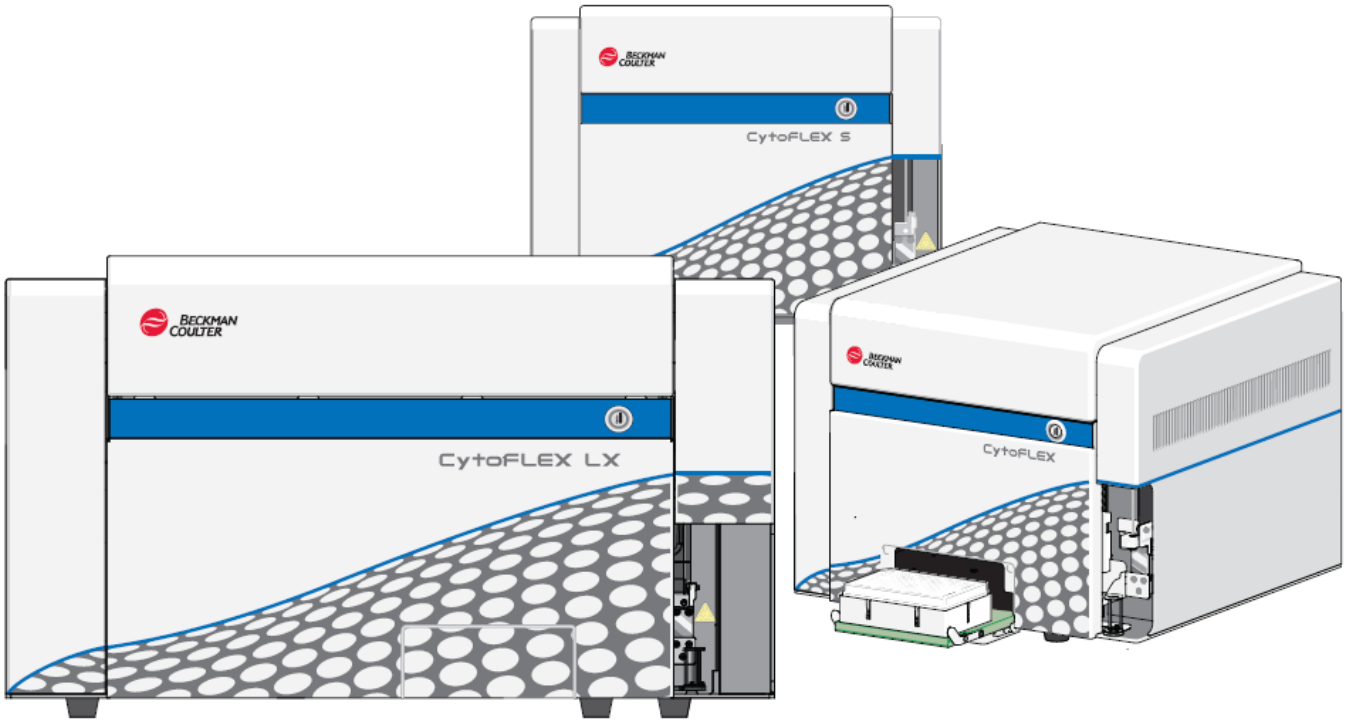
CytoFLEX™ Flow Cytometer中文操作手冊



BECKMAN COULTER
Life Sciences

美商貝克曼庫爾特公司生命科學部

http://www.beckmancoulter.com.tw

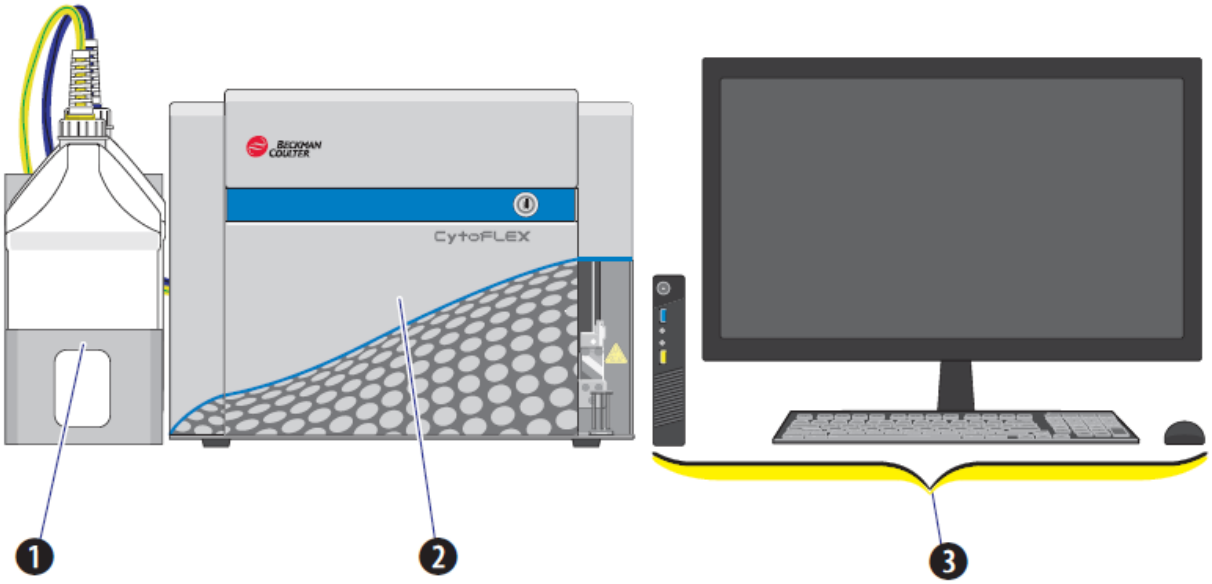
Software

CytExpert 儀器操控軟體，與機器連線收取 Flow Data 及進行數據分析

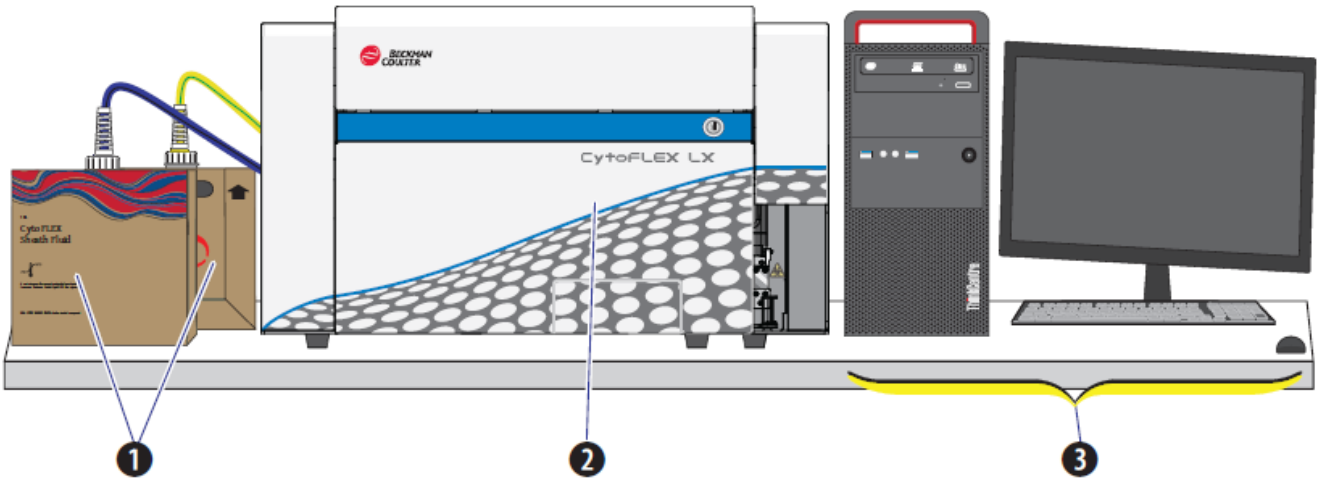
目　　錄

一、CytoFLEX Flow Cytometer儀器外觀

[CytoFLEX & CytoFLEX S]



[CytoFLEX LX]



(1) Fluid Containers(藍色管路Sheath Fluid, 黃色管路Waste Bottle), (2) Cytometer and (3) Workstation.

二、CytoFLEX使用之Channels及對應螢光染劑

[CytoFLEX]

Laser	Filter	Channel Names	Dyes
488nm	525/40	FITC	FITC, AlexaFluor™ 488, CFSE, Fluo-3
	585/42	PE	PE, PI
	610/20	ECD	ECD, PE-Texas Red®, PE-CF594, PI
	690/50	PC5.5	PerCP, PerCP-Cy5.5, PE-Cy5, PE-Cy5.5
	780/60	PC7	PE-Cy7
638nm	660/20	APC	APC, AlexaFluor™ 647, eFluor™ 660
	712/25	APC-A700	APC-A700, AlexaFluor™ 700
	780/60	APC-A750	APC-A750, APC-Cy7, APC-H7, APC-eFluor™ 780
405nm	450/45	PB450	Pacific Blue, V450, eFluor™ 450, BV421, DAPI, Hoechst
	525/40	KO525	Krome Orange, AmCyan, V500, BV510
	610/20	Violet610	BV605, Qdot® 605, eF605
	660/20	Violet660	BV650, Qdot® 655
	780/60	Violet780	BV786, Qdot® 800

[CytoFLEX S] 375 Laser

Laser	Filter	Channel Names	Dyes
488nm	525/40	FITC	FITC, AlexaFluor™ 488, CFSE, Fluo-3
	585/42	PE	PE, PI
	610/20	ECD	ECD, PE-Texas Red®, PE-CF594, PI
	690/50	PC5.5	PC5, PC5.5, PerCP, PerCP-Cy5.5, PE-Cy5, PE-Cy5.5
	780/60	PC7	PC7, PE-Cy7
638nm	660/20	APC	APC, AlexaFluor™ 647, eFluor™ 660, Cy®5
	712/25	APC-A700	APC-A700, AlexaFluor™ 700, Cy®5.5
	780/60	APC-A750	APC-A750, APC-Cy7, APC-H7, APC-eFluor™ 780
405nm	450/45	PB450	Pacific Blue, V450, eFluor™ 450, BV421, DAPI, Hoechst
	525/40	KO525	Krome Orange, AmCyan, V500, BV510
	610/20	Violet610	BV605, Qdot® 605, eF605
375nm	450/45	DAPI	DAPI, HoechstBlue
	675/30	HoechstRed	HoechstRed

[CytoFLEX S] 561 Laser

Laser	Filter	Channel Names	Dyes
488nm	525/40	FITC	FITC, AlexaFluor™ 488, CFSE, Fluo-3
	690/50	PC5.5	PC5, PC5.5, PerCP, PerCP-Cy5.5, PE-Cy5, PE-Cy5.5
561nm	585/42	PE	PE, PI
	610/20	ECD	ECD, PE-Texas Red®, PE-CF594, PI
	690/50	PC5.5	PC5, PC5.5, PerCP, PerCP-Cy5.5, PE-Cy5, PE-Cy5.5
	780/60	PC7	PC7, PE-Cy7
638nm	660/20	APC	APC, AlexaFluor™ 647, eFluor™ 660
	712/25	APC-A700	APC-A700, AlexaFluor™ 700
	780/60	APC-A750	APC-A750, APC-Cy7, APC-H7, APC-eFluor™ 780
405nm	450/45	PB450	Pacific Blue, V450, eFluor™ 450, BV421, DAPI, Hoechst
	525/40	KO525	Krome Orange, AmCyan, V500, BV510
	610/20	Violet610	BV605, Qdot® 605, eF605
	660/20	Violet660	BV650, Qdot® 655

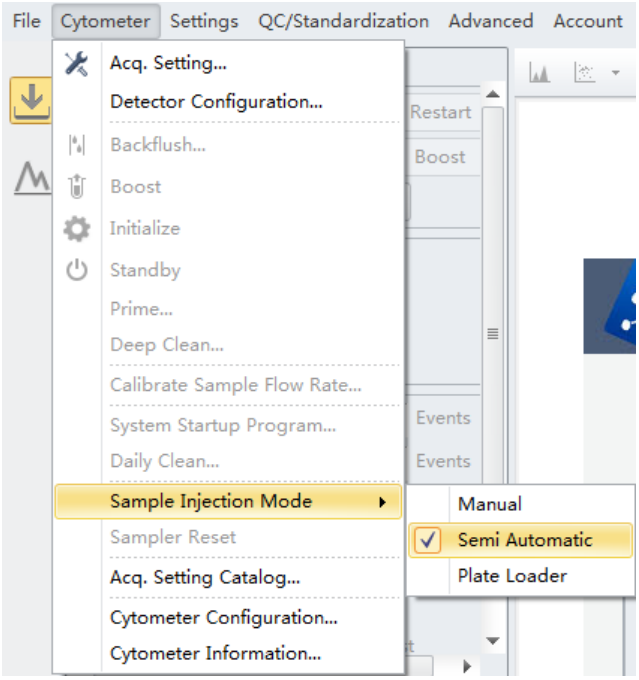
[CytoFLEX LX]

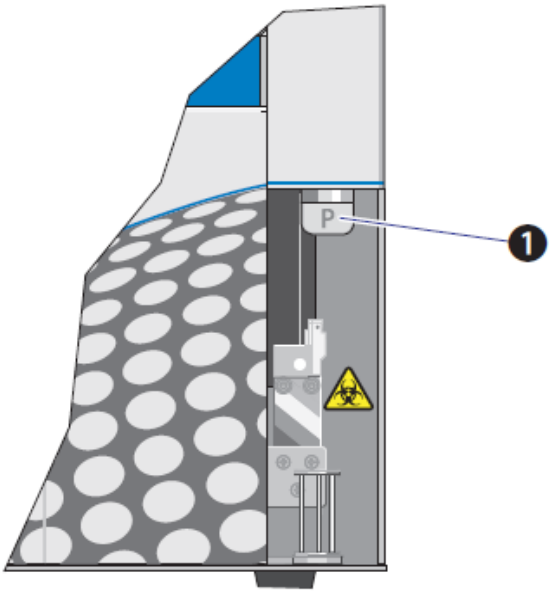
Laser	Filter	Channel Names	Dyes
405nm	450/45	V450-PB450	Pacific Blue, V450, eFluor™ 450, BV421, DAPI, Hoechst
	525/40	V525-KrO	Krome Orange, AmCyan, V500, BV510
	610/20	V610	BV605, Qdot® 605, eF605
	660/20	V660	BV650, Qdot® 655
	763/43	V763	BV785, Qdot® 800
488nm	525/40	B525-FITC	FITC, AlexaFluor™ 488, CFSE, Fluo-3
	690/50	B690-PC5.5	PC5, PC5.5, PerCP, PerCP-Cy5.5, PE-Cy5, PE-Cy5.5
	610/20	B610-ECD	ECD, PE-Texas Red®, PE-CF594, PI
561nm	610/20	Y610-Mcherry	Mcherry, ECD, PE-CF594
	585/42	Y585-PE	PE, DsRed
	675/30	Y675-PC5	PC5, mPlum
	710/50	Y710-PC5.5	PC5.5, PE-AF680
	763/43	Y763-PC7	PC7
638nm	660/20	R660-APC	APC, AlexaFluor™ 647, eFluor™ 660, Cy®5
	712/25	APC-A700	APC-A700, AlexaFluor™ 700, Cy®5.5
	763/43	R763-APC-A750	APC-A750, APC-Cy7, APC-H7, APC-eFluor™ 780
808 nm	840/20	IR840-A790	Alexa Fluor®790
	885/40	IR885	PromoFluor-840, IR fixable viability dye

三、[Semi-Automatic sample模式] 開機步驟與軟體主畫面說明

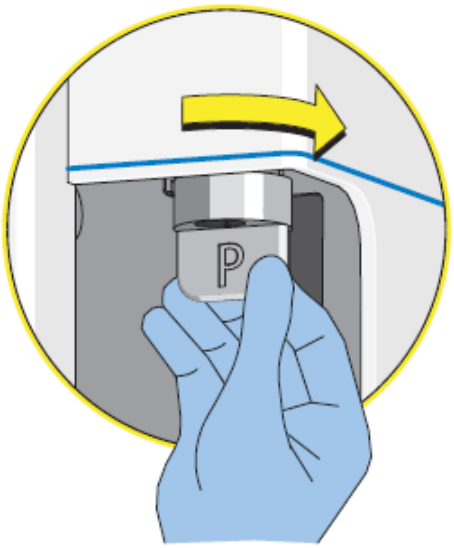
1. 開啟 CytoFLEX 背面(左側)電源。
2. 確認 Sheath Fluid 足夠，並且清空廢液筒，不要鎖緊蓋子。
3. 開啟電腦主機以及螢幕電源，依常規模式進入 Windows 系統。
4. 點選桌面上 CytExpert 軟體 

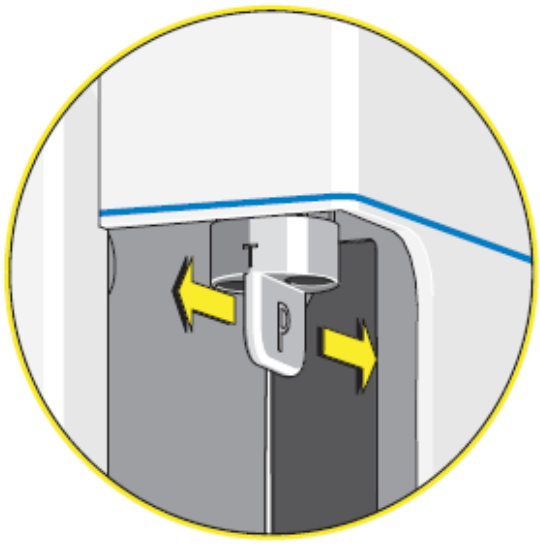
，進入操作軟體。
5. 點選 Cytometer，選擇 Sample Injection Mode，點選 **Semi-Automatic** 單管式自動上樣，再開啟 CytoFLEX 背面(左邊)電源，使儀器自動校正上樣區位置：





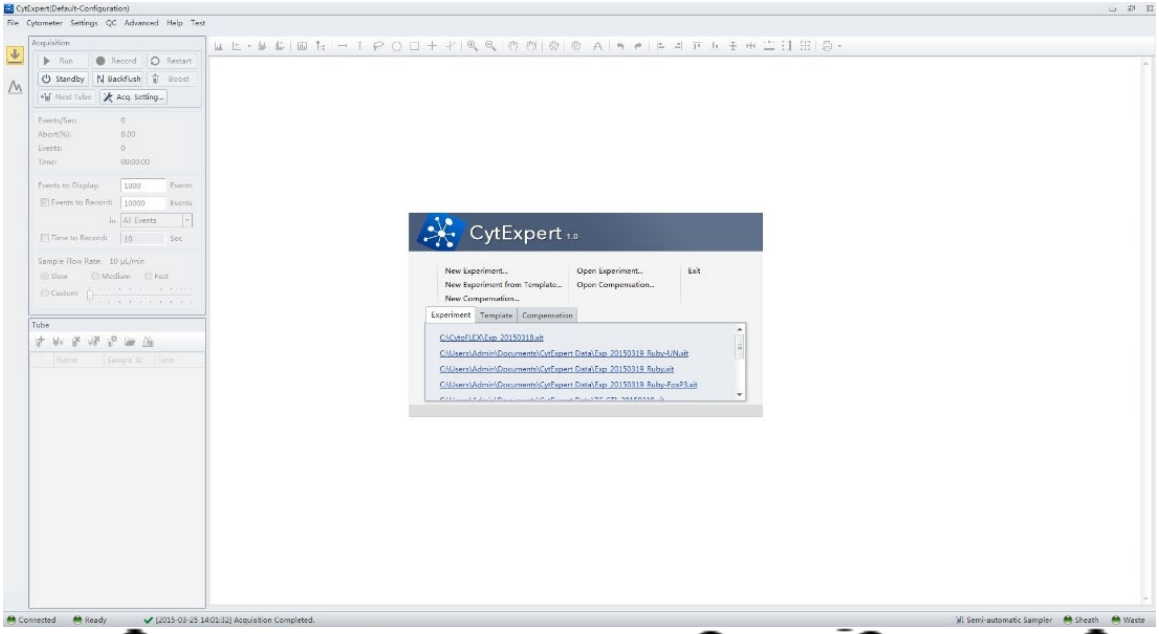
6. Cytometer 儀器上樣區開關旋鈕模組①Switch module knob 旋轉至 T 位置，如下圖顯示：





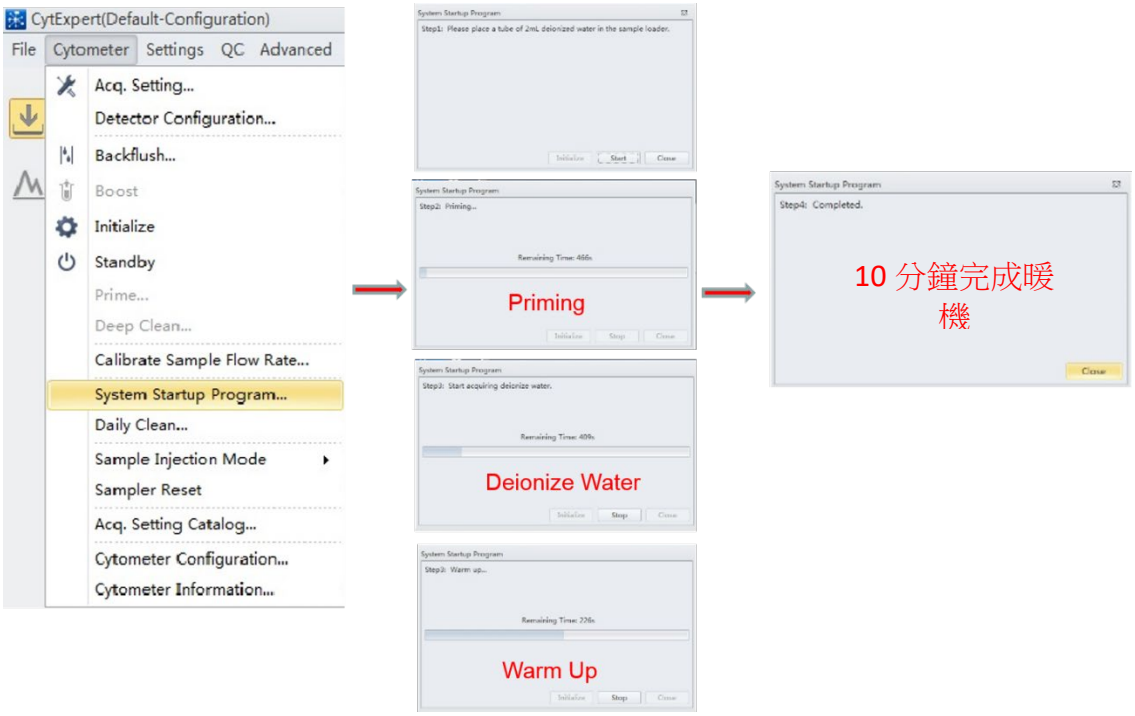
7. ※若有開啟**[User Management功能]**，此時可見以下畫面：

8. ※選取專屬的Username，接著在Password欄位輸入密碼並按下  繼續。
9. 此時進入軟體歡迎主畫面，確認左下方 Connected 及 Ready 和右下方 Sheath 及 Waste 為綠燈，表示電腦與機器連線完成。

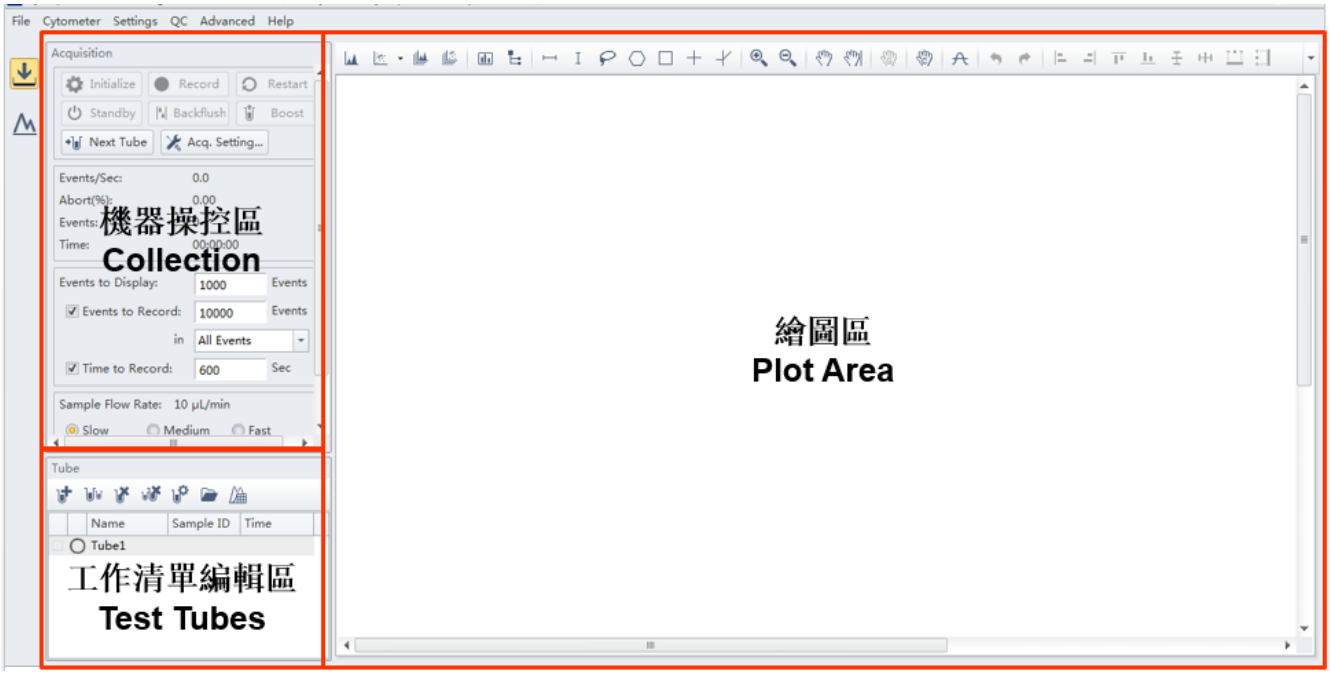


1. Communication connection status.
2. Instrument status information.
3. Laser status.
4. Sampler status.
5. Fluid status information.

10. 由 Cytometer 進入”System Start Up Program”，放上 2 mL 去離子水，點擊 Start，CytoFLEX 執行 Priming、沖洗去離子水及 Warm Up，約 10 分鐘完成開機及暖機動作，點擊 Close。

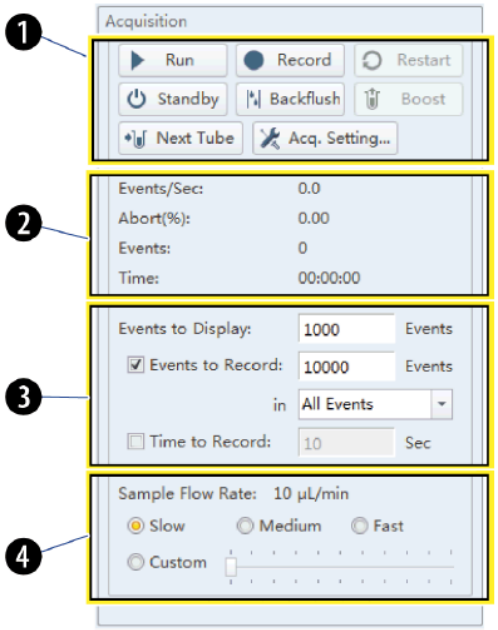


11. 由 File 進入(或起始頁面)，點選 New Experiment 並儲存實驗檔案名稱，即可見到軟體的工作區，如下圖：



1. **Collection.** Establishes control over data recording options and displays the acquisition status.
2. **Test tubes.** Allows you to configure and duplicate sample tubes, set display attributes, manage experimental data and compensation.
3. **Plot area.** Includes plot and gating controls, as well as an area for drawing plots and generating graphs.

機器操控區(Collection)



1. Acquisition control. Controls sample loading/unloading and data acquisition and recording.

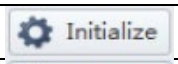
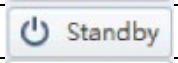
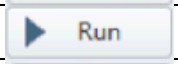
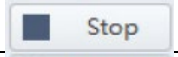
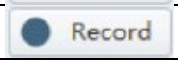
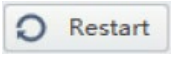
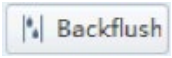
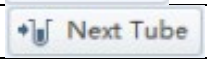
2. Acquisition status. Displays such information as the acquisition rate (Events/Sec), cell count, duration and abort (%).

3. Acquisition conditions. Sets the necessary conditions for recording data.

- **Events to Record.** Used to set the number of events to record in the specified population.
- **Time to Record.** Used to set the collection time duration in seconds.

4. Sample flow rate. Sets the acquisition rate for data collection.

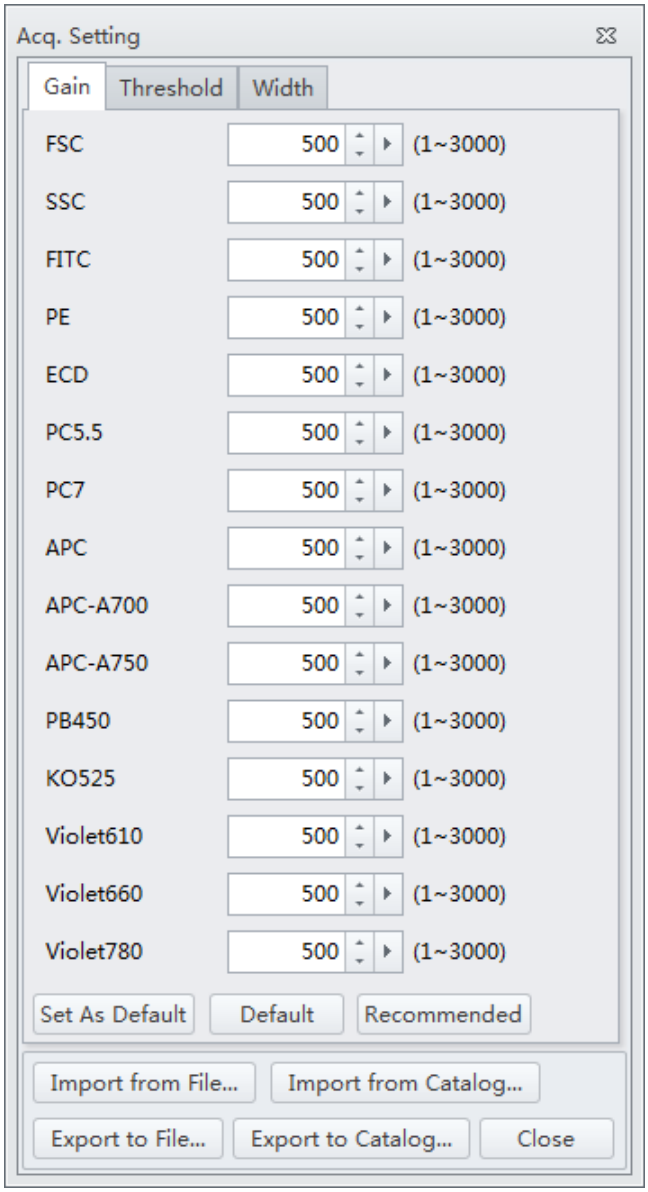
- **Slow :** 10 µL/min - **Medium :** 30 µL/min - **High :** 60 µL/min
- **Custom :** 10 - 240 µL/min

	Put the instrument in initialized state.
	Put the instrument in standy state.
	Start acquisition or continue an acquistion if previosly stop.
	Stop the acquistion of the current sample and output the results.
	Used to set the collection conditions for sample recording.
	Reset the current acquired events to zero and clear the current data in memory. Acquisition restarts at zero events.
	Flush the sample line and flow cell with sheath fluid to remove bubbles.
	To transfer the sample to the flow cell.
	Switch to the next sample tube.
	Display the acquisition setting dialog box to adjust the Cytometer settings.

※ 

Acquisition Setting 為儀器條件設定操控視窗，包含：

1. Gain：調整偵測器訊號放大程度。

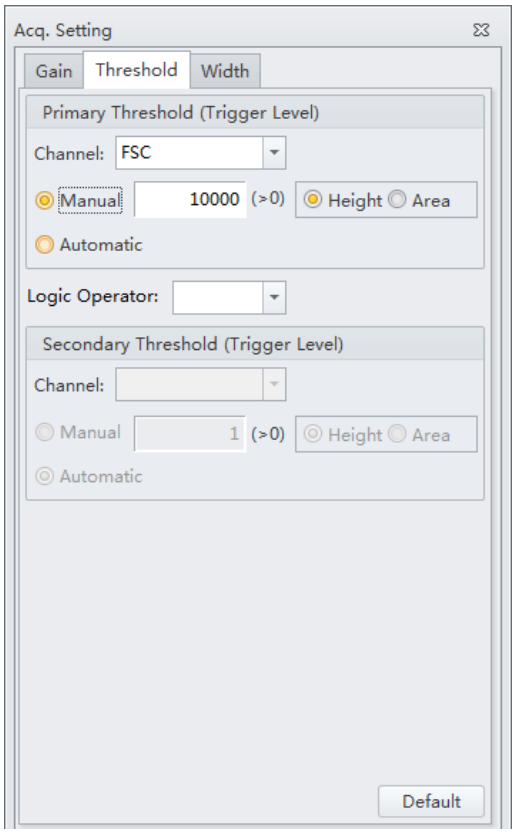


Note：

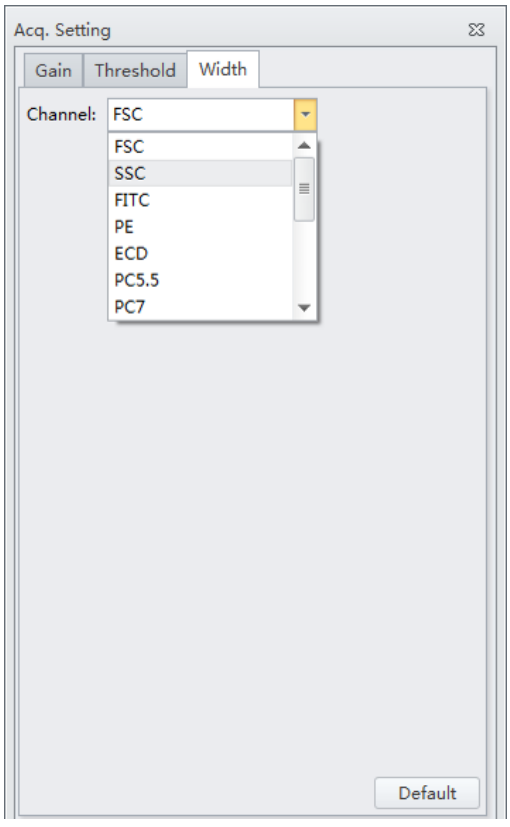
電壓值須注意不要過低，不要低於個位數。

一般細胞樣本 Background 建議設定在 10^2 – 10^4 之間。

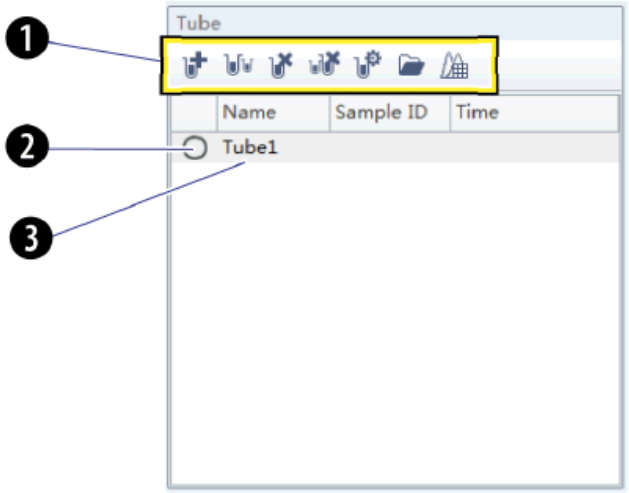
2. Threshold：排除雜訊的門檻，建議使用 Automatic 設定。

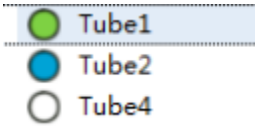


3. Width：訊號通過的時間參數，可選擇所要偵測的參數值時間。



工作清單編輯區(Test Tubes)





1. **Test tube management controls.** Manages sample tubes. Used to add, copy, or delete attributes, open the tube property, and open the compensation matrix.
2. **Test tube status indication.** Displays a colored symbol in front of each tube indicating the status of the tube processing.
3. **Test tube list.** Displays the sample tubes used in the experiment. Right-click a tube in the list to perform additional operations.

	New Tube	Create a new tube.
	Duplicate without data	Create a blank row at the end of the current experiment.
	Delete Tube	Delete one currently selected tube.
	Delete Multiple Tubes	Delete the multiple selected tubes.
	Property	Sample tube basic information.
	Open the folder	Open the experiment folder.
	Compensation Matrix	Open the compensation matrix.

白	Indicates that the tube data was not collected.
藍	Indicates that the tube data was acquired by selecting **Run** but not saved, and the tube data can be overwritten.
綠	Indicates that the tube data was saved by selecting **Record** and that this data cannot be overwritten.

繪圖區(Plot area)

	Histogram	Create a Histogram Plot and specify the plot properties.
	Dot Plot	Create a Dot Plot and specify the plot properties.
	Density Plot	Create a Density Plot and specify the plot properties.
	Pseudo Color Plot	Create a Pseudo Color Plot and specify the plot properties.
	Contour Plot	Create a Contour Plot and specify the plot properties.
	New Histograms	Create multiple Histogram Plots and specify the plot properties.
	New 2-D Plots	Create multiple Dot Plots and specify the plot properties.
	Statistics	Create Statistical charts.
	Population Hierarchy	Create Hierarchical charts.
	Line Segment	Insert a Linear gating of plots.
	Vertical	Insert a Vertical gating of plots.
	Lasso	Insert a Lasso gating into a dual parameter plots.
	Polygon	Insert a Polygon gating into a dual parameter plots.
	Rectangle	Insert a Rectangle gating into a dual parameter plots.
	Four Quadrant	Insert a Four Quadrant gating into a dual parameter plots.
	Hinged	Insert a Hinged gating into a dual parameter plots.
	Auto Line Segment	Creat an Auto Line Segment around the selected population on a plot.
	Auto Polygon	Creat an Auto Polygon around the selected population on a plot.
	Zoom In	For Zooming in.
	Zoom Out	For Zooming out.
	Pan	For scaling axis ranges in the plots.
	Single Side Pan	For scaling single axis range in the plots.
	Adjust Gain	For increasing and lowering gain adjustments on the plots.
	Adjust Compensation	For adjusting compensation of either of the parameters on a 2-D histogram.
	Threshold	For setting the minimum particle size limit or flurescence intensity that acquisition will allow.
	Undo	For undoing an action in the drawing area.
	Redo	For redoing an action in the drawing area.

	Align Left	Align all the selected items to the left of the selection area.
	Align Right	Align all the selected items to the right of the selection area.
	Align Top	Align all the selected items to the top of the selection area.
	Align Bottom	Align all the selected items to the bottom of the selection area.
	Vertical Distribute	Align all the selected items to the vertical distribution.
	Horizontal Distribute	Align all the selected items to the horizontal distribution.
	Make Same Width	Resize the selected items to all be the same width as the reference item.
	Make Same Hight	Resize the selected items to all be the same hight as the reference item.
	Make Same Size	Resize the selected items to all be the same size as the reference item.
	Rearrange	For restoring the plots to the default positions.
	Print	For printing and previewing the plot area.
	Print Preview	Used to access the Preview screen.
	Page Setup	Used to adjust the page settings.
	Batch Print	Used to print data for multiple tubes.
	Batch Export to PDF File	Used to print a PDF of the data for multiple tubes.

四、流式細胞儀品管流程

1. CytoFLEX Daily QC Fluorospheres 螢光品管微球

意義與目的：

CytoFLEX Daily QC Fluorospheres (Part # B53230)是一種大小和螢光強度均一而穩定的螢光球懸浮液。用於 CytoFLEX 流式細胞儀每日光學系統(散射光及螢光)及液流系統的調校與確效。

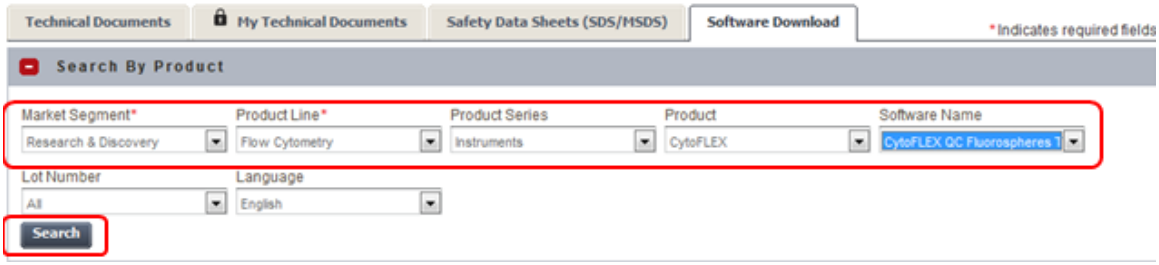
本產品為約 3 μm 大小的螢光球，可被 488 nm 藍光雷射、638 nm 紅光雷射及 405 nm 紫光雷射所激發，發射 410 nm 至 800 nm 波長的螢光，用來評估前向散射光(Forward Scatter, FSC)、側向散射光(Side Scatter, SSC)以及 FL1 - FL13 的螢光參數。

CytoFLEX Daily QC 品管液所測得的前向散射光、側向散射光以及每個螢光參數 FL1 - FL13 都會依照下列原廠規範條件作檢測：

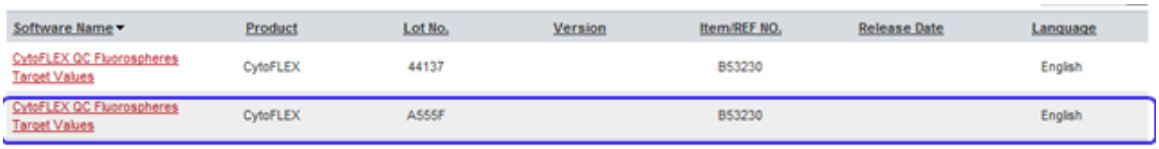
- The gain differences must must be≦20% from the target gain.
- The median fluorescense intensity (MFI) differences must be≦5% from the target MFI.
- The rCV must be≦5%.

每次開機後，分析樣品前，務必先分析 CytoFLEX Daily QC 品管液以確認儀器處於穩定狀態，或是當懷疑儀器故障或不穩定時，也可先分析一管 CytoFLEX Daily QC 品管液進行初步檢查。

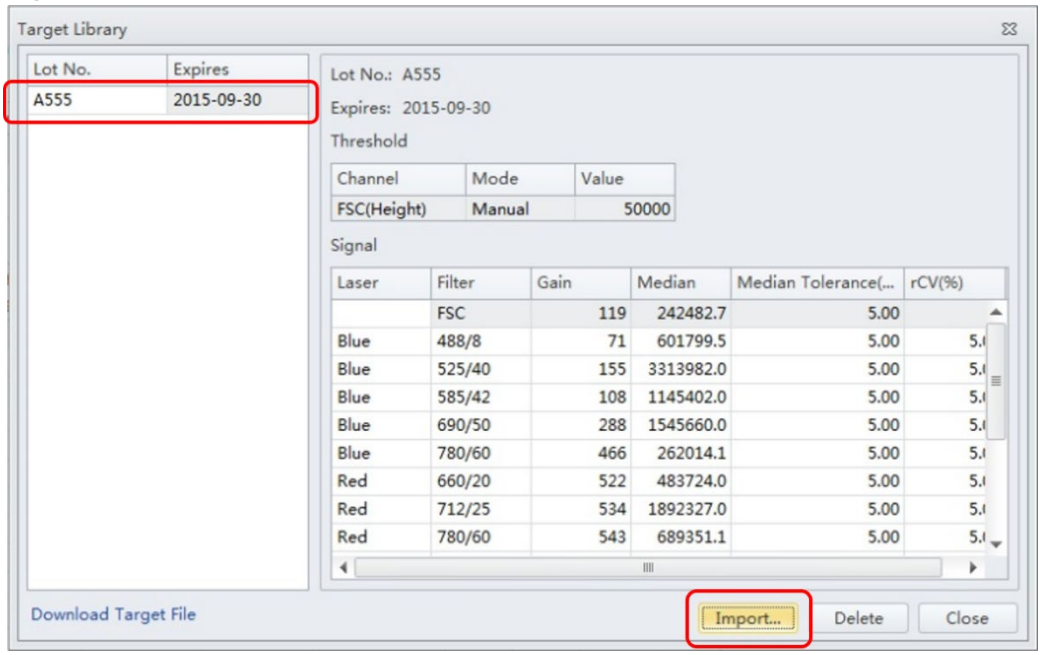
2. 下載及輸入 CytoFLEX Daily QC Fluorospheres 的 Target Value。
購買新一批 CytoFLEX Daily QC Fluorospheres，需到下列網址下載其 Target Value 並輸入 CytoFLEX 軟體中。
https://www.beckmancoulter.com/wsrportal/page/softwareDownloadSearch
(1). 如下圖選擇後點擊 Search。



(2). 選擇購買 CytoFLEX Daily QC Fluorospheres 的批號(例如：A555F)，下載存在行動裝置中(A555.tgt)。



(3). 由 QC 進入 Start QC，再由 Settings 進入 Target Library，Import 下載的 Target Value。



3. 執行 QC 流程，建議每天完成開機暖機後即進行此步驟，以瞭解儀器狀態。

(1). 將 CytoFLEX Daily QC Fluorospheres 自冰箱取出，先震盪讓沉澱的 QC Fluorospheres 均勻混合。

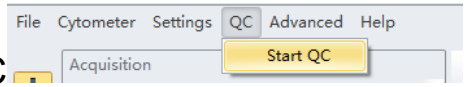
(2). 製備 CytoFLEX Daily QC Fluorospheres 品管液：

[Semi-Automatic sample 模式]

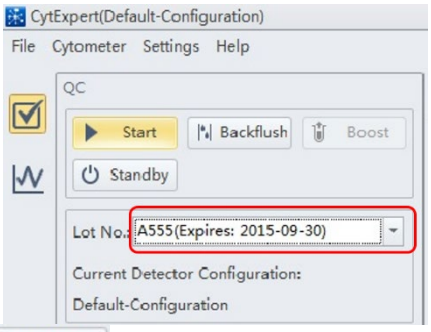
滴 3 滴於 tube 中(12 x 75 mm tube 或 1.5/2 mL 離心管)，再加入 1 mL 去離子水均勻混合(此製備好的品管液可於 4ºC 冰箱避光保存 5 天)，放置於上樣架準備上機。

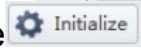
[Plate Loader 模式]

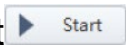
滴 1 滴於 well 中，再加入 200 µL 去離子水均勻混合，放置於盤式上樣架準備上機，點擊 **Load**。

(3). 由 QC 進入 Start QC 

(4). 選擇 CytoFLEX Daily QC 的 Lot No.例如：A555(Expires: 2015-09-30)。

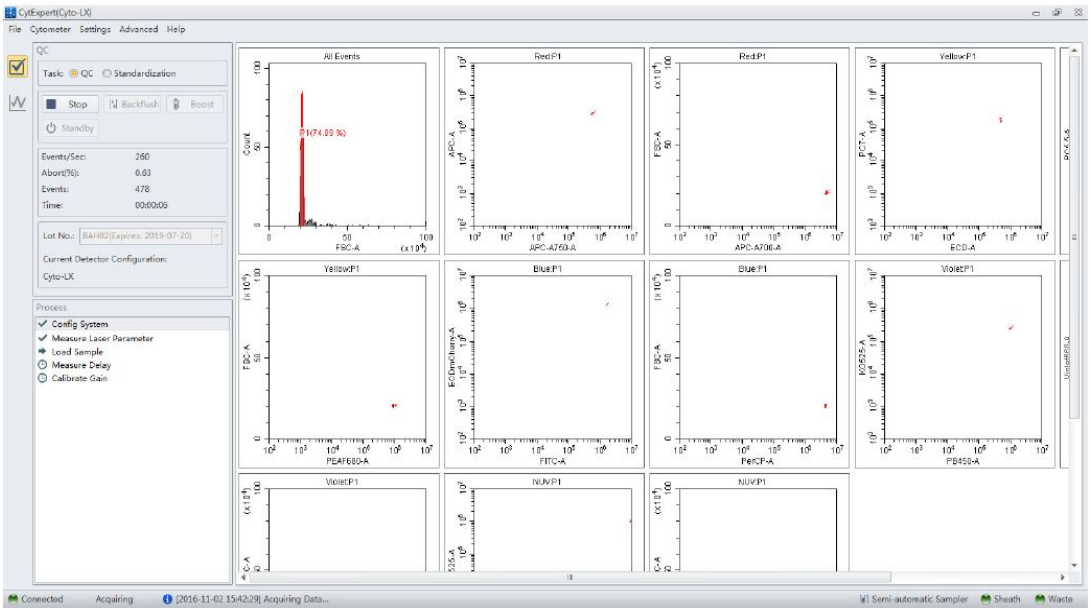


(5). 點擊 Initialize 

啟動儀器，再點擊 Start 

即執行 CytoFLEX 的 QC 步驟，開始品管液分析。

如下圖顯示：



(6). 檢視 QC Report 結果，CytoFLEX Daily QC 品管液所測得的前向散射光、側向散射光以及每個螢光參數 FL1 - FL13 是否都通過原廠規範條件呈現綠色打勾 Pass。此時表示機器穩定無問題，可繼續進行樣品分析。

QC Report 結果如下：

QC Report

Bead Lot No.:	A555		
Bead Expires:	2015-09-30	QC Date:	2015-01-28 14:38
Cytometer Name:	DxFLEX	Cytometer SN:	AS05034
Detector Configuration:	Default-Configuration		
Loader Type:	Semi Automatic		

Threshold

Channel: FSC(Height) Mode: Manual Value: 50000

Laser

Laser	Delay(μs)	Power(mW)	Target Power(mW)	Result
Blue laser	0.48	52	40-60	✓
Red laser	-40.00	44	40-60	✓
Violet laser	44.16	84	70-120	✓

Signal Value

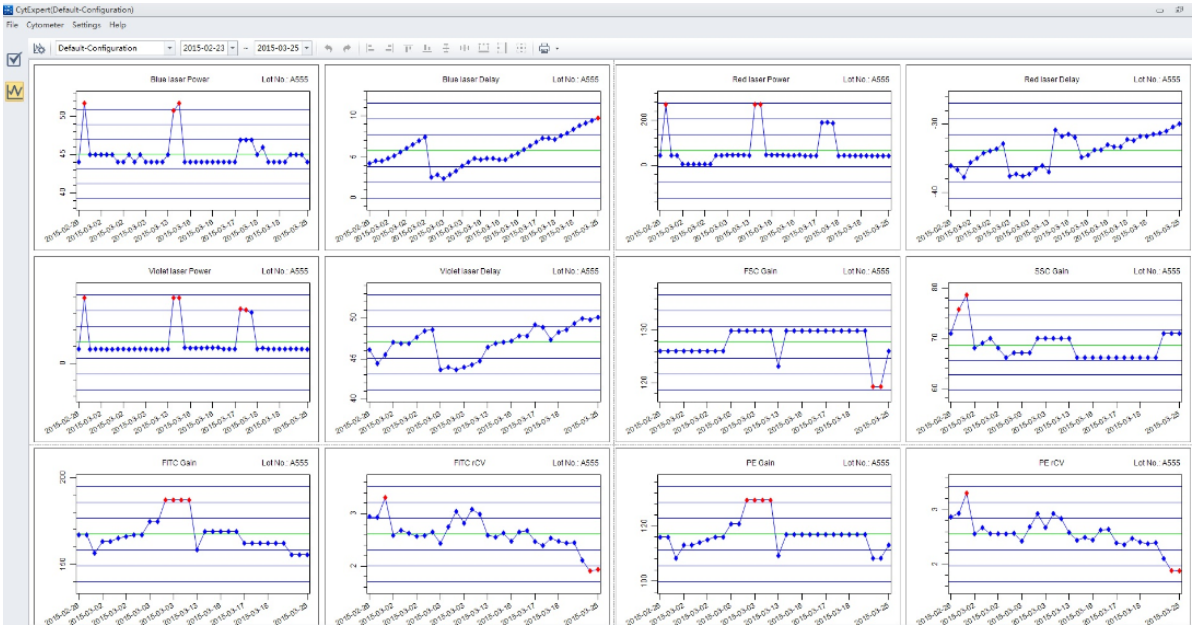
Parameter	Gain	Target Gain	%Difference Target Gain	Median	Target Median	%Difference Target Median	rCV(%)	Target rCV(%)	Width	Result
FSC	80	80	0.00	245720.7	242482.7	1.34	-	-	980.0	✓
SSC	202	202	0.00	624664.8	601799.5	3.80	-	-	1067.0	✓
FITC	165	165	0.00	3344234.0	3313982.0	0.91	1.00	5.00	1090.3	✓
PE	134	134	0.00	1155391.0	1145402.0	0.87	0.90	5.00	1095.0	✓
ECD	196	196	0.00	624280.3	620159.8	0.66	1.02	5.00	1085.3	✓
PC5.5	322	322	0.00	1562936.0	1545660.0	1.12	1.04	5.00	1091.9	✓
PC7	419	419	0.00	263379.3	262014.1	0.52	1.32	5.00	1085.6	✓
APC	479	500	-4.20	483638.6	483724.0	-0.02	1.45	5.00	1611.5	✓
APC-A700	500	500	0.00	1857151.0	1892327.0	-1.86	1.32	5.00	1613.1	✓
APC-A750	475	475	0.00	694970.5	689351.1	0.82	1.58	5.00	1613.7	✓
PB450	94	94	0.00	725048.2	718632.1	0.89	2.52	5.00	1260.5	✓
KO525	53	53	0.00	143906.7	143298.4	0.42	2.61	5.00	1261.1	✓
Violet610	324	324	0.00	93317.3	92769.6	0.59	2.87	5.00	1262.9	✓
Violet660	253	253	0.00	45540.2	45071.9	1.04	3.06	5.00	1260.4	✓
Violet780	296	296	0.00	68841.3	68488.8	0.51	3.17	5.00	1264.6	✓

Result

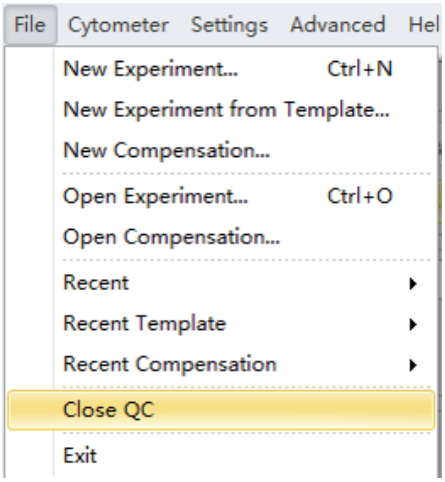
QC Passed.

當儀器穩定時呈現綠色打勾 Pass，當儀器異常時呈現紅色交叉 Failed。

(7). 可進一步檢測第二頁面的 Levey-Jennings Charts，CytoFLEX Daily QC 品管液是否落於信賴區間內。如下圖顯示 ：



(8). 離開 QC Report 可由 File 進入，點擊 Close QC。



(9). 若品管結果超出範圍顯示紅色交叉 Failed，必須執行 Prime 以及 Deep Clean 步驟進行管路阻塞排除，或者執行 Daily Clean 管路清洗流程，再重新分析一管 CytoFLEX Daily QC 品管液。

(10). 若品管結果還是超出範圍，請通知原廠工程師或者產品專員。

五、[Semi-Automatic sample模式] 設定新的Experiment

雙染(FITC/PE)Surface Markers設定

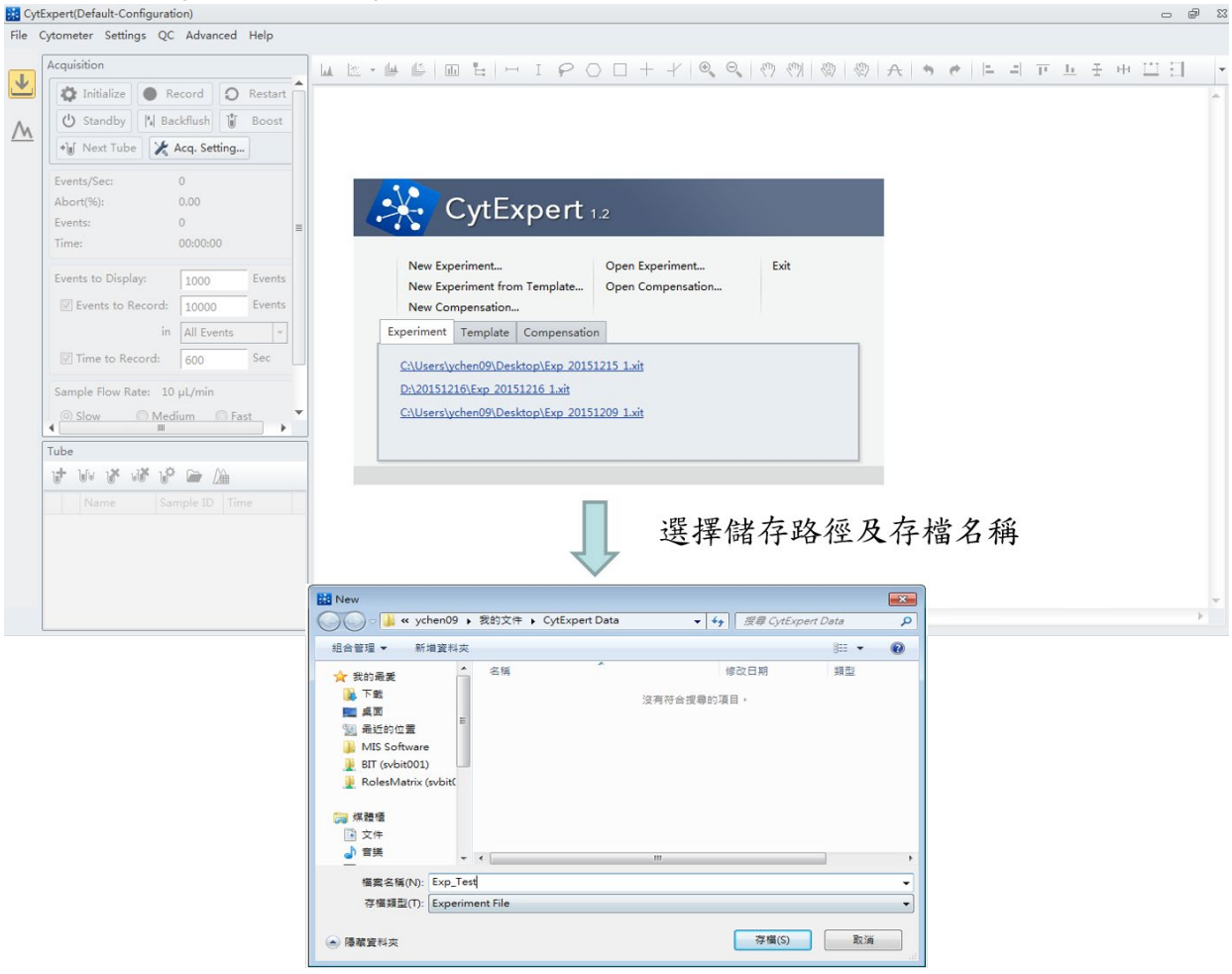
✧ 以CD3-FITC / CD4-PE為示範，需準備四管樣品用以調整儀器的設定值：

1. 陰性樣品：未染色的細胞，或以Isotype抗體染色的細胞
2. 單染FITC的陽性樣品
3. 單染PE的陽性樣品
4. 雙染的陽性樣品

➢ 操作步驟：

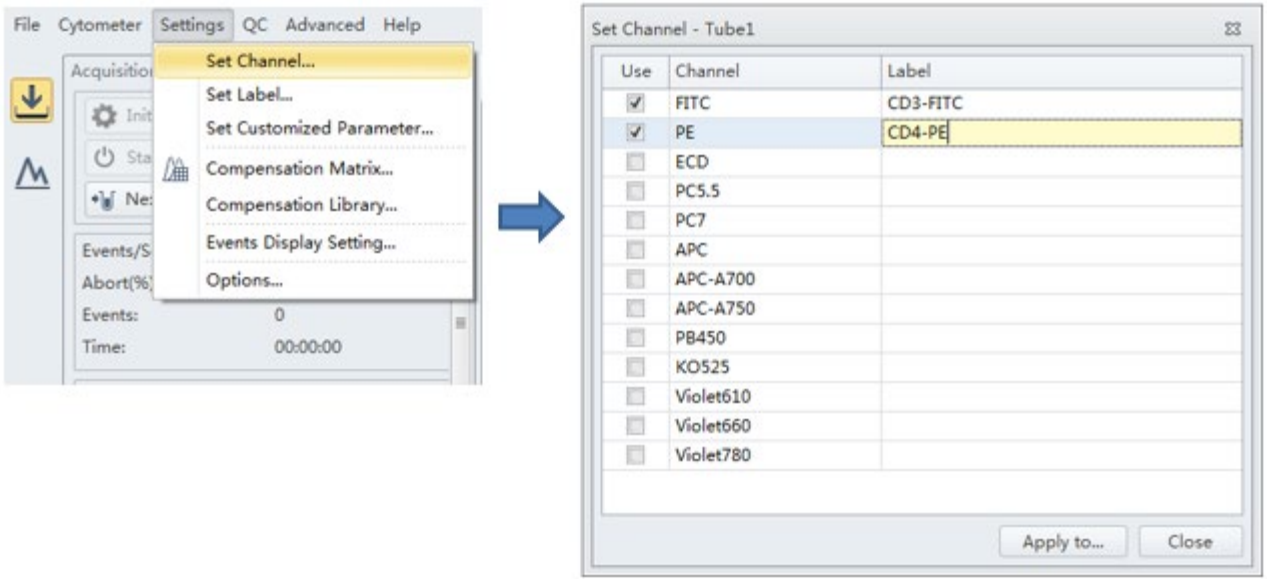
a. 開啟一個新的Experiment：

由File進入(或歡迎頁面)，點選New Experiment並儲存實驗檔案名稱。



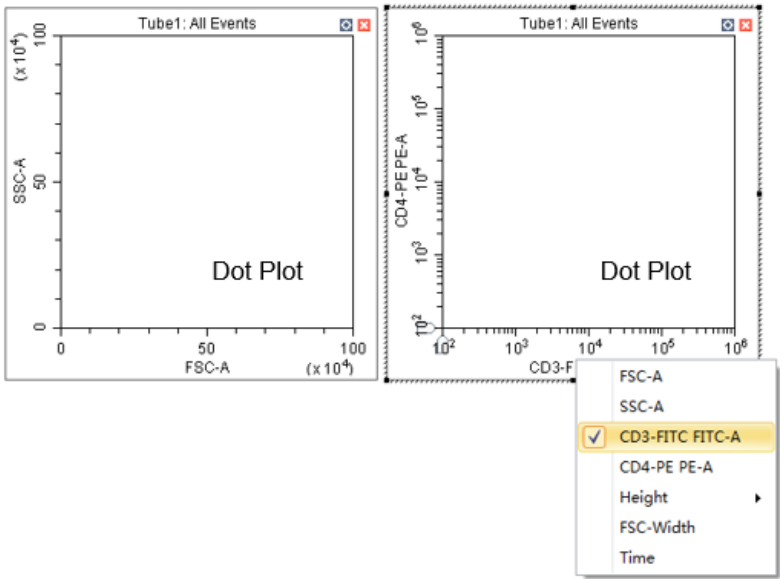
b. 選擇希望收取的參數：

點擊Settings，選擇Set Channel，勾選Channels及標示抗體染劑名稱，點擊Close。



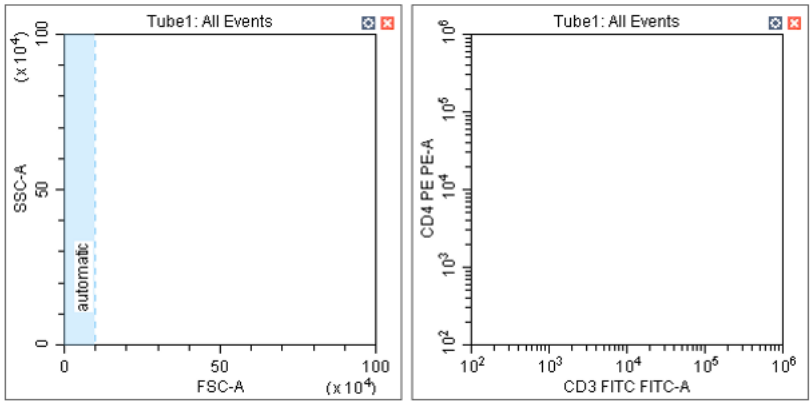
c. 利用已勾選的參數繪製希望分析的圖形：

在繪圖工具列中點選Dot Plots，於圖形上的X/Y軸點滑鼠左鍵，選擇想要標示的抗體螢光參數。



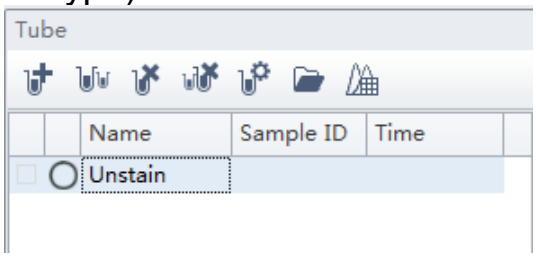
d. 確認Threshold是否設定完成：

在繪圖工具列中點選Threshold，此時會於FSC/SSC圖形上出現automatic的藍色虛線，Surface Marker的實驗中，建議將Threshold設於FSC第一個刻度位置。

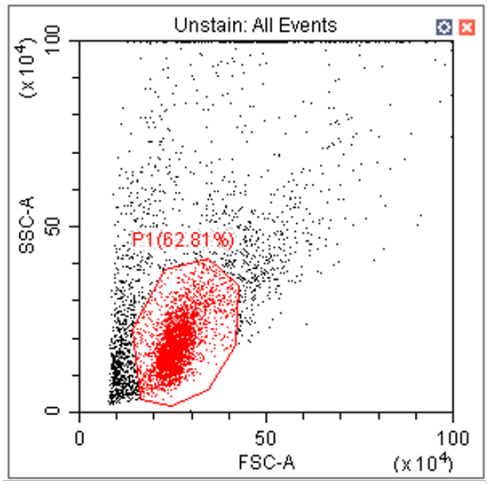


e. 分析陰性樣品(可使用未染色的樣品，或以Isotype抗體染色的陰性樣品)，依下列方式調整各個偵測器的Gain值：

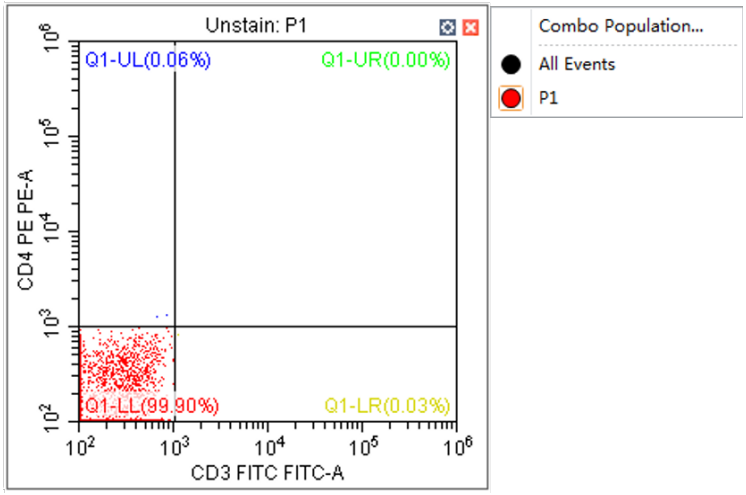
1. 左下角Tube表格雙擊Name欄位，輸入樣品名稱(例如Unstain / Isotype)



2. 按下儀器操控區中的Run，此時機器開始收集樣品數據(此時所收集的數據會於樣品名稱顯示閃爍的藍色圓圈，表示暫存，圖形中的數據呈現動態的變動)，並顯示在剛才畫好的圖形上，使用繪圖區的Gain 調整FSC/SSC的Gain值，或使用Pan 或Single Side Pan 調整X/Y的Scale，找到FSC/SSC中想要分析的細胞族群。使用圈選工具列 ，對FSC/SSC的細胞群以多邊形Polygonal Gate 圈選P1 Gate。

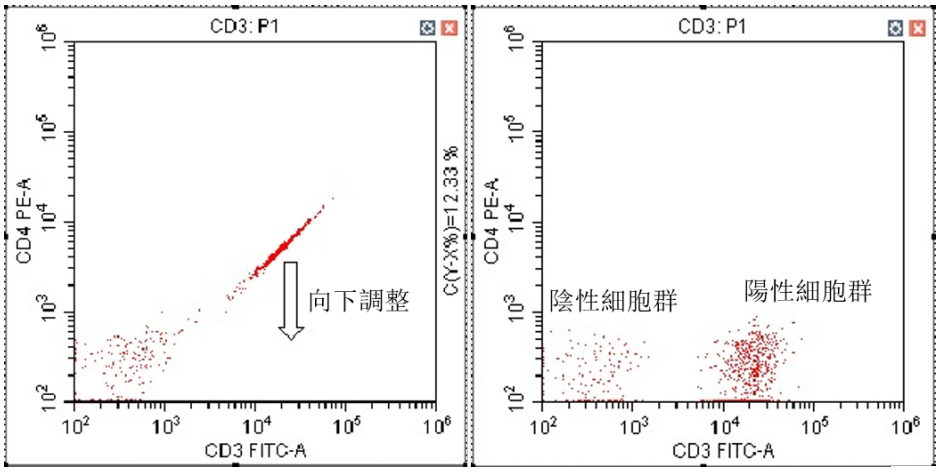


3. 接著調整FL1、FL2的Gain值，於螢光圖上方點選左鍵，選擇P1 Gate觀察，接著以十字象限 定義Negative位置，使FL1/FL2雙參數圖形的細胞落在左下角第一個Log位置。

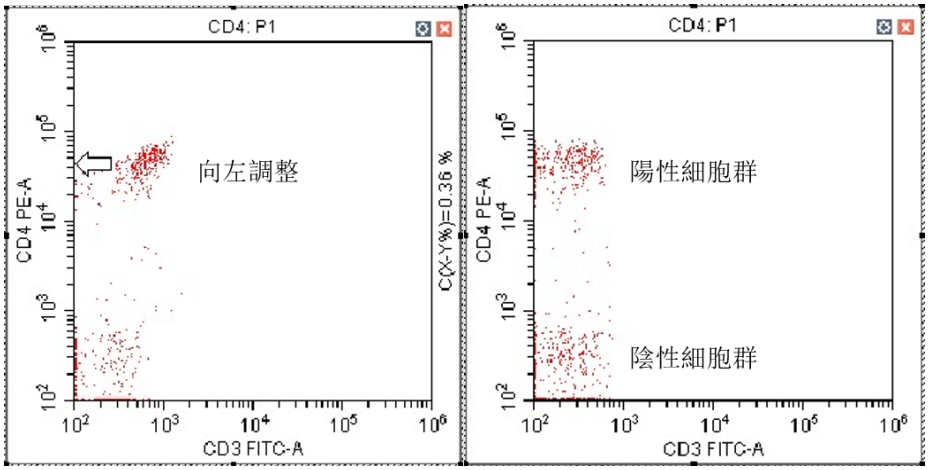


f. 分析單染的陽性樣品調整螢光補償值(Compensation)：
使用兩管樣品：以FL1單染樣品調整FL2-%FL1
以FL2單染樣品調整FL1-%FL2

1. 上樣CD3-FITC單染樣品，左下角Tube表格新增1管，雙擊Name欄位輸入樣品名稱(例如CD3)。點擊Run，接著使用繪圖區的Compensation，調整螢光補償。直接在Plot上拖拉細胞群即可設定螢光補償。



2. 上樣CD4-PE單染樣品，左下角Tube表格再新增1管，雙擊Name欄位輸入樣品名稱(例如CD4)。點擊Run，調整螢光補償Compensation。直接在Plot上拖拉細胞群即可設定螢光補償。



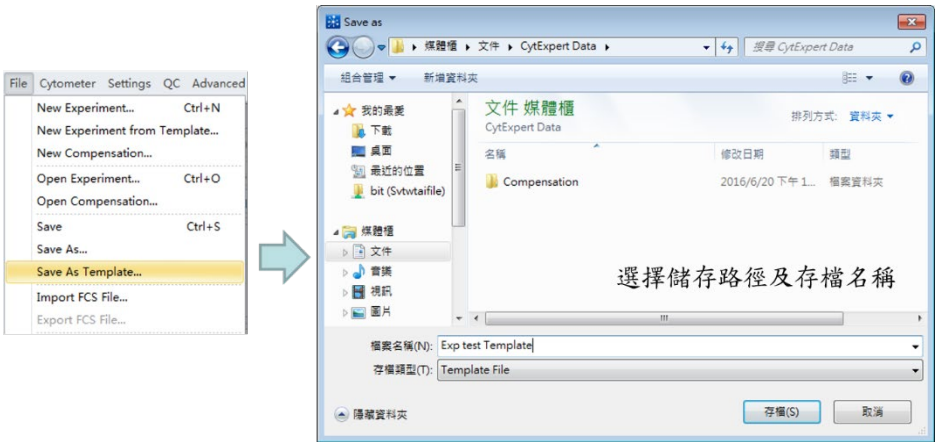
3. 分析雙染樣品，左下角Tube表格再新增1管，雙擊Name欄位輸入樣品名稱(例如CD3-CD4)。點擊Run，即以設定好的條件分析樣品，點擊Record 正式收取樣品數據(此時所收集的數據會於樣品名稱顯示閃爍的綠色圓圈)。

將Experiment儲存為Template

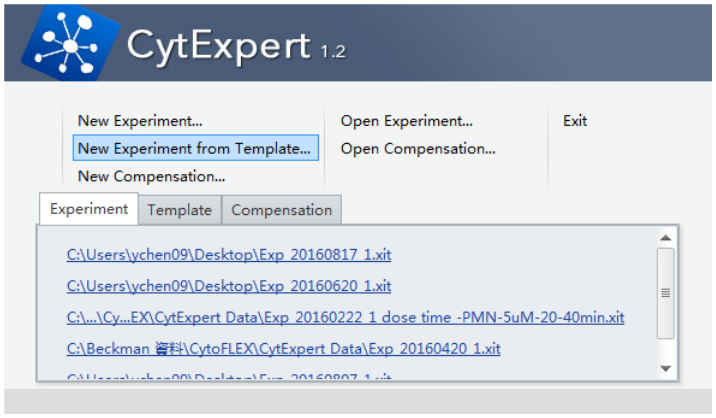
✧ 如將已設定好的Experiment，之後需再進行使用時可以儲存此Template。

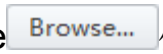
➢ 操作步驟：

1. 於已設定好的Experiment中，點選File，選擇Save as Template並儲存實驗檔案名稱。

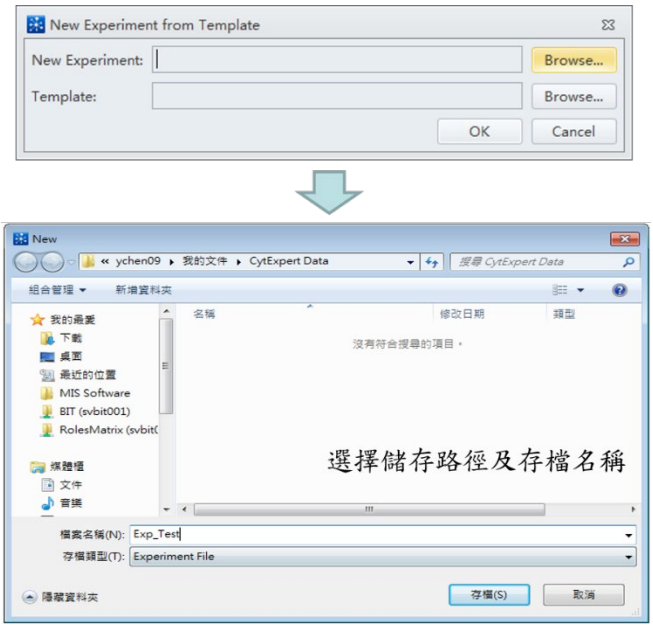


2. 之後需進行相同Experiment時，由File進入(或歡迎頁面)，點選New Experiment from Template。

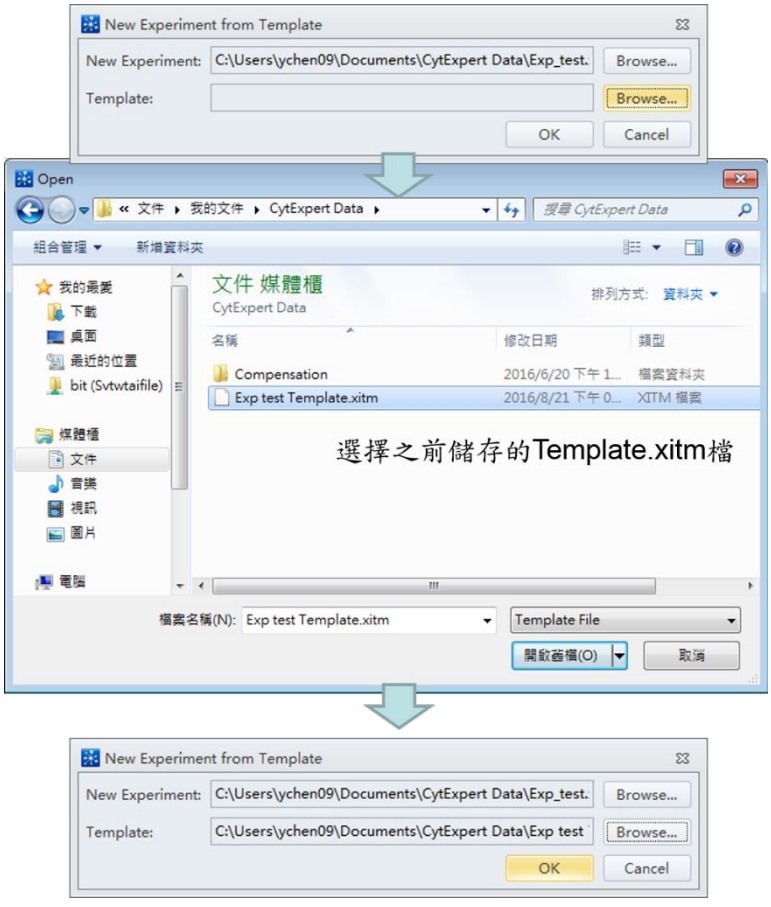


3. 點選New Experiment的Browse 

儲存新實驗檔案名稱。



4. 再點選Template的Browse選擇之前儲存的Template.xitm檔。

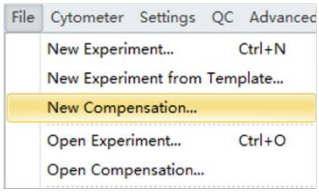


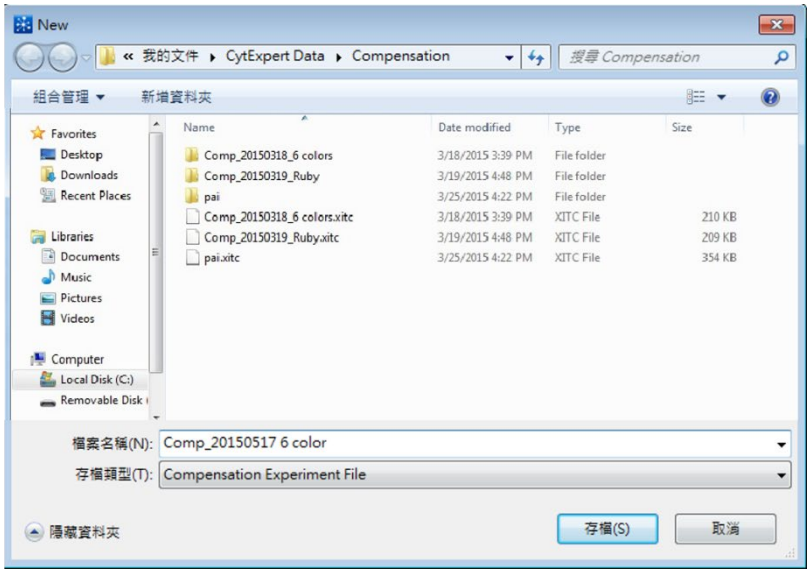
5. 選擇完成後點擊OK，開啟既有Template。

自動螢光補償設定

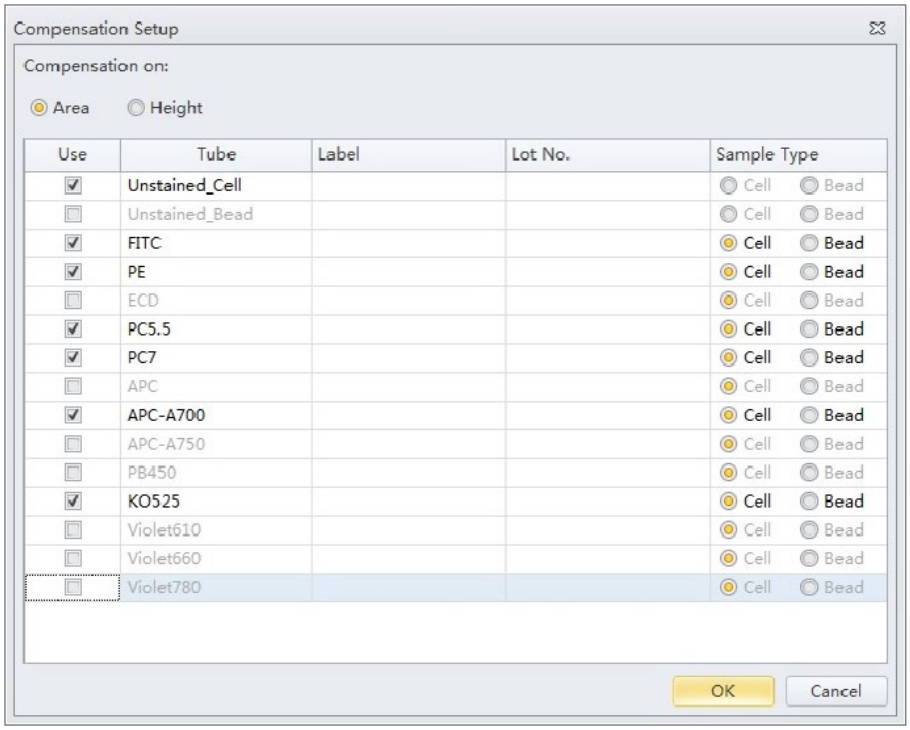
以CD3-FITC / CD4-PE / CD19-PC5.5 / CD16-PC7及CD56-PC7 / CD8-APCA700 / CD45-KO 6色染色為示範，共有8管樣品分別為Unstained / Isotype、各抗體單色染色和6色染色樣品。

1. 由File進入(或起始頁面)，點選New Compensation並儲存實驗檔案名稱。

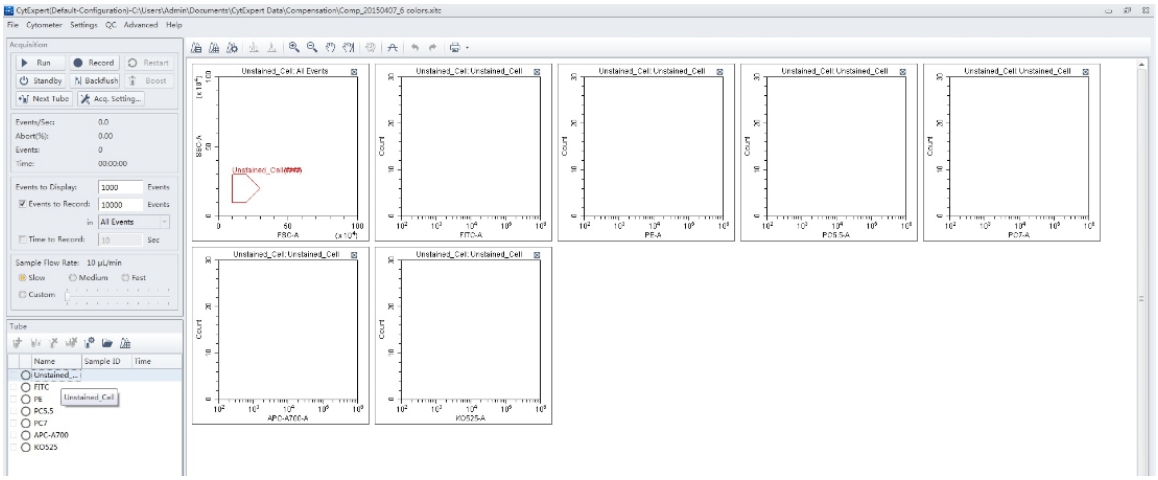




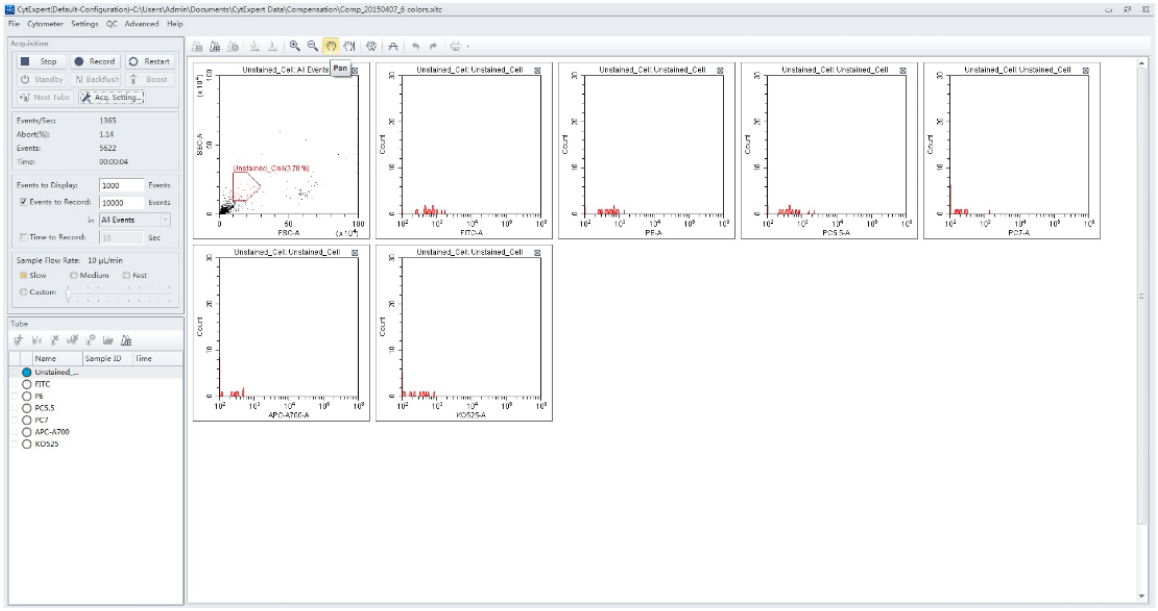
2. 依照軟體導引，勾選使用細胞或珠子以及使用染劑，點擊OK。



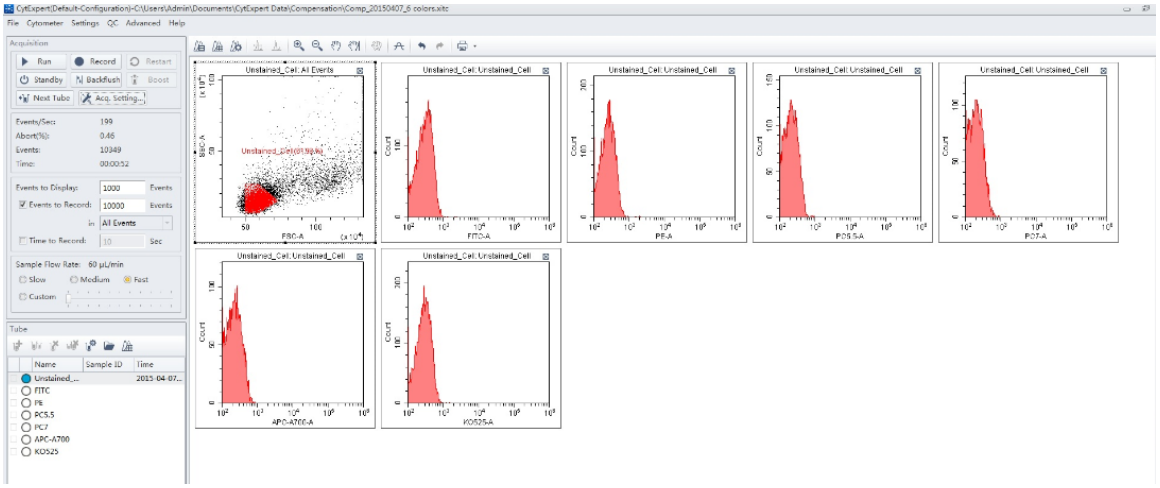
3. 此時軟體會根據所勾選染劑自動畫圖，並且左下角Tube表格自動設定準備上樣之Unstained / Isotype及單色染色樣品管。



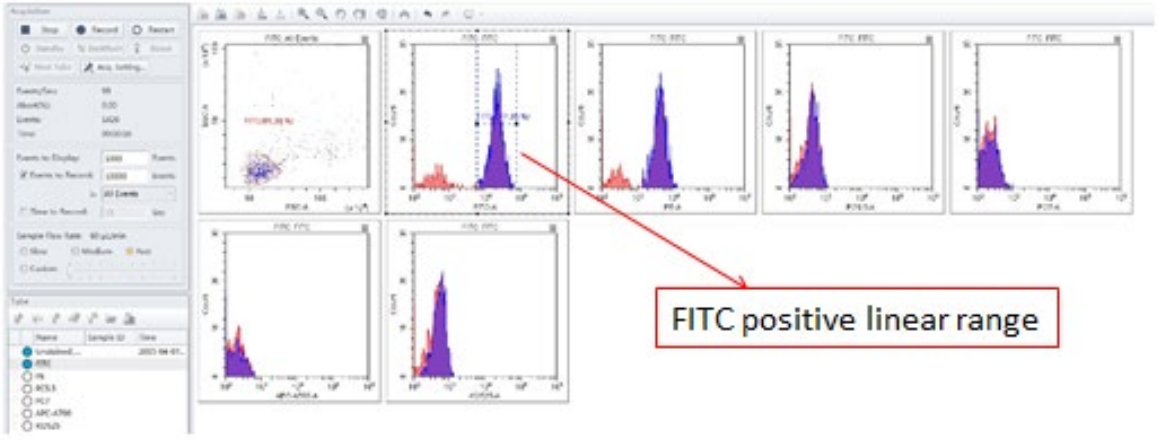
4. 先上Unstained / Isotype樣品管，點擊Run。

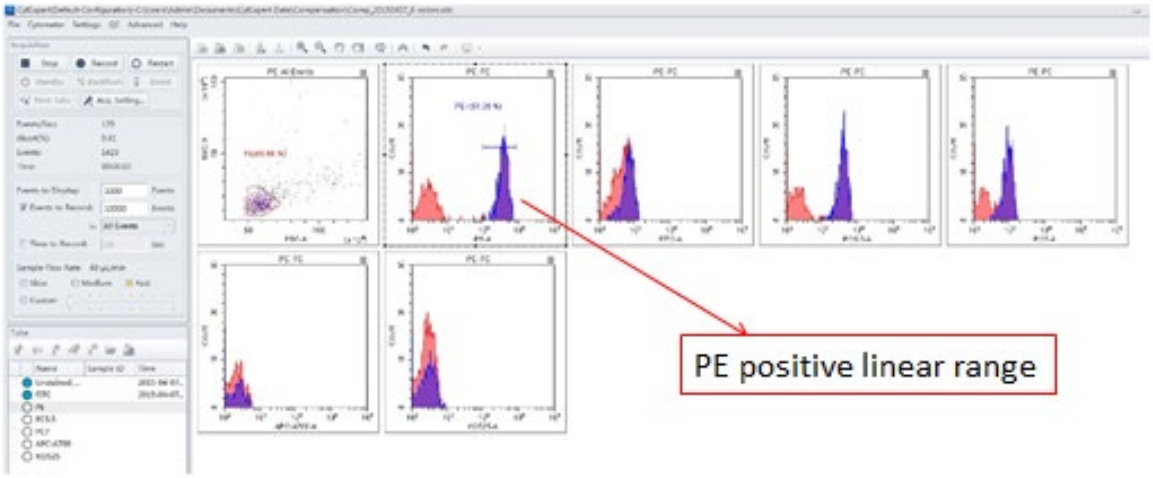


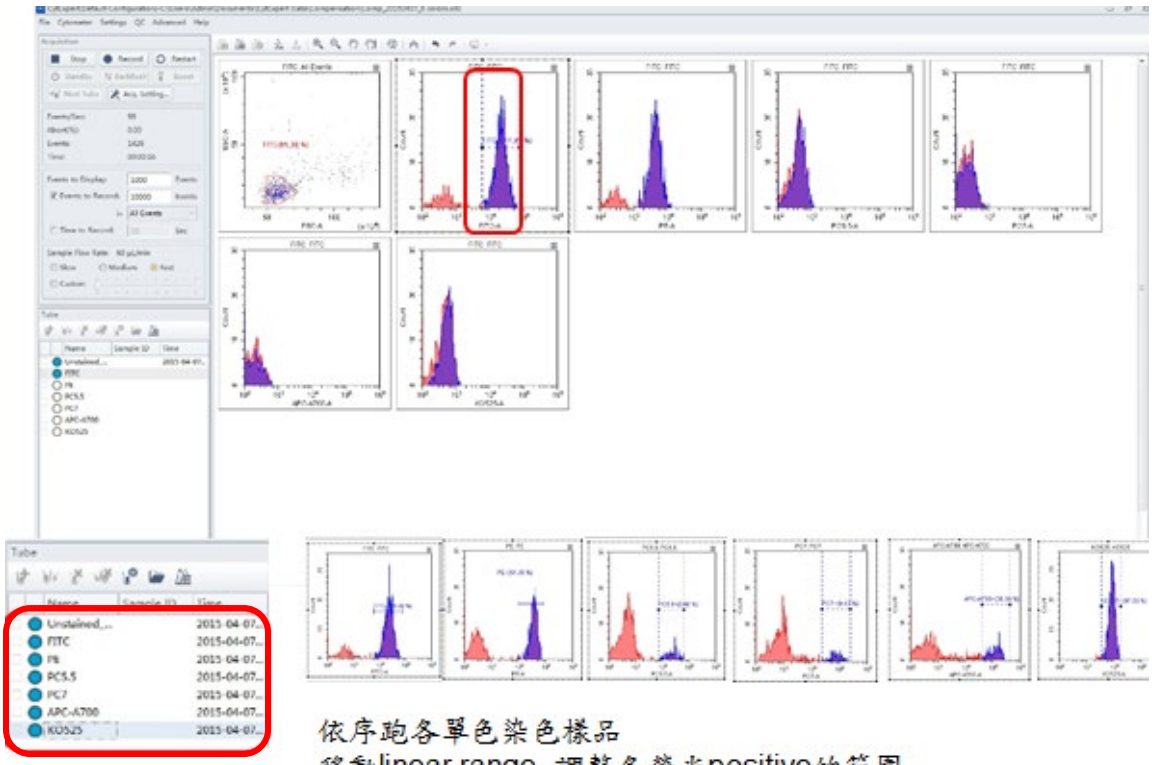
5. 調整FSC/SSC設定(使用Scale  ，FSC/SSC五角型圈選，Threshold  及Gain )將FSC/SSC圖形中調整到可以看見主要細胞群。



6. 分別再上樣單色FITC、PE、PC5.5、PC7、APC-A700及Krome Orange，並且調整單色染色的Positive區域中的Linear Rang。







依序跑各單色染色樣品
移動linear range, 調整各螢光positive的範圍

7. 再Double Check並微調各個單色染色的Positive區域中的Linear Rang。點擊快捷工具列Compensation Calculation  ，6色Compensation Matrix計算完成。

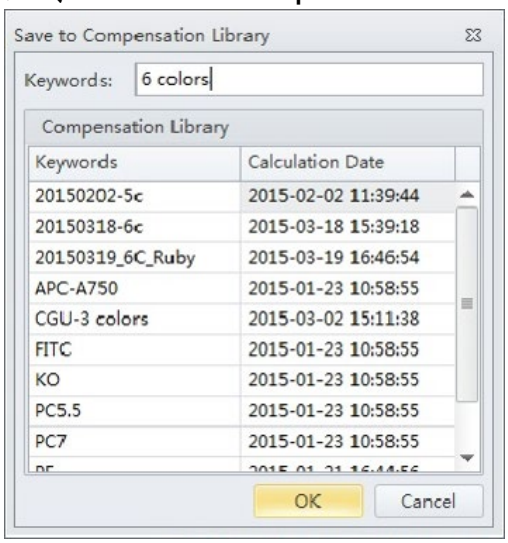
Compensation Matrix

Use | Show Autofluorescence | Area

Cha...	-FIT...	-PE%	-EC...	-PC...	-PC...	-AP...	-AP...	-AP...	-PB...	-KO...	-Vio...	-Vio...	-Viole...
FITC		1.08	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.66	0.00	0.00	0.00
PE	19.06		0.00	2.34	2.30	0.00	0.00	0.00	0.00	0.46	0.00	0.00	0.00
ECD	22.23	148....		3.32	3.54	0.00	0.00	0.00	0.00	0.76	0.00	0.00	0.00
PC5.5	1.33	9.97	0.00		0.84	0.00	1.01	0.00	0.00	0.00	0.00	0.00	0.00
PC7	0.34	2.16	0.00	63.64		0.00	0.97	0.00	0.00	0.00	0.00	0.00	0.00
APC	0.00	0.00	0.00	2.55	0.06		15.18	0.00	0.00	0.00	0.00	0.00	0.00
APC...	0.00	0.00	0.00	33.23	0.06	0.00		0.00	0.00	0.00	0.00	0.00	0.00
APC...	0.00	0.00	0.00	33.41	13.54	0.00	107....		0.00	0.00	0.00	0.00	0.00
PB4...	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		6.07	0.00	0.00	0.00
KO5...	1.37	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00
Viol...	0.81	12.56	0.00	0.00	0.22	0.00	0.00	0.00	0.00	253....		0.00	0.00
Viol...	0.09	1.46	0.00	0.19	0.02	0.00	0.59	0.00	0.00	38.29	0.00		0.00
Viol...	0.08	0.28	0.00	6.31	10.17	0.00	4.31	0.00	0.00	8.60	0.00	0.00	

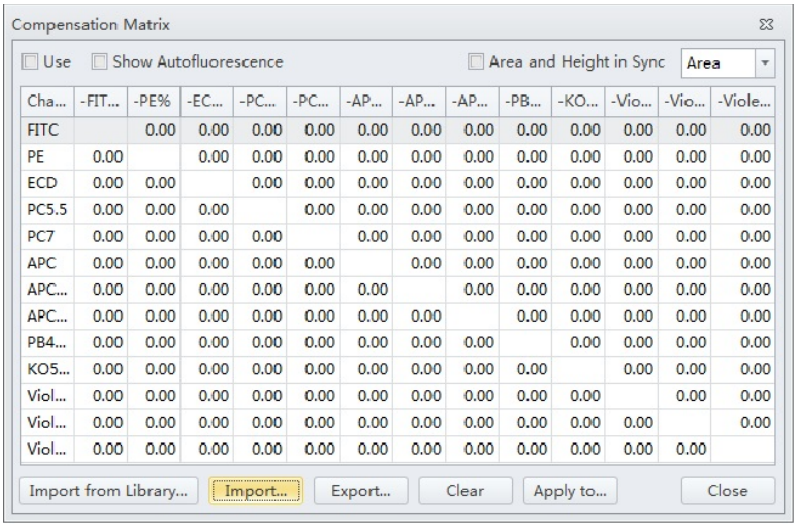
Save to Compensation Library... | Save As... | Close

8. 點擊Save to Compensation Library，給予檔名後點擊OK。

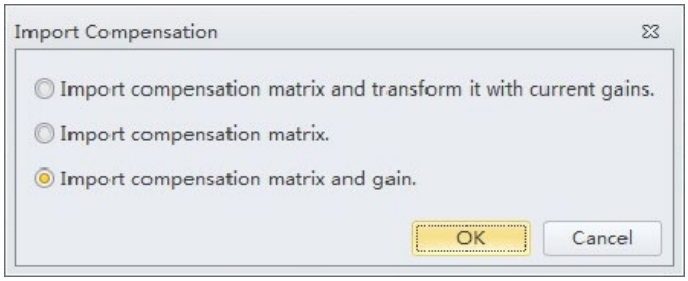


9. 點擊Save As，儲存此Compensation Matrix，此Matrix可以套用於其後相同染色的 Experiment，例如以下例子。

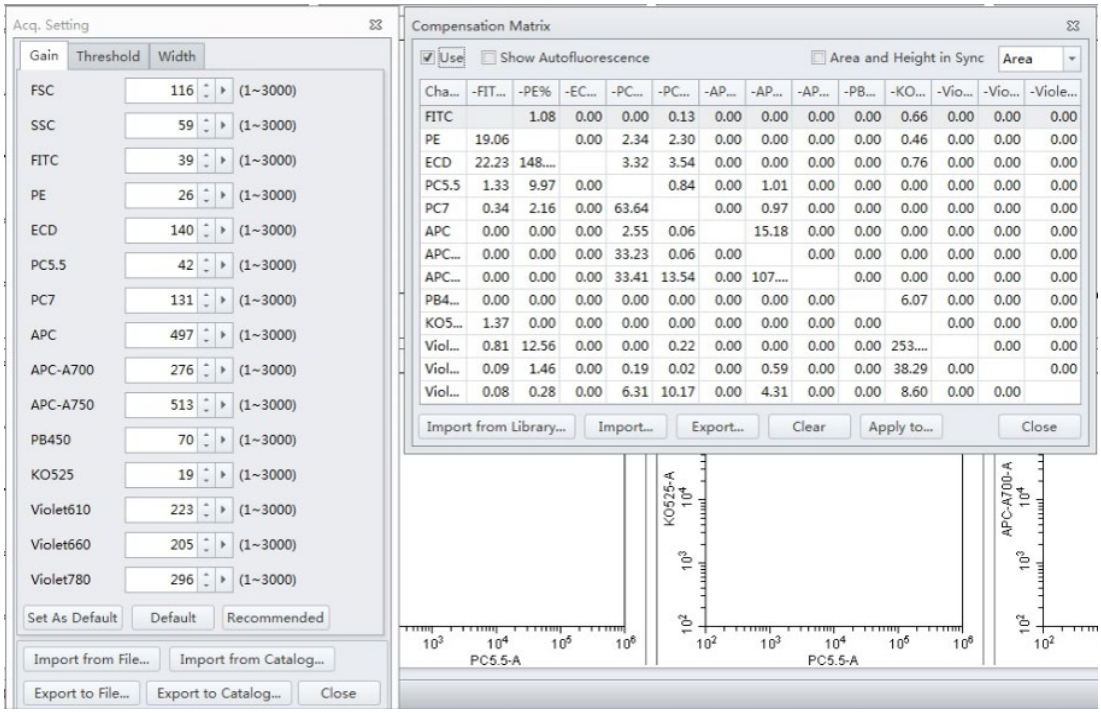
10. New Experiment，在Tube表格上的工具列點擊Compensation Matrix  後，再點擊Import。



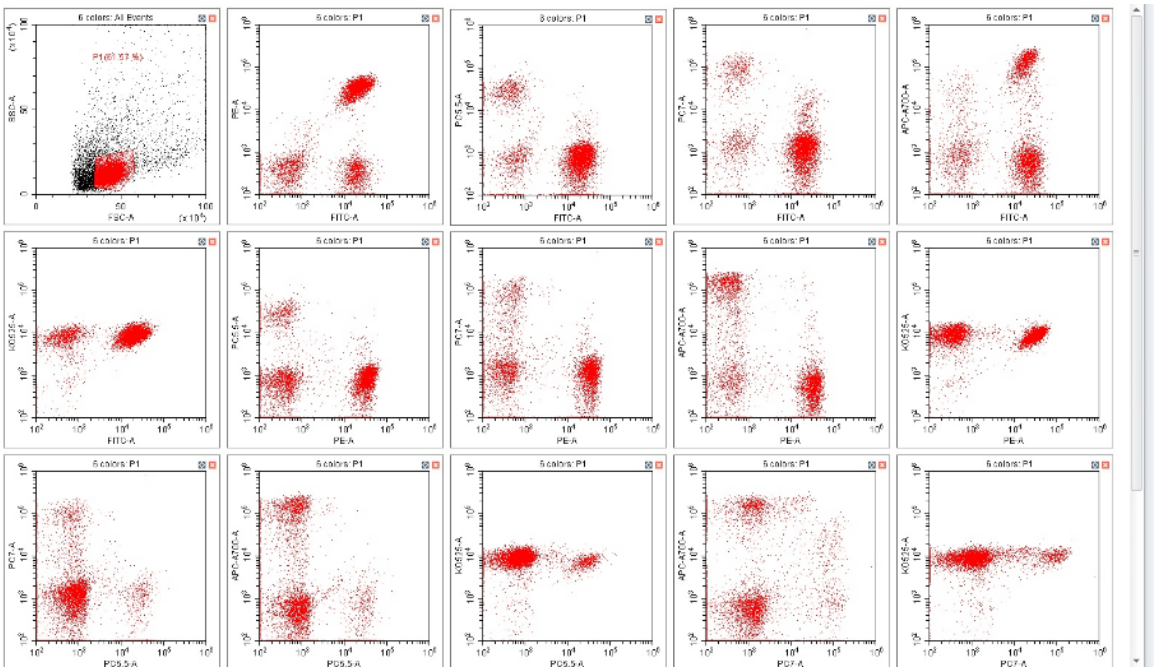
11. 選擇已經儲存的Compensation Matrix，勾選”Import compensation matrix and gain”，再點擊OK。



12. 先針對這個Experiment畫圖(Dot Plots、Histogram)再跑樣品，此實驗會使用這個Compensation Matrix所設定的電壓(Gain)及螢光補償值跑樣品。



13. 所得結果如下：

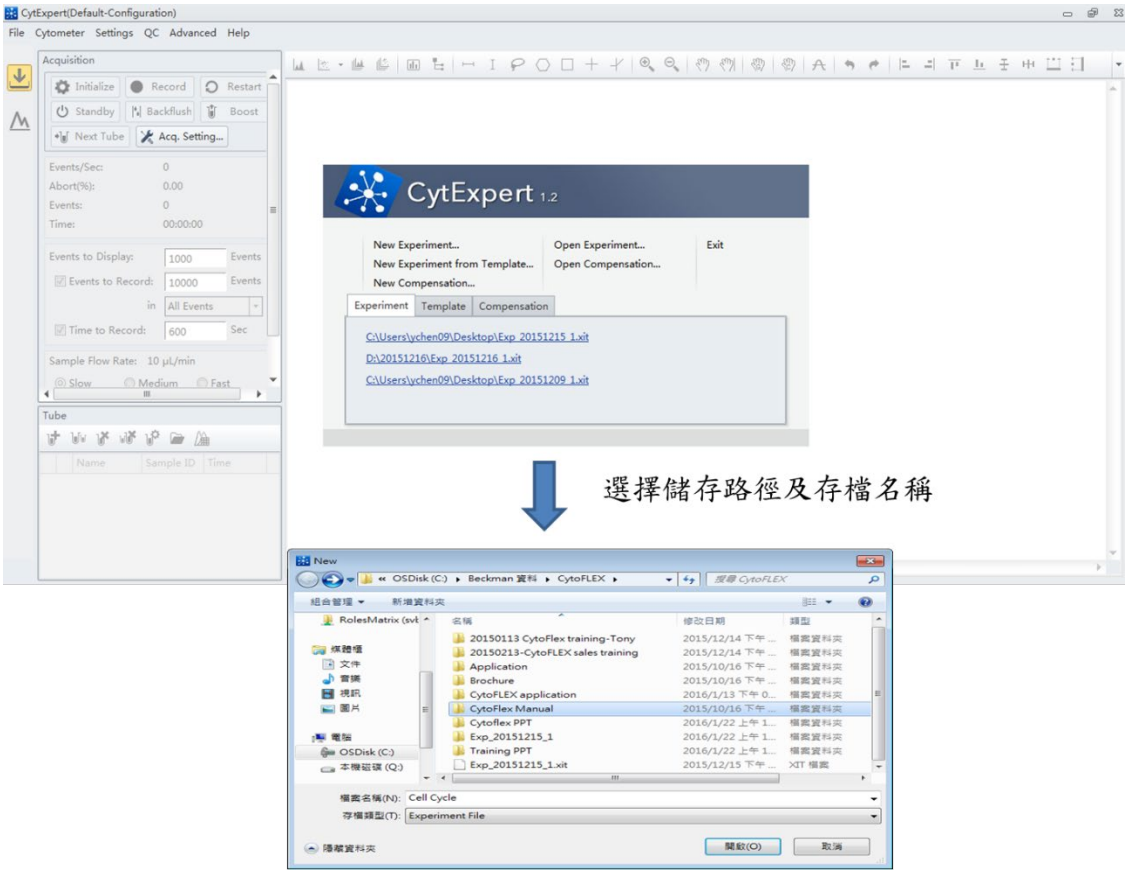


Cell Cycle 設定

- 操作步驟：

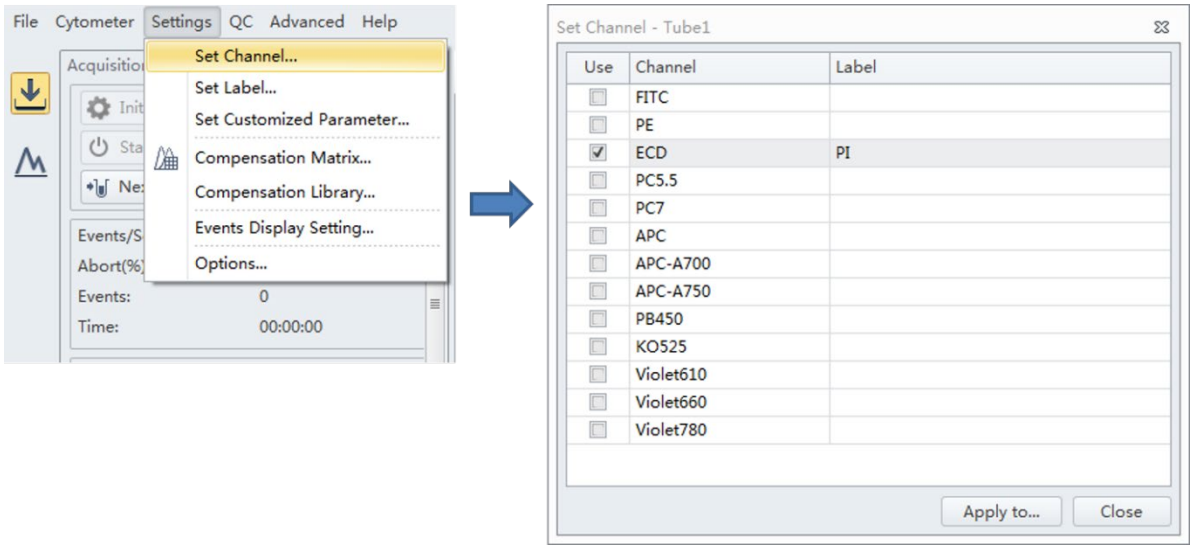
a. 開啟一個新的Experiment：

由File進入(或起始頁面)，點選New Experiment並儲存實驗檔案名稱。



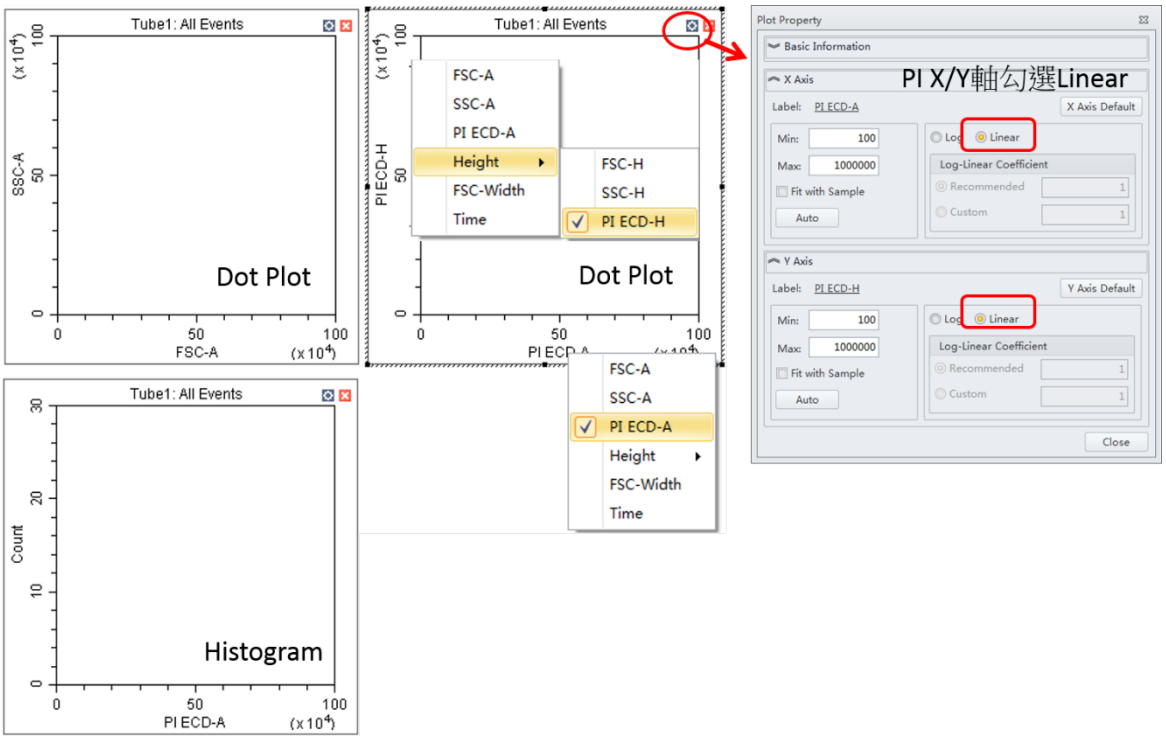
b. 選擇希望收取的參數：

點擊Settings，選擇Set Channel，勾選Channels及標示染劑名稱，點擊Close。



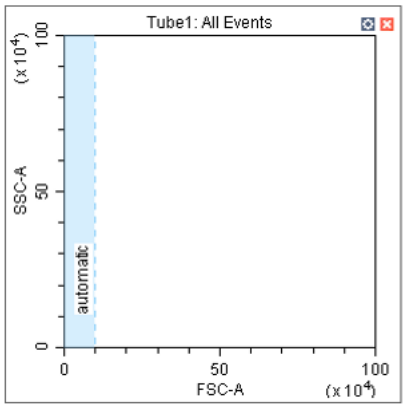
c. 利用已勾選的參數繪製Cell Cycle所需分析的圖形如下：

在繪圖工具列中點選Dot Plots及Histogram，於圖形上的X/Y軸點滑鼠左鍵，選擇想要標示的染劑螢光參數PI-A / PI-H，並點選圖形的右上角螺絲，將PI X/Y軸選為線性Linear。



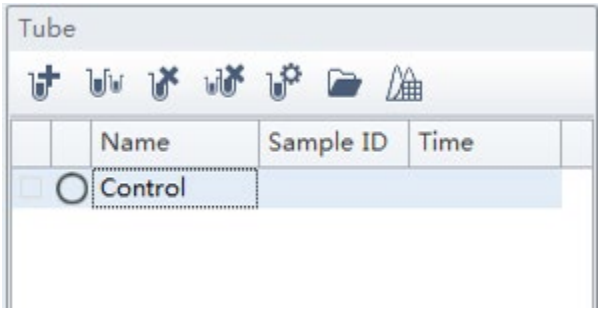
d. 確認Threshold是否設定完成：

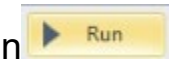
在繪圖工具列中點選Threshold，此時會於FSC/SSC圖形上出現Automatic的藍色虛線，Cell Cycle的實驗中，建議將Threshold設於FSC第一個刻度位置。



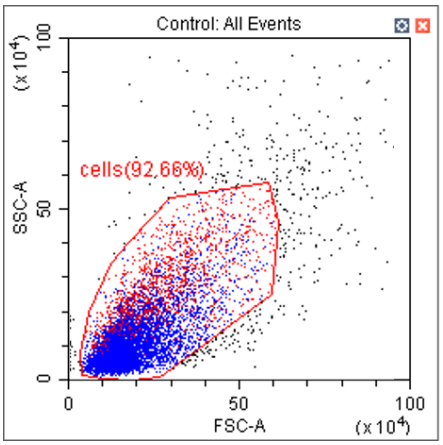
e. 分析陽性樣品(已固定且以PI染色的健康細胞)，依下列方式調整各個偵測器的Gain值：

1. 左下角Tube表格雙擊Name欄位，輸入樣品名稱(例如Control)。

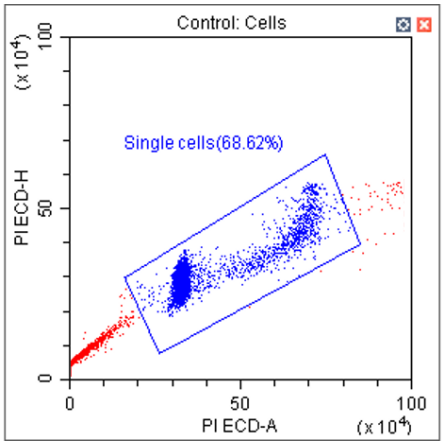


2. 按下儀器操控區中的Run 

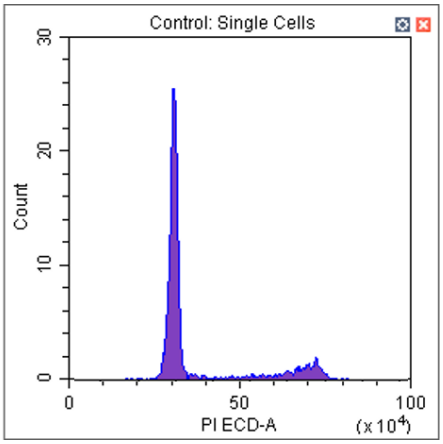
，此時機器開始收集樣品數據(此時所收集的數據會於樣品名稱顯示閃爍的藍色圓圈，表示暫存，圖形中的數據呈現動態的變動)，並顯示在剛才畫好的圖形上。



使用繪圖區的 Gain  ，調整 FSC/SSC 的 Gain 值或使用 Pan  調整 X/Y 軸的 Scale，找到 FSC/SSC 要分析的主要細胞族群，以多邊形 Polygonal Gate  圈選 Cell Gate。

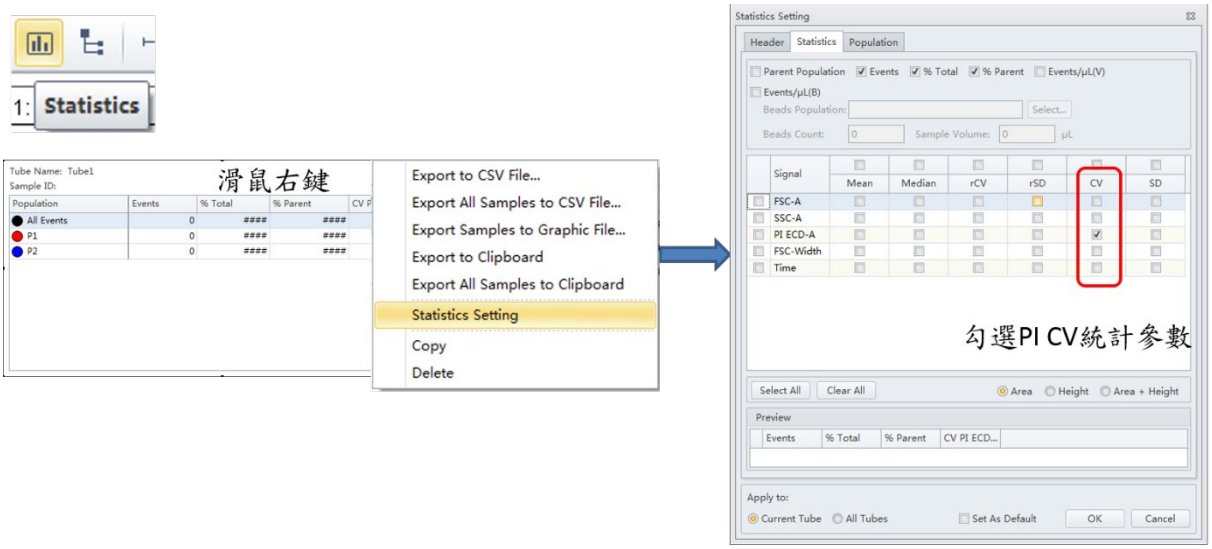


調整雙參數點圖 PI-A / PI-H Gain  ，使單顆細胞分佈在由左下到右上的 45 度角，以多邊形 Polygonal Gate  圈選 Single cells Gate。



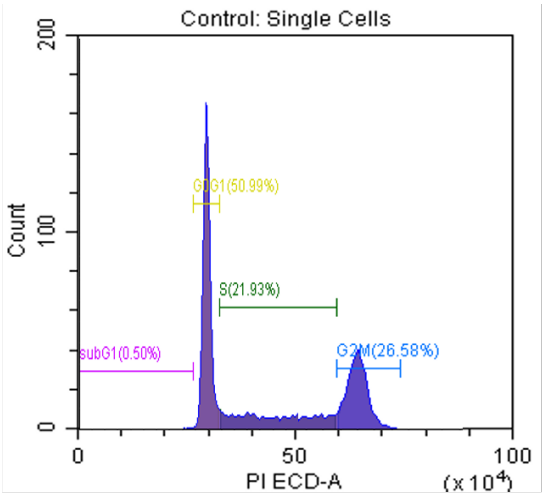
最後檢視單參數 PI-A 微調 Gain 值，使 G0/G1 Phase 主要位於第二或第三個刻度位置，此時即可得到 Cell Cycle 最後圖形。

3. 以Line Segment圈選Cell Cycle Phase 後點選統計圖表Statistics，出現統計數值表後點選滑鼠右鍵，選擇 Statistics Setting，選取第二頁面Statistic，即可勾選PI CV統計參數。



Note：

1. 步驟中切記觀察細胞是否有群聚現象，若細胞無法拍散，則需過篩或重新置備樣品。
2. 結果評估：數據收取後，通常會檢視 G0G1 Phase 的變異係數(CV 值)，用以評估細胞的固定、染色過程是否完善。一般來說，會要求 Control 組的 G0/G1 Phase 的 **CV 值必須小於 8**，代表細胞的固定過程良好，並具有均一的染色結果，如下圖。CV 值大於 8 的數據，必須捨棄不用。



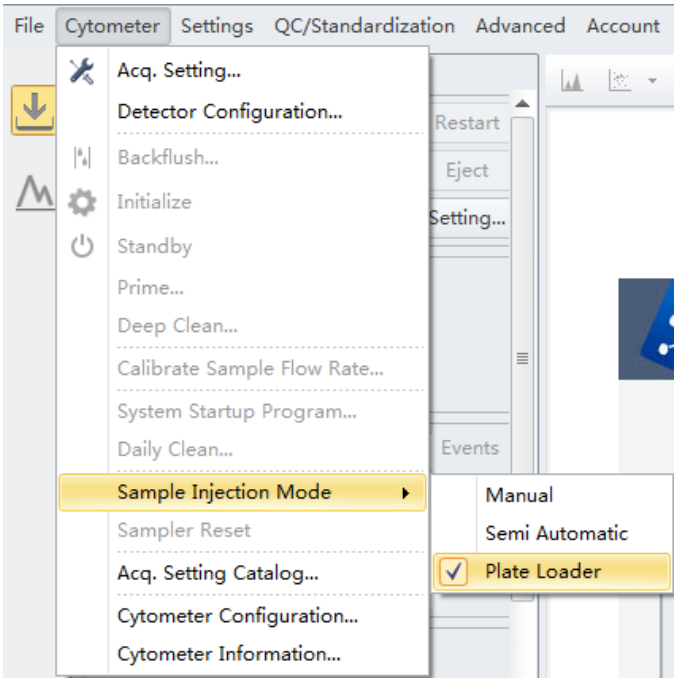
Population	Events	% Total	% Parent	Mean PI ECD-A	CV PI ECD-A
All Events	14468	100.00%	100.00%	3991389.3	50.05%
subG1	49	0.34%	0.50%	1367337.4	21.42%
G0G1	5036	34.81%	50.99%	1980746.5	2.56%
S	2166	14.97%	21.93%	2844225.8	18.90%
G2M	2625	18.14%	26.58%	3803745.5	3.02%
P1	12404	85.73%	85.73%	2911448.8	33.56%
P2	9876	68.26%	79.62%	2651626.5	14.94%

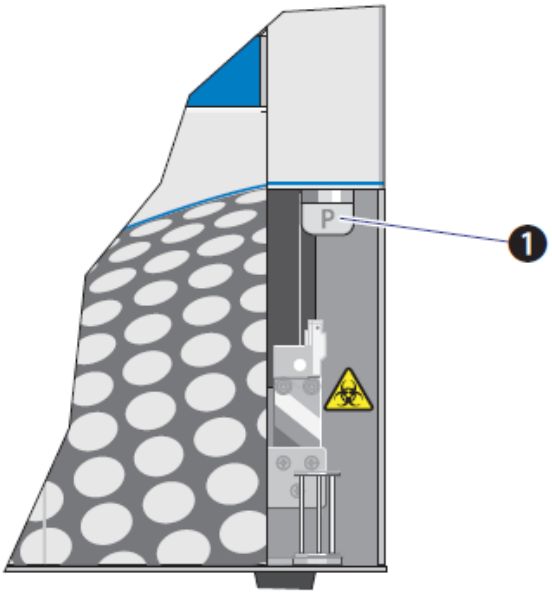
3. CV 值不好的結果可能肇因於：
   - 細胞固定步驟不良 → 改善固定步驟，請教有經驗者。
   - 使用了不當的固定試劑 → 更換固定試劑。
   - 染色時間不足 → 增加染色時間。
   - RNase 處理時間不足 → 增加 RNase 處理時間。有時候可將 CV 值太高的樣品再放入 37℃中反應 10 分鐘，即可改善結果。

六、[Plate Loader模式] 開機步驟與軟體主畫面說明

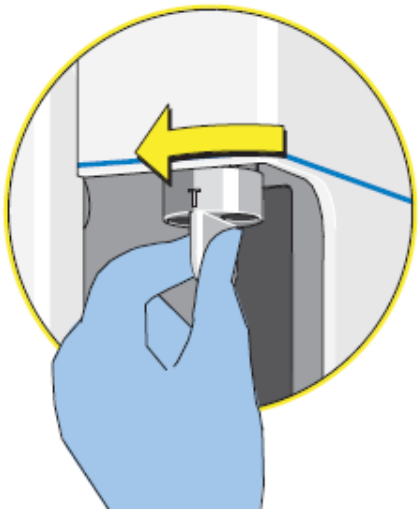
1. 開啟 CytoFLEX 背面(左側)電源。
2. 確認 Sheath Fluid 足夠，並且清空廢液筒，不要鎖緊蓋子。
3. 開啟電腦主機以及螢幕電源，依常規模式進入 Windows 系統。
4. 點選桌面上 CytExpert 軟體 

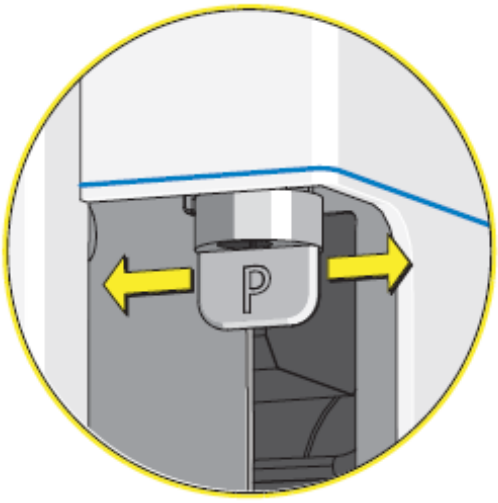
，進入操作軟體。
5. 點選 Cytometer，選擇 Sample Injection Mode，點選 **Plate Loader** 盤式上樣，再開啟 CytoFLEX 背面(左邊)電源，使儀器自動校正盤式上樣區位置：





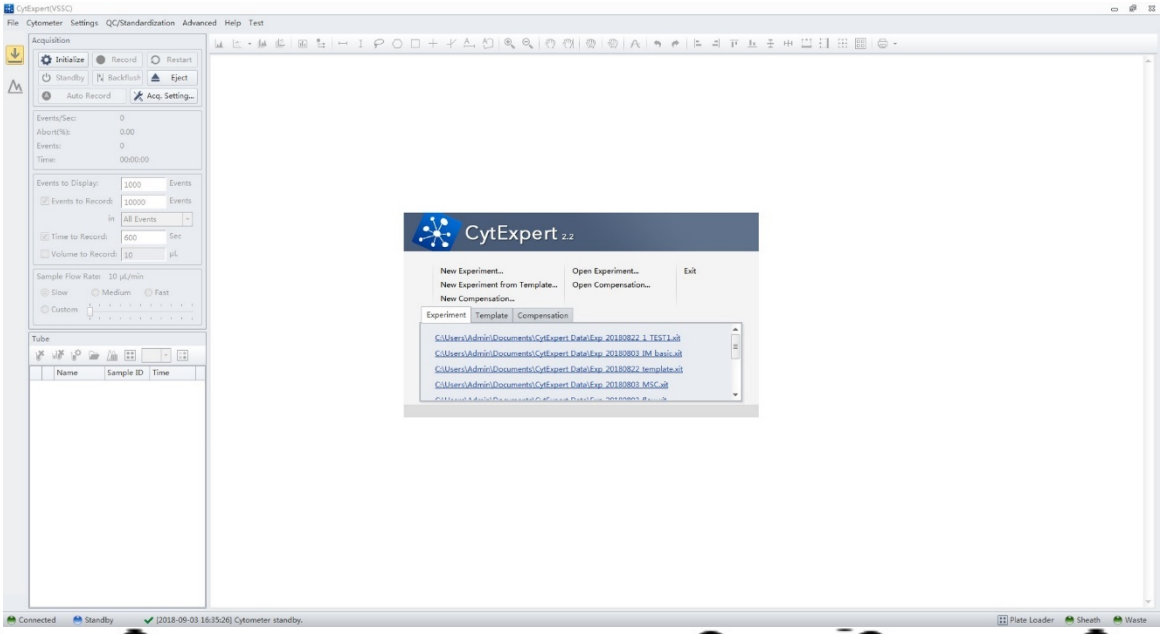
6. Cytometer 儀器上樣區開關旋鈕模組①Switch module knob 旋轉至 P 位置，如下圖顯示：





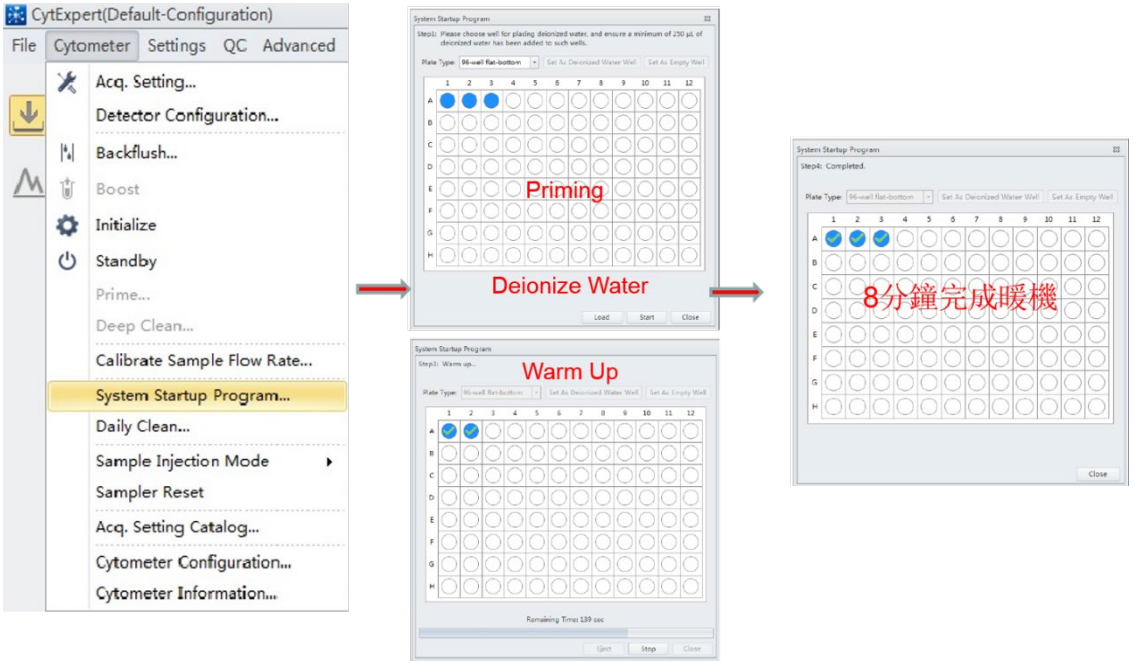
7. ※若有開啟**[User Management功能]**，此時可見以下畫面：

8. ※選取專屬的Username，接著在Password欄位輸入密碼並按下  繼續。
9. 此時進入軟體歡迎主畫面，確認左下方 Connected 及 Ready 和右下方 Sheath 及 Waste 為綠燈，表示電腦與機器連線完成。

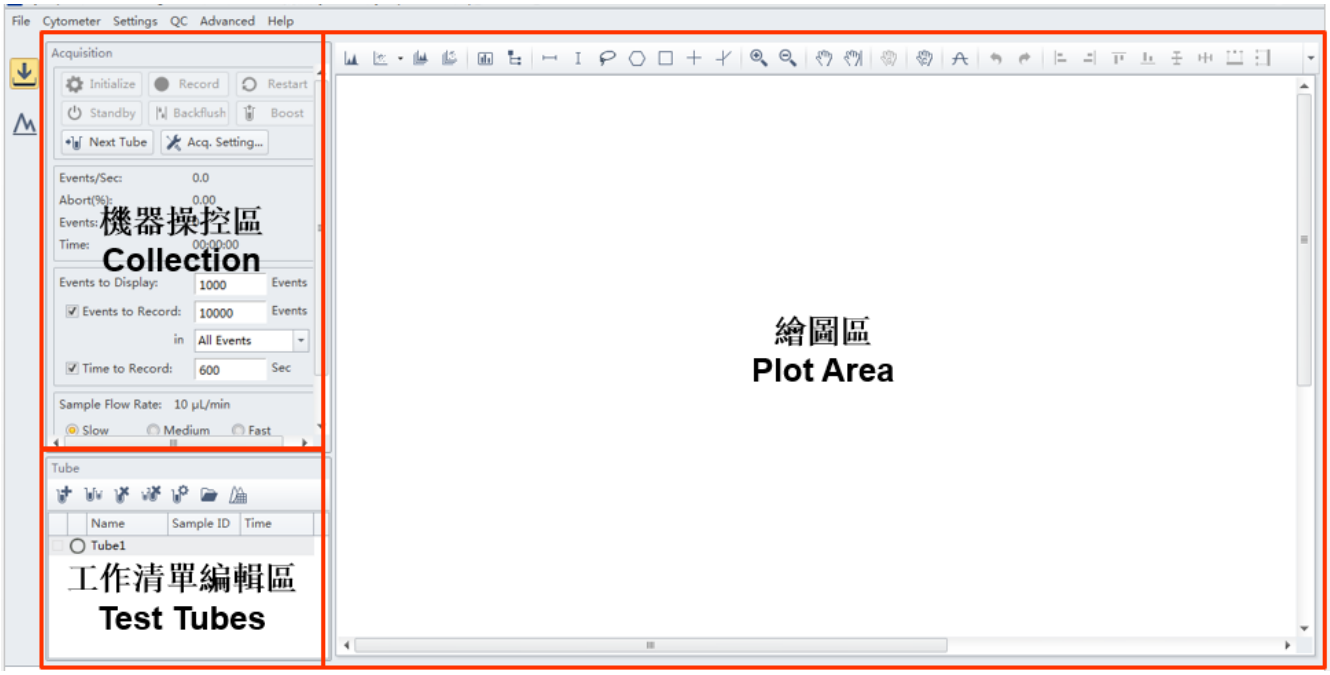


1. Communication connection status.
2. Instrument status information.
3. Laser status.
4. Sampler status.
5. Fluid status information.

10. 由 Cytometer 進入”System Start Up Program”，放上 3 wells (250 µL/well)去離子水，選擇盤子形式，點擊 Load 及 Start，CytoFLEX 執行 Priming、沖洗去離子水及 Warm Up，約 10 分鐘完成開機及暖機動作，點擊 Close。

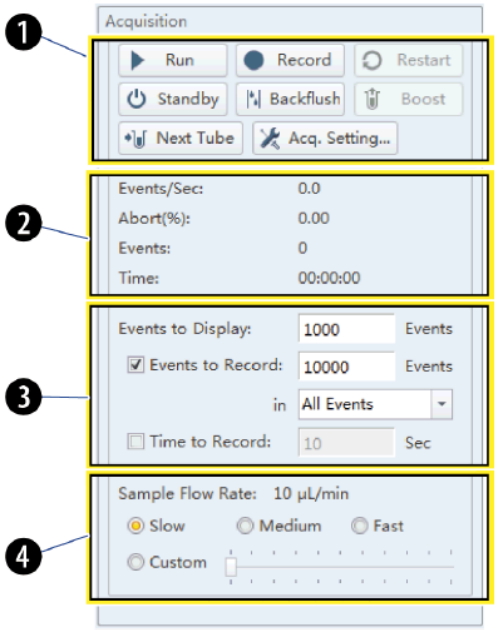


11. 由 File 進入(或起始頁面)，點選 New Experiment 並儲存實驗檔案名稱，即可見到軟體的工作區，如下圖：



4. **Collection.** Establishes control over data recording options and displays the acquisition status.
5. **Test tubes.** Allows you to configure and duplicate sample tubes, set display attributes, manage experimental data and compensation.
6. **Plot area.** Includes plot and gating controls, as well as an area for drawing plots and generating graphs.

機器操控區(Collection)



1. **Acquisition control.** Controls sample loading/unloading and data acquisition and recording.

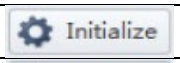
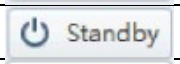
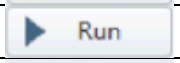
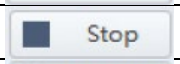
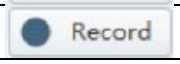
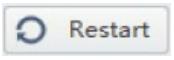
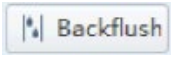
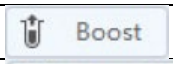
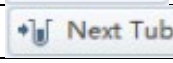
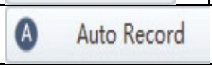
2. Acquisition status. Displays such information as the acquisition rate (Events/Sec), cell count, duration and abort (%).

3. Acquisition conditions. Sets the necessary conditions for recording data.

- **Events to Record.** Used to set the number of events to record in the specified population.
- **Time to Record.** Used to set the collection time duration in seconds.

4. Sample flow rate. Sets the acquisition rate for data collection.

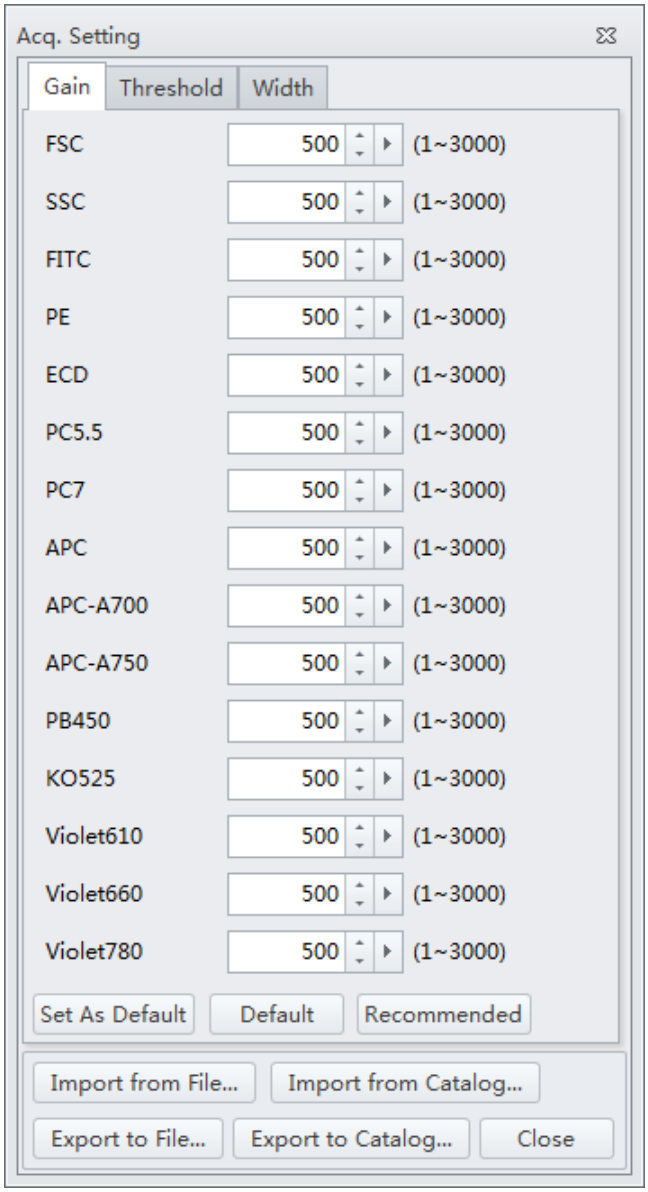
- **Slow :** 10 µL/min - **Medium :** 30 µL/min - **High :** 60 µL/min
- **Custom :** 10 - 240 µL/min

	Put the instrument in initialized state.
	Put the instrument in standy state.
	Start acquisition or continue an acquistion if previosly stop.
	Stop the acquistion of the current sample and output the results.
	Used to set the collection conditions for sample recording.
	Reset the current acquired events to zero and clear the current data in memory. Acquisition restarts at zero events.
	Flush the sample line and flow cell with sheath fluid to remove bubbles.
	To transfer the sample to the flow cell.
	Switch to the next sample tube.
	Display the acquisition setting dialog box to adjust the Cytometer settings.
	Open the plate holder.
	Sample acquisition occurs in the order indicated by the numbers

※ 

Acquisition Setting 為儀器條件設定操控視窗，包含：

1. Gain：調整偵測器訊號放大程度。

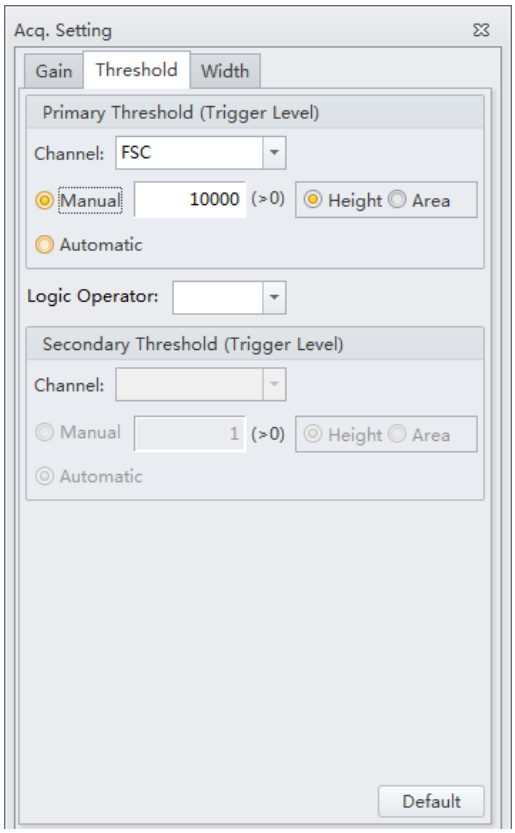


Note：

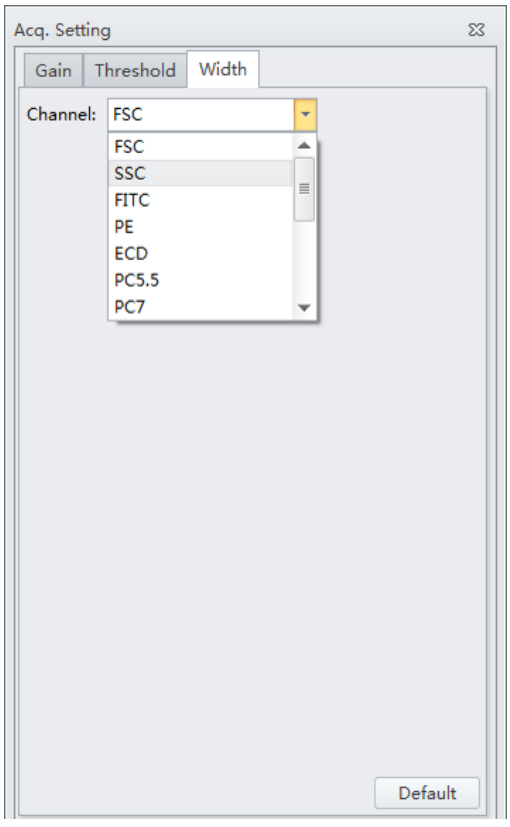
電壓值須注意不要過低，不要低於個位數。

一般細胞樣本 Background 建議設定在 10^2 – 10^4 之間。

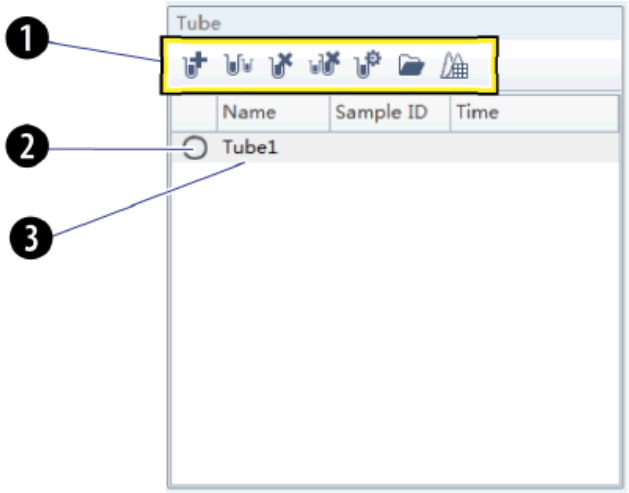
2. Threshold：排除雜訊的門檻，建議使用 Automatic 設定。

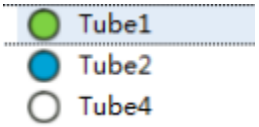


3. Width：訊號通過的時間參數，可選擇所要偵測的參數值時間。



工作清單編輯區(Test Tubes)





1. **Test tube management controls.** Manages sample tubes. Used to add, copy, or delete attributes, open the tube property, and open the compensation matrix.
2. **Test tube status indication.** Displays a colored symbol in front of each tube indicating the status of the tube processing.
3. **Test tube list.** Displays the sample tubes used in the experiment. Right-click a tube in the list to perform additional operations.

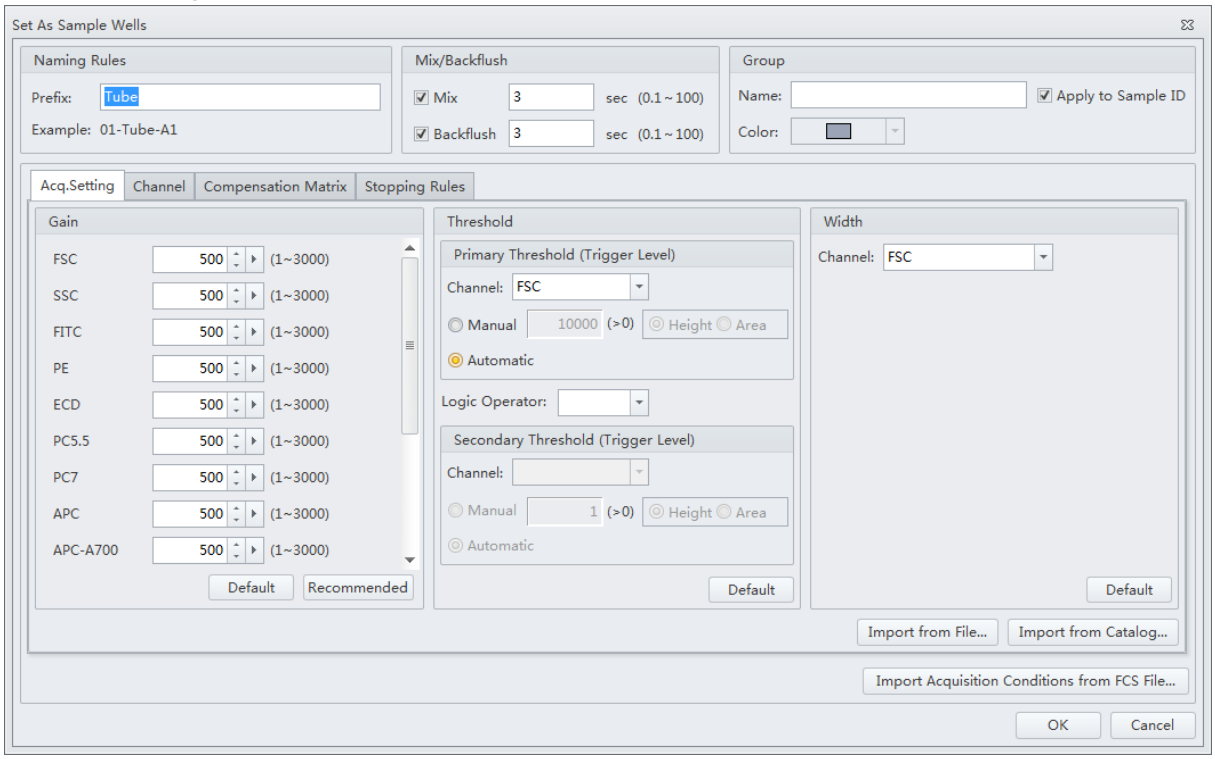
	Plate	Open the plate window.
	Add Plate	Add one new plate.
	Add Plate from Template	Add a plate template with preset settings.
	Duplicate Current Plate without Data	Creat a copy of the selected plate without data.
	Delete Plate	Delete one plate.
	Save Plate Template	Save the plate condition as a template.
	Set As Sample Wells	Set selected wells as sample wells.
	Set As Cleaning Agent Wells	Set selected wells as cleaning wells.
	Set As Deionized Water Wells	Set selected wells as Deionized Water wells.
	Set As Empty Wells	Reset selected wells as empty.
	Set Acquisition Condition	Select the desired acquisition settings.
	Set Auto Acquisition	Set the selected wells for auto record.
	Cancel Auto Acquisition	Remove the auto record setting from the selected wells.
	Heat Map	Open Heat Map window.
	New Heat Map	Add one new plate with Heat Map.

	Duplicate	Duplicate current Heat Map.
	Modify Current Heat Map Setting	Modify existing Heat Map settings.
	Delete Current Heat Map	Delet a single Heat Map from the list of Heat Map window.
	Delete Multiple Heat Maps	Delet multiple Heat Maps from the list of Heat Map window.
	Refresh	Refresh a single Heat Map from the list of Heat Map window.
	Refresh All	Refresh all Heat Maps from the list of Heat Map window.
	Export to Graphic File	Export a Heat Map as a Graphics File(.bmp or .emf)
	Export to Clipboard	Export a Heat Map to a Clipboard file(.bmp)

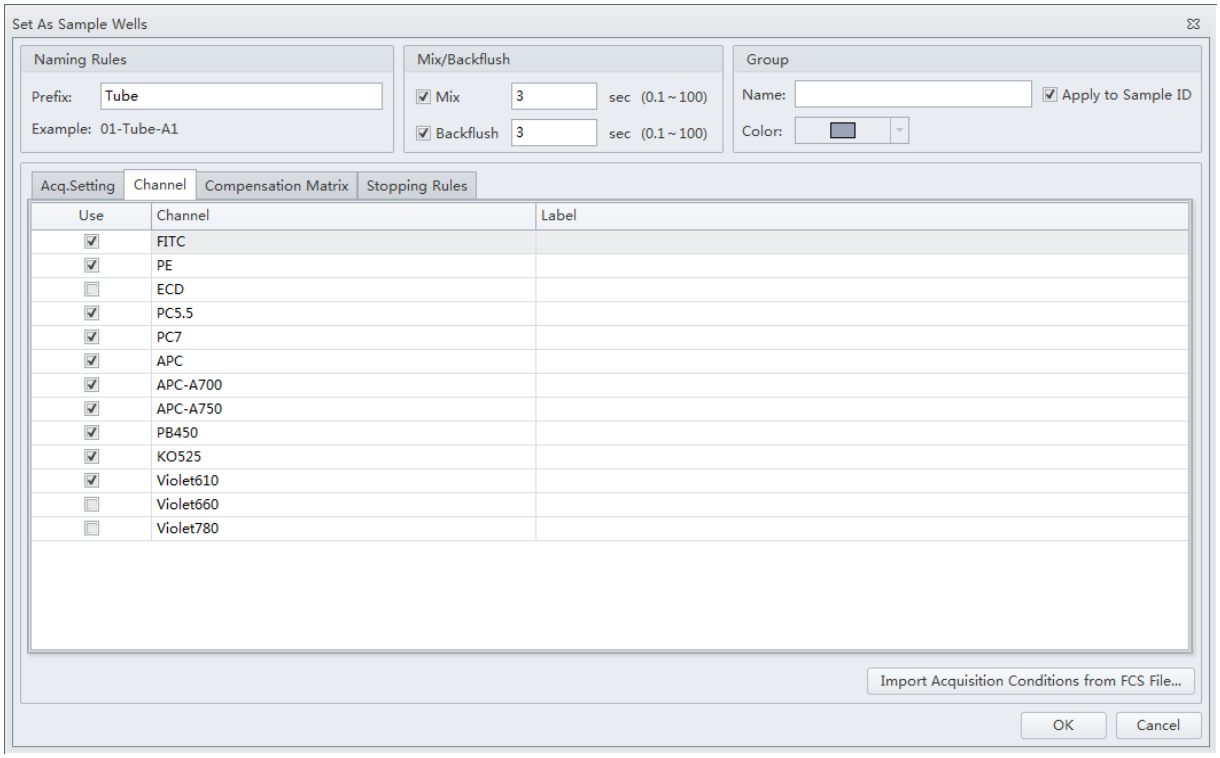
 藍勾	A blue check mark means the data is acquired, not recorded.
 綠勾	A green check mark means the data is recorded.
 紅叉	A red cross mark means the data is recorded. But the acquisition is terminated abnormally. For example, the well is skipped or the acquisition is manually stopped.

Set As Sample Wells 為儀器設定操控視窗，包含：

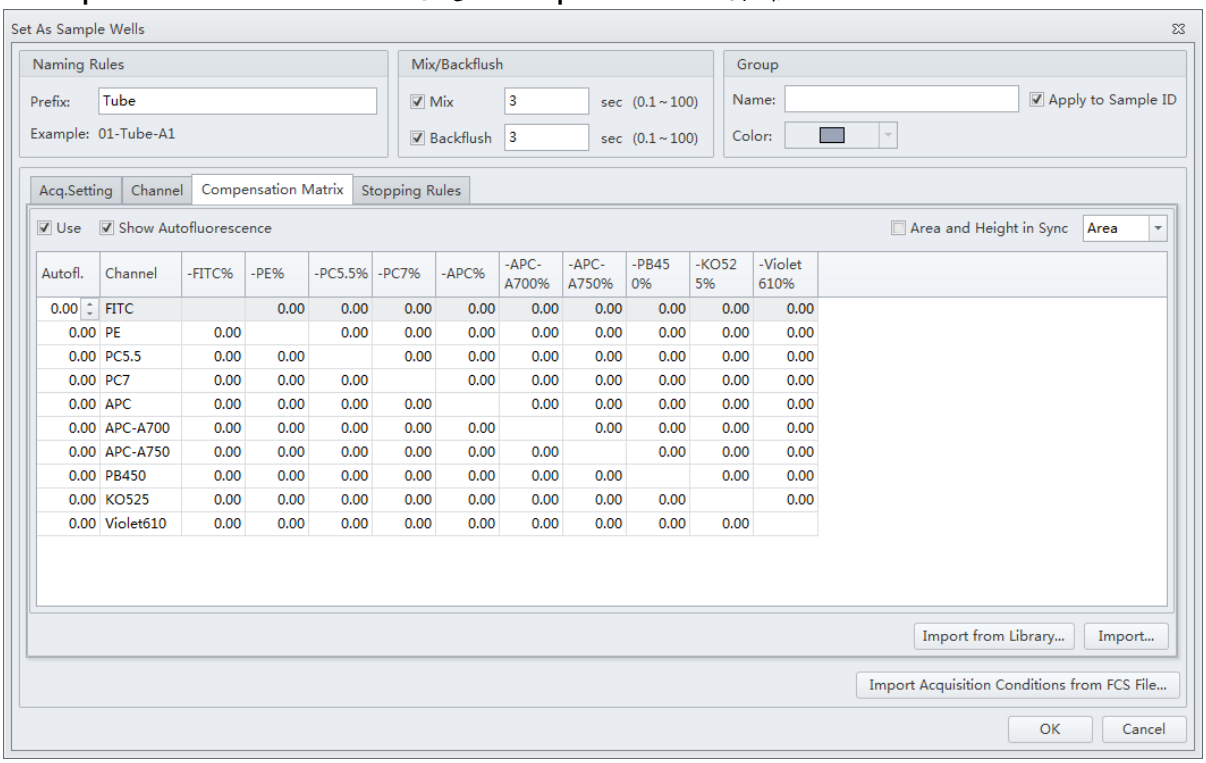
1. Naming Rules、Mix/Backflush 及 Group
   Acq. Setting：調整 Gain、Threshold 及 Width。



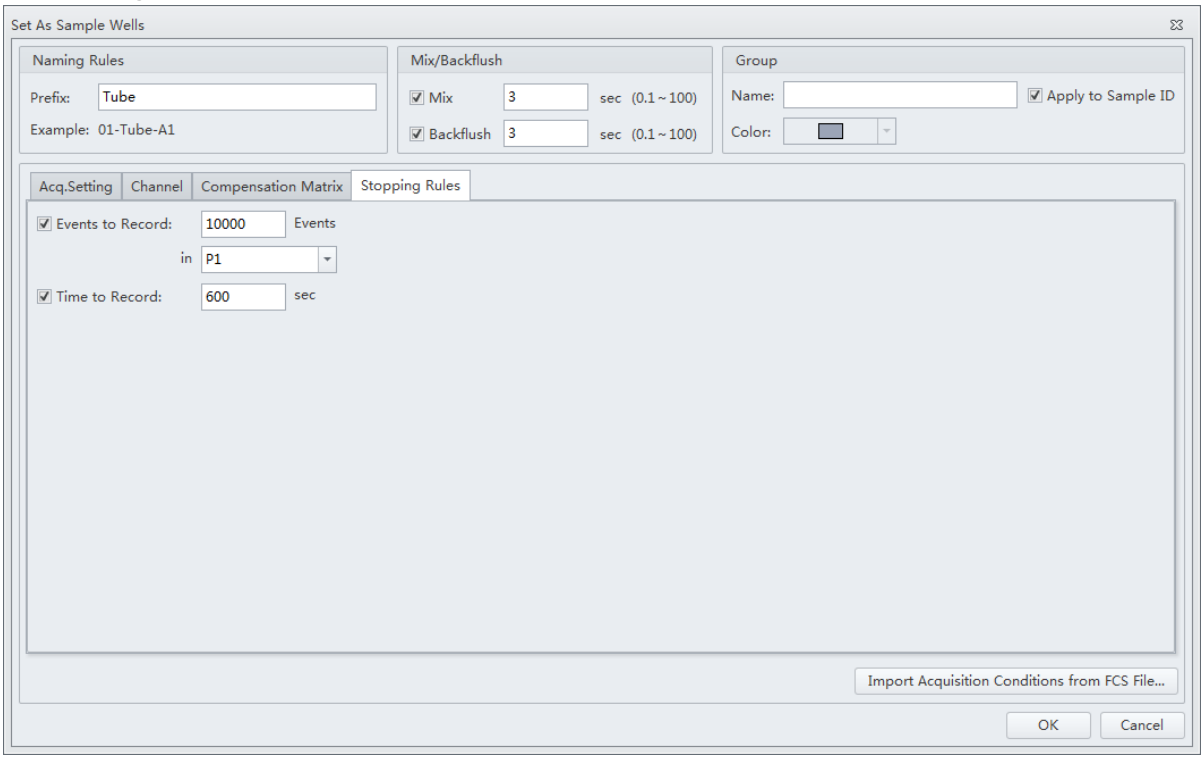
2. Channel：勾選 Channels 及標示抗體染劑名稱。



3. Compensation Matrix：設定 Compensation 數值。

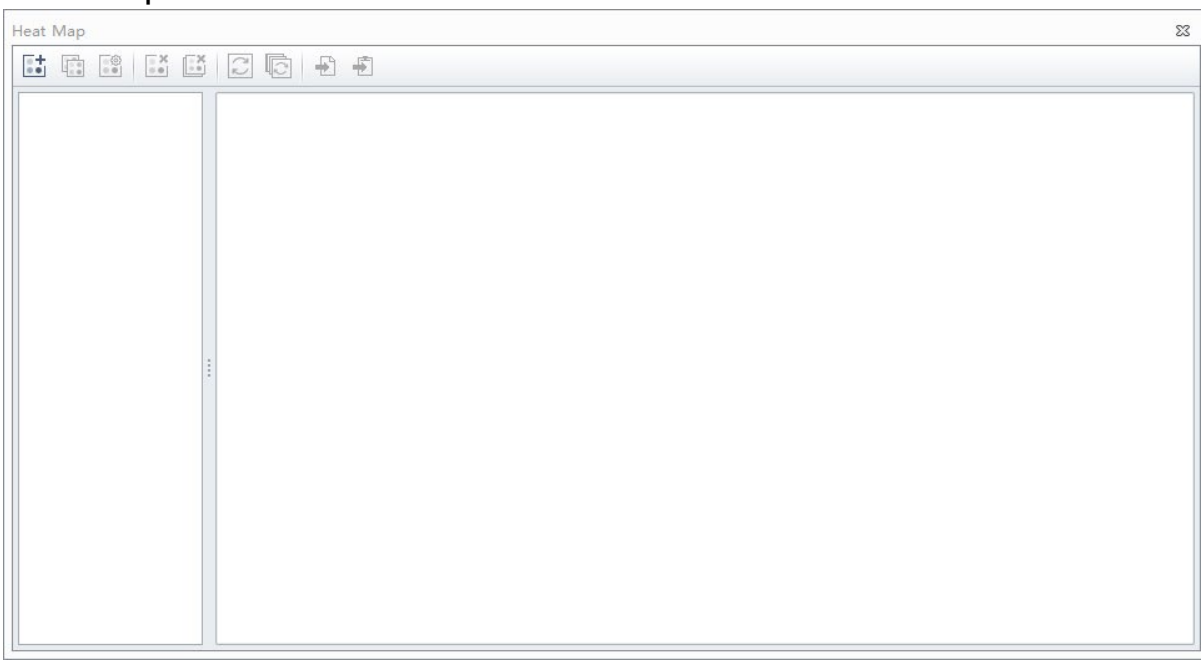


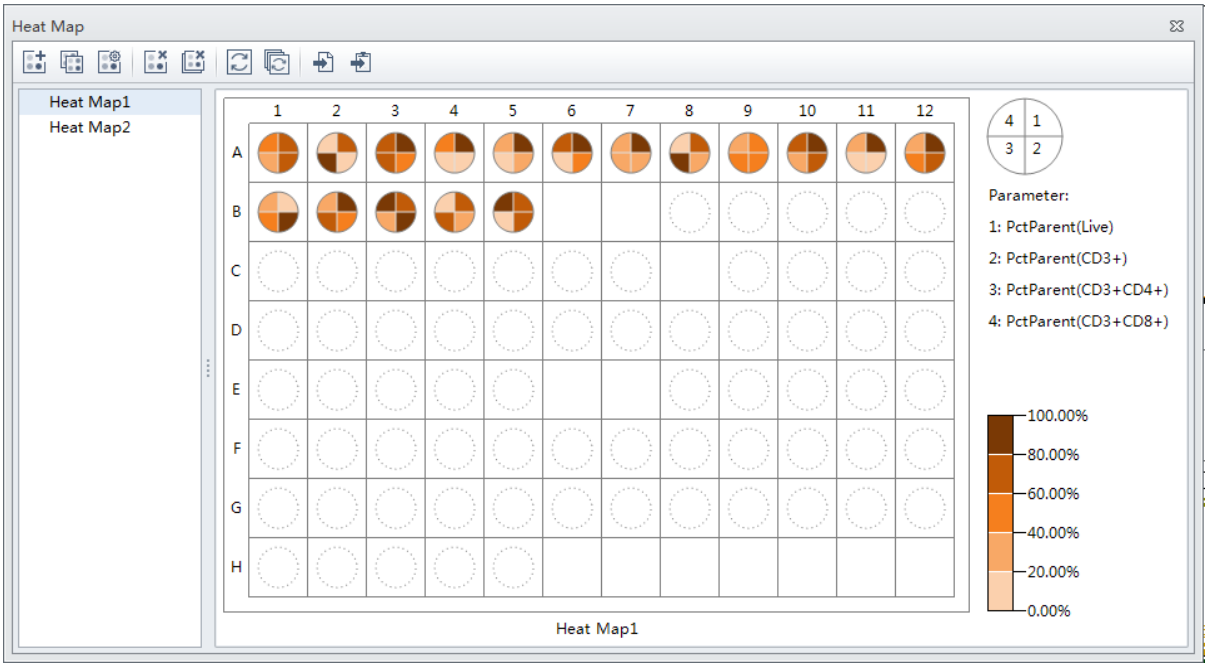
4. Stopping Rules：設定儲存條件。



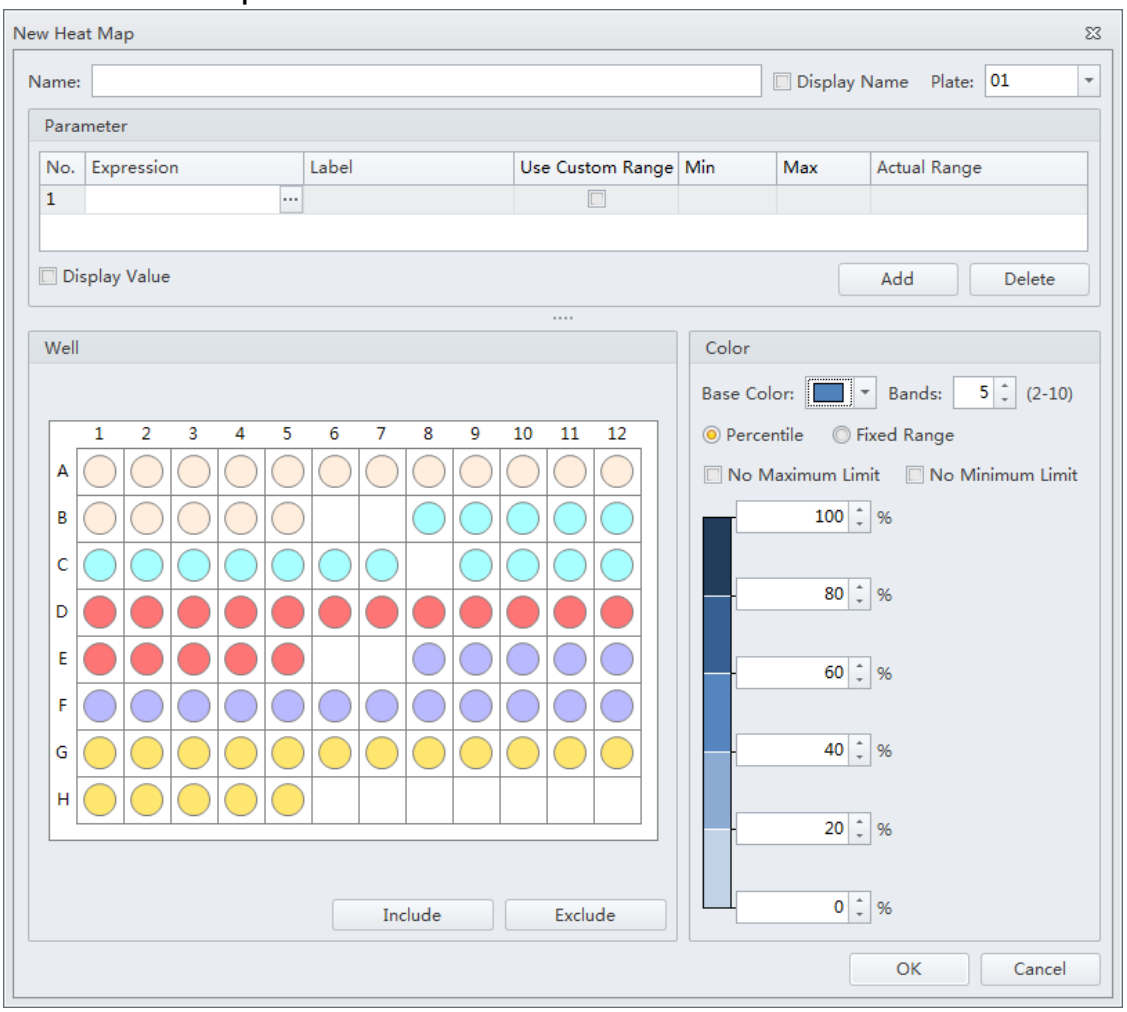
Heat Map 設定操控視窗，包含：

5. Heat Map Window。





6. New Heat Map。



繪圖區(Plot area)

	Histogram	Create a Histogram Plot and specify the plot properties.
	Dot Plot	Create a Dot Plot and specify the plot properties.
	Density Plot	Create a Density Plot and specify the plot properties.
	Pseudo Color Plot	Create a Pseudo Color Plot and specify the plot properties.
	Contour Plot	Create a Contour Plot and specify the plot properties.
	New Histograms	Create multiple Histogram Plots and specify the plot properties.
	New 2-D Plots	Create multiple Dot Plots and specify the plot properties.
	Statistics	Create Statistical charts.
	Population Hierarchy	Create Hierarchical charts.
	Line Segment	Insert a Linear gating of plots.
	Vertical	Insert a Vertical gating of plots.
	Lasso	Insert a Lasso gating into a dual parameter plots.
	Polygon	Insert a Polygon gating into a dual parameter plots.
	Rectangle	Insert a Rectangle gating into a dual parameter plots.
	Four Quadrant	Insert a Four Quadrant gating into a dual parameter plots.
	Hinged	Insert a Hinged gating into a dual parameter plots.
	Auto Line Segment	Creat an Auto Line Segment around the selected population on a plot.
	Auto Polygon	Creat an Auto Polygon around the selected population on a plot.
	Zoom In	For Zooming in.
	Zoom Out	For Zooming out.
	Pan	For scaling axis ranges in the plots.
	Single Side Pan	For scaling single axis range in the plots.
	Adjust Gain	For increasing and lowering gain adjustments on the plots.
	Adjust Compensation	For adjusting compensation of either of the parameters on a 2-D histogram.
	Threshold	For setting the minimum particle size limit or flurescence intensity that acquisition will allow.
	Undo	For undoing an action in the drawing area.
	Redo	For redoing an action in the drawing area.

	Align Left	Align all the selected items to the left of the selection area.
	Align Right	Align all the selected items to the right of the selection area.
	Align Top	Align all the selected items to the top of the selection area.
	Align Bottom	Align all the selected items to the bottom of the selection area.
	Vertical Distribute	Align all the selected items to the vertical distribution.
	Horizontal Distribute	Align all the selected items to the horizontal distribution.
	Make Same Width	Resize the selected items to all be the same width as the reference item.
	Make Same Hight	Resize the selected items to all be the same hight as the reference item.
	Make Same Size	Resize the selected items to all be the same size as the reference item.
	Rearrange	For restoring the plots to the default positions.
	Print	For printing and previewing the plot area.
	Print Preview	Used to access the Preview screen.
	Page Setup	Used to adjust the page settings.
	Batch Print	Used to print data for multiple tubes.
	Batch Export to PDF File	Used to print a PDF of the data for multiple tubes.

七、[Plate Loader模式] 設定新的Experiment

雙染(FITC/PE)Surface Markers設定

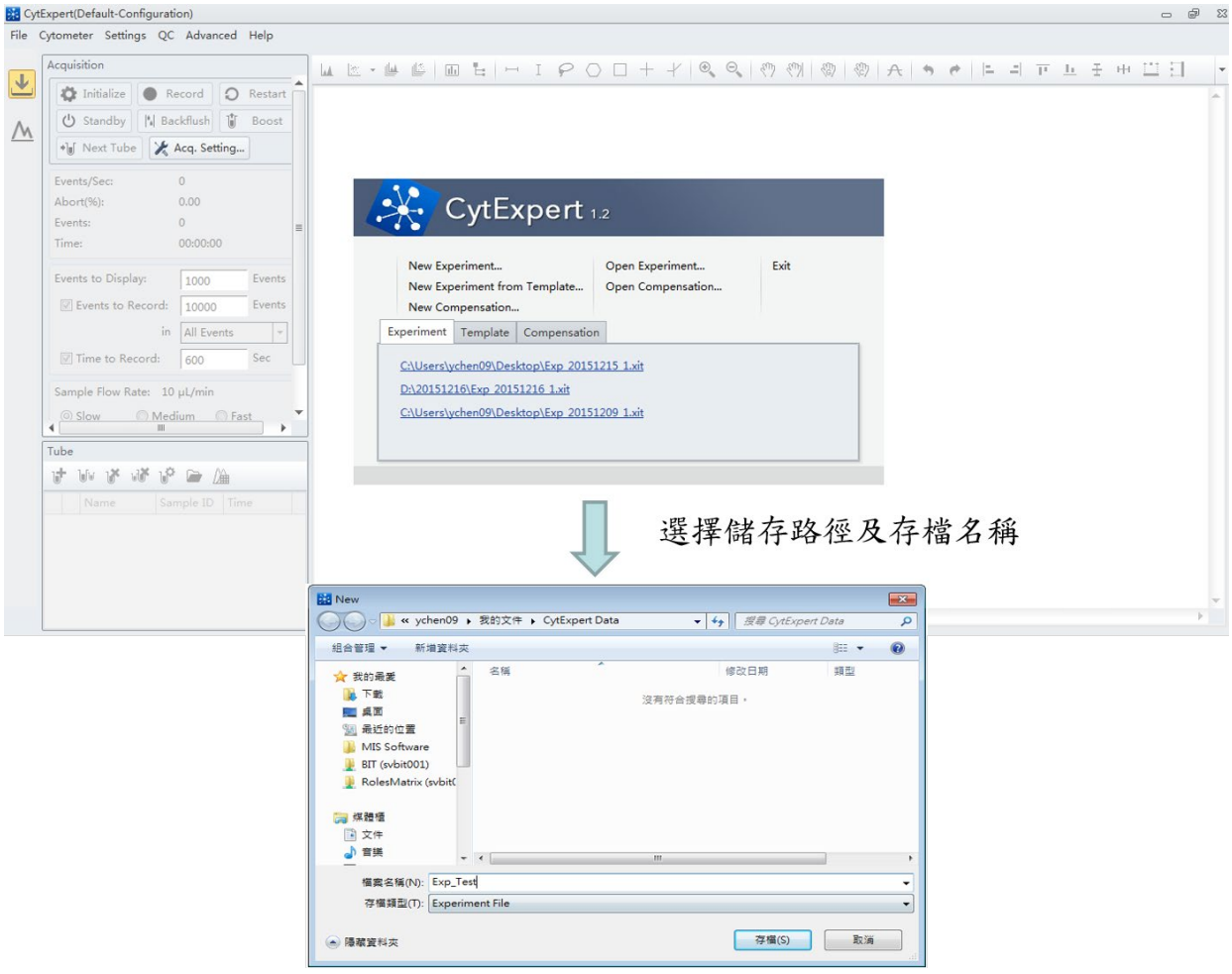
✧ 以CD3-FITC / CD4-PE為示範，需準備四管樣品用以調整儀器的設定值：

1. 陰性樣品：未染色的細胞，或以Isotype抗體染色的細胞
2. 單染FITC的陽性樣品
3. 單染PE的陽性樣品
4. 雙染的陽性樣品

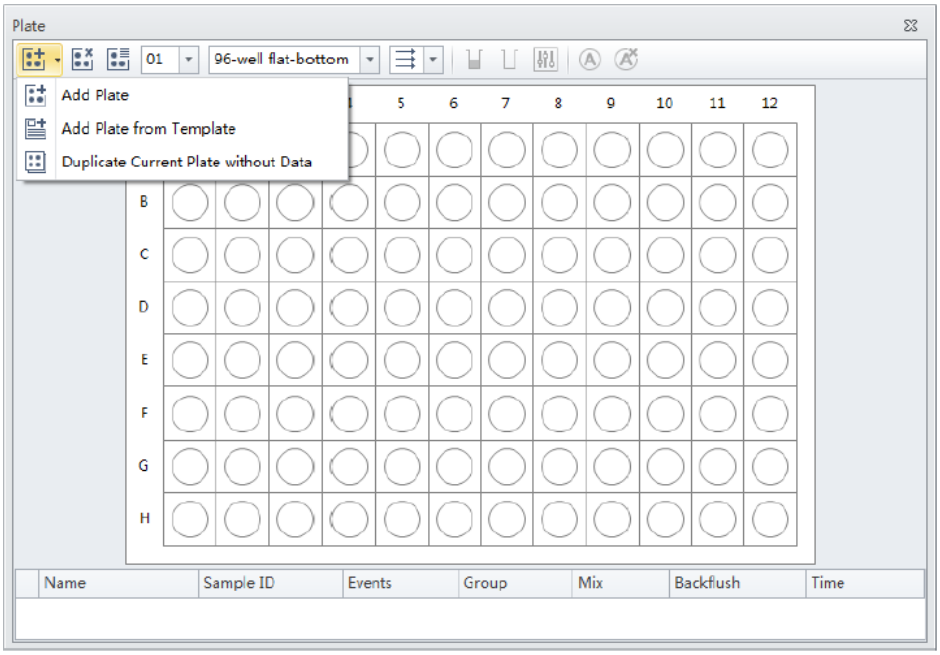
➢ 操作步驟：

a. 開啟一個新的Experiment：

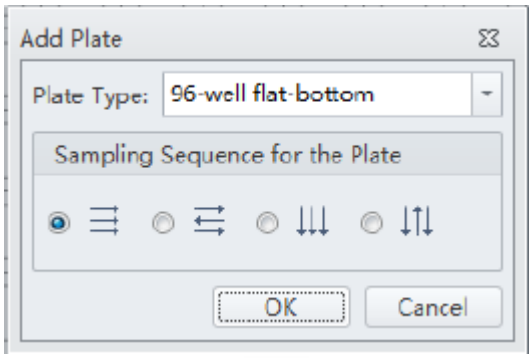
由File進入(或起始頁面)，點選New Experiment並儲存實驗檔案名稱。



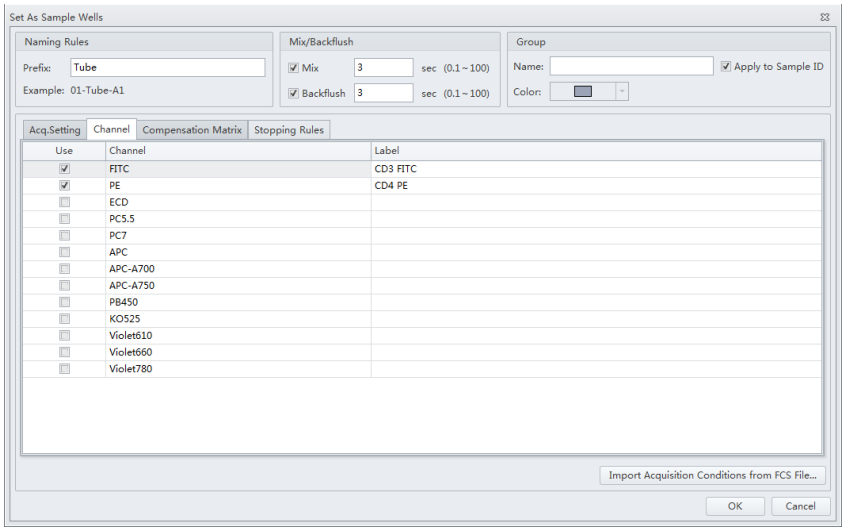
b. 點選Plate 並新增一個空白Plate 。



1. 選擇96 well盤式規格(平底、V底或U底)及Sample跑的方向順序，點擊OK。

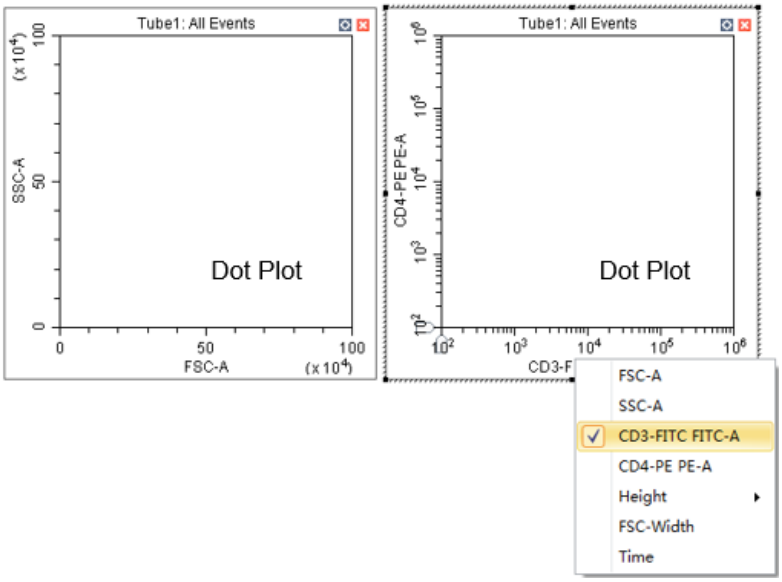


2. 選擇指定well，點擊 Set As Sample Wells，選擇Set Channel，勾選Channels及標示抗體染劑名稱。



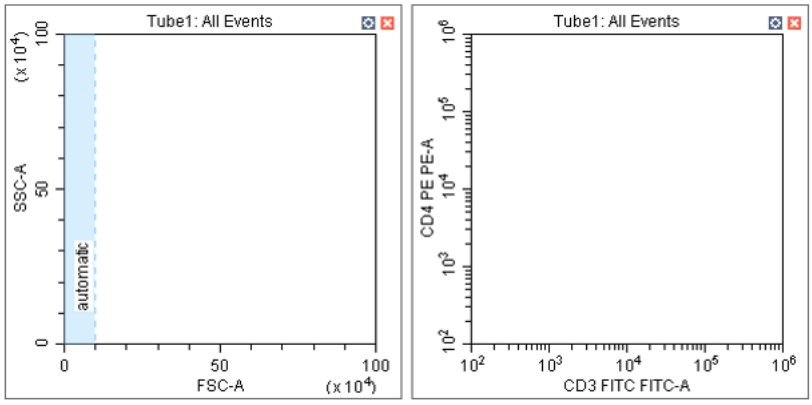
c. 利用已勾選的參數繪製希望分析的圖形：

在繪圖工具列中點選Dot Plots，於圖形上的X/Y軸點滑鼠左鍵，選擇想要標示的抗體螢光參數。



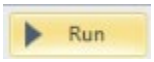
d. 確認Threshold是否設定完成：

在繪圖工具列中點選Threshold，此時會於FSC/SSC圖形上出現automatic的藍色虛線，Surface Marker的實驗中，建議將Threshold設於FSC第一個刻度位置。

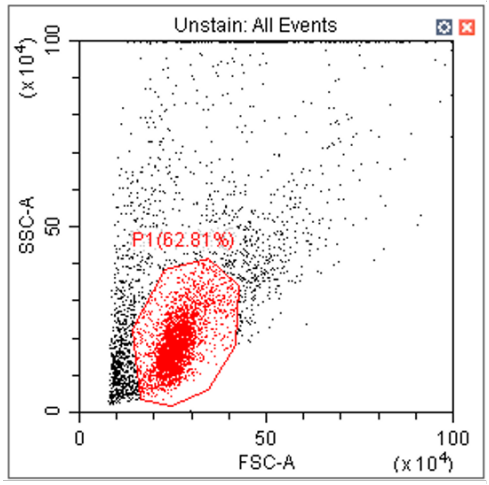


e. 分析陰性樣品(可使用未染色的樣品，或以isotype抗體染色的陰性樣品)，依下列方式調整各個偵測器的Gain值：

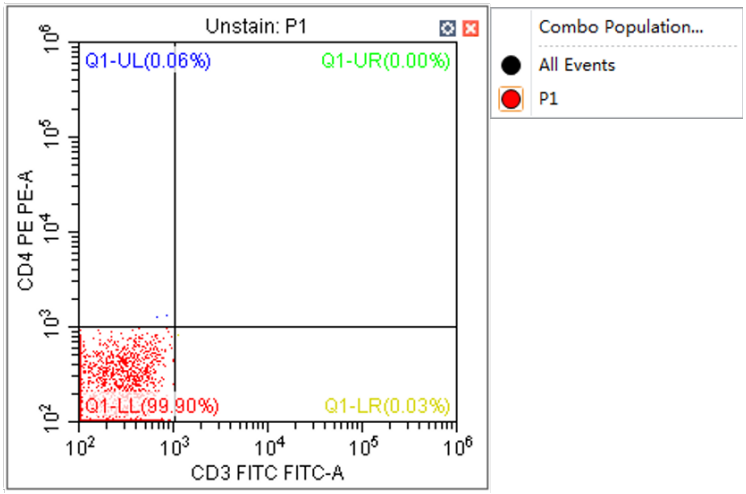
1. 輸入樣品名稱(例如Unstain / Isotype)

2. 按下儀器操控區中的Run 

，此時機器開始收集樣品數據(此時所收集的數據會於樣品名稱顯示閃爍的藍色圓圈，表示暫存，圖形中的數據呈現動態的變動)，並顯示在剛才畫好的圖形上，使用繪圖區的Gain 
調整FSC/SSC的Gain值，或使用Pan 
或Single Side Pan 
調整X/Y的Scale，找到FSC/SSC中想要分析的細胞族群。使用圈選工具列 ，對FSC/SSC的細胞群以多邊形Polygonal Gate 圈選P1 Gate。



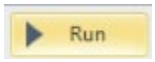
3. 接著調整FL1、FL2的Gain值，於螢光圖上方點選左鍵，選擇P1 Gate觀察，接著以十字象限定義Negative位置，使FL1/FL2雙參數圖形的細胞落在左下角第一個Log位置。

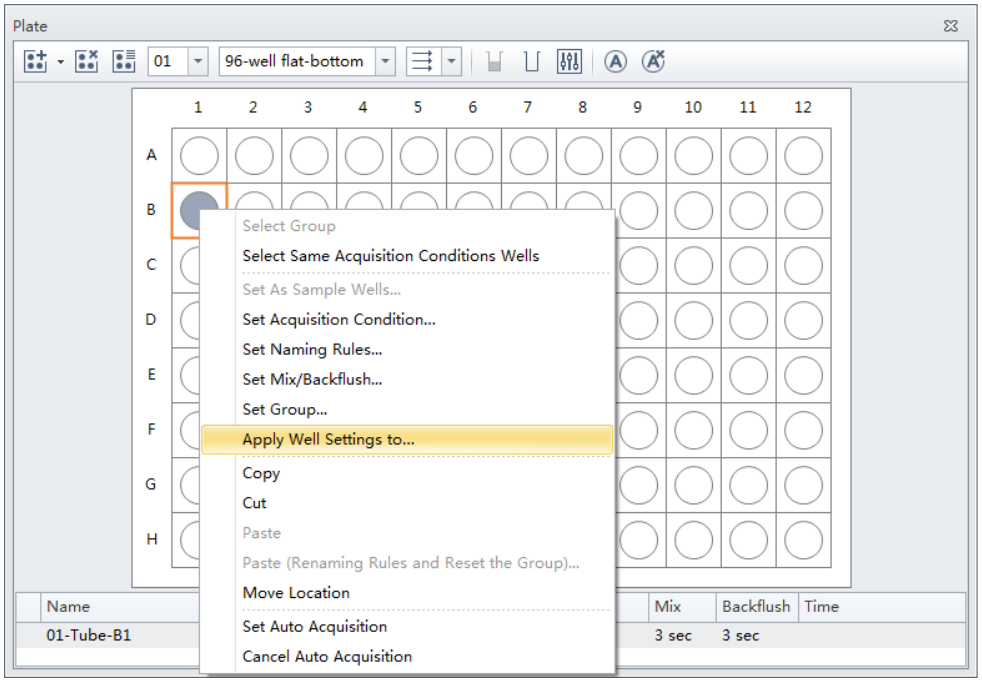


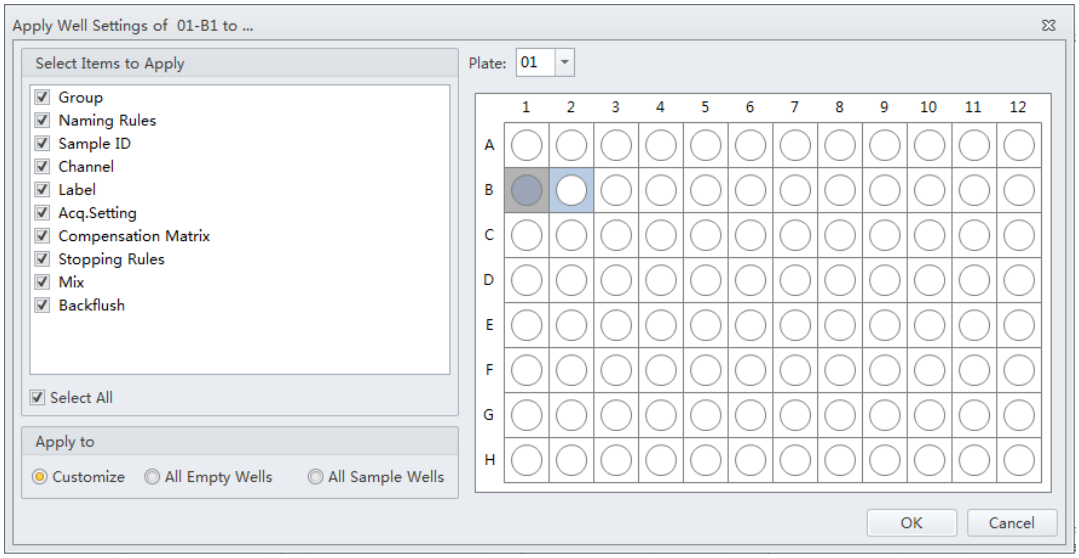
f. 分析單染的陽性樣品調整螢光補償值(Compensation)：

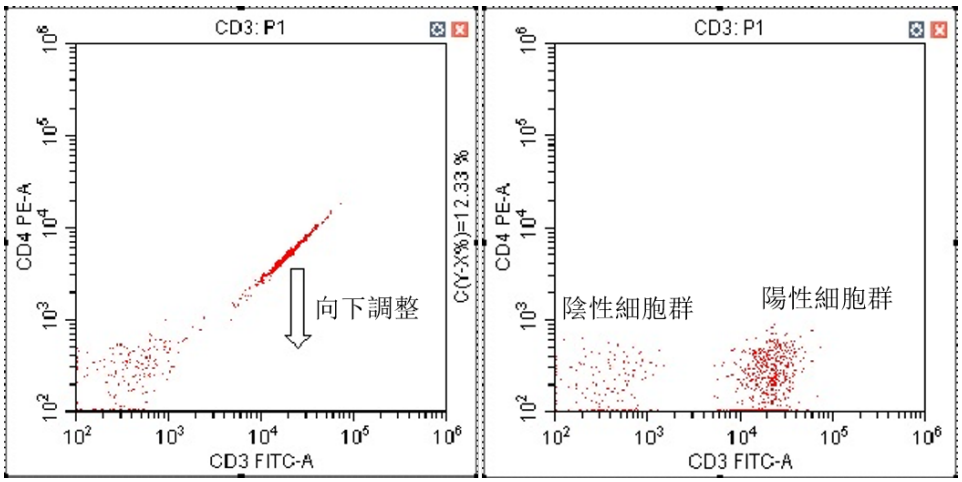
使用兩管樣品：以FL1單染樣品調整FL2-%FL1

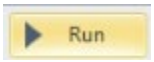
以FL2單染樣品調整FL1-%FL2

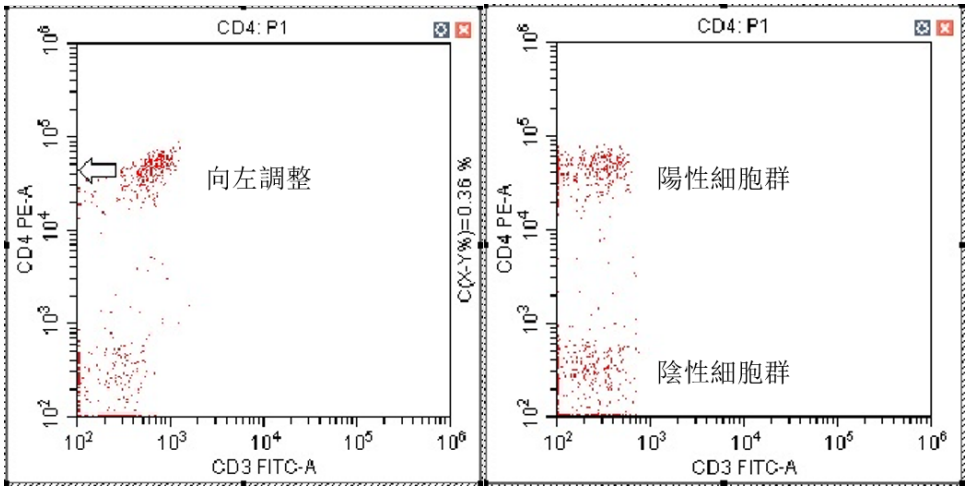
1. 於Blank well按右鍵，點選Apply Well Setting to上樣CD3-FITC單染樣品well，點擊OK，左下Name欄位輸入樣品名稱(例如CD3)。點擊Run  ，接著使用繪圖區的Compensation  ，調整螢光補償。直接在Plot上拖拉細胞群即可設定螢光補償。

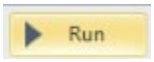






2. 點選Apply Well Setting to上樣CD4-PE單染樣品，左下角Name欄位輸入樣品名稱(例如CD4)。點擊Run  ，調整螢光補償Compensation  。直接在Plot上拖拉細胞群即可設定螢光補償。



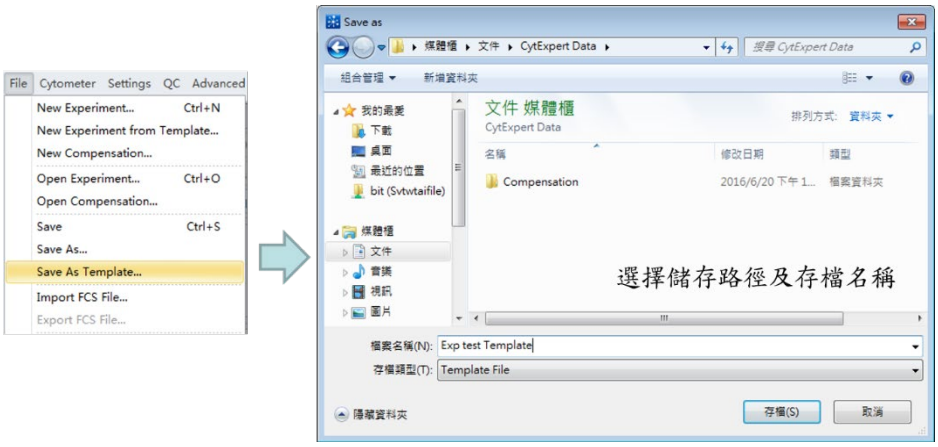
3. 點選Apply Well Setting to雙染樣品，左下角Name欄位輸入樣品名稱(例如CD3-CD4)。點擊Run  ，即以設定好的條件分析樣品，點擊Auto Record  正式收取樣品數據。

將Experiment儲存為Template

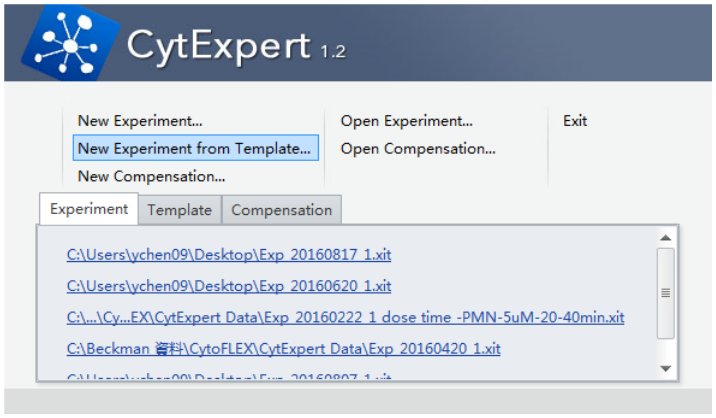
✧ 如將已設定好的Experiment，之後需再進行使用時可以儲存此Template。

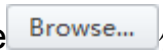
➢ 操作步驟：

1. 於已設定好的Experiment中，點選File，選擇Save as Template並儲存實驗檔案名稱。

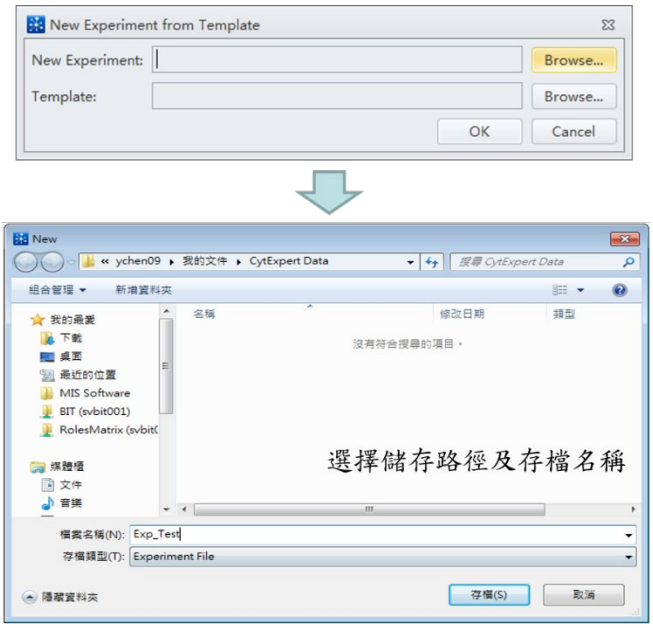


2. 之後需進行相同Experiment時，由File進入(或歡迎頁面)，點選New Experiment from Template。

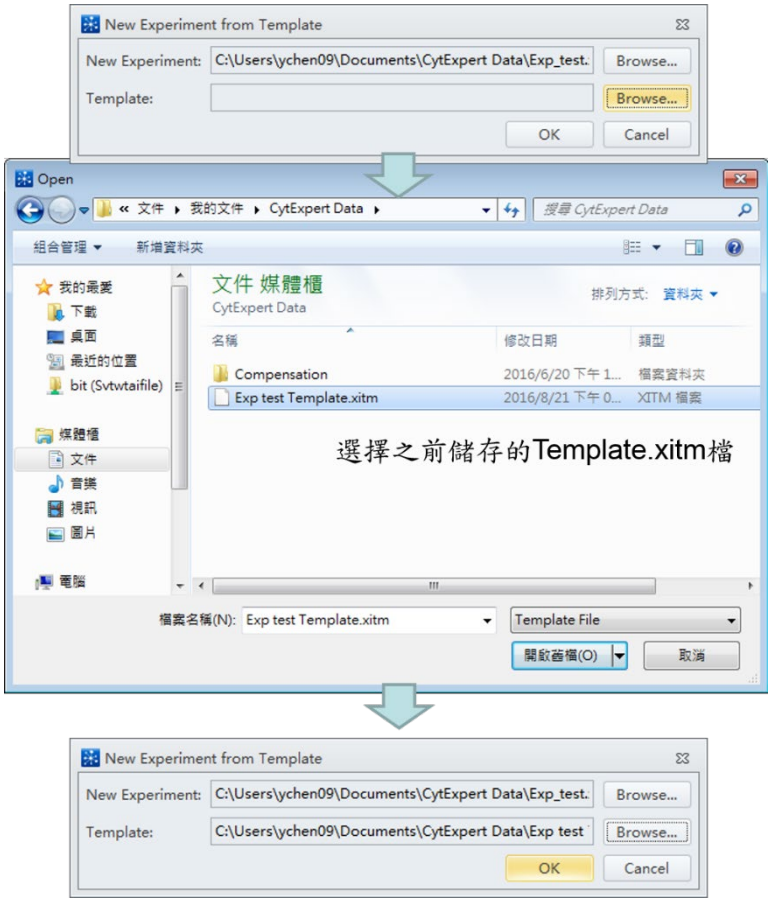


3. 點選New Experiment的Browse 

儲存新實驗檔案名稱。



4. 再點選Template的Browse選擇之前儲存的Template.xitm檔。

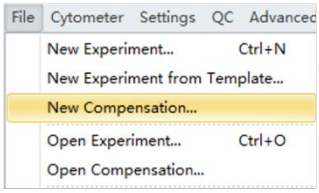


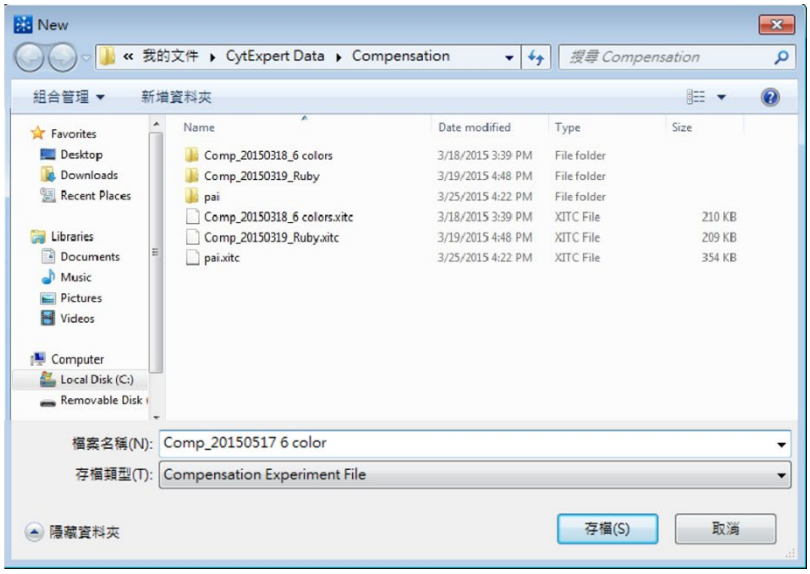
5. 選擇完成後點擊OK，開啟既有Template。

自動螢光補償設定

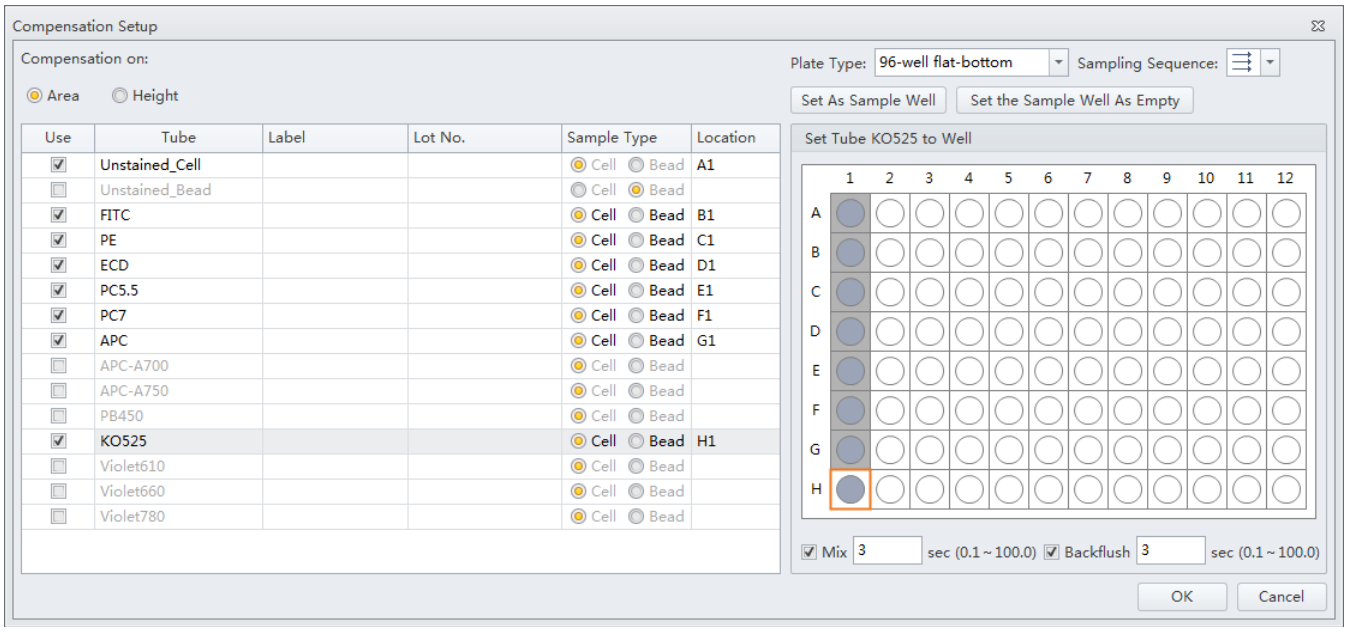
以CD3-FITC / CD4-PE / CD19-PC5.5 / CD16-PC7及CD56-PC7 / CD8-APCA700 / CD45-KO 6色染色為示範，共有8管樣品分別為Unstained / Isotype、各抗體單色染色和6色染色樣品。

1. 由File進入(或起始頁面)，點選New Compensation並儲存實驗檔案名稱。

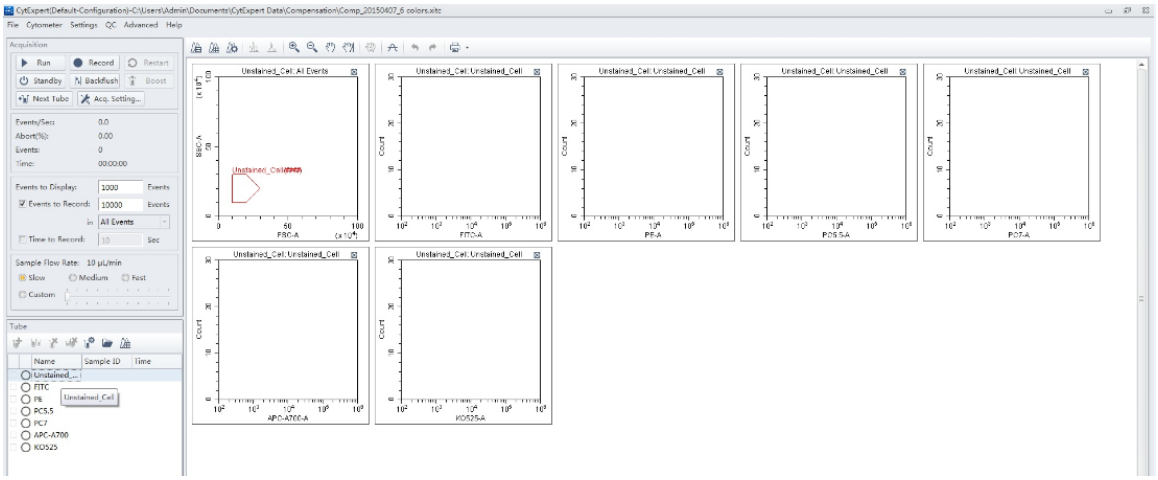




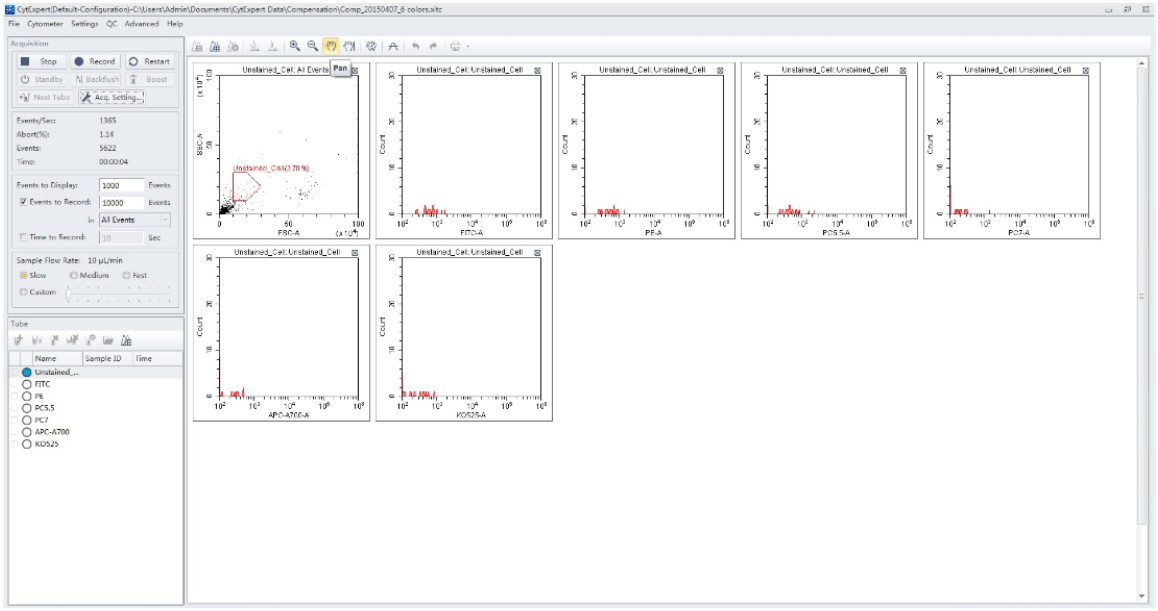
2. 選擇盤子類型，依照軟體導引，勾選使用細胞或珠子以及使用染劑，指定sample位置，點擊Set As Sample Well設定Location位置，點擊OK。



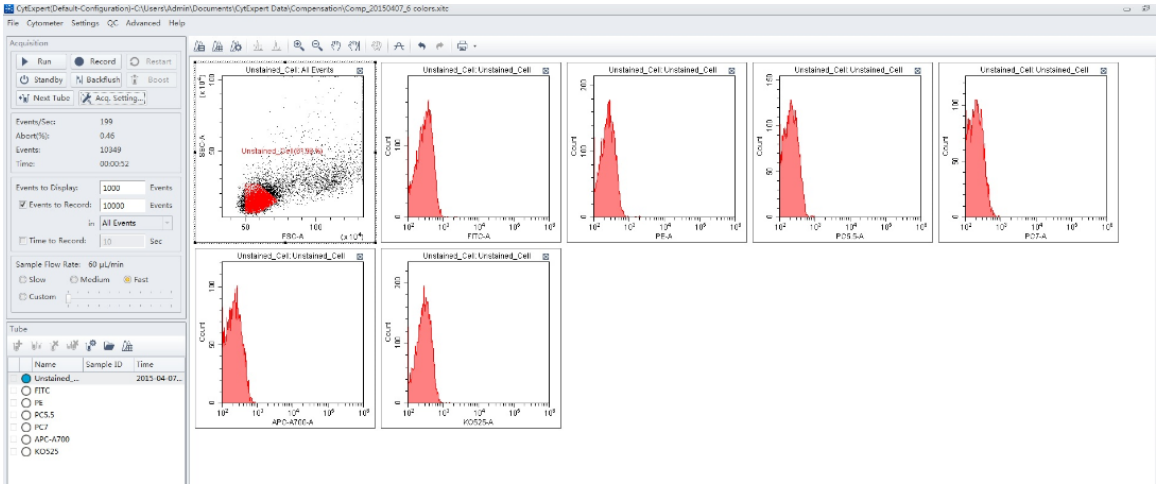
3. 此時軟體會根據所勾選染劑自動畫圖，並且左下角Tube表格自動設定準備上樣之Unstained / Isotype及單色染色樣品管。



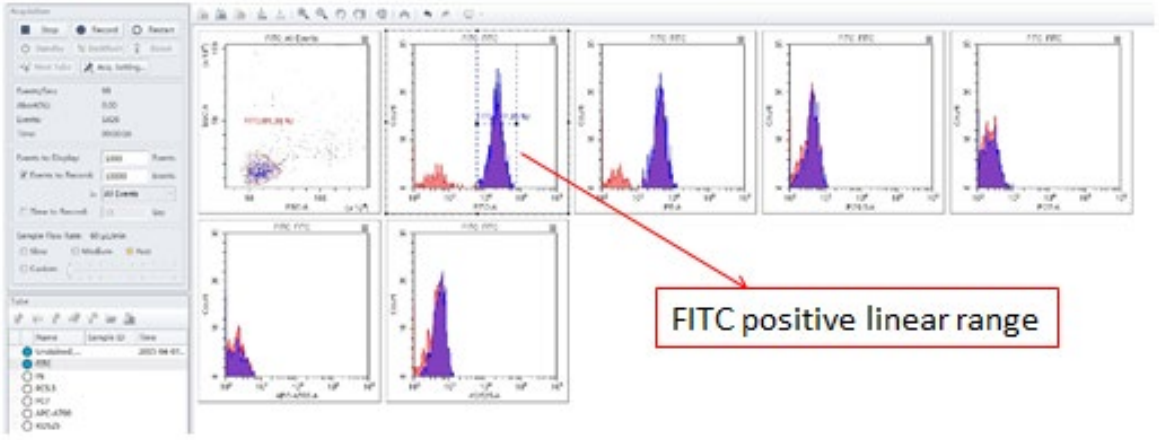
4. 先上Unstained / Isotype樣品管，點擊Run。

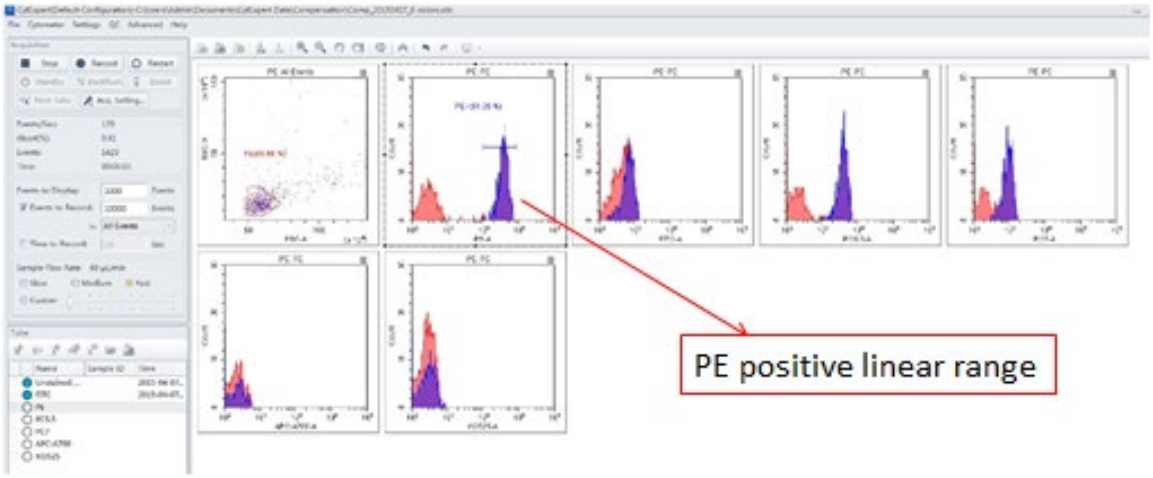


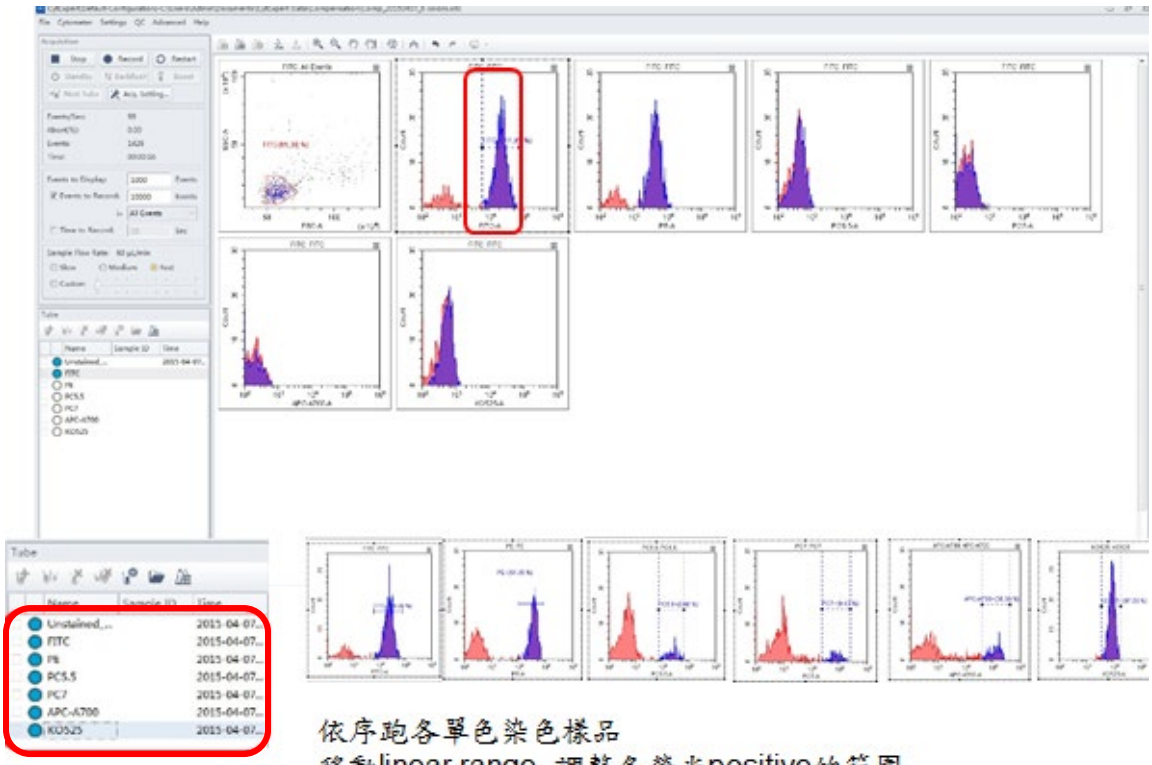
5. 調整FSC/SSC設定(使用Scale  ，FSC/SSC五角型圈選，Threshold  及Gain )將FSC/SSC圖形中調整到可以看見主要細胞群。



6. 分別再上樣單色FITC、PE、PC5.5、PC7、APC-A700及Krome Orange，並且調整單色染色的Positive區域中的Linear Rang。







依序跑各單色染色樣品
移動linear range, 調整各螢光positive的範圍

7. 再Double Check並微調各個單色染色的Positive區域中的Linear Rang。點擊快捷工具列Compensation Calculation  ，6色Compensation Matrix計算完成。

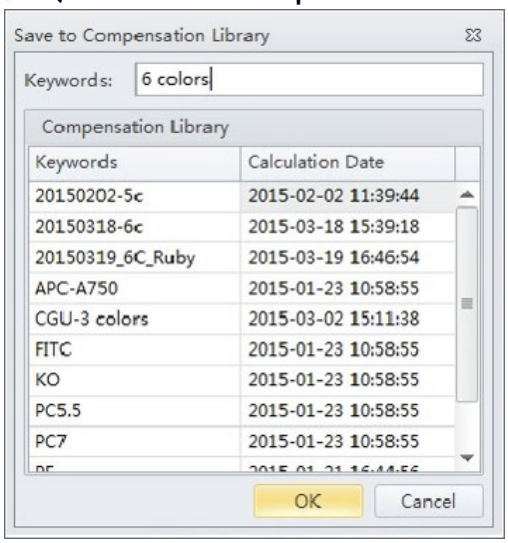
Compensation Matrix

Use | Show Autofluorescence | Area

Cha...	-FIT...	-PE%	-EC...	-PC...	-PC...	-AP...	-AP...	-AP...	-PB...	-KO...	-Vio...	-Vio...	-Viole...
FITC		1.08	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.66	0.00	0.00	0.00
PE	19.06		0.00	2.34	2.30	0.00	0.00	0.00	0.00	0.46	0.00	0.00	0.00
ECD	22.23	148....		3.32	3.54	0.00	0.00	0.00	0.00	0.76	0.00	0.00	0.00
PC5.5	1.33	9.97	0.00		0.84	0.00	1.01	0.00	0.00	0.00	0.00	0.00	0.00
PC7	0.34	2.16	0.00	63.64		0.00	0.97	0.00	0.00	0.00	0.00	0.00	0.00
APC	0.00	0.00	0.00	2.55	0.06		15.18	0.00	0.00	0.00	0.00	0.00	0.00
APC...	0.00	0.00	0.00	33.23	0.06	0.00		0.00	0.00	0.00	0.00	0.00	0.00
APC...	0.00	0.00	0.00	33.41	13.54	0.00	107....		0.00	0.00	0.00	0.00	0.00
PB4...	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		6.07	0.00	0.00	0.00
KO5...	1.37	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00
Viol...	0.81	12.56	0.00	0.00	0.22	0.00	0.00	0.00	0.00	253....		0.00	0.00
Viol...	0.09	1.46	0.00	0.19	0.02	0.00	0.59	0.00	0.00	38.29	0.00		0.00
Viol...	0.08	0.28	0.00	6.31	10.17	0.00	4.31	0.00	0.00	8.60	0.00	0.00	

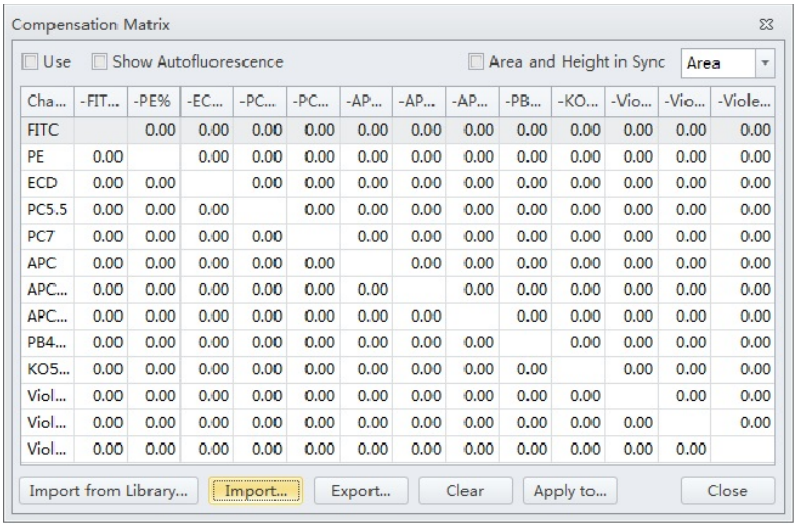
Save to Compensation Library... | Save As... | Close

8. 點擊Save to Compensation Library，給予檔名後點擊OK。

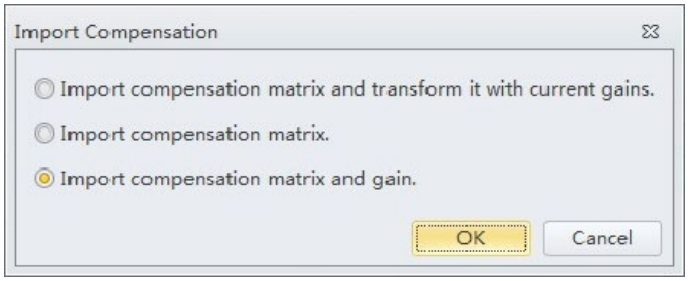


9. 點擊Save As，儲存此Compensation Matrix，此Matrix可以套用於其後相同染色的 Experiment，例如以下例子。

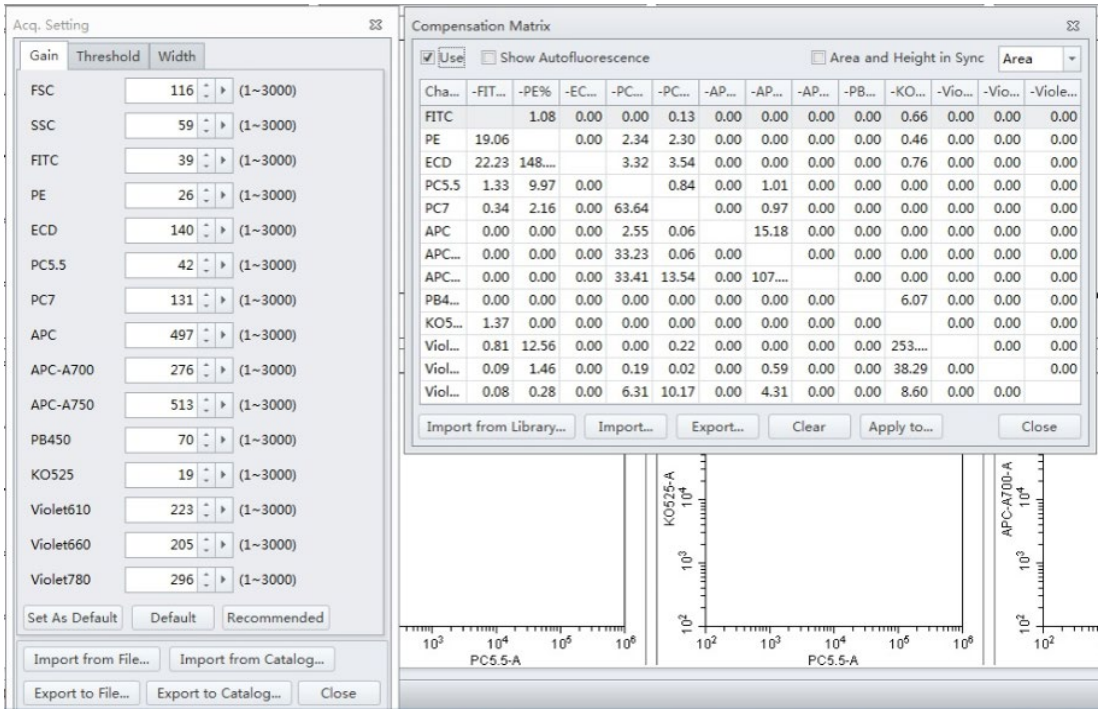
10. New Experiment，在Tube表格上的工具列點擊Compensation Matrix  後，再點擊Import。



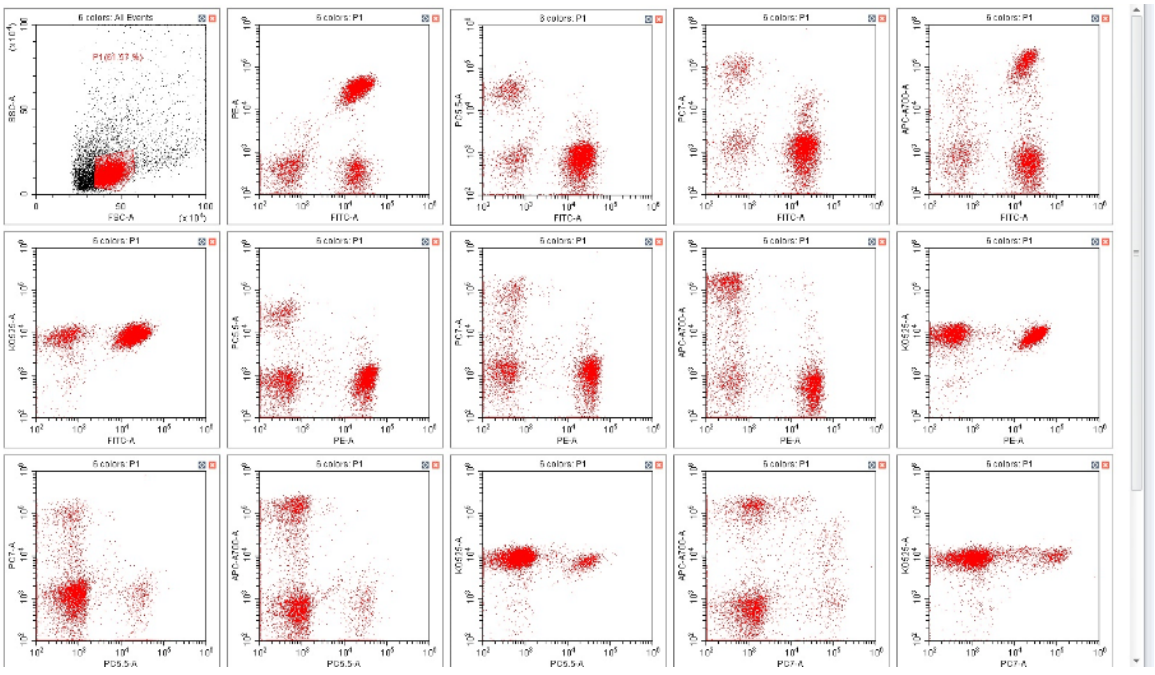
11. 選擇已經儲存的Compensation Matrix，勾選”Import compensation matrix and gain”，再點擊OK。



12. 先針對這個Experiment畫圖(Dot Plots、Histogram)再跑樣品，此實驗會使用這個Compensation Matrix所設定的電壓(Gain)及螢光補償值跑樣品。



13. 所得結果如下：

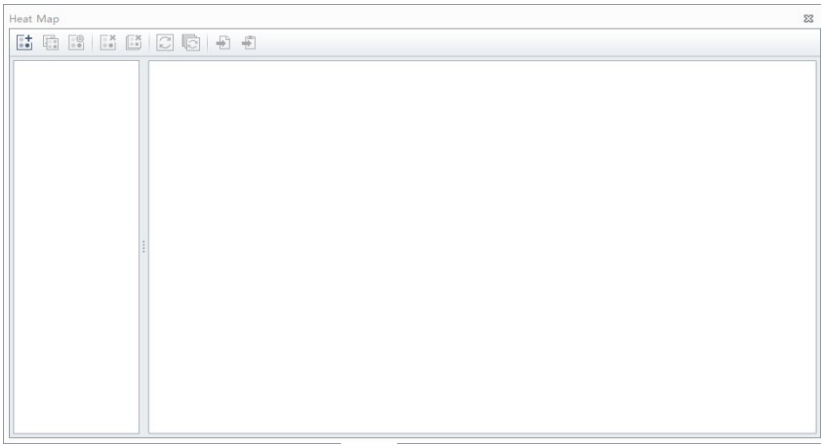


Heat Map功能設定

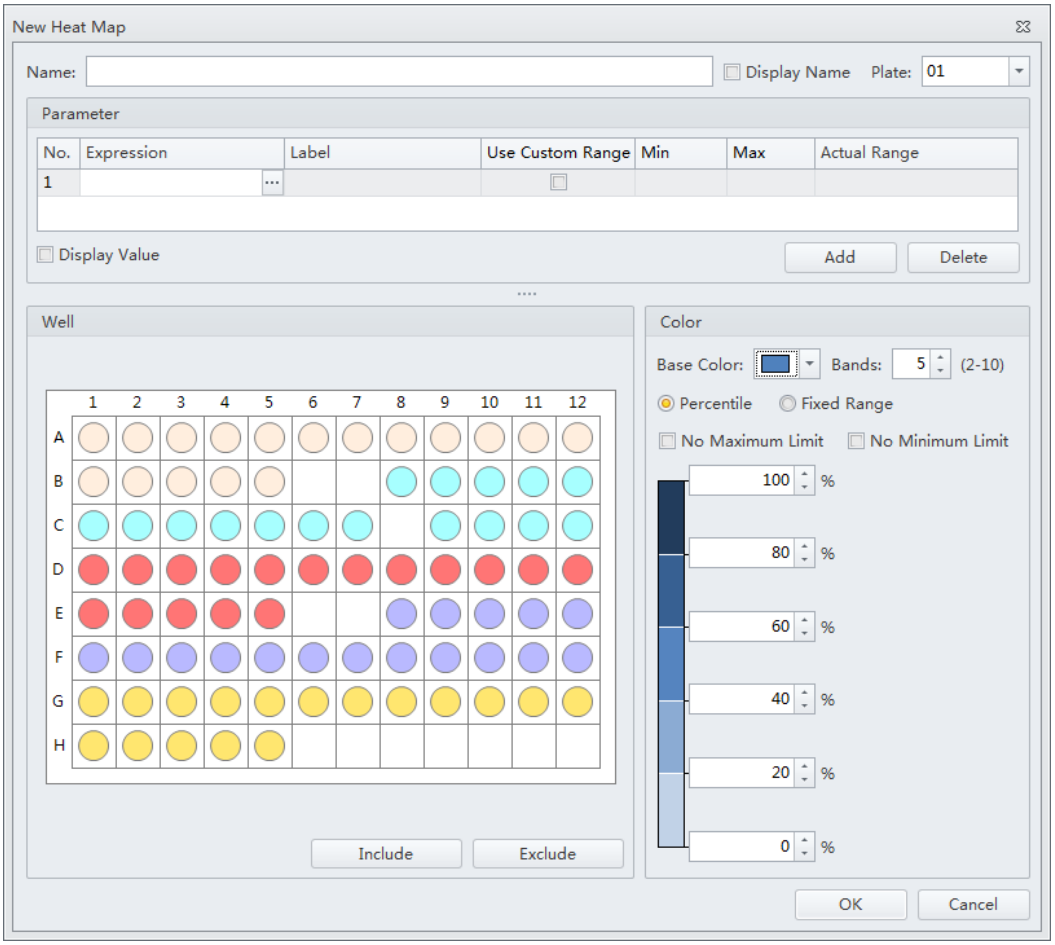
✧ 欲開啟Heat Map功能，需有一組含有至少2 wells以上盤式數據：

➢ 操作步驟：

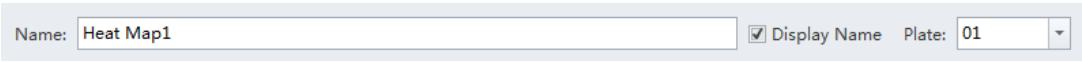
a. 在已知盤式數據的Experiment中，點選Heat Map 開啟Heat Map Window。



b. 點選New Heat Map 開啟新Heat Map設定視窗。

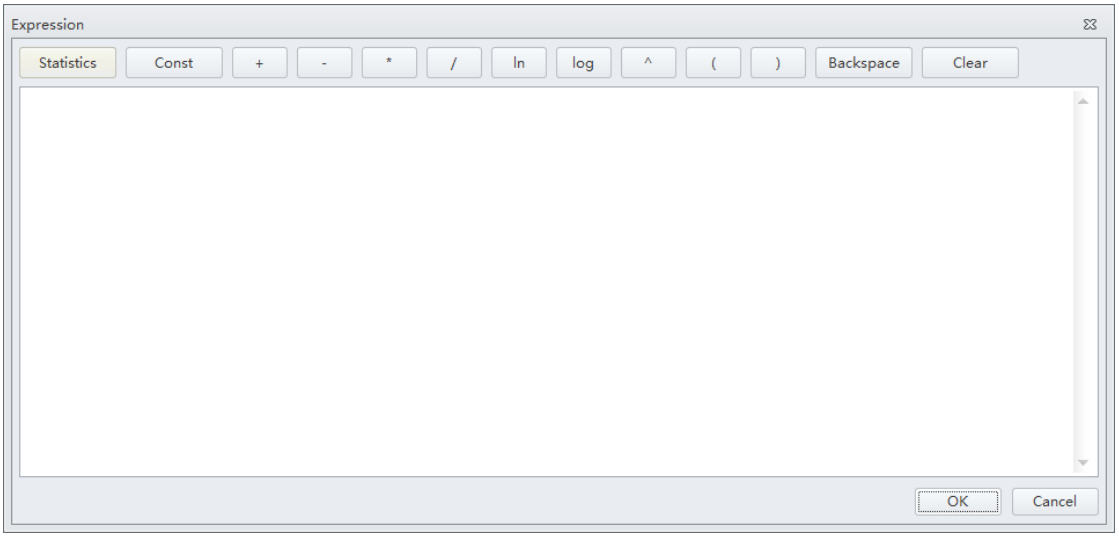


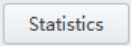
c. 輸入欲命名Heat Map名稱：

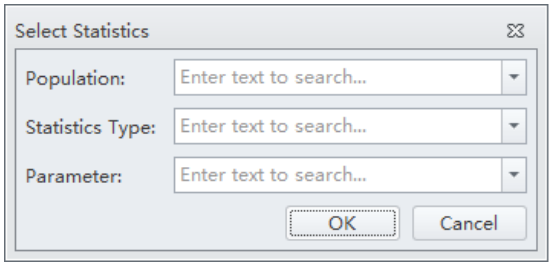


d. 點選Add  新增欲分析的參數欄位(最多可選擇6組參數)：

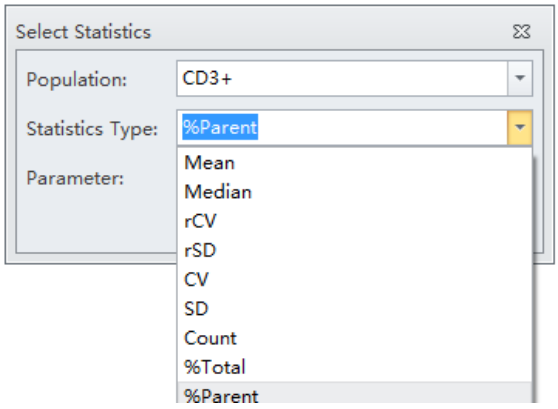
1. 點選  ，顯示欲表現參數數據視窗：



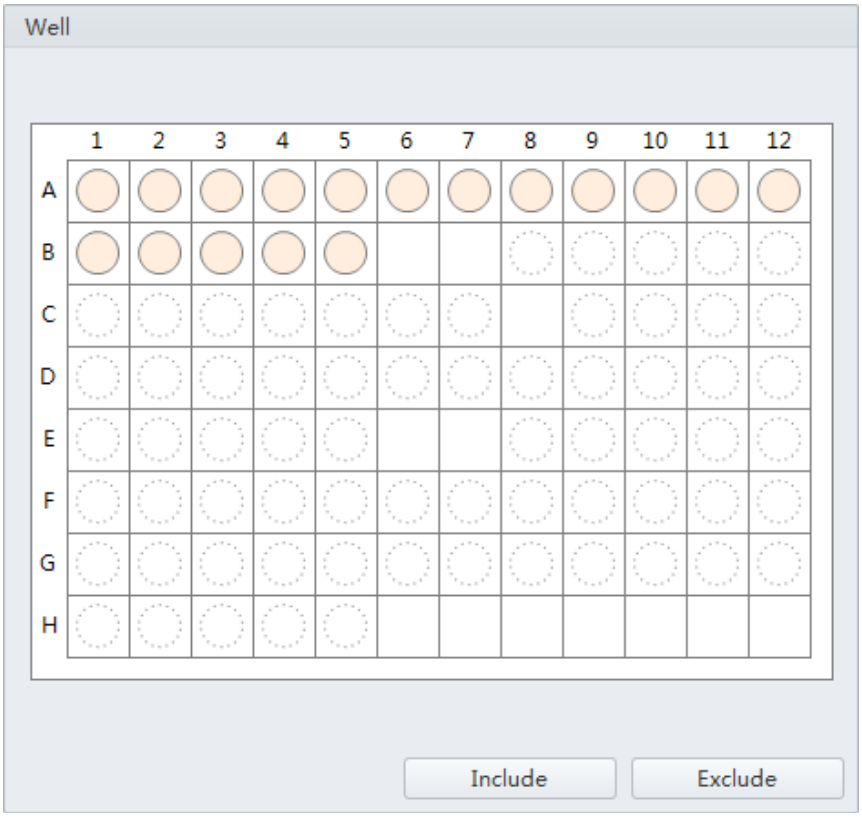
2. 點選Statistic  ，選擇欲分析的參數數據後，點選OK：



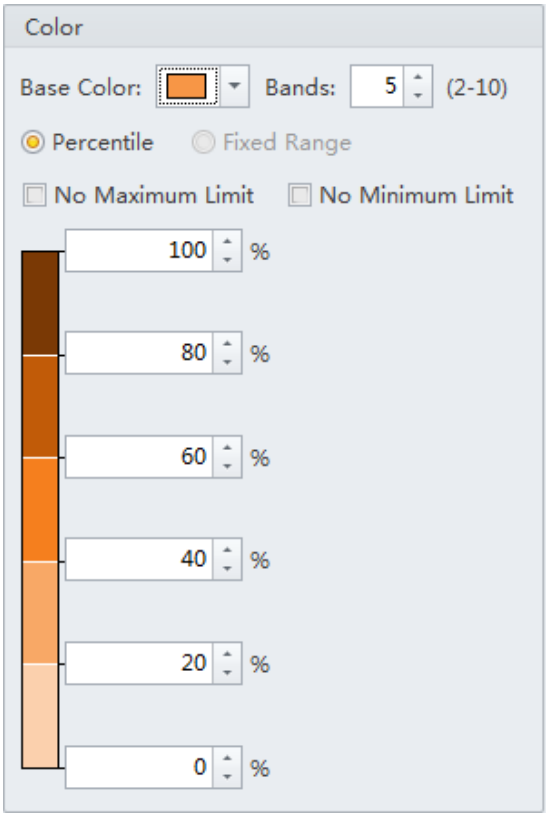
- **Population**：選擇分析族群
- **Statistic Type**：選擇統計數值
- **Parameter**：選擇統計參數



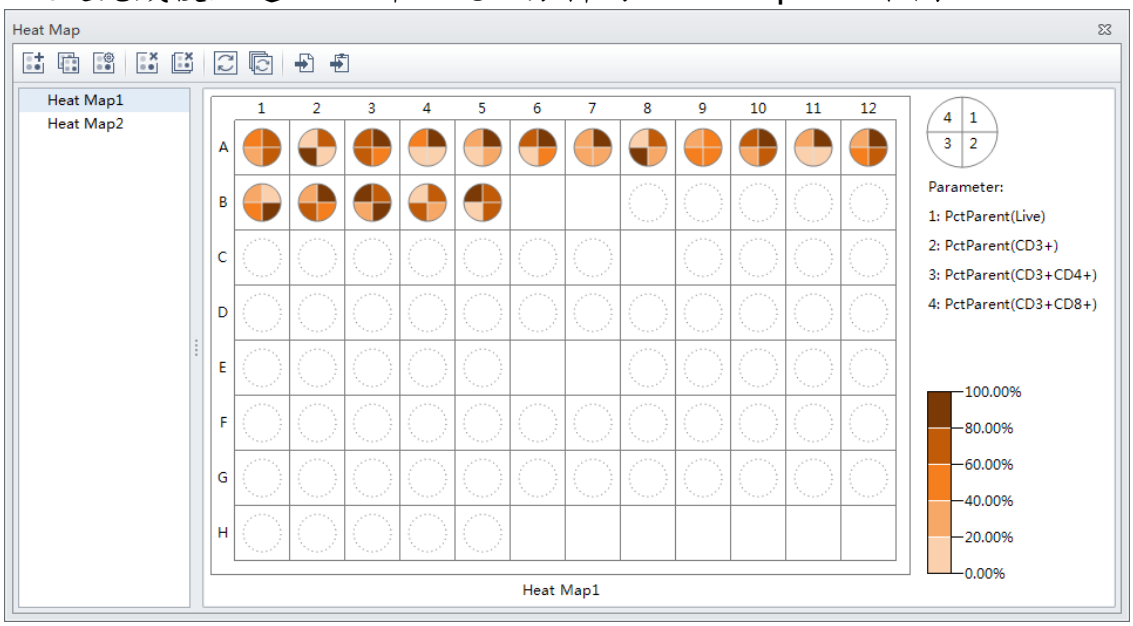
e. 選擇欲分析的well個數，可點選Include或Exclude增減well數：



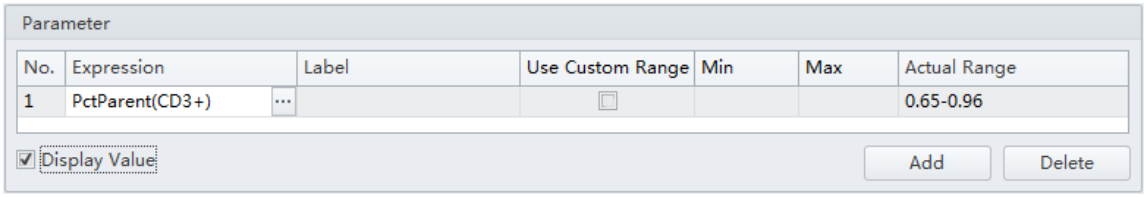
f. 選擇Heat Map顯示的顏色及色澤百分比表現：



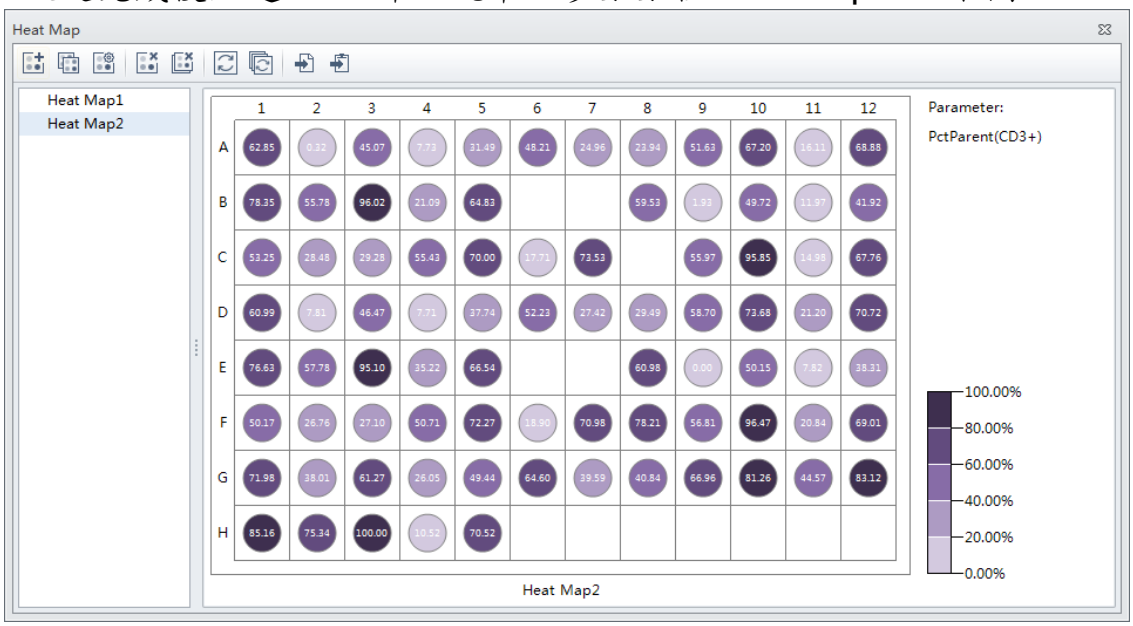
g. 設定完成後點選OK，即呈現欲分析的Heat Map，如下圖：



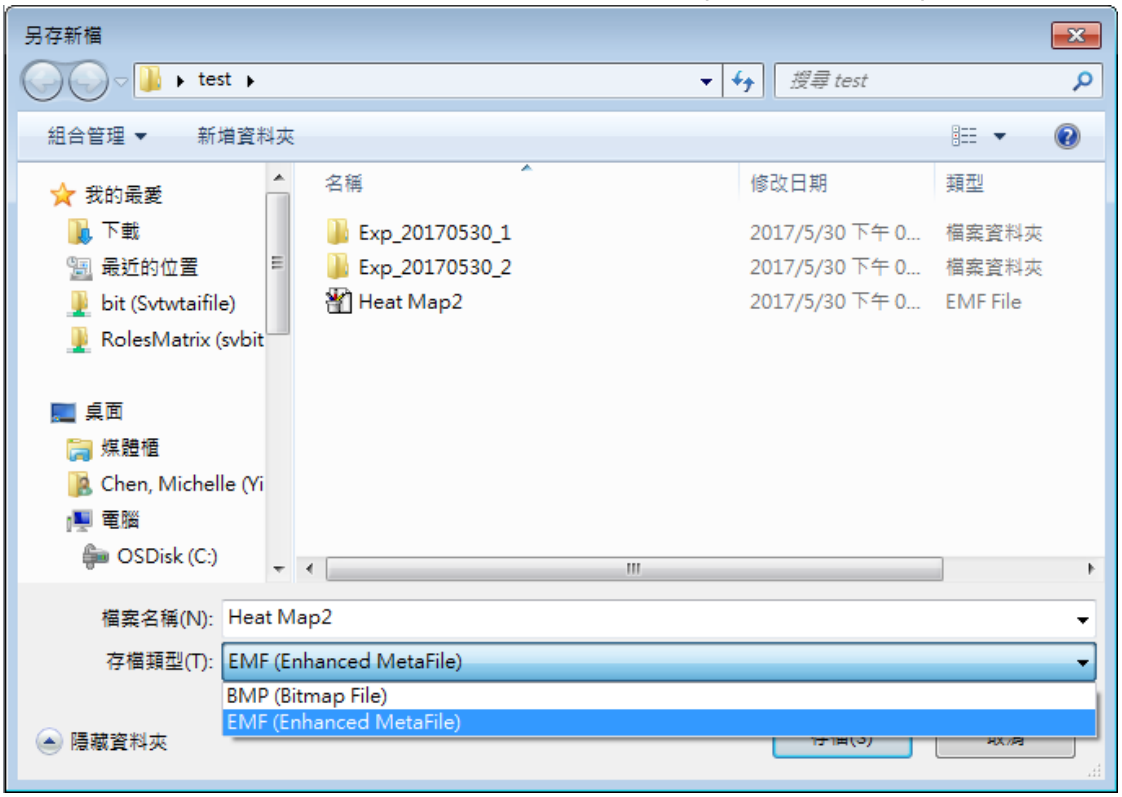
h. 若欲直接呈現Heat Map單一參數數值，選擇欲分析的單一參數後，勾選Display Value。



i. 設定完成後點選OK，即呈現單一參數數值Heat Map，如下圖：



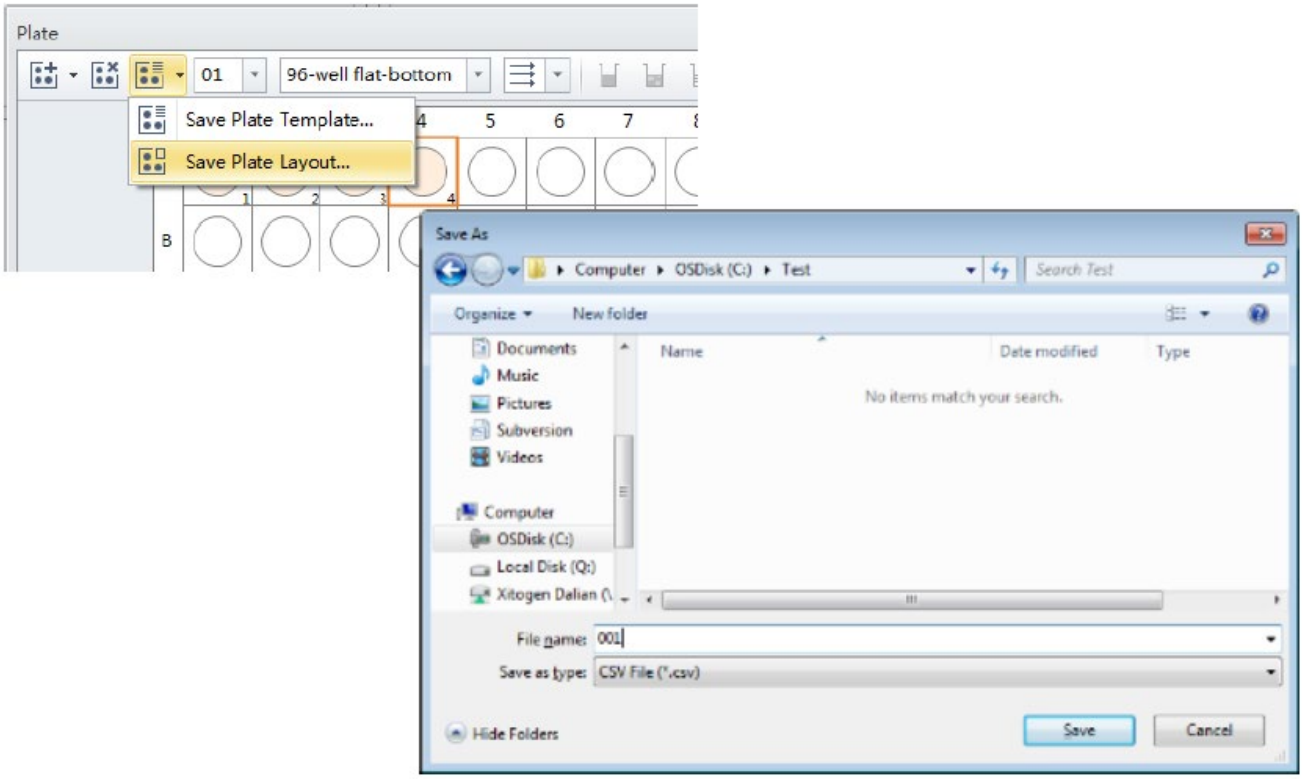
j. 將Heat Map輸出，點選Export to Graphic File 或Export to Clipboard ，可將Heat Map輸出成圖檔(.bmp or .emf)。



輸出和輸入樣品資訊

輸出

a. 點擊Save Plate Layout...並選擇檔案存取路徑，例如：001.csv。



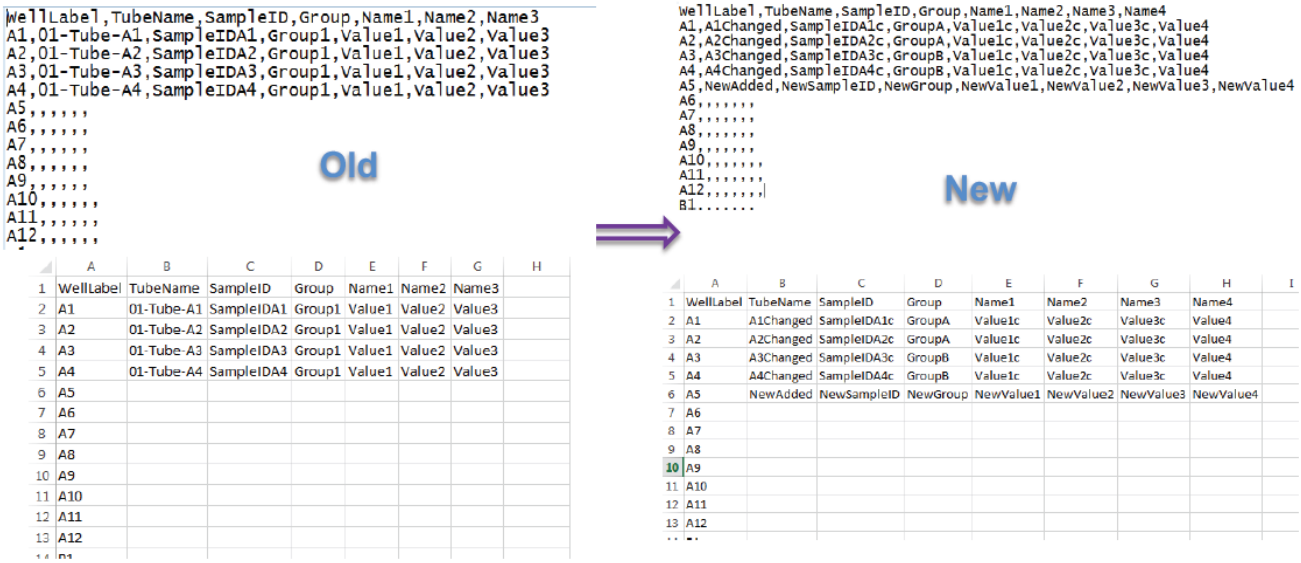
b. 使用記事本或Excel來開啟001.csv檔進行檢視。

```
WellLabel,TubeName,SampleID,Group,Name1,Name2,Name3
A1,01-Tube-A1,SampleIDA1,Group1,Value1,Value2,Value3
A2,01-Tube-A2,SampleIDA2,Group1,Value1,Value2,Value3
A3,01-Tube-A3,SampleIDA3,Group1,Value1,Value2,Value3
A4,01-Tube-A4,SampleIDA4,Group1,Value1,Value2,Value3
A5,,,,,,
A6,,,,,,
A7,,,,,,
A8,,,,,,
A9,,,,,,
A10,,,,,,
A11,,,,,,
A12,,,,,,
```

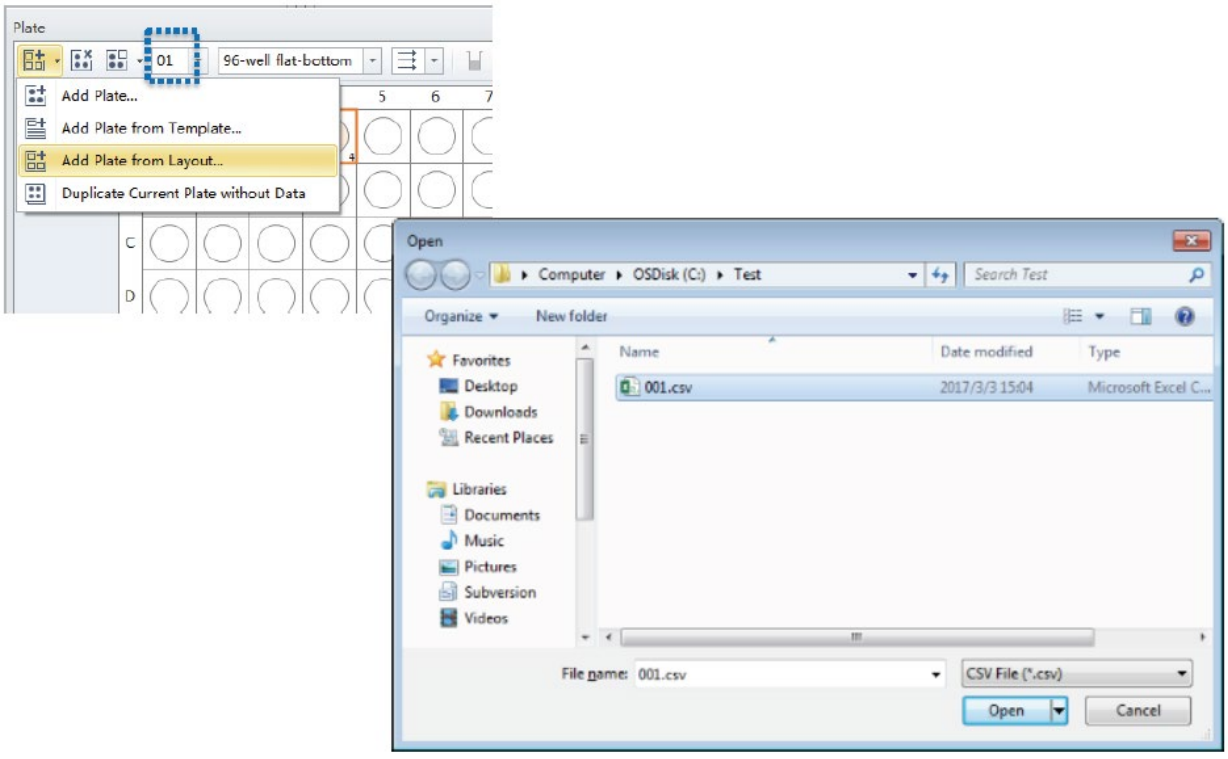
	A	B	C	D	E	F	G	H
1	WellLabel	TubeName	SampleID	Group	Name1	Name2	Name3	
2	A1	01-Tube-A1	SampleIDA1	Group1	Value1	Value2	Value3	
3	A2	01-Tube-A2	SampleIDA2	Group1	Value1	Value2	Value3	
4	A3	01-Tube-A3	SampleIDA3	Group1	Value1	Value2	Value3	
5	A4	01-Tube-A4	SampleIDA4	Group1	Value1	Value2	Value3	
6	A5							
7	A6							
8	A7							
9	A8							
10	A9							
11	A10							
12	A11							
13	A12							

輸入

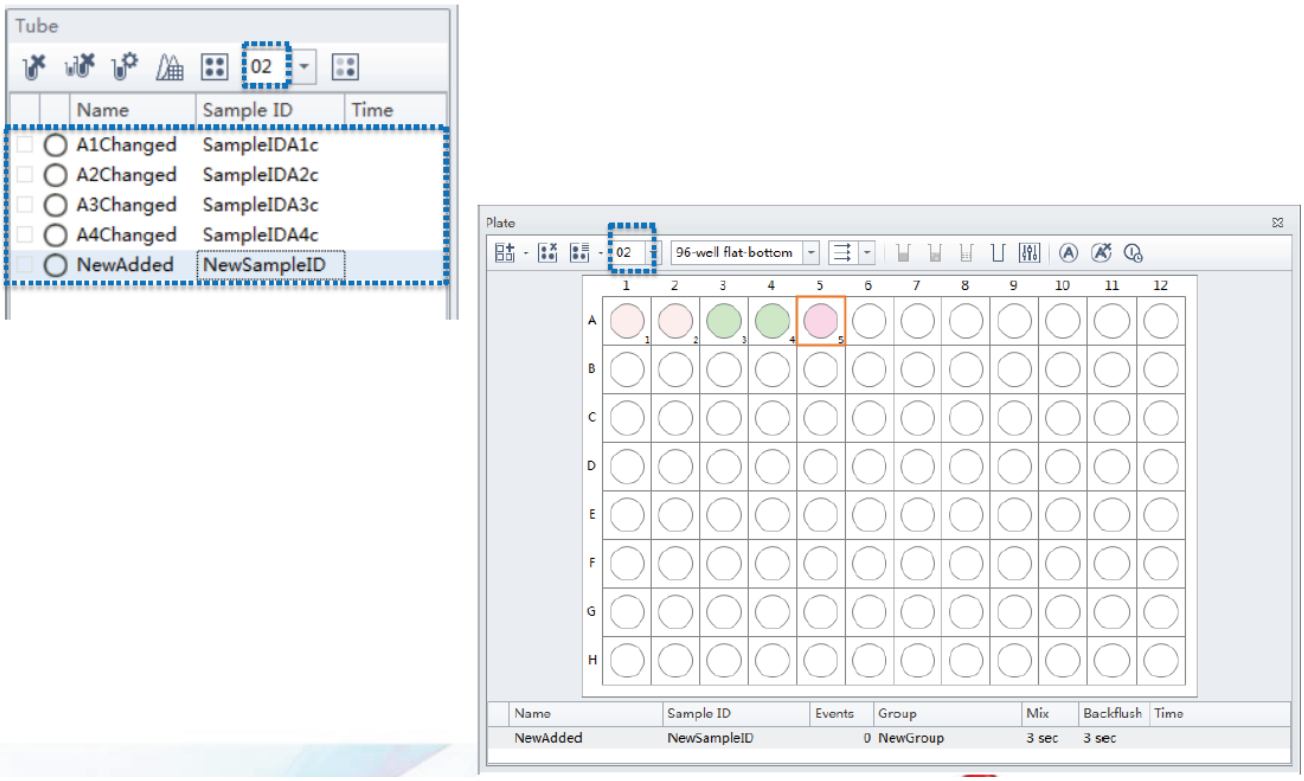
a. 使用記事本或Excel來開啟csv檔進行以下資訊編輯：
   例如：001.csv
   1. 更改Tube name
   2. 更改Sample ID和Group
   3. 更改Custom metadata
   4. 在A5位置增加一個新樣品
   5. 增加新的Custom metadata，例如：Name4



b. 點擊Add Plate from Layout…，選擇已修改過的001.csv檔並點選Open。



c. 軟體會依據001.csv檔於New Plate中呈現新的樣品資訊。



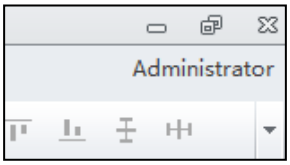
八、建立新的使用者

※開啟**[User Management 功能]**

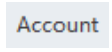
在 CytExpert 軟體中，使用者可以設定自己專屬的帳號，藉由：

1. 點選桌面 CytExpert 軟體 

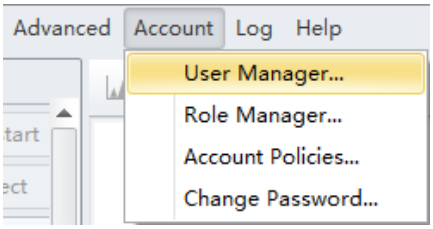
。
2. 輸入程式管理員"**Admin**"，接著在 Password 欄位輸入密碼"**password1234**"後，按下  繼續進入管理員模式 Administrator。



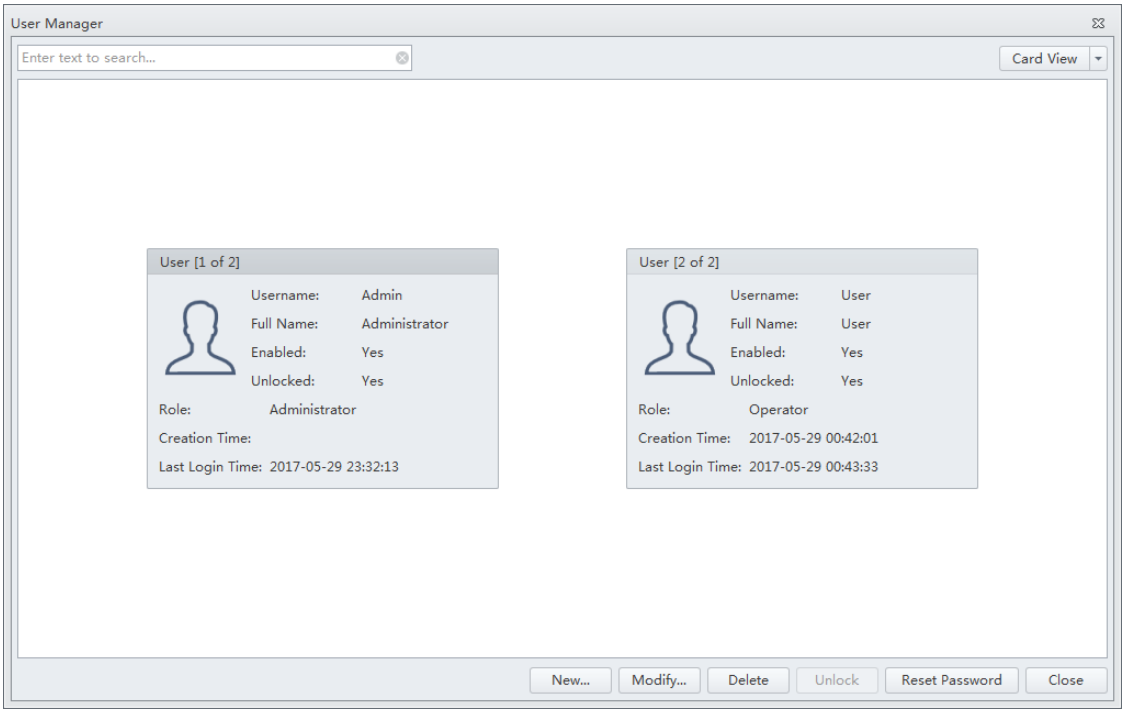
軟體右上方會呈現 Administrator 模式

3. 點選左上 Menu 中的 **Account** 

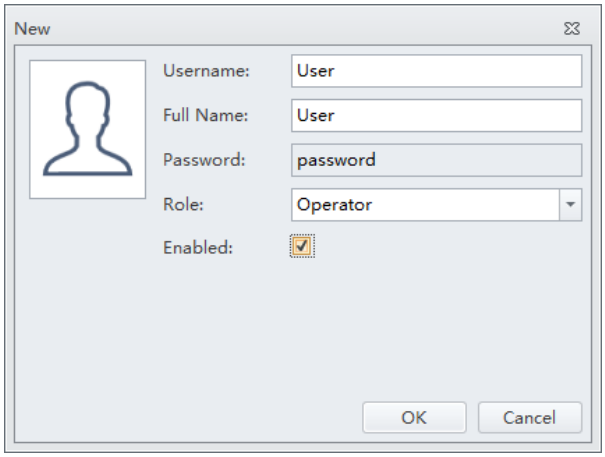
>User Manager，出現以下畫面：



- **New**：新增一個使用者
- **Modify**：修改已經存在的使用者
- **Delete**：刪除一個使用者
- **Unlock**：將系統鎖住的使用者解鎖
- **Reset Password**：重新設定使用者密碼

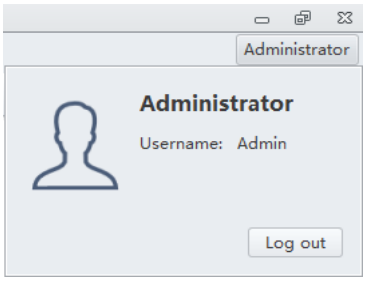


4. 點選”New”按鍵，可看到以下畫面：

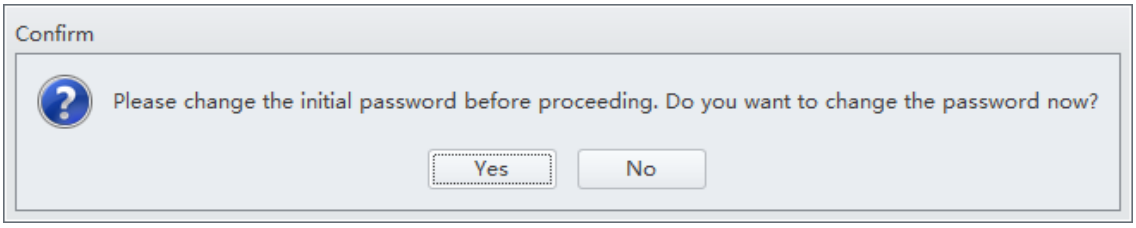


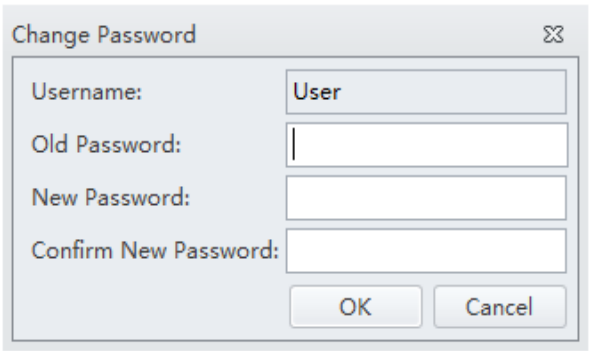
- **Username**：在此輸入使用者名稱
- **Full Name**：在此輸入使用者名稱 (可相同)
- **Password**：系統預設為 password
- **Role**：選擇使用者 Operator
- **Enabled**：勾選，選擇啟用此新使用者

5. 點選 OK 後，點選 Close。
6. 選擇軟體右上方 Administrator，選擇 Log out 登出，輸入**新使用者名稱**及系統預設密碼”**password**”，按下 ▶ 繼續。



7. 點選 Yes 確認變更使用者密碼，輸入 **Old Password :** 系統預設密碼”**password**”，設定新密碼”**必須含有英文及數字**”，確認後點選 OK 完成。

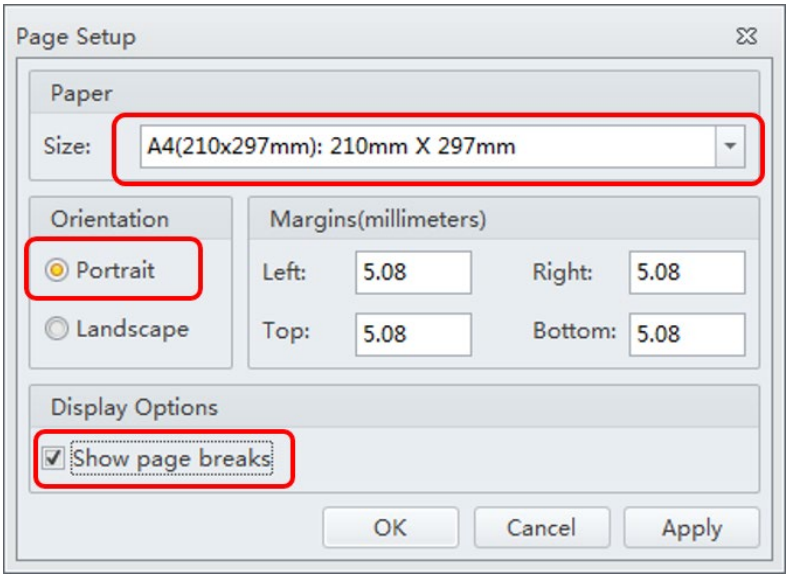




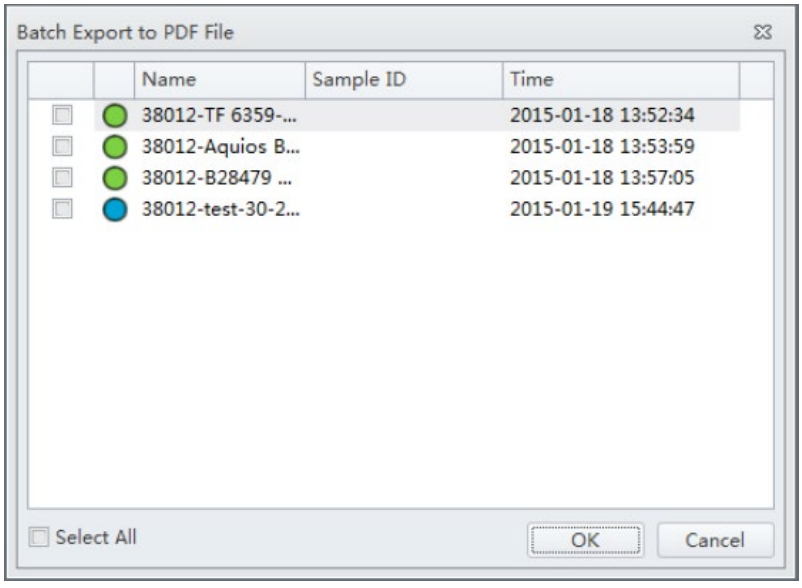
九、數據輸出

A. 轉換成PDF檔案格式

1. 實驗結果分析完成後可點擊Print 右側箭頭中的Page Setup ，Size可選擇A4頁面輸出方式，點選Portrait以直式方式輸出，並勾選Show page breaks，使繪圖區顯示A4大小頁面排列。

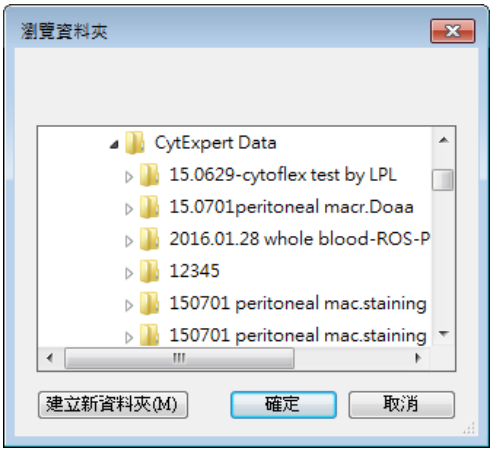


2. 於繪圖區排列好欲輸出圖形格式，再點擊Batch Export to PDF File ，勾選欲輸出的樣品管後，點選OK。



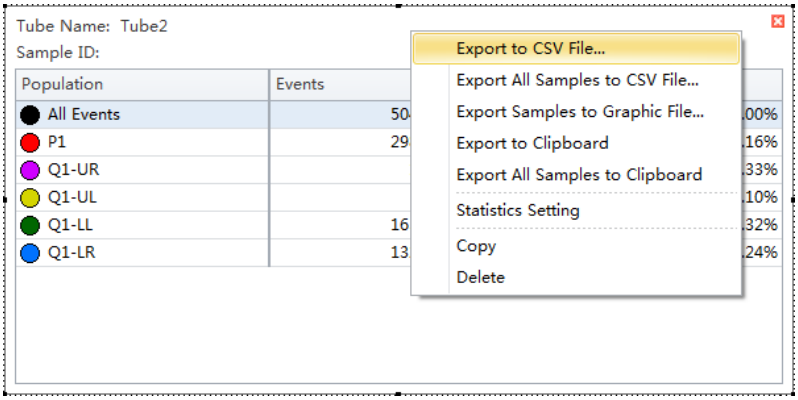
(※如欲輸出多檔案可勾選多筆樣品資料，但檔案輸出會以單一筆樣品數據為一個PDF File)

3. 選擇欲儲存的資料夾路徑後，點選確定，則檔案會依路徑自動儲存成PDF File。 

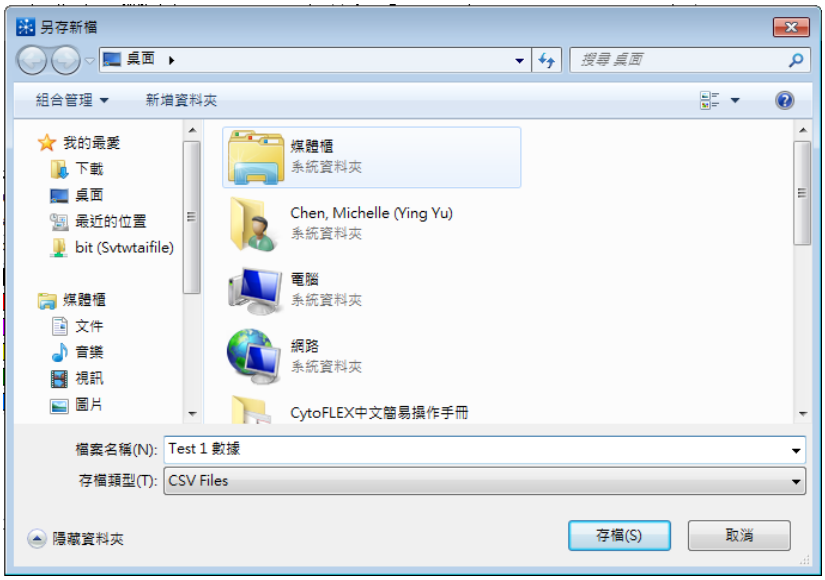


B. 數據輸出CSV檔案格式

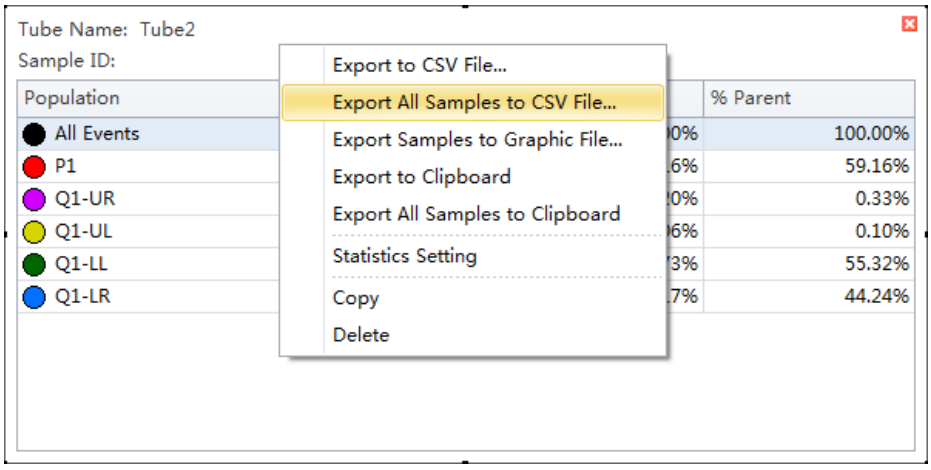
1. 於實驗結果分析完成的統計數值表Statistics，點選滑鼠右鍵，選擇Export to CSV File。



2. 選擇存檔名稱及路徑位置，點選存檔，則檔案會依路徑自動儲存成單一個CSV File。 



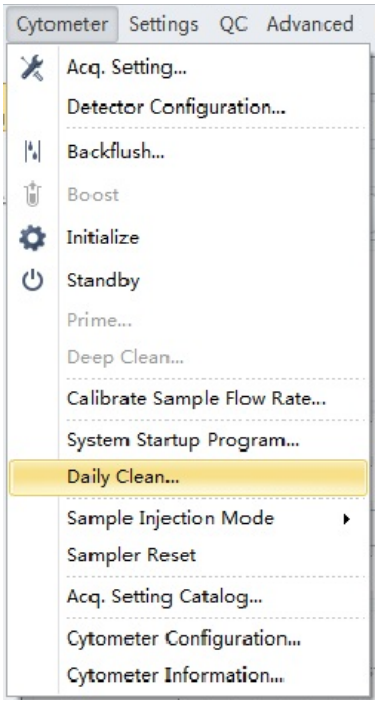
(※如欲輸出多筆樣品檔案可選擇Export All Samples to CSV File，檔案輸出會以多筆樣品數據為一個CSV File)



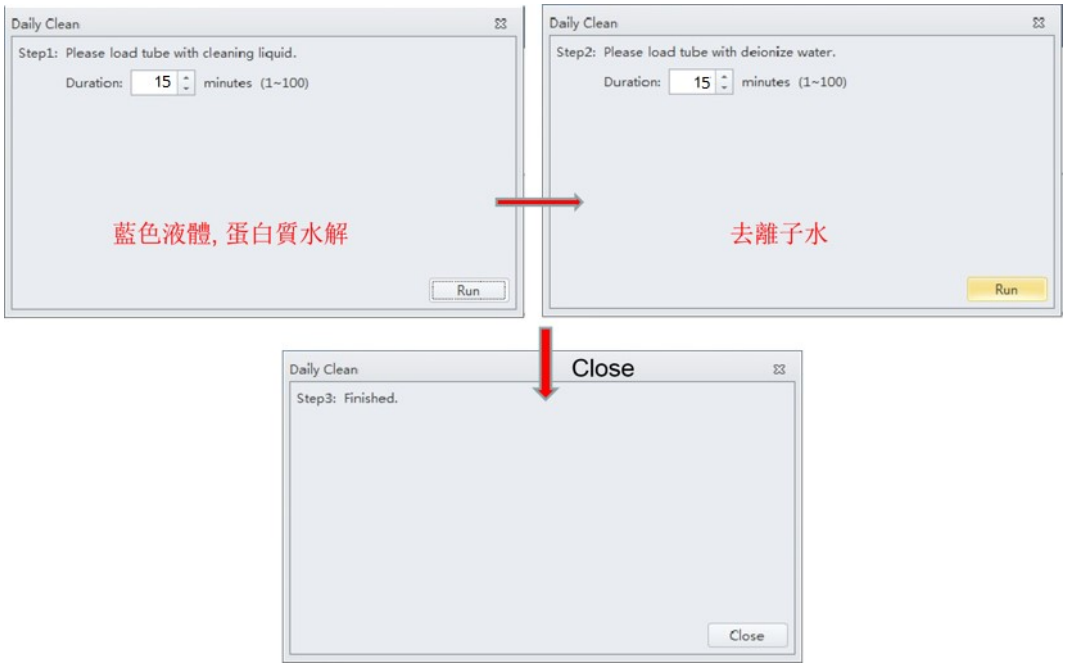
十、關機流程

[Semi-Automatic sample模式]

1. 在收取Data的Experiment裡，新增1 tube，上1管2 mL 10%漂白水以最高流速240 µL/min進行上樣，沖洗5分鐘
2. 執行Daily Clean。由左上方Cytometer進入，點擊Daily Clean。



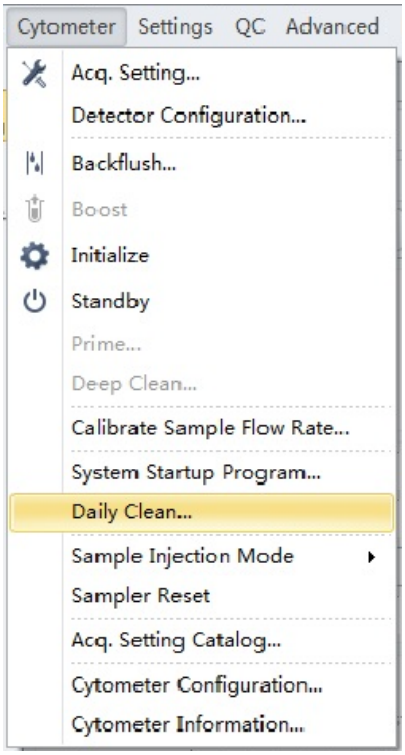
3. 先洗1管FlowClean Cleaning Agent (3 mL蛋白質水解酵素，藍色液體)，15分鐘；再洗1管去離子水，15分鐘。點擊Close。



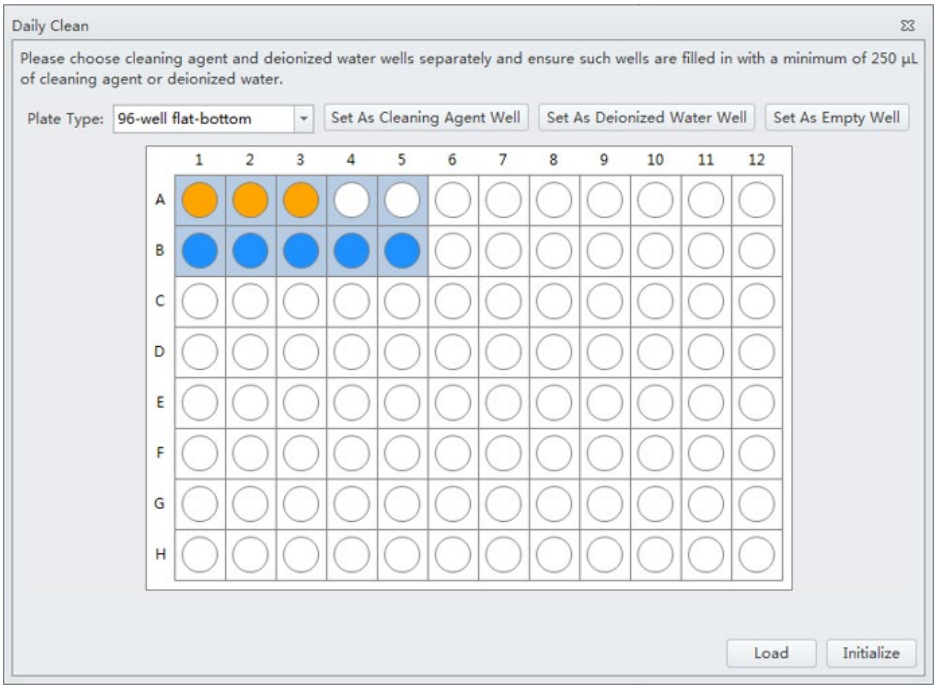
4. 關閉CytExpert軟體，放置樣品試管架會自動收回機器內，關閉儀器左後方的電源鍵即完成關機。

[Plate Loader模式]

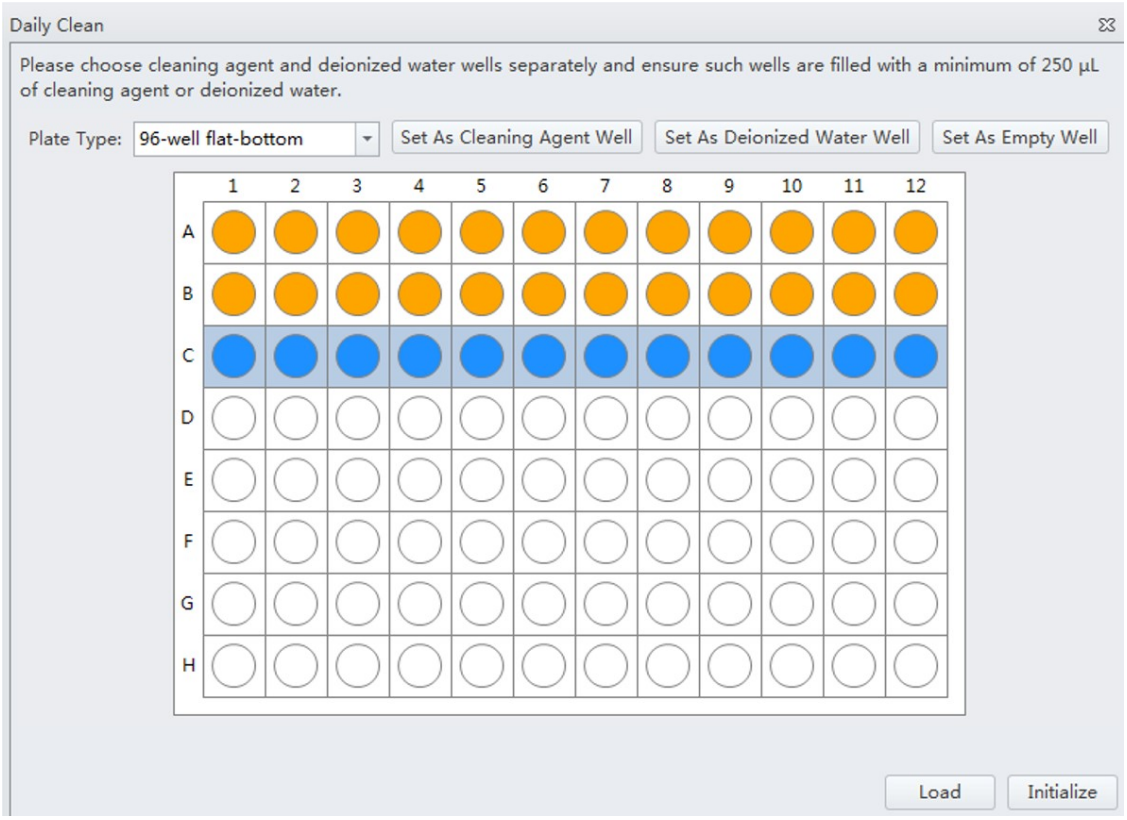
1. 執行Daily Clean。由左上方Cytometer進入，點擊Daily Clean。



2. 放上6 wells (250 µL/well) 10%漂白水、12 wells (250 µL/well) FlowClean Cleaning Agent及12 wells ((250 µL/well)去離子水，選擇盤子形式，將儀器原始設定10 wells點選 Set As Empty Well 清空well。



再將6 wells 10%漂白水及12 wells FlowClean Cleaning Agent設定成 Set As Cleaning Agent Well，12 wells去離子水設定成 Set As Deionized Water Well 點擊Load及Start，進行清洗步驟，點擊Close。

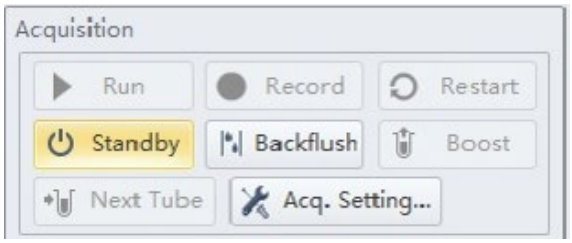


3. 關閉CytExpert軟體，關閉儀器左後方的電源鍵即完成關機。

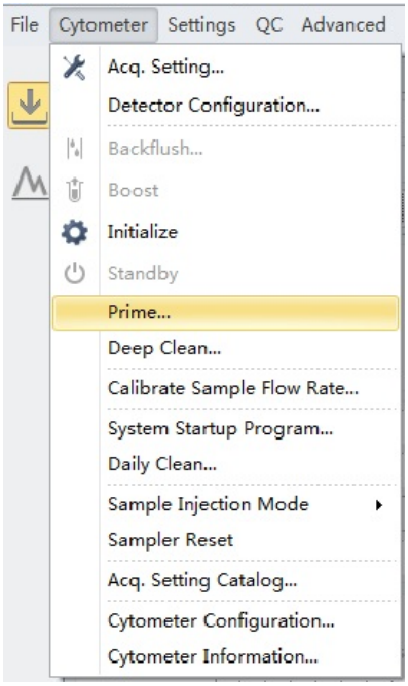
十一、 簡易故障排除

1. Prime：當機器在收集樣品數據時，如果收集的速度越來越慢，可能有堵塞情況，此時請執行 Prime。

(1)先點選 Acquisition 之中的指令 Standby。



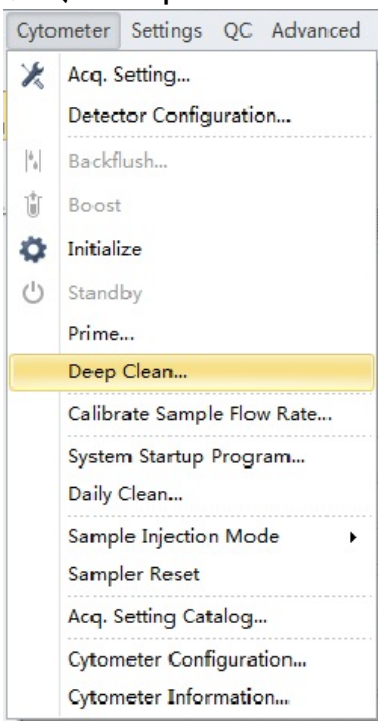
(2)由 Cytometer 進入，點擊 Prime。



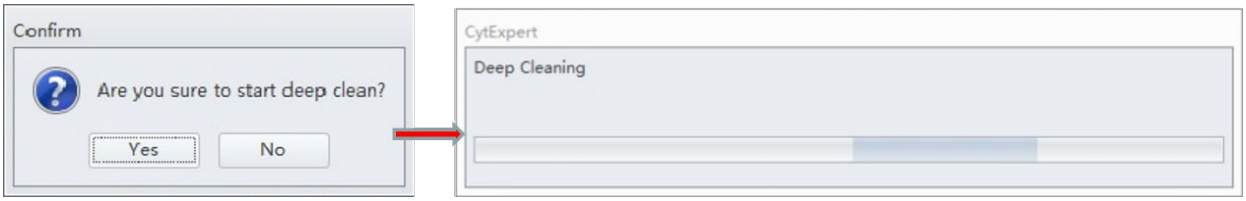
(3)點擊 Yes 即開始執行 Prime。

2. Deep Clean：發現機器嚴重塞管時，執行 Prime 也無法排除，請執行 Deep Clean。

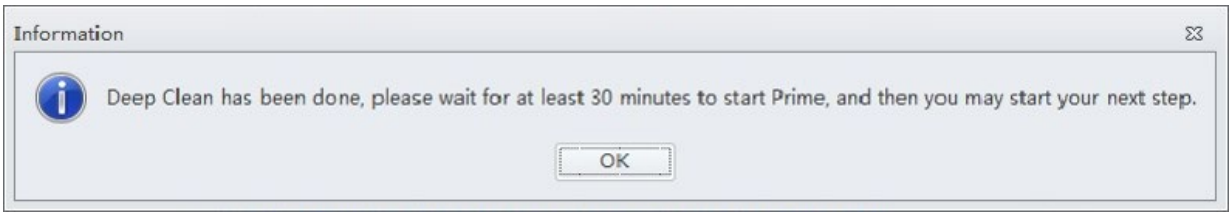
(1)由 Cytometer 進入，點擊 Deep Clean



(2)點擊 Yes 後即以 Contrad 70 Cleaning Solution (diluted KOH)清洗管路及 Flow Cell。



(3)浸泡至少 30 分鐘後再執行 Prime，完成 Deep Clean 動作。



附錄1： Surface Marker樣品之製備及染色

《方法一》取白血球再染色

1. 取得含抗凝劑(EDTA or HEPARIN)的血，放置室溫。
2. 用等量的 PBS 和 Blood 混合來稀釋 (最大稀釋比 PBS:Blood = 2 : 1)
3. 取 3 c.c Ficoll-Hypaque(density 1.077) 放入 15 mL tube
   (or 取 12 c.c Ficoll-Hypaque 放入 50 mL tube)
4. 慢慢放入稀釋血 8mL (or 30mL)在 Ficoll-Hypaque 之上方，不要混淆到下層。
5. 小心 balance 後，離心 400g，25min，RT
6. 小心取得 mononuclear layer cells
7. 加入 2 倍量的 PBS 來稀釋 mononuclear layer cells
8. 200g，10min，去上清液
9. 重複 Step7、8
10. 計算細胞數 and resuspend (細胞濃度調整到接近 $1x10^7$cells/ mL)
11. 保存在 4℃，一直到使用
12. 取 100uL cells＋20uL mAb，Mix
13. Incubation 30 min
14. Resuspend to 500uL～1mL with PBS (含 0.5% paraformaldehyde)
15. 分析 within 6 hours

《方法二》直接全血染色法

1. 100ul whole blood＋20ul mAb.
2. Incubation **15-20 min**
3. 500ul OptiLyse C, Mix
4. incubation **10 min, RT**
5. 500ul PBS, Mix
6. After at least 10 min
7. 分析 on instrument

附錄2： 常用的Cell Cycle固定染色法（酒精固定法及PI染色）

方法一、

1. 置備懸浮細胞液，最後濃度調整約為 2×10^6 cells/ mL。若細胞株為 Attached cell Line，先以 Trypsin 將細胞打下，再調整濃度（注意 Trypsin 處理不可過頭）。
2. 取 1 mL 細胞液，以冰冷的 PBS 清洗細胞一次，離心後，倒除上清液。
3. 以剩餘的上清液將細胞打散（必須確定細胞已完全打散）。
4. 在震盪器上（轉速不可開太快）一邊震盪一邊逐一滴入 3 mL 70% 冰冷的酒精，（注意觀察細胞，不要讓細胞發生 aggregate 現象）。
5. 置於–20℃冰箱中固定至少一小時（ 建議固定隔夜以上為佳 ）。
6. 染色前，將細胞從–20℃冰箱取出，300 g 離心 5 分鐘，去除上清液。
7. 以剩餘的上清液將細胞打散，加入 5 mL PBS，靜置 3 分鐘後離心，去除上清液。
8. 重複步驟 7，以 5 mL PBS 再清洗細胞一次。
9. 加入 1 mL PI / Triton X-100 (Final Conc. PI = 20 μg /mL, Triton-X 100 = 0.1%, RNase A = 0.2mg/ml)，均勻打散細胞，避光染色至少 30 分鐘。
10. 上機前打散細胞並以 30~40 μm 尼龍篩網過濾細胞樣本即可上機進行分析。

方法二、

Materials :

1. Propidium iodide (sigma) : 10 mg/ml in water, store at 4 degrees.

   Note : solution made at RT will fall out of solution in fridge. No problem. Just mix it up and squirt the cloud into the staining mix. It will go into solution there.
2. RNaseA (Sigma), 10 mg/ml in water, store at 4 degrees (long term 20 degrees)
3. PBS : at 4 degrees
4. Ethanol (100%, store at -20 degress)

Fixation :

(1) Spin cells out of media – 1500 rpm x 5 minutes

(2) Wash once with PBS

(3) Collect 2 x 10^6 cells.

(4) Pellet cells by spinning at 1500 rpm, 4℃ for 5 minutes.

(5) Resuspend cell pellet in 300ul of cold PBS. Vortex.

(6) Fix cells by adding 700ul of -20℃ absolute ethanol. (drop by drop initially)

(7) Store cells at -20℃ in this fixation buffer until ready for analysis. (No more than 2 weeks)

Staining :

(1) Centrifuge (as above) fixed cells and resuspend pellet in 1 ml of PBS.

(2) Add 100ul of 200 ug/ml DNase-free, RNase-A and incubate at 37℃ for 30 minutes.

(3) Add 100ul of 1 mg/ml propidium iodide (light sensitive) and incubate at room temperature for 5-10 minutes.

(4) Place samples in 12x75mm tubes and read on Flow cytometry

Reference :

Experimental Cell Research **207**, 142-151.

附錄3： CytoFLEX 常用耗材貨號

CytoFLEX 常用耗材	貨號
Flow tubes	2523749
Flow Clean (500ml)	A64669
Coulter Clenz (1L)	8448188
Coulter Clenz (5L)	8448222
CONTRAD® 70	81911
CytoFLEX Sheath Fluid	B51503
CytoFLEX Daily QC Fluorospheres	B53230