General Rules:

- No gloves

- No propidium iodide (PI) or ethidium bromide

- When using **intercalating agents**: wash after measure 1 min with Water, 3% hypochlorite and again water. Then Daily Clean

- Sheath fluid:
  - at least 2h old MilliQ water from error free water stations
- **<u>40 µl of sample are lost</u>**! 30 µl for initial boost, and ~10 µl (V-bottom) are necessary to take up sample.

- When you are **<u>FIRST</u>**:
  - System Start UP
  - Daily QC
  - Daily Clean

- When you **<u>finished</u>** your experiment
  - Daily clean
  - If sheath fluid is quite low: Fill up and empty waste

- When you are the **<u>LAST:</u>**
  - **2x** Daily clean
  - Refill sheath
  - Empty waste and add 50 ml 6% Na-hypochlorite

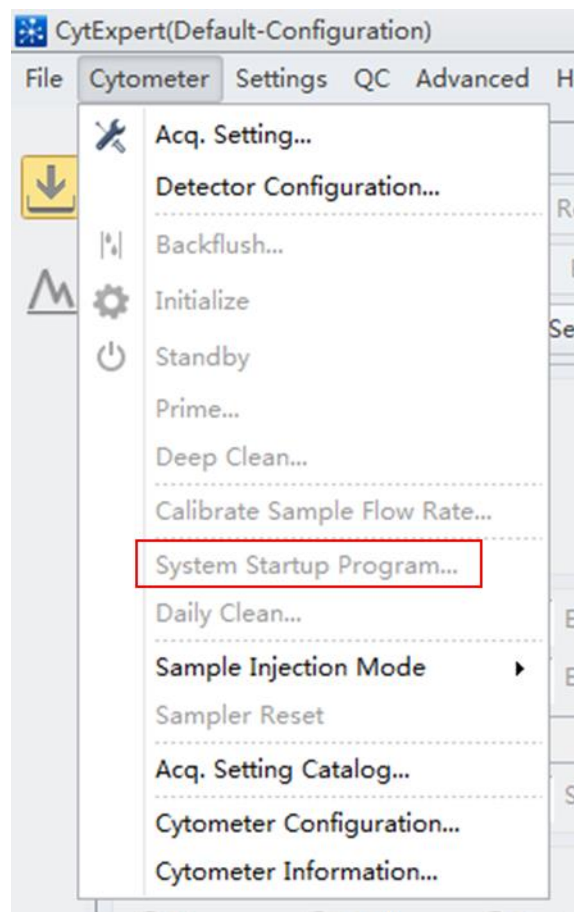
System Start-Up

When:

Every time you start the cytometer and every 24 hours (mandatory from the software)

How:

- Turn on the cytometer and the PC. Start CytExpert. The order does not matter.
- Klick on Cytometer → System Startup Program…
- Pipet **200 µl MiliQ H_2O** in 3 wells. <u>Use a Clean/QC Plate</u>.
- Define the wells that you used and klick “Start” or “Initialize”→”Start”



Next steps:

- Quality Control – QC
- Daily Clean

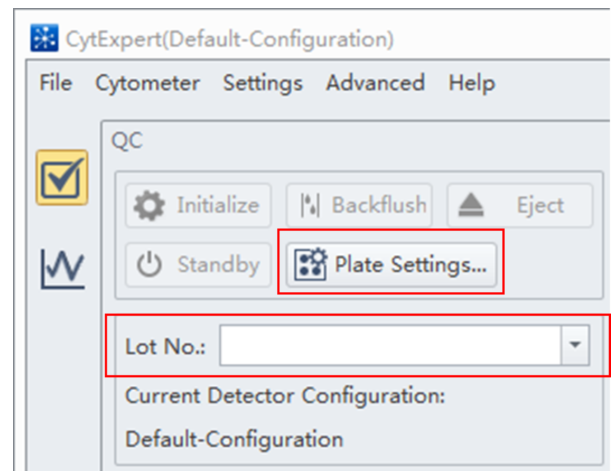
Quality Control – QC

When:

Once a day, when you first use the cytometer. After System startup

How:

- Klick QC → Start QC
- Vortex the FACS tube containing **diluted QC beads** (3 drops/ml MiliQ H_2O) **well**!
- Pipet **200 µl diluted QC beads** in the corresponding well
- Enter Lot No. (written on the side of bead package) AND define the well containing QC beads in Plate settings



- After a successful QC pipet the **beads** from the well **back into the tube** to reuse them and wash the well with water.
- Klick "Start" or "Initialize"→"Start"

Troubleshooting:

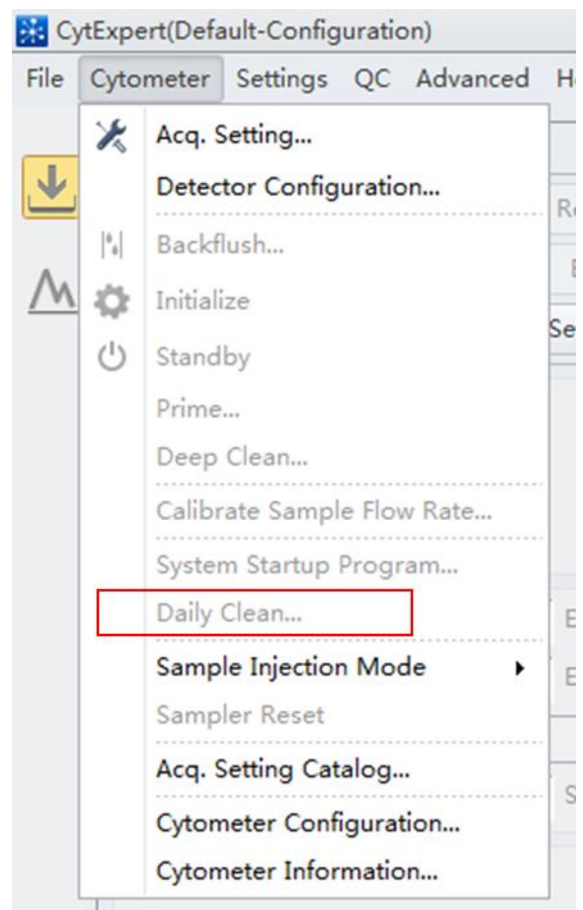
- If QC beads are empty generate new ones (3 drops/ml MiliQ H_2O)
- Sample rate to low: vortex beads better
- Laser delay to high/low: no problem
- Laser power 0/low: restart cytometer
- **Laser too high: STANDBY! Call for help**
- Other fails:
  - Did you vortex well enough?
  - Did you choose the correct well?
  - Use another well.
  - Prime the cytometer (see→ refilling sheath fluid)
  - Repeat QC.
  - Restart Cytometer

Daily Clean

When:

- After QC
- After you finished your measurement
- When shutting down: **2 times**
- When you feel like the Cytoflex may be dirty and needs to be cleaned
    - After blood
    - After bacteria
    - After sticky things that we don't want in the system

How:



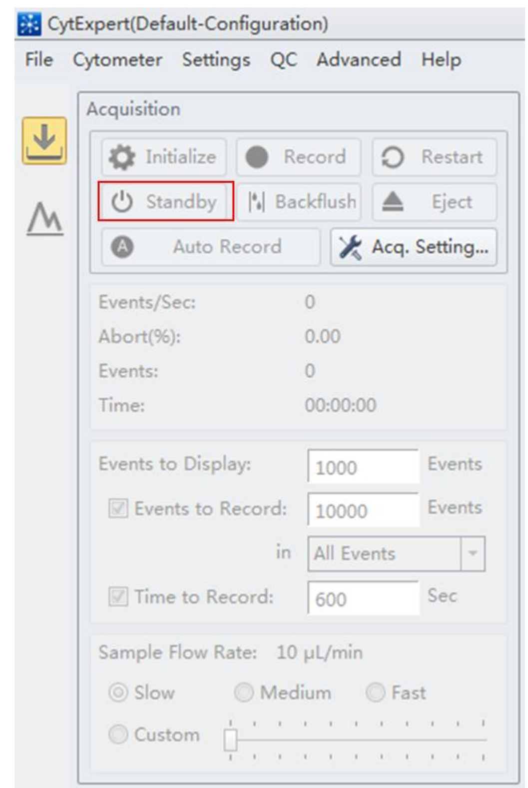
- Klick on Cytometer→Daily Clean **NOT deep clean**
- Pipet **200 µl blue Cleaning solution** in 3 wells and **200 µl MiliQ H_2O** in 5 wells. Use the Clean/QC Plate.
- Define the wells you used.
    - **Attention!** The blue cleaning solution is depicted orange, while water is depicted blue in CytExpert.
- Klick "Start" or "Initialize"→"Start"

Refilling Sheath fluid during measurement

When:

When it’s about to get empty or when the cytometer is already crying because it is empty.

How:



- Mute alert.
    - Klick on “mute” on the right bottom corner
- Don’t panic, you can still measure a few samples
- Klick on “Standby” or “Pause” and then “Stop” when in automated measurement.
- refill the sheath fluid without generating air bubbles in the tank
- Empty the waste and add 50 ml 6% Na-hypochlorite
- Klick Cytometer--> "Prime". You are sure that you want to prime, so klick "Yes"

NB:

- **Always refill the sheath fluid in standby-modus**. Otherwise the cytometer will slowly, but surely suck in air, until the pump can no longer pump any fluid.
- Take care to not generate any air bubbles! This cytometer is quite sensitive, especially the yellow laser (561 nm). Air bubbles disturb the flow, cause QC failures, unspecific events and so on. Therefore, decant the sheath fluid into the tank without generating any and perform a “Prime” afterwards to flush the tubings.

Shutting Down

When:

When you are the last person who will use it today.

How:

- Perform **a double** Daily Clean (6x cleaning, 10x water; 2 columns)
- Close Cytexpert
- Turn off the cytometer
- Turn off the PC
- **Empty** waste, add 50 ml 6% Na-hypochlorite and **refill** Sheath-fuid
- Have fun with your data ☺

Spectra viewer:	https://www.bdbiosciences.com/sg/research/multicolor/spectrum_viewer/index.jsp															
	3. LASER (80 µs)		2. LASER (40 µs)			4. LASER (120 µs)						1. LASER (0 µs)				
Laser	**488**		**638**			**405**						**561**				
Filter	525/40 BP	690/50 BP	660/20 BP	712/25 BP	780/60 BP	405/10 BP	450/45 BP	525/40 BP	610/20 BP	660/20 BP	780/60 BP	561/10	610/20 BP	585/42 BP	780/60 BP	690/50 BP
Bandwidth	505-545	665-715	650-670	699.5-724.5	750-810	400-410	427.5-472.5	505-545	600-620	650-670	750-810	556-566	600-620	564-606	750-810	665-715
Fluorochromes	FITC AF488 CFSE DHR TO	PC PC5.5 PerCP PerCP-Cy5.5 PC5 (7-AAD)	APC AF647 eF660	APC-eF700 AF700	APC-AF750 APC-Cy7 APC-H7 APC-eF780		PacBlue V450 eF450 BV421 DAPI	Krome Orange AmCyan V500 BV510	Violet610 BV605 Qdot605 mCherry	Violet660 BV650 Qdot655			ECD PE-TxRed PE-CF594 mCherry eF610	PE dsRed tdTomato	PE-Cy7	PC PC5.5 PerCP PerCP-Cy5.5 PC5 7-AAD
Channel Name	**FITC**	**PerCP**	**APC**	**APC-A700**	**APC-A750**	**VSSC**	**PB450**	**KO525**	**Violet610**	**Violet660**			**ECD**	**PE**	**PC7**	**PC5.5**