



FlowJo Software Analysis

- Basic & Advanced Function

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Flow Data Analysis

■ 分析圈選階層 (Population Hierarchy)

▼ Panel 6 10 color Sirigen_Tube_001_020.fcs

▼ Lymphocytes

▼ CD3

▼ Q1: CD4-, CD8+

▼ CM

Q1: CD27-, CD28+

Q2: CD27+, CD28+

Q3: CD27+, CD28-

Q4: CD27-, CD28-

► EM

► Naive

► TEMRA

Q2: CD4+, CD8+

▼ Q3: CD4+, CD8-

Q1: CD45RA-, CD45RO+

Q2: CD45RA+, CD45RO+

Q3: CD45RA+, CD45RO-

Q4: CD45RA-, CD45RO-

Q5: CD45RA-, CD197+

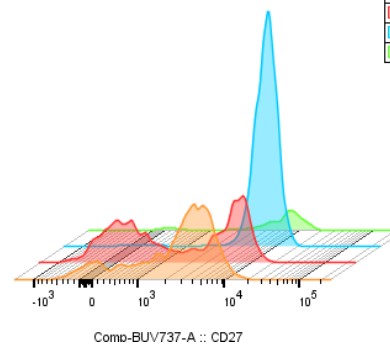
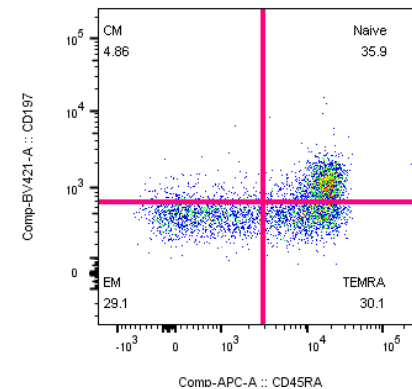
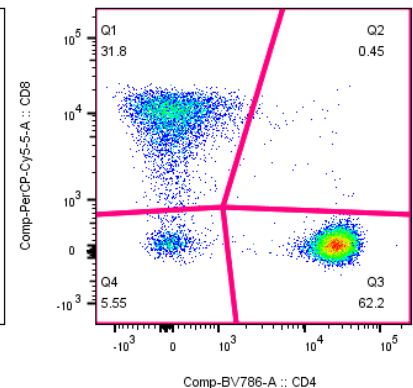
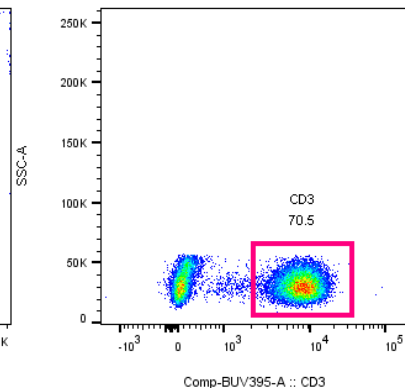
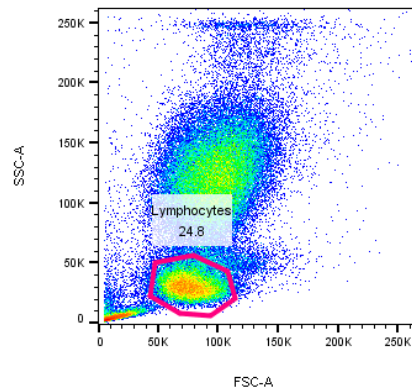
Q6: CD45RA+, CD197+

Q7: CD45RA+, CD197-

Q8: CD45RA-, CD197-

Treg

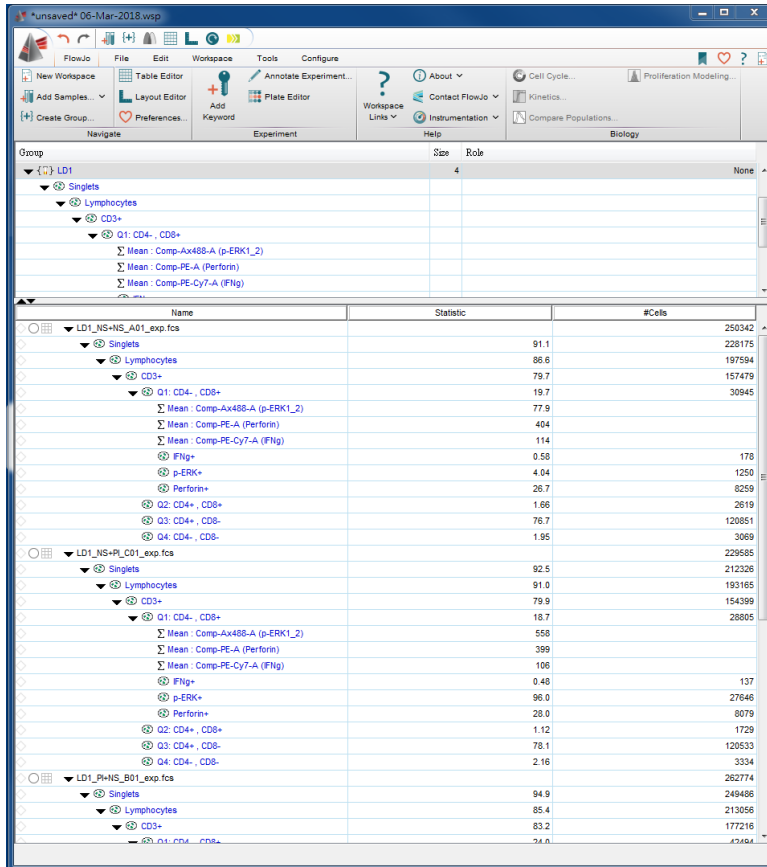
Q4: CD4-, CD8-



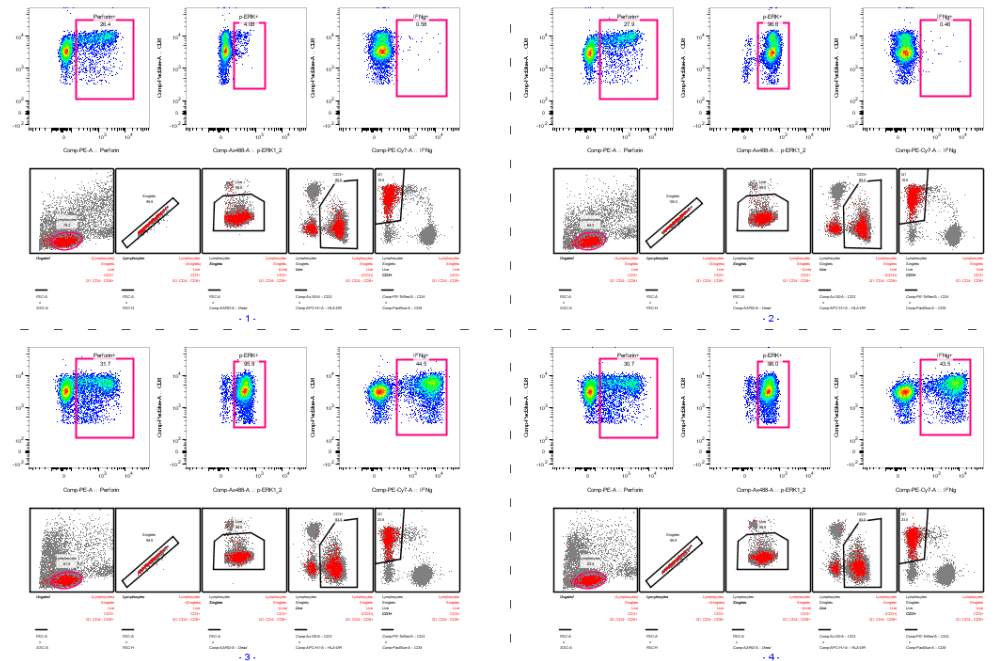
Sample Name	Subset Name	Count
Panel 6 10 color Sirigen_Tube_001_020.fcs	EM	1346
Panel 6 10 color Sirigen_Tube_001_020.fcs	TEMRA	1393
Panel 6 10 color Sirigen_Tube_001_020.fcs	Naive	1661
Panel 6 10 color Sirigen_Tube_001_020.fcs	CM	225

Flow Data Analysis

■ 儲存分析模板 (Workspace Template)



■ 批次分析報告輸出 (Batch Report)



■ 統計輸出/Heat Map 統計

Ancestry Subset Statistic For	p-ERK1/2 Mean	Perforin Mean	IFN- γ Mean	CD8 %	CD4 %
LD1_NS+NS_A01_exp.fcs	78.0	402	114	19.6	77.0
LD1_NS+PI_C01_exp.fcs	562	397	108	18.6	78.3
LD1_PI+NS_B01_exp.fcs	414	463	3020	23.9	72.6
LD1_PI+PI_D01_exp.fcs	411	463	3018	23.5	73.0
Mean	366	431	1564	21.4	75.2
SD	205	36.7	1680	2.69	2.85



FlowJo is now a wholly owned subsidiary of BD.

<https://www.flowjo.com/solutions/flowjo>

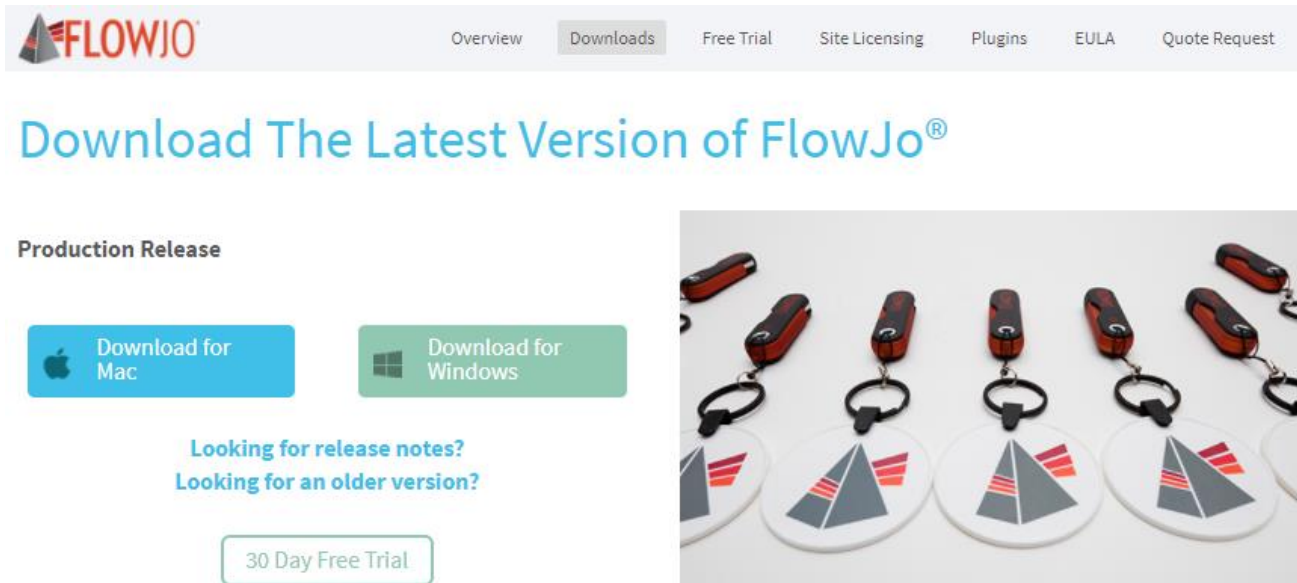
BD於2017年11月收購FlowJo, LLC
目前FlowJo是BD的全資子公司

有FlowJo購買需求可直接聯絡BD台灣分
公司 或撥 0800-737-842



Download FlowJo on website

1. 進入 FlowJo 網站下載最新版的安裝檔
2. **FlowJo** 網站 → **Download** → **FlowJo**



Mac Release Notes (10.4.2):

New Features

- FlowJo icons on high resolution monitors are now correctly sized.
- FACSLytic data now gets its own badge in FlowJo's cytometer preferences and utilizes standard BD transformations by default.

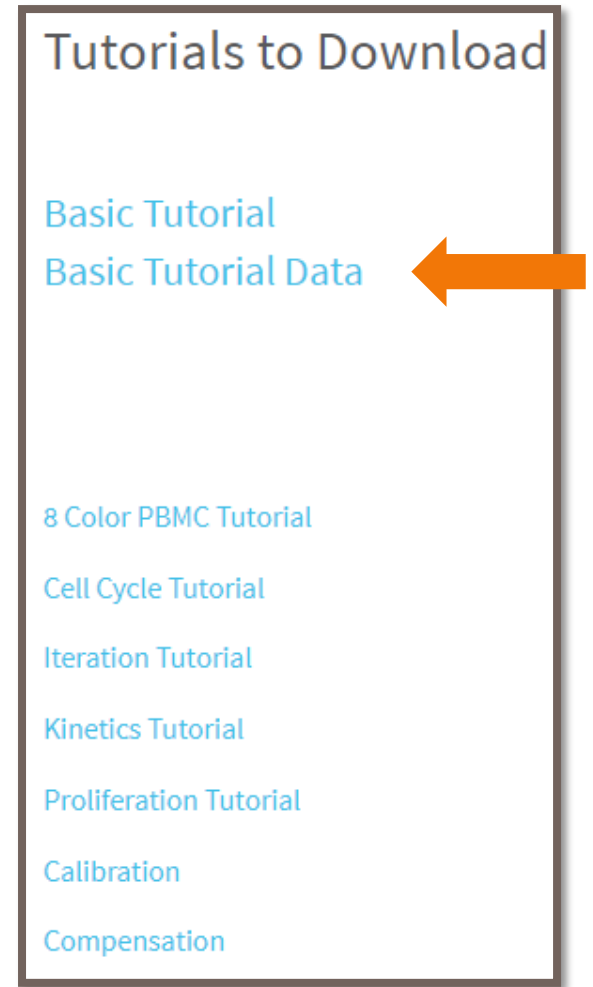
可下載的安裝檔：

- Mac版
- Windows版 (32/64bit)

Download DEMO data

1. **FlowJo** 網站 → **Download** (頁面下方)
2. 點擊  下載數據
3. 即可看到左邊 Tutorials to Download 頁面

練習操作需使用的數據：
Basic Tutorial Data



Today's Demo Data Set: Phospho-Flow + Intracellular Cytokine Staining (PFICS)

Polyclonal PFICS Assay:

- Thaw and rest cryopreserved human PBMC overnight
- Stimulate with PMA+Ionomycin (PI) for 2 hours or rest (NS) while blocking protein secretion → signaling and cytokines
- Stain for viability (AARD) and surface antigens (CD3, CD4, CD8, CD38 and HLA-DR)
- Stimulate PI for 20 minutes or NS rest
- Fix, perm and stain for intracellular antigens (phospho-ERK1/2, IFN- γ and Perforin)

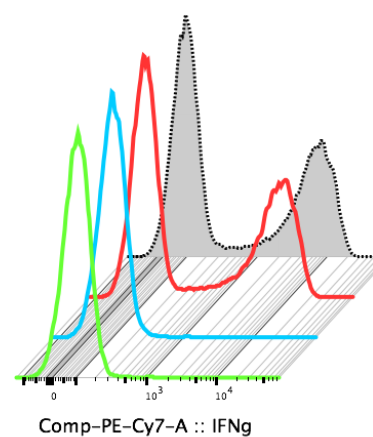
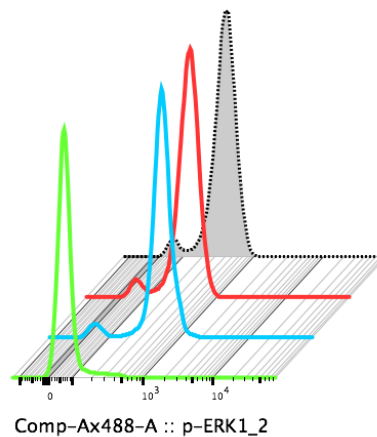
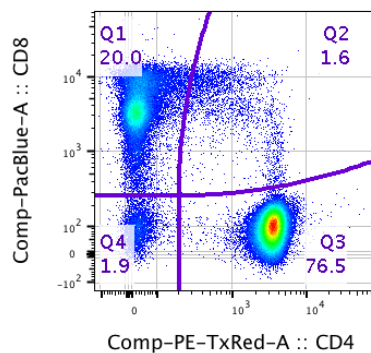
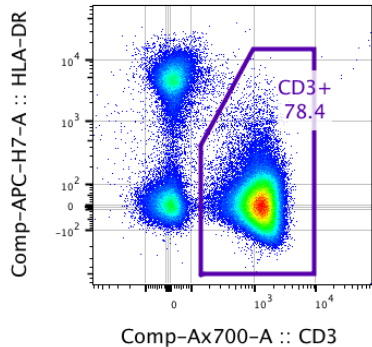
Crawford, T. Q. et. al. (2014) Cytometry A.



PFICS Stim Conditions

- 2 Stimulations → 4 potential *STIM combinations/conditions

*STIM Condition	Total *STIM Time	phospho-ERK Response	IFN- γ Response
NS+NS	0 min	-	-
NS+PI	20 min	++++	-
PI+NS	120 min	+++	+++
PI+PI	140 min	+++	+++



Sample Name
LD1_NS+NS_A01_exp.fcs
LD1_NS+PI_C01_exp.fcs
LD1_PI+NS_B01_exp.fcs
LD1_PI+PI_D01_exp.fcs

工作介面 The FlowJo v10 Workspace

- An interface to organize your data and initiate actions.

The screenshot displays the FlowJo v10 Workspace interface. The top ribbon contains tabs for File, Edit, Workspace, Tools, and Configure. Below the ribbon, there are several tool buttons including 'New Workspace', 'Table Editor', 'Layout Editor', 'Add Samples...', 'Create Group...', 'Annotate Experiment...', 'Plate Editor', 'Cell Cycle...', 'Kinetics...', 'Proliferation Modeling...', 'Compare Populations...', and 'Help'. The main workspace area is divided into three panes: 'Navigate' (left), 'Experiment' (middle), and 'Biology' (right). The 'Navigate' pane shows a hierarchical tree view of sample groups, including 'All Samples', 'All Stain', 'Compensation', 'FMOs', 'Master Gates', 'Time', 'Singlets', 'Lymphocytes', 'Live', 'CD3+', 'Q1: CD4-, CD8+', 'Median: Comp-Ax488-A (p-ERK1_2)', 'Median: Comp-PE-Cy7-A (IFNg)', 'IFNg+', 'Perf+', 'pERK+', and 'IFNg+Perf-pERK+'. The 'Experiment' pane shows a table of sample statistics, including 'Name', 'Statistic', '#Cells', '*PID', '*STIM', and 'WELL ID'. The 'Biology' pane shows a table of sample statistics, including 'Name', 'Statistic', '#Cells', '*PID', '*STIM', and 'WELL ID'.

Name	Statistic	#Cells	*PID	*STIM	WELL ID
LD1_NS+NS_A01_exp.fcs		250342	LD1	NS+NS	A01
LD1_NS+PI_C01_exp.fcs		229585	LD1	NS+PI	C01
LD1_PI+NS_B01_exp.fcs		262774	LD1	PI+NS	B01
LD1_PI+PI_D01_exp.fcs		244977	LD1	PI+PI	D01
LD2_NS+NS_A02_exp.fcs		330780	LD2	NS+NS	A02
LD2_NS+PI_C02_exp.fcs		286306	LD2	NS+PI	C02
LD2_PI+NS_B02_exp.fcs		279202	LD2	PI+NS	B02
LD2_PI+PI_D02_exp.fcs		275465	LD2	PI+PI	D02
Time	99.7	274625			
Singlets	96.6	265165			
Lymphocytes	91.0	241425			
Live	75.2	181586			
CD3+	83.0	150726			
Q1: CD4-, CD8+	24.8	37327			
Median: Comp-Ax488-A (p-ERK1_2)	451				
Median: Comp-PE-Cy7-A (IFNg)	3591				
IFNg+	62.4	23286			
Perf+	49.3	18386			
pERK+	91.9	34306			
IFNg-	37.6	14041			
Perf-	50.7	18941			
pERK-	8.09	3021			
IFNg+Perf+pERK+	44.2	16497			
IFNg+Perf+pERK-	0.55	206			
IFNg+Perf-pERK+	17.4	6495			

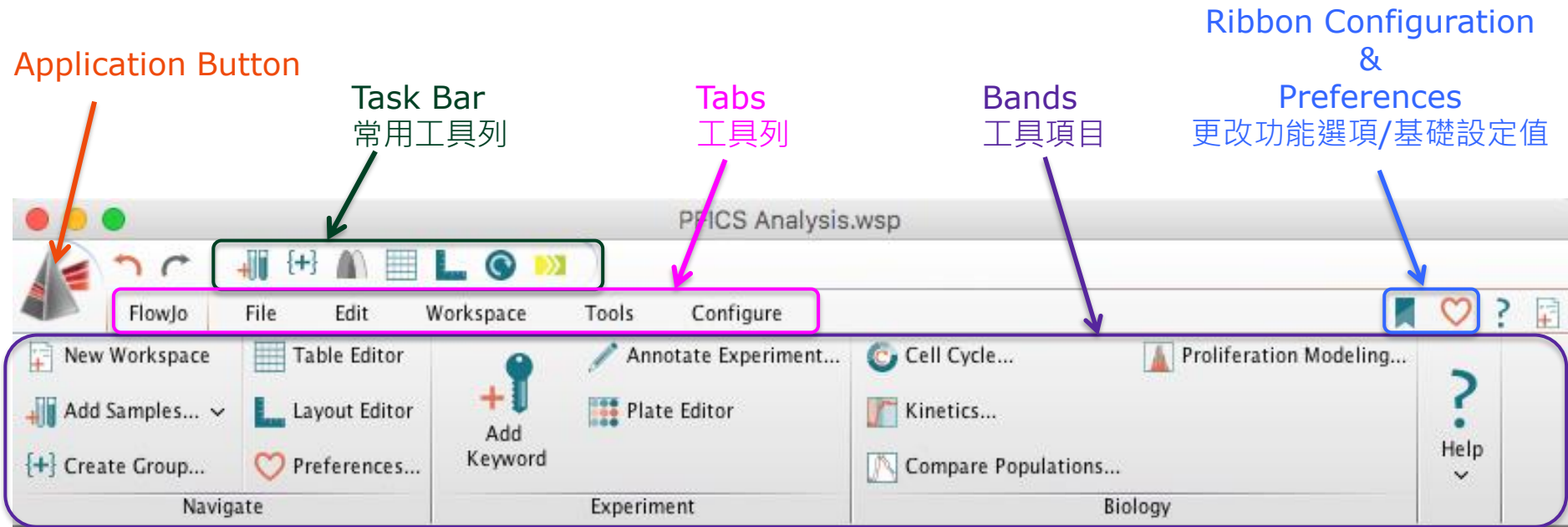
Ribbon
Tabs and Bands
功能介面

Groups and Group
Analysis
群組介面

Samples and
sample analysis
樣品介面

功能介面 Ribbons, Tabs and Bands

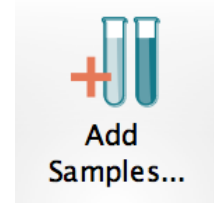
- Ribbon organization allows easy visual navigation of workspace functions.



- Tabs group similar Bands together.
- Bands group similar Actions together.

輸入數據 Importing Data

- Drag and drop into Samples Pane or click the Add Samples button.



Group	Size	Role
{ } All Samples	0	Test
{ } Compensation	0	Compensation

Drag Samples Here

Group	Size	Role
{ } All Samples	46	Test
{ } Compensation	12	Compensation
{ } PFICSComp	46	Test

Name	Statistic	#Cells
☐ Bead Comps_4 PE-TR_F01_exp.fcs		19202
☐ Bead Comps_8 PB_F02_exp.fcs		14969
☐ Bead Comps_38 PE-Cy5_F03_exp.fcs		17603
☐ Bead Comps_DR APC-H7_F04_exp.fcs		18907
☐ Bead Comps_US Beads +FP_F05_exp.fcs		30000
☐ Bead Comps_ERK A488_F06_exp.fcs		24114
☐ Bead Comps_IFN PE-Cy7_F07_exp.fcs		30000
☐ Bead Comps_Perforin PE_F08_exp.fcs		19212
☐ Bead Comps_US Beads No FP_F09_exp.fcs		10290
☐ Cell Comps_AARD_E01_exp.fcs		145743
☐ Cell Comps_CD3 A700_E02_exp.fcs		129537
☐ Cell Comps_US Cells_E03_exp.fcs		158360
☐ LD1_NS+NS_A01_exp.fcs		250342
☐ LD1_PI+NS_B01_exp.fcs		262774
☐ LD1_NS+PI_C01_exp.fcs		229585
☐ LD1_PI+PI_D01_exp.fcs		244977
☐ LD2_NS+NS_A02_exp.fcs		330780
☐ LD2_PI+NS_B02_exp.fcs		279202
☐ LD2_NS+PI_C02_exp.fcs		286306
☐ LD2_PI+PI_D02_exp.fcs		275465
☐ LD4_NS+NS_A03_exp.fcs		222740
☐ LD4_PI+NS_B03_exp.fcs		224146

群組介面 Group Pane

- The Group area lists all groups in the Workspace, # of samples in each group (Size), and the Role of that group (ex. Test, Compensation, Controls) .
- Groups act like folders to organize your samples, allows master gating and unique report generation.

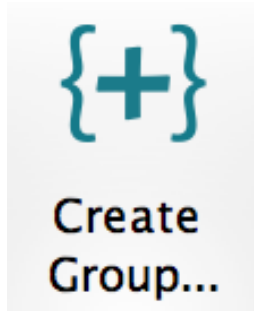
Group	Size	Role
{ } All Samples	46	Test
{ } AllStain	20	Test
{ } Compensation	12	Compensation
{ } FMOs	14	Controls
▼ { } MasterGates	34	None
▼ { } Time		
▼ { } Singlets		
▼ { } Lymphocytes		
▼ { } Live		
▼ { } CD3+		
▼ { } Q1: CD4-, CD8+		
Σ Geometric Mean : Ax488-A (p-ERK1_2)		
Σ Geometric Mean : PE-A (Perforin)		
Σ Geometric Mean : PE-Cy7-A (IFNg)		
▼ { } IFNg+		
Σ Freq. of Parent		
{ } Perf+		
{ } pERK+		

- Group owned analysis gains the group color.

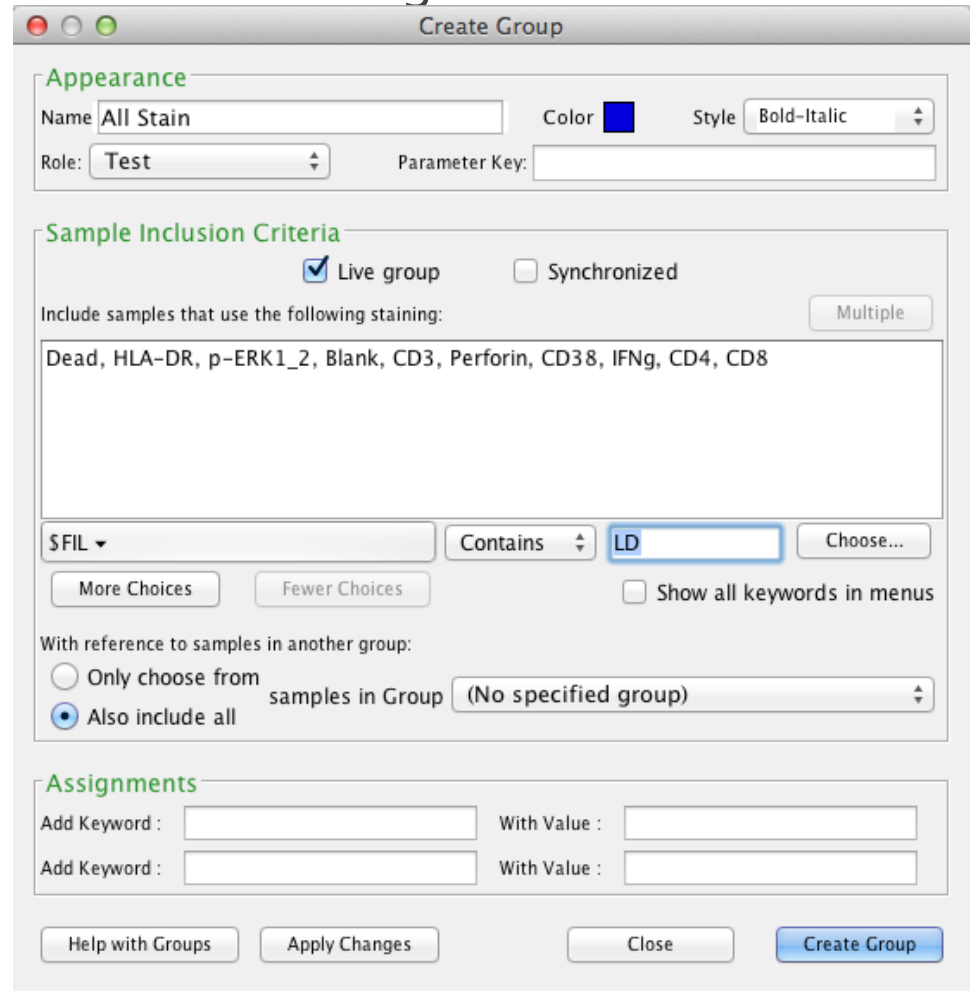
建立/編輯族群

Creating and Editing Groups

- Click the Create Group Icon located in either the task bar at the top of the workspace, or within the Navigate band.



- Double click on an existing group to edit its properties.



Create Group

Appearance

Name: Color: Style:

Role: Parameter Key:

Sample Inclusion Criteria

☒ Live group ☐ Synchronized

Include samples that use the following staining:

\$FIL Contains

☐ Show all keywords in menus

With reference to samples in another group:

☐ Only choose from

☒ Also include all

Assignments

Add Keyword : With Value :

Add Keyword : With Value :

樣品選取標準 Sample Inclusion Criteria

- **Live groups** automatically include samples based on user-defined **Sample Inclusion Criteria**.

- Sample Inclusion Criteria could include the staining panel, characters in the \$FIL (file name), a user defined Keyword attribute, or a combination of features.

- 可利用關鍵字將Flow數據快速地分類到群組內。

Modify Group

Appearance

Name: Color: Style:

Role: Parameter Key:

Sample Inclusion Criteria

☒ Live group ☐ Synchronized

Include samples that use the following staining:

\$FIL LD

And Lacks

And =

☐ Show all keywords in menus

With reference to samples in another group:

☐ Only choose from

☒ Also include all

Assignments

Add Keyword : With Value :

Add Keyword : With Value :

添加關鍵字 Add New Keywords

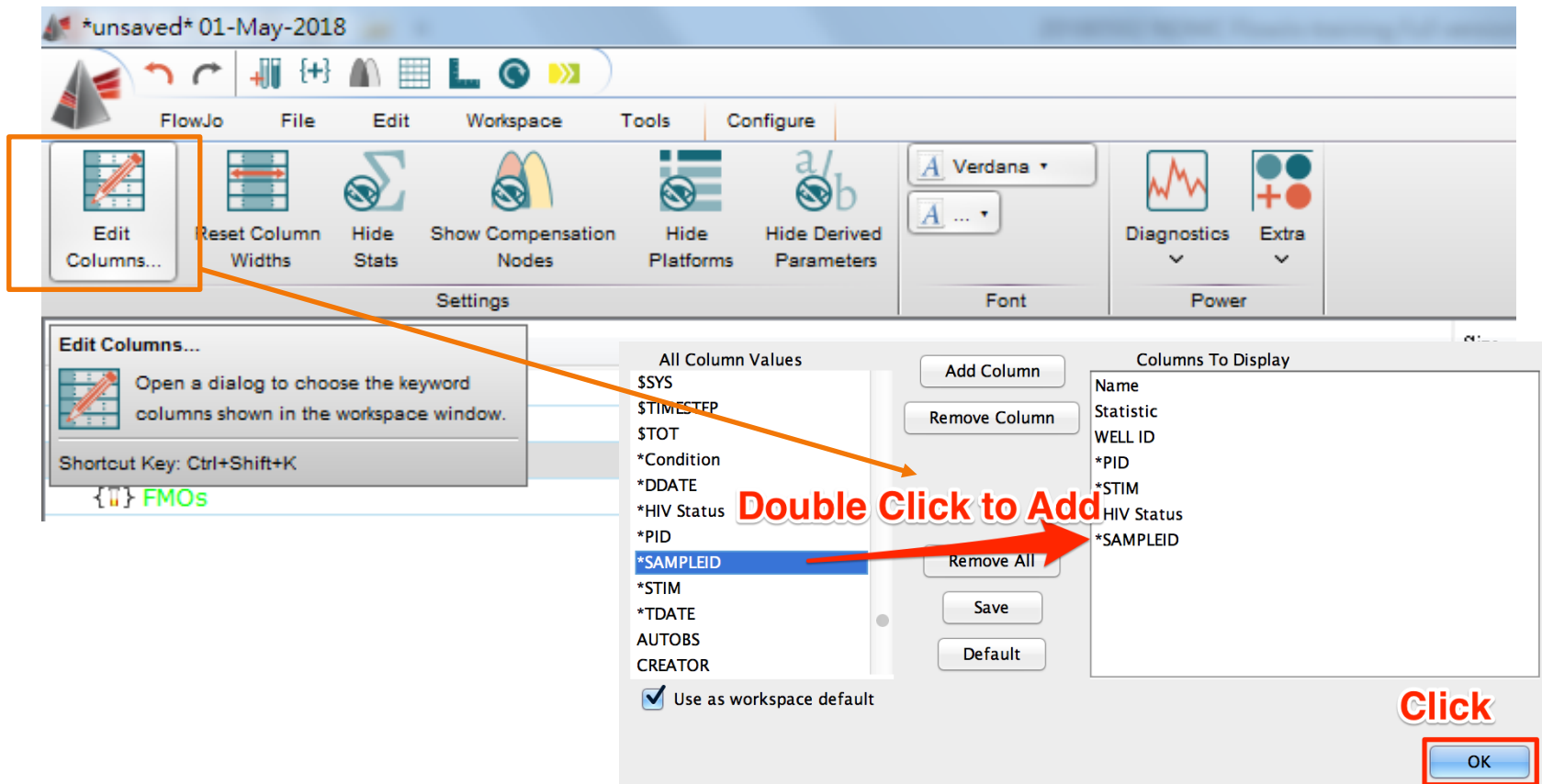
- Keywords attach descriptive metadata to samples
- Examples:
 - FCS file standard required keywords (ex. \$FIL, \$PnS)
 - User defined descriptive Keywords (ex. Patient ID, Timepoint)
- Workspace Tab → Keywords Band → **Add Keyword** allows user to define new Keywords and add metadata

The screenshot shows the FlowJo software interface. The 'Workspace' tab is selected in the top menu. The 'Keywords' band is highlighted with a red box. Below the main interface, a table lists sample names, statistics, reagents, and keywords.

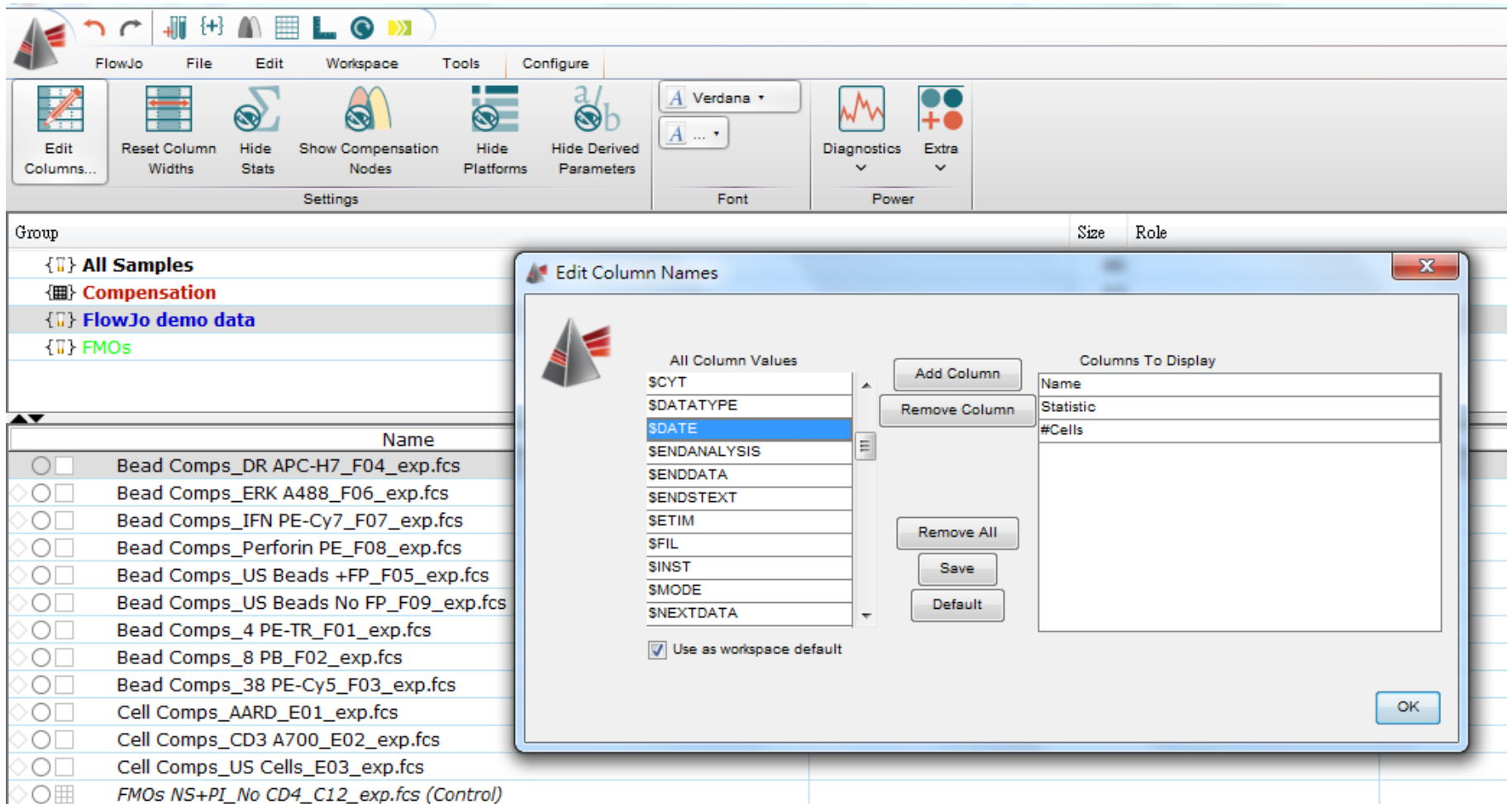
Name	Statistic	PacBlue-A reagent	Patient ID	Timepoint
LD1_NS+NS_A01_exp.fcs		CD8	LD1	Day 0
LD1_NS+PI_C01_exp.fcs		CD8	LD1	Day 0
LD1_PI+NS_B01_exp.fcs		CD8	LD1	Day 0
LD1_PI+PI_D01_exp.fcs		CD8 LD1		Day 0
LD2_NS+NS_A02_exp.fcs		CD8	LD2	Day 0
LD2_NS+PI_C02_exp.fcs		CD8	LD2	Day 0
LD2_PI+NS_B02_exp.fcs		CD8	LD2	Day 0
LD2_PI+PI_D02_exp.fcs		CD8	LD2	Day 0

呈現關鍵字 Display Existing Keywords

- Configure Tab → Settings Band → **Edit Columns** allows user to display Keywords as columns in the workspace samples pane



編輯Column Names可顯示FCS file本身帶有的Key word內容
EX: \$DATE=數據收集時間



添加完畢後就可以看到data視窗出現 \$DATE資訊

Statistic	# Cells	\$DATE
	18907	18-JAN-2013
	24114	18-JAN-2013
	30000	18-JAN-2013
	19212	18-JAN-2013
	30000	18-JAN-2013
	10290	18-JAN-2013
	19202	18-JAN-2013
	14969	18-JAN-2013
	17603	18-JAN-2013
	145743	18-JAN-2013
	129537	18-JAN-2013
	158360	18-JAN-2013
	219336	18-JAN-2013
	269027	18-JAN-2013
	281200	18-JAN-2013
	249824	18-JAN-2013
	276464	18-JAN-2013
	254708	18-JAN-2013
	286676	18-JAN-2013

樣品介面 Samples and Sample Analysis

- Displays the sample list and associated analysis of the currently selected group.
- Statistic and #Cells columns are displayed by default. Additional Keyword attributes can be displayed as columns.

Name	Statistic	#Cells	*HIV Status	*PID	*STIM
LD1_NS+NS_A01.fcs		250342	Neg	LD1	NS+NS
LD1_NS+PI_C01.fcs		229585	Neg	LD1	NS+PI
LD1_PI+NS_B01.fcs		262774	Neg	LD1	PI+NS
Time	99.7	261964			
Singlets	96.2	252097			
Lymphocytes	93.7	236200			
Live	96.2	227167			
CD3+	81.4	184893			
Q1: CD4-, CD8+	24.0	44355			
Q2: CD4+, CD8+	1.13	2090			
Q3: CD4+, CD8-	72.7	134352			
Q4: CD4-, CD8-	2.22	4096			
LD1_PI+PI_D01.fcs		244977	Neg	LD1	PI+PI

- Double click on a sample to open a Graph Window and add gates.

作圖視窗 The Graph Window

- Facilitates data visualization and gating.

Gating Tools
圈選工具

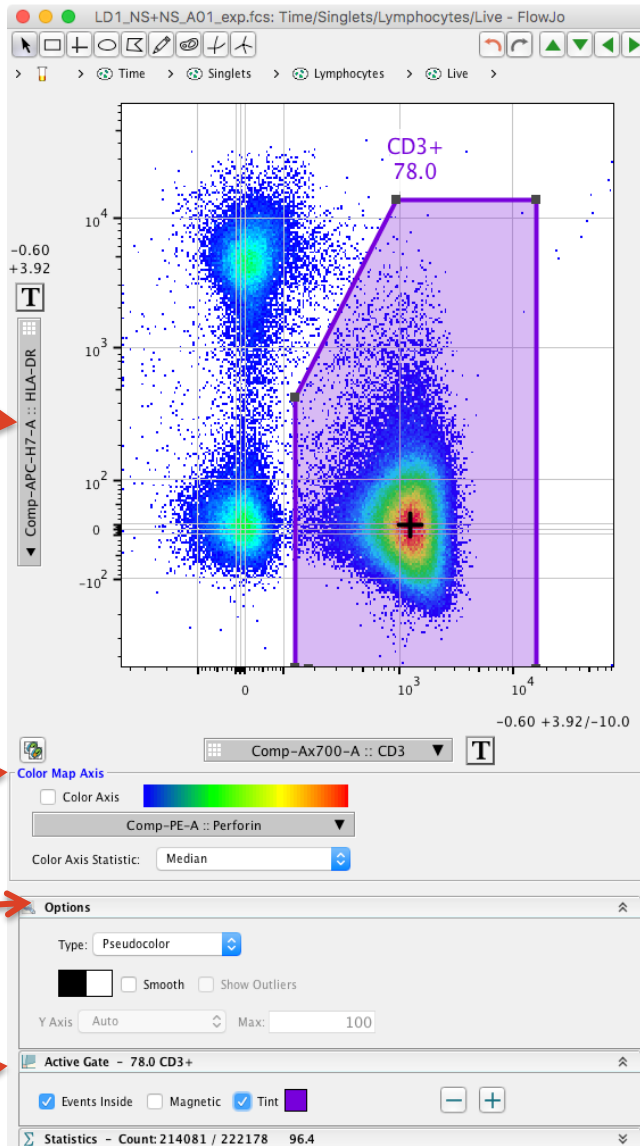
Navigation Buttons
瀏覽數據

Parameter Selection
選擇參數

Color Map 3rd Parameter
色彩圖參數

Plot View Options
圖其他呈現方式

Active Gate Options
圈選呈現方式

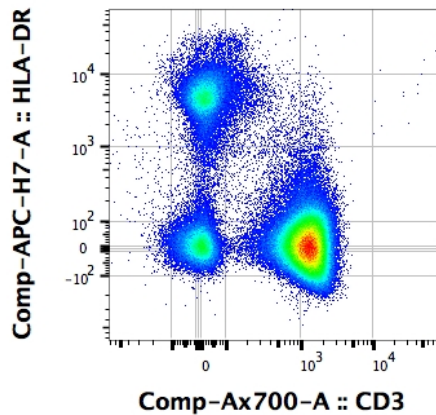


- Several different plot types are available to display flow data.
- Click on the Options Menu below the graph image and select Graph Type from the dropdown menu.

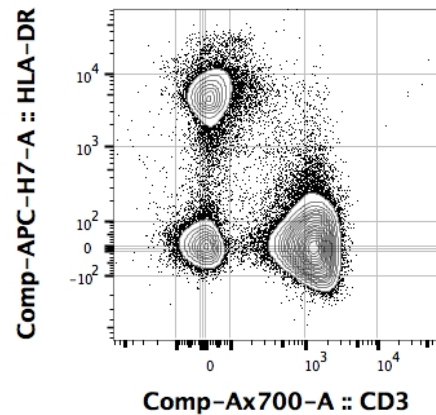
作圖呈現方式 Graph Display Options

- Try them all and pick what pleases you, or best represents your data.

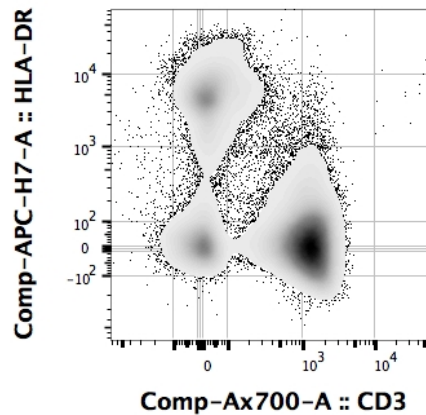
Pseudocolor



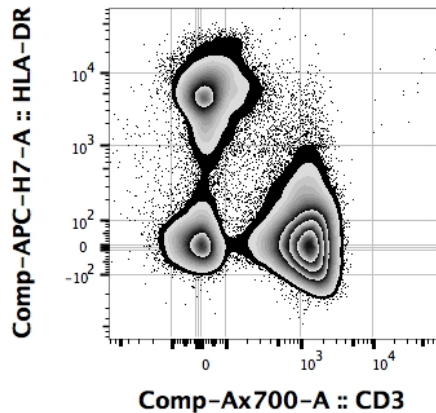
Contour



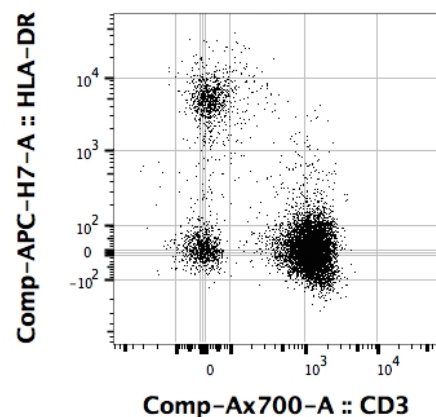
Density



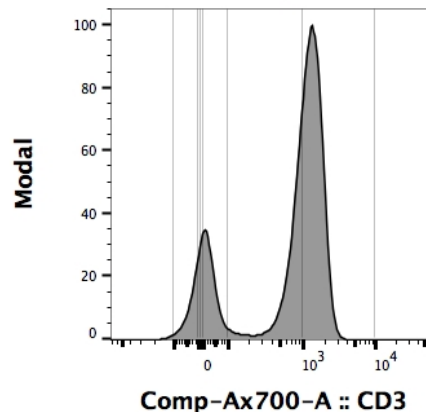
Zebra



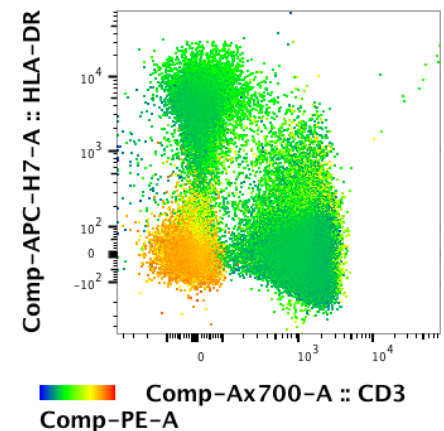
Dot Plot



Histogram



Color Map 3rd Parameter

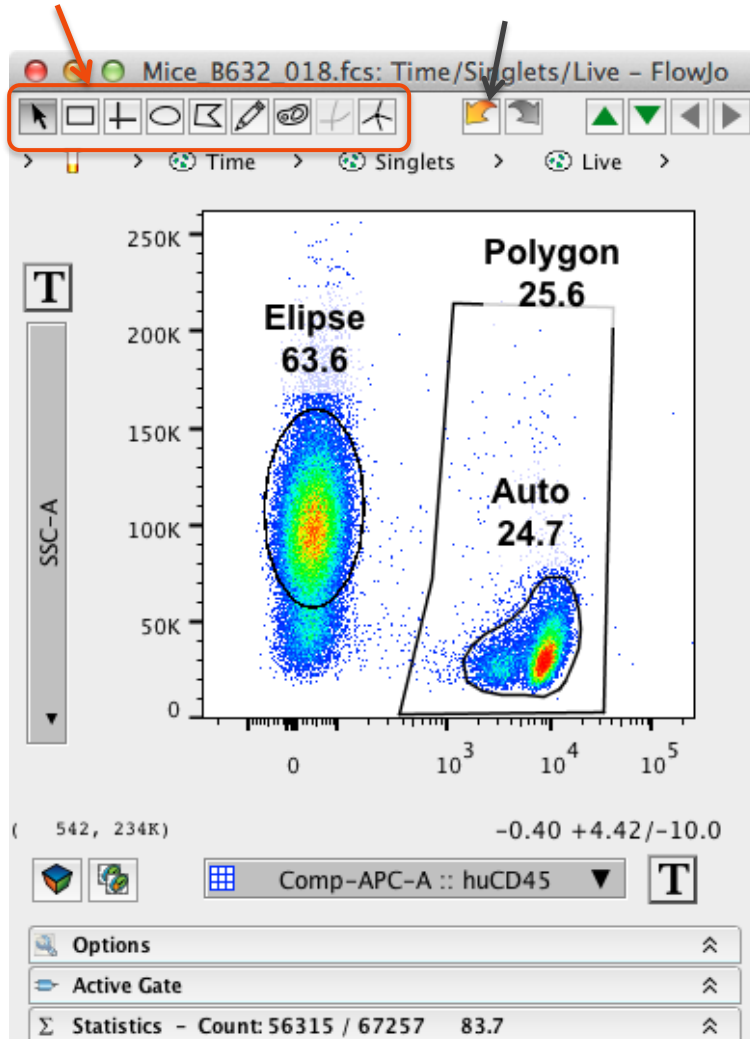


圈選工具 Gating tools

- Are located at the top left in a Graph Window.

Gating Tools 圈選工具

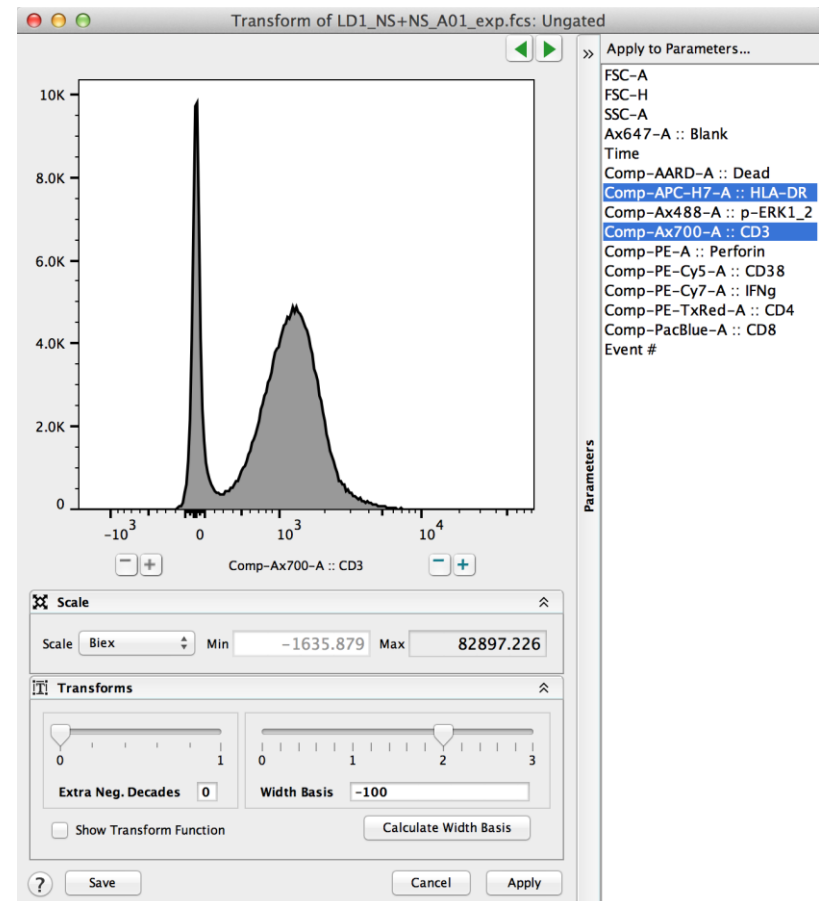
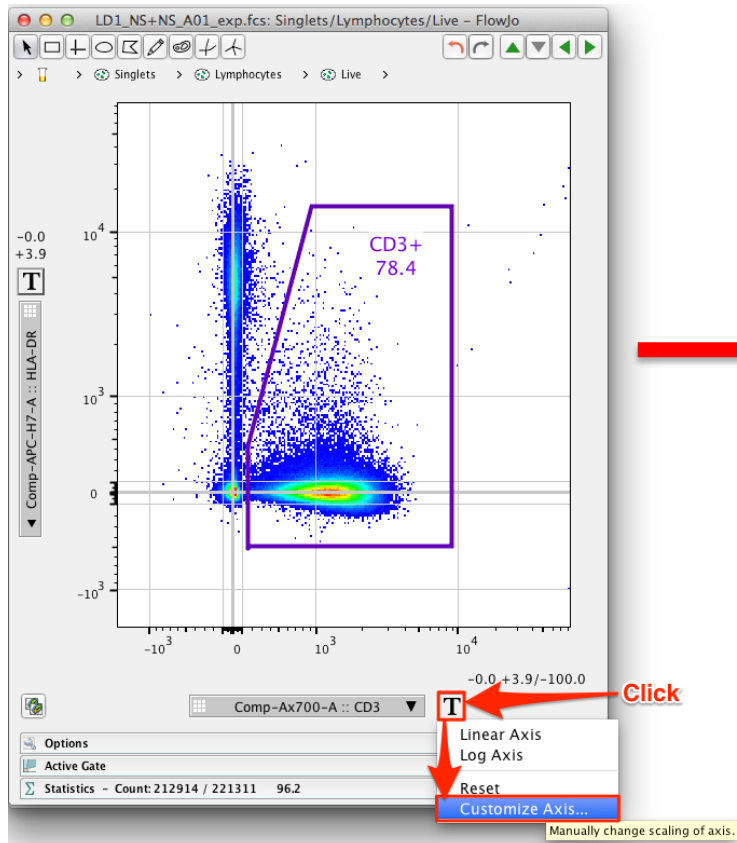
Undo!! 還原動作



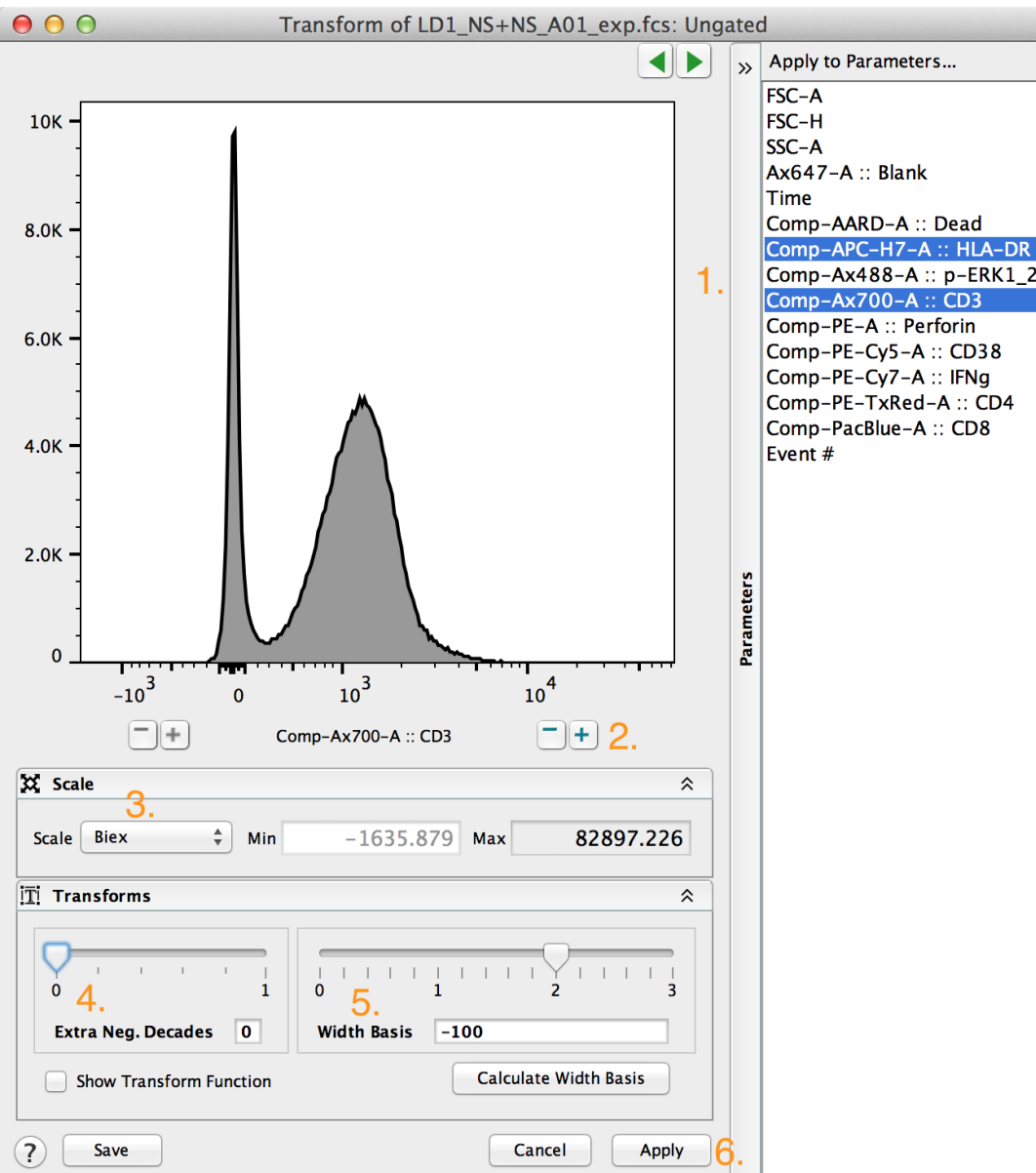
- Gates can always be modified or removed, so don't be shy.
- Explore the gating options and pick what works best for you.

數值轉換功能 Transforming Data

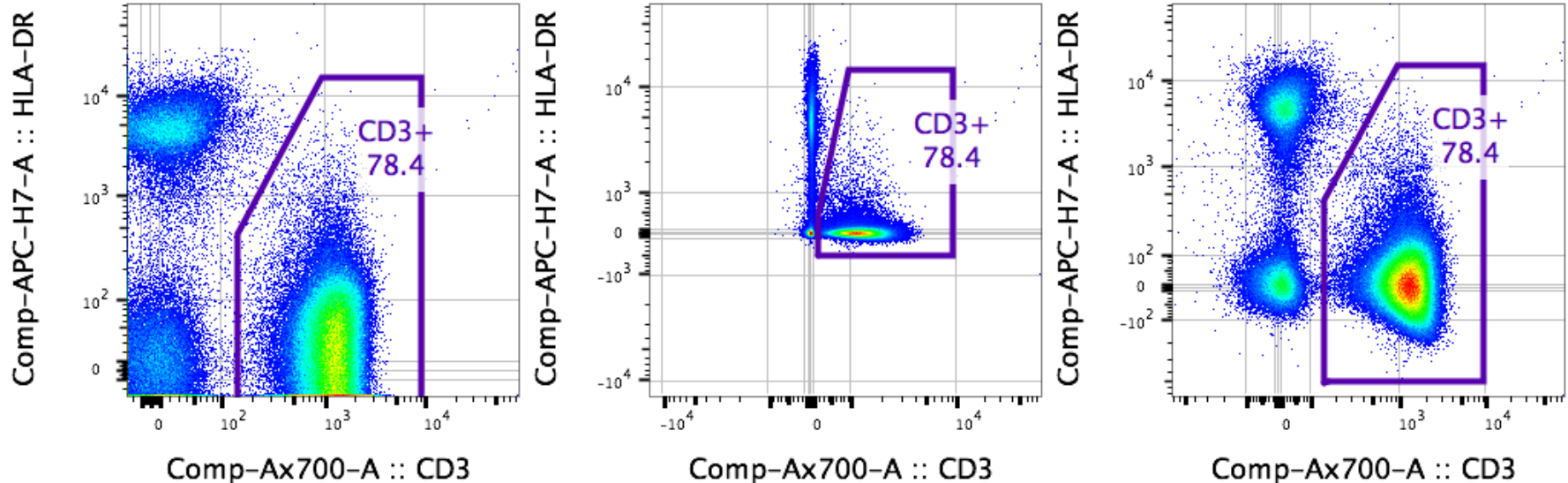
- Your data may initially look 'squished'.
- Click the Transformation [T] button and Select Customize Axis... to change the visual display.



數值轉換選項 Transform Options



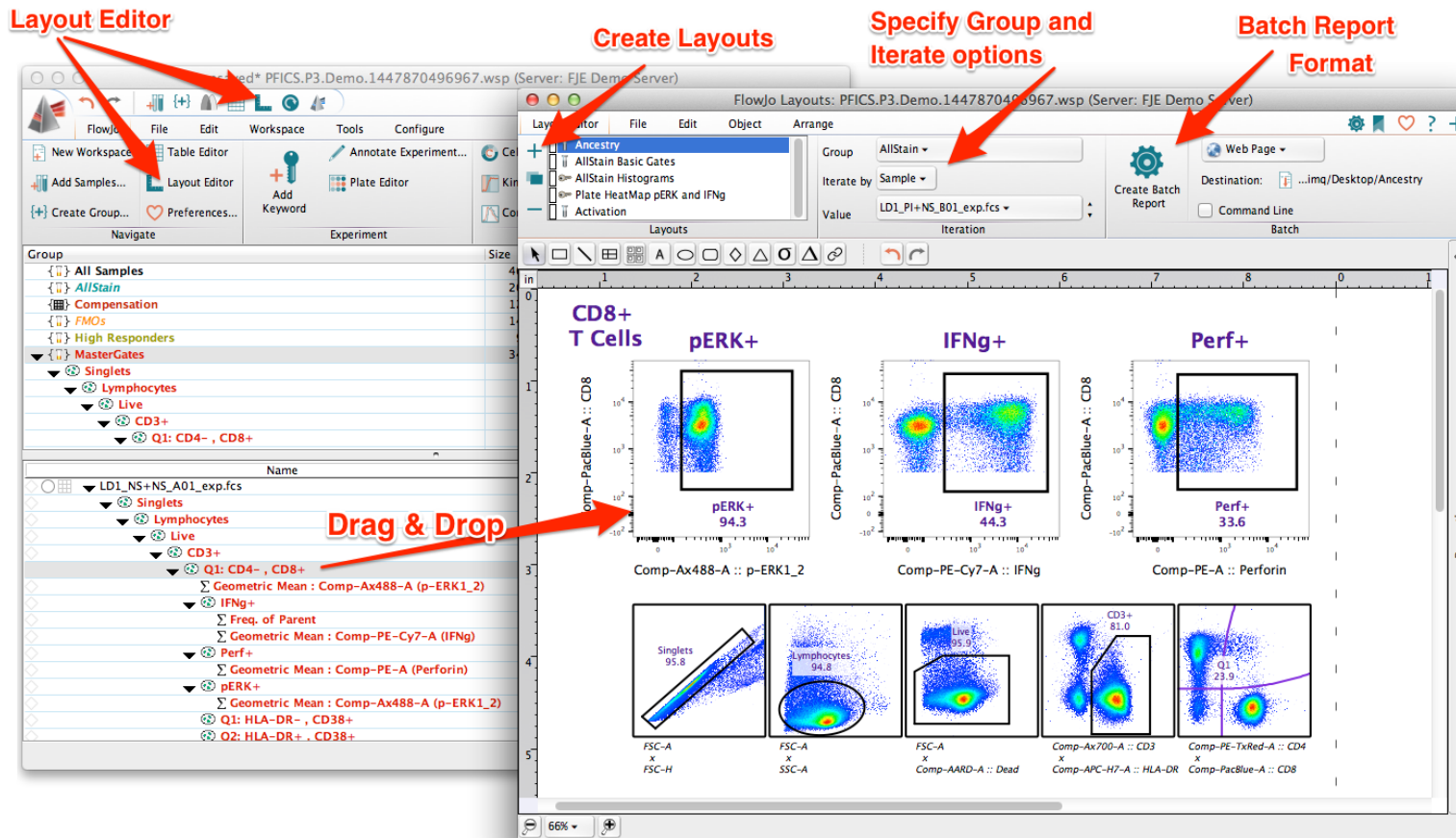
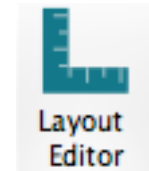
數值轉換效果 Effects of Transformation



1. Gets rid of the “squishing” of cells.
2. Ensures the visual population center better correlates with the statistical center (median).
3. Makes high resolution compensated digital cytometry data more appealing to the eye. 調整數據的視覺尺度表現，一般來說不影響統計數據本身

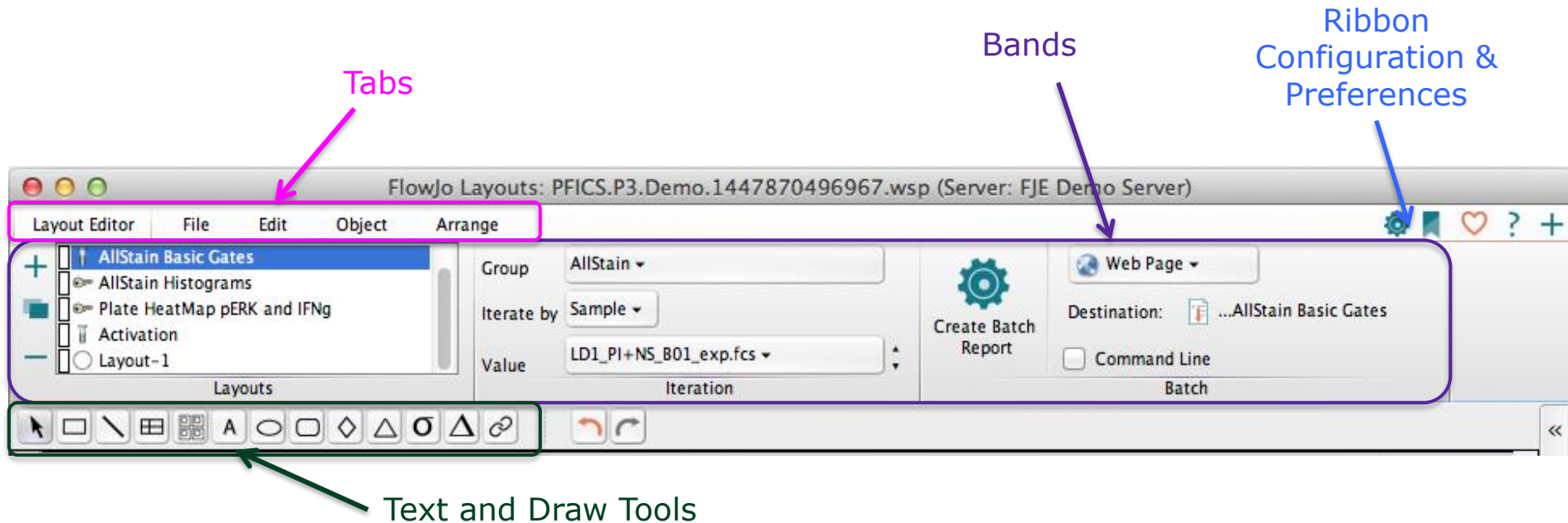
作圖輸出 The Layout Editor

- A tool for creating graphical reports.
- Click on the Layout Editor icon.
- Drag populations from a sample to Layout Editor.



作圖輸出 Working in Layout Editor

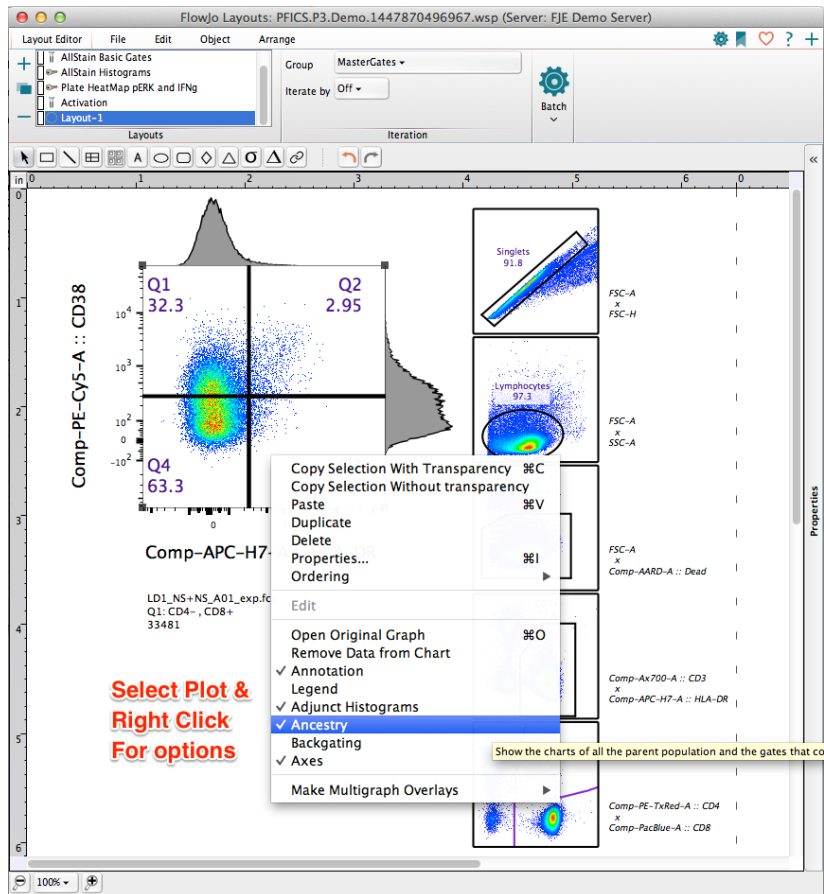
- Similar to the Workspace. Layout Editor has its own customizable Ribbon with Tabs and Bands to organize actions.



- Try clicking on the different tabs to see what types of actions are available.

作圖輸出 Within Layout Editor

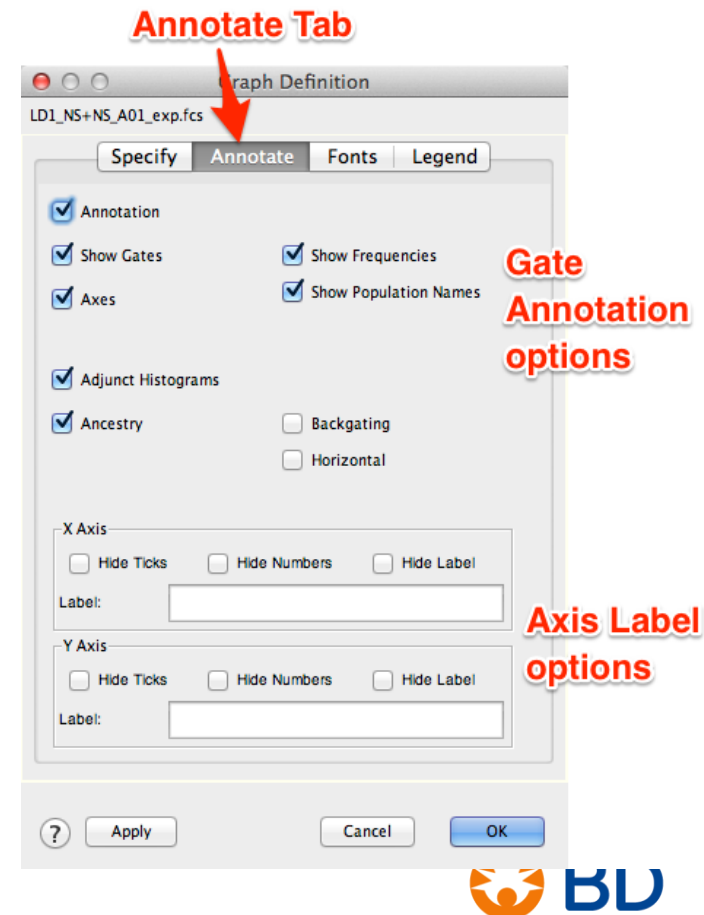
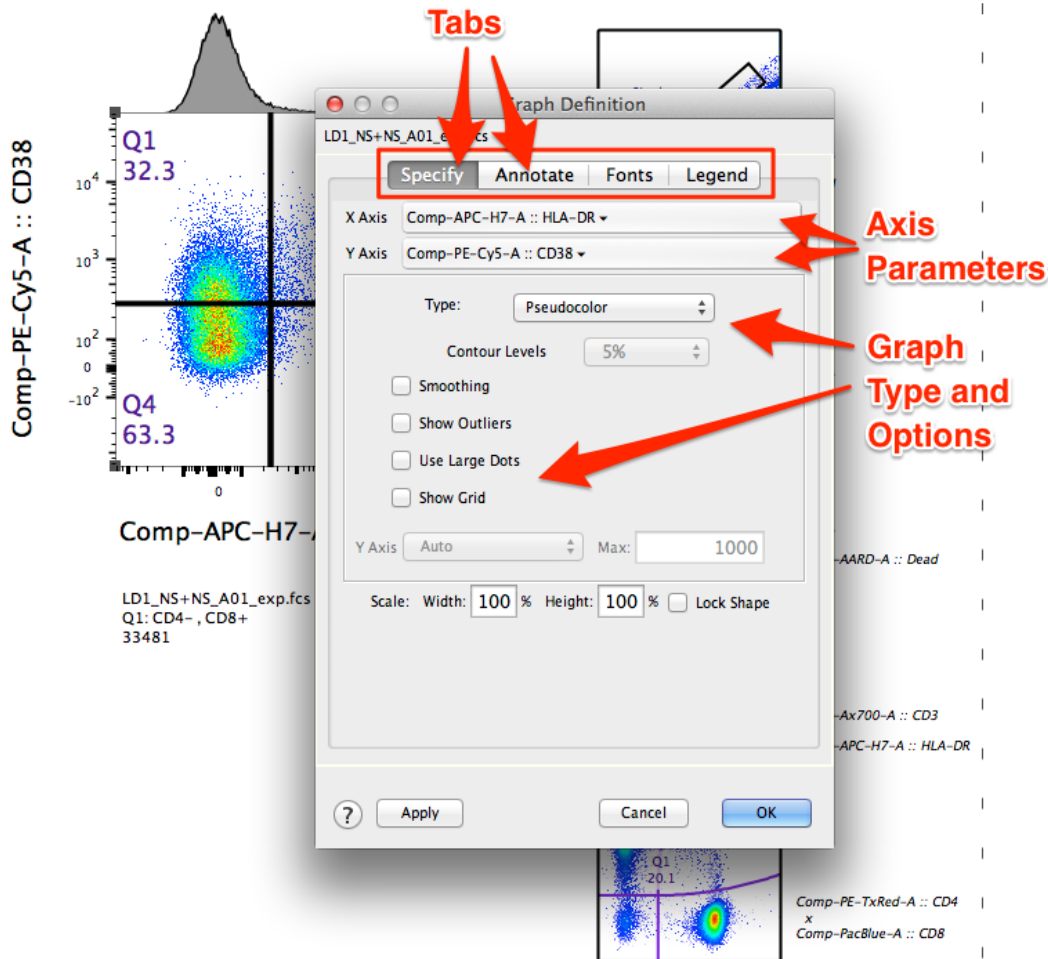
- Graphs can be organized and re-formatted.
- Statistics, keywords, text and even shapes or objects can be added to illustrate your analysis.



- Right Click on a graph plot for Ancestry and Backgating options
- Right click and select Properties for additional graph formatting

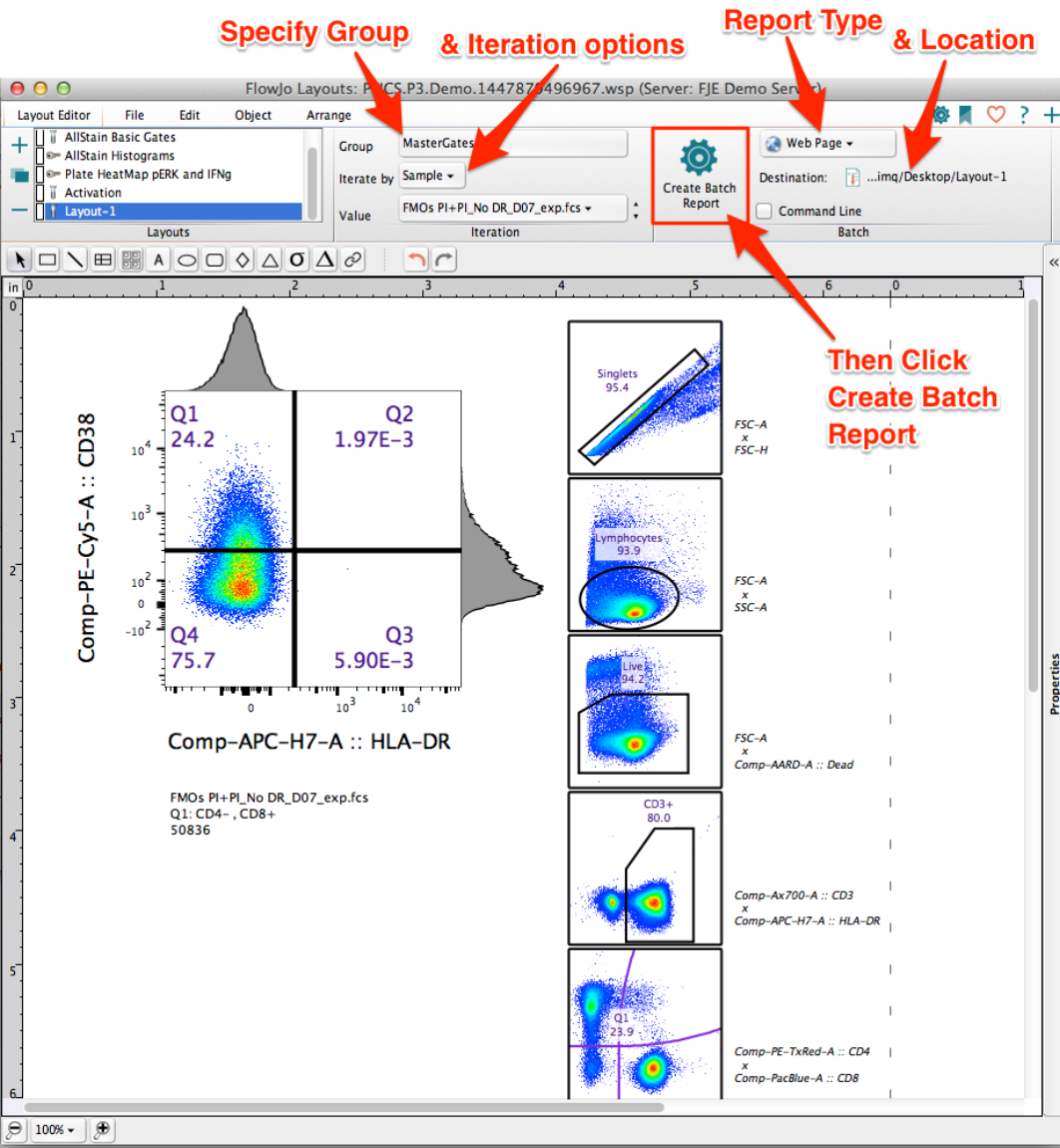
作圖輸出 Working in Layout Editor

- Double Click a graph to change its properties/formatting with 4 tabs of Graph Definition options

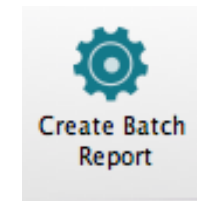


批次報告輸出

Batch Analysis of Layout Editor Graphics

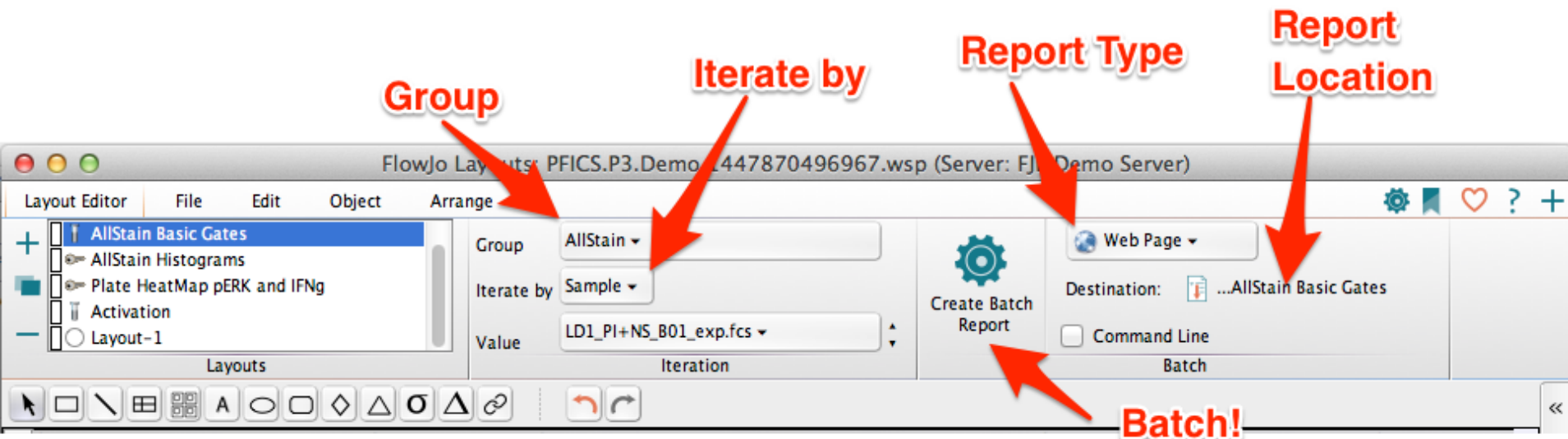
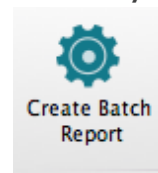


- Batch operations perform repetitive analysis on multiple samples, applying the layout to an entire set of samples.
- Specify Group, Iterate by, Report type and Location, then Click Create Batch Report .



批次報告輸出 Batch Report Layouts

- Specify Group
- Choose Iterate by option
 - Sample
 - Panel
 - Keyword
 - Iterate By (must be Same for all samples displayed in layout)
 - Discriminator (must be Different for all samples displayed in layout)
- Specify type of Report
- Specify Destination to write report



增加統計 Adding Statistics

- Add a statistic to any gated population selected within a sample gating hierarchy.
- Statistic nodes can be group-applied just like a gate.

The screenshot illustrates the steps to add a statistic in FlowJo:

- 1. Select Population:** In the 'Group' tree on the left, select the 'Q1: CD4+, CD8+' population under the 'Live' gate.
- 2. Select Add Statistic...:** Click the 'Add Statistic...' button in the 'Tools' menu.
- 3. Choose Statistic:** In the 'Add Statistic...' dialog, select 'Geometric Mean' from the list of statistics.
- 4. Choose Parameter:** In the 'Add Statistic...' dialog, select 'Comp-PE-A :: Perforin' from the list of parameters.
- 5. Click Add:** Click the 'Add' button in the 'Add Statistic...' dialog to apply the statistic to the selected population.

The 'Statistics' table in the main window shows the following data:

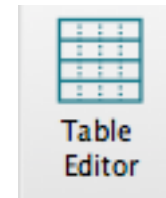
Group	Count	Test
All Samples	46	Test
AllStain	20	Test
Compensation	12	Compensation
FMOs	14	Controls
MasterGates	34	None
PFICSComp	46	Test
PI+PI	5	Test

The 'Statistics' table in the 'Add Statistic...' dialog shows the following data:

Statistic	Count	Test
Median	0.49	162
Mean	0.015	5
Geometric Mean	0.41	136
Robust CV	2.94	980
Robust SD	26.6	8867
CV	1.59	530
SD	67.9	22654
Percentile	1.44	2394
Median Abs Dev	76.6	127512
Freq. of Parent		
Freq. of Grandparent		
Freq. of Total		
Count		
Mode		

統計資料輸出 The Table Editor

- A tool for creating statistical reports.
- Type ⌘ T, or click on the Table Editor icon.
- Drag Populations & Statistics to Table Editor.



Open Table Editor

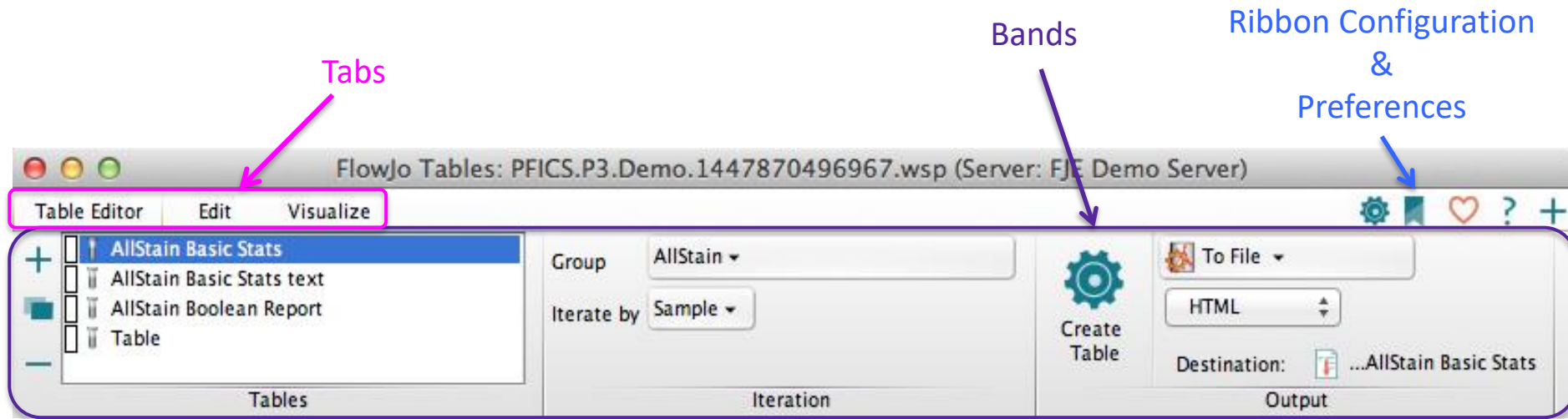
Drag Populations & Statistics

FlowJo Tables: PFICS.P3.Demo.1447870496967.wsp (Server: FJE Demo Server)

Col...	Population	Statistic	Parameter	Name
1	*PID			
2	*STIM			
3	Singlets/Lymphocytes/Live/CD3+/Q1: CD4-, CD8+	Geometric Mean	Comp-Ax488-A	pERK GMF
4	Singlets/Lymphocytes/Live/CD3+/Q1: CD4-, CD8+/IFNγ	Freq. of Parent	% IFNγ+	
5	Singlets/Lymphocytes/Live/CD3+/Q1: CD4-, CD8+/Perf+	Freq. of Parent	% Perf+	
6	Formula			CD4/CD8...
7	Singlets/Lymphocytes/Live/CD3+/Q1: CD4-, CD8+/Q2: HLA-DR+, CD38+	Freq. of Parent	HLA-DR+, ...	
8	Singlets/Lymphocytes/Live/CD3+/Q1: CD4-, CD8+/pERK+	Freq. of Parent	% pERK+	
9	Singlets/Lymphocytes/Live/CD3+/Q1: CD4-, CD8+/IFNγ	Geometric Mean	Comp-PE-Cy7-A	IFNγ GMF
10	Singlets/Lymphocytes/Live/CD3+/Q1: CD4-, CD8+/Perf+	Geometric Mean	Comp-PE-A	Perf GMF
11	Singlets/Lymphocytes/Live	Freq. of Parent	Viability	
12	Singlets/Lymphocytes/Live/CD3+	Freq. of Parent	% CD3+	
13	Singlets/Lymphocytes/Live/CD3+/Q1: CD4-, CD8+	Freq. of Parent	% CD8+	
14	Singlets/Lymphocytes/Live/CD3+/Q3: CD4+, CD8-	Freq. of Parent	% CD4+	

統計資料輸出 Within Table Editor

- Again, the Table Editor has its own customizable Ribbon with Tabs and Bands to organize actions.

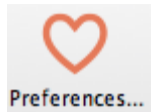


- Specify the group you wish to batch, and how to iterate the batch process, then in the Output band, specify where you want the batch output to go.

視覺化工具 Table Editor Visualize Tools

- Table formatting/visualization options such as heat mapping are contained within the Visualize Tab.

- Highlight row(s), then select the visualization.
- Expected Ranges can be set within Preferences



→ Ranges



FlowJo Tables: PFICS.P3.Demo.1447870496967.wsp (Server: FJE Demo Server)

Table Editor Edit **Visualize**

Heat Map **2. Apply visualization tool**

Standard Deviation

Expected Range NK Cells

Time Series Correlation 3D Plot

Formatting Plots

C...	Population	Statistic	Parameter	Name
1	*PID			
2	*STIM			
3	Singlets/Lymphocytes/Live/CD3+/Q1: CD4- , CD8+	Geometric Mean	Comp-Ax488-A	pERK GMF
4	Singlets/Lymphocytes/Live/CD3+/Q1: CD4- , CD8+ /IFNg+	Freq. of Parent		% IFNg+
5	Singlets/Lymphocytes/Live/CD3+/Q1: CD4- , CD8+ /Perf+	Freq. of Parent		% Perf+
6	Formula			CD4/CD8 R...
7	Singlets/Lymphocytes/Live/CD3+/Q1: CD4- , CD8+/Q2: HLA-DR+ , CD38+	Freq. of Parent		HLA-DR+ ,C...
8	Singlets/Lymphocytes/Live/CD3+/Q1: CD4- , CD8+ /pERK+	Freq. of Parent		% pERK+
9	Singlets/Lymphocytes/Live/CD3+/Q1: CD4- , CD8+ /IFNg+	Geometric Mean	Comp-PE-Cy7-A	IFNg GMF
10	Singlets/Lymphocytes/Live/CD3+/Q1: CD4- , CD8+ /Perf+	Geometric Mean	Comp-PE-A	Perf GMF
11	Singlets/Lymphocytes/Live	Freq. of Parent		Viability
12	Singlets/Lymphocytes/Live/CD3+	Freq. of Parent		% CD3+
13	Singlets/Lymphocytes/Live/CD3+/Q1: CD4- , CD8+	Freq. of Parent		% CD8+
14	Singlets/Lymphocytes/Live/CD3+/Q3: CD4+ , CD8-	Freq. of Parent		% CD4+

1. Highlight Rows

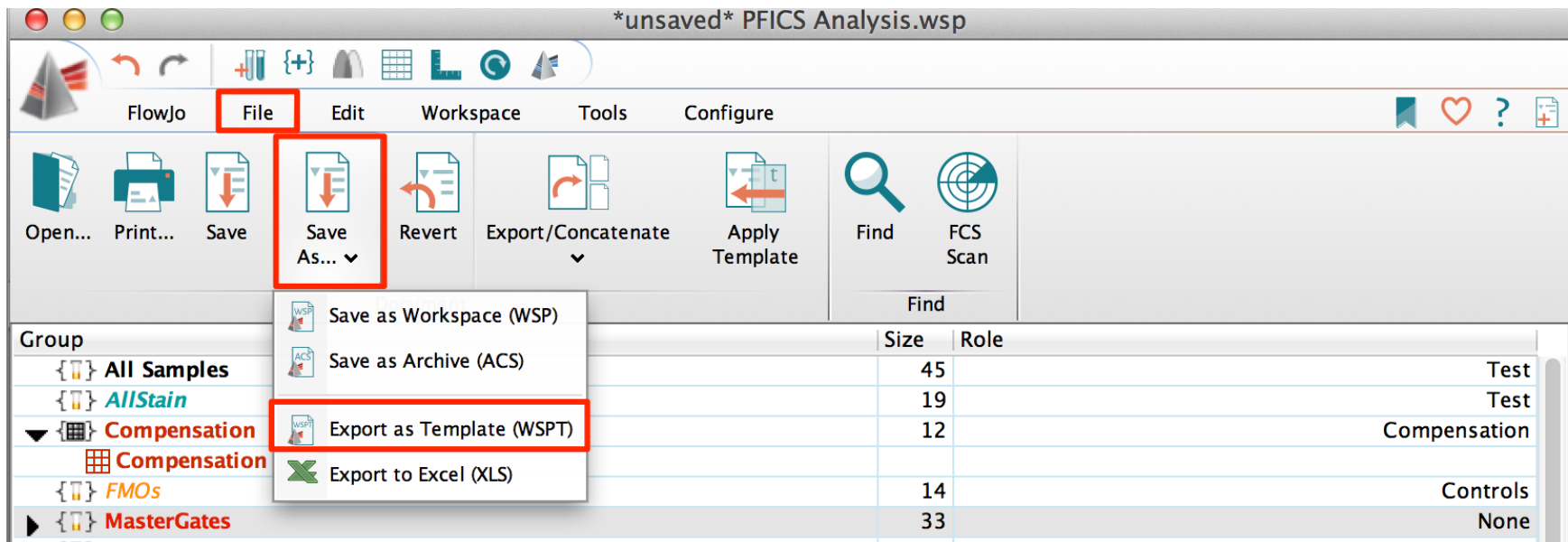
視覺化統計表 Table Editor Output

- Formatting/visualization options are maintained when a table is batched to either Display or HTML formats.
- Other file types (ex. Text, CSV, Excel) produce statistics tables lacking visualization formatting.

Ancestry Subset Statistic For	*PID	*STIM	pERK GMF	% IFNg+	% Perf+	CD4/CD8 Ratio	HLA-DR+,	% pERK+	IFNg GMF	Perf GMF
LD1_NS...	LD1	NS+NS	74.1	1.09	30.2	▲ 3.81	2.95	4.70	642	812
LD1_NS...	LD1	NS+PI	503	0.96	30.0	▲ 4.13	2.72	94.9	504	809
LD1_PI+...	LD1	PI+NS	375	44.3	33.6	▲ 3.04	2.26	94.3	4917	807
LD1_PI+...	LD1	PI+PI	373	43.8	32.7	▲ 3.06	1.94	94.5	4907	816
LD2_NS...	LD2	NS+NS	75.6	1.83	55.9	2.80	2.07	0.45	509	818
LD2_NS...	LD2	NS+PI	496	1.91	53.4	▲ 3.01	1.87	91.0	425	752
LD2_PI+...	LD2	PI+NS	420	64.0	52.1	▲ 2.86	1.27	92.6	5894	739
LD2_PI+...	LD2	PI+PI	407	63.7	51.4	▲ 2.91	1.46	92.7	5768	734
LD4_NS...	LD4	NS+NS	86.6	1.05	21.1	1.52	2.71	8.08	494	740
LD4_NS...	LD4	NS+PI	596	1.74	23.6	1.52	2.80	97.1	403	775
LD4_PI+...	LD4	PI+NS	456	28.2	23.8	▼ 1.21	1.74	96.8	5298	577
LD4_PI+...	LD4	PI+PI	449	26.5	22.6	▼ 1.22	1.48	96.4	5035	566
LD12_N...	LD12	NS+NS	67.5	0.74	37.5	▲ 3.64	2.93	4.14	755	440
LD12_N...	LD12	NS+PI	414	0.50	35.3	▲ 4.28	3.19	89.3	683	444
LD12_PL...	LD12	PI+NS	327	45.3	40.8	1.94	1.50	84.8	4632	408
LD12_PL...	LD12	PI+PI	319	46.1	41.4	1.94	1.64	83.7	4793	403
LD14_N...	LD14	NS+NS	72.4	0.50	14.3	2.11	1.90	4.11	689	811
LD14_N...	LD14	NS+PI	483	0.45	13.8	2.30	2.19	95.5	595	829
LD14_PL...	LD14	PI+NS	366	17.7	18.2	1.66	1.21	94.8	3708	650
LD14_PL...	LD14	PI+PI	351	17.0	18.3	1.67	1.10	93.2	3565	644
Mean			336	20.4	32.5	2.53	2.05	70.7	2711	679
SD			167	23.0	13.4	0.96	0.65	39.5	2259	152

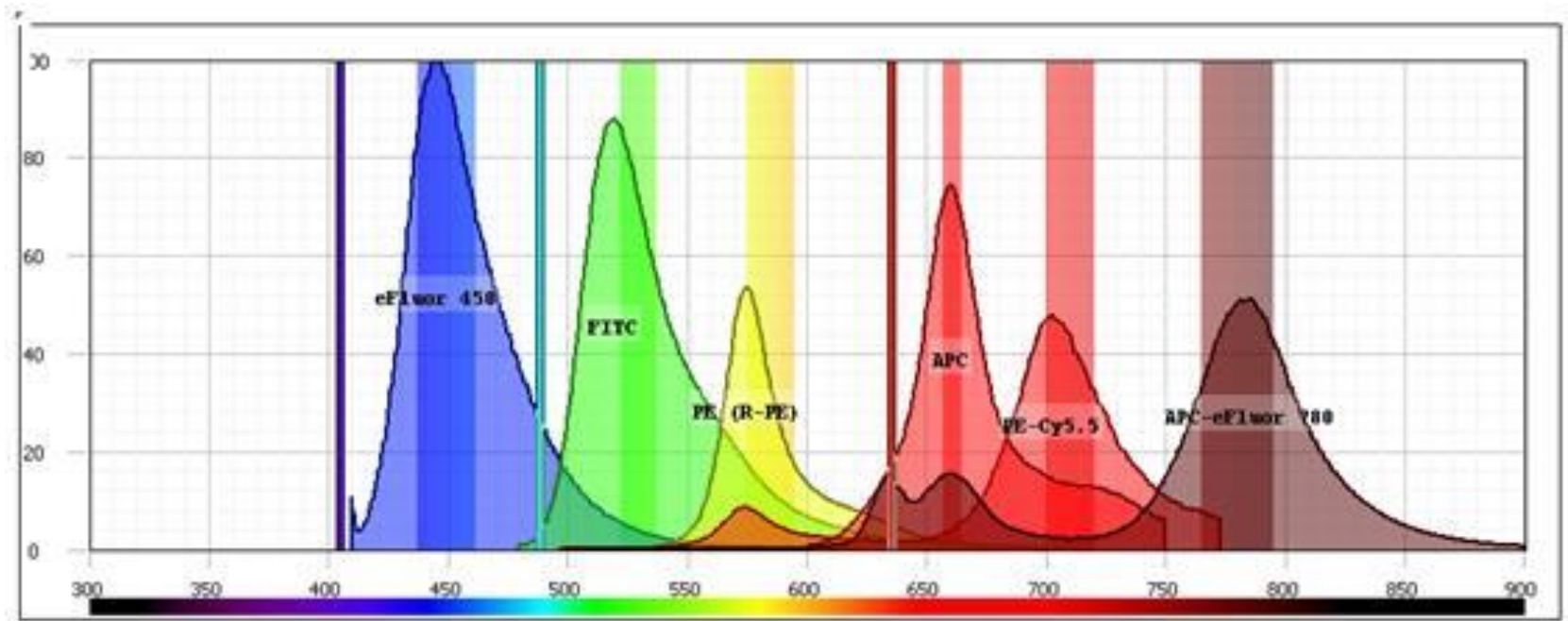
儲存分析模板 Workspace Templates

- Allows saving all analysis reports in your workspace without data.
- Streamlines repetitive analysis of multiple runs using the same staining panel(s).
- File Tab → Document Band → Save As... → **Export as a Template (WSPT)**



螢光補償 Compensation

- Compensation corrects for spillover between fluorochrome emission spectra.



- Compensation is essential for multicolor panels.

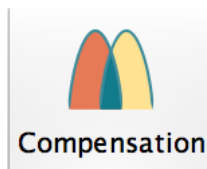
Three Rules of Compensation

- First, there must be a single stained control for every fluorescent parameter in the experiment!
- In Addition, there are three *rules* for 'good' compensation controls.
 1. Controls need to be at least as bright or brighter than any sample the compensation will be applied to.
 2. Background fluorescence should be the same for the positive and negative control.
 3. Compensation controls **MUST** match the exact experimental fluorochrome.

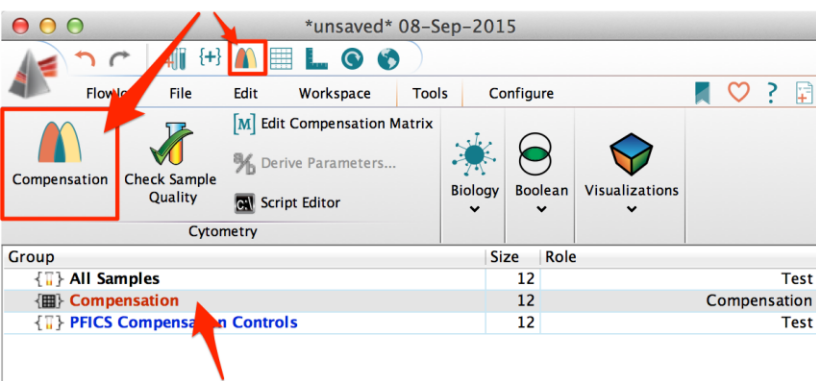
http://flowjo.typepad.com/the_daily_dongle/2011/09/three-rules-for-compensation-controls.html

螢光補償 Compensation I

- Select a Compensation Group in the groups window, then click in the task bar.



2. Click the Compensation Tool



1. Highlight Compensation Group

	Name	Statistic	
☐	Bead Comps_DR APC-H7_F04_exp.fcs (Control)		
☐	Bead Comps_ERK A488_F06_exp.fcs (Control)		
☐	Bead Comps_IFN PE-Cy7_F07_exp.fcs (Control)		
☐	Bead Comps_Perforin PE_F08_exp.fcs (Control)		
☐	Bead Comps_US Beads +FP_F05_exp.fcs (Control)	30000	
☐	Bead Comps_US Beads No FP_F09_exp.fcs (Control)	10290	
☐	Bead Comps_4 PE-TR_F01_exp.fcs (Control)	19202	
☐	Bead Comps_8 PB_F02_exp.fcs (Control)	14969	
☐	Bead Comps_38 PE-Cy5_F03_exp.fcs (Control)	17603	
☐	Cell Comps_AARD_E01_exp.fcs (Control)	145743	
☐	Cell Comps_CD3 A700_E02_exp.fcs (Control)	129537	
☐	Cell Comps_US Cells_E03_exp.fcs (Control)	158360	

The wizard auto gates samples

Group	Size	Role
{ } All Samples	12	Test
▼ { } Compensation	12	Compensation
{ } PFICS Compensation Controls	12	Test

Name	Statistic	#Cells
▼ Bead Comps_DR APC-H7_F04_exp.fcs (Control)		18907
Size	60.3	11396
APC-H7-A+	42.8	4873
▼ Bead Comps_ERK A488_F06_exp.fcs (Control)		24114
Size	66.8	16113
Ax488-A+	47.1	7593
▼ Bead Comps_IFN PE-Cy7_F07_exp.fcs (Control)		30000
Size	70.4	21132
PE-Cy7-A+	52.5	11095
▼ Bead Comps_Perforin PE_F08_exp.fcs (Control)		19212
Size	71.0	13645
PE-A+	55.4	7559
▼ Bead Comps_US Beads +FP_F05_exp.fcs (Control)		30000
Size	70.7	21206
Ax647-A+	100.0	21197
▼ Bead Comps_US Beads No FP_F09_exp.fcs (Control)		10290
Size	76.4	7859
▼ Bead Comps_4 PE-TR_F01_exp.fcs (Control)		19202
Size	66.1	12699
PE-TxRed-A+	48.9	6205
▼ Bead Comps_8 PB_F02_exp.fcs (Control)		14969
Size	66.7	9988

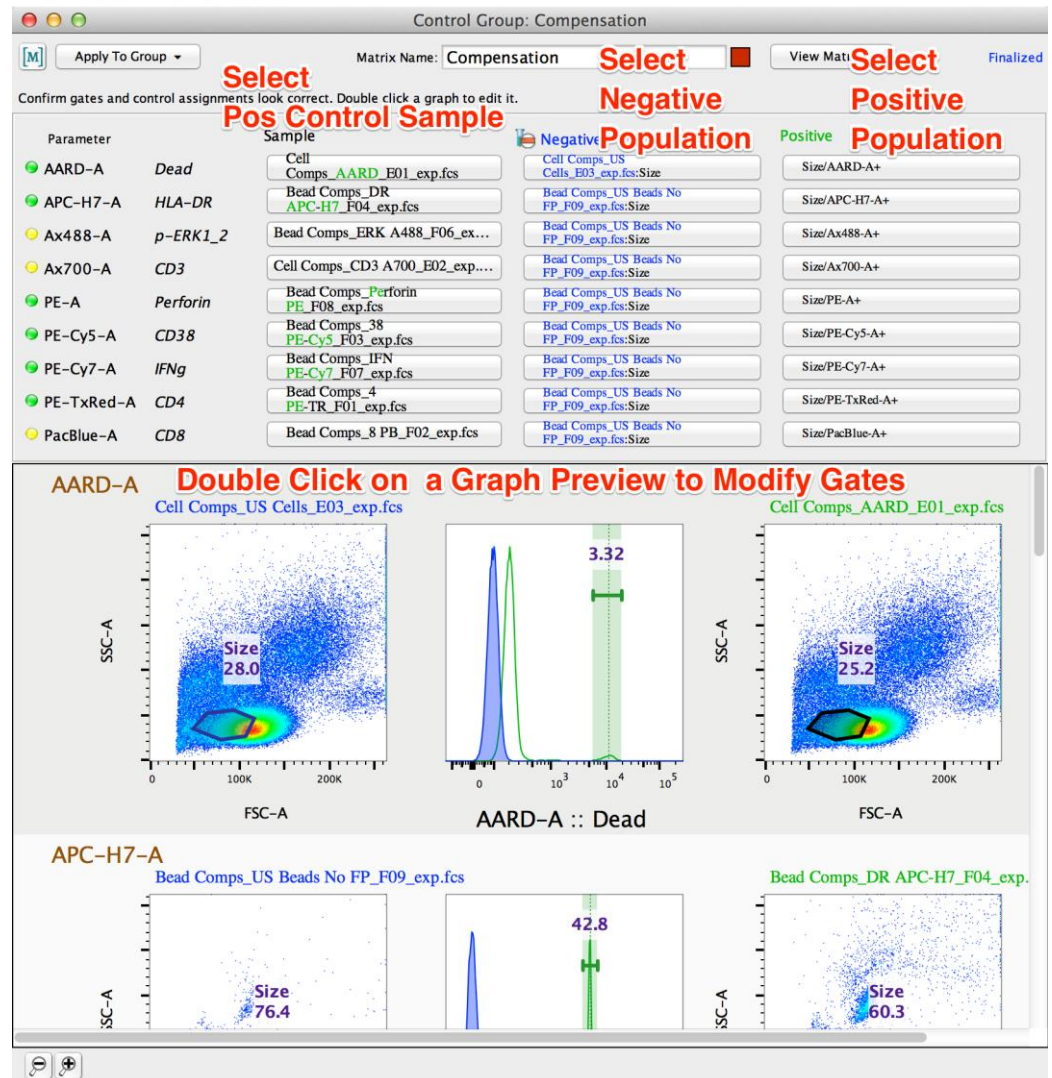
螢光補償 Compensation II

- Define positive and negative sample populations.

- Choose from the dropdown lists for each parameter, or drag and drop to populate fields.

- Double click graph preview to modify gates.

For each Parameter



Control Group: Compensation

[M] Apply To Group ▾ Matrix Name: Compensation View Matrix... Finalized

Confirm gates and control assignments look correct. Double click a graph to edit it.

Parameter	Sample	Negative	Positive
AARD-A Dead	Cell Comps AARD_E01_exp.fcs	Cell Comps_US Cells_E03_exp.fcs:Size	Size/AARD-A+

Use Sample drop down list

to select Pos Control Sample and

Choose or Remove Parameters

Bead Comps_DR APC-H7_F04_exp.fcs
Bead Comps_ERK A488_F06_exp.fcs
Bead Comps_IFN PE-Cy7_F07_exp.fcs
Bead Comps_Perforin PE_F08_exp.fcs
Bead Comps_US Beads +FP_F05_exp.fcs
Bead Comps_US Beads No FP_F09_exp.fcs
Bead Comps_4 PE-TR_F01_exp.fcs
Bead Comps_8 PB_F02_exp.fcs
Bead Comps_38 PE-Cy5_F03_exp.fcs
Cell Comps_AARD_E01_exp.fcs
Cell Comps_CD3 A700_E02_exp.fcs
Cell Comps_US Cells_E03_exp.fcs

<Clear>
<Remove This Parameter>
Choose Parameters
<Reset All>
<Reset This Parameter>

Use Negative drop down list

to Select Negative Sample or Population

Bead Comps_DR APC-H7_F04_exp.fcs :: Size
Bead Comps_ERK A488_F06_exp.fcs :: Size
Bead Comps_IFN PE-Cy7_F07_exp.fcs :: Size
Bead Comps_Perforin PE_F08_exp.fcs :: Size
Bead Comps_US Beads +FP_F05_exp.fcs :: Size
Bead Comps_US Beads No FP_F09_exp.fcs :: Size
Bead Comps_4 PE-TR_F01_exp.fcs :: Size
Bead Comps_8 PB_F02_exp.fcs :: Size
Bead Comps_38 PE-Cy5_F03_exp.fcs :: Size
Cell Comps_AARD_E01_exp.fcs :: Size
Cell Comps_US Cells_E03_exp.fcs :: Size

Size
Size/AARD-A+
<Clear>

Use Positive drop down list

to Choose Positive population

Size
Size/AARD-A+
<Clear>

- Note that you can always create your own gates on a sample and then choose those from the drop down menus.
- When set up is complete, select View Matrix (top right) to Modify, Apply, Save or Preview the matrix you've created.

螢光補償 Compensation III

- Preview and apply the calculated matrix

Select Color **Name Matrix** **Edit Matrix** **Save a copy of the Matrix**

Apply Matrix with Drag and Drop onto Group or Sample

Applied Matrix Badge is Color Coded

Add a Matrix from file

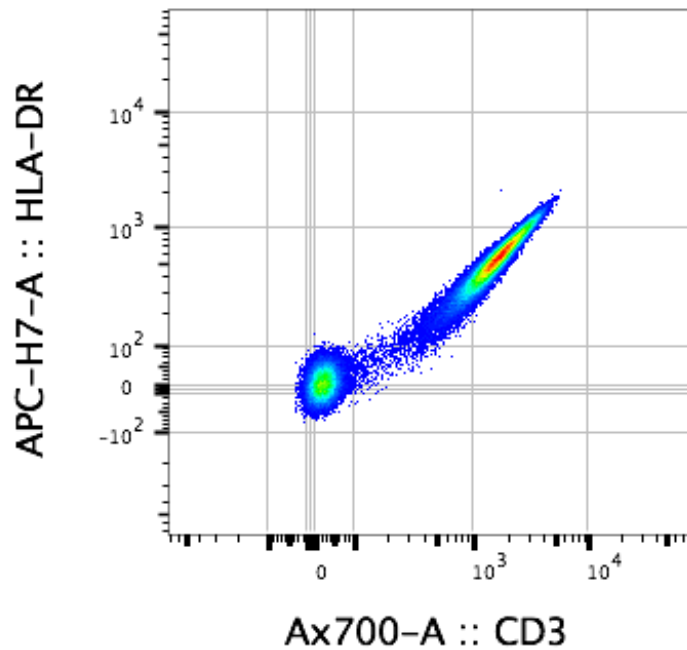
Preview Matrix affect on a sample

Matrix	AARD-A :: Dead	APC-H7-A :: HLA-DR	Ax488-A :: p-ERK1_2	Ax700-A :: CD3	PE-A :: Perforin	PE-Cy5-A :: CD38	PE-Cy7-A :: IFNg	PE-TxRed-A :: CD4	PacBlue-A :: CD8
AARD-A :: Dead	100	0.0351	0.3746	0.0685	0.0382	0.1447	0.0399	0.064	24.1599
APC-H7-A :: HLA-DR	0	100	0	3.2511	0.0169	0.8078	39.6125	0.056	0
Ax488-A :: p-ERK1_2	1.8492	0	100	0	0.0119	0	0	0	0
Ax700-A :: CD3	0.1713	34.835	0.1071	100	0	1.0301	10.2007	0	0.0443
PE-A :: Perforin	0	0.0125	0.3404	0.0375	100	14.4881	1.3119	37.6694	0
PE-Cy5-A :: CD38	0	3.0045	0.0253	7.7547	1.6106	100	12.018	0.7082	0
PE-Cy7-A :: IFNg	0	5.7598	0.0603	0.3117	1.8877	0.368	100	0.8245	0
PE-TxRed-A :: CD4	0	0.0291	0.1118	0.0572	23.9323	52.786	6.0459	100	0
PacBlue-A :: CD8	16.9144	0	0.0597	0	0.0076	0	0	0.0063	100

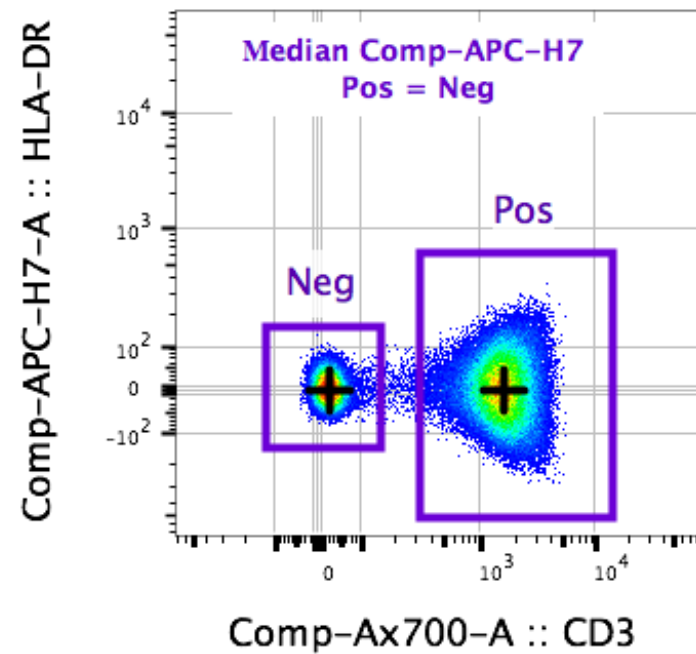
螢光補償 Compensation - Effects



Uncompensated



Compensated



Thank you!



新加坡商必帝公司 生物科學部
Product Specialist 產品專員
Mail: mandy.chiang@bd.com
Website: www.bdbiosciences.com