

WHAT IS CLAIMED IS:

1. A method for determining a location of one or more analytes in a biological sample, the method comprising:
 - 5 (a) providing an array comprising a plurality of capture probes, wherein a capture probe of the plurality of capture probes comprises (i) a spatial barcode and (ii) a capture domain;
 - (b) providing one or more semi-porous materials, wherein the one or more semi-porous materials are disposed between a biological sample and the array;
 - 10 (c) applying an electric field to the biological sample, the one or more semi-porous materials, and the array, wherein the electric field promotes the migration of the one or more analytes in the direction of the array, wherein the capture domain of the capture probe specifically binds to a first analyte of the one or more analytes; and
 - 15 (d) determining (i) all or a portion of the sequence of the spatial barcode, or a complement thereof, and (ii) all or a portion of the sequence of the first analyte, or a complement thereof, and using the sequences of (i) and (ii) to determine the location of the first analyte in the biological sample.
- 20 2. The method of claim 1, wherein the capture probe further comprises one or more of: a cleavage domain, a functional domain, and a unique molecular identifier.
3. The method of claim 1 or 2, wherein the first analyte is mRNA.
- 25 4. The method of any one of claims 1-3, wherein further comprising obtaining an image of the biological sample.
5. The method of any one of claims 1-4, wherein a first semi-porous material of the one or more semi-porous materials retains one or more second analytes.
- 30 6. The method of claim 5, wherein the first semi-porous material comprises a plurality of analyte-binding moieties, wherein an analyte-binding moiety of the plurality specifically binds to a second analyte of the one or more second analytes.

7. The method of claim 6, wherein the analyte binding moiety is an antibody or an antigen-binding fragment thereof.
8. The method of claim 6 or 7, wherein the second analyte is a protein.
- 5 9. The method of any one of claims 5-8, further comprising determining the location of the second analyte in the first semi-porous material.
- 10 10. The method of claim 9, wherein determining the location of the second analyte in the first semi-porous material comprises the use of immunofluorescence staining.
11. The method of claim 10, wherein determining the location of the second analyte in the first semi-porous material comprises:
obtaining an image of the first semi-porous material; and
15 correlating the immunofluorescence staining in the image of the first semi-porous material with an image of the biological sample.
12. The method of any one of claims 1-11, wherein the one or more semi-porous materials comprises a second semi-porous material, wherein the second semi-porous material retains one or more third analytes.
- 20 13. The method of claim 12, wherein the second semi-porous material comprises a plurality of analyte-binding moieties for the one or more third analytes.
- 25 14. The method of claim 13, wherein an analyte-binding moiety of the plurality of analyte binding moieties specifically binds to a third analyte of the one or more third analytes.
15. The method of claim 14, wherein the third analyte is a nucleic acid.
- 30 16. The method of claim 15, wherein the nucleic acid is miRNA.
17. The method of any one of claims 12-16, further comprising determining the location of the third analyte in the second semi-porous material.

18. The method of claim 17, wherein determining the location of the third analyte in the second semi-porous material comprises the use of in situ hybridization.
19. The method of claim 18, wherein determining the location of the third analyte
5 comprises:
obtaining an image of the second semi-porous material; and
correlating the in situ hybridization in the image of the second semi-porous material with an image of the biological sample.
20. The method of claim 1, wherein the one or more semi-porous materials separate the
10 one or more analytes from other molecules in the biological sample.
21. The method of any one of claims 1-20, wherein the one or more semi-porous materials comprise a hydrogel.
22. The method of any one of claims 1-20, wherein the one or more semi-porous materials comprise a permeable membrane.
23. The method of any one of claims 1-22, wherein at least one of the one or more semi-
20 porous materials has non-uniform pore sizes.
24. The method of any one of claims 1-22, wherein at least one of the one or more semi-porous materials has a substantially uniform pore size.
25. The method of any one of claims 1-24, wherein the electric field is applied to a discrete area of the biological sample.
26. The method of any one of claims 1-24, wherein the electric field is applied to a discrete area of the array.
27. The method of any one of claims 1-26, wherein the one or more semi-porous materials comprise one or more fiducial markers that align the one or more semi-porous materials to the image of the biological sample.
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28. The method of any one of claims 1-27, wherein the biological sample, the one or more semi-porous materials, and the array are in direct contact with a buffer.
29. The method of claim 5, further comprising crosslinking the one or more second analytes to the first semi-porous material.
30. The method of claim 12, further comprising crosslinking the one or more third analytes to the second semi-porous material.
31. The method of claim 5, further comprising removing the first semi-porous material after the first semi-porous material retains the one or more second analytes.
32. The method of claim 12, further comprising removing the second semi-porous material after the second semi-porous material retains the one or more third analytes.
33. The method of claim 12, wherein the method further comprises:
removing the first semi-porous material after the first semi-porous material retains the one or more second analytes; and
disposing the second semi-porous material between the biological sample and the array.
34. The method of claim 33, wherein the electric field is applied after removal of the first semi-porous material and disposal of the second semi-porous material between the biological sample and the array.
35. The method of claim 34, wherein the second semi-porous material retains one or more third analytes.
36. The method of any one of claims 1-35, wherein the biological sample is a tissue section.
37. The method of any one of claims 1-35, wherein the biological sample is a formalin-fixed paraffin-embedded biological sample.

38. The method of any one of claims 1-35, further comprising a step of fixing the biological sample.
39. The method of any one of claims 1-35, further comprising a step of permeabilizing the biological sample.
40. A method for determining a location of one or more analytes in a biological sample, the method comprising:
- (a) providing a first array comprising a plurality of first capture probes, wherein a first capture probe of the plurality of first capture probes comprises (i) a first spatial barcode and (ii) a first capture domain;
 - (b) providing a semi-porous material, wherein the semi-porous material is disposed between the biological sample and the first array;
 - (c) applying an electric field to the biological sample, the semi-porous material, and the first array, wherein the electric field promotes the migration of the one or more analytes in the direction of the first array, wherein the first capture domain specifically binds to a first analyte of the one or more analytes, wherein the semi-porous material retains a second analyte of the one or more analytes;
 - (d) determining (i) all or a portion of the sequence of the first spatial barcode, or a complement thereof, and (ii) all or a portion of the sequence of the first analyte, or a complement thereof, and using the sequences of (i) and (ii) to determine the location of the first analyte in the biological sample;
 - (e) providing a second array comprising a plurality of second capture probes, wherein a second capture probe of the plurality of second capture probes comprises (i) a second spatial barcode and (ii) a second capture domain;
 - (f) applying an electric field to the semi-porous material and the second array, wherein the electric field promotes the migration of the second analyte in the direction of the second array, wherein the second capture domain specifically binds to the second analyte; and
 - (g) determining (i) all or a portion of the sequence of the second spatial barcode, or a complement thereof, and (ii) all or a portion of the sequence of the second analyte, or a complement thereof, and using the sequences of (i) and (ii) to determine the location of the second analyte in the biological sample.

41. The method of claim 40, wherein the capture probe further comprises one or more of:
a cleavage domain, a functional domain, and a unique molecular identifier.
42. The method of claim 40 or 41, wherein the first analyte is mRNA.
- 5 43. The method of any one of claims 40-42, wherein the method further comprises
obtaining an image of the biological sample.
44. The method of any one of claims 40-43, wherein the semi-porous material comprises
10 one or more analyte binding moieties.
45. The method of claim 44, wherein the one or more analyte binding moieties
specifically bind to a nucleic acid.
- 15 46. The method of claim 45, wherein the nucleic acid is miRNA.
47. The method of claim 40, wherein the semi-porous material retains one or more
analytes from the biological sample.
- 20 48. The method of any one of claims 40-47, wherein the semi-porous material comprises
a hydrogel.
49. The method of any one of claims 40-47, wherein the semi-porous material comprises
a permeable membrane.
- 25 50. The method of any one of claim 40-49, wherein the semi-porous material has non-
uniform pore sizes.
51. The method of any one of claims 40-49, wherein the semi-porous material has a
30 substantially uniform pore size.
52. The method of any one of claims 40-51, wherein an electric field is applied to a
discrete area of the biological sample.

53. The method of any one of claims 40-51, wherein the electric field is applied to a discrete area of the first array.
54. The method of any one of claims 40-53, wherein the electric field is applied to a discrete area of the second array.
55. The method of claim 43, wherein the semi-porous material comprises one or more fiducial markers that align the semi-porous material to the image of the biological sample.
56. The method of any one of claims 40-55, wherein, in step (c), the biological sample, the semi-porous material, and the first array are in direct contact with a buffer.
57. The method of any one of claims 40-56, wherein, in step (f), the semi-porous material and the second array are in direct contact with a buffer.
58. The method of any one of claims 40-57, further comprising crosslinking the second analyte to the semi-porous material.
59. The method of claims 40-58, wherein the biological sample is a tissue section.
60. The method of claims 40-58, wherein the biological sample is formalin-fixed and paraffin-embedded.
61. The method of any one of claims 40-58, further comprising a step of fixing the biological sample.
62. The method of any one of claims 40-58, further comprising a step of permeabilizing the biological sample.
63. A method for determining a location of one or more analytes in a biological sample, the method comprising:

- (a) providing a first array comprising a plurality of first capture probes, wherein a first capture probe of the plurality of first capture probes comprises (i) a first spatial barcode and (ii) a first capture domain;
- 5 (b) providing a semi-porous material, wherein the semi-porous material is disposed between the biological sample and the first array, wherein the semi-porous material comprises an analyte capture agent comprising (i) an analyte binding moiety, (ii) analyte binding moiety barcode, and (iii) an analyte capture sequence;
- 10 (c) applying an electric field to the biological sample, the semi-porous material, and the first array, wherein the electric field promotes the migration of the one or more analytes in the direction of the first array, wherein the first capture domain specifically binds to a first analyte of the one or more analytes, and wherein the analyte binding moiety specifically binds to a second analyte of the one or more analytes, and the semi-porous material retains the second analyte;
- 15 (d) determining (i) all or a portion of the sequence of the first spatial barcode, or a complement thereof, and (ii) all or a portion of the sequence of the first analyte, or a complement thereof, and using the sequences of (i) and (ii) to determine the location of the first analyte in the biological sample;
- 20 (e) providing a second array comprising a plurality of second capture probes, wherein a second capture probe of the plurality of second capture probes comprises (i) a second spatial barcode and (ii) a second capture domain;
- 25 (f) applying an electric field to the semi-porous material and the second array, wherein the electric field promotes the migration of the second analyte in the direction of the second array, wherein the second capture domain specifically binds to the analyte capture sequence ;
- (g) determining (i) all or a portion of the sequence of the second spatial barcode, or a complement thereof, and (ii) all or a portion of the sequence of the analyte binding moiety barcode, or a complement thereof, and using the sequences of (i) and (ii) to determine the location of the second analyte in the biological sample.
- 30 64. The method of claim 63, wherein one or both of the first capture probe and the second capture probe further comprises one or more of a cleavage domain, a functional domain, and a unique molecular identifier.

65. The method of claim 63 or 64, wherein the first analyte is mRNA.

66. The method of any one of claims 63-65, wherein the method further comprises obtaining an image of the biological sample.
- 5 67. The method of any one of claims 63-66, wherein the analyte capture agent is crosslinked to the semi-porous material.
68. The method of any one of claims 63-66, wherein the second analyte is a protein.
- 10 69. The method of any one of claims 63-67, wherein the analyte binding moiety comprises an antibody or an antigen-binding fragment thereof.
70. The method of any one of claims 63-69, wherein the semi-porous material separates one or more analytes from other molecules in the biological sample.
- 15 71. The method of any one of claims 63-70, wherein the semi-porous material comprises a hydrogel.
72. The method of any one of claims 63-70, wherein the semi-porous material comprises a permeable membrane.
- 20 73. The method of any one of claims 63-72, wherein the semi-porous material has non-uniform pore sizes.
- 25 74. The method of any one of claims 63-72, wherein the semi-porous material has a substantially uniform pore size.
75. The method of any one of claims 63-74, wherein the electric field is applied to a discrete area of the biological sample.
- 30 76. The method of any one of claims 63-75, wherein the electric field is applied to a discrete area of the first array.

77. The method of any one of claims 63-76, wherein the electric field is applied to a discrete area of the second array.
- 5 78. The method of claim 66, wherein the semi-porous material comprises one or more fiducial markers that align the semi-porous material to the image of the biological sample.
- 10 79. The method of any one of claims 63-74, wherein, during step (c), the biological sample, the semi-porous material, and the first array, are in direct contact with a buffer.
80. The method of any one of claims 63-75, wherein, during step (f), the semi-porous material and the second array are in direct contact with a buffer.
- 15 81. The method of any one of claims 63-76, further comprising crosslinking the second analyte to one or both of the semi-porous material and the analyte capture agent.
82. The method of claims 63-81, wherein the biological sample is a tissue section.
- 20 83. The method of claims 63-81, wherein the biological sample is formalin-fixed and paraffin-embedded.
84. The method of claims 63-81, further comprising a step of fixing the biological sample.
- 25 85. The method of claims 63-81, further comprising a step of permeabilizing the biological sample.
- 30 86. A system comprising:
(a) an array comprising a plurality of capture probes, wherein a capture probe of the plurality of capture probes comprises (i) a spatial barcode and (ii) a capture domain;
(b) one or more semi-porous materials disposed between a biological sample and the array; and

(c) an electric field, wherein the electric field promotes the migration of one or more analytes in the direction of the array, wherein the capture domain of the capture probe binds to a first analyte of the one or more analytes.

5 87. The system of claim 86, wherein a first semi-porous material of the one or more semi-porous materials retains one or more second analytes.

88. The system of claim 86 or 87, wherein the first semi-porous material comprises a plurality of analyte-binding moieties, wherein an analyte-binding moiety of the
10 plurality specifically binds to a second analyte of the one or more second analytes.

89. The system of claim 88, wherein the analyte binding moiety is an antibody or antigen-binding fragment thereof.

15 90. The system of claim 88 or 89, wherein the second analyte is a protein.

91. The system of any one of claims 87-90, further comprising determining the location of the second analyte in the first semi-porous material.

20 92. The system of claim 91, wherein determining the location of the second analyte in the first semi-porous material comprises immunofluorescence staining.

93. The system of claim 92, wherein determining the location of the second analyte in the first semi-porous material comprises:
25 obtaining an image of the first semi-porous material; and
correlating the immunofluorescence staining in the image of the first semi-porous material with an image of the biological sample.

94. The system of any one of claims 86-93, wherein the one or more semi-porous
30 materials comprise a second semi-porous material, wherein the second semi-porous material retains one or more third analytes.

95. The system of claim 94, wherein the second semi-porous material comprises a plurality of analyte-binding moieties for the one or more third analytes.
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96. The system of claim 95, wherein an analyte-binding moiety of the plurality of analyte binding moieties specifically binds to a third analyte of the one or more third analytes.
97. The system of claim 96, wherein the third analyte is a nucleic acid.
- 5 98. The system of claim 97, wherein the nucleic acid is miRNA.
99. The system of any one of claims 94-98, further comprising determining the location of the third analyte in the second semi-porous material.
- 10 100. The system of claim 99, wherein determining the location of the third analyte in the second semi-porous material comprises the use of in situ hybridization.
101. The system of claim 100, wherein determining the location of the third analyte comprises:
- 15 obtaining an image of the second semi-porous material; and
correlating the in situ hybridization in the image of the second semi-porous material with an image of the biological sample.
102. The system of claim 94, further comprising crosslinking the one or more third analytes to the second semi-porous material.
103. The system of claim 88, further comprising cross-linking the one or more second analytes to the first semi-porous material.
- 25 104. The system of claim 102, further comprising removing the second semi-porous material after the second semi-porous material retains the one or more third analytes.
105. The system of claim 102, further comprising:
- 30 removing the first semi-porous material after the first semi-porous material retains the one or more second analytes; and
disposing the second semi-porous material between the biological sample and the array.

106. The system of claim 105, wherein the electric field is applied after removal of the first semi-porous material and disposal of the second semi-porous material between the biological sample and the array.
- 5 107. The system of claim 106, wherein the second semi-porous material retains one or more third analytes.
108. The system of any one of claims 86-107, wherein the capture probe further comprises one or more of: a cleavage domain, a functional domain, and a unique
10 molecular identifier.
109. The system of any of claims 86-108, further comprising imaging the biological sample.
- 15 110. The system of any one of claims 86-109, wherein the first analyte is mRNA
111. The system of any one of claims 86-110, wherein the semi-porous material comprises a hydrogel.
- 20 112. The system of any one of claims 86-110, wherein the semi-porous material comprises a permeable membrane.
113. The system of any one of claims 86-112, wherein the semi-porous material has non-uniform pore sizes.
- 25 114. The system of any one of claims 86-112, wherein the semi-porous material has a substantially uniform pore size.
115. The system of any one of claims 86-114, wherein the one or more semi-porous materials comprise one or more fiducial markers that align the one or more semi-porous materials to the image of the biological sample.
- 30 116. The system of any one of claims 86-115, wherein the biological sample is a tissue section.
- 35 117. The system of any one of claims 116, wherein the biological sample is a formalin-fixed paraffin-embedded biological sample.

118. The system of any one of claims, further comprising a step of fixing the biological sample.
- 5 119. The system of any one of claims 86-118, further comprising a step of permeabilizing the biological sample.
120. The system of any one of claims 86-119, wherein the second analyte is protein.
- 10 121. The system of any one of claims 86-120, wherein an electric field is applied to a discrete area of the biological sample.
122. The system of any one of claims 86-120, wherein the electric field is applied to a discrete area of the array.
- 15 123. The system of any one of claims 86-122, wherein, the biological sample, the semi-porous material, and the array, are in direct contact with a buffer.
124. The system of any one of claims 86-123, wherein the system further comprises determining (i) all or a portion of the sequence of the spatial barcode, or a complement thereof, and (ii) all or a portion of the sequence of the first analyte, or a complement thereof, and using the sequences of (i) and (ii) to determine the location of the first analyte in the biological sample.
- 20 125. A system comprising:
- 25 (a) a first array comprising a plurality of first capture probes, wherein a first capture probe of the plurality of first capture probes comprises (i) a first spatial barcode and (ii) a first capture domain;
- 30 (b) a semi-porous material, wherein the semi-porous material is disposed between the biological sample and the first array;
- (c) a first electric field, wherein the first electric field promotes the migration of the one or more analytes in the direction of the first array, wherein the first capture domain specifically binds to a first analyte of the one or more analytes, wherein the semi-porous material retains a second analyte of the one or more analytes;
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- (d) a second array comprising a plurality of second capture probes, wherein a second capture probe of the plurality of second capture probes comprises (i) a second spatial barcode and (ii) a second capture domain;
- (e) a second electric field, wherein the second electric field promotes the migration of the second analyte in the direction of the second array, wherein the second capture domain specifically binds to the second analyte; and
- (f) determining (i) all or a portion of the sequence of the second spatial barcode, or a complement thereof, and (ii) all or a portion of the sequence of the second analyte, or a complement thereof, and using the sequences of (i) and (ii) to determine the location of the second analyte in the biological sample.

126. The system of claim 125, wherein the capture probe further comprises one or more of: a cleavage domain, a functional domain, and a unique molecular identifier.
127. The system of claim 124 or 125, wherein the first analyte is mRNA.
128. The system of any one of claims 125-127, wherein the system further comprises obtaining an image of the biological sample.
129. The system of any one of claims 125-128, wherein the semi-porous material comprises a hydrogel.
130. The system of any one of claims 125-128, wherein the semi-porous material comprises a permeable membrane.
131. The system of any one of claims 125-130, wherein the semi-porous material has non-uniform pore sizes.
132. The system of any one of claims 125-130, wherein the semi-porous material has a substantially uniform pore size.
133. The system of any one of claims 125-132, wherein the one or more semi-porous materials comprise one or more fiducial markers that align the one or more semi-porous materials to the image of the biological sample.

134. The system of any one of claims 125-133, wherein the biological sample is a tissue section.
- 5 135. The system of any one of claims 125-134, wherein the biological sample is a formalin-fixed paraffin-embedded biological sample.
136. The system of any one of claims 125-135, further comprising a step of fixing the biological sample.
- 10 137. The system of any one of claims 125-136, further comprising a step of permeabilizing the biological sample.
138. The system of any one of claims 125-137, wherein the semi-porous material retains one or more analytes from the biological sample.
- 15 139. The system of any one of claims 125-138, wherein the first electric field, the second electric field, or both, is applied to a discrete area of the biological sample.
140. The system of any one of claims 125-139, wherein the first electric field is applied to a discrete area of the first array.
- 20 141. The system of any one of claims 125-140, wherein the second electric field is applied to a discrete area of the second array.
- 25 142. The system of any one of claims 125-141, wherein the biological sample, the semi-porous material, and the first array are in direct contact with a buffer.
143. The system of any one of claims 125-142, wherein the biological sample, the semi-porous material and the second array are in direct contact with a buffer.
- 30 144. The system of any one of claims 125-142, wherein the semi-porous material separates one or more analytes from other molecules in the biological sample.
145. The system of any one of claims 125-144, wherein the semi-porous material comprises one or more analyte binding moieties.
- 35 146. The system of any one of claims 125-145, wherein the one or more analyte binding moieties bind a nucleic acid.

147. The system of claim 146, wherein the nucleic acid is miRNA.
148. The system of any one of claims 145-147, wherein the analyte binding moiety is
5 crosslinked to the semi-porous material.
149. The system of any one of claims 125-144, wherein the semi-porous material
comprises one or more analyte capture agents.
- 10 150. The system of claim 149, wherein the one or more analyte capture agents comprises
(i) an analyte binding moiety, (ii) analyte binding moiety barcode, and (iii) an
analyte capture sequence.
151. The system of claim 149 or 150, wherein the second analyte is protein.
- 15 152. The system of any one of claims 149-151, wherein the analyte capture agent is
crosslinked to the semi-porous material.
153. The system of any one of claims 149-152, wherein the analyte binding moiety
20 comprise an antibody or an antigen-binding fragment thereof.
154. The system of any one claims 151-155, wherein the analyte binding moiety barcode
identifies the analyte capture agent.
- 25 155. The system of any one of claims 149-154, wherein the analyte capture sequence
binds to the second capture domain of the second capture probe.
156. The system of any one of claims 125-155, wherein the system further comprises:
(a) determining (i) all or a portion of the sequence of the first spatial barcode, or a
30 complement thereof, and (ii) all or a portion of the sequence of the first analyte, or a
complement thereof, and using the sequences of (i) and (ii) to determine the location
of the first analyte in the biological sample; and
(b) determining (i) all or a portion of the sequence of the second spatial barcode, or a
complement thereof, and (ii) all or a portion of the sequence of the analyte binding
35 moiety barcode, or a complement thereof, and using the sequences of (i) and (ii) to
determine the location of the second analyte in the biological sample.