

We claim:

Claim 1: A method of generating an image of a region of interest in a biological sample comprising the steps of:

- 1) generating a first image including said region of interest of said biological sample having undergone a first protocol but not a second protocol; and
- 2) generating a second image including said region of interest of said biological sample after having undergone a second protocol;

wherein said region of interest is smaller than said sample and wherein the second protocol includes immunofluorescence detection of a target protein or fluorescence detection of at least one target nucleic acid sequence by hybridizing a probe with the target nucleic acid sequence.

Claim 2: The method of claim 1, wherein the region of interest is selected by comparing the first image of the biological sample to pre-determined criterion.

Claim 3: The method of any of the preceding claims, wherein the first protocol includes immunofluorescence detection of a target protein.

Claim 4: The method of claim 3, wherein the immunofluorescence detection includes physically binding an antibody with the target protein.

Claim 5: The method of claim 4, wherein said antibody is labeled with a fluorophore.

~~Claim 6: The method of any of claims 3-5, wherein said target protein is selected from the group consisting of cytokeratin, cyclin D1, cyclin B1, Ki67, ER, AR, PI3K, cMET, EGFR, cMyc, PTEN, MAPK, EGFR and HER2.~~

Claim 6: The method of any of claims 3-5, wherein the first protocol further includes immunofluorescence detection of at least one additional protein that provides morphological information.

~~Claim 8: The method of claim 7, wherein said at least one additional protein comprise keratin 15, 19, E-cadherin, Na+K+ATPase, Claudin 1, Collagen IV, EPCAM, fibronectin, glut 1, SMA, vimentin, smooth muscle actin, CD31 and pan-cytokeratin.~~

Claim 9: The method of any of claims 7-8, wherein said morphological information comprises tissue origin, cell origin, tumor, normal, and stromal regions, cell types, and subcellular structure such as cell membrane, cytoplasm, nucleus.

Claim 10: The method of any of claims 7-9, wherein said morphological information comprises information about cytoplasm location of cells of epithelial origin.

Claim 11: The method of any of the preceding claims, wherein the second protocol includes immunofluorescence detection of a target protein or fluorescence detection of at least one target nucleic acid sequence by hybridizing a probe with the target nucleic acid sequence.

Claim 12: The method of claim 11, wherein 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 13: The method of any of claims 11-12, wherein said hybridizing reaction is FISH, and the reaction further comprises probes that identify each of the chromosomes on which the nucleic acid sequences of interest reside.

Claim 14: The method of any of claims 11-13, wherein said probe comprises a nucleic acid probe, a peptide nucleic acid probe, a locked nucleic acid (LNA) probe, mRNA probe, miRNA probe or siRNA probe.

Claim 15: The method of any of claims 11-14, wherein said probe is fluorescently labeled.

Claim 16: The method of any of claims 11-15, wherein said target nucleic acid sequences comprise nucleic acid sequences for EGFR, TOP2A, cMyc, ALK, FGFR1 and HER2.

Claim 17: The method of any of the preceding claims, wherein the first or the second protocol comprises staining the sample with a fluorescent marker that provides morphological information.

~~Claim 18: The method of claim 17, wherein said fluorescent marker comprises 4',6-diamidino-2-phenylindole (DAPI), Eosin, Hoechst 33258 and Hoechst 33342 (two bisbenzimidides), Propidium Iodide, Quinacrine, Fluorescein-phalloidin, Chromomycin A 3, Acridine-Feulgen reaction, Auramine-O-Feulgen reaction and Ethidium Bromide.~~

~~Claim 19: The method of any of claims 17-188, wherein said fluorescent marker stains the nucleus of a cell.~~

~~Claim 120: The method of any of the preceding claims, wherein the first or the second protocol comprises detecting autofluorescence of the biological sample.~~

~~Claim 121: The method of any of the preceding claims, wherein the first and second images are fluorescent images.~~

~~Claim 122: The method of claim 121, 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 23: The method of claim 22, wherein the brightfield type images resemble a simulated H&E image.~~

~~Claim 24: The method of claim 22, wherein the brightfield type images resemble a simulated DAB image.~~

~~Claim 1325: The method of any of the preceding claims, further comprising generating a composite image that includes at least the regions of interest from each of the first and the second images.~~

~~Claim 1426: The method of claim 1325, wherein generating of the composite image comprises registering locations of selected signals obtained during the generation of the first image with locations of selected signals obtained during the generation of the second image.~~

Claim ~~1527~~: The method of any of claims ~~1325-1426~~, wherein the locations of selected signals are from within the region of interest.

Claim ~~1628~~: A method of analyzing a biological sample, comprising providing an image of the biological sample according to claim 1, and analyzing the biological sample from the image.

~~Claim 29: The method of claim 28, wherein the first protocol includes protein expression and the second protocol includes detection of a nucleic acid sequence of interest.~~

~~Claim 30: The method of any of claims 28-29, wherein said sample comprises a cell or a tissue sample.~~

~~Claim 31: The method of any of claims 28-29, wherein said sample comprises a Formalin-Fixed, Paraffin-Embedded (FFPE) tissue sample.~~

Claim ~~1732~~: A method of generating an image of a biological sample, comprising the steps of:

- 1) generating a first image of said biological sample after a first protocol and before a second protocol is performed on said biological sample; and
- 2) Imaging a region of interest of said sample after the second protocol has been performed on the region of interest of said biological sample, said region of interest being smaller than said sample.

Claim ~~1833~~: A method of imaging a biological sample, comprising the steps of

- 1) generating a first image of said biological sample after performing a first protocol but prior to performing a second protocol thereon; and
- 2) generating a second image of a region of interest of said sample after performing said second protocol on said region of interest, wherein said region of interest is smaller than said sample.

Claim ~~1934~~: The method of claim 1, wherein said second image covers a smaller area of said biological sample compared to an area covered by said first image.