

example, the analyzing comprises the determination of at least one clinical decision. Such clinical decision, in one example, includes determining disease prognosis, predicting recurrence of disease, predicting targets of therapy of disease, inclusion of subjects of clinical trials, or therapeutic treatment strategy for at least one subject.

In one aspect, the present disclosure provides a method of determining a prognosis of a cancer in a subject, comprising assessing representative data from a subject, wherein the representative data are generated in a method comprising analyzing a ~~processed~~ homogenate composition derived from ~~a heterogeneous one~~ or more fixed surgical resection tissue samples and/or one or more fixed residual surgical tissue samples, wherein any heterogeneity of cells within the obtained one or more fixed surgical resection tissue samples and/or the one or more fixed residual surgical tissue samples is substantially uniformly distributed within the homogenate composition, and wherein any aliquot removed from the homogenate composition comprises one or more populations of cellular structures at a proportion at which they existed within the one or more fixed surgical resection tissue samples and/or the one or more fixed residual surgical tissue samples, wherein the analyzing comprises generating quantitative and/or qualitative data for one or more markers in one or more aliquots of the homogenate composition ~~comprising substantially homogeneously distributed cellular structures, wherein a ratio of cellular structures in each subset of the homogenate is substantially similar to the ratio of cellular structures in the tissue sample.~~

In another aspect, the present disclosure provides a method of staining cells, comprising:

a. obtaining one or more fixed surgical resection tissue samples;

- b. homogenizing the one or more fixed surgical resection tissue samples to provide a homogenized sample, wherein any subpopulations of cells that were originally spatially segregated within the one or more fixed surgical resection tissue samples are homogeneously distributed throughout the homogenized sample, and wherein a ratio of cellular structures in each subset of the homogenized sample is substantially similar to the ratio of cellular structures in the one or more fixed surgical resection tissue samples;
- c. isolating intact cells from at least a first aliquot of the homogenized sample; and
- d. staining at least a portion of the isolated intact cells in the first aliquot.

In another aspect, the present disclosure provides a method of sorting cells within a tumor from a subject, comprising:

- (a) preparing a representative sample from one or more fixed but not paraffin embedded specimens from the tumor of the subject, wherein the representative sample is prepared by mechanically dissociating the one or more fixed but not paraffin embedded specimens, wherein any subpopulations of cells that were originally spatially segregated within the obtained one or more fixed specimens are substantially homogeneously distributed throughout the representative sample; and
- (b) sorting dissociated single cells from a first aliquot of the prepared representative sample into at least a first dissociated cell population, wherein the sorting is performed using a fluorescence-activated cell sorter (FACS) or multicellular tissue fragment sorting (MTFS).

BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

5 **FIGS. 1A-1C** are schematic representations of the disclosed methods for sample acquisition in pathology. **FIG. 1A** illustrates sample acquisition of a colon; **FIG. 1B** illustrates signal acquisition of lung tissue; **FIG. 1C** illustrates sample acquisition of kidney tissue.

10 **FIGs. 2A and 2B illustrate** a schematic representation of the homogenate of the disclosure. **FIG. 2A** is an illustration of how the disclosed homogenization methods generate a representative sample that contains subclones at the proportion at which they existed within the solid tumor. **FIG. 2B** is an illustration of how the homogenate facilitates detection of low-prevalence subclones.

15 **FIG. 3** is a flow chart depicting schematically the disclosed protocol using physical, mechanical, biochemical methods for generating representative tumor samples that are representative of heterogeneity within a tumor.

FIG. 4 is a graph showing the results from size fractionation of the representative sample.

20 **FIGS. 5A-C** show H&E staining of the flow through and retained fractions collected following series filtration of the biochemically-digested representative sample. **FIG. 5A** illustrates H&E staining of the fraction retained in the mesh (top) and the flow through (bottom) of the micron mesh. **FIG. 5B** illustrates H&E staining of the fraction retained in the mesh (top) and the flow through (bottom) of the micron mesh. **FIG 5C** illustrates H&E staining of the fraction retained in the
25 mesh (top) and the flow through (bottom) of the micron mesh.

WHAT IS CLAIMED IS:

1. A method of determining a prognosis of a cancer in a subject, comprising assessing representative data from a subject, wherein the representative data are generated in a method comprising analyzing a ~~processed~~ homogenate composition derived from a heterogeneous one or more fixed surgical resection tissue samples and/or one or more fixed residual surgical tissue samples, wherein any heterogeneity of cells within the obtained one or more fixed surgical resection tissue samples and/or the one or more fixed residual surgical tissue samples is substantially uniformly distributed within the homogenate composition, and wherein any aliquot removed from the homogenate composition comprises one or more populations of cellular structures at a proportion at which they existed within the one or more fixed surgical resection tissue samples and/or the one or more fixed residual surgical tissue samples, wherein the analyzing comprises generating quantitative and/or qualitative data for one or more markers in one or more aliquots of the homogenate composition comprising substantially homogeneously distributed cellular structures, wherein a ratio of cellular structures in each subset of the homogenate is substantially similar to the ratio of cellular structures in the tissue sample.

~~2. The method of claim 1, wherein the analyzing comprises generating quantitative and/or qualitative data for a marker in the homogenate composition.~~

32. The method of claim 21, wherein the marker comprises a DNA, a protein, an RNA, a lipid, a cell organelle, a metabolite, or a cell.

43. The method of claim 1, further comprising comparing the representative data to a predetermined value for the data.

54. The method of claim 43, wherein the predetermined value is selected from clinical trial data, a data for a subject, a data from scientific literature, a data for a biologic or small molecule under clinical development.

65. The method of claim 1, wherein the representative data comprises a representative oncology data, wherein the representative oncology data comprises quantitative and/or qualitative data of at least one tumor marker from a first biological sample, said tumor marker is associated with the presence of a tumor, the method further comprising:
- a. measuring the tumor marker in the first biological sample, wherein the measured values of the tumor marker is normalized;
 - b. obtaining a composite score based on the normalized measured value; and
 - c. comparing the composite score to a predetermined score to determine a cancer risk value of the subject.

~~7. The method of claim 1, wherein the tissue sample is selected from the group of: a tumor, a lymph node, a metastasis, a polyp, a cyst, a resection, an organ, or a fraction thereof.~~

~~8. The method of claim 7, wherein the tissue sample comprises spatially segregated cellular structures.~~

96. The method of claim 81, wherein the cellular structures comprise a cell cluster, an individual cell, a fragment of a cell, an organelle, a peptide, a nucleic acid, a lipid, a metabolite, or a combination thereof.

~~107.~~ The method of ~~any one of claims 7 to 9~~claim 6, wherein the homogenate composition comprises up to 25%, up to 50%, up to 75% or up to 100% of the cellular structures from the obtained one or more fixed surgical resection tissue samples and/or the one or more fixed residual surgical tissue sampletissue sample.

~~118.~~ The method of ~~any one of claims 7 to 10~~claim 6, wherein the homogenate composition comprises 100% of the cellular structures from the obtained one or more fixed surgical resection tissue samples and/or the one or more fixed residual surgical tissue sampletissue sample.

~~129~~. The method of any one of claims ~~7 to 116~~ to ~~8~~, wherein the homogenate further comprises one or more of a non-human cell, a human cell, a non-native protein, a nucleic acids, or a small molecule, a dye, a virus, a bacterium, a parasite, protozoan, or a chemical.

~~1310~~. The method of claim ~~129~~, wherein the small molecule comprises a hapten, a peptide tag, a protein tag, a fluorescent tag, a nucleic acid tag, and combination thereof.

~~1411~~. The method of claim 1, further comprising calculating a quantitative score for the prognosis of the cancer, wherein the prognosis is classified based on the quantitative score.

~~1512~~. The method of claim 1, wherein the representative data comprises information about number, types, states, and/or percentage of the ~~cell-cellular~~ structures in the homogenate.

~~1613~~. The method of claim ~~1512~~, wherein the ~~cell-cellular~~ structures comprise stem cells, epithelial cells, blood cells, fat cells, skin cells, endothelial cells, cancer cells, or immune cells.

~~1714~~. The method of claim ~~1613~~, wherein the immune cells comprises neutrophils, monocytes, macrophages, dendritic cells, natural killer cells, T-cells, and/or B-cells,

~~1815~~. The method of claim ~~1714~~, wherein the T-cells comprise killer T cells, helper T cells, regulatory T cells, pan T cells, naïve T cells, activated T cells, and/or gamma delta T-cells.

~~1916~~. The method of claim ~~1512~~, wherein the states of ~~cell-cellular~~ structures comprise proliferation, apoptosis, necrosis, migration, epithelial-mesenchymal transition (“EMT”), mitosis, cell cycle arrest, S-phase, senescence, and/or differentiation.

[2017](#). The method of claim 1, wherein the representative data comprises information about a marker in the homogenate [composition](#).

5 [2118](#). The method of claim [2017](#), wherein the marker is selected from the group of a DNA, a protein, an RNA, a lipid, a cell organelle, a metabolite, or a cell.

[2219](#). The method of claim [2017](#), wherein the marker comprises one or more of [Her2](#), ~~bRaf~~, ~~ERBB2~~, ~~PI3KCA~~, ~~FGFR2~~, ~~p53~~, ~~BRCA~~, ~~CCND1~~, ~~MAP2K4~~, ~~ATR~~, ~~AFP~~, ~~ALK~~, ~~BCR-ABL~~, ~~BRCA1/BRCA2~~, ~~BRAF~~, ~~V600E~~, ~~Ca-125~~, ~~CA19.9~~, ~~EGFR~~, ~~Her-2~~, ~~KIT~~, ~~PSA~~, ~~S100~~, ~~KRAS~~, ~~ER/Pr~~, ~~UGT1A1~~, ~~CD30~~, ~~CD20~~, ~~F1P1L1~~, ~~PDGFR α~~ , ~~PDGFR~~, ~~TMPT~~, ~~TMPRSS2~~, ~~ABCB5~~, ~~AFP-L3~~, Alpha-fetoprotein, Alpha-methyl acyl-CoA racemase, ~~BRCA1~~, ~~BRCA2~~, ~~CA-15-3~~, ~~CA-242~~, ~~Ca-27-29~~, ~~CA-125~~, ~~CA15-3~~, ~~CA19-9~~, Calcitonin, Carcinoembryonic antigen, Carcinoembryonic antigen peptide-1, Des-gamma carboxy prothrombin, Desmin, Early prostate cancer antigen-2, Estrogen receptor, Fibrin degradation product, Glucose-6-phosphate isomerase, ~~vE6~~, ~~E7~~, ~~L1~~, ~~L2~~ or ~~p16INK4a~~, Human chorionic gonadotropin, ~~IL-6~~, Keratin 19, Lactate dehydrogenase, Leucyl aminopeptidase, Lipotropin, Metanephrines, Neprilysin, ~~NMP22~~, Normetanephrine, ~~PCA3~~, Prostate-specific antigen, Prostatic acid phosphatase, Synaptophysin, Thyroglobulin, ~~TNF~~, a transcription factor selected from ~~ERG~~, ~~ETV1 (ER81)~~, ~~FLI1~~, ~~EST1~~, ~~EST2~~, ~~ELK1~~, ~~ETV6~~, ~~ETV7~~, ~~GABP α~~ , ~~ELF1~~, ~~ETV4~~, ~~ETV5~~, ~~ERF~~, ~~PEA3/E1AF~~, ~~PU.1~~, ~~ESE1/ESX~~, ~~SAP1 (ELK4)~~, ~~ETV3 (METS)~~, ~~EWS/FLI1~~, ~~ESE1~~, ~~ESE2 (ELF5)~~, ~~ESE3~~, ~~PDEF~~, ~~NET (ELK3; SAP2)~~, ~~NERF (ELF2)~~, or ~~FEV~~. ~~XXX~~, Tumor associated glycoprotein 72, ~~e-kit~~, ~~SCF~~, ~~pAKT~~, ~~pc-kit~~, Vimentin, ~~CTLA-4~~, ~~PDL1~~, ~~PDL2~~, ~~PD1~~, ~~B7-H3~~, ~~B7-H4~~, ~~BTLA~~, ~~HVEM~~, ~~KIR~~, ~~TIM3~~, ~~GAL9~~, ~~GITR~~, ~~LAG3~~, ~~VISTA~~, ~~KIR~~, ~~2B4~~, ~~TRPO2~~, ~~CD160~~, ~~CGEN-15049~~, ~~CHK-1~~, ~~CHK2~~, ~~A2aR~~, ~~TL1A~~, ~~CTLA-4~~, ~~PDL1~~, ~~PDL2~~, ~~PD1~~, ~~B7-H3~~, ~~B7-H4~~, ~~BTLA~~, ~~HVEM~~, ~~TIM3~~, ~~GAL9~~, ~~LAG3~~, ~~VISTA~~, ~~KIR~~, ~~2B4~~, ~~CD160~~, ~~CGEN-15049~~, ~~CHK1~~, ~~CHK2~~, ~~A2aR~~, ~~B-7 family~~, and the combination thereof.

2320. The method of claim 2017, wherein the marker is selected from the group of: a protein modification, said modification is selected from a group consisting of acetylation, ADP-ribosylation, acylation, ADP-ribosylation, amidation, covalent attachment of a flavin, covalent attachment of a heme, ~~covalent attachment of a nucleotide or a nucleotide derivative, covalent attachment of a lipid or lipid derivative,~~ covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, arginylation, and ubiquitination.

2421. The method of claim 2017, wherein the marker is selected from the group of: a genomic polymorphism, a pharmacogenomics single nucleotide polymorphism (SNP), a genomic SNP, a somatic polymorphism, and differential expression of a protein, a lipid, and/or a cellular organelle, a single nucleotide position; an intragenic region or an intergenic region; an exon or an intron, ~~or a fragment thereof;~~ a coding region or a non-coding region; a promoter, an enhancer, a 5' untranslated region (5' UTR), or a 3' untranslated region (3' UTR), ~~or a fragment thereof;~~ a cDNA ~~or a fragment thereof;~~ an SNP; a somatic mutation, a germ line mutation or both; a point or a single mutation; a deletion mutation; an in-frame deletion, an intragenic deletion, a full gene deletion; an insertion mutation; an intragenic insertion; an inversion mutation; an intra-chromosomal inversion; a linking mutation; a linked insertion mutation; an inverted duplication mutation; a tandem duplication; an intrachromosomal tandem duplication; a translocation; a chromosomal translocation, a non-reciprocal translocation; a rearrangement; a genomic rearrangement; a rearrangement of one or more introns, ~~or a fragment thereof;~~ a rearranged intron; a 5'- or a 3'-UTR, an altered nucleotide sequence, encodes an altered amino acid sequence, a chromosomal translocation, an intra-chromosomal inversion, a change in copy number, a change in expression level, a change in protein level, a change in protein

activity, or a change in methylation status, in a cancer tissue or cancer cell, as compared to a normal, healthy tissue or cell.

5 22. The method of claim 1, wherein the quantitative data and/or the qualitative data is generated from flow cytometry analysis of at least a first aliquot of the homogenate composition.

23. The method of claim 1, wherein the quantitative data and/or the qualitative data is generated from sequencing the cellular components within at least first aliquot of the homogenate composition.

10 24. The method of claim 23, wherein the sequencing of the cellular components comprises next generation sequencing, single cell sequencing, or single nucleus sequencing.

25. The method of claim 1, wherein the generated representative data comprises an identification, detection, or characterization of one or more low prevalence genetic events.

15 26. The method of claim 25, wherein the one or more low prevalence genetic events are selected from a point mutation, a deletion, an addition, a translocation, a genetic fusion, or an amplification of a gene.

27. The method of claim 1, wherein the generated representative data comprises an identification of one or more subclones.

28. A method of staining cells, comprising:

20 a. obtaining one or more fixed surgical resection tissue samples;

b. homogenizing the one or more fixed surgical resection tissue samples to provide a homogenized sample, wherein any subpopulations of cells that were originally spatially segregated within the one or more fixed surgical resection tissue samples are homogeneously distributed throughout the homogenized sample, and wherein a ratio of cellular structures in each subset of the
25 homogenized sample is substantially similar to the ratio of cellular structures in the one or more fixed surgical resection tissue samples;

c. isolating intact cells from at least a first aliquot of the homogenized sample;
and

30 d. staining at least a portion of the isolated intact cells in the first aliquot.

29. The method of claim 28, wherein the isolated intact cells are immunoenzymatically stained for the presence of one or more biomarkers.

30. The method of claim 28, further comprising sorting the stained cells into two or more populations of stained cells.

5 31. A method of sorting cells within a tumor from a subject, comprising:

(a) preparing a representative sample from one or more fixed but not paraffin embedded specimens from the tumor of the subject, wherein the representative sample is prepared by mechanically dissociating the one or more fixed but not paraffin embedded specimens, wherein any subpopulations of cells that were
10 originally spatially segregated within the obtained one or more fixed specimens are substantially homogeneously distributed throughout the representative sample; and

(b) sorting dissociated single cells from a first aliquot of the prepared representative sample into at least a first dissociated cell population, wherein
15 the sorting is performed using a fluorescence-activated cell sorter (FACS) or multicellular tissue fragment sorting (MTFS).

32. The method of claim 31, wherein the cells are sorted based on a fluorescent detection of a tumor cell marker.

33. The method of claim 31, wherein the cells are sorted based on a fluorescent detection
20 of one or more cell markers.