

SYSTEMS AND METHODS FOR USING AN IMMUNOSTAINING MASK TO SELECTIVELY REFINE ISH ANALYSIS RESULTS

FIELD

[0001] This disclosure relates generally to the detection of protein expression and target nucleic acid sequences in a biological sample, and more particularly, to systems and methods for using an immunostaining mask to selectively refine in situ hybridization (ISH) analysis results.

BACKGROUND

[0002] Analysis of proteins in histological sections and other cytological preparations may be performed using fluorescence in situ hybridization (FISH). FISH is a cytogenetic technique for detecting and localizing the presence or absence of specific DNA sequences on chromosomes. FISH uses fluorescent probes that bind to only those parts of the chromosome with which they show a high degree of sequence complementarity. Fluorescence microscopy can be used to find out where the fluorescent probe is bound to the chromosomes by counting the fluorescent dots present in an image.

[0003] For FISH analysis in carcinoma tumor samples, the measurement of the FISH dot counts is relevant only for the epithelial cells in a sample. However, conventional FISH analysis measures the FISH signal for all cells in a field of view because there lacks an effective way of limiting the analysis to a defined subset of cells. In the absence of manual intervention, this can lead to many non-epithelial cells (such as stromal cells) contributing to the FISH dot counting statistics, which leads to less accurate dot counting measurements.

SUMMARY

[0004] According to a first aspect, there is provided a computer-implemented method of processing image data representing biological units in a tissue sample, the computer including a processor, the method comprising: receiving, by the processor, a

first image of the tissue sample containing signals from an immunofluorescent (IF) morphological marker, wherein the tissue sample is stained with the IF morphological marker; receiving, by the processor, a second image of the same tissue sample containing signals from a fluorescent probe, wherein the tissue sample is hybridized *in situ* with the fluorescent probe; registering, by the processor, locations of signals from the IF morphological marker in the first image with locations of signals from the fluorescent probe in the second image to produce a registered image; classifying, by the processor, each biological unit in the tissue sample into one of at least two classes based on a mean intensity of the signals from the IF morphological marker in the first image; performing, by the processor, a fluorescence in situ hybridization (FISH) analysis of the tissue sample in the second image to obtain results therefrom; and filtering, by the processor, the results of the FISH analysis to produce a subset of the results pertaining to biological units classified in one of the at least two classes only.

[0004a] ~~According to one embodiment,~~ Also disclosed is a computer-implemented method of processing image data representing biological units in a tissue sample, where the computer includes a processor, includes receiving, by the processor, a first image of

the tissue sample containing signals from an immunofluorescent (IF) morphological marker, wherein the tissue sample is stained with the IF morphological marker, and receiving, by the processor, a second image of the same tissue sample containing signals from a fluorescent probe, wherein the tissue sample is hybridized in situ with the fluorescent probe. The method further includes classifying, by the processor, each biological unit in the tissue sample into one of at least two classes based on a mean intensity of the signals from the IF morphological marker in the first image, performing, by the processor, a fluorescence in situ hybridization (FISH) analysis of the tissue sample in the second image to obtain results therefrom, and filtering, by the processor, the results of the FISH analysis to produce a subset of the results pertaining to biological units classified in one of the at least two classes only. In some embodiments, the classes may include epithelial and non-epithelial cells.

[0005] ~~In some embodiments~~ As mentioned in the above first aspect, the method ~~may further include~~ registering, by the processor, locations of signals from the IF morphological marker in the first image with locations of signals from the fluorescent probe in the second image to produce a registered image. In some embodiments, the method may further include segmenting, by the processor, the second image to identify nuclei in the tissue sample based on the locations of signals from the fluorescent probe in the second image. In some embodiments, each biological unit may correspond to respective nuclei identified in the tissue sample. In some embodiments, the act of segmenting the nuclei may include applying, by the processor, a wavelet transform to the second image.

[0006] In some embodiments, the method may further include generating, by the processor, a Voronoi partition and rings generated from a threshold distance map from the nuclei in the second image in which each respective ring is constrained to at least partially surround only one nucleus in the tissue sample. In some embodiments, the act of generating the Voronoi partition in the second image may enable generating local background regions from the second image using nuclei identified in the tissue sample as seeds of the Voronoi partition multiplied by a mask defined by each ring.

[0007] In some embodiments, the IF morphological marker may be configured to target a cytokeratin (CK) protein. The method may further include thresholding the

first image using a threshold value in conjunction with Otsu's thresholding method and estimating a minimum intensity level of an epithelial region in the first image. In some embodiments, the method may further include changing the threshold value interactively by a user while observing a change in classification of each biological unit.

[0008] In some embodiments, each nucleus may be classified as epithelial or non-epithelial by computing a mean cytokeratin (CK) intensity within the ring surrounding the nucleus and comparing the mean CK intensity to the estimated minimum intensity level of the epithelial region. In some embodiments, the IF morphological marker may include a 4',6-diamidino-2-phenylindole (DAPI) stain. In some embodiments, the IF morphological marker may be configured to target at least one of a cytokeratin (CK) protein and a Human Epidermal Growth Factor Receptor 2 (HER2) protein. In some embodiments, the fluorescent probe may be configured to bind to at least one of a HER2 gene and a Chromosome 17 centrometric repeat.

[0008a] According to a second aspect, there is provided a non-transitory computer-readable medium having stored thereon computer-executable instructions that when executed by a computer cause the computer to: receive a first image of a tissue sample containing signals from an immunofluorescent (IF) morphological marker, wherein the tissue sample is stained with the IF morphological marker; receive a second image of the same tissue sample containing signals from a fluorescent probe, wherein the tissue sample is hybridized *in situ* with the fluorescent probe; register locations of signals from the IF morphological marker in the first image with locations of signals from the fluorescent probe in the second image to produce a registered image; classify each biological unit in the tissue sample into one of at least two classes based on a mean intensity of the signals from the IF morphological marker in the first image; perform a fluorescence in situ hybridization (FISH) analysis of the tissue sample in the second image to obtain results therefrom; and filter the results of the FISH analysis to produce a subset of the results pertaining to biological units classified as epithelial only.

[0009] ~~According to one embodiment,~~Also disclosed is a non-transitory computer-readable medium ~~has stored thereon~~ computer-executable instructions that when executed by a computer cause the computer to receive a first image of a tissue sample containing signals from an immunofluorescent (IF) morphological marker, wherein the tissue sample is stained with the IF morphological marker, and receive a second image of the same tissue sample containing signals from a fluorescent probe, wherein the tissue sample is hybridized in situ with the fluorescent probe. The computer readable medium further includes computer-executable instructions that when executed by the computer cause the computer to classify each biological unit in the tissue sample into one of at least two classes based on a mean intensity of the signals from the IF morphological marker in the first image, perform a fluorescence in situ hybridization (FISH) analysis of the tissue sample in the second image to obtain results therefrom, and filter the results of the FISH analysis to produce a subset of the results pertaining to biological units classified as epithelial only.

[0010] ~~In some embodiments,~~As mentioned in the above second aspect the computer readable medium may further include computer-executable instructions that when executed by the computer cause the

computer to register locations of signals from the IF morphological marker in the first image with locations of signals from the fluorescent probe in the second image to produce a registered image. In some embodiments, the computer readable medium may further include computer-executable instructions that when executed by the computer cause the computer to segment the second image to identify nuclei in the tissue sample based on the locations of signals from the fluorescent probe in the second image.

[0010a] According to a third aspect, there is provided a system for processing image data representing biological units in a tissue sample, the system comprising: a processor; an input in electrical communication with the processor and configured to receive the image data; and a memory in electrical communication with the processor, the memory including computer-executable instructions that when executed by the processor cause the processor to: receive a first image of a tissue sample containing signals from an immunofluorescent (IF) morphological marker, wherein the tissue sample is stained with the IF morphological marker, receive a second image of the same tissue sample containing signals from a fluorescent probe, wherein the tissue sample is hybridized *in situ* with the fluorescent probe; register locations of the signals from the IF morphological marker in the first image with locations of signals from the fluorescent probe in the second image to produce a registered image; classify each biological unit in the tissue sample into one of at least two classes based on a mean intensity of the signals from the IF morphological marker in the first image; perform a fluorescence in situ hybridization (FISH) analysis of the tissue sample in the second image to obtain results therefrom; and filter the results of the FISH analysis to produce a subset of the results pertaining to biological units classified in one of the at least two classes only.

[0011] ~~According to one embodiment,~~Also disclosed is a system for processing image data representing biological units in a tissue sample includes a processor, an input in electrical communication with the processor and configured to receive the image data, and a memory in electrical communication with the processor. The memory includes computer-executable instructions that when executed by the processor cause the processor to receive a first image of a tissue sample containing signals from an immunofluorescent (IF) morphological marker, wherein the tissue

sample is stained with the IF morphological marker, receive a second image of the same tissue sample containing signals from a fluorescent probe, wherein the tissue sample is hybridized in situ with the fluorescent probe, classify each biological unit in the tissue sample into one of at least two classes based on a mean intensity of the signals from the IF morphological marker in the first image, perform a fluorescence in situ hybridization (FISH) analysis of the tissue sample in the second image to obtain results therefrom, and filter the results of the FISH analysis to produce a subset of the results pertaining to biological units classified in one of the at least two classes only. In some embodiments, the classes may include epithelial and non-epithelial cells.

[0012] ~~In some embodiments,~~ As mentioned in the above third aspect, the memory may further include computer-executable instructions that when executed by the processor cause the processor to register locations of signals from the IF morphological marker in the first image with locations of signals from the fluorescent probe in the second image to produce a registered image. In some embodiments, the memory may further include computer-executable instructions that when executed by the processor cause the processor to segment the second image to identify nuclei in the tissue sample based on the

CLAIMS

What is claimed is:

1. A computer-implemented method of processing image data representing biological units in a tissue sample, the computer including a processor, the method comprising:

receiving, by the processor, a first image of the tissue sample containing signals from an immunofluorescent (IF) morphological marker, wherein the tissue sample is stained with the IF morphological marker;

receiving, by the processor, a second image of the same tissue sample containing signals from a fluorescent probe, wherein the tissue sample is hybridized *in situ* with the fluorescent probe;

classifying, by the processor, each biological unit in the tissue sample into one of at least two classes based on a mean intensity of the signals from the IF morphological marker in the first image;

performing, by the processor, a fluorescence *in situ* hybridization (FISH) analysis of the tissue sample in the second image to obtain results therefrom; and

filtering, by the processor, the results of the FISH analysis to produce a subset of the results pertaining to biological units classified in one of the at least two classes only.

2. The method of claim 1, wherein the at least two classes include epithelial and non-epithelial.

3. The method of claim 1, further comprising registering, by the processor, locations of signals from the IF morphological marker in the first image with locations of signals from the fluorescent probe in the second image to produce a registered image.

4. The method of claim 3, further comprising segmenting, by the processor, the second image to identify nuclei in the tissue sample based on the locations of signals from the fluorescent probe in the second image.
5. The method of claim 4, wherein each biological unit corresponds to respective nuclei identified in the tissue sample.
6. The method of claim 4, wherein segmenting the nuclei includes applying, by the processor, a wavelet transform to the second image.
7. The method of claim 6, further comprising generating, by the processor, a Voronoi partition and rings generated from a threshold distance map from the nuclei in the second image in which each respective ring is constrained to at least partially surround only one nucleus in the tissue sample.
8. The method of claim 7, wherein generating the Voronoi partition in the second image enables generating local background regions from the second image using nuclei identified in the tissue sample as seeds of the Voronoi partition multiplied by a mask defined by each ring.
9. The method of claim 7, wherein the IF morphological marker is configured to target a cytokeratin (CK) protein, and wherein the method further comprises thresholding the first image using a threshold value in conjunction with Otsu's thresholding method and estimating a minimum intensity level of an epithelial region in the first image.
10. The method of claim 9, further comprising changing the threshold value interactively by a user while observing a change in classification of each biological unit.
11. The method of claim 9, wherein each nucleus is classified as one of epithelial and non-epithelial by computing a mean cytokeratin (CK) intensity within the ring surrounding the nucleus and comparing the mean CK intensity to the estimated minimum intensity level of the epithelial region.

12. The method of claim 1, wherein the IF morphological marker includes a 4',6'-diamidino-2-phenylindole (DAPI) stain.
13. The method of claim 1, wherein the IF morphological marker is configured to target at least one of a cytokeratin (CK) protein and a Human Epidermal Growth Factor Receptor 2 (HER2) protein.
14. The method of claim 1, wherein the fluorescent probe is configured to bind to least one of a HER2 gene and a Chromosome 17 centromeric repeat.
15. A non-transitory computer-readable medium having stored thereon computer-executable instructions that when executed by a computer cause the computer to:
- receive a first image of a tissue sample containing signals from an immunofluorescent (IF) morphological marker, wherein the tissue sample is stained with the IF morphological marker;
 - receive a second image of the same tissue sample containing signals from a fluorescent probe, wherein the tissue sample is hybridized *in situ* with the fluorescent probe;
 - classify each biological unit in the tissue sample into one of at least two classes based on a mean intensity of the signals from the IF morphological marker in the first image;
 - perform a fluorescence *in situ* hybridization (FISH) analysis of the tissue sample in the second image to obtain results therefrom; and
 - filter the results of the FISH analysis to produce a subset of the results pertaining to biological units classified as epithelial only.
16. The computer readable medium of claim 15, further comprising computer-executable instructions that when executed by the computer cause the computer to:

register locations of signals from the IF morphological marker in the first image with locations of signals from the fluorescent probe in the second image to produce a registered image.

17. The computer readable medium of claim 16, further comprising computer-executable instructions that when executed by the computer cause the computer to:

segment the second image to identify nuclei in the tissue sample based on the locations of signals from the fluorescent probe in the second image.

18. A system for processing image data representing biological units in a tissue sample, the system comprising:

a processor;

an input in electrical communication with the processor and configured to receive the image data; and

a memory in electrical communication with the processor, the memory including computer-executable instructions that when executed by the processor cause the processor to:

receive a first image of a tissue sample containing signals from an immunofluorescent (IF) morphological marker, wherein the tissue sample is stained with the IF morphological marker;

receive a second image of the same tissue sample containing signals from a fluorescent probe, wherein the tissue sample is hybridized *in situ* with the fluorescent probe;

classify each biological unit in the tissue sample into one of at least two classes based on a mean intensity of the signals from the IF morphological marker in the first image;

perform a fluorescence *in situ* hybridization (FISH) analysis of the tissue sample in the second image to obtain results therefrom; and

filter the results of the FISH analysis to produce a subset of the results pertaining to biological units classified in one of the at least two classes only.

19. The system of claim 18, wherein the at least two classes include epithelial and non-epithelial.

20. The system of claim 18, wherein the memory further includes computer-executable instructions that when executed by the processor cause the processor to:

register locations of signals from the IF morphological marker in the first image with locations of signals from the fluorescent probe in the second image to produce a registered image.

21. The system of claim 20, wherein the memory further includes computer-executable instructions that when executed by the processor cause the processor to:

segment the second image to identify nuclei in the tissue sample based on the locations of signals from the fluorescent probe in the second image.

22. The system of claim 21, wherein each biological unit corresponds to respective nuclei identified in the tissue sample.

23. The system of claim 21, wherein the memory further includes computer-executable instructions that when executed by the processor cause the processor to apply a wavelet transform to the second image to segment the nuclei in the tissue sample.

24. The system of claim 23, wherein the memory further includes computer-executable instructions that when executed by the processor cause the processor to generate a Voronoi partition and rings generated from a threshold distance map from the nuclei in the second image in which each respective ring is constrained to at least partially surround only one nucleus in the tissue sample.

25. The system of claim 23, wherein the memory further includes computer-executable instructions that when executed by the processor cause the processor to

generate local background regions from the second image using nuclei identified in the tissue sample as seeds of the Voronoi partition multiplied by a mask defined by each ring.

26. The system of claim 18, wherein the IF morphological marker includes a 4',6-diamidino-2-phenylindole (DAPI) stain.

27. The system of claim 18, wherein the IF morphological marker is configured to target at least one of a cytokeratin (CK) protein and a Human Epidermal Growth Factor Receptor 2 (HER2) protein.

28. The system of claim 18, wherein the fluorescent probe is configured to bind to least one of a HER2 gene and a Chromosome 17 centrometric repeat.

CLAIMS

What is claimed is:

1. A computer-implemented method of processing image data representing biological units in a tissue sample, the computer including a processor, the method comprising:

receiving, by the processor, a first image of the tissue sample containing signals from an immunofluorescent (IF) morphological marker, wherein the tissue sample is stained with the IF morphological marker;

receiving, by the processor, a second image of the same tissue sample containing signals from a fluorescent probe, wherein the tissue sample is hybridized *in situ* with the fluorescent probe;

registering, by the processor, locations of signals from the IF morphological marker in the first image with locations of signals from the fluorescent probe in the second image to produce a registered image;

classifying, by the processor, each biological unit in the tissue sample into one of at least two classes based on a mean intensity of the signals from the IF morphological marker in the first image;

performing, by the processor, a fluorescence *in situ* hybridization (FISH) analysis of the tissue sample in the second image to obtain results therefrom; and

filtering, by the processor, the results of the FISH analysis to produce a subset of the results pertaining to biological units classified in one of the at least two classes only.

2. The method of claim 1, wherein the at least two classes include epithelial and non-epithelial.

3. The method of claim 1, further comprising segmenting, by the processor, the second image to identify nuclei in the tissue sample based on the locations of signals from the fluorescent probe in the second image.
4. The method of claim 3, wherein each biological unit corresponds to respective nuclei identified in the tissue sample.
5. The method of claim 3, wherein segmenting the nuclei includes applying, by the processor, a wavelet transform to the second image.
6. The method of claim 5, further comprising generating, by the processor, a Voronoi partition and rings generated from a threshold distance map from the nuclei in the second image in which each respective ring is constrained to at least partially surround only one nucleus in the tissue sample.
7. The method of claim 6, wherein generating the Voronoi partition in the second image enables generating local background regions from the second image using nuclei identified in the tissue sample as seeds of the Voronoi partition multiplied by a mask defined by each ring.
8. The method of claim 6, wherein the IF morphological marker is configured to target a cytokeratin (CK) protein, and wherein the method further comprises thresholding the first image using a threshold value in conjunction with Otsu's thresholding method and estimating a minimum intensity level of an epithelial region in the first image.
9. The method of claim 8, further comprising changing the threshold value interactively by a user while observing a change in classification of each biological unit.
10. The method of claim 8, wherein each nucleus is classified as one of epithelial and non-epithelial by computing a mean cytokeratin (CK) intensity within the ring surrounding the nucleus and comparing the mean CK intensity to the estimated minimum intensity level of the epithelial region.

11. The method of claim 1, wherein the IF morphological marker includes a 4',6-diamidino-2-phenylindole (DAPI) stain.

12. The method of claim 1, wherein the IF morphological marker is configured to target at least one of a cytokeratin (CK) protein and a Human Epidermal Growth Factor Receptor 2 (HER2) protein.

13. The method of claim 1, wherein the fluorescent probe is configured to bind to least one of a HER2 gene and a Chromosome 17 centrometric repeat.

14. A non-transitory computer-readable medium having stored thereon computer-executable instructions that when executed by a computer cause the computer to:

receive a first image of a tissue sample containing signals from an immunofluorescent (IF) morphological marker, wherein the tissue sample is stained with the IF morphological marker;

receive a second image of the same tissue sample containing signals from a fluorescent probe, wherein the tissue sample is hybridized *in situ* with the fluorescent probe;

register locations of signals from the IF morphological marker in the first image with locations of signals from the fluorescent probe in the second image to produce a registered image;

classify each biological unit in the tissue sample into one of at least two classes based on a mean intensity of the signals from the IF morphological marker in the first image;

perform a fluorescence in situ hybridization (FISH) analysis of the tissue sample in the second image to obtain results therefrom; and

filter the results of the FISH analysis to produce a subset of the results pertaining to biological units classified as epithelial only.

15. The computer-readable medium of claim 14, further comprising computer-executable instructions that when executed by the computer cause the computer to:

Segment the second image to identify nuclei in the tissue sample based on the locations of signals from the fluorescent probe in the second image.

16. A system for processing image data representing biological units in a tissue sample, the system comprising:

a processor;

an input in electrical communication with the processor and configured to receive the image data; and

a memory in electrical communication with the processor, the memory including computer-executable instructions that when executed by the processor cause the processor to:

receive a first image of a tissue sample containing signals from an immunofluorescent (IF) morphological marker, wherein the tissue sample is stained with the IF morphological marker,

receive a second image of the same tissue sample containing signals from a fluorescent probe, wherein the tissue sample is hybridized *in situ* with the fluorescent probe;

register locations of the signals from the IF morphological marker in the first image with locations of signals from the fluorescent probe in the second image to produce a registered image;

classify each biological unit in the tissue sample into one of at least two classes based on a mean intensity of the signals from the IF morphological marker in the first image;

perform a fluorescence in situ hybridization (FISH) analysis of the tissue sample in the second image to obtain results therefrom; and

filter the results of the FISH analysis to produce a subset of the results pertaining to biological units classified in one of the at least two classes only.

17. The system of claim 16, wherein the at least two classes include epithelial and non-epithelial.

18. The system of claim 16, wherein the memory further includes computer-executable instructions that when executed by the processor cause the processor to:

Segment the second image to identify nuclei in the tissue sample based on the locations of signals from the fluorescent probe in the second image.

19. The system of claim 18, wherein each biological unit corresponds to respective nuclei identified in the tissue sample.

20. The system of claim 18, wherein the memory further includes computer-executable instructions that when executed by the processor cause the processor to apply a wavelet transform to the second image to segment the nuclei in the tissue sample.

21. The system of claim 20, wherein the memory further includes computer-executable instructions that when executed by the processor cause the processor to generate a Voronoi partition and rings generated from a threshold distance map from the nuclei in the second image in which each respective ring is constrained to at least partially surround only one nucleus in the tissue sample.

22. The system of claim 20, wherein the memory further includes computer-executable instructions that when executed by the processor cause the processor to generate local background regions from the second image using nuclei identified in the tissue sample as seeds of the Voronoi partition multiplied by a mask defined by each ring.

23. The system of claim 16, wherein the IF morphological marker includes a 4', 6-diamidino-2-phenylindole (DAPI) stain.

24. The system of claim 16, wherein the IF morphological marker is configured to target at least one of a cytokeratin (CK) protein and a human epidermal Growth factor receptor 2 (HER2) protein.

25. The system of claim 16, wherein the fluorescent probe is configured to bind to least one of a HER2 gene and a Chromosome 17 centrometric repeat.