

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

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High-throughput screening (HTS) and isolation of single cell, in clonal level, for biochemical, genomic, epigenomic, transcriptomic, proteomic, or metabolomic studies has been recently 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, Professor L. A. Herzenbergs, who received the Kyoto prize (Japanese equivalent to Nobel Prize) in Advanced Technology in 2006. Using the innovative capacity of High-Dimensional FACS (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 (μ W) (100 μ m) (Figures 1–5 and Table 1), and {B} using high-resolution optical lens to improve the precision and the sensitivity of μ W sort (Figure 4). HT- μ W-HiD-FASCS is the most innovative technology for clonal level studies of the fine subsets of cells.

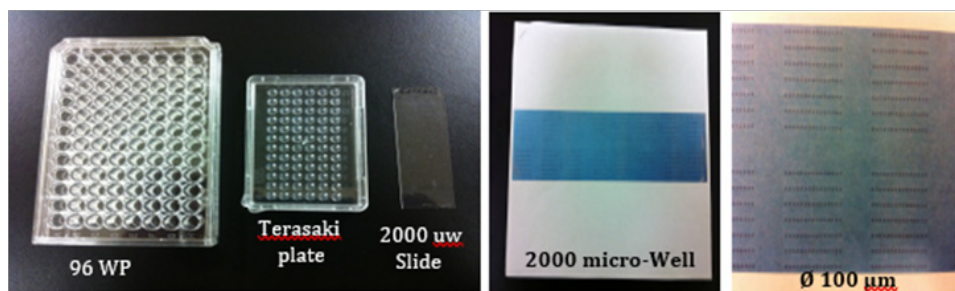


Figure 1 From High Throughput (96 well and Terasaki, 72 well) plate to HT (2000 micro-well) slide.

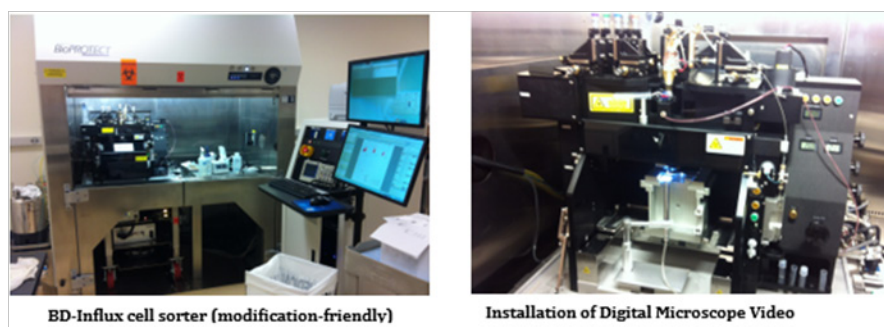


Figure 2 Development of High-Throughput (HT) microWell (μ W) Fluorescence Activated Single Cell Sorting (FASCS).

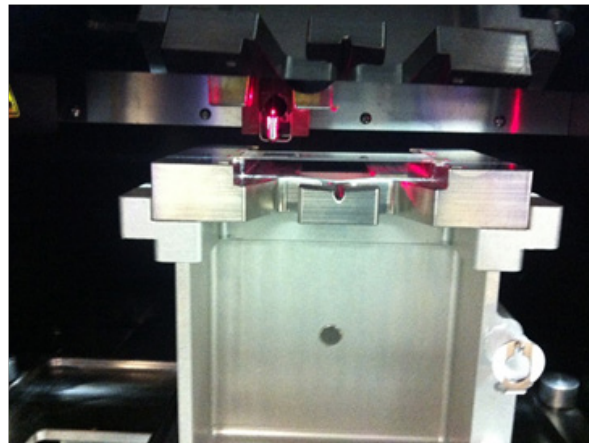


Figure 3 Increase accuracy of single cells deposition on slide by avoiding air currents in the flow chamber, creating a vertical trajectory, re-positioning the waste stream catcher off center, and positioning the slide closer to the sorter nozzle.

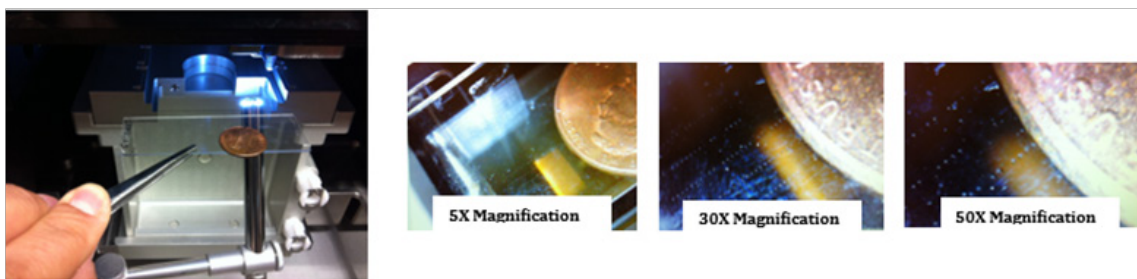


Figure 4 Direct verification of successful drop deposition by inverted microscope with high-resolution optical lens: Andonstar Digital Microscope Video. Magnification: +100X, DSP: High Power Digital Image Monarch Processor; Sensor: high-quality CMOS sensor, 2M pixel; Resolution: 640x480, 1600x1200; Frame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

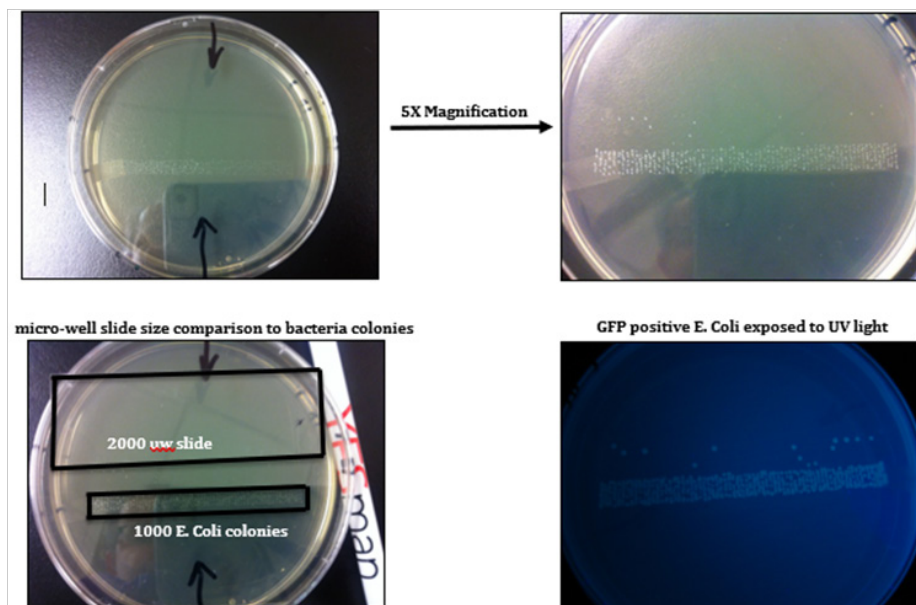


Figure 5 Single bacterial sorted on agar plate to verify the feasibility of HT-μW-HiD-FASCS.

Table 1 Key Criteria for a successful optimization of sort setting for HT- μ W-HiD-FACS

| 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 μ m) | 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.