CLAIMS

WHAT IS CLAIMED IS:

1. A method for processing a biological sample, comprising:

(a) providing a container comprising said biological sample comprising an analyte within a synthetic three-dimensional (3D) matrix, which synthetic 3D matrix comprises an attachment moiety; and

(b) directing a tethering molecule into said biological sample such that said tethering molecule (i) flows through said synthetic 3D matrix and comes in contact with said analyte, and (ii) attaches to said analyte and said attachment moiety, thereby attaching said analyte to said synthetic 3D matrix.

2. The method of claim 1, wherein said tethering molecule comprises a tethering moiety that has specificity for said attachment moiety.

3. The method of claim 1 or claim 2, wherein said synthetic 3D matrix encapsulates said biological sample.

4. The method of any one of claims 1-3, further comprising, prior to (a), providing a matrix-forming material in said container, and using said matrix-forming material to generate said synthetic 3D matrix.

5. The method of claim 4, wherein said synthetic 3D matrix is generated by polymerization of said matrix-forming material.

6. The method of claim 4, wherein said synthetic 3D matrix is generated by cross-linking of said matrix-forming material.

7. The method of any one of claims 1-6, further comprising, subsequent to (b), identifying said analyte within said synthetic 3D matrix.

8. The method of any one of claims 1-7, wherein said tethering molecule comprises a nucleic acid sequence complementary to a sequence of said analyte.

9. The method of claim 8, wherein said tethering molecule comprises a poly-deoxythymidine (dT) sequence.

10. The method of claim 8 or claim 9, further comprising, in (b), contacting said biological sample with a hybridization reaction enhancing agent, wherein said hybridization reaction enhancing agent enhances a rate of hybridization between said nucleic acid sequence and said sequence of said analyte.

11. The method of claim 10, wherein said hybridization reaction enhancing agent comprises a functional group that facilitates inactivation of said hybridization reaction enhancing agent.

12. The method of claim 10 or claim 11, further comprising subsequent to contacting said biological sample with said hybridization reaction enhancing agent, subjecting said functional group to conditions sufficient to inactivate said hybridization reaction enhancing agent.

13. The method of any one of claims 10-12, further comprising inactivating said hybridization reaction enhancing agent.

14. The method of any one of claims 11-13, wherein said functional group is a hydrating group.

15. The method of claim 14, wherein said hydrating group is an ionic, electrolytic, or hydrophilic group.

16. The method of any one of claims 11-15, wherein said functional group further comprises a cleavable linker, which cleavable linker links said functional group to a polymer backbone of said hybridization reaction enhancing agent.

17. The method of claim 16, wherein said cleavable linker comprises an alpha-hydroxy acid, a beta-keto acid, or a disulfide linkage.

18. The method of claim 16 or claim 17, further comprising cleaving said functional group from said polymer backbone of said hybridization reaction enhancing agent.

19. The method of claim 18, further comprising removing said functional group from said synthetic 3D matrix.

20. The method of any one of claims 10-19, further comprising removing said hybridization reaction enhancing agent from said synthetic 3D matrix.

21. The method of claim 20, wherein said removing comprises washing away said hybridization reaction enhancing agent from said synthetic 3D matrix.

22. The method of any one of claims 1-21, further comprising, subsequent to (b), contacting said biological sample with a protease.

23. The method of any one of claims 1-22, wherein said analyte comprises (i) a nucleic acid molecule or (ii) a polypeptide molecule.

24. The method of claim 23, wherein said analyte comprises one or more polypeptide molecules, and wherein said analyte is bound by an antibody or fragment thereof.

25. The method of claim 24, wherein said antibody or fragment thereof is conjugated to a nucleic acid barcode, and wherein said tethering molecule comprises a nucleic acid sequence complementary to said nucleic acid barcode.

26. The method of claim 23, wherein said analyte comprises one or more polypeptide molecules, and wherein said tethering molecule is an antibody or fragment thereof conjugated to a nucleic acid barcode.

27. The method of claim 26, wherein said nucleic acid barcode comprises a tethering moiety, which tethering moiety attaches to said attachment moiety of said synthetic 3D matrix.

28. The method of claim 23, wherein said analyte is said nucleic acid molecule, and wherein said tethering molecule comprises a primer sequence complementary to a sequence of a nucleic acid molecule of said one or more nucleic acid molecules.

29. The method of claim 28, further comprising, in (b), hybridizing said primer sequence to said sequence of said nucleic acid molecule.

30. The method of claim 29, further comprising, subsequent to (b), extending said primer sequence hybridized to said sequence of said nucleic acid molecule to generate an extension product attached to said synthetic 3D matrix.

31. The method of claim 30, further comprising hybridizing a probe to said extension product in presence of an additional hybridization reaction enhancing agent.

32. The method of claim 31, wherein said probe is a padlock probe or a molecular inversion probe.

33. The method of claim 31 or 32, further comprising inactivating said additional hybridization reaction enhancing agent or removing said additional hybridization reaction enhancing agent from said synthetic 3D matrix.

34. The method of claim 33, further comprising circularizing said probe.

35. The method of claim 34, wherein said circularizing comprises ligating a 3' end and a 5' end of said probe.

36. The method of any one of claims 31-35, further comprising subjecting said probe to an amplification reaction to generate an amplification product.

37. The method of claim 36, wherein said amplification reaction is rolling circle amplification.

38. The method of claim 36 or 37, wherein said amplification reaction incorporates a modified base to said amplification product.

39. The method of claim 38, wherein said modified base is 5-azidomethyl-dUTP.

40. The method of claim 36, wherein said amplification reaction is a non-enzymatic reaction.

41. The method of claim 40, wherein said non-enzymatic reaction is a hybridization chain reaction (HCR) or a branched DNA reaction.

42. The method of any one of claims 1-41, wherein said biological sample is a cell or a tissue.

43. The method of claim 42, wherein said cell or said tissue is fixed.

44. The method of claim 42 or 43, wherein said cell or said tissue is permeabilized.

45. A method for processing a biological sample, comprising:

(a) contacting said biological sample comprising an analyte with a tethering molecule and a hybridization reaction enhancing agent, wherein said tethering molecule comprises a tethering moiety and a nucleic acid sequence complementary to a sequence of said analyte, and wherein said hybridization reaction enhancing agent enhances a rate of hybridization between said nucleic acid sequence and said sequence of said analyte;

(b) hybridizing said nucleic acid sequence of said tethering molecule to said sequence of said analyte;

(c) contacting said biological sample having said tethering molecule hybridized to said analyte with a matrix-forming material; and

(d) using said matrix-forming material to form a synthetic three-dimensional (3D) matrix with said tethering molecule attached thereto via said tethering moiety.

46. The method of claim 45, wherein said synthetic 3D matrix preserves an absolute or relative spatial position of said analyte within said biological sample.

47. The method of claim 45, wherein said synthetic 3D matrix further comprises an attachment moiety.

48. The method of claim 47, wherein said tethering moiety of said tethering molecule has specificity for said attachment moiety.

49. The method of claim 48, further comprising, in (d), attaching said tethering moiety to said attachment moiety.

50. The method of any one of claims 45-49, wherein said hybridization reaction enhancing agent comprises a functional group that facilitates inactivation of said hybridization reaction enhancing agent.

51. The method of claim 50, further comprising, subsequent to (d), subjecting said functional group to conditions sufficient to inactivate said hybridization reaction enhancing agent.

52. The method of claim 51, further comprising inactivating said hybridization reaction enhancing agent.

53. The method of any one of claims 50-52, wherein said functional group is a hydrating group.

54. The method of claim 53, wherein said hydrating group is an ionic, electrolytic, or hydrophilic group.

55. The method of any one of claims 50-54, wherein said functional group further comprises a cleavable linker, which cleavable linker links said functional group to a polymer backbone of said hybridization reaction enhancing agent.

56. The method of claim 55, wherein said cleavable linker comprises an alpha-hydroxy acid, a beta-keto acid, or a disulfide linkage.

57. The method of claim 55 or 56, further comprising cleaving said functional group from said polymer backbone of said hybridization reaction enhancing agent.

58. The method of claim 57, further comprising removing said functional group from said synthetic 3D matrix.

59. The method of any one of claims 45-49, further comprising removing said hybridization reaction enhancing agent from said synthetic 3D matrix.

60. The method of claim 59, wherein said removing comprises washing away said hybridization reaction enhancing agent from said synthetic 3D matrix.

61. The method of any one of claims 45-60, further comprising, subsequent to (d), contacting said biological sample encapsulated within said synthetic 3D matrix with a protease.

62. The method of any one of claims 45-61, wherein said analyte comprises (i) a nucleic acid molecule or (ii) a polypeptide molecule.

63. The method of claim 62, wherein said analyte is one or more polypeptide molecules, wherein said analyte is bound by an antibody or fragment thereof.

64. The method of claim 63, wherein said antibody or fragment thereof is conjugated to a nucleic acid barcode, and wherein said tethering molecule comprises a nucleic acid sequence complementary to said nucleic acid barcode.

65. The method of claim 62, wherein said analyte is one or more nucleic acid molecules, and wherein said tethering molecule comprises a primer sequence complementary to a sequence of a nucleic acid molecule of said one or more nucleic acid molecules.

66. The method of claim 65, further comprising, subsequent to (d), extending said primer sequence hybridized to said sequence of said nucleic acid molecule to generate an extension product attached to said synthetic 3D matrix.

67. The method of claim 66, further comprising hybridizing a probe to said extension product in presence of an additional hybridization reaction enhancing agent.

68. The method of claim 67, wherein said probe is a padlock probe or a molecular inversion probe.

69. The method of claim 67 or 68, further comprising inactivating said additional hybridization reaction enhancing agent or removing said additional hybridization reaction enhancing agent from said synthetic 3D matrix.

70. The method of claim 69, further comprising circularizing said probe.

71. The method of claim 70, wherein said circularizing comprises ligating a 3' end and a 5' end of said probe.

72. The method of any one of claims 67-71, further comprising subjecting said probe to an amplification reaction to generate an amplification product.

73. The method of claim 72, wherein said amplification reaction is rolling circle amplification.

74. The method of claim 72 or 73, wherein said amplification reaction incorporates a modified base to said amplification product.

75. The method of claim 74, wherein said modified base is 5-azidomethyl dUTP.

76. The method of claim 72, wherein said amplification reaction is a non-enzymatic reaction.

77. The method of claim 76, wherein said non-enzymatic reaction is a hybridization chain reaction (HCR) or a branched DNA reaction.

78. The method of any one of claims 45-77, wherein said biological sample is a cell or a tissue.

79. The method of claim 78, wherein said cell or said tissue is fixed.

80. The method of claim 78 or 79, wherein said cell or said tissue is permeabilized.

81. A composition for processing a biological sample comprising an analyte, comprising:

said biological sample encapsulated within a synthetic three-dimensional (3D) matrix;

an attachment moiety coupled to said synthetic 3D matrix;

a tethering probe, wherein said tethering probe comprises a nucleic acid sequence complementary to a sequence of said analyte, and wherein said nucleic acid sequence is configured to hybridize to said sequence of said analyte; and

a hybridization reaction enhancing agent, which hybridization reaction enhancing agent enhances a rate of hybridization between said nucleic acid sequence and said sequence of said analyte.

82. The composition of claim 81, wherein said attachment moiety is configured to preserve an absolute or relative spatial position of said analyte within said synthetic 3D matrix.

83. The composition of claim 81 or claim 82, wherein said analyte is a nucleic acid molecule or a polypeptide molecule.

84. The composition of any one of claims 81-83, wherein said biological sample is a cell or a tissue.

85. The composition of any one of claims 81-84, wherein said cell or said tissue is fixed.

86. The composition of claim 84 or 85, wherein said cell or said tissue is permeabilized.

87. The composition of any one of claims 81-86, wherein said hybridization reaction enhancing agent comprises a functional group that facilitates inactivation of said hybridization reaction enhancing agent.

88. The composition of claim 87, wherein said functional group is a hydrating group.

89. The composition of claim 88, wherein said hydrating group is an ionic, electrolytic, or hydrophilic group.

90. The composition of any one of claims 87-89, wherein said functional group further comprises a cleavable linker, which cleavable linker links said functional group to a polymer backbone of said hybridization reaction enhancing agent.

91. A method for processing a biological sample, comprising:

(a) contacting said biological sample comprising a polypeptide analyte with a tethering molecule, wherein said tethering molecule comprises (i) an antibody or fragment thereof conjugated to a nucleic acid barcode and (ii) a tethering moiety;

(b) binding said polypeptide analyte with said tethering molecule;

(c) contacting said biological sample having said tethering molecule bound to said polypeptide analyte with a matrix-forming material; and

(d) using said matrix-forming material to form a synthetic three-dimensional (3D) matrix with said tethering molecule attached thereto via said tethering moiety.

92. The method of claim 91, wherein said synthetic three-dimensional (3D) matrix comprises an attachment moiety.

93. The method of claim 91 or claim 92, wherein said tethering moiety is an azide.

94. The method of claim 93, wherein said attachment moiety comprises an alkyne and (b) comprises reacting said alkyne with said azide.

95. The method of any one of claims 91-94, wherein, in (b), said tethering molecule attaches to said attachment moiety via a click chemistry reaction.

96. The method of any one of claims 91-94, wherein said attachment moiety and said tethering moiety are configured to couple to one another in a click chemistry reaction.

97. The method of any one of claims 91-96, further comprising, subsequent to (b), degrading said analyte.

98. The method of claim 97, further comprising, subsequent to (b), contacting said biological sample with a protease.

99. The method of any one of claims 91-98, wherein, in (a), said biological sample comprises an additional analyte within said synthetic 3D matrix, which synthetic 3D matrix comprises an additional attachment moiety.

100. The method of claim 99, further comprising contacting an additional tethering molecule with said biological sample such that said additional tethering molecule (i) flows through said synthetic 3D matrix and comes in contact with said additional analyte, and (ii) attaches to said additional analyte and said additional attachment moiety, thereby attaching said additional analyte to said synthetic 3D matrix.

101. The method of claim 99 or claim 100, wherein said polypeptide analyte and said additional analyte are different analytes.

102. The method of claim 101, wherein said polypeptide analyte and said additional analyte are different types of analytes.

103. The method of any one of claims 99-102, wherein said additional analyte is a nucleic acid molecule.

104. The method of claim 103, wherein said nucleic acid is a ribonucleic acid molecule.

105. The method of claim any one of claims 99-104, further comprising identifying said polypeptide analyte and said additional analyte.

106. The method of any one of claims 103-105, wherein said additional tethering molecule comprises a primer sequence complementary to a sequence of said nucleic acid molecule.

~~107. The method of claim 106, further comprising, hybridizing said primer sequence to said sequence of said nucleic acid molecule.~~

~~108. The method of claim 107, further comprising, extending said primer sequence hybridized to said sequence of said nucleic acid molecule to generate an extension product attached to said synthetic 3D matrix.~~

~~109. The method of claim 108, further comprising hybridizing a probe to said extension product.~~

~~110. The method of claim 109, wherein said probe is a padlock probe or a molecular inversion probe.~~

~~111. The method of claim 109 or claim 110, further comprising circularizing said probe.~~

~~112. The method of claim 111, wherein said circularizing comprises ligating a 3' end and a 5' end of said probe.~~

~~113. The method of any one of claims 109-112, further comprising subjecting said probe to an amplification reaction to generate an amplification product.~~

~~114. The method of claim 113, wherein said amplification reaction is rolling circle amplification.~~

~~115. The method of claim 113 or claim 114, wherein said amplification reaction incorporates a modified base to said amplification product.~~

~~116. The method of claim 115, wherein said modified base is 5-azidomethyl-dUTP.~~

~~117. The method of claim 113, wherein said amplification reaction is a non-enzymatic reaction.~~

~~118. The method of claim 117, wherein said non-enzymatic reaction is a hybridization chain reaction (HCR) or a branched DNA reaction.~~

~~119. The method of any one of claims 91-118, wherein said biological sample is a cell or a tissue.~~

~~120. The method of claim 119, wherein said cell or said tissue is fixed.~~

~~121. The method of claim 119 or claim 120, wherein said cell or said tissue is permeabilized.~~

~~122. The method of any one of claims 99-118, wherein said additional analyte is attached to said additional attachment moiety prior to said polypeptide analyte is attached to said attachment moiety.~~

~~123. The method of any one of claims 99-122, wherein said attachment moiety and said additional attachment moiety are different.~~

124. The method of claim 123, wherein said additional attachment moiety is added to said 3D matrix after (d).

125. The method of claim 123, wherein said additional attachment moiety is added to said 3D matrix after during or before (d).

126. The method of any one of claims 123-125, wherein said tethering moiety comprises an acryloyl group and said additional tethering moiety is an azide.

127. A method for processing a biological sample, comprising:

(a) providing said biological sample comprising a polypeptide analyte within a synthetic three-dimensional (3D) matrix, which synthetic 3D matrix comprises an attachment moiety;

(b) contacting said biological sample with a tethering molecule, wherein said tethering molecule comprises (i) an antibody or fragment thereof conjugated to a nucleic acid barcode and (ii) a tethering moiety; and

(c) binding said polypeptide analyte with said tethering molecule; thereby attaching said analyte to said synthetic 3D matrix via said attachment moiety.

128. The method of claim 127, wherein, in (a), said biological sample comprises an additional analyte within said synthetic 3D matrix.

129. The method of claim 127 or 128, wherein said synthetic 3D matrix comprises an additional attachment moiety.

130. The method of claim 129, wherein said additional attachment moiety is provided to said synthetic 3D matrix after it is formed in (a).

131. The method of claim 129, wherein said additional attachment moiety is provided to said synthetic 3D matrix before or during it is formed in (a).

132. The method of any one of claims 127-131, wherein (b) is performed simultaneously or concurrently with forming of the synthetic 3D matrix.

133. The method of any one of claims 127-131, wherein (b) is performed after forming of the synthetic 3D matrix.

134. The method of any one of claims 128-133, wherein said additional analyte is a nucleic acid molecule.

135. The method of claim 134, further comprising providing an additional tethering molecule, wherein said additional tethering molecule comprises a primer sequence complementary to a sequence of said nucleic acid molecule.

136. The method of claim 135, further comprising, hybridizing said primer sequence to said sequence of said nucleic acid molecule.

~~137. The method of claim 136, further comprising, extending said primer sequence hybridized to said sequence of said nucleic acid molecule to generate an extension product.~~

~~138. The method of claim 137, wherein said extension product is attached to said synthetic 3D matrix.~~

~~139. The method of claim 137 or claim 138, further comprising hybridizing a probe to said extension product.~~

~~140. The method of claim 139, wherein said probe is a padlock probe or a molecular inversion probe.~~

~~141. The method of claim 139 or claim 140, further comprising circularizing said probe.~~

~~142. The method of claim 141, wherein said circularizing comprises ligating a 3' end and a 5' end of said probe.~~

~~143. The method of any one of claims 139-142, further comprising subjecting said probe to an amplification reaction to generate an amplification product.~~

~~144. The method of claim 143, wherein said amplification reaction is rolling circle amplification.~~

~~145. The method of claim 143 or claim 144, wherein said amplification reaction incorporates a modified base to said amplification product.~~

~~146. The method of claim 145, wherein said modified base is 5-azidomethyl-dUTP.~~

~~147. The method of any one of claims 129-146, wherein said attachment moiety and said additional attachment moiety are different.~~

~~148. The method of any one of claims 135-147, wherein said additional tethering molecule (i) flows through said synthetic 3D matrix and comes in contact with said additional analyte, and (ii) attaches to said additional analyte and said additional attachment moiety, thereby attaching said additional analyte to said synthetic 3D matrix.~~

~~149. The method of any one of claims 135-148, wherein said tethering moiety comprises an acryloyl group and said additional tethering moiety is an azide.~~

1. A method for processing a biological sample comprising a polypeptide analyte and a ribonucleic acid (RNA) analyte, wherein the biological sample is a cell or tissue sample, comprising:

(a) contacting said biological sample with a tethering molecule, wherein said tethering molecule comprises (i) an antibody or fragment thereof that binds to said polypeptide analyte and (ii) a nucleic acid barcode conjugated to said antibody or

fragment thereof, wherein said nucleic acid barcode identifies said antibody or fragment thereof and wherein said nucleic acid is conjugated to an acryloyl group;

(b) contacting said biological sample with a primer complementary to a sequence of said RNA molecule and extending said primer to generate a complementary deoxyribonucleic acid (cDNA) molecule;

(c) contacting said biological sample having said tethering molecule bound to said polypeptide analyte with a matrix-forming material comprising a click chemistry moiety and using said matrix-forming material to form a synthetic three-dimensional (3D) matrix comprising said click chemistry moiety wherein said acryloyl group is incorporated into said synthetic 3D matrix, thereby tethering said nucleic acid barcode to said synthetic 3D matrix;

(d) subjecting said biological sample to a clearing process;

(e) hybridizing a probe to said cDNA molecule;

(f) circularizing said probe;

(g) amplifying said circularized probe using a modified base to generate an amplification product comprising said modified base, wherein said modified base comprises an additional click chemistry moiety;

(h) attaching said amplification product to said synthetic 3D matrix via a click chemistry reaction;

(i) detecting said nucleic acid barcode to identify said polypeptide analyte in said biological sample; and

(j) detecting said amplification product to identify said RNA analyte in said biological sample.

2. The method of claim 1, wherein said click chemistry moiety comprises an alkyne, wherein said modified base comprises an azide, and wherein said click chemistry reaction comprises reacting said alkyne with said azide.

3. The method of claim 2, wherein said modified base is 5-azidomethyl-dUTP.

4. The method of any one of claims 1-3, wherein said cell or tissue sample is fixed.

5. The method of claim 4, further comprising, prior to (a), contacting said biological sample with a fixative.

6. The method of any one of claims 1-5, wherein said cell or said tissue sample is permeabilized.

7. The method of claim 6, further comprising, prior to (a), contacting said biological sample with a permeabilization agent.

8. The method of any one of claims 1-7, wherein said probe is a padlock probe or a molecular inversion probe.

9. The method of claim 8, wherein circularizing said probe comprises ligating a 3' end and a 5' end of said probe.

10. The method of any one of claims 1-9, wherein said primer comprises an additional acryloyl group and wherein said additional acryloyl group is incorporated into said synthetic 3D matrix.

11. The method of any one of claims 1-10, wherein said clearing process comprises contacting said biological sample with an enzyme capable of degrading a protein.

12. The method of claim 11, wherein said enzyme capable of degrading a protein comprises a protease.

13. The method of any one of claims 1-12, wherein said clearing process comprises contacting said biological sample with a surfactant or a denaturant.

14. The method of any one of claims 1-13, wherein said clearing process comprises contacting said biological sample with an enzyme capable of degrading a nucleic acid, wherein said capable of degrading a nucleic acid is optionally an RNase.

15. The method of any one of claims 1-14, wherein detecting said nucleic acid barcode comprises sequencing said nucleic acid barcode and wherein detecting said amplification product comprises sequencing at least a portion of said amplification product.

16. The method of any one of claims 1-15, wherein detecting said nucleic acid barcode comprises hybridizing a labeled probe to said nucleic acid barcode and detecting said labeled probe and wherein detecting said amplification product comprises hybridizing an additional labeled probe to said amplification product and detecting said additional labeled probe.