staining the sample with a fluorescent marker that provides morphological information; (c) detecting, by fluorescence, signals from the first binder and the fluorescent marker; (d) generating the first image of at least part of the sample from the detected fluorescent signals. The step of generating the second image of the biological sample comprising the steps of: (a) contacting the same sample from step (1) with a probe for each of at least one target nucleic acid sequence thus hybridizing the probes with the target nucleic acid sequence; (b) optionally, staining the sample with the fluorescent marker; (c) detecting, by fluorescence, signals from the probes for each of the target nucleic acid sequences and the fluorescent marker; and (d) generating the second image of at least part of the sample from the detected fluorescent signal. In certain embodiments, the composite image is generated by combining one or more signals from the first image with one or more signals from the second image.

~~In one aspect, t~~There is provided a method for providing a composite image of a single biological sample, comprising the steps of:

(1) generating a first image of the biological sample, comprising the steps of:
   a. contacting the sample on a solid support with a first binder for a target protein;
   b. staining the sample with a fluorescent marker that provides morphological information;
   c. detecting, by fluorescence, signals from the first binder and the fluorescent marker;
   d. generating the first image of at least part of the sample from the detected fluorescent signals; and then

(2) generating a second image of the biological sample, comprising the steps of:
   a. contacting the same sample from step (1) with a probe for each of at least one target nucleic acid sequence thus hybridizing the probes with the target nucleic acid sequence;
   b. optionally, staining the sample with the fluorescent marker;
   c. detecting, by fluorescence, signals from the probes for each of the target nucleic acid sequences and the fluorescent marker; and
   d. generating the second image of at least part of the sample from the detected fluorescent signal; and

(3) generating a composite image that provides the relative location of both the target protein and the target nucleic acid.

In a first aspect, there is provided a method for providing a composite image of a single biological sample, comprising the steps of:

(1) generating a first image of the biological sample, comprising the steps of:

e. contacting the sample on a solid support with a first binder for a target protein;

f. staining the sample with a fluorescent marker that provides morphological information;

g. detecting, by fluorescence, signals from the first binder and the fluorescent marker;

h. generating the first image of at least part of the sample from the detected fluorescent signals; and then

(2) generating a second image of the biological sample, comprising the steps of:

a. contacting the same sample from step (1) with a probe for each of at least one target nucleic acid sequence thus hybridizing the probes with the target nucleic acid sequence;

b. staining the sample with the fluorescent marker;

c. detecting, by fluorescence, signals from the probes for each of the target nucleic acid sequences and the fluorescent marker; and

d. generating the second image of at least part of the sample from the detected fluorescent signal; and

(3) generating a composite image that provides the relative location of both the target protein and the target nucleic acid,

whereby the composite image allows direct comparison of results obtained from the first image with those from the second image on a cell to cell basis.

Another embodiment of the disclosure provides a method for generating a first and a second image of a single biological sample, comprising the steps of: (1) generating the first image of the biological sample, comprising the steps of: (a) contacting the sample on a solid support with a first binder for a target protein; (b) staining the sample with a fluorescent marker that provides morphological information; (c) detecting, by fluorescence, signals from the first binder and the fluorescent marker; (d) generating a first image of at least part of the sample from the detected fluorescent signals; and then (2) generating the second image of the biological sample, comprising the steps of: (a) contacting the same sample from step (1) with a probe for each of at least one target nucleic acid sequence thus hybridizing the probes with the target nucleic acid sequence; (b) optionally, staining the sample with the fluorescent marker; (c) detecting, by fluorescence, signals from the probes for each of the target nucleic acid sequences and the fluorescent marker; and (d) generating a second image of at least part of the sample from the detected fluorescent signal.

In another aspect, there is provided a method of analyzing a biological sample, comprising providing a composite image of the biological sample as defined above, and analyzing the expression of the protein and the nucleic acid sequences of interest from the composite image.

~~In another aspect, t~~There is provided a kit for fluorescent detection of a protein as well as fluorescent detection of a target nucleic acid sequence on the same biological sample. Thus, an embodiment of the disclosure provides a kit that includes components for performing the novel method of the disclosure.

In another aspect, there is provided a method for generating a first and a second image of a single biological sample, comprising the steps of:

(1) generating the first image of the biological sample, comprising the steps of:

a. contacting the sample on a solid support with a first binder for a target protein;

b. staining the sample with a fluorescent marker that provides morphological information;

c. detecting, by fluorescence, signals from the first binder and the fluorescent marker;

d. generating a first image of at least part of the sample from the detected fluorescent signals; and then

(2) generating the second image of the biological sample, comprising the steps of:

a. contacting the same sample from step (1) with a probe for each of at least one target nucleic acid sequence thus hybridizing the probes with the target nucleic acid sequence;

b. optionally, staining the sample with the fluorescent marker;

c. detecting, by fluorescence, signals from the probes for each of the target nucleic acid sequences and the fluorescent marker; and

d. generating a second image of at least part of the sample from the detected fluorescent signal,

whereby a composite image allows direct comparison of results obtained from the first image with those from the second image on a cell to cell basis.

Claims

Claim 1: A method for providing a composite image of a single biological sample, comprising the steps of:

(1) generating a first image of the biological sample, comprising the steps of:

a. contacting the sample on a solid support with a first binder for a target protein;
b. staining the sample with a fluorescent marker that provides morphological information;
c. detecting, by fluorescence, signals from the first binder and the fluorescent marker;
d. generating the first image of at least part of the sample from the detected fluorescent signals; and then

(2) generating a second image of the biological sample, comprising the steps of:

a. contacting the same sample from step (1) with a probe for each of at least one target nucleic acid sequence thus hybridizing the probes with the target nucleic acid sequence;
b. ~~optionally,~~ staining the sample with the fluorescent marker;
c. detecting, by fluorescence, signals from the probes for each of the target nucleic acid sequences and the fluorescent marker; and
d. generating the second image of at least part of the sample from the detected fluorescent signal; and

(3) generating a composite image that provides the relative location of both the target protein and the target nucleic acid,

whereby the composite image allows direct comparison of results obtained from the first image with those from the second image on a cell to cell basis.

Claim 2: The method of claim 1, wherein generation of the composite image comprises; a) using signal information acquired in the generation of the first image and the generation of the second image to generate the composite image; or b) registering the location of signals from the fluorescent marker acquired in step 1 with the location of signals from the fluorescent marker acquired in step 2.

~~Claim 3: The method of claim 1, wherein generation of the composite image comprises registering the location of signals from the fluorescent marker acquired in step 1 with the location of signals from the fluorescent marker acquired in step 2.~~

Claim 43. The method of any of claims ~~1-3~~ 2, wherein step (1)(d) comprises,

(i) generating an initial image of at least part of the sample from the detected fluorescent signals; and

(ii) selecting a region of interest from the initial image, and detecting by fluorescence, signals from at least the first binder and the fluorescent marker to generate the first image at a higher resolution than the initial image.

Claim ~~54~~. The method of claim 43, wherein the composite image is generated by combining signal information from the higher resolution image and the second image.

Claim ~~65~~. The method of any of claims ~~4-53~~-4, comprising registering the location of signals from the fluorescent marker in the higher resolution image with the location of signals from the fluorescent marker in the second image.

Claim ~~7-6~~: The method of any of the preceding claims, wherein generating the first image and/or generating the second image comprises generating a brightfield type image that resembles a brightfield stain, wherein said brightfield type images resemble a simulated H&E image or a simulated DAB image.

Claim ~~8~~ 7: The method of claim ~~7~~ 6, wherein the regions of interests are selected at least in part based on morphological information from the brightfield type image of the first image.

Claim ~~9~~8: The method of any of the preceding claims, wherein said binder is an antibody specific for the target protein.

Claim ~~10~~9: The method of claim ~~9~~8, wherein said antibody is labeled with a fluorophore.

Claim ~~11~~10: The method of any of the preceding claims, wherein in step (1)(a), the sample is also contacted with at least one additional binder that provides additional morphological information; and in step (1)(c), signals from the at least one additional binder is also detected by fluorescence.

Claim ~~12~~11: The method of any of the preceding claims, further comprising digesting the sample by a proteinase prior to step (2)(a).

Claim ~~13~~12: The method of any of the preceding claims, wherein in step (2)(a), said hybridizing reaction is selected from the group consisting of FISH, IQ-FISH, in-situ PCR, rolling circle amplification and primed in situ labeling.

Claim ~~14~~13: The method of any of the preceding claims, wherein after step (1)(d), fluorescent signal from the first binder is modified.

Claim ~~15~~14: The method of claim ~~14~~13, wherein steps (1)(a) through (1)(d) are repeated with another binder for a different protein.

Claim ~~16~~15: The method of any of the preceding claims, wherein after step (2)(d), fluorescent signal from the probes is modified by oxidation, stripping, photobleaching, or a mixture thereof, and steps (2)(a) through (2)(d) are repeated with probes for additional nucleic acid sequences of interest.

Claim ~~17~~16: A method of analyzing a biological sample, comprising providing a composite image of the biological sample according to claim 1, and analyzing the expression of the protein and the nucleic acid sequences of interest from the composite image.

Claim ~~18~~17: The method of claim ~~17~~16, wherein in step (1)(a), the sample is also contacted with at least one additional binder that provides additional morphological information; and in step (1)(c), signals from the at least one additional binder is also detected by fluorescence, and the method further comprising creating a color blended composite image for the first image, said composite image includes the image of the target protein, the morphological information represented by the at least one additional binder, and the fluorescent marker.

~~Claim 19: A kit, comprising components for fluorescent detection of a protein as well as fluorescent detection of a target nucleic acid sequence on the same biological sample.~~

~~Claim 20: A system, comprising means for performing fluorescent detection of a protein as well as a target nucleic acid sequence on the same biological sample.~~

Claim ~~2~~1~~8~~: A method for generating a first and a second image of a single biological sample, comprising the steps of:

(1) generating the first image of the biological sample, comprising the steps of:

a. contacting the sample on a solid support with a first binder for a target protein;

b. staining the sample with a fluorescent marker that provides morphological information;

c. detecting, by fluorescence, signals from the first binder and the fluorescent marker;

d. generating a first image of at least part of the sample from the detected fluorescent signals; and then

(2) generating the second image of the biological sample, comprising the steps of:

a. contacting the same sample from step (1) with a probe for each of at least one target nucleic acid sequence thus hybridizing the probes with the target nucleic acid sequence;

b. optionally, staining the sample with the fluorescent marker;

c. detecting, by fluorescence, signals from the probes for each of the target nucleic acid sequences and the fluorescent marker; and

d. generating a second image of at least part of the sample from the detected fluorescent signal,

whereby a composite image allows direct comparison of results obtained from the first image with those from the second image on a cell to cell basis.