CLAIMS

What is claimed is:

1. A method for profiling RNAs being translated in a cell, the method comprising:

a) contacting the cell with one or more sets of probes, wherein each set of the one or more sets of probes comprises a first probe, a second probe, and a third probe, wherein

i) the first probe comprises an oligonucleotide portion that is complementary to a portion of the second probe, a first oligonucleotide barcode sequence, an oligonucleotide portion that is complementary to a portion of the third probe, an oligonucleotide portion that is complementary to an RNA of interest, and a second oligonucleotide barcode sequence;

ii) the second probe comprises a portion that recognizes a ribosome and an oligonucleotide portion that is complementary to a portion of the first probe; and

iii) the third probe comprises an oligonucleotide portion that is complementary to the RNA of interest, an oligonucleotide portion that is complementary to a portion of the first probe, and an oligonucleotide barcode sequence, wherein the oligonucleotide barcode sequence of the third probe is complementary to the first oligonucleotide barcode sequence of the first probe;

b) ligating the 5′ end and the 3′ end of the first probe together to produce a circular oligonucleotide;

c) performing rolling circle amplification to amplify the circular oligonucleotide using the third probe as a primer to produce one or more concatenated amplicons;

d) embedding the one or more concatenated amplicons in a polymeric matrix; and

e) sequencing the concatenated amplicons embedded in the polymeric matrix to determine an identity and location of each RNA of interest in the cell.

2. The method of claim 1, wherein the portion of the second probe that recognizes the ribosome comprises 1) an agent that binds an antibody, or an antibody variant or fragment; 2) an antibody, or an antibody variant or fragment; or 3) an oligonucleotide that is complementary to a portion of rRNA within the ribosome.

3. The method of claim 2, wherein the portion of the second probe that recognizes the ribosome comprises an antibody, wherein the antibody is a secondary antibody, and wherein the method further comprises contacting the cell with a primary antibody that recognizes the ribosome and can be bound by the secondary antibody of the second probe.

4. The method of claim 3, wherein the primary antibody is an anti-40S ribosomal protein S3 (RPS3) antibody or an anti-60S ribosomal protein L4 (RPL4) antibody.

5. The method of claim 2, wherein the portion of the second probe that recognizes the ribosome comprises an oligonucleotide that is complementary to a portion of an 18S rRNA.

6. The method of any one of claims 1-5, wherein the second probe further comprises a polymerization blocker.

7. The method of any one of claims 1-6, wherein the oligonucleotide barcode sequences are 5-15, 6-14, 7-13, 8-12, or 9-11 nucleotides in length.

8. The method of any one of claims 1-7, wherein

(i) the first probe comprises the structure:

5′-[portion complementary to portion of second probe]-[first barcode sequence]-[portion complementary to portion of third probe]-[portion complementary to RNA of interest]-[second barcode sequence]-3′;

(ii) the second probe comprises the structure:

5′-[portion recognizing ribosome]-[portion complementary to portion of first probe]-3′; and/or

(iii) the third probe comprises the structure:

5′-[portion complementary to RNA of interest]-[portion complementary to portion of first probe]-[barcode sequence]-3′.

9. The method of any one of claims 1-7, wherein the portion of the first probe that is complementary to a portion of the second probe is split between the 5′ end and the 3′ end of the first probe.

10. The method of any one of claims 1-9, wherein RNAs being translated are profiled in multiple cells simultaneously.

11. The method of claim 10, wherein the multiple cells comprise a plurality of cell types.

12. The method of any one of claims 1-11, wherein the cell is present within an intact tissue.

13. The method of any one of claims 1-12, wherein translation of more than 1, more than 2, more than 3, more than 4, more than 5, more than 10, more than 20, more than 30, more than 40, more than 50, more than 100, more than 200, more than 500, more than 1000, more than 2000, more than 3000, more than 4000, or more than 5000 RNAs are profiled simultaneously.

14. The method of any one of claims 1-13, wherein the second oligonucleotide barcode sequence of the first probe is a gene specific sequence used to identify an RNA of interest.

15. The method of any one of claims 1-14, wherein the step of sequencing comprises performing sequencing with error-reduction by dynamic annealing and ligation (SEDAL).

16. The method of any one of claims 1-15, wherein the step of performing rolling circle amplification further comprises providing amine-modified nucleotides, wherein the amine-modified nucleotides are incorporated into the one or more concatenated amplicons.

17. The method of claim 16, wherein the step of embedding the one or more concatenated amplicons in the polymeric matrix comprises reacting the amine-modified nucleotides of the one or more concatenated amplicons with acrylic acid N-hydroxysuccinimide ester and co-polymerizing the one or more concatenated amplicons and the polymeric matrix.

18. The method of any one of claims 1-17 further comprising:

(i) profiling additional molecules within the cell;

(ii) determining a cell type of the profiled cell, or cell types of multiple profiled cells, by comparing an RNA translation profile of the cell or cells to reference data comprising RNA translation profiles of cells of various cell types;

(iii) determining a cell state of the profiled cell, or cell states of multiple profiled cells, by comparing an RNA translation profile of the cell or cells to reference data comprising RNA translation profiles of cells of various cell states; and/or

(iv) overexpressing or knocking out one or more genes in the cell to determine whether the one or more genes are involved in regulating translation of the RNA of interest.

19. The method of any one of claims 1-18, wherein the method does not disrupt spatial information of subcellular structure, cell morphology, and/or tissue organization.

20. A method for profiling RNAs being translated in a cell, the method comprising:

a) contacting the cell with one or more pairs of probes, wherein each pair of the one or more pairs of probes comprises a first probe and a second probe, wherein

i) the first probe comprises an oligonucleotide portion that is complementary to a portion of the second probe, an oligonucleotide portion that is complementary to an RNA of interest, and an oligonucleotide barcode sequence; and

ii) the second probe comprises a portion that recognizes a ribosome and an oligonucleotide portion that is complementary to a portion of the first probe;

b) ligating the 5′ end and the 3′ end of the first probe together to produce a circular oligonucleotide;

c) performing rolling circle amplification to amplify the circular oligonucleotide using the second probe as a primer to produce one or more concatenated amplicons;

d) embedding the one or more concatenated amplicons in a polymeric matrix; and

e) sequencing the concatenated amplicons embedded in the polymeric matrix to determine the identity and location of each RNA of interest in the cell.

21. A pair of probes comprising a first probe and a second probe, wherein:

i) the first probe comprises an oligonucleotide portion that is complementary to a portion of the second probe, an oligonucleotide portion that is complementary to an RNA of interest, and an oligonucleotide barcode sequence; and

ii) the second probe comprises a portion that recognizes the ribosome and an oligonucleotide portion that is complementary to a portion of the first probe.

22. A set of probes comprising a first probe, a second probe, and a third probe, wherein:

i) the first probe comprises an oligonucleotide portion that is complementary to a portion of the second probe, a first oligonucleotide barcode sequence, an oligonucleotide portion that is complementary to a portion of the third probe, an oligonucleotide portion that is complementary to an RNA of interest, and a second oligonucleotide barcode sequence;

ii) the second probe comprises a portion that recognizes a ribosome and an oligonucleotide portion that is complementary to a portion of the first probe; and

iii) the third probe comprises an oligonucleotide portion that is complementary to the RNA of interest, an oligonucleotide portion that is complementary to a portion of the first probe, and an oligonucleotide barcode sequence, wherein the oligonucleotide barcode sequence of the third probe is complementary to the first oligonucleotide barcode sequence of the first probe.

23. A plurality of probes comprising multiple pairs of probes of claim 21 or multiple sets of probes of claim 22, wherein each pair of probes or set of probes comprises oligonucleotide portions that are complementary to a different RNA of interest.

24. A system for profiling RNAs being translated in a cell comprising

a) a cell;

b) one or more pairs of probes comprising a first probe and a second probe, wherein

i) the first probe comprises an oligonucleotide portion that is complementary to a portion of the second probe, an oligonucleotide portion that is complementary to an RNA of interest, and an oligonucleotide barcode sequence; and

ii) the second probe comprises a portion that recognizes a ribosome and an oligonucleotide portion that is complementary to a portion of the first probe;

c) a microscope; and

d) a computer.

25. A system for profiling RNAs being translated in a cell comprising

a) a cell;

b) one or more sets of probes comprising a first probe, a second probe, and a third probe, wherein

i) the first probe comprises an oligonucleotide portion that is complementary to a portion of the second probe, a first oligonucleotide barcode sequence, an oligonucleotide portion that is complementary to a portion of the third probe, an oligonucleotide portion that is complementary to an RNA of interest, and a second oligonucleotide barcode sequence;

ii) the second probe comprises a portion that recognizes a ribosome and an oligonucleotide portion that is complementary to a portion of the first probe; and

iii) the third probe comprises an oligonucleotide portion that is complementary to the RNA of interest, an oligonucleotide portion that is complementary to a portion of the first probe, and an oligonucleotide barcode sequence, wherein the oligonucleotide barcode sequence of the third probe is complementary to the first oligonucleotide barcode sequence of the first probe;

c) a microscope; and

d) a computer.