sample. The photoreaction step can include applying an electron transfer agent, *e.g.,* a borate salt, and initiating a photoreaction, *e.g.*, by irradiating the sample with visible light, to inactivate the signal generator, *e.g.*, fluorescent dye. The photoreaction step may further include an additive which prevents target modification caused by the photoreaction by-product, e.g., free radicals and singlet oxygen.

[00010] In some embodiments, advantages of the disclosed methods may include the rapid destruction of signal in each cycle. For example, in some instances, quenching is observed in about 100 milliseconds as compared to more than 15 minutes in conventional methods. In some embodiments, the disclosed methods also may be characterized by the absence of residual fluorescence even in high expression targets resulting, *e.g.,* in increased signal-to-noise ratio. Also, the disclosed methods do not damage the biological sample or its components, *e.g.*, the epitopes, such that the same sample may be used for many dozens of cycles. Also, in some embodiments, when compared to direct photobleaching of fluorescent dyes, the disclosed methods are advantageous because they do not require high power light which may damage biological sample components.

[00011] ~~In one aspect, t~~The present disclosure is 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) 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 and an additive which prevents target modification during step (d);

(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

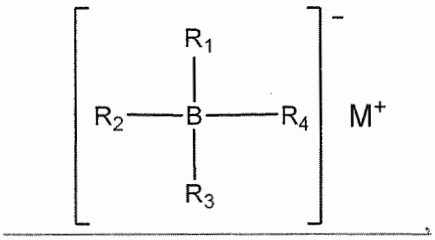
(f) detecting a signal from the probe bound in step (e).

[00011a] In an aspect, there is provided 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) 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, wherein the electron transfer reagent is a borate salt represented by the following structural formula:



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.

and an additive which prevents target modification in step (d) below wherein said additive is selected from a free radical scavenger or a quencher for singlet oxygen ;

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

[00033] In some embodiments, the components of the biological sample that are different from the probe are not significantly modified.

[00034] In some embodiments, no detectable signal is detected after step (d).

[00035] In some embodiments, the signal generator comprises a chromophore, or a Raman-active tag.

[00036] In some embodiments, the additive which prevents target modification is a free radical scavenger. In a preferred embodiment, the free radical scavenger is selected from the group consisting of ascorbic acid, n-propyl gallate, mercaptoethanol, cysteine hydrochloride, t-butyl hydroxy toluene, cycloheptatriene, dioctyl phthalate, 1,4-Dihydro-*o*-toluamide, a-tocopherol and trolox.

[00037] In other embodiments, the additive which prevents target modification is a quencher for singlet oxygen. In a preferred embodiment, the quencher for singlet oxygen is selected from the group consisting of ascorbic acid, a-tocopherol, curcurmin and DABCO.

[00038] In some embodiments, the method of probing multiple targets in a biological sample further comprises, after step (d), washing the sample with a wash solution that effectively removes residual electron transfer reagents from the sample. In some embodiments one or more enablers may be added to the wash solution that may facilitate removal of residual electron transfer reagent by increasing it solubility in the wash solution. In some embodiments these enablers include organic solvent, cationic reagents, chaotropes, detergents or a combination thereof. In preferred embodiments the enabler is ethanol. In the most preferred embodiment the enabler is 70% ethanol.

[00039] In another ~~aspect~~embodiment, the present disclosure is a method of probing multiple targets in a biological sample comprising:

(a) binding multiple probes to multiple targets present in the biological sample including multiple targets;

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

(c) contacting the sample comprising the bound probes of step (a) with an electron transfer reagent and an additive which prevents target modification in step (d);

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

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

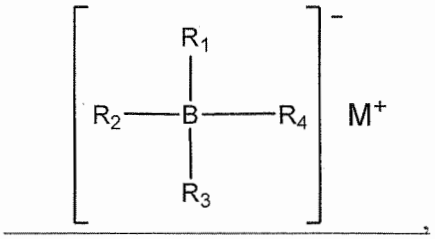
(f) detecting the second set of signals.

[00039a] In another aspect, there is provided a method of probing multiple targets in a biological sample comprising:

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

(b) 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 wherein the electron transfer reagent is a borate salt represented by the following structural formula:



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.

and an additive which prevents target modification in step (d) below wherein said additive is selected from a free radical scavenger or a quencher for singlet oxygen;

(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) detecting the second set of signals.

[00040] In some embodiments, irradiation of sample in step (d) initiates a photoreaction that substantially inactivates the signal generator by photoactivated chemical bleaching. In some embodiments, the photoreaction comprises intermolecular electron transfer. In other embodiments, the photoreaction comprises intramolecular electron transfer.

[00041] In some embodiments, the signal generator is irreversibly modified. In some embodiments, the signal generator is irreversibly modified by a photoreaction that inactivates the signal generator by photoactivated chemical bleaching.

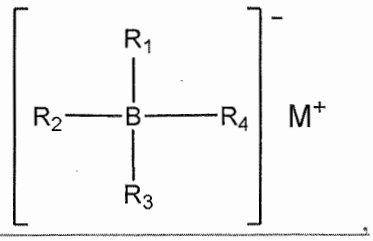
[00042] In some embodiments, the method of probing multiple targets in a biological sample further comprises, after step (d), washing the sample with a wash solution that effectively removes residual electron transfer reagents from the sample. In some embodiments, the wash solution contains ethanol.

[00043] In another ~~aspect~~embodiment, the present disclosure is 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 an additive which prevents target modification and irradiation of the biological sample.

[00043a] In another aspect, there is provided 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 wherein the electron transfer reagent is a borate salt represented by the following structural formula:



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.

and an additive which prevents target modification and irradiation of the biological sample wherein said additive is selected from a free radical scavenger or a quencher for singlet oxygen.

[00044] In some embodiments, the high throughput multiplexing biological sample analysis method comprises, in each cycle, washing the sample with a wash solution that effectively removes residual electron transfer reagents from the sample. In some embodiments, the wash solution contains ethanol.

[00045] In some embodiments, the method allows rapid signal cycling without significantly modifying the components of the biological sample that are different from the probe.

[00046] In another aspect, the present disclosure is a kit for bleaching a signal for probing multiple targets in a biological sample, comprising:

an electron transfer reagent that, when contacted with a signal generator, is capable of bleaching the signal generator upon irradiation; and

an additive which prevents target modification during photoactivated chemical bleaching of the signal generator.

[00047] In certain embodiments, the kit for bleaching a signal may further include additional components for probing multiple targets in a biological sample. For example, the kit may include an antigen retrieval solution. The kit may also include a solution that blocks non-specific binding of a probe to the biological sample. In other embodiments, kit may also include an enabler, a reagent when added to wash solution helps removal of residual borate after signal removal.

[00048] In another aspect, the present disclosure is a method for using the kit to bleach a signal for the purpose of enabling a signal cycling process for probing multiple targets in a biological sample, comprising: after detecting a signal from at least one probe bound to one or more targets present in a biological sample, contacting the sample with the electron transfer reagent and the additive which prevents target modification; and irradiating the sample.

[00049] In some embodiments, the present invention is a kit for probing multiple targets in a biological sample comprising:

multiple probes comprising a binder coupled to a signal generator;

an electron transfer reagent that, when contacted with the signal generator, is capable of bleaching the signal generator upon irradiation; and

an additive which prevents target modification during photoactivated chemical bleaching of the signal generator.

[00050] In certain embodiments, the kits further include an instruction for using the kit.

[00051] In ~~another aspect~~another embodiment, the present disclosure is 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) 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 and an additive which prevents target modification in step (d);

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

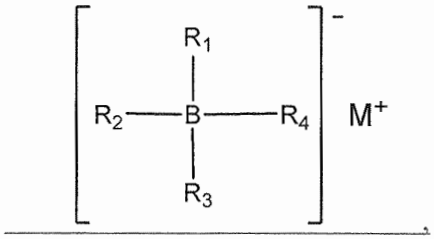
[00051a] In another aspect, there is provided a series of at least two images depicting optically labeled biological targets wherein:

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) 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 wherein the electron transfer reagent is a borate salt represented by the following structural formula:



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.

and an additive which prevents target modification in step (d) below wherein said additive is selected from a free radical scavenger or a quencher for singlet oxygen;

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

[00052] In another embodiment~~aspect~~, the present disclosure is a method of probing 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) detecting a signal from the probe bound in step (a);

(c) contacting the sample comprising the bound probe of step (a) with an electron transfer reagent and an additive which prevents target modification in step (d); and

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

[00053] In another embodiment~~aspect~~, the present disclosure is 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) binding at least one control probe to one or more targets present in the sample;

(c) detecting a signal from the probe bound in step (a) and a control signal from the control probe bound in step (b);

(d) contacting the sample in step (c) with an electron transfer reagent that is capable of selectively reacting with the probe and not the control probe and an additive which prevents target modification in step (e);

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

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

(g) detecting a signal from the probe bound in step (f).

[00054] In some embodiments, the steps (a) and (b) are performed simultaneously. In some embodiments, the step (g) also comprises detecting a signal from the control probe bound in step (b).

[00055] In some embodiments, the method of probing multiple targets in a biological sample further comprises, after step (e), washing the sample with a wash solution that effectively removes residual electron transfer reagents from the sample. In some embodiments, the wash solution contains ethanol.

[00056] In another ~~aspect~~embodiment, the present disclosure is an automated process for photoactivated chemical bleaching of a biological sample loaded/captured in a flow cell device, comprising the following automated steps of

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

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

c) filling the flow cell with an electron transfer reagent and optionally an additive which prevents target modification during subsequent sample irradiation;

d) irradiating the sample by exposure to light to inactivate signals from the probe; and

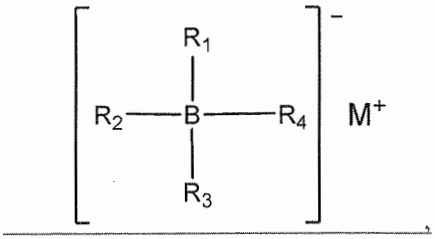
e) repeating steps a) and b) with at least one other probe, for another round of imaging.

[00056a] In another aspect, there is provided an automated process for photoactivated chemical bleaching of a biological sample loaded/captured in a flow cell device, comprising the following automated steps of

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

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

filling the flow cell with an electron transfer reagent wherein the electron transfer reagent is a borate salt represented by the following structural formula:



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.

c) and optionally an additive which prevents target modification during subsequent sample irradiation wherein said additive is selected from a free radical scavenger or a quencher for singlet oxygen;

d) irradiating the sample by exposure to light to inactivate signals from the probe;

e) optionally washing out the electron transfer reagent and the additive; and

f) repeating steps a) and b) with at least one other probe, for another round of imaging.

[00057] In certain embodiments, after sample irradiation, the automated process also includes an optional wash step to wash out the electron transfer reagent and the additive. In other embodiments, the electron transfer reagent and the additive may be washed out during the subsequent probe binding step a). In other embodiments, the electron transfer reagent and the additive may be washed out during an optional step that removed excess probe before subsequent signal detection step b). These latter embodiments are particularly suited for high solubility borate electron transfer reagents.

[00058] In certain embodiments of the automated process for photoactivated chemical bleaching, sample irradiation is accomplished by exposing specific regions of the sample to light using optical filters, a microscope objective, and a translation stage. In other embodiments, sample irradiation is accomplished by exposing the entire sample at once to light.

DESCRIPTION OF THE FIGURES

[00059] FIGURE 1 is a grayscale image of a graph showing absorbance of Cy3 dye at 550 nm after incubation with different concentrations of triphenylbutyl borate lithium salt and irradiation for 4 or 10 minutes.

[00060] FIGURE 2 shows grayscale images of samples stained with Cy3-conjugated cytokeratin before and after photoactivated chemical bleaching.

[00061] FIGURE 3 shows grayscale images of samples stained with Cy5-conjugated pan cadherin before and after photoactivated chemical bleaching.

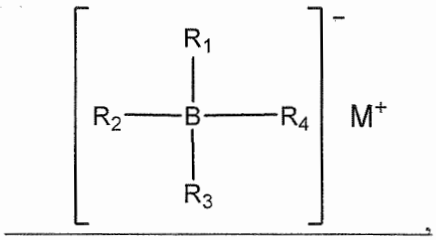
CLAIMS

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) 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, wherein the electron transfer reagent is a borate salt represented by the following structural formula:



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.

and an additive which prevents target modification in step (d) below wherein said additive is selected from a free radical scavenger or a quencher for singlet oxygen ;

(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) 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 detected in step (b) is an optical signal.

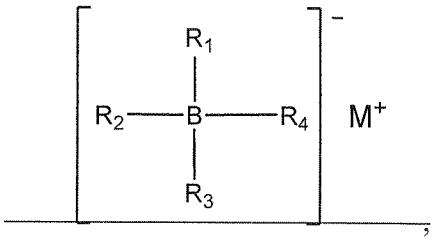
3. The method of claim 2, wherein the probe in step (a) comprises a fluorescent signal generator, and the signal detected in step (b) is a fluorescent signal.

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 irradiating the sample in step (d) is accomplished by exposing the sample to light of 350 nm - 1.3 μM in wavelength.

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

~~7. The method of claim 1, wherein the electron transfer reagent is a borate salt represented by the following structural formula:~~



~~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.~~

~~8~~7. The method of claim ~~7~~1, wherein each R_1, R_2, and R_3 is an optionally substituted aryl and R_4 is an optionally substituted alkyl.

~~9~~8. The method of claim ~~8~~7, 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.

~~10~~9. The method of claim 1, wherein said probe is a morphological stain.

~~11. The method of claim 3, wherein the electron transfer reagent is a high water solubility borate salt.~~

~~12. The method of claim 11, wherein the high water solubility borate salt is a tetra-n-butylborate salt, a Phenyl-Tris-2-(4-methoxyPEG(10)methylphenyl)ethyl borate salt or a diphenyl-bis-2-(4-(methoxyPEG(10)methyl)phenyl)ethyl borate salt.~~

~~13~~10. The method of claim 1, wherein steps (c)-(f) are repeated one or more times.

~~14~~11. The method of claim 1, wherein the steps (c) and (d) are performed for about 100 milliseconds to about 15 minutes.

~~15~~12. The method of claim 1, further comprising, after step (d), washing the sample with a wash solution that effectively removes residual electron transfer reagents from the sample.

~~16~~13. The method of claim ~~15~~12, wherein said wash solution contains an enabler.

~~17~~14. 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).

~~18~~15. 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.

~~19~~16. The method of claim 1, wherein no detectable signal is detected after step d).

~~20~~17. The method of claim 1, wherein the additive which prevents target modification is a free radical scavenger.

~~21~~18. The method of claim ~~20~~17, wherein the free radical scavenger is selected from the group consisting of ascorbic acid, n-propyl gallate, mercaptoethanol, cysteine hydrochloride, t-butyl hydroxy toluene, cycloheptatriene, dioctyl phthalate, 1,4-Dihydro-*o*-toluamide, a-tocopherol and trolox.

~~22~~19. The method of claim 1, wherein the additive which prevents target modification is a quencher for singlet oxygen.

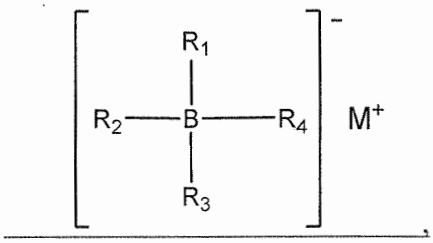
~~23~~20. The method of claim ~~22~~19, wherein the quencher for singlet oxygen is selected from the group consisting of ascorbic acid, a-tocopherol, curcurmin and DABCO.

~~24~~21. A method of probing multiple targets in a biological sample comprising:

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

(b) 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 wherein the electron transfer reagent is a borate salt represented by the following structural formula:



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.

and an additive which prevents target modification in step (d) below wherein said additive is selected from a free radical scavenger or a quencher for singlet oxygen;

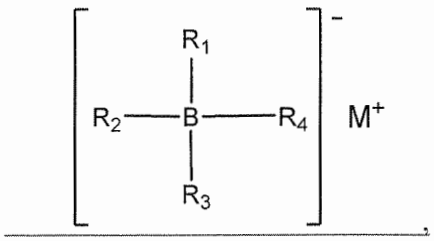
(d) irradiating the sample of step (c);

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

(f) detecting the second set of signals.

~~25~~22. 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 wherein the electron transfer reagent is a borate salt represented by the following structural formula:



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.

and an additive which prevents target modification and irradiation of the biological sample wherein said additive is selected from a free radical scavenger or a quencher for singlet oxygen.

~~26~~23. The method of claim ~~25~~22, wherein the method allows rapid signal cycling without significantly modifying the components of the biological sample that are different from the probe.

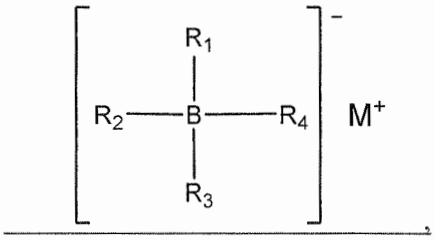
~~27~~24. A series of at least two images depicting optically labeled biological targets wherein:

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) 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 wherein the electron transfer reagent is a borate salt represented by the following structural formula:



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.

and an additive which prevents target modification in step (d) below wherein said additive is selected from a free radical scavenger or a quencher for singlet oxygen;

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

~~28~~25. The method according to claim 1, wherein the binder and signal generator are embodied in a single entity.

~~29~~26. The method according to claim ~~28~~25, wherein the single entity is a small molecule probe.

~~30~~27. The method according to claim ~~29~~26, wherein the small molecule probe is an organic dye.

~~31~~28. The method according to claim ~~30~~27, wherein the organic dye binds specific structures or proteins in the sample.

~~32~~29. A kit for bleaching a signal for probing multiple targets in a biological sample, comprising:

an electron transfer reagent that, when contacted with a signal generator, is capable of bleaching the signal generator upon irradiation; and

an additive which prevents target modification during photoactivated chemical bleaching of the signal generator.

~~33~~30. A method for using the kit of claim ~~32~~29 to bleach a signal for the purpose of enabling a signal cycling process for probing multiple targets in a biological sample, comprising: after detecting a signal from at least one probe bound to one or more targets present in a biological

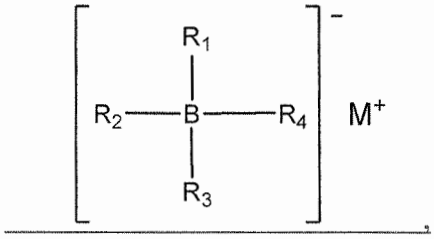
sample, contacting the sample with the electron transfer reagent and the additive which prevents target modification; and irradiating the sample.

~~34~~31. An automated process for photoactivated chemical bleaching of a biological sample loaded/captured in a flow cell device, comprising the following automated steps of

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

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

filling the flow cell with an electron transfer reagent wherein the electron transfer reagent is a borate salt represented by the following structural formula:



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.

c) and optionally an additive which prevents target modification during subsequent sample irradiation wherein said additive is selected from a free radical scavenger or a quencher for singlet oxygen;

d) irradiating the sample by exposure to light to inactivate signals from the probe;

e) optionally washing out the electron transfer reagent and the additive; and

f) repeating steps a) and b) with at least one other probe, for another round of imaging.

~~35~~32. The automated process for photoactivated chemical bleaching according to claim ~~34~~31. wherein sample irradiation is accomplished by exposing specific regions of the sample to light using optical filters, a microscope objective, and a translation stage.

~~36~~33. The automated process for photoactivated chemical bleaching according to claim ~~34~~31. wherein sample irradiation is accomplished by exposing the entire sample at once to light.