applying an electric field to the biological sample, the one or more semi-porous materials, and the array, where the electric field promotes the migration of the one or more analytes in the direction of the array, where the capture domain of the capture probe specifically binds to a first analyte of the one or more analytes; and 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.

In some examples, the capture probe further includes one or more of: a cleavage domain, a functional domain, and a unique molecular identifier. In some examples, the first analyte is mRNA.

In some examples, the method includes obtaining an image of the biological sample. In some examples, a first semi-porous material of the one or more semi-porous materials retains one or more second analytes. In some examples, the first semi-porous material includes a plurality of analyte-binding moieties, where an analyte-binding moiety of the plurality specifically binds to a second analyte of the one or more second analytes.

In some examples, the analyte binding moiety is an antibody or an antigen-binding fragment thereof. In some examples, the second analyte is a protein.

In some examples, the method includes determining the location of the second analyte in the first semi-porous material. In some examples, determining the location of the second analyte in the first semi-porous material includes the use of immunofluorescence staining. In some examples, determining the location of the second analyte in the first semi-porous material includes: 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.

In some examples, the one or more semi-porous materials includes a second semi-porous material, where the second semi-porous material retains one or more third analytes. In some examples, the second semi-porous material includes a plurality of analyte-binding moieties for the one or more third analytes. In some examples, the plurality of analyte binding moieties specifically binds to a third analyte of the one or more third analytes. In some examples, the third analyte is a nucleic acid. In some examples, the nucleic acid is miRNA. In some examples, the method includes determining the location of the third analyte in the second semi-porous material. In some examples, determining the location of the third analyte in the second semi-porous material includes the use of in situ hybridization. In some examples, determining the

location of the third analyte includes: 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.

In some examples, the one or more semi-porous materials separate the one or more analytes from other molecules in the biological sample. In some examples, the one or more semi-porous materials include a hydrogel. In some examples, the one or more semi-porous materials includes a permeable membrane. In some examples, at least one of the one or more semi-porous materials has non-uniform pore sizes. In some examples, at least one of the one or more semi-porous materials has a substantially uniform pore size. In some examples, the electric field is applied to a discrete area of the biological sample. In some examples, the electric field is applied to a discrete area of the array.

In some examples, the one or more semi-porous materials includes one or more fiducial markers that align the one or more semi-porous materials to the image of the biological sample.

In some examples, the biological sample, the one or more semi-porous materials, and the array are in direct contact with a buffer. In some examples, the one or more second analytes to the first semi-porous material. In some examples, the method includes crosslinking the one or more third analytes to the second semi-porous material. In some examples, the method includes removing the first semi-porous material after the first semi-porous material retains the one or more second analytes. In some examples, the method includes removing the second semi-porous material after the second semi-porous material retains the one or more third analytes.

In some examples, the method includes: 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.

In some examples, 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.

In some examples, the second semi-porous material retains one or more third analytes.

In some examples, the biological sample is a tissue section. In some examples, the biological sample is a formalin-fixed paraffin-embedded biological sample. In some examples, the method includes a step of fixing the biological sample.

In some examples, the method includes a step of permeabilizing the biological sample.

Also provided herein are methods for determining a location of one or more analytes in a biological sample, the method including: (a) providing a first array including a plurality of first capture probes, where a first capture probe of the plurality of first capture probes includes (i) a first spatial barcode and (ii) a first capture domain; (b) providing a semi-porous material, where 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, where the electric field promotes the migration of the one or more analytes in the direction of the first array, where the first capture domain specifically binds to a first analyte of the one or more analytes, where 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 including a plurality of second capture probes, where a second capture probe of the plurality of second capture probes includes (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, where the electric field promotes the migration of the second analyte in the direction of the second array, where 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.

In some examples, the capture probe further includes one or more of: a cleavage domain, a functional domain, and a unique molecular identifier. In some examples, the first analyte is mRNA.

In some examples, the method includes obtaining an image of the biological sample. In some examples, the semi-porous material includes one or more analyte binding moieties. In some examples, the one or more analyte binding moieties specifically bind to a nucleic acid. In some examples, where the nucleic acid is miRNA. In some examples, the semi-porous material retains one or more analytes from the biological sample. In some examples, the semi-porous material includes a hydrogel. In some examples, the semi-porous material includes a permeable membrane. In some

examples, the semi-porous material has non-uniform pore sizes. In some examples, the semi-porous material has a substantially uniform pore size.

In some examples, an electric field is applied to a discrete area of the biological sample. In some examples, the electric field is applied to a discrete area of the first array. In some examples, the electric field is applied to a discrete area of the second array. In some examples, the semi-porous material includes one or more fiducial markers that align the semi-porous material to the image of the biological sample.

In some examples, in step (c), the biological sample, the semi-porous material, and the first array are in direct contact with a buffer. In some examples, in step (f), the semi-porous material and the second array are in direct contact with a buffer.

In some examples, the method includes crosslinking the second analyte to the semi-porous material.

In some examples, the biological sample is a tissue section. In some examples, the biological sample is formalin-fixed and paraffin-embedded. In some examples, the method includes a step of fixing the biological sample. In some examples, the method includes a step of permeabilizing the biological sample.

Also provided herein are methods for determining a location of one or more analytes in a biological sample, the method including: (a) providing a first array including a plurality of first capture probes, where a first capture probe of the plurality of first capture probes includes (i) a first spatial barcode and (ii) a first capture domain; (b) providing a semi-porous material, where the semi-porous material is disposed between the biological sample and the first array, where the semi-porous material includes an analyte capture agent including (i) an analyte binding moiety, (ii) analyte binding moiety barcode, and (iii) an analyte capture sequence; (c) applying an electric field to the biological sample, the semi-porous material, and the first array, where the electric field promotes the migration of the one or more analytes in the direction of the first array, where the first capture domain specifically binds to a first analyte of the one or more analytes, and where 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; (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 including a plurality of second capture probes, where a second capture probe of the plurality of second capture probes includes (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, where the electric field promotes the migration of the second analyte in the direction of the second array, where 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.

In some examples, one or both of the first capture probe and the second capture probe further includes one or more of a cleavage domain, a functional domain, and a unique molecular identifier. In some examples, the first analyte is mRNA.

In some examples, the method includes obtaining an image of the biological sample. In some examples, the analyte capture agent is crosslinked to the semi-porous material. In some examples, the second analyte is a protein.

In some examples, the analyte binding moiety includes an antibody or an antigen-binding fragment thereof.

In some examples, the semi-porous material separates one or more analytes from other molecules in the biological sample. In some examples, the semi-porous material includes a hydrogel. In some examples, the semi-porous material includes a permeable membrane. In some examples, the semi-porous material has non-uniform pore sizes. In some examples, the semi-porous material has a substantially uniform pore size. In some examples, the electric field is applied to a discrete area of the biological sample. In some examples, the electric field is applied to a discrete area of the first array. In some examples, the electric field is applied to a discrete area of the second array.

In some examples, the semi-porous material includes one or more fiducial markers that align the semi-porous material to the image of the biological sample.

In some examples, during step (c), the biological sample, the semi-porous material, and the first array, are in direct contact with a buffer. In some examples, during step (f), the semi-porous material and the second array are in direct contact with a buffer.

In some examples, the method includes crosslinking the second analyte to one or both of the semi-porous material and the analyte capture agent.

In some examples, the biological sample is a tissue section. In some examples, the biological sample is formalin-fixed and paraffin-embedded. In some

examples, the method includes a step of fixing the biological sample. In some examples, the method includes a step of permeabilizing the biological sample.

Also, provided herein are systems including: (a) an array including a plurality of capture probes, where a capture probe of the plurality of capture probes includes (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, where the electric field promotes the migration of one or more analytes in the direction of the array, where the capture domain of the capture probe binds to a first analyte of the one or more analytes.

In one aspect, the present disclosure refers to 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, and wherein the first analyte is a nucleic acid.

In some examples, a first semi-porous material of the one or more semi-porous materials retains one or more second analytes. In some examples, the first semi-porous material includes a plurality of analyte-binding moieties, where an analyte-binding moiety of the plurality specifically binds to a second analyte of the one or more second analytes. In some examples, the analyte binding moiety is an antibody or antigen-binding fragment thereof. In some examples, the second analyte is a protein.

In some examples, the method includes determining the location of the second analyte in the first semi-porous material. In some examples, the method includes determining the location of the second analyte in the first semi-porous material includes immunofluorescence staining.

In some examples, determining the location of the second analyte in the first semi-porous material includes: 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.

In some examples, the one or more semi-porous materials includes a second semi-porous material, where the second semi-porous material retains one or more third analytes. In some examples, the second semi-porous material includes a plurality of analyte-binding moieties for the one or more third analytes. In some examples, an analyte-binding moiety of the plurality of analyte binding moieties specifically binds to a third analyte of the one or more third analytes. In some examples, the third analyte is a nucleic acid. In some examples, the nucleic acid is miRNA.

In some examples, the method includes determining the location of the third analyte in the second semi-porous material. In some examples, determining the location of the third analyte in the second semi-porous material includes the use of in situ hybridization. In some examples, determining the location of the third analyte includes: 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.

In some examples, the method includes crosslinking the one or more third analytes to the second semi-porous material. In some examples, the method includes cross-linking the one or more second analytes to the first semi-porous material. In some examples, the method includes removing the second semi-porous material after the second semi-porous material retains the one or more third analytes.

In some examples, the method includes 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.

In some examples, 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.

In some examples, the second semi-porous material retains one or more third analytes.

In some examples, the capture probe further includes one or more of: a cleavage domain, a functional domain, and a unique molecular identifier.

In some examples, the method includes imaging the biological sample.

In some examples, the first analyte is mRNA

In some examples, the semi-porous material includes a hydrogel. In some examples, the semi-porous material includes a permeable membrane. In some examples, the semi-porous material has non-uniform pore sizes. In some examples, the semi-porous material has a substantially uniform pore size.

In some examples, the one or more semi-porous materials include one or more fiducial markers that align the one or more semi-porous materials to the image of the biological sample.

In some examples, the biological sample is a tissue section. In some examples, the biological sample is a formalin-fixed paraffin-embedded biological sample. In some examples, the method includes a step of fixing the biological sample. In some examples, the method includes a step of permeabilizing the biological sample.

In some examples, the second analyte is protein.

In some examples, an electric field is applied to a discrete area of the biological sample. In some examples, the electric field is applied to a discrete area of the array. In some examples, the biological sample, the semi-porous material, and the array, are in

direct contact with a buffer. In some examples, the system further includes 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.

Also provided herein are system including: (a) a first array including a plurality of first capture probes, where a first capture probe of the plurality of first capture probes includes (i) a first spatial barcode and (ii) a first capture domain; (b) a semi-porous material, where the semi-porous material is disposed between the biological sample and the first array; (c) a first electric field, where the first electric field promotes the migration of the one or more analytes in the direction of the first array, where the first capture domain specifically binds to a first analyte of the one or more analytes, where the semi-porous material retains a second analyte of the one or more analytes; (d) a second array including a plurality of second capture probes, where a second capture probe of the plurality of second capture probes includes (i) a second spatial barcode and (ii) a second capture domain; (e) a second electric field, where the second electric field promotes the migration of the second analyte in the direction of the second array, where 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.

In one aspect, the present disclosure refers to a system comprising:

(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;

(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 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 semi-porous material retains a second analyte of the one or more analytes;

(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 an analyte capture sequence; and

(f) determining (i) 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.

In some examples, the capture probe further includes one or more of: a cleavage domain, a functional domain, and a unique molecular identifier. In some examples, the first analyte is mRNA.

In some examples, the system further includes obtaining an image of the biological sample.

In some examples, the semi-porous material includes a hydrogel. In some examples, the semi-porous material includes a permeable membrane. In some examples, the semi-porous material has non-uniform pore sizes. In some examples, the semi-porous material has a substantially uniform pore size.

In some examples, the one or more semi-porous materials include one or more fiducial markers that align the one or more semi-porous materials to the image of the biological sample.

In some examples, the biological sample is a tissue section. In some examples, the biological sample is a formalin-fixed paraffin-embedded biological sample.

In some examples, the method includes a step of fixing the biological sample. In some examples, the method includes a step of permeabilizing the biological sample.

In some examples, the semi-porous material retains one or more analytes from the biological sample.

In some examples, the first electric field, the second electric field, or both, is applied to a discrete area of the biological sample. In some examples, the first electric field is applied to a discrete area of the first array. In some examples, the second electric field is applied to a discrete area of the second array.

In some examples, the biological sample, the semi-porous material, and the first array are in direct contact with a buffer. In some examples, the biological sample, the semi-porous material and the second array are in direct contact with a buffer. In some examples, the semi-porous material separates one or more analytes from other molecules in the biological sample. In some examples, the semi-porous material includes one or more analyte binding moieties.

In some examples, the one or more analyte binding moieties bind a nucleic acid. In some examples, the nucleic acid is miRNA. In some examples, the analyte binding moiety is crosslinked to the semi-porous material.

In some examples, the semi-porous material includes one or more analyte capture agents. In some examples, the one or more analyte capture agents includes (i) an analyte binding moiety, (ii) analyte binding moiety barcode, and (iii) an analyte capture sequence. In some examples, the second analyte is protein.

In some examples, the analyte capture agent is crosslinked to the semi-porous material.

In some examples, the analyte binding moiety include an antibody or an antigen-binding fragment thereof.

In some examples, the analyte binding moiety barcode identifies the analyte capture agent. In some examples, the analyte capture sequence binds to the second capture domain of the second capture probe.

In some examples, the system further includes: (a) 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; 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 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.

All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, patent application, or item of information was specifically and individually indicated to be incorporated by reference. To the extent publications, patents, patent applications, and items of information incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

Where values are described in terms of ranges, it should be understood that the description includes the disclosure of all possible sub-ranges within such ranges, as well as specific numerical values that fall within such ranges irrespective of whether a specific numerical value or specific sub-range is expressly stated.

The term "each," when used in reference to a collection of items, is intended to identify an individual item in the collection but does not necessarily refer to every item in the collection, unless expressly stated otherwise, or unless the context of the usage clearly indicates otherwise.

Various examples of the features of this disclosure are described herein. However, it should be understood that such examples are provided merely by way of example, and numerous variations, changes, and substitutions can occur to those skilled in the art without departing from the scope of this disclosure. It should also be understood that various alternatives to the specific examples described herein are also within the scope of this disclosure.

DESCRIPTION OF DRAWINGS

The following drawings illustrate certain embodiments of the features and advantages of this disclosure. These embodiments are not intended to limit the scope of the appended claims in any manner. Like reference symbols in the drawings indicate like elements.

FIG. 1 is a schematic diagram showing an example of a barcoded capture probe, as described herein.

FIG. 2 is a schematic diagram of an exemplary analyte capture agent.

FIG. 3 is a schematic diagram depicting an exemplary interaction between a feature-immobilized capture probe **324** and an analyte capture agent **326**.