OPEN ACCESS

Technical Note

Vaccines and Immunology Open Access Journal

High-throughput microwell high-dimensional fluorescence activated Single cell sorting (HT-µW-HiD-FASCS)

Kartoosh Heydari

Director of Li Ka Shing Center FACS Core Facility, Department of Molecular Cell Biology / Cancer Research Laboratory, UC Berkeley, CA

Correspondence: Dr. Kartoosh Heydari, Director of Li Ka Shing Center FACS Core Facility, Department of Molecular Cell Biology / Cancer Research Laboratory, UC Berkeley, CA, Tel 6503877646, Email kartooshheydari@berkeley.edu

Received: February 03, 2018 | **Published:** February 15, 2018

High-throughput screening (HTS) and isolation of single cell, in clonal level, for biochemical, genomic, epigenomic, transcriptomic, proteomic, or metabolomic studies has beenrecently addressed by many scientific communities in field of Cancer Immuno-biology/-therapy and Regenerative Medicine.Fluorescence Activated Cell Sorting (FACS) has been the most innovative technology in life sciences and cell therapy since its invention in 1969 by my former postdoctoral advisers at Stanford University, ProfessorL. A. Herzenbergs, who received the Kyoto prize (Japanese equivalent to Nobel Prize) in Advanced Technology in 2006. Using theinnovative capacity of High-DimensionalFACS (HiD-FACS) and HiD-Fluorescence Activated Single Cell Sorting (HiD-FASCS), we have added a novel concept in cell biology and regenerative medicine fields by describing a novel cell subset with unique function we named "Privileged Cells".[1] We have now extended this innovative capacity of FASCS by {A} high-throughput(HT) single cell sorting (2000 wells/slid) in microWell (µW)(100 µm)(Figures 1–5 and Table 1), and {B} using high-resolution optical lens to improve the precision and the sensitivity of µW sort (Figure 4). HT-µW-HiD-FASCS is the most innovative technology for clonal level studies of the fine subsets of cells.

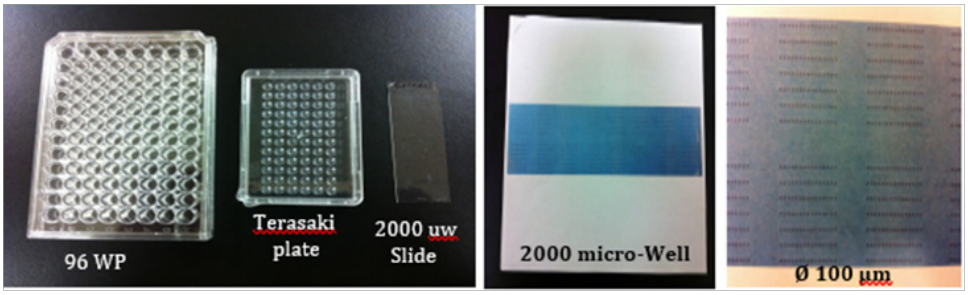

Figure 1 From High Throughput (96 well and Terasaki, 72 well) plate to HT (2000 micro-well) slide.

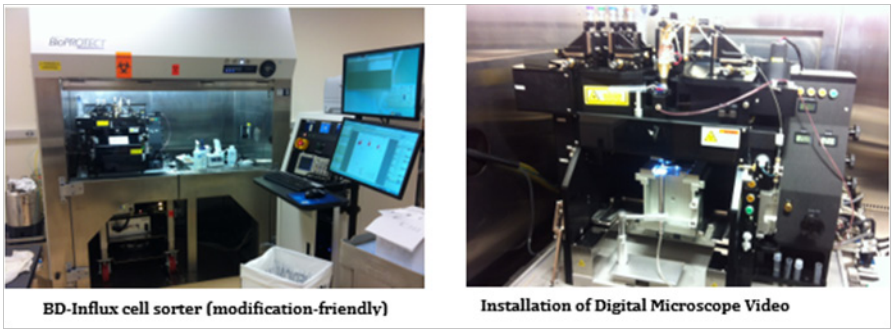

Figure 2 Development of High-Throughput (HT) microWell (µW) Fluorescence Activated Single Cell Sorting (FASCS).

Citation: Heydari K. High-throughput microwell high-dimensional fluorescence activated Single cell sorting (HT-µW-HiD-FASCS). *Vaccine Immunol Open Access J.* (2018);1(1):5–7. DOI: 10.30881/vioaj.00002

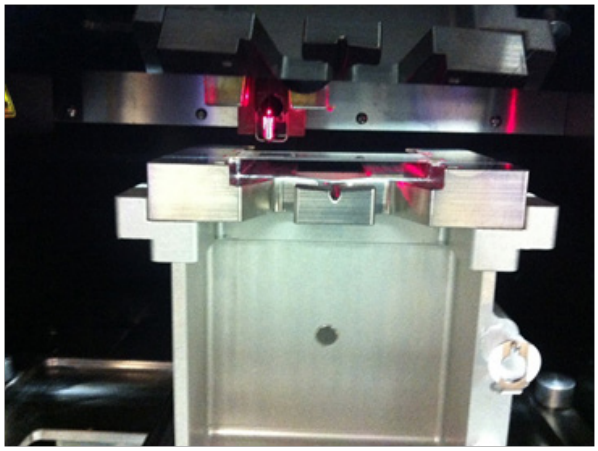

Figure 3 Increase accuracy of single cells deposition on slide by avoiding air currents in the flow chamber, creating a vertical trajectory, re-positioning the waste stream catcher off center, and positioning the slide closer to the sorter nozzle.

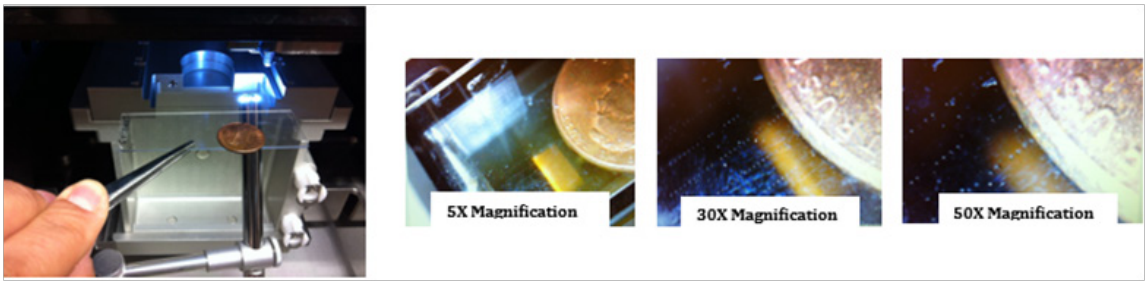

Figure 4 Direct verification of successful drop deposition by inverted microscope with high-resolution optical lens: Andonstar Digital Microscope Video. Magnification: +100X, DSP: High Power Digital Image Monarch Processor; Sensor: high-quality CMOS sensor, 2M pixel; Resolution: 640x480,1600x1200; Frame rate: 640x480 resolution=30 frames/sec,1600x1200=5frames/sec; Focal distance: 5mm–30mm.

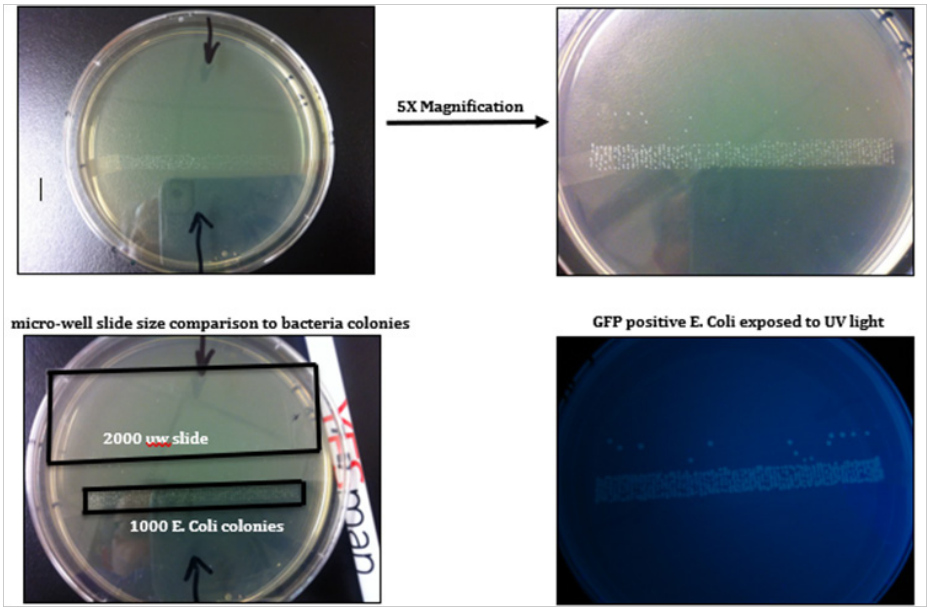

Figure 5 Single bacterial sorted on agar plate to verify the feasibility of HT-μW-HiD-FASCS.

Citation: Heydari K. High-throughput microwell high-dimensional fluorescence activated Single cell sorting (HT-μW-HiD-FASCS). *Vaccine Immunol Open Access J*. (2018);1(1):5–7. DOI: 10.30881/vioaj.00002

Table 1 Key Criteria for a successful optimization of sort setting for HT-μW-HiD-FASCS

Instrument setup	Sample Preparation
1. Nozzle choice / sheath pressure	1. Single cell suspension (2-5% protein, EDTA 2-5mM)
2. Laser alignment and delay (Calibration beads)	2. Samples with lots of dead cells (use DNAse, no EDTA)
3. Drop delay adjustment (Accudrop beads)	3. Match the cell concentration to the instrument setup and cell type
4. Gating strategy to gate out noise, debris and doublets	4. Stain cocktail should be fully worked up prior to sorting
5. Fsc / Ssc in log (cells & organelles < 1 um)	5. Proper single color control (beads better than cells)
6. Sort precision (single cell)	
7. Flow rate (for single cell <100 cell/s)	
8. Collection vessel targeting	
9. Sort Process	

References

1. Guo S, Zi X, Schulz VP, et al. Nonstochastic reprogramming from a privileged somatic cell state. *Cell*. 2014;156(4):649–662.

Citation: Heydari K. High-throughput microwell high-dimensional fluorescence activated Single cell sorting (HT-μW-HiD-FASCS). *Vaccine Immunol Open Access J*. (2018);1(1):5–7. DOI: 10.30881/vioaj.00002