

WHAT IS CLAIMED IS:

~~1. A method of analyzing immune cell infiltration in a cancer stromal region of a biological sample, the method comprising:~~

~~(a) identifying a cancerous region or an analyte associated with the cancerous region in the biological sample;~~

~~(b) identifying a stromal region or an analyte associated with the stromal region in the biological sample;~~

~~(c) identifying one or more immune cells or an analyte associated with an immune cell in one or more locations in the biological sample; and~~

~~(d) using (i) the identified cancerous region and stromal region or associated analytes thereof in the biological sample and (ii) the identified one or more immune cells or associated analytes thereof to analyze immune cell infiltration in the cancer stromal region of the biological sample.~~

~~2. The method of claim 1, wherein the identifying the cancerous region, the identifying the stromal region, and/or the identifying immune cells comprises:~~

~~(a) generating a dataset from the biological sample, wherein the dataset comprises one or more of:~~

~~(i) analyte data for a plurality of analytes captured from a plurality of spatial locations in the biological sample;~~

~~(ii) image data comprising images of the plurality of spatial locations of the biological sample; and~~

~~(iii) registration data linking the analyte data to the image data; and~~

~~(b) using the dataset to identify the cancerous region, the stromal region, and/or the immune cells in the biological sample.~~

~~3. The method of claim 2, wherein (b) comprises providing the dataset to a trained machine learning module, wherein the trained machine learning module is trained at least in part from training data comprising reference analyte datasets from one or more reference samples, wherein the one or more reference samples comprise (1) one or more reference cancerous regions, (2) one or more reference stromal regions, and (3) one or more reference immune cells.~~

~~4. The method of claim 3, wherein the abundance of immune cells is determined via the trained machine learning module.~~

~~5. The method of any one of the preceding claims, wherein the cancerous region comprises one or more of a benign tumor, a pre-metastatic tumor, a malignant tumor, and one or more inflammatory cells.~~

~~6. The method of any one of the preceding claims, wherein the stromal region comprises one or more of connective tissue, blood vessels, and inflammatory cells.~~

~~7. The method as in any one of the preceding claims, further comprising permeabilizing the biological sample.~~

~~8. The method of any one of the preceding claims, wherein the analyte associated with the cancerous region, an analyte associated with the stromal region, and/or an analyte associated with an immune cell is a nucleic acid.~~

~~9. The method of claim 8, wherein the nucleic acid is RNA.~~

~~10. The method of claim 9, wherein the RNA is an mRNA.~~

~~11. The method of any one of the preceding claims, wherein the analyte associated with the cancerous region, the analyte associated with the stromal region, and/or the analyte associated with an immune cell is detected by the steps comprising:~~

~~contacting the biological sample with a substrate comprising a plurality of capture probes, wherein a capture probe of the plurality of capture probes comprises a spatial barcode and a capture domain;~~

~~hybridizing the analyte associated with the cancerous region, the analyte associated with the stromal region, and/or the analyte associated with an immune cell to the capture probe; and~~

~~determining (i) all or a part of a sequence corresponding to the analyte associated with the cancerous region, the analyte associated with the stromal region, and/or the analyte associated with an immune cell, or a complement thereof, and (ii) all or a part of a sequence corresponding to the spatial barcode, or a complement thereof, and using the determined~~

~~sequence of (i) and (ii) to identify the abundance and/or spatial location of the analyte associated with the cancerous region, the analyte associated with the stromal region, and/or the analyte associated with an immune cell, or a complement thereof in the biological sample.~~

~~12.—The method of claim 11, wherein the determining step comprises sequencing.~~

~~13.—The method of any one of claims 1-7, wherein the analyte associated with the cancerous region, an analyte associated with the stromal region, and/or an analyte associated with an immune cell is a protein.~~

~~14.—The method of claim 13, wherein the analyte associated with the cancerous region, the analyte associated with the stromal region, and/or the analyte associated with an immune cell is detected by the steps comprising:~~

~~attaching the biological sample with a plurality of analyte capture agents, wherein an analyte capture agent of the plurality of analyte capture agents comprises:~~

~~(i) — an analyte binding moiety that binds specifically to the analyte associated with the cancerous region, the analyte associated with the stromal region, and/or the analyte associated with an immune cell;~~

~~(ii) — an analyte binding moiety barcode; and~~

~~(iii) — an analyte capture sequence, wherein the analyte capture sequence binds specifically to a capture domain;~~

~~contacting the biological sample with a substrate, wherein the substrate comprises a plurality of capture probes, wherein a capture probe of the plurality of capture probes comprises (i) the capture domain and (ii) a spatial barcode;~~

~~hybridizing the analyte associated with the cancerous region, the analyte associated with the stromal region, and/or the analyte associated with an immune cell to the capture probe; and~~

~~determining (i) all or a part of a sequence corresponding to the analyte associated with the cancerous region, the analyte associated with the stromal region, and/or the analyte associated with an immune cell, or a complement thereof, and (ii) all or a part of a sequence corresponding to the spatial barcode, or a complement thereof, and using the determined sequence of (i) and (ii) to identify the abundance and/or spatial location of the analyte associated with the cancerous region, the analyte associated with the stromal region, and/or the analyte associated with an immune cell, or a complement thereof in the biological sample.~~

15. —The method of claim 14, wherein the determining step comprises:
sequencing (i) all or a part of a sequence corresponding to the analyte associated with the cancerous region, the analyte associated with the stromal region, and/or the analyte associated with an immune cell, or a complement thereof, and (ii) all or a part of a sequence corresponding to the spatial barcode, or a complement thereof, and using the determined sequence of (i) and (ii) to identify the abundance and/or spatial location of the analyte associated with the cancerous region, the analyte associated with the stromal region, and/or the analyte associated with an immune cell, or a complement thereof in the biological sample.

16. —The method of claim 14 or 15, wherein the analyte binding moiety is an antibody or antigen-binding fragment thereof, a cell surface receptor binding molecule, a receptor ligand, a small molecule, a T-cell receptor engager, a B-cell receptor engager, a pro-body, an aptamer, a monobody, an affimer, or a darpin.

17. —The method of any one of the preceding claims, wherein the analyte associated with the cancerous region, the analyte associated with the stromal region, and/or the analyte associated with an immune cell is detected using *in situ* sequencing.

—18. —The method of any one of the preceding claims, wherein the analyte associated with the cancerous region, the analyte associated with the stromal region, and/or the analyte associated with an immune cell is detected using an antibody.

19. —The method of any one of one of the preceding claims, further comprising contacting the biological sample with one or more stains.

20. —The method of claim 19, wherein the one or more stains comprises hematoxylin and eosin.

21. —The method of claim 19 or 20, wherein the one or more stains comprise one or more optical labels.

22.—The method of claim 21, wherein the one or more optical labels are selected from the group consisting of: fluorescent, radioactive, chemiluminescent, calorimetric, or colorimetric labels.

23.—The method of any one of claims 19-22, further comprising identifying one or more cancerous regions in the biological sample using the one or more stains specific to a cancer marker.

24.—The method of claim 23, wherein the cancer marker is pancytokeratin (Pan-CK).

25.—The method of any one of claims 19-24, further comprising identifying one or more stromal regions within the one or more cancerous regions using the one or more stains specific to a stromal marker.

26.—The method of claim 25, wherein the stromal marker is CD45.

27.—The method of any one of claims 2-26, wherein the image data is generated by obtaining an image of the biological sample.

28.—The method of claim 27, further comprising registering the image data to a spatial location.

29.—The method of claim 27 or 28, further comprising identifying (1) the one or more cancerous regions and/or (2) the one or more stromal regions based on the image data.

30.—The method of any one of claims 27-29, further comprising identifying the one or more immune cells based on the image data.

31.—The method of any one of claims 2-30, further comprising identifying the one or more cancerous regions via the trained machine learning module.

32.—The method of any one of claims 2-31, further comprising identifying the one or more stromal regions via the trained machine learning module.

~~33.—The method of any one of claims 2-32, further comprising identifying the one or more immune cells via the trained machine learning module.~~

~~34.—The method of any one of the preceding claims, wherein the analysis of immune cell infiltration in the cancer stromal region of the biological sample comprises determining abundance of immune cells in the cancer stromal region in the biological sample.~~

~~35.—The method of any one of claim 11-34, wherein identifying the one or more cancer regions comprises:~~

~~(i)—obtaining an image and registering the image data to the spatial location,~~

~~(ii)—using the spatial location of the determined sequences, or~~

~~(ii)—obtaining an image and registering the image data to the spatial location, and using the spatial location of the determined sequences;~~

~~identifying the one or more stromal regions comprises:~~

~~(i)—obtaining an image and registering the image data to the spatial location,~~

~~(ii)—using the spatial location of the determined sequences, or~~

~~(iii)—obtaining an image and registering the image data to the spatial location, and using the spatial location of the determined sequences; and~~

~~identifying the one or more immune cells or associated analytes thereof in one or more locations in the biological sample comprises:~~

~~(i)—obtaining an image and registering the image data to the spatial location,~~

~~(ii)—using the spatial location of the determined sequences, or~~

~~(iii)—obtaining an image and registering the image data to the spatial location, and using the spatial location of the determined sequences.~~

~~36.—The method of claim 34, wherein the abundance of immune cells in the cancer stromal region is determined as a percentage of cells in the cancer stroma area that are immune cells or a percentage of area of the cancer stroma that is occupied by immune cells.~~

37. — The method of claim 36, wherein the abundance of immune cells in the cancer stromal region is determined using the spatial location of the determined sequence of the one or more cancerous regions, one or more stromal regions, and one or more immune cells.

38. — The method of claim 37, wherein the using the spatial location of the determined sequences comprises determining the sequence using *in situ* sequencing.

39. — The method of claim 36, wherein the abundance of immune cells in the cancer stromal region is determined using segmenting and

- (i) — obtaining an image and registering the image data to the spatial location;
- (ii) — using the spatial location of the determined sequences, or
- (iii) — obtaining an image and registering the image data to the spatial location, and using the spatial location of the determined sequences.

40. — The method of any one of the preceding claims, wherein the determining comprises:

- (a) — identifying the amount of genes associated with immune infiltrating cells compared to known housekeepers normalized by number of cells per spatial location;
- (b) — identifying the ratio of one or more tumor infiltrating lymphocytes (TILs) to one or more tumor infiltrating B cells (TIBs); and/or
- (c) — calculating the abundance of tumor infiltrating immune cells in the biological sample based on the percentage of spatial locations comprising analytes associated with an immune infiltrating cells.

41. — The method of any one of the preceding claims, wherein the identification of the one or more immune cells comprises segmenting immune cells from the image data.

42. — The method of one of the preceding claims, further comprising determining a cancer prognosis based on the immune infiltration.

43. — The method of one of the preceding claims, further comprising scoring or determining the severity of the cancer in the subject based on the immune infiltration score.

44.—The method of one of the preceding claims, wherein the determining comprises identifying the ratio of one or more tumor infiltrating lymphocytes (TILs) to one or more tumor infiltrating B cells (TIBs) or one or more tumor infiltrating T cells to one or more tumor infiltrating B cells (TIBs).

45.—The method of one of the preceding claims, further comprising administering a therapeutic treatment, wherein the therapeutic treatment comprises surgery, chemotherapeutic agents, growth inhibitory agents, cytotoxic agents, agents used in radiation therapy, anti-angiogenesis agents, cancer immunotherapeutic agents, apoptotic agents, antitubulin agents, or a combination thereof.

46.—The method as in any one of the preceding claims, wherein the biological sample is obtained from a biopsy from a subject.

47.—The method as in any one of the preceding claims, wherein the biological sample is obtained from a surgical excision from a subject.

48.—The method of claim 46 or 47, wherein the biological sample is collected during an endoscopy or colonoscopy from a subject.

49.—The method as in any one of the preceding claims, wherein the biological sample is a tissue section.

50.—The method as in any one of the preceding claims, wherein the biological sample is a tissue section on a slide.

51.—The method as in any one of the preceding claims, wherein the biological sample is a formalin-fixed, paraffin-embedded (FFPE) sample, a frozen sample, or a fresh sample.

52.—The method as in any one of the preceding claims, wherein the biological sample is an FFPE sample.

53.—The method of any one of the preceding claims, wherein the immune cells are selected from a B cell, a T cell, an NK cell, a monocyte, a macrophage, a neutrophil, a granulocyte, an innate lymphoid cell, or a dendritic cell or combinations thereof.

54.—The method of any one of the preceding claims, wherein the analyte associated with the cancerous region is selected from an analyte from the AKT pathway, an analyte from the JAK-STAT pathway, and an analyte from the Notch pathway or combinations thereof.

55.—The method of any one of the preceding claims, wherein the analyte associated with the cancerous region is selected from SCGB2A1, MKI67, BRCA1, BRCA2, PIKCD, CALML6, MYC, TP53, PALB2, RAD51, and MSH2 or combinations thereof.

56.—The method of any one of the preceding claims, wherein the analyte associated with the cancerous region is selected from SCGB2A1, MKI67, BRCA1, BRCA2, PIK3CD, and CALML6 or combinations thereof.

57.—The method of any one of the preceding claims, wherein the analyte associated with the cancerous region is selected from PRKCI, VTCN1, MECOM, TOP2A, SHDH, XPO1, TERC, FUT8, SOX17, PBX1, EIF42, WT1, byproducts, precursors, and degradation products thereof, and any combination thereof.

58.—The method of any one of the preceding claims, wherein the analyte associated with the cancerous region is selected from VTCN1, MECOM, TOP2A, XPO1, FUT8, SOX17, PBX1, EIF42, WT1, byproducts, precursors, and degradation products thereof, and any combination thereof.

59.—The method of any one of the preceding claims, wherein the analyte associated with the cancerous region is TOP2A, and byproducts, precursors, and degradation products thereof.

60.—The method of any one of the preceding claims, wherein the analyte associated with the cancerous region is XPO1, and byproducts, precursors, and degradation products thereof.

61. —The method of any one of the preceding claims, wherein the analyte associated with the stromal region is selected from VIM, EPCAM, FAP, and CDH1.

62. —The method of any one of the preceding claims, wherein the analyte associated with the stromal region is selected from FAP, VCAN, ACTA2, and PDGFRB.

63. —The method of any one of the preceding claims, wherein the analyte associated with an immune cell is selected from BLK, CD19, FCRL2, MS4A1, KIAA0125, TNFRSF17, TCL1A, SPIB, PNOC, PTRPC, PRF1, GZMA, GZMB, NKG7, GZMH, KLRK1, KLRB1, KLRD1, CTSW, GNLY, CCL13, CD209, HSD11B1, LAG3, CD244, EOMES, PTGER4, CD68, CD84, CD163, MS4A4A, TPSB2, TPSAB1, CPA3, MS4A2, HDC, FPR1, SIGLEC5, CSF3R, FCAR, FCGR3B, CEACAM3, S100A12, KIR2DL3, KIR3DL1, KIR3DL2, IL21R, XCL1, XCL2, NCR1, CD6, CD3D, CD3E, SH2D1A, TRAT1, CD3G, TBX21, FOXP3, CD8A, CD8B, CD79A, CD79B, CD4, IGHA1, IGHG2, JCHAIN, IGKC, CD27, CD38, CD16, IL17RB, FANK1, CTLA4, MSR1, MRC1, NKG7, FCN1, TIGIT/LAG3.

64. —The method as in any one of the preceding claims, wherein the one or more immune cells is selected from:

- (i) —a CD3⁺ and CD4⁺ T cell;
- (ii) —a CD3⁺ and CD8⁺ T cell;
- (iii) —a regulatory T cell comprising one or more of: CD4, Foxp3, IL17RB, CTLA4, FANK1, HAVCR1, CD25, CTLA-4, GITR, LAG-3, and CD127;
- (iv) —a TH1 cell comprising one or more of: CD4, CD3D, S100A4, IL7R, and IFNG;
- (v) —a TH2 cell comprising one or more of: CD4, IL7R, ICOS, CTLA4, TNFRSF4, and TNFRS18;
- (vi) —a TH17 cell comprising one or more of: CD4, CD3D, IL17A, GZMA, and S100A4;
- (vii) —a cytotoxic T cell comprising one or more of: CD8, CD3D, S100A4, IFNG, GZMB, GZMA, and IL2RB;
- (viii) —a plasma cell comprising: one or more JCHAIN, MZB1, IGHA1, IGHG1, and IGKC;
- (ix) —a monocyte comprising CD14⁺ CD16⁻;
- (x) —a monocyte comprising CD14⁻ CD16⁺; and

(xi) — a natural killer cell comprising NKG7.

65. — The method of any one of the preceding claims, wherein the immune infiltrating cells is a tumor infiltrating B cell (TIB).

66. — The method of claim 65, wherein the TIB is selected from:

(i) — a plasma cell comprising one or more of: MZB1, IGLL5, IGHA1, IGHG1, JCHAIN, IGKC, IGHA2, IGLC2, IGLV3-1, and IGLV2-14;

(ii) — an Ig⁺ B cells comprising one or more of: IGHV3-74, SOCS3, JCHAIN, and SPARC;

(iii) — an activated B cell comprising: CD79B, HMGB2, HMGB1, HMGN1, and RGS13;

(iv) — a B cell comprising one or more of: MEF2B, RGS13, and MS4A1; and

(v) — a B cell comprising CD79A and CD79B.

67. — A method of determining immune cell infiltration in a biological sample comprising one or more cancerous regions and one or more stromal regions in a subject, the method comprising:

— (a) — generating a dataset from the biological sample obtained from the subject, wherein the dataset comprises:

(i) — analyte data for a plurality of analytes captured from a plurality of spatial locations of the biological sample, wherein an analyte in the plurality of analytes is an analyte associated with the cancerous region, an analyte associated with the stromal region, and/or an analyte associated with an immune cell;

— (b) — providing the dataset to a trained machine learning module, wherein the trained machine learning module comprises reference analyte datasets from one or more reference samples, wherein the one or more reference samples comprises (i) a cancerous region from one or more cancerous regions, (2) a stromal region from one or more stromal regions, and (3) an immune cells from one or more immune cells; and

— (c) — determining, via the trained machine learning module, the immune cell infiltration in the biological sample obtained from the subject.

68.—A method of determining immune cell infiltration in a biological sample comprising one or more cancerous regions and one or more stromal regions, the method comprising:

——(a)——generating a dataset from the biological sample obtained from a subject, wherein the dataset comprises:

(i)——analyte data for a plurality of analytes captured from a plurality of spatial locations of the biological sample, wherein an analyte in the plurality of analytes is an analyte associated with the cancerous region, an analyte associated with the stromal region, and/or an analyte associated with an immune cell;

(ii)——image data comprising images of the plurality of spatial locations of the biological sample; and

(iii)——registration data linking the analyte data to the image data;

——(b)——providing the dataset to a trained machine learning module, wherein the trained machine learning module comprises reference analyte datasets from one or more reference samples, wherein the one or more reference samples comprises (i) a cancerous region from one or more cancerous regions, (2) a stromal region from one or more stromal regions, and (3) an immune cells from one or more immune cells; and

——(c)——determining, via the trained machine learning module, the immune cell infiltration in the biological sample.

69.—The method of claim 67 or 68, wherein the trained machine learning module is at least one of a supervised learning module, a semisupervised learning module, an unsupervised learning module, a regression analysis module, a reinforcement learning module, a self-learning module, a feature learning module, a sparse dictionary learning module, an anomaly detection module, a generative adversarial network, a convolutional neural network, or an association rules module.

70.—The method of any one of claims 67-69, wherein generating the dataset comprises:

contacting a biological sample having cancer with a substrate comprising a plurality of capture probes, wherein in the biological sample comprises (1) one or more cancerous regions, (2) one or more stromal regions, and (3) one or more tumor infiltrating immune cells, and wherein a capture probe of the plurality of capture probes comprises a spatial barcode and a capture domain;

~~attaching an analyte from the biological sample to the capture probe;~~
~~determining (i) all or a part of a sequence corresponding to the analyte, or a complement thereof, and (ii) all or a part of a sequence corresponding to the spatial barcode, or a complement thereof, and using the determined sequence of (i) and (ii) to identify the spatial location and abundance of the analyte in the biological sample; and~~
~~identifying a spatial location as being part of a cluster based on the determined sequences corresponding to the analytes at the spatial location and using the clusters to analyze immune cell infiltration in the cancer stroma of the subject having cancer.~~

~~71.—The method of claim 70, wherein a cluster one or more immune cells is identified using one of the methods selected from: nonlinear dimensionality reduction, t-distributed stochastic neighbor embedding (t-SNE), global t-distributed stochastic neighbor embedding (g-SNE), and uniform manifold approximation and projection (UMAP).~~

~~72.—The method of any one of claims 67-71, wherein generating the dataset comprises:~~

~~attaching the biological sample with a plurality of analyte capture agents, wherein an analyte capture agent of the plurality of analyte capture agents comprises:~~

~~(i) —an analyte binding moiety that binds specifically to the analyte associated with the cancerous region, the analyte associated with the stromal region, and/or the analyte associated with an immune cell;~~

~~(ii) —an analyte binding moiety barcode; and~~

~~(iii) —an analyte capture sequence, wherein the analyte capture sequence binds specifically to a capture domain;~~

~~contacting the biological sample with a substrate, wherein the substrate comprises a plurality of capture probes, wherein a capture probe of the plurality of capture probes comprises (i) the capture domain and (ii) a spatial barcode;~~

~~hybridizing the analyte associated with the cancerous region, the analyte associated with the stromal region, and/or the analyte associated with an immune cell to the capture probe; and~~

~~determining (i) all or a part of a sequence corresponding to the analyte associated with the cancerous region, the analyte associated with the stromal region, and/or the analyte associated with an immune cell, or a complement thereof, and (ii) all or a part of a sequence corresponding to the spatial barcode, or a complement thereof, and using the determined~~

~~sequence of (i) and (ii) to identify the abundance and/or spatial location of the analyte associated with the cancerous region, the analyte associated with the stromal region, and/or the analyte associated with an immune cell, or a complement thereof in the biological sample.~~

~~73. — The method of any one of claims 66-71, wherein the analyte data is generated using *in situ* sequencing.~~

~~74. — A kit comprising:~~

- ~~(a) — an antibody that specifically binds to an antigen on an infiltrating immune cell;~~
- ~~(b) — a substrate comprising a plurality of capture probe, wherein a capture probe of the plurality of capture probes comprises a capture domain; and~~
- ~~(c) — instructions for performing the method of any one of claims 1-72.~~

~~751. A kit comprising:~~

- ~~(a) an antibody that specifically binds to an antigen in or on an infiltrating immune cell;~~
- ~~(b) a second antibody that specifically binds to an antigen in or on a stromal cell;~~
- ~~(c) a substrate comprising a plurality of capture probes, wherein a capture probe of the plurality of capture probes comprises a capture domain; and a spatial barcode; and~~
- ~~(d) instructions for performing a method of analyzing tumor infiltrating immune cells in a cancer stromal region of a biological sample, the method ~~of claim 1-72~~ comprising:~~
 - ~~(i) identifying a cancerous region or an analyte associated with the cancerous region in the biological sample;~~
 - ~~(ii) identifying a stromal region or an analyte associated with the stromal region in the biological sample;~~
 - ~~(iii) identifying one or more immune cells or an analyte associated with an immune cell in one or more locations in the biological sample; and~~
 - ~~(iv) using (A) the identified cancerous region or the analyte associated with the cancerous region in the biological sample, (B) the identified stromal region or the analyte associated with the stromal region in the biological sample, and (C) the identified one or more immune cells or the analyte associated with the immune cell to analyze tumor infiltrating immune cells in the cancer stromal region of the biological sample.~~

2. The kit of claim 1, wherein the analyte associated with the cancerous region, the analyte associated with the stromal region, and/or the analyte associated with an immune cell is a nucleic acid.

3. The kit of claim 2, wherein the nucleic acid is mRNA.

4. The kit of any one of claims 1-3, wherein the analyte associated with the cancerous region, the analyte associated with the stromal region, and/or the analyte associated with the immune cell is a protein.

5. The kit of claim 4, further comprising a plurality of analyte capture agents, wherein an analyte capture agent of the plurality of analyte capture agents comprises:
an analyte binding moiety that binds specifically to the analyte associated with the cancerous region, the analyte associated with the stromal region, and/or the analyte associated with an immune cell;
an analyte binding moiety barcode; and
an analyte capture sequence, wherein the analyte capture sequence binds specifically to a capture domain.

6. The kit of claim 5, wherein the analyte binding moiety is an antibody or antigen-binding fragment thereof, a cell surface receptor binding molecule, a receptor ligand, a small molecule, a T-cell receptor engager, a B-cell receptor engager, a pro-body, an aptamer, a monobody, an affimer, or a darpin.

7. The kit of any one of claims 1-6, wherein the analyte associated with the cancerous region is selected from SCGB2A1, MKI67, BRCA1, BRCA2, PIKCD, CALML6, MYC, TP53, PALB2, RAD51, MSH2, PRKCI, VTCN1, MECOM, TOP2A, SHDH, XPO1, TFRC, FUT8, SOX17, PBX1, EIF42, WT1, TOP2A, XPO1, VIM, EPCAM, FAP, CDH1, and combinations thereof.

8. The kit of any one of claims 1-7, wherein the analyte associated with the stromal region is selected from FAP, VCAN, ACTA2, PDGFRB, and combinations thereof.

9. The kit of any one of claims 1-8, wherein the analyte associated with an immune cell is selected from BLK, CD19, FCRL2, MS4A1, KIAA0125, TNFRSF17, TCL1A, SPIB, PNOC, PTRPC, PRF1, GZMA, GZMB, NKG7, GZMH, KLRK1, KLRB1, KLRD1, CTSW, GNLY, CCL13, CD209, HSD11B1, LAG3, CD244, EOMES, PTGER4, CD68, CD84, CD163, MS4A4A, TPSB2, TPSAB1, CPA3, MS4A2, HDC, FPR1, SIGLEC5, CSF3R, FCAR, FCGR3B, CEACAM3, S100A12, KIR2DL3, KIR3DL1, KIR3DL2, IL21R, XCL1, XCL2, NCR1, CD6, CD3D, CD3E, SH2D1A, TRAT1, CD3G, TBX21, FOXP3, CD8A, CD8B, CD79A, CD79B, CD4, IGHA1, IGHG2, JCHAIN, IGKC, CD27, CD38, CD16, IL17RB, FANK1, CTLA4, MSR1, MRC1, NKG7, FCN1, TIGIT/LAG3, and combinations thereof.

~~76~~10. A computer implemented method comprising:

- (a) generating a dataset ~~of from~~ a plurality of biological samples, wherein the dataset comprises, for ~~each a~~ biological sample of the plurality of biological samples:
 - (i) analyte data for a plurality of analytes captured at a plurality of spatial locations of a reference biological sample;
 - (ii) image data of the reference biological sample; and
 - (iii) registration data of the imaged data linking to the analyte data according to ~~the one or more~~ spatial locations ~~of in~~ the reference biological sample; wherein the reference biological sample comprises:
 - (1) ~~one or more a~~ cancerous regions in the reference biological sample,
 - (2) ~~one or more a~~ stromal regions within the ~~one or more~~ cancerous regions, and
 - (3) ~~a plurality of tumor infiltrating lymphocytes (TILs)~~ one or more immune cells;
- (b) training a machine learning module with the dataset, thereby generating a trained machine learning module; and
- (c) determining tumor infiltrating immune cells ~~infiltration in a the~~ biological sample ~~via using~~ the trained machine learning module.

11. The computer implemented method of claim 10, wherein generating the trained machine learning module comprises identifying the cancerous regions, the stromal regions, and/or the one or more immune cells via the trained machine learning module.

12. The computer implemented method of claim 10 or claim 11, wherein the determining tumor infiltrating immune cells in the biological sample comprises:

(a) identifying abundance of analytes associated with immune infiltrating cells compared to known housekeepers normalized by number of cells per spatial location;

(b) identifying a ratio of one or more tumor infiltrating lymphocytes (TILs) to one or more tumor infiltrating B cells (TIBs), wherein the immune cells comprise the TILs and the TIBs; and/or

(c) calculating the abundance of tumor infiltrating immune cells in the biological sample based on the percentage of spatial locations comprising analytes associated with an immune infiltrating cells.

13. The kit or computer implemented method of any one of claims 1-12, wherein the cancerous region comprises one or more of a benign tumor, a pre-metastatic tumor, a malignant tumor, and one or more inflammatory cells.

14. The kit or computer implemented method of any one of claims 1-13, wherein the stromal region comprises one or more of connective tissue, blood vessels, and inflammatory cells.

15. The kit or computer implemented method of any one of claims 1-14, wherein the tumor infiltrating immune cells comprise a B cell, a T cell, an NK cell, a monocyte, a macrophage, a neutrophil, a granulocyte, an innate lymphoid cell, or a dendritic cell or combinations thereof.

16. The kit or computer implemented method of any one of claims 1-15, wherein the tumor infiltrating immune cells express CD19, CD79A, CD79B, CD3D, CD3E, CD4 and/or CD8A.

17. The kit or computer implemented method of any one of claims 1-16, wherein the biological sample is obtained from a biopsy from a subject.

18. The kit or computer implemented method of any one of claims 1-17, wherein the biological sample is a tissue section.

19. The kit or computer implemented method of any one of claims 1-18, wherein the biological sample is a fixed sample, a frozen sample, or a fresh sample.

7720. A system comprising:

(a) a storage element operable to store a dataset of a plurality of biological samples, wherein the dataset comprises: analyte data for a plurality of analytes captured at a plurality of spatial locations of a reference biological sample; image data of the biological sample; and registration data of the imaged data linking to the analyte data according to the spatial locations of the reference biological sample; wherein the biological sample comprises (1) ~~one or more~~a cancerous regions in the reference biological sample, (2) ~~one or more a~~ stromal regions within the ~~one or more~~-cancerous regions, and (3) the a plurality of tumor infiltrating ~~lymphocytes (TILs)~~immune cells; and

(b) a processor operable to process the dataset through a machine learning module to train the machine learning module, to determine immune cell infiltration in a biological sample.