

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~~the bead and a modified probe to generate an optically tagged bead, wherein ~~said~~the bead comprises a binding sequence, wherein ~~said-the~~the modified probe hybridizes to ~~said-the~~the binding sequence, wherein ~~said-the~~the modified probe comprises a fluorescent tag and a 3' chain-terminating modification at a 3' end of the modified probe, and wherein ~~said-modified-probe hybridized to said bead is substantially unreactive to one or more chemical reactions~~the 3' chain-terminating modification at the 3' end of the modified probe renders the modified probe hybridized to the bead at least 60% less reactive in an extension reaction as compared to an equivalent unmodified nucleic acid probe; (b) detecting one or more signals indicative of ~~said-the~~the fluorescent tag from ~~said-the~~the 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 wherein the optically tagged bead comprises: i) a molecule comprising the binding sequence hybridized to the modified probe, and ii) a nucleic acid barcode molecule, wherein the molecule is different from the nucleic acid barcode molecule.~~

**[0006b]** In ~~one another~~ aspect, provided herein is a kit, comprising: a bead comprising: (i) a molecule comprising a binding sequence and (ii) a nucleic acid barcode molecule, wherein the molecule is different from the nucleic acid barcode molecule; and a plurality of modified probes configured to hybridize to the bead, wherein a modified probe of ~~said-the~~the plurality of modified probes comprises a fluorescent tag and a 3' chain-terminating modification at a 3' end of the modified probe, wherein ~~said-the~~the modified probe is configured to hybridize to ~~said-the~~the binding sequence, and wherein ~~said-the~~the modified probe, when hybridized to ~~said-the~~the 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 configured to be at least 60% less reactive in an extension reaction as compared to an equivalent unmodified nucleic acid 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.

## CLAIMS

### 1. A method for processing a bead, comprising:

- (a) hybridizing ~~a~~the bead and a modified probe to generate an optically tagged bead, wherein ~~said the~~ bead comprises a binding sequence, wherein ~~said the~~ modified probe hybridizes to ~~said the~~ binding sequence, wherein ~~said the~~ modified probe comprises a fluorescent tag and a 3' chain-terminating modification at a 3' end of the modified probe, and wherein ~~said modified probe hybridized to said bead is substantially unreactive to one or more chemical reactions the 3' chain-terminating modification at the 3' end of the modified probe renders the modified probe hybridized to the bead at least 60% less reactive in an extension reaction as compared to an equivalent unmodified nucleic acid probe;~~
- (b) detecting one or more signals indicative of ~~said the~~ fluorescent tag from ~~said the~~ 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 wherein the optically tagged bead comprises: i) a molecule comprising the binding sequence hybridized to the modified probe, and ii) a nucleic acid barcode molecule, wherein the molecule is different from the nucleic acid barcode molecule.~~

2. The method of claim 1, wherein the modified probe comprises a base that is mismatched with a base of the binding sequence when the modified probe is hybridized to the binding sequence.

3. The method of claim 1, wherein the modified probe further comprises a fluorophore at the 3' end of the modified probe, wherein the fluorophore is in addition to the fluorescent tag.

4. The method of claim 1, wherein the modified probe comprises the fluorescent tag at a 5' end of the modified probe.

5. The method of claim 1, further comprising, prior to (a), subjecting a nucleic acid probe comprising the fluorescent tag to conditions sufficient to modify the nucleic acid probe to yield the modified probe.

6. The method of claim 5, further comprising adding the 3' chain-terminating modification to the nucleic acid probe.

7. The method of claim 5, further comprising adding a moiety to the nucleic acid probe, wherein the moiety is selected from the group consisting of a carbon spacer at a 3' end, a biotin molecule at a 3' end, an inverted terminal base at a 3' end, and an unlocked nucleic acid.

8. The method of claim 7, wherein the carbon spacer comprises a 3 carbon atom spacer.

9. The method of claim 1, further comprising (c) conducting the extension reaction with or in a presence of the optically tagged bead.

10. The method of claim 9, wherein (b) is performed prior to (c).

11. The method of claim 9, wherein (b) is performed subsequent to (c).

12. The method of claim 1, wherein the optically tagged bead comprises a gel bead.

13. The method of claim 1, further comprising determining a parameter of the optically tagged bead based on the one or more signals.

14. The method of claim 13, wherein the optically tagged bead comprises a plurality of primers and wherein the parameter is a concentration of the plurality of primers on the optically tagged bead, and wherein the modified probe comprises a probe sequence configured to hybridize to a sequence common to the plurality of primers.

15. The method of claim 13, wherein the parameter is a size of the optically tagged bead.

16. The method of claim 1, further comprising, subsequent to (a), partitioning the optically tagged bead in a partition among a plurality of partitions.

17. The method of claim 16, wherein the partition is a droplet or a well.

18. The method of claim 16, further comprising using the one or more signals to assess bead occupancy of the partition.

19. The method of claim 1, further comprising, subsequent to (a), co-partitioning the optically tagged bead with an analyte in a partition among a plurality of partitions.

~~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.~~

~~1420.~~ A kit, comprising:

a bead comprising: (i) a molecule comprising a binding sequence and (ii) a nucleic acid barcode molecule, wherein the molecule is different from the nucleic acid barcode molecule; and

a plurality of modified probes configured to hybridize to the bead, wherein a modified probe of ~~said the~~ plurality of modified probes comprises a fluorescent tag and a 3' chain-terminating modification at a 3' end of the modified probe, wherein ~~said the~~ modified probe is configured to hybridize to ~~said the~~ binding sequence, and wherein ~~said the~~ modified probe, when hybridized to ~~said the~~ 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 configured to be at least 60% less reactive in an extension reaction as compared to an equivalent unmodified nucleic acid probe.

~~1521.~~ The kit of claim ~~1420~~, wherein ~~said the~~ modified probe comprises ~~said the~~ fluorescent tag at a 5' end.