

WHAT IS CLAIMED IS:

1. 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 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.

10. A computer implemented method comprising:

- (a) generating a dataset from a plurality of biological samples, wherein the dataset comprises, for 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 one or more spatial location in the reference biological sample;wherein the reference biological sample comprises:
 - (1) a cancerous region in the reference biological sample,
 - (2) a stromal region within the cancerous region, and
 - (3) 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 in the biological sample 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.

20. 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) a cancerous region in the reference biological sample, (2) a stromal region within the cancerous region, and (3) the a plurality of tumor infiltrating 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.