High content DuraClone reagents on the CytoFLEX flow cytometer: Instrument setup using the CytoFLEX Compensation Library

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PRINCIPLE OF THE TECHNIQUE

SUMMARY

Compensation values traditionally have been tied to photomultiplier tube (PMT) settings, making frequent repetitions of single stained tubes necessary. The use of fiber array photo diodes which show a virtually linear response across their full detection range enables the CytoFLEX* to recalculate compensation automatically for any gain setting. Therefore, lot-specific single stains can be acquired once and stored within the compensation library for future use without the need to re-run a whole compensation experiment if gains are adjusted.

INTRODUCTION

Tandem dyes play an important role in high content flow cytometry. However, degradation of tandem dyes over time due to light and oxygen exposure as well as inappropriate storage conditions can severely affect their emission spectra and may result in the need for frequent compensation adjustment. Furthermore, manufacturer-dependent spectral variability may occur in tandem dyes that could require lot-specific compensation settings.

With the DuraClone format Beckman Coulter introduced a new proprietary dry reagent formulation that secures long-term fluorochrome stability while stored at room temperature. Instrument set-up including accurate and precise compensation using dry, lot-matched tandem dyes is easy and can be managed by non-experts. The following paragraphs describe this procedure for CytoFLEX flow cytometers. (Please assure the filter configurations match the dye combinations).

MATERIAL

Each DuraClone IM kit includes tubes of reagents in dry format for the multicolor panel as well as the single color conjugates in order to accomplish detector and compensation settings. For a list of currently available panels, please refer to table on page 11 or on-line at http://www.duraclone.com/im/.

Additional materials: Beckman Coulter Reagents
- CytoFLEX Daily QC Fluorospheres
- VersaLyse Lysing Solution
- IOTest 3 Fixative Solution
- VersaComp Antibody Capture Kit

SAMPLE STAINING AND INSTRUMENT PREPARATION

Sample Staining:

1. Please refer to the Instructions For Use (IFU) included in the DuraClone IM kit for specific staining instructions.
2. Single color staining for compensation setup using DuraClone Compensation Kit and VersaComp beads:
   a. Add 1 drop of well mixed VersaComp Antibody Capture Negative Beads and 1 drop of well mixed VersaComp Antibody Capture Positive Beads to the bottom of each tube of the lot-specific Compensation Kit included in the DuraClone IM Kit. Vortex thoroughly.
   b. Incubate in the dark at room temperature (20 –30°C) for 15 min.
   c. Add 1mL of buffer (see VersaComp IFU) to each tube containing VersaComp beads.
   d. Vortex.
   e. Centrifuge at 300 x g for 6 min. Decant the supernatant.
   f. Resuspend the beads in 600 µL of buffer. Store bead solution in the dark until acquisition.

**CytoFLEX instrument preparation:**

3. Run the CytoFLEX System Startup Program.
4. Run quality control (QC) procedure according to the CytoFLEX user manual.
5. Verify the detector configuration.

GENERATE CYTOFLEX COMPENSATION LIBRARY

Perform the steps outlined in this section for every new lot of the DuraClone IM kit.

1. Select "New Compensation" from the File menu for the acquisition of the single stains (Figure 1).
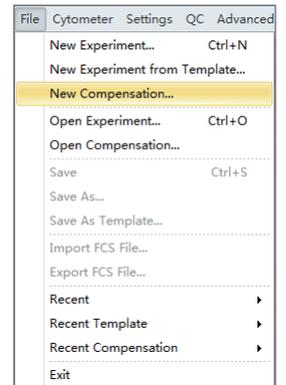

2. Select a file location and save the new compensation as "DuraClone xxx Lot yyy", replacing xxx by the name of the panel, e.g. "IM T Cell" and yyy with the Lot number of the DuraClone IM kit (Figure 2).
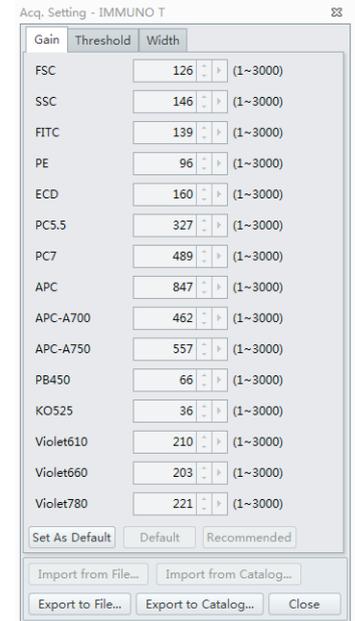
Figure 2
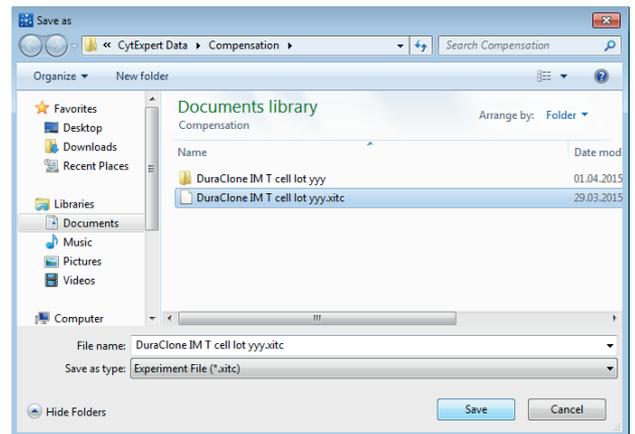
3. In the 'Compensation Setup' window (Figure 3), select tubes for every fluorochrome used, include the antibody used for staining as 'Label' and include the Lot no. for easy identification. Choose 'Bead' as sample type.
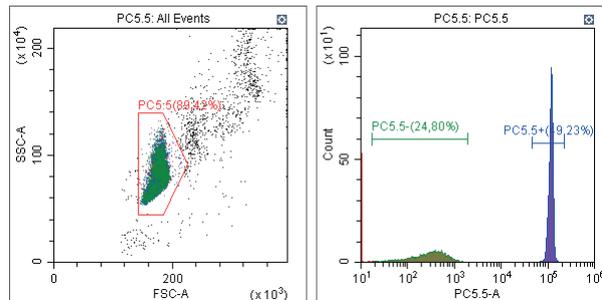
Figure 3
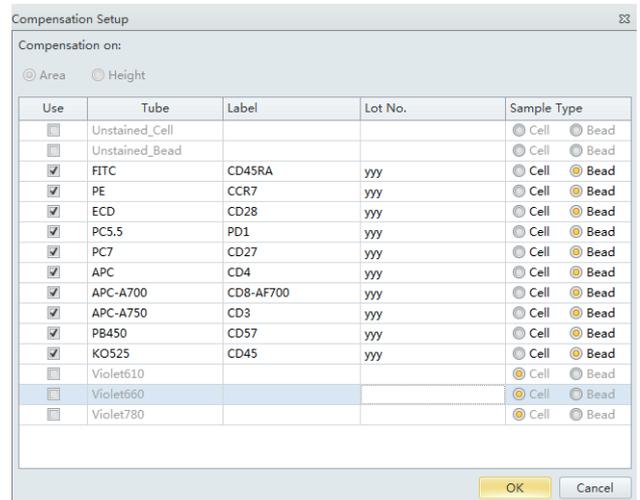
Figure 4

4. Import the recommended gain settings:

    a. Select "Acq. Setting…" on the left side of the screen. The Acq. Setting window appears (Figure 4).

    b. Select the "Gain" tab in the Acq. Setting window.

    c. Select "Recommended" to work with the instrument's QC settings.

5. Run the single positive control samples:

    a. Place the single positive tube in sample loading position.

    b. Select the appropriate, corresponding tube in the CytExpert software.

    c. Move the gate in the FSC/SSC plot so that it encloses the desired population. If necessary, move the positive gate so that it encloses the positive population (Figure 5).

NOTE: Adjust the threshold in the first tube by using the threshold tool. Use the "Auto" function in the Plot Properties Menu to facilitate the display of the VersaComp beads.
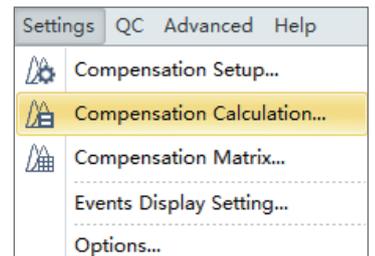
Figure 5

6. Check the data from all acquired sample tubes and confirm that the gating is appropriate.

Figure 6

7. Select "Compensation Calculation" in the Compensation menu (Figure 6) to calculate the compensation values.

The Compensation Matrix window (Figure 7) appears, displaying the calculated compensation values.

Figure 7

Cha...	-FIT...	-PE%	-EC...	-PC...	-PC...	-AP...	-AP...	-AP...	-PB...	-KO...	-Vio...	-Vio...	-Viole...
FITC		1,32	0,75	0,14	0,00	0,07	0,16	0,08	0,19	0,67	0,00	0,00	0,00
PE	14,80		15,25	1,62	0,00	0,01	0,07	0,03	0,07	0,42	0,00	0,00	0,00
ECD	6,09	56,34		0,93	0,00	0,00	0,03	0,00	0,00	0,26	0,00	0,00	0,00
PC5.5	1,39	15,06	39,68		0,00	0,23	1,33	0,01	0,00	0,09	0,00	0,00	0,00
PC7	1,19	10,05	31,88	40,00		0,22	4,58	2,74	0,23	0,41	0,00	0,00	0,00
APC	0,00	0,00	0,69	4,43	0,00		0,86	7,23	0,00	0,08	0,00	0,00	0,00
APC...	0,00	0,00	0,11	45,61	0,00	9,95		0,95	0,00	0,05	0,00	0,00	0,00
APC...	0,00	0,02	0,23	35,62	0,00	7,95	104,...		0,00	0,10	0,00	0,00	0,00
PB4...	0,03	0,00	0,00	0,00	0,00	0,02	0,11	0,03		3,36	0,00	0,00	0,00
KO5...	2,91	0,06	0,36	0,00	0,00	0,04	0,15	0,09	16,97		0,00	0,00	0,00
Viol...	0,44	6,46	10,38	0,07	0,00	0,06	0,08	0,06	1,17	55,41		0,00	0,00
Viol...	0,33	3,29	6,82	0,97	0,00	3,10	0,14	0,25	0,66	35,91	0,00		0,00
Viol...	0,93	1,64	6,10	26,21	0,00	1,50	23,94	18,28	1,01	21,01	0,00	0,00	

8. Select "Save as…" and choose a location and a name to save the generated compensation matrix, e.g. "DuraClone xxx Lot yyy".

9. Select "Save to Compensation Library…" and assign a key word, e.g. "DuraClone xxx Lot yyy" to save the single color compensation values to the compensation library.

FLOW CYTOMETRY ACQUISITION: INITIAL APPLICATION SETUP

1. Select "File - "New Experiment".

2. Select location and a name, e.g. "DuraClone xxx", replacing xxx by the name of the panel, e.g. "IM T Cell".

3. Select "Set Label" (Figure 8) from the Settings menu, and enter the antibodies used for staining as label description.

Figure 8

Channel	Label
FITC	CD45RA-FITC
PE	CCR7-PE
ECD	CD28-ECD
PC5.5	CD279-PC5.5
PC7	CD27-PC7
APC	CD4-APC
APC-A700	CD8-AF700
APC-A750	CD3-APCA750
PB450	CD57-Pacblue
KO525	CD45-KrO
Violet610	CD95-BV605
Violet660	CD25-BV-650
Violet780	CD127-BV785

Apply to:
◉ Current Tube ◯ All Tubes

Figure 9

4. Import the gains and compensation matrix by selecting "Compensation Matrix" from the "settings" menu (Figure 9) and selecting the "Import" function (Figure 10) and selecting the lot specific DuraClone compensation matrix. Choose to import the compensation matrix and gain as shown in Figure 11.
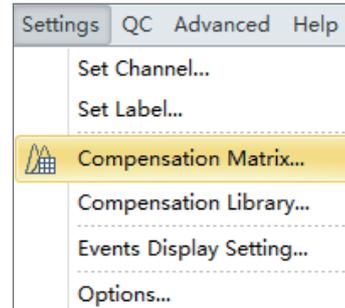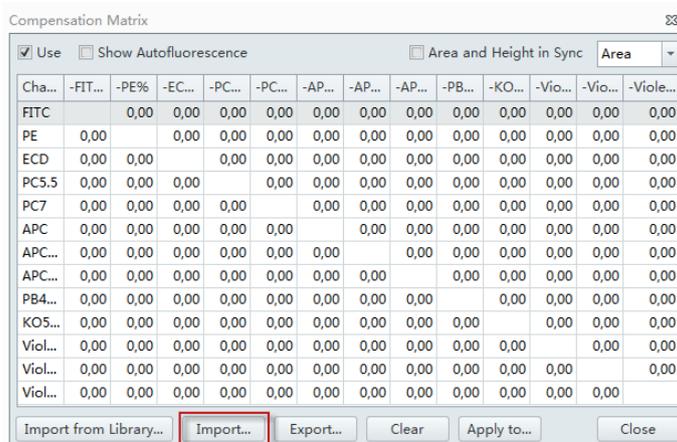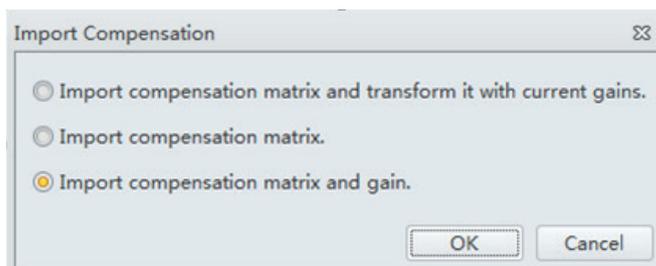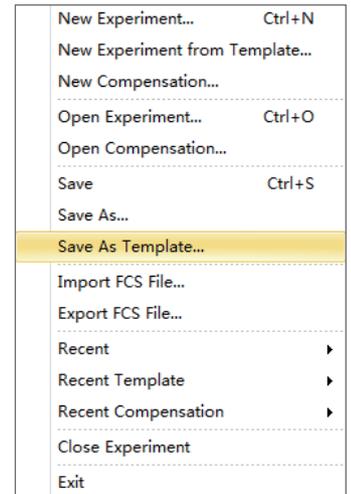

Figure 10

Figure 11

5. Generate the desired plots & gating strategy. Please make sure to include the plots of interest for the intended application and include suitable stopping conditions.

6. Run a tube stained with the full antibody panel and check for plausibility of patterns and populations.

7. If necessary, adjust the gain setting of each channel under the Gain tab in the Acq. Setting window. Raising the gain increases the signal. Lowering the gain reduces the signal.

8. Alternatively, use the Gain Control button on the toolbar in the graphic control area to adjust the gain values for cell population data to their desired levels, directly on the plots where the data appears during data collection. To save the adjusted gain settings as default go to "Cytometer" → "Acq. Settings…" → "Gain" → "Set as default".

Note: If changes are made to gain settings the compensation has to be imported from the compensation library and transformed with current gains before any samples should be recorded. See CytoFLEX IFUs and section V.4 for details.

9 Select "File" → "Save as template…" to generate an Experiment template for Routine use, e.g. "DuraClone xxx", replacing xxx by the name of the panel, e.g. "IM T Cell" (Figure 12).

Figure 12

FLOW CYTOMETRY ACQUISITION: ROUTINE USE

1. Open the "New Experiment from Template" dialogue box in the File menu (Figure 13).

2. Select the previously saved template (e.g. "DuraClone xxx") and define a name and file location for the new experiment, e.g. "DuraClone xxx date".

3. Open the "Acq. Settings" window and select "Gain".

    a. Select "Recommended" to work with the QC settings.

    b. Select "Default" to work with previously defined settings.

4. Import Compensation from Library.

    a. Open the Compensation Matrix Window (Figure 14).

    b. Choose "Import from Library…" (Figure 15).
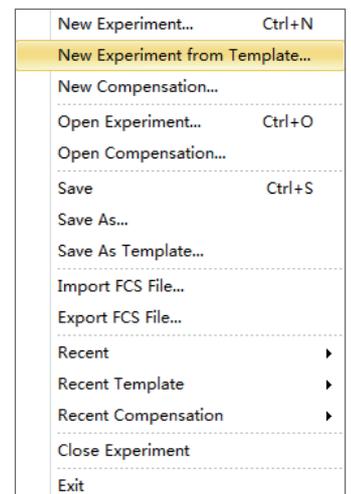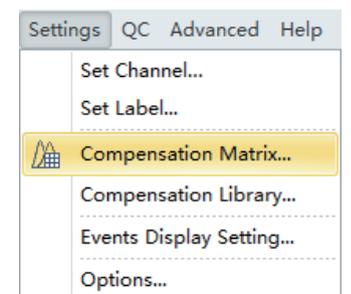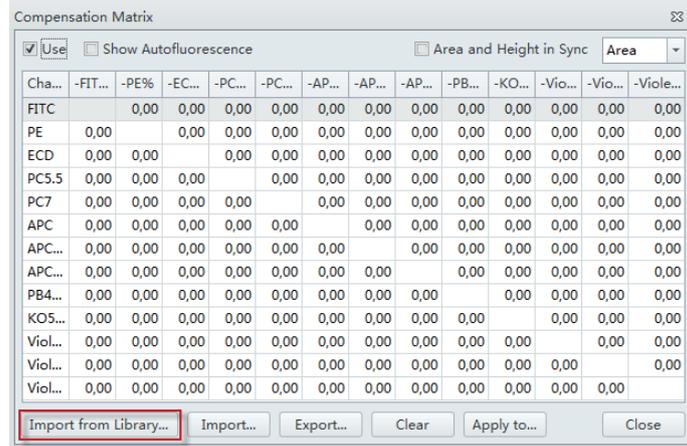
Figure 13

Figure 14

Figure 15

Figure 16

5. Choose the appropriate key word ("DuraClone xxx Lot yyy"), load the single stains and click "OK" (Figure 16).
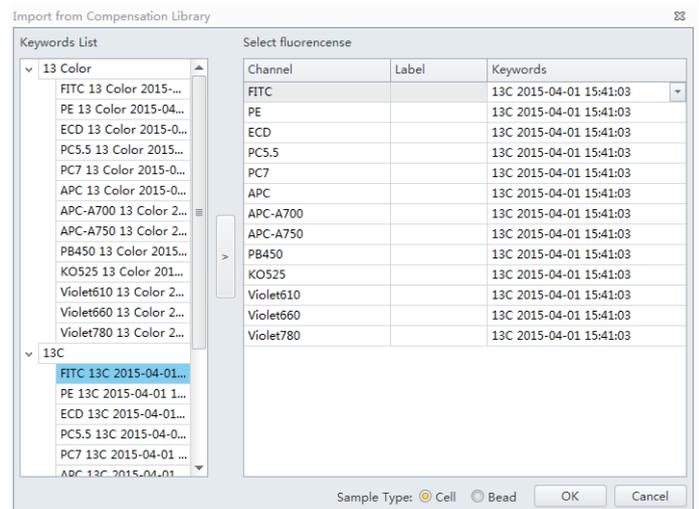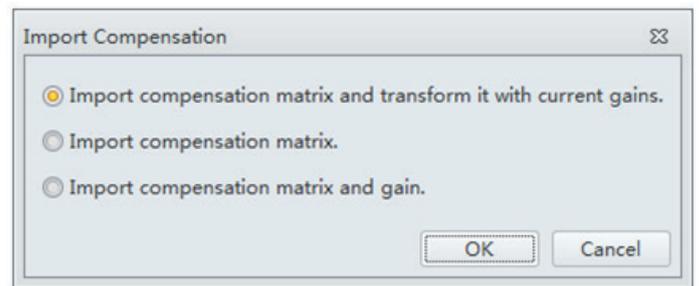

Figure 17

6. Select "Import compensation matrix and transform with current gain" and click "OK" to import the compensation values (Figure 17).

7. Add tubes to the experiment and label as desired.

8. Start sample acquisition.

RESULTS

The procedure outlined here allows for the generation of reproducible high content data using DuraClone IM Kits on the CytoFLEX flow cytometer utilizing the innovative CytoFLEX compensation library. The dry reagent formulation of the DuraClone IM kits guarantees for long term fluorochrome stability at room temperature, and increases reproducibility of results by reducing even complex panels to only a few pipetting steps. Together with the innovative CytoFLEX compensation library that automatically recalculates compensation without the need to re-run single color control tubes, this offers a unique and reliable solution for basic and clinical research applications.

REFERENCES

- CytoFLEX Flow Cytometer Instructions for Use PN B49006AB
- Weit N., Kapinsky M. & Böhmler A. (2015): Advanced analysis of human T cell subsets on the CytoFLEX flow cytometer using a 13 color tube based on DuraClone dry reagent technology. Beckman Coulter Application Information Bulletin # FLOW-817APP03.15-A.

NOTES

The results demonstrated in this application sheet represent those generated on the Beckman Coulter CytoFLEX Flow Cytometer with 488 nm / 638 nm / 405 nm laser configuration. As differences exist in the performance between analyzers, the authors cannot guarantee a similar appearance with the use of other Flow Cytometers.

Laser configuration on the CytoFLEX:

- 405-nm, 80-mW solid-state diode laser
- 488-nm, 50-mW solid-state diode laser
- 638-nm, 50-mW solid-state diode laser

Figure 18 Filter configuration
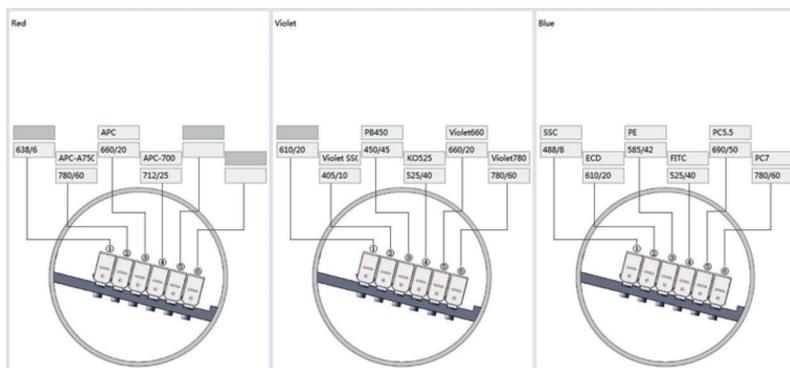
REAGENT DETAILS

The following DuraClone IM kits are available and applicable to the described procedure:

PN	Panels	488 nm					633 nm			405 nm	
		FITC	PE	ECD	PC5.5	PC7	APC / A647 [4]	A700 [1] / APC-A700 [2]	APC-A750 [3]	Pacific Blue**	Krome Orange
B53309	**DuraClone IM Phenotyping Basic Tube** RUO - 25 tests	CD16	CD56	CD19	-	CD14	CD4 / -	CD8 / -	CD3	-	CD45
B53318	**DuraClone IM B cell Tube** RUO - 25 tests	IgD	CD21	CD19	-	CD27	CD24		CD38	IgM	CD45
B53328	**DuraClone IM T cell subsets Tube** RUO - 25 tests	CD45RA	CCR7	CD28	PD1	CD27	CD4	CD8 / -	CD3	CD57	CD45
B53351	**DuraClone IM Dendritic cell Tube** RUO - 25 tests	CD16	Lineage§	-	CD1c	CD11c	Clec9A / -	- / CD123	-	HLA-DR	CD45
B53340	**DuraClone IM TCRs Tube** RUO - 25 tests	TCRγδ	TCRαβ	HLA-DR	-	TCRVδ1	CD4 / -	CD8 / -	CD3	TCRVδ2	CD45

§ CD3 / CD19 / CD20 / CD14 / CD56

(1) Alexa Fluor* 700 (2) APC-Alexa Fluor* 700 (3) APC-Alexa Fluor* 750 (4) Alexa Fluor* 647

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