

CLAIMS

What is claimed is:

1. A method, comprising:
 - 5 exposing a sample to a plurality of nucleic acid probes;
for each of the nucleic acid probes, capturing images of the sample using at least 2 detectors focused on different focal planes within the sample;
determining binding of the nucleic acid probes within the sample using the images; and
 - 10 determining an abundance and/or a spatial distribution of nucleic acids within the sample corresponding to the binding of the plurality of nucleic acid probes.
2. The method of claim 1, comprising capturing images of the sample using at least 4 detectors focused on different focal planes within the sample.
- 15 3. The method of any one of claims 1 or 2, comprising capturing images of the sample using at least 8 detectors focused on different focal planes within the sample.
4. The method of any one of claims 1-3, comprising capturing images of the sample using at least 16 detectors focused on different focal planes within the sample.
- 20 5. The method of any one of claims 1-4, comprising capturing images of the sample using at least 32 detectors focused on different focal planes within the sample.
- 25 6. The method of any one of claims 1-5, wherein each of the different focal planes is focused at no more than 1000 nm from a neighboring focal plane.
7. The method of any one of claims 1-6, comprising passing light from the sample through a plurality of beamsplitters to the at least 4 detectors.
- 30 8. The method of any one of claims 1-7, wherein at least some of the detectors are cameras.

9. The method of any one of claims 1-8, wherein at least some of the detectors are point detectors.
10. The method of any one of claims 1-9, wherein at least some of the detectors are photodetectors.
11. The method of any one of claims 1-10, wherein at least some of the detectors are avalanche photodiodes.
12. The method of any one of claims 1-11, wherein at least some of the detectors are one dimensional arrays.
13. The method of any one of claims 1-12, wherein at least some of the detectors are line detectors.
14. The method of any one of claims 1-13, comprising determining z positions of at least some of the nucleic acid probes in the sample at a resolution better than 300 nm.
15. The method of any one of claims 1-14, comprising exposing the nucleic acid probes to primary amplifier nucleic acids able to bind to the nucleic acid probes, wherein a maximum number of primary amplifier nucleic acids is able to bind to a nucleic acid probe; and
exposing the primary amplifier nucleic acids to secondary amplifier nucleic acids able to bind to the primary amplifier nucleic acids, wherein a maximum number of secondary amplifier nucleic acids is able to bind to the primary amplifier nucleic acids.
16. The method of claim 15, comprising exposing the primary amplifier nucleic acids to secondary amplifier nucleic acids able to bind to the primary amplifier nucleic acids, wherein the binding of primary amplifier nucleic acids and secondary amplifier nucleic acids to a target is saturable.
17. The method of any one of claims 15 or 16, comprising exposing the primary amplifier nucleic acids to secondary amplifier nucleic acids able to bind to the primary

amplifier nucleic acids, wherein the secondary amplifier nucleic acids bind to the primary amplifier nucleic acids within a fixed distance.

18. The method of any one of claims 15-17, wherein no more than 20 primary amplifier nucleic acids is able to bind to the nucleic acid probe.
19. The method of any one of claims 15-18, wherein the primary amplifier nucleic acids have an average length of less than 300 nucleotides.
20. The method of any one of claims 15-19, further comprising exposing the primary amplifier nucleic acids to secondary amplifier nucleic acids able to bind to the primary amplifier nucleic acids, wherein a maximum number of secondary amplifier nucleic acids is able to bind to the primary amplifier nucleic acids.
21. The method of claim 20, wherein the secondary amplifier nucleic acids comprise a fluorescent signaling entity.
22. The method of any one of claims 1-21, further comprising:
creating codewords binding of the nucleic acid probes within the sample; and
for at least some of the codewords, matching the codeword to a valid codeword optionally wherein, if no match is found, applying error correction to the codeword to form a valid codeword.
23. The method of any one of claims 1-22, comprising exposing the sample to at least 5 different nucleic acid probes.
24. The method of any one of claims 1-23, comprising sequentially exposing the sample to a plurality of nucleic acid probes.
25. The method of any one of claims 1-24, wherein the plurality of nucleic acid probes comprises a combinatorial combination of nucleic acid probes with different sequences.

26. The method of any one of claims 1-25, wherein the plurality of nucleic acid probes have an average length of between 10 and 300 nucleotides.
27. The method of any one of claims 1-26, wherein at least some of the plurality of nucleic acid probes comprises a target sequence and one or more read sequences.
28. The method of claim 27, wherein the target sequence of the plurality of nucleic acid probes has an average length of between 10 and 200 nucleotides.
29. The method of any one of claims 27 or 28, wherein the target sequence binds to a target via specific binding.
30. The method of any one of claims 27-29, wherein the plurality of read sequences are distributed on the plurality of nucleic acid probes so as to define an error-correcting code.
31. The method of any one of claims 1-30, wherein the plurality of nucleic acid probes defines a code space with a Hamming distance of at least 2.
32. The method of any one of claims 1-31, comprising determining binding of the nucleic acid probes using fluorescence imaging.
33. The method of any one of claims 1-32, comprising determining binding of the nucleic acid probes using multi-color fluorescence imaging.
34. The method of any one of claims 1-33, comprising determining binding of the nucleic acid probes using a super-resolution fluorescence imaging technique.
35. The method of claim 34, comprising determining binding of the nucleic acid probes using stochastic optical reconstruction microscopy (STORM).
36. The method of any one of claims 1-35, wherein the sample comprises a cell.
37. The method of claim 36, wherein the cell is a human cell.

38. The method of any one of claims 36 or 37, wherein the cell is fixed.
39. A method, comprising:
- 5 exposing a sample to a plurality of nucleic acid probes;
for each of the nucleic acid probes, exposing the nucleic acid probes to
amplifier nucleic acids able to bind thereto, wherein a maximum finite number of
amplifier nucleic acids is able to directly or indirectly bind to a nucleic acid probe;
and
- 10 for each of the nucleic acid probes, capturing images of the sample using at
least 4 cameras focused on different focal planes within the sample.