

applications, such as quality control purposes during product development, or optimization of process flows. Hence, there is a need to develop methods and systems for processing the bead for direct characterization.

[0006] The present disclosure provides methods and systems for generating and processing optically tagged beads. The optically tagged beads can be generated without chemically modifying, or without substantial modification to, the beads. A nucleic acid probe comprising a fluorescent tag may be hybridized to a bead to generate an optically tagged bead. The nucleic acid probe hybridized to the optically tagged bead may be detectable by one or more signals (e.g., fluorescent signals) and may be substantially unreactive to one or more chemical reactions performed with, or in the presence of, the optically tagged bead.

[0006a] In one aspect, provided is a method for processing a bead, comprising: (a) hybridizing a bead and a modified probe to generate an optically tagged bead, wherein said bead comprises a binding sequence, wherein said modified probe hybridizes to said binding sequence, wherein said modified probe comprises a fluorescent tag and a 3' chain-terminating modification, and wherein said modified probe hybridized to said bead is substantially unreactive to one or more chemical reactions; (b) detecting one or more signals indicative of said fluorescent tag from said optically tagged bead; and (c) conducting a chemical reaction of said one or more chemical reactions with, or in a presence of, said optically tagged bead.

[0006b] In one aspect, provided herein is a kit, comprising: a bead comprising a binding sequence; and a plurality of modified probes configured to hybridize to the bead, wherein a modified probe of said plurality of modified probes comprises a fluorescent tag and a 3' chain-terminating modification, wherein said modified probe is configured to hybridize to said binding sequence, and wherein said modified probe, when hybridized to said binding sequence, is substantially unreactive to one or more chemical reactions conducted with, or in a presence of, said bead hybridized to said modified probe.

[0007] In an example, provided is a method for processing a bead, comprising: (a) hybridizing a bead and a modified probe to generate an optically tagged bead, wherein the bead comprises a binding sequence, wherein the modified probe hybridizes to the binding sequence, wherein the modified probe comprises a fluorescent tag, and wherein the modified probe hybridized to the bead is substantially unreactive to one or more chemical reactions; (b) detecting one or more signals indicative of the fluorescent tag from the optically tagged bead; and (c) conducting a chemical reaction of the one or more chemical reactions with, or in a presence of, the optically tagged bead.

- [0008] In some examples, the method comprises, prior to hybridizing, subjecting a nucleic acid probe comprising the fluorescent tag under conditions sufficient to modify the nucleic acid probe to yield the modified probe.
- [0009] In some examples, the nucleic acid probe comprises the fluorescent tag at a 5' end.
- [0010] In some examples, the conditions comprise adding a 3' chain-terminating modification to the nucleic acid probe.
- [0011] In some examples, the conditions comprise adding a fluorophore to a 3' end of the nucleic acid probe, wherein the fluorophore is in addition to the fluorescent tag.
- [0012] In some examples, the conditions comprise adding a 3 carbon spacer to a 3' end of the nucleic acid probe. In some examples, the conditions comprise adding a 6 carbon spacer to a 3' end of the nucleic acid probe. In some examples, the conditions comprise adding a 9 carbon spacer to a 3' end of the nucleic acid probe. In some examples, the conditions comprise adding a 18 carbon spacer to a 3' end of the nucleic acid probe.
- [0013] In some examples, the conditions comprise adding a biotin molecule to a 3' end of the nucleic acid probe.
- [0014] In some examples, the nucleic acid probe comprises a probe sequence configured to hybridize to the binding sequence. In some examples, the modified probe comprises the probe sequence.
- [0015] In some examples, the conditions comprise inverting a terminal base at a 3' end of the probe sequence.
- [0016] In some examples, the conditions comprise modifying the probe sequence to destabilize hybridization between the bead and the modified probe.
- [0017] In some examples, the hybridization comprises a mismatched base pair. In some examples, the hybridization comprises two mismatched base pairs.
- [0018] In some examples, the modified probe comprises unlocked nucleic acid.
- [0019] In some examples, the modified probe comprises the fluorescent tag at a 5' end.
- [0020] In some examples, the detecting is performed prior to conducting the one or more chemical reactions. In some examples, the detecting is performed subsequent to conducting the one or more chemical reactions.
- [0021] In some examples, the bead is a gel bead.
- [0022] In some examples, the bead comprises a plurality of barcode molecules. In some examples, the plurality of barcode molecules comprise the binding sequence. In some examples, the bead comprises a molecule comprising the binding sequence separate from the plurality of barcode molecules.

[0023] In some examples, the method further comprises determining a parameter of the bead based on the one or more signals. In some examples, the bead comprises a plurality of primers and wherein the parameter is a concentration of the plurality of primers on the bead, wherein the modified probe comprises a probe sequence configured to hybridize to a sequence common to the plurality of primers. In some examples, the parameter is a size of the bead.

[0024] In some examples, the method further comprises, subsequent to the hybridizing, partitioning the optically tagged bead in a partition. In some examples, the partition is a droplet. In some examples, the partition is a well.

[0025] In some examples, the method further comprises determining a parameter of the partition or contents thereof based on the one or more signals. In some examples, the parameter is a cell occupancy of the partition. In some examples, the parameter is a bead occupancy of the partition. In some examples, the parameter is a concentration of a reagent in the partition. In some examples, the parameter is a size of the partition. In some examples, the partition comprises a cell, and the parameter is a cell phenotype.

[0026] In some examples, the method further comprises, subsequent to the hybridizing, co-partitioning the optically tagged bead and a biological analyte. In some examples, the method further comprises, subsequent to the hybridizing, co-partitioning the optically tagged bead and a cell bead.

[0027] In some examples, the method further comprises, prior to the hybridizing, generating the modified probe. In some examples, the generating comprises selecting the fluorescent tag from a plurality of fluorophores.

[0028] In some examples, the modified probe is hybridized to a selected region of the bead, wherein the selected region comprises the binding sequence. In some examples, the method further comprises, prior to the hybridizing, selecting the selected region.

[0029] In some examples, a plurality of modified probes, including the modified probe, is hybridized to the selected region of the bead, wherein the selected region comprises a plurality of binding sequences, including the binding sequence, configured to hybridize to the plurality of modified probes.

[0030] In some examples, the hybridizing comprises hybridizing a plurality of modified probes, including the modified probe, to the optically tagged bead, wherein the plurality of modified probes comprises a plurality of fluorescent tags, including the fluorescent tag. In some examples, the method further comprises, prior to the hybridizing, selecting the plurality of fluorescent tags.

[0031] In some examples, the plurality of modified probes are hybridized to one or more selected regions of the bead. In some examples, the method further comprises, prior to the hybridizing, selecting the one or more selected regions. In some examples, the method further comprises, prior to the hybridizing, selecting a density or concentration, or ranges thereof, of modified probes hybridized to the one or more selected regions.

[0032] In another example, provided is a kit, comprising: a plurality of modified probes configured to hybridize to a bead comprising a binding sequence, wherein a modified probe of the plurality of modified probes comprises a fluorescent tag, wherein the modified probe is configured to hybridize to the binding sequence, and wherein the modified probe, when hybridized to the binding sequence, is substantially unreactive to one or more chemical reactions conducted with, or in a presence of, the bead hybridized to the modified probe.

[0033] In some examples, the method further comprises the bead.

[0034] In some examples, a fluorescent signal from the fluorescent tag is detectable prior to the one or more chemical reactions. In some examples, a fluorescent signal from the fluorescent tag is detectable subsequent to the one or more chemical reactions.

[0035] In some examples, the modified probe comprises the fluorescent tag at a 5' end.

[0036] In another example, provided is a kit, comprising: a plurality of nucleic acid probes configured to hybridize to a bead, wherein the plurality of nucleic acid probes, when hybridized to the bead, are substantially reactive to one or more chemical reactions conducted with, or in a presence of, the bead hybridized to the plurality of nucleic acid probes; a plurality of fluorophores; and reagents for modifying the plurality of nucleic acid probes to generate a plurality of modified probes, wherein the plurality of modified probes are (i) configured to hybridize to the bead, (ii) comprises the plurality of fluorophores, and (iii) when hybridized to the bead, are substantially unreactive to one or more chemical reactions conducted with, or in a presence of, the bead hybridized to the plurality of modified probes.

[0037] In some examples, the kit further comprises the bead.

[0038] In some examples, a fluorescent signal from the plurality of fluorophores is detectable prior to the one or more chemical reactions. In some examples, a fluorescent signal from the plurality of fluorophores is detectable subsequent to the one or more chemical reactions.

[0039] In some examples, the plurality of nucleic acid probes comprises the plurality of fluorophores. In some examples, the plurality of nucleic acid probes comprises the plurality of fluorophores at a 5' end.

[0040] In some examples, the plurality of modified probes comprises the plurality of fluorophores at a 5' end.

[0041] In some examples, the reagents comprise reagents configured to add a 3' chain-terminating modification to the plurality of nucleic acid probes.

[0042] In some examples, the reagents comprise reagents configured to add a fluorophore to a 5' end of the plurality of nucleic acid probes. In some examples, the reagents comprise reagents configured to add a fluorophore to a 3' end of the plurality of nucleic acid probes.

[0043] In some examples, the reagents comprise reagents configured to add a 3' chain-terminating modification to the plurality of nucleic acid probes, wherein the plurality of nucleic acid probes comprises the plurality of fluorophores at a 5' end.

[0044] In some examples, the reagents comprise reagents configured to add a 3 carbon spacer to a 3' end of the plurality of nucleic acid probes. In some examples, the reagents comprise reagents configured to add a 6 carbon spacer to a 3' end of the plurality of nucleic acid probes. In some examples, the reagents comprise reagents configured to add a 9 carbon spacer to a 3' end of the plurality of nucleic acid probes. In some examples, the reagents comprise reagents configured to add a 18 carbon spacer to a 3' end of the plurality of nucleic acid probes.

[0045] In some examples, wherein the reagents comprise reagents configured to add a biotin molecule to a 3' end of the plurality of nucleic acid probes.

[0046] In some examples, the reagents comprise reagents configured to invert a terminal base at a 3' end of the plurality of nucleic acid probes.

[0047] In some examples, the plurality of modified probes comprises a probe sequence configured to hybridize to a binding sequence of a bead. In some examples, the probe sequence and the binding sequence comprises a mismatched base pair. In some examples, the probe sequence and the binding sequence comprises two mismatched base pairs.

[0048] In some examples, the modified probes comprise unlocked nucleic acid.

[0049] Another example of the present disclosure provides a non-transitory computer readable medium comprising machine executable code that, upon execution by one or more computer processors, implements any of the methods above or elsewhere herein.

[0050] In an example, the present disclosure provides a method for processing a bead. The method may comprise hybridizing a bead and a first nucleic acid probe to generate an optically tagged bead, wherein the bead may comprise a first binding sequence, and the first nucleic acid probe may hybridize to the first binding sequence, and the first nucleic acid probe may comprise a first fluorescent tag. The method may further comprise detecting one or more signals indicative of the first fluorescent tag from the optically tagged bead; and using the one or more signals to determine the presence of the first binding sequence on the bead.

[0051] In some examples, the method may further comprise, hybridizing the bead with a second nucleic acid probe before detecting the one or more signals indicative of the first fluorescent tag from the optically tagged bead. The bead may comprise a second binding sequence which may be different than the first binding sequence, and the second nucleic acid probe may hybridize to the second binding sequence, and the second nucleic acid probe may comprise a second fluorescent tag which may be different than the first fluorescent tag. In some examples, the method further comprises detecting one or more additional signals indicative of the second fluorescent tag from the optically tagged bead and using the one or more additional signals to determine the presence of the second binding sequence on the bead.

[0052] In some examples, the detecting of said one or more signals comprises using a flow cytometer, a fluorescence-activated cell sorting (FACS) instrument, or a combination thereof.

[0053] In some examples, the method further comprises processing each bead of a population of beads through (a)-(c), and subsequent to (c), using the one or more signals from each bead of the population of beads to determine a quality of the population of beads. In some examples, the quality of population of beads comprises a loading efficiency of the binding sequence to the population of beads, an efficiency of hybridization of the modified probe or the first nucleic acid probe to the binding sequence, homogeneity or bead-to-bead variability of the population of beads, or combinations thereof. In some examples, the

homogeneity or bead-to-bead variability may be quantified or characterized by a coefficient of variance of the one or more signals detected for the population of beads.

[0054] Another example of the present disclosure provides a system comprising one or more computer processors and computer memory coupled thereto. The computer memory comprises machine executable code that, upon execution by the one or more computer processors, implements any of the methods above or elsewhere herein.

[0055] Additional examples and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative examples of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different examples, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

INCORPORATION BY REFERENCE

[0056] 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. To the extent publications and patents or patent applications 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.

BRIEF DESCRIPTION OF THE DRAWINGS

[0057] 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 (also “Figure” and “FIG.” herein), of which:

[0058] **FIG. 1** shows an example of a microfluidic channel structure for partitioning individual biological particles.

[0059] **FIG. 2** shows an example of a microfluidic channel structure for delivering barcode carrying beads to droplets.

[0060] **FIG. 3** shows an example of a microfluidic channel structure for co-partitioning biological particles and reagents.

CLAIMS

1. A method for processing a bead, comprising:

- (a) hybridizing a bead and a modified probe to generate an optically tagged bead, wherein said bead comprises a binding sequence, wherein said modified probe hybridizes to said binding sequence, wherein said modified probe comprises a fluorescent tag and a 3' chain-terminating modification, and wherein said modified probe hybridized to said bead is substantially unreactive to one or more chemical reactions;
- (b) detecting one or more signals indicative of said fluorescent tag from said optically tagged bead; and
- (c) conducting a chemical reaction of said one or more chemical reactions with, or in a presence of, said optically tagged bead.

2. The method of claim 1, further comprising, prior to (a), subjecting a nucleic acid probe comprising said fluorescent tag under conditions sufficient to modify said nucleic acid probe to yield said modified probe, optionally wherein:

- (i) said nucleic acid probe comprises said fluorescent tag at a 5' end;
- (ii) said conditions comprise adding a 3' chain-terminating modification to said nucleic acid probe;
- (iii) said conditions comprise adding a fluorophore to a 3' end of said nucleic acid probe, wherein said fluorophore is in addition to said fluorescent tag;
- (iv) said conditions comprise adding a carbon spacer to a 3' end of said nucleic acid probe, optionally wherein said carbon spacer comprises a 3 carbon spacer, a 6 carbon spacer, a 9 carbon spacer, or a 18 carbon spacer;
- (v) said conditions comprise adding a biotin molecule to a 3' end of said nucleic acid probe;
- (vi) said conditions comprise inverting a terminal base at a 3' end of said probe sequence; or
- (vii) said conditions comprise modifying said probe sequence to destabilize hybridization between said bead and said modified probe, optionally wherein said hybridization comprises a mismatched base pair or two mismatched base pairs.

3. The method of claim 1 or claim 2, wherein said modified probe comprises:
- (i) an unlocked nucleic acid; or
 - (ii) said fluorescent tag at a 5' end.
4. The method of any one of claims 1-3, wherein (b) is performed prior to (c), or wherein (b) is performed subsequent to (c)
5. The method of any one of claims 1-4, wherein:
- (i) said fluorescent tag comprises a fluorophore;
 - (ii) said bead is a gel bead; and/or
 - (iii) said bead comprises a plurality of barcode molecules, optionally wherein:
 - (a) said plurality of barcode molecules comprise said binding sequence; or
 - (b) said bead comprises a molecule comprising said binding sequence separate from said plurality of barcode molecules.
6. The method of any one of claims 1-5, further comprising determining a parameter of said bead based on said one or more signals, optionally wherein:
- (i) said bead comprises a plurality of primers and wherein said parameter is a concentration of said plurality of primers on said bead, wherein said modified probe comprises a probe sequence configured to hybridize to a sequence common to said plurality of primers; or
 - (ii) said parameter is a size of said bead.
7. The method of any one of claims 1-6, further comprising, subsequent to (a), partitioning said optically tagged bead in a partition, optionally:
- (i) wherein said partition is a droplet or a well; or
 - (ii) further comprising determining a parameter of said partition or contents thereof based on said one or more signals, optionally wherein:
 - (a) said parameter is a cell occupancy of said partition;
 - (b) said parameter is a bead occupancy of said partition;
 - (c) said parameter is a concentration of a reagent in said partition;

- (d) said parameter is a size of said partition; or
- (e) said partition comprises a cell, and said parameter is a cell phenotype.

8. The method of any one of claims 1-7, further comprising:

- (i) subsequent to (a), co-partitioning said optically tagged bead and a biological analyte;
- (ii) subsequent to (a), co-partitioning said optically tagged bead and a cell bead; or
- (iii) prior to (a), generating said modified probe, optionally wherein said generating comprises selecting said fluorescent tag from a plurality of fluorophores.

9. The method of any one of claims 1-8, wherein said modified probe is hybridized to a selected region of said bead, wherein said selected region comprises said binding sequence, optionally:

- (i) further comprising, prior to (a), selecting said selected region; or
- (ii) wherein a plurality of modified probes, including said modified probe, is hybridized to said selected region of said bead, wherein said selected region comprises a plurality of binding sequences, including said binding sequence, configured to hybridize to said plurality of modified probes.

10. The method of any one of claims 1-9, wherein said hybridizing comprises hybridizing a plurality of modified probes, including said modified probe, to said optically tagged bead, wherein said plurality of modified probes comprises a plurality of fluorescent tags, including said fluorescent tag, optionally:

- (i) further comprising, prior to (a), selecting said plurality of fluorescent tags; and/or
- (ii) wherein said plurality of modified probes are hybridized to one or more selected regions of said bead, optionally further comprising, prior to (a):
 - (i) selecting said one or more selected regions; or
 - (ii) selecting a density or concentration, or ranges thereof, of modified probes hybridized to said one or more selected regions.

11. The method of any one of claims 1 to 10, wherein said detecting of said one or more signals comprises using a flow cytometer or a fluorescence-activated cell sorting (FACS) instrument, or a combination thereof.

12. The method of any one of claims 1 to 11, further comprising processing each bead of a population of beads through (a)-(c), and subsequent to (c), using said one or more signals from each bead of said population of beads to determine a quality of said population of beads.

13. The method of claim 12, wherein said quality of said population of beads comprises a loading efficiency of said binding sequence to said population of beads, an efficiency of hybridization of said modified probe or said first nucleic acid probe to said binding sequence, a homogeneity or bead-to-bead variability of said population of beads, or combinations thereof, optionally wherein said homogeneity or bead-to-bead variability is quantified or characterized by a coefficient of variance of said one or more signals detected for said population of beads.

14. A kit, comprising:

a bead comprising a binding sequence; and

a plurality of modified probes configured to hybridize to the bead, wherein a modified probe of said plurality of modified probes comprises a fluorescent tag and a 3' chain-terminating modification, wherein said modified probe is configured to hybridize to said binding sequence, and wherein said modified probe, when hybridized to said binding sequence, is substantially unreactive to one or more chemical reactions conducted with, or in a presence of, said bead hybridized to said modified probe.

15. The kit of claim 14, wherein said modified probe comprises said fluorescent tag at a 5' end.