FlowJo Software Analysis
- Basic & Advanced Function

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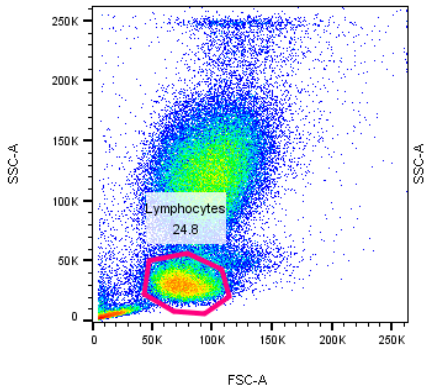
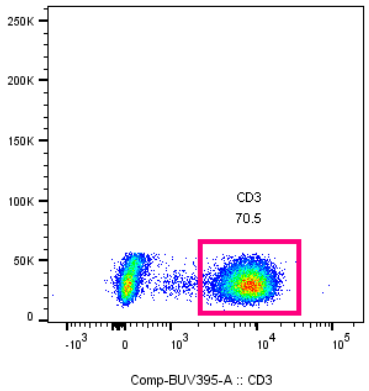
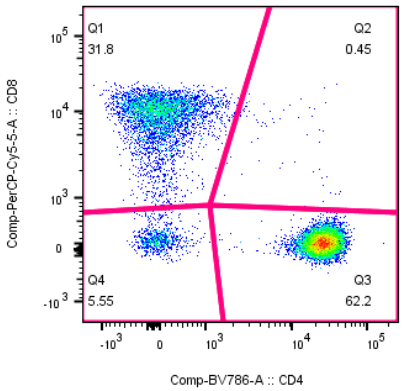
Flow Data Analysis

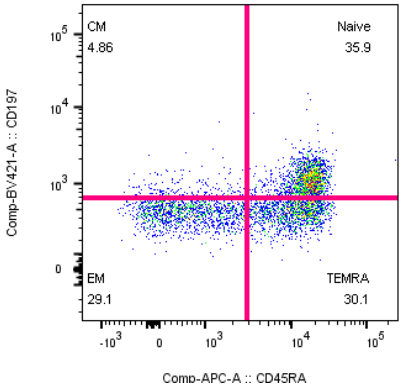
- **分析圈選階層**
  (Population Hierarchy)

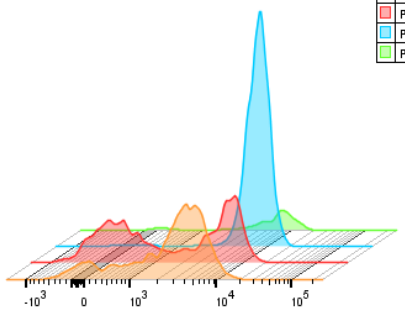
- Panel 6 10 color Sirigen_Tube_001_020.fcs
  - Lymphocytes
    - CD3
      - Q1: CD4- , CD8+
        - CM
          - Q1: CD27- , CD28+
          - Q2: CD27+ , CD28+
          - Q3: CD27+ , CD28-
          - Q4: CD27- , CD28-
        - EM
        - Naive
        - TEMRA
      - Q2: CD4+ , CD8+
      - 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-







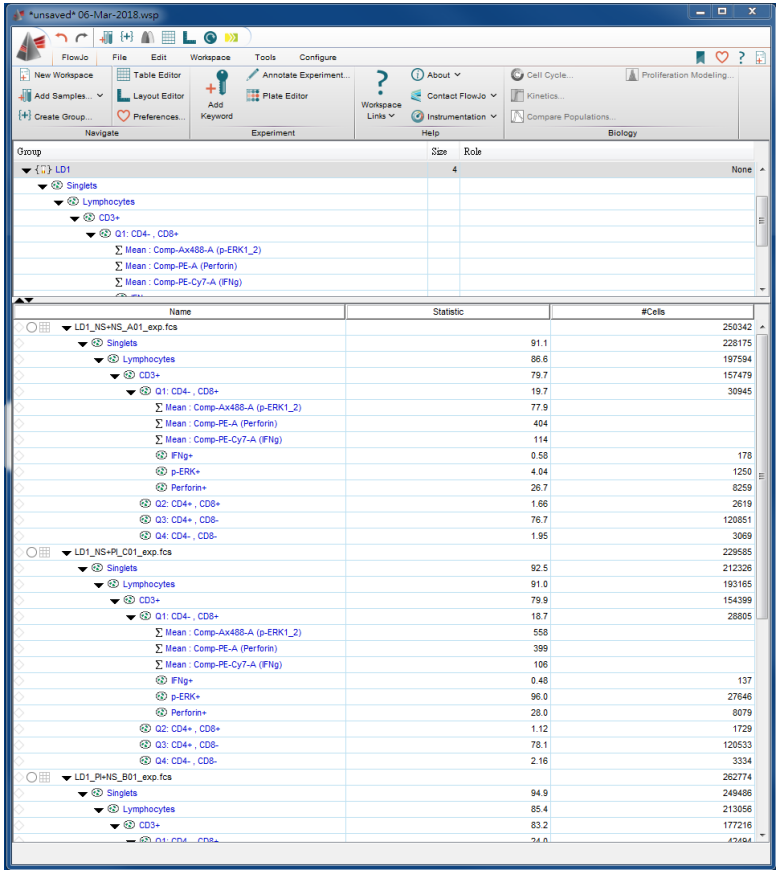




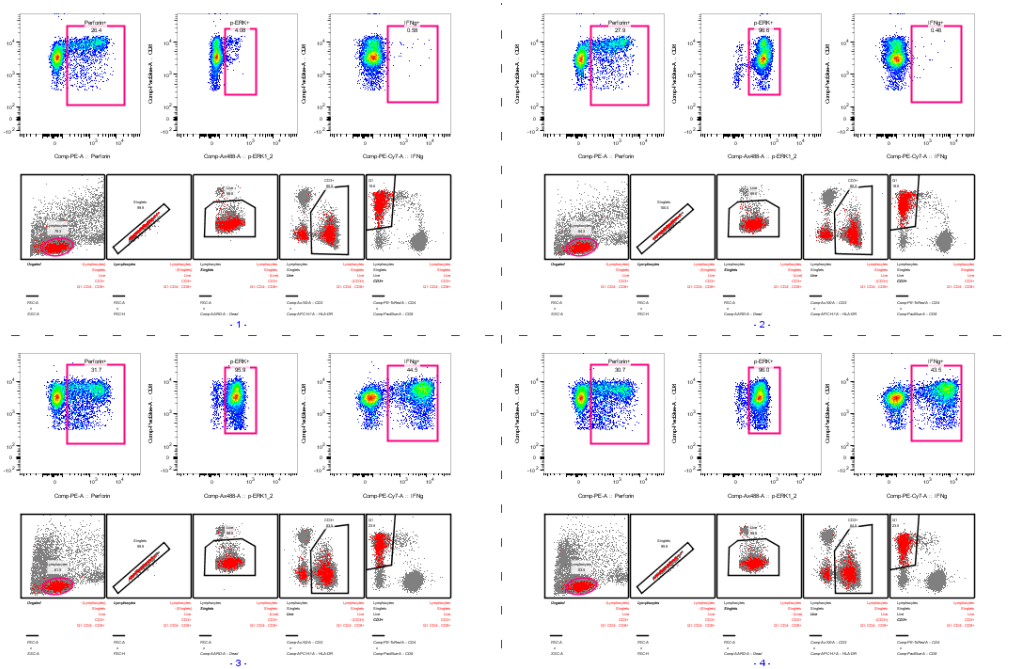
	Sample Name	Subset Name	Count
	Panel 6 10 color Sirigen_Tube_001_020.fcs	EM	1346
	Panel 6 10 color Sirigen_Tube_001_020.fcs	TEMRA	1393
	Panel 6 10 color Sirigen_Tube_001_020.fcs	Naive	1661
	Panel 6 10 color Sirigen_Tube_001_020.fcs	CM	225

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-R 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	106	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*

BD

FLOWJO, LLC

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FlowJo is now a wholly owned subsidiary of BD.

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

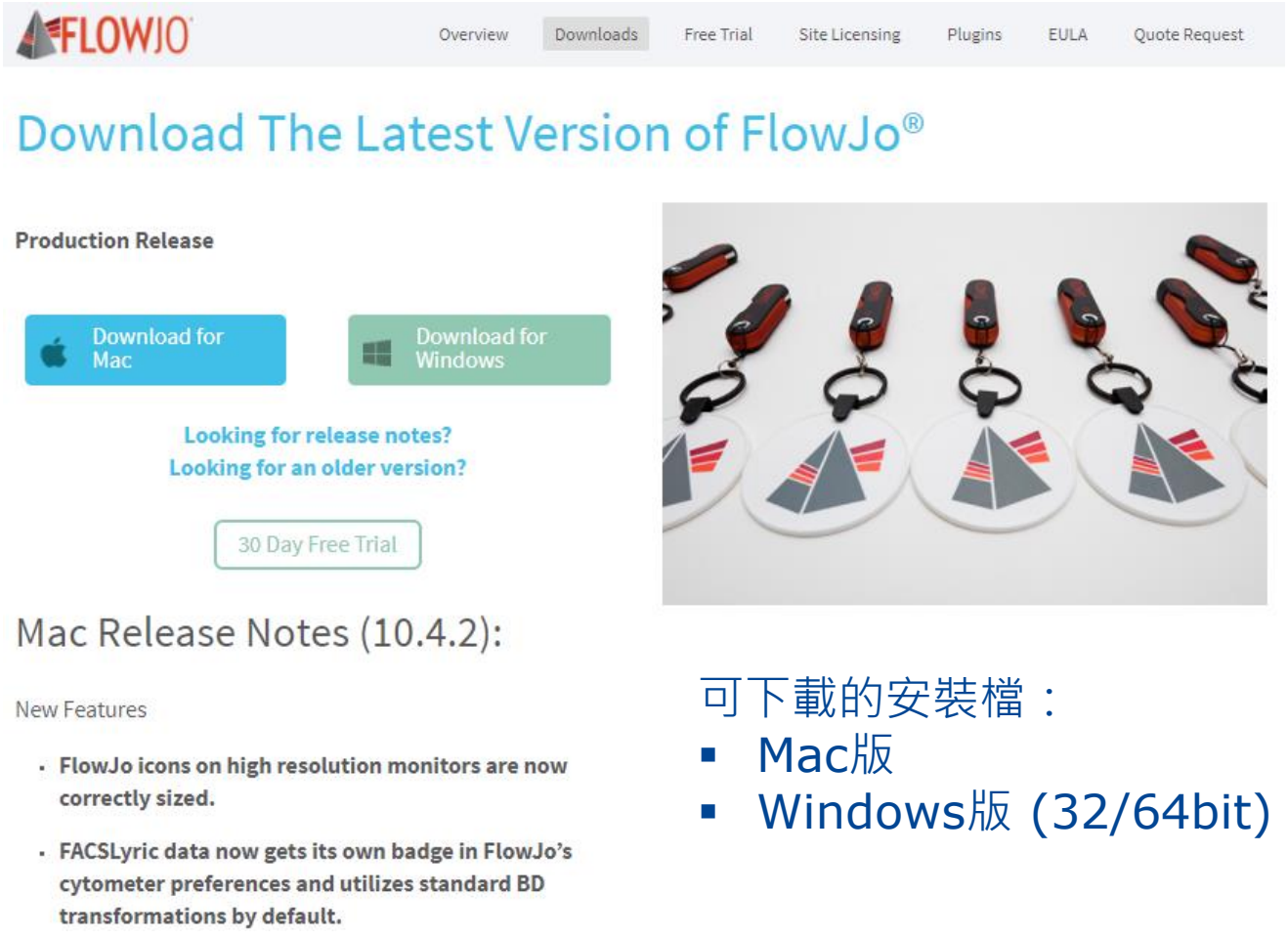
BD於2017年11月收購FlowJo, LLC
目前FlowJo是BD的全資子公司

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

BD

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**

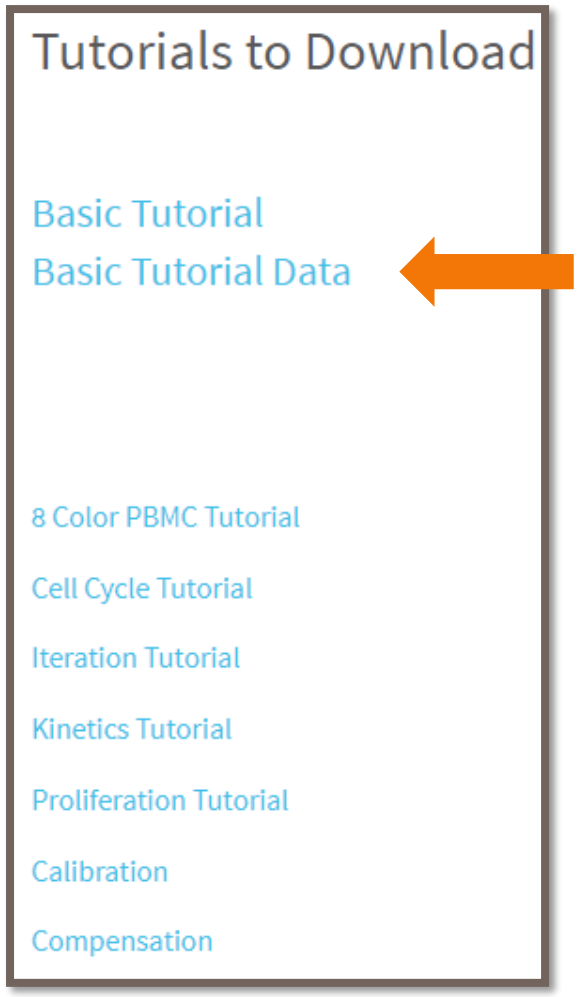


可下載的安裝檔：

- Mac版
- Windows版 (32/64bit)

Download DEMO data

1. **FlowJo 網站 → Download** (頁面下方)
2. 點擊 Tutorials to Download 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

Todays Demo Data Set: Phospho-Flow + Intracellular Cytokine Staining (PFICS)

Polyclonal PFICS Assay:

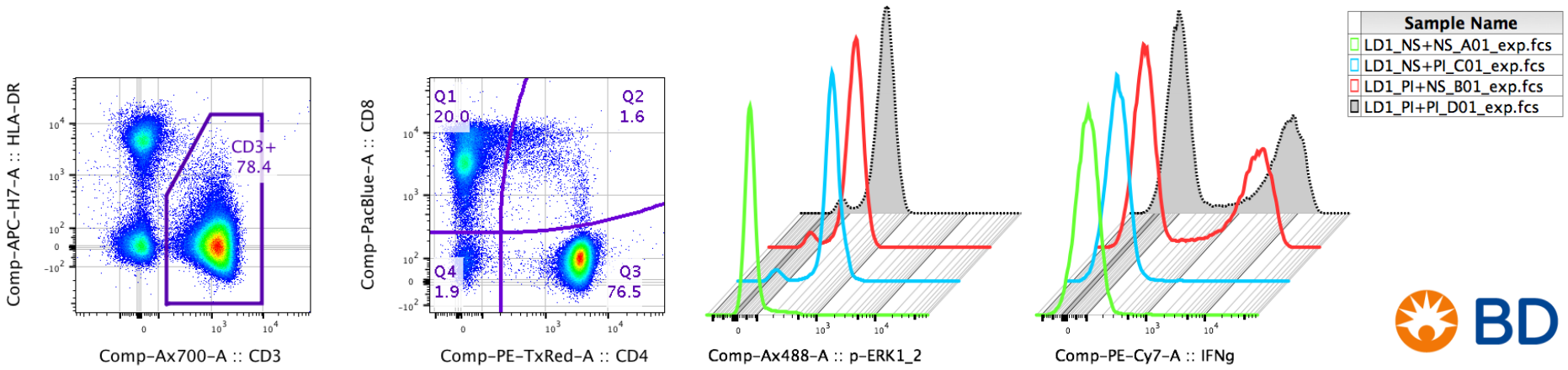
- Thaw and rest cryopreserved human PBMC overnight
- Stimulate with PMA+Ionomycin (PI) for 2 hours or rest (NS) while blocking protein secretion → signaling and cytokines
- Stain for viability (AARD) and surface antigens (CD3, CD4, CD8, CD38 and HLA-DR)
- Stimulate PI for 20 minutes or NS rest
- Fix,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

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

PFICS Stim Conditions

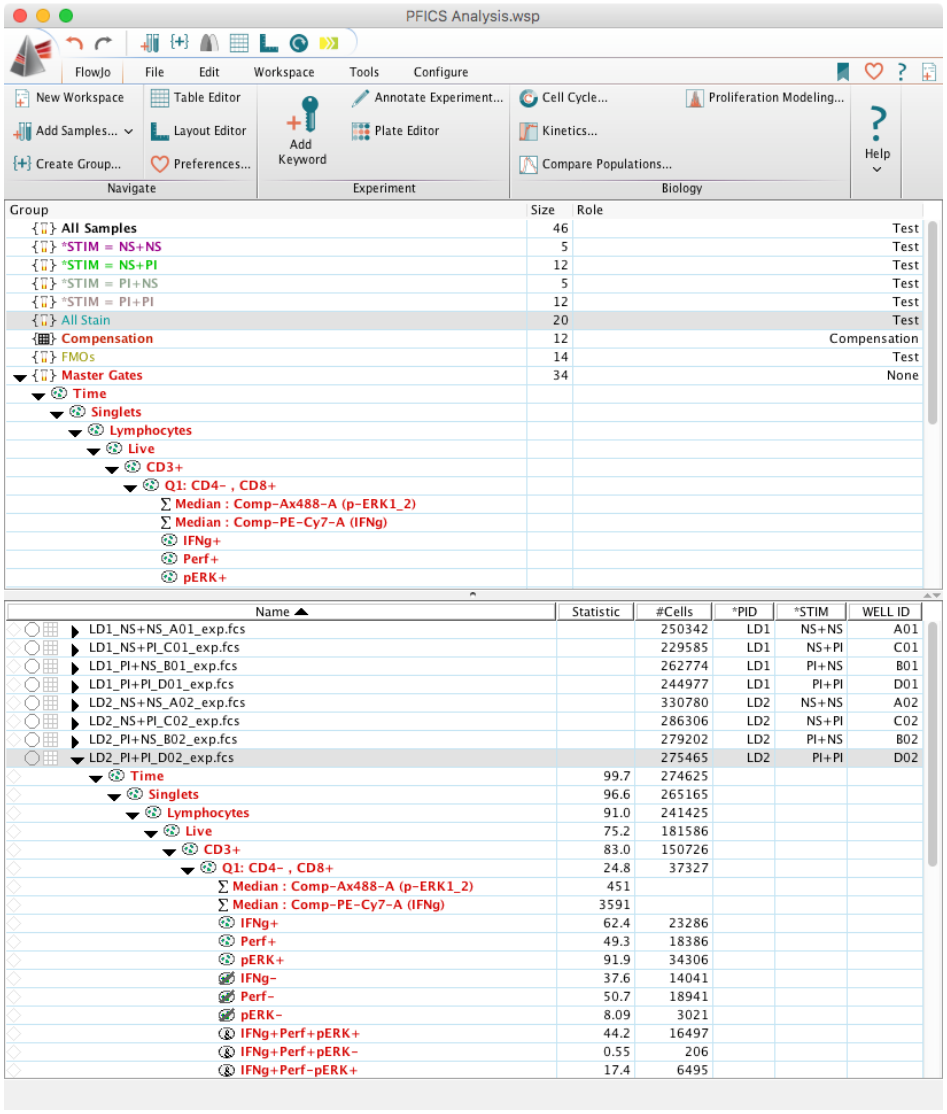
- 2 Stimulations → 4 potential *STIM combinations/conditions

*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	+++	+++



工作介面 The FlowJo v10 Workspace

- An interface to organize your data and initiate actions.



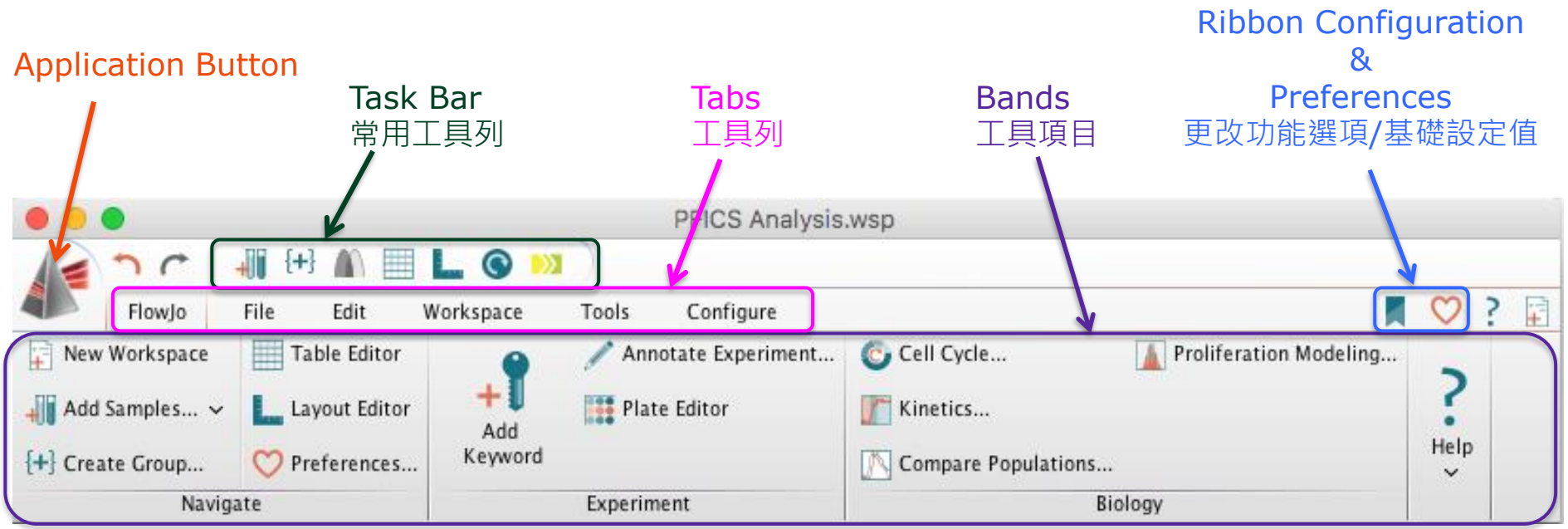
Ribbon
Tabs and Bands
功能介面

Groups and Group Analysis
群組介面

Samples and sample analysis
樣品介面

功能介面 Ribbons, Tabs and Bands

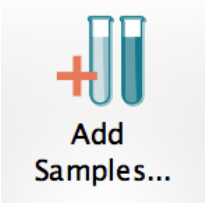
- Ribbon organization allows easy visual navigation of workspace functions.

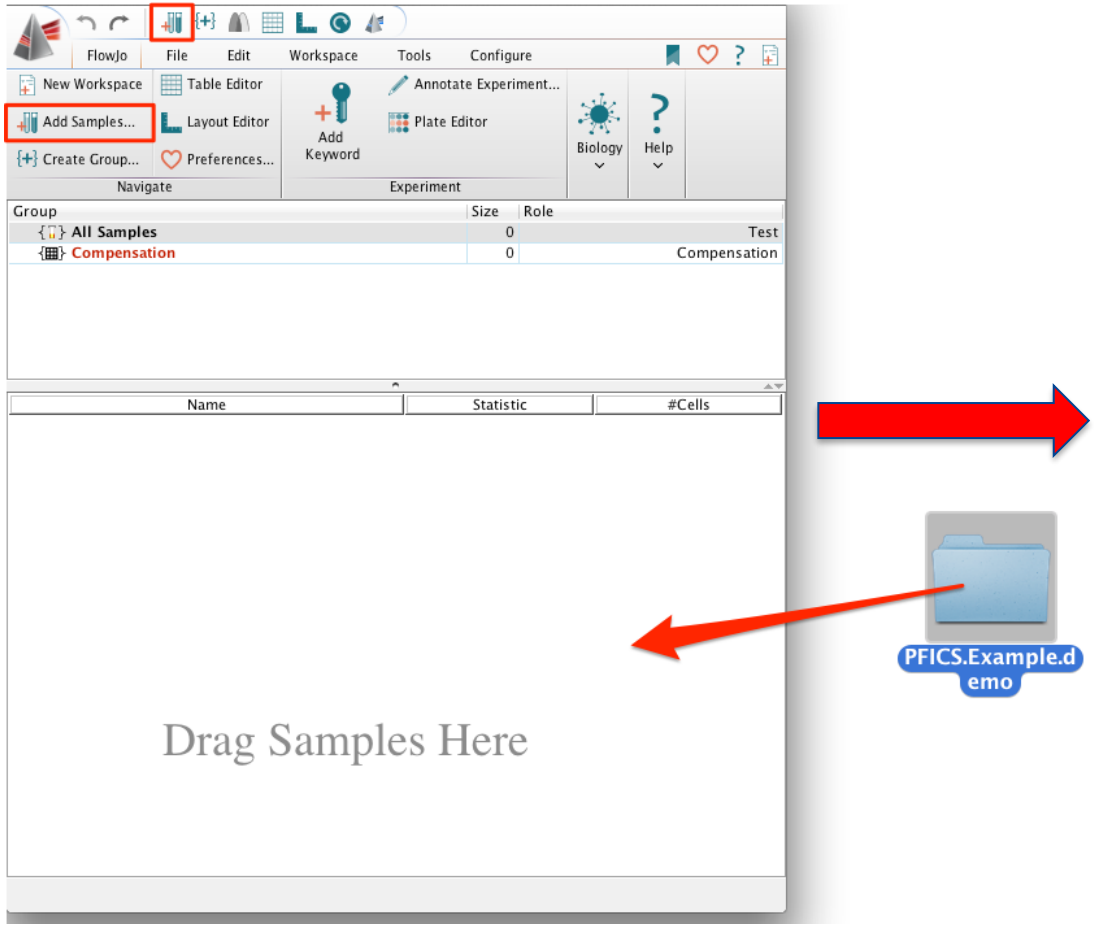


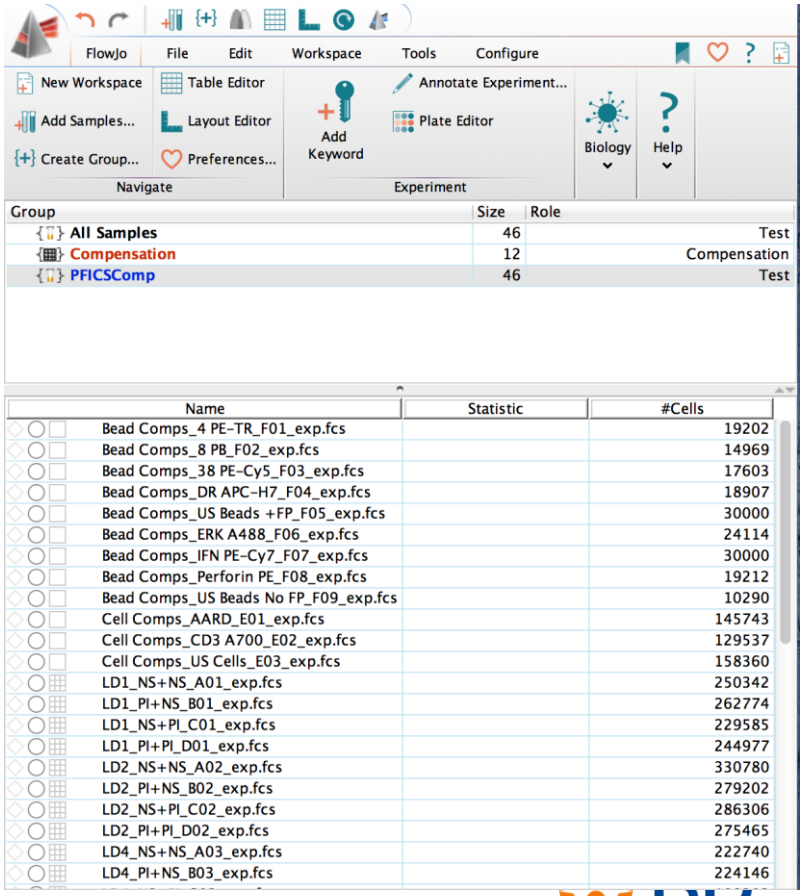
- Tabs group similar Bands together.
- Bands group similar Actions together.

輸入數據 Importing Data

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

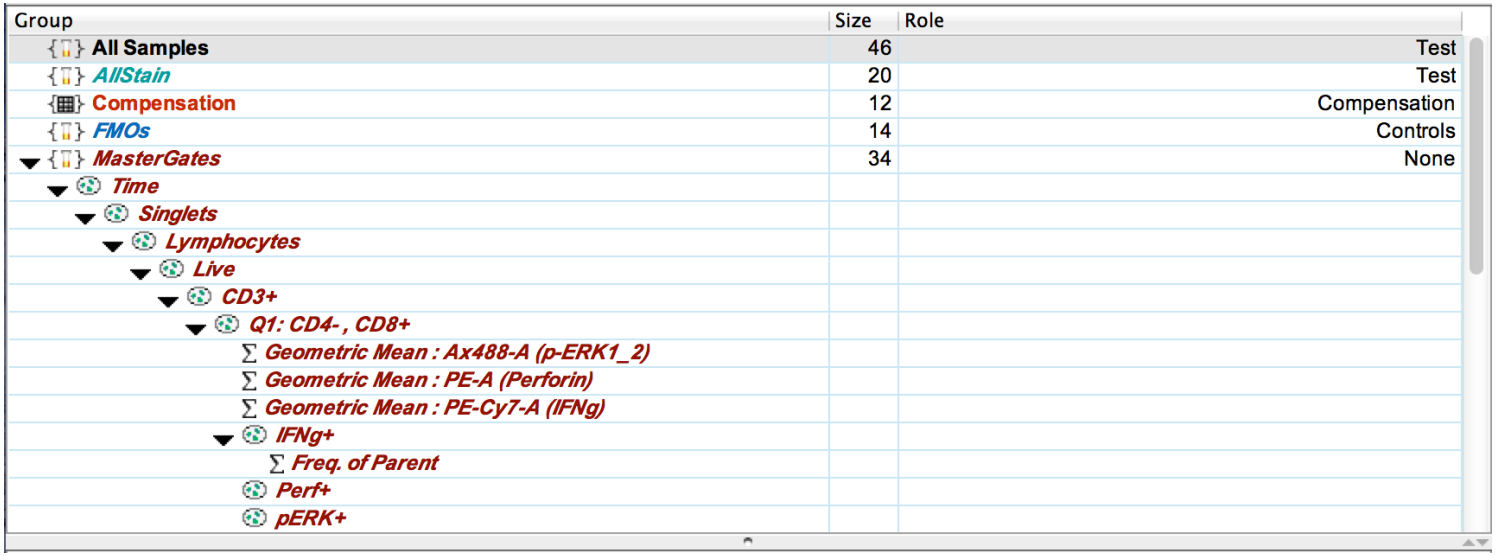






群組介面 Group Pane

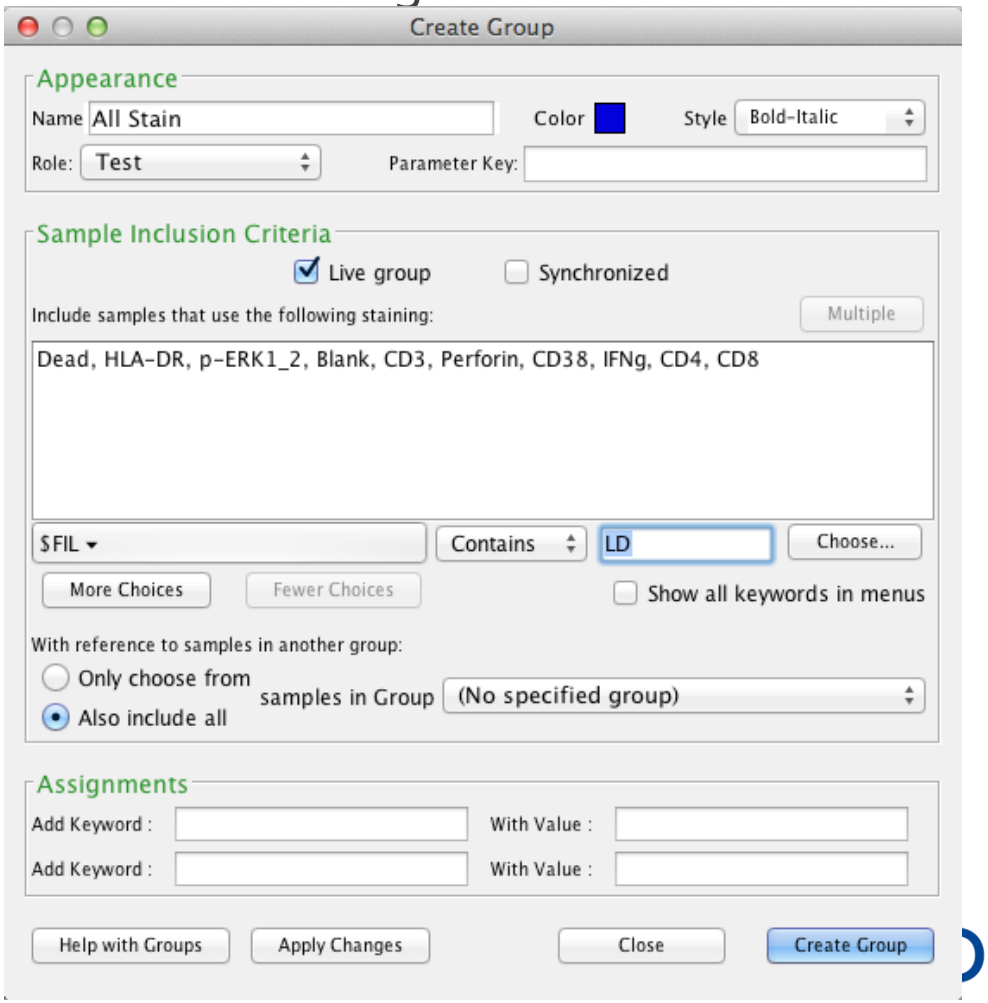
- The Group area lists all groups in the Workspace, # of samples in each group (Size), and the Role of that group (ex. Test, Compensation, Controls) .
- Groups act like folders to organize your samples, allows master gating and unique report generation.



- Group 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.



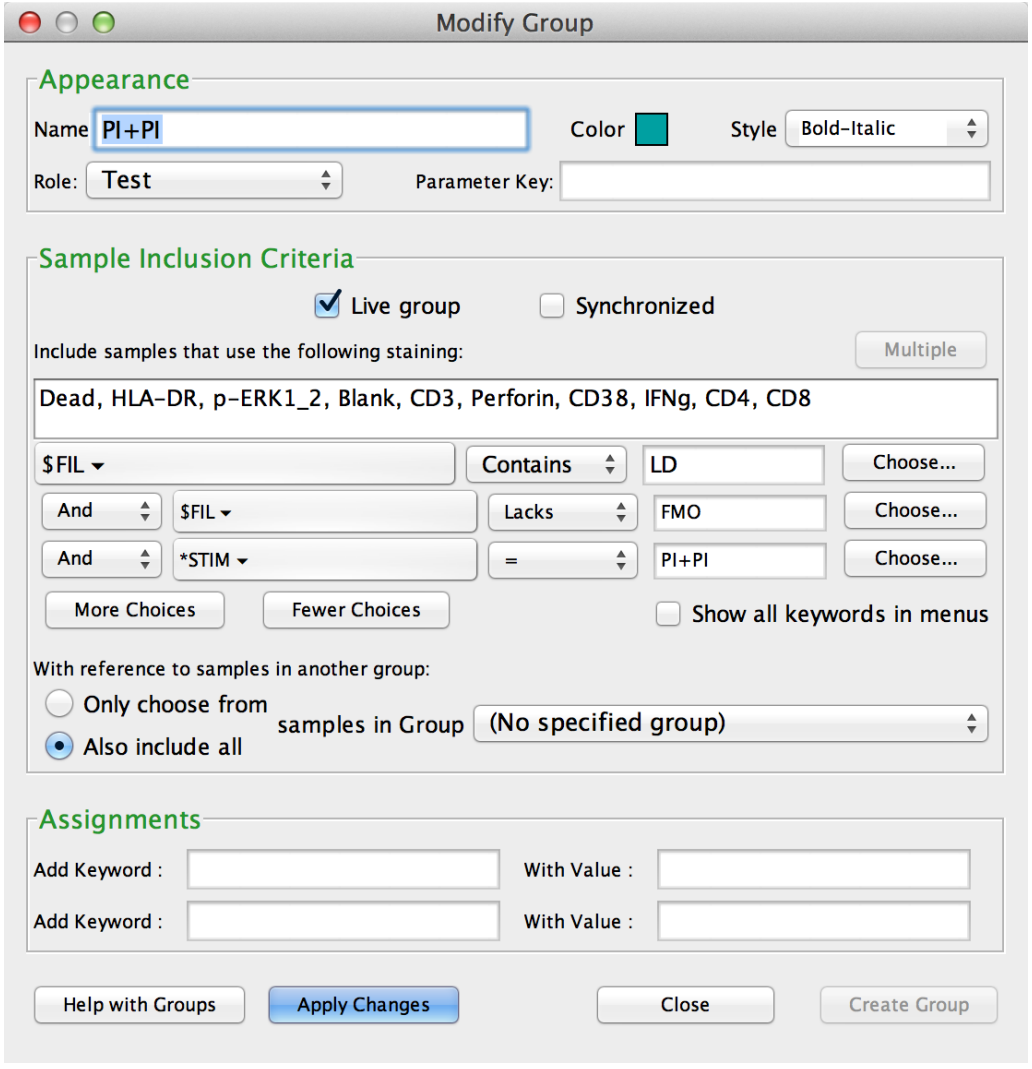
- Double click on an existing group to edit its properties.

樣品選取標準 Sample Inclusion Criteria

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

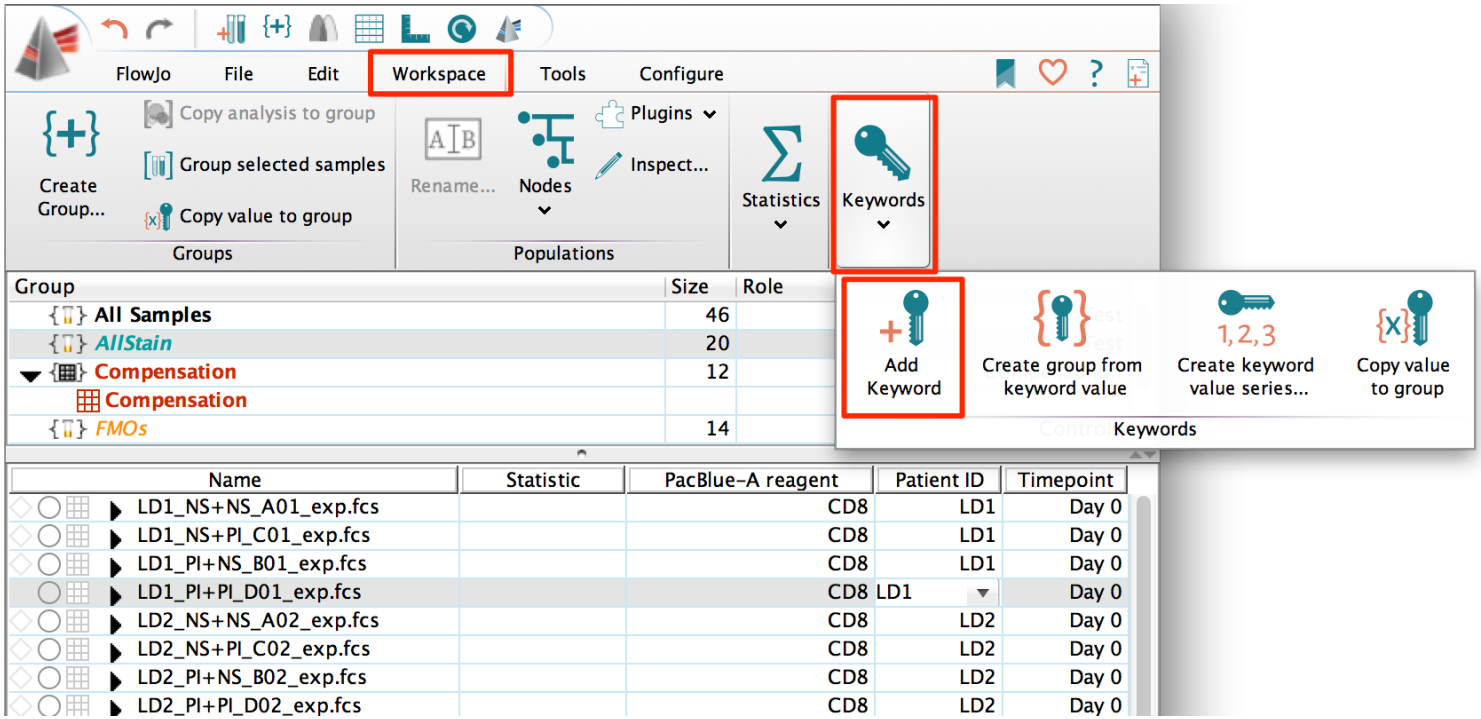
  - Sample Inclusion Criteria could include the staining panel, characters in the $FIL (file name), a user defined Keyword attribute, or a combination of features.

  - 可利用關鍵字將Flow數據快速地分類到群組內.



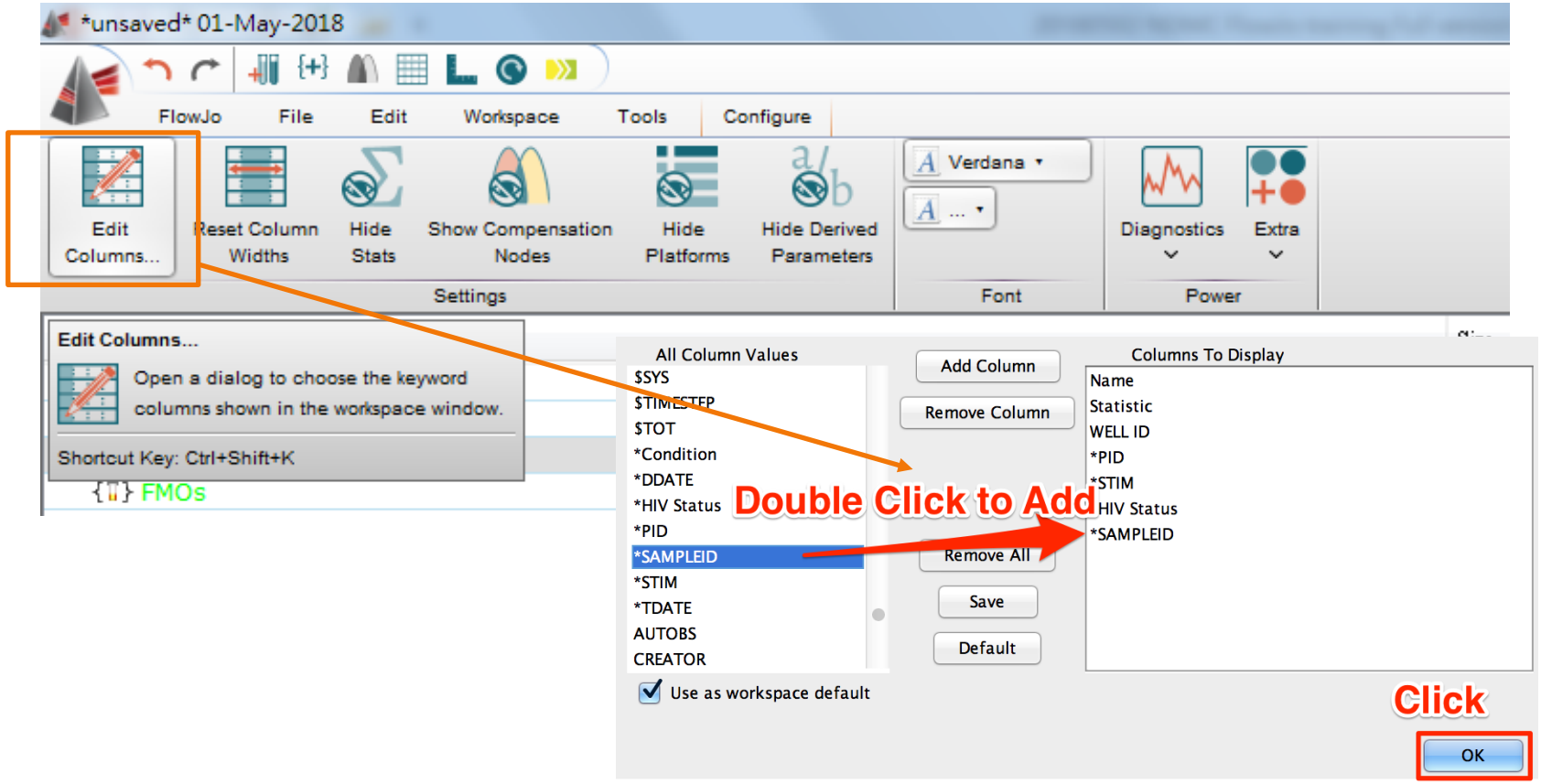
添加關鍵字 Add New Keywords

- 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

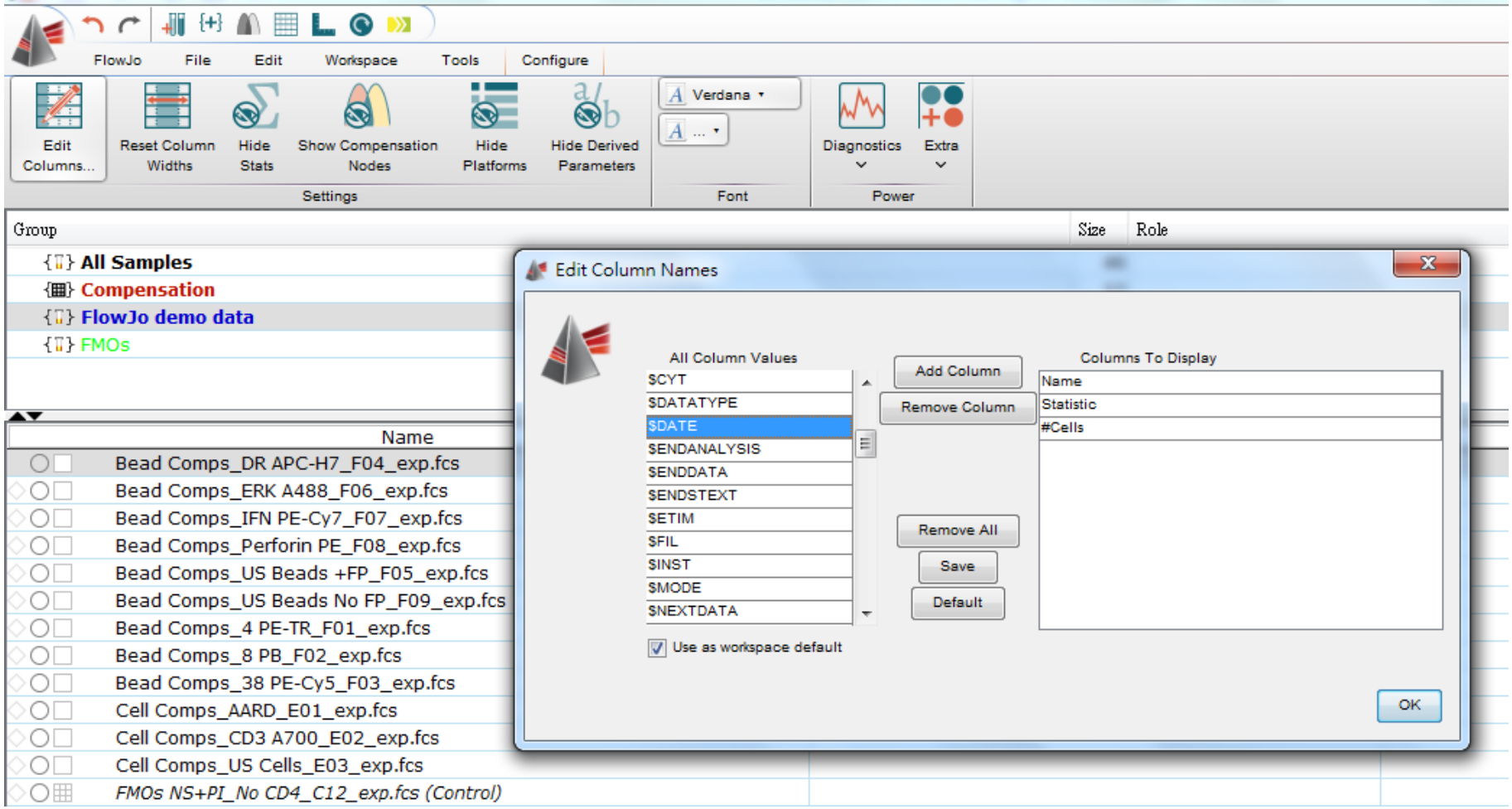


呈現關鍵字 Display Existing Keywords

- 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.

BD

作圖視窗 The Graph Window

- Facilitates data visualization and gating.

Gating Tools
圈選工具

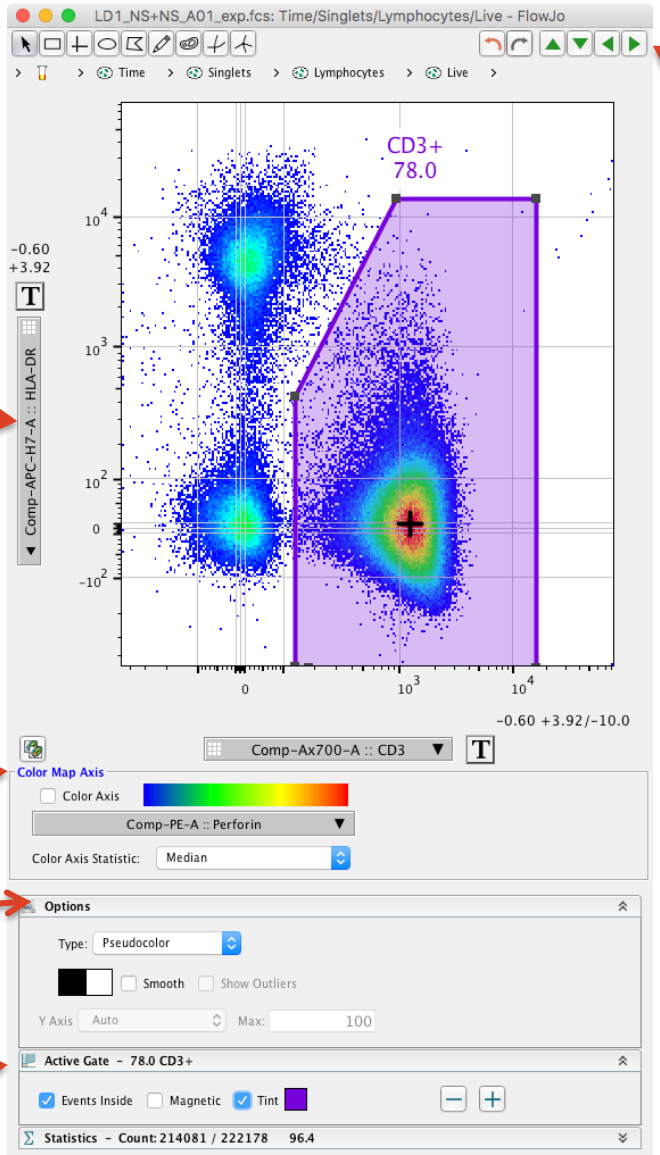
Navigation Buttons
瀏覽數據

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

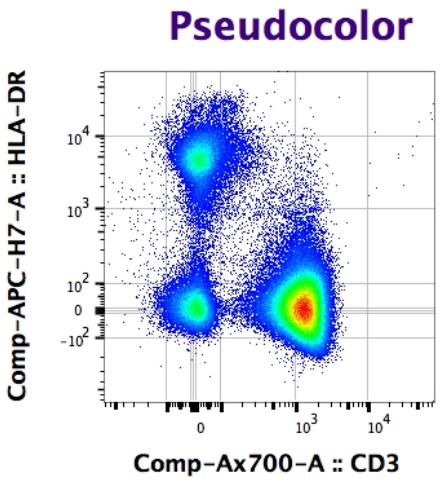


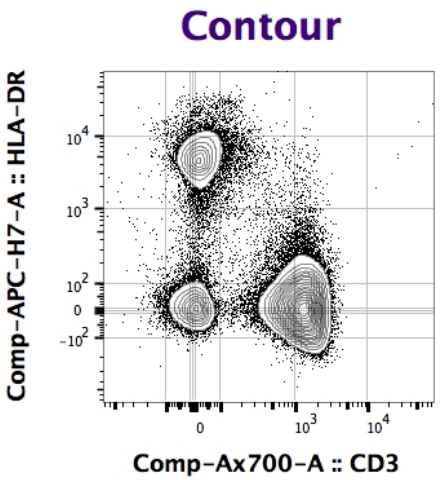
- Several different plot types are available to display flow data.

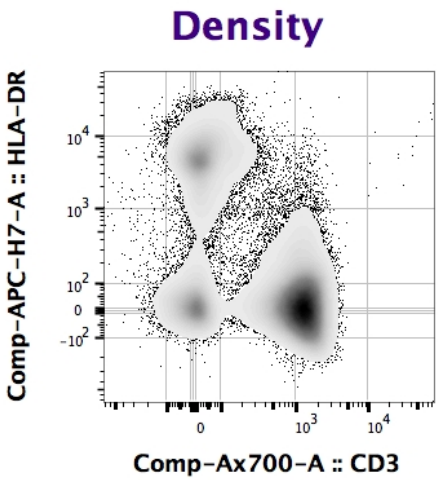
- Click on the Options Menu below the graph image and select Graph Type from the dropdown menu.

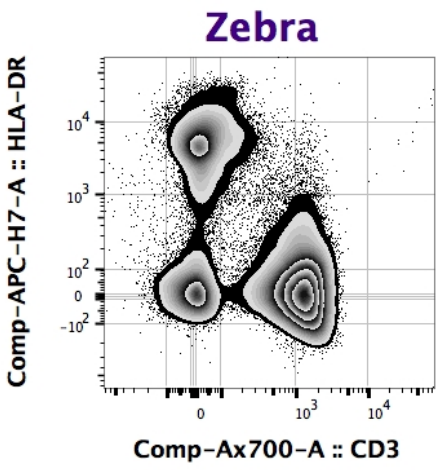
作圖呈現方式 Graph Display Options

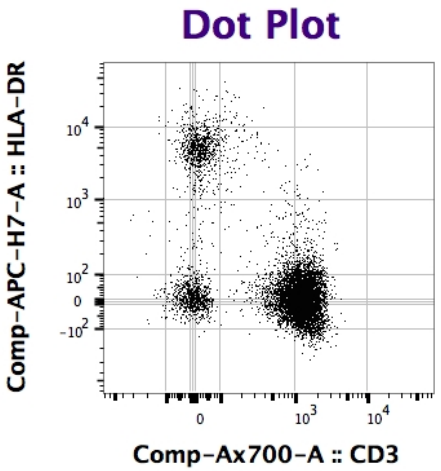
- Try them all and pick what pleases you, or best represents your data.

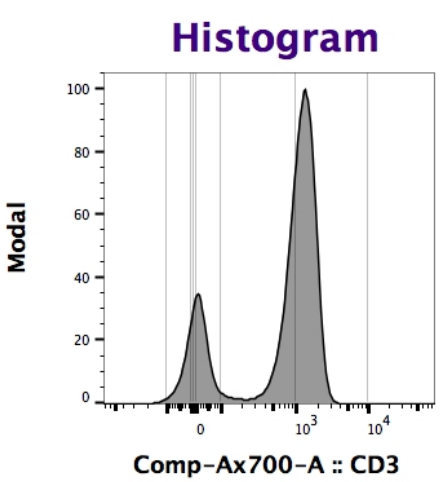


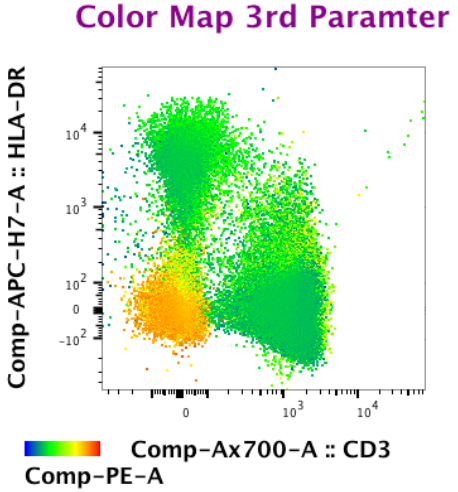






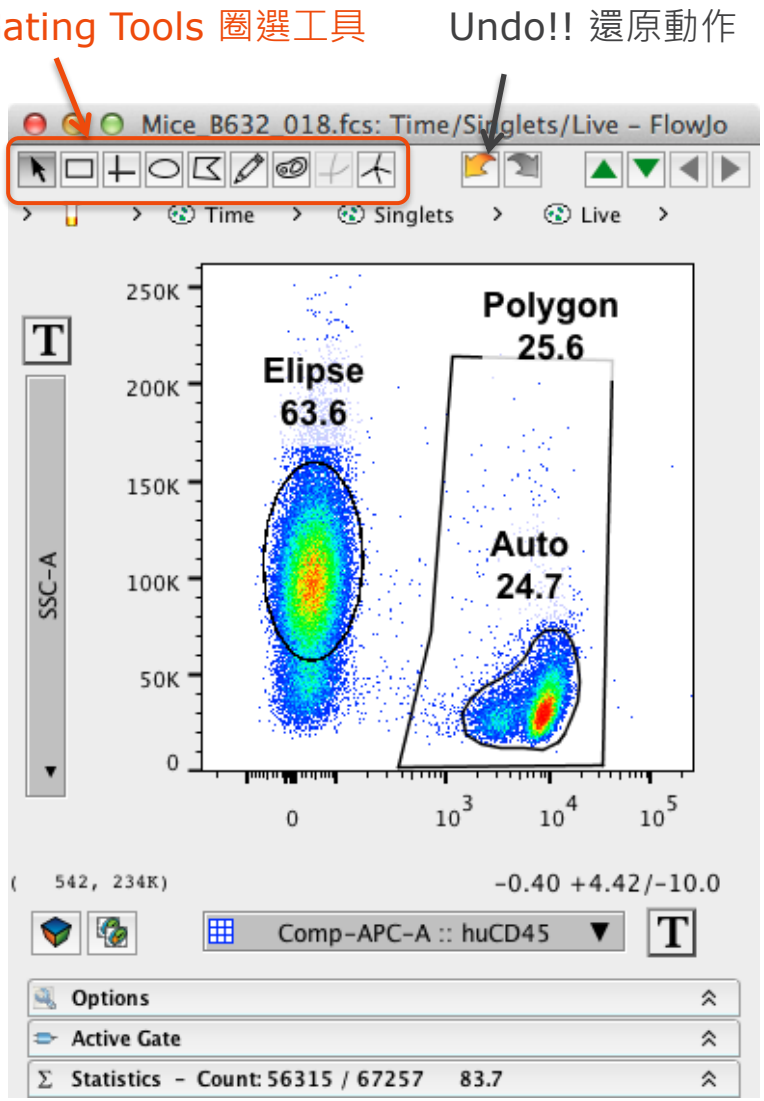






圈選工具 Gating tools

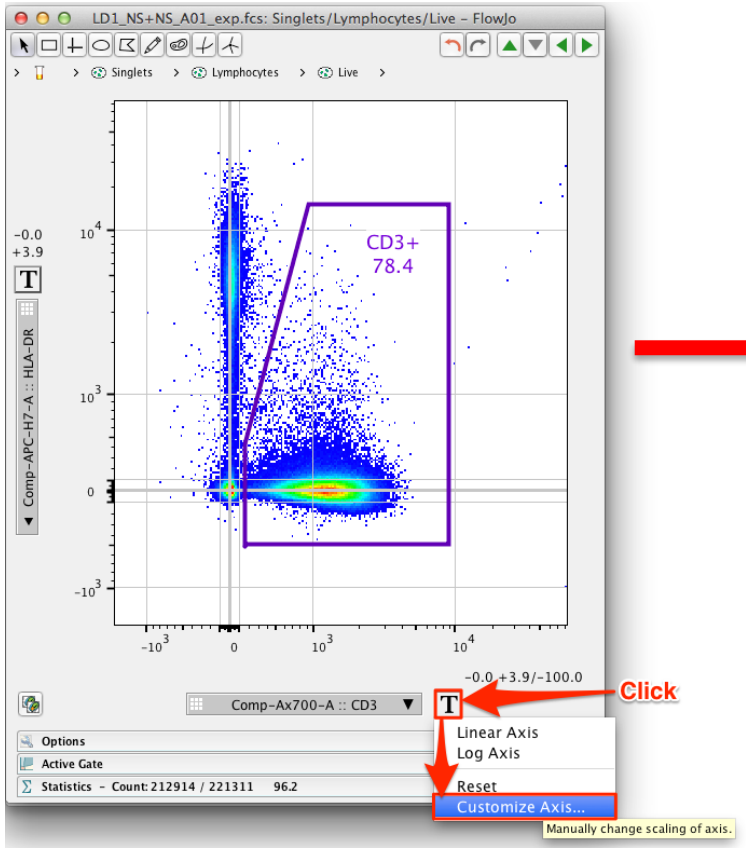
- Are located at the top left in a Graph Window.

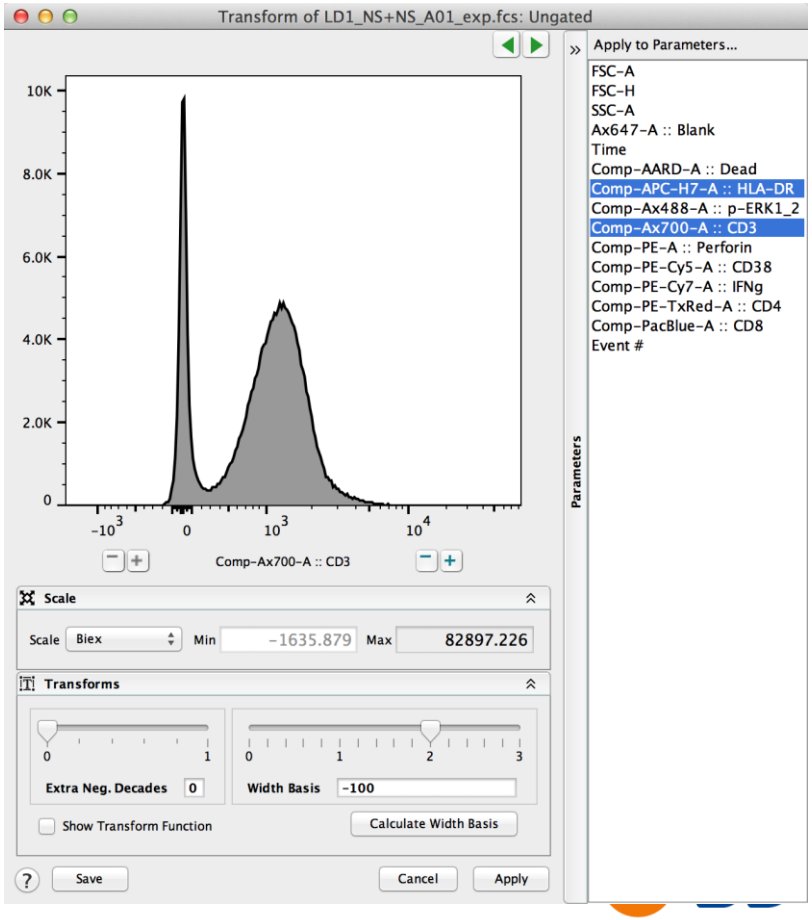


- Gates can always be modified or removed, so don’t be shy.
- Explore the gating options and pick what works best for you.

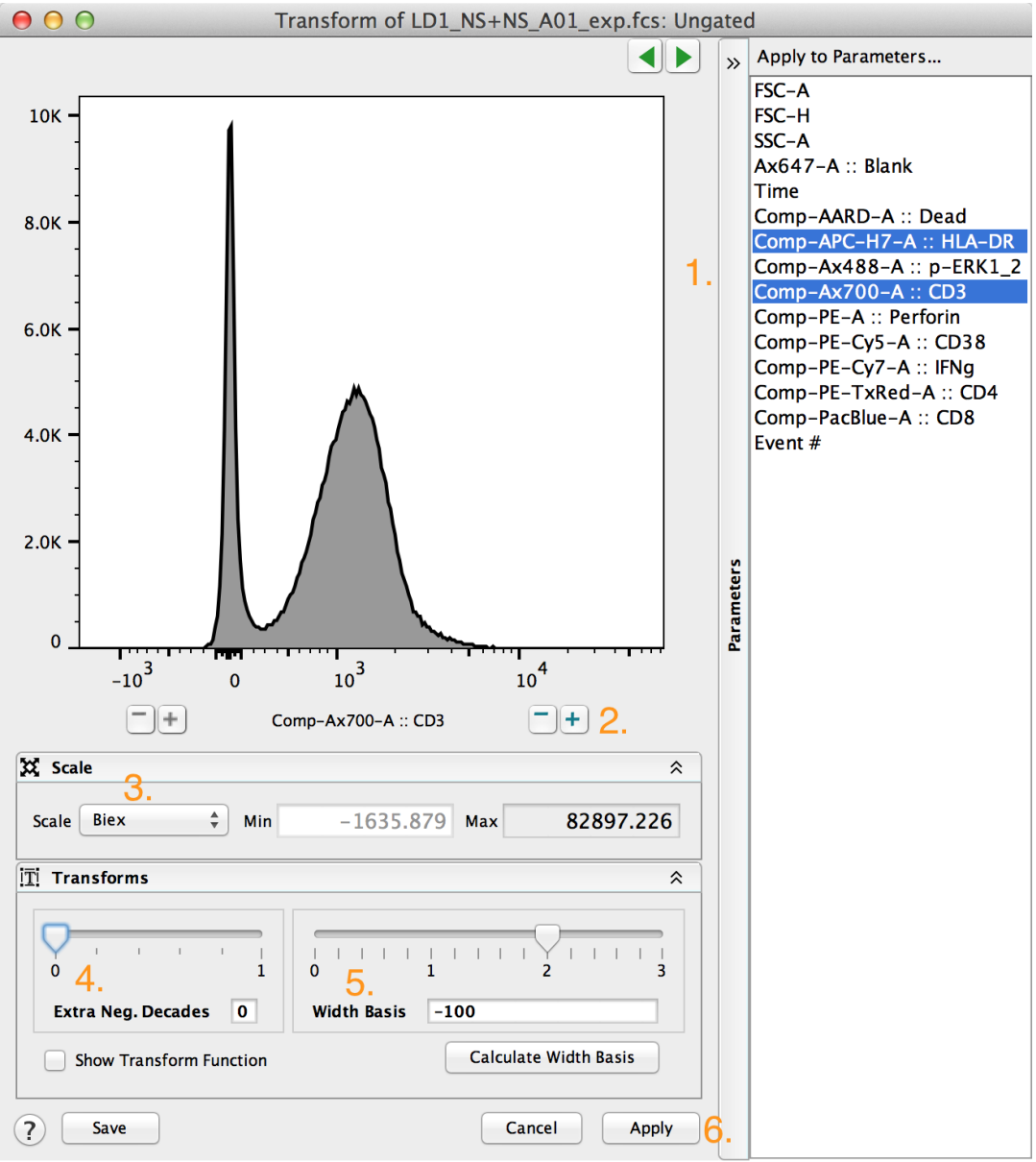
數值轉換功能 Transforming Data

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



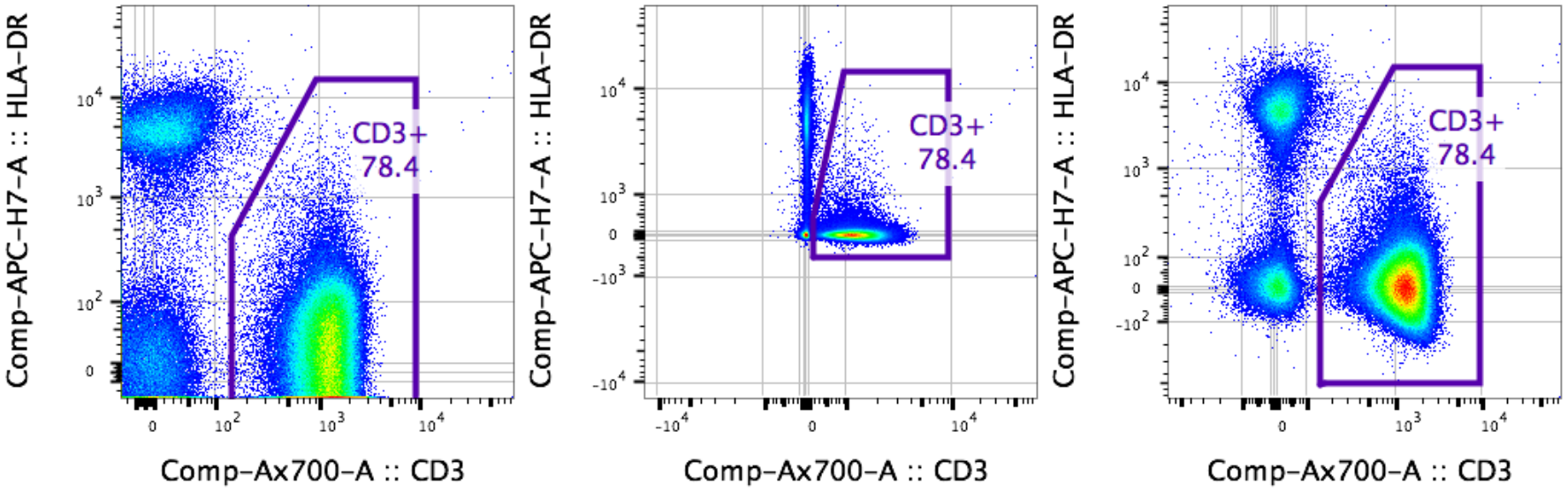


數值轉換選項 Transform Options



1. Select parameter(s)
2. Add or remove extra Pos. decades/range on top end
3. Select scale (Biex displays linear around zero and log further out)
4. Add or remove extra Neg. decades/range on bottom end
5. Width Basis scales how much visual display is given to linear vs. log range of the Biex scale
6. Applies the transformation settings to all selected parameters

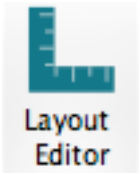
數值轉換效果 Effects of Transformation

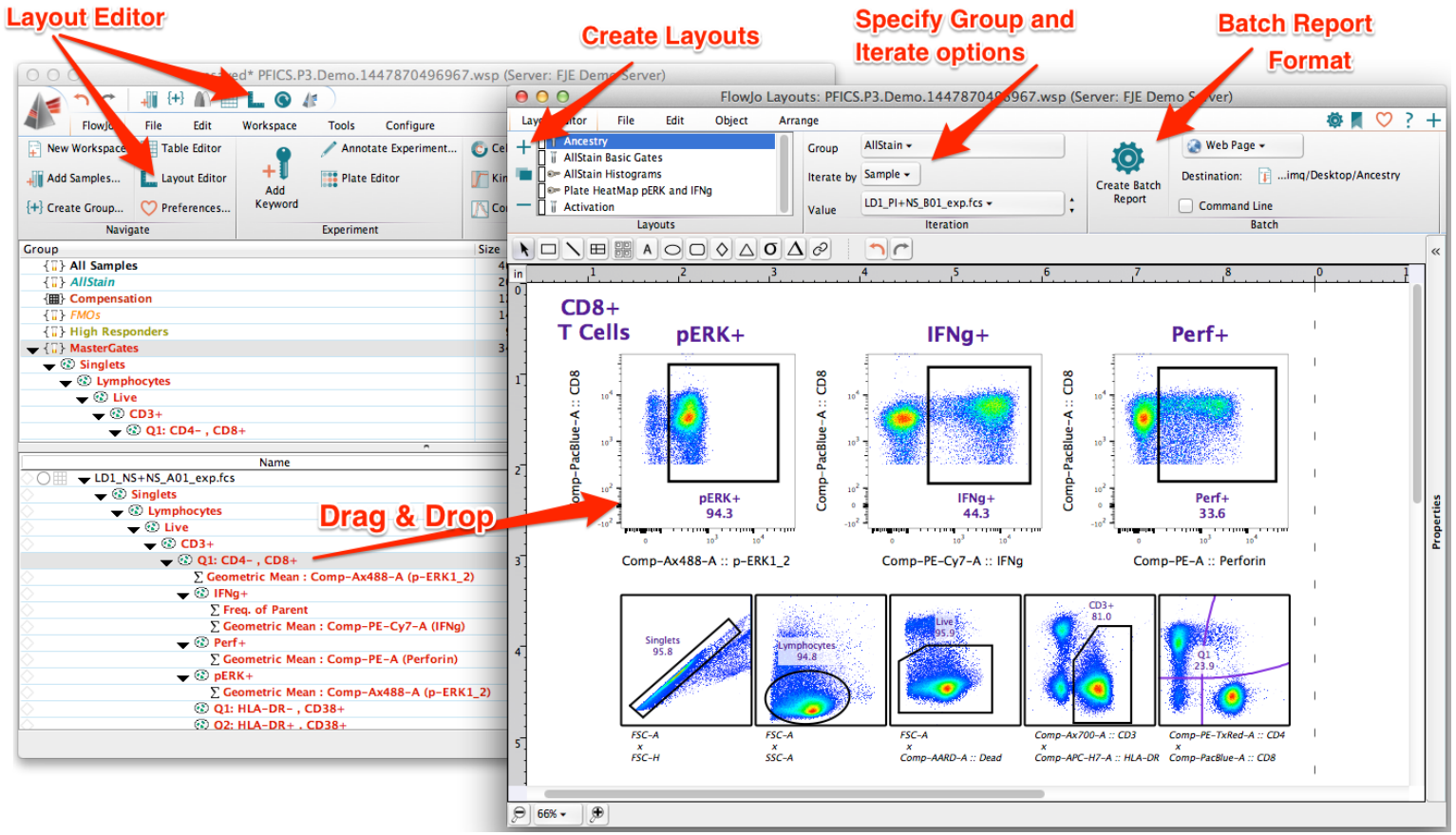


1. Gets rid of the "squishing" of cells.
2. Ensures the visual population center better correlates with the statistical center (median).
3. Makes high resolution compensated digital cytometry data more appealing to the eye.調整數據的視覺尺度表現, 一般來說不影響統計數據本身

作圖輸出 The Layout Editor

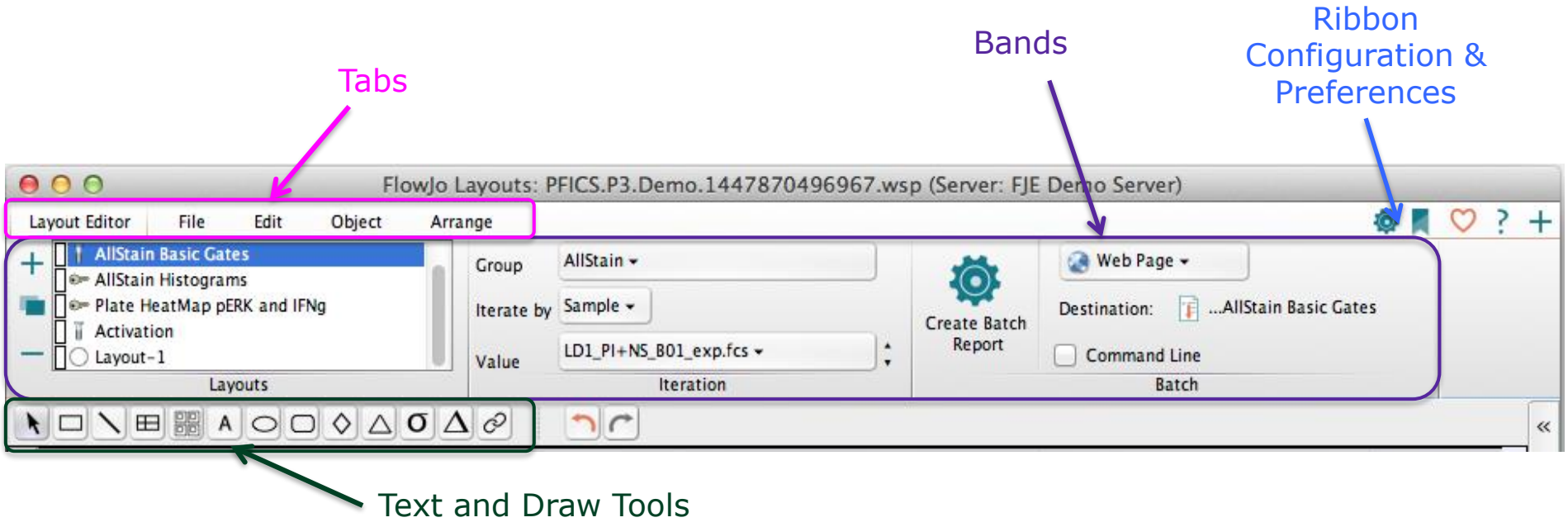
- A tool for creating graphical reports.
- Click on the Layout Editor icon.
- Drag populations from a sample to Layout Editor.





作圖輸出 Working in Layout Editor

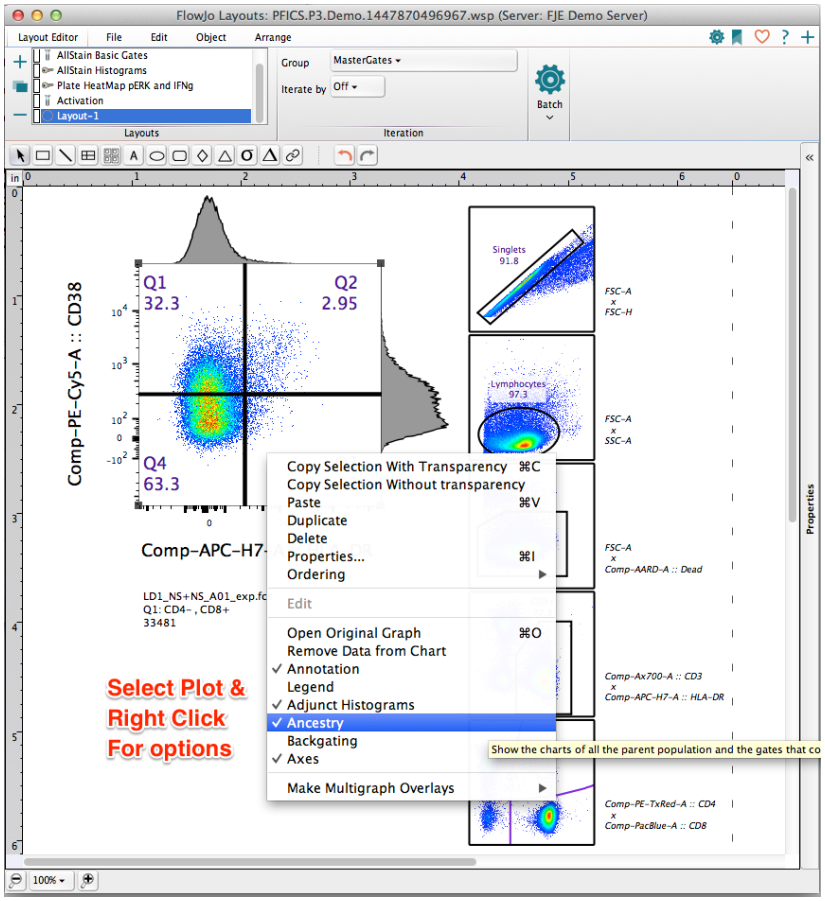
- Similar to the Workspace. Layout Editor has its own customizable Ribbon with Tabs and Bands to organize actions.



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

作圖輸出 Within Layout Editor

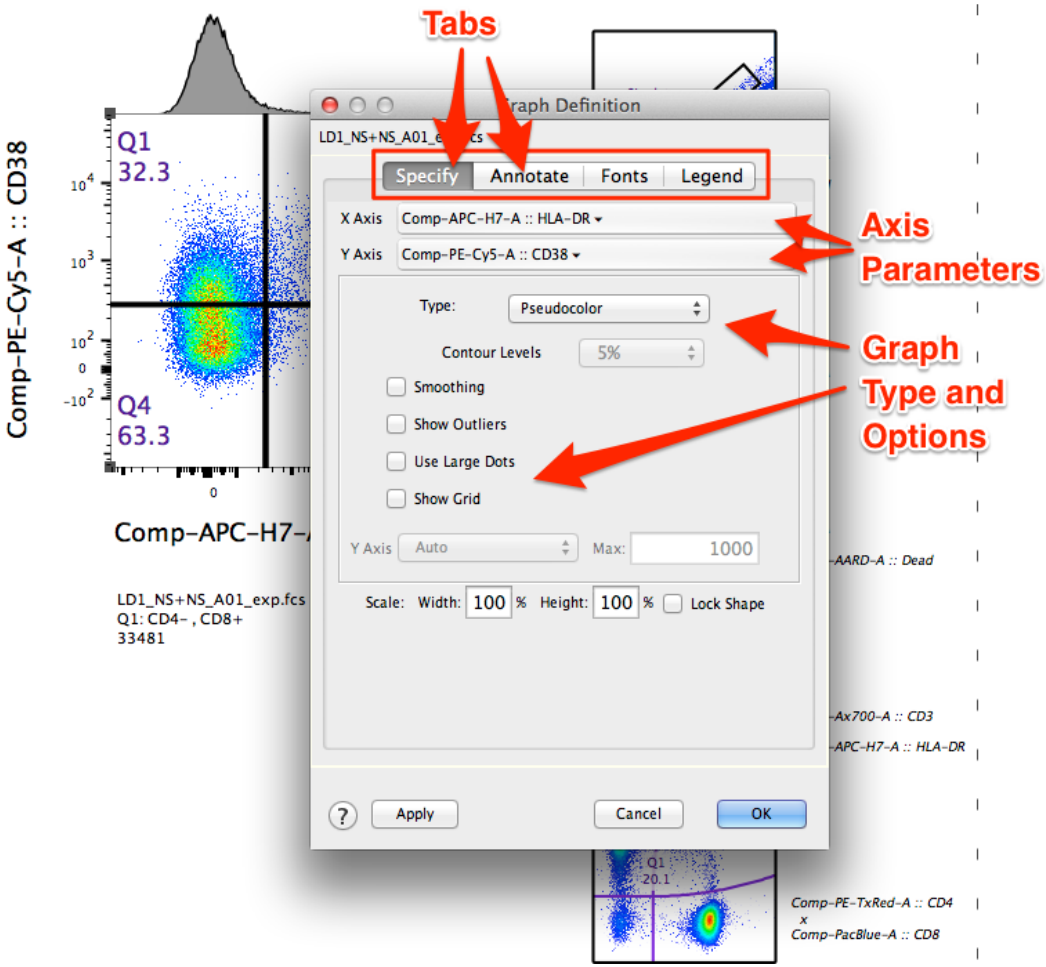
- Graphs can be organized and re-formatted.
- Statistics, keywords, text and even shapes or objects can be added to illustrate your analysis.

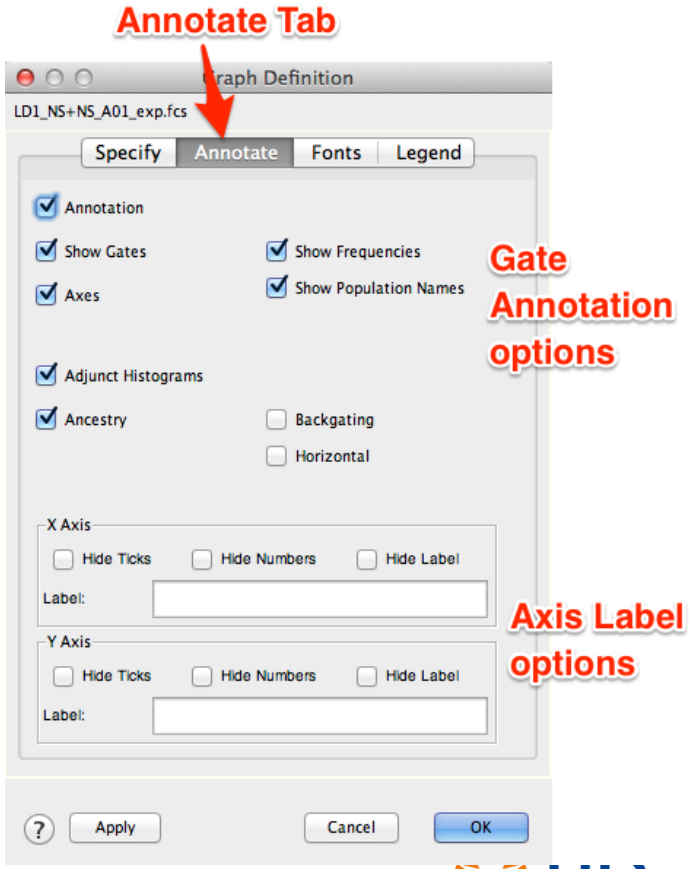


- Right Click on a graph plot for Ancestry and Backgating options
- Right click and select Properties for additional graph formatting

作圖輸出 Working in Layout Editor

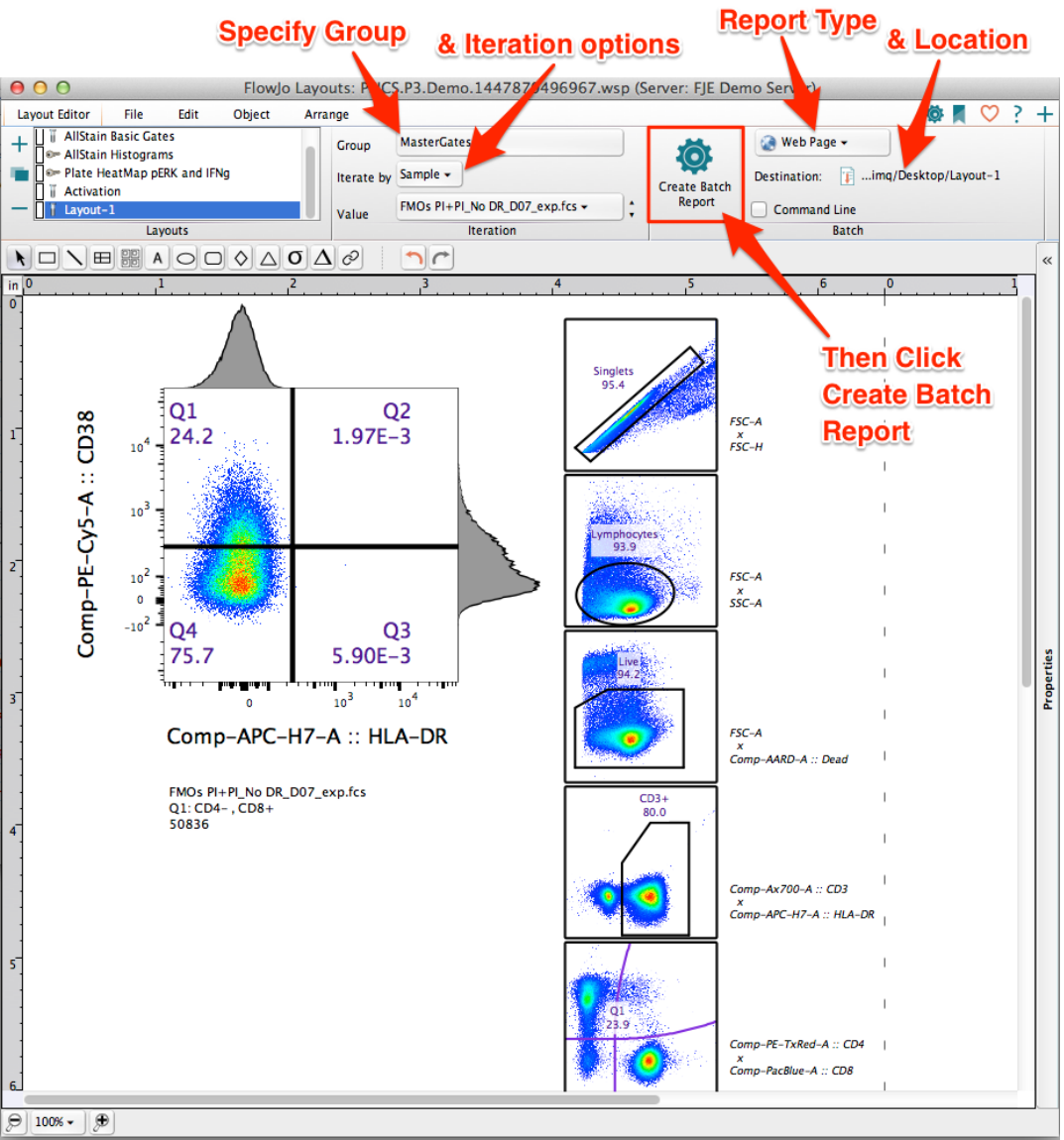
- Double Click a graph to change its properties/formatting with 4 tabs of Graph Definition options





批次報告輸出

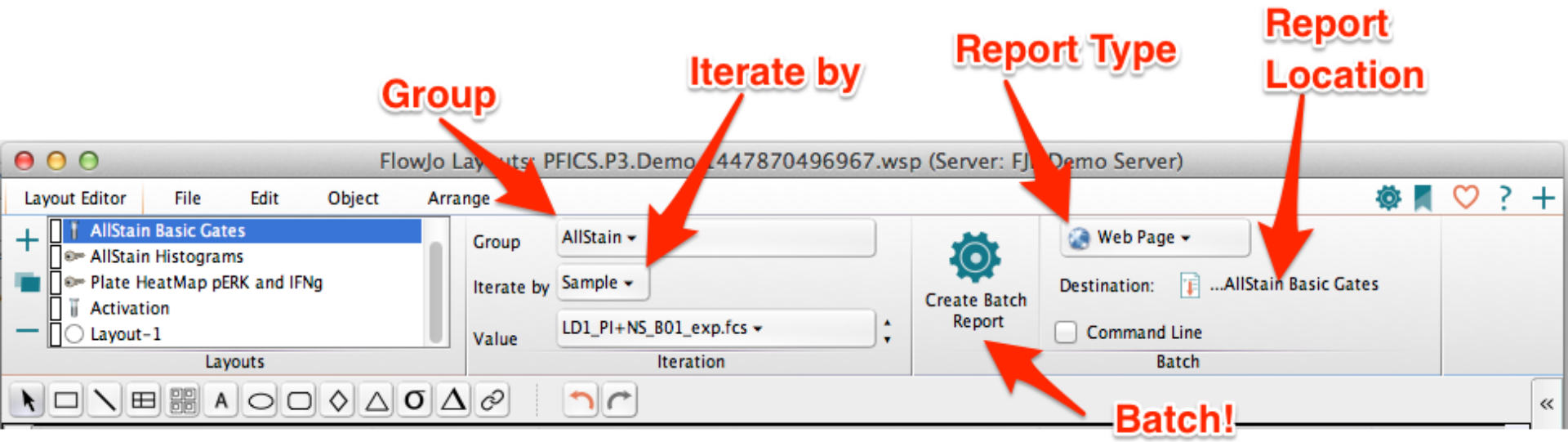
Batch Analysis of Layout Editor Graphics



- 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 .

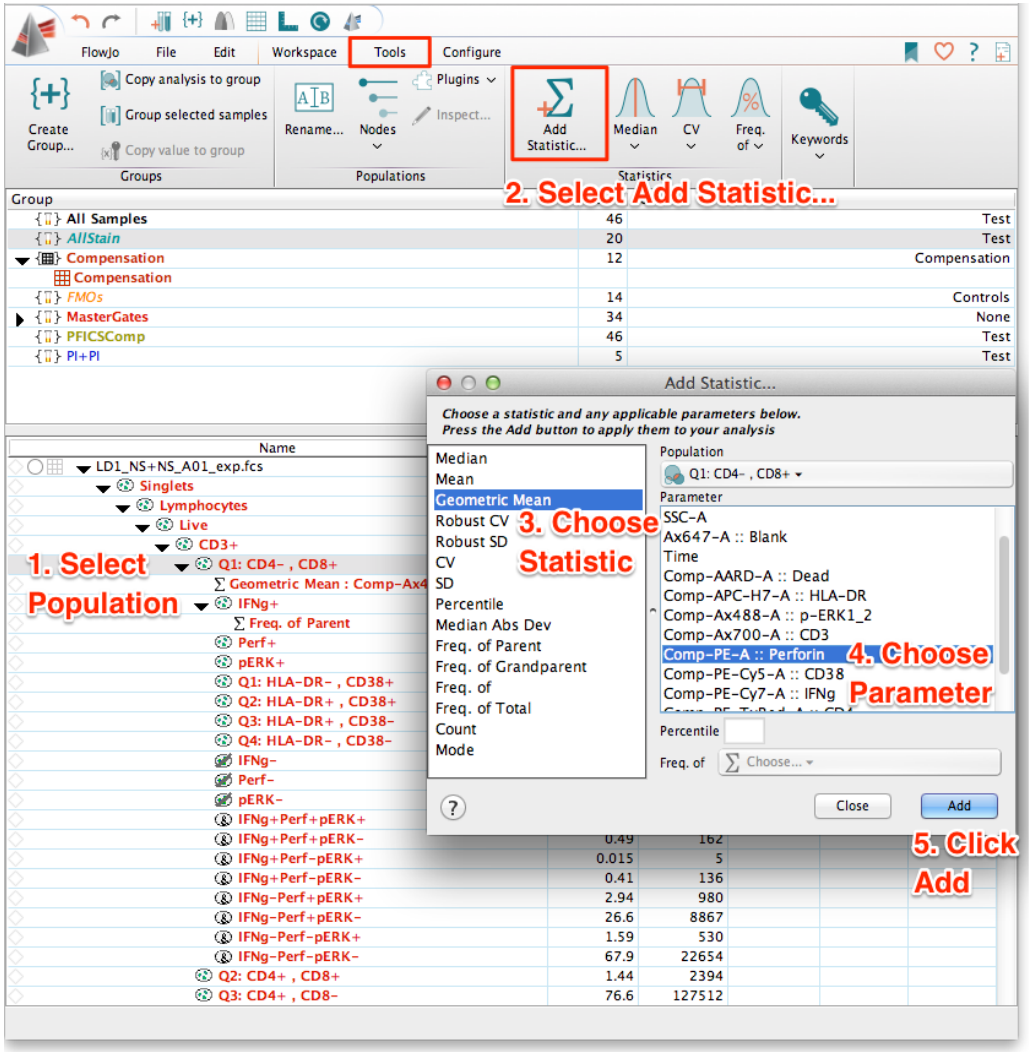
批次報告輸出 Batch Report Layouts

- 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



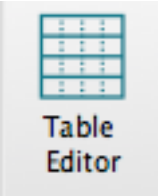
增加統計 Adding Statistics

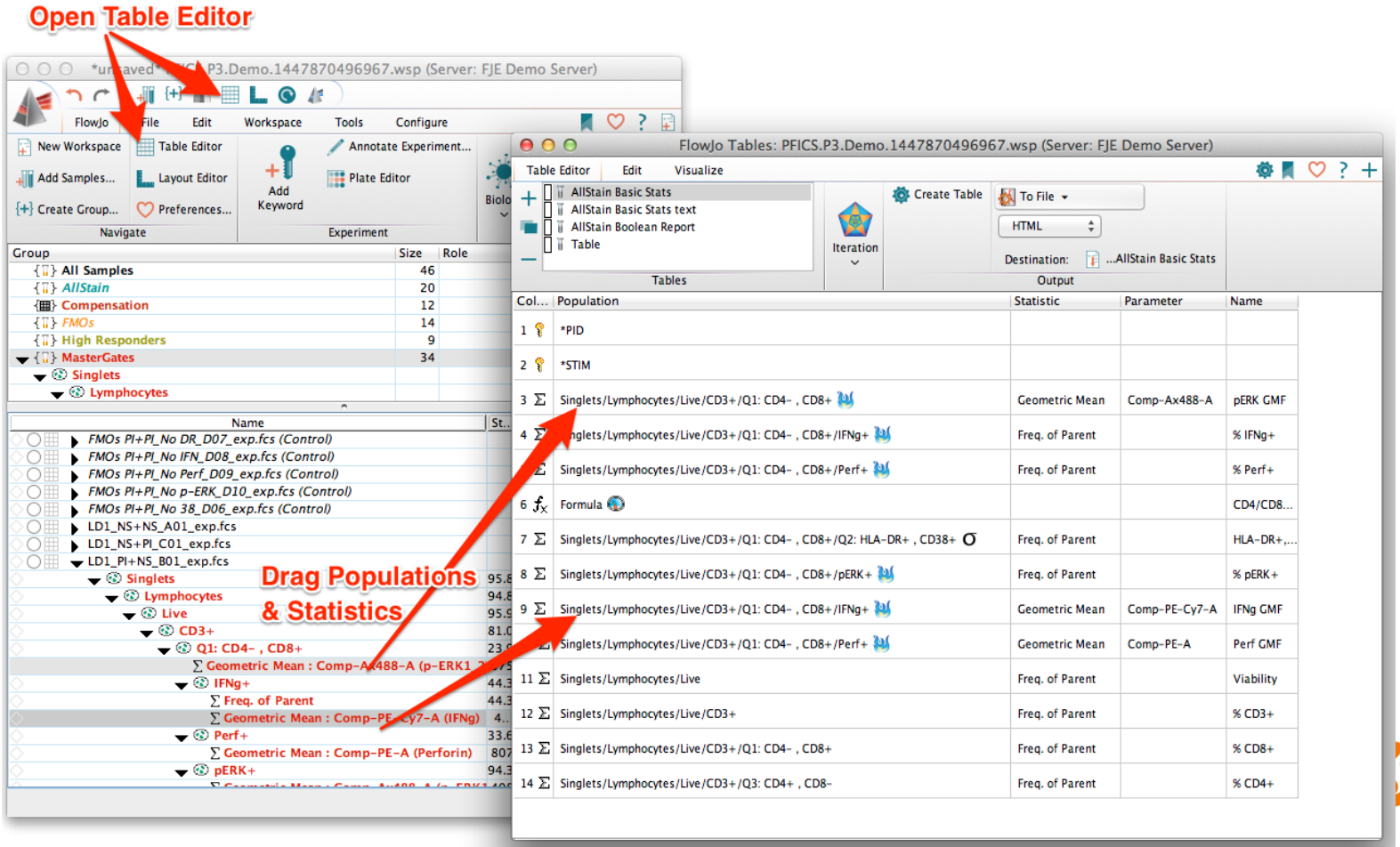
- Add a statistic to any gated population selected within a sample gating hierarchy.
- Statistic nodes can be group-applied just like a gate.



統計資料輸出 The Table Editor

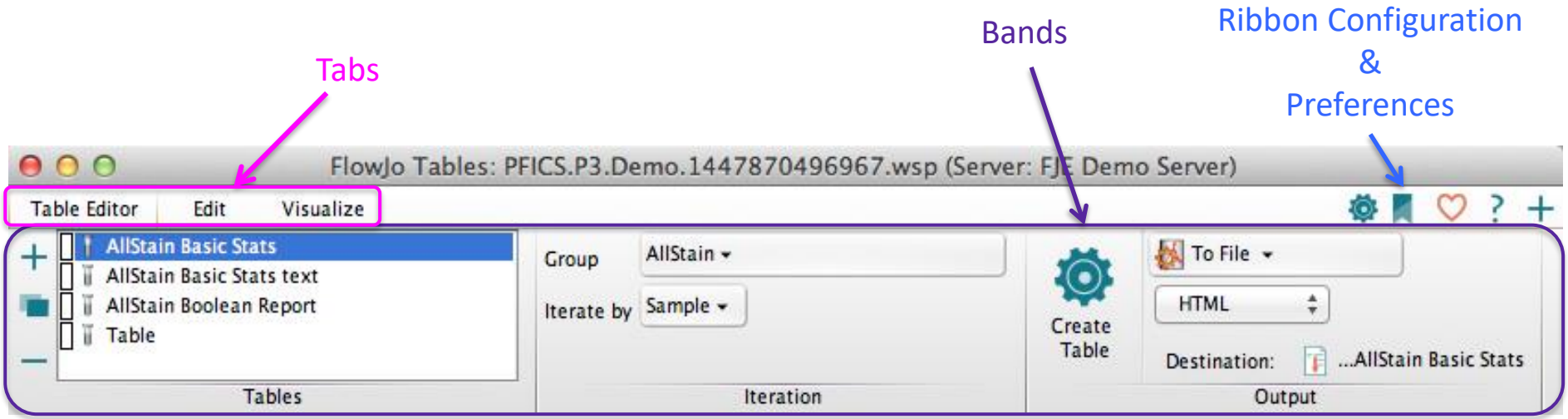
- A tool for creating statistical reports.
- Type ⌘ T, or click on the Table Editor icon.
- Drag Populations & Statistics to Table Editor.





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.

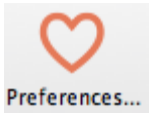


- Specify the group you wish to batch, and how to iterate the batch process, then in the Output band, specify where you want the batch output to go.

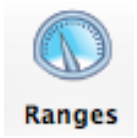
視覺化工具 Table Editor Visualize Tools

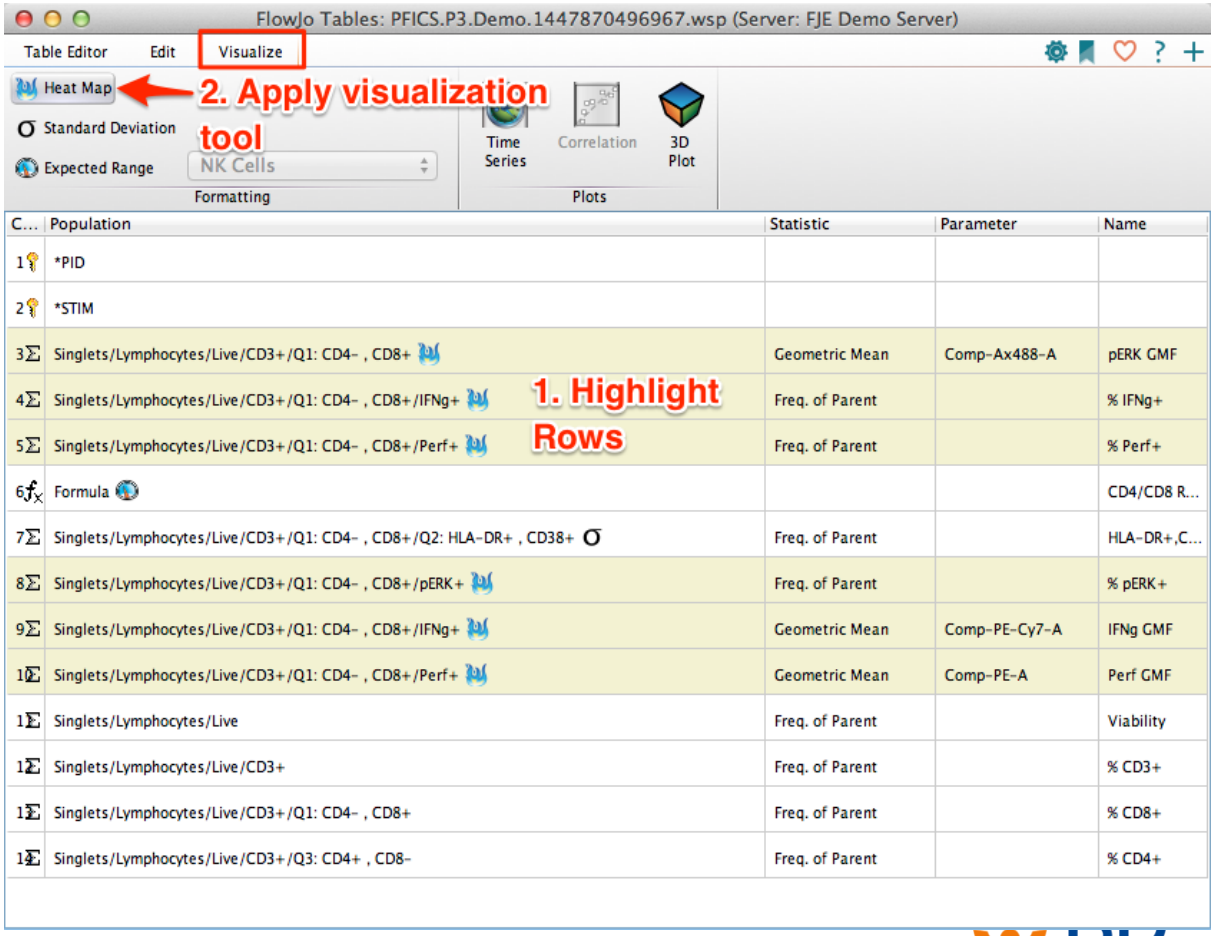
- Table formatting/visualization options such as heat mapping are contained within the Visualize Tab.

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



→ Ranges





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.

Table – AllStain Basic Stats

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_PI...	LD12	PI+NS	327	45.3	40.8	1.94	*1.50*	84.8	4632	408
LD12_PI...	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_PI...	LD14	PI+NS	366	17.7	18.2	1.66	*1.21*	94.8	3708	650
LD14_PI...	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

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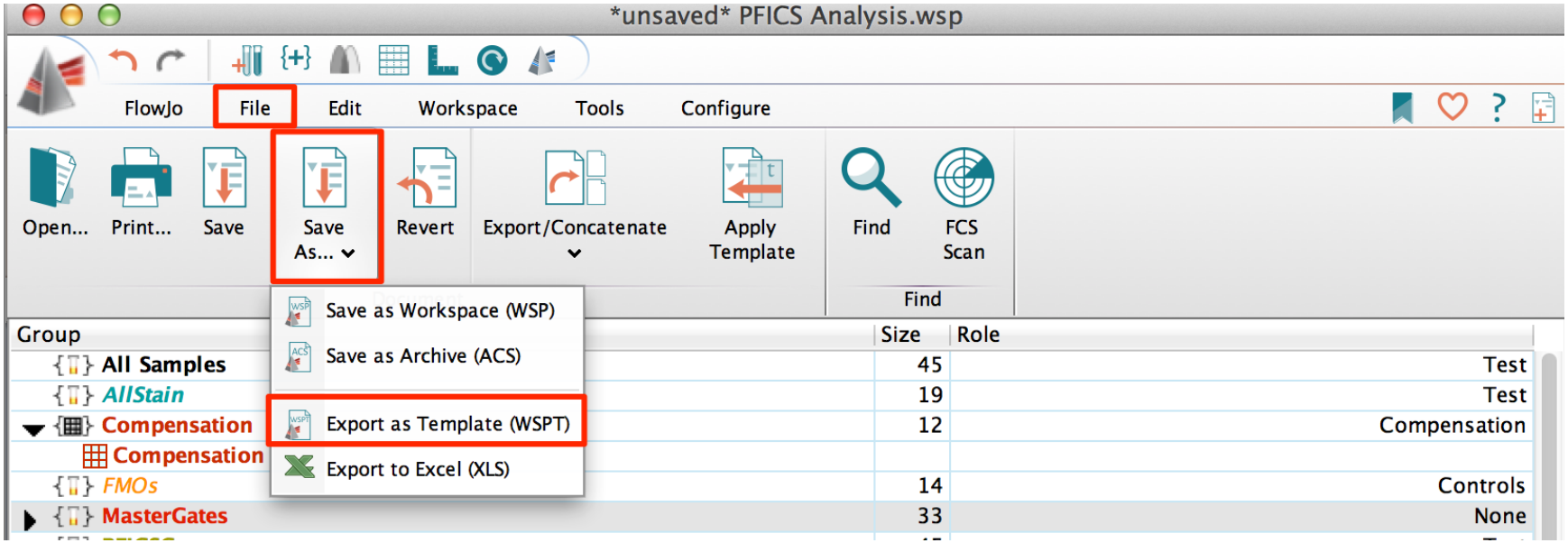
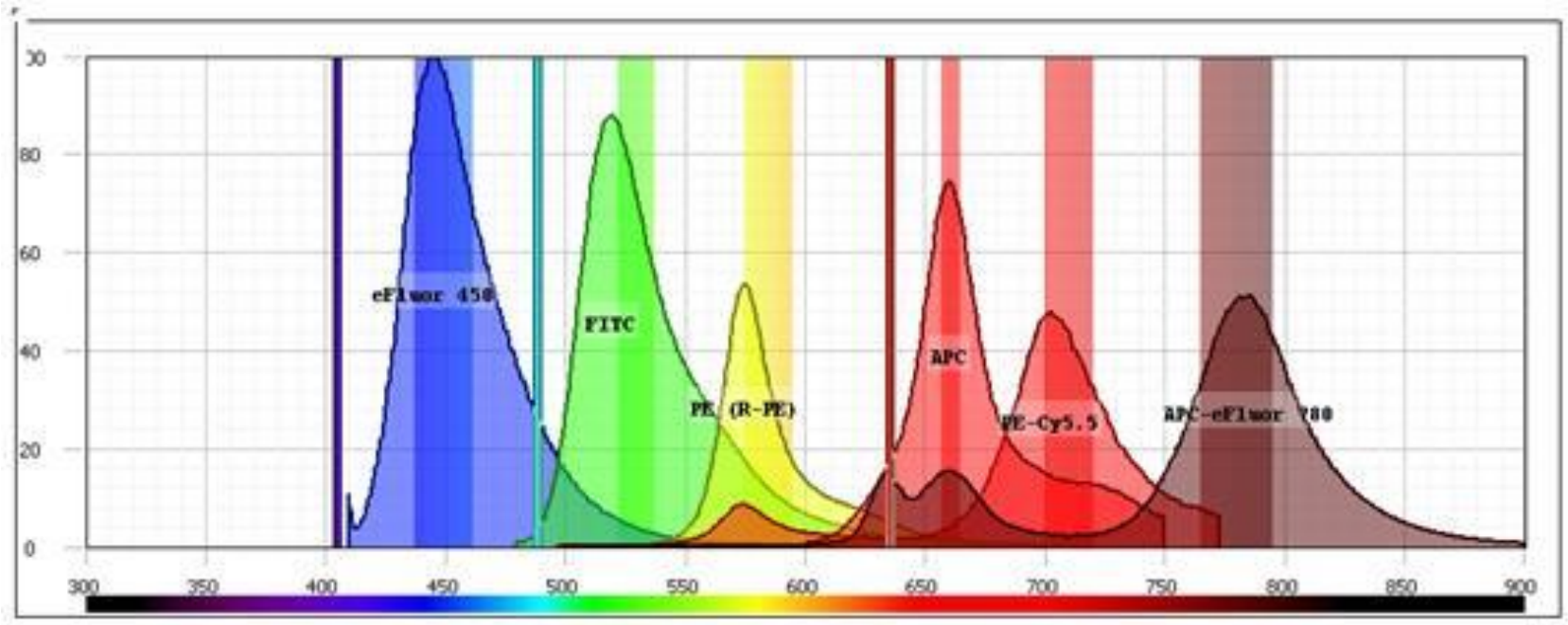
儲存分析模板 Workspace Templates

- 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

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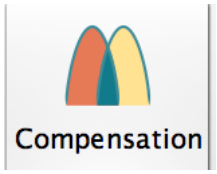
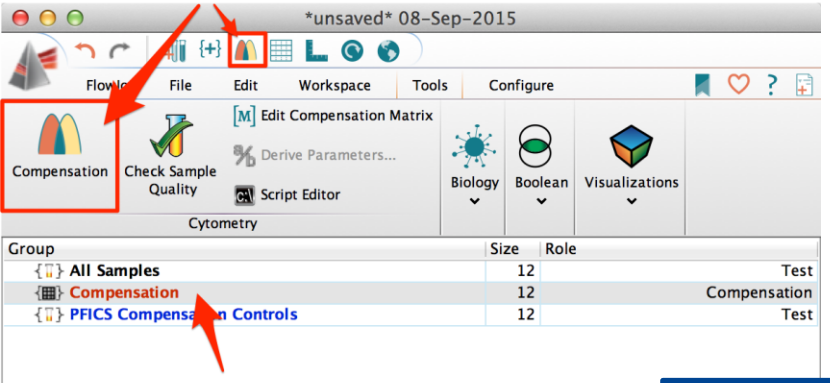
螢光補償 Compensation I

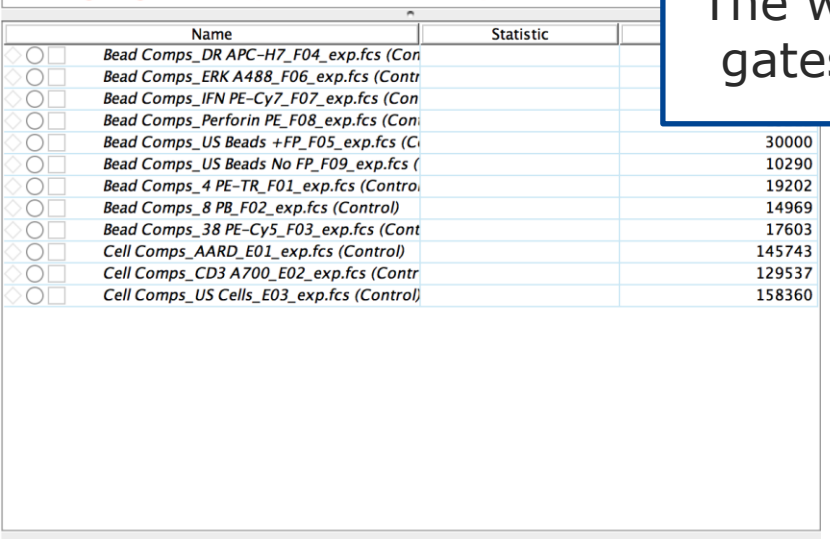
- Select a Compensation Group in the groups window, then click in the task bar.



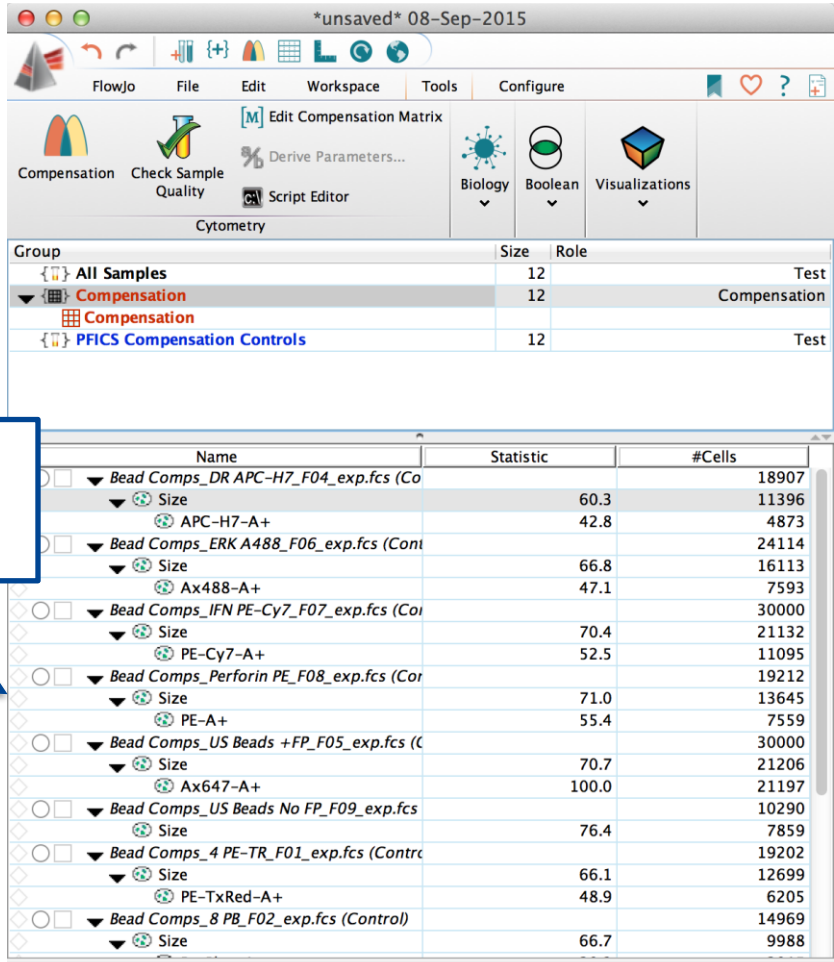
2. Click the Compensation Tool



1. Highlight Compensation Group



The wizard auto gates samples

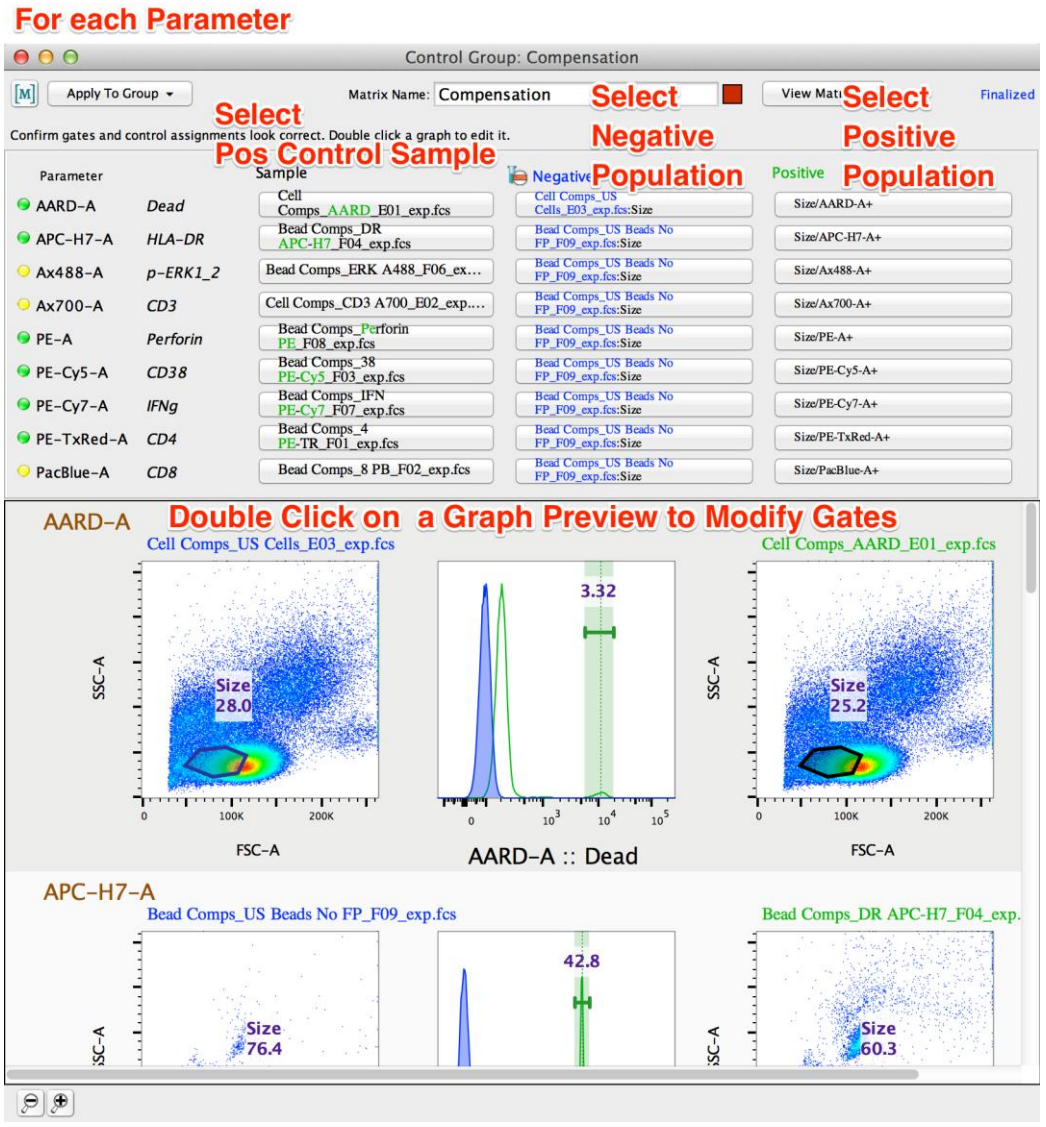


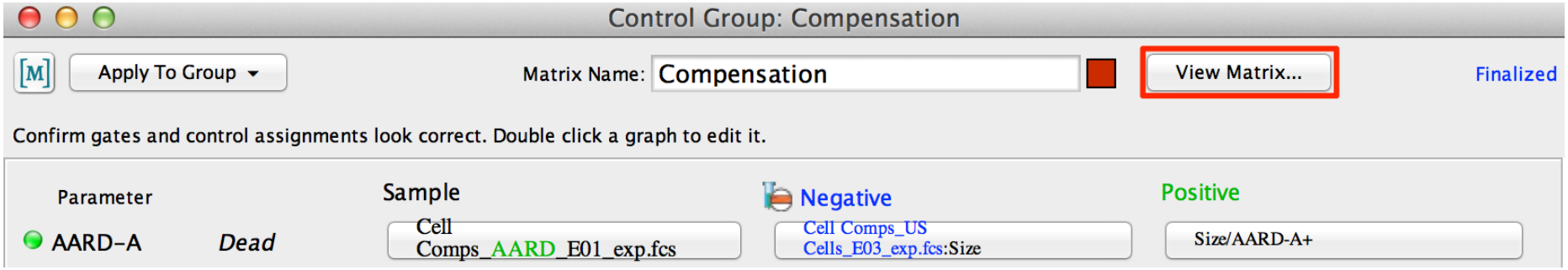
螢光補償 Compensation II

- 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.

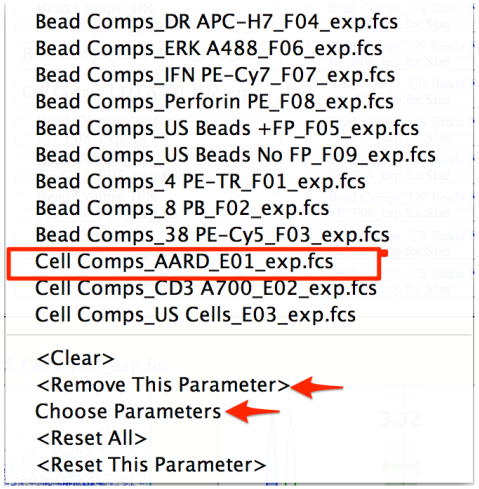




Use Sample drop down list

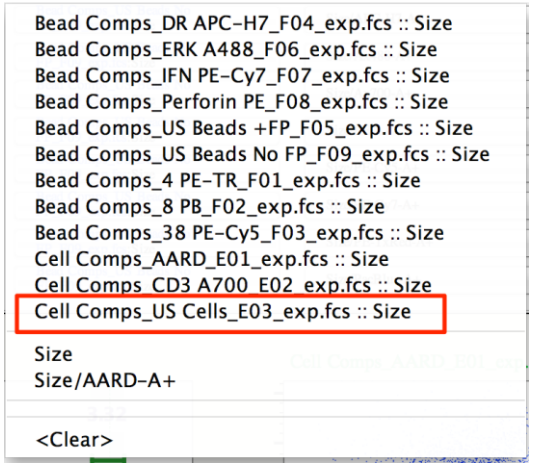
to select Pos Control Sample and

Choose or Remove Parameters



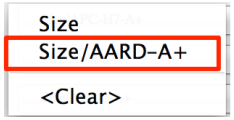
Use Negative drop down list

to Select Negative Sample or Population



Use Positive drop down list

to Choose Positive population



- 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.

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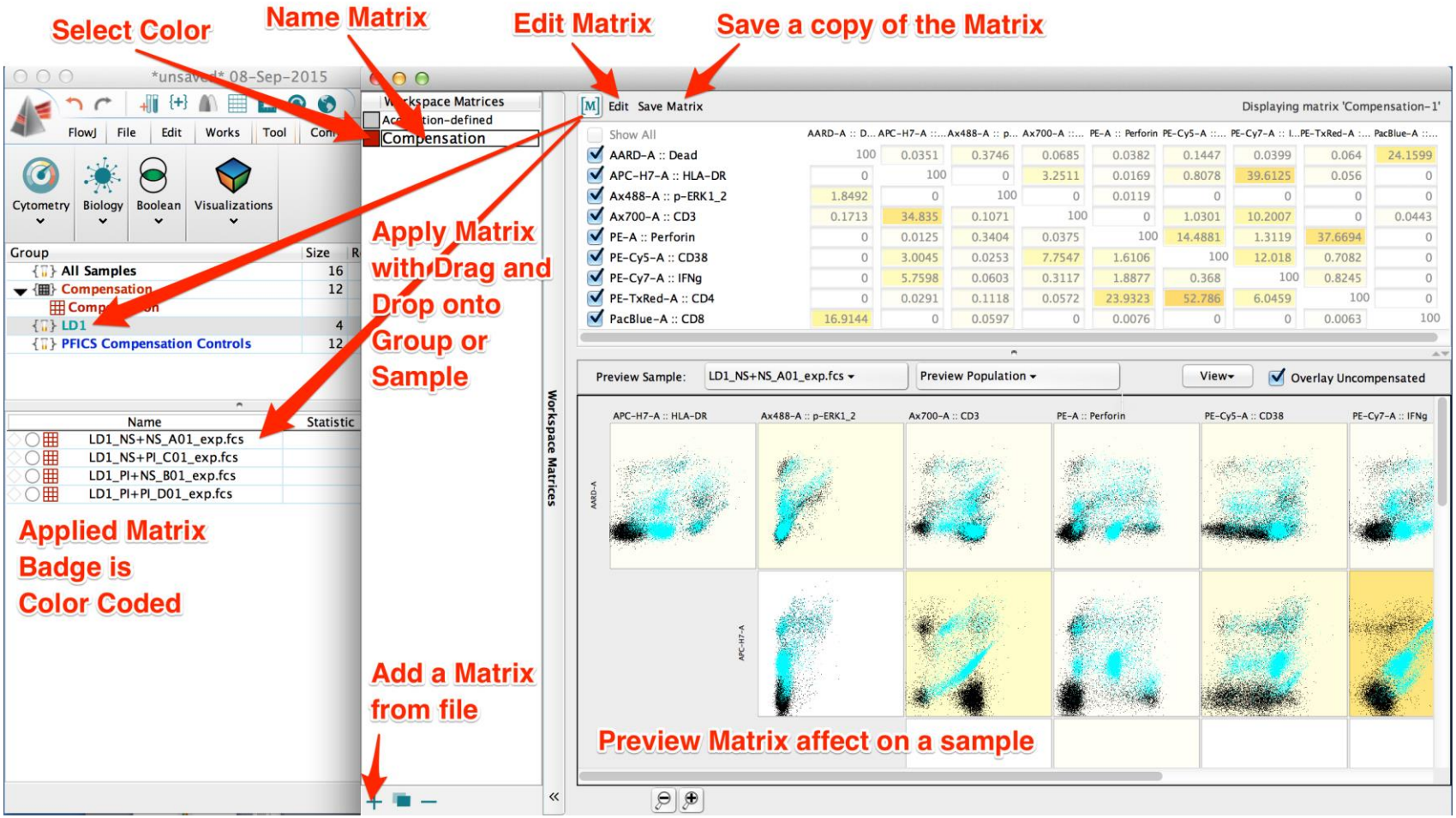
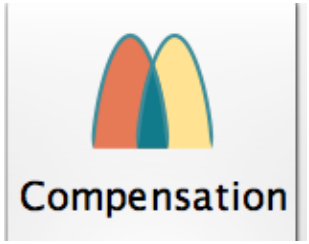
螢光補償 Compensation III

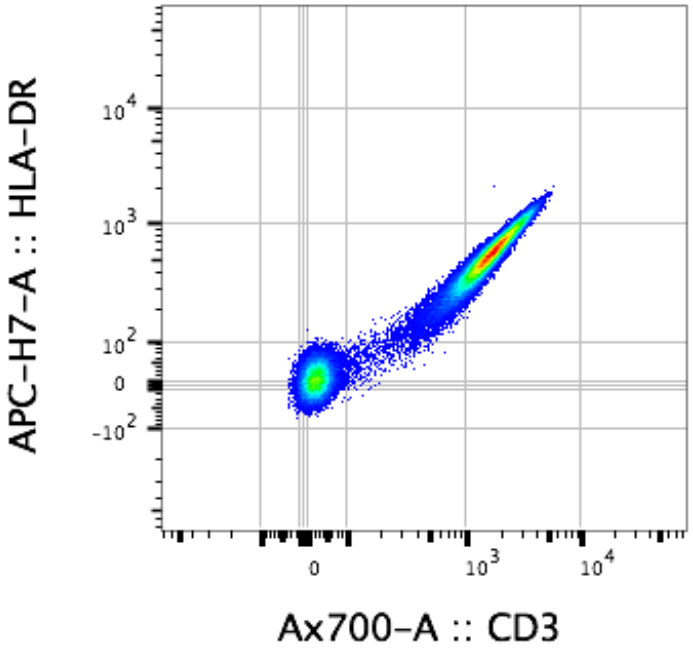
- Preview and apply the calculated matrix



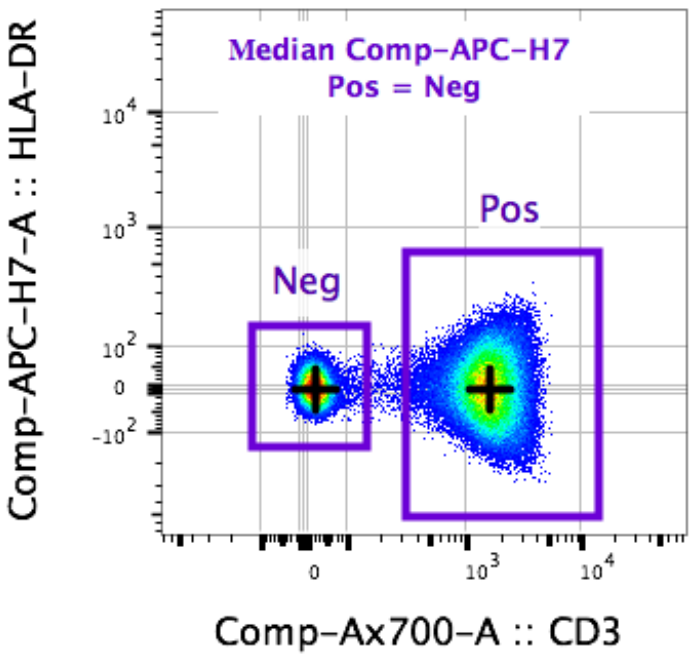
螢光補償 Compensation - Effects



Uncompensated



Compensated



Thank you!

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