

# Standard Operating Procedure Of The CytoFLEX S Flow Cytometer At The Institute Of Virology

## Before starting with the CytoFLEX S Flow Cytometer:

1. A new user needs to get an introduction to the flow cytometer by Dr. Christopher Kornfeld (Beckman Coulter, e-mail: [ckornfeld@beckman.com](mailto:ckornfeld@beckman.com), tel. +41 (0) 78 637 8 549). Besides, it is recommended that an inexperienced FC user follows a course on general aspects of flow cytometry data acquisition and analysis offered by the Flow Cytometry Core Facility of the University of Zurich ([link](#))
2. Book the Flow Cytometer through the room/ device booking platform of the Vetsuisse Faculty:  
<http://vetdek-web.uzh.ch/vsfinfrabookings/week.php?year=2018&month=10&day=09&area=1&room=120>
3. The booking time should not exceed 3 hours. If more extended periods are required, the booking time needs to be justified and discussed with the FC operator.

**IMPORTANT:** Please, respect your booked time. If you do not need your reserved time slot (either partially or entirely), please readjust your booking accordingly – at the earliest convenience to ensure that others users can use the instrument. **In case of any schedule conflict, internal users of the Institute of Virology have the priority.**

## To Switch ON the machine:

1. Turn ON the computer
2. Turn ON the CytoFLEX S FC by pressing the switch bottom at the back left side.
3. Start the CytExpert software, set/ load your protocol, save your data...and start acquiring your data. Take into consideration that you have two modes of acquisition:
  - i) Manual Injection mode.
  - ii) Semi-automatic injection mode.

## Using the manual injection mode:

1. Select **Sample Injection Mode>Manual** in the FC menu to change the sample injection mode selection. The sampler status icon located in the bottom right side of the screen changes to display *Manual Sampler*.
2. Manually swing the sample tube holder out from the standby position to the sample loading position
3. Select **initialize**
4. Load the sample tube. Note: the sample tube holder accommodates 1.5 ml, 2.0 ml and 12 x 75 mm sample tubes.

5. Manually swing the sample tube holder gently back to the standby position
6. Manually raise the sample tube holder gently to the sample acquisition position and hold them in that position.
7. Select **Boost** to transfer the sample to the flow cell
8. Select **Run**. **NOTE:** *The sample runs at the designated flow rate, and the Cytometer begins to acquire data.*
9. When you are satisfied with the appearance of the data, select **Record** to record the data.
10. Wait for the data acquisition to finish or select **Stop**. Then, manually lower the sample tube holder and move it to the sample loading position
11. Select **Backflush** to clean the probe.

### Using Plate Loader Injection Mode

The plate Loader Injection mode can be used for running small volumes using the following plates: 96-well flat bottom, 96-well V-bottom, and 96-well U-bottom.

1. Select **Sample Injection Mode> Plate Loader** in the Cytometer menu to change the Sample Injection mode selection
2. The restart warning prompt appears on the screen. Select **OK**. **NOTE:** *The restart warning only appears when switching to and from the Plate Loader sample injection mode.*
3. Turn the Cytometer's main power switch **OFF**.
4. Remove the single tube sample probe and replace it with the plate loader **PEEK** tubing.
5. Turn the Cytometer's main power switch **ON**. The sampler status icon located in the bottom right side of the screen changes to display *Plate Loader*.
6. Select **Initialize**.
7. Select **Eject**.
8. Place the plate flat on the plate holder and ensure that it is secure. **NOTE:** *Ensure that plate well A1 aligns with the position A1 on the plate holder.*
9. Select **Load** to load the plate
10. Select **Run**. The plate loader automatically loads the plate holder stage and begins to acquire data.
11. When you are satisfied with the appearance of the data, select **Record** to record data.
12. Wait for the data acquisition to finish or select **Stop**. The Cytometer back flushes the sample probe.
13. Select **Eject** to eject the sample loader.

A **USER MANUAL** for CytoFLEX S is at your disposition on the side of the instrument's computer.

**IMPORTANT:** Always filter your samples right before acquisition (i.e., 35 µm filter tubes, FALCON Ref. 3522535) to avoid clogging the system. This is a strict requirement for all samples in any case.

### After your data acquisition:

1. Once you finished with your data acquisition proceed with the cleaning of the instrument.

For that:

#### When using the manual injection mode:

1. Select **Daily Clean** in the Cytometer menu
2. Add 2 ml of FlowClean solution to an unused sample tube
3. Add 2 ml of DI water to an unused sample tube.
4. Insert the sample tube with 2 ml of FlowClean solution into the sample holder and select **Run**. **NOTE: The default cleaning time is 3 minutes.**
5. Remove the Flow Clean tube
6. Insert the sample tube with 2 ml of DI water into the sample holder and select **Run** to perform the second step of the cleaning process. **NOTE: The default cleaning time is 5 minutes.**
7. After the process has been completed, remove the sample tube and close the Daily Clean window.

#### When using the plate loader mode:

1. Select **Daily Clean** in the **Cytometer** menu. The Daily Clean window appears. The plate loader automatically ejects the plate holder stage.
2. Follow the on-screen software prompts and select the desired wells for cleaning agent (3 wells) and deionized water (5 wells).

#### IMPORTANT:

- (a) *Select the desired wells for cleaning agent and select **Set As Cleaning Agent Well**.*
- (b) *Select the desired wells for the deionized water and select **Set As Deionized Water Well**.*

**NOTE:** To deselect water wells, select the desired well and select Set As Empty Well.

3. Select **Load** to load the plate
4. Select **Start** to start the cleaning procedure. The message *Please confirm that the correct plate is appropriately placed and press OK* appears. Select **OK**
5. Select **Close**.

#### Sheath Fluid/empty waste:

Before starting your analysis: check that at least one full Sheath Fluid container is at disposition. If not please prepare new Sheath Fluid (see recipe below) and make a quote "missing Sheath fluid" in the logbook. If the Sheath Fluid is empty or the waste container is full during the acquisition, a notification will appear in the acquisition panel of the CytExpert software followed by a beeping sound. This signal means that the acquisition needs to stop immediately and replace the Sheath fluid container (back card with blue tube) and/or the waste container

(front white plastic container with a yellow tube). Once replaced, click the bottom in the CytExpert control panel and proceed with the acquisition.

**Preparation of SHEATH FLUID:** Use the empty Sheath fluid container, add 100 ml of Bronopol stock solution (2.5 g/l) to the canister and fill up with filtered water. The filtered water is to be taken from the device above the sink in the room TPV 00.24. Mix well and close the container with the lid and put it next to the Fluidic installment.

**WASTE replacement:** Add 1% (40 ml) Kohrsolin FF disinfectant to the full waste container. Label the **WASTE** container with tape that indicates your NAME and DATE. Place the container below the bench. At 24 h post-disinfectant addition, empty the content of the **WASTE** container into the sink, rinse it with water and put the empty **WASTE** container back below the bench of the CytoFLEX S FC.

**IMPORTANT:** It should always be at least one container with SHEATH fluid ready next to the fluidic Installment. **NEVER MIX UP SHEATH AND WASTE CONTAINER.**

Exit the CytExpert software and sign in the logbook on the desk, stating the **DATE**, your **NAME** and **INSTITUTE** and any **EVENT** (if happened—such as “missing Sheath Fluid”).

#### **DATABASE:**

- Save you Data exclusively on the **Windows (C:)\CytoFLEX S Data\Users** and Compensation data are stored in **Windows(C:)\CytoFLEX S Data\Compensation\compensation users**.
- Export your data via an external hard drive or via the network institute server using your UZH username and password.

**IMPORTANT:** Previous the use your external hard-drive or memory stick, check it for any virus or Trojan.

#### **REPLACING THE OPTICAL FILTER:**

When it is required to use an optical filter with non-standard wavelength, you must replace the optical filter yourself.

1. Confirm that the instrument is in the standby state or that the instrument is turned off.
2. Confirm the laser corresponds to the channel in which the optical filter is to be replaced.
3. Open the top cover of the instrument.
4. Press the spring piece of the WDM cap corresponding to the laser, and open the WDM cap. **DO NOT TOUCH THE OPTICAL FILTER GLASS PIECE.**
5. Use vertical force to remove the optical filter to be replaced, and note the color identification and wavelength identification on the optical filter bracket.

6. Insert the optical filter to be installed vertically into the corresponding position, taking care to align the wavelength identification with the left, and that the brackets are inserted into the bottom
7. Close the WDM cap and the instrument top cover.
8. Turn on the Cytometer and open the software.
9. Select Detector Configuration in the Cytometer menu and create a new instrument configuration based on the settings of the new optical filter.
10. For more details, refer at the user manual pg 11-57/11-60

**IMPORTANT:** Once finished with your measurements, return the filters to the standard position.

**A**



**B**



The standard position of the optical filters in the Cytoflex S with **(A)** and without **(B)** the WDM caps.

For further information or help, please contact Catherine Eichwald (e-mail: [ceichwald@vetvir.uzh.ch](mailto:ceichwald@vetvir.uzh.ch), Tel. extension: 58711) or Bernd Vogt (e-mail: [bernd.vogt@uzh.ch](mailto:bernd.vogt@uzh.ch), Tel. extension: 58714).