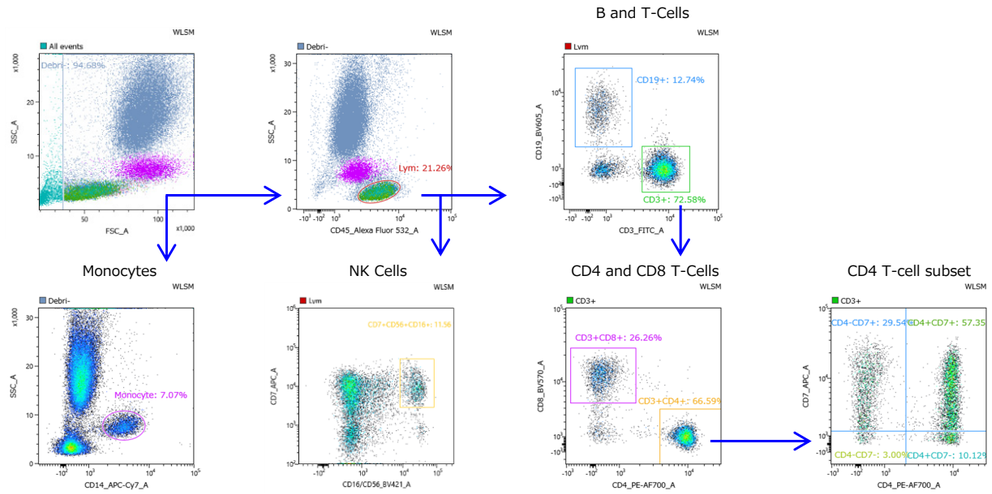
(D)



(E)

Population	Parent	Obtained	Expected Results	
		%	%	±
CD3+	LY %	72.8	74	9
CD3+/CD4+	LY %	48.3	50	9
CD3+/CD8+	LY %	19.4	23	6
CD14+	MO %	78.7	81	20
CD19+	LY %	12.9	13	5
CD7+	LY %	74.3	73	18

Figure 9. Phenotyping of TBNK cells using 3 lasers (405/488/638 nm).
(A) Staining panel for 8-color TBNK immunophenotyping. (B) Emission spectra of fluorochromes listed in (A). (C) Spectra of each single stained cell sample acquired by SA3800. Region from 617 nm to 662 nm in the 32ch PMT is shielded to prevent 638 nm laser shining into the PMT by inserting a physical mask. (D) All lymphocytes and monocytes were identified by staining with CD45. From the CD45$^+$ population B cells (CD19) and T cells (CD3) were identified. The T cell population was further analyzed to determine relative percentages of effector T cells (CD8) and helper T cells (CD4). NK cells (CD16/CD56) were identified from lymphocytes. CD7$^+$ cells were identified from CD4$^+$ T cells. Data was analyzed with Weighted Least Square Method (WLSM) to unmix multi-stained sample. (E) The percentages of cells obtained were comparable to data obtained from Beckman Coulter, Inc.

Summary and References

Novel spectral flow cytometer equipped with 4 lasers, 3D AutoSampler, and 32 channel array PMT showed significant stability for more than 100 days. Additional stability comparisons among 3 instruments show measurement reproducibility and enhanced fluorescent resolution with spectral unmixing. In addition, the 3D AutoSampler with mixing and cooling functions maintained stability, low carryover, and high throughput throughout testing. Results from a multicolor TBNK study were consistent with control values and spectral graphs show panel specific cell fingerprints for each cell subset.

[1]Futamura, Koji, et al. “Novel Full-Spectral Flow Cytometry with Multiple Specrally-Adjacent Fluorescent Proteins and Fluorochromes and Visualization of In Vivo Cellular Movement .” *Cytometry Part A* in press.
[2]Hoffman, Robert A., et al. “Characterization of Flow Cytometer Instrument Sensitivity.” *Current Protocols in Cytometry* 1.20.1 (2007) 1.20.18.
[3]Milush, Jeffrey M., et al. "CD56negCD16$^+$ NK cells are activated mature NK cells with impaired effector function during HIV-1 infection." *Retrovirology* 10.1 (2013): 158.

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SONY

Evaluation results of SA3800: Introducing a novel spectral analyzer for high-throughput single-cell analysis

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Abstract

Single cell analysis has proven to be an integral part of life science research. Cell heterogeneity, the significance of rare populations, and the complexity of existing biological variances have each contributed to an increased demand for high-throughput single-cell analysis tools. Spectral analyzers are such a tool. They offer a revolutionary and innovative approach to flow cytometry. Sony spectral analyzers are unlike conventional flow cytometers because their approach to the detection of fluorescence utilizes a linear array of 32 PMTs and a custom prism array[1]. This engineering design enables a maximum amount of information to be captured from each interrogated cell because it negates the use of interference filters and therefore a loss of collected photons. At Sony, we have worked to engineer and develop a high-throughput spectral analyzer, the SA3800. This 4 laser analyzer is built on the DNA of other Sony instruments for the biotechnology marketplace. This presentation reports the result of instrument evaluation which includes long time stability and multicolor analysis.

Materials and Methods

Materials

AlignCheck, 8-Peak, and SH800 Setup Beads, blocking buffer and staining buffer from Sony Biotechnology. All indicated fluorescent conjugated antibodies were from Sony Biotechnology or Life Technologies. IMMUNO-TROL™ Cells and lysing buffer were from Beckman Coulter, Inc.

Methods

1. QC/System Performance

MESF, Q value, and B value were calculated by the Hoffman method[2].

2. 3D AutoSampler Study

AlignCheck beads and Jurkat cells were run on an instrument prototype alternating 8 times and then average carryover was calculated.

3. 8-color TBNK Human Control Leukocyte Immunophenotyping

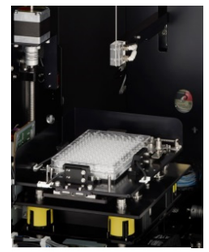
IMMUNO-TROL cells were lysed with lysing buffer. After lysis, cells were washed twice and incubated with blocking buffer for 30 min on ice. Then cells were stained with the antibody conjugated indicated in the figures for 15 min on ice and rinsed with staining buffer twice before data acquisition. Data was analyzed with Sony prototype software.

Introduction

SA3800 is a novel spectral analyzer which has 4 lasers (405, 488, 561, 638 nm), 10 prisms, 32 channel array PMT, 2 independent PMTs, and 3D AutoSampler. The SA3800’s 3D AutoSampler has a stationary probe and a plate that moves in three dimensions (X, Y, Z axes), speeding sample acquisition and reducing the delay necessary for cleaning. The probes built-in self-washing function with relatively shorter tubing reduces carryover (spec <0.1%). Other features include 3D mixing and external plate cooling option.

(A)

(B)



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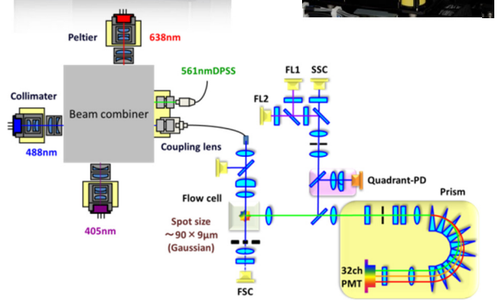

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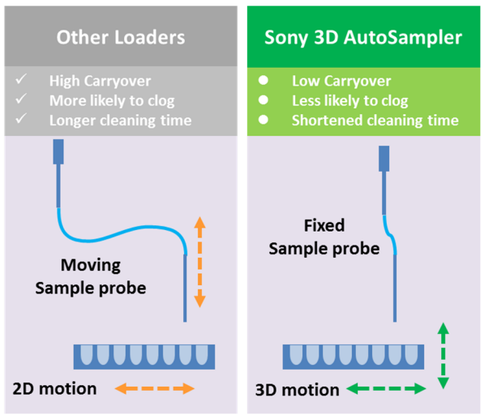

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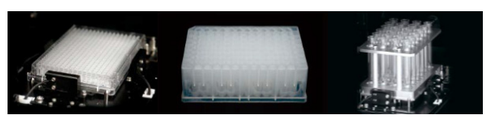

Figure 1. Introduction of SA3800 with 3D AutoSampler.
(A) Exterior of SA3800. (B) Picture of 3D AutoSampler. (C) Optical design of SA3800. (D) Feature comparison of Sony’s 3D AutoSampler. Low carryover, less clogging, and shorter cleaning times are achieved by both the 3D motion of the system and shorter tubing. (E) The SA3800 supports 12x75 mm tubes, standard height 384- and 96- well plates with round, flat, v- and conical shapes in addition to 96-well half deep and deep bottom plate.

Results

rCV of AlignCheck QC over time in both Normal/Low flow modes

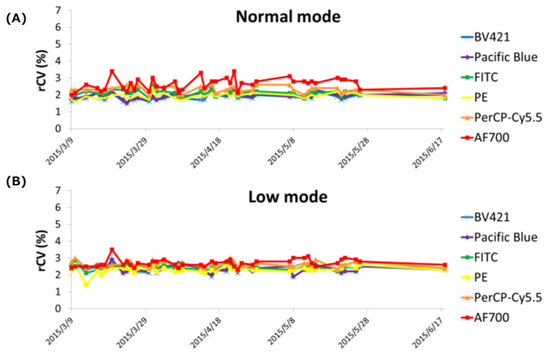

Figure 2. Levey-Jennings (LJ) charts of rCV analysis over 100 days
AlignCheck beads with an emission profile between 400-800 nm were run on a prototype instrument over 100 days at 2 different flow speeds. rCVs of BV421™ (V1), Pacific Blue™ (V2), FITC (ch5-10 in 32ch PMT), PE (ch14-18), PerCP-Cy5.5(ch25-27), and Alexa Fluor® 700 (ch27-29) were calculated and tracked over 100 days. (A) LJ chart of rCV in Normal flow mode (5 m/s). (B) LJ chart of rCV in low flow mode (3 m/s) .

3D AutoSampler with mixing and cooling function was engineered to reduce carryover, increase throughput, and maintain sample consistency

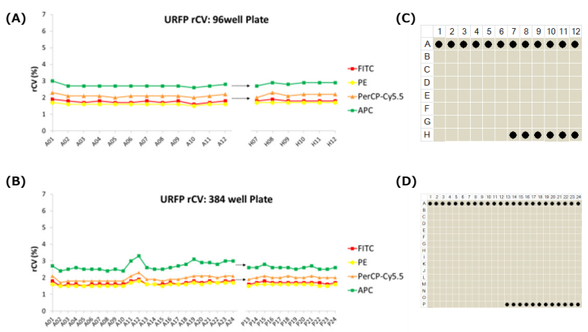

Figure 5. Stable results with the 3D AutoSampler using AlignCheck beads in 96/384 well.
(A) rCVs of FITC, PE, PerCP-Cy5.5, APC acquired from A01-A12 and H07-12 wells from a 96 well plate. (B) rCVs acquired from A01-A24 and P13-24 wells from a 384 well plate were plotted. (C, D) Acquisition and calculations were carried out from all wells, only wells with black circle were plotted.

Stability testing: Long term QC using 8-peak beads

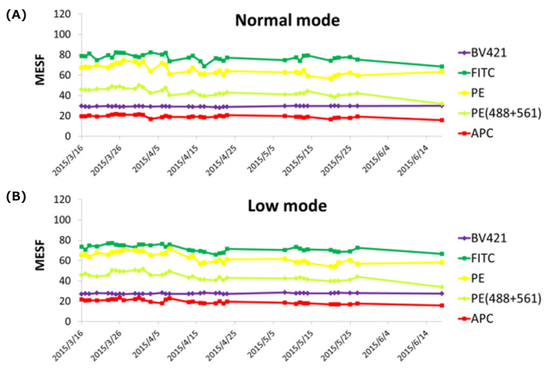

Figure 3. LJ charts of MESF using 8-peak beads over 100 days
8-peak beads were run on a prototype of the instrument over 100 days at 2 different flow speeds. (A) MESF in Normal flow mode (5 m/s). (B) MESF in low flow mode (3 m/s).

Specificity: Low carryover (3 days, 3 instruments)

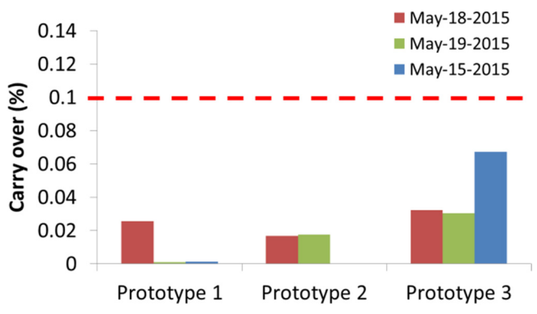

Figure 6. Carryover study
Carryover study with beads and Jurkat cells was carried out on 3 days among 3 different instruments. The average of the percentage were under 0.1 % (red dash line).

Comparison among 3 prototype instruments

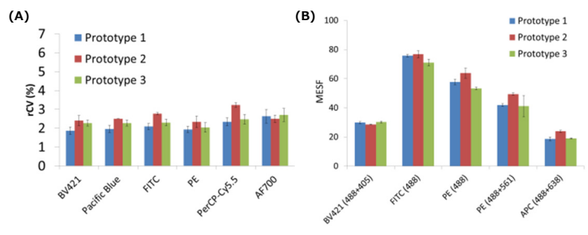

Figure 4. Performance comparison among 3 prototype instruments
AlignCheck beads and 8-peak beads were run on 3 prototypes in Normal flow mode. Standard deviations were calculated and shown from over 100 days results for Prototype 1 and 3 days results for Prototype 2&3. (A) rCV.(B) MESF.

Sampling Reproducibility: Mixing function performance

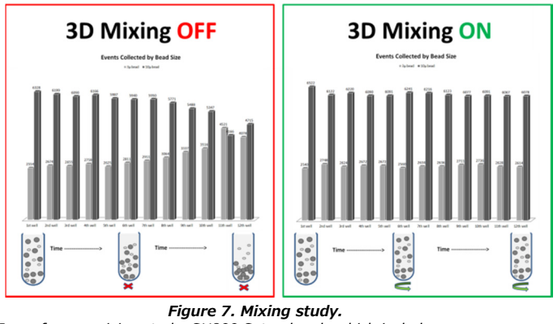

Figure 7. Mixing study.
To perform a mixing study, SH800 Setup beads which include 3 nm and 10 nm beads were used. 10 nm beads are likely to settle rather than 3 nm beads. Without mixing (OFF) the ratio of events of 3 nm and 10 nm beads changed likely due to settling of 10 nm beads. With mixing (ON), the ratio remained consistent showing robust well-to –well reproducibility.

*Sample Reproducibility: Cooling unit performance

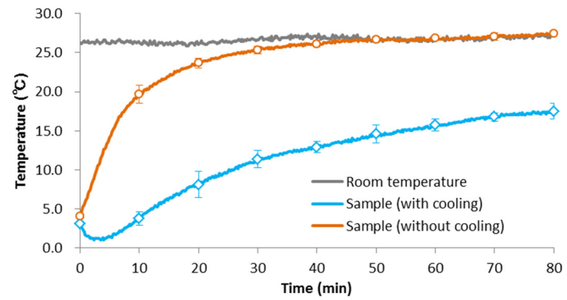

Figure 8. Cooling performance.
The temperature of deionized water (DIW) inside 96 well plates was measured. The temperature without cooling (orange) increased up to 20 degrees after 10 min. With cooling (blue) DIW maintained a temperature of around 15 degrees even after 80 min. Each graph was the average of 4 wells and the bar shows standard deviation.

8-color TBNK Immunophenotyping

TBNK (B-Cell, T-Cell, and natural killer cells) panels are frequently used to examine major leukocyte populations. All of these populations are important for normal immune function. With the ability to analyze more fluorochromes, the SA3800 allows additional subsets to be defined making the most of your precious samples. In this example two additional markers were added to the traditional panel, CD14 (a monocyte marker) and CD7. CD7 identifies a population of activated NK cells that is negative for CD56. The NK cell population is traditionally defined by CD16 and CD56 expression, but is heterogeneous. The addition of CD7 further defines the NK population[3]. CD7 is also expressed on T-cell subsets, but not on B-cells.

(A)

Specificity	Dye
CD16/CD56	BV421
CD3	FITC
CD45	Alexa Fluor® 532
CD8	BV570
CD19	BV605
CD7	APC
CD4	PE-Alexa Fluor® 700
CD14	APC-Cy7