CLAIMS

What is claimed is:

1. A method of probing multiple targets in a biological sample comprising:

(a) binding at least one probe to one or more targets present in the biological sample including multiple targets;

(b) ~~observing~~ detecting a signal from the at least one probe bound in step (a);

(c) contacting the sample comprising the bound probe of step (a) with an electron transfer reagent;

(d) irradiating the sample of step (c);

(e) binding at least one probe to one or more targets present in the sample of step (d); and

(f) ~~observing~~ detecting a signal from the probe bound in step (e).

2. The method of claim 1, wherein the probe in step (a) comprises an optical signal generator, and the signal observed in step (b) is an optical signal.

3. The method of claim 2, wherein the probe in step (a) comprises a fluorescent signal generator, and the signal observed in step (b) is a fluorescent signal.

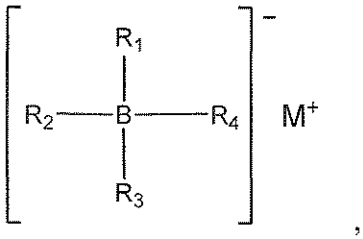
4. The method of claim 1, wherein irradiating the sample in step (d) is carried out in the presence of a buffer at pH of 5-9.

~~5. The method of claim 1, wherein steps (c) and (d) are carried out at the temperature of 4-50 °C.~~

~~6~~5. The method of claim 1, wherein irradiating the sample in step (d) is accomplished by exposing the sample to light of 350 nm - 1.3 μM in wavelength.

~~7~~6. The method of claim ~~6~~5, wherein irradiating the sample in step (d) is accomplished by exposing the sample to light of 400-700 nm in wavelength.

87. The method of claim 1, wherein the electron transfer reagent is a borate salt represented by the following structural formula:



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wherein:

each R_1, R_2, and R_3 is, independently, an alkyl, an alkenyl, an akynyl, an aryl or a heteroaryl, wherein said alkyl, alkenyl, alkynyl, aryl or heteroaryl is optionally substituted with one or more substituents selected from the group consisting of (C1-C4)alkyl, (C1-C4)alkoxy, (C1-C4)alkylamino, amino, hydroxyl, cyano, halogen, or nitro,

R_4 is an alkyl, an alkenyl, or an akynyl, wherein said alkyl, alkenyl, or alkynyl is optionally substituted with one or more substituents selected from the group consisting of (C1-C4)alkyl, (C1-C4)alkoxy, (C1-C4)alkylamino, amino, hydroxyl, cyano, halogen, or nitro, and

M^+ is selected from the group consisting of organic and inorganic cations.

98. The method of claim 87, wherein each R_1, R_2, and R_3 is an optionally substituted aryl and R_4 is an optionally substituted alkyl.

109. The method of claim 98, wherein each R_1, R_2, and R_3 is unsubstituted phenyl and R_4 is unsubstituted butyl, and the borate salt is triphenylbutyl borate salt.

1110. The method of claim 87, wherein M^+ is an inorganic cation selected from the group consisting of Li^+, Na^+ or K^+.

~~12. The method of claim 3, wherein the fluorescent signal generator comprises a cyanine dye.~~

~~13. The method of claim 12, wherein the cyanine dye is Cy3 or Cy5.~~

~~14~~11. The method of claim 1, wherein steps (c)-(f) are repeated one or more times.

~~15. The method of claim 1, wherein the steps (c) and (d) are performed for about 20 seconds to about 15 minutes.~~

~~16. The method of claim 1, further comprising measuring one or more intensity values of the signal observed in observing step (b), step (f), or steps (b) and (f).~~

~~17. The method of claim 16, further comprising correlating the intensity value with an amount of target present in the sample.~~

~~18~~12. The method of claim 1, wherein the probe in step (a) and the probe in step (e) each comprise a signal generator, wherein the signal generator in step (a) is different from the signal generator in step (e).

~~19~~13. A method of probing multiple targets in a biological sample comprising:

(a) binding multiple probes to multiple targets present in the biological sample, wherein the multiple probes include a first set of probes and a second set of probes;

(b) ~~observing~~ detecting a first set of signals from the first set of probes bound in step (a);

(c) contacting the sample comprising the bound probe of step (a) with an electron transfer reagent;

(d) irradiating the sample of step (c);

(e) generating a second set of signals from the second set of probes bound in step (a);

(f) ~~observing~~ detecting the second set of signals.

~~20~~14. The method of claim 1, wherein irradiation of sample in step (d) initiates a photoreaction that substantially inactivates the signal generator by photoactivated chemical bleaching.

~~21. The method of claim 1, wherein the signal generator is irreversibly modified.~~

~~22~~15. The method of claim 1, wherein no detectable signal is observed after step (d).

~~23~~16. A high throughput multiplexing biological sample analysis method, the method comprising:

a signal cycling process, wherein in each cycle, staining and imaging is followed by applying an electron transfer reagent and irradiation of the biological sample.

~~24~~17. The method of claim ~~23~~16, wherein the method allows rapid signal cycling without significantly modifying the components of the biological sample that are different from the probe.

~~25~~18. A series of at least two images depicting optically labeled biological targets wherein:

the images are obtained in the process of probing multiple targets in a biological sample, wherein the process comprises:

(a) binding at least one optical probe to one or more targets present in the biological sample including multiple targets;

(b) ~~observing~~ detecting a signal from the optical probe bound in step (a);

(c) contacting the sample comprising the bound optical probe of step (a) with an electron transfer reagent;

(d) irradiating the sample of step (c);

(e) binding at least one optical probe to one or more targets present in the sample of step (d); and

(f) ~~observing~~ detecting a signal from the optical probe bound in step (e).