

What is claimed is:

1. ~~—A method for spatially detecting at least one target analyte in at least one cell from a tissue sample comprising:~~

~~(1) contacting at least one target analyte in at least one cell in a tissue sample with at least one probe comprising a target binding domain and an identifier oligonucleotide, wherein the identifier oligonucleotide comprises a unique nucleic acid sequence which identifies the target analyte bound to the target binding domain;~~

~~(2) providing a force to a location of the tissue sample sufficient to release the identifier oligonucleotide;~~

~~(3) collecting the released identifier oligonucleotide;~~

~~(4) hybridizing to the released identifier oligonucleotide a first nucleic acid probe and a second nucleic acid probe,~~

~~wherein the first nucleic acid probe comprises:~~

~~— a nucleic acid complementary to a portion of the identifier oligonucleotide,~~

~~— a nucleic acid sequence comprising a unique molecular identifier,~~

~~a first amplification primer binding site, and~~

~~wherein the second nucleic acid probe comprises:~~

~~a nucleic acid complementary to a portion of the identifier oligonucleotide, and~~

~~a second amplification primer binding site, and~~

~~wherein the first and the second nucleic acid probes hybridize to the identifier oligonucleotide such that the first and the second nucleic acid probes are adjacent but not overlapping;~~

~~(5) ligating the hybridized first and second nucleic acid probes together;~~

~~(6) amplifying the ligation product produced in step (5); and~~

~~(7) identifying the released identifier oligonucleotide by sequencing the amplified products produced in step (6), thereby spatially detecting the at least one target analyte in the at least one cell in a tissue sample.~~

2. ~~The method of claim 1, wherein amplifying the ligation product comprises performing PCR.~~

3. ~~The method of claim 2, wherein performing PCR comprises a first amplification primer and second amplification primer,~~

~~wherein the first amplification primer comprises:~~

~~a first flow cell binding site;~~

~~a first nucleic acid sequence which identifies the specific location of the tissue sample from which the identifier oligonucleotide was released; and~~

~~a nucleic acid sequence complementary to the second amplification primer binding site; and~~

~~wherein the second amplification primer comprises:~~

~~a second flow cell binding site;~~

~~a second nucleic acid sequence which identifies the specific location of the sample from which the identifier oligonucleotide was released; and~~

~~a nucleic acid sequence complementary to the first amplification primer binding site.~~

4. ~~The method of claim 3, wherein at least one of the amplification primers comprises an affinity molecule.~~

5. ~~The method of claim 1, wherein the unique nucleic acid sequence which identifies the target analyte bound to the target binding domain comprises between about 10 nucleotides and about 50 nucleotides~~

6. ~~The method of claim 1, wherein at least one of the amplification primer binding sites comprises about 20 to about 50 nucleotides.~~

71. A method for spatially detecting the abundance of at least one target analyte in at least one cell from a tissue sample comprising, wherein the at least one target analyte is a nucleic acid molecule:

(1) contacting at least one target analyte in at least one cell in a tissue sample with at least one three probes, thereby tiling the probes on the at least one target analyte,

wherein the probes are oligonucleotide probes,

wherein each of the probes comprising comprise:

a target binding domain that binds to at least one target analyte, and
 a single-stranded identifier oligonucleotide, wherein the single-stranded identifier oligonucleotide comprises:

- a unique nucleic acid sequence which identifies the target analyte bound to the target binding domain,
- a nucleic acid sequence comprising a unique molecular identifier,
- a first amplification primer binding site, and
- a second amplification primer binding site;

wherein each of the probes comprise a photo-cleavable linker between the single-stranded identifier oligonucleotide and the target binding domain;

(2) ~~providing a force-exciting to~~ a location of the tissue sample with light of sufficient wavelength capable of cleaving the photo-cleavable linker, thereby to release-releasing the single-stranded identifier oligonucleotides;

(3) collecting the released single-stranded identifier oligonucleotides from the location of the tissue sample excited in step (2);

(4) amplifying the collected single-stranded identifier oligonucleotides, wherein amplifying the collected single-stranded identifier oligonucleotides comprises performing PCR, wherein performing PCR comprises a first amplification primer and a second amplification primer;

wherein the first amplification primer comprises:

a first flow cell binding site;

a first nucleic acid sequence which identifies the specific location of the tissue sample from which the identifier oligonucleotide was released; and

a nucleic acid sequence complementary to the second amplification primer binding site; and

wherein the second amplification primer comprises:

a second flow cell binding site;

a second nucleic acid sequence which identifies the specific location of the sample from which the identifier oligonucleotide was released; and

a nucleic acid sequence complementary to the first amplification primer binding site;

(5) identifying the released single-stranded identifier oligonucleotides by sequencing the amplified products produced in step (4), thereby spatially detecting the abundance of the at least one target analyte in the at least one cell in a tissue sample.

8. ~~— The method of claim 7, wherein amplifying the collected identifier oligonucleotide comprises performing PCR.~~

9. ~~— The method of claim 8, wherein performing PCR comprises a first amplification primer and second amplification primer,~~

~~— wherein the first amplification primer comprises:~~

~~a first flow cell binding site;~~

~~a first nucleic acid sequence which identifies the specific location of the tissue sample from which the identifier oligonucleotide was released; and~~

~~a nucleic acid sequence complementary to the second amplification primer binding site; and~~

~~— wherein the second amplification primer comprises:~~

~~— a second flow cell binding site;~~

~~a second nucleic acid sequence which identifies the specific location of the sample from which the identifier oligonucleotide was released; and~~

~~a nucleic acid sequence complementary to the first amplification primer binding site.~~

2. The method of claim 1, wherein step (a) comprises contacting the at least one target analyte in at least one cell in a tissue sample with at least five probes.

403. The method of claim ~~3~~ or ~~9~~ 1 or 2, wherein at least one of the flow cell binding sites comprises a flow cell adapter sequence suitable for sequencing.

444. The method of ~~claim 3 or 9~~ any one of claims 1 to 3, wherein at least one of the flow cell binding sites comprises a P5 flow cell adapter sequence, wherein the P5 flow cell adapter sequence comprises the sequence set forth in SEQ ID NO: 3.

~~125.~~ The method of ~~claim 3 or 9~~ any one of claims 1 to 4, wherein at least one of the flow cell binding sites comprises a P7 flow cell adapter sequence, wherein the P7 flow cell adapter sequence comprises the sequence set forth in SEQ ID NO: 4.

~~136.~~ The method of ~~claim 3 or 9~~ any one of claims 1 to 5, wherein at least one of the flow cell binding sites comprises about 15 to about 40 nucleotides.

7. The method of claim 6, wherein:

(a) at least one of the flow cell binding sites comprises about 29 nucleotides; or

(b) at least one of the flow cell binding sites comprises about 24 nucleotides.

~~148.~~ The method of ~~claim 7~~ any one of claims 1 to 7, wherein the unique nucleic acid sequence which identifies the target analyte bound to the target binding domain comprises between about 5 nucleotides and about 25 nucleotides.

9. The method of claim 8, wherein the unique nucleic acid sequence which identifies the target analyte bound to the target binding domain comprises about 12 nucleotides.

~~1510.~~ The method of ~~claim 7~~ any one of claims 1 to 9, wherein at least one of the amplification primer binding sites comprises about 10 to about 40 nucleotides.

11. The method of claim 11, wherein:

(a) at least one of the amplification primer binding sites comprises about 34 nucleotides;

or

(b) at least one of the amplification primer binding sites comprises about 33 nucleotides.

~~1612.~~ The method of ~~claim 1 or 7~~ any one of claims 1 to 11, wherein at least one of the amplification primer binding sites comprises an i7 sequence, wherein the i7 sequence comprises the sequence set forth in SEQ ID NO: 1.

~~1713.~~ The method of ~~claim 1 or 7~~ any one of claims 1 to 12, wherein at least one of the amplification primer binding sites comprises an i5 sequence, wherein the i5 sequence comprises the sequence set forth in SEQ ID NO: 2.

~~1814.~~ The method of ~~claim 3 or 9~~ any one of claims 1 to 13, wherein at least one of the nucleic acid sequences which identify the specific location of the tissue sample from which the identifier oligonucleotide was released comprises about 1 to about 20 nucleotides.

15. The method of claim 14, wherein at least one of the nucleic acid sequences which identify the specific location of the tissue sample from which the identifier oligonucleotide was released comprises about 8 nucleotides.

~~1916.~~ The method of ~~claim 1 or 7~~ any one of claims 1 to 15, wherein the nucleic acid sequence comprising a unique molecular identifier comprises about 5 to about 20 nucleotides.

17. The method of claim 16, wherein the nucleic acid sequence comprising a unique molecular identifier comprises about 14 nucleotides.

~~2018.~~ The method of ~~claim 7~~ any one of claims 1 to 17, wherein the target binding domain comprises between about 10 nucleotides to about 70 nucleotides.

19. The method of claim 18, wherein the target binding comprises between about 35 to about 50 nucleotides.