

[0012] The following presents a summary of the present disclosure in order to provide a basic understanding of some of the aspects of the present disclosure. This summary is not an extensive overview of the present disclosure. It is not intended to identify key/critical elements of the present disclosure or to delineate the scope of the present disclosure. Its sole purpose is to present some of the concepts of the present disclosure in a simplified form as a prelude to the more detailed description that is presented later.

[0013] The present disclosure provides systems and methods for tissue classification. An image of a tissue sample (*e.g.*, from a subject) on a substrate is obtained as a plurality of pixels in electronic form (*e.g.*, using transmission light microscopy). In some embodiment the plurality of pixels comprises at least 1000, 10,000, 100,000, 1×10^6 , 2×10^6 , 3×10^6 , 4×10^6 , 5×10^6 , 8×10^6 , or 15×10^6 pixels. The image includes fiducial markers on the substrate boundary. Pixels are assigned to a first class, indicating tissue sample, or a second class, indicating background. The assigning uses the fiducial markers to define a bounding box within the image and removes pixels outside the box. Then, heuristic classifiers are applied to the pixels: for each respective pixel in the plurality of pixels, each heuristic classifier votes for the respective pixel between the first and second class, thereby forming an aggregated score for each pixel that is one of first class, likely first class, likely second class, and obvious second class. *E.g.*, each pixel receives an aggregate score from the heuristic classifiers indicating obvious tissue, likely tissue, likely background, or obvious background. The aggregated score and intensity of each pixel is applied to a segmentation algorithm such as a graph cut algorithm (*e.g.*, a GrabCut segmentation algorithm) to assign a probability to each pixel of being tissue sample or background.

[0013a] According to an aspect, the present disclosure provides a tissue classification method. The method comprises: A) obtaining an image of a tissue sample overlayed on a substrate, as a plurality of pixels in electronic form, wherein the image includes a plurality of fiducial markers on an outer boundary of the substrate surrounding a capture spot array and the tissue sample overlayed on the substrate; and B) assigning each respective pixel in the plurality of pixels to a first class or a second class, wherein the first class indicates overlay of the tissue sample on the substrate and the second class indicates background, and wherein the plurality of pixels comprises at least 100,000 pixels, by a procedure that comprises: (i) using the plurality of fiducial markers to define a bounding box within the image, (ii) using the bounding box to remove respective pixels falling outside the bounding box from the plurality of pixels, (iii) running, after the removing (ii), a plurality of heuristic classifiers on the plurality of pixels, wherein, for each respective pixel in the plurality of pixels, each respective

heuristic classifier in the plurality of heuristic classifiers casts a vote for the respective pixel between the first class and the second class, thereby forming a corresponding aggregated score for each respective pixel in the plurality of pixels, wherein each corresponding aggregated score is a class in a set of classes comprising obvious first class, likely first class, likely second class, and obvious second class, and (iv) applying the aggregated score and intensity of each respective pixel in the plurality of pixels to a segmentation algorithm to independently assign a probability to each respective pixel in the plurality of pixels of being tissue sample or background.

[0014] One embodiment of the present disclosure provides a tissue classification method. The method comprises obtaining an image of a sectioned tissue sample on a substrate (*e.g.*, from a subject) as a plurality of pixels in electronic form. The image includes a plurality of fiducial markers (*e.g.*, on an outer boundary of the substrate). In some embodiments the plurality of fiducial markers comprises more than 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 500, 1000, or 5000 markers. In some embodiments, the plurality of fiducial markers consists of less than 1000, 2000, 3000, 4000, or 5000 fiducial markers. The image is acquired using transmission light microscopy. Each respective pixel in the plurality of pixels is assigned a class. For instance, in some embodiments, each pixel is assigned to a first class or a second class. In some embodiments, each pixel is assigned to any one of a plurality of classes, where the plurality of classes consists of N

What is claimed:

1. A tissue classification method comprising:

A) obtaining an image of a tissue sample overlayed on a substrate, as a plurality of pixels in electronic form, wherein the image includes a plurality of fiducial markers on an outer boundary of the substrate surrounding a capture spot array and the tissue sample overlayed on the substrate; and

B) assigning each respective pixel in the plurality of pixels to a first class or a second class, wherein the first class indicates overlay of the tissue sample on the substrate and the second class indicates background, and wherein the plurality of pixels comprises at least 100,000 pixels, by a procedure that comprises:

(i) using the plurality of fiducial markers to define a bounding box within the image,

(ii) using the bounding box to remove respective pixels falling outside the bounding box from the plurality of pixels,

(iii) running, after the removing (ii), a plurality of heuristic classifiers on the plurality of pixels, wherein, for each respective pixel in the plurality of pixels, each respective heuristic classifier in the plurality of heuristic classifiers casts a vote for the respective pixel between the first class and the second class, thereby forming a corresponding aggregated score for each respective pixel in the plurality of pixels, wherein each corresponding aggregated score is a class in a set of classes comprising obvious first class, likely first class, likely second class, and obvious second class, and

(iv) applying the aggregated score and intensity of each respective pixel in the plurality of pixels to a segmentation algorithm to independently assign a probability to each respective pixel in the plurality of pixels of being tissue sample or background.

2. The method of claim 1, the method further comprising:

overlaying a mask on the image, wherein the mask causes each respective pixel in the plurality of pixels of the image that has been assigned a greater probability of being tissue to be assigned a first attribute and each respective pixel in the plurality of pixels that has been assigned a greater probability of being background to be assigned a second attribute.

3. The method of claim 2, wherein the first attribute is a first color and the second attribute is a second color.

4. The method of claim 3, wherein the first color is one of red and blue and the second color is the other of red and blue.

5. The method of claim 2, wherein the first attribute is a first level of brightness or opacity and the second attribute is a second level of brightness or opacity.

6. The method of claim 2, wherein:

the capture spot array comprises a set of capture spots in the form of a two-dimensional array of positions on the substrate,

each respective capture spot in the set of capture spots is (i) at a different position in the two-dimensional array and (ii) associates with one or more analytes from the tissue, and

each respective capture spot in the set of capture spots is characterized by at least one unique spatial barcode in a plurality of spatial barcodes, and wherein the method further comprises:

assigning each respective capture spot in the plurality of capture spots the first attribute or the second attribute based upon the assignment of pixels in the vicinity of the capture spot in the image to the first attribute or the second attributed by the overlaying.

7. The method of claim 6, wherein a capture spot in the set of capture spots comprises a capture domain.

8. The method of claim 6, wherein a capture spot in the set of capture spots comprises a cleavage domain.

9. The method of claim 6, wherein each capture spot in the set of spots is attached directly or attached indirectly to the substrate.

10. The method of claim 6, wherein the one or more analytes comprise five or more analytes, ten or more analytes, fifty or more analytes, one hundred or more analytes, five hundred or more analytes, 1000 or more analytes, 2000 or more analytes, or between 2000 and 100,000 analytes.

11. The method of claim 6, wherein the unique spatial barcode encodes a unique predetermined value selected from the set {1, ..., 1024}, {1, ..., 4096}, {1, ..., 16384}, {1, ..., 65536}, {1, ..., 262144}, {1, ..., 1048576}, {1, ..., 4194304}, {1, ..., 16777216}, {1, ..., 67108864}, or {1, ..., 1×10^{12} }.
12. The method of any one of claims 6-11, wherein each respective capture spot includes 1000 or more capture probes, 2000 or more capture probes, 10,000 or more capture probes, 100,000 or more capture probes, 1×10^6 or more capture probes, 2×10^6 or more capture probes, or 5×10^6 or more capture probes.
13. The method of claim 12, wherein each capture probe in the respective capture spot includes a poly-A sequence or a poly-T sequence and the unique spatial barcode that characterizes the respective capture spot.
14. The method of claim 12, wherein each capture probe in the respective capture spot includes the same spatial barcode from the plurality of spatial barcodes.
15. The method of claim 12, wherein each capture probe in the respective capture spot includes a different spatial barcode from the plurality of spatial barcodes.
16. The method of any one of claims 1-15, wherein the tissue sample has a depth of 100 microns or less.
17. The method of claim 6, wherein
- the one or more analytes is a plurality of analytes,
 - a respective capture spot in the set of capture spots includes a plurality of capture probes,
 - each capture probe in the plurality of capture probes including a capture domain that is characterized by a capture domain type in a plurality of capture domain types, and
 - each respective capture domain type in the plurality of capture domain types is configured to bind to a different analyte in the plurality of analytes.

18. The method of claim 17, wherein the plurality of capture domain types comprises between 5 and 15,000 capture domain types and the respective capture probe plurality includes at least five, at least 10, at least 100, or at least 1000 capture probes for each capture domain type in the plurality of capture domain types.

19. The method of claim 6, wherein

the one or more analytes is a plurality of analytes,

a respective capture spot in the set of capture spots includes a plurality of capture probes, each capture probe in the plurality of capture probes including a capture domain that is characterized by a single capture domain type configured to bind to each analyte in the plurality of analytes in an unbiased manner.

20. The method of claim 6, wherein each respective capture spot in the set of capture spots is contained within a 100 micron by 100 micron square on the substrate.

21. The method of claim 6, wherein a distance between a center of each respective spot to a neighboring capture spot in the set of capture spots on the substrate is between 50 microns and 300 microns.

22. The method of any one of claims 6-21, wherein a shape of each capture spot in the set of capture spots on the substrate is a closed-form shape.

23. The method of claim 22, wherein the closed-form shape is circular, elliptical, or an N-gon, where N is a value between 1 and 20.

24. The method of 22, wherein the closed-form shape is hexagonal.

25. The method of claim 22, wherein the closed-form shape is circular and each capture spot in the set of capture spots has a diameter of 80 microns or less.

26. The method of claim 25, wherein the closed-form shape is circular and each capture spot in the set of capture spots has a diameter of between 30 microns and 65 microns.

27. The method of claim 26, wherein a distance between a center of each respective capture spot to a neighboring capture spot in the set of capture spots on the substrate is between 50 microns and 80 microns.

28. The method of any one of claims 1-27, wherein the plurality of heuristic classifiers comprises a first heuristic classifier that identifies a single intensity threshold that divides the plurality of pixels into the first class and the second class, thereby causing the first heuristic classifier to cast a vote for each respective pixel in the plurality of pixels for either the first class or the second class, and wherein the single intensity threshold represents a minimization of intra-class intensity variance between the first and second class or a maximization of inter-class variance between the first class and the second class.

29. The method of claim 28, wherein the plurality of heuristic classifiers comprises a second heuristic classifier that identifies local neighborhoods of pixels with the same class identified using the first heuristic classifier and applies a smoothed measure of maximum difference in intensity between pixels in the local neighborhood thereby causing the second heuristic classifier to cast a vote for each respective pixel in the plurality of pixels for either the first class or the second class.

30. The method of any one of claims 1-29, wherein the plurality of heuristic classifiers comprises a third heuristic classifier that performs edge detection on the plurality of pixels to form a plurality of edges in the image, morphologically closes the plurality of edges to form a plurality of morphologically closed regions in the image and assigns pixels in the morphologically closed regions to the first class and pixels outside the morphologically closed regions to the second class, thereby causing the third heuristic classifier to cast a vote for each respective pixel in the plurality of pixels for either the first class or the second class.

31. The method of claim 30, wherein:

the plurality of heuristic classifiers consists of the first, second, and third heuristic classifier, each respective pixel assigned by each of the heuristic classifiers in the plurality of classifiers to the second class is labelled as obvious second class, and

each respective pixel assigned by each of the plurality of heuristic classifiers as the first class is labelled as obvious first class.

32. The method of any one of claims 1-30, wherein the segmentation algorithm is a GrabCut segmentation algorithm.

33. The method of any one of claim 1-32, wherein the method further comprises acquiring the image using transmission light microscopy.

34. A computer system comprising:

one or more processors;

memory; and

one or more programs, wherein the one or more programs are stored in the memory and configured to be executed by the one or more processors, the one or more programs for tissue classification, the one or more programs including instructions for:

A) obtaining an image of a tissue sample overlaid on a substrate, as a plurality of pixels in electronic form, wherein the image includes a plurality of fiducial markers on an outer boundary of the substrate surrounding a capture spot array and the tissue sample overlaid on the substrate; and

B) assigning each respective pixel in the plurality of pixels to a first class or a second class, wherein the first class indicates overlay of the tissue sample on the substrate and the second class indicates background, and wherein the plurality of pixels comprises at least 100,000 pixels, by a procedure that comprises:

(i) using the plurality of fiducial markers to define a bounding box within the image,

(ii) using the bounding box to remove respective pixels falling outside the bounding box from the plurality of pixels,

(iii) running, after the removing (ii), a plurality of heuristic classifiers on the plurality of pixels, wherein, for each respective pixel in the plurality of pixels, each respective heuristic classifier in the plurality of heuristic classifiers casts a vote for the respective pixel between the first class and the second class, thereby forming a corresponding aggregated score for each respective pixel in the plurality of pixels, wherein each corresponding aggregated score is a class in a set of

classes comprising obvious first class, likely first class, likely second class, and obvious second class, and

(iv) applying the aggregated score and intensity of each respective pixel in the plurality of pixels to a segmentation algorithm to independently assign a probability to each respective pixel in the plurality of pixels of being tissue sample or background.

35. A computer readable storage medium storing one or more programs, the one or more programs comprising instructions, which when executed by an electronic device with one or more processors and a memory cause the electronic device to perform tissue classification by a method comprising:

A) obtaining an image of a tissue sample overlaid on a substrate, as a plurality of pixels in electronic form, wherein the image includes a plurality of fiducial markers on an outer boundary of the substrate surrounding a capture spot array and the tissue sample overlaid on the substrate; and

B) assigning each respective pixel in the plurality of pixels to a first class or a second class, wherein the first class indicates overlay of the tissue sample on the substrate and the second class indicates background, and wherein the plurality of pixels comprises at least 100,000 pixels, by a procedure that comprises:

(i) using the plurality of fiducial markers to define a bounding box within the image,

(ii) using the bounding box to remove respective pixels falling outside the bounding box from the plurality of pixels,

(iii) running, after the removing (ii), a plurality of heuristic classifiers on the plurality of pixels, wherein, for each respective pixel in the plurality of pixels, each respective heuristic classifier in the plurality of heuristic classifiers casts a vote for the respective pixel between the first class and the second class, thereby forming a corresponding aggregated score for each respective pixel in the plurality of pixels, wherein each corresponding aggregated score is a class in a set of classes comprising obvious first class, likely first class, likely second class, and obvious second class, and

(iv) applying the aggregated score and intensity of each respective pixel in the plurality of pixels to a segmentation algorithm to independently assign a probability to each respective pixel in the plurality of pixels of being tissue sample or background.