CLAIMS

What is claimed is:

1. A method for mapping gene and protein expression in a cell, the method comprising:

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

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

ii) the second oligonucleotide probe comprises a portion that is complementary to the nucleic acid of interest, a portion that is complementary to the first oligonucleotide probe, and a barcode sequence, wherein the barcode sequence of the second oligonucleotide probe is complementary to the second barcode sequence of the first oligonucleotide probe;

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

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

d) contacting the cell with one or more detecting agents, wherein each detecting agent binds to a protein of interest;

e) embedding the one or more concatenated amplicons and the one or more detecting agents in a polymeric matrix;

f) contacting the one or more concatenated amplicons embedded in the polymeric matrix with a third oligonucleotide probe comprising a sequence that is complementary to the first barcode sequence of the first oligonucleotide probe; and

g) imaging the one or more concatenated amplicons embedded in the polymeric matrix and the one or more detecting agents embedded in the polymeric matrix to determine the location of the nucleic acids of interest and the proteins of interest within the cell and, optionally, map gene and protein expression.

2. The method of claim 1, wherein gene and protein expression are profiled in multiple cells, optionally wherein the cells comprise a plurality of cell types, optionally wherein the cell types are selected from the group consisting of stem cells, progenitor cells, neuronal cells, astrocytes, dendritic cells, endothelial cells, microglia, oligodendrocytes, muscle cells, myocardial cells, mesenchymal cells, epithelial cells, immune cells, and hepatic cells, wherein the cell is a permeabilized cell and/or wherein the cell is present within an intact tissue, optionally wherein the intact tissue is a fixed tissue sample.

3. The method of claim 1 or 2, wherein the nucleic acid of interest is DNA or RNA, optionally wherein the RNA is mRNA, wherein gene expression for more than 100, more than 200, more than 500, more than 1000, more than 2000, or more than 3000 nucleic acids of interest is mapped, optionally wherein gene expression for up to one million nucleic acids of interest is mapped.

4. The method of any one of claims 1-3, wherein the barcode sequences on the first and second oligonucleotide probes are 5-15, 6-14, 7-13, 8-12, or 9-11 nucleotides in length; and/or wherein the barcode sequences on the first and second oligonucleotide probes are 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 nucleotides in length, optionally wherein the barcode sequences on the first and second oligonucleotide probes are 10 nucleotides in length; and/or wherein the first barcode sequence of the first oligonucleotide probe is a gene-specific sequence used to identify a nucleic acid of interest.

5. The method of any one of claims 1-4, wherein the first and second oligonucleotide probes bind different portions of the nucleic acid of interest, optionally wherein the first oligonucleotide probe comprises the structure:

5′-[portion complementary to second probe]-[portion complementary to nucleic acid of interest]-[first barcode sequence]-[second barcode sequence]-3′, and/or

the second oligonucleotide probe comprises the structure:

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

6. The method of any one of claims 1-5, wherein the second barcode sequence of the first oligonucleotide probe increases specificity of the detection of the nucleic acid of interest

, and/or wherein the second barcode sequence of the first oligonucleotide probe reduces non-specific amplification.

7. The method of any one of claims 1-6, wherein the third oligonucleotide probe comprises a detectable label, optionally wherein the detectable label is a fluorophore.

8. The method of any one of claims 1-7, wherein the one or more detecting agents are selected from antibodies that each comprise a detectable label, antibodies conjugated to an oligonucleotide sequence, and small molecules, optionally wherein the antibodies comprise anti-p-Tau antibodies and/or wherein the small molecule is X-34, and optionally wherein the method further comprises contacting the detecting agents with a secondary detecting agent that is a secondary antibody or an oligonucleotide sequence conjugated to a detectable label, preferably wherein the detectable label is a fluorophore.

9. The method of any one of claims 1-8, wherein the step of imaging comprises fluorescence imaging, optionally wherein the step of imaging comprises confocal microscopy or epifluorescence microscopy; and/or wherein the locations of the nucleic acids of interest and the proteins of interest are determined in the same round of imaging; or wherein the locations of the nucleic acids of interest and the proteins of interest are determined in separate rounds of imaging.

10. The method of any one of claims 1-9, wherein the step of contacting each of the one or more detecting agents embedded in the polymeric matrix with a secondary detecting agent is performed after the step of performing rolling circle amplification to amplify the circular oligonucleotide.

11. The method of any one of claims 1-10, wherein the step of contacting the cell with one or more detecting agents is performed before the step of embedding.

12. The method of any one of claims 1-11, wherein the polymeric matrix is a hydrogel, optionally wherein the hydrogel is a polyvinyl alcohol hydrogel, a polyethylene glycol hydrogel, a sodium polyacrylate hydrogel, an acrylate polymer hydrogel, or a polyacrylamide hydrogel.

13. The method of any one of claims 1-12, 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, optionally wherein the step of embedding the one or more concatenated amplicons in the polymer matrix comprises reacting the amine-modified nucleotides of the one or more amplicons with acrylic acid *N*-hydroxysuccinimide ester and co-polymerizing the one or more concatenated amplicons and the polymeric matrix.

14. The method of any one of claims 1-13, wherein the method is performed at subcellular resolution, optionally wherein the method is performed at a subcellular resolution of 200 nm, 150 nm, 100 nm, 50 nm, 40 nm, 30 nm, 20 nm, or 10 nm, preferably wherein the method is performed at a subcellular resolution of 200 nm.

15. A method for mapping gene expression in a cell, the method comprising:

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

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

ii) the second oligonucleotide probe comprises a portion that is complementary to the nucleic acid of interest, a portion that is complementary to the first oligonucleotide probe, and a barcode sequence, wherein the barcode sequence of the second oligonucleotide probe is complementary to the second barcode sequence of the first oligonucleotide probe;

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

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

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

e) contacting the one or more concatenated amplicons embedded in the polymeric matrix with a third oligonucleotide probe comprising a sequence that is complementary to the first barcode sequence of the first oligonucleotide probe; and

f) imaging the one or more concatenated amplicons embedded in the polymeric matrix to determine the location of the nucleic acids of interest within the cell and, optionally, map gene expression.

16. A method for mapping gene and protein expression in a cell, the method comprising:

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

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

ii) the second oligonucleotide probe comprises a portion that is complementary to the nucleic acid of interest and a portion that is complementary to the first oligonucleotide probe;

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

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

d) contacting the cell with one or more detecting agents, wherein each detecting agent binds to a protein of interest;

e) embedding the one or more concatenated amplicons and the one or more detecting agents in a polymeric matrix;

f) contacting the one or more concatenated amplicons embedded in the polymeric matrix with a third oligonucleotide probe comprising a sequence that is complementary to the barcode sequence of the first oligonucleotide probe; and

g) imaging the one or more concatenated amplicons embedded in the polymeric matrix and the one or more detecting agents embedded in the polymeric matrix to determine the location of the nucleic acids of interest and the proteins of interest within the cell and, optionally, map gene and protein expression.

17. A plurality of oligonucleotide probes comprising a first oligonucleotide probe and a second oligonucleotide probe, wherein

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

ii) the second oligonucleotide probe comprises a portion that is complementary to the nucleic acid of interest, a portion that is complementary to the first oligonucleotide probe, and a barcode sequence,

wherein the barcode sequence of the second oligonucleotide probe is complementary to the second barcode sequence of the first oligonucleotide probe.

18. The plurality of oligonucleotide probes of claim 17, wherein the barcode sequences on the first and second oligonucleotide probes are 5-15, 6-14, 7-13, 8-12, or 9-11 nucleotides in length; and/or, wherein the barcode sequences on the first and second oligonucleotide probes are 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 nucleotides in length, optionally wherein the barcode sequences on the first and second oligonucleotide probes are 10 nucleotides in length; and/or wherein the first barcode sequence of the first oligonucleotide probe is a gene-specific sequence used to identify a nucleic acid of interest, wherein the first oligonucleotide probe comprises the structure:

5′-[portion complementary to second probe]-[portion complementary to nucleic acid of interest]-[first barcode sequence]-[second barcode sequence]-3′; and/or

wherein the second oligonucleotide probe comprises the structure:

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

19. A kit comprising the plurality of oligonucleotide probes of claim 17 or 18, optionally wherein the kit further comprises one or more antibodies, wherein each antibody binds to a protein of interest, optionally wherein the antibodies comprise an anti-p-Tau antibody; and/or wherein the kit further comprises a small molecule detecting agent, optionally wherein the small molecule detecting agent is X-34; and/or wherein the kit further comprises a third oligonucleotide probe, optionally wherein the third oligonucleotide probe comprises a sequence that is complementary to the first barcode sequence of the first oligonucleotide

probe, and optionally wherein the third oligonucleotide probe comprises a detectable label, preferably wherein the detectable label is a fluorophore.

20. A system comprising:

a) a cell; and

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

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

ii) the second oligonucleotide probe comprises a portion that is complementary to the nucleic acid of interest, a portion that is complementary to the first oligonucleotide probe, and a barcode sequence, wherein the barcode sequence of the second oligonucleotide probe is complementary to the second barcode sequence of the first oligonucleotide probe; and, optionally, one or more of

c) a microscope, optionally wherein the microscope is a confocal microscope;

a computer; and one or more additional cells, optionally wherein the cells are part of a tissue sample.