CLAIMS

We claim:

1. A method comprising:

attaching a single DNA template molecule to each of a plurality of attachment elements on a sample container, wherein the average distance between adjacent elements is less than Abbe's limit;

applying a stochastic photo-switching chemistry to all of the molecules at the same time to cause the attached molecules to fluoresce in on and off events in up to four different colors by stochastic photo-switching; and

imaging the on and off events in a color channel for each color in real-time as the on and off events are occurring for the attached molecules.

2. The method of claim 1, wherein each of the plurality of attachment elements on the sample container is within a field of view of an imager used to image the on and off events such that imaging of the on and off events occurs at the same time for the attached molecules at the plurality of attachment elements.

3. The method of claim 1, applying a stochastic photo-switching chemistry to all of the attached molecules at the same time comprises applying a stochastic optical reconstruction microscopy, a DNA Points Accumulation for Imaging in Nanoscale Topography, or a direct stochastic optical reconstruction microscopy stochastic photoswitching chemistry to all of the molecules at the same time.

4. The method of claim 1, wherein due to the stochastic nature of the photo switching, the probability that an on event for a given base for a given molecule will occur at the same time as an on event for the same base at a molecule adjacent to the given molecule is less than about 0.5%.

5. The method of claim 1, wherein concentrations of reagents for the stochastic photo switching are sufficient such that the probability that an on event for a given base for a given molecule will occur at the same time as an on event for the same base at a molecule adjacent to the given molecule is less than about 0.5%.

6. The method of claim 1, further comprising, controlling a rate at which the on and off events occur to control a probability that an on event for a given base for a given molecule will occur at the same time as an on event for the same base at a molecule adjacent to the given molecule.

7. The method of claim 6, wherein controlling the rate at which the on and off events occur comprises adjusting concentrations of nucleotides and enzyme in the stochastic photo-switching chemistry.

8. The method of claim 6, wherein controlling the rate at which the on and off events occur comprises adjusting the on and off times so that the probability that an on event for a given base for a given molecule will occur at the same time as an on event for the same base at a molecule adjacent to the given molecule is lower than a determined error rate in a sequencing application in which the method is applied.

9. The method of claim 1, further comprising, determining whether an illumination intensity of a detected on event in a color channel is greater than a predetermined threshold.

10. The method of claim 1, further comprising, determining whether a spot size of a detected on event in a color channel is greater than a predetermined threshold.

11. The method of claim 1, wherein the average distance between adjacent elements is less than about 20 nm.

12. The method of claim 1, wherein the average distance between adjacent elements is within a range of about 2 nm to about 20 nm.

13. An imaging system comprising:

a sample container comprising a plurality of attachment elements wherein a single DNA template molecule is attached to each of the attachment elements, and further wherein the average distance between adjacent attachment elements is less than Abbe's limit; and

an imager positioned to image photo-switching occurring at the plurality of attachment elements by capturing on and off events in a plurality of color channels at the same time as the on and off events are occurring for the attached molecules when a stochastic photo-switching chemistry is applied to all of the attached molecules at the same time causing the attached molecules to fluoresce in the on and off events in up to four different colors.

14. The imaging system of claim 13, wherein the sample container comprises a flowcell that comprises the plurality of attachment elements at a plurality of sample locations.

15. The imaging system of claim 13, wherein each of the plurality of attachment elements on the sample container is within a field of view of the imager used to image the photo-switching such that the capturing of the on and off events occurs at the same time for the attached molecules at the plurality of attachment elements.

16. The imaging system of claim 13, the stochastic photo-switching chemistry applied to all of the attached molecules at the same time comprises a stochastic optical reconstruction microscopy, a DNA Points Accumulation for Imaging in Nanoscale Topography, or a direct stochastic optical reconstruction microscopy stochastic photoswitching chemistry.

17. The imaging system of claim 13, wherein concentrations of reagents for the stochastic photo switching are sufficient such that the probability that an on event for a given base for a given molecule will occur at the same time as an on event for the same base at a molecule adjacent to the given molecule is less than 0.5%.

18. The imaging system of claim 13, wherein a rate at which the on and off events occur yields a probability that an on event for a given base for a given molecule will occur at the same time as an on event for the same base at a molecule adjacent to the given molecule is lower than a determined error rate in a sequencing application in which the method is applied.

19. The imaging system of claim 13, further comprising, determining whether an illumination intensity or a spot size of a detected on event in a color channel is greater than a predetermined threshold.

20. The imaging system of claim 13, wherein the average distance between adjacent elements is less than about 20 nm.

21. A method comprising:

(a) providing an array of nucleotide sequences anchored to a solid support, wherein the average distance between adjacent anchors is less than Abbe's limit;

(b) providing a mixture to the array comprising an enzyme capable of coupling nucleotides, a deblocking agent, a nucleic acid bound to a strand of nucleotides having a sequence complimentary to the nucleotide sequences anchored to the solid support, and more than one nucleotide analog comprising a base with a label moiety and corresponding quencher moiety bound thereto wherein the label moieties are correlated with a specific base moiety; and

(c) allowing sequential addition of a plurality of the nucleotide analogs to the nucleic acid to proceed via several reaction cycles in the mixture while concurrently imaging the label moieties within the array;

wherein each reaction cycle comprises:

(i) the polymerase adding a nucleotide analog to the nucleic acid by cleaving the quencher moiety and forming a transient nucleic acid species comprising the label moiety; and

(ii) the deblocking agent modifying the transient nucleic acid species to remove the label moiety.

22. The method of claim 21, wherein the average distance between adjacent anchors is less than about 20 nm.

23. The method of claim 21, wherein the average distance between adjacent anchors is within a range of about 2 nm to about 20 nm.

24. The method of claim 21, wherein the nucleotide analog comprises a pentose moiety having a 3' carbon and the label moiety is attached to the nucleotide at the 3' carbon.

25. The method of claim 21, wherein the nucleotide analog comprises a triphosphate moiety and wherein the quencher moiety is attached to the triphosphate moiety.

26. The method of claim 21, wherein the deblocking agent comprises a phosphoesterase enzyme.

27. The method of claim 26, wherein the phosphoesterase selectively removes a phosphodiester moiety or the phosphotriester moiety from the transient nucleic acid species.

28. The method of claim 26, wherein the phosphoesterase is selected from the group consisting of Endonuclease IV and AP endonuclease.

29. The method of claim 21, wherein the transient nucleic acid species is present for at least 1 millisecond before the deblocking agent modifies the transient nucleic acid species to remove the label moiety.

30. The method of claim 29, wherein the transient nucleic acid species is present for no more than 30 seconds before the deblocking agent modifies the transient nucleic acid species to remove the label moiety.

31. The method of claim 21, wherein the several reaction cycles comprises at least 100 reaction cycles, whereby the nucleic acid is extended by addition of at least 100 nucleotide analogs.

32. The method of claim 21, wherein the enzyme capable of coupling nucleotides comprises a polymerase, a myosin, or a kinase.