SYSTEM AND METHOD FOR TISSUE ANALYSIS

STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

[0001] None.

REFERENCE TO RELATED APPLICATIONS

[0002] This application claims the benefit of the priority date of United States provisional application 63/556,364, filed February 21, 2024, the contents of which are incorporated herein in their entirety.

BACKGROUND

[0003] The isolation of single cells, nuclei and other subcellular organelles from fresh or fixed tissue is an area of interest to investigators in the life sciences. Processing of fixed tissue, such as FFPE tissue, can involve removal of the preservative, for example by dissolving in an organic solvent, and rehydrating the released tissue. Processing of fresh or rehydrated tissue can involve dissociating tissue to disrupt extracellular matrix, for example in the presence of enzymatic solutions that break chemical bonds in the extracellular matrix or with mechanical grinding to release cells and/or nuclei and other subcellular organelles. The released cells, nuclei and/or other subcellular organelles can then be collected for analysis, for example, genomic analysis of single cells.

[0004] Automated methods of isolating single cells, nuclei and/or other subcellular organelles from fresh or preserved tissue speeds up this process and makes them more predictable.

BRIEF DESCRIPTION OF THE DRAWINGS

[0005] The accompanying drawings, which are incorporated herein and form a part of the specification, illustrate exemplary embodiments and, together with the description, further serve to enable a person skilled in the pertinent art to make and use these embodiments and others that will be apparent to those skilled in the art. The invention will be more particularly described in conjunction with the following drawings wherein:

[0006] **Figure 1** shows a Sample processing System that processes specimens into biocomponents such as single cells or nuclei for bioanalysis.

[0007] **Figure 2** shows an overview of a Tissue Processing System and some exemplary modules. Tissue specimens or other specimens are processed into single cells, nuclei, nucleic acids, single-cell libraries, and other biologicals through the use of one or more cartridges and one or more of the Physical Dissociation Subsystem, the Enzymatic and Chemical Dissociation

Subsystem, the Measurement Subsystem, the Fluidic Subsystem, the Control Subsystem, and a Magnetic Module.

[0008] **Figure 3** shows the overall design concept for a prototype showing functional system and a few example modalities of mechanical disruption and examples of chemicals and enzymes to dissociate FFPE tissue specimens into single cells, nuclei, and other biomolecules.

[0009] **Figure 4** shows an example of a Single-Sample Tissue Processing System with mechanical disruption in a single cartridge with a bank of enzymes and reagents located in the instrument to dissociate solid tissue specimens into single cells, nuclei, and other biomolecules.

[00010] **Figure 5** shows another example of a Single-Sample Tissue Processing System with mechanical disruption in a single cartridge with a bank of enzymes and reagents located separately from the instrument in a reagent module.

[00011] **Figure 6** shows the front of an example of a Single-Sample Tissue Processing System used to dissociate tissue specimens into suspensions of single nuclei, and other biomolecules, using a cartridge.

[00012] **Figure 7** shows the back of an example of the Single-Sample Tissue Processing System.

[00013] **Figures 8 A-C** shows an example of a cartridge with processing, post-processing, and vacuum trap chambers for processing tissue specimens into single nuclei, single cells, and other biomolecules.

[00014] **Figures 9 A-D** show an example of adding reagents to a cartridge mixing the reagents, removing the reagents, and mechanically disrupting the tissue for processing tissue specimens into single cells, nuclei, and other biomolecules.

[00015] **Figure 10** shows an exemplary computer system.

[00016] **Figure 11** shows a cartridge architecture using pinch valves to direct liquid flows.

[00017] **Figure 12** shows an exemplary cartridge fluidic architecture.

[00018] **Figure 13A** shows a cutaway view of an exemplary cartridge. **Figure 13B** shows a cartridge comprising a filter positioned in the post-processing chamber.

[00019] **Figure 14** shows a top-down view of an exemplary cartridge.

[00020] **Figure 15** shows an exemplary grinder assembly.

[00021] **Figure 16** shows a cutaway view of an exemplary cartridge with a grinder assembly positioned for insertion into the processing chamber.

[00022] **Figure 17** shows an exemplary cartridge with a grinder assembly inserted into the processing chamber.

[00023] **Figures 18 A-B** show an exemplary cartridge with a feature designed to center the head of the rotor in the processing chamber and set the bottom gap and side gaps between the rotor and the wall of the processing chamber.

[00024] **Figures 19 A-D** show a port cover with a low durometer over a port secured by a port cover retaining cylinder, or a crimp, or a heat staked port cover retaining cylinder.

[00025] **Figures 20 A-E** show a cap engaging with a rotary motor adapter and with a cartridge with processing, post-processing, and vacuum trap chambers for processing tissue specimens into single cells, nuclei, and other organelles or biomolecules. Figure 20A shows actuator before engagement with the plunger assembly. Figures 20B and 20C show the engaged in two different rotational orientations. Figures 20D and 20E show the plunger assembly in partially and fully depressed states.

[00026] **Figure 21** shows an exemplary workflow to deparaffinize and rehydrate FFPE specimens followed by nuclei isolation.

[00027] **Figure 22** shows a picture of cartridge adapted for processing preserved tissue.

[00028] **Figure 23** shows a drawing of a cartridge adapted for processing preserved tissue, including ports for engaging a cartridge interface of an instrument.

[00029] **Figure 24** shows a cut-away view of a cartridge adapted for processing preserved tissue.

[00030] **Figure 25** shows a cut-away view of a cartridge adapted for processing preserved tissue.

[00031] **Figure 26** shows a top-down view of a cartridge.

[00032] **Figure 27** shows an exemplary reagent module including caddy holding reagent containers and a waste container.

[00033] **Figure 28** shows a side view of an exemplary reagent module.

[00034] **Figure 29** shows an exemplary system.

[00035] **Figures 30A** and **30B** show a cutaway view of a processing chamber including a plunger assembly (disruption head) being translated along the Z axis (1) and hitting the stator (2).

[00036] **Figures 31A-C** show a mechanical assembly with a force sensor to monitor force on the rotor face in the z-axis.

[00037] **Figure 32** shows a mechanical assembly for actuating the plunger assembly from a different aspect.

[00038] **Figure 33** shows a graph of force on the rotor as a function of position along the Z axis. Force increases slightly when the motor engages the plunger assembly and reaches an inflection point with dramatic increase in force when the rotor touches the bottom of the processing chamber.

[00039] **Figure 34** shows a schematic of a mechanical assembly of an actuator for translating a plunger assembly along a z-axis.

SUMMARY

[00040] Provided herein are systems, kits and methods for isolating cells, nuclei and/or subcellular organelles from fresh, frozen, or preserved tissue.

[00041] Systems are provided that engage different cartridges, adapted either for processing preserved tissue or for dissociating cells, nuclei and/or other subcellular organelles from tissue. In some embodiments, both types of cartridges comprise a processing chamber in fluidic communication with a post-processing chamber. The processing chambers of both cartridges are adapted to accommodate a plunger assembly that comprises a rotor. In the case of the preserved tissue cartridge, the rotor has a circumference such that when the rotor is inserted into the processing chamber the gap between the walls of the processing chamber and the rotor is small enough to prevent passage of pieces of tissue containing cells, nuclei and/or other subcellular organelles from passing between the rotor and the chamber wall. In contrast, for the dissociation cartridge the rotor has a circumference such that when the rotor is inserted into the processing chamber the gap between the walls of the processing chamber and the rotor is large enough to allow passage of cells, nuclei and/or other subcellular organelles between the rotor and the chamber wall. More particularly, for the tissue dissociation cartridge, certain rotors may have a circumference that allows passage of cells, nuclei, and other subcellular organelles, and other rotors may have a circumference that allows passage of nuclei and/or other subcellular organelles, but not whole cells.

[00042] Furthermore, in cartridges adapted for tissue dissociation, the processing chamber can comprise a stator comprising grinding features, and, the rotor can comprise a face facing the stator that also comprises grinding features. Such grinding features can be absent from the stator and the rotor face of the preserved tissue processing cartridge.

[00043] Cartridges for processing preserved tissue, on the one hand, and releasing cells and/or subcellular organelles from tissue, on the other hand, have geometries such that features of each can engage the same element in a system to which the cartridges can engage. So, for example, both cartridges can have the following elements for engaging ports in the instrument once engaged: (1) a plunger configured to fit through a top orifice into a processing chamber that engages an actuator in the system, (2) a processing chamber port that engages a port in the cartridge interface, (3) a post processing chamber port that engages a port in the cartridge

interface and (4) a vacuum port. Each of these can be relatively positioned in each of the preserved tissued processing cartridge and the tissue dissociation cartridge such that these ports all engage the same ports/actuator in the instrument. Also, the cartridge interface of the instrument can comprise alignment features to properly align a cartridge for engagement. Accordingly, both the preserved tissue processing cartridge and the tissue dissociation cartridge can comprise alignment, guide, or registration features complementary to the alignment, guide, or registration feature in the same cartridge interface such that each cartridge can be engaged. This might be, for example, a hole for a pin or vice versa, a groove for guide rib or key or vice versa, or a dovetail.

[00044] The post-processing chambers of both cartridges can be configured for different purposes. Both post-processing chambers can communicate with a source of vacuum to pull liquid from the processing chamber into the post-processing chamber. In the tissue dissociation cartridge, the post-processing chamber can be configured to collect the cells in the chamber without being pulled out of the cartridge by the source of vacuum. In the preserved tissue processing cartridge, the post-processing chamber can be configured to pull liquid from the processing chamber into, and then out of, the post-processing chamber. This can be accomplished by, for example, including a same-positioned port in the post-processing chamber. In the tissue dissociation cartridge, there also can be a vacuum chamber communicating with the post-processing chamber and connected to a source of vacuum in the instrument. In the preserved tissue processing cartridge, the same-positioned port can be connected to a source of vacuum, and positioned to function as a drain in the post-processing cartridge to collect liquid moved into the post-processing cartridge when vacuum is applied. In one embodiment, the port can function as a drain when a floor of the post-processing cartridge is positioned at about the level of the port.

[00045] A system for performing both processing of preserved tissue and dissociating tissue can comprise the following features. For dissociating tissue the instrument can comprise a cartridge interface to engage the cartridge and that comprises connectors, e.g., configured as cannulas positioned to engage ports in the cartridge that are in fluid communication with the processing chamber, the post-processing chamber and/or a separate vacuum chamber. The system can further comprise a fluidic subsystem comprising reservoirs adapted to contain reagents used in tissue dissociation, such as enzymatic solutions. The system can further comprise one or more sources of positive and/or negative pressure configured to move liquids from the fluidic subsystem into and/or out of the cartridge. The system can further comprise a mechanical subsystem comprising an actuator configured to actuate the plunger assembly. Actuation can be either or both of rotational (in either clockwise or counterclockwise direction) and linear, which is to say, to move the rotor up and/or down within the processing chamber (*i.e.,* along the Z axis).

[00046] The system can also comprise a force sensor operatively linked to the rotor configured to measure, in real time, the force on the rotor. Using feedback from the force sensor, and a computer program configured to control pressure on the rotor, the mechanical assembly can control motion of the plunger assembly in the Z axis. So, for example, when the force sensor senses a force on the rotor consistent with the rotor hitting the stator, the software can instruct the mechanical assembly to cease downward motion of the rotor. Similarly, when the force sensor senses a force on the rotor consistent with hitting tissue in the processing chamber, the software can instruct the mechanical assembly to pause downward motion and begin rotating the rotor. The system can store in computer memory a position of the rotor along the Z axis that corresponds to a force measurement consistent with the rotor touching a tissue sample and/or touching the floor of the chamber. Then, in subsequent grinding operations, either in the currently used cartridge in the same run, or in different cartridges, the rotor can be depressed to the relevant position stored in memory.

[00047] For processing preserved tissue, the instrument can comprise a reversibly engageable tissue processing subsystem that comprises a caddy for holding a plurality of containers and one or more elements selected from (A) a containment barrier configured to contain liquids within the caddy, (B) a plurality of containers in the caddy including at least one container for containing a reagent liquid, and a waste container, (C) a scale configured to measure the weight of the waste container, (D) an optical sensor that senses liquid in the bottom of the caddy, and (E) a plurality of fluidic conduits that connect the containers with ports through couplings in a sensing mechanism that senses when a proper coupling has been made. The instrument further comprises fluidic conduits that connect the couplings with ports in the cartridge interface and one or more sources of pressure for moving liquids in either direction between the containers in the caddy and chambers in the cartridge.

DETAILED DESCRIPTION

I. Introduction

[00048] **Figure 1** shows a Sample processing System **50** that can input specimen **101** and process them to produce biologicals such as single cells **1000** or nuclei **1050**, microtissues **6001**, organoids **6002**, or other biocomponents comprised of subcellular components **1060**, and biomolecules **1070** such as macromolecules **1071** and nucleic acids **1072,** comprised of DNA **1073** and RNA **1074**; proteins **1075**; carbohydrates **1076**; lipids **1077**; biomolecules **1070** with multiple types of macromolecules **1071**; metabolites **1078**; and other biological components, including natural products **1079** for bioanalysis.

[00049] Referring to **Figure 2** , in many embodiments, the Tissue Processing System **110** processing is performed in cartridges **200** in the system. Tissue samples, *e.g.*, fresh tissue **101**, or preserved tissue samples, such as FFPE tissue specimens **150** or OCT (optimal cutting

temperature) tissue specimens **160,** or other specimens **101** are converted to single nuclei **1050**, single cells **1000**, or other organelles, or biomolecules or single cell libraries **1200** or bulk libraries **1210** through the use of cartridge **200** with one or more of the Physical Dissociation Subsystem **300**, the Enzymatic and Chemical Dissociation Subsystem **400**, the Measurement Subsystem **500**, the Fluidic Subsystem **600**, the Control Subsystem **700**, the Magnetic Module **900**, and the Temperature Subsystem **1475**.

[00050] The Physical Dissociation Subsystem **300** can perform mixing or perform physical disruption by one or more of passing the specimen through orifices, grinding, rotating a rotor with features to dissociate tissue, forcing tissue through screens or mesh, sonication, ultrasonics, blending, homogenization, bead beating, and other methods known to one skilled in the art to physically disrupt tissue to help produce single cells.

[00051] The Enzymatic and Chemical Dissociation Subsystem **400** can perform deparaffinization by adding organic solvents, such as xylene or xylene substitutes, to the cartridge and perform rehydration by adding mixtures of ethanol with increasing amounts of water or buffer. The Enzymatic and Chemical Dissociation Subsystem **400** can perform crosslink reversal and/or enzymatic disruption by using heat or adding formulations of reagents or mixture of components comprised of but not limited to proteinase K, collagenases (*e.g.*, collagenases type I, II, III, IV, and others), elastase, trypsin, papain, hyaluronidase, chymotrypsin, neutral protease, clostripain, caseinase, neutral protease (Dispase®), DNAse, protease XIV, RNase inhibitors, or other enzymes, biochemicals, or chemicals such as EDTA, protease inhibitors, buffers, acids, or base.

[00052] Another aspect of the Enzymatic and Chemical Dissociation Subsystem **400** is that it can perform chemical disruption or chemical and enzymatic disruption by adding formulations of chemicals that can disrupt tissue or cellular integrity, such as Triton X-100, Tween, Nonident P40, octyl glucoside, polyoxyethylene(9)dodecyl ether, digitonin, IGEPAL™ CA630 octylphenyl polyethylene glycol, n-octyl-beta-Dglucopyranoside (betaOG), n-dodecyl-beta, Tween™. 20, polyethylene glycol sorbitan monolaurate, Tween™ 80 polyethylene glycol sorbitan monooleate, polidocanol, n-dodecyl beta- D-maltoside (DDM), NEMO nonylphenyl polyethylene glycol, C12E8 (octaethylene glycol ndodecyl monoether), hexaethyleneglycol mono-n-tetradecyl ether (C14E06), octyl-betathioglucopyranoside (octyl thioglucoside, OTG), Emulgen, and polyoxyethylene 10 lauryl ether other surfactants, or detergents or chemicals that can dissociate tissue into cells or produce nuclei or other organelles.

[00053] In other embodiments, different reagents or mixtures of reagents are applied sequentially to dissociate the FFPE or OCT sample or specimen into single cells **1000** or nuclei. **1050**. The physical and enzymatic/chemical dissociation systems can be separate from each other, or they can be co-located (*e.g.*, acting upon the sample simultaneously or sequentially).

[00054] In some embodiments, the amount of dissociation is monitored at intervals during the dissociation or at the endpoint, and in some instances the viability is determined during processing using a Measurement Subsystem **500**. The Measurement Subsystem **500** can be an optical imaging device to image cells or nuclei or tissue using brightfield, phase contrast, fluorescence, chemiluminescence, near-field, or other optical readouts, or an electrical measurement, such as an impedance measurement of the change in conductivity when a cell passes through a sensor, or other types of measurement.

[00055] The addition and movement of fluids can be performed by a Fluidic Subsystem **600**. The Fluidic Subsystem **600** can use syringe pumps, piezopumps, on-cartridge pumps and valves, vacuum (negative pressure), pressure, pneumatics, or other components well known to one skilled in the art.

[00056] The Tissue Processing System **110** can be controlled by software in a Control Subsystem **700** which can be comprised of a user interface **740** through a monitor, embedded display, or a touch screen **730** to communicate with and control devices, modules, subsystems, instruments, and systems. In some instances the Control Subsytem **700** can include interfaces to smart devices, laboratory information management systems, other instruments, analysis software, display software, databases, email, and other applications. The Control Subsystem **700** can include control software **725** and scripts that control the operation and in some embodiments the scripts can be revised, created, or edited by the operator.

[00057] In another aspect provided herein is a device for the dissociation of a biological sample, the device comprising: (i) a biological sample or specimen **101**; (ii) a cartridge **200** capable of dissociating tissue; (iii) an instrument to operate the cartridge **200** and provide fluids as needed (iv) a measurement module **500** such as an optical imager to measure titer, clumping, and/or viability, (v) pumps and containers for exchange of dissociation solution for buffer or growth media at the desired titer, and (vi) output vessels such as a chamber in the cartridge, 8-well strip tubes, microtiter plates, Eppendorf tubes, conical centrifuge tubes, or other vessels capable of receiving cell suspensions.

[00058] In another aspect provided herein is a device for the dissociation of a biological sample and the production of single-cell **1000** or nuclei **1050** suspensions or matched bulk nucleic acids **1010** or single cell libraries **1200** or matched bulk libraries **1210**, the device comprising: (i) a chamber or area to input a biological sample or specimen either directly or in a device;(ii) a cartridge capable of dissociating tissue or specimen; (iii) an instrument to operate the cartridge and provide fluids as needed (iv) a measurement module such as an optical imager to measure titer, clumping, and/or viability, necrosis, cytotoxicity, apoptosis, *etc.* (v) sources of positive and/or negative pressure for exchange of dissociation solution for buffer or growth media at the desired titer, and (vi) output vessels such as 8 well strip tubes, microtiter

plates, Eppendorf tubes, a chamber in the cartridge, or other vessels capable of receiving cell suspensions.

[00059] Referring to **Figure 4,** a Magnetic processing module **900** can use magnetic processing of magnetic and paramagnetic particles or beads or surfaces of other sizes and shapes, referred to as beads, to separate single cells **1000**, or cell types, or nuclei **1050**, or other biocomponents comprised of subcellular components **1060**, and biomolecules **1070** such as macromolecules **1071** and nucleic acids **1072,** comprised of DNA **1073** and RNA **1074**; proteins **1075**; carbohydrates **1076**; lipids **1077**; biomolecules **1070** with multiple types of macromolecules **1071**; metabolites **1078**; and other biological components, including natural products **1079** for bioanalysis. In some embodiments the beads have a surface chemistry that facilitates the purification of the biologicals in conjunction with the chemical conditions. In other embodiments the beads have affinity molecules comprised of antibodies, aptamers, biomolecules, *etc.* that specifically purify certain biologicals such as cell types, nucleic acids, nuclei **1050**, or other components of tissue or samples.

[00060] The basic elements of the Tissue Processing System **110** can be configured in multiple ways depending on the specimen(s) **101** or FFPE tissue specimens **150** or OCT tissue specimens **160** and analytes to be analyzed. In the following example, one of the numerous configurations is described in detail but in no way is the invention limited to these configurations as will be obvious to one skilled in the art. The Tissue Processing System **110** can accommodate many different types of specimens **101**, comprised of fresh tissue; snap-frozen tissue; microtome slices (cryo, laser or vibrating) of tissue; fixed tissue; bulk material obtained by surgical excision, biopsies, fine needle aspirates; samples from surfaces, and other matrices, or FFPE tissue specimens **150**.

[00061] The instant disclosure teaches how to produce a system that processes FFPE tissue specimens **150** and OCT tissue specimens **160** and other samples into preferentially nuclei **1050** or into single-cells **1000**. The process may require adapting to the widely varying starting types of FFPE tissue specimens **150**, with different requirements depending on the tissue, species, age, and state.

[00062] In the instant invention, many embodiments are possible and are incorporated by reference from patent application PCT/US2017/063811 filed November 29, 2017 (Jovanovich, Chear, McIntosh, Pereira, and Zaugg, "Method and Apparatus for processing Tissue Samples") and from patent application PCT/US19/35097 filed June 1, 2019 (Jovanovich, Chear, Leisz, Eberhart, and Bashkin, "Method and Apparatus for processing Tissue Samples"); the contents of all of which are incorporated herein in their entirety as well as the number system used therein except where there is conflict the numbering herein predominates.

[00063] This disclosure describes how to automate, integrate, and, importantly, standardize the complete process to create single-nuclei **1050** in a single sample Tissue processing **110** system embodiment using a novel mechanism to retain the tissue and a novel cartridge design. It is clear to one skilled in the art that multi-sample embodiments can be accomplished with the same instant invention. The Tissue Processing System **110** will greatly enable basic researchers, students, and translational researchers as well as clinicians and others with its ease of use and high performance.

II. Cartridge designs

[00064] Cartridges **200** can be used to process tissue into single-cell **1000** suspensions or nuclei **1050** and can be for single-use.

[00065] Referring to **Figure 3**, cartridge **200** will input specimen **101** (*e.g.*, fresh tissue, FFPE tissue specimen **150** or OCT tissue specimen **160**) and output single cells **1000** or nuclei **1050**. The Tissue Processing System **110** as shown conceptually in **Figure 3** combines the mechanical disruption of specimen **101** on cartridge **200**, adds reagents such as chemicals, detergents, enzymatic or chemical dissolution solutions **410** and other fluids according to the protocols, and controls sample movement, pressures, and temperature. The Tissue Processing System **110** can move or rotate mechanical tissue disruptor elements comprised of, without limitation, a syringe plunger, pestle, Dounce pestle, or grinder, using a z axis stepper **2110** with a rotary motor **2120** coupled through the cap **210**.

[00066] Cartridges **200** can be designed for 3D printing, injection molding in plastics with single or double pulls and low labor assembly, or layered assembly of fluidic and other layers, combinations of methods, and other methods well known to one skilled in the art. Fluids can be delivered to cartridge **200** by pumps such as a syringe pump **2130** or by vacuum or can be preloaded onto cartridge **200** or by any combination of the foregoing. In some embodiments, flexible tubing **493** can connect chambers and create simple pinch valves **491** to direct flow. In other embodiments, channels are created in the cartridge **200** and valves can be incorporated such as pneumatic valves, or other valves.

A. Tissue Dissociation Cartridge

[00067] Referring to **Figure 8C**, the term plunger, or plunger assembly, is at times used to refer to combination of shaft/piston **216** and rotor **218** with optional disruption features (*e.g.,* teeth) **355** with spring **213** in sheath **212**.

[00068] In an embodiment, the mechanical tissue disruptor elements have features **355** on the bottom of the rotor or grinder that can mechanically disrupt tissue at the bottom or floor of processing chamber **440** which in some embodiments may have complementary features **355** to aid in the disruption of the tissue. In some embodiments, the mechanical tissue disruptor elements does not have features **355** on the bottom of the rotor or grinder but can be flat and

mechanically disrupt tissue against a flat surface at the bottom or floor of processing Chamber **440.** Disruption also occurs in the 'side gap' between the rotor and the side wall of processing Chamber **440** in some embodiments.

[00069] **Figures 13-17** show an exemplary cartridge of this disclosure. **Figure 13** shows a cutaway view of an exemplary cartridge. The cartridge **200** includes a processing chamber **440** comprising a stator comprising teeth arranged in an annular array. The processing chamber further comprises a first processing port from which a cell, nuclei organelle suspension can be removed from the processing chamber. Also shown are post-processing chamber **460** and a vacuum chamber. The vacuum chamber **468** comprises a vacuum port **467**. **Figures 15 and 16** show an exemplary grinder assembly **345** of this disclosure. The grinder assembly **345** includes a plunger comprising a piston **216** and a rotor **218** positioned at an end of the piston. The rotor comprises on a bottom surface, grinding elements, *e.g.*, teeth **355**, including a central tooth **356** and an annular array of three rings of teeth. The teeth **355** can have a blunt or sharp shape. They may take the shape of a trapezoid in cross section. The outermost ring of teeth **355** is positioned at the edge of the rotor. The grinding assembly **345** further includes a sleeve or sheath **212** around the piston **216**. The grinding assembly **345** further includes a cap **210** to position the plunger in the processing chamber. The cap **210** further comprises a slot or other mechanism configured to engage a key of an actuator to actuate the grinder. Not shown here is a spring which biases the rotor **218** toward the cap **210** so that positive pressure must be asserted on the plunger by the actuator to press the rotor **218** against the stator. The annular rings of teeth **355** in the rotor and the stator are positioned complementary to one another so that when the grinder is pressed against the stator the rings of the stator mesh with the rings of the rotor (*e.g.,* are staggered against). That is, in an exemplary embodiment, teeth in the stator do not touch teeth in the rotor. This configuration facilitates rotation of the rotor against the stator so that teeth from one part do not collide with teeth from another part. The number of rings of teeth in each of the rotor and the stator can be determined by a skilled artisan. Factors influencing the determination include the total surface area of the stator and the face of the rotor, as well as the size of the teeth. In certain embodiments the number of rings of teeth in the stator and/or the rotor **218** can be any of none, one, two, three, four, five, or six. In one embodiment teeth can have a trapezoidal cross-section. The processing chamber can have a cylindrical shape. The stator can have a radius between, for example, 5 mm and 25 mm, *e.g.*, about 12 mm. The processing chamber can have a volume less than 1 ml, or between about 1 mL and 50 mL, for example, between about 10 mL and about 30 mL, e.g., about 15 mL. The rotor and the sidewalls of the processing chamber can be configured so that when the plunger is inserted into the processing chamber there is a gap between the sidewall of the processing chamber and an edge of the rotor. The size of the gap can be optimized to allow passage of whole cells, nuclei or organelles between the sidewall and the rotor. In certain embodiments, the teeth can have a height of about 500 microns and a width of about 1 mm to 2 mm.

[00070] Spin rates for the dissociation can be 10-200 rpm. Total revolutions of the grinding element can be 5-500. In an exemplary protocol, the spin rate is about 45 rpm (slow) or about 150 rm (fast), with about 4 seconds of revolution, about 1-2 second pause, then about another 4 seconds, then repeat (about 16 seconds total rotation time) at each vertical displacement step of the stepper motor, sequentially going lower towards the bottom of the cartridge, about 9 vertical displacements in all, and at the bottom-most step, there are about 3 repetitions of the rotation periods rather than 2.

B. Preserved Tissue Processing Cartridge

[00071] It is desirable that disposable cartridge **200** process multiple types of preserved FFPE **150** or OCT **160** tissues with mechanical disruption and enzymatic or chemical dissociation that can be adjusted according to the tissue type and condition of the FFPE tissue, such as age, or chemical process. The cartridge **200** can be designed to process tissue as quickly and as gently as possible, not expose the operator to the tissue being processed, and be manufacturable at low cost. Multiple mechanical methods may be needed to accommodate the wide range of tissues and their individual requirements: designs are shown that can be readily adapted to multiple different mechanical disruption methods comprising variable orifice **490**, grinding with rotating plungers **336**, pestles **361**, and straining and filtering using a plunger **362** as well as other mechanical methods without limitation.

[00072] **Figures 22-26** show an exemplary embodiment of a cartridge for preserved tissue processing. The cartridge of this embodiment shares many similar elements with the tissue dissociation cartridge described above with adaptations for processing of preserved tissue.

[00073] Cartridge **2500** comprises processing chamber **2540** and post-processing chamber **2560**. Processing chamber **2540** comprises first processing chamber port **2504** including nipple **2571** that is fluidically connected to post-processing chamber **2560** through fluidic conduit **2553**. Processing chamber **2540** further comprises a second processing chamber port **2570** which can be used to introduce liquids into the processing chamber. First processing chamber port **2504** and second processing chamber port **2570** can be placed in the same position as ports **1604** and **470** of exemplary tissue dissociation cartridge in order to meet with the same ports in the cartridge interface of the instrument.

[00074] Processing chamber **2540** can have inserted therein, plunger assembly **2510**. Plunger assembly **2510** can differ from a plunger assembly used in a tissue dissociation cartridge. More particularly, the rotor in a tissue dissociation cartridge has a circumference that provides a space between the outer wall of the rotor and the inner wall of the processing chamber. This space is selected to allow cells, nuclei, and/or subcellular organelles to pass around the rotor and collect on top of it when the rotor is fully depressed. In a cartridge configured for preserved tissue processing, the space is selected to be small enough so that

spent liquid, but not pieces of tissue or in some embodiments released cells, nuclei and/or subcellular organelles (depending on what is to be collected) can pass. In certain embodiments, this gap can be no more than any of 200 μm, 100 μm, 50 μm, and 25 μm.

[00075] In the cartridge for processing preserved tissue, the face of the rotor and the stator of the processing chamber can comprise or can not comprise grinding features.

[00076] First processing chamber port **2504** can be positioned on a side wall of the cartridge such that when the rotor of plunger assembly **2510** is fully depressed at least part of the port is positioned to drain liquid collecting above the top of the rotor. In some embodiments, part of the port is below the top of the fully depressed rotor. In other embodiments, all of the port is positioned above the top of the rotor. In any case, the port can be positioned such that the expected volume of liquid displaced above the top of the rotor contacts the port and can be removed via the port.

[00077] Post-processing chamber **2560** comprises an inlet **2554**. Inlet **2554** can be positioned in a lid or cover **2562** that covers orifice **2566**. Inlet **2554** can be fluidically connected to the first processing chamber through fluidic conduit **2553** and port **2504**. A first post-processing port **2585** can be placed in the same position as first post-processing port **485** of the exemplary tissue dissociation cartridge described herein.

[00078] Post-processing chamber **2560** can further comprise floor **2580** positioned such that liquid collecting in the post-processing chamber can drain to first post-processing port **2585**. In certain embodiments, floor **2580** may be slanted so that liquid pools at first post-processing port **2585**.

[00079] In alternative embodiments, fluidic conduit **2553** can connect directly with a port in the cartridge connectable to a source of pressure in the instrument, for example, either first post-processing port **2585**, or a port corresponding in position to vacuum port **467**.

[00080] In operation, both the tissue dissociation cartridge and the preserved tissue processing cartridge can have ports positioned to engage the same cartridge interface connector in the instrument. So, for example, in a preserved tissue processing step, processing reagents can be introduced into processing chamber **2540** through second processing chamber port **2570**. After the rotor is fully depressed in the processing chamber, spent reagents collect above the rotor and can be pulled by a vacuum exerted at first post-processing port **2585** through fluid line **2553** and into the post-processing chamber. From here, the spent reagents can be pulled from the post-processing chamber.

[00081] Similarly, in a tissue dissociation process, dissociation reagents can be introduced into processing chamber **440** through second processing chamber port **470**, positioned to engage the same connector in the cartridge interface as port 2570. After tissue dissociation, the rotor is depressed and liquid including released cells, nuclei and/or other subcellular organelles

collect above the top of the rotor. Because first processing chamber port **1604** is positioned in fluid communication with the region above the top of the fully depressed rotor, the cells, nuclei, in or other subcellular organelles can be pulled through fluidic line **453** and into the post-processing chamber. In contrast to the use of the preserved tissue processing cartridge, suction can be pulled from vacuum port **467** which is configured to apply vacuum to the post-processing chamber. However, because vacuum port **467** is positioned well above the floor of the post-processing chamber and can be separated by a wall almost reaching the top of the chamber, liquid comprising cells, nuclei, and/or other subcellular organelles is not pulled through vacuum port **467**.

III. Tissue Processing System

[00082] In one embodiment of the Sample processing System **50** as a Tissue Processing System **110**, as shown in **Figure 1,** the Tissue Processing System **110** can perform powerful integrated tissue-to-genomics or sample-to-other answer (genomic, proteomic, metabolomic, or epigenetic, multi-omics, etc.) analysis functionality for scientists to simply and standardize the production and or analysis of single-cell **1000** or nuclei **1050** suspensions, affinity purified single cells **1100,** affinity purified nuclei **1105**, nucleic acids **1072**, and bulk libraries **1210** from solid or liquid tissues. As will be obvious to one skilled in the art, the biological materials produced such as single cells **1000,** nuclei **1050**, nucleic acids **1072**, single cell libraries **1200**, single nuclei libraries **1250**, bulk libraries **1210**, or other biocomponents comprised of subcellular components **1060**, or biomolecules **1070** such as macromolecules **1071** and nucleic acids **1072,** comprised of DNA **1073** and RNA **1074,** can also be used for many genomic, cell biology, proteomics, metabolomics, and other analytical methods.

[00083] The Tissue Processing System **110** can integrate the preparation of biological materials from preserved tissue samples, e.g., FFPE tissue specimens **150** or OCT tissue specimen **160**, with measurement subsystems **500** that perform an analysis selected from one or more of: DNA or RNA sequencing, next generation DNA or RNA sequencing, next next generation DNA or RNA sequencing of nucleic acids and their adducts (such as epigenetic modifications); nanopore sequencing of nucleic acids and their adducts; single cell DNA sequencing of nucleic acids and their adducts; single nuclei RNA sequencing of nucleic acids and their adducts; PCR, digital droplet PCR, qPCR, RT-qPCR; genomic analysis, gene expression analysis, gene mapping, DNA fragment mapping; imaging including optical and mass spectrometry imaging; DNA or RNA microarray analysis; fluorescent, Raman, optical, mass spectrometry and other detection modalities of nucleic acids and their adducts with and without labels; proteomic analysis including fluorescent, Raman, optical, mass spectrometry, protein sequencing, and other detection modalities of proteins and peptides and their adducts and modifications with and without labels; carbohydrate characterization and profiling including sequencing, fluorescent, Raman, optical, mass spectrometry, and other detection modalities of

carbohydrates and their adducts and other covalent polymers with and without labels; lipid characterization and profiling including sequencing, fluorescent, Raman, optical, mass spectrometry, and other detection modalities of lipids and their adducts and other covalent polymers with and without labels; flow cytometry; characterization of cells and profiling including fluorescent, Raman, optical, mass spectrometry, and other detection modalities of cells and their adducts and other covalent polymers with and without labels; metabolic profiling including sequencing, fluorescent, Raman, optical, mass spectrometry, and other detection modalities of metabolites and their adducts and other covalent polymers with and without labels; functional analysis including protein-protein interactions, protein-lipid interactions, protein-DNA interactions, RNA-DNA interactions, and other interactions between molecules derived from biological materials, with and without labels; bioinformatic analysis of cells, organelles, and biomolecules; and mass spectrometry and other analytical methods. In some embodiments the measurement system 500 can be physically integrated and fluids transferred by robotic pipetting, fluid flow through tubing or capillaries, centrifugal methods, or other methods.

[00084] Referring to **Figure 4**, in this embodiment mechanical and enzymatic dissociation is performed in single-use cartridges **200** in one or more processing chambers **440** to produce nuclei suspensions **1050,** single-cell suspension **1000** or, nucleic acids **1072**, biomolecules **1070**, subcellular components **1060**, or other products. The samples can then be processed in the one or more post-processing chamber(s) **460** by optional bead-based affinity purification of cell types by surface antigens to produce affinity purified single-cell suspensions **1000** or nuclear suspensions **1050** by nuclear antigens or nucleic acids **1072**, biomolecules **1070**, subcellular components **1060** can be further processed into purified mRNA, NGS libraries, or other sample types. In some embodiments, one or more of the processing **440** and post-processing chambers **460** and strain chambers **450** and vacuum trap chambers **468** and waste chambers **430** or other chambers can be combined.

IV. Instrument and System

A. Instrument

[00085] The Tissue Processing System **110** can mechanically disrupt tissue and enzymatically dissociate and reverse crosslinks of the disrupted tissue in a cartridge **200** into single cells **1000** or nuclei **1050**. As shown in **Figure 4,** a Single Sample Tissue Processing System **2010** can combine the Physical Dissociation Subsystem **300** and the Enzymatic and Chemical Dissociation Subsystem **400** to produce single-cell **1000** or nuclei **1050** suspensions. The instrument provides the mechanical motion and fluidics to the cartridge which in turn mechanically and enzymatically or chemically process the FFPE tissue specimen **150** into single cells **1000** or nuclei **1050**. Multiple reagents **411** can be stored on the instrument or reagent module **1430** with cooling as needed.

[00086] A 3D CAD representation of one embodiment of a Single-Sample Tissue processing **2010** design packaged with a 'skin' is shown in **Figure 4** and another embodiment is shown in **Figures 6** and **7.** Both embodiments have a two axis mechanical motion (Z axis stepper **2110** and rotary motor **2120**) integrated with fluidics based on a syringe pump ,for example, with 1.6 µL resolution with a six-way valve (C2400MP, TriContinent) controlled by control software **725**.

[00087] Referring to **Figure 4**, a computer **720** with an operating system, for example, such as Windows 10 and 85 Gbytes HD (Beelink, AP42) can run control software **725** to control the system with display on a 10" touchscreen **730** (eleduino, Raspberry Pi10) or on a tablet **750** such as a Windows Surface Pro 6. Chassis **1010** provides the framework to mount components and the exterior case of the system.

B. Fluidic Subsystem

[00088] The embodiment of the Single-Sample Tissue Processing System **2010** shown in **Figure 4** has a fluidic subsystem **600** with a single syringe pump **2130** with a single six-way valve **2140** to supply liquids, pressure, or vacuum to cartridge **200** from reagent block **415**. In one embodiment, cartridge **200** has two processing chambers **440** and a single post-processing chamber **460**. In an embodiment, magnetic processing module **900** can apply magnetic force to cartridge **200** under software control to enable the use of paramagnetic beads, paramagnetic surfaces, paramagnetic nanoparticles, and other magnetic or paramagnetic particles to purify and analyze single cells **1000,** nuclei **1050**, nucleic acids **1072**, biomolecules **1070**, subcellular components **1060**, or other products.

[00089] An embodiment of the Single-Sample Tissue processing System **2010** with a case on is shown in **Figure 5.** This embodiment has a reagent module **1430** which can be separate from Single Sample Tissue processing Instrument **2010** as shown in **Figure 4** with power and control provided by Single Sample Tissue processing Instrument **2010** or a separate power source and processor can be used, or as shown in **Figure 4** reagent module **1430** can be integrated inside a single instrument case.

[00090] Referring to **Figures 27** and **28**, the system can comprise a reagent block or reagent subsystem configured for handling reagents in the processing of preserved tissue. Such reagents typically include organic solvents, such as xylene, for removing preservatives from preserved tissue, such as paraffin, as well as alcohols in aqueous solutions for rehydrating tissues from which preservatives have been removed. The reagent block can comprise a caddy configured to hold one or a plurality of containers, including reagent containers and a waste container. The caddy can include a containment barrier to contain any liquid spilled into the caddy. The caddy also can contain optical sensors that sense the presence of liquids at the bottom of the caddy.

[00091] Certain reagents used for removing preservatives from preserved tissue may be considered hazardous materials. Accordingly, tracking and controlling these materials can provide a benefit to the user. In certain embodiments, the caddy includes a scale on which the waste container rests. The scale can measure the weight of the waste container. Software in the system receives such measurements and can determine when the waste container is becoming full. In this case, the system can be configured to alert the user and/or stop moving liquids into the waste container.

[00092] Fluidic conduits from the waste container and/or reagent containers in the caddy can connect with couplings in the fluidic subsystem of the instrument. The couplings can include sensors, for example, that use the Hall effect, to confirm that a connector is properly seated within the coupling.

C. Force Sensor

[00093] Referring to **Figure 6**, in an embodiment, Single Sample Tissue processing Instrument **1020** has a linear driver motor, such as a z-axis stepper motor **2110**, which may have an optional encoder, that controls the vertical position of rotary motor **2120** mounted on z-axis stepper slide **2111** attached to the inverted 'U' shaped structural frame **1020** mounted on chassis **1010**.

[00094] A force gauge can be incorporated into the z-stage stepper **2110** to provide force-feedback control of the mechanical force on the specimen **101** or below cantilevered cartridge slide **1450**; this can help ensure very gentle mechanical processing steps and prevent application of high force by the rotor **218** onto the bottom of processing chamber **440**. Syringe pump **2130** connects fluidically with tubing or capillaries or microchips or other fluidic connectors with six-way valve **2141** and six-way valve **2140** to supply reagents, pressure, or vacuum to cartridge **200** (not shown) from reagent module **1430**.

[00095] Referring to **Figures 30-34**, in some embodiments, the force feedback mechanism can be configured to determine the position of the face of the rotor in the processing chamber. More specifically, force on the rotor increases somewhat when the actuator engages the plunger assembly (first inflection point in Figure **33**). Also, the force on the rotor increases significantly when the rotor encounters the stator, or floor, of the processing chamber (steep inflection point in Figure **33**). When the force gauge measures a force consistent with the rotor encountering tissue, the operating software can commence a grinding routine. The grinding routine can include a combination of rotating the rotor in one or both of clockwise or counterclockwise directions, and further depressing the rotor when the force measurement indicates to the operating software that more forceful grinding is necessary. A force measurement consistent with the rotor contacting the bottom of the chamber, can indicate to the

operating software that no further grinding is necessary and that liquid that is collected above the top of the rotor is ready for movement into the processing chamber.

[00096] The force sensor can be used to calibrate the plunger for operation in the same or different cartridges. In the first cartridge, position of the rotor and the z-axis consistent with tissue contact in or state or contact can be received into memory as calibrated positions. In the same cartridge, in the execution of a tissue dissociation or preserved tissue processing protocol, the plunger can be depressing the z-axis two positions in memory consistent with tissue contact or stator contact. Calibration can be performed on each cartridge engaged with the cartridge interface. Alternatively, a single calibration run can be performed in the calibrated positions used in a plurality of different cartridges engaged with the interface.

D. Cartridge Interface

[00097] Cartridge **200** is placed into cartridge receiver tray **1510** on cartridge slide **1450** which is designed to hold cartridge **200** precisely, with the center of processing chamber **440** concentric with the center of rotary motor shaft **2121** of rotary motor **2120** within a distance or 1 or, 5, or 10, or 15, or 20, or 25, or 50, or 100, or 250 μm, or more when inserted by moving cartridge **200** in cartridge receiver tray **1510** on cartridge slide **1450** on cartridge slide rail **1480** until spring-loaded cartridge slide knob **1452** locks into place into a hole in cartridge slide **1450** with cartridge **200** held in place near or in contact with the thermal transfer plate **1470** and making fluidic connections with the pogo pins **1415** of cartridge interface **1510**.

E. Temperature Sensor

[00098] The temperature regulating subsystem **1475** can set the thermal transfer plate **1470** to a given temperature by cartridge Peltier **1440** or other temperature regulating device such as strip resistive heaters, circulating fluids, *etc.* to set the cartridge temperature in the processing chamber **440** and post-processing chamber **460** under control of board **2250**. In some embodiments, the temperature of processing chamber **440** and post-processing chamber **460** can be set independently. In some embodiments the temperature regulating system can use a thermocouple, or thermistor, or IR camera to set the temperature of the thermal transfer plate **1470** or the outside of cartridge **200.**

[00099] In an embodiment, fluidic ports on cartridge **200** dock with spring-loaded pogo pins **1415** to connect fluids, gases, or vacuum to cartridge **200** on cartridge insertion. In another embodiment, pogo pins **1415** or canula **1416** are moved to connect with cartridge **200** after cartridge insertion. In another embodiment, canula **1416** connected to fluidic lines from syringe pump **2130** are held rigidly attached to the thermal transfer plate **1470** or other part of instrument and cartridge **200** has flexible materials on cartridge ports that seal with the canula(s) **1416** after cartridge insertion, as described below. Cartridge ports are ports opening out of a cartridge. A cartridge port may communicate directly with a chamber by being a port in

the chamber, or indirectly, *e.g.*, through another chamber comprising the port and communicating with the chamber in question.

F. Magnetic Processing Module

[000100] The embodiment of the single-sample Tissue Processing System **2010** shown in **Figure 6** has a Magnetic processing Module **900** and magnet **910** is moved by magnetic actuator **935** mounted on inverted 'U' shaped structural frame **1020** under control of control software **725** using controller **2122**. Magnet **910** can be far from cartridge **200** as shown in **Figure 9** and not interact with any magnetic beads **685** in cartridge **200** or in an extended position magnet **910** is moved to be near cartridge **200** for magnetic capture and processing of magnetic beads **685**. Many embodiments of configurations of the geometric relationship of the Magnetic processing Module **900** and magnet **910** and cartridge 2**00** are possible.

[000101] Referring to **Figure 7**, in an embodiment, the Single-Sample Singulator System **2000** has a back structural frame **1021** on structural frame **1020** that mounts electronics **710** comprising rotary motor controller **2122**, z-axis stepper controller **2112**, 24 V to 5 V step down power supply **2230** and 24 V to 12 V step down power supply **2225**. Power can be supplied to single-sample Tissue processing System **2010** by plugging a 24 V power supply into plug **762** connecting to fuse **761** and power switch **760.** Six way valves **2140** and **2141** are controlled by boards **2210** and **2212**. Reagent Peltier relay board **2240** can control reagent Peltier.

[000102] Systems that process one or more cartridges simultaneously are within the scope of the present invention. The cartridge **200** can have one or more processing Chamber(s) **440** and none, one, or more Post-Processing Chamber(s) **460** as well as none, one or more other chambers such as cartridge waste chamber **435** or vacuum trap chamber **468**.

[000103] In one embodiment, illustrated in **Figure 8**, cap **210,** alternatively referred to as a tissue disruptor, is placed on top of processing chamber **440** after specimen **101** is added into processing chamber **440** of cartridge **200**. After cartridge **200** is inserted into the instrument, pogo pins **1415,** canula **1416,** or other fluidic connectors can connect with none, one, or more of cartridge ports **470** to supply reagents to processing chamber **440**, first post-processing port **485** to supply reagents or vacuum to post-processing chamber **460**, cartridge vacuum trap port **467** to supply vacuum to vacuum trap chamber **468,** or cartridge waste port **2355** to supply vacuum or reagents to cartridge waste line **2351**.

[000104] An embodiment of cartridge **200** for processing tissue specimen **150** illustrated in **Figure 8** fluidically connects processing chamber **440** to post-processing chamber **460** using fluidic line **453**, which can be tubing, connecting from processing chamber nipple **471** to port **452** (connected through nipple **454)** positioned over a strainer **2711**inserted into post-processing chamber **460**. In other embodiments, no strainer can be used or strainer **2711** can be incorporated as an in-line filter, for example in a Swinny filter holder attached to the output of

processing chamber **440** or in fluidic line **453** or attached to lid **462.** In an embodiment, dual or triple or more filters are used in strainer **2711**, for example, a 145 micron filter followed by a 40 micron filter followed by a 20 micron filter; other combinations are envisioned.

[000105] Lid **462** produces a vacuum tight seal of post-processing chamber **460** and vacuum trap chamber **468** when cap **465** is sealed on lid **462.** Lid **462** can be attached to cartridge body **201** by ultrasonic welding, glue, epoxy, adhesives, and other methods to produce a vacuum tight seal . The permanent attachment of lid **462** ensures single usage of cartridge **200** to eliminate cross sample contamination by preventing changing of strainer **2711**.

[000106] In some embodiments, cartridge **200** can have on-cartridge valves which can be pinch valves on fluidic lines such as fluidic line **453** which the instrument actuates to open and close lines, or by using a 'T' junction and two lines, route fluids down different paths such as to on cartridge waste or to an a optics imaging system **520**, or to multi'omics processing of another workflow or analysis method. In another embodiment, fluidic lines such as fluidic line **453** can be partially closed to create a variable orifice that can disrupt partially dissociated tissue. Actuators can open and pinch close tubing in the cartridge **200**, or operate the variable orifice using variable orifice device when desired. In other embodiments, cartridge **200** can have on-cartridge valves which can be miniaturized pneumatic valves, or microvalves. In some embodiments, microfluidics or microchips are used for fluidic lines. In an embodiment there are no valves on the cartridge **200,** with all fluidic control coming from the instrument.

[000107] Referring to **Figure 8A**, **Figure** 13 and **Figure** 14, when vacuum is applied to vacuum trap port **467** or to first post-processing port **485**, liquids including single cell suspensions **1000**, nuclei **1050**, and other subcellular components **1060**, and biomolecules **1070** are pulled from processing chamber **440** through fluidic line **453** and strainer **2711** into strain drain **451** and into output collector region **461** of post-processing chamber **460**. Strainer **2711** can have pore sizes no more than any of 2, 5, 10, 15, 20, 25, 30, 40, 50, 70, 100, 125, 200 µm, or larger to filter the suspension of biological material. Multiple in-line or stacked strainers **2711** can be employed to successively remove different sized components of the dissociated tissue specimen **110**. Cap **210** with cap coupler **211,** and head **218** (also referred to as "rotor" **253**) is shown ready to be inserted into sample inlet port **425**. Head **218** can have a surface for disrupting tissue that can comprise raised features **355** that aid in mechanically disrupting a tissue, organ, microtissue **6001**, organoid **6002** or other biological material.

[000108] Referring to **Figure 8B** and **Figure 8C**, the cap coupler (also referred to as "drive head") **211** is held inside cap sheath **212** which in one embodiment has cap sheath hole **214**. Cap coupler **211** is attached to cap shaft **216** which passes through cap sheath hole **214** and is attached to the head **218** which can be a rotor **353** with grinding teeth **355**. The assembly of cap coupler **211** attached to cap shaft **216** and head **218** are referred to as a plunger assembly which is a type of moveable mechanical tissue disruptor **345**.

[000109] Referring to **Figure 18A**, in a preferred embodiment, head **218** attached to cap shaft **216** has a outwardly annular beveled head feature **356** designed to improve centricity of head **218** inside processing chamber **440** and thereby the uniformity of side gap **221** at the bottom of travel. When z-axis stepper motor **2110** lowers and cap coupler **211** is pushed down by rotary motor coupler **2125**, head **218** will lower until outwardly annular beveled feature **356** engages with inwardly annular beveled preprocessor chamber feature **357** on the inside wall of processing chamber **440** to produce a centered head **218** as shown in **Figure 18B**. The centering of head **218** will produce a uniform side gap **221** between head **218** and the inner wall of processing chamber **440**. In addition, if the height of head **211** is less than the height of the processing chamber **440** below inwardly beveled feature **357,** the engagement of outwardly annular beveled head feature **356** with inwardly annular beveled preprocessor chamber feature **357** will set a uniform bottom gap **222**. The size of the side gap and the bottom gap can be optimized for different cell types or for different sized nuclei or subcellular organelles, or multicellular structures such as intestinal crypts. In addition, to allow passage of disrupted tissue when head **218** is seated on inwardly annular beveled preprocessor chamber feature **357,** the inwardly annular beveled preprocessor chamber feature **357**can be fluted to have sections with the same or different depths. The side gap **221** between the head **218** of moveable mechanical disruptor **345** and the inside wall is preferably greater than or equal to any of 1 μm, or 2 μm, or 5 μm, or 10 μm, or 15 μm, or 20 μm, or 25 μm, or 30 μm, or 40 μm, or 50 μm, or 75 μm, or 100 μm, or 150 μm, or 200 μm, or 250 μm, or 500 μm, and 1000 μm or more, as well as any size in between. In a preferred embodiment, the side gap 221 is greater than 50 microns and less than 150 microns for nuclei and other subcellular organelles and is greater than 75 microns and less than 250 microns for cells. A gap size for isolation of nuclei can be, for example, between about 30 microns and about 200 microns, e.g., about 40 microns and about 150 microns, or about 100 μm to about 125 μm. A gap size for isolation of cells can be, for example, between about 50 μm and about 400 μm, e.g., about 200 μm to about 300 μm, or about 250 μm. The bottom gap **222** between the bottom of head **218** of moveable mechanical disruptor **345** and the bottom of processing chamber **440** is preferably greater than or equal to 1 μm, or 2 μm, or 5 μm, or 10 μm, or 15 μm, or 20 μm, or 25 μm, or 30 μm, or 40 μm, or 50 μm, or 75 μm, or 100 μm, or 150 μm, or 200 μm, or 250 μm, or 500 μm, and 1000 μm or more, as well as any size in between. In some embodiments, different heads can be selected to be used with the same diameter processing chamber **440** to produce different side gaps **221** or bottom gaps **222** to simplify manufacturing and inventory management requirements. A bottom gap between a flat surface of the head and the flat bottom surface of the processing chamber can also be limited by the position of the flutes, or half domes, or other structures that prevent or define gaps between a flat surface of the head and the flat bottom surface of the processing chamber.

[000110] Referring to **Figure 19**, none, one, or more of the ports to cartridge **200** can have flexible or low durometer port covers **442**, for example without limitation 40 to 100 durometer. As illustrated in **Figure 19A** and in cutout **Figure 19B**, port cover **442** can be inserted into the space between the port and port cover retaining cylinder **441** to secure the port cover **442** in place over, for example as shown, reagent addition port **470**. A fluidic canula **1416** or fluidic pogo pin **1415** with an outside diameter larger than port cover center hole **446** can engage the port covered by port cover **442** and, because of the relatively low durometer, the port cover **442** will be deformed by fluidic canula **1416** or fluidic pogo pin **1415** to create a seal around the fluidic canula **1416** or fluidic pogo pin **1415**. In some configurations, the deformation can be used to eliminate the need for springs and the use of the fluidic pogo pin **1415** can be replaced by a non-movable fluidic canula **1416**. **Figure 19C** shows port cover **442** retained by crimp seal **443. Figure 19D** shows port cover **442** retained by forming port cover retaining cylinder **441** higher than the port cover **442** and melting the port cover retaining cylinder **441** to form a heat stake lip **444** that retains port cover **442.**

[000111] In a preferred embodiment the Single Sample Singulator Instrument **2050** has an actuator for mechanical processing that has a stepper motor **2110** that controls the vertical position of rotary motor **2120** and rotary motor shaft **2121** attached to rotary motor coupler **2125** that in turn can mechanically couples with cap coupler **211** of the cap **210** when inserted into cartridge **200.** The coupler can have a drive head that takes any appropriate form, such as a slot, a Phillips head, a quadrex, atri-wing, a spanner or a hex. Rotary motor coupler **2125** has one or more facets that reversibly engage cap coupler **211** by actions such as moving downward and slowly rotating. As shown in **Figure 20 A**, in a preferred embodiment, rotary motor coupler **2125** has a single blade to engage cap coupler **211** as stepper motor **2110** lowers, the rotary motor coupler **2125** attached to rotary motor shaft **2121** engages cap coupler **211** in cap **210** and if the rotary motor coupler **2125** is not lined up with cap coupler groove **217,** the rotary motor coupler **2125** can not directly insert into the cap coupler groove **217**. In a preferred embodiment, cap coupler **211** has two surfaces on either side of cap coupler groove **217** which slope in opposite directions across the cap coupler **211** such that each side has a higher and lower wall on either side of cap coupler groove **217.** When rotary motor shaft **2121** turns in the clockwise direction (looking from above), rotary motor coupler **2125** blade spins in the clockwise direction and encounters the high side of the wall of cap coupler groove **217** and begins to rotate cap coupler **211** clockwise. As stepper motor **2110** lowers, the rotary motor coupler **2125** will engage the cap coupler groove **217**, as shown in **Figure 20 C.** As shown in **Figure 20 D,** as stepper motor **2110** continues to lower, the rotary motor **2120** and rotary motor shaft **2121** attached to rotary motor coupler **2125** will lower, pushing on cap coupler groove **217** and the cap coupler **211** will compress cap spring **213** against the bottom of cap sheath **212** and lower head **218**. As shown in **Figure 20 E**, head **218** can be lowered close to or in contact with the bottom of processing chamber **440**, which can be a stator **354,** and head **218** can be rotated

to disrupt tissue. When stepper motor **2110** raises, rotary motor **2120** and rotary motor coupler **2125** raise up and cap spring **213** decompresses to push cap coupler **211** against rotary motor coupler **2125** to continue engagement.

[000112] In another embodiment of the Single Sample Singulator Instrument **2050**, stepper motor **2110** controls the vertical position of rotary motor **2120** which is magnetically coupled to moveable disruptor **345** with a magnetic or paramagnetic element embedded with cap **210** as part of cap coupler **211** or as part of moveable disruptor **345** or head **218.**

[000113] When rotary motor coupler **2125** is engaged with cap coupler **211** by mechanical coupling, magnetic coupling, pneumatic, or fluidic coupling, or other coupling methods, and rotary motor **2120** rotates, moveable disruptor **325** and head **218** are rotated. Stepper motor **2110** controls the vertical position of the rotary motor **2120** and thereby the vertical position of rotary motor coupler **2125,** to raise or lower moveable disruptor **345** and head **218** in processing chamber **440.** Combining rotation of rotary motor **2120** and movement of stepper motor **2110** enables many patterns of motion of moveable tissue disruptor **345** and head **218.**

[000114] The inside walls of processing chamber **440** can be embodied in many different shapes. The inside walls of processing chamber **440** can be fluted to have sections with different depths. In a preferred embodiment, the inside wall can have a circular profile with the largest gap between the head **218** of moveable mechanical tissue disruptor **345** and the inside wall of preferably greater than or equal to any of 1 μm, or 2 μm, or 5 μm, or 10 μm, or 15 μm, or 20 μm, or 25 μm, or 30 μm, or 40 μm, or 50 μm, or 75 μm, or 100 μm, or 150 μm, or 200 μm , or 250 μm, or 500 μm, and 1000 μm or more, as well as any size in between.

[000115] Moveable tissue disruptor **345** can be embodied in many different shapes with many different profiles. In one embodiment, moveable tissue disruptor **345** can have a head **218** which is a rotor **353** with optional features, for example, grinding teeth **355** on the bottom of rotor **353** and grinding teeth **355** on stator **354** which is on the top surface of the bottom of the processing chamber **440** to assist in disruption of large pieces of tissue specimens **120** into smaller pieces or assist in the dissociation into single cells **1000** or nuclei **1050** or biomolecules **1070**. As shown in **Figures 8** and **18**, the sides of head **218** can be a cylinder to create an inside gap **221** with the inside wall over the length of the cylinder. By raising and lowering head **218** without turning head **218**, thereby using it as a moveable disruptor **345,** the system can process specimen **101** by trituration. In another embodiment the sides of the head **218** can form a ball-like structure to create a gap with the inside wall in a small area and the bottom of processing chamber **440** can be rounded to match the ball-like structure to create a Dounce-like mechanical tissue disruptor **345**. In other embodiments, multiple regions with gaps of the same or different sizes can be created by varying the side profile of moveable tissue disruptor **345** and the inner wall of processing chamber **440**. In other embodiments, the stator can be stationary or movable (e.g., rotating). In other embodiments, the stator is non-porous. In other

embodiments, either or both of the processing chamber or the rotor can have a circular or non-circular cross-section. In the case of a non-circular cross-section, the rotor is configured to rotate within the processing chamber. In its rotation, a portion of a wall of the rotor will have a gap between the rotor and the processing chamber of between about **150** and **250** microns.

V. Methods of Use

[000116] Methods of isolating dissociated single cells, nuclei or other subcellular organelles from preserved tissue can proceed as follows. A first part of the process involves processing preserved tissue samples to remove preservatives and, typically, rehydrate the tissue, and optionally to further treat the deparaffinized, rehydrated sample with enzymatic processing, or heat processing or chemical processing. A second part of the process involves disrupting the rehydrated tissue to release individual cells, nuclei, and/or subcellular organelles. These two parts can be performed in different cartridges. The first part can be performed in a cartridge adapted for processing preserved tissue samples, as described herein. The second part can be performed in a cartridge adapted for dissociating tissue samples, also as described herein.

A. Processing Preserved Tissue Samples

1. Removal of Preservatives

[000117] A preserved tissue sample, such as an FFPE sample or an OTC sample is placed into the bottom of the processing chamber of a preserved tissue processing cartridge. The cartridge is engaged with the cartridge interface of the instrument. Fluidic connections from reagent containers are checked to confirm proper seating. Xylene, or another appropriate organic solvent, is moved from a reagent container, through fluidic lines and a cartridge interface connector, into the processing chamber through the second processing chamber port **2570**. The rotor is pumped up and down to facilitate dissolving of preservatives. After sufficient time, the actuation system presses the rotor until it encounters the tissue sample. This pushes spent liquid reagents above the top of the rotor. In one embodiment the rotor is lowered to a position such that the top of the rotor is below first processing chamber port **2504** and is rotated to mix the solvent. or moves to a fixed position above the bottom of the chamber and below the first processing chamber port **2504**. As discussed herein, the gap between the side of the rotor and the internal wall of the processing chamber has been selected so that liquid can pass between the rotor and the wall but, tissue pieces or cells, nuclei, and/or subcellular organelles cannot. If the intention is to isolate cells, the gap can be of a size too small to pass cells, even if it is large enough to pass nuclei and other subcellular organelles. Using vacuum, applied at first post-processing port **2585**, liquid is pulled from first processing chamber port **2504** into the processing chamber. Liquids collecting at first post-processing port **2585** are then further moved, typically by vacuum, through the cartridge interface connector, and through fluidic lines into the waste container located, for example, in the caddy. In an alternative embodiment, liquid

is pulled through first processing chamber port **2504**, through fluidic conduit **2553**, and out a reagent port, *e.g.*, positioned at first post-processing port **2585**. This process can be repeated until sufficient preservatives are removed from the preserved tissue.

2. Rehydration of preserved tissue

[000118] Preserved tissue from which preservatives have been removed can now be rehydrated. In this process, one or more concentrated alcohol solutions are sequentially exchanged with less concentrated alcohol solutions and then with buffer solutions until the tissue is sufficiently rehydrated. This process can involve moving alcohol-containing solutions in the same manner into the processing chamber through the second processing chamber port. Again, the rotor can be pumped up and down to facilitate dissolving of preservatives. After sufficient time, the actuation system presses the rotor until it encounters the tissue sample. This pushes spent liquid reagents above the top of the rotor. After first incubation with an alcohol-containing solution, the rotor can be sufficiently depressed to push the spent solution to position above the top of the rotor.

[000119] In one embodiment the rotor is lowered to a position such that the top of the rotor is below first processing chamber port **2504** and is rotated to mix the solvent, or moves to a fixed position above the bottom of the chamber and below the first processing chamber port **2504**. As previously, liquid is pulled from the first processing chamber port out the reagent port, depending on configuration (and optionally through into the post-processing chamber), and into the waste container. This process is repeated with increasingly more dilute alcohol solutions until tissue is fully rehydrated.

[000120] The process can be continued with rehydration in a buffer, e.g., PBS, using the same processing methods of mixing with the rotor in a position that prevents pieces of tissue from being pulled out of the processing chamber.

3. Post-processing of deparaffinized and rehydrated tissues.

[000121] It may be desirable to further process the deparaffinized and rehydrated tissue in the preserved tissue cartridge. In some embodiments, an enzymatic solution is used to loosen the cells and nuclei in the tissue. In one embodiment, Liberase TH (Roche) can be added and incubated for 45 min at a low temperature, such as 8 °C, to post-process the tissue while minimizing degradation of nucleic acids. In other embodiments, heat can be applied to assist in antigen presentation. In another embodiment, buffers can be applied to postprocess the deparaffinized and rehydrated tissue.

B. Disruption of Tissue Samples to Release Cells, Nuclei, and/or Other Subcellular Organelles

[000122] Rehydrated tissue can now be subjected to disruption to release individual cells, nuclei, and/or other subcellular organelles. The process involves moving the rehydrated tissue sample from the preserved tissue processing cartridge into the processing chamber of the cartridge adapted for tissue dissociation.

[000123] Disruption of tissue can include a plurality of disruption steps, each involving positioning the head a different distance from floor of the chamber to produce gaps of different sizes. Typically, at each position, the head will rotate, further facilitating disruption or mixing. In certain embodiments, an organ can be auto-minced by the disruptor before tissue disruption into single cells **1000** or nuclei **1050** or other biological materials. Such a method can involve a first disruption step, which can include setting the head at a plurality of different distances from the floor of the chamber and rotating at each gap distance, to provide tissue with greater surface area and less distance for access by enzymes. A next step can involve incubating the auto-minced organ with enzymes or chemicals for tissue disruption into single cells **1000** or nuclei **1050**. A next step can involve a second disruption step, which, in turn, can include setting the head at a plurality of different distances from the floor of the chamber and rotating the head.

[000124] Processes described here can be performed using one or more computer systems that can be networked together. Calculations can be performed in a cloud computing system in which data on the host computer is communicated through the communications network to a cloud computer that performs computations and that communicates, or outputs, results to a user through a communications network. For example, nucleic acid sequencing can be performed on sequencing machines located at a user site. The resulting sequence data files can be transmitted to a cloud computing system where the sequence classification algorithm performs one or more operations of the methods described herein. At any step a cloud computing system can transmit results of calculations back to the computer operated by the user.

[000125] Data can be transmitted electronically, e.g., over the Internet. Electronic communication can be, for example, over any communications network include, for example, a high-speed transmission network including, without limitation, Digital Subscriber Line (DSL), Cable Modem, Fiber, Wireless, Satellite and, Broadband over Powerlines (BPL). Information can be transmitted to a modem for transmission, e.g., wireless or wired transmission, to a computer such as a desktop computer. Alternatively, reports can be transmitted to a mobile device. Reports may be accessible through a subscription program in which a user accesses a website which displays the report. Reports can be transmitted to a user interface device accessible by the user. The user interface device could be, for example, a personal computer, a laptop, a smart phone or a wearable device, *e.g.*, a watch, for example worn on the wrist.

VI. Computer Systems

[000126] Methods provided herein can be executed by programmable digital computer.

[000127] **Figure 10** shows an exemplary computer system. The computer system **9901** includes a central processing unit (CPU, also "processor" and "computer processor" herein) **9905**, which can be a single core or multi core processor, or a plurality of processors for parallel processing. The computer system **9901** also includes memory or memory location **9910** (e.g., random-access memory, read-only memory, flash memory), electronic storage unit **9915** (e.g., hard disk), communication interface **9920** (*e.g.*, network adapter) for communicating with one or more other systems, and peripheral devices **9925**, such as cache, other memory, data storage and/or electronic display adapters. The computer readable memory **9910**, storage unit **9915**, interface **9920** and peripheral devices **9925** are in communication with the CPU **9905** through a communication bus (solid lines), such as a motherboard. The storage unit **9915** can be a data storage unit (or data repository) for storing data. The computer system **9901** can be operatively coupled to a computer network ("network") **9930** with the aid of the communication interface **9920**. The network **9930** can be the Internet, an internet and/or extranet, or an intranet and/or extranet that is in communication with the Internet. The network **9930** in some cases is a telecommunication and/or data network. The network **9930** can include one or more computer servers, which can enable distributed computing, such as cloud computing.

[000128] The CPU **9905** can execute a sequence of machine-readable instructions, which can be embodied in a program or software (code). The instructions may be stored in a memory location, such as the computer readable memory **9910**. The instructions can be directed to the CPU **9905**, which can subsequently program or otherwise configure the CPU **9905** to implement methods of the present disclosure.

[000129] The storage unit **9915** can store files, such as drivers, libraries, and saved programs. The storage unit **9915** can store user data, e.g., user preferences, log files, video or other images, and user programs. The computer system **9901** in some cases can include one or more additional data storage units that are external to the computer system **9901**, such as located on a remote server that is in communication with the computer system **9901** through an intranet or the Internet.

[000130] The computer system **9901** can communicate with one or more remote computer systems through the network **9930**.

[000131] Methods as described herein can be implemented by way of machine (*e.g.,* computer processor) executable code stored on an electronic storage location of the computer system **9901**, such as, for example, on the computer readable memory **9910** or electronic storage unit **9915**. The machine executable or machine-readable code can be provided in the form of software. During use, the code can be executed by the processor **9905**. In some

cases, the code can be retrieved from the storage unit **9915** and stored on the memory **9910** for ready access by the processor **9905**. In some situations, the electronic storage unit **9915** can be precluded, and machine-executable instructions are stored on memory **9910**. The code can be used to communicate and issue instructions to electronic devices, e.g., circuit boards **9940**, modules, or subsystems, on the instrument ,for example, the rotary DC motor relay board **2134** or the heater relay board **2240** driving Peltier **1440** to accomplish tasks such as rotating a motor or controlling the temperature of the cartridge **200**.

[000132] Machine-executable code can be stored on an electronic storage unit, such as memory (*e.g.,* read-only memory, random-access memory, flash memory) or a hard disk. "Storage" type media can include any or all of the tangible memory of the computers, processors or the like, or associated modules thereof, such as various semiconductor memories, tape drives, disk drives and the like, which may provide non-transitory storage at any time for the software programming. All or portions of the software may at times be communicated through the Internet or various other telecommunication networks.

[000133] The computer system **9901** can include or be in communication with an electronic display **9935** that comprises a user interface (UI) **9940** for providing, for example, input parameters for methods described herein. Examples of UIs include, without limitation, a graphical user interface (GUI) and web-based user interface.

EXEMPLARY EMBODIMENTS

[000134] 1. A cartridge for processing preserved tissue, comprising:

(a) a processing chamber comprising: (i) a stator, (ii) a side wall, (iii) a top orifice, (iv) a first processing chamber port positioned in the side wall, and (v) an optional second processing chamber port positioned in the side wall; and

(b) a plunger assembly positioned in the processing chamber through the top orifice, wherein the plunger assembly comprises a rotor, optionally attached to a plunger, the rotor comprising a face facing the stator; wherein the rotor is rotatable and movable toward and away from the stator; and wherein the rotor has a circumference such that a space between the rotor and the side wall prevents passage of pieces of tissue, cells, nuclei, and other sub-cellular organelles between the rotor and the side wall.

[000135] 2. The cartridge of embodiment 1, wherein the plunger assembly comprises a plunger attached to the rotor.

[000136] 3. The cartridge of embodiment 1, wherein the space between the rotor and the side wall is no more than any of 200 μm, 100 μm, 50 μm, and 25 μm.

[000137] 4. The cartridge of embodiment 1, wherein:

(i) the first processing chamber port is positioned in the side wall of the processing chamber, such that, when the rotor is fully depressed in the processing chamber, at least part of the first processing chamber port is positioned above a top of the rotor.

[000138] 5. The cartridge of embodiment 4, further comprising:

(c) a post-processing chamber comprising an inlet, a side wall, a floor, and a first post-processing port positioned in the side wall, and, optionally, wherein the inlet is positioned in a cover covering an orifice of the post-processing chamber;

wherein:

(i) the post-processing chamber is in fluidic communication with the first processing chamber port through a fluidic conduit; and

(ii) the first post-processing port is positioned to drain fluid that collects in the post-processing chamber.

[000139] 6. The cartridge of embodiment 5, wherein the floor of the post-processing chamber is slanted.

[000140] 7. The cartridge of embodiment 4, further comprising:

(c) a first post-processing port in fluidic communication with the first processing chamber port through a fluidic conduit, and configured to engage a cartridge interface connector (e.g., in the form of a cannula) of a cartridge interface of an instrument.

[000141] 8. An instrument comprising:

(i) at least one cartridge interface configured to engage a cartridge of any of embodiments 1-7, wherein the cartridge interface comprises at least a first cartridge interface connector configured to engage a first post-processing port in the cartridge that is fluidically connected, directly or indirectly, to the first processing chamber port;

(ii) a reagent subsystem comprising at least a waste container; and

(iii) a fluidic subsystem comprising:

(1) one or more fluid lines connecting the waste container to the first cartridge interface connector (e.g., in the form of a cannula); and sources of pressure configured to move liquids from the processing chamber, through the first post-processing port and the first cartridge interface connector, and into the waste container.

[000142] 9. The instrument of embodiment 8, further comprising:

(iv) a mechanical subsystem comprising an actuator that engages the plunger assembly, and one or more motors that move the actuator in an up-down

(Z axis) direction of the processing chamber, and rotates the actuator around the Z axis.

[000143] 10. The instrument of embodiment 9, further comprising:

(v) a control subsystem comprising a digital computer comprising a processor and memory, wherein the memory comprises code that, when executed by the processor, instructs the system to perform one or more operations.

[000144] 11. A kit comprising:

(I) a first cartridge for processing preserved tissue comprising:

(a) a processing chamber comprising: (i) a stator, (ii) a side wall, (iii) a top orifice, (iv) a first processing chamber port positioned in the side wall, and (v) an optional second processing chamber port positioned in the side wall; and

(b) a plunger assembly positioned in the processing chamber through the top orifice, wherein the plunger assembly comprises a rotor, optionally attached to a plunger, the rotor comprising a face facing the stator; wherein the rotor is rotatable and movable toward and away from the stator; and wherein the rotor has a circumference such that a space between the rotor and the side wall prevents passage of pieces of tissue, cells, nuclei, and other sub-cellular organelles between the rotor and the side wall and, optionally, wherein a face of the rotor does not comprise grinding features; wherein:

(i) the first processing chamber port is positioned in the side wall of the processing chamber, such that, when the rotor is fully depressed in the processing chamber, at least part of the first processing chamber port is positioned above a top of the rotor; and

(c) a first post-processing port fluidically connected to the first processing chamber port, e.g., through a fluidic conduit;

(d) wherein the cartridge optionally comprises a post-processing chamber comprising an inlet, a side wall, a floor, wherein the first post-processing port is positioned in the side wall, and, optionally, a cover covering an orifice in the post-processing chamber and comprising the inlet; wherein:

(i) the post-processing chamber is in fluidic communication with the first processing chamber port through the fluidic conduit; and

(ii) the first post-processing port is positioned to drain fluid that collects in the post-processing chamber; and

(II) a second cartridge for dissociating cells, nuclei and/or other sub-cellular organelles from tissue comprising:

(a) a processing chamber comprising: (i) a stator comprising grinding features, (ii) a side wall, (iii) a top orifice, (iv) a first processing chamber port positioned in the side wall; and (v) an optional second processing chamber port positioned in the side wall; and

(b) a plunger assembly positioned in the processing chamber through the top orifice, wherein the plunger assembly comprises a rotor, optionally attached to a plunger, the rotor comprising a face facing the stator, wherein the face comprises grinding features, optionally wherein the rotor has a circumference such that when the rotor is inserted into the processing chamber a gap between the walls of the processing chamber and the rotor is large enough to allow passage of cells, nuclei and/or other subcellular organelles between the rotor and the chamber wall;

(c) a post-processing chamber comprising (i) a floor, (ii) a side wall, (iii) an inlet, and (iv) a first post-processing port positioned in the side wall, and (v) an optional second post-processing chamber port; and

(d) a cartridge pressure port configured to apply negative pressure to the processing chamber through the post-processing chamber, directly or through a vacuum chamber;

wherein:

the first post-processing port of the first cartridge, and the first post-processing port of the second cartridge are configured to engage, separately, with the same first cartridge interface connector of a cartridge interface of an instrument, and, optionally, the second processing chamber port of the first cartridge in the second processing chamber port of the second cartridge are configured to engage separately with the same second cartridge interface connector of a cartridge interface of an instrument.

[000145] 12. The kit of embodiment 11, further comprising:

(a) an instrument comprising:

(i) at least one cartridge interface configured to engage the first cartridge and, separately, the second cartridge, wherein the cartridge interface comprises at least a first cartridge interface connector (e.g., in the form of a cannula) configured to engage the first post-processing port of the first cartridge, and the first post-processing port of the second cartridge;

(ii) a reagent subsystem comprising at least one reagent container containing a reagent and at least one waste container;

(iii) a fluidic subsystem comprising:

(1) at least one fluid line connecting the reagent container with the first cartridge interface connector (e.g., in the form of a cannula), and at

least one fluid line connecting the waste container to the first cartridge interface connector (e.g., in the form of a cannula); and

(2) one or more pressure sources configured to apply positive or negative pressure to move liquids and/or gasses through the one or more fluid lines;

(iv) a mechanical subsystem comprising an actuator, a linear driver (e.g., a stepper motor or a pneumatic driver) that drives the actuator in an up-down (Z axis) direction, and a rotary motor that rotates the actuator around the Z axis; and

(v) a control subsystem comprising a digital computer comprising a processor and memory, wherein the memory comprises code that, when executed by the processor, instructs the system to perform one or more operations; and

wherein, when the first or second cartridge is engaged with the cartridge interface:

(A) the first post-processing port engages the same first cartridge interface connector; and

(B) optionally, the second processing chamber port engages the same second cartridge interface connector.

[000146] 13. A method of isolating cells and/or subcellular organelles from preserved tissue comprising:

(a) providing a kit of embodiment 11 or 12, wherein the first cartridge is engaged with the cartridge interface, wherein the processing chamber of the first cartridge comprises a preserved tissue sample;

(b) processing the preserved tissue sample by:

(i) using the actuator to depress the rotor to push liquid to the level of the first processing chamber port;

(ii) using the fluidic subsystem, moving processing reagents into the processing chamber to remove preservative compounds from the preserved tissue ;

(iii) using the fluidic subsystem to move the liquid out of the processing chamber (e.g., through the first post-processing port (optionally through the post-processing chamber) and the first cartridge interface connector into a waste container fluidically connected therewith);

(iv) optionally, using the fluidic subsystem, moving rehydrating reagents into the processing chamber to rehydrate the preserved tissue; and

(v) using the fluidic subsystem, moving rehydrating reagents out of the processing chamber (e.g., through the first post-processing port (optionally through the post-processing chamber) and the first cartridge interface connector into a waste container fluidically connected therewith);

(c) moving the processed tissue from the first cartridge into the processing chamber of the second cartridge, and, engaging the second cartridge with one of the cartridge interfaces;

(d) dissociating cells and/or subcellular organelles from the processed tissue by:

(i) using the fluidic subsystem, moving tissue dissociation reagents into the processing chamber;

(ii) using the actuator to depress and rotate the rotor to release cells and/or nuclei from the processed tissue and to push liquid to move the released cells and/or subcellular organelles to the level of the first processing chamber port;

(iii) using the fluidic subsystem to move the cells and/or subcellular organelles from the processing chamber into the post-processing chamber; and

(iv) collecting the released cells and/or subcellular organelles from the post-processing chamber.

[000147] 14. A system for processing tissue comprising:

(a) an instrument comprising:

(i) at least one cartridge interface for engaging a cartridge, wherein the cartridge interface comprises at least first, second and third cartridge interface connectors configured to engage ports in an engaged cartridge;

(ii) a reversibly engageable processing reagent subsystem comprising one or more of:

(A) a caddy for holding a plurality of containers, wherein the carriage comprises a containment barrier configured to contain liquids inside the carriage,

(B) a plurality of containers held by the carriage, wherein the containers include at least one container containing a reagent for processing paraffinized tissue, and at least one waste container,

(C) a scale configured to measure the weight of the waste container;

(D) one or more optical sensors that senses liquid in the bottom of the caddy;

(E) a plurality of fluidic conduits connecting the containers with one or more couplings of a fluidic subsystem of the instrument, wherein the instrument comprises a sensing mechanism that senses when the fluidic conduits and couplings are properly connected; and properly seated;

(F) optionally a sensor that e.g., uses the Hall effect to sense the connection between the caddy and the rest of the instrument

(iii) a fluidics subsystem comprising:

(A) the one or more couplings connected to a plurality of fluidic conduits, wherein the fluidic conduits communicate with the cartridge interface connectors; and

(B) one or more pressure sources for moving reagents from the one or more containers through one or more of the cartridge interface connectors, and for moving liquids from cartridge interface connectors into the waste container.

[000148] 15. A method for releasing cells and/or subcellular organelles from tissue comprising:

(a) providing a cartridge comprising:

(1) a processing chamber comprising: (i) a stator, (ii) a side wall, (iii) a top orifice, and (iv) a first processing chamber port positioned in the side wall; and a second processing chamber port positioned in the side wall;

(2) a plunger assembly positioned in the processing chamber through the top orifice, wherein the plunger assembly comprises a plunger comprising a rotor, the rotor comprising a face facing the stator, wherein the face comprises grinding features; wherein the rotor is rotatable and movable toward and away from the stator; and wherein the plunger assembly is operatively connected to a pressure sensor configured to measure pressure against the face of the rotor;

(b) providing a tissue sample in the processing chamber;

(c) depressing the rotor in the processing chamber until the pressure sensor measures a target pressure consistent with contact between the face of the rotor and the tissue sample;

(d) rotating the rotor against the tissue sample while maintaining the target pressure, to dissociate cells and/or subcellular organelles from the tissue sample;

(e) stopping depression of the rotor when the pressure sensor measures a pressure consistent with the face of the rotor contacting the stator.

[000149] 16. A method of calibrating a plunger for cartridge comprising:

(a) while depressing a plunger comprising a shaft attached to a rotor, within a chamber of a cartridge, measuring and recording force on the plunger at different positions along the Z axis of the plunger and, determining a calibrated fully depressed position consistent with the rotor contacting a floor of the chamber.

[000150] 17. The method of embodiment 16, further comprising:

(b) while depressing the plunger within a chamber of the cartridge, determining a calibrated tissue engagement position consistent with the rotor contacting tissue in the chamber.

[000151] 18. The method of embodiment 16 or 17, further comprising:

(c) performing a dissociation protocol on tissue in the chamber wherein the protocol comprises depressing the plunger to the determined fully depressed position and/or the determined contacting tissue position.

[000152] 19. The method of embodiment 16 or 17, comprising using the calibrated fully depressed position and/or the determined contacting tissue position with a second, different cartridge.

[000153] As used herein, the following meanings apply unless otherwise specified. The words "can" and "may" are used in a permissive sense (i.e., meaning having the potential to), rather than the mandatory sense (i.e., meaning must). The words "include", "including", and "includes" and the like mean including, but not limited to. The singular forms "a," "an," and "the" include plural referents. Thus, for example, reference to "an element" includes a combination of two or more elements, notwithstanding use of other terms and phrases for one or more elements, such as "one or more." The phrase "at least one" includes "one", "one or more", "one or a plurality", and, therefore, contemplates the use of the term "a plurality". The term "or" is, unless indicated otherwise, non-exclusive, i.e., encompassing both "and" and "or." The term "any of' between a modifier and a sequence means that the modifier modifies each member of the sequence. So, for example, the phrase "at least any of 1, 2 or 3" means "at least 1, at least 2 or at least 3". The term "about" refers to a range that is 5% plus or minus from a stated numerical value within the context of the particular usage. The term "consisting essentially of" refers to the inclusion of recited elements and other elements that do not materially affect the basic and novel characteristics of a claimed combination.

[000154] It should be understood that the description and the drawings are not intended to limit the invention to the particular form disclosed, but to the contrary, the intention is to cover all modifications, equivalents, and alternatives falling within the spirit and scope of the present invention as defined by the appended claims. Further modifications and alternative embodiments of various aspects of the invention will be apparent to those skilled in the art in view of this description. Accordingly, this description and the drawings are to be construed as illustrative only and are for the purpose of teaching those skilled in the art the general manner of carrying out the invention. It is to be understood that the forms of the invention shown and described herein are to be taken as examples of embodiments. Elements and materials may be substituted for those illustrated and described herein, parts and processes may be reversed or omitted, and certain features of the invention may be utilized independently, all as would be apparent to one skilled in the art after having the benefit of this description of the invention. Changes may be made in the elements described herein without departing from the spirit and scope of the invention as described in the following claims.

[000155] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

WHAT IS CLAIMED IS:

1. A cartridge for processing preserved tissue, comprising:

(a) a processing chamber comprising: (i) a stator, (ii) a side wall, (iii) a top orifice, (iv) a first processing chamber port positioned in the side wall, and (v) an optional second processing chamber port positioned in the side wall; and

(b) a plunger assembly positioned in the processing chamber through the top orifice, wherein the plunger assembly comprises a rotor, optionally attached to a plunger, the rotor comprising a face facing the stator; wherein the rotor is rotatable and movable toward and away from the stator; and wherein the rotor has a circumference such that a space between the rotor and the side wall prevents passage of pieces of tissue, cells, nuclei, and other sub-cellular organelles between the rotor and the side wall.

2. The cartridge of claim **1**, wherein the plunger assembly comprises a plunger attached to the rotor.

3. The cartridge of claim **1**, wherein the space between the rotor and the side wall is no more than any of 200 μm, 100 μm, 50 μm, and 25 μm.

4. The cartridge of claim **1**, wherein:

(i) the first processing chamber port is positioned in the side wall of the processing chamber, such that, when the rotor is fully depressed in the processing chamber, at least part of the first processing chamber port is positioned above a top of the rotor.

5. The cartridge of claim **4**, further comprising:

(c) a post-processing chamber comprising an inlet, a side wall, a floor, and a first post-processing port positioned in the side wall, and, optionally, wherein the inlet is positioned in a cover covering an orifice of the post-processing chamber;

wherein:

(i) the post-processing chamber is in fluidic communication with the first processing chamber port through a fluidic conduit; and

(ii) the first post-processing port is positioned to drain fluid that collects in the post-processing chamber.

6. The cartridge of claim **5**, wherein the floor of the post-processing chamber is slanted.

7. The cartridge of claim **4**, further comprising:

(c) a first post-processing port in fluidic communication with the first processing chamber port through a fluidic conduit, and configured to engage a cartridge interface connector (e.g., in the form of a cannula) of a cartridge interface of an instrument.

8. An instrument comprising:

(i) at least one cartridge interface configured to engage a cartridge of any of claims **1-7**, wherein the cartridge interface comprises at least a first cartridge interface connector configured to engage a first post-processing port in the cartridge that is fluidically connected, directly or indirectly, to the first processing chamber port;

(ii) a reagent subsystem comprising at least a waste container; and

(iii) a fluidic subsystem comprising:

(1) one or more fluid lines connecting the waste container to the first cartridge interface connector (e.g., in the form of a cannula); and sources of pressure configured to move liquids from the processing chamber, through the first post-processing port and the first cartridge interface connector, and into the waste container.

9. The instrument of claim **8**, further comprising:

(iv) a mechanical subsystem comprising an actuator that engages the plunger assembly, and one or more motors that move the actuator in an up-down (Z axis) direction of the processing chamber, and rotates the actuator around the Z axis.

10. The instrument of claim **9**, further comprising:

(v) a control subsystem comprising a digital computer comprising a processor and memory, wherein the memory comprises code that, when executed by the processor, instructs the system to perform one or more operations.

11. A kit comprising:

(I) a first cartridge for processing preserved tissue comprising:

(a) a processing chamber comprising: (i) a stator, (ii) a side wall, (iii) a top orifice, (iv) a first processing chamber port positioned in the side wall, and (v) an optional second processing chamber port positioned in the side wall; and

(b) a plunger assembly positioned in the processing chamber through the top orifice, wherein the plunger assembly comprises a rotor, optionally attached to a plunger, the rotor comprising a face facing the stator; wherein the rotor is rotatable and movable toward and away from the stator; and wherein the rotor has a circumference such that a space between the rotor and the side wall prevents passage of pieces of tissue, cells, nuclei, and other sub-cellular organelles between the rotor and the side wall and, optionally, wherein a face of the rotor does not comprise grinding features; wherein:

(i) the first processing chamber port is positioned in the side wall of the processing chamber, such that, when the rotor is fully depressed in the

processing chamber, at least part of the first processing chamber port is positioned above a top of the rotor; and

(c) a first post-processing port fluidically connected to the first processing chamber port, e.g., through a fluidic conduit;

(d) wherein the cartridge optionally comprises a post-processing chamber comprising an inlet, a side wall, a floor, wherein the first post-processing port is positioned in the side wall, and, optionally, a cover covering an orifice in the post-processing chamber and comprising the inlet; wherein:

(i) the post-processing chamber is in fluidic communication with the first processing chamber port through the fluidic conduit; and

(ii) the first post-processing port is positioned to drain fluid that collects in the post-processing chamber; and

(II) a second cartridge for dissociating cells, nuclei and/or other sub-cellular organelles from tissue comprising:

(a) a processing chamber comprising: (i) a stator comprising grinding features, (ii) a side wall, (iii) a top orifice, (iv) a first processing chamber port positioned in the side wall; and (v) an optional second processing chamber port positioned in the side wall; and

(b) a plunger assembly positioned in the processing chamber through the top orifice, wherein the plunger assembly comprises a rotor, optionally attached to a plunger, the rotor comprising a face facing the stator, wherein the face comprises grinding features, optionally wherein the rotor has a circumference such that when the rotor is inserted into the processing chamber a gap between the walls of the processing chamber and the rotor is large enough to allow passage of cells, nuclei and/or other subcellular organelles between the rotor and the chamber wall;

(c) a post-processing chamber comprising (i) a floor, (ii) a side wall, (iii) an inlet, and (iv) a first post-processing port positioned in the side wall, and (v) an optional second post-processing chamber port; and

(d) a cartridge pressure port configured to apply negative pressure to the processing chamber through the post-processing chamber, directly or through a vacuum chamber;

wherein:

the first post-processing port of the first cartridge, and the first post-processing port of the second cartridge are configured to engage, separately, with the same first cartridge interface connector of a cartridge interface of an instrument, and, optionally, the second processing chamber port of the first cartridge in the second processing chamber port of the second cartridge are configured to

engage separately with the same second cartridge interface connector of a cartridge interface of an instrument.

12. The kit of claim **11**, further comprising:

(a) an instrument comprising:

(i) at least one cartridge interface configured to engage the first cartridge and, separately, the second cartridge, wherein the cartridge interface comprises at least a first cartridge interface connector (e.g., in the form of a cannula) configured to engage the first post-processing port of the first cartridge, and the first post-processing port of the second cartridge;

(ii) a reagent subsystem comprising at least one reagent container containing a reagent and at least one waste container;

(iii) a fluidic subsystem comprising:

(1) at least one fluid line connecting the reagent container with the first cartridge interface connector (e.g., in the form of a cannula), and at least one fluid line connecting the waste container to the first cartridge interface connector (e.g., in the form of a cannula); and

(2) one or more pressure sources configured to apply positive or negative pressure to move liquids and/or gasses through the one or more fluid lines;

(iv) a mechanical subsystem comprising an actuator, a linear driver (e.g., a stepper motor or a pneumatic driver) that drives the actuator in an up-down (Z axis) direction, and a rotary motor that rotates the actuator around the Z axis; and

(v) a control subsystem comprising a digital computer comprising a processor and memory, wherein the memory comprises code that, when executed by the processor, instructs the system to perform one or more operations; and

wherein, when the first or second cartridge is engaged with the cartridge interface:

(A) the first post-processing port engages the same first cartridge interface connector; and

(B) optionally, the second processing chamber port engages the same second cartridge interface connector.

13. A method of isolating cells and/or subcellular organelles from preserved tissue comprising:

(a) providing a kit of claim 11 or **12**, wherein the first cartridge is engaged with the cartridge interface, wherein the processing chamber of the first cartridge comprises a preserved tissue sample;

(b) processing the preserved tissue sample by:

(i) using the actuator to depress the rotor to push liquid to the level of the first processing chamber port;

(ii) using the fluidic subsystem, moving processing reagents into the processing chamber to remove preservative compounds from the preserved tissue;

(iii) using the fluidic subsystem to move the liquid out of the processing chamber (e.g., through the first post-processing port (optionally through the post-processing chamber) and the first cartridge interface connector into a waste container fluidically connected therewith);

(iv) optionally, using the fluidic subsystem, moving rehydrating reagents into the processing chamber to rehydrate the preserved tissue; and

(v) using the fluidic subsystem, moving rehydrating reagents out of the processing chamber (e.g., through the first post-processing port (optionally through the post-processing chamber) and the first cartridge interface connector into a waste container fluidically connected therewith);

(c) moving the processed tissue from the first cartridge into the processing chamber of the second cartridge, and, engaging the second cartridge with one of the cartridge interfaces;

(d) dissociating cells and/or subcellular organelles from the processed tissue by:

(i) using the fluidic subsystem, moving tissue dissociation reagents into the processing chamber;

(ii) using the actuator to depress and rotate the rotor to release cells and/or nuclei from the processed tissue and to push liquid to move the released cells and/or subcellular organelles to the level of the first processing chamber port;

(iii) using the fluidic subsystem to move the cells and/or subcellular organelles from the processing chamber into the post-processing chamber; and

(iv) collecting the released cells and/or subcellular organelles from the post-processing chamber.

14. A system for processing tissue comprising:

(a) an instrument comprising:

(i) at least one cartridge interface for engaging a cartridge, wherein the cartridge interface comprises at least first, second and third cartridge interface connectors configured to engage ports in an engaged cartridge;

(ii) a reversibly engageable processing reagent subsystem comprising one or more of:

(A) a caddy for holding a plurality of containers, wherein the carriage comprises a containment barrier configured to contain liquids inside the carriage,

(B) a plurality of containers held by the carriage, wherein the containers include at least one container containing a reagent for processing paraffinized tissue, and at least one waste container,

(C) a scale configured to measure the weight of the waste container;

(D) one or more optical sensors that senses liquid in the bottom of the caddy;

(E) a plurality of fluidic conduits connecting the containers with one or more couplings of a fluidic subsystem of the instrument, wherein the instrument comprises a sensing mechanism that senses when the fluidic conduits and couplings are properly connected; and properly seated

(F) optionally a sensor that e.g., uses the Hall effect to sense the connection between the caddy and the rest of the instrument

(iii) a fluidics subsystem comprising:

(A) the one or more couplings connected to a plurality of fluidic conduits, wherein the fluidic conduits communicate with the cartridge interface connectors; and

(B) one or more pressure sources for moving reagents from the one or more containers through one or more of the cartridge interface connectors, and for moving liquids from cartridge interface connectors into the waste container.

15. A method for releasing cells and/or subcellular organelles from tissue comprising:

(a) providing a cartridge comprising:

(1) a processing chamber comprising: (i) a stator, (ii) a side wall, (iii) a top orifice, and (iv) a first processing chamber port positioned in the side wall; and a second processing chamber port positioned in the side wall;

(2) a plunger assembly positioned in the processing chamber through the top orifice, wherein the plunger assembly comprises a plunger comprising a rotor, the rotor comprising a face facing the stator, wherein the face comprises grinding features; wherein the rotor is rotatable and movable toward and away from the stator; and wherein the plunger assembly is operatively connected to a pressure sensor configured to measure pressure against the face of the rotor;

(b) providing a tissue sample in the processing chamber;

(c) depressing the rotor in the processing chamber until the pressure sensor measures a target pressure consistent with contact between the face of the rotor and the tissue sample;

(d) rotating the rotor against the tissue sample while maintaining the target pressure, to dissociate cells and/or subcellular organelles from the tissue sample;

(e) stopping depression of the rotor when the pressure sensor measures a pressure consistent with the face of the rotor contacting the stator.

16. A method of calibrating a plunger for cartridge comprising:

(a) while depressing a plunger comprising a shaft attached to a rotor, within a chamber of a cartridge, measuring and recording force on the plunger at different positions along the Z axis of the plunger and, determining a calibrated fully depressed position consistent with the rotor contacting a floor of the chamber.

17. The method of claim 16, further comprising:

(b) while depressing the plunger within a chamber of the cartridge, determining a calibrated tissue engagement position consistent with the rotor contacting tissue in the chamber.

18. The method of claim 16 or **17**, further comprising:

(c) performing a dissociation protocol on tissue in the chamber wherein the protocol comprises depressing the plunger to the determined fully depressed position and/or the determined contacting tissue position.

19. The method of claim 16 or **17**, comprising using the calibrated fully depressed position and/or the determined contacting tissue position with a second, different cartridge.