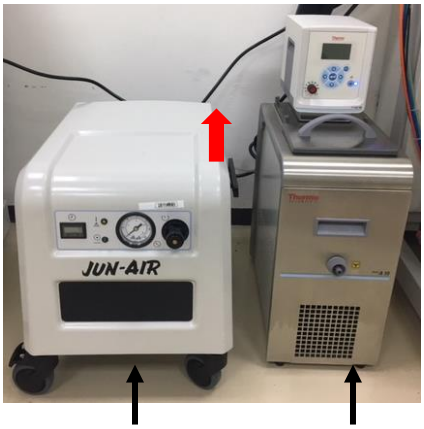
FACSMelody 細胞分選儀 操作流程

Check Fludics 確認液流

1. 確認 Sheath Tank 是否填滿 FACSheath Buffer (鋼瓶八分滿處)

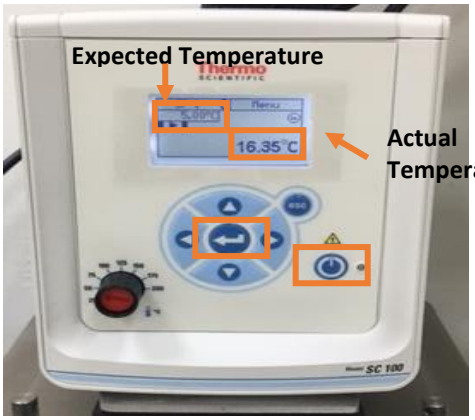
2. 確認 Waste Tank 是清空的，並裝 1L 漂白水

3. 若分選下來之細胞需要溫控，調整預期之溫度後，點擊中間箭頭紐，水浴槽即會開始調節溫度



Air Compressor　Water Bath

Waste Tank　Sheath Tank



Start Up System 儀器開機

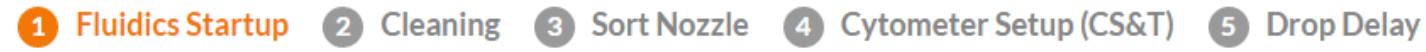
1. 開啟電腦及空氣壓縮機(開關於儀器右後方)，進入 windows 介面後，開啟 Tera Term 程式

2. 開啟 FACSMelody 儀器開關，待 Tera Term 和儀器連線完成，會顯示 **End of DataAcqBootUp**

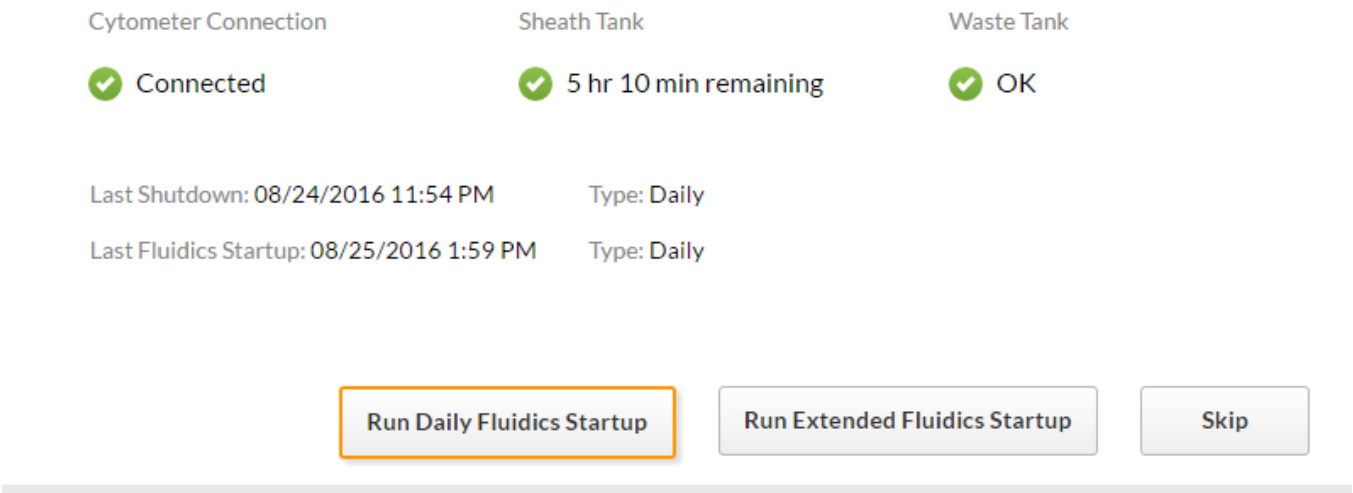
3. 開啟 FACSChorus 軟體，鍵入個人之帳號及密碼

4. 待 FACSChorus 軟體連線成功 Cytometer Connection 顯示 Connected

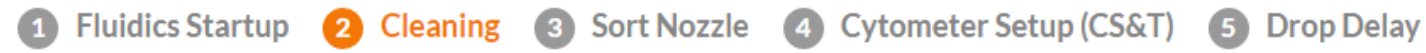
Fluidics Startup 液流啟動



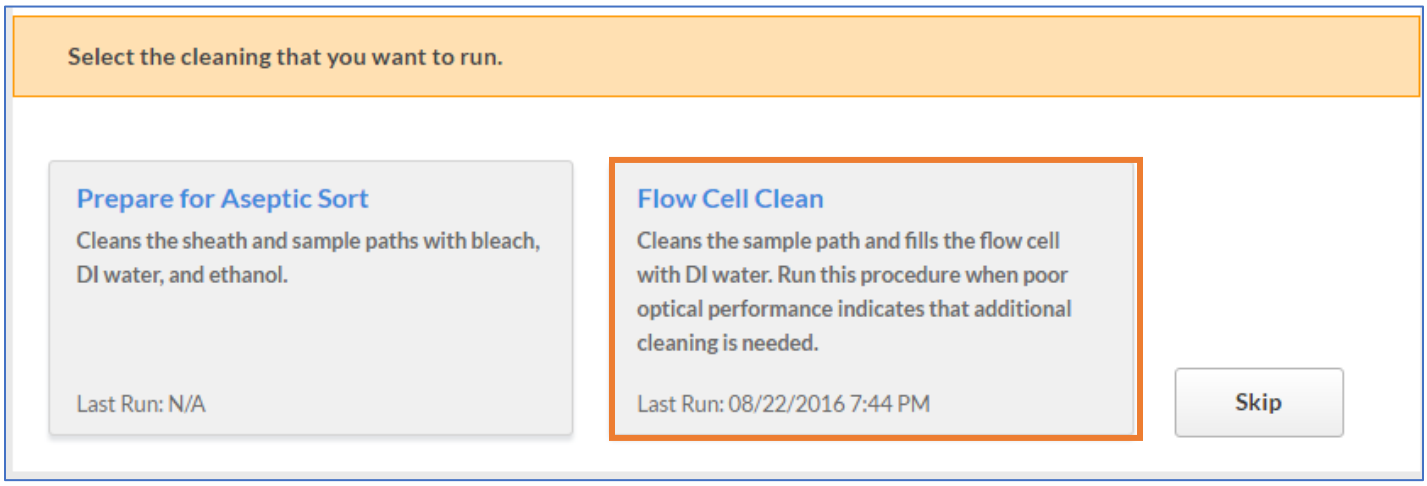
1. 執行 ① Fluidics Startup：執行 Run Daily Fluidics Startup



Cleaning 清洗



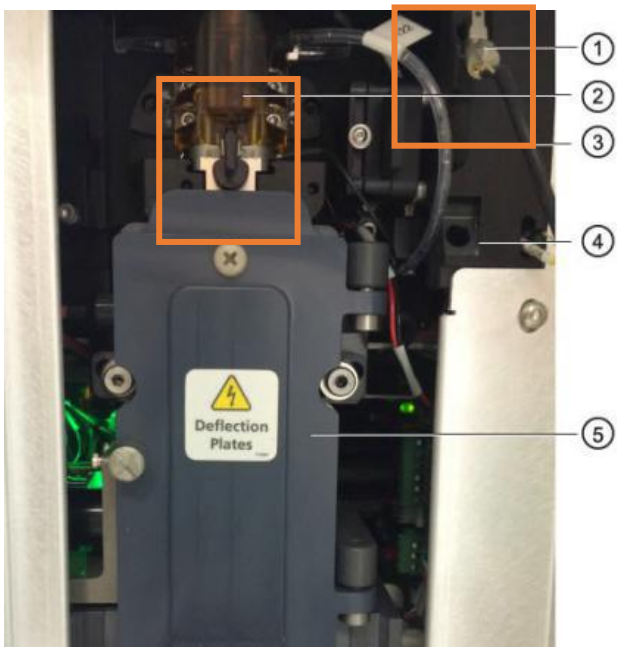
2. ② Cleaning：若是接續上一位使用者，請執行 Flow Cell Clean，否則請點選 Skip



Sort Nozzle 分選噴嘴

1 Fluidics Startup　2 Cleaning　3 Sort Nozzle　4 Cytometer Setup (CS&T)　5 Drop Delay

1. ③Sort Nozzle ：



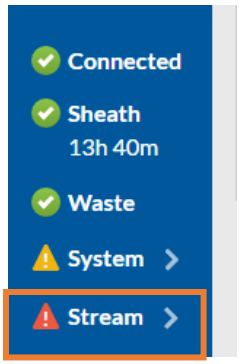
No.	Description
1	Nozzle holder with closed loop nozzle
2	Flow cell
3	Forward scatter detector with neutral density filter
4	Nozzle holder
5	Sort block

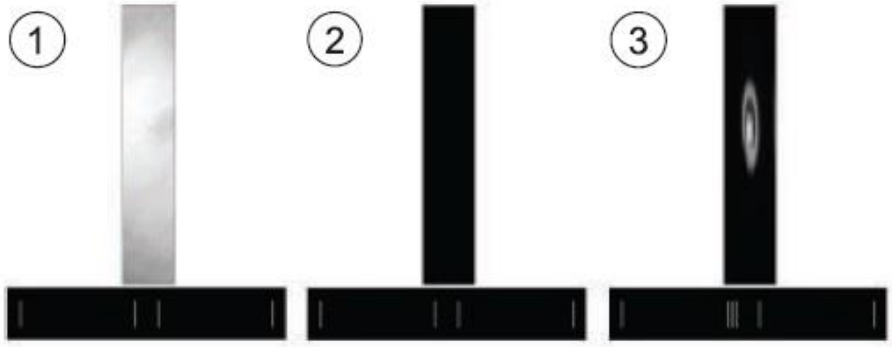
2. 將 Closed Loop Nozzle 移除，放入 Closed Loop Nozzle Holder，並將 100um nozzle 放置 Flow cell 下方

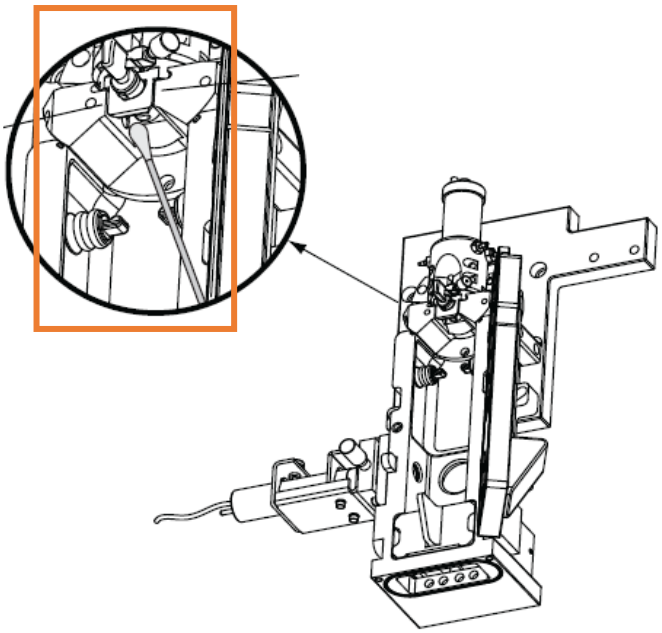
*重要: 請輕輕抽出或放置 Closed Loop Nozzle 及 100um Nozzle，並注意上方紅色 O ring 是否有脫落，若紅色 O ring 不見，請立即告知管理者)

3. 放上偏極板，放上偏極板前，請先確認偏極版上無鹽類結晶，可使用 ddH2O 清洗及使用拭淨紙擦拭

4.點擊左邊下方 Stream，確認 Stream 影像乾淨(如 1)，若影像不乾淨(如 2.3)，請用拭淨紙或棉棒擦拭鏡頭下方







5. 關上 Sort Block Door，點擊 Continue，即開啟 Stream

6. 確認 Stream 確實進入廢液槽中，若沒有以中心位置進入廢液槽(下圖 2 之紅色箭頭指示)中，可以利用六角起子將 Sort Block 左右之旋鈕(下圖 1 之紅色箭頭指示)轉鬆，將位置對好後即可轉緊

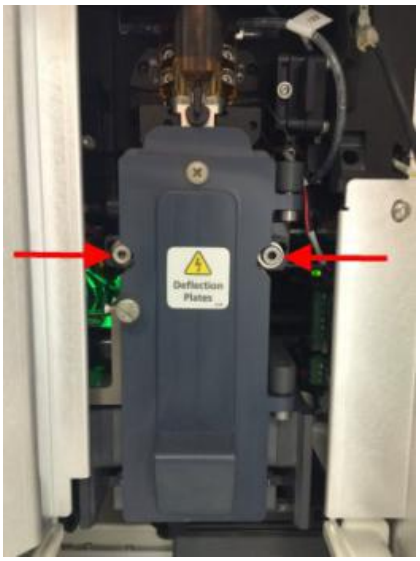


圖 1.

圖 2.

Cytometer Setup (CS&T) 儀器 QC

1 Fluidics Startup　2 Cleaning　3 Sort Nozzle　4 Cytometer Setup (CS&T)　5 Drop Delay

1. ④ Cytometer Setup (CS&T)：此步驟管理者操作，請點選 Skip

Drop Delay 分選 QC

1 Fluidics Startup　2 Cleaning　3 Sort Nozzle　4 Cytometer Setup (CS&T)　5 Drop Delay

1. ⑤ Drop Delay：放上 Accudrop Beads，500ul PBS + 1 drop Accudrop beads

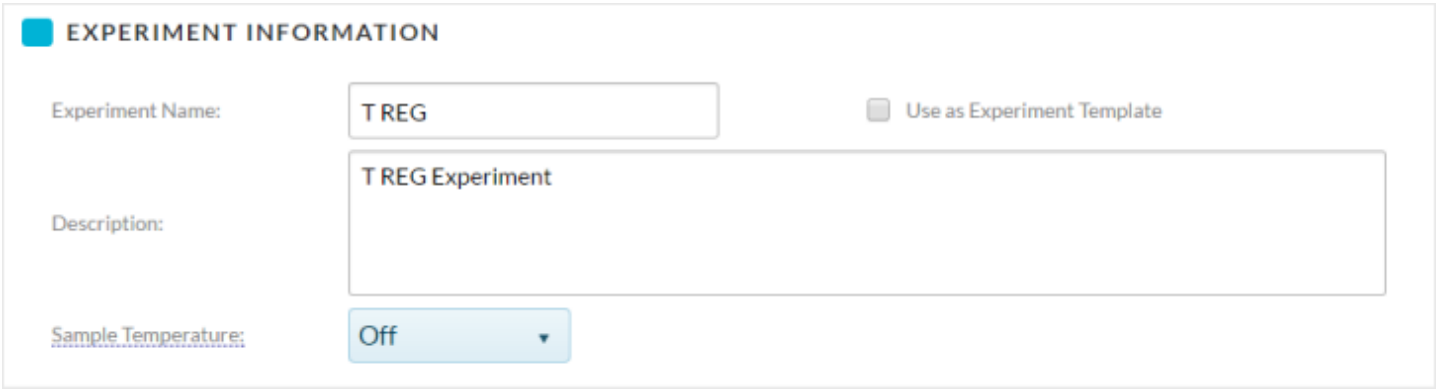
以上步驟完成，即可進入實驗設定部分

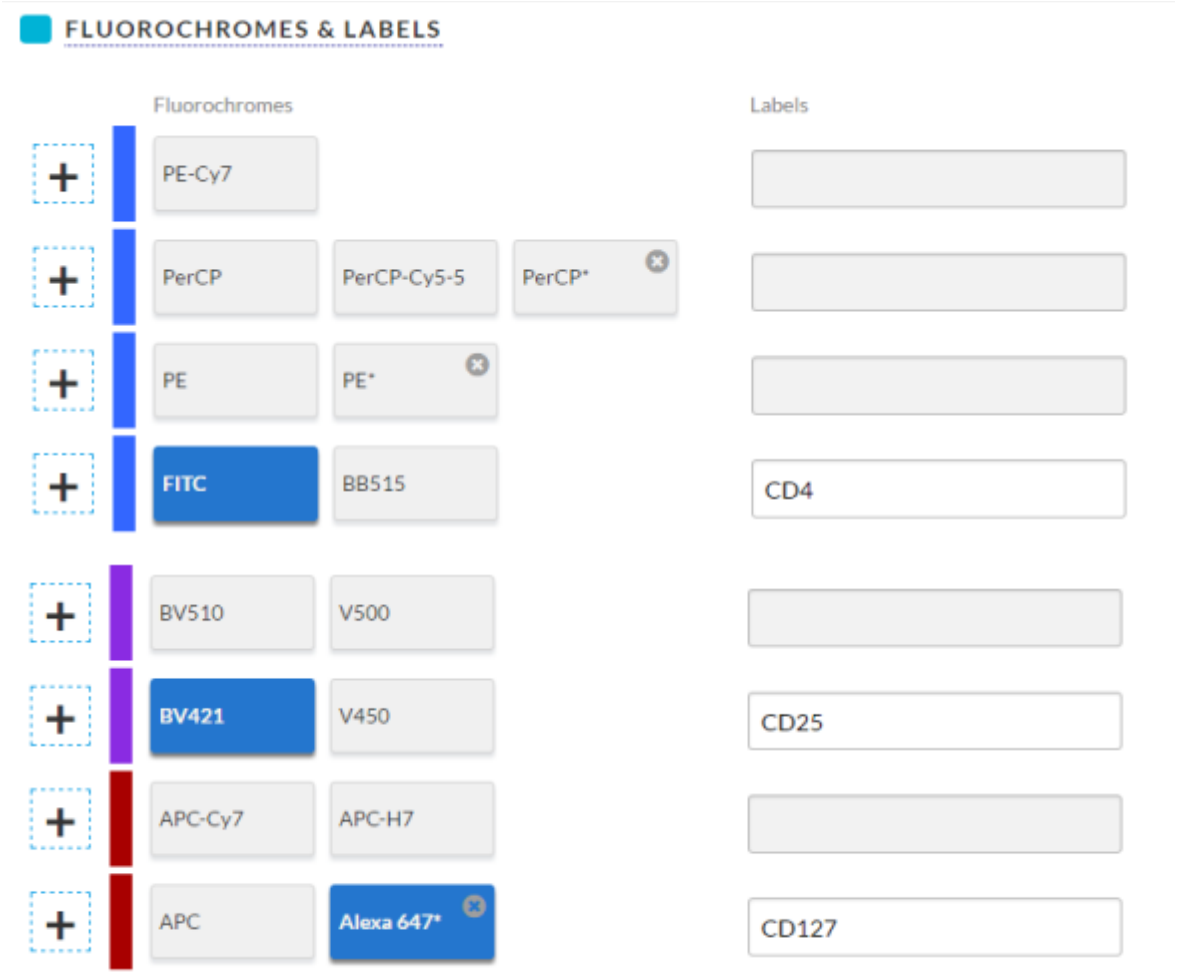
Design Experiment 設計實驗

1. ① Design Experiment：點選 Design Experiment 進行實驗設定

2. 點選 New Experiment 建立新的實驗

3. 設定實驗名稱、實驗內容敘述、上樣溫控及選擇所需之參數，可將 CD marker 或 Protein 名稱輸入置 Label 欄位裡





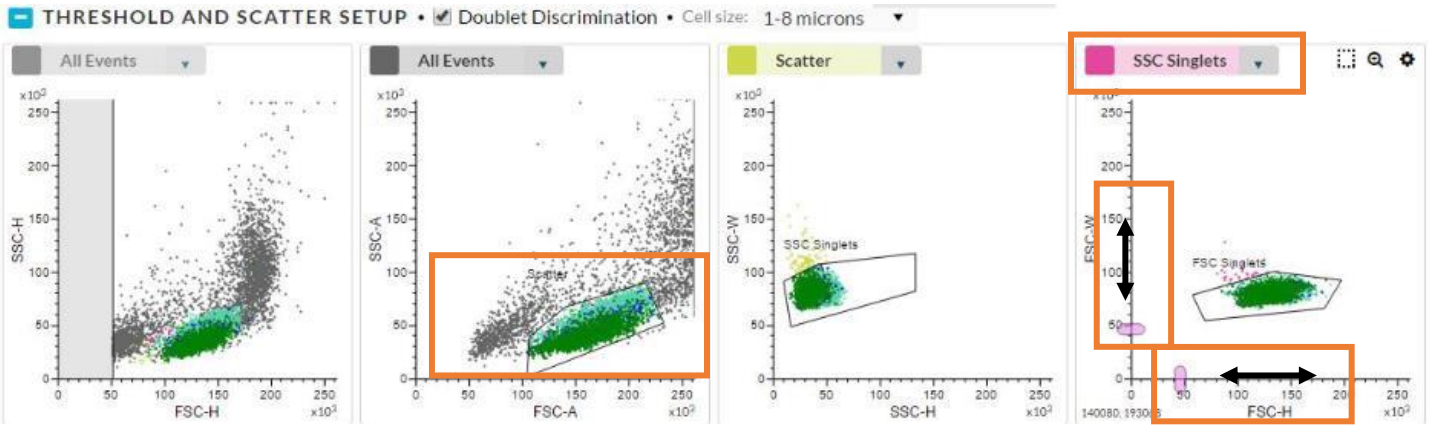
View Data 檢視數據

1. ② View Data：點選 View Data

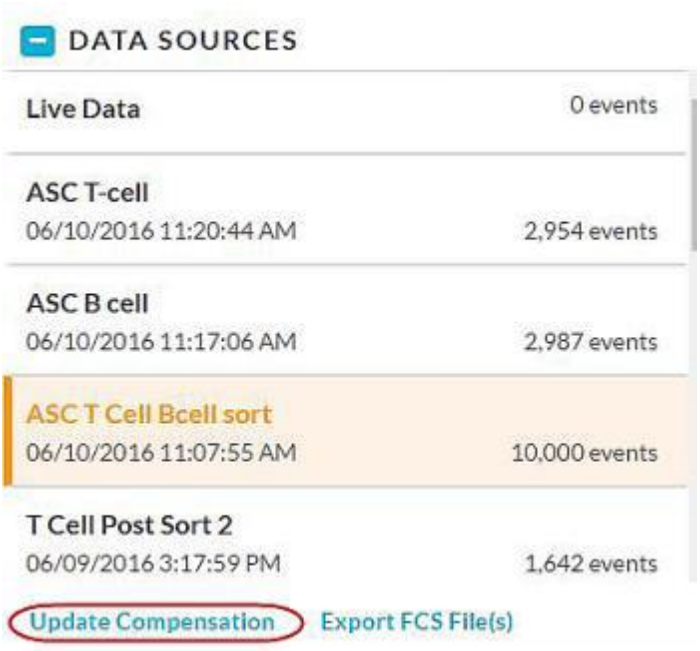
2. 將樣品放置上樣區，於 Acquisition Dashboard 點選 Load Sample，調整適當流速

3. 利用 XY 軸之粉紅色滑標來調動電壓

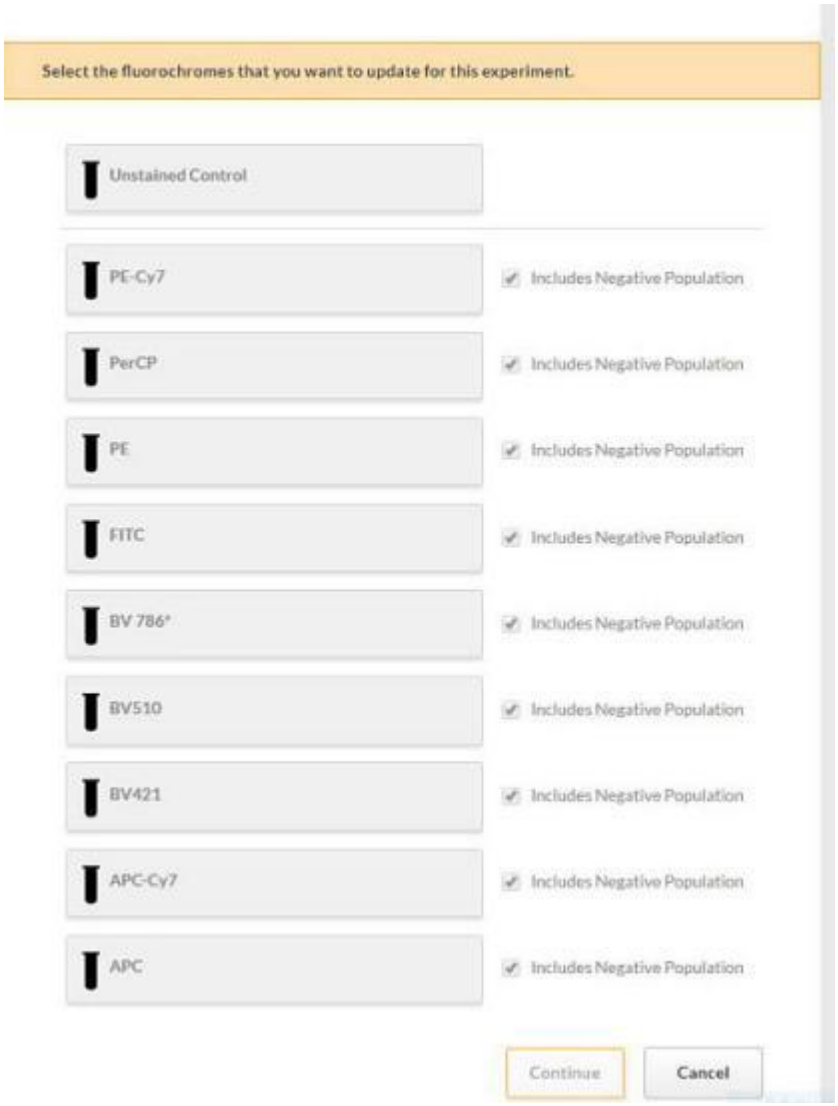
4. 調整 Gate 於適當位置，確認 Dot Plot 所呈現之細胞群



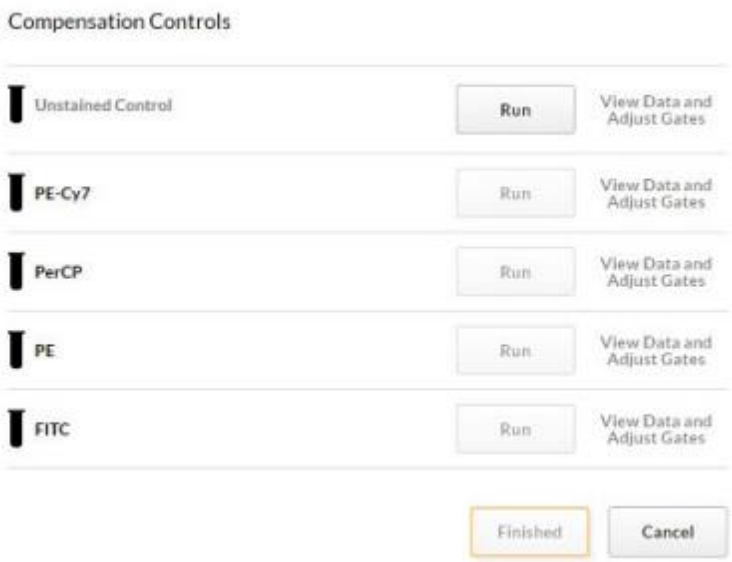
5. 如果需要使用單染樣品調整螢光補償，點擊 Update Compensation，**目前若有使用到 561nm Yellow Green Laser，如 PE, PE 系列染劑 (PE-Texas, PE-Cy5, PE-Cy7..)需自備單染，並在自己的實驗內更新螢光補償 (update compensation)**。若細胞自體螢光較高，建議使用 BD CompBeads 來置備單染樣品，調整螢光補償



6. 選擇需要更新之單染樣品，點擊 Continue

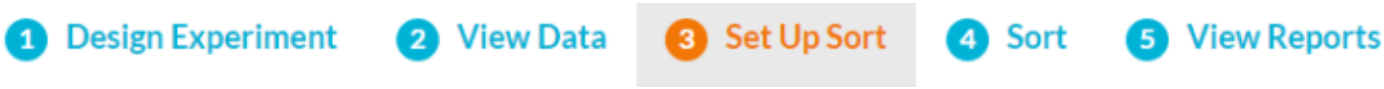


7. 將單染樣品上樣紀錄數據並調整 Negative population/ Positve population 圈選位置，完成後點擊 Finished



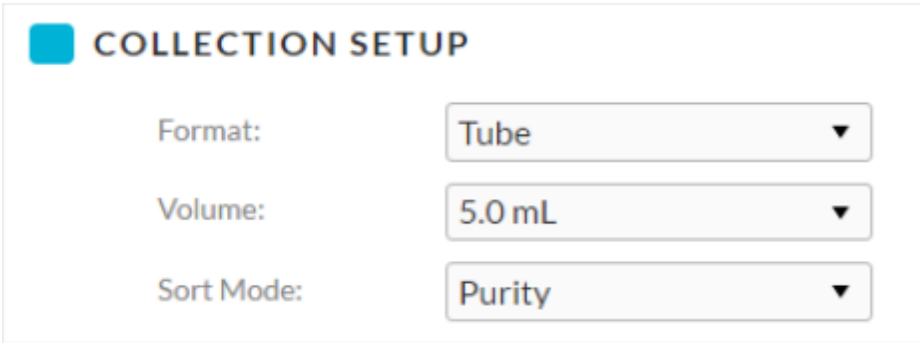
6. 於 Acquisition Dashboard 中 Recording Criteria 設定數據收取之細胞數，並點擊 Start Recording 紀錄分選前的數據

Set Up Sort 分選設定

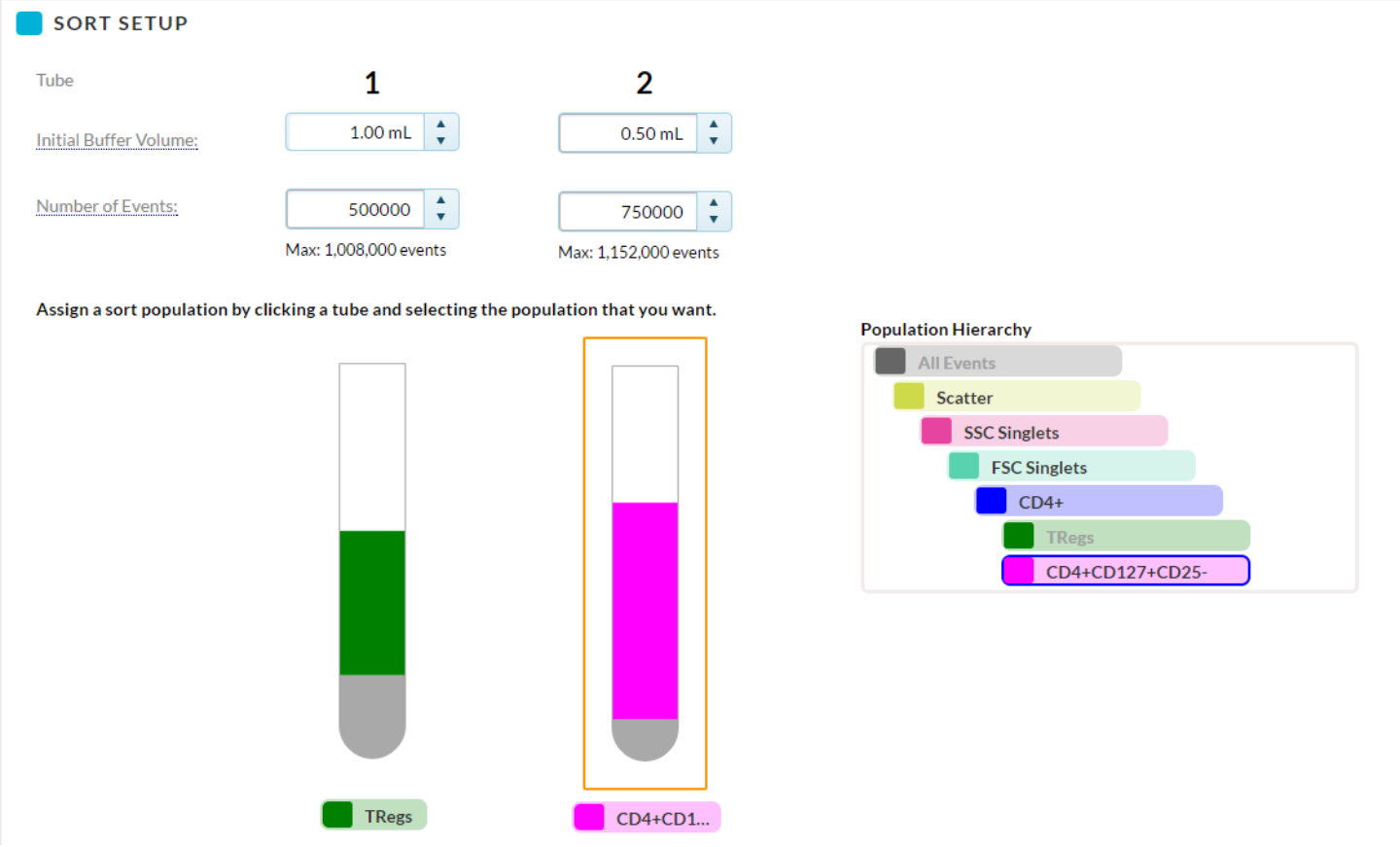


1. ③Set Up Sort：點擊 Set Up Sort，進入分選設定

2. 選擇使用之收集管，Tube, Plate 或 Slide；選擇收集管之體積；選擇分選模式，Yield (產率), purity (純度) 或 single cell (單顆細胞)

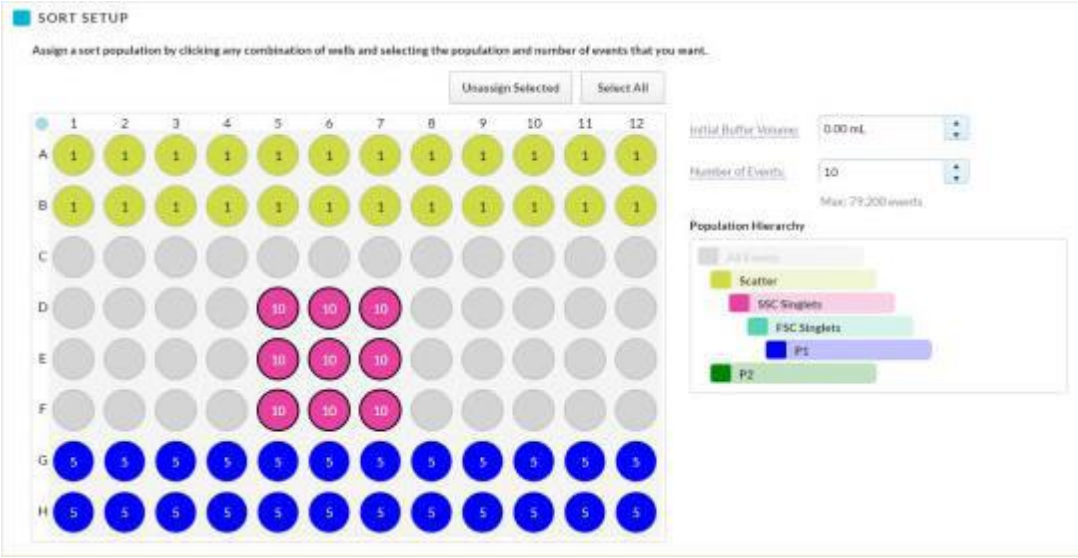


Tube



輸入收集管內預填之 buffer 體積及預期分選之細胞數，分別點擊分選收集管，再由細胞群階層內點擊欲分選之細胞

Plate and Slide



輸入收集管內預填之 buffer 體積及預期分選之細胞數，分別點擊分選收集管，再由細胞群階層內點擊欲分選之細胞

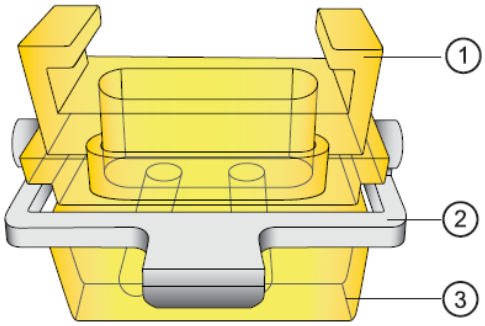
*若選用 Plate/Slide，須加裝 splash shield 於 Sort Block 下方，並點擊 Eject，ACDU 裝置會到 Sort Block 下方，即可放上 Plate/Slide

Sort 分選

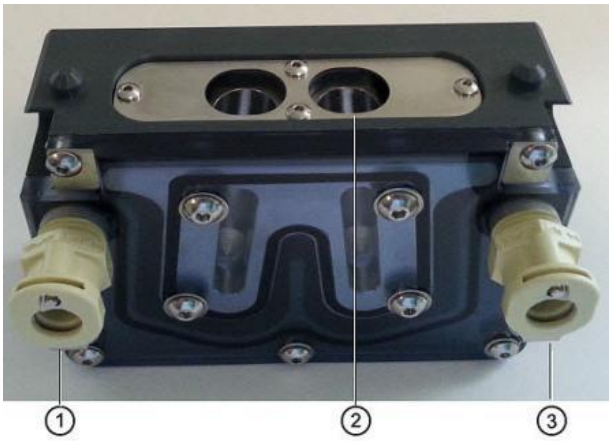
1. ④Sort：點擊 Sort，進入分選視窗

2. 將分選收集管放置在適當之 Tube Holder 中，Tube Holder 亦可接溫控水管維持特定溫度

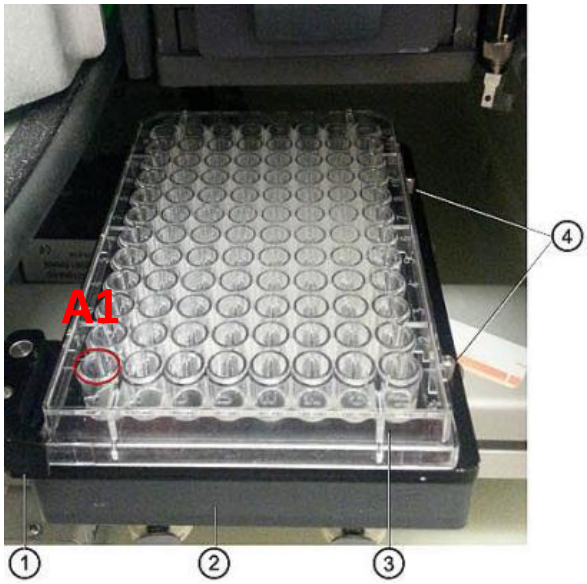
Tube



No.	Description
1	Adapter
2	Handle
3	Tube holder

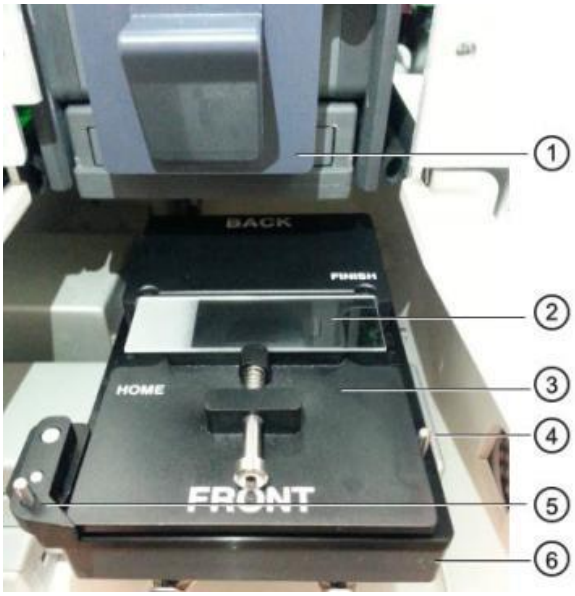


Plate



No.	Description
1	Locking lever
2	Stage platform
3	96-well plate
4	Guiding pins

Slide



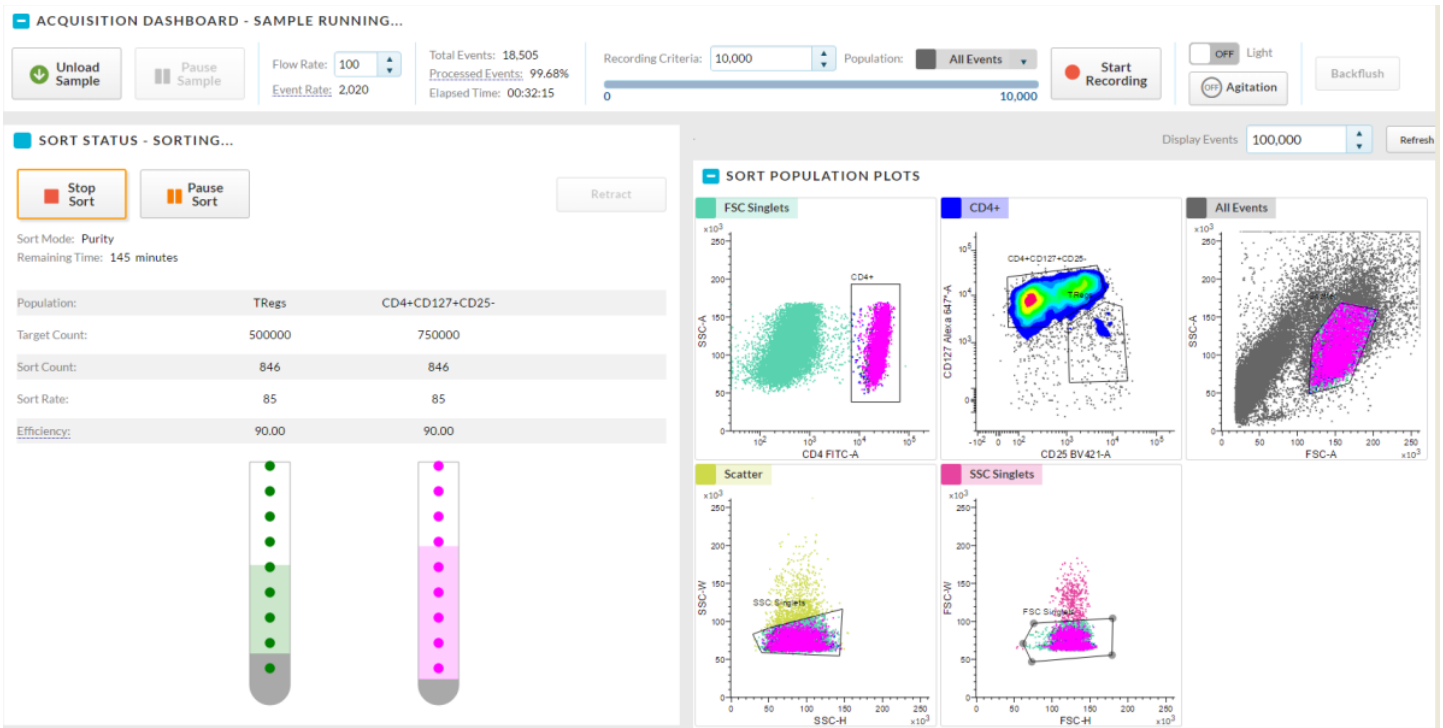
No.	Description
1	Sort block
2	Slide
3	Slide holder
4	Guiding pin
5	Locking lever
6	Stage platform

3. 若細胞仍在進樣倉中，即可點擊 Resume Sample，否則點擊 Load Sample

4. (Optional) 點擊 Start Recording 紀錄數據

5. 點擊 Start Sort

6. 利用分選狀態及分選細胞群圖監測分選狀態



View Reports 觀看分選報告

1.⑤ View Report：點擊 View Report，觀看分選報告

2. 可查看分選資訊及以 PDF 檔格式輸出報告，點擊 Export Report

Data Export 檔案輸出

1. 於 Data Resoruce 下方點選 Export FCS Files，將檔案輸出

2. 按 Ctrl+P 可將 View Data 中的 Dot Plot、階層圖、統計輸出成 PDF 檔。

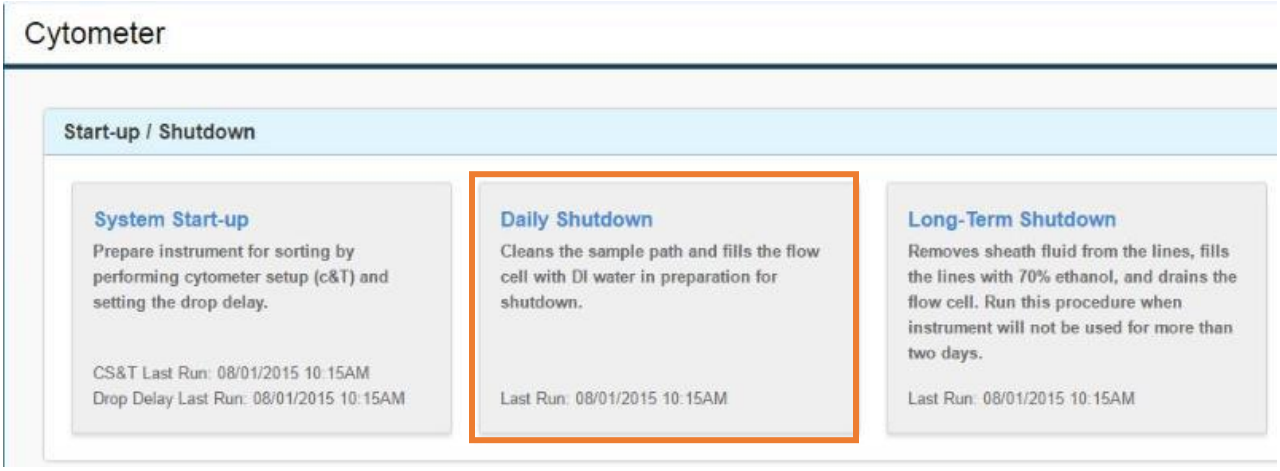
Shutdown System 關機流程

1. 跳至 View Data，放上 3ml FACSRinse，點擊 Load Sample，並以最高流速 100 跑 10 分鐘

2. (同上) 放上 3ml FACSClean，以最高流速 100 跑 10 分鐘

3. (同上) 放上 3ml ddH2O，以最高流速 100 跑 10 分鐘

4. 執行 Fludics shutdown



5. 點擊軟體左方主要工具列 Cytometer

6. 點擊 Daily Shutdown，依照指示完成儀器關機

7. 清洗噴嘴：請將 100um Nozzle 放置裝 ddH2O 之管內，放置超音波震盪機震盪 1 分鐘，並用拭淨紙將多餘水分吸乾，勿用擦拭方式，可能會導致 O ring 之耗損

8. 請將偏極版拆下，利用 ddH2O 清洗，並擦拭乾淨或擺置下方陰乾即可

9. 將 Sheath tank 洩壓，可利用卸壓閥讓 Sheath Tank 完全洩壓，並將 Sheath Tank 填置八分滿

10. Waste tank 請倒入水槽，並加入 1L 漂白水

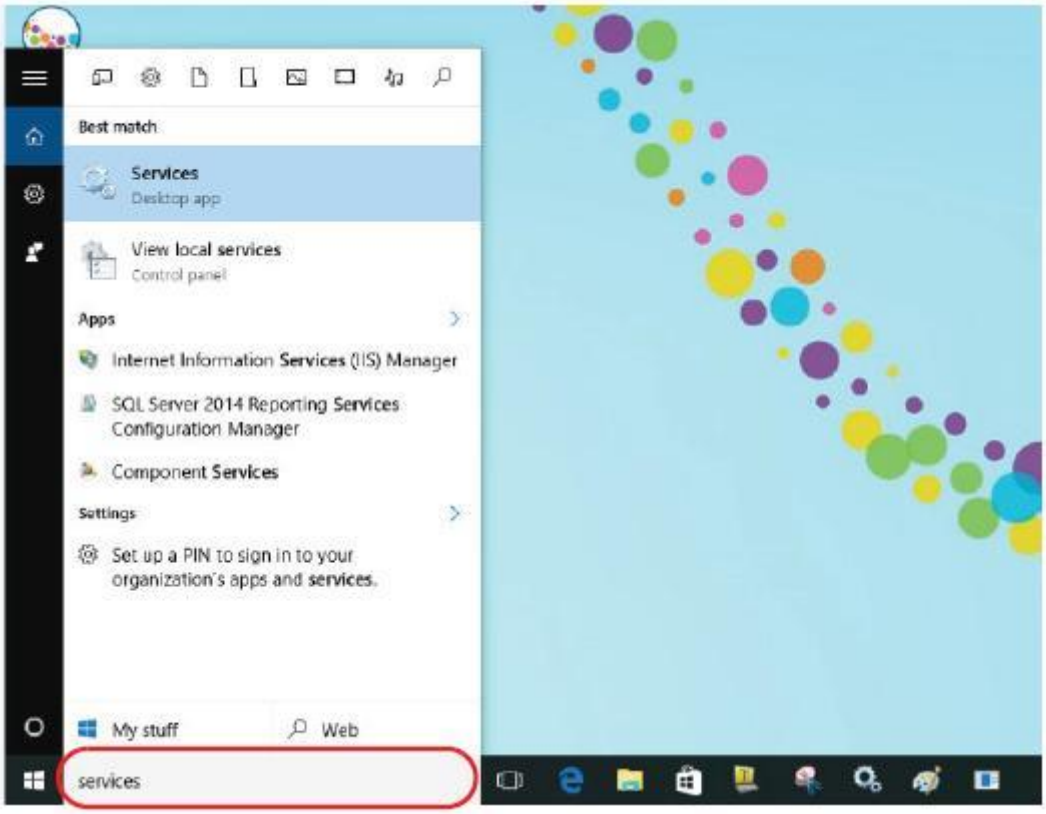
Troubleshooting:

FACSChorus Software Disconnected to FACSMelody

1. Caused by BD FACSChorus Instrument Service is Stop

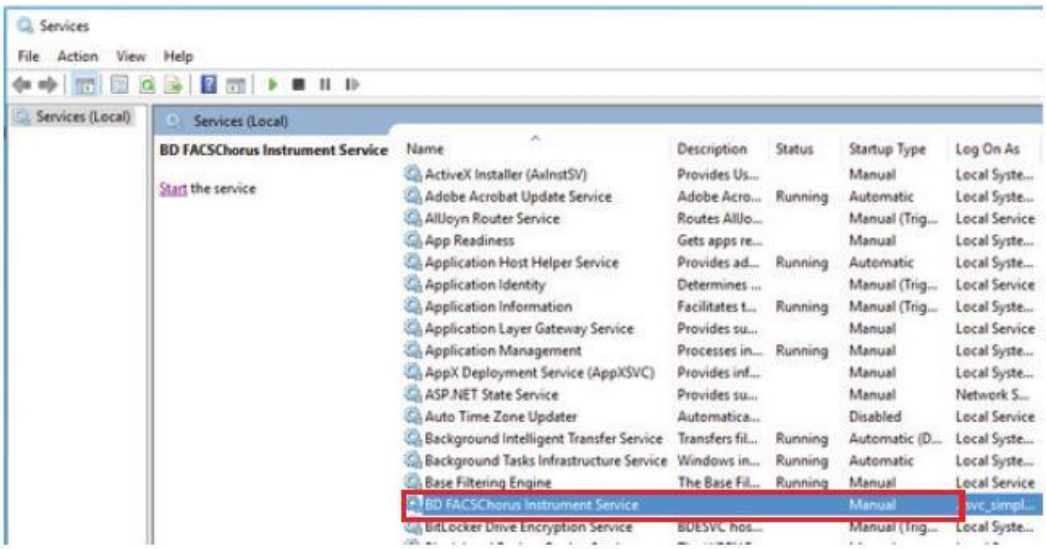
   Solutions: Follow up below procedure to start the BD FACSChorus Instrument Service

a. On the Desktop, type Services in the Search for Web and Windows field to locate the Services app.

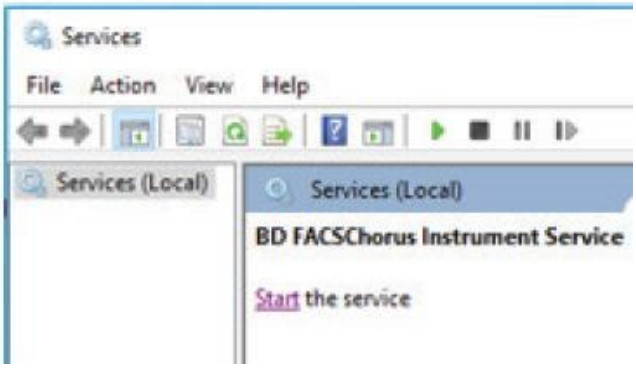


b. Double-click the Services app as above.

c. Locate and click the BD FACSChorus Instrument Service.exe file.



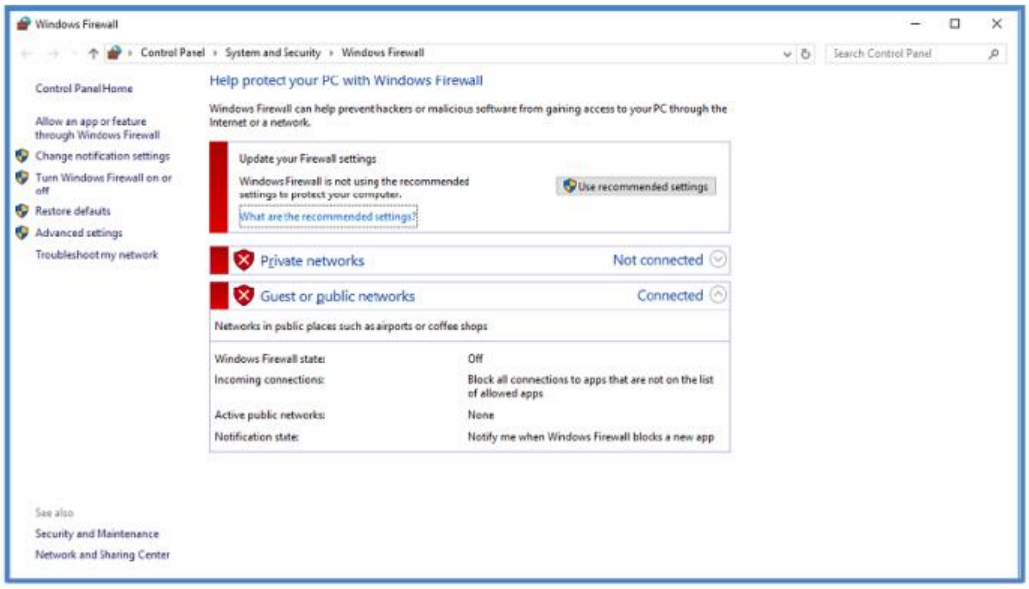
d. Click Start the service as below.



2. Caused by Firewall in ON.

   FACSChorus software can only connect to the FACSMelody instrument if all firewalls are disabled in Windows10.

   Solutions: Open the Windows Firewall setting and turn Windows firewall OFF.



3. Caused by Configurations files are damaged.

   Solutions: Call BD service engineer (0800-737842) to restore configuration files.