

[0009] 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.

[0010] One aspect of the present disclosure provides a method for identifying a morphological pattern. The method comprises, at a computer system comprising one or more processing cores, a memory, and a display, obtaining a discrete attribute value dataset associated with a plurality of probe spots having a first spatial arrangement. Each probe spot in the plurality of probe spots is assigned a unique barcode in a plurality of barcodes and the plurality of probe spots comprises at least 1000 probe spots. The discrete attribute value dataset comprises a plurality of spatial projections of a biological sample (*e.g.*, tissue sample). The discrete attribute value dataset further comprises at least a first spatial projection for a first tissue section of the biological sample and a second spatial projection for a second tissue section of the biological sample. The discrete attribute value data set further comprises one or more two-dimensional images, for the first spatial projection in the plurality of spatial projections. Each two-dimensional image in the one or more two-dimensional images is taken of a first tissue section, obtained from the biological sample, overlaid on a substrate (*e.g.*, a slide, coverslip, semiconductor wafer, chip, *etc.*) having the plurality of probe spots arranged in the first spatial arrangement. Also, each two-dimensional image in the one or more two-dimensional images comprises a plurality of at least 100,000 pixel values. The discrete attribute value dataset further comprises a corresponding plurality of discrete attribute values for each respective probe spot in the plurality of probe spots obtained from spatial sequencing of the first tissue section. Each respective discrete attribute value in the corresponding plurality of discrete attribute values is for a different locus in a plurality of loci. Each such corresponding plurality of discrete attribute values for each respective probe spot in the plurality of probe spots comprises at least 500 discrete attribute values.

[0011] The method further comprises obtaining a corresponding cluster assignment for each respective probe spot in the plurality of probe spots associated with the discrete attribute value dataset using a clustering analysis of the plurality of probe spots across the plurality of spatial projections, wherein the corresponding cluster assignment assigns the respective probe spot to a corresponding cluster in a plurality of clusters and is based on the corresponding plurality of discrete attribute values of the respective probe spot, or a

corresponding plurality of principal component values derived from the corresponding plurality of discrete attribute values of the respective probe spot.

[0012] The method further comprises displaying, in a first window on the display, a set of pixel values, in the plurality of at least 100,000 pixel values, corresponding to all or a portion of a first two-dimensional image in the one or more two-dimensional images of the first spatial projection.

[0013] The method further comprises overlaying on the first two-dimensional image and co-aligned with the first two-dimensional image (i) first indicia for each probe spot in the plurality of probe spots that have been assigned to a first cluster in the plurality of clusters and (ii) second indicia for each probe spot in the plurality of probe spots that have been assigned to a second cluster in the plurality of clusters, thereby identifying the morphological pattern.

[0014] In some embodiments, the one or more spatial projections is a plurality of spatial projections of the biological sample, the plurality of spatial projections comprises the first spatial projection for a first tissue section of the biological sample, and the plurality of spatial projections comprises a second spatial projection for a second tissue section of the biological sample. In some such embodiments, the one or more two-dimensional images for the first spatial projection comprises a first plurality of two-dimensional images, and the second spatial projection comprises a second plurality of two-dimensional images.

[0015] In some embodiments, each two-dimensional image in the first plurality two-dimensional images is taken of the first tissue section of the biological sample, and each two-dimensional image in the second plurality two-dimensional images is taken of a second tissue section of the biological sample.

[0016] In some embodiments, each two-dimensional image of the first plurality of two-dimensional images is displayed, co-aligned with the (i) first indicia for each probe spot in the plurality of probe spots that have been assigned to the first cluster and (ii) second indicia for each probe spot in the plurality of probe spots that have been assigned to the second cluster. In some such embodiments, the method further comprises, responsive to receiving user display instructions, displaying or removing from display one or more two-dimensional images in the first plurality of two-dimensional images.

[0038] Still another aspect of the present disclosure provides a non-transitory computer readable storage medium storing one or more programs for identifying a morphological pattern. The one or more programs are configured for execution by a computer. The one or more programs collectively encode computer executable instructions for performing any of the methods disclosed above. Still another aspect of the present disclosure provides a visualization system comprising one or more processing cores, a memory, and a display. The memory stores instructions for performing any of the methods disclosed above.

[0039] As disclosed herein, any embodiment disclosed herein when applicable can be applied to any aspect.

[0040] Various embodiments of systems, methods, and devices within the scope of the appended claims each have several aspects, no single one of which is solely responsible for the desirable attributes described herein. Without limiting the scope of the appended claims, some prominent features are described herein. After considering this discussion, and particularly after reading the section entitled “Detailed Description” one will understand how the features of various embodiments are used.

INCORPORATION BY REFERENCE

[0041] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference in their entireties to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0042] The implementations disclosed herein are illustrated by way of example, and not by way of limitation, in the figures of the accompanying drawings. Like reference numerals refer to corresponding parts throughout the several views of the drawings.

[0043] Figures 1A and 1B are an example block diagram illustrating a computing device in accordance with some embodiments of the present disclosure.

CLAIMS

What is claimed:

1. A method for identifying a morphological pattern, the method comprising:
at a computer system comprising one or more processing cores, a memory, and a display:
A) obtaining a discrete attribute value dataset associated with a plurality of probe spots having a first spatial arrangement, wherein each probe spot in the plurality of probe spots is assigned a unique barcode in a plurality of barcodes and the plurality of probe spots comprises at least 1000 probe spots, the discrete attribute value dataset comprising:
 - (i) a plurality of spatial projections of a biological sample, comprising at least a first spatial projection for a first tissue section of the biological sample and a second spatial projection for a second tissue section of the biological sample,
 - (ii) one or more two-dimensional images, for the first spatial projection in the plurality of spatial projections, each two-dimensional image in the one or more two-dimensional images (a) taken of a first tissue section, obtained from the biological sample, overlaid on a substrate having the plurality of probe spots arranged in the first spatial arrangement and (b) comprising a plurality of at least 100,000 pixel values, and
 - (iii) a corresponding plurality of discrete attribute values for each respective probe spot in the plurality of probe spots obtained from a spatial sequencing of the first tissue section, wherein each respective discrete attribute value in the corresponding plurality of discrete attribute values is for a different locus in a plurality of loci and wherein the corresponding plurality of discrete attribute values for each respective probe spot in the plurality of probe spots comprises at least 500 discrete attribute values;
B) obtaining a corresponding cluster assignment for each respective probe spot in the plurality of probe spots associated with the discrete attribute value dataset using a clustering analysis of the plurality of probe spots across the plurality of spatial projections, wherein the corresponding cluster assignment assigns the respective probe spot to a corresponding cluster in a plurality of clusters and is based on the corresponding plurality of discrete attribute values of the respective probe spot, or a corresponding plurality of principal component values derived from the corresponding plurality of discrete attribute values of the respective probe spot;

C) displaying, in a first window on the display, a set of pixel values, in the plurality of at least 100,000 pixel values, corresponding to all or a portion of a first two-dimensional image in the one or more two-dimensional images of the first spatial projection; and

D) overlaying on the first two-dimensional image and co-aligned with the first two-dimensional image (i) first indicia for each probe spot in the plurality of probe spots that have been assigned to a first cluster in the plurality of clusters and (ii) second indicia for each probe spot in the plurality of probe spots that have been assigned to a second cluster in the plurality of clusters, thereby identifying the morphological pattern.

2. The method of claim 1, wherein

the one or more two-dimensional images for the first spatial projection comprises a first plurality of two-dimensional images, and

the second spatial projection comprises a second plurality of two-dimensional images.

3. The method of claim 2, wherein

each two-dimensional image in the first plurality of two-dimensional images is taken of the first tissue section of the biological sample, and

each two-dimensional image in the second plurality of two-dimensional images is taken of the second tissue section of the biological sample.

4. The method of claim 2 or 3, wherein each two-dimensional image of the first plurality of two-dimensional images is displayed, co-aligned with the (i) first indicia for each probe spot in the plurality of probe spots that have been assigned to the first cluster and (ii) second indicia for each probe spot in the plurality of probe spots that have been assigned to the second cluster.

5. The method of claim 4, the method further comprising, responsive to receiving user display

instructions, displaying or removing from display one or more two-dimensional images in the first plurality of two-dimensional images.

6. The method of any one of claims 2-5, wherein each respective two-dimensional image in the first plurality of two-dimensional images is acquired from the first tissue section using a different wavelength or different wavelength band.

7. The method of claim 1, wherein

the one or more two-dimensional images of the first spatial projection is a plurality of two-dimensional images,

a first two-dimensional image in the plurality of two-dimensional images is a bright-field image of the first tissue section,

a second two-dimensional image in the plurality of two-dimensional images is a first immunohistochemistry (IHC) image of the first tissue section taken at a first wavelength or a first wavelength range, and

a third two-dimensional image in the plurality of two-dimensional images is a second immunohistochemistry (IHC) image of the first tissue section taken at a second wavelength or a second wavelength range that is different than the first wavelength or the first wavelength range.

8. The method of claim 7, wherein

the first two-dimensional image is acquired using Haemotoxylin and Eosin, a Periodic acid-Schiff reaction stain, a Masson's trichrome stain, an Alcian blue stain, a van Gieson stain, a reticulin stain, an Azan stain, a Giemsa stain, a Toluidine blue stain, an isamin blue/eosin stain, a Nissl and methylene blue stain, a sudan black and/or osmium staining of the biological sample.

9. The method of claim 1, the method comprising

storing the first two-dimensional image in a first schema, wherein the first schema comprises a first number of tiles; and

storing the first two-dimensional image in a second schema, wherein the second schema comprises a second number of tiles, wherein the second number of tiles is less than the first number of tiles.

10. The method of claim 9, responsive to receiving display instructions for a user, switching from the first schema to the second schema in order to display all or a portion of the first two-dimensional image, or

switching from the second schema to the first schema in order to display all or a portion of the first two-dimensional image.

11. The method of claim 9, wherein:

at least a first tile in the first number of tiles comprises a first predetermined tile size, at least a second tile in the first number of tiles comprises a second predetermined tile size, and

at least a first tile in the second number of tiles comprises of a third predetermined tile size.

12. The method of claim 1, wherein the discrete attribute value dataset redundantly represents a first discrete attribute value for each respective locus in the plurality of loci of each probe spot in the plurality of probe spots and a corresponding second discrete attribute value for each respective probe spot in the plurality of probe spots for the first spatial projection in the plurality of spatial projections in both a compressed sparse row format and a compressed sparse column format in which first and second discrete attribute values that have a null discrete attribute data value are discarded.

13. The method of claim 1, wherein the obtaining B) comprises clustering all or a subset of the probe spots in the plurality of probe spots across the plurality of spatial projections using the discrete attribute values assigned to each respective probe spot in each of the plurality of spatial projections as a multi-dimensional vector, wherein the clustering is configured to load less than the entirety of the discrete attribute value dataset into a non-persistent memory during the clustering thereby allowing the clustering of the discrete attribute value dataset having a size that exceeds storage space in a non-persistent memory allocated to the discrete attribute value dataset.

14. The method of claim 1, wherein each respective cluster in the plurality of clusters consists of a unique subset of the plurality of probe spots.

15. The method of claim 1, wherein at least one probe spot in the plurality of probe spots is assigned to more than one cluster in the plurality of clusters with a corresponding probability value indicating a probability that the at least one probe spot belongs to a respective cluster of the plurality of clusters.

16. The method of claim 1, wherein

each locus in the plurality of loci is a respective gene in a plurality of genes, and

each discrete attribute value in the corresponding plurality of discrete attribute values is a count of UMI that map to a corresponding probe spot and that also map to a respective gene in the plurality of genes.

17. The method of claim 16, wherein the discrete attribute value dataset represents a whole transcriptome sequencing experiment that quantifies gene expression in counts of transcript reads mapped to the plurality of genes.

18. The method of claim 16, wherein the discrete attribute value dataset represents a targeted transcriptome sequencing experiment that quantifies gene expression in UMI counts mapped to probes in the plurality of probes.

19. The method of claim 1, wherein

the first indicia is a first graphic or a first color, and

the second indicia is a second graphic or a second color.

20. The method of claim 13, wherein the clustering all or a subset of the probe spots comprises k-means clustering with K set to a predetermined value between one and twenty-five.

21. The method of claim 1, wherein

each locus in the plurality of loci is a respective feature in a plurality of features,

each discrete attribute value in the corresponding plurality of discrete attribute values is a count of UMI that map to a corresponding probe spot and that also map to a respective feature in the plurality of features, and

each feature in the plurality of features is an open-reading frame, an intron, an exon, an entire gene, an RNA transcript, a predetermined non-coding part of a reference genome, an enhancer, a repressor, a predetermined sequence encoding a variant allele, or any combination thereof.

22. The method of claim 1, wherein the plurality of loci comprises more than 1000 loci.

23. The method of claim 1, wherein each unique 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} }.

24. The method of any one of claims 1-23, wherein the plurality of loci include one or more loci

on a first chromosome and one or more loci on a second chromosome other than the first chromosome.

25. The method of any one of claims 1-24, wherein cells in the first tissue section that map to the probe spots of the first cluster are a first cell type and cells in the first tissue section that map to the probe spots of the second cluster are a second cell type.

26. The method of claim 25, wherein the first cell type is diseased cells and the second cell type is lymphocytes.

27. The method of any one of claims 1-24, wherein cells in the first tissue section that map to the first cluster are a first tissue type and cells in the first tissue section that map to the second cluster are a second tissue type.

28. The method of claim 27, wherein the first tissue type is healthy tissue and the second tissue type is diseased tissue.

29. The method of any one of claims 1-28, wherein the morphological pattern is a spatial arrangement of probe spots assigned to the first cluster relative to probe spots assigned to the second cluster.

30. The method of claim 1, wherein the method further comprises:

in response to a first user selection of a first subset of probe spots using the displayed pixel values of the first two-dimensional image, assigning the first subset of probe spots to the first cluster; and

in response to receiving a second user selection of a second subset of probe spots using the displayed pixel values of the first two-dimensional image, assigning the second subset of probe spots to the second cluster.

31. The method of claim 1, wherein the method further comprises:

in response to a first user selection of a first subset of probe spots using displayed discrete

attribute values of an active list of genes superimposed on the first two-dimensional image, assigning the first subset of probe spots to the first cluster; and

in response to a second user selection of a second subset of probe spots using displayed discrete attribute values of an active list of genes superimposed on the first two-dimensional image, assigning the second subset of probe spots to the second cluster.

32. The method of claim 1, wherein

the discrete attribute value dataset further comprises one or more two-dimensional images for the second spatial projection, each two-dimensional image in the one or more two-dimensional images of the second spatial projection (a) taken of a second tissue section, obtained from the biological sample, overlaid on a substrate having the plurality of probe spots arranged in the first spatial arrangement and (b) comprising a plurality of at least 100,000 pixel values, the method further comprising:

displaying, in a second window on the display, pixel values of all or a portion of a first two-dimensional image in the one or more two-dimensional images of the second projection.

33. The method of claim 32, the method further comprising linking cluster selection, cluster creation, loci selection, cluster membership, or cluster indicia selection between the first window and the second window.

34. The method of any one of claims 1-33, wherein a file size of the discrete attribute value dataset is more than 100 megabytes.

35. The method of any one of claims 1-34, wherein the discrete attribute value dataset further comprises a first layer map comprising a corresponding first plurality of layer map pixel values, wherein the first plurality of layer map pixel values of the first layer map are derived by a first processing of at least the plurality of pixel values of the first two-dimensional image in the one or more two-dimensional images of the first spatial projection or the corresponding plurality of discrete attribute values for each probe spot in the plurality of probe spots associated with the discrete attribute value dataset, the method further comprising:

superimposing and displaying on the first two-dimensional image on the display the

portion of the first layer map that corresponds to the displayed portion of the first two-dimensional image.

36. The method of claim 35, wherein the discrete attribute value dataset further comprises a second layer map comprising a corresponding second plurality of layer map pixel values, wherein the second plurality of layer map pixel values of the second layer map are derived by a second processing of at least the plurality of pixel values of the first two-dimensional image in the one or more two-dimensional images of the first spatial projection or the corresponding plurality of discrete attribute values for each probe spot in the plurality of probe spots associated with the discrete attribute value dataset, the method further comprising:

superimposing and displaying on the first two-dimensional image on the display the portion of the second layer map that corresponds to the displayed portion of the first two-dimensional image.

37. The method of claim 36, wherein the first processing and the second processing are different.

38. The method of claim 36, wherein the first and second processing are each independently selected from the group consisting of a cell segmentation, a stain analysis, an analysis of a combination of stains, an identification of a morphological structure in the first two-dimensional image, an identification of a tissue structure in the first two-dimensional image, a classification of pathology within the first two-dimensional image, and an output of a trained machine learning algorithm in response to input of the first two-dimensional image.

39. The method of claim 35, wherein the first layer map comprises the same number of pixels as the first two-dimensional image.

40. The method of claim 35, wherein the first layer map comprises a different value for each probe spot in the plurality of probe spots.

41. A non-transitory computer-readable medium storing one or more computer programs

executable by a computer for identifying a morphological pattern, the computer comprising a memory, the one or more computer programs collectively encoding computer executable instructions for performing a method comprising:

A) obtaining a discrete attribute value dataset associated with a plurality of probe spots having a first spatial arrangement, wherein each probe spot in the plurality of probe spots is assigned a unique barcode in a plurality of barcodes and the plurality of probe spots comprises at least 1000 probe spots, the discrete attribute value dataset comprising:

(i) a plurality of spatial projections of a biological sample, comprising at least a first spatial projection for a first tissue section of the biological sample and a second spatial projection for a second tissue section of the biological sample,

(ii) one or more two-dimensional images, for the first spatial projection in the plurality of spatial projections, each two-dimensional image in the one or more two-dimensional images (a) taken of a first tissue section, obtained from the biological sample, overlaid on a substrate having the plurality of probe spots arranged in the first spatial arrangement and (b) comprising a plurality of at least 100,000 pixel values, and

(iii) a corresponding plurality of discrete attribute values for each respective probe spot in the plurality of probe spots obtained from a spatial sequencing of the first tissue section, wherein each respective discrete attribute value in the corresponding plurality of discrete attribute values is for a different locus in a plurality of loci and wherein the corresponding plurality of discrete attribute values for each respective probe spot in the plurality of probe spots comprises at least 500 discrete attribute values;

B) obtaining a corresponding cluster assignment for each respective probe spot in the plurality of probe spots associated with the discrete attribute value dataset using a clustering analysis of the plurality of probe spots across the plurality of spatial projections, wherein the corresponding cluster assignment assigns the respective probe spot to a corresponding cluster in a plurality of clusters and is based on the corresponding plurality of discrete attribute values of the respective probe spot, or a corresponding plurality of principal component values derived from the corresponding plurality of discrete attribute values of the respective probe spot; and

C) displaying, in a first window on a display, a set of pixel values, in the plurality of at

least 100,000 pixel values, corresponding to all or a portion of a first two-dimensional image in the one or more two-dimensional images of the first spatial projection; and

D) overlaying on the first two-dimensional image and co-aligned with the first two-dimensional image (i) first indicia for each probe spot in the plurality of probe spots that have been assigned to a first cluster in the plurality of clusters and (ii) second indicia for each probe spot in the plurality of probe spots that have been assigned to a second cluster in the plurality of clusters, thereby identifying the morphological pattern.

42. A visualization system comprising one or more processing cores, a memory, and a display, wherein the memory stores instructions for performing a method for identifying a morphological pattern, the method comprising:

A) obtaining a discrete attribute value dataset associated with a plurality of probe spots having a first spatial arrangement, wherein each probe spot in the plurality of probe spots is assigned a unique barcode in a plurality of barcodes and the plurality of probe spots comprises at least 1000 probe spots, the discrete attribute value dataset comprising:

(i) a plurality of spatial projections of a biological sample, comprising at least a first spatial projection for a first tissue section of the biological sample and a second spatial projection for a second tissue section of the biological sample,

(ii) one or more two-dimensional images, for the first spatial projection in the plurality of spatial projections, each two-dimensional image in the one or more two-dimensional images (a) taken of a first tissue section, obtained from the biological sample, overlaid on a substrate having the plurality of probe spots arranged in the first spatial arrangement and (b) comprising a plurality of at least 100,000 pixel values, and

(iii) a corresponding plurality of discrete attribute values for each respective probe spot in the plurality of probe spots obtained from a spatial sequencing of the first tissue section, wherein each respective discrete attribute value in the corresponding plurality of discrete attribute values is for a different locus in a plurality of loci and wherein the corresponding plurality of discrete attribute values for each respective probe spot in the plurality of probe spots comprises at least 500 discrete attribute values;

B) obtaining a corresponding cluster assignment for each respective probe spot in the plurality of probe spots associated with the discrete attribute value dataset using a clustering analysis of the plurality of probe spots across the plurality of spatial projections, wherein the corresponding cluster assignment assigns the respective probe spot to a corresponding cluster in a plurality of clusters and is based on the corresponding plurality of discrete attribute values of the respective probe spot, or a corresponding plurality of principal component values derived from the corresponding plurality of discrete attribute values of the respective probe spot; and

C) displaying, in a first window on a display, a set of pixel values, in the plurality of at least 100,000 pixel values, corresponding to all or a portion of a first two-dimensional image in the one or more two-dimensional images of the first spatial projection; and

D) overlaying on the first two-dimensional image and co-aligned with the first two-dimensional image (i) first indicia for each probe spot in the plurality of probe spots that have been assigned to a first cluster in the plurality of clusters and (ii) second indicia for each probe spot in the plurality of probe spots that have been assigned to a second cluster in the plurality of clusters, thereby identifying the morphological pattern.