

WHAT IS CLAIMED IS:

1. A method for determining the sequence of a target polynucleotide, comprising:
 - (a) contacting a primer polynucleotide with a mixture comprising one or more of four different types of nucleotide conjugates, wherein a first type of nucleotide conjugate comprises a first label, a second type of nucleotide conjugate comprises a second label, and a third type of nucleotide conjugate comprises a third label, wherein each of the first label, the second label, and the third label is spectrally distinct from one another, and wherein the primer polynucleotide is complementary to at least a portion of the target polynucleotide;
 - (b) incorporating a nucleotide conjugate from the mixture in the primer polynucleotide to produce an extended primer polynucleotide;
 - (c) performing a first imaging event using a first excitation light source and collecting a first emission signal from the extended primer polynucleotide;
 - (d) performing a second imaging event using a second excitation light source and collecting a second emission signal from the extended primer polynucleotide;wherein the first excitation light source and the second excitation light source have different wavelengths; and wherein first emission signal and the second emission signal are collected in a single emission detection channel.
2. The method of claim 1, wherein the fourth type of nucleotide conjugate is not labeled or is labeled with a fluorescent moiety that does not emit a detectable signal from either the first imaging event or the second imaging event.
3. The method of claim 1 or 2, wherein the incorporation of the first type of the nucleotide conjugate is determined by a signal state in the first imaging event and a dark state in the second imaging event.
4. The method of any one of claims 1 to 3, wherein the incorporation of the second type of the nucleotide conjugates is determined by a dark state in the first imaging event and a signal state in the second imaging event.
5. The method of any one of claims 1 to 4, wherein the incorporation of the third type of the nucleotide conjugates is determined by a signal state in both the first imaging event and the second imaging event.

6. The method of any one of claims 1 to 5, wherein the incorporation of the fourth type of the nucleotide conjugates is determined by a dark state in both the first imaging event and the second imaging event.

7. The method of any one of claims 1 to 6, wherein the four types of nucleotide conjugates comprise dATP, dCTP, dGTP and dTTP or dUTP, or non-natural nucleotide analogs thereof.

8. The method of claim 7, wherein each of the four types of nucleotide conjugates in the mixture has a 3' hydroxyl blocking group.

9. The method of any one of claims 1 to 8, further comprising: (e) removing the 3' hydroxyl blocking group and the label from the incorporated nucleotide conjugate after the second imaging event, and prior to the next sequencing cycle.

10. The method of claim 9, further comprising:
repeating steps (a)-(e) for multiple cycles; and
determining the sequence of the target polynucleotide based on the sequentially incorporated nucleotide conjugates.

11. The method of claim 10, wherein steps (a)-(e) are repeated for at least 50 cycles.

12. The method of any one of claims 1 to 11, wherein each of the first excitation light source and the second excitation light source comprises a laser, a light-emitting diode (LED), or a combination thereof.

13. The method of any one of claims 1 to 12, wherein the first excitation light source has a wavelength of about 400 nm to about 480 nm.

14. The method of claim 13, wherein the first excitation light source has a wavelength of about 450 nm to about 460 nm.

15. The method of any one of claims 1 to 14, wherein the second excitation light source has a wavelength of about 490 nm to about 550 nm.

16. The method of claim 15, wherein the second excitation light source has a wavelength of about 510 nm to about 530 nm.

17. The method of any one of claims 1 to 12, wherein the first excitation light source has a wavelength of about 490 nm to about 550 nm.

18. The method of claim 17, wherein the first excitation light source has a wavelength of about 510 nm to about 530 nm.

19. The method of any one of claims 1 to 12, 17 and 18, wherein the second excitation light source have a wavelength of about 400 nm to about 480 nm.

20. The method of claim 19, wherein the second excitation light source have a wavelength of about 450 nm to about 460 nm.

21. The method of any one of claims 1 to 20, wherein the single emission detection channel has a detection spectrum range above 560 nm.

22. The method of any one of claims 1 to 21, wherein the method does not comprise a chemical modification of any nucleotide conjugates in the mixture between the first imaging event and the second imaging event.

23. The method of any one of claims 1 to 22, wherein the target polynucleotide is immobilized to a solid support.

24. The method of claim 23, wherein the solid support comprises a plurality of immobilized target polynucleotides.

25. The method of claim 23 or 24, wherein the solid support comprises a patterned flow cell.

26. The method of claim 25, wherein the patterned flow cell comprises a plurality of nanowells and each nanowell comprises an immobilized target polynucleotide.

27. The method of any one of claims 23 to 26, wherein the solid support further comprises a complementary metal-oxide-semiconductor (CMOS) chip.

28. A kit for sequencing application, comprising:

- a first type of nucleotide conjugate comprises a first label;
- a second type of nucleotide conjugate comprises a second label; and
- a third type of nucleotide conjugate comprises a third label;

wherein each of the first label, the second label, and the third label is spectrally distinct from one another, the first label and the third label are excitable using a first light source wavelength, the second label and the third label are excitable using a second light source wavelength that is different from the first light source wavelength; and

wherein each of the first label, the second label and the third label has an emission spectrum that is detectable in a single detection channel.

29. The kit of claim 28, further comprising a fourth type of nucleotide, and wherein the fourth type of nucleotide is unlabeled (dark).

30. The kit of claim 28 or 29, wherein the first light source has a wavelength of about 400 nm to about 480 nm.

31. The kit of claim 30, wherein the first light source has a wavelength of about 450 nm to about 460 nm.

32. The kit of any one of claims 28 to 31, wherein the second light source has a wavelength of about 490 nm to about 550 nm.

33. The kit of claim 32, wherein the second light source has a wavelength of about 510 nm to about 530 nm.

34. The kit of any one of claims 28 to 33, wherein the single emission detection channel has a detection spectrum range of greater than about 560 nm.

35. The kit according to any one of claims 28 to 34, further comprising a DNA polymerase and one or more buffer compositions.