

CLAIMS

1. A method for sequencing polynucleotides comprising:
 - attaching a first DNA template molecule to a first attachment element of a plurality of attachment elements of a sample container and attaching a second DNA template molecule to a second attachment element of the plurality of attachment elements, wherein the distance between the first attachment element and the second attachment element is less than Abbe's limit;
 - providing a stochastic photo-switching chemistry to the sample container, the stochastic photo-switching chemistry comprising a set of molecules that have a 5' diphosphate quencher molecule and a 3' phosphate block with a label moiety, a polymerase to incorporate a nucleotide of the set of nucleotides and cleave the 5' diphosphate quencher molecule, and an enzyme to cleave the 3' phosphate block with the label moiety;
 - imaging a series of on and off events for the first DNA template molecule in real-time as the on and off events are occurring, each on event occurring when the polymerase incorporates a nucleotide of the set of nucleotides and cleaves the 5' diphosphate quencher molecule and each off event occurring when the enzymes cleaves the 3' phosphate block with the label moiety; and
 - imaging a series of on and off events for the second DNA template in real-time as the on and off events are occurring;
 - wherein the series of on and off events for the first DNA template molecule and the series of on and off events for the second DNA template molecule are stochastic and not synchronised.

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 first DNA template molecule and second DNA template molecule.
3. 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%.
4. 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%.
5. 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 first nucleotide base for the first DNA template molecule will occur at the same time as an on event for the nucleotide base for the second DNA template molecule based on a concentration of the first nucleotide base of the set of nucleotides and a concentration of the enzyme.
6. The method of claim 1, further comprising, (i) determining whether an illumination intensity of a detected on event in a color channel is greater than a predetermined threshold; or (ii) determining whether a spot size of a detected on event in a color channel is greater than a predetermined threshold.

7. The method of claim 1, wherein the distance between the first attachment element and the second attachment element is less than about 20 nm.
8. The method of claim 7, wherein the distance between the first attachment element and the second attachment element is within a range of 2 nm to 20 nm.
9. An imaging assembly for performing the method of claim 1 comprising:
a sample container comprising a plurality of attachment elements wherein the first DNA template molecule is attached to the first attachment element and the second DNA template molecule is attached to the second attachment element, 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 first attachment element and the second attachment element 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 first DNA template molecule and the second DNA template molecule when the stochastic photo-switching chemistry is applied.
10. The imaging assembly of claim 9, wherein the sample container comprises a flowcell that comprises the plurality of attachment elements at a plurality of sample locations.
11. The imaging assembly of claim 9, 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.
12. The imaging assembly of claim 9, 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%.

13. The imaging assembly of claim 9, 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.

14. The imaging assembly of claim 9, wherein the average distance between adjacent elements is less than 20 nm.