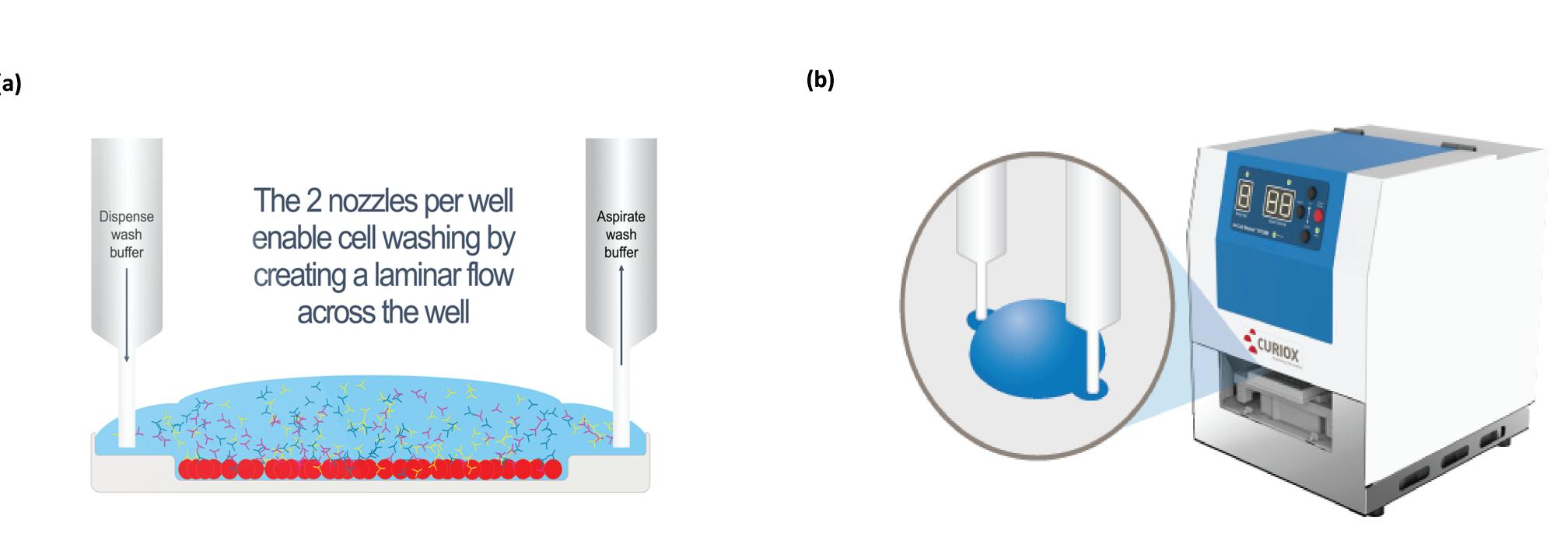


# Laminar Wash™ AUTO system: a reliable walk-away sample preparation solution for better TILs recovery without centrifugation

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#### CURIOX'S LAMINAR WASH TECHNOLOGY AND FULL AUTOMATION OF SAMPLE PREPARATION WITH LW™ AUTO1000



(c)

Fig 1 Curiox's centrifuge-less cell preparation platform is enabled by our wall-less plate and laminar flow washer.

(a) The Laminar Wash™ (LW) plate (96 well) or strip (16 well) consists of an array of hydrophilic spots surrounded by hydrophobic surface, which functions as a virtual wall that separates each spot. Each spot can process from a single cell to as many as 10 million cells without the mechanical stress and cell losses associated with centrifugation.

(b) HT1000 washes 96 wells in a fully automated process with individual nozzles for each well.

(c) The Laminar Wash™ AUTO 1000 software interface allows modifications to sample volume, buffer volume, antibody volume, number of washes, incubation time and temperature. In addition, it comes with pre-programmed protocols to ensure user consistency. User-friendly Graphical Interface (GUI): The software prompts the user to conveniently enter the parameters of existing SOPs or protocols with no coding or programming of the liquid handling system.

# LAMINAR WASH™ IMPROVES RETENTION and BETTER RETAINS ENDOGENOUS PROTEIN EXPRESSION

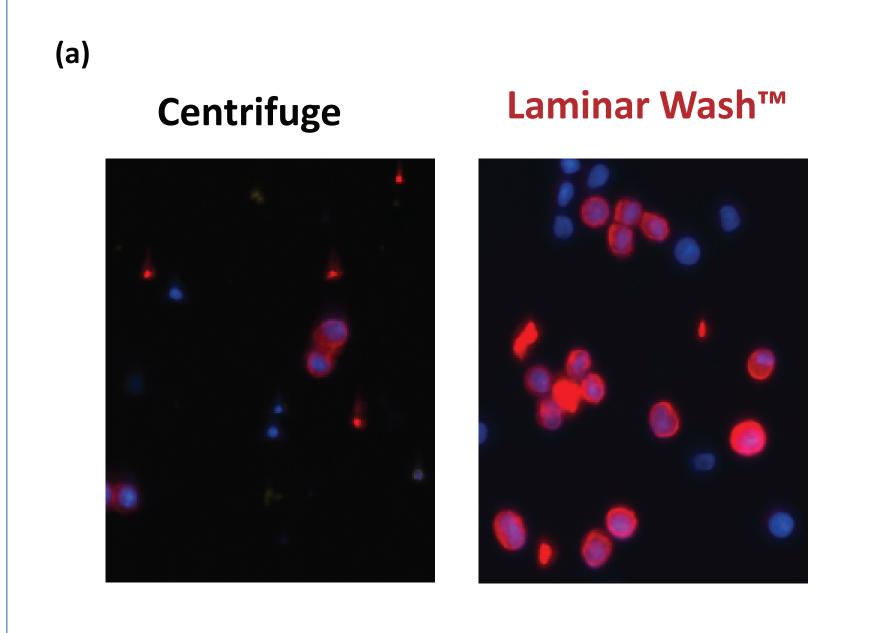


Fig 3(a) In processing of low cell numbers (50-100), LW retained higher cell count with better cellular morphology and brighter endogenous fluorescent expression. The dim protein expression is evidence of mechanic stresses caused by centrifugation.

Data from a biotech company in San Diego

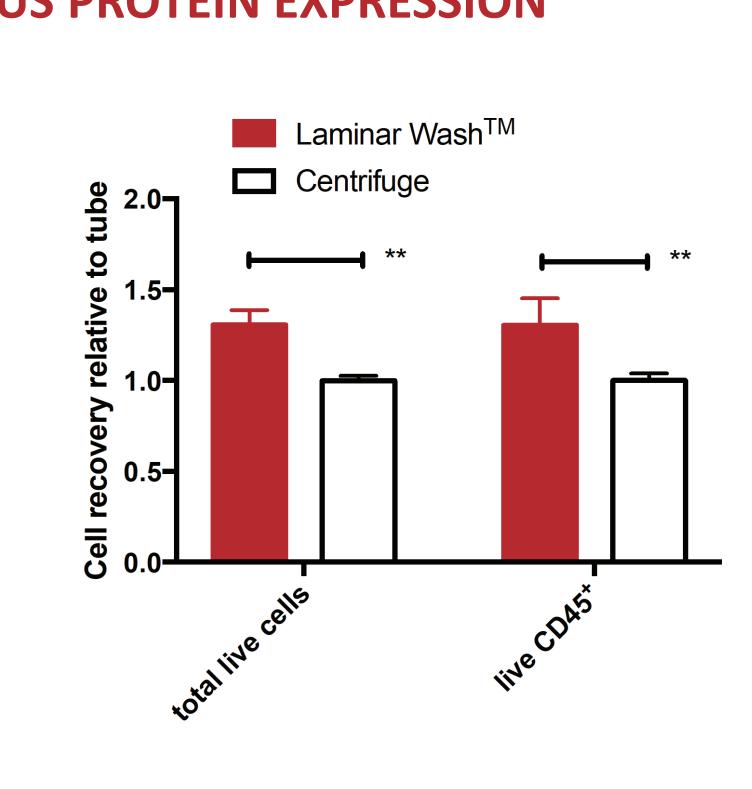


Fig 3(b) In subcutaneous murine melanoma tumor samples, LW retained 40% more live cells than centrifuge, with less tumor debris and better antibody staining.

### LAMINAR WASH™ INCREASES % LIVE CELLs for TILs and LEADS TO HIGHER hCD45+ POPULATION

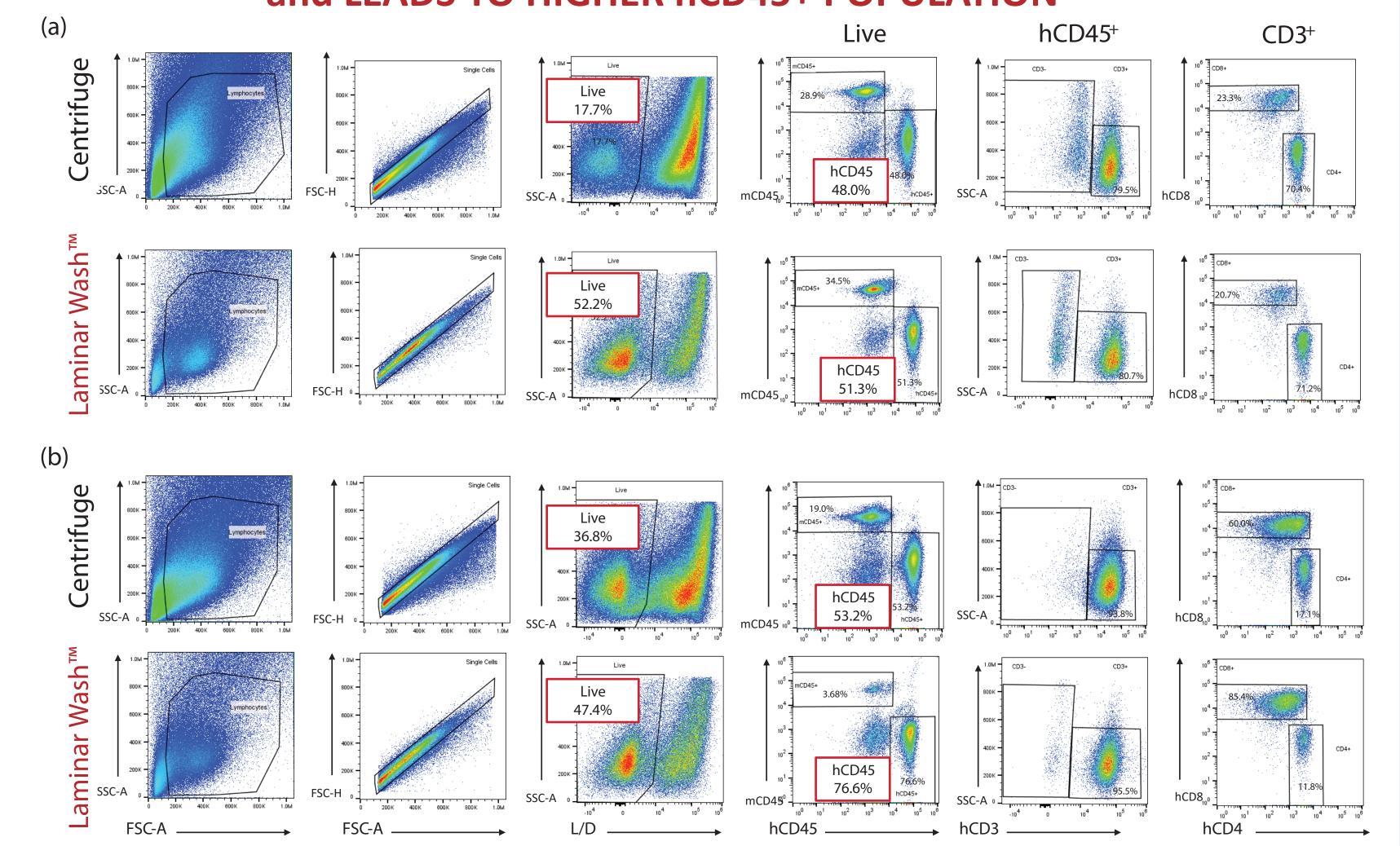


Fig 4 A549-inoculated humanized mouse (a) unchallenged tumors (b) challenged tumors

### LAMINAR WASH™ ENABLES ACCURATE IDENTIFICATION of TUMOR INFILTRATING LYMPHOCYTE (TIL) POPULATION

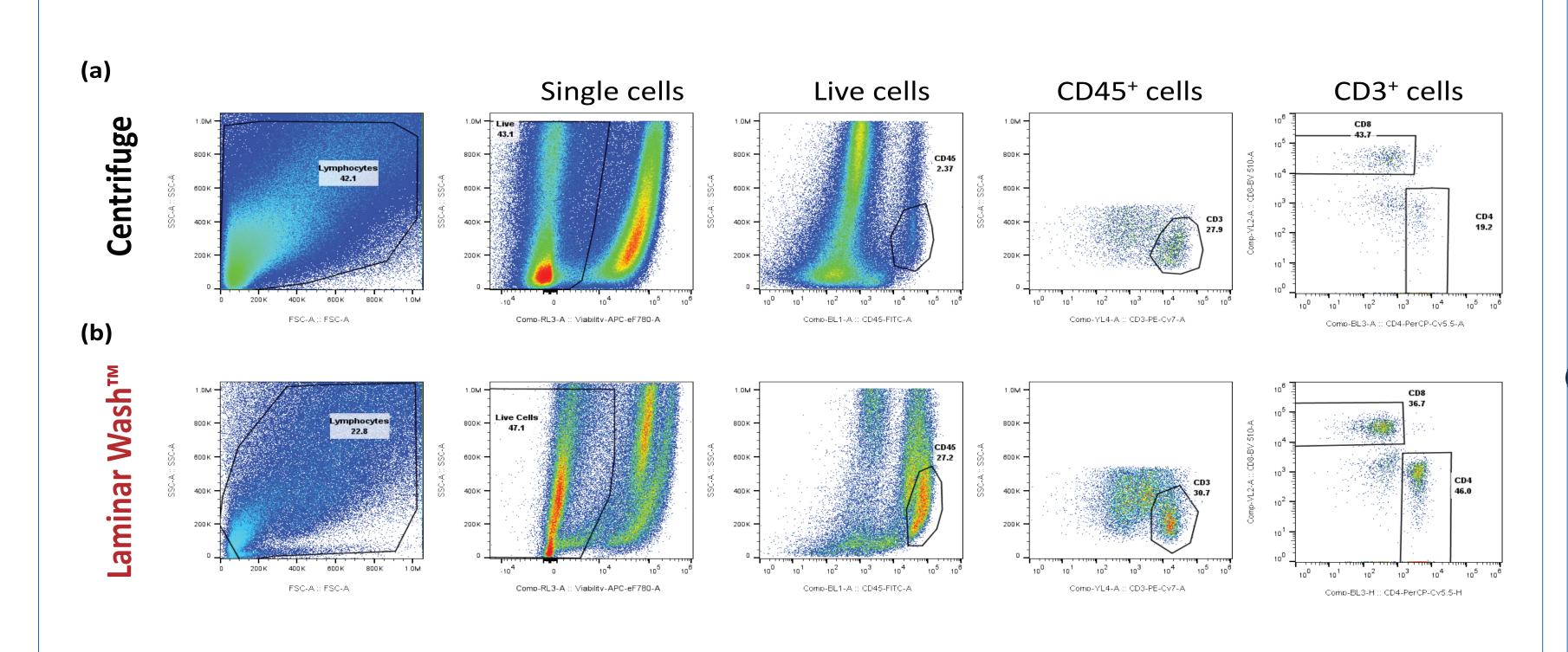
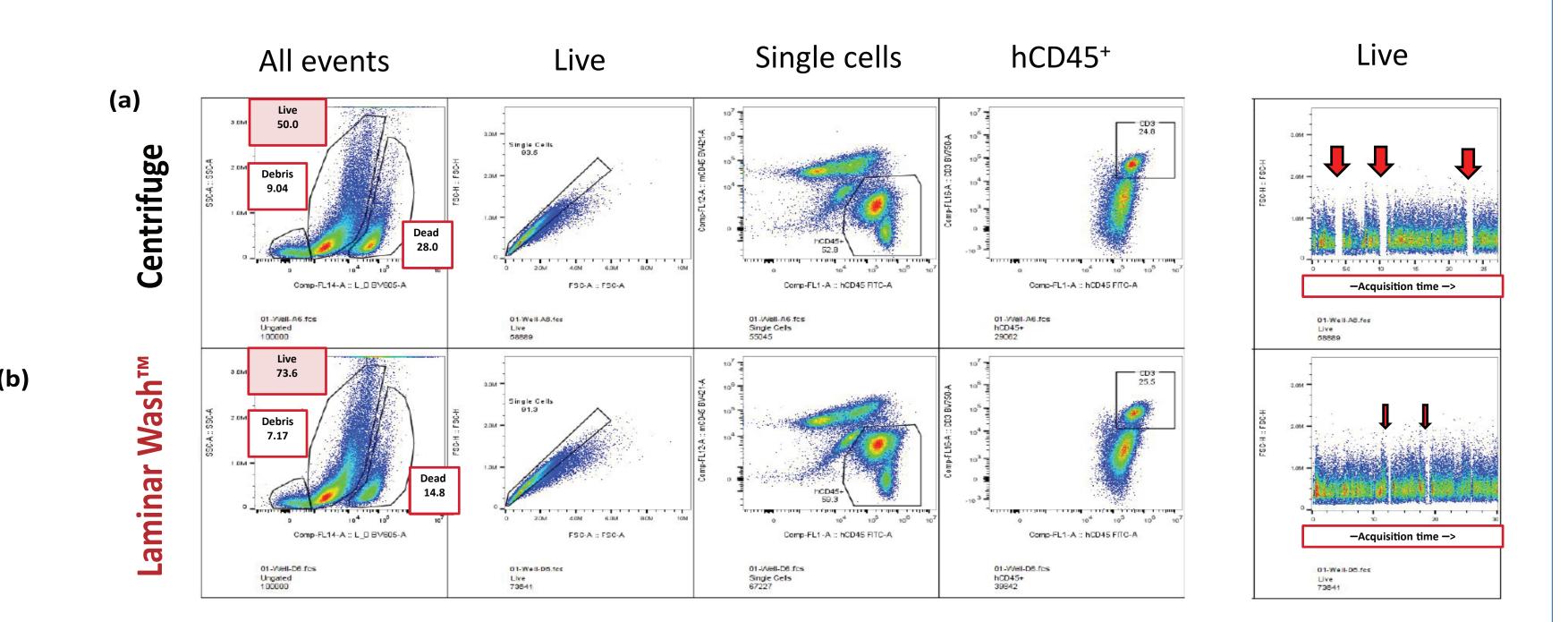


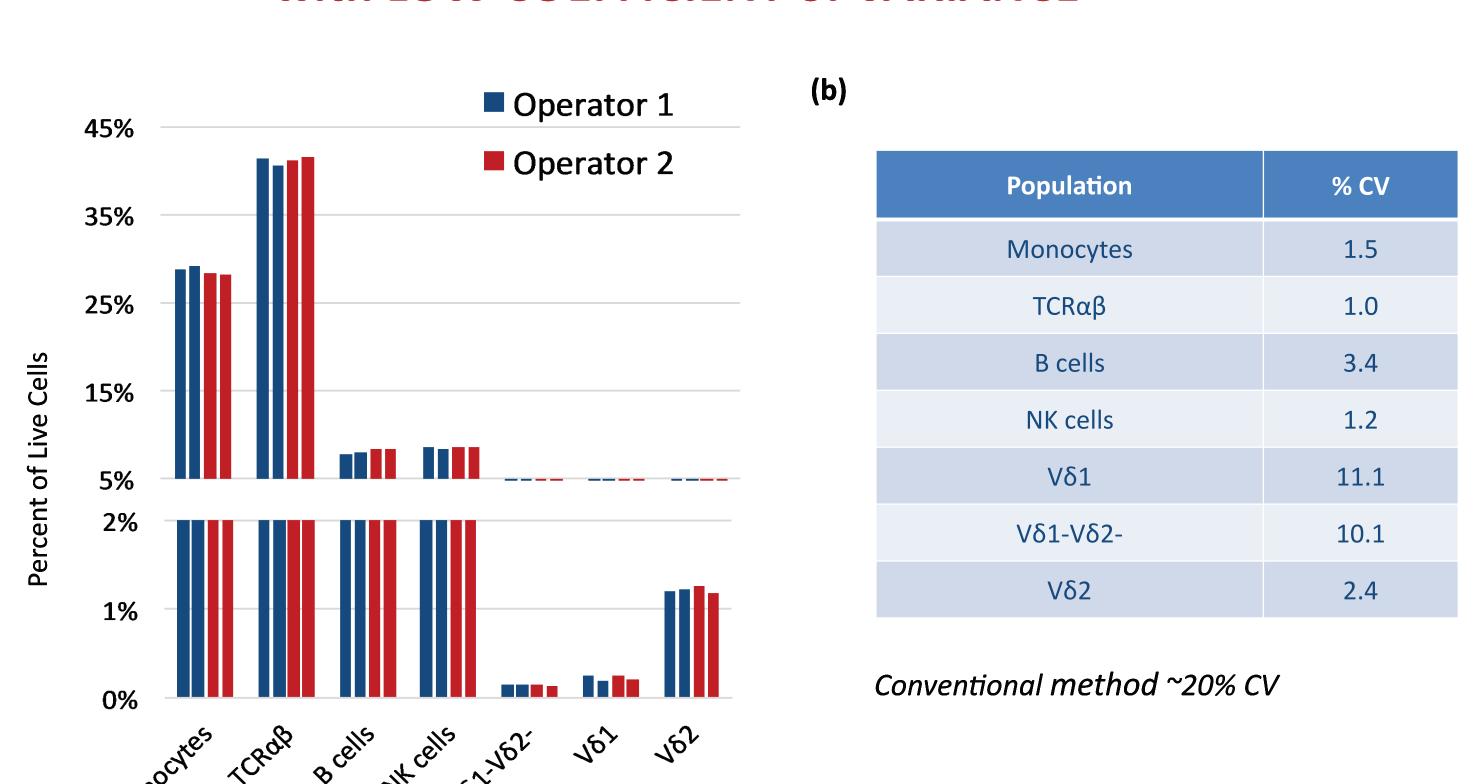
Fig 7(a) Centrifuge wash method – cell loss and mechanical stress through sequential pelleting and resuspension Fig 7(b) Laminar Wash™ method – less tumor debris, but higher retention of TILs and better resolution of populations

## LAMINAR WASH™ LEADS to IMPROVED CELL VIABILITY of TILs and LESS CYTOMETER CLOGGING



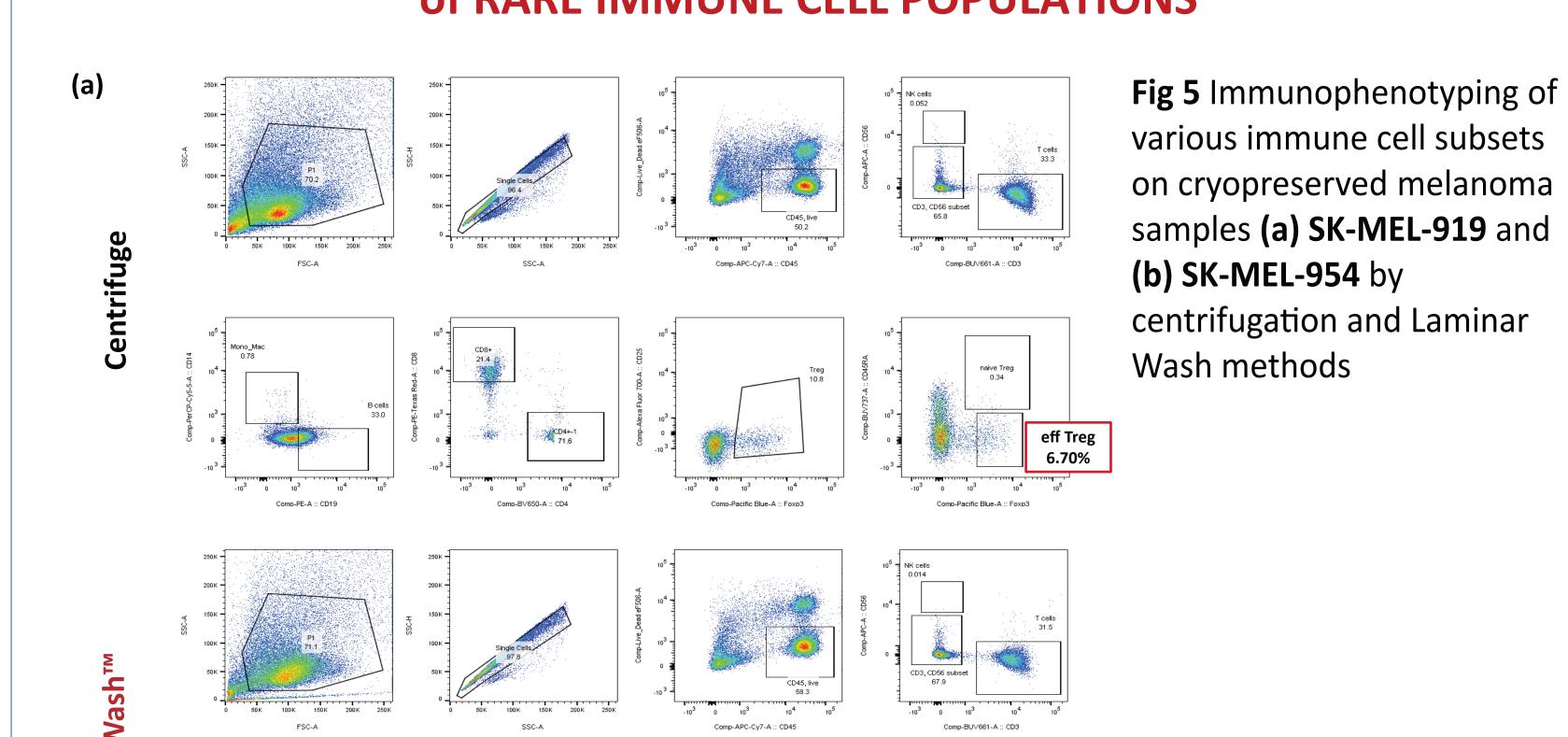
**Fig 8** FACS plots by **(a) centrifuge** vs. **(b) Laminar Wash™** methods. The dissociated tumor samples from Raji-inoculated Balb/c mice showed higher viability and increased debris removal with Laminar Wash™, leading to less clogging and more consistent reading.

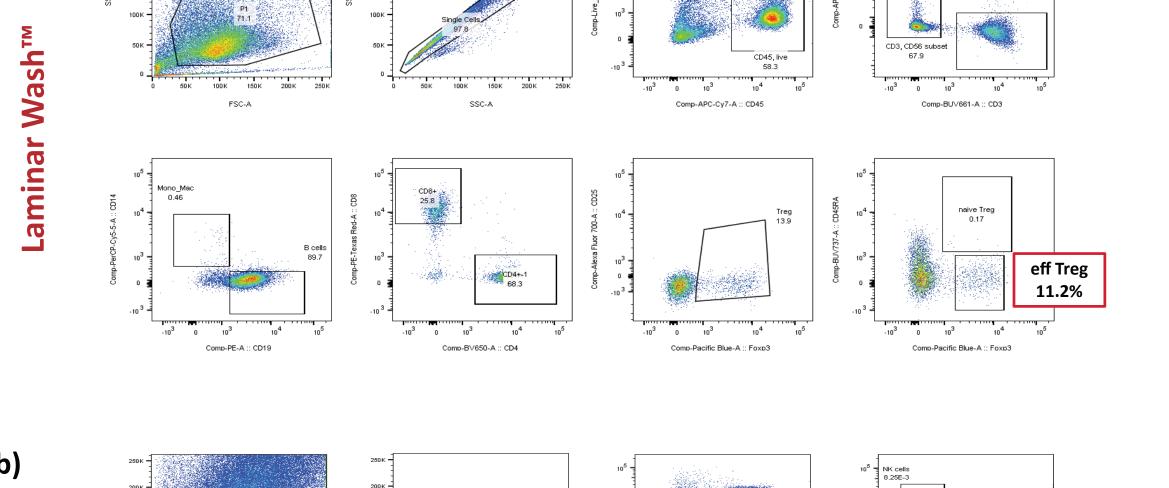
### AUTOMATED LAMINAR WASH™ PRODUCES CONSISTENT DATA with LOW COEFFICIENT of VARIANCE



**Fig 2** By using Laminar Wash™ in a single wash step during immunophenotyping of human PBMCs, the user achieved **(a)** consistency and **(b)** lower coefficient of variance even between different operators. *Webinar available from www.curiox.com* 

## LW<sup>TM</sup> LEADS TO MORE EFFECTIVE INTRACELLULAR STAINING of RARE IMMUNE CELL POPULATIONS





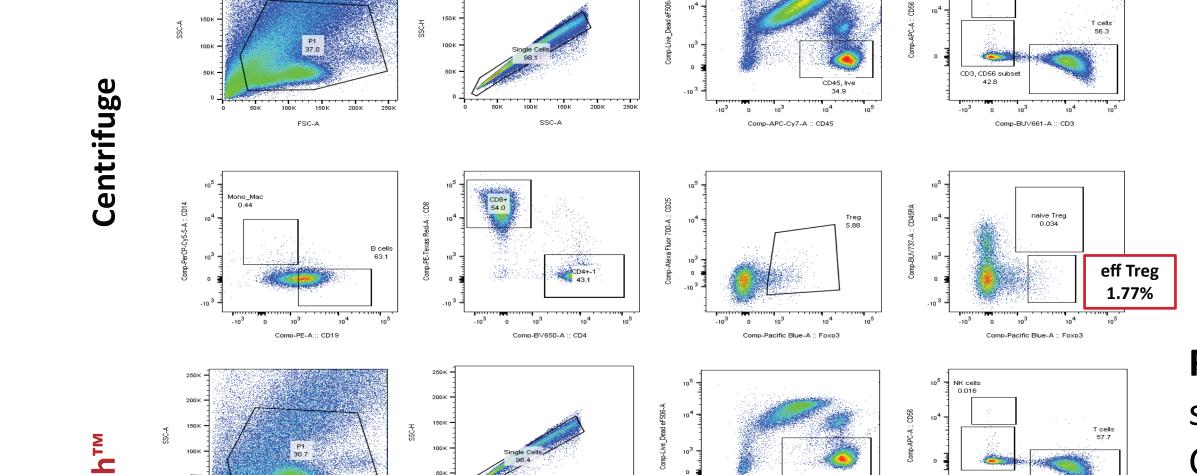
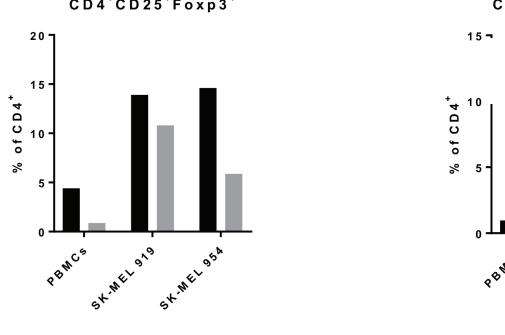
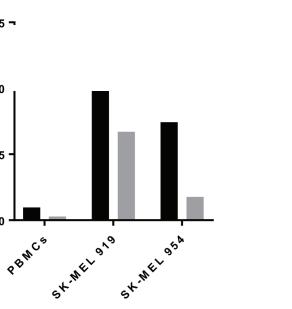
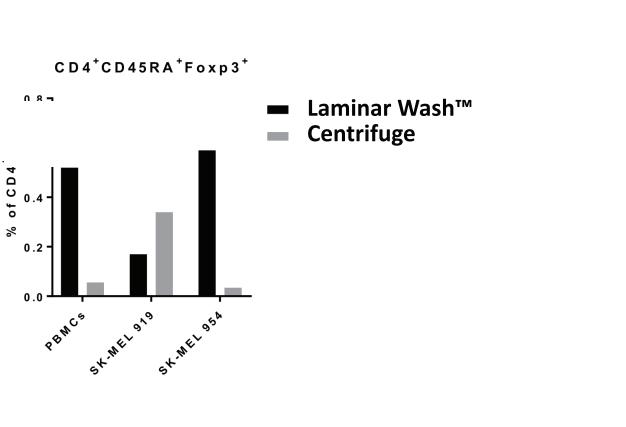


Fig 5(c) Percentages of CD4+ subsets: CD25+Foxp3+, CD45RA-Foxp3hi, and CD45RA+Foxp3+ cells in PBMCs and melanoma samples processed by Laminar Wash (black) vs. Centrifugation (grey) methods

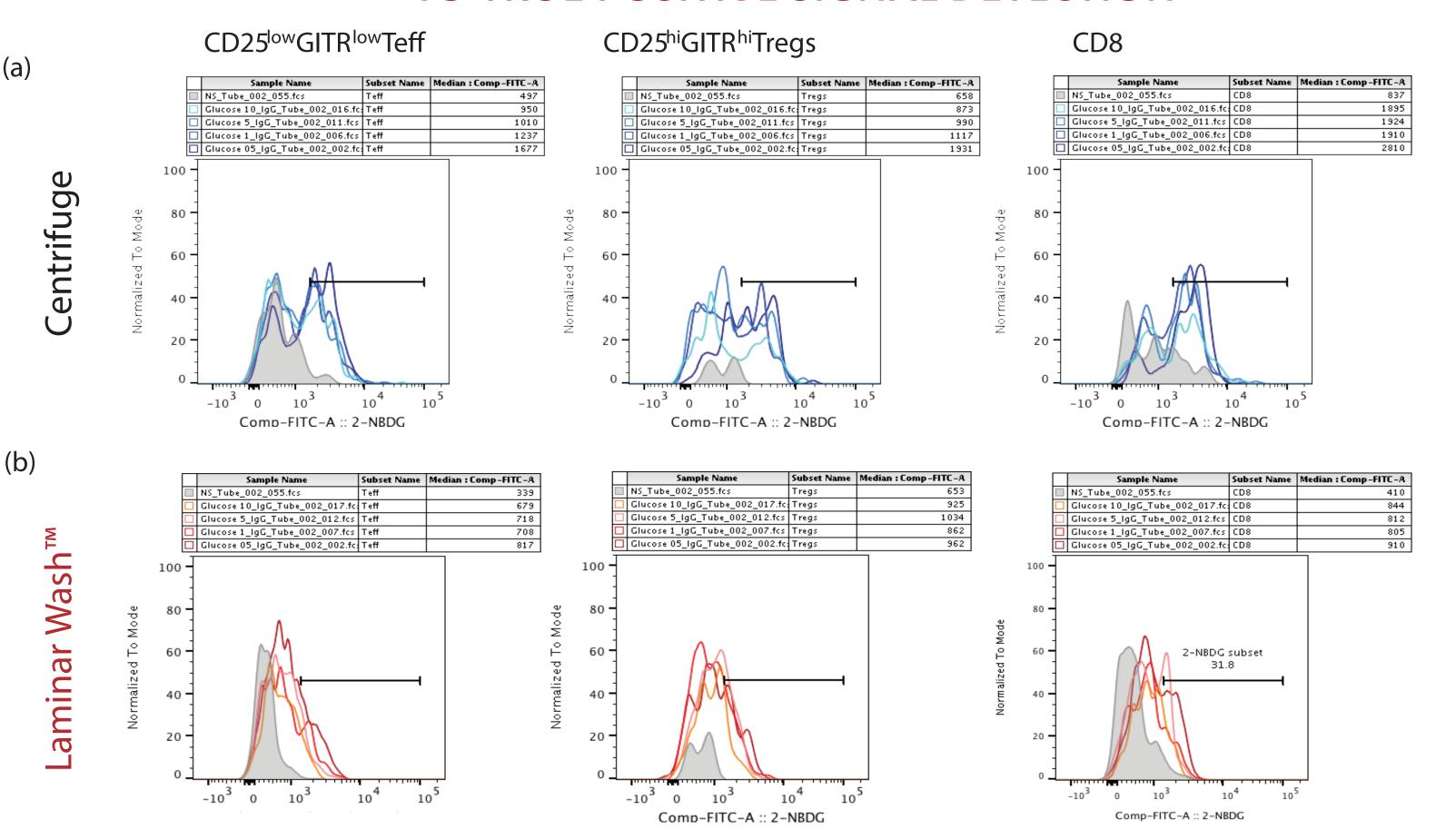






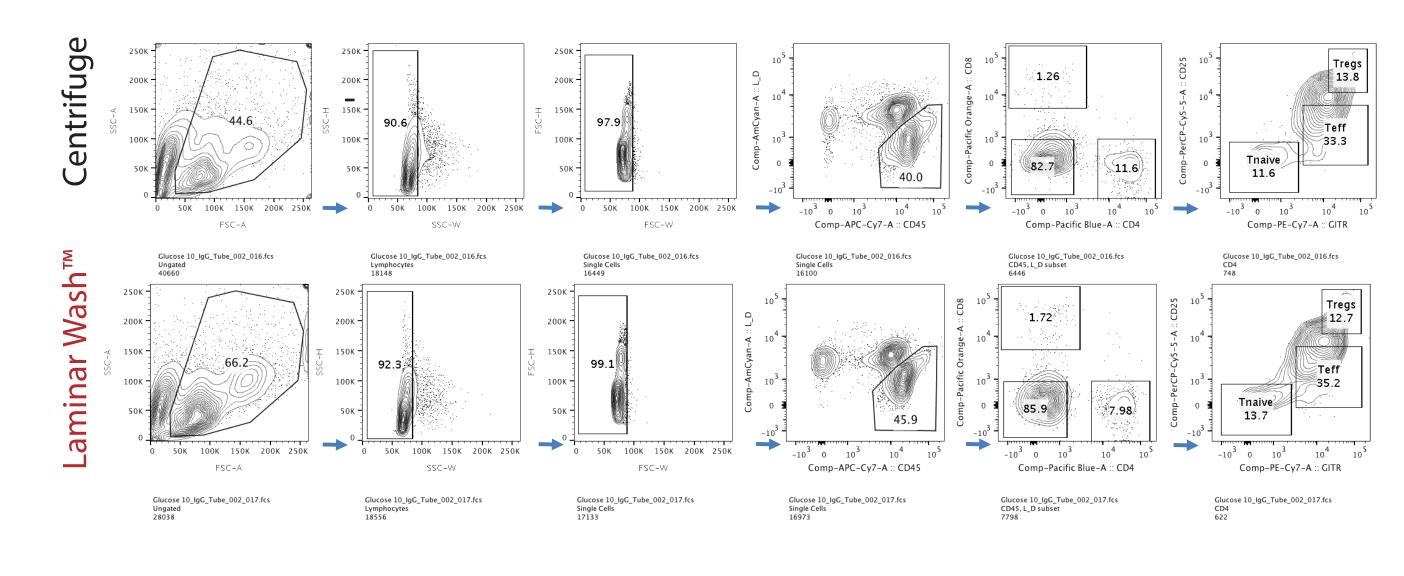
# Memorial Sloan Kettering Cancer Center

### LW<sup>™</sup> BETTER REMOVES STAINING BACKGROUND LEADING TO TRUE POSITIVE SIGNAL DETECTION



**Fig 6** Splenocytes from 4T1 bearing BALB/c activated for 24hrs with soluble aCD3 (0.5μg/ml) in the presence of glucose [0.5 10 mM]. Cultures were then washed and incubated with 2-NBDG (final concentration 100mM). Cells were washed by (a) Centrifuge vs. (b) Laminar Wash method. \*NS= non stimulated, but fully stained

#### GATING STRATEGIES for the 2-NBDG ASSAY



 After extensive washing, cells were stained with a panel of fluorochrome conjugated antibodies for surface markers to identify CD8 and CD4 effector T cells as well as Tregs

### CONCLUSIONS

#### Laminar Wash™

- 1. produces consistent data with low coefficient of variance through automation
- 2. improves retention and better retains endogenous protein expression
- 3. increases % live cells for TILs and leads to higher hCD45+ population
- 4. more effectively removes signal background leading to true positive signal detections
- 5. enables accurate identification of TILs
- 6. leads to more effective intracellular staining of rare immune cell populations

#### **ACKNOWLEDGEMENTS**

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