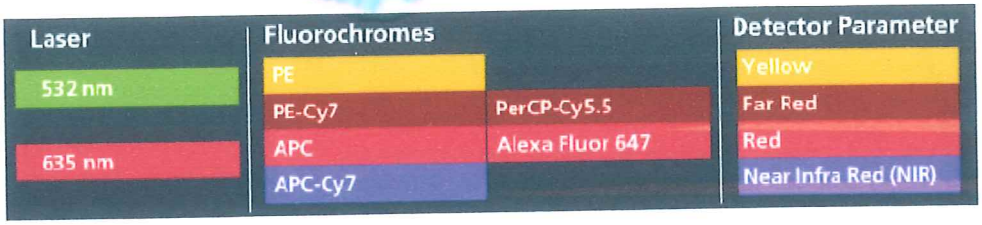
FACS ARRAY USER GUIDE

General: NO FITC!!!
Colors that can be used:
PerCP-Cy5.5 or PE-Cy7
PE , APC-Cy7, APC



FACS Sheath: special for FACS Array

Starting Up procedure

1. Power Switch on the backside of the machine in the middle
2. Power Switch on the front side
3. Switch on the computer
   Username: Administrator
   Password: BDIS

Click on BD Facs Array System Software
Login name: Administrator
Password: welcome

BD Facs Array Software

Open *Experiment Wizard* and select a Wizard session: 

- Screening clones
- CBA
- HLA typing
- Tetramer Titration

Next
Choose template: ***? colors I)*** or *CBA II)*
Next

I) Normal FACS analysis

Follow the protocol of the Wizard step by step:
Instrument Setup and optical Spillover (automatic compensation) – **Yes**
Next
Click on the **colors** you will use
Next
Number of samples – settings not included !
Next
Sample identification – you can add a name
Next
Number of wells per sample
Next
Name of well
Next

Fluorophore labels – **enter the name of the antibodies**

Next

Keyword

Next

Adjusting the plate layout – **continuous horizontal without separator**

Next

Loader Settings: **Sample flow rate 2.0 µl/sec**
Sample volume: 60µl if you have 100µl in the well
Mixing volume 50 µl
Mixing speed 200µl
Number of mix 3
Wash volume 200 µl

Next

Acquisition settings – **how many events**

Next

Experiment name - **Initials (2 letters) followed by the date**

Next

Saving Wizard session – Yes, if you like to save the experiment as template in the Experiment Wizard

Next

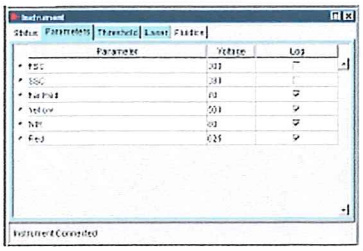
A summary of your designed experiment appears on the screen. If you like to modify go <Back> - otherwise

Finish

Your Experience appears on the workspace 

Setting layout on the plate: unstained
PerCP-Cy5.5/Pe-Cy7
PE
APC-Cy7
APC

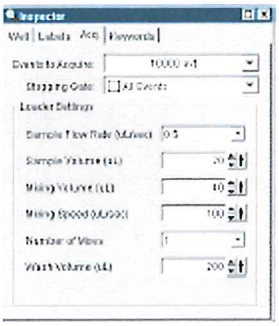
Open *Setup Tools* – *Instrument/Parameters* to open compensation parameters 



Load your plate (click load)

In the Workspace go on *plate_001/ Display: Acquire*

In *Select* click on **None** (wells will turn white)



Click in the Acquire Sheet on well **A1** (it turn green)

Click on Inspector  *Acquisition - Experiment* and change the flow rate to **0.5ul/sec and the sample volume to 100 µl** for the settings

Click on *Setup* and measure a **few** events – click on **setup again to stop**

Settings:

Click on **plate_001/ Acquire/ Select/ None**

Adjust the FSC and SSC voltage to place the population of interest on the scale in the plot. Adjust the voltage of each fluorochrome to ~300 (baseline) and press enter.

Click on the next well **A2**: Setup

- Adjust gate in dot plot to lymphocytes
- Adjust voltage of the fluorochrome if necessary
- Adjust Gate P2 to the peak if necessary

Repeat the procedure for each color

Save Settings to calculate the compensation

Go back to well **A1** click on **Acquire**, the samples will pass automatically and the compensation will be calculated

Make sure that you have enough sample volume in each well!!!!!

Acquire your samples

Go in *Select* to **AUTO**

Click in *Acquisition* on **Acquire** – all programmed wells will be acquired and saved automatically.

If you have more than one plate unload the first, measured one and load the second plate and click on acquired.

Export data

Click on the Browser
Select Experiment
File/BDExport/LICR/Month folder
FCS – Check FCS format :2.0 for Cellquest; 3.0 for FACS Diva

II) CBA-Experiments

Open *Experiment Wizard*
Next
Choose template: **BD CBA Kit**
OKay
Follow the protocol of the Wizard step by step, - **P1 as acquisition stop!!**

NO SETTINGS AND NO CONTROLS ARE NEEDED !!

Export data in folder **Export>CBA**

Cleaning Procedure and Shut down

Go in Display/ Acquire and click on Unload

1) Use the **Clean** function in the *Instrument* menu

or

2) Add the samples at the end your of your samples on the plate

Put in 96 well plate **4** wells FACS Clean followed by **4** wells **dH_2O**

Go to Display *Prepare and a*dd the samples manually by clicking on the first well after your last sample

Display: Acquire/plate_001/Load - **Acquire**

When finish: Display: Acquire/plate_001/**Unload**

Close your Experiment

Click on **Shutdown Fluidics** on the Instrument menu

Wait till the fluidics is shut down - message appears in the Status box – very fast!

Close the BD FACS Array System Software

Shut down the computer and switch of the **two** power buttons

Fill up the Sheath

Empty the Waste each time after use