CLAIMS

WHAT IS CLAIMED IS:

1. A method of isolating biomolecules from at least one region of interest on a planar tissue section on a solid substrate for sequencing or analysis, comprising:

(a) circumscribing defined regions of interest on the planar tissue section on a solid substrate with a polymer to form a nonphysical fluid barrier, wherein the region of interest is smaller than 1mm in diameter; and

(b) selectively disrupting in situ cells within the at least one defined region of interest.

2. A method of isolating biomolecules from at least one region of interest on a planar tissue section on a solid substrate for sequencing or analysis, comprising:

applying to the planar tissue section a chemical mask circumscribing the at least one region of interest in the planar tissue section on a solid substrate,

wherein the chemical mask forms a barrier suitable for selective disruption of cells within the at least one region of interest, wherein the chemical mask is not a physical fluid barrier.

~~3. A method of isolating biomolecules from at least one region of interest on a planar tissue section on a solid substrate for sequencing or analysis, comprising:~~

~~dispensing a solvent suitable for disruption of cells within the region of interest in the planar tissue section on a solid substrate to form a droplet containing liberated cellular components,~~

~~wherein the droplet is isolated from fluid communication from tissue outside the region of interest without a physical barrier.~~

~~4.~~3. A method of isolating and detecting biomolecules from a plurality of regions of interest in a tissue section, comprising:

applying a hydrophobic mask to a tissue section such that at least 1000 regions of interest in the planar tissue section are isolated from fluid communication with each other without a physical barrier and

detecting a plurality of proteins or nucleic acids liberated in situ from cells within the at least 1000 regions of interest, such that the spatial location information of the proteins or nucleic acids is retained.

~~5.~~4. The method of any one of claims 1-~~3~~4, wherein the method is capable of isolating regions of interest smaller than or equal to about 100 microns in diameter.

~~6.~~5. The method of any one of claims 1-~~4~~5, wherein the tissue section is an FFPE tissue section.

~~7.~~6. The method of any one of claims 1-~~5~~6, comprising detecting a plurality of proteins or nucleic acids liberated in situ from the cells within the region of interest, wherein detecting comprises sequencing the nucleic acids liberated in situ from the cells.

~~8.~~7. The method of claim ~~6~~7, wherein sequencing comprises tagging the nucleic acids liberated in situ with barcodes that identify the locations of the regions of interest.

~~9.~~8. The method of claim ~~7~~8, wherein the barcodes are not linked to a surface.

~~10.~~9. The method of claim ~~3~~8, comprising detecting a plurality of proteins or nucleic acids liberated in situ from the cells within the region of interest, and wherein detecting comprises sequencing the nucleic acids liberated in situ from the cells, and wherein sequencing comprises tagging the nucleic acids liberated in situ with barcodes that identify the locations of the regions of interest, and wherein tagging comprises tagging RNA liberated in situ and involves

(a) applying oligonucleotides containing unique nucleic acid sequences to the at least 1000 regions of interest; and

(b) performing 1st and 2nd-strand cDNA synthesis on the at least 1000 regions of interest such that the regions of interest are isolated from each other.

~~11.~~10. The method of claim ~~9~~10, wherein there is reduced cross-contamination of the unique nucleic acid sequences between cDNA molecules synthesized from the regions of interest.

~~12.~~11. The method of claim ~~9~~10, wherein the 1st –strand cDNA synthesis is performed in situ.

~~13.~~12. The method of claim ~~9~~10, wherein the 1st and 2nd-strand cDNA synthesis is performed in situ.

~~14.~~13. The method of claim ~~6~~7, wherein sequencing the nucleic acids liberated in situ from the cells comprises sequencing both mRNA and genomic DNA.

~~15.~~14. The method of claim ~~6~~14, wherein sequencing the nucleic acids liberated in situ from the cells comprises sequencing mRNA.

~~16.~~15. The method of claim ~~14~~15, wherein sequencing mRNA comprises a pre-amplification step performed on cDNA generated from the mRNA.

~~17.~~16. The method of claim 15~~16~~, wherein the pre-amplification step is LM-PCR, PCR with random hexamer primers, PCR with poly-A specific primers, or any combination thereof

~~18.~~17. The method of claim 6~~14~~, wherein sequencing the nucleic acids liberated in situ from the cells comprises sequencing genomic DNA.

~~19.~~18. The method of claim 17~~18~~, wherein sequencing genomic DNA comprises a whole genome amplification (WGA) step performed on the genomic DNA.

~~20.~~19. The method of claim 18~~19~~, wherein the WGA step is degenerate-oligonucleotide-primed (DOP) PCR, multiple-displacement-amplification (MDA), Multiple Annealing and Looping–Based Amplification Cycles (MALBAC), PicoPlex, isothermal amplification with self-degenerate primers, or a combination thereof.

~~21.~~20. The method of claim 2~~any one of the preceding claims~~, wherein the chemical mask comprises at least a layer of cyanoacrylate polymer.

~~22.~~21. The method of claim 2 or claim 20~~any one of the preceding claims~~, wherein the chemical mask comprises at least a non-silylated fluoroalkyl layer.

~~23.~~22. The method of claim 21~~22~~, wherein the non-silylated fluoroalkyl layer comprises a fluoroacrylate or non-silylated perfluoroalkane compound.

~~24.~~23. The method of any one of claims 20-22~~21-23~~, wherein the chemical mask comprises at least a perfluoroalkylsilane layer.

~~25.~~24. The method of claim 23~~24~~, wherein the perfluoroalkylsilane layer comprises a perfluoroalkyltrichlorosilane or a perfluoroalkyltris(dimethylamino)silane.