experimented on the combination of immunohistochemistry (IHC) and chromogenic in situ hybridization (CISH) for HER2 on a single sample. Am J Clin Pathol 2012; 137:102-110; doi: 10.1309/AJCPLNHINN9O6YSF. While the authors were able to obtain signal by first imaging CISH sample, followed by imaging IHC sample, they found that better images were obtained when not only IHC is performed after CISH, also the sample was only imaged until the completion of all wet lab experimentation (i.e. without an intermediate imaging step). Importantly, Reisenbichler et al. could not obtain CISH signal when they first performed IHC on the sample, then performed CISH.

Gaiser et al. described a method for the analysis of tissue structures localized by protein expression for gene amplification within the same tissue sample. The authors first detected CD133 expression in colon cancer tissue samples, using monoclonal antibody and FITC-labeled secondary antibody. Using 40x magnification, regions of interest were manually selected and recorded. After imaging, FISH analysis was performed simultaneously for amplified cMyc oncogene and ZNF217. The sample was again imaged in the same regions at 40 x magnification. An automated microscope and custom designed classifier for tile based FISH analysis was used to analyze the images. Anal Cell Pathol (Amst). 2010; 33(2): 105–112; doi: 10.3233/ACP-CLO-2010-0532).

There is still a need for improved methods for the detection of multiple targets in the same biological sample and the creation of a composite image to compare expression on a cell by cell basis, allowing the inclusion or exclusion of cells based on protein expression, apoptosis etc.

Summary ~~of the Invention~~

In one ~~aspect~~embodiment of the ~~invention~~disclosure, it is provided a method for the fluorescent detection of a protein as well as a target nucleic acid sequence on the same biological sample. Thus, another~~one~~ embodiment ~~of the invention~~ provides a method for providing a composite image of a single biological sample which comprises the following ordered steps: (1) generating a first image of the biological sample; (2) generating a second image of the biological sample; and (3) generating a composite image that provides the relative location of both the target protein and the target nucleic acid. The step of 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 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, 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.

Another embodiment of the ~~invention~~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 ~~of the invention~~, ~~it~~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 ~~still~~ another aspect ~~of the invention~~, ~~it~~there is provided a kit for ~~the~~ 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 ~~invention~~disclosure provides a kit that includes components for performing the novel method of the ~~invention~~disclosure.

In yet another aspect ~~of the invention~~, ~~it~~there is provided a system for performing ~~the~~ fluorescent detection of a protein as well as a target nucleic acid sequence on the same biological sample. Thus, one embodiment of the ~~invention~~disclosure provides a system that includes means for performing the novel method of the ~~invention~~disclosure.

Brief Description of the Drawings

Figure 1 is a flowchart showing the key steps for an embodiment of the invention.

Figure 2A: Pseudocolor image illustrating a breast cancer tissue imaged at 10x. Tissue section was stained using Cy3 labeled Cytokeratin antibodies, Cy5 labeled Her2 antibody and DAPI. Immunofluorescence images were collected with monochrome camera, digitally overlaid and colored as follows: DAPI, blue; Cytokeratins, green; Her2, red. Selection of an ROI, shown in Figure 2 is shown with a light blue rectangle.

Figure 2B: DAPI and cytokeratin Images from figure 1A and an image representing background fluorescence in green channel were converted to form a virtual H&E image as described.

Figure 2C: Virtual DAB image generated from DAPI and Her2 images in 1A.

Figure 3: Combined Her2 Immunofluorescence (yellow), DAPI (blue), Her2 FISH (red) and Centromere 17 FISH (green) of a 40x ROI selected from tissue shown in Figure 2.

Detailed Description of the Invention

Definitions:

To more clearly and concisely describe and point out the subject matter of the claimed invention, the following definitions are provided for specific terms that are used in the following description and the claims appended hereto.

The singular forms "a" "an" and "the" include plural referents unless the context clearly dictates otherwise. Approximating language, as used herein throughout the specification and claims, may be applied to modify any quantitative representation that could permissibly vary without resulting in a change in the basic function to which it is related. Accordingly, a value