CLAIMS

What is claimed is:

1. A method of anchoring fragmented target nucleic acid within a matrix and clearing non-target cellular components comprising:
    a. contacting a tissue sample with at least two anchoring agents, wherein the first anchoring agent forms a covalent bond with the fragmented target nucleic acid and the second anchoring agent comprises an oligonucleotide that hybridizes with the fragmented target nucleic acid;
    b. embedding the sample in a polymer matrix wherein the first and second anchoring agents each form a covalent bond with the polymer matrix; and,
    c. clearing the non-target cellular components from the polymer matrix wherein the fragmented target nucleic acid remains anchored in the polymer matrix to form a matrix anchored target nucleic acid sample.
2. The method of claim 1, wherein the first anchoring agent is an alkylating agent.
3. The method of claim 2, wherein the alkylating agent is selected from the group consisting of Altretamine, Bendamustine, Busulfan, Carboplatin, Carmustine, Chlorambucil, Cisplatin, Cyclophosphamide, Dacarbazine, Ifosfamide, Lomustine, Mechlorethamine, Melphalan, Oxaliplatin, Temozolomide, Thiotepa and Trabectedin.
4. The method of claim 1, wherein the second anchoring agent comprises alternating dT and locked dT portions that hybridize with the fragmented target nucleic acid.
5. The method of claim 1, wherein the second anchoring agent comprises a poly-dT portion that hybridizes to the fragmented target nucleic acid.
6. The method of claim 1, wherein the first anchoring agent and the second anchoring agent each comprise an acrydite moiety that covalently binds the polymer matrix.
7. The method of claim 1, wherein the first anchoring agent is an alkylating agent derivatized with an acrydite moiety.

8. The method of claim 1, wherein the second anchoring agent comprises a poly-dT portion that hybridizes to the fragmented target nucleic acid and an acrydite moiety that covalently binds the polymer matrix.
9. The method of claim 1, wherein the fragmented target nucleic acid is RNA.
10. The method of claim 1, wherein the fragmented target nucleic acid is DNA.
11. The method of claim 1, further comprising contacting the anchored target nucleic acid sample with one or more primary oligonucleotide probes that hybridize to the fragmented target nucleic acids.
12. The method of claim 11, wherein the primary oligonucleotide probes are single molecule (sm)FISH probes or multiplexed error robust fluorescence in situ hybridization (MERFISH) probes.
13. The method of claim 11, wherein the one or more primary oligonucleotide probes comprise a first portion comprising a target sequence and a second portion comprising one or more read sequences.
14. The method of claim 13, further comprising determining read sequences based on contacting the one or more primary oligonucleotide probes with a plurality of secondary nucleic acid probes comprising a recognition sequence that hybridizes to the read sequence of the primary nucleic acid probe.
15. The method of claim 1, further comprising imaging using multiplexed fluorescence in situ hybridization comprising contacting the anchored target nucleic acid sample with one or more primary oligonucleotide probes that hybridize to the fragmented target nucleic acids and comprising one or more sequential steps of adding a plurality of secondary nucleic acid probes comprising a label moiety.
16. The method of claim 1, further comprising imaging using multiplexed error robust fluorescence in situ hybridization (MERFISH) probes comprising contacting the anchored target nucleic acid sample with one or more MERFISH probes that hybridize to the fragmented target nucleic acids.
17. The method of claim 13, further comprising imaging using multiple rounds of fluorescence in situ hybridization wherein, in each round, one or more different

secondary nucleic acid probes, each conjugated to a spectrally distinct fluorescent label are used to readout out multiple readout sequences simultaneously.

18. A method of anchoring fragmented target RNA within a matrix and clearing non-target cellular components comprising:
    a. contacting a tissue sample with at least two anchoring agents, wherein the first anchoring agent comprises an alkylating agent that forms a covalent bond with the fragmented target nucleic acid and the second anchoring agent comprises a polyT sequence that is complementary and hybridizes to the fragmented target RNA and wherein the first anchoring agent and the second anchoring agent each comprise an acrydite moiety that covalently binds to the matrix;
    b. embedding the sample in a polymer matrix wherein the first and second anchoring agents each form a covalent bond with the polymer matrix; and,
    c. clearing the non-target cellular components from the polymer matrix wherein the fragmented target RNA remains anchored in the polymer matrix to form a matrix anchored target RNA sample.
19. The method of claim 18, wherein the alkylating agent is selected from the group consisting of Altretamine, Bendamustine, Busulfan, Carboplatin, Carmustine, Chlorambucil, Cisplatin, Cyclophosphamide, Dacarbazine, Ifosfamide, Lomustine, Mechlorethamine, Melphalan, Oxaliplatin, Temozolomide, Thiotepa and Trabectedin.
20. A method for imaging fragmented target nucleic acid within a matrix and clearing non-target cellular components comprising:
    a. contacting a tissue sample with at least two anchoring agents, wherein the first anchoring agent forms a covalent bond with the fragmented target nucleic acid and the second anchoring agent comprises an oligonucleotide that hybridizes with the fragmented target nucleic acid;
    b. embedding the sample in a polymer matrix wherein the first and second anchoring agents each form a covalent bond with the polymer matrix;

c. clearing the non-target cellular components from the polymer matrix wherein the fragmented target nucleic acid remains anchored in the polymer matrix to form a matrix anchored target nucleic acid sample; and,
d. contacting the anchored target nucleic acid sample with one or more primary oligonucleotide probes that hybridize to the fragmented target nucleic acids and a plurality of secondary nucleic acid probes comprising a fluorescent label and a recognition sequence that hybridizes to a sequence of the primary nucleic acid probe and imaging the fragmented target nucleic acids.