What is claimed is:

1. A method for in situ gene sequencing of a target nucleic acid in a cell in an intact tissue, the method comprising:
(a) contacting a fixed and permeabilized intact tissue sample with at least a pair of oligonucleotides under conditions to allow for specific hybridization,
wherein the pair of oligonucleotides comprises a first oligonucleotide and a second oligonucleotide; wherein each of the first oligonucleotide and the second oligonucleotide comprises a first complementarity region, a second complementarity region, and a third complementarity region; wherein the second oligonucleotide further comprises a barcode sequence; wherein the first complementarity region of the first oligonucleotide is complementary to a first portion of the target nucleic acid, wherein the second complementarity region of the first oligonucleotide is complementary to the first complementarity region of the second oligonucleotide, wherein the third complementarity region of the first oligonucleotide is complementary to the third complementarity region of the second oligonucleotide, wherein the second complementarity region of the second oligonucleotide is complementary to a second portion of the target nucleic acid, and wherein the first complementarity region of the first oligonucleotide is adjacent to the second complementarity region of the second oligonucleotide;
(b) adding ligase to ligate the second oligonucleotide 5' and 3' ends to generate a closed nucleic acid circle;
(c) preincubating the tissue sample with a DNA polymerase for a sufficient time to allow uniform diffusion of the DNA polymerase throughout the tissue sample before performing rolling circle amplification;
(d) performing rolling circle amplification by contacting the tissue sample with deoxyribonucleotide triphosphates such that one or more amplicons are generated, wherein the ligated second oligonucleotide serves as a template and the first oligonucleotide serves as a primer for the DNA polymerase;
(e) embedding the tissue sample in a hydrogel before or after any one of steps (a)-(d);
(f) cross-linking the one or more amplicons to the hydrogel;
(g) clearing the hydrogel to enhance hydrogel transparency;
(h) contacting the one or more hydrogel-embedded amplicons having the barcode sequence with a read primer and a fluorescently labeled probe, wherein the probe comprises an oligonucleotide comprising a first thiol group covalently linked to a fluorophore comprising a second thiol group by a disulfide bond between the first thiol group and the second thiol group;
(i) ligating the read primer and the fluorescently labeled probe, wherein the ligation only occurs when both the read primer and the fluorescently labeled probe are complementary to adjacent sequences of the same amplicon;
(j) contacting the hydrogel with an anti-fade buffer comprising an antioxidant;

(k) imaging the one or more hydrogel-embedded amplicons to determine positioning of the target nucleic acid in the cell in the intact tissue that is undergoing in situ gene sequencing, wherein imaging is performed in presence of the anti-fade buffer;
(l) contacting the hydrogel with a reducing agent resulting in reduction of the disulfide bond and cleavage of the fluorophore from the probe;
(m) removing the fluorophore from the hydrogel; and
(n) reiterating steps (h)-(m).

2. The method of claim 1, wherein the anti-oxidant is N-propyl gallate.

3. The method of claim 1 or 2, wherein RNAs endogenous to the tissue sample are first attached to the hydrogel prior to step (a).

4. The method of any one of claims 1-3, wherein said clearing the hydrogel is performed before or after any one of steps (a)-(d).

5. The method of any one of claims 1-4, wherein the contacting the fixed and permeabilized intact tissue comprises hybridizing a plurality of oligonucleotide primers having specificity for different target nucleic acids.

6. The method of any one of claims 1-5, further comprising sectioning the intact tissue and placing a section of the intact tissue flat onto a surface.

7. The method of claim 6, wherein a metal ring is pressed down onto the section and used to transfer the section flat onto the surface.

8. The method of any one of claims 1-7, wherein the reducing agent is TCEP.

9. The method of any one of claims 1-8, wherein the hydrogel is contacted with the reducing agent in step (l) for 30 minutes.

10. The method of any one of claims 1-9, further comprising exposing the read primer and the fluorescently labeled probe to a stripping buffer comprising 80% formamide prior to step (l).