BRIEF DESCRIPTION

[0005] Disclosed herein are novel methods for high-throughput multiplexing sample analysis. The methods employ, *e.g.*, a signal cycling process wherein in each cycle, a photoreaction step allows the same signal generators, *e.g.*, fluorophores, to be reused in the subsequent cycle to detect additional markers, *e.g.*, proteins. These methods can be employed, *e.g.*, for sequentially analyzing a biological sample to discern, among other things, the presence, absence, concentration, and/or spatial distribution of multiple biological targets in a biological 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.

[0006] 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 20 seconds 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.

[0006a] In a first 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;

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

[0007] In some embodiments, the present ~~invention~~ 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) observing 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;

as the signal generator in step (e). In other embodiments, the signal generator in step (a) is different from the signal generator in step (e).

[0027] In some embodiments, the signals observed in step (b) and step (f) are both detectable in a single detection channel. In other embodiments, the signal observed in step (b) or step (f) is independently detectable in different detection channels.

[0028] In some embodiments, the components of the biological sample that are different from the probe are not significantly modified.

[0029] In some embodiments, no detectable signal is observed after step (d).

[0030] In some embodiments, the signal generator comprises a chromophore, or a Raman-active tag.

[0030a] In a second 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, wherein the multiple probes include 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;

(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.

[0031] In some embodiments, the present ~~invention~~ 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;

(b) observing 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;

(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 the second set of signals.

[0032] 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.

[0033] 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.

[0034] In some embodiments, the present ~~invention~~ 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 irradiation of the biological sample.

[0035] In some embodiments, the method allows rapid signal cycling without significantly modifying the components of the biological sample that are different from the probe.

[0036] In some embodiments, the present ~~invention~~ disclosure 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.

[0036a] In a third 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;

(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).

[0037] In some embodiments, the present ~~invention~~ 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) observing 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 a signal from the optical probe bound in step (e).

[0038] In some embodiments, the present ~~invention~~ 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) observing 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

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

[0039] In some embodiments, the present ~~invention~~ 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) observing 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;

(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