

CLAIMS:

1. A system for identifying a nucleotide in a nucleic acid bound to a substrate, comprising:

- a first detector configured to detect a first range of wavelengths of light;
- a second detector configured to detect a second range of wavelengths of light;
- a light source comprising a laser or a light-emitting diode which outputs light at an optical frequency; and

- a processor configured to:

- control the light source to generate light at the optical frequency to stimulate an emission from a single nucleotide in a nucleic acid on the substrate if the nucleic acid comprises a fluorescent label, wherein one fluorescent label or no fluorescent label is attached to the nucleotide; and

- identify the nucleotide in the nucleic acid based on whether the emission is detectable by the first detector, the second detector, both the first and second detectors, or neither the first nor second detectors.

2. The system of Claim 1, further comprising:

- a first nucleotide coupled to a first fluorescent label;
- a second nucleotide coupled to a second fluorescent label;
- a third nucleotide coupled to a third fluorescent label; and
- a fourth nucleotide coupled to no fluorescent label,

wherein the light source is configured to:

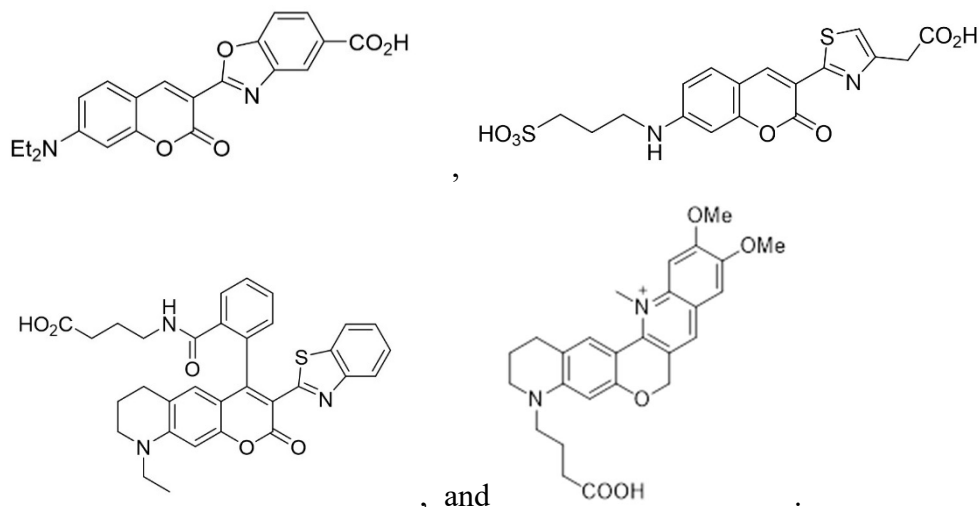
- excite the first fluorescent label to emit light to be detectable by the first detector;

- excite the second fluorescent label to emit light to be detectable by the second detector; and

- excite the third fluorescent label to emit light to be detectable by both the first and second detectors.

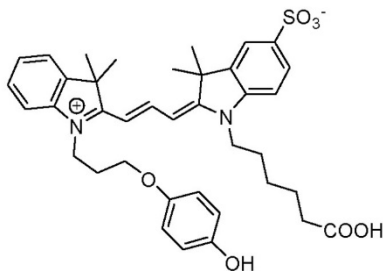
3. The system of Claim 1 or 2, wherein any fluorescent label is selected from the group consisting of polymethine derivatives, coumarin derivatives, benzopyran derivatives, and chromenoquinoline derivatives.

4. The system of any one of Claims 1-3, wherein any fluorescent label is selected from the group consisting of:



5. The system of Claim 2, further comprising an additional first nucleotide coupled to no fluorescent label; or further comprising an additional first nucleotide coupled to an alternative fluorescent label, wherein the alternative fluorescent label cannot be excited by the light source to emit light to be detectable by the first detector; or further comprising an additional first nucleotide coupled to an alternate fluorescent label, wherein the alternate fluorescent label can be excited by the light source to emit light to be detectable by the first detector, and wherein the alternate fluorescent label emits dimmer light as compared to the first fluorescent label.

6. The system of Claim 5, wherein the alternative fluorescent label is



7. The system of Claim 2, wherein the first fluorescent label has a Stokes shift between 20 nm–50 nm, the second fluorescent label has a Stokes shift between 100 nm–130 nm, and the third fluorescent label has a Stokes shift between 60 nm–90 nm.

8. The system of any one of Claims 2-7, wherein the first fluorescent label is not detectable by the second detector, and wherein the second fluorescent label is not detectable by the first detector; or wherein the first fluorescent label is also detectable by the second detector, or wherein the second fluorescent label is also detectable by the first detector .

9. The system of any one of Claims 1-8, wherein the first range of wavelengths and the second range of wavelengths do not overlap.

10. The system of any one of Claims 1-9, wherein the optical frequency corresponds to a wavelength in a predefined range of wavelengths of light, wherein the predefined range comprises at least one wavelength that is shorter than all of the wavelengths in the first range and in the second range; or wherein the predefined range comprises at least one wavelength that is longer than some of the wavelengths in the first range or in the second range.

11. The system of Claim 10, wherein the predefined range comprises 405 nm–460 nm.

12. The system of any one of Claims 1-11, wherein the detectors comprise complementary metal-oxide-semiconductor image sensors, charge-coupled device image sensors, photomultiplier tubes, photodiodes, or any combination thereof.

13. The system of any one of Claims 1-12, further comprising one or more optical filter materials, one or more diffraction gratings, one or more light dispersing elements, or any combination thereof.

14. The system of any one of Claims 1-13, wherein the substrate comprises a plurality of chemically functionalized regions, a plurality of cavities, a plurality of optical resonators, a plurality of optical waveguides, or any combination thereof.

15. The system of any one of Claims 2-14, wherein the nucleotides are selected from the group consisting of an analog of dGTP, an analog of dTTP, an analog of dUTP, an analog of dCTP, and an analog of dATP.

16. The system of any one of Claims 2-15, wherein the first nucleotide is a first reversibly blocked nucleotide triphosphate (rbNTP), the second nucleotide is a second rbNTP, the third nucleotide is a third rbNTP, and the fourth nucleotide is a fourth rbNTP.

17. A method for determining the sequence of a polynucleotide, comprising:

emitting light at an optical frequency from a light source onto a polynucleotide; and
identifying the sequence of the polynucleotide based on whether there is a detectable emission stimulated by the light, from a single nucleotide in the polynucleotide in a first range of wavelengths of light, a second range of wavelengths of light, both the first and second ranges of wavelengths of light, or neither the first nor the second range of wavelengths of light, wherein one fluorescent label or no fluorescent label is coupled to the nucleotide.

18. The method of Claim 17, wherein

determining the nucleotide to be a first type of nucleotide if it is coupled to a first fluorescent label;

determining the nucleotide to be a second type of nucleotide if it is coupled to a second fluorescent label;

determining the nucleotide to be a third type of nucleotide if it is coupled to a third fluorescent label; and

determining the nucleotide to be a fourth type of nucleotide if it is coupled to no fluorescent label.

19. The method of Claim 17 or 18, wherein any coupled fluorescent label is selected from the group consisting of polymethine derivatives, coumarin derivatives, benzopyran derivatives, and chromenoquinoline derivatives.

20. The method of Claim 18 or 19, wherein the first fluorescent label has a Stokes shift between 20 nm–50 nm, the second fluorescent label has a Stokes shift between 100 nm–130 nm, and the third fluorescent label has a Stokes shift between 60 nm–90 nm.