CLAIMS

1. A method for analyzing a biological sample, comprising:

(a) contacting the biological sample with a plurality of probes each comprising a target-specific barcode sequence,

wherein a first probe of the plurality of probes comprises a first probe-resolution barcode sequence and a second probe of the plurality of probes comprises a second probe-resolution barcode sequence, and

wherein the first probe targets a first molecule of a target analyte and the second probe targets a second molecule of the target analyte at a first location and a second location in the biological sample, respectively, and the target-specific barcode sequence corresponds to the target analyte;

(b) detecting a plurality of signals associated with the target-specific barcode sequences of the plurality of probes;

(c1) detecting a signal associated with the first probe-resolution barcode sequence at the first location; and

(c2) detecting a signal associated with the second probe-resolution barcode sequence at the second location,

wherein the signals of steps (c1) and (c2) are associated with the target analyte.

2. The method of claim 1, wherein the plurality of signals detected in step (b) comprise overlapping signals that are not spatially resolved into individual puncta in step (b), and for overlapping signals that are associated with the target-specific barcode sequence, an overlapping signal is associated with the signal of step (c1) or the signal of step (c2) but not both, thereby resolving the overlapping signals into signals associated with the first and second probes, respectively.

3. The method of any of claims 1-2, wherein the signals in steps (b), (c1), and/or (c2) are detected using detectable probes that directly or indirectly bind to the target-specific barcode sequence or a complement thereof, the first probe-resolution barcode sequence or a complement thereof, and the second probe-resolution barcode sequence or a complement thereof, respectively, and optionally the detection comprises rolling circle amplification (RCA),

hybridization chain reaction (HCR), linear oligonucleotide hybridization chain reaction (LO-HCR), or primer exchange reaction (PER), or any combination thereof.

4. The method of any of claims 1-3, wherein the target-specific barcode sequence is about 20 nucleotides in length, and the first and second probe-resolution barcode sequences are about 5 nucleotides in length.

5. The method of any of claims 1-4, wherein the first and/or second probes further comprise an anchor sequence that is common among probes targeting different target analytes in the biological sample.

6. The method of any of claims 1-5, wherein the first and/or second probe-resolution barcode sequences are separated from the 5' or 3' nucleotide of the target-specific barcode sequence by 0, 1, 2, 3, 4, 5, or more nucleotide residues.

7. The method of any of claims 1-6, wherein the first probe-resolution barcode sequences is common among two or more probes each targeting a different target analyte in the biological sample and/or the second probe-resolution barcode sequence is common among two or more probes each targeting a different target analyte in the biological sample.

8. The method of claim 1, wherein the first molecule of the target analyte is of a first species of organism and the second molecule of the target analyte is of a second species of organism different from the first species of organism, and wherein the first and second probe-resolution barcode sequences are associated with the first and second species of organism, respectively.

9. The method of claim 1, wherein the first probe comprises a first target binding sequence complementary to a first nucleic acid sequence of the target analyte and the second probe comprises a second target binding sequence complementary to a second nucleic acid sequence of the target analyte.

10. The method of claim 1, wherein the first and second probes are circular probes or circularizable probes or probe sets.

11. The method of claim 10, wherein the first and second probes are circularized by ligation using a nucleic acid sequence in the target analyte and/or a splint as a template.

12. The method of claim 11, further comprising generating products of the circularized first probe and the circularized second probe *in situ* in the biological sample, wherein the products are amplification products generated using rolling circle amplification (RCA).

13. The method of claim 12, wherein the method comprises imaging the biological sample to detect the products *in situ* by sequential hybridization, sequencing by hybridization, sequencing by ligation, sequencing by synthesis, sequencing by binding, or a combination thereof.

14. The method of any of claims 12-13, wherein the products are rolling circle amplification (RCA) products and are detected by:

contacting the biological sample with one or more detectably-labeled probes that directly or indirectly hybridize to the RCA products, and

dehybridizing the one or more detectably-labeled probes from the RCA products,

optionally wherein the contacting and dehybridizing steps are repeated with the one or more detectably-labeled probes and/or one or more other detectably-labeled probes that directly or indirectly hybridize to the RCA products.

15. The method of any of claims 12-14, wherein the products are rolling circle amplification (RCA) products and are detected by:

contacting the biological sample with one or more intermediate probes that directly or indirectly hybridize to the RCA products, wherein the one or more intermediate probes are detectable using one or more detectably-labeled probes, and

dehybridizing the one or more intermediate probes and/or the one or more detectably-labeled probes from the RCA products,

optionally wherein the contacting and dehybridizing steps are repeated with the one or more intermediate probes, the one or more detectably-labeled probes, one or more other intermediate probes, and/or one or more other detectably-labeled probes.

16. The method of claim 15, wherein the one or more intermediate probes each comprises a sequence that hybridizes to one of the RCA products and one or more overhangs that hybridize to a detectably-labeled probe but not to the RCA product.

17. The method of any of claims 1-16, comprising:

(i) contacting the biological sample with detectable probes that hybridize to the target-specific barcode sequence or complement thereof;

(ii) imaging the biological sample to detect the plurality of signals of step (b);

(iii) optionally removing the detectable probes from the target-specific barcode sequence or complement thereof;

(iv) contacting the biological sample with detectable probes that hybridize to the first and second probe-resolution barcode sequences or complements thereof;

(v) imaging the biological sample to detect the signal of step (c1) in a first detection channel;

(vi) imaging the biological sample to detect the signal of step (c2) in a second detection channel that is different from the first detection channel; and

(vii) optionally removing the detectable probes from the first and second probe-resolution barcode sequences or complements thereof.

18. The method of claim 17, further comprising registering images of the imaging steps for detecting the plurality of signals of step (b), the signal of step (c1), and the signal of step (c2), wherein the plurality of signals of step (b), the signal of step (c1), and the signal of step (c2) are associated using the registered images.

19. A method for analyzing a biological sample, comprising:

(a) contacting the biological sample with a plurality of circular or circularizable probes comprising a first circular or circularizable probe and a second circular or circularizable probe,

wherein the first circular or circularizable probe comprises a target-specific barcode sequence and a first probe-resolution barcode sequence, and the second circular or circularizable probe comprises the target-specific barcode sequence and a second probe-resolution barcode sequence, and

wherein the plurality of circular or circularizable probes hybridize to different nucleic acid molecules in the biological sample, and the target-specific barcode sequence corresponds to a target nucleic acid;

(b) generating rolling circle amplification (RCA) products of the first and second circular or circularizable probes;

(c) contacting the biological sample with detectable probes that hybridize to the RCA products at the complement of the target-specific barcode sequence;

(d) detecting signals associated with the target-specific barcode sequence;

(e) contacting the biological sample with detectable probes that hybridize to the RCA products at the complement of the first probe-resolution barcode sequence and with detectable probes that hybridize to the RCA products at the complement of the second probe-resolution barcode sequence; and

(f) detecting, in separate detection channels, a signal associated with the first probe-resolution barcode sequence and a signal associated with the second probe-resolution barcode sequence.

20. A method for analyzing a biological sample, comprising:

(a) contacting the biological sample with a plurality of probes each comprising a target-specific barcode sequence associated with a target analyte,

wherein a first probe of the plurality of probes comprises a first probe-resolution barcode sequence associated with a first species of organism and a second probe of the plurality of probes comprises a second probe-resolution barcode sequence associated with a second species of organism, and

wherein the first probe targets a first nucleic acid sequence of the target analyte of the first species of organism and the second probe targets a second nucleic acid sequence of the target analyte of the second species of organism, and the target-specific barcode sequence corresponds to the target analyte, wherein the first nucleic acid sequence and the second nucleic acid sequence are homologs of the target analyte in the first and second species of organism, respectively;

(b) detecting a plurality of signals associated with the target-specific barcode sequences of the plurality of probes;

(c1) detecting a signal associated with the first probe-resolution barcode sequence; and

(c2) detecting a signal associated with the second probe-resolution barcode sequence,

wherein the signals of steps (c1) and (c2) are associated with the target analyte.

21. A kit for analyzing a biological sample, comprising:

(i) a plurality of probes each comprising a target-specific barcode sequence,

wherein a first probe of the plurality of probes comprises a first probe-resolution barcode sequence and a second probe of the plurality of probes comprises a second probe-resolution barcode sequence, and

wherein the plurality of probes target different molecules of a target analyte in the biological sample, and the target-specific barcode sequence corresponds to the target analyte;

(ii) detectable probes that directly or indirectly bind to the target-specific barcode sequence or complement thereof;

(iii) detectable probes that directly or indirectly bind to the first probe-resolution barcode sequence or complement thereof; and

(iv) detectable probes that directly or indirectly bind to the second probe-resolution barcode sequence or complement thereof.