

sample to detect CD8; **FIG. 9E** DAB- IHC analysis of the representative human lung sample to detect Ep-Cam.

FIGS. 10A, 10B and 10C illustrates an exemplary DAB ICC protocol, set forth in steps 1-102, for protein detection in representative samples. In this particular example, the protocol was used to detect Her2.

FIG. 11 provides an exemplary fluorescence ICC protocol (set forth in steps 1-38) for protein detection in representative samples.

FIG. 12 shows the detection of CD20, which demarcates B-cells, using automated DAB ICC in a representative sample prepared from a mixture of animal tissue and human tonsil specimens. CD20 was detected in cells from the human tonsil tissue contained in the representative sample.

FIGS. 13A and 13B show the detection of Her2-positive Calu-3 cells present in a representative sample prepared from tonsil tissue and a Her-2 positive xenograft tumor using fluorescence ICC. **FIG. 13A** illustrates a representative sample containing Calu-3 cells incubated with secondary antibody only (negative control). The background signal in Calu-3 cells generated by the secondary antibody is designated by the dashed line arrow. **FIG. 13B** illustrates a representative sample containing Calu-3 cells incubated with a Her2 antibody (4B5) prior to addition of the secondary antibody

FIGS. 14A, 14B, 14C, 14D and 14E provides an exemplary multiplex chromogenic ICC protocol (set forth in steps 1-225) for detection of multiple proteins in a representative sample.

FIG. 15 shows the detection of Ki-67, CD20, and CD3 using multiplex chromogenic IHC in the representative sample generated from human tonsil specimens.

FIG. 16 shows the detection of b-Raf-positive cells, present at a prevalence of about 0.015% in a representative tonsil sample.

FIG. 17 shows a 5x magnification of homogenized tonsil tissue. Note the scale bar indicating 50 microns in length.

FIGS 18A and 18B show images of the residual surgical material. **FIG. 18A** illustrates material from a colon resection that still contains an eight (8) cm colon adenocarcinoma ; **FIG. 18B** illustrates residual tissue from a partial nephrectomy of a kidney containing a papillary urothelial kidney tumor .

Fig. 30C utilizes DAPI. EpCAM positive cells correspond to cells with higher DNA content based on the DAPI intensity plot.

FIGS. 31A, and FIGS. 31B, 31C and 31D show tonsil dissociated cells sorting using magnetic beads. **FIG. 31A** shows that FSC-A vs. FSC-H was used for doublet discrimination. Fluorescent cells above certain threshold were used in the comparison. **FIG. 31B-31D** illustrates bar graphs showing the percentage of fluorescent cells (CD3 or CD8 positive cells) in the total cell population before and after depletion.

FIG. 32 shows sonication of multicellular aggregates. The sonication of multicellular aggregates (population size between 12 and 18 μ m) gives single cells (population size between 5.5 and 9.3 μ m). The size of the generated single cells corresponds to that of tumor cells.

FIG. 33A-33D show multicellular aggregates treated with collagenase then sonicated. **FIG. 33A** shows size distribution of collagenase treated cell aggregates with sonication; **FIG. 33B** shows size distribution of collagenase treated cell aggregates without sonication. **FIG. 33C** illustrates images of the samples displayed in **FIG. 33A**; **FIG. 33D** illustrates images of the samples displayed in **FIG. 33B**.

FIG. 34 shows RNA and DNA liberated from homogenates made from Representative samples (Rep) and fresh (Trad) tonsil tissue..

FIGS 35A-35D-35C show RNA stability in different storage conditions over 6 months. **FIG. 35A** illustrates that tissue from a pancreatic well-differentiated neuroendocrine neoplasm was incubated in standard cell storage solutions as needed; **FIG. 35B** illustrates that tissue from a papillary urothelial carcinoma was incubated in standard cell storage solutions as needed; **FIG. 35C** illustrates that tissue from a colon adenocarcinoma was incubated in standard cell storage solutions as indicated.

FIGS. 36A and 36B show protein stability in different storage conditions over 6 months. **FIG. 36A** illustrates that protein, as measured by IHC for C-met, is stable in papillary urothelial carcinoma at all storage conditions and temperatures; **FIG. 36B** illustrates that protein, as measured by IHC for C-met, is stable in colon adenocarcinoma at all storage conditions and temperatures.

antibody (Neg. control), and right panels show staining with anti-cytokeratin (red) primary antibodies. DAPI staining (blue) marks nuclei. 2 ms exposure was used for all images.

5 **FIG. 50** shows positive cytokeratin (CK) staining distinguishes tumor nuclei from normal. Histograms of flow cytometry data for nuclei isolated from a colon tumor (top panel) or a lung tumor (lower panel) representative samples.

FIGS. 51A-51C show cellular composition of tumors. **FIG 51A** illustrates a fraction of nuclei isolated from a colon adenocarcinoma (389); **FIG. 51B** illustrates a fraction of nuclei isolated from a lung squamous carcinoma (528) that
10 were designated normal vs. tumor using flow cytometry

FIG. 52 illustrates a graph showing quantification of the DNA yield from nuclei isolated from representative samples

FIGS. 53A and 53B are whole slide images of a histological section taken from an intact tonsil stained with a pan-keratin antibody. **FIG. ~~54A-53A~~** depicts a
15 traditional histological section of a normal tonsil detected by DAB for Pan-Keratin. **FIG. ~~54B-53B~~** depicts a section from a representative sample of tonsil detected by DAB for Pan-Keratin.

FIGS. ~~55A-54A~~ and ~~55B-54B~~ are images of samples of homogenized tonsil is embedded in paraffin and sectioned. **FIG. ~~55A-54A~~** depicts a traditional
20 histological section of a normal tonsil detected by DAB for CD8. **FIG. ~~55B-54B~~** depicts a section from a representative sample of tonsil detected by DAB for CD8.

FIG. ~~56-55~~ is a diagram of the workflow of the current disclosure

DETAILED DESCRIPTION

25 It is to be understood that the present disclosure is not limited to particular aspects described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only, and is not intended to be limiting, since the scope of the present disclosure will be limited only by the appended claims.

30 Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this technology belongs. Although any methods and materials similar