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) contacting a cell or a tissue with a detectable nucleotide conjugate under conditions suitable to form a binding complex within said cell or said tissue, wherein said cell or said tissue comprises a plurality of nucleic acid molecules comprising said target nucleic acid sequence that is primed, wherein said detectable nucleotide conjugate comprises at least two nucleotide moieties, and wherein said binding complex forms between (i) said 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; (b) detecting said binding complex; and (c) performing (a) to (b) 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 someexamples, 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,

## CLAIMS

WHAT IS CLAIMED:

**1.** A method for analyzing a target nucleic acid sequence in situ, the method comprising:

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

(b) detecting said binding complex; and

(c) performing (a) and (b) 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

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

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

(iii) any combination of (i) and (ii).

**6.** The method of claim 1, wherein said identifying said target nucleic acid sequence in (c) 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.

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

**8.** 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 (c).

**9.** 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 (c).

**10.** 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.

**11.** The method of claim 10, wherein said common core is spheroidal.

**12.** The method of claim 11, wherein said detectable nucleotide conjugate further comprises a detectable moiety.

**13.** The method of claim 12, wherein said detectable moiety is coupled to said common core.

**14.** The method of claim 1, wherein in (a) 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.

**15.** The method of claim 14, wherein said different types of said nucleotide moieties optionally comprises at least three different types of said nucleotide moieties.

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

**17.** 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.

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

**19.** The method of claim 18, wherein said cell is a cancerous cell or said tissue is a cancerous tissue.