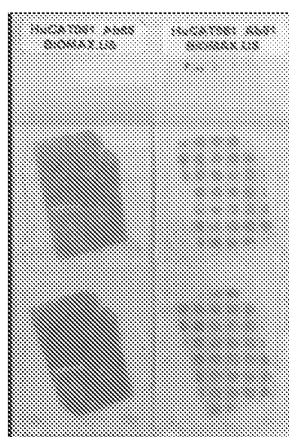
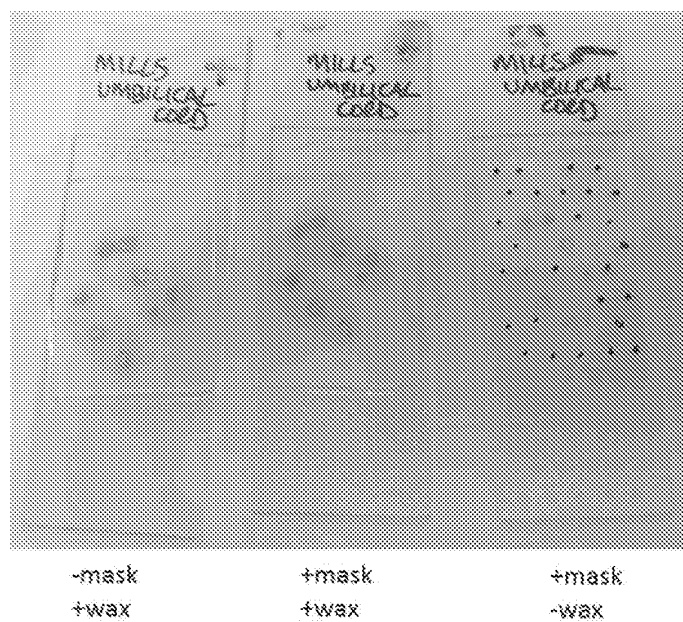


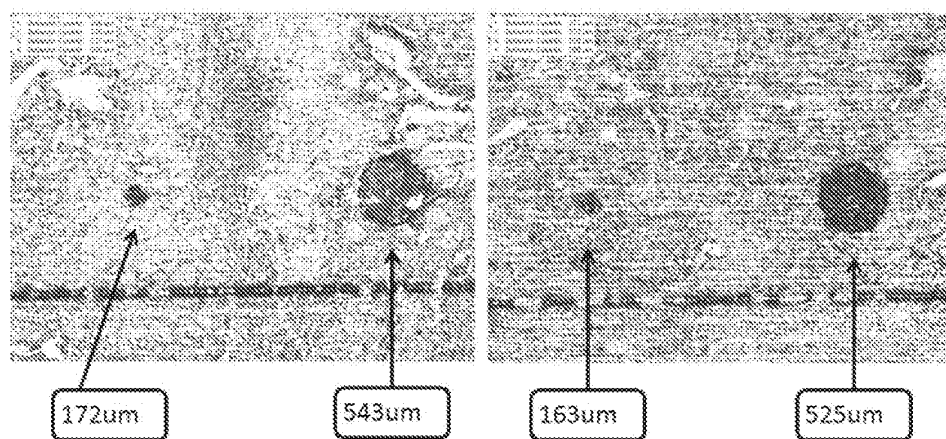
FIGURE 1



Slide on the left: stained microtomed liver tissue

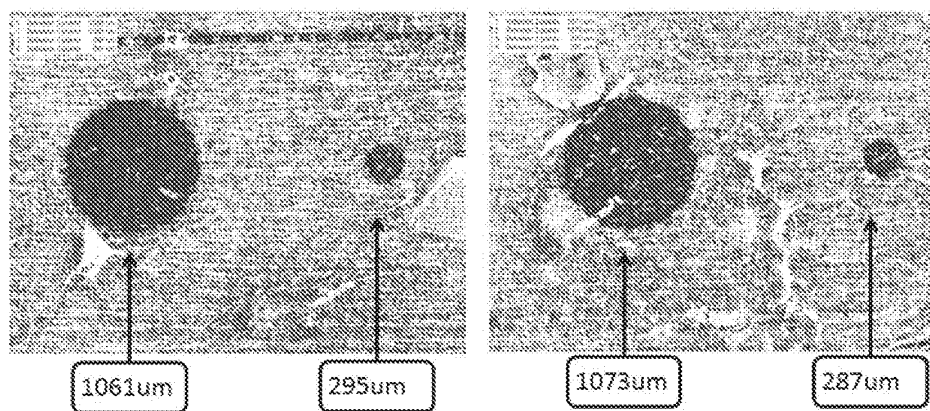
Slide on the right: patterned microtomed liver tissue, stained post patterning

FIGURE 2A



125um and 500um features on Liver

FIGURE 2B



1mm and 250um features on Liver

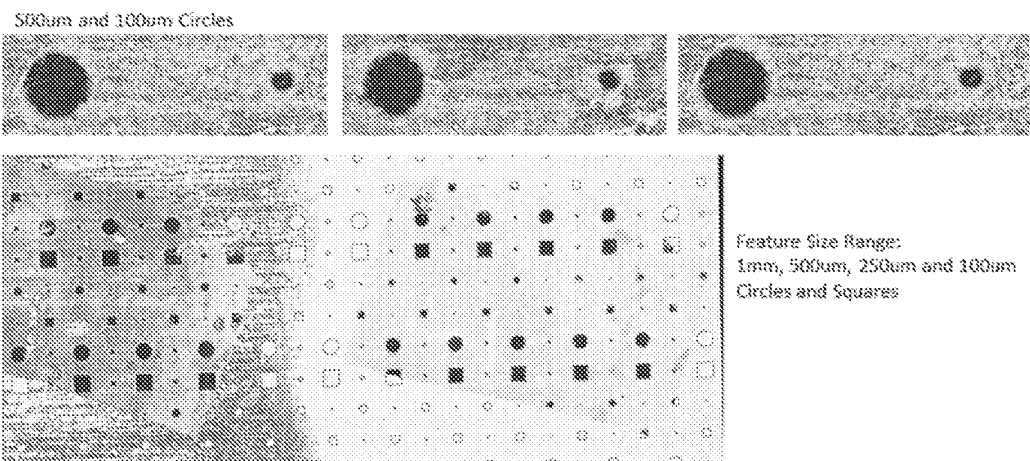
FIGURE 2C



Custom Patterns

FIGURE 2D

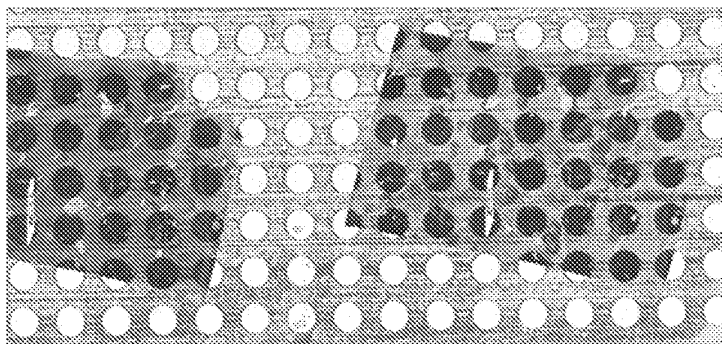
- On FFPE liver microtome slides



Patterned Slide: Liver Tissue

FIGURE 2E

PATTERNED SLIDE: 2MM FEATURES ON LIVER



Patterned Slide: 2mm features on Liver

FIGURE 2F

25 micron features on liver

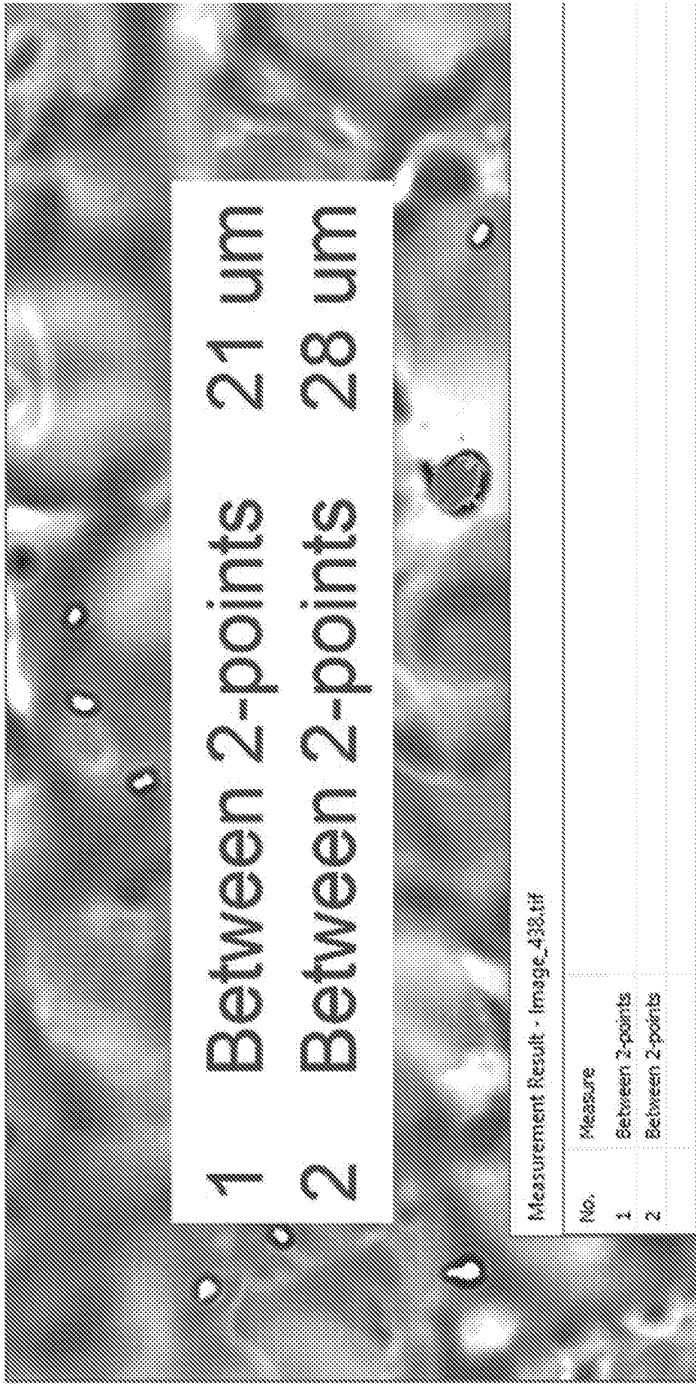


FIGURE 3

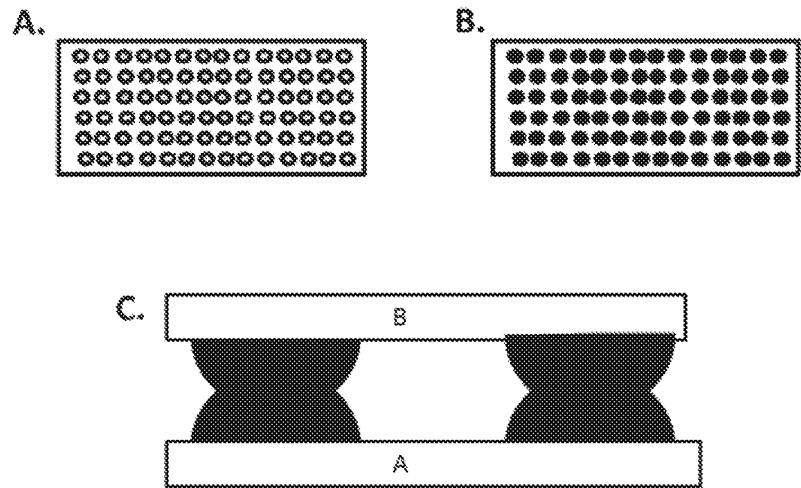
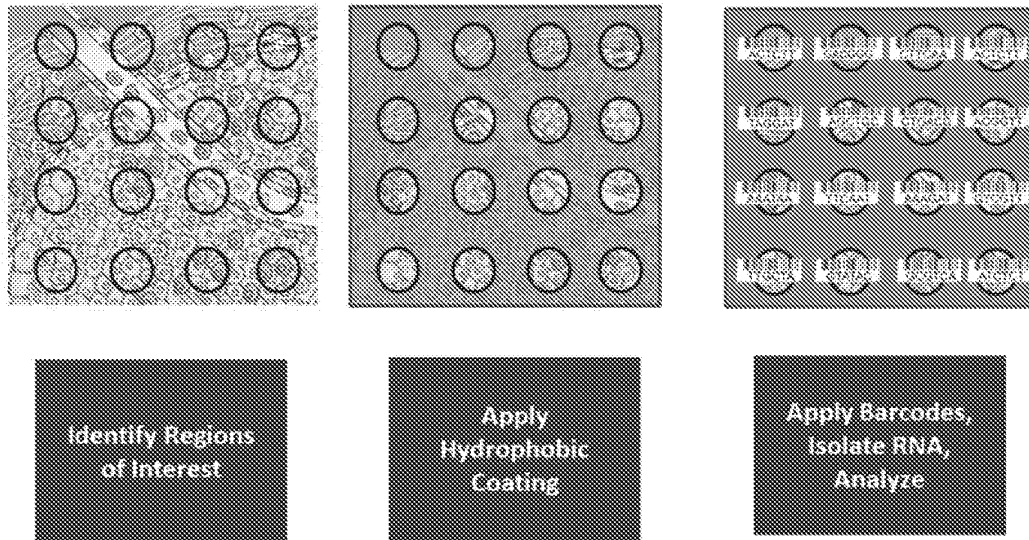


FIGURE 4

Hydrophobic Masking Process Allows Isolation and
Tagging of Tissue Regions without Specialized Equipment
(Microfluidics, Lasers)



For the box on the rightmost side, some additional steps before “Analyze”

- Lyse and apply Barcodes
- First strand Synthesis
- Second strand Synthesis

Next step: Pool prior to sequencing

FIGURE 5

Freeform Delineation of Hydrophobic Mask Also Allows for Isolation of Particular Regions of Interest

- Custom ROIs may be defined e.g by microscopy combined with inkjet printing

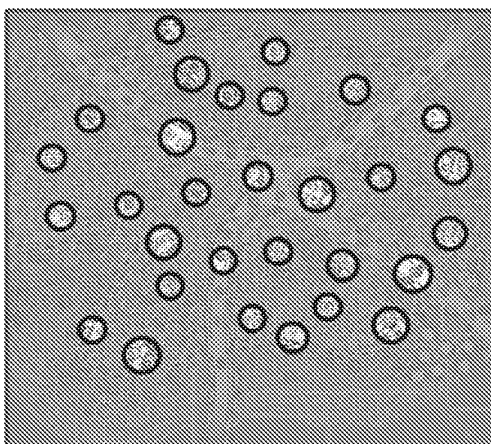


FIGURE 6

Application of Spatially-Aware Barcodes Allow Deconvolution of Gene Expression by Cell Type in Tissue

	Cell Type	Gene Expression Data	Sequence Data	Location
	Myeloid	1.Fasta	1.Seq	A1
	Malignant	2.Fasta	2.Seq	C22
	Fibroblast	3.Fasta	3.Seq	G5
	T lymphocyte	4.Fasta	4.Seq	H8
	Dendritic	5.Fasta	5.Seq	N4
	B and T Lymphocyte	6.Fasta	6.Seq	T9
	T Lymphocyte	n.Fasta	n.Seq	F5

FIGURE 7

H and E Stain

IHC Stain HER2+

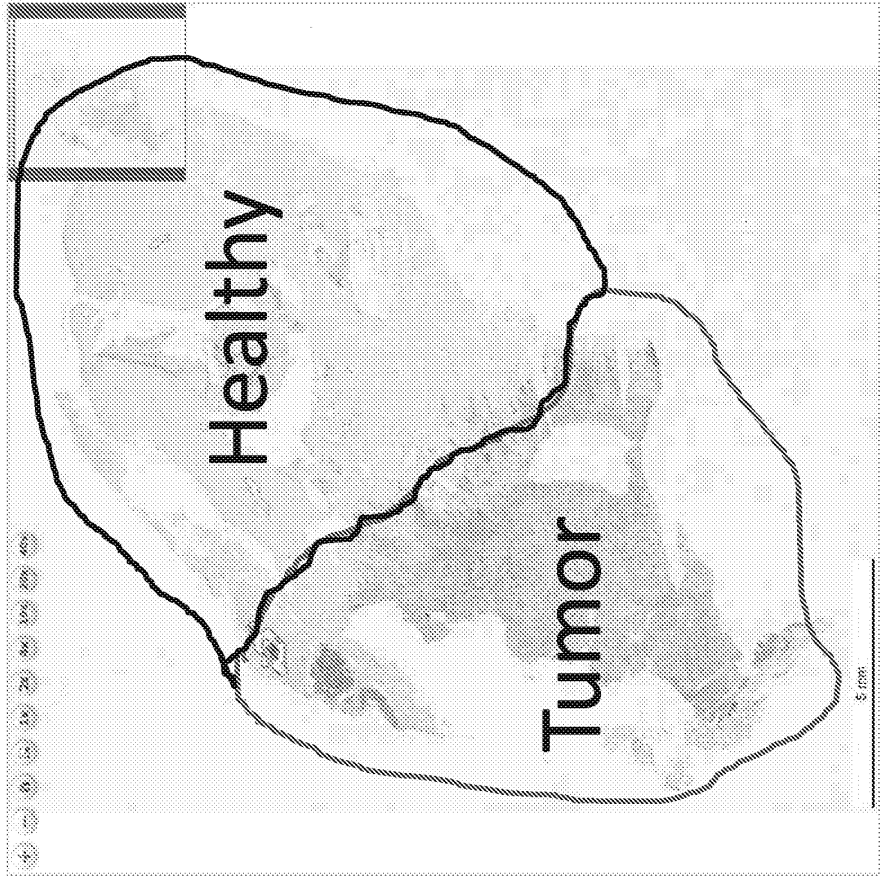
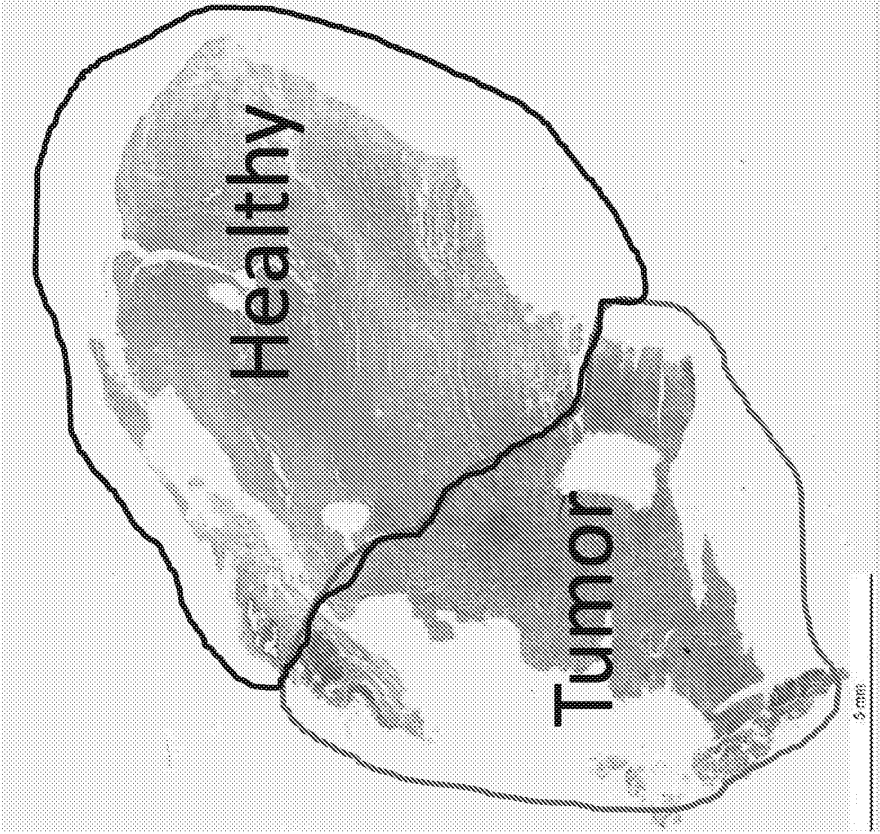


FIGURE 8

H and E Stain

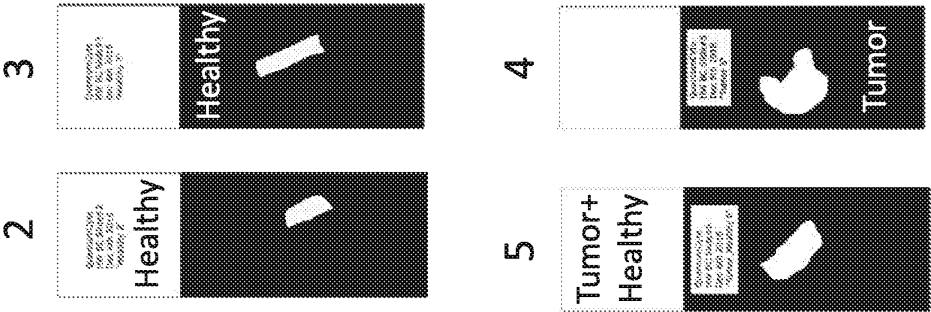
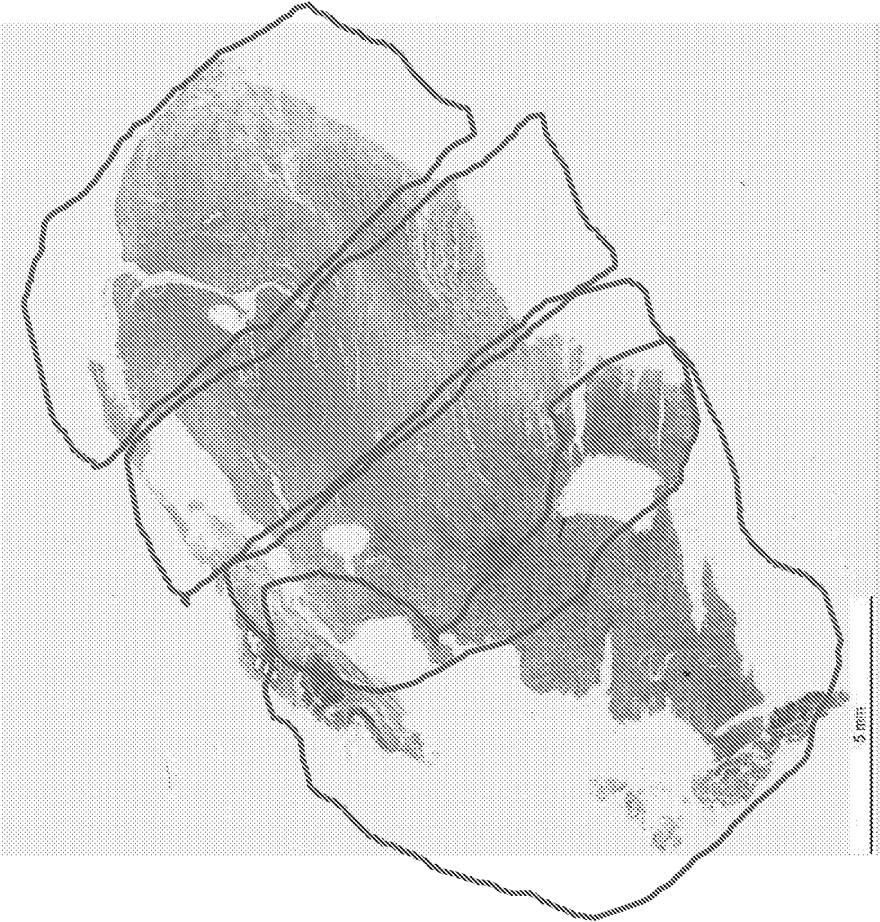


FIGURE 9

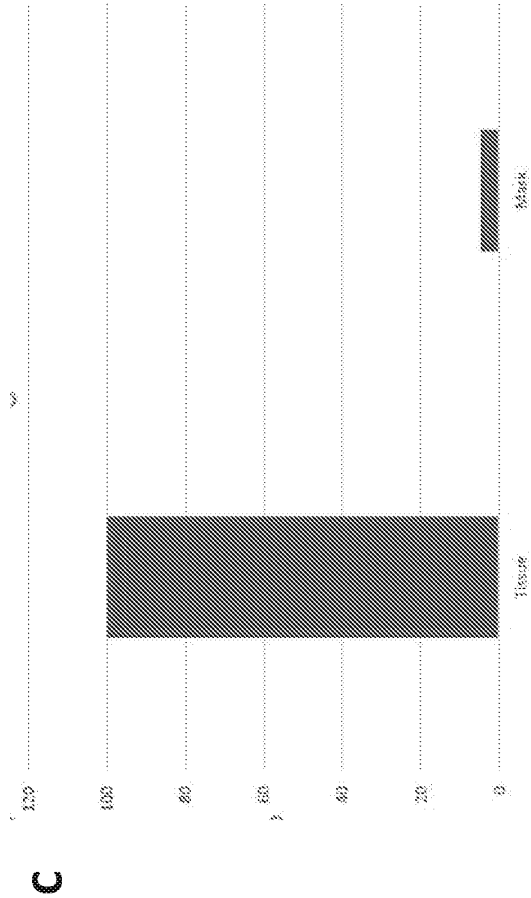
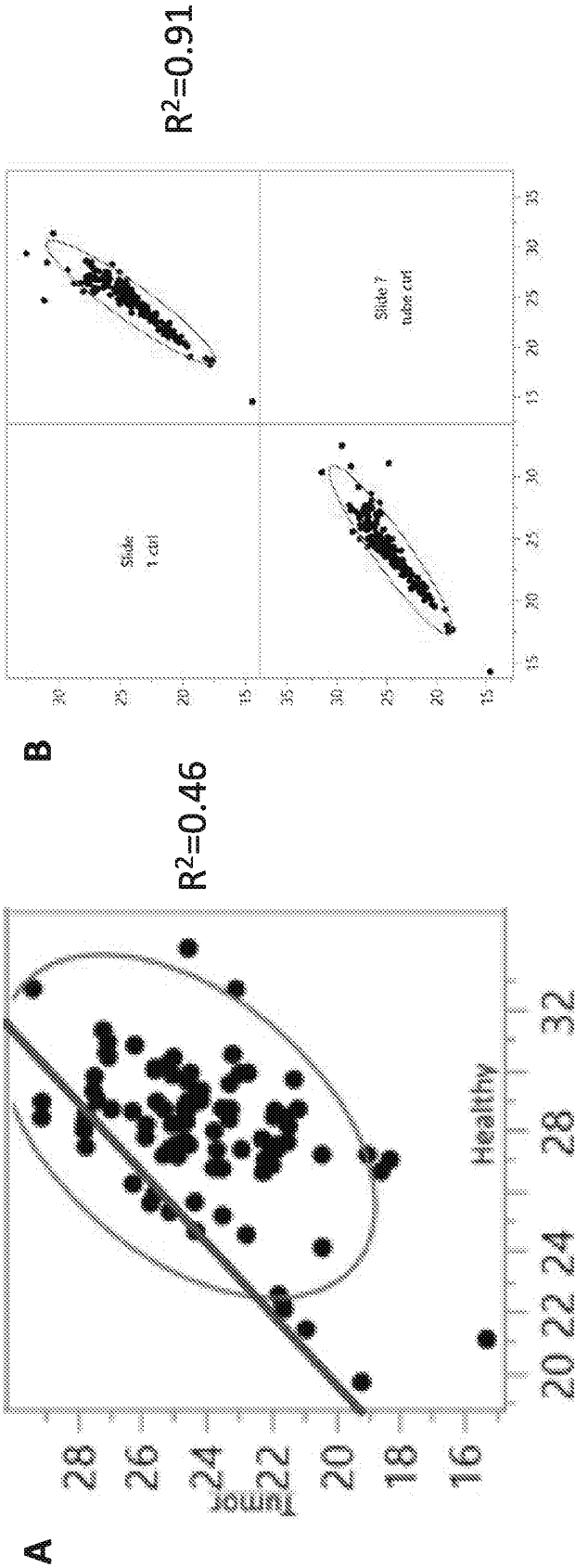
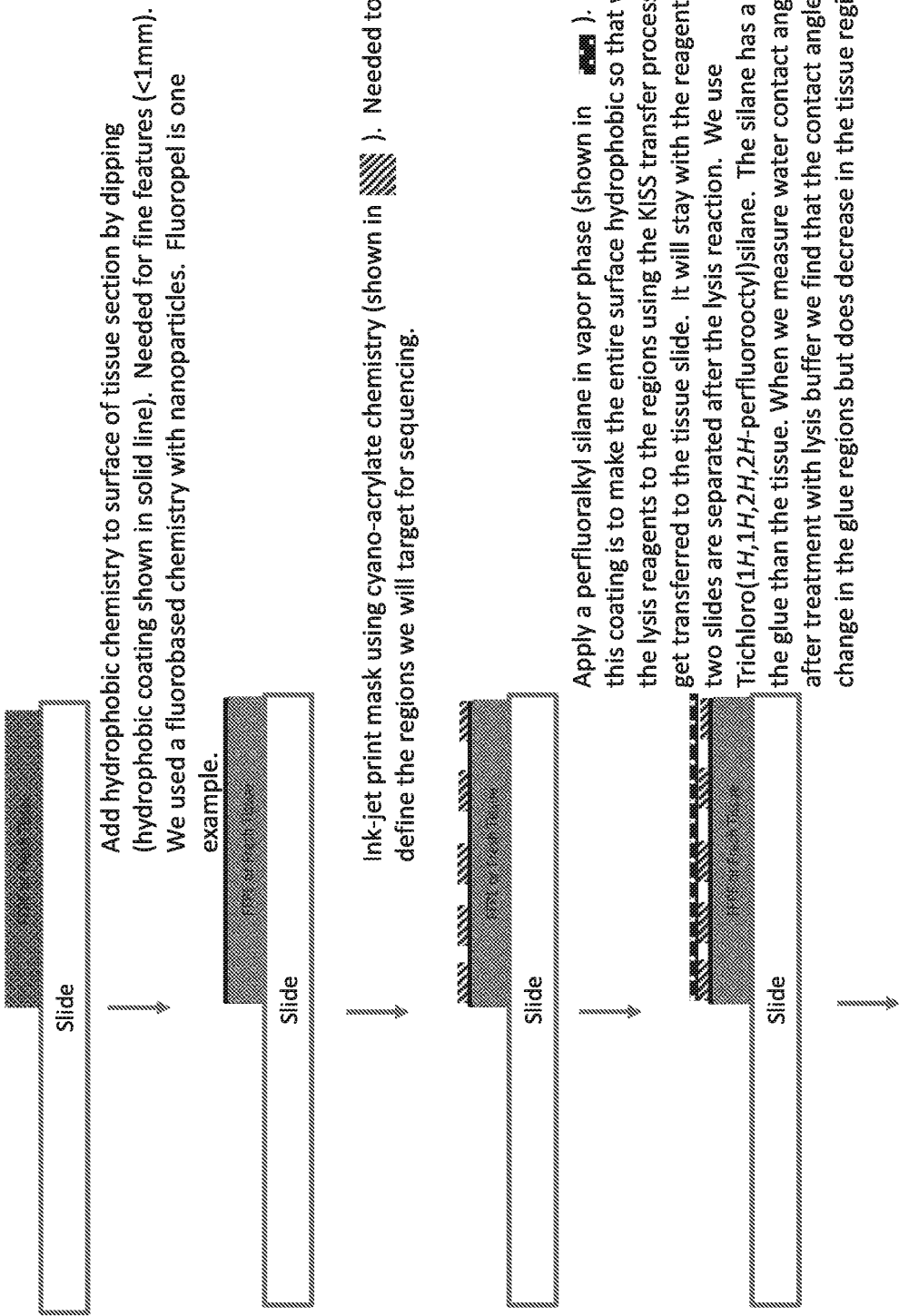


FIGURE 10A



Add hydrophobic chemistry to surface of tissue section by dipping (hydrophobic coating shown in solid line). Needed for fine features (<1mm). We used a fluorobased chemistry with nanoparticles. Fluoropel is one example.

Ink-jet print mask using cyano-acrylate chemistry (shown in ▨). Needed to define the regions we will target for sequencing.

Apply a perfluoralkyl silane in vapor phase (shown in ▨). The purpose of this coating is to make the entire surface hydrophobic so that when we deliver the lysis reagents to the regions using the KISS transfer process the drop does get transferred to the tissue slide. It will stay with the reagent slide when the two slides are separated after the lysis reaction. We use Trichloro(1H,1H,2H,2H-perfluorooctyl)silane. The silane has a tighter bond to the glue than the tissue. When we measure water contact angles before and after treatment with lysis buffer we find that the contact angle does not change in the glue regions but does decrease in the tissue regions.

See next slide

FIGURE 10B

