by the ability to isolate, tag, and prepare nucleic acids for sequencing. Likewise, there are limitations associated with the need to transfer prepared libraries to separate instruments, systems, or locations in order to carry out sequencing steps. This provides a practical limitation on sequencing throughput of approximately 50,000 cells per sequencing run, which, given the vastly larger number of cells present in a diagnostically relevant sample of a tissue, secretion, excretion, or exudate, or a microbiome sample, places strict limits on the sensitivity and utility of these assays. A level of addressability may be achieved simply by physically isolating samples and performing isolations, library preparation, and sequencing reactions in a known sequence. However, this process is labor intensive and time consuming, making it impractical as a means of screening large numbers of patients or as a means of deploying systematic screening methods.

[0005] Accordingly, there is a need for compositions and methods that can increase the accuracy and throughput of cellularly addressable sequencing methods, as well as cellularly or spatially addressable sequencing methods that obviate the aforementioned limitations of existing technologies.

SUMMARY

[0005a] In one aspect, provided herein is a method for analyzing a target nucleic acid sequence in situ, the method comprising: (a) providing a cell or a tissue comprising a plurality of nucleic acid molecules, wherein said plurality of nucleic acid molecules comprises said target nucleic acid sequence; (b) contacting a subset of said plurality of nucleic acid molecules with a detectable nucleotide conjugate under conditions suitable to form a binding complex between (i) at least two nucleotide moieties of said detectable nucleotide conjugate and (ii) at least one nucleotide of each of at least two of said target nucleic acid sequence of said plurality of nucleic acid molecules within said cell or said tissue, wherein said subset of said plurality of nucleic acid molecules is primed; (c) detecting said binding complex; and (d) performing (b) to (c) for at least two other nucleotides of said at least two of said target nucleic acid sequence of said plurality of nucleic acid molecules, thereby identifying said target nucleic acid sequence.

[0006] Examples disclosed herein provide methods for analyzing a biological sample comprising: (a) detecting a multivalent binding complex formed in a presence of a biological sample or derivative thereof between a target nucleic acid sequence of a target nucleic acid molecule or derivative thereof and a detectable polymer-nucleotide conjugate; and (b) determining an origin of said target nucleic acid sequence in said biological sample or derivative thereof. In some examples, determining in (b) is performed at least in part by analyzing a relative three-dimensional relationship between said target nucleic acid sequence and a point of reference of said biological sample or derivative thereof. In some

examples, methods further comprise contacting said biological sample or derivative thereof with said detectable polymer-nucleotide conjugate in said presence of the biological sample. In some examples, methods further comprise coupling at least a portion of said target nucleic acid sequence to a capture oligonucleotide molecule coupled to a surface of a substrate. In some examples, said surface has a water contact angle of less than or equal to 45 degrees. In some examples, coupling comprises hybridizing in a presence of a hybridization buffer comprising: (i) a first polar aprotic solvent having a dielectric constant that is no greater than 40 and having a polarity index of 4-9; and (ii) a second polar aprotic solvent having a dielectric constant that is less than or equal to 115. In some examples,

methods further comprise immobilizing said biological sample or derivative thereof on said surface in a manner that is sufficient to fix said relative three-dimensional relationship. In some examples, methods further comprise amplifying said target nucleic acid sequence on said surface of said substrate, optionally, using rolling circle amplification. In some examples, an image of said surface in said presence of said biological sample or derivative thereof exhibits a contrast-to-noise ratio of greater than or equal to about 5 as measured by: (a) contacting said surface with a fluorescently labeled nucleotide molecule comprising a nucleic acid sequence that is complementary to at least a portion of a capture oligonucleotide immobilized to said surface; and (b) following (a), imaging said surface using an inverted microscope and a camera under non-signal saturating conditions while said surface is immersed in a buffer. In some examples, methods further comprise performing a nucleotide binding reaction between a nucleotide moiety coupled to said polymer-nucleotide conjugate and said target nucleic acid molecule or derivative thereof. In some examples, said target nucleic acid molecule or derivative thereof is a deoxyribonucleic acid (DNA) molecule. In some examples, said biological sample or derivative thereof comprises a fluid biological sample. In some examples, said origin is a cancerous tissue.

[0007] Examples disclosed herein provide methods for identifying at least a portion of a sub-cellular component within a cell or tissue *in situ*, the method comprising: (a) detecting a signal from a multivalent binding complex between said sub-cellular component or derivative thereof and a detectable polymer-nucleotide conjugate; and (b) processing at least said signal detected in (a) to identify said at least said portion of said sub-cellular component or derivative thereof. In some examples, said sub-cellular component or derivative thereof is a nucleic acid. In some examples, said nucleic acid is DNA. In some examples, methods further comprise: (c) immobilizing said cell or said tissue on a surface of a substrate. In some examples, methods further comprise: (d) coupling at least a portion of said sub-cellular component to a capture molecule coupled to a said surface. In some examples, methods further comprise: (e) permeabilizing said tissue or lysing said cell prior to detecting in (a). In some examples, said surface has a water contact angle of less than or equal to 45 degrees. In some examples, coupling in (d) comprises hybridizing said capture molecule with said at least said portion of said sub-cellular component in a presence of a hybridization buffer comprising: (i) a first polar aprotic solvent having a dielectric constant that is no greater than 40 and having a polarity index of 4-9; and (ii) a second polar aprotic solvent having a dielectric constant that is less than or equal to 115. In some examples, an image

of said surface exhibits a contrast-to-noise ratio of greater than or equal to about 5 as measured by: (a) contacting said surface with a fluorescently labeled nucleotide molecule comprising a nucleic acid sequence that is complementary to at least a portion of a capture oligonucleotide immobilized to said surface; and (b) following (a), imaging said surface using an inverted microscope and a camera under non-signal saturating conditions while said surface is immersed in a buffer. In some examples, detecting said signal from said multivalent binding complex in (a) comprises performing a nucleotide binding reaction between a nucleotide moiety coupled to said polymer-nucleotide conjugate and said sub-cellular component or derivative thereof. In some examples, said tissue is from a tumor.

[0008] A system for analyzing a biological sample comprising: a substrate comprising a surface having coupled thereto a polymer layer suitable to immobilize said biological sample to said surface, wherein: said biological sample or derivative thereof comprises a target nucleic acid molecule or derivative thereof; said polymer layer is configured to couple with (i) said biological sample or derivative thereof, or (ii) said target nucleic acid molecule or derivative thereof; said target nucleic acid molecule or derivative thereof is configured to couple with a nucleotide moiety comprising a detectable label; and an image of said surface exhibits a contrast-to-noise ratio of greater than or equal to about 5 when said image of said surface is obtained using an inverted microscope and a camera under non-signal saturating conditions while said surface is immersed in a buffer and wherein said detectable label is a fluorescent dye. In some examples, said polymer layer is hydrophilic. In some examples, systems further comprise a fixing agent that fixes said biological sample to said surface when said biological sample is contacted with said fixing agent while adjacent to said surface. In some examples, said fixing agent comprises formaldehyde or glutaraldehyde. In some examples, said target nucleic acid molecule is a concatemer. In some examples, said target nucleic acid molecule comprises a universal sequence region comprising a spatial barcode sequence or a sample barcode sequence configured to retain an origin of said target nucleic acid molecule in said biological sample. In some examples, an image of said surface exhibits a contrast-to-noise ratio of greater than or equal to about 10 when said image of said surface is obtained. In some examples, said substrate is a flow cell device comprising a first flow channel and, optionally, a second flow channel. In some examples, said substrate is a planar substrate that is reflective, transparent, or translucent. In some examples, said flow cell device is a capillary flow cell device.

[0009] Examples disclosed herein comprise systems for analyzing nucleic acid sequence information in a biological sample or derivative thereof, the system comprising: one or more computer processors programed to: (a) detect a signal from a multivalent binding complex formed in a presence of said biological sample or derivative thereof between a target nucleic acid sequence of a target nucleic acid molecule or derivative thereof and a detectable polymer-nucleotide conjugate, wherein said signal is indicative of an identity of a nucleotide in said target nucleic acid sequence; and (b) determine an origin of said target nucleic acid sequence in said biological sample. In some examples, said one or more computer processors is programed to determine said origin of said target nucleic acid sequence in (b) by analyzing a relative three-dimensional relationship between said target nucleic acid molecule or derivative thereof and said biological sample or derivative thereof. In some examples, said system further comprises a database configured to store three-dimensional data related to said origin of said target nucleic acid sequence. In some examples, said database is further configured to store sequencing data comprising said identity of said nucleotide in said target nucleic acid sequence. In some examples, (b) is performed by associating said sequencing data and said three-dimensional data. In some examples, said one or more computer processors is programed to identify said target nucleic acid sequence in less than 60 minutes by repeating (a) to (b). In some examples, said one or more computer processors is programed to perform (a) to (b) with an accuracy of base-calling that is characterized by a Q-score of greater than 25 for at least 80% of nucleotides identified. In some examples, said detectable polymer-nucleotide conjugate comprises: (a) a polymer core; and (b) two or more nucleotide moieties attached to said polymer core, wherein said polymer-nucleotide conjugate is configured to form a multivalent binding complex between said two or more nucleotide moieties and said target nucleic acid molecule or derivative thereof. In some examples, said one or more nucleotide moieties comprises a nucleotide, a nucleotide analog, a nucleoside, or a nucleoside analog. In some examples, said polymer core comprises a polymer that has a star, comb, cross-linked, bottle brush, or dendrimer configuration. In some examples, said polymer core comprises a branched polyethylene glycol (PEG) molecule. In some examples, systems further comprise an optical imaging system comprising a field-of-view (FOV) greater than 1.0 mm^2.

[0010] Examples disclosed herein provide kits comprising: (a) a detectable polymer-nucleotide conjugate comprising: (i) a polymer core; and (ii) (ii) two or more nucleotide moieties attached to said polymer core; and (b) instructions for identifying at least a portion

of a sub-cellular component within a cell or tissue *in situ* by contacting said detectable polymer-nucleotide conjugate with said sub-cellular component under conditions sufficient to form a multivalent binding complex between said two or more nucleotide moieties and said sub-cellular component. In some examples, kits comprise 4 types of said detectable polymer-nucleotide conjugate, wherein each of said 4 types has a different nucleotide moiety attached thereto.

[0011] Examples disclosed herein comprise kits comprising: (a) a substrate comprising a surface having coupled thereto a polymer layer suitable to immobilize a biological sample or derivative thereof to said surface; and (b) instructions for determining a target nucleic acid sequence and an origin of said target nucleic acid sequence in said biological sample or derivative on said surface. In some examples, kits further comprise: (a) a hybridization buffer comprising: (i) a first polar aprotic solvent having a dielectric constant that is no greater than 40 and having a polarity index of 4-9; and (ii) a second polar aprotic solvent having a dielectric constant that is less than or equal to 115; and (b) instructions for hybridizing at least a portion of said target nucleic acid sequence to at least a portion of a capture oligonucleotide coupled to said surface.

INCORPORATION BY REFERENCE

[0012] 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, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0014] **Figure 1** is a schematic illustration of one embodiment of the low binding support comprising a glass substrate and alternating layers of hydrophilic coatings which are covalently or non-covalently adhered to the glass, and which further comprises chemically-

CLAIMS

WHAT IS CLAIMED:

1. A method for analyzing

a target nucleic acid sequence in situ, the method comprising:

(a) providing a cell or a tissue comprising a plurality of nucleic acid molecules, wherein said plurality of nucleic acid molecules comprises said target nucleic acid sequence;

(b) contacting a subset of said plurality of nucleic acid molecules with a detectable nucleotide conjugate under conditions suitable to form a binding complex between (i) at least two nucleotide moieties of said detectable nucleotide conjugate and (ii) at least one nucleotide of each of at least two of said target nucleic acid sequence of said plurality of nucleic acid molecules within said cell or said tissue, wherein said subset of said plurality of nucleic acid molecules is primed;

(c) detecting said binding complex; and

(d) performing (b) to (c) for at least two other nucleotides of said at least two of said target nucleic acid sequence of said plurality of nucleic acid molecules, thereby identifying said target nucleic acid sequence.

2. The method of claim 1, wherein said cell or said tissue is immobilized to an interior surface of a flow cell comprising, optionally, one or more hydrophilic polymer layers.

3. The method of claim 2, wherein said one or more hydrophilic polymer layers comprises:

(a) a polymer comprising polyethylene glycol (PEG);

(b) a branched polymer; or

(c) any combination of (a) and (b).

4. The method of claim 2, wherein said interior surface has a water contact angle comprising less than or equal to 45 degrees.

5. The method of claim 1, further comprising

(a) permeabilizing said tissue or lysing said cell prior to said contacting in (b);

(b) washing said cell or said tissue following (c) to remove said detectable nucleotide conjugate from said cell or said tissue; or

(c) any combination of (a) and (b).

6. The method of claim 2, wherein an image of said interior surface exhibits a contrast-to-noise ratio (CNR) of greater than or equal to about 10 when said CNR is measured by:

(a) contacting said interior surface with a fluorescently labeled nucleotide molecule comprising a nucleic acid sequence that is complementary to at least a portion of a capture oligonucleotide immobilized to said interior surface; and

(b) following (a), imaging said interior surface using an inverted microscope and a camera under non-signal saturating conditions while said interior surface is immersed in a buffer.

7. The method of claim 1, wherein said identifying said target nucleic acid sequence in (d) is performed with an accuracy of base-calling that is characterized by a Q-score of greater than 25 for at least 80% of nucleotides identified.

8. The method of claim 1, further comprising determining a spatial location of said target nucleic acid sequence within said cell or said tissue.

9. The method of claim 1, further comprising determining a cell or tissue type of said cell based, at least in part, on said identifying said target nucleic acid sequence in (d).

10. The method of claim 1, further comprising determining a tissue type of said tissue based, at least in part, on said identifying said target nucleic acid sequence in (d).

11. The method of claim 1, wherein said detectable nucleotide conjugate comprises:

(a) a common core; and

(b) said at least two nucleotide moieties coupled to said common core, wherein said common core comprises a polymer, a micelle, a liposome, a microparticle, a nanoparticle, or a quantum dot.

12. The method of claim 11, wherein said common core is spheroidal.

13. The method of claim 12, wherein said detectable nucleotide conjugate further comprises a detectable moiety.

14. The method of claim 13, wherein said detectable moiety is coupled to said common core.

15. The method of claim 1, wherein in (b) said detectable nucleotide conjugate is included in a mixture of a plurality of detectable nucleotide conjugates, wherein each of said plurality of detectable nucleotide conjugates comprises different types of nucleotide moieties from each other.

16. The method of claim 15, wherein said different types of said nucleotide moieties optionally comprises at least three different types of said nucleotide moieties.

17. The method of claim 1, wherein said at least two nucleotide moieties do not comprise a blocking group coupled thereto.

18. The method of claim 1, wherein said plurality of nucleic acid molecules comprises a blocking group sufficient to prevent incorporation of said at least two nucleotide moieties into said at least two of said target nucleic acid sequence.

19. The method of claim 1, wherein said detecting said binding complex comprises imaging said cell or said tissue us in one or more image sensors, wherein said one or more image sensors optionally comprises a photodetector array.

20. The method of claim 19, wherein said cell is a cancerous cell or said tissue is a cancerous tissue.