CLAIMS

WHAT IS CLAIMED IS:

1. A method for processing a biological sample comprising a polypeptide analyte and a ribonucleic acid (RNA) analyte, wherein the biological sample is a cell or tissue sample, comprising:

(a) contacting said biological sample with a tethering molecule, wherein said tethering molecule comprises (i) an antibody or fragment thereof that binds to said polypeptide analyte and (ii) a nucleic acid barcode conjugated to said antibody or fragment thereof, wherein said nucleic acid barcode identifies said antibody or fragment thereof and wherein said nucleic acid is conjugated to an acryloyl group;

(b) contacting said biological sample with a primer complementary to a sequence of said RNA molecule and extending said primer to generate a complementary deoxyribonucleic acid (cDNA) molecule;

(c) contacting said biological sample having said tethering molecule bound to said polypeptide analyte with a matrix-forming material comprising a click chemistry moiety and using said matrix-forming material to form a synthetic three-dimensional (3D) matrix comprising said click chemistry moiety wherein said acryloyl group is incorporated into said synthetic 3D matrix, thereby tethering said nucleic acid barcode to said synthetic 3D matrix;

(d) subjecting said biological sample to a clearing process;

(e) hybridizing a probe to said cDNA molecule;

(f) circularizing said probe;

(g) amplifying said circularized probe using a modified base to generate an amplification product comprising said modified base, wherein said modified base comprises an additional click chemistry moiety;

(h) attaching said amplification product to said synthetic 3D matrix via a click chemistry reaction;

(i) detecting said nucleic acid barcode to identify said polypeptide analyte in said biological sample; and

(j) detecting said amplification product to identify said RNA analyte in said biological sample.

2. The method of claim 1, wherein said click chemistry moiety comprises an alkyne, wherein said modified base comprises an azide, and wherein said click chemistry reaction comprises reacting said alkyne with said azide.
3. The method of claim 2, wherein said modified base is 5-azidomethyl-dUTP.
4. The method of any one of claims 1-3, wherein said cell or tissue sample is fixed.
5. The method of claim 4, further comprising, prior to (a), contacting said biological sample with a fixative.
6. The method of any one of claims 1-5, wherein said cell or said tissue sample is permeabilized.
7. The method of claim 6, further comprising, prior to (a), contacting said biological sample with a permeabilization agent.
8. The method of any one of claims 1-7, wherein said probe is a padlock probe or a molecular inversion probe.
9. The method of claim 8, wherein circularizing said probe comprises ligating a 3' end and a 5' end of said probe.
10. The method of any one of claims 1-9, wherein said primer comprises an additional acryloyl group and wherein said additional acryloyl group is incorporated into said synthetic 3D matrix.
11. The method of any one of claims 1-10, wherein said clearing process comprises contacting said biological sample with an enzyme capable of degrading a protein.
12. The method of claim 11, wherein said enzyme capable of degrading a protein comprises a protease.
13. The method of any one of claims 1-12, wherein said clearing process comprises contacting said biological sample with a surfactant or a denaturant.
14. The method of any one of claims 1-13, wherein said clearing process comprises contacting said biological sample with an enzyme capable of degrading a nucleic acid, wherein said capable of degrading a nucleic acid is optionally an RNase.
15. The method of any one of claims 1-14, wherein detecting said nucleic acid barcode comprises sequencing said nucleic acid barcode and wherein detecting said amplification product comprises sequencing at least a portion of said amplification product.
16. The method of any one of claims 1-15, wherein detecting said nucleic acid barcode comprises hybridizing a labeled probe to said nucleic acid barcode and detecting said labeled probe and wherein detecting said amplification product comprises hybridizing

an additional labeled probe to said amplification product and detecting said additional labeled probe.