the original heterogeneous tissue sample. The disclosed composition addresses and overcomes the limitations of prior art methods that fail to account for tissue heterogeneity in clinical samples, especially samples for use in clinical oncology.

The homogenate composition is derived or sourced from various tissues, organs or samples thereof, for example a lymph node, a metastasis, a polyp, a cyst, a resection, an organ, or a fraction thereof, or spatially segregated cells. In one embodiment, the homogenate comprises from about 25% to about 100% of the cellular structures of the tissue sample. The homogenate may comprise a protein fraction, lipid, nucleic acids or other moieties which are present in the starting tissue, e.g., a whole tumor, lymph nodes or metastases used to derive the homogenate thereof, wherein the relative proportions of such substituents components are representative of the starting tissue. The substantially homogenous cellular structures may comprise single cells or a plurality of cell clusters isolated from a normal or abnormal tissue. In certain embodiments, the homogenate may comprise a liquid or non-liquid tissue sample, obtained by a variety of methods such as a cytology needle aspirate, effusion sample or a pap smear.

In other embodiments, the homogenate may be isolated from preserved tissue, for example formalin fixed tissue. In other embodiments the tissue sample is not preserved or fixed, and/or comprises live cells. The heterogeneous tissue sample may be isolated from one or more tissues obtained from one or more patients.

The homogenate is suitable for use in various diagnostic, prognostic and clinical applications, including but not limited to generating representative data including representative oncology data, cancer staging, identification and assessment of prognosis of diseases (e.g., tumor staging), selection and design of appropriate treatment regimens, clinical trial matching, marker identification and characterization, tissue profiling, and storage of the homogenate composition. The homogenate composition is also useful for screening therapeutics or to generate therapeutics in treating patients, e.g., as vaccines or in the manufacture of cancer vaccines or immune cell-based therapies, as the representative samples contain a diverse array of the antigens expressed by a given tumor or tumors.

The disclosure also provides methods for generating a homogenate composition that is representative of the heterogeneous tissue sample, e.g., a

representative sample. More particularly, the disclosure comprise applying mechanical, chemical and/or biochemical, e.g., enzymatic, dissociation methods to intact tissue-containing samples, e.g., whole organs, tumors, lymph nodes, metastasized tissues or combinations of any of the foregoing (from the same or different patients) in order to provide an accurate representative sample despite spatial heterogeneity within the original tissue or organ, e.g., a tumor, thereby increasing detection likelihood for minor sub-clone populations and/or low prevalence events.

In one embodiment, the disclosure generally relates to the development of a methodology for generating representative tissue samples of, for example, whole organs, tumors, lymph nodes, metastases, or combinations thereof in order to address the issue of heterogeneity, e.g., tumor heterogeneity, in clinical specimens, especially clinical specimens for use in clinical oncology, and the use of such representative samples or portions thereof in various diagnostic and therapeutic methods as well as compositions comprising such representative samples for use in diagnosis and therapy, especially oncology.

Moreover, , representative samples from different patients or different tissues of single or different patients may each be labeled with unique identifying labels, e.g., a hapten, and the labeled samples of different patients or tissues combined and used in desired assay methods.

Representative samples derived by exemplary embodiments of the disclosure may be utilized to improve the accuracy of detecting, diagnosing, and/or staging of different tumor types, irrespective of tumor tissue type, location, size or volume. The presently disclosed methods are useful for the production of a representative sample from normal tissue or putative precancerous tissues (e.g., obtained from subjects at higher risk of developing cancer because of a genetic risk or a prior cancer) to identify rare cell.

In one embodiment, the disclosure provides a method for producing a biological sample suitable for assessing heterogeneity of cells within a tumor, lymph node, or metastases and/or assessing the prognosis of a particular cancerous condition in a subject and/or determining an appropriate therapeutic protocol for a subject with a cancerous condition. This method comprises (i) obtaining a tissue (such as a tumor

sample or a lymph node or metastases) that comprises spatially distinct regions of the tissue or which comprises a whole tumor or a substantial portion thereof, and (ii) homogenizing the tissue such that the heterogeneity of the cells is substantially homogeneously distributed within the resultant homogenate or a portion or fraction thereof. The sample or samples optionally may be fixed and/or preserved, e.g., formalin fixed, ethanol fixed, frozen or freeze-dried, stored in wax (such as paraffin), etc. before or after homogenization of an entire or substantially entire tumor, lymph node, metastases, or even an entire organ such as a kidney.

In another embodiment, the disclosure provides a method for producing a biological sample suitable for assessing heterogeneity of cells within a sample (such as a tumor sample, lymph node, metastases or a combination thereof) and/or assessing the prognosis of a particular cancerous condition in a subject comprising: (i) obtaining one or more intact samples from a solid tumor or a lymph node, preferably wherein each intact sample comprises at least about 100-200; 200-1,000; 1,000-5,000; 10,000-100,000; 100,000-1,000,000; 1,000,000-5,000,000; 5,000,000-1,000,000,000; 1,000,000,000-5,000,000,0000, or more cells, or alternatively at least 1,000; 10,000; 100,000; 1,000,000; 5,000,000; 10,000,000; 50,000,000; 100,000,000; 500,000,000; 1,000,000,000; 5,000,000,000; 10,000,000,000; 50,000,000,000; 100,000,000,000; 500,000,000,000; 1,000,000,000,000; 5,000,000,000,000; 10,000,000,000,000; 50,000,000,000,000; 100,000,000,000,000 or more cells, and optionally fixed or preserved (such as a formalin, paraffin, or ethanol fixed or preserved sample), and (ii) separately or in combination homogenizing the one or more samples, wherein the one or more homogenates each substantially homogeneously express the heterogeneity of the respective sample or samples. In one embodiment, the intact sample or samples from the solid tumor or the lymph node comprise or alternatively consist essentially of, or yet further consist of a portion of the solid tumor or the lymph node. In another embodiment, the intact sample or samples from the solid tumor or the lymph node comprise, or alternatively consist essentially of, or yet further consist of substantially portions of the solid tumor or the lymph node. In a further embodiment, the intact sample or samples from the solid tumor or the lymph node

comprise, or alternatively consist essentially of, or yet further consist of the entire solid tumor or the entire lymph node.

The representative samples optionally may be further dissociated and/or treated to remove or isolate specific types of molecules such as specific cell types, proteins, nucleic acids, or lipids, and the like and using, for example, CAVA computational analysis of the Illumina sequencing output to be used in diagnostic and therapeutic methods.

In yet another embodiment, the disclosure provides a method for producing a biological sample suitable for assessing heterogeneity of cells within a tumor or lymph node or metastases or combination thereof comprising (i) obtaining one or more biopsy samples from a solid tumor or a lymph node or metastases, preferably wherein each biopsy sample comprises at least about 100-200; 200-1,000; 1,000-5,000; 10,000-100,000; 100,000-1,000,000; 1,000,000-5,000,000; 5,000,000-1,000,000,000; 1,000,000,000-5,000,000,0000, or alternatively at least 1,000; 10,000; 100,000; 1,000,000; 5,000,000; 10,000,000; 50,000,000; 100,000,000; 500,000,000; 1,000,000,000; 5,000,000,000; 10,000,000,000; 50,000,000,000; 100,000,000,000; 500,000,000,000; 1,000,000,000,000; 5,000,000,000,000; 10,000,000,000,000; 50,000,000,000,000; 100,000,000,000,000 or more cells, and optionally fixed or preserved (such as a formalin, paraffin, or ethanol fixed or preserved sample), and (ii) separately or in combination homogenizing the one or more biopsy samples, under conditions wherein the resultant homogenate or homogenates are substantially dissociated into individual cells and the resultant homogenate or homogenates are substantially homogeneous.

In another embodiment, the disclosure provides a method for producing a biological sample suitable for assessing whether a subject has or is at risk of developing a virulent form of a particular cancer and/or whether a has a virulent form cancer comprising (i) obtaining one or more intact biopsy samples from a solid tumor or a lymph node or metastases or precancerous cyst, preferably wherein each biopsy sample comprises at least about 100-200; 200-1,000; 1,000-5,000; 10,000-100,000; 100,000-1,000,000; 1,000,000-5,000,000; 5,000,000-1,000,000,000; 1,000,000,000-5,000,000,0000, or more cells, or alternatively at least 1,000; 10,000; 100,000; 1,000,000; 5,000,000; 10,000,000; 50,000,000;

100,000,000; 500,000,000; 1,000,000,000; 5,000,000,000; 10,000,000,000; 50,000,000,000; 100,000,000,000; 500,000,000,000; 1,000,000,000,000; 5,000,000,000,000; 10,000,000,000,000; 50,000,000,000,000; 100,000,000,000,000 or more cells, and optionally fixed or preserved (such as a formalin, paraffin, or ethanol fixed or preserved sample), and (ii) separately or in combination homogenizing the one or more biopsy samples, wherein the resultant one or more homogenates each substantially homogeneously contains the heterogeneity of the respective biopsy sample or samples, and optionally isolating or detecting the presence of at least one biomarker. In this embodiment, the presence or absence of the biomarker is indicative of a virulent form of cancer, or alternatively the upregulation or downregulation of the biomarker is associated with a virulent form of the particular cancer.

In yet another embodiment, the disclosure provides a method for characterizing a landscape within a heterogeneous tumor, lymph nodes or metastases or precancerous cyst and/or detecting genetically distinct subclones within a heterogeneous tumor lymph nodes or metastases or precancerous cyst and/or identifying low prevalence events within a tumor lymph nodes or metastases or precancerous cyst and/or determining the prevalence of targets within a tumor lymph nodes or metastases or precancerous cyst comprising (i) obtaining a sample or samples of the tumor lymph nodes or metastases or precancerous cyst that encompasses spatially distinct regions of the tumor lymph nodes or metastases or precancerous cyst, which is or are optionally fixed or preserved prior to homogenization e.g., with formalin, paraffin and/or ethanol, and (ii) homogenizing the tumor lymph nodes or metastases or precancerous cyst sample or samples separately, thereby producing a set of homogenates that is representative of the landscape of the heterogeneity within the tumor, lymph nodes, metastases, or precancerous cyst and is suitable for characterizing the landscape of the tumor and/or detecting genetically distinct subclones within a heterogeneous tumor lymph nodes or metastases or precancerous cyst and/or identifying low prevalence events within a tumor or lymph nodes or metastases or precancerous cyst and/or determining the prevalence of targets within a tumor lymph nodes or metastases or precancerous cyst. These landscapes relate to the genomic diversity (eg. the

number of point mutations, insertions, and deletions within the tumor), the diversity in tumor phenotypes (eg. amount of the tumor that has undergone epithelial to mesenchymal transition), diversity in the host immune response (eg. the diversity of the expression of immune checkpoint regulators in the tumor and immune cells), the diversity of all potential resistance mechanisms (eg. the number and diversity of the tumor mutations that confer resistance to targeted therapy), the diversity of tissue histologies (eg. amount of the tumor that is squamous vs. adenocarcinoma in lung cancer), the diversity of neo-antigens expressed by the tumor, and/or other complex phenotypic, morphological, histological, genomic, proteomic, metabolomic landscapes across all affected, or potentially affected tissue that is resected from a subject.

In yet another embodiment, the disclosure provides a method for detecting precancerous cells or cancerous cells in supposed normal tissues or putative precancerous tissues in a patient, e.g., one at risk of developing cancer because of a genetic mutation or previous cancer, or a patient with precancerous cysts or polyps comprising (i) obtaining a sample or samples of supposed normal tissues or putative precancerous tissues such as precancerous cysts or polyps that encompass spatially distinct regions of the supposed normal tissues or putative precancerous tissues of the patient, which is optionally fixed or preserved prior to homogenization, and (ii) homogenizing the sample or samples, thereby producing a homogenate that is representative of the supposed normal tissues or putative precancerous tissues and which is suitable for detecting rare cancerous cells or cancer stem cells, e.g., even before any sign of disease has manifested in the patient.

In another embodiment, the disclosure provides methods of using representative samples and portions thereof produced by the any of the foregoing methods in different assay formats, wherein these assays may be effected in high throughput, performed simultaneously or at different times or different locations, and/or by automation (fully automated or semi-automated).

In another embodiment, the disclosure provides for representative samples or portions thereof produced by the any of the foregoing methods which are stored for

future use, non-limiting examples of such include e.g., frozen, formalin-fixed, paraffin-embedded, processed with ethanol, or freeze-dried.

In another embodiment, the disclosure provides for representative samples or portions thereof produced by the any of the foregoing methods are used to derive (and optionally purify) antibodies or antigens specific to a particular antigen from a cancer cell or cell types in a patient sample, which antibodies or antigens potentially may be used in personalized medicine, i.e., in the production of therapeutic or prophylactic cancer vaccines.

The homogenization step in all of the above-mentioned methods may be effected by a method which preserves the integrity of the cells within the sample, i.e., the bulk of the cells within the homogenized sample or samples are not lysed and whereby the resultant homogenate and portions thereof are "representative" of the sample or samples. Therefore, the cells within the sample or a portion thereof reflect the percentages of the different cell types within the entirety of the tissue sample or samples, e.g., a solid tumor or a lymph node. This may be accomplished, for example, by mechanical dissociation of the tumor sample or a portion thereof (such as mechanical dissociation performed with or without the addition of liquid to the tumor sample or a portion thereof) and/or chemical or enzymatic dissociation of the tumor sample or a portion thereof (such as treatment with an enzyme that selectively or preferentially or primarily acts upon extracellular matrix proteins as compared to membrane-associated proteins). Alternatively, the homogenization methods may result in the dissociation of the cells while still generating a sample that is representative of the starting tissue, such as a whole tumor. The homogenized representative samples optionally may be further dissociated and/or treated to remove or isolate specific types of molecules such as specific cell types, proteins, nucleic acids or lipids, and the like thereby generating other representative samples which may be used in diagnostic and therapeutic methods.

Any of the above methods may include detecting the expression of at least one biomarker, e.g., at least one lipid, protein, or nucleic acid biomarker, in the homogenate or a portion or fraction thereof. Additionally, the methods may further include detecting the percentage of tumor cells in the homogenate or a

each tumor are used to assess the similarities and/or differences of the two or more tumors or disease condition of different patients. In a further embodiment, the homogenates from one or more subjects can be combined for the purposes of a multiple-subject representative sample.

The homogenates may be derived from two or more putative normal or precancerous tissues, e.g., breast, cervical, colorectal, or precancerous cysts or polyps obtained from a subject or multiple subjects, e.g., one with a BRCA mutation, and the resultant homogenates or fractions thereof used to assess whether any abnormal cells or disease biomarkers are present.

In addition, non-human cells (such as insect cells and/or mouse cells) or other foreign proteins, nucleic acids, or small molecules may be added to the homogenate to create an internal control for positive protein or nucleic acid detection.

Small molecules (such as haptens, peptide tags, protein tags, fluorescent tags, and/or nucleic acid tags) may be added to the sample and used to provide spatial information in the representative sample. For example, a sample (such a tumor or lymph node) may be sectioned, e.g., cut into quadrants, and a different hapten (or other suitable small molecule) may be "doped" into each section prior to homogenizing the sections to generate a representative sample. It should be understood that the number of sections that can be generated from each sample for "doping" prior to homogenization is not limited but, rather, likely selected in scale with the size of the sample, i.e., the larger the sample, the greater the number of sections that can be "tagged" with a small molecule prior to homogenization. In this way, spatial information can be maintained in the resultant homogenates or fractions thereof.

In one embodiment, small molecules can also be added to the sample prior to combining the sample with a different sample from another patient or the same patient and, thus, provides a means to differentiate samples when run in a multiplex assay format.

The samples which are homogenized may be preserved, e.g., formalin fixed, or may or treated with ethanol before or after homogenization. Because of safety concerns, tissue samples are generally formalin or otherwise fixed prior to

or may not be affected under conditions that maintain the viability and integrity of the cells within the sample, e.g., under some homogenization conditions the cells are substantially not lysed.

In one embodiment, homogenization processes comprise the use of a mechanical process, non-limiting examples of such include mortar & pestle, a dounce homogenizer or tissue grinder, a hand held electronic rotary blade tissue homogenizer (such as Omni-TH available from Thomas Scientific), a bead beating homogenizer (such as a bullet blender or a Burton Precellys 24 Tissue Homogenizer or a Bead Ruptor available from OMNI), optionally at a speed of between about 100 and about 75,000 RPM for rotational homogenizers or a speed of about 0.5 m/s to about 2.5 m/s for bead beaters, and for a length of about 30 second to about 5 minutes, about 5 minutes to about 10 minutes, about 10 minutes to about 30 minutes, or about 30 minutes to about 60 minutes. As noted herein, the mechanical homogenization process can be used alone or in combination with other processes.

In another embodiment, homogenization comprises, alone or in combination with other processes, the use of an enzyme preparation, non-limiting examples of such include for example, interstitial collagenase, Gelatinase-A, Stromelysin 1, Matrilysin, Neutrophil collagenase, Gelatinase-B, Stromelysin 2, Stromelysin 3, Macrophage metalloelastase, Collagenase 3, MT1-MMP, MT2-MMP, MT3- MMP, MT4-MMP, Collagenase 4, Enamelysin, X-MMP, CA-MMP, MT5-MMP, MT6-MMP, Matrilysin-2, MMP-22, endoproteinase, trypsin, chymotrypsin, endoproteinase Asp-N, endoproteinase Arg-C, endoproteinase Glu-C (V8 protease), endoproteinase Lys-C, pepsin, thermolysin, elastase, papain, proteinase K, subtilisin, clostripain, exopeptidase, carboxypeptidase A, carboxypeptidase B, carboxypeptidase P, carboxypeptidase Y, cathepsin C, acylamino-acid-releasing enzyme, pyroglutamate aminopeptidase, or any combination thereof, optionally at a concentration of about 0.001 μg/ml to about 1000 mg/ml, and for a length of about 1 minute to about 120 minutes.

The tumor or other sample used in the disclosed methods that encompasses spatially distinct regions of the tumor or other tissue may comprise at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, at least 85%, at least 95%, at least 96%, at

detect a low prevalence genetic event (such as a genetic event that occurs at 20% prevalence, 15% prevalence, 10% prevalence, 5% prevalence, 2% prevalence, 1% prevalence, 0.5% prevalence, 0.1% prevalence, 0.001% prevalence, 0.001% prevalence, 0.0001% prevalence, 0.00001% prevalence, 0.000001% prevalence or less). Exemplary genetic events include a point mutation, a deletion, an addition, a translocation, a genetic fusion, or an amplification of a gene. Likewise, the methods can also involve detecting genetic or epigenetic heterogeneity within the tumor sample or a portion thereof and/or detecting cells comprising rare genetic or epigenetic variations. Such cells may be present in the tumor sample at a frequency of less than 5%, less than 1%, less than 0.5%, less than 0.1%, less than 0.05%, or less than 0.01%.

The detected rare cells may comprise one or more genetic or epigenetic differences that confer resistance to a therapy, a sensitivity to one therapy over another, an anti-cancer therapy and/or promote metastasis. Therefore, in one embodiment, the detection of such cells will facilitate cancer prognosis as well as the selection of an appropriate therapeutic regimen such as chemotherapy, combination targeted therapy, and/or the use of biologics.

The foregoing methods may also include the use of at least one detectable label selected from fluorescent molecules or fluorochromes such as 4-acetamido-4'-isothiocyanatostilbene-2,2'disulfonic acid, acridine and derivatives such as acridine and acridine isothiocyanate, 5-(2'-aminoethyl)aminonaphthalene-1-sulfonic acid (EDANS), 4-amino-N-[3-vinylsulfonyl)phenyl]naphthalimide-3,5 disulfonate (Lucifer Yellow VS), N-(4-anilino-1-naphthyl)maleimide, anthranilamide, Brilliant Yellow, coumarin and derivatives such as coumarin, 7-amino-4-methylcoumarin (AMC, Coumarin 120), 7-amino-4-trifluoromethylcouluarin (Coumaran 151); cyanosine; 4',6-diaminidino-2-phenylindole (DAPI); 5',5''-dibromopyrogallol-sulfonephthalein (Bromopyrogallol Red); 7- diethylamino-3-(4'-isothiocyanatophenyl)-4-methylcoumarin; diethylenetriamine pentaacetate; 4,4'-diisothiocyanatodihydro-stilbene-2,2'-disulfonic acid; 4,4'-diisothiocyanatostilbene-2,2'- disulfonic acid; 5-[dimethylamino]naphthalene-1-sulfonyl chloride (DNS, dansyl chloride); 4-(4'-dimethylaminophenylazo)benzoic acid (DABCYL); 4-

dimethylaminophenylazophenyl-4'- isothiocyanate (DABITC); eosin and derivatives such as eosin and eosin isothiocyanate; erythrosin and derivatives such as erythrosin B and erythrosin isothiocyanate; ethidium; fluorescein and derivatives such as 5-carboxyfluorescein (FAM), 5-(4,6-dichlorotriazin-2-yl)aminofluorescein (DTAF), 2'7'-dimethoxy-4'5'-dichloro-6-carboxyfluorescein (JOE), fluorescein, fluorescein isothiocyanate (FITC), and QFITC (XRITC); 2',7'-difluorofluorescein (OREGON GREEN®); fluorescamine; IR144; IR1446; Malachite Green isothiocyanate; 4- methylumbelliferone; ortho cresolphthalein; nitrotyrosine; pararosaniline; Phenol Red; B- phycoerythrin; o-phthaldialdehyde; pyrene and derivatives such as pyrene, pyrene butyrate and succinimidyl 1-pyrene butyrate; Reactive Red 4 (Cibacron.RTM. Brilliant Red 3B-A); rhodamine and derivatives such as 6-carboxy-X-rhodamine (ROX), 6-carboxyrhodamine (R6G), lissamine rhodamine B sulfonyl chloride, rhodamine (Rhod), rhodamine B, rhodamine 123, rhodamine X isothiocyanate, rhodamine green, sulforhodamine B, sulforhodamine 101 and sulfonyl chloride derivative of sulforhodamine 101 (Texas Red); N,N,N',N'-tetramethyl-6- carboxyrhodamine (TAMRA); tetramethyl rhodamine; tetramethyl rhodamine isothiocyanate (TRITC); riboflavin; rosolic acid and terbium chelate derivatives, thiol-reactive europium chelates which emit at approximately 617 nm

The disclosed methods may be automated, in whole or in part. For example, steps (i) and (ii) may be automated, but any subsequent steps, e.g., steps (iii) and (iv), are manual. Alternatively, by way of example, steps (i) and (ii) may be manual, whereas subsequent steps, e.g., steps (iii) and (iv), are automated. Additionally, all steps encompassed by the methods may be automated, such that the methods are fully automated.

The disclosed methods may be used, alone or in combination with other known methods (such as TNM), for tumor staging. In one embodiment, the methods further comprise evaluating one or more embodiments of the representative sample of the tumor, and the extent to which tumor cells have spread to the regional lymph nodes through analysis of the representative sample of the resected lymph nodes to predict the likelihood of the disease recurrence and/or progression.

appropriate therapeutic regimen for a cancer patient The therapeutic regimen can include gene therapy, chemotherapy, a targeted small molecule, other targeted therapies, immunomodulator administration, radiation, cytokine administration, surgery, or a combination thereof.

Moreover, the disclosed methods can be used to select at least one therapeutic agent (such as gene therapy (e.g., CRISPR), T cell therapy (e.g., CAR T cell), an antibody, nucleic acid, small molecule, or polypeptide, which antagonizes, inhibits, or blocks the expression or functional activity of at least one detected biomarker) suitable for use in a subject whose sample or tumor was the source for the representative sample generated by the methods provided.

In an additional embodiment, the present disclosure pertains to a method for preparing a representative sample for analysis, comprising (1) obtaining a surgical resection tissue sample from at least one subject; and (2) homogenizing the surgical resection tissue sample to obtain a homogenized sample. In an aspect, there is provided an in vitro method for preparing a representative sample for analysis, comprising: a. providing a surgical resection tissue sample from at least one subject; and b.homogenizing the surgical resection tissue sample to obtain a homogenized sample. In one embodiment, at least a portion of the surgical resection tissue sample is fixed. In an additional embodiment, the method further comprises processing a first portion of the surgical resection sample and generating one or more fixed, embedded tissue blocks and further homogenizing a second portion of the remaining surgical tissue resection sample. A portion of the one or more fixed, embedded tissue blocks may be processed by micrototomy to produce one or more tissue thin sections for morphological analysis. In addition, the at least one of the one or more fixed, embedded tissue blocks may be homogenized. In one embodiment the surgical resection tissue sample includes one or more separate pieces of tissue. In an additional embodiment, the one or more separate pieces of tissue comprise at least a portion of one or more primary solid tumor tissue masses resected from a subject to obtain the surgical resection sample. In another embodiment, the one or more separate pieces of tissue comprise at least a portion of one or more lymph nodes resected from the subject.

In an additional embodiment, the method further comprises separately homogenizing at least a portion of the separate pieces of tissue to yield separate homogenized samples. In an additional embodiment, the surgical resection tissue sample comprises a single tissue mass which may be further divided into two or

more pieces of the single tissue mass. Additionally, at least one of the two or more pieces of the single tissue mass may be homogenized and preserved. In one embodiment, the homogenization may comprise physical separation, such as cutting, dicing, or mincing. In another embodiment, the homogenization may comprise mechanical disassociation, such as blending or juicing. In yet another embodiment, the homogenization is accomplished by biochemical disassociation, for instance with a protease.

In an additional embodiment, one or more biomolecules may be purified from at least a portion of the homogenate, such biomolecules may include DNA, RNA, proteins, lipids, and metabolites. The biomolecules may then be analyzed, for instance by PCR, mass spectrometry, next generation sequencing, or ELISA. Such analysis produces at least one dataset.

In an additional embodiment, at least a portion of the homogenized sample may be embedded in paraffin. In an additional embodiment, the method further comprises preparing one or more thin sections of the paraffin embedded homogenized sample and performing histological analysis on the sample. Such histological analysis may include H&E staining, IHC staining, ISH staining, and FISH staining. The histological analysis may be interpreted by a human or quantified on an automated device. In an additional embodiment, the interpretation or quantification produces at least one dataset.

In one embodiment, the disclosure also pertains to further processing at least a portion of the homogenate to generate cellular fragments. Such processing may include physical, mechanical, chemical, or enzymatic methods. Such cellular fragments may include nuclei, cellular membranes, and cellular organelles. In another embodiment, at least a portion of the cellular fragments are affixed to at least one glass slide and optionally subjected to histological analysis. Such histological analysis may include H&E staining, IHC staining, ISH staining, or FISH staining. The analysis may be interpreted by a human or quantified by an automated device. The interpretation or quantification results in the creation of at least one dataset.

In an additional embodiment, at least a portion of the cellular fragments is analyzed by flow cytometry, FACS, or particle analyzer, wherein such analysis produces a data set. In one embodiment, at least one cellular fragment from the at least a

portion of the cellular fragments is purified. Such purification may occur, for example, by FACS, affinity purification, size exclusion differential centrifugation, filtration, or electrophoresis. In another embodiment, biomolecules may be isolated from the purified at least one cellular fragment from the at least a portion of the cellular fragments. The biomolecules may be analyzed by PCR, mass spectrometry, next generation sequencing, or ELISA. In one embodiment the analysis produces at least one dataset.

In yet another embodiment, the method of the present disclosure further comprises further processing at least a portion of the homogenate to generate at least one disassociated cell, for instance by physical, mechanical, chemical, or enzymatic. The disassociated cell is a normal cell, a cancer cell, or a bacterial cell. In one embodiment, the disassociated cell is affixed to at least one glass slide and subjected to histological analysis. Such analysis may include, for example, H&E staining, IHC staining, ISH staining, or FISH staining. In an additional embodiment, the analysis is interpreted by a human or quantified by an automated device. In an additional embodiment, the interpretation or quantification produces at least one dataset. In an additional embodiment, at least one cell from the at least one disassociated cell is purified by such means as FACS, affinity purification, size exclusion differential centrifugation, filtration, or electrophoresis. In an additional embodiment, biomolecules may be isolated from the purified at least one cell from the at least one disassociated cell. In an additional embodiment, the biomolecules may be analyzed, for example by PCR, mass spectrometry, next generation sequencing, or ELISA. In an additional embodiment, such analysis produces at least one dataset.

In an additional embodiment, the purified at least one cell from the at least one disassociated cell is affixed to at least one glass slide, and optionally is subjected to histological analysis. In an additional embodiment, the histological analysis is H&E staining, IHC staining, ISH staining, or FISH staining. Such analysis may be interpreted by a human or quantified by an automated device. In an additional embodiment, the analysis or interpretation produces at least one dataset.

In an additional embodiment, the datasets produced by the above-disclosed methods are further analyzed. In one embodiment, the analysis comprises the determination of a biomarker diversity or phenotypic diversity. In an additional

embodiment, the analyzing comprises the determination of at least one clinical decision. Such clinical decision, in one embodiment, 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.

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.

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.

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 facilities detection of low-prevalence subclones.

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.

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 mesh (top) and the flow through (bottom) of the micron mesh.

WHAT IS CLAIMED IS:

1. An in vitro method for preparing a representative sample for analysis, comprising:
   a. providing a surgical resection tissue sample from at least one subject; and
   b. homogenizing the surgical resection tissue sample to obtain a homogenized sample.

2. The method of claim 1, further comprising fixing at least a portion of the surgical resection tissue sample.

3. The method of claim 1, further comprising processing a first portion of the surgical resection sample and generating one or more fixed, embedded tissue blocks, optionally further comprising homogenizing a second portion of the remaining surgical tissue resection sample.

4. The method of claim 3, further comprising processing at least a portion of the one or more fixed, embedded tissue blocks by micrototomy to produce one or more tissue thin sections for morphological analysis.

5. The method of claim 4, further comprising deparaffinizing at least one of the one or more fixed, embedded tissue blocks and homogenizing the tissue from the one or more deparaffinized fixed, embedded tissue blocks.

6. The method of claim 1, wherein the surgical resection tissue sample includes one or more separate pieces of tissue, wherein the one or more separate pieces of tissue comprise at least a portion of one or more primary solid tumor tissue masses previously resected from a subject to obtain the surgical resection sample and/or at least a portion of one or more lymph nodes previously resected from the subject.

7. The method of claim 6, further comprising separately homogenizing at least a portion of the separate pieces of tissue to yield separate homogenized samples.

8. The method of claim 1, wherein the surgical resection tissue sample comprises a single tissue mass, wherein the single tissue mass is further divided into two or more pieces of the single tissue mass, and at least one of the two or more pieces of the single tissue mass is homogenized and at least one of the remaining two or more pieces of the single tissue mass are preserved.

9. The method of claim 1, wherein the homogenizing comprises physical separation by cutting, dicing, or mincing, or wherein the homogenizing comprises mechanical disassociation by blending or juicing, or wherein the homogenizing is by biochemical disassociation by a protease.

10. The method of claim 1, further comprising purifying and analyzing one or more biomolecules from at least a portion of the homogenized sample, wherein the one or more biomolecules are selected from the group consisting of DNA, RNA, proteins, lipids, and metabolites, wherein the analyzing the one or more biomolecules is by PCR, mass spectrometry, next generation sequencing, or ELISA, and wherein the analyzing produces at least one dataset.

11. The method of claim 1, further comprising embedding at least a portion of the homogenized sample in paraffin, preparing one or more thin sections of the paraffin embedded homogenized sample, and performing histological analysis on thin sections of the paraffin embedded homogenized sample by H&E staining, IHC staining, ISH staining, or FISH staining, wherein the histological analysis on thin sections of the paraffin embedded homogenized sample is interpreted by a human, or wherein the histological analysis on thin sections of the paraffin embedded homogenized sample is quantified by an automated device.

12. The method of claim 1, further comprising further processing at least a portion of the homogenized sample by physical, mechanical, chemical, or enzymatic methods to generate cellular fragments, wherein the cellular fragments are selected from the group consisting of nuclei, cellular membranes, and cellular organelles.

13. The method of claim 12, wherein at least a portion of the cellular fragments are affixed to at least one glass slide and subjected to histological analysis by H&E staining, IHC staining, ISH staining, or FISH staining, wherein the histological analysis on at least a portion of the cellular fragments affixed to at least one glass slide is interpreted by a human, or wherein the histological analysis on at least a portion of the cellular fragments affixed to at least one glass slide is quantified by an automated device.

14. The method of claim 12, wherein at least a portion of the cellular fragments is analyzed by flow cytometry, FACS, or particle analyzer, wherein the analysis produces a dataset.

15. The method of claim 12, further comprising purifying at least one cellular fragment from the at least a portion of the cellular fragments by FACS, affinity purification, size exclusion differential centrifugation, filtration, or electrophoresis,, isolating biomolecules from the purified at least one cellular fragment from the at least a portion of the cellular fragments, analyzing the biomolecules by PCR, mass spectrometry, next generation sequencing, or ELISA, wherein the analysis produces at least one dataset.

16. The method of claim 1, further comprising further processing at least a portion of the homogenized sample to generate at least one disassociated cell, wherein the processing of at least a portion of the homogenate is physical, mechanical, chemical, or enzymatic,

and wherein the at least one disassociated cell is a normal cell, a cancer cell, or a bacterial cell.

17. The method of claim 16, wherein the at least one disassociated cell is affixed to at least one glass slide and subjected to histological analysis by H&E staining, IHC staining, ISH staining, or FISH staining, wherein the histological analysis on the at least one disassociated cell affixed to at least one glass slide is interpreted by a human, or wherein the histological analysis on the at least one disassociated cell affixed to at least one glass slide is quantified by an automated device.

18. The method of claim 16, wherein the at least one disassociated cell is analyzed by flow cytometry, FACS, or particle analyzer, and wherein the analysis produces a data set.

19. The method of claim 16, further comprising purifying at least one cell from the at least one disassociated cell

by FACS, affinity purification, size exclusion differential centrifugation, filtration, or electrophoresis.

20. The method of claim 19, further comprising isolating biomolecules from the purified at least one cell from the at least one disassociated cell and analyzing the biomolecules by PCR, mass spectrometry, next generation sequencing, or ELISA, wherein the analysis produces at least one dataset.

21. The method of claim 19, wherein the purified at least one cell from the at least one disassociated cell is affixed to at least one glass slide and subjected to histological analysisby H&E staining, IHC staining, ISH staining, or FISH staining, wherein the histological analysis on the purified at least one cell from the at least one disassociated cell affixed to at least one glass slide is interpreted by a human,

or wherein the histological analysis on the purified at least one cell from the at least one disassociated cell affixed to at least one glass slide is quantified by an automated device.

22. The method of any one of claims 11, 13, 17 and 21, wherein the interpretation by a human produces at least one dataset, or wherein the quantification by an automated device produces at least one dataset.

23. The method of any one of claims 10, 14, 15,18, 20 and 22, further comprising analyzing the at least one dataset from the at least one subject, wherein the analyzing comprises the determination of a biomarker diversity or phenotypic diversity dataset, or the determination of the prevalence of at least one distinct biomarker or phenotype, or the determination of at least one clinical decision, wherein the clinical decision is 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.