

ELECTROPHORESIS CASSETTES AND INSTRUMENTATION

CROSS-REFERENCE TO RELATED APPLICATION

- 5 This application claims the benefit of U.S. Patent Application Serial No. 63/026,975 titled ELECTROPHORESIS CASSETTES AND INSTRUMENTATION, filed May 19, 2020, the disclosure of which is hereby incorporated by reference in its entirety.

BACKGROUND

- 10 Cells within a tissue have differences in cell morphology and/or function due to varied analyte levels (e.g., gene and/or protein expression) within the different cells. The specific position of a cell within a tissue (e.g., the cell's position relative to neighboring cells or the cell's position relative to the tissue microenvironment) can affect, e.g., the cell's morphology, differentiation, fate, viability, proliferation, behavior, signaling, and cross-talk
15 with other cells in the tissue.

- Spatial heterogeneity has been previously studied using techniques that typically provide data for a handful of analytes in the context of intact tissue or a portion of a tissue (e.g., tissue section), or provide significant analyte data from individual, single cells, but fails to provide information regarding the position of the single cells from the originating
20 biological sample (e.g., tissue).

- Various methods have been used to prepare a biological sample for analyzing analyte data in the sample. In some applications, a biological sample can be permeabilized to facilitate transfer of analytes out of the sample, and/or to facilitate transfer of species (such as capture probes) into the sample.
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SUMMARY

 This document generally relates to electrophoretic devices, apparatuses, systems, instruments, and methods for analyte migration.

- In general, electrophoresis can be used to migrate analytes from a sample toward a
30 substrate that includes capture probes for spatial transcriptomics applications in order to enhance sensitivity and/or resolution. The present disclosure provides various implementations of electrophoretic devices, systems and instruments to improve electrophoresis and ease of use. Electrophoretic systems and instruments described herein

provide different designs for electrode geometry and/or placement, and further provide different approaches for electrical interfacing between components. For example, various configurations of electrophoretic systems and instruments described herein utilize different electrode designs that can result in uniform electromigration of analytes. Further, the

5 electrophoretic systems described herein consider that, where a sample tissue is very thin, relatively small angles of the electric field has no or little effect on drift of analyte migration. Moreover, various implementations of electrophoretic systems and instrumentations optimize the size and/or location (x, y, z directions) of electrodes (e.g., cathode) relative to a sample, thereby increasing uniformity of electric field and reducing or minimizing drift of analyte

10 migration. In addition, the electrophoresis systems and instruments described herein provides a solution to conveniently check for electrical connections in the systems and instruments, and alert users of potential improper electrical connections, prior to performing electrophoresis.

Particular implementations of the present disclosure described herein provide an

15 electrophoretic system for analyte migration. The system includes a substrate, a substrate cassette, a cathode assembly, and a power supply. The substrate may include a plurality of regions. Each region may be electrically conductive and further configured to comprise one or more capture probes and a biological sample comprising an analyte. The substrate may include a first electrode contact. The first electrode contact may be electrically connected to

20 at least one of the plurality of regions. The substrate cassette may be disposed at the substrate and include a plurality of apertures corresponding to the plurality of regions of the substrate. The plurality of apertures may define a plurality of buffer chambers comprising the plurality of regions of the substrate. The substrate cassette may comprise a connection interface electrically coupled to the first electrode contact of the substrate. In some implementations,

25 the substrate cassette may comprise the connection interface that exposes the first electrode contact of the substrate. The cathode assembly may include a plurality of electrodes positioned within the plurality of buffer chambers of the substrate cassette, respectively. The cathode assembly may include a second electrode contact. The second electrode contact may be electrically connected to at least one of the plurality of electrodes. The power supply may

30 be electrically connected with the first electrode contact of the substrate at the connection interface of the substrate cassette, and electrically connected with the second electrode contact of the cathode assembly. The power supply may generate an electric field between the plurality of regions and the plurality of electrodes, respectively, such that the analyte in the biological sample migrates toward the capture probe on the substrate.

In some implementations, the system described herein can include one or more of the following features. The capture probe may be coated, spotted or printed on each of the plurality of regions. The biological sample may be placed in contact with one or more of the plurality of regions on the substrate. The substrate cassette may include a substrate holder including a substrate mount for securing the substrate, and a gasket including a plurality of gasket apertures configured to align with the plurality of regions when the substrate is secured by the substrate holder. The plurality of apertures may include the plurality of gasket apertures. The substrate holder may include a plurality of holder apertures configured to align with the plurality of gasket apertures when the substrate is secured by the substrate holder.

5 The plurality of apertures may include the plurality of gasket apertures and the plurality of holder apertures. The substrate may include a glass slide coated with a conductive material. The conductive material may include at least one of tin oxide (TO), indium tin oxide (ITO), a transparent conductive oxide (TCO), aluminum doped zinc oxide (AZO), fluorine doped tin oxide (FTO), or any combination thereof. The electrophoretic system may include a first

10 electrical wire connecting the power supply to the first electrode contact, and a second electrical wire connecting the power supply to the second electrode contact. The plurality of buffer chambers may receive a buffer. The buffer may include a permeabilization reagent. The electrophoretic system may include a light configured to illuminate onto the plurality of regions to permeabilize the biological sample on the plurality of regions. The sample may be

15 permeabilized using a detergent before, after, or during enzymatic treatment. The sample may be permeabilized with a lysis reagent being added to the sample. The sample may be permeabilized by exposing the sample to a protease.

The substrate cassette may include a body defining a cavity configured to partially receive the substrate. The connection interface of the substrate cassette may include a slit

25 defined at the body and configured for the substrate to partially extend out from the body such that the first electrode contact of the substrate is positioned outside the body. The body may include a main face and a lateral face extending from a periphery of the main face. The plurality of the apertures may be defined at the main face of the body. The slit may be defined at the lateral face of the body.

30 The substrate cassette may include a body defining a cavity configured to receive the substrate. The connection interface of the substrate cassette may include a contact opening defined at the body and configured to expose the first electrode contact of the substrate there through. The body may include a main face and a lateral face extending from a periphery of the main face. The plurality of the apertures may be defined at the main face of the body. The

contact opening may be defined at the main face adjacent the lateral face. The contact opening may be defined further at the lateral face of the body to extend from the main face to the lateral face.

5 The electrophoretic system may include a first contact pin electrically attached to the substrate and providing the first electrode contact. The substrate cassette may include a body defining a cavity configured to receive the substrate. The connection interface of the substrate cassette may include a contact hole defined at the body and configured to expose the first contact pin therethrough. The body may include a main face and a lateral face extending from a periphery of the main face. The plurality of the apertures may be defined at the main face of
10 the body. The contact hole may be defined at the main face.

The substrate cassette may include a body defining a cavity configured to receive the substrate, and a contact pin extending through a wall of the body and having a first end and a second end opposite to the first end. The first end may be arranged exterior of the body, and the second end may be arranged interior of the body and configured to electrically contact
15 with the first electrode contact of the substrate. The electrophoretic system may include a contact bracket that electrically engages with the substrate and provides the first electrode contact of the substrate. The second end of the contact pin may electrically contact with the contact bracket.

The cathode assembly may include a cathode body configured to position at least
20 partially on the substrate cassette. The plurality of electrodes may project from the cathode body. Each of the plurality of electrodes may include at least one of a conductive wire, a conductive rod, or an array of conductive wires. Each of the plurality of electrodes may include a conductive plate at a distal end of the at least one of the conductive wire, the conductive rod, or the array of conductive wires. The conductive plate may be configured as
25 at least one of a circular plate, a square plate, or a ring. The cathode body may include the second electrode contact.

The substrate cassette may include a second aperture corresponding to the first electrode contact of the substrate and configured to define a second buffer chamber on the first electrode contact of the substrate. The plurality of buffer chambers may contain a first
30 buffer. The second chamber may contain a second buffer different from the first buffer. The second buffer may have an electrolyte strength greater than the first buffer. The second buffer may include a sodium chloride solution. The cathode assembly may include a cathode body configured to position at least partially on the substrate cassette. The plurality of electrodes may project from the cathode body and be configured to position within the plurality of

buffer chambers, respectively. The cathode assembly may include a second electrode projecting from the cathode body and configured to position within the second chamber. The cathode body may include an anode contact that is electrically connected to the second electrode. The power supply may be electrically connected to the anode contact of the
5 cathode body so that the power supply is electrically connected with the first electrode contact of the substrate via the second electrode that positions within the second chamber containing the second buffer on the first electrode contact of the substrate.

The substrate cassette may include a cassette body defining a cavity configured to receive the substrate. The cassette body may include the cathode assembly such that the
10 plurality of electrodes are configured to position within the plurality of buffer chambers. The substrate cassette may include a plurality of cathode contact pins extending through a wall of the cassette body and electrically contacting with the second electrode contact such that the cathode contact pin is electrically connected to the at least one of the plurality of electrodes. Each of the plurality of electrodes may include at least one of a conductive wire, a conductive
15 rod, or an array of conductive wires. Each of the plurality of electrodes may include a conductive plate at a distal end of the at least one of the conductive wire, the conductive rod, or the array of conductive wires. The cassette body may include an anode contact pin extending through the wall of the body and having a first end and a second end opposite to the first end. The first end may be arranged exterior of the cassette body and the second end
20 may be arranged interior of the cassette body and configured to electrically contact with the first electrode contact of the substrate. The electrophoretic system may include a contact bracket that electrically engages with the substrate and provides the first electrode contact of the substrate. The second end of the anode pin may electrically contact with the contact bracket.

25 The body may include the cathode assembly such that the plurality of electrodes are configured to position within the plurality of buffer chambers. The substrate cassette may include a cathode contact pin extending through a wall of the body and electrically contacting with the second electrode contact such that the cathode contact pin is electrically connected to the at least one of the plurality of electrodes. The substrate cassette may include an anode
30 contact pin extending from the body, and a conductive wire having a first end and an opposite second end. The first end may be electrically connected to the anode contact pin, and the second end may be electrically connected to the first electrode contact that is positioned outside the body.

The electrophoretic system may include an anode cassette body and an anode contact pin. The anode cassette body may define a cavity configured to receive the first electrode contact of the substrate that is positioned outside the body of the substrate cassette. The anode contact pin may extend through a wall of the anode cassette body and have a first end and a second end opposite to the first end. The first end may be arranged exterior of the anode cassette body and the second end may be arranged interior of the anode cassette body and configured to electrically contact with the first electrode contact of the substrate. The electrophoretic system may include an anode contact bracket that electrically engages with the substrate and provides the first electrode contact of the substrate. The second end of the anode contact pin may electrically contact with the anode contact bracket.

The electrophoretic system may include a system housing including the power supply, a cassette tray that extends from the system housing and is configured to receive the substrate cassette thereon, and a cassette cover that extends from the system housing above the cassette tray.

The electrophoretic system may include a system housing including the power supply, a cassette tray that extends from the system housing and is configured to receive the substrate cassette thereon, and a cassette cover that extends from the system housing above the cassette tray. The cassette cover may include a cathode connector and an anode connector. The cathode connector may electrically engage with the cathode contact pin of the substrate cassette. The anode connector may electrically engage with the anode contact pin of the substrate cassette.

The electrophoretic system may include a system housing including the power supply, a cassette tray that extends from the system housing and is configured to receive the substrate cassette thereon, and a cassette cover that extends from the system housing above the cassette tray and includes the cathode assembly. The plurality of electrodes may project from the cassette cover toward the plurality of chambers of the substrate cassette. The cassette cover may include an anode connector configured to electrically engage with the contact pin of the substrate cassette.

Particular implementations of the present disclosure described herein provide a method for analyte migration. The method may include loading a substrate comprising capture probes with a substrate cassette into an electrophoresis instrument, the substrate cassette including a plurality of buffer chambers, the substrate including a plurality of regions each comprising one or more capture probes and a biological sample containing an analyte; arranging a cathode to place a plurality of electrodes within the plurality of buffer chambers,

respectively; electrically connecting a power supply to the substrate; electrically connecting the power supply to the cathode; applying a first voltage between the substrate and the cathode; detecting an output parameter in response to the first voltage; determining whether the output parameter meets a threshold value; and based on the output parameter meeting the threshold value, applying a second voltage to generate electric fields between the plurality of regions of the substrate and the plurality of electrodes through the plurality of buffer chambers to cause the analytes in the biological samples to move toward the capture probes on the substrate.

In some implementations, the method described herein can optionally include one or more of the following features. The output parameter may be an impedance. The threshold value may be indicative of a propriety of at least one of an electrical connection of the power supply to the substrate, an electrical connection of the power supply to the cathode, or an electrical property of the buffers. The method may include, based on the output parameter not meeting the threshold value, generating a notification to inform an electrical connection is improper; and ceasing to apply the second voltage to generate the electric fields. The threshold value may be a predetermined range of value. The method may include, prior to loading the substrate with the substrate cassette, placing the biological samples in contact with the capture probes on the substrate; arranging the substrate cassette onto the substrate to align a plurality of apertures of the substrate cassette with a plurality of regions of the substrate and define the plurality of buffer chambers on the plurality of regions; and supplying buffers in the plurality of buffer chambers.

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 embodiments of the features of this disclosure are described herein. However, it should be understood that such embodiments 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 embodiments 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.

FIGS. 1A-B are schematic side and top views of an example electrophoretic system for analyte migration.

FIG. 2 is a schematic perspective view of an example substrate.

FIG. 3 illustrates an example configuration of a substrate and a sample undergoing electrophoretic process using the electrophoretic system of **FIGS. 1A-B**.

FIG. 4A is a schematic top view of an example substrate cassette.

FIG. 4B is a schematic top view of an example substrate for use with the substrate cassette of **FIG. 4A**.

FIG. 4C is a schematic top view of the substrate cassette of **FIG. 4A** that receives the substrate of **FIG. 4B**.

FIG. 4D is a schematic perspective view of an example substrate for use with the substrate cassette of **FIG. 4A**.

FIG. 5 illustrates an example of the substrate cassette of **FIG. 4A**.

FIG. 6 is a bottom cross sectional, perspective view of the substrate cassette, taken along line A-A of **FIG. 5**.

FIG. 7A shows a top view of an example substrate cassette in an open position.

FIG. 7B shows a side view of a latitudinal side of the substrate cassette of **FIG. 7A**.

FIG. 7C shows a cross sectional view of the latitudinal side of the substrate cassette of **FIG. 7B**.

FIG. 8 illustrates an example placement of the substrate into the substrate cassette of **FIG. 7A**.

FIG. 9 is a schematic top view of another example substrate cassette.

FIG. 10A is a schematic top view of yet another example substrate cassette.

FIG. 10B is a schematic top view of yet another example substrate for use with the substrate cassette of **FIG. 10A**.

5 **FIG. 10C** is a schematic top view of the substrate cassette of **FIG. 10A** that receives the substrate of **FIG. 10B**.

FIG. 10D is a schematic cross sectional side view of the substrate cassette of **FIG. 10A** that receives the substrate of **FIG. 10B**.

FIG. 11A is a schematic top view of yet another example substrate cassette.

10 **FIG. 11B** is a schematic top view of yet another example substrate for use with the substrate cassette of **FIG. 11A**.

FIG. 11C is a schematic top view of the substrate cassette of **FIG. 11A** that receives the substrate of **FIG. 11B**.

15 **FIG. 11D** is a schematic cross sectional side view of the substrate cassette of **FIG. 11A** that receives the substrate of **FIG. 11B**.

FIG. 12A is a schematic top view of an example cathode assembly.

FIG. 12B is a schematic top view of an example substrate cassette that receives an example substrate.

20 **FIG. 12C** is a schematic top view of the cathode assembly of **FIG. 12A** that is disposed on the substrate cassette and the substrate of **FIG. 12B**.

FIG. 12D is a schematic cross sectional side view of the cathode assembly, the substrate cassette, and the substrate of **FIG. 12C**.

25 **FIG. 12E** is a schematic cross sectional side view of the cathode assembly, the substrate cassette, and the substrate of **FIG. 12C**, in which the cathode assembly has another example electrode.

FIG. 12F is a schematic cross sectional side view of the cathode assembly, the substrate cassette, and the substrate of **FIG. 12C**, in which the cathode assembly has yet another example electrode.

30 **FIG. 12G** is a schematic cross sectional side view of the cathode assembly, the substrate cassette, and the substrate of **FIG. 12C**, in which the cathode assembly has yet another example electrode.

FIG. 13A is a schematic top view of another example cathode assembly.

FIG. 13B is a schematic top view of an example substrate.

FIG. 13C is a schematic top view of an example substrate cassette.

FIG. 13D is a schematic top view of the cathode assembly of **FIG. 13A** that is disposed on the substrate cassette of **FIG. 13B** and the substrate of **FIG. 13C**.

FIG. 13E is a schematic cross sectional side view of the cathode assembly, the substrate cassette, and the substrate of **FIG. 13D**, which illustrates an example buffer chamber.

FIG. 13F is a schematic cross sectional side view of the cathode assembly, the substrate cassette, and the substrate of **FIG. 13D**, which illustrates an example anode chamber.

FIG. 14A is a schematic top view of yet another example substrate cassette that receives a substrate.

FIG. 14B is a schematic cross sectional side view of the substrate cassette and the substrate of **FIG. 14A**.

FIG. 14C is a schematic cross sectional side view of a variation of the substrate cassette and the substrate of **FIG. 14A**.

FIG. 15A is a schematic top view of yet another example substrate cassette that receives a substrate.

FIG. 15B is a schematic cross sectional side view of the substrate cassette and the substrate of **FIG. 15A**.

FIG. 16A is a schematic top view of an example electrical connection configuration among a substrate cassette, a substrate, and a control system.

FIG. 16B is a schematic top view of another example electrical connection configuration among a substrate cassette, a substrate, and a control system.

FIG. 16C is a schematic cross sectional side view of part of the electrical connection configuration of **FIG. 16B**.

FIG. 17A is a schematic perspective view of an example instrument for electrophoresis.

FIG. 17B is a schematic cross sectional side view of an example cassette cover of the instrument that receives an example set of substrate cassette and substrate.

FIG. 17C is a schematic cross sectional side view of another example cassette cover of the instrument that receives an example set of substrate cassette and substrate.

FIG. 18 is a flowchart of an example process for analyte migration.

FIG. 19 is a block diagram of computing devices that may be used to implement the systems and methods described in this document, as either a client or as a server or plurality of servers.

FIG. 20A shows a schematic of an example analytical workflow in which electrophoretic migration of analytes is performed after permeabilization.

FIG. 20B shows a schematic of an example analytical workflow in which electrophoretic migration of analytes and permeabilization are performed simultaneously.

5 **FIG. 21A** shows an example perpendicular, single slide configuration for use during electrophoresis.

FIG. 21B shows an example parallel, single slide configuration for use during electrophoresis.

FIG. 21C shows an example multi-slide configuration for use during electrophoresis.

10 **FIG. 22A-B** are schematics illustrating expanded (**FIG. 22A**) and side views (**FIG. 22B**) of an electrophoretic transfer system configured to direct transcript analytes toward a spatially-barcoded capture probe array.

FIG. 23 is a schematic illustrating an exemplary workflow protocol utilizing an electrophoretic transfer system.

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DETAILED DESCRIPTION

As described herein, electrophoresis can be used to migrate analytes from a sample toward a substrate that includes capture probes for spatial transcriptomics applications in order to enhance sensitivity and/or resolution. The present disclosure provides various
20 implementations of electrophoretic systems and instruments to improve electrophoresis and ease of use. Electrophoretic systems and instruments described herein provide different designs for electrode geometry and/or placement, and further provide different approaches for electrical interfacing between components. For example, various configurations of electrophoretic systems and instruments described herein utilize different electrode designs
25 that can result in uniform electromigration of analytes. Further, the electrophoretic systems described herein consider that, where a sample tissue is very thin, relatively small angles of the electric field has no or little effect on drift of analyte migration. Moreover, various implementations of electrophoretic systems and instrumentations optimize the size and/or location (x, y, z directions) of electrodes (e.g., cathode) relative to a sample, thereby
30 increasing uniformity of electric field and reducing or minimizing drift of analyte migration. In addition, the electrophoresis systems and instruments described herein provide a solution to conveniently check for electrical connections in the systems and instruments, and alert users of potential improper electrical connections, prior to performing electrophoresis.

FIGS. 1A-B are schematic side and top views of an example electrophoretic system **100** for analyte migration. In some implementations, the system **100** is configured similarly to electrophoretic systems described herein, for example with reference to **FIGS. 20A-B** and **21A-C**. The system **100** can be used to actively cause analytes described herein (e.g., nucleic acids, proteins, charged molecules, etc.) in a biological sample to migrate to capture probes on a substrate. In some implementations, the system **100** can include a substrate **102** as a first electrode (e.g., anode), a second electrode **104** (e.g., cathode), and a spacer **110** disposed between the substrate **102** and the second electrode **104**.

Referring also to **FIG. 2**, the substrate **102** is configured to place a biological sample **112** that contains one or more analytes **314** (not shown in **FIG. 2**; *see* **FIG. 3**). By way of example, the biological sample **112** can be one or more cells or a tissue sample including one or more cells. The substrate **102** can include one or more regions **116** for placing or containing a sample **112** thereon. In some implementations, the substrate **102** can further comprise one or more capture probes **118** on a substrate region **116**. The capture probes **118** can be placed on the substrate region **116** in a variety of ways described herein. For example, the capture probes **118** can be attached (e.g., directly or indirectly, reversibly or non-reversibly) to a region on an array. Alternatively or in addition, the capture probes **118** can be immobilized on the substrate region **116** of the substrate **102**. The sample **112** can be placed and manipulated on the substrate **102** in various ways described herein. In some implementations, the biological sample can be placed in contact with the capture probes on each region. Alternatively, the biological samples do not make contact with the capture probes. For example, an intermediate layer (e.g., gels) can be disposed between the substrate and the sample, which is compatible with electrophoresis.

In some implementations, the substrate **102** is configured to be used as a first electrode in the system **100**. For example, the substrate **102** can be used as an anode. In another example, the substrate **102** can be used as a cathode. The substrate **102** can be configured to be conductive at least in the substrate region **116**. In some implementations, the substrate **102** can be configured as a conductive substrate described herein. For example, the substrate **102** can include one or more conductive materials that permit the substrate **102** to function as an electrode (e.g., the anode). Examples of such a conductive material include tin oxide (TO), indium tin oxide (ITO), a transparent conductive oxide (TCO), aluminum doped zinc oxide (AZO), fluorine doped tin oxide (FTO), or any combination thereof. Alternatively or in addition, other materials may be used to provide desired conductivity to the substrate **102**. In some implementations, the substrate **102** can be coated with the

conductive material. For example, the substrate **102** can include a conductive coating **109** (**FIG. 1A**) on the surface of the substrate **102**, and a sample **112** is provided on the coating **109** of the substrate **102**. In other implementations, the substrate **102** can be made partially or entirely of a conductive material. For example, a sample (e.g., tissue section) can be directly placed on a conductive material (e.g., graphite, silicon, other semiconductive material, etc.). The substrate **102** can be made of glass or other transparent material for imaging purposes.

Although the substrate **102** is illustrated to include a single substrate region **116** in **FIG. 2**, other implementations of the substrate **102** can include a plurality of substrate regions (e.g., two substrate regions in **FIGS. 1A-B**, eight substrate regions in **FIG. 4**, etc.) configured to place multiple samples thereon, respectively. Each of such multiple substrate regions can be configured similarly to the first substrate region **116** described herein.

Referring to **FIGS. 1A-B**, where the substrate **102** is used as the anode, the second electrode **104** is configured as a cathode. In this example, therefore, the second electrode **104** can also be referred to as the cathode **104**. As described herein, the cathode **104** can be provided in various configurations.

In some implementations, the substrate **102** and the cathode **104** can be arranged within a container (e.g., substrate cassettes described herein) that defines a buffer chamber **122** between the substrate **102** and the cathode **104**. The buffer chamber **122** is configured to contain a buffer **124**. In some implementations, the substrate **102** and the cathode **104** can be fully immersed into the buffer **124**. In alternative implementations, either or both of the substrate **102** and the cathode **104** can be partially inserted into the buffer **124** located in the container.

The buffer **124** can be of various types. In some implementations, the buffer **124** includes a permeabilization reagent. In some embodiments, the buffer **124** is contained in the buffer chamber **122** throughout the electrophoretic process. The permeabilization reagent can permeabilize the sample before and/or during electrophoresis. In addition or alternatively, the sample can be permeabilized using other methods described herein, independently or in conjunction with permeabilization using the permeabilization reagent.

The spacer **110** can be disposed between the substrate **102** and the cathode **104** to separate the substrate **102** and the cathode **104** by a distance **D**. The spacer **110** can include a non-conductive material, such as plastic, glass, porcelain, rubber, silicone, etc. The distance **D** can be determined to provide a desired level of spatial resolution based on several factors, such as the strength and/or duration of an electric field generated between the substrate **102**

and the cathode **104**, and other parameters described herein. The spacer **110** can define at least part of the buffer chamber **122** between the substrate **102** and the cathode **104**.

The control system **130** can generate an electric field (-E) between the substrate **102** and the cathode **104**. The control system **130** can include a controller **132** configured to
5 apply a voltage between the substrate **102** and the cathode **104** using a power supply **134**. The power supply **134** can include a high voltage power supply. The controller **132** can be electrically connected to the substrate **102** and the cathode **104**, for example using electrical wires. The control system **130** can include a user interface **136** configured to receive a user
10 input of starting or stopping the electrophoretic process. The user interface **136** can include various types of input devices, such as a graphic user interface, physical or virtual buttons, switches, keypads, keyboard, etc., configured to receive a user input for adjusting operating parameters of the system **100** or for accessing other information (e.g., instructions) associated with the system **100**. Examples of operating parameters can include, but are not limited to, voltage applied, duration of voltage application, etc. In some implementations, the input
15 devices can be used to select a subset of substrate regions **116** on the sample **112** so that the subset of substrate regions **116** are electrically activated to generate an electric field between the subset of substrate regions **116** and the cathode **104**. In addition, the user interface **136** can include an output device, such as a display, lamps, etc., configured to output the operating parameters of the system **100** or other information associated with the system **100**.
20 **FIG. 3** illustrates an example configuration of a substrate and a sample undergoing electrophoresis using the electrophoretic system **100**. The configuration and process illustrated in **FIG. 3** may be similar to the electrophoretic processes described herein, for example with reference to **FIGS. 21A-C**. Referring to **FIG. 3**, the application of an electric field (-E) causes the analytes **314** (e.g., negatively charged analytes) to move towards the
25 capture probes **318** (e.g., positively charged analytes) in the direction of the arrows shown. In some implementations, the analytes **314** include a protein or a nucleic acid. In some embodiments, the analytes **314** are negatively charged proteins or nucleic acids. In some embodiments, the analytes **314** include a positively charged protein or a nucleic acid. In some embodiments, the analytes **314** includes a negatively charged transcript. For example,
30 the analytes **314** include a polyA transcript. In some implementations, a detergent or other reagent can be added to change the charge of the analytes. For example, SDS as used in SDS-PAGE can coat the proteins and substantially provide a uniform negative charge on the proteins. Other implementations can be used to change the charge of molecule with a

covalent attachment of another charged molecule. Yet another option is to change the pH of the solution.

In some embodiments, the capture probes **318** are configured as a coating that is in contact with the substrate **302** (e.g., a coating provided on the surface of the substrate **302**).

5 In some embodiments, the capture probes **318** can include a feature array, or can be replaced by a feature array. In some implementations, the feature array can be the same as the substrate region described herein. In alternative embodiments, the feature array can be different from the substrate region described herein. In some embodiments, the analytes **314** move towards the capture probes **318** for a distance (h). In some embodiments, the buffer **324** (e.g., permeabilization reagent) can be in contact with the sample **312**, the substrate **302**, the cathodes **304**, or any combination thereof. The buffer **324** can include any of the permeabilization reagents disclosed including but not limited to a dried permeabilization reagent, a permeabilization buffer, a buffer without a permeabilization reagent, a permeabilization gel, and a permeabilization solution.

15 Referring to **FIGS. 4-16**, various configurations of an electrophoretic system **100** are described. In general, as described herein, the system **100** can include a substrate, a substrate cassette, a cathode assembly (e.g., **FIGS. 12-13**), and a control system. The substrate can be configured similarly to the substrate **102** or other substrate described herein. The substrate cassette can be configured to be disposed on the substrate and define buffer chambers (e.g., the buffer chambers **122**) on the substrate. The cathode assembly is configured to implement the cathode **104** described herein. For example, the cathode assembly can include a plurality of electrodes configured to position within the plurality of buffer chambers of the substrate cassette, respectively. The control system can be configured similarly to the control system **130**. For example, the control system includes a power supply electrically connected with the
25 substrate and the cathode assembly and can supply power to generate electric field between the substrate (e.g., respective substrate regions thereof) and the cathode assembly (e.g., the plurality of electrodes thereof) such that the analytes in biological samples migrate toward capture probes on the substrate.

Referring to **FIGS. 4A-D**, an example of a substrate cassette **404** is described. The
30 substrate cassette **404** includes a body **410** and a plurality of apertures **412**. The body **410** can be configured to mount onto the substrate **402**, or receive the substrate **402**. For example, the body **410** is configured to define a cavity that can receive at least a portion of the substrate **402**. The plurality of apertures **412** are configured and arranged in the body **410** such that, when the substrate cassette **404** is disposed on, or receives, the substrate **402**, the

plurality of apertures **412** are aligned with a plurality of substrate regions **416** (**FIG. 4B**) of the substrate **402** and define a plurality of buffer chambers on the plurality of substrate regions **416** of the substrate **402**.

Referring also to **FIG. 4D**, the substrate **402** can be configured similarly to the substrate **102**. The substrate **402** is configured to place a plurality of biological samples **413** that contains one or more analytes. By way of example, each biological sample **413** can be one or more cells or a tissue sample including one or more cells. The substrate **402** can include a plurality of substrate regions **416** for placing or containing the samples **413** thereon. In some implementations, the substrate **402** can further comprise one or more capture probes **418** on each substrate region **416**. The capture probes **418** can be placed on the substrate region **416** in a variety of ways described herein. For example, the capture probes **418** can be directly attached (e.g., reversibly or non-reversibly) to a feature on an array. In another example, the capture probes **418** can be indirectly attached (e.g., reversibly or non-reversibly) to a feature on an array. Alternatively or in addition, the capture probes **418** can be immobilized on the substrate region **416** of the substrate **402**. The sample **413** can be prepared on the substrate **402** in various ways described herein.

In some implementations, the substrate **402** is configured to be used as a first electrode in the electrophoretic system **100**. For example, the substrate **402** can be used as an anode. In another example, the substrate **402** can be used as a cathode. The substrate **402** can be configured as a conductive substrate described herein. For example, the substrate **402** can include one or more conductive materials that permit the substrate **402** to function as an electrode (e.g., the anode). Examples of such a conductive material include tin oxide (TO), indium tin oxide (ITO), a transparent conductive oxide (TCO), aluminum doped zinc oxide (AZO), fluorine doped tin oxide (FTO), and any combination thereof. Alternatively or in addition, other materials may be used to provide desired conductivity to the substrate **402**. In some implementations, the substrate **402** can be coated with the conductive material. For example, the substrate **402** can include a conductive coating on the surface of the substrate or each substrate region of the substrate, and the sample **413** is provided on the coating of the substrate **402** or each substrate region thereof.

Referring back to **FIG. 4B**, the substrate **402** includes a first electrode contact **420** for electrical connection to the control system **408** (e.g., a power supply thereof). For example, the first electrode contact **420** can be electrically connected with a first wire **422** (**FIG. 4C**) extending from the control system **408**. The first electrode contact **420** is electrically connected to the plurality of substrate regions **416**.

The substrate cassette **404** further includes a connection interface **414** configured to expose the first electrode contact **420** of the substrate **402** for electrical connection to the control system **408**. For example, the substrate cassette **404** can include a slit **417** as the connection interface **414**. The slit **417** can be defined at the body **410**. As illustrated in **FIG. 4C**, where the substrate **402** is received in the substrate cassette **404**, the slit **417** of the substrate cassette **404** can permit the substrate **402** to partially extend out from the body **410** such that the first electrode contact **420** of the substrate **402** is positioned outside the body **410**. The control system **408** can be connected to the substrate **402** via the first electrode contact **420** exposed from the substrate cassette **404**. For example, the first wire **422** extending from the control system **408** can be electrically connected to the first electrode contact **420** outside the substrate cassette **404**. The first wire **422** can include a connecting pin or clip **424** that can be removably attached to the first electrode contact **420** of the substrate **402**. Other types of fastening mechanisms can be used to connect the first wire **422** to the first electrode contact **420** of the substrate **402**.

Referring to **FIGS. 5-8**, various examples of a substrate cassette **204** are illustrated. A substrate cassette **204** can also be referred to as a substrate holder.

FIG. 5 illustrates an example of the substrate cassette **504**. This embodiment of the substrate holder **504** can advantageously provide a single-piece component that can be arranged in an open configuration or closed configuration, when desired. In particular, **FIG. 5** shows a top surface **593** of the substrate holder **504** in a closed position. The substrate holder **504** includes the plurality of apertures **512**. For example, the substrate holder **504** includes the body **510** having a main side **503** (e.g., a main surface or face), opposite longitudinal sides **505**, and opposite latitudinal sides **507**. The longitudinal sides **505** and the latitudinal sides **507** can constitute a lateral side (e.g., a lateral surface or face) extending from a periphery of the main side **503**. The plurality of apertures **512** can be provided at the main side **503** of the body **510**.

The substrate holder **504** can include a substrate loading mechanism for loading and holding the substrate. For example, the substrate loading mechanism can include a first tab **550a** and a second tab **550b**. The first tab **550a** and the second tab **550b** can protrude from one of the longitudinal sides **505** of the substrate holder **504**. In some embodiments, any type of fastener or engagement feature that allows releasable engagement can be used instead of the first and second tabs **550a** and **550b**, such as, for example, screws and press fit type connectors. In some embodiments, the substrate holder **504** includes 5 tabs or less (e.g., 4 tabs or less, 3 tabs or less, 2 tabs or less, or 1 tab). In some embodiments, the substrate

holder **504** is a single molded unit. Any suitable plastic or polymer can be used as a suitable molding material.

FIG. 6 is a bottom cross sectional, perspective view of the substrate holder **504**, taken along line A-A of **FIG. 5**. The substrate holder **504** includes a gasket **654** and defines a cavity **652** configured to receive the substrate **502**. In some embodiments, the substrate holder **504** is a single molded unit that includes the gasket **654**. That is, in some embodiments, the substrate holder **504** and the gasket **654** are one part. In some embodiments, the substrate holder **504** is overmolded with the gasket **654**. For example, the substrate holder **504** is a first injection molded plastic part with a second part (e.g., a pliable material) molded onto it to create the gasket **654**. In some embodiments, the pliable material is an elastomer. In some embodiments, the pliable material is silicone rubber. In some embodiments, the gasket **654** is a separate part that is not molded with the substrate holder **504**.

Referring to **FIGS. 5-6**, the substrate holder **504** includes the slit **516** defined at the lateral face of the body **510**. As described herein, the slit **516** is used as an example of the connection interface **514** for exposing the first electrode contact **420** of the substrate **502**. In some implementations, the slit **516** is defined at one of the latitudinal sides **507** and configured for the substrate **502** to partially extend out from the body **510** at the one of the latitudinal sides **507**, so that the first electrode contact **620** of the substrate **502** is positioned outside the body **510**. In other implementations, the slit **516** can be defined at a different side of the body **510**, such as any suitable one of the main side **503**, the longitudinal sides **505** and the latitudinal sides **507**.

FIG. 7A shows a top view of a substrate holder **704** in an open position. The opening and closing mechanism of the substrate holder **704** is a hinged mechanism. A bottom component **762** of the substrate holder **704** can be hinged to a top component **764** of the substrate holder **704** via a hinge **760**. In some embodiments, the hinge **760** can be a living hinge. In some embodiments, the substrate holder **704** includes 10 hinges or less (e.g., 9 hinges or less, 8 hinges or less, 7 hinges or less, 6 hinges or less, 5 hinges or less, 3 hinges or less, 2 hinges or less, or 1 hinge). Non-limiting examples of hinges that the substrate holder **704** can include, include a straight or flat living hinge, a butterfly living hinge, a child safe hinge, a double living hinge, and a triple living hinge.

The substrate holder **704** further includes one or more engagement features, such as a first notch **758a** and a second notch **758b**. The first and second notches **758a** and **758b** can engage the first and second tabs **750a** and **750b**, respectively, when pressed together. The

first and second notches **758a** and **758b** can protrude from a longitudinal side **705** of the top component **764** of the substrate holder **704**. In some embodiments, the substrate holder **704** includes three, four, five, six, seven, eight, nine, ten or more notches. In some embodiments, the notches protrude from a latitudinal side **707** of the substrate holder **704**. In some
5 embodiments, the first and second notches **758a** and **758b** are rigid and do not flex when engaging the first and second tabs **750a** and **750b**, respectively. In some embodiments, the first and second notches **758a** and **758b**, respectively, can be flexible.

FIG. 7B shows a side view of a latitudinal side **707**. The first and second notches **758a** and **758b** can project upward and include a notch ledge **766** that engages a tab ledge **768**, as shown in **FIG. 7C**. Alternatively, in some embodiments, the substrate holder **704**
10 includes a snap fit locking mechanism for releasably receiving and releasably securing the substrate. Non-limiting examples of other types of fasteners to be used in locking mechanisms of the substrate holder **704** include a catch, a projection, a male connector, and a female connector.

15 **FIGS. 7A** and **8** illustrate the placement of the substrate **702** into the substrate holder **704**. In some embodiments, the substrate **702** can be “loaded” onto an inner rim or an inner edge of the bottom component **762** of the substrate holder **704**, while a portion of the substrate **702** including the first electrode contact **720** is positioned exterior of the substrate holder **704**. Once loaded, the top component **764** is closed by pressing the first notch **758a**
20 and the second notch **758b** against the first and second tabs **750a** and **750b**, respectively, thereby forming a tight seal with the substrate **702**. In some embodiments, the substrate does not have to be tilted under tabs **750** or any other tabs. In some embodiments, the substrate holder **704** includes one or more tabs to help load the slide onto the inner rim or inner edge of the bottom component **762** of substrate holder **704**. When the substrate **702** is loaded in the
25 substrate holder **703**, the first electrode contact **720** of the substrate **702** is exposed from the substrate holder **704** through the slit **716**.

 In other implementations, the hinge **760** can be provided at one of the latitudinal sides **707**. An example of this hinge arrangement is described in PCT/US20/79843, titled **IMAGING SUPPORT DEVICES**, filed April 24, 2020, the disclosure of which is
30 incorporated herein by reference in its entirety.

 Referring to **FIG. 9**, another example of the substrate cassette **904** can be configured similarly to the substrate cassettes **204**, **504**, and **704** described in **FIGS. 4-8**, except for the connection interface **214**. Unlike the substrate cassette **204**, **504**, and **704** of **FIGS. 3** and **5-8**, in this example, the substrate cassette **904** can be configured to fully receive the substrate

902. Further, instead of a slit, the substrate cassette 904 includes a contact opening 900 as the connection interface 914. The contact opening 900 is defined at the body 910 and configured to expose the first electrode contact 920 of the substrate 902.

The contact opening 900 can be defined at the main side 903 of the body 910. In some implementations, the contact opening 900 is defined at the main side 903 adjacent the lateral side of the body 910. As illustrated in FIG. 9, for example, the contact opening 900 is defined at the main side 903 adjacent one of the latitudinal sides 907. The contact opening 900 can be defined at both the main side 903 and the latitudinal side 907, so as to extend from the main side 903 to the latitudinal side 907 of the body 910.

Referring to FIGS. 10A-D, yet another example of a substrate cassette 1004 is described, which includes the body 1010 and the plurality of apertures 1012 as described herein. The substrate cassette 1004 in this example is similarly configured to the substrate cassette described in FIGS. 4-8, except for the connection interface 1014. Unlike the substrate cassette 204, 504, and 704 of FIGS. 3 and 5-8, in this example, the substrate cassette 1004 can be configured to fully receive the substrate 1002.

In this example, as illustrated in FIG. 10B, the substrate 1002 includes a substrate contact pin 1015 that provides the first electrode contact 1020. The substrate contact pin 1015 can extend from a surface of the substrate 1002 that includes a plurality of substrate regions 1026. The substrate contact pin 1015 is electrically connected to the substrate 1002. In embodiments where the substrate 1002 includes the conductive coating 1009, the substrate contact pin 1015 can be electrically connected to the conductive coating 1009, as illustrated in FIG. 10D.

The substrate cassette 1004 further includes a contact hole 1000 as the connection interface 1014. The contact hole 1000 can be defined at the body 1010 and configured to expose the substrate contact pin 1015 of the substrate 1002. The contact hole 1000 can be defined at the main side 1003 of the body 1010. The contact hole 1000 can be positioned to align with the substrate contact pin 1015 when the substrate 1002 is received in or held by the substrate cassette 1004.

Referring to FIGS. 11A-D, a fourth example of the substrate cassette 1104 is described, which includes the body 1110 and the plurality of apertures 1112 as described herein. The substrate cassette 1104 in this example is similarly configured to the substrate cassette described in FIGS. 4-8, except for the connection interface 1114. Unlike the substrate cassette 1104, 504, and 704 of FIGS. 3 and 5-8, in this example, the substrate cassette 1104 can be configured to fully receive the substrate 1102.

The substrate cassette **1104** includes a cassette contact pin **1130** as the connection interface **1114**. The cassette contact pin **1130** can extend through the body **1110** (e.g., the main side **1103** of the body **1110**) such that a first end **1132** of the cassette contact pin **1130** is placed exterior of the body **1110**, and an opposite, second end **1134** of the cassette contact pin **1130** is placed interior of the body **1110**. The cassette contact pin **1130** is configured to electrically connect to the substrate **1102** received in the substrate cassette **1104**. In embodiments where the substrate **1102** includes the conductive coating **1109**, the cassette contact pin **1130** can be electrically connected to the conductive coating **1109**. For example, the second end **1134** of the cassette contact pin **1130** is configured to electrically connect to the conductive coating **1109**, as illustrated in **FIG. 11D**.

In some implementations, the system **100** can include a substrate contact bracket **1136** configured to engage with the substrate **1102** and electrically connected to the substrate **1102**. For example, the substrate contact bracket **1136** can be engaged with a portion of the substrate **1102** and electrically contact the conductive coating **1109** of the substrate **1102**, as shown in **FIG. 11D**. The cassette contact pin **1130** can be configured to electrically connect to the substrate contact bracket **1136** when the substrate **1102** is received in the substrate cassette **1104**. For example, the second end **1134** of the cassette contact pin **1130** is configured to contact with the substrate contact bracket **1136** based on the substrate **1102** held in the substrate cassette **1104**. Thus, the cassette contact pin **1130** can be electrically connected to the substrate **1102** and further provide the connection interface **1114** at the exterior of the substrate cassette **1104**. The first wire **1122** extending from the control system **1108** can simply be connected to the cassette contact pin **1130** (e.g., the first end **1132** thereof) so that the control system **1108** can be electrically connected to the substrate **1102** that can function as one (e.g., anode) of the electrodes in electrophoresis.

Referring to **FIGS. 12A-D**, an example of the cathode assembly **1206** is described. The cathode assembly **1206** can include a cathode body **1250** configured to be positioned at least partially on the substrate cassette **1204**. The cathode assembly **1206** can include a plurality of electrodes **1252** that project from the cathode body **1250**. The plurality of electrodes **1252** are configured to position within the plurality of apertures **1212** of the substrate cassette **1204**, respectively, when the cathode body **1250** is positioned on the substrate cassette **1204**. Thus, the electrodes **1252** is disposed within the respective buffer chambers that are defined by the apertures **1212** of the substrate cassette **1204** on the substrate regions **1216** of the substrate **1202**. Each of the electrodes **1252** functions as a cathode in each of the buffer chambers.

The cathode assembly **1206** can include a cathode contact **1254** configured for electrical connection to the control system **1208**. For example, a second wire **1226** extending from the control system **1208** is electrically connected to the cathode contact **1254**. The second wire **1226** can include a connecting pin or clip **1228** that can be removably attached to the cathode contact **1254** of the cathode assembly **1206**. Other types of fastening mechanisms can be used to connect the second wire **1226** to the cathode contact **1254** of the cathode assembly **1206**. The cathode contact **1254** is electrically connected to the plurality of electrodes **1252**. In some implementations, as shown in **FIG. 12D**, the cathode assembly **1206** includes a conductive layer **1258** that is electrically connected to both the cathode contact **1254** and the plurality of electrodes **1252**. The cathode contact **1254** can be exposed at the cathode body **1250** so as to be easily accessible exterior of the cathode body **1250**.

As shown in **FIG. 12C**, the cathode assembly **1206** is disposed on the substrate **1204** that receives or holds the substrate **1202**. The cathode assembly **1206** is oriented and arranged such that the plurality of electrodes **1252** are inserted into the respective apertures **1212** of the substrate cassette **1204** and thus disposed on the respective substrate regions **1216** of the substrate **1202**. The cathode assembly **1206** can be used with substrates and substrate cassettes of various configurations. In the illustrated example of **FIG. 12C**, the cathode assembly **1206** is used with the substrate **1202** and the substrate cassette **1204** described in **FIGS. 4-8**. In other examples, the cathode assembly **1206** can be used with the substrates **1202** and the substrate cassettes described in **FIGS. 9-11**.

The electrode **1252** can be of various shapes. For example, the electrode **1252** can be configured as a conductive wire **1255** (**FIG. 12D**), a conductive rod **1256** (**FIG. 12E**), or an array of conductive wires **1257** (**FIG. 12F**). Alternatively, as shown in **FIG. 12G**, the electrode **1252** can include a conductive extension **1261** and a conductive plate **1263** at a distal end (i.e., a free end) of the conductive extension **1261**. The conductive extension **1261** can be of various shapes, such as the conductive wire **1255**, the conductive rod **1256**, the conductive wire array **1257**, or other suitable shapes. The conductive plate **1263** can be of various shapes, such as a circular plate **1263A**, a ring **1263B**, a square plate **1263C**, or other suitable shapes.

Referring to **FIGS. 13A-E**, other examples of the substrate **1302**, the substrate cassette **1304**, and the cathode assembly **1306** are described. Referring to **FIG. 13B**, the substrate **1302** in this example is configured similarly to the substrate **1302** described herein. In this example, the substrate **1302** further includes an electrode region **1303** that functions as the first electrode contact **320**. The electrode region **1302** is configured to electrophoretically

connect to the control system **1308** so that the substrate **1302** can be used as an electrode (e.g., anode) in the system **100**. In embodiments where the substrate **1302** includes the conductive coating **1309**, the electrode region **1303** is electrically connected to the conductive coating **1309**. For example, the electrode region **1303** can be a hole that exposes a portion of the conductive coating **1309** of the substrate **1302** to a second buffer **1307**, as illustrated in **FIG. 13F**.

Referring to **FIG. 13C**, the substrate cassette **1304** in this example is configured similarly to the substrate cassette **1304** described herein. The substrate cassette **1304** can include an electrode aperture **1301**, as well as the plurality of apertures **1312** described herein. The electrode aperture **1301** is configured to be aligned with the electrode region **1303** of the substrate **1302** when the substrate cassette **1304** receives the substrate **1302**. As illustrated in **FIG. 13C**, the substrate cassette **1304** is configured to fully receive the substrate **1302**. Further, as illustrated in **FIGS. 13E-F**, the plurality of apertures **1312** and the electrode aperture **1301** of the substrate cassette **1304** are arranged on the plurality of substrate regions **1316** and the electrode region **1303** of the substrate **1302**, respectively. As described herein, the plurality of apertures **1312** (either independently or in cooperation with the gasket **1354** as shown in **FIG. 13E**) can define a plurality of first buffer chambers (e.g., the buffer chambers **122**) on the plurality of substrate regions **1316**, which are configured to receive first buffers **1305** (e.g., the buffer **124**) for analyte migration under electrophoresis. In addition, the electrode aperture **1301** (either independently or in cooperation with the gasket **1354** as shown in **FIG. 13F**) can define a second buffer chamber on the electrode region **1303**, which is configured to receive a second buffer **1307**. In some implementations, the second buffer **1307** is different from the first buffer **1305**. The second buffer **1307** can have an electrolyte strength that is greater than the first buffer **1305**. For example, the second buffer **1307** can be selected to have ionic strength that ranges from 1 to 1300 mmol/L. One example of the second buffer **1307** is a sodium chloride solution. Other examples of the second buffer **1307** include sodium hydroxide, potassium chloride, or any buffer with a high concentration of ions, which can include the first buffer **1305** at a higher concentration. Further examples of the first and second buffers can be found in Reijenga, et al., Buffer Capacity, Ionic Strength and Heat Dissipation in Capillary Electrophoresis, Journal of Chromatography A, 744 (1996) 1347-153, the disclosure of which is incorporated herein by reference in its entirety.

Referring to **FIG. 13A**, the cathode assembly **1306** includes the cathode body **1350** configured to be positioned at least partially on the substrate cassette **1304**. The cathode

assembly **1306** can include a plurality of electrodes **1352** that project from the cathode body **1350**. The plurality of electrodes **1352** are configured to position within the plurality of apertures **1312** of the substrate cassette **1304**, respectively, when the cathode body **1350** is positioned on the substrate cassette **1304**. Thus, the electrodes **1352** is disposed within the
5 respective buffer chambers that are defined by the apertures **1312** of the substrate cassette **1304** on the substrate regions **316** of the substrate **1302**. Each of the electrodes **1352** functions as a cathode in each of the buffer chambers.

The cathode assembly **1306** can include a second electrode contact **1354** configured for electrical connection to the control system **1308**. The second electrode contact **1354** can
10 be used for a cathode contact. For example, a second wire **1326** extending from the control system **1308** is electrically connected to the cathode contact **1354**. The second wire **326** can include a connecting pin or clip **1328** that can be removably attached to the cathode contact **1354** of the cathode assembly **1306**. Other types of fastening mechanisms can be used to connect the second wire **1326** to the cathode contact **1354** of the cathode assembly **1306**.
15 The cathode contact **1354** is electrically connected to the plurality of electrodes **1352**. In some implementations, as shown in **FIG. 13E**, the cathode assembly **1306** includes a conductive layer **1358** that is electrically connected to both the cathode contact **1354** and the plurality of electrodes **1352**. The cathode contact **1354** can be exposed at the cathode body **1350** so as to be easily accessible exterior of the cathode body **1350**.

In this example, the cathode assembly **1306** further includes a second electrode **1310**
20 that projects from the cathode body **1350**. As illustrated in **FIG. 13F**, the second electrode **1310** is configured to position within the electrode aperture **1301** of the substrate cassette **1304** when the cathode body **1350** is positioned on the substrate cassette **1304**. Thus, the second electrode **1310** is disposed within the second buffer chamber that is defined by the
25 electrode aperture **1301** of the substrate cassette **1304** (independently or in cooperation with the gasket **354**) on the electrode region **1303** of the substrate **1302**. The second electrode **1310** can function as part of the anode in electrophoresis.

The cathode assembly **1306** can include an anode contact **1313** configured for electrical connection to the control system **1308**. For example, the first wire **322** extending
30 from the control system **1308** is electrically connected to the anode contact **1313**. The first wire **1322** can include a connecting pin or clip **1328** that can be removably attached to the anode contact **1313** of the cathode assembly **1306**. Other types of fastening mechanisms can be used to connect the first wire **1322** to the anode contact **1313** of the cathode assembly **1306**. The anode contact **1313** is electrically connected to the second electrode **1310**. In

some implementations, as shown in **FIG. 13F**, the cathode assembly **1306** includes a conductive layer **1314** that is electrically connected to both the anode contact **1313** and the second electrode **1310**. As illustrated in **FIG. 13D**, the anode contact **1313** can be exposed at the cathode body **1350** so as to be easily accessible exterior of the cathode body **1350**.

5 As illustrated in **FIG. 13F**, with the control system **1308** being electrically connected to the anode contact **1313** of the cathode assembly **1306**, the control system **1308** is electrophoretically connected to the electrode region **1303** (i.e., the first electrode contact **320**) of the substrate **1302** via the second electrode **1310** that is positioned within the second chamber containing the second buffer **1307** on the electrode region **1303** of the substrate
10 **1302**.

Referring to **FIGS. 14A-C**, yet another example of a substrate cassette **1404** is described. As described herein, the substrate cassette **1404** includes the body **1410** and the plurality of apertures **1412**. The substrate cassette **1404** in this example is configured similarly to the substrate cassette **404** described in **FIGS. 4-8**. In addition, the substrate
15 cassette **1404** is configured to incorporate at least part of the cathode assembly **1406** described herein. For example, as illustrated in **FIG. 14B**, the substrate cassette **1404** includes a plurality of electrodes **1403** that function as the electrodes **1452** described herein. The plurality of electrodes **1403** are configured to position within the buffer chambers **1405** (similar to the buffer chambers **122** described herein) defined at least by the apertures **1412** of
20 the substrate cassette **1404**. The electrode **1403** can be of various configurations, similarly to the electrodes **1452**.

Referring to **FIG. 14B**, the substrate cassette **1404** can include a plurality of cathode contact pins **1406** that extend through a wall of the cassette body **1410** and electrically connect to the plurality of electrodes **1403**. The plurality of cathode contact pins **1406** are
25 electrically connected to the second electrode contact or cathode contact **1454** that is exposed at the cassette body **1410**. Alternatively, one of the cathode contact pins **1456** can be exposed exterior of the cassette body **1410** and used as the cathode contact **1454**. As described herein, the cathode contact **1454** is used to connect to the control system **1408**, such as via the second wire **1426** extending from the control system **1408**.

30 In this example, the substrate cassette **1404** is used with the substrate **202** described in **FIGS. 4-8**. In other examples, the substrate cassette **1404** can be used with the substrates **902** described in **FIGS. 9-13**.

The electrode **1403** can be of various configurations, similarly to the electrodes **1452**. For example, the electrode **1403** can be configured as a conductive wire (similar to the

conductive wire **1255** in **FIG. 12D**), a conductive rod (similar to the conductive rod **1256** in **FIG. 12E**), or an array of conductive wires (similar to the conductive wire array **1257** in **FIG. 12F**). Alternatively, the electrode **1403** can be configured similarly to the electrode **1252** shown in **FIG. 12G**, (e.g., extensions with various tips such as a circular plate, a ring, a square plate, or other suitable shapes). Referring to **FIG. 14C**, each buffer chamber **1405** can be provided with two or more electrodes **1403**.

Referring to **FIGS. 15A-B**, yet another example of a substrate cassette **1504** is described. The substrate cassette **1504** in this example is configured similarly to the substrate cassette **1504** of **FIGS. 14A-C**. In addition, the substrate cassette **1504** includes the connection interface **1514** shown in **FIGS. 11A-D**. In particular, the substrate cassette **1504** in this example further includes the cassette contact pin **1530** as the connection interface **1514**. The cassette contact pin **1530** can extend through the body **1510** such that the first end **1532** of the cassette contact pin **1530** is placed exterior of the body **1510**, and the opposite, second end **1534** of the cassette contact pin **1530** is placed interior of the body **1510**. The cassette contact pin **1530** is configured to electrically connect to the substrate **1502** received in the substrate cassette **1504**. In embodiments where the substrate **1502** includes the conductive coating **1509**, the cassette contact pin **1530** can be electrically connected to the conductive coating **1509**. For example, the second end **1534** of the cassette contact pin **1530** is configured to electrically connect to the conductive coating **1509**, as illustrated in **FIG. 15B**.

In some implementations, the system **1500** can include the substrate contact bracket **1536** configured to engage with the substrate **1502** and electrically connected to the substrate **1502**. For example, the substrate contact bracket **1536** can be engaged with a portion of the substrate **1502** and electrically contact the conductive coating **1509** of the substrate **1502**, as shown in **FIG. 15B**. The cassette contact pin **1530** can be configured to electrically connect to the substrate contact bracket **1536** when the substrate **1502** is received in the substrate cassette **1504**. For example, the second end **1534** of the cassette contact pin **1530** is configured to contact with the substrate contact bracket **1536** based on the substrate **1502** held in the substrate cassette **1504**. Thus, the cassette contact pin **1530** can be electrically connected to the substrate **1502** and further provide the connection interface **1514** at the exterior of the substrate cassette **1504**.

Accordingly, the control system **1508** can be easily connected to the system **1500**. For example, the first wire **1522** extending from the control system **1508** can simply be connected to the cassette contact pin **1530** (e.g., the first end **1532** thereof) so that the control

system **1508** can be electrically connected to the substrate **1502** that can function as one (e.g., anode) of the electrodes in electrophoresis. Further, the second wire **1526** extending from the control system **1508** can simply be connected to the cathode contact **1554** so that the control system **1508** can be electrically connected to the electrodes **602** (e.g., cathodes) in the electrophoresis system.

Referring to **FIGS. 16A-C**, example connection schemes are described. Referring to **FIG. 16A**, the substrate cassette **1604** and the substrate **1602** are configured similarly to the substrate cassette **1404** and the substrate **1402** shown in **FIGS. 14A-C**. In addition, the substrate cassette **1604** includes an anode contact pin **1630** extending from the cassette body **1610**. Further, a conductive wire **1632** is provided to electrically connect the anode contact pin **1630** to the first electrode contact **1620** of the substrate **1602**. For example, the conductive wire **1632** includes a first end **1634** and an opposite second end **1635**. The first end **1634** can be electrically connected to the anode contact pin **1630**, and the second end **1635** can be electrically connected to the first electrode contact **1620** that is positioned outside the body **1610**. Connecting pins, clips, or other suitable fastening mechanisms can be provided at the first and second ends **1634**, **1635** to connect the conductive wire **1632** to the anode contact pin **1630** and the first electrode contact **1620**, respectively.

Referring to **FIG. 16B**, the substrate cassette **1604** and the substrate **1602** are configured similarly to the substrate cassette **1404** and the substrate **1402** shown in **FIGS. 14A-C**. In addition, the substrate cassette **1604** includes an anode cassette body **1650**. The anode cassette body **1650** can define a cavity configured to receive a portion of the substrate **1602**. For example, the anode cassette body **1650** can be configured to receive at least the first electrode contact **1620** of the substrate **1602** that is positioned exterior of the cassette body **1610** of the substrate cassette **1604**.

The anode cassette body **1650** can include a connection interface **1614** that is similar to the connection interface **1114** described in **FIGS. 11A-D**. In particular, as shown in **FIG. 16C**, the anode cassette body **1650** includes a cassette contact pin **1630** as the connection interface **1614**. The cassette contact pin **1630** can extend through anode cassette body **1650** such that a first end **1631** of the cassette contact pin **1630** is placed exterior of anode cassette body **1650**, and an opposite, second end **1634** of the cassette contact pin **1630** is placed interior of anode cassette body **1650**. The cassette contact pin **1630** is configured to electrically connect to the substrate **1602** received in the anode cassette body **1650**. In embodiments where the substrate **1602** includes a conductive coating **1609**, the cassette contact pin **1630** can be electrically connected to the conductive coating **1609**. For example,

the second end **1634** of the cassette contact pin **1630** is configured to electrically connect to the conductive coating **1609**, as illustrated in **FIG. 16C**.

In some implementations, the system **100** can include a substrate contact bracket **1636** configured to engage with the substrate **1602** and electrically connected to the substrate **1602**.
5 For example, the substrate contact bracket **1636** can be engaged with a portion of the substrate **1602** and electrically contact the conductive coating **1609** of the substrate **1602**, as shown in **FIG. 16C**. The cassette contact pin **1630** can be configured to electrically connect to the substrate contact bracket **1636** when the substrate **1602** is received in the anode cassette body **1650**. For example, the second end **1634** of the cassette contact pin **1630** is
10 configured to contact with the substrate contact bracket **1636** based on the substrate **1602** held in the anode cassette body **1650**. Thus, the cassette contact pin **1630** can be electrically connected to the substrate **1602** and further provide the connection interface **1614** at the exterior of the anode cassette body **1650**. The first wire **1622** extending from the control system **1608** can simply be connected to the cassette contact pin **1630** (e.g., the first end **1631**
15 thereof) so that the control system **1608** can be electrically connected to the substrate **1602** that can function as one (e.g., anode) of the electrodes in electrophoresis.

Referring to **FIGS. 17A-C**, an example instrument **1700** for electrophoresis is described. The instrument **1700** can be used with one or more components of the electrophoretic system **100** described herein. In some implementations, the instrument **1700**
20 is configured to automate at least part of the electrophoretic process that uses the substrate **1702**, the substrate cassette **1704**, the cathode assembly **1707**, and the control system **1708**.

Referring to **FIG. 17A**, the instrument **1700** has an instrument housing **1703**, a cassette tray **1704**, and a cassette cover **1706**. The instrument housing **1703** is configured to receive at least one or all of the components of the electrophoretic system **100**. For example,
25 the instrument housing **1703** is configured to include the control system **1708**. In some implementations, the instrument housing **1703** can include the controller **132** and the power supply **134**. The power supply **134** can be connected to an electrical outlet external to the instrument housing **1703**. Alternatively or in addition, the power supply **134** can include a battery that is received in the instrument housing **1703**. The instrument housing **1703** can
30 further include the user interface **136** of the control system **108**. The user interface **136** can be placed in a location (e.g., adjacent the cassette tray **1705**) suitable for a user to control the instrument **1700**.

The cassette tray **1705** is configured to receive the substrate cassette **1704**. For example, the substrate cassette **1704**, which receives the substrate **1702**, can be placed on the

cassette tray **1705**. In some implementations, the cassette tray **1705** extends from the instrument housing **1703** so that the substrate cassette **1704** can be conveniently placed on the cassette tray **1705**. In some implementations, the cassette tray **1705** is retractable into the instrument housing **1703** so that electrophoresis can be performed within the instrument housing **1703**. Once the electrophoresis is completed, the cassette tray **1705** is extended from the instrument housing **1703** so that the substrate cassette **1704** can be removed from the cassette tray **1705**. Alternatively, the cassette tray **1705** can remain exterior of the instrument housing **1703** before, during, and after electrophoresis is performed.

The cassette tray **1705** can be configured to receive various implementations of the substrate cassette **1704** described herein. Referring to **FIG. 17B**, in one example, the substrate cassette **1704** described in **FIGS. 15A-B** is placed on the cassette tray **1705**. In this example, the cassette cover **1706** is configured to be located above the cassette tray **1705** and provide electrical connections between the substrate cassette **1704** and the control system **1708**. Referring to **FIG. 17B**, in embodiments where the substrate cassette **1704** described in **FIGS. 15A-B** is placed on the cassette tray **1705**, the cassette cover **1706** can include a cathode connector **1710** and an anode connector **1711**. The cathode connector **1710** is configured to electrically engage with the cathode contact **1754** (or an end of the cathode contact pin **606**) of the substrate cassette **1704**. The cathode connector **1710** is further electrically connected to the control system **1708**. For example, the cathode connector **1710** is electrically connected to the second wire **1726** extending from the control system **1708**. The anode connector **1711** is configured to electrically engage with the anode contact (i.e., the first end **1732** of the cassette contact pin **1730**) of the substrate cassette **1704**. The anode connector **1711** is further electrically connected to the control system **1708**. For example, the anode connector **1711** is electrically connected to the first wire **1722** extending from the control system **1708**.

In some implementations, the cassette cover **1706** can be movable between a raised position and a lowered position. In the raised position, the cassette cover **1706** is positioned at a first predetermined distance from the cassette tray **1705** so that the substrate cassette **1704** can be placed on the cassette tray **1705** without interference with the cassette cover **1706**. Once the substrate cassette **1704** is placed on the cassette tray **1705**, the cassette cover **1706** can be moved to the lowered position in which the cassette cover **1706** is positioned closer to the cassette tray **1705** and positioned at a second predetermined distance (shorter than the first predetermined distance) from the cassette tray **1705**. In the lowered position, the cathode connector **1710** and the anode connector **1711** are engaged with the cathode

contact **1754** and the anode contact (i.e., the first end **1732** of the cassette contact pin **1730**) of the substrate cassette **1704**, respectively. The cathode connector **1710** and the anode connector **1711** can be positioned so as to be aligned with the cathode contact **1754** and the anode contact (i.e., the first end **1732** of the cassette contact pin **1730**) of the substrate cassette **1704** when the cassette cover **1706** is positioned in the lowered position relative to the substrate cassette **1704**. In some implementations, the cassette cover **1706** can be manually operated between the raised position and the lowered position. Alternatively or in addition, the cassette cover **1706** is automatically actuated using an actuator (e.g., a motor) that is controlled by a controller (e.g., the control system **1708**). For example, a user can place the substrate cassette **1704** on the cassette tray **1705** and select a menu via a user interface (e.g., the user interface **136** (**FIG. 1**)) to lower the cassette cover **1706**, and the control system **1708** operates the actuator to move the cassette cover **1706** so that the cassette cover **1706** engages with the substrate cassette **1704** and makes necessary electrical connection with the substrate cassette **1704** as described above.

Referring to **FIG. 17C**, in another example, the substrate cassette **1704** described in **FIGS. 11A-D** is placed on the cassette tray **1705**. In this example, the cassette cover **1706** is configured to be located above the cassette tray **1705** and provide at least part of the cathode assembly **1707**. For example, the cassette cover **1706** can include a plurality of electrodes **1720** that project from the cassette cover **1706** and configured to extend into the plurality of buffer chambers **1723** (defined at least by the plurality of apertures **1712** of the substrate cassette **1704**). The cassette cover **1706** can further include a cathode connector **1724** that electrically connects the plurality of electrodes **1720** and is further configured to electrically connect the control system **1708** via, for example, the second wire **1726** extending from the control system **1708**.

Further, the cassette cover **1706** can include an anode connector **726**. The anode connector **1727** is configured to electrically engage with the anode contact (i.e., the first end **1732** of the cassette contact pin **1730**) of the substrate cassette **1704**. The anode connector **1727** is further electrically connected to the control system **1708**. For example, the anode connector **726** is electrically connected to the first wire **1722** extending from the control system **1708**.

As described above, the cassette cover **1706** can be movable between the raised position and the lowered position. Once the substrate cassette **1704** is placed on the cassette tray **1705** with the cassette cover **1706** being in the raised position, the cassette cover **1706** can be moved to the lowered position. In the lowered position, the plurality of electrodes

1720 are positioned within the buffer chambers 1723, respectively. Further, in the lowered position, the anode connector 1711 are engaged with the anode contact (i.e., the first end 1732 of the cassette contact pin 1730) of the substrate cassette 1704.

FIG. 18 is a flowchart of an example process 1800 for analyte migration. The process 1800 can be performed using the electrophoretic system 100 (including the instrument 1700) described herein. The process 1800 can be used to check for electrical connections in the system 100 prior to execution of electrophoresis.

In some implementations, the process 1800 can include loading a substrate (e.g., the substrate 1002) to an electrophoresis instrument (e.g., the instrument 1700) (1802). For example, as described herein, the substrate 1002 comprises capture probes on the substrate regions 1016, and one or more biological samples can be placed on the substrate 1002 so that the biological samples contact with the capture probes. Alternatively, the biological samples do not make contact with the capture probes. For example, an intermediate layer (e.g., gels) can be disposed between the substrate and the sample, which is compatible with electrophoresis. A substrate cassette (e.g., the substrate cassette 1004) can be arranged on the substrate 1002 so that the plurality of apertures 1012 of the substrate cassette 1004 are aligned with the substrate regions 1016 of the substrate 1002 and define a plurality of buffer chambers (e.g., the buffer chambers 122, 1723) on the substrate regions 1016 of the substrate 1002. Then, buffers (e.g., the buffers 124) are supplied in the buffer chambers.

The process 1800 can include arranging a cathode relative to the substrate (1804). For example, the cathode can include a plurality of electrodes (e.g., the electrodes 1252, 1720) that can be placed within the plurality of chambers, respectively.

The process 1800 can include electrically connecting a power supply to the substrate and the cathode (1806). As described herein, the power supply can be included in and controlled by the control system 1008. The power supply (or the control system) can be electrically connected to the substrate (as the anode) and the cathode (e.g., the plurality of electrodes) in various ways described herein, such as using various connection interfaces implemented in the substrate 1002, the substrate cassette 1004, the cathode assembly 1006, the cassette cover 1706, etc.

The process 1800 can include applying a test voltage between the substrate (i.e., the anode) and the cathode (1808). The test voltage can be lower than a voltage necessary for executing electrophoresis in the instrument 1700. The test voltage can be applied to ensure that electrical connections are made in the electrophoretic system 100 (including the instrument 1700) before electrophoresis is performed. Such electrical connections can

include at least one of an electrical connection of the power supply to the substrate, an electrical connection of the power supply to the cathode, or an electrical property of the buffers.

5 The process **1800** can include detecting an output parameter in response to the test voltage (**1810**). In some implementations, the output parameter can be an impedance. For example, the test voltage can allow a current to flow through the system **100** (including the instrument **1700**), and the process **1800** can measure the current in the system **100**, and calculate an impedance based on the measured current and the test voltage being applied. In other implementations, other parameters in the system **100** can be used.

10 The process **1800** can include determining whether the output parameter meets a threshold value (**1812**). The threshold value can be a value that is indicative of proper electrical connection in the system **100** (including the instrument **1700**). For example, the threshold value can be an impedance of the system **100** when proper electrical connections are made in the system **100**, such as the electrical connection of the power supply to the
15 substrate, the electrical connection of the power supply to the cathode, the electrical property of the buffers, etc. The threshold value can be a single value. Alternatively, the threshold value can be a range of value.

The process **1800** can include, upon determining that the output parameter meets the threshold value, applying a voltage for analyte migration (**1814**). For example, when the
20 output parameter meets the threshold value, the electrical connections in the system can be considered to be proper. Therefore, an actual voltage for electrophoresis can be applied to generate electric fields between the substrate (e.g., the plurality of substrate regions of the substrate) and the cathode (e.g., the plurality of electrodes) through one or a plurality of buffer chambers. The electrical fields can cause the analytes in the biological samples to
25 migrate toward the capture probes on the substrate.

The process **1800** can include, upon determining that the output parameter does not meet the threshold value, ceasing to apply the voltage for analyte migration (**1816**). For example, when the output parameter does not meet the threshold value, the electrical connections in the system can be considered to be improper. Thus, the instrument **1700** can
30 stop applying the actual voltage if it has already started it, or does not proceed with applying the actual voltage if it hasn't started yet.

The process **1800** can include, upon determining that the output parameter does not meet the threshold value, generating a notification of a potential electrical connection issue (**1818**). The notification can be output to the instrument **1700** or other devices that the user

can access. For example, the notification can be output via the user interface **136**. The notification can include information that alerts users of an improper electrical connection in the system **100** (including the instrument **1700**). The notification can be output before advancing to subsequent steps for electrophoresis (e.g., applying the actual voltage for electrophoresis), so that the user can decide to take any action to investigate and/or remediate such improper connection. For example, based on the notification, the user can examine physical connections between the power supply and the substrate or between the power supply and the cathode, and check for the buffers. The user can then adjust the physical connections, or replace/refill the buffers, to resolve any issue. Alternatively, the notification can be output during or after performing such subsequent steps for electrophoresis (e.g., applying the actual voltage for electrophoresis). The user can consider such possibility of connection issues when analyzing the analytes captured by the electrophoresis.

FIG. 19 is a block diagram of computing devices **1900** that may be used to implement the systems and methods described in this document, as either a client or as a server or plurality of servers. Computing device **1900** is intended to represent various forms of digital computers, such as laptops, desktops, workstations, personal digital assistants, servers, blade servers, mainframes, and other appropriate computers. Computing device **1950** is intended to represent various forms of mobile devices, such as personal digital assistants, cellular telephones, smartphones, and other similar computing devices. The components shown here, their connections and relationships, and their functions, are meant to be examples only, and are not meant to limit implementations described and/or claimed in this document.

Computing device **1900** includes a processor **1902**, memory **1904**, a storage device **1906**, a high-speed interface **1908** connecting to memory **1904** and high-speed expansion ports **1910**, and a low speed interface **1912** connecting to low speed bus **1914** and storage device **1906**. Each of the components **1902**, **1904**, **1906**, **1908**, **1910**, and **1912**, are interconnected using various busses, and may be mounted on a common motherboard or in other manners as appropriate. The processor **1902** can process instructions for execution within the computing device **1900**, including instructions stored in the memory **1904** or on the storage device **1906** to display graphical information for a graphic user interface (GUI) on an external input/output device, such as display **1916** coupled to high-speed interface **1908**. In other implementations, multiple processors and/or multiple buses may be used, as appropriate, along with multiple memories and types of memory. Also, multiple computing devices **1900** may be connected, with each device providing portions of the necessary operations (e.g., as a server bank, a group of blade servers, or a multi-processor system).

The memory **1904** stores information within the computing device **1900**. In one implementation, the memory **1904** is a volatile memory unit or units. In another implementation, the memory **1904** is a non-volatile memory unit or units. The memory **1904** may also be another form of computer-readable medium, such as a magnetic or optical disk.

5 The storage device **1906** is capable of providing mass storage for the computing device **1900**. In one implementation, the storage device **1906** may be or contain a computer-readable medium, such as a floppy disk device, a hard disk device, an optical disk device, or a tape device, a flash memory or other similar solid state memory device, or an array of devices, including devices in a storage area network or other configurations. A computer
10 program product can be tangibly embodied in an information carrier. The computer program product may also contain instructions that, when executed, perform one or more methods, such as those described above. The information carrier is a computer- or machine-readable medium, such as the memory **1904**, the storage device **1906**, or memory on processor **1902**.

15 The high-speed controller **1908** manages bandwidth-intensive operations for the computing device **1900**, while the low speed controller **1912** manages lower bandwidth-intensive operations. Such allocation of functions is an example only. In one implementation, the high-speed controller **1908** is coupled to memory **1904**, display **1916** (e.g., through a graphics processor or accelerator), and to high-speed expansion ports **1910**, which may accept various expansion cards (not shown). In the implementation, low-speed
20 controller **1912** is coupled to storage device **1906** and low-speed expansion port **1914**. The low-speed expansion port, which may include various communication ports (e.g., USB, Bluetooth, Ethernet, wireless Ethernet) may be coupled to one or more input/output devices, such as a keyboard, a pointing device, a scanner, or a networking device such as a switch or router, e.g., through a network adapter.

25 The computing device **1900** may be implemented in a number of different forms, as shown in the figure. For example, it may be implemented as a standard server **1920**, or multiple times in a group of such servers. It may also be implemented as part of a rack server system **1924**. In addition, it may be implemented in a personal computer such as a laptop computer **1922**. Alternatively, components from computing device **1900** may be combined
30 with other components in a mobile device (not shown). Each of such devices may contain one or more of computing device **1900**, and an entire system may be made up of multiple computing devices **1900** communicating with each other.

Additionally computing device **1900** can include Universal Serial Bus (USB) flash drives. The USB flash drives may store operating systems and other applications. The USB

flash drives can include input/output components, such as a wireless transmitter or USB connector that may be inserted into a USB port of another computing device.

Various implementations of the systems and techniques described here can be realized in digital electronic circuitry, integrated circuitry, specially designed ASICs (application specific integrated circuits), computer hardware, firmware, software, and/or combinations thereof. These various implementations can include implementation in one or more computer programs that are executable and/or interpretable on a programmable system including at least one programmable processor, which may be special or general purpose, coupled to receive data and instructions from, and to transmit data and instructions to, a storage system, at least one input device, and at least one output device.

These computer programs (also known as programs, software, software applications or code) include machine instructions for a programmable processor, and can be implemented in a high-level procedural and/or object-oriented programming language, and/or in assembly/machine language. As used herein, the terms “machine-readable medium” “computer-readable medium” refers to any computer program product, apparatus and/or device (e.g., magnetic discs, optical disks, memory, Programmable Logic Devices (PLDs)) used to provide machine instructions and/or data to a programmable processor, including a machine-readable medium that receives machine instructions as a machine-readable signal. The term “machine-readable signal” refers to any signal used to provide machine instructions and/or data to a programmable processor.

To provide for interaction with a user, the systems and techniques described here can be implemented on a computer having a display device (e.g., a CRT (cathode ray tube) or LCD (liquid crystal display) monitor) for displaying information to the user and a keyboard and a pointing device (e.g., a mouse or a trackball) by which the user can provide input to the computer. Other kinds of devices can be used to provide for interaction with a user as well; for example, feedback provided to the user can be any form of sensory feedback (e.g., visual feedback, auditory feedback, or tactile feedback); and input from the user can be received in any form, including acoustic, speech, or tactile input.

The systems and techniques described herein can be implemented in a computing system that includes a back end component (e.g., as a data server), or that includes a middleware component (e.g., an application server), or that includes a front end component (e.g., a client computer having a graphical user interface or a Web browser through which a user can interact with an implementation of the systems and techniques described here), or any combination of such back end, middleware, or front end components. The components

of the system can be interconnected by any form or medium of digital data communication (e.g., a communication network). Examples of communication networks include a local area network (“LAN”), a wide area network (“WAN”), peer-to-peer networks (having ad-hoc or static members), grid computing infrastructures, and the Internet.

5 The computing system can include clients and servers. A client and server are generally remote from each other and typically interact through a communication network. The relationship of client and server arises by virtue of computer programs running on the respective computers and having a client-server relationship to each other.

10 Active capture methods

In some of the methods described herein, an analyte in a biological sample (e.g., in a cell or tissue section) can be transported (e.g., passively or actively) to a capture probe (e.g., a capture probe affixed to a substrate (e.g., a substrate or bead)).

For example, analytes can be transported to a capture probe (e.g., an immobilized capture probe) using an electric field (e.g., using electrophoresis), pressure, fluid flow, gravity, temperature, and/or a magnetic field. For example, analytes can be transported through, e.g., a gel (e.g., hydrogel), a fluid, or a permeabilized cell, to a capture probe (e.g., an immobilized capture probe) using a pressure gradient, a chemical concentration gradient, a temperature gradient, and/or a pH gradient. For example, analytes can be transported through a gel (e.g., hydrogel), a fluid, or a permeabilized cell, to a capture probe (e.g., an immobilized capture probe).

In some examples, an electrophoretic field can be applied to analytes to facilitate migration of analytes towards a capture probe. In some examples, a sample containing analytes contacts a substrate having capture probes fixed on the substrate (e.g., a slide, cover slip, or bead), and an electric current is applied to promote the directional migration of charged analytes towards capture probes on a substrate. An electrophoresis assembly (e.g., an electrophoretic chamber), where a biological sample is in contact with a cathode and capture probes (e.g., capture probes fixed on a substrate), and where the capture probes are in contact with the biological sample and an anode, can be used to apply the current.

30 In some embodiments, methods utilizing an active capture method can employ a
conductive substrate (e.g., any of the conductive substrates described herein). In some
embodiments, a conductive substrate includes paper, a hydrogel film, or a glass slide having a
conductive coating. In some embodiments, a conductive substrate (e.g., any of the conductive
substrates described herein) includes one or more capture probes.

FIGS. 20A and 20B show different example analytical workflows of active capture methods using an electric field (e.g., using electrophoresis). In some examples, a biological sample **2002** (e.g., a tissue sample) can be in contact with a first substrate **2004**. In some embodiments, first substrate **2004** can have one or more coatings (e.g., any of the conductive substrates described herein) on its surface. Non-limiting examples of coatings include, nucleic acids (e.g., RNA) and conductive oxides (e.g., indium tin oxide). In some embodiments, first substrate **2004** can have a functionalization chemistry on its surface. In the examples shown in **FIG. 20A and 20B**, first substrate **2004** is overlaid with a first coating **2006**, and first coating **2006** (e.g., a conductive coating) is further overlaid with a second coating **2008**. In some embodiments, first coating **2006** is an indium tin oxide (ITO) coating. In some embodiments, second coating **2008** is a lawn of capture probes (e.g., any of the capture probes described herein). In some embodiments, a substrate can include an ITO coating. In some embodiments, a substrate can include capture probes or capture probes attached to features on the substrate.

Biological sample **2002** and second coating **2008** (e.g., a lawn of capture probes) can be in contact with a permeabilization solution **2010**. Non-limiting examples of permeabilization solutions includes enzymes (e.g., proteinase K, pepsin, and collagenase), detergents (e.g., sodium dodecyl sulfate (SDS), polyethylene glycol tert-octylphenyl ether, polysorbate 80, and polysorbate 20), ribonuclease inhibitors, buffers optimized for electrophoresis, buffers optimized for permeabilization, buffers optimized for hybridization, or combinations thereof. Permeabilization reagents can also include but are not limited to a dried permeabilization reagent, a permeabilization buffer, a buffer without a permeabilization reagent, a permeabilization gel, and a permeabilization solution. In some examples, biological samples (e.g., tissue samples) can be permeabilized first and then be subjected to electrophoresis.

FIG. 20A shows an example analytical workflow including a first step **2012** in which biological sample **2002** can be permeabilized prior to subjecting the sample **2002** to electrophoresis. Any of the permeabilization methods disclosed herein can be used during first step **2012**. Biological sample **2002** includes an analyte **2014**. In some embodiments, the analyte **2014** is a negatively charged analyte. First substrate **2004** can include a capture probe **2016** that is fixed or attached to the first substrate **2004** or attached to features (e.g., beads) **2018** on the substrate. In some embodiments, capture probe **2016** can include any of the capture probes disclosed herein. In some embodiments, capture probes are directly or

indirectly attached to the first substrate **2004**. In some embodiments, the capture probe **2016** is positively charged.

In step **2020**, after permeabilization of biological sample **2002** concludes, the sample **2002** can be subjected to electrophoresis. During electrophoresis, the biological sample **2002** is subjected to an electric field that can be generated by sandwiching biological sample **2002** between the first substrate **2004** and a second substrate **2022**, connecting each substrate to a cathode and an anode, respectively, and running an electric current through the substrates. The application of the electric field “-E” causes the analyte **2004** (e.g., a negatively charged analyte) to migrate towards the substrate **2004** and capture probe **2016** (e.g., a positively charged capture probe) in the direction of the arrows shown in **FIG. 20A**. In some embodiments, the analyte **2014** migrates towards the capture probe **2016** for a distance “h.” In some embodiments, the analyte **2014** migrates towards a capture probe **2016** through one or more permeabilized cells within the permeabilized biological sample (e.g., from an original location in a permeabilized cell to a final location in or proximal to the capture probe **2016**). Second substrate **2022** can include the first coating **2006** (e.g., a conductive coating), thereby allowing electric field “-E” to be generated.

In some embodiments, the analyte **2014** is a protein or a nucleic acid. In some embodiments, the analyte **2014** is a negatively charged protein or a nucleic acid. In some embodiments, the analyte **2014** is a positively charged protein or a nucleic acid. In some embodiments, the capture probe **2016** is a protein or a nucleic acid. In some embodiments, the capture probe **2016** is a positively charged protein or a nucleic acid. In some embodiments, the capture probe **2016** is a negatively charged protein or a nucleic acid. In some embodiments, the analyte **2014** is a negatively charged transcript. In some embodiments, the analyte **2014** is a poly(A) transcript. In some embodiments, the capture probe **2016** is attached to a feature in a feature array. In some embodiments, permeabilization reagent **2010** can be in contact with sample **2002**, first substrate **2004** second substrate **2022**, or any combination thereof.

FIG. 20B shows an example analytical workflow in which biological sample **2002** can be permeabilized and subjected to electrophoresis simultaneously. In some embodiments, simultaneous permeabilization and electrophoresis of biological sample **2002** can decrease the total duration of the analytical workflow translating into a more efficient workflow.

In some embodiments, the permeabilization reaction is conducted at a chilled temperature (e.g., about 4°C). In some embodiments, conducting the permeabilization reaction at a chilled temperature controls the enzyme activity of the permeabilization

reaction. In some embodiments, the permeabilization reaction is conducted at a chilled temperature in order to minimize drift and/or diffusion of the analyte **2014** from an original location (e.g., a location in a cell of the biological sample **2002**) until a user is ready to initiate the permeabilization reaction. In some embodiments, the permeabilization reaction is conducted at a warm temperature (e.g., a temperature ranging from about 15°C to about 37°C or more) in order to initiate and/or increase the rate of the permeabilization reaction. In some embodiments, once electrophoresis is applied and/or the permeabilization reaction is heated, the permeabilization reaction allows for analyte migration from an original location (e.g., a location in a cell of the biological sample **2002**) to the capture probe **2016** on the first substrate **2004**.

Referring generally to **FIGS. 21A-C**, example substrate configurations for use in the active migration of analytes from a first location to a second location via electrophoresis are shown. **FIG. 21A** shows an example substrate configuration for use in electrophoresis in which the first substrate **2104** and the second substrate **2122** are aligned at about 90 degrees with respect to each other. In this example, the first substrate **2104** including biological sample **2102** is placed beneath second substrate **2122**. Both the first substrate **2104** and the second substrate **2122** can be connected to electrical wires **2124** that direct an electric current from a power supply to the substrates, thereby generating an electric field between the substrates. **FIG. 21B** shows an additional example substrate configuration for use during electrophoresis in which the first substrate **2104** and the second substrate **2122** are parallel with respect to each other. In this example, the first substrate **2104** including biological sample **2102** is also placed beneath second substrate **2122**.

FIG. 21C shows yet an additional example substrate configuration for use in electrophoresis in which the second substrate **2122** and a third substrate **2126** are aligned at about 90 degrees with respect to the first substrate **2104**. Thus, in this example, a first biological sample **2102a** and a second biological sample **2102b** can be subjected to electrophoresis simultaneously. In some embodiments, 3, 4, 5, 6, 7, 8, 9, 10, or more biological samples can be placed on a same substrate and be subjected to electrophoresis simultaneously. In some embodiments, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more top substrates can be placed above a same bottom substrate containing one or more samples in order to simultaneously subject the one or more samples to electrophoresis. In some embodiments, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more top substrates can be perpendicularly placed (e.g., at about 90 degrees) above a same bottom substrate containing one or more samples in order to simultaneously subject the one or more samples to electrophoresis. In some embodiments, 2,

3, 4, 5, 6, 7, 8, 9, 10, or more top substrates can be placed at a particular angle (e.g., at about 10, 20, 30, 40, 45, 50, 60, 70, or 80 degrees) with respect to a bottom substrate containing one or more samples in order to simultaneously subject the one or more samples to electrophoresis. In some embodiments, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more top substrates can be placed in a parallel orientation above a same bottom substrate containing one or more biological samples in order to simultaneously subject the one or more samples to electrophoresis. In some embodiments, a configuration of top substrates can be arranged above a same bottom substrate containing one or more biological samples in order to simultaneously subject the one or more samples to electrophoresis. In some embodiments, a first configuration of top substrates can be arranged above a second array of bottom substrates containing one or more biological samples in order to simultaneously subject the one or more biological samples to electrophoresis. In some embodiments, simultaneously subjecting two or more biological samples on a same substrate to electrophoresis can provide the advantage of a more effective workflow. In some embodiments, one or more of the top substrates can contain the biological sample.

In some embodiments, methods utilizing an active capture method can include one or more solutions between the biological sample and the substrate (e.g., a substrate including capture probes). In some embodiments, the one or more solutions between the biological sample and the substrate including capture probes can include a permeabilization buffer (e.g., any of the permeabilization buffers described herein). In some embodiments, the one or more solutions between the biological sample and the substrate including capture probes can include an electrophoresis buffer.

In some embodiments, actively capturing analytes can include one or more porous materials between the biological sample and the substrate including capture probes. In some embodiments, the one or more porous materials between the biological sample and substrate including capture probes can include a paper or a blotting membrane. In some embodiments, the one or more porous materials between the biological sample and the substrate including capture probes can include a gel containing one or more solutions. For example, in a non-limiting way, the gel can be a SDS-PAGE gel. In some embodiments, the one or more porous materials between the biological sample and the substrate including capture probes can contain a permeabilization buffer. In some embodiments, the one or more porous materials between the biological sample and the substrate including capture probes can contain an electrophoresis buffer. In some embodiments, actively capturing analytes can include one or

more solutions and one or more porous materials between the biological sample and the substrate including capture probes.

- In some embodiments, the one or more porous materials between the biological sample and the substrate including capture probes (e.g., an array) can act as a filter to separate analytes (e.g., analytes of interest) from other molecules or analytes present in the biological sample. In some embodiments, the analytes (e.g., analytes of interest) are RNA transcripts. In some embodiments, the one or more porous materials between the biological sample and the substrate including capture probes can act as a filter to separate RNA transcripts from other molecules (e.g., analytes) such as proteins, lipids and/or other nucleic acids. In some embodiments, the one or more porous materials between the biological sample and the substrate including capture probes can act as a filter to separate the analytes and other molecules based on physicochemical properties. For example, in a non-limiting way, analytes can be separated on properties such as charge, size (e.g., length, radius of gyration, effective diameters, etc.), hydrophobicity, hydrophilicity, molecular binding (e.g., immunoaffinity), and combinations thereof. In some embodiments, the one or more porous materials between the biological sample and the substrate including capture probes can separate the analytes from other molecules to reduce non-specific binding near the capture probes and therefore improve binding between the analytes and the capture probes, thus improving subsequent assay performance.
- In some embodiments, the one or more porous materials between the biological sample and the substrate including capture probes can act as molecular sieving matrices for electrophoretic analyte separation. For example, in a non-limiting way, separation of analytes can occur based on physicochemical properties such as charge, size (e.g., length, radius of gyration, and effective diameters, etc.), electrophoretic mobility, zeta potential, isoelectric point, hydrophobicity, hydrophilicity, molecular binding (e.g., immunoaffinity), and combinations thereof. In some embodiments, the one or more porous materials between the biological sample and the substrate including capture probes can be of a uniform pore size. In some embodiments, the one or more porous materials between the biological sample and the substrate including capture probes can have discontinuities in pore sizes, as generally used in different gel electrophoresis schemes. In some embodiments, the one or more porous materials between the biological sample and the substrate including capture probes can have gradients in pore sizes. For example, the one or more porous materials (e.g., a hydrogel) can have a gradient of pore sizes such that the gradient separates the analytes as the analytes migrate to the substrate including capture probes (e.g., an array).

In some embodiments, the one or more porous materials between the biological sample and the substrate including capture probes can separate the analytes based on length. For example, shorter analytes will have a higher electrophoretic mobility, and therefore migrate faster towards the capture probes relative to longer analytes in an electrophoretic setup. In some embodiments, the one or more porous materials between the biological sample and the substrate including capture probes separate the analytes based on length, such that only shorter analytes can migrate through the one or more porous materials to reach the capture probes, while longer analytes cannot reach the capture probes.

In some embodiments, specific subsets of analytes (e.g., a subset of transcripts) can be captured by applying an electrophoretic field for a certain amount of time. In some embodiments, specific subsets of analytes (e.g., a subset of transcripts) can be captured by selecting different porous materials (e.g., porous materials with different compositions) between the biological sample and the substrate including capture probes. In some embodiments, specific subsets of analytes (e.g., a subset of transcripts) can be captured by applying an electrophoretic field for a certain amount of time and selecting different porous materials between the biological sample and the substrate including capture probes (e.g., an array).

In some embodiments, the one or more porous materials between the biological sample and the substrate including capture probes can have discontinuities in pore sizes that can cause an increase in the concentration of the migrating analytes (e.g., “stacking”). For example, the one or more porous materials (e.g., a hydrogel) between the biological sample and the substrate including capture probes can have discontinuities in pore sizes that can cause an increase in the concentration of the analytes near the capture probes resulting in favorable binding kinetics and increased sensitivity. In some embodiments, the one or more porous materials between the biological sample and the substrate including capture probes can have discontinuities in pore sizes that enhance the separation between migrating analytes of different sizes and/or lengths. In some embodiments, the one or more porous materials between the biological sample and the substrate including capture probes can include a first porous material and a second porous material, with the first porous material having a larger pore size than the second porous material. In some embodiments, the first porous material is located on the surface, or near the surface, of the biological sample. In some embodiments, the second porous material (e.g., second porous material with a smaller pore size than the first porous material) can be placed on the surface, or near the surface, of the first porous material. In some embodiments, as analytes migrate (e.g., migrate via electrophoresis) from the

biological sample through the first porous material and the second porous material sequentially, the migrating analytes can collect (e.g., “stack”) at the interface between the first porous material and the second porous material.

In some embodiments, the one or more porous materials between the biological sample and the substrate including capture probes can include a gradient in pore sizes for continuous stacking as analytes migrate through decreasing pore sizes (e.g., decreasing pore diameter). In some embodiments, the one or more porous materials between the biological sample and the substrate including capture probes can include a gradient in pore sizes such that the pores decrease in diameter as the analytes migrate from the biological sample to the substrate including capture probes. In some embodiments, the pore size gradient can increase the resolution among analytes of different sizes. In some embodiments, the pore size gradient can increase the concentration of the analytes near the capture probes. In some embodiments, the pore size gradient can continuously reduce the speed at which the analytes migrate and collect (e.g., “stack”) as the analytes migrate through the gradient of decreasing pore sizes (e.g., decreasing pore diameter).

In some embodiments, the one or more porous materials between the biological sample and the substrate including capture probes can include a gradient gel for continuous stacking as analytes migrate through decreasing pore sizes (e.g., decreasing pore diameter) of the gradient gel. In some embodiments, the gradient gel can have pores with a decreasing diameter as the analytes migrate toward the capture probes. In some embodiments, the gradient gel can increase the separation resolution among analytes of different sizes. In some embodiments, the gradient gel can increase the concentration of analytes near the capture probes. In some embodiments, the gradient gel can continuously reduce the speed at which analytes migrate and collect (e.g., “stack”) as the analytes migrate through the gradient gel of decreasing pore sizes (e.g., decreasing in diameter).

In some embodiments, a biological sample can be placed in a first substrate holder (e.g., a substrate holder described herein). In some embodiments, a spatially-barcoded capture probe array (e.g., capture probes, barcoded array) can be placed on a second substrate holder (e.g., a substrate holder described herein). In some embodiments, a biological sample can be placed in a first substrate holder that also contains capture probes. In some embodiments, the first substrate holder, the second substrate holder, or both can be conductive (e.g. any of the conductive substrates described herein). In some embodiments, the first substrate holder including the biological sample, the second substrate holder including capture probes, or both, can be contacted with permeabilization reagents (e.g., a permeabilization buffer) and

analytes can be migrated from the biological sample toward the barcoded array using an electric field.

In some embodiments, electrophoresis can be applied to a biological sample on a barcoded array while in contact with a permeabilization buffer. In some embodiments, electrophoresis can be applied to a biological sample on a barcoded array while in contact with an electrophoresis buffer (e.g. a buffer that lacks permeabilization reagents). In some embodiments, the permeabilization buffer can be replaced with an electrophoresis buffer after a desired amount of time. In some embodiments, electrophoresis can be applied simultaneously with the permeabilization buffer or electrophoresis buffer. In some embodiments, electrophoresis can be applied after a desired amount of time of contact between the biological sample and the permeabilization buffer or electrophoresis buffer.

In some embodiments, the biological sample can be placed on a substrate (e.g., a porous membrane, a hydrogel, paper, etc.). In some embodiments, the biological sample placed on the substrate can have a gap (e.g., a space) between the substrate and the substrate holder (e.g., conductive substrate holder). In some embodiments, the barcoded array can be placed on a substrate (e.g., a porous membrane, a hydrogel, paper, etc.). In some embodiments, the barcoded array can have a gap between the substrate and substrate holder (e.g., conductive substrate holder). In some embodiments, the barcoded array can be placed in direct proximity to the biological sample or at a desired distance from the biological sample. In some embodiments, a buffer reservoir can be used between the substrate holder (e.g., conductive substrate holder) and the barcoded array, the biological sample, or both. This setup allows the analytes to be migrated to a barcoded array while not in proximity with the electrodes (e.g. conductive substrate holder), thus resulting in more stable electrophoresis.

In some embodiments, a combination of at least two buffers with different ionic compositions can be used to differentially migrate analytes based on their ionic mobility (e.g., isotachopheresis (ITP)). For example, using two or more buffers with different ionic compositions can increase the concentration of analytes prior to contact with a barcoded array. Isotachopheresis includes at least two buffers that contain a common counter-ion (e.g., ions that have different charge sign than the analytes) and different co-ions (e.g., ions that have the same charge sign as the analytes) (Smejkal P., et al., Microfluidic isotachopheresis: A review, *Electrophoresis*, 34.11 1493-1509, (2013) which is incorporated herein by reference in its entirety). In some embodiments, one buffer can contain a co-ion with a higher ionic mobility (e.g. speed at which they travel through solution in an electric field) than the analytes (e.g., the “leading” buffer). In some embodiments, a second buffer can contain a co-

ion with a lower ionic mobility than the analytes (e.g., the “trailing” buffer). In some embodiments, a third buffer can contain a co-ion with an ionic mobility that is between the electric mobility of the analytes. In some embodiments, a biological sample can be placed on a first substrate holder (e.g., a conductive substrate holder) and the barcoded array can be placed on a second substrate holder (e.g., a second conductive substrate holder) and contacted with a permeabilization buffer and the analytes can be migrated away from the biological sample and toward the barcoded array using an electric field. As the electric field is applied to the biological sample the analytes can be concentrated in the buffer as they are migrated toward the capture probes. In some embodiments, isotachopheresis can be used with gel-based separations (e.g., any of the gel-based separations described herein).

In some embodiments, a permeabilization buffer can be applied to a region of interest (e.g., region of interest as described herein) in a biological sample. In some embodiments, permeabilization reagents (e.g. a hydrogel containing permeabilization reagents) can be applied to a region of interest in a biological sample. For example, a region of interest can be a region that is smaller in area relative to the overall area of the biological sample. In some embodiments, the permeabilization buffer or permeabilization reagents can be contacted with the biological sample and a substrate including capture probes (e.g., an array). In some embodiments, the biological sample can have more than one region of interest (e.g. two, three). In some embodiments, the biological sample, the substrate including capture probes, or both, can be placed in a conductive substrate holder. In some embodiments, analytes can be released from the region(s) of interest and migrated from the biological sample toward the capture probes with an electric field.

In some embodiments, electrophoretic transfer of analytes can be performed while retaining the relative spatial locations of analytes in a biological sample while minimizing passive diffusion of an analyte away from its location in a biological sample. In some embodiments, an analyte captured by a capture probe (e.g., capture probes on a substrate) retains the spatial location of the analyte present in the biological sample from which it was obtained (e.g., the spatial location of the analyte that is captured by a capture probe on a substrate when the analyte is actively migrated to the capture probe by electrophoretic transfer can be more precise or representative of the spatial location of the analyte in the biological sample than when the analyte is not actively migrated to the capture probe). In some embodiments, electrophoretic transport and binding process is described by the Damköhler number (Da), which is a ratio of reaction and mass transport rates. The fraction of analytes bound and the shape of the biological sample will depend on the parameters in the

Da. There parameters include electromigration velocity U_e (depending on analyte electrophoretic mobility μ_e and electric field strength E), density of capture probes (e.g., barcoded oligonucleotides) p_0 , the binding rate between probes (e.g., barcoded oligonucleotides) and analytes k_{on} , and capture area thickness L .

$$5 \quad Da \sim \frac{k_{on} p_0 L}{\mu_e E}$$

Fast migration (e.g., electromigration) can reduce assay time and can minimize molecular diffusion of analytes.

In some embodiments, electrophoretic transfer of analytes can be performed while retaining the relative spatial alignment of the analytes in the sample. As such, an analyte captured by the capture probes (e.g., capture probes on a substrate) retains the spatial information of the cell or the biological sample from which it was obtained. Applying an electrophoretic field to analytes can also result in an increase in temperature (e.g., heat). In some embodiments, the increased temperature (e.g., heat) can facilitate the migration of the analytes towards a capture probe.

15 In some examples, a spatially-addressable microelectrode array is used for spatially-constrained capture of at least one charged analyte of interest by a capture probe. For example, a spatially-addressable microelectrode array can allow for discrete (e.g., localized) application of an electric field rather than a uniform electric field. The spatially-addressable microelectrode array can be independently addressable. In some embodiments, the electric field can be applied to one or more regions of interest in a biological sample. The electrodes may be adjacent to each other or distant from each other. The microelectrode array can be configured to include a high density of discrete sites having a small area for applying an electric field to promote the migration of charged analyte(s) of interest. For example, electrophoretic capture can be performed on a region of interest using a spatially-addressable
25 microelectrode array.

A high density of discrete sites on a microelectrode array can be used. The surface can include any suitable density of discrete sites (e.g., a density suitable for processing the sample on the conductive substrate in a given amount of time). In one embodiment, the surface has a density of discrete sites greater than or equal to about 500 sites per 1 mm². In some
30 embodiments, the surface has a density of discrete sites of about 100, about 200, about 300, about 400, about 500, about 600, about 700, about 800, about 900, about 1,000, about 2,000, about 3,000, about 4,000, about 5,000, about 6,000, about 7,000, about 8,000, about 9,000,

about 10,000, about 20,000, about 40,000, about 60,000, about 80,000, about 100,000, or about 500,000 sites per 1 mm². In some embodiments, the surface has a density of discrete sites of at least about 200, at least about 300, at least about 400, at least about 500, at least about 600, at least about 700, at least about 800, at least about 900, at least about 1,000, at least about 2,000, at least about 3,000, at least about 4,000, at least about 5,000, at least about 6,000, at least about 7,000, at least about 8,000, at least about 9,000, at least about 10,000, at least about 20,000, at least about 40,000, at least about 60,000, at least about 80,000, at least about 100,000, or at least about 500,000 sites per 1 mm².

Schematics illustrating an electrophoretic transfer system configured to direct nucleic acid analytes (e.g., mRNA transcripts) toward a spatially-barcoded capture probe array are shown in **FIG. 22A** and **FIG. 22B**. In this exemplary configuration of an electrophoretic system, a sample **2202** is sandwiched between the cathode **2201** and the spatially-barcoded capture probe array **2204, 2205**, and the spatially-barcoded capture probe array **2204, 2205** is sandwiched between the sample **2202** and the anode **2203**, such that the sample **2202** is in contact with the spatially-barcoded capture probes **2207**. When an electric field is applied to the electrophoretic transfer system, negatively charged nucleic acid analytes **2206** will be pulled toward the positively charged anode **2203** and into the spatially-barcoded array **2204, 2205** containing the spatially-barcoded capture probes **2207**. The spatially-barcoded capture probes **2207** interact with the nucleic acid analytes (e.g., mRNA transcripts hybridize to spatially-barcoded nucleic acid capture probes forming DNA/RNA hybrids) **2207**, making the analyte capture more efficient. The electrophoretic system set-up may change depending on the target analyte. For example, proteins may be positive, negative, neutral, or polar depending on the protein as well as other factors (e.g., isoelectric point, solubility, etc.). The skilled practitioner has the knowledge and experience to arrange the electrophoretic transfer system to facilitate capture of a particular target analyte.

FIG. 23 is an illustration showing an exemplary workflow protocol utilizing an electrophoretic transfer system. In the example, **Panel A** depicts a flexible spatially-barcoded feature array being contacted with a sample. The sample can be a flexible array, wherein the array is immobilized on a hydrogel, membrane, or other flexible substrate. **Panel B** depicts contact of the array with the sample and imaging of the array-sample assembly. The image of the sample/array assembly can be used to verify sample placement, choose a region of interest, or any other reason for imaging a sample on an array as described herein. **Panel C** depicts application of an electric field using an electrophoretic transfer system to aid in efficient capture of a target analyte. Here, negatively charged mRNA target analytes migrate

toward the positively charged anode. **Panel D** depicts application of reverse transcription reagents and first strand cDNA synthesis of the captured target analytes. **Panel E** depicts array removal and preparation for library construction (**Panel F**) and next-generation sequencing (**Panel G**).

5

Spatial analysis methodologies and compositions described herein can provide a vast amount of analyte and/or expression data for a variety of analytes within a biological sample at high spatial resolution, while retaining native spatial context. Spatial analysis methods and compositions can include, e.g., the use of a capture probe including a spatial barcode (e.g., a
10 nucleic acid sequence that provides information as to the location or position of an analyte within a cell or a tissue sample (e.g., mammalian cell or a mammalian tissue sample) and a capture domain that is capable of binding to an analyte (e.g., a protein and/or a nucleic acid) produced by and/or present in a cell. Spatial analysis methods and compositions can also include the use of a capture probe having a capture domain that captures an intermediate
15 agent for indirect detection of an analyte. For example, the intermediate agent can include a nucleic acid sequence (e.g., a barcode) associated with the intermediate agent. Detection of the intermediate agent is therefore indicative of the analyte in the cell or tissue sample.

Non-limiting aspects of spatial analysis methodologies and compositions are described in U.S. Patent Nos. 10,774,374, 10,724,078, 10,480,022, 10,059,990, 10,041,949,
20 10,002,316, 9,879,313, 9,783,841, 9,727,810, 9,593,365, 8,951,726, 8,604,182, 7,709,198, U.S. Patent Application Publication Nos. 2020/239946, 2020/080136, 2020/0277663, 2020/024641, 2019/330617, 2019/264268, 2020/256867, 2020/224244, 2019/194709, 2019/161796, 2019/085383, 2019/055594, 2018/216161, 2018/051322, 2018/0245142, 2017/241911, 2017/089811, 2017/067096, 2017/029875, 2017/0016053, 2016/108458,
25 2015/000854, 2013/171621, WO 2018/091676, WO 2020/176788, Rodriques et al., Science 363(6434):1463-1467, 2019; Lee et al., Nat. Protoc. 10(3):442-458, 2015; Trejo et al., PLoS ONE 14(2):e0212031, 2019; Chen et al., Science 348(6233):aaa6090, 2015; Gao et al., BMC Biol. 15:50, 2017; and Gupta et al., Nature Biotechnol. 36:1197-1202, 2018; the Visium Spatial Gene Expression Reagent Kits User Guide (e.g., Rev C, dated June 2020), and/or the
30 Visium Spatial Tissue Optimization Reagent Kits User Guide (e.g., Rev C, dated July 2020), both of which are available at the 10x Genomics Support Documentation website, and can be used herein in any combination. Further non-limiting aspects of spatial analysis methodologies and compositions are described herein.

Some general terminology that may be used in this disclosure can be found in Section (I)(b) of WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663.

Typically, a “barcode” is a label, or identifier, that conveys or is capable of conveying information (e.g., information about an analyte in a sample, a bead, and/or a capture probe).

- 5 A barcode can be part of an analyte, or independent of an analyte. A barcode can be attached to an analyte. A particular barcode can be unique relative to other barcodes. For the purpose of this disclosure, an “analyte” can include any biological substance, structure, moiety, or component to be analyzed. The term “target” can similarly refer to an analyte of interest.

- 10 Analytes can be broadly classified into one of two groups: nucleic acid analytes, and non-nucleic acid analytes. Examples of non-nucleic acid analytes include, but are not limited to, lipids, carbohydrates, peptides, proteins, glycoproteins (N-linked or O-linked), lipoproteins, phosphoproteins, specific phosphorylated or acetylated variants of proteins, amidation variants of proteins, hydroxylation variants of proteins, methylation variants of proteins, ubiquitylation variants of proteins, sulfation variants of proteins, viral proteins (e.g.,
15 viral capsid, viral envelope, viral coat, viral accessory, viral glycoproteins, viral spike, etc.), extracellular and intracellular proteins, antibodies, and antigen binding fragments. In some embodiments, the analyte(s) can be localized to subcellular location(s), including, for example, organelles, e.g., mitochondria, Golgi apparatus, endoplasmic reticulum, chloroplasts, endocytic vesicles, exocytic vesicles, vacuoles, lysosomes, etc. In some
20 embodiments, analyte(s) can be peptides or proteins, including without limitation antibodies and enzymes. Additional examples of analytes can be found in Section (I)(c) of WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663. In some embodiments, an analyte can be detected indirectly, such as through detection of an intermediate agent, for example, a ligation product or an analyte capture agent (e.g., an
25 oligonucleotide-conjugated antibody), such as those described herein.

- A “biological sample” is typically obtained from the subject for analysis using any of a variety of techniques including, but not limited to, biopsy, surgery, and laser capture microscopy (LCM), and generally includes cells and/or other biological material from the subject. In some embodiments, a biological sample can be a tissue section. In some
30 embodiments, a biological sample can be a fixed and/or stained biological sample (e.g., a fixed and/or stained tissue section). Non-limiting examples of stains include histological stains (e.g., hematoxylin and/or eosin) and immunological stains (e.g., fluorescent stains). In some embodiments, a biological sample (e.g., a fixed and/or stained biological sample) can

be imaged. Biological samples are also described in Section (I)(d) of WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663.

5 In some embodiments, a biological sample is permeabilized with one or more permeabilization reagents. For example, permeabilization of a biological sample can facilitate analyte capture. Exemplary permeabilization agents and conditions are described in Section (I)(d)(ii)(13) or the Exemplary Embodiments Section of WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663.

10 Array-based spatial analysis methods involve the transfer of one or more analytes from a biological sample to an array of features on a substrate, where each feature is associated with a unique spatial location on the array. Subsequent analysis of the transferred analytes includes determining the identity of the analytes and the spatial location of the analytes within the biological sample. The spatial location of an analyte within the biological sample is determined based on the feature to which the analyte is bound (e.g., directly or indirectly) on the array, and the feature's relative spatial location within the array.

15 A "capture probe" refers to any molecule capable of capturing (directly or indirectly) and/or labelling an analyte (e.g., an analyte of interest) in a biological sample. In some embodiments, the capture probe is a nucleic acid or a polypeptide. In some embodiments, the capture probe includes a barcode (e.g., a spatial barcode and/or a unique molecular identifier (UMI)) and a capture domain). In some embodiments, a capture probe can include a cleavage domain and/or a functional domain (e.g., a primer-binding site, such as for next-generation sequencing (NGS)). See, e.g., Section (II)(b) (e.g., subsections (i)-(vi)) of WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663. Generation of capture probes can be achieved by any appropriate method, including those described in Section (II)(d)(ii) of WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663.

25 In some embodiments, more than one analyte type (e.g., nucleic acids and proteins) from a biological sample can be detected (e.g., simultaneously or sequentially) using any appropriate multiplexing technique, such as those described in Section (IV) of WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663.

30 In some embodiments, detection of one or more analytes (e.g., protein analytes) can be performed using one or more analyte capture agents. As used herein, an "analyte capture agent" refers to an agent that interacts with an analyte (e.g., an analyte in a biological sample) and with a capture probe (e.g., a capture probe attached to a substrate or a feature) to identify the analyte. In some embodiments, the analyte capture agent includes: (i) an analyte binding

moiety (e.g., that binds to an analyte), for example, an antibody or antigen-binding fragment thereof; (ii) analyte binding moiety barcode; and (iii) an analyte capture sequence. As used herein, the term “analyte binding moiety barcode” refers to a barcode that is associated with or otherwise identifies the analyte binding moiety. As used herein, the term “analyte capture
5 sequence” refers to a region or moiety configured to hybridize to, bind to, couple to, or otherwise interact with a capture domain of a capture probe. In some cases, an analyte binding moiety barcode (or portion thereof) may be able to be removed (e.g., cleaved) from the analyte capture agent. Additional description of analyte capture agents can be found in
10 Section (II)(b)(ix) of WO 2020/176788 and/or Section (II)(b)(viii) U.S. Patent Application Publication No. 2020/0277663.

There are at least two methods to associate a spatial barcode with one or more neighboring cells, such that the spatial barcode identifies the one or more cells, and/or contents of the one or more cells, as associated with a particular spatial location. One method is to promote analytes or analyte proxies (e.g., intermediate agents) out of a cell and towards
15 a spatially-barcoded array (e.g., including spatially-barcoded capture probes). Another method is to cleave spatially-barcoded capture probes from an array and promote the spatially-barcoded capture probes towards and/or into or onto the biological sample.

In some cases, capture probes may be configured to prime, replicate, and consequently yield optionally barcoded extension products from a template (e.g., a DNA or
20 RNA template, such as an analyte or an intermediate agent (e.g., a ligation product or an analyte capture agent), or a portion thereof), or derivatives thereof (see, e.g., Section (II)(b)(vii) of WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663 regarding extended capture probes). In some cases, capture probes may be configured to form ligation products with a template (e.g., a DNA or RNA template, such as
25 an analyte or an intermediate agent, or portion thereof), thereby creating ligations products that serve as proxies for a template.

As used herein, an “extended capture probe” refers to a capture probe having additional nucleotides added to the terminus (e.g., 3’ or 5’ end) of the capture probe thereby extending the overall length of the capture probe. For example, an “extended 3’ end”
30 indicates additional nucleotides were added to the most 3’ nucleotide of the capture probe to extend the length of the capture probe, for example, by polymerization reactions used to extend nucleic acid molecules including templated polymerization catalyzed by a polymerase (e.g., a DNA polymerase or a reverse transcriptase). In some embodiments, extending the capture probe includes adding to a 3’ end of a capture probe a nucleic acid sequence that is

complementary to a nucleic acid sequence of an analyte or intermediate agent specifically bound to the capture domain of the capture probe. In some embodiments, the capture probe is extended using reverse transcription. In some embodiments, the capture probe is extended using one or more DNA polymerases. The extended capture probes include the sequence of the capture probe and the sequence of the spatial barcode of the capture probe.

In some embodiments, extended capture probes are amplified (e.g., in bulk solution or on the array) to yield quantities that are sufficient for downstream analysis, e.g., via DNA sequencing. In some embodiments, extended capture probes (e.g., DNA molecules) act as templates for an amplification reaction (e.g., a polymerase chain reaction).

Additional variants of spatial analysis methods, including in some embodiments, an imaging step, are described in Section (II)(a) of WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663. Analysis of captured analytes (and/or intermediate agents or portions thereof), for example, including sample removal, extension of capture probes, sequencing (e.g., of a cleaved extended capture probe and/or a cDNA molecule complementary to an extended capture probe), sequencing on the array (e.g., using, for example, in situ hybridization or in situ ligation approaches), temporal analysis, and/or proximity capture, is described in Section (II)(g) of WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663. Some quality control measures are described in Section (II)(h) of WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663.

Spatial information can provide information of biological and/or medical importance. For example, the methods and compositions described herein can allow for: identification of one or more biomarkers (e.g., diagnostic, prognostic, and/or for determination of efficacy of a treatment) of a disease or disorder; identification of a candidate drug target for treatment of a disease or disorder; identification (e.g., diagnosis) of a subject as having a disease or disorder; identification of stage and/or prognosis of a disease or disorder in a subject; identification of a subject as having an increased likelihood of developing a disease or disorder; monitoring of progression of a disease or disorder in a subject; determination of efficacy of a treatment of a disease or disorder in a subject; identification of a patient subpopulation for which a treatment is effective for a disease or disorder; modification of a treatment of a subject with a disease or disorder; selection of a subject for participation in a clinical trial; and/or selection of a treatment for a subject with a disease or disorder.

Spatial information can provide information of biological importance. For example, the methods and compositions described herein can allow for: identification of transcriptome

and/or proteome expression profiles (e.g., in healthy and/or diseased tissue); identification of multiple analyte types in close proximity (e.g., nearest neighbor analysis); determination of up- and/or down-regulated genes and/or proteins in diseased tissue; characterization of tumor microenvironments; characterization of tumor immune responses; characterization of cells
5 types and their co-localization in tissue; and identification of genetic variants within tissues (e.g., based on gene and/or protein expression profiles associated with specific disease or disorder biomarkers).

Typically, for spatial array-based methods, a substrate functions as a support for direct or indirect attachment of capture probes to features of the array. A “feature” is an entity that
10 acts as a support or repository for various molecular entities used in spatial analysis. In some embodiments, some or all of the features in an array are functionalized for analyte capture. Exemplary substrates are described in Section (II)(c) of WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663. Exemplary features and geometric attributes of an array can be found in Sections (II)(d)(i), (II)(d)(iii), and (II)(d)(iv) of WO 2020/176788
15 and/or U.S. Patent Application Publication No. 2020/0277663.

Generally, analytes and/or intermediate agents (or portions thereof) can be captured when contacting a biological sample with a substrate including capture probes (e.g., a substrate with capture probes embedded, spotted, printed, fabricated on the substrate, or a substrate with features (e.g., beads, wells) comprising capture probes). As used herein,
20 “contact,” “contacted,” and/or “contacting,” a biological sample with a substrate refers to any contact (e.g., direct or indirect) such that capture probes can interact (e.g., bind covalently or non-covalently (e.g., hybridize)) with analytes from the biological sample. Capture can be achieved actively (e.g., using electrophoresis) or passively (e.g., using diffusion). Analyte capture is further described in Section (II)(e) of WO 2020/176788 and/or U.S. Patent
25 Application Publication No. 2020/0277663.

In some cases, spatial analysis can be performed by attaching and/or introducing a molecule (e.g., a peptide, a lipid, or a nucleic acid molecule) having a barcode (e.g., a spatial barcode) to a biological sample (e.g., to a cell in a biological sample). In some embodiments, a plurality of molecules (e.g., a plurality of nucleic acid molecules) having a plurality of
30 barcodes (e.g., a plurality of spatial barcodes) are introduced to a biological sample (e.g., to a plurality of cells in a biological sample) for use in spatial analysis. In some embodiments, after attaching and/or introducing a molecule having a barcode to a biological sample, the biological sample can be physically separated (e.g., dissociated) into single cells or cell

groups for analysis. Some such methods of spatial analysis are described in Section (III) of WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663.

In some cases, spatial analysis can be performed by detecting multiple oligonucleotides that hybridize to an analyte. In some instances, for example, spatial analysis
5 can be performed using RNA-templated ligation (RTL). Methods of RTL have been described previously. See, e.g., Credle *et al.*, *Nucleic Acids Res.* 2017 Aug 21;45(14):e128. Typically, RTL includes hybridization of two oligonucleotides to adjacent sequences on an analyte (e.g., an RNA molecule, such as an mRNA molecule). In some instances, the oligonucleotides are DNA molecules. In some instances, one of the oligonucleotides includes
10 at least two ribonucleic acid bases at the 3' end and/or the other oligonucleotide includes a phosphorylated nucleotide at the 5' end. In some instances, one of the two oligonucleotides includes a capture domain (e.g., a poly(A) sequence, a non-homopolymeric sequence). After hybridization to the analyte, a ligase (e.g., SplintR ligase) ligates the two oligonucleotides together, creating a ligation product. In some instances, the two oligonucleotides hybridize to
15 sequences that are not adjacent to one another. For example, hybridization of the two oligonucleotides creates a gap between the hybridized oligonucleotides. In some instances, a polymerase (e.g., a DNA polymerase) can extend one of the oligonucleotides prior to ligation. After ligation, the ligation product is released from the analyte. In some instances, the ligation product is released using an endonuclease (e.g., RNase H). The released ligation
20 product can then be captured by capture probes (e.g., instead of direct capture of an analyte) on an array, optionally amplified, and sequenced, thus determining the location and optionally the abundance of the analyte in the biological sample.

During analysis of spatial information, sequence information for a spatial barcode associated with an analyte is obtained, and the sequence information can be used to provide
25 information about the spatial distribution of the analyte in the biological sample. Various methods can be used to obtain the spatial information. In some embodiments, specific capture probes and the analytes they capture are associated with specific locations in an array of features on a substrate. For example, specific spatial barcodes can be associated with specific array locations prior to array fabrication, and the sequences of the spatial barcodes
30 can be stored (e.g., in a database) along with specific array location information, so that each spatial barcode uniquely maps to a particular array location.

Alternatively, specific spatial barcodes can be deposited at predetermined locations in an array of features during fabrication such that at each location, only one type of spatial barcode is present so that spatial barcodes are uniquely associated with a single feature of the

array. Where necessary, the arrays can be decoded using any of the methods described herein so that spatial barcodes are uniquely associated with array feature locations, and this mapping can be stored as described above.

When sequence information is obtained for capture probes and/or analytes during
5 analysis of spatial information, the locations of the capture probes and/or analytes can be determined by referring to the stored information that uniquely associates each spatial barcode with an array feature location. In this manner, specific capture probes and captured analytes are associated with specific locations in the array of features. Each array feature location represents a position relative to a coordinate reference point (e.g., an array location, a
10 fiducial marker) for the array. Accordingly, each feature location has an “address” or location in the coordinate space of the array.

Some exemplary spatial analysis workflows are described in the Exemplary Embodiments section of WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663. See, for example, the Exemplary embodiment starting with “In some non-
15 limiting examples of the workflows described herein, the sample can be immersed...” of WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663. See also, e.g., the Visium Spatial Gene Expression Reagent Kits User Guide (e.g., Rev C, dated June 2020), and/or the Visium Spatial Tissue Optimization Reagent Kits User Guide (e.g., Rev C, dated July 2020).

20 In some embodiments, spatial analysis can be performed using dedicated hardware and/or software, such as any of the systems described in Sections (II)(e)(ii) and/or (V) of WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663, or any of one or more of the devices or methods described in Sections *Control Slide for Imaging, Methods of Using Control Slides and Substrates for, Systems of Using Control Slides and Substrates for*
25 *Imaging, and/or Sample and Array Alignment Devices and Methods, Informational labels of* WO 2020/123320.

Suitable systems for performing spatial analysis can include components such as a chamber (e.g., a flow cell or sealable, fluid-tight chamber) for containing a biological sample. The biological sample can be mounted for example, in a biological sample holder. One or
30 more fluid chambers can be connected to the chamber and/or the sample holder via fluid conduits, and fluids can be delivered into the chamber and/or sample holder via fluidic pumps, vacuum sources, or other devices coupled to the fluid conduits that create a pressure gradient to drive fluid flow. One or more valves can also be connected to fluid conduits to regulate the flow of reagents from reservoirs to the chamber and/or sample holder.

The systems can optionally include a control unit that includes one or more electronic processors, an input interface, an output interface (such as a display), and a storage unit (e.g., a solid state storage medium such as, but not limited to, a magnetic, optical, or other solid state, persistent, writeable and/or re-writeable storage medium). The control unit can
5 optionally be connected to one or more remote devices via a network. The control unit (and components thereof) can generally perform any of the steps and functions described herein. Where the system is connected to a remote device, the remote device (or devices) can perform any of the steps or features described herein. The systems can optionally include one or more detectors (e.g., CCD, CMOS) used to capture images. The systems can also
10 optionally include one or more light sources (e.g., LED-based, diode-based, lasers) for illuminating a sample, a substrate with features, analytes from a biological sample captured on a substrate, and various control and calibration media.

The systems can optionally include software instructions encoded and/or implemented in one or more of tangible storage media and hardware components such as application
15 specific integrated circuits. The software instructions, when executed by a control unit (and in particular, an electronic processor) or an integrated circuit, can cause the control unit, integrated circuit, or other component executing the software instructions to perform any of the method steps or functions described herein.

In some cases, the systems described herein can detect (e.g., register an image) the
20 biological sample on the array. Exemplary methods to detect the biological sample on an array are described in PCT Application No. 2020/061064 and/or U.S. Patent Application Serial No. 16/951,854.

Prior to transferring analytes from the biological sample to the array of features on the substrate, the biological sample can be aligned with the array. Alignment of a biological
25 sample and an array of features including capture probes can facilitate spatial analysis, which can be used to detect differences in analyte presence and/or level within different positions in the biological sample, for example, to generate a three-dimensional map of the analyte presence and/or level. Exemplary methods to generate a two- and/or three-dimensional map of the analyte presence and/or level are described in PCT Application No. 2020/053655 and
30 spatial analysis methods are generally described in WO 2020/061108 and/or U.S. Patent Application Serial No. 16/951,864.

In some cases, a map of analyte presence and/or level can be aligned to an image of a biological sample using one or more fiducial markers, e.g., objects placed in the field of view of an imaging system which appear in the image produced, as described in the *Substrate*

Attributes Section, *Control Slide for Imaging* Section of WO 2020/123320, PCT Application No. 2020/061066, and/or U.S. Patent Application Serial No. 16/951,843. Fiducial markers can be used as a point of reference or measurement scale for alignment (e.g., to align a sample and an array, to align two substrates, to determine a location of a sample or array on a substrate relative to a fiducial marker) and/or for quantitative measurements of sizes and/or distances.

While this specification contains many specific implementation details, these should not be construed as limitations on the scope of any inventions or of what may be claimed, but rather as descriptions of features specific to particular implementations of particular inventions. Certain features that are described in this specification in the context of separate implementations can also be implemented in combination in a single implementation. Conversely, various features that are described in the context of a single implementation can also be implemented in multiple implementations separately or in any suitable sub-combination. Moreover, although features may be described above as acting in certain combinations and even initially claimed as such, one or more features from a claimed combination can in some cases be excised from the combination, and the claimed combination may be directed to a sub-combination or variation of a sub-combination.

Similarly, while operations are depicted in the drawings in a particular order, this should not be understood as requiring that such operations be performed in the particular order shown or in sequential order, or that all illustrated operations be performed, to achieve desirable results. In certain circumstances, multitasking and parallel processing may be advantageous. Moreover, the separation of various system components in the implementations described above should not be understood as requiring such separation in all implementations, and it should be understood that the described program components and systems can generally be integrated together in a single software product or packaged into multiple software products.

Thus, particular implementations of the subject matter have been described. Other implementations are within the scope of the following claims. In some cases, the actions recited in the claims can be performed in a different order and still achieve desirable results. In addition, the processes depicted in the accompanying figures do not necessarily require the particular order shown, or sequential order, to achieve desirable results. In certain implementations, multitasking and parallel processing may be advantageous.