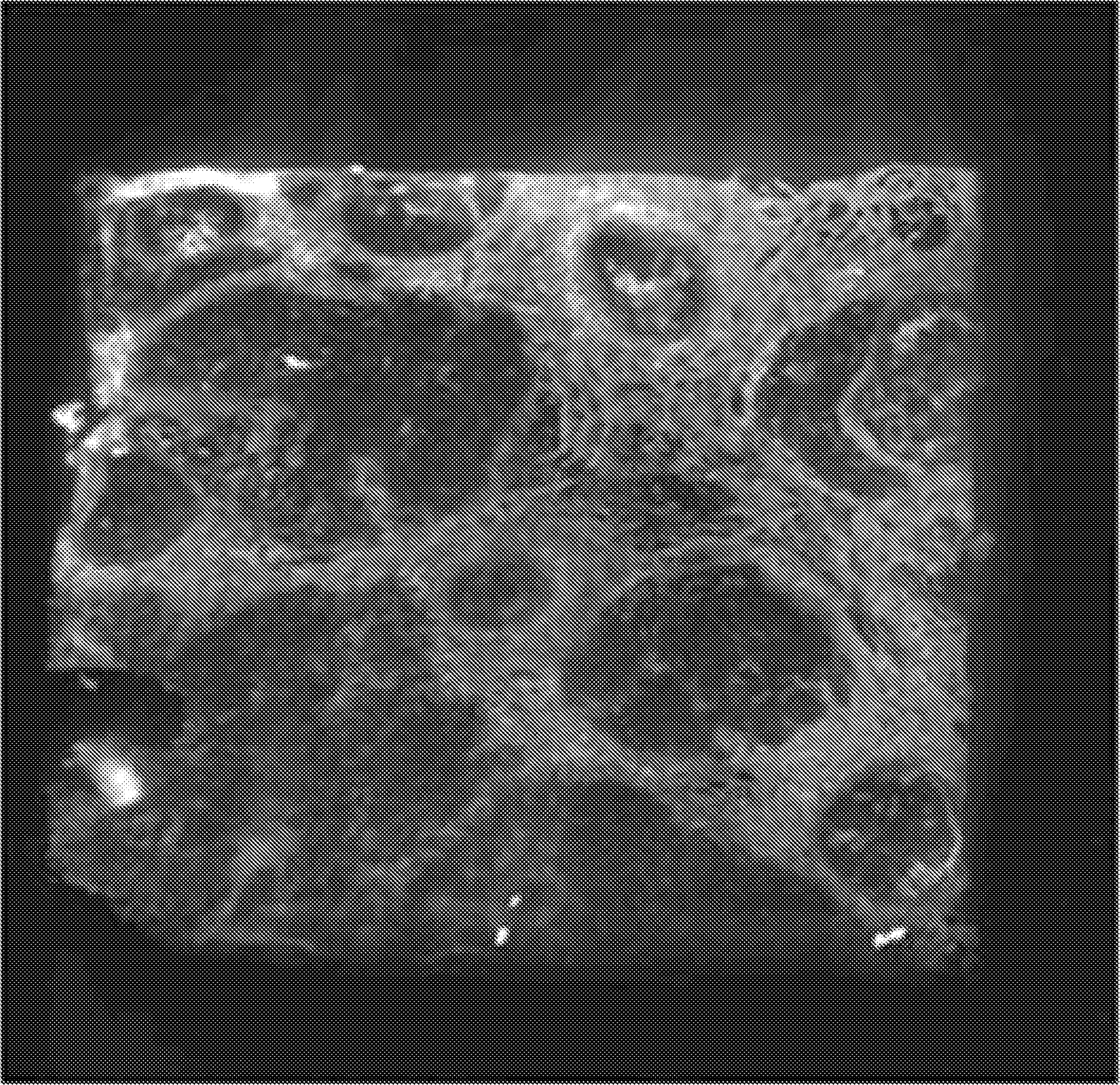


Figure 38B

FIG. 38C: CD3 Protein Quantification

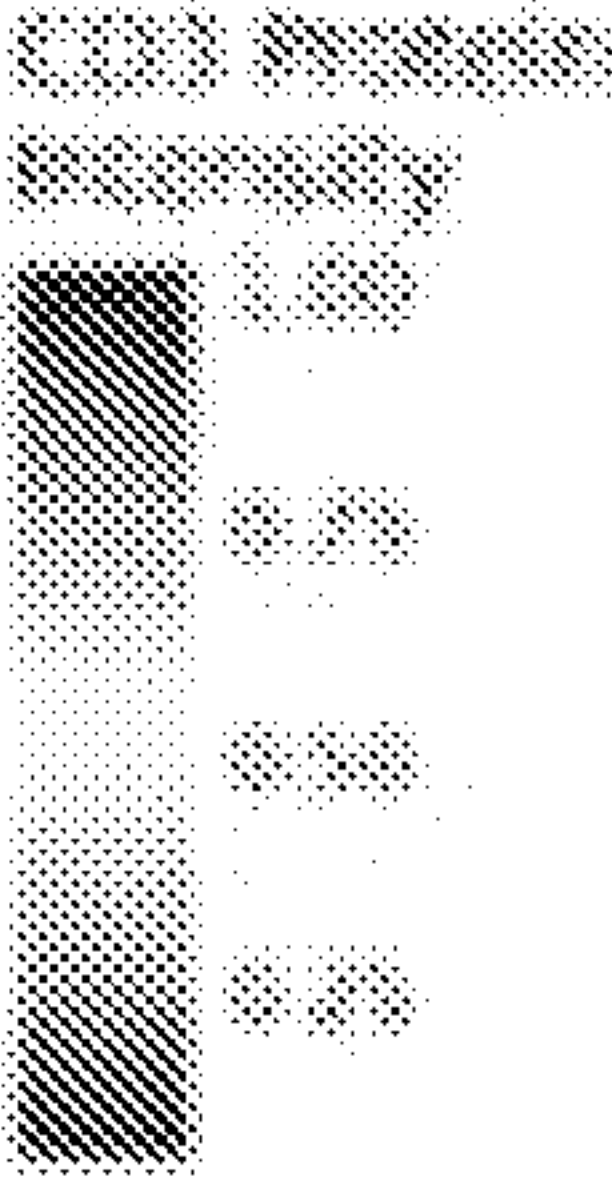
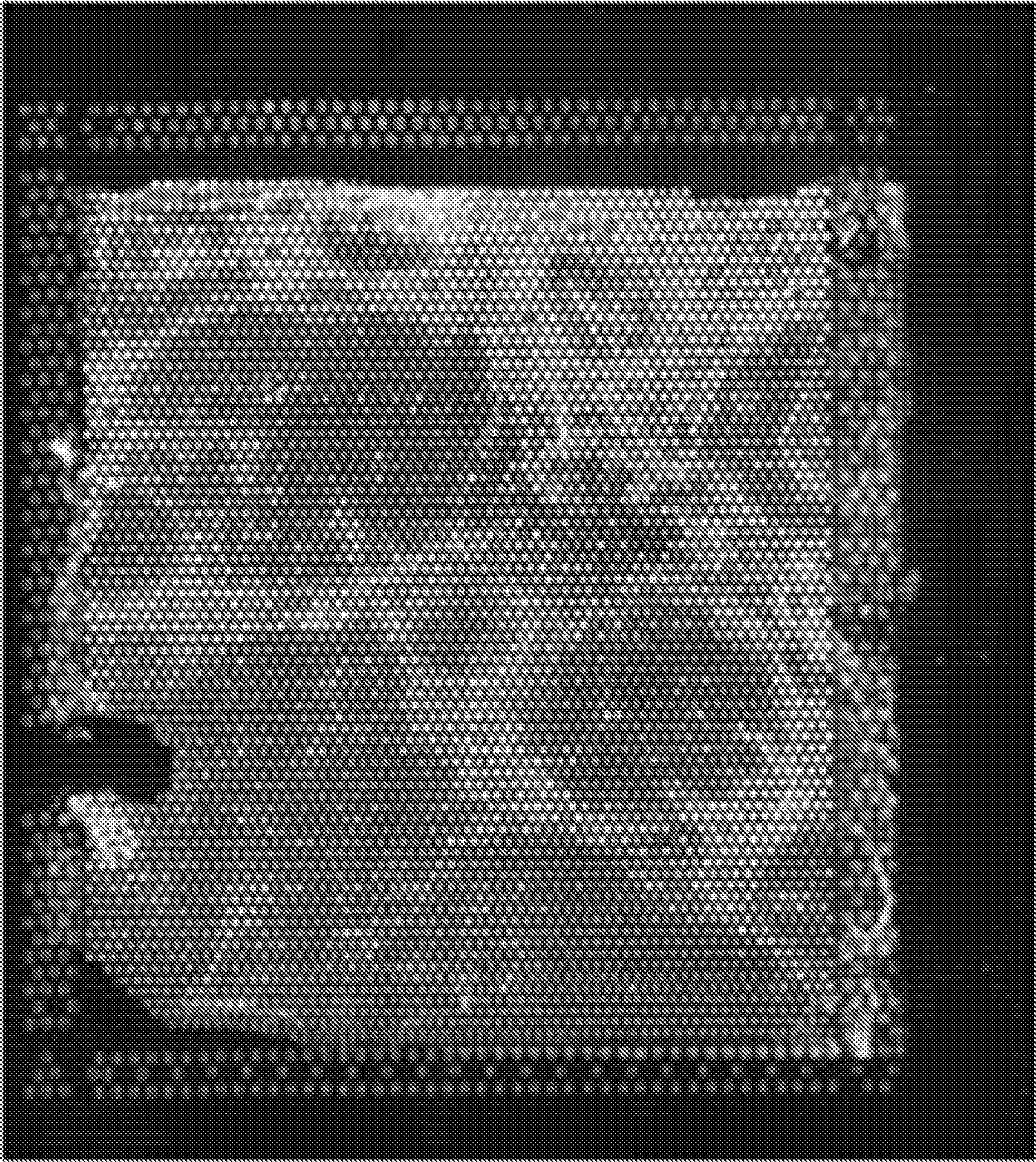


Figure 38C

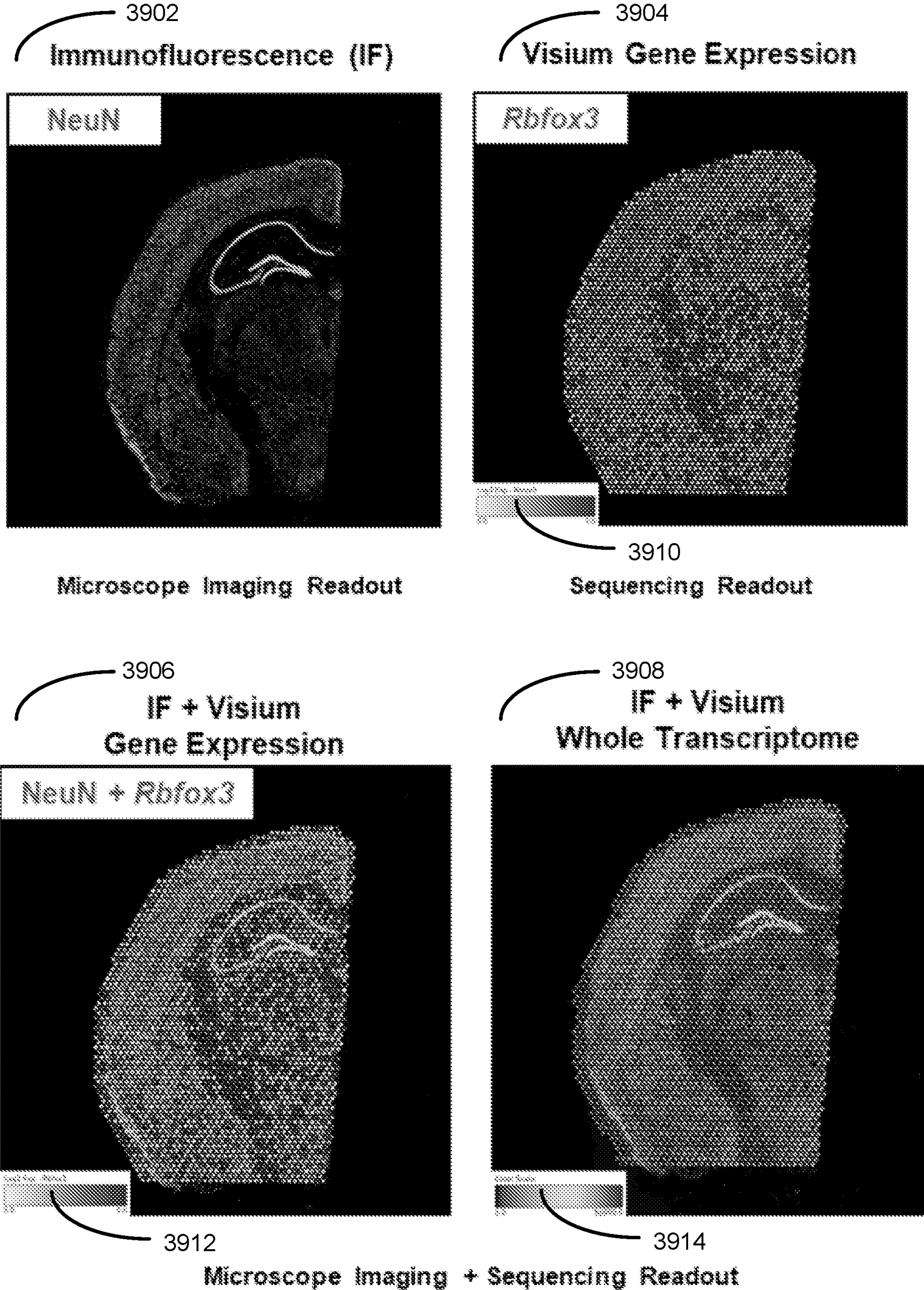


Figure 39

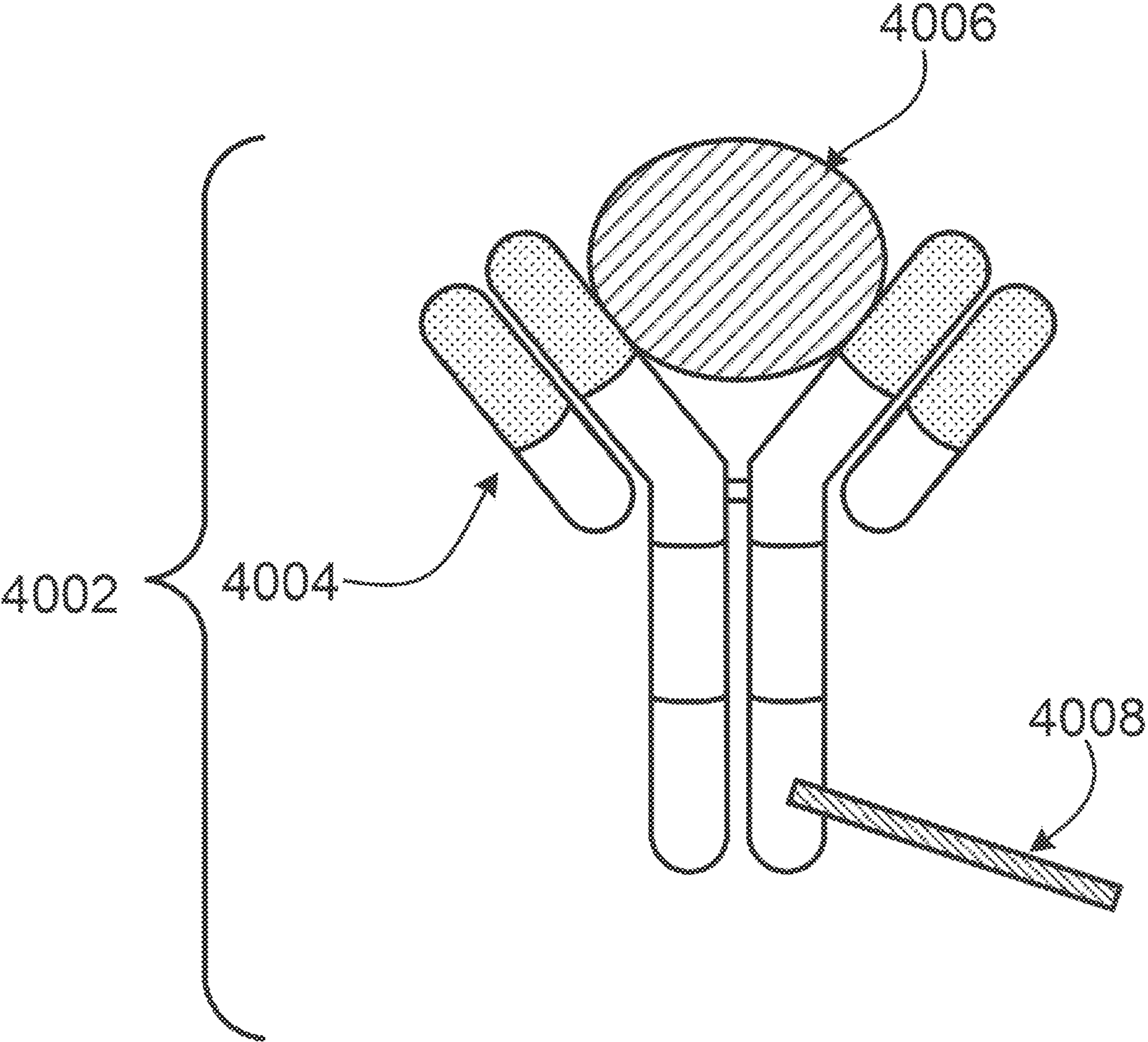


Figure 40

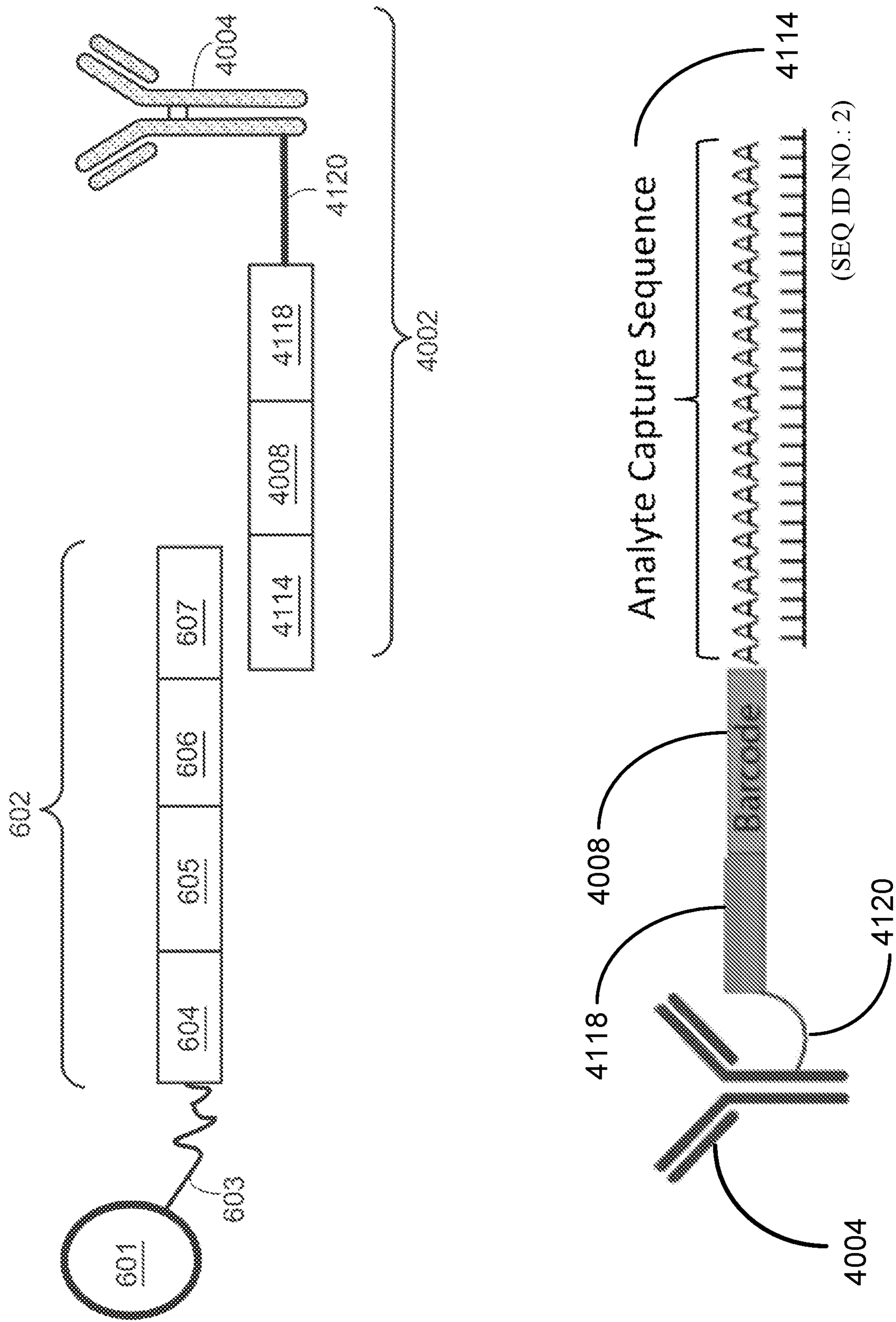


Figure 41A

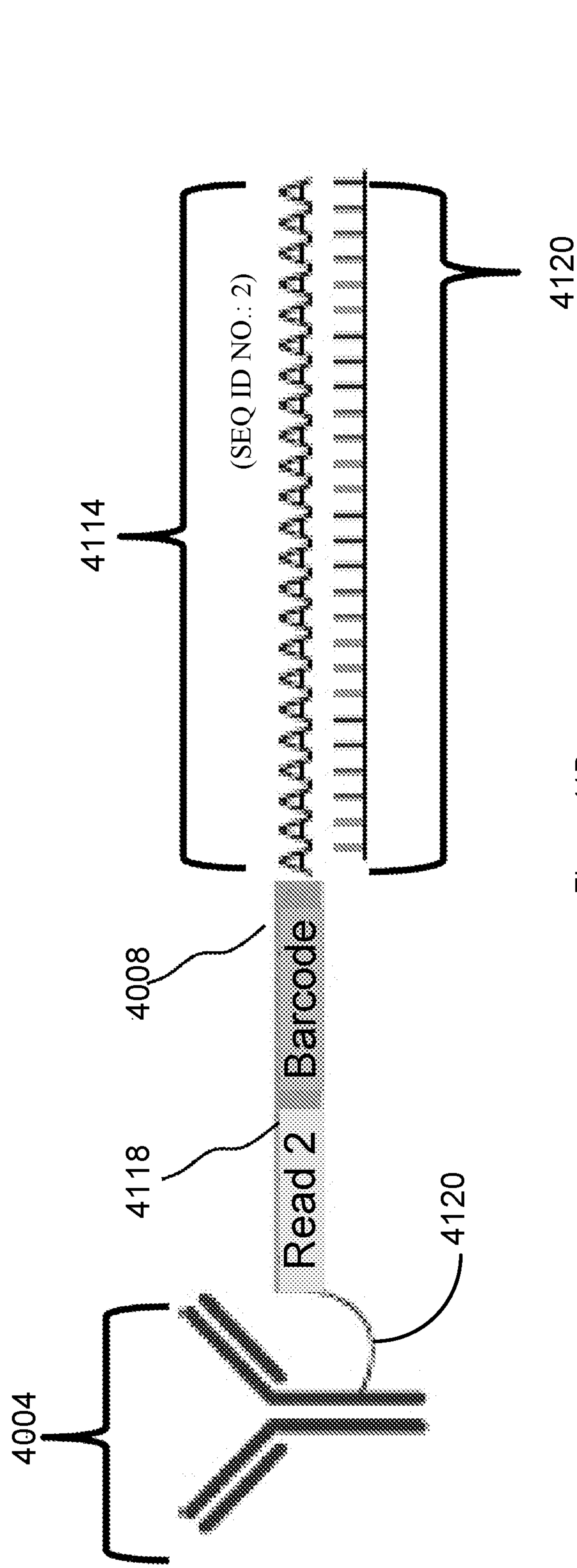


Figure 41B

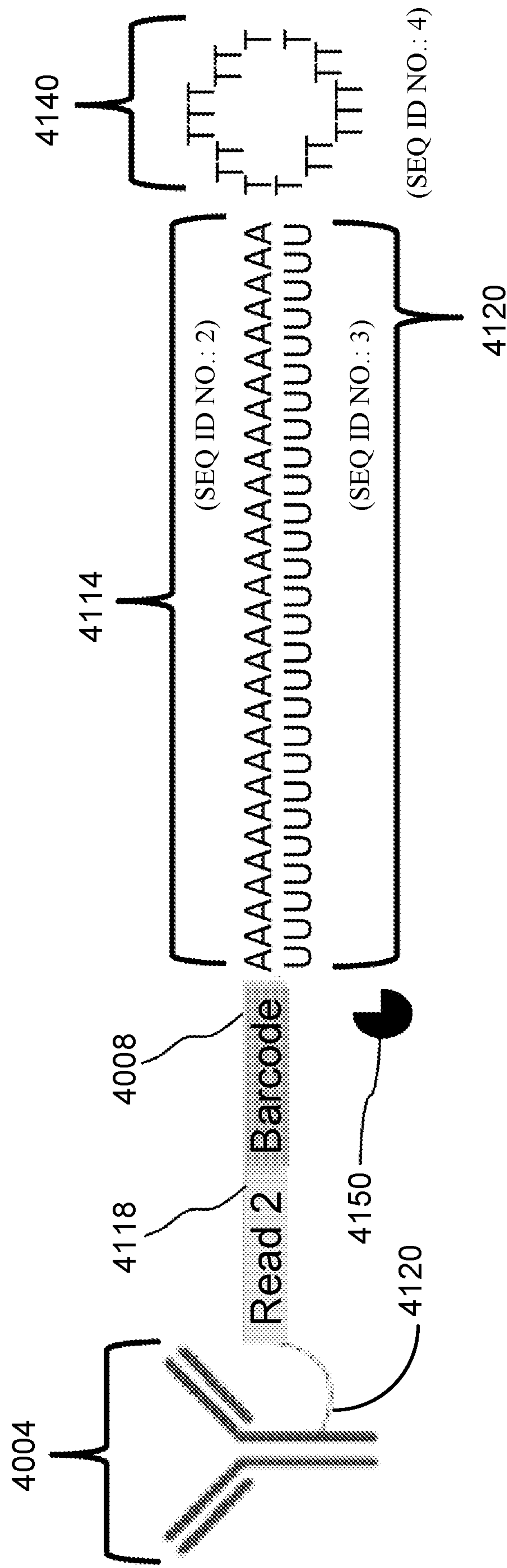


Figure 41C

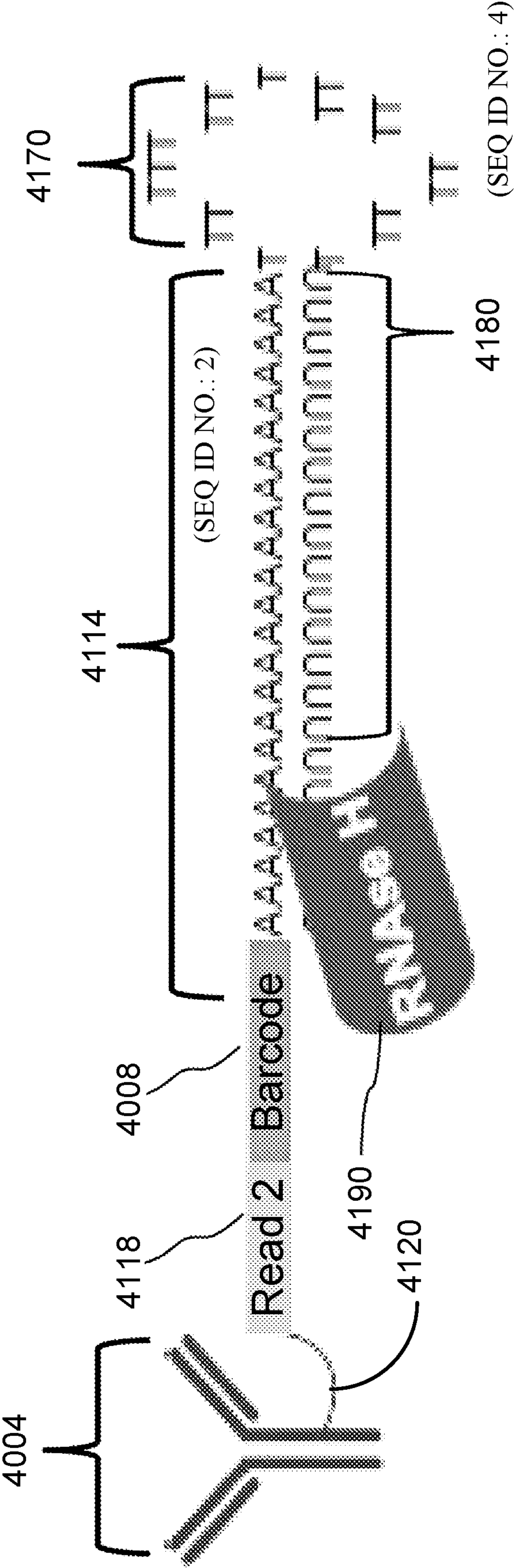


Figure 41D

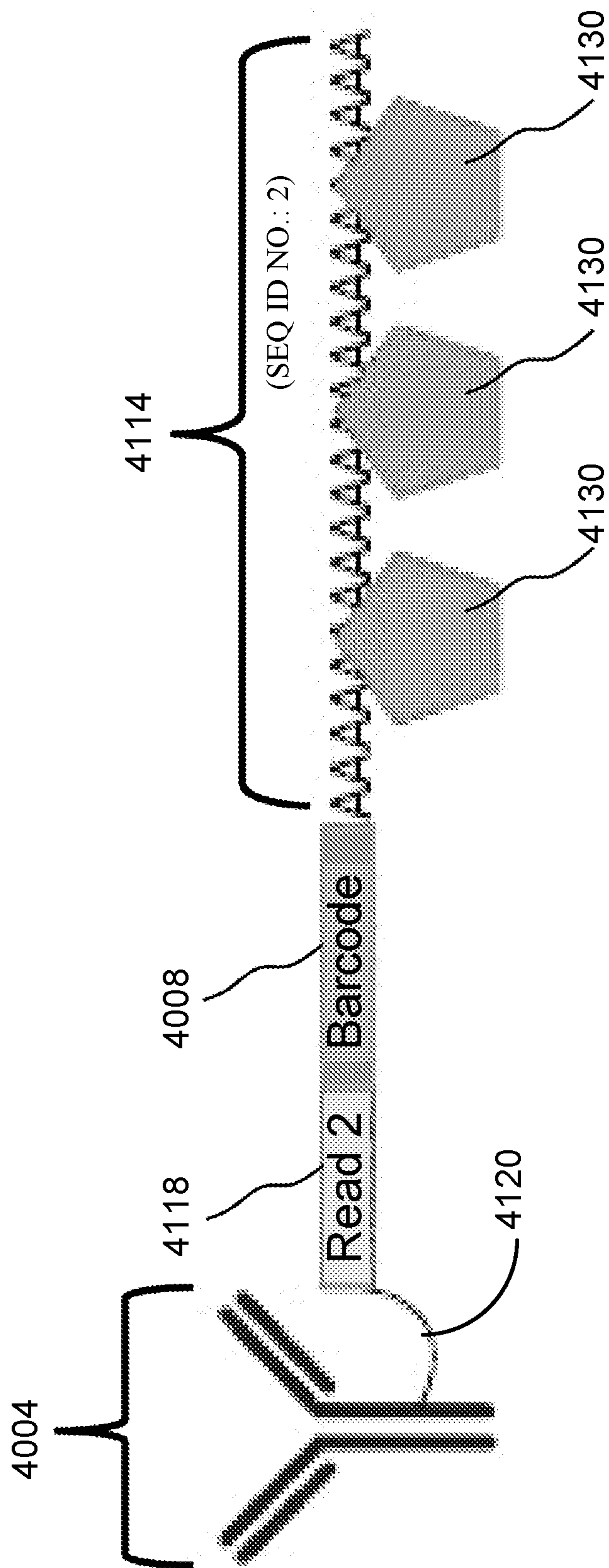


Figure 41E

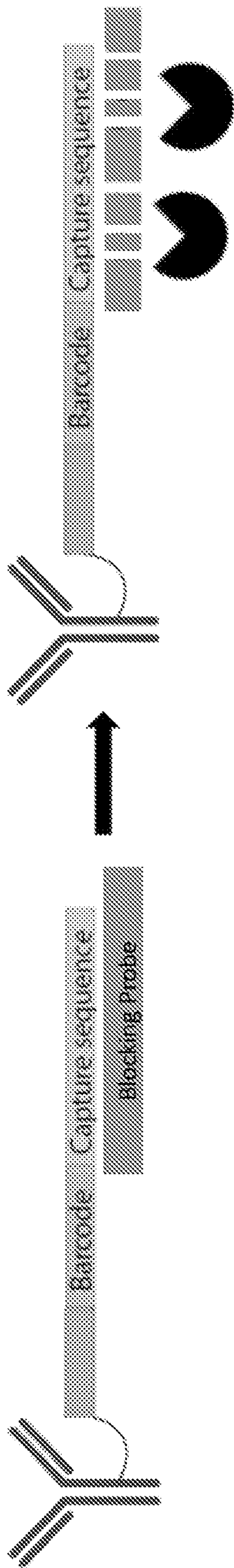


Figure 42

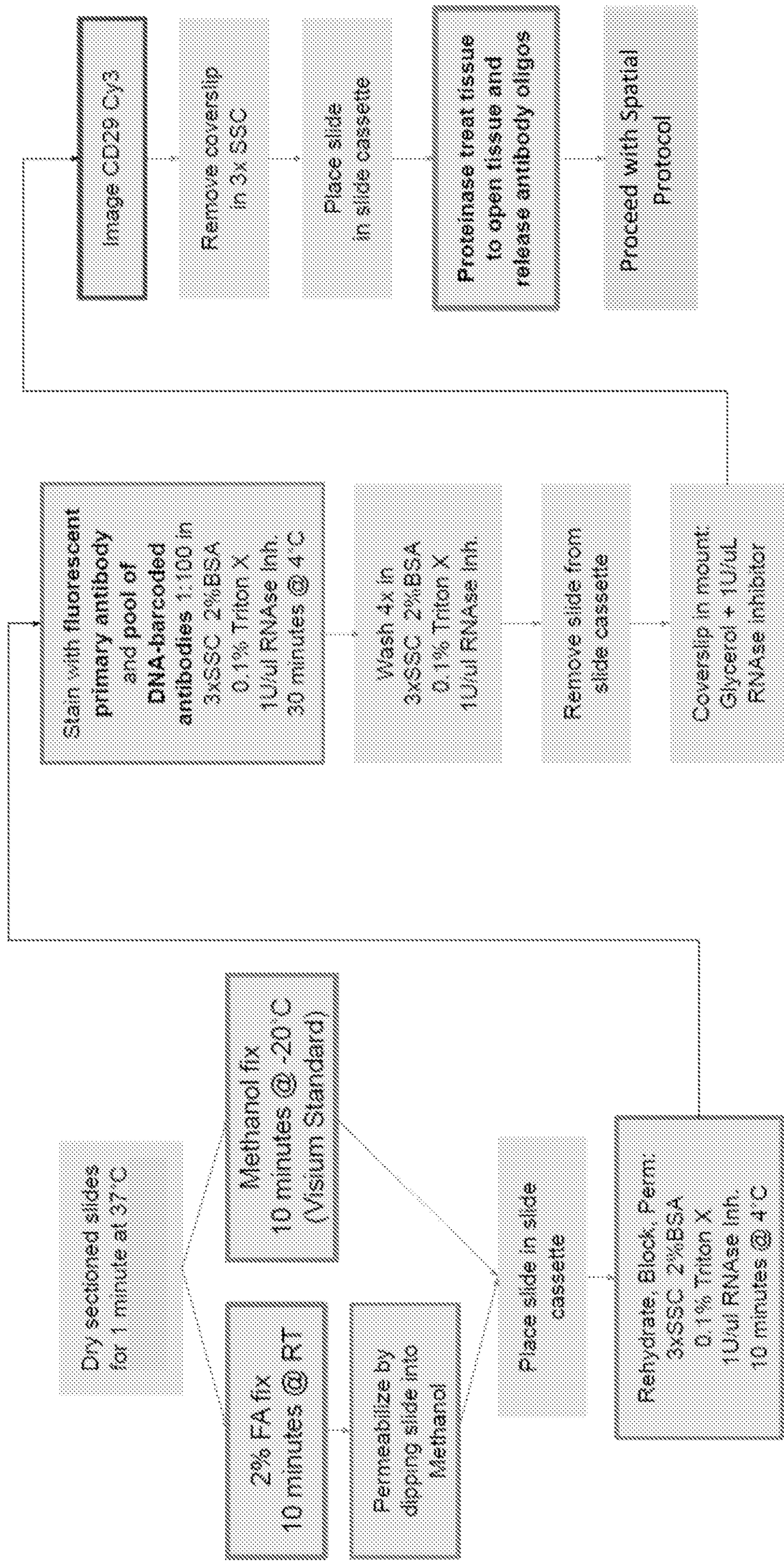


Figure 43

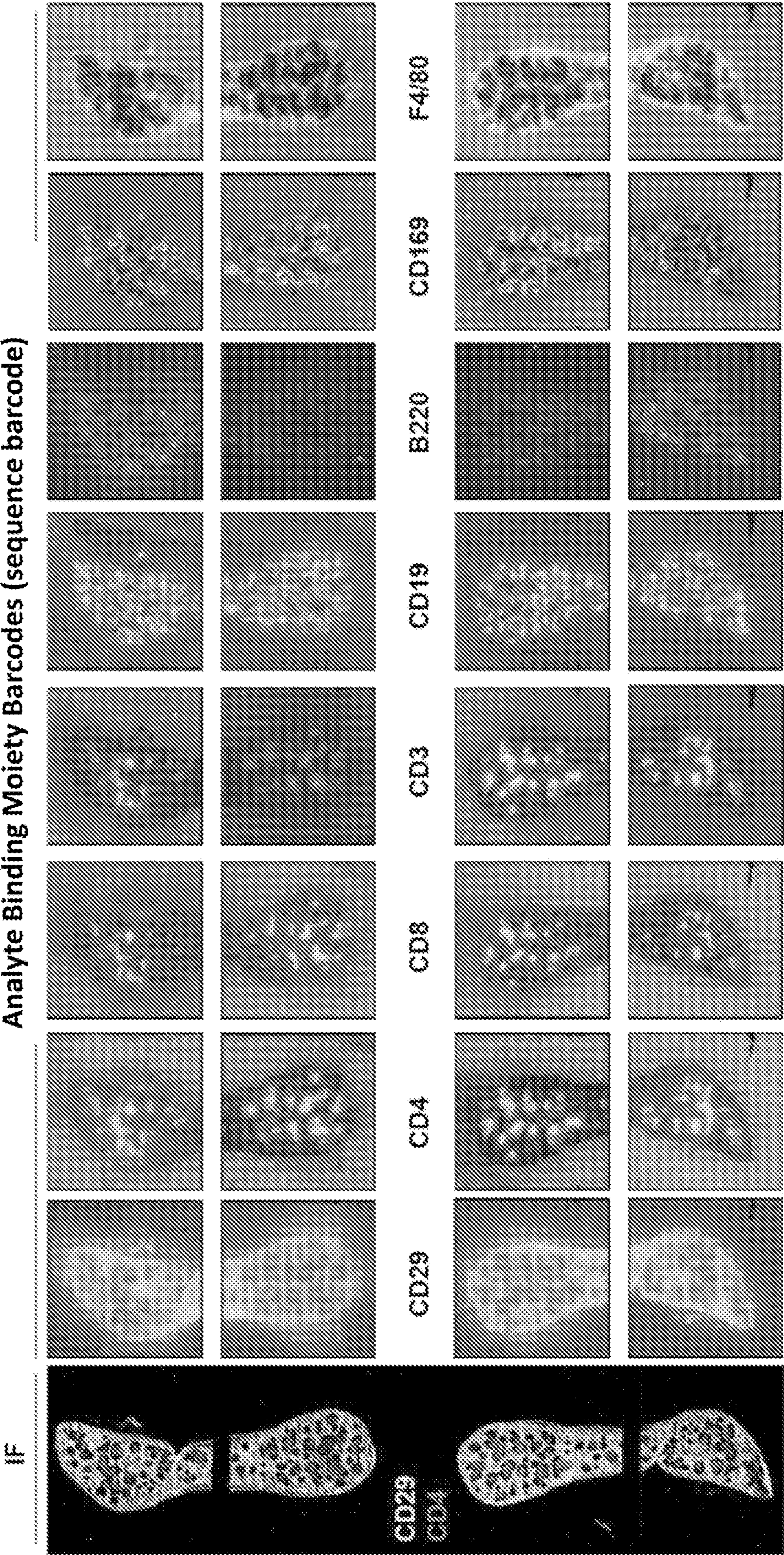


Figure 44

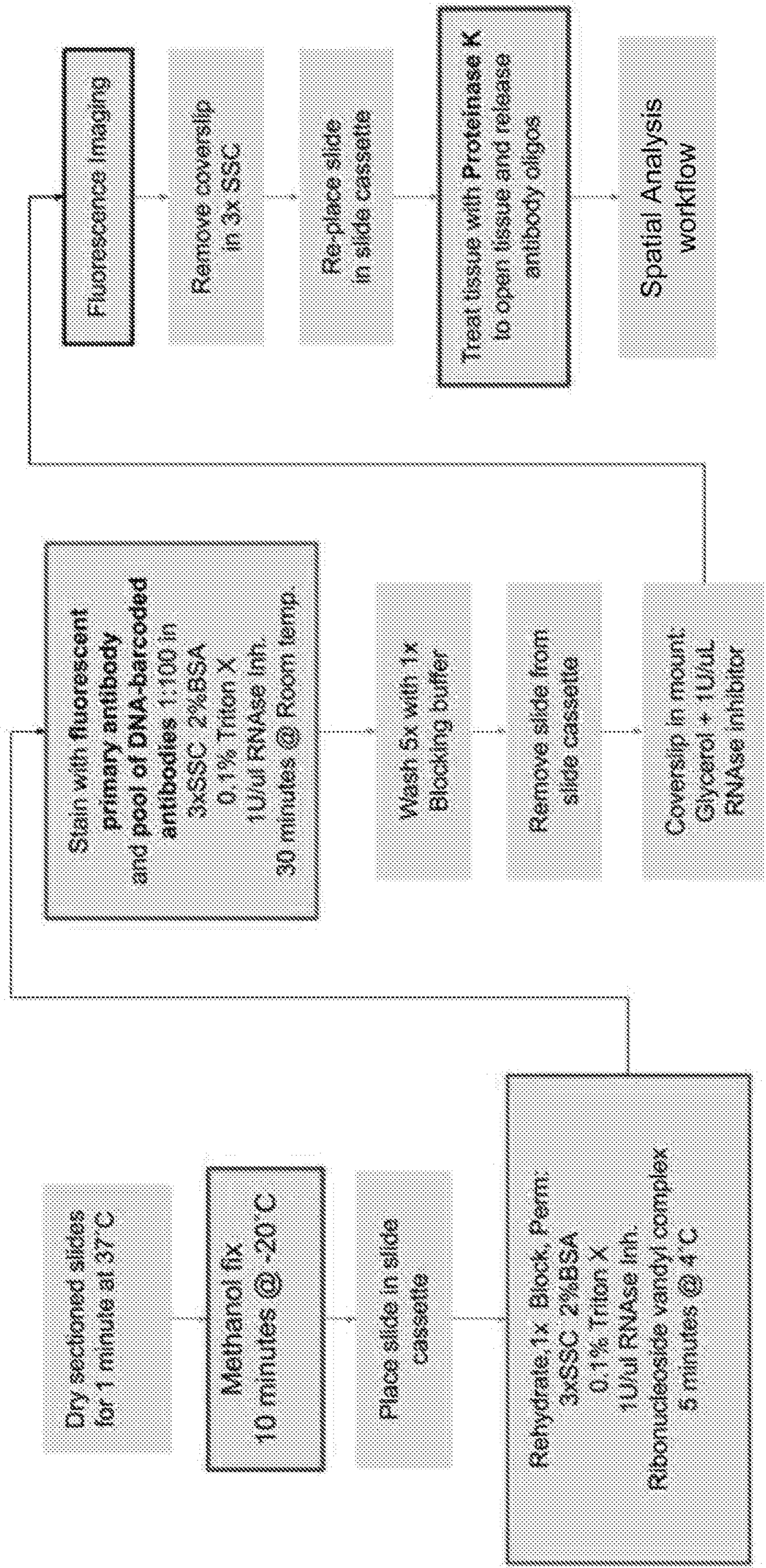


Figure 45

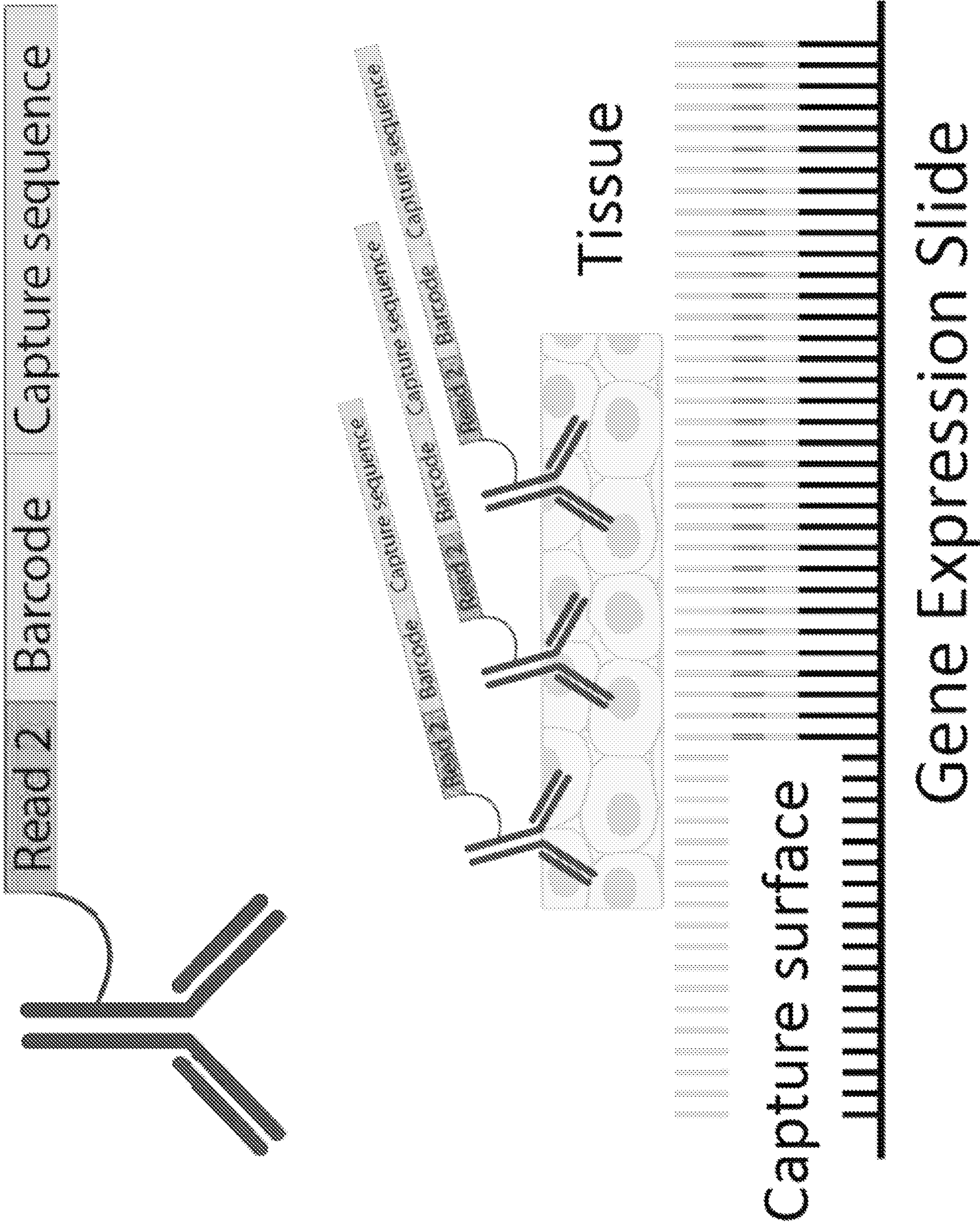


Figure 46A

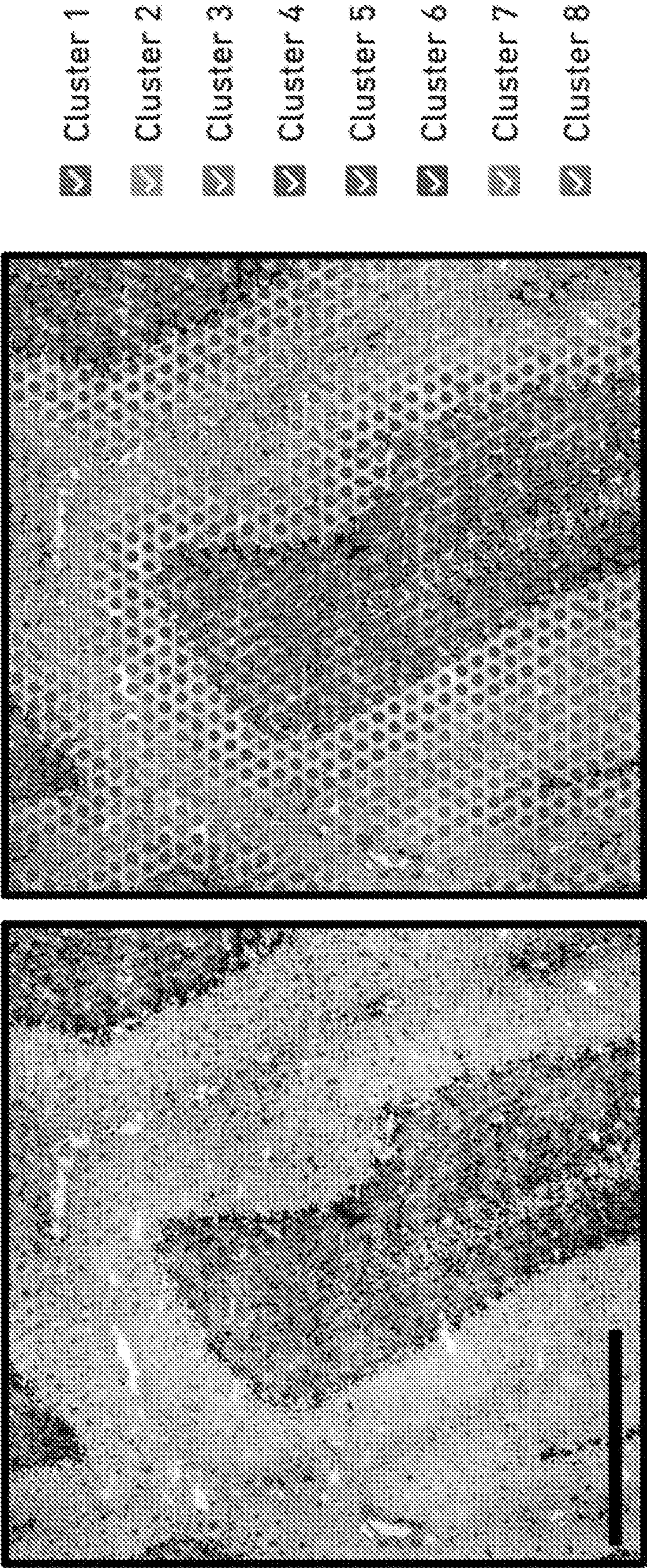


Figure 46C

Figure 46B



Figure 46D

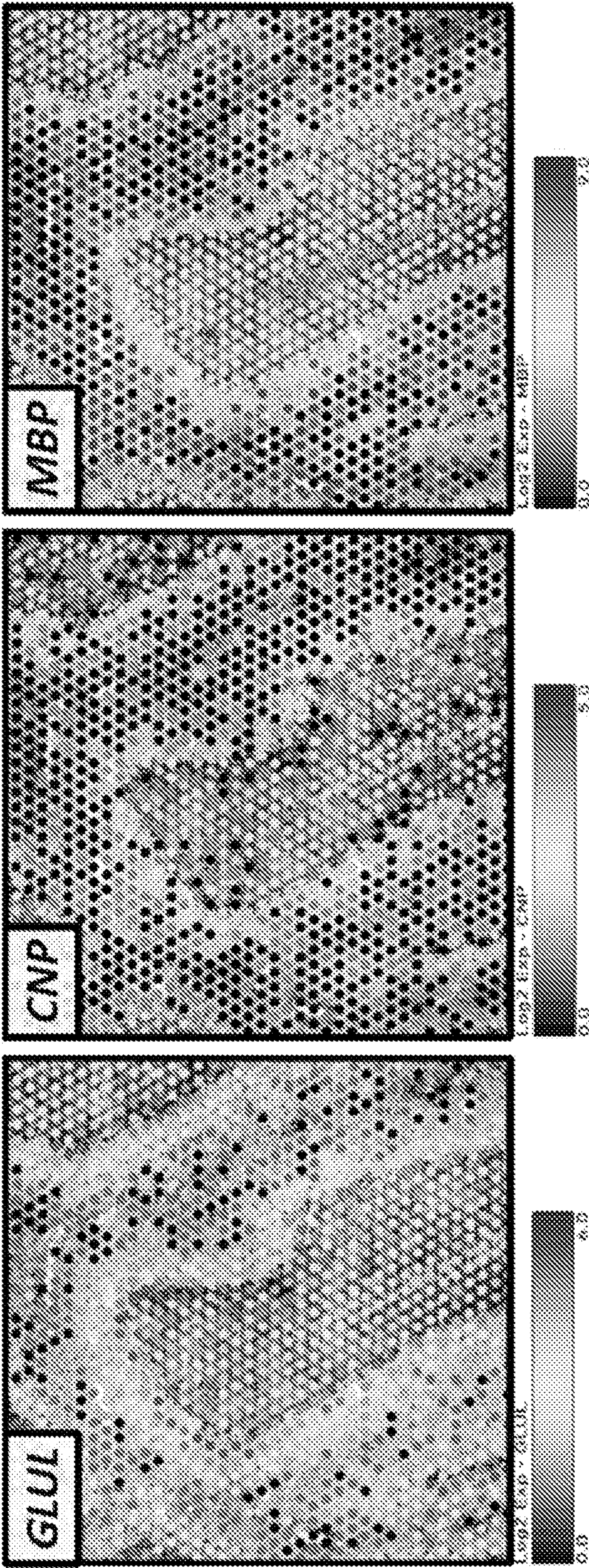


Figure 46E

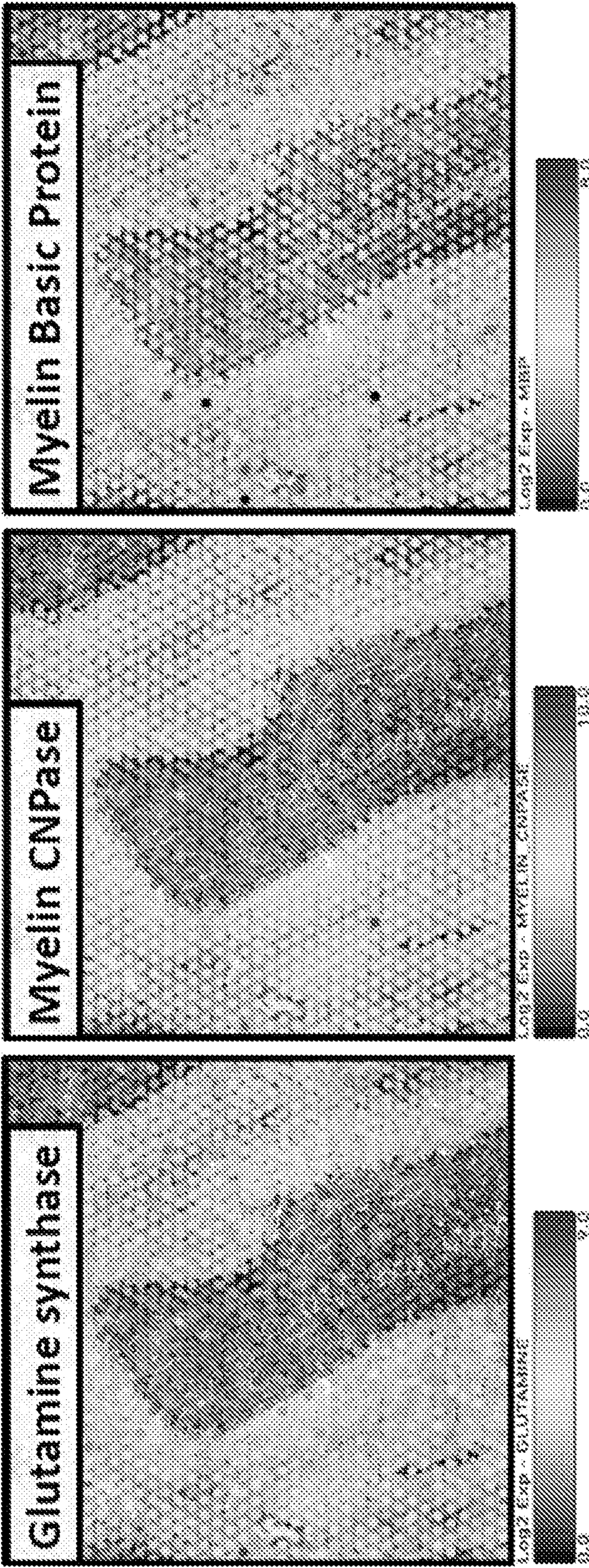


Figure 46F

Figure 46G

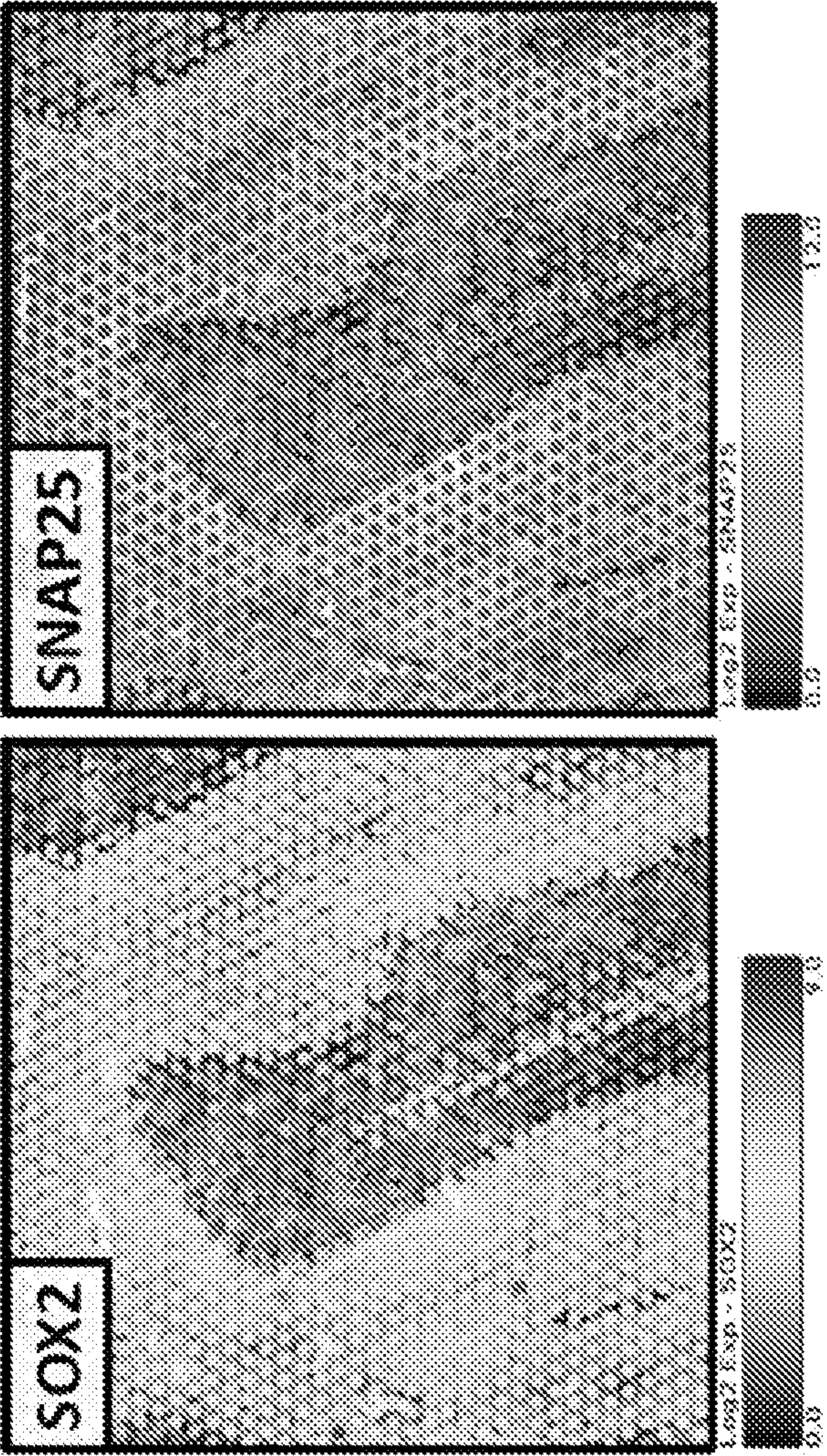
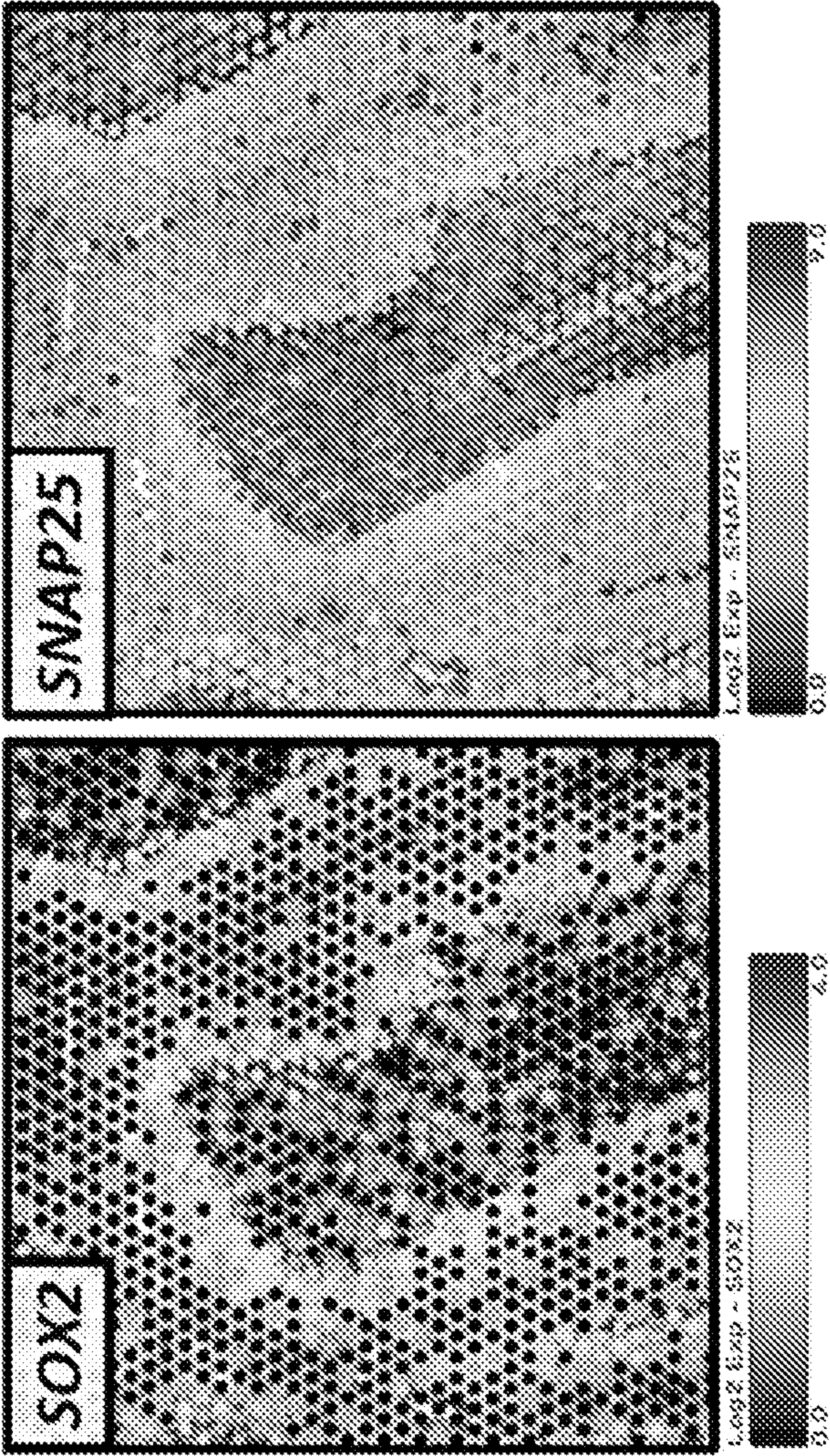


Figure 46H

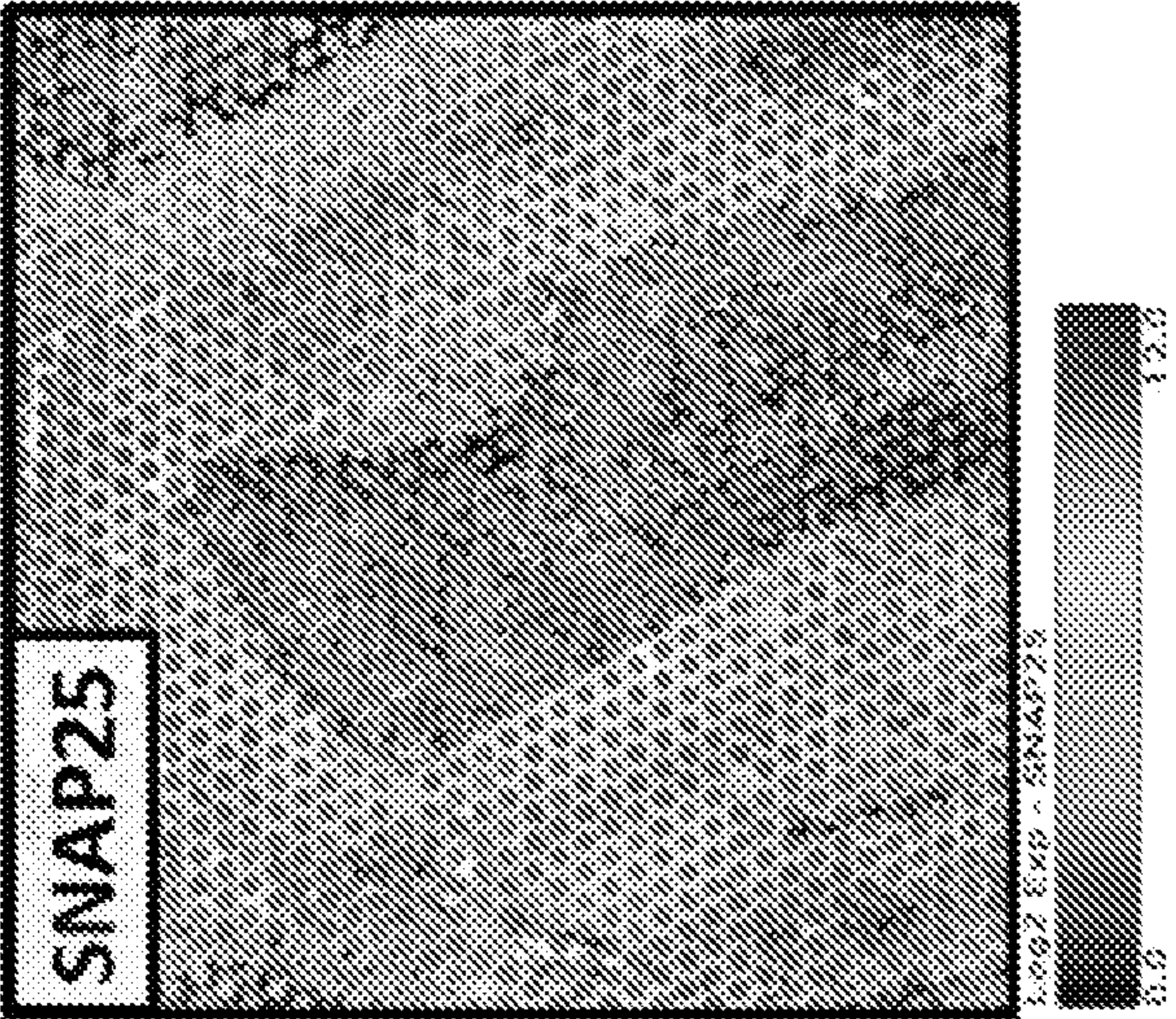
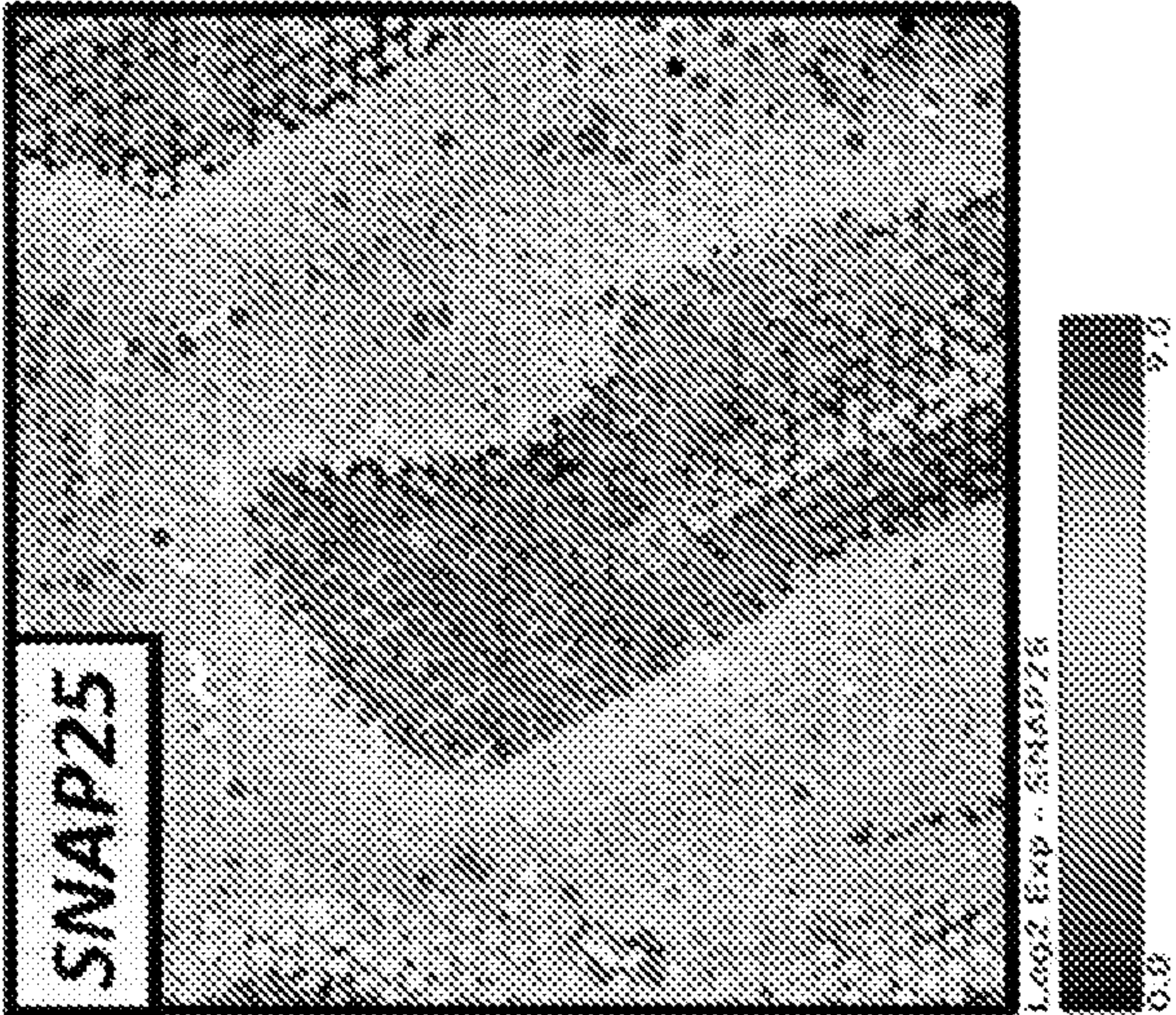


Figure 46I

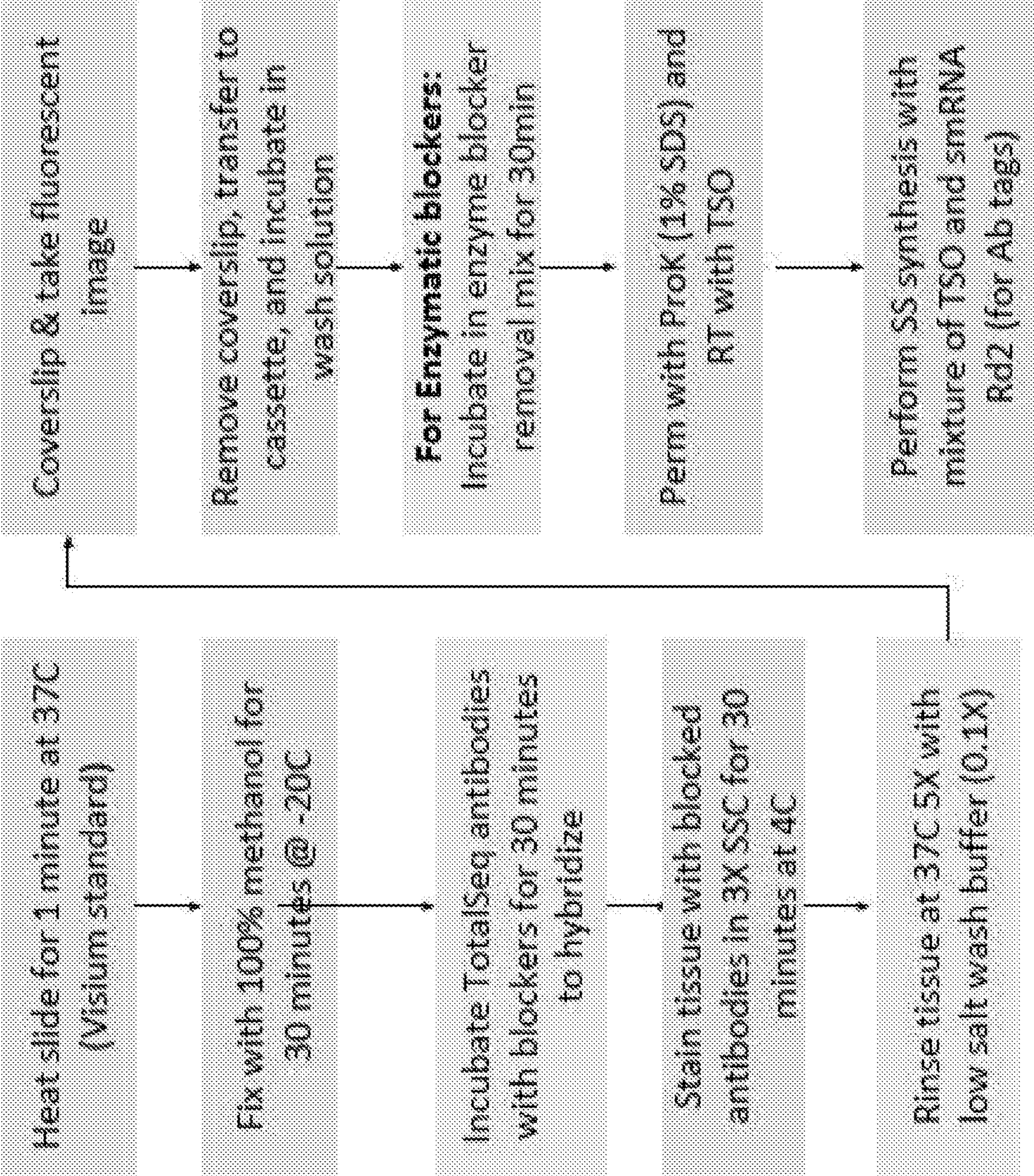


Figure 47