[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 embodiment, the present disclosure is a kit for bleaching a signal for probing multiple targets in a biological sample, comprising:

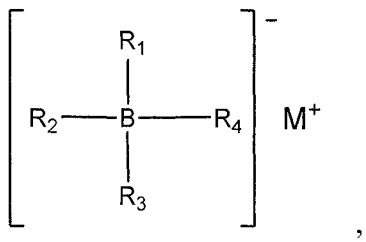
an electron transfer reagent that, when contacted with a signal generator, is capable of bleaching the signal generator upon irradiation; and

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

[00046a] In another aspect, there is provided 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, 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,

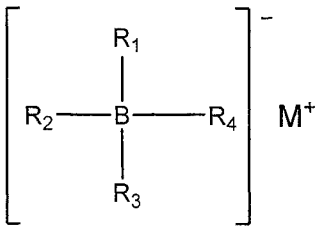
R_4 is an alkyl, an alkenyl, or an akynyl, wherein said alkyl, alkenyl, or alkynyl is optionally substituted with one or more substituents selected from the group consisting of (C1-C4)alkyl, (C1-C4)alkoxy, (C1-C4)alkylamino, amino, hydroxyl, cyano, halogen, or nitro, and

M^+ is selected from the group consisting of organic and inorganic cations, and
wherein said additive is selected from a free radical scavenger or a quencher for singlet oxygen.

[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 embodiment, 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.

[0048a] In another aspect, there is provided a method for using the kit as defined above 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, 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
wherein said additive is selected from a free radical scavenger or a quencher for singlet oxygen.

[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.

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.

23. The method of claim22, wherein the method allows rapid signal cycling without significantly modifying the components of the biological sample that are different from the probe.

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

25. The method according to claim 24, wherein the single entity is a small molecule probe.

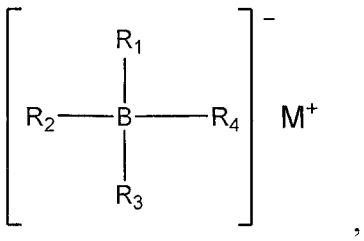
26. The method according to claim25, wherein the small molecule probe is an organic dye.

27. The method according to claim 26, wherein the organic dye binds specific structures or proteins in the sample.

28. 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, 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,

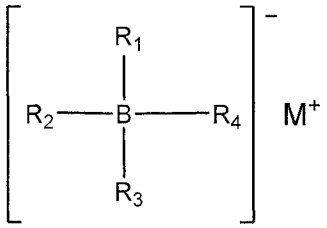
R_4 is an alkyl, an alkenyl, or an akynyl, wherein said alkyl, alkenyl, or alkynyl is optionally substituted with one or more substituents selected from the group consisting of

(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 wherein said additive is selected from a free radical scavenger or a quencher for singlet oxygen.

29. A method for using the kit of claim 28 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, 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

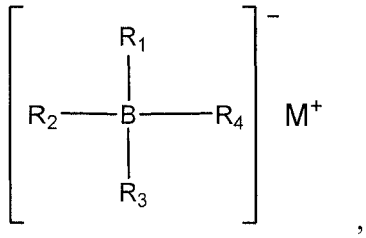
M^+ is selected from the group consisting of organic and inorganic cations, and
wherein said additive is selected from a free radical scavenger or a quencher for singlet oxygen.

30. 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; optionally washing out the electron transfer reagent and the additive; and
e) repeating steps a) and b) with at least one other probe, for another round of imaging.

31. The automated process for photoactivated chemical bleaching according to claim 30. wherein sample irradiation is accomplished by exposing specific regions of the sample to light using optical filters, a microscope objective, and a translation stage.

32. The automated process for photoactivated chemical bleaching according to claim 30. wherein sample irradiation is accomplished by exposing the entire sample at once to light.