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~~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 ~~0~~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~~ 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 0-4, wherein the cell is present in a population of cells.~~

~~6. The method of claim 5, wherein the population of cells comprises a plurality of cell types.~~

~~7. The method of any one of claims 0-6, wherein the target nucleic acid is RNA or DNA.~~

~~8. The method of claim 7, wherein the RNA is mRNA.~~

~~9. The method of any one of claims 0-8, wherein the second oligonucleotide comprises a padlock probe.~~

~~10. The method of any one of claims 0-9, wherein the first complementarity region of the first oligonucleotide has a length of 19-25 nucleotides.~~

11. The method of any one of claims 0-10, wherein the second complementarity region of the first oligonucleotide has a length of 6 nucleotides.

12. The method of any one of claims 0-0, wherein the third complementarity region of the first oligonucleotide has a length of 6 nucleotides.

13. The method of any one of claims 0-0, wherein the first complementarity region of the second oligonucleotide has a length of 6 nucleotides.

14. The method of any one of claims 0-0, wherein the second complementarity region of the second oligonucleotide has a length of 19-25 nucleotides.

15. The method of any one of claims 0-0, wherein the third complementarity region of the second oligonucleotide has a length of 6 nucleotides.

16. The method of any one of claims 0-0, wherein the first complementarity region of the second oligonucleotide comprises the 5' end of the second oligonucleotide.

17. The method of any one of claims 0-0, wherein the third complementarity region of the second oligonucleotide comprises the 3' end of the second oligonucleotide.

18. The method of any one of claims 0-0, wherein the first complementarity region of the second oligonucleotide is adjacent to the third complementarity region of the second oligonucleotide.

19. The method of any one of claims 1-0, wherein the barcode sequence of the second oligonucleotide provides barcoding information for identification of the target nucleic acid.

20<u>5</u>. The method of any one of claims 1-<u>4</u>0, wherein the contacting the fixed and permeabilized intact tissue comprises hybridizing a plurality of oligonucleotide primers having specificity for different target nucleic acids.

21. The method of claim 0, wherein the second oligonucleotide is provided as a closed nucleic acid circle, and the step of adding ligase is omitted.

22. The method of any one of claims 0-21 , wherein the deoxynucleotide triphosphates comprise an amine-modified deoxynucleotide triphosphate.

~~23. The method of claim 22, wherein the amine-modified deoxynucleotide triphosphate comprises an acrylic acid N-hydroxysuccinimide moiety modification.~~

~~24. The method of any one of claims 0-23, wherein the embedding comprises copolymerizing the one or more amplicons with acrylamide.~~

~~25. The method of any one of claims 0-24, wherein the target nucleic acid is substantially retained in the hydrogel after the clearing.~~

~~26. The method of any one of claims 1 -25, wherein the clearing comprises substantially removing a plurality of cellular components from the hydrogel-embedded tissue sample.~~

~~27. The method of claim 25 or 26, wherein the clearing comprises substantially removing lipids and proteins from the hydrogel-embedded tissue sample.~~

~~28. The method of any one of claims 0-27, wherein the imaging comprises imaging the one or more hydrogel-embedded amplicons using confocal microscopy, two-photon microscopy, light-field microscopy, intact tissue expansion microscopy, and/or CLARITY™-optimized light sheet microscopy (COLM).~~

~~29. The method of any one of claims 1-28, wherein the intact tissue has a thickness of 5-20 μm.~~

~~30. The method of any one of claims 1-28, wherein the intact tissue has a thickness of 50-200 μm.~~

~~31~~6. The method of any one of claims 1-~~30~~5, further comprising sectioning the intact tissue and placing a section of the intact tissue flat onto a surface.

~~32~~7. The method of claim ~~31~~ 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).

~~33~~11. A fluidic system, comprising:

~~h~~a) a device comprising an unpressurized sample chamber;

b~~i~~) a lid, wherein the lid covers the top of the sample chamber;

c~~j~~) an interface tube, wherein a first end of the interface tube is held by the lid, and a second end of the interface tube is positioned above a tissue sample in the sample chamber sufficiently close to a bottom edge of the sample chamber such that liquid within the sample chamber remains in contact with the bottom of the interface tube as long as liquid remains in the sample chamber, optionally, wherein the interface tube is placed at a polar angle relative to the tissue sample plane;

d~~k~~) a fluidic line coupled to the first end of the interface tube such that fluid flows through the fluidic line into the interface tube and flows out of the second end of the interface tube onto the tissue sample in the sample chamber;

e~~L~~) a pump, optionally, wherein the pump is a syringe pump;

~~m~~f) an imaging system; and

g~~n~~) a processor unit configured to perform the method of any one of claims 1~~0-32~~10.

~~34. The fluidic system of claim 33, wherein the sample chamber is a well of a multiwell plate or a slide chamber.~~

~~35. The fluidic system of claim 33 or 34, wherein the interface tube is placed at a polar angle relative to the tissue sample plane.~~

~~36~~12. The fluidic system of ~~any one of claims 33-35,~~claim 11 further comprising a cryostat that maintains the sample chamber at a cryogenic temperature.

~~37~~13. The fluidic system of ~~any one of claims 33-36~~claim 11 or 12, further comprising a multiway selector valve interfaced with the pump.

~~38~~14. The fluidic system of claim ~~37~~13, further comprising a reagent tray comprising one or more containers or wells comprising reagents for performing in situ gene sequencing of a target nucleic acid in a cell in an intact tissue, wherein said reagents are fluidically connected to the multiway selector valve.

~~39~~15. The fluidic system of claim ~~38~~14, further comprising an adjustable stage supporting the reagent tray, a chiller, and a position sensor that allows movement of the adjustable stage to allow selection of a reagent from the reagent tray.

~~40. The fluidic system of any one of claims 37-39, further comprising a buffer tray comprising one or more containers or wells comprising buffers, wherein the buffers are fluidically connected to the multiway selector valve.~~

~~41~~16. The fluidic system of ~~any one of claims 38-40~~claim 14 or 15, wherein the reagent tray and/or the buffer tray are disposable.

~~42. The fluidic system of any one of claims 37-41 , further comprising a waste container fluidically connected to the multiway selector valve.~~

~~43. The fluidic system of any one of claims 33-42, wherein the pump is a syringe pump.~~

~~44. The fluidic system of any one of claims 33-43, wherein the imaging system comprises a microscope that performs confocal microscopy, spinning disk confocal microscopy, light sheet microscopy, lattice light sheet microscopy, or light field microscopy.~~

~~45~~17. The fluidic system of any one of claims ~~33-44~~11-16, further comprising a container enclosing the fluidic system, wherein the container blocks ambient light.

~~46~~18. The fluidic system of claim ~~45~~17, wherein the container enclosing the fluidic system comprises one or more doors or drawers.

~~47~~19. The fluidic system of claim ~~45 or 46~~17 or 18, wherein the container enclosing the fluidic system is an acoustic dampening container.

~~48~~20. The fluidic system of any one of claims ~~33-47~~11-19, further comprising means for cryosectioning a tissue sample.