Standardization capability on spectral flow cytometer

***Instruments A, B, F** Japan, **C** US, **D, E** Europe*

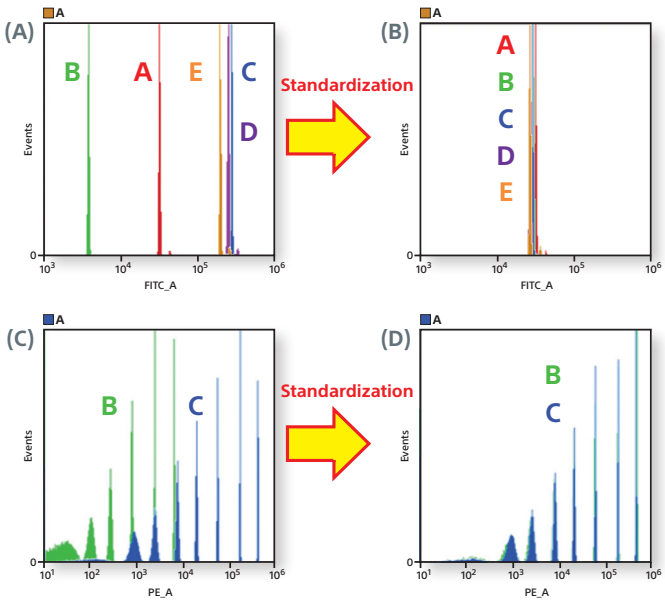


***Figure 1.** Histogram of AlignCheck beads and 8-peak beads.*

*(**A**) Histogram of FITC region (channel: 5 - 10 in 32ch PMT) of AlignCheck beads acquired with normal mode with PMT voltage: 60% on 5 instruments (**A** and **B** from Japan, **C** from US, and **D** and E from Europe) which equip different sensitive PMT each including prototype instruments. (**B**) Histogram of FITC region acquired with standardization mode with ST value: 4.00 (ST value was introduced for the standardization mode as alternative for PMT voltage). Obtained signal intensity was almost identical. (**C**) Histogram of PE region (channel: 14 - 18) of 8-peak beads acquired with normal mode with PMT voltage: 60% on 2 instruments (**B** and **C**). (**D**) Histogram of PE region acquired with standardization mode with ST value: 5.00. All peaks matched very well even the peak of negative population.*

Introduction

Sony has developed a standardization function, that globally standardizes Sony spectral instruments. The function ensures researchers have consistent operating parameters from instrument to instrument.

This technical note discusses and shows how standardization worked successfully with both beads and cells on six Sony SA3800 spectral analyzer instruments located in the United States, Europe, and Japan.

Flow Cytometers are used in studies that utilize, multiple instruments located in different geographical locations, require researchers to find ways to standardize instrument measurement. The need for a simple way to ensure absolute instrument standardization has grown rapidly however other solutions fall short of the simple one click global standardization – offered in Sony Biotechnology systems.

Materials

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

Methods

1. **Multi – Instrument Standardization**
   Standardization capability with 6 instruments in the world AlignCheck beads and 8-peak beads were run on 6 instruments using the same protocol with/without standardization mode.

2. **Day-by-day stability with Standardization**
   Day-by-day comparison was carried out with 3 instruments in Japan. Median fluorescence intensities obtained from 8-peak beads in FITC region were plotted.

3. **Multicolor TB Human Control Leukocyte Immunophenotyping with Standardization.**
   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 acquired on 3 instruments in Japan with standardization mode.

   Comparison between Normal mode and Standardization mode with AlignCheck beads and 8-peak beads on 6 instruments

Multi- instrument comparison of Area and Height.

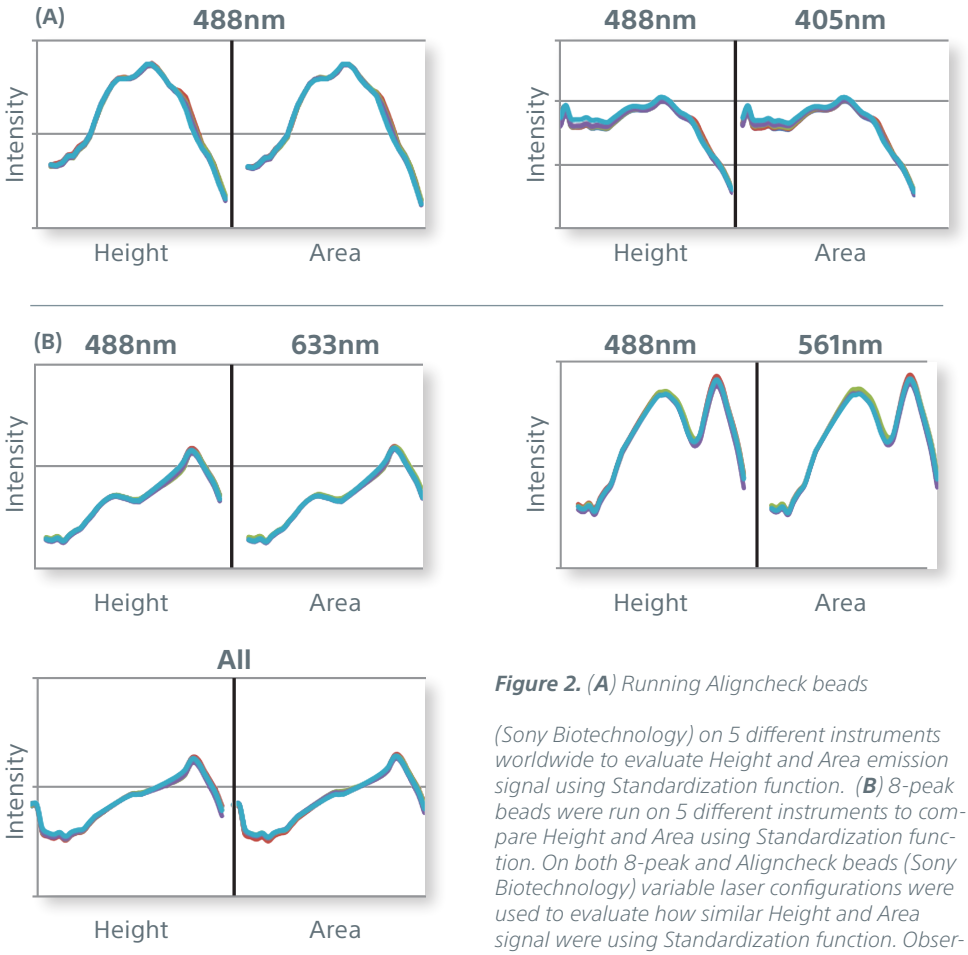


Figure 2. *(**A**) Running Aligncheck beads (Sony Biotechnology) on 5 different instruments worldwide to evaluate Height and Area emission signal using Standardization function. (**B**) 8-peak beads were run on 5 different instruments to compare Height and Area using Standardization function. On both 8-peak and Aligncheck beads (Sony Biotechnology) variable laser configurations were used to evaluate how similar Height and Area signal were using Standardization function. Observations showed that all instruments matched on Height and Area using both form of beads.*

Day-by-day comparison of Standardization mode approx. 100 days

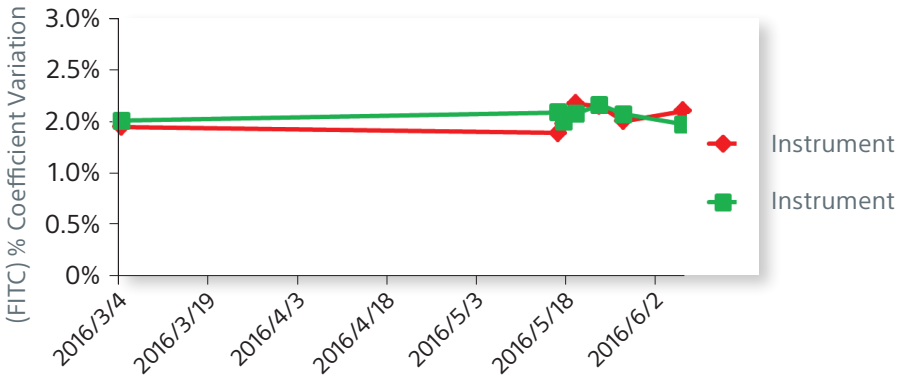


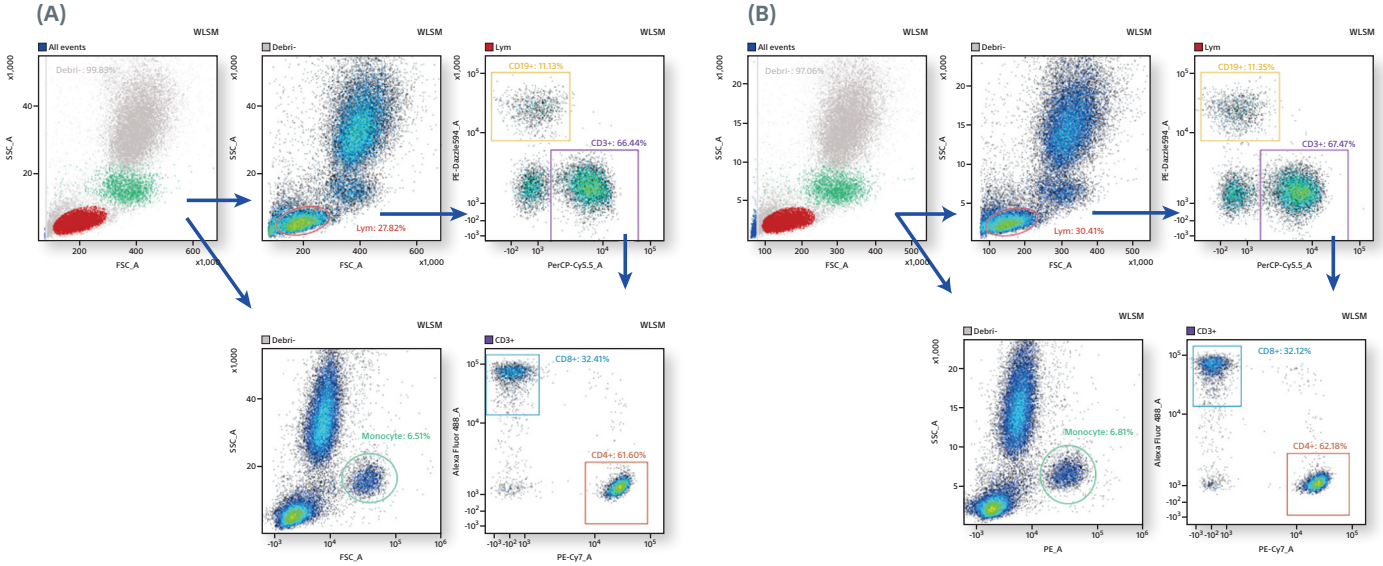
Figure *Stability results from day-by-day comparison.*

8-peak beads were run on 2 instruments for 100 days. Median intensities of FITC region of brightest peak were calculated and also tracked for 100 days. These median intensity values match the result shown in Figure 1 (B). CV of these intensity values were less than 3.00%

5-color T-cell B-cell analysis

(A)

(B)



(C)

Population	Parent	Instrument A	Instrument B	Expected Results	
		%	%	%	±
$CD3^+$	LY %	66.4	67.5	74	9
$CD3^+/CD4^+$	LY %	40.9	42.0	50	9
$CD3^+/CD8^+$	LY %	21.5	21.7	23	6
$CD14^+$	MO %			81	20
$CD19^+$	LY %	11.1	11.4	13	5

Figure *Phenotyping of TB cells on 2 instruments (**A** and **B**) in Japan with Standardization mode.*

*5-color staining was done with Alexa Fluor 488-CD8a, PE-CD14, PE-Dazzle594- CD19, PerCP-Cy5.5-CD3, PE-Cy7-CD4. All lymphocytes were identified by FSC/SSC. Monocytes were identified by staining with CD14. From the Lymphocyte 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). Data was analyzed with Weighted Least Square Method to unmix multi-stained sample. (A) Sample acquired by the instrument **A**. (B) Sample acquired by the instrument **B**. (C) The percentages of cells obtained were comparable to data obtained from Beckman Coulter, Inc.*

Conclusion

In this study, standardization capability on spectral flow cytometers was evaluated using beads and cells. Even though the sensitivity of the detection of each instrument was different from each other, the signals matched almost perfectly in Standardization mode. In addition, the day-by-day study showed high stability for approximately 100 days using Standardization mode. All data demonstrated that the Standardization feature is a reliable and easy method, and has the potential to accelerate international multicenter research programs.

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