COMPOSITIONS AND METHODS FOR ANALYTE DETECTION

CROSS-REFERENCE

[0001] This application claims priority to U.S. Provisional Patent Application No. 62/967,362, filed January 29, 2020, which application is herein incorporated by reference in its entirety for all purposes.

BACKGROUND

[0002] Fluorescent in situ sequencing (FISSEQ) can be used to detect target molecules within a sample (e.g., a biological sample) in situ. During FISSEQ, a three-dimensional (3D) matrix can be generated within the sample to immobilize the target molecules or derivatives thereof. Nucleic acid target molecules may be subsequently amplified and sequenced within the 3D matrix. The 3D matrix with the attached nucleic acid molecules can provide an information storage medium where the nucleic acid molecules represent stored information which can be read within the 3D matrix.

[0003] FISSEQ may be used to detect one or more fluorescent signals emanating from each sequencing template within a FISSEQ library over more than one cycle of fluorescence detection, wherein the fluorescent signals over the totality of detection cycles may comprise an information construct, which may be mapped to molecular identification or otherwise provide information about the nature of the detected molecule. The 3D matrix can allow reagent exchange and removal of background molecules without losing the target molecules.

SUMMARY

[0004] Recognized herein is a need for a robust protocol for processing a sample for fluorescent in situ sequencing (FISSEQ). The methods and systems provided herein can be used to immobilize target nucleic acid molecules on the three-dimensional (3D) matrix and allow efficient buffer exchange and clearing of background molecules within the 3D matrix.

[0004a] In an aspect, the present disclosure provides 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.

[0005] In an aspect, the present disclosure provides a method for processing a biological sample, comprising: (a) providing a container comprising the biological sample comprising an analyte within a synthetic three-dimensional (3D) matrix, which synthetic 3D matrix comprises an attachment moiety; and (b) directing a tethering molecule into the biological sample such that the tethering molecule (i) flows through the synthetic 3D matrix and comes