Document Number:	**SASoM/EQUIP/105.v1**
Title:	**Maintenance and Operation of the BD CytoFlex Flow Cytometer**
Version:	**v1**
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SOP History		
Number	Date	Reason for Change
v1	04/02/2021	Original

1.0 Purpose –

The purpose of this SOP is to outline the Maintenance and Operation of the BD CytoFlex Flow Cytometer in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to the Maintenance and Operation of the BD CytoFlex Flow Cytometer within the SASoM.

3.0 Responsibilities –

It is the responsibility of all users of the BD CytoFlex Flow Cytometer within the SASoM to comply with this SOP. Use of the instrument is restricted to those who have received formal training.

4.0 Procedure –

[A] Preparation:

- Before turning on the instrument, (i) check that the 'sheath fluid' container is full, and (ii) the 'waste' container is empty. Fill / empty as required. Sheath fluid product number B51503
- If you are running biological samples through the machine you need to add a bleach tablet with a little DI water to the waste container before running samples.
- Turn on the CytoFlex by flicking the switch at the back of the instrument and 'login' to the computer (the username is "beckman", contact a member of the Harrison lab group for the password).
- Open the CytoSmart software. By default the machine will be in 'Plate Mode'. To switch it to 'Tube Mode', turn the metal switch adjacent to the tube loader by 90 degrees anticlockwise to show a "T" symbol for "tube" (it displays a "P" in plate mode).

[B] System 'Startup':

- Before running any samples, perform a 'Startup' procedure to 'prime' the system with sheath fluid and warm up the lasers. Only when this has been completed should you run (i) Quality Control (QC) calibration beads, and then (ii) test samples. The Startup procedure should be performed each time the instrument is switched on (even it is has been used previously in the day). This can be done as follows:

- Select: Cytometer → click 'Startup' and place a FACS tube with 2 ml DI water in the arm when prompted. If using Plate Mode, fill any 3 wells with at least 200µl DI water. Follow the prompts and mark which wells you have put water in.
- 'Startup' should take about 5 minutes

[C] Calibrition / Quality Control (QC) runs:

Perform QC to confirm that the gain settings and time delays between the lasers are correct. This process requires the use of fluorochrome-conjugated calibration beads (product code: B53230) that are registered with Beckman:

- QC should be performed before every run.
- Prepare a diluted suspension of calibration beads by first shaking the bottle and then adding 3 drops to 1 ml of DI water / sheath fluid. Vortex immediately before carrying out QC in either Tube or Plate format. If performing QC from a plate, it is possible to simply add 1 drop of QC beads to 250 µl of DI water/sheath fluid directly into a well.
- QC bead aliquots can be stored for up to a week at 4°C in the dark.
- Click "Initialise" to start pumping sheath fluid through the system (note: the machine will prompt you to do this before starting a QC run anyway).
- Select QC (tab at top) → Start QC / Standardisation.
- There will be a drop-down box which asks for the LOT number of the beads you are using. **MAKE SURE WHAT YOU ENTER MATCHES WITH WHAT IS ON THE BOX!!!**

- Click Start and load the tray / tube when prompted after which the QC will run automatically. Keep an eye on the event rate; if it is consistently below 100 events / second then this is an indication of a blockage and the QC process will fail.
- If the QC process does fail, perform a daily clean cycle (see below) and then try again. If it continues to fail after a thorough clean, contact a member of the Harrison lab group.
- The instrument will automatically generate a Pass / Fail report at the end of the procedure. The summary will also display the "acceptable ranges" for each parameter. Everything should 'Pass' before running a sample. For reference, there is a PDF file on the desktop which shows the results of QC performed on the 'Day of Installation'. Refer to these values and if they are different by a large margin then contact Ollie or a member of the Harrison lab group.
- If everything has passed click File → Close QC / Standardisation.

After completing the QC calibration check, put the instrument into "Standby" mode (there is a button in the software saying "standby which you click) until you are ready to run your samples.

[D] Running Samples:

Before commencing a run make sure within your samples you have the appropriate controls including single stained controls (one for each colour you are using).

In plate mode:

- Load your samples into a flat-bottomed 96 well plate (200 μl per well max)
  - It is a good idea to make note of which sample is where on the lid of the plate and take a picture of it for when you analyse/write up later
- Go file → new experiment, to generate a new experiment file. give your experiment an appropriate name
- On the left side of the screen it will show a box which will list all your tubes/wells, at the moment this will be empty
- Click "add plate" and select which wells you are using and what direction you want the machine to sample in (by rows, by columns, left to right etc.)
- You can block highlight a particular set of wells to assign them a "group" which may be useful for better keeping track of certain experimental conditions
- The machine will prompt you to assign parameters for your samples including:
  - Whether or not the wells need mixing and or a backflush
  - What filters you want to look in (i.e. what colours are you using)
  - Stopping rules (i.e. do you want to keep recording samples for a certain length of time or until a specific number of events is reached etc)
  - Application of any previously calculated compensation grids (this is more relevant to multicolour panels where signals can overlap)
- Once you are happy with the plate layout and parameters click "Okay". Your assigned wells should then be listed in the "Samples" box
  - Note: at any time you can add more wells/samples or edit parameters

- Use the blank space in the middle/right side of the screen to add your desired plots e.g. dot plots, histograms etc, along with any gates you want.
- If the machine is in standby then click “initialise”
- Highlight a sample (ideally a control) and click “run”. The machine will begin to aspirate and acquire cells in you sample but will not record it i.e. it will not save any data.
- At this point, adjust your gain settings, plots and gates to best visualise your samples (i.e. altering the axis of your plots or adjusting the shape/size of your gates).
- When you are happy hit “record”. The machine will then start saving the data for each event that goes through the machine until it reaches the first stopping condition.
    - During this time you can still edit your plots and gates but you cannot make any adjustments to the gain for your respective channels.
- Once it reaches the stopping condition the machine will stop aspirating your sample and will save all the data for that well as an FCS file.
- You can then either manually select each sample → run → record if you feel you need to make adjustments to what is displayed or you can click “autorecord” which will tell the machine to go through each of your samples sequentially
    - Once again, any analysis or adjustment of plots and gates can be done retrospectively of acquiring your sample however you cannot alter the gains once a sample has been recorded.
- When the machine finishes the run it will eject your plate
- Perform a daily clean or alternatively you can assign wells at the start of your run to contain cleaning solution and DI water and the machine will automatically clean itself at the end of the run.

In tube mode:

- Click “add tube” above the sample box and enter your parameters and stopping conditions as you would with a plate.
- How to acquire, visualise and analyse samples is the same as that with a plate except there is no auto-function, for each separate sample in the same experiment you need to manually replace the tubes once each one finishes recording data.
- Perform a daily clean once you have finished acquiring samples.

[E] Daily Clean:

NOTE: A ‘Daily Clean’ cycle should be performed at the end of EVERY run of samples. The machine should NEVER be switched off before first carrying out a ‘Daily Clean’ cycle.

In plate mode:

- Fill at least 3 wells with blue cleaning solution (FlowClean cleaning agent, product code A64669) and at least 3 wells with DI water (doesn’t matter about the exact volume as long as its roughly >200 µl
- Click Cytometer tab → Daily Clean.

- A pop-up will be displayed where you have to define the plate format i.e. assign (i) which wells you have used, and (ii) what solution is in each well.
- Once you have correctly assigned the wells and loaded the plate, hit 'Start'.
- The length of time it takes to perform a clean cycle depends on the number of wells you are using, but typically lasts anything upwards of 12 minutes.
- The Plate will be automatically ejected once the clean cycle is finished.

In tube mode:

- Fill one FACS tube with 5 ml of 'Cleaning Solution' (blue in colour) and another tube with 5 ml of DI water.
- Click Cytometer tab → Daily Clean.
- The instrument will then ask you to insert the Cleaning Solution tube. Load the tube and click Start. After a certain period of time the machine will eject the tube and ask for the next tube (containing water). Load this second tube, and click 'Continue'.
- The tube will again automatically be ejected automatically once the Clean Cycle is finished (approximately 10-12 minutes).

[F] Shutdown procedure:

Once you have finished running samples and performed a 'Daily Clean Cycle', place the instrument in "Standby Mode " (how) before closing the software and switching off the instrument at the back.

- Data Analysis can be performed at any time without the need to operate the instrument simply by opening the software.

[G] Deep Clean Procedure:

A deep clean should be performed once a month. Currently this is occurring on the 20th of every month.

- Put the machine on "Standby" mode
- Select Cytometer → Deep Clean
- Click Start

The Deep Clean cycle will then pump Contrad 70 (product code 81911), an intensive cleaning solution, through the fluidics system of the machine. Whilst the process of pumping the fluid is very quick, it is recommended that this procedure should be carried out for a period of 30 minutes.

After 30 minutes, click on "Initialise" to perform a 'Daily Clean' cycle (as described above) before (i) running samples or (ii) simply switching the instrument off.

5.0 Personal protection –

A Howie coat must be worn at all times. Gloves must be worn when handling solutions and running samples.

6.0 Training –

All users have to be trained by a designated person before using the Instrument.

7.0 Related documents –

7.1 CHARM _ 22100_Antibody Expression by Flow Cytometry
7.2 Manual for the instrument (available online).
7.3 SASoM-METHOD-128-Antibody Detection by Flow Cytometry

8.0 Approval and sign off –

Author:

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Signature: Date: 04/02/2021

Management Approval:

Name: Peter Mullen

Position: Research Fellow

Signature: Date: 04/02/2021

QA release by:

Name: Alex MacLellan

Position: QA Manager

Signature: Date:

STANDARD OPERATING PROCEDURE

Please sign below to indicate you have read this S.O.P and understand the procedures involved.

NAME	POSITION HELD	SIGNATURE	DATE