complementary to that of a target region of a nucleic acid molecule of interest (e.g., target nucleic acid molecule). A method may comprise hybridizing a first probe and a second probe to first and second target regions of the nucleic acid molecule, linking the first and second probes to provide a probe-linked nucleic acid molecule, and barcoding the probe-linked nucleic acid molecule. A method may comprise hybridizing a first probe to a first target region of a nucleic acid molecule, barcoding the probe, and hybridizing a second probe to a second target region of the nucleic acid molecule to generate a barcoded, probe-linked nucleic acid molecule. In some aspects, the method may comprise hybridizing a probe to a nucleic acid molecule attached to a feature-binding moiety to provide a probe-binding moiety complex and barcoding the probe. One or more processes of the methods provided herein may be performed within a partition such as a droplet or well. The methods of the present disclosure be useful, for example, in controlled analysis and processing of analytes such as biological particles, nucleic acids, and proteins. One or more of the methods described herein may allow for genomic, transcriptomic, or exomic profiling with higher sensitivity. The methods of the present disclosure may be useful in detecting variants and characterizing nucleic acid molecules, e.g., for assessment of single nucleotide polymorphisms (SNPs), alternative splice junctions, insertions, deletions, V(D)J rearrangements, etc. The methods of the present disclosure may be useful for multiplexed analysis of nucleic acids and proteins while minimizing reagent usage, e.g., by decreasing the number of unoccupied partitions for analysis.

[0006a] In one aspect, disclosed herein is a method of analyzing a cell, comprising: (a) providing a sample comprising a cell, wherein the cell comprises: (i) a feature, wherein the feature comprises a protein, a peptide, a lipid, or a carbohydrate; and (ii) a nucleic acid molecule; and (b) contacting the cell with a feature binding group and coupling the feature binding group to the feature, wherein the feature binding group comprises an oligonucleotide comprising a feature nucleic acid sequence that identifies the feature binding group; (c) hybridizing a first nucleic acid probe to the nucleic acid molecule; wherein the first nucleic acid probe comprises (i) a hybridizing sequence that hybridizes to a first target sequence of the nucleic acid molecule and (ii) a non-hybridizing sequence; (d) using the feature nucleic acid sequence and a first barcode molecule comprising a first barcode sequence to generate a first barcoded nucleic acid molecule comprising (i) the feature nucleic acid sequence or reverse complement thereof, and (ii) the first barcode sequence or reverse complement thereof; and (e) coupling the first nucleic acid probe and a second barcode molecule comprising a second barcode sequence; and (f) using the non-hybridizing sequence of the first nucleic acid probe and the second barcode molecule to generate a second barcoded nucleic acid molecule comprising (i) the first target sequence or reverse complement thereof and (ii) the second barcode sequence or reverse complement thereof.

CLAIMS

WHAT IS CLAIMED IS:

1. A method of analyzing a cell, comprising:

(a) providing a sample comprising a cell, wherein the cell comprises:

(i) a feature, wherein the feature comprises a protein, a peptide, a lipid, or a carbohydrate; and

(ii) a nucleic acid molecule; and

(b) contacting the cell with a feature binding group and coupling the feature binding group to the feature, wherein the feature binding group comprises an oligonucleotide comprising a feature nucleic acid sequence that identifies the feature binding group;

(c) hybridizing a first nucleic acid probe to the nucleic acid molecule; wherein the first nucleic acid probe comprises (i) a hybridizing sequence that hybridizes to a first target sequence of the nucleic acid molecule and (ii) a non-hybridizing sequence;

(d) using the feature nucleic acid sequence and a first barcode molecule comprising a first barcode sequence to generate a first barcoded nucleic acid molecule comprising (i) the feature nucleic acid sequence or reverse complement thereof, and (ii) the first barcode sequence or reverse complement thereof; and

(e) coupling the first nucleic acid probe and a second barcode molecule comprising a second barcode sequence; and

(f) using the non-hybridizing sequence of the first nucleic acid probe and the second barcode molecule to generate a second barcoded nucleic acid molecule comprising (i) the first target sequence or reverse complement thereof and (ii) the second barcode sequence or reverse complement thereof.

2. The method of claim 1, wherein the feature binding group comprises an antibody.

3. The method of claim 1, wherein the feature comprises a cell surface receptor or an intracellular protein.

4. The method of claim 1, further comprising, prior to or during (c), permeabilizing the cell.

5. The method of claim 1, further comprising, prior to (d), providing a splint molecule, wherein the splint molecule comprises a first splint sequence and a second splint sequence, wherein a first portion of the first barcode molecule hybridizes to the first splint sequence in the splint molecule, and a second portion of the oligonucleotide hybridizes to the second splint sequence on the splint molecule.

6. The method of claim 5, wherein the first splint sequence and the second splint sequence are adjacent on the splint molecule.

7. The method of claim 5, wherein the first splint sequence and the second splint sequence are separated by a gap region of at least 1 nucleotide on the splint molecule.

8. The method of claim 5, wherein (d) comprises linking the first barcode molecule and the oligonucleotide together to generate the first barcoded nucleic acid molecule.

9. The method of claim 1, wherein the first barcode sequence in the first barcode molecule identifies the sample.

10. The method of claim 1, further comprising (g) using the first barcoded nucleic acid molecule and a third barcode molecule comprising a third barcode sequence to generate a third barcoded nucleic acid molecule comprising (i) the feature nucleic acid sequence or reverse complement thereof, (ii) the first barcode sequence or reverse complement thereof; and (iii) the third barcode sequence or reverse complement thereof.

11. The method of claim 10, wherein (g) occurs in a partition among a plurality of partitions.

12. The method of claim 11, wherein the third barcode sequence identifies the partition from other partitions of the plurality of partitions.

13. The method of claim 10, wherein (g) comprises hybridizing a first capture sequence in the third barcode molecule to a first capture binding sequence in the first barcoded nucleic acid molecule; and extending the third barcode molecule to generate the third barcoded nucleic acid molecule.

14. The method of claim 13, wherein the third barcode molecule is coupled to a bead.

15. The method of claim 10, further comprising detecting the third barcoded nucleic acid molecule, thereby identifying (i) the feature nucleic acid sequence, (ii) the first barcode sequence; and (iii) the third barcode sequence.

16. The method of claim 1, wherein the nucleic acid molecule is an RNA transcript.

17. The method of claim 1, wherein (c) further comprises hybridizing a second nucleic acid probe to a second target sequence of the nucleic acid molecule.

18. The method of claim 17, wherein, in (c), wherein the first target sequence and the second target sequence are adjacent on the nucleic acid molecule.

19. The method of claim 17, wherein, in (c), the first target sequence and the second target sequence are separated by a gap region of at least 1 nucleotide on the nucleic acid molecule.

20. The method of claim 17, further comprising, linking the first nucleic acid probe and the second nucleic acid probe together, thereby generating a linked nucleic acid molecule comprising (i) the first nucleic acid probe and (ii) the second nucleic acid probe.

21. The method of claim 20, wherein the linked nucleic acid molecule comprises a fourth barcode sequence that identifies the sample.

22. The method of claim 21, wherein the fourth barcode sequence is the same as the first barcode sequence.

23. The method of claim 21, wherein (f) comprises using the non-hybridizing sequence in the linked nucleic acid molecule and the second barcode molecule to generate the second barcoded nucleic acid molecule, wherein the second barcoded nucleic acid molecule comprises (i) the first target sequence or reverse complement thereof, (ii) the second barcode sequence or reverse complement thereof, and (iii) the fourth barcode sequence or reverse complement thereof.

24. The method of claim 1, wherein (f) occurs in a partition among a plurality of partitions.

25. The method of claim 24, wherein the second barcode sequence identifies the partition among the plurality of partitions.

26. The method of claim 23, wherein (e) comprises hybridizing a second capture sequence in the second barcode molecule to a second capture binding sequence in the non-hybridizing sequence of the first nucleic acid probe; and (f) comprises extending the second barcode molecule to generate the second barcoded nucleic acid molecule.

27. The method of claim 26, wherein the second barcode molecule is coupled to a bead.

28. The method of claim 23, further comprising detecting the second barcoded nucleic acid molecule, thereby identifying (i) the first target sequence, (ii) the second barcode sequence, and (iii) the fourth barcode sequence.

29. The method of claim 1, wherein (d) is performed in a partition among a plurality of partitions.

30. The method of claim 29, wherein the first barcode sequence identifies the partition from other partitions of the plurality of partitions.