

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. 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. The method of any one of claims 1-4, wherein the method is capable of isolating regions of interest smaller than or equal to about 100 microns in diameter.
6. The method of any one of claims 1-5, wherein the tissue section is an FFPE tissue section.
7. The method of any one of claims 1-6, 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. The method of claim 6, wherein sequencing comprises tagging the nucleic acids liberated *in situ* with barcodes that identify the locations of the regions of interest.
9. The method of claim 8, wherein the barcodes are not linked to a surface.
10. The method of claim 8, 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. The method of claim 10, wherein there is reduced cross-contamination of the unique nucleic acid sequences between cDNA molecules synthesized from the regions of interest.
12. The method of claim 10, wherein the 1st-strand cDNA synthesis is performed *in situ*.
13. The method of claim 10, wherein the 1st and 2nd-strand cDNA synthesis is performed *in situ*.
14. The method of claim 6, wherein sequencing the nucleic acids liberated *in situ* from the cells comprises sequencing both mRNA and genomic DNA.
15. The method of claim 14, wherein sequencing the nucleic acids liberated *in situ* from the cells comprises sequencing mRNA.
16. The method of claim 15, wherein sequencing mRNA comprises a pre-amplification step performed on cDNA generated from the mRNA.
17. The method of claim 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. The method of claim 14, wherein sequencing the nucleic acids liberated *in situ* from the cells comprises sequencing genomic DNA.
19. The method of claim 18, wherein sequencing genomic DNA comprises a whole genome amplification (WGA) step performed on the genomic DNA.

20. The method of claim 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. A tissue section, comprising thereupon at least 1000 aqueous droplets that are isolated from fluid communication with each other without a physical barrier.
22. The tissue section of claim 21, comprising a hydrophobic mask isolating the 1000 aqueous droplets from fluid communication with each other
23. The tissue section of claim 21 or 22, wherein the at least 1000 aqueous droplets comprise at least 1000 unique oligonucleotides.
24. The tissue section of any one of claims 21-23, wherein the hydrophobic mask comprises at least a layer of cyanoacrylate polymer.
25. The tissue section of any one of claims 21-24, wherein the hydrophobic mask comprises at least a non-silylated fluoroalkyl layer.
26. The tissue section of claim 24, wherein the non-silylated fluoroalkyl layer comprises a fluoroacrylate or non-silylated perfluoroalkane compound.
27. The tissue section of any one of claims 21-26, comprising at least a perfluoroalkylsilane layer.
28. The tissue section of claim 27, wherein the perfluoroalkylsilane layer comprises a perfluoroalkyltrichlorosilane or a perfluoroalkyltris(dimethylamino)silane
29. The tissue section of any one of claims 23-28, wherein the oligonucleotides are not linked to a surface.
30. A method of isolating cellular components from at least one region of interest in a planar tissue section on a solid substrate, comprising:
 - (a) 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;
 - (b) solubilizing cellular components within the at least one region of interest by dispensing a solution comprising one or more extraction agents onto the region of interest, thereby selectively lysing cells circumscribed within the at least one region of interest.
31. The method of claim 30, wherein dispensing the solution onto the region of interest comprises:

(a) dispensing the solution comprising one or more extraction agents onto an agent transfer device comprising at least one agent transfer array element positioned on a surface of a second solid substrate;

(b) contacting the at least one agent transfer array element positioned on the surface of the second solid substrate to the planar tissue section, such that the at least one agent transfer array element spatially corresponds to the at least one region of interest in the planar tissue section, wherein the contacting allows the solution comprising the one or more extraction agents to be transferred to the at least one region of interest in the planar tissue section, thereby solubilizing the cellular components in the at least one region of interest in the solution comprising the one or more extraction agents.

32. The method of claim 30, further comprising

isolating at least one solubilized cellular component from the at least one region of interest in the planar tissue section on the solid substrate.

33. The method of any one of claims 30-32, further comprising

sequencing nucleic acids among the cellular components solubilized from the at least one region of interest.

34. The method of any one of claims 30-33, further comprising

performing tandem mass spectrometry on proteins among the cellular components solubilized from the at least one region of interest.

35. The method of any one of claims 30-34, wherein the solution in (b) further comprises a soluble tag, wherein the soluble tag corresponds to the at least one region of interest.

36. The method of claim 35, wherein the soluble tag is an oligonucleotide.

37. The method of claim 36, wherein the oligonucleotide comprises a sample index sequence corresponding to the region of interest and, optionally, a unique molecular identifier sequence.

38. The method of claim 36, wherein the oligonucleotide comprises a charge tag corresponding to the region of interest.

39. The method of claim 36 or 37, wherein the oligonucleotide is double-stranded.

40. The method of any one of claims 36-38, wherein the oligonucleotide is conjugated to a bead.

41. The method of claim 35, wherein the soluble tag is a Tandem Mass Tag.

42. The method of any one of claims 30-41, wherein the one or more extraction agents comprise one or more surfactants, proteases, tonicity adjusting agents, chaotropes, nucleases, buffers, protease inhibitors, phosphatase inhibitors, or nuclease inhibitors.

43. The method of any one of claims 30-42, wherein the chemical mask has a contact angle of 60-155 degrees.
44. The method of any one of claims 30-43, wherein the chemical mask is applied via a hydrophobic mask solution comprising a fluoropolymer or a fluoroacrylic polymer.
45. The method of claim 44, wherein the chemical mask solution is applied via a solution comprising an acrylate or cyano-acrylate.
46. The method of claim 45, wherein the chemical mask solution further comprises a solvent.
47. The method of claim 46, wherein the solvent is a propylene glycol derivative, a fluorocarbon, or an alcohol.
48. The method of claim 46, wherein the solvent is perfluorooctane, perfluoro-2-methylpentane, perfluoro-1,3-dimethylcyclohexane, perfluorodecalin, or 1,3-difluoropropane.
49. The method of any one of claims 30-48, wherein the at least one region of interest is circular, and the diameter of the region of interest is 1mm or less, 500 microns or less, 250 microns or less, 125 microns or less, 100 microns or less, 80 microns or less, 50 microns or less, 25 microns or less, or 15 microns or less.
50. The method of any one of claims 30-44, wherein the at least one region of interest is less than about 7.8×10^5 square microns in area.
51. The method of any one of claims 31-50, wherein the at least one agent transfer array element positioned on the surface of the second solid substrate comprises at least one hydrophilic region circumscribed by a hydrophobic region.
52. The method of claim 51, wherein the at least one agent transfer array element positioned on the surface of the second solid substrate is capable of delivering a volume of solution 10,000 picoliters or less, 1,000 picoliters or less, 500 picoliters or less, 250 picoliters or less, 100 picoliters or less, 50 picoliters or less, 10 picoliters or less, or 2 picoliters or less.
53. The method of claim 51, wherein the hydrophobic region circumscribing the at least one hydrophilic region has a contact angle of 60-155 degrees.
54. The method of any one of claims 30-53, comprising solubilizing cellular components from more than one region of interest in the planar tissue section.
55. The method of any one of claims 30-53 comprising solubilizing cellular components from at least 10, at least 100, at least 1000, or at least 10,000 regions of interest in the planar tissue section.
56. The method of any one of claims 30-55, wherein the hydrophobic mask circumscribing the at least one region of interest in the planar tissue section comprises a grid pattern.

57. The method of any one of claims 30-55, wherein the hydrophobic mask circumscribing the at least one region of interest in the planar tissue section comprises a circle.
58. The method of any one of claims 30-57, wherein the hydrophobic mask circumscribing the at least one region of interest in the planar tissue section on a solid substrate is applied to the planar tissue section using a piezoelectric ink-jet delivery device.
59. The method of any one of claims 30-58, wherein the planar tissue section is about 2 to about 50 μm in thickness.
60. The method of any one of claims 30-58, wherein the planar tissue section is about 1 to about 15 μm in thickness.
61. The method of any one of claims 30-58, wherein the planar tissue section is a formalin fixed, paraffin embedded (FFPE) tissue section.
62. The method of any one of claims 30-58, wherein the planar tissue section is an unfixed tissue section.
63. A system for isolating cellular components from at least one region of interest in a planar tissue section on a first solid support, comprising:
- (a) the planar tissue section on a first solid support comprising the at least one region of interest circumscribed with a hydrophobic mask;
 - (b) a second solid support comprising at least one agent transfer array element positioned on the surface of the solid substrate so as to align with the at least one region of interest in the planar tissue section, the at least one agent transfer array element including a solution comprising one or more extraction agents; and
 - (c) a motorized stage coupled to the solid support comprising the at least one agent transfer array element and capable of translating the solid support such that the at least one agent transfer array element positioned on the surface of the solid substrate can contact the at least one region of interest in the planar tissue section circumscribed with a hydrophobic mask.
64. The system of claim 63, further comprising
- (i) a computer system configured to control the position of the motorized stage such that the at least one agent transfer array element positioned on the surface on the second solid substrate contacts the at least one region of interest in the tissue section.
65. The system of claim 63 or 64, wherein the hydrophobic mask solution is applied by a system comprising

- (i) a piezoelectric ink-jet delivery device capable of delivering a hydrophobic mask solution to the tissue section on the solid support and
 - (ii) a computer system configured to control the piezoelectric ink-jet delivery device to circumscribe at least one region of interest in the planar tissue section with the hydrophobic mask solution to generate a hydrophobic mask.
66. The system of claims 63-65, wherein the at least one agent transfer array element positioned on the surface of the second solid substrate comprises at least one hydrophilic region circumscribed by a hydrophobic region.
67. The system of any one of claims 58-66, wherein the hydrophobic mask solution comprises at least one of C7F15CH2OCOC(CH3)=CH2, FC-722, PerFluoroCoat, or FluoroPel.
68. The system of any one of claims 58-67, wherein the hydrophobic mask solution comprises at least one of an acrylate or cyanoacrylate.
69. The system of any one of claims 58-68, wherein the at least one agent transfer array element positioned on the surface of the second solid substrate is capable of delivering a volume of solution 10,000 picoliters or less, 1,000 picoliters or less, 500 picoliters or less, 250 picoliters or less, 100 picoliters or less, 50 picoliters or less, 10 picoliters or less, or 2 picoliters or less.
70. The system of any one of claims 58-68, wherein the contact angle of the hydrophobic region circumscribing the at least one hydrophilic region is 60-155 degrees.
71. The system of any one of claims 65-70, wherein the system for hydrophobic mask solution application comprises a UV light source capable of polymerizing said hydrophobic mask solution after application to the planar tissue section.
72. The system of any one of claims 65-71, wherein the system for hydrophobic mask solution application comprises an apparatus for chemical vapor deposition of an perfluoroalkylsilane.
73. A kit for isolating cellular components from at least one region of interest in a planar tissue section, comprising any of the elements of any one of claims 63-72.
74. A tissue section, comprising thereupon from bottom to top at least one cyanoacrylate layer followed by at least one perfluoroalkylsilane layer.
75. The tissue section of claim 74, comprising at least one non-silylated fluoroalkyl layer below the at least one cyanoacrylate layer.
76. The tissue section of claim 75, wherein the non-silylated fluoroalkyl layer comprises a fluoroacrylate or a non-silylated perfluoroalkane compound.

77. The tissue section of any one of claims 74-76, wherein the perfluoroalkylsilane layer comprises a perfluoroalkyltrichlorosilane or a perfluoroalkyltris(dimethylamino)silane.
78. The tissue section of any one of claims 74-77, comprising at least one region lacking the cyanoacrylate layer circumscribed by regions possessing the cyanoacrylate layer.
79. The tissue section of claim 78, wherein the region is substantially elliptical, polygonal, or free-form.
80. The tissue section of any one of claims 78-79, wherein application of an aqueous solution of detergent to the tissue section is capable of solubilizing cells within the region.
81. The tissue section of any one of claims 78-80, wherein the region is less than about 1mm in size.
82. The tissue section of claim 81, wherein the region is less than about 100 microns in size.
83. The tissue section of any one of claims 74-82, wherein the tissue section is a formalin-fixed paraffin-embedded (FFPE) tissue section.
84. The tissue section of claim 83, wherein the tissue section is a deparaffinized FFPE tissue section.