

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 the 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 a 3' chain-terminating modification at a 3' end of the modified probe, and wherein 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 the fluorescent tag from the 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 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 the plurality of modified probes comprises a fluorescent tag and a 3' chain-terminating modification at a 3' end of the modified probe, wherein the modified probe is configured to hybridize to the binding sequence, and wherein the modified probe, when hybridized to the binding sequence, is

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 the 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 a 3' chain-terminating modification at a 3' end of the modified probe, and wherein 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 the fluorescent tag from the 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.

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

21. The kit of claim 20, wherein the modified probe comprises the fluorescent tag at a 5' end.