

CLAIMS

1. A method, comprising:
 - providing a sample, the sample including a first nucleotide and a second nucleotide;
 - contacting the sample with a first fluorescent dye associated with a first complementary nucleotide to the first nucleotide and a second fluorescent dye associated with a second complementary nucleotide to the second nucleotide, the first fluorescent dye emitting first emitted light within a first wavelength band responsive to a first excitation illumination light, the second fluorescent dye emitting second emitted light within a second wavelength band responsive to a second excitation illumination light, wherein a first mean wavelength is defined for a first wavelength band of the first fluorescent dye and a second mean wavelength is defined for a second wavelength band of the second fluorescent dye, the first and second mean wavelengths having at least a predefined separation between 50nm and 100nm;
 - simultaneously collecting, using one or more image detectors, multiplexed fluorescent light comprising the first emitted light and the second emitted light, the first emitted light being a first color channel corresponding to the first wavelength band and the second emitted light being a second color channel corresponding to the second wavelength band; and
 - identifying the first nucleotide based on the first wavelength band of the first color channel and the second nucleotide based on the second wavelength band of the second color channel.
2. The method as in claim 1, wherein the first wavelength band corresponds to a blue color and the second wavelength band corresponds to a green color.
3. The method as in claims 1 or 2, wherein the first wavelength band is included within a range of ~~about~~ 450 nm to ~~about~~ 525 nm, and wherein the second wavelength band is included within a range of ~~about~~ 525 nm to ~~about~~ 650 nm.

~~4. The method as in any of claims 1-3, wherein a first mean or peak wavelength is defined for a first emission spectrum of the first fluorescent dye, and a second mean or peak wavelength is defined for a second emission spectrum of the second fluorescent dye, the first and second mean or peak wavelengths having at least a predefined separation from each other.~~

45. The method as in any of claims 1-~~34~~, wherein the first wavelength band has shorter wavelengths than the second wavelength band, wherein the second wavelength band is associated with a first wavelength, and wherein a wavelength emission separation between the first fluorescent dye and the second fluorescent dye is defined so that an emission spectrum of the first fluorescent dye includes at most a predefined amount of light at or above the first wavelength.

56. The method as in any of claims 1-~~45~~, wherein simultaneously collecting the multiplexed fluorescent light includes:

- detecting the first emitted light using a first optical subsystem for the first color channel, and
- detecting the second emitted light using a second optical subsystem for the second color channel,

wherein an emission dichroic filter directs the first emitted light of the first color channel to the first optical subsystem and the second emitted light of the second color channel to the second optical subsystem.

67. The method as in claim 56, wherein at least one of the first optical subsystem and the second optical subsystem includes an angled optical path.

78. The method as in any of claims 1-67, wherein an emission spectrum of the first fluorescent dye has a peak in the first wavelength band.

89. The method as in any of claims 1-78, wherein the sample further includes a third nucleotide, and

wherein the method further comprises:

contacting the sample with a third fluorescent dye emitting third emitted light within the first wavelength band responsive to the first excitation illumination light, and emitting fourth emitted light within the second wavelength band responsive to the second excitation illumination, wherein the multiplexed fluorescent light further comprises the third emitted light and the fourth emitted light; and

identifying the third nucleotide based on the first wavelength band of the first color channel and on the second wavelength band of the second color channel.

949. The method as in any of claims 1-78, wherein the sample further includes a third nucleotide, and

wherein the method further comprises:

contacting the sample with a third fluorescent dye emitting third emitted light within a third wavelength band responsive to a third excitation illumination light, wherein the multiplexed fluorescent light further comprises the third emitted light; and

identifying the third nucleotide based on the third wavelength band.

1044. An apparatus, comprising:

a flow cell containing a sample, the sample including a first nucleotide and a second nucleotide, wherein the first nucleotide is coupled to a first fluorescent dye, wherein the second nucleotide is coupled to a second fluorescent dye, the first fluorescent dye emitting first emitted light within a first wavelength band responsive to a first excitation illumination light, the second fluorescent dye emitting second emitted light within a second wavelength band responsive to a second excitation illumination light;

an illumination system simultaneously providing the first excitation illumination light and the second excitation illumination light to the flow cell; and a light collection system simultaneously collecting multiplexed fluorescent light comprising the first emitted light and the second emitted light, the first emitted light being a first color channel corresponding to the first wavelength band and the second emitted light being a second color channel corresponding to the second wavelength band, wherein a first mean wavelength is defined for a first wavelength band of the first fluorescent dye and a second mean wavelength is defined for a second wavelength band of the second fluorescent dye, the first and second mean wavelengths having at least a predefined separation between 50nm and 100nm.

1112. The apparatus as in claim 1011, wherein the first wavelength band corresponds to a blue color and the second wavelength band corresponds to a green color.

1213. The apparatus as in claims 1011 or 1112, wherein the first wavelength band is included within a range of ~~about~~ 450 nm to ~~about~~ 525 nm, and wherein the second wavelength band is included within a range of ~~about~~ 525 nm to ~~about~~ 650 nm.

~~14.~~ The apparatus as in any of claims ~~11-13~~, wherein ~~a first mean or peak wavelength is defined for a first emission spectrum of the first fluorescent dye, and a second mean or peak wavelength is defined for a second emission spectrum of the second fluorescent dye, the first and second mean or peak wavelengths having at least a predefined separation from each other.~~

1315. The apparatus as in any of claims 1011-1214, wherein the first wavelength band has shorter wavelengths than the second wavelength band, wherein the second wavelength band is associated with a first wavelength, and wherein a wavelength emission separation between the first fluorescent dye and the second fluorescent dye is defined so that an emission spectrum of the first fluorescent dye includes at most a predefined amount of light at or above the first wavelength.

1416. The apparatus as in any of claims ~~1011-1315~~, wherein the light collection system includes:

5 a first optical subsystem for the first color channel detecting the first emitted light, and

a second optical subsystem for the second color channel detecting the second emitted light,

10 wherein an emission dichroic filter directs the first emitted light of the first color channel to the first optical subsystem and the second emitted light of the second color channel to the second optical subsystem.

1517. The apparatus as in claim ~~1416~~, wherein at least one of the first optical subsystem and the second optical subsystem includes an angled optical path.

15 1618. The apparatus as in any of claims ~~1011-1517~~, wherein an emission spectrum of the first fluorescent dye has a peak in the first wavelength band.

1719. The apparatus as in any of claims ~~1011-1618~~, wherein the sample further includes a third nucleotide coupled to a third fluorescent dye emitting third emitted light within the first wavelength band responsive to the first excitation illumination light, and emitting fourth emitted light within the second wavelength band responsive to the second excitation illumination, and wherein the multiplexed fluorescent light further comprises the third emitted light and the fourth emitted light.

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1820. The apparatus as in any of claims ~~1011-1618~~, wherein the sample further includes a third nucleotide coupled to a third fluorescent dye emitting third emitted light within a third wavelength band responsive to a third excitation illumination light, wherein the multiplexed fluorescent light further comprises the third emitted light.

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