CLAIMS

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

(a) contacting the biological sample with a first reaction mixture, wherein:

the biological sample comprises a circular nucleic acid comprising a hybridization region,

the first reaction mixture comprises a polymerase, wherein the biological sample comprises cells or is a tissue sample,

the circular nucleic acid or the polymerase is prebound to a polynucleotide comprising a sequence complementary to the hybridization region, and

the polymerase activity of the polymerase is inhibited; and

(b) contacting the biological sample with a second reaction mixture to allow the polymerase to extend the polynucleotide hybridized to the hybridization region using the circular nucleic acid as a template,

wherein a rolling circle amplification product of the circular nucleic acid is generated in the biological sample.

2. The method of claim 1, wherein the first reaction mixture is substantially free of deoxynucleoside triphosphates (dNTPs) and/or nucleoside triphosphates (NTPs).

3. The method of claim 1 or 2, wherein the first reaction mixture comprises a di-cation that is not a cofactor of the polymerase, optionally wherein the di-cation is Sr^{2+} or Ca^{2+}.

4. The method of claim 1 or 2, wherein the first reaction mixture is substantially free of a cofactor of the polymerase, optionally wherein the cofactor comprises Mg^{2+}, Co^{2+}, and/or Mn^{2+}.

5. The method of claim 4, wherein the first reaction mixture comprises a chelating agent, optionally wherein the chelating agent comprises EDTA, EGTA, BAPTA, DTPA, or a combination thereof.

6. The method of any of claims 1-5, wherein the polynucleotide comprises a 3′ protective group.

7. The method of claim 6, wherein the polynucleotide is 3′ thiophosphate-protected, thereby protecting the polynucleotide from 3'→5' exonuclease degradation by the polymerase while allowing priming by the polymerase.

8. The method of any of claims 1-7, wherein the polynucleotide is a primer, and the primer is prebound to the polymerase in the first reaction mixture prior to contacting the biological sample in step (a).

9. The method of any of claims 1-8, wherein the hybridization region in the circular nucleic acid is a primer hybridization region that hybridizes to the primer, and the circular nucleic acid further comprises a target hybridization region that hybridizes to a target nucleic acid.

10. The method of any of claims 1-8, wherein the polynucleotide is a target nucleic acid, and the target nucleic acid is prebound to the circular nucleic acid in the biological sample prior to and/or during contacting the first reaction mixture in step (a).

11. The method of claim 9 or 10, further comprising removing one or more molecules of the polymerase that are not bound to the circular nucleic acid from the biological sample prior to contacting the biological sample with the second reaction mixture.

12. The method of any of claims 1-11, wherein the second reaction mixture:

(a) comprises a deoxynucleoside triphosphate (dNTP) and/or a nucleoside triphosphate (NTP); and

(b) comprises a cofactor of the polymerase, optionally wherein the cofactor is a di-cation.

13. The method of any of claims 1-12, wherein the second reaction mixture is substantially free of the polymerase and/or other polymerases.

14. The method of any of claims 1-13, wherein the method comprises imaging the biological sample to detect the rolling circle amplification product.

15. The method of any of claim 1-14, wherein a sequence of the rolling circle amplification product is analyzed *in situ* in the biological sample.

16. The method of any of claim 1-15, wherein the circular nucleic acid is formed in the biological sample from a probe or a probe set for a target molecule.

17. The method of any of claims 1-16, wherein the biological sample is a tissue sample.

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

(a) contacting the biological sample with a binding mixture, wherein:

the biological sample comprises a plurality of circular nucleic acids each comprising a primer hybridization region,

the binding mixture comprises a plurality of complexes each comprising a polymerase bound to a primer, wherein the primer comprises a sequence complementary to the primer hybridization region of one or more circular nucleic acids, wherein the biological sample

comprises cells or is a tissue sample, and

the polymerase activity of the polymerase is inhibited,

thereby allowing the plurality of complexes to hybridize to the plurality of circular nucleic acids; and

(b) contacting the biological sample with a primer extension reaction mixture to allow the polymerase to extend the primer hybridized to the primer hybridization region,

thereby synchronizing rolling circle amplification the plurality of circular nucleic acids in the biological sample.

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

(a) contacting the biological sample with a binding mixture, wherein:

the biological sample comprises a plurality of circular nucleic acids each comprising a hybridization region hybridized to a polynucleotide,

the binding mixture comprises a polymerase, wherein the biological sample

comprises cells or is a tissue sample, and

the polymerase activity of the polymerase is inhibited,

thereby allowing the polymerase to bind to the plurality of circular nucleic acids; and

(b) contacting the biological sample with a primer extension reaction mixture to allow the polymerase to extend the polynucleotide hybridized to the hybridization region,

thereby synchronizing rolling circle amplification (RCA) of the plurality of circular nucleic acids in the biological sample.

20. The method of claim 19, wherein:

(a) the standard deviation of the diameter of the plurality of RCA products is smaller than the standard deviation of the diameter of RCA products generated using a non-synchronized method;

(b) the mean, average, or medium peak intensity value of the plurality of RCA products is 2 to 10 times greater than a mean, average, or medium peak intensity value of RCA products formed without synchronizing RCA of the plurality of circular nucleic acids in the biological sample; or

(c) the distribution of the relative observed signal of RCA products formed with synchronizing RCA of the plurality of circular nucleic acids in the biological sample is narrower than the distribution of the relative observed signal of RCA products formed without synchronizing RCA of the plurality of circular nucleic acids in the biological sample.