CLAIMS

1. A method for determining nucleotide sequences of target polynucleotides, comprising:

(a) performing a sequencing reaction that comprises one or more cycles of:

(i) incorporating four different types of nucleotide conjugates into a plurality of polynucleotides complementary to the target polynucleotides to produce extended polynucleotides, wherein a first type of nucleotide conjugates comprises a first photoswitchable label, a second type of nucleotide conjugates comprises a second photoswitchable label, and a third type of nucleotide conjugates comprises a third label;

(ii) detecting a first collection of signals from the extended polynucleotides in each cycle via a first imaging event;

(iii) irradiating the extended polynucleotides with a light source to cause changes in emission signals of the first photoswitchable label and the second photoswitchable label;

(iv) detecting a second collection of signals from the extended polynucleotides via a second imaging event;

(b) determining the sequences of the target polynucleotides based on the sequentially incorporated nucleotide conjugates.

2. The method of claim 1, wherein the incorporation of the first type of the nucleotide conjugates is determined from a signal state in the first imaging event and a dark state in the second imaging event.

3. The method of claim 1 or 2, wherein the incorporation of the second type of the nucleotide conjugates is determined from a dark state in the first imaging event and a signal state in the second imaging event.

4. The method of any one of claims 1 to 3, wherein the incorporation of the third type of the nucleotide conjugates is determined from a signal state in the first imaging event and the second imaging event.

5. The method of any one of claims 1 to 4, wherein the incorporation of a fourth type of the nucleotide conjugates is determined from a dark state in the first imaging event and the second imaging event.

6. The method of any one of claims 1 to 5, wherein the four different types of nucleotide conjugates are simultaneously present and compete for incorporation during each cycle.

7. The method of any one of claims 1 to 6, wherein the step (a) is repeated at least 50 times.

8. The method of any one of claims 1 to 7, wherein the incorporation of the nucleotide conjugates is performed by a polymerase.

9. The method of any one of claims 1 to 8, wherein the different types of nucleotide conjugates comprise reversible terminator moieties.

10. The method of claim 9, wherein step (a) further comprises cleaving the reversible terminator moieties from incorporated nucleotide conjugates prior to the next incorporation cycle.

11. The method of any one of claims 1 to 10, wherein the nucleotide conjugates comprise nucleotide types selected from the group consisting of dATP, dTTP, dUTP, dCTP, dGTP, and non-natural nucleotide analogs thereof.

12. The method of any one of claims 1 to 11, wherein the labels of the first type of nucleotide conjugates and the third type of nucleotide conjugates comprise the same fluorescent moieties.

13. The method of any one of claims 1 to 11, wherein the labels of the first type of nucleotide conjugates, the second type of nucleotide conjugates, and the third type of nucleotide conjugates comprise different fluorescent moieties.

14. The method of claim 13, wherein the different fluorescent moieties are detected using the same emission filter.

15. The method of any one of claims 1 to 14, wherein the fourth type of nucleotide conjugates is not labeled with a fluorescent moiety.

16. The method of any one of claims 1 to 15, wherein the irradiating in step (a)(iii) does not substantially change the signal detected from the third label of the third type of nucleotide conjugates.

17. The method of any one of claims 1 to 16, wherein the irradiating light source in step (a)(iii) comprises a laser, a light-emitting diode (LED), or a combination thereof.

18. The method of any one of claims 1 to 17, wherein the irradiating light source in step (a)(iii) has a different wavelength than an excitation wavelength used in the first imaging event.

19. The method of any one of claims 1 to 18, wherein the irradiating light source in step (a)(iii) has a wavelength of about 350 nm to about 450 nm.

20. The method of claim 19, wherein the irradiating light source in step (a)(iii) has a wavelength of about 405 nm.

21. The method of any one of claims 1 to 20, wherein the first imaging event and the second imaging event have substantially the same excitation wavelength.

22. The method of claim 21, wherein the first imaging event and the second imaging event have an excitation wavelength of about 550 nm to 650 nm.

23. The method of claim 22, wherein the first imaging event and the second imaging event have an excitation wavelength of about 633 nm.

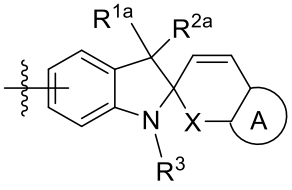
24. The method of any one of claims 1 to 23, wherein the first photoswitchable label comprises a first fluorescent moiety covalently bonded, optionally via a first linker, to a first photochromic moiety.

25. The method of claim 24, wherein the first fluorescent moiety comprises a fluorescent moiety emitting a red light.

26. The method of claim 24 or 25, wherein the first fluorescent moiety comprises a silicon rhodamine moiety.

27. The method of any one of claims 24 to 26, wherein the first photochromic moiety comprises a spiropyrano or spirothiopyrano moiety.

28. The method of any one of claims 24 to 27, wherein the first photochromic moiety comprises a structure of formula (I):



(I)

wherein:

X is O (oxygen) or S (sulfur);

each of R^{1a} and R^{2a} is independently selected from the group consisting of H, halo, optionally substituted C_{1-6} alkyl, optionally substituted C_{2-6} alkenyl, optionally substituted C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, and C_{1-6} haloalkoxy;

R^3 is selected from the group consisting of H, C_{1-6} alkyl, and $–(CH_2)_n-R^4$;

R^4 is selected from the group consisting of C_{6-10} aryl, 5 to 10 membered heteroaryl, 3 to 7 membered carbocyclyl, and 3 to 7 membered heterocyclyl, each optionally substituted;

ring A is C_{6-10} aryl or 5 to 10 membered heteroaryl, each substituted with at least one electron withdrawing group;

n is an integer of 1 to 6; and

wherein the irradiating step (a)(iii) causes a break of the spiro C—X bond.

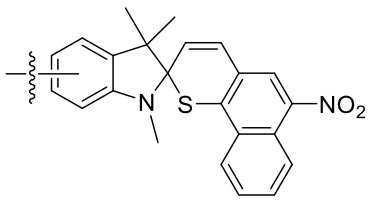
29. The method of claim 28, wherein X is S.

30. The method of claim 28 or 29, wherein each R^{1a} and R^{2a} is C_{1-6} alkyl.

31. The method of any one of claims 28 to 30, wherein ring A is phenyl or naphthyl substituted with at least one electron withdrawing group.

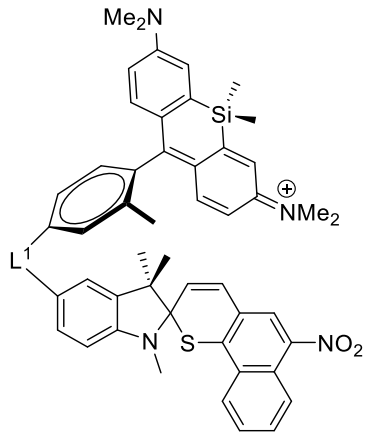
32. The method of claim 31, wherein the electron withdrawing group is selected from the group consisting of nitro, cyano, fluoro, bromo, $-S(O)_2OH$, $-S(O)_2CF_3$, ammonium, alkyl ammonium, and C_{1-6} alkyl substituted with one or more fluoro or bromo.

33. The method of any one of claims 24 to 32, wherein the first photochromic moiety comprises the structure of formula (Ia):



(Ia).

34. The method of any one of claims 24 to 33, wherein the first photoswitchable label comprise the structure:



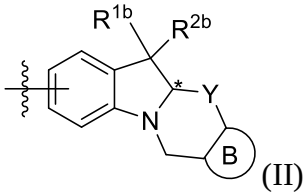
, wherein L^1 is the first linker.

35. The method of any one of claims 1 to 34, wherein the second photoswitchable label comprises a second fluorescent moiety covalently bonded to a second photochromic moiety, optionally via a second linker.

36. The method of claim 35, wherein the second fluorescent moiety comprises a coumarin moiety.

37. The method of claim 35 or 36, wherein the second photochromic moiety comprises an oxazine moiety or a thiazine moiety.

38. The method of any one of claims 35 to 37, wherein the second photochromic moiety comprises a structure of formula (II):



(II)

wherein:

Y is O (oxygen) or S (sulfur);

each of R^{1b} and R^{2b} is independently selected from the group consisting of H, halo, optionally substituted C_{1-6} alkyl, optionally substituted C_{2-6} alkenyl, optionally substituted C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, and C_{1-6} haloalkoxy;

ring B is C_{6-10} aryl or 5 to 10 membered heteroaryl; each substituted with at least one electron withdrawing group; and

wherein * indicates the attachment point to the fluorescent moiety, and the irradiating step (a)(iii) causes a break of the carbon*—Y bond.

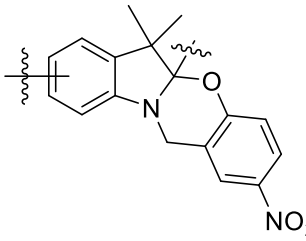
39. The method of claim 38, wherein Y is O.

40. The method of claim 38 or 39, wherein each R^{1b} and R^{2b} is C_{1-6} alkyl.

41. The method of any one of claims 38 to 40, wherein ring B is phenyl or naphthyl substituted with at least one electron withdrawing group.

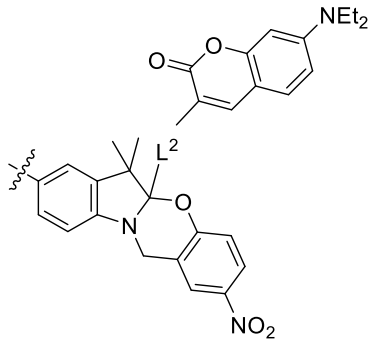
42. The method of claim 41, wherein the electron withdrawing group is selected from the group consisting of nitro, cyano, fluoro, bromo, $-S(O)_2OH$, $-S(O)_2CF_3$, ammonium, alkyl ammonium, and C_{1-6} alkyl substituted with one or more fluoro or bromo.

43. The method of any one of claims 35 to 42, wherein the second photochromic moiety comprises the structure of formula (IIa):



(IIa).

44. The method of any one of claims 35 to 43, wherein the second photoswitchable label comprises the structure:



, wherein L^2 is the second linker.

45. The method of any one of claims 1 to 44, wherein the plurality of polynucleotides are attached to a substrate.

46. The method of claim 45, wherein detecting the first collection of signals and the second collection of signals comprises obtaining images of the substrate.

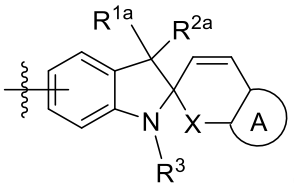
47. A nucleotide conjugate comprising a photoswitchable label, wherein the photoswitchable label comprises a fluorescent moiety covalently bonded, optionally via a linker, to a photochromic moiety.

48. The nucleotide conjugate of claim 47, wherein the fluorescent moiety emits red light upon excitation.

49. The nucleotide conjugate of claim 47 or 48, wherein the fluorescent moiety comprises a silicon rhodamine moiety.

50. The nucleotide conjugate of claim 48 or 49, wherein the photochromic moiety comprises a spiropyrano or spirothiopyrano moiety.

51. The nucleotide conjugate of any one of claims 48 to 50, wherein the photochromic moiety comprises a structure of formula (I):



(I)

wherein:

X is O (oxygen) or S (sulfur);

each of R^{1a} and R^{2a} is independently selected from the group consisting of H, halo, optionally substituted C_{1-6} alkyl, optionally substituted C_{2-6} alkenyl, optionally substituted C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, and C_{1-6} haloalkoxy;

R^3 is selected from the group consisting of H, C_{1-6} alkyl, and $–(CH_2)_n-R^4$,

R^4 is selected from the group consisting of C_{6-10} aryl, 5 to 10 membered heteroaryl, 3 to 7 membered carbocyclyl, and 3 to 7 membered heterocyclyl, each optionally substituted;

ring A is C_{6-10} aryl or 5 to 10 membered heteroaryl, each substituted with at least one electron withdrawing group; and

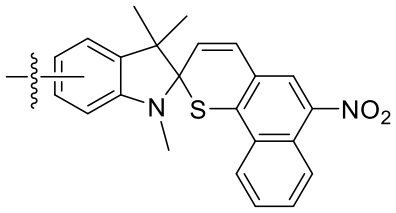
n is an integer of 1 to 6.

52. The nucleotide conjugate of claim 51, wherein X is S.

53. The nucleotide conjugate of claim 51 or 52, wherein each R^{1a} and R^{2a} is C_{1-6} alkyl.

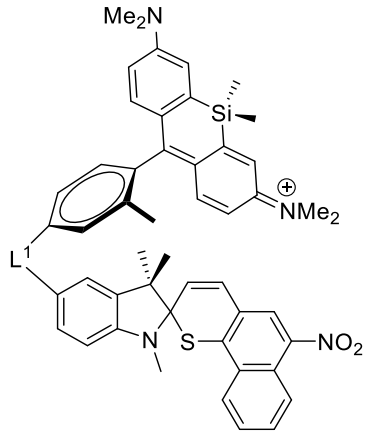
54. The nucleotide conjugate of any one of claims 51 to 53, wherein ring A is phenyl or naphthyl substituted with at least one electron withdrawing group selected from the group consisting of nitro, cyano, fluoro, bromo, $-S(O_2)OH$, , $-S(O)_2CF_3$, ammonium, alkyl ammonium, and C_{1-6} alkyl substituted with one or more fluoro or bromo.

55. The nucleotide conjugate of any one of claims 48 to 54, wherein the photochromic moiety comprises the structure of formula (Ia):



(Ia).

56. The nucleotide conjugate of any one of claims 49 to 55, wherein the photoswitchable label comprise the structure:

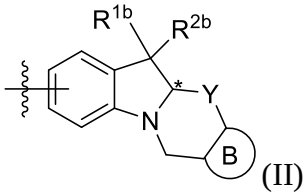


, wherein L^1 is a linker.

57. The nucleotide conjugate of claim 47 or 48, wherein the fluorescent moiety comprises a coumarin moiety.

58. The nucleotide conjugate of claim 57, wherein the photochromic moiety comprises an oxazine moiety or a thiazine moiety.

59. The nucleotide conjugate of claim 57 or 58, wherein the photochromic moiety comprises a structure of formula (II):



(II)

wherein:

Y is O (oxygen) or S (sulfur);

each of R^{1b} and R^{2b} is independently selected from the group consisting of H, halo, optionally substituted C_{1-6} alkyl, optionally substituted C_{2-6} alkenyl, optionally substituted C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, and C_{1-6} haloalkoxy; and

ring B is C_{6-10} aryl or 5 to 10 membered heteroaryl; each substituted with at least one electron withdrawing group; and

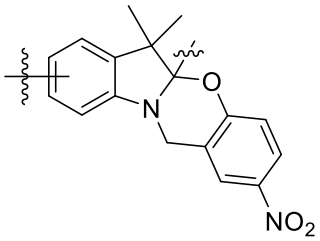
wherein * indicates the attachment point to the fluorescent moiety.

60. The nucleotide conjugate of claim 59, wherein Y is O.

61. The nucleotide conjugate of claim 59 or 60, wherein each R^{1b} and R^{2b} is C_{1-6} alkyl.

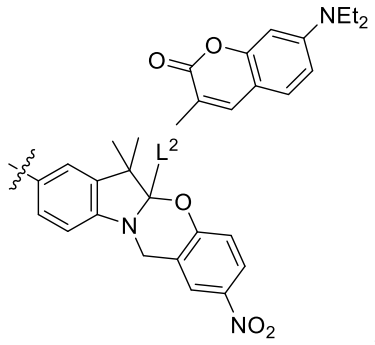
62. The nucleotide conjugate of any one of claims 59 to 61, wherein ring B is phenyl or naphthyl substituted with at least one electron withdrawing group selected from the group consisting of nitro, cyano, fluoro, bromo, $-S(O_2)OH$, $-S(O)_2CF_3$, ammonium, alkyl ammonium, and C_{1-6} alkyl substituted with one or more fluoro or bromo.

63. The nucleotide conjugate of any one of claims 56 to 62, wherein the photochromic moiety comprises the structure of formula (IIa):



(IIa).

64. The nucleotide conjugate of any one of claims 56 to 63, wherein the photoswitchable label comprises the structure:



, wherein L^2 is a linker.

65. The nucleotide conjugate of any one of claims 47 to 64, wherein the photoswitchable label is attached to a nucleobase through a cleavable linker.

66. The nucleotide conjugate of claim 64, wherein the photoswitchable label is attached to the C5 position of a pyrimidine base or the C7 position of a 7-deaza purine base.

67. An oligonucleotide comprising the nucleotide conjugate of any one of claims 47 to 66.

68. A kit comprising two or more types of nucleotides, wherein a first type of nucleotides is a labeled nucleotide conjugate according to any one of claims 48 to 56, and a second type of nucleotides is a labeled nucleotide conjugate according to any one of claims 57 to 64.

69. The kit of claim 68, comprising four nucleotides wherein a third type of nucleotides comprises a label that emits at the same wavelength as the first type of nucleotides, and a fourth type of nucleotides is unlabeled.

70. The kit of claim 69, wherein the first type of nucleotides, the second type of nucleotides, and the third type of nucleotides can be detected at the same emission wavelength.

71. The kit according to any one of claims 68 to 70, for use on an automated sequencing instrument, wherein the automated sequencing instrument comprises two lasers operating at different excitation wavelengths, and a detection system having a single detection channel set to a fixed emission wavelength.