

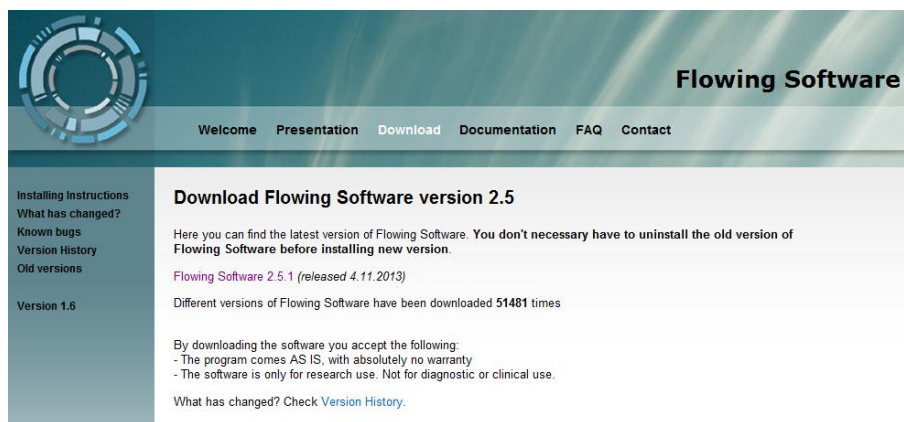
# Flowing Software 簡易軟體操作手冊

Flowing Software 為 Cell Imaging Core, Turku Centre for Biotechnology 的 Perttu Terho 特為 High through put data analyze 高通量數據分析所發展的免費軟體計畫。

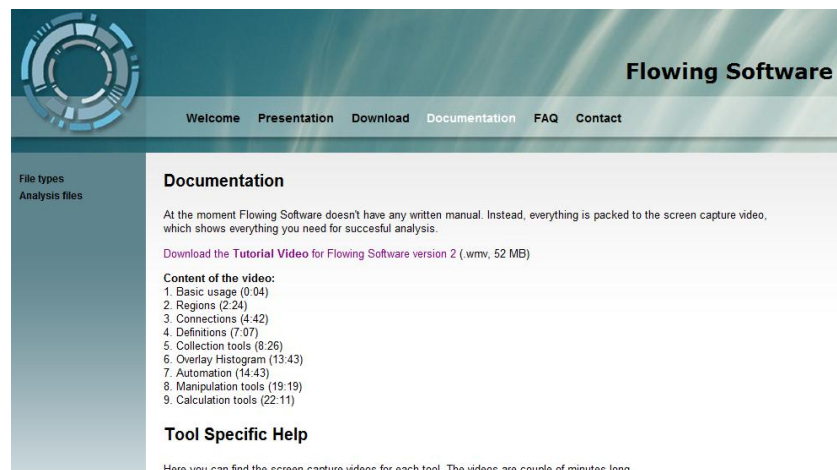
Flowing Software 網頁 <http://www.flowingsoftware.com/index.php?page=31>

適用環境 Windows XP, Vista, 7, 8

下載及安裝, 請至 Flowing Software 官網 Download 頁下載最新版本軟體



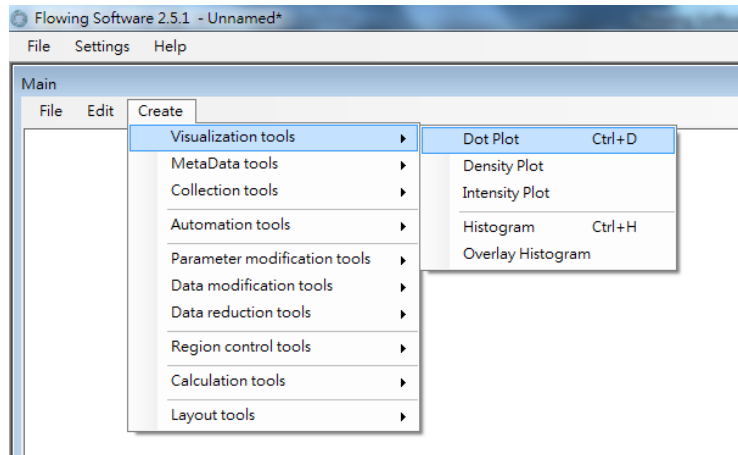
教學影片下載



## 1. 基本操作說明

### 1-1 Dot Plot

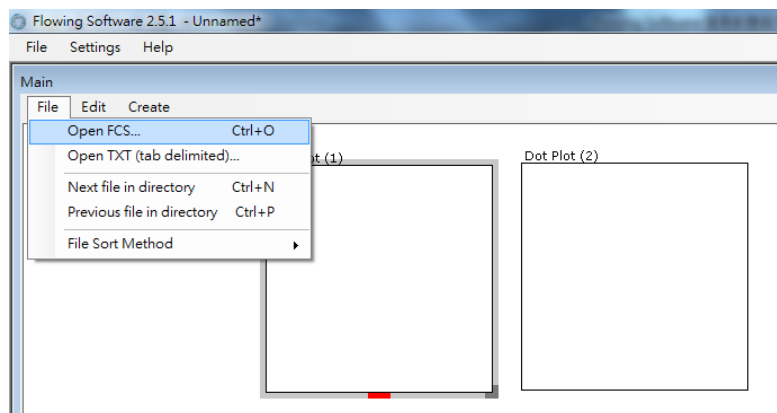
產生 Dot Plot: 於 Main 主視窗>Create>Visualization tools>Dot Plot (Ctrl+D)

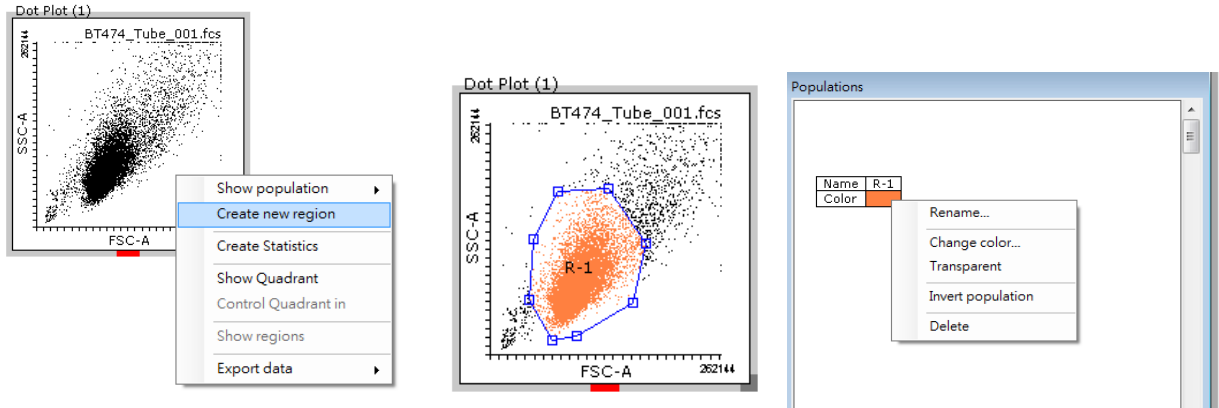


File>Open FCS (Ctrl+O) 開啟欲分析之檔案後, 可在圖上右鍵>Create new region

圈選目標細胞族群(自動命名為 R-1), 軟體主視窗右側 Population 出現 R-1 族群

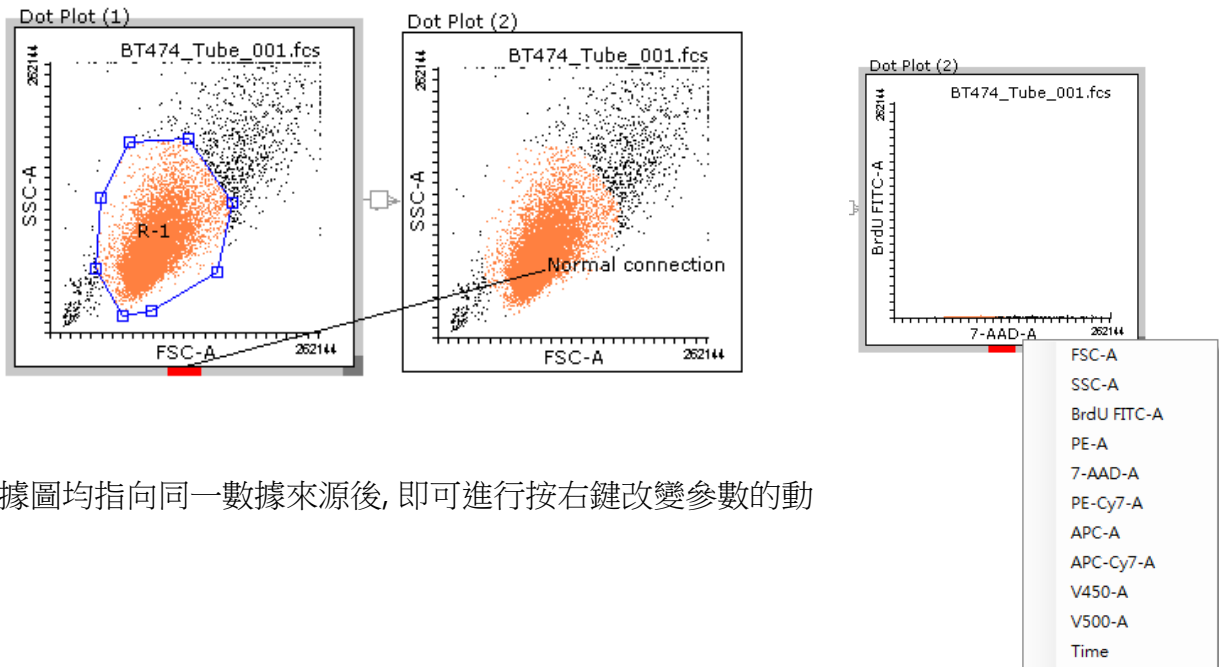
右鍵可對 R-1 進行重新命名, 更換顏色等動作





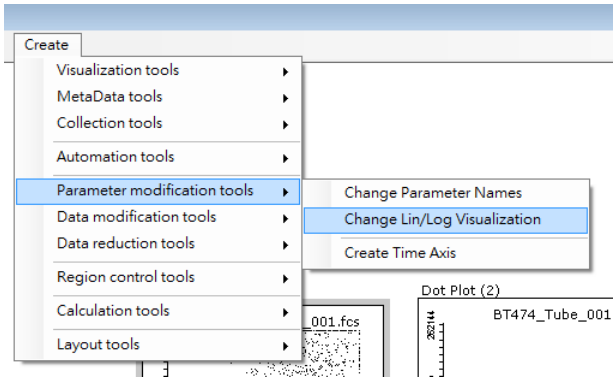
## 1-2 圖形之間的連動 (Global), 改變數據圖的參數

畫出所需數量的 Dot Plot 後, 可利用來源圖下方紅色區域拉出箭頭連結所有圖之間的關係, 使其均指向同一數據來源(FCS raw data)

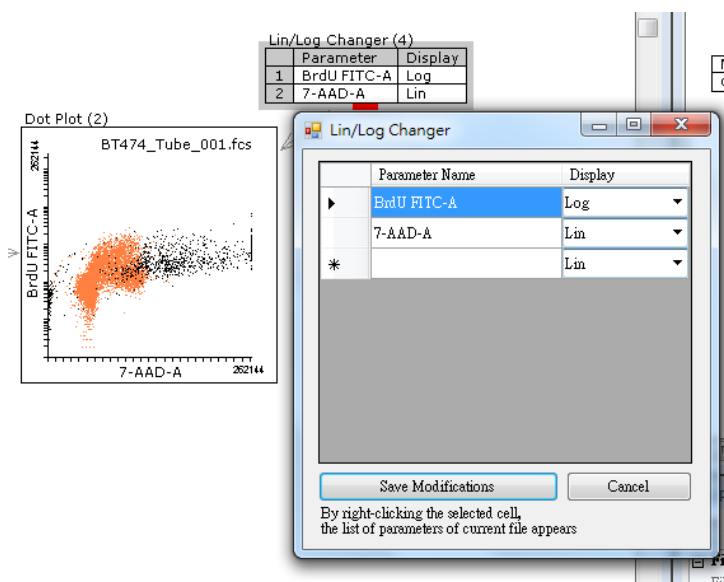
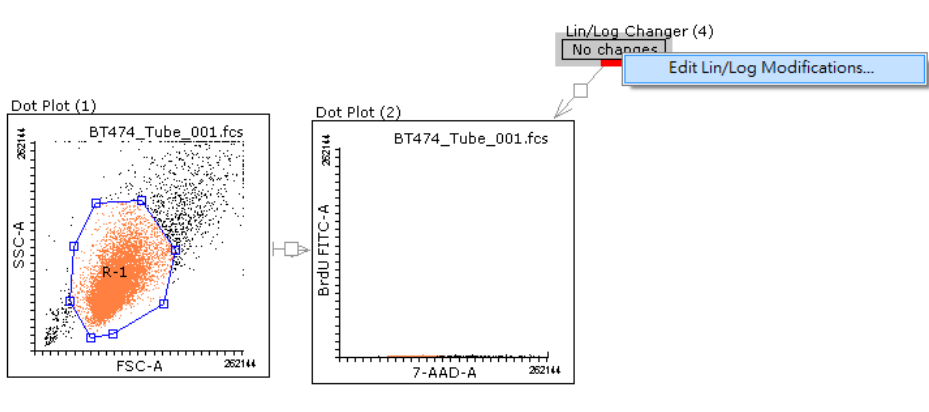


數據圖均指向同一數據來源後, 即可進行按右鍵改變參數的動作

如要改變原始數據參數名稱或者顯示方式可由 Main>Create>Parameter modification tools>Change Parameter Names or Change Lin/Log Visualization



欲更改參數 Lin/Log 表現, 新增一 Lin/Log Changer 控制視窗後將之連結到欲調整的數據圖上, 在控制視窗上按右鍵>Edit Lin/Log Modification

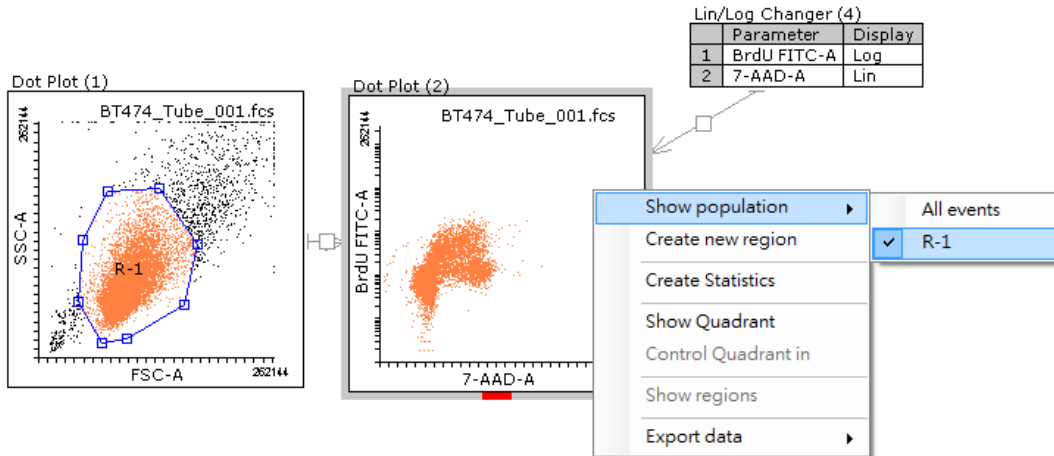


填入欲改變顯示方式的參數名

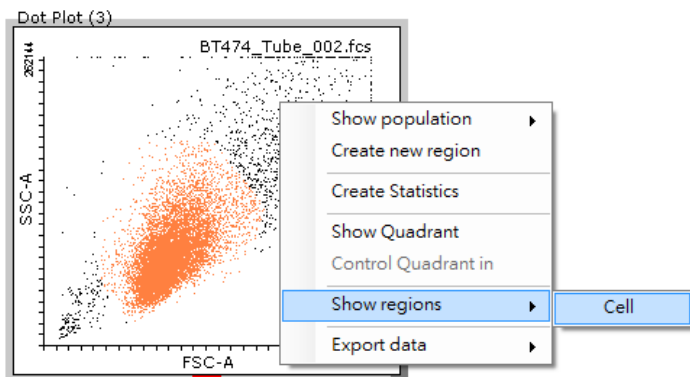
注意參數名稱需完全一致

### 1-3 顯示特定族群

在欲改變顯示的圖上按右鍵>Show population>選擇所欲顯示的族群 (例:R-1)



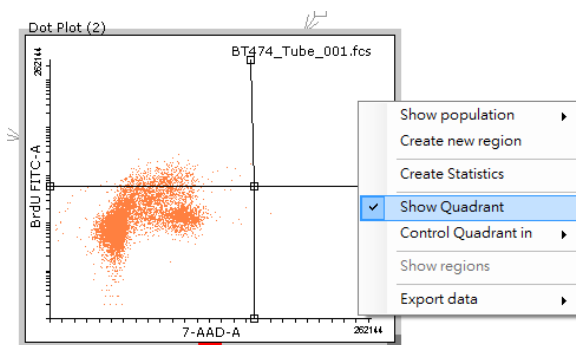
### 1-4 不同數據圖中顯示同樣的圈選區域(Gating)



在不同數據圖中, 若先前的數據圖已有圈選區域, 當在相同的參數組合下, 可於圖上右鍵

Show regions> 選擇相對應圈選區域即可顯示出 Gating

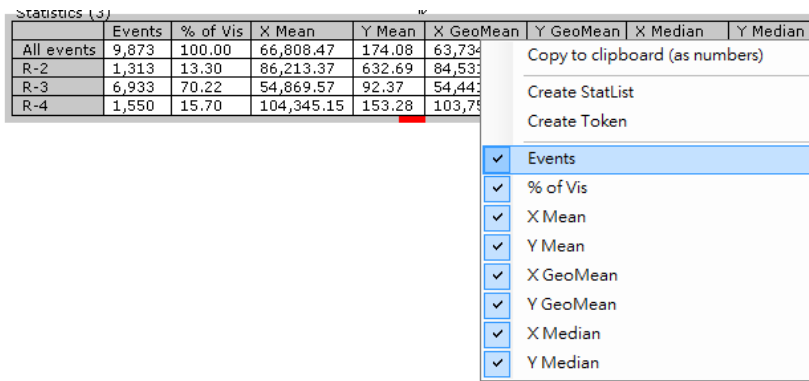
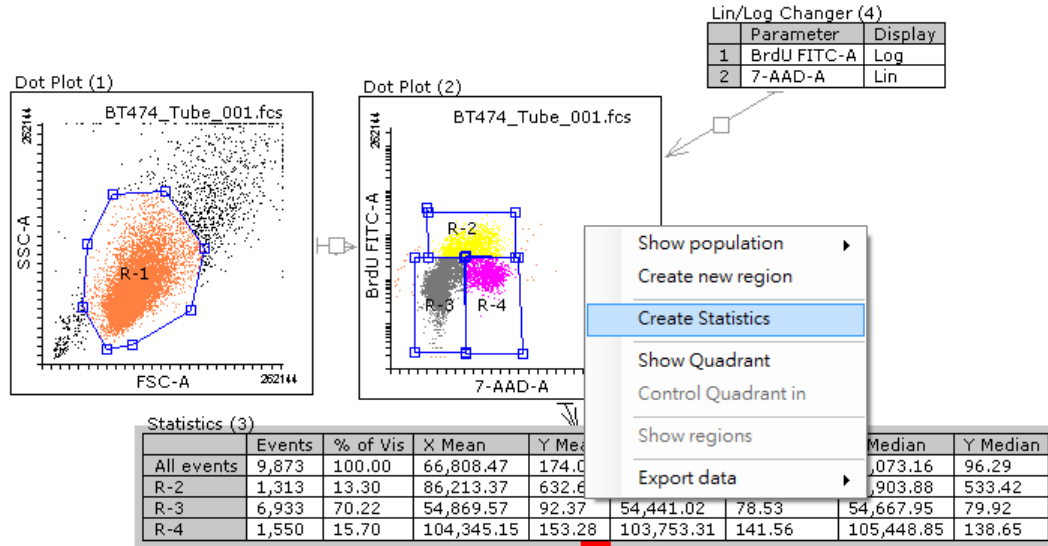
### 1-5 Quadrant (四象限圈選工具)



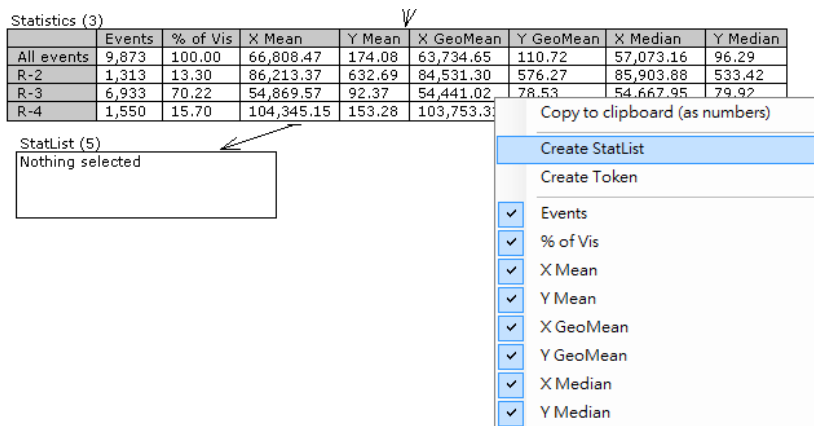
在欲使用 Quadrant 圈選工具圖上按右鍵, Show Quadrant 即可叫出四象限圈選工具並依需求調整之

## 1-6 統計表

畫出欲分析的統計區域後, 於圖上按右鍵>Create Statistics 即可叫出統計表



在統計表上按右鍵可以編輯顯示的統計項目(Events, % of Vis, X Mean, Y Mean 等)



Create StatList 可以將特定族群的特定統計項目歸納在同一表格內

Statistics (3)					
	Events	% of Vis	X Mean	Y Mean	X GeoMean
All events	9,873	100.00	66,808.47	174.08	63,734.65
R-2	1,313	13.30	86,213.37	632.69	84,531.30
R-3	6,933	70.22	54,869.57	92.37	54,441.02
R-4	1,550	15.70	104,345.15	153.28	103,753.31

StatList (5)			
	R-3: % of Vis	R-4: % of Vis	R-2: % of Vis
BT474_Tube_001.fcs	70.22	15.70	13.30

- All events: Y Median
- R-2: Events
- R-2: % of Vis
- R-2: X Mean
- R-2: Y Mean
- R-2: X GeoMean
- R-2: Y GeoMean
- R-2: X Median
- R-2: Y Median
- R-3: Events
- R-3: % of Vis
- R-3: X Mean
- R-3: Y Mean
- R-3: X GeoMean

在 StatList 上按右鍵將欲收集的族群-統計項目收集在同一個表格中

Statistics (7)		
	% of Vis	CV
All events	100.00	32.67
H-5	70.72	10.93
H-6	11.63	8.21
H-7	16.57	8.15

StatList (9)	
	H-5: % of
BT474_Tube_003.fcs	70.72

- Copy to clipboard (as numbers)
- Clear
- View type
- Heatmap
- All events: % of Vis
- All events: CV

在 Statistics 或 StatList 上右鍵  
 >Copy to clipboard (as number)  
 可複製數據, 貼至 excel 表中

## 1-7 批次分析 (Folder Runner)

Create>Automation tools>Folder Runner>Run through current folder

利用此工具可以批次將同一個資料夾中的檔案分析完畢並得到所有檔案的統計數據  
(搭配 StatList 等小工具使用)

The screenshot shows the FlowJo software interface. The 'Main' window has the 'Create' menu open, with 'Automation tools' selected. A sub-menu is open for 'Folder Runner', showing options: 'Run through current folder', 'Forward Jumper', 'Update Counter', 'Update Only in Column X', and 'Update when File Name contains'. The 'Run through current folder' option is highlighted. In the background, a dot plot is visible with a gate labeled 'Cell' and a table titled 'Lin/Log Changer (4)' with the following data:

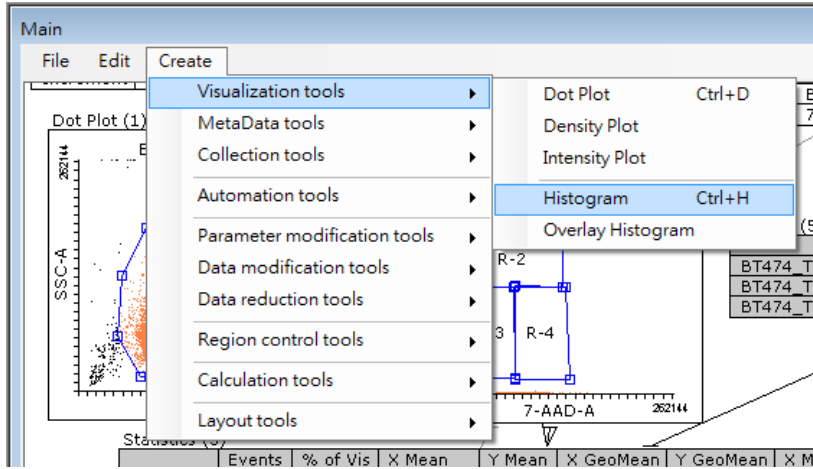
Parameter	Display
1 BrdU FITC-A	Log
2 7-AAD-A	Lin

Below the main window, a detailed view of the 'Folder Runner (8)' dialog box is shown. It includes fields for 'File name' (BT474\_Tube\_003.fcs) and 'Increment' (1). A 'Run through current folder' button is highlighted. Below the dialog, two dot plots are shown. The first is 'Dot Plot (1)' for 'BT474\_Tube\_003.f' showing a gate labeled 'Cell' on an SSC-A vs FSC-A plot. The second is a plot for 'BrdU FITC-A' vs '7-AAD-A' showing gates R-2, R-3, and R-4. To the right, a 'StatList (5)' window displays a list of files: BT474\_Tube\_003.fc, BT474\_Tube\_001.fc, and BT474\_Tube\_002.fc.

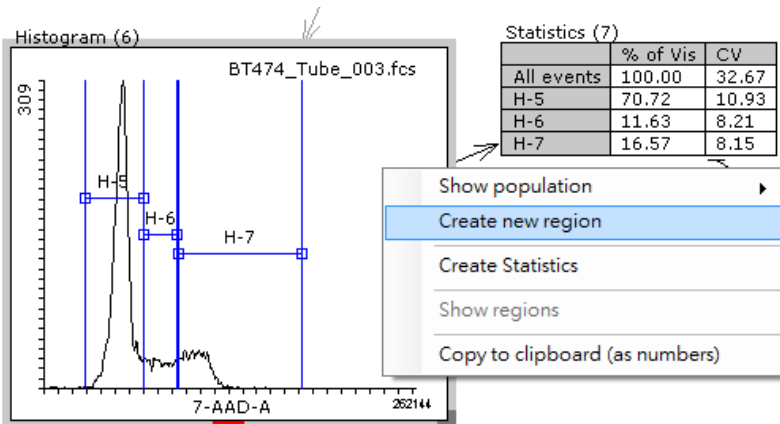


## 2. 直方圖

### 2-1 基本操作



Create>Visualization  
tools>Histogram



將直方圖同樣與其他數據圖  
做連結後, 右鍵>Create new  
region 即可畫出 interval  
gate, 其餘統計表產生方式與  
Dot plot 圖相同

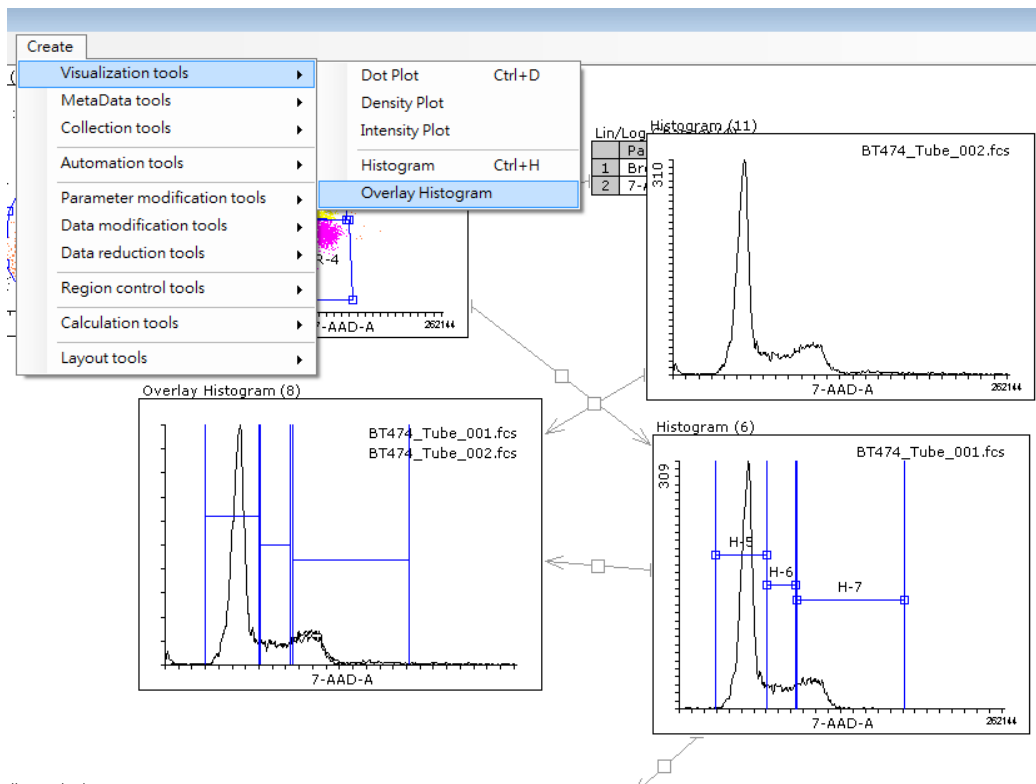
## 2-2 Overlay Histogram 直方圖疊圖

先畫出欲疊圖的直方圖並設定其數據來源 (選圖後 Open FCS or Ctrl+O)

再叫出重疊直方圖工具

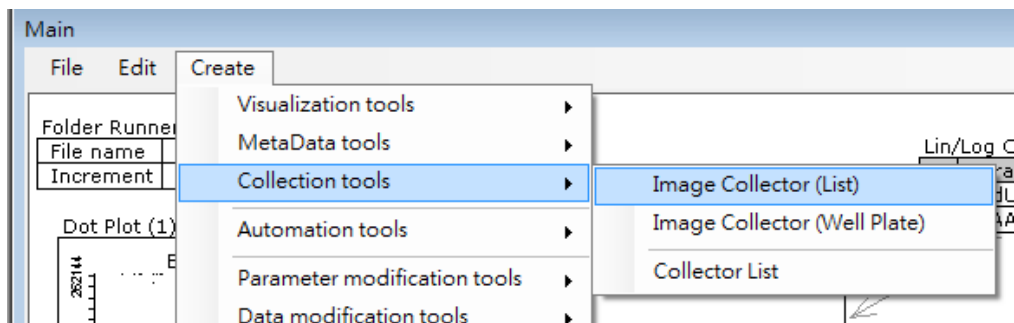
Create>Visualized tools> Overlay Histogram

將來源直方圖下方紅色區域拉出箭頭指向此疊圖後即可將直方圖重疊在一起

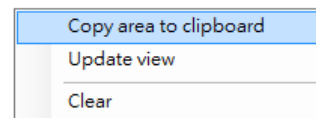
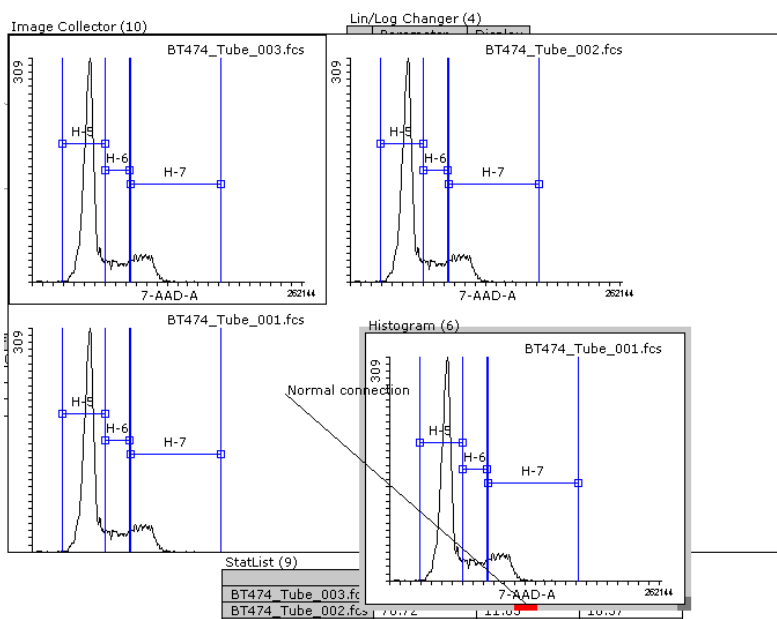


### 3. Collection tools (圖檔收集器)

Create>Collection tools>Image Collector (List)



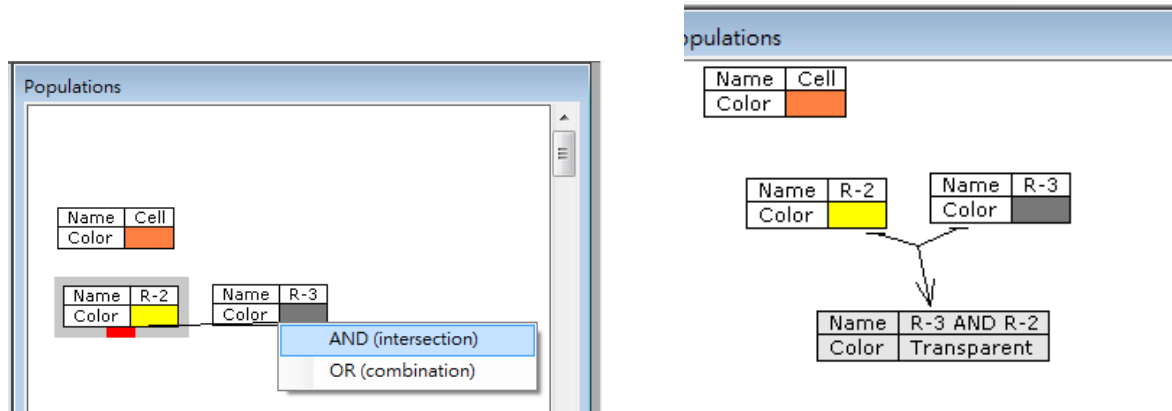
將欲收集的圖連結到 Image Collector 中, 利用更換數據來源或者 Folder Runner 等方式收集所有欲收集的圖檔



Copy area to clipboard  
可以複製圖檔到 ppt 等

#### 4. Population 布林邏輯

連結欲做布林邏輯的 Population 即可產生 AND, OR 的交集聯集等邏輯



#### 5. Meta Data Viewer/ Compensation Matrix/Parameter data viewer

The screenshot shows the 'Main' menu with 'MetaData tools' expanded to 'Metadata Viewer'. A 'Metadata Viewer (8)' window is open, displaying a table of metadata.

Metadata Viewer (8)	Value
Cytometer	BD FACSVers
Total Events	11116
Parameters	11
Comments	
Date	16-Apr-2014
Begin time	16:23:40
End time	16:24:43
Time (sec)	63
Events/sec	176.4

Compensation Matrix: 可顯示螢光補償資訊

Parameter data viewer: 可顯示各參數數值